Biosensors and Other Applications

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Lecture: 7/7

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Outline

Now you know many techniques and fundamental theory related to surfaces.

Here we will look at some applications related to polymers on surfaces, in particular resistance to *protein adsorption*.

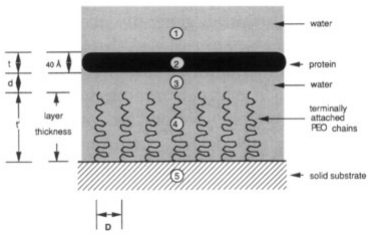
Also, the *surface force apparatus* (SFA) will be introduced.

Another interesting application that combines the previous lectures is *affinity biosensors*.

Preventing Protein Adsorption

Hydrophilic and neutral polymer brushes prevent protein adsorption.

Why this is the case has been debated for a very long time...



Jeon et al.
Journal of Colloid and Interface Science **1991**, 142 (1), 149-158.

Protein–Surface Interactions in the Presence of Polyethylene Oxide

I. Simplified Theory

S. I. JEON,[1] J. H. LEE, J. D. ANDRADE, AND P. G. DE GENNES*

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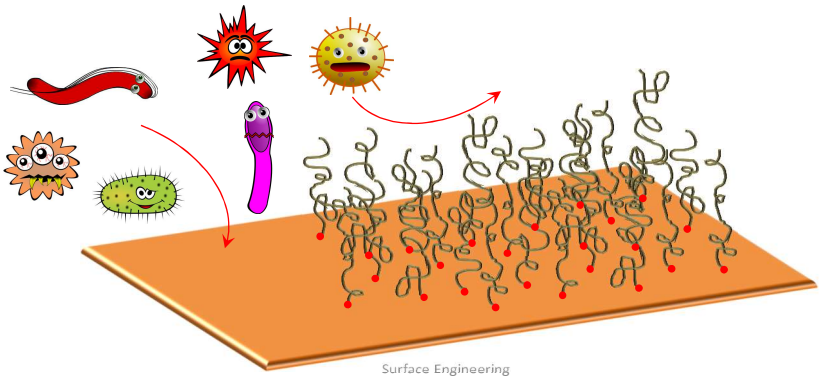
Received May 1, 1989; accepted August 21, 1990

The protein resistance character of polyethylene oxide (PEO) chains terminally attached to a hydrophobic solid substrate is theoretically studied. Steric repulsion, van der Waals attraction, and hydrophobic interaction free energies are considered. The results are dependent on the chain length and surface density of PEO. The protein approaches the PEO surface by diffusion and is affected by the van der Waals attraction between the PEO surface and protein through water. Further approach of the protein initiates the compression of PEO chains, which induces a steric repulsion effect; an additional van der Waals attraction becomes important between the substrate and protein through the water solvated PEO layer. The van der Waals component with the substrate decreases with increasing surface density and chain length of terminally attached PEO chains. Other synthetic polymers were also studied, indicating that the protein resistance character is related to the refractive index, with PEO having the lowest refractive index of the common water-soluble synthetic polymers. The osmotic and elastic constants of steric repulsion for terminally attached PEO were estimated as ~0.007 and 0.02, respectively, from literature data for PEO adsorbed to mica. The steric repulsion free energy and the combined steric repulsion and hydrophobic interaction free energies were calculated as a function of surface density and chain length of PEO. The free energy calculations as a function of surface density and chain length of PEO reveal that a high surface density and long chain length of terminally attached PEO should exhibit optimal protein resistance, with the attainment of high surface density of PEO being more important than long chain length. These theoretical results should be helpful in the design and development of materials resistant to protein adsorption. © 1991 Academic Press, Inc.

Preventing Biofouling

In biointerface science, one often wants to make surfaces inert towards protein adsorption and cell attachment.

Very important for medical applications! Even if it works, a remaining challenge is brush stability over time.



Do Proteins Adsorb on Solid Surfaces and Why?

It depends on protein type and it is far from obvious, but there are some general trends.

On hydrophobic surfaces:

• Hydrophobic interactions (with regions of the protein).

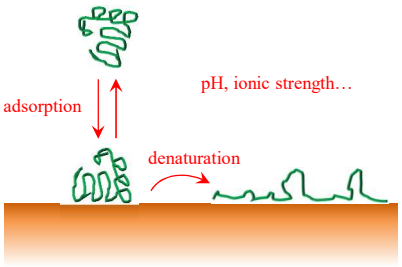
• Usually denaturation and irreversible binding.

On hydrophilic surfaces:

• Electrostatic interactions (counterion release).

• Sometimes reversible adsorption or no binding.

• Often pH dependant.



Where are the Proteins?

If adsorption still happens there are three scenarios:

• *Primary* adsorption, to the underlying surface.

• *Secondary* adsorption, on top of the brush.

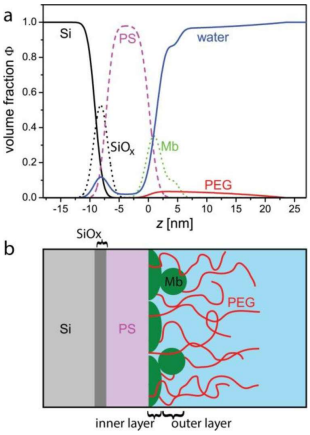
• *Ternary* adsorption, inside the brush.

Hard to distinguish from each other except by *neutron reflectometry*, a quite complicate method:

• Specular reflection of neutrons with wavelengths of a few Å.

• Momentum change from interactions with nuclei (not electrons).

• Isotope labelling can give additional information.



Schneck et al.
Langmuir **2013**, 29 (46), 14178-14187.

Probing Protein Exclusion by SPR

The SPR technique can show that proteins do not enter a brush.

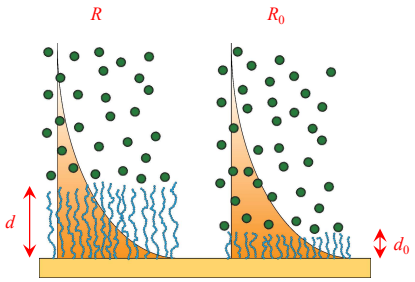
Inject high concentration of proteins and measure the response due to changes in the refractive index of the bulk liquid.

An *exclusion height* can be determined by comparing with a reference surface if the field decay length is known:

$$d = \delta \times \log\left(\frac{R_0}{R}\right) + d_0$$

Response $R < R_0$.

Schoch, Lim
Langmuir **2013**, 29 (12), 4068-4076.

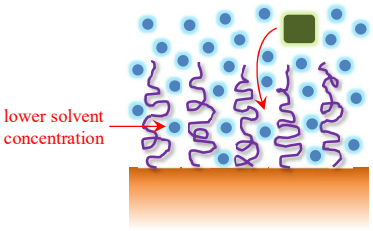


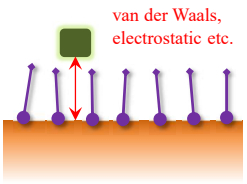
Why not Enter a Brush?

Size of particle relative to grafting density is important. It is debated which repelling effects dominate, examples are:

- Osmotic pressure.
- Conformational entropy loss of the coils (loss of available volume).
- Hydration shell around the polymers (icelike water).
- Electrostatic repulsion if brush is charged.

For very thin "brushes" (or alkanethiol monolayers), some forces may act through the layer. Attractive forces may give secondary adsorption.

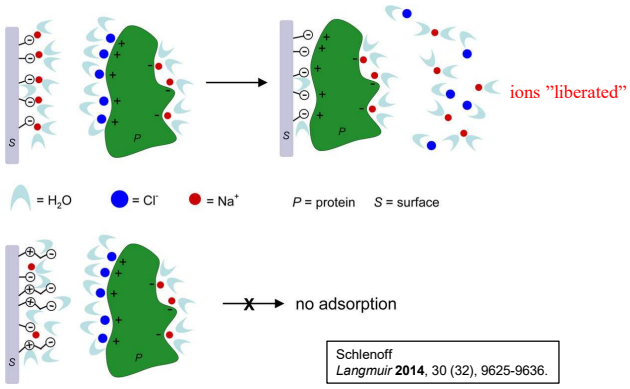


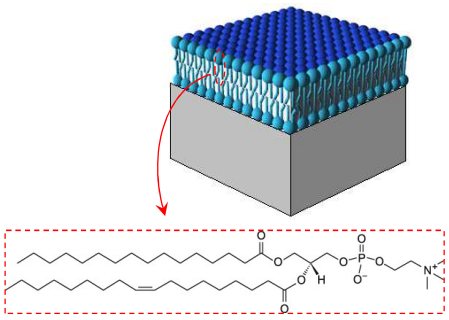


Zwitterionic Surfaces

Zwitterionic surfaces also repel proteins, even if they are very thin.

Ideal system should be a zwitterionic brush!





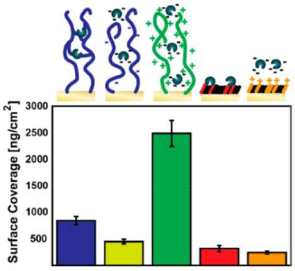
Schlenoff
Langmuir **2014**, 30 (32), 9625-9636.

Sticky Brushes

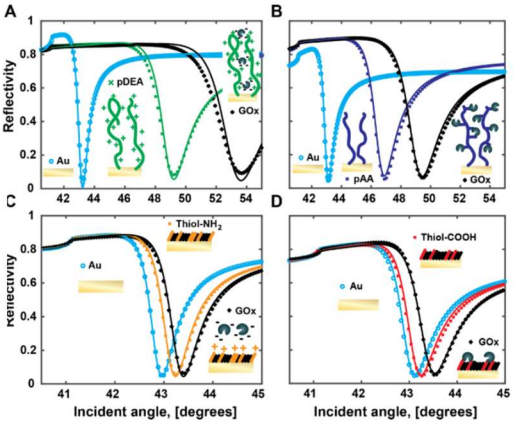
Sometimes you actually do want to bind proteins to a surface! For instance, immobilized enzymes are great for catalysis.

Brushes are good for several reasons:

- They are "soft", protein structure and activity is often preserved.
- They can bind proteins in 3D, high capacity.



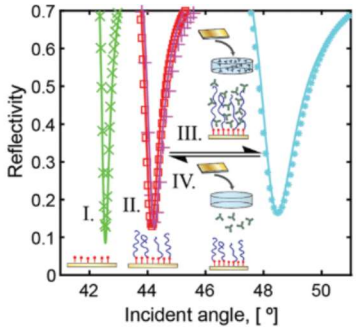
Ferrand-Drake del Castillo et al.
Langmuir **2019**, 35 (9), 3479-3489.

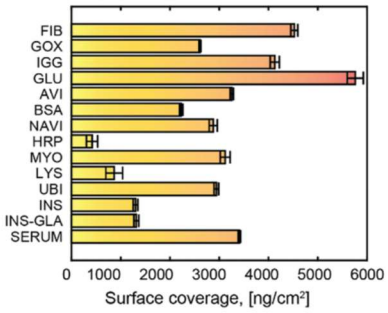


What Makes Proteins Bind?

Important favorable interactions between polymer brushes and proteins:

- Electrostatic (conventional)
- Hydrophobic (not good for structure preservation)
- Hydrogen bonds (!?)





Ferrand-Drake del Castillo et al. *Chem. Comm.* **2020**, 56 (44), 5889-5892.

Mechanical Properties of Polymer Brushes

Assume good solvent so that $H > R$ in order to minimize the free energy.

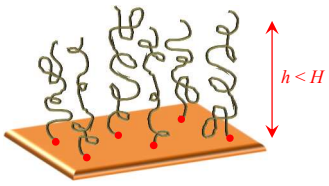
The brush is like a collection of aligned springs. A trampoline!

$$G_{\text{tot}}(h) = \frac{3k_{\text{B}}Th^2}{2Nab} + \frac{\Gamma k_{\text{B}}TN^2v}{h} + \text{constant}$$

$$F(h) = -\frac{\partial G_{\text{tot}}}{\partial h} = \frac{\Gamma k_{\text{B}}TN^2v}{h^2} - \frac{3k_{\text{B}}Th}{Nab}$$

Opposite signs for excluded volume and conformational entropy terms.

Here we assume that $h < H$ as if a blunt object tries to push down the brush.



Biltema
http://www.biltema.se/

Polymer Brushes as Actuators

What is the pressure required to compress a polymer brush by a blunt object?

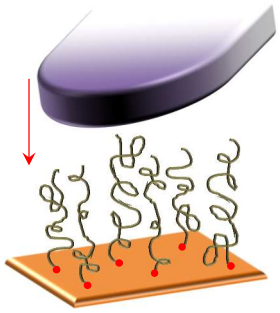
We have the force-distance relation for one coil which occupies an area $1/\Gamma$ by deriving $G(h)$. The pressure exerted is thus:

$$P(h) = F(h)\Gamma = k_B T\left[\frac{\Gamma^2 N^2 v^2}{h^2} - \frac{3h\Gamma}{abN}\right]$$

Not perfectly accurate (for instance due to monomer density profile) but gives an estimate.

Excluded volume term will dominate at small h.

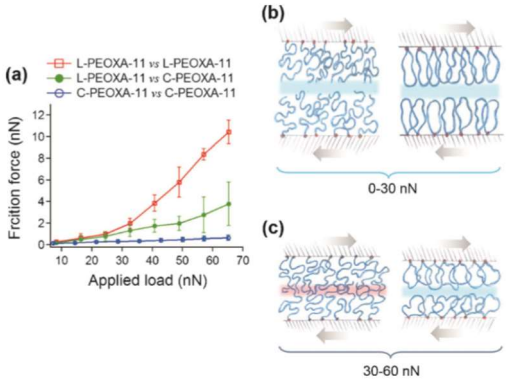
Now Γ is a much more "important" parameter, which is because it determines the volume fraction in the brush!



Polymer Brushes Reduce Friction

Since the self-repulsion is so strong, the brushes strongly reduce friction in liquid.

Ideally the chains should not intertwine.



Morgese et al.
Angewandte Chemie International Edition **2016**, 55 (50), 1-7.

Measuring Repulsion from Polymer Brushes

By atomic force microscopy in liquid one can probe the repulsive force from a brush.

Contact with brush is detected in *indentation mode*.

The solid surface can be imaged by pushing the tip through the brush.

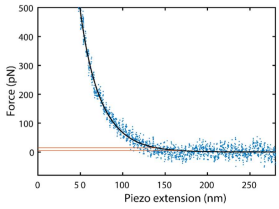


Figure S2. Example of a force-distance curve measured on a planar gold surface polymerized 2.5 min in PBS at room temperature and the fitted curve. As expected the contact point is hard to identify. The dashed lines indicate a threshold force of 5 or 15 pN.

Emilsson et al.
Applied Surface Science **2017**, 396, 384-392.

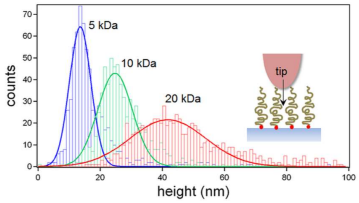
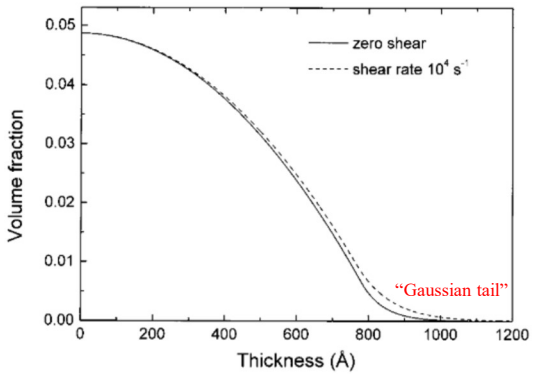


Figure 3. Atomic force microscopy histogram of measurements of brush heights for three sizes of poly(ethylene glycol) grafted in 0.9 M Na_2SO_4. The tip is pushed through the brush until it contacts the hard surface.

Emilsson et al.
ACS Applied Materials & Interfaces **2015**, 7 (14), 7505-7515.

Influence of Shear Forces?

Flow does not have a significant influence on *H* (neutron reflectivity results).

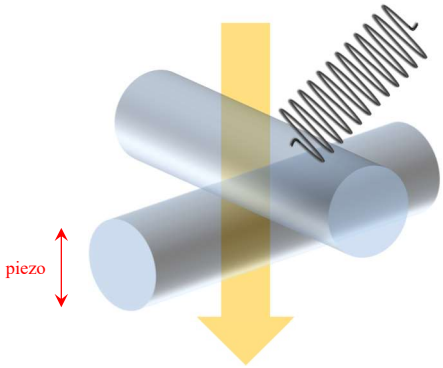


Baker et al.
Macromolecules **2000**, 33 (4), 1120-1122.

Measuring Surface Forces

In the surface force apparatus (SFA), two large cylinders are used (radius a few cm) perpendicular to each other. This is equivalent to the interaction between a sphere of the same radius and a planar surface.

- Piezo control to move one cylinder in z.
- Distance d measured by light interference.
- Mechanical spring measures force F.



SFA Pros and Cons

- Force resolution 10^{-8} N, not like AFM, but better resolution in $W(d)$.
- Distance is absolute, not relative!
- Requires very planar (on the atomic scale) surfaces.
- Only a few instruments exist in the world and they are in research labs…

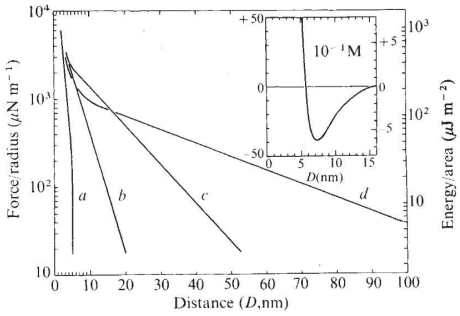
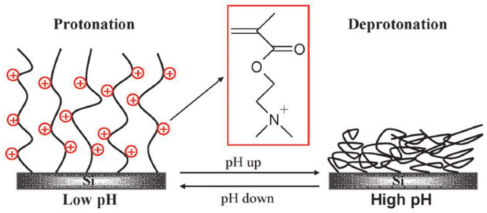


Fig. 2 Experimental results of direct measurement of force as a function of separation between two crossed mica cylinders of radius R in various KNO_3 solutions. The right-hand ordinate gives the interaction energy per unit area for two parallel plates, calculated according to the 'Derjaguin approximation'[7]. The ordinate of the inset is linear. The magnitude of the repulsive forces was found to be different for different mica samples. The results shown here are for those samples in which the repulsion was relatively weak and where a secondary minimum was clearly observed. The scatter in the experimental points is ~ 10 μN m^{-1} for the force/radius. *a*, 10^{-1} M; *b*, 10^{-2} M; *c*, 10^{-3} M; *d*, 10^{-4} M.

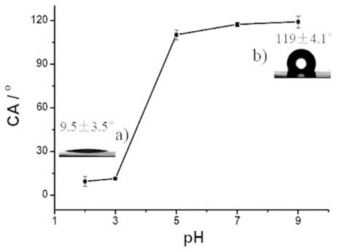
Israelachvili, Adams
Nature **1976**, 262 (5571), 774-776.

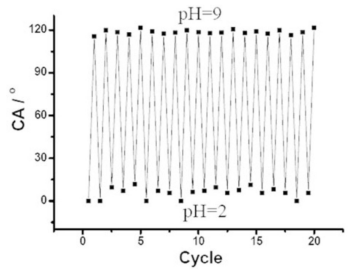
Responsive Interfaces

Polymers that undergo coil-globule transitions in response to environmental changes are called responsive polymers. They can be used as sensors or actuators and for "switchable" properties in general.



Zhang et al.
Chemical Communications **2008**, (10), 1199-1201.





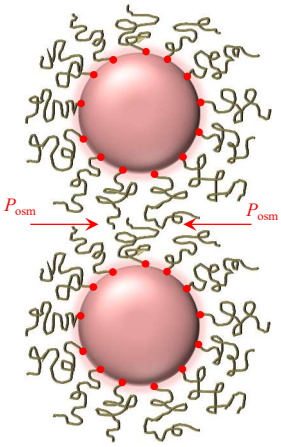
Polymers on Colloids

Colloids tend to aggregate due to van der Waals attraction etc.

Polymers grafted to the colloids can stabilize a suspension!

However, if the solvent is poor for the polymer, bridges are formed and aggregation is promoted.

Flocculation and sedimentation can be induced.



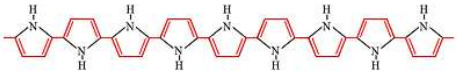
Organic Electronics

Electronic components on chips like light-emitting diodes and transistors can now be made of polymers!

Polymers containing delocalized (π-conjugated) electrons are central because they conduct electricity. Note that this does not necessarily mean aromatic compounds (as in having benzene rings).

The films can be prepared by *electropolymerization* directly on a conducting surface.

Since the polymers are soft they are excellent for flexible electronic devices!



polypyrrole

Video: Acreo Display

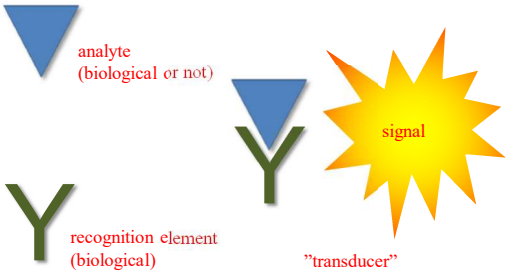
Display made of polymers (no metals or semiconductors).

What is a Biosensor?

One definition can be found in the handbook from the International Union for Pure and Applied Chemistry (IUPAC):

A device that uses specific biochemical reactions mediated by isolated enzymes, immunosystems, tissues, organelles or whole cells to detect chemical compounds usually by electrical, thermal or optical signals.

The use of recognition elements (or *receptors*) is sometimes referred to as ”affinity based” biosensing.

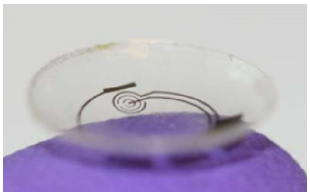


Biosensors in Everyday Life

Not many! New biosensor technologies are more common in research environments.

Glucose sensor changed the life of diabetes patients. By some considered to be the only truly successful biosensor and still developing. Quantitative!

Pregnancy tests (a *lateral flow assay*) detect human chorionic gonadotropin from urine. (Also for ovulation.) Qualitative!



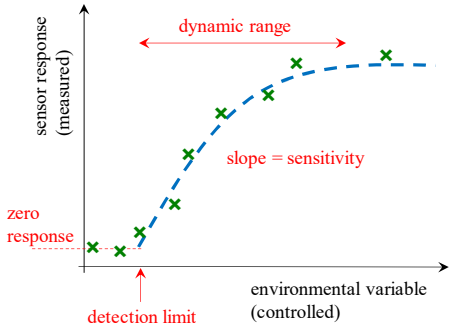
Yao et al.
Biosensors and Bioelectronics **2011**, 26 (7), 3290-3296.

Clearblue
http://www.clearblue.com/

Sensor Terminology

Most sensors, not the least biosensors, need to *calibrated*. In a calibration experiment the response to known doses of the variable of interest is measured.

The *sensitivity*, *dynamic range* and *detection limit* are defined from the calibration curve.

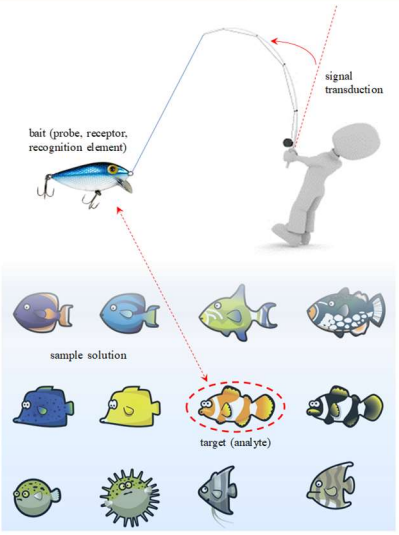


The Challenge of Specificity

Most biosensors need to operate *label-free* to be useful. This means that they work even if the analyte does not carry any artificial label.

When operating label-free, the biggest problems in biosensor technology is arguably false positive results.

When we search for analytes in biological samples we will always have a lot of other molecules present that can interfere with the detection.



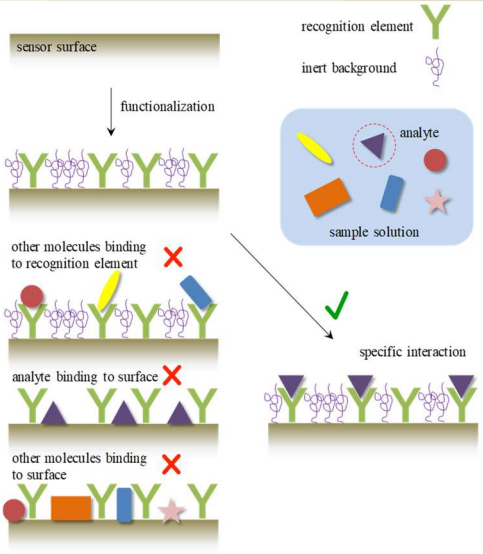
Label-Free Surface-Based Detection

The surface must be chemically functionalized such that only the analyte binds.

Polymer brushes are great for an inert starting point.

Then introduce receptors.

Then use the generic surface sensitive techniques!



Video: SPR Biosensor

Dedicated to biomolecular interaction analysis in controlled environments.

Know Your Techniques!

Sometimes things go wrong when researchers use machines they know nothing about…

Review

Journal of Molecular Recognition

Received: 23 February 2011, Accepted: 25 February 2011, Published online in Wiley Online Library: 2011

(wileyonlinelibrary.com) DOI: 10.1002/jmr.1138

Survey of the 2009 commercial optical biosensor literature

Rebecca L. Rich[a] and David G. Myszka[a]*

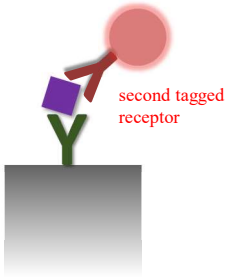
We took a different approach to reviewing the commercial biosensor literature this year by inviting 22 biosensor users to serve as a review committee. They set the criteria for what to expect in a publication and ultimately decided to use a pass/fail system for selecting which papers to include in this year's reference list. Of the 1514 publications in 2009 that reported using commercially available optical biosensor technology, only 20% passed their cutoff. The most common criticism the reviewers had with the literature was that "the biosensor experiments could have been done better." They selected 10 papers to highlight good experimental technique, data presentation, and unique applications of the technology. This communal review process was educational for everyone involved and one we will not soon forget. Copyright © 2011 John Wiley & Sons, Ltd.

Rich, Myszka
Journal of Molecular Recognition **2011**, 24 (6), 892-914.

Sandwich Assays

Use secondary receptor and signal amplification post binding.

Improves detection limit, but excludes real time analysis.



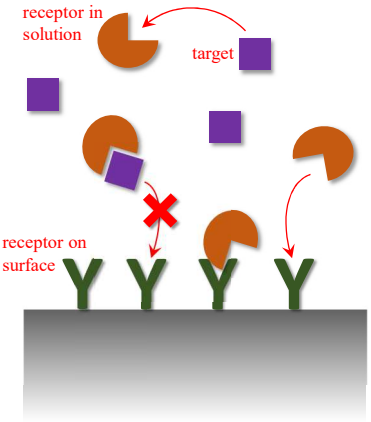
Competitive Assays

Measure the reduction in receptor binding to the surface, which contains a receptor for the receptor.

Target binding to the receptor in solution blocks binding site for receptor on surface.

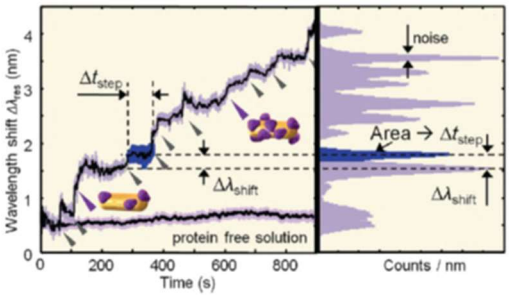
Excellent for small molecules and interaction occurs in solution!

But again no k_{on} and k_{off}!

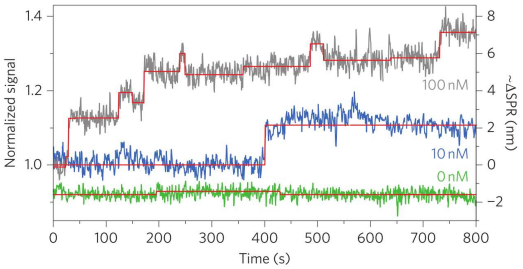


Resolving Single Molecules

Remember the plasmonic nanoparticles. Large proteins adsorbing directly on nanoparticles can be detected individually.



Ament et al.
Nano Letters **2012**, 12 (2), 1092-1095.



Zijlstra et al.
Nature Nanotechnology **2012**, 7 (6), 379-382.

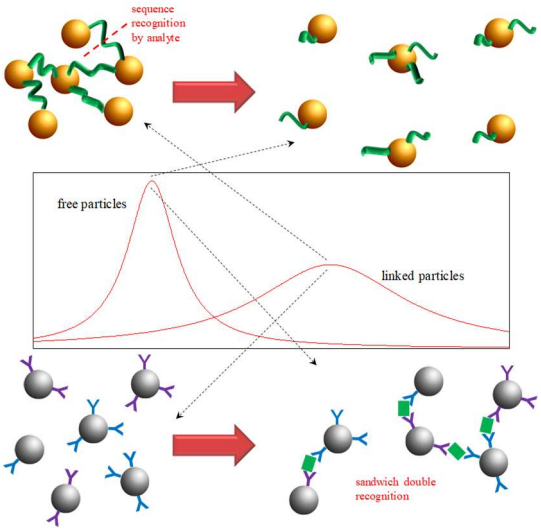
Cool, but is it useful?

Detection Based on Particle Coupling

Nanoparticles close to each other will change their plasmon resonances.

The spectral changes are much larger and the sensitivity is higher.

However, this requires that the analyte either cleaves the link between particles or couples them together.



Other Uses of Nanoparticles

Gold and silver nanoparticles have been used throughout history for “improving health”.

Also, they are great labels since they do not bleach!

Wikipedia: *Argyria*

Checklist 7

- Preventing biofouling
- Protein-polymer interactions
- Responsive polymer brushes
- Mechanical properties of polymer brushes
- Surface force apparatus
- Biosensors
- Applications of nanoparticles in biology

Exercise 7.1

A biosensor uses antibodies on a surface to capture a target from solution. Assume equilibrium is established with C_0 ten times lower than K_D. The sensor response is translated to $\Gamma = 10$ ng/cm^2 and the analyte is a protein with mass 40 kg/mol. What is the area occupied by one antibody? Is it a densely packed layer?

→

Each receptor occupies 61 nm^2 which is a quite dense but not unreasonable antibody layer considering their size.