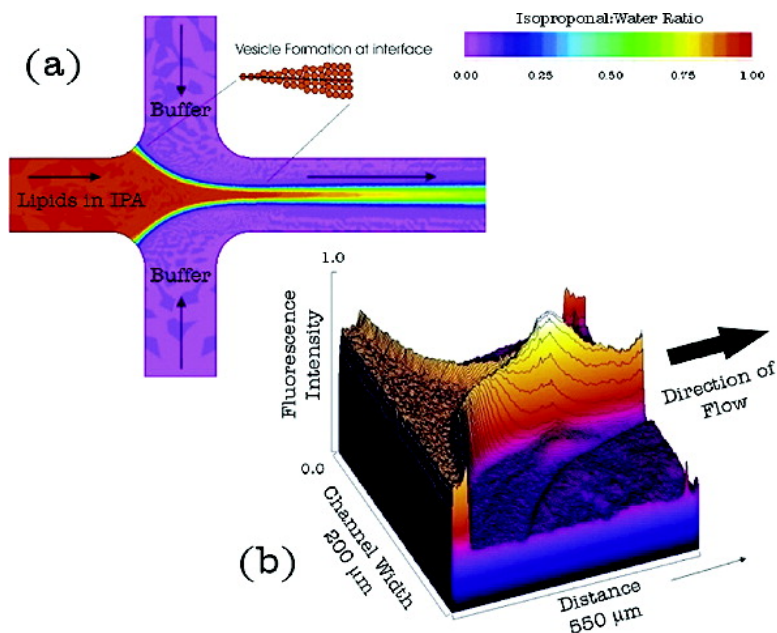Communication

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Controlled Vesicle Self-Assembly in Microfluidic Channels with Hydrodynamic Focusing

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There are a growing number of applications for nanoscale particles in biochemistry that include interrogating,[1] perturbing,[2] and stimulating[3] the cellular environment. The design and production of nanometer-scale objects, such as quantum dots, colloidal particles, and vesicles, can be accomplished in bulk either by chemical synthesis or self-assembly processes. In a cell, chemical synthesis and self-assembly processes are exquisitely controlled by the closely regulated local environment to ensure the reproducible production of nanometer-scale components such as proteins and vesicles. In bulk chemical production methods, the local environment is not well controlled, leading to significant chemical fluctuations, or electrical, mechanical perturbations that often result in inhomogeneous populations of nanoparticles.

Liposomes, a class of nanoparticles that are cellular mimetics, are composed of a lipid bilayer membrane that encapsulates and sequesters an aqueous volume. Of critical importance to the successful implementation of liposomes in vivo for applications such as targeted drug delivery and DNA transfection[4] is the ability to control the liposome size and size distribution, as size influences the clearance rate from the body and ultimately determines the drug dosage. Conventional bulk production modes of liposome preparation require the mixing of two or more phases, liquid−liquid or liquid−solid, resulting in their spontaneous self-assembly into a spherical membrane.[5] These self-assembly processes typically occur in a system with a characteristic length on the order of centimeters, resulting in chemical and/or mechanical conditions that are highly heterogeneous on the length scale of a liposome. This variation often leads to liposome preparations with large polydispersity with respect to size and lamellarity.

Reproducible nanometer-scale synthesis and self-assembly processes require environments that are controllable on the dimension of the particle itself. Microfluidic systems have several characteristics that allow process control at this level. First, in these systems, interfacial forces dominate and bulk inertial forces are often negligible, leading to enhanced heat- and diffusional mass-transfer properties that are naturally observed at the cellular level. Second, the laminar flow conditions in microfluidic channels can be used to create a well-defined and predictable interfacial region between two fluids—a feature that has been used to focus fluid streams hydrodynamically to submicrometer dimensional scales for rapid mixing and patterning.[6] These characteristics allow us to impart control on the nanometer-length scale in a regime that was previously difficult to access experimentally.

Here we report the use of microfluidics to elicit control over the spontaneous self-assembly of liposomes from a solution of dissolved phospholipids. In this work we hydrodynamically focus a stream of lipid tincture at a microchannel cross junction between two aqueous buffer streams as depicted in Figure 1a. In a typical
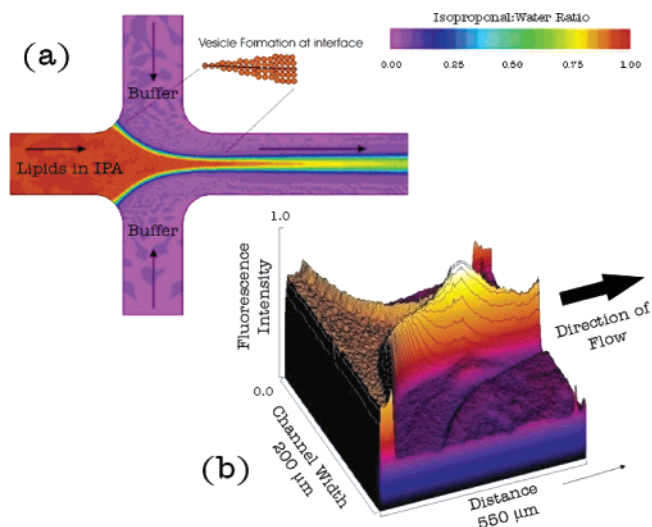
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Figure 1. (a) Schematic of liposome formation process in the microfluidic channel. Color contours represent the concentration ratios of IPA to aqueous buffer. (b) 3-D color contour map of $DiIC_{18}$ fluorescence intensity at focused region during liposome formation.

procedure, isopropyl alcohol (IPA) containing the dissolved lipids plus a fluorescent dye ($DiIC_{18}$) flows through the center inlet channel, and an aqueous phosphate-buffered saline solution flows through the two side inlet channels. $DiIC_{18}$ is a membrane-intercalating dye that exhibits enhanced quantum efficiency when trapped in a lipid membrane as compared to the quantum efficiency of the dissolved dye. The flow rates of the IPA and buffer channels are adjusted to control the degree of hydrodynamic focusing and the width of the center stream, thus controlling the IPA/buffer dilution process. Liposome formation is energetically favorable at points in the system where the concentration of the IPA/buffer mixture reaches a critical condition where lipid solubility is low. As shown in the microchannel fluorescence profile in Figure 1b, the liposomes form initially (as is manifested by the increased fluorescence of the $DiIC_{18}$) along the boundary between the IPA and buffer where a ridge of increased fluorescence is clearly visible. Fluorescence intensity increases to its maximum value immediately downstream of the minimum width of the IPA stream, where the stream is highly focused, indicating the highest concentration of liposomes. Two effects lead to the high liposome concentration here in the system: (i) liposomes formed along the interfacial region follow streamlines and are directed to collect at the center point in the channel, and (ii) at this point the majority of the focused IPA stream has diluted to the critical concentration favoring formation of the more stable liposome vesicle. According to two-dimensional modeling of this flow field with Navier−Stokes and the species convection−diffusion equations, this increase in fluorescence
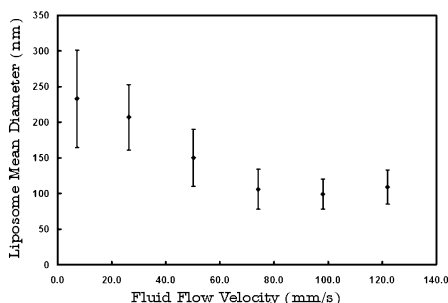
Figure 2. Liposome mean diameter measured by light scattering vs mean bulk fluid flow velocity in the center outlet channel (the combined flow velocities of all inlets). IPA inlet velocity is maintained at 2.4 mm/s while the inlet velocity of each buffer channel is varied from 2.4 to 59.8 mm/s.

intensity is coincident with a maximum isopropyl alcohol concentration of approximately 40 wt % across the microchannel.

Most importantly, we have determined that one can control the liposome size by altering the ratio of the flow rate in the side inlet channels compared to the center inlet channel. As this ratio increases, the magnitude of the shear stresses applied to the liposomes as they self-assemble also increases. This results in a decrease in both the mean size and range (polydispersity) of liposome diameter, as can be seen in Figure 2. Thus, by tuning the flow rates in the microfluidic channel, the physical characteristics of the resultant liposome preparation can be facilely controlled over the range of 100−300 nm. Further, the liposome preparations are more monodisperse in size than liposomes prepared by traditional bulk methods. To our knowledge, this is the first demonstration of the fine control that can be obtained by using microfluidic interfaces to manipulate nanoparticle assembly.

One important characteristic of liposomes is their ability to stably encapsulate molecules in their aqueous cavity. To demonstrate and automate the encapsulation process, a fluorescent dye, carboxyfluorescein (CF), was incorporated into the buffer streams at a concentration of 1 mM. Upon formation of the liposomes at the interface between IPA and buffer, the liposomes encapsulate a discrete volume of buffer, and with that, should entrap dissolved CF. Figure 3 provides the details of the encapsulation process shown in three different images taken of the same microfluidic flow field. In panel (a), immediately apparent is the visible refractive index change at the interface between the alcohol and aqueous phases that dissipates as the two phases interdiffuse. Panel (b) shows the fluorescence of the $DiIC_{18}$ in the microfluidic network. The hydrodynamic focusing of this stream is clearly visible, as is the bright fluorescent spot that corresponds to maximum liposome concentration. It is noted that the $DiIC_{18}$ stream does not get significantly wider as it migrates down the microchannel due to the incorporation of the low-molecular weight $DiIC_{18}$ into liposomes, which have diffusion coefficients that are orders of magnitude lower than the dissolved lipids and dye molecules. Panel (c) shows CF contained in the buffer phase in the side inlet streams. We have determined that liposomes that self-assemble in this simple process encapsulate high concentrations of CF as determined by confocal fluorescence microscopy (presented in Supporting Information).
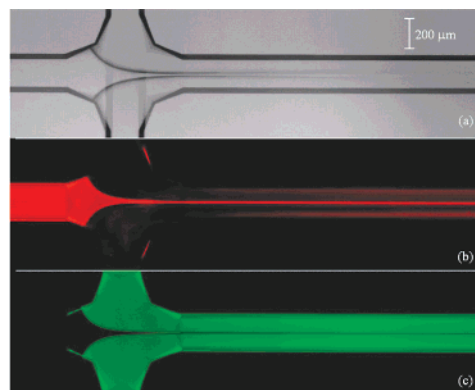

Figure 3. (a) White light image of hydrodynamic focusing of IPA by buffer streams. Fluorescence images of (b) $DiIC_{18}$ in IPA stream and (c) CF in buffer streams. Silicon/glass microchannels have trapezoidal cross sections with the following dimensions; depth = 40 μm, maximum width = 200 μm, minimum width = 147 μm.

In conclusion, we have determined that by manipulating the length scale and the shear forces applied to liposomes upon formation using microfluidics, tightly controlled liposome populations are produced without the need for subsequent processing steps to modify liposome size. The liposome self-assembly strategy described here could be implemented for on-demand drug encapsulation and delivery and is readily scaled up with the development of multichannel microfluidic systems. We predict that the synthesis and self-assembly of nanoscale particles for other applications in nanotechnology will also greatly benefit from adaptations of this approach.

Supporting Information Available: Liposome formulation, microchannel fabrication, flow control (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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JA0318030