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Nanoplasmonic upconverting nanoparticles as orientation sensors for single particle microscopy

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Statement of the Problem: Protein-DNA interactions are the center of many important biological pathways including DNA replication, transcription, recombination, and repair. Dynamic movements of proteins on DNA include jumping, hopping and quasi-1D curvilinear movement during which the protein maintains continuous DNA contact (Fig. 1). While some work has been done on translational motion of proteins on DNA, the 3D rotational motion of the proteins on DNA, is less explored, due to limitations in current nanoprobes. Recent studies have shown that rare earth upconverting nanoparticles (UCNP) offer an attractive alternative method for tracking orientation due to their inherent excitation polarization dependence. UCNPs are excited in the near infrared and fluoresce via anti-stokes emission in the visible energy range (400–800 nm), making them attractive for use in biological settings due to their low-energy excitation photon energy. UCNPs are excellent fluorescent probes, as they have no blinking, bleaching, or fluorescence background due to their near infrared excitation. These properties allow dynamic molecular interactions to be tracked continuously in real time. By coating disk-shaped UCNPs with a metal layer (NP-UCNP), they gain a large anisotropy in the fluorescence yield if illuminated with polarized light. This fluorescence anisotropy of the NP-UCNP probe renders them as excellent orientation probes in both linear and 3D tracking.

Methodology & Theoretical Orientation: We have designed and demonstrate proof of concept of single particle orientation and rotation tracking of NP-UCNP probes. We apply 1) predictive modeling to design and optimize NP- UCNPs for anisotropic fluorescence intensity with orientation, 2) perform correlated structural and optical single nanoparticle spectroscopy of nanofabricated NP-UCNPs to validate model predictions, and 3) analyzed the diffusional characteristics of a single NP-UCNP tumbling in solution between coverslip and slide to confirm that the orientation dependent fluorescence of the single NP-UCNP can be used to track single molecules.

Findings: It was found that the shape asymmetry of the UCNP itself contributes strongly to the orientation and excitation polarization dependence of the emission intensity. The presence of a gold shell enhances the intensity contrast between flat and edge orientations. We analyzed a particle tumbling in solution to show that the diffusional constant of a single particle can be determined.

Conclusion & Significance: The proposed new orientation sensitive platform based on NP-UCNP probes that can be coupled to proteins has wide-ranging applications in the future analysis and compilation of protein dynamics in any biological system. This model will open new opportunities for the biomedical research community to develop novel technologies for early diagnosis, control, and treatment of a wide-range of human diseases.

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