SUPPORTING INFORMATION

Table S1. Predicted and average surface exposure, antigenicity and maximum homology for individual amino acids and 14-mer epitopes of MrkA.

Residue #	Residue	Predicted quantities: Amino acid: Pred. Antigen.	Pred. Surf.-Exp.	Avg. Surf.-Exp.	Epitope (14-mer), starting at residue #: Avg. Antigen.	Max Homol. (%)
1	M	0	1	29	0	57
2	K	0	1	21	0	64
3	K	0	1	14	0	71
4	V	0	1	7	0	71
5	L	0	0	0	0	71
6	L	0	0	0	0	64
7	S	0	0	0	0	57
8	A	0	0	0	0	57
9	A	0	0	0	0	57
10	M	0	0	0	0	57
11	A	0	0	0	7	57
12	T	0	0	0	14	57
13	A	0	0	7	21	57
14	F	0	0	7	29	57
15	F	0	0	14	36	57
16	G	0	0	21	43	64
17	M	0	0	21	50	71
18	T	0	0	29	50	71
19	A	0	0	29	50	71
20	A	0	0	36	50	71
21	H	0	0	36	50	64
22	A	0	0	43	50	64
23	A	0	0	43	50	57
24	D	1	0	50	50	64
25	T	1	0	50	43	64
26	T	1	1	57	36	64
27	V	1	0	57	29	64
28	G	1	1	64	21	64
29	G	1	1	64	14	64
30	G	1	0	57	7	50
31	Q	0	1	64	0	64
32	V	0	0	57	0	64
33	N	0	1	64	0	64
34	F	0	0	64	0	57
35	F	0	1	71	7	57
36	G	0	0	71	14	57
37	K	0	1	79	21	64
38	V	0	0	79	29	64
39	T	0	1	86	36	64
40	D	0	1	86	43	57
41	V	0	1	79	50	57
42	S	0	1	79	50	57
43	C	0	0	71	50	64
44	T	0	1	79	50	57
45	V	0	0	71	50	57
46	S	0	1	79	50	57
47	V	0	1	79	50	71
48	N	1	1	71	57	64
49	G	1	1	71	50	64
50	Q	1	1	71	50	71
51	G	1	1	71	50	57
52	S	1	1	71	50	64
53	D	1	1	64	50	57
54	A	1	0	64	50	57
87	C	1	0	86	86	57
88	Q	1	1	86	79	57
89	A	1	1	86	71	57
90	A	1	1	79	64	57
91	D	1	1	71	57	71
92	G	1	1	64	50	57
93	T	1	1	64	43	57
94	K	1	1	57	36	64
95	Q	1	1	50	36	64
96	D	1	1	50	36	64
97	D	1	1	50	36	57
98	V	1	0	43	36	57
99	S	0	1	50	36	57
100	K	0	1	50	43	57
101	L	0	0	50	50	57
102	G	0	1	57	57	57
103	V	0	0	57	64	64
104	N	0	0	64	71	64
105	W	0	0	64	79	57
106	T	0	1	71	86	64
107	G	0	0	71	93	57
108	G	1	0	71	100	57
109	N	1	1	71	100	57
110	L	1	1	71	100	64
111	L	1	0	71	100	64
112	A	1	1	79	100	64
113	G	1	1	79	100	64
114	A	1	1	79	100	57
115	T	1	1	79	100	71
116	S	1	1	79	100	71
117	K	1	1	71	100	57
118	Q	1	0	71	93	64
119	Q	1	1	71	86	71
120	G	1	1	64	79	71
121	Y	1	0	57	71	57
122	L	1	0	57	64	57
123	A	1	1	57	57	57
124	N	1	1	50	50	57
125	T	1	1	43	43	64
126	E	1	1	43	36	57
127	A	1	1	43	36	57
128	S	1	1	43	36	57
129	G	1	1	43	36	57
130	A	1	0	43	36	57
131	Q	0	1	50	36	64
132	N	0	0	50	43	64
133	I	0	0	57	50	64
134	Q	0	0	64	57	64
135	L	0	0	71	64	71
136	V	0	0	71	71	57
137	L	0	0	79	79	57
138	S	0	0	86	86	57
139	T	0	1	93	93	64
140	D	1	1	93	100	64
173	T	0	1	43	14	64
174	Y	0	0	43	14	64
175	Y	0	0	50	14	64
176	V	0	0	57	14	64
177	G	0	0	64	14	64
178	Y	0	0	64	14	71
179	A	0	0	71	14	71
180	T	0	0	71	14	71
181	S	0	1	79	14	64
182	A	0	1	71	14	57
183	P	0	1	71	14	57
184	T	1	1	64	14	57
185	T	1	1	57	7	57
186	V	0	0	50	0	50
187	T	0	1	57	0	57
188	T	0	1	50	0	50
189	G	0	1	50	0	50
190	V	0	1	---	---	---
191	V	0	0	---	---	---
192	N	0	1	---	---	---
193	S	0	0	---	---	---
194	Y	0	1	---	---	---
195	A	0	0	---	---	---
196	T	0	1	---	---	---
197	Y	0	0	---	---	---
198	E	0	0	---	---	---
199	I	0	0	---	---	---
200	T	0	1	---	---	---
201	Y	0	0	---	---	---
202	Q	0	1	---	---	---

55	N	0	1	71	50	64	141	N	1	1	93	100	64
56	V	0	0	71	57	64	142	A	1	1	93	100	57
57	Y	0	1	79	64	64	143	T	1	1	93	100	64
58	L	0	0	79	71	64	144	A	1	1	93	100	71
59	S	0	1	86	79	71	145	L	1	1	93	100	64
60	P	0	1	86	86	71	146	T	1	1	93	100	64
61	V	1	0	86	93	64	147	N	1	1	93	100	57
62	T	0	1	93	93	64	148	K	1	1	93	100	57
63	L	1	1	93	100	71	149	I	1	0	93	100	57
64	T	1	1	93	100	71	150	I	1	1	100	100	57
65	E	1	1	93	100	71	151	P	1	1	100	100	64
66	V	1	0	93	93	64	152	G	1	1	100	100	64
67	K	1	1	93	86	57	153	D	1	1	100	100	64
68	A	1	1	93	79	57	154	S	1	1	100	100	57
69	A	1	1	86	71	57	155	T	1	1	100	100	64
70	A	1	1	86	64	57	156	Q	1	1	100	100	64
71	A	1	1	79	57	64	157	P	1	1	93	100	64
72	D	1	1	79	50	64	158	K	1	1	93	93	57
73	T	1	1	79	43	64	159	A	1	1	86	86	64
74	Y	1	1	71	43	50	160	K	1	1	86	79	64
75	L	1	1	71	43	57	161	G	1	1	79	71	64
76	K	1	1	71	43	57	162	D	1	1	71	64	64
77	P	1	1	71	43	57	163	A	1	1	64	57	64
78	K	1	1	71	43	57	164	S	1	1	57	50	71
79	S	0	1	71	43	57	165	A	1	1	50	43	71
80	F	0	0	71	50	57	166	V	1	1	43	36	64
81	T	0	1	79	57	57	167	A	1	1	36	29	64
82	I	0	0	79	64	50	168	D	1	1	36	21	64
83	D	0	1	86	71	57	169	G	1	1	36	14	57
84	V	0	0	86	79	57	170	A	1	0	36	7	64
85	S	0	1	86	86	57	171	R	0	1	43	7	57
86	N	0	1	86	86	57	172	F	0	0	43	14	57

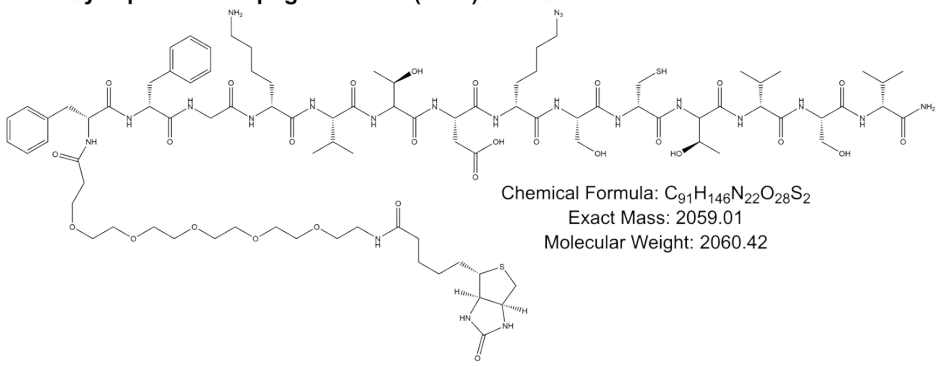
Table S2. Isocratic points and relative affinities of macrocyclic analogues of lead MrkA ligand cy(LLFFF).

ELISA Assay #	Compound #	N-terminal tag	Cycle sequence	Isocratic point (%)	Relative affinity*	[MrkA] for ELISA	Modification
ELISA 1	Ref. compound	Bio-Peg10-peg10-	cy(X-LLFFF-Z)	74.53	1.00	N/A	Reference
ELISA 2	Ref. compound	Bio-peg10-	cy(X-LLFFF-Z)	75.88	1.00	25 nM	Reference
	2	Bio-peg10-	cy(X-ALFFF-Z)	65.55	0.53	25 nM	L-to-A substitution
	3	Bio-peg10-	cy(X-LAFFF-Z)	66.00	0.26	25 nM	L-to-A substitution
	4	Bio-peg10-	cy(X-LLAFF-Z)	70.97	0.59	25 nM	F-to-A substitution
	5	Bio-peg10-	cy(X-LLFAF-Z)	66.00	0.10	25 nM	F-to-A substitution
	6	Bio-peg10-	cy(Z-LLFFA-X)	64.43	0.04	25 nM	F-to-A substitution
ELISA 3	Ref. compound	Bio-peg10-	cy(X-LLFFF-Z)	75.88	1.00	50 nM	Reference
	7	Bio-peg10-	cy(X-AAFFF-Z)	54.65	0.15	50 nM	LL-to-AA substitution
	8	Bio-peg10-	cy(X-LFFF-Z)	61.84	0.46	50 nM	L removal
	10	Bio-peg10-	cy(X-LLFFF-(Z3))	76.89	0.89	50 nM	Z-to-Z3 substitution (remove one methylene group)
	11	Bio-peg10-	cy(X-LLFF4-Z)	75.53	0.88	50 nM	F-to-4 substitution
ELISA 4	Ref. compound	Bio-peg5-	cy(X-LLFFF-Z)	78.85	1.00	100 nM	Reference
	13	Bio-peg5-	cy(X-TTFFF-Z)	53.40	0.04	100 nM	LL-to-TT substitution
ELISA 5	Ref. compound	Bio-peg10-	cy(X-LLFFF-Z)	75.88	1.00	25 nM	Reference
	16	Bio-peg10-R3-peg10-	cy(X-LLFFF-Z)	45.02	0.90	25 nM	Add -Peg10-R3 tag
	17	Bio-peg10-R6-peg10-	cy(X-LLFFF-Z)	39.82	1.22	25 nM	Add -Peg10-R6 tag
	18	Bio-peg10-R9-peg10-	cy(X-LLFFF-Z)	37.03	0.58	25 nM	Add -Peg10-R9 tag
ELISA 6	Ref. compound	Bio-peg10-	cy(X-LLFFF-Z)	75.88	1.00	25 nM	Reference
	19	Bio-peg10-	cy(X-HLFFF-Z)	47.52	0.81	25 nM	Cationic residue substitution
	20	Bio-peg10-	cy(X-DLFFF-Z)	66.33	0.21	25 nM	Anionic residue substitution
	21	Bio-peg10-	cy(X-LLFF-Z)	64.99	0.14	25 nM	Residue removal
	22	Bio-peg10-	cy(X-LLF-Z)	56.45	0.09	25 nM	Double residue removal
	23	Bio-peg10-	cy(X-FFF-Z)	60.27	0.14	25 nM	Double residue removal

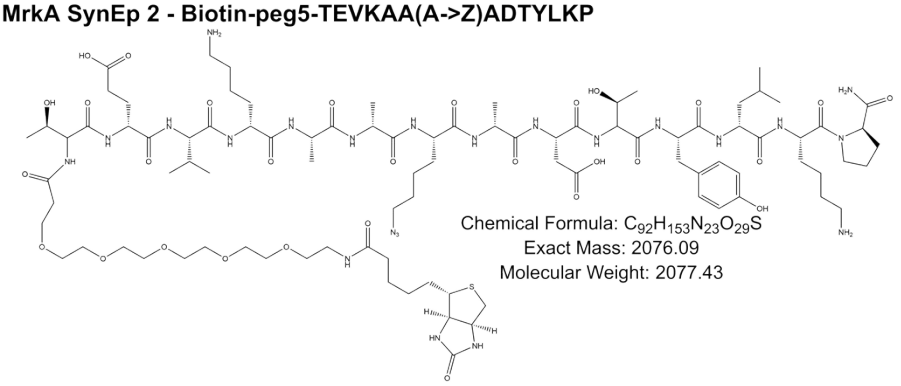
*"*Relative affinity is the ratio of ELISA signal for a given compound versus that obtained from cy(LLFFF) under the same assay conditions (e.g., MrkA concentration). A Relative Affinity value of <1 reflects lower affinity to MrkA versus cy(LLFFF).*

**cy() indicates cyclization, X = propargylglycine, Z = Azidolysine, Z3 = azidonorvaline, 4 = 4-fluoro-phenylalanine Bio = biotin, R*i* = polyarginine tag with *i* arginine residues, peg*i* = polyethylene glycol chain of length *i*. All amino acids represented by single-letter amino acid codes.

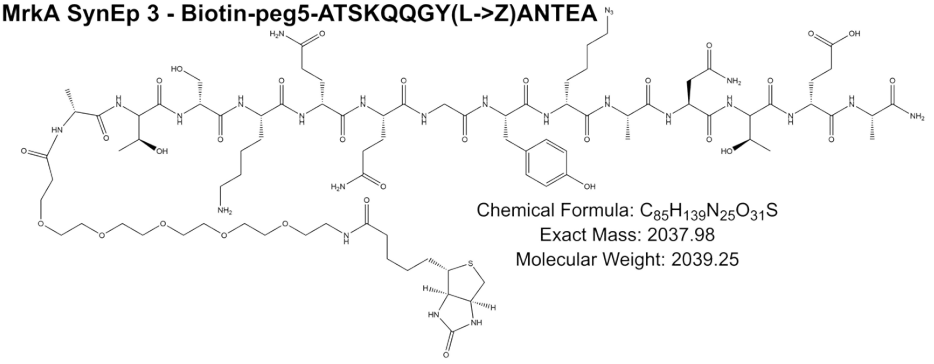
MrkA SynEp 1 - Biotin-peg5-FFGKVT(V->Z)SCTVSV



MrkA SynEp 2 - Biotin-peg5-TEVKAA(A->Z)ADTYLKP



MrkA SynEp 3 - Biotin-peg5-ATSKQQGY(L->Z)ANTEA



MrkA SynEp 4 - Biotin-peg5-STQPK(A->Z)KGDASAVA

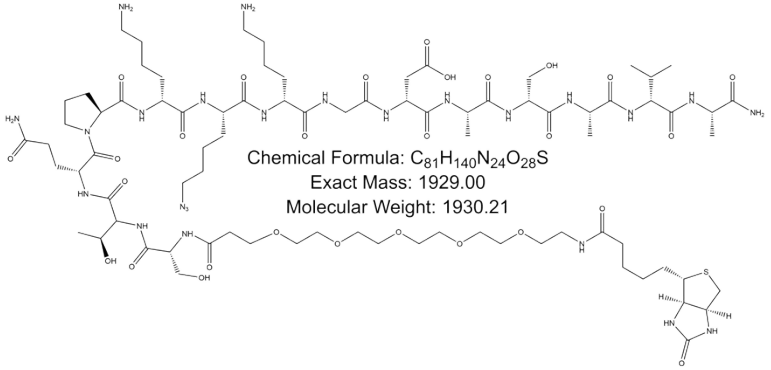


Figure S1. Molecular structures and parameters for synthetic epitopes used for PCC combinatorial screens in this study.

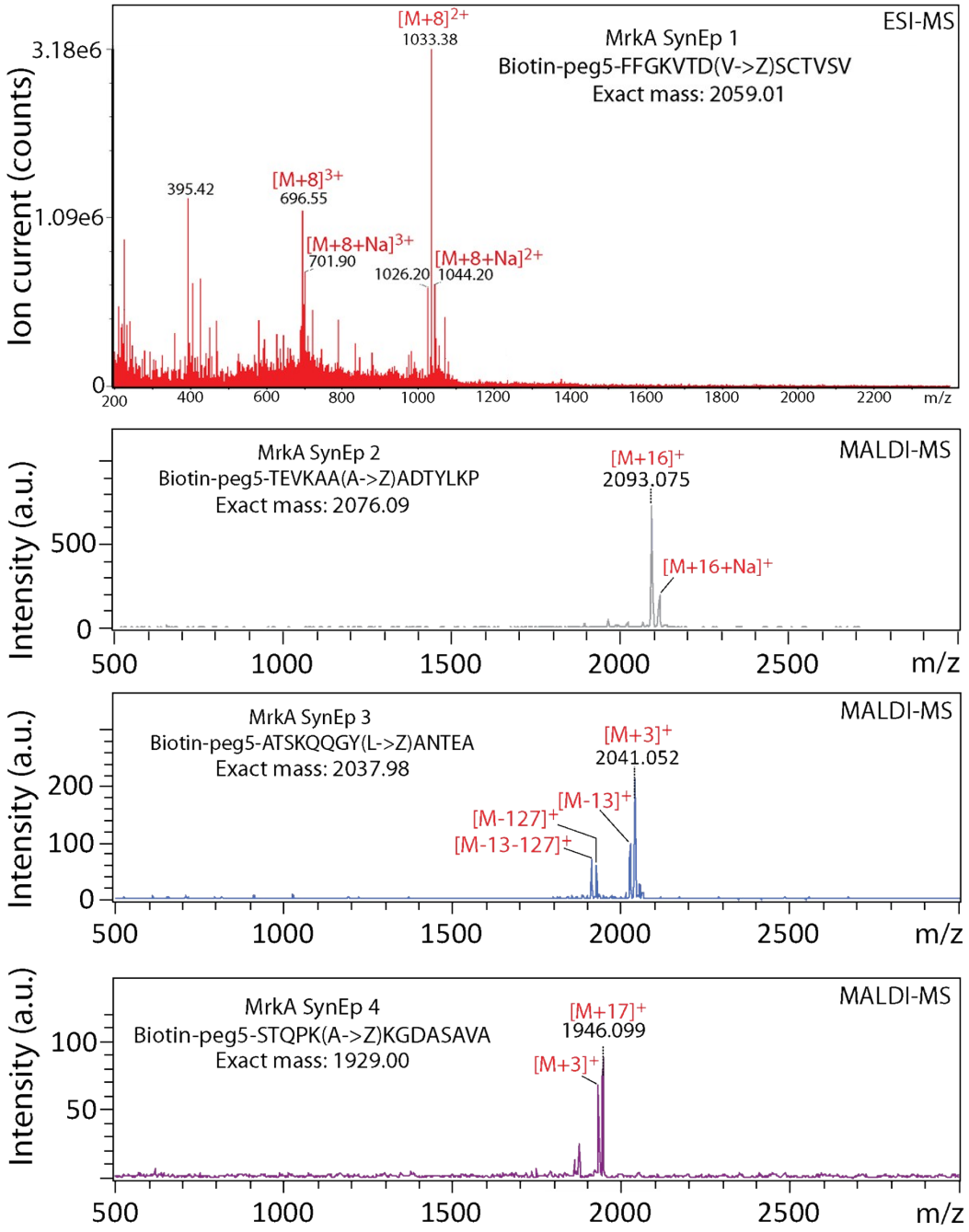
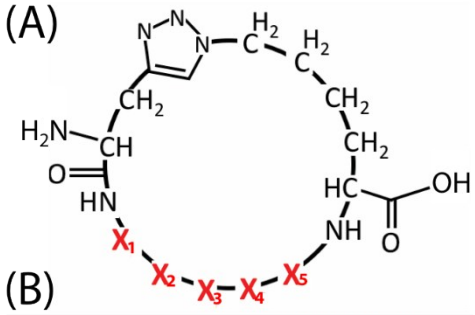


Figure S2. Representative mass spectrometric characterization data for synthetic epitopes (SynEps) used for PCC combinatorial screening. SynEp 1 was purified by using a Waters Autopurification system that employs electrospray ionization to detect and purify compounds, while SynEps 2-4 were purified by HPLC and then matrix-assisted laser desorption ionization spectroscopy. Multiple HPLC runs were used to purify each compound, and the mass spectrometry data shown here are acquired from a single run or fraction that was subsequently pooled. Masses with +16 likely arise from oxidation of the SynEp.



(B)

Hit #	Protein	HPraGly	X1	X2	X3	X4	X5	Az4
1	MrkA		F	Y	T	K	G	
2	MrkA		E	Y	E	G	K	
3	MrkA		P	W	N	K	G	
4.1	MrkA		S	W	T	G	E	
4.2	MrkA		R	H	P	G	E	
5	MrkA		N	R	T	G	P	
6	MrkA		P	R	E	G	P	
7	MrkA		S	N	F	G	P	
8	MrkA		E	K	T	P	G	
9	MrkA		K	G	F	P	G	
10	MrkA		G	G	F	N	A	
11	MrkA		N	G	P	V	H	
12	MrkA		W	Y	K	G	P	
13	MrkA		W	D	Y	K	G	
14	MrkA		Y	R	H	L	G	
15	MrkA		G	V	H	R	L	
16	MrkA		G	V	V	E	K	
17	MrkA		G	L	T	H	A	
18	MrkA		S	L	G	L	T	
19	MrkA		L	L	F	F	F	
20	MrkA		T	T	F	F	F	
21	MrkA		K	P	A	G		
22	MrkA		A	K	P	E	P	
23	MrkA		E	W	V	S	A	
24	MrkA		E	F	S	G	V	
25	MrkA		D	G	T	A	L	
26.1	MrkA		V	V	N	L	P	
26.2	MrkA		T	P	N	L	P	
26.3	MrkA		R	P	E	G	P	

Figure S3. (A) Chemical structure of macrocyclic peptide ligands to MrkA, in which each red X_i represents one of 17 common amino acids (excluding methionine, cysteine, and isoleucine). **(B)** Table with the sequences of the 26 different macrocyclic peptide ligands from the in situ click screen of four target epitopes on MrkA.

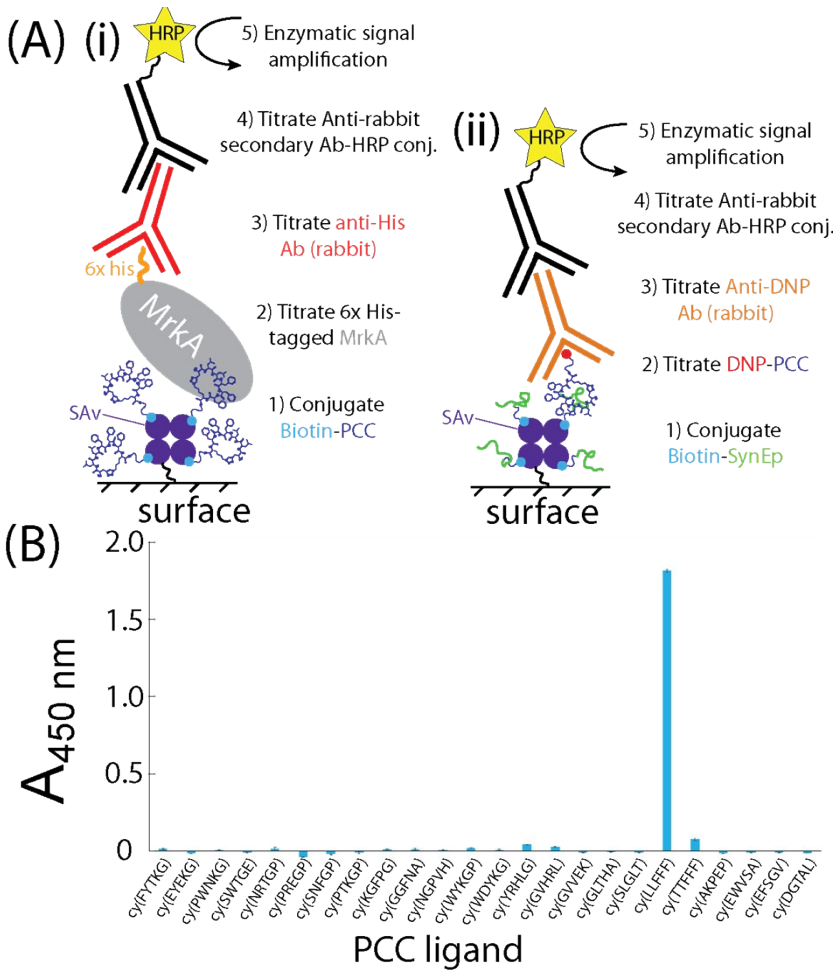


Figure S4. (A) Schematic diagrams of the sandwich ELISA assay formats used to test the binding affinities of PCC ligands to (i) full-length MrkA and (ii) SynEps. The relative sizes of the molecules in these illustrations are not to scale. **(B)** Bar graph showing the absorbances obtained from an enzyme-linked Immunoassay (ELISA) that tests the ability of immobilized PCC peptides to capture full-length MrkA at 100 nM from aqueous solution. The strong absorbance associated with immobilized *cy(LLFFF)* demonstrates strong binding of *cy(LLFFF)* to MrkA. Moderate binding is also observed for *cy(TTFFF)*, *cy(YRHLG)* and *cy(GVHRL)*. Measurements were done in duplicate and error bars indicate the span of the data. Intensities in (B) are shown after subtraction of a dummy ligand Biotin-peg5-cy(HNGPT) background.

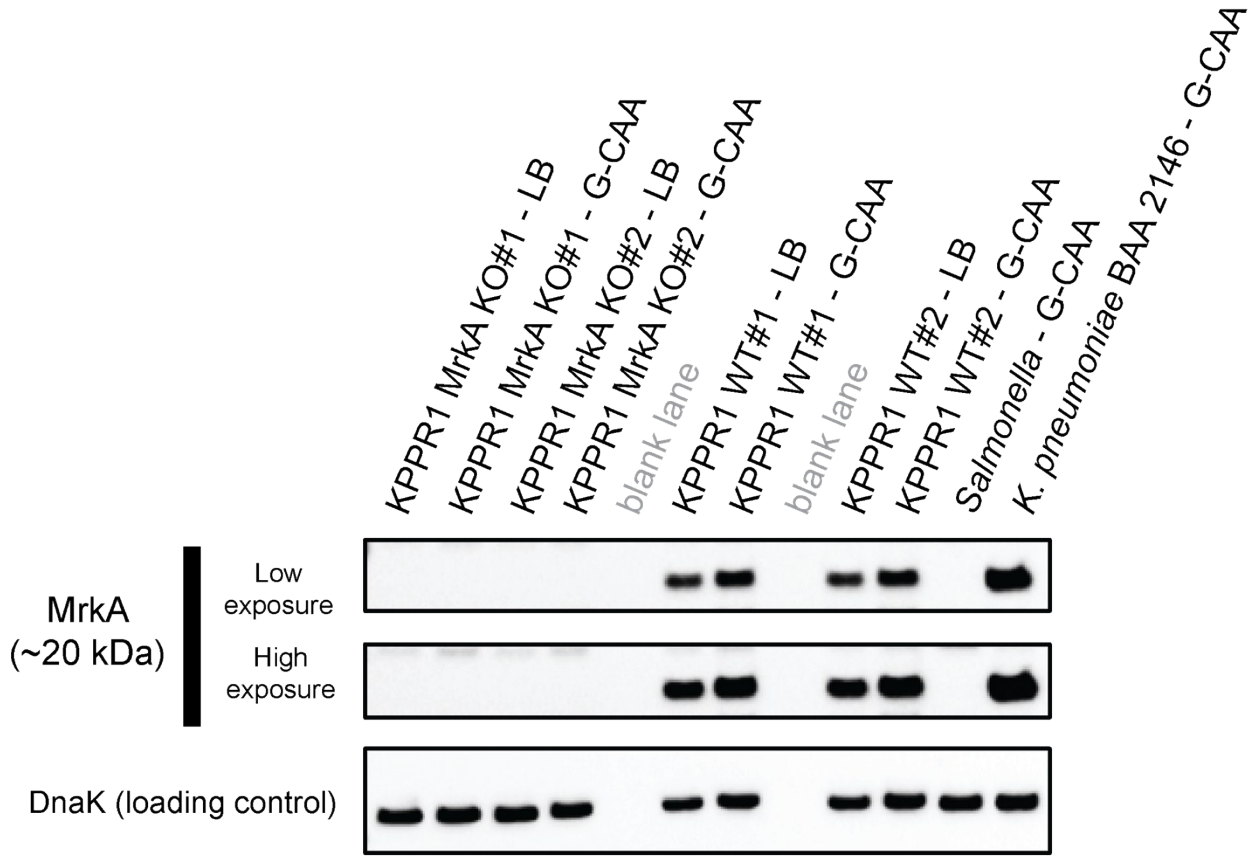


Figure S5. Western blot to detect MrkA protein in wild type and MrkA/B/C knockout (KO) versions of *K. pneumoniae* strain ATCC 43816 ("KPPR1"), grown in either LB (LB) or Glycerol Casamino acid (G-CAA) meida. KPPR1 MrkA KO grown in either LB or G-CAA media shows no detectable signal, confirming no MrkA expression. By comparison, wild type KPRR1 cultured in either LB or G-CAA yields strong signals that establish high MrkA expression under either growth condition. A negative control of *Salmonella*, which does not express MrkA, does not show any signal, while a positive control of *K. pneumoniae* strain BAA2146 cultured in G-CAA shows a strong signal.

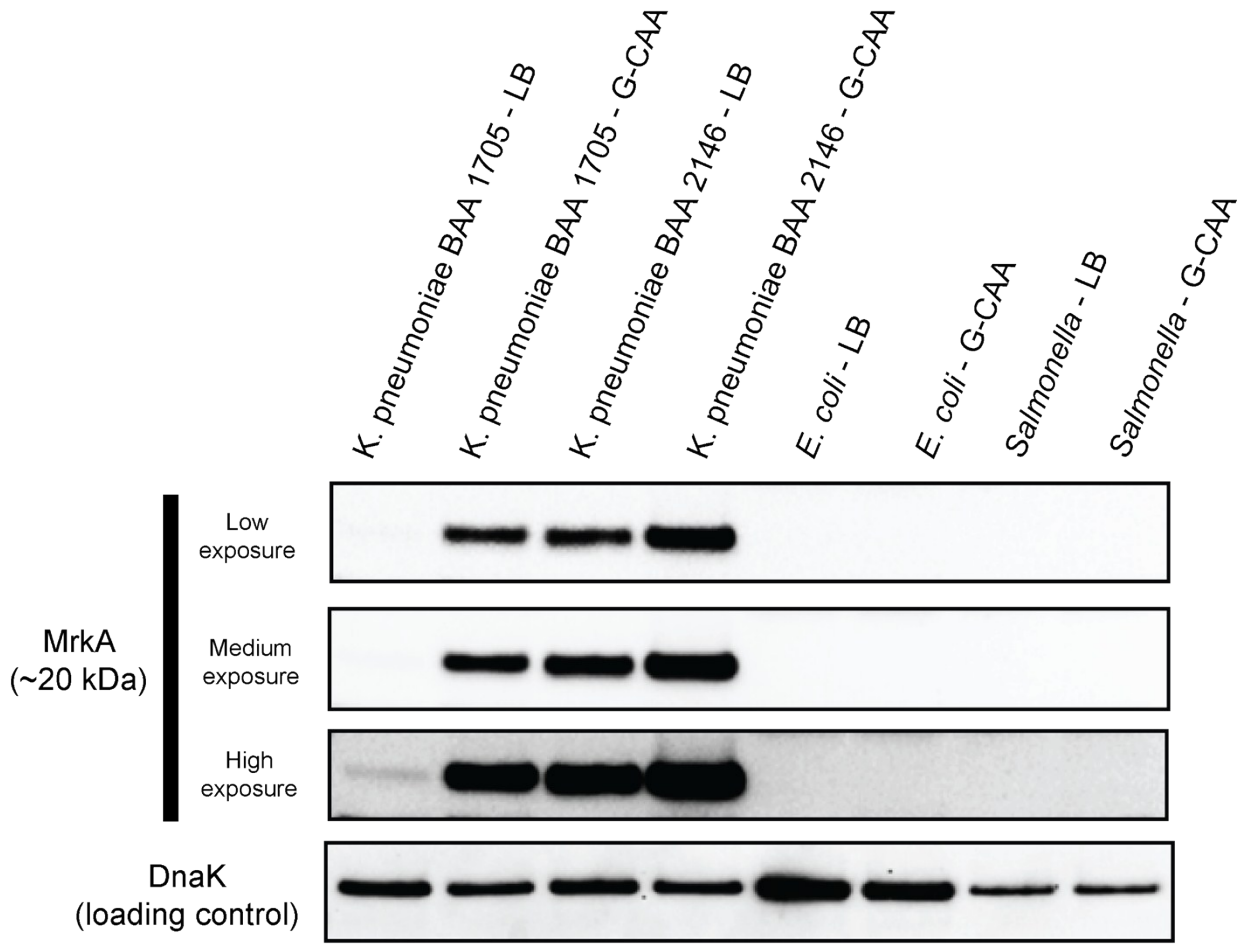


Figure S6. Western blot to detect MrkA protein in *Klebsiella pneumoniae*, *Escherichia coli* and *Salmonella typhimurium* grown in either LB (LB) or Glycerol Casamino acid (G-CAA) solutions. *K. pneumoniae* BAA1705 grown in LB shows very faint signals associated with MrkA protein, while substantially higher signals associated with MrkA are observed for the same cells grown in NG-CAA. MrkA was highly expressed in *K. pneumoniae* BAA 2146 in either LB or GCAA. No detectable MrkA was observed in either *E. coli* or *S. typhimurium* under any growth conditions tested.

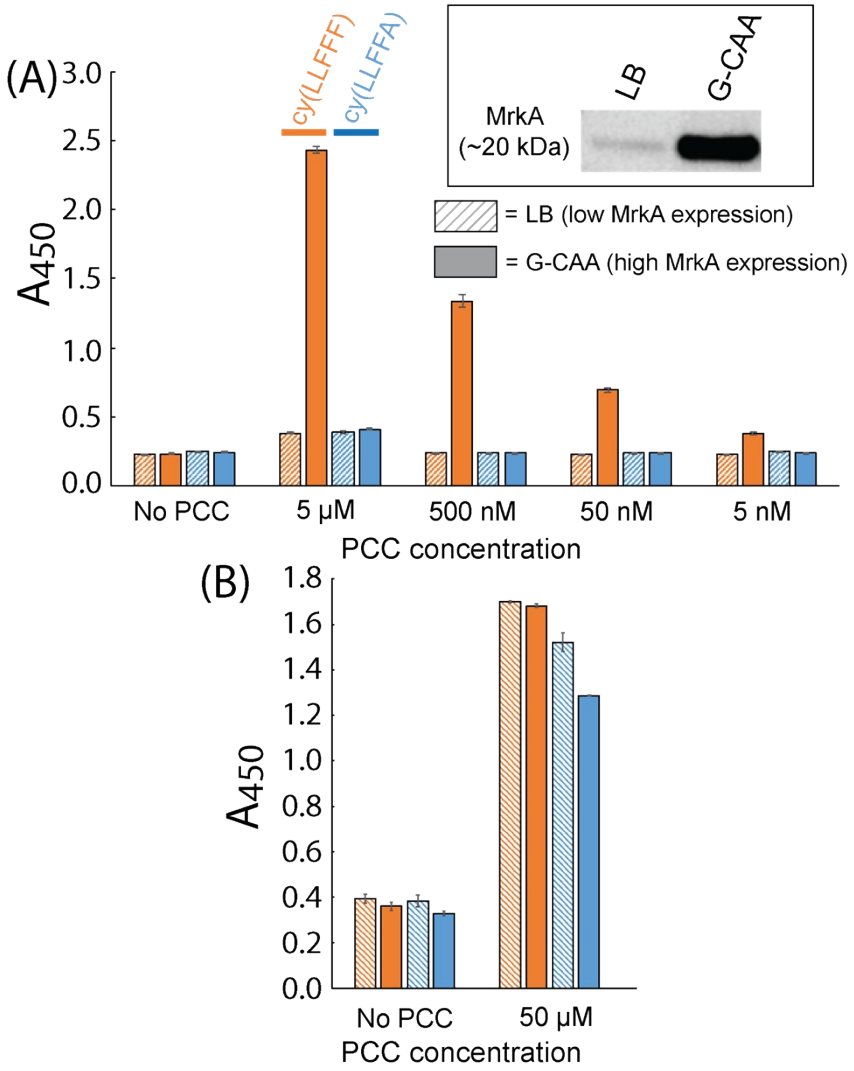


Figure S7. Plots of 450 nm absorbance from whole-cell ELISAs conducted on *K. pneumoniae* (strain ATCC BAA-1705) cells cultured in either LB (dashed bars) or G-CAA media (solid bars) and treated biotinylated cy(LLFFF) and cy(LLFFA) PCCs at various concentrations. The inset in 5(A) shows cropped western blot lanes (full western blot in Figure S6) that establish low MrkA expression for bacteria grown in LB and high levels for bacteria cultured in G-CAA media. Samples that were not treated with biotinylated PCCs, but with secondary antibody, establish a background absorbance (leftmost bars, "No PCC"). **(A)** Strong absorbances are observed from samples treated with biotinylated cy(LLFFF) at concentrations from 5 μM to 5 nM (solid orange bars), while near-baseline absorbances are observed from cells cultured in LB (orange dashed bars). Thus, cy(LLFFF) preferentially binds MrkA-expressing cells. By comparison, both wild type and MrkA-knockout samples treated with cy(LLFFA) at all concentrations tested yield near-baseline absorbances (solid and striped blue bars), indicating nearly no binding. **(B)** Cells cultured in either LB or G-CAA that were treated at higher 50 μM concentrations of cy(LLFFF) and cy(LLFFA) produce strong and comparable ELISA signals, demonstrating that both compounds bind *K. pneumoniae* cells regardless of MrkA-expression. Such binding may occur through non-specific hydrophobic type interactions with cell surface components.

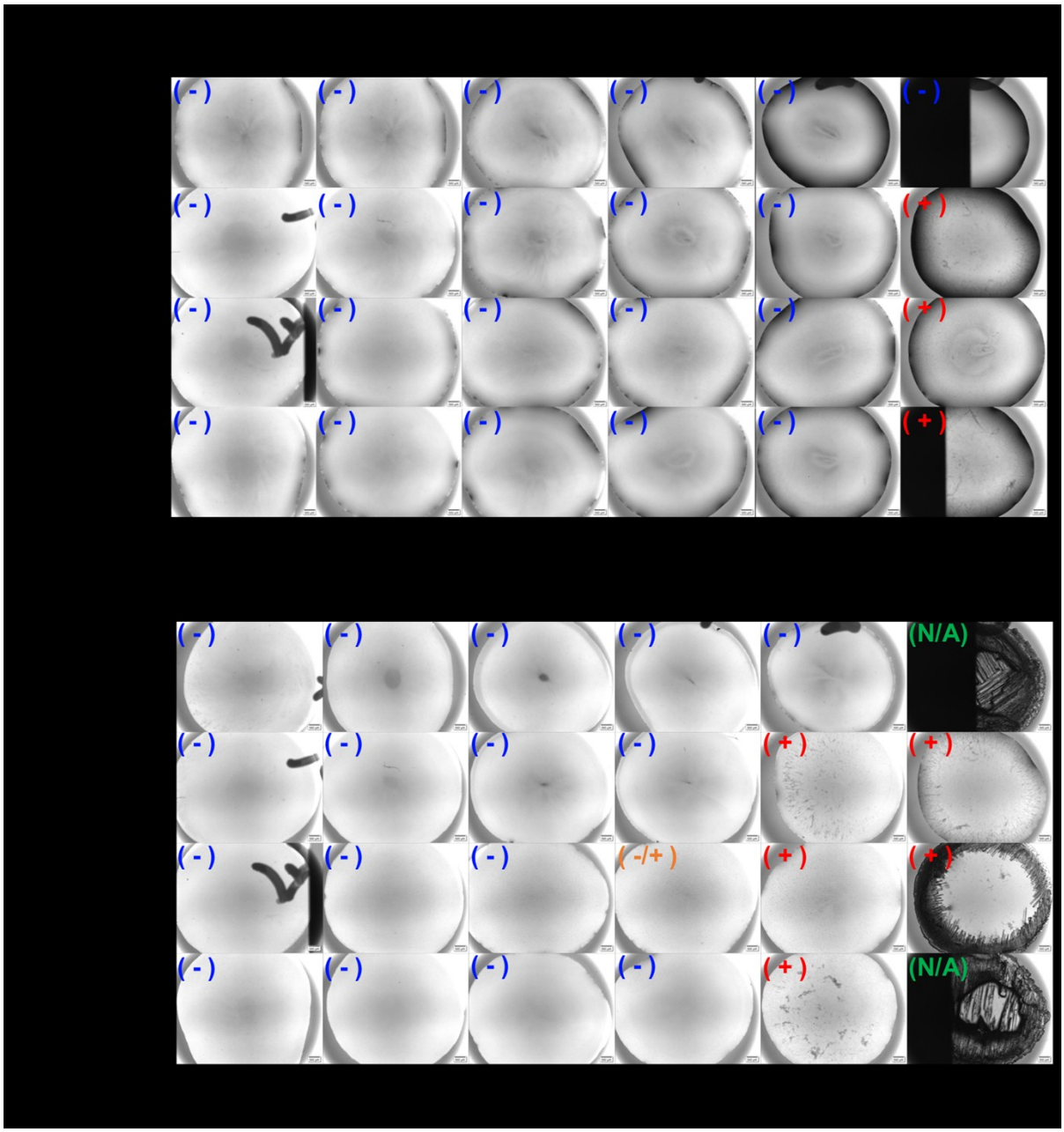


Figure S8. Images of droplets of phosphate buffered solution containing live bacteria and various concentrations of $(NH_4)_2SO_4$ at 10 m or 30 m following mixing. Aggregation in each droplet was assessed by visual inspection and is indicated as non-aggregated "(-)", low levels of aggregation "(-/+)", and extensive aggregation "(+)". All droplets were on a single glass slide and image acquisition of all droplets took less than a minute for each 10 m and 30 m time point. Crystallization was observed at 30 m times, indicated by "N/A", that precluded assessment of aggregation. These results show that *K. pneumoniae* strains aggregate at similar or higher salt concentrations than *E. coli* and *S. typhimurium*, indicating similar or lower cell surface hydrophobicity for these strains of *K. pneumoniae* versus *E. coli* and *S. typhimurium*.

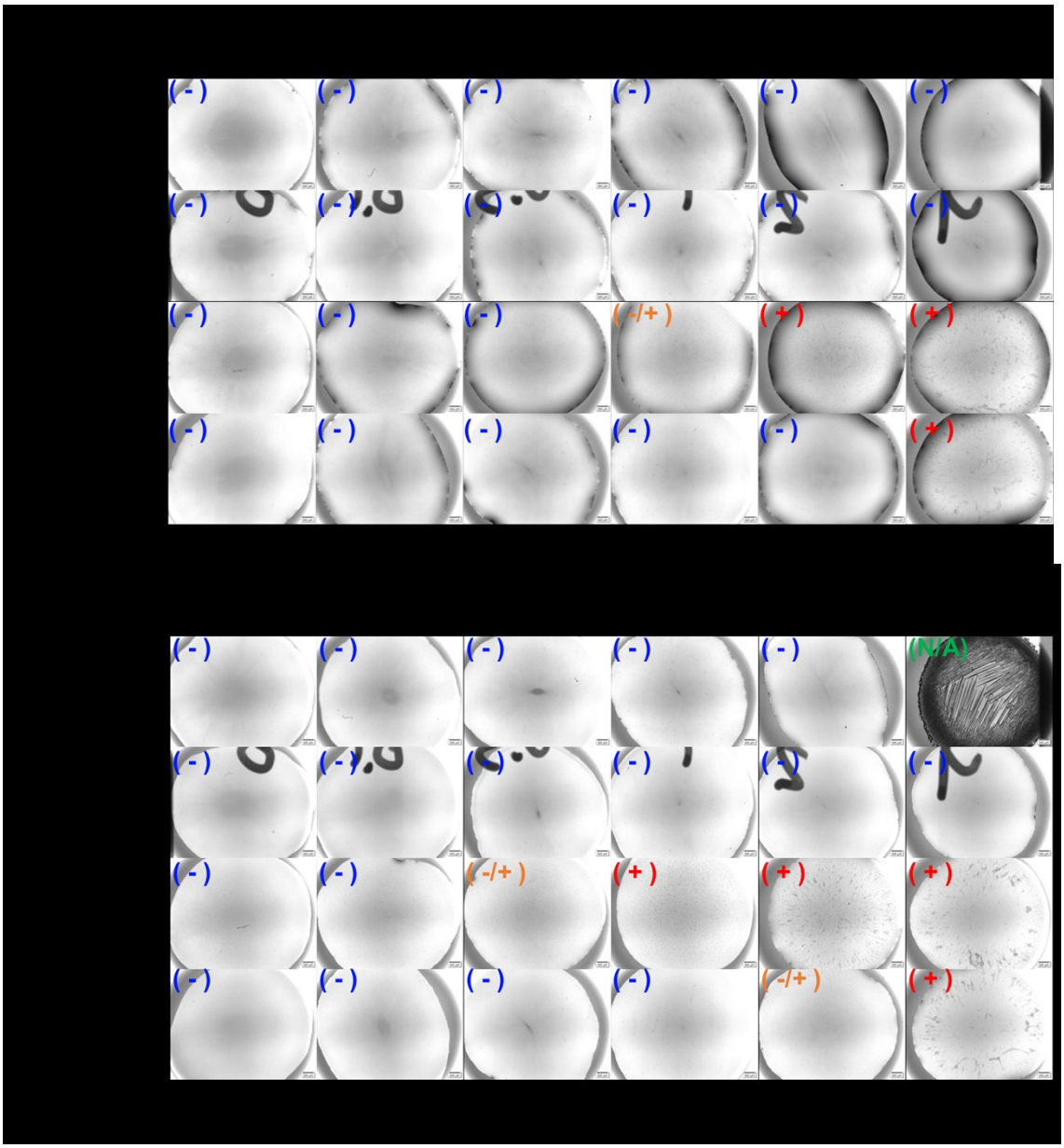


Figure S9. Images of droplets of phosphate buffered solution containing heat-killed bacteria and various concentrations of $(NH_4)_2SO_4$ at 10 m or 30 m following mixing. Aggregation in each droplet was assessed by visual inspection and is indicated as non-aggregated "(-)", low levels of aggregation "(-/+)", and extensive aggregation "(+)". All droplets were on a single glass slide and image acquisition of all droplets took less than a minute for each 10 m and 30 m time point. Crystallization was observed at 30 m times, indicated by "N/A", that precluded assessment of aggregation. These results corroborate the similar cell surface hydrophobicities of *K. pneumoniae* strains used here and *E. coli* and *S. Typhimurium*, as indicated by salt aggregation tests on live bacteria.

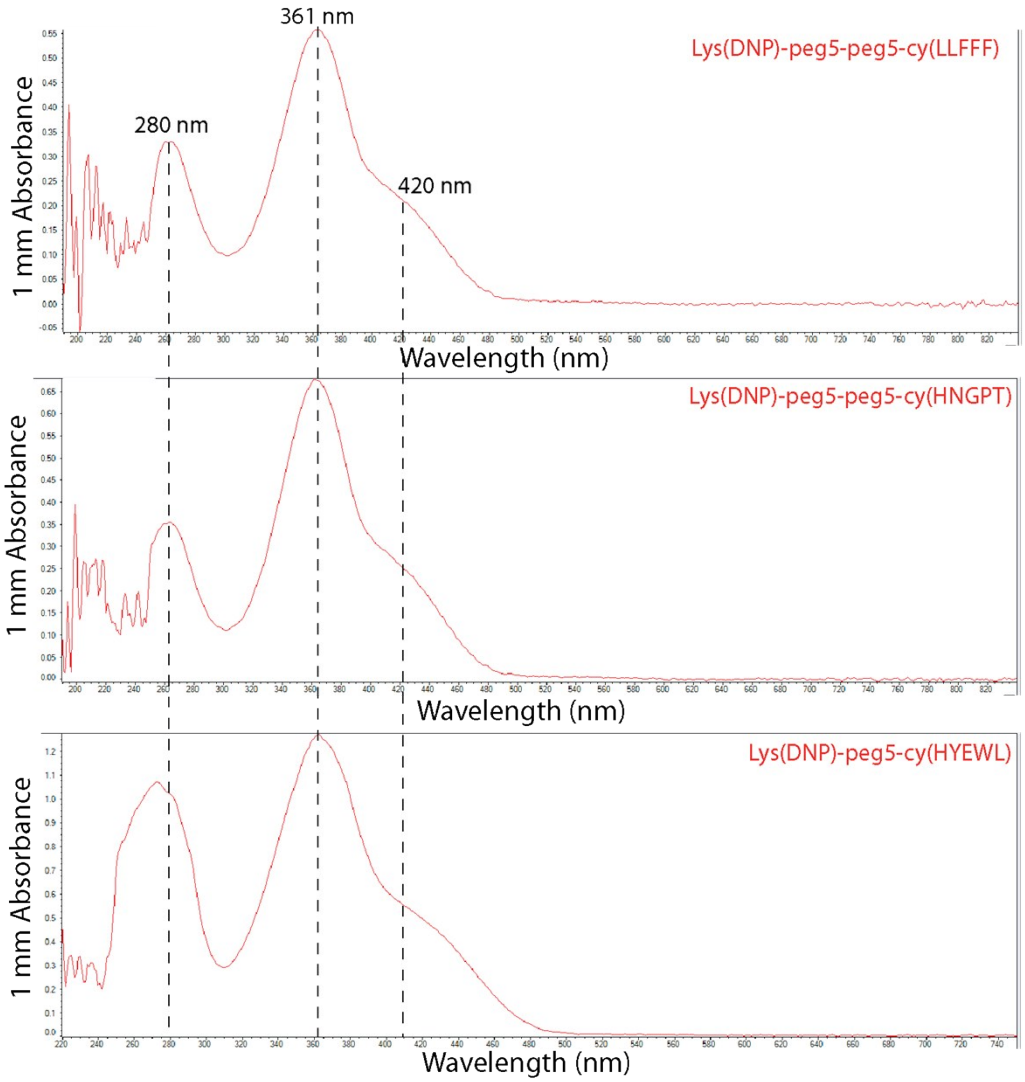


Figure S10. UV-visible absorbance spectra for representative dinitrophenyl(DNP)-conjugated compounds, including cy(LLFFF), cy(HNGPT) and cy(HYEWL), solubilized in dimethylsulfoxide, all of which show characteristic absorbance peak at 361 nm and shoulder at 420 nm that are associated with DNP. An absorbance peak at 280 nm is also present in each compound, which likley has contributions from the lys(DNP) residue and other aromatic residues in these compounds. DNP was conjugated to the macrocyclic peptide ligand via a C-terminal DNP-modified lysine residue, separated by either a peg5 or peg5-peg5 linker.

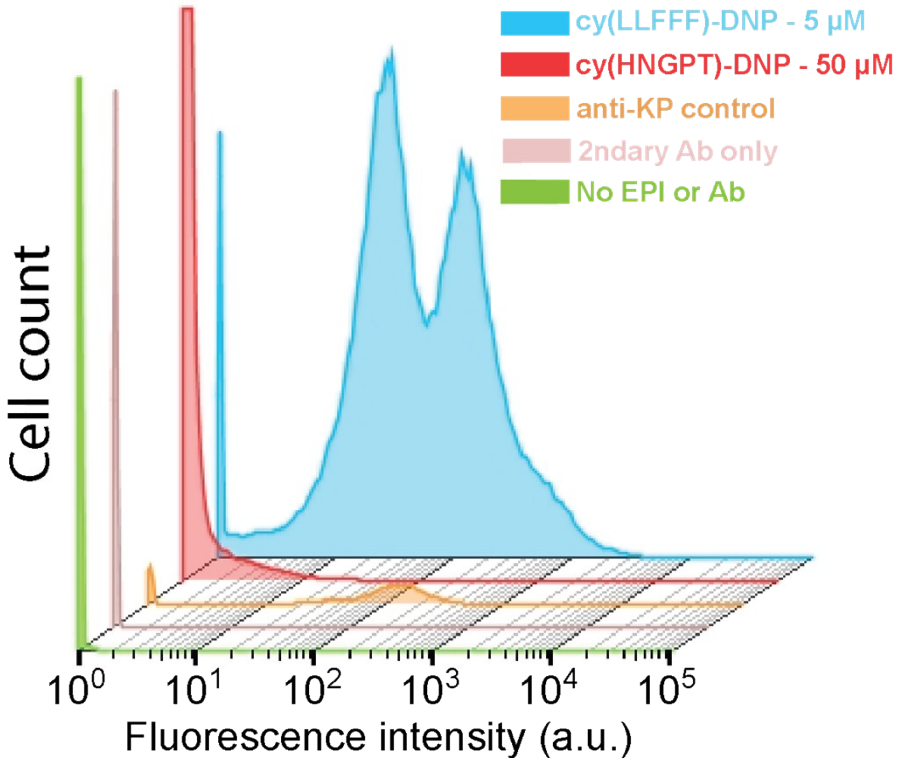


Figure S11. Plot of the fluorescence intensity versus cell count for *K. pneumoniae* (strain BAA 1705) opsonized by DNP-conjugated AR-PCC ligands and fluorescently-tagged anti-DNP antibodies. Cells exposed to cy(LLFFF) at 5 μM (blue) concentrations showed much higher fluorescence than cells incubated with 50 μM of the control cy(HGNPT)-DNP conjugate plus fluorescent anti-DNP (red). Cells exposed only to only *Klebsiella pneumoniae* antiserum (orange), secondary antibody (i.e., fluorescently tagged anti-DNP, pink), or neither AR-PCCs or antibodies (green) showed the least fluorescence. Cytometry measurements were gated for single cells and each histogram comprises >4,000 cells.

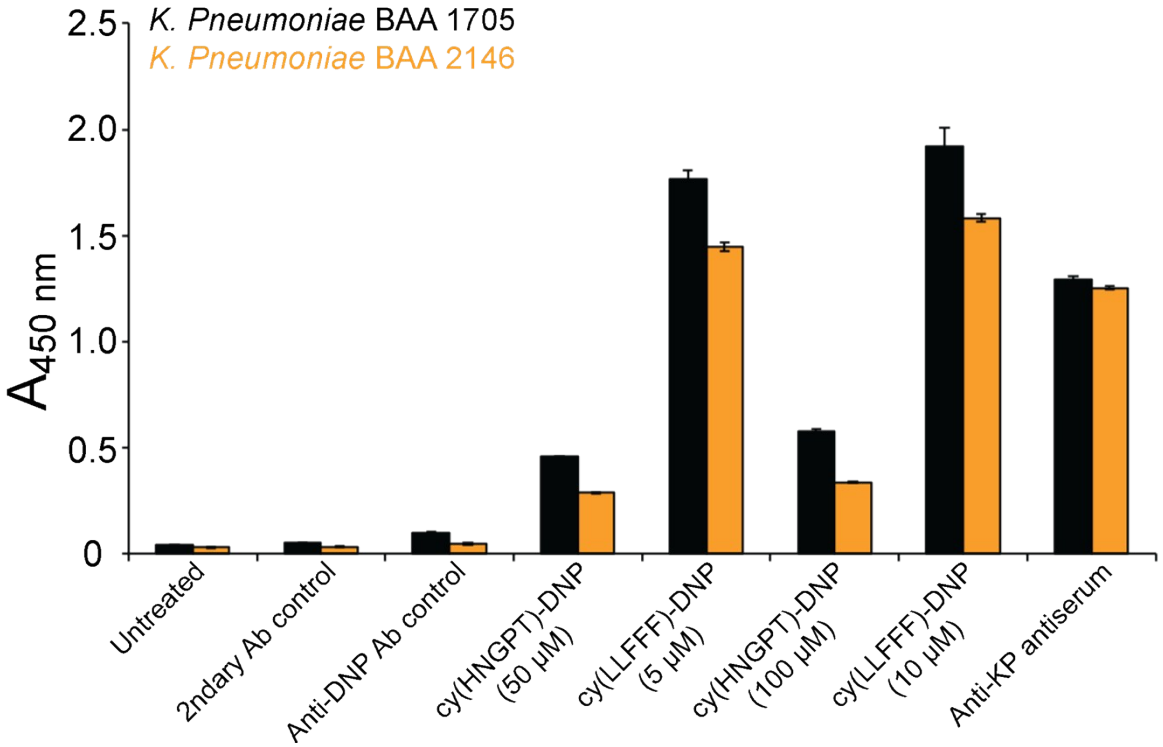


Figure S12. Plot of 450 nm absorbances obtained from a cell-based ELISA assay conducted on two strains of *K. pneumoniae* cells, BAA 1705 (black) and BAA 2146 (orange), that were either untreated or opsonized by AR-PCCs and/or antibodies. Very low absorbances were observed for *K. pneumoniae* cells that were untreated, or treated either with secondary antibody or anti-DNP controls. By comparison, much larger signal is observed for *K. pneumoniae* opsonized with cy(LLFFF) plus anti-DNP at either 5 μM or 10 μM AR-PCC concentrations (anti-DNP concentration fixed). This establishes AR-PCC-driven opsonization by cy(LLFFF)-DNP. Much smaller signals were observed from *K. pneumoniae* cells opsonized with a dummy AR-PCC ligand cy(HNGPT)-DNP at a much greater concentrations of 50 and 100 μM. This indicates opsonization by cy(LLFFF)-DNP is specific and is driven by the ligand cy(LLFFF), rather than the Lys(DNP) moiety.

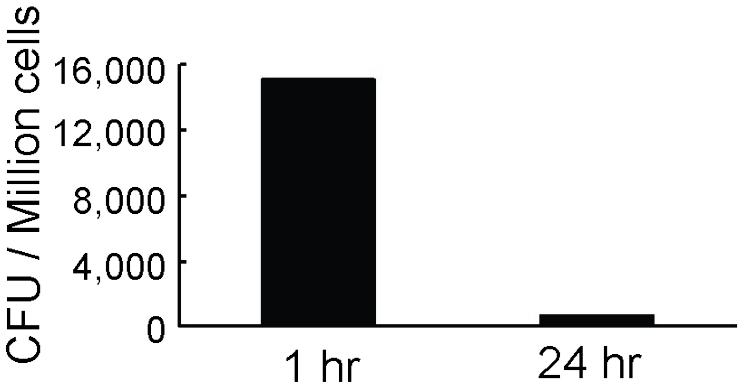


Figure S13. Plot of CFU counts per million cells obtained by plating and growing macrophage lysate after exposure of macrophages to *K. pneumoniae* bacteria for either 1 or 24 h. Larger cell counts (~15,000 CFU) were observed in samples prepared from macrophages harvested at 1 h, while very low counts (~1,000 CFU) were detected in samples from macrophages harvested at 24 h. This demonstrates that after 2 h, bacteria remain viable inside the phagosome, while after 24 h most bacteria were rendered inviable.

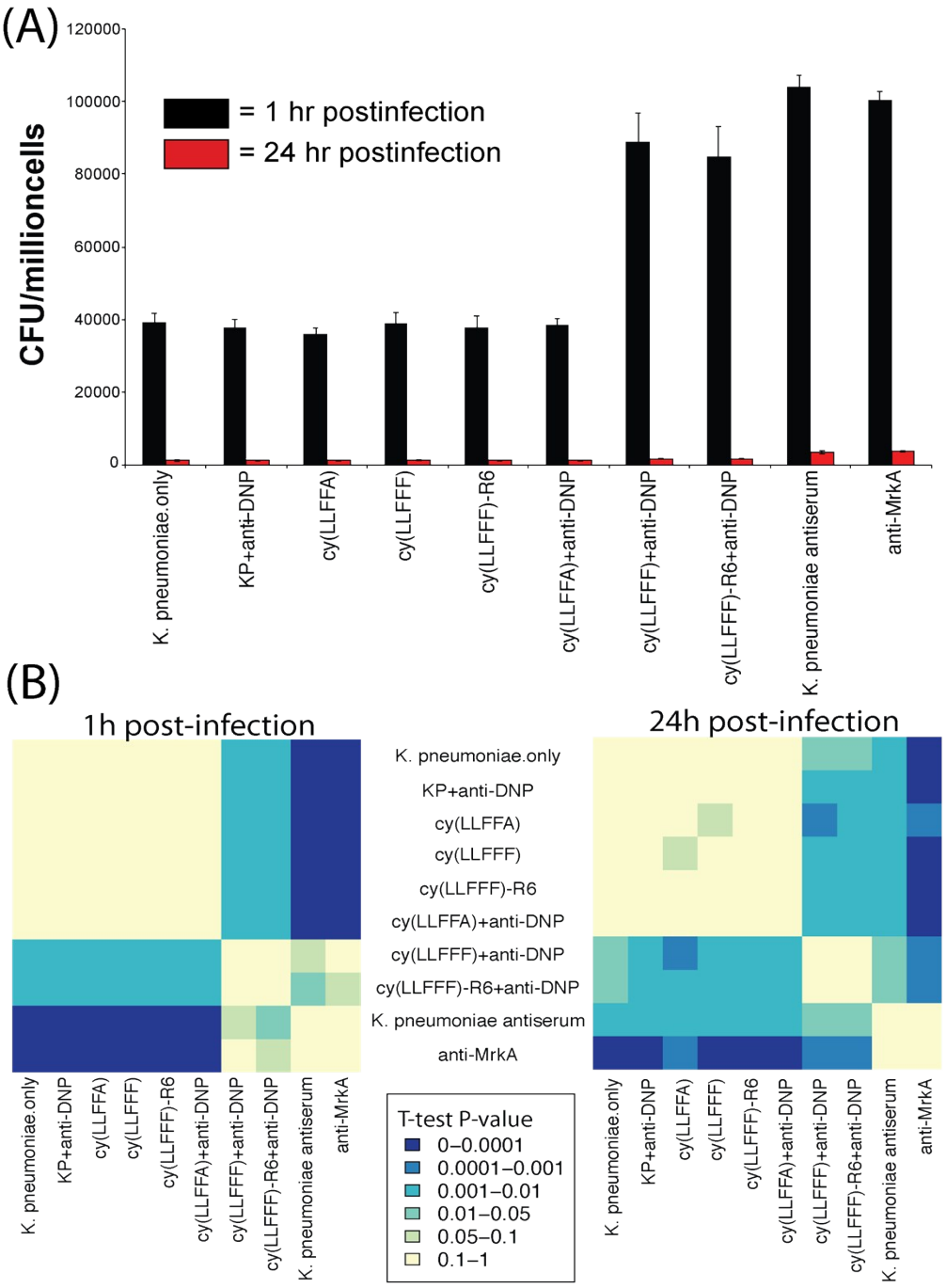


Figure S14. T-test analyses to assess the statistical significance of the phagocytosis and opsonophagocytic killing (OPK) assays performed on *K. pneumoniae* without or with exposure to various compounds and antibodies, as shown in Figure 5 of the main text. The OPK assay data of Figure 5 in the main text is reproduced in (A), while (B) shows a heatmap of the results of a T-test conducted of the colony forming units (CFU) counts between all samples in this dataset. Ranges for P-values are indicated by different colors (see legend), with cooler colors associated with lower P-values. For example, in the 1h post-infection heatmap, intersection of cy(LLFFF)-R6-anti-DNP on the x-axis and cy(LLFFF)-R6 is dark teal, indicating a P-value of 0.001-0.01, illustrating that the difference between the CFU counts for these samples is statistically significant.

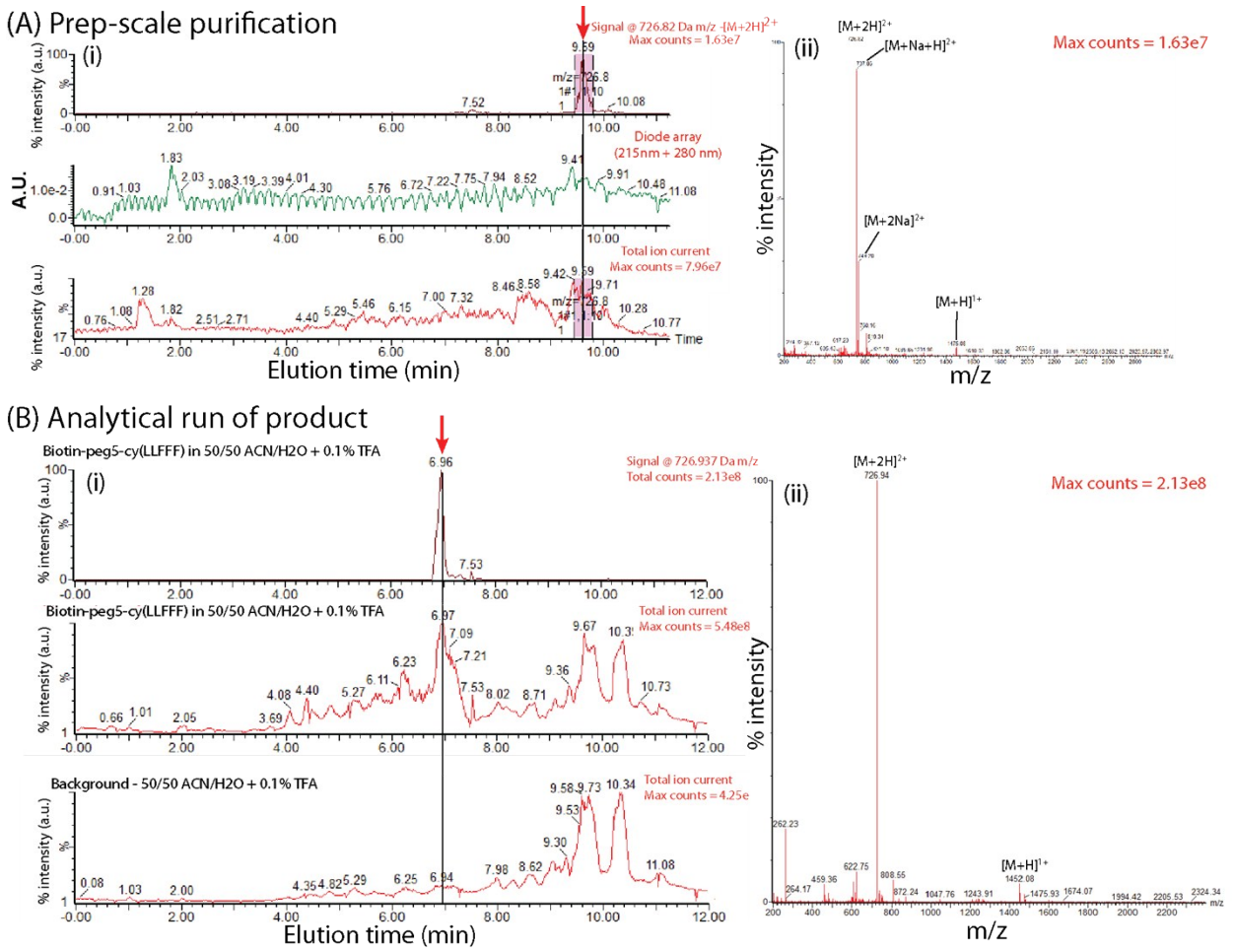


Figure S15. (A) Example purification and purity evaluation of the compound cy(LLFFF) conjugated with a peg5-biotin moiety. (i) HPLC-MS chromatograms of the total ion current (lower) and ion current at 726.82 +/- 2 Da (top), which is the $[M+2H]^{2+}$ mass of the desired product, and the absorbance (A280 + A215) (middle). The corresponding mass spectrum recorded at the elution time indicated by the red arrow in (i), which shows peaks associated with the $[M+H]^{+}$, $[M+2H]^{2+}$, $[M+Na+H]^{2+}$, $[M+2Na]^{2+}$. (B) (i) Example HPLC-MS chromatograms (top two) of the product purified in (A) in DMSO at 10 mM alongside a run on the same column but with 50/50 ACN/H_2O without compound (lower). Shown in (ii) is a mass spectrum obtained at the elution time indicated in the red arrow in (i) which shows peaks associated with the $[M+H]^{+}$ and $[M+2H]^{2+}$.