Characterization of micrometer thick layers of spin coated chitin

Multi-Parametric Surface Plasmon Resonance (MP-SPR) instruments can measure optical properties and address surface interactions from very thin films (down to Ångstroms) up to micrometer thick layers. The measurement of thicker sensor coatings is based on a SPR waveguide mode.

Micrometer thick chitin layers were spin coated onto gold (Au) and polystyrene (PS) substrates and subsequently assessed through MP-SPR waveguide signal. Thickness and refractive index (RI) of chitin layer were determined in dry and liquid conditions using the same instrument set-up (Figure 1). The polymer thickness was almost 2 µm in air but less than 200 nm in buffer and in addition it was thicker on gold when compared to polystyrene. The affinity constant of a chitin binding domain of Bacillus circulans (CBD-IN$_N$) was determined and evidenced to be higher on chitin deposited on the polystyrene-coated sensor when compared to chitin

Introduction

After cellulose, chitin is the second most abundant biopolymer in nature. As a renewable resource, it has gained popularity as a biomaterial due to its biodegradability, biocompatibility, low immunogenicity and low toxicity. It can be easily bioengineered and thus is a promising material in various application fields, such as food science, medicine and agriculture.

Optical biosensor techniques based on evanescent field sensing, like Surface Plasmon Resonance (SPR), have typically an effective detection depth of a few hundred nanometers from the sensing surface. With traditional SPR instrumentation, coatings thicker than the penetration depth, as frequently encountered in the case of ceramics, porous materials and biopolymers, can be difficult to evaluate. The unique optical system of Multi-Parametric Surface Plasmon Resonance (MP-SPR) instruments enables characterization of films up to a few micrometers thick thanks to so-called SPR waveguide mode (Granqvist *et al.*, Langmuir, 2013). Optical waveguide is a phenomenon that can occur in structures able to capture and internally reflect light, especially when a dielectric guiding layer with sufficiently high refractive index (n) and thickness (d) is present between metal and sample medium (Figure 2). MP-SPR provides a suitably wide scanning range for registering the plasmon excitations associated with the waveguide phenomenon, observed as one or more SPR minima below the main SPR dip (Figure 3). These minimas will respond to modification of layer optical properties (e.g. thickness increase or surface binding), allowing characterization of interactions and conformation changes within much thicker layers as normally assessed by the traditional SPR technique.

Materials and methods

Chitin polymer derived from shrimp shell was spin coated *ex situ* onto bare gold sensors slides and onto gold sensor slides pre-coated with 10 nm polystyrene. The solution for spin coating was prepared by dissolving chitin in dimethylacetamide (DMA) and 5% LiCl to a final concentration of 0.1% (w/v).

Measurements were performed on the MP-SPR Navi™ 200 OTSO instrument and carried out at 20°C. First, the MP-SPR spectrum of the sensor surface was registered in air (dry state measurement). The running buffer (20 mM TEA,

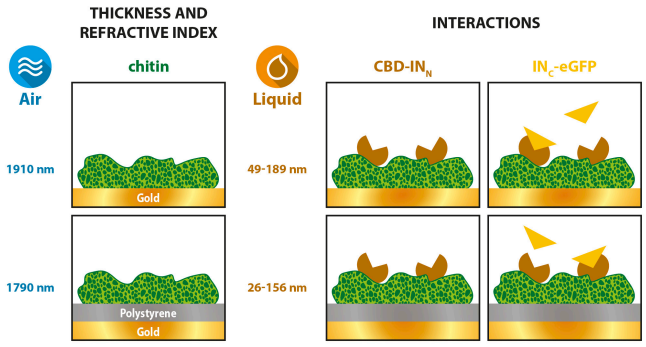

Figure 1. A chitin layer was spin coated on gold and on polystyrene to characterize layer properties and protein interactions. CBD-IN$_N$ (chitin binding domain of *Bacillus circulans*) protein is split-intein pair with IN$_C$-eGFP protein fraction.

1 mM EDTA, 1 mM DTT, 1 mM NaN3, pH=8.0) was subsequently introduced at a flow rate of 20 µl/min to ensure surface assessment in the wet state. The obtained MP-SPR curves from both dry and wet state measurements were fitted using MP-SPR Navi™ LayerSolver software to solve thickness and refractive index (RI).

Recombinant proteins CBD-IN$_N$ and IN$_C$-eGFP were expressed in E. Coli cells, where CBD corresponded to the Chitin Binding Domain found in *Bacillus circulans*. CBD-IN$_N$ was loaded to chitin surfaces deposited on gold and polystyrene at increasing concentrations of 490, 981, 1961, 3922 and 7854 nM. Subsequently, the split-intein IN$_C$-eGFP protein was injected on CBD-IN$_N$ for 5 min at increasing concentrations of 17.5, 175, 350, 701, 1401 and 2803 nM. Binding affinity and kinetics were calculated using TraceDrawer™ for MP-SPR Navi™.

More details in the original publication Casteleijn *et.al.* 2018.

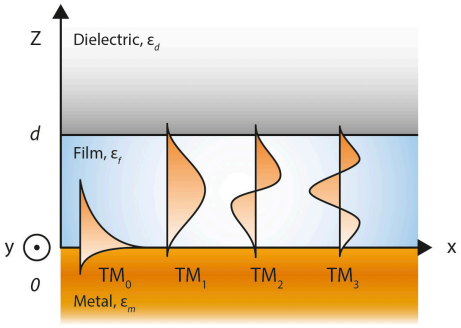

Figure 2. SPR waveguide phenomenon at the interface of guiding layer and metal.

Results and discussion

Bare gold and polystyrene-coated gold surfaces were assessed using MP-SPR before and after spin-coating of chitin polymer solution, both in air (dry state) and buffer flow (wet state) conditions (Table 1). The SPR curves of basic Au and Au-PS substrate (before coating) were fitted first to get the exact thickness of the gold and the PS layers. SPR curves measured after the chitin deposition were fitted to evaluate thickness and optical properties of chitin layer using blank sensor slide values as background. Two wavelength (670 nm and 785 nm) data were analyzed to obtain both thickness and RI of the chitin layers in air and buffer (Figure 3). Dry state measurement resulted in waveguide

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by using the dedicated LayerSolver™ software. A slightly thicker (1910 nm) layer of chitin was formed on gold as compared to the film deposited on polystyrene substrate (1790 nm). Upon the introduction of buffer, the thickness of chitin layers clearly decreased down to 49.2 nm on gold and 26.4 nm on polystyrene surface.

Data analysis enabled calculation of the refractive index of chitin film overlaying the gold and polystyrene substrate in both air and buffer conditions (Table 1). The refractive index of chitin layer in air resulted to be lower (1.36-1.39) as compared to bulk material of shrimp chitin (1.61), which was in accordance with previously reported studies with spin coated chitin. The biopolymer appeared to be more stable and uniform on a polystyrene coated sensor while chitin deposited on gold surface demonstrated large build-up structures, probably due to exposure of gold to lithium ions from spin-coating solution.

Once chitin coated sensors were loaded with the running buffer, the interactions with chitin binding protein (CBD-IN_N) was measured and followed by injections of a specific analyte (IN_C-eGFP protein). TraceDrawer™ for MP-SPR Navi™ was used to calculate affinity and kinetics constants for both interaction experiments (Table 2). The affinity of CBD-IN_N to chitin layer was obtained according to the one-to-two binding model, indicating that protein was bound to the biopolymer with a specific and a non-specific interaction component. The chitin binding domain was tightly bound to chitin generated on both gold and polystyrene surface since a dissociation phase was not clearly observed. However, the affinity appeared to be higher and the binding more specific in the case of chitin coated onto the polystyrene layer. Indeed, the interaction of CBD resulted in lower K_D and higher association rates, which was likely due to the more stable and uniform chitin layer formed on the polystyrene surface.

Binding of IN_C-eGFP protein fraction specific to CBD-INN captured over chitin layers was also measured. MP-SPR analysis enabled to determine the kinetics constants of interaction and distinguish the binding difference for chitin generated over gold and polystyrene surface (Table 2). In the first case, the K_D values resulted to be higher but the specific binding appeared to be more pronounced. On the other hand, binding of protein onto polystyrene-chitin was stronger but demonstrated slightly higher level on non-specific interactions.

Conclusions

In this application note, we demonstrated how MP-SPR can be used to determine optical properties (refractive index and thickness) of up to micrometer thick light non-absorbing layers based on optical waveguide mode reflection curves. The detection of SPR waveguide mode requires a wide angular range measurement uniquely provided by MP-SPR technology. Advantageous optical arrangement enables layer characterization in air and in liquid without any change in the instrument setup. Real-time monitoring of interactions with chitin binding proteins revealed differences in functionality of the two surfaces used in the study. The SPR waveguide mode phenomenon can be highly advantageous, especially in biomaterial studies of various dielectrics when thin layer preparation of the target material is difficult or impossible to achieve.

Original publication

Casteleijn *et al.*, Colloids and surfaces (539), 2018

Reference:

Granqvist *et al.*, Lagmuir (29), 2013

Recommended instrumentation for reference assay experiments

MP-SPR Navi™ 200 OTSO, 400 KONTIO, 210A VASA, 410A KAURIS, 220A NAALI and 420A ILVES with additional wavelengths - L

Sensors surfaces: Au, other metal or inorganic coating

Software: MP-SPR Navi™ Control, DataViewer, LayerSolver™ and TraceDrawer™ for MP-SPR Navi™

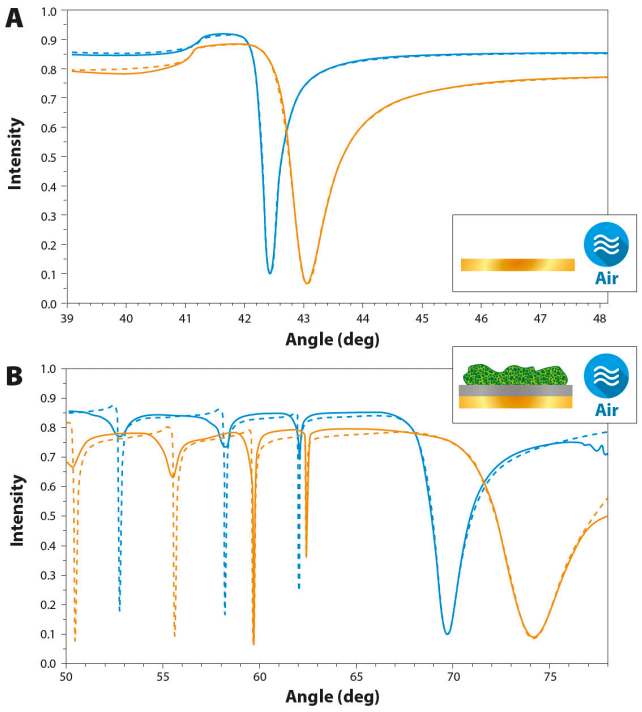

Figure 2. Full SPR curves registered in air at two wavelengths (670 nm – orange lines, 785 nm – blue lines). (A) uncoated gold and (B) chitin layer on polystyrene and gold. Dashed lines are the fitted curves from LayerSolver™ analysis.

Layer	Media	Thickness (nm)	RI (670nm)	RI (785nm)
polystyrene		10.7	1.6056	1.5971
		5.5	1.6056	1.5971
chitin		1910	1.308	1.3885
		49.2-188.5	1.3617	1.3595
chitin		1790	1.3678	1.3651
		26.4-155.6	1.3379	1.3350

Table 1. Thickness and refractive index of polystyrene and chitin layers. Data analysis provided by LayerSolver™.

Protein	Surface	K_{D1} (nM)	K_{D2} (nM)
CBD-IN_N		76.3	0.0425
		<0.001	0.0052
IN_C-eGFP	0.025 nM CBD-IN_N	90.3	0.0026
	0.025 nM CBD-IN_N	13.8	0.571
	0.025 nM CBD-IN_N	42.5	<0.001
	0.025 nM CBD-IN_N	1.63	0.0509

Table 2. Kinetic constants obtained by TraceDrawer™ analysis (one-to-two binding model) from interactions of chitin layer with chitin binding protein (CBD-IN_N) and subsequent specific recognition of IN_C-eGFP protein.

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