Supporting Information

Integration of Quartz Crystal Microbalance-Dissipation and Reflection-Mode Localized Surface Plasmon Resonance Sensors for Biomacromolecular Interaction Analysis

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**Experimental Section**

**Vesicle Preparation.** Vesicles composed of 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) (Avanti Polar Lipids, Alabaster, AL, USA) were prepared by the extrusion method\(^1\). Briefly, lipids dissolved in chloroform were treated with a stream of nitrogen gas to form a dried lipid film. The film was rehydrated in aqueous buffer solution (10 mM Tris [pH 7.5] with 150 mM NaCl) at a nominal lipid concentration of 5 mg/mL, followed by vortexing. Extrusion was then performed using 30-nm diameter track-etched polycarbonate membranes. Vesicles were diluted in buffer solution immediately before experiment and used within 24 h of preparation. All aqueous solutions and buffers were prepared with Milli-Q-treated water with a minimum resistivity of 18.2 MΩ·cm (Millipore, Billerica, MA, USA).

**Dynamic Light Scattering.** Dynamic light scattering (DLS) was performed on a 90Plus Particle Size Analyzer and the results were analyzed using digital autocorrelator software (Brookhaven Instruments Corporation, New York, USA). All measurements were taken at a scattering angle of 90° where the reflection effect is minimized.

**Additional Details of Measurement Operation.** The combined QCM-D and LSPR setup is comprised of the Q-Sense E1 system (Biolin Scientific, Stockholm, Sweden) together with the Insplorion Acoulyte (Insplorion AB, Gothenburg, Sweden), which provides an optical connection between the QCM-D measurement chamber and the Insplorion X-Nano optics unit (Insplorion AB, Gothenburg, Sweden). Measurements were performed on the Acoulyte quartz crystal sensor, which is a modified version of the regular Q-Sense quartz crystal sensor whereby the top electrode is coated with a silicon dioxide spacer layer on which randomly distributed gold nanodisks (height and diameter of \(~20\) and \(~100\) nm, respectively) were fabricated by hole-mask colloidal lithography\(^2\) and sputter-coated with a thin layer of titanium oxide (thickness \(~10\) nm). The data was collected at multiple odd overtones, and the QCM-D signals presented in this work.
were collected at the 5th (n=5) odd overtone and normalized according to the overtone number. Prior to use, the sensor was soaked in a 1% v/v sodium dodecyl sulfate (SDS) solution for 30 min, and then rinsed with water and ethanol, respectively. After drying with a stream of nitrogen gas, the sensor was cleaned using an oxygen plasma cleaner for at least 30 s, before immediately fixing the treated sensor chip in the Q-Sense QWM401 window module, which was then mounted into the E1 chamber. The solution outlet was connected to a Reglo Digital peristaltic pump (Ismatec, Glattbrugg, Switzerland) in order to control the introduction of fluid samples. Samples were introduced under continuous flow at a flow rate of 100 µL/min. The window module provides optical access to the sensor via the branched fiber probe of the Acoulyte adaptor, which is comprised of connectors to the lamp and the spectrometer (both contained within the Insplorion X-Nano optics unit), which join to form the probe end of the fiber.

**Data Analysis.** QCM-D data analysis was performed using the Voigt-Voinova model available on the Q-Tools software package (Biolin Scientific). For the model fitting, the thickness and effective acoustic mass of the adsorbed vesicle layer and SLB were calculated by assuming the film density to be 1000 kg/m³ and the viscosity of the bulk aqueous solution to be 0.001 Pa/s. The LSPR data analysis was performed with the Insplorer software package (Insplorion AB). The time resolution was 1 Hz. The spectral resolution of the plasmon resonance was determined by high-order polynomial fitting, and the centroid position, which is denoted as the LSPR peak position in this work, was calculated from the fit. The effective optical mass \( \Gamma_{\text{LSPR}} \) was calculated based on the Lorenz-Lorentz relation, by using the following equation:

\[
\Gamma_{\text{LSPR}} = \frac{3\tau(n_{\text{film}} - n_{\text{buffer}})(n_{\text{film}} + n_{\text{buffer}})}{(n_{\text{film}}^2 + 2)[\tau(n_{\text{buffer}}^2 + 2) - \nu(n_{\text{buffer}}^2 - 1)]}
\] (1)

where \( \tau \) is the film thickness obtained from Voigt-Voinova model fitting, \( n_{\text{film}} \) is the average refractive index of the film, and \( n_{\text{buffer}} \) is the refractive index of the buffer. The latter was
measured using an Abbe refractometer and was determined to be 1.336. The specific refractivity, $r$, and specific volume, $v$, of the adsorbed layer were taken to be 0.286 cm$^3$/g and 0.98 cm$^3$/g, respectively$^5$. The average refractive index of the film $n_{\text{film}}$ was determined by using the following equation

$$n_{\text{film}} = \frac{(n_{\text{eff}} - n_{\text{buffer}})}{1 - e^{-t/L}} + n_{\text{buffer}}$$

(2)

where $n_{\text{eff}}$ is the effective refractive index within the sensing volume and $L$ is the decay length of the LSPR field intensity (i.e., sensing penetration depth), both of which were obtained using the following equation

$$\Delta \lambda = S(n_{\text{eff}} - n_{\text{buffer}}) = S[1 - e^{(-t/L)}](n_{\text{SLB}} - n_{\text{buffer}})$$

(3)

where $S$ is the bulk refractive index sensitivity of the sensor, experimentally determined to be 150 nm/RIU, and $n_{\text{SLB}}$ is the refractive index of the SLB, previously reported to be 1.45$^5$. 

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References