Weakly Luminescent Nanocrystals that Make Exceptional Single-Molecule Imaging Probes

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Imaging complex materials at the single-molecule level reveals heterogeneities that are lost in ensemble imaging experiments. An ongoing challenge is the development of probes with the photostability, brightness, and continuous emission necessary at higher single-molecule excitation powers. Lanthanide-doped upconverting nanoparticles (UCNPs) overcome problems of photostability and continuous emission, and their upconverted emission can be excited with biologically benign NIR light at powers orders of magnitude lower than those required for conventional multiphoton imaging probes *(1,2)*. But the brightness of UCNPs has been limited by a poor understanding energy transfer and relaxation within individual nanocrystals and unavoidable trade-offs between brightness and size *(3)*. We have developed UCNPs under 10 nm in diameter that are over an order of magnitude brighter under single-particle imaging conditions than the brightest ensemble compositions, allowing us to visualize single upconverting nanoparticles as small as fluorescent proteins *(4)*. We use a combination of advanced characterization (single-nanocrystal lifetimes and full emission spectra) and advanced theoretical modeling *(5)* to find that surface effects become critical at $d < 20$ nm, and that the higher fluences used in single-molecule imaging fundamentally change the factors that determine nanocrystal brightness. We find that factors known to increase brightness in bulk experiments are unimportant at higher excitation powers, and that, paradoxically, the brightest probes under single-molecule excitation are barely luminescent at the ensemble level. Our results address key obstacles for optimizing nanocrystals as single-molecule probes and suggest a single-molecule probe development strategy involving iterative rounds of kinetic modeling and detailed nanocrystal characterization.

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