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On the role of electrostatics on protein-protein interactions

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Abstract

The role of electrostatics on protein-protein interactions and binding is reviewed in this article. A brief outline of the computational modeling, in the framework of continuum electrostatics, is presented and basic electrostatic effects occurring upon the formation of the complex are discussed. The role of the salt concentration and pH of the water phase on protein-protein binding free energy is demonstrated and indicates that the increase of the salt concentration tends to weaken the binding, an observation that is attributed to the optimization of the charge-charge interactions across the interface. It is pointed out that the pH-optimum (pH of optimal binding affinity) varies among the protein-protein complexes, and perhaps is a result of their adaptation to particular subcellular compartment. At the end, the similarities and differences between hetero- and homo-complexes are outlined and discussed with respect to the binding mode and charge complementarity.

Keywords

electrostatics; protein-protein interactions; pH; salt; nsSNPs; missense mutations

1. Introduction

The living cell is an extremely complicated system and is comprised of hundreds of thousands of types of biological macromolecules, which constantly interact with each other to maintain the function of the cell, reflecting the dynamics of cellular networks [1, 2]. The interactions are very specific and frequently a particular protein macromolecule is able to recognize its partner among hundreds of thousands of candidates [3]. At the same time, the recognition process is fast and thus some protein-protein interactions may be electrostatically guided, perhaps through a long-range force that selects and brings the interacting partners together [4–6]. The best candidate for such a guiding long-range force is the electrostatic force [7–12]. A rough estimate of the electrostatic energy of interaction between two molecules carrying a unit net charge and positioned at a distance 10A away from each other results in almost 1 [KJ/mol], which is much more than any other energy component contributes to the binding at such distances.

Thus, electrostatic forces and energies are essential for the interactions of virtually all biological macromolecules [13–15] (see also excellent review [16]). The central role of electrostatics is due to the fact that most biological macromolecules, especially DNA and RNA, are highly charged. However, they are not easy to calculate because the association occurs in a water phase at specific salt concentration and pH. In this review we first briefly outline the current continuum methods for computing the electrostatic component of the

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binding free energy. In addition to contributing to the binding free energy, the long-range electrostatic interactions can steer protein molecules toward their pre-binding orientations. However, some complexes are formed of identical macromolecules (homo-complexes), while others involve different entities (hetero-complexes). The main difference between these two cases is the net charge of the monomers, which for homo-dimers is the same for both monomers, while for hetero-complexes the monomers frequently carry opposite net charges. Such a difference is expected to result in different roles of electrostatics on the protein-protein recognition at very large distance, at which the distribution of the charges is not important but the net charge is. As it was mentioned above, charged groups are essential for the interactions of macromolecules, but their probability to be charged (directly connected to their pKa's) is frequently perturbed in proteins and RNAs due mostly to electrostatic effects. Moreover, the binding itself can induce ionization changes resulting in proton uptake/release. In addition, the ionization phenomena are strongly affected by the pH and ion concentration in the water phase, which are specific for each subcellular compartment. Here we review the progress made in understanding the role of pH and salt on the macromolecular binding and the possibility that the macromolecular association is adapted to the subcellular microenvironment. At the end, we outline the findings related to the effects of single amino acid substitutions (resulting from either disease-causing mutations or non-synonymous single nucleoside polymorphisms, nsSNPs, found in the general population) on the electrostatic component of the binding free energy.

2. Modeling the electrostatic component of binding free energy

To investigate the role of electrostatics on protein-protein association, the electrostatic component of the binding free energy needs to be accurately calculated. However, there are three major obstacles in *in silico* modeling of the binding free energy of biological macromolecules: (1) the binding occurs in a water phase consisting of millions of water molecules whose positions and orientations vary dynamically [17–19]; (2) the unbound and bound monomers are also dynamical structures existing as an ensemble of structures, and these ensembles could be quite different for unbound versus bound monomers [20–25], and (3) the charged states of the ionizable groups may be non standard in the unbound and bound monomers and even may change due to the binding [26–30]. Taking into account rigorously the above effects in the computational protocol is a challenge.

Computational methods for evaluating the binding free energy can be grouped into two major classes with respect to the treatment of the water phase: explicit and implicit. Explicit models consider the water phase as a sea of explicit water molecules which are allowed to sample different positions and orientation during the calculations of the binding free energy. Typically the corresponding monomers and protein-protein complex are allowed to explore different conformations as well. While such an approach is assumed to better represent the physical reality of protein-protein binding, in this review we will focus on implicit methods of computing the electrostatic component of the binding energy (see Ref. [31–34] for comparison between explicit and implicit treatment). The focus on the implicit model is due to the speed of calculations, the ability to handle large systems (large protein-protein complexes), and the lack of convergence problems [35]. It should be mentioned that hybrid approaches have also been developed that treat bound waters and ions explicitly while the bulk water and ions are modeled as homogenous medium with the corresponding ion concentration [36–39].

In a continuum framework, the electrostatic potential in a system comprised of biological macromolecules (modeled as low dielectric entities) immersed in water (high dielectric medium) and in the presence of mobile ions obeys the Poisson-Boltzmann equation (PBE) [40]. However, because of the irregular shape of the proteins and protein-protein complexes,

the PBE must be solved by the means of numerical algorithms [41]. As an alternative to the PBE, the Generalized Born (GB) approach can be applied as well [42–46], although with less impressive accuracy [47]. A typical scheme for computing the electrostatic component of the binding free energy is shown in Fig. 1. For each state (the unbound and bound monomers) the electrostatic energy components are obtained, which in terms of PBE formalism are typically the Coulombic energy of interaction; the interaction energy solvent-water (solvation energy) and the interaction energy of solvent-ions (termed ionic contribution to the energy of the system) [48]. In the case of solving the non linear PBE, the osmotic pressure and electrostatic stress terms must be added to the total electrostatic energy as well [49]. Currently there are many available computer programs designed to deliver these energy terms such as DelPhi [48, 50], Adaptive Poisson-Boltzmann Solver (APBS) and its variants [51–53], and many others [54], including GB based solutions [55–58]. In particular, the DelPhi program provides very convenient output of the abovementioned energies, and thus their individual contributions can be evaluated separately [48, 50] and because of this, DelPhi is extensively used for modeling electrostatic interactions [58–60].

In applying such computational protocols, the conformational changes can either be taken into account or not (Fig. 1). In the first case, the method is termed unbound-bound calculations, while if the structures of unbound monomers are considered to be the same as in the bound state, the protocol is called bound-bound calculations. In both cases, however, the 3D structure of the protein-protein complex must be known by either experimental means (X-ray or NMR experiments) or must be predicted *in silico* [61, 62], however, the last case was not extensively explored in the past due to the significant structural imperfections generated in the models predicted by *ab-initio* docking methods [63, 64]. As an alternative, accurate models of the 3D structures of protein-protein complexes can be delivered by homology methods using highly homologous templates [65–69]. In the bound-bound approach, sometimes termed “rigid body approach”, the structures of unbound monomers are taken from the protein-protein complex and no conformational changes upon the binding are assumed (solid lines in Fig. 1). The advantage of such an approach is that the internal mechanical energy remains the same for bound and unbound states and needs not to be calculated.

Instead, only the non-bonded energies must be accounted for, the most important one being electrostatics. The disadvantage is that such a model is quite simplified and may not be able to capture all important effects associated with protein-protein association. The bound-unbound approach is much more appropriate (solid lines for the complex and dashed lines for the unbound monomers in Fig. 1), but involves changes of the internal energy of the interacting partners, which is difficult to calculate. However, if we are only interested in the electrostatic component, then the computation is much easier provided that the bound and unbound structures are available. In addition, we are not interested in the absolute energies, but either salt or pH dependence (for most cases). Thus, the change of the internal energy from a bound to unbound state will be a constant contribution that will not affect salt and pH dependence.

The role of protonation states (ionization states) of titratable groups on the calculations of the electrostatic component of the binding energy is obvious. An inappropriate charge assignment to a given ionizable group could affect the calculations dramatically. Therefore, in the best case scenario, pKa calculations must be carried out on the bound and unbound monomers and charges assigned according to the predicted pKa’s. Such predictions are typically not performed prior to the calculations of the electrostatic component of the binding energy because of the assumptions that titratable groups of unbound monomers are charged at neutral pH and do not alter their protonation states upon the binding. Such a protocol can be termed “rigid” charge protocol. While the presumption that all (or most of)

titratable groups are ionized in unbound monomers holds in many cases, it frequently may not be valid for the bound state (the protein-protein complex), especially for the titratable groups at the interface of the complex. Thus, the net charge of the complex and unbound monomers may differ resulting in proton uptake/release upon the binding. A protocol that takes into account such a possibility can be termed a "flexible" charge approach (Fig. 1), as for example used in Ref. [26].

3. Role of pH and salt concentration

The role of salt and pH on protein-protein binding was recently reviewed [70]. The importance of proton uptake/release in receptor-ligand interactions is demonstrated by the experimental observation that the vast majority of receptor-ligand interactions are pH-dependent [71–76]. A variation of several pHs can result in binding free energy changes of several KJ/mol [76, 77] or even can change the ligand binding preferences [78]. Even more, different binding interactions can occur at different pHs; for example, as found in the case of beta-lactoglobulin that is a dimer at low pH but forms a tetramer at high pH [79]. Similar phenomena were found in the case of calmodulin, whose domains adopt compact arrangement at low pH while at high pH form "dumbbell" shaped structures [80–82]. From a practical perspective, the ability to re-engineer enzymatic pH-activity profiles is important for the industrial application of enzymes [83]. This possibility has been theoretically and experimentally explored to re-engineer enzymatic pH-activity profiles and pH-dependence of kinetic parameters by changing active site pKa values using point mutations [55, 84–87].

The effect of salt concentration on protein-protein binding free energy and its electrostatic component can also be significant. To the best of our knowledge, the E9 Dnase – Im9 complex exhibits the largest sensitivity to the salt concentration, resulting in $\delta\Delta\Delta G(I)/\Delta\ln(I) = 8[\mathrm{KJ/mol}^2]$ [88, 89] (where $\Delta\Delta G(I)$ is the change of the binding free energy and I is the ionic strength). Thus, a small variation of the salt concentration alters the binding energy by several KJ/mol. Another example is of the complex formed between β-Lactamase (TEM-1) with its protein inhibitor (BLIP), in which the association rate decreases an order of magnitude as salt concentration increases from zero to 0.5M [90]. The list of examples can be extended further to include other complexes with experimentally measured salt dependence of the binding energy [91–94], indicating that most of the protein-protein interactions are affected by the ion concentration in the water phase.

There are significant similarities and at the same time, significant differences of the origin of pH and salt dependence effects (Fig. 2). The overall proton uptake/release induced by protein-ligand association originates from individual pKa shifts of titratable groups induced by the binding. These shifts are typically associated with titratable groups located at the interface of the complex and thus experiencing either significant desolvation energy (Fig. 2a) or being involved in new interactions upon the complex formation (Fig. 2b). In contrast, the salt dependence of the binding originates from the difference of the charges-ions interaction prior to and after binding, i.e. mostly from the difference of the solvent exposure of the charges. This effect is similar to the desolvation effect and thus was termed the de-saltation effect [95] (Fig. 2c). However, the effect depends on charge-charge interactions as well, since new charge interactions can alter the ion distribution in the water phase and thus change the charges-ions interaction (Fig. 2d). Even more complicated cases can occur, when the binding induces ionization change, which in turn affects the salt dependence of the binding affinity.

Salt dependence of protein-protein binding was modeled in the past and compared with experimental data to assess the accuracy of the computational methods. It was shown that Poisson-Boltzmann formalism is capable of capturing non specific salt effects with very

good accuracy [95]. The limited cases examined indicated that almost always the increase of the salt concentration makes binding weaker, both experimentally and computationally. Sequential *in silico* studies on a much larger dataset comprised of 1482 complexes confirmed such a tendency for hereto-complexes, and to a certain extent for homo-complexes as well [96]. The observation that the increase of the salt concentration weakens the protein-protein association in the vast majority of the cases was attributed to the charge-charge optimization at the interface of the interacting partners [97, 98]. Thus, the increase of the salt concentration increases the screening of these favorable interactions and weakens the binding, despite of the net charge of monomers.

The pH-dependence of protein-protein binding is a result of the pKa shifts induced by the binding. Such pKa changes were investigated *in silico* for the binding of antibiotics to small ribosome units [99], for the enzyme active sites [27], for the kinase-ligand binding [100] and for the protein kinase systems [101]. The pKa changes were calculated to occur upon hirudin-thrombin binding [49] and the binding of small ligands to HIV protease [40, 102]. A recent study on 37 protein-protein complexes showed that in a vast majority of the cases the average pKa shifts for acidic residues induced by the complex formation were negative, indicating that complex formation stabilizes their ionizable states, whereas the histidines were predicted to destabilize the complex [103]. PROPKA method was used on 75 protein-protein complexes, and their corresponding free forms, to model changes in the protonation state of individual residues, and net changes in the protonation state of the complex relative to the unbound proteins [28]. It was concluded that protein-protein binding at neutral pH is often associated with changes in the protonation state of amino acid residues and with changes in the net protonation state of the proteins. Similar observations were made and outlined in a recent review article [104], including the proton uptake/release at pH different from neutral pH. It should be mentioned that proton uptake/release is a pH dependent effect and the pH-dependence profiles are different for each protein-protein complex, perhaps, reflecting the adaptation of each interaction to the environment where the binding is supposed to occur. The possibility of such an adaptation toward the subcellular environment is discussed in the next paragraph.

4. Adaptation of protein-protein interactions to subcellular characteristic pH

The typical pH-dependence profile of protein-ligand free energy of binding is bell shaped and has an absolute minimum which is termed the pH-optimum of binding. At pH-optimum proton uptake/release is zero and the binding affinity is maximal as indicated by the general formulation of proton binding [70] $\Delta G(pH)=2.3RT\int_{pH_0}^{pH_1}\Delta Q(pH)d(pH)$. To perform their function, protein complexes should be able to tolerate small fluctuations of pH of the environment, which will require that ΔQ be zero or close to zero at the characteristic pH of the subcellular location where the protein complex is supposed to be formed. The characteristic pH varies among cellular organelles as shown in Fig. 3 (a collection of characteristic subcellular pHs can be found in the works of Warwicker and coworkers [105, 106]) and thus the proteins and protein-protein complexes existing in there should have evolved to perform their function at these characteristic pHs (see excellent review [107]). Such a possibility was investigated on a set of 37 proteins, using rigid body approach and it was found that the pH-optimum of binding is correlated with the pH-optimum of stability of individual monomers [103]. Later, similar investigation was carried out, but accounting for the conformational changes induced on the binding and it was confirmed that the correlation does exist between pH-dependent properties of the complex and unbound monomers [108]. It was noticed that the pH-optimum, either of binding or of stability, may not necessarily

have to be similar among the binding partners and the complex, but rather they should have similar pH region in which their properties are almost not affected by small fluctuations of the local pH (termed pH-flat). The existence of such a pH-flat region would indicate that the protein-protein complexes are insensitive to small changes of the local characteristic pH, however, there are examples of proteins and protein-protein complexes which function is to sense and respond to the changes in cellular pH [109–111]. Obviously, such protein-protein interactions must not to be independent of pH and should not have pH-flat region, instead their affinity should be strongly affected by the pH changes [109, 112, 113].

5. nsSNP modulating of the electrostatic component of the binding free energy

Non-synonymous single nucleoside polymorphism (nsSNP) and in general, any missense mutation results in a change of the amino acid composition of the target protein. Frequently, such changes involve charged or polar amino acids and thus affect the net charge or the dipole moment of the protein. Specifically, if the site of the mutation is located at the protein-protein interface, even a minor change of the charge distribution or the volume of the side chain can induce large effects on the electrostatic component of the binding free energy. Recently an analysis of 264 protein-protein complexes with nsSNPs located at the protein-protein interface was reported [114], with the goal to discriminate the effects caused by mutations found in the general population (non-OMIM) versus mutations known to cause diseases (OMIM). It was found that disease-causing mutations tend to electrostatically destabilize the corresponding protein-protein complexes, while non-OMIM mutations do not exhibit such a trend. An opposite effect was predicted for the HER2 receptor binding to herceptin, where the deleterious nsSNP introduced an additional 2 hydrogen bonds compared with the wild type and thus enhanced electrostatic interactions across the interface of the complex [115]. In contrast, series of charged amino acid missense mutations in the DDR2 gene were found to reduce the ability of the corresponding protein to bind collagen [116]. Since all mutations involve charged residues, the reduction of affinity should be due to change of the electrostatic component of binding free energy. Reduced binding was found to occur also in the case of introducing extra charge on the SHH gene, which in turn affects the corresponding protein affinity to GAS1 [117]. Reduced affinity was found as well in the case of a missense mutation in the BRCA1 gene, which results in an extra positive charge in the binding pocket [118]. Another report on missense mutations in the same gene, the BRCA1 gene, indicated that a charged residues replacement can either reduce or increase binding affinity to p53 [119]. Our recent study on spermidine sythase (SMS) dimerization showed that even a missense mutation introducing polar residue at the interface of the dimer can greatly reduce dimer affinity [120]. Further *in silico* analysis on the dimerization of the SMS suggested that the effect of the mutation depends not only on the change in charge, but can be amplified by the local electrostatic field created by other, even distant, charges (Zhang *et al*, PLoS Comp. Biol., submitted). This limited set of examples indicates that the effect of nsSNPs on the electrostatic component of the binding free energy depends on many factors, with the primary being the polarity of the charge, the local potential and the ability of the newly introduced side chain to rearrange and form new interactions. However, in terms of being disease-causing or harmless, any significant deviation of the wild type binding affinity (decrease or increase of the affinity) was found to cause disease.

6. The role of electrostatics in the cases of hetero- and homo-dimers

The major difference between hetero- and homo-complexes is that hetero-complexes are made of different proteins, which can carry opposite net charge, while homo-complexes being comprised of two identical units can not. Because of this, the role of electrostatics in steering the monomers toward each other is distinctively different for hetero- and homo-

complexes. Since in most of the cases the monomers forming a hetero-complex carry opposite net charge, the electrostatics will cause an attraction, while it will be just the opposite for homo-complexes. However, this statement should be clarified since bringing two proteins in close proximity of space does not necessarily help the binding, since binding interfaces may not be positioned properly. Thus, at short distances, the role of electrostatics will depend on the charge distribution, rather on the global net charge of the monomers. Finally, the electrostatic component of the binding free energy will depend on the delicate balance between desolvation energy and pair wise interactions across the interface. A recent study on 260 hetero- and 2148 homo-complexes revealed that electrostatics is predicted to oppose binding in about 80% of the homo-complexes, and this percentage varies from 43% up to 85% for hetero-complexes, depending on the protocol and force field used to make the predictions [121]. The difference is not very significant. However, it should be mentioned that homo-complexes can be formed by two distinctive binding modes: (a) a binding mode that results in the formation of the interface made of identical amino acid interactions across the interface and (b) a binding mode resulting in non-identical interfaces, similar to the interfaces found in hetero-complexes. Much more detailed classification, comprised of (a) cyclic-oligomer, (b) twisted-dimer, (c) dimer-parallel, (d) dimer-perpendicular and (e) dimer-circular was introduced in Ref. [122]. The abovementioned study [121] did not distinguish between these two cases, and perhaps this is the main reason that the predicted effects for hetero- and homo-complexes are not significantly different. In addition, many proteins are know to form both hetero- and homo-complexes at the same time [123–126] and the role of electrostatics will depend on the type of complex formed and the corresponding interface. It was suggested that electrostatics is specifically optimized to avoid homo-complex interactions in the case of LIN-2/7 domains [127]. The role of electrostatics interactions was also demonstrated for homo- and hetero-tetrameric hemoglobin formation [128]. A recent study on 393 non-redundant homo-oligomer interfaces emphasizes the importance of electrostatic complementarity at the interfaces, rather than the global properties on the monomers [122]. This study also delivers electrostatic rules for discriminating the biological-interface from the crystallographic-interface. Apparently the role of electrostatics will depend not only on the type of the complex, hetero- or homo-, but also on the type of binding modes in the case of homo-complex formation (see Ref. [129] for the evolutionary preferences of homo-complexes to form symmetrical and asymmetrical interfaces). It can be expected that in the case of a parallel homo-complex, the electrostatics will always oppose the binding.

5. Conclusions

In this review we outlined the continuum methods for modeling the electrostatic component of the binding free energy and its role on protein interactions and binding. Using a continuum electrostatics framework instead of an explicit water model is typically justified by the need to calculate large number of cases to provide the statistical significance of the obtained results. Even nowadays computing binding free energies with explicit water model for several hundreds or thousand protein-protein complexes is computationally infeasible. Thus, continuum methods must be applied, which despite their macroscopic nature were shown to be quite accurate in describing the electrostatic component of the energy. At the same time, intrinsic limitations of continuum models will result in non accurate energies when explicit waters or ions play significant roles in protein-protein binding. Perhaps, the tradeoff is to use hybrid models [37, 38, 130], which treat the important waters and ions explicitly, while the rest of the water phase is described with the continuum approach.

This review demonstrated that salt- and pH-dependent phenomena, which are mostly electrostatic in origin, can greatly affect protein-protein interactions. Such effects can naturally be modeled with the continuum electrostatics approach as implemented in the PB

equation in conjunction with a corresponding pKa algorithm, as with Multi Conformation Continuum Electrostatics (MCCE) [131–133]. As an alternative, the explicit water models, for example constant pH MD methods [134–136], require much more computational resources since the calculations must be repeated at each pH. In addition, the pKa calculations based on the continuum model are still believed to be more accurate than the corresponding algorithms using explicit water representation [137, 138]. However, if necessary to treat some waters or ions explicitly, hybrid approaches do exist, which treat the buried ions and water molecules explicitly within the calculations of the ionizable states [131–133].

Since the characteristic salt concentration and pH vary within subcellular compartments, the pH and salt dependence of protein-protein binding free energy can be associated with a subcellular localization of protein-protein complexes. Protein-protein complexes existing in a particular environment should be able to tolerate its characteristic pH and salt concentration. This review indicates that there is growing evidence that the monomers forming the complex should have similar pH properties and this can be used to transfer subcellular annotations from one partner to another.

The progress in genome sequencing offers vast amounts of data regarding missense mutations, both disease-causing and harmless mutations. It was shown that such mutations may affect the electrostatic component of the binding free energy and thus affect the cellular interaction network. The ability to model such effects will be crucial in understanding the molecular effects associated with disease-causing mutations and in the long run, assist in developing treatments for the corresponding diseases.

This review also outlines the similarities and differences of the electrostatic interactions in hetero- and homo-complexes. While the hetero-complexes are formed by two different molecules, the homo-complexes result from the binding of two identical (on a sequence level) molecules. Frequently, each molecule in the homo-dimer has its own reaction cite, but the biological function occurs only after the dimer is formed. Perhaps the analysis of the electrostatic interactions within the homo-complexes, as contrasted to hetero-complexes, may shed light and may provide some clue for such cases. It may be speculated that the formation of the homo-complex is necessary in order to provide favorable electrostatic environments for the corresponding biological reaction.

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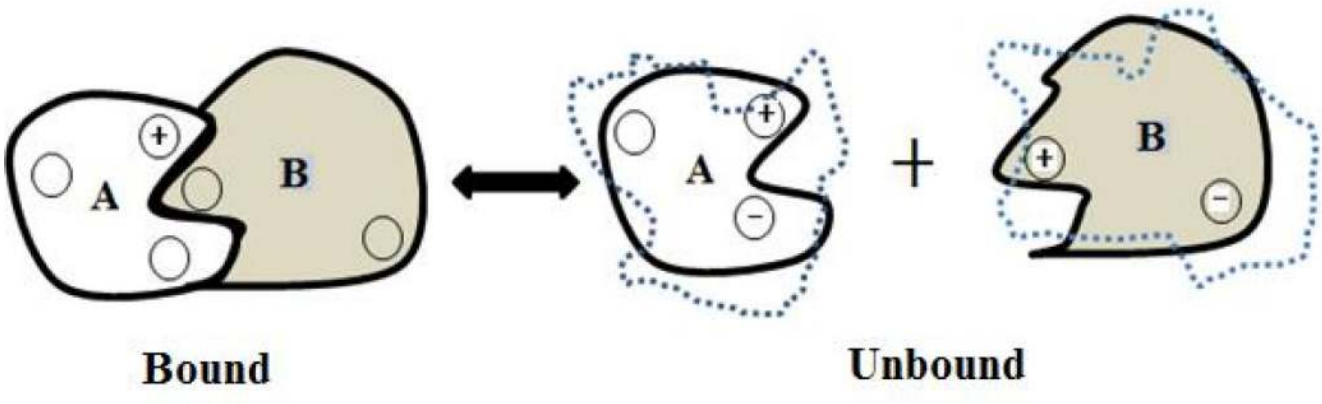

Fig. 1.
Schematic representation of "rigid" body binding concept (solid lines) and "flexible" binding (dash lines).

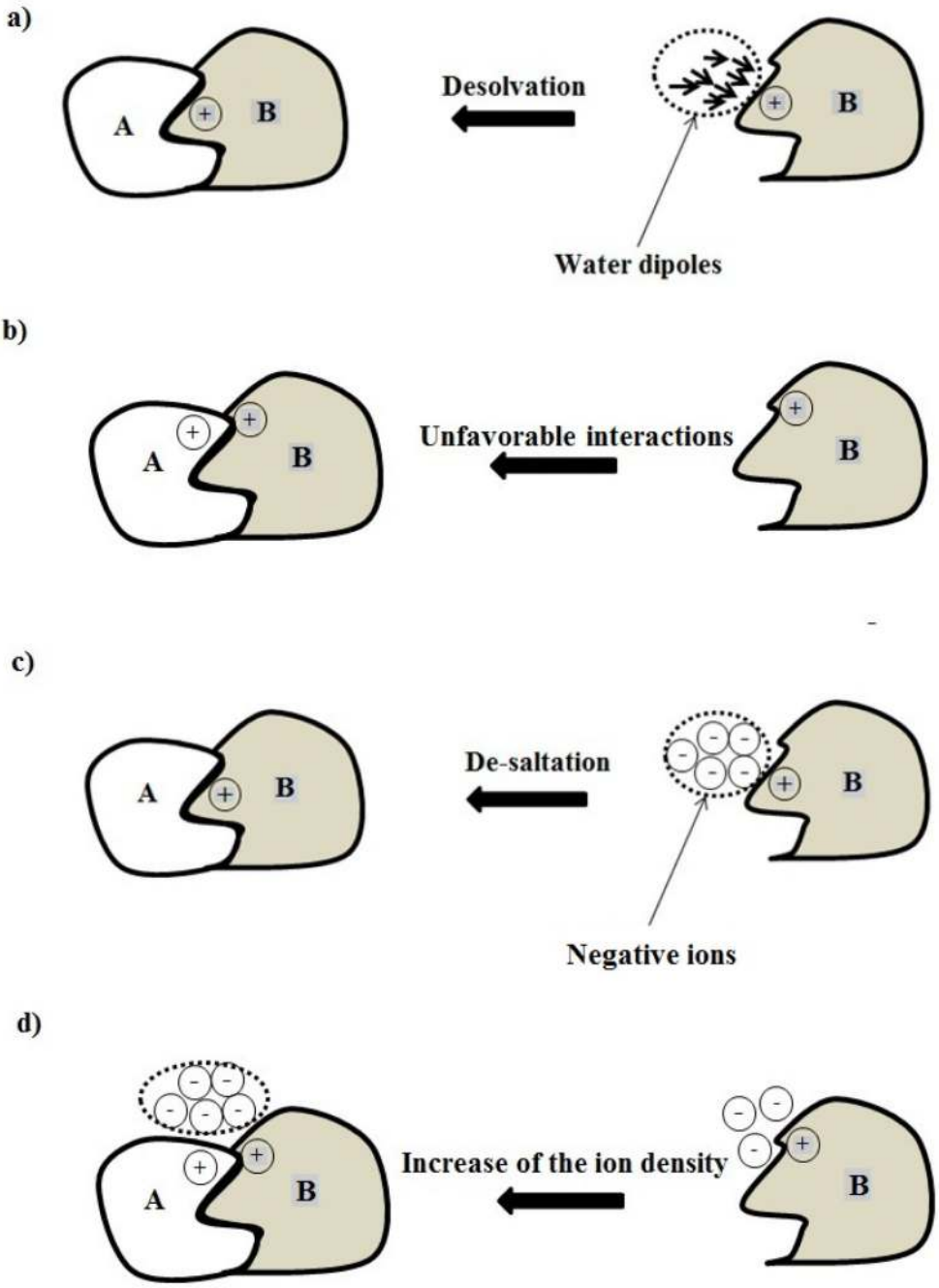

Fig. 2.

Schematic representation of the major effects associated with the calculation of pH and salt dependence of the electrostatic component of the binding energy.

(a) desolvation effect, which originates from the removal of the charge-water interactions upon binding.

(b) establishment of new interactions upon complex formation.

(c) de-saltation effect, which originates from the removal of favorable charge-ion interactions upon the binding.

(d) change of the ion distribution in the water phase upon complex formation.

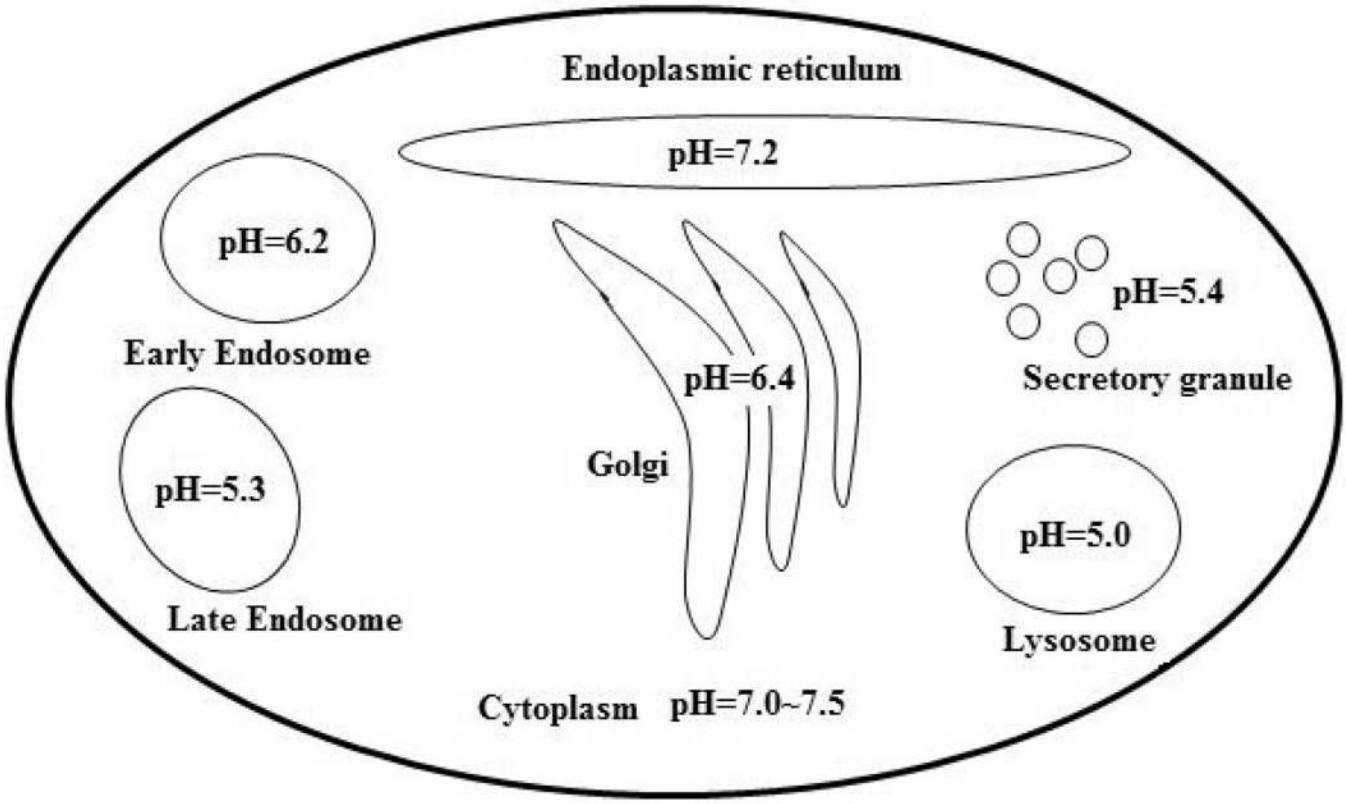

Fig. 3.
Schematic representation of a cell with several compartments with the corresponding characteristic pHs.