POLITECNICO DI MILANO

Protein adsorption on the TiO_2 polymorphs and on CNT at different curvature: a molecular modeling study

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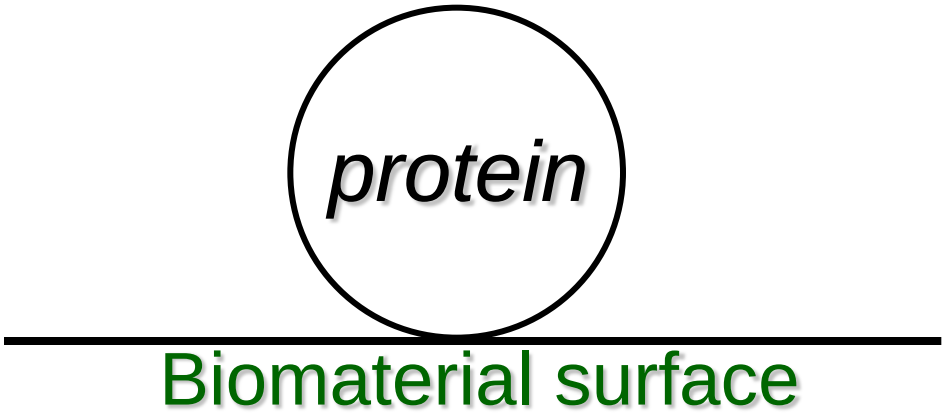
Simulation study

of protein adsorption on biomaterials surfaces:

- TiO_2
- Carbon Nanotubes (CNT)

comparing our theoretical results with experimental data

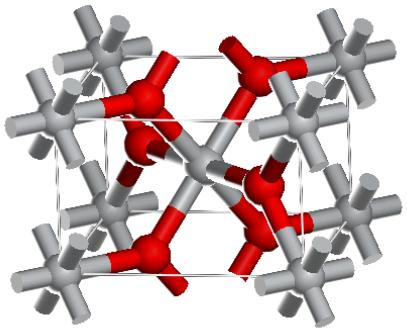


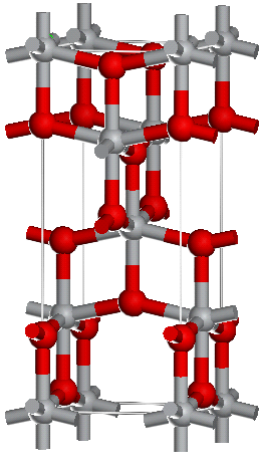
Theoretical work about adsorption on TiO_2 surfaces

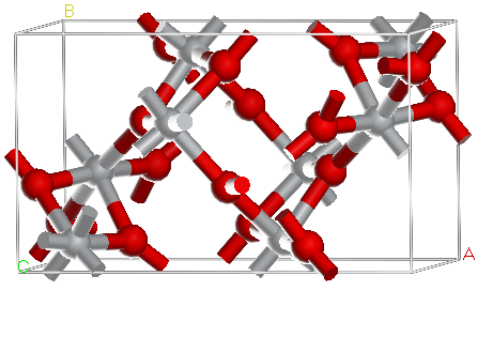
- **Ti :** widely used in medical *implants and prostheses* due to its **bulk and surface properties**.

- It interacts with biological fluids through a **passivating TiO_2 film**
  ↳We wish to control the film **thickness**, **morphology** and **crystal structure**

  - Three TiO_2 polymorphs: **rutile**, **anatase** and **brookite**

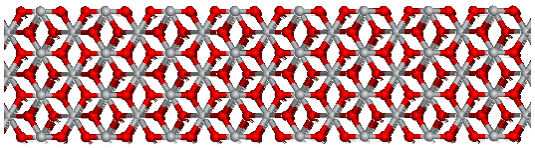




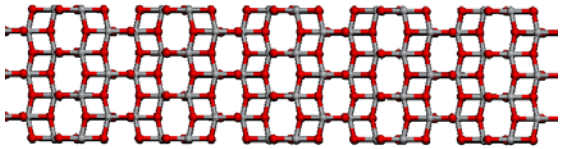


They expose **unlike surfaces**, which may ***differently*** **affect protein adsorption**
↳hence ***biocompatibility*** and ***performance*** of the biomaterial.

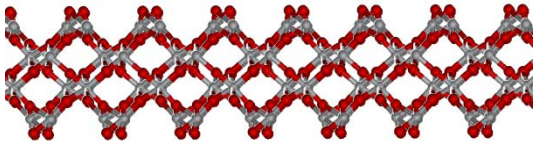
Protein adsorption on ***ideal surfaces*** of the TiO_2



Rutile (001)



Anatase (100)

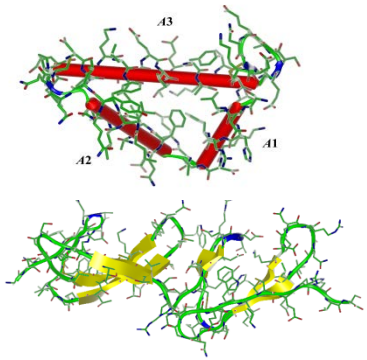


Brookite (100)

- ✓ only bridging oxygen and Ti atoms are exposed
- ✓ *Similar surface chemistry :*
  in this case we can study the effect of the **nanoscale topography** only

Protein fragments:

- ✓ ALB = albumin subdomain (α-helices)
- ✓ FIB = fibronectin modules (β-sheets)



- A **key issue**: **at a fixed surface chemistry**,
  does the **surface topography affects protein adsorption**?

Current simulation methods based on molecular mechanics and molecular dynamics
can (*correctly*) predict significant differences?

Simulation methods and proposed strategy

Molecular studies at atomistic level based on
Molecular Mechanics and **Molecular Dynamics**

Molecular Mechanics

Energy minimization with respect to all the variables (the atomic coordinates) of protein fragments near different surfaces

- **geometry** of interaction (*conformational changes*)
- **strength** of interaction (interaction energy E_{int}, strain energy E_{strain})
- **surface coverage (*total* or *partial*)** and **film formation**

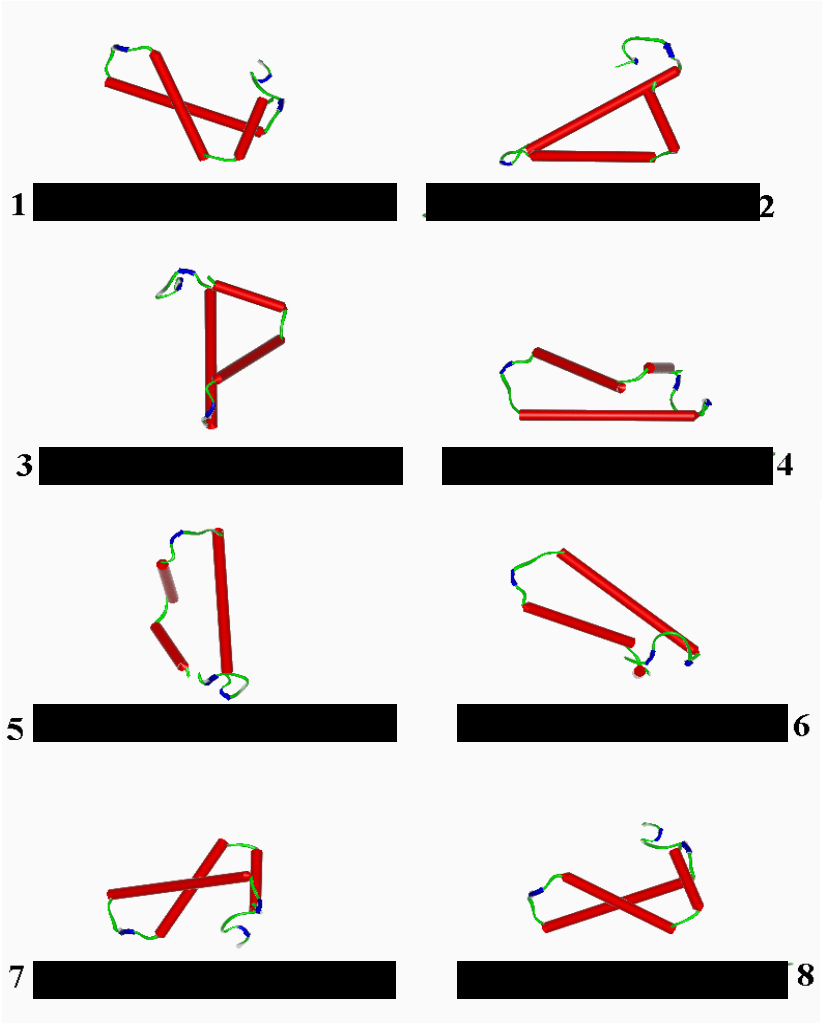
Molecular Dynamics

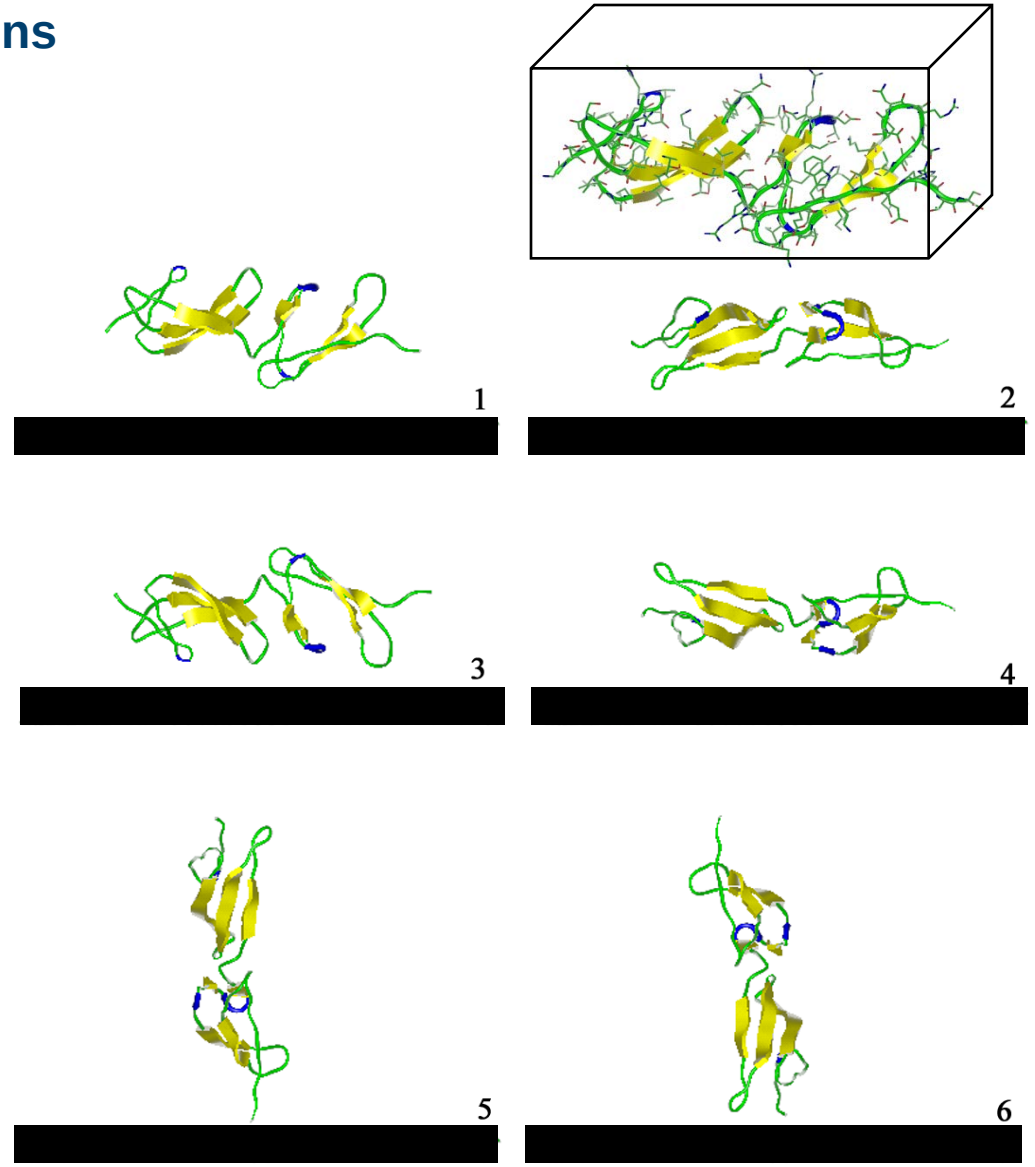
Time evolution of the system *at constant (average) T* solving the classical equations of motions (Newton) for each atom

- **kinetics** of adsorption process (*kinetics of spreading*)
- **mobility** on the surface
- **possible surface ordering** induced by the surface

1. First step of proposed *simulation protocol*

Starting with different trial orientations (*random approach from solution*)

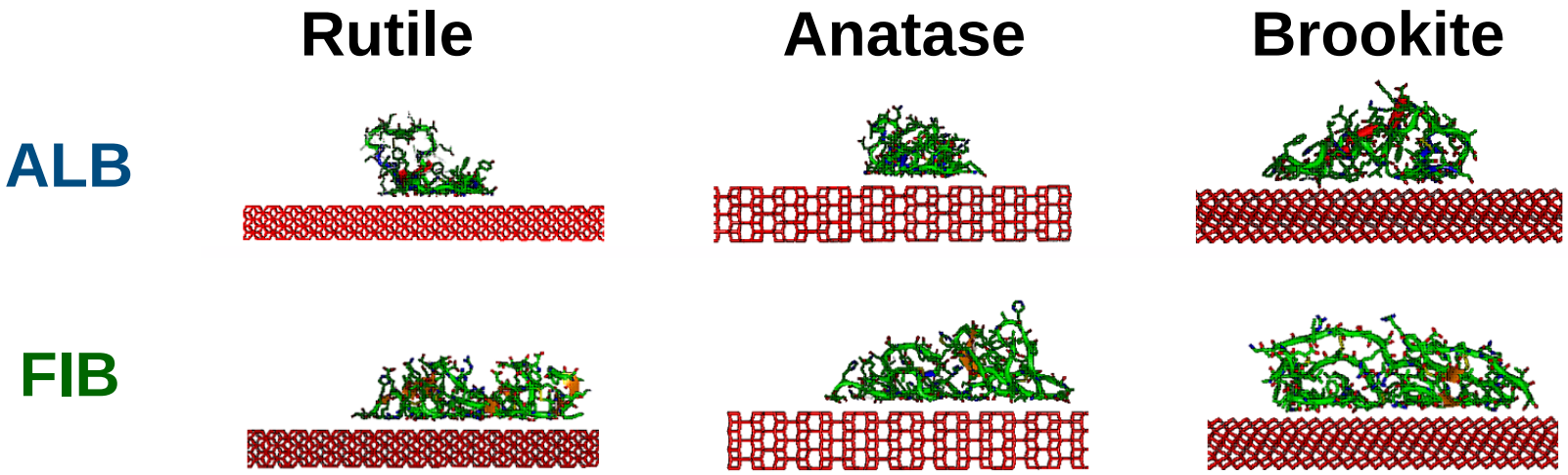




1. MM ⇒ *Initial* adsorption stage

Starting with different trial orientations

The energy minimizations yield the most stable *initial* geometries:



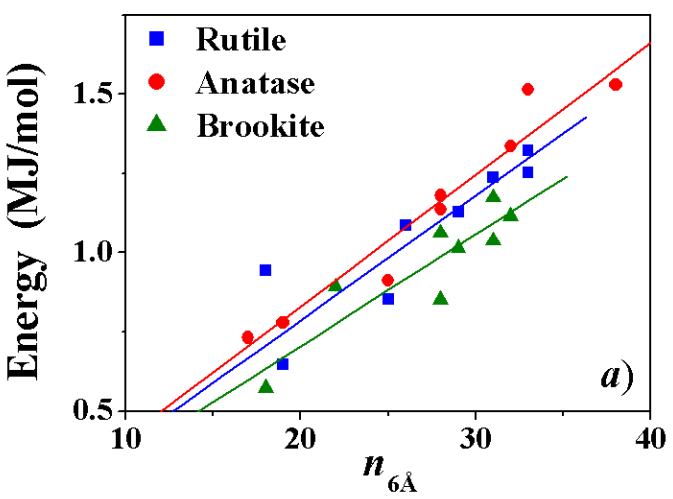
Local deformations to enhance the contact surface and *local* loss of secondary structure

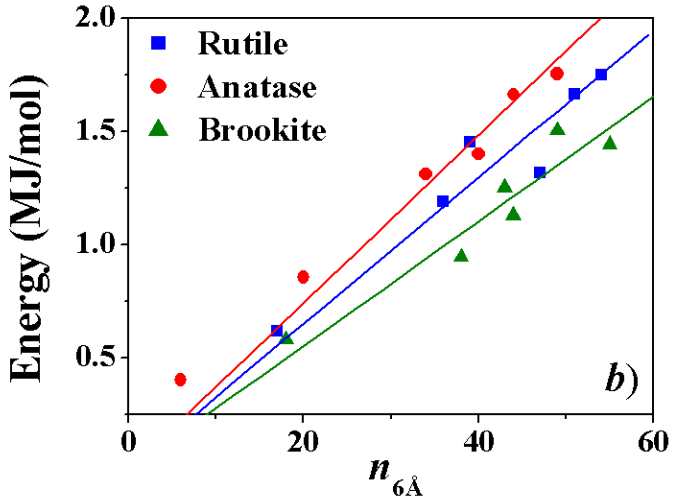
Other higher energy geometries are present (local energy minima)

For all initial geometries the E_{int} and E_{strain} are calculated…

1. MM - Initial adsorption stage

Interaction energy (E_{int}) plotted as a function of $n_{6Å}$ = no. of residues in contact with the surface





ALB **FIB**

For both proteins we find:

- ✓ **More favorable interaction on anatase surface**
- ✓ **Brookite shows a significantly weaker interaction**
- ✓ **Strain energy** (*broken H-bonds, ...*) **increases more slowly than E_{int}**

  ↳ This behavior implies that **larger deformations may enhance the interaction strength…**

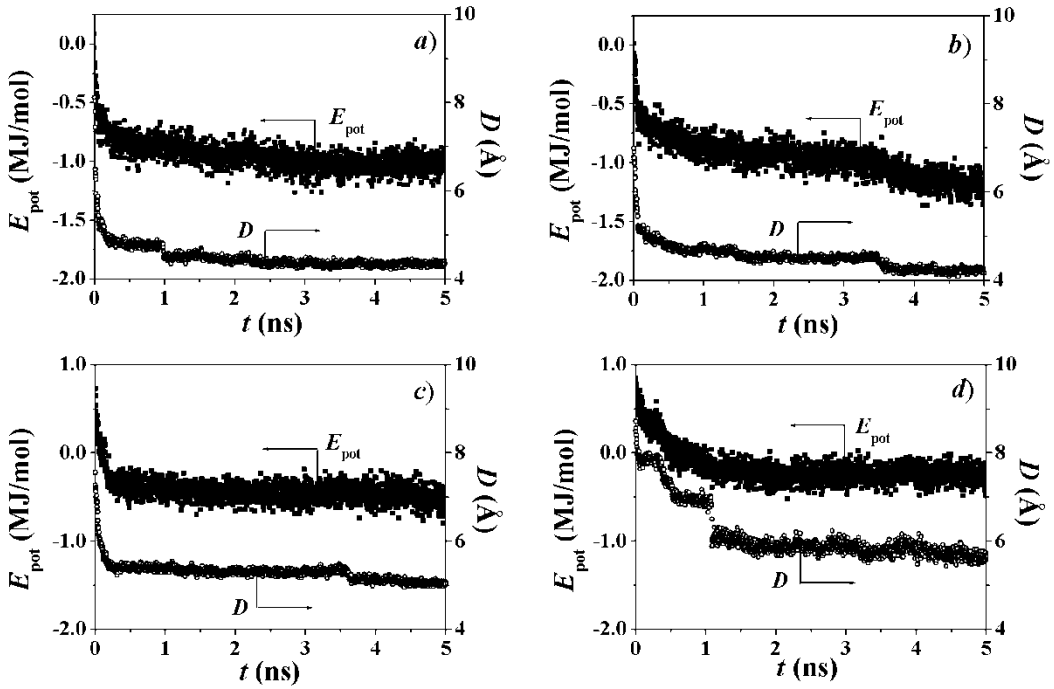
2. Second step of proposed *simulation protocol*
MD : Kinetics of spreading

E_{pot} and D = distance of proteins center of mass from the surface as a function of time

Rutile **Anatase**

ALB

FIB



When the protein fragments spread on the surface:

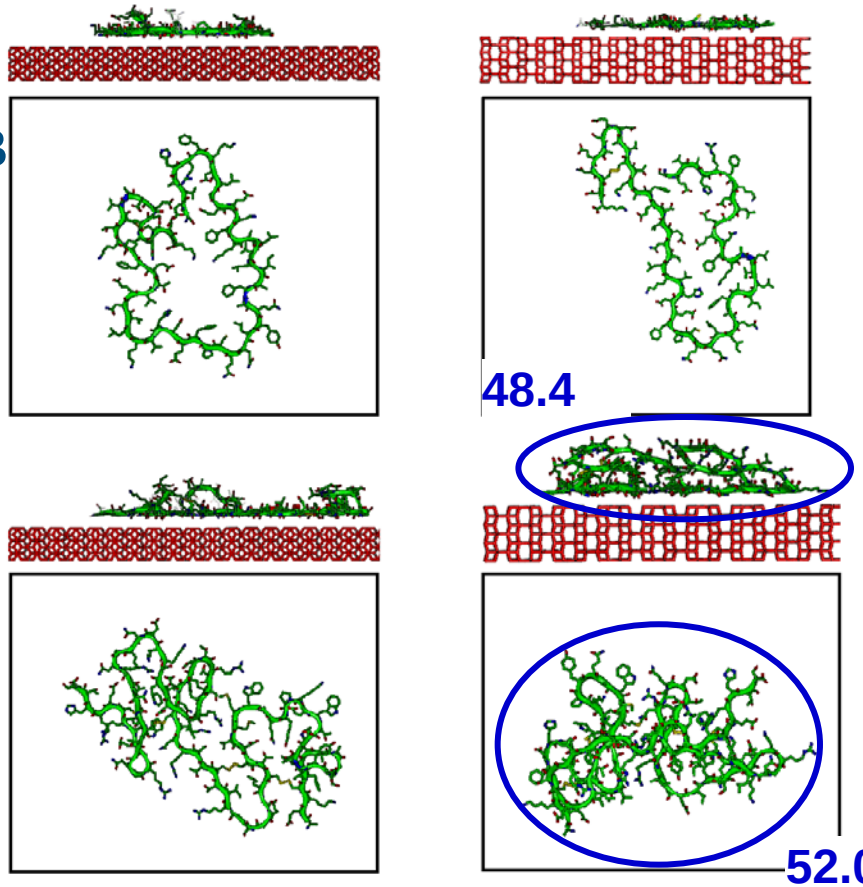
1. the potential energy decreases smoothly

2. while the distance can show some jumps due to conformational changes

Faster spreading for albumin (a '*soft*' protein)

3. Third step of proposed *simulation protocol*
MM after MD ⇒ *Final* adsorption stage

Most stable final geometries



- Surface spreading and flattening: more residues interact with the surface
  - ↳ Stronger interaction with optimization of both:
    - protein-surface interactions
    - intra-molecular interactions (that can take place at longer time)

- Interaction energy per residue in contact with the surface (**intrinsic E_{int} kJ/mol**):
  - ✓ **For both protein fragments Stronger adsorption on anatase** *as an effect of the surface topography*!

On anatase, stronger interaction with FIB that keeps a secondary structure similar to the native one

Comparing Theoretical results – Experimental data

- Considering the intrinsic E_{int} and the conformation, **the fibronectin fragments**:

1. show **stronger interaction with anatase surface *than albumin***

in agreement with competitive adsorption experiments HSA and Fn on TiO_2

HSA adsorption is faster (larger diffusivity) but is then replaced by Fn

SR Sousa et al. J. Biomed. Mat. Res. A, 84, 281-290, 2008

2. **Retain their secondary and tertiary structure**

↳ then, their functionality, that is to mediate the cell adhesion
- then, important for osteoblast adhesion and osteointegration

in agreement with experiments on TiO_2 polymorphs

Anatase shows better osteoblast adhesion (driven by Fn adsorption!)
and osteointegration *than rutile*

R Chiesa et al., *J Appl Biomater Biomech*, 1, *91-107*, 2003
R Chiesa et al., *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*, 103, *745-756*, 2007

.. about Protein Adsorption on

- **CNT surfaces**

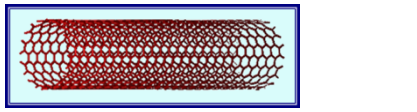
C allotropes

substrates with same surface chemistry
but different curvature, then topography

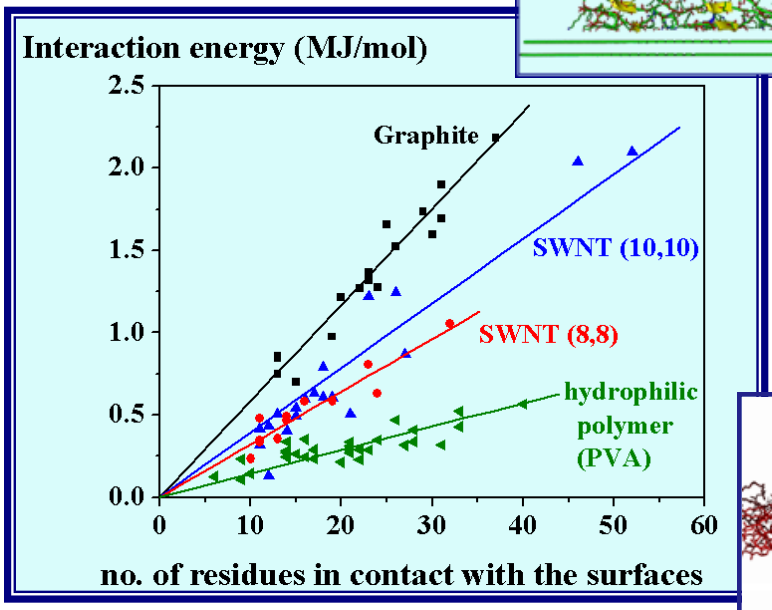
using the same methodology

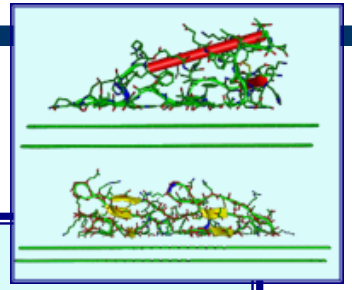
comparing theoretical results with experimental data

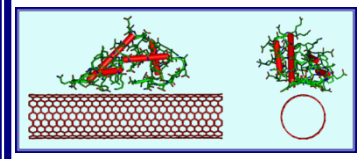
I[st] result: Initial Adsorption stage on (10,10) and (8,8) CNT

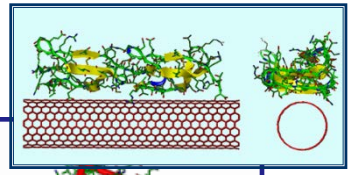


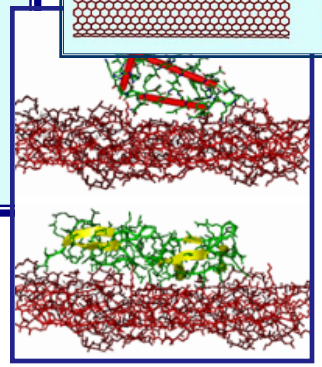
Outer convex surfaces
SWNT (10,10) ∅ = 13.6 Å
(8,8) ∅ = 10.8 Å







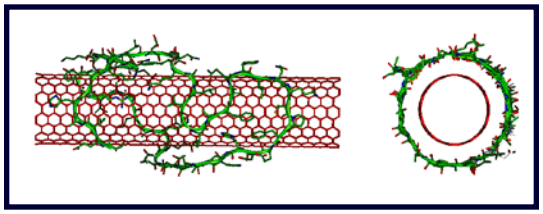


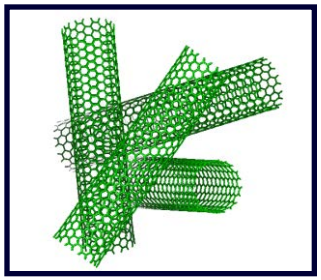


- The interaction energy increases in the order (8,8) CNT < (10,10) CNT < flat GRAPHITE
  - Then, a larger curvature yields a weaker interaction!
    - *Still, it is stronger than on* Hydrophilic PVA amorphous surface

... Results reported in my PhD Thesis In Materials Engineering at the Politecnico of Milan

IInd result: Final adsorption stage on OUTER CNT surface

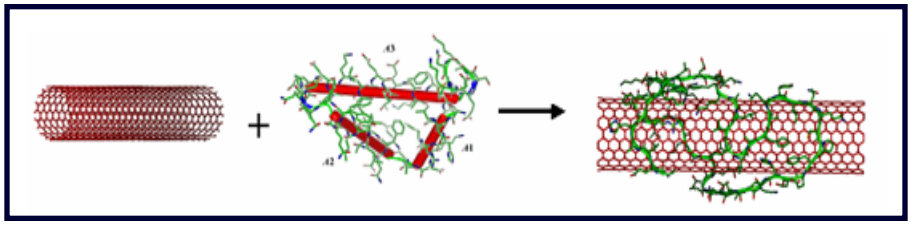




The energetic cost to detach a CNT from a random aggregate

is less than the energy gain due to adsorption

(calculated after MM and MD runs in the most stable adsorption geometry)

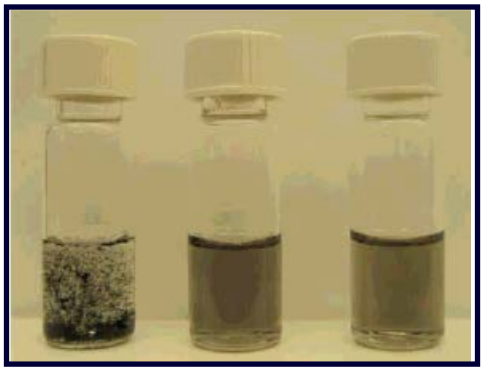


⇒ hence we find that CNT can be solubilized in water by proteins through non covalent interactions

... AS INDEED EXPERIMENTALLY FOUND

JS Dordick, RS Kane et al

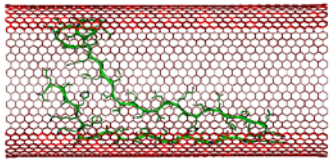
Langmuir, 22, 1392-1395 (2006)

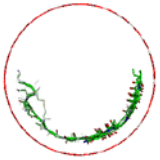


in water **with BSA** **with MJL**

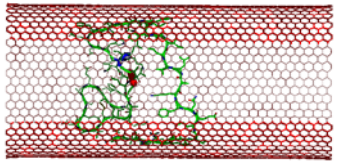
III[rd] result: Final adsorption stage on INTERNAL CNT surface

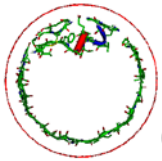
Two molecular conformations within nanotubes – (30,30) CNT





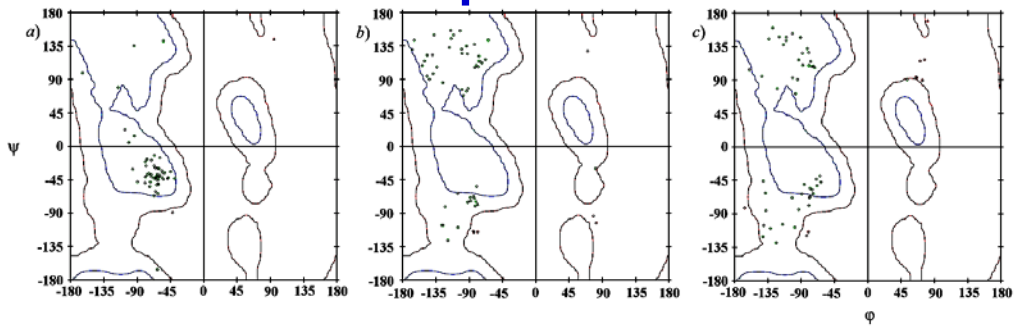
hairpin
most stable

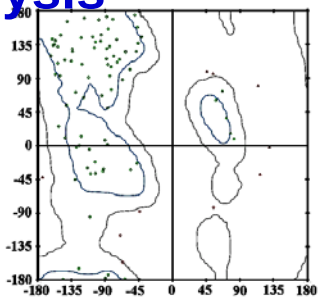




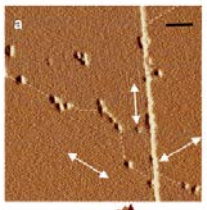
ring-like
less stable
(fewer surface contacts)

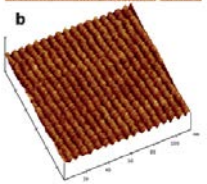
Ramachandran plots for conformational analysis





Native ALB (α-helices) **hairpin** **ring-like** **native FIB (β-sheets)**

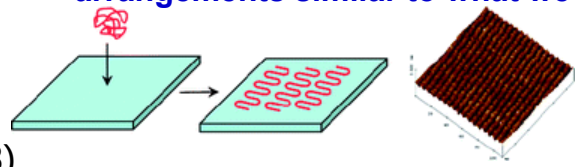




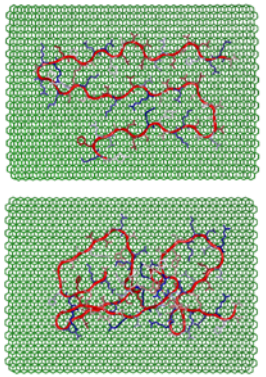
AFM measurements on graphite:

hen egg white lysozyme, β-lactoglobulin, BSA, bovine pancreas insulin

Experimental results are explained with parallel disposition of the backbone strands
→ arrangements similar to what we obtained



O. Cavalleri et al.,
Soft Matter, **4**, 965-967 (2008)



… Finally, recent results about

competitive adsorption:

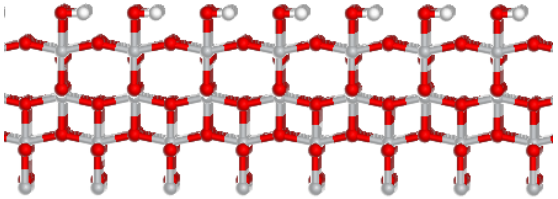
- on TiO_2 anatase nanocrystal surface
- *of small molecules* *(quinoline molecules)…*

Work in progress …

Anatase TiO_2 can exposed different crystal surfaces

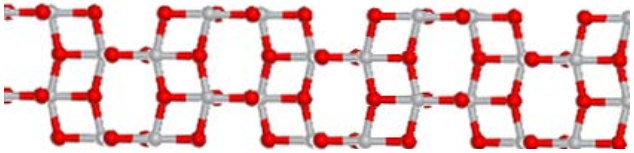
…. Considering ideal TiO_2 anatase nanocrystal:

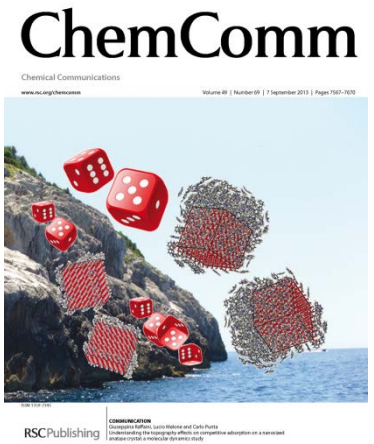
Anatase (001) *hydroxylated surface*



Anatase (00-1) *Titanium atoms exposed*

Anatase (100) *bridging oxygens*





G. Raffaini, L. Melone, C. Punta, *Chem. Commun.,* (2013)

GENERAL CONCLUSIONS

- **MM** and **MD simulations** are **most useful to study protein adsorption:**
  with atomistic details ⇒
  we can study the formation, structure and stability of the physisorbed layer

- We can model the effect of the nanoscale topography (roughness, curvature, …).

- The interaction strength is related also to the protein size and rigidity, and the nature of aminoacids in contact with the specific surface (hydrophilic or hydrophobic).

- TiO_2 polymorphs: anatase leads to a stronger adsorption than rutile, in particular for fibronectin which also preserves the native structure and functionality.

- CNT: strong adsorption dependent on the curvature (concavity or convexity)
- proteins can solubilize CNT in water through non-covalent interactions
- concave surfaces of appropriate curvature provide a stronger adsorption

- Ordered surfaces may induce an intramolecular parallel arrangement.

- F. Ganazzoli, G. Raffaini, Computer simulation of polypeptide adsorption on biomaterials, *Phys. Chem., Chem. Phys*. 2005
- G. Raffaini, F. Ganazzoli, Molecular modeling study of protein adsorption on the surface of titanium dioxide polymorphs, *Phil. Trans. R. Soc. A* 2012.
- G. Raffaini, F. Ganazzoli, Surface Topography Effects in Protein Adsorption on Nanostructured Carbon Allotropes, *Langmuir* 2013
- G. Raffaini, L. Melone, C. Punta, Understanding the topography effects on competitive adsorption on a nanosized anatase crystal: a molecular dynamics study, *Chem. Comm.* 2013.

ACKNOWLEDGMENTS

Prof. F. Ganazzoli
Prof. G. Marletta

Prof. A. Cigada
Prof. C. Migliaresi

Dr. L. De Nardo
Dr. C. Punta
Dr. L. Melone

Dr. G. Candiani **(SAST Surface Associated Selective-Transfection – FIRB 2008)**