Tip-Specific Functionalization of Gold Nanorods for Plasmonic Biosensing

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Abstract

Plasmonic biosensors based on functionalized metal nanoparticles are being investigated for the many possibilities opened up by using a nano-object as a label-free sensor.[1] For instance, they give access to miniaturization and multiplexing of devices or to biosensing inside live cells. Also, the nanometric volume probed by a single metal nanoparticle is ideal for detection at single-molecule level.[2,3] The probed volume is defined by the region of enhanced electric field surrounding the plasmonic particle. For metal nanorods, this enhancement is strongly concentrated at the tips (Figure 1A). The adsorption of a biomacromolecule at the tips is transduced into a larger frequency shift of the surface plasmon resonance making easier its optical detection. Tip-specific functionalization gives practically the same sensor response as full-surface functionalization and it avoids undesirable line broadening by chemical interface damping.[4] For single-molecule detection, the better sensitivity from specifically functionalizing the tips makes it possible to detect every single molecule that binds and unbinds to the nanorod.[2] In ensemble conditions, it is also advantageous as it maximizes the plasmon shift by concentrating all the analyte in the region with the highest field.
We have developed a chemical procedure for tip-specific functionalization of gold nanorods. This procedure was used to attach biotin receptors to nanorods with ensemble-average dimensions of 9 nm by 37 nm, and a longitudinal plasmon in water at approximately 760 nm. The functionalized gold nanorods were used as a proof-of-concept for plasmonic biosensing of streptavidin (Figure 1B). The red-shift of the plasmon peak was followed over time to trace the binding kinetics of streptavidin-biotin at the nanorods' surface (Figure 1C). The response to increasing streptavidin concentrations afforded its binding affinity (Figure 1D). We find dissociation constants (K_d) in the range of nM that are around five orders of magnitude higher than in solution. Similarly high values of K_d have been reported in the literature with some degree of dependency on the biotin-linker length and its surface density. We have also explored different biotin-linker lengths (13.5, 29 and 56 Angström) and compared assays on nanorods that were fully functionalized with biotin or specifically at the tip. The longer linker in combination with a sparse tip-functionalization yielded the largest plasmon shift, ca. 10 nm, at surface saturation. This result suggests that steric hindrance is most likely interfering with binding affinity of streptavidin-biotin at the surface. The binding kinetics seem to also support this picture by showing strong deviations from first order exponential kinetics, although other effects may also interfere e.g., non-specific adsorption. In this contribution, we will discuss these effects to elucidate the role of surface chemistry in the design and performance of plasmonic biosensors.

References

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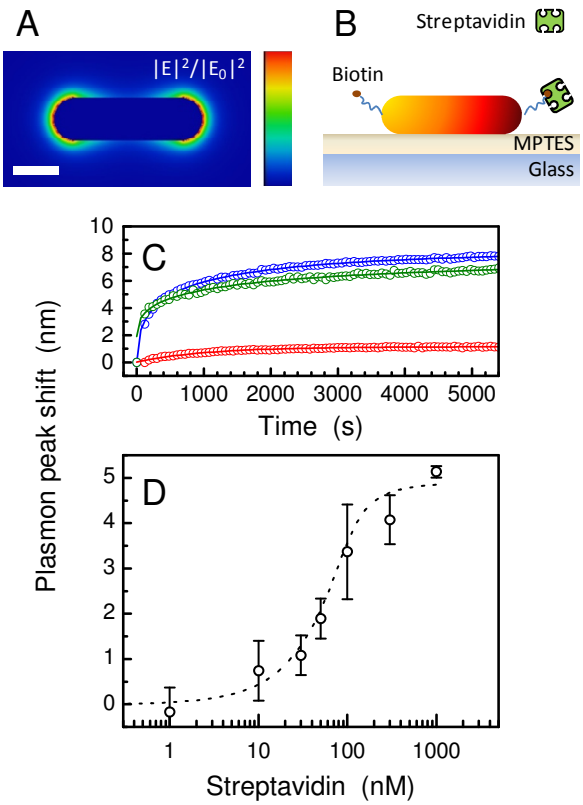

Figure 1 – (A) Near field enhancement calculated with discrete dipole approximation for a gold nanorod of size 31 nm × 9 nm at its longitudinal surface plasmon resonance – white bar corresponds to 10 nm and color scale spans squared field amplitudes from 1 (blue) to 3000 (red); (B) Scheme depicting tip-specific functionalization of a gold nanorod with biotin and binding of streptavidin protein; (C) Kinetic traces of tip-functionalized gold nanorods upon adding a solution of streptavidin 100 nM in PBS buffer – biotin-linker lengths of 13.5 Å (red), 29 Å (green) and 56 Å (blue); (D) Dose-response curve of tip-functionalized gold nanorods with a biotin-linker length of 13.5 Å for streptavidin binding in PBS buffer.