Supplementary information for:

Nanobiosensors for therapeutic drug monitoring

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Supplementary information:

Surface plasmon resonance (SPR)

An incident light is directed onto the metal film through a prism at an angle that allows total internal reflection (Figure 2, left). If all the conditions are met, including p-polarization, negative real dielectric constant and small imaginary dielectric constant for the metal, [1, 2] then, at a given angle-wavelength pair, the light wavevector (Equation SI1) can be equal to the wavevector of the oscillating electrons of the metal film (plasmons) (Equation SI2) resulting in resonance of the surface plasmons. [2]

$$k_x = \frac{2\pi}{\lambda}\eta_D sin(\theta) \quad \text{(SI1)}$$

$$k_{sp} = \frac{2\pi}{\lambda}\sqrt{\frac{\varepsilon_m(\lambda)\varepsilon_s(\lambda)}{\varepsilon_m(\lambda) + \varepsilon_s(\lambda)}} \quad \text{(SI2)}$$

This will be read at the detector by a loss in reflectance at a certain angle-wavelength pair, which will give the characteristic SPR band (Figure 2, right). In SPR biosensing, when a recognition event occurs between a receptor bound to the metallic surface and its specific target, the change in the refractive index near the metal film will affect the oscillating plasmons and lead to a change in the absorption wavelength. This shift is directly proportional to the quantity of material adsorbed onto the surface and can therefore be used for precise quantification of an analyte.

Localized surface plasmon resonance (LSPR)

As with SPR sensing, the wavelength shifts in LSPR remains proportional to the size of the molecule (or the thickness of the adsorbed layer) as shown in Equation SI3 where m is the sensitivity of the nanoparticles (bulk refractive index response), Δn is the change in the refractive index due to the adsorption of a molecule on the surface of the nanoparticle, d is the effective thickness of the adsorbed molecule and l_d is the decay length of the electromagnetic field.

$$\Delta\lambda_{max} = m\Delta n\,[\,1 - e^{-2d/l_d}\,] \qquad \text{(SI3)}$$

Although this equation gives a good approximation of the relationship between LSPR wavelength and field decay, it does not take into account the variation in the electromagnetic field when the size and shape of the nanoparticles differ from a perfect sphere. It is therefore important to remember when using LSPR that the plasmon band wavelength can be tuned by changing the size and the shape of the nanoparticles and that these changes can affect the sensitivity of the technique.[3]

Surface enhanced Raman scattering (SERS)

The SERS phenomenon is generally accepted to be the result of both electromagnetic and chemical enhancement mechanisms. [4] Since Raman scattering intensity is proportional to the electrical field, excitation of the localized plasmons of a nanostructure will produce an amplification of 10^4 of the Raman signal. The extinction spectrum of an arbitrarily shaped nanoparticle is described by Equation SI4, where ε_{out} is the dielectric constant of the external

environment, ε_r and ε_i are the real and imaginary components of the dielectric constant of the metal nanoparticle ε_{in}, and χ is the shape factor that accounts for any difference from the spherical geometry of a nanoparticle.

$$E(\lambda) = \frac{24\pi^2 N a^3 \varepsilon_{out}{}^{3/2}}{\lambda \ln(10)} \left[\frac{\varepsilon_i(\lambda)}{(\varepsilon_r(\lambda) + \chi\varepsilon_{out})^2 + \varepsilon_i(\lambda)^2} \right] \quad \text{(SI4)}$$

Additional amplification (~10^2) can be attributed to the localized electronic resonance of the molecule adsorbed onto the Raman substrate or to the resonance of the charge transfer between the metal substrate and the adsorbed molecule. [4] Thus, to have an efficient SERS substrate, the material must support a surface plasmon resonance, have as many SERS active sites as possible and have a high electromagnetic field enhancement. It is also worth noting that the SERS phenomenon is distance dependant and that the analyte such as therapeutic drugs does not have to be in direct contact with the SERS surface, but must be close enough to benefit from the surface enhancement. The SERS intensity distance dependence is described by Equation SI5 [4] where a is the average size of the enhancing metallic feature and r the distance between the analyte and the metallic surface. Thus, any biosensing scheme involving SERS must favour the capture of analyte as close as possible for direct detection. Otherwise, dimerization or aggregation assays must be considered to generate a large SERS response from an analyte.

$$I_{SERS} = \left(\frac{a + r}{a}\right)^{-10} \quad \text{(SI5)}$$

Förster resonance energy transfer (FRET)

The efficiency of the energy transfer depends on the distance between the donor and the acceptor and is described by Equation SI6 where R_0 is the Förster distance (energy transfer probability of 50% at that distance) and R is the distance between the donor and the acceptor when the transfer occurs.

$$E = \frac{R_0^6}{R^6 + R_0^6} \quad \text{(SI6)}$$

This technology can be used to facilitate the study of living cells in their environment, in reaction to an anticancer drug for example, by building biosensors with fluorescent proteins. The

ability to study cells in their natural environment is crucial to better comprehend the pharmacodynamics and effect of a drug on specific cells and ensure optimal treatment conditions or to improve our understanding of disease progression. [5, 6] The advantage of using a fluorescent protein over a fluorescent organic molecule is that the protein can easily be expressed in cells and is stable in this environment, allowing the study of cells without disturbing their natural surroundings. [5]

There are two types of FRET sensors: intermolecular and intramolecular. Aoki et al. [5] have discussed in detail the process of developing FRET biosensors and their principles, as illustrated in Figure 4. Briefly, a FRET sensor is composed of one energy donor and one energy acceptor, that can be attached or not by a linker. The energy donor must have an emission spectrum overlapping the excitation spectrum of the acceptor. When the donor and the acceptor are brought into close contact, the energy transfer occurs and the emission wavelength measured now corresponds to the emission wavelength of the acceptor, contrary to the donor emission wavelength before the merger.

Quartz crystal microbalance (QCM)

These frequency changes are measured by an oscillator, which is connected to a frequency counter connected to a computer. Equations SI7 and SI8 [7] describes the relationship between the change in the oscillating frequency of the crystal and the quantity of material adsorbed on the surface where Δf is the resonance frequency change (Hz), C_f is the integrated QCM/mass sensitivity, $f_0$is the fundamental frequency of an AT-cut crystal (Hz), Δm is the change in mass (g), A is the area of the electrode, $\Delta\eta_L$ is the viscosity of the liquid and $\Delta\rho_L$ its density, μ_q is the shear modulus of an AT-cut quartz crystal and ρ_q its density.

$$\Delta f = C_f f_0^2 \frac{\Delta m}{A} + C_f f_0^{3/2} (\Delta\eta_L \Delta\rho_L)^{1/2} \quad \text{(SI7)}$$

$$C_f = \frac{-2}{(\mu_q \rho_q)^{1/2}} \quad \text{(SI8)}$$

This equation describes measurements that are done in the liquid phase, which has recently been developed since older measurements using QCM could only been done in the dry phase.[7] This technique is very sensitive, which implies that the crystal has to be perfectly polished to prevent false signals due to the solution entering asperities in the surface. [7] The laminar flow of the solutions in contact with the microbalance also has to be optimized to minimize mechanical perturbations that can affect the frequency of the sensor. Also, it is sensitive to nonspecific binding since it responds to mass adsorption, which is a universal characteristic.

References

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