

Appendix 2C

Supplemental Tables

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DNA Patterning step	Solution Loading Method (new)	Tubing Method (old)
Polylysine loading	12 min	> 24 min
Polylysine flow setting	10 min	20 min
DNA tubing preparation	n.a.	30 min
DNA loading	22 min	50 min

Table A2C.1. A comparison of the various steps associated with the Solution Loading Method (reported here) and the previously reported Tubing Method for preparing microfluidic flow-patterned ssDNA barcodes. By using the solution loading method, 20-25 devices are handled within one hour as opposed to 8-10 devices with individual tubing for each amine DNA.

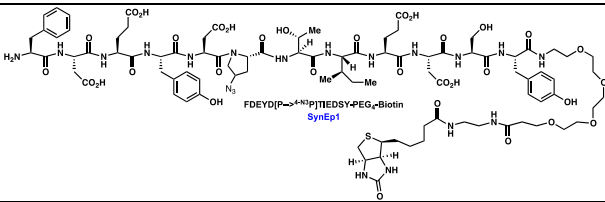
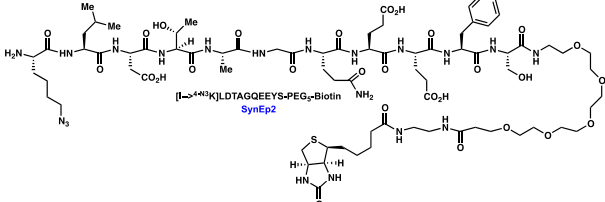
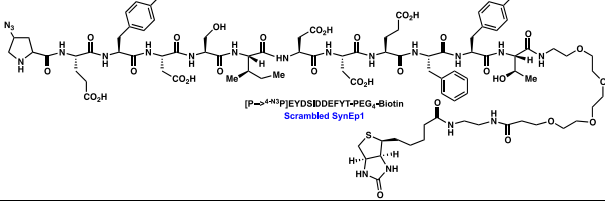
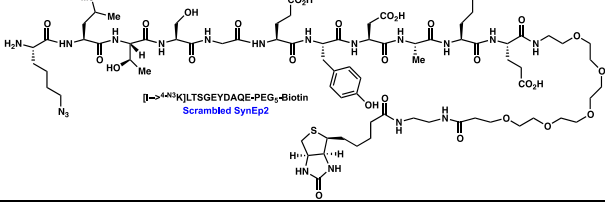
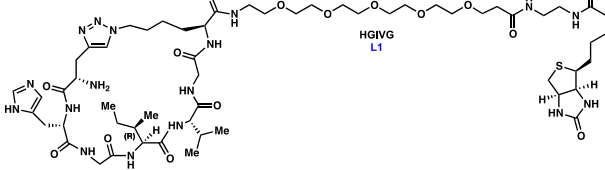
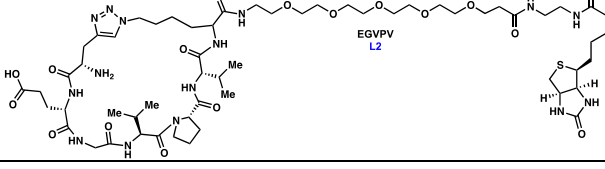
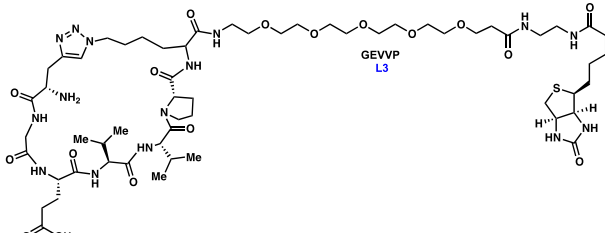
DNA i.d.	Sequence
B	5'-NH2-C6-AAA AAA AAA AAA AGC CTC ATT GAA TCA TGC CTA-3'
B'	5'-NH2-C6-AAA AAA AAA ATA GGC ATG ATT CAA TGA GGC-3'
C	5'-NH2-C6-AAA AAA AAA AAA AGC ACT CGT CTA CTA TCG CTA-3'
C'	5'-NH2-C6-AAA AAA AAA ATA GCG ATA GTA GAC GAG TGC-3'
D	5'-NH2-C6-AAA AAA AAA AAA GGT CGA GAT GTC AGA GTA-3'
D'	5'-NH2-C6-AAA AAA AAA ATA CTC TGA CAT CTC GAC CAT-3'
E	5'-NH2-C6-AAA AAA AAA AAA AAT GTG AAG TGG CAG TAT CTA-3'
E'	5'-NH2-C6-AAA AAA AAA ATA GAT ACT GCC ACT TCA CAT-3'
F	5'-NH2-C6-AAA AAA AAA AAA AAT CAG GTA AGG TTC ACG GTA-3'
F'	5'-NH2-C6-AAA AAA AAA ATA CCG TGA ACC TTA CCT GAT-3'
G	5'-NH2-C6-AAA AAA AAA AAA AGA GTA GCC TTC CCG AGC ATT-3'
G'	5'-NH2-C6-AAA AAA AAA AAA TGC TCG GGA AGG CTA CTC-3'
H	5'-NH2-C6-AAA AAA AAA AAA AAT TGA CCA AAC TGC GGT GCG-3'
H'	5'-NH2-C6-AAA AAA AAA ACG CAC CGC AGT TTG GTC AAT-3'
I	5'-NH2-C6-AAA AAA AAA AAA ATG CCC TAT TGT TGC GTC GGA-3'
I'	5'-NH2-C6-AAA AAA AAA ATC CGA CGC AAC AAT AGG GCA-3'
K	5'-NH2-C6-AAA AAA AAA AAA ATA ATC TAA TTC TGG TCG CGG-3'
K'	5'-NH2-C6-AAA AAA AAA ACC GCG ACC AGA ATT AGA TTA-3'
L	5'-NH2-C6-AAA AAA AAA AAA AGT GAT TAA GTC TGC TTC GGC-3'
L'	5'-NH2-C6-AAA AAA AAA AGC CGA AGC AGA CTT AAT CAC-3'
M	5'-NH2-C6-AAA AAA AAA AAA AGT CGA GGA TTC GTA ACC TGT-3'
M'	5'-NH2-C6-AAA AAA AAA AAC AGG TTC AGA ATC CTC GAC-3'
N	5'-NH2-C6-AAA AAA AAA AAA AGT CCT CGC TTC GTC TAT GAG-3'
N'	5'-NH2-C6-AAA AAA AAA ACT CAT AGA CGA AGC GAG GAC-3'
O	5'-NH2-C6-AAA AAA AAA AAA ACT TCG TGG CTA GTC TGT GAC-3'
O'	5'-NH2-C6-AAA AAA AAA AGT CAC AGA CTA GCC ACG AAG-3'
P	5'-NH2-C6-AAA AAA AAA AAA ATC GCC GTT GGT CTG TAT GCA-3'
P'	5'-NH2-C6-AAA AAA AAA ATG CAT ACA GAC CAA CGG CGA-3'
Q	5'-NH2-C6-AAA AAA AAA AAA ATA AGC CAG TGT GTC GTG TCT-3'
Q'	5'-NH2-C6-AAA AAA AAA AGA CAC GAC ACA CTG GCT TAT-3'

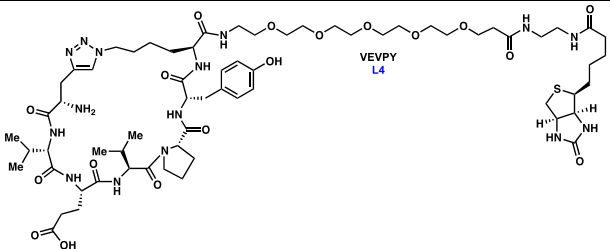
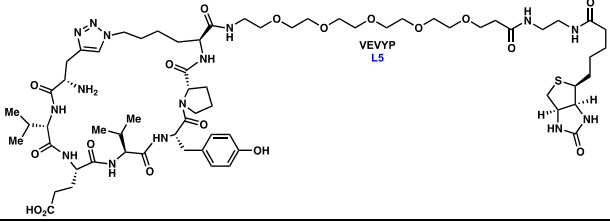
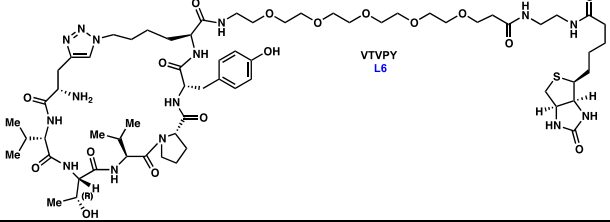
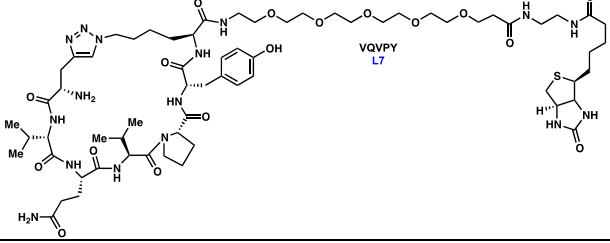
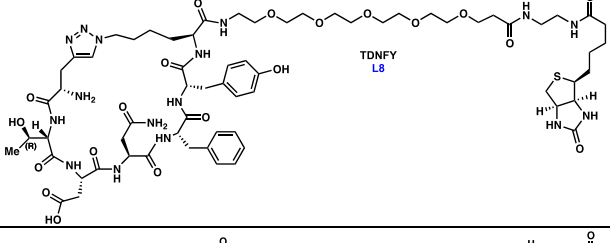
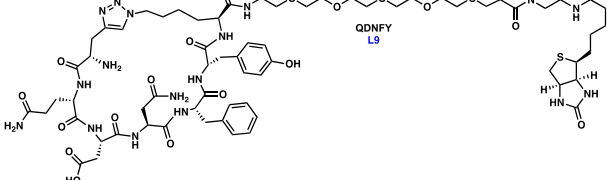
Table A2C.2. Table of ssDNA sequences used for SAC-DNA conjugation and DNA barcode patterning. The rows shaded in grey are used for conjugation to SAC protein.

Lane	B'	C'	D'	E'	F'	G'	H'	I'
original scan (F_{532})	59024.03	53476.07	54155.37	56759.83	52075.97	51375.13	49365.43	55336.00
six month rescan (F_{532})	55831.77	51549.57	50095.27	52715.00	49412.37	48684.57	45213.60	51664.53
Difference (F_{532})	3192.27	1926.50	4060.10	4044.83	2663.60	2690.57	4151.83	3671.47
% Difference	5.41	3.60	7.50	7.13	5.11	5.24	8.41	6.63
Lane	K'	L'	M'	N'	O'	P'	Q'	
original scan (F_{532})	52048.67	56120.87	52744.73	53185.87	52884.73	52943.00	52287.07	
six month rescan (F_{532})	48675.67	53388.87	48104.17	50050.87	49501.07	48818.27	49845.17	
Difference (F_{532})	3373.00	2732.00	4640.57	3135.00	3383.67	4124.73	2441.90	
% Difference	6.48	4.87	8.80	5.89	6.40	7.79	4.67	

Table A2C.3. Analysis of the degradation in barcode quality during extended storage. The data above is from a barcode validated two months after its preparation and a subsequent re-scan of the validation region six months later. The average difference in F_{532} output was 3348.80, and the average % difference was 6.26%.

Table A2C.4. Synthetic epitopes and PCC ligands characterization table.

Structure	Chemical Formula	HPLC Ret. Time (min)	Exp. mass (amu)	Obs. mass (amu)
	$C_{90}H_{128}N_{20}O_{33}S$	32:30-33:30	[M+H]= 2049.88 [M+Na]= 2071.188	2074.32
	$C_{77}H_{124}N_{20}O_{29}S$	28:30-29:30	[M+H]= 1825.864	1826.22 2
	$C_{90}H_{128}N_{20}O_{33}S$	32:00-33:00	[M+H]= 2049.875 [M+2 Pip-3 OH+H]= 2167.030	2166.39
	$C_{77}H_{124}N_{20}O_{29}S$	25:00-26:00	[M+H]= 1825.649, [M-OH+H]= 1809.99 1	1810.51
	$C_{57}H_{95}N_{17}O_{15}S$	3:30-4:30 (f1), 36:00-38:00 (f2)	[M+H]= 1290.699 [M+Na]= 1312.681	1310.81 (f1), 1289.31 (f2)
	$C_{58}H_{97}N_{15}O_{17}S$	2:30-5:00	[M+H]= 1308.699 [M+Na]= 1330.682	1329.9
	$C_{58}H_{97}N_{15}O_{17}S$	3:00-5:00 (f1), 41:30-43:00 (f2)	[M+H]= 1308.699 [M+Na]= 1330.681	1330.16 (f1), 1312.34 (f2)

Structure	Chemical Formula	HPLC Retention Time (min)	Expected mass (amu)	Observed mass (amu)
	$C_{65}H_{103}N_{15}O_{18}S$	36:30 - 39:30 (f1), 40:00 - 41:00 (f2)	[M+H]= 1414.740 [M+Na]= 1436.722	1414.38 (f1), 1435.89 (f2)
	$C_{65}H_{103}N_{15}O_{18}S$	37:00 - 39:00 (f1), 39:00 - 40:00 (f2)	[M+H]= 1414.740 [M+Na]= 1436.722	1413.47 (f1), 1435.88 (f2)
	$C_{64}H_{103}N_{15}O_{17}S$	37:30 - 39:00	[M+H]= 1386.746 [M+Na]= 1408.727	1387.36
	$C_{65}H_{104}N_{16}O_{17}S$	36:00 - 38:30 (f1), 39:00 - 41:30 (f2)	[M+H]= 1413.722 [M+Na]= 1435.738	1412.29 (f1), 1434.80 (f2)
	$C_{66}H_{98}N_{16}O_{20}S$	36:30 - 38:00	[M+H]= 1467.694 [M+Na]= 1489.676	1488.37
	$C_{67}H_{99}N_{17}O_{20}S$	4:30-5:30 (f1), 37:00 - 38:30 (f2)	[M+H]= 1494.705 [M+Na]= 1516.687	1514.14 (f1), 1494.04 (f2)

Lig.	Full Lane			Full Centroid			Left Part Centroid			Middle Part Centroid			Right Part Centroid		
	Avg F ₆₃₅	Std-dev	%CV	Avg F ₆₃₅	Std-dev	%CV	avg F ₆₃₅	Std-dev	%CV	avg F ₆₃₅	Std-dev	%CV	avg F ₆₃₅	Std-dev	%CV
L6	15968	4436	27.8	17836	3037	17.0	15645	2058	13.2	19382	2578	13.3	18546	3072	16.6
L4a	10434	3093	29.6	11363	2491	21.9	10179	1444	14.2	11181	2208	19.8	12764	2935	23.0
L4b	25079	5502	21.9	27245	4173	15.3	23307	3555	15.3	27398	2128	7.8	31147	2030	6.5
L7	48537	9262	19.1	53496	5310	9.9	48599	4548	9.4	54620	3690	6.8	57411	3113	5.4
L3a	7151	2185	30.6	8117	1291	15.9	7137	1106	15.5	8430	952	11.3	8811	1171	13.3
L3b	15157	3057	20.2	16158	2040	12.6	15070	1916	12.7	16390	2085	12.7	17046	1615	9.5
L5a	7851	2139	27.2	8341	1748	21.0	7918	1268	16.0	8920	1975	22.1	8196	1826	22.3
L5b	31581	5350	16.9	32390	2859	8.8	31947	2750	8.6	32789	2700	8.2	32448	3133	9.7
L7a	32992	7831	23.7	36651	5433	14.8	32020	2774	8.7	37152	4709	12.7	40918	4399	10.8
L7b	14791	4605	31.1	16195	3322	20.5	12956	1819	14.0	16944	1899	11.2	18781	2914	15.5
L9a	16612	4630	27.9	18391	3310	18.0	14966	1107	7.4	20262	2145	10.6	20045	3023	15.1
L9b	41812	7828	18.7	45500	5838	12.8	39380	2637	6.7	47791	5200	10.9	49508	3078	6.2
L1a	18768	3903	20.8	20134	2815	14.0	17838	2497	14.0	20725	2225	10.7	21906	1983	9.1
L1b	22519	4786	21.3	24340	3430	14.1	21889	1763	8.1	24245	2629	10.8	26959	3580	13.3
Bk	14498	3225	22.2	15102	2350	15.6	12609	891	7.1	15486	1525	9.8	17285	1505	8.7

Region	average %CV
Full Lane	23.9
Full centroid	15.5
left part centroid	11.4
middle centroid	11.9
right part centroid	12.3

Table A2C.5. A pixel by pixel analysis of variance along a barcode lane. The %CV values for the entire barcode set shown in Figure S15 using the full lane, full centroid, left part of the centroid, middle part of the centroid, and right part of the centroid are displayed in the top table. The average %CV values for each set are displayed in the bottom table.

log[KRAS]	L1a	L1b	L2	L3a	L4a	L4b	L5a
-3.54	28.57	736.24	47.37	38.32	78.34	–	78.36
-3.84	55.65	61.13	73.45	10.80	–	–	23.71
-4.14	63.67	95.71	135.76	61.12	–	129.09	25.89
-4.62	41.32	78.02	37.31	29.92	113.59	18.39	83.24
-5.01	75.01	104.74	91.75	37.41	129.43	126.70	35.44
-5.32	72.72	62.92	311.26	68.47	–	75.72	33.16
-6.01	66.87	58.07	116.58	68.77	129.48	45.88	–
-6.32	43.25	34.28	74.47	117.95	94.72	21.05	114.95
-6.62	27.30	83.53	383.23	219.70	208.89	51.85	93.22
-7.01	91.85	79.90	298.37	112.63	59.01	182.28	88.78
avg %CV	56.62	139.45	156.95	76.51	116.21	81.37	64.08
log[KRAS]	L5b	L6	L7a	L7b	L8	L9a	L9b
-3.54	30.85	20.29	8.43	17.55	2.82	16.76	58.89
-3.84	24.60	80.02	12.77	9.85	3.79	26.82	31.45
-4.14	15.86	73.89	27.32	2.45	2.70	29.67	43.65
-4.62	38.87	–	14.52	10.08	6.82	31.29	34.73
-5.01	41.45	294.01	26.54	28.29	4.31	21.32	34.12
-5.32	29.12	65.36	179.54	59.14	23.74	22.19	38.54
-6.01	52.54	41.04	13.50	87.28	51.17	32.95	42.65
-6.32	69.24	29.65	22.00	178.75	125.33	29.51	70.74
-6.62	152.61	36.53	76.64	79.07	66.46	61.52	36.05
-7.01	16.40	134.31	69.97	16.02	–	30.45	48.13
avg %CV	47.15	86.12	45.12	48.85	31.90	30.25	43.89

Table A2C.6. The %CV data for the ELISAs. The %CV measurements are given as the |%CV|. Any %CV>=300% has been marked with an “–”. The global average %CV for the assays was 73.2%.

centroid	L6	L4a	L4b	L7	L3a	L3b	L5a	L5b	L7a	L7b	L9a	L9b	L1a	L1b	Bk
L6	—	***	***	***	***	***	***	***	***	***	n.s.	***	***	***	***
L4a		—	***	***	***	***	***	***	***	***	***	***	***	***	***
L4b			—	***	***	***	***	***	***	***	***	***	***	***	***
L7				—	***	***	***	***	***	***	***	***	***	***	***
L3a					—	***	n.s.	***	***	***	***	***	***	***	***
L3b						—	***	***	***	n.s.	***	***	***	***	**
L5a							—	***	***	***	***	***	***	***	***
L5b								—	***	***	***	***	***	***	***
L7a									—	***	***	***	***	***	***
L7b										—	***	***	***	***	**
L9a											—	***	***	***	***
L9b												—	***	***	***
L1a													—	***	***
L1b														—	***
Bk															—
Full lane	L6	L4a	L4b	L7	L3a	L3b	L5a	L5b	L7a	L7b	L9a	L9b	L1a	L1b	Bk
L6	—	***	***	***	***	*	***	***	***	*	n.s.	***	***	***	**
L4a		—	***	***	***	***	***	***	***	***	***	***	***	***	***
L4b			—	***	***	***	***	***	***	***	***	***	***	***	***
L7				—	***	***	***	***	***	***	***	***	***	***	***
L3a					—	***	**	***	***	***	***	***	***	***	***
L3b						—	***	***	***	n.s.	**	***	***	***	n.s.
L5a							—	***	***	***	***	***	***	***	***
L5b								—	n.s.	***	***	***	***	***	***
L7a									—	***	***	***	***	***	***
L7b										—	***	***	***	***	n.s.
L9a											—	***	***	***	***
L9b												—	***	***	***
L1a													—	***	***
L1b														—	***
Bk															—

Table A2C.7. Calculated p-values for the pixel by pixel full-lane and centroid scatterplots. Symmetric 2-tail, unequal variance p-value matrixes for the centroid and full-lane scatterplots in Figure S15. The p-values are denoted: n.s. $p > 0.05$, * $0.05 < p < 0.005$, ** $0.005 < p < 0.0005$, *** $p < 0.0005$.

	L1a	L1b	L2	L3a	L3b	L4a	L4b	L5a	L5b	L6	L7a	L7b	L8	L9a	L9b	BN-A ₂₀ -Cy3
Fig. 3	0.91	0.92	0.95	0.94	0.81	0.91	0.8	0.97	0.85	0.92	0.95	0.85	0.96	0.94	0.96	N/A
Fig. S16	0.95	0.95	0.96	0.96	0.83	0.93	0.87	0.98	0.88	0.94	0.97	0.89	0.98	0.95	0.97	0.66
Fig. S17	0.56	0.18	0.45	0.73	N/A	N/A	N/A	0.53	0.78	0.73	0.93	0.96	0.97	0.81	0.72	N/A

Table A2C.8. The goodness of fit measurements for the allosteric KRas binding curves. The r-squared values for the curves after a double background correction in Figures 3 and S17, and a single background correction in Figure S16.