



# From Molecules to Clusters to Particles to Products: the Case for Modelling

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- Modelling has a significant role to play in the design of product formulations for solids.
- Product design, from first principles, requires both modelling and experimental approaches to be applied across length and time scales.
- Molecular modelling approaches address the shortest length and time scales and provide information about, for example:
  - Selection of solid-state form (crystallisation/precipitation)
  - Purity of solids as a function of growth environment
  - Particle shape and surface properties
  - Particle dissolution

## Molecules, Clusters and Crystals: A Multi-scale Approach to Understanding Kinetic Pathways in Crystal Nucleation from Solution

*Collaborative research project at Leeds and Manchester Universities funded by an EPSRC Critical Mass grant (PI Leeds- K.J. Roberts, PI Manchester-R.J. Davey)*

- **Experimental :**
  - Determination of thermodynamic parameters via solution-state studies
  - Nucleation and growth kinetics from solution
  - Single crystal growth and characterisation
  - *In-situ* cell development
  - XANES predictions and data collection
  - SAXS/WAXS data collection
- **Computational:**
  - Modelling molecular association in solution
  - DFT for predicting XANES (CASTEP) linked to experiment
  - Growth interface modelling.
  - Crystal morphology predictions

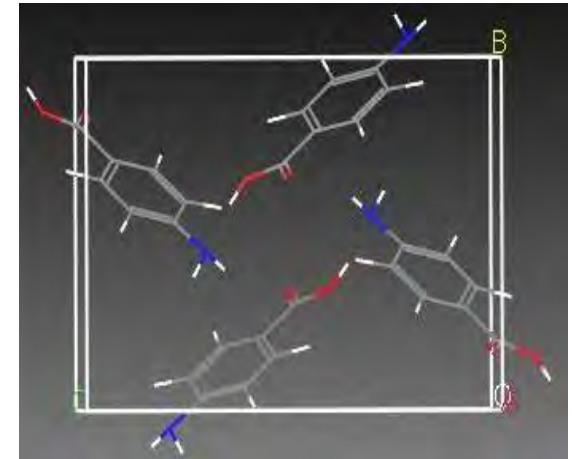
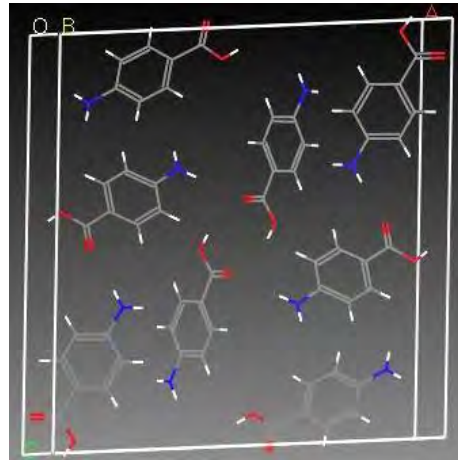
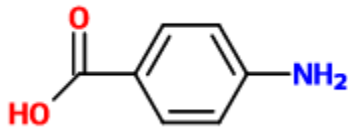


- Remit to employ molecular modelling approaches to better understand molecular self-assembly and material properties of particulate systems.
- Further to combine experimental measurement (XANES) and molecular modelling to generate and the refine structural models describing molecular self-assembly, and crystal nucleation from solution.
- Provide molecular scale information for meso-scale modelling.

# Study Material: Para Aminobenzoic Acid (PABA)



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- Compound with two known polymorphs  $\alpha$  and  $\beta$  with an enantiotropic relationship.
- Transition temperature between  $13.8^\circ\text{C}$  and  $16^\circ\text{C}$ .<sup>1,2</sup>
- Observed needle like morphology.
- $\alpha$ -form: Strong H-bonding carboxylic acid  $\text{OH}\dots\text{O}$  interactions and  $\pi$ - $\pi$  stacking.
- High temperature stable-form
- $\alpha$  crystallises with 2 molecules in the asymmetric unit and 8 molecules in the unit cell.
- Observed prismatic plate like crystals.
- $\beta$ -form: H-bonding ring made up of pairs of  $\text{OH}\dots\text{N}$  and  $\text{NH}\dots\text{O}$  interactions
- Low temperature form  $\beta$  crystallises with 1 molecule in the asymmetric unit and 4 molecules in the unit cell.

1. H. Hao, M. Barrett, Y. Hu, W. Su, S. Ferguson, B. Wood, B. Glennon,, 16 (2011) 35-41
2. M. Svard, F.L. Nordstrom, E.-M. Hoffmann, B. Aziz, A.C. Rasmuson,, CrystEngComm, (2013).

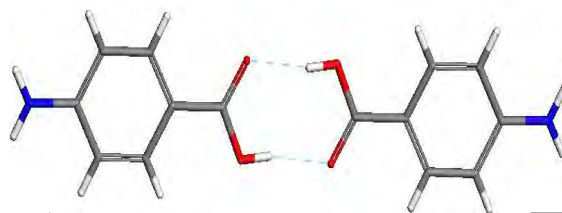
# Hierarchical method: Explore the properties of the study material



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PABA

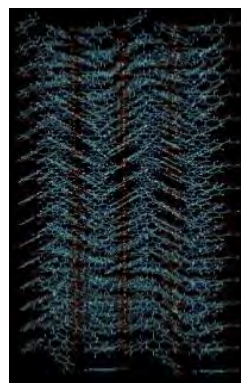
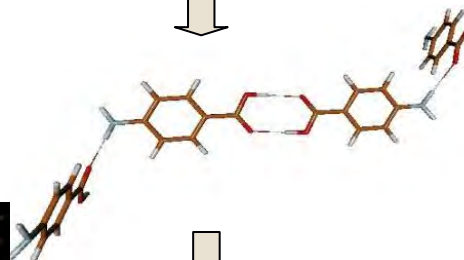
Monomers vs Dimers



Single  
molecule



Small Cluster  
Analysis



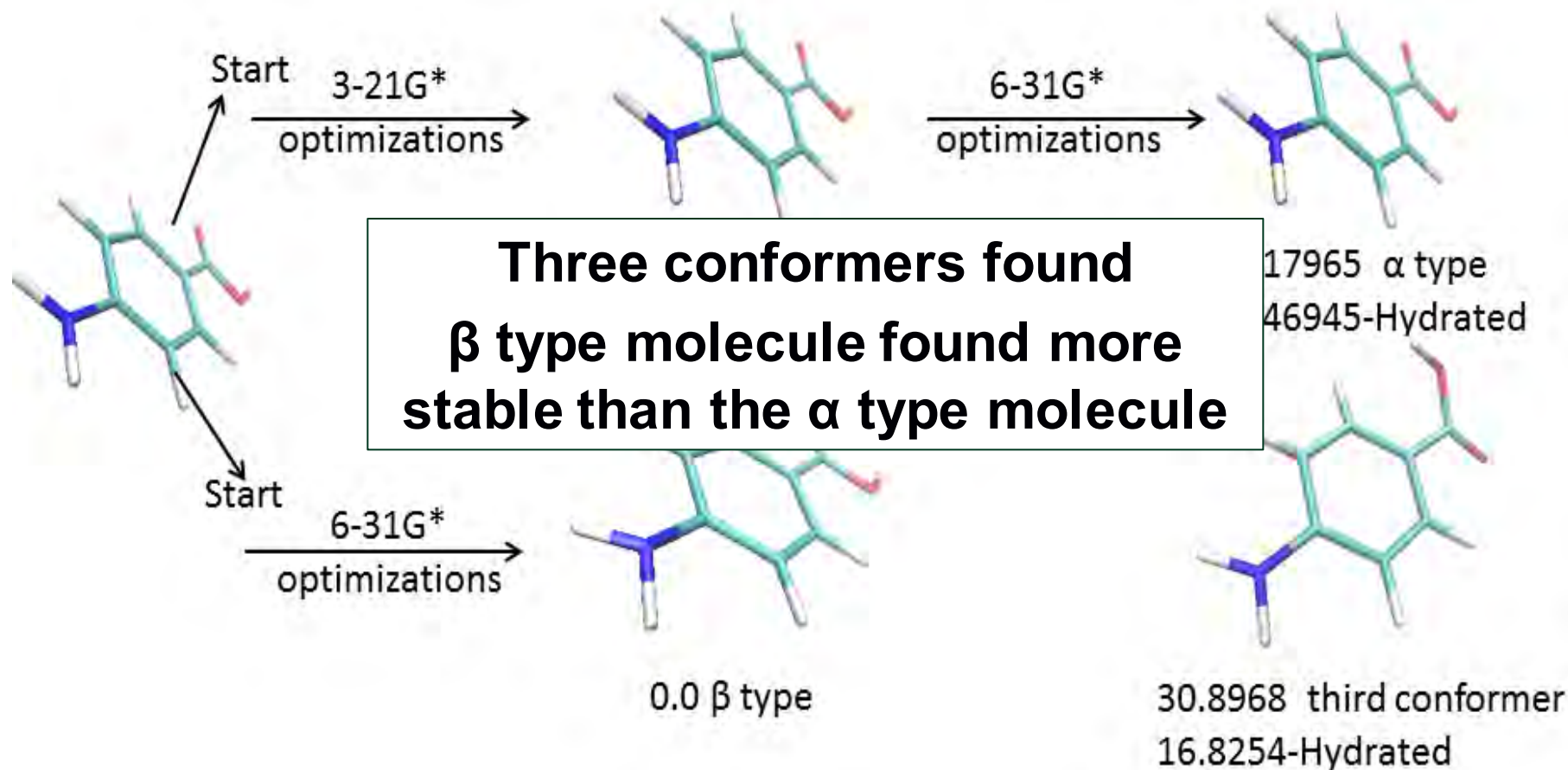
Nano-crystals

# Conformational Analysis of p-amino benzoic 1



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- Starting points for optimisation were molecular geometry taken from, separately, crystal structure of  $\alpha$  and  $\beta$  polymorph



# Conformational Analysis of p-amino benzoic 2



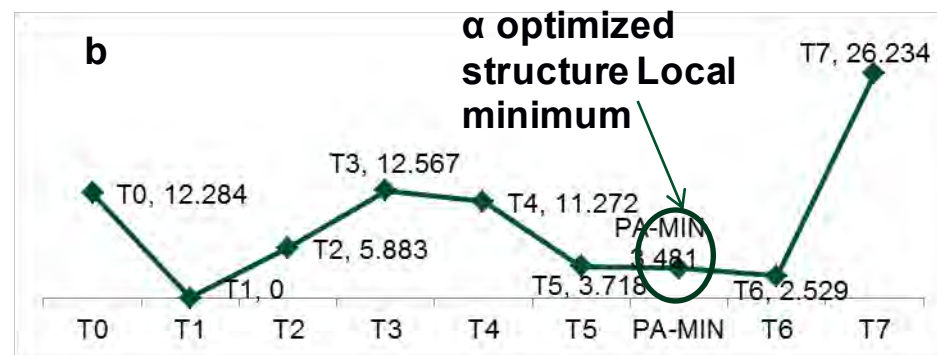
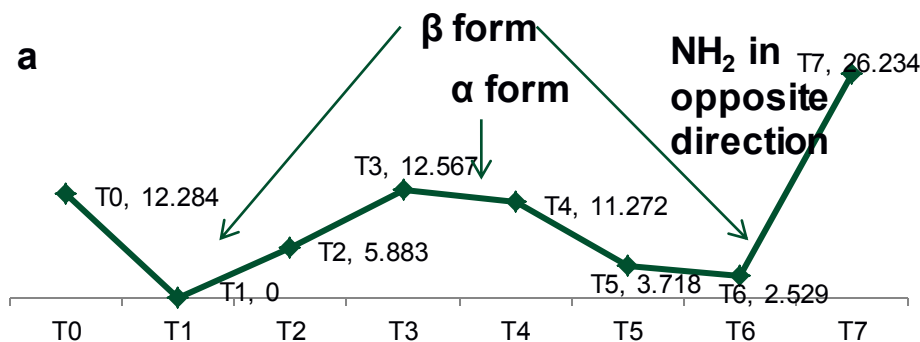
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- Degree of pyramidal character in  $\text{-NH}_2$  group adjusted in reaction coordinate calculation. Starting from geometry in  $\alpha$  polymorph find a local energy minimum  $\sim 3.5$  kJ/mol less stable than global minimum reached from geometry in  $\beta$  polymorph

two molecules in asymmetric unit



$\alpha$  polymorph 3.5 kJ/mol less stable than global minimum reached from  $\beta$  polymorph  
 $\alpha$  type conformer is essentially a transition state.





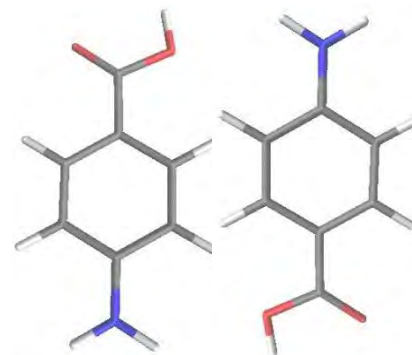
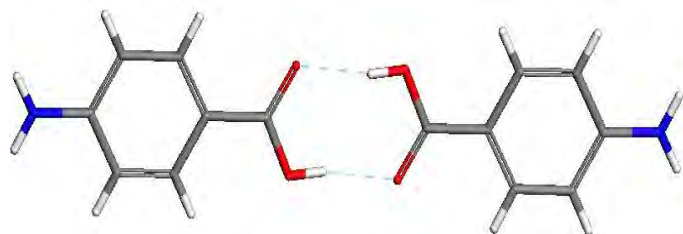
# Solvation free energies of PABA: Single molecule- dimers



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## Computational Experiments: Research Questions

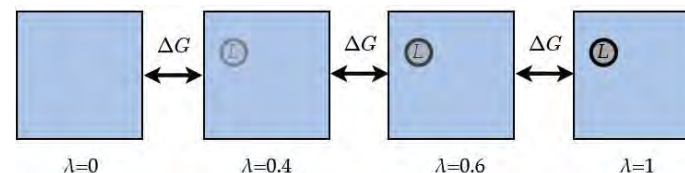
- What is the preference of molecules to form monomers or dimers in solution.
- Monitor the interactions occurring of PABA molecules with solvent.
- Application of different methodologies for the prediction of solvation free energies i.e. quantum chemical and atomistic molecular dynamics simulations to test on efficiency and transferability between the two methods.



Two fundamental methods were used in order to explore the solvation free energies of PABA in solution:

1. QM calculations: SMD solvation continuum model at the density functional theory level used.

2. MD using thermodynamic integration technique:



The interactions between the ligand and the solvent are turned 'slowly' on (or off) .

- Minimisation using the steepest descents method.

- Topology files, force field parameters were derived from the GAFF force field. RESP charges derived ab-initio calculations at different levels of theory.

- The initial state ( $\lambda=0$ ) is defined by turning off the electrostatic and VDW interactions between the solute and the solvent.

# Solvation free energies of the single PABA molecule



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## Quantum Chemical calculations

	Energies in kJ/mol		
Functional	B3LYP	M06	B97D
Basis set used:			
6-31+G*	-44.984	-51.519	-50.337
aug-cc-pvdz			

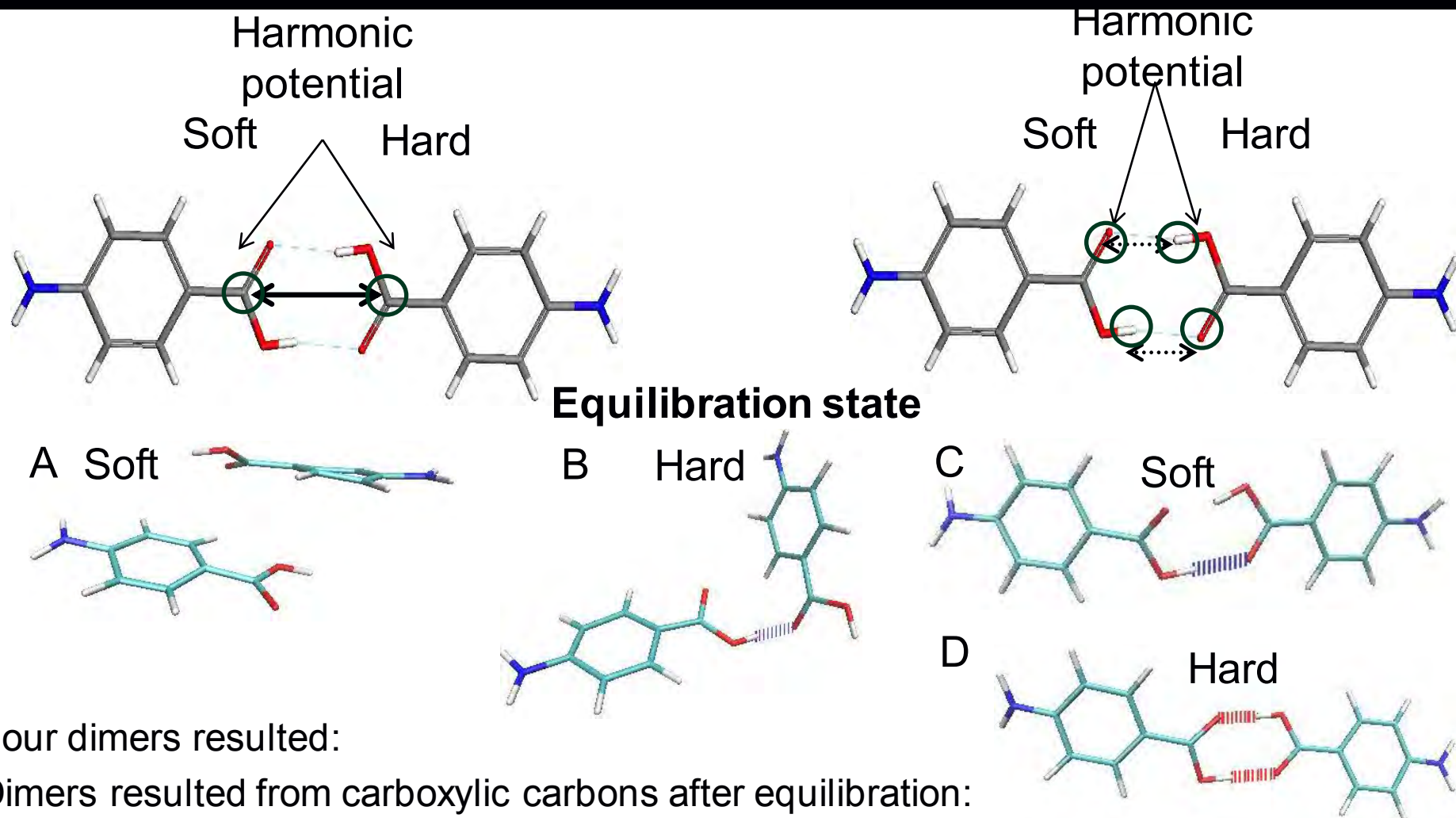
**Results from both theoretical methods used are in agreement for the solvation free energy of the single molecule of PABA when using high level of theory.**

MP2/6-31+G*-AMBER	-39.05	-45.18
MP2-aug-cc-pvdz-AMBER	-46.08	-42.38
MP2-aug-cc-pvTz-AMBER	-46.66	-42.31

# Prediction of the solvation free energy of carboxylic acid dimers of PABA



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Four dimers resulted:

Dimers resulted from carboxylic carbons after equilibration:

Two separated dimers (A), Catemer (B)

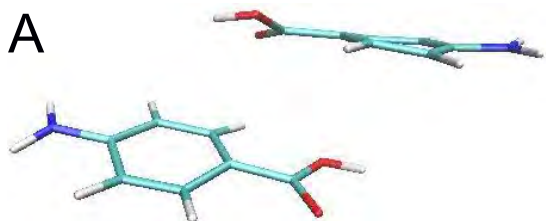
Catemer (closer to carboxylic dimer)(C), Carboxylic dimer (D)

# Prediction of the solvation free energy of carboxylic acid dimers of PABA



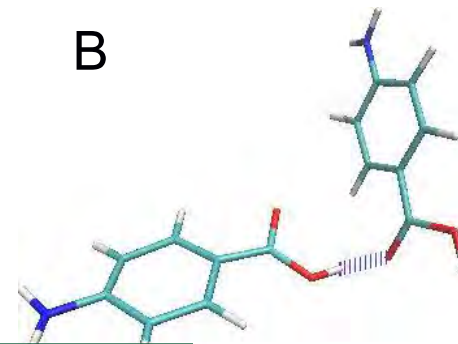
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A

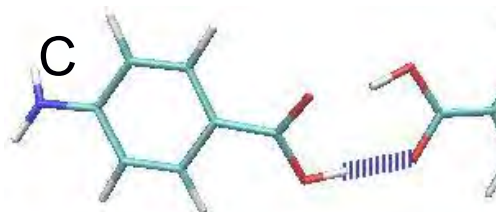


Method at 5° C

B



C



Gradually the free energy of solvation increases from separated monomers to a carboxylic acid dimer



0.0



-2.37



-8.79



-11.43

A

B

C

D

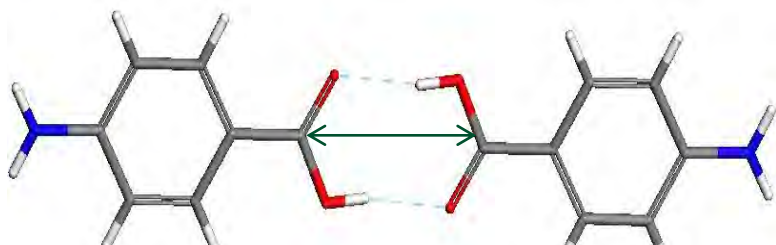
Relative energies in kJ/mol

# Solvation free energy of PABA in non-aqueous solvents

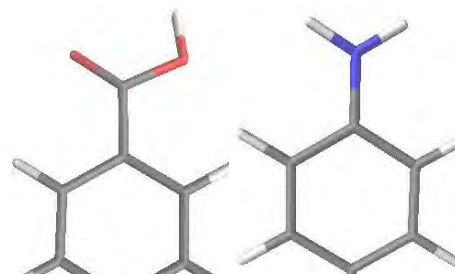


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Two molecules of PABA that are forming, a carboxylic acid dimer



Two non-interacting molecules in solution



**Two isolated molecules strongly preferred over the carboxylic acid dimer for PABA in IPA, balanced in Ethyl acetate whilst in acetonitrile the dimers are preferred**

**Results in agreement with experimental data**

Acetonitrile	-90.14 +/- 1.23	-95.82 +/- 1.75	-4.68
Ethyl acetate	-102.13 +/- 1.96	-101.86 +/- 1.84	+0.27
IPA	-95.10 +/- 1.72	-84.34 +/- 1.61	+10.96



# Conclusions

## **- Monitor the interactions dominating PABA molecules with solvent**

Calculated solvated free energies showed the strength of the interactions between solute and solvent of isolated molecules and carboxylic acid dimers of PABA in four different solvents.

## **- What is the preference of molecules to form monomers or dimers in solution.**

For PABA in water the solvation free energy increases from two isolated molecules to a carboxylic acid dimer. Two isolated molecules strongly preferred over the carboxylic acid dimer for PABA in IPA, balanced in Ethyl acetate whilst in acetonitrile dimers are preferred.

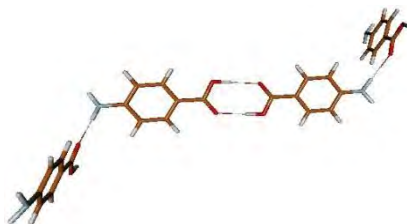
## **-QM and MD simulations to test on efficiency and transferability between the two methods.**

QM calculations and MD simulations showed good agreement for the monomers and carboxylic acid dimers for PABA in aqueous environment.

# Energetics and stability p-amino benzoic acid clusters Using Gas-Phase and Solvent Continuum Calculations



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## COMPUTATIONAL EXPERIMENTS-RESEARCH

What is the influence of solvent on which polymorph of PABA crystallises?

What are the relative stabilities of the single molecule and small clusters of PABA based on the crystal structure within the COSMO dielectric?



# Stability of p-amino benzoic acid clusters using Solvent Continuum Calculations



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In DFT the energy of a system is given by:

$$E_{\text{DFT}} = E_{\text{NE(R)}} + E_{\text{T}} + E_{\text{NE(A)}} + E_{\text{coul}} + \boxed{E_{\text{exch}} + E_{\text{corr}}}$$

**THE EXCHANGE AND CORRELATION TERMS CAN BE DETERMINED BY DIFFERENT FUNCTIONALS.**

Functionals differ in using either only the electron density (**local methods**) or the density and its gradients (**gradient corrected methods or GGA**) or mixtures of DFT and Hartree-Fock exchange energies are used (**hybrid functionals**).

## Computational details

Molecules and clusters selected of PABA based on the crystal structures  
DFT/COSMO calculation using:

1. DMol3 with DNP basis set and PBE functional.
2. Gaussian with 6-31G\* basis set and B3LYP and M06 functional.

Continuum solvent calculations using:

H<sub>2</sub>O, ACN, EtOH, MeOH, CH<sub>3</sub>NO<sub>2</sub>, EtOAc and DMSO.



Implicit conducting solvent dielectric approach to solvation.

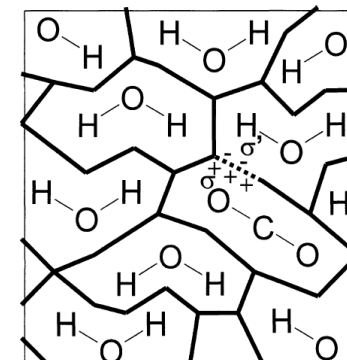
Cavity created around solute molecule/cluster in dielectric.

DFT/QM calculation optimising solute within the dielectric.

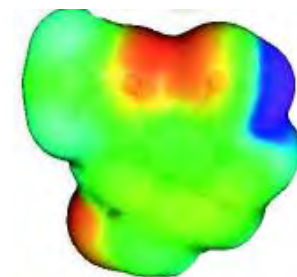
Statistical thermodynamics calculation based on interacting polar and non polar “segments” of the solute.

Statistical thermodynamics step accounts for H-bonding and VdW interactions within the solution

Allows for direct comparison of the population of two conformers in solution.



**Figure 1: Schematic representation of solute cavity within H<sub>2</sub>O dielectric medium.**



**Figure 2: Visual representation of surface polarity of a molecule created by COSMO calculation.**

1. Klamt, A.; Schuurmann, G., COSMO: a new approach to dielectric screening in solvents with explicit expressions for the screening energy and its gradient. *Journal of the Chemical Society, Perkin Transactions 2* **1993**, 0, 799-805

2. Klamt, A., CONDUCTOR-LIKE SCREENING MODEL FOR REAL SOLVENTS - A NEW APPROACH TO THE QUANTITATIVE CALCULATION OF SOLVATION PHENOMENA. *Journal of Physical Chemistry* **1995**, 99, 2224-2235.

# MOLECULAR CLUSTERS OF TWO, FOUR & EIGHT MOLECULES FROM CRYSTAL STRUCTURE OF PABA

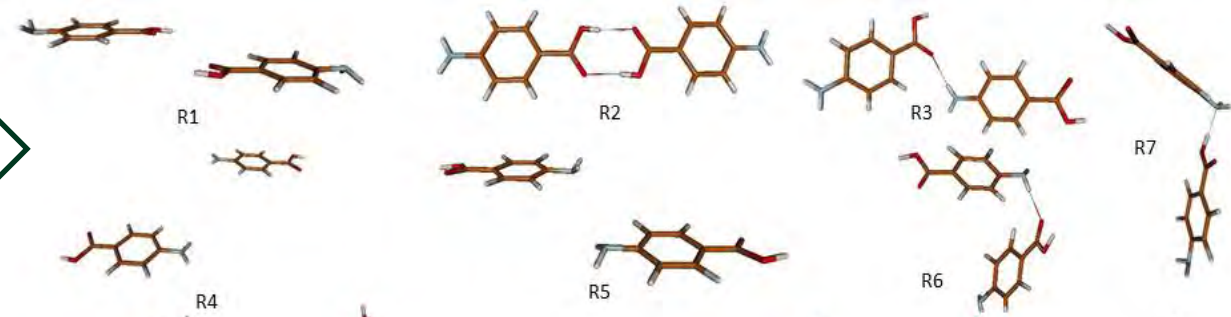


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Dimers: R1-R7

R1,R5,R6,R7( $\beta$ -polymorph)

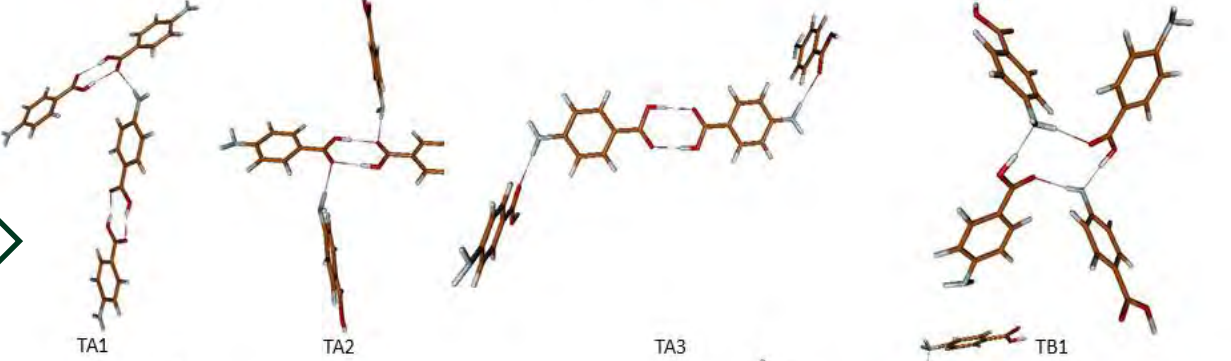
R2,R3,R4 ( $\alpha$ -polymorph)  $\Rightarrow$



Tetramers:TA1,TA2,TA3,TB1

TA1,TA2,TA3( $\alpha$ -polymorph)

TB1 ( $\beta$ -polymorph)  $\Rightarrow$



Octamers :OA1, OB1

OA1( $\alpha$ -polymorph)

OB1( $\beta$ -polymorph)  $\Rightarrow$



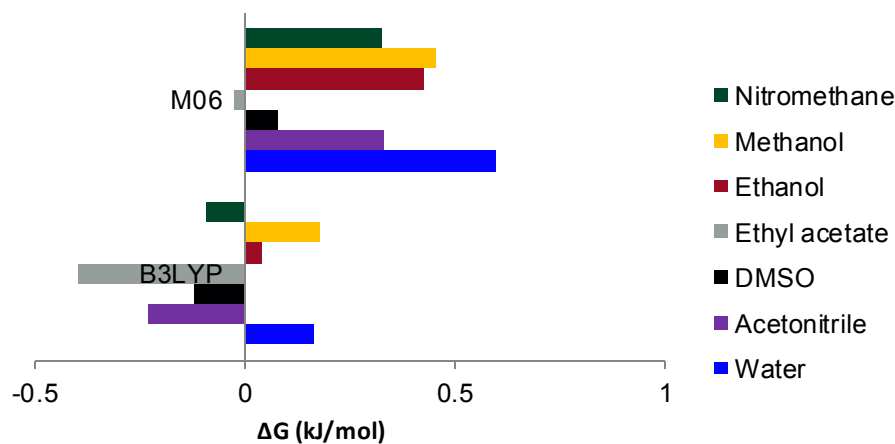
# COSMO: Relative Energies of Solvated PABA clusters: Dimers



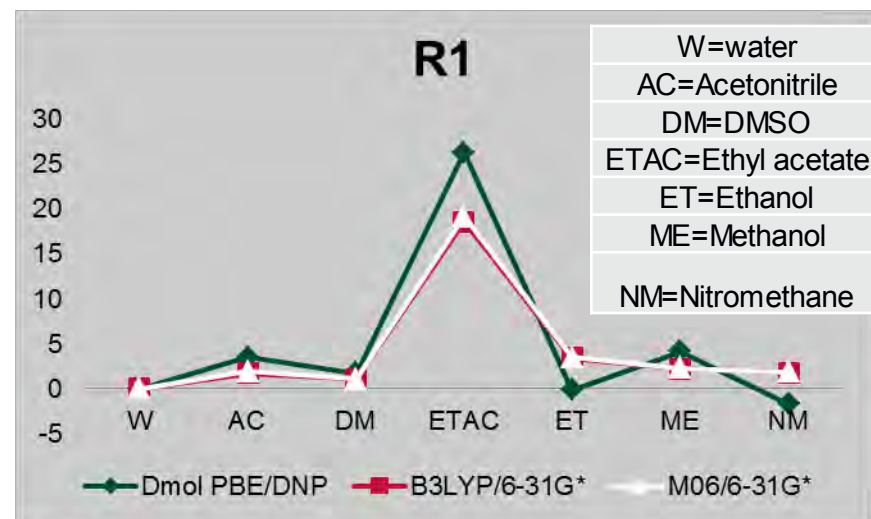
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Relative Energies in kJ/mol

## $\Delta G$ clusters R6-R3

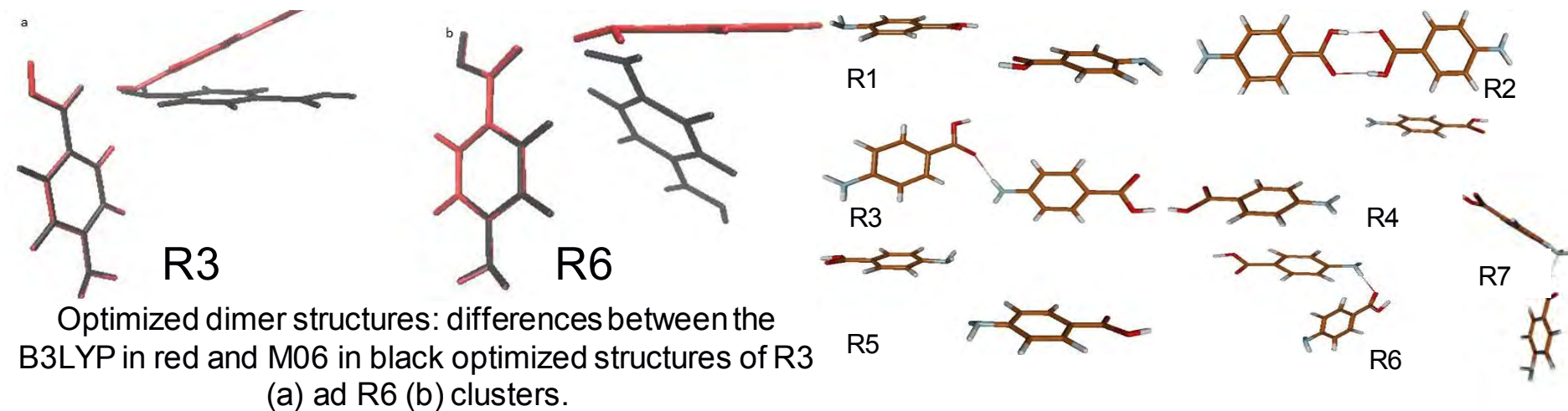


Relative Energies in kJ/mol



W=water  
AC=Acetonitrile  
DM=DMSO  
ETAC=Ethyl acetate  
ET=Ethanol  
ME=Methanol  
NM=Nitromethane

— Dmol PBE/DNP — B3LYP/6-31G\* — M06/6-31G\*



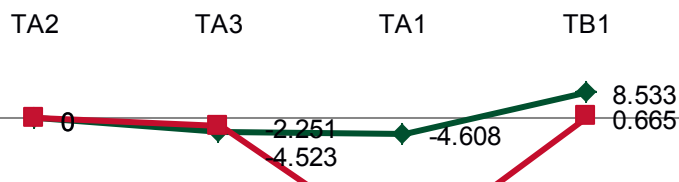
# COSMO: Relative Energies of Solvated PABA clusters: Tetramers and Octamers



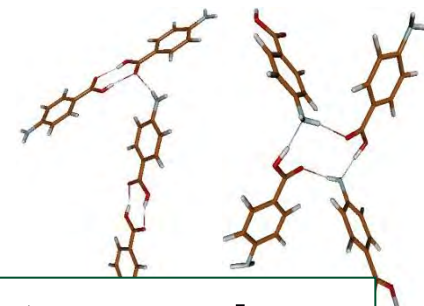
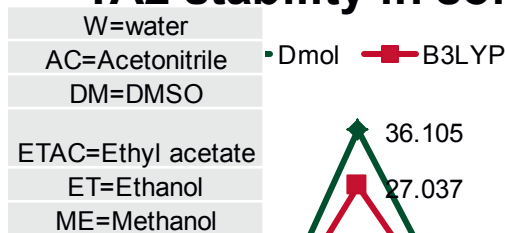
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## Tetramers in water

—◆— Dmol —■— B3LYP



## TA2 stability in solvents

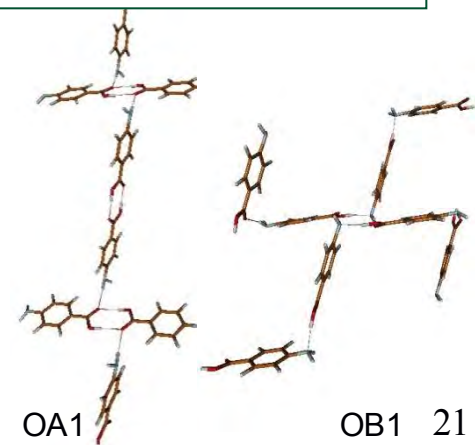


**TA1 cluster always appear the most stable tetramer in energy**

**Clusters extracted from  $\alpha$  polymorph found most stable**  
**All clusters found most stable in water**

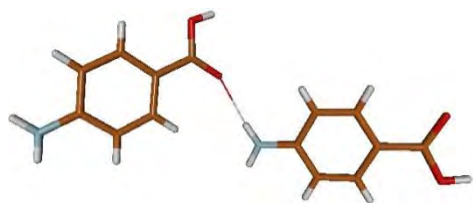
The  $\alpha$  type cluster is the most stable in energy in all cases.

Tetramers that contain two carboxylic acid dimers (TA1) is the most stable cluster in solution.

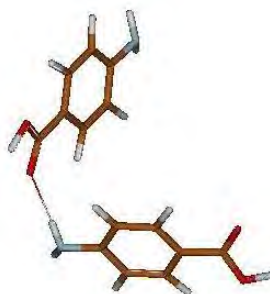




# R3-R6 CLUSTER COMPARISONS



$\alpha$  R3



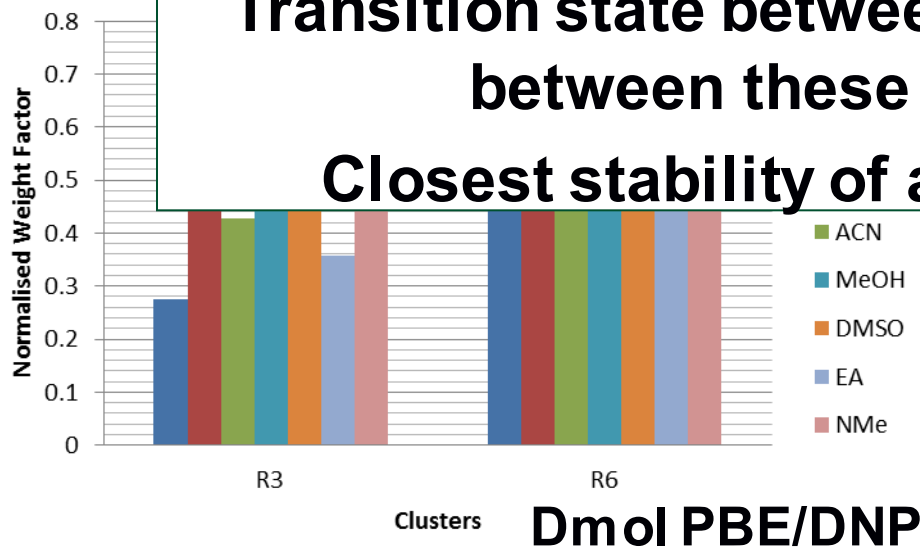
$\beta$  R6



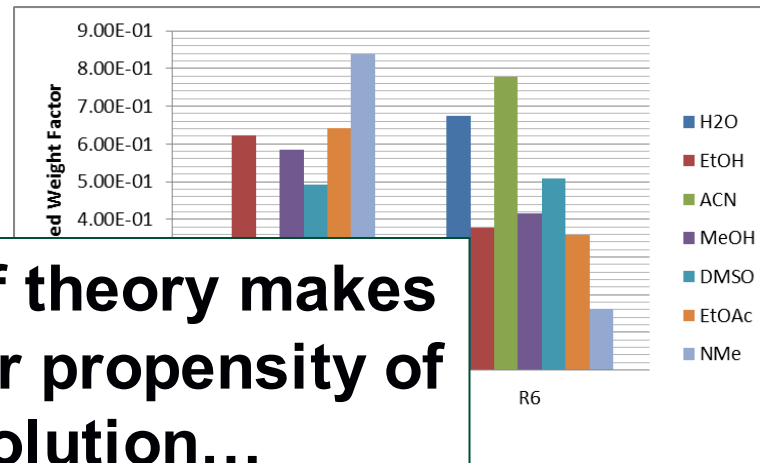
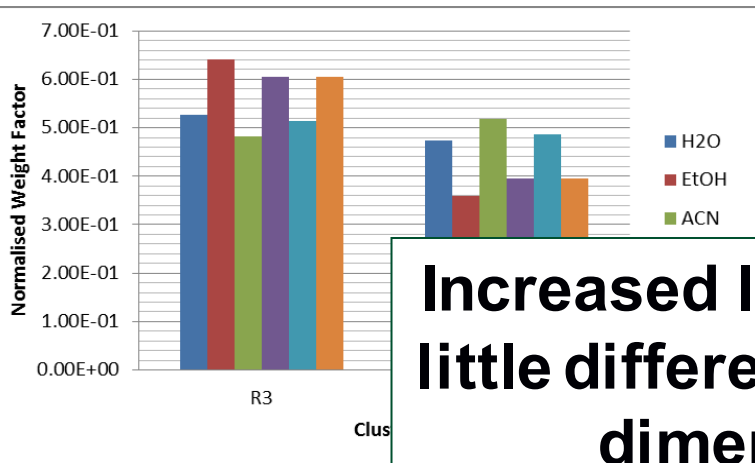
$\beta$  R7

**Transition state between  $\alpha$  and  $\beta$  could be between these two dimers**

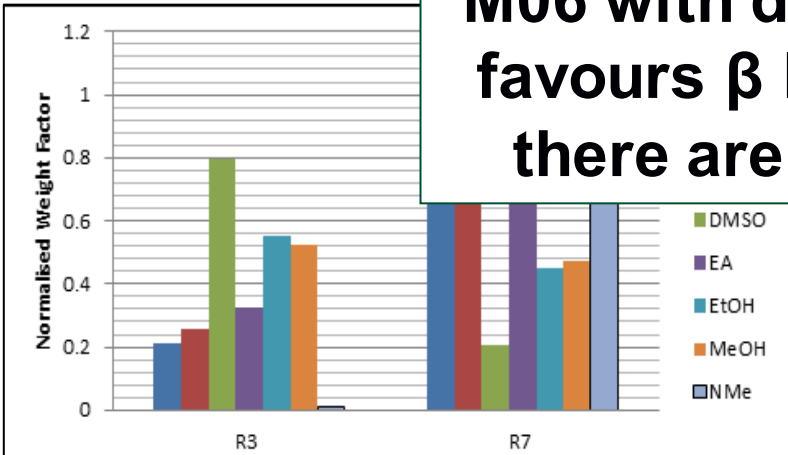
**Closest stability of all dimers tested**



- $\text{H}_2\text{O}$  shows most stability for the  $\beta$  reflecting experimental work<sup>5</sup>.
- Potential building block for formation of the  $\beta$  polymorph
- If the dimers could be blocked.
- Possible transition state from  $\alpha$  to  $\beta$ .



**Increased level of theory makes little difference for propensity of dimers in solution...  
M06 with dispersion correction favours  $\beta$  building block when there are no dimers present**



ich has

- R6  $\beta$  dimer more stable with M06 dispersive correction when carboxylic acid dimers are not present. Especially in water.
  - Reflecting experimental observations



# Conclusions

**What are the relative stabilities of the single molecule and small clusters of PABA based on the crystal structure within the COSMO dielectric**

Quantum chemical calculations revealed the importance of the carboxylic acid dimers and how they can be stabilised in solution.

**What is the influence of solvent on which polymorph of PABA crystallises**

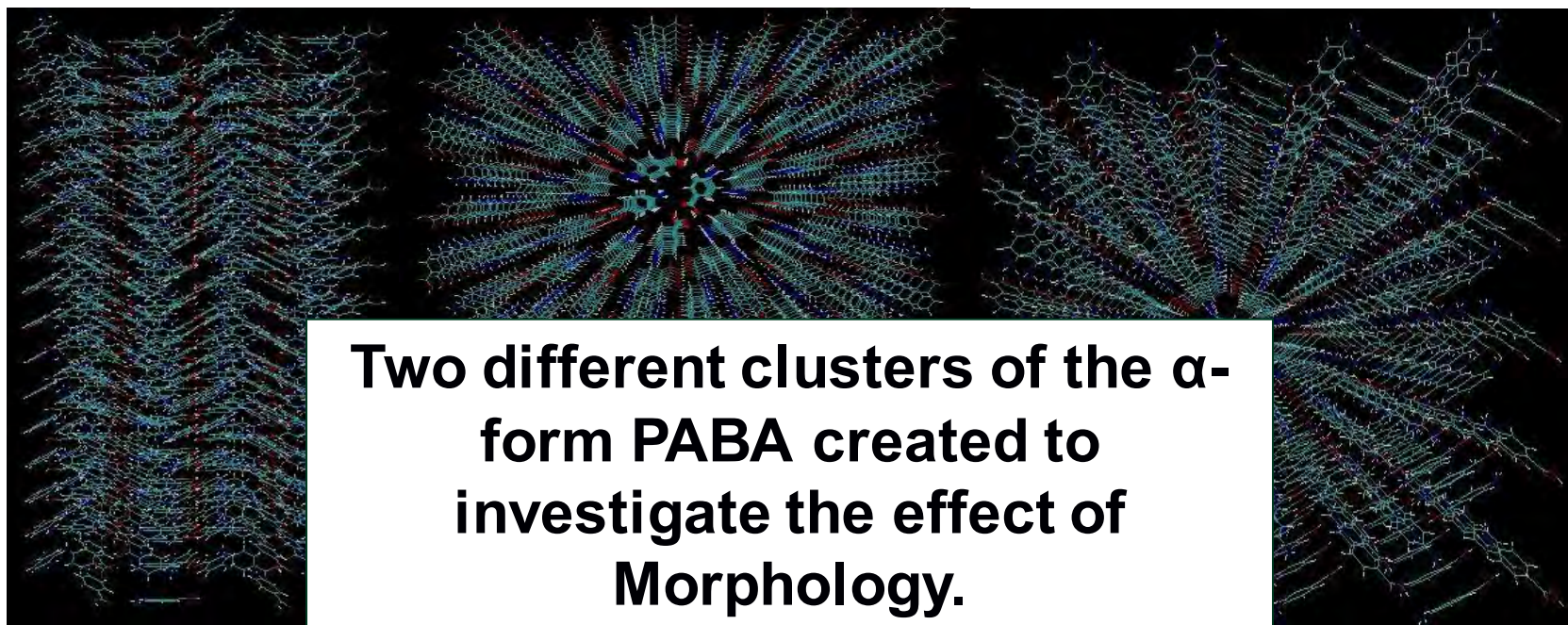
Depending of the level of theory specific clusters are more stable in the presence of a solvent. Particular interesting are the clusters that contain an NH---O interaction (R3 and R6) where their stability change in different solvent environment and also the OH---N interaction (R7 cluster).





## Computational Experiments: Research Questions

- Q1: What is the fitness and transferability of the potential used to perform simulations of molecular clusters in solution as a function of temperature?
- Q2: What are sufficiently large clusters to confer structural stability (taking crystal structure as the starting point) and what influence does the shape of the cluster have?
- Q3: Consider possible outcomes from the simulations e.g. (i) clusters retain order (ii) cluster becomes liquid like but retain order (iii) clusters break-up i.e. dissolve



2 x 16 x 2 anhydrous  
crystal of PABA  $\alpha$ -form

The 3 x 7 x 3 anhydrous  
crystal of PABA  $\alpha$ -form

6 x 5 x 4 anhydrous crystal  
of PABA  $\beta$ -form

Two different clusters of  $\alpha$ -form PABA created to investigate the effect of Morphology.



- Molecular dynamics simulations carried out using program GROMACS.
- Employed the Generalised Amber Force Field (GAFF) calculations set-up using the *antechamber* utility.
- Molecular clusters of PABA solvated by water treated using the TIP3P potential.

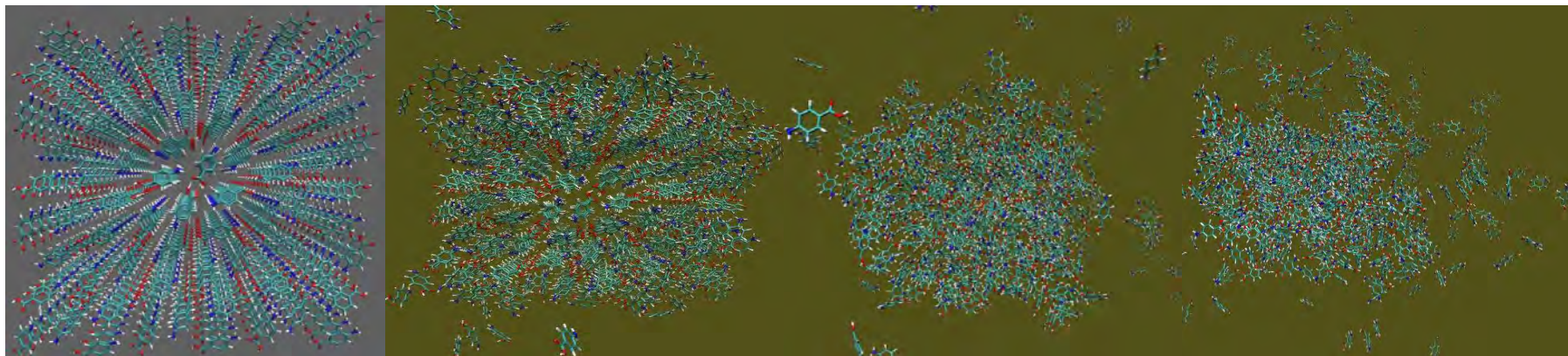
## Computational approach

- Initialised with energy minimization of the solvated clusters for 2000 steps using the steepest descents. System equilibration (100ps) in the NVT ensemble.
- Second period of equilibration (100ps) in the NPT ensemble.
- Production MD for 5 ns in the NPT ensemble at one atm. pressure and using time-step of 2fs.
- The electrostatic interactions are described with RESP charges based on density functional theory.

# MD Simulations of 'Nano-crystal' of Alpha Polymorph of PABA in Water

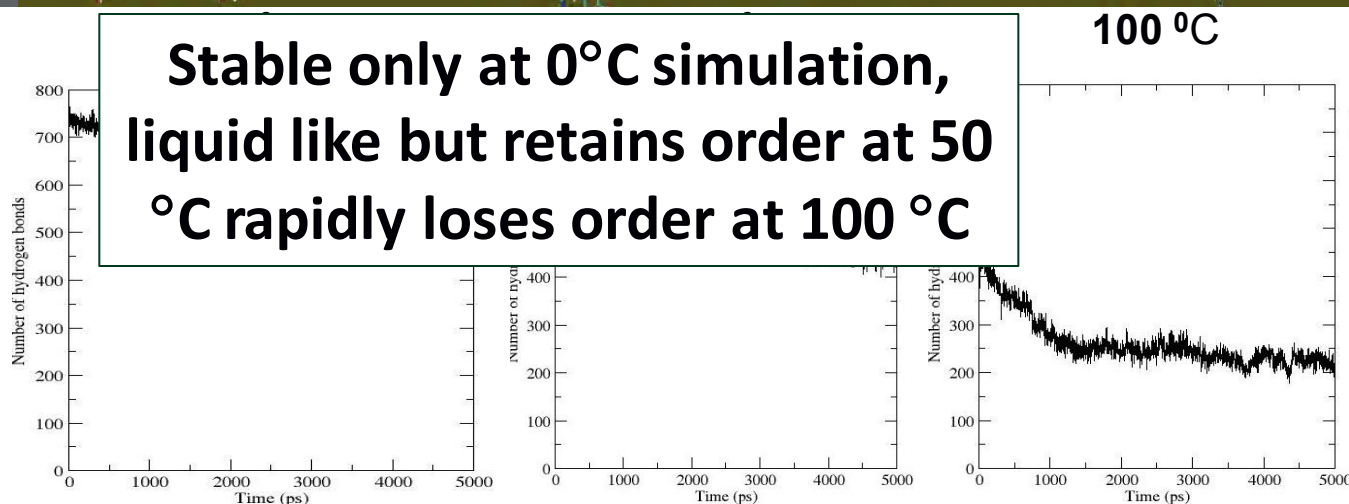


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Starting structure

Number of solute-solute Hydrogen bonds

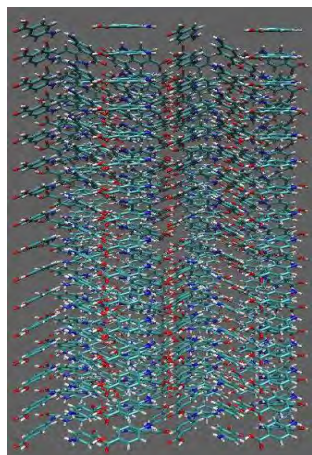


The 3 x 7 x 3  $\alpha$  cluster was totally stable only in the 0°C simulation, in the 50°C simulation cluster retains order for about 3ns and in the 100°C simulation cluster rapidly loses order

# MD Simulations of 'Nano-crystal' of Alpha Polymorph of PABA in Water

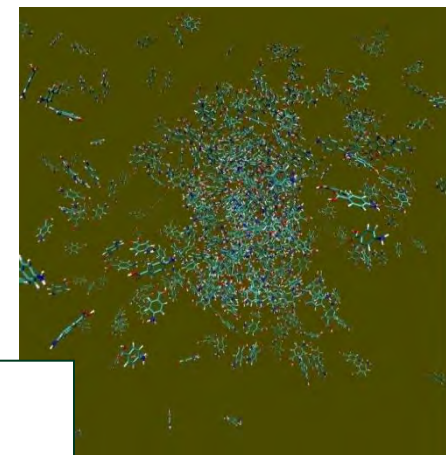
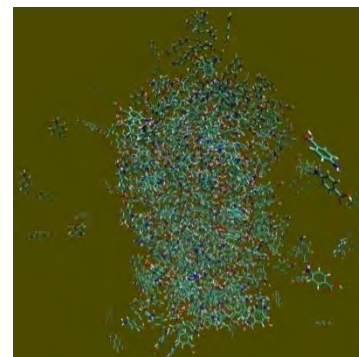
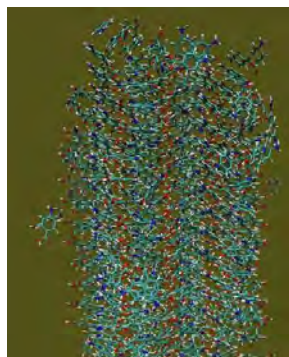


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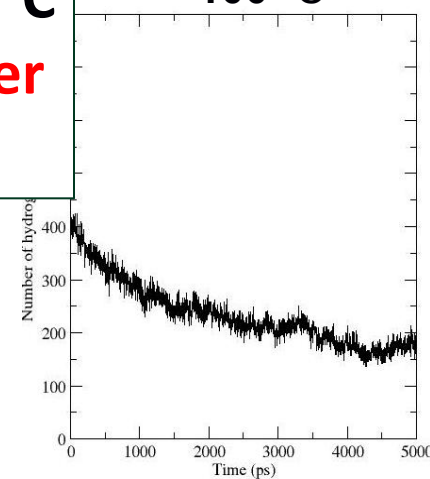
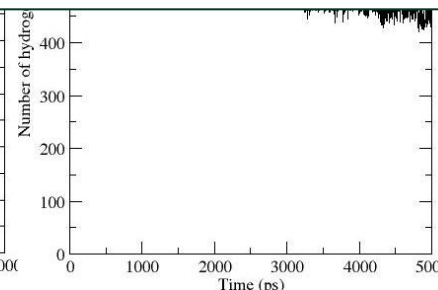
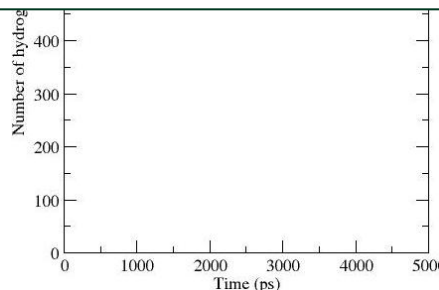
Starting structure

Number of solute-solute Hydrogen bonds



100 °C

Stable only at 0°C simulation, liquid like but retains order at 50 °C loses order at 100 °C: **retains order longer at 50 °C and at 100 °C**

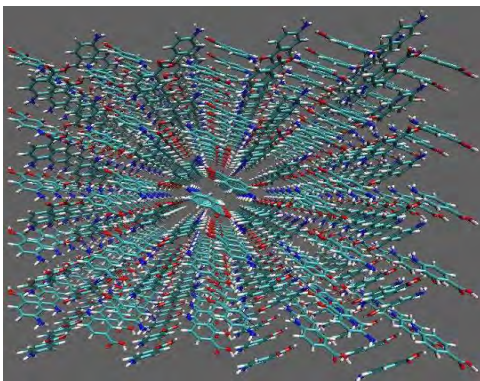


The 2 x 16 x 2  $\alpha$  cluster was totally stable only in the 0°C simulation, in the 50°C simulation cluster retains order for about 4ns and in the 100°C simulation cluster rapidly loses order

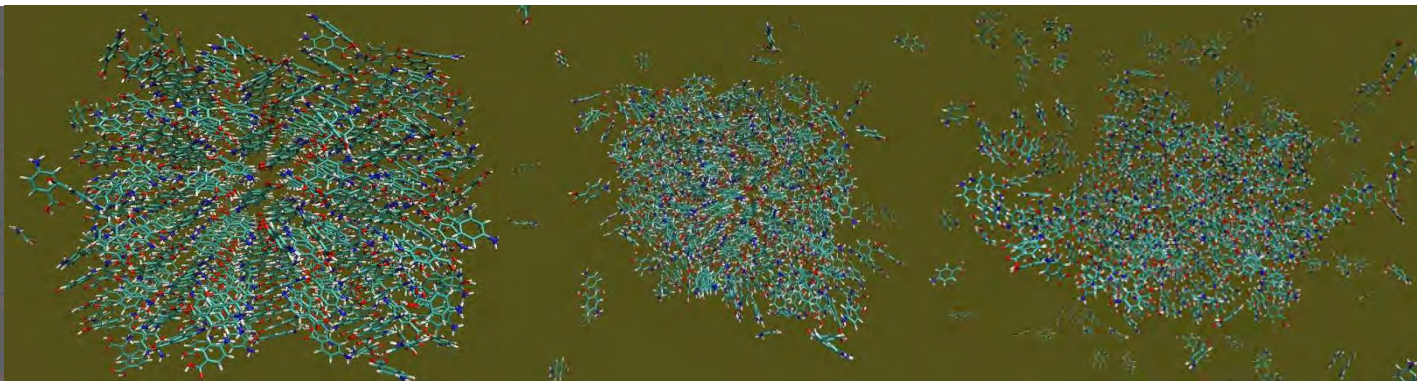
# MD Simulations of 'nano-crystal' of Beta Polymorph of PABA



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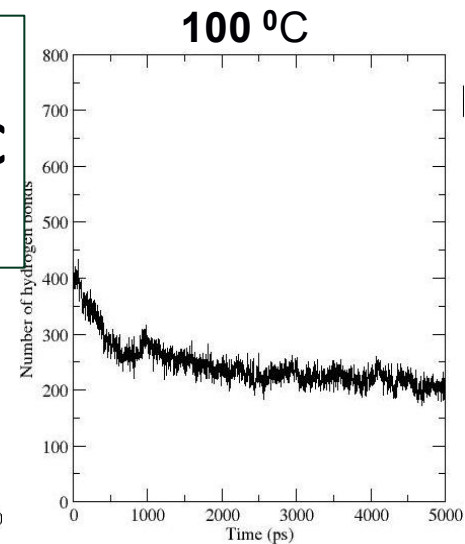
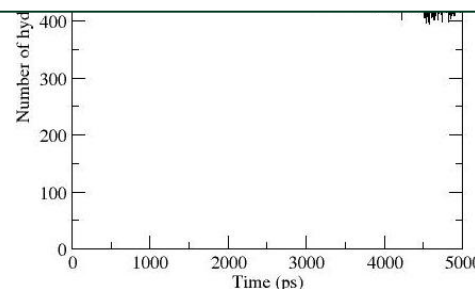
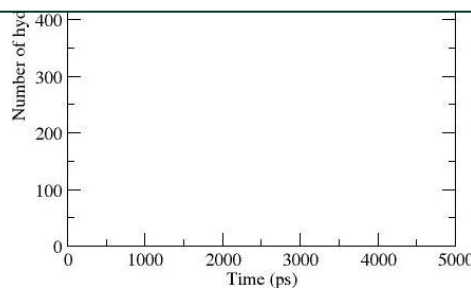


Starting structure



Stable only at 0°C simulation,  
liquid like but retains order at 50 °C  
loses order at 100 °C

Number of  
solute-solute  
Hydrogen bonds

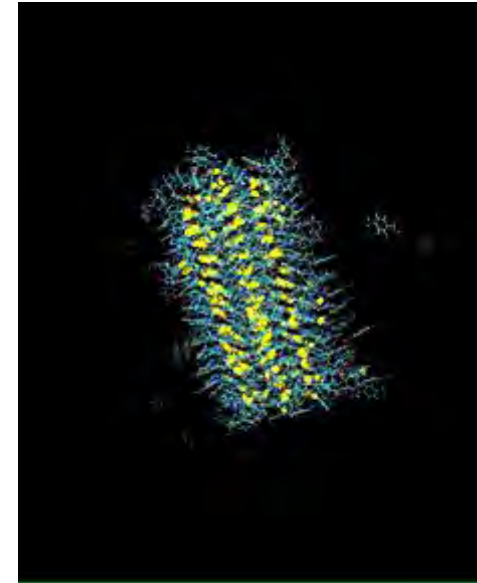
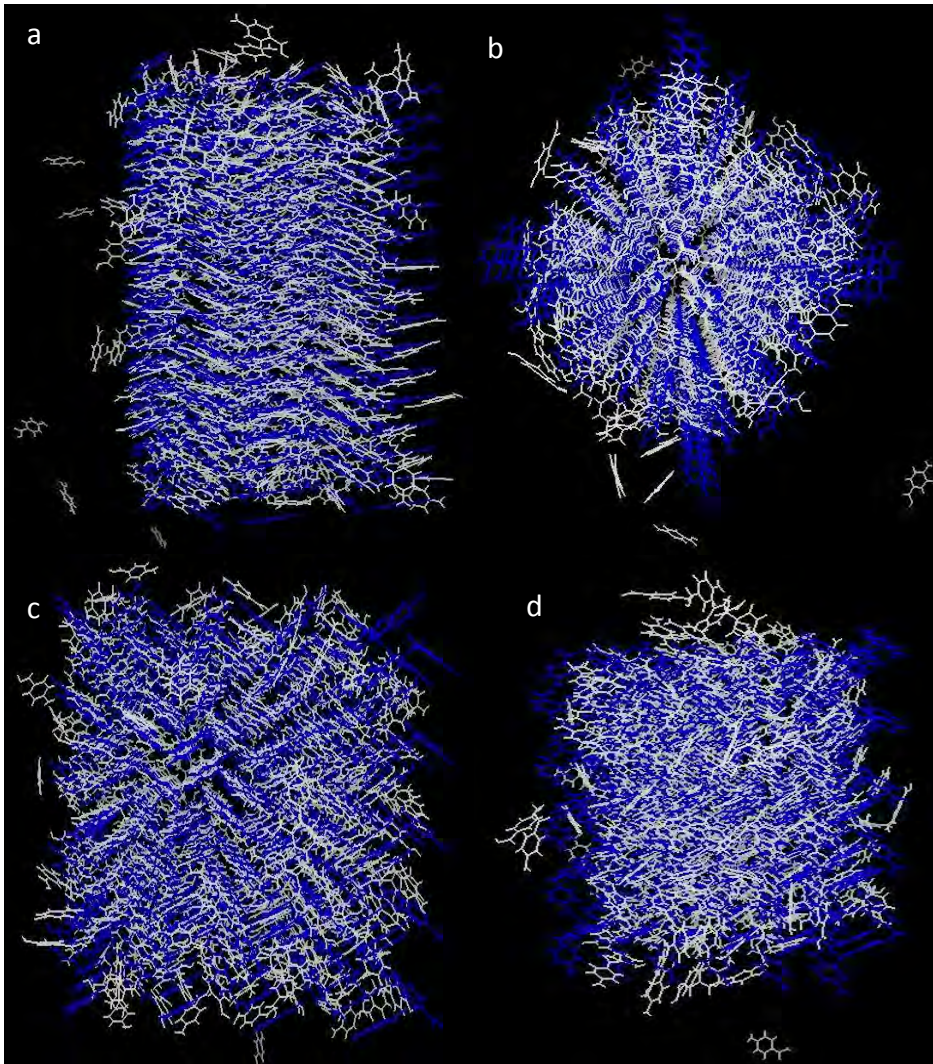


The 6 x 5 x 4  $\beta$  cluster remained stable at 0°C. At 50°C core of cluster retained structural integrity, with detachment of some surface PABA molecules. The 100°C simulation manifested almost complete dissolution of the cluster in the initial short equilibration period.

# Comparison of the cluster before and after 5ns simulation



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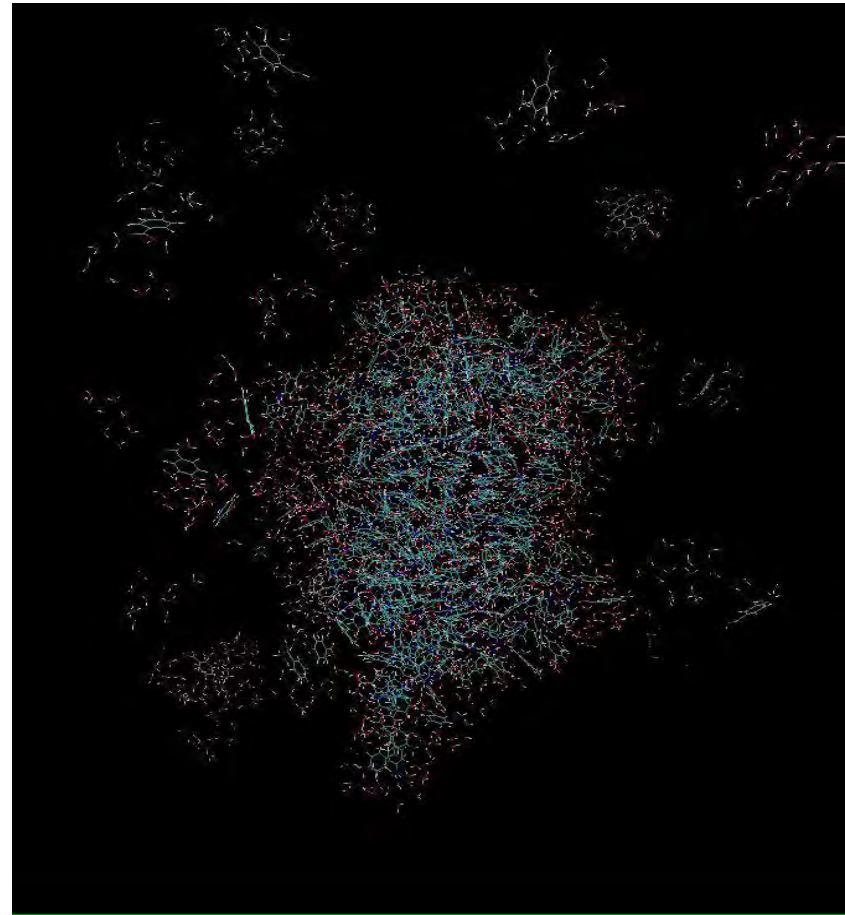
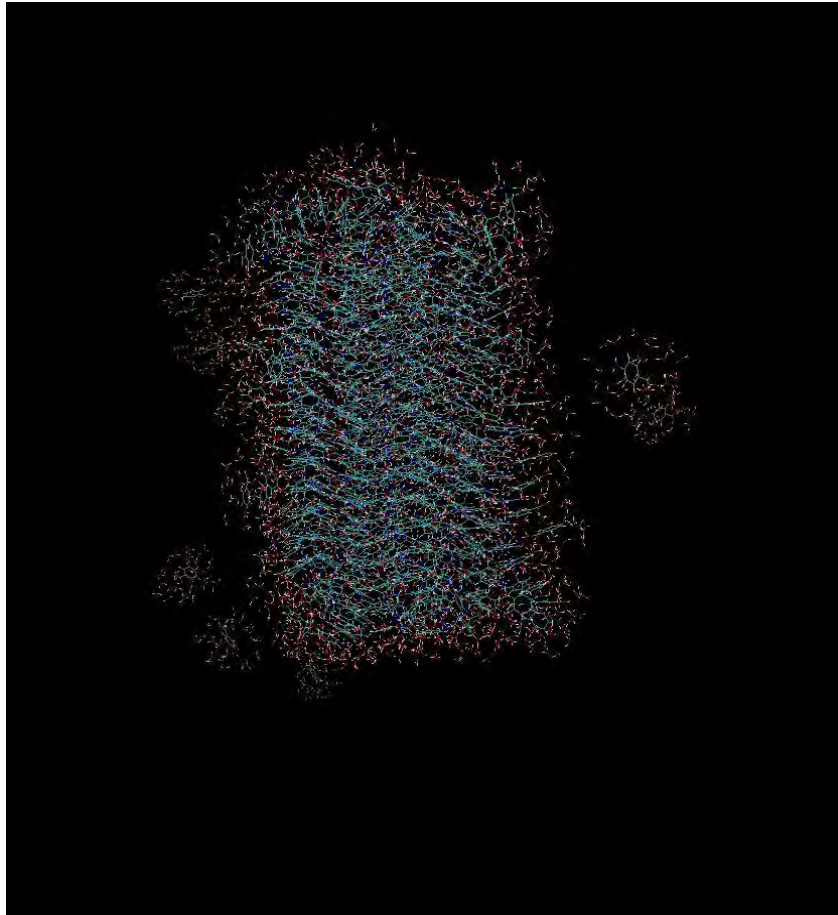


Overlap of the starting  $2 \times 16 \times 2$  crystal structure of the  $\alpha$ -form (a) side view (b) top view and  $6 \times 5 \times 4$  crystal structure (c) side view (d) top view with the crystal structure image taken after 5ns simulation at  $0\text{C}^\circ$ . PABA molecules detached first at the edges and corners

# Ingress of Water into Clusters



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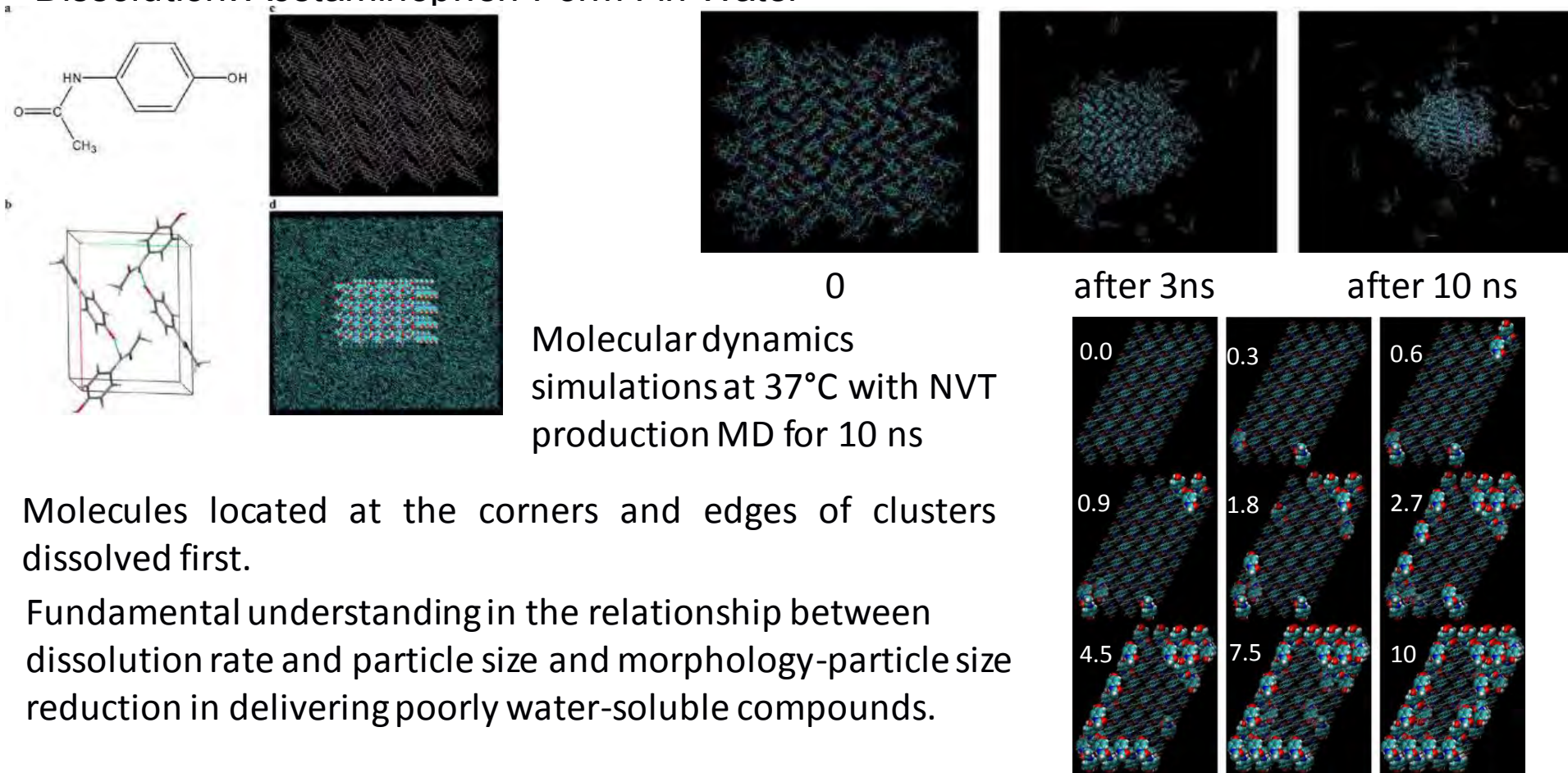
Water in all cases ingress from edges and corners.  
The central core of the cluster retains the long range order.  
More obvious in the simulations performed at high temperatures.





# Comparison: Similar Recent Work

## Molecular Dynamics of Drug Crystal Dissolution: Acetaminophen Form I in Water<sup>1</sup>



Molecules located at the corners and edges of clusters dissolved first.

Fundamental understanding in the relationship between dissolution rate and particle size and morphology-particle size reduction in delivering poorly water-soluble compounds.

1.Y. Gao, W.Olsen Mol. Pharmaceutics 2013, 10, 905–917



- **Q1: What is the fitness and transferability of the potential used in the formations of molecular clusters in solution in different**  
The potential used was successfully reproduced the formation of clusters in aqueous environment at 0 °C. When temperature increases perhaps larger clusters required.
- **Q2: Sufficiently large clusters are taken above the critical size?**  
At 0 °C cluster for both polymorphs remain stable. At 100 °C they rapidly losing their order which implicates that may have to built large cluster.
- **Q3: Possible outcomes from the simulations? (i) clusters retain order (ii) cluster becomes liquid like but retain order (iii) clusters**

Simulations revealed that at three different temperatures the three different possible outcomes states were observed.



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**The Critical Mass Project teams at Manchester and Leeds Universities.**

**Thank you for your attention**