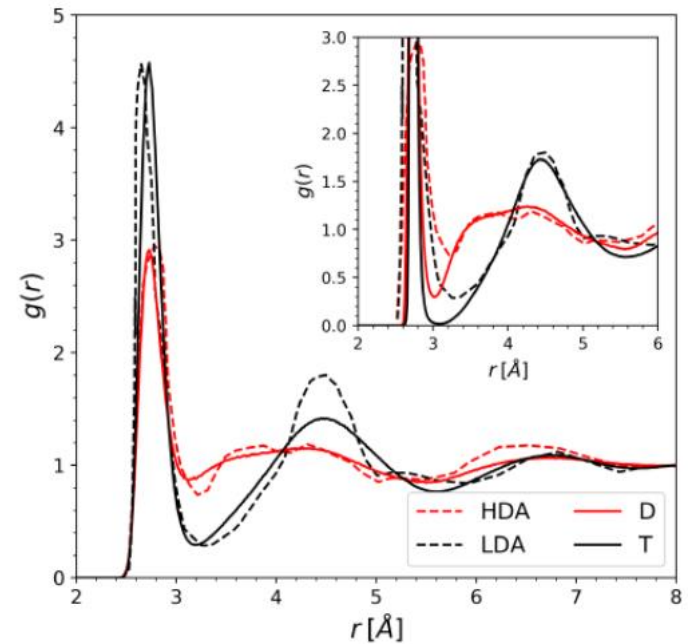
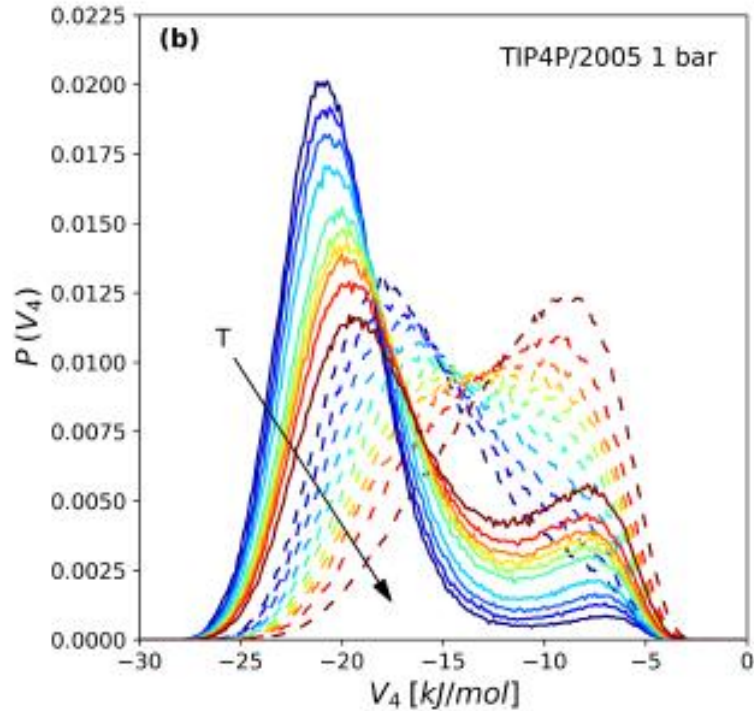


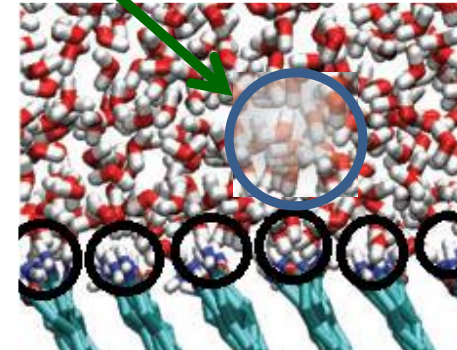
# Recap

## $V_4$ indicator:



Bulk conditions

$r=3.3\text{\AA}$



Self-assembled monolayer (SAM: functionalized alkyl chains)

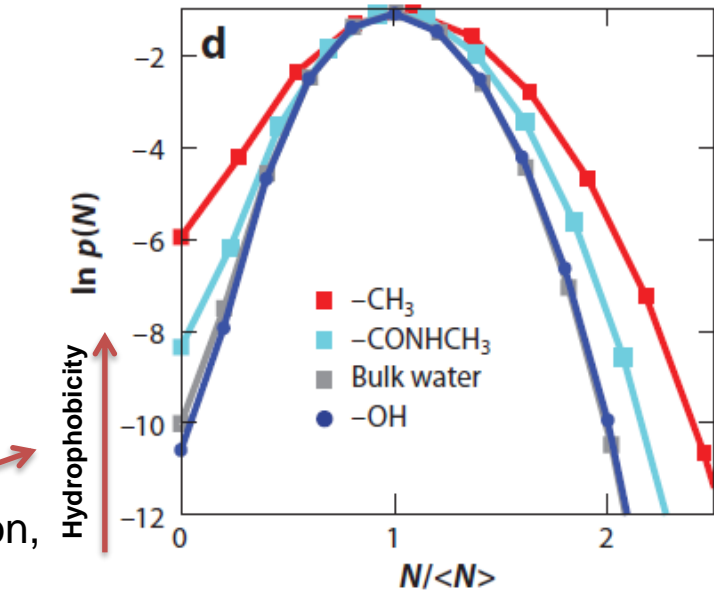
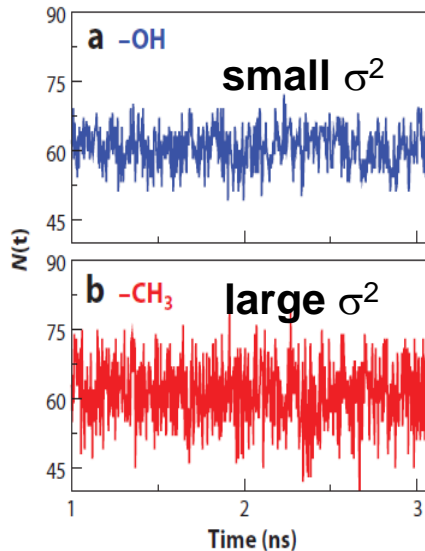
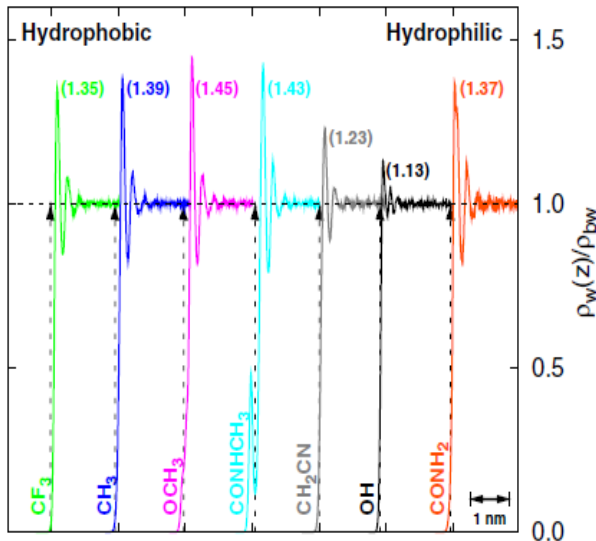
S. Jamadagni, R. Godawat, and S. Garde Annu. Rev. Chem. Biomol. Eng. (2011).

# Hydration and nanoconfined water

## Quantifying local hydrophobicity. Water density fluctuations

**Water “abhors” vacuum** ~ hydration,  $\delta$  profiles/layering.

Density fluctuations enhanced at hydrophobic surfaces. Generic contexts with controlled chemistry and geometry in order to determine how chemical topology and topography define the local hydrophobicity. Functionalized SAMs.



$$\mu \approx k_b T \langle N \rangle^2 / 2\sigma^2$$

$$\mu = -k_b T \ln[p(N = 0)]$$

(free-energy of cavity creation at small volume)

$\uparrow \sigma^2 / \langle N \rangle^2$ , cavity creation, vacating probability

## TWO-LIQUID SCENARIO (LLCP):

Water as a mixture of two competing liquid states: LDL / HDL

LDL: structured, low local density, low mobility.

HDL: unstructured (defects), high density, higher mobility.

This may explain why it expands when freezing, increases its mobility at high  $\rho$  or in constricted (nanometric) spaces (superflux in ion channels, aquaporins, CNTs, ec.), increased fluctuations at Widom line (maxima in  $C_p$ ,  $K_T$ ), etc.

Two local molecular arrangements, not only in supercooled regime but also above the melting temperature ( $T_M$ ).

Unlike normal liquids, water expands its local structure to improve energy (directional interactions, HBs)  $\rightarrow$  HB network. **But we will see that this is only part of the story. DEFECTS**

**LCCP: Dominant paradigm in the description of the anomalous behavior of water.**

**However** (J.L. Finney, J. Chem. Phys. 160, 060901 (2024)):

*“While the two-liquid description would be relevant in supercooled water, it is not clear that it is important to our understanding of the behavior of water at room temperature or of its interactions in real-life situations”.*

# Hydration and nanoconfined water

Contrary to normal liquids, water expands its structure (its second neighbor shell) in order to improve HB-coordination. Liquid water tends to form an extended HB network (HB).

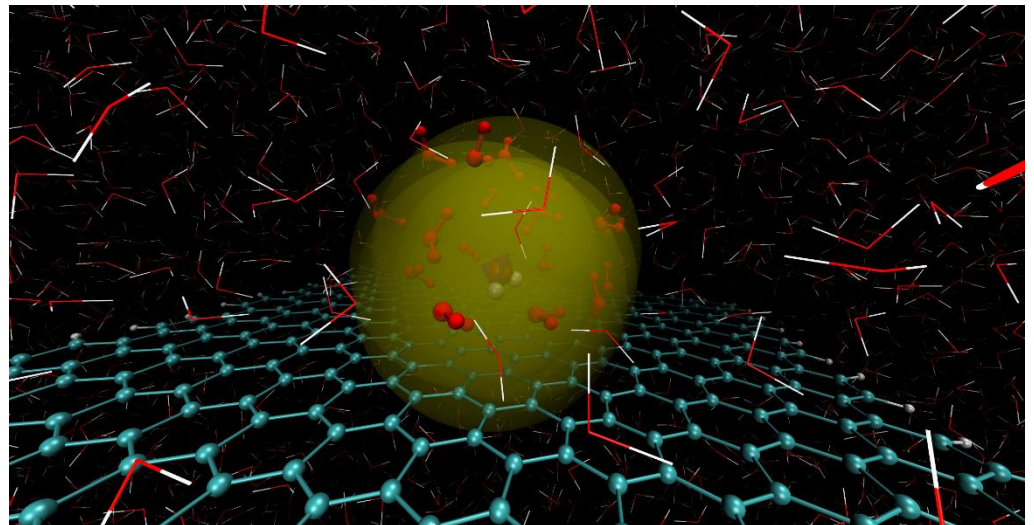
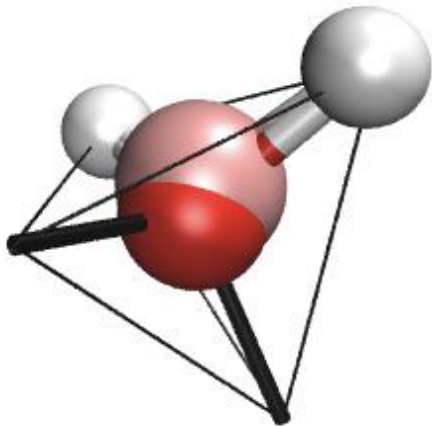
But this is only part of the story, as we shall see. Defects.

**Ansatz:** Since water presents a strong reluctance to lose its tetrahedrally-directed coordination, anytime a water molecule resigns a HB, some directional energetic compensation would be demanded.

Such a tendency to retain (a certain extent of) tetrahedral interaction results from the need to avoid the otherwise uncompensated partial molecular charges.

## New metric: $V_{4s}$

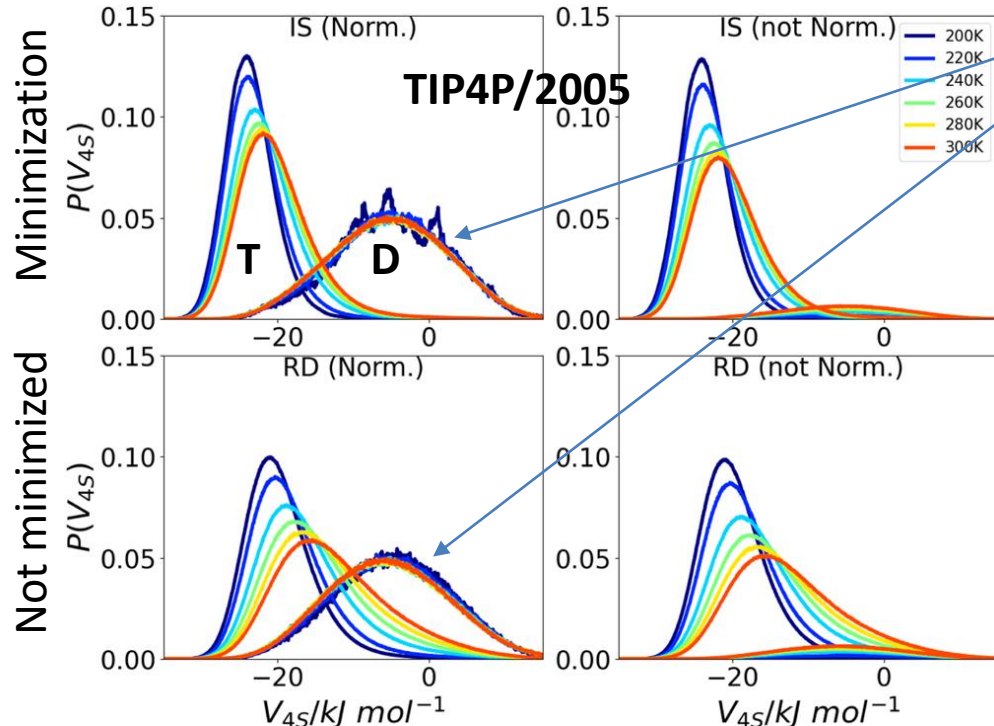
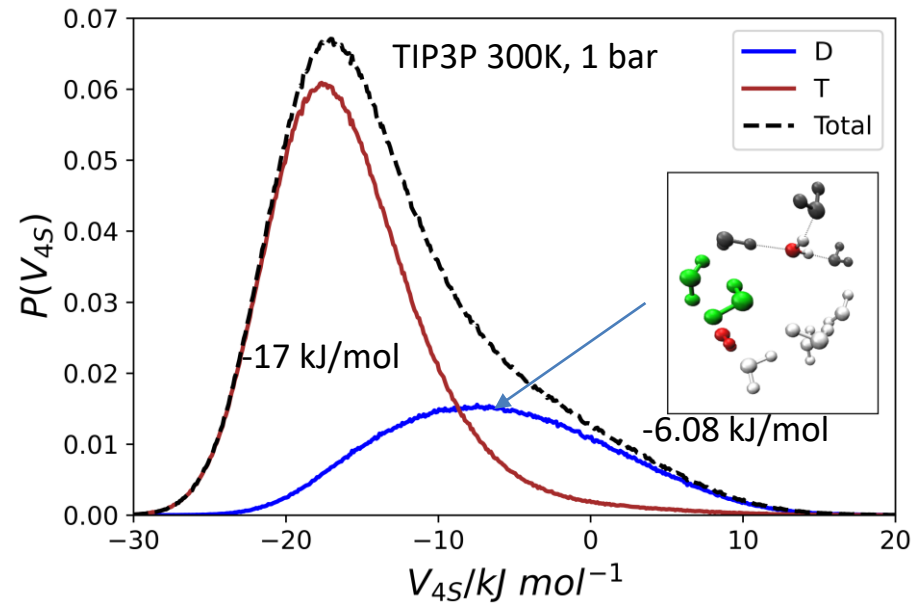
Partition space in four tetrahedral sites/regions. Compute adding up all the interactions (Coulomb, LJ) at each four sites within a sphere of 6 Å ( $\sim 2^{\text{nd}}$  peak O-O RDF). Order them in decreasing intensity:  $V_{1s} \dots V_{4s}$



## BULK WATER:

**Fine-tuned structure/energy interplay** between the first and second neighbor shells:

- Expansion 2<sup>nd</sup> shell and 1<sup>st</sup> shell HB interaction energy. LDA-like (T).
- HB loss: Contraction of second shell for partial energy compensation. HDA-like (D), mean value: -6.08 kJ/mol (quite attractive  $\gg k_B T$ ).



Rather insensitive to temperature changes and to minimization

**Two demands or requirements: satisfy HB, or partially compensate HB-lacking site.**

Enables to establish extended HB network while allowing for coordination defects (otherwise extremely costly within an expanded structure).

**Evidences molecular principle underlying the existence of two local molecular arrangements.**

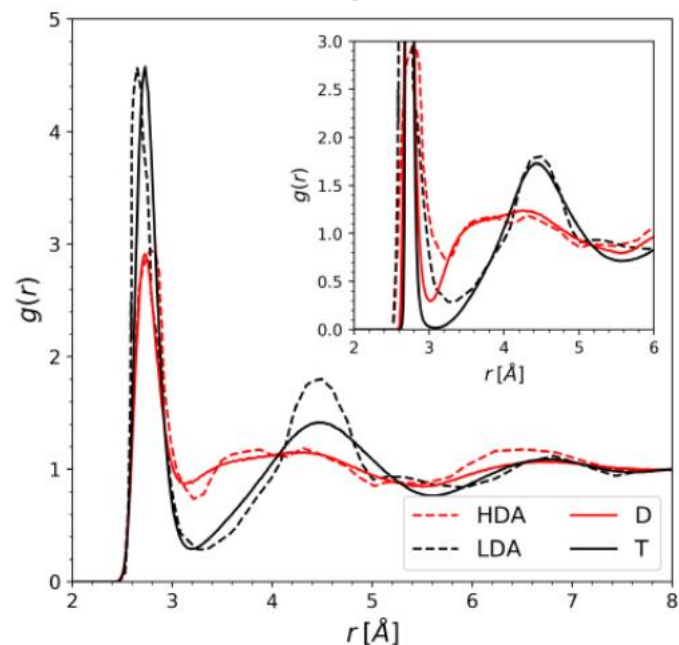


First response to Finney (JCP 2024): “*while the two-liquid description would be relevant to the behavior of supercooled water, it is not clear that it would also be significant to our understanding of water and its interactions at room temperature and in real-life situations.*”

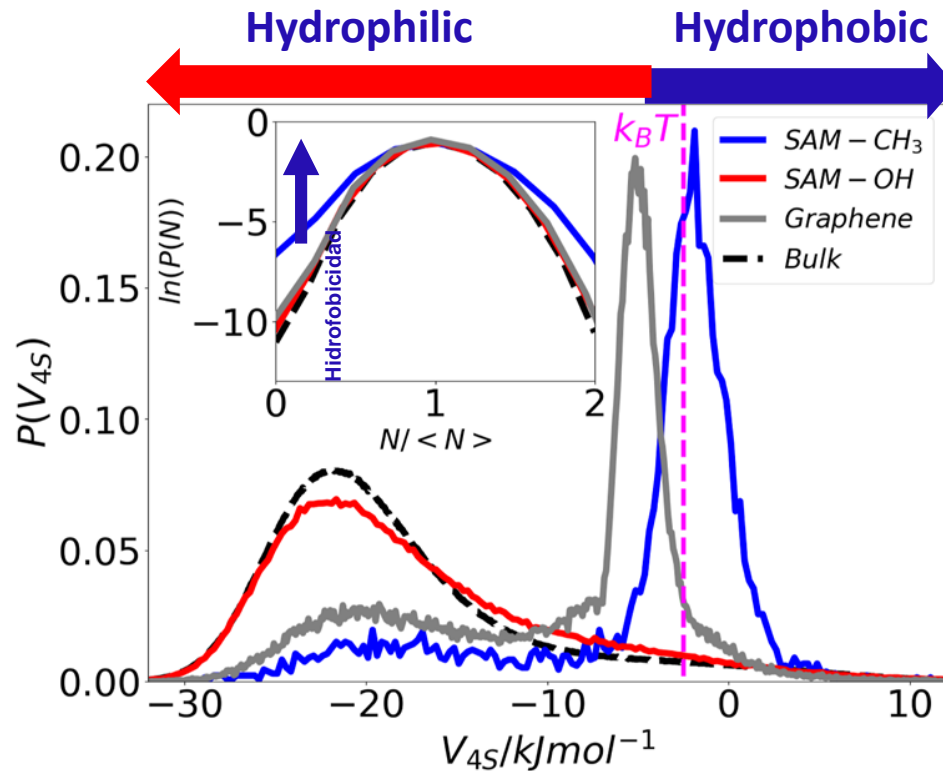
We elucidated the existence of a fine-tuned interplay between local structure and energetics that allows water to establish an extended hydrogen bond network while compensating for uncoordinated sites. **This molecular mechanism is operational at all temperatures (from supercooling to room temperature).** We also showed that it underlies the existence of two molecular arrangements compatible with water’s two-liquids scenario, even at room temperature.

However, while compatible with the two-liquids scenario, we note that our results only rely on the existence of HDA-like and LDA-like molecules, as we had already shown.

N. A. Loubet, A. R. Verde and G. A. Appignanesi,  
J. Chem. Phys. 160, 144502 (2024)  
and arXiv:2311.08087v2 [cond-mat.soft] (2023).



# HIDRATION WATER AND NANOSCALE CONFINEMENT:



- The new structural index,  $V_{4s}$ , might provide us with an appropriate measure of hydrophobicity.
- We shall also see that the molecular principle valid in bulk conditions is also operative at hydration/nanoconfinement.
- This might extend the validity of the two-liquids scenario for water.
- Thus, we shall provide a response to Finney's second question (Finney, JCP 2024): Interactions, "real-life" situations.

...in other words:

- As we saw, there are two requirements in bulk water (HB or defect compensation). Thus, from "*Water: One substance, two liquids*" → *One simple molecule, two interaction demands*
- We shall see that these two requirements also verify at hydration → two hydrophilicity thresholds (*hydros: water; philos: love; water: two ways to love*).  
Molecular fingerprints of hydrophilicity; hydrophob/hydrophil scale absolute values

# First applications to solve a pair of intriguing conundrums:

## 1) Unexpected hydrophilicity of graphene

Dense C-network. **Apolar  $\neq$  Hydrophobic.**

But weaker interactions than SAM-OH.

Loses 1 HB, W-C recovers only 35-45%!

As we saw before in: *Fluid Phase Equil.* **362**, 81 (2014);  
*J. Chem. Phys.* 143, 154704 (2015)

N. A. Loubet, A. R. Verde and G. A. Appignanesi, *J. Chem. Phys.* 160, 144502 (2024) and arXiv preprint; arXiv:2311.08087v2 [cond-mat.soft] (2023).

## $V_{4s}$ Distributions:

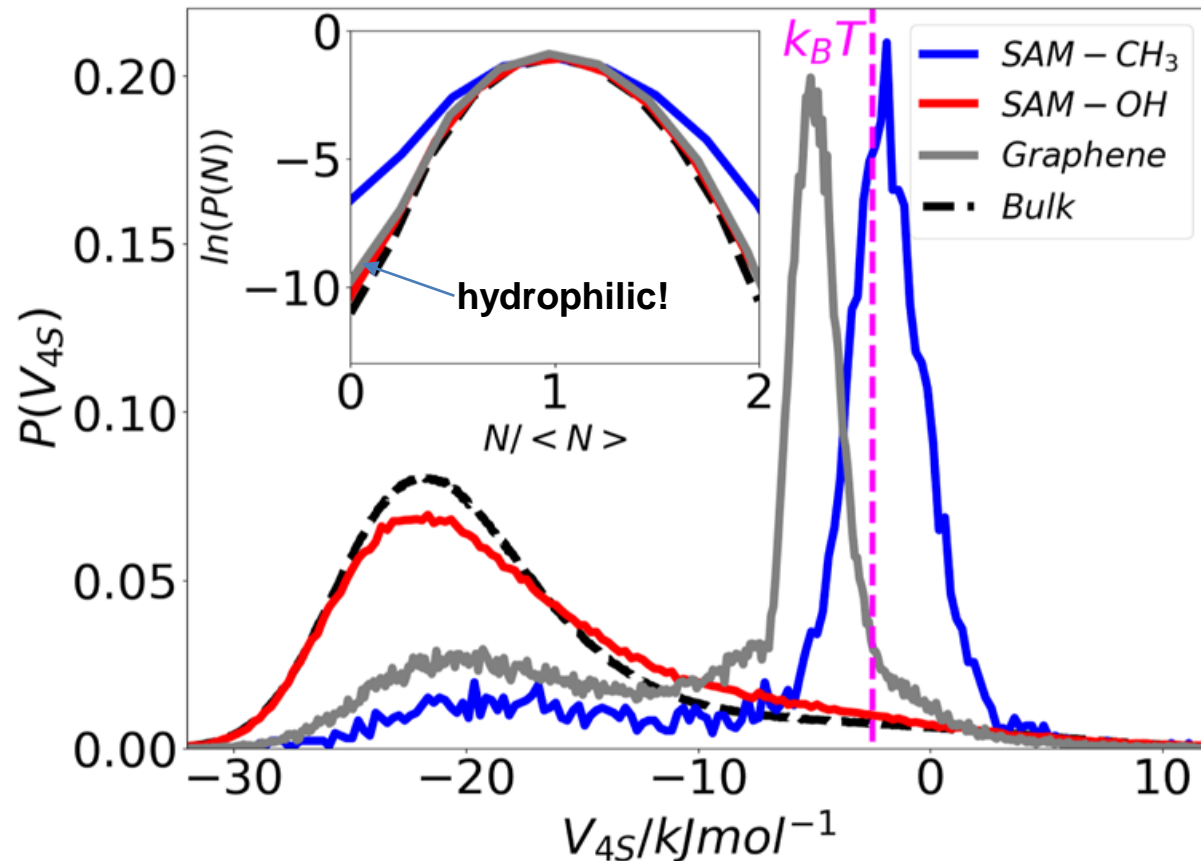
The system must mimic the behavior of HDA- or LDA-like (two-liquids) to allow hydrophilic wetting.

### Hydrophilic threshold:

It is not necessary to recover a full HB (LDL environment).

It suffices to mimic an HDL environment ( $<-6.08$  kJ/mol)

Energetic environment able to locally grow an stable HB network around molecules akin to LDL or HDL. On the contrary (lower attraction), then more affinity for bulk.



$$\langle V_{4s} \rangle_{\text{graphene}} = -8.08 \text{ kJ/mol} < -6.08 \text{ kJ/mol} \approx \text{HDL}$$

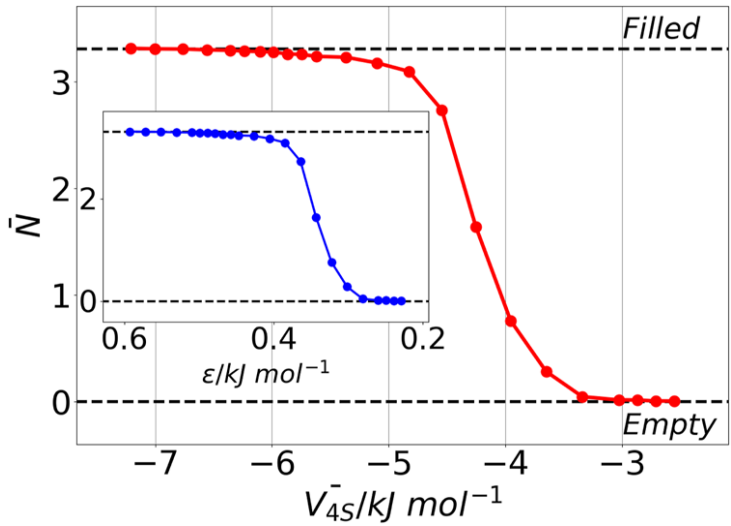
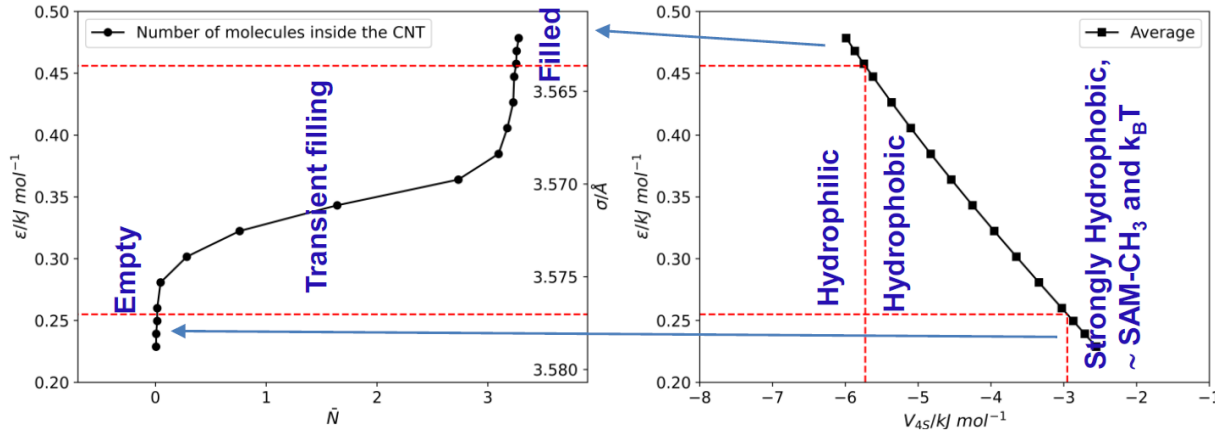
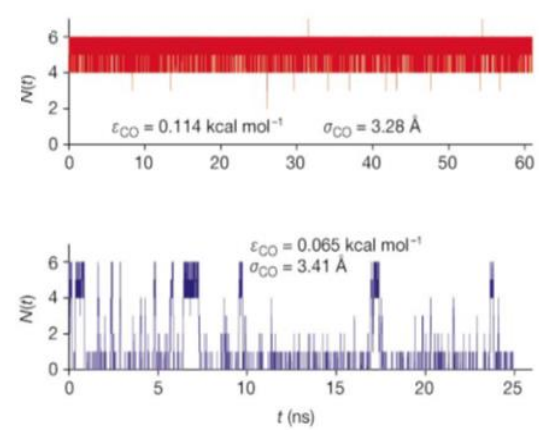


## 2) “Surprising” penetration in CNT of subnanometric section

Water enters in spite of 55-65% energetic loss!

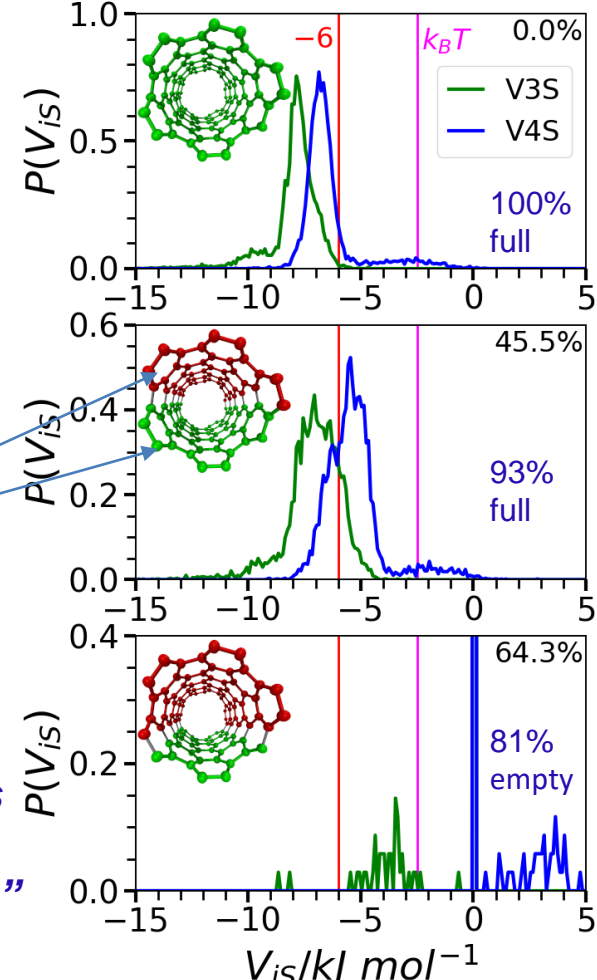
A decrease in water-wall attractions ( $\epsilon$ ) in MD CNT desorbs (Hummer et al. Science 2001)

$V_{4s}$  (and  $V_{3s}$ ): precise prediction of filling/drying transitions (hydrophilicity threshold)



Homogeneously and gradually weakened

**Energy sacrifice.**  
 ¿How many interactions for wetting?  
 No more than one <“HDL”>



# HYDROPHOBICITY AND GEOMETRY

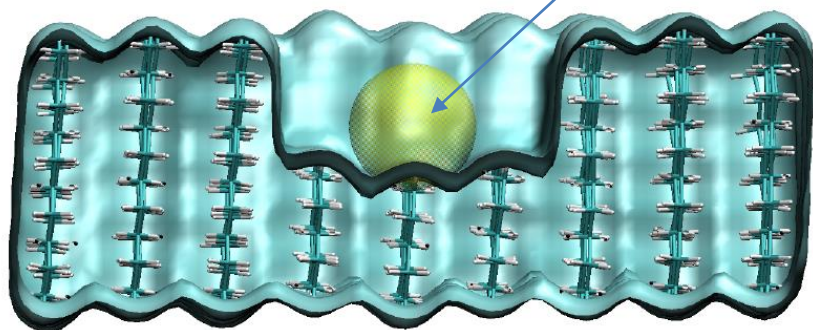
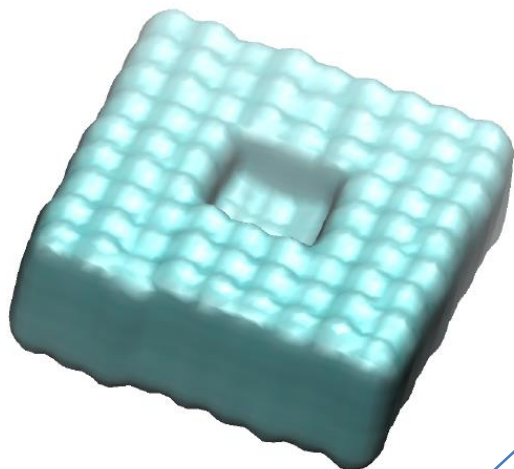
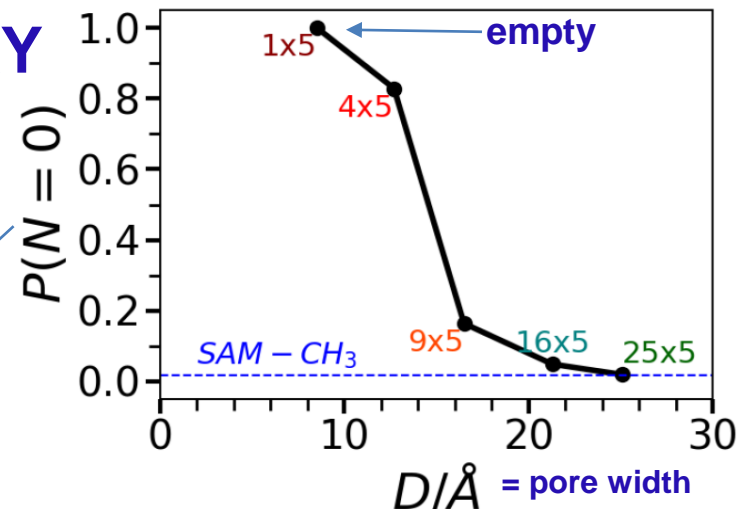


FIG. 1: Schemes of one of the pores carved in the model hydrophobic SAM ( $9 \times 5$  pore). In the lateral view we indicate the observation sphere we consider at the bottom of the cavity. For better visualization, we do not show the hydrating water molecules.

S. R. Accordino, L. M. Alarcón, N. A. Loubet and G. A. Appignanesi, J. Chem. Phys. 161, 044504 (2024).



Water vacating probability (of the corresponding observation sphere) as a function of the pore size. We also depict the value for the SAM-CH<sub>3</sub> in dashed line.

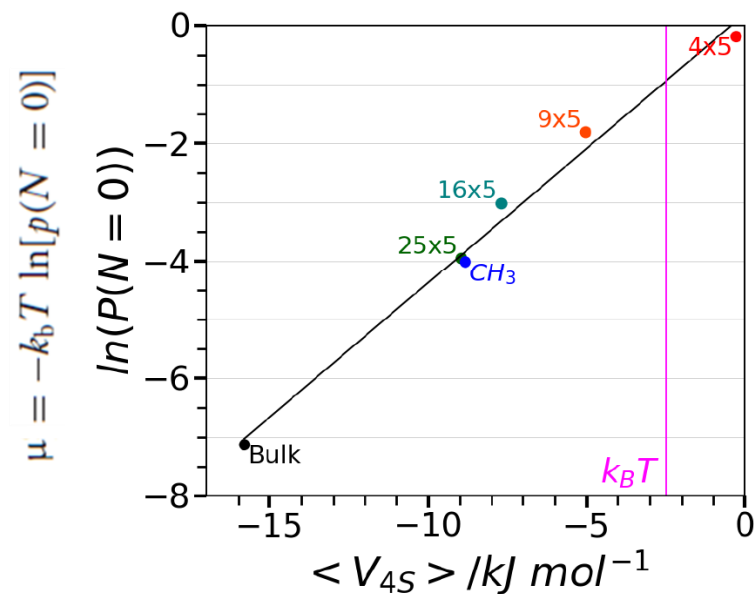


FIG. 6: Water vacating probability, logarithm of  $P(N=0)$ , as a function of the mean value of  $V_{4S}$  for the molecules inside the observation spheres of the different pores studied. We also include the case of the SAM-CH<sub>3</sub>.

# EFFECT OF THE DENSE C NETWORK: "SLIDING ON GRAPHENE"

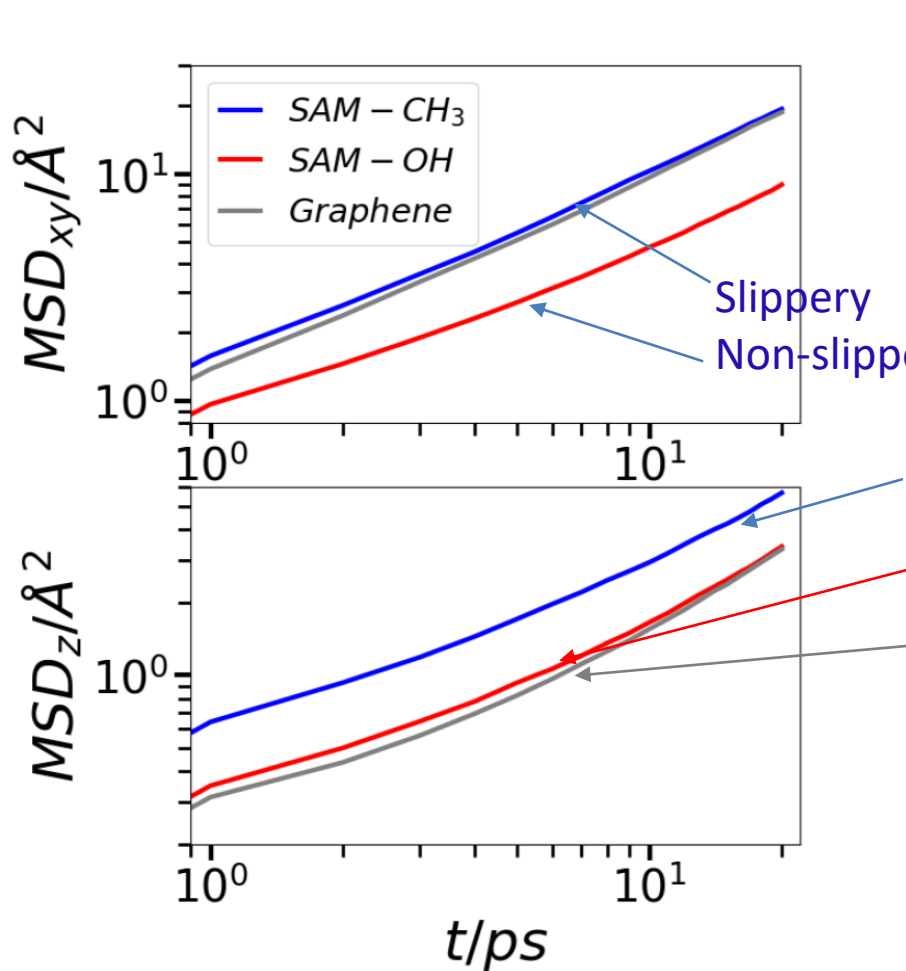
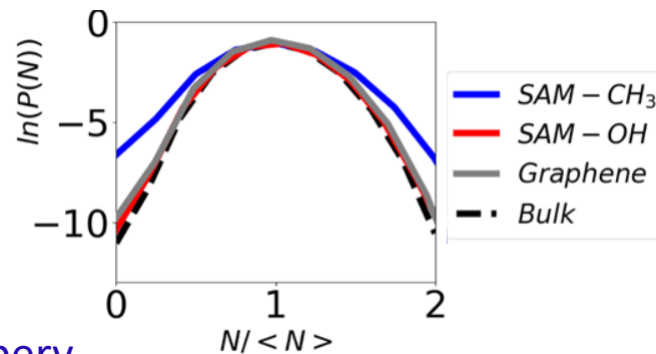


FIG. 9: TOP: Mean square displacement in the direction parallel to the surface or (x,y) plane. BOTTOM: MSD in the normal (z) direction. We plot the cases of the hydrophilic and hydrophobic SAMs and graphene.



Positive entropy of hydration derived from an enhanced water translational diffusivity  
 High hydration enthalpy. Difficult to remove  
 Dense C network. Intermediate hyd. enthalpy.  
 Smooth exchange of the several weak interactions

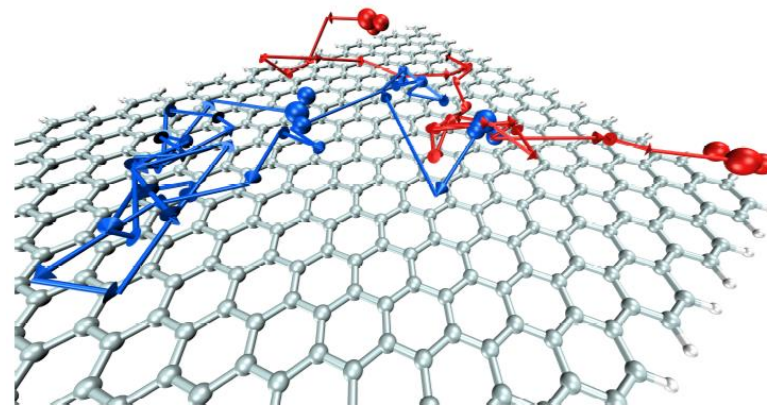
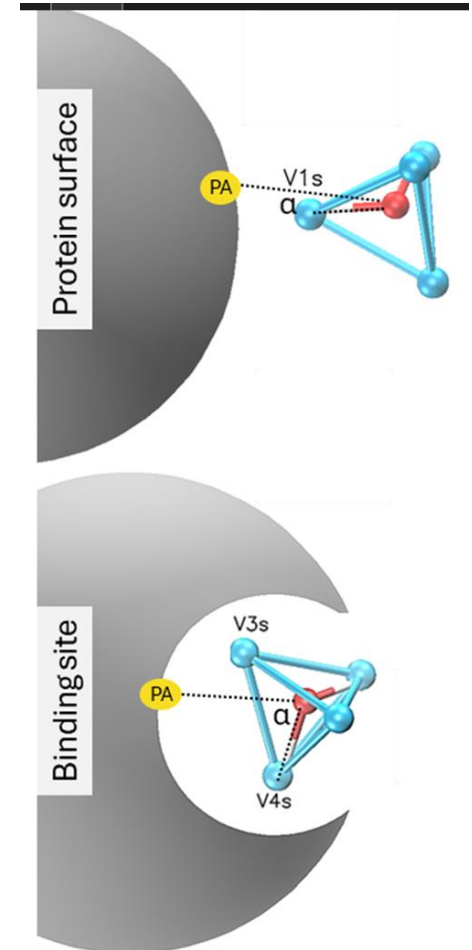
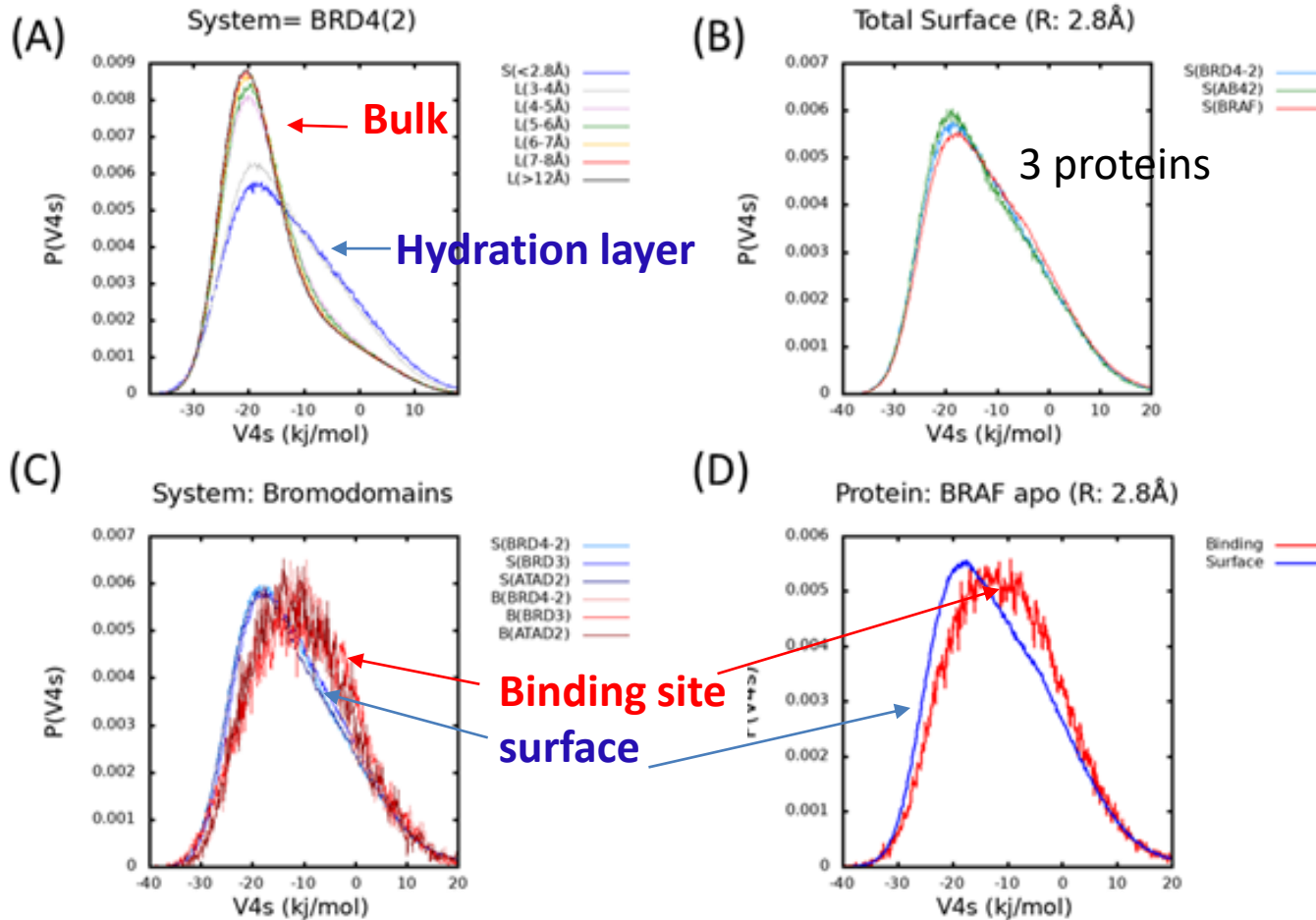


FIG. 10: Two trajectories (indicated by consecutive green and red vectors for each case) illustrating the typical behavior of the water molecules over graphene. Each vector represents the movement during 1 ps.

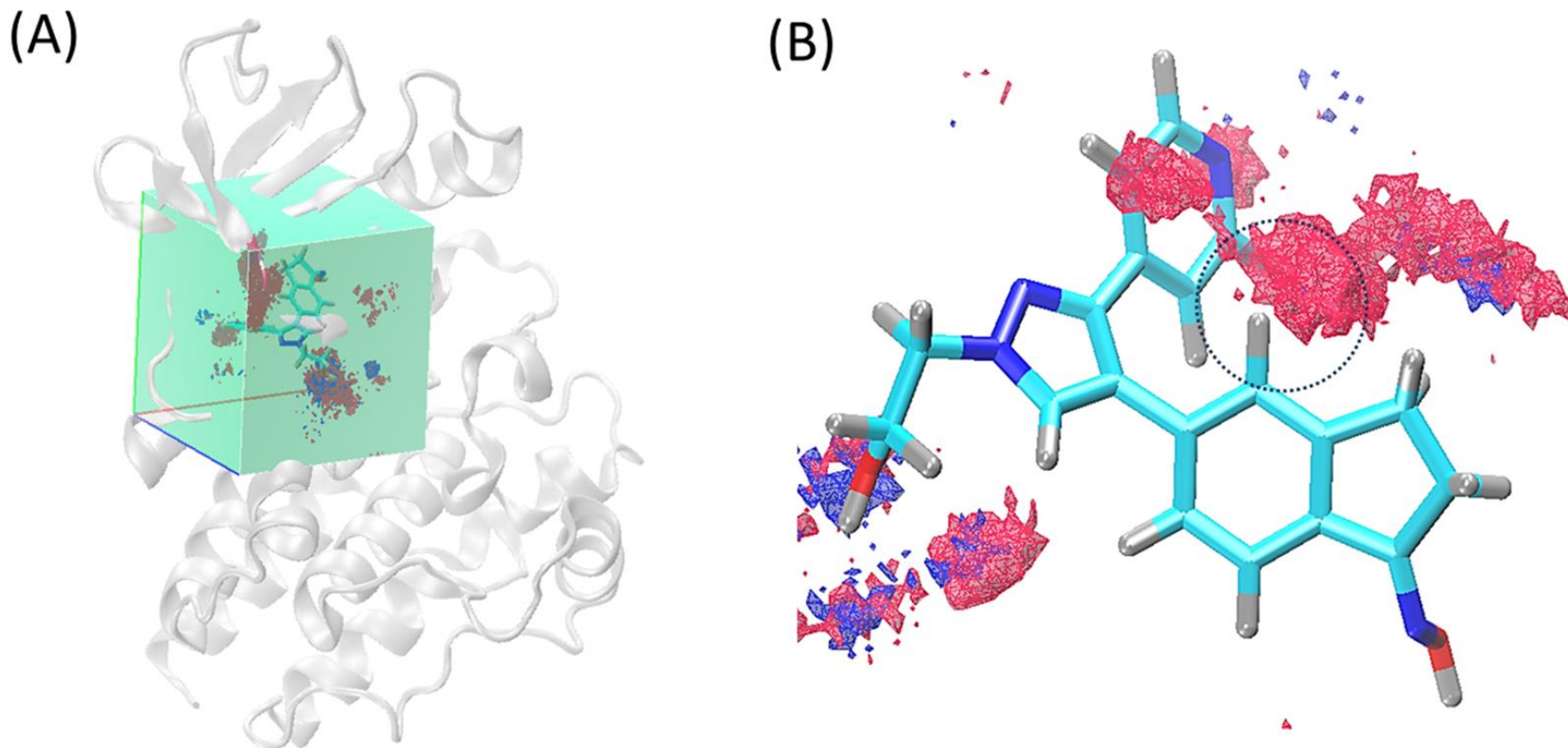
**Good for adsorption and conduction (CNT)**

# Protein Hydration Water : Focus On Binding Site Description



**Figure 1- Characterization of protein hydration water through the V4S index: an analysis of its probability distribution.** **A)** Comparison between protein hydration water in blue (water molecules located at a distance  $R$  lower than  $2.8 \text{ \AA}$  relative to any heavy atom of the protein) and bulk water in black (water molecules located at a distance  $R$  higher than  $12 \text{ \AA}$  relative to the protein surface). In addition, a progressive cutoff distance with respect to the protein was considered forming layers: for instance, between  $3$  and  $4 \text{ \AA}$  (gray);  $4$  and  $5 \text{ \AA}$  (plumb),  $5$  and  $6 \text{ \AA}$  (green), etc. **B)** Comparison of the hydration properties exhibited by three proteins belonging to different families: BRD4(2) (blue), BRAF (red) and AB42 (green). **C)** Characterization of the hydration properties exhibited by the binding site in three proteins belonging to the Bromodomains family (red palette) and comparison with global protein hydration properties (blue palette). **D)** Comparison between binding site hydration for Braf kinase protein (red) and global protein hydration properties (blue).





**Figure 4- Superimposition of the water energetic maps estimated for Braf apo with the small molecule GDC0879 (PDBid 7SHV).** In both figures, grid points with high cumulative number (4X) of easy to remove water (higher than -6.5 kJ/mol) are colored in red and grid points with high cumulative number (4X) of tightly bounded water are colored in blue. **A)** Braf protomer A is shown in gray and the binding site is highlight in green. **B)** GDC0879 small molecule in its bounded configuration (PDBid= 7SHV).

C. A. Menendez, S. R. Accordino, N. A. Loubet and G. A. Appignanesi, submitted (2024).



# Nanoscale Water Behavior and Its Impact on Adsorption: CNTs and Diclofenac

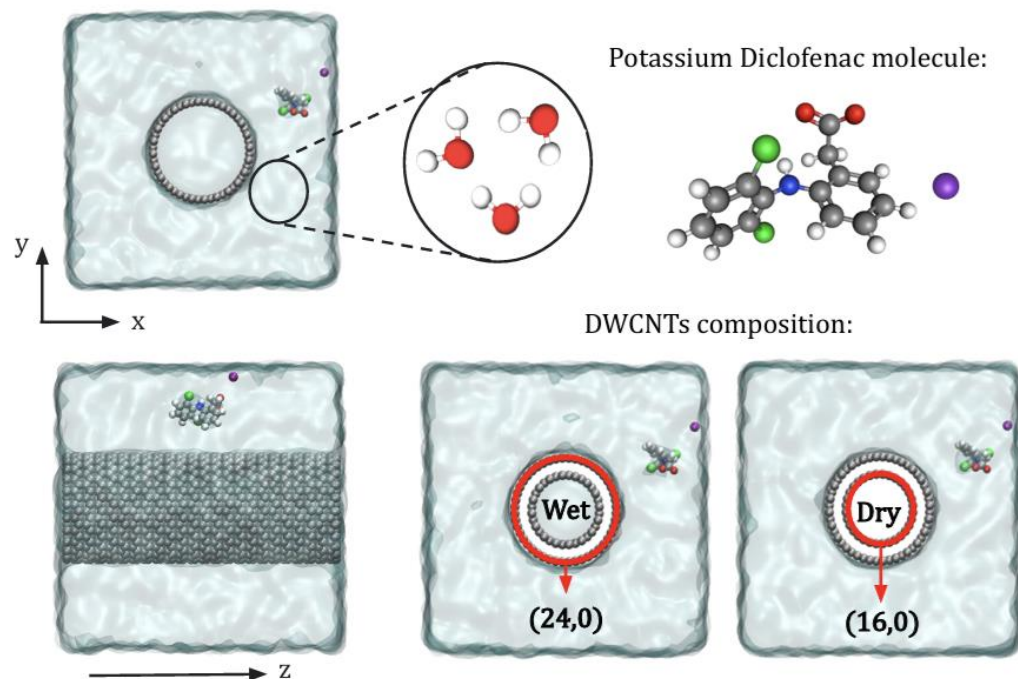


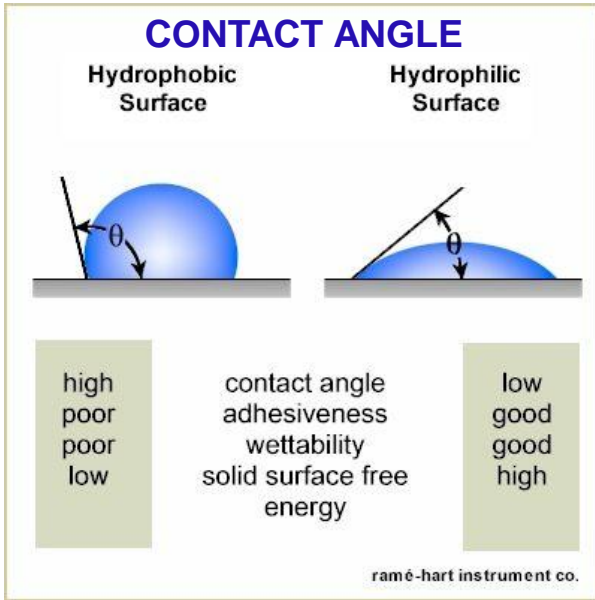
FIG. 1. Depictions of simulated systems, diclofenac molecule, and CNTs orientation. The color scheme for atoms follows: red is Oxygen, white is Hydrogen, gray is Carbon, green is Chloride, blue is Nitrogen, and purple is the Potassium ion.

Double vs single-walled CNT  $\langle V_{4_s} \rangle$  : -7.17 vs. -6.81 kJ/mol; In P(N=0): -9.35 vs -8.93  
Water more retained at DWCNT, thus more difficult to replace by the Diclofenac molecule.

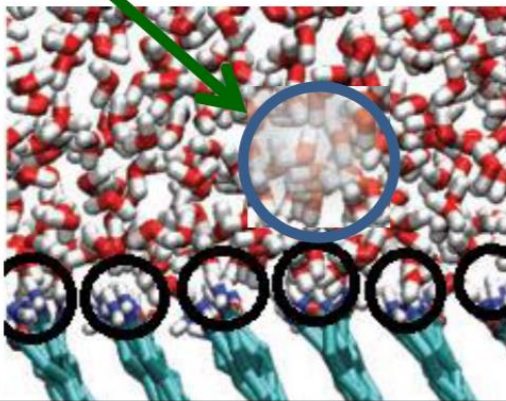
*P. R. B. Côrtes, N. A. Loubet, C. A. Menéndez, G. A. Appignanesi, M. H. Köhler, J. R. Bordin, submitted and arXiv:2407.16631 [cond-mat.soft] (2024)*

# HYDRATION AND NANOCONFINED WATER:

## Searching for the signatures of hydrophilicity



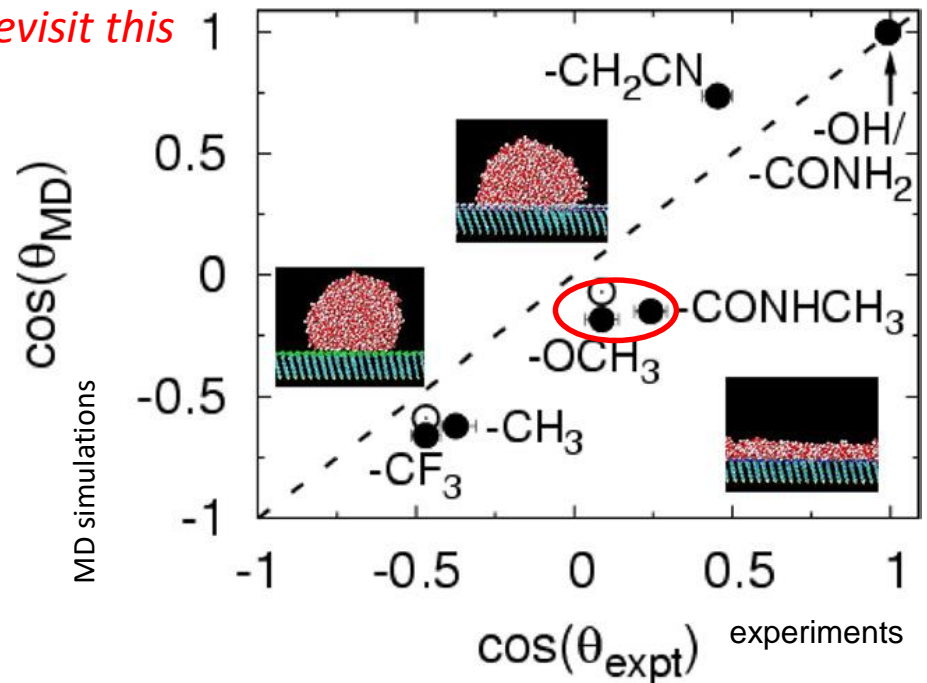
$r=3.3\text{\AA}$



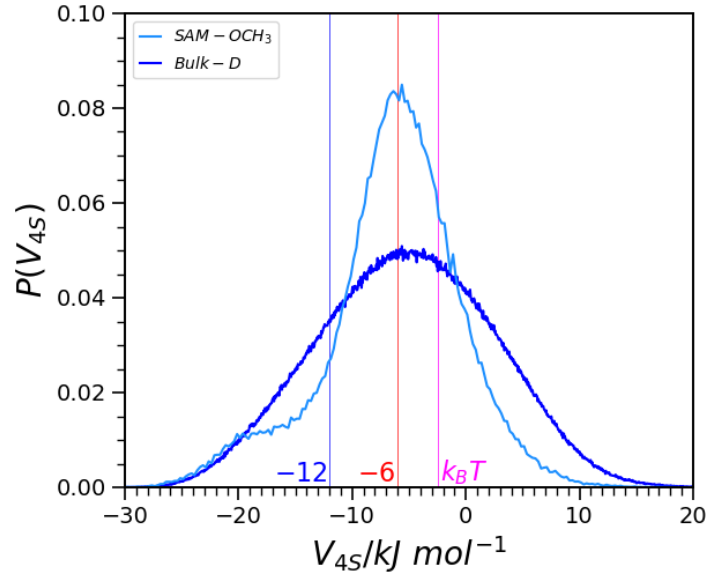
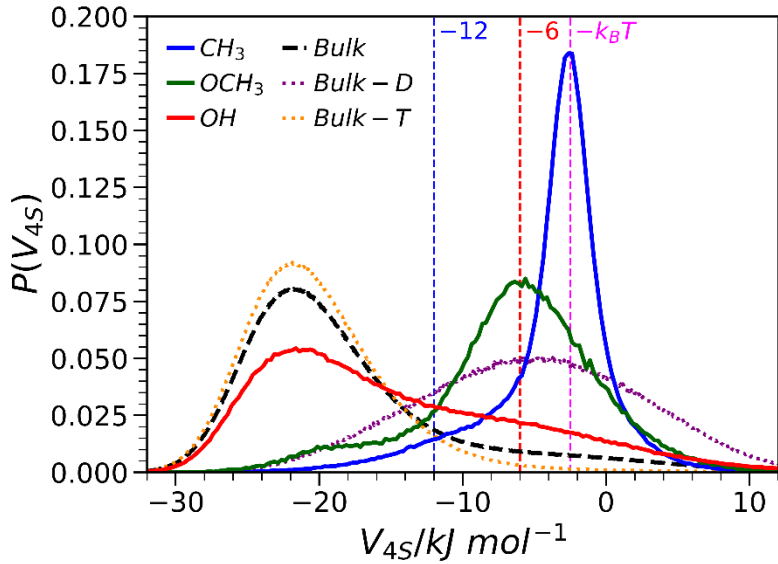
Self-assembled monolayer  
(SAM: functionalized alkyl chains)

**EXPERIMENTAL MEASURE ON SOLID SURFACES:**  
Garnick&Bae (Science (2008)): “Hydrophobicity depends on the eye of the beholder... One common definition is that water droplets on a planar hydrophobic surface possess a contact angle larger than  $90^\circ$ ; but given that nothing dramatic changes when the contact angle falls below this or any other point, it is just a convenient but arbitrary definition” (remember also relative hydrophobicity scales).

*We hereby revisit this*

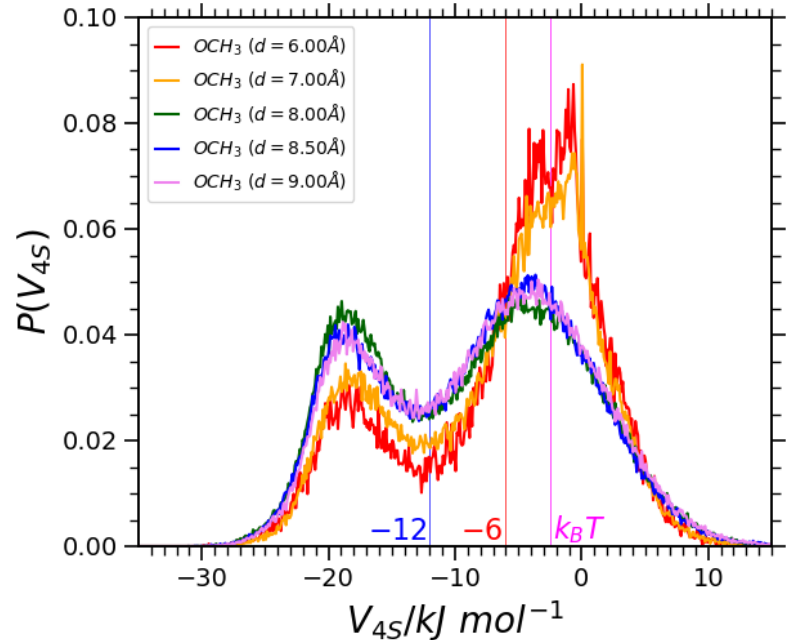
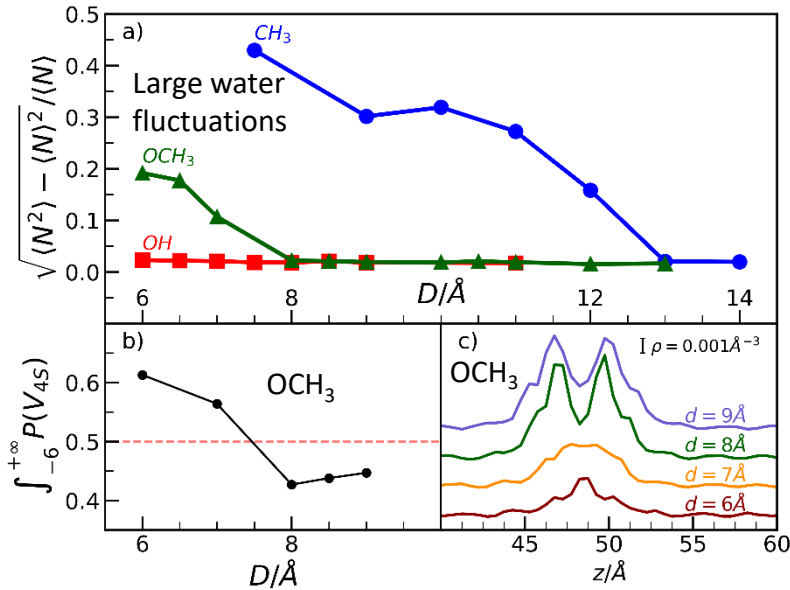


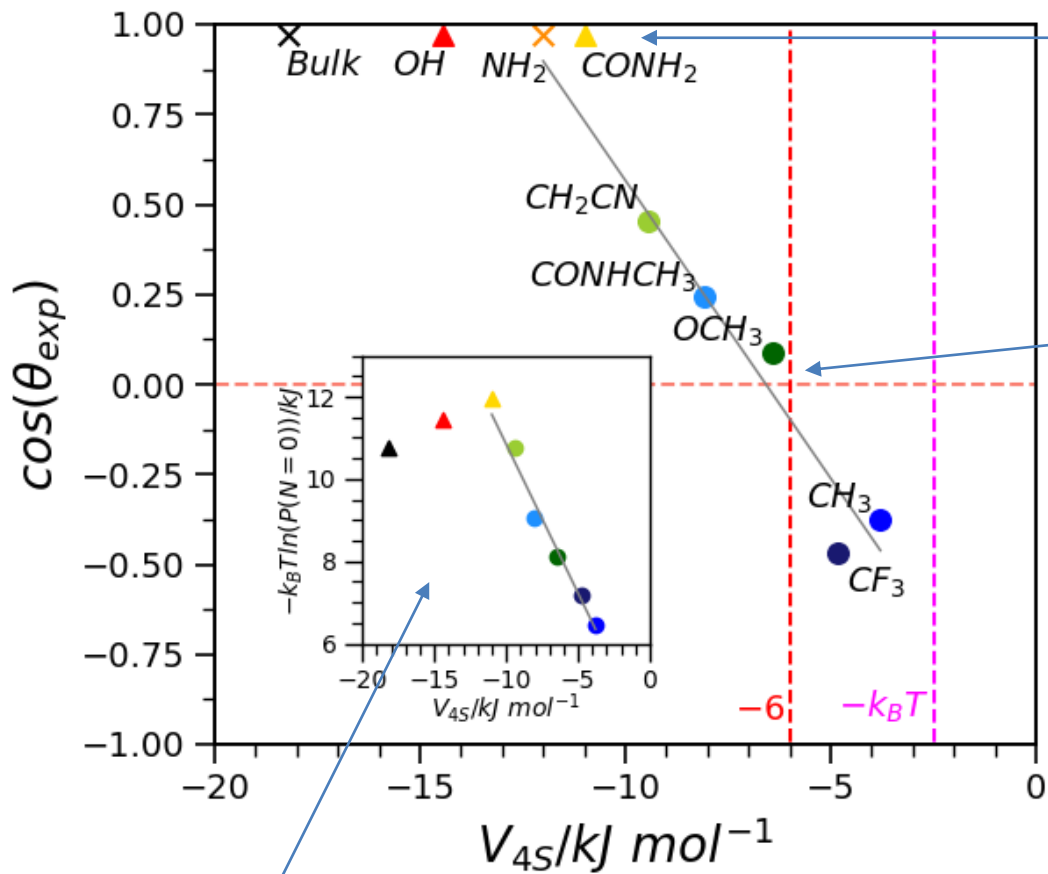
## SAMs with different functionalization:



$\text{OCH}_3$  slightly hydrophilic. Very similar to D

## SAMs. Parallel plates (confined water)



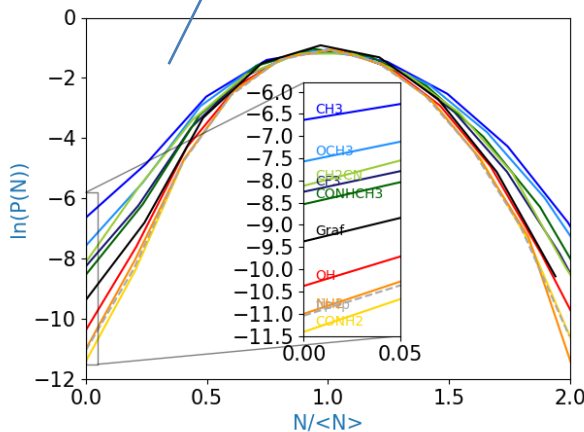


Saturation, strong hydrophilic limit

Transition hydrophobic-hydrophilic

$V_{4S}$  Classifies correctly all SAMs

Excellent correlations



Also excellent correlations between  $V_{4S}$  and water vacating probabilities

N. A. Loubet, A.R. Verde and G. A. Appignanesi, submitted and arXiv:2409.00437 [cond-mat.soft] (2024).

Water is good at establishing interactions. You do not have necessarily to fulfill the optimal expectation (fully replace HBs); it gives you a much modest alternative, it is enough to reach a partial compensation level (defect level).

*One simple molecule, two interaction demands (two ways of love).*

*In turn, these requirements translate into two limit wetting behaviors: hydrophilicity saturation (linear HB) and hydrophilic threshold (transition hydrophobic-hydrophilic).*

$\theta=90^\circ$  ( $\cos\theta = 0$ ) when  $V_{4s} = -6\text{kJ/mol}$  (bulk D molecules). If the surface cannot favorably compete with bulk, then hydrophobic.

**Fingerprints of hydrophilicity at  $\theta=90^\circ$ !**

*From signatures of hydrophilicity to establishment of absolute (not just relative) values for hydrophobicity/hydrophilicity.*



-We elucidated the existence of a fine-tuned interplay between local structure and energetics that allows water to establish an extended hydrogen bond network while compensating for uncoordinated sites. We also showed that this molecular mechanism underlies water's two-liquids scenario, thus providing a detailed molecular rationale for this relevant description.

-We showed that the same principles are operative at hydration and nanoconfinement conditions, thus extending the validity of the two-liquids scenario to contexts of paramount relevance for which the behavior of water is determinant.

-We defined conditions for wettability, thus providing an accurate measure of hydrophobicity and a reliable predictor of water filling and drying transitions. These goals have been out of reach in the absence of a proper molecular rationalization of the wetting process.

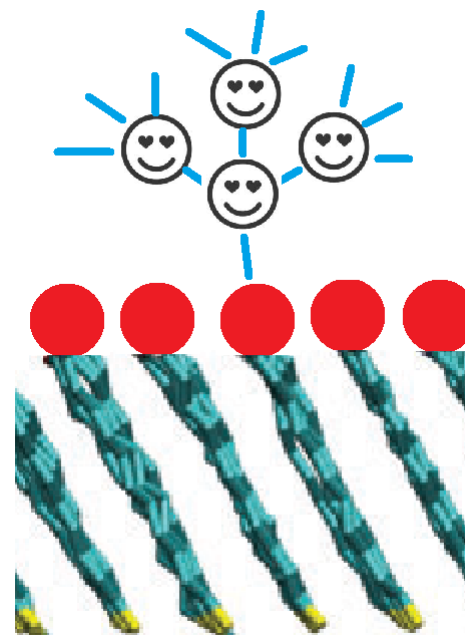
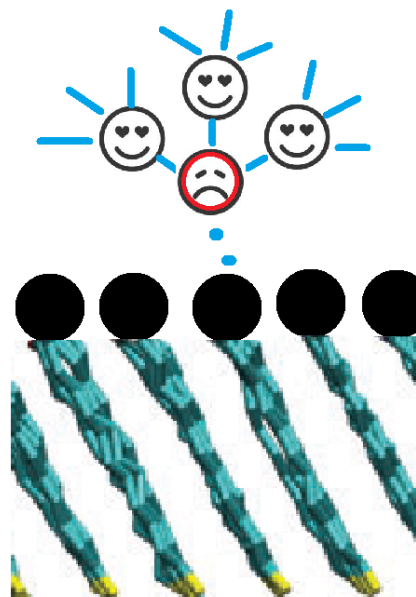
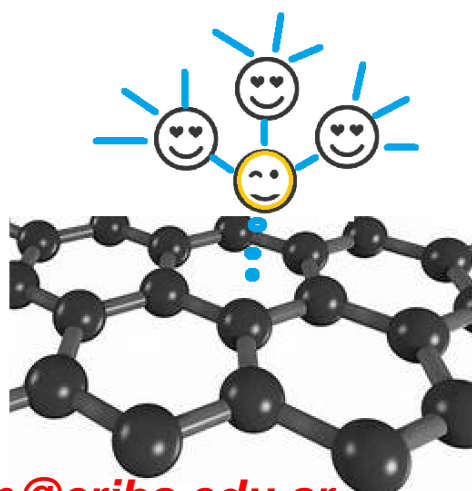
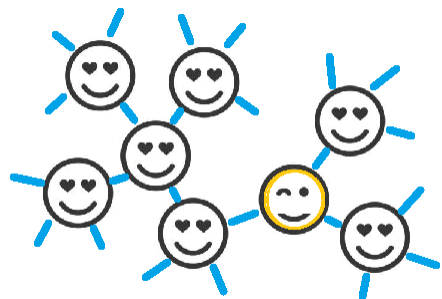
-We provided a structural metric that, unlike previous indicators that were only valid for bulk conditions, is suitable for generic contexts and could therefore open the possibility of elucidating the active role of water in broad fields of biophysics and materials science. This made possible to find the fingerprints of hydrophilicity, while enabling an absolute measure of hydrophobicity/hydrophilicity (not just relative values).

-First applications of this method: unexpected high hydrophilicity of graphene-like systems, "surprising" penetration of water in small-radius carbon nanotubes, effect of geometry on hydrophobicity, role in adsorption and detection of protein binding sites.

## Bahía Blanca (Argentina):

*Cintia Menéndez, Sebastián Accordino,  
Laureano Alarcón, Alejandro Verde, J.  
M. Montes de Oca (now at The  
University of Chicago, USA) and*

*Nicolás Loubet*



***“Laudato si, mi Signore, per sor’aqua, la cuale è molto utile et humilde et pretiosa et casta.”*** Francesco d'Assisi (*Canticle of the Creatures*)

*“Praised be You, my Lord, for **sister water**, who is very useful and **humble** and precious and pure.”*

*Hints on water’s behavior seemed to in fact be already hidden in Saint Francis’s prayer: Simplicity (humility) and Interactions (sister water, sor’aqua). The relevance of interaction to bring in complexity (a complex, beautiful and most useful behavior) to a simple system.*

*But humility and interactions are of course extremely relevant beyond physics to life in general:*

*Encyclicals from  
Pope Francis*

*Laudato Si*



*Fratetlli tutti*

Saint Francis mastered simplicity, he mastered humility. And he also lived in a deep level of interaction: fraternity. Francis genuinely felt as a brother to all men and women. Thence, sister water, brother sun, sister moon, sister earth, brother birds, brother trees and herbs, ... even brother wolf.

*"God is sphere, whose center is everywhere and whose circumference is nowhere."*

*from "Pascal's sphere", by Jorge Luis Borges.*

Indeed, Francis deeply felt such sacred center in every single being or entity around him.

***Thank you!***