**Self-assembly in amphiphilic macro-molecules with solvent exposed hydrophobic moieties**

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**Abstract:**

Self-assembly at nanometer length by amphiphilic molecules with solvent exposed hydrophobic groups are relevant in neurodegenerative disorders as well as in nano-bio technology. Here we study such self-assembly in these systems using a model system of spherical particles having charge at core but solvent repelling surface, using Monte-Carlo simulations and mean field treatment. We find that solvophobicity mediated attraction leads aggregation, while electrostatic repulsions control stability of finite clusters. The aggregation threshold relates the parameters of two interactions through an algebraic dependence. The study also qualitatively explains experimental observations on aggregation of misfolded proteins and can be useful guide to tune stability of nm sized self-assembly in systems with exposed hydrophobic groups.

Self-assembled structures at nanometer (nm) length scale are often formed by amphiphilic macromolecules with hydrophobic parts exposed to water [1-3](#_ENREF_1" \o "Dehsorkhi, 2014 #773), just opposite to their normal tendencies. Such self-assembled structures have applications in material science [4-6](#_ENREF_4" \o "Moreno-Gonzalez, 2011 #3266), and bio-molecular systems as well [6-9](#_ENREF_6). Potential applications of these systems require control over cluster sizes. Macro-molecular self-assembly is describe by colloidal self-assembly and understood as balance of competing forces, namely, short-ranged attraction and long ranged repulsion (SALR) [10](#_ENREF_10), [11](#_ENREF_11). However, experimental observations on stability of self-assembled structures with solvent exposed hydrophobic parts, like temperature dependence of cluster sizes, cannot be explained in terms of colloidal models. This calls for appropriate models of competing interactions in these systems to capture stability of self-assembled structures.

Amphiphilic bio-macromolecules [12](#_ENREF_12), like proteins have natural tendencies to expose their hydrophilic moieties to water and bury hydrophobic moieties to avoid water in native state. Clustering in native proteins, like lysozyme in aqueous medium [13](#_ENREF_13) has been explained using colloidal self-assembly. The short-ranged attraction arises due to osmotic pressure difference due to solvent depletion [10](#_ENREF_10), [11](#_ENREF_11) and the long ranged repulsion is electrostatic. Sometimes due to mutation, physiological stress and changes in physio-chemical conditions proteins undergo deviations from native structures, exposing the hydrophobic parts as well to water [6](#_ENREF_6), [14](#_ENREF_14). Extended fibril structure of peptide aggregations with exposed hydrophobic groups, known as amyloids, has been widely studied due to their relevance in neurodegenerative diseases [6-9](#_ENREF_6" \o "Vetri, 2015 #787). Neuro-toxicity of amyloids depends on their sizes: Large amyloids are not neuro-toxic [6-9](#_ENREF_6" \o "Vetri, 2015 #787). Experimental studies indicate that hydrophobic parts of the peptides are largely responsible for amyloid formation [15](#_ENREF_15). However, the size of amyloids also depends on salt concentration and pH of the medium [15](#_ENREF_15), [16](#_ENREF_16). These experiments suggest that the competing forces in these systems are attraction between hydrophobic moieties, known to lead to hydrophobic collapse [17](#_ENREF_17), [18](#_ENREF_18) and electrostatic repulsion. Although self-assembly in these systems results from balance of attractive and repulsive forces as in colloidal self-assembly, there are experimental observations different in these two classes of systems. For instance, the cluster size of amyloids decreases both at high and low temperatures unlike in colloidal clusters which disintegrate with temperature. Colloids [19-23](#_ENREF_19) with attractive patches on particle surface have been used to model self-assembled structures of macromolecules with exposed hydrophobic moieties. However, there has been yet no attempt to the best of our knowledge to understand the experimental observations on such systems, like amyloids, as balance of forces between hydrophobic and hydrophilic sites. It is not even clear if such structures are formed in equilibrium or driven by kinetics [24](#_ENREF_24).

Solvophobic particles much larger than the solvent molecules have been considered in the past [25-29](#_ENREF_25" \o "Berne, 2009 #3261). These particles repel solvent molecules stabilizing vapour phase of low density around them. The vapour phase grows thicker as the liquid-gas phase coexistence is approached in subcritical condition [30](#_ENREF_30). The solvent mediated effective interaction between two such solutes arises due to difference in Laplace pressure at the liquid-vapour interface [31](#_ENREF_31). The attraction can be represented by a harmonic potential with spring constant [31](#_ENREF_31). Physically, harmonic dependence originates from stability of configuration where solvent molecules are pushed away by both the solvophobic surfaces. Here we consider model particles with spherical surface repelling solvent molecules which mimics exposed hydrophobic groups and a charge placed at the centre to mimic the hydrophilic (charged) sites. In this qualitative study we do not explicitly take solvent, rather the solvent effects are taken implicitly through effective pair potentials between the model solute particles, routinely done for soft matter systems [32](#_ENREF_32), [33](#_ENREF_33). The effective pair potential between the particles, harmonic potential for interaction between solvophobic surfaces and effective screened electrostatic repulsion between the core charges with screening length given by used for charge-stabilized colloids [34-36](#_ENREF_34" \o "Schmitz, 2000 #3258). Our Monte Carlo simulations studies coupled with mean field analysis show that finite nm sized clusters are stable below a threshold value of , while the system forms aggregates above the threshold for a given . The threshold shows algebraic dependence between and with exponent, independent of system parameters. The experimental observations, including the nontrivial temperature dependence of clusters in misfolded proteins, are qualitatively understood from the model.

*Monte-Carlo (MC) simulations of model system:*

The interaction potential between two solvophobic particles of radius *R*, surrounded by vapour of radius , has been derived in Ref. [31](#_ENREF_31). Excluding the linear terms, the effective interaction,, so far as the surface-to-surface distance [31](#_ENREF_31). Here is an effective spring constant where is surface tension at the gas-liquid interface of width [31](#_ENREF_31). We take screened electrostatic repulsion between the core charges, known as Derjaguin–Landau–Verwey–Overbeek (DLVO) potential [37](#_ENREF_37) with the form, . The pre-factor of the electrostatic repulsion is considered as and is the inverse of Debye screening length. Here is the number of charges present in a model particle, the electronic charge, dielectric constant of solvent, the electric permittivity in vacuum, the valence of each type of ion present in the solution, including contributions from salt as well as from the model particles, the Boltzmann constant, the ionic concentration in mol/L and the Avogadro’s Number. , excluding the particle charge, gives ionic strength. The electrostatic repulsion gets exponentially damped with a length scale.

We consider particle radius as the length unit and energy unit at room temperature (= 300). The MC simulations have been performed in a cubic box of dimension = 14.0 nm with the periodic boundary conditions in all three directions. There are 1000 model particles of diameter 1.0 nm and charge 28, typical, for instance, in proteins in neutral solvent at room temperature. The volume fraction (volume of a particle times the number density) of the model particles is 0.15. The particle positions are updated according to the Metropolis algorithm [38](#_ENREF_38). The simulations are run for 100,000 MC steps out of which first ~30,000 MC steps are discarded for equilibration and different quantities are averaged for last 70,000 configurations [38](#_ENREF_38). We identify clusters formed by the particles by arbitrarily choosing th particle and then calculate distance for all of th particles with respect to th particle over equilibrium trajectories. If is less than a certain distancethen these th particles are considered to belong to the same cluster as the th [38](#_ENREF_38) particle and total number of particles belong to that particular cluster gives cluster size, . The process is repeated for other particles. We calculate number of clusters of different sizes in equilibrium configurations to yield distribution of cluster size) and compute the mean value ().

Since we do not consider explicitly the solvent molecules, we tune parameters of the effective potential to mimic effects of changing solvent conditions in our calculations. Let us consider the case of room temperature. Water at room temperature is in subcritical condition [39](#_ENREF_39). We take 1.27 observed for Lennard-Jones systems in earlier studies in a subcritical liquid near phase-existence [31](#_ENREF_31). Using experimental value [40](#_ENREF_40), [41](#_ENREF_41) of at room temperature, we obtain that = = 3.0. We observe that in presence of solvophobicity mediated attraction only, particles tend to form aggregated structure, along with ~ spanning the system. In order to ensure equilibrium of our model system, we generate for different windows, each consisting of 5000 configurations. We find that for all of the cases, shows identical values, indicating equilibrium state of our system. This aggregation of the order of system size is analogous to hydrophobic collapse [17](#_ENREF_17), [18](#_ENREF_18) known in the literature. Similar system spanning clusters have been reported in SALR colloidal models as well [42](#_ENREF_42), [43](#_ENREF_43).

Next we consider effect of the electrostatic repulsion which competes with the solvophobic attraction. The harmonic potential will bring particles close together, but the electrostatic repulsion will take them apart. The self-assembly is due to balance between these forces. We increase for fixed at the room temperature. Experimentally, is increased by increasing salt concentration for a fixed temperature, solvent dielectric constant and charge at the centre of the solute. Some representative cases are shown in Figs. 1(a)–(c). ) has sharply peaked structures, showing uniformity in size distribution of clusters. We find that at = 3.0, for low (= 2.0), ) is unimodal (Fig. 1(a)) and ~ 2.0, indicating formation of small clusters. If we further increase screening ( = 6.0), ) (Fig. 1(b)) is bimodal with a strong peak around 100 and a much lower peak around 800 *~ N*. This is indicative of predominant presence of finite but large clusters at this screening. Finally, at = 13.0, ) is primarily unimodal (Fig. 1(c)) with ~ . Thus, there is a threshold , below which finite clusters are stable, while above this the system show system spanning clusters.

We further compute the structural pair correlation functions [44](#_ENREF_44) , which gives the probability of finding a neighbor atom around a central atom within a spherical shell of radii and. is computed by binning the separation between different pair of particles averaged over equilibrium configurations. The data for different cases are shown in insets of Figs. 1(a)-(c). The data show only a few peaks indicating that the correlations decay within a finite distance. Such short-ranged order is typical for amorphous structures. Considering amorphous structure at mean scaled density in our simulation, typical radius of a cluster of 100 particles is approximately 5 nm.

Earlier [30](#_ENREF_30) studies show that at a given temperature in the subcritical region, the gas-liquid interface decraeses as one moves away from the gas-liquid phase co-existence line by increasing liquid density. Thus decrease in corresponds to increase in liquid solvent density away from the gas-liquid phase coexistence at a given subctritical temperrature. We vary to mimic the effect of changing solvent density at room temperature. We show in vs plot (Fig. 2(a)), the regions of stability of finite clusters vis-a-vis aggregations spanning the system. The values for different values of are shown by the symbols. The curves joining the lines are the best fits which we call the aggregation lines. The aggregation line is the boundary between two different self-assembled structures. Above the aggregation lines the particles form aggregates, while below it the particles form finite clusters. We find two different regimes of aggregation lines: For lower , we find from the slope of the log-log plot that giving the equation of the aggregation line. For larger , the dependence is much weaker, the aggregation curve being .

We also consider cluster size variation by tuning the electrostatic repulsion at room temperature upon changing dielectric constant and charge of the particles, at a given salt concentration, while keeping the hydrophobic part unchanged. Dielectric constant of solvent is known [45](#_ENREF_45), [46](#_ENREF_46) to control self-assembly of charged colloids. Here, we model different solvent implicitly by changing dielectric constant, . We show for different values of and in Fig. 2(b). We observed that is longer ranged for 60 than that for 40 for the same value of (28). The pre-factor of the electrostatic repulsion, *X*, decreases with increasing . However, inverse Debye screening length also decreases with increasing which renders the range of the repulsion longer and outweighs the decrease in *X.* We observe (Fig. 2(c)) that for = 3.0 the cluster size decreases as we increase The enhancement of electrostatic repulsion, compared to the hydrophobicity mediated attraction, (inset of Fig. 2(b)) leads to decrease in cluster size. Similar enhancement in range of electrostatic repulsion by decreasing charge at the center (Fig. 2(b)) leads to decrease in cluster size (inset of Fig. 2(c)). Here also, the increase in *X*, is outweighed by increase in the Debye screening length with *Z*, leading to decrease in electrostatic potential compared to the hydrophobic attraction.

We now turn our attention to temperature dependence of self-assembly in our model. Since the dielectric constant of a normal liquid varies inversely with temperature [44](#_ENREF_44) the electrostatic repulsion is not sensitive to temperature. However, the hydrophobic potential is sensitive to temperature [31](#_ENREF_31). The width of liquid-vapour interface around a solvophobic particle increases with temperature in a subcritical solvent due to increasing compressibility [41](#_ENREF_41), [47](#_ENREF_47). On the other hand, the surface tension decreases with temperature [40](#_ENREF_40). These two effects compete so that has non-monotonic dependence on temperature as shown earlier [29](#_ENREF_29), [31](#_ENREF_31), [48](#_ENREF_48). We use known experimental values of surface tension of water at different temperatures [40](#_ENREF_40). The experimental values of isothermal compressibility at different temperatures [41](#_ENREF_41) of water have been employed to calculate the interfacial width using relation derived in Ref. 2[6](#_ENREF_29). Ref. 2[6](#_ENREF_29) considers Lennard-Jones fluid, not polar water. However, compressibilty is related to long wave-length fluctations of density [44](#_ENREF_44) primarily governed by the positions of the oxygen atoms. Hence, orientation of water molecules in the solvent would not be important for compressibility. The data in Fig. 2(d) indicates that for small value of (= 2.0), the cluster size shows non-monotonic dependence over temperature with maximum at an intermediate temperature.

*Case of misfolded protein clusters:* Since misfolded proteins form an important class of self-assembled structures with exposed hydrophobic parts [1-3](#_ENREF_1" \o "Dehsorkhi, 2014 #773), it is interesting to compare our model to self-assembly of misfolded proteins.Themost important feature of the model is the presence of gas bubble surrounding the solvophobic particles. Formation of gas bubble [49](#_ENREF_49), [50](#_ENREF_50) has been observed at interface of hydrophobic solid and water. The solvation behaviors of proteins are complex due to presence of hydrophilic residues in the vicinity of hydrophobic moieties. We perform full atomistic molecular dynamics (MD) simulations of polypeptides in several functional proteins [51-55](#_ENREF_51) (details of the peptide, simulation protocol and the secondary structures along with root mean squared deviation plots of the peptides are in Supplemental Table S1, Supplemental material and in Figs. S1(a)-(j) respectively) having both hydrophobic and hydrophilic amino acid residues in water in physiological condition to calculate solvent distribution around different residues. The simulations have been carried out using the Amber99ILDN [56](#_ENREF_56) force field parameters in the Gromacs package [57](#_ENREF_57), [58](#_ENREF_58).

We compute water distribution around different types of residues by binning the separation *r* between oxygen atom of water and backbone carbon atom of the polypeptide over equilibrated trajectories. Fig. 3(a) shows the distributions around acidic, basic, polar and hydrophobic residues. The figure indicates that water molecules gather around acidic and basic residues, while polar and hydrophobic residues are having weaker water distribution. Thus the picture of stabilization of water vapour holds near the hydrophobic residues of polypeptides. There is one more caveat: Most common end product of misfolded protein aggregation is extended β-amyloid fibril [59](#_ENREF_59), [60](#_ENREF_60) having β sheets parallel to fibril axis. Our model amounts to representing the water exposed hydrophobic residues by a smooth solvophobic surface of a sphere of diameter of a few nanometers, comparable to protein size. The charge at the centre mimics the charged residues. The model ignores shape anisotropy and inherent roughness of the protein surface. The model also does not take into account the distribution of charged sites over the molecular surface.

Let us examine how much of the experimental results on amyloids can be reproduced by this simple model. The phase diagram in Fig. 2(a) shows that the aggregates are stable with increasing for a given . This is qualitatively consistent with observations that the stretch of hydrophobic parts of the peptides favors amyloid formation [15](#_ENREF_15). Fluorescence correlation spectroscopic (FCS) studies [15](#_ENREF_15) indicate that size of clusters increases with increasing ionic strength of the solution by adding NaCl. We find from our simulation that the cluster size increases monotonically for a fixed value of below . The vs plot in Fig. 3(b) for = 3.0 shows ~ dependence below (=13.0). However, the experiments in Ref [15](#_ENREF_15) neither ascertain the dependence of cluster size on ionic strength, nor report a threshold . Scattering measurement shows that salts of divalent metal ions at micro-molar concentration [16](#_ENREF_16) leads β-amyloid to precipitate, forming large sized assembly. This is consistent with our results, for increase in valance of metal ion increases which would result in larger clusters.

The variation of pH of the medium leads to changes in charge state of the residues. FCS studies [15](#_ENREF_15) also show that increase in residue charges due to decrease in solution pH leads to smaller clusters. The reduction in cluster size with increasing, observed in our model system, is qualitatively consistent with these experimental results. Moreover, isothermal titration calorimetric experiments, fluorescence measurement and CD spectra performed on 2 amyloid fibril reveal that the cluster size of amyloid decreases both for high and low temperatures [61](#_ENREF_61" \o "Adachi, 2018 #3280); this observation is also qualitatively explained in our model.

*Mean field theory:* We account for the aggregation line from a simple theoretical frame work. The structural information of thermodynamically stable system is given by the scattering function [44](#_ENREF_44) . Clustering in a system is represented by peak at low wave vector of , while for aggregation the peak shifts to limit. *s*(*q*) is connected to of liquid correlation function [44](#_ENREF_44), via for a system of density . The peak of corresponds to that of as well. We examine the low peak of from a mean field treatment. At low density, the mean field approximation [62](#_ENREF_62) , where and is the Fourier Transform of the interaction potential. In the mean field approximation for our system, . Here is Fourier Transform of direct correlation function for DLVO potential and is that for solvophobic term. This is valid for long-ranged electrostatic potential, namely low. Since, the solvophobicity mediated term operates till , we use mean field treatment for this term also. As (details in Supplemental Material), . Here }, where is function of . , depends on pre-factor of DLVO potential (), for a fixed temperature depends on *R* and .

We find that has a minimum at 0, if > 0 and a maximum at 0, if < 0. Hence, the condition for aggregation is = 0, which yields that ~ . The mean field analysis reveals that the stability of the aggregated phase is in qualitative agreement to that obtained from our numerical simulation for low . This is not surprising for the mean field treatment is valid for longer ranged potential. This qualitative agreement suggests that the stability of the structures is achieved in thermodynamic sense. However, in this analysis we overestimate stability of the aggregated phase which could be due to mean field nature of the analysis, ignoring fluctuations.

Our model rightly captures the competition between attraction between hydrophobic parts and electrostatic repulsion in stabilizing self-assembled structures in macromolecules with exposed hydrophobic groups, although we have not incorporated system details, like shape anisotropy, surface roughness, surface charge distribution.The most nontrivial feature is non-monotonic temperature dependence of cluster size in our model in contrast to colloidal SALR models where clusters disintegrate at elevated temperatures. The contrasting feature lies in attractive part of interaction. In SALR models, the short ranged attraction is due to depletion of solvent molecules, also known as the Asakura-Oosawa model depletion [10](#_ENREF_10), [11](#_ENREF_11). To the contrary, the range of attraction in our model is given by radius of vapour around the particles, and is not necessarily short-ranged, depending on the thermodynamic condition of the solvent. For instance, the interfacial width of the gas-liquid interface increases as the gas-liquid phase coexistence is approached, the width being divergent near gas-liquid critical point [26](#_ENREF_26" \o "Meyer, 2006 #3262).

System spanning clusters, similar to our model, have been observed in SALR models as well [42](#_ENREF_42), [43](#_ENREF_43). In addition, we predict the existence of aggregation line, demarking the system spanning clusters with finite clusters. There is algebraic dependence between effective parameters for the hydrophobic and electrostatic parts, namely, and respectively over the line. This prediction can be verified by experiments on misfolded proteins. The exponent of the dependence is independent of system details. The exponent is determined by gross properties, like surface tension, compressibility, dielectric constant, the Debye screening length and overall charge on the macromolecule. Thus our results should hold for amphiphilic macro-molecules of nm size with solvent exposed hydrophobic groups similar to amyloids.Ignoring the distribution of charged residues amounts to neglecting multi-pole moments of the charge distribution, which may be important for anisotropic self-assembled structures.

To summarize, our studies describe a model to capture stability of nm sized clusters over aggregation in macromolecules with solvent exposed hydrophobic moieties. The finite clusters are stable below a threshold value of the inverse Debye screening length and aggregates are stable above the threshold. The experimental observations on stability of amyloid clusters including the temperature dependence are qualitatively explained by this model. Our model may provide useful guideline to control self-assembled structures formed by macromolecules with exposed hydrophobic sites.

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**Supplemental Material:**

Details of peptides, MD simulation along with mean field analysis.

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**Figure Captions:**

Figure 1. Probability distribution of cluster size of model system, in presence of a fixed solvophobicity mediated attraction ( = 3.0) and different . (a) Finite size cluster for = 2.0. Inset: Radial distribution function vs . (b) Coexisting small clusters and aggregation for = 6.0. Inset: vs . (c) Large aggregation at = 13.0. Inset: vs .

Figure 2. (a) Phase diagram in and in plane showing finite size cluster and large aggregated structure. The solid line shows dependence, while dashed line represents for aggregation threshold. (b) vs for different values of and . Black solid: 40, = 28, Gray solid: 60 and = 28. Black dashed: 80, = 50 and Gray dashed: 80, = 90. Inset: for a fixed value of (= 3.0). (c) Increase of with decrease of polarity of the solvent. Inset: Decrease in cluster size with increasing charge of the core . (d) Decrease of with increase of temperature .

Figure 3. (a) Distribution functions of water around hydrophilic and hydrophobic residues of protein**,** black solid line;acidic, gray solid line; basic, gray dashed; hydrophobic and black dashed; polar residues. (b) vs plot to show cluster size variation with ionic strength.



Figure 1.



Figure 2



Figure 3