Biomolecular nanotechnology: protein immobilization at the nanoscale

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Overview

- Introduction to nanobioscience
- Thin film techniques
- Chemical self-assembling
- Langmuir-Blodgett technique
- Layer-by-Layer self-assembling
- Monolayer engineering technique

Nanobioscience

The possibility to manipulate, arrange and investigate biological components at the molecular level in a biomimetic situation

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Organization of biomolecules in a two or three dimensional space

↓

Thin film techniques

Thin film techniques

Allow the use of biomolecules as elementary building blocks to develop self-assembled films of predefined geometry and just one molecule thick.

These methods have been used to immobilize biologically active species.

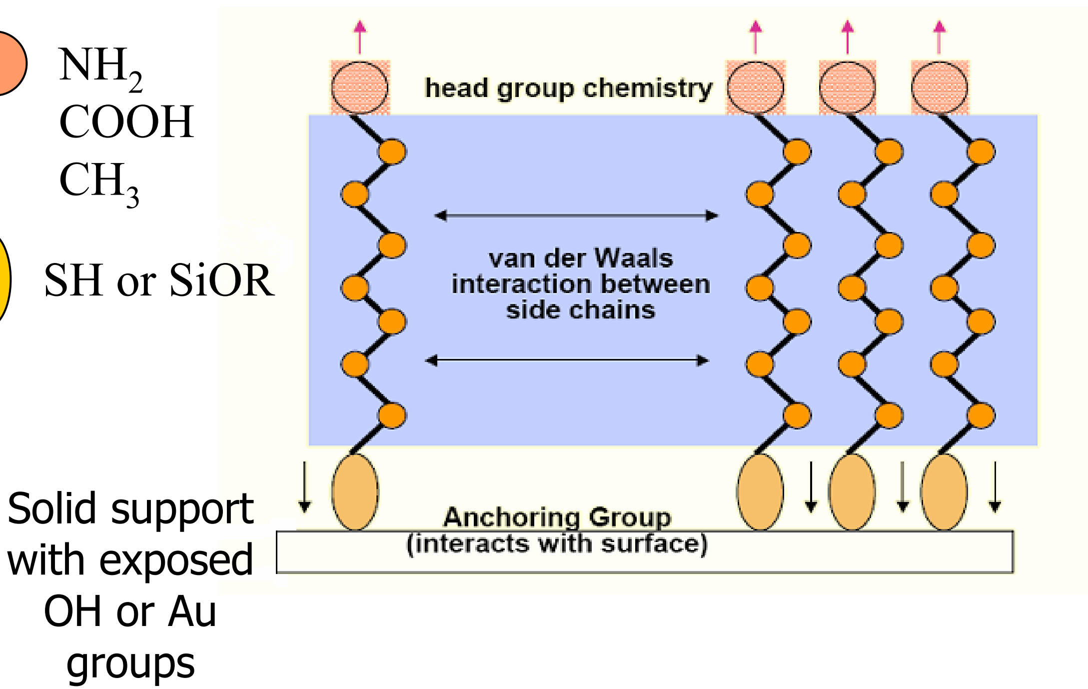
Thin films in diagnostics-nanoassays

- Process control at the molecular scale level
- Miniaturization
- Improved response
- Feasibility
  - mass of a self-assembled monolayer = $2x10^{-7}$ g/cm^2

Chemical Self-Assembly

Activation of inorganic supports for subsequent immobilization of biomolecules:

- Functionalization via silanisation
- Functionalisation via thiol-groups (R-SH) on Au-surfaces



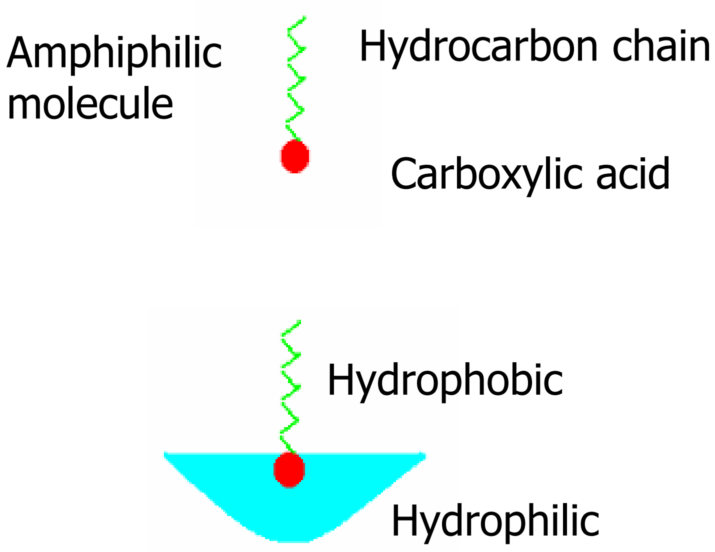
Disadvantages:

- limited to few substrates
- substrate quality
- layer stability (unstable to oxidation)
- strong interaction with biomolecules that can cause denaturation

Langmuir-Blodgett technique

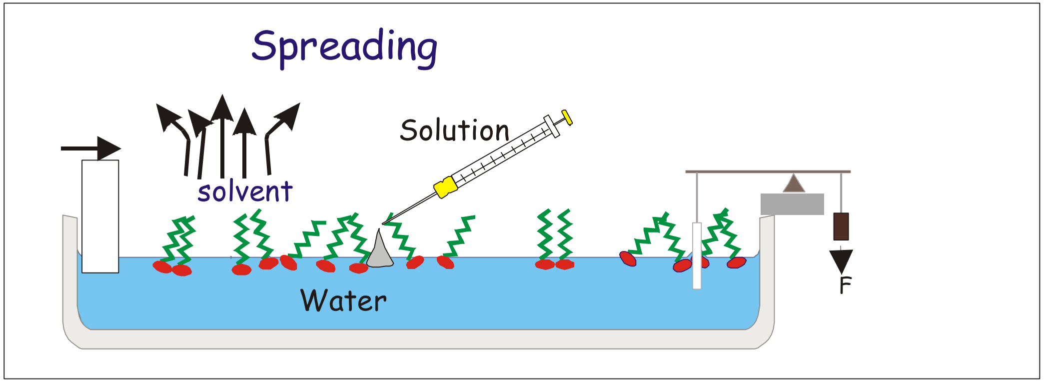
The Langmuir-Blodgett technique enables to form highly ordered monomolecular amphiphilic films at the air-water interface and to subsequently transfer them onto the surface of a solid support.

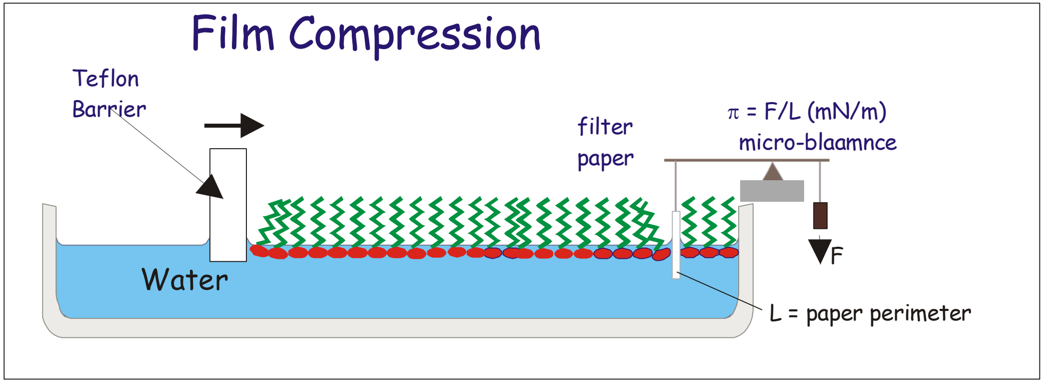
Amphiphilic molecules consist of a hydrophilic (water soluble) and a hydrophobic (water insoluble) part. This amphiphilic nature of molecules is responsible for their association behaviour in solution and their accumulation at interfaces.



Monolayer formation

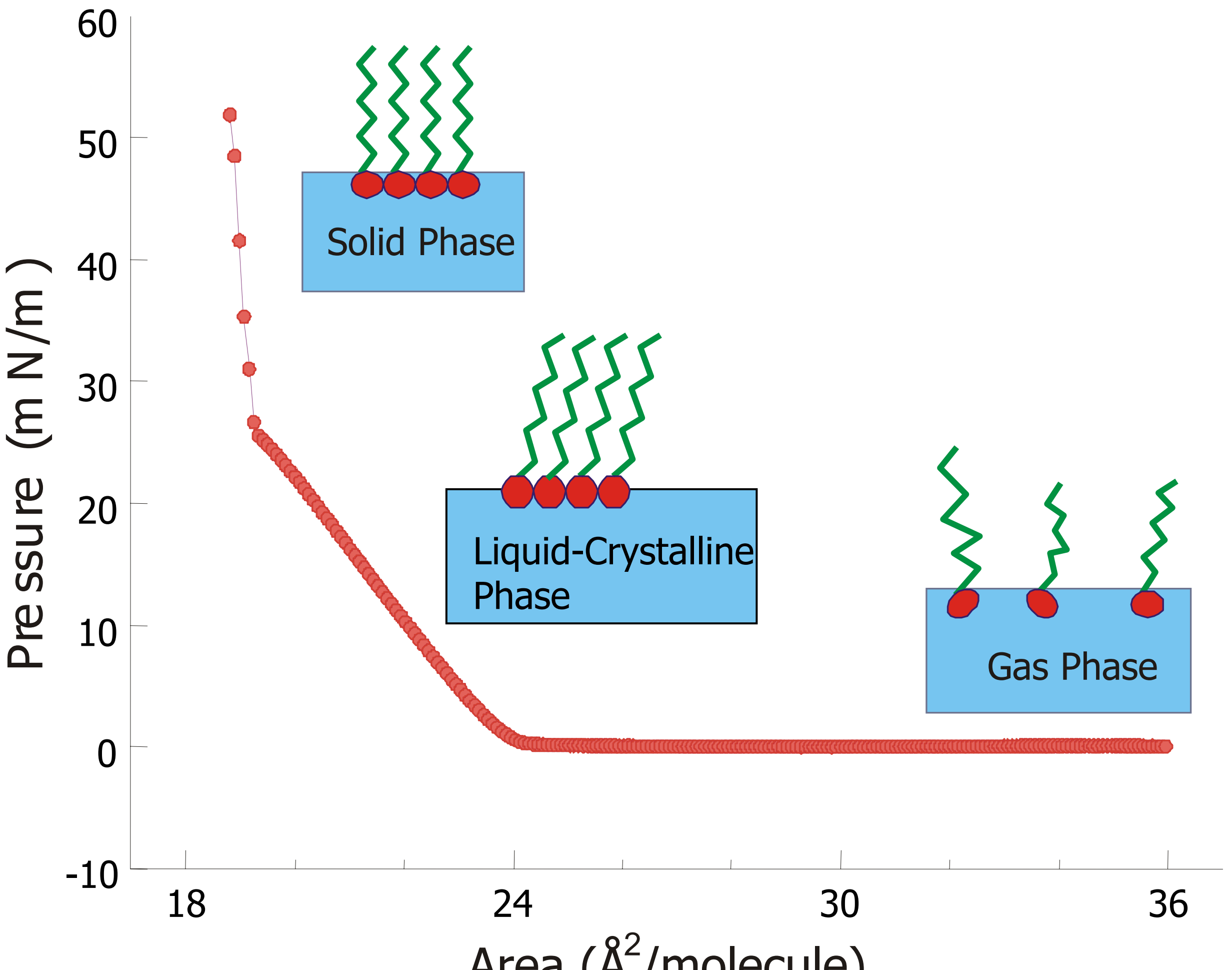
Spreading and Compressing





Π-A Isotherm

Dependence of surface pressure on the area per molecule

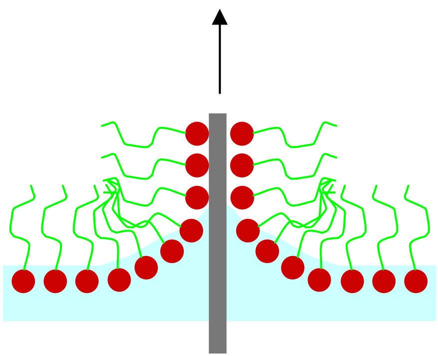


Surface pressure:

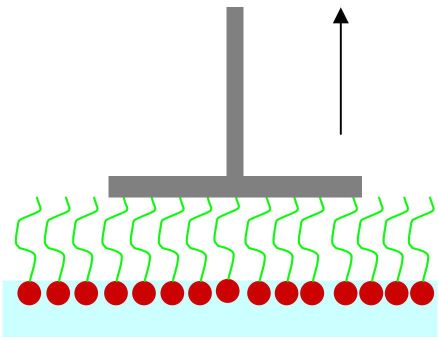
$$\Pi = \theta_{H_2O} - \theta_{ml}$$

Monolayer deposition

Deposition techniques: (a) Langmuir-Blodgett method; (b) Langmuir-Schaefer method

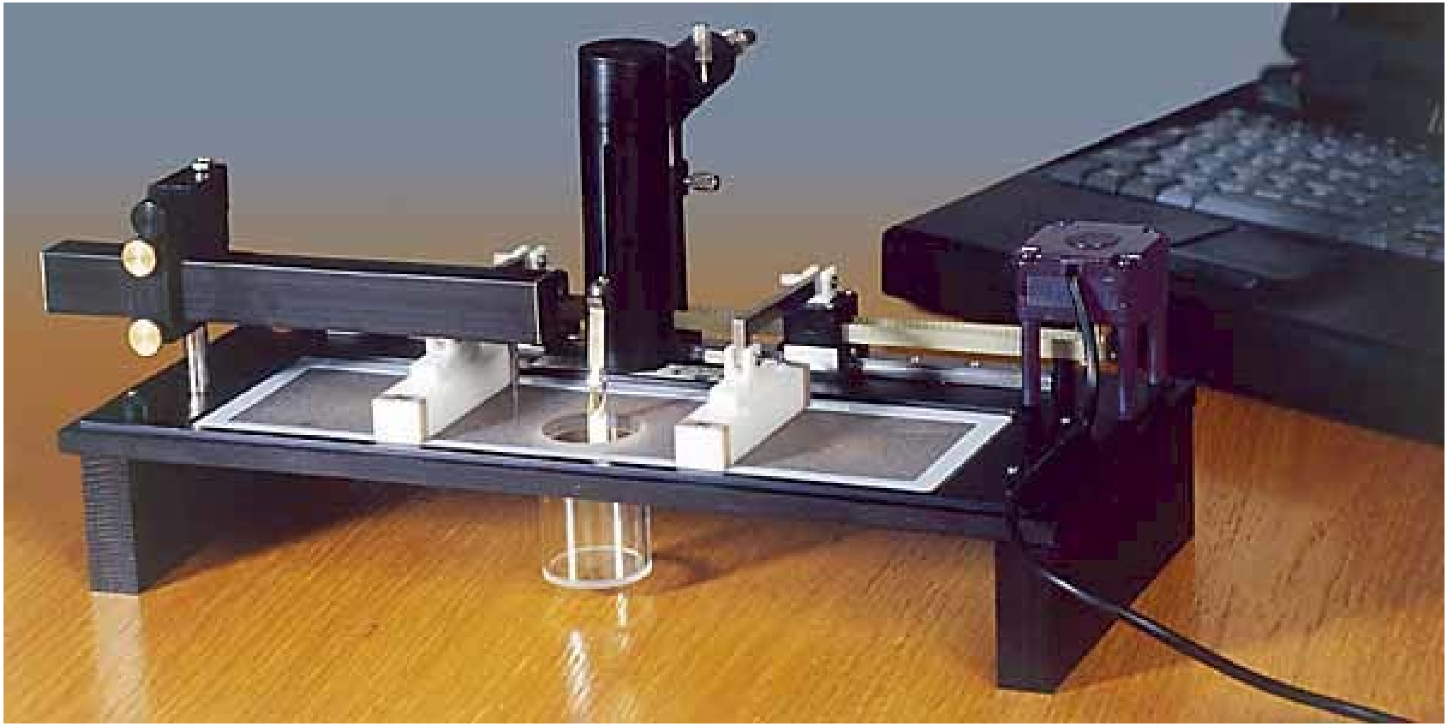


(a)



(b)

Langmuir trough



Characterization Techniques

- Pressure-Area isotherms: provides information on the miscibility of heterogeneous phases.
- Brewster Angle Microscopy: provides information on the monolayer structure at the air-water interface.
- Spectroscopy: provides information on the chemical composition of the films.
  - FTIR
  - NMR
  - UV
- X-ray diffraction: provides insights as to the arrangement of heterogeneous films.
- Ellipsometry: provides information on the film thickness.
- AFM microscopy: enables to get a visual representation of the film surface.
- Other parameters include changes in conductivity, use of fluorescent probes…

LB monolayers in bioscience

LB technique for the investigation of biological systems:

- Interaction of specific molecules with biological membranes.
- Deposition of monolayers of oriented biomolecules onto solid supports.
- Deposition of complicated structures (e.g. donor/acceptor molecules) to study biological processes such as photosynthesis.

Protein monolayers

Main difficulties:

- Proteins are soluble in water.
  - Decreasing the temperature.
  - Increasing the ionic strength.
- Proteins can be strongly affected by the air-water surface tension.
  - Diminishing the time of exposure of the monolayer to the surface tension.

Each case must be considered and verified separately

Film properties:

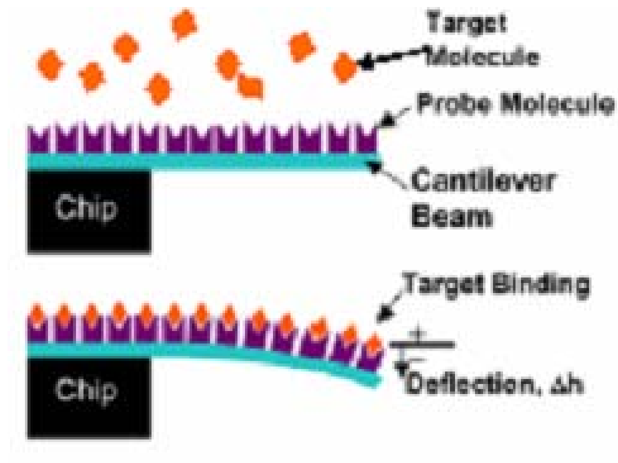
- Orientation - proteins can be oriented by the use of an electric field during monolayer formation.
- Stability - proteins in LB films have demonstrated an increase thermal stability

Applications

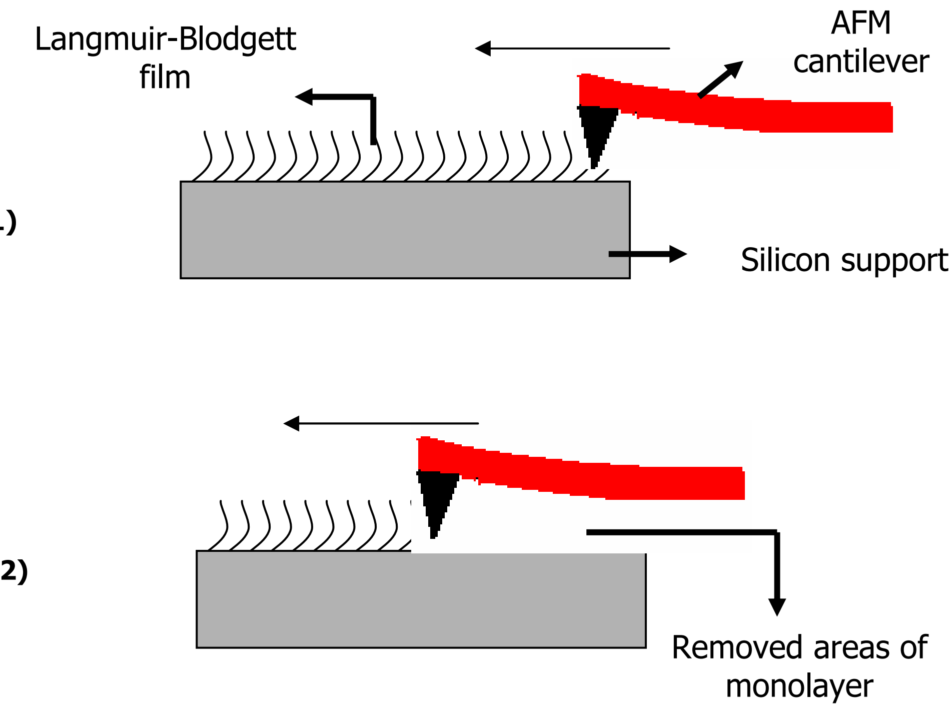
- Films of antibodies:
  - Immunosensors, IgG monolayers onto solid supports preserve their antigen recognition functionality.
- Films of enzymes:
  - Biosensors (e.g. glucose sensor, cholesterol sensor).
  - Biocatalytic films (e.g based on lipase).
- Films of photosensitive proteins.
  - Optical storage and processes based on bacteriorhodopsin

AFM related applications

Nanosensors



Nanolithography



Conclusions on LB films

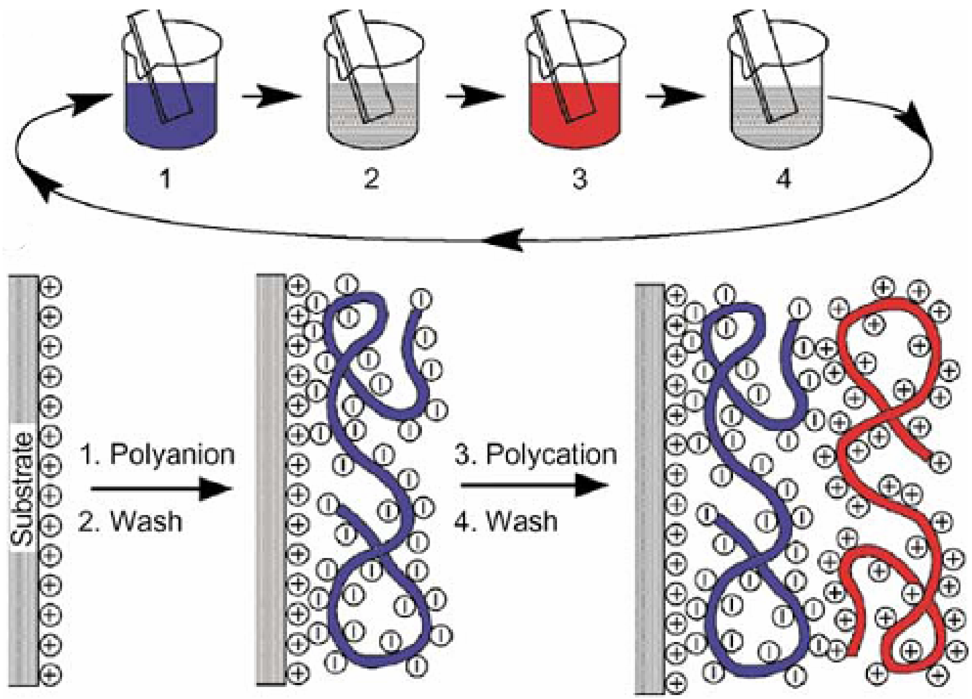
- LB technique allows the formation of highly ordered monomolecular layers.
- Films of active and oriented proteins can be deposited.
- Promising method for protein manipulation.

Layer-by-Layer Self Assembly

This technique makes use of the alternate adsorption of oppositely charged macromolecules to build up multilayered structures.

- Definite and predetermined knowledge of their molecular composition.
- Predetermined thickness ranging from 5 to 1000 nm.
- Precision 1 nm.
- Insoluble in buffer solutions.

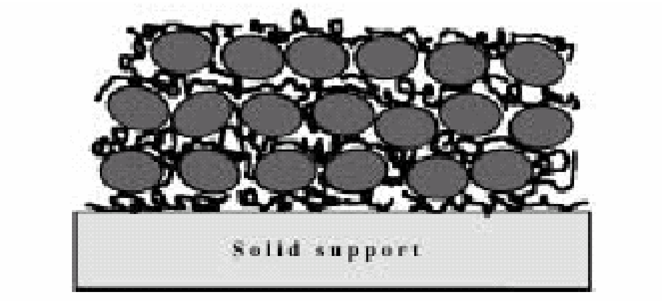
Assembly procedure



A positive solid support, is immersed into a solution of an anionic polyelectrolyte for the adsorption of a monolayer, and then it is rinsed. Then the support is immersed into a solution of a cationic polyelectrolyte for the adsorption of a monolayer, then it is rinsed.

Layer constituents

- Synthetic polyelectrolytes
- Inorganic nanoparticles
- Lipids
- Ceramics
- <u>Biomolecules</u>



Schematic rappresentation of the protein-polyion multilayer

Protein/polyion multilayers

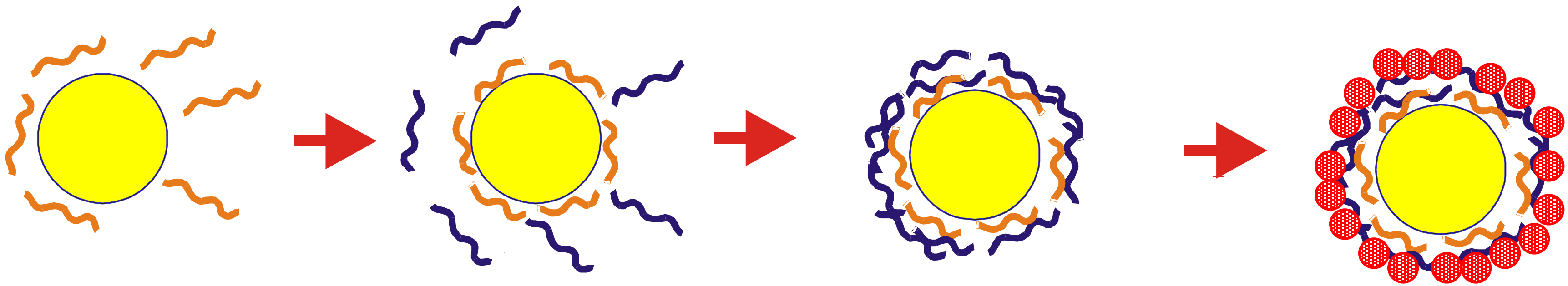
- Complex biofunctional architectures
- Enhanced functional stability

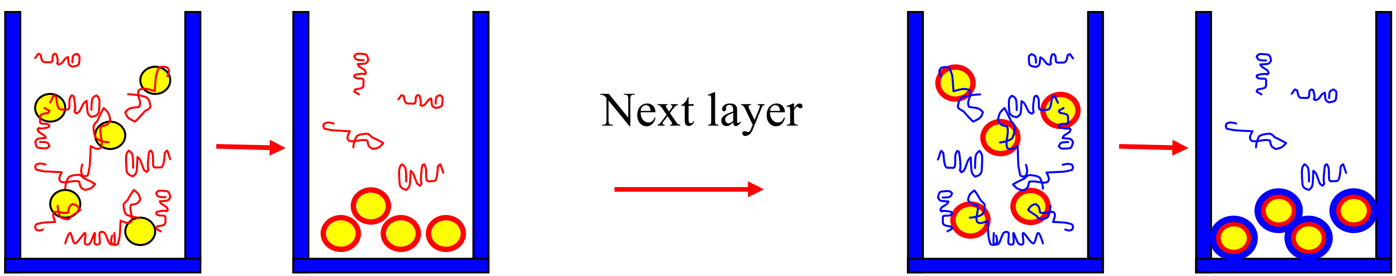
Protein multilayers

Protein	Molecular weight	Isoelect point	pH used	Charge	Alternate with	Mass coverage mg/m2	Thickness of protein+ polyion bilayer nm
Cytochrome	12400	10.1	4.5	**+**	-	3.6	2.4+1.6
Albumin	68000	4.9	8.0	-	+	23	16.0+1.0
Urease	489000	5.0	7.0	-	+	23	Bilayer 16
Hemoglobin	64000	6.8	4.6	+	-	26	17.5+3.0
Lysozyme	14000	11	4.0	+	-	3.5	2.3+1.9
Pepsin	35000	1.0	6.0	-	+	4.5	3.0+0.6

Bionanoparticles

Protein shell assembly on a latex sphere (d = 20-500nm)



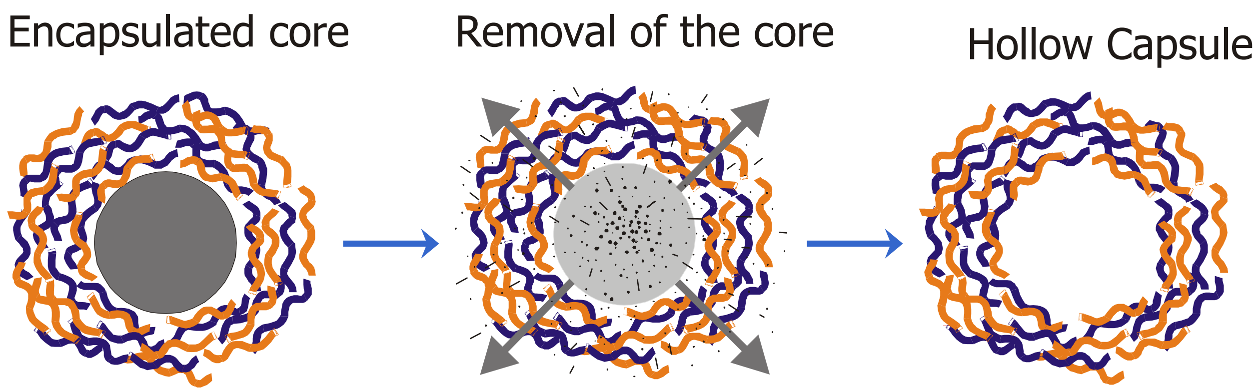


Centrifugation

Nanocapsules

Preparation

Core: e.g.
melamine
ormamide
particles
d=100nm



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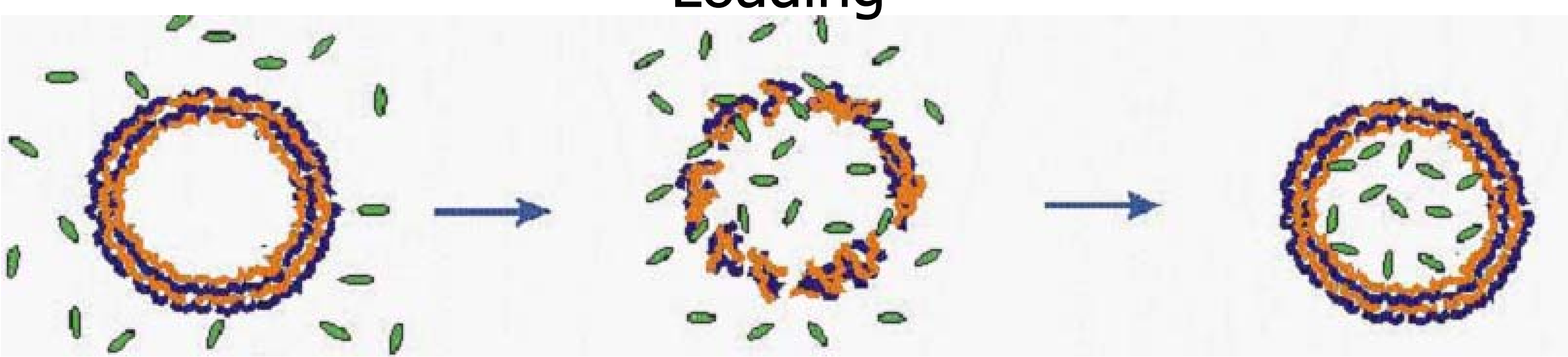


Characterization Techniques

– Quartz Crystal Microbalance; Surface Plasmon Resonance: provides information on the kinetics of the adsorption process.
– Uv-vis Spectroscopy: enables to control the assembly procedure.
– Z-potential: provides a control on the +/- charge alternation of the outermost layer.
– X-ray diffraction: provides information on the film structure and thickness.
– Ellipsometry: provides information on the film thickness.
– AFM, SEM, confocal microscopies: enables to get a visual representation of the film surface.

Applications

- Biosensors:
  - assembly of immunoglobulins or enzymes onto the surface of transducers.
- Biocompatible Nanofilms:
  - assembly of biomolecules onto biomaterial surfaces in order to promote specific cellular responses (nerve tissue regeneration).
- Bio/Nano-Reactors:
  - Loading of enzymes into hollow nanocapsules.
- Controlled Drug Release:
  - encapsulation of therapeutic agents for controlled and targeted release.

AFM related applications

- Sensing layers
- Characterization of mechanical properties of nanocapsules.
- TASNANO Task 3.3: Manipulation-processes at the molecular level.
  - LbL multilayers of polyoxometalates/polyelectrolytes have been prepared for studies on nanoelectrochemistry.

Conclusions on LbL films

- LbL technique allows the formation of protein/polyions multilayers in an easy and general process.
- Not only limited to planar supports.
- Promising method for molecular architecture realization.

Monolayer engineering

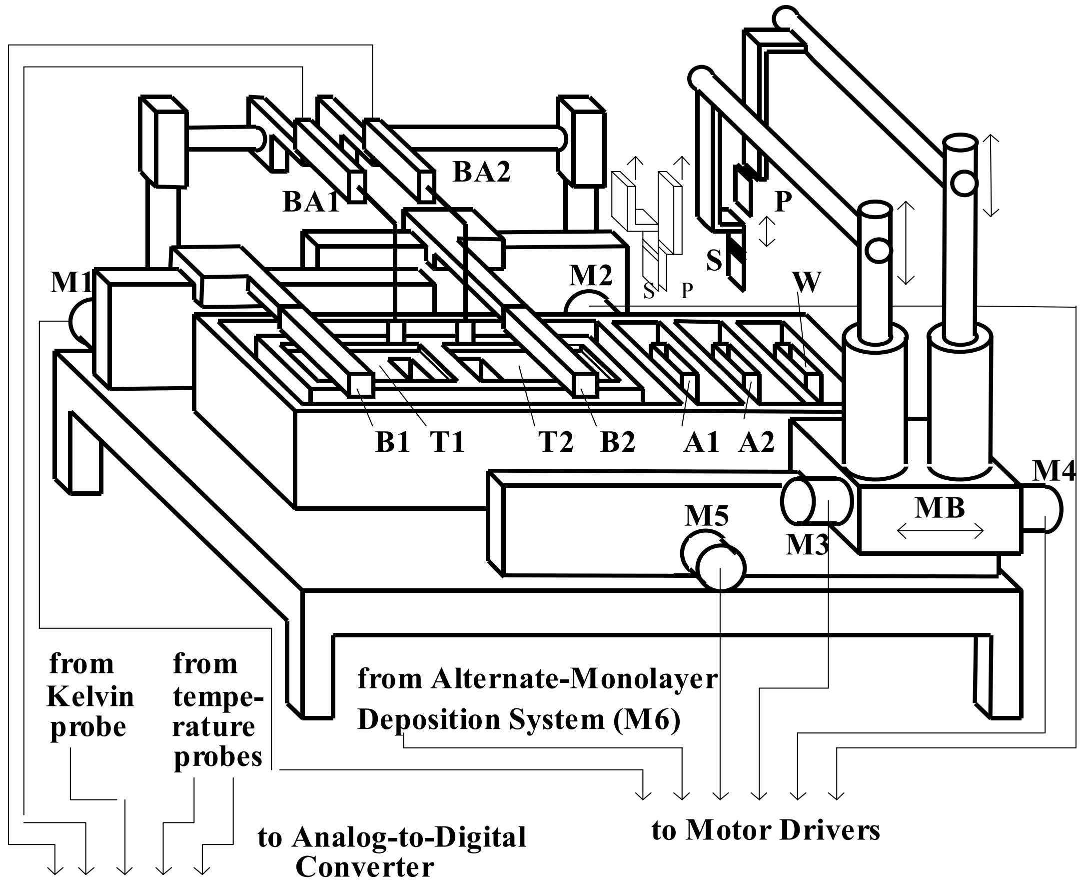
Method of immobilization at nano-scale level, which includes elements of Langmuir-Blodgett technique, self assembly and layer-by-layer techniques.

GOAL

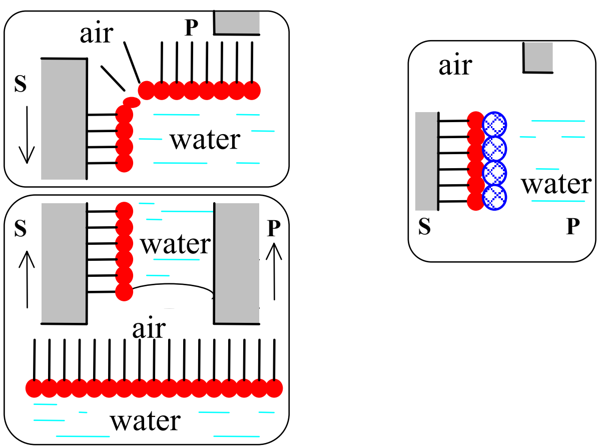
Development of materials of complex layered structure which include protein layers.

Experimental set up

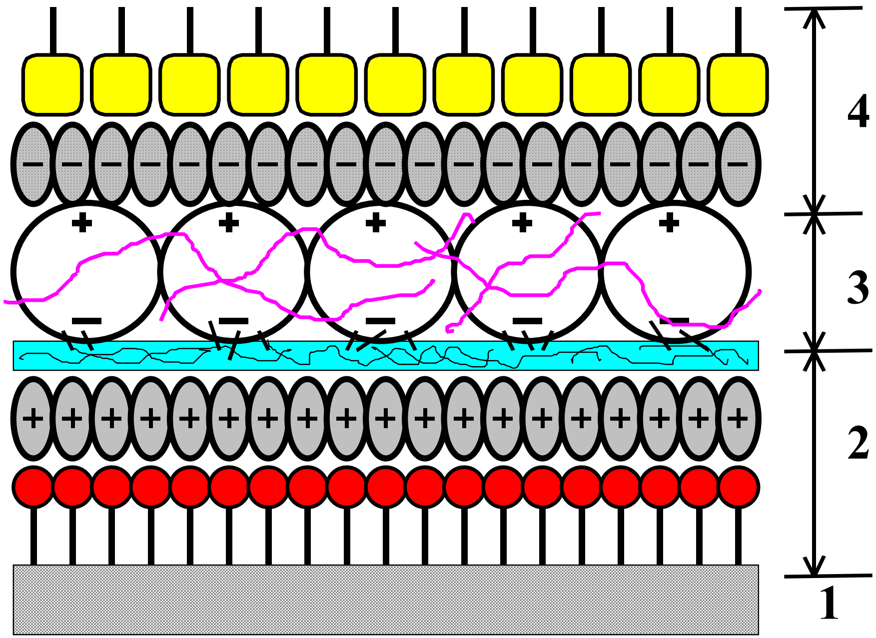
M1-M6: stepper motors; BA1, BA2: Wilhelmy balances; B1,B2: barriers; T1,T2: Langmuir troughs; A1, A2: compartments for adsorption; W: compartment for washing; S: substrate MB: mobile block



Sample holder:



Structure of the multilayer



1. Solid support.
2. Bottom layer which ensure orientation of biomolecules and high adhesion between the active layer and the support.
3. Active layer of biomolecules with stabilizing compound.
4. Protective layer.

Conclusions

Thin film techniques provide a simple method for the fuctionalization of surfaces using nanogram amount of material, giving a high level of control of the process, creating a biomimetic environment, thereby helping to stabilize biomolecules.