Supplementary information

for

High-speed, high-purity separation of gold nanoparticle-DNA origami constructs using centrifugation

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Experimental

Disclaimer: Certain commercial equipment, instruments, or materials are identified in order to specify the experimental procedure adequately. Such identification is not intended to imply recommendation or endorsement by the National Institute of Standards and Technology, nor is it intended to imply that the materials or equipment identified are necessarily the best available for the purpose.

Materials: We purchased single strand M13mp18 and thiol conjugated single-stranded DNA strands commercially. We purchased all unmodified and poly-A labeled DNA staple strands commercially, and used them without further purification. We purchased colloidal solutions of AuNPs and all other chemicals commercially. 1× TAE/Mg^{2+} buffer contains 40 mmol L^{-1} (mM) tris(hydroxymethyl)aminomethane (tris), 20 mmol L^{-1} (mM) acetic acid, 2 mmol L^{-1} (mM) ethylenediaminetetraaceticacid (EDTA), and 12.5 mmol L^{-1} (mM) magnesium acetate, pH 8.0 and 0.5× TBE buffer has 45 mmol L^{-1} (mM) tris(hydroxymethyl)aminomethane (tris), 45 mmol L^{-1} (mM) Boric acid, and 1 mmol L^{-1} (mM) ethylenediaminetetraaceticacid (EDTA).

Self-assembly of DNA origami: We assembled rectangular DNA origami according to the method of Rothemund.[1] We mixed a long single strand of M13mp18 and staple strands at a molar ratio of 1 to 5 in 1× TAE/Mg^{2+} buffer and slowly annealed the strands at 1 °C min^{-1}

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from 95 °C to room temperature using a DNA thermal cycler. We removed excess staple strands by washing four to five times with 1× TAE/Mg^{2+} buffer (400 μL) in a "100 000 molecular weight cut-off (MWCO) filter" (100 kDa MWCO) microcentrifuge filter at an acceleration of 600 rad s^{-1} (5 730 rpm, 34 355 m s^{-2}, 3 500 × *g*) for 2 min. We omitted staple strands on vertical edges to avoid stacking of origami along vertical edges.

Functionalization of AuNP with ssDNA: We stabilized AuNPs by adsorption of bis(parasulfonatophenyl) phenylphosphine dihydrate dipotassium salt (phosphine salt). Typically, we added 10 mg of phosphine salt to 50 mL of AuNP solution, shaking the mixture overnight at room temperature. We then precipitated the AuNPs by adding sodium chloride until the color turned bluish purple. After centrifugation at 850 rad s^{-1} (68 670 m s^{-2}, 7 000 × *g*) for several minutes, we removed the supernatant with a pipette, and re-dispersed the AuNP precipitate in 2.5 mmol L^{-1} (mM) phosphine solution. We mixed dithiolated DNA and phosphine-protected AuNPs at various molar ratios, which depended on the size of AuNPs, and pre-incubated for an hour. We added solutions of TBE buffer (10×), SDS (0.01%) and NaCl (1 mol L^{-1}) to pre-incubated AuNPs and ssDNA and brought the final concentrations of TBE, SDS, and NaCl to 0.5×, 0.01%, and 0.05 mol L^{-1} (M), respectively. We left the solution of AuNP/DNA in buffer for two hours at room temperature. We slowly increased the NaCl concentration of the solution to 0.5 mol L^{-1} (M) by sequential additions of 0.5× TBE buffer (0.01% SDS)/1 mol L^{-1} (M) NaCl and incubated the mixture for 24 h at room temperature. We purified conjugates of ssDNA-AuNP by spin column in a microcentrifuge.

Preparation of AuNP-DNA Origami Constructs: We mixed DNA origami solution with ssDNA functionalized AuNP solution at 1.3× to 2× molar ratio to the binding locations on an origami and slowly annealed the solution from 37 °C to room temperature using a DNA thermal cycler.

Centrifugation of AuNP-DNA Origami Constructs: We used a medium of nine-layers of different concentrations of iodixanol (10%, 15%, …, 50%) for centrifugation of AuNP-DNA origami constructs. We denote the different constructs as *n*AuNP-origami, where *n* is the number of AuNP bound to an origami. We prepared iodixanol solutions of different concentrations as follows: we first diluted commercial (Optiprep™, Sigma-Aldrich), 60% (w/v) iodixanol in water by 6× TAE/Mg^{2+} (*i.e.*, 6 times more concentrated than 1× TAE/Mg^{2+}) to 50% iodixanol in 1× TAE/Mg^{2+}. We prepared all other solutions by sequential dilution of 50% iodixanol in 1× TAE/Mg^{2+} with 1× TAE/Mg^{2+}. We prepared the nine-layer density gradient medium in a centrifuge tube a day prior to centrifugation. We loaded the sample solution on top of the density gradient in the centrifuge tube, and then centrifuged it at various speeds (in an Eppendorf Microcentrifuge, Model 5417R) depending on the size of AuNP to get separation of bands. We used a swing bucket rotor (Eppendorf A-8-11) in all experiments and the centrifuge temperature was set to 20 °C with an uncertainty, as specified by the vendor, of ± 2 °C. We recovered each band from the medium after sedimentation of the constructs was complete by pipetting. We buffer-exchanged the iodixanol medium for 1× TAE/Mg^{2+} buffer by spin column.

Scanning Electron Microscope (SEM) Characterization of AuNP-DNA Origami Constructs: A silicon wafer was treated with oxygen plasma before SEM imaging. We loaded a solution of AuNP-DNA origami (5 μL) onto an oxygen plasma-treated Si wafer, allowed it to rest for a minute, and quickly washed the wafer with water. We imaged the sample by SEM with an incident electron energy of 1.0 kV.

Dynamic Light Scattering (DLS) Measurements of AuNP-DNA Origami Constructs: We performed dynamic light scattering measurements using non-invasive backscatter optics with 45 μL samples in low-volume quartz batch cuvette. We set the temperature to 20 °C.

Results and Analysis

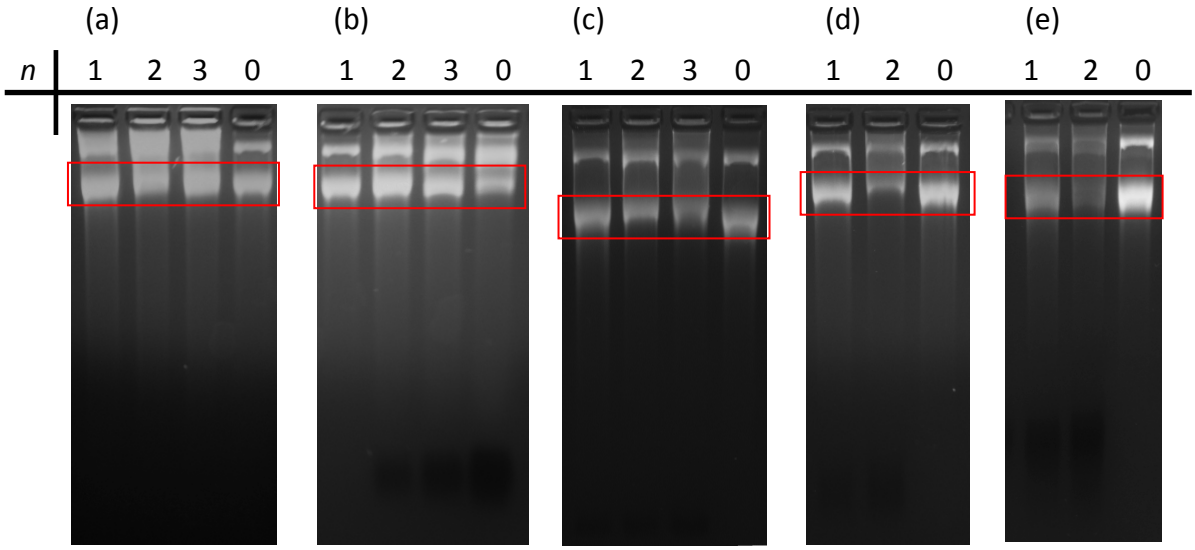

Figure S1. 1.5% agarose gel of (a) 5 nm AuNP-origami, (b) 10 nm AuNP-origami, (c) 15 nm AuNP-origami, (d) 20nm AuNP-origami, and (e) 30 nm AuNP-origami conjugates. The gels were pre-stained by ethidium bromide to visualize the band of the products. *n* represents the desired number of AuNP attached to DNA origami. The bands of desired product are highlighted by red boxes. The slower moving bands are probably cross-linked origami, as indicated by a previous study.[2]

Table S1. Dynamic viscosity and density of centrifugation media at 20.0 ± 0.1 °C (average ± limit of uncertainty)

Notes	Volume fraction of iodixanol	Dynamic viscosity	Density
	(%)	(mPa s)	(g cm^{-3})
in 1× TAE/Mg^{2+} buffer	0	1.02	1.000
"	5	1.14	1.027
"	10	1.31	1.054
"	15	1.49	1.079
"	20	1.75	1.107
"	25	2.10	1.134
"	30	2.54	1.160
"	35	3.21	1.188
"	40	4.11	1.214
"	45	5.42	1.241
"	50	6.97	1.262
in water (OptiPrep™)	60	14.33	1.320

The limits of uncertainty on dynamic viscosity and density are ± 0.01 mPa s and ± 0.001 g cm^{-3} respectively

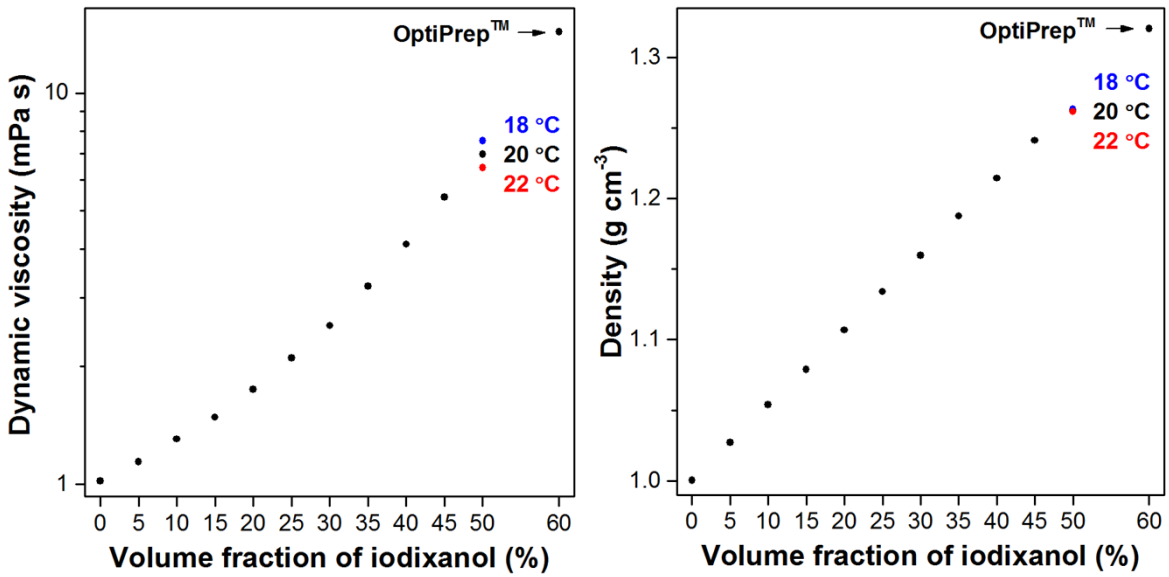

Figure S2. Dynamic viscosity and density increase as a function of volume fraction of iodixanol in water. The limits of uncertainty for all variables are smaller than the data points. A 2 °C variation in temperature around 20 °C, corresponds to the uncertainty in medium temperature during centrifugation, and results in an ≈ 8% variation in dynamic viscosity.

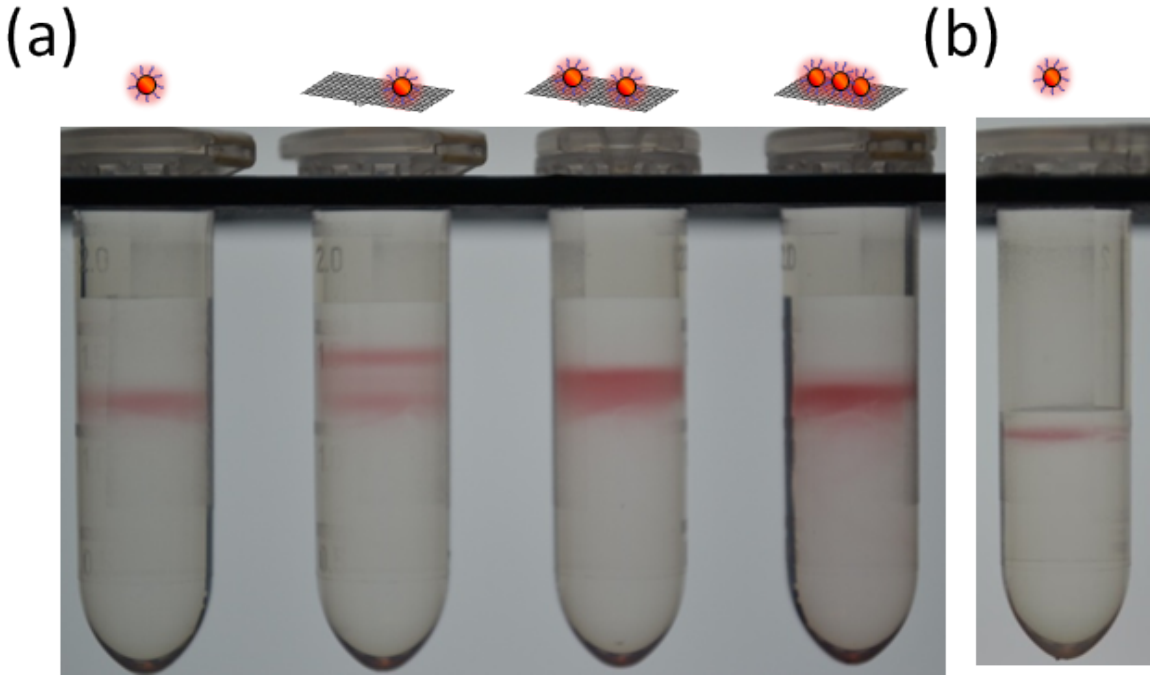

Figure S3. Separation of 10 nm AuNP constructs by centrifugation (a) after 240 min in 50% glycerol. Different constructs (2AuNP-origami and 3AuNP-origami) were not separated from free AuNPs. (b) 10 nm AuNP after 60 min of centrifugation in 50 % iodixanol. The layer of AuNP was not stable during centrifugation. The centrifugation speed was 850 rad s^{-1} (8 100 rpm, 68 600 m s^{-2}, 7 000 × *g*) for both.

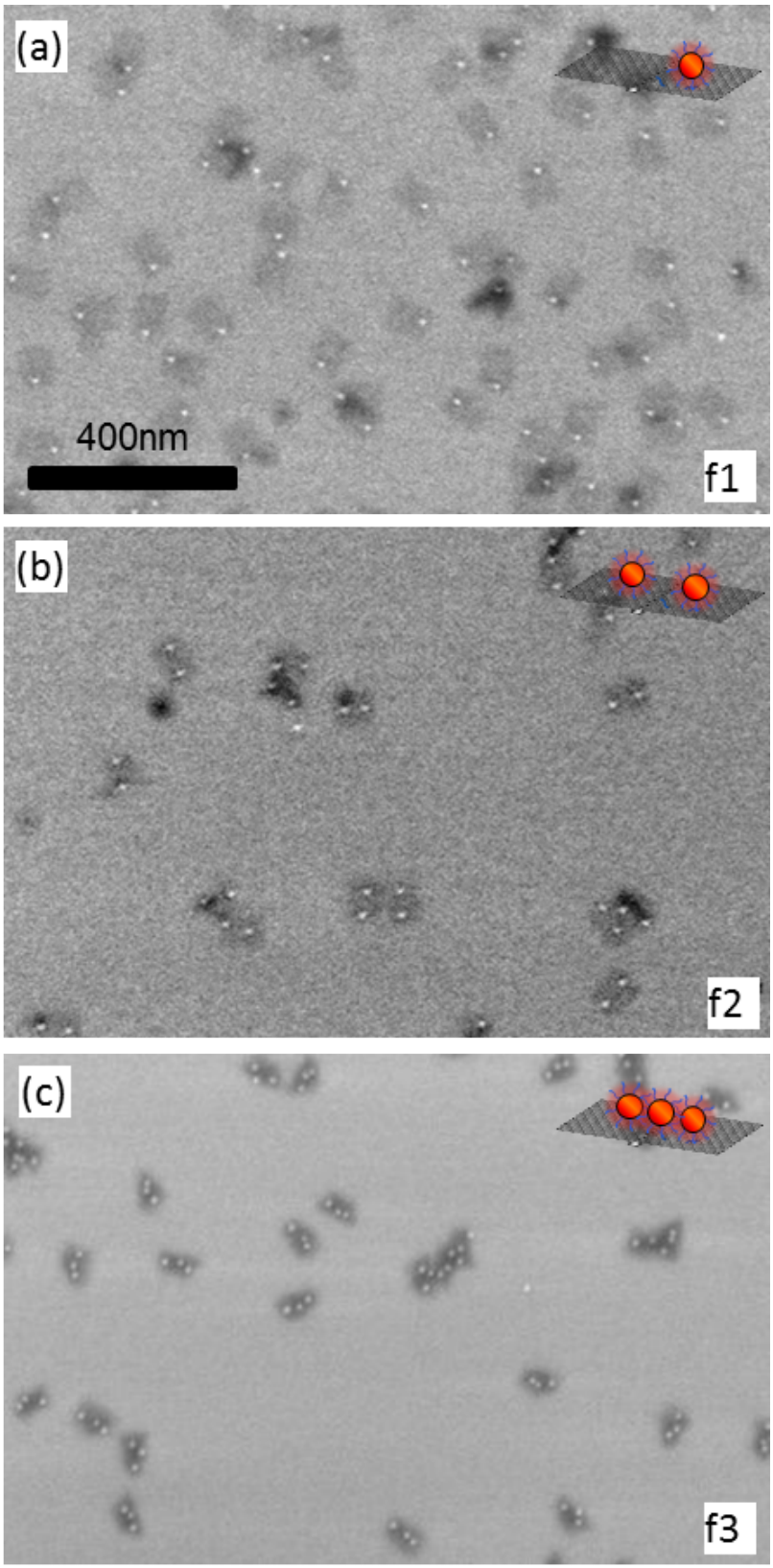

Figure S4. Typical SEM images of different fractions of *n*10 nm AuNP-origami conjugates as labeled in Figure 2 (a) after centrifuge purification. The fraction f1 is (96.1 ± 0.5)% 1AuNP-origami, f2 is (94.6 ± 1.0)% 2AuNP-origami, and f3 is (92.9 ± 1.3)% 3AuNP-origami.

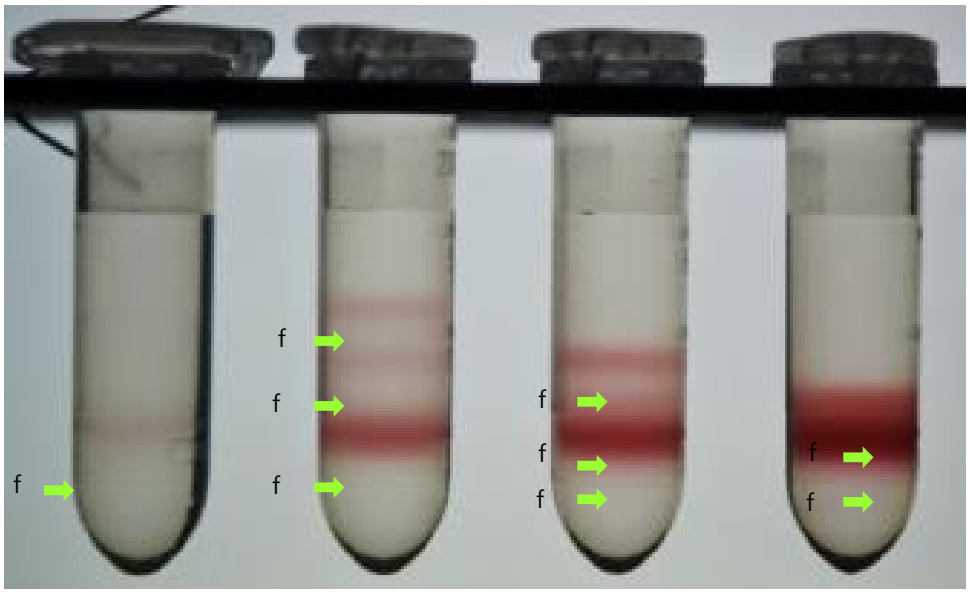

Figure S5. Separation of *n*10 nm AuNP constructs by centrifugation. Different fractions of *n*10 nm AuNP-origami constructs are labeled the same as Figure 2a. The centrifugation speed was 850 rad s^{-1} (8 100 rpm, 68 600 $m \cdot s^{-2}$, 7 000 × *g*).

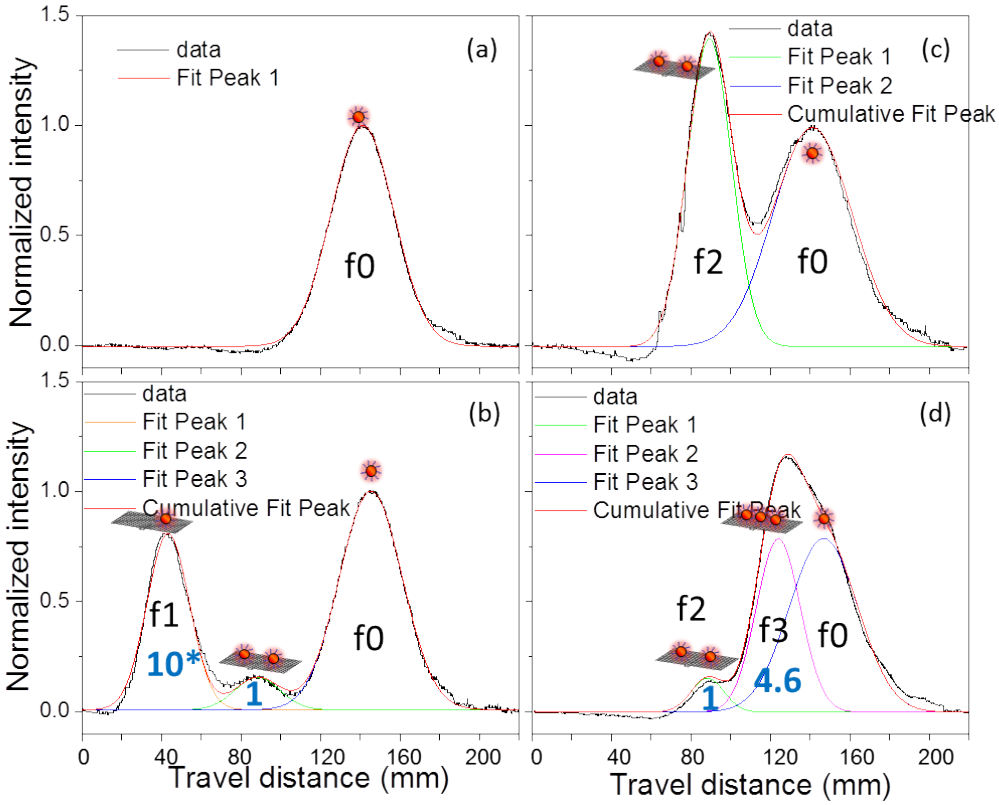

Figure S6. Centrifugrams of *n*10 nm AuNP-origami conjugates of each tube from the left to the right ((a) to (d)) in Figure 2 (a). Each band has been labeled as Figure 2 and fitted by a Gaussian function. The blue numbers in (b) and (d) are the values of the calculated area of the coresponding peak divided by the number of AuNP. These and the following Gaussian fits are not quantitative, due to variability in background illumination intensity, but rather provide a qualitative estimate of the relative fractions of the various constructs present.

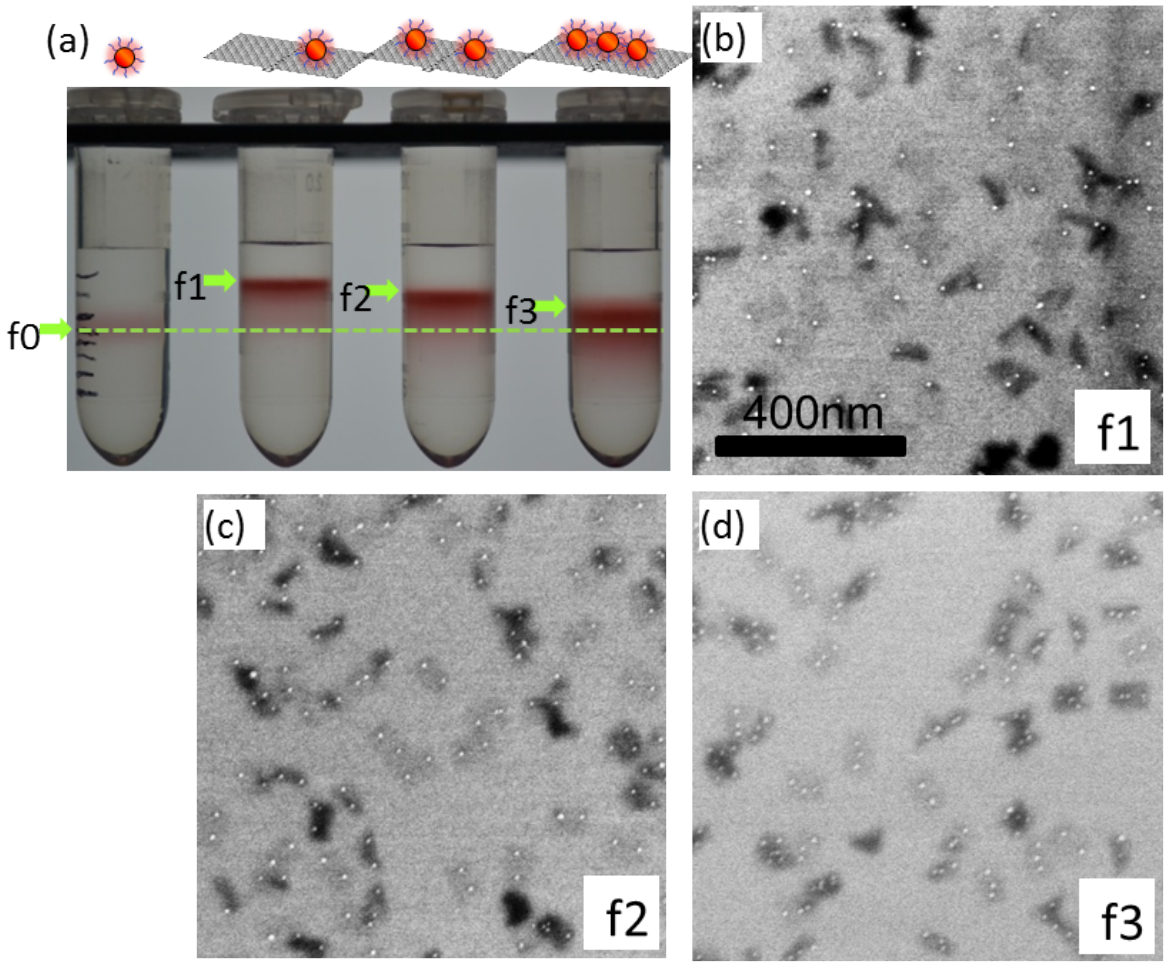

Figure S7. (a) Photographic image of the centrifuge tubes containing different conjugates of 5 nm AuNP-DNA origami after spinning for 150 min at 1100 rad s^{-1} (10 500 rpm, 114 660 m s^{-2}, 11 700 × *g*). (b)-(d) SEM images of different fractions of AuNP-origami conjugates as labeled in (a). The yields are ≈ 100%; within our measurement uncertainty.

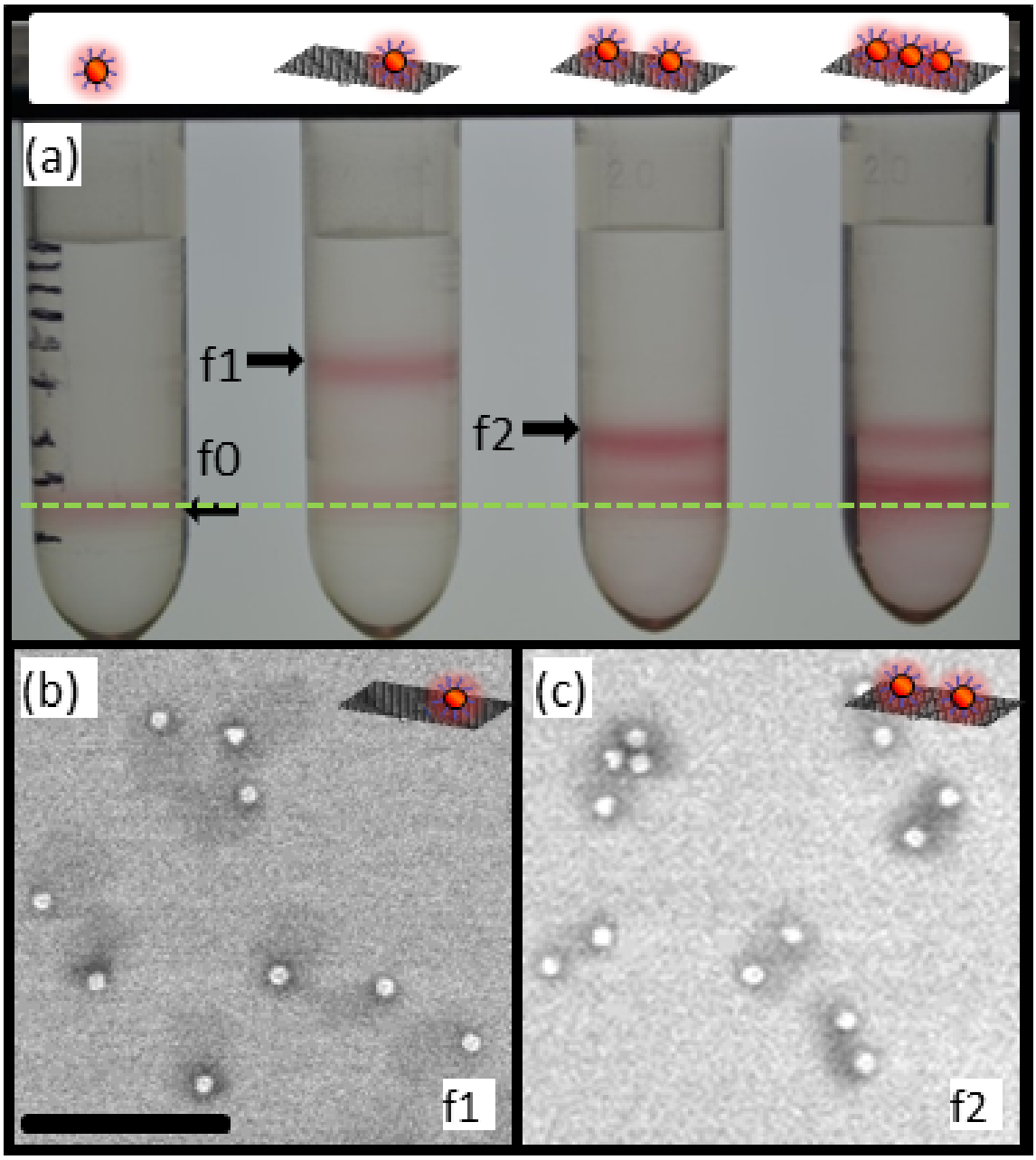

Figure S8. Separation of *n*15 nm AuNP-DNA origami. (a) Photograph of centrifuge tubes containing different constructs after spinning for 3 h at 681 rad s^{-1} (6 500 rpm, 44 100 m s^{-2}, 4500 × *g*). (b)-(d) Typical SEM images of different fractions of AuNP-origami constructs as labeled in (a). Schematics of the target product are shown for each tube. The fraction labeled f0 corresponds to free AuNPs. The SEM scale bar is 200 nm. Each construct (*n* = 1 and 2) is clearly separated, based on the differences in sedimentation coefficients. Analysis of the SEM micrographs shows that the slowest fraction, f1, is (96.9 ± 1.1)% 1AuNP-origami and the fraction f2 is (95.8 ± 1.3)% 2AuNP-origami.

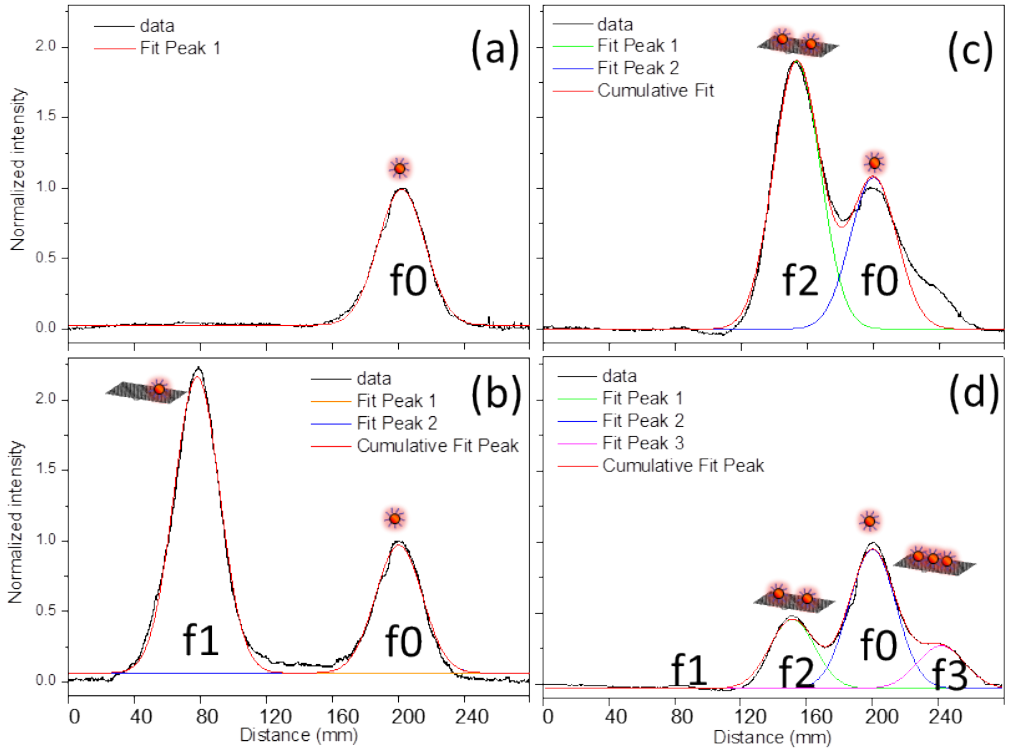

Figure S9. Centrifugram of *n*15 nm AuNP-origami conjugates of each tube from the left to the right ((a) to (d)) in Figure 3 (a). Each band has been labeled as in Figure S7 and fitted by a Gaussian function.

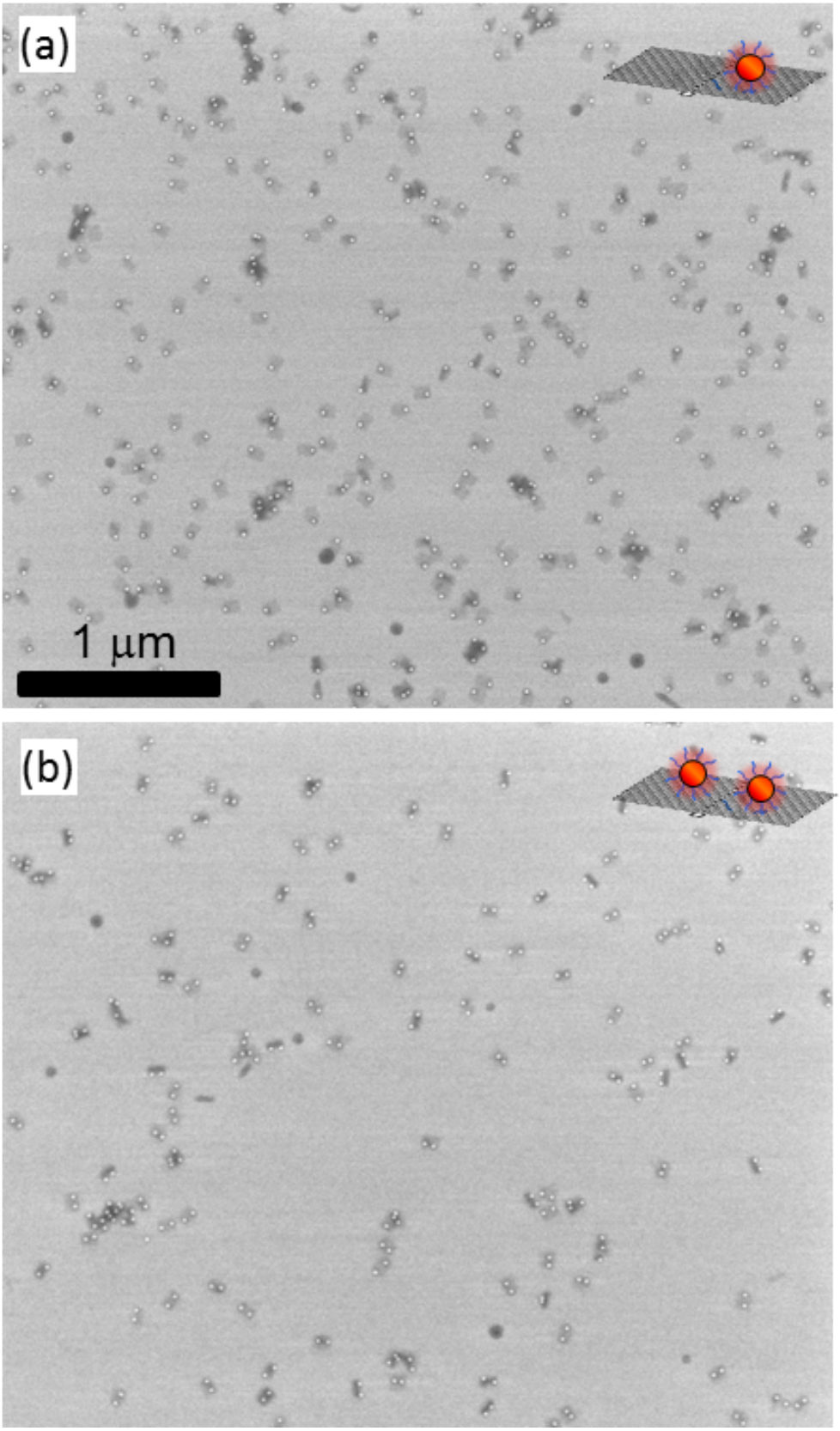

Figure S10. Typical SEM images of different fractions of *n*15 nm AuNP-origami conjugates after centrifuge purification. Each construct (*n* = 1 and 2) is clearly separated, based on the differences in sedimentation coefficients. Analysis of the SEM micrographs shows that the slowest fraction f1 contains (96.9 ± 1.1)% 1AuNP-origami and f2 is (95.8 ± 1.3)% 2AuNP-origami.

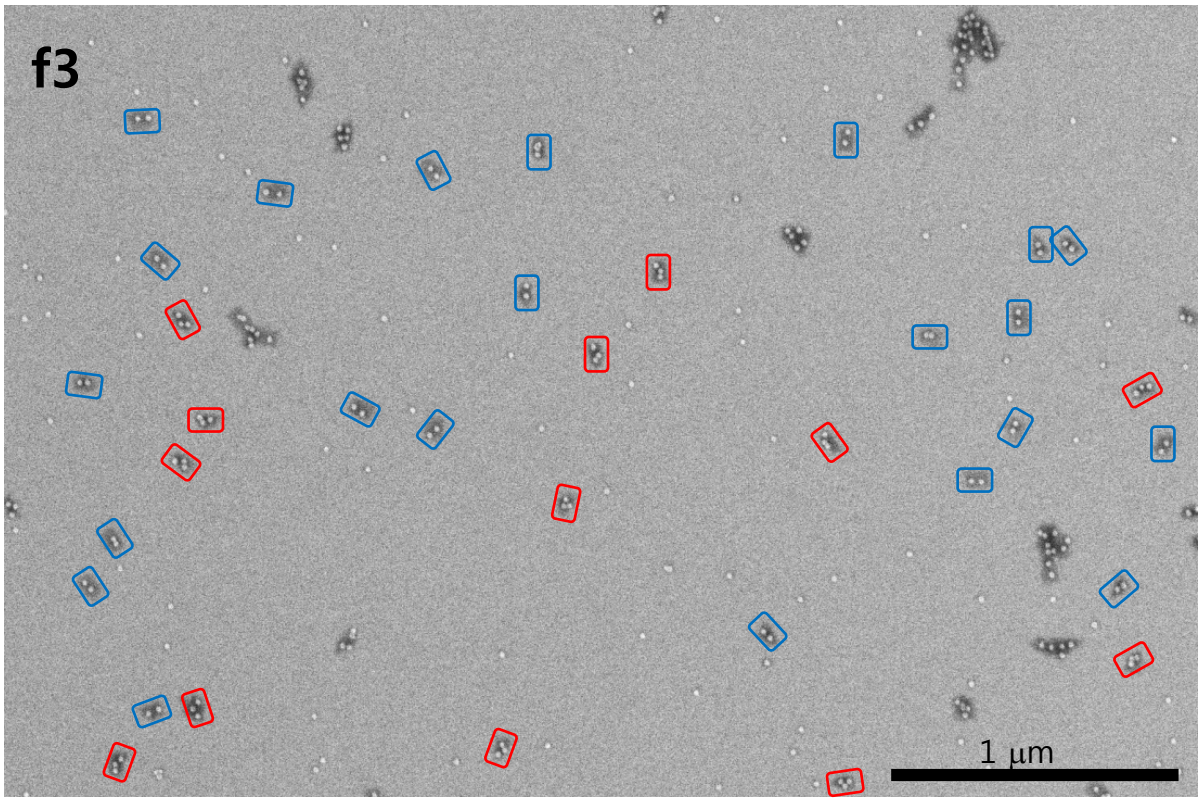

Figure S11. SEM images of the band f3 (Figure *S7*) separated from the centrifugation tube targeting 15 nm 3AuNP-origami. A blue rectangle highlights 2AuNP-origami and a red rectangle shows 3AuNP-origami. The fractions of 2AuNP-origami and 3AuNP-origami are (62 ± 8)% and (37 ± 8)%, respectively.

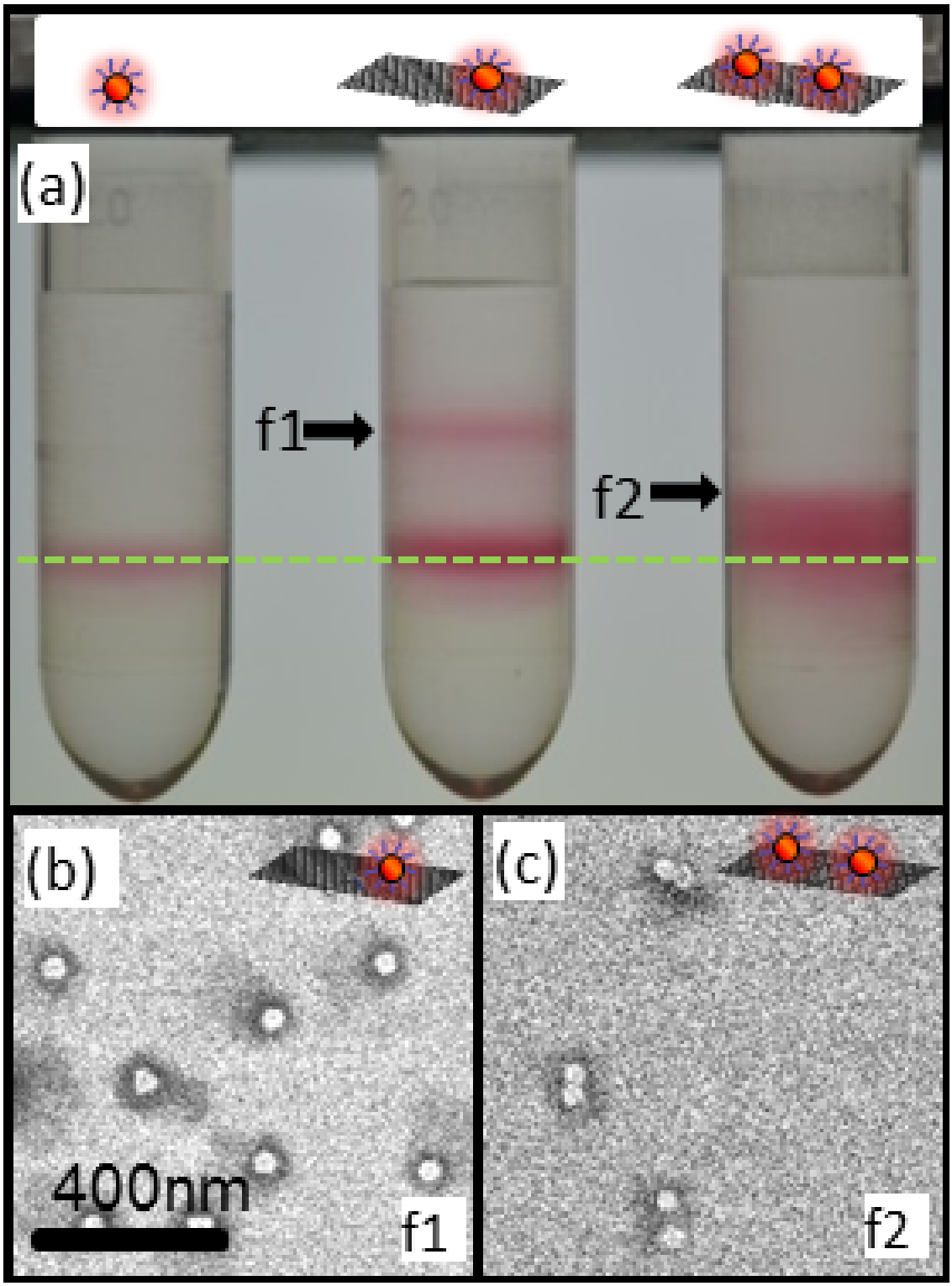

Figure S12. Separation of *n*20 nm AuNP-DNA origami. (a) Photograph of centrifuge tubes containing different constructs after spinning for 2 hours at 508 rad s^{-1} (4 850 rpm, 24 500 m s^{-2}, 2 500 × *g*). (b)-(d) Typical SEM images of different fractions of AuNP-origami constructs as labeled in (a). Schematics of the target product are shown for each tube. The SEM scale bar is 200 nm.

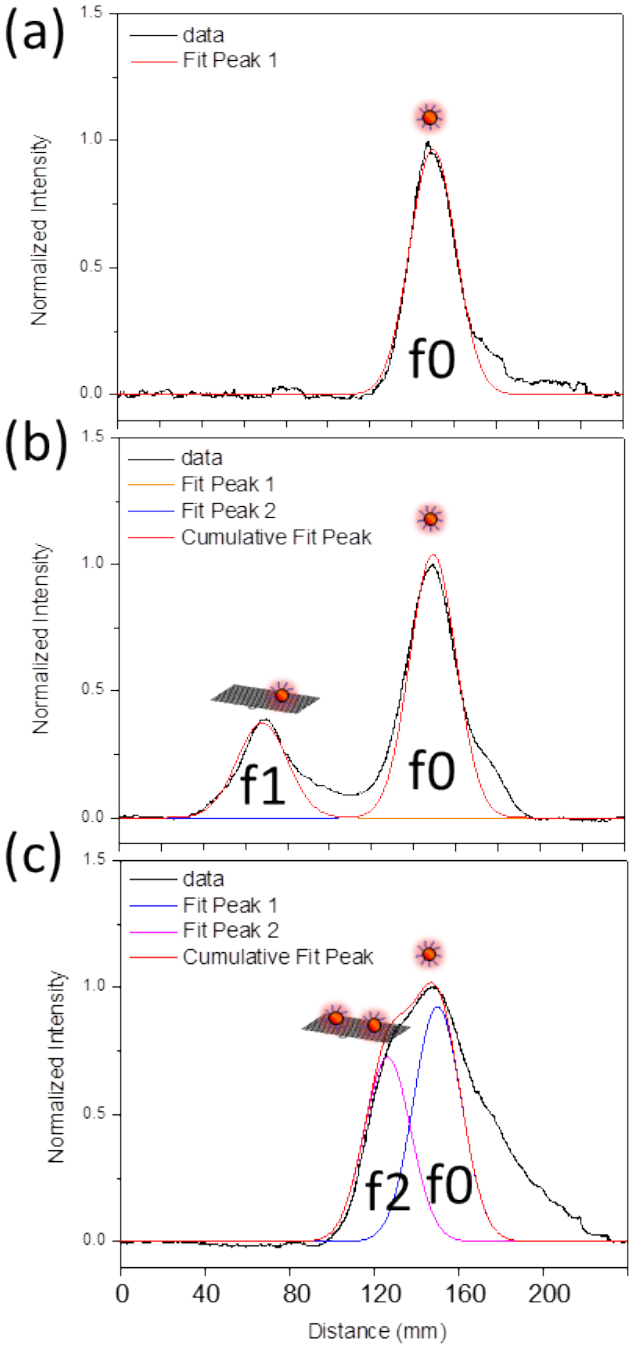

Figure S13. Centrifugram of *n*20 nm AuNP-origami conjugates of each tube from the left to the right ((a) to (c)) in Figure S11 (a). Each band has been labeled as Figure S11 and fitted by a Gaussian function.

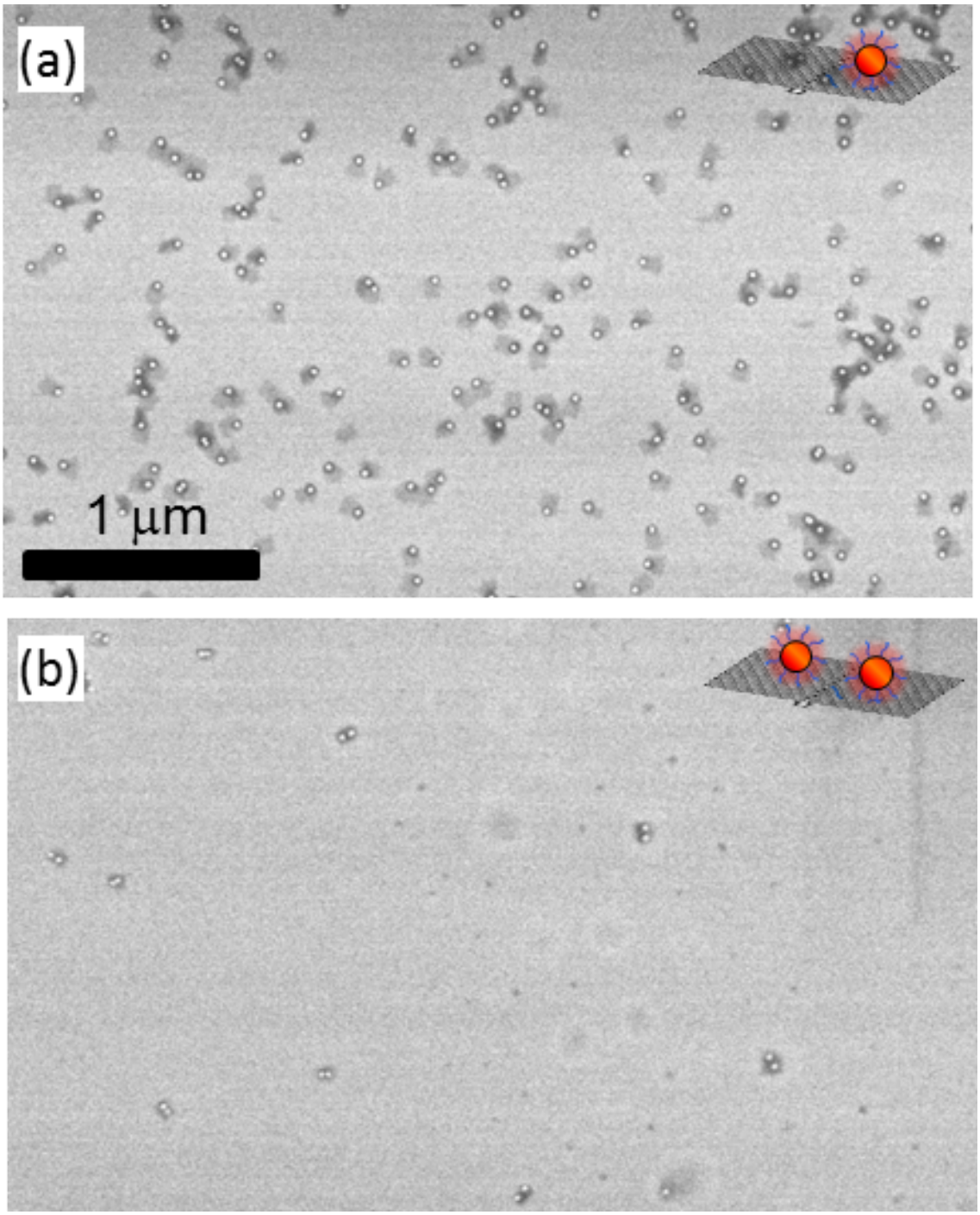

Figure S14. Typical SEM images of different fractions of *n*20 nm AuNP-origami conjugates purified by centrifugation. The extracted fractions, f1 and f2 are 1AuNP-origami, (97.0 ± 1.6)% and 2AuNP-origami, (94.3 ± 5.8)%, respectively, as determined from an analysis of SEM images.

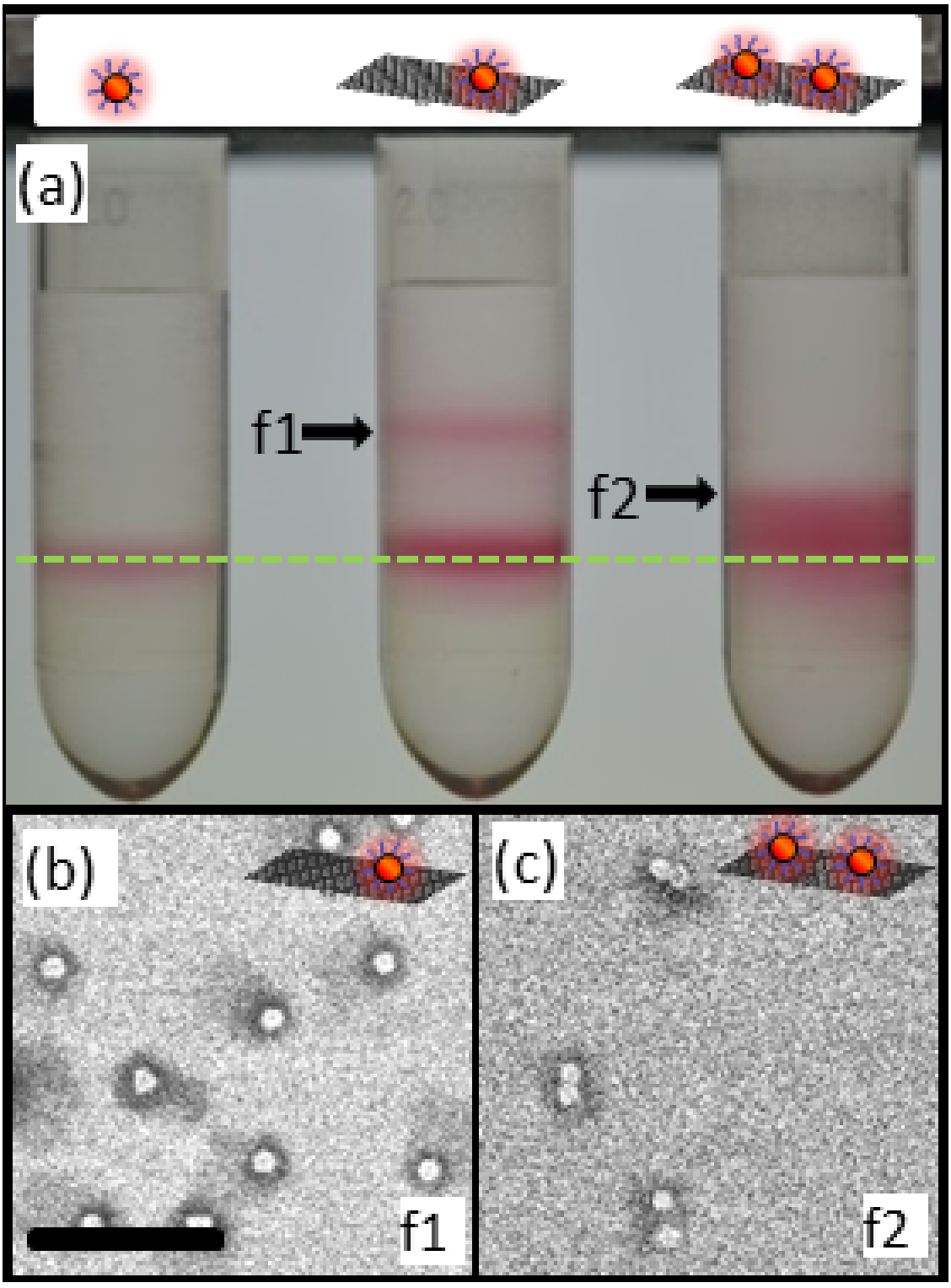

Figure S15. (a) Photograph of the centrifuge tubes containing *n*30 nm AuNP-DNA origami after spinning for 2 h at 287 rad s^{-1} (2 740 rpm, 7 840 m s^{-2}, 800 × g). (b)-(c) Typical SEM images of a fractions of AuNP-origami conjugates as labeled in (a).

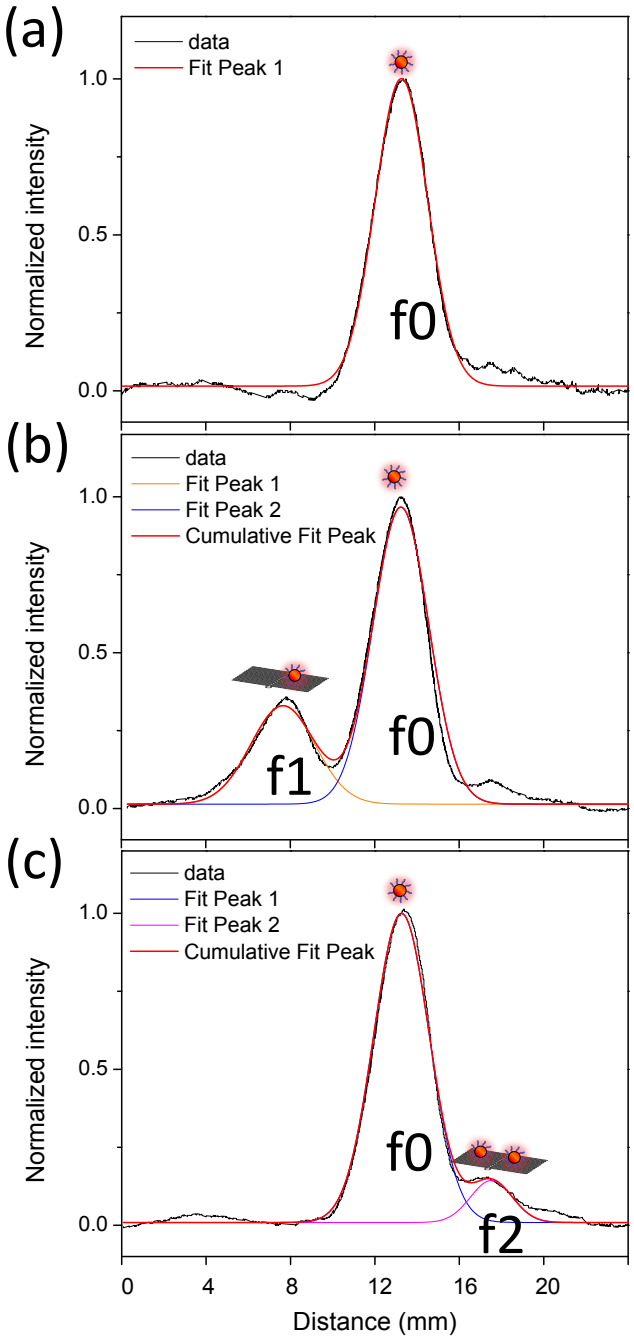

Figure S16. Centrifugram of *n*30 nm AuNP-origami conjugates of each tube from the left to the right ((a) to (c)) in Figure S14 (a). Each band has been labeled as Figure S14 and fitted by a Gaussian function.

Centrifugation Modeling Equations

We assume that AuNPs or *n*AuNP-origami (*n* is the number of AuNP bound to an origami) are isolated objects in solution, *i.e.*, "infinitely dilute solutions", in which the objects experience only three forces during centrifugation in the reference frame of the centrifuge tube: a centrifugal force F_C, a buoyant force F_B, and a drag force F_D. The centrifugal force F_C is given by

$$F_C = m\omega^2 r\,, \qquad (1)$$

where ω is the angular velocity of the centrifuge, m is the mass of the object, and r is the distance of the object from the rotation axis. The buoyant force F_B is opposite in direction to the centrifugal force and is proportional to the mass m_0 of fluid displaced by the object *via*[5]

$$F_B = -m_o\omega^2 r\,, \qquad (2)$$

where ω is the angular velocity and r is the distance of the object from the rotation axis. The drag force, F_D is expressed as the following,

$$F_D = -6\pi\eta_s R_H u \qquad (3)$$

where u is the velocity of the object, η_s is the fluid viscosity, and R_H is the hydrodynamic radius of the object; collectively the terms $6\pi\eta_s R_H$ are the drag coefficient for an object in a fluid medium.

Approximating, $u = \frac{1}{2}(m-m_0)\omega^2 r t^2$, and setting R_H proportional to a, the object radius, and $(m-m_0)$ proportional to a^3, we see that the time taken to reach the terminal velocity is proportional to $a/\sqrt{(3\pi\eta)}$. Thus, for small objects, terminal velocity is reached in a very short time (less than 10^{-6} s) during centrifugation, meaning that the object is always at its instantaeous terminal velocity throughout the separation medium. We therefore assume

$$F_C + F_B + F_D = 0\,, \qquad (4)$$

which implies

$$u_t = \frac{(m - m_o)\omega^2 r}{6\pi\eta_s R_H}, \qquad (5)$$

where u_t stands for terminal velocity.

Assuming that we know all of the quantities on the right-hand side of Equation (5), we may predict the total distance r_f that an object travels in during a fixed time interval by solving the equation

$$t = \int_{r_0}^{r_0 + r_f} u_t^{-1}(r) dr, \qquad (6)$$

where u_t^{-1} is the pace of the object (which depends on r via η_s and the fluid density ρ_{fluid}), t is the total time the object is allowed to fall, and r_0 is the initial position of the object relative to the axis of rotation. The physics of Equation (6) is straightforward: the time to travel a distance r_f is the sum over the infinitesimal paces of the object times the distance it travels at each pace. The difference in masses m-m_0 can be written in the form

$$m - m_0 = V_{or}\Delta\rho_{or} + n(V_{au}\Delta\rho_{au} + V_{sh}\Delta\rho_{sh}), \quad (7)$$

where V_{or}, V_{au}, and V_{sh} are the volumes of the DNA origami, AuNP, and DNA shell surrounding the AuNP, respectively; likewise, the $\Delta\rho_{or}$, $\Delta\rho_{au}$, and $\Delta\rho_{sh}$ are the densities of origami, AuNP, and shells surrounding the AuNP *relative* to the surrounding medium (i.e., $\Delta\rho_{or} = \rho_{or} - \rho_{fluid}$, etc.). Combining Equation (5) – (7) gives

$$t = \int_{r_0}^{r_0 + r_f} \frac{6\pi R_H \eta_s}{\omega^2 r[V_{or}\Delta\rho_{or} + n(V_{au}\Delta\rho_{au} + V_{sh}\Delta\rho_{sh})]} dr, \qquad (8)$$

where η_s and the $\Delta\rho$ depend on r.

In order to actually compute Equation (8), we assume that the AuNPs and DNA shells are spherical, which implies

$$V_{au} = \frac{4\pi a^3}{3} \qquad (9)$$

$$V_{sh} = \frac{4\pi\left(a + a_d\right)^3}{3} - \frac{4\pi a^3}{3} \qquad (10)$$

where a is the radius of the AuNP and a_d is the thickness of the DNA shell. We also take V_{or} =14,000 nm^3 (100 nm × 70 nm × 2 nm) and assume that the fluid density and viscosity can be approximated as exponential functions of r. These are derived by first fitting the data in Table S1 to determine η and ρ as a function of iodixanol concentration and then fitting the values of η and ρ as a function of r. The integral in Equation (8) is computed numerically to find r_f.

The most significant sources of experimental error affecting this analytical model probably relate to uncertainties in the actual dynamic viscosity profile in the centrifuge tube. The principle sources for these are pipetting errors during the construction of the gradient, diffusion of the gradient prior to and during centrifugation, and temperature deviations from the set point during centrifugation.

Band Width Analysis

We compute the widths of the sedimentation bands, which we assume to be approximately Gaussian, by calculating the sedimentation distances, r_f, for particles of radius $\bar{a}$, $(\bar{a} + \sigma)$ and $(\bar{a} - \sigma)$, where $\bar{a}$ is the mean radius, and σ is the standard deviation in particle radius determined from TEM measurements. The normalized band width for each nanoparticle or construct is:

$$\frac{r_{f(\bar{a}+\sigma)} - r_{f(\bar{a}-\sigma)}}{r_{f(\bar{a})}} \qquad (11)$$

The same procedure is followed for *n*AuNP-origami constructs, but we assume that the variations in AuNP size are uncorrelated, and therefore divide σ by $\sqrt{n}$, where n is 1, 2, or 3 to calculate the variation in the mass of nanoparticles attached to each construct. As noted in the main text, we assume a constant hydrodynamic radius, R_H, for all constructs equal to that measured by DLS for free origami.

Finally, we note that the absolute values of the measured and calculated distances do not agree. A sensitivity analysis of the possible sources of error suggests that the most probable cause of deviation is the likely difference between the actual and estimated dynamic viscosity profile along the centrifuge tube, as described above.

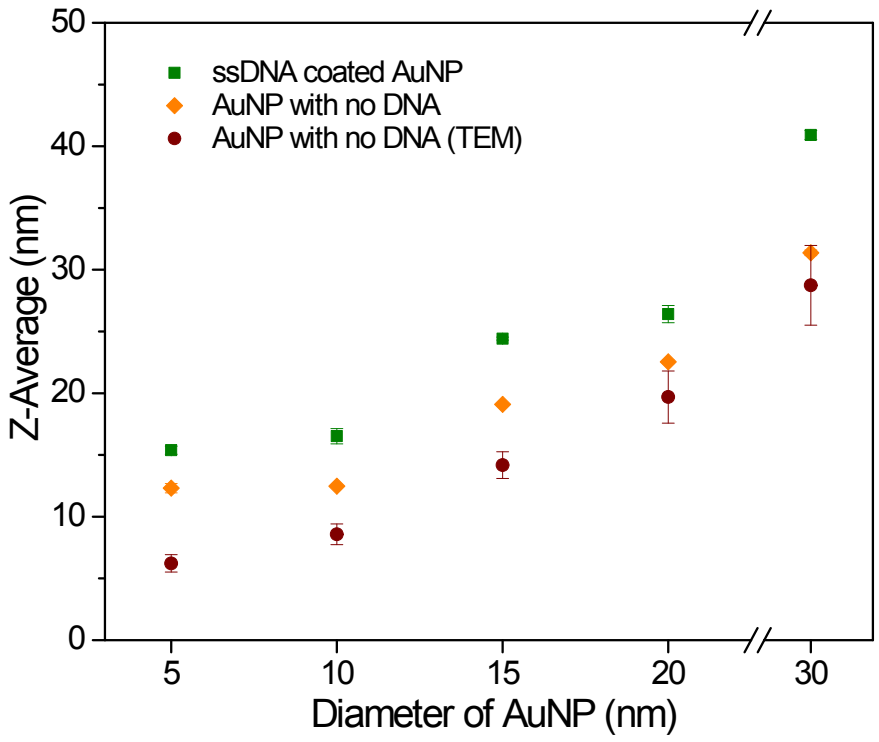

Figure S17. Diameter of AuNPs with or without a DNA coating measured by DLS and TEM. DLS measurements are performed in solution and report the hydrodynamic size of the particles. TEM measurements are performed in vacuum and quantify the size of the AuNPs alone, and are not sensitive to the presence of DNA or other surface functionalization. Vertical bars are one standard deviation.

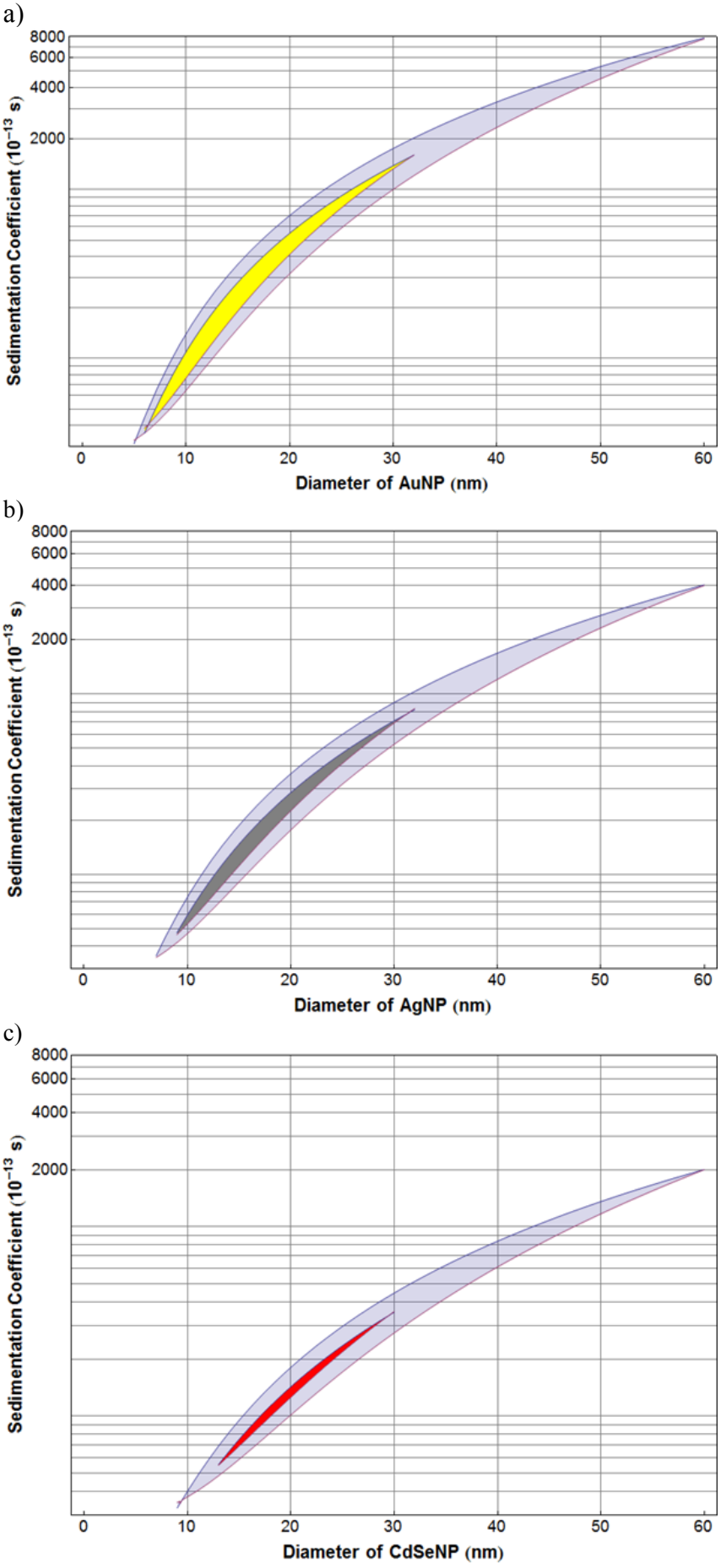

Figure S18. Comparison of sedimentation coefficients *vs.* particle size for free gold nanoparticles and 1AuNP-origami constructs, free silver nanoparticles and 1AgNP-origami constructs, and free CdSe nanoparticles and 1CdSeNP-origami constructs. The larger shaded area represents the range of particle sizes over which separations can be achieved, assuming a monodisperse size distribution. The small shaded area represents the range of particle sizes over which separations can be achieved, assuming a size distribution which varies by ± 10 % about the average. As the nanoparticle density decreases, the nanoparticle size must increase in order to add appreciable mass and thus change the sedimentation coefficient of the NP-origami complex. The densities of the three materials are: Au, 19.3 $\times 10^3$ kg m^{-3}; Ag, 10.5 $\times 10^3$ kg m^{-3}; CdSe, 5.8 $\times 10^3$ kg m^{-3}

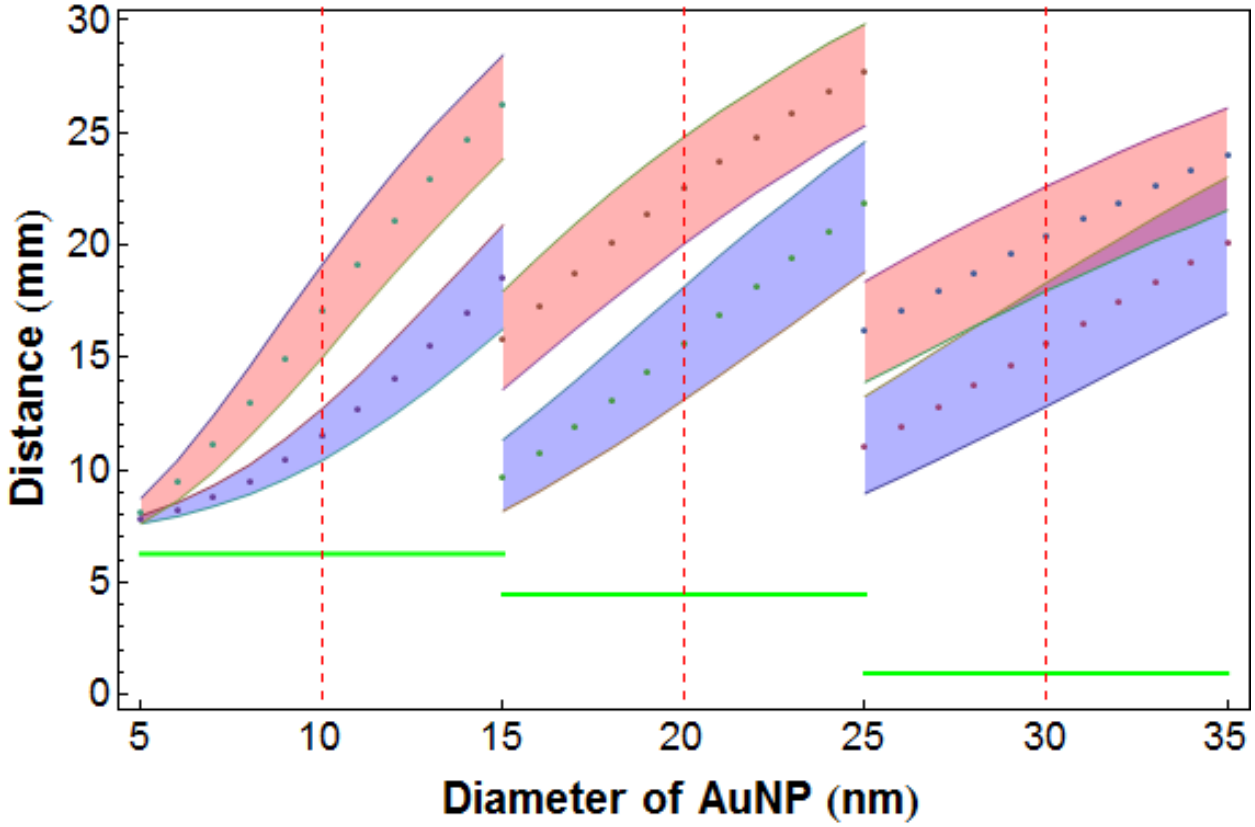

Figure S19. Sedimentation distance versus particle size for free gold nanoparticles and 1AuNP-origami constructs. The red bands represent the distance traveled by free gold nanoparticles assuming a nanoparticle size variation of ± 10 %, and the blue bands the distance traveled by 1AuNP-origami constructs. The horizontal green lines are the distances traveled by undecorated origami.

References

1. P. W. K. Rothemund, *Nature*, 2006, **440**, 302.
2. S. Pal, Z. Deng, B. Ding, H. Yan, Y. Liu, *Angew. Chem. Int. Ed.,* **2010**, *49*, 2704.
3. F. C. Klebaner, *Introduction to Stochastic Calculus with Applications (Second Edition)*, Imperial College Press, London, England **2005**.

Sequence of single stranded M13mp18 can be found at the website of New England BioLabs Inc.;

https://www.neb.com/~/media/NebUs/Page%20Images/Tools%20and%20Resources/Interactive%20Tools/DNA%20Sequences%20and%20Maps/Text%20Documents/m13mp18gbk.txt

Sequences of unmodified & modified staple strands

In order to capture T18 coated AuNPs, specific staple strands at pre-determined locations were extended with 22 Adenines. The following strands are the modified strands at different binding sites.

14, 61, 85, 87, 119, 121, 145, 147, 171

T18 strand: 5‘-/5DTPA/TTT TTT TTT TTT TTT TTT -3’

Table S2. Sequences of unmodified staple strands

Name	Sequence (5’ → 3’)
1	CAAGCCCAATAGGAAC CCATGTACAAACAGTT
2	AATGCCCCGTAACAGT GCCCGTATCTCCCTCA
3	TGCCTTGACTGCCTAT TTCGGAACAGGGATAG
4	GAGCCGCCCCACCACC GGAACCGCGACGGAAA
5	AACCAGAGACCCTCAG AACCGCCAGGGGTCAG
6	TTATTCATAGGGAAGG TAAATATT CATTCAGT
7	CATAACCCGAGGCATA GTAAGAGC TTTTTAAG
8	ATTGAGGGTAAAGGTG AATTATCAATCACCGG
9	AAAAGTAATATCTTAC CGAAGCCCTTCCAGAG
10	GCAATAGCGCAGATAG CCGAACAATTCAACCG
11	CCTAATTTACGCTAAC GAGCGTCTAATCAATA
12	TCTTACCAGCCAGTTA CAAAATAAATGAAATA
13	ATCGGCTGCGAGCATG TAGAAACCTATCATAT
14	CTAATTTATCTTTCCT TATCATTCATCCTGAA
15	GCGTTATAGAAAAAGC CTGTTTAG AAGGCCGG
16	GCTCATTTTCGCATTA AATTTTTG AGCTTAGA
17	AATTACTACAAATTCT TACCAGTAATCCCATC
18	TTAAGACGTTGAAAAC ATAGCGATAACAGTAC
19	TAGAATCCCTGAGAAG AGTCAATAGGAATCAT
20	CTTTTACACAGATGAA TATACAGTAAACAATT
21	TTTAACGTTCGGGAGA AACAATAATTTTCCCT
22	CGACAACTAAGTATTA GACTTTACAATACCGA
23	GGATTTAGCGTATTAA ATCCTTTGTTTTCAGG
24	ACGAACCAAAACATCG CCATTAAA TGGTGGTT
25	GAACGTGGCGAGAAAG GAAGGGAA CAAACTAT
26	TAGCCCTACCAGCAGA AGATAAAAACATTTGA
27	CGGCCTTGCTGGTAAT ATCCAGAACGAACTGA
28	CTCAGAGCCACCACCC TCATTTTCCTATTATT
29	CTGAAACAGGTAATAA GTTTTAACCCCTCAGA
30	AGTGTACTTGAAAGTA TTAAGAGGCCGCCACC
31	GCCACCACTCTTTTCA TAATCAAACCGTCACC

32	GTTTGCCACCTCAGAG CCGCCACCGATACAGG
33	GACTTGAGAGACAAAA GGGCGACAAGTTACCA
34	AGCGCCAACCATTTGG GAATTAGATTATTAGC
35	GAAGGAAAATAAGAGC AAGAAACAACAGCCAT
36	GCCCAATACCGAGGAA ACGCAATAGGTTTACC
37	ATTATTTAACCCAGCT ACAATTTTCAAGAACG
38	TATTTTGCTCCCAATC CAAATAAGTGAGTTAA
39	GGTATTAAGAACAAGA AAAATAATTAAAGCCA
40	TAAGTCCTACCAAGTA CCGCACTCTTAGTTGC
41	ACGCTCAAAATAAGAA TAAACACCGTGAATTT
42	AGGCGTTACAGTAGGG CTTAATTGACAATAGA
43	ATCAAAATCGTCGCTA TTAATTAACGGATTCG
44	CTGTAAATCATAGGTC TGAGAGACGATAAATA
45	CCTGATTGAAAGAAAT TGCGTAGACCCGAACG
46	ACAGAAATCTTTGAAT ACCAAGTTCCTTGCTT
47	TTATTAATGCCGTCAA TAGATAATCAGAGGTG
48	AGATTAGATTTAAAAG TTTGAGTACACGTAAA
49	AGGCGGTCATTAGTCT TTAATGCGCAATATTA
50	GAATGGCTAGTATTAA CACCGCCTCAACTAAT
51	CCGCCAGCCATTGCAA CAGGAAAAATATTTTT
52	CCCTCAGAACCGCCAC CCTCAGAACTGAGACT
53	CCTCAAGAATACATGG CTTTTGATAGAACCAC
54	TAAGCGTCGAAGGATT AGGATTAGTACCGCCA
55	CACCAGAGTTCGGTCA TAGCCCCCGCCAGCAA
56	TCGGCATTCCGCCGCC AGCATTGACGTTCCAG
57	AATCACCAAATAGAAA ATTCATATATAACGGA
58	TCACAATCGTAGCACC ATTACCATCGTTTTCA
59	ATACCCAAGATAACCC ACAAGAATAAACGATT
60	ATCAGAGAAAGAACTG GCATGATTTTATTTTG
61	TTTTGTTTAAGCCTTA AATCAAGAATCGAGAA
62	AGGTTTTGAACGTCAA AAATGAAAGCGCTAAT
63	CAAGCAAGACGCGCCT GTTTATCAAGAATCGC
64	AATGCAGACCGTTTTT ATTTTCATCTTGCGGG
65	CATATTTAGAAATACC GACCGTGTTACCTTTT
66	AATGGTTTACAACGCC AACATGTAGTTCAGCT
67	TAACCTCCATATGTGA GTGAATAAACAAAATC
68	AAATCAATGGCTTAGG TTGGGTTACTAAATTT
69	GCGCAGAGATATCAAA ATTATTTGACATTATC
70	AACCTACCGCGAATTA TTCATTTCCAGTACAT
71	ATTTTGCGTCTTTAGG AGCACTAAGCAACAGT
72	CTAAAATAGAACAAAG AAACCACCAGGGTTAG
73	GCCACGCTATACGTGG CACAGACAACGCTCAT
74	GCGTAAGAGAGAGCCA GCAGCAAAAAGGTTAT
75	GGAAATACCTACATTT TGACGCTCACCTGAAA
76	TATCACCGTACTCAGG AGGTTTAGCGGGGTTT
77	TGCTCAGTCAGTCTCT GAATTTACCAGGAGGT
78	GGAAAGCGACCAGGCG GATAAGTGAATAGGTG
79	TGAGGCAGGCGTCAGA CTGTAGCGTAGCAAGG
80	TGCCTTTAGTCAGACG ATTGGCCTGCCAGAAT
81	CCGGAAACACACCACG GAATAAGTAAGACTCC
82	ACGCAAAGGTCACCAA TGAAACCAATCAAGTT
83	TTATTACGGTCAGAGG GTAATTGAATAGCAGC

84	TGAACAAACAGTATGT TAGCAAACTAAAAGAA
85	CTTTACAGTTAGCGAA CCTCCCGACGTAGGAA
86	GAGGCGTTAGAGAATA ACATAAAAGAACACCC
87	TCATTACCCGACAATA AACAACATATTTAGGC
88	CCAGACGAGCGCCCAA TAGCAAGCAAGAACGC
89	AGAGGCATAATTTCAT CTTCTGACTATAACTA
90	TTTTAGTTTTTCGAGC CAGTAATAAATTCTGT
91	TATGTAAACCTTTTTT AATGGAAAAATTACCT
92	TTGAATTATGCTGATG CAAATCCACAAATATA
93	GAGCAAAAACTTCTGA ATAATGGAAGAAGGAG
94	TGGATTATGAAGATGA TGAAACAAAATTTCAT
95	CGGAATTATTGAAAGG AATTGAGGTGAAAAAT
96	ATCAACAGTCATCATA TTCCTGATTGATTGTT
97	CTAAAGCAAGATAGAA CCCTTCTGAATCGTCT
98	GCCAACAGTCACCTTG CTGAACCTGTTGGCAA
99	GAAATGGATTATTTAC ATTGGCAGACATTCTG
100	TTTT TATAAGTA TAGCCCGGCCGTCGAG
101	AGGGTTGA TTTT ATAAATCC TCATTAAATGATATTC
102	ACAAACAA TTTT AATCAGTA GCGACAGATCGATAGC
103	AGCACCGT TTTT TAAAGGTG GCAACATAGTAGAAAA
104	TACATACA TTTT GACGGGAG AATTAACTACAGGGAA
105	GCGCATTA TTTT GCTTATCC GGTATTCTAAATCAGA
106	TATAGAAG TTTT CGACAAAA GGTAAAGTAGAGAATA
107	TAAAGTAC TTTT CGCGAGAA AACTTTTTATCGCAAG
108	ACAAAGAA TTTT ATTAATTA CATTTAACACATCAAG
109	AAAACAAA TTTT TTCATCAA TATAATCCTATCAGAT
110	GATGGCAA TTTT AATCAATA TCTGGTCACAAATATC
111	AAACCCTC TTTT ACCAGTAA TAAAAGGGATTCACCA GTCACACGTTTT
112	CCGAAATCCGAAAATC CTGTTTGAAGCCGGAA
113	CCAGCAGGGGCAAAAT CCCTTATAAAGCCGGC
114	GCATAAAGTTCCACAC AACATACGAAGCGCCA
115	GCTCACAATGTAAAGC CTGGGGTGGGTTTGCC
116	TTCGCCATTGCCGGAA ACCAGGCATTAAATCA
117	GCTTCTGGTCAGGCTG CGCAACTGTGTTATCC
118	GTTAAAATTTTAACCA ATAGGAACCCGGCACC
119	AGACAGTCATTCAAAA GGGTGAGAAGCTATAT
120	AGGTAAAGAAATCACC ATCAATATAATATTTT
121	TTTCATTTGGTCAATA ACCTGTTTATATCGCG
122	TCGCAAATGGGGCGCG AGCTGAAATAATGTGT
123	TTTTAATTGCCCGAAA GACTTCAAAACACTAT
124	AAGAGGAACGAGCTTC AAAGCGAAGATACATT
125	GGAATTACTCGTTTAC CAGACGACAAAAGATT
126	GAATAAGGACGTAACA AAGCTGCTCTAAAACA
127	CCAAATCACTTGCCCT GACGAGAACGCCAAAA
128	CTCATCTTGAGGCAAA AGAATACAGTGAATTT
129	AAACGAAATGACCCCC AGCGATTATTCATTAC
130	CTTAAACATCAGCTTG CTTTCGAGCGTAACAC
131	TCGGTTTAGCTTGATA CCGATAGTCCAACCTA
132	TGAGTTTCGTCACCAG TACAAACTTAATTGTA
133	CCCCGATTTAGAGCTT GACGGGGAAATCAAAA
134	GAATAGCCGCAAGCGG TCCACGCTCCTAATGA
135	GAGTTGCACGAGATAG GGTTGAGTAAGGGAGC

136	GTGAGCTAGTTTCCTG TGTGAAATTTGGGAAG
137	TCATAGCTACTCACAT TAATTGCGCCCTGAGA
138	GGCGATCGCACTCCAG CCAGCTTTGCCATCAA
139	GAAGATCGGTGCGGGC CTCTTCGCAATCATGG
140	AAATAATTTTAAATTG TAAACGTTGATATTCA
141	GCAAATATCGCGTCTG GCCTTCCTGGCCTCAG
142	ACCGTTCTAAATGCAA TGCCTGAGAGGTGGCA
143	TATATTTTAGCTGATA AATTAATGTTGTATAA
144	TCAATTCTTTTAGTTT GACCATTACCAGACCG
145	CGAGTAGAACTAATAG TAGTAGCAAACCCTCA
146	GAAGCAAAAAAGCGGA TTGCATCAGATAAAAA
147	TCAGAAGCCTCCAACA GGTCAGGATCTGCGAA
148	CCAAAATATAATGCAG ATACATAAACACCAGA
149	CATTCAACGCGAGAGG CTTTTGCATATTATAG
150	ACGAGTAGTGACAAGA ACCGGATATACCAAGC
151	AGTAATCTTAAATTGG GCTTGAGAGAATACCA
152	GCGAAACATGCCACTA CGAAGGCATGCGCCGA
153	ATACGTAAAAGTACAA CGGAGATTTCATCAAG
154	CAATGACACTCCAAAA GGAGCCTTACAACGCC
155	AAAAAAGGACAACCAT CGCCCACGCGGGTAAA
156	TGTAGCATTCCACAGA CAGCCCTCATCTCCAA
157	GTAAAGCACTAAATCG GAACCCTAGTTGTTCC
158	AGTTTGGAGCCCTTCA CCGCCTGGTTGCGCTC
159	AGCTGATTACAAGAGT CCACTATTGAGGTGCC
160	ACTGCCCGCCGAGCTC GAATTCGTTATTACGC
161	CCCGGGTACTTTCCAG TCGGGAAACGGGCAAC
162	CAGCTGGCGGACGACG ACAGTATCGTAGCCAG
163	GTTTGAGGGAAAGGGG GATGTGCTAGAGGATC
164	CTTTCATCCCCAAAAA CAGGAAGACCGGAGAG
165	AGAAAAGCAACATTAA ATGTGAGCATCTGCCA
166	GGTAGCTAGGATAAAA ATTTTTAGTTAACATC
167	CAACGCAATTTTTGAG AGATCTACTGATAATC
168	CAATAAATACAGTTGA TTCCCAATTTAGAGAG
169	TCCATATACATACAGG CAAGGCAACTTTATTT
170	TACCTTTAAGGTCTTT ACCCTGACAAAGAAGT
171	CAAAAATCATTGCTCC TTTTGATAAGTTTCAT
172	TTTGCCAGATCAGTTG AGATTTAGTGGTTTAA
173	AAAGATTCAGGGGGTA ATAGTAAACCATAAAT
174	TTTCAACTATAGGCTG GCTGACCTTGTATCAT
175	CCAGGCGCTTAATCAT TGTGAATTACAGGTAG
176	CGCCTGATGGAAGTTT CCATTAAACATAACCG
177	TTTCATGAAAATTGTG TCGAAATCTGTACAGA
178	ATATATTCTTTTTTCA CGTTGAAAATAGTTAG
179	AATAATAAGGTCGCTG AGGCTTGCAAAGACTT
180	CGTAACGATCTAAAGT TTTGTCGTGAATTGCG
181	ACCCAAATCAAGTTTT TTGGGGTCAAAGAACG
182	TGGACTCCCTTTTCAC CAGTGAGACCTGTCGT
183	TGGTTTTTAACGTCAA AGGGCGAAGAACCATC
184	GCCAGCTGCCTGCAGG TCGACTCTGCAAGGCG
185	CTTGCATGCATTAATG AATCGGCCCGCCAGGG
186	ATTAAGTTCGCATCGT AACCGTGCGAGTAACA
187	TAGATGGGGGGTAACG CCAGGGTTGTGCCAAG

188	ACCCGTCGTCATATGT ACCCCGGTAAAGGCTA
189	CATGTCAAGATTCTCC GTGGGAACCGTTGGTG
190	TCAGGTCACTTTTGCG GGAGAAGCAGAATTAG
191	CTGTAATATTGCCTGA GAGTCTGGAAAACTAG
192	CAAAATTAAAGTACGG TGTCTGGAAGAGGTCA
193	TGCAACTAAGCAATAA AGCCTCAGTTATGACC
194	TTTTTGCGCAGAAAAC GAGAATGAATGTTTAG
195	AAACAGTTGATGGCTT AGAGCTTATTTAAATA
196	ACTGGATAACGGAACA ACATTATTACCTTATG
197	ACGAACTAGCGTCCAA TACTGCGGAATGCTTT
198	CGATTTTAGAGGACAG ATGAACGGCGCGACCT
199	CTTTGAAAAGAACTGG CTCATTATTTAATAAA
200	GCTCCATGAGAGGCTT TGAGGACTAGGGAGTT
201	ACGGCTACTTACTTAG CCGGAACGCTGACCAA
202	AAAGGCCGAAAGGAAC AACTAAAGCTTTCCAG
203	GAGAATAGCTTTTGCG GGATCGTCGGGTAGCA
204	ACGTTAGTAAATGAAT TTTCTGTAAGCGGAGT
205	TTTT CGATGGCC CACTACGTAAACCGTC
206	TATCAGGG TTTT CGGTTTGC GTATTGGGAACGCGCG
207	GGGAGAGG TTTT TGTAAAAC GACGGCCATTCCCAGT
208	CACGACGT TTTT GTAATGGG ATAGGTCAAAACGGCG
209	GATTGACC TTTT GATGAACG GTAATCGTAGCAAACA
210	AGAGAATC TTTT GGTTGTAC CAAAAACAAGCATAAA
211	GCTAAATC TTTT CTGTAGCT CAACATGTATTGCTGA
212	ATATAATG TTTT CATTGAAT CCCCCTCAAATCGTCA
213	TAAATATT TTTT GGAAGAAA AATCTACGACCAGTCA
214	GGACGTTG TTTT TCATAAGG GAACCGAAAGGCGCAG
215	ACGGTCAA TTTT GACAGCAT CGGAACGAACCCTCAG
216	CAGCGAAAA TTTT ACTTTCA ACAGTTTCTGGGATTT TGCTAAAC TTTT
Loop1	AACATCACTTGCCTGAGTAGAAGAACT
Loop2	TGTAGCAATACTTCTTTGATTAGTAAT
Loop3	AGTCTGTCCATCACGCAAATTAACCGT
Loop4	ATAATCAGTGAGGCCACCGAGTAAAAG
Loop5	ACGCCAGAATCCTGAGAAGTGTTTTT
Loop6	TTAAAGGGATTTTAGACAGGAACGGT
Loop7	AGAGCGGGAGCTAAACAGGAGGCCGA
Loop8	TATAACGTGCTTTCCTCGTTAGAATC
Loop9	GTACTATGGTTGCTTTGACGAGCACG
Loop10	GCGCTTAATGCGCCGCTACAGGGCGC