

Supplementary Information for:

A Simple Platform for the Rapid Development of Antimicrobials

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Abstract: Recent infectious outbreaks highlight the need for platform technologies that can be quickly deployed to develop therapeutics needed to contain the outbreak. We present a simple concept for rapid development of new antimicrobials. The goal was to produce in as little as one week thousands of doses of an intervention for a new pathogen. We tested the feasibility of a system based on antimicrobial synbodies. The system involves creating an array of 100 peptides that have been selected for broad capability to bind and/or kill viruses and bacteria. The peptides are pre-screened for low cell toxicity prior to large scale synthesis. Any pathogen is then assayed on the chip to find peptides that bind or kill it. Peptides are combined in pairs as synbodies and further screened for activity and toxicity. The lead synbody can be quickly produced in large scale, with completion of the entire process in one week.

Supplementary Table S1. Microorganisms used in this study.

Organism	Strain	Classification	Inactivation	Screened on		
				10k	275	100 array
<i>Acinetobacter baumannii</i>	ATCC 15149	Gram-negative	None	X	X	X
<i>Bacillus subtilis</i>		Gram-positive	None	X	X	
<i>Burkholderia pseudomallei</i>	ANG-BURK003	Gram-negative	G.	X	X	
<i>Escherichia coli</i> O111:B4	ATCC 12015	Gram-negative	None	X	X	
<i>E. coli</i> O157:H7	ATCC 1883	Gram-negative	None	X	X	X
<i>Francisella tularensis</i>	Schu S4	Gram-negative	G. At.	X	X	
<i>Pseudomonas aeruginosa</i>	PAO1	Gram-negative	None	X	X	
<i>Rickettsia prowazekii</i>	Cairo	Gram-negative	G.	X		
<i>R. prowazekii</i>	Madrid	Gram-negative	G.	X	X	
<i>Staphylococcus aureus</i>	UAB637	Gram-positive	None	X	X	
<i>S. epidermidis</i>	MN8	Gram-positive	None			X
<i>Streptococcus mutans</i>	UAB149	Gram-positive	None	X	X	
<i>S. pneumoniae</i>	STREP5	Gram-positive	None	X	X	
Adenovirus	Adenoid 6	Non-enveloped, dsDNA				X
Cytomegalovirus	AD169	Enveloped, dsDNA	BPL	X	X	
Dengue Virus	Type 2	Enveloped, +ssRNA	BPL	X	X	
Herpes Virus	HSV-2G	Enveloped, dsDNA	Ag.	X	X	
Influenza H1N1	A/PR/8/34	Enveloped, -ssRNA	None	X	X	X
Influenza H1N1	A/CA/07/2009	Enveloped, -ssRNA	None			X
Influenza H3N2	A/Uruguay/716/2007	Enveloped, -ssRNA	At.	X	X	
Influenza B	B/Brisbane/60/2008	Enveloped, -ssRNA	At.	X		
Norovirus	Norwalk GI.1	Non-Enveloped, +ssRNA	VLP	X		
Rotavirus	SA-11	Non-enveloped, dsRNA				X

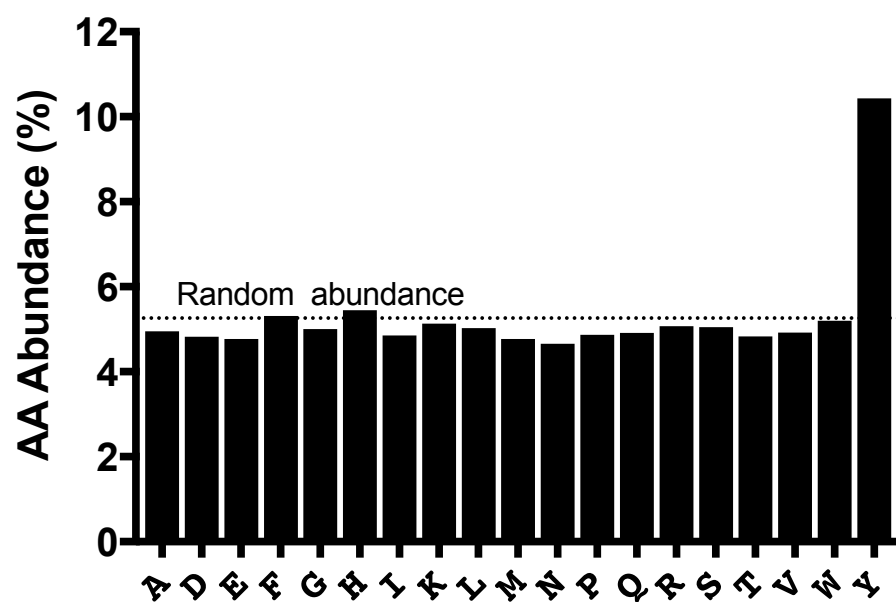
Rubella Virus	HPV77	Enveloped, +ssRNA	UV	X	X	X
SARS Coronavirus	Urbani	Enveloped, +ssRNA	UV	X	X	
Vaccinia Virus	Lister	Enveloped, dsDNA	P+UV	X	X	
Varicella Zoster Virus	ROD	Enveloped, dsDNA	UV?	X	X	
Venezuelan Equine Encephalitis Virus	Trinidad 1A/B	Enveloped, +ssRNA	BPL + G			

BPL = b-propiolactone inactivation. UV = UV inactivation. P+UV = psoralen + UV inactivation.

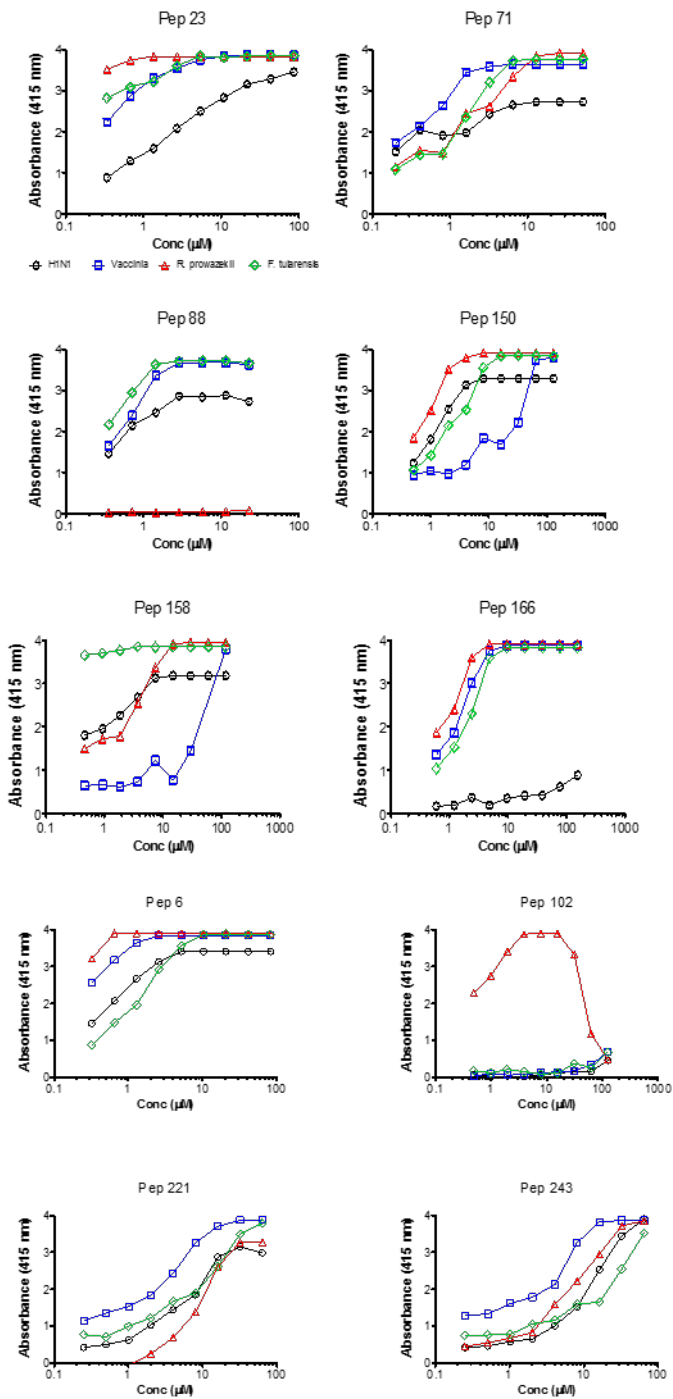
G = gamma irradiation. At. = attenuated organism. Ag. = surface antigen. VLP = virus-like particle.

Supplementary Table S2. Peptides sequences of 100 peptide library.

Peptide Number	Peptide Sequence	Peptide Number	Peptide Sequence
2	CSGCRRAGRPLLVCHPAHGG	123	CSGHLMHIHYWHKPGTPYPM
3	CSGDFLSHKDKMKHKWKWDE	124	CSGHLNTRHLFHGFQPIHHP
5	CSGDMYEYNPFQGNHIYNKK	125	CSGHNIYAQYGYPYDHMYEG
8	CSGEGWYRDEAPQLNDKMK	127	CSGHYADDKKNMTEKYKMHY
20	CSGLGKNQAKHLHGTYSKI	128	CSGHYGSAFDKEMDTKGHRA
23	CSGNSDYNAIWQKHVEAHKE	129	CSGHYNRQYNNYYQGKYRSL
36	CSGVGWKSLQQGPELPPQ	134	CSGKDHNADQESVHWKYKG
38	CSGWALRIREHADIDNYVKR	137	CSGKKHGPKYYSMSDRVVNQ
40	CSGYADQYMYKQSPMYPKTD	145	CSGKRRKQANPRRNALASE
43	CSGYNWSRKKHKYYPKLIAY	147	CSGKRYLQKGKALRGLYIF
45	CSGYVINGIMTSGAMAGSHK	149	CSGKSQEIQDPPDIWNQMKW
47	CSGAEVYLQAHFEGWASKAK	150	CSGKTEHYMPNNNTFGYEYE
50	CSGALTVMKQCHKLGTVLP	151	CSGKVHRWKYHSRQNYTYRP
52	CSGAMHGFPATTPAIGRYPW	155	CSGLFPVKTWKYWKAYSIA
53	CSGANPWEKEDDRYSYKHK	156	CSGLIPKRAEYKQHFQMHRT
54	CSGAQEWAAKSYKWNKDGYL	157	CSGLLHELDDYKINPQKY
58	CSGAVNYQEVFNKARMKKR	158	CSGLLYHFVGLRTMKISMM
63	CSGDEFGMHAFGHKLQAIKN	163	CSGMCQMYQPMCKKYYRLL
67	CSGDLPPSEHASMPDVRKKQ	165	CSGMIHYHMGYQKYDTSNH
68	CSGDMHWGYQDGKTLVPTSK	166	CSGMKQPKHNKINDNPKAYE
70	CSGDNQGFNKMYPKSMGFVH	169	CSGMVIHDVAPKQPKPHGWS
71	CSGDPTHATEPKRYEAYNDH	170	CSGMWRHSKKKEDPYDLKNW
72	CSGDPVQLIHPMDWEGQKYH	172	CSGNDKKGNKLYASNAKY
73	CSGDRENDKSFQKRKDAGVI	174	CSGNETAPDNTYRYKQSAQK
74	CSGDSKSIHIMPVHMIFFPD	176	CSGNKVHPKSKEQKVYINYP
75	CSGDSPMGYHQKTSPWADK	179	CSGNPNTWQINYPHLYTHRA
79	CSGDVAQFTSSGMYRPANKM	181	CSGNQKTDKHKHYQWWEIY
83	CSGEDSDEPYWQPPKHWHK	183	CSGNWSEWKKVKAPHNQPK
84	CSGEKMTKYFFEKYGAAMP	186	CSGPENEMKSIVIFPEKKDH
85	CSGELFFTRDNNMHNHMHKM	189	CSGPGQQWIGNDWKAVQKGK
86	CSGELYSPRMKAIYSYHWHK	190	CSGPHFMFEPSPVVRPNYKQA
88	CSGEMWAIMPPIIKPDNKGH	192	CSGPHPQYRHGPKHYIRTQM
91	CSGEPSPQKYKLGKGLNEH	204	CSGQFSAKKYWEIKPMDYWK
93	CSGEWPDIKHKYNMNQFVSR	206	CSGQITSHQKYKSVFNQHM
94	CSGEWRQKMPKSAFNKPKQ	207	CSGQPSNHRKMSMIYPKE
95	CSGEYMLKTEPHEDHRDKYW	210	CSGQYSQQSSSYQQMFKKE
96	CSGFKDFDDYFNVSKYIYW	216	CSGRPHAHVERMKTDRQYAW
98	CSGFKNYDMVEDKHNKHAYA	219	CSGRQSKRYKEFGKDPTKAH
101	CSGFMPKVKMWFEEMVQCHK	223	CSGSAFDQKDSADASKWGYK
104	CSGFRKHPWKHPRHKFHKPW	227	CSGSFNQYFPYPMIDYLLK
105	CSGFRKYDIHAMYKPRNKYQ	228	CSGYDVHMGPMNDNHYFKK
107	CSGFWRKHPFRWKHPRPKH	233	CSGSLVWNNGDYKYNPKMPS
110	CSGGAKSKEKGYQVPLSHS	241	CSGTANELLYKNGVKNPK
111	CSGGAVNKYHYDYKTKKKPY	247	CSGTVEPTPGKGPVYRRK
113	CSGGKDSQDAYSMQMIRS	252	CSGVDSNSKYEIGKEHDLKK
114	CSGGKGPVYRRKHQELQAM	256	CSGVLQTVHGPEAVGLSKVK
116	CSGGSLLTAIKTRAPQELKFL	263	CSGWLMSVIKADRKAHRKEQ
119	CSGHELEEAKPPSQRWEHK	268	CSGYGGYHEQFGMMEHPSSK
121	CSGHKFNHFLNEHAHWLSGR	272	CSGYLDRKELGRCAQAQMNK
122	CSGHKQCHKLGTVLPESFC	274	CSGYNVHRPFIDPARDHPM

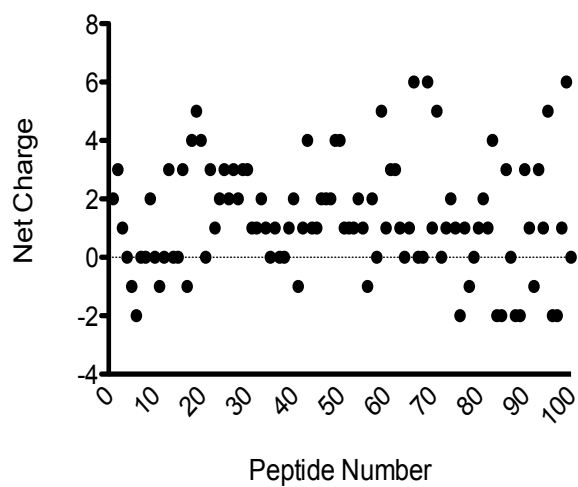


Supplementary Figure S1. Amino acid abundance for the peptides in the 10,000 peptide microarray. The dashed line indicates the percent abundance if each amino acid was of random distribution.

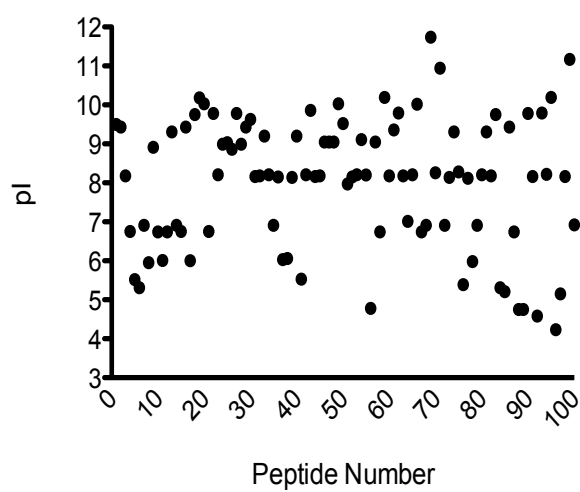


Supplementary Figure S2. ELISA screening for the 275 peptides library. Four pathogens (Vaccinia virus, Influenza virus A/PR/8/34 (H1N1), *F. tularensis* or *R. prowazekii* Madrid) were tested against the 275 peptides sequences to identify those with broad binding spectrum by ELISA.

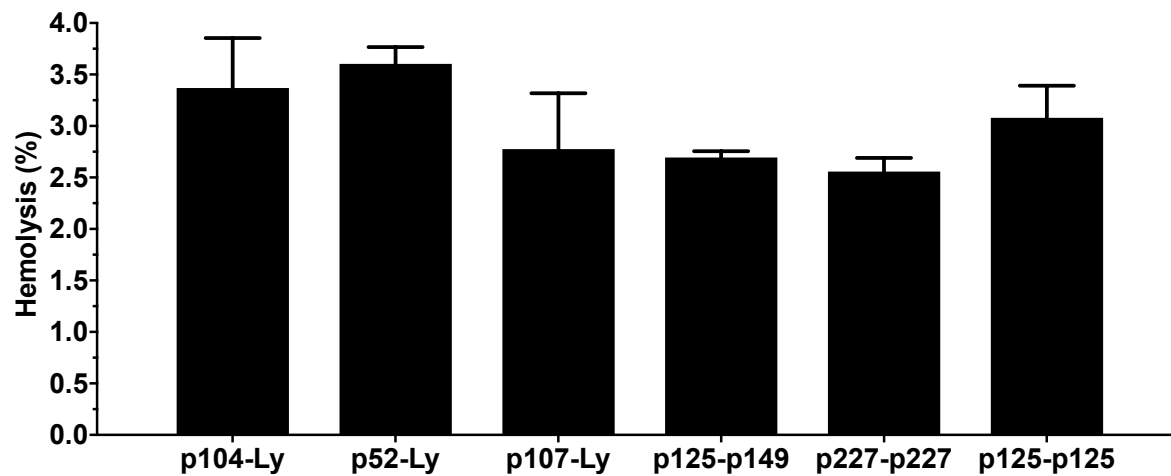
A)



B)



Supplementary Figure S3. Properties of 100 peptide library. Distribution of **A)** net charge and **B)** pI of peptide library.



Supplementary Figure S4. Evaluation of hemolysis by the pathogen-specific synbodies. The synbodies selected (125 μ M) against Influenza virus (p125-p149, p227-p227) and *S. epidermidis* (p104-Ly, p52-Ly, p107-Ly) were incubated with mice red blood cells and hemolysis was measured after 60 minutes.