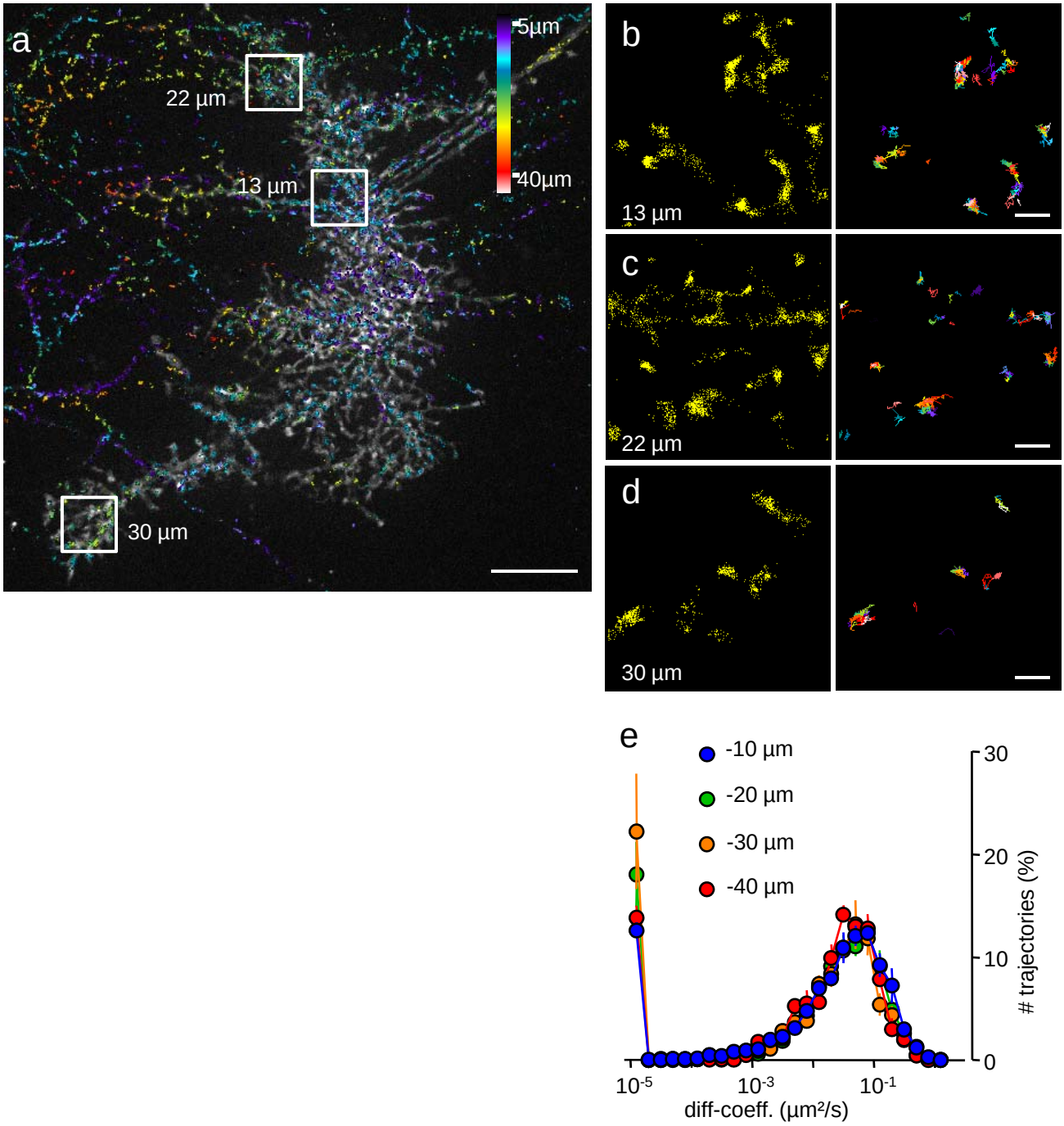
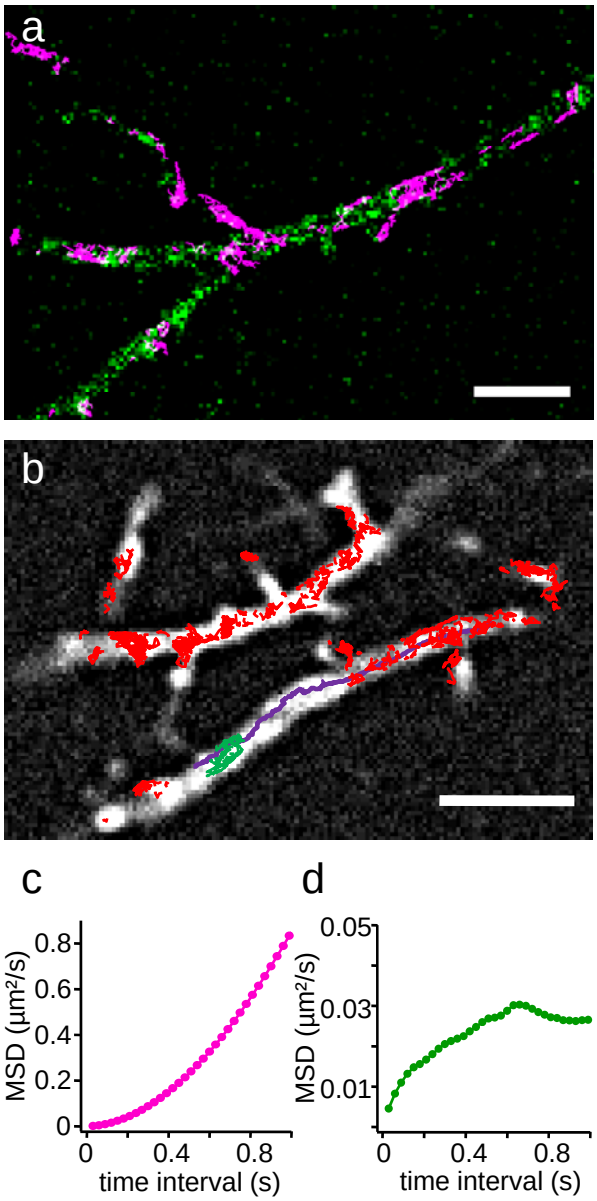


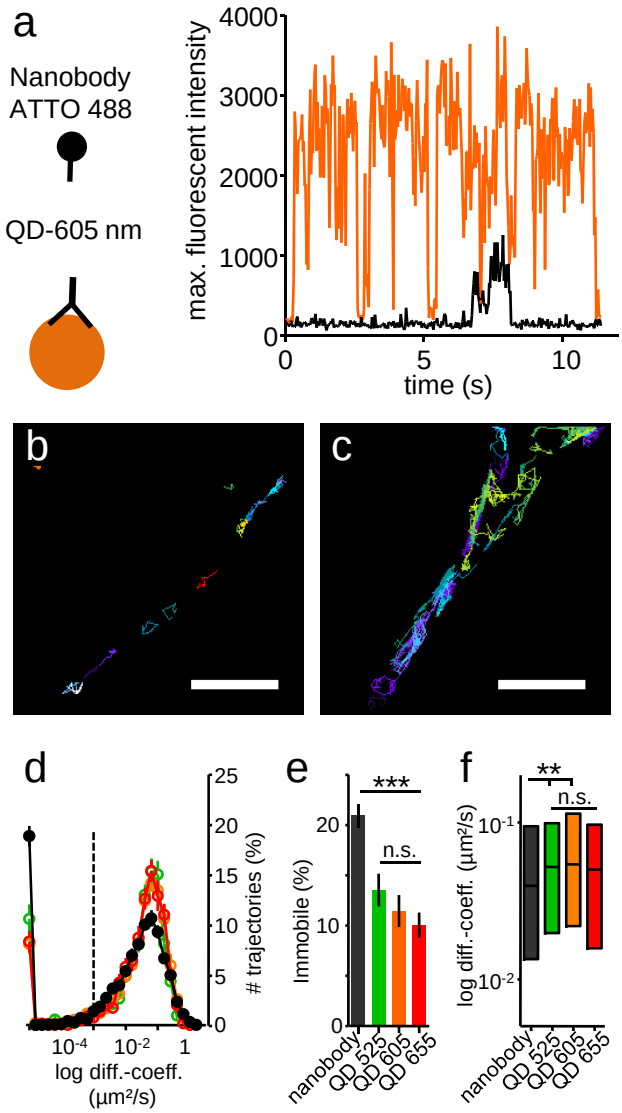
Supplementary Figure S1. GPI-GFP mobility in cell membrane of glia cells.

a) Example of a GPI-GFP transfected glial cell labelled with QD-anti-GFP. The maximum intensity projection (represents 400 image plains with a 0.1 µm increment in z-direction) of the GFP-fluorescence (gray) is overlaid with the localisation of anti-GFP-QDs (coloured). The scale bar represents 10 µm. **b-d)** Magnified view of positions at different depths, as indicated in (a). **e)** QD positions are shown in yellow and the corresponding trajectories >12 points are provided on the right hand side. The scale bar represents 1µm. e) Distribution of the diffusion coefficient of GPI-GFP expressed in glial cells. Mobile fractions were not different (Kruskal-Wallis test, $p > 0.05$), dotted line indicate threshold for diffusion coefficient for mobile population (0.008 µm²/s). Each bin in the distribution is given as mean ± s.e.m. for the different depth.



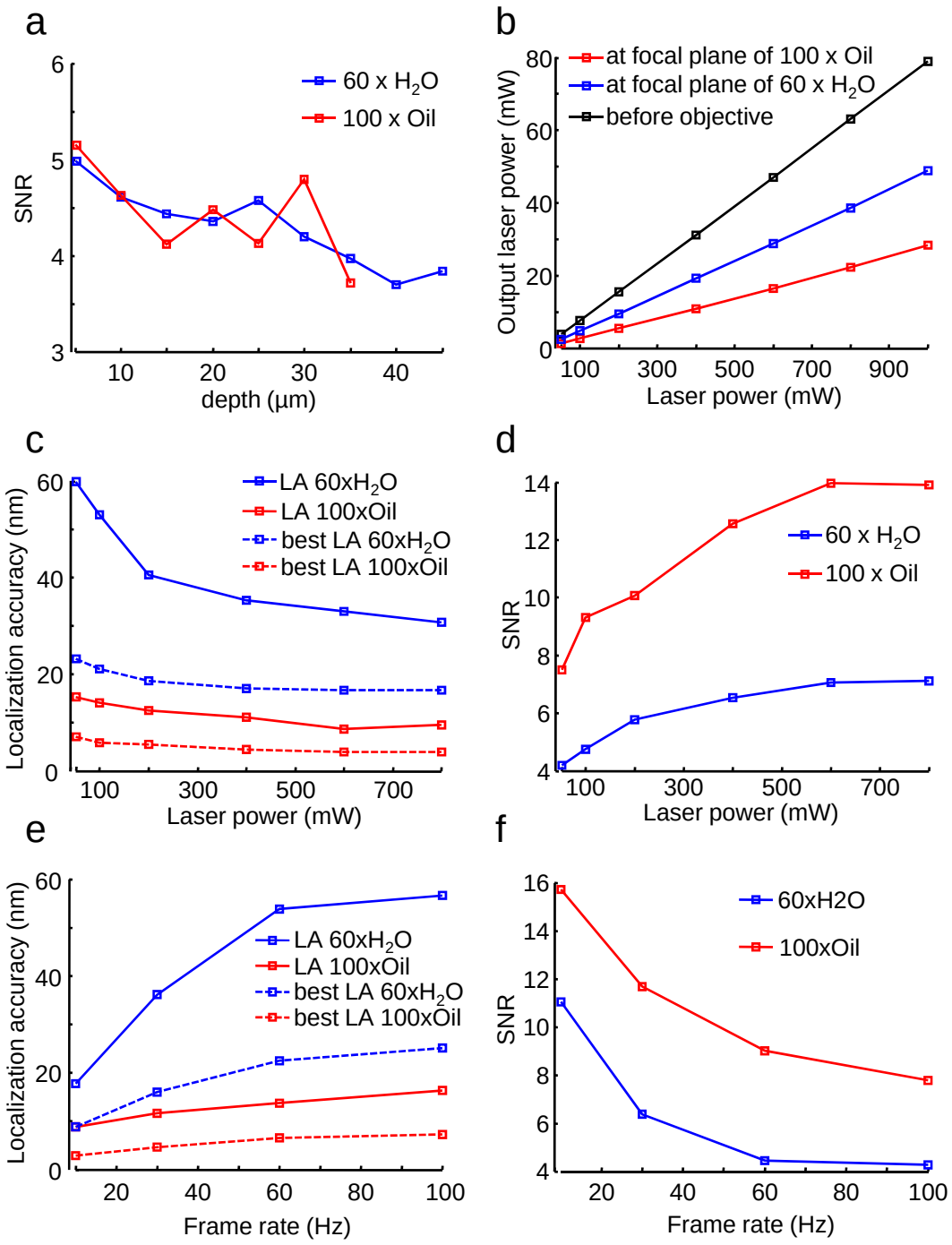
Supplementary Figure S2. Reconstructed trajectories of supplementary movies.

a) The overlay of GPI-GFP fluorescence and reconstructed trajectories (magenta) are shown. **b)** Overlay of the computed trajectories (red) on the GPI-GFP labelled dendrites (white). The green trajectory represents a QD with confined motion and a directly transported QD is shown in magenta. The corresponding MSD-plots over time are given in **c-d)** for confined and direct motion, respectively. The scale bars represent 1µm.



Supplementary Figure S3. Comparison of diffusion properties for QDs and nanobodies.

a) Maximum fluorescent intensity of an individual anti-GFP nanobody (black) in comparison to QD605 (orange). Note the differences in intensity between the different probes. **b-c)** Exemplary trajectories along neurits are plotted for individual nanobodies and QDs (scale bar 1µm). **d)** Distribution of diffusion coefficients of nanobodies (black) and QDs of different size; green (525 nm emission), orange (605 nm emission) and red (655 nm emission). The error bars represent ± s.e.m.. **e)** Immobile fraction of nanobody and QD labelled GPI-GFP molecules. The differences are tested by one-way ANOVA followed by a Bonferronis multiple comparison test, ***$p < 0.0001$. The error bars represent ± s.e.m. **f)** Median of diffusion coefficient of mobile fraction of nanobody and QD labelled GPI-GFP molecules with interquartile range, differences are tested by Kruskal-Wallis test followed by a Dunn's multiple comparison test, ***$p < 0.001$. Data are from 5062 trajectories for nanobody-ATTO488 labelled GPI-GFP molecules, 4150 trajectories for QD-525 labelled molecules, 5382 trajectories for QD-605 labelled molecules and 2788 trajectories for QD-655 labelled molecules recorded from two different cultures.



Supplementary Figure S4. Localisation accuracy of different objectives.

a) SNR as a function of the depth, computed from immobile QDs in the tissue. The sampling rate was 30Hz and the laser power was set to 400 and 100mW for the 60x water and 100x oil objective, respectively. **b)** The laser power directly after the CSU and at the focal planes of the objectives shows linear dependency on the input power. **c-f)** Localisation accuracy and SNR as a function of the laser input power and the sampling rate for different objectives. The data are obtained from immobile QDs dried on a cover slip. The dashed line denotes the theoretical limit of the localisation accuracy and the straight line represents the localisation accuracy computed from Gaussian fits to repeated measurements.

Input resistance (MΩ)	236.7 ± 65.9
Membrane potential (mV)	-57.2 ± 1.5
Max. firing frequency (Hz)	73.2 ± 15.3
Steady state frequency (Hz)	26.6 ± 8.7
Spike amplitude (mV)	98.8 ± 13.5
Spike half width (ms)	3.3 ± 0.8
Spike threshold (mV)	-50.1 ± 2.1
EPSC amplitude (pA)	14 ± 2
EPSC frequency (Hz)	0.5 ± 0.1

Supplementary table S1. Electrophysiological properties of neurons in cultured slices.

Data are from n = 6 out of 4 slices and represent mean ± s.e.m..