Engineering Quantum Dots for Live-Cell Single Molecule Imaging

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Recent advances in single molecule imaging have shed light on a broad range of biological processes, revealing the dynamics of protein-protein interactions and the mechanics of macromolecular machines. These studies have primarily made use of organic dyes and fluorescent proteins, which unfortunately suffer from poor stability during photo-excitation, limiting the duration of experiments to just several seconds. Our research is focused on engineering quantum dots (QDs) to overcome these limitations. QDs are nanoparticles composed of semiconductor materials that exhibit exceptional resistance to photon-induced degradation, with fluorescence brightness that exceeds dyes and proteins by a factor of 10-40. These attributes are very attractive for single molecule imaging applications, especially for live-cell imaging. However the large hydrodynamic size of conventional QDs (15-35 nm) has hindered their use in living cells due to severely restricted diffusion and steric hindrance. We are developing a new generation of QDs with hydrodynamic sizes similar to globular proteins (5-15 nm) that exhibit enhanced stability and a strong resistance to nonspecific adsorption. We postulate that minimizing the size of quantum dots will be critical for single-molecule imaging in living cells. We have engineered QDs with a variety of hydrodynamic sizes and we have imaged the diffusion of these particles in living cells using real-time multicolor single-molecule imaging to find that there is a size cutoff for free diffusion in the cytoplasm and other crowded macromolecular environments, indicating that minimizing the size of nanoparticle probes and therapeutic agents is important to maximize efficacy.