Supporting Information

High affinity protein surface binding through co-engineering of nanoparticles and proteins

*Moumita Ray,[a] Giorgia Brancolini,[b] David C. Luther,[a] Ziwen Jiang,[a] Roberto Cao-Milán,[a] Alejandro M. Cuadros,[a] Andrew Burden,[a] Vincent Clark,[a] Subinoy Rana,[a] Rubul Mout,[a] Ryan F. Landis,[a] Stefano Corni[b,c] and Vincent M. Rotello[*a]*

[a] Department of Chemistry, University of Massachusetts, 710 North Pleasant Street, Amherst, MA 01003, USA

[b] Center S3, CNR Institute of Nanoscience, via Campi 213/A, 41125 Modena, Italy

[c] Department of Chemical Science, University of Padova, via Marzolo 1, 35131 Padova, Italy

* Corresponding author e-mail: rotello@umass.edu

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Size and ζ-potential of AuNPs

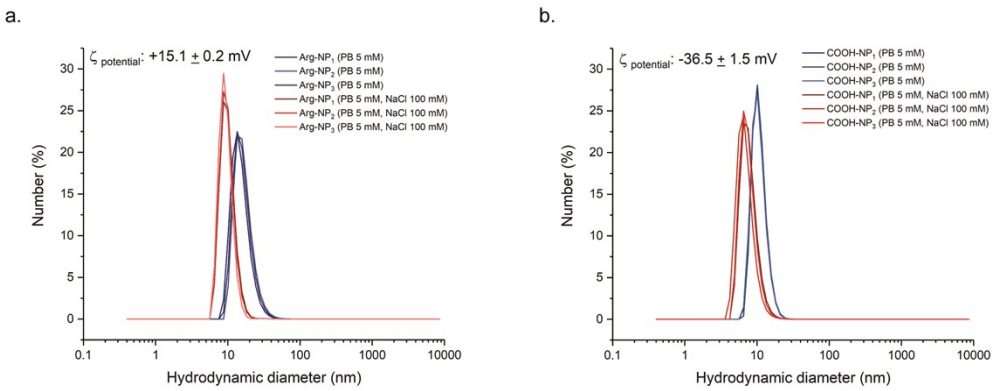

Figure S1. ζ-potential and dynamic light scattering (DLS) diameter of (a) Arg-NPs and (b) COOH-NPs, measured at 5 mM PB, and 5 mM PB (NaCl, 150 mM), pH 7.4. Each nanoparticle solution was prepared to final concentrations of 0.1 µM.

Protein Sequences

*wt*GFP Sequence:

MRGSHHHHHHGSMASKGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGKLPVPWP
TLVTTFSYGVQCFSRYPDHMKRHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDFK
EDGNILGHKLEYNYNSHNVYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLST
QSALSKDPNEKRDHMVLLEFVTAAGITHGMDEYK

-30GFP Sequence

MGHHHHHHGGASKGEELFDGVVPILVELDGDVNGHEFSVRGEGEGDATEGELTLKFICTTGELPVPWPTLV
TTLTYGVQCFSDYPDHMDQHDFFKSAMPEGYVQERTISFKDDGTYKTRAEVKFEGDTLVNRIELKGIDFKED
GNILGHKLEYNFNSHDVYITADKQENGIKAEFEIRHNVEDGSVQLADHYQQNTPIGDGPVLLPDDHYLSTESA
LSKDPNEDRDHMVLLEFVTAAGIDHGMDELYK

+15GFP sequence with Thrombin cleavage site:

MRGSGHHHHHHGSLVPRGSGGASKGERLFTGVVPILVELDGDVNGHKFSVRGEGEGDATRGKLTLKFICTT
GKLPVPWPTLVTTLTYGVQCFSRYPKHMKRHDFFKSAMPEGYVQERTISFKKDGTYKTRAEVKFEGRTLVNRI
ELKGRDFKEKGNILGHKLEYNFNSHNVYITADKRKNGIKANFKIRHNVKDGSVQLADHYQQNTPIGRGPVLLP
RNHYLSTRSALSKDPKEKRDHMVLLEFVTAAGITHGMDELYK

+36GFP sequence with Thrombin cleavage site:

MRGSHHHHHHGSLVPRGSGGASKGERLFRGKVPILVELKGDVNGHKFSVRGKGKGDATRGKLTLKFICTTG
KLPVPWPTLVTTLTYGVQCFSRYPKHMKRHDFFKSAMPKGYVQERTISFKKDGKYKTRAEVKFEGRTLVNRIK
LKGRDFKEKGNILGHKLRYNFNSHKVYITADKRKNGIKAKFKIRHNVKDGSVQLADHYQQNTPIGRGPVLLPR
NHYLSTRSKLSKDPKEKRDHMVLLEFVTAAGIKHGRDERYK

Radial distribution functions of AuNPs at varied ionic strength

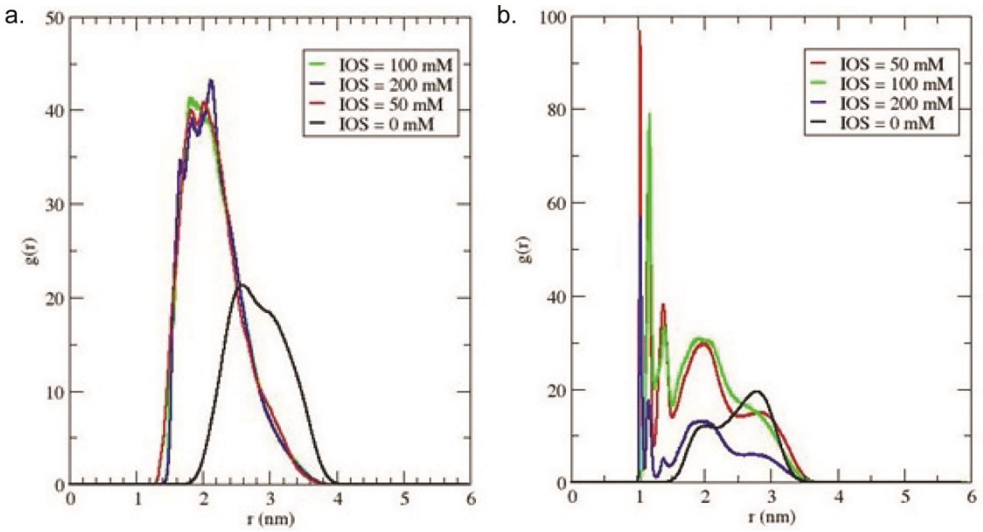

Figure S2. - Radial distribution functions of (a.) Arg-NPs and (b.) COOH-NPs from 100 ns MD simulations including explicit water at 0 mM and Na+/Cl- ions to simulate ionic strength conditions (IOS) at 0 mM, 50 mM, 100 mM and 200 mM, respectively.

Size determination of GFP: NPs assemblies

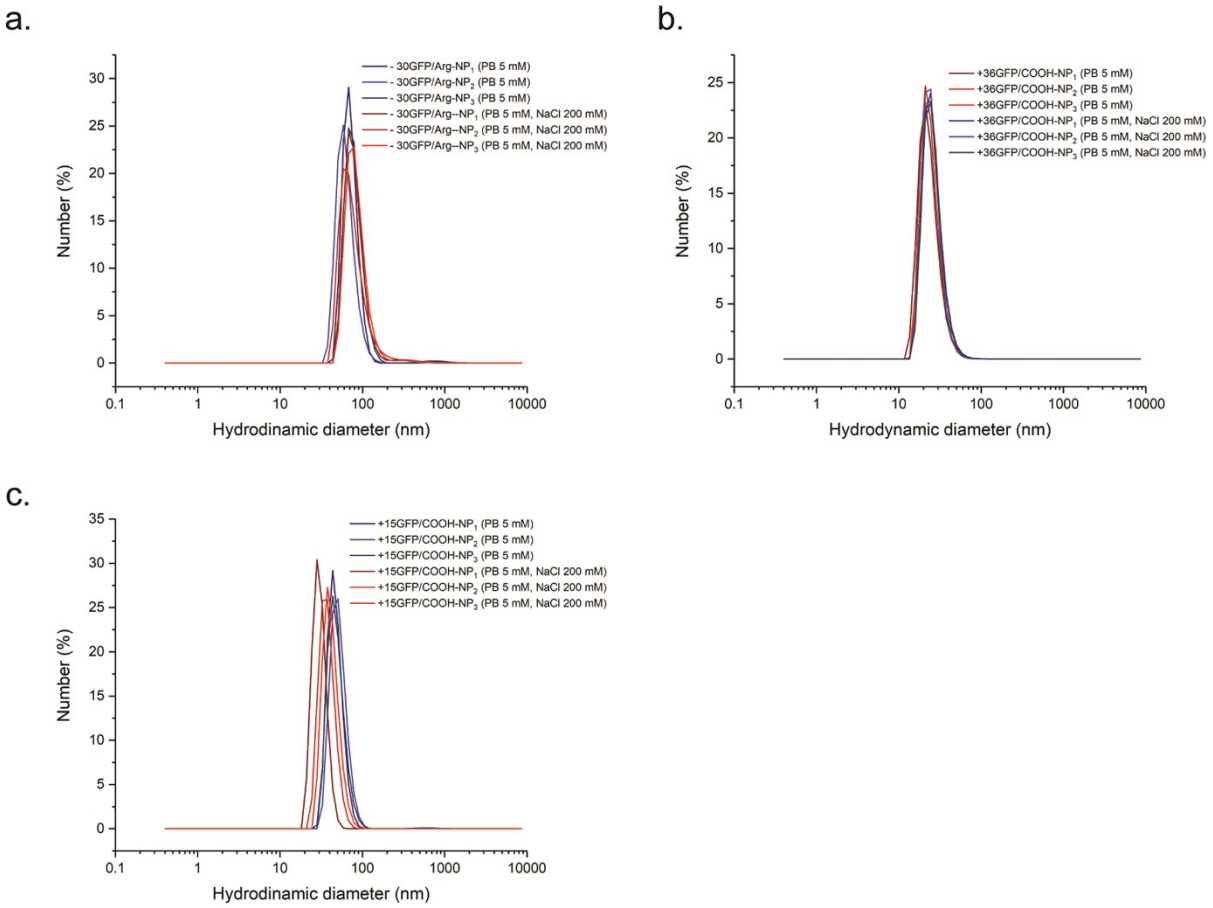

Figure S3*:* To study the formation of assemblies by DLS, 1 mL of stock solutions containing each GFP mutant, -30GFP (a), +36GFP (b), and +15GFP (c) at 100 nM in PB (5 mM) and PB with NaCl (200 mM) were prepared. 0.5 mL of each solutions were added into 0.5 mL solutions of the corresponding NP (COOH-NP and Arg-NP) solution, previously dissolved in the same buffer conditions to form the assemblies. Subscript represents technical replicates. Protein: NP ratios used were those necessary to bind all protein in solution, in accordance with titration experiments (-30GFP: Arg-NP equal to 1:4, +36GFP: COOH-NP and +15GFP: COOH-NP equal to 1:1). The mixture was let to stabilize for at least 10 min. before performing DLS analyses. Size determinations of these assemblies were based on number. These results demonstrate the formation of discrete assemblies between each supercharged GFP and its complementary NP.

Size determination of GFP: (excess) NPs

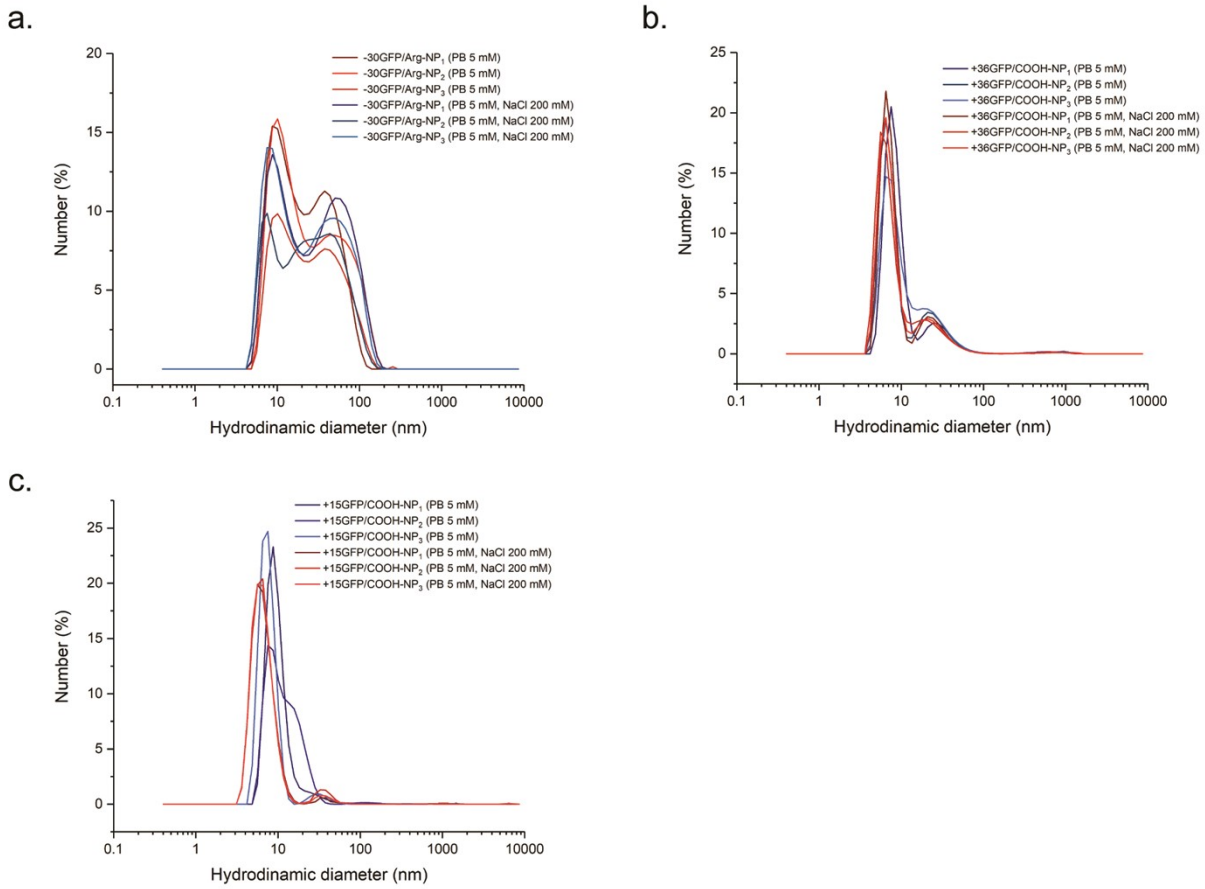

Figure S4*:* To study the formation of assemblies in an excess of NPs using DLS, 1 mL of stock solutions containing each GFP mutant, -30GFP (a), +36GFP (b), and +15GFP (c) at 100 nM in PB (5 mM) and PB with NaCl (200 mM) were prepared. 0.5 mL of each solution was added into 0.5 mL solutions of the corresponding NP (COOH-NP or Arg-NP) solution, previously dissolved in the same buffer conditions to form the assemblies. Protein: NP ratios used were the necessary amount to bind all protein in solution, in accordance with titration experiments (+30GFP: ArgNP equal to 1:6, -36GFP: COOHNP and -15GFP: COOH-NP equal to 1:4). The mixture was let to stabilize for 10 min before analysis. Size determinations of this assemblies were based on number. These results demonstrate the formation of discrete assemblies between supercharged GFPs and the complementary NPs even in the presence of excess NPs. Excess NPs were observed in DLS as a second population of free NPs.

Binding constants of -30GFP: Arg-NPs at varied temperature

Table S1*:* Binding constant values derived from fluorescence titrations between Arg-NPs and -30GFP (50 nM), performed parametrically at varying temperature (22-37°C) and salt concentrations (NaCl, 0-200 mM) in 5 mM phosphate buffer.

	K_b					
	22 °C	**25 °C**	**28 °C**	**31 °C**	**34 °C**	**37 °C**
NaCl (mM) in 5mM PB	$K_b \times 10^9/M^{-1}$					
0	2.741 ± 0.147	2.809 ± 0.411	4.718 ± 0.256	4.711 ± 0.512	4.372 ± 1.210	5.024 ± 0.433
25	2.923 ± 0.344	5.696 ± 1.676	7.764 ± 1.213	3.441 ± 0.422	4.176 ± 0.433	4.829 ± 0.419
50	3.257 ± 0.201	11.032 ± 2.210	7.685 ± 0.920	2.538 ± 0.502	3.948 ± 0.710	4.135 ± 0.817
100	13.220 ± 3.213	6.686 ± 0.311	5.481 ± 0.105	2.638 ± 0.305	3.236 ± 0.214	3.963 ± 0.088
125	6.691 ± 0.707	6.149 ± 0.119	3.043 ± 0.377	2.159 ± 0.499	2.984 ± 0.812	2.149 ± 0.309
150	3.331 ± 0.166	4.732 ± 0.561	2.437 ± 0.712	1.257 ± 0.215	2.096 ± 0.464	1.686 ± 0.005
200	3.139 ± 0.198	2.734 ± 0.953	1.133 ± 0.180	1.271 ± 0.409	2.007 ± 0.166	1.062 ± 0.013

Titration of -30GFP with Arg-NPs at varied temperature

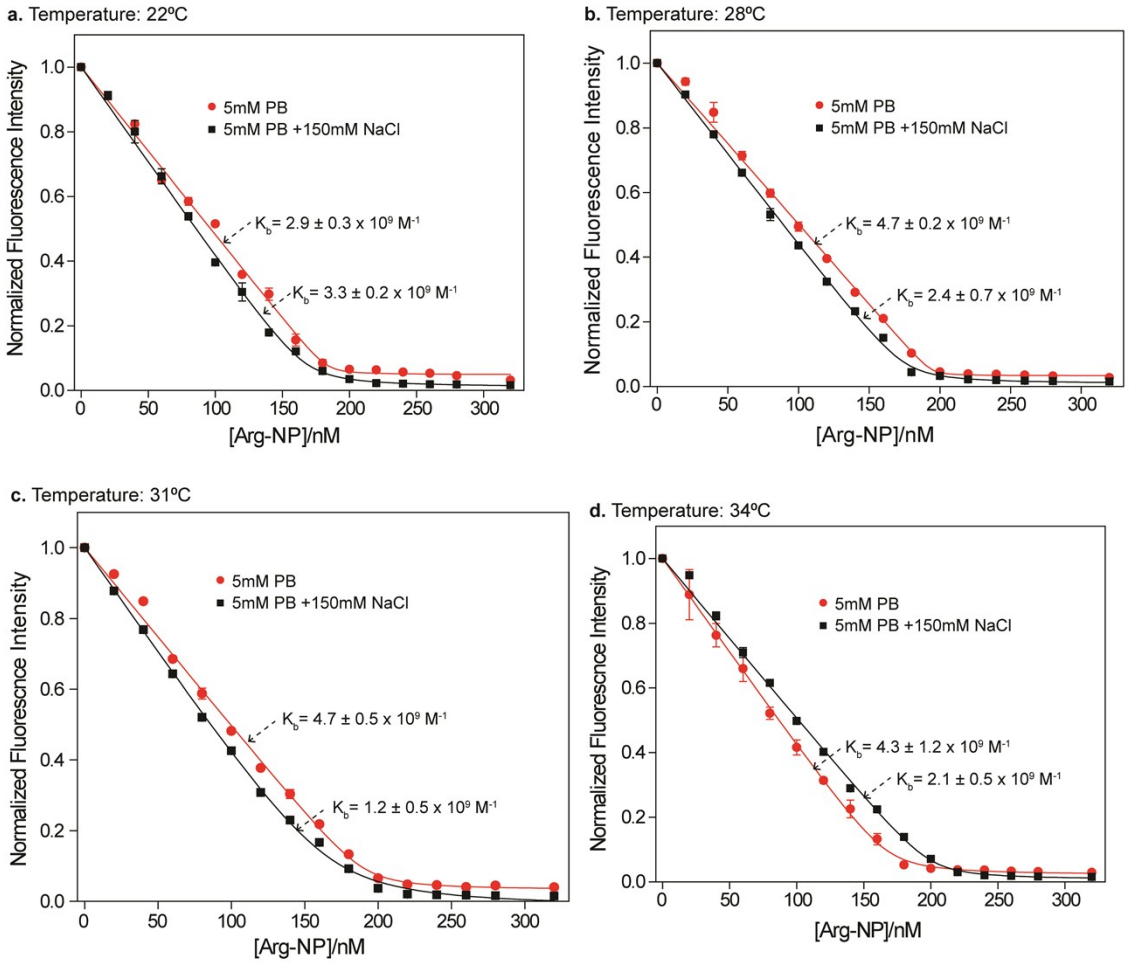

Figure S5*:* Fluorescence titrations between Arg-NPs and -30GFPs (50 nM) in 5 mM phosphate buffer and in 150 mM NaCl containing 5 mM phosphate buffer (pH 7.4) at (a) 22°C, (b) 28°C, (c) 31°C and (d) 34°C. The complex binding constant (K_b) was determined using previously reported method.

Binding constants of +36GFP: COOH-NPs at varied temperature

Table S2*:* Binding constant values derived from fluorescence titrations between COOH-NPs and +36GFP (50 nM), performed parametrically at varying temperature (22-37°C) and salt concentrations (NaCl, 0-200 mM) in 5 mM phosphate buffer.

	K_b					
	22 °C	**25 °C**	**28 °C**	**31 °C**	**34 °C**	**37 °C**
NaCl (mM) in 5mM PB	$K_b \times 10^9/M^{-1}$					
0	0.214 ± 0.114	0.265 ± 0.302	0.191 ± 0.026	0.405 ± 0.050	0.275 ± 0.022	0.465 ± 0.050
25	0.525 ± 0.034	0.564 ± 0.646	0.341 ± 0.213	0.328 ± 0.043	0.473 ± 0.046	0.339 ± 0.042
50	0.983 ± 0.021	0.574 ± 0.210	0.509 ± 0.090	0.153 ± 0.054	0.414 ± 0.071	0.345 ± 0.082
100	2.976 ± 0.221	2.093 ± 0.032	0.673 ± 0.012	0.279 ± 0.031	0.252 ± 0.022	0.392 ± 0.009
125	0.579 ± 0.170	0.238 ± 0.011	0.377 ± 0.038	0.149 ± 0.050	0.224 ± 0.080	0.166 ± 0.031
150	0.584 ± 0.017	0.109 ± 0.053	0.200 ± 0.074	0.128 ± 0.030	0.242 ± 0.056	0.153 ± 0.000
200	0.588 ± 0.020	0.176 ± 0.090	0.321 ± 0.012	0.144 ± 0.041	0.202 ± 0.017	0.187 ± 0.001

Titrations of -7GFP (*wt*GFP) with Arg-NPs at varied ionic strength

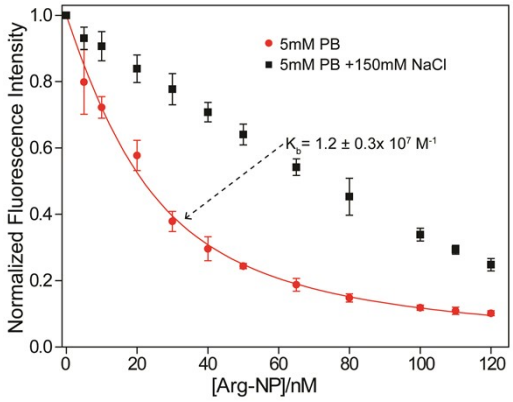

Figure S6*:* Fluorescence titrations between Arg-NPs and *wt*GFP (100 nM) in 5 mM phosphate buffer and in 150 mM NaCl containing 5 mM phosphate buffer (pH 7.4) at 25°C. Complex binding constant (K_b) was determined using previously reported method.

Titrations of +36GFP with COOH-NPs at varied temperatures and ionic strength

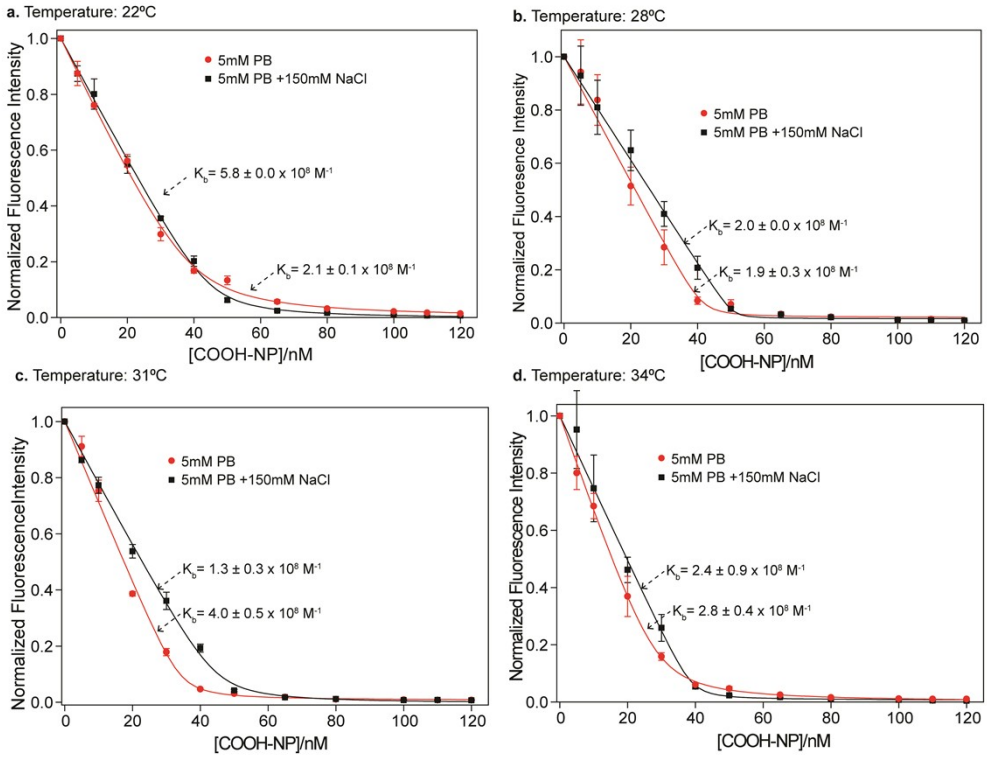

Figure S7*:* Fluorescence titrations between COOH-NPs and +36GFPs (100 nM) in 5 mM phosphate buffer and in 150 mM NaCl containing 5 mM phosphate buffer (pH 7.4) at (a) 22°C, (b) 28°C, (c) 31°C and (d) 34°C. The complex binding constant (K_b) was determined using previously reported method.

Binding constant of +15GFP and COOH-NPs

Table S3*:* Binding constant values derived from fluorescence titrations between COOH-NPs and +15GFP (100 nM), performed parametrically at varying temperature (22-37°C) and salt concentrations (NaCl, 0-200 mM) in 5 mM phosphate buffer.

	K_b					
	22 °C	**25 °C**	**28 °C**	**31 °C**	**34 °C**	**37 °C**
NaCl (mM) in 5mM PB	$K_b \times 10^9/M^{-1}$					
0	0.068 ± 0.001	0.056	0.060	0.021	0.030	0.016
25	0.062	0.056	0.089	0.038	0.031	0.015
50	0.075	0.057	0.089 ± 0.002	0.055	0.014	0.026
100	0.154 ± 0.002	0.139	0.126	0.096	0.058	0.047
125	0.106 ± 0.001	0.043	0.057	0.028	0.034	0.032
150	0.097	0.030	0.024	0.034	0.029	0.049
200	0.054	0.047	0.017	0.017	0.011	0.012

Titrations of +15GFP with COOH-NPs at varied temperatures and ionic strength

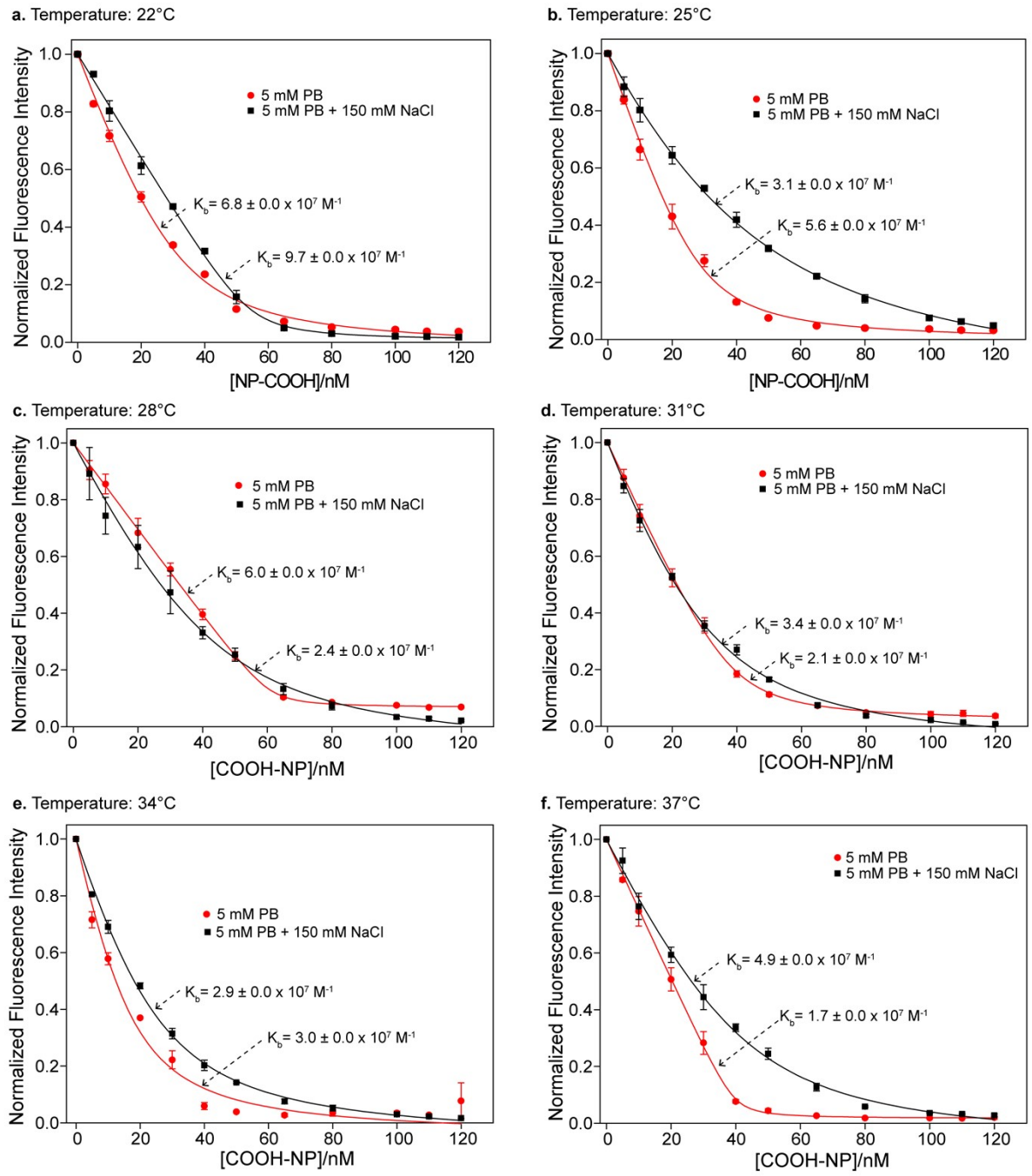

Figure S8*:* Fluorescence titrations between COOH-NPs and +15GFPs (100 nM) in 5 mM phosphate buffer and in 150 mM NaCl containing 5 mM phosphate buffer (pH 7.4) at (a) 22°C, (b) 25°C, (c) 28°C, (d) 31°C (e) 34°C and (f) 37°C. Complex binding constant (K_b) was determined using a previously reported method.

Cumulative number of counterions within AuNP monolayers

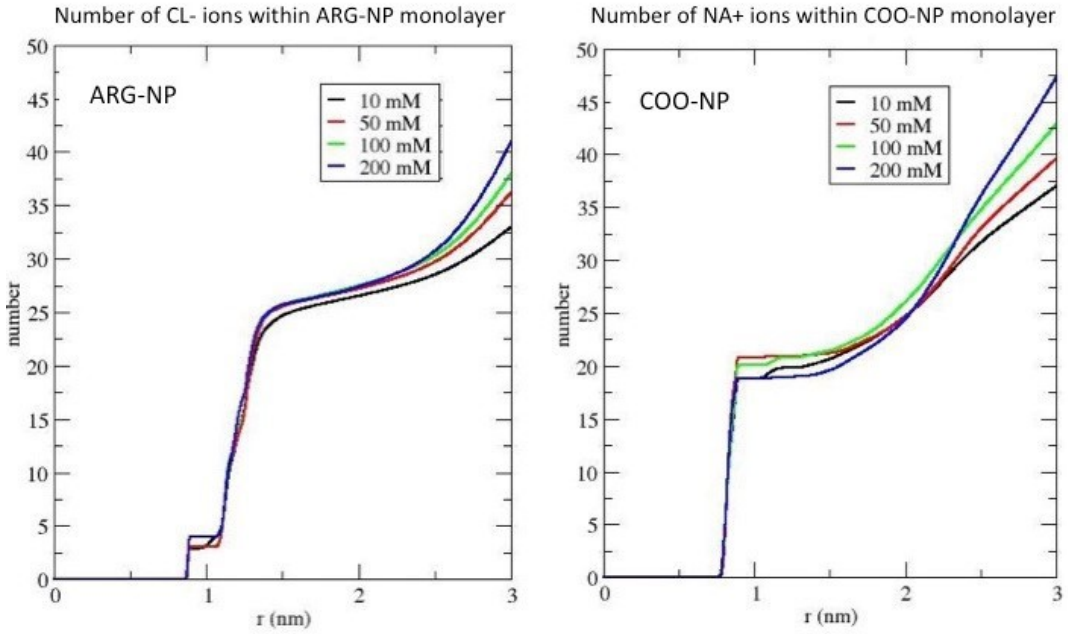

Figure S9: Cumulative number RDF (Radial Distribution Function) from MD, corresponding to the average number of Cl^- (or Na^+) ions within a distance *r* from the center-of-mass of gold core. The NPs monolayer was approximated with the radius of gyration of Arg-NP (r= 2.5 nm) and COO-NP (r= 2 nm), respectively. Results were obtained from 200 ns MD trajectories of Arg-NP and COOH-NP in water.

Computational model of +36GFP and *wt*GFP with COOH-NP

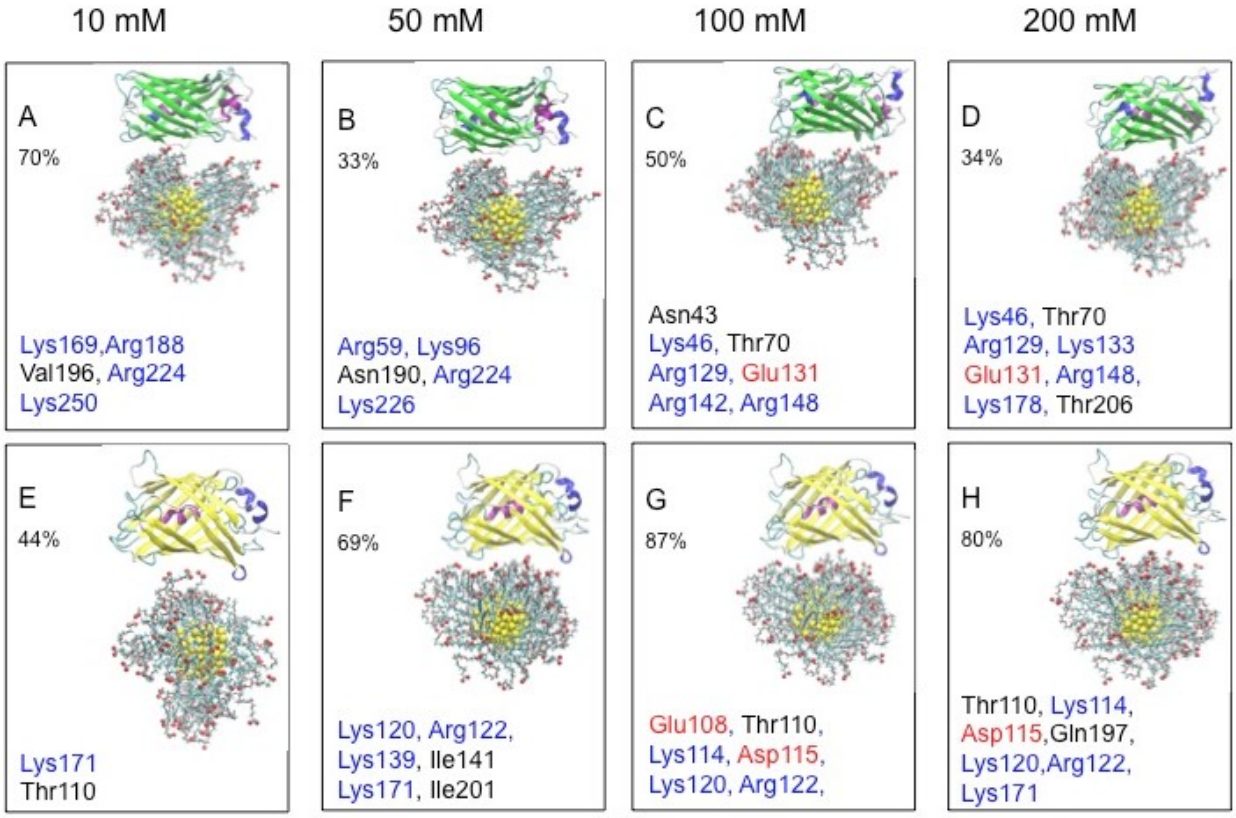

Figure S10: Representative structure of the most populated complexes of +36GFP on anionic gold nanocluster $Au_{144}[L_{60}]^{-60}$ [$L=S(CH_2)_9(OC_2H_4)_4COO^-$] at different ionic strengths, obtained from BD simulation. The relative population of the selected clusters is reported in percentage. The protein residues contacting the nanoparticle at the short distances (less than 3.5 Å) are reported with different color, indicating neutral residues (black), positively charged residues (blue) and negatively charged residues (red). The protein backbone is shown in cartoon representation. The ligand and the gold nanoparticle are shown in Van der Waals representation. Below: Representative structure of the most populated complexes of *wt*GFP on anionic gold nanocluster $Au_{144}[L_{60}]^{-60}$ at different ionic strengths, obtained from BD simulation.

Docking results of +36GFP and *wt*GFP with COOH-NP

Table S4*:* Summarized docking results of +36GFP: COOH-NP and *wt*GFP: COOH-NP interaction at varied ionic strength (IS); these represent the most populated complexes, which are ranked by size. (a) Relative population of the cluster (b) U_{Repr}: total interaction energy of the representative of the given cluster, in kT with T= 300K (c) U_{EP}: total electrostatic energy of the representative complex, in kT (d) U^{e}_{ds}: electrostatic desolvation energy of the representative complex, in kT (e) U^{h}_{ds}: non-polar (hydrophobic) desolvation energy of the representative complex, in kT (f) RMSD of the structures within the cluster with respect to the representative complex.

Label	RelPop [a] %	U_{Repr} [b]	U_{EP} [c]	U^{e}_{ds} [d]	U^{h}_{ds} [e]	spread [f]
+36GFP : COOH-NPs						
IS = 10 mM						
a1	70	-52.942	-42.384	5.452	-16.010	3.643
a2	9	-53.740	-44.251	7.585	-17.074	0.869
a3	11	-53.077	-40.761	7.067	-19.382	7.863
a4	5	-54.251	-38.431	8.288	-24.108	0.509
a5	5	-54.559	-40.977	11.417	-25.000	0.477
IS = 50 mM						
b1	33	-35.193	-22.054	4.923	-18.062	2.154
b2	24	-35.442	-21.123	6.233	-20.551	1.489
b3	20	-37.296	-22.588	9.845	-24.552	0.987
b4	22	-36.552	-20.223	7.004	-23.332	0.820
IS = 100 mM						
c1	50	-32.189	-16.800	7.113	-22.502	1.049
c2	26	-34.418	-17.851	6.518	-23.085	1.138
c3	12	-31.348	-17.103	4.572	-18.817	4.990
c4	11	-32.149	-15.719	6.328	-22.757	0.757
IS = 200 mM						
d1	34	-29.228	-13.254	8.166	-24.140	1.108
d2	18	-27.651	-13.992	7.150	-20.809	1.729
d3	31	-29.147	-12.892	7.268	-23.523	0.872
d4	16	-28.370	-12.408	5.932	-21.895	0.627
***wt*GFP : COOH-NPs**						
IS = 10 mM						
e1	44	-11.024	-6.454	5.485	-10.056	5.815
e2	37	-10.722	-4.434	5.219	-11.507	6.123
e3	16	-11.116	-7.496	6.672	-10.292	6.559
e4	3	-10.615	-6.014	5.515	-10.117	6.102
IS = 50 mM						
f1	69	-11.512	-5.103	5.407	-11.816	12.369
f2	22	-13.897	-3.669	5.988	-16.216	2.210
f3	8	-13.945	-5.013	4.549	-13.481	1.281
f4	1	-11.419	-7.370	5.518	-9.566	2.509
IS = 100 mM						
g1	87	-15.020	-4.353	4.661	-15.328	2.606
g2	10	-12.906	-3.161	2.388	-12.132	4.707
g3	2	-14.025	2.122	1.356	-17.503	7.523
g4	1	-14.340	-6.592	5.718	-13.465	1.067
IS = 200 mM						
h1	80	-13.447	-3.868	4.999	-14.578	7.153
h2	11	-12.240	0.210	2.639	-15.089	2.549
h3	6	-12.033	-3.010	4.185	-13.207	6.742
h4	3	-12.060	-3.892	2.733	-10.901	3.915