

Ion-specific weak adsorption of salts and water/octanol transfer free energy of a model amphiphilic hexapeptide

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Supplementary information

Water/octanol peptide partitioning: Fluorescence spectroscopy

To get further insight about a possible structuring, the coexisting phases were separated and analyzed by light scattering. DLS of both phases gave an apparent size of about 1.5 nm indicating that there was no significant further organization of the fluids. This analysis was completed by an emission spectroscopy measurement. Fluorescence spectra of octanol phases after peptide partitioning were comparable to spectra in pure octanol: excitation at 257 nm gave the typical 282 nm emission peak due to phenylalanine aromatic group (Figure 1, right). In contrast, fluorescence of aqueous phases have changed: the emission signal is now partially shifted at 304 nm (Figure 1, left), evidencing that peptides self-assemble via π -stacking interactions involving phenyl groups ^{1,2}.

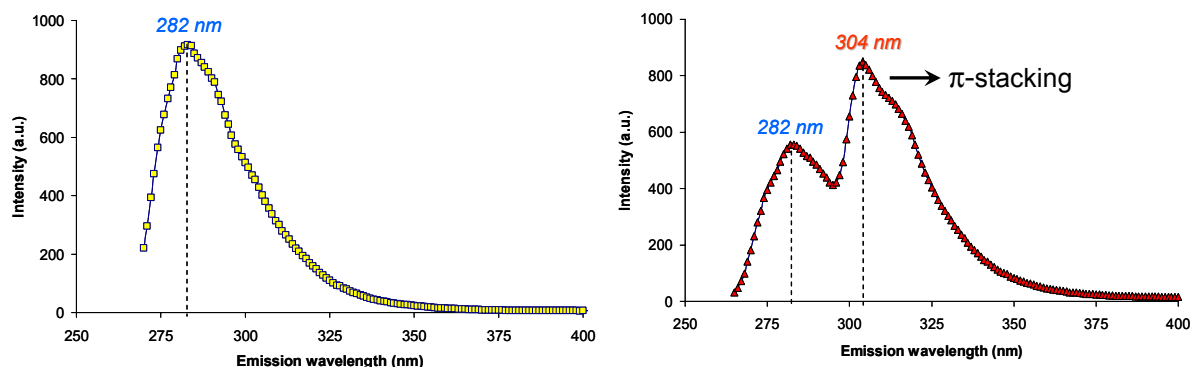


Figure 1. Fluorescence emission spectra of 5 mM (0.33%) peptide in aqueous phase before (right) and after (left) contact with octanol, evidencing π -stacking in the latter case. Excitation wavelength was 257 nm.

Apparent size from DLS hints towards the formation of dimers only by this amphiphilic peptide. This shows that peptide dimerization is not spontaneous in water alone and are favoured by the presence of dissolved octanol in water (octanol solubility in pure water is about 4.5 mM³). Moreover, although in octanol dimerization probably involves only intermolecular H-bonds, increased hydrophobic interactions in water induce aromatic stacking. This kind of π -stacking is usual for peptides bearing aromatic side-chains and is thought to be one of the driving forces for β -amyloid like fibres formation⁴⁻⁶. The question lies whenever peptide dimers interact also *via* intermolecular H-bonding in water. The H-bond contribution in log-P has recently been introduced by other groups studying partition coefficients of model substances between a water solution and the lipophilic core of direct micelles formed by either anionic or cationic surfactants (SDS and CTAB, respectively)^{7,8}. It came out that increasing H-bonds led to an increased hydrophilicity and a lower logP value. In our case, peptide's logP increases upon increasing concentration. This shows that less and less H-bonds between peptide and water are formed inducing an increase of the peptide global hydrophobicity. As a consequence dimerization observed in water involves inter-peptide H-bonding, in addition to π -stacking interactions.

With a view to evidencing whether these different salts can modify the peptide dimerization we analyzed the phases by emission spectroscopy. In all cases no π -stacking was observed in the organic phase. Regarding the aqueous fluid, fluorescence measurement could not be realized with NaNO₃ due to nitrate UV absorption in this range. Concerning NaCl and LiCl, aqueous phase's fluorescence revealed π -stacking, whereas in NaOH this organization could

not be observed. This point out that packing via π -stacking involved also carboxylic acids of the head group that required being at least partially protonated to allow this interaction. A possible dimer structure involving both π -stacking and H-bonding is represented in figure 2. In all cases, the approximation of monomers or dimers stacked via carboxylate associated via hydrogen binding or stacking as globular colloids remains valid.

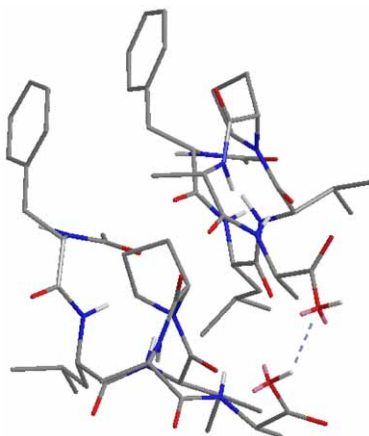


Figure 2. Possible structure representing peptide dimerization involving both π -stacking between phenylalanines and H-bonding between C-ter carboxylic acids.

Peptide net charge considering a coulombic perturbation of van der Waals interactions

Figure 3 below illustrates how the net adsorbed charge is derived as a perturbation from a decrease in the apparent Hamaker constant.

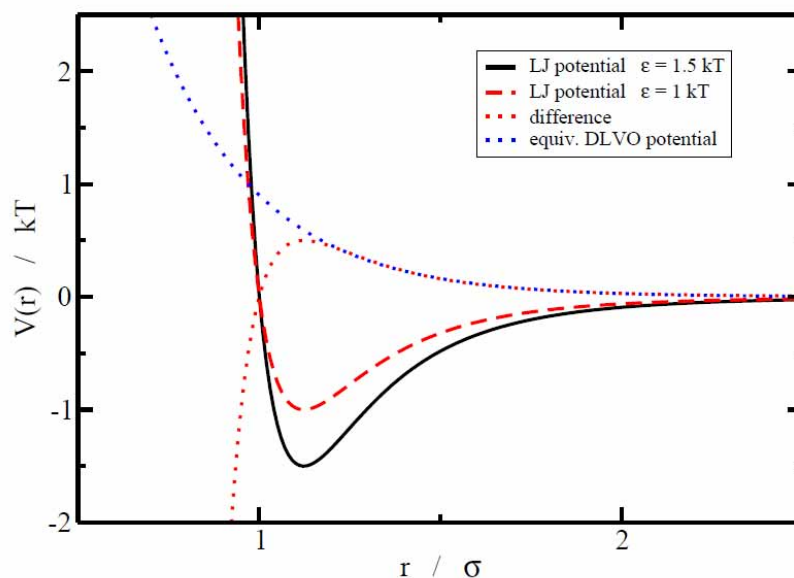


Figure 3. Lennard-Jones and DLVO potentials as a function of the relative distance

The Lennard-Jones potentials with an apparent Hamaker constant reduced from $1.5 k_B T$ to $1 k_B T$ are shown as a black line and a red dashed line respectively. The difference between these two potentials as shown in red is due to an electrostatic repulsive interaction due to the weakly adsorbed ions, considering the case of NaCl as neutral, *i.e.* no electrostatic contribution due to excess of adsorption of chloride or sodium.

The equivalent DLVO potential is the one producing the same miscibility gap in liquid-gas separation, *i.e.* the same virial coefficient. The effective charge which is derived as the only adjustable parameter is used to evaluate surface potential and enthalpy associated to the weak ion adsorption process.

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