

## Supporting information

### Mapping the relationship between total and functional antibodies conjugated to nanoparticles with spectrally-resolved direct stochastic optical reconstruction microscopy (dSTORM)

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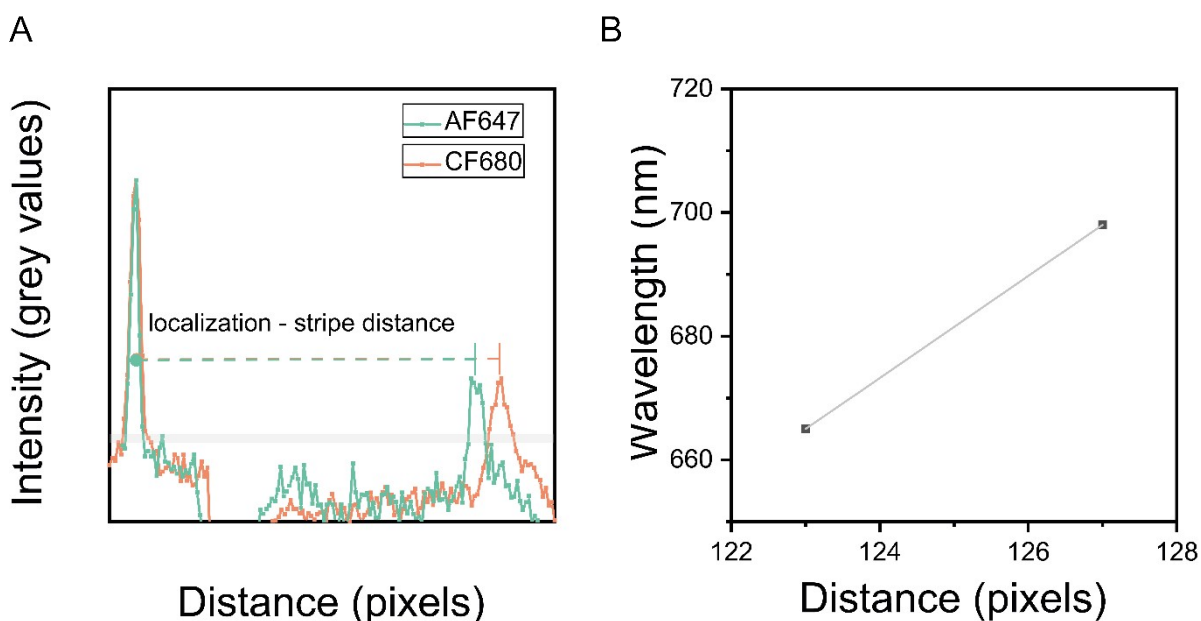
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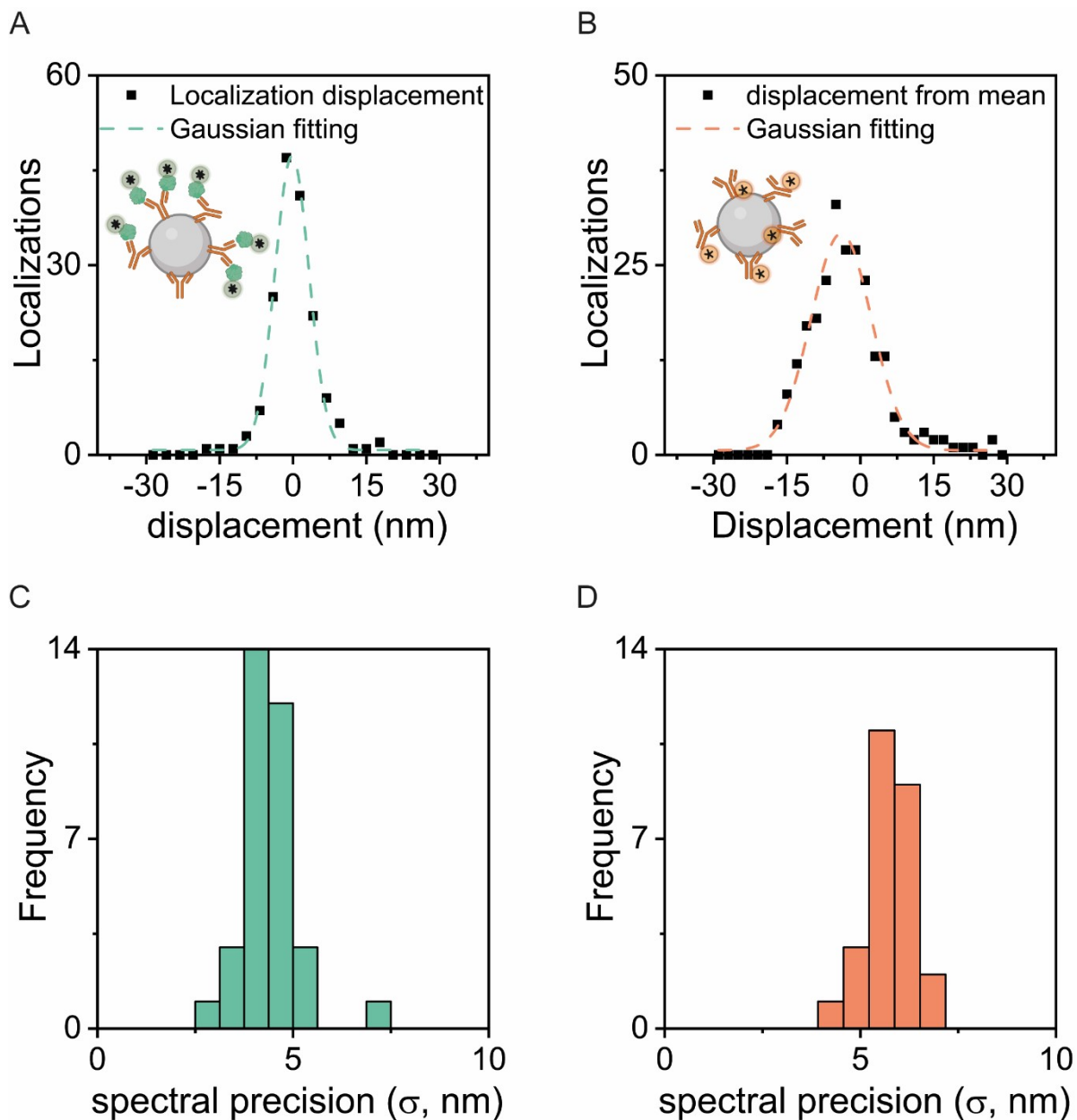
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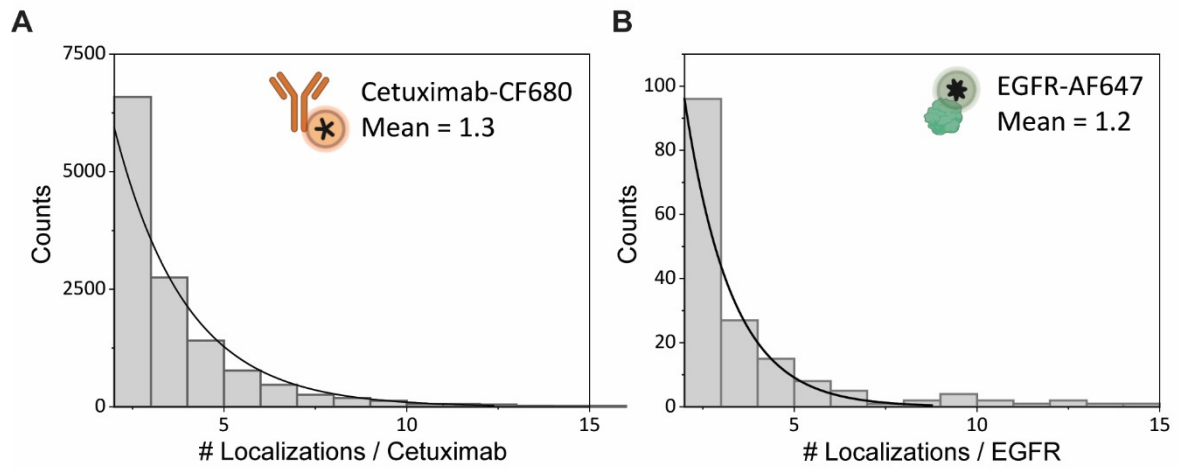
#### Supplementary Figures



**Supplementary Figure S1.** A) Representative raw intensity profile of two single-localizations on a single nanoparticle (AF647 in green and CF680 in orange). Each fluorophore has a unique spectral peak which is reflected in pixel distance from localization in spatial region. B) The localization - stripe pixel distance can be calibrated to extract the pixel to wavelength ratio.



**Supplementary Figure S2.** A) Wavelength peak displacements from the mean value for a single EGFR-AF647-labelled nanoparticle. B) Wavelength peak displacements from the mean value for a single cetuximab-CF680-labelled nanoparticle. Each square corresponds to a single localization. The single-particle spectral precision ( $\sigma$ ) can be calculated by fitting a Gaussian profile. C) Histogram of spectral precisions from 40 EGFR-AF647-labelled nanoparticles. D) Histogram of spectral precisions from 40 cetuximab-CF680-labelled nanoparticles.



**Supplementary Figure S3.** Quantification of single-molecule localizations per A) cetuximab-CF680 and B) EGFR-AF647 molecules. The histogram of the number of localizations per cetuximab was fitted with an exponential decay function to obtain the mean.