

Supporting Information

Phage Wrapping with Cationic Polymers Eliminates Non-specific Binding between M13 Phage and High pI Target Proteins

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Table S1. Estimated pI values of proteins targeted by published molecular display experiments.*

Target	Estimated pI	Ref.
Nuclear factor- κ B (residues 463-472)	3.56	1
Hepatitis virus B core antigen (HBcAg)	4.21	2
H7-flagellan of <i>E. coli</i>	4.74	3
Fc-domain	4.85	4
Bcl-X _L	4.86	5
β -glucosidase (<i>A. faecalis</i>)	5.15	6
EF-Tu	5.30	7
Carboxypeptidase B (porcine)	5.30	6
ML-IAP	5.43	8
Concanavalin A	5.43	9,10
Hexokinase (<i>S. cerevisiae</i>)	5.56	6
HMGB1 (<i>H. sapiens</i>)	5.61	11
Alcohol dehydrogenase (<i>S. cerevisiae</i>)	5.62	6
MMP-9 (gelatinase B)	5.70	12
Ribonuclease S	5.70	13
Factor VIIa	5.74	14
Mouse serum albumin	5.75	15
G-protein (G- α -i1)	5.75	16,17
Stromelysin and matrilysin	5.77	18
Erythroblastic leukemia viral oncogene homolog-2	5.80	19
Rabbit serum albumin	5.85	15
Anthrax protective antigen	5.88	20
Serine repeat antigen 5 (<i>P. falciparum</i>)	5.88	21
Prolyl tRNA synthetase	5.92	6
H5N1 hemagglutinin (Influenza virus A Vietnam/1203/04)	6.01	22
Human serum albumin	6.05	15
Streptavidin	6.06	23
Human serum albumin	6.05	24,25

Vinculin	6.08	26
NADH oxidase (Thermophilus)	6.11	27
Tryptase	6.12	28
Protein Secretion Chaperone CsaA (<i>A. tumefaciens</i>)	6.14	29
H5N1 neuraminidase (Influenza virus A Vietnam/1203/04)	6.26	22
Subtilisin BPN, Factor Xa	6.30	30
Complement protein C3b	6.39	31
Tumor necrosis factor- α	6.44	19,32
Prostate specific membrane antigen	6.50	33
Human Tryptase-beta-1	6.62	34
c-Src tyrosine kinase	6.62	35
Lyn tyrosine kinase	6.70	35
Glycogen phosphorylase A (rabbit)	6.77	6
Erythroblastic leukemia viral oncogene homolog-4 (fused to Fc IgG)	6.98	19
Blk tyrosine kinase	7.05	35
Protein A (<i>S. aureus</i>)	7.06	36
Erythroblastic leukemia viral oncogene homolog-1 (fused to Fc IgG)	7.42	19
alpha-chymotrypsin	7.60	37
West Nile Virus envelope protein	7.66	38
Calmodulin	7.92	39,40
Fragment crystallizable of human IgG1	7.93	19
Tyrosine kinase (pp60-src)	7.95	41
Trypsin	8.13	42
Urease (<i>Helicobacter pylori</i>)	8.03	43
α -bungarotoxin	8.31	44
Syk tyrosine kinase	8.32	35
Human plasma kallikrein	8.60	45
Na ⁺ -citrate symporter CitS	8.67	46
Penicillin-binding protein 2a (<i>S. aureus</i>)	8.61	47
Ribonuclease A (bovine)	8.68	48
cAMP-dependent protein kinase, catalytic subunit	8.84	49
Tyrosyl tRNA synthetase (<i>H. influenza</i>)	8.90	6
Angiotensin converting enzyme	9.08	50
Transcription factor GCN4	9.31	51
Colicin E9 DNase	9.50	52
Cathepsin G (<i>H. sapiens</i>)	11.19	45

*This updated version of a previously reported table⁵³ lists the pIs of proteins successfully targeted in phage, ribosome and mRNA display experiments. With the exception of one outlier (Cathepsin G), the targeted proteins have a range of pI values between 3.56 and 9.50. The sequences of the listed target

proteins were obtained from the NCBI protein database or other sources, and the pIs were calculated using the ProtParam tool from the ExPASy Proteomics Server.⁵⁴ As noted previously, high pI proteins compose 35% of the human proteome.

Table S2. The viability of phage and bacteria following treatment with cationic polymers.*

Table S2A: Kanamycin Plate				Table S2B: Kanamycin Plate				Table S2C: Kanamycin Plate	
Polymer Concentration (μ M)	Polymer			Polymer Concentration (μ M)	Polymer			Polymer Concentration (μ M)	Polymer
	2	2	3		3	1	1		4
100									
10									
1									
0.1									
0.01									
0.001									
0.0001									

Table S2D: Tetracycline Plate				Table S2E: Tetracycline Plate				Table S2F: Tetracycline Plate	
Polymer Concentration (μ M)	Polymer			Polymer Concentration (μ M)	Polymer			Polymer Concentration (μ M)	Polymer
	2	2	3		3	1	1		4
100									
10									
1									
0.1									
0.01									
0.001									
0.0001									

*After wrapping with the indicated concentrations of polymers **1**, **2**, **3** or **4**, M13-KO7 phage (harboring a gene conferring kanamycin resistance) were allowed to infect a tetracycline resistant strain of *E. coli* at a MOI of 1. A 10- μ L aliquot of the infected bacterial culture was then spotted at the indicated positions on either kanamycin or tetracycline LB plates. Each spot, thus, provides a small bacterial lawn to quantitate the number of viable, infective phage or bacteria resulting from each condition. Tables S2A, S2B and S2C illustrate the viability of polymer-wrapped phage by quantifying the number of bacteria, which were conferred kanamycin resistance by M13-KO7 phage infection. Polymers **2** and **3** reduced or eliminated phage infectivity at high polymer concentrations (10-100 μ M), whereas **1** and **4** demonstrated no effect on phage infectivity under the same conditions. In Tables S2D, S2E and S2F, M13-KO7-infected bacteria grown on tetracycline plates indicate bacterial survival in the presence of each polymer. In this experiment, the titers were performed in duplicate for polymers **1**, **2** and **3** (polymer **3** was spotted on separate plates due to space limitations), and in triplicate for polymer **4**.

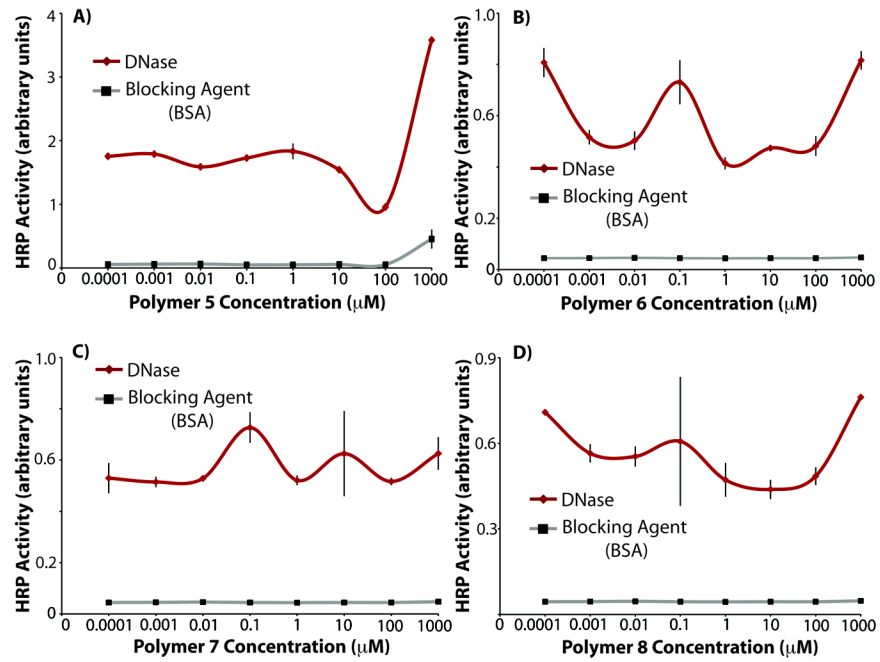


Figure S1. Inefficient phage wrapping polymers. In these phage-based ELISAs, M13-KO7 phage (1 nM) was wrapped with polymers (A) 5, (B) 6, (C) 7 or (D) 8 before assay for binding to either DNase or BSA (negative control). Such polymers failed to wrap phage effectively, as demonstrated by the strong binding to DNase. Though bearing positive charge, polymer 5 failed to wrap the phage as effectively as polymers 1 through 4.

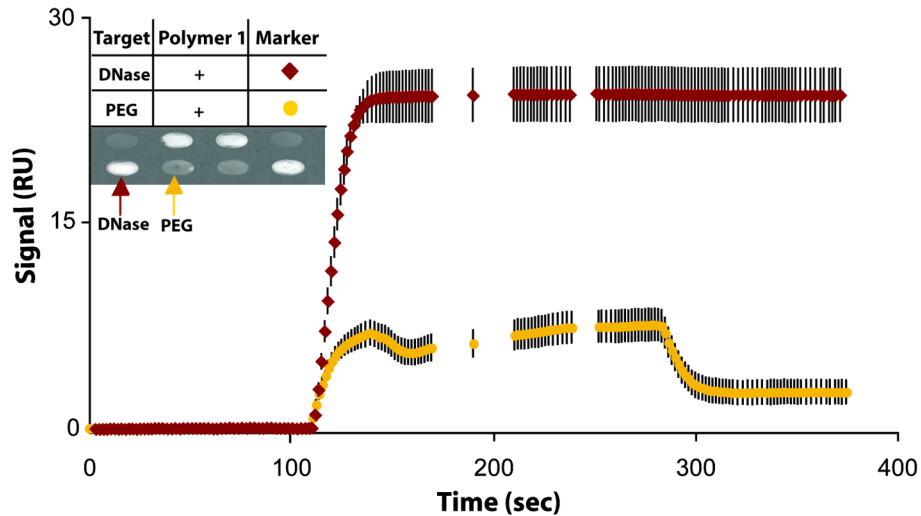


Figure S2. SPR imaging measurements with phage-displayed DNase-1 wrapped with polymer 1. The inset shows imaging of DNase-coated spots compared to PEG-coated spots. During the time-course experiment, DNase-1 phage was exposed to the surface at ~ 100 seconds, followed by rinsing with PBS at ~ 280 seconds; during this rinsing step, non-specific binding to PEG was greatly reduced. Markers denote the average of four spots with error bars indicating standard error.

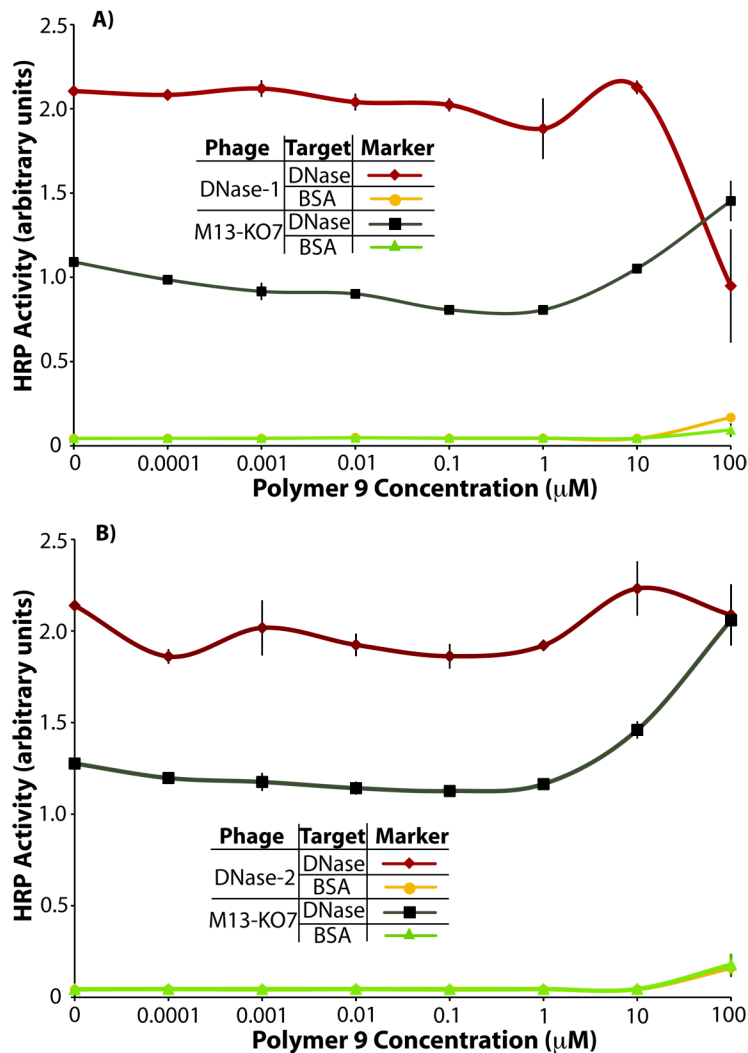


Figure S3. ELISAs demonstrating failed wrapping of phage-displayed (A) DNase-1 and (B) DNase-2 by the negatively charged polymer 9. In both experiments, phage (1 nM) were incubated with the indicated concentrations of polymer 9 before assay for binding to DNase. As expected, polymer 9 failed to wrap both phage, and block nonspecific binding to DNase at polymer concentrations $\leq 10 \mu\text{M}$. At a higher polymer concentration (100 μM), M13-KO7 binding increased slightly, presumably due to a similar cross-linking effect as observed with the cationic polymers. Polymer 9 decreased slightly the binding of DNase-1 to DNase at this high polymer concentration, perhaps by blocking the phage-displayed DNase-1 ligand from binding to DNase.

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