Rational Design and Absolute Quantum yields of NIR-Fluorescent Nanoparticles for Bioanalytical Applications

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Fluorescence-based techniques are amongst the most widespread tools in the material and life sciences, with new applications continuously emerging. Concepts for signal amplification and multiplexing strategies to increase the overall detection sensitivity and to enable the ratiometric or simultaneous detection of several analytes or species are current trends in the life sciences. [1]

This can be elegantly achieved by particles loaded or labeled with fluorophores. Particularly beneficial is the encapsulation of hydrophobic fluorophores with low solubility in aqueous media, which enables their application in biologically relevant environments. Particles loaded with chromophores emitting in the visible or near-infrared (NIR) spectral region allows the fluorescence detection within the diagnostic window between ca. 650 to 950 nm. [2] For the majority of *in vitro* or *in vivo* applications, detection within this region is mandatory especially for deep tissue imaging, to minimize scattering and absorption from water, tissue, and blood components and signal contributions from their autofluorescence. [3] Fluorophore incorporation in micro and nano particles can furthermore reduce unspecific interactions between the dye molecules and the surrounding medium and can minimize cytotoxic effects. Moreover, the encapsulation of NIR emitting dyes can enhance their photochemical and thermal stability as well as their usually low fluorescence quantum yields in polar and protic solvents like water. [4]

Here, we present the preparation and characterization of bright, differently sized, NIR-fluorescent polystyrene nano particles using a simple and versatile one-step staining procedure, which can be easily adapted to various dye classes. [5] The influence of the amount of incorporated dye on the absolute fluorescence quantum yield and brightness of differently sized fluorescent particles in suspension was studied with a custom-made calibrated integrating sphere setup. [5] The resulting particles with various surface functions and spectral properties have great potential in cellular imaging or biomarker targeting. Applications as ratiometric nano sensors for several analytes, e.g. oxygen, [6] and labels for lifetime multiplexing detection schemes will be exemplary shown.

References: [1] U. Resch-Genger et al., *Nature Methods* **2008**, 5, 763. [2] J. Yan et al., *NanoToday* **2007**, *2*, 44. [3] K.E. Adams et al., *Journal of Biomedical Optics*, **2007**. 12(2): 024017-1 - 024017-9. [4] V. Buschmann et al., *Bioconj. Chem*. **2003**, 14, 195. [5] T. Behnke et al., *Dyes and Pigments*, **2012**. 94(2): 247-257. [6] J. Napp et al., *Analytical Chemistry*, **2011**. 83(23): 9039-9046.