SUPPLEMENTARY INFORMATION

Nano-dynamic imaging: NANOSPACER as a low-cost optical method for real-time visualization of nanoparticle disassembly and functionalization

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Table S1. Cost of other nanoparticle imaging techniques compared to NanoSpacer.

Technique	Approximate. Instrument Cost	Per-Sample Cost	Expertise Needed	Time to Result	Dynamic Imaging Capability	Notes
Transmission Electron Microscopy (TEM)	£400k–£1.6M	£40–£160	Very High	Minutes-Hours	No	High spatial resolution (<1 nm). Static.
Cryo-TEM	£800k–£2.4M	£150–£320	Very High	Hours	No	High spatial resolution, hydrated samples. Static
Liquid-Phase TEM	£1.2M–£2.4M	£160+	Very High	Hours	Yes	Real-time in liquid, but expensive and radiation-damaging.
Atomic Force Microscopy (AFM)	£150k–£400k	~£20–30 per sample (tip + substrate)	High	Hours	Yes	Dynamic surface imaging; liquid, slow.
In situ liquid cell SEM	£400k–£1.2M	£120–£250	High	Hours	Yes	Hard for organic samples.
Interferometric Scattering Microscopy (iSCAT)	£240k–£640k	< £5	Very High	Hours	Yes	Complex optics and individual set-ups
Dark-field Microscopy	£8k–£80k	< £1	Moderate	Minutes	Yes	Simple, limited resolution for organic NPs.
Nanoparticle Tracking Analysis (NTA)	£60k–£120k	< £1	Moderate	Minutes	Yes	Particle tracking; limited below 60 nm.

NanoSpacer + Olympus BX53M (this work)	**£3-20k microscope**	**~£1-10**	**Low**	**Minutes**	**Yes (Real Time)**	Enables label-free dynamic imaging using standard microscope optics.

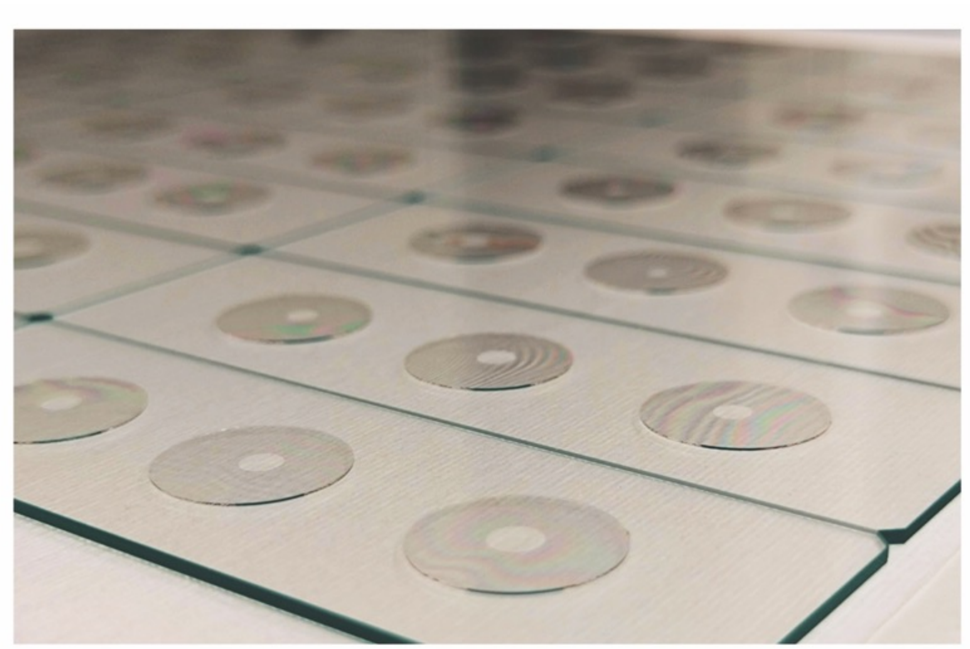

Figure S1. Image of the NanoSpacers after the assembly of the cover slip and glass slides.

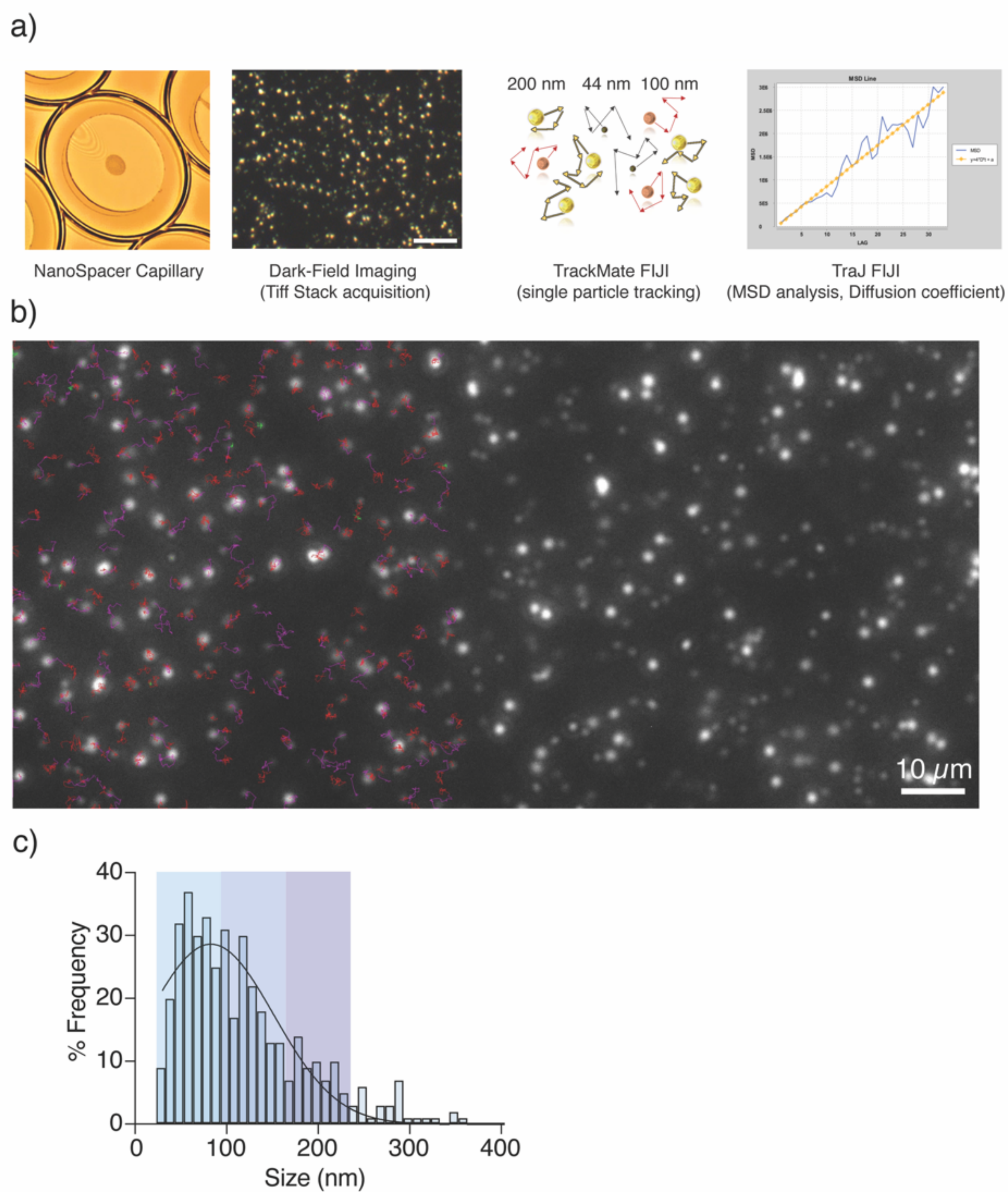

Figure S2. Images of polystyrene nanoparticles, as well as sizing data across a number of sizes, 44, 100, and 200 nm. a) Cartoon of the overall process of sizing. b) Image of all sizes (44, 100, and 200 nm) of polystyrene particles; (left) Example of TraJ tracks overlay; (right) TraJ tracks not plotted to show original data. c) Frequency plot of the polystyrene nanoparticles of 44, 100, and 200 nm over complete field of view. (Individual particle tracks were used for the size histogram generation.)

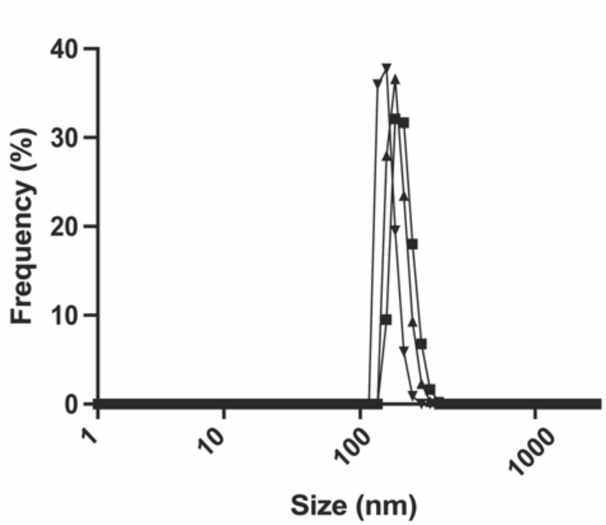

Figure S3. Dynamic light scattering (DLS) of indocyanine green J-aggregate nanoparticles in deionized water three different measurements were performed, and together demonstrate and average particle size of 196 nm with a PDI of 0.28.

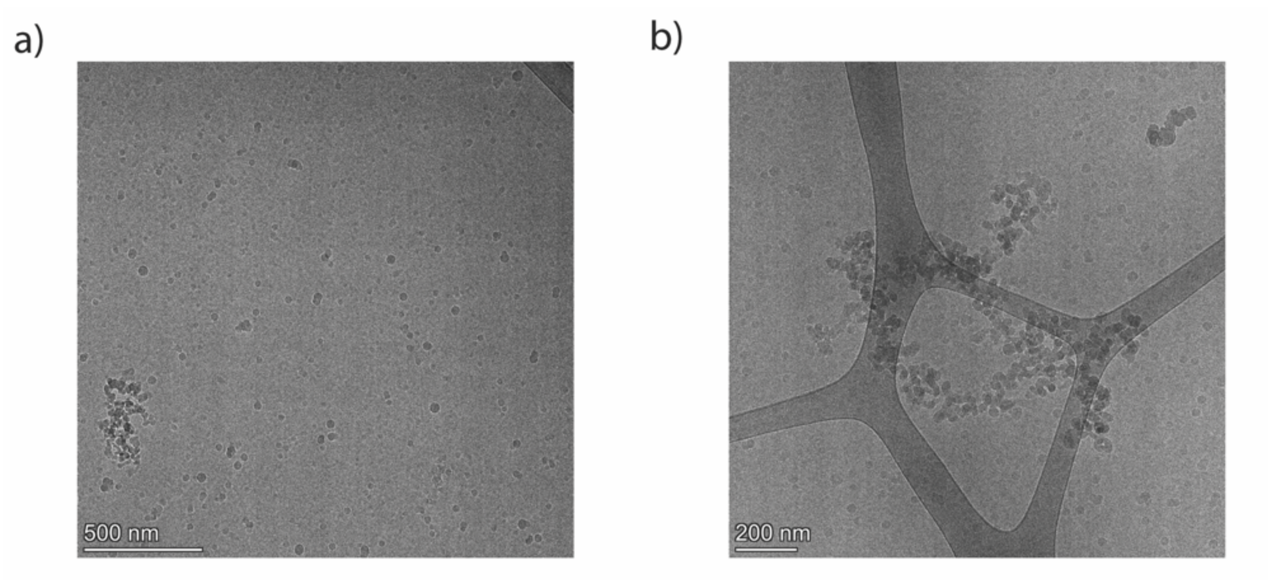

Figure S4. Cryo-TEM images of Indocyanine green J-aggregate nanoparticles using different fields of view a) 500 nm and b) 200 nm scale bar.

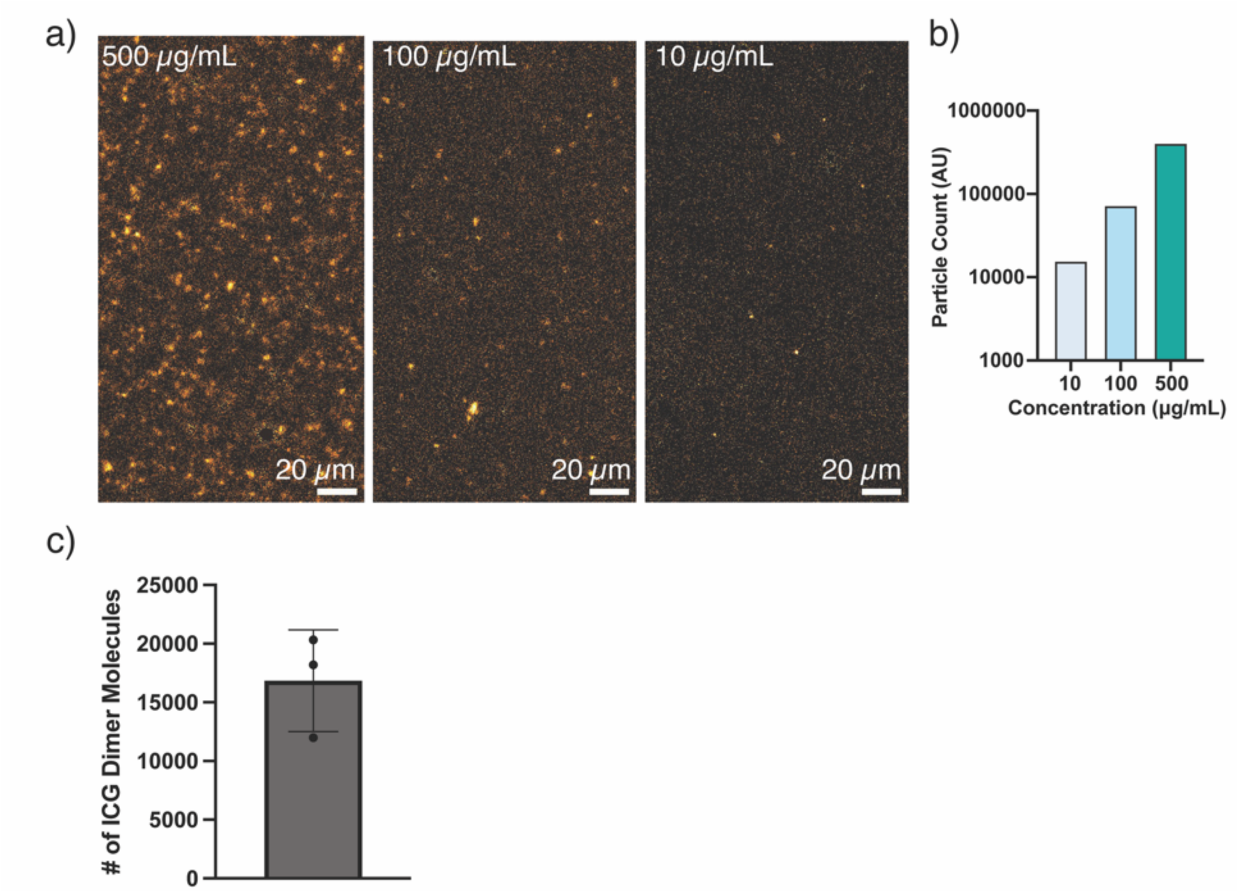

Figure S5. Quantification analysis of ICG J-aggregate nanoparticles using the NanoSpacer. a) Images of different dilutions used for quantification. b) Plot of particle count (AU) to concentration. c) Average calculation from the three measurements across the range of concentrations.

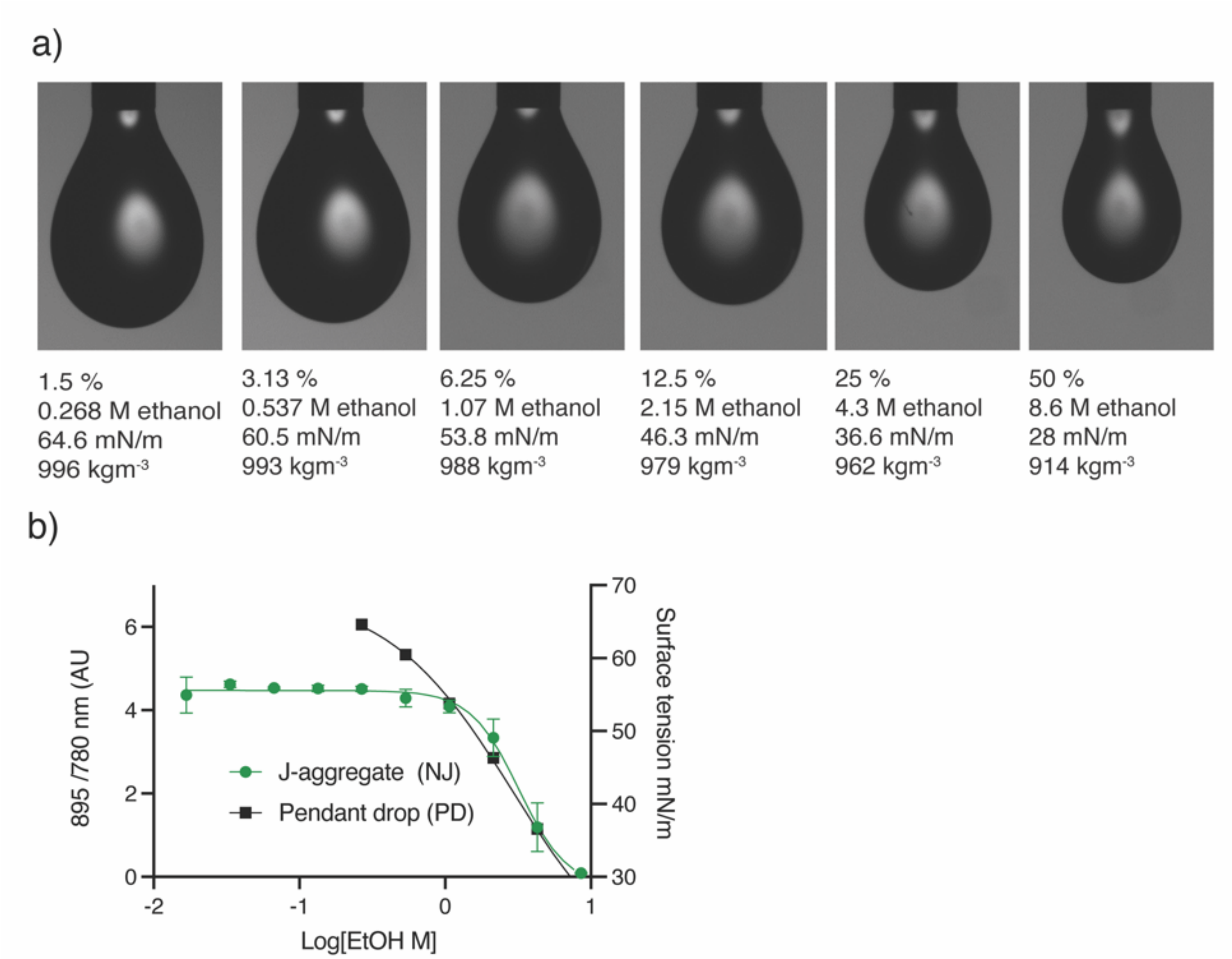

Figure S6. ICG J-aggregate Nanoparticle disassembly in solvents UV-VIS as well as surface tension measurements of drop shape**.** a) Pendant drop analysis of different ethanol concentrations. b) Overlay of UV-VIS monitored disassembly process with ethanol, and corresponding surface tension measurements.

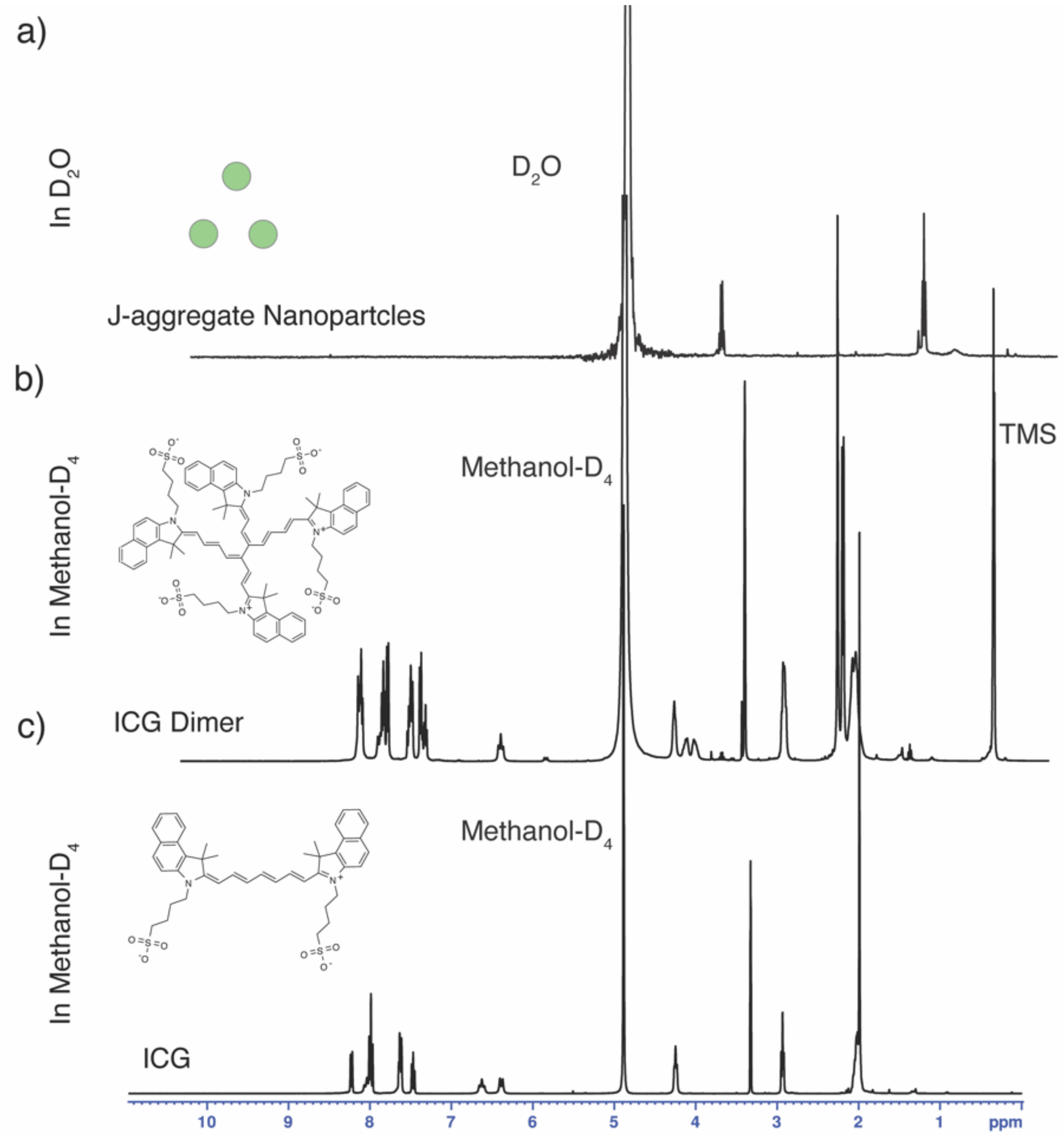

Figure S7. Indocyanine Green J-aggregate disassembly in deuterated methanol determined by NMR. a) ^{1}H NMR spectrum of nanoparticles J-aggregates in D_2O. b) ^{1}H NMR of nanoparticle J-aggregates obtained using deuterated methanol (CD_3OD), revealing dimer structure as particles dissemble. c) ^{1}H NMR of ICG monomer in deuterated methanol (CD_3OD).

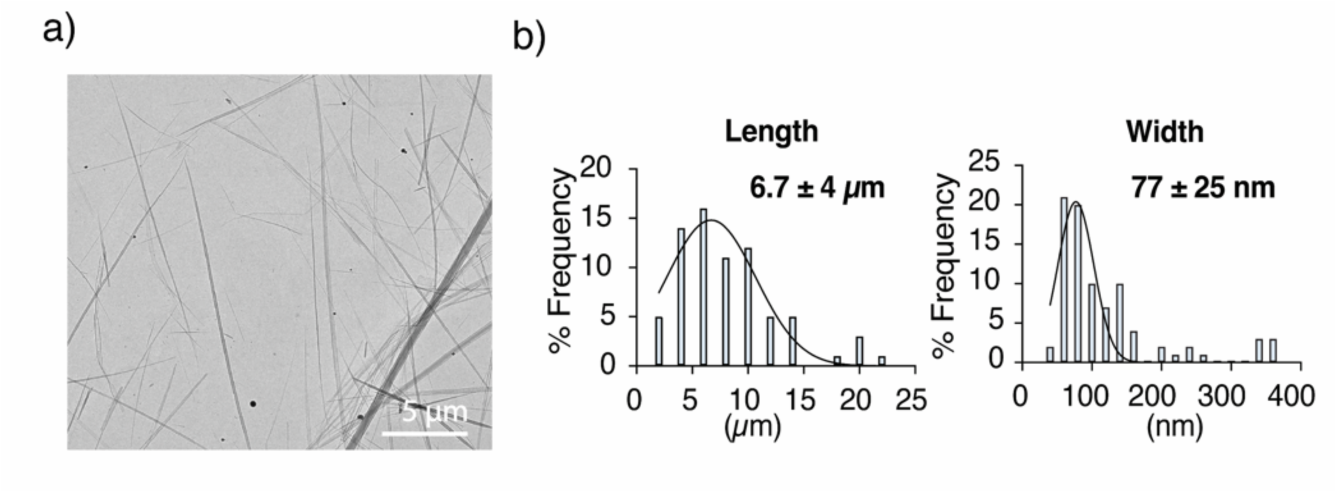

Figure S8. a) Transmission electron microscopy images of ICG and ICG-azide nanorod J-aggregate. b) Average nanorod width and length of distributions obtained from TEM images using Fiji.

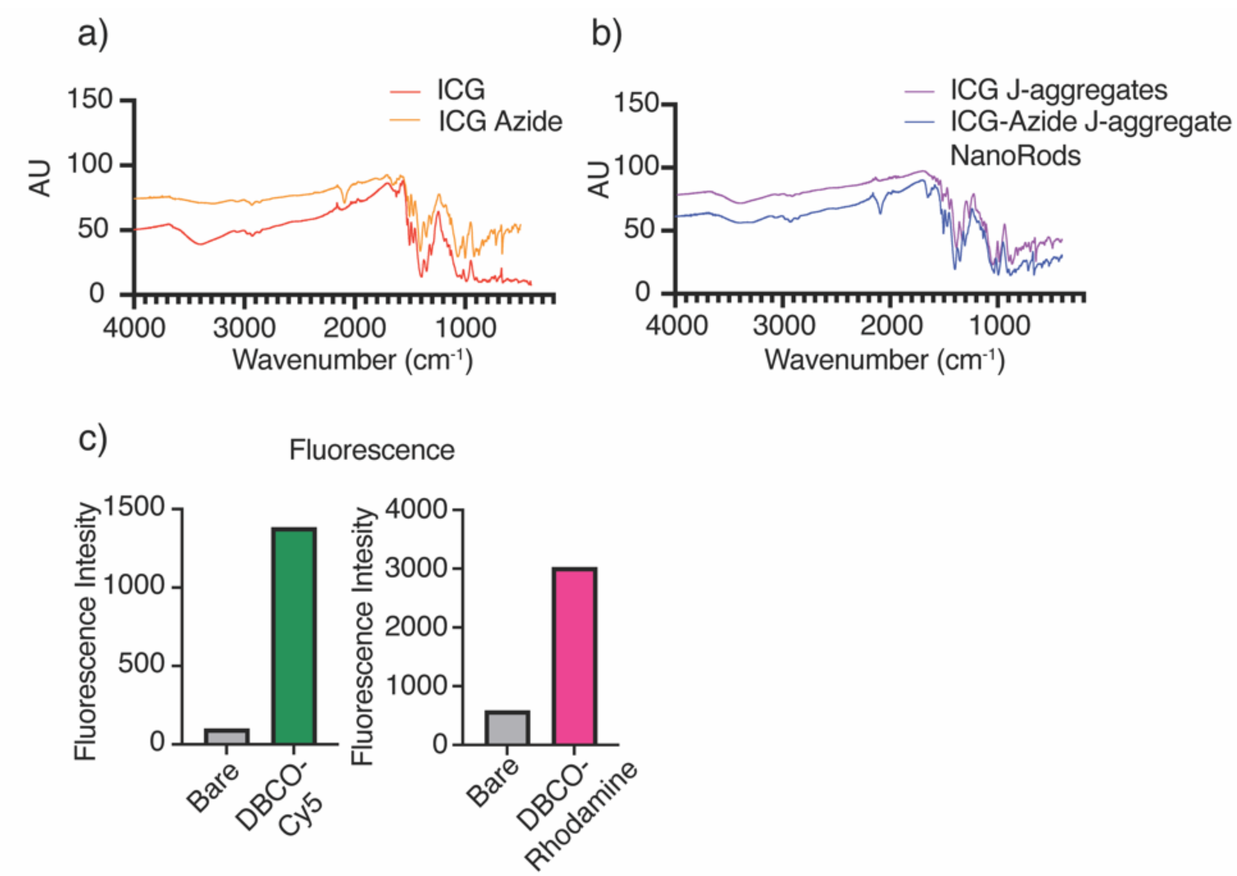

Figure S9. Confirmation of azide present in ICG-azide J-aggregate nanorods. a) Fourier transform infrared spectroscopy (FTIR) spectra of both ICG as well as ICG azide. b) FTIR spectra of ICG J-aggregates and ICG-ICG-azide J-aggregates. c) Fluorescence of washed ICG-ICG azide hybrid nanorods before and after the addition of DBCO- cy5 and DBCO-rhodamine, and three subsequent washes.

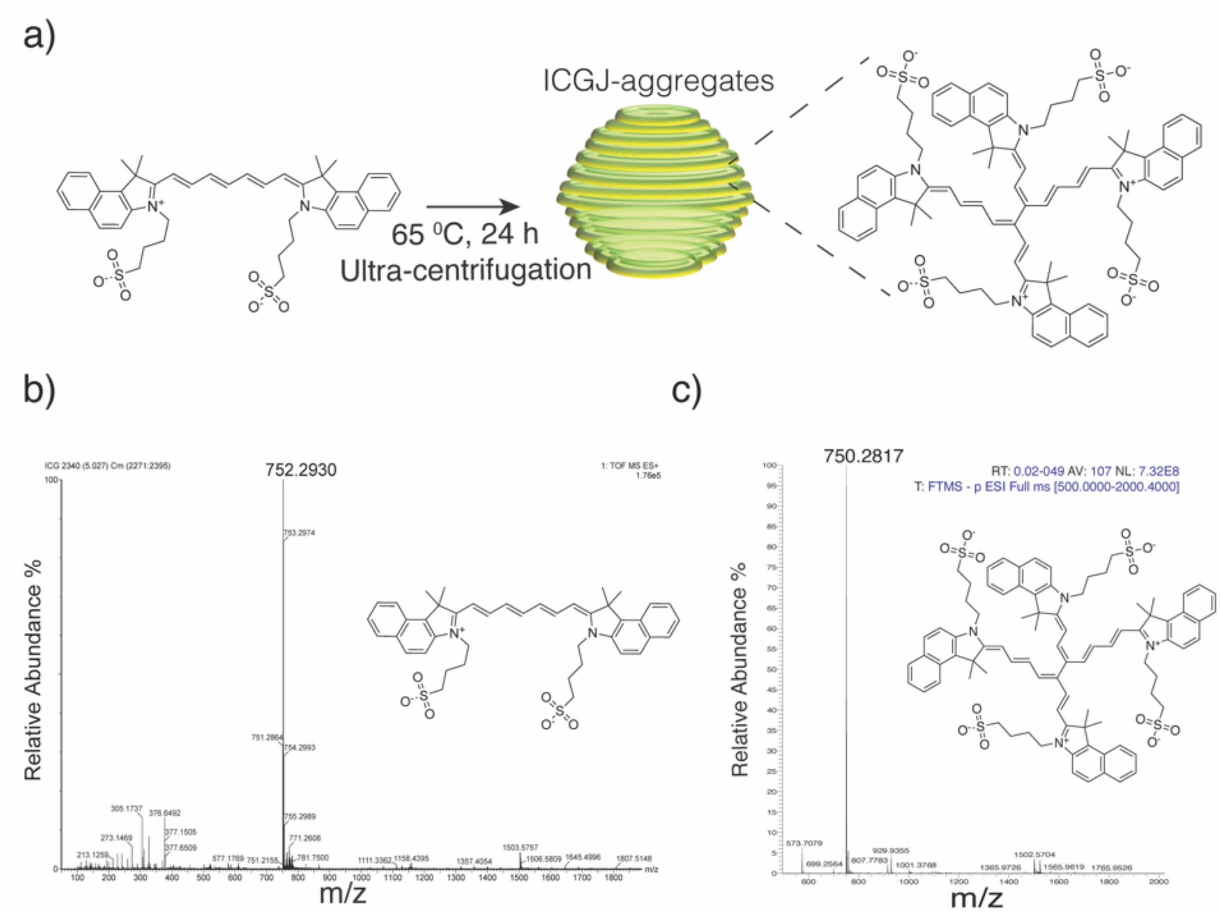

Figure S10. Mass spectrometry analysis of normal ICG J-aggregates demonstrating they are composed of dimers of ICG. a) Cartoon of Nanoparticle synthesis and resulting structure. b) Mass spectra of ICG, Mass predicted from $C_{43}H_{47}N_{2}O_{6}S_{2}^{-}$ $(M+H)^{+}$:752.29; Found:752.2930. c) Mass spectra of normal ICG J-aggregates after disassembly of the Nanoparticles in methanol mass predicted from $C_{86}H_{92}N_{4}O_{12}S_{4}^{2-}$: 750.28; Found:750.2817.

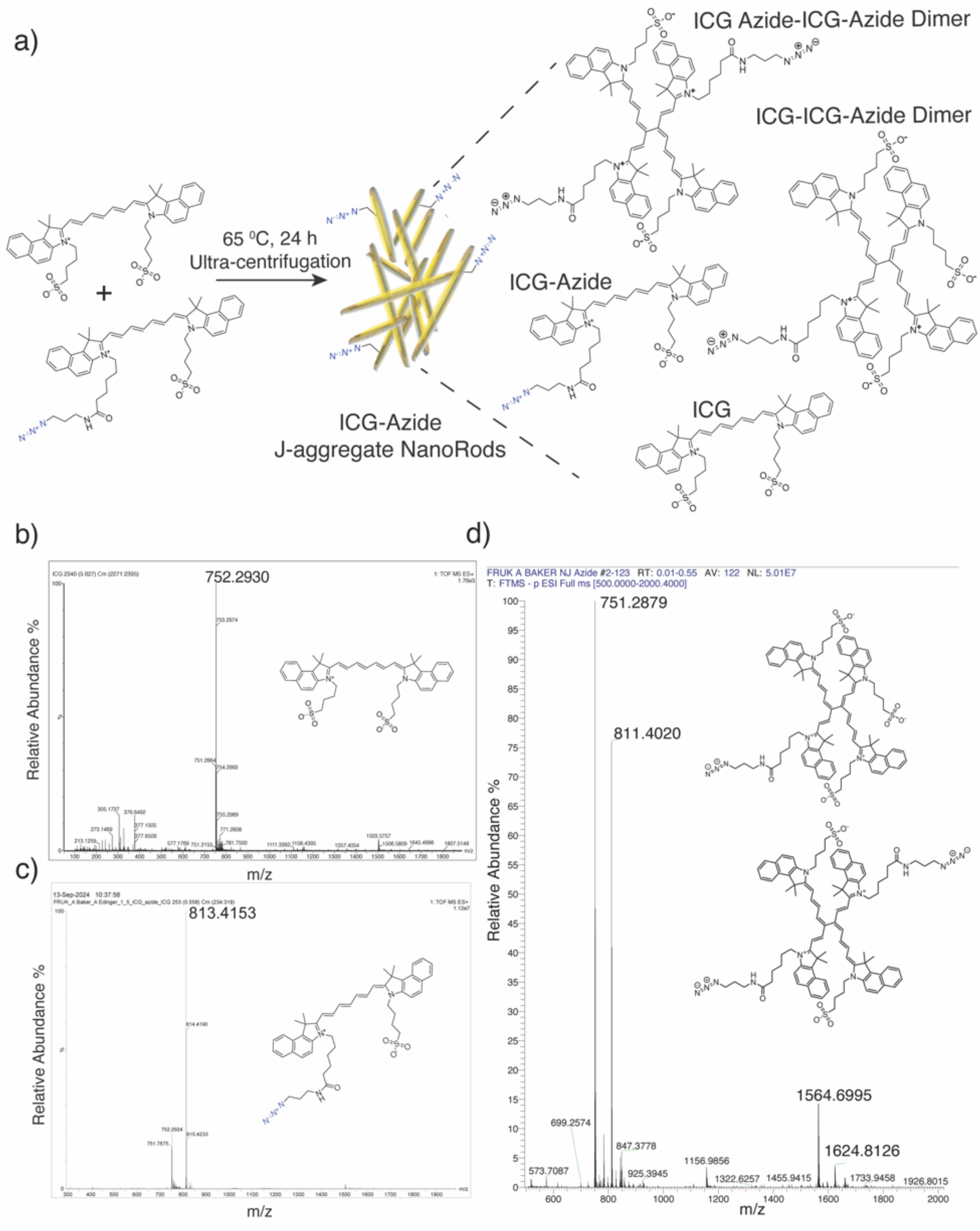

Figure S11. Mass spectrometry analysis of ICG-Azide J-aggregate NanoRods of Nanoparticle synthesis and assembly. a) Cartoon of ICG Azide NanoRod synthesis. b) Mass spectra of ICG Mass spectra of ICG Mass predicted from C43H47N2O6S2- $(M+H)^+$:752.29; Found:752.2930. c) Mass spectra of ICG Azide Mass predicted from C48H56N6O4S $(M+H)^+$: 813.41; Found: 813.4153. d) Mass spectra of ICG ICG-Azide NanoRods after dissolving in methanol, ICG-ICG Azide dimer predicted mass from $C91H101N8O10S3^-$ $(M+2H)^+$: 1564.68; Found: 1564.6995. ICG Azide-ICG Azide dimer predicted mass from C96H110N12O8S2 (M+H): 1624.80; Found 1624.8126.

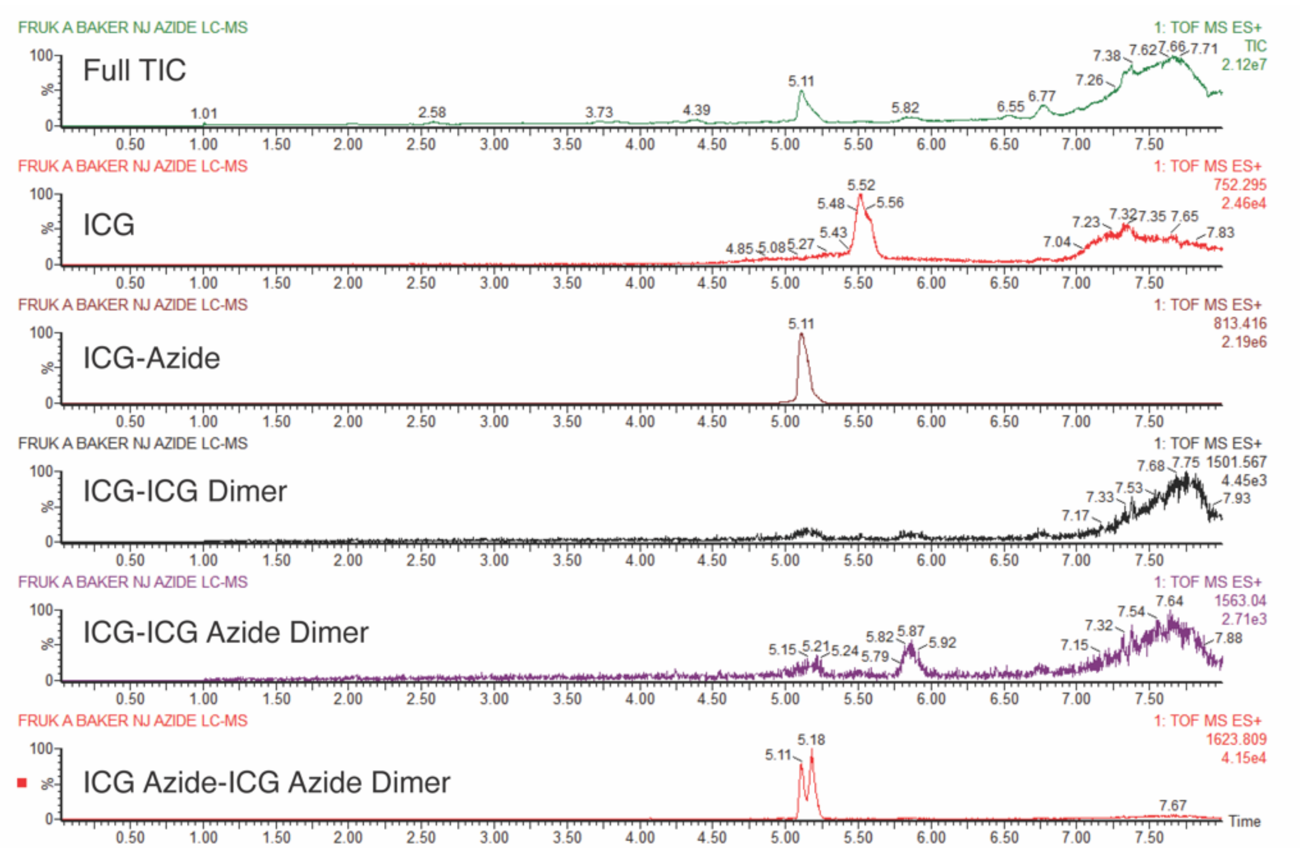

Figure S12. HPLC chromatogram for ICG-ICG Azide J-aggregate NanoRods analyzed for specific masses for each species in the reaction. ICG 752.2 m/z, ICG-azide 813.4, ICG-ICG dimer 1501.6 m/z, ICG-ICG-azide dimer 1563 m/z, and ICG azide-ICG azide dimer 1623.8 m/z.

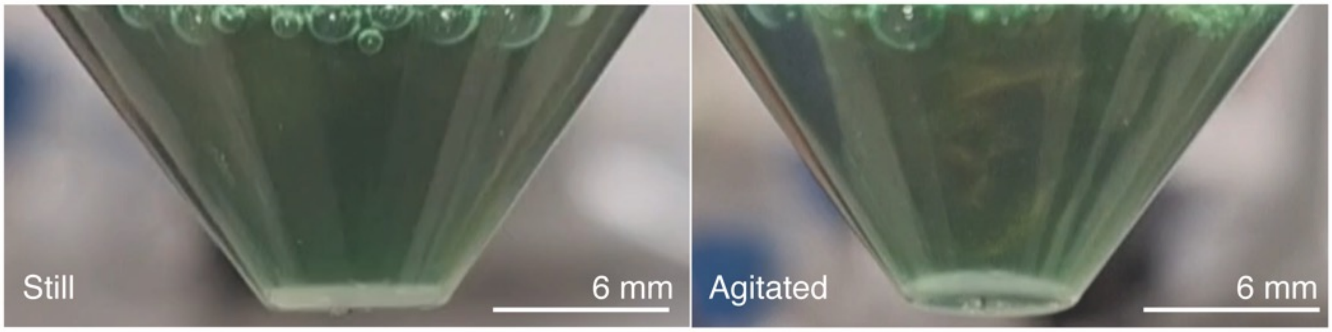

Figure S13. Hybrid ICG and ICG azide J-aggregate NanoRods scatter in bulk solution before and after agitation.

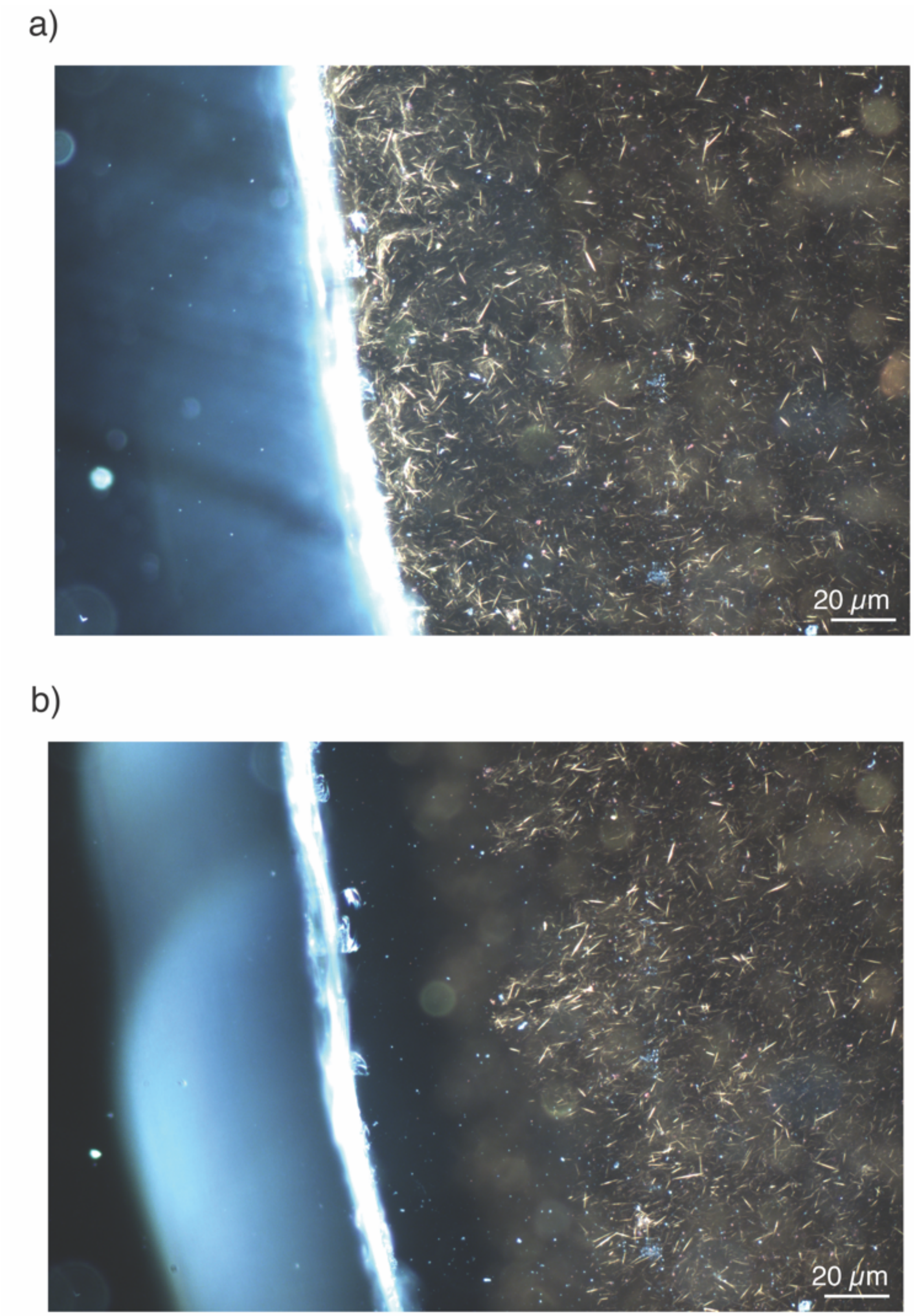

Figure S14. ICG Azide Nanorod disassembly as viewed from the MicroSpacer. a) Before, and b) after ethanol addition.

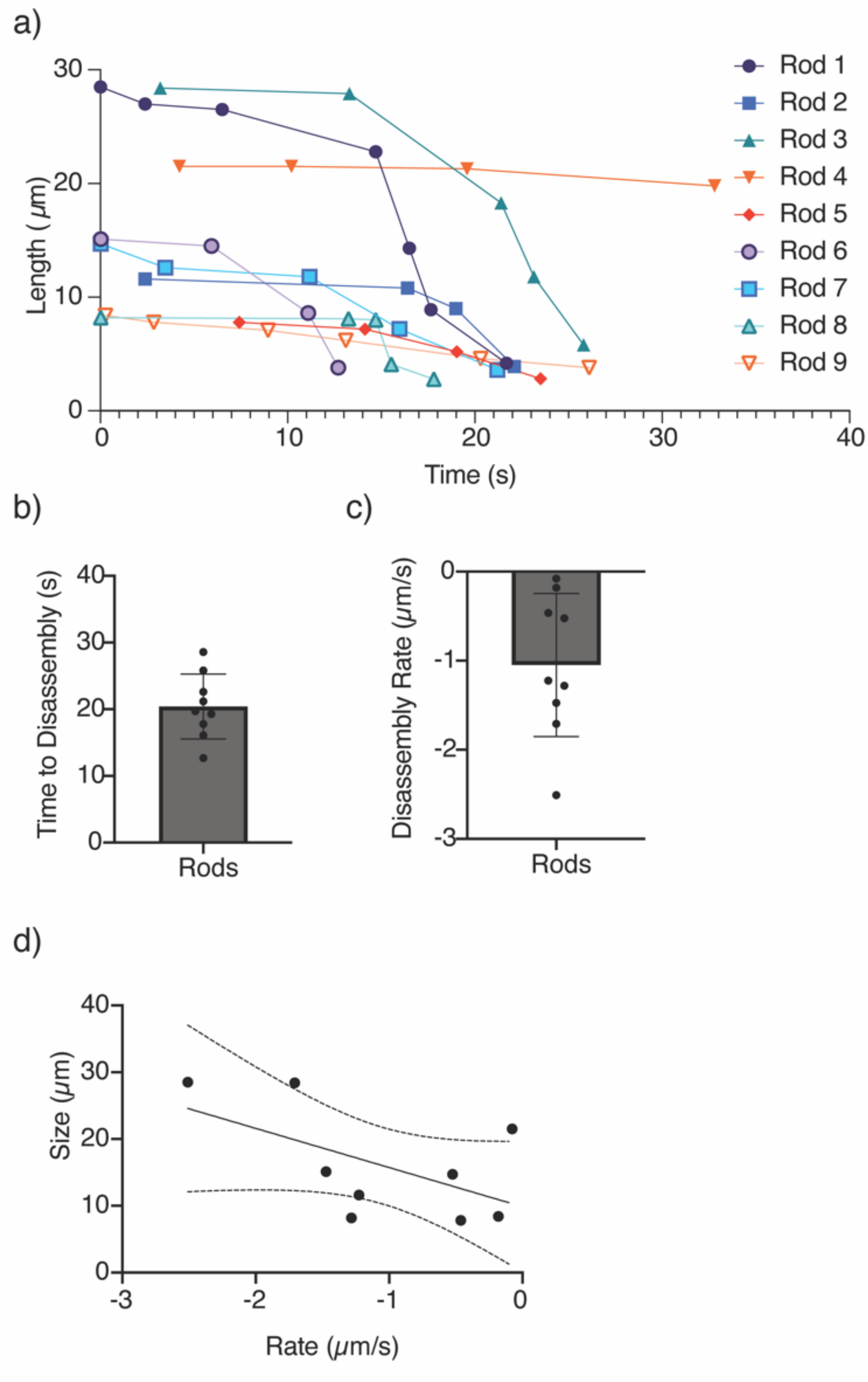

Figure S15. Single nanorod disassembly kinetics. a) individual traces of rod length over time for 9 individual rods. b) Time to disassembly for the individual rods Data represent as mean ± SD. c) Rate of disassembly for n=9 individual rods Data represent as mean ± SD. d) Size versus Time to disassembly for n=9 individual rods ($p=0.1102$, $R^2=0.323$).

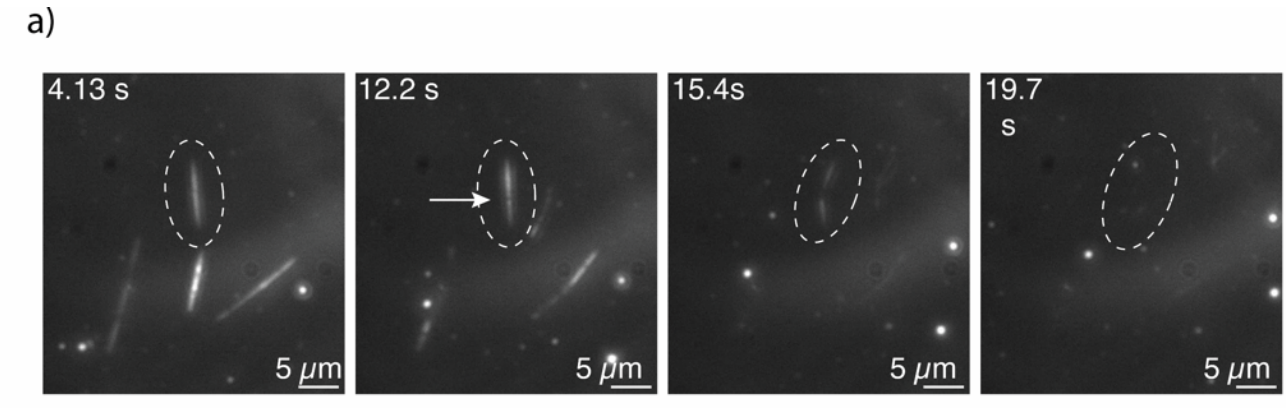

Figure S16. Images from videos demonstrating the breaking point first appears as an area of less density on the nanorods at 12.2 s.

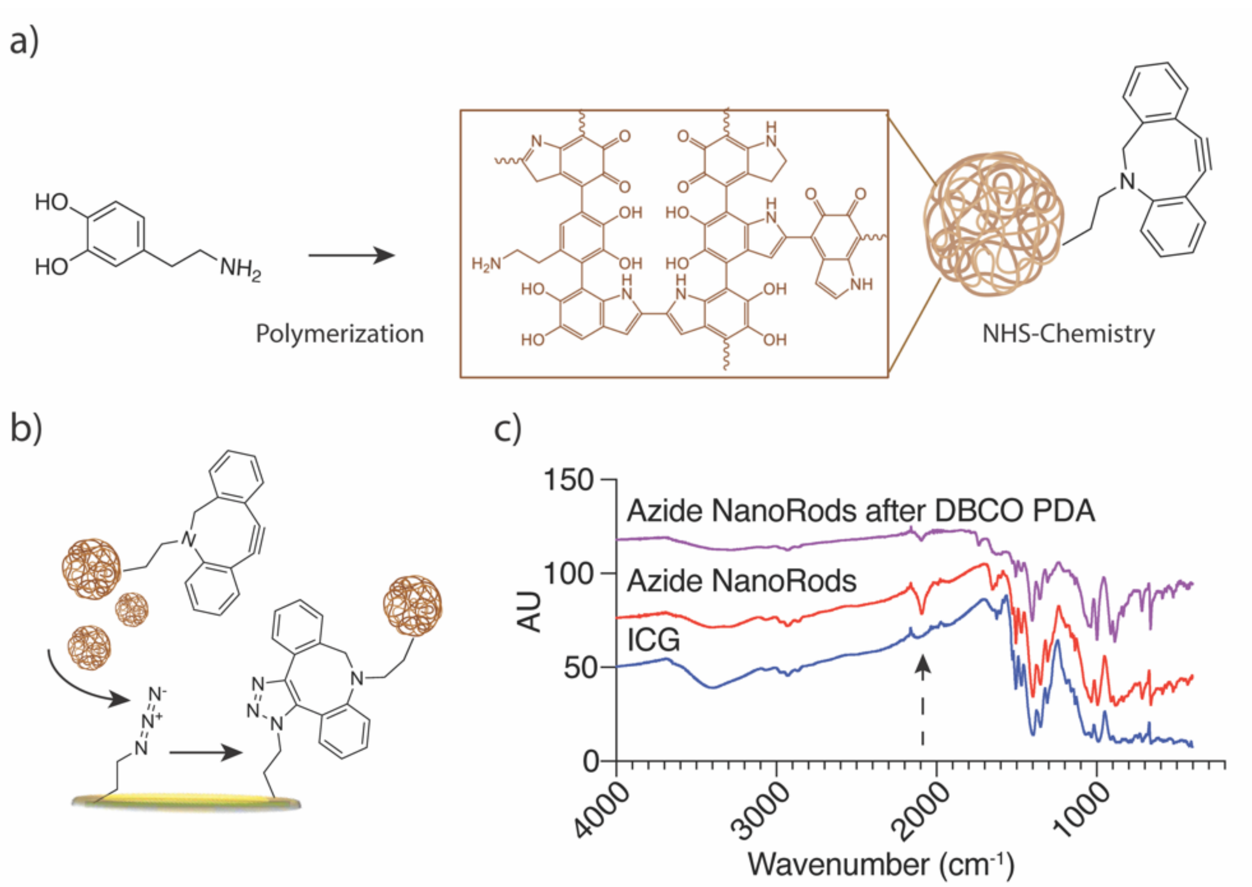

Figure S17. Cartoon of polydopamine modification with DBCO. a) overview of PDA reaction, and DBCO modification on primary surface amines. b) Cartoon of ICG azide nanorod attaching to polydopamine modified with DBCO. c) Fourier transform infrared spectroscopy (FTIR) spectra of ICG, ICG azide NanoRods as well as ICG azide NanoRods after reaction with polydopamine particles modified with DBCO.

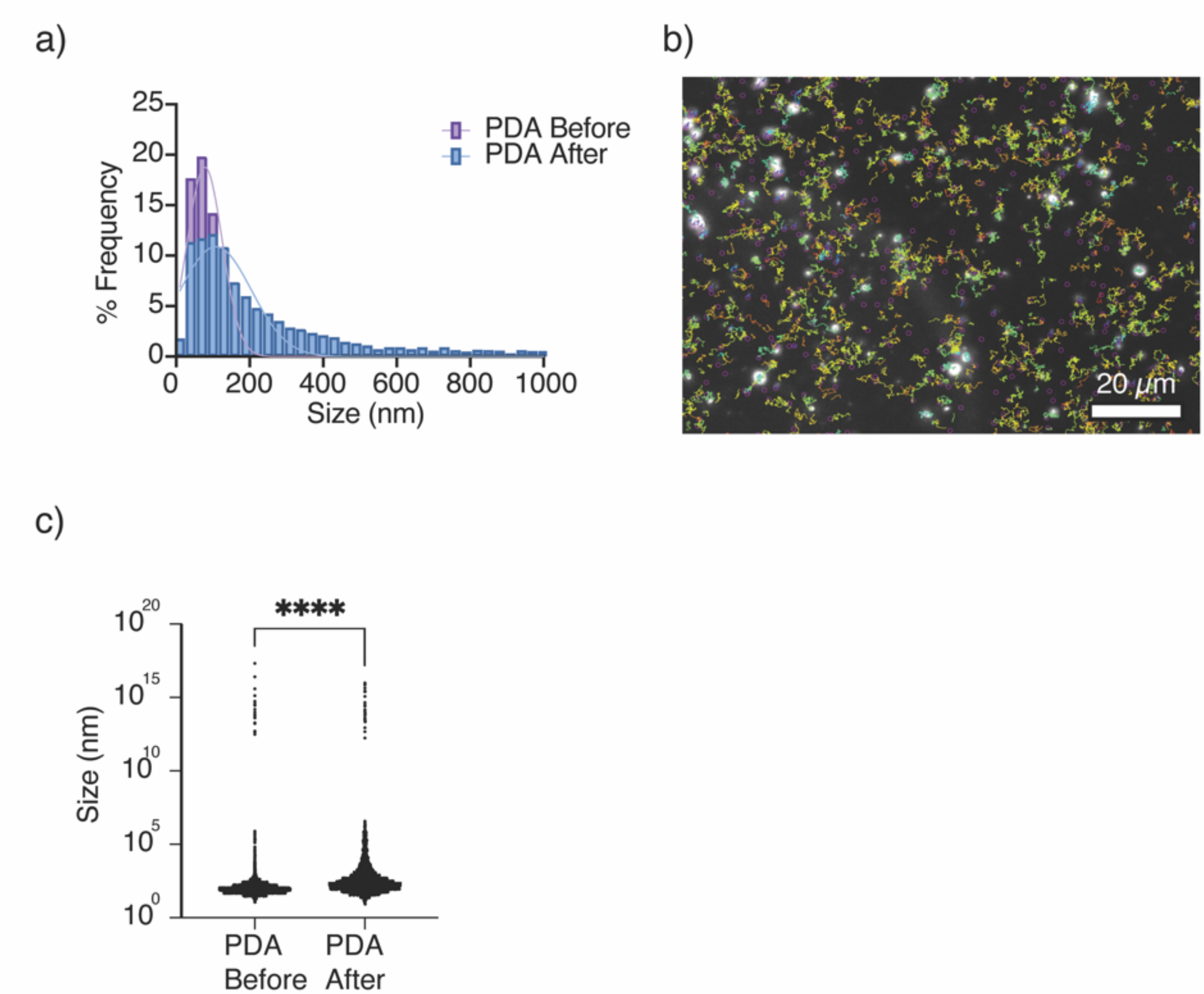

Figure S18. Polydopamine size analysis. a) Measured sizes of PDA both before and after click reaction, 89 nm before (n=10221 events) to 216 nm after (3310 events), a significant change ($p<0.0001$), analyzed using Mann Whitney Test. b) Track traces of PDA speed (nm/s) of PDA nanoparticles without the presence of the ICG azide nanorods. c) Plot of PDA size both before and after reaction analyzed using Mann Whitney Test to calculate the significance ($p<0.0001$), (*p<.05, **p<.01, ***p<.001, ****p<.0001).

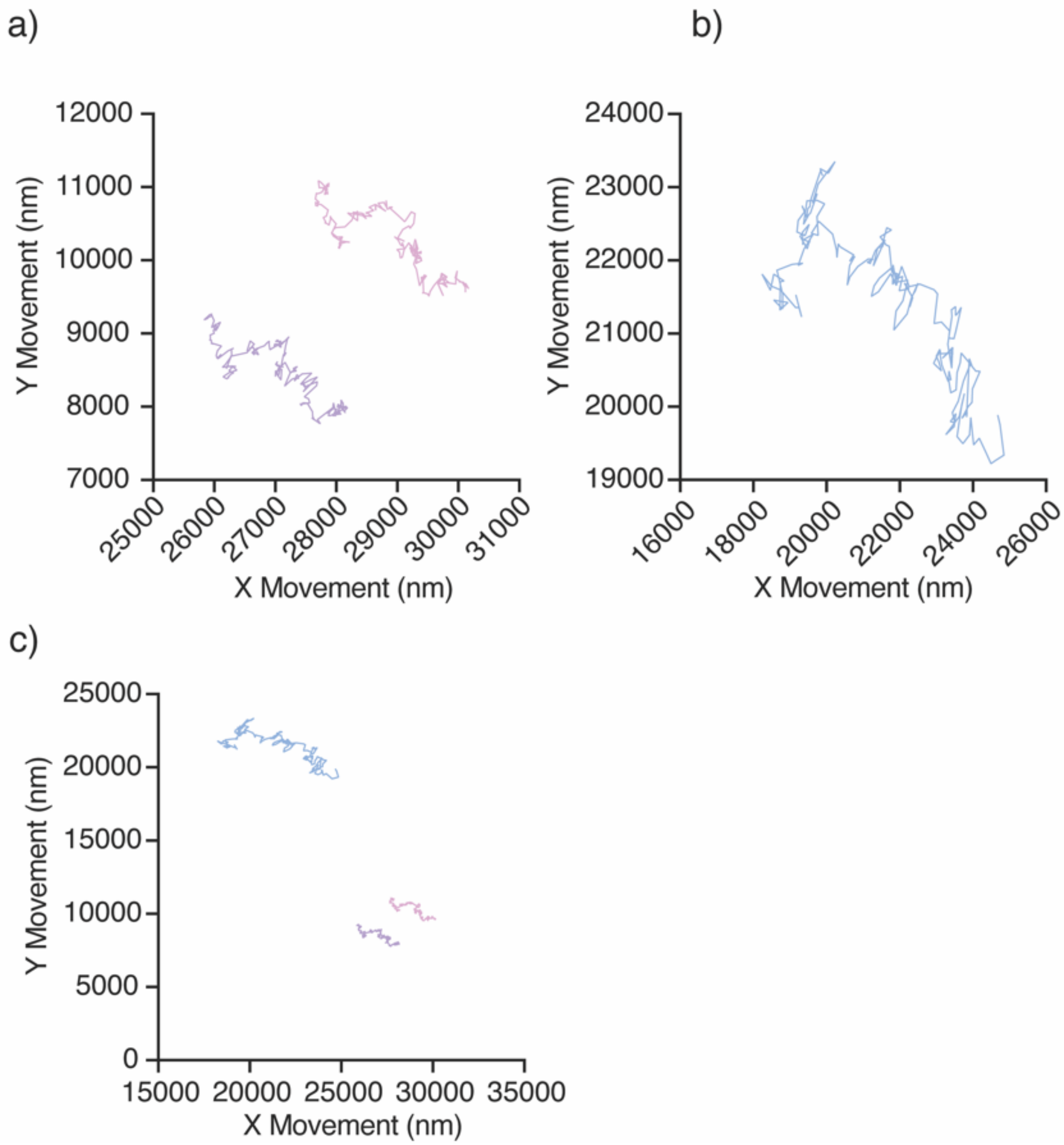

Figure S19. Tracks of single polydopamine particles. a) Nanoparticle tracks of particles attached to the same nanorod and b) freely diffusing. c) Replotted on the same axis to demonstrate scale comparison between the different traces.