Supplementary Information

Studies of Surface Preparation for the Fluorosequencing of Peptides

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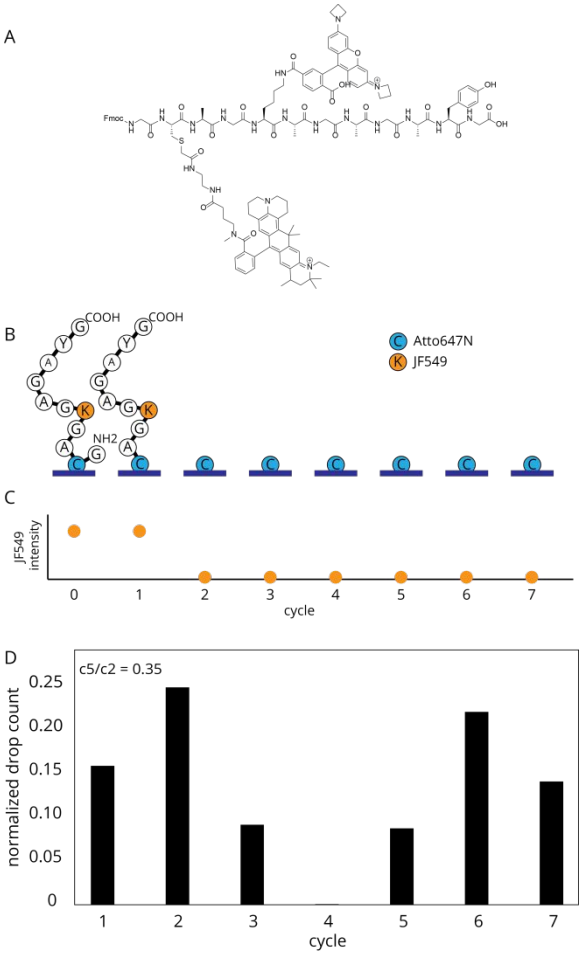
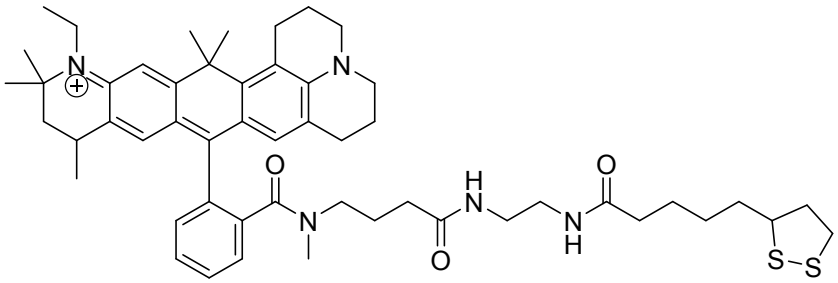


FIGURE S1. A) Structure of peptide PS1 B) Expected sequence changes of a single PS1 peptide attached though the Atto647N after rounds of Edman degradation C) The relative fluorescence changes for Atto647N on JF549 attached peptide P2 where signal loss is correlated to liberation of the remaining peptide after Edman degradation at the labeled lysine. D) Fluorosequencing results for peptides PS1, with a peak drop count after the second Edman cycle (normalized to total counts , n=6450).

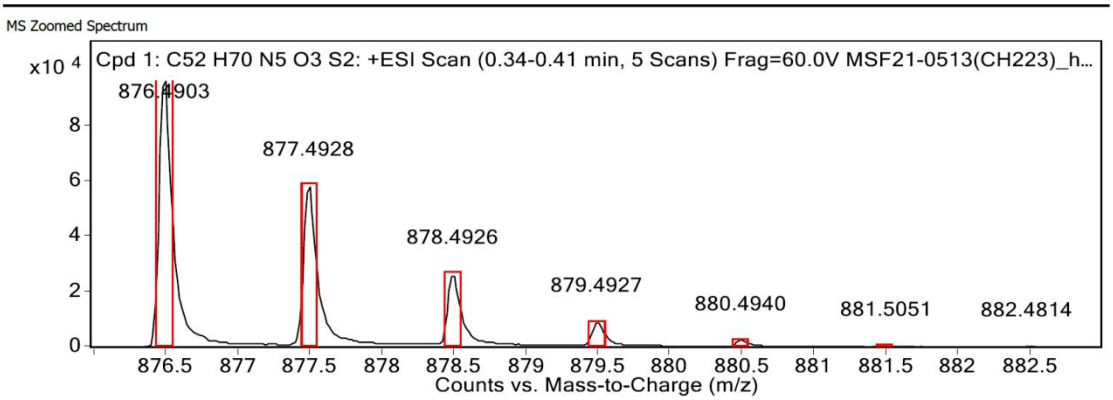
Figure S2: HiRes MS data for modified Atto647N for aldehyde labeling



Chemical Formula: $C_{52}H_{70}N_5O_3S_2^+$

Exact Mass: 876.49

m/z: 876.49 (100.0%), 877.50 (57.2%), 878.50 (16.7%), 878.49 (11.0%), 879.49 (5.3%), 879.50 (3.9%), 877.49 (3.4%), 880.49 (1.6%)



MS Spectrum Peak List

Obs. m/z	Calc. m/z	Charge	Abundance	Formula	Ion Species	Tgt Mass Error (ppm)
876.4903	876.4915	1	97795	C52H70N5O3S2	M+	1.28
877.4928	877.4946	1	58535	C52H70N5O3S2	M+	2.04
878.4926	878.4942	1	26609	C52H70N5O3S2	M+	1.87
879.4927	879.4947	1	9192	C52H70N5O3S2	M+	2.26
880.4940	880.4951	1	2453	C52H70N5O3S2	M+	1.19
881.5051	881.4956	1	455	C52H70N5O3S2	M+	-10.77
882.4814	882.4963	1	229	C52H70N5O3S2	M+	16.96

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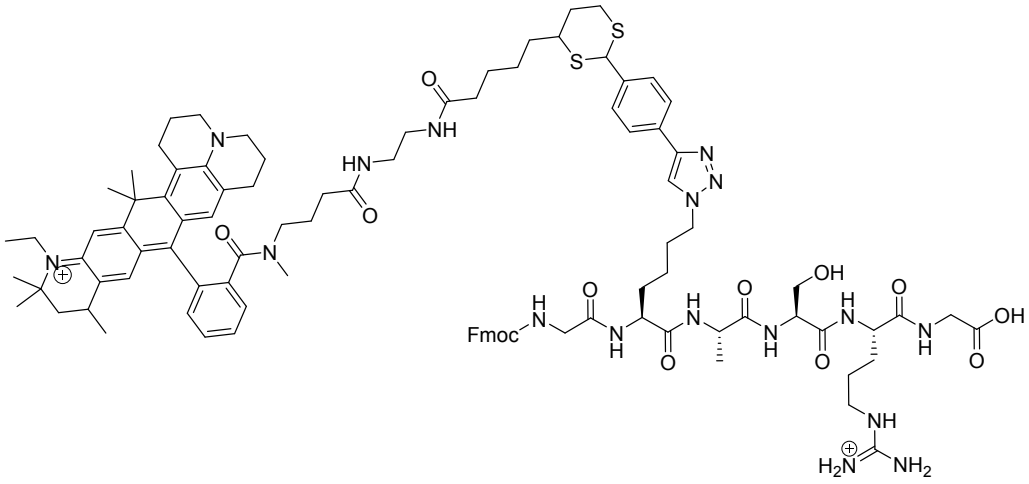
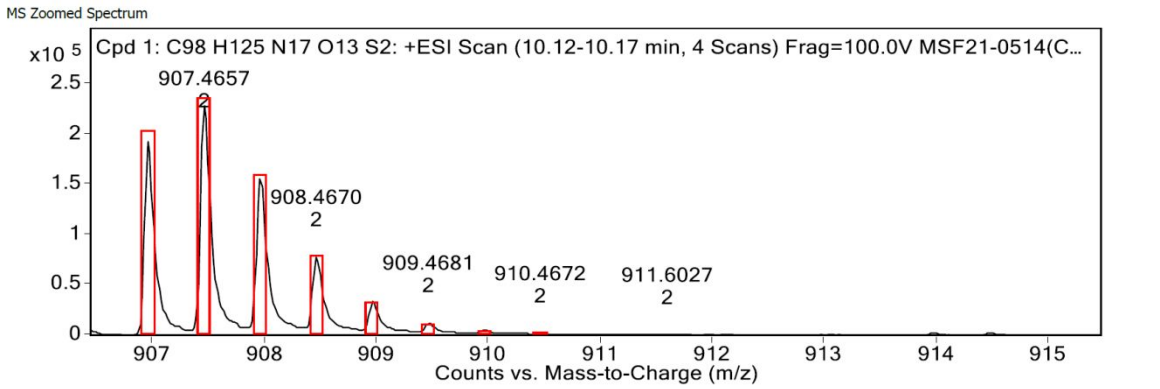
Figure S3: HiRes MS data for fluorophore labeling of P1



Chemical Formula: $C_{98}H_{127}N_{17}O_{13}S_2^{2+}$
Exact Mass: 1813.92
m/z: 907.46 (100.0%), 906.96 (92.6%), 907.96 (57.6%), 908.46 (25.3%), 907.96 (14.8%), 908.46 (9.8%), 907.46 (7.3%), 908.96 (6.6%), 908.97 (6.4%), 909.46 (2.4%), 909.47 (1.6%), 908.96 (1.2%)



MS Spectrum Peak List

Obs. m/z	Calc. m/z	Charge	Abundance	Formula	Ion Species	Tgt Mass Error (ppm)
605.3137			300642			
906.9647	906.9615	2	194673	C98H125N17O13S2	(M+2H)+2	-3.55
907.4657	907.4630	2	230659	C98H125N17O13S2	(M+2H)+2	-2.99
907.9664	907.9639	2	157803	C98H125N17O13S2	(M+2H)+2	-2.77
908.4670	908.4646	2	78569	C98H125N17O13S2	(M+2H)+2	-2.65
908.9686	908.9652	2	34409	C98H125N17O13S2	(M+2H)+2	-3.77
909.4681	909.4658	2	12950	C98H125N17O13S2	(M+2H)+2	-2.51
909.9609	909.9664	2	5052	C98H125N17O13S2	(M+2H)+2	6.12
910.4672	910.4671	2	2436	C98H125N17O13S2	(M+2H)+2	-0.09
911.0305	910.9678	2	1523	C98H125N17O13S2	(M+2H)+2	-68.76

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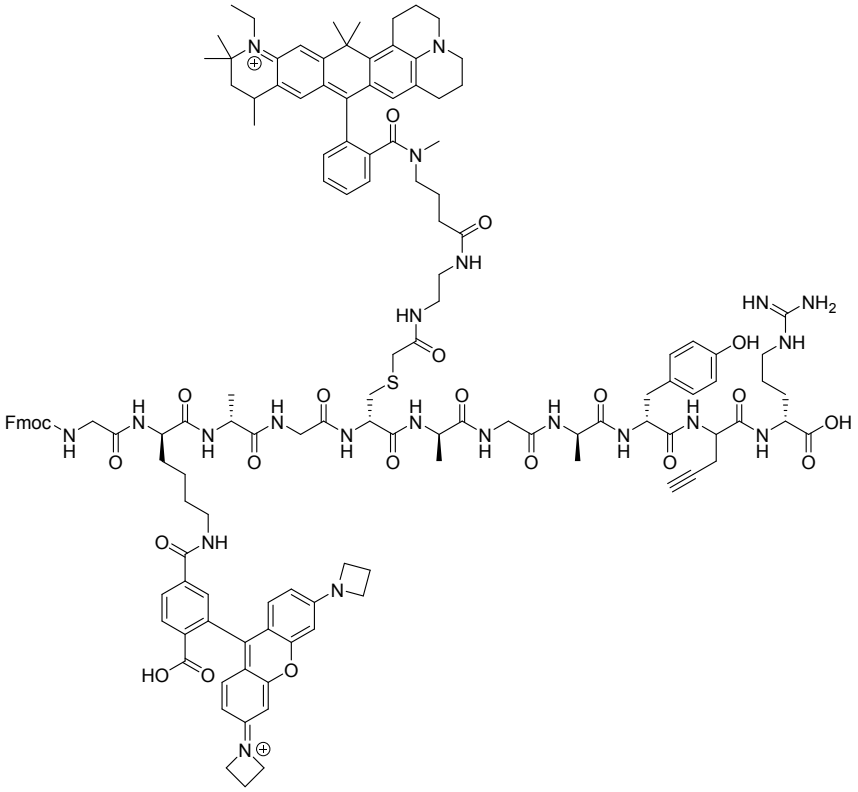
Figure S4: HiRes MS data for Fluorophore Labeling of P2



Chemical Formula: $C_{132}H_{158}N_{22}O_{22}S^{2+}$
Exact Mass: 2435.16
m/z: 1218.08 (100.0%), 1218.58 (76.2%), 1217.58 (68.8%), 1219.08 (43.9%), 1219.59 (15.5%), 1218.58 (11.3%), 1219.58 (6.8%), 1218.08 (6.1%), 1220.09 (5.2%), 1219.08 (5.1%), 1220.08 (3.1%), 1219.09 (1.9%), 1220.59 (1.8%)
Elemental Analysis: C, 65.06; H, 6.54; N, 12.65; O, 14.44; S, 1.32

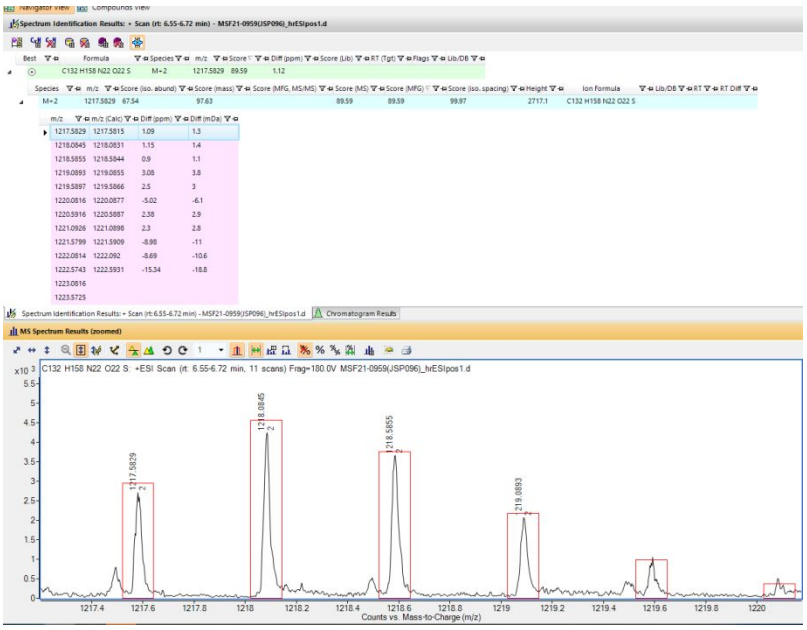
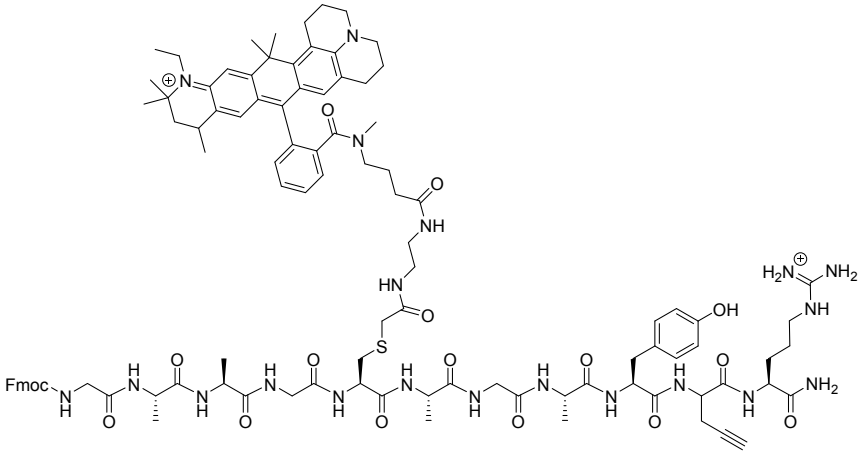
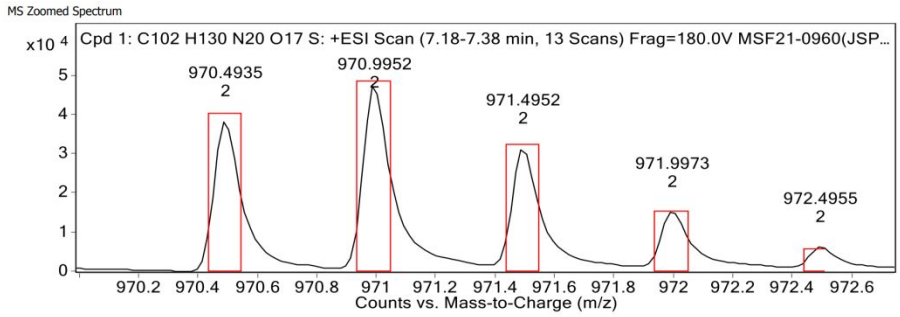


Figure S5: HiRes MS data for Fluorophore Labeling of P3



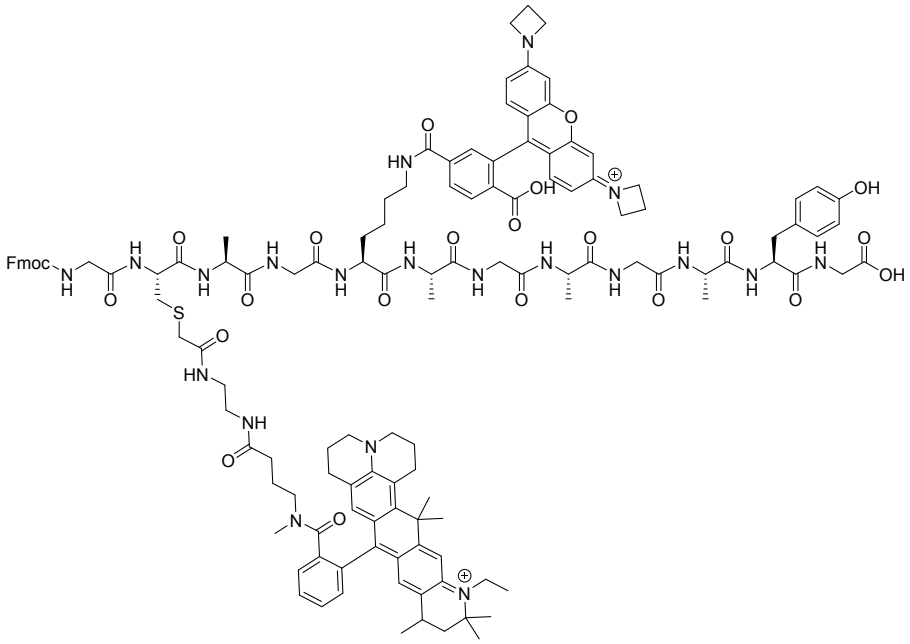
Chemical Formula: $C_{102}H_{132}N_{20}O_{17}S^{2+}$
Exact Mass: 1940.98
m/z: 970.99 (100.0%), 970.49 (83.9%), 971.49 (52.6%), 971.99 (23.2%), 971.49 (14.5%), 971.99 (8.6%), 972.49 (8.6%), 972.49 (3.2%), 972.99 (2.1%), 970.99 (1.3%), 973.00 (1.2%)
Elemental Analysis: C, 63.07; H, 6.85; N, 14.42; O, 14.00; S, 1.65



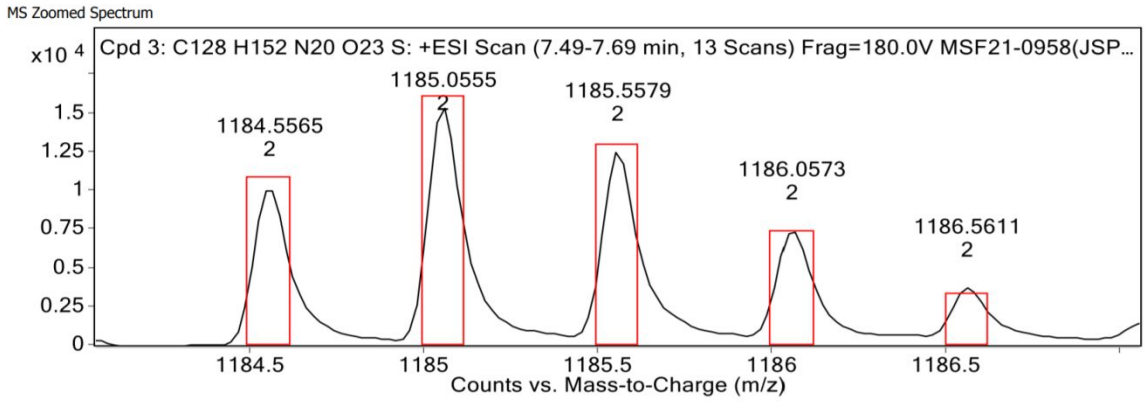
MS Spectrum Peak List

Obs. m/z	Calc. m/z	Charge	Abundance	Formula	Ion Species	Tgt Mass Error (ppm)
265.1787			242900			
970.4935	970.4895	2	38608	C102H130N20O17S	(M+2H)+2	-4.15
970.9952	970.9909	2	47953	C102H130N20O17S	(M+2H)+2	-4.42
971.4952	971.4921	2	31633	C102H130N20O17S	(M+2H)+2	-3.12
971.9973	971.9931	2	15476	C102H130N20O17S	(M+2H)+2	-4.35
972.4955	972.4941	2	6530	C102H130N20O17S	(M+2H)+2	-1.51
973.0350	972.9950	2	2973	C102H130N20O17S	(M+2H)+2	-41.12
973.4877	973.4960	2	1248	C102H130N20O17S	(M+2H)+2	8.52

Figure S6: HiRes MS data for Fluorophore Labeling of PS1



Chemical Formula: $C_{128}H_{152}N_{20}O_{23}S^{2+}$
Exact Mass: 2369.10
Molecular Weight: 2370.80
m/z: 1185.05 (100.0%), 1185.55 (79.4%), 1184.55 (70.5%), 1186.06 (32.3%), 1186.05 (15.1%), 1186.56 (14.7%), 1186.55 (6.3%), 1187.06 (5.4%), 1185.05 (5.2%), 1185.55 (3.4%), 1187.05 (2.3%), 1185.56 (1.7%), 1187.56 (1.4%)
Elemental Analysis: C, 64.85; H, 6.46; N, 11.82; O, 15.52; S, 1.35



MS Spectrum Peak List

Obs. m/z	Calc. m/z	Charge	Abundance	Formula	Ion Species	Tgt Mass Error (ppm)
797.7037			109711			
1184.5565	1184.5524	2	10248	C128H152N20O23S	M+2	-3.41
1185.0555	1185.0540	2	15393	C128H152N20O23S	M+2	-1.3
1185.5579	1185.5553	2	12527	C128H152N20O23S	M+2	-2.21
1186.0573	1186.0564	2	7438	C128H152N20O23S	M+2	-0.76
1186.5611	1186.5575	2	3739	C128H152N20O23S	M+2	-3
1187.0540	1187.0586	2	1453	C128H152N20O23S	M+2	3.89
1187.5691	1187.5596	2	724	C128H152N20O23S	M+2	-8.01
1188.0141	1188.0607	2	356	C128H152N20O23S	M+2	39.25
1188.5406	1188.5618	2	257	C128H152N20O23S	M+2	17.82

Table S1: Average difference between initial and TFA3 of samples in Figure 4.

Average difference between initial and TFA3

	EDC	Buffer only
None	1067 ± 121	357 ± 73
Hexyl	1162 ± 153	340 ± 226
n-propyl	1419 ± 67	357 ± 172
t-butyl	1480 ± 237	508 ± 154
PEG-3	1514 ± 129	185 ± 57

Table S2: Average difference between initial and TFA3 of samples in Figure 5

Average difference between initial and TFA3

	EDC	Buffer only
0 PEG-3	2123 ± 75	1432 ± 78
0.75 PEG-3	623 ± 61	132 ± 16
1.5 PEG-3	1249 ± 367	141 ± 48
2.0 PEG-3	1194 ± 251	224 ± 32
2.5 PEG-3	936 ± 245	262 ± 27
0 PEG-3	1354 ± 188	327 ± 53
0.75 PEG-3	1053 ± 155	178 ± 51
1.5 PEG-3	895 ± 101	211 ± 50
2.0 PEG-3	1287 ± 78	274 ± 59
2.5 PEG-3	1064 ± 109	345 ± 70
0 PEG-3	149 ± 12	103 ± 15
0 PEG-3	532 ± 48	516 ± 95
0.75 PEG-3	439 ± 59	128 ± 20
1.5 PEG-3	799 ± 38	300 ± 15
2.0 PEG-3	538 ± 217	280 ± 69
2.5 PEG-3	857 ± 69	170 ± 20
0 PEG-3	1034 ± 52	775 ± 73
0 PEG-3	373 ± 28	219 ± 29
0.75 PEG-3	1289 ± 166	825 ± 52
1.5 PEG-3	434 ± 52	332 ± 39
2.0 PEG-3	609 ± 82	529 ± 58

Table S3: Average difference between initial and TFA3 of samples in Figure 6

	Average difference between initial and TFA3	
	EDC	Buffer only
0PEG0APTES_1	1581 ± 73	857 ± 122
0PEG0APTES_2	1110 ± 245	376 ± 61
0PEG0APTES_3	1578 ± 107	1399 ± 71
0PEG1APTES_1	619 ± 80	496 ± 43
0PEG1APTES_2	164 ± 127	892 ± 65
0PEG1APTES_3	241 ± 60	214 ± 31
2PEG0APTES_1	1234 ± 141	713 ± 80
2PEG0APTES_2	1874 ± 57	716 ± 80
2PEG0APTES_3	1262 ± 75	829 ± 67
2PEG1APTES_1	694 ± 48	732 ± 44
2PEG1APTES_2	750 ± 55	727 ± 51
2PEG1APTES_3	817 ± 74	589 ± 38