Supporting Information

Nanoscopic visualization of microgel-immobilized cytochrome P450 enzymes and their local activity

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Contents

1 Dynamic light scattering (DLS)

The hydrodynamic radii of the microgels were measured by dynamic light scattering experiments on an ALV setup using a 633 nm HeNe laser (JDS Uniphase, 35 mW). The setup included a goniometer (ALV, CGS-8F), two avalanche photodiodes (Perkin Elmer SPCM-CD2969), a digital hardware correlator (ALV 5000), and light scattering electronics (ALV LSE-5003). To avoid multiple scattering, samples were highly diluted in KPi buffer (50 mM, pH 7.5). The temperature was controlled using an external thermostat (Julabo F32) and a toluene bath, which was also used to match the refractive index of the cuvette glass. The scattering angle was varied from 30° to 110° in 10° steps. Samples were measured in triplicate at 20 and 22 °C. The diffusion coefficient was obtained by linear regression of the first cumulant against q^2. The hydrodynamic radius was obtained by means of the Stokes-Einstein equation.

Table S1 Hydrodynamic radii of poly(VCL-*co*-Vim) microgels with and without Cytochrome P450 BM3 measured by dynamic light scattering in KPi buffer (50 mM, pH 7.5).

Microgel	R_h 20 °C / nm	R_h 22 °C / nm
P(VCL-*co*-Vim)	428 ± 6	424 ± 7
P(VCL-*co*-Vim) with Cytochrome P450 BM3	359 ± 6	346 ± 5

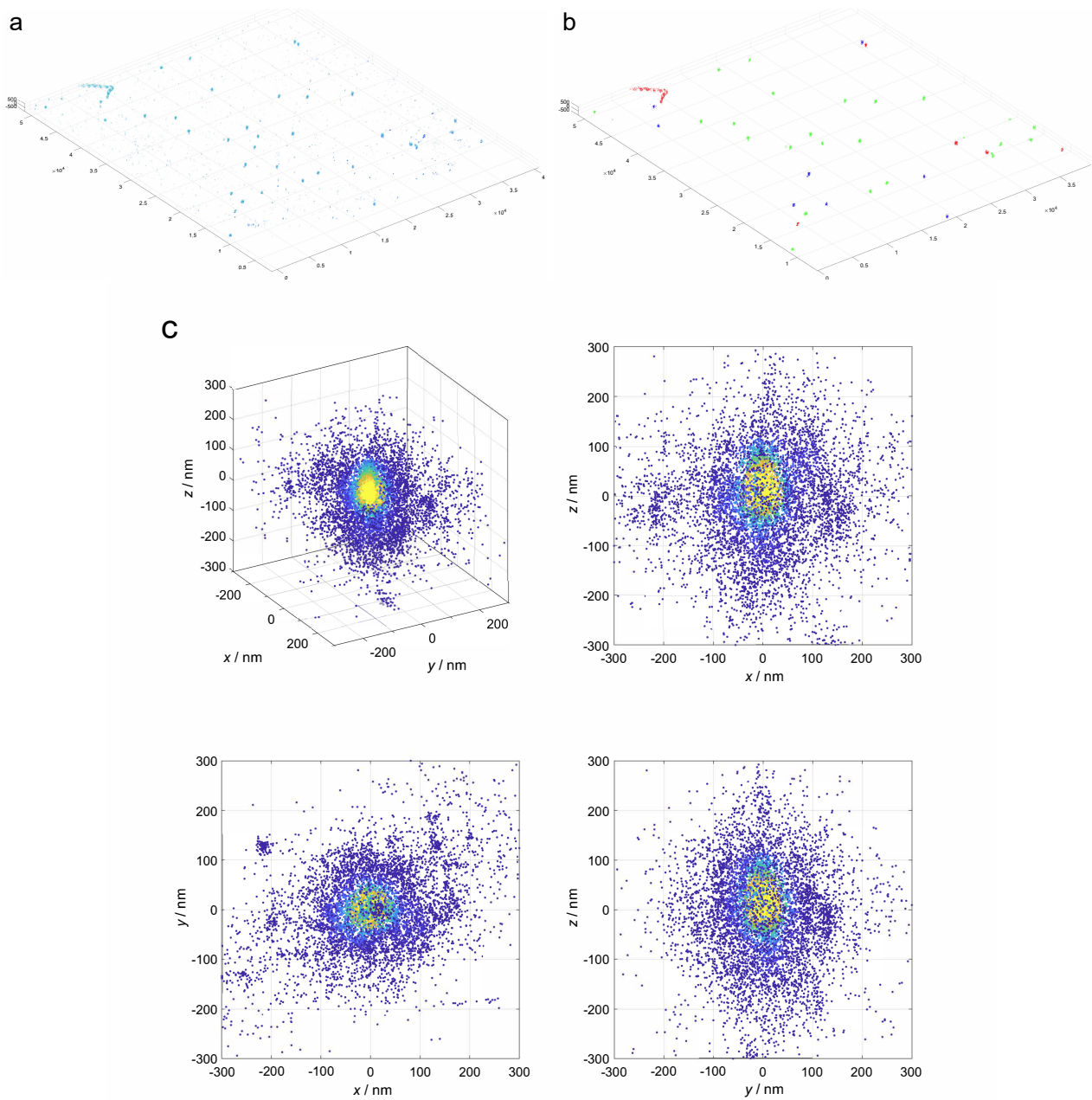# 2   Localization-based super-resolution fluorescence microscopy

Figure S1 Exemplary field of view of PAINT measurements: (a) all localizations obtained by the Thun-derSTORM localization are presented. Within them, (b) the clusters belonging to microgels were identified and chosen as representative point clouds (green) or discarded (blue and red). (c) The chosen point clouds were overlaid to construct a representative microgel PAINT point cloud. This point cloud is presented as pseudo-3D graph and as projections on the y-, z- and x-plane. All further analyses presented in the main paper are based on this representative point cloud.
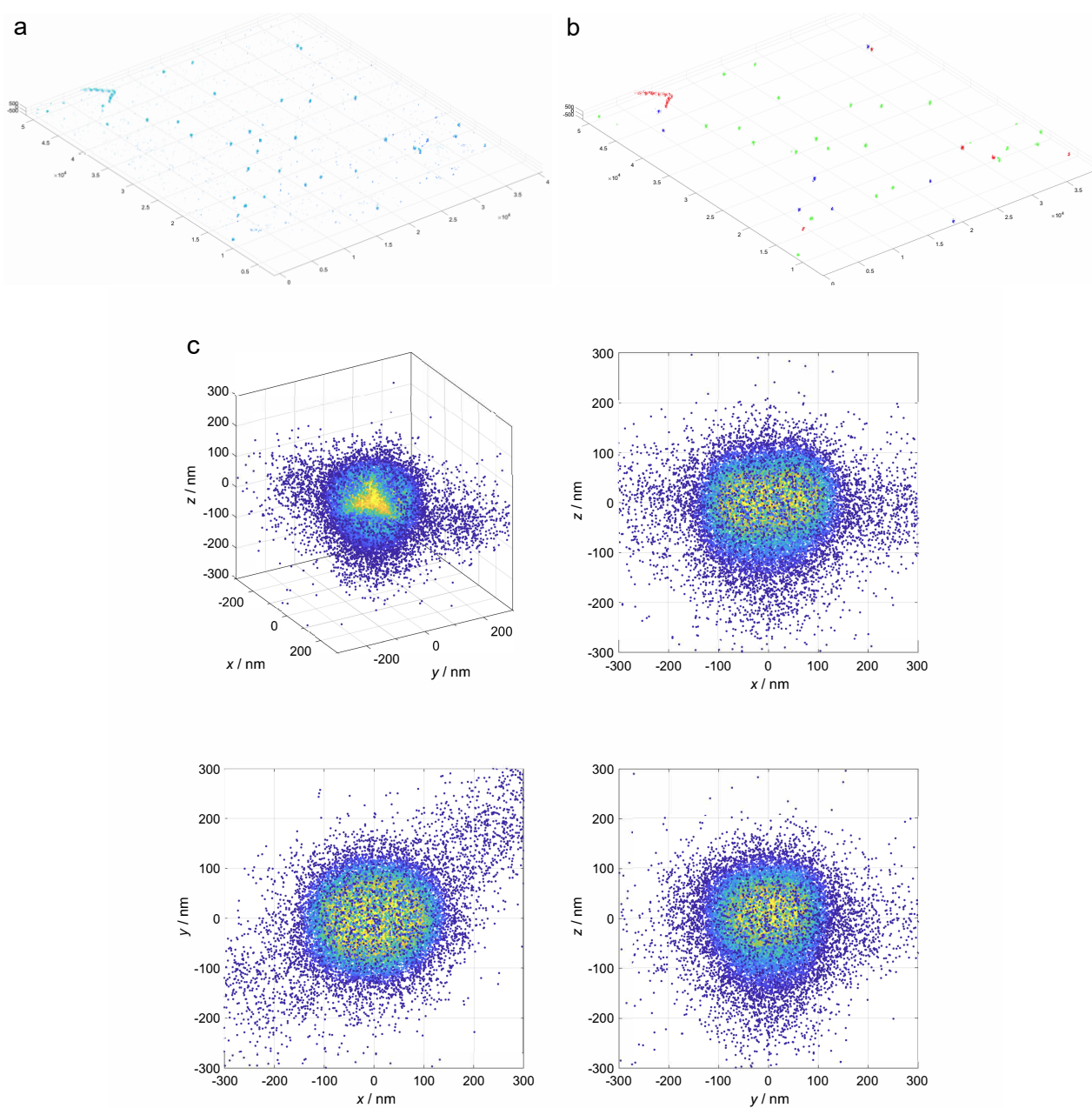
Figure S2 Exemplary field of view of dSTORM measurements: (a) all localizations obtained by the ThunderSTORM localization are presented. Within them, (b) the clusters belonging to microgels were identified and chosen as representative point clouds (green) or discarded (blue and red). (c) The chosen point clouds were overlaid to construct a representative microgel PAINT point cloud. This point cloud is presented as pseudo-3D graph and as projections on the y-, z- and x-plane. All further analyses presented in the main paper are based on this representative point cloud.
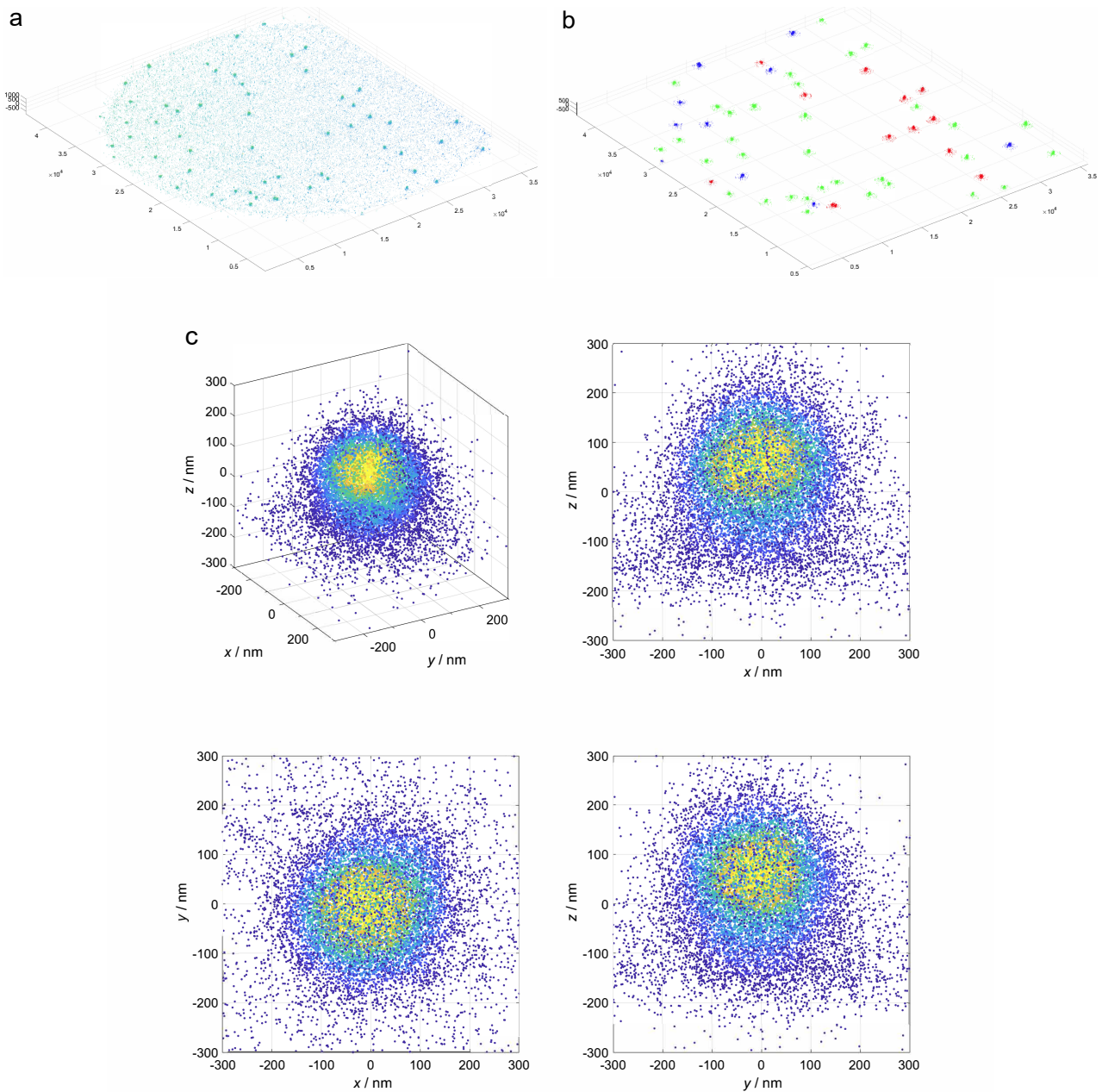
Figure S3 Exemplary field of view of NASCA measurements: (a) all localizations obtained by the ThunderSTORM localization are presented. Within them, (b) the clusters belonging to microgels were identified and chosen as representative point clouds (green) or discarded (blue and red). (c) The chosen point clouds were overlaid to construct a representative microgel PAINT point cloud. This point cloud is presented as pseudo-3D graph and as projections on the y-, z- and x-plane. All further analyses presented in the main paper are based on this representative point cloud.