

## 2011 - Summary report

The process of molecular association induces local and global changes to the molecules involved, which alter their physical and chemical behaviour in solution. The  $\pi$ - $\pi$  stacked aromatic interactions have been found to govern diverse supramolecular organizations and aggregates in both the solution and the solid state. In comparison to more conventional interactions, such as hydrophobic interactions and hydrogen bonds, the  $\pi$ - $\pi$  stacked interaction is not so clear and no commonly used model has been built to interpret the experimental observations. Therefore, the study on the variety of systems involved in the  $\pi$ - $\pi$  stacked interaction is important to fully understand this type of noncovalent interaction.

### *Analysis by indefinite non-cooperative (isodesmic) model*

The isodesmic model of solute self-association is based on the assumption that solute molecules associate to form stacks (dimers, trimers etc.) where the equilibrium constant  $K$  for each step is the same.

Thus



It can be shown that:

$$[A_0] = [A]/(1 - K[A])^2 \quad (2)$$

where  $[A_0]$  is the total concentration of solute  $A$  and  $[A]$  is the concentration of the monomer in solution. Further it is assumed that the proton chemical shifts changes observed for a solute molecule binding at the end of a stack are half those observed for a molecule associating in the middle of a stack

$$\delta_j = \frac{(j-1)\delta_i + \delta_m}{j} = \delta_{A_j} \quad (3)$$

where  $\delta_j$  is the proton chemical shift of the  $j$ -mer,  $\delta_m$  is the proton chemical shift of the monomer, *i. e.* at infinite dilution and  $\delta_i$  is the maximal proton chemical shift of solute  $A$  present in a stack. Owing to rapid exchange in self-association reactions, the observed chemical shift of all species present in solution is given by:

$$\delta_{obs} = \frac{1}{[A_0]} \left\{ [A]\delta_m + \sum_{n=2}^{\infty} n[A_n]\delta_{A_n} \right\} \quad (4)$$

Substituting equation (2) into equation (4) gives:

$$\delta_{obs} = \left( \frac{\sqrt{1+4K[A_0]}-1}{2K[A_0]} \right) \delta_m + \left( 1 + \frac{1-\sqrt{1+4K[A_0]}}{2K[A_0]} \right) \delta_i \quad (5)$$

We note that a dimerization model assuming only the shift values  $\delta_m$  and  $\delta_{dimer}$ , with an association constant  $K_{dimer}$ , gives an identical equation the only differences being that  $K$  is replaced by  $2 K_{dimer}$  and  $(\delta_i - \delta_m)$  is replaced by  $(\delta_{dimer} - \delta_m)$  which is equivalent to  $(\delta_i - \delta_m)/2$ , since a molecule in a dimer is effectively at the end of a stack of two.

Therefore, NMR chemical shift data cannot be used to distinguish dimerization from stacking, if the isodesmic model is applicable. The parameters  $\delta_m$ ,  $\delta_i$ , and  $K$  in equation (5) were calculated from a least-squares fitting of the data to the isodesmic model.

### *Attenuated model*

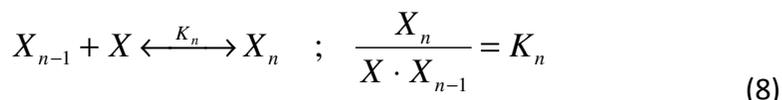
In the attenuated model successive equilibrium constants for self – association taper off according to the prescription  $K_i = K/i$ . The weak point of this method consists in the fact that the asymptotic value of the association constant for oligomers of higher order approaches zero. Following the same procedure as for the above mentioned model, the obtained results are:

$$[A_0] = [A]e^{K[A]} \quad (6)$$

$$\delta_{obs} = \frac{1}{K[A_0]} \{ (e^{K[A]} - 1)\delta_m + (e^{K[A]}(K - 1) + 1)\delta_i \} \quad (7)$$

### *Attenuated model based on entropic effects*

The indefinite self-association process of molecules in solution can be described by the following set of equations:



Accordingly, the oligomers total concentration  $X_a$  (including monomers) and the total concentration of monomers,  $X_t$  are given by:

$$X_a = \sum_{n=1} X_n \quad ; \quad X_t = \sum_{n=1} nX_n \quad (9)$$

The  $^1\text{H}$  NMR signals of different protons of the monomer are affected in a different manner by the self-association process, depending on the strength of their interaction with other monomers in the stack. The generally accepted hypothesis is that for a linear self-assembling process the influence is mainly limited to the first neighbors. If the monomer architecture has an intrinsic asymmetry then the chemical shift of the external monomers can be also asymmetrically affected by the left or right neighbor respectively,  $\Delta\delta_{i,r} = \delta_m - \delta_{i,r}$ . This influence is experienced by the first and last molecule from the aggregate. Any other monomer cumulates the effect coming from both left

and right neighbors  $\Delta\delta = \Delta\delta_l + \Delta\delta_r$  and its chemical shift becomes  $\delta_i = \delta_m - \Delta\delta$ . Consequently the mean chemical shift per monomer in an  $n$ -mer is obtained by the weighted average contribution coming from the external and internal monomers respectively

$$\delta_n = \frac{\delta_l + \delta_r + (n-2)\delta_i}{n} = \delta_m - \frac{(n-1)\Delta\delta}{n} \quad ; \quad \delta_n \xrightarrow{n \rightarrow \infty} \delta_i \quad (10)$$

The measured NMR chemical shift is just the weighted average contribution coming from the aggregates of any order which populate the solution

$$\delta_{obs} = \frac{\delta_m X + \sum_{n=2} n X_n \delta_n}{X_t} = \delta_m - \Delta\delta \left( 1 - \frac{X_a}{X_t} \right) \quad (11)$$

This equation has a general validity and is suitable for any self-association process. A particular model, describing the process lead to a specific dependence of  $X_a$  on  $X_t$  and specify also the variation of  $\delta_{obs}$  with  $X_t$ . Our proposed model consider that the entropy modifications associated with the addition of each monomer to the aggregate lead to a gradually decrease starting from  $K_2$  and goes to  $K$ , at very large dimensions of oligomers according to

$$K_n = \frac{K}{n} \left( \frac{n}{n-1} \right)^3 \quad (12)$$

The developed theoretical approach was tested on the NMR data obtained from ciprofloxacin self-association experiments (J. Phys. Chem. B 2012, 116, 6488 – 6498). Our theoretical predictions, relying only on firm statistical thermodynamical arguments, are able to fit very accurately the measured data.