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for

Chemical and Genetic Wrappers for Improved Phage and RNA Display

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Table S1. The pIs of target proteins successfully used in phage, ribosome and mRNA display experiments. Target proteins are a subset of those reviewed previously.[40,41] Target protein sequences were found on NCBI protein database (when specific protein sequences could not be found, proteins sequences from an alternate source was used) and estimated pIs were calculated.[42]

Target	Estimated pI	Ref.
Hepatitis virus B core antigen (HBcAg)	4.21	[1]
H7-flagellan of E. coli	4.74	[2]
Fc-domain	4.85	[3]
Bcl-X_L	4.86	[4]
β-glucosidase (A. faecalis)	5.15	[5]
EF-Tu	5.3	[6]
Carboxypeptidase B (porcine)	5.3	[5]
ML-IAP	5.43	[7]
Concanavalin A	5.43	[8,9]
Hexokinase (S. cervisiae)	5.56	[5]
Alcohol dehydrogenase (S. cervisiae)	5.62	[5]
MMP-9 (gelatinase B)	5.7	[10]
Ribonuclease S	5.7	[11]

Factor VIIa	5.74	[12]
Mouse serum albumin	5.75	[13]
G-protein (G-α-i1)	5.75	[14,15]
Stromelysin and matrilysin	5.77	[16]
Rabbit serum albumin	5.85	[13]
Anthrax protective antigen	5.88	[17]
Prolyl tRNA synthetase	5.92	[5]
Human serum albumin	6.05	[13]
Streptavidin	6.06	[18]
Human serum albumin	6.05	[19]
Vinculin	6.08	[20]
NADH oxidase (Thermophilus)	6.11	[21]
Tryptase	6.12	[22]
Subtilisin BPN, Factor Xa	6.3	[23]
Complement protein C3b	6.39	[24]
Tumor necrosis factror-α	6.44	[25]
Prostate specific membrane antigen	6.5	[26]
Human Tryptase-beta-1	6.62	[27]
c-Src tyrosine kinase	6.62	[28]
Lyn tyrosine kinase	6.7	[28]
Glycogen phosphorylase A (rabbit)	6.77	[5]
Blk tyrosine kinase	7.05	[28]
alpha-chymotrypsin	7.6	[29]
Calmodulin	7.92	[30,31]
Tyrosine kinase (pp60-src)	7.95	[32]
Trypsin	8.13	[33]
Urease (Heliobactor pylori)	8.03	[34]
α-bungarotoxin	8.31	[35]
Syk tyrosine kinase	8.32	[28]
Penicillin-binding protein 2a (S. aureus)	8.61	[36]
Tyrosil tRNA synthetase (H. influenza)	8.9	[5]
Angiotensin converting enzyme	9.08	[37]
Transcription factor GCN4	9.31	[38]
colicin E9 DNase	9.5	[39]

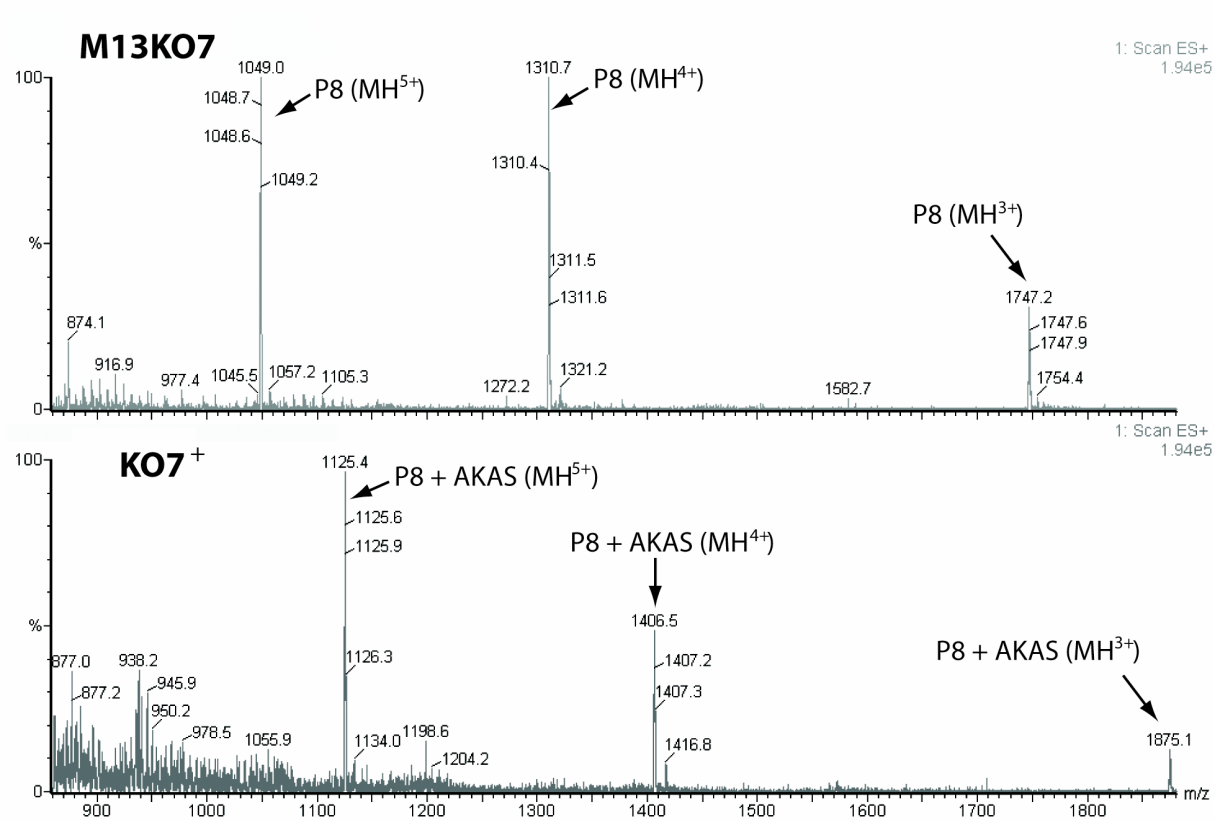

Figure S1. LC-MS of M13KO7 and KO7^{+} P8 coat proteins. Display of the amino acid sequence AKAS on every copy of KO7^{+} P8 was confirmed. The observed mass (*m/z*) for P8 coat proteins matched expected values within 0.015% as follows: M13KO7 P8, observed 1747.2 (MH^{3+}), 1310.7 (MH^{4+}) and 1049.0 (MH^{5+}) (calculated mass: 5238.0); and KO7^{+} P8, observed 1875.1 (MH^{3+}), 1406.5 (MH^{4+}) and 1125.4 (MH^{5+}) (calculated mass: 5622.6). KO7^{+} and M13KO7 phage were shaken separately in 50% isopropanol for 30 min prior to injection in the LC-MS.[43,44]

A) B)

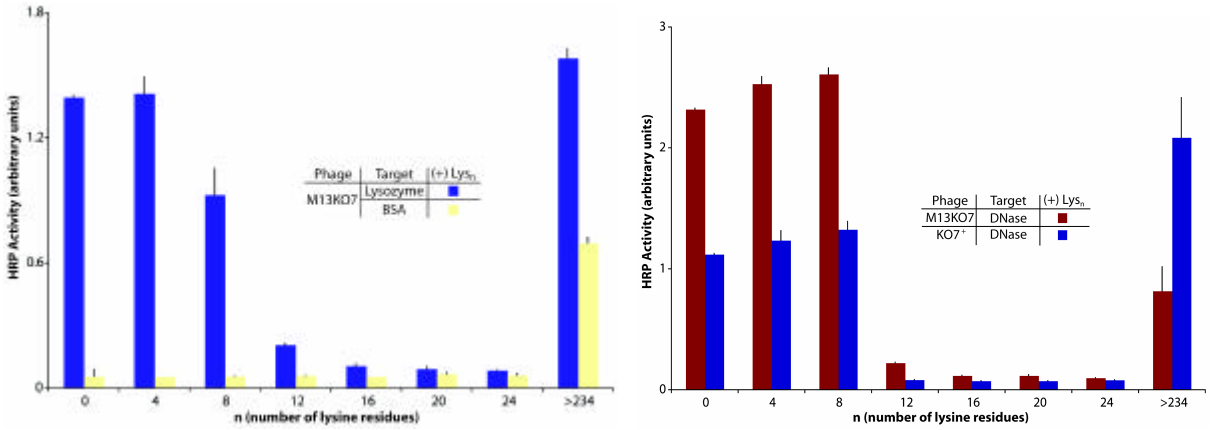

Figure S2. Phage-based ELISAs demonstrating oligolysine wrapping of M13KO7 and KO7^{+} phage, and blocking of non-specific binding to lysozyme (**A**) and DNase (**B**), respectively. (**A**) Different length oligolysine peptides (at 10 μM each) were incubated with M13KO7 phage and exposed to lysozyme. (**B**) M13KO7 and KO7^{+} phage were wrapped by oligolysine peptides varying in length (at 10 μM each) and tested for blocking non-specific binding to DNase. In both experiments, oligolysines between 12 and 24 residues in length eliminated background binding of phage to the target. Commercial poly-lysine (~234 lysines) failed to block spurious interactions.

Table S2. Titers of *E. coli* colony-forming units (CFU) infected by Lys_{18}-wrapped phage.

Lys_{18} Concentration [μM]	CFU Count
100	800 ± 20
10	940 ± 40
1	1000 ± 20
0	1300 ± 100

Measurement of *E. coli* Infection by Lys_{18}-Wrapped Phage. M13 phage (final concentration of 85 fM) were incubated with serial dilutions of Lys_{18} in PBT buffer (0.05% Tween-20, 0.2% BSA in PBS; 100?L final volume) and shaken for 30 min. Log-phase CJ236 *E. coli* (100 ?L) was added to each Lys_{18}-phage mixture and shaken (150 rpm) at 37 ºC for 30 min. A 10 μL aliquot of each sample was spotted on LB plates supplemented with 50 μg mL^{-1} carbenicillin, and incubated overnight at

37 ºC. The resultant colony-forming units shown in Table S2 represent the average of two experiments.

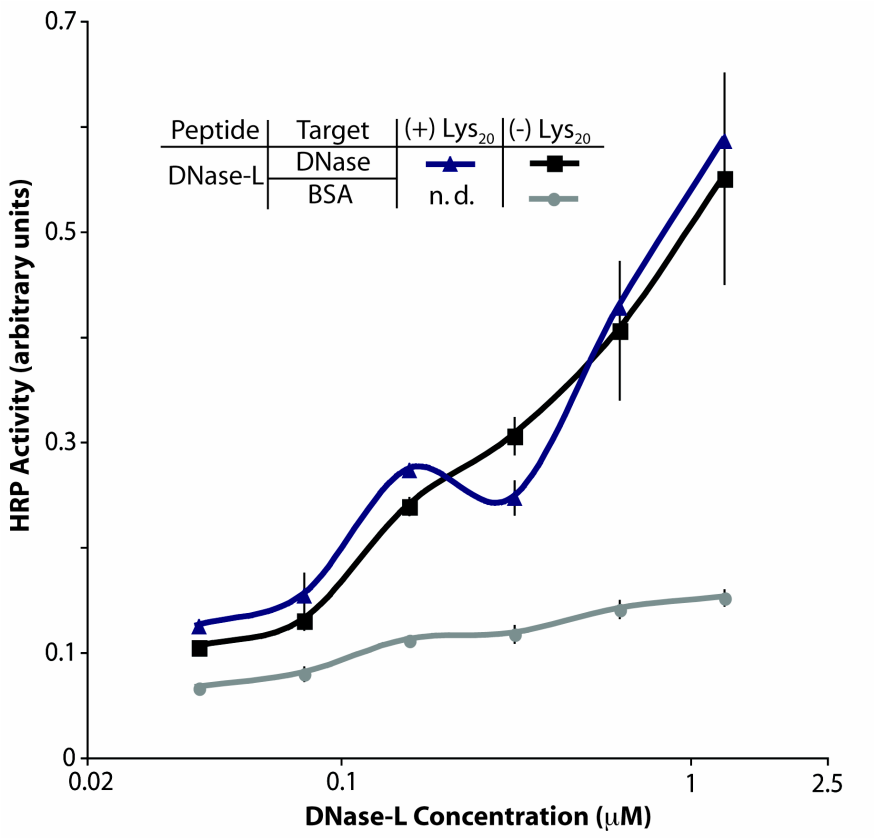

Figure S3. ELISA demonstrating high affinity binding by chemically synthesized DNase-L to DNase. DNase-L was synthesized with a C-terminal biotin to allow detection with HRP-conjugated streptavidin. The addition of Lys_{20} has no effect on the DNase-L binding to DNase. High DNase-L concentrations (>1.25 μM) produced variable results, perhaps due to the low solubility of the peptide (data not shown).

Synthesis of DNase-L peptide. DNase-L was synthesized at 0.1 mmol scale by using conventional solid phase peptide synthesis protocols on a Biotin-PEG-Nova-Tag™ resin (Novabiochem), which yields a C-terminally biotinylated peptide. After the synthesis, the DNase-L N terminus was acetylated with a capping solution (0.8 mL acetic anhydride, 1.6 mL diisopropylethylamine, 5.6 mL DMF) for 30 min, and the peptide was cleaved from the resin with a cleaving solution (9.5 mL trifluoroacetic acid, 0.25 mL trisisopropyl silane, 0.25 mL H_2O, 10 mg dithiothreitol) for 4 h. DNase-L was then purified by standard HPLC protocols.

Chemically synthesized DNase-L ELISA. Maxisorp plates were coated with DNase and blocked with BSA as described in the Experimental Section. Prior to the experiment, the DNase-L peptide was incubated in wash buffer supplemented with 10% DMSO to ensure complete oxidation of cysteines.[45] A test with Ellman's reagent revealed no free thiols after overnight oxidation. For wells with Lys_{20} added, the DNase-L peptide was incubated in the presence of Lys_{20} (1 μM) for 1 h. The DNase-L peptides were transferred in wash buffer to the DNase-coated plate and incubated for 1 h, followed by removal of the peptide solution and rinsing twice with wash buffer. HRP-conjugated streptavidin (0.25 μg/mL in PBT) was added for 15 min before washing four times with wash buffer and once with PBS. HRP activity in the presence of OPD solution was measured as described in the Experimental Section.

Synthesis and purification of oligolysine peptides. Oligolysine peptides with C-terminal carboxamides were synthesized using standard solid-phase peptide synthesis protocols on Rink-Amide resin from Novabiochem. Fmoc-Lys(Boc)-OH was also obtained from Novabiochem. The coupling agent *O*-(Benzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HBTU) was obtained from GL Biochem (Shanghai) Ltd, *N,N*-diisopropylethylamine from Acros organics, and 4-methylpiperidine from Fluka for Fmoc deprotection.[46]

All peptides were purified by HPLC using an XTerra MS C_{18} column (10 x 50 mm with 2.5 μm pore size). The crude peptide was dissolved in Nanopure doubly distilled H_2O to a concentration of 6 to 10 mg/mL, and purified by HPLC, using a constant flow rate of 5 mL/min, with 0.4 – 0.6 mL injection volumes. After injection, the Lys_4, Lys_8, Lys_{12} and Lys_{16} peptides were subjected to isocratic HPLC for 5 min period in Buffer A (95% H_2O, 5% acetonitrile, 0.1% TFA). Next, the peptides were subjected to a gradient of 0 to 15% Buffer C (95% acetonitrile, 5% H_2O, 0.1% TFA) over 15 min. Purified Lys_4 was obtained from the initial 100% Buffer A fraction with a retention time of 0.2 – 0.4 min. Purified Lys_8 was obtained from the initial 100% Buffer A fraction with a retention time of 0.68 – 0.9 min. Purified Lys_{12} was obtained from the initial 100% Buffer A fraction with a retention time of 0.63 – 1.0 min. Purified Lys_{16} was obtained from the initial 100% Buffer A fraction with a retention time of 0.8 – 1.2 min.

HPLC purification of Lys_{20} began with isocratic HPLC for 5 min in Buffer B (99% H_2O, 1% acetonitrile, 0.1% TFA), followed by a gradient of 0% to 4% Buffer C over 8 min. Fractions containing pure Lys_{20} were collected at 1.9 to 2.8% Buffer C. The HPLC purification of Lys_{24} began with isocratic HPLC for 5 min in Buffer B, followed by a gradient of 0% to 6% Buffer C over 6 min. Fractions containing Lys_{24} were collected at 4.3 to 5.4% Buffer B.

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