Caffeine self-association in aqueous solution: from the supramolecular to atomic scale clustering

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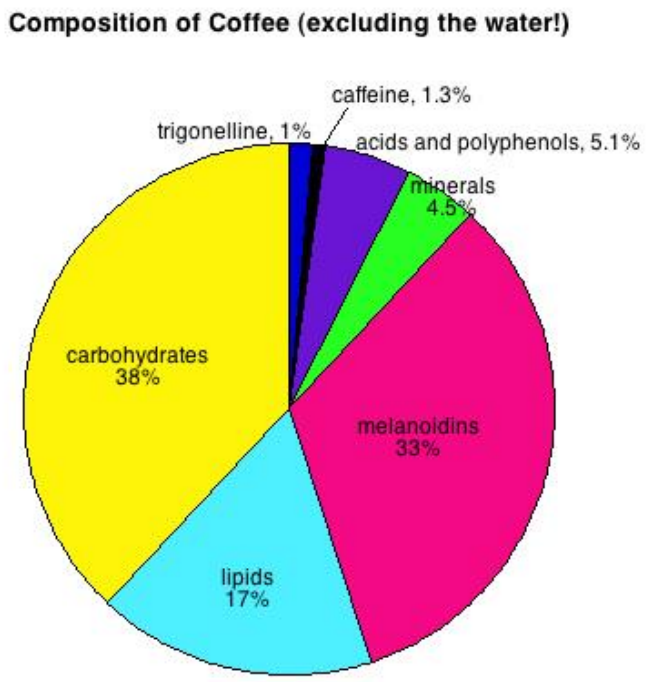
Conference on Atomistic Simulations of Biomolecules: towards a Quantitative Understanding of Life Machinery
ICTP 6th – 10th March 2017

Caffeine in coffee beans

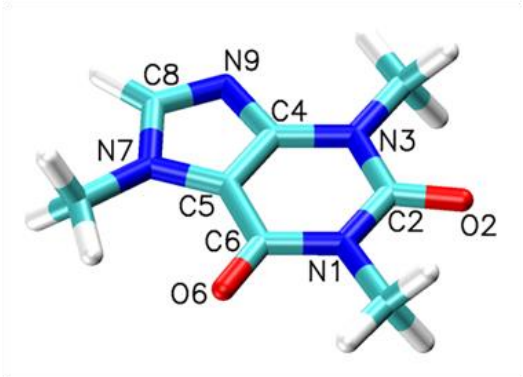


Coffee contains at least 1500 different compounds

Achieve a better knowledge on how water molecules interact with food biomolecules and how this affects the association of food biomolecules in aqueous solution, in order to understand the fundamental role of active substances in the food properties

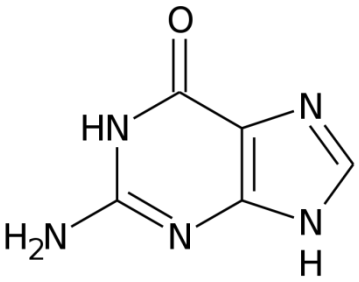
In an espresso coffee c=1 – 1.5 mg/ml

Caffeine as a purine molecule

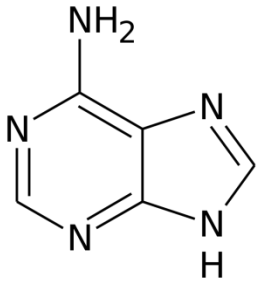


1,3,7-trimethyl xanthine
CAFFEINE

DNA nucleotide bases

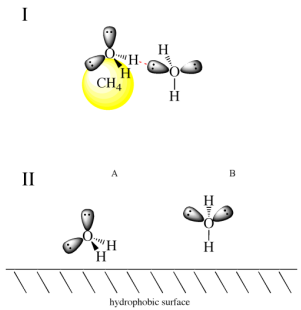


Guanine

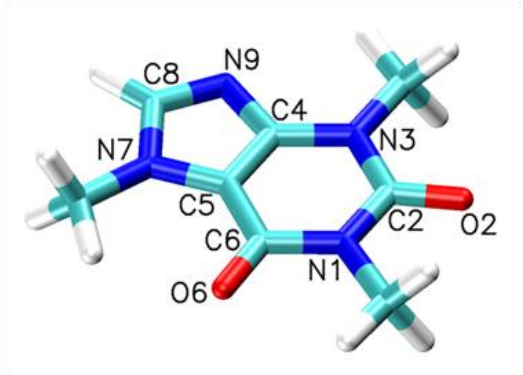


Adenine

Test theory of hydrophobic hydration



Outline



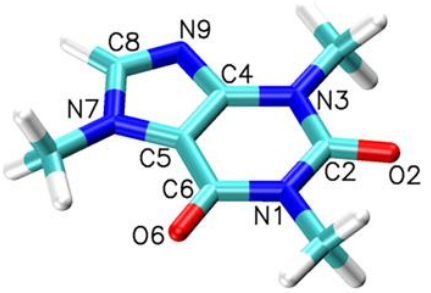
1,3,7-trimethyl xanthine
CAFFEINE

HOMOTACTIC INTERACTIONS AND HYDRATION
MD simulations
vs
NDIS experiments
SANS experiments
Resonance Raman scattering

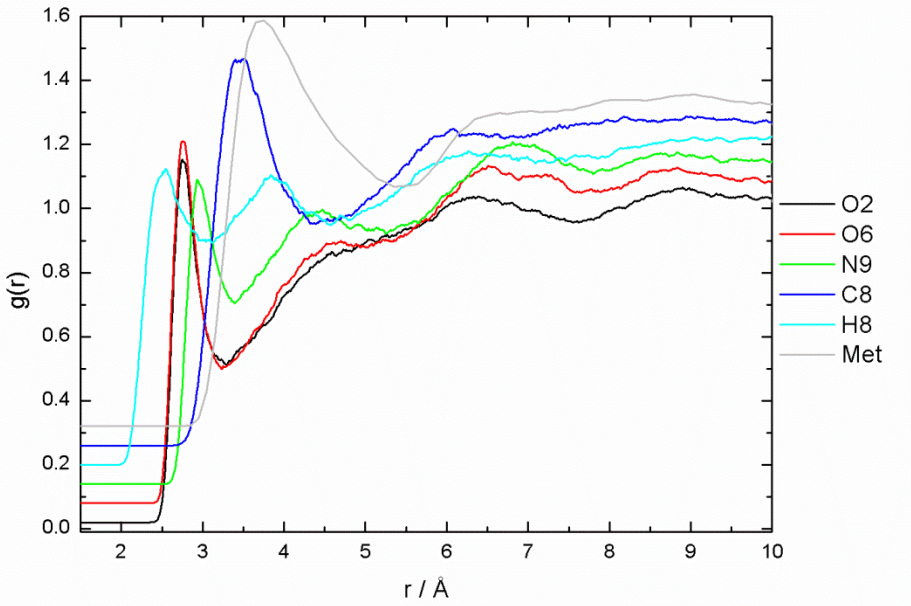
HETEROTACTIC INTERACTIONS
MD simulations
vs
^{1}H-NMR

Caffeine hydration

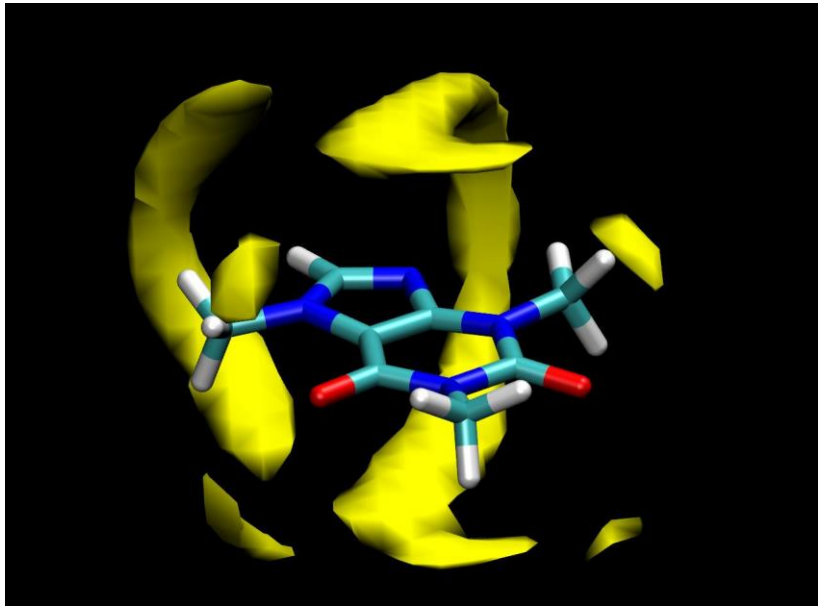
1. **Caffeine force field development**
2. **Water structuring**



Pair distribution functions for water oxygen atoms

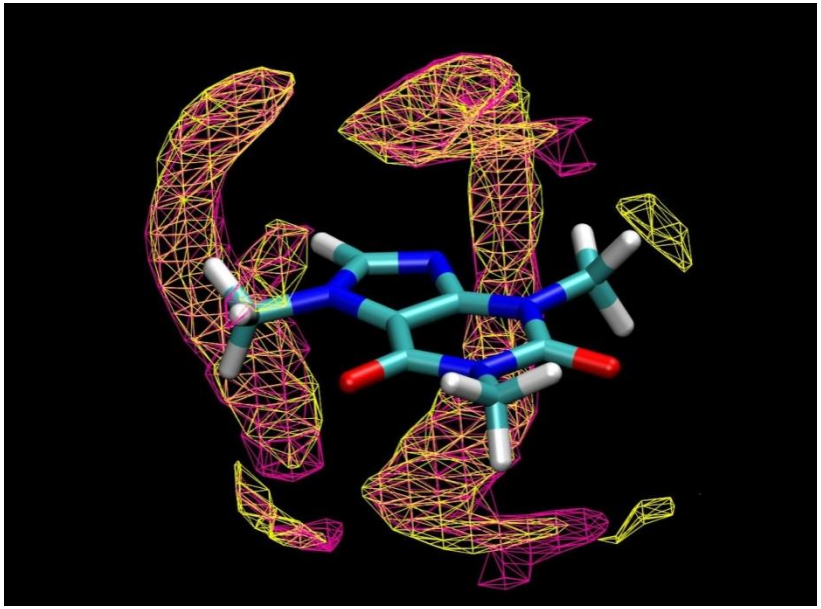


Water oxygen atom density (1.3 x bulk)



Caffeine hydration

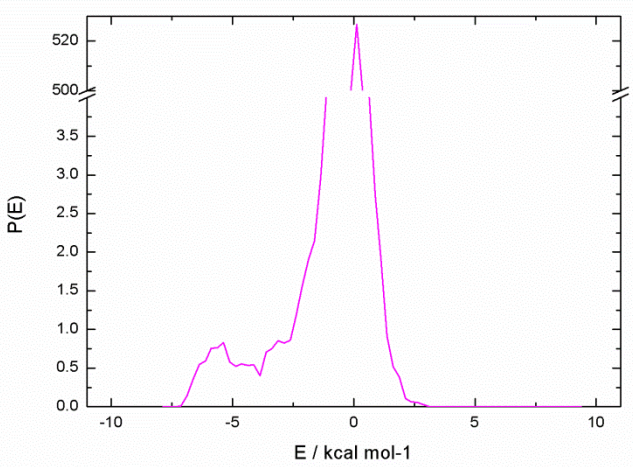
Comparing different water models…TIP4P TIP3P

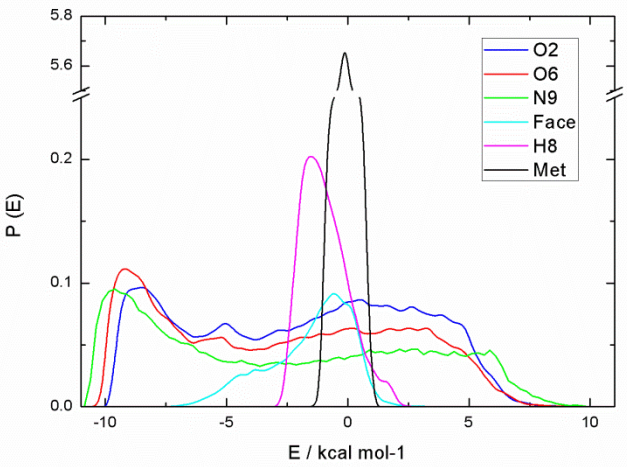


Clouds of water oxygen atom density

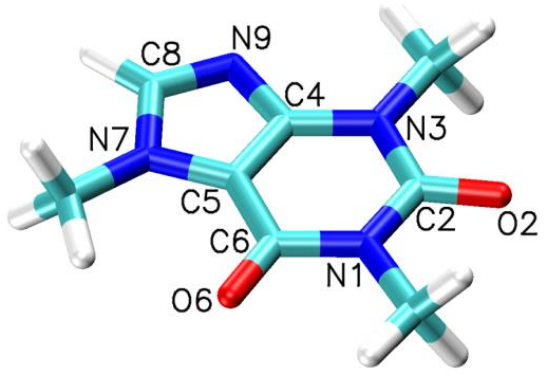
1.3 times bulk density

Caffeine hydration



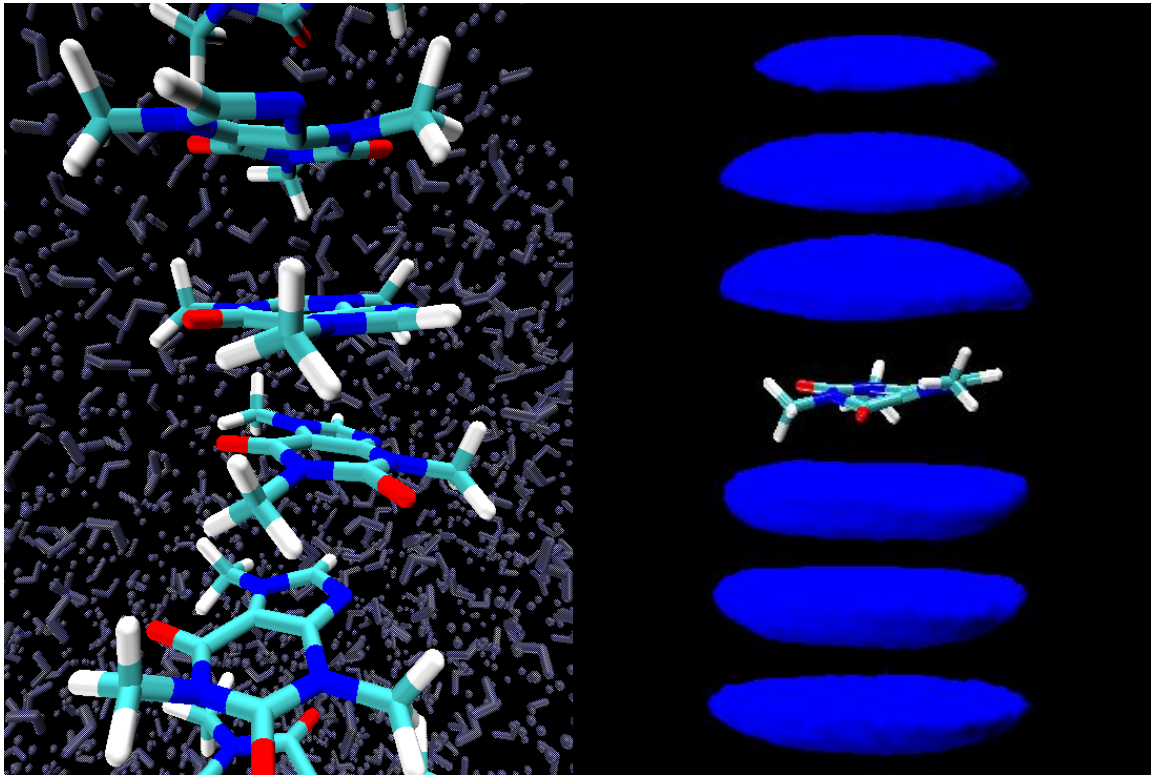


O2	Distance O_{H20}-$O2_{caff}$ < 4	4.4
O6	Distance O_{H20}-$O6_{caff}$ < 4	3.9
N9	Distance O_{H20}-$N9_{caff}$ < 4	3.4
H8	Distance O_{H20}-$C8_{caff}$ < 4	1.9
Met	4 < Distance O_{H20}-$CMet_{caff}$ < 5	4.3
Face	O_{H2O} in the parallelepiped with base formed by N1 and N9 atom positions and height 5	1.3
Bulk	Everything else	639.2



Caffeine self-association

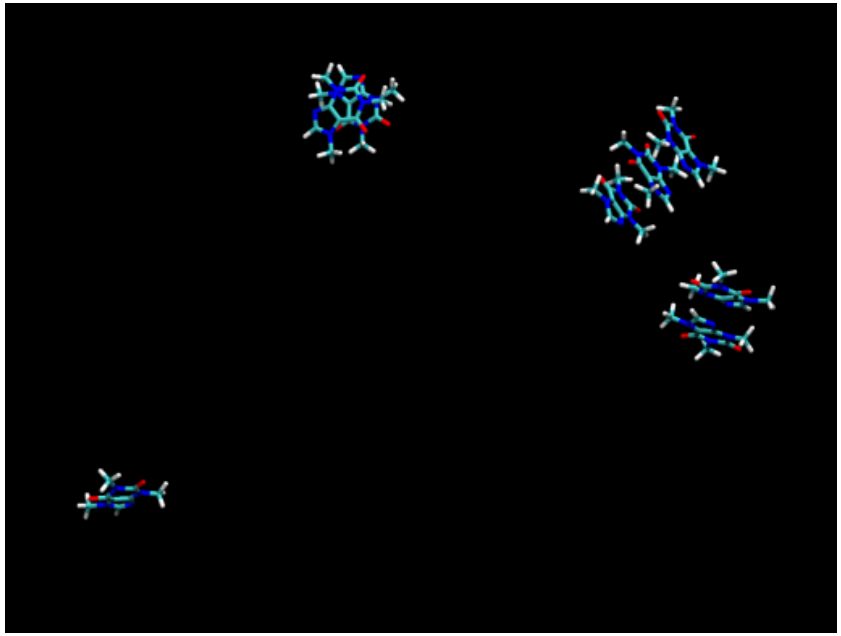
MD simulation 8 caffeine molecules in TIP4P water (0.1 m) at 298 K

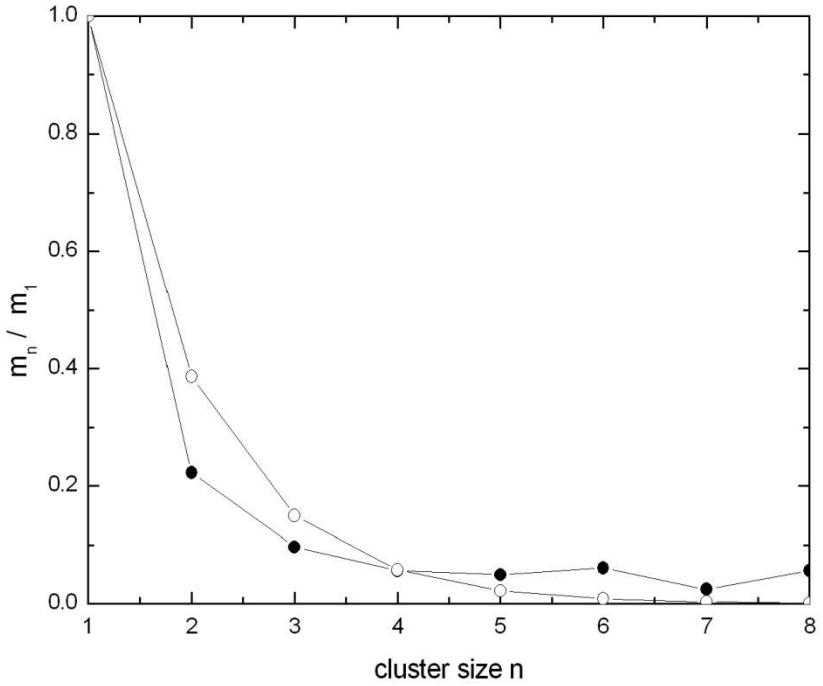


Contours of caffeine density enclosing regions with a caffeine atom density of 10 times bulk density

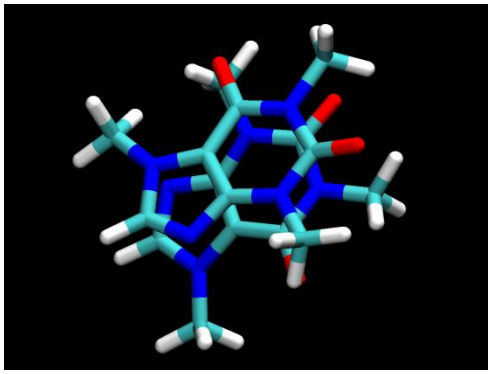
Caffeine self-association

Distribution of cluster sizes from the simulation (•) vs isodesmic model (o)

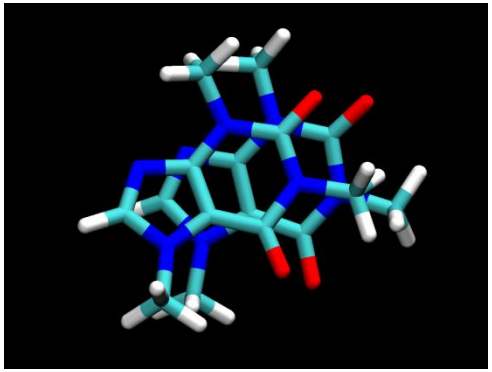




Caffeine self-association

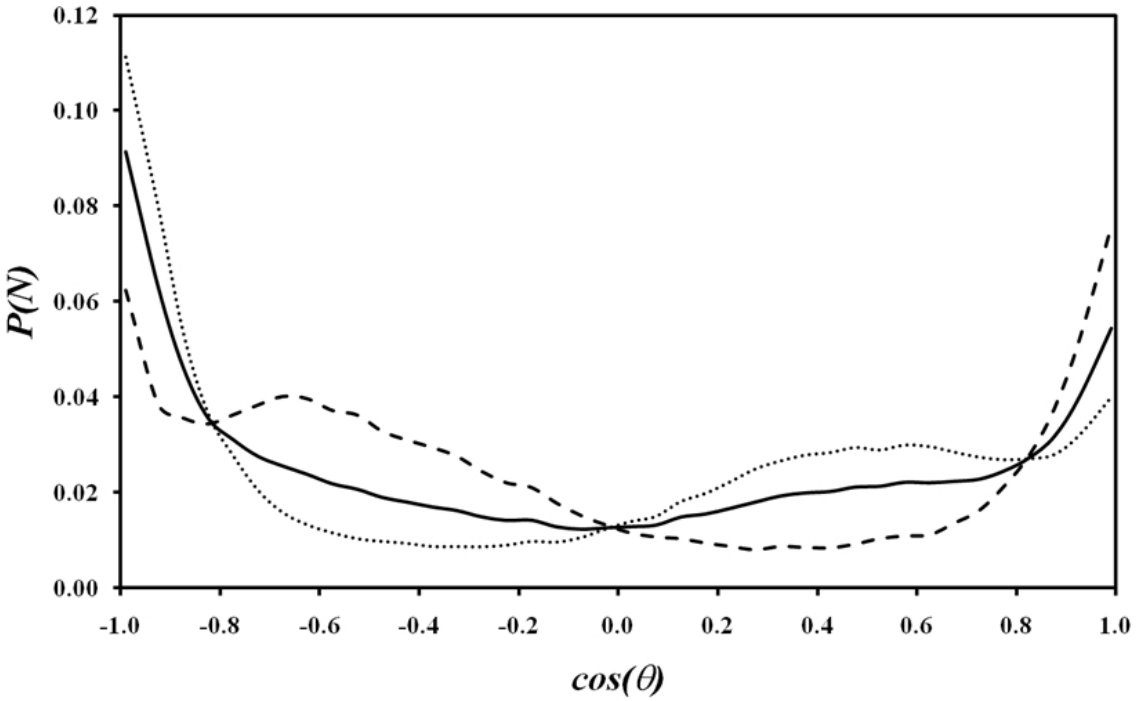


Flipped

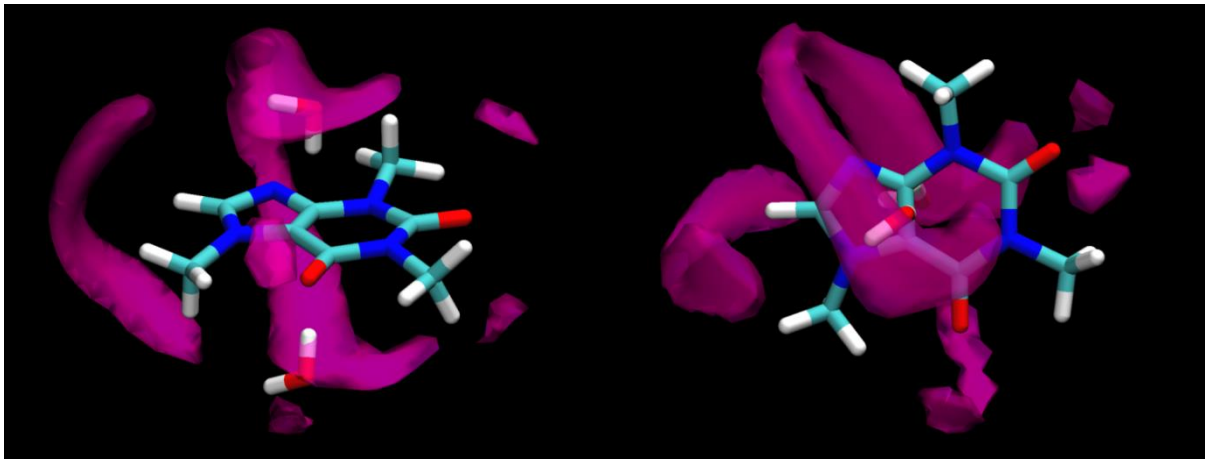


Non-flipped

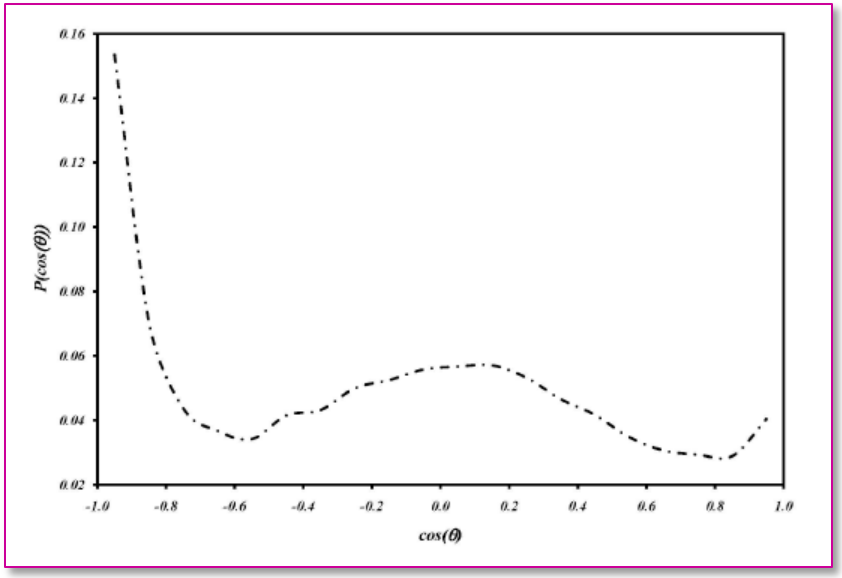
Geometry of association

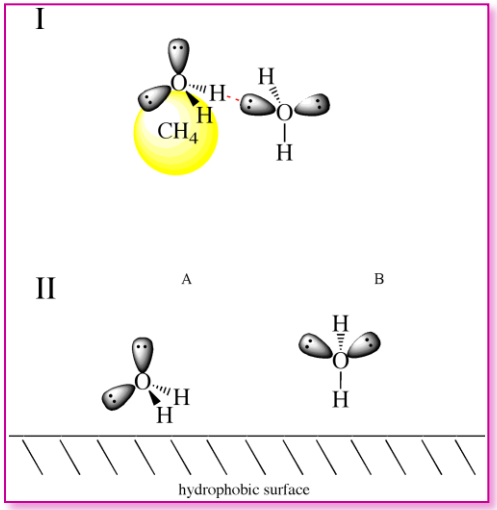


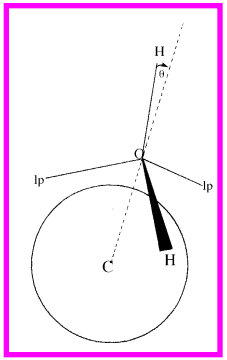
Role of hydration



Probability of observing an angle cos θ between the water bond vectors and the normal to the caffeine surface plane



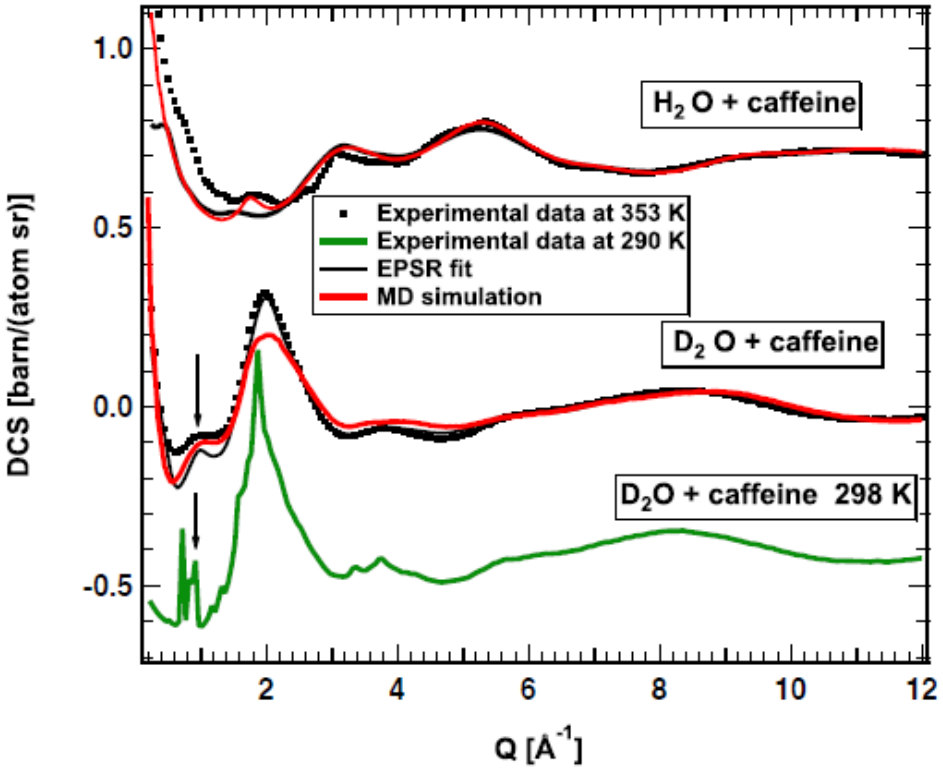




Chandler, D. Nature, 2005, 437(29), 640-647
Tavagnacco, L. et al. J. Phys. Chem. B, 2011, 115(37), 10957-10966

Caffeine hydration

Neutron diffraction with isotopic substitution experiments

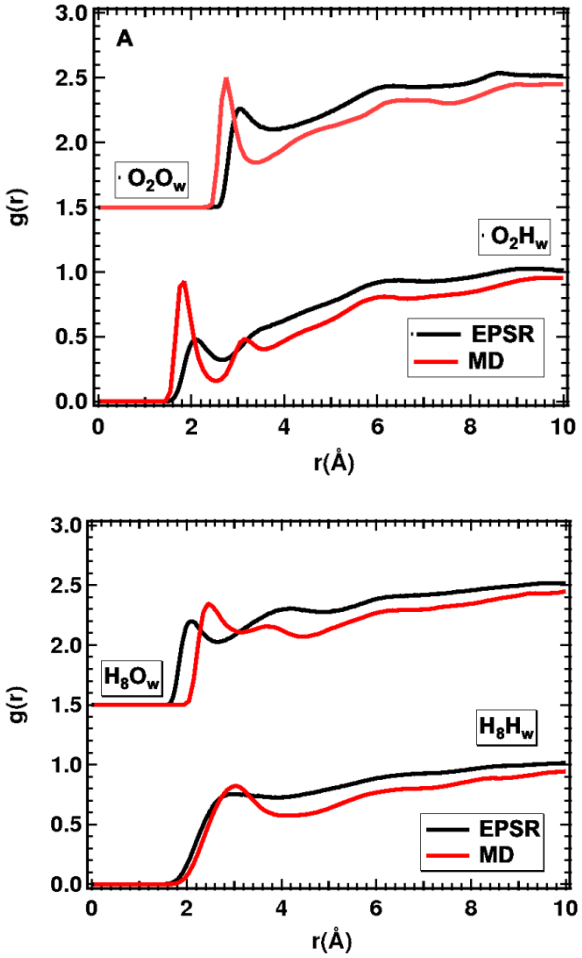


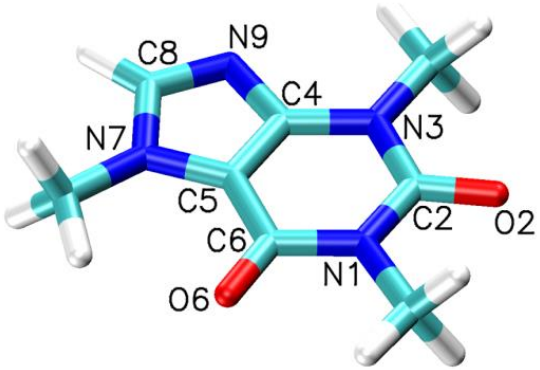
1 m caffeine aqueous solution
$H_2O/D_2O/HDO$
at 80°C

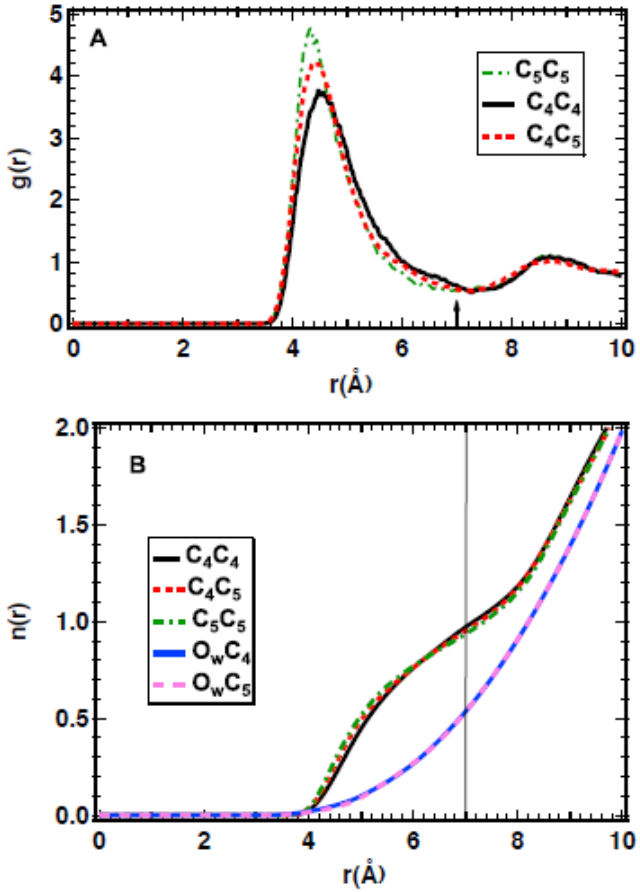
SANDALS diffractometer
at the ISIS spallation neutron source

Tavagnacco, L. et al. J. Phys. Chem. B, 2015, 119(37), 13294-13301

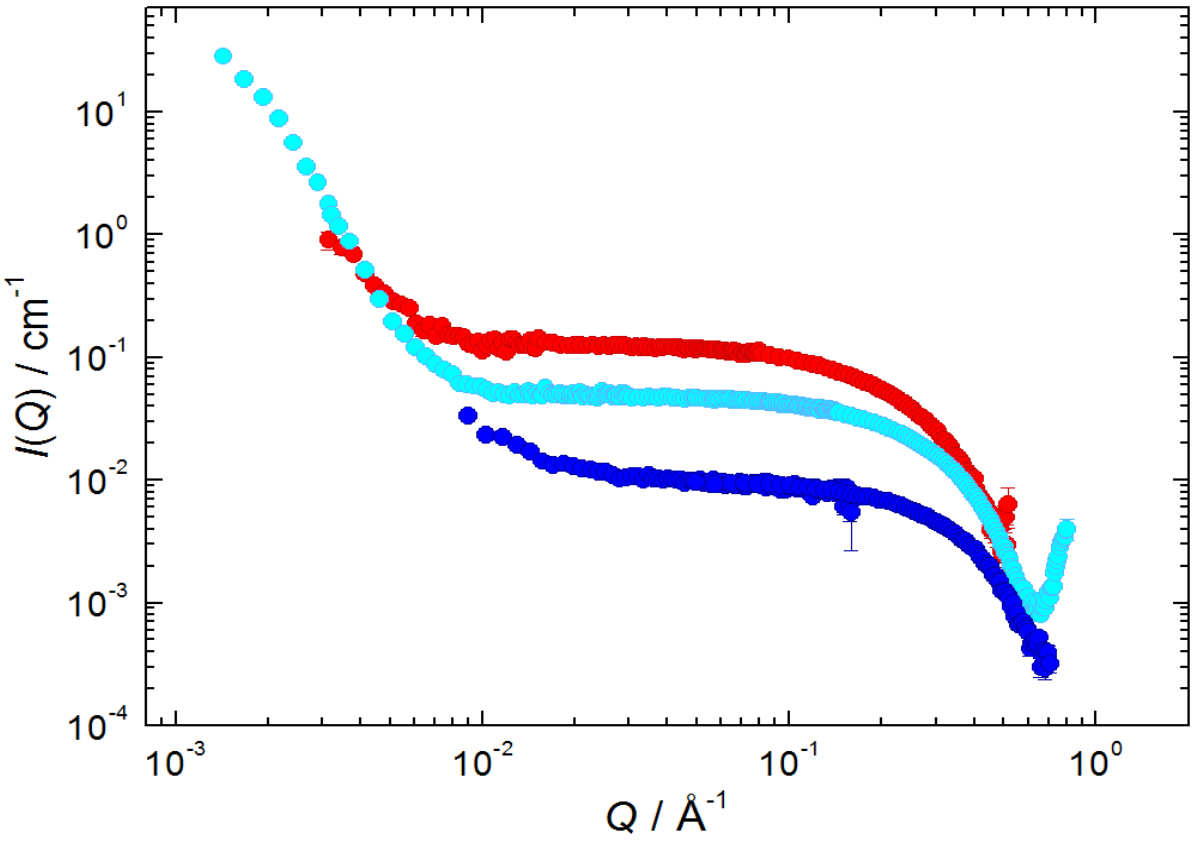
Caffeine hydration







Caffeine self-association

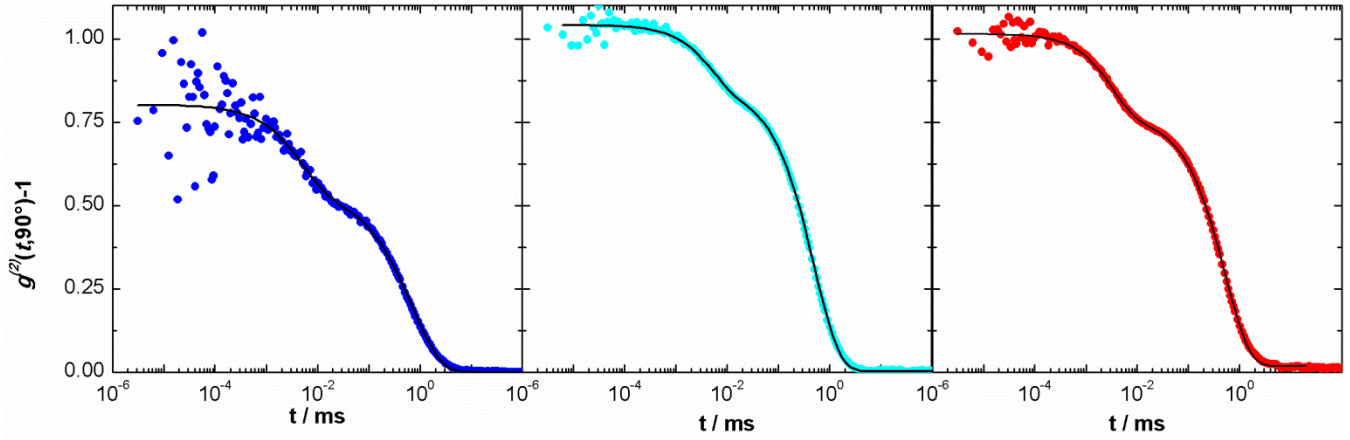


0.1m @ 25°C
0.4m @ 45°C
1.0m @ 80°C

SANS experiments
D11 and D22 diffractometers at ILL

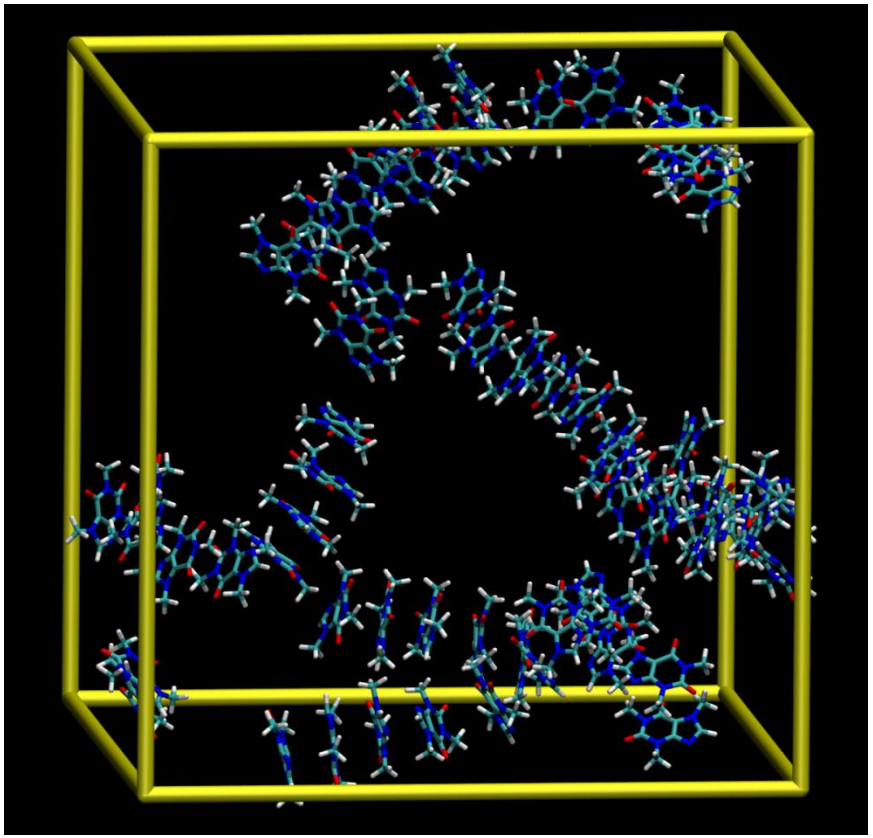
Caffeine self-association

Small particles in solution whose size is increasing with concentration and temperature coexistent with larger structures



	T (ºC)	$\langle R_h^{(1)} \rangle$ (Å)	$\langle R_h^{(2)} \rangle$ (Å)	$R_g^{(1)}$ (Å)	$R_g^{(2)}$ (Å)	$\frac{R_g^{(1)}}{\langle R_h^{(1)} \rangle}$	$\frac{R_g^{(2)}}{\langle R_h^{(2)} \rangle}$
		DLS	DLS	SANS	SLS		
0.1 m	25	3.8 ± 0.5	1100 ± 200	5.1 ± 0.1	1600 ± 100	1.3 ± 0.2	1.5 ± 0.4
0.4 m	43	6.3 ± 0.1	1380 ± 40	6.1 ± 0.1	1500 ± 100	0.97 ± 0.03	1.1 ± 0.1
1.0 m	80	9.6 ± 0.2	3700 ± 100	8.0 ± 0.1	2500 ± 200	0.83 ± 0.02	0.67 ± 0.07

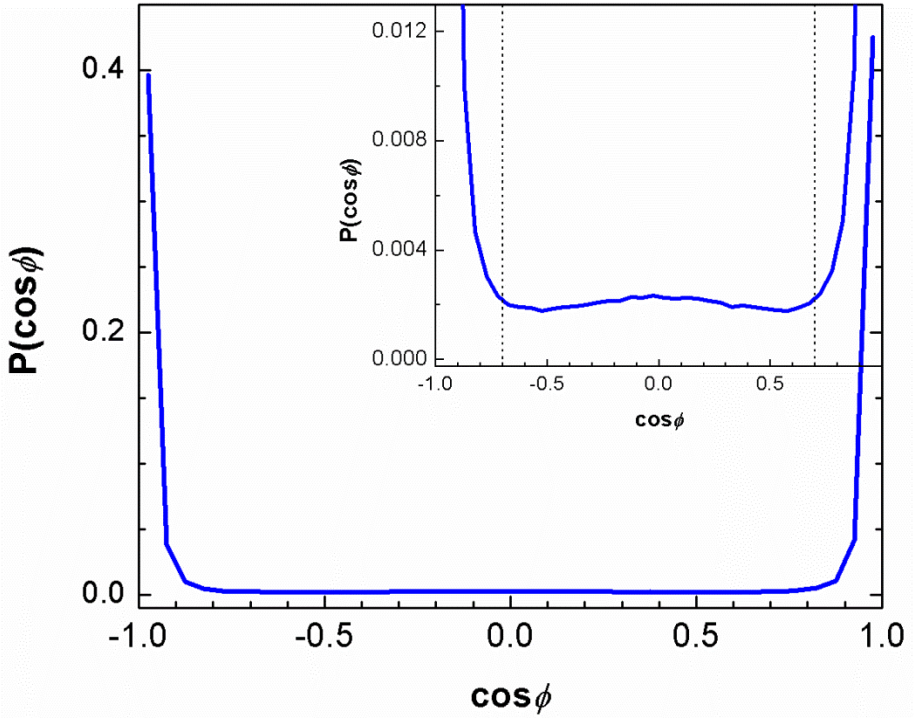
Caffeine self-association

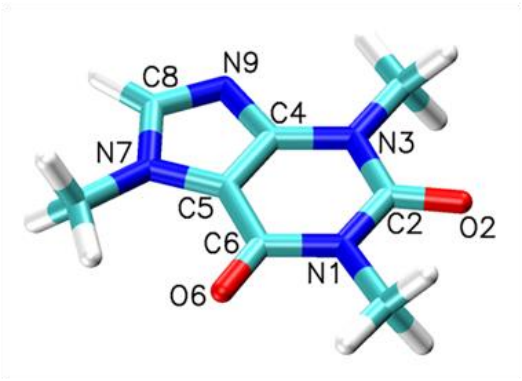


MD simulation 1 m at 80°C

MD clearly shows the presence of *small clusters* and *branched structures*

Stacked vs branched clusters

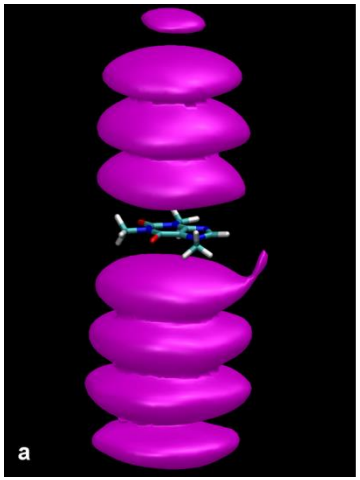


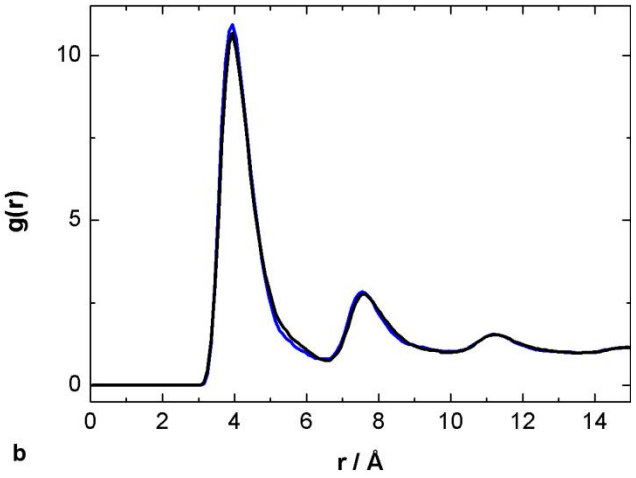


Stacked cluster
dC4-C4 < 6.5 Å and dC5-C5 < 6.5 Å
cosϕ < -0.7 or cosϕ > 0.7

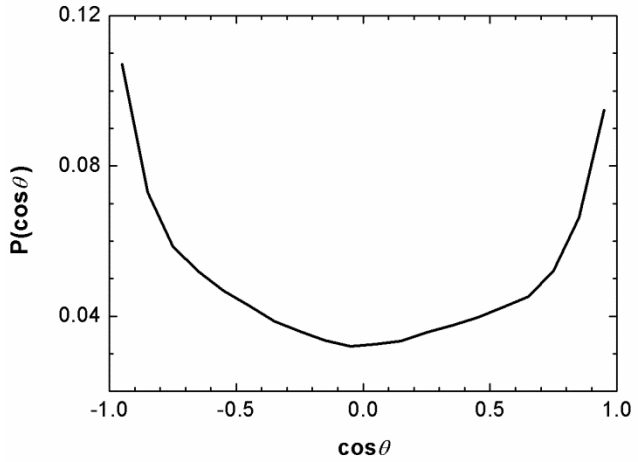
Branched aggregate
dC4-C4 < 6.5 Å and dC5-C5 < 6.5 Å
-0.7 < cosϕ > 0.7

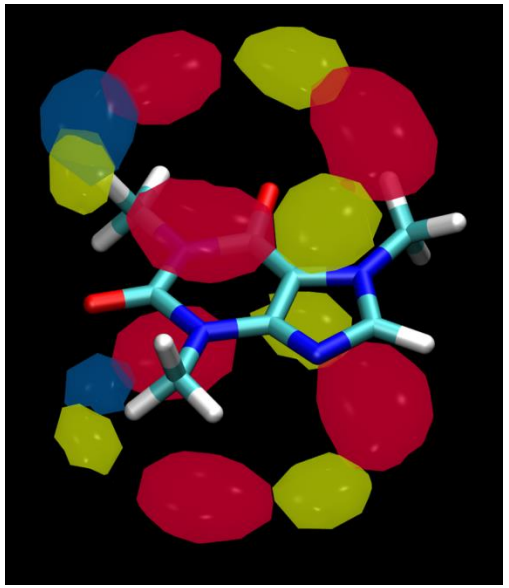
Stacked clusters





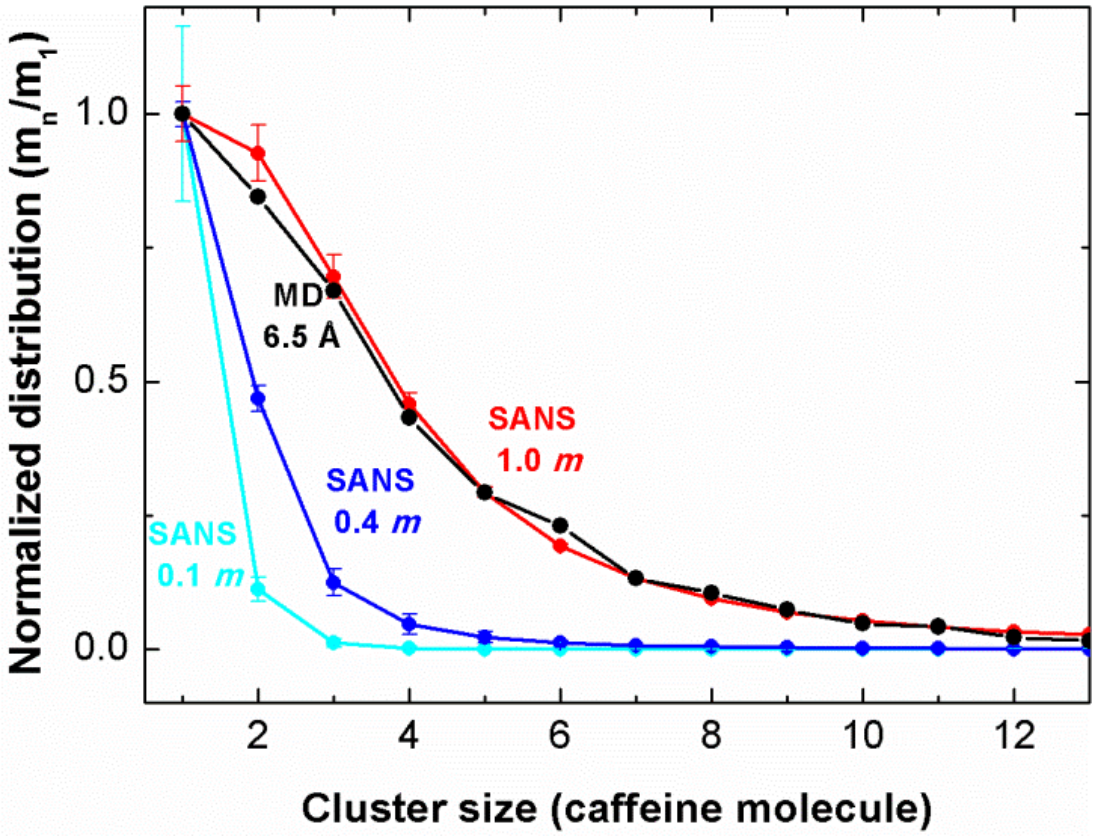
Pair distribution function
C4-C4
C5-C5





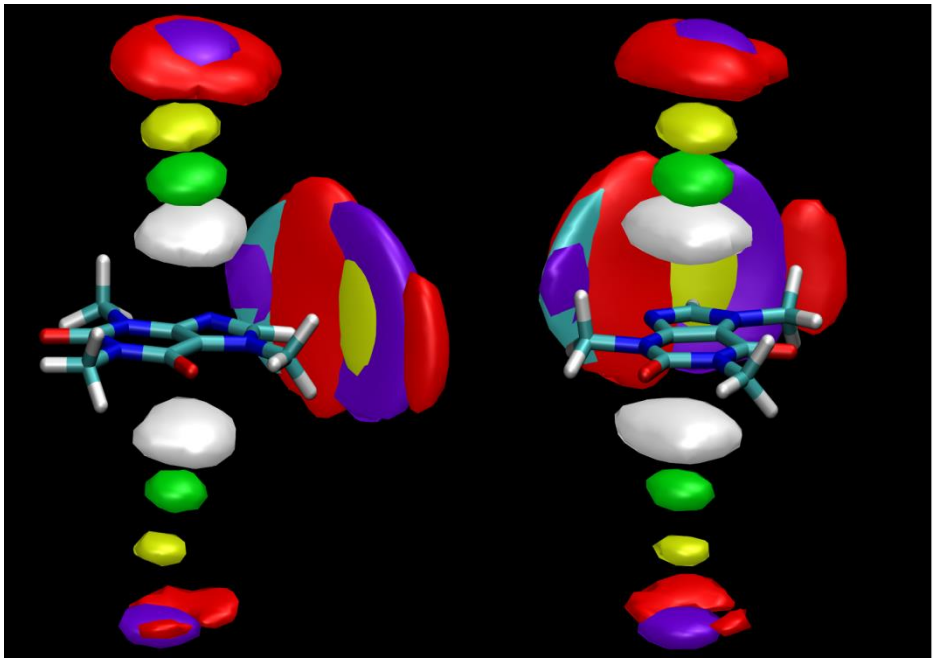
Cosine of the average angle θ between two consecutive stacked caffeine dipole vectors

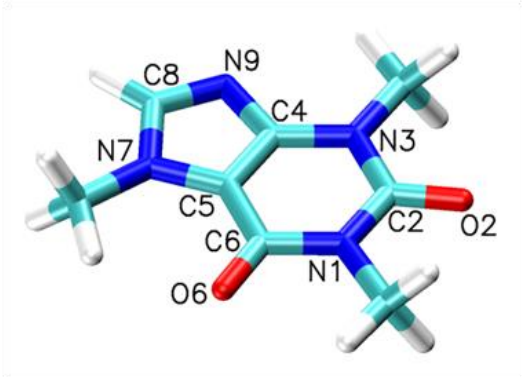
Stacked clusters



Tavagnacco, L. et al. J. Phys. Chem. B, 2016, 120 (37), 9987-9996

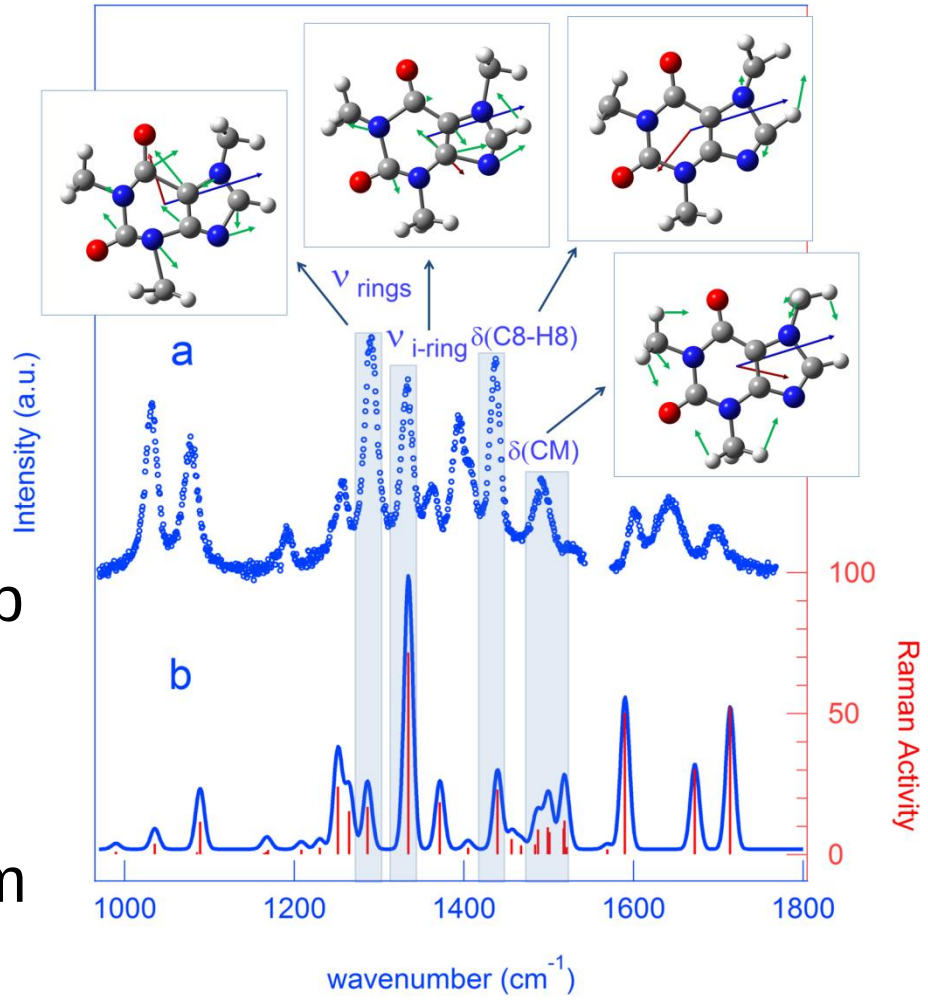
Branched aggregates





O2 and O6
C1M, C3M and C7M
C4 and C5
N1 C2 and N3
N7 C8 and N9
H8 white

Caffeine stacking



Exp

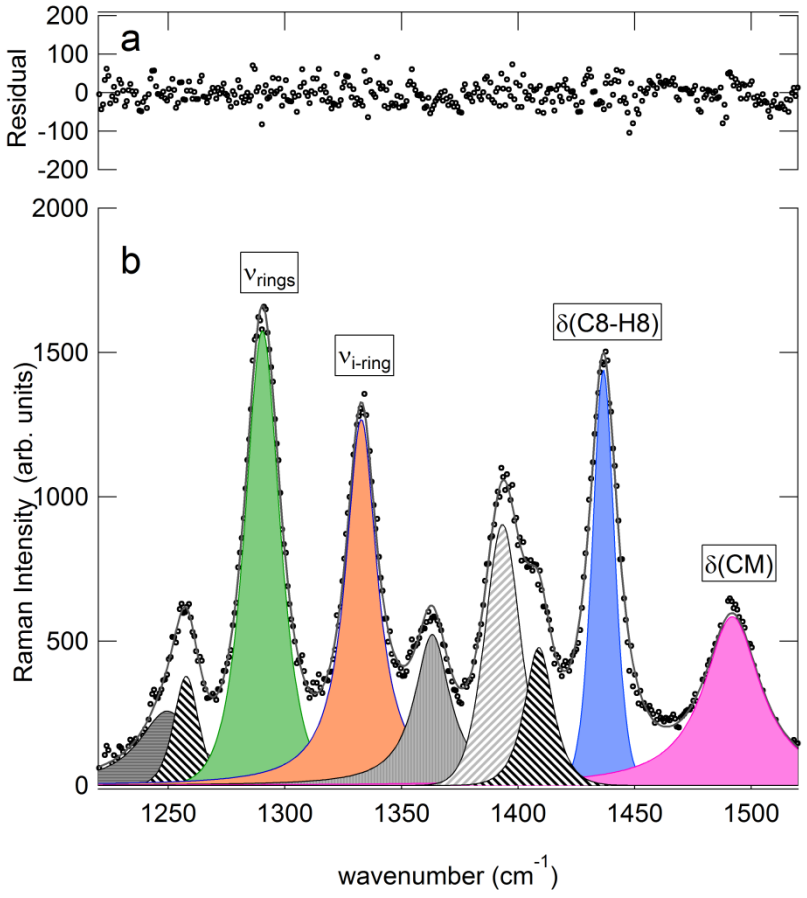
Sim

UV Resonance Raman scattering experiments

IUVS beamline Elettra

Tavagnacco, L. et al. Phys. Chem. Chem. Phys., 2016, 18, 13478 – 13486

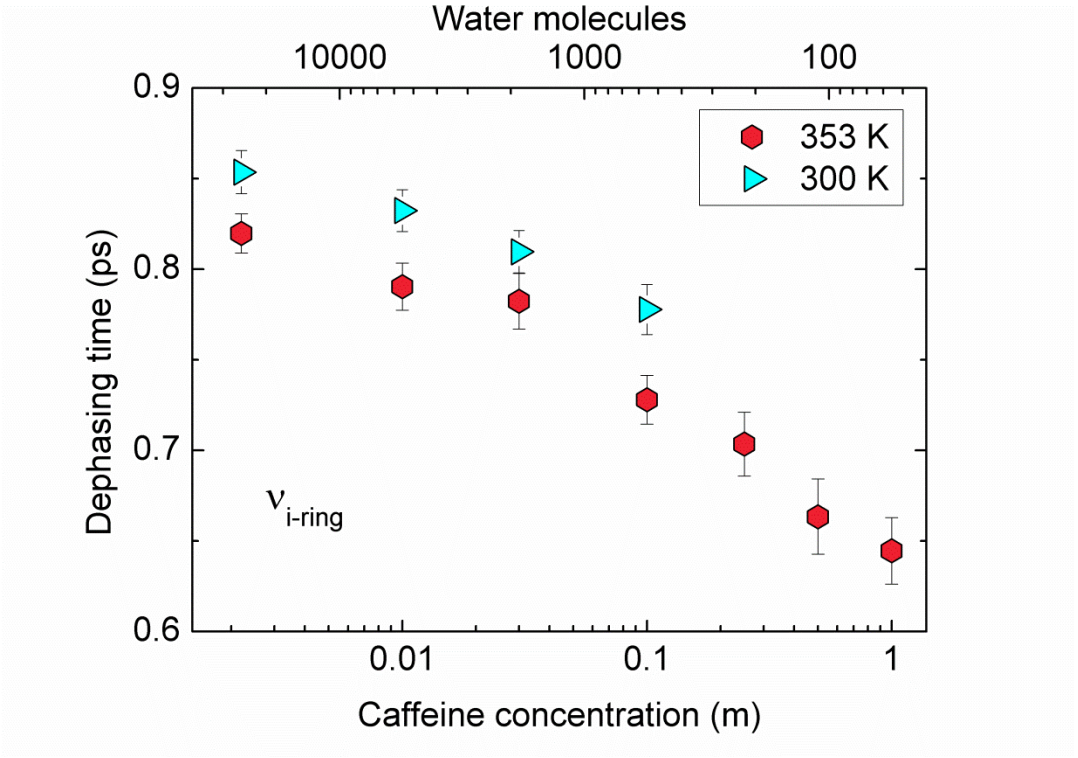
Caffeine stacking

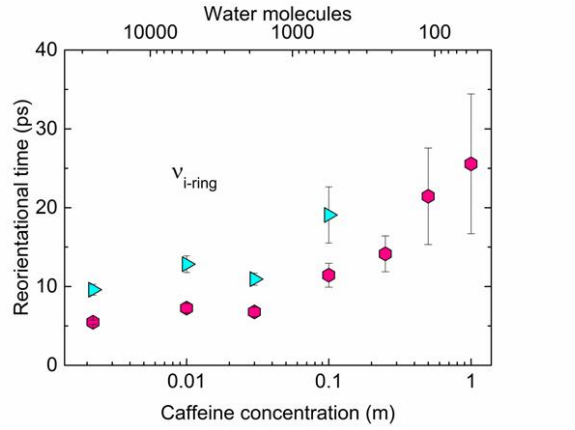


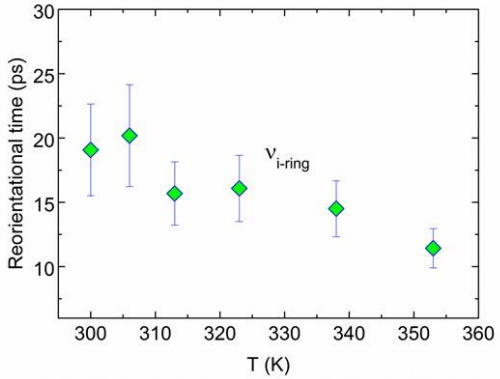
Data were analyzed using the Kubo Anderson framework (KAF).
This model allows the determination of the vibrational dephasing relaxation time and the reorientational relaxation time.

The non coincidence effect was also studied as a function of the temperature and the concentration.

Caffeine stacking

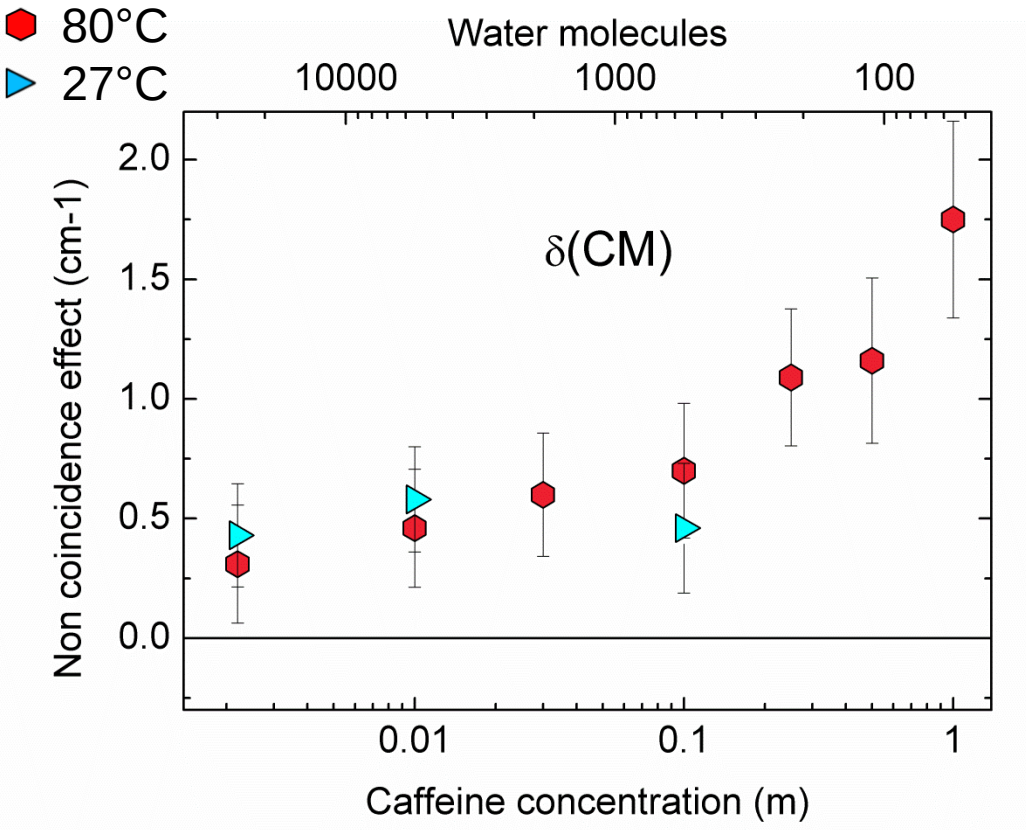


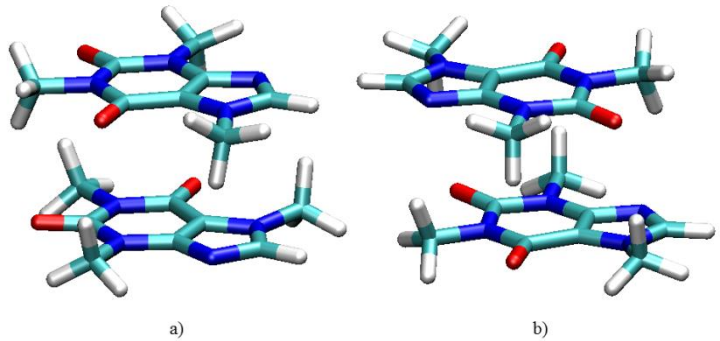




Ea ~2 kcal mol^{-1}

Role of dipolar interaction



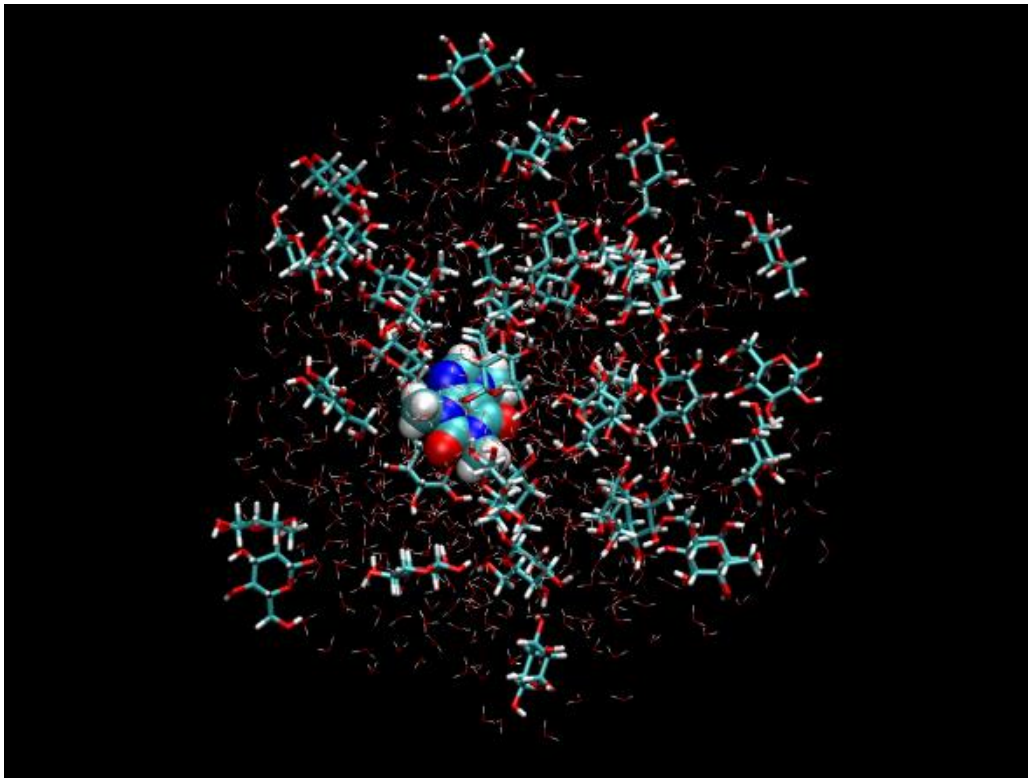


DFT calculations of the isotropic and anisotropic Raman activity of caffeine dimers in parallel and antiparallel stacking show positive NCE

The non coincidence effect, NCE, is defined as the non-coincidence of the position of the maxima of the isotropic and anisotropic Raman components:

$$\Delta\nu_{NCE} = \nu_{aniso} - \nu_{iso}$$

Heterotactic Interactions



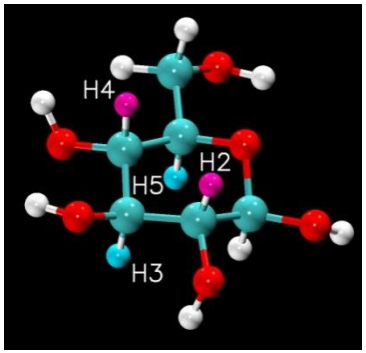
MD

	β-gluc.	α-gluc.	Sucr.	Sorb
N° caffeine molec.	1	1	1	1
N° sugar molec.	36	36	13	13
N° water molec.	667	667	666	666
Box size [Å]	30.03	30.03	29.26	28.4
Caff. conc. / m	0.083	0.083	0.083	0.083
Sug. conc. / m	3.0	3.0	1.08	1.08
Time [ns]	80	80	100	80

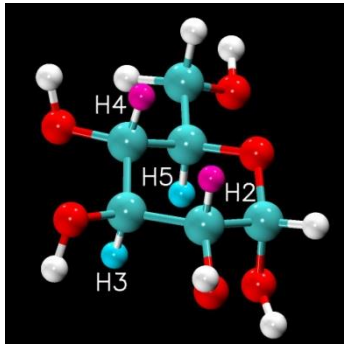
^{1}H-NMR titration exp.

Tavagnacco, L. et al. J. Phys. Chem. B, 2012, 116(38), 11701-11711
Tavagnacco, L. et. al. Food Biophys., 2013, 8(3) , 216-222.

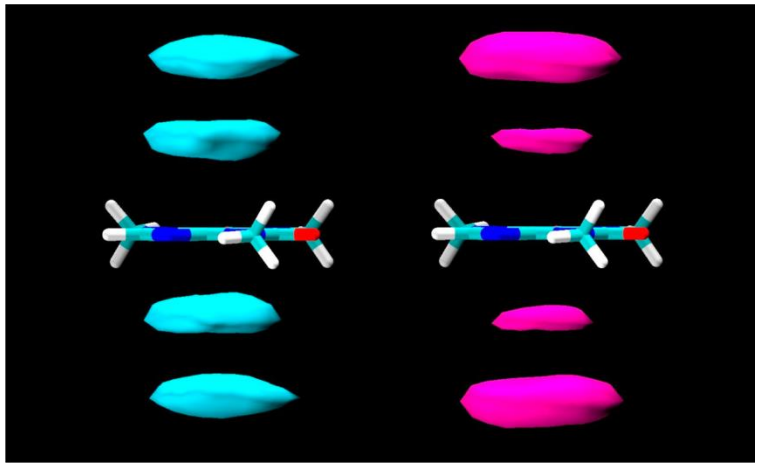
Caffeine – Glucose Interaction

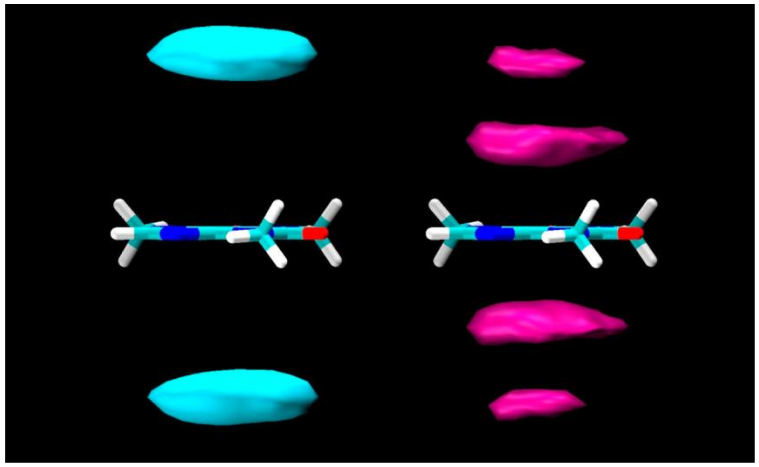


β-D-glucopyranose



α-D-glucopyranose

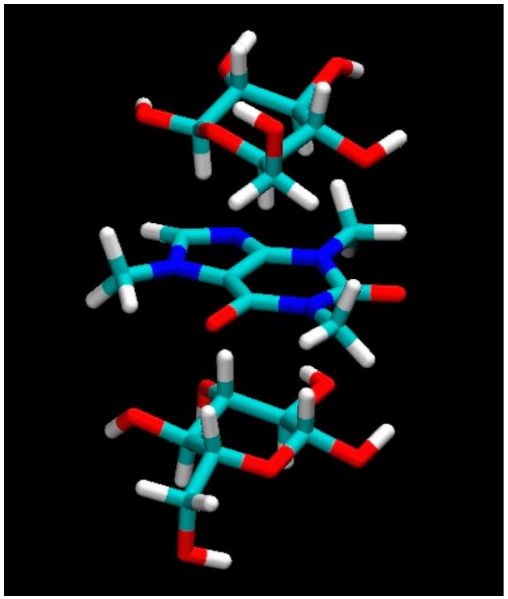




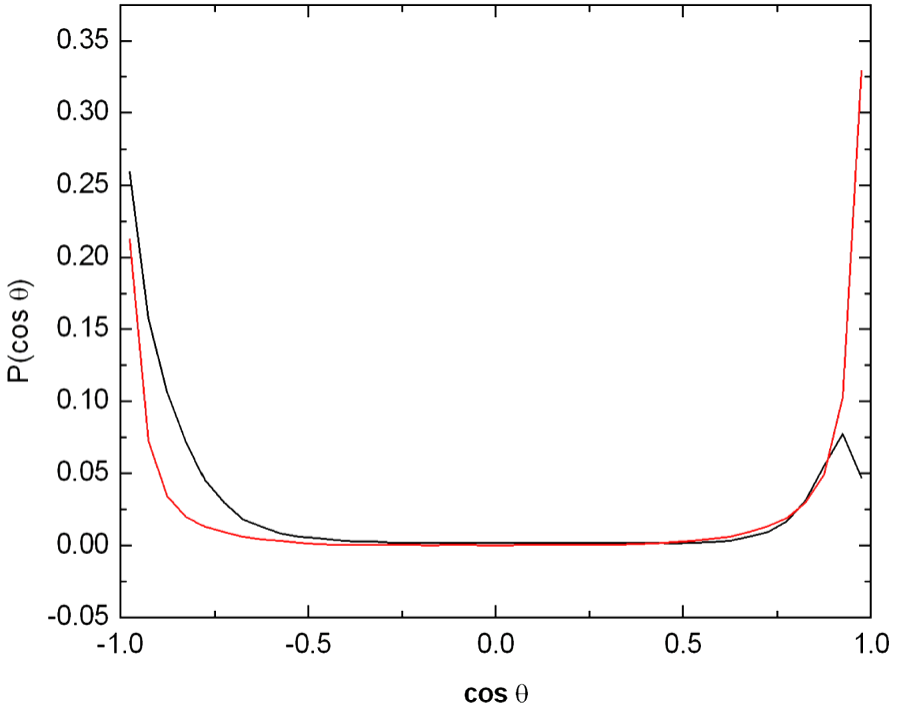
Protons H3 H5 - Protons H2 H4

The contours enclose regions with proton density 3 times those of the bulk solution.

Caffeine – Glucose Interaction



Probability of the cosine of the angle between the normal vector to the caffeine plane and the normal vector to the glucose plane



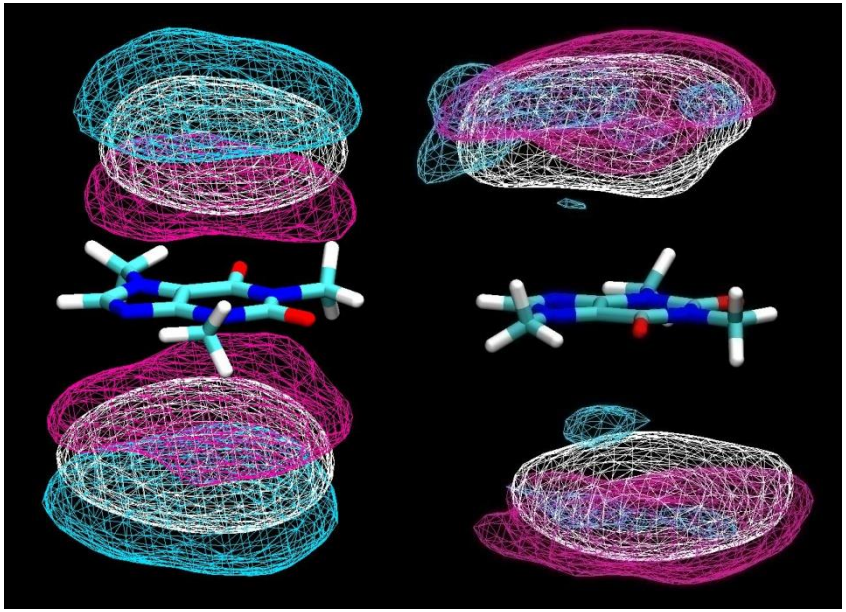
Protons **H3 H5** pointing away from the caffeine plane

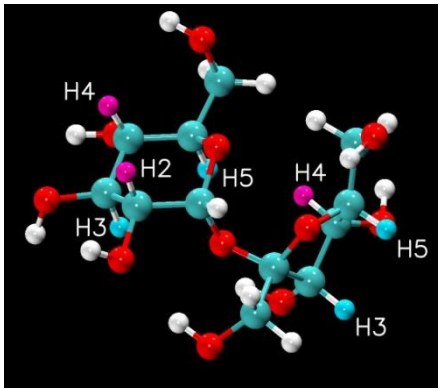
α anomer **β anomer**

Protons **H3 H5** pointing toward the caffeine plane

Caffeine – Sucrose Interaction

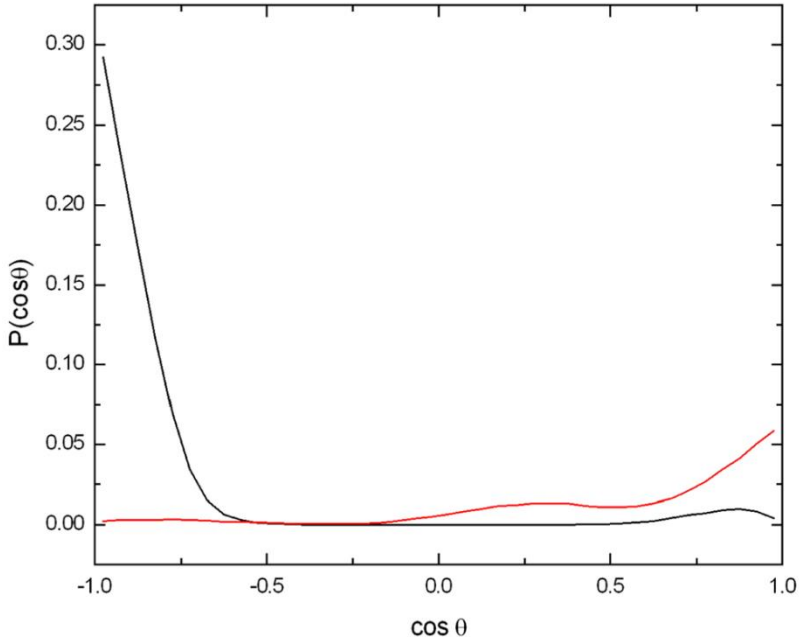
Glucose **Fructose**





H3 H5
RING
H2 H4

Probability of the cosine of the angle between the normal vector to the caffeine plane and the normal vector to the sucrose monomer plane

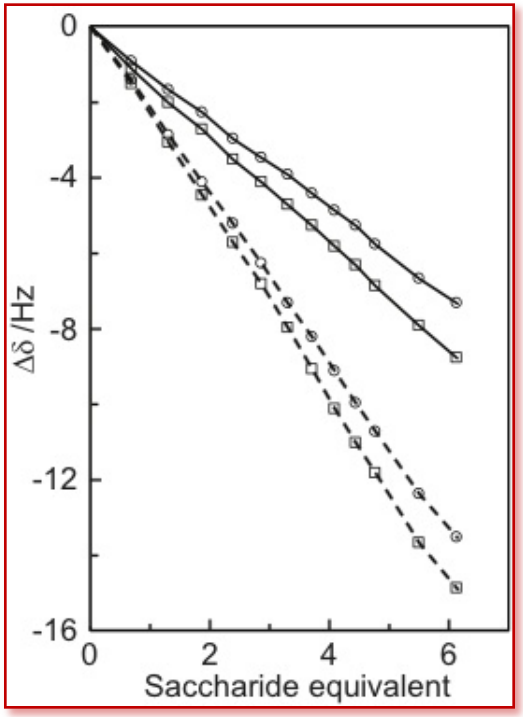


Protons **H2 H4** pointing toward the caffeine plane

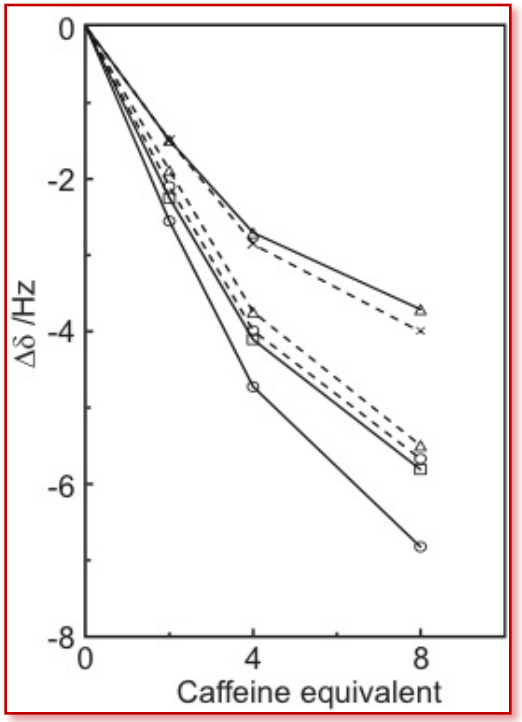
Protons **H2 H4** pointing away from the caffeine plane

NMR titration experiments

1



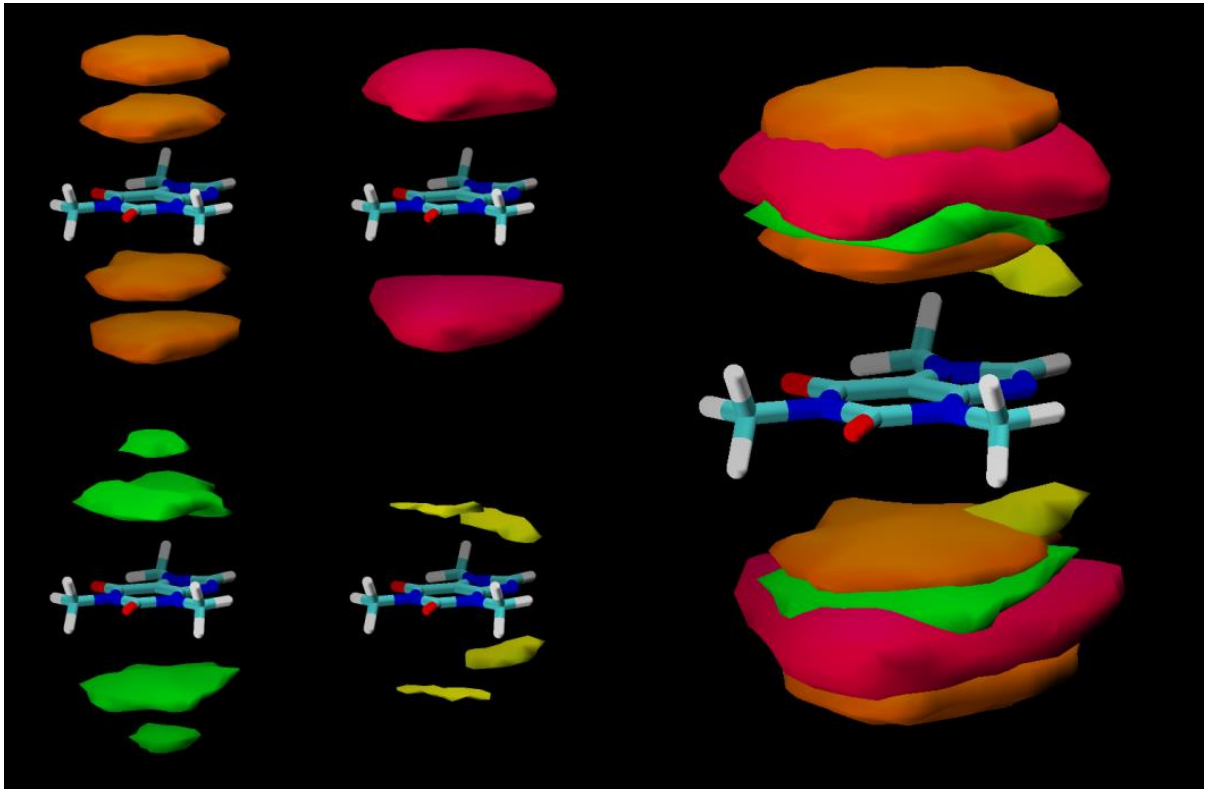
2

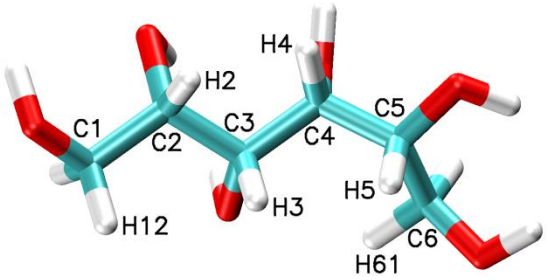


1 **^{1}H NMR chemical shift changes of protons H8 (○) and Me1 (□) upon addition of D-glucose (solid lines) or sucrose (dashed lines).**

2 **^{1}H NMR chemical shift changes of sucrose protons upon addition of caffeine for H1*g* (○), H2*g* (□) and H3*g* (Δ) of the glucose residue (solid lines) and for H1*f* (○), H3*f* (D) and H4*f* (x) of the fructose residue (dashed lines).**

Caffeine – Sorbitol interaction



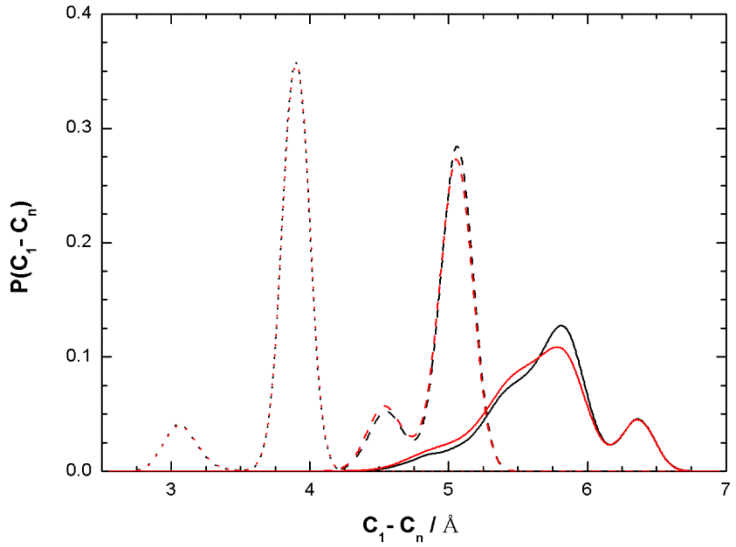


Density maps calculated for the individual sorbitol atoms

Orange: aliphatic protons
Red: carbon atoms
Green: oxygen atoms
Yellow: hydroxyl protons

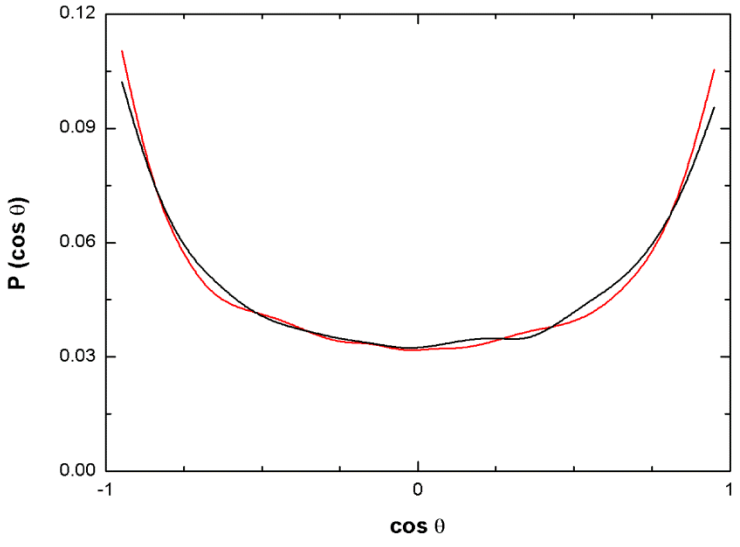
Caffeine – Sorbitol interaction

Probability of the distance between the C1 and Cn atom positions



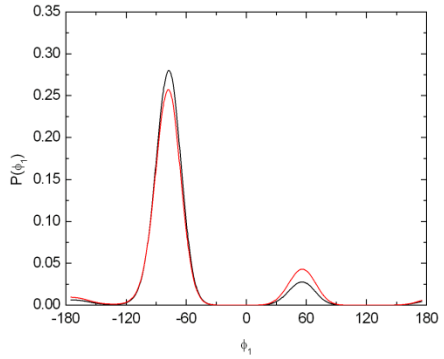
Black: bound sorbitol molecules
Red: free sorbitol molecules
Dotted lines: atoms C1-C4
Dashed lines: atoms C1-C5
Solid lines: atoms C1-C6

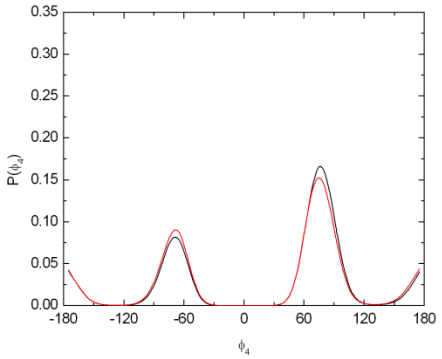
Probability of the cosine of the angle between the dipole moment vector of the caffeine molecule and the bound sorbitol chain vector

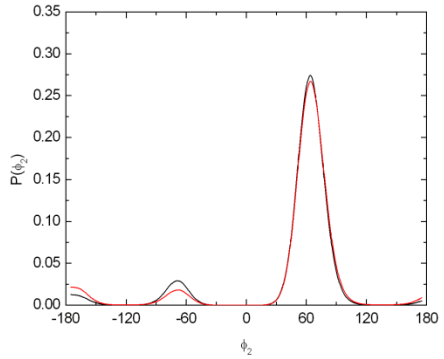


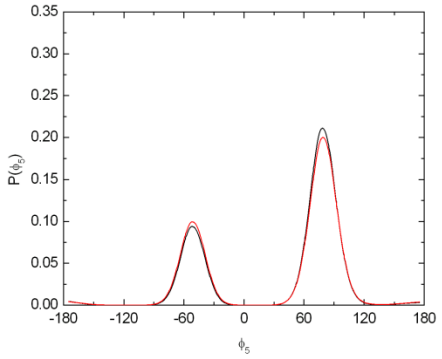
Black: C1 C4 atom positions
Red: C1 C5 atom positions

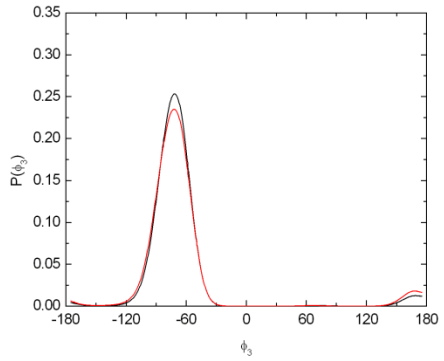
Caffeine – Sorbitol interaction



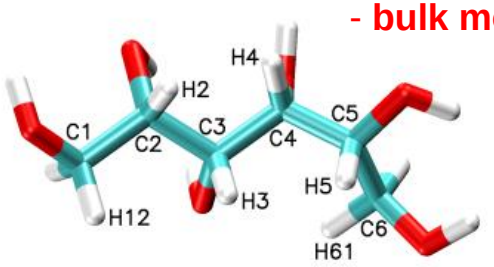








- **interacting molecules**
- **bulk molecules**



Summary

- ✓ **MD simulations complementary to different experimental approaches allowed to characterize the hydration and association properties of a food biomolecule**
- ✓ **Caffeine self-aggregation promotes the formation of two types of clusters: linear aggregates of stacked molecules and disordered branched aggregates.**
- ✓ **The water structuring explains the caffeine enthalpy-driven hydrophobic association.**
- ✓ **Dipolar interactions play an important role in the formation of caffeine aggregates.**
- ✓ **Caffeine weakly binds sugars by face-to-face stacking**

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