

Supporting Information

of

Super-resolution Localization and Defocused Fluorescence Microscopy on Resonantly Coupled Single-Molecule Single-Nanorod Hybrids

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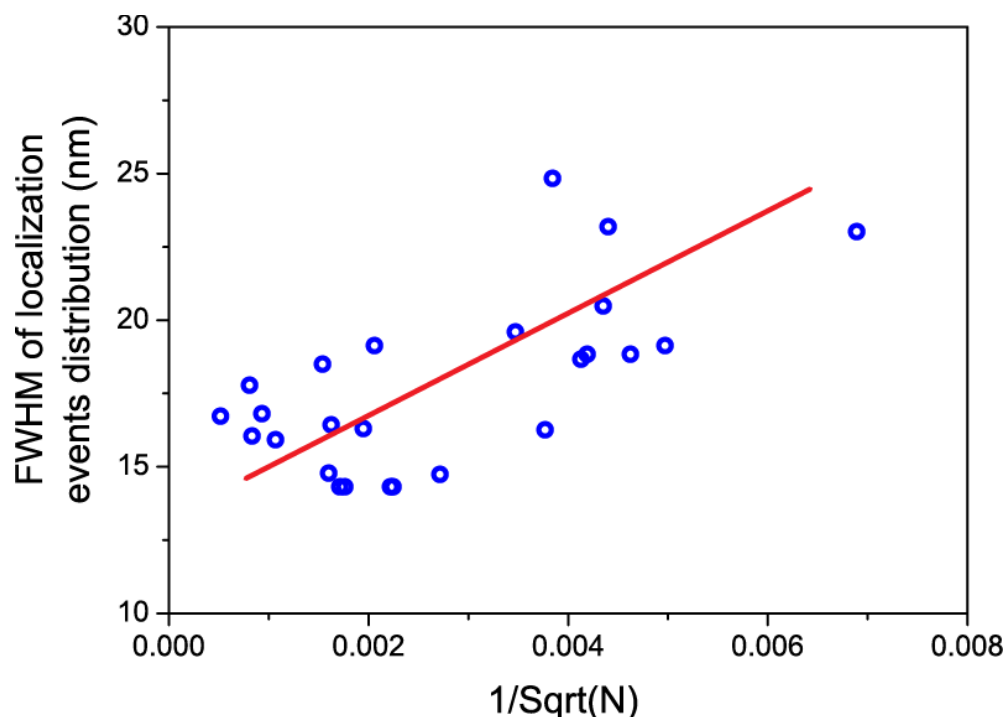


Figure S1. Correlation between the FWHMs of localization events distribution measured on each nanorods and their maximum fluorescence burst intensities (N). We use the inverse of the square root of the maximum fluorescence burst intensity (N) to demonstrate the dependence on localization results and the fluorescence enhancements. The blue cycles represent measurements on each individual nanorod. The red solid line is guide for eye.

In the following, we comment on the correlation between the localization event distributions and the fluorescence enhancement on each nanorod.

Firstly, we comment on the distributions in nanorod dimensions and in plasmon resonances. We note that the geometries of the nanorods are similar but with standard deviations of 7 nm in lengths and of 5 nm in diameters. The longitudinal plasmon resonances, which contributes the most to the fluorescence enhancement in our study, show strong correlation with aspect ratios (the length divided by the diameter) of nanorods.¹⁻² Therefore, slight differences in dimensions can lead to significant differences in aspect ratios, resulting in plasmon resonances of individual nanorods ranging from 580 nm to 700 nm in glycerol.

Secondly, we comment on the surface plasmon dependent fluorescence enhancements. As reported in previous studies,³ the plasmon induced fluorescence enhancement strongly relies on the resonant coupling between surface plasmon resonances of individual nanorods and the molecule's absorption/emission spectra, leading to significant differences in the observed intensities on each nanorod.³ We found that different nanorods can result in different enhanced fluorescence intensities of more than 10 fold differences.

Finally, we explain how the differences in enhanced fluorescence intensities can

influence the localization accuracy in our experiment. In super-resolution localization microscopy, the localization accuracy relies on the signal to noise ratio which in turn depends strongly on the number of collected photons. Generally, the accuracy is inversely correlated with the square root of the photons collected during each blinking event,⁴ the enhanced fluorescence burst intensities in our case. Therefore, the localization is expected to vary depending on fluorescence enhancements by individual nanorods, which differs due to differences in plasmon resonances. We would estimate the localization accuracies in our experiment can vary with a factor about 3 times due to the 10-fold differences in enhanced fluorescence burst intensities. The aforementioned correlation is shown in figure S1. The FWHM of localization event distribution narrows when the fluorescence enhancement gets stronger.

1. Link, S.; Mohamed, M. B.; El-Sayed, M. A., Simulation of the Optical Absorption Spectra of Gold Nanorods as a Function of Their Aspect Ratio and the Effect of the Medium Dielectric Constant. *J. Phys. Chem. B* **1999**, *103*, 3073-3077.
2. Kou, X. S.; Zhang, S. Z.; Tsung, C. K.; Yeung, M. H.; Shi, Q. H.; Stucky, G. D.; Sun, L. D.; Wang, J. F.; Yan, C. H., Growth of Gold Nanorods and Bipyramids Using Cteab Surfactant. *J. Phys. Chem. B* **2006**, *110*, 16377-16383.
3. Khatua, S.; Paulo, P. M. R.; Yuan, H. F.; Gupta, A.; Zijlstra, P.; Orrit, M., Resonant Plasmonic Enhancement of Single-Molecule Fluorescence by Individual Gold Nanorods. *Acs Nano* **2014**, *8*, 4440-4449.
4. Huang, B.; Bates, M.; Zhuang, X. W., Super-Resolution Fluorescence Microscopy. *Annu. Rev. Biochem.* **2009**, *78*, 993-1016.