Electronic Supplementary Information for the Manuscript Entitled

Functionalized nanoscale through microscale polypeptide stabilized emulsions for display of biomolecules

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Experimental:

General: NeutrAvidin (Pierce), FITC-NeutrAvidin (Pierce), biotin-PEG(3,400)-FITC (Nanocs), triton x-100 (Aldrich), and biotin-NHS ester (Aldrich) were used as received. All other reagents, solvents, and PDMS oil were reagent grade and used without purification. K_{55}(*rac*-L)$_{20}$ was synthesized according to a previous protocol.[1] $^{1}H\{^{13}C\}$ NMR spectra were recorded on a Bruker AVANCE 400 MHz spectrometer. Ultrapure (18 MΩ) deionized water (DI water) was prepared using a Millipore Milli-Q Biocel A10 purification unit. Microscale emulsion droplets were generated using a PowerGen 125 handheld homogenizer. Nanoscale emulsions were prepared with a Misonix Microson XL2000 ultrasonic homogenizer. Droplet sizes were determined using dynamic light scattering (Beckman Coulter N4-Plus). Fluorescence and DIC microscopy was performed on a Zeiss Axiovert 200 microscope.

Synthesis of biotin-K_{55}(*rac*-L)$_{20}$. To a 20 mL scintillation vial was added a stir bar, followed by K_{55}(*rac*-L)$_{20}$ (75 mg, 6.7 x 10^{-3} mmol) that was dispersed in 5.0 mL of a 0.10 M $NaHCO_3$ aqueous solution. Next, NHS (570 µL of a 20 mg/mL solution in water) and EDC (950 µL of a 20 mg/mL solution in water) were added, followed by the addition of biotin-NHS (680 µL of a 10 mg/mL solution in water). The reaction was stirred overnight and then acidified to pH = 3 by addition of 0.10 M HCl. The polypeptide was then transferred to a Spectra/Por dialysis bag (2,000 MWCO), dialyzed against DI water for 3 days (changed twice daily) and then the product was

isolated by freeze drying (70 mg, 93 % yield). Degree of biotinylation was determined using peak integrations from ^{1}H-NMR spectra of the biotinylated copolymer recorded in d-TFA. ^{1}H-NMR (400 MHz, d-TFA, 25 ^{o}C): δ=7.22 (br s, 1H), 5.35-4.89 (br m, 1H), 4.55-4.50 (br m, 2H), 3.73-3.85 (br m, 1H), 3.56 (br s, 2H), 3.38-3.00 (br m, 2H), 2.42-1.56 (br m, 11H), 1.31-1.20 (br m, 12H). The ratio of integrals from the 3.56 (lysine side chain, 2H) and 4.55-4.50 (biotin ring, 2H) resonances was found to be 17.1 to 1, indicating 5.0 mol% biotinylation, or 3 biotin groups per chain.

Microscale biotin-K_{55}(*rac*-L)$_{20}$ stabilized emulsions. To a 2 dram vial, 600 µL of a biotin-K_{55}(*rac*-L)$_{20}$ (1.0 mM) solution and 600 µL of K_{55}(*rac*-L)$_{20}$ (1.0 mM) solution in DI water were added by pipet to afford final concentrations of 0.5 mM for each surfactant. The solution was vortexed to mix the surfactants thoroughly. Next, 300 µL of 10 cSt PDMS (ϕ = 0.20) containing pyrene (50 mM), a hydrophobic fluorescent dye, was added. The solution was emulsified using a handheld homogenizer for 30 seconds to form microscale oil in water (O/W) droplets. A 200 µL aliquot was removed and then centrifuged (3 times at 2,500 rpm for 5 minutes each cycle) to remove excess biotinylated surfactant and then redispersed each cycle with 0.50 mM K_{55}(*rac*-L)$_{20}$ + 0.05 % Triton x-100 in 0.010 M PBS to obtain final volumes of 200 µL. Other emulsions with different biotin-K_{55}(*rac*-L)$_{20}$/ K_{55}(*rac*-L)$_{20}$ ratios were prepared in a similar manner except the amounts of polypeptide surfactant stock solutions added were adjusted accordingly. For example, 300 µL of a biotin-K_{55}(*rac*-L)$_{20}$ (1.0 mM) solution and 900 µL of K_{55}(*rac*-L)$_{20}$ (1.0 mM) solution afforded final concentrations of 0.25 mM biotin-K_{55}(*rac*-L)$_{20}$ + 0.75 mM K_{55}(*rac*-L)$_{20}$.

Binding of FITC-NeutrAvidin to biotin-K_{55}(*rac*-L)$_{20}$ emulsions. A suspension of biotin-K_{55}(*rac*-L)$_{20}$ stabilized emulsion droplets (20 µL) was added to a 1.5 mL centrifuge tube, followed by addition of excess FITC-NeutrAvidin solution (100 µL of a 1.0 mg/mL in 0.010 M PBS). The

suspension was vortexed and allowed to incubate for 30 minutes. The suspension was then centrifuged (3 times at 2,500 rpm for 5 minutes each cycle) and redispersed each cycle with fresh 0.50 mM K_{55}(*rac*-L)$_{20}$ + 0.05 % Triton x-100 in 0.010 M PBS to obtain final volumes of 120 µL.

Binding of FITC-PEG-biotin to NeutrAvidin coated emulsions. A suspension of NeutrAvidin coated emulsion droplets (30 µL) (prepared using above procedure) was added to a 1.5 mL centrifuge tube, followed by addition of excess FITC-PEG-biotin solution (150 µL of a 1.0 mg/mL in 0.010 M PBS). The suspension was vortexed and incubated for 30 minutes. To remove unattached FITC-PEG-biotin, the suspension was centrifuged (3 times at 2,500 rpm for 5 minutes each cycle) and redispersed each cycle with fresh 0.50 mM K_{55}(*rac*-L)$_{20}$ + 0.05 % Triton x-100 in 0.010 M PBS to obtain final volumes of 180 µL.

Fluorescence imaging of emulsion assemblies. Double sided tape was first applied to a microscope slide to act as a spacer, followed by attachment of a coverslip. An aliquot of emulsion suspension, approximately 10 µL, was then applied between the coverslip and microscope slide. The samples were imaged using a Zeiss Axiovert 200 fluorescence microscope having an ultraviolet filter set (λ_{ex} = 365 nm, λ_{em} = 420-470 nm) and blue filter (λ_{ex} = 450-490 nm, λ_{em} = 515-565 nm).

Nanoscale biotin-K_{55}(*rac*-L)$_{20}$ stabilized emulsions. To a 1.5 mL centrifuge tube,a biotin-K_{55}(*rac*-L)$_{20}$ solution (200 µL, 1.0 mM) in deionized water was added. To this solution, 10 cSt PDMS (50 µL , ϕ = 0.20) was added and the mixture emulsified using an ultrasonic homogenizer (Misonix Microson XL2000) for 60 seconds.

Dynamic Light Scattering (DLS). To a 4.0 mL 4 sided quartz cuvet was added a biotin-K_{55}(*rac*-L)$_{20}$ nanoscale emulsion sample (10 µL), followed by approximately 3.0 mL of DI water. A cap was placed on the cuvet and the solution gently mixed.the sample was then analyzed by DLS at 90˚ for 180 seconds. The autocorrelation data was fitted using CONTIN algorithm to determine the approximate average diameters of the nanoemulsion droplets (droplet diameter = 390 nm, PDI = 0.22).

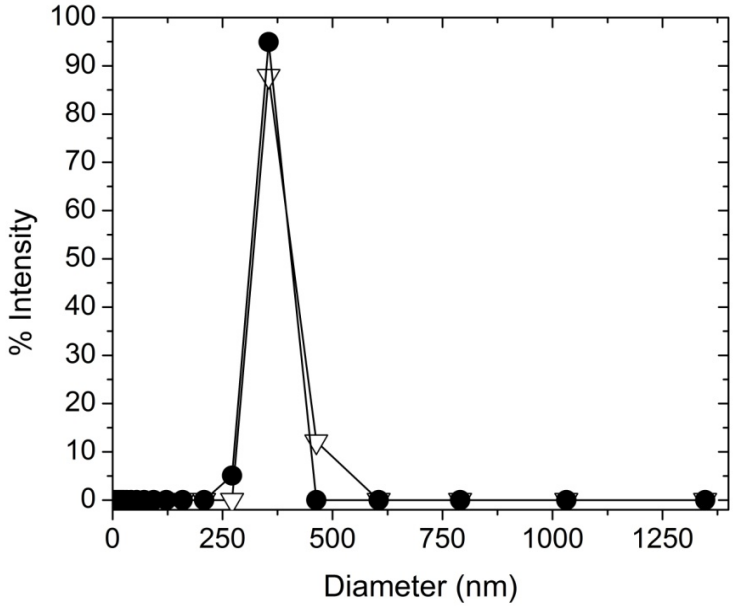

Figure S1: Dynamic light scattering data of nanoscale emulsion droplets stabilized with 1.0 mM biotin-K_{55}(*rac*-L)$_{20}$ at t = 0 days (●) and t = 24 days (▽). Surfactant concentration: 1.0 mM biotin-K_{55}(*rac*-L)$_{20}$; oil phase: 10 cSt PDMS; ϕ = 0.20; emulsification: ultrasonic homogenizer = 60 seconds.

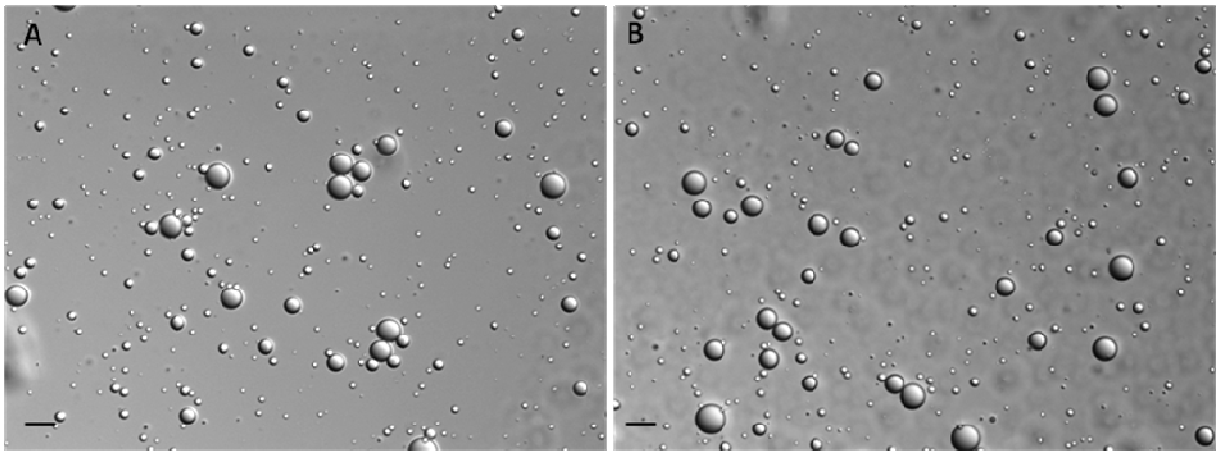

Figure S2 DIC Optical microscopy images of microscale emulsion droplets stabilized with 1.0 mM biotin-K_{55}(*rac*-L)$_{20}$ after A) initial preparation (t = 0 days) and B) t = 24 days. As can be seen, the initial as formed droplets are polydisperse in diameter, ranging from *ca.* 1 to 20 microns. Surfactant concentration: 1.0 mM biotin-K_{55}(*rac*-L)$_{20}$; oil phase: 10 cSt PDMS; ϕ = 0.20; emulsification: handheld homogenizer = 30 seconds. Scale bar = 20 μm.

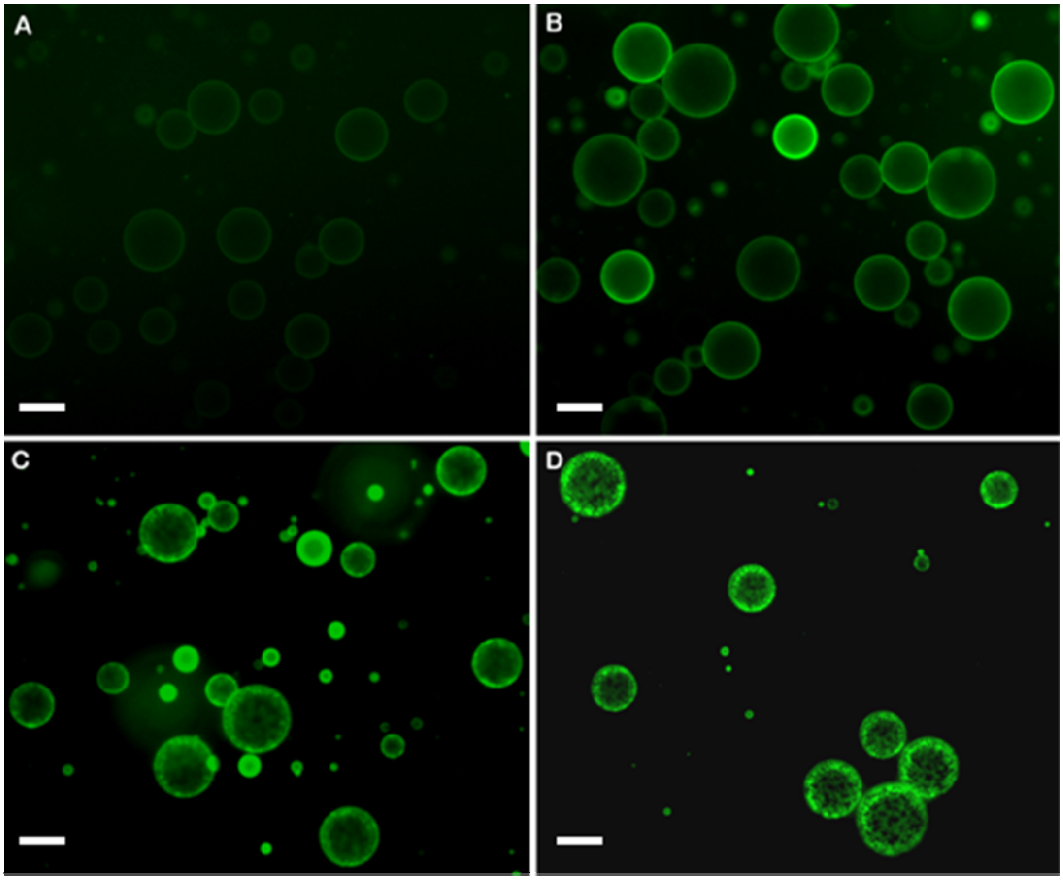

Figure S3 Fluorescence microscopy images showing FITC-NeutrAvidin interactions with microscale emulsion droplets containing different ratios of biotin-K_{55}(*rac*-L)$_{20}$ and unmodified K_{55}(*rac*-L)$_{20}$. For all emulsions: oil phase: 10 cSt PDMS; ϕ = 0.20; emulsification: handheld homogenizer = 30 seconds. A) Image of 0.25 mM biotin-K_{55}(*rac*-L)$_{20}$ + 0.75 mM K_{55}(*rac*-L)$_{20}$ droplets after addition of FITC-NeutrAvidin followed by centrifugation and resuspension. B) Image of 0.5 mM biotin-K_{55}(*rac*-L)$_{20}$ + 0.5 mM K_{55}(*rac*-L)$_{20}$ droplets after addition of FITC-NeutrAvidin followed by centrifugation and resuspension. C) Image of 0.75 mM biotin-K_{55}(*rac*-L)$_{20}$ + 0.25 mM K_{55}(*rac*-L)$_{20}$ droplets after addition of FITC-NeutrAvidin followed by centrifugation and resuspension. D) Image of 1.0 mM biotin-K_{55}(*rac*-L)$_{20}$ droplets after addition of FITC-NeutrAvidin followed by centrifugation and resuspension. Green = FITC-NeutrAvidin. Droplets were diluted approximately tenfold prior to imaging. Scale bar = 20 μm.

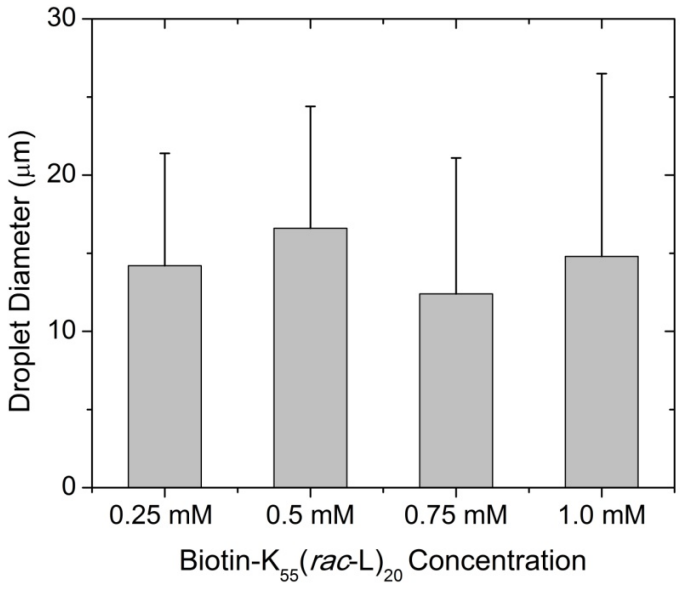

Figure S4 Droplet diameters from fluorescence micrographs of microscale droplets stabilized with varying concentrations of biotin-K_{55}(*rac*-L)$_{20}$. Microscale emulsion droplets were prepared using different ratios of biotin-K_{55}(*rac*-L)$_{20}$ and unmodified K_{55}(*rac*-L)$_{20}$, where the total surfactant concentration was 1.0 mM. For all emulsions: oil phase: 10 cSt PDMS; ϕ = 0.20; emulsification: handheld homogenizer = 30 seconds. Average droplet diameters were determined by counting 100-200 droplets per sample. The error bars represent the standard deviation.

References:

1) Hanson, J.A.; Chang, C.; Graves, S.; Li, Z.; Mason, T.G.; and Deming, T.J. *Nature*, **2008**, *455*, 85-88.