Orientation of Protein Determined in QD-Bioconjugate Assembly

One class of hybrid nanomaterials that bridges the interface between materials science and biology is the protein–nanomaterial composite. Beneficial protein properties imparted by design to such nanocomposites include self-assembly assistance, biorecognition, and catalysis. The interaction between protein bioreceptors and the inorganic nanocomponents is fundamentally important. Recently, quantum dots (QDs) were bound to maltose-binding proteins (MBPs) to make solution-phase biosensors that function by changes in fluorescence resonance energy transfer (FRET). Although the biosensors' activities were evident, the nature of the MBP-QD interaction was unknown. In the work discussed here, I.L. Medintz, J.H. Konnert, and co-workers at the Naval Research Laboratory in Washington, D.C., have developed a FRET-based modeling technique to determine the orientation of MBP coordinated to the QD surface.

As reported in the June 29 issue of the *Proceedings of the National Academy of Sciences* (p. 9612), the researchers prepared MBP-QD assemblies from CdSe-ZnS core–shell QDs and six different MBP mutants in which single cysteine substitutions were spatially distributed on the protein surface and labeled with rhodamine red (RR) dye. The core–shell QDs were made water soluble by using bidentate dihydrolipoic acid to replace the organic capping shell left over from the colloidal synthesis process. MBP, an ellipsoidal protein with dimensions of about 30 Å × 40 Å × 65 Å, coordinates to the Zn-S surface of each spherical QD (core–shell diameter, ~60 Å) by a C-terminal oligohistidine segment. The researchers determined from FRET efficiency data the distances, d_i, from the energy-donating QD to the six different RR-acceptor locations. These distances were used in conjunction with the MBP crystal structure to model the orientation of MBP with respect to the QD surface using a method the researchers liken to a nanoscale global positioning system, that is, triangulating a point on the globe from orbiting satellites. The protein's dye-acceptor positions relative to a spherical QD are analogous to satellite positions about the earth. The researchers said that this approach should, in principle, allow determination of the QD orientation with respect to MBP with as few as four non-coplanar locations instead of the 21 parameters that define the six MBP-dye acceptor positions and the QD center. The researchers, however, resorted to an iterative method that minimized the mean-square deviations of the measured and computed d_i values.

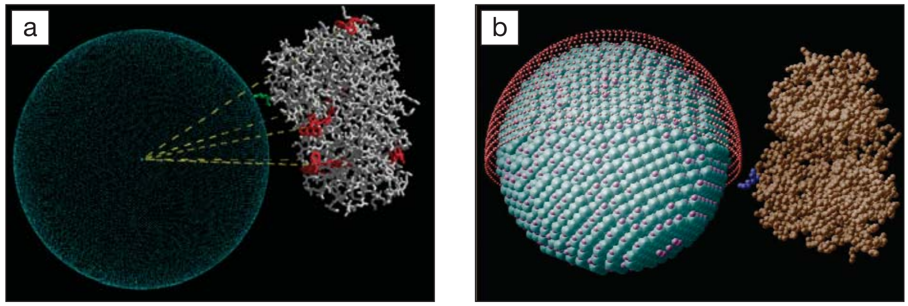

Figure 1. Refined maltose-binding protein (MBP) orientation relative to a quantum dot (QD). (a) Side view with all six rhodamine red structures positioned (in red). The refined distances to the center of the QD are shown in yellow. The distance from the nearest MBP atom is shown in green. (b) View of the final refined orientation of MBP relative to the QD, with QD surface sulfur atoms in teal and zinc atoms in pink. The red shell shows the estimated outer radius of the dihydrolipoic acid ligand. Reproduced with permission from PNAS; *© National Academy of Sciences, USA 2004.*

The research team found that MBP has a preferred orientation on the QD surface (see Figure 1), although it appears that MBP is free to rotate about the pentahistidine that attaches to the QD. Medintz, Konnert, and their colleagues believe that their approach can be used to determine the orientation of other proteins on a QD or any other spherical nanoparticle and, indeed, may be extended to other labeled biomaterials attached to any nanoparticle surface.

STEVEN TROHALAKI

Surface Plasmons Utilized to Achieve High-Density Nanolithography

The semiconductor industry is under constant pressure to reduce feature size in the photolithographic processing of integrated circuits in order to increase transistor density and computing power; however, using new lithography techniques that overcome the diffraction limit of the light are still costly. W. Srituravanich, X. Zhang, and colleagues at the University of California, Los Angeles, have developed an economically viable process called plasmonic nanolithography (PNL) that utilizes surface plasmons transmitted through subwavelength two-dimensional hole-array masks.

The researchers reported in the June 9 issue of *Nano Letters* (p. 1085) the fabrication of 90 nm dot-array patterns on a 170 nm period using PNL, which is well below the diffraction limit of far-field optical lithography. They observed strong UV light transmission and wavelength-dependent transmission in their far-field transmission measurements, confirming surface plasmons as active contributors in the lithography process.

The researchers designed a plasmonic Al mask perforated with 2D periodic hole arrays surrounded by two dielectric layers with quartz as the supporting substrate and a UV-transparent poly(methyl methacrylate) (PMMA) spacer layer (30–80 nm thick) with comparable dielectric constants of 2.18 and 2.30 at the exposure wavelength of 365 nm. They fabricated 40-nm-wide hole-array masks with 170 nm, 220 nm, or 250 nm periods on 80-nm-thick Al films using focused ion-beam (FIB) milling, with the 220 nm period corresponding to (1,0) and (0,1) resonance modes.

After FIB milling, a 30-nm-thick spacer layer of PMMA was spun on, followed by a layer of photoresist (SU-8). After development, atomic force microscopy revealed feature sizes as small as 90 nm, which is equivalent to ~$\lambda/4$ of the used exposure light wavelength, and with a pattern period of $<\lambda/2$, which is far beyond the diffraction limit of far-field lithography. The optimal exposure time was only 9 s, corresponding to a fluence of 72 mJ/cm^2. This is comparable to typical exposure fluences used in conventional lithography, with large features implying strong near-field transmission enhancement by surface plasmons, which gives it great economic potential. Even smaller features can be realized by reducing the spacer layer thickness, opening up high-resolution, high-density nanolithography with high transmittance using conventional light sources, which would eliminate the need for complex vacuum setups such as are needed for extreme UV lithography.

The PNL technique may find application in biosensing, photonic crystals, and high-density magnetic storage. The plasmon concept applies not only to periodic

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