SUPER-RESOLUTION IMAGING OF SINGLE-MOLECULE DNA INTERACTIONS WITH PLASMONIC NANOPARTICLES

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Plasmonic nanoparticle-sensors are being developed for label-free detection of single analyte molecules binding to receptors on the surface of the particle[1,2]. The response of a plasmonic sensor strongly depends on the position where an analyte binds due to the spatially heterogeneous near-field[1]. Also, binding kinetics are expected to vary across a nanoparticle surface due to geometry dependent fluid accessibility[3]. It however remains unknown how the location of binding of an analyte correlates with binding kinetics and sensor response. Here we employ DNA-PAINT to establish the location of analyte binding at the single molecule, single-particle level by super-resolution localization of DNA hybridization events.

We use a fluorophore-coupled oligo which transiently binds to an oligo functionalized gold bipyramid (Fig. 1, top). To prevent near-field coupling between the fluorophore and the plasmon resonance[4] we employ a dye (ATTO532, $\lambda_{emission}$ = 540 nm) that is spectrally detuned far away from the plasmon wavelength (λ_{LSPR} = 815 nm). The large ~275 nm detuning minimizes plasmon coupling effects that would otherwise bias the fluorophore localization to the location of particle. The number of complementary bases and the analyte concentration are optimized to ensure that at most a single dye-coupled oligo is bound to a particle at any time. We localize the events by fitting the intensity distribution on a CCD camera, with hundreds of bipyramids across the field of view monitored in parallel. A resulting time trace showing fluorescence bursts from DNA hybridization events on the surface of a single bipyramid is shown in Fig. 1 (bottom).

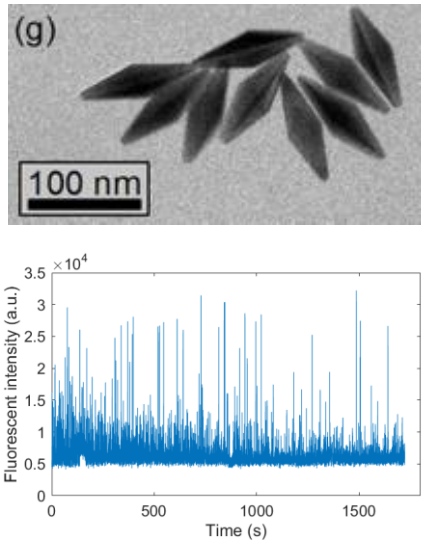

Figure 1: (Top) TEM image of gold bipyramids used for localisation. (Bottom) Time trace showing stochastic single fluorophore binding to a single gold bipyramid.

The super-resolution localization of each binding event will enable us to establish a connection between binding location, binding kinetics, and sensor response at the single-molecule level. This will further our understanding of nanoparticle-based biosensors and give insight into geometry-dependent interaction kinetics.

[1] Beuwer, M. A., Prins, M. W. J., & Zijlstra, P. "Stochastic protein interactions monitored by hundreds of single-molecule plasmonic biosensors", *Nano Letters*, 15(5), 3507 (2015).
[2] Rosman, C., Prasad, J., Neiser, A., Henkel, A., Edgar, J., & Sönnichsen, C., "Multiplexed plasmon sensor for rapid label-free analyte detection". *Nano Letters*, 13(7), 3243 (2013).
[3] Antosiewicz, T. J., & Käll, M., "A Multiscale Approach to Modeling Plasmonic Nanorod Biosensors", *The Journal of Physical Chemistry C*, 120(37), 20692-20701 (2016).
[4] Blythe, K. L., & Willets, K. A. (2015). "Super-Resolution Imaging of Fluorophore-Labeled DNA Bound to Gold Nanoparticles: A Single-Molecule, Single-Particle Approach". *The Journal of Physical Chemistry C*, 120(2), 803-815.