Protein adsorption on the TiO$_2$ polymorphs and on CNT at different curvature: a molecular modeling study

Giuseppina Raffaini

*Dipartimento di Chimica, Materiali e Ing. Chimica “G. Natta”*  
*Politecnico di Milano - Italy*
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$_2$

- 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 affect 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)*

![Images of different trial orientations](image-url)
1. MM \(\Rightarrow\) Initial adsorption stage

Starting with different trial orientations

\(\because\) The energy minimizations yield the most stable initial geometries:

Rutile

Anatase

Brookite

\(\because\) Local deformations to enhance the contact surface and local loss of secondary structure

\(\because\) Other higher energy geometries are present (local energy minima)

For all initial geometries the \(E_{\text{int}}\) and \(E_{\text{strain}}\) are calculated...
1. MM - Initial adsorption stage

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

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 $E_{\text{int}}$
  - This behavior implies that larger deformations may enhance the interaction strength…
2. Second step of proposed simulation protocol

MD: Kinetics of spreading

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

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**


  
  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 and osteointegration than rutile**

  R Chiesa et al., *J Appl Biomater Biomech*, 1, 91-107, 2003
  
.. 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
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
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
IIIrd 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.,
... Finally, recent results about competitive adsorption:

- on TiO$_2$ anatase nanocrystal surface
- of small molecules (quinoline molecules)…
Anatase TiO₂ can expose different crystal surfaces

.... Considering ideal TiO₂ anatase nanocrystal:

**Anatase (001)** hydroxylated surface

**Anatase (00-1)** Titanium atoms exposed

**Anatase (100)** bridging oxygens

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.

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)