Plasmonic Biosensors

Outline

About plasmonic biosensors:

• What is a surface-based biosensor?

• Plasmons in nanoparticles and on surfaces.

• Examples of plasmonics in biotechnology.

The connection to binding kinetics to surfaces: We can now measure $J$!
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

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Clearblue

http://www.clearblue.com/
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.

Sensor Terminology

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 (fish) in biological samples we will always have a lot of other molecules (fishes) present that can interfere with the detection.
Detect anything that binds to a surface! Several instruments exist, most are based on optical measurements, some mechanical techniques and also a few other.

In this course you will learn about two techniques: Surface Plasmon Resonance (SPR) and Quartz Crystal Microbalance (QCM).

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**Surface Sensitive Techniques**

**Know Your Techniques!**

You will learn the physics behind the techniques. The instruments will not be “black boxes” to you when you encounter them in the future!

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

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Survey of the 2009 commercial optical biosensor literature

Rebecca L. Rich1 and David G. Myszka2*

We took a different approach to reviewing the commercial biosensor literature this year by inviting 22 biosensor users to serve as a cadre of editors. They set the criteria for what to expect in a publication and ultimately decided on a pass/fail system for selecting which papers to include in this year’s survey list. Unlike (unpublished) a 2009 paper reporting on commercially available optical biosensor technologies, this 2010 report does not cover all of the technology. This year we decided to focus our attention on the numbers and what we could do better.” They selected 10 papers to highlight good experimental technique, data presentation, and unique applications of the technology. This continues the review process we described in the 2009 survey and we will not soon forget. Copyright © 2011 John Wiley & Sons, Ltd.
The surface must be chemically functionalized such that only the analyte binds.

The techniques work in real-time, which gives information about binding kinetics.

Optical techniques like SPR often enable multiplexing, detection of multiple targets, by imaging mode.

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**Biosensor Scenarios**

*In vivo*: Inside the living organism. Not for plasmonic biosensors!

*In situ*: Biological sample analyzed in artificial setting. Possible but difficult!

*In vitro*: Artificial setting, bottom-up synthetic biology. Perfect!
Implications from Binding Kinetics

Remember the models for binding kinetics to surfaces! Let the A molecules represent receptors and the B molecules targets (analytes). \( A + B \rightarrow AB \)

If the surface is a sensor that gives a measurable response proportional to \( \Gamma \):

- Real-time operation needed to determine \( k_{on} \) and \( k_{off} \) and to confirm equilibrium. Measuring the equilibrium coverage gives only \( K_D \) from \( C_0 \) or vice versa.

- Diffusion models can estimate performance limits: A molecule can diffuse to the sensor surface without binding to it, but not bind to the surface without first diffusing to it.

- Flow is often needed to prevent diffusion effects and reach Langmuir behavior.

- Small sensors give a higher diffusive flux due to edge effects. But the capture efficiency is poor under flow (many molecules will be lost).

What is a Plasmon?

Physical understanding: Plasmons are collective oscillations in the free electrons of metals.

Mathematical understanding: Plasmons are solutions to Maxwell's equations for certain metal-dielectric geometries.

For a metal nanoparticle, the polarization enhanced at certain frequencies of light. For noble metals, this occurs for visible light, which gives strong colors.

The Lycurgus Cup is the oldest example (year ~400) of this kind of staining.
Absorption and Scattering

The polarizability ($\alpha$) of the particle determines the absorption and scattering cross sections ($\sigma$). The extinction is the sum of absorption and scattering.

For gold nanoparticles (small and spherical):
- **Blue** light is absorbed (true for all gold).
- **Green** light is absorbed and scattered (by the plasmon).
- **Red** light is transmitted (low extinction).

$$\sigma_{\text{ext}} = k \, \text{Im}(\alpha)$$
$$\sigma_{\text{sca}} = \frac{k^4}{6\pi} |\alpha|^2$$
$$\sigma_{\text{abs}} = \sigma_{\text{ext}} - \sigma_{\text{sca}}$$

Here $k$ is the incident wavevector ($k = \frac{2\pi}{\lambda}$) and $\alpha$ is defined as a volume. The cross sections are areas (shadows)!

Electrostatic Approximation

By considering the electric field of light as static, the polarizability can be calculated easily for a sphere:

$$\alpha_0 = 3V \left( \varepsilon(\lambda) - \varepsilon_{\infty} \right) \frac{\varepsilon_{\infty}}{\varepsilon(\lambda) + 2\varepsilon_{\infty}}$$

This is valid for particles that are small compared to the wavelength of light (<50 nm).

The symbol $\varepsilon$ represents (relative) permittivity at optical frequencies.

For simple transparent materials like water, $\varepsilon$ is roughly the square of the refractive index ($\varepsilon = n^2$).

For metals, the permittivity is complex (energy absorption) and dispersive ($\lambda$ dependent).

[Diagram of geometric cross section areas]
The Optical Near-Field

The absorption and scattering cross sections correspond to the “far field” properties of the nanoparticles, i.e. they describe what happens to a light wave that passed the particle.

One can also talk about the “near-field”, i.e. the local electromagnetic field distribution on the nanoscale. This is very important when using the particles as biosensors!

The field is strongest at the metal and typically extends a distance approximately equal to the radius of the particle.

Electrostatic theory can be used to calculate the near field as well:

\[
\vec{E}(x, y, z) = E_0 \left[ \frac{x}{r} - \text{Re}\left(\frac{3x}{r} \left(\frac{x}{r} y + y z\right)\right) \right]
\]

Surface Plasmons

Surface plasmons are similar to ordinary light (electromagnetic plane wave) but confined to the interface between a metal (conductor) and a dielectric (insulator).

The surface plasmon propagates along the interface (like a wave on a water surface). Eventually the wave energy has dissipated (normally by heating the metal).

The wave must have transverse magnetic polarization.
The Dispersion Relation

There exists a relation between the momentum and energy of a surface plasmon. In contrast to nanoparticles, surface plasmons exist in a continuum of frequencies!

Problem: Momentum is always lacking for the incident light! The dispersion relation does not cross that of photons in the dielectric medium!

Excitation of Surface Plasmons

The additional momentum needed can be added in several ways.

One is to introduce a periodic grating on the surface. The grating wavevector adds to that of the incident photons. The condition for excitation is:

\[ \text{Re} \left( \frac{\varepsilon(\lambda_n) k_m}{\varepsilon(\lambda_n) + \varepsilon_m} \right) = n_m \sin(\theta) + j \frac{j \lambda_n}{A} \]

One can also use a high refractive index material, a thin metal film and total internal reflection configuration. The resonance condition is then:

\[ \text{Re} \left( \frac{\varepsilon(\omega) k_m}{\varepsilon(\omega) + \varepsilon_m} \right) = n_t \sin(\theta) \]
**Total Internal Reflection**

When measuring the reflected light one sees a minimum, representing SPR! One can vary either the angle or the wavelength of incident light (spectroscopy at fixed angle).

![Graph showing Fresnel coefficients](image)

- $\theta_0 \approx 630 \text{ nm}$
- $\lambda_{SP} = 4 \text{ nm}$

**Surface Plasmon Near-Field**

At any time, the surface plasmon has negative and positive poles on the metal surface.

The electric field has two components in the $xz$ plane. Magnetic field only in $y$.

The time-averaged field only depends on the distance from the surface due to symmetry.

The field extends approximately half of the wavelength of light used to excite the surface plasmon.
Checklist

You do not have to learn any equations for calculating stuff related to the optics, such as how to predict the resonance of a certain nanoparticle etc. However, you need to understand the basic physics.

Checklist of some important concepts and the differences between nanoparticle and surface plasmons:

• Electrostatic approximation.

• Extinction, absorption and scattering cross sections.

• Dispersion relation and momentum of incident light.

• How to excite plasmons by light and spectroscopy methodology.

• Electromagnetic field extension, difference between near-field and far-field.

Biosensing with Plasmonics

The most common plasmonic biosensor principle is refractometric detection:

• When a molecule binds to the surface, the refractive index changes. All molecules of interest have a refractive index which is higher than water.

• The properties of the plasmon are changed because they depend on the refractive index close to the metal.

• By optical spectroscopy, changes in intensity of light for different wavelengths can then be detected. The resonance shifts in the spectrum.

This holds both for surface plasmons (the SPR technique) and nanoparticle plasmons (lab exercise 2).
Commercial SPR

- First paper published in 1983.
- Pharmacia Biosensor started shortly after. Became Biacore later, which is now part of GE Healthcare.
- SPR is now the most established biosensor technology for studying biomolecular interactions.
- But not used for medical diagnostics...
- The instruments are expensive, but primarily because they contain efficient liquid handling and temperature stabilization etc. SPR can be cheap!

Liedberg et al.
Sensors and Actuators 1983

SPR Imaging

SPR can be operated in imaging mode which enables multiplexing: Several interactions can be probed simultaneously using the same sample solution.

One molecule in solution: See how it interacts with different receptors on different spots. Ideal for proteomics and in principle for drug development.

Several molecules in solution: Detect the presence of multiple analytes in one sample using different recognition elements. High risk of problems from nonspecific binding.
Other Optical Techniques

Plasmons is definitely not the only way to go!

- Ellipsometry (often for air environment).
- Optical waveguide lightmode spectroscopy.
- Dual polarization interferometry.

Very similar since all are based on refractometric detection!

Importance of Field Extension

If the electromagnetic field extends very little, molecules far away will not be detected.

If the field extends far, only a part of the detection capability is utilized and the system becomes more sensitive to “bulk effects” far away from the surface.
Miniaturized Nanoparticle Sensors

If SPR is so great, why bother with plasmonic biosensors based on nanoparticles?

One reason is that people want to resolve individual molecules binding to the surface.

SPR is hard to make sufficiently small, but through measurements on single nanoparticles one can perhaps reach single molecule resolution?

Resolving Single Molecules

Very large proteins adsorbing directly on the surface.

Help of complementary techniques can make it slightly better…

Cool, but is it useful?
**Sandwich Assays**

Use secondary receptor and signal amplification post binding.

Improves detection limit, but excludes real time analysis to get $k_{on}$ and $k_{off}$.

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**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 $k_{off}$!
Detection Based on Particle Coupling

Instead of detecting changes in refractive index on the metal surface, one can utilize the fact that nanoparticles close to each other will change their plasmon resonances.

In general, the spectral changes are much larger and the sensitivity is better.

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!
Reflections & Questions

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