Supporting Information

Protein-sized Dye-loaded Polymer Nanoparticles for Free Particle Diffusion in Cytosol

Andreas Reisch,* Doriane Heimburger, Pauline Ernst, Anne Runser, Pascal Didier, Denis Dujardin, and Andrey S. Klymchenko*
Figure S1. Transmission electron microscopy (TEM) images of particles made by nanoprecipitation for different percentages and type of charged groups. NPs were loaded with 10 wt% of R18/F5-TPB. Where not mentioned, nanoprecipitation was carried out in milliQ water. For PMMA-SO₃H at 1% nanoprecipitations were carried out in different NaCl concentrations as noted. Scale bars correspond to 50 nm.
Figure S2. Histograms of sizes of particles made by nanoprecipitation as a function on percentage and type of charged groups as determined by TEM. Mean values of sizes determined by transmission electron microscopy are given for NPs loaded with 10 wt% of R18/F5-TPB. At least 200 NPs were analyzed per condition. Error bars give full width at half maximum of the distribution.
Table S1. Particle sizes as determined by TEM and estimated number of charged groups per nm² of particle surface and total number per particle.

| %  | COOH | SO₃H | NMe₃ |
|----|------|------|------|
| size (nm) | charged groups per nm² | size (nm) | charged groups per nm² | size (nm) | charged groups per nm² |
| 1   | 42   | 0.4  | 2300 | 17   | 0.17 | 150  | 18   | 0.18 |
| 2   | 41   | 0.8  | 4300 | 12   | 0.24 | 107  | 12   | 0.24 |
| 5   | 37   | 1.9  | 8042 | 9    | 0.43 | 109  | 7    | 0.34 |
| 7.5 | 35   | 2.6  | 9400 | 8    | 0.56 | 112  | 7    | 0.53 |
| 10  | 29   | 2.9  | 7800 | 7    | 2.9  | 7780 | 7    | 0.66 |

Figure S3. Influence of the precipitation medium on the size of nanoparticles made by nanoprecipitation of carboxylate bearing polymers. Mean values of sizes determined by transmission electron microscopy are given for NPs loaded with 10 wt% of R18/F5-TPB. At least 200 NPs were analyzed per condition. Error bars give full width at half maximum of the distribution.
Table S2. Steady state fluorescence properties of selected nanoparticles loaded with 10 wt% R18/F5-TPB. Measurements were carried out in a FlouroMax-4 spectrofluorometer with an excitation at 530 nm and an intensity of about 1 mW/cm².

| Charged groups | Size (nm) | Fluorophores per NP | QY (%) | Brightness (M.cm⁻¹) | Fluorescence cross section (cm²) |
|----------------|-----------|---------------------|--------|---------------------|---------------------------------|
| PMMA-COOH 1    | 42        | 1700                | 59 ± 2 | 1.0 x 10⁸           | 3.8 x 10⁻¹³                     |
| PMMA-COOH 10   | 29        | 560                 | 59 ± 2 | 4.3 x 10⁷           | 1.3 x 10⁻¹³                     |
| PMMA-SO₃H 1    | 17        | 110                 | 61 ± 3 | 6.9 x 10⁶           | 2.6 x 10⁻¹⁴                     |
| PMMA-SO₃H 2    | 12        | 40                  | 55 ± 1 | 2.2 x 10⁶           | 8.4 x 10⁻¹⁵                     |
| PMMA-SO₃H 5    | 9         | 18                  | 41 ± 2 | 7.0 x 10⁵           | 2.7 x 10⁻¹⁵                     |
| PMMA-NMe₃ 1    | 18        | 120                 | 35 ± 3 | 4.3 x 10⁵           | 1.6 x 10⁻¹⁴                     |
Figure S4. Single-particle fluorescence microscopy. The nanoparticles were immobilized in solutions of polyvinylalcohol (PVA) in water with a PVA concentration of 15 wt%. Images were acquired using an excitation at 532 nm with an intensity of 1 W/cm². The conditions for excitation, recording and presentation are identical for all images. The mean number of emitted photons per particle per second and the per particle fluorescence cross-sections are given. At least 50 particles were analyzed per condition.
Figure S5. Microinjection of nanoparticles in living HeLa cells. The left hand images are maximum projections of fluorescence micrographs collected over 1 min (50 ms per frame) of nanoparticles made from different polymers, loaded with 10 wt% of R18/F5-TPB and coated with Tween 80. Right hand images are phase-contrast images of the corresponding cells. Scale bars correspond to 10 µm.