LSPR TRANSDUCERS FOR SENSING PROTEIN-CARBOHYDRATE INTERACTIONS

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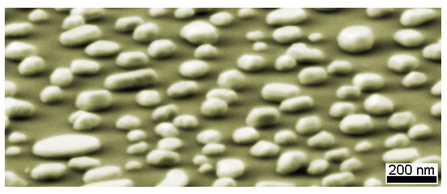
Introduction

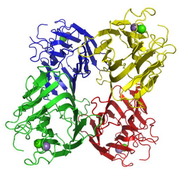
The present work combines preparation of ultrathin gold island films with automated synthesis of carbohydrates in order to develop simple and inexpensive localized surface plasmon resonance (LSPR) transducers for monitoring protein–carbohydrate interactions. The specific recognition between Concanavalin A (Con A) and mannose is used as a model system.

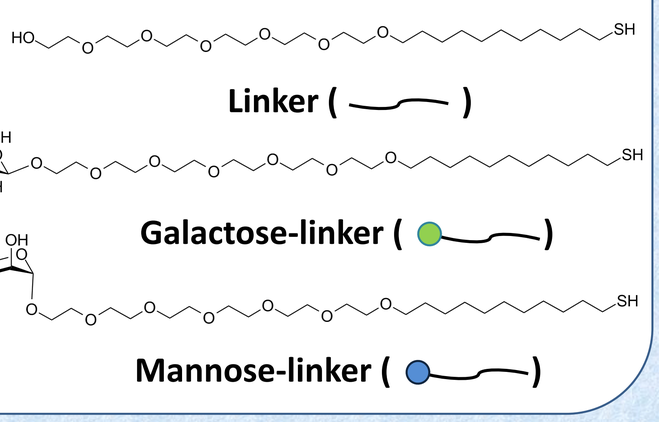
Materials



Discontinuos gold thin film ( ) 10 nm Au evaporated on glass, annealed 10 h / 570 °C

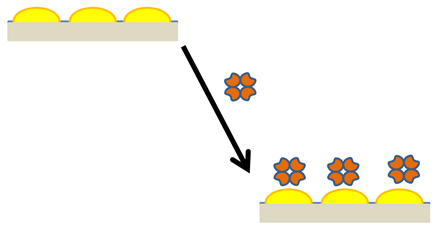
Concanavalin A ( )

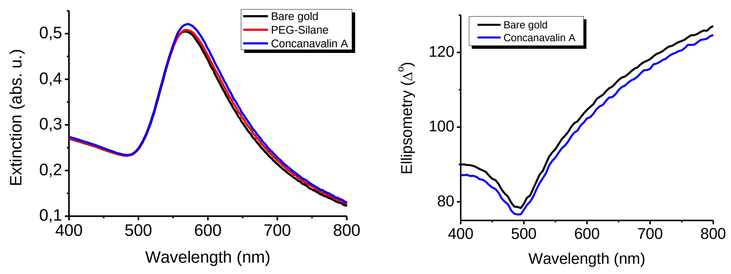




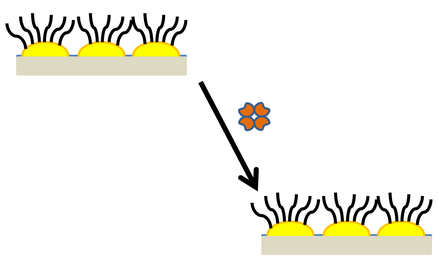
Self-assembly (SA) on gold of carbohydrate monolayers and specific recognition of Concanavalin A (Con A)

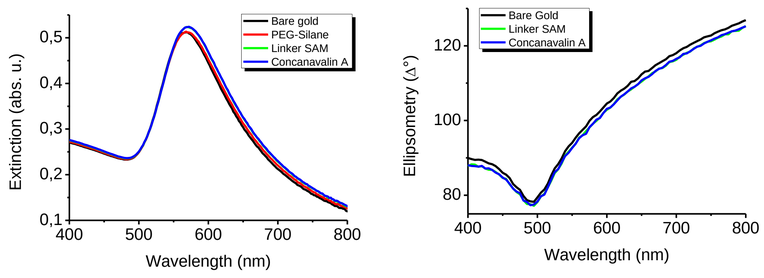
A) Non-specific binding of Con A on **bare gold** surface



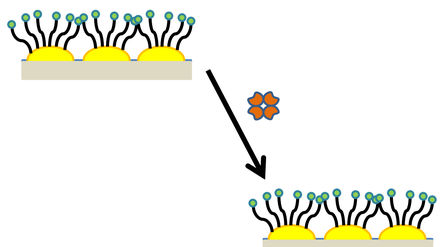


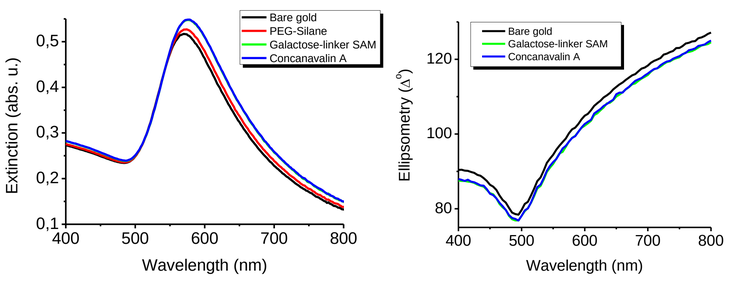
B) SA of a **linker** monolayer – NO recognition of Con A



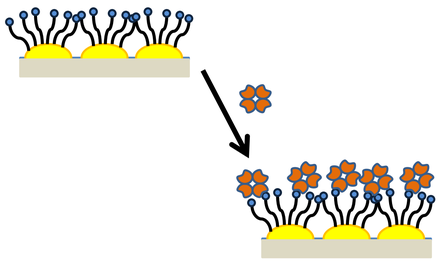


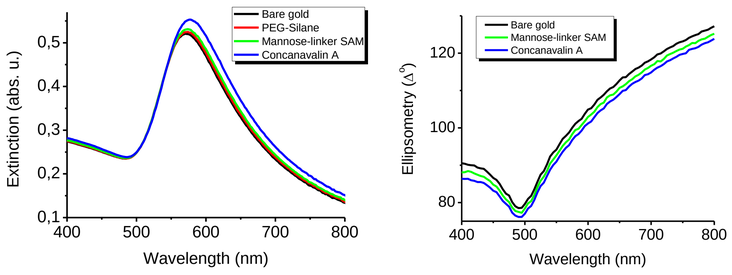
C) SA of a **galactose-linker** monolayer – NO recognition of Con A





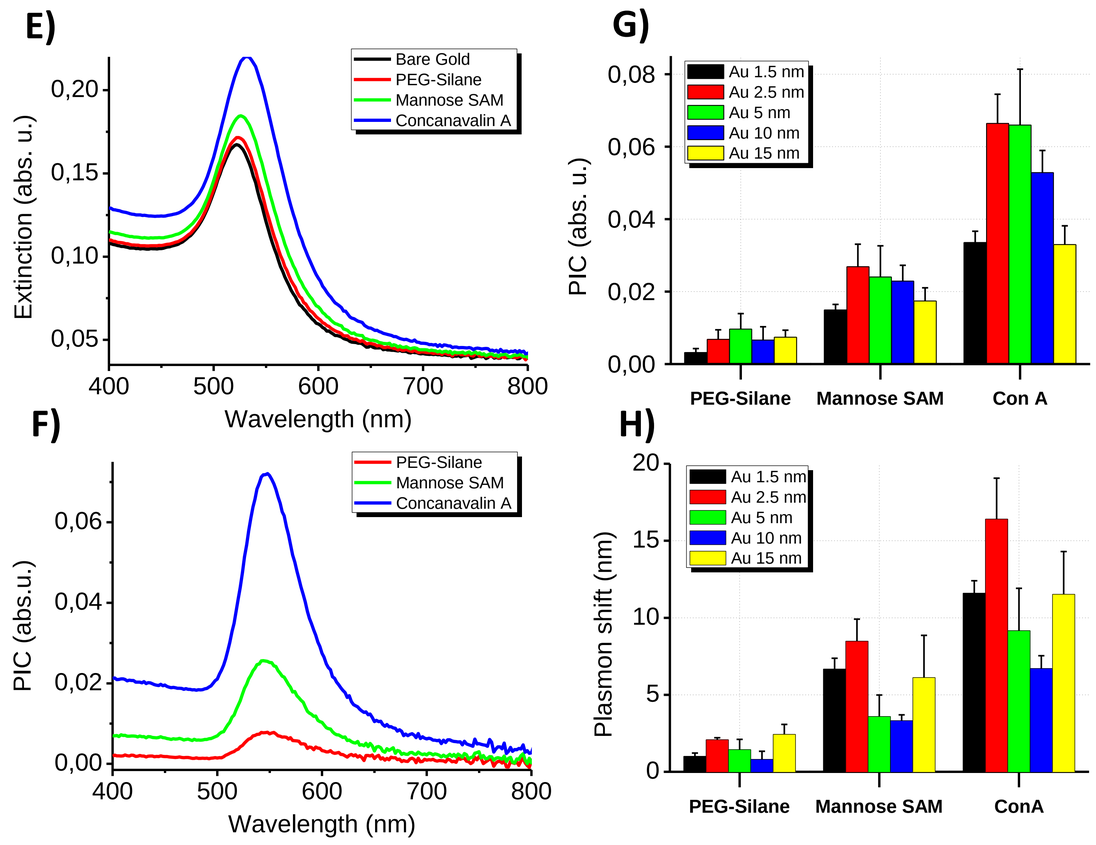
D) SA of a **mannose-linker** monolayer – **recognition and binding of Con A**





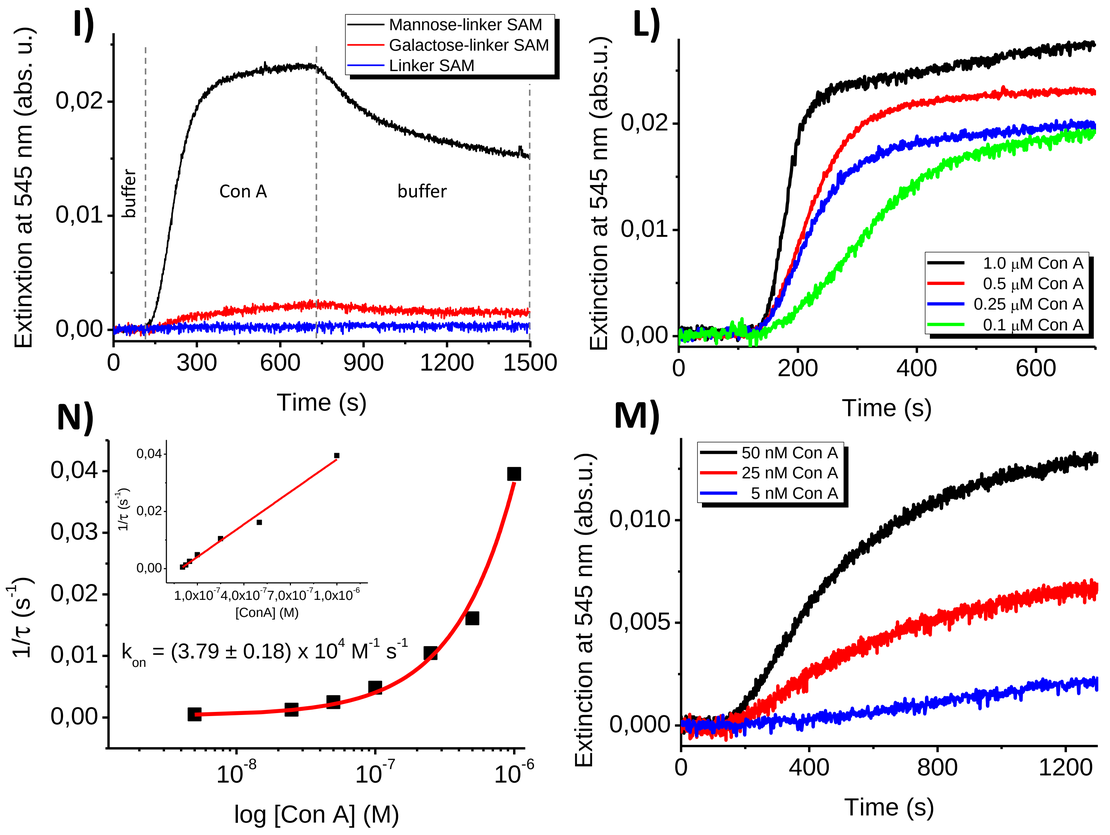
Left: schematic representation of the process. **Center and right:** transmission spectra of the Au island tranducer (nominal thickness, 10 nm) and ellipsometric measuraments performed on a continuous Au film (nominal thickness, 100 nm) corresponding to the different steps.

Optimization of the LSPR transducer



Left: Absolute (**E**) and differential (**F**) changes in the LSPR band accompanying the sequence of recognition steps (Au nominal thickness, 5 nm). **Right**: The response of transducers of different Au nominal thicknesses, shown as change in the PIC (**G**) and plasmon wavelength shift (**H**). [PIC is plasmon intensity change, obtained by subtracting the bare Au spectrum from the other spectra in **E**.]

Con A binding kinetics on the LSPR transducer



Top left: flow-cell kinetics of Con A recognition on different SAMs (**I**). **Right**: Con A binding kinetics to the mannose-linker SAM, performed at different protein concentrations (**L**-**M**). **Bottom left**: plots of the Con A association rate constant (k_{on}) to the mannose-linker SAM (**N**).

Conclusions

The present work shows that (i) the combined use of gold island films and automatically-synthesized carbohydrates allows convenient development of specific LSPR tranducers for sensing protein-carbohydrate interaction; (ii) optimization of the gold island structure improves significantly the transducer response; (iii) such tranducers can be applied to the determination of kinetic parameters associated with protein-carbohydrates interactions.