Imaging of single upconversion nanoparticles in aqueous buffers

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Single-particle microscopy is a powerful method to extract information that is not accessible by ensemble methods. Upconversion nanoparticles (UCNPs) are attracting emissive labels for single-particle microscopy, because they offer very high signal to noise ratio, due their anti-Stokes emission. Moreover, they are extremely photostable and do not blink, giving the unique possibility to track events on time scales several orders of magnitude greater than conventional organic dyes [1]. However, ensemble measurements of diluted aqueous dispersions of UCNPs have recently shown luminescence instability over time due to particle dissolution-related effects [2, 3]. This can be especially detrimental for single-particle experiments, but this effect has never been estimated at the single particle level. To investigate this point, we used quantitative wide-field microscopy to monitor the luminescence response of individual UCNPs under aqueous conditions [4]. The particles were found to rapidly loose their luminescence and exhibit large changes in their spectral response, which led to considerable heterogeneity in their luminescence and band intensity ratio. These changes were clearly attributed to the dissolution of the particles, as a result of their low concentration in the buffer that forces the dissolution equilibrium towards total dissolution. The dissolution of the UCNPs and the subsequent development of their heterogeneity can be largely slowed down by adding millimolar concentrations of sodium fluoride in the buffer or addition of appropriate shells. Therefore, quantitative experiments based on intensity/spectral response from individual UCNPs in aqueous buffers need to be corrected for these effects or even better, be performed under conditions that carefully prevent these effects. This will be illustrated using an exemple of application on live cells.

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