Supplementary Material (ESI) for Molecular BioSystems

Supporting Information

Supramolecular Assembly and Small Molecule Recognition by Genetically Engineered Protein Block Polymers Composed of Two SADs

Jennifer S. Haghpanah,[a] Carlo Yuvienco,[a] Eric W. Roth,[b] Alice Liang,[b] Raymond S. Tu[c] and Jin Kim Montclare[a,d]

[a] *Polytechnic Institute of New York University, 6 Metrotech Center, Brooklyn, NY 11201 USA Fax: 718-260-3676; Tel: 718-260-3679; jmontcla@poly.edu*

[b] *New York University School of Medicine, 550 First Avenue, New York, NY, 10016 USA.*

[c] *The City College of New York, 160 Convent Avenue, New York, NY 10031 USA.*

[d] *SUNY Downstate Medical Center, 450 Clarkson Avenue, Brooklyn, NY 11203 USA.*

Expression & Purification. DNA constructs containing the C, EC, CE and ECE genes were transformed into phenylalanine auxotrophic *E.coli* strain AF-IQ containing the plasmid IQ, which expresses the *lacI*^{q} repressor[1, 2]. Protein polymers were expressed in a 2L flask containing 200 mL of M9 minimal medium with 200 µL of each of the following: 1M $MgSO_4$, 0.1M $CaCl_2$, 35 mg/ mL chloramphenicol, 200 mg/ mL ampicillin, and 35 mg/ mL vitamin B. The M9 medium also contained 8 mL of 1g/L 20 amino acid solution and 2 mL of a 40% glucose solution. After 6 hours of pre-induction at 37°C and 350 rpm an OD_{600} of 1.0 was reached. The cell culture was then centrifuged at 4000 rpm and cell pellet was washed twice with cold 0.9% NaCl and then re-suspended in a 2L flask with 200 mL fresh M9 medium containing all the ingredients above with 200 µL of 200 mg/ mL Isopropyl β-D-1-thiogalactopyranoside. Expression was induced for 3 hours at 37°C and 350 rpm. Cells were centrifuged at 8000 rpm, and stored in the -80°C. Cells were subjected to osmotic shock[3] and then lysed via French press at 23600 psi. To remove cell debris, samples were centrifuged at 20,000 RCF at 4°C. The proteins in the supernatant was purified under denaturing conditions via IMAC HighTrap FF column (GE Life Sciences) on FPLC and were eluted in 50 mM sodium phosphate dibasic and 6M urea pH = 8.0 with an imdazole gradient from 20 mM to 1 M across 10 mL.

Expression for E was carried out using methods from our previous paper[4]. A 1 L pellet of E was re-suspended in 40 mL of lysis buffer (115 mM monobasic sodium phosphate, 8 M urea, 10 mM Tris-HCl, pH 8.0). The lysate was thawed at 4 °C and homogenized with a French press (40K-cell; Thermo Scientific) at 23 600 psi. Lysate was then centrifuged at 20,000 rpm for 75 min at 4 °C. The crude extract was allowed to bind on a rotating mixer to Ni-NTA agarose beads (2.5 mL, Qiagen) for 1 h. The beads with the crude extract were then centrifuged for 8 min at 1000 rpm and 25 mL of cell lysate, which was not bound to the beads, was poured off. This process of washing the beads was repeated two more times, and the last 25 mL was packed into 2 mL column (Pierce) and eluted with a decreasing step-wise pH gradient of lysis buffer, pH 8.0 – 5.0.

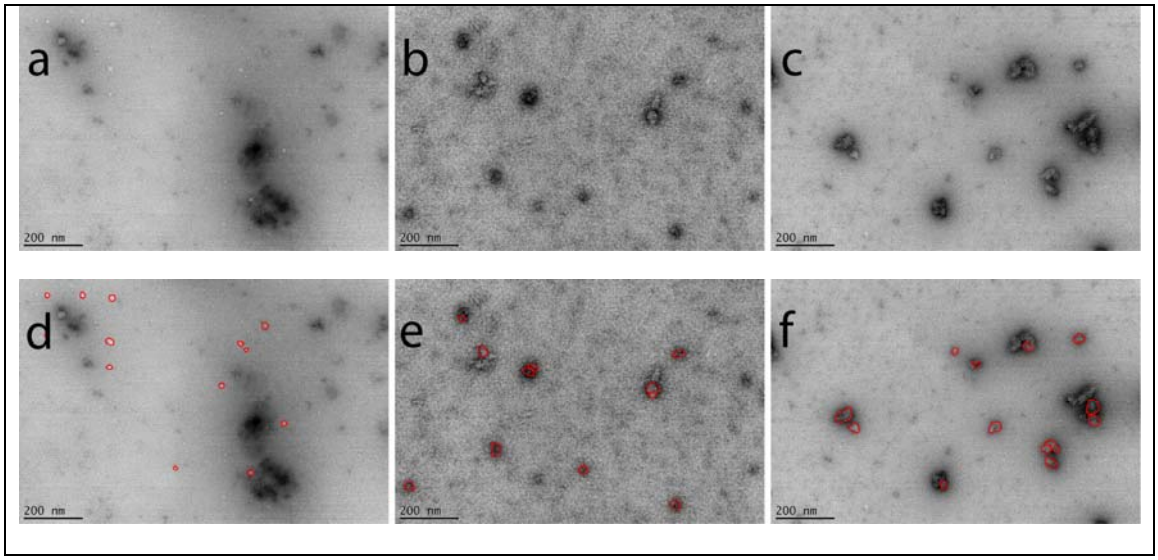

Figure S1: x 110,000 magnification micrographs of a) elasin b) COMP and c) elastin/COMP mixture protein samples with identified particles (d-f, marked in red) used for length measurements using ImageJ.

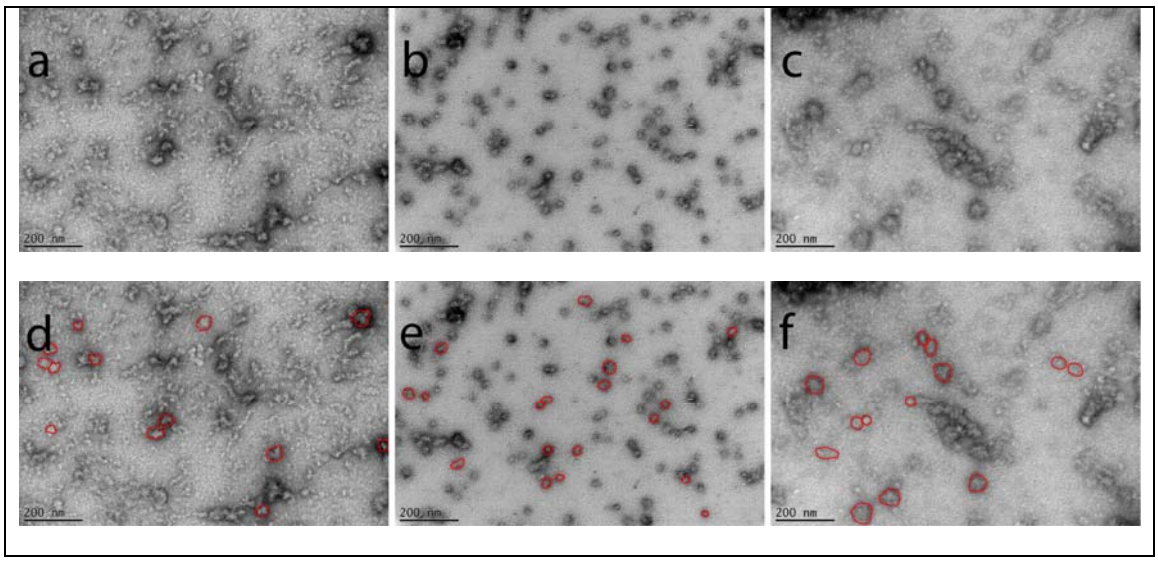

Figure S2: x 110,000 magnification micrographs of a) EC b) CE and c) ECE block protein samples with identified particles (d-f, marked in red) used for length measurements using ImageJ.

Table S1: Particle size measurements performed on particles identified in Figures S1 and S2 using ImageJ software.

Elastin					
Particle	**Area (nm^2)**	**Major (nm)**	**Minor (nm)**	**Angle**	**Circularity**
1	454.444	29.008	19.947	155.815	0.888
2	230.667	20.897	14.054	176.508	0.906
3	263.333	20.454	16.392	97.748	0.885
4	354.222	21.498	20.979	135.262	0.952
5	198.556	16.464	15.355	116.973	0.941
6	210	19.411	13.775	76.187	0.917
7	241.556	20.712	14.849	15.666	0.906
8	279.667	19.186	18.56	151.201	0.947
9	121.333	13.483	11.458	136.809	0.876
10	307.667	21.788	17.98	11.765	0.924
11	407.667	24.147	21.496	105.924	0.942
12	131.444	14.213	11.775	15.812	0.725
13	204	19.433	13.366	156.552	0.763
Mean	261.889	20.053	16.153	104.017	0.89
SD	98.946	4.032	3.376	57.776	0.07
Min	121.333	13.483	11.458	11.765	0.725
Max	454.444	29.008	21.496	176.508	0.952

COMP					
Particle	**Area (nm^2)**	**Major (nm)**	**Minor (nm)**	**Angle**	**Circularity**
1	881.222	39.365	28.502	119.12	0.858
2	369.222	26.36	17.834	173.843	0.885
3	717.667	40.156	22.755	152.618	0.851
4	498.667	35.834	17.718	148.497	0.752
5	1064.778	46.877	28.921	82.392	0.726
6	736.667	34.883	26.888	152.032	0.879
7	662.333	33.525	25.154	157.678	0.863
8	759.778	36.922	26.201	139.08	0.852
9	363.333	23.401	19.769	9.433	0.919
10	1095.556	44.141	31.601	1.4	0.862
11	692.444	40.438	21.802	13.876	0.793
Mean	712.879	36.537	24.286	104.543	0.84
SD	243.011	6.997	4.665	66.276	0.059
Min	363.333	23.401	17.718	1.4	0.726
Max	1095.556	46.877	31.601	173.843	0.919

Elasin+COMP Mixture					
Particle	**Area (nm^2)**	**Major (nm)**	**Minor (nm)**	**Angle**	**Circularity**
1	824	35.837	29.276	139.606	0.916
2	1877.556	63.614	37.58	32.11	0.804
3	955.444	46.094	26.392	145.963	0.799
4	740	34.456	27.345	17.141	0.902
5	949	55.596	21.733	136.459	0.637
6	1112.778	43.365	32.672	26.982	0.81
7	370.222	24.507	19.235	66.991	0.841
8	402	27.784	18.422	164.435	0.819
9	846.556	38.793	27.785	11.565	0.852
10	1669.778	52.163	40.757	75.829	0.852
11	439.556	30.31	18.464	102.3	0.816
12	594	35.34	21.401	153.14	0.824
13	1104.778	44.302	31.751	138.525	0.893
Mean	914.282	40.936	27.139	93.157	0.828
SD	457.051	11.397	7.233	57.131	0.069
Min	370.222	24.507	18.422	11.565	0.637
Max	1877.556	63.614	40.757	164.435	0.916

Elastin-COMP Fusion					
Particle	**Area (nm^2)**	**Major (nm)**	**Minor (nm)**	**Angle**	**Circularity**
1	1852.778	53.99	43.694	41.701	0.886
2	1517.556	51.247	37.704	9.178	0.916
3	1709.444	58.075	37.478	1.583	0.844
4	694.111	32.253	27.401	48.6	0.811
5	1423.778	48.617	37.288	179.674	0.855
6	1043.889	43.392	30.63	39.274	0.803
7	1297.222	46.131	35.804	167.573	0.884
8	1106.667	41.35	34.076	166.651	0.674
9	2110.333	56.63	47.448	32.937	0.872
10	2456.556	58.189	53.752	30.928	0.717
11	1199	42.391	36.013	109.754	0.783
12	1320.778	45.791	36.725	13.188	0.906
13	731.778	35.486	26.257	162.602	0.873
Mean	1420.299	47.196	37.251	77.203	0.833
SD	511.988	8.307	7.603	68.97	0.073
Min	694.111	32.253	26.257	1.583	0.674
Max	2456.556	58.189	53.752	179.674	0.916

COMP-Elastin Fusion					
Particle	**Area (nm^2)**	**Major (nm)**	**Minor (nm)**	**Angle**	**Circularity**
1	685.222	33.935	25.709	61.166	0.906
2	457.556	29.081	20.033	1.962	0.893
3	832.444	36.583	28.972	65.284	0.877
4	626.667	33.206	24.028	162.521	0.877
5	723.222	35.582	25.88	43.876	0.879
6	558.889	27.455	25.919	52.623	0.962
7	522.667	27.978	23.786	25.01	0.885
8	484	28.207	21.847	4.597	0.932
9	345.556	23.947	18.373	164.335	0.938
10	1146.556	42.29	34.519	125.285	0.919
11	462	25.891	22.719	116.478	0.96
12	1080.222	42.855	32.094	31.822	0.929
13	1124	44.859	31.903	173.786	0.898
14	935.444	42.414	28.082	5.258	0.905
15	1569.444	46.665	42.822	137.578	0.933
16	886.444	48.356	23.341	26.063	0.752
17	694.333	30.658	28.836	22.745	0.945
18	780.444	39.701	25.03	11.999	0.879
19	517.778	27.312	24.138	147.393	0.95
Mean	759.626	35.104	26.738	72.62	0.906
SD	307.751	7.75	5.679	61.943	0.047
Min	345.556	23.947	18.373	1.962	0.752
Max	1569.444	48.356	42.822	173.786	0.962

Elastin-COMP-Elastin Fusion					
Particle	**Area (nm^2)**	**Major (nm)**	**Minor (nm)**	**Angle**	**Circularity**
1	2852.778	64.818	56.038	124.477	0.908
2	1592.778	57.24	35.429	78.581	0.848
3	1397.444	46.251	38.47	111.622	0.937
4	2663.222	59.331	57.153	71.374	0.936
5	2886.778	64.697	56.812	133.936	0.924
6	1329.444	45.184	37.463	128.985	0.944
7	831.111	33.855	31.257	149.163	0.94
8	840.778	36.2	29.572	138.046	0.945
9	2936.444	68.23	54.797	6.554	0.87
10	3833.444	71.402	68.358	85.639	0.899
11	1707.889	53.579	40.586	150.243	0.93
12	1564.222	49.393	40.322	163.727	0.95
13	2274.333	77.611	37.311	171.742	0.762
14	2152.778	59.09	46.387	36.583	0.872
Mean	2061.675	56.206	44.997	110.762	0.905
SD	885.835	12.928	11.672	48.785	0.052
Min	831.111	33.855	29.572	6.554	0.762
Max	3833.444	77.611	68.358	171.742	0.95

Table S2: Summary of particle size measurements shown in Table S1.

Sample	**Avg. axis length (nm)**	**Std. Dev.**
Elastin	18.103	3.704
COMP	30.4115	5.831
CE Fusion	30.921	6.7145
Elastin+COMP Mixture	34.0375	9.315
EC Fusion	42.2235	7.955
ECE Fusion	50.6015	12.3

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3. Magnusdottir, A., Johansson, I., Lars-Goran, D., Nordlund, P., Berglund, H., *Nature Methods*, **2009,** 6, 7, 477-478
4. J. S. Haghpanah, C. Yuvienco, D. E. Civay, H. Barra, P. J. Baker, S. Khapli, N. Voloshchuk, S. K. Gunasekar, M. Muthukumar and J. K. Montclare, *Chembiochem*, 2009, **10**, 2733-2735.