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Engineering Functional Polymer Capsules Towards Smart Nanoreactors

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Engineering Functional Polymer Capsules Towards Smart Nanoreactors

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1. Introduction

The design and construction of integrated chemical ensembles for studying complex biological systems is attracting considerable interest in a wide range of research communities.¹ Amongst these studies, one of the most successful stories is the construction of hollow compartments, *i.e.* in the form of nano-or microscale capsules with an aqueous core surrounded by an hydrophobic domain.² Such a constructed model is of considerable interest due to promising applications in materials science, biomedicine, and catalysis.³⁻⁴ The domain of the hollow compartments can either be of inorganic nature or can be made up of organic molecules such as lipids (*e.g.* liposomes) and polymers.⁵ To date, a great variety of polymer-based compartments has been demonstrated, including polymeric capsules⁶⁻⁸ and vesicles of an ordered membrane of amphiphilic block-copolymers.⁹⁻¹¹ In comparison with other reported hollow compartments,^{5,12} the main difference lying in polymeric vesicles is their polymer membrane which endows the structure with stability, flexibility, and functionality. It should be noted that a specific block length ratio in the block-copolymers is essential for the formation of vesicles. For a fixed hydrophilic part, with an increasing hydrophobic section of the amphiphilic block-copolymer, first micelles are formed, then worms, vesicles and finally tubular vesicles.¹³ Depending on the origin of the block-copolymer, the vesicles are called polymersomes^{2,14} (for non-branched synthetic polymers),

1
2
3 dendrimersomes¹⁵ (for dendrimers) or recently reported proteinosome¹⁶⁻¹⁷ (for
4 synthetic proteins) either as mono- or multi-compartment capsules.¹⁸⁻¹⁹ Most
5
6 commonly, polymersome is also used as the governing term for the named
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8 structures. In comparison to their lipid counterparts, polymersomes exhibit a
9
10 low critical aggregate concentration (CAC), meaning that there is only an
11
12 insignificant number of individual polymer chains in the solution after reaching
13
14 CAC.²⁰ This means that polymersomes are non-ergodic, have no exchange of
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16 block-copolymer chains between separate vesicles and show a greatly
17
18 enhanced mechanical stability over liposomes.^{11,21}

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20
21 With respect to their functionality, the recent development of polymer
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23 chemistry allows the tailor-made adjustment of membrane properties,
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25 including that they can be functionalized at the outer surface for targeting
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27 approaches and possess stimuli-responsiveness for release of encapsulated
28
29 compounds "on demand".²²⁻²⁶ Besides their application as
30
31 drug-delivery-systems, these properties make polymeric vesicles ideal
32
33 candidates to be nanoreactors by encapsulating active compounds (enzymes,
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35 proteins, enzyme mimics) with the dual role of protecting the compounds from
36
37 proteolytic attack and serving as a reaction space.^{10,27-30} The necessary
38
39 exchange of substrates/products with the environment to support in situ
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41 reactions occurs through porous, synthetic membranes or by the insertion of
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43 channel proteins depending on the chemical nature of the copolymer.³¹⁻³³ A
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45 significant step beyond this is also realized by the exploitation of the designed
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3 nanoreactors to be artificial organelles.³⁴⁻³⁶ Such synthetically constructed
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6 models represent a new emerging field, since on the one hand they show
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9 promising potential for therapeutic applications in order to actively produce
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12 drugs to fight disease (enzyme replacement therapy) and to enhance the
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14 activity of existing organelles in cases of cellular stress; and on the other hand
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16 they could be exploited to be an artificial cell to contribute to the view of origin
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19 of life.

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21 In this regard, a number of reviews have been published covering a number
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23 of structures from polymersomes,^{2,37} biohybrid polymer capsules³⁸⁻⁴⁰ to
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25 multi-compartmentalized polymeric system¹⁸⁻¹⁹ with the application in the field
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27 of drug delivery, biomedicine or biomimicry *etc.* In this review, we specially
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29 focus our interest on the recently published papers about the engineering of a
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31 wide range of polymer-based capsules towards smart nanoreactors and
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33 artificial organelles. We start with a discussion on the preparation of polymer
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35 vesicles based on different techniques, including self-assembly,
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37 template-based polymerisation, in situ polymerisation, emulsion
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39 polymerisation, microfluidics or Pickering emulsion *etc.* (Section 2).
40
41 Layer-by-layer method is not included, since it has been widely summarized
42
43 very recently by Caruso *et al.*⁴¹⁻⁴⁴ Then the engineering of polymer capsules
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45 to be nanoreactors from the membrane domain-based design to core
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47 domain-based design is discussed in Section 3. Subsequently, we highlight the
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49 potential application of the constructed polymer capsule-based nanoreactors
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3 in controlled enzyme catalysis, polymerisation, nanoparticles synthesis and
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6 artificial organelles in Section 4.
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10 11 **2. Synthetic Functional Polymer Capsules** 12

13 14 2.1. Formation of Polymersomes 15

16 Polymersomes are, as stated in the introduction, hollow structures with an
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18 ordered polymer membrane. These can be formed of an amphiphilic AB
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20 block-copolymers to yield a polymer bilayer or of ABA/ABC
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22 triblock-copolymers forming a mono/multilayer. Both versions have the
23
24 hydrophobic block (A or C) at the outer surface and in the centre of the
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26 membrane. The first challenge when working with polymersomes is thus
27
28 finding a method that ensures a controlled membrane formation with the
29
30 amphiphilic block-copolymer of interest. As polymeric structures (micelles and
31
32 vesicles) are generally very stable once formed, it is even more important to
33
34 target the correct self-assembly structure directly. Due to their stability
35
36 polymersomes are unlikely to rearrange themselves into an energetically more
37
38 stable one due to a high kinetic barrier. This barrier prevents single polymer
39
40 chains to go into solution and interchange with other structures formed. Such a
41
42 behaviour results in a low critical aggregation concentration (CAC) and a low
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44 rate of molecules interchange which is described as ergodicity. Polymeric
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46 aggregates are consequently non-ergodic, which distinguishes them from lipid
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48 vesicles and micelles, which show a considerable amount of ergodicity. It
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4 should be noted that this lack of ergodicity can also be shown in the lack of
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6 mobility in polymeric membranes as compared to lipid ones. Given the correct
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8 combination of polymer and self-assembly method, it is then possible to trigger
9
10 the formation of custom-made assemblies, even of mixtures from the classical
11
12 micelles, worms, vesicles and tubes to give round-bottom flask-like assemblies,
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14 for example.⁴⁵
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19 There are generally three ways to form polymersomes. One way is to start
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21 from a thin polymer film. In here the bulk material is brought into contact with
22
23 water and then swells selectively to form the final structures. This is a “top
24
25 down” approach and it is commonly referred to as thin film rehydration.²⁰ The
26
27 opposite of this approach is then logically the “bottom up” approach starting
28
29 from a completely dissolved amphiphilic block-copolymer and then triggering
30
31 the self-assembly process.²⁰ The third way has only been reported very
32
33 recently by Armes *et al.*⁴⁶ and describes polymersomes formation during
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35 polymerisation. The authors named it a polymerisation-induced self-assembly
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37 or PISA process. All of these approaches will now be covered in detail in the
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39 following sections.
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49 2.1.1 Formation from Bulk Material

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51 In this approach, the polymersomes are formed from a previously created
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53 polymeric thin film which is then rehydrated with a suitable solvent, which is
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55 why this method is also referred to as film-rehydration. To create the initial film,
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4 the polymer, an amphiphilic block-copolymer, is dissolved in a good solvent for
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6 both blocks in a concentration of 5-10 mg/ml. When the solvent is then
7
8 removed, the polymer creates a thin film on the vessel wall (Figure 1).^{20,47-49}
9
10 This dry film is now wetted with a good solvent for exclusively one of the blocks.
11
12 In the case of water based systems, this is done with either plain water or
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14 buffer solution such as phosphate buffered saline (PBS), while organic solvent
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16 systems are also known.⁵⁰⁻⁵² Water now diffuses into the film, causing the
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18 hydrophilic part to swell. It is very important to ensure that the polymer stays as
19
20 a film during the hydration and that no small patches of bulk material get into
21
22 the solution as the necessary shear force from the stirring must be present at
23
24 all times.^{21,49} A feasible method to prevent this is to avoid the use of low
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26 molecular-weight polymers (below 10 kg/mol classical film rehydration is not
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28 reported) as they are less entangled, leading them to detaching from one
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30 another easier than longer polymers.⁵³⁻⁵⁴
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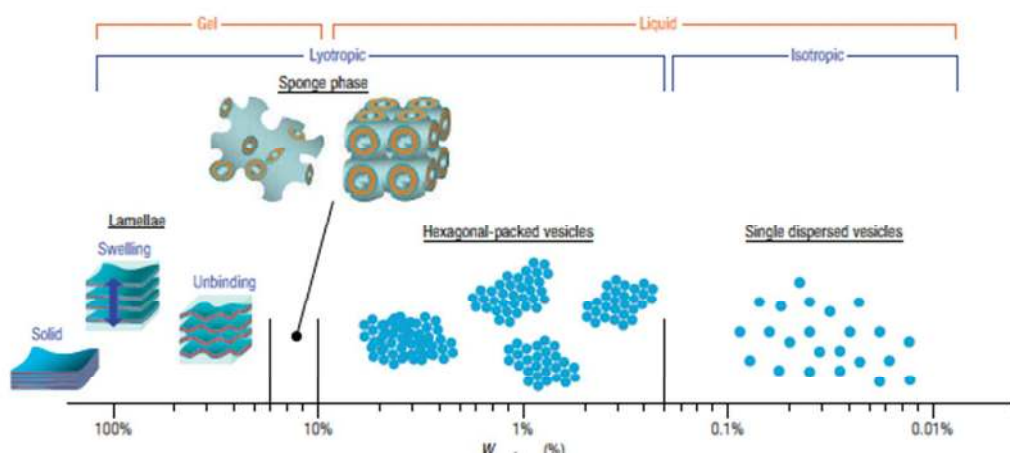


Figure 1. The evolution of a polymersome from a thin film to a complete vesicle shown in dependence of the water/polymer ratio and molecular weight. After the first lamellae

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4 phases are formed, packed vesicles (sponge phase) are formed initially and later on free
5
6 ones appear in solution.⁴⁹ (Reproduced with permission from reference 49. Copyright
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8 (2005) Nature Publishing Group).

9
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11
12 Eventually, pre-vesicle structures such as tubes and genius-like particles
13
14 detach from the edge of the film into the solution.^{21,49,55-57} When they now
15
16 develop further the hydrophobic part of the polymer needs to be shielded off by
17
18 the hydrophilic block.^{9,54,58} The polymer now slowly arranges into a structure
19
20 where this shielding is achieved best, depending on the block length ratio (see
21
22 introduction). It can only happen slowly since the arrangement of the polymer
23
24 chains happens through the reportedly slow diffusion of them. Literature knows
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26 this process to take up to 8 weeks, but may be speeded up by the application
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28 of ultrasound.⁵⁹⁻⁶¹

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35 Film rehydration is thus a long process and not suitable for short-term
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37 evaluation whether a polymer is feasible to form vesicles, or for polymers that
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39 show hindered diffusion due to high glass transition or crystallinity like
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41 polystyrene (PS) or poly(L-lactic acid) (PLLA).^{20-21,49} Also, hydrolysable
42
43 polymers are not feasible as they may degrade during the long formation
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45 process. Besides this general approach to film rehydration, some derived
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47 methods have been developed further by Battaglia *et al.*, to make it also
48
49 feasible for shorter polymer chains. Battaglia *et al.* showed that if the polymer
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51 chain is short enough (*e.g.* below 5 kg/mol), no stirring is necessary and the
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53 vesicle evolution process occurs just by putting the polymer in contact with
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4 water. If mixed with a fluorescent dye, the process can now actually be
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6 monitored by fluorescence microscopy.⁴⁹ The same group now went a step
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8 further and developed a film rehydration from a patterned surface.⁶² When
9
10 altering the surface hydrophobicity in a 1000 or 2000 mesh grid (1.0 and 2.0
11
12 mm grid spacing), the size of polymersomes created is homogeneous and
13
14 within micrometre range. This is in contrast to classic film rehydration which
15
16 leads to a broad size range of vesicles.⁶²
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21 Electroformation is another form of film rehydration to create polymersomes.
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23 It has been developed originally for liposome formation where the building
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25 blocks (lipids) have a significantly smaller molecular weight.⁶³⁻⁶⁴ In this method
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27 the film is put on a conductive surface (indium tin oxide (ITO) on glass) in an
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29 alternating current at varying frequencies and voltages. Both parameters have
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31 to be adapted for the polymer system used. However, while it has been shown
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33 that polymersomes can be formed that way,⁶⁵ it has proven difficult to detach
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35 the polymers from the substrate, yielding dome-like structures in many cases,
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37 probably due to their higher molecular weight compared to lipids (Figure 2).⁶⁶
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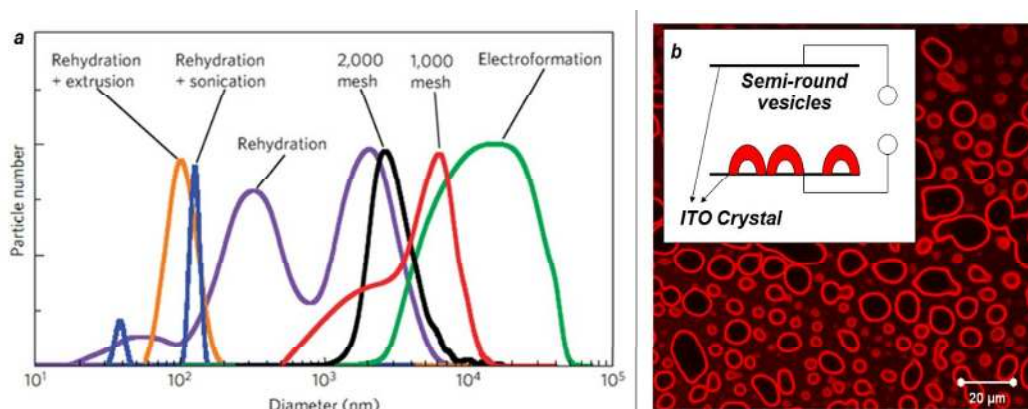


Figure 2. Vesicle sizes achieved by different kinds of film rehydration, controlled and uncontrolled and also using electroformation.⁶² Electroformation itself is shown on the left where dome-like structures evolve with PEG-PDEA.⁶⁶ (Part (a) reproduced with permission from reference 62. Copyright (2009) Nature Publishing Group.)

In summary, thin film rehydration can yield vesicles of a broad variety in size, from 100 nm until 100 micrometers, depending on the specific conditions used and the polymer applied.

2.1.2 Formation from Solution

Besides the “top down” approach just described, the “bottom up” way to form polymersomes from dissolved unimeric polymer chains is also widely used. Depending on the amphiphilic block-copolymer structure, this switch can be accomplished by either exchanging an organic solvent to water⁶⁷ or by changing the properties of the solvent (pH, temperature)^{13,56} which yields in the one block of the polymer becoming insoluble. The assembly transitions through micelles, octopus-like assemblies to worms and vesicles via different

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3 pathways which occur simultaneously (Figure 3).
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6 The principle of these formations is always the same and was one of the first
7 methods used for polymersome preparation by Discher and Eisenberg.^{2,14}
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9 First of all, the whole polymer is dissolved in an organic solvent suitable for
10 both blocks. Once the polymer is dissolved into single polymer chains, *e.g.*
11 unimers, the polarity is slowly raised by the means of adding water. At a certain
12 point (which depends on the polymer and solvent used), the water content of
13 the solution becomes too large to dissolve the hydrophobic part of the polymer.
14 From this point onwards polymer-polymer interactions are favoured over
15 polymer-solvent interactions and the self-assembly process is induced. One
16 can see from this approach that not all organic solvents are suitable for this
17 approach, but that it has to be miscible with water.^{14,20} Hence, only polymers
18 that are soluble in water-miscible organic solvents can be used applying this
19 organic solvent-to-water solvent switch.
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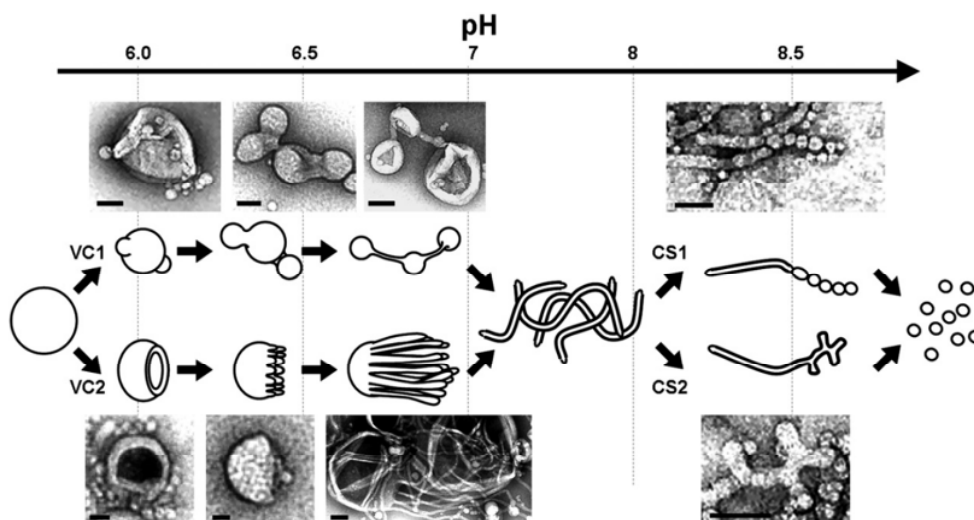


Figure 3. Possible structures observed when using a pH guided assembly of poly((butyl)methacrylate)-poly(methacrylic acid) PBMA-PMA via 2 pathways of vesicle creation (VC1 and VC2) and the final creation of spheres (CS1 and CS2).⁶⁸ (Reproduced with permission from reference 68. Copyright (2009) Royal Society of Chemistry).

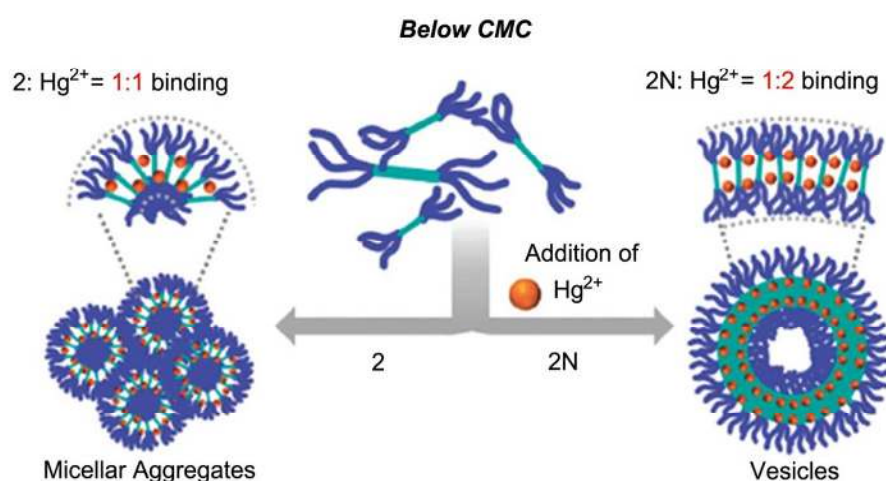
Once the polymer begins to self-assemble, it is necessary to make a very slow transition process in order to yield the thermodynamically stable polymersomes and not kinetically favoured micelles.^{2,69} It is the stability of polymer aggregates that hinders their transition into the thermodynamically more stable polymersomes. This is mainly due to the amount of energy needed to dissolve a single chain from the aggregates, also reflected by the very low critical aggregation concentration (CAC) of polymers in comparison to lipids.^{64,70-71} A slow solvent transition will thus favour the thermodynamically stable state of the vesicle.

Apart from switching the polarity of the solvent, one can also change the pH or temperature of the solvent to induce self-assembly, if suited polymers are

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3 used in the hydrophobic part. Here, the whole polymer is dissolved in water in
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5 a state where it is completely soluble, using sensitive polymers. Temperature
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7 sensitive polymers like PNIPAM with an LCST for example, are soluble at low
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9 temperatures. With rising temperatures, the hydrogen bonds keeping the
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11 polymer in solution break and thus induce the self-assembly process into
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13 polymersomes.⁷²⁻⁷³ Temperature-sensitive peptidosomes can, of course, be
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15 formed in the same way.⁷⁴ Kiick *et al.* report a diethylene glycol functionalised
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17 polymethacrylate, PDEGMEMMA, which shows a temperature-dependent
18
19 solubility switch when conjugated with a collagen-like polymer.⁷⁵ In case of
20
21 pH-sensitive polymers it is logically the pH switch which induces the
22
23 self-assembly process.^{59,66,76} Again, the changes in pH or temperature need to
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25 be done slowly due to the non-ergodic behaviour of polymers as stated above.
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27 Also for polyionic polymers it has been shown that immediate complexation by
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29 counterions does induce vesicle formation. After complexation the charges get
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31 masked and the previously water-soluble polyion becomes hydrophobic,
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33 inducing the self-assembly into vesicles.⁷⁷ Essentially, not even charges are
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35 necessary, but the polymer must be able to complex the ions added. Lee *et al.*
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37 showed this by the complexation of Hg²⁺ ions by polyimidazoles. The binding
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39 forces of the ligand-metal complex were enough to induce vesicle and micelle
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41 formation. The morphology of the aggregates form is governed the amount of
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43 mercury added (Figure 4).⁷⁸ Independent of the actual external trigger used,
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45 the polymersomes produced in that approach are always around 100-200 nm
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4 in size.

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6 The complexing agent does not necessarily have to be a salt, but can also
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8 be an oppositely charged polyion. These complexes do form vesicles and are
9
10 known as polymer-ionic-complex-vesicles (PICsomes).⁷⁹⁻⁸⁰ In contrast to the
11
12 examples just discussed, the second polyion does not need to be added slowly,
13
14 which is a notable exception from other vesicle formation methods. PICsomes
15
16 will form if the hydrophilic-to-hydrophobic balance is correct. Once formed,
17
18 PICsomes have similar properties as classic polymersomes.^{27,79,81-82}



40
41 **Figure 4.** Schematic representation of the Hg²⁺-induced self-assembly of polyions
42
43 towards micelles and vesicles through complexation.⁷⁸ (Reproduced with permission from
44
45 reference 78. Copyright (2014) Royal Society of Chemistry).
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48
49 There is also an extrusion-based way of performing a stepwise solvent
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51 switch which was developed by Lecommandoux *et al.* (Figure 5). Here, water
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53 is added to a solution of the polymer in an organic solvent which is not miscible
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55 with water (Toluene). A similar process for liposomes was the role-model for
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4 polymersomes made this way.⁸³ The polymer now aggregates at the
5
6 water-organic solvent interface, stabilising the water droplet and thus forming a
7
8 water-in-oil emulsion.^{18,84} Hence, this method is also referred to as forming
9
10 polymersomes by emulsion. The size of the droplets in the emulsion
11
12 determines the size of the final polymersomes formed, making large or giant
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14 polymersomes of several micrometers possible. The water-oil-emulsion is now
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16 extruded into an aqueous solution with higher density than the organic solvent.
17
18 Adjusting the density is achieved by using a sucrose solution. Once the water
19
20 droplets of the emulsion reach the sucrose-solution/oil interface, the polymer
21
22 bilayer originally stabilising this interface assembles around the
23
24 polymer-stabilised water droplet. As a result, the water droplet has a second
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26 polymer layer around it and is in an aqueous environment, hence it is now a
27
28 polymersome.⁸⁴⁻⁸⁵ The water used to create the emulsion can already contain
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30 small polymersomes produced by a different process which could in this way
31
32 now be encapsulated in a bigger polymersome. Lecommandoux *et al.*, who
33
34 pioneered the emulsion/extrusion technique, have shown the production of
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36 such polymersomes-in-polymersomes aggregates.⁸⁶
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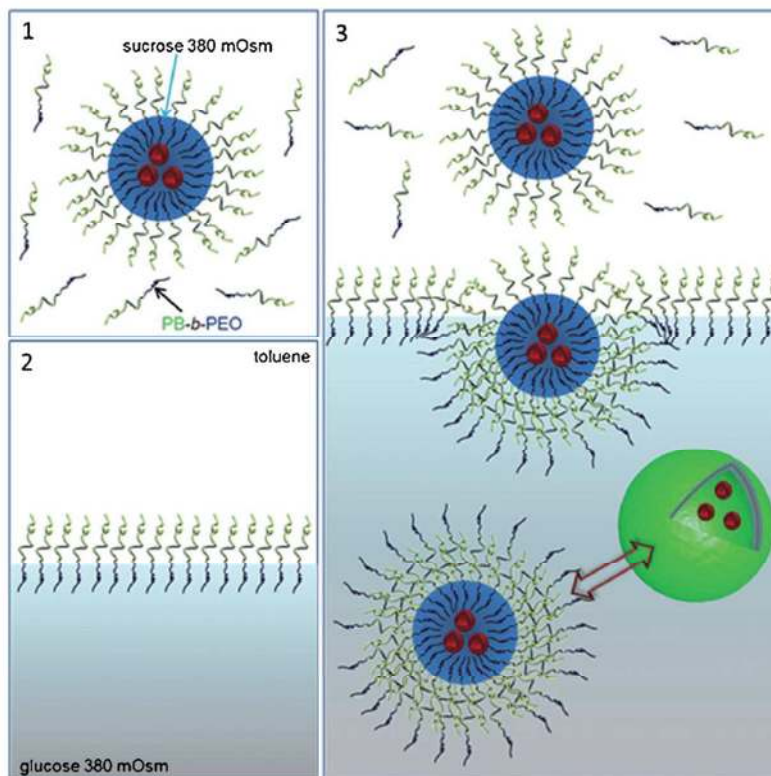


Figure 5. Production of vesicles using the emulsion method result in a layer-by-layer formation of the vesicle. If the first water droplet already contains small polymersomes (red), the production of small vesicles enclosed into larger vesicles (green) is possible using this approach.⁸⁵ (Reproduced with permission from reference 85. Copyright (2012) John Wiley and Sons).

Preparing round droplets (and eventually form polymer nanoparticles) from emulsions is something which was achieved by microfluidics ever since it was invented.⁸⁷ In here, the fluids flow through nanometer to micrometer sized channels and meet at a definite time point, forming their aggregates. Consequently, this approach can also be used to form polymersomes (Figure 6). Initial results by Weitz and Battaglia *et al.* did not only proof that

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4 polymersomes can be formed in that way, but that encapsulating drugs can be
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6 done in the same step.⁸⁸ The advantage of microfluidics is its high yield of
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8 encapsulation which comes with the nature of microfluidics. Each channel
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10 hosts one part of the original material which means that the cargo is injected
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12 directly into the vesicles. The lack of a consecutive cleaning step is of course a
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14 positive point for microfluidics. So far, polymers of various molecular weights
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16 have been used not only to make polymersomes, but also to create small
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18 polymersomes encapsulated in large polymersomes.⁸⁹⁻⁹¹ With regard to the
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20 polymers applied, the methacrylic-based poly(methacrylic phosphoryl
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22 choline)-poly(diisopropylaminoethyl methacrylate) (PMPC-PDPA)⁸⁸ as well as
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24 the ROP based PEG-PLLA⁹⁰⁻⁹² have both been applied, showing that there is
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26 in principle no restriction towards the polymers. The examples show further
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28 that microfluidics can be expanded from being just a sophisticated tool to an
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30 effective emulsion-based method for polymersomes formation. It is now also
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32 possible to use it for the production of multicompartment capsules
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34 encapsulating polymersomes as already mentioned for the emulsion-based
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36 method,^{86,91,93} but also capable of mimicking other techniques. PMPC-PDPA
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38 based polymersomes, for example, are created using an in-situ pH switch,
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40 hence a way of solvent switch.⁸⁸ So while the solvent switch is limited to
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42 polymers soluble in water-miscible solvents and the emulsion-based way is
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44 limited to water-insoluble solvents, microfluidics can do both. As with every
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46 method, there are also disadvantages involved in microfluidics. Although scale
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up by numbering up is easy, the slow production speed of vesicles through microfluidics does hinder a larger scale application. It should also be noted that mainly large polymersomes (above 50 μm) have been reported so far when prepared in microfluidics. Furthermore, no further application of polymersomes made by microfluidics (drug delivery or nanoreactors) has been shown so far which is why the scope of this polymersome making method is not clear at the moment.²⁰

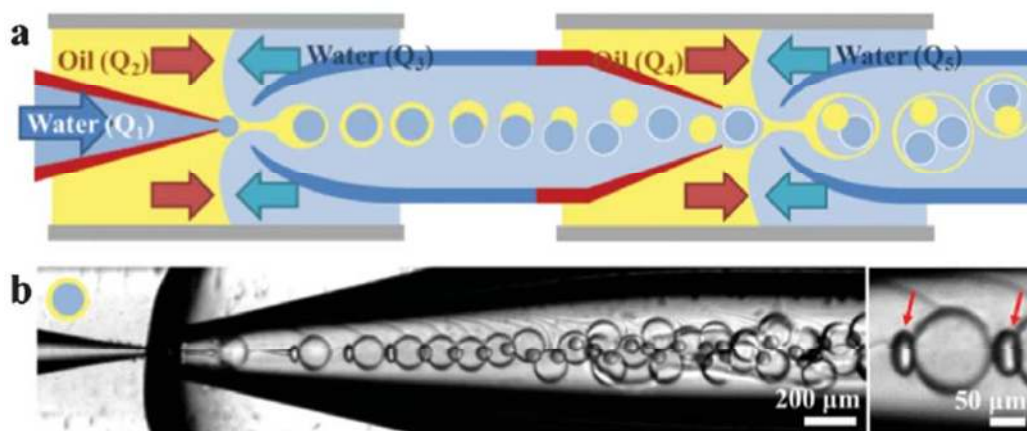


Figure 6. (a) Schematic illustration of the microfluidic device for preparation of multi-compartmentalised polymersomes containing several inner polymersomes. (b) Optical microscope image showing injection of polymersomes into the innermost droplets of double-emulsion drops. For the formation of vesicles the breaking of the middle phase is triggered by the core droplets of the aqueous suspension.⁸⁹ (Reproduced with permission from reference 89. Copyright (2013) Royal Society of Chemistry).

2.1.3 Formation during Polymerisation

A very recent method to form polymersomes is to produce them from a hydrophilic macroinitiator, e.g. while the hydrophobic part of the final

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amphiphilic block-copolymer is produced. Armes *et al.* now showed various examples of this polymerisation-induced self-assembly (PISA). The most important prerequisite for PISA is that the monomers of the hydrophobic part of the final polymer are soluble in water. Due to this solubility switch, an originally homogeneous solution gives an amphiphilic block-copolymer which can then self-assemble *in situ* (Figure 7).^{46,94} The polymerisation of hydroxypropylmethacrylate (HPMA) from a macroinitiator is widely reported by the group as the monomer HPMA is water-soluble, but the polymer PHPMA is not. Interestingly, the polymerisation method used by the group, *reversible addition fragmentation transfer polymerisation* (RAFT) works without a metal catalyst starting from a water-soluble pre-polymer as chain transfer agent (macro-CTA).^{46,94-95} It is not clear so far, whether this is a necessary prerequisite or if other living polymerisation techniques are also feasible for this approach.

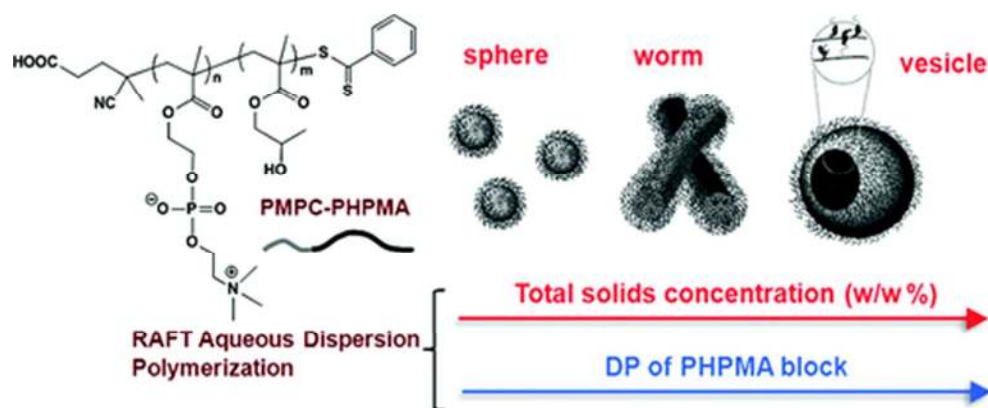


Figure 7. PMPC-PHPMA vesicles are synthesised using PISA. With increasing block length of the building block-copolymer, the self-assembly structures change from spheres

(micelles) to worms to vesicles.⁹⁴ (Reproduced with permission from reference 94. Copyright (2011) American Chemical Society).

Within PISA, the polymerisation can be stopped at any time point, resulting in the structure that is stable at that specific hydrophilic to hydrophobic aspect ratio. Due to the dynamics in the system during polymerisation, the micelle-polymersome transition hindered for polymersomes formed by solvent switch is allowed for the PISA approach. This was repeatedly used to generate phase diagrams of different block-copolymers showing which self-assembly structure is favoured for which block-copolymer ratio from micelles (or spheres) over worms to vesicles (Figure 8).^{46,96-97}

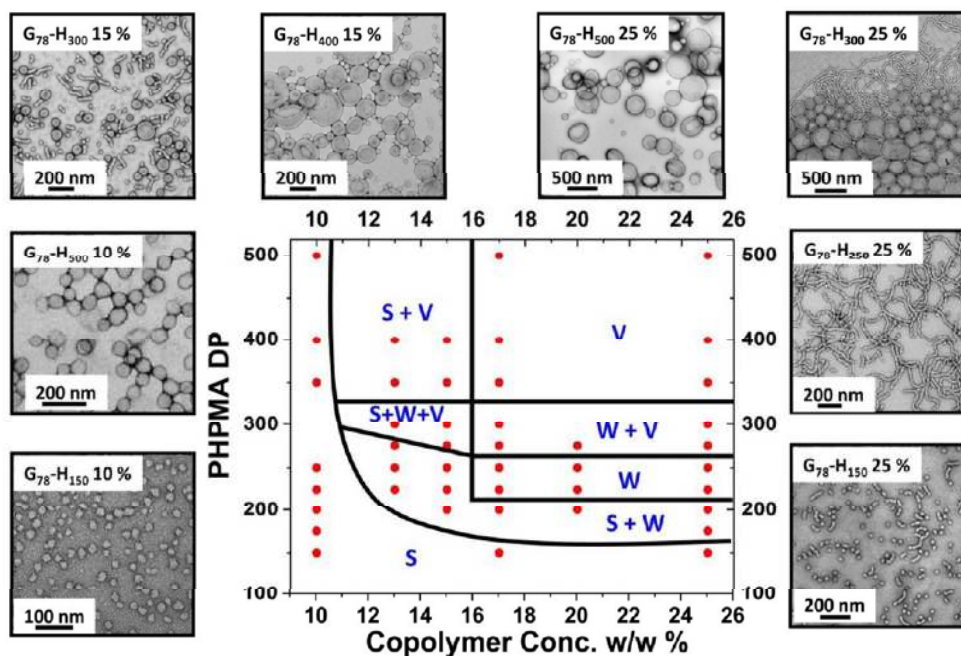


Figure 8. Phase diagram with corresponding block lengths for a poly(glycidyl methacrylate)-poly(hydroxypropyl) (G-H) block copolymer prepared by PISA forming

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3 spheres (micelles), worms and vesicles.⁴⁶ (Reproduced with permission from reference
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6 46. Copyright (2012) American Chemical Society).
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13 Once the vesicles are formed, no further transition of the self-assembly
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15 structures occurs. However, an ever-growing polymer results in an ever longer
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17 hydrophobic block of the block-copolymer. With no phase change occurring, a
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19 thickening of the membrane is the only option left for the polymer and this is
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21 what happens. It is not obvious from the start in which direction the membrane
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23 will be growing. The membrane now either grows to the inside, to the outside,
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25 or do both simultaneously.⁹⁵⁻⁹⁶ The first option would lead to vesicles of
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27 decreasing surface area, but stable size, and the second option to ones with
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29 increasing surface area and size and the combination of both growing
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31 directions would lead to a slight increase in size and a stable surface area. For
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33 their PHPMA based system, Armes *et al.* report a growth to the inside only, *e.g.*
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35 a decrease in surface area but stable size. Decreasing the surface size means
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37 that the inner surface area is shrinking constantly with the hydrophilic part of
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39 the polymer getting very crowded on the inner side of the membrane.⁹⁸ For this
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41 polymer, it is suggested that the constraint block-copolymer chains flip through
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43 the membrane so that they are finally on the outside of the vesicle. Due to the
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45 convex nature of the outside of the vesicle, this is energetically favoured over a
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47 crowded concave inner part of the vesicle. So far, it is not clear whether this is
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49 true for other polymerisation achieved with PISA as well.⁹⁵⁻⁹⁶
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4 PISA is, of course, not limited to water-borne systems only. Whenever a
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6 monomer turns from solvophilic to solvophobic during polymerisation,
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8 self-assembly to polymersomes and other structures occurs (Figure 9). Armes
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10 *et al.* reported PISA in ethanol using poly(benzyl) methacrylate) (PBzMA) from
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12 a poly(acrylic acid) (PAA) macro-CTA. The resulting PAA-PBzMA is then
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14 reported to assume all known polymeric self-assembly structures from micelles
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16 to vesicles.⁵¹ Further chain extension with a semi-fluorinated polymer
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18 poly((2,2,2-trifluoroethyl) methacrylate) (PTFEMA) gave an ABC
19
20 triblock-copolymer. Polymersomes of this PISA-approach then showed a
21
22 definite composition of the inner and outer membrane.⁵⁰ In a very similar
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24 approach Lowe *et al.* also applied PISA in ethanol using 2-phenylethyl
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26 methacrylate as the monomer with switching solubility. It should be noted that
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28 the group used poly(2-(dimethylamino)ethyl methacrylate) (PDMA) as a
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30 starting CTA.⁵² Being a solvophilic block in ethanol, it is rapidly applied as
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32 solvophobic block in waterborne polymersomes.⁹⁹⁻¹⁰¹ Besides in ethanol, PISA
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34 was also attempted successfully in n-dodecane with poly(lauryl methacrylate)-
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36 poly(benzyl methacrylate) [PLMA-PBzMA] again with PBzMA as the switching
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38 block.¹⁰²
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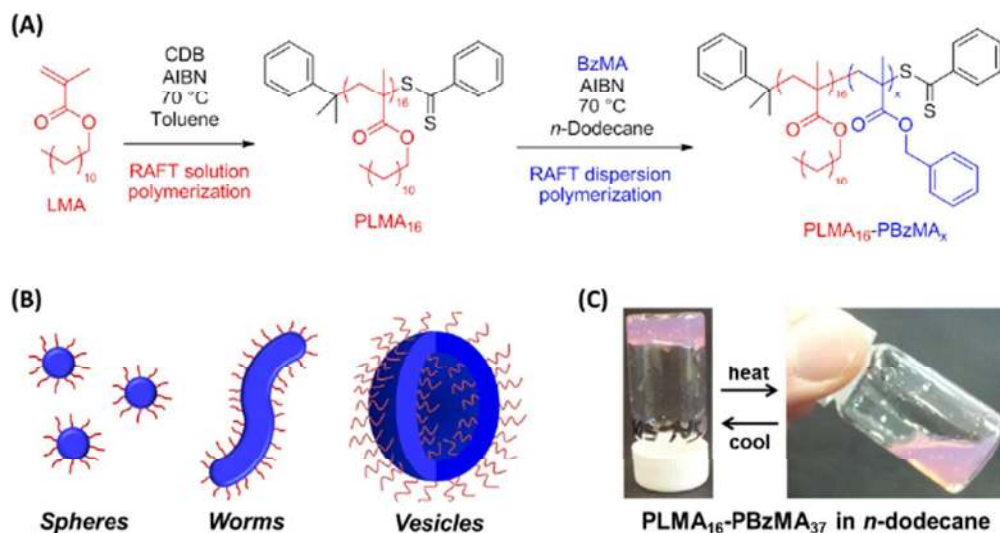


Figure 9. PISA, performed in *n*-dodecane using poly(lauryl methacrylate)-poly(benzyl methacrylate) (PLMA-PBzMA) as solvophilic and solvophobic parts.¹⁰² (Reproduced with permission from reference 102. Copyright (2014) American Chemical Society (Creative Commons CC-BY)).

2.2 Bulk Polymeric Capsules

Recently, polymeric capsules, with a polymer membrane domain and an empty core domain, as another type of polymeric compartmentalized model are also attracting considerable attention. Differing from above-mentioned polymersome of which the polymer in the membrane should be strictly amphiphilic to form a bilayer structure, the polymer in the polymeric capsules can be varied freely which endow us with more freedom to design different functionalised polymeric capsules as desired introducing also controlled permeability of the membrane and surface functionality. Tailoring morphology

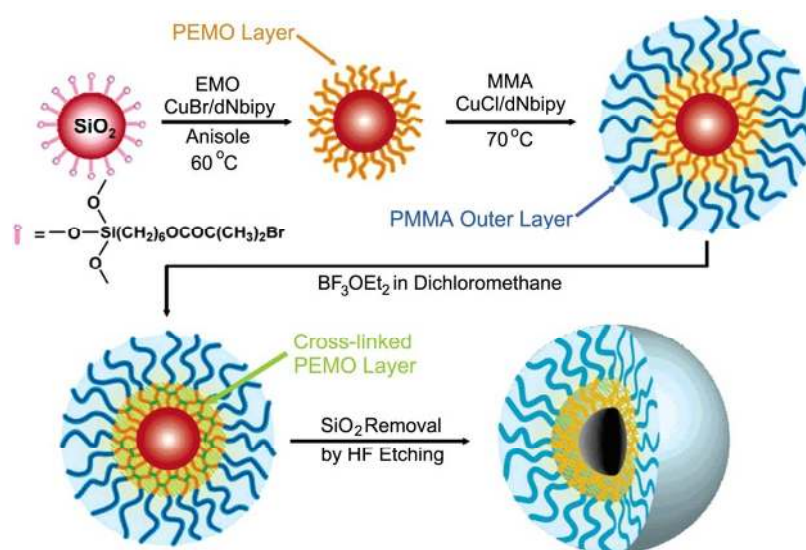
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4 and physicochemical properties is of great interest to improve and broaden the
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6 potential of polymeric capsules for advanced applications in drug delivery,
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8 nanoreactor and biotechnology. To date, significant progress has been made
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10 in the design and fabrication of such models, and various routes leading to
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12 hollow polymer capsules have been explored. Here, only the template-based
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14 polymerisation, emulsion polymerisation and distillation–precipitation
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16 polymerisation will be discussed. The additionally widely used layer-by-layer
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18 approach has been reviewed extensively recently⁴¹⁻⁴⁴ and will not be repeated
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20 here.
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28 29 2.2.1 Template-Based Polymerisation

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31 In recent years, the colloidal template method, involving the coating of a
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33 sacrificial inorganic/organic template with polymer followed by stabilization of
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35 the polymer shell via a cross-linking reaction, is especially popular.^{6,19} The
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37 template is typically selected to be a readily removable nanoparticle, e.g.,
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39 made from silica, gold or other organic compounds. Thus, by combining this
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41 method with surface-initiated polymerisation (SIP) technique, an elegant and
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43 versatile route to form polymeric capsules with tailored properties has been
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45 widely studied. Usually, the polymeric capsule is obtained after cross-linking
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47 the shell and removing the core.¹⁰³⁻¹⁰⁵ The obvious advantages of this
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49 template-based polymerisation strategy to synthesize the polymeric capsules
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51 are: (i) the size of capsules can be easily tuned by choosing different sized
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4 nanoparticles as templates, (ii) the thickness of the shell can be controlled by
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6 controlling the molecular weight of the anchored polymers, (iii) the composition
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8 of the polymer shell can be easily varied by using different functional
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10 monomers, (iv) and the post-functionalisation on the surface of the polymeric
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12 capsules can proceed from the active end-site of the grafted polymer chain.
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14 Recently, the rapid development in polymer chemistry¹⁰⁶⁻¹⁰⁷ laid a solid
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16 foundation for the prosperity of SIP in the synthesis of polymeric capsules. In
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18 this regards, the pioneering example on this topic was shown by Hawker *et*
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20 *al.*¹⁰³ who employed surface-initiated controlled nitroxide mediated radical
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22 polymerisation (NMRP) to grow polystyrene from the surface of silica
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24 nanoparticles and cross-linked the capsules either thermally by the
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26 incorporation of vinyl benzocyclobutene groups or chemically by the reaction
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28 of maleic anhydride repeat units with a diamino crosslinker. Hereafter, in
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30 subsequent publications, surface-initiated atom transfer radical polymerisation
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32 (ATRP) was also successfully employed to synthesize polymeric capsules by
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34 Fukuda¹⁰⁴ and Kang *et al.*¹⁰⁵ For instance, by surface-initiated ATRP strategy,
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36 an oxetane-functionalised block copolymer was built on the surface of SNP.
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38 Taking advantage of the ring-opening reactivity of oxetane for the shell
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40 cross-linking and dissolving the core, a monodisperse hollow capsule was
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42 obtained(Figure 10).¹⁰⁴ The created almost monodisperse polymeric capsules
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44 can form well-ordered close-packed colloidal crystals that diffract light.¹⁰⁸ Also,
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46 based on this surface-initiated ATRP method, Kang and coworkers prepared a
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4 block copolymer of PS-*b*-PMMA grafted silica nanoparticle. Ultraviolet
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6 exposure of the hybrid particles resulted in cross-linking of the PS intermediate
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8 layer, then after the decomposition of the PMMA outermost layer and
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10 subsequent removal of the silica core by HF etching provided cross-linked PS
11
12 hollow capsules.¹⁰⁵ In the following study, the authors also demonstrated the
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14 membrane of the PS hollow capsules can be functionalized by hydrophobic
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16 polystyrene with terminal azide groups (PS-N₃) and hydrophilic poly(ethylene
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18 glycol) with terminal thiol group (PEG-SH) via alkyne-azide and thiol-ene
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20 surface click reactions, and produced a hairy PS hollow capsules with binary
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22 polymer brushes on the surface.¹⁰⁹



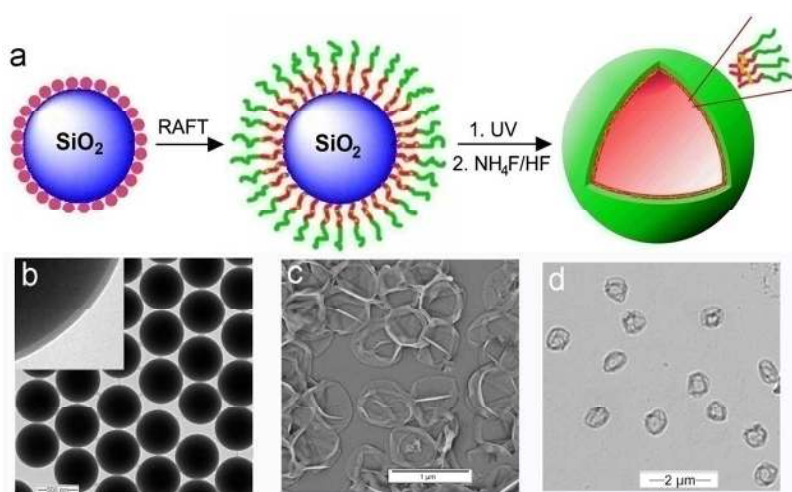
49 **Figure 10.** Schematic representation for the synthesis of polymeric capsules by SIP.

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51 Employing 3-ethyl-3-(methacryloyloxy)methyloxetane (EMO), and methyl methacrylate
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53 (MMA) as monomers. PEMO-*b*-PMMA grafted silica nanoparticles (SNPs) were obtained
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55 via surface-initiated ATRP. After crosslinking the PEMO layer of (PEMO-*b*-PMMA)-SNP
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4 by cationic ring-opening reaction of the oxetane groups of the EMO moieties the core is
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6 removed by HF etching. This process gave cross-linked (PEMO-*b*-PMMA) polymeric
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8 capsules.¹⁰⁴ (Reproduced with permission from reference 104. Copyright (2007) American
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10 Chemical Society).
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16 It is well-known that RAFT has emerged as a promising controlled radical
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18 polymerisation technique due to its versatility and simplicity, and the
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20 advantage that the resulting polymer is free from the contamination of metal
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22 catalyst.¹⁰⁷ Also, RAFT is compatible with almost all of the conventional radical
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24 polymerisation monomers. As shown in a recent study by Voit and coworkers,
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26 taking advantage of surface-initiated reversible addition–fragmentation
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28 chain-transfer (RAFT) polymerisation, polymeric capsules can also be
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30 successfully synthesized, and the general procedure is shown in Figure 11.¹¹⁰
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32 After anchoring a dithiocarbonate RAFT agent onto the silica nanoparticle,
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34 block-copolymers P*t*BMA-*co*-PDMIPM-*b*-PHPMA were grown from the surface
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36 by RAFT polymerisation. 2,3-Dimethylmaleic imidopropyl methacrylate as a
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38 photo-cross-linker was incorporated in the polymer chain to allow crosslinking
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40 the shell, and after removal of silica templates by HF buffer solution (pH 5.0)
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42 well-dispersed polymeric capsules could be obtained. In the following study,
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44 an intelligent polymeric capsule with an ultrathin membrane was developed by
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46 using a pH sensitive monomer diethylaminoethyl methacrylate (DEAEMA) as
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48 well as a disulphide containing monomer pyridyldisulphide ethylmethacrylate
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4 (PDSM) as main units.¹¹¹ Cross-linking was successfully achieved by using
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6 dithiols of different lengths and led to stable polymeric capsules after removal
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8 of the silica template. For this constructed polymeric capsule
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10 (PDEAEMA-co-PPDSM), its permeability of membrane could be well tuned by
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12 adjusting the pH and using different lengths of the cross-linkers. Also, due to
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14 the disulphide cross-linker, degradation of the capsules using glutathione as
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16 the disulphide cross-linker, degradation of the capsules using glutathione as
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18 reducing agent can be achieved which is further significantly promoted at more
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20 acidic environment. Using a rather long-chain dithiol cross-linker it could be
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22 demonstrated that a protein-sized and cationic hyperbranched polymer can
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24 successfully go through the cationic membrane in the swollen state which
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26 makes this polymeric capsule be a very promising candidate in the application
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28 as nanoreactor or nanocarrier.¹¹¹



50 **Figure 11.** (a) Schematic illustration of the procedure to form polymeric capsules based
51 on the surface-initiated RAFT polymerisation from silica nanoparticles as templates, (b)
52 TEM image of polymer grafted silica nanoparticles (inset is a magnification of (b)), (c)
53 SEM and (d) TEM images of the synthesized polymeric capsules.¹¹⁰ (Reproduced with
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4 permission from reference 110. Copyright (2011) American Chemical Society).
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9 Among these studies, the surface initiating photo-polymerisation has also
10 been widely used as another simple, versatile and effective approach to
11 generate polymeric capsules.¹¹²⁻¹¹³ As shown in Figure 12, Yin and coworkers
12 immobilised crosslinked PDMAEMA shell containing co-initiator amino groups
13 on the surface of silica particles, which can initiate MMA in the presence of
14 photoinitiator under UV-irradiation.¹¹³ Polymeric capsules with well-defined
15 outer PMMA shell were obtained after removing the silica cores. It is
16 noteworthy that this surface initiating polymerisation technique is easy and
17 robust for the synthesis of different kinds of polymeric capsules by changing
18 the used monomer. One example shown by Yang *et al.* was that an optically
19 active polymer can be incorporated into the polymer shell by copolymerisation
20 of optically active N-propargylamide copolymers and maleic anhydride and
21 vinyl acetate.¹¹² Accordingly, each constructed polymeric capsule can be
22 considered as a single chiral resolution vessel due to its inner cavity and the
23 helical polymer chains grafted in the shell. Apart from chiral recognition and
24 chiral resolution functions, such polymeric capsules are also expected to show
25 other applications involving chiral catalysis, controlled release, and chiroptical
26 materials.
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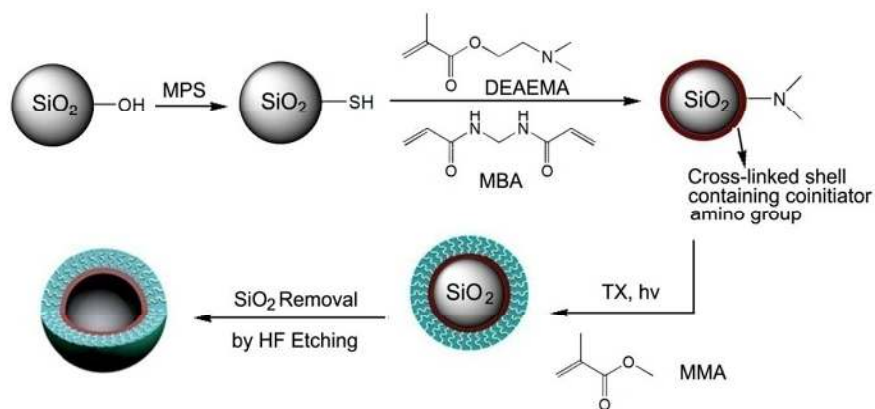


Figure 12. The process for synthesis of polymeric vesicles with well-defined PMMA

brushes from a SiO₂ nanoparticle.¹¹³ (Reproduced with permission from reference 113.

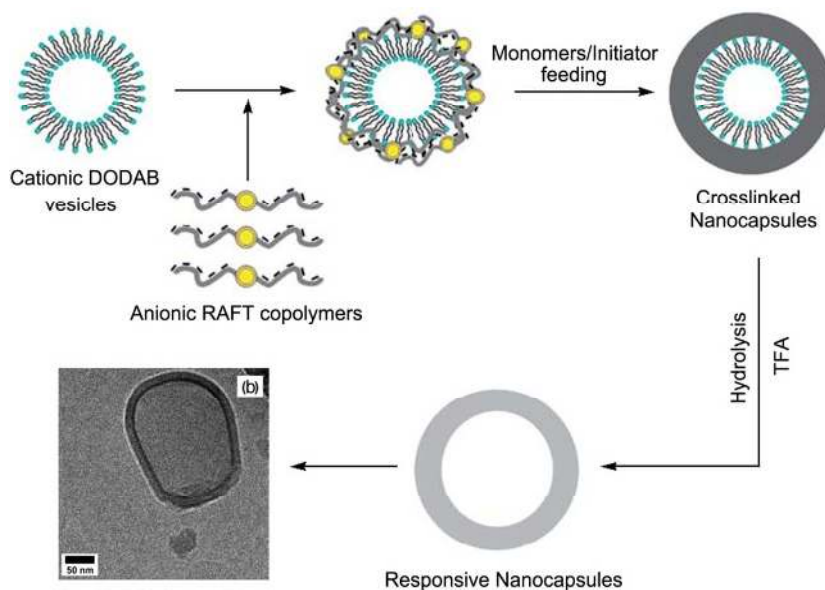
Copyright (2010) Royal Society of Chemistry).

In addition to the above-mentioned “grafting from” method to anchor polymers onto the template surface, Boyer and coworkers showed an approach based on “grafting to” method to anchor polymers onto the surface of gold nanoparticles to synthesize polymeric capsules.¹¹⁴ In detail, two different functional diblock polymers were made by RAFT polymerisation with a cross-linkable segment comprised of an alternating copolymer of styrene and maleic anhydride. It has been well confirmed that the polymers synthesized via RAFT polymerisation can be used to assemble around gold nanoparticles via the high affinity of RAFT end-groups for gold surfaces as demonstrated for the first time by McCormick, Lowe, and Sumerlin *et al.*¹¹⁵⁻¹¹⁶ Accordingly, for the obtained gold nanoparticle polymer brush, after cross-linking of the polymer layer by the addition of ethylene diamine in the presence of 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide, and the removal of gold

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4 cores using aqua regia, well-defined polymeric capsules was obtained. Also,
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6 the data showed that the use of aqua regia had no negative effect on the
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8 integrity of the polymer chains in the shell.
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11 In general, in these discussed template-based polymerisation strategies
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13 solid templates such as silica or gold nanoparticles are employed which means
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15 harsh chemical reagents (e.g. acids, organic solvents) are required to dissolve
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17 the template,^{103,110,114} consequently placing some limitations when chemically
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19 sensitive materials such as biomolecules are present. Also, utilisation of solid
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21 particles as templates means that the substances of interest have to be
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23 encapsulated into the capsule by diffusion after removal of the core, which is
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25 inefficient. Taking these issues into account, some soft templates have
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27 attracted significant interest such as emulsion droplets or vesicles, which can
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29 be readily removed under relatively mild conditions (e.g., aqueous alcohol
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31 solutions) and which can be easily pre-loaded with substances.¹¹⁷ For example,
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33 Ali and coworkers employed vesicles as templates to construct polymeric
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35 capsules.¹¹⁸⁻¹¹⁹ As depicted in Figure 13, the approach simply requires the
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37 adsorption of short-chain negatively charged copolymers, poly(butyl
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39 acrylate-co-acrylic acid) containing a RAFT group, on the oppositely charged
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41 unilamellar vesicles of dimethyldioctadecylammonium bromide (DODAB).¹¹⁸
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4 methyl methacrylate, tertiary butyl acrylate and a divinyl crosslinker ethylene
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6 glycol dimethacrylate, to form a crosslinked polymer shell. After hydrolysing
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8 the tertiary butyl ester groups of the shell, a pH responsive polymeric capsule
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10 was finally obtained. Although, due to colloidal instability, a vesicle template
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12 might not be that suitable in the final application, and this soft templating
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14 approach still represents a promising method for the synthesis of polymeric
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16 capsules due to the mild conditions for “core dissolution” and the easy
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18 encapsulation of the substance of interest.
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44 **Figure 13.** (a) Schematic representation of the synthesis of vesicle-templated
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46 pH-responsive nanocapsules by aqueous starved feed emulsion polymerisation using
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48 RAFT copolymers as stabilisers, and (b) CryoTEM micrographs of a single synthesized
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50 nanocapsule.¹¹⁸ (Reproduced with permission from reference 118. Copyright (2011)
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54 Royal Society of Chemistry).
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2.2.2 Miniemulsion Polymerisation

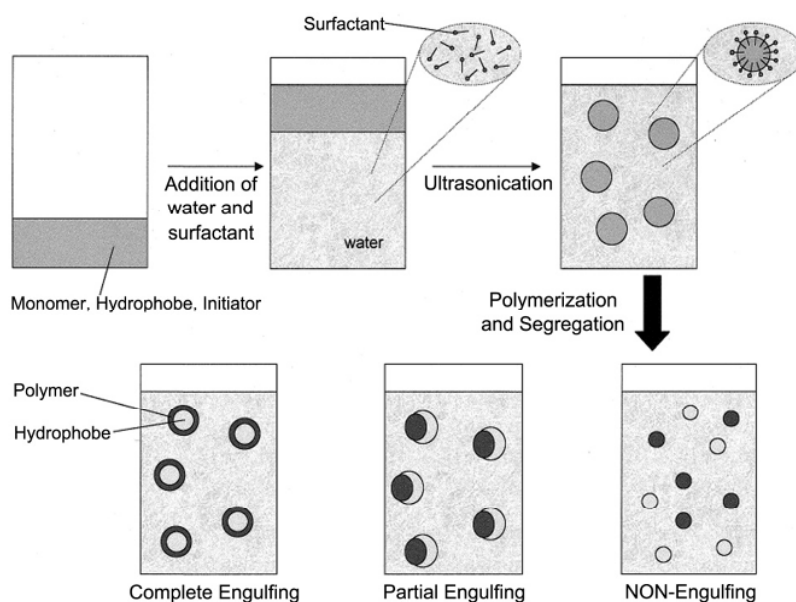
An alternative approach to generate polymeric capsules, miniemulsion polymerisation, is of special interest and is attracting more and more attention.¹²⁰⁻¹²¹ The method uses a mixture of two immiscible phases and leads to the formation of monodisperse stable droplets either as oil-in-water or water-in-oil miniemulsion in the presence of high shear stress including ultrasonication or high speed homogenizer. The size of the obtained droplets can be varied within the range between 50 and 500 nm depending on the type and effective amount of the surfactant or co-surfactant. There are almost unlimited choices of monomers/polymers and chemical reactions to generate the polymeric capsules with a hydrophilic or hydrophobic core. Furthermore, the surface of polymeric capsules can be easily functionalised, for example, with biomolecules for specific targeting or biosensing. Therefore, there is no doubt that this technique gives another significant opportunity to produce polymeric capsules with desired properties for a broad range of bioapplications.

Up to now, a host of excellent reports from Landfester's group have shown the versatility of miniemulsion polymerisations as a tool for the fabrication of polymeric capsules composed of different kinds of polymers obtained by a variety of polymerisation types ranging from radical¹²² and anionic¹²³ polymerisations to polyaddition¹²⁴ or polycondensation methods.¹²⁵⁻¹²⁶ Basically, they can be divided into three categories:

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4 (i) Formation of polymeric capsules by phase separation:
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7 In phase separation process, normally an organic liquid is chosen as a
8 dispersed phase of the direct miniemulsion which is a solvent for the
9 monomers but a non-solvent for the polymers. By tuning the surface tensions
10 of the participating interfaces in the system, phase separation occurs and the
11 non-solvent is engulfed by the growing polymeric shell, leading to the
12 formation of polymeric capsules with the encapsulated organic liquid in the
13 core(Figure 14). For example, when using the more hydrophobic styrene, the
14 copolymerisation with hydrophilic monomers such as acrylic acid,¹²²
15 methacrylic acid,¹²⁷ or N-isopropylacrylamide (NIPAAm),¹²⁸ a large fraction of
16 capsules could form. Their properties including size and thickness of the shell
17 could be adjusted by changing the ratio of co-surfactant to monomer. In
18 another study, Landfester and coworkers showed a system to encapsulate the
19 volatile fragrance in poly(methyl methacrylate), polystyrene, or acrylic
20 copolymer capsules. When the content of the fragrance was about 25 wt%, a
21 particle morphology was obtained consisting of a matrix composed of the
22 fragrance in the polymer, while only at larger quantities of the fragrance, small
23 microdomains could be clearly formed with homogeneous distribution of the
24 fragrance.¹²⁹ Such varied hollow polymeric capsule morphology was also
25 observed in a system applying the biodegradable surfactant lecithin and the
26 eco-friendly hydrophobe Neobee M5 (triglyceride).¹³⁰ Additionally,
27 nanocapsule formation via the nitroxide-mediated cross-linking
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4 copolymerisation of styrene and divinyl benzene, stabilised by
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6 poly(vinylalcohol) (PVA) to fabricate polymeric capsules was also
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8 demonstrated by Zetterlund and Okubo *et al.*¹³¹
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34 **Figure 14.** Schematic view of the growing polymeric capsules by phase separation in
35 miniemulsion.¹²² (Reproduced with permission from reference 122. Copyright 2001
36 American Chemical Society).
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43 (ii) Formation of polymeric capsules by interfacial polymerisation:

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46 Interfacial polymerisation is another highly efficient approach to generate
47 polymeric capsules. On the one hand, this can be realised either by using
48 interfacially active initiators or water soluble initiators generating amphiphilic
49 species, “anchoring” the growing polymeric chain to the interface. On the other
50 hand, this can be also realised if the hydrophilic monomer is present in the
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3 aqueous phase and the other hydrophobic monomer in the organic phase, and
4 these monomeric species only meet and react to give polymers at the interface.
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9 By far, three types of polymerisation strategies including interfacial controlled
10 radical copolymerisation¹³²⁻¹³³, anionic polymerisation¹³⁴, and interfacial
11 polyaddition reactions are mostly used to synthesize polymeric
12 capsules.^{125,135-136}
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19 One representative for the formation of polymeric capsules by interfacial
20 controlled radical polymerisation process is to use styrene for the
21 encapsulation of isooctane and potassium peroxydisulfate as anchoring
22 agent.¹³⁷ The formation of a polymeric capsule morphology, especially that of a
23 liquid core and a polymeric shell, was extremely dependent on both the type of
24 RAFT and the initiator. Different RAFT agents lead to different polymerisation
25 rates, thus resulting in different chain lengths as a function of time. In the study,
26 the two exploited RAFT agents, phenyl 2-propyl phenyl dithioacetate (PPPDTA)
27 led to very fast polymerisation reactions, while phenyl 2-propyl dithiobenzoate
28 (CDB) retarded the polymerisation reaction. This could influence viscosity and
29 consequently chain mobility and therefore could cause a deviation from the
30 desired morphology. Also, the type of the used initiator which can influence the
31 surface activity of entering oligomers, was another important factor in obtaining
32 the correct structure. Using PPPDTA living polymerisation with KPS and AIBN,
33 only with KPS the polymeric capsules could be generated. In the case of CDB
34 and KPS, no polymeric capsules could be generated, since the viscosity of the
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4 growing polymeric shell was probably not high enough to reduce the chain
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6 mobility sufficiently, also allowing diffusion of the growing polymer into the
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8 droplet.
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10
11 Taking advantage of an amphiphilic poly(acrylic acid)-*block*-polystyrene
12
13 RAFT agent as a surfactant and polymerisation mediator, Luo and coworkers
14
15 demonstrated that highly uniform polymeric capsules could be obtained.¹³⁸⁻¹³⁹
16
17 While, instead of acrylic acid, the authors also incorporated the monomer
18
19 maleic anhydride into the oligomer RAFT agent.¹⁴⁰ Given that the
20
21 ammonolysed oligomer RAFT agent would self-assemble on the interface of
22
23 water/droplets, such self-assembly property combining with the RAFT living
24
25 polymerisation chemistry rendered the polymer chains to grow inwards
26
27 gradually in particles, leading to the formation of a polymer shell (Figure 15).
28
29 Accordingly, via tuning of the hydrophilicity of the oligomer RAFT agent by the
30
31 structure of oligomer RAFT agent or the degree of ammonolysis, the final
32
33 morphology of the synthesized polymeric capsules can be easily varied.¹⁴⁰ In
34
35 this study, when the molar ratio of ammonia to anhydride groups was 0.9 and
36
37 with 0.5 wt% SDS as a co-surfactant, optimised polymeric capsules were
38
39 obtained. Also, the incorporation of anhydride groups in polymer shell offers
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41 great opportunities to functionalise the surface properties of the capsules.
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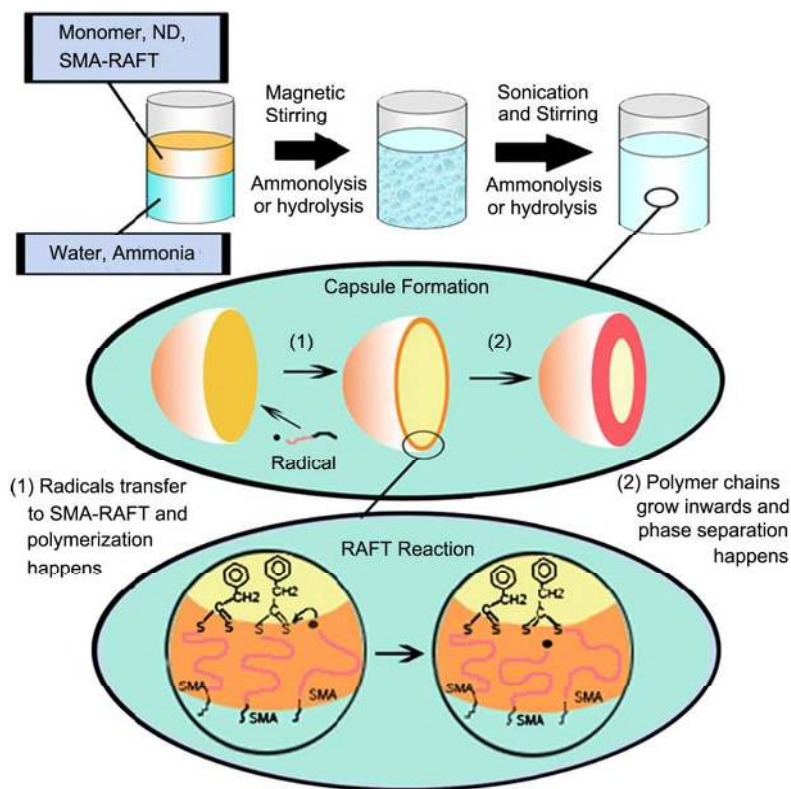


Figure 15. Schematic illustration of the procedure to generate polymer capsules based on RAFT interfacial miniemulsion polymerisation by using an amphiphilic RAFT oligomer (SMA-RAFT) as a surfactant.¹⁴⁰ (Reproduced with permission from reference 140. Copyright(2007) Elsevier Ltd.).

Matyjaszewski and coworkers¹⁴¹ also demonstrated the synthesis of polymeric capsules through an interfacially confined copolymerisation of a monovinyl monomer and a divinyl cross-linker via an *activator generated by electron transfer atom transfer radical polymerisation* (AGET ATRP) in a miniemulsion system using an amphiphilic block-copolymer poly(ethylene oxide)-*b*-poly(*n*-butyl methacrylate) (PEO-PBMACI), as a stabiliser and macroinitiator. The average diameter of the resulting capsules was about

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4 200–300 nm with good stability and dispersion in some common organic
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6 solvents. The introduction of various degradable cross-linking agents into the
7
8 system resulted in the formation of polymeric capsules that were cleaved
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10 under specific conditions.
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14 Besides the RAFT and ATRP polymerisation, anionic polymerisation can
15
16 also be carried out at the droplet's interface to generate polymeric capsules
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18 with encapsulated either aqueous or organic phases as core.^{120,134} In both
19
20 cases, the reaction is exclusively initiated at the droplet's interface, employing
21
22 alkyl cyanoacrylates as a monomer. Since the combination of two
23
24 electron-withdrawing groups (ester and nitrile) bonded to the same carbon
25
26 atom renders the alkyl cyanoacrylate highly reactive, the interfacial anionic
27
28 polymerisation of alkyl cyanoacrylate monomers is a very efficient way to
29
30 obtain biodegradable capsules. In this regard, poly(alkyl cyanoacrylate)-based
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32 capsules with different payloads including paclitaxel,¹⁴² antiepileptic drugs¹⁴³
33
34 and insulin¹⁴⁴ have been demonstrated.
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42 In addition, polymeric capsules could also be synthesized through interfacial
43
44 polyaddition or polycondensation reactions by using hydrophilic and
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46 hydrophobic precursors, which were dissolved in two different (continuous or
47
48 disperse) phases and reacted at the droplets interface. For example,
49
50 Landfester and coworkers demonstrated the synthesis of polyurea,
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52 polythiourea, or polyurethane capsules with an aqueous core performing the
53
54 interfacial polycondensation reactions in an inverse miniemulsion
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4 system.^{125,135-136,145} In this case, the size of the capsules, the thickness and
5
6 functionality of the shell were studied thoroughly. The size of the capsule was
7
8 dependent not only on the concentration of the surfactant but also on the time
9
10 of addition of the second monomer. The thickness of the wall could be
11
12 controlled by the amount of monomers employed. The functionality of the shell
13
14 was governed by the nature of the monomers and the ratio between the two
15
16 monomers introduced in the miniemulsion. Also after cross-linking, the
17
18 polymeric capsules can be easily transferred into the aqueous phase, whereby
19
20 the non-reacted diisocyanate groups (from a cross-linker) will hydrolyse,
21
22 resulting in the formation of primary amine groups on the capsule's surface.
23
24 Thus further modification of the capsule's surface was realised with targeted
25
26 molecules such as folic acid,¹⁴⁶ oligomannose¹⁴⁷ or antibodies.¹⁴⁸
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36 (iii) Formation of capsules by precipitation of polymer on preformed droplets:

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38 Recently, via the controlled polymer nanoprecipitation onto inverse
39
40 miniemulsion droplets, the so-called nanoprecipitation method was developed
41
42 by Landfester and coworkers which was also able to be exploited to fabricate
43
44 polymeric capsules.¹⁴⁹⁻¹⁵⁰ The deposition of a polymeric shell, *e.g.*, PMMA,
45
46 PCL or PMA, from the organic continuous phase onto the stable nanodroplets
47
48 dispersed phase was achieved by changing the gradient of the
49
50 solvent/nonsolvent mixture of dichloromethane/cyclohexane under mild
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52 evaporation. After re-dispersion of the nanocapsules in an aqueous medium,
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4 stable polymeric capsules with a chlorohexidine digluconate loaded aqueous
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6 core re-dispersed in the aqueous continuous phase were obtained for the first
7
8 time.¹⁴⁹⁻¹⁵⁰
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10
11 In all, miniemulsion as a tool for the formation of polymeric capsules is one of
12
13 the most commonly exploited techniques, especially given its versatility,
14
15 simplicity, productivity, and ease of application on small scales. Moreover, the
16
17 characteristic feature in the miniemulsion process is that stable, small, and
18
19 narrowly distributed droplets are formed which could retain ideally identical
20
21 throughout the polymerisation process. This enables the possibility for the
22
23 encapsulation of different materials, ranging from liquid to solid, from organic
24
25 to inorganic, and from molecularly dissolved to aggregated species into
26
27 polymeric capsules. There is no doubt that such technique provides a wide
28
29 platform for the construction of various novel functional nanomaterials as
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31 required.
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41 2.3 Proteinosomes

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44 Very recently, being rather different from polymersomes and polymeric
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46 capsules, a new type of membrane-bounded capsule model based on the
47
48 spontaneous interfacial assembly of amphiphilic recombinant proteins¹⁷ or
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50 protein–polymer conjugates has been reported.¹⁶ For example, the
51
52 protein–polymer conjugates, which consisted of about three polymer chains
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54 per protein, assembled at water droplet/oil interfaces into micrometer-scale
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4 water-filled capsules termed proteinosomes. The proteinosomes were
5
6 delineated by a closely packed monolayer of conjugated protein–polymer
7
8 building blocks that could be crosslinked such that the micro-compartments
9
10 were transferred into water without fragmentation (Figure 16). As a
11
12 consequence, a wide range of guest molecules from small molecule
13
14 fluorescence dyes to colloidal particles (~500 nm in diameter) could be
15
16 encapsulated at high efficiency inside the micro-compartments. Moreover, the
17
18 protein–polymer membrane was sufficiently elastic and robust to withstand
19
20 partial dehydration and rehydration, and remained structurally intact when held
21
22 at a temperature of 70 °C for 90 min, suggesting that the proteinosomes could
23
24 provide a useful protocell model system for studying the microscale
25
26 confinement of thermophilic enzymes, or for developing artificial cells capable
27
28 of undertaking temperature cycling procedures, such as PCR-mediated
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30 amplification of entrapped genetic polymers.
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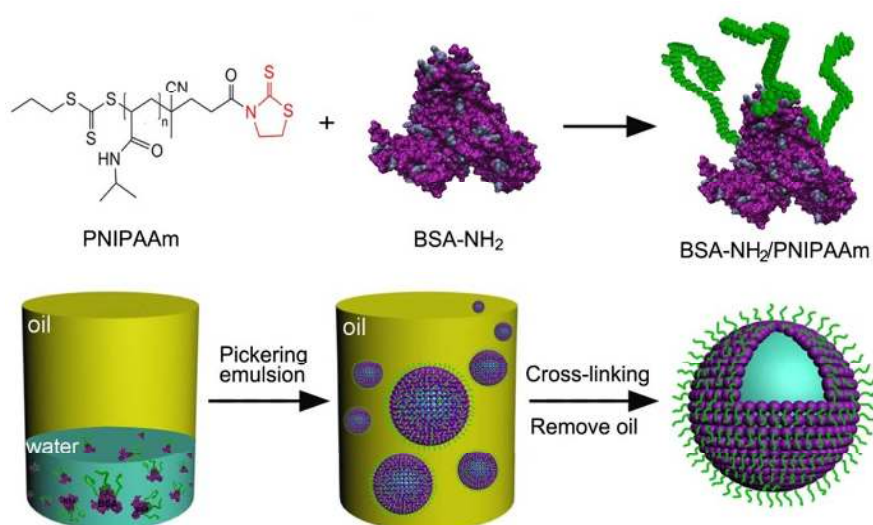


Figure 16. Schematic illustration of a general procedure for preparation of proteinosomes.

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4 Top row: coupling of activated PNIPAAm polymer chains with primary amine groups of
5
6 cationised BSA-NH₂ to produce protein-polymer nano-conjugates (BSA-NH₂/PNIPAAm).
7

8
9 Bottom row: Use of protein-polymer building blocks for the spontaneous assembly of
10
11 proteinosome micro-compartments in oil, and their transfer into a bulk water phase.¹⁶
12

13
14 (Reproduced with permission from reference 16. Copyright (2013) Nature Publishing
15
16 Group).
17
18

19
20
21 A key advantage of the proteinosome system lies with the ability to rationally
22
23 design the structure and function of the proteinosome membrane by controlling
24
25 the chemistry of the protein-polymer nanoscale building blocks. This makes
26
27 the system an attractive candidate for the development of a range of functional
28
29 proteinosomes. For example, recent studies indicated that the proteinosome
30
31 membrane was semipermeable, stimulus-responsive, enzymatically active,
32
33 and mechanically elastic.¹⁶ It also exhibited size-selective permeability, only
34
35 allowing molecules less than 40 kDa in molecular mass to enter or leave the
36
37 proteinosome interior. Moreover, conjugation of the temperature sensitive
38
39 polymer, poly(N-isopropylacrylamide) (PNIPAAm) to the protein molecules
40
41 generated a thermally gated system that operated via the swelling or
42
43 deswelling (at $T > 33$ °C) of the polymer chains present on the outer surface of
44
45 the proteinosome membrane. As a consequence, enzyme-mediated
46
47 peroxidation within the proteinosomes of a small molecule initially present in
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49 the external solution was switched on or off by thermally gating the diffusion of
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the substrate through the polymer shell barrier layer (Figure 17).¹⁶

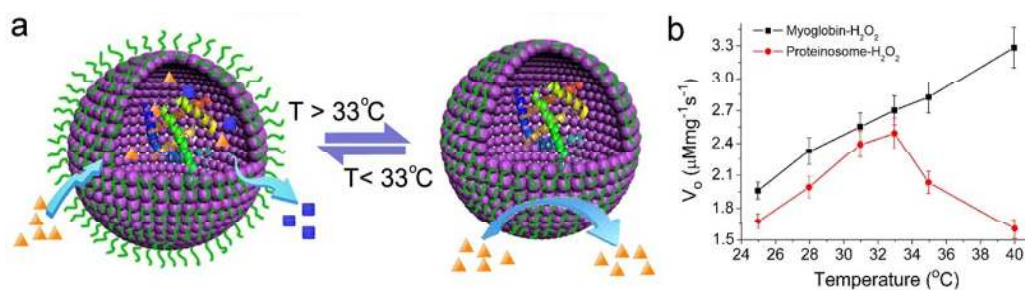


Figure 17.(a) Scheme showing polymer chain gating of enzyme reactions inside proteinosomes. (b) The corresponding temperature-dependent catalytic reaction rates of free myoglobin (black lines) and proteinosome-encapsulated myoglobin (red lines) in the presence of both reactants (2-methoxyphenol and H_2O_2).¹⁶ (Reproduced with permission from reference 16 Copyright (2013) Nature Publishing Group).

As a further extension of these ideas, the construction of a proteinosome suited for a membrane-mediated cascade reaction was demonstrated.¹⁵¹ Three cascade enzymes, glucose amylase (GA), glucose oxidase (GO) and horseradish peroxidase (HRP) have been used to prepare protein-PNIPAAm amphiphilic nano-conjugates to construct proteinosomes comprising an enzyme triad capable of undertaking a multi-step membrane-mediated cascade reaction using starch as the starting substrate.¹⁵¹ The tandem reaction can be regulated by the positional assembly and spatial separation of the three enzymes within the membrane of the proteinosome (Figure 18), as

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4 well as by temperature-dependent changes in the conformation of the
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6 covalently attached polymer chains located on the external surface.
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9 Furthermore, in a recent work, proteinosomes with new types of higher-order
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11 functionality based on controlled membrane disassembly, internalised
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13 assembly of a cytoskeletal-like matrix, and self-production of a
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15 membrane-enclosing outer wall have been described.¹⁵² DNA strands were
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17 encapsulated in the proteinosomes and their release was triggered by
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19 protease-mediated hydrolysis or chemical cleavage of the membrane building
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21 blocks. In addition, enzyme-mediated amino acid dephosphorylation was used
22
23 to generate a supramolecular amino acid hydrogel within the interior of the
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25 proteinosome. As a consequence, the mechanical properties of the
26
27 proteinosome were considerably enhanced. For example, the spherical
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29 morphology of the hydrogel-containing proteinosomes was quickly
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31 re-established after the protocells were mechanically deformed or deflated due
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33 to dehydration. Interestingly, prolonged growth of the encapsulated hydrogel
34
35 resulted in penetration of the amino acid nanofilaments through the porous
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37 shell of the proteinosome to produce a continuous, protease-resistant outer
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39 wall that totally enclosed the protein–polymer membrane,¹⁵² analogous in
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41 some respects to the cell wall/cell membrane layered structure of bacterial
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43 cells.
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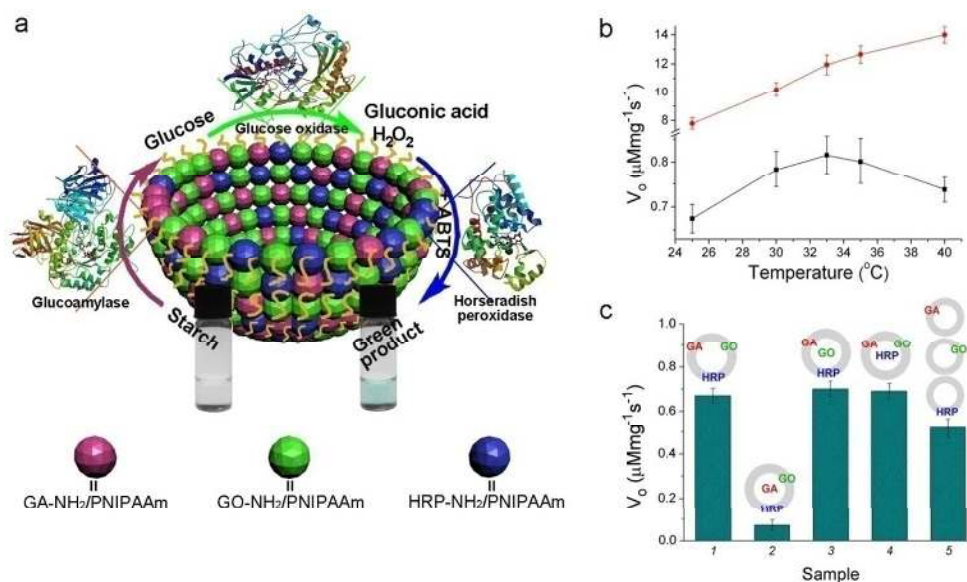


Figure 18. (a) Schematic illustration showing a proteinosome membrane-mediated three-stage cascade reaction in water by using three enzymes, glucose amylase (GA), glucose oxidase (GO) and horseradish peroxidase (HRP), as building blocks. (b) Plots of temperature-dependent initial three-step cascade reaction rates (V_0) (red line: aqueous mixture of GA, GO and HRP enzyme-polymer nanoconjugates; black line dispersion of multi-enzyme proteinosomes comprising membrane building blocks) and (c) summary chart of the catalytic activity of enzyme-polymer proteinosomes prepared with different membrane and internal spatial organisations.¹⁵¹ (Reproduced with permission from reference 151. Copyright (2014) Royal Society of Chemistry).

3. Engineering Functional Polymer Capsules Towards Smart

Nanoreactors

3.1 Membrane-Based Functionalisation of Polymer Capsules

3.1.1 Channel Proteins as Transporters

The improved stability of polymersomes over liposomes does become an

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4 issue when it comes to membrane permeability. Especially polymersomes
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6 designed as nanoreactors need to have some way to make their enclosed
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8 active cargo like an enzyme accessible without destroying the vesicle. Natural
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10 and artificial liposomes contain channel proteins to gate transmembrane traffic,
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12 making them an obvious choice also for polymersomes.^{4,83,153}
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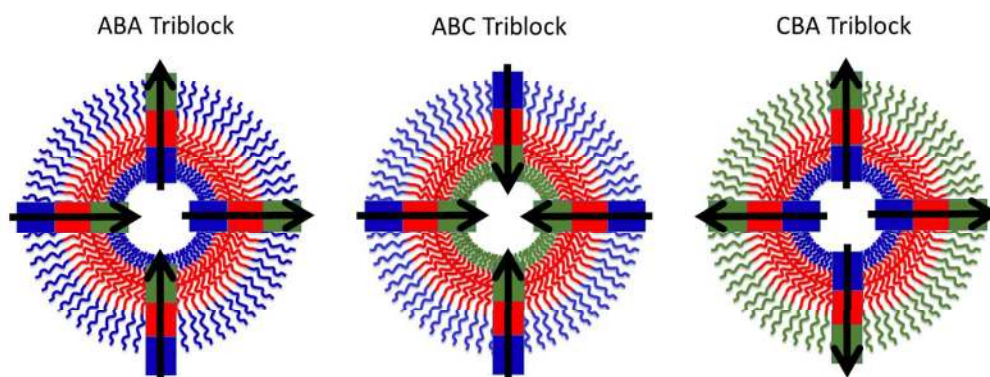
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17 First trials in this direction have studied whether a natural protein will have
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19 the ability to retain its structure and with this its functionality in an artificial
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21 environment. Initial trials were done with polymer-fortified liposomes which
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23 were strengthened by cross-linked poly(butyl methacrylate) (PBMA).¹⁵⁴ The
24
25 protein, the bacterial outer-membrane-protein F (OmpF), continued to gate the
26
27 activity of beta-lactamases and proved that natural proteins can continue to
28
29 work in an artificial membrane made from polymers.¹⁵⁴
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34 It was then a logical consequence to incorporate trans-membrane-proteins
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36 into the membranes of polymersomes. From a polymer point of view the soft
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38 poly(dimethylsiloxane) (PDMS) as hydrophobic part in combination with
39
40 poly(methyloxazoline) is applied repeatedly and nearly solely for
41
42 transmembrane protein incorporation.^{34,155} The block lengths of the polymers
43
44 vary, but all have a polymersome forming ratio as proven by TEM micrographs.
45
46 The proteins inserted range from simple pore proteins^{31,156-158} to receptors¹⁵⁹
47
48 and pumping proteins.¹⁶⁰ Because channel-proteins are amphiphilic, they may
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50 influence the shape, although the aim is of course that the polymersomes
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52 remain as vesicles. Walz *et al.* tested this with varying amounts of Aquaporin Z,
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1
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3 a channel protein, incorporated into the block-copolymer membrane.¹⁵⁶ From
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5
6 15 polymer chains per protein onwards, vesicles were formed. When more
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8
9 protein was present, the vesicular shape started to break up and more
10
11 complex structures were formed. These include planar membranes and
12
13 smaller vesicles. However, the protein remained active until it started to
14
15 crystallise at a 1/1 protein to polymer ratio.¹⁵⁶ It should be noted that proteins
16
17 generally also insert into polymer bilayer membranes, which do not form
18
19 vesicles, e.g. as shown for alpha hemolysin in a solid-substrate supported
20
21 PDMS-PMOXA-PDMS membrane.¹⁶¹
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26 Another important aspect to consider is the orientation of the protein.
27
28 Especially if the protein does only transport in one direction, the orientation
29
30 becomes of great importance to control specific inward and outward transport.
31
32 Meier *et al.* tested this by including Aquaporin 0 into vesicles of the ABA
33
34 polymer mentioned above and an ABC triblock-copolymer of
35
36 PEG-PDMS-PMOXA. The authors used polymers with different aspect ratios
37
38 to ensure the production of vesicles with PEG on the outside as well as ones
39
40 with PMOXA on the outside, known from previous studies.¹⁶² Whichever
41
42 polymer is supposed to go to the inside of the vesicle is considerably shorter
43
44 than the one going to the outside as it needs to cover less volume on the
45
46 concave side of the membrane. In a comparison also with lipid membranes,
47
48 only vesicles from asymmetric ABC triblock-copolymers resulted in a directed
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50 insertion of the Aquaporin 0 examined. They could even show that vesicles
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4 with the opposite membrane structure (PEG-PDMS-PMOXA from outside to
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6 inside as compared to PMOXA-PDMS-PEG from outside to inside), also yield
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8 the opposite channel protein insertion into them (Figure 19).¹⁶³
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24 **Figure 19.** Polymersomes with symmetric (ABA) and asymmetric (ABC) triblock
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26 copolymer membranes which hold a channel protein. The protein inserts either with a
27
28 prominent orientation for the asymmetric ABC triblock copolymers and at random for the
29
30 symmetric ABA block copolymers.¹⁶³
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34
35 Polymersomes with channel proteins were also brought into service as
36
37 nanoreactors or other functional nanocarriers. Generally it has to be stated that
38
39 all applications of channel protein-equipped polymersomes were done with the
40
41 symmetric PMOXA-PDMS-PMOXA ABA triblock copolymer, hence without any
42
43 control of protein orientation. One of the first examples from Meier *et al.* shows
44
45 the insertion of LamB, which is not only a transmembrane protein, but also acts
46
47 as a receptor for the bacteriophage lambda. Once docked to the receptor, the
48
49 phage is now able to empty it itself and transfer its cargo (DNA) into the
50
51 polymersome.¹⁵⁹ The same group later showed that vesicles including
52
53 Aquaporin Z had a significantly higher permeability towards ions than plain
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4 polymersomes. Both systems were exposed to a positive ion gradient and only
5
6 the vesicles including the channel proteins reacted towards this change. Van
7
8 Gelder *et al.* conducted a study where OmpF was inserted into the membrane,
9
10 but the group went a step further and also included the ViVax nucleoside
11
12 hydrolase (TvNH) into their vesicles as it can activate prodrugs based on
13
14 riboside.¹⁶⁴ A number of such prodrugs were now inserted and the riboside
15
16 was cleaved off. It should be noted that both examples rely on a non-oriented
17
18 insertion of channel proteins to ensure transport in and out of the vesicles and
19
20 to avoid the build-up of osmotic pressure from the inside as diffusion barrier.¹⁶⁴
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26 Montemagno *et al.* managed to achieve a significant milestone by rebuilding
27
28 a vesicle that can produce ATP.¹⁶⁰ The polymer, PEtOxa-PDMS-PEtOxa is
29
30 very similar to the methyl analogue discussed so far, so it does not pose a
31
32 significant change, especially as it is also well characterised.¹⁶⁵ The
33
34 significance of this work is that the authors needed to include
35
36 bacteriorhodopsin (BR) to establish the proton gradient, but also the ATPase to
37
38 use that very gradient for the production of ATP. It is remarkable that the
39
40 authors used a symmetric block-copolymer and thus lacked control over
41
42 protein orientation. The ATPase has the rotating outer bit, which may be too
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44 bulgy to insert the “wrong” way, but only nanoreactors where a vast majority of
45
46 BR is inserted correctly will work, as the wrongly inserted BR proteins would
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48 reduce the proton gradient built up by the other proteins. The authors were
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50 able to show that the nanoreactor is working as intended and does produce
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ATP from ADP and phosphate.¹⁶⁵ Despite this achievement, nothing is stated on the yield of these nanoreactors, which may explain why this work was not continued so far.

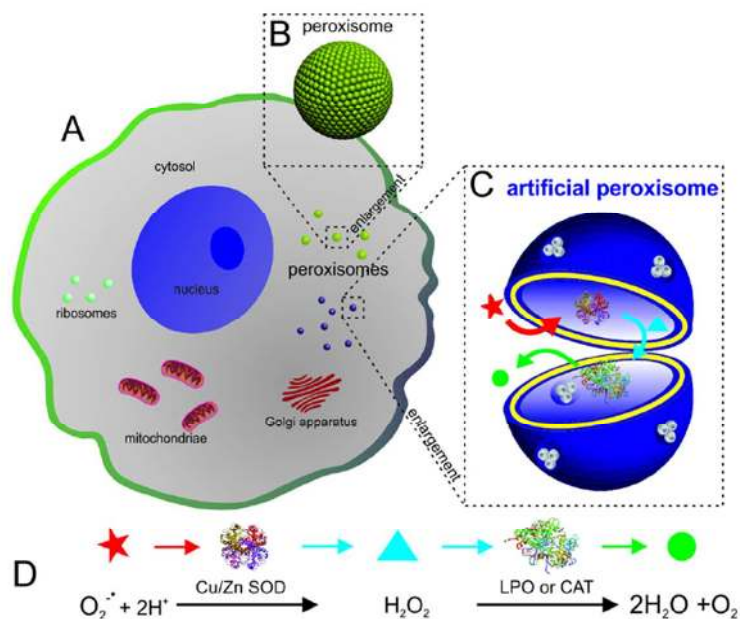


Figure 20. An artificial peroxisome (C) containing OmpF as a channel protein and enclosed proteins to act as nanoreactors within the cell (A). The polymersomes aid the natural peroxisomes (B) naturally present in the cells. The separate reaction steps are shown in part D.¹⁵⁷ (Reproduced with permission from reference 157. Copyright(2013) American Chemical Society).

Hunziker and Palivan *et al.* did a series of consecutive studies on polymersomes of the ABA block-copolymer (PMOXA-PDMS-PMOXA) with the channel protein OmpF to create a biologically functional nanoreactors. Their final goal was the creation of a synthetic organelle, specifically an artificial

1
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3 peroxisome, e.g. vesicles that clear its surroundings of superoxides.^{158,166-167}

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6 As proof of concept, acid phosphatase was included in the polymersome. The
7
8 phosphatase is too big for the OmpF pores, so it stays inside, but its substrates
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10 and products can enter and leave the vesicle.³¹ Amplex Red was a suitable
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12 substrate as its dephosphorylated version is fluorescent, while its
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14 phosphorylated version is not. In a sense, this nanoreactor was rightfully
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16 described as pH sensitive, simply because the acid phosphatase is only active
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18 at acidic pH values. However, the pores are functioning independent of the pH
19
20 value, hence the pH does not gate the entrance to the vesicle.³¹ The proof of
21
22 concept was not a peroxisome as yet, but a first one was achieved late without
23
24 transmembrane proteins, exploiting the limited permeability of the plain
25
26 polymersomes towards very small molecules like hydrogen peroxide.¹⁶⁷ The
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28 authors now had a proof of concept of a OmpF-based nanoreactors and an
29
30 initial peroxisome. Both concepts were then combined to yield a far better
31
32 functioning peroxisome than the initial one. The group equipped the vesicles
33
34 with a complex system of proteins inside and the OmpF in the membrane. First
35
36 of all, the stress-inducing Xantine was oxidised by Xantine Oxidase and the
37
38 product then reacted further to hydrogen peroxide with the aid of Cu/Zn and
39
40 superoxidase. Hydrogen peroxide would still be toxic, so a third enzyme,
41
42 lactoperoxidase, then transformed it into water and oxygen.¹⁶⁶ This artificial
43
44 peroxisome proved to work extracellular but also within cells. As a last step,
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46 this nanoreactors was now not only included in cells but the HeLa cells were
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4 also treated to experience oxidative stress. When the cells were previously
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6 treated with the artificial peroxisomes mentioned, they could withstand stress
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8 levels that proved to be toxic to untreated cells.¹⁵⁸ To the best of our
9
10 knowledge, this represents the first example of polymersomes as artificial
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12 organelles within a cell that actually aid the cells survival using enclosed
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14 enzymes as protected drugs. This marks a significant milestone in combining
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16 the applications of polymersomes as nanoreactors and drug delivery systems.
17
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20
21 (Figure 20)
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26 3.1.2 pH Responsive Polymersomes

27
28 One of the easiest ways to produce a responsive polymersome is to include
29
30 a pH sensitive monomer in the hydrophobic block. Upon change in pH this part
31
32 becomes hydrophilic, leading to a then entirely hydrophilic block-copolymer.
33
34 Such a polymer does not form polymersomes anymore, inducing a
35
36 disassembly of the vesicles.^{20,168-169} Not only is the pH easy to control and can
37
38 be changed once the polymersome is formed, it is also known to change
39
40 during biological processes like endocytosis. The latter one makes pH
41
42 sensitive polymersomes perfect candidates of drug delivery systems, which is
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44 widely reported.¹⁷⁰⁻¹⁷¹ A pH responsive polymer for polymersomes can, of
45
46 course, be responsive towards a basic pH (*e.g.* boronic acid)³³ or towards the
47
48 acidic region (*e.g.* tertiary amines).^{66,76,172} For some amine-based pH sensitive
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50 polymersomes, the term CO₂-sensitive polymersome is also used in
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3 literature.¹⁷³⁻¹⁷⁴ In these examples the addition of CO₂ is used as a means to
4 drop the pH of the solution, e.g. exploiting the formation of carbonic acid
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6 leading to a protonation of the substrate. Albeit the addition of CO₂ being a
7
8 neat way to change the pH of the solution, the polymers are responding to the
9
10 pH change of the solution and are thus pH-responsive rather than
11
12 CO₂-responsive.
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18
19 With respect to pH sensitive polymersomes, it can be stated that all of them
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21 are produced by controlled radical polymerisation and thus are based either on
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23 acrylates, methacrylates or other vinyl-based polymers with a respective side
24
25 chain. The reason for this is quite simple, because pH sensitivity comes along
26
27 with protonation and thus a lone electron pair exists. This lone pair could act as
28
29 an initiator in ring opening polymerisation (ROP) or anionic polymerisation,
30
31 making these tough methods to introduce pH sensitivity or any functionality
32
33 with a lone electron pair and require at least the use of protecting groups in
34
35 corresponding monomers.¹⁷⁵ This has only been accomplished so far with
36
37 trityl-protected polyhistidine, where after deprotection PEG-polyhistidine
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39 block-copolymers were self-assembled into pH-sensitive polymersomes
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41 (Figure 21).¹⁷⁶⁻¹⁷⁷ The pKa of these polymersomes and whether disassembly
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43 or a conformation change is reached with decreasing the pH value, can be
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45 tuned if some poly(lactic acid) is polymerised alongside the polyhistidine in the
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47 hydrophobic block.¹⁷⁸
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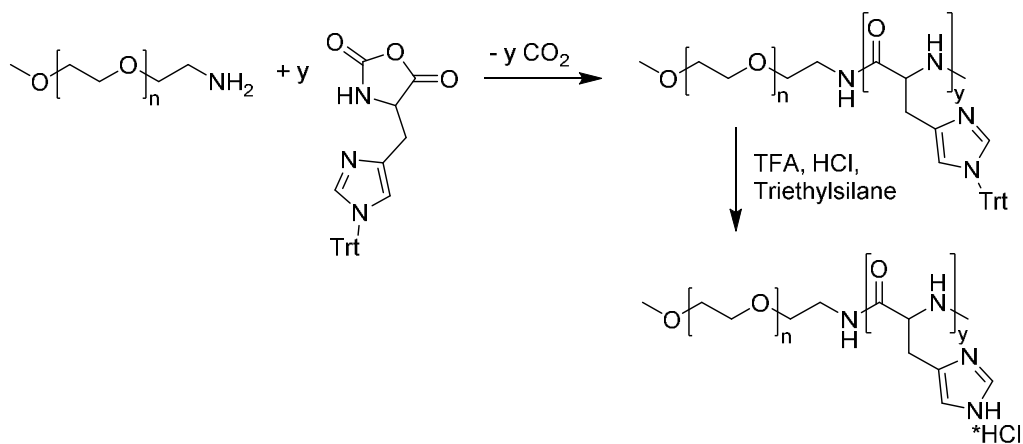


Figure 21. Synthesis of PEG-PHis *via* an NCA-based Ring-Opening-Polymerisation with a consecutive deprotection (cleavage of trityl group) of the polyhistidine.¹⁷⁷

The vast majority of pH sensitive polymersomes responds to a change towards an acidic medium, *e.g.*, are amine-based. However, van Hest *et al.* did also report a system based on poly(styrene boronic acid) (PSBA)- which responds towards a change into basic pH (Figure 22).³³ Being a Lewis acid, the boronic units of the PEG₂₂-*b*-PSBA₉₃ bind a hydroxyl ion in a basic pH range. The negative charges present are responsible for solubility switch and the polymersome disassembles. For the system reported, the pKa is between 9 and 10.

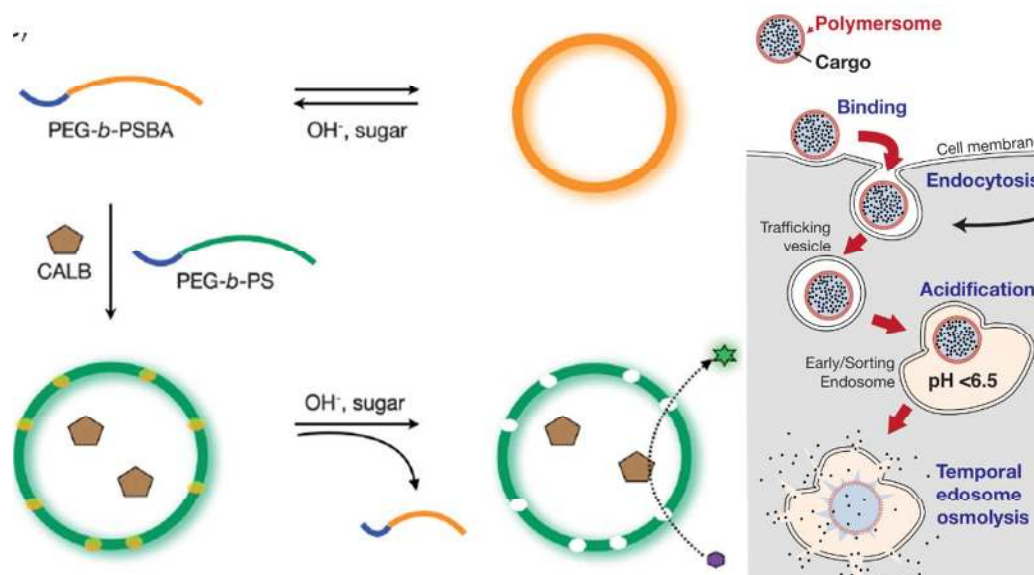


Figure 22. pH sensitive polymersomes using PEG-PS and PEG-PSBA (structure in Table at the end of the Review) as nanoreactors for enzymatic reactions where the membrane can only be passed at pH 9 or lower (left).³³ PDPA-containing pH sensitive polymersomes used as drug-delivery-systems exploit the endosomal pH switch to release their cargo (right, redrawn from reference with permission).¹⁷⁹ (Left part reproduced with permission from reference 33. Copyright (2009) John Wiley and Sons; right part reproduced with permission from reference 179. Copyright (2009) Royal Society of Chemistry).

The tertiary amines used in pH sensitive block-copolymers are mainly attached to a methacrylic backbone. A variety of authors use poly(diisopropylaminoethyl methacrylate) PDPA^{13,172,180-183} or its ethyl (PDEA)^{56-57,66,76,111,184-187} and methyl (PDMA)^{52,100,188} derivatives. It should be noted that a change in the substitution from isopropyl units towards methyl units causes a significant change in the pKa of the polymer. While PDPA has a pKa of 6.4 at room temperature,¹³ that of PDEA is already at 7.3^{10,58} and PDMA

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4 has a pKa of about 7.5^{10,58} at room temperature. PDMA is also temperature
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6 sensitive, thus a dual-responsive polymer, but this dual property has only been
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8 applied rarely so far by Lecommandoux *et al.*⁹⁹ Both other derivatives are
9
10 used extensively by Battaglia *et al.* (PDPA) and Voit *et al.* (PDEA) in their
11
12 respective polymersome systems. PDPA has been proven to be a perfect
13
14 candidate for drug delivery applications. It is used to exploit the pH drop
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16 occurring after endocytosis in the early and late endosomes. The
17
18 polymersomes are stable in cell media as well as the bloodstream (pH 7.4) but
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20 disassemble in the early endosomes (pH 6.0) and release their payload at this
21
22 stage (Figure 22). What happens to the single polymer chains is not known so
23
24 far. It should be noted that polymersomes of this specific polymer system of
25
26 Battaglia *et al.* (POEGMA-PDPA) are able to cross the blood-brain-barrier, thus
27
28 enabling polymersomes of POEGMA-PDPA to deliver drugs to the brain, an
29
30 especially protected part of the body.
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39 On the other hand, polymersomes containing PDEA proved to have a pKa
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41 suitable for application as nanoreactors, especially cross-linked ones. In
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43 contrast to drug delivery systems, enzyme-based nanoreactors need to be
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45 operational at close-to-neutral pH values to avoid denaturation of the enclosed
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47 enzyme at low pH. In their cross-linked state (maleimide-based cross-linker),
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49 the polymersomes show a reversible swelling upon hitting the pKa. It means
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51 that the disassembly process of the polymersomes is hindered by the
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53 cross-linking bonds leaving a swollen and permeable polymersome.⁶⁶ It should
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4 be noted that the “switching point” of these polymersomes decreases with
5
6 increasing cross-linking density. The PEG-PDEA-PDMIBM polymer applied by
7
8 Voit *et al.* to form cross-linked nano reactors shows a pKa of 7.0(Figure 23).¹⁸⁶
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10
11 The authors showed that single, but also cascade reactions can be controlled
12
13 with these nanoreactors. In all examples, reactions take place at an acidic pH
14
15 only, when the membrane is swollen. When the membrane was in a
16
17 non-swollen state (basic pH), no reaction was observed.¹⁸⁶⁻¹⁸⁷ Again with a
18
19 fully reversible pH switch at 7.0, the same group applied very similar
20
21 polymersomes (with a benzophenone cross-linker) also as drug delivery
22
23 system.⁷⁶ The drug, doxorubicin, is released in different speeds depending on
24
25 the cross-linking density and pH and also targeting towards specific cell types
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27 has been introduced. An application on cells did not only proof it being
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29 non-toxic but the drug effect was also proven on different cell types.¹⁸⁹
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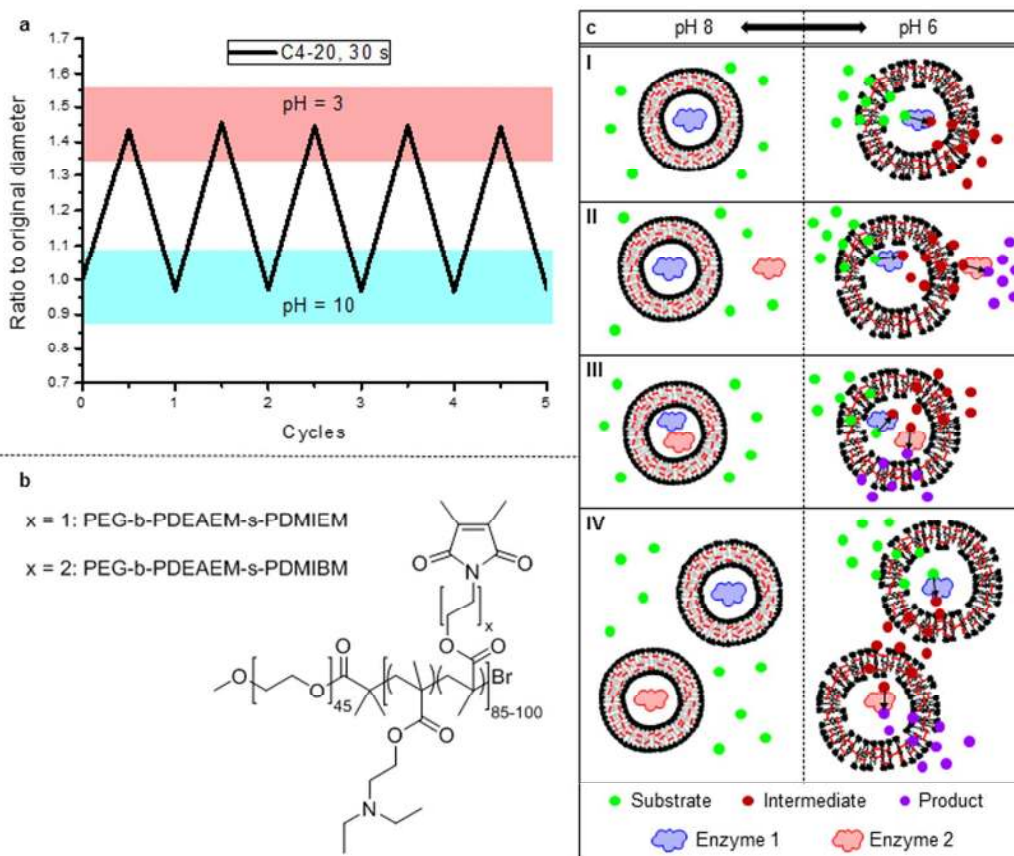
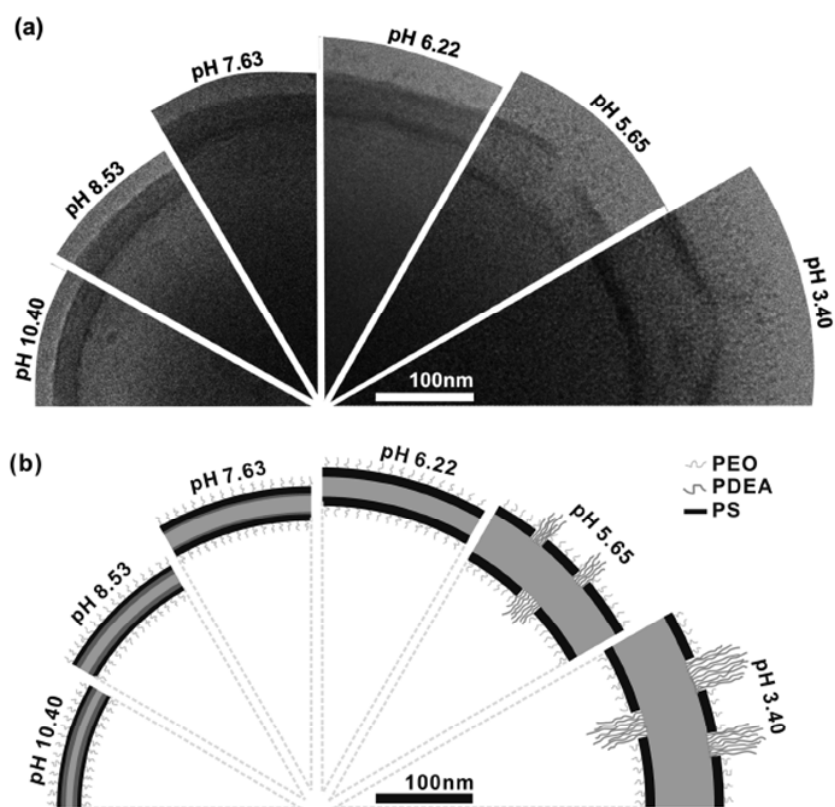


Figure 23. Cross-linked and pH sensitive polymersomes show a reproducible swelling-deswelling behaviour (a)¹⁸⁶ from a bifunctional PEG-PDEA-s-PDMI(E/B)M block-copolymer (b). The vesicles allow for single and cascade enzymatic reactions, also across separate vesicles (c).¹⁸³

Apart from the system just discussed, there are a number of other polymersomes with a reversible swelling behaviour (pH induced) reported. The main difference is that other systems use physical cross-linking with polystyrene as reported by Eisenberg *et al.* (Figure 24)¹⁸⁴ rather than chemical cross-linking. Using this glassy polymer, the adhesive forces in the polystyrene

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4 part are stronger than the repulsive forces resulting from the protonation. The
5
6 polymersome stays intact, but does show a swelling (“breathing” as described
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8 by the authors) as already discussed for cross-linked vesicles. When van Hest
9
10 *et al.* mixed their PEG-PSBA with PEG-PS, they saw a similar effect. Just with
11
12 10w% of PEG-PSBA in the mixture the adhesive forces of PS were strong
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14 enough to keep the polymersome intact. Again, the change in pH leads to a
15
16 swelling of the vesicle, which the authors exploit to gate enzymatic activity
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18 inside the polymersome.³³
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51 **Figure 24.** Breathing polymersomes of an ABC triblock copolymer (PEG-PS-PDEA). The
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53 membrane thickens (swells) upon acidification, but the strong interactions in the
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3 polystyrene part keeps the vesicles intact.¹⁸⁴ (Reproduced with permission from reference
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6 184. Copyright (2009) American Chemical Society).
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10 Studies including a block-copolymer of PS and PDEA as hydrophobic
11 components have proven this concept.¹⁸⁴ Initially the reversible swelling of this
12 physical crosslinking was reached by adding acid and base, respectively, but
13 was then shown to work also by adding CO₂ and N₂ to reach the desired pH
14 switch. In the cross-linking examples discussed so far, PEG was the
15 hydrophilic component, but using PNIPAM a similar result was achieved.¹⁷⁴
16
17 With PCL as the hydrophobic part (and PDMA as pH sensitive part), the
18 reversible switching is quite remarkable because PCL has lower van der Waals
19 interactions than polystyrene. This makes it unexpected that the
20 polymersomes stay in shape upon the pH change and do not show a transition
21 to micelles. Again the pH switch for PNIPAM-*b*-PCL-*b*-PDMA was achieved
22 with the consecutive addition of CO₂ and N₂ and the system was claimed to be
23 CO₂ responsive rather than pH responsive. Until now, however, no
24 nanoreactors have been reported using physical cross-linked systems.^{100,174}
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45 However, as soon as a block-copolymer with a statistical mix of 2 monomers
46 in the hydrophobic block is used (PS and PDEA), the physical interactions
47 exerted by PS become too weak to hold the polymersome together. With
48 ongoing protonation of a PEG-*b*-PDEA-*r*-PS block copolymer and consecutive
49 lack of stabilisation of the residual hydrophobic part, the polymersomes
50 become instable. Protonation makes the polymer hydrophilic and shifts the
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4 hydrophilic-to-hydrophobic block length ratio. The self-assembly structures
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6 change in accordance with previously published results from vesicles to worms
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8 and micelles (Figure 25).¹⁹⁰ The very same principle has also been applied to
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10 block-copolymers containing guanidine and amidine derivatives as pH
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12 sensitive part.¹⁹¹⁻¹⁹² Literature discusses them as CO₂-sensitive, but as the
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14 addition of CO₂ merely changes the pH, these polymers are discussed as pH
15
16 sensitive in this review. What does make these systems special, is that the
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18 positive charge it is now stabilised over several bonds by mesomeric
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20 resonance structures. It is reasonable to assume that a classic pH switch
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22 would result in the same behaviour.¹⁹¹⁻¹⁹² These vesicles have also been
23
24 deployed to induce a pH-induced switch in morphology, from polymersomes to
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26 worms and micelles, depending on the block-length ratio used.¹⁹¹⁻¹⁹²
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49 **Figure 25.** Vesicle-to-micelle transition of a PEG-PDEA-PS with PDEA and PS being
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51 incorporated in a random block copolymer. Acidification disturbs the
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53 hydrophilic-to-hydrophobic balance, forcing a switch in the self-assembly structures.¹⁹⁰
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4 (Reproduced with permission from reference 190. Copyright (2009) Royal Society of
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6 Chemistry).
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10 Apart from the discussed addition of acid or CO₂ to change to pH, very
11 recently it was reported by Gu *et al.*, that the vesicles themselves are also able
12 induce a pH change. A reaction cascade of enclosed glucose oxidase and
13 catalase induces a pH drop as soon as the substrate, glucose, is added to the
14 solution. The ketal units in the hydrophobic block now get hydrolysed in acidic
15 conditions, forcing vesicle disassembly.¹⁹³
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27 3.1.3 Temperature Responsive Vesicles

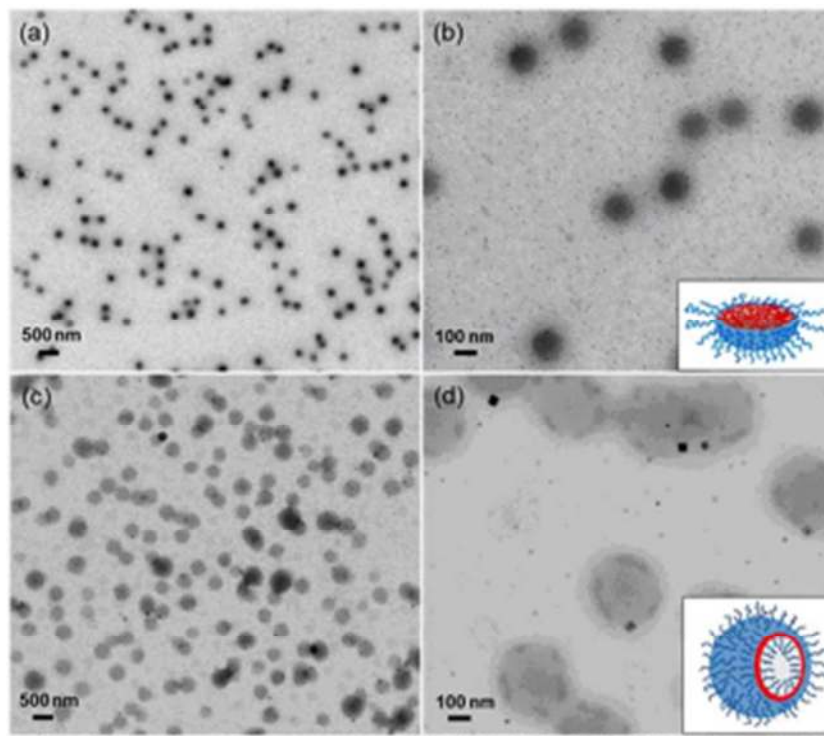
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29 The best known example for a temperature sensitive polymer is PNIPAM
30 with its LCST around 33°C and it is, of course, also used for temperature
31 responsive polymersomes.⁷² A LCST behaviour means that the polymer is
32 water soluble below the LCST (hence feasible as hydrophilic component) and
33 water insoluble above it (then suitable as hydrophobic component). One of the
34 first examples is thus logically a PEG-PNIPAM block-copolymer where the
35 vesicles are only stable above the LCST of PNIPAM where it is hydrophobic.⁷³
36
37 Later applications including PNIPAM use it in combination with other functional
38 monomers. Cross-linking was the first property to be combined with PNIPAM.
39
40 The authors produced a PCMA-PNIPAM diblock-copolymer which does then
41 form vesicles. After cross-linking, cooling the PNIPAM down did not lead to a
42 destruction of the vesicles, but only to a swelling as already discussed of pH
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3 sensitive vesicles. The model compound 4-aminopyridine was released from
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5 swollen vesicles at low temperatures only.¹⁹⁴ A similar approach was used later
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7 on using PEG-PAA-PNIPAM polymers where the PAA part was then
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9 enzymatically cross-linked. Interestingly, the use of different buffers influenced
10
11 the LCST. When conducting the experiment in PBS, a LCST of 38°C was
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13 observed, while experiments in MES buffer gave a LCST of 32°C. The authors
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15 showed that vesicles from this polymer show cellular uptake, no toxicity and a
16
17 variety of drugs were released successfully.¹⁹⁵
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24 A rather unexpected polymer to study a temperature sensitive behaviour is
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26 PEG itself, especially as it is a standard hydrophilic part for polymersomes. It
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28 was shown that the PEG shell is temperature sensitive, although not in a
29
30 physiological range.¹⁹⁶ When heated to 75°C the hydrophilic PEG chains
31
32 collapse and cannot support the membrane any longer. Once collapsed, the
33
34 vesicles turned to dense nano-objects where the hydrophilic and hydrophobic
35
36 segments form small patches within one another. The collapsing process also
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38 means that vesicles of about 200-300 nm turn into objects of about 100 nm.¹⁹⁶
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44 Tertiary amines are also temperature sensitive, which is mainly due to the
45
46 pKa of the polymers being dependent on temperature. This dependency was
47
48 shown recently for the PMPC-PDPA polymer where the pKa at 4°C is at 7.5 but
49
50 goes down to 5.6 at 50°C. No dependency on the chain length of the
51
52 temperature sensitive part (PDPA) was found by the authors.¹³ The
53
54 methyl-counterpart PDMA, on the other hand, has been integrated into
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4 polymersomes to exploit the temperature sensitivity around 35-40°C.¹⁹⁷
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30 **Figure 26.** A typical temperature responsive system (here PVCL-*b*-PVPON). Upon
31 heating the polymer assembles into micelles (a) and b): 155-164 resp. block length of
32 PVPON) or vesicles (c and d: 155-404 resp. block length).¹⁹⁸ (Reproduced with
33 permission from reference 198. Copyright (2014) John Wiley and Sons).
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41 Other, less common cyclic or linear amides also show an LCST behaviour
42 and this was also applied in the production of responsive vesicles. PVCL-PVP
43 samples of various block-lengths are reported by Kharlampieva *et al.* which
44 can form micelles and polymersomes amongst others. However, they only
45 form above 45°C when the PVCL part is hydrophobic. The actual LCST of
46 PVCL is around 35°C, but when conjugated with PVP, the observed cloud point
47 rises to 40-45°C, because the soluble PVP holds the polymer in solution at
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4 higher temperatures. Stable self-assembled structures are reported for 50°C
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6 for a variety of different block lengths tested. TEM confirmation for vesicles
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8 was obtained for a PVCL₁₅₅-*b*-PVPON₁₆₄ polymer (Figure 26).¹⁹⁸ Another
9
10 temperature sensitive amide is trans-N-(2-ethoxy-1,3-dioxan-5-yl) acrylamide
11
12 (tNEA). Similar to PNIPAM, the polymer is totally dehydrated, e.g. hydrophobic
13
14 at 37°C. It is reported as a hydrophobic block in polymersomes where a
15
16 temperature drop can thus be used to trigger polymersome disassembly and
17
18 drug release.¹⁹⁹ A good example for linear amide is aspartamide, and polymers
19
20 of it with an LCST behaviour were used in vesicles.²⁰⁰⁻²⁰¹ Especially
21
22 poly(α,β -N-substituted-DL-aspartamide)s are reported to show a wide range of
23
24 possible LCST values, ranging from 20°C up to 50°C. Although the polymer
25
26 structure does not change (block-copolymer of substituted and unsubstituted
27
28 aspartamides), the LCST can be adjusted precisely by changing the salt
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30 concentration and the pH of the external solution.²⁰⁰

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39 It comes unexpectedly that the hydrophobic poly(benzyl-L-glutamate)
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41 (PBLG) in conjugation with PEG or polylysine, shows a temperature sensitive
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43 behaviour as well. In recent work, it was shown that PBLG containing polymers
44
45 show a temperature dependent release profile of an enclosed drug (e.g.
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47 doxorubicin). In contrast to previous examples, the membrane becomes more
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49 permeable when heated for this polymer.²⁰²⁻²⁰³

56 57 3.1.4 Light Responsive Polymersomes 58 59 60

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4 Light is non-invasive and can be applied in a locally confined area, making
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6 light-responsive materials very attractive all across science. When it comes to
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8 vesicles, two main classes of light-responsive materials are known to literature.
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11 The first class uses light to cross-link the membrane, while the second one
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13 uses it to change the physical properties of the polymer chains.
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16 The photo cross-linked systems known are combined with pH or
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18 temperature sensitive materials and have been discussed at the
19
20 corresponding sections with respect to their additional functionality. Here, more
21
22 emphasis is laid on the cross-linking part (Figure 27). Cross-linking agents
23
24 reported are based on cinnamoylic acid,²⁰⁴ dimethylmaleimide^{66,186-187,205-206} or
25
26 benzophenone.^{76,189} The first two systems rely on a [2+2] cycloaddition which
27
28 is only allowed photo chemically but not thermally.²⁰⁷⁻²⁰⁸ The resulting structure
29
30 is thus defined and the cross-linking moiety remains non-reactive during the
31
32 polymerisation. The downside of this process is that it requires 2 similar
33
34 reactive units to form the cross-linking bond hampering the efficiency of the
35
36 process. Using benzophenone yields radical crosslinking, meaning a less
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38 controlled process. However, since the radical can react with almost any part
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40 of a neighbouring polymer chain and does not rely on meeting the same unit,
41
42 cross-linking is more efficient. All of these light-based cross-linking methods
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44 are more efficient and yield more stable polymersomes than other examples of
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46 cross-linking achieved by hydrolysis of siloxanes²⁰⁹ or the addition of an
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48 external agent (e.g. diamine, Figure 27).²¹⁰
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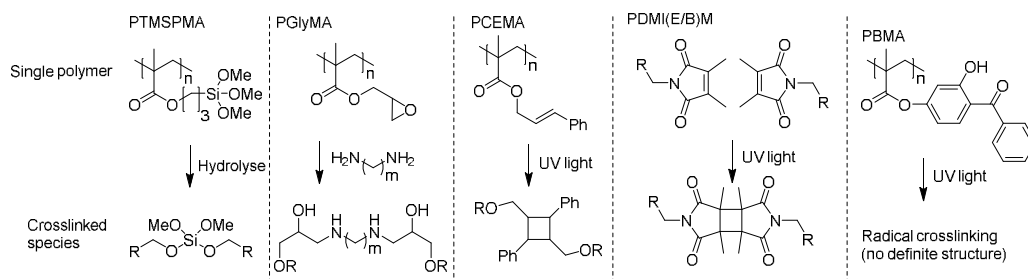


Figure 27. Cross-Linking reactions used in literature exploiting hydrolysis,²⁰⁹ external reagents,²¹⁰ UV light for controlled [2+2] cycloadditions^{66,186,194} and radical cross-linking.^{75,185}

Independent of the cross-linker used, the amount of cross-linking (e.g. cross-linking density) was shown to affect the final properties of the polymersome. Cross-linking density is known to regulate drug-release,⁷⁵ cellular toxicity,²⁰⁵ degree of swelling^{75,205} as well as the stiffness of the actual membrane.²⁰⁶

The second type of photoresponsive polymers change the physical properties of the membrane without cross-linking it. One class is based on azobenzene derived compounds which change conformation from trans to cis upon irradiation. Polymersomes containing azobenzene derived compounds in the side-chain of the hydrophobic part (azobenzene-modified methacrylate) can be achieved with the functional polymer as part of a linear methacrylate-based polymer as reported by Pei *et al.*,¹⁰¹ as part of the main chain as recently shown by Huang *et al.*,²¹¹ or also as star-like structures as reported by Moretto *et al.*²¹² and Oriol *et al.*²¹³⁻²¹⁴ The latter ones combine a diazo-containing hydrophobic methacrylate with a PEG-based star polymer

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2
3 (PEG₁₂)₃ in the hydrophilic part.²¹⁰ The resulting structure is thus a photo
4 responsive miktoarm star polymer. The structural change in the hydrophobic
5 part leads to a disorder in the membrane which can no longer supports its
6 original shape. As a consequence of this conformational stress, drugs start to
7 be able to pass the membrane after irradiation. Moretto *et al.* use the same
8 principle on a polyamide-based polymer.²¹² Both systems thus allow for a
9 light-triggered drug release (Figure 28).^{212,210} The same holds true for vesicles
10 of a polymer with the reverse architecture of a single PEG-chain connected a
11 dendron containing azo functionality. Irradiation of the sample also leads to
12 membrane distortion and drug release.²⁰⁹ The conformational change within
13 diazo polymers can also be used to change the stability of the supramolecular
14 pillar[6]arene-diazobenzene complex. Huang *et al.* equipped this complex with
15 hydrophilic and hydrophobic units to trigger self-assembly in water.²¹⁵ Upon
16 irradiation and the consecutive trans-cis change, the complex becomes
17 instable and the vesicles disassemble.
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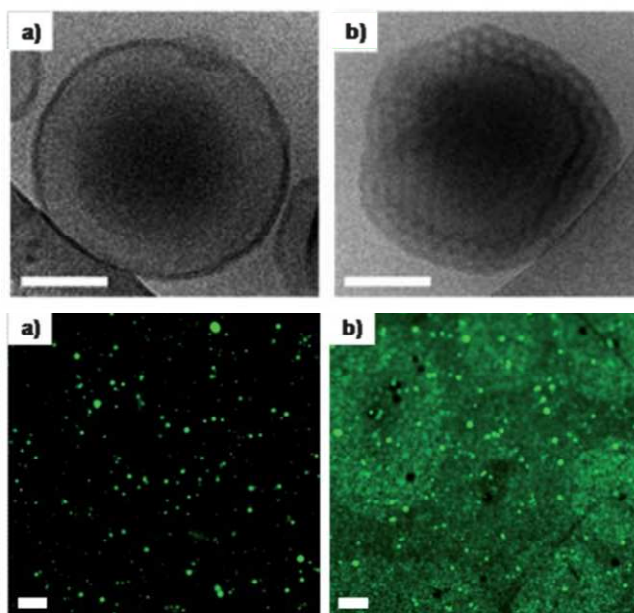


Figure 28. Diazobenzene-based block-copolymer PEG-b-d16isoAZOb for light triggered release. The conformational change distorts the membrane (a to b), making it open for transmembrane diffusion of an encapsulated drug.²¹⁴ (Reproduced with permission from reference 214. Copyright (2013) Royal Society of Chemistry).

Besides inducing conformation changes, light is also known to trigger the degradation of some polymers, for example ones based on nitrobenzene. When it comes to drug delivery, light can be used for a localised drug release with such systems. Meier *et al.* incorporated a photo cleavable linker between PAA and poly(methylcaprolactone) (PmCL). Cleaving the chains leads to separated PAA and PmCL segments where the latter ones aggregate into small 20 nm-sized particles (Figure 29).²¹⁶ Drugs were not incorporated so far into this system, leaving its potential for biological applications uncertain. Dong *et al.* also report the use of ortho-nitrobenzyl units, but incorporated them into

the side chain of their PEG-polyamide block-copolymer. Irradiation leads to the side chains being cleaved off, thus reducing the molecular weight of the hydrophobic block and forcing a switch towards either small vesicles or micelles, depending on the polymer used.²¹⁷

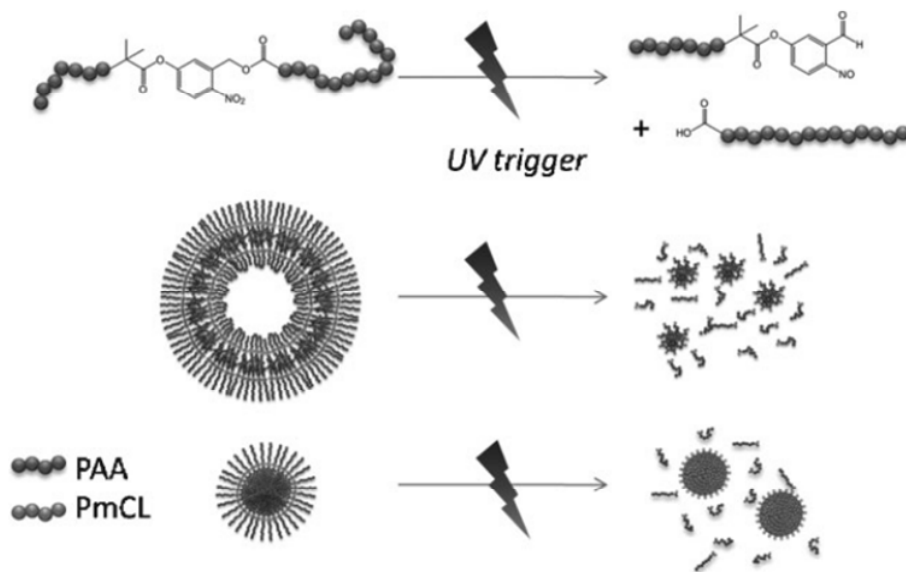


Figure 29. Nitrobenzene units linking PAA and PmCL get cleaved upon radiation; the resulting shift in hydrophilic-to-hydrophobic balance forces vesicle disassembly and micelle formation.²¹⁶ (Reproduced with permission from reference 216. Copyright (2010) John Wiley and Sons).

3.1.5 Reduction and Oxidation Sensitive Polymersomes

Sulphur containing polymers are readily known to be reduction or oxidation sensitive, depending on their structure. Disulphide bonds can be reduced to two thiols,^{61,185,218-219} or oxidized vice versa,²¹⁷ and a single sulphur atom can be oxidised towards a sulfoxide.^{217,220} Both possibilities are reported for their

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4 use in polymersomes for triggered drug release and will be discussed in the
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6 following section.
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9 In the majority of the reported examples, the disulphide bond is located at
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11 the border between the hydrophilic and the hydrophobic part of the polymer
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13 with the hydrophilic part being PEG in all cases. A commercially available or
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15 custom-synthesised PEG-SH is then conjugated to an initiator or a complete
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17 polymer. This can be butyl methacrylate or a cholesterol-modified
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19 methacrylate,²¹⁵ as examples for radical polymerisation with cholesterol giving
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21 the advantage of the additional functionality. With ring-opening polymerisation,
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23 poly(propylene glycol)(PPG),⁶¹ PCL¹⁸⁵ and poly(propylene sulphide) (PPS)²¹⁸
24
25 have been prepared. Apart from PPG these polymers also carry additional
26
27 functionality with PCL being biodegradable and PPS can be oxidised, although
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29 this was not discussed by the authors in this instance. The perhaps most
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31 sophisticated approach in this respect is known from Zhao *et al.*, who created
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33 an AB₂C₂miktoarm star polymer with A being PEG, B being PCL and the C
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35 polymers are either PNIPAM or PDEA to introduce an additional functionality
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37 by radical polymerisation. The C-polymers are attached via a disulphide bond
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39 to the core moiety. As one can see from the papers mentioned, the reduction
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41 sensitivity is usually combined with another functionality. This ranges from
42
43 biodegradability to sensitivity towards pH or temperature, amongst others. All
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45 of these papers report a complete cleavage of the disulphide bonds within 10
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47 minutes of incubation either into cells or treatment with DTT. The PEG corona
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is chopped off, leaving a plain hydrophobic polymer which does no longer support vesicles leading to complete drug release (Figure 30). Biodegradable polymers like PCL are of course useful, as the residual chunks can now be digested safely *in vivo*.²²¹⁻²²² The miktoarm star, however, does not disassemble directly, but the lower amount of hydrophobic polymer forces a conformational change from vesicles to micelles. But also here the addition of DTT leads to a significant increase in drug release.

In a prominent exception, the disulphide bond is located within the PEG itself. Once cleaved, the hydrophilic part of the amphiphilic block-copolymer gets shorted, hence disturbing the hydrophilic-to-hydrophobic balance. The authors use this shift to promote a change from micelles into polymersomes upon disulphide cleavage.⁸¹

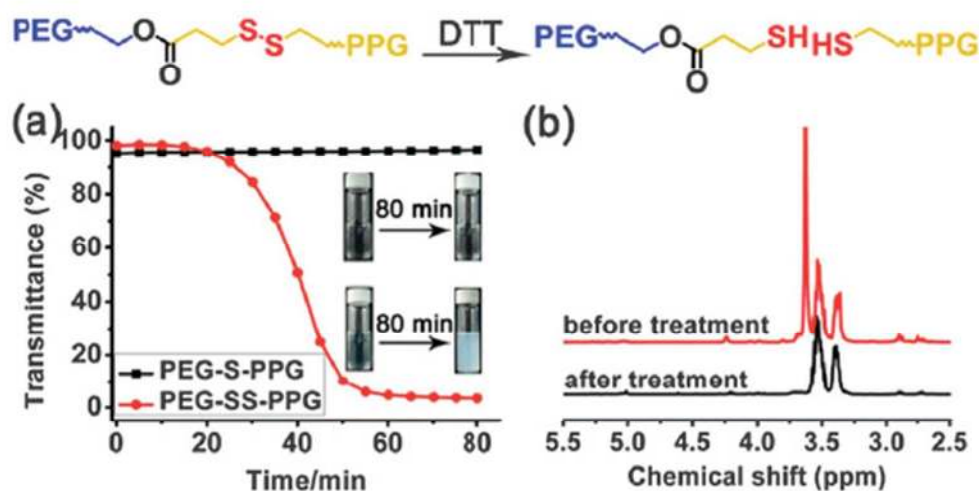


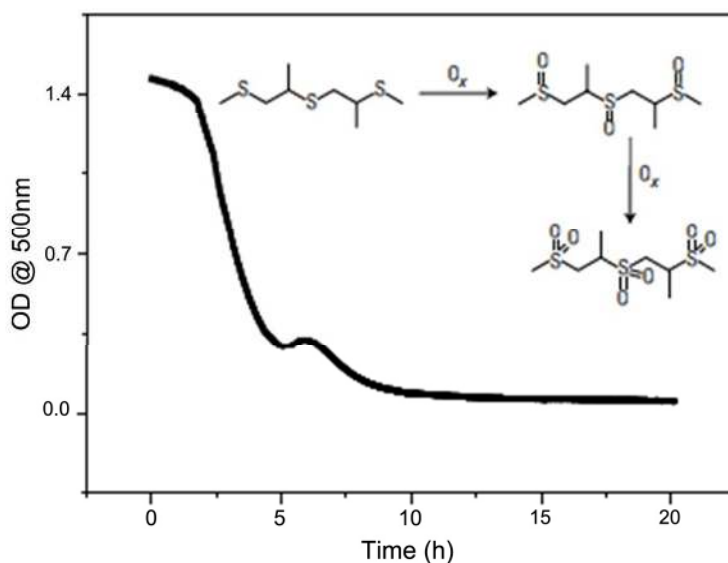
Figure 30. A typical disulphide-containing sensitive polymersome: the hydrophilic part (here PEG) is linked to the hydrophobic part (here PPG) via a disulphide bond, which can be cleaved using DTT or enzymes, leading to a destruction of the vesicles. A disulphide

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4 bond is necessary for vesicles destruction as opposed to a thioether (a). Bond cleavage
5
6 also leads to a distinctive change in the NMR spectrum (b).⁶¹ (Reproduced with
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8 permission from reference 61. Copyright (2014) Royal Society of Chemistry).
9

10
11 Besides reduction of a disulphide bond, oxidation of thiols to create
12
13 disulphides is also an option (Figure 31).²²⁰ To achieve this in a controlled
14
15 fashion the thiols need to be protected beforehand to prevent autooxidation.
16
17 Dong *et al.* reported polypeptides with o-nitrophenyl units which could be
18
19 cleaved photo chemically as discussed in the corresponding section.²¹⁷ The
20
21 resulting thiols were then used to crosslink the residual polymeric micelles by
22
23 adding DTT and dilute H₂O₂. Besides crosslinking the polymersome
24
25 membrane, some inter-micelle cross-linking was also reported by the authors,
26
27 but not to the extent of resulting in precipitating particles. Reversing the steps
28
29 was also attempted by the authors, *e.g.* first oxidising the sulphur. In this case,
30
31 a sulfone was created ultimately decreasing the hydrophobicity of the polymer.
32
33 Although it did not destroy the polymersomes, it did result in shrinkage of
34
35 them.²¹⁷ Hubbell *et al.* pioneered the work of oxidising polymersomes (Figure
36
37 31).²²⁰ In their simple PEG-PPS-PEG system, stable vesicles were formed
38
39 initially and ultimately destroyed by oxidising the sulphur atoms of the PPS to
40
41 sulfoxides and further to sulfones. Both versions are far more hydrophilic than
42
43 the initial PPS, leading to a complete disassembly of the vesicles towards
44
45 single polymer chains.²²⁰
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57 Alike pH sensitive vesicles, ones from oxidation/reduction block-copolymers
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4 can of course also be crosslinked. Yoshida *et al.* achieved this by incorporating
5
6 a Ruthenium initiator for controlled radical polymerisation and thus the ability to
7
8 crosslink methacrylamide units within the polymer. Once this is achieved, the
9
10 polymersomes show a swelling-deswelling behaviour similar to cross-linked
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12 pH sensitive vesicles, although less reproducible in terms of size.²²³



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35 **Figure 31.** Rapid increase in transmission upon oxidation of the PEG-PPS
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37 block-copolymer due to oxidation of the sulphur atoms. This leads to a disassembly of the
38
39 polymersomes.²²⁰ (Reproduced with permission from reference 220. Copyright (2004)
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41 Nature Publishing Group).
42
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45

46 Replacing existing thiols by new ones merely by stoichiometric excess of the
47
48 new ones, can also induce a vesicle response if the new thiol has distinctively
49
50 different properties than the old one. O'Reilly *et al.* exploit this to force a
51
52 conformation change from micelles to vesicles purely by thiol exchange.²²⁴
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56 The new thiol is of aromatic nature and more hydrophobic than the old one
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3 with an ethanol residue. This pushes the hydrophilic to hydrophobic ratio
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6 towards the hydrophobic fraction, forcing the change.
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10 3.1.6 Solvent Responsive Vesicles

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12 Besides the physical stimuli just discussed, polymersomes can also react to
13
14 a change in the solvent surrounding them. The solvent polarity must of course
15
16 not fall below the threshold where both polymer blocks are soluble and reverse
17
18 the solvent-switch method to create polymersomes. But if one has a closer
19
20 look at this solvent switch method, there is an important issue to be aware of.
21
22 When water is added slowly to the polymer solution and the vesicles form,
23
24 there is of course some organic solvent in the mixture, which is then dialysed
25
26 away. It is this slight switch in solvent polarity to which some polymersomes
27
28 react with a change in morphology to produce totally collapsed or “kippah”
29
30 vesicles. The authors chose this nickname in line with previous terms in
31
32 science which are derived from mythology or religion, such as Janus
33
34 particles.²²⁵ The term has since spread to pure silica vesicles²²⁶ and
35
36 silica/organic hybrid nanospheres.²²⁷
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46 The first example of kippah vesicles from Eisenberg *et al.* describes a
47
48 PAA₄₇-PS₄₃₄ system where vesicles are prepared in multiple variations of the
49
50 solvent switch method.²²⁵ The crucial bit is always when the residual amount of
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52 organic solvent is removed. If a water-miscible solvent is used, as it has to be
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54 for most cases of a solvent switch (see according section), it leaves the
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4 polymersome through the membrane and is supposedly replaced by pure
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6 water to keep the internal volume of the polymersome constant. For
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8 polystyrene, however, the water cannot diffuse back because the membrane is
9
10 too glassy and hydrophobic to allow water to travel through it. The internal
11
12 volume of the polymersome shrinks in that consequence and the membrane
13
14 bends in – as proposed by the authors.²²⁵ Further, more extensive studies by
15
16 van Hest *et al.* use a PEG₄₅-PS₂₃₀ system, so also the glassy polystyrene is
17
18 necessary. In a first publication, the authors are able to confirm the previously
19
20 purely speculative mechanism proposed above, by showing TEM images at
21
22 various stages of the process.²²⁸ This left the question whether the process is
23
24 reversible to some extent and whether the solvent mixture used has an effect
25
26 on the process. Reversibility was proven by adding a small amount of organic
27
28 solvent, which made the membrane just a small bit permeable and allowed it to
29
30 inflate to give the energetically favoured vesicle structure (Figure 32).⁶⁵ This
31
32 opens the question why this does not happen beforehand when the kippah
33
34 vesicles form, as a small amount of organic solvent is left in the solution until
35
36 the last moment.^{67,228} The answer is that some solvents have plasticising and
37
38 some deplastisising effects on polystyrene. It was shown that dioxane results
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40 in a more rigid PS membrane while THF makes it permeable towards organic
41
42 solvents and water.⁶⁵ Interesting enough, this leads to the possibility to create
43
44 kippah vesicles as well as flat, oblate-like structures and normal vesicles just
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46 by changing the solvent the polymersomes are dialysed against.⁶⁵ Very
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recently, the authors demonstrated that this transition in shape can be monitored using magnetic birefringence.²²⁹ or even can be used to control the shape using magnetic fields.²³⁰

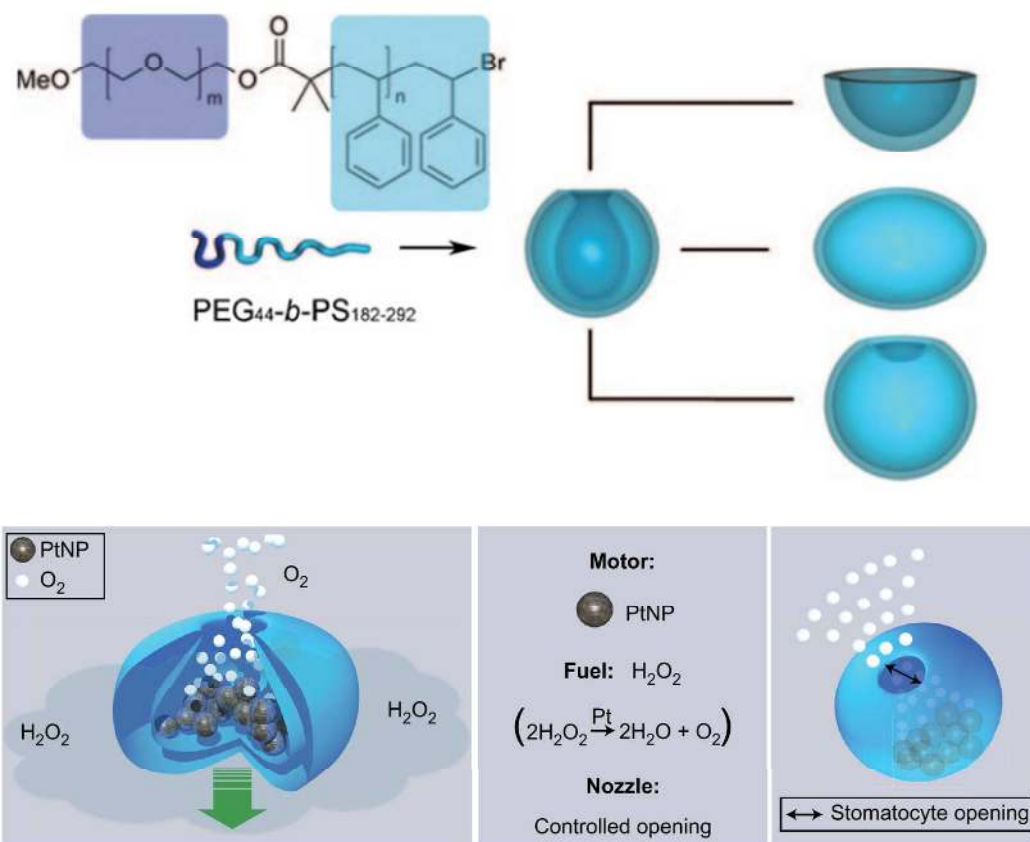


Figure 32. The formation of kippah vesicle (top), oblates and vesicles with indentations from the solvent responsive PEG-PS system.⁶⁵ Once formed, they can host Pt nanoparticles (bottom) which can be used to catalyse the decomposition of hydrogen peroxide to propel the nanoparticle.²³¹ (Reproduced with permission from reference 65 and 231. Copyright (2011) John Wiley and Sons and Copyright (2012) Nature Publishing Group, respectively).

Once formed, these collapsed vesicles (also called stomatocytes) can

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2
3 perform multiple tasks due to the cavity created. Just like normal
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6 polymersomes, stomatocytes can enclose hydrophilic substances in their core.
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9 The main difference is that the shell is now open to one side allowing for the
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11 diffusion of small molecules while prohibiting the diffusion of larger ones.²³² It
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13 was shown, for example that reactive enzymes as well as metal nanoparticles
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15 can be enclosed into the inner cavity (or stomach) of the stomatocytes during
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17 their formation process.²³¹⁻²³² With respect to nanoparticles, the stomatocytes
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19 can even act as template for their growth.²³² It has been shown that the
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21 enclosed Pt nanoparticle can now catalyse a reaction, like the decomposition
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23 of H₂O₂, and in this case the formed gaseous O₂ can propel the vesicle.²³¹
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26 As one can expect, the amount of fuel governs the speed of the vesicles.²³³
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31 It should be noted that the mixing of two immiscible polymers can also lead
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33 to changes in the topology of the polymersome surface. Similar indentations as
34
35 for kippah vesicles are reported for a PAA₄₀-PBD₁₁₀ and PEG₄₅-PBD₁₁₀
36
37 mixtures, also by the van Hest group.²³⁴ However, such phase separations are
38
39 also known to work the other way around and result in protrusions on the
40
41 outside of the vesicle as reported for a great variety of PEG, PEE and PMPC
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43 mixtures by Battaglia *et al.*²³⁵⁻²³⁷
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51 3.1.7 Combined Responsiveness

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53 Until here, we focussed on the many different ways of how to include
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55 sensitivity into vesicles. The current trend is towards multiple responsiveness,
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4 e.g. to combine responsiveness towards different stimuli into one vesicle.
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6 Many of the examples discussed do so, although we only highlighted one of
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8 the functionalities until now. If one has a look at statistics, the number of
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10 possible combinations of the above mentioned ways of responsiveness leads
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12 to a large number of possible copolymers, either as blocks or as random
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14 copolymers. The principle of how the then formed vesicles work, however,
15
16 does not deviate and is always very similar. Upon a first response, the
17
18 hydrophobic part of the polymersome becomes shorter or is weakened,
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20 triggering the transformation into micelles. A second switch then yields the
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22 disassembly into unimers.^{95,96,186} Due to this, we will not cover them in detail
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24 within this review.
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31 The story changes slightly for the cross-linked polymersomes which are also
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33 already covered extensively in the sections above. Their first responsiveness,
34
35 the one towards light is irreversible, and cross-links the vesicles. The second
36
37 one, which can be of any nature (pH, temperature or oxidation) is reversible
38
39 and causes a reversible swelling of the polymersomes as discussed. Due to
40
41 their popularity, the combination of temperature and pH sensitivity into
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43 polymersomes is discussed separately. One way to achieve this is to use
44
45 PDMAEM in the polymer as it is pH sensitive and temperature sensitive (see
46
47 above). One can, of course, also use two different polymers to get the dual
48
49 responsiveness. Typically, PNIPAM is the temperature-sensitive polymer and
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51 one of the amine containing polymers serves as the pH sensitive part.^{186,238-239}
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4 It should be noted that the amount of responsiveness is of course not limited
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6 to two functionalities. Zhao *et al.*¹⁹⁵ and Pei *et al.*¹⁰¹ included three
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8 functionalities into polymersomes which led to temperature and pH sensitivity,
9
10 and in addition, either light responsiveness (diazo bond)¹⁰¹ or redox sensitivity
11
12 (disulphide bond).¹⁹⁵ This allows to cut a fine balance between controlling the
13
14 self-assembly state and permeability of the membrane as it has been shown in
15
16 the corresponding sections of each of the sensitivities.
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24 3.2 Core-Based Design

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27 It has been widely accepted that the unique structure of hollow polymer
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29 capsules consist of a polymer membrane domain and an empty core domain.
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31 To engineer functional polymer capsules towards smart nanoreactors, in
32
33 addition to the above-mentioned modulation of the polymer membrane, the
34
35 other significant aspect is the modulation of the empty core domain.
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37 Subsequently, in this subsection, we will focus on the discussion about what
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39 can be done for modifying the core domain, including the incorporation of
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41 another small polymer compartment or inorganic particles into the core.
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48 3.2.1 Multi-Compartment Model

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50 Being inspired by biological cells which are able to perform multiple complex
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52 reactions within confined environments owing to their internal
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54 sub-compartment structure, the development of multi-compartment polymer
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56 capsules is now attracting more and more researchers' attention.²⁴⁰⁻²⁴⁴ The
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4 constructed multi-compartment polymer capsules, to be considered as a new
5
6 generation of polymer capsules, definitely will bring a new way for the
7
8 application of the polymer capsules as nanoreactors. The concept of
9
10 multi-compartment polymer morphology was first raised by Ringsdorf²⁴⁵ twenty
11
12 years ago, and then was widely developed.²⁴⁶⁻²⁴⁸ Up to now, the created
13
14 multi-compartment polymeric capsules include: vesosome,²⁴⁹ a liposome in
15
16 liposome structure formed by smaller vesicles encapsulated within a bigger
17
18 vesicle; polymer vesosome,^{85,250} a polymersome in a polymersome structure
19
20 formed by using different polymersomes as the carrier and sub-compartment
21
22 structures; dendrimersomes formed by the self-assembly of Janus dendrimers
23
24 in solution with predictable size and number of bilayers;^{15,251-258}
25
26 capsosomes^{243,259-260} formed by the LbL method to encapsulate
27
28 sub-compartments in the shell or in the core of a polymeric capsule; and other
29
30 polymer capsules in polymer capsule models synthesized by self-assembly,²⁶¹
31
32 surface-initiated polymerisation,²⁶² double emulsion²⁶³⁻²⁶⁴ or microfluidic
33
34 methodology *etc.*⁹³

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36 For example, using a simple method based on sequential self-assembly
37
38 Nallani and coworkers fabricated larger polymersomes of
39
40 poly(styrene)-*b*-poly(L-isocyanoalanine(2-thiophen-3-yl-ethyl) amide)
41
42 (PS-*b*-PIAT) with an encapsulated suspension of smaller polymersomes in the
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44 core.²⁶¹ While the encapsulation efficiency of the smaller polymersome in the
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46 core was relative low, in order to improve this, as a powerful alternative method
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4 emulsions or double emulsions technique was developed. Using a double
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6 emulsion technique in a water/oil/water system, Chiu and coworkers
7
8 demonstrated to generate a multi-compartment capsule by encapsulating
9
10 small vesicles within large polymersomes with pH-responsive membranes.²⁶³
11
12 In detail, copolymers were dissolved in an organic phase, followed by
13
14 emulsification and evaporation of the organic solvent (THF and CHCl₃) to form
15
16 polymer vesicles. These preformed vesicles were subsequently introduced
17
18 into a water phase of the second-stage double-emulsion process to form the
19
20 multicompartment assembly in size range from 1 to 15 μm. The size of these
21
22 vesicles can be tuned by varying the ratio of THF to CHCl₃ and by changing
23
24 the content of the copolymers. In this system, the outer and inner
25
26 compartments are composed of poly(acrylic acid)-co-poly(distearin acrylate)
27
28 (poly(AAc-co-DSA)) polymersomes equipped with pH-responsive
29
30 transmembrane channels (Figure 33a), allowing the controlled release of
31
32 encapsulated cargo in response to environmental pH changes. Interestingly,
33
34 such pH-responsive process is reversible in the range of pH 5 to 8. Even
35
36 though these polymersomes are impermeable to small hydrophilic solutes,
37
38 such as calcein at pH 5 (Figure 33b), a change in the ionisation state of the
39
40 acrylic acid units at pH 8 could result in the formation of pores and
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42 subsequently allows the transport of the encapsulated cargo across the
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44 membranes (Figure 33c).²⁶³
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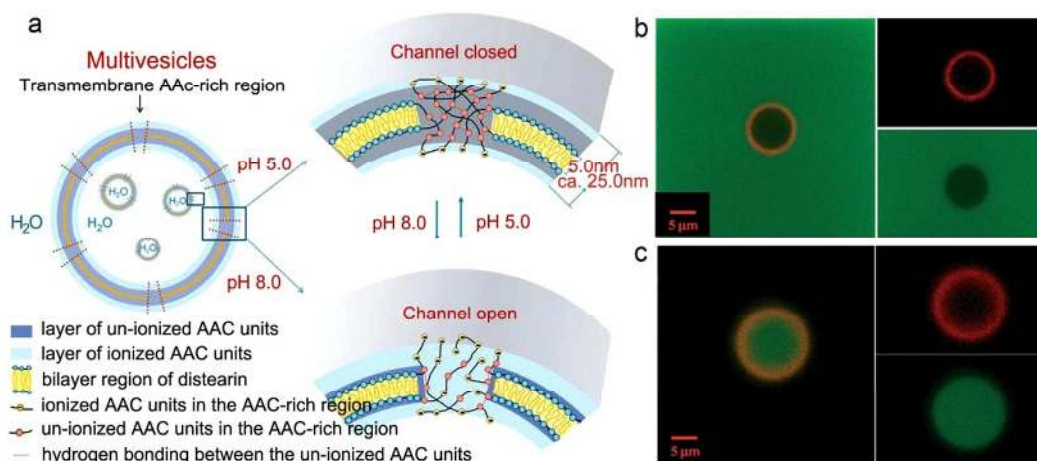


Figure 33. (a) Illustration of multi-compartment assemblies equipped with pH-responsive transmembrane channels from a two-stage double emulsion vesicle formation process using poly(AAc-co-DSA). (b) Confocal laser scanning microscopy images of Nile red-stained polymersomes with the addition of calcein at pH 5.0 (calcein cannot diffuse into polymersomes), and (c) after adjusting the pH to 8 (calcein diffuses into polymersomes) and replacement with fresh pH 5 buffer (calcein is confined with the polymersomes).²⁶³ (Reproduced with permission from reference 263. Copyright (2008) John Wiley and Sons).

To develop multi-compartment capsules, such emulsion process was further improved by Weitz and coworkers by combining emulsion with microfluidic technique.²⁶⁵ As demonstrated in section 2.1, the main advantage of this technique is the ability to control the number as well as the size of the compartments in the assembled models simply by tuning the flow rates of the phases in the microfluidic channels and changing the capillary diameter, respectively. The size of the multi-compartment polymer capsules could be

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4 varied ranging from 50 to 200 μm in diameter, with containing 2–8 inner
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6 compartments. Also the composition of the membrane can be changed freely
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8 by using different polymers as building blocks. Namely, both, the external and
9
10 internal polymer capsules could be composed of biocompatible poly(ethylene
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12 glycol)-*b*-poly(lactic acid) (PEG-*b*-PLA) diblock copolymers, with the controlled
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14 release of the encapsulated cargo induced by the introduction of mechanical
15
16 strain or selective dissociation of the polymersome membranes by
17
18 hydrolysis.^{93,266}

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24 Very recently, as a further development in this field it was demonstrated by
25
26 Lecommandoux and co-workers that a polymersome in a polymersome model
27
28 was generated using a facile, versatile, reproducible, and low-time and product
29
30 consuming technique based on the emulsion-centrifugation method (please
31
32 also refer to section 2.1.2).^{18,85,250} The inner polymersomes were formed by
33
34 nanoprecipitation of poly(trimethylene carbonate)-*b*-poly(L-glutamic acid)
35
36 (PTMC-*b*-PGA).²⁶⁷ This suspension was then loaded in larger polymersomes
37
38 of polybutadiene-*b*-poly(ethylene oxide) (PB-*b*-PEO) by
39
40 emulsion-centrifugation.²⁶⁸

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46 Due to the distinctive structure of multi-compartment polymeric capsules
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48 with multiple hollow core domains and a tunable polymer shell, one can easily
49
50 foresee promising applications in different fields.^{249,269} For example, multiple
51
52 sub-compartments in a polymeric capsule might be considered as individual
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54 nanoreactors able to perform multiple complex reactions within confined
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4 environments to simulate simple steps of cell activity; capsules that contain
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6 multiple batches of drug carriers in compartments of different hierarchies can
7
8 serve as a depot for highly controlled and sustained release of drugs. In this
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10 regards Caruso and coworkers have shown the construction of a serial of
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12 multi-compartment polymeric capsules based on the LbL method and using
13
14 liposomes or small size polymeric capsules as sub-compartments.^{242,259,270-273}

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18 These model capsules were applied as nanoreactors and drug carriers by
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20 encapsulation of an enzyme,²⁵⁰ pDNA²⁷⁴ or hydrophobic²⁷⁰ and hydrophilic²⁷⁵
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22 moieties within the liposomal sub-compartments. It was also possible to
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24 perform a two-step enzymatic catalytic reaction, whereby reduction of a
25
26 substrate is exploited to subsequently trigger cargo release.²⁴⁴ As
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28 demonstrated in Figure 34, the authors encapsulated glutathione reductase
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30 into liposomes, which were then embedded into polymer carrier capsules to
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32 form capsosomes. The architecture of the capsosomes enables a temperature
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34 triggered conversion of oxidised glutathione to its reduced sulfhydryl form by
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36 the encapsulated glutathione reductase. The reduced glutathione
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38 subsequently induces the release of the encapsulated oligopeptides from the
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40 capsosomes by reducing the disulphide linkages of the conjugates. This study
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42 well highlights the potential of capsosomes to continuously generate a potent
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44 antioxidant while simultaneously releasing small molecule therapeutics.
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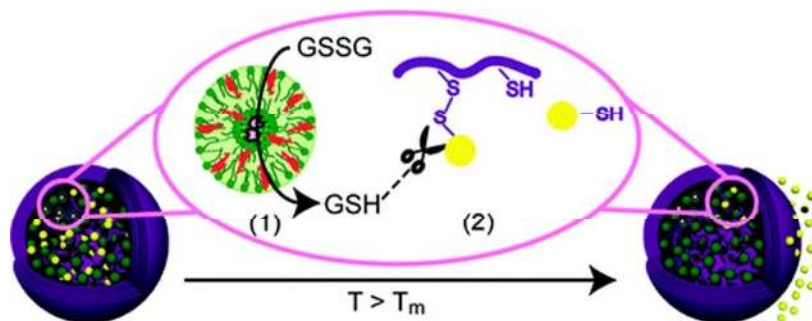


Figure 34. Schematic illustration of a temperature-triggered catalysis of an encapsulated glutathione reductase in the “Free-Floating” liposomal subcompartments of capsosomes. In there glutathione disulphide (GSSG) bonds are reduced to its sulfhydryl form (GSH). Consecutive release of encapsulated oligopeptides was triggered by the catalytic activity of glutathione reductase within the capsosomes.²⁴⁴ (Reproduced with permission from reference 244. Copyright (2011) American Chemical Society).

3.2.2 “Yolk-Shell” Model

Apart from the incorporation of the sub-compartment structure into the core, to engineer the core domain of polymer capsules, another effective way is to directly encapsulate a functional component in the core, subsequently in the unique hollow polymer structure, a “rattle” or “yolk-shell” morphology is developed. For example, Kang and coworkers synthesized narrowly-dispersed silver@silica@poly(methacrylic acid) (Ag@SiO₂@PMAA) core–double shell hybrid nanoparticles via distillation–precipitation polymerisation.²⁷⁶ Selective removal of the inorganic silica inner-shell from the Ag@SiO₂@PMAA core–double shell hybrid nanoparticles by HF etching produced the Ag@air@PMAA hybrid nanorattles with a silver nanocore, PMAA shell and

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4 free space in between. The as-synthesized Ag@air@PMAA hybrid nanorattles
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6 were also explored as a nanoreactor system for confined catalytic reduction of
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8 *p*-nitrophenol, of which the rate of catalytic reaction can be further regulated by
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10 controlling molecule diffusion in and out of the stimuli-responsive PMAA shell
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12 through the simple variation of environmental stimuli, such as salt (NaCl)
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14 concentration in the medium.²⁷⁶
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19 As another special type of multi-compartment models, onion-like capsules
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21 consisting of concentric capsules with uniform spacing between the walls are
22
23 also attracting more and more attention. The dense onion-like structures were
24
25 first found in bulk block-copolymer blends over twenty years ago.²⁷⁷ Later such
26
27 onion-like structures had been formed in solution and various building blocks
28
29 were employed ranging from small molecule surfactant systems²⁷⁸ to block
30
31 copolymers²⁷⁹⁻²⁸⁰ and to very recently reported Janus dendrimers.²⁵³ For
32
33 example, onion-like dendrimersomes reported by Percec *et al.* were obtained
34
35 via self-assembly by simple injection of a solution of Janus dendrimers into a
36
37 water-miscible solvent, into water or into buffer.²⁵³ Significantly, the size and
38
39 the number of bilayers of the onion-like capsules were predictable by the final
40
41 concentration of the Janus dendrimers. At low concentration, double-bilayer
42
43 capsules were formed, whereas at higher concentrations they formed
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45 multibilayer onion-like capsules. Undoubtedly, such onion-like
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47 dendrimersomes provide simple and easily accessible mimics of various
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49 double-bilayer and multibilayer biological membranes of different cells and
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organelles, such as Gram-negative bacteria or multilamellar bodies *etc.*

4. Application of the Polymer Capsule Nanoreactors

A typical polymer capsule as described above has an aqueous lumen sequestered from the external aqueous environment by a polymer membrane and therefore can encapsulate both hydrophilic and hydrophobic cargo either into the membrane domain or the core domain. As has been discussed above, a wide range of techniques can be applied to generate a diversity of stable and robust polymer capsules including polymersomes, polymeric capsules or proteinosome *etc.*^{14,38,40,281-282} The unique structure of polymer capsules is of special interest and is attracting more and more researchers' attention, in view of their promising application as nanoreactors. Generally, to exploit polymer capsules as nanoscale reaction vessels, two main requirements have to be considered.¹⁰ One is that catalytically active guests need to be encapsulated within their inner compartments to confine the reaction into the polymer capsules, and the other one is that the semipermeable polymer membrane, which separates the inner aqueous compartment from the outer bulk solution, should allow the exchange of substrates between the outside and the inside of polymer capsules.^{10,18,30,283} Here, the former requirement can be easily met, since the strong encapsulating ability is one of the characteristic features of polymer capsules, especially for the ones which were synthesized by self-assembly or miniemulsion method. For example, the reported

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4 proteinosome model showed that a GFP cell-free gene expression system
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6 containing a hundred or so components could be integrally encapsulated into
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8 the core without affecting the GFP expression within the proteinosome internal
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10 volume.¹⁶ For the latter requirement, in comparison to liposomes, polymer
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12 capsule membranes are often several times thicker because of the higher
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14 molecular weights of copolymers compared to those of typical phospholipids
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16 which makes the membrane, especially for the polymersome system, even
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18 less permeable to small water-soluble molecules. However, with the advances
19
20 made in polymer chemistry in recent years, it is becoming much easier to tune
21
22 the permeability of the membrane as well as their surface functionality by both
23
24 chemical and physical strategies. By the chemical design and synthesis of
25
26 various smart copolymers as building block (see section 3.1), the permeability
27
28 of the polymer capsules can be well modulated by responding to external
29
30 stimuli including pH, light, redox and solvent *etc.*^{47,132,284-288} Alternatively, as
31
32 demonstrated very recently, tuning the permeability of the membrane was also
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34 studied by some physical strategies through incorporating channel proteins
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36 within membranes, choosing different length of bifunctional compound as
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38 cross-linkers or controlling the shear-rate *etc.*^{31,111,186,277} Taken together, it is
39
40 no doubt that these well-developed strategies provide an effective way to
41
42 construct different functional nanoreactors. In the following section, the
43
44 discussion will be given based on the application of the polymer capsule
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46 nanoreactors in enzyme catalysis, polymerisation, nanoparticles synthesis and
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4 as artificial organelles *etc.*
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8 9 4.1 Application of the Polymer Capsule Nanoreactors for Enzyme Catalysis

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11 For the application of polymer capsules as nanoreactors, a straightforward
12 way is to encapsulate active guests within the inner compartment of polymer
13 capsules.^{10,29,35} For example, a very recent study showed that an enzymatic
14 nanoreactor was constructed by incorporating β -galactosidase into a polyion
15 complex vesicles (PICsomes), as shown in Figure 35.²⁷ In this system, by
16 tuning the hydrophobicity of the aliphatic side chains in the pendant group of
17 the constituent polyelectrolytes, the non-cross-linked PICsomes demonstrated
18 sufficient stability at physiological salinity and temperature. Even in the
19 presence of trypsin in the exterior, the activity of the encapsulated
20 β -galactosidase was well maintained.²⁷ Up to now, a host of polymer
21 capsule-based nanoreactors was constructed by encapsulating
22 peroxidases,^{47,289-290} penicillin acylase,²⁹¹ superoxide dismutase,^{32,283,284,158}
23 catalase,^{32,158,292-293} myoglobin,^{111,174,186,294} galactosidase,²⁷ lysozyme,²⁸⁵
24 lipase,^{33,86} phenylacetone monooxygenase,⁸¹ alcalase,⁸¹ alcohol
25 dehydrogenase,^{81,295} laccase²⁹⁶ or alkaline phosphatase,²⁹⁷ respectively, in the
26 core. Within the confined space provided by the polymer capsule, reactions
27 can occur with higher selectivity or less side reactions.²⁹⁸ Furthermore, it has
28 been shown that reactions and proteins could be monitored on the single
29 molecule level using nanoreactors.²⁹⁹⁻³⁰⁰ Cascade reactions of two or more
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4 catalytic species become more efficient due to the close spatial proximity and
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6 precise positioning of the catalysts.^{166,301-302} Considering that most
7
8 enzyme-loaded nanoreactors need to be able to perform enzymatic reactions
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10 in situ with membrane diffusion of substrates and products, the
11
12 above-mentioned successful tuned permeabilisation strategies (in section 3.1)
13
14 will enable a controlled enzyme-mediated catalytic behaviour for the
15
16 constructed nanoreactor.^{107,182} For example, Voit and coworkers loaded
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18 myoglobin into a pH sensitive poly(diethylaminoethylmethacrylate) (PDEAEM)
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20 capsules¹⁰⁷ (see also Figure 23).¹⁸⁷ As anticipated, to be a nanoreactor, its
21
22 catalytic efficiency can be well modulated by pH. Since at high pH value (pH
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24 8.0), the PDEAEM membrane is deprotonated and fully hydrophobic, the
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26 hydrophilic substrate hydrogen peroxide cannot easily diffuse through the
27
28 hydrophobic membrane to reach the encapsulated myoglobin in the core, thus
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30 showed a relative low catalytic activity. While, after adjusting the pH value of
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32 the measured solution to pH 6.0, an obviously enhanced activity was observed.
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34 This is due to the fact that the nanocapsule membrane is now in a swollen
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36 state and totally hydrophilic which makes the transmembrane diffusion
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38 possible, thereby allowing the substrates both guaiacol and hydrogen peroxide
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40 to reach the myoglobin in the core and accelerate the reaction.
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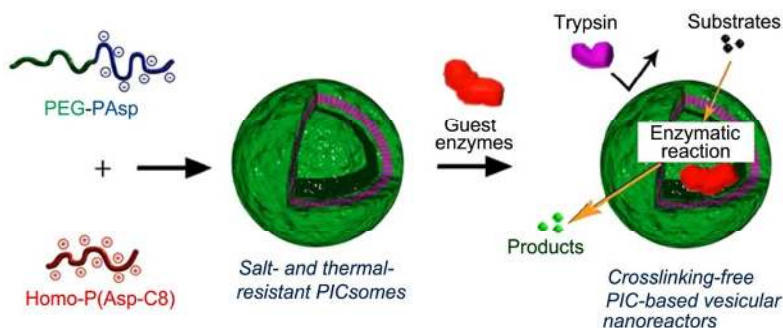


Figure 35. Schematic illustration of the formation of a salt- and thermal-resistant polyion complex vesicle as an enzymatic nanoreactor by encapsulating β -galactosidase in the core.²⁶ (Reproduced with permission from reference 26. Copyright (2014) American Chemical Society).

In another effort to develop such enzyme-containing nanoreactors which only contain a single type of enzyme, more complex systems have been proposed involving two different cascade enzymes loaded inside nanoreactors^{260,293} or occupying different locations in the nanoreactor system (inside, within the membrane, or attached onto the membrane of the polymersome).²⁹² For instance, van Hest and co-workers constructed biohybrid polymersome nanoreactors in which three different enzymes were spatially positioned, and precisely ordered (Figure 36a).²⁹² These enzymes were incorporated into the membrane (Candida antarctica lipase B, CalB), encapsulated in the inner aqueous compartment (glucose oxidase, GO) and attached to the surface of the polymersome (horseradish peroxidase, HRP), respectively. Accordingly, the constructed nanoreactor was capable of performing a three-step cascade reaction, in which glucose acetate was converted by CalB to glucose, which

was then oxidised by GO into gluconolactone in the second step. The hydrogen peroxide produced was used by HRP to oxidise 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) to green colour $ABTS^+$. Furthermore, the authors also reported an interesting enzyme-filled Pickering emulsion that was stabilised by fully packed crosslinked polymersomes at the water/oil interface (Figure 36b-d). By loading a model enzyme CalBin different ways, either in the aqueous phase or inside the lumen of the polymersome to maximise the contact area at the interface between two phases, this polymersome-stabilised colloidosome architecture could be efficiently and repeatedly applied in biphasic enzymatic transformations.²⁸⁵

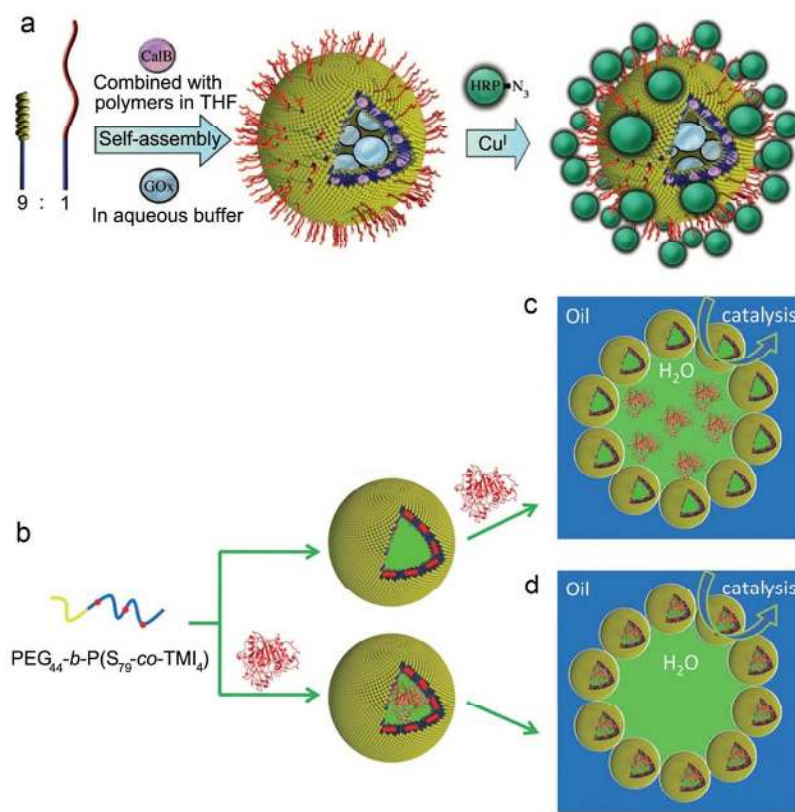


Figure 36. (a) Schematic representation of positional assembly of enzymes in a

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4 polymersome, with GO encapsulated in the inner compartment, CalB trapped in the
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6 polymeric bilayer and the third enzyme HRP immobilised on the polymersomal perimeter
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8 though a covalent linkage.²⁹² (Reproduced with permission from reference 292. Copyright
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10 (2009) John Wiley and Sons). (b) Representation of the crosslinking process to prepare
11
12 stabilised polymersomes. (c) and (d) Schematic representation of a
13
14 polymersome-stabilised Pickering emulsion with the enzyme in the water phase (c), or with
15
16 the enzyme inside the polymersome lumen (d).²⁸⁵ (Reproduced with permission from
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18 reference 285. Copyright (2012) John Wiley and Sons).
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26 With the tremendously successful design of single compartments-based
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28 nanoreactors, investigations of the corresponding higher-order architectures,
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30 in particular bilayer-based multi-compartment capsules-based nanoreactors, is
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32 now gaining more and more attention. As illustrated in Figure 37a, a
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34 multi-compartmentalised nanoreactor was reported for that enzyme-filled
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36 polystyrene-*b*-poly(3-(isocyanato-lalanyl-aminoethyl)thiophene) (PS-*b*-PIAT)
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38 nanoreactors were encapsulated together with free enzymes and substrates in
39
40 a larger polybutadiene-*b*-poly(ethylene oxide) (PB-*b*-PEO) polymersome.⁸⁶ By
41
42 deliberate placing the enzymes needed for each separate cascade step in
43
44 different subcompartments, the reaction intermediates were forced to cross
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46 over compartmental boundaries. Thereby, truly confined reactions and local or
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48 gradient-like generation of reaction products, as it occurs in nature, could be
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50 observed. In this pathway, the profluorescent substrate **1** (Figure 37b) first
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4 undergoes oxidation by the solvent- and temperature-stable Baeyer–Villiger
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6 monooxygenase, PAMO, which uses NADPH as a cofactor. The resulting
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8 ester moiety is then hydrolysed by CalB, and subsequent oxidation of the
9
10 resulting alcohol to an aldehyde is achieved by an NAD⁺-dependent ADH.
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12 Finally, the last reaction yields fluorescent resorufin (5) through a spontaneous
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14 non-enzymatic beta-elimination step.⁸⁶ Recently, Nallani and coworkers
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16 showed another construction of a multi-compartment polymersome
17
18 nanoreactor where a passive protein channel OmpF was embedded into the
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20 inner membrane, forming a passive diffusion channel to allow for the exchange
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22 of small molecules and ions.²⁶⁹ Selective encapsulation of GO in the outer
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24 compartment and HRP in the inner compartment resulted in an efficient
25
26 enzymatic cascade nanoreactor system. Such successful demonstration of
27
28 this multi-compartment structure functionalised with membrane proteins is a
29
30 significant step forward to tap into the selectivity displayed by such
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32 membranes, opening the possibility of engineering truly specialised
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34 multi-compartment nanoreactors.
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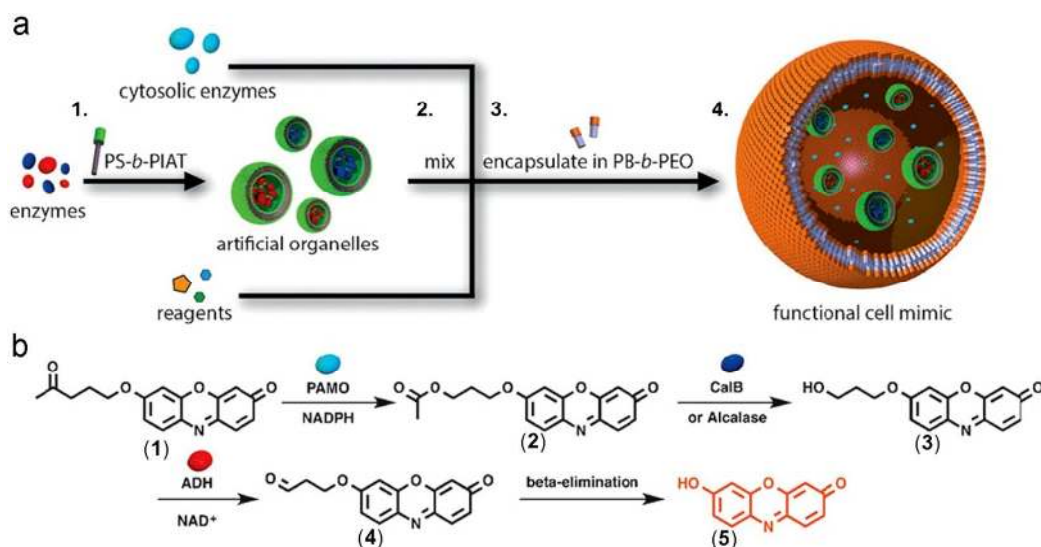


Figure 37. (a) Schematic illustration of the procedure to construct a multi-compartmentalised nanoreactor. The initial encapsulation of different enzymes in polystyrene-*b*-poly(3-(isocyanato-lalanyl-amino-ethyl)-thiophene) (PS-*b*-PIAT) nanoreactors (1), followed by mixing of cytosolic enzymes, and other reagents (2), before encapsulation of the reaction mixture in polybutadiene-*b*-poly(ethylene oxide) (PB-*b*-PEO) vesicles (3) to create the multi-compartmentalised model (4), inside which enzymatic multi-compartment catalysis takes place. (b) A defined cascade reaction scheme happened in the constructed multi-compartmentalised model.⁸⁶ (Reproduced with permission from reference 86. Copyright (2014) John Wiley and Sons).

Recently, other reports showed that apart from the application in catalysis, the constructed nanoreactors can also be used in theranostic approaches via installing fluorescent photo sensitizer signals and simultaneously producing reactive oxygen species(ROS) efficiently “on demand”,³⁰³ or can be used to protect the encapsulated guests from degrading agents and enzymes outside

of the polymer capsules.²⁸¹ This is especially important for biomedical applications of nanoreactors, where an encapsulated enzyme could produce a drug or scavenge toxic substances in intra- or extracellular fluids.^{283,291,304} For example, laccases (Lac)²⁹⁶ are oxidised enzymes with a broad range of applications, in soil remediation, as bleaching agent in the textile industry, and for cosmetics. Protecting laccases against degradation and inhibition is of great importance for many of these applications. With these objectives in mind, Bruns and coworkers demonstrated that encapsulation of Lac in the poly(N-vinylpyrrolidone)-*block*-poly(dimethylsiloxane)-*block*-poly(N-vinylpyrrolidone) (PNVP-*b*-PDMS-*b*-PNVP) triblock copolymers polymersomes significantly protected the enzyme against enzymatic degradation and against small inhibitors (Figure 38).²⁹⁶

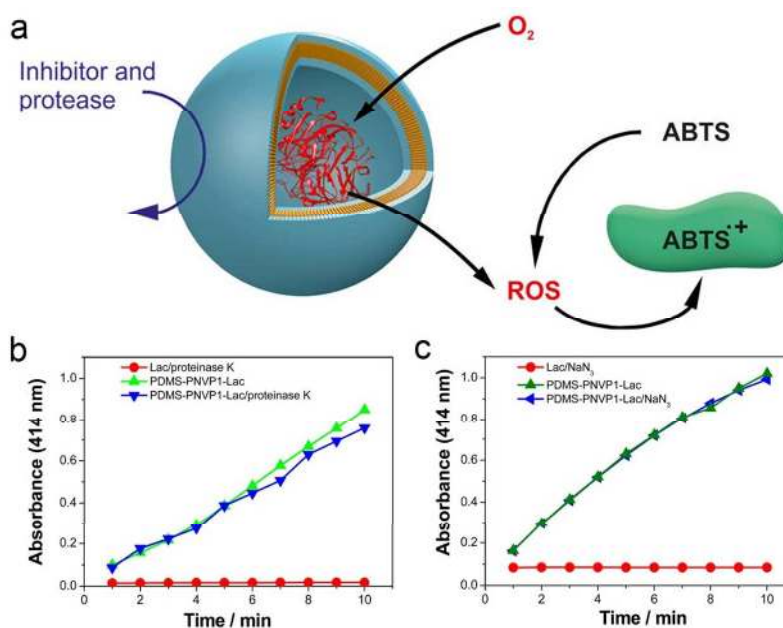


Figure 38.(a) Schematic illustration of a poly(N-vinylpyrrolidone)-poly(dimethylsiloxane)-based polymersome nanoreactors for laccase-catalysed biotransformations. Protection of

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4 Lac in the PDMS-PNVP polymersomes against an externally added protease (b) and
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6 inhibitor NaN_3 (c).²⁹⁶ (Reproduced with permission from reference 296. Copyright (2014)
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8 American Chemical Society).
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10 11 12 13 14 4.2 Application the Polymer Capsule Nanoreactors for Polymerisation 15

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17 Apart from the above-discussed incorporation of enzymes into the polymer
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19 capsules as nanoreactors, the hollow core domain itself has showed the ability
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21 to be an ideal confined reaction space for efficient polymerisation,³⁰⁵⁻³⁰⁷ and
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23 compartmentalization. For instance, physical confinement of reactants to
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25 monomer droplets or polymer capsules interior, enables the nanoreactors to
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27 show a marked beneficial effect on the progression of a controlled/living radical
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29 polymerisation based on the persistent radical effect. Up to now, although
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31 much work have been demonstrated in the field of polymerisation in
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33 nanoreactors, most of them are contingent on the radical polymerisation
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35 involving ATRP, RAFT or nitroxide-mediated radical polymerisation (NMP)
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37 taking place in emulsion,³⁰⁶ miniemulsion,³⁰⁸⁻³⁰⁹ or microemulsion.³¹⁰ The
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39 influence of compartmentalisation on the kinetics of conventional radical
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41 emulsion polymerisation systems has been clearly demonstrated. The
42
43 polymerisation rate and the obtained polymer molecular weight in an emulsion
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45 polymerisation are generally higher than in the corresponding homogeneous
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47 (bulk/solution) polymerisation, as a consequence of compartmentalisation of
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49 propagating radicals leading to reduced bimolecular termination rates. Two
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4 fundamental types of compartmentalisation effects were adopted: the
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6 segregation effect and the confined space effect.³¹¹ The segregation effect
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8 refers to two species located in separate particles being unable to react, and
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10 the confined space effect refers to two species located in the same
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12 compartment reacting at a higher rate in a small space than in a large space.
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14 Overall, the segregation effect on bimolecular termination results in an
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16 increase in livingness (higher end-functionality), whereas the confined space
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18 effect on deactivation may result in an increase in the level of control over the
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20 molecular weight distribution.
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26 For example, Zhang and co-workers presented a study about RAFT
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28 polymerisation of a typical hydrophobic monomer of styrene within a hollow
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30 capsule suspended in water (Figure 39).³⁰⁷ A hollow capsule containing a
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32 hydrophilic corona of poly(methacrylic acid) (PMAA) and a cross-linked
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34 polystyrene shell was synthesized within which the typical hydrophobic
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36 monomer of styrene, the RAFT agent S-benzylidithiobenzoate and the initiator
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38 2,2'-azobisisobutyronitrile can be encapsulated and RAFT polymerisation of
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40 styrene took place in well controlled manner in water. In comparison of the
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42 homogeneous bulk polymerisation, it is found that the resultant polymer of
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44 polystyrene has a competitively low dispersity index and its number-average
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46 molecular weight linearly increases with monomer conversion. Altogether, as
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48 outlined in the reported models, compartmentalisation in nanoreactors of living
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50 radical polymerisation, as long as the deactivator concentration is sufficiently
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low, can offer a means of improving both the level of control over the molecular weight distribution and the end-functionality.

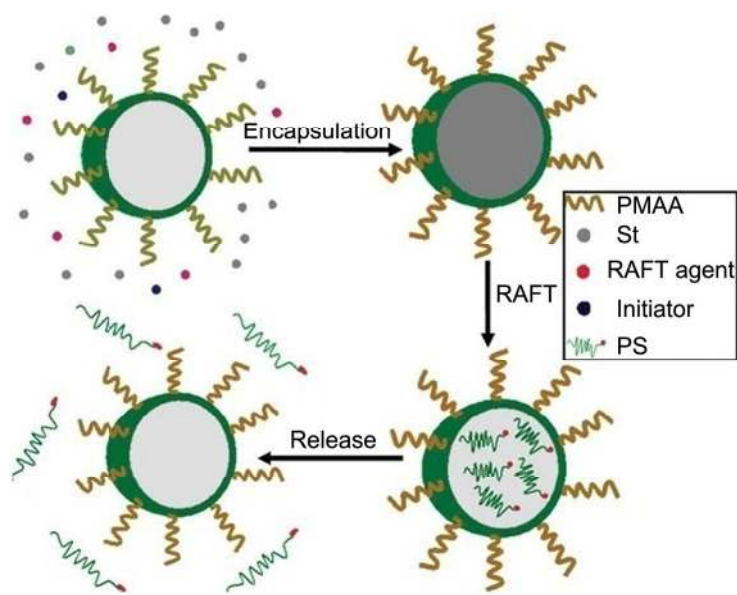


Figure 39. Schematic nanoreactors formation when-RAFT polymerisation of styrene in water is used.³⁰⁷ (Reproduced with permission from reference 307. Copyright (2010) Wiley Periodicals, Inc.).

4.3 Application of the Polymer Capsule Nanoreactors for Nanoparticles Synthesis

Some reports demonstrated that polymer capsules-based nanoreactors can also be exploited for promoting nanoparticles synthesis, since the empty core domain has shown the ability to carry reagents and that it was able to create a favourable environment to optimise the reaction. In this regard, microemulsions should be one of the first types of individual microreactors explored for nanosynthesis. Different preparation procedures to fabricate both direct and reverse emulsions have been developed and studied.³¹²⁻³¹⁵ These

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4 procedures allow the preparation of monodisperse, stable microemulsions of
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6 different sizes. Currently, a wide range of inorganic nanoparticles have been
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8 obtained in microemulsion systems involving the preparation of SiO_2 ,³¹⁵⁻³¹⁶
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10 TiO_2 ³¹⁷ or CaCO_3 ³¹⁸ *etc.* in reverse water-in-oil emulsions. Despite the
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12 significant success in spatially confined nanosynthesis inside emulsions,
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14 alternatively, polymer capsules such as constructs based on layer-by-layer
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16 strategy, as another elegant system for catalysing nanosynthesis was also
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18 developed.³¹⁹ One of the significant advantages of this polymer capsule is the
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20 semipermeable shell which is permeable for small ions but not permeable for
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22 polymers or larger particles.³²⁰ In view of the fact that some polymers can
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24 suppress the growth of inorganic crystals, this allows the formation of inorganic
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26 particle strictly inside the core domain of the capsules keeping the suppressing
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28 polymers outside.³¹⁴ Thus, the growth of calcium carbonate and barium
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30 carbonate particles only inside the polymer capsules was obtained.
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39 Another way to initiate the growth of particles in the capsule interior is to
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41 change pH of the whole system slightly over the threshold of solubility. The
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43 inner shell of the capsules might serve for nucleation.³²¹ It is possible to
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45 synthesize functional materials in the capsule, and to modify the permeation
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47 properties of the polymer capsule walls by deposition polymers within the
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49 capsule walls.³²² Moreover, Sukhorukov and coworkers also demonstrated the
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51 influence of pH gradient on the chemical reaction in the capsule interior. The
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53 precipitation of acidic dyes in a polymer capsule filled with negatively charged
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3 poly(styrene sulfonate) (PSS) buffering the pH around 2-2.5 was studied.³²³
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6 Once the carboxylic dyes went through the membrane, the low pH inside the
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8 capsules resulted in precipitation. Accordingly, the capsules were filled with
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10 dye molecules. Based on such strategy, the successful synthesis of other
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12 inorganic materials including polytungstic acid and nanosized WO_3 in the
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14 capsules was demonstrated.³²⁴ In contrast, the authors also showed that if
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16 using cationic poly(allyl amine hydrochloride) (PAH) instead of anionic PSS, an
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18 alkaline environment was created inside the capsule which was able to
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20 catalyse the synthesis of nanosized Fe_2O_3 , TiO_2 gel or magnetic ferrites within
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22 the capsule interior.³²⁵⁻³²⁶
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31 4.4 Artificial Organelles 32 33 34 35

36 Cell organelles are membrane-delineated intracellular components with
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38 specialised functions.^{29,34-35} They have unique biochemical microenvironments,
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40 characterised by a specific set of enzymes, and are enclosed by a selective
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42 membrane, allowing the subcellular spatial control of different functions. They
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44 are thus responsible for crucial cellular activities, for example mitochondria are
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46 involved in cellular respiration, ribosomes in protein production *etc.* Recently,
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48 the design of simplified, synthetic organelles capable of functioning inside of
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50 cells for the purpose of both producing drugs and enhancing the function of
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52 natural organelles in pathological conditions by using small surfactant such as
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4 a lipid as a building block has gathered considerable interest.³²⁷⁻³²⁸ In this
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respect, it is not surprising that polymer capsules, in consideration of their aqueous cavities which can serve as a reaction space,³²⁹ and the semipermeable membrane which allows transport of necessary substrates and products of the enzymatic reactions, have been taken as one of the most popular scaffolds to develop artificial organelles. Up to now, pioneering attempts have been made by Meier and von Hest *et. al.* to exploit polymer compartments into artificial organelles by loading enzymes or mimics of them inside (Figure 40). Meanwhile, basic requirements to design artificial organelles have been concluded:^{28,33} (i) polymer compartments of appropriate size for cell-uptake, (ii) activity of encapsulated enzymes maintained in situ, (iii) a polymer membrane, which is stable and permeable to small molecules, (iv) integrity and activity preserved in cells, and (v) biocompatibility and biodegradability of polymer compartments.

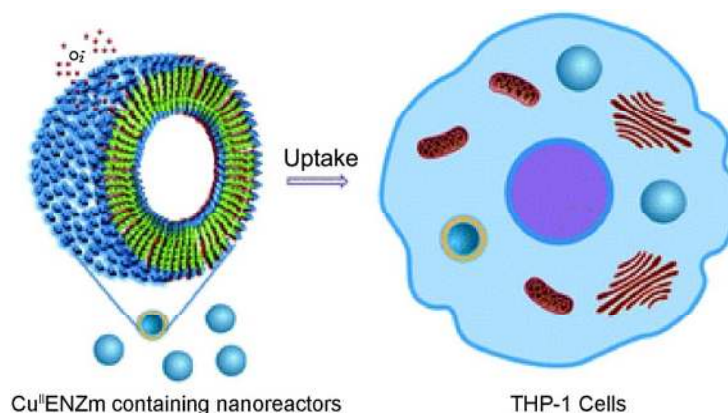


Figure 40. Schematic representation of the construction of polymeric nanoreactors towards artificial organelles by incorporating a dual-enzyme mimic of superoxide

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dismutase and catalase. The uptake of these loaded polymeric nanoreactors into THP-1 cells.¹⁶⁷ (Reproduced with permission from reference 167. Copyright (2011) Royal Society of Chemistry).

With these objectives in mind, Meier and Palivan showed a series of beautiful work on exploiting polymersome nanoreactors into artificial organelles by encapsulating enzymes/enzyme mimics into the core.^{30,156,166-167} As discussed in section 3.1.1 (Figure 20), the successful construction of an artificial model represents a milestone in the development of *in vivo* cell implants and a new frontier in medical therapy. Undoubtedly, this encourages researchers for designing various artificial organelles. Recently, also van Hest and coworkers made a great effort to exploit enzyme-loaded polymersomes into artificial organelles.³⁵ As illustrated in Figure 41,²⁸⁹ a horseradish peroxidase-filled polymersome was presented, which was capable of protecting its contents from proteolytic degradation.²⁸⁹⁻²⁹⁰ To promote the cellular uptake, cell-penetrating peptides were covalently anchored onto the surface of the model. It was found that the cellular delivery of polymersomes was restricted to cells with an intrinsically high phagocytic activity, and the catalytic activity conferred to the cells was maintained at levels that were significantly higher than those reported for soluble enzymes.

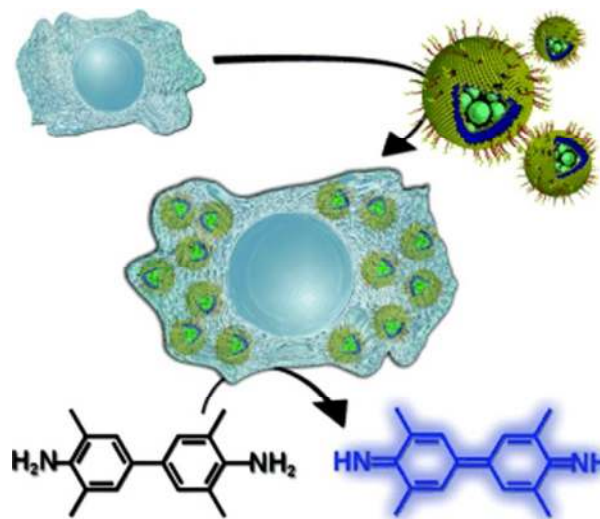
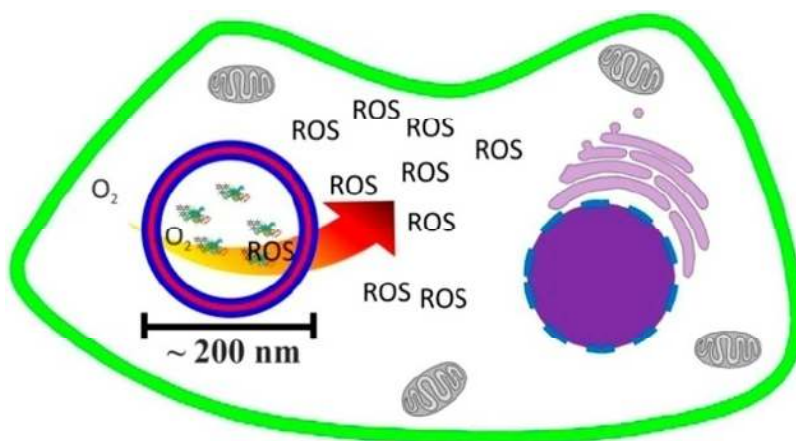


Figure 41. Schematic illustration of a polystyrene-*block*-poly[L-isocyanoalanine (2-thiophen-3-ylethyl) amide] (PS-PIAT)-based polymersome nanoreactor functionalised with Tat-peptide. This protein is known to mediate cellular uptake and thus also to the artificial organelle. Encapsulated HRP converts the substrate tetramethylbenzidine, which allows the activity of the nanoreactor to be measured by absorption.²⁸⁹ (Reproduced with permission from reference 289. Copyright (2010) John Wiley and Sons).

Last but not least, it is interesting to mention that a cellular Trojan horse acting as a source of reactive oxygen species (ROS) “on demand” inside cells was successfully reported by Palivan *et al.*(Figure 42).³³⁰ The Trojan horse was engineered by optimizing a light responsive model based on a highly active photosensitiser encapsulated in two different amphiphilic block copolymers (PMOXA-PDMS-PMOXA and PNVP-PDMS-PNVP) polymersomes. The models based on PMOXA-PDMS-PMOXA polymersomes with diameters of around 200 nm were the most efficient in terms of photo

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4 sensitiser encapsulation and size to fulfil the requirements for high retention
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6 and permeability. These constructed models also showed the highest uptake
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8 and stability in HeLa cells. While nontoxic in dark conditions, they produced
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10 ROS upon illumination in conditions specific for photodynamic therapy.
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12 Intracellular ROS levels increased by a factor of 40, which was sufficient to
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14 significantly reduce cell viability in the region containing illuminated Trojan
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16 horse models.
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Figure 42. Schematic representation of a light-responsive nanoreactor containing photosensitisers to act as a cellular Trojan horse by producing ROS inside cells “on demand”.³³⁰ (Reproduced with permission from reference 330. Copyright 2014, American Chemical Society).

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Despite tremendous efforts and the existence of exciting synthetic models, the studies reported on the application of polymer capsule-based nanoreactors as actual artificial organelles in vitro and eventually in vivo, are still mainly

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4 proofs of concept, and there are many challenges ahead before the full
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6 realisation of the implantation in cells to treat pathological conditions or to
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8 support the design of artificial cells will be seen. As major prerequisites which
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10 need to be realized in polymeric capsules as artificial organelles, introduction
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12 of specific targeting of cell types of interest and subsequent effective
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14 internalised by those cells have to be named. Furthermore, the designed
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16 models should be biocompatible without causing side effects by interactions
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18 with cells. Also, localisation of artificial organelles after cellular uptake is still an
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20 issue of intense research, given that polymersomes are reported to be routed
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22 to endosomes and lysosomes after internalisation. Escaping from the
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24 endocytotic machinery is therefore another key step towards fully functional
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26 artificial organelles.
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36 **5. Conclusions**

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39 In this review, we have put a spotlight on recent developments in polymer
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41 capsules with special emphasis on the field of polymersomes and their use as
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43 nanoreactors. After the first publications of Discher and Eisenberg on polymer
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45 vesicles in 1999 and 2002,^{2,14} the field has advanced rapidly. There are now
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47 multiple ways to prepare vesicles from amphiphilic block-copolymers in a
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49 defined way and further equip them with multiple functionalities and
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51 responsiveness to realize effective drug delivery systems, nanoreactors and
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53 even artificial organelles.
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4 Polymersome-making procedures are now available for functional and
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6 non-functional block-copolymers, being rubbery, of glassy nature, or crystalline,
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8 covering film rehydration, solvent, pH, or temperature switch, as well as
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10 emulsion, centrifugation or microfluidic techniques. This variety in methods
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12 allows for targeted and highly effective polymersome production, whilst
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14 avoiding or minimizing other aggregates like micelles and worm-like and
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16 tubular vesicles. The latest development in this respect, the formation of
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18 vesicles during polymerisation (so-called PISA) now even allows for in-situ
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20 vesicle production. Altogether one can conclude that it is now possible, by
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22 choosing the fitting method, to prepare vesicles of all amphiphilic
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24 block-copolymers which have the correct block-length ratio, regardless of the
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26 exact polymer composition involved. Recent developments in proteinosomes
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28 and dendrimersomes are very good examples in that respect. Especially
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30 proteinosomes caught up very quickly with their counterparts of purely artificial
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32 polymers. The future development of methods faces the challenge to be more
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34 robust or quicker than the current ones, if they are to be established in the
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36 field.
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46 The preparation methods for polymer capsules not requiring amphiphilic
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48 block-copolymers of the right ratio, like microfluidics and template methods,
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50 are also briefly highlighted. Here, the main challenge is to remove the central
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52 core without damaging the polymer membrane. Success in this regard is
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54 reported but clearly polymersomes dominate so far the field of nanoreactors.
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4 The main reason is that functionality of almost any kind can easily be included
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6 into polymersomes to make the membrane responsive and permeable. The
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8 inclusion of transmembrane proteins for this purpose is developed to a stage
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10 that cellular organelles can be mimicked and also replaced. Especially the
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12 development of pH sensitive polymersomes is very popular. The introduction
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14 of a variety of tertiary amines and acetal cleavages gives pH (or sometimes
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16 CO₂) sensitive systems with some variety of the pKa, which is even more
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18 diverse if boronic acid is taken into account as well. Temperature sensitive
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20 systems have also been realized and are recently more driven towards
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22 application. Also a great variety of cross-linking methods have been developed
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24 for stabilizing the capsule membrane. They cover photochemical, radical and
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26 condensation reactions as well as physical cross-linking. Cross-linked systems
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28 are often combined with redox, temperature and pH sensitive function to give
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30 vesicles with reversible membrane swelling.
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39 In this review we focused on the application of polymersomes, or more
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41 general polymeric capsules, as nanoreactors. For a nanoreactor to work, an
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43 enzyme or any other active cargo need to be enclosed and the substrate and
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45 reagents need to reach it. This principle is now available for cross-linked and
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47 non-cross-linked vesicles to give nanoreactors of high complexity allowing for
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49 multi-component and multi-stage reaction cascades. Most prominent are the
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51 development of artificial cell organelles, vesicle-in-vesicle nanoreactors and
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53 reactions across different vesicles. Today, vesicles can also be used as
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nanoreactors to produce new polymers or to enhance polymerisation procedures, making this the starting point of self-replicating polymersomes.

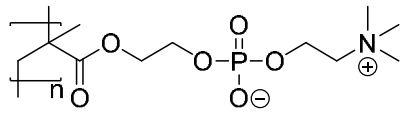
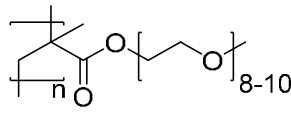
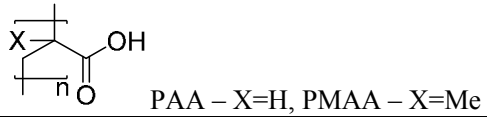
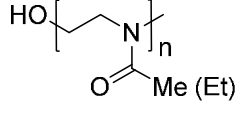
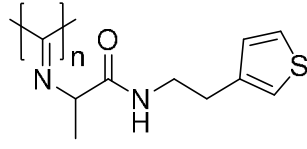
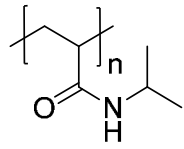
Clearly, exiting progress has been made in the recent years in engineering functional polymer capsules towards smart nanoreactors. High potential can be seen, e.g., in exploiting biohybrid approaches as in the reported proteinosomes mimicking even better biological processes and selective reaction schemes. Still, more robust preparation procedures with the potential for scale-up and further application studies beyond the proof of principles shown so far are needed to fully exploit the high promise of polymer capsule based nanoreactors in biomedicine, bio-catalysis or biotechnology.

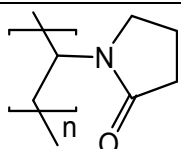
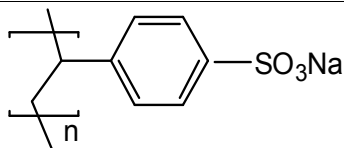
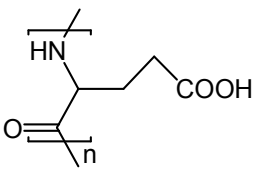
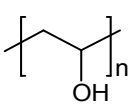
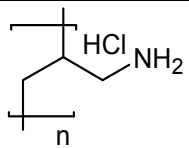
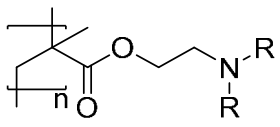
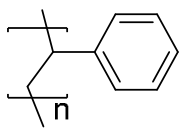
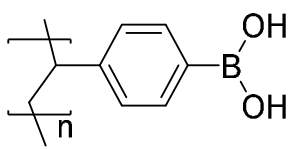
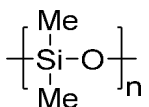
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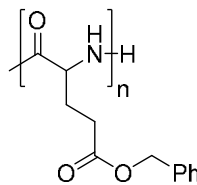
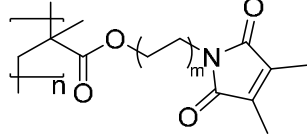
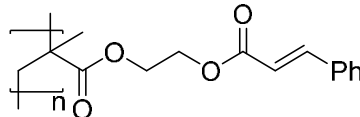
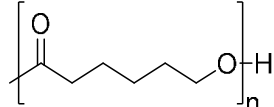
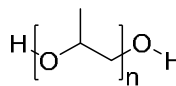
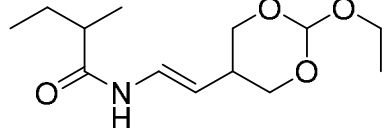
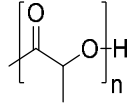
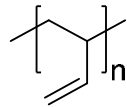
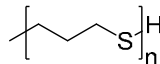
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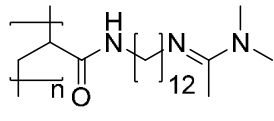
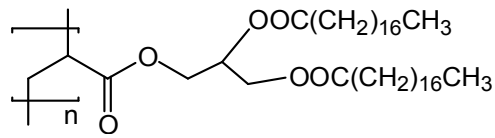
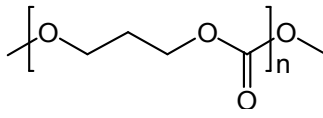
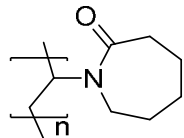
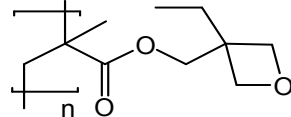
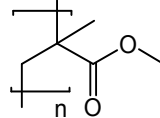
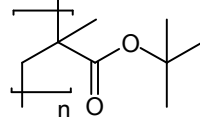
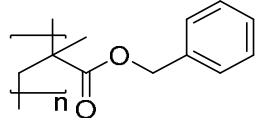
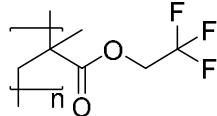
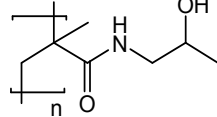
Table 1: List of polymer structures

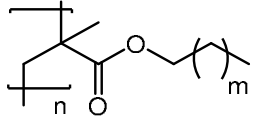
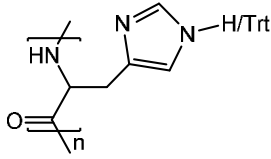
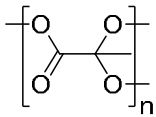
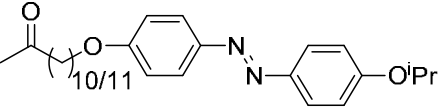
W/ L ⁱ	Name	Abbreviation/ Property	Formula
W	Poly(ethylene oxide)	PEO or PEG	$\text{H} \left[\text{O} \text{---} \text{CH}_2 \text{---} \text{CH}_2 \right]_n \text{O} \text{---} \text{H}$

	orPoly (ethylene glycol)	Non-Immunogenic	
W	Poly(methacrylic phosphoryl choline)	PMPC Non-Immunogenic	
W	Poly(oligo(ethylene glycol)methacrylate)	POEGMA Non-Immunogenic PDEGMEMMA for 2 PEG units	
W	Poly((meth)acrylic acid)	P(M)AA	
W	Poly(methyl oxazoline)	PMOxA Non-Immunogenic PEtOxa - Ethyl derivative	
W	Poly(L - isocyanoalanine(2 - thio- phen - 3 - yl - ethyl)amide)	PIAT	
W	Poly(N-isopropylacrylamid e)	PNIPAM, Temperature sensitive	

1 2 3 4 5 6 7	W	poly(N-vinylpyrrolidone)	PNVP/PVP/PVPO N	
8 9 10 11 12	W	poly(styrene sulfonate)	PSS	
13 14 15 16 17 18 19	W	poly(L-glutamic acid)	PGA	
20 21 22	W	poly(vinylalcohol)	PVA	
23 24 25 26 27	W	poly(allyl amine hydrochloride)	PAH	
28 29 30 31 32 33 34 35 36 37 38 39 40	L	Poly(dimethyl/ethyl/isopropyl/ aminoethyl methacrylate)	PD(M/E/P)A pH sensitive (for "M", also Temperature sensitive)	 <p>M: R = Me E: R = Et P: R = iPr</p>
41 42 43 44 45	L	Polystyrene	PS High Stiffness	
46 47 48 49 50 51	L	Poly(styrene-p-boronic acid)	PSBA, pH-sensitive	
52 53 54 55 56 57 58 59 60	L	Poly(dimethyl siloxane)	PDMS	

1 2 3 4 5 6 7 8 9	L	Poly(benzyl-L-glutamate)	PBLG	
10 11 12 13 14 15 16	L	Poly(dimethylmaleicimido(ethyl/butyl) methacrylate)	PDMI(E/B)M Photo-Cross-linkable	 E-m=1, B-m=2
17 18 19 20 21	L	Poly(cinnamoyl ethyl methacrylate)	PCEMA	
22 23 24 25 26	L	Polycaprolactone	PCL, Biodegradable	
27 28 29 30	L	Poly(propylene) glycol	PPG	
31 32 33 34 35	L	(trans-N-(2-ethoxy-1,3-dioxan-5-yl) acrylamide)unit	t(NEA)	
36 37 38 39 40 41 42 43	L	Poly(L-)lactic acid	PLLA, Biodegradable High Stiffness	
44 45 46 47 48 49 50	L	Polybutadiene	PBD (PB) Can be Hydrogenated	
51 52 53 54 55 56 57 58 59 60	L	Poly(propylene sulphide)	PPS Oxidation-sensitive	

1 2 3 4 5 6 7 8	L	poly(N-amidino)dodecyl acrylamide	PAD pH sensitive	
9 10 11 12 13 14 15	L	poly(distearin acrylate)	PDSA	
16 17 18 19 20	L	poly(trimethylene carbonate)	PTMC	
21 22 23 24 25	L	Poly(N-vinylcaprolactone)	PVCL Temp. Sensitive	
26 27 28 29 30	L	poly(3-ethyl-3-(methacrylo yloxy)methyloxetane)	PEMO	
31 32 33 34 35	L	poly(methyl methacrylate)	PMMA	
36 37 38 39 40	L	poly(<i>tert</i> -butylmethacrylate)	PtBMA	
41 42 43 44 45	L	Poly(benzylmethacrylate)	PBzMA	
46 47 48 49 50	L	Poly((2,2,2-trifluoroethyl methacrylate)	PTFEMA	
51 52 53 54 55 56 57 58 59 60	L	Poly(2-hydroxypropyl methacrylamide)	PHPMA Non-Immunogenic	

L	Poly(n-butyl methacrylate) poly(lauryl methacrylate)	PBMA, m=2 PLMA, m=10	
L	Poly(histidine) (trityl protect- ted)	Phis(-Trt)	
L	Azobenzene-containing dendrons d16isoAZO Light-sensitive	Dendritic unit  Azo-Endcap 	

¹ W = Hydrophilic (Water soluble) / L = Hydrophobic (Lipid soluble)

Abbreviations

ABTS	2,2'-Azinobis(3-ethylbenzothiazoline-6-sulfonic acid)
AGET ATRP	<i>Electron transfer atom transfer radical polymerisation</i>
AIBN	2,2'-Azobisisobutyronitrile
ATP/ADP	Adenosine tri(di-)phosphate
ATRP	Atom transfer radical polymerisation
BR	Bacterial rhodopsin
CAC	Critical aggregate concentration
CalB	Candida antarctica lipase B
CDB	Phenyl 2-propyl dithiobenzoate

CTA	Chain transfer agent
DBTTC	Dibenzyltrithiocarbonate
DEAEMA	Diethylaminoethyl methacrylate
DNA	Desoxyribonucleic acid
DODAB	Dimethyldioctadecylammonium bromide
DTT	Dithiothreitol
GA	Glucose amylase,
GFP	Green fluorescent protein
GO	Glucose oxidase
GSH	Glutathione
GSSG	Glutathione disulphide
HRP	Horseradish peroxidase
ITO	Indium tin oxide
KPS	Potassium persulfate
Lac	Laccase
LCST	Lower critical solution temperature
MES	2-(N-morpholino)ethanesulfonic acid buffer
NADPH	Nicotinamide adenine dinucleotide phosphate
NCA	N-carboxyanhydrid
NMRP	Nitroxide mediated radical polymerisation
OmpF	Outer-membrane-protein F
PBS	Phosphate buffered saline

PDSM	Pyridyldisulphideethylmethacrylate
PISA	Polymerisation induced self-assembly
PPPDTA	Phenyl 2-propyl phenyl dithioacetate
RAFT	Reversible addition–fragmentation chain-transfer
ROS	Reactive oxygen species
ROP	Ring opening polymerisation
SIP	Surface-initiated polymerisation
TEM	Transmission electron microscopy
TvNH	Vivax nucleoside hydrolyse

References

- (1) Mann, S. "Systems of Creation: The Emergence of Life from Nonliving Matter" *Acc. Chem. Res.* **2012**, *45*, 2131-2141.
- (2) Discher, D. E.; Eisenberg, A. "Polymer vesicles" *Science* **2002**, *297*, 967-973.
- (3) Schwille, P. "Bottom-up synthetic biology: engineering in a tinkerer's world" *Science* **2011**, *333*, 1252-1254.
- (4) Szostak, J. W.; Bartel, D. P.; Luisi, P. L. "Synthesizing life" *Nature* **2001**, *409*, 387-390.
- (5) Dinsmore, A. D.; Hsu, M. F.; Nikolaides, M. G.; Marquez, M.; Bausch, A. R.; Weitz, D. A. "Colloidosomes: Selectively Permeable Capsules Composed of Colloidal Particles" *Science* **2002**, *298*, 1006-1009.
- (6) Meier, W. "Polymer nanocapsules" *Chem. Soc. Rev.* **2000**, *29*, 295-303.

- 1
2
3
4 (7) Caruso, F.; Caruso, R. A.; Möhwald, H. "Nanoengineering of inorganic and hybrid hollow
5
6 spheres by colloidal templating" *Science* **1998**, *282*, 1111-1114.
7
8
9 (8) Utada, A. S.; Lorenceau, E.; Link, D. R.; Kaplan, P. D.; Stone, H. A.; Weitz, D. A.
10
11 "Monodisperse Double Emulsions Generated from a Microcapillary Device" *Science* **2005**,
12
13 *308*, 537-541.
14
15
16 (9) Blanz, A.; Armes, S. P.; Ryan, A. J. "Self-Assembled Block Copolymer Aggregates: From
17
18 Micelles to Vesicles and their Biological Applications" *Macromol. Rapid Commun.* **2009**, *30*,
19
20 267-277.
21
22
23 (10) Renggli, K.; Baumann, P.; Langowska, K.; Onaca, O.; Bruns, N.; Meier, W. "Selective and
24
25 Responsive Nanoreactors" *Adv. Funct. Mater.* **2011**, *21*, 1241-1259.
26
27
28 (11) Letchford, K.; Burt, H. "A review of the formation and classification of amphiphilic block
29
30 copolymer nanoparticulate structures: micelles, nanospheres, nanocapsules and
31
32 polymersomes" *Eur. J. Pharm. Biopharm.* **2007**, *65*, 259-269.
33
34
35 (12) Kisak, E.; Coldren, B.; Evans, C.; Boyer, C.; Zasadzinski, J. "The vesosome-a
36
37 multicompartiment drug delivery vehicle" *Curr. Med. Chem.* **2004**, *11*, 199-219.
38
39
40 (13) Pearson, R. T.; Warren, N. J.; Lewis, A. L.; Armes, S. P.; Battaglia, G. "Effect of pH and
41
42 temperature on PMPC-PDPA copolymer self-assembly" *Macromolecules* **2013**, *46*,
43
44 1400-1407.
45
46
47 (14) Discher, B. M.; Won, Y. Y.; Ege, D. S.; Lee, J. C. M.; Bates, F. S.; Discher, D. E.; Hammer, D.
48
49 A. "Polymersomes: Tough vesicles made from diblock copolymers" *Science* **1999**, *284*,
50
51 1143-1146.
52
53
54 (15) Percec, V.; Wilson, D. A.; Leowanawat, P.; Wilson, C. J.; Hughes, A. D.; Kaucher, M. S.;
55
56
57
58
59
60

- 1
2
3
4 Hammer, D. A.; Levine, D. H.; Kim, A. J.; Bates, F. S.; Davis, K. P.; Lodge, T. P.; Klein, M. L.;
- 5
6 DeVane, R. H.; Aqad, E.; Rosen, B. M.; Argintaru, A. O.; Sienkowska, M. J.; Rissanen, K.;
- 7
8
9 Nummelin, S.; Ropponen, J. "Self-Assembly of Janus Dendrimers into Uniform
- 10
11 Dendrimersomes and Other Complex Architectures" *Science* **2010**, *328*, 1009-1014.
- 12
13 (16) Huang, X.; Li, M.; Green, D. C.; Williams, D. S.; Patil, A. J.; Mann, S. "Interfacial assembly
- 14
15 of protein-polymer nano-conjugates into stimulus-responsive biomimetic protocells" *Nat.*
- 16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
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41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
- (17) Vargo, K. B.; Parthasarathy, R.; Hammer, D. A. "Self-assembly of tunable protein
- suprastructures from recombinant oleosin" *Proc. Natl. Acad. Sci. U.S.A* **2012**, *109*,
- 11657-11662.
- (18) Marguet, M.; Bonduelle, C.; Lecommandoux, S. "Multicompartmentalized polymeric systems:
- towards biomimetic cellular structure and function" *Chem. Soc. Rev.* **2013**, *42*, 512-529.
- (19) Huang, X.; Voit, B. "Progress on multi-compartment polymeric capsules" *Polym. Chem.* **2013**,
- 4*, 435-443.
- (20) Messenger, L.; Gaitzsch, J.; Chierico, L.; Battaglia, G. "Novel aspects of encapsulation and
- delivery using polymersomes" *Curr. Opin. Pharmacol.* **2014**, *18*, 104-111.
- (21) Battaglia, G.; Ryan, A. J. "Pathways of polymeric vesicle formation" *J. Phys. Chem. B* **2006**,
- 110*, 10272-10279.
- (22) Wang, S.; Wang, H.; Liu, Z.; Wang, L.; Wang, X.; Su, L.; Chang, J. "Smart pH- and
- reduction-dual-responsive folate-PEG-coated polymeric lipid vesicles for tumor-triggered
- targeted drug delivery" *Nanoscale* **2014**, *6*, 7635-7642.
- (23) Lv, L.-P.; Landfester, K.; Crespy, D. "Stimuli-Selective Delivery of two Payloads from Dual

- 1
2
3
4 Responsive Nanocontainers" *Chem. Mater.* **2014**, *26*, 3351-3353.
- 5
6 (24) Ru, C.; Fenghua, M.; Chao, D.; Klok, H. A.; Zhiyuan, Z. "Dual and multi-stimuli responsive
7
8 polymeric nanoparticles for programmed site-specific drug delivery" *Biomaterials* **2013**, *34*,
9
10 3647-3657.
- 11
12 (25) Kost, J.; Langer, R. "Responsive polymeric delivery systems" *Adv. Drug Delivery Rev.* **2012**,
13
14 *64*, 327-341.
- 15
16 (26) Egli, S.; Schlaad, H.; Bruns, N.; Meier, W. "Functionalization of Block Copolymer Vesicle
17
18 Surfaces" *Polymers* **2011**, *3*, 252-280.
- 19
20 (27) Chuanoi, S.; Anraku, Y.; Hori, M.; Kishimura, A.; Kataoka, K. "Fabrication of Polyion
21
22 Complex Vesicles with Enhanced Salt and Temperature Resistance and Their Potential
23
24 Applications as Enzymatic Nanoreactors" *Biomacromolecules* **2014**, *15*, 2389-2397.
- 25
26 (28) Dobrunz, D.; Toma, A. C.; Tanner, P.; Pfohl, T.; Palivan, C. G. "Polymer Nanoreactors with
27
28 Dual Functionality: Simultaneous Detoxification of Peroxynitrite and Oxygen Transport"
29
30 *Langmuir* **2012**, *28*, 15889-15899.
- 31
32 (29) Tanner, P.; Baumann, P.; Enea, R.; Onaca, O.; Palivan, C.; Meier, W. "Polymeric Vesicles:
33
34 From Drug Carriers to Nanoreactors and Artificial Organelles" *Acc. Chem. Res.* **2011**, *44*,
35
36 1039-1049.
- 37
38 (30) Kim, K. T.; Meeuwissen, S. A.; Nolte, R. J.; van Hest, J. C. "Smart nanocontainers and
39
40 nanoreactors" *Nanoscale* **2010**, *2*, 844-858.
- 41
42 (31) Broz, P.; Driamov, S.; Ziegler, J.; Ben-Haim, N.; Marsch, S.; Meier, W.; Hunziker, P. "Toward
43
44 intelligent nanosize bioreactors: A pH-switchable, channel-equipped, functional polymer
45
46 nanocontainer" *Nano Lett.* **2006**, *6*, 2349-2353.
- 47
48
49
50
51
52
53
54
55
56
57
58
59
60

- 1
2
3
4 (32) Louzao, I.; van Hest, J. C. M. "Permeability Effects on the Efficiency of Antioxidant
5
6 Nanoreactors" *Biomacromolecules* **2013**, *14*, 2364-2372.
7
- 8
9 (33) Kim, K. T.; Cornelissen, J. J. L. M.; Nolte, R. J. M.; van Hest, J. C. M. "A Polymersome
10
11 Nanoreactor with Controllable Permeability Induced by Stimuli-Responsive Block
12
13 Copolymers" *Adv. Mater.* **2009**, *21*, 2787-2791.
14
15
- 16 (34) Tanner, P.; Egli, S.; Balasubramanian, V.; Onaca, O.; Palivan, C. G.; Meier, W. "Can
17
18 polymeric vesicles that confine enzymatic reactions act as simplified organelles?" *FEBS Lett.*
19
20
21 **2011**, *585*, 1699-1706.
22
- 23 (35) Peters, R. J. R. W.; Louzao, I.; van Hest, J. C. M. "From polymeric nanoreactors to artificial
24
25 organelles" *Chem. Sci.* **2012**, *3*, 335-342.
26
27
- 28 (36) Kamat, N. P.; Katz, J. S.; Hammer, D. A. "Engineering Polymersome Protocells" *J. Phys.*
29
30
31 *Chem. Lett.* **2011**, *2*, 1612-1623.
32
- 33 (37) Discher, D. E.; Ahmed, F. "POLYMERSOMES" *Annu. Rev. Biomed. Eng.* **2006**, *8*, 323-341.
34
35
- 36 (38) van Dongen, S. F. M.; de Hoog, H.-P. M.; Peters, R. J. R. W.; Nallani, M.; Nolte, R. J. M.; van
37
38
39 Hest, J. C. M. "Biohybrid Polymer Capsules" *Chem. Rev.* **2009**, *109*, 6212-6274.
40
- 41 (39) Sukhorukov, G.; Fery, A.; Möhwald, H. "Intelligent micro-and nanocapsules" *Prog. Polym.*
42
43
44 *Sci.* **2005**, *30*, 885-897.
45
- 46 (40) Tong, W.; Song, X.; Gao, C. "Layer-by-layer assembly of microcapsules and their biomedical
47
48
49 applications" *Chem. Soc. Rev.* **2012**, *41*, 6103-6124.
50
- 51 (41) Cui, J.; van Koeveden, M. P.; Muellner, M.; Kempe, K.; Caruso, F. "Emerging methods for
52
53
54 the fabrication of polymer capsules" *Adv. Colloid Interface Sci.* **2014**, *207*, 14-31.
55
- 56 (42) del Mercato, L. L.; Ferraro, M. M.; Baldassarre, F.; Mancarella, S.; Greco, V.; Rinaldi, R.;
57
58
59
60

- 1
2
3
4 Leporatti, S. "Biological applications of LbL multilayer capsules: From drug delivery to
5
6 sensing" *Adv. Colloid Interface Sci.* **2014**, *207*, 139-154.
7
- 8
9 (43) Liu, P. "Stabilization of layer-by-layer engineered multilayered hollow microspheres" *Adv.*
10
11 *Colloid Interface Sci.* **2014**, *207*, 178-188.
12
- 13
14 (44) Parakhonskiy, B. V.; Yashchenok, A. M.; Konrad, M.; Skirtach, A. G. "Colloidal micro- and
15
16 nano-particles as templates for polyelectrolyte multilayer capsules" *Adv. Colloid Interface Sci.*
17
18 **2014**, *207*, 253-264.
19
- 20
21 (45) Ueda, M.; Uesaka, A.; Kimura, S. "Selective disruption of each part of Janus molecular
22
23 assemblies by lateral diffusion of stimuli-responsive amphiphilic peptides" *Chem. Comm.*
24
25 **2015**, *51*, 1601-1604.
26
27
- 28
29 (46) Blanazs, A.; Ryan, A. J.; Armes, S. P. "Predictive Phase Diagrams for RAFT Aqueous
30
31 Dispersion Polymerization: Effect of Block Copolymer Composition, Molecular Weight, and
32
33 Copolymer Concentration" *Macromolecules* **2012**, *45*, 5099-5107.
34
35
- 36
37 (47) Spulber, M.; Najer, A.; Winkelbach, K.; Glaied, O.; Waser, M.; Pieleles, U.; Meier, W.; Bruns, N.
38
39 "Photoreaction of a hydroxyalkylphenone with the membrane of polymersomes: a versatile
40
41 method to generate semipermeable nanoreactors" *J. Am. Chem. Soc.* **2013**, *135*, 9204-9212.
42
43
- 44
45 (48) Lomas, H.; Canton, I.; MacNeil, S.; Du, J.; Armes, S. P.; Ryan, A. J.; Lewis, A. L.; Battaglia,
46
47 G. "Biomimetic pH sensitive polymersomes for efficient DNA encapsulation and delivery"
48
49 *Adv. Mater.* **2007**, *19*, 4238-4246.
50
- 51
52 (49) Battaglia, G.; Ryan, A. J. "The evolution of vesicles from bulk lamellar gels" *Nat Mater* **2005**,
53
54 *4*, 869-876.
55
- 56
57 (50) Semsarilar, M.; Penfold, N. J. W.; Jones, E. R.; Armes, S. P. "Semi-crystalline diblock
58
59
60

- 1
2
3
4 copolymer nano-objects prepared via RAFT alcoholic dispersion polymerization of stearyl
5
6 methacrylate" *Polym. Chem.* **2015**, *6*, 1751-1757.
7
8
9 (51) Gonzato, C.; Semsarilar, M.; Jones, E. R.; Li, F.; Krooshof, G. J. P.; Wyman, P.; Mykhaylyk, O.
10
11 O.; Tuinier, R.; Armes, S. P. "Rational Synthesis of Low-Polydispersity Block Copolymer
12
13 Vesicles in Concentrated Solution via Polymerization-Induced Self-Assembly" *J. Am. Chem.*
14
15 *Soc.* **2014**, *136*, 11100-11106.
16
17
18 (52) Pei, Y.; Lowe, A. B. "Polymerization-induced self-assembly: ethanolic RAFT dispersion
19
20 polymerization of 2-phenylethyl methacrylate" *Polym. Chem.* **2014**, *5*, 2342-2351.
21
22
23 (53) Battaglia, G.; Tomas, S.; Ryan, A. J. "Lamellarsomes: metastable polymeric multilamellar
24
25 aggregates" *Soft Matter* **2007**, *3*, 470-475.
26
27
28 (54) Smart, T.; Lomas, H.; Massignani, M.; Flores-Merino, M. V.; Perez, L. R.; Battaglia, G.
29
30 "Block copolymer nanostructures" *Nano Today* **2008**, *3*, 38-46.
31
32
33 (55) Robertson, J. D.; Yealland, G.; Avila-Olias, M.; Chierico, L.; Bandmann, O.; Renshaw, S. A.;
34
35 Battaglia, G. "pH-Sensitive Tubular Polymersomes: Formation and Applications in Cellular
36
37 Delivery" *ACS Nano* **2014**, *8*, 4650-4661.
38
39
40 (56) Adams, D. J.; Butler, M. F.; Weaver, A. C. "Effect of Block Length, Polydispersity, and Salt
41
42 Concentration on PEO-PDEAMA Block Copolymer Structures in Dilute Solution" *Langmuir*
43
44 **2006**, *22*, 4534-4540.
45
46
47
48 (57) Adams, D. J.; Adams, S.; Atkins, D.; Butler, M. F.; Furzeland, S. "Impact of mechanism of
49
50 formation on encapsulation in block copolymer vesicles" *J Control Release* **2008**, *128*,
51
52 165-170.
53
54
55 (58) Onaca, O.; Enea, R.; Hughes, D. W.; Meier, W. "Stimuli-Responsive Polymersomes as
56
57
58
59
60

- 1
2
3
4 Nanocarriers for Drug and Gene Delivery" *Macromol. Biosci.* **2009**, *9*, 129-139.
- 5
6 (59) Pegoraro, C.; MacNeil, S.; Battaglia, G. "Transdermal drug delivery: from micro to nano"
7
8
9 *Nanoscale* **2012**, *4*, 1881-1894.
- 10
11 (60) Liao, J. F.; Wang, C.; Wang, Y. J.; Luo, F.; Qian, Z. Y. "Recent Advances in Formation,
12
13
14
15
16
17 (61) Tong, R.; Lu, X. L.; Xia, H. S. "A facile mechanophore functionalization of an amphiphilic
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32 (63) Angelova, M.; Dimitrov, D. "Liposome electroformation" *Faraday Discuss. Chem. Soc.* **1986**,
33
34
35
36
37 (64) Wesolowska, O.; Michalak, K.; Maniewska, J.; Hendrich, A. B. "Giant unilamellar vesicles - a
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57 (67) Meeuwissen, S. A.; Kim, K. T.; Chen, Y.; Pochan, D. J.; van Hest, J. C. M. "Controlled Shape
58
59
60

- 1
2
3
4 Transformation of Polymersome Stomatocytes" *Angew. Chem. Int. Ed.* **2011**, *50*, 7070-7073.
- 5
6 (68) Fernyhough, C.; Ryan, A. J.; Battaglia, G. "pH controlled assembly of a
7
8 polybutadiene-poly(methacrylic acid) copolymer in water: packing considerations and kinetic
9
10 limitations" *Soft Matter* **2009**, *5*, 1674-1682.
- 11
12 (69) Themistou, E.; Battaglia, G.; Armes, S. P. "Facile synthesis of thiol-functionalized amphiphilic
13
14 polylactide-methacrylic diblock copolymers" *Polym. Chem.* **2014**, *5*, 1405-1417.
- 15
16 (70) Liu, K. K.; Wan, K. T. "Mechanics of biomimetic/biological vesicles" *Mater. Sci. Found.* **2008**,
17
18 *41-43*, 115-133.
- 19
20 (71) Canton, I.; Battaglia, G. "Endocytosis at the nanoscale" *Chem. Soc. Rev.* **2012**, *41*, 2718-2739.
- 21
22 (72) Hong, C. Y.; You, Y. Z.; Pan, C. Y. "Synthesis and characterization of well-defined diblock and
23
24 triblock copolymers of poly(N-isopropylacrylamide) and poly(ethylene oxide)" *J. Polym. Sci.*
25
26 *A Polym. Chem* **2004**, *42*, 4873-4881.
- 27
28 (73) Chen, X. R.; Ding, X. B.; Zheng, Z. H.; Peng, Y. X. "A self-assembly approach to
29
30 temperature-responsive polymer nanocontainers" *Macromol. Rapid Commun.* **2004**, *25*,
31
32 1575-1578.
- 33
34 (74) Park, W. M.; Champion, J. A. "Thermally Triggered Self-Assembly of Folded Proteins into
35
36 Vesicles" *J. Am. Chem. Soc.* **2014**, *136*, 17906-17909.
- 37
38 (75) Luo, T. Z.; He, L. R.; Theato, P.; Kiick, K. L. "Thermoresponsive Self-Assembly of
39
40 Nanostructures from a Collagen-Like Peptide-Containing Diblock Copolymer" *Macromol.*
41
42 *Biosci.* **2015**, *15*, 111-123.
- 43
44 (76) Yassin, M.; Appelhans, D.; Mendes, R.; Rummeli, M.; Voit, B. "pH-dependent Release of
45
46 Doxorubicin from Fast Photo Cross-linkable Polymersomes Based on Benzophenone Units"
47
48
49
50
51
52
53
54
55
56
57
58
59
60

- 1
2
3
4 *Chem. Eur. J.* **2012**, *18*, 12227-12231.
- 5
6 (77) Wei, J.; Ju, X.-J.; Zou, X.-Y.; Xie, R.; Wang, W.; Liu, Y.-M.; Chu, L.-Y.
7
8 "Multi-Stimuli-Responsive Microcapsules for Adjustable Controlled-Release" *Adv. Funct.*
9
10 *Mater.* **2014**, *24*, 3312-3323.
- 11
12 (78) Kim, I.; Lee, N. E.; Jeong, Y. J.; Chung, Y. H.; Cho, B. K.; Lee, E. "Micellar and vesicular
13
14 nanoassemblies of triazole-based amphiphilic probes triggered by mercury(II) ions in a 100%
15
16 aqueous medium" *Chem. Comm.* **2014**, *50*, 14006-14009.
- 17
18 (79) Anraku, Y.; Kishimura, A.; Oba, M.; Yamasaki, Y.; Kataoka, K. "Spontaneous Formation of
19
20 Nanosized Unilamellar Polyion Complex Vesicles with Tunable Size and Properties" *J Am*
21
22 *Chem Soc* **2010**, *132*, 1631-1636.
- 23
24 (80) Schrage, S.; Sigel, R.; Schlaad, H. "Formation of amphiphilic polyion complex vesicles from
25
26 mixtures of oppositely charged block ionomers" *Macromolecules* **2003**, *36*, 1417-1420.
- 27
28 (81) Dong, W. F.; Kishimura, A.; Anraku, Y.; Chuanoi, S.; Kataoka, K. "Monodispersed Polymeric
29
30 Nanocapsules: Spontaneous Evolution and Morphology Transition from Reducible
31
32 Hetero-PEG PICmicelles by Controlled Degradation" *J Am Chem Soc* **2009**, *131*, 3804-+.
- 33
34 (82) Anraku, Y.; Kishimura, A.; Yamasaki, Y.; Kataoka, K. "Living Unimodal Growth of Polyion
35
36 Complex Vesicles via Two-Dimensional Supramolecular Polymerization" *J Am Chem Soc*
37
38 **2013**, *135*, 1423-1429.
- 39
40 (83) Noireaux, V.; Libchaber, A. "A vesicle bioreactor as a step toward an artificial cell assembly"
41
42 *Proc. Natl. Acad. Sci. U.S.A* **2004**, *101*, 17669-17674.
- 43
44 (84) Marguet, M.; Sandre, O.; Lecommandoux, S. "Polymersomes in "Gelly" Polymersomes:
45
46 Toward Structural Cell Mimicry" *Langmuir* **2011**, *28*, 2035-2043.
- 47
48
49
50
51
52
53
54
55
56
57
58
59
60

- 1
2
3
4 (85) Marguet, M.; Edembe, L.; Lecommandoux, S. "Polymersomes in polymersomes: multiple
5
6 loading and permeability control" *Angew. Chem. Int. Ed.* **2012**, *51*, 1173-1176.
7
8
9 (86) Peters, R. J. R. W.; Marguet, M.; Marais, S.; Fraaije, M. W.; van Hest, J. C. M.;
10
11 Lecommandoux, S. "Cascade Reactions in Multicompartmentalized Polymersomes" *Angew.*
12
13 *Chem. Int. Ed.* **2014**, *53*, 146-150.
14
15
16 (87) Teh, S. Y.; Lin, R.; Hung, L. H.; Lee, A. P. "Droplet microfluidics" *Lab on a Chip* **2008**, *8*,
17
18 198-220.
19
20
21 (88) Brown, L.; McArthur, S. L.; Wright, P. C.; Lewis, A.; Battaglia, G. "Polymersome production
22
23 on a microfluidic platform using pH sensitive block copolymers" *Lab on a Chip* **2010**, *10*,
24
25 1922-1928.
26
27
28 (89) Thiele, J.; Abate, A. R.; Shum, H. C.; Bachtler, S.; Forster, S.; Weitz, D. A. "Fabrication of
29
30 Polymersomes using Double-Emulsion Templates in Glass-Coated Stamped Microfluidic
31
32 Devices" *Small* **2010**, *6*, 1723-1727.
33
34
35 (90) Kim, S. H.; Kim, J. W.; Kim, D. H.; Han, S. H.; Weitz, D. A. "Polymersomes Containing a
36
37 Hydrogel Network for High Stability and Controlled Release" *Small* **2013**, *9*, 124-131.
38
39
40 (91) Kim, S. H.; Nam, J.; Kim, J. W.; Kim, D. H.; Han, S. H.; Weitz, D. A. "Formation of
41
42 polymersomes with double bilayers templated by quadruple emulsions" *Lab on a Chip* **2013**,
43
44 *13*, 1351-1356.
45
46
47 (92) Kim, S. H.; Kim, J. W.; Kim, D. H.; Han, S. H.; Weitz, D. A. "Enhanced-throughput
48
49 production of polymersomes using a parallelized capillary microfluidic device" *Microfluidics*
50
51 *and Nanofluidics* **2013**, *14*, 509-514.
52
53
54 (93) Kim, S.-H.; Shum, H. C.; Kim, J. W.; Cho, J.-C.; Weitz, D. A. "Multiple Polymersomes for
55
56
57
58
59
60

- 1
2
3
4 Programmed Release of Multiple Components" *J. Am. Chem. Soc.* **2011**, *133*, 15165-15171.
- 5
6 (94) Sugihara, S.; Blanazs, A.; Armes, S. P.; Ryan, A. J.; Lewis, A. L. "Aqueous Dispersion
7
8 Polymerization: A New Paradigm for in Situ Block Copolymer Self-Assembly in
9
10 Concentrated Solution" *J. Am. Chem. Soc.* **2011**, *133*, 15707-15713.
- 11
12
13 (95) Warren, N. J.; Mykhaylyk, O. O.; Mahmood, D.; Ryan, A. J.; Armes, S. P. "RAFT Aqueous
14
15 Dispersion Polymerization Yields Poly(ethylene glycol)-Based Diblock Copolymer
16
17 Nano-Objects with Predictable Single Phase Morphologies" *J. Am. Chem. Soc.* **2014**, *136*,
18
19 1023-1033.
- 20
21
22
23 (96) Ratcliffe, L. P. D.; Blanazs, A.; Williams, C. N.; Brown, S. L.; Armes, S. P. "RAFT
24
25 polymerization of hydroxy-functional methacrylic monomers under heterogeneous conditions:
26
27 effect of varying the core-forming block" *Polym. Chem.* **2014**, *5*, 3643-3655.
- 28
29
30
31 (97) Chambon, P.; Blanazs, A.; Battaglia, G.; Armes, S. P. "Facile Synthesis of Methacrylic ABC
32
33 Triblock Copolymer Vesicles by RAFT Aqueous Dispersion Polymerization" *Macromolecules*
34
35 **2012**, *45*, 5081-5090.
- 36
37
38 (98) Warren, N. J.; Mykhaylyk, O. O.; Ryan, A. J.; Williams, M.; Doussineau, T.; Dugourd, P.;
39
40 Antoine, R.; Portale, G.; Armes, S. P. "Testing the Vesicular Morphology to Destruction: Birth
41
42 and Death of Diblock Copolymer Vesicles Prepared via Polymerization-Induced
43
44 Self-Assembly" *J. Am. Chem. Soc.* **2015**, *137*, 1929-1937.
- 45
46
47
48 (99) Agut, W.; Brûlet, A.; Schatz, C.; Taton, D.; Lecommandoux, S. b. "pH and Temperature
49
50 Responsive Polymeric Micelles and Polymersomes by Self-Assembly of
51
52 Poly[2-(dimethylamino)ethyl methacrylate]-b-Poly(glutamic acid) Double Hydrophilic Block
53
54 Copolymers" *Langmuir* **2010**, *26*, 10546-10554.
- 55
56
57
58
59
60

- 1
2
3
4 (100) Liu, B. W.; Zhou, H.; Zhou, S. T.; Zhang, H. J.; Feng, A. C.; Jian, C. M.; Hu, J.; Gao, W. P.;
5
6 Yuan, J. Y. "Synthesis and Self-Assembly of CO₂-Temperature Dual Stimuli-Responsive
7
8 Triblock Copolymers" *Macromolecules* **2014**, *47*, 2938-2946.
9
10
11 (101) Guo, W. J.; Wang, T. S.; Tang, X. D.; Zhang, Q.; Yu, F. Q.; Pei, M. S. "Triple
12
13 Stimuli-Responsive Amphiphilic Glycopolymers" *J. Polym. Sci. A Polym. Chem* **2014**, *52*,
14
15 2131-2138.
16
17
18 (102) Fielding, L. A.; Lane, J. A.; Derry, M. J.; Mykhaylyk, O. O.; Armes, S. P. "Thermo-responsive
19
20 Diblock Copolymer Worm Gels in Non-polar Solvents" *J. Am. Chem. Soc.* **2014**, *136*,
21
22 5790-5798.
23
24
25 (103) Blomberg, S.; Ostberg, S.; Harth, E.; Bosman, A. W.; Van Horn, B.; Hawker, C. J. "Production
26
27 of crosslinked, hollow nanoparticles by surface - initiated living free - radical
28
29 polymerization" *J. Polym. Sci. A Polym. Chem* **2002**, *40*, 1309-1320.
30
31
32
33 (104) Morinaga, T.; Ohkura, M.; Ohno, K.; Tsujii, Y.; Fukuda, T. "Monodisperse silica particles
34
35 grafted with concentrated oxetane-carrying polymer brushes: Their synthesis by
36
37 surface-initiated atom transfer radical polymerization and use for fabrication of hollow
38
39 spheres" *Macromolecules* **2007**, *40*, 1159-1164.
40
41
42
43 (105) Fu, G.; Shang, Z.; LIANG, H.; Kang, E.; Neoh, K. "Preparation of cross-linked polystyrene
44
45 hollow nanospheres via surface-initiated atom transfer radical polymerizations"
46
47 *Macromolecules* **2005**, *38*, 7867-7871.
48
49
50 (106) Matyjaszewski, K.; Tsarevsky, N. V. "Nanostructured functional materials prepared by atom
51
52 transfer radical polymerization" *Nat. Chem.* **2009**, *1*, 276-288.
53
54
55 (107) Boyer, C.; Bulmus, V.; Davis, T. R.; Ladmiral, V.; Liu, J.; Perrier, S. "Bioapplications of
56
57
58
59
60

- RAFT polymerization" *Chem. Rev.* **2009**, *109*, 5402-5436.
- (108) Xu, X.; Asher, S. A. "Synthesis and utilization of monodisperse hollow polymeric particles in photonic crystals" *J. Am. Chem. Soc.* **2004**, *126*, 7940-7945.
- (109) Li, G. L.; Xu, L. Q.; Tang, X.; Neoh, K.; Kang, E. "Hairy Hollow Microspheres of Fluorescent Shell and Temperature-Responsive Brushes via Combined Distillation-Precipitation Polymerization and Thiol-ene Click Chemistry" *Macromolecules* **2010**, *43*, 5797-5803.
- (110) Huang, X.; Appelhans, D.; Formanek, P.; Simon, F.; Voit, B. "Synthesis of Well-Defined Photo-Cross-Linked Polymeric Nanocapsules by Surface-Initiated RAFT Polymerization" *Macromolecules* **2011**, *44*, 8351-8360.
- (111) Huang, X.; Appelhans, D.; Formanek, P.; Simon, F.; Voit, B. "Tailored Synthesis of Intelligent Polymer Nanocapsules: An Investigation of Controlled Permeability and pH-Dependent Degradability" *ACS Nano* **2012**, *6*, 9718-9726.
- (112) Zhou, K.; Tong, L.; Deng, J.; Yang, W. "Hollow polymeric microspheres grafted with optically active helical polymer chains: Preparation and their chiral recognition ability" *J Mater Chem* **2010**, *20*, 781-789.
- (113) Chen, F.; Jiang, X.; Liu, R.; Yin, J. "Polymeric vesicles with well-defined poly(methyl methacrylate) (PMMA) brushes via surface-initiated photopolymerization (SIPP)" *Polym. Chem.* **2011**, *2*, 614-618.
- (114) Boyer, C.; Whittaker, M. R.; Nouvel, C.; Davis, T. P. "Synthesis of hollow polymer nanocapsules exploiting gold nanoparticles as sacrificial templates" *Macromolecules* **2010**, *43*, 1792-1799.
- (115) Lowe, A. B.; Sumerlin, B. S.; Donovan, M. S.; McCormick, C. L. "Facile Preparation of

- 1
2
3
4 Transition Metal Nanoparticles Stabilized by Well-Defined (Co)polymers Synthesized via
5
6 Aqueous Reversible Addition-Fragmentation Chain Transfer Polymerization†" *J. Am. Chem.*
7
8 *Soc.* **2002**, *124*, 11562-11563.
- 9
10
11 (116) Roth, P. J.; Theato, P. "Versatile Synthesis of Functional Gold Nanoparticles: Grafting
12
13 Polymers From and Onto" *Chem. Mater.* **2008**, *20*, 1614-1621.
- 14
15
16 (117) Hotz, J.; Meier, W. "Vesicle-templated polymer hollow spheres" *Langmuir* **1998**, *14*,
17
18 1031-1036.
- 19
20
21 (118) Ali, S. I.; Heuts, J. P. A.; van Herk, A. M. "Vesicle-templated pH-responsive polymeric
22
23 nanocapsules" *Soft Matter* **2011**, *7*, 5382-5390.
- 24
25
26 (119) Ali, S. I.; Heuts, J. P. A.; van Herk, A. M. "Controlled Synthesis of Polymeric Nanocapsules
27
28 by RAFT-Based Vesicle Templating" *Langmuir* **2010**, *26*, 7848-7858.
- 29
30
31 (120) Musyanovych, A.; Landfester, K. "Polymer Micro- and Nanocapsules as Biological Carriers
32
33 with Multifunctional Properties" *Macromol. Biosci.* **2014**, *14*, 458-477.
- 34
35
36 (121) Landfester, K.; Musyanovych, A.; Mailänder, V. "From polymeric particles to multifunctional
37
38 nanocapsules for biomedical applications using the miniemulsion process" *J. Polym. Sci. A*
39
40 *Polym. Chem* **2010**, *48*, 493-515.
- 41
42
43 (122) Tiarks, F.; Landfester, K.; Antonietti, M. "Preparation of polymeric nanocapsules by
44
45 miniemulsion polymerization" *Langmuir* **2001**, *17*, 908-918.
- 46
47
48 (123) Musyanovych, A.; Landfester, K. "Synthesis of poly (butylcyanoacrylate) nanocapsules by
49
50 interfacial polymerization in miniemulsions for the delivery of DNA molecules" *Prog. Colloid*
51
52 *Polym. Sci.* **2008**, 120-127.
- 53
54
55 (124) Jagielski, N.; Sharma, S.; Hombach, V.; Mailänder, V.; Rasche, V.; Landfester, K.
56
57
58
59
60

- 1
2
3
4 "Nanocapsules synthesized by miniemulsion technique for application as new contrast agent
5
6 materials" *Macromol. Chem. Phys.* **2007**, *208*, 2229-2241.
7
8
9 (125) Crespy, D.; Stark, M.; Hoffmann-Richter, C.; Ziener, U.; Landfester, K. "Polymeric
10
11 nanoreactors for hydrophilic reagents synthesized by interfacial polycondensation on
12
13 miniemulsion droplets" *Macromolecules* **2007**, *40*, 3122-3135.
14
15
16 (126) Torini, L.; Argillier, J. F.; Zydowicz, N. "Interfacial Polycondensation Encapsulation in
17
18 Miniemulsion" *Macromolecules* **2005**, *38*, 3225-3236.
19
20
21 (127) Luo, Y.; Zhou, X. "Nanoencapsulation of a hydrophobic compound by a miniemulsion
22
23 polymerization process" *J. Polym. Sci. A Polym. Chem* **2004**, *42*, 2145-2154.
24
25
26 (128) Cao, Z.; Shan, G. "Synthesis of polymeric nanocapsules with a crosslinked shell through
27
28 interfacial miniemulsion polymerization" *J. Polym. Sci. A Polym. Chem* **2009**, *47*, 1522-1534.
29
30
31 (129) Theisinger, S.; Schoeller, K.; Osborn, B.; Sarkar, M.; Landfester, K. "Encapsulation of a
32
33 Fragrance via Miniemulsion Polymerization for Temperature-Controlled Release" *Macromol.*
34
35 *Chem. Phys.* **2009**, *210*, 411-420.
36
37
38 (130) Romio, A. P.; Sayer, C.; Araújo, P. H. H.; Al-Haydari, M.; Wu, L.; da Rocha, S. R. P.
39
40 "Nanocapsules by Miniemulsion Polymerization with Biodegradable Surfactant and
41
42 Hydrophobe" *Macromol. Chem. Phys.* **2009**, *210*, 747-751.
43
44
45 (131) Zetterlund, P. B.; Saka, Y.; Okubo, M. "Gelation and Hollow Particle Formation in
46
47 Nitroxide-Mediated Radical Copolymerization of Styrene and Divinylbenzene in
48
49 Miniemulsion" *Macromol. Chem. Phys.* **2009**, *210*, 140-149.
50
51
52 (132) Utama, R. H.; Drechsler, M.; Förster, S.; Zetterlund, P. B.; Stenzel, M. H. "Synthesis of
53
54 pH-Responsive Nanocapsules via Inverse Miniemulsion Periphery RAFT Polymerization and
55
56
57
58
59
60

- 1
2
3
4 Post-Polymerization Reaction" *ACS Macro Lett.* **2014**, *3*, 935-939.
- 5
6 (133) Utama, R. H.; Stenzel, M. H.; Zetterlund, P. B. "Inverse Miniemulsion Periphery RAFT
7
8 Polymerization: A Convenient Route to Hollow Polymeric Nanoparticles with an Aqueous
9
10 Core" *Macromolecules* **2013**, *46*, 2118-2127.
- 11
12 (134) Zhang, Y.; Zhu, S.; Yin, L.; Qian, F.; Tang, C.; Yin, C. "Preparation, characterization and
13
14 biocompatibility of poly(ethylene glycol)-poly(n-butyl cyanoacrylate) nanocapsules with oil
15
16 core via miniemulsion polymerization" *Eur. Polym. J.* **2008**, *44*, 1654-1661.
- 17
18 (135) Baier, G.; Friedemann, K.; Leuschner, E.-M.; Musyanovych, A.; Landfester, K. "pH Stability
19
20 of Poly(urethane/urea) Capsules Synthesized from Different Hydrophilic Monomers via
21
22 Interfacial Polyaddition in the Inverse Miniemulsion Process" *Macromol. Symp.* **2013**,
23
24 *331-332*, 71-80.
- 25
26 (136) Rosenbauer, E.-M.; Landfester, K.; Musyanovych, A. "Surface-Active Monomer as a
27
28 Stabilizer for Polyurea Nanocapsules Synthesized via Interfacial Polyaddition in Inverse
29
30 Miniemulsion" *Langmuir* **2009**, *25*, 12084-12091.
- 31
32 (137) van Zyl, A. J. P.; Bosch, R. F. P.; McLeary, J. B.; Sanderson, R. D.; Klumperman, B.
33
34 "Synthesis of styrene based liquid-filled polymeric nanocapsules by the use of
35
36 RAFT-mediated polymerization in miniemulsion" *Polymer* **2005**, *46*, 3607-3615.
- 37
38 (138) Lu, F.; Luo, Y.; Li, B. "A Facile Route to Synthesize Highly Uniform Nanocapsules: Use of
39
40 Amphiphilic Poly(acrylic acid)-block-polystyrene RAFT Agents to Interfacially Confine
41
42 Miniemulsion Polymerization" *Macromol. Rapid Commun.* **2007**, *28*, 868-874.
- 43
44 (139) Luo, Y.; Gu, H. "A General Strategy for Nano-Encapsulation via Interfacially Confined
45
46 Living/Controlled Radical Miniemulsion Polymerization" *Macromol. Rapid Commun.* **2006**,
47
48
49
50
51
52
53
54
55
56
57
58
59
60

- 1
2
3
4 27, 21-25.
5
6 (140) Luo, Y.; Gu, H. "Nanoencapsulation via interfacially confined reversible addition
7
8 fragmentation transfer (RAFT) miniemulsion polymerization" *Polymer* **2007**, *48*, 3262-3272.
9
10
11 (141) Li, W.; Matyjaszewski, K.; Albrecht, K.; Möller, M. "Reactive surfactants for polymeric
12
13 nanocapsules via interfacially confined miniemulsion ATRP" *Macromolecules* **2009**, *42*,
14
15 8228-8233.
16
17
18 (142) Huang, C.-Y.; Chen, C.-M.; Lee, Y.-D. "Synthesis of high loading and encapsulation efficient
19
20 paclitaxel-loaded poly(n-butyl cyanoacrylate) nanoparticles via miniemulsion" *Int. J. Pharm.*
21
22 **2007**, *338*, 267-275.
23
24
25
26 (143) Fresta, M.; Cavallaro, G.; Giammona, G.; Wehrli, E.; Puglisi, G. "Preparation and
27
28 characterization of polyethyl-2-cyanoacrylate nanocapsules containing antiepileptic drugs"
29
30 *Biomaterials* **1996**, *17*, 751-758.
31
32
33
34 (144) Aboubakar, M.; Puisieux, F.; Couvreur, P.; Vauthier, C. "Physico-chemical characterization of
35
36 insulin-loaded poly(isobutylcyanoacrylate) nanocapsules obtained by interfacial
37
38 polymerization" *Int. J. Pharm.* **1999**, *183*, 63-66.
39
40
41
42 (145) Baier, G.; Musyanovych, A.; Dass, M.; Theisinger, S.; Landfester, K. "Cross-Linked Starch
43
44 Capsules Containing dsDNA Prepared in Inverse Miniemulsion as "Nanoreactors" for
45
46 Polymerase Chain Reaction" *Biomacromolecules* **2010**, *11*, 960-968.
47
48
49 (146) Baier, G.; Baumann, D.; Siebert, J. M.; Musyanovych, A.; Mailänder, V.; Landfester, K.
50
51 "Suppressing Unspecific Cell Uptake for Targeted Delivery Using Hydroxyethyl Starch
52
53 Nanocapsules" *Biomacromolecules* **2012**, *13*, 2704-2715.
54
55
56 (147) Freichels, H.; Wagner, M.; Okwieka, P.; Meyer, R. G.; Mailänder, V.; Landfester, K.;
57
58
59
60

- 1
2
3
4 Musyanovych, A. "(Oligo)mannose functionalized hydroxyethyl starch nanocapsules: en route
5
6 to drug delivery systems with targeting properties" *J. Mater. Chem. B* **2013**, *1*, 4338-4348.
7
8
9 (148) Paiphansiri, U.; Dausend, J.; Musyanovych, A.; Mailänder, V.; Landfester, K. "Fluorescent
10
11 Polyurethane Nanocapsules Prepared via Inverse Miniemulsion: Surface Functionalization for
12
13 Use as Biocarriers" *Macromol. Biosci.* **2009**, *9*, 575-584.
14
15
16 (149) Paiphansiri, U.; Tangboriboonrat, P.; Landfester, K. "Antiseptic Nanocapsule Formation via
17
18 Controlling Polymer Deposition onto Water-in-Oil Miniemulsion Droplets" *Macromol. Symp.*
19
20 **2007**, *251*, 54-62.
21
22
23 (150) Paiphansiri, U.; Tangboriboonrat, P.; Landfester, K. "Polymeric Nanocapsules Containing an
24
25 Antiseptic Agent Obtained by Controlled Nanoprecipitation onto Water-in-Oil Miniemulsion
26
27 Droplets" *Macromol. Biosci.* **2006**, *6*, 33-40.
28
29
30 (151) Huang, X.; Li, M.; Mann, S. "Membrane-mediated cascade reactions by enzyme-polymer
31
32 proteinosomes" *Chem. Comm.* **2014**, *50*, 6278-6280.
33
34
35 (152) Huang, X.; Patil, A. J.; Li, M.; Mann, S. "Design and construction of higher-order structure
36
37 and function in proteinosome-based protocells" *J. Am. Chem. Soc.* **2014**, *136*, 9225-9234.
38
39
40 (153) Kumar, C. V.; Chaudhari, A. "Proteins immobilized at the galleries of layered alpha-zirconium
41
42 phosphate: Structure and activity studies" *J. Am. Chem. Soc.* **2000**, *122*, 830-837.
43
44
45 (154) Graff, A.; Winterhalter, M.; Meier, W. "Nanoreactors from Polymer-Stabilized Liposomes"
46
47 *Langmuir* **2001**, *17*, 919-923.
48
49
50 (155) Gunkel-Grabole, G.; Sigg, S.; Lomora, M.; Lorcher, S.; Palivan, C. G.; Meier, W. P.
51
52 "Polymeric 3D nano-architectures for transport and delivery of therapeutically relevant
53
54 biomacromolecules" *Biomater. Sci.* **2015**, *3*, 25-40.
55
56
57
58
59
60

- 1
2
3
4 (156) Kumar, M.; Habel, J. E. O.; Shen, Y. X.; Meier, W. P.; Walz, T. "High-Density Reconstitution
5
6 of Functional Water Channels into Vesicular and Planar Block Copolymer Membranes" *J. Am.*
7
8 *Chem. Soc.* **2012**, *134*, 18631-18637.
9
- 10
11 (157) Kumar, M.; Grzelakowski, M.; Zilles, J.; Clark, M.; Meier, W. "Highly permeable polymeric
12
13 membranes based on the incorporation of the functional water channel protein Aquaporin Z"
14
15 *Proc. Natl. Acad. Sci. U.S.A* **2007**, *104*, 20719-20724.
16
17
- 18
19 (158) Tanner, P.; Balasubramanian, V.; Palivan, C. G. "Aiding Nature's Organelles: Artificial
20
21 Peroxisomes Play Their Role" *Nano Lett.* **2013**, *13*, 2875-2883.
22
23
- 24 (159) Graff, A.; Sauer, M.; Van Gelder, P.; Meier, W. "Virus-assisted loading of polymer
25
26 nanocontainer" *Proc. Natl. Acad. Sci. U.S.A* **2002**, *99*, 5064-5068.
27
28
- 29 (160) Choi, H. J.; Montemagno, C. D. "Artificial organelle: ATP synthesis from cellular mimetic
30
31 polymersomes" *Nano Lett.* **2005**, *5*, 2538-2542.
32
33
- 34 (161) Zhang, X. Y.; Fu, W. Y.; Palivan, C. G.; Meier, W. "Natural channel protein inserts and
35
36 functions in a completely artificial, solid-supported bilayer membrane" *Scientific Reports*
37
38 **2013**, *3*, 7.
39
- 40
41 (162) Stoenescu, R.; Meier, W. "Vesicles with asymmetric membranes from amphiphilic ABC
42
43 triblock copolymers" *Chem. Comm.* **2002**, 3016-3017.
44
45
- 46 (163) Stoenescu, R.; Graff, A.; Meier, W. "Asymmetric ABC-triblock copolymer membranes induce
47
48 a directed insertion of membrane proteins" *Macromol. Biosci.* **2004**, *4*, 930-935.
49
50
- 51 (164) Ranquin, A.; Versees, W.; Meier, W.; Steyaert, J.; Van Gelder, P. "Therapeutic Nanoreactors:
52
53 Combining Chemistry and Biology in a Novel Triblock Copolymer Drug Delivery System"
54
55
56 *Nano Lett.* **2005**, *5*, 2220-2224.
57
58
59
60

- 1
2
3
4 (165) Choi, H. J.; Brooks, E.; Montemagno, C. D. "Synthesis and characterization of nanoscale
5
6 biomimetic polymer vesicles and polymer membranes for bioelectronic applications"
7
8
9 *Nanotechnology* **2005**, *16*, S143-S149.
- 10
11 (166) Tanner, P.; Onaca, O.; Balasubramanian, V.; Meier, W.; Palivan, C. G. "Enzymatic Cascade
12
13 Reactions inside Polymeric Nanocontainers: A Means to Combat Oxidative Stress" *Chem. Eur.*
14
15
16 *J.* **2011**, *17*, 4552-4560.
- 17
18 (167) Balasubramanian, V.; Onaca, O.; Ezhevskaya, M.; Van Doorslaer, S.; Sivasankaran, B.;
19
20 Palivan, C. G. "A surprising system: polymeric nanoreactors containing a mimic with
21
22 dual-enzyme activity" *Soft Matter* **2011**, *7*, 5595-5603.
- 23
24 (168) De Oliveira, H.; Thevenot, J.; Lecommandoux, S. "Smart polymersomes for therapy and
25
26 diagnosis: fast progress toward multifunctional biomimetic nanomedicines" *Wiley Interdiscip*
27
28
29
30
31 *Rev Nanomed Nanobiotechno* **2012**, *4*, 525-546.
- 32
33 (169) Pawar, P. V.; Gohil, S. V.; Jain, J. P.; Kumar, N. "Functionalized polymersomes for biomedical
34
35 applications" *Polym. Chem.* **2013**, *4*, 3160-3176.
- 36
37 (170) Canton, I.; Massignani, M.; Patikarnmonthon, N.; Chierico, L.; Robertson, J.; Renshaw, S. A.;
38
39 Warren, N. J.; Madsen, J. P.; Armes, S. P.; Lewis, A. L.; Battaglia, G. "Fully synthetic polymer
40
41 vesicles for intracellular delivery of antibodies in live cells" *Faseb J.* **2013**, *27*, 98-108.
- 42
43 (171) Akinc, A.; Battaglia, G. "Exploiting Endocytosis for Nanomedicines" *Cold Spring Harb*
44
45
46
47 *Perspect Biol.* **2013**, *5*, DOI: doi:10.1101/cshperspect.a016980.
- 48
49 (172) Lomas, H.; Massignani, M.; Abdullah, K. A.; Canton, I.; Lo Presti, C.; MacNeil, S.; Du, J. Z.;
50
51
52
53
54
55
56
57
58
59
60
Blanz, A.; Madsen, J.; Armes, S. P.; Lewis, A. L.; Battaglia, G. "Non-cytotoxic polymer
vesicles for rapid and efficient intracellular delivery" *Faraday Discuss* **2008**, *139*, 143-159.

- 1
2
3
4 (173) Itel, F.; Al-Samir, S.; Oberg, F.; Chami, M.; Kumar, M.; Supuran, C. T.; Deen, P. M. T.; Meier,
5
6 W.; Hedfalk, K.; Gros, G.; Endeward, V. "CO₂ permeability of cell membranes is regulated by
7
8 membrane cholesterol and protein gas channels" *Faseb J.* **2012**, *26*, 5182-5191.
9
10
11 (174) Yan, Q.; Wang, J.; Yin, Y.; Yuan, J. "Breathing Polymersomes: CO₂ - Tuning Membrane
12
13 Permeability for Size - Selective Release, Separation, and Reaction" *Angew. Chem. Int. Ed.*
14
15 **2013**, *52*, 5070-5073.
16
17
18 (175) Nuyken, O.; Pask, S. D. "Ring-Opening Polymerization-An Introductory Review" *Polymers*
19
20 **2013**, *5*, 361-403.
21
22
23 (176) Lee, E. S.; Shin, H. J.; Na, K.; Bae, Y. H. "Poly(L-histidine)-PEG block copolymer micelles
24
25 and pH-induced destabilization" *J Control Release* **2003**, *90*, 363-374.
26
27
28 (177) Mavrogiorgis, D.; Bilalis, P.; Karatzas, A.; Skoulas, D.; Fotinogiannopoulou, G.; Iatrou, H.
29
30 "Controlled polymerization of histidine and synthesis of well-defined stimuli responsive
31
32 polymers. Elucidation of the structure-aggregation relationship of this highly multifunctional
33
34 material" *Polym. Chem.* **2014**, *5*, 6256-6278.
35
36
37 (178) Li, Z.; Qiu, L. P.; Chen, Q.; Hao, T. N.; Qiao, M. X.; Zhao, H. X.; Zhang, J.; Hu, H. Y.; Zhao,
38
39 X. L.; Chen, D. W.; Mei, L. "pH-sensitive nanoparticles of
40
41 poly(L-histidine)-poly(lactide-co-glycolide)-tocopheryl polyethylene glycol succinate for
42
43 anti-tumor drug delivery" *Acta Biomater* **2015**, *11*, 137-150.
44
45
46 (179) LoPresti, C.; Lomas, H.; Massignani, M.; Smart, T.; Battaglia, G. "Polymersomes: nature
47
48 inspired nanometer sized compartments" *J Mater Chem* **2009**, *19*, 3576-3590.
49
50
51 (180) Giacomelli, C.; Le Men, L.; Borsali, R.; Lai-Kee-Him, J.; Brisson, A.; Armes, S. P.; Lewis, A.
52
53
54 L. "Phosphorylcholine-based pH-responsive diblock copolymer micelles as drug delivery
55
56
57
58
59
60

- 1
2
3
4 vehicles: Light scattering, electron microscopy, and fluorescence experiments"
5
6 *Biomacromolecules* **2006**, *7*, 817-828.
7
8
9 (181) Lomas, H.; Du, J. Z.; Canton, I.; Madsen, J.; Warren, N.; Armes, S. P.; Lewis, A. L.; Battaglia,
10
11 G. "Efficient Encapsulation of Plasmid DNA in pH-Sensitive PMPC-PDPA Polymersomes:
12
13 Study of the Effect of PDPA Block Length on Copolymer-DNA Binding Affinity" *Macromol.*
14
15 *Biosci.* **2010**, *10*, 513-530.
16
17
18 (182) Canton, I.; Battaglia, G. "Polymersomes-mediated delivery of fluorescent probes for targeted
19
20 and long-term imaging in live cell microscopy" *Methods Mol Biol* **2013**, *991*, 343-351.
21
22
23 (183) Colley, H. E.; Hearnden, V.; Avila-Olias, M.; Cecchin, D.; Canton, I.; Madsen, J.; MacNeil, S.;
24
25 Warren, N.; Hu, K.; McKeating, J. A.; Armes, S. P.; Murdoch, C.; Thornhill, M. H.; Battaglia,
26
27 G. "Polymersome-Mediated Delivery of Combination Anticancer Therapy to Head and Neck
28
29 Cancer Cells: 2D and 3D in Vitro Evaluation" *Mol Pharmaceut* **2014**, *11*, 1176-1188.
30
31
32 (184) Yu, S. Y.; Azzam, T.; Rouiller, I.; Eisenberg, A. "'Breathing" Vesicles" *J. Am. Chem. Soc.*
33
34 **2009**, *131*, 10557-10566.
35
36
37 (185) Wang, X. Y.; Sun, H. L.; Meng, F. H.; Cheng, R.; Deng, C.; Zhong, Z. Y.
38
39 "Galactose-Decorated Reduction-Sensitive Degradable Chimaeric Polymersomes as a
40
41 Multifunctional Nanocarrier To Efficiently Chaperone Apoptotic Proteins into Hepatoma
42
43 Cells" *Biomacromolecules* **2013**, *14*, 2873-2882.
44
45
46 (186) Gaitzsch, J.; Appelhans, D.; Wang, L.; Battaglia, G.; Voit, B. "Synthetic Bio - nanoreactor:
47
48 Mechanical and Chemical Control of Polymersome Membrane Permeability" *Angew. Chem.*
49
50 *Int. Ed.* **2012**, *51*, 4448-4451.
51
52
53 (187) Grafe, D.; Gaitzsch, J.; Appelhans, D.; Voit, B. "Cross-linked polymersomes as nanoreactors
54
55
56
57
58
59
60

- 1
2
3
4 for controlled and stabilized single and cascade enzymatic reactions" *Nanoscale* **2014**, *6*,
5
6 10752-10761.
7
8
9 (188) Hong, L. Z.; Zhang, Z. H.; Zhang, Y.; Zhang, W. A. "Synthesis and Self-Assembly of
10
11 Stimuli-Responsive Amphiphilic Block Copolymers Based on Polyhedral Oligomeric
12
13 Silsesquioxane" *J. Polym. Sci. A Polym. Chem* **2014**, *52*, 2669-2683.
14
15
16 (189) Yassin, M. A.; Appelhans, D.; Wiedemuth, R.; Formanek, P.; Boye, S.; Lederer, A.; Temme, A.;
17
18 Voit, B. "Overcoming Concealment Effects of Targeting Moieties in the PEG Corona:
19
20 Controlled Permeable Polymersomes Decorated with Folate-Antennae for Selective Targeting
21
22 of Tumor Cells" *Small* **2015**, *11*, 1580-1591.
23
24
25
26 (190) Liu, H. B.; Guo, Z. R.; He, S.; Yin, H. Y.; Fei, C. H.; Feng, Y. J. "CO₂-driven vesicle to
27
28 micelle regulation of amphiphilic copolymer: random versus block strategy" *Polym. Chem.*
29
30 **2014**, *5*, 4756-4763.
31
32
33
34 (191) Lin, F.; Wu, J. L.; Liu, J. G. "The study of far UV irradiation of 2-methoxycytosine in
35
36 phosphate solution and its novel photoproduct" *J. Photochem. Photobiol. A-Chem.* **2000**, *131*,
37
38 49-53.
39
40
41 (192) Amos, R. C.; Nazemi, A.; Bonduelle, C. V.; Gillies, E. R. "Tuning polymersome surfaces:
42
43 functionalization with dendritic groups" *Soft Matter* **2012**, *8*, 5947-5958.
44
45
46 (193) Tai, W. Y.; Mo, R.; Di, J.; Subramanian, V.; Gu, X.; Buse, J. B.; Gu, Z. "Bio-Inspired Synthetic
47
48 Nanovesicles for Glucose-Responsive Release of Insulin" *Biomacromolecules* **2014**, *15*,
49
50 3495-3502.
51
52
53
54 (194) Chen, X. R.; Ding, X. B.; Zheng, Z. H.; Peng, Y. X. "Thermosensitive cross-linked polymer
55
56 vesicles for controlled release system" *New Journal of Chemistry* **2006**, *30*, 577-582.
57
58
59
60

- 1
2
3
4 (195) Miao, K.; Liu, H. H.; Zhao, Y. L. "Thermo, pH and reduction responsive coaggregates
5
6 comprising AB(2)C(2) star terpolymers for multi-triggered release of doxorubicin" *Polym.*
7
8 *Chem.* **2014**, *5*, 3335-3345.
- 9
10
11 (196) Hocine, S.; Cui, D.; Rager, M. N.; Di Cicco, A.; Liu, J. M.; Wdzieczak-Bakala, J.; Brulet, A.;
12
13 Li, M. H. "Polymersomes with PEG Corona: Structural Changes and Controlled Release
14
15 Induced by Temperature Variation" *Langmuir* **2013**, *29*, 1356-1369.
- 16
17
18 (197) Lu, C. L.; Urban, M. W. "Tri-Phasic Size- and Janus Balance-Tunable Colloidal Nanoparticles
19
20 (JNPs)" *Acs Macro Lett.* **2014**, *3*, 346-352.
- 21
22
23 (198) Liang, X.; Kozlovskaya, V.; Cox, C. P.; Wang, Y.; Saeed, M.; Kharlampieva, E. "Synthesis and
24
25 Self-Assembly of Thermosensitive Double-Hydrophilic
26
27 Poly(N-vinylcaprolactam)-b-poly(N-vinyl-2-pyrrolidone) Diblock Copolymers" *J. Polym. Sci.*
28
29 *A Polym. Chem* **2014**, *52*, 2725-2737.
- 30
31
32 (199) Qiao, Z. Y.; Ji, R.; Huang, X. N.; Du, F. S.; Zhang, R.; Liang, D. H.; Li, Z. C. "Polymersomes
33
34 from Dual Responsive Block Copolymers: Drug Encapsulation by Heating and
35
36 Acid-Triggered Release" *Biomacromolecules* **2013**, *14*, 1555-1563.
- 37
38
39 (200) Hsu, S. P.; Chu, I. M.; Yang, J. D. "Thermo- and pH-Responsive Polymersomes of
40
41 Poly(alpha,beta-N-substituted-DL-aspartamide)s" *J. Appl. Polym. Sci.* **2012**, *125*, 133-144.
- 42
43
44 (201) Lai, M. H.; Jeong, J. H.; DeVolder, R. J.; Brockman, C.; Schroeder, C.; Kong, H. "Ellipsoidal
45
46 Polyaspartamide Polymersomes with Enhanced Cell-Targeting Ability" *Adv. Funct. Mater.*
47
48 **2012**, *22*, 3239-3246.
- 49
50
51 (202) Zhuang, Z. L.; Cai, C. H.; Jiang, T.; Lin, J. P.; Yang, C. Y. "Self-assembly behavior of
52
53 rod-coil-rod polypeptide block copolymers" *Polymer* **2014**, *55*, 602-610.
- 54
55
56
57
58
59
60

- 1
2
3
4 (203) Iatrou, H.; Dimas, K.; Gkikas, M.; Tsimblouli, C.; Sofianopoulou, S. "Polymersomes from
5
6 Polypeptide Containing Triblock Co- and Terpolymers for Drug Delivery against Pancreatic
7
8 Cancer: Asymmetry of the External Hydrophilic Blocks" *Macromol. Biosci.* **2014**, *14*,
9
10 1222-1238.
11
12
13 (204) Hu, H.; Liu, G. J. "Miktoarm Star Copolymer Capsules Bearing pH-Responsive
14
15 Nanochannels" *Macromolecules* **2014**, *47*, 5096-5103.
16
17
18 (205) Gaitzsch, J.; Canton, I.; Appelhans, D.; Battaglia, G.; Voit, B. "Cellular Interactions with
19
20 Photo-Cross-Linked and pH-Sensitive Polymersomes: Biocompatibility and Uptake Studies"
21
22 *Biomacromolecules* **2012**, *13*, 4188-4195.
23
24
25 (206) Gaitzsch, J.; Appelhans, D.; Janke, A.; Stempel, M.; Schwille, P.; Voit, B. "Cross-linked and
26
27 pH sensitive supported polymer bilayers from polymersomes - studies concerning thickness,
28
29 rigidity and fluidity" *Soft Matter* **2014**, *10*, 66-73.
30
31
32
33 (207) Gupta, S.; Kuckling, D.; Kretschmer, K.; Choudhary, V.; Adler, H. J. "Synthesis and
34
35 characterization of stimuli-sensitive micro- and nanohydrogels based on photocrosslinkable
36
37 poly(dimethylaminoethyl methacrylate)" *J. Polym. Sci. A Polym. Chem* **2007**, *45*, 669-679.
38
39
40 (208) Hoffmann, R.; Woodward, R. B. "SELECTION RULES FOR CONCERTED
41
42 CYCLOADDITION REACTIONS" *J. Am. Chem. Soc.* **1965**, *87*, 2046-&.
43
44
45 (209) Du, J. Z.; Armes, S. P. "pH-responsive vesicles based on a hydrolytically self-cross-linkable
46
47 copolymer" *J. Am. Chem. Soc.* **2005**, *127*, 12800-12801.
48
49
50 (210) Chambon, P.; Blanazs, A.; Battaglia, G.; Armes, S. P. "How Does Cross-Linking Affect the
51
52 Stability of Block Copolymer Vesicles in the Presence of Surfactant?" *Langmuir* **2012**, *28*,
53
54 1196-1205.
55
56
57
58
59
60

- 1
2
3
4 (211) Zhao, Q.; Wang, Y.; Yan, Y.; Huang, J. B. "Smart Nanocarrier: Self-Assembly of Bacteria-like
5
6 Vesicles with Photoswitchable Cilia" *ACS Nano* **2014**, *8*, 11341-11349.
7
8
9 (212) Mazzier, D.; Maran, M.; Perucchin, O. P.; Crisma, M.; Zerbetto, M.; Causin, V.; Toniolo, C.;
10
11 Moretto, A. "Photoresponsive Supramolecular Architectures Based on Polypeptide Hybrids"
12
13 *Macromolecules* **2014**, *47*, 7272-7283.
14
15
16 (213) Blasco, E.; Schmidt, B. V. K. J.; Barner-Kowollik, C.; Pinol, M.; Oriol, L. "A Novel
17
18 Photoresponsive Azobenzene-Containing Miktoarm Star Polymer: Self-Assembly and
19
20 Photoresponse Properties" *Macromolecules* **2014**, *47*, 3693-3700.
21
22
23 (214) Blasco, E.; del Barrio, J.; Sanchez-Somolinos, C.; Pinol, M.; Oriol, L. "Light induced
24
25 molecular release from vesicles based on amphiphilic linear-dendritic block copolymers"
26
27 *Polym. Chem.* **2013**, *4*, 2246-2254.
28
29
30 (215) Xia, D. Y.; Yu, G. C.; Li, J. Y.; Huang, F. H. "Photo-responsive self-assembly based on a
31
32 water-soluble pillar[6]arene and an azobenzene-containing amphiphile in water" *Chem. Comm.*
33
34 **2014**, *50*, 3606-3608.
35
36
37 (216) Cabane, E.; Malinova, V.; Meier, W. "Synthesis of Photocleavable Amphiphilic Block
38
39 Copolymers: Toward the Design of Photosensitive Nanocarriers" *Macromol. Chem. Phys.*
40
41 **2010**, *211*, 1847-1856.
42
43
44 (217) Liu, G.; Zhou, L. Z.; Guan, Y. F.; Su, Y.; Dong, C. M. "Multi-Responsive Polypeptidosome:
45
46 Characterization, Morphology Transformation, and Triggered Drug Delivery" *Macromol.*
47
48 *Rapid Commun.* **2014**, *35*, 1673-1678.
49
50
51 (218) Cerritelli, S.; Velluto, D.; Hubbell, J. A. "PEG-SS-PPS: Reduction-Sensitive Disulfide Block
52
53 Copolymer Vesicles for Intracellular Drug Delivery" *Biomacromolecules* **2007**, *8*, 1966-1972.
54
55
56
57
58
59
60

- 1
2
3
4 (219) Jia, L.; Cui, D.; Bignon, J.; Di Cicco, A.; Wdzieczak-Bakala, J.; Liu, J. M.; Li, M. H.
5
6 "Reduction-Responsive Cholesterol-Based Block Copolymer Vesicles for Drug Delivery"
7
8 *Biomacromolecules* **2014**, *15*, 2206-2217.
9
10
11 (220) Napoli, A.; Valentini, M.; Tirelli, N.; Mueller, M.; Hubbell, J. A. "Oxidation-responsive
12
13 polymeric vesicles" *Nat Mater* **2004**, *3*, 183-189.
14
15
16 (221) Atzet, S.; Curtin, S.; Trinh, P.; Bryant, S.; Ratner, B. "Degradable Poly(2-hydroxyethyl
17
18 methacrylate)-co-polycaprolactone Hydrogels for Tissue Engineering Scaffolds"
19
20 *Biomacromolecules* **2008**, *9*, 3370-3377.
21
22
23 (222) Lee, J. S.; Feijen, J. "Biodegradable polymersomes as carriers and release systems for
24
25 paclitaxel using Oregon Green (R) 488 labeled paclitaxel as a model compound" *J Control*
26
27 *Release* **2012**, *158*, 312-318.
28
29
30
31 (223) Tamate, R.; Ueki, T.; Yoshida, R. "Self-Beating Artificial Cells: Design of Cross-Linked
32
33 Polymersomes Showing Self-Oscillating Motion" *Adv. Mater.* **2015**, *27*, 837-842.
34
35
36 (224) Mabire, A. B.; Robin, M. P.; Willcock, H.; Pitto-Barry, A.; Kirby, N.; O'Reilly, R. K. "Dual
37
38 effect of thiol addition on fluorescent polymeric micelles: ON-to-OFF emissive switch and
39
40 morphology transition" *Chem. Comm.* **2014**, *50*, 11492-11495.
41
42
43 (225) Azzam, T.; Eisenberg, A. "Fully Collapsed (Kippah) Vesicles: Preparation and
44
45 Characterization" *Langmuir* **2010**, *26*, 10513-10523.
46
47
48 (226) Kao, K.-C.; Tsou, C.-J.; Mou, C.-Y. "Collapsed (kippah) hollow silica nanoparticles" *Chem.*
49
50 *Comm.* **2012**, *48*, 3454-3456.
51
52
53 (227) Chen, T.; Du, B.; Fan, Z. "Facile Fabrication of Polymer Nanocapsules with Cross-Linked
54
55 Organic-Inorganic Hybrid Walls" *Langmuir* **2012**, *28*, 11225-11231.
56
57
58
59
60

- 1
2
3
4 (228) Kim, K. T.; Zhu, J. H.; Meeuwissen, S. A.; Cornelissen, J. J. L. M.; Pochan, D. J.; Nolte, R. J.
5
6 M.; van Hest, J. C. M. "Polymersome Stomatocytes: Controlled Shape Transformation in
7
8 Polymer Vesicles" *J. Am. Chem. Soc.* **2010**, *132*, 12522-12524.
9
10
11 (229) Rikken, R. S. M.; Kerkenaar, H. H. M.; Nolte, R. J. M.; Maan, J. C.; van Hest, J. C. M.;
12
13 Christianen, P. C. M.; Wilson, D. A. "Probing morphological changes in polymersomes with
14
15 magnetic birefringence" *Chem. Comm.* **2014**, *50*, 5394-5396.
16
17
18 (230) Rikken, R. S. M.; Nolte, R. J. M.; Maan, J. C.; van Hest, J. C. M.; Wilson, D. A.; Christianen,
19
20 P. C. M. "Manipulation of micro- and nanostructure motion with magnetic fields" *Soft Matter*
21
22 **2014**, *10*, 1295-1308.
23
24
25 (231) Wilson, D. A.; Nolte, R. J. M.; van Hest, J. C. M. "Autonomous movement of platinum-loaded
26
27 stomatocytes" *Nature Chemistry* **2012**, *4*, 268-274.
28
29
30 (232) Wilson, D. A.; Nolte, R. J. M.; van Hest, J. C. M. "Entrapment of Metal Nanoparticles in
31
32 Polymer Stomatocytes" *J Am Chem Soc* **2012**, *134*, 9894-9897.
33
34
35 (233) Wilson, D. A.; de Nijs, B.; van Blaaderen, A.; Nolte, R. J. M.; van Hest, J. C. M. "Fuel
36
37 concentration dependent movement of supramolecular catalytic nanomotors" *Nanoscale* **2013**,
38
39 *5*, 1315-1318.
40
41
42 (234) Meeuwissen, S. A.; Bruekers, S. M. C.; Chen, Y. C.; Pochan, D. J.; van Hest, J. C. M.
43
44 "Spontaneous shape changes in polymersomes via polymer/polymer segregation" *Polym.*
45
46 *Chem.* **2014**, *5*, 489-501.
47
48
49 (235) LoPresti, C.; Massignani, M.; Fernyhough, C.; Blanazs, A.; Ryan, A. J.; Madsen, J.; Warren, N.
50
51
52 J.; Armes, S. P.; Lewis, A. L.; Chirasatitsin, S.; Engler, A. J.; Battaglia, G. "Controlling
53
54 Polymersome Surface Topology at the Nanoscale by Membrane Confined Polymer/Polymer
55
56
57
58
59
60

- 1
2
3
4 Phase Separation" *ACS Nano* **2011**, *5*, 1775-1784.
- 5
6 (236) Massignani, M.; LoPresti, C.; Blanazs, A.; Madsen, J.; Armes, S. P.; Lewis, A. L.; Battaglia, G.
7
8 "Controlling Cellular Uptake by Surface Chemistry, Size, and Surface Topology at the
9
10 Nanoscale" *Small* **2009**, *5*, 2424-2432.
- 11
12 (237) Ruiz-Perez, L.; Madsen, J.; Themistou, E.; Gaitzsch, J.; Armes, S. P.; Battaglia, G. "Nanoscale
13
14 detection of metal-labeled copolymers in patchy polymersomes" *Poly. Chem.* **2015**, *6*,
15
16 2065-2068.
- 17
18 (238) Liu, Q. M.; Chen, S.; Chen, J.; Du, J. Z. "An Asymmetrical Polymer Vesicle Strategy for
19
20 Significantly Improving T-1 MRI Sensitivity and Cancer-Targeted Drug Delivery"
21
22 *Macromolecules* **2015**, *48*, 739-749.
- 23
24 (239) Xu, W.; Ledin, P. A.; Plamper, F. A.; Synatschke, C. V.; Muller, A. H. E.; Tsukruk, V. V.
25
26 "Multiresponsive Microcapsules Based on Multilayer Assembly of Star Polyelectrolytes"
27
28 *Macromolecules* **2014**, *47*, 7858-7868.
- 29
30 (240) Städler, B.; Price, A. D.; Chandrawati, R.; Hosta-Rigau, L.; Zelikin, A. N.; Caruso, F.
31
32 "Polymer hydrogel capsules: en route toward synthetic cellular systems" *Nanoscale* **2009**, *1*,
33
34 68-73.
- 35
36 (241) Chandrawati, R.; Chong, S. F.; Zelikin, A. N.; Hosta-Rigau, L.; Städler, B.; Caruso, F.
37
38 "Degradation of liposomal subcompartments in PEGylated capsosomes" *Soft Matter* **2011**, *7*,
39
40 9638-9646.
- 41
42 (242) Hosta-Rigau, L.; Chung, S. F.; Postma, A.; Chandrawati, R.; Städler, B.; Caruso, F.
43
44 "Capsosomes with "Free - Floating" Liposomal Subcompartments" *Adv. Mater.* **2011**, *23*,
45
46 4082-4087.
- 47
48
49
50
51
52
53
54
55
56
57
58
59
60

- 1
2
3
4 (243) Chandrawati, R.; van Koeverden, M. P.; Lomas, H.; Caruso, F. "Multicompartment Particle
5
6 Assemblies for Bioinspired Encapsulated Reactions" *J. Phys. Chem. Lett.* **2011**, *2*, 2639-2649.
7
8
9 (244) Chandrawati, R.; Odermatt, P. D.; Chong, S. F.; Price, A. D.; Städler, B.; Caruso, F. "Triggered
10
11 Cargo Release by Encapsulated Enzymatic Catalysis in Capsosomes" *Nano Lett.* **2011**, *11*,
12
13 4958-4963.
14
15
16 (245) Ringsdorf, H. **3rd EURESCO Conference on Supramolecular Chemistry: Molecular**
17
18 **Recognition and Drug-Receptor Interactions, Salamanca (Spain), Aug 29 - Sept 3, 1996.**
19
20
21 (246) Li, Z.; Kesselman, E.; Talmon, Y.; Hillmyer, M. A.; Lodge, T. P. "Multicompartment micelles
22
23 from ABC miktoarm stars in water" *Science* **2004**, *306*, 98.
24
25
26 (247) Gröschel, A. H.; Walther, A.; Löbbling, T. I.; Schmelz, J.; Hanisch, A.; Schmalz, H.; Müller, A.
27
28 H. E. "Facile, Solution-Based Synthesis of Soft, Nanoscale Janus Particles with Tunable Janus
29
30 Balance" *J. Am. Chem. Soc.* **2012**, *134*, 13850-13860.
31
32
33 (248) Lutz, J. F.; Laschewsky, A. "Multicompartment Micelles: Has the Long - Standing Dream
34
35 Become a Reality?" *Macromol. Chem. Phys.* **2005**, *206*, 813-817.
36
37
38 (249) Paleos, C. M.; Tsiourvas, D.; Sideratou, Z.; Pantos, A. "Formation of artificial
39
40 multicompartment vesosome and dendrosome as prospected drug and gene delivery carriers" *J*
41
42 *Control Release* **2013**, *170*, 141-152.
43
44
45 (250) Marguet, M.; Sandre, O.; Lecommandoux, S. "Polymersomes in "Gelly" Polymersomes:
46
47 Toward Structural Cell Mimicry" *Langmuir* **2012**, *28*, 2035-2043.
48
49
50 (251) Zhang, S.; Moussodia, R.-O.; Vertesy, S.; Andre, S.; Klein, M. L.; Gabius, H.-J.; Percec, V.
51
52 "Unraveling functional significance of natural variations in structural aspects of a human
53
54 galectin by glycodendrimersomes with programmable glycan surface" *Proc. Natl. Acad. Sci.*
55
56
57
58
59
60

- 1
2
3
4 *U.S.A* **2015**, *112*, 5585-5590.
- 5
6 (252) Zhang, S.; Moussodia, R.-O.; Murzeau, C.; Sun, H.-J.; Klein, M. L.; Vertesy, S.; Andre, S.;
7
8 Roy, R.; Gabius, H.-J.; Percec, V. "Dissecting Molecular Aspects of Cell Interactions Using
9
10 Glycodendrimersomes with Programmable Glycan Presentation and Engineered Human
11
12 Lectins" *Angew. Chem. Int. Ed.* **2015**, *54*, 4036-4040.
- 13
14
15
16 (253) Zhang, S.; Sun, H.-J.; Hughes, A. D.; Moussodia, R.-O.; Bertin, A.; Chen, Y.; Pochan, D. J.;
17
18 Heiney, P. A.; Klein, M. L.; Percec, V. "Self-assembly of amphiphilic Janus dendrimers into
19
20 uniform onion-like dendrimersomes with predictable size and number of bilayers" *Proc. Natl.*
21
22 *Acad. Sci. U.S.A* **2014**, *111*, 9058-9063.
- 23
24
25
26 (254) Zhang, S.; Sun, H.-J.; Hughes, A. D.; Draghici, B.; Lejnieks, J.; Leowanawat, P.; Bertin, A.;
27
28 De Leon, L. O.; Kulikov, O. V.; Chen, Y.; Pochan, D. J.; Heiney, P. A.; Percec, V.
29
30 ""Single-Single" Amphiphilic Janus Dendrimers Self-Assemble into Uniform
31
32 Dendrimersomes with Predictable Size" *ACS Nano* **2014**, *8*, 1554-1565.
- 33
34
35
36 (255) Zhang, S.; Moussodia, R.-O.; Sun, H.-J.; Leowanawat, P.; Muncan, A.; Nusbaum, C. D.;
37
38 Chelling, K. M.; Heiney, P. A.; Klein, M. L.; Andre, S.; Roy, R.; Gabius, H.-J.; Percec, V.
39
40 "Mimicking Biological Membranes with Programmable Glycan Ligands Self-Assembled from
41
42 Amphiphilic Janus Glycodendrimers" *Angew. Chem. Int. Ed.* **2014**, *53*, 10899-10903.
- 43
44
45
46 (256) Percec, V.; Leowanawat, P.; Sun, H.-J.; Kulikov, O.; Nusbaum, C. D.; Tran, T. M.; Bertin, A.;
47
48 Wilson, D. A.; Peterca, M.; Zhang, S.; Kamat, N. P.; Vargo, K.; Mook, D.; Johnston, E. D.;
49
50 Hammer, D. A.; Pochan, D. J.; Chen, Y.; Chabre, Y. M.; Shiao, T. C.; Bergeron-Brelek, M.;
51
52 Andre, S.; Roy, R.; Gabius, H.-J.; Heiney, P. A. "Modular Synthesis of Amphiphilic Janus
53
54 Glycodendrimers and Their Self-Assembly into Glycodendrimersomes and Other Complex
55
56
57
58
59
60

- 1
2
3 Architectures with Bioactivity to Biomedically Relevant Lectins" *J. Am. Chem. Soc.* **2013**,
4
5
6 *135*, 9055-9077.
7
- 8
9 (257) Peterca, M.; Percec, V.; Leowanawat, P.; Bertin, A. "Predicting the Size and Properties of
10
11 Dendrimersomes from the Lamellar Structure of Their Amphiphilic Janus Dendrimers" *J. Am.*
12
13 *Chem. Soc.* **2011**, *133*, 20507-20520.
14
- 15
16 (258) Rosen, B. M.; Wilson, C. J.; Wilson, D. A.; Peterca, M.; Imam, M. R.; Percec, V.
17
18 "Dendron-mediated self-assembly, disassembly, and self-organization of complex systems"
19
20 *Chem. Rev.* **2009**, *109*, 6275-6540.
21
- 22
23 (259) Städler, B.; Chandrawati, R.; Price, A. D.; Chong, S. F.; Breheney, K.; Postma, A.; Connal, L.
24
25 A.; Zelikin, A. N.; Caruso, F. "A Microreactor with Thousands of Subcompartments: Enzyme
26
27 - Loaded Liposomes within Polymer Capsules" *Angew. Chem. Int. Ed.* **2009**, *48*, 4859-4862.
28
- 29
30 (260) Städler, B.; Chandrawati, R.; Goldie, K.; Caruso, F. "Capsosomes: subcompartmentalizing
31
32 polyelectrolyte capsules using liposomes" *Langmuir* **2009**, *25*, 6725-6732.
33
- 34
35 (261) Fu, Z.; Ochsner, M. A.; De Hoog, H. P. M.; Tomczak, N.; Nallani, M.
36
37 "Multicompartmentalized polymersomes for selective encapsulation of biomacromolecules"
38
39 *Chem. Comm.* **2011**, *47*, 2862-2864.
40
- 41
42 (262) Li, G.; Lei, C.; Wang, C.; Neoh, K.; Kang, E.; Yang, X. "Narrowly dispersed double-walled
43
44 concentric hollow polymeric microspheres with independent pH and temperature sensitivity"
45
46 *Macromolecules* **2008**, *41*, 9487-9490.
47
- 48
49 (263) Chiu, H.-C.; Lin, Y.-W.; Huang, Y.-F.; Chuang, C.-K.; Chern, C.-S. "Polymer Vesicles
50
51 Containing Small Vesicles within Interior Aqueous Compartments and pH-Responsive
52
53 Transmembrane Channels" *Angew. Chem. Int. Ed.* **2008**, *47*, 1875-1878.
54
55
56
57
58
59
60

- 1
2
3
4 (264) Thompson, K. L.; Chambon, P.; Verber, R.; Armes, S. P. "Can Polymersomes Form
5
6 Colloidosomes?" *J. Am. Chem. Soc.* **2012**, *134*, 12450-12453.
7
8
9 (265) Shum, H. C.; Zhao, Y.; Kim, S. H.; Weitz, D. A. "Multicompartment polymersomes from
10
11 double emulsions" *Angew. Chem. Int. Ed.* **2011**, *50*, 1648-1651.
12
13
14 (266) Nisisako, T.; Okushima, S.; Torii, T. "Controlled formulation of monodisperse double
15
16 emulsions in a multiple-phase microfluidic system" *Soft Matter* **2005**, *1*, 23-27.
17
18
19 (267) Sanson, C.; Schatz, C.; Le Meins, J.-F. o.; Brûlet, A.; Soum, A.; Lecommandoux, S. b.
20
21 "Biocompatible and Biodegradable Poly(trimethylene carbonate)-b-Poly(l-glutamic acid)
22
23 Polymersomes: Size Control and Stability" *Langmuir* **2009**, *26*, 2751-2760.
24
25
26 (268) Mabrouk, E.; Cuvelier, D.; Pontani, L.-L.; Xu, B.; Levy, D.; Keller, P.; Brochard-Wyart, F.;
27
28 Nassoy, P.; Li, M.-H. "Formation and material properties of giant liquid crystal
29
30 polymersomes" *Soft Matter* **2009**, *5*, 1870-1878.
31
32
33 (269) Siti, W.; de Hoog, H.-P. M.; Fischer, O.; Shan, W. Y.; Tomczak, N.; Nallani, M.; Liedberg, B.
34
35 "An intercompartmental enzymatic cascade reaction in channel-equipped
36
37 polymersome-in-polymersome architectures" *J. Mater. Chem. B* **2014**, *2*, 2733-2737.
38
39
40 (270) Hosta-Rigau, L.; Städler, B.; Yan, Y.; Nice, E. C.; Heath, J. K.; Albericio, F.; Caruso, F.
41
42 "Capsosomes with multilayered subcompartments: assembly and loading with hydrophobic
43
44 cargo" *Adv. Funct. Mater.* **2010**, *20*, 59-66.
45
46
47 (271) Hosta-Rigau, L.; Chandrawati, R.; Saveriades, E.; Odermatt, P. D.; Postma, A.; Ercole, F.;
48
49 Breheney, K.; Wark, K. L.; Städler, B.; Caruso, F. "Noncovalent Liposome Linkage and
50
51 Miniaturization of Capsosomes for Drug Delivery" *Biomacromolecules* **2010**, *11* 3548-3555.
52
53
54 (272) Chandrawati, R.; Städler, B.; Postma, A.; Connal, L. A.; Chong, S. F.; Zelikin, A. N.; Caruso,
55
56
57
58
59
60

- 1
2
3
4 F. "Cholesterol-mediated anchoring of enzyme-loaded liposomes within disulfide-stabilized
5
6 polymer carrier capsules" *Biomaterials* **2009**, *30*, 5988-5998.
7
8
9 (273) Zelikin, A. N.; Quinn, J. F.; Caruso, F. "Disulfide cross-linked polymer capsules: en route to
10
11 biodeconstructible systems" *Biomacromolecules* **2006**, *7*, 27-30.
12
13
14 (274) Lomas, H.; Johnston, A. P. R.; Such, G. K.; Zhu, Z.; Liang, K.; van Koeverden, M. P.;
15
16 Alongkornchotikul, S.; Caruso, F. "Polymersome-Loaded Capsules for Controlled Release of
17
18 DNA" *Small* **2011**, *7*, 2109-2119.
19
20
21 (275) Lee, M. H.; Hribar, K. C.; Brugarolas, T.; Kamat, N. P.; Burdick, J. A.; Lee, D. "Harnessing
22
23 Interfacial Phenomena to Program the Release Properties of Hollow Microcapsules" *Adv.*
24
25 *Funct. Mater.* **2012**, *22*, 131-138.
26
27
28 (276) Li, G. L.; Tai, C. A.; Neoh, K.; Kang, E.; Yang, X. "Hybrid nanorattles of metal core and
29
30 stimuli-responsive polymer shell for confined catalytic reactions" *Polym. Chem.* **2011**, *2*,
31
32 1368-1374.
33
34
35 (277) Kinning, D. J.; Winey, K. I.; Thomas, E. L. "Structural transitions from spherical to
36
37 nonspherical micelles in blends of poly(styrene-butadiene) diblock copolymer and polystyrene
38
39 homopolymers" *Macromolecules* **1988**, *21*, 3502-3506.
40
41
42 (278) Zipfel, J.; Lindner, P.; Tsianou, M.; Alexandridis, P.; Richtering, W. "Shear-Induced Formation
43
44 of Multilamellar Vesicles ("Onions") in Block Copolymers" *Langmuir* **1999**, *15*, 2599-2602.
45
46
47 (279) Shen, H.; Eisenberg, A. "Control of Architecture in Block-Copolymer Vesicles" *Angew. Chem.*
48
49 *Int. Ed.* **2000**, *39*, 3310-3312.
50
51
52 (280) Li, L.; Rosenthal, M.; Zhang, H.; Hernandez, J. J.; Drechsler, M.; Phan, K. H.; Rütten, S.; Zhu,
53
54
55 X.; Ivanov, D. A.; Möller, M. "Light-Switchable Vesicles from Liquid-Crystalline
56
57
58
59
60

- 1
2
3
4 Homopolymer–Surfactant Complexes" *Angew. Chem. Int. Ed.* **2012**, *51*, 11616-11619.
- 5
6 (281) Mai, Y.; Eisenberg, A. "Self-assembly of block copolymers" *Chem. Soc. Rev.* **2012**, *41*,
7
8 5969-5985.
- 9
10
11 (282) Li, M.; Huang, X.; Tang, T. Y. D.; Mann, S. "Synthetic cellularity based on non-lipid
12
13 micro-compartments and protocell models" *Curr. Opin. Chem. Biol.* **2014**, *22*, 1-11.
- 14
15
16 (283) Palivan, C. G.; Fischer-Onaca, O.; Delcea, M.; Itef, F.; Meier, W. "Protein-polymer
17
18 nanoreactors for medical applications" *Chem. Soc. Rev.* **2012**, *41*, 2800-2823.
- 19
20
21 (284) Liang, K.; Such, G. K.; Johnston, A. P. R.; Zhu, Z.; Ejima, H.; Richardson, J. J.; Cui, J.;
22
23 Caruso, F. "Endocytic pH-Triggered Degradation of Nanoengineered Multilayer Capsules"
24
25 *Adv. Mater.* **2014**, *26*, 1901-1905.
- 26
27
28 (285) Liu, G.; Wang, X.; Hu, J.; Zhang, G.; Liu, S. "Self-Immolative Polymersomes for
29
30 High-Efficiency Triggered Release and Programmed Enzymatic Reactions" *J. Am. Chem. Soc.*
31
32
33 **2014**, *136*, 7492-7497.
- 34
35
36 (286) Viger, M. L.; Sheng, W.; Dore, K.; Alhasan, A. H.; Carling, C.-J.; Lux, J.; Lux, C. d. G.;
37
38 Grossman, M.; Malinow, R.; Almutairi, A. "Near-Infrared-Induced Heating of Confined Water
39
40 in Polymeric Particles for Efficient Payload Release" *ACS Nano* **2014**, *8*, 4815-4826.
- 41
42
43 (287) Yi, Q.; Sukhorukov, G. B. "UV light stimulated encapsulation and release by polyelectrolyte
44
45 microcapsules" *Adv. Colloid Interface Sci.* **2014**, *207*, 280-289.
- 46
47
48 (288) Zhang, Y.; Teo, B. M.; Goldie, K. N.; Stadler, B.
49
50 "Poly(N-isopropylacrylamide)/Poly(dopamine) Capsules" *Langmuir* **2014**, *30*, 5592-5598.
- 51
52
53 (289) van Dongen, S. F. M.; Verdurmen, W. P. R.; Peters, R. J. R. W.; Nolte, R. J. M.; Brock, R.; van
54
55 Hest, J. C. M. "Cellular Integration of an Enzyme-Loaded Polymersome Nanoreactor" *Angew.*

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2
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46
47
48
49
50
51
52
53
54
55
56
57
58
59
60
- Chem. Int. Ed.* **2010**, *49*, 7213-7216.
- (290) Kuiper, S. M.; Nallani, M.; Vriezema, D. M.; Cornelissen, J. J. L. M.; van Hest, J. C. M.; Nolte, R. J. M.; Rowan, A. E. "Enzymes containing porous polymersomes as nano reaction vessels for cascade reactions" *Org. Biomol. Chem.* **2008**, *6*, 4315-4318.
- (291) Langowska, K.; Palivan, C. G.; Meier, W. "Polymer nanoreactors shown to produce and release antibiotics locally" *Chem. Comm.* **2013**, *49*, 128-130.
- (292) Onaca, O.; Hughes, D. W.; Balasubramanian, V.; Grzelakowski, M.; Meier, W.; Palivan, C. G. "SOD Antioxidant Nanoreactors: Influence of Block Copolymer Composition on the Nanoreactor Efficiency" *Macromol. Biosci.* **2010**, *10*, 531-538.
- (293) Axthelm, F.; Casse, O.; Koppenol, W. H.; Nauser, T.; Meier, W.; Palivan, C. G. "Antioxidant nanoreactor based on superoxide dismutase encapsulated in superoxide-permeable vesicles" *J. Phys. Chem. B* **2008**, *112*, 8211-8217.
- (294) Wang, Z.; van Oers, M. C. M.; Rutjes, F. P. J. T.; van Hest, J. C. M. "Polymersome Colloidosomes for Enzyme Catalysis in a Biphasic System" *Angew. Chem. Int. Ed.* **2012**, *51*, 10746-10750.
- (295) Meeuwissen, S. A.; Rioz-Martinez, A.; de Gonzalo, G.; Fraaije, M. W.; Gotor, V.; van Hest, J. C. M. "Cofactor regeneration in polymersome nanoreactors: enzymatically catalysed Baeyer-Villiger reactions" *J Mater Chem* **2011**, *21*, 18923-18926.
- (296) Spulber, M.; Baumann, P.; Saxer, S. S.; Pielas, U.; Meier, W.; Bruns, N. "Poly (N-vinylpyrrolidone)-poly (dimethylsiloxane)-based polymersome nanoreactors for laccase-catalyzed biotransformations" *Biomacromolecules* **2014**, *15*, 1469-1475.
- (297) Yildiz, U. H.; De Hoog, H.-P. M.; Fu, Z.; Tomczak, N.; Parikh, A. N.; Nallani, M.; Liedberg,

- 1
2
3
4 B. "Third-Party ATP Sensing in Polymersomes: A Label-Free Assay of Enzyme Reactions in
5
6 Vesicular Compartments" *Small* **2014**, *10*, 442-447.
7
8
9 (298) Vriezema, D. M.; Comellas Aragonès, M.; Elemans, J. A. A. W.; Cornelissen, J. J. L. M.;
10
11 Rowan, A. E.; Nolte, R. J. M. "Self-Assembled Nanoreactors" *Chem. Rev.* **2005**, *105*,
12
13 1445-1490.
14
15
16 (299) Rosenkranz, T.; Katranidis, A.; Atta, D.; Gregor, I.; Enderlein, J.; Grzelakowski, M.; Rigler,
17
18 P.; Meier, W.; Fitter, J. "Observing Proteins as Single Molecules Encapsulated in
19
20 Surface-Tethered Polymeric Nanocontainers" *ChemBioChem* **2009**, *10*, 702-709.
21
22
23 (300) Comellas-Aragones, M.; Engelkamp, H.; Claessen, V. I.; Sommerdijk, N. A. J. M.; Rowan, A.
24
25 E.; Christianen, P. C. M.; Maan, J. C.; Verduin, B. J. M.; Cornelissen, J. J. L. M.; Nolte, R. J.
26
27 M. "A virus-based single-enzyme nanoreactor" *Nat. Nano.* **2007**, *2*, 635-639.
28
29
30 (301) van Dongen, S. F. M.; Nallani, M.; Cornelissen, J. L. L. M.; Nolte, R. J. M.; van Hest, J. C. M.
31
32 "A Three-Enzyme Cascade Reaction through Positional Assembly of Enzymes in a
33
34 Polymersome Nanoreactor" *Chem. Eur. J.* **2009**, *15*, 1107-1114.
35
36
37 (302) Vriezema, D. M.; Garcia, P. M. L.; Sancho Oltra, N.; Hatzakis, N. S.; Kuiper, S. M.; Nolte, R.
38
39 J. M.; Rowan, A. E.; van Hest, J. C. M. "Positional Assembly of Enzymes in Polymersome
40
41 Nanoreactors for Cascade Reactions" *Angew. Chem. Int. Ed.* **2007**, *46*, 7378-7382.
42
43
44 (303) Baumann, P.; Balasubramanian, V.; Onaca-Fischer, O.; Sienkiewicz, A.; Palivan, C. G.
45
46 "Light-responsive polymer nanoreactors: a source of reactive oxygen species on demand"
47
48
49 *Nanoscale* **2013**, *5*, 217-224.
50
51
52 (304) Brinkhuis, R. P.; Rutjes, F. P. J. T.; van Hest, J. C. M. "Polymeric vesicles in biomedical
53
54 applications" *Polym. Chem.* **2011**, *2*, 1449-1462.
55
56
57
58
59
60

- 1
2
3
4 (305) Valade, D.; Jeon, Y.; Kessel, S.; Monteiro, M. J. "Influence of the Z - group on the RAFT -
5
6 mediated polymerizations in nanoreactors" *J. Polym. Sci. A Polym. Chem* **2012**, *50*,
7
8 4762-4771.
9
- 10
11 (306) Zetterlund, P. B. "Controlled/living radical polymerization in nanoreactors:
12
13 compartmentalization effects" *Polym. Chem.* **2011**, *2*, 534-549.
14
15
- 16 (307) Zhang, M.; Zhang, W. "Reversible addition - fragmentation chain transfer polymerization of a
17
18 typical hydrophobic monomer of styrene within microreactor of shell - corona hollow
19
20 microspheres suspending in water" *J. Polym. Sci. A Polym. Chem* **2010**, *48*, 5446-5455.
21
22
- 23 (308) Asua, J. M. "Miniemulsion polymerization" *Prog. Polym. Sci.* **2002**, *27*, 1283-1346.
24
25
- 26 (309) Landfester, K. "Polyreactions in Miniemulsions" *Macromol. Rapid Commun.* **2001**, *22*,
27
28 896-936.
29
30
- 31 (310) Chow, P. Y.; Gan, L. M. "Microemulsion Polymerizations and Reactions" *Adv. Polym. Sci.*
32
33 **2008**, *175*, 257-298.
34
35
- 36 (311) Zetterlund, P. B.; Kagawa, Y.; Okubo, M. "Controlled/Living Radical Polymerization in
37
38 Dispersed Systems" *Chem. Rev.* **2008**, *108*, 3747-3794.
39
40
- 41 (312) Kent, P.; Saunders, B. R. "The Role of Added Electrolyte in the Stabilization of Inverse
42
43 Emulsions" *J. Colloid Interface Sci.* **2001**, *242*, 437-442.
44
45
- 46 (313) Landfester, K.; Willert, M.; Antonietti, M. "Preparation of Polymer Particles in Nonaqueous
47
48 Direct and Inverse Miniemulsions" *Macromolecules* **2000**, *33*, 2370-2376.
49
50
- 51 (314) Sukhorukov, G. B.; Susha, A. S.; Davis, S.; Leporatti, S.; Donath, E.; Hartmann, J.; Möhwald,
52
53 H. "Precipitation of Inorganic Salts inside Hollow Micrometer-Sized Polyelectrolyte Shells" *J.*
54
55 *Colloid Interface Sci.* **2002**, *247*, 251-254.
56
57
58
59
60

- 1
2
3
4 (315) Li, W.; Sha, X.; Dong, W.; Wang, Z. "Synthesis of stable hollow silica microspheres with
5
6 mesoporous shell in nonionic W/O emulsion" *Chem. Comm.* **2002**, 2434-2435.
7
8
9 (316) Jesionowski, T. "Influence of aminosilane surface modification and dyes adsorption on zeta
10
11 potential of spherical silica particles formed in emulsion system" *Colloid Surf. A-Physicochem.*
12
13 *Eng. Asp.* **2003**, 222, 87-94.
14
15
16 (317) Andersson, M.; Österlund, L.; Ljungström, S.; Palmqvist, A. "Preparation of Nanosize
17
18 Anatase and Rutile TiO₂ by Hydrothermal Treatment of Microemulsions and Their Activity
19
20 for Photocatalytic Wet Oxidation of Phenol" *J. Phys. Chem. B* **2002**, 106, 10674-10679.
21
22
23 (318) Li, M.; Mann, S. "Emergent Nanostructures: Water-Induced Mesoscale Transformation of
24
25 Surfactant-Stabilized Amorphous Calcium Carbonate Nanoparticles in Reverse
26
27 Microemulsions" *Adv. Funct. Mater.* **2002**, 12, 773-779.
28
29
30 (319) Horecha, M.; Kaul, E.; Horechyy, A.; Stamm, M. "Polymer microcapsules loaded with Ag
31
32 nanocatalyst as active microreactors" *J. Mater. Chem. A* **2014**, 2, 7431-7438.
33
34
35 (320) Sukhorukov, G. B.; Brumen, M.; Donath, E.; Möhwald, H. "Hollow Polyelectrolyte Shells:
36
37 Exclusion of Polymers and Donnan Equilibrium" *J. Phys. Chem. B* **1999**, 103, 6434-6440.
38
39
40 (321) Sukhorukov, G.; Dähne, L.; Hartmann, J.; Donath, E.; Möhwald, H. "Controlled Precipitation
41
42 of Dyes into Hollow Polyelectrolyte Capsules Based on Colloids and Biocolloids" *Adv. Mater.*
43
44 **2000**, 12, 112-115.
45
46
47 (322) Ghan, R.; Shutava, T.; Patel, A.; John, V. T.; Lvov, Y. "Enzyme-Catalyzed Polymerization of
48
49 Phenols within Polyelectrolyte Microcapsules" *Macromolecules* **2004**, 37, 4519-4524.
50
51
52 (323) Shehukin, D. G.; Sukhorukov, G. B. "Nanoparticle synthesis in engineered organic nanoscale
53
54 reactors" *Adv. Mater.* **2004**, 16, 671-682.
55
56
57
58
59
60

- 1
2
3
4 (324) Shchukin, D. G.; Dong, W.; Sukhorukov, G. B. "Spatially Confined Tungstate Ion
5
6 Polymerization in Microcapsules" *Macromol. Rapid Commun.* **2003**, *24*, 462-466.
7
8
9 (325) Shchukin, D. G.; Schattka, J. H.; Antonietti, M.; Caruso, R. A. "Photocatalytic Properties of
10
11 Porous Metal Oxide Networks Formed by Nanoparticle Infiltration in a Polymer Gel
12
13 Template" *J. Phys. Chem. B* **2003**, *107*, 952-957.
14
15
16 (326) Radtchenko, I. L.; Giersig, M.; Sukhorukov, G. B. "Inorganic Particle Synthesis in Confined
17
18 Micron-Sized Polyelectrolyte Capsules" *Langmuir* **2002**, *18*, 8204-8208.
19
20
21 (327) Elani, Y.; Law, R. V.; Ces, O. "Vesicle-based artificial cells as chemical microreactors with
22
23 spatially segregated reaction pathways" *Nat. Commun.* **2014**, *5*, 5305.
24
25
26 (328) Mansy, S. S.; Schrum, J. P.; Krishnamurthy, M.; Tobe, S.; Treco, D. A.; Szostak, J. W.
27
28 "Template-directed synthesis of a genetic polymer in a model protocell" *Nature* **2008**, *454*,
29
30 122-U110.
31
32
33 (329) Nardin, C.; Thoeni, S.; Widmer, J.; Winterhalter, M.; Meier, W. "Nanoreactors based on
34
35 (polymerized) ABA-triblock copolymer vesicles" *Chem. Comm.* **2000**, 1433-1434.
36
37
38 (330) Baumann, P.; Spulber, M.; Dinu, I. A.; Palivan, C. G. "Cellular trojan horse based polymer
39
40 nanoreactors with light-sensitive activity" *J. Phys. Chem. B* **2014**, *118*, 9361-9370.
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Jens Gaitzsch received his PhD from the Technische Universität Dresden (TUD) in 2013 under the guidance of Prof Brigitte Voit, conducting the research at the Leibniz Institute of Polymer Research (IPF) Dresden. The thesis was funded by a studentship of the Rosa-Luxemburg-Foundation. He then worked as a PDRA in the Group of Prof Giuseppe Battaglia at University College London before moving into a Fellowship of the German Research Association (DFG) in 2014 but remaining in the same group. In September 2015 he started working at the University of Basel in the Group of Prof. Meier. His research is focussed on the development on functional biomimetic and amphiphilic block-copolymers for biomedical applications.



Xin Huang received his PhD from Jilin University, China, in 2009. Then he did

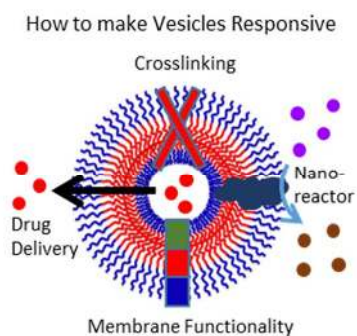
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4 one year postdoctoral research at Centre for Advanced Macromolecular
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6 Design, UNSW, Australia. In 2010, he carried out his postdoctoral research
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8 work supported by Alexander von Humboldt foundation under the guidance of
9
10 Prof. Brigitte Voit at Leibniz Institute of Polymer Research Dresden, Germany.
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12 After that, in 2012 he was granted Marie Curie Research Fellowship and did
13
14 two years' research in Prof. Stephen Mann's group in University of Bristol, UK.
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16 Since July 2014, he was appointed as professor at Harbin Institute of
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18 Technology, China. His main research interests lie in the construction of
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20 polymer/protein-based compartment towards protocells, artificial organelles or
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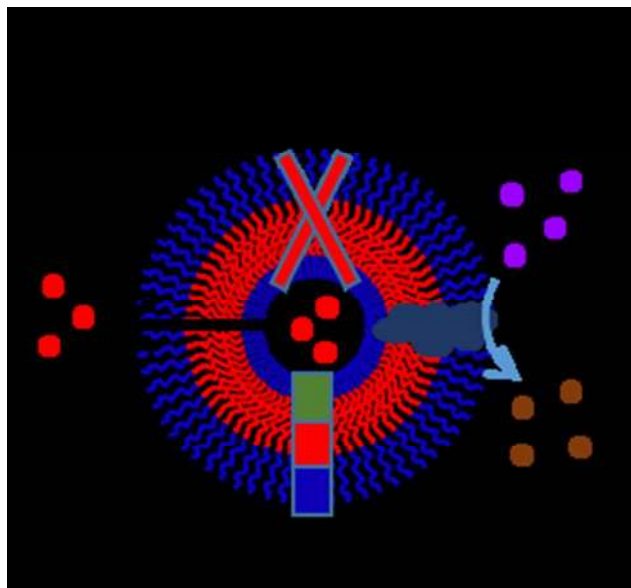
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50 **Brigitte Voit** received her PhD in Macromolecular Chemistry in 1990 from
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52 University Bayreuth, Germany. After a postdoctoral work at Eastman Kodak in
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54 Rochester, USA, she went in 1992 to Technische Universität München
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56 receiving her habilitation degree in Macromolecular Chemistry there in 1996.
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4 In 1997 Brigitte Voit was appointed head of the Institute of Macromolecular
5
6 Chemistry at the Leibniz Institute of Polymer Research (IPF) Dresden, as well
7
8 as Professor for "Organic Chemistry of Polymers" at Technische Universität
9
10 Dresden (TUD). Since 2002 she is in addition heading the IPF Dresden as
11
12 Managing Director and Chief Scientific Officer. Her major research interest is in
13
14 the synthesis of new functional polymer architectures covering topics like
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16 dendritic polymers, functional block and graft copolymers, responsive
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18 hydrogels and capsules, as well as thermo- and photo labile polymers.
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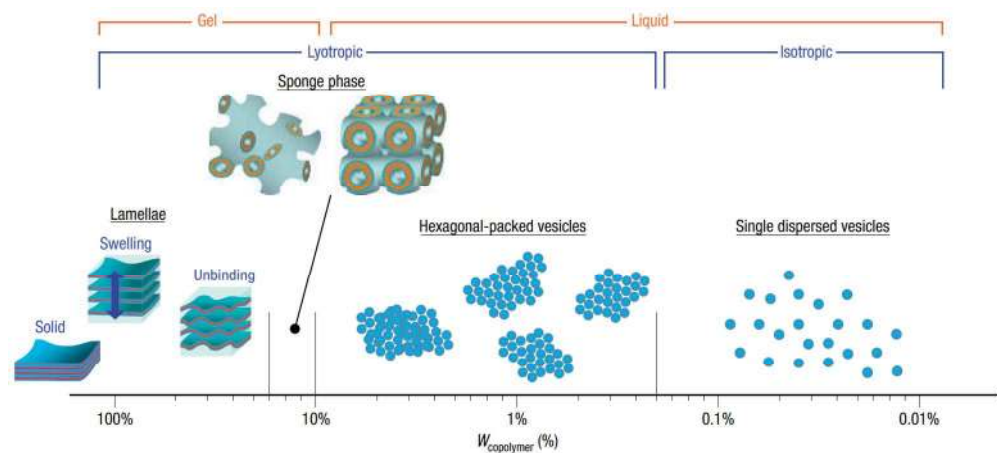
TOC graphic



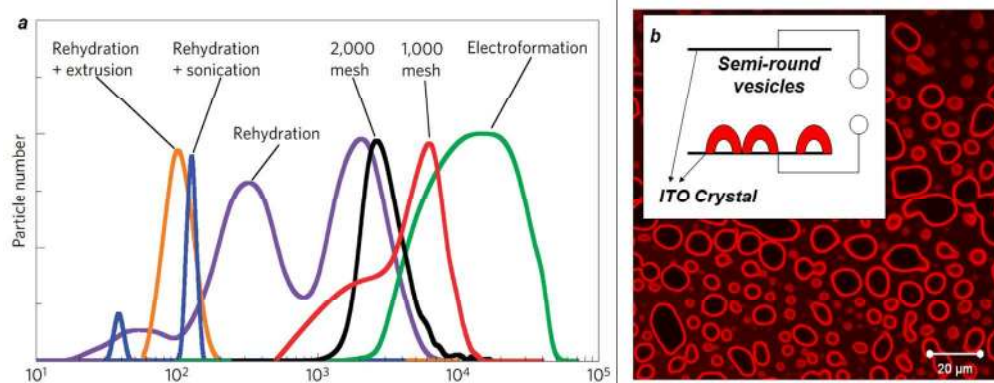
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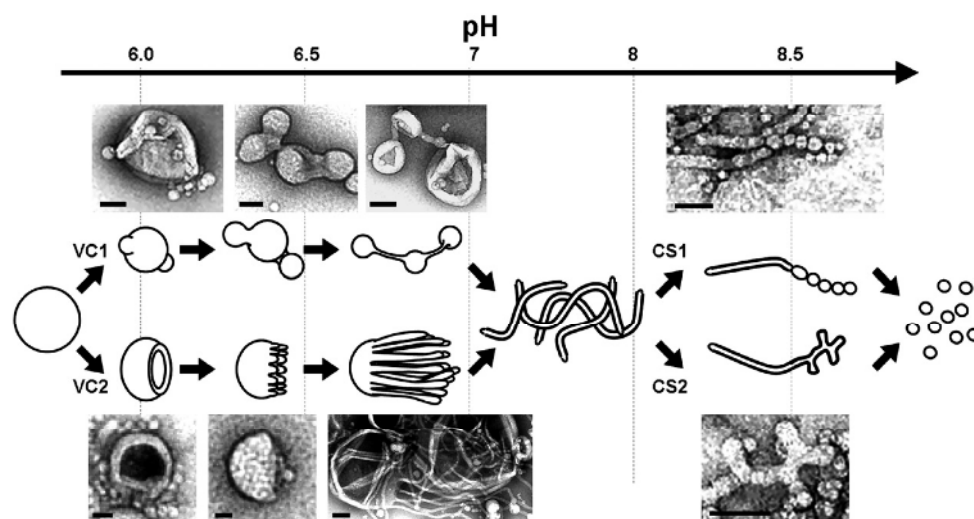
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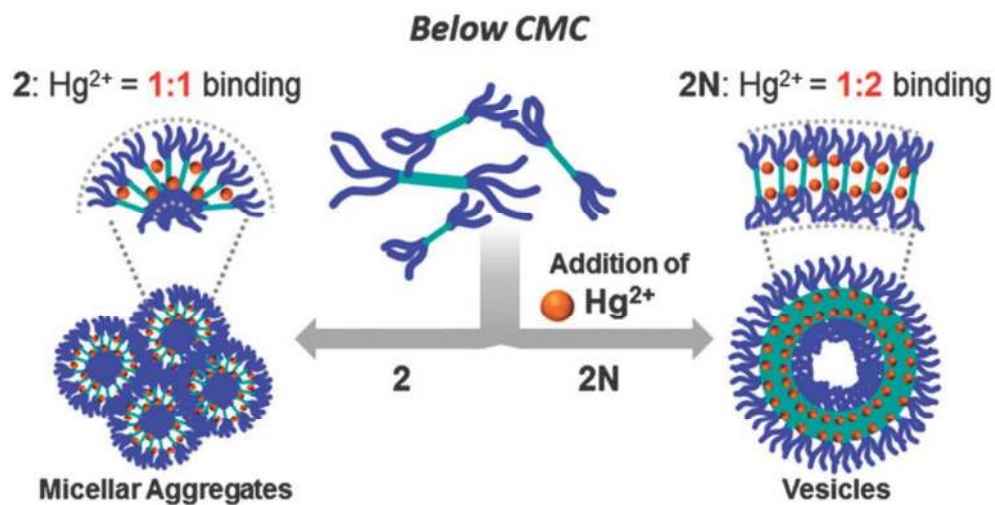
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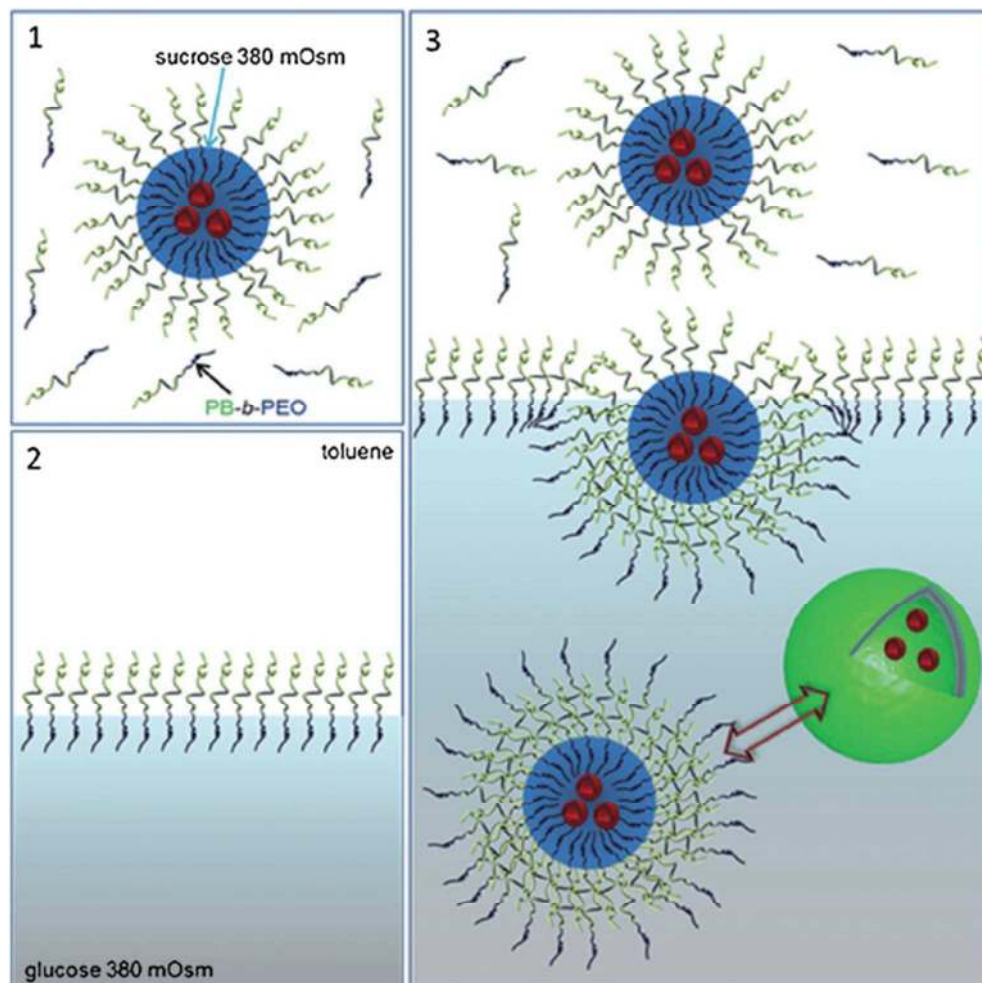
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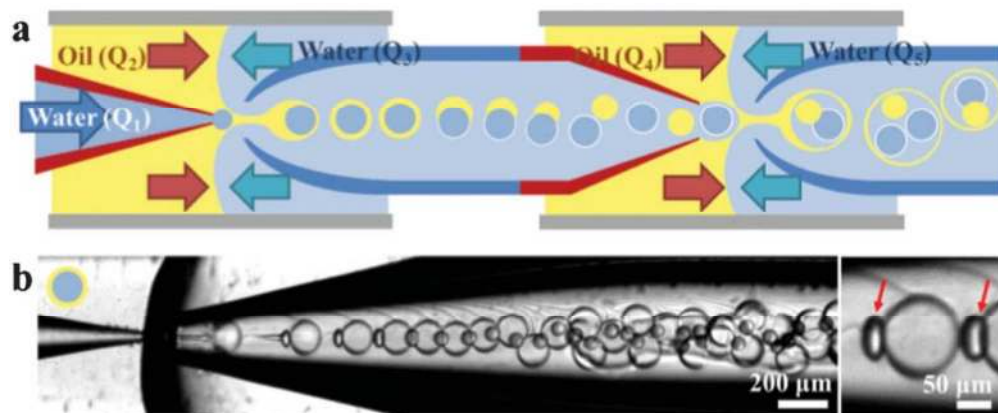
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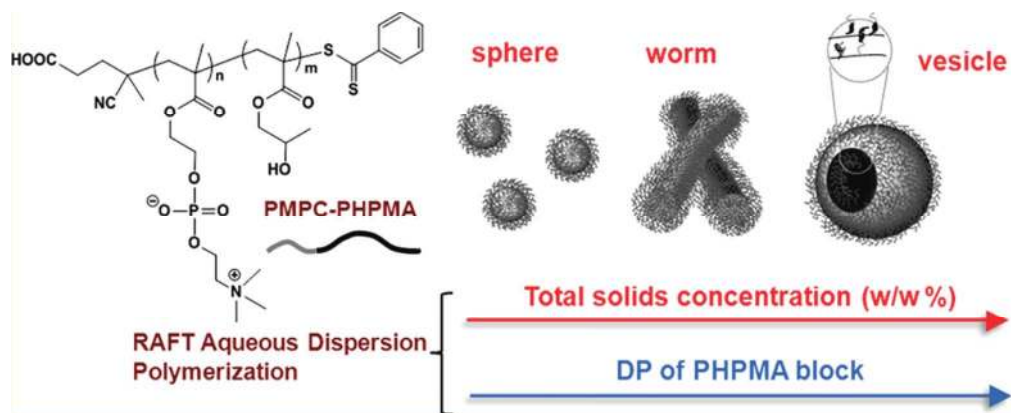
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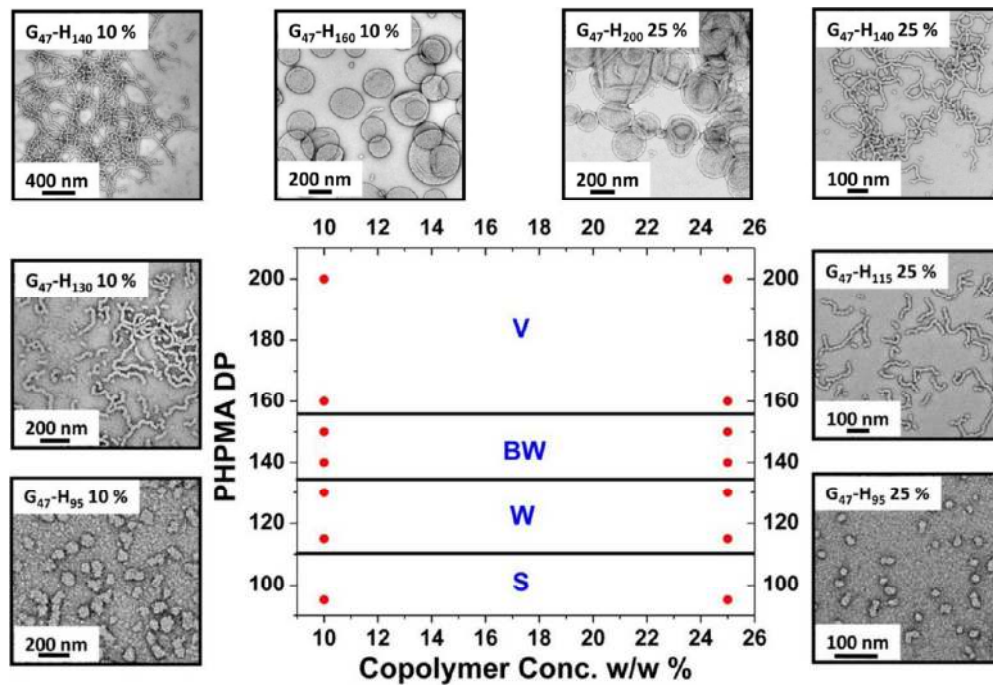
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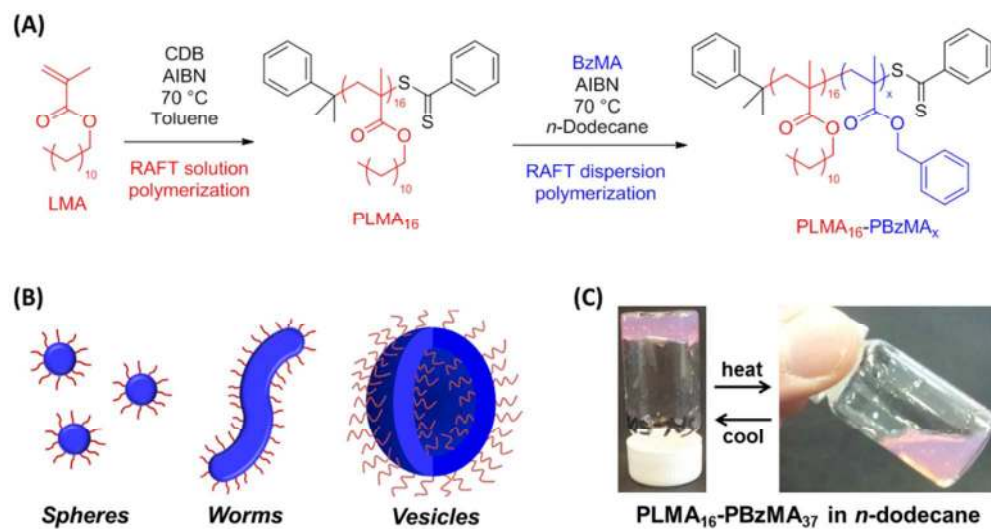
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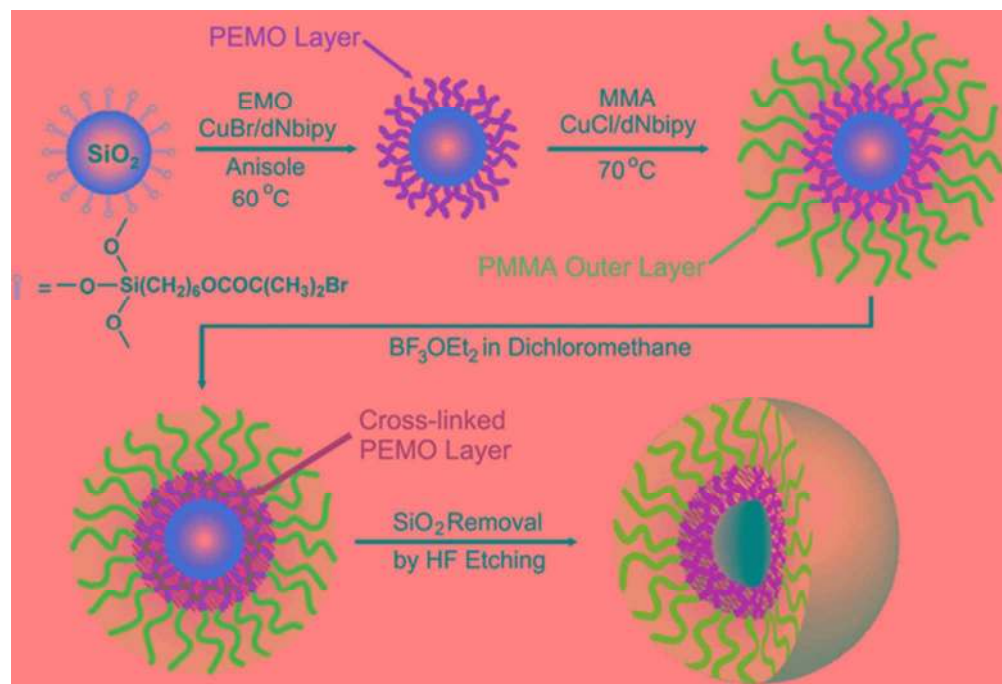
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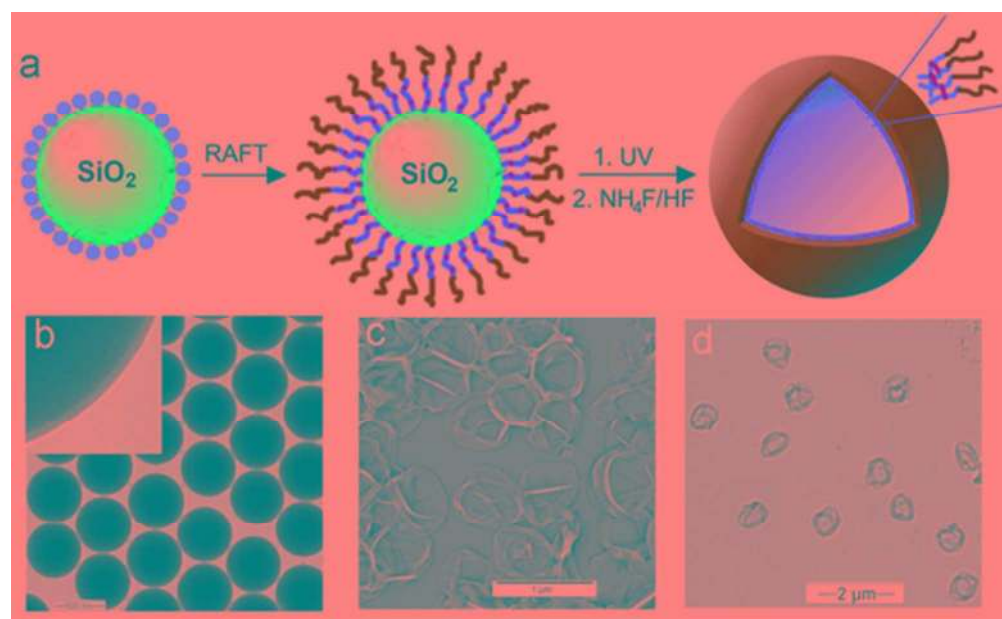
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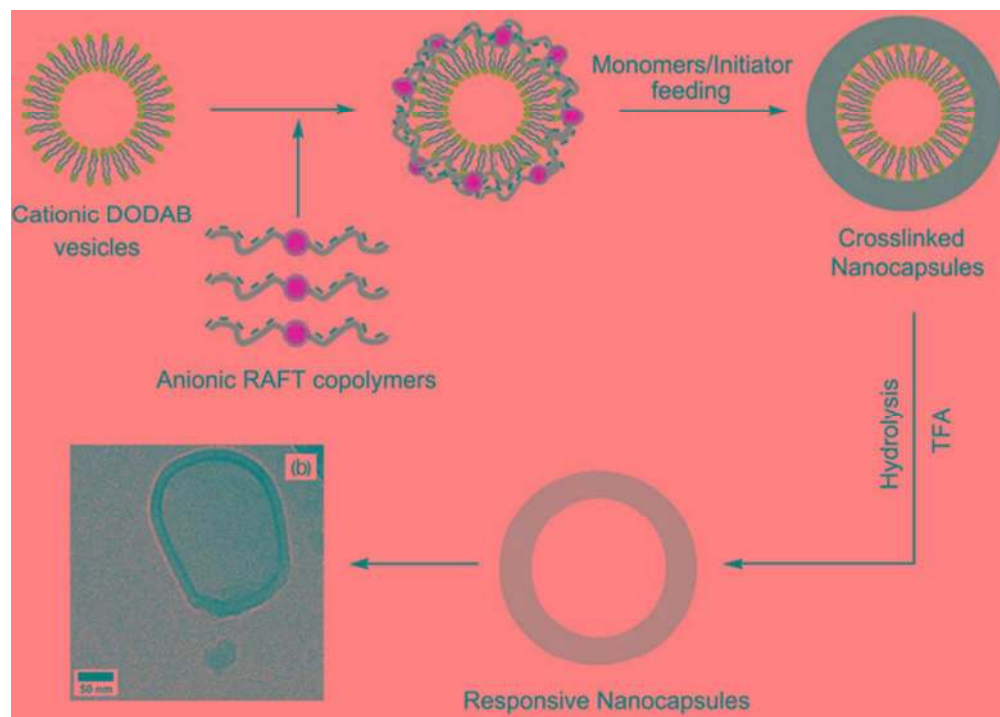
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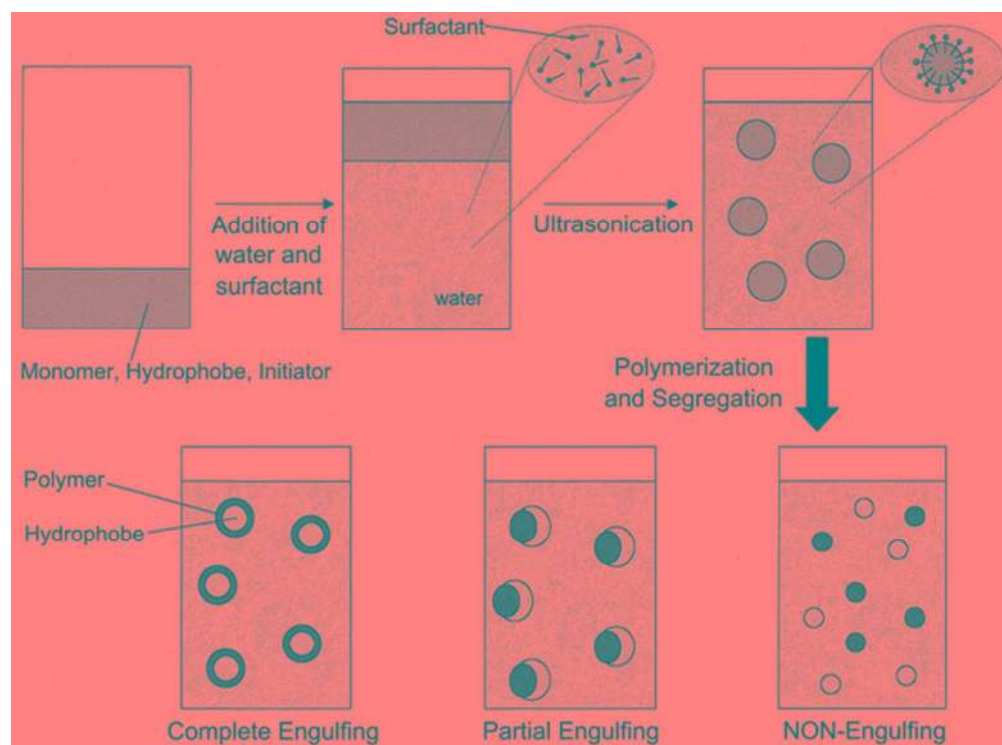
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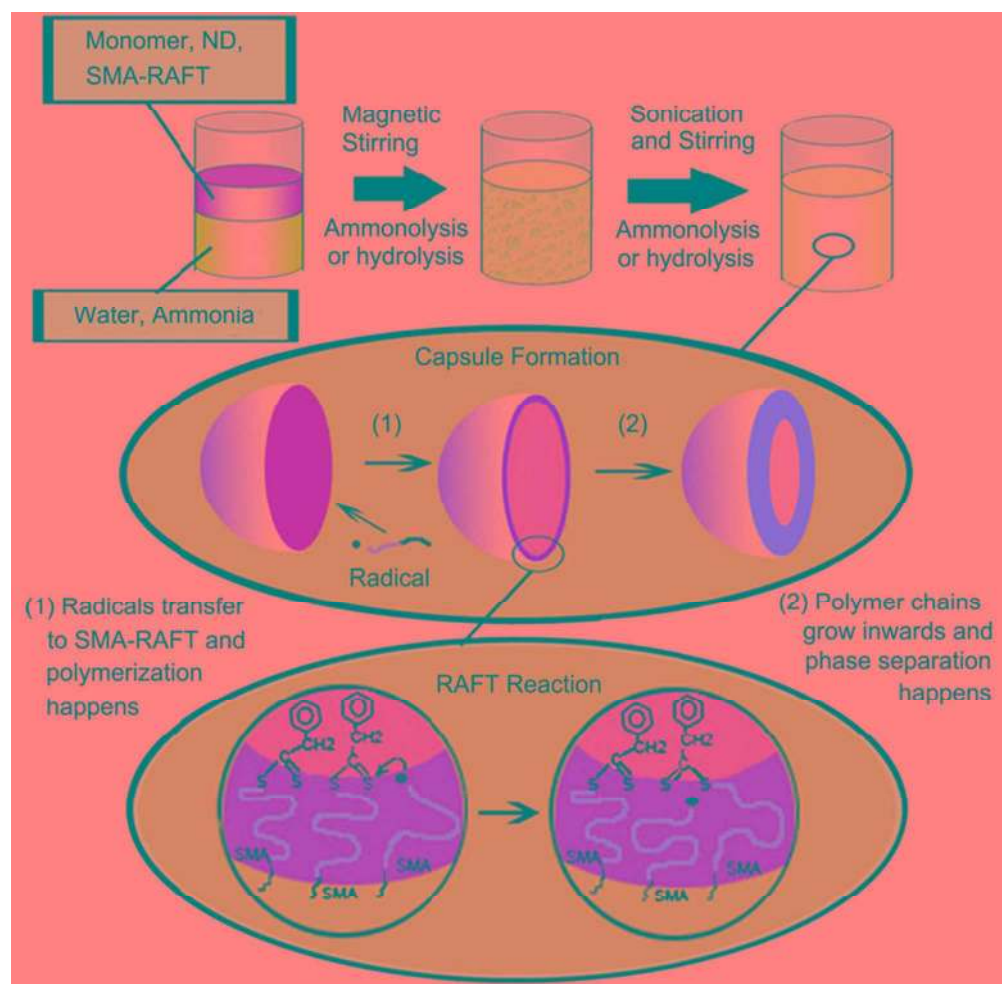
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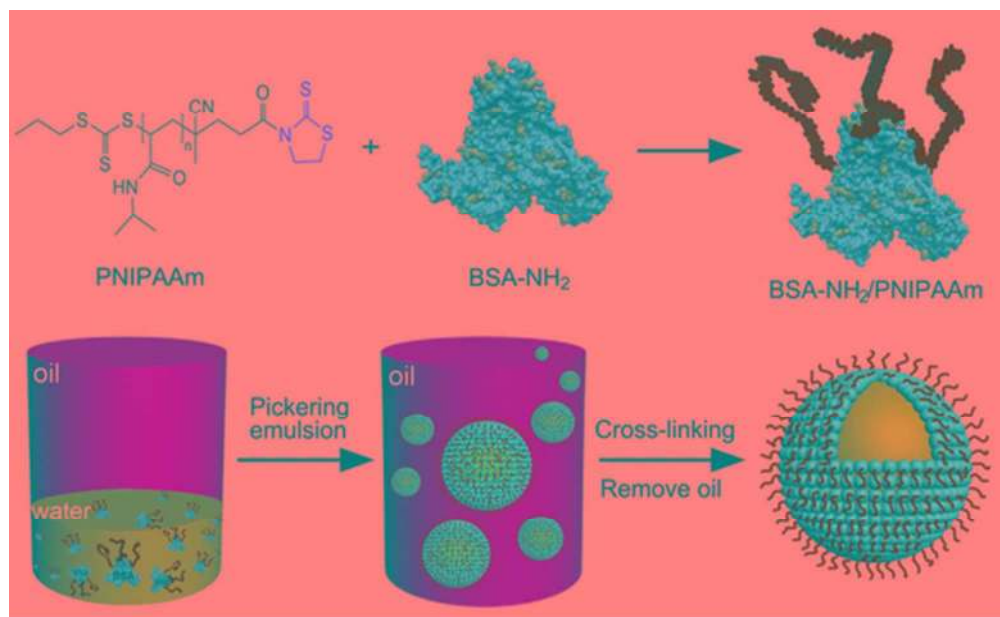
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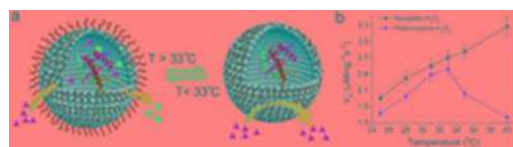
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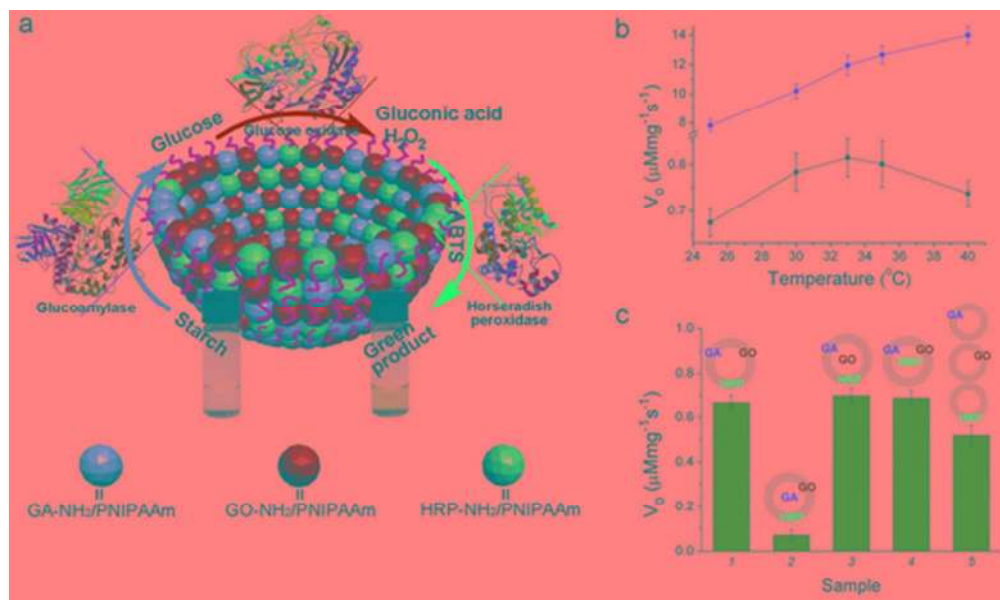
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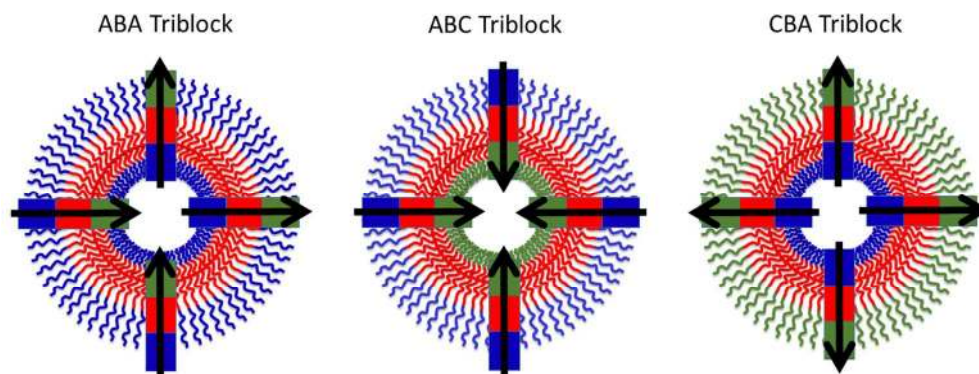
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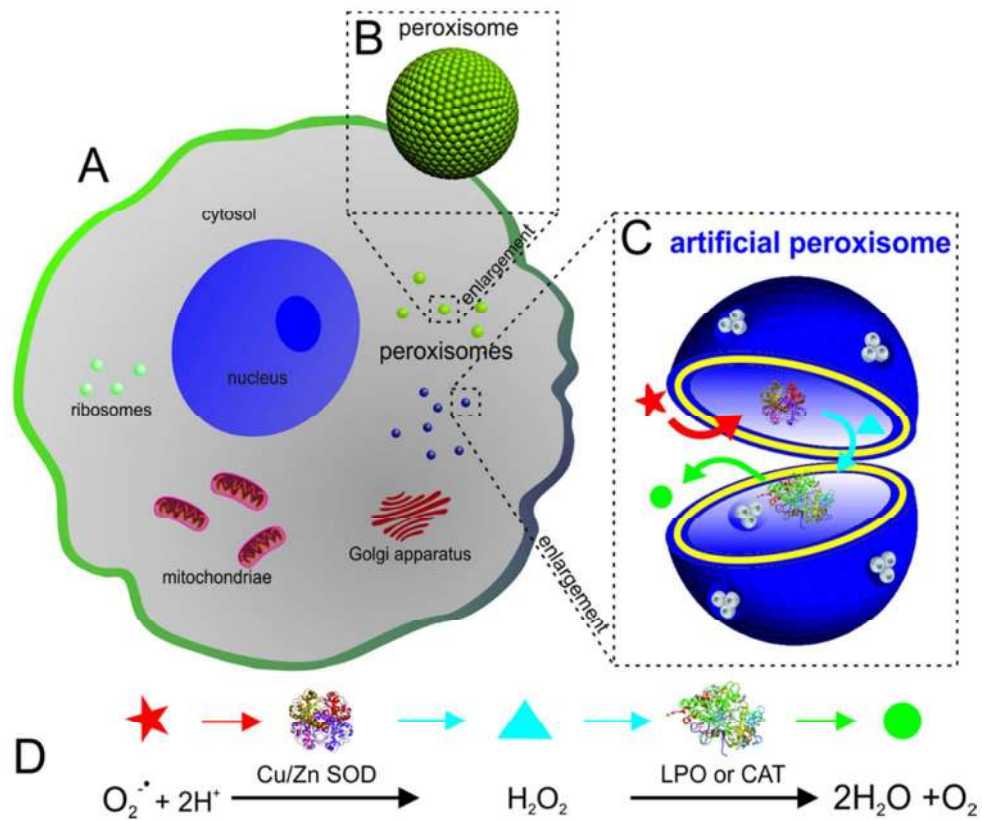


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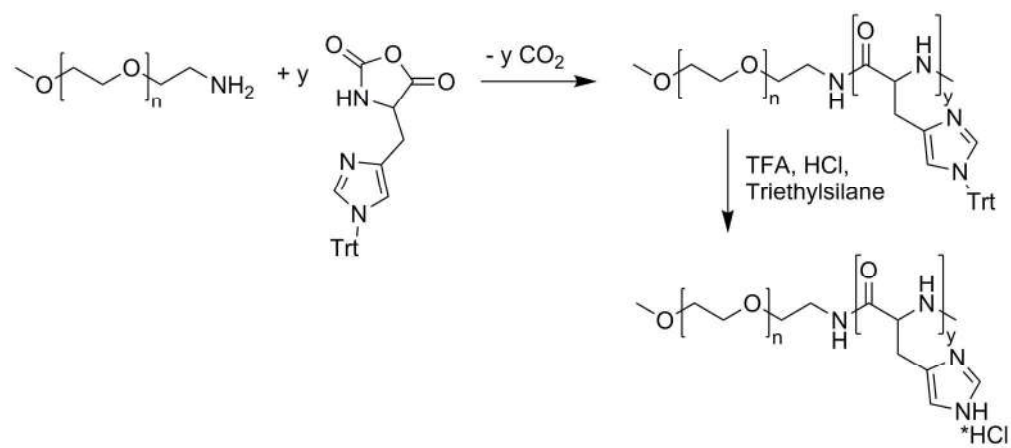


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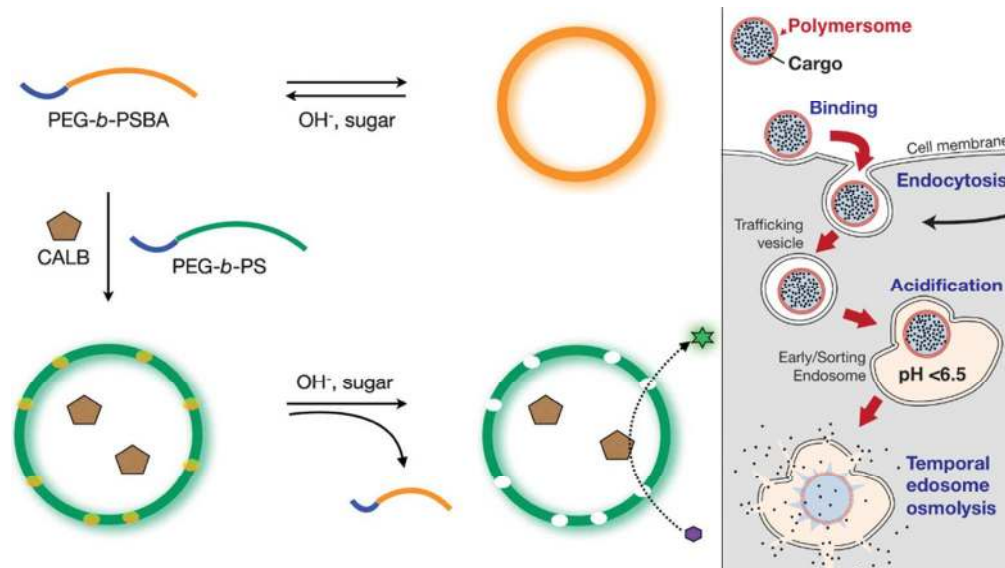




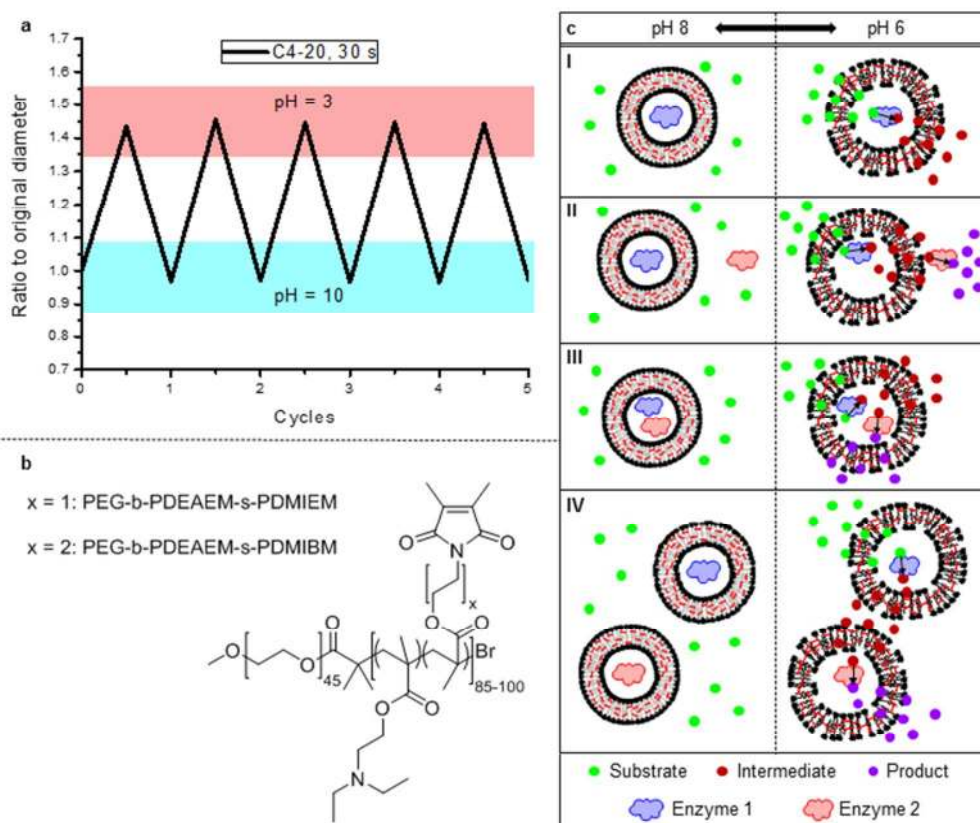
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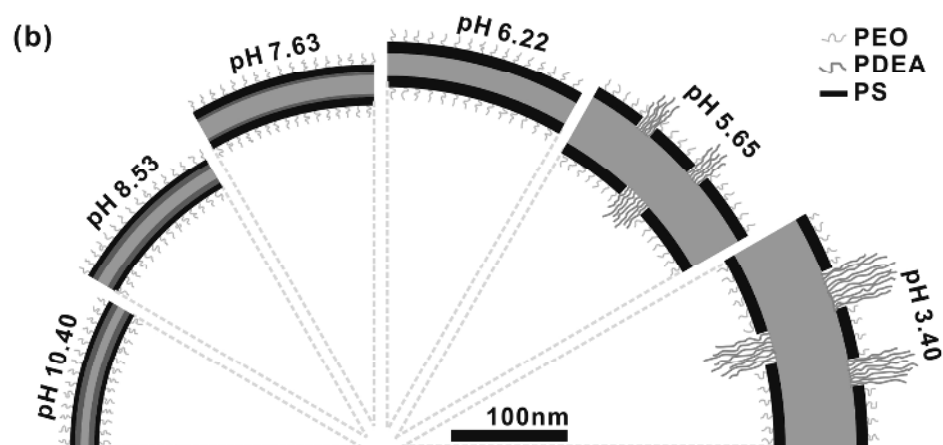
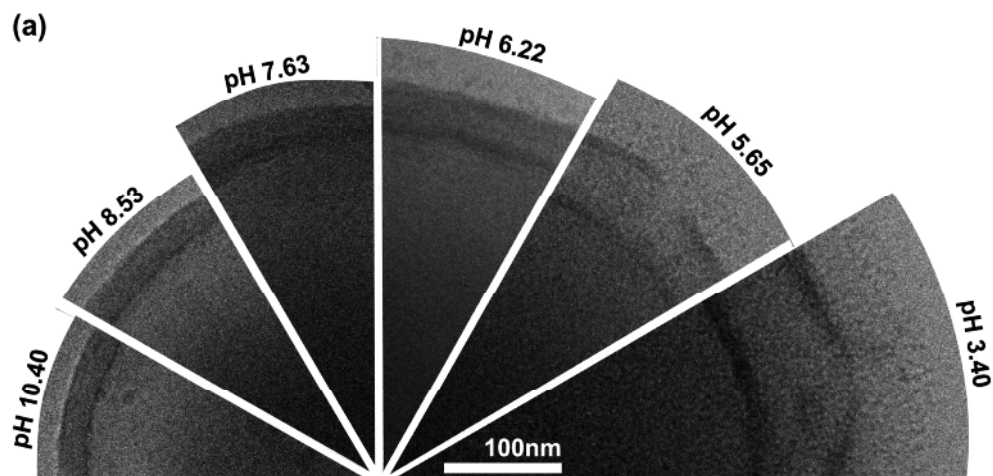
179x79mm (300 x 300 DPI)



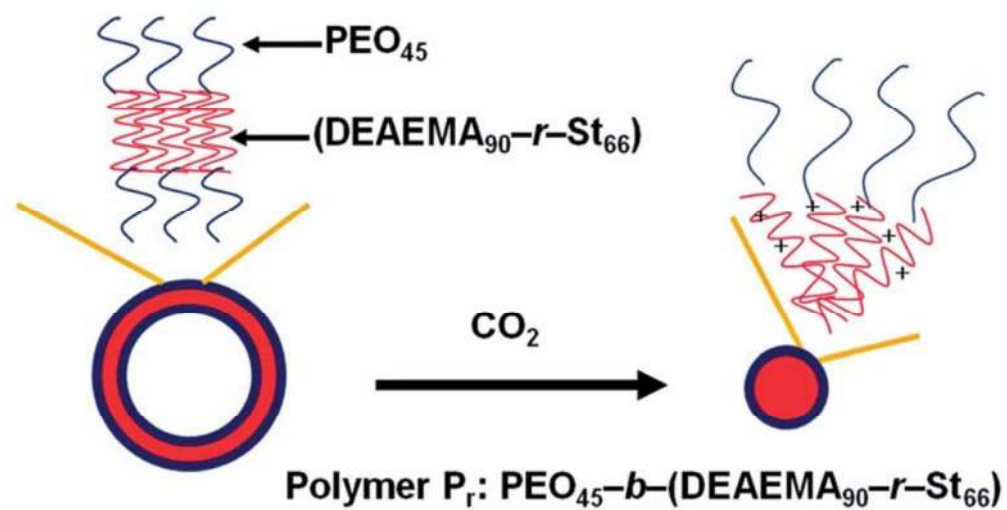
338x190mm (96 x 96 DPI)



190x160mm (96 x 96 DPI)

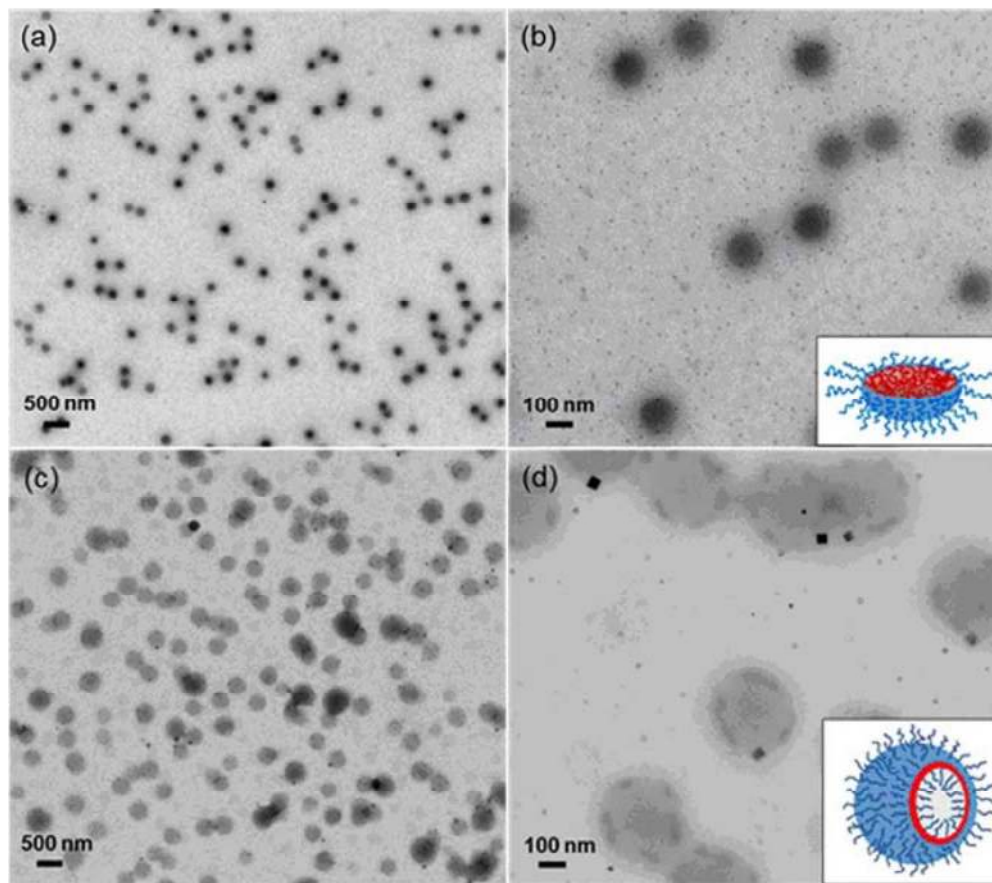


688x666mm (96 x 96 DPI)

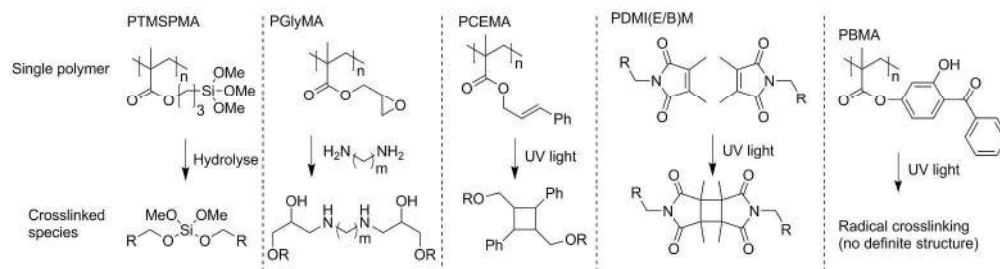


271x139mm (96 x 96 DPI)

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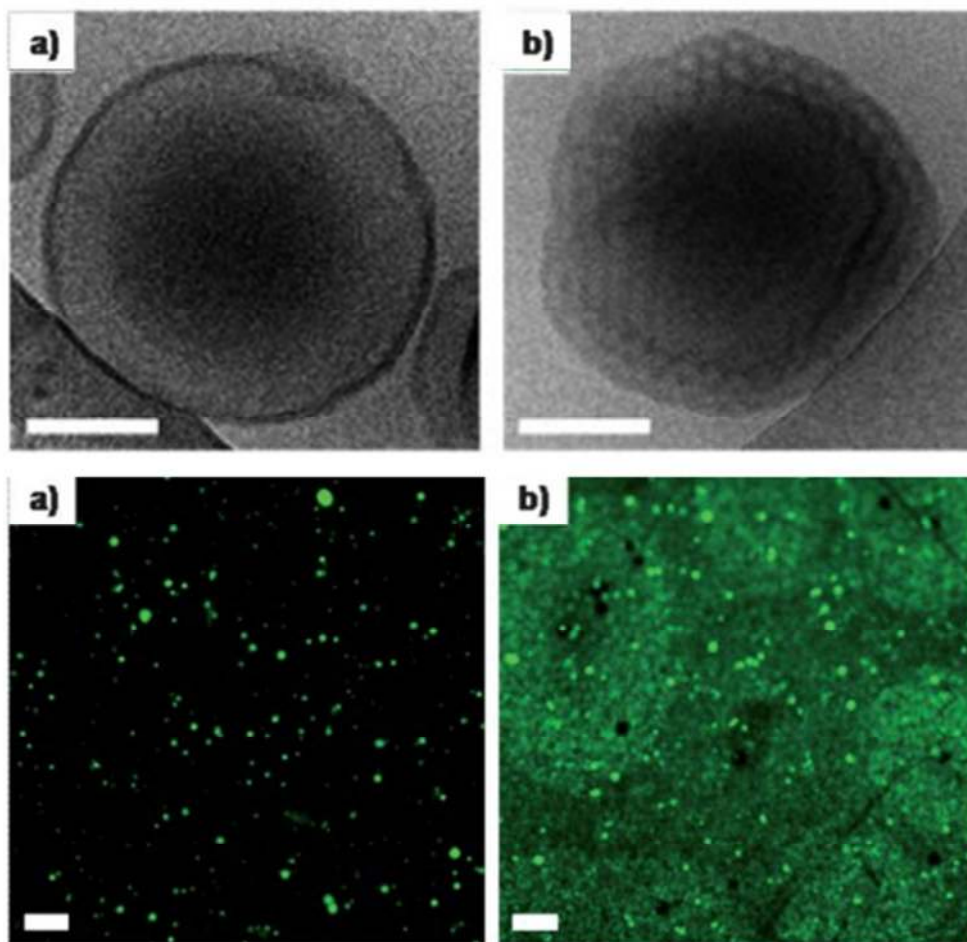


339x299mm (96 x 96 DPI)

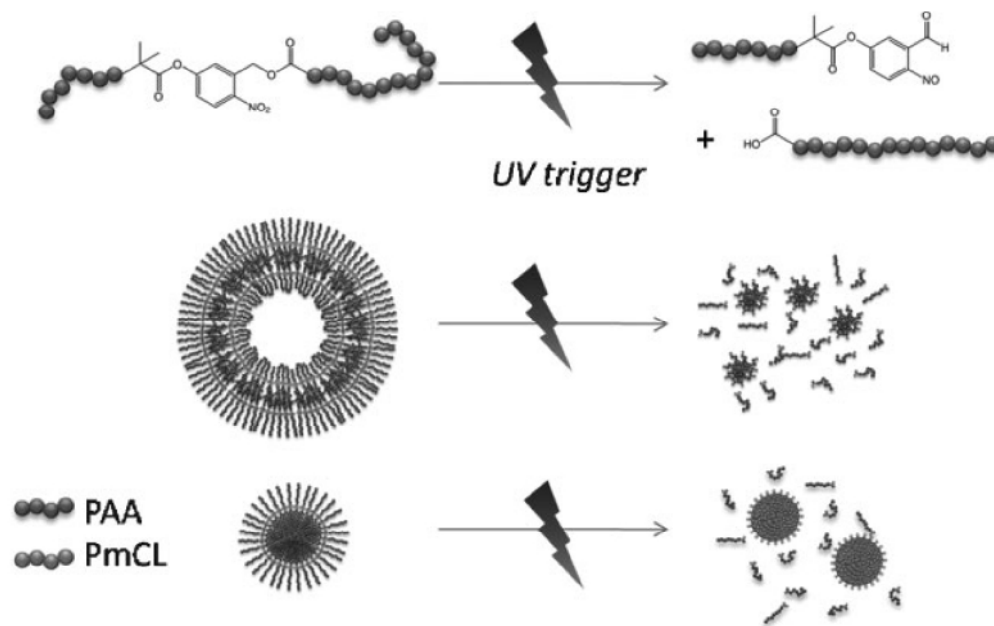


240x64mm (300 x 300 DPI)

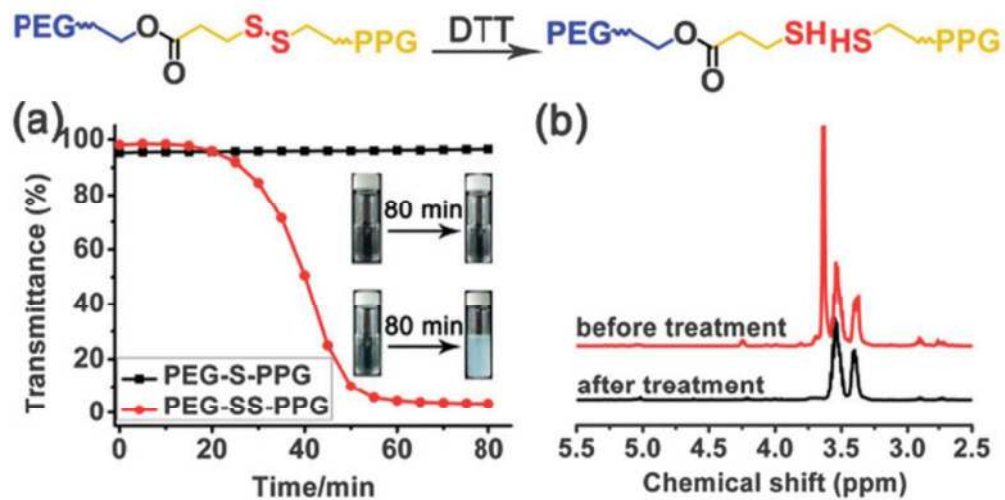
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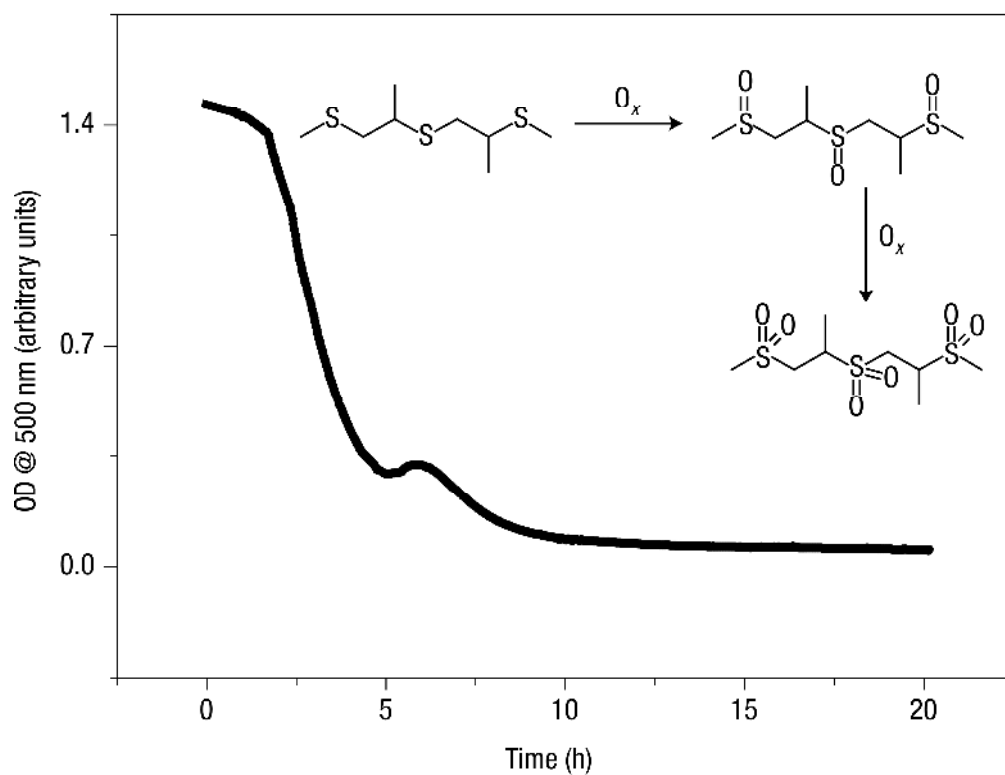
166x160mm (96 x 96 DPI)

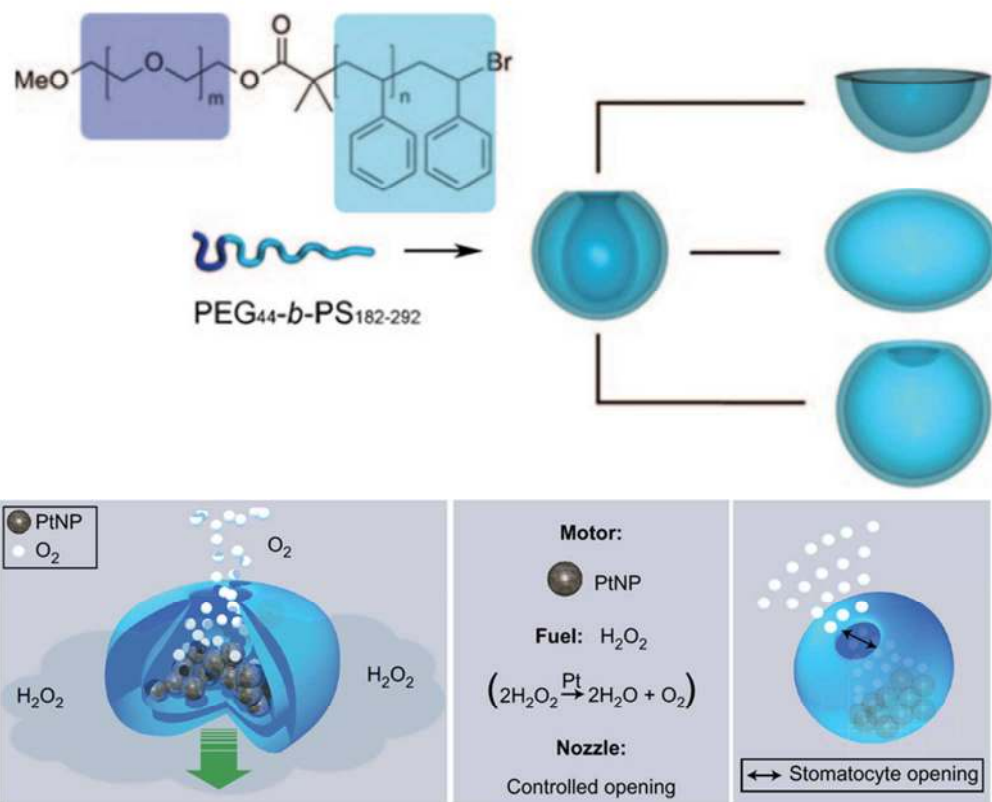


331x209mm (96 x 96 DPI)

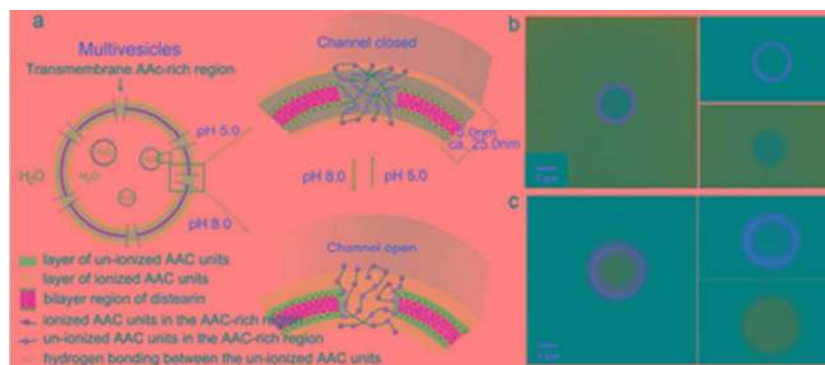


282x144mm (96 x 96 DPI)



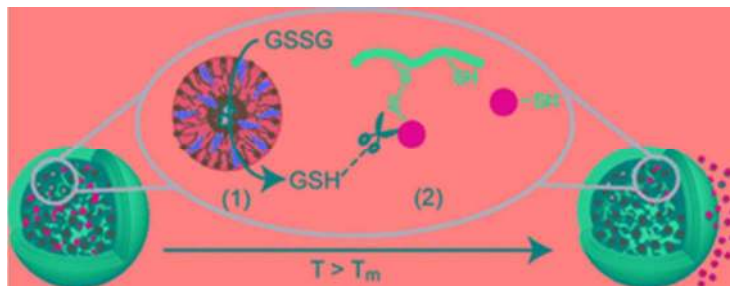


229x190mm (96 x 96 DPI)

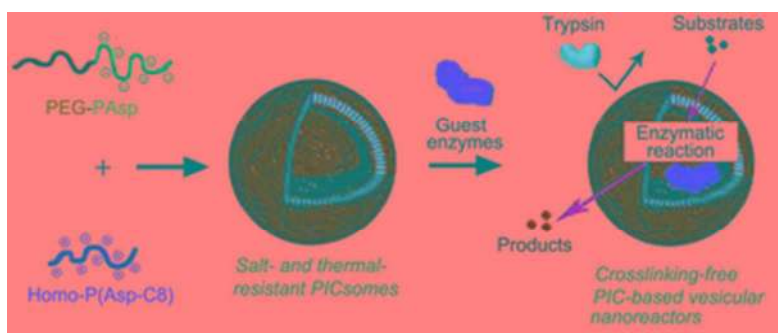


35x15mm (300 x 300 DPI)

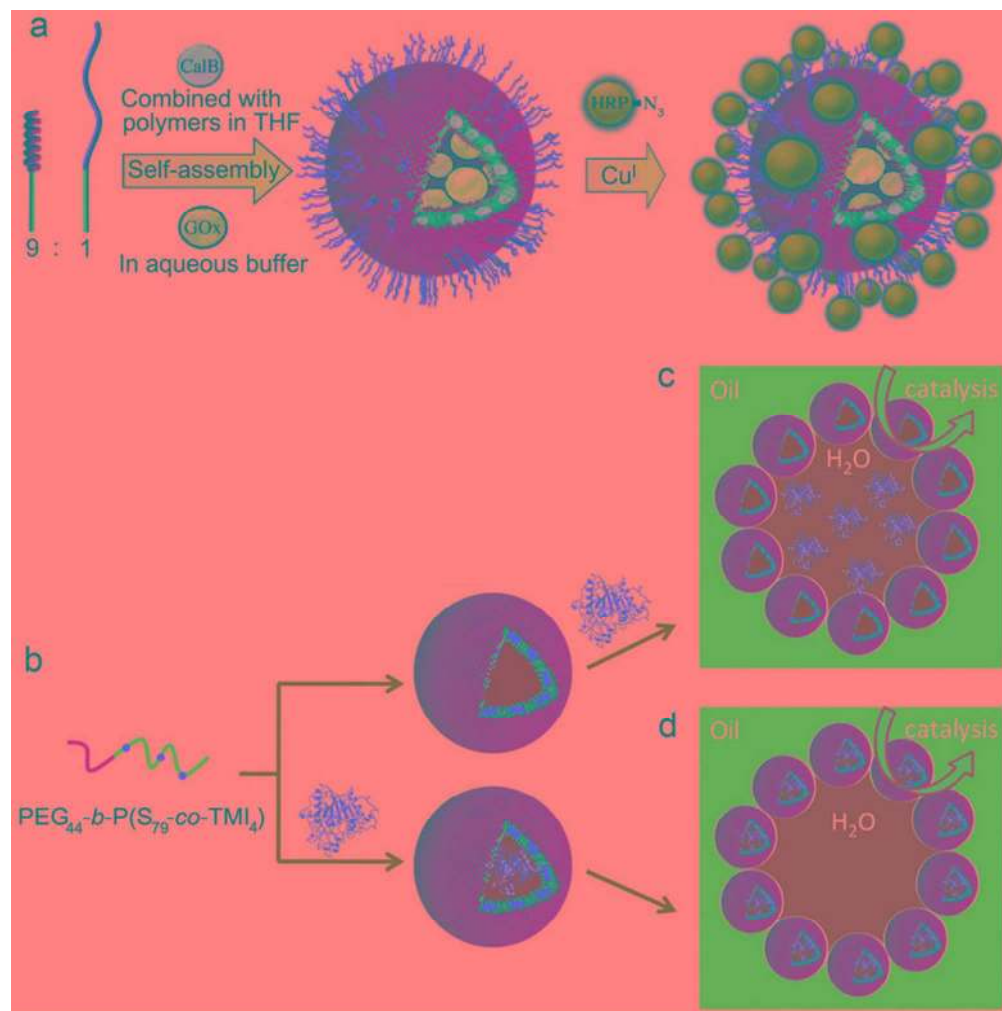
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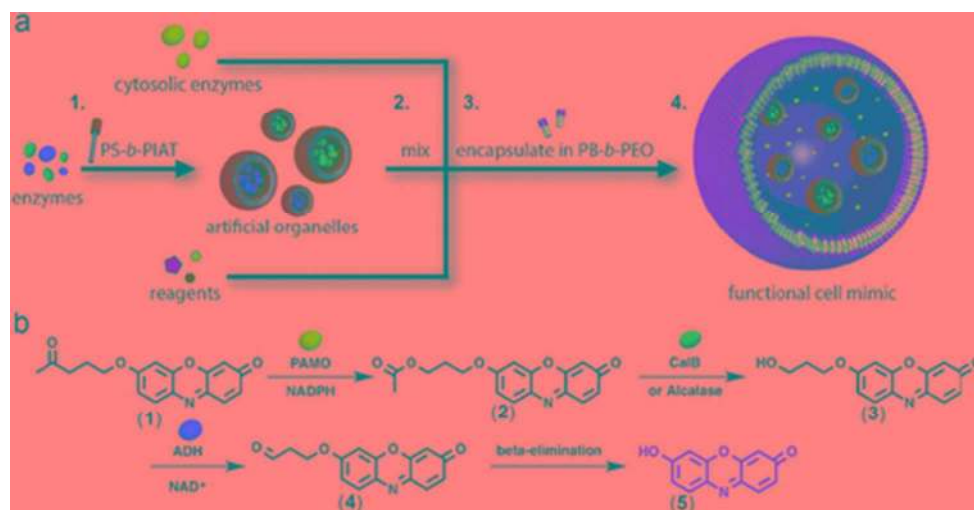
30x11mm (300 x 300 DPI)



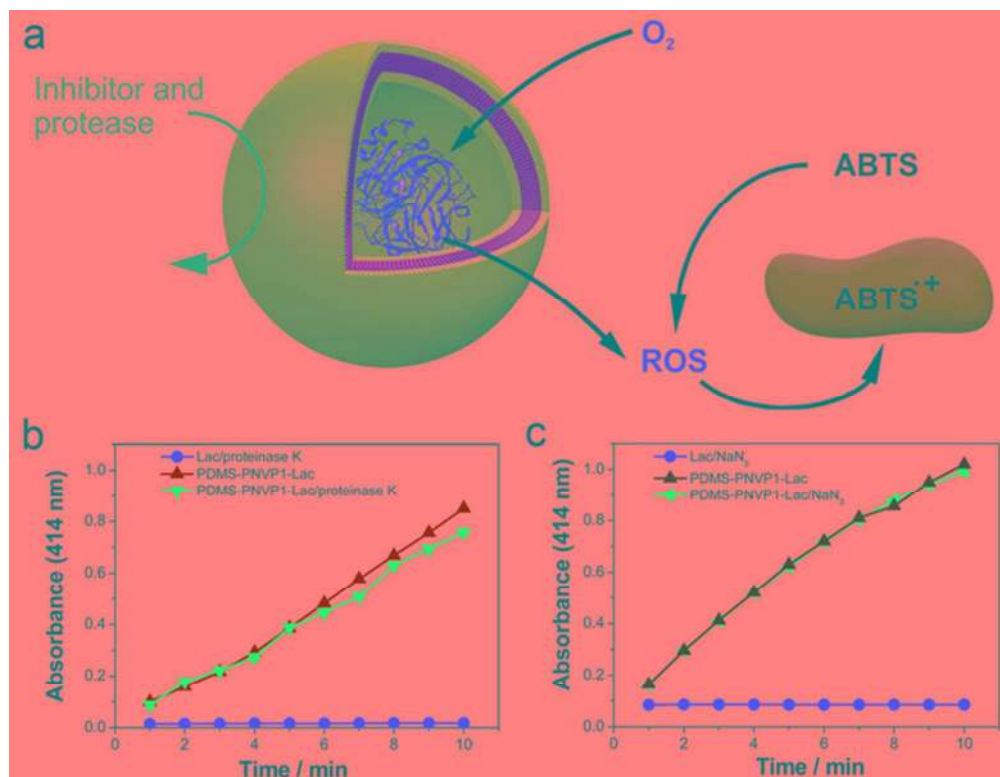
33x13mm (300 x 300 DPI)



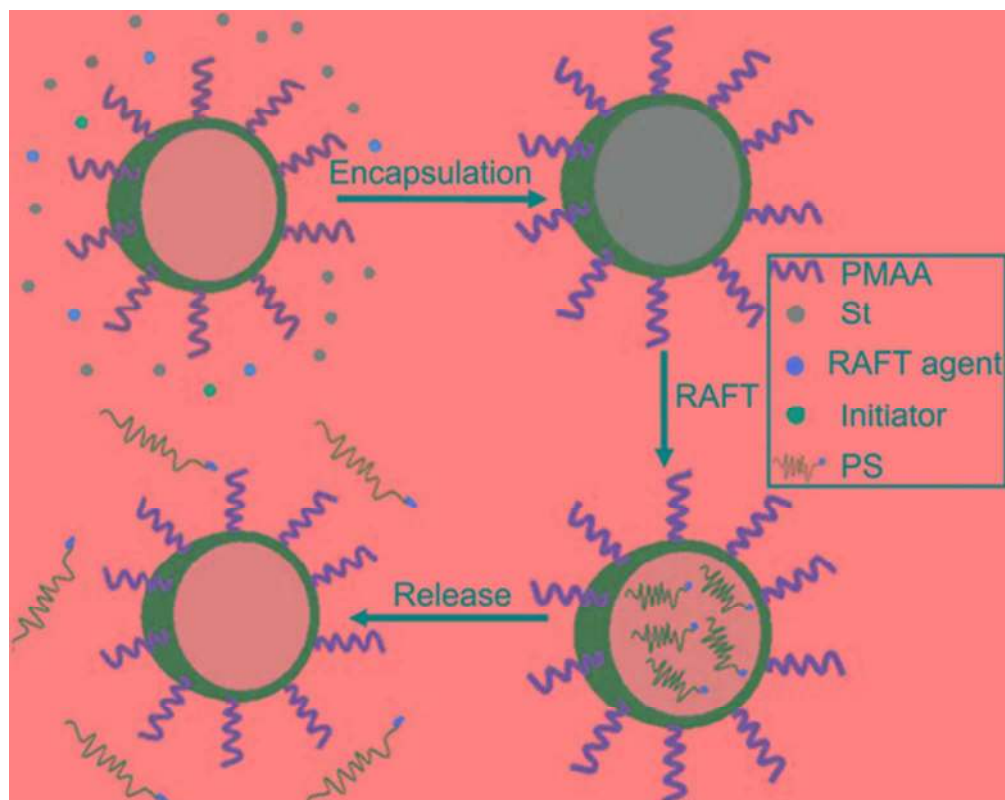
80x81mm (300 x 300 DPI)



41x21mm (300 x 300 DPI)

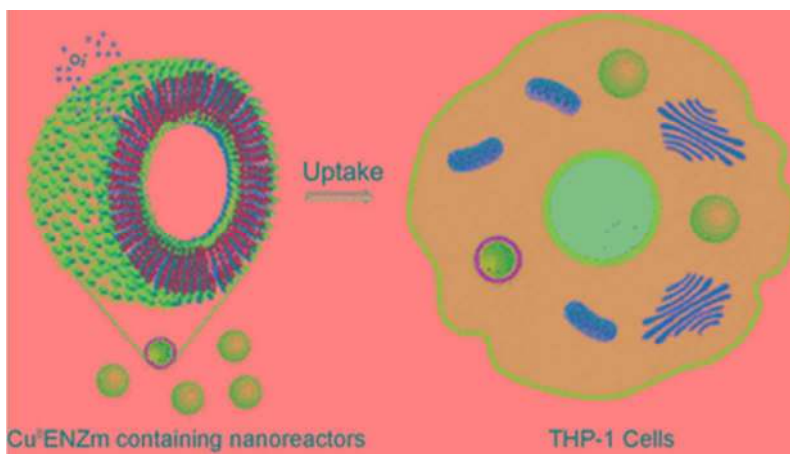


61x47mm (300 x 300 DPI)

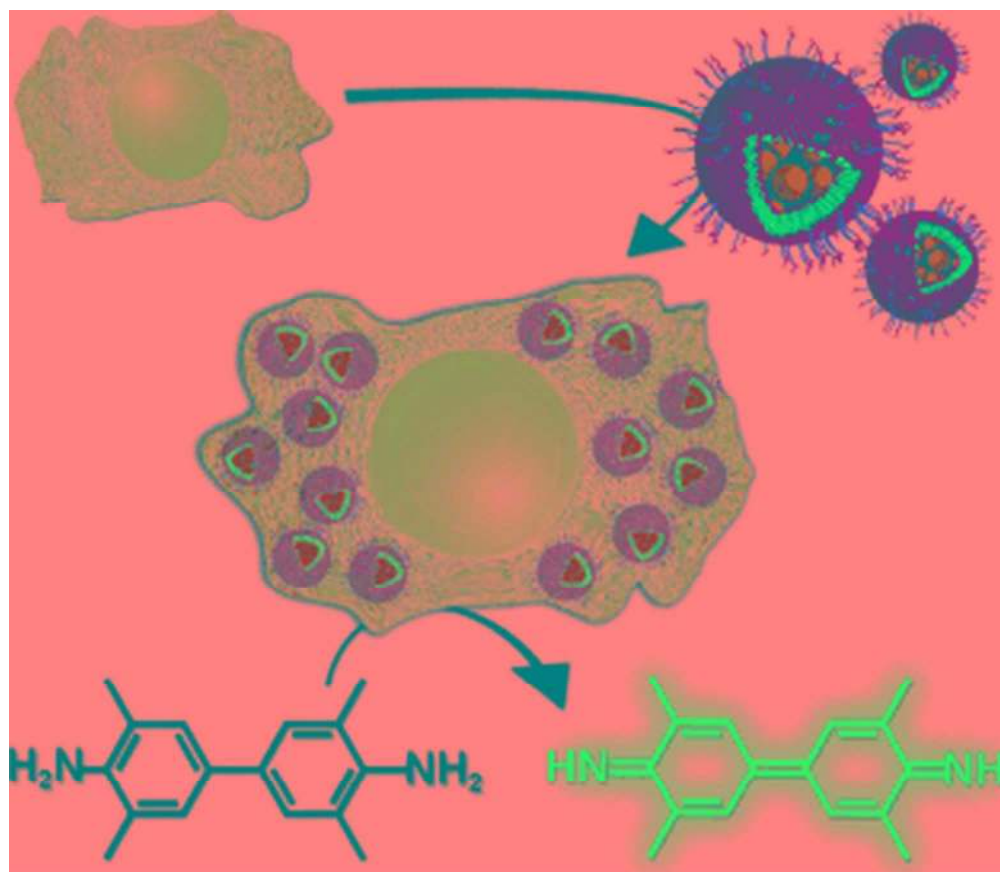


47x37mm (300 x 300 DPI)

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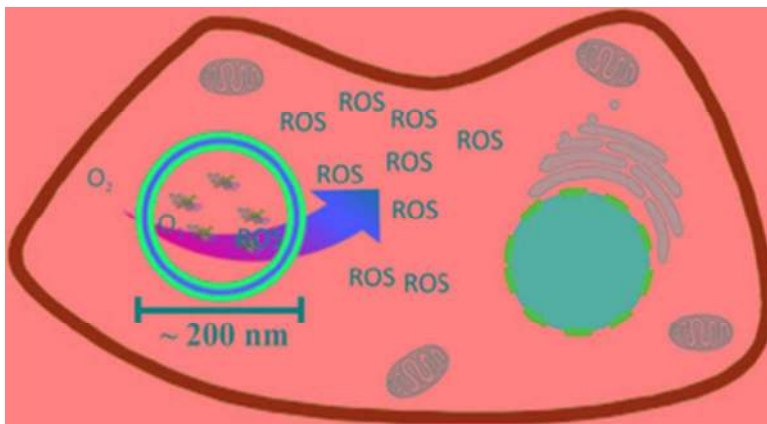


33x18mm (300 x 300 DPI)



51x44mm (300 x 300 DPI)

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32x17mm (300 x 300 DPI)



author picture Gaitzsch
54x54mm (72 x 72 DPI)

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author picture Huang
34x46mm (300 x 300 DPI)

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author picture Voit
138x193mm (300 x 300 DPI)