

# Chemical and Genetic Wrappers for Improved Phage and RNA Display

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An Achilles heel inherent to all molecular display formats, background binding between target and display system introduces false positives into screens and selections. For example, the negatively charged surfaces of phage, mRNA, and ribosome display systems bind with unacceptably high nonspecificity to positively charged target molecules, which represent an estimated 35% of proteins in the human proteome. Here we report the first systematic attempt to understand why a broad class of molecular display selections fail, and then solve the underlying problem for both phage and RNA display. Firstly, a genetic strategy was used to introduce a short, charge-neutralizing peptide into the solvent-

exposed, negatively charged phage coat. The modified phage (K07<sup>+</sup>) reduced or eliminated nonspecific binding to the problematic high-pI proteins. In the second, chemical approach, nonspecific interactions were blocked by oligolysine wrappers in the cases of phage and total RNA. For phage display applications, the peptides Lys<sub>n</sub> (where n=16 to 24) emerged as optimal for wrapping the phage. Lys<sub>8</sub>, however, provided effective wrappers for RNA binding in assays against the RNA binding protein HIV-1 Vif. The oligolysine peptides blocked nonspecific binding to allow successful selections, screens, and assays with five previously unworkable protein targets.

## Introduction

Molecular display leverages large numbers of different molecules (often > 10<sup>9</sup>) for the discovery, affinity maturation, and dissection of protein binding interactions.<sup>[1–6]</sup> Display technologies must physically link the displayed molecule to an encoding sequence. Ribosome and mRNA display fuse the displayed peptide to RNA,<sup>[7]</sup> whereas phage display attaches the displayed protein to the surface of a virus.<sup>[8]</sup> In both cases, the negatively charged presentation scaffolds—RNA or phage coat<sup>[9]</sup>—can bind directly to the target, bypassing the displayed protein. Such “background binding” is a weakness inherent to all molecular display systems.

Background binding (that is, wild-type phage coat proteins interacting with the target) derails selections, as all library members can then become candidates for amplification. Under conditions causing high background binding, phage lacking a displayed protein will be amplified preferentially, due to their less demanding requirements for growth. The resultant false positives confound both screens and selections.

To avoid this pitfall, we routinely begin phage display projects by testing for binding between the target and control M13K07 phage, which displays no proteins on its surface. For example, as reported previously, M13K07 nonspecifically binds with low affinity to the caveolin-1 scaffolding domain (CSD),<sup>[10]</sup> which complicates phage-displayed shotgun scanning and the discovery of ligands targeting CSD. The 20-residue CSD binds and regulates the activity of several key signaling proteins, including protein kinase A (PKA), endothelial and neuronal nitric oxide synthase (eNOS and nNOS, respectively), and adenylyl cyclase.<sup>[11]</sup>

In many molecular display experiments, this spurious binding occurs with target proteins possessing high isoelectric

points (pIs), typically > 9. A survey of successful phage, ribosome, and mRNA display experiments reveals a previously unreported cutoff around this pI, together with an optimal pI range from about four to nine (see Table S1 in the Supporting Information). In our experience, conventional additives for reducing nonspecific interactions, such as blocking agents and detergents, usually prove insufficient to overcome such background binding because of the extremely strong interactions between the negatively charged surfaces of phage and RNA and the positively charged, high-pI target proteins. Attempts to increase wash stringency and to vary salt concentrations or pH are typically futile: hence the few examples of successful selection against proteins with pI > 9. In addition, such protocols require the target to remain stable under harsh conditions.

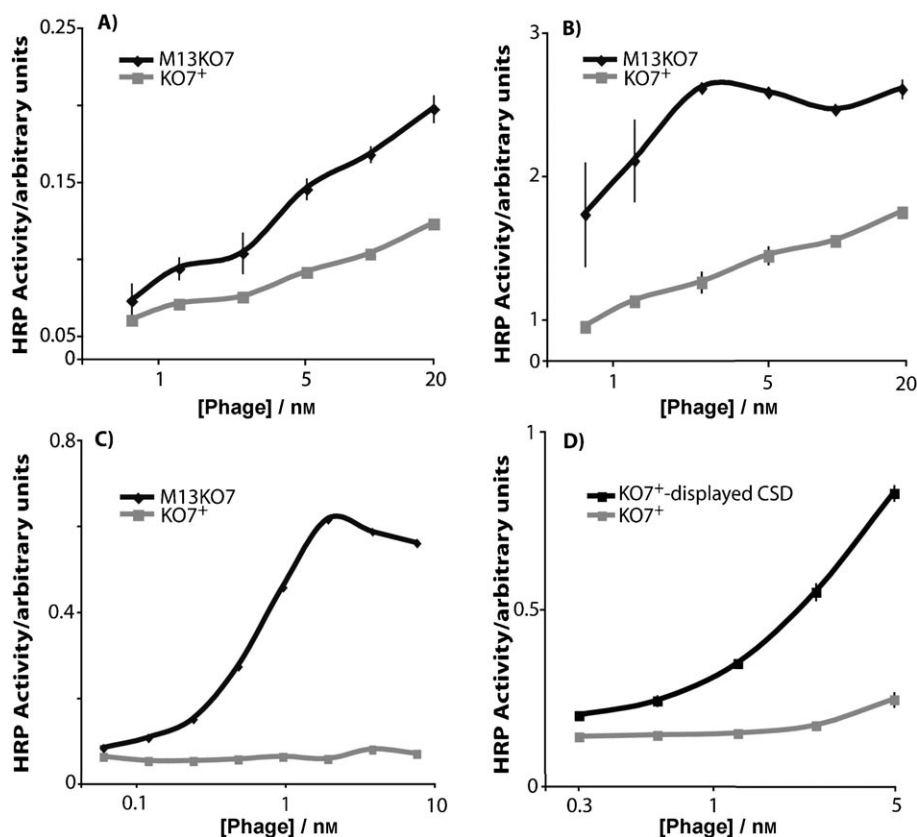
This paper presents new methods to address the unresolved and largely underappreciated problem of background interactions inherent to all molecular display systems. First, we used a

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Supporting information for this article is available on the WWW under <http://www.chembiochem.org> or from the author.



**Figure 1.** Phage-based ELISAs demonstrating spurious binding by M13KO7 phage and avoidance of background binding by the genetically modified KO7<sup>+</sup> phage to A) Vif, pI 9.5, B) DNase, pI 9.5, and C) Gag p55, pI 9.2. Such high background makes these proteins nonstarters for phage display experiments. The problem persists, but can be solved by KO7<sup>+</sup> helper phage for phage-displayed ligands, as in D) CSD, pI 9.5. As shown, CSD displayed on the surface of KO7<sup>+</sup> dimerizes with surface-immobilized synthetic CSD, and KO7<sup>+</sup> lacking displayed CSD completely eliminates the nonspecific binding observed previously with M13KO7.<sup>[10]</sup> Error bars indicate standard error in all experiments.

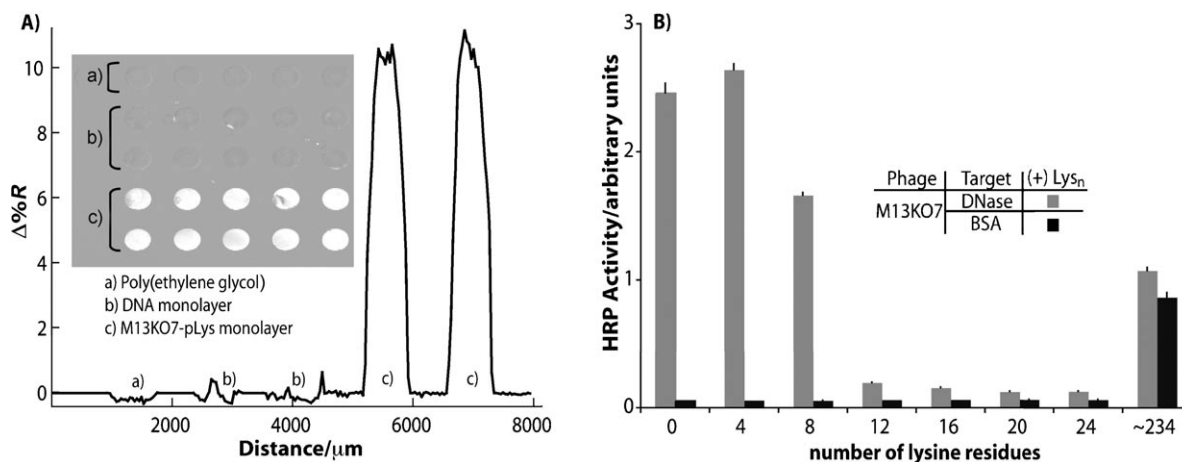
modified M13 phage with a positively charged peptide inserted between Gly3 and Asp4 of P8, the major coat protein. This phage scaffold, termed KO7<sup>+</sup>, reduced or eliminated nonspe-

cific binding to a number of high-pI proteins and worked well for screens and ELISA-based measurements (Figure 1). Secondly, chemical wrapping of the M13KO7 phage by short oligolysine peptides (Figure 2B) eliminated the spurious binding of M13KO7 phage to other challenging high-pI proteins and allowed rapid, conventional selection of high-affinity ligands to colicin E9 DNase (Figure 4, below). In addition, oligolysine wrapping was also capable of eliminating background binding between total RNA and the RNA-binding protein HIV-1 Vif (Figure 5, below). The approach provides a general solution to a challenging problem associated with at least three formats of molecular display: mRNA, ribosome, and phage display.

## Results and Discussion

### Use of KO7<sup>+</sup> phage with positively charged target proteins

Filamentous phage such as M13KO7 present three solvent-exposed acidic residues within



**Figure 2.** A) SPR imaging measurement of M13KO7 adsorption to a pLys monolayer. The graph shows experiments without (a and b) and with (c) pLys on a surface. B) Oligolysine wrapping of phage shown by ELISA. Oligolysines of different length (Lys<sub>n</sub>) were synthesized to determine optimal lengths for wrapping to suppress nonspecific phage binding to DNase. Note the unacceptably high background resulting from use of commercial polylysine (~234 lysines).

dues. To address this problem, we synthesized a modified M13KO7 phage, termed KO7<sup>+</sup>, with an added lysine residue incorporated into every copy of P8. This design follows the example of Scott and co-workers, who demonstrated that the related f1 phage could tolerate the presence of an Ala-Lys-Ala-Ser (AKAS) peptide inserted between P8 residues Gly3 and Asp4.<sup>[12]</sup> Transplantation of this peptide insert from f1.K into a helper phage system allows good yields of phage-displayed proteins produced through trans infection by KO7<sup>+</sup>. Complete display of the P8 AKAS insert upon the KO7<sup>+</sup> surface was confirmed by LC-MS and DNA sequencing (Figure S1).

In phage-based ELISAs, KO7<sup>+</sup> reduced or eliminated nonspecific interactions between phage and HIV-1 Vif (pI 9.5, Figure 1A), colicin E9 DNase (pI 9.5, Figure 1B), HIV-1 Gag p55 (pI 9.2, Figure 1C), and CSD (pI 9.5, Figure 1D), providing a basis for screens and assays. The last example includes phage display of CSD for assay of CSD dimers, which were previously observed by NMR.<sup>[13]</sup> Against the four targets, the exceptionally strong binding to conventional M13KO7 helper phage precludes such binding assays. Despite these results, selections against multiple targets with use of KO7<sup>+</sup> as a helper phage resulted in selectants harboring an empty phagemid. In addition, the stability of KO7<sup>+</sup> phage was significantly reduced in relation to that of wild-type M13KO7. Refrigeration of KO7<sup>+</sup> for short periods (one to two weeks) resulted in decomposition of the phage particles, with storage at -78 °C in 10% glycerol only slowing decay. Yields and infectivity of KO7<sup>+</sup> phage were diminished as well, as demonstrated by two- to threefold reductions in plaque and colony-forming units, respectively.

### Lysine wrappers for phage

To address the background binding problem chemically, we designed wrappers to coat the phage and to block unwanted phage interactions. For example, the negatively charged phage coat adheres avidly to high-pI poly-L-lysine (pLys), as demonstrated by SPR imaging (Figure 2A). SPR imaging can detect adsorption onto microarrays formed on chemically modified gold thin films through changes in the local refractive index. The SPR image in Figure 2A shows the specific adsorption of M13KO7 phage onto the ten array elements that have been modified with a monolayer of pLys electrostatically adsorbed onto a DNA monolayer. No phage adsorption was observed either onto the ten array elements possessing a DNA monolayer without pLys, or onto the five array elements modified with a poly(ethylene glycol) monolayer instead of DNA. However, in ELISA experiments, pLys blocked nonspecific interactions only poorly, and appeared actually to cross-link phage with target proteins and blocking agents nonspecifically (for example, the enhanced phage binding to BSA, commonly used as a blocking agent; Figure 2B). More consistent wrapping and effective elimination of background binding required specific lengths of oligolysine. The optimal peptide length was determined by wrapping M13KO7 phage with Lys<sub>n</sub> peptide variants, followed by assay for binding to DNase (Figure 2B). Oligolysines containing between 12 and 24 residues were able to wrap the phage completely and prevent nonspecific binding. As de-

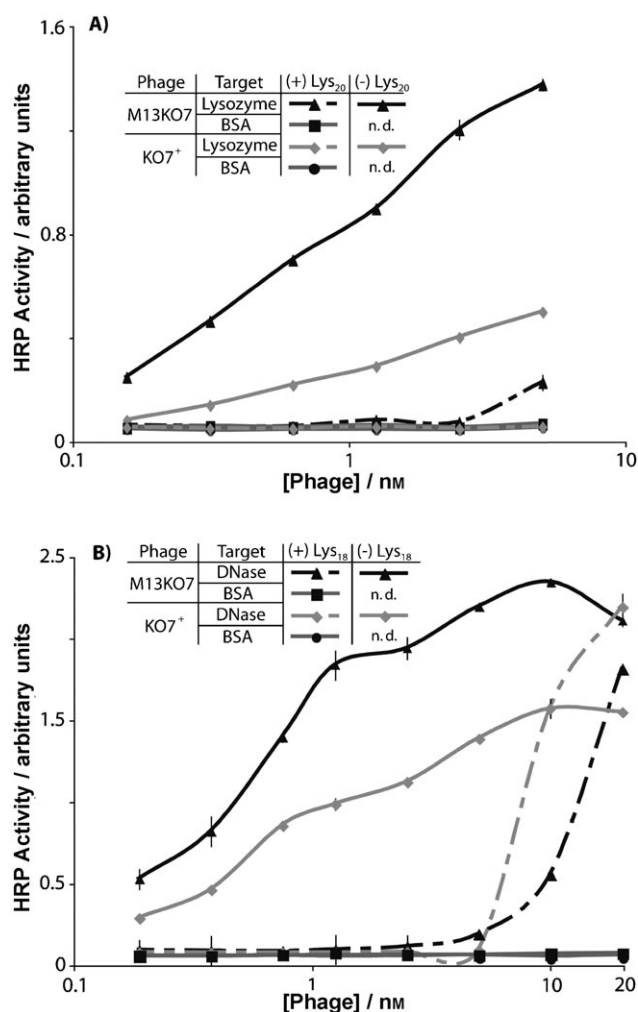
scribed above, pLys, a heterogenous mixture of different lengths and attachments (isopeptidic and peptidic), failed to block nonspecific binding to DNase completely. Taken together, the results demonstrate that the oligolysine wrapping requires an avidity-based, critical mass of positive charge for complete phage wrapping. Though the concentration of lysine residues remained fixed for each Lys<sub>n</sub> variant (25 μg mL<sup>-1</sup>), only peptides with twelve or more Lys residues were able to bind to the phage to block background binding. Furthermore, similar results were observed for wrapping of KO7<sup>+</sup> and M13KO7 phage to DNase and lysozyme (pI 9.5), respectively (Figure S2). The generality of the approach demonstrates the specificity of oligolysines for wrapping the phage, not interacting with the target.

Wrapping M13KO7 phage with Lys<sub>18</sub> diminished phage infectivity by ~40% at high Lys<sub>18</sub> concentrations (100 μM; Table S2), which is much less than the two- to threefold reductions in infectivity associated with KO7<sup>+</sup>. We speculate that, in infecting *E. coli*, the negatively charged surface of M13KO7 provides long-range attraction to the host F-pilus, which is composed of the high-pI F-pilin (pI 9.3). Then, interaction of the phage minor coat protein P3 and the F-pilin tip leads to phage entry into the cell.<sup>[14-16]</sup> Wrapping M13KO7 phage with oligolysine or adding a lysine residue to the KO7<sup>+</sup> coat alters the negatively charged surface of the phage, thus diminishing its infectivity. Other viruses employ charged coat proteins to infect their hosts: the cucumber mosaic virus, for example, requires an aspartate-rich domain in one of its viral capsid proteins to infect its host,<sup>[17]</sup> whereas aspartate residues in the DAG motif of the coat protein of tobacco etch virus are necessary for viral infectivity.<sup>[18]</sup>

In ELISA experiments using lysozyme and DNase as targets, wrapping with Lys<sub>20</sub> and with Lys<sub>18</sub> peptides, respectively, abolished nonspecific binding to these high-pI proteins (Figure 3A and B). As expected, KO7<sup>+</sup> phage showed less nonspecific binding than wild-type M13KO7 in the absence of lysine peptide. However, when wrapped by Lys<sub>18</sub> or Lys<sub>20</sub>, background binding of both M13KO7 and KO7<sup>+</sup> phage to lysozyme and DNase was reduced about tenfold, for phage concentrations of 5 nM or less. A 200-fold molar excess of oligolysine is required to wrap the phage completely, as spurious binding returns at higher phage concentrations (> 5 nM).

Lys<sub>18</sub> wrapping of a phage-displayed peptide library was used to select for ligands to DNase, which has therapeutic potential in ADEPT systems.<sup>[19]</sup> A naive library of 10<sup>9</sup> different peptides displayed on the surface of conventional M13KO7 was subjected to four rounds of selection in the presence of a constant concentration of Lys<sub>18</sub>. Low concentrations of phage (5 nM) and excess Lys<sub>18</sub> (1 μM) ensured complete wrapping. These otherwise conventional phage selections yielded five high-affinity DNase ligands, with four out of the five containing the sequence X<sub>2</sub>CX<sub>8</sub>CX<sub>2</sub> (Table 1). Several sequences featured one or more glutamic acid residues at the N terminus; this suggests that the conditions also selected for interaction with the added Lys<sub>18</sub> peptide.

The ligand DNase-L demonstrated the best display levels and highest affinity for DNase both in the presence and in the

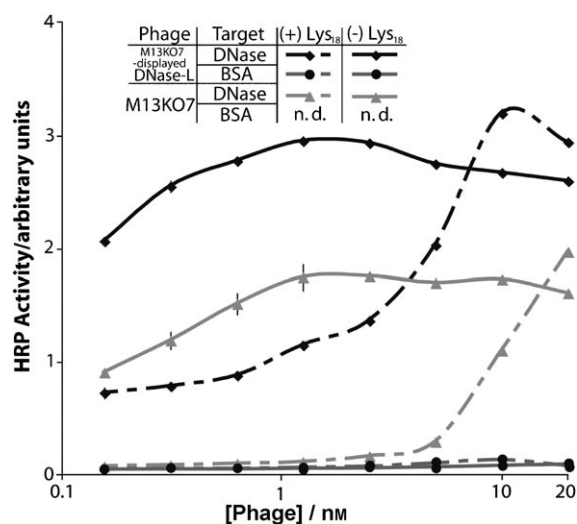


**Figure 3.** Wrapping eliminates M13KO7 and KO7<sup>+</sup> phage background binding to A) lysozyme (wrapping with Lys<sub>20</sub>), and B) DNase (Lys<sub>18</sub>). In Figures 3 and 4, n.d. indicates "not determined in this experiment." However, in experiments run under identical or similar conditions (not shown), binding between BSA and the phage partner was undetectable.

Peptide name	Sequence	Abundance after four rounds [%] <sup>[a]</sup>
DNase-A	AQCVSFESAFYCWV	73
DNase-G	EVCVTLDFGVWCLQ	2.4
DNase-J	EFCVTMDQWMVCVP	2.4
DNase-L	EEWYCLRQGTFTLYCFW	7.3
DNase-M	FPCGECVIARLCPA	2.4

[a] Unreadable sequences and sequences containing stop codons comprised about 10% of the selectants.

absence of Lys<sub>18</sub> (Figure 4). Without the addition of Lys<sub>18</sub>, binding of phage-displayed DNase-L to DNase is stronger than that seen with M13KO7, because the displayed peptide adds binding energy to the phage–DNase interaction. As expected for an interaction mediated by the DNase-L peptide, phage-dis-



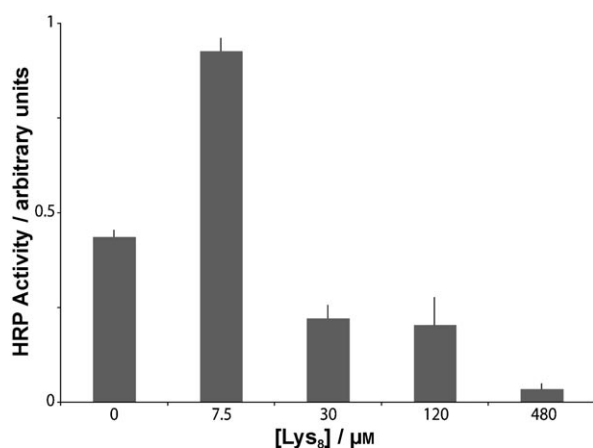
**Figure 4.** Binding to DNase by the M13KO7-displayed DNase-L peptide in the presence or absence of Lys<sub>18</sub> demonstrates successful selection against a previously challenging and marginal target.

played DNase-L retains significant affinity, despite the oligo-lysine wrapper. Experiments with chemically synthesized, non-phage-displayed DNase-L further demonstrated the strong binding of DNase-L to the target DNase (Figure S3).

Thus, the addition of Lys<sub>18</sub> drops M13KO7 background binding to a minimum at phage concentrations of <5 nM. As described above for KO7<sup>+</sup> phage display of CSD, the wrapper does not interfere with the successful display and binding of a ligand on the phage surface. Although successful selections for DNase ligands from M13KO7-displayed libraries have been reported, the exceedingly stringent washing conditions (40 washes)<sup>[20]</sup> could potentially denature the target protein or the displayed proteins and typically lead to low efficiency selections. Wrapping of phage with oligo-lysine enables conventional, rapid selections and assays with high-pI protein targets under mild conditions.

### Lysine wrappers for RNA

To demonstrate the generality of this approach to other molecular display systems, we next applied the oligo-lysine wrapping procedure to RNA. Total RNA extracts from mouse livers provided a mixture of mRNA, rRNA, and tRNA, which were labeled with biotinylated psoralen. As a particularly stringent test for the wrapper, the RNA-binding protein Vif was used as a target. To wrap RNA, shorter oligo-lysines (Lys<sub>8</sub>) than the phage wrappers blocked nonspecific binding more effectively (data not shown). Analogously with the phage wrapping experiments, excess Lys<sub>8</sub> was required to block RNA binding to Vif (480 μM Lys<sub>8</sub>), as demonstrated by ELISA (Figure 5). RNA showed exceptionally strong binding at low Lys<sub>8</sub> concentrations (for example, 7.5 μM Lys<sub>8</sub>), likely due to simultaneous interactions between Lys<sub>8</sub> and both RNA and Vif. Low levels of Lys<sub>8</sub>-dependent binding to Vif and streptavidin-HRP were subtracted as background.



**Figure 5.** The strategy of wrapping with Lys<sub>8</sub> also blocked nonspecific binding by total RNA to the RNA-binding protein Vif.

Since mRNA is not readily amenable to backbone modifications to remove the negative charge (that is, the strategy for ameliorating background binding demonstrated with KO7<sup>+</sup>), the addition of oligolysine to RNA could provide a viable approach by which to control background binding for mRNA and ribosome display systems. However, our results do not demonstrate suppressed background binding in actual mRNA or ribosome display experiments, as total RNA extracts were used. The efficacy of oligolysine wrapping in such RNA-based display systems remains to be determined.

## Conclusions

In our experience, spurious background binding confounds screens and selections. We report a general solution to this problem, and demonstrate its effectiveness in both phage and RNA systems. Application of molecular display to positively charged proteins is especially important for structural proteomics and *in vivo* selections,<sup>[21,22]</sup> as proteins with  $pI > 9$  represent approximately 35% of the human proteome (estimated from ref. [23]). In addition to the identification and optimization of ligands, molecular display systems are finding an increasing number of uses directly in biosensor applications.<sup>[24–31]</sup> The chemical and genetic approaches described here provide advantages for such applications, both through evasion of background binding and through device construction by simple adherence of the display system to oligolysine-coated surfaces. Furthermore, the straightforward solution described here could find broad applicability for many challenging selections otherwise stymied by false positives from background binding.

## Experimental Section

### Oligonucleotides used for M13KO7 helper phage genome mutagenesis

KO7-P8-AKAS-fwd: 5'-CTTATTCGCTAGCGACGATCCCGCAAAGCG-3'.  
 KO7-P8-AKAS-rev: 5'-TAAAGAGTCTAGCTTTTGACCCTCAGCAGCG-AAAGAC-3'.

KO7-F1: 5'-TGTA AACGACGGCCAGTGCCTTCGTAGTGGCATT A-3'.

KO7-R1: 5'-CAGGAAACAGCTATGACACAGTTTCAGCGGAGTGA-3'.

M13-F1: 5'-TGTA AACGACGGCCAGT-3'.

**M13KO7 helper phage genome mutagenesis:** Synthetic oligonucleotide primers for mutagenic PCR were designed to introduce the AKAS insert of the Scott lab's f1.K into the M13KO7 major coat protein (P8). A PCR (50 μL reaction) with Herculase™ DNA polymerase (Stratagene) with use of single-stranded M13KO7 DNA as template and the KO7-P8-AKAS-fwd/rev primer pair was prepared and thermocycled according to the manufacturer's protocol. PCR products were affinity purified with the DNA Clean & Concentrator kit (Zymo Research). Purified PCR product was subjected to NheI endonuclease digestion (at the restriction site underlined above), purified by agarose gel electrophoresis, and isolated by use of a Zymoclean Gel DNA Recovery kit (Zymo Research). Digested DNA was ligated with T4 DNA ligase according to the manufacturer's guidelines. Ligated DNA was transformed into chemically competent *E. coli* XL10-Gold<sup>[32]</sup> and plated on Luria–Bertani broth (LB)/kanamycin (10 μg mL<sup>-1</sup>) agar plates. Individual transformants were used to inoculate cultures (2 mL LB containing 10 μg mL<sup>-1</sup> kanamycin), which were shaken overnight at 37 °C. Double-stranded cccDNA was recovered from cultures by alkaline lysis (miniprep, Zippy Plasmid Miniprep kit) and tested for inclusion of the insert by digestion with NheI prior to DNA sequencing, by use of an initial PCR reaction with the KO7-F1/R1 primer pair, followed with a sequencing reaction with use of the M13-F1 sequencing primer. The resultant KO7<sup>+</sup> dsDNA was transformed into chemically competent *E. coli* XL1-Blue.<sup>[32]</sup> Individual transformants were used to inoculate cultures (1 mL 2YT containing 25 μg mL<sup>-1</sup> kanamycin), which were shaken for 9 h at 37 °C. Starter cultures (250 μL) were transferred to 2YT media (5 mL) as above. After 15.5 h incubation at 37 °C, cells were removed by centrifugation for 10 min at 10000g, and the supernatant was transferred to a second centrifuge tube containing PEG/NaCl (20% poly(ethylene glycol) 8000, 2.5 M NaCl, 1 mL) and incubated at 4 °C. Phage were harvested from the supernatant by centrifugation (10 min at 10000g) and re-suspended in PBS (pH 7.2, 200 μL). Mutated phage harboring the AKAS insert were verified by DNA sequencing as described above and by mass spectrometry (Figure S1). Plaque preparations were used to prepare phage stocks and to test phage packaging viability.

**SPR imaging measurements:** A SPR imager (GWC Technologies) was used for investigating adsorption of phage onto poly-L-lysine-modified (pLys-modified) gold thin films. The gold thin films (45 nm) were prepared by vapor deposition of gold onto SF-10 glass slides (18 mm × 18 mm) by use of a Denton DV-502A metal evaporator with a 1 nm underlayer of chromium. Patterns of poly-L-lysine-modified surface were created by the photopatterning of adsorbed 11-mercaptoundecanoic acid (MUA; Sigma–Aldrich) and pLys ( $M_w$  34300; Sigma–Aldrich) as described elsewhere.<sup>[33,34]</sup> The patterned surface was exposed to a solution of phage in water that was allowed to adsorb electrostatically onto the surfaces. The difference images were obtained by subtracting images acquired before and after adsorption of the phage, and then a line profile was taken across the image to create the plot shown in Figure 2A.

**Synthesis of oligolysine and DNase-L peptides:** Oligolysine peptides were synthesized with carboxamide C termini and unblocked amine functionalities on the N termini. DNase-L was synthesized with a C-terminal PEG-biotin tag and an acetylated N terminus. Conventional peptide synthesis and purification protocols are provided in the Supporting Information. The identities and purities of oligolysine and DNase-L peptides were determined by MALDI-MS.

Lys<sub>4</sub>: expected  $m/z$  529.72; observed  $m/z$  553.01  $[M+Na]^+$ . Lys<sub>8</sub>: expected  $m/z$  1042.43; observed  $m/z$  1043.41  $[M+H]^+$  and 1065.04  $[M+Na]^+$ . Lys<sub>12</sub>: expected  $m/z$  1555.12; observed  $m/z$  1555.79  $[M+H]^+$ , 1578.31  $[M+Na]^+$ , and 1593.56  $[M+K]^+$ . Lys<sub>16</sub>: expected  $m/z$  2067.82; observed  $m/z$  2090.90  $[M+Na]^+$ . Lys<sub>20</sub>: expected  $m/z$  2580.26; observed  $m/z$  2580.76  $[M+H]^+$  and 2603.47  $[M+Na]^+$ . Lys<sub>24</sub>: expected  $m/z$  3092.90; observed  $m/z$  3115.61  $[M+Na]^+$ . DNase-L: expected  $m/z$  2844.67; observed  $m/z$  2845.00  $[M+H]^+$

**Protein expression and purification:** HIV-1 Vif, colicin E9 DNase (a S49C variant), and lysozyme (variant with an added GKC peptide before residue 1) were produced by conventional bacterial protein expression and purification. Recombinant HIV-1 Gag p55 from yeast expression was obtained through the NIH AIDS Research & Reference Reagent Program.

**Phage ELISAs:** With the exception of the washes, all binding steps were carried out in an orbital shaker (150 rpm) and at room temperature unless otherwise noted. Target proteins were diluted (to  $5 \mu\text{g mL}^{-1}$ ) in sodium carbonate buffer (50 mM Na<sub>2</sub>CO<sub>3</sub>, pH 9.6) prior to coating on a Nunc Maxisorp plate (100  $\mu\text{L}$  per well) for 2 h (or overnight at 4 °C). After removal of the coating solution, the wells were blocked with blocking buffer (0.2% BSA in PBS, 400  $\mu\text{L}$ ) for 30 min and later rinsed three times with wash buffer [0.05% Tween 20 (*v/v*, all others *w/v*) in PBS, 200  $\mu\text{L}$ ]. For oligolysine-wrapping experiments, phage samples were incubated with the oligolysine peptides ( $2.5\text{--}25 \mu\text{g mL}^{-1}$ ) or commercial polylysine (Sigma,  $M_w > 30000$ ,  $2.5\text{--}25 \mu\text{g mL}^{-1}$ ) in PBT buffer (0.2% BSA in wash buffer) for 1 h. Control phage lacking the oligolysine or polylysine wrapping were incubated in PBT buffer instead. Phage samples were then transferred to the target protein-coated wells (100  $\mu\text{L}$  per well) and incubated for 1 h, followed by removal of the phage solutions and rinsing with wash buffer (five times, 200  $\mu\text{L}$  per well). A 1:4000 dilution of HRP-conjugated anti-M13 phage antibody (Amersham Biosciences) in PBT buffer (100  $\mu\text{L}$ ) was added to each well, for 30 min. After removal of the antibody solution and a washing step (rinsing three times with wash buffer and once with PBS), the wells were treated with *o*-phenylenediamine (OPD) dihydrochloride/H<sub>2</sub>O<sub>2</sub> solution (1 mg mL<sup>-1</sup> OPD and 0.02% H<sub>2</sub>O<sub>2</sub>, 100  $\mu\text{L}$ ) in citric acid buffer (50 mM citric acid, 50 mM Na<sub>2</sub>HPO<sub>4</sub>, pH 5.0). HRP activity was measured at 450 nm in a 96-well microtiter plate reader (Bio-Tek Instruments, Inc.).

**Biopanning for phage-displayed DNase ligands:** A phage-displayed library was constructed by use of an optimized<sup>[35]</sup> oligonucleotide-directed mutagenesis protocol.<sup>[36]</sup> The oligonucleotide GCT ACA AAT GCC TAT GCA NNS NNS NNS TGC NNS NNS NNS NNS NNS NNS NNS TGC NNS NNS GGT GGA GGA TCC GGC GGA (where N = A/G/C/T, 25% each; S = G/C, 50% each) encoded all 20 naturally occurring amino acids and was used to prime the synthesis of a DNA strand complementary to the phagemid template (pM1165a).

In selection experiments, *E. coli* DNase was coated onto 48 wells of a Maxisorp plate, followed by a blocking step with BSA as described above. A phage-displayed library of 10<sup>9</sup> peptide variants fused to wild-type P8 was diluted (to 5 nM) in PBT and incubated in the presence of Lys<sub>18</sub> ( $2.5 \mu\text{g mL}^{-1}$ ) for 1 h. The phage-Lys<sub>18</sub> mixture was transferred to the DNase-coated wells (100  $\mu\text{L}$  per well) and incubated for 1 h. The wells were rinsed four times (200  $\mu\text{L}$  of wash buffer), and the selected phage was eluted with HCl (0.1 M, 100  $\mu\text{L}$  per well) and vigorous shaking (250 rpm), followed by neutralization with Tris (1 M, pH 8.0, 33  $\mu\text{L}$  per well). In each subsequent round, the number of washes after the binding step was increased by two, and the blocking solution was alternated between BSA and ovalbumin (0.2% in PBS). The eluted phage were added

to log-phase XL1-Blue *E. coli* (5 mL), and incubated at 37 °C with shaking for 30 min, followed by M13KO7 helper phage infection (10<sup>10</sup> phage per mL) for 20 min. The infected culture was transferred to 2YT media (100 mL) supplemented with carbenicillin (50  $\mu\text{g mL}^{-1}$ ) and kanamycin (20  $\mu\text{g mL}^{-1}$ ) for phagemid selection, and grown overnight at 37 °C with shaking. Phage libraries were harvested as described above. Four rounds of selection were carried out before assays with individual clones as described above.

**RNA ELISA:** All RNA buffers were supplemented with Human Placental RNase Inhibitor (EMD). Total RNA samples (20  $\mu\text{g mL}^{-1}$  in TAE) were mixed with psoralen/PEO<sub>3</sub>/biotin (0.6 mM, Pierce) and cross-linked by UV irradiation (365 nm) for 30 min. Excess biotin was removed by precipitation through addition of potassium acetate (0.2 M) in ethanol (two volumes) and centrifugation at 14 krpm for 20 min. The biotinylated RNA pellet was washed with ethanol (70%) and resuspended in PBS. To demonstrate biotinylation of the RNA, an aliquot of the resuspended pellet was used to coat control wells of the ELISA described below. The RNA sample was then diluted (to 10  $\mu\text{g mL}^{-1}$ ) for incubation with Lys<sub>8</sub> in wash buffer for 1 h.

Maxisorp plates were coated with HIV-1 Vif (20  $\mu\text{g mL}^{-1}$ ), blocked, and washed as described above. The RNA-Lys<sub>8</sub> mixture was added to the Vif-coated wells (100  $\mu\text{L}$  per well), and incubation was carried out for 2 h before rinsing once with wash buffer. HRP-conjugated streptavidin (0.25  $\mu\text{g mL}^{-1}$  in PBT) was added for 15 min before washing twice with wash buffer and once with PBS. HRP activity in the presence of OPD solution was measured as described above.

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