Homogeneous biosensing assay based on plasmonic photothermal fluorescence quenching

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Abstract

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Molecular sensors combine specific molecular recognition schemes with a physicochemical signaling mechanism in order to report the presence or quantify the concentration of a specific molecule, the analyte. Homogeneous sandwich immunoassays are decidedly attractive because they provide high sensitivities for the determination of low concentrations of proteins and hormones, by means of simple measurement protocols. Because they do not involve any separation or purification steps, a natural challenge for these kind of assays consists of filtering out the specific signal from background contributions. FRET (Förster resonance energy transfer)[1] as well fluorescence quenching by metallic nanoparticles[2] have been successfully applied for this purpose. Here we present a new homogeneous sensing scheme based on the combination of temperature-dependent molecular fluorescence and plasmonic heating of gold nanorods.

In the sandwich assay, the analyte brings fluorophores into the near vicinity of gold nanorods. Plasmonic heating generates a nanometric region of high temperature around the gold nanorods which reports specifically the surface binding reaction by a quantitative reduction of fluorescence emission. With this all-optical sensor sub-nanomolar concentrations are easily detected in a biotin-streptavidin model system.

References

- [1] Y. Ohiro, R. Arai, H. Ueda, and T. Nagamune, *Anal. Chem.*, vol. 74, no. 22, pp. 5786–5792, Nov. 2002.
- [2] S. Mayilo, M. a Kloster, M. Wunderlich, A. Lutich, T. a Klar, A. Nichtl, K. Kürzinger, F. D. Stefani, and J. Feldmann, *Nano Lett.*, vol. 9, no. 12, pp. 4558–63, Dec. 2009.

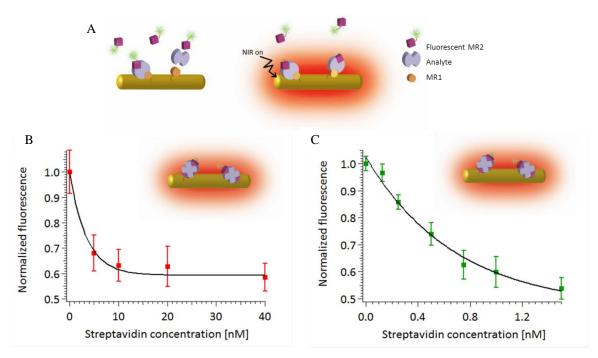


Figure 1 A: The assay consists of gold nanorods surface-functionalized (which present a strong longitudinal plasmon absorption band at around 800 nm) with molecular recognition units (MR1) for the analyte, and a different molecular recognition unit (MR2) which is fluorescently labeled with AlexaFluor 546. B and C) Normalized fluorescence emission under NIR illumination as a function of streptavidin concentration obtained for assays composed of (b) 1nM biotin-AuNRs + 80 nM biotin-AlexaFluor546, and (b) 1 nM biotin-AuNRs + 5 nM biotin-AlexaFluor546. Lines are exponential fits.