Counterion-enhanced emission approach to develop ultrabright cyanine-loaded fluorescent polymer nanoparticles for cell barcoding

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Fluorescent organic nanoparticles are particularly interesting as tools for bioimaging due to their high brightness and potential biodegradability, which is one of their key advantages over inorganic fluorescent nanoparticles.[1] However, major challenges in the field of fluorescent organic nanoparticles are poor encapsulation of loaded dyes and aggregation-caused quenching (ACQ). As it was recently reported by our group,[2,3] combining a cationic dye, rhodamine B octadecyl ester (R18), with a bulky hydrophobic counterion can strongly decrease ACQ and increase encapsulation efficiency of the dyes inside nanoparticles. Moreover, bulky counterions not only prevented fluorescence quenching, but also induced interfluorophore communication, making whole particles behave like single emitters – turning on and off (blinking) as a whole particle.

In this work we extended the *counterion-enhanced emission* approach to cyanines, one of the largest families of dyes, covering a spectral region from green to NIR. We synthesized ion pairs of hydrophobic dioctadecyl-substituted cyanines DiO, DiI, DiD, Cy5.5 and Cy7.5 with several tetraphenylborates of different size and fluorination degree, and encapsulated those ion pairs inside PLGA (poly(lactide-*co*-glycolide)) nanoparticles at up to 50 mM dye/polymer concentration.

Our results have confirmed that bulky hydrophobic counterions can increase encapsulation and prevent self-quenching for all tested cyanines, the most effective being the most bulky and fluorinated tetraphenylborate, F12. Interestingly, quantum yields of nanoparticles increased when going from the NIR to the green region for the same dye loading and counterion. Moreover, nanoparticles loaded with different dyes but the same counterion appeared to have the same size and surface properties.

Taking advantage of these properties of our nanoparticles, we created a multicolor cell barcoding system using nanoparticles of three distinct absorption and emission bands (DiO, DiI and DiD dyes with F12 counterion). These nanoparticles can spontaneously enter cells through endocytosis and show no leakage inside the cells, being a good tool for cell labeling. Moreover, it was found, that due to nearly identical surface properties and no difference in cellular uptake, mixing these nanoparticles of three colors in different ratios can generate at least 13 distinguishable color codes for cell labeling. This method was applied to different cell lines (HeLa, KB, 293T, CHO, RBL, U97 and D2A1) and it can be used for simultaneous tracking of co-cultured color-coded cell populations for more than 2 weeks. Applications for cell tracking in culture and in live zebrafish have been validated.

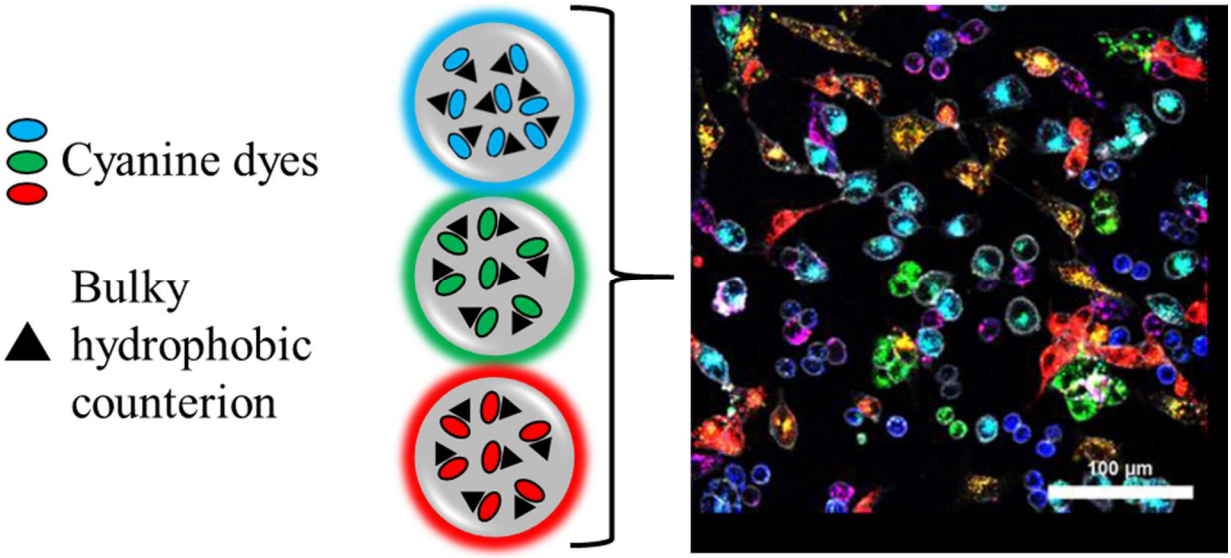

Figure 1. Illustration of multicolor cell barcoding principle.

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References:

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