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Chemical and Genetic Wrappers for Improved Phage and RNA Display

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Abstract

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 non-specificity to positively charged target molecules, which represent an estimated 35% of proteins in

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the human proteome. 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. First, a genetic strategy introduced a short charge neutralizing peptide into the solvent-exposed, negatively charged phage coat. The modified phage ($KO7^+$) reduced or eliminated non-specific binding to the problematic high pI proteins. In the second, chemical approach, oligolysine wrappers for phage and total RNA blocked non-specific interactions. For phage display applications, the peptides Lys_n (where n = 16 to 24) emerged as optimal for wrapping the phage. Lys_8, however, provided effective wrappers for RNA binding in assays against the RNA binding protein HIV-1 Vif. The oligolysine peptides blocked non-specific binding to allow successful selections, screens, and assays with five previously unworkable protein targets.

Keywords

electrostatic interactions; high pI proteins; lysine; non-specific binding; phage display

Introduction

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

Background binding (i.e., 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 preferentially amplified 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 binding between the target and control M13KO7 phage, which displays no proteins on its surface. For example, as reported previously, M13KO7 non-specifically binds with low affinity to the caveolin-1 scaffolding domain (CSD)[10], 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 signalling proteins, including protein kinase A (PKA), endothelial and neuronal nitric oxide synthase (eNOS and nNOS, respectively), and adenylyl cyclase.[11] In many molecular display experiments, this spurious binding occurs with target proteins having high isoelectric points (pIs), typically > 9. A survey of successful phage, ribosome and mRNA display experiments reveals a previously unreported cutoff around this pI, and an optimal pI range from about 4 to 9 (Supporting Information, Table S1). In our experience, conventional additives for reducing nonspecific interactions, such as blocking agents and detergents, usually prove insufficient to overcome such background binding due to the extremely strong interactions between the negatively charged surfaces of phage and RNA, and positively charged, high pI target proteins. Attempts to increase wash stringency and 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 under-appreciated problem of background interactions inherent to all molecular display systems. First, we used a modified M13 phage with a positively charged peptide inserted between Gly3 and Asp4 of the major coat protein, P8. This phage scaffold, termed $KO7^+$, reduced or eliminated non-

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specific binding to a number of high pI proteins, and worked well for screens and ELISA-based assays (Figure 1). Second, 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). In addition, oligolysine wrapping could eliminate background binding between total RNA and the RNA-binding protein HIV-1 Vif (Figure 5). 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$^+$ Phage with Positively Charged Target Proteins

Filamentous phage like M13KO7 present three solvent-exposed acidic residues within the N-terminal five positions of the ~2700 copy, major coat protein, P8. This acidic patch lends a net negative charge to the phage surface, and can cause nonspecific interactions with proteins having an abundance of positively charged residues. To address this problem, we synthesized a modified M13KO7 phage, termed KO7$^+$, 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 an Ala-Lys-Ala-Ser (AKAS) peptide inserted between P8 residues Gly3 and Asp4.[12] Transplanting this peptide insert from f1 into a helper phage system allows good yields of phage-displayed proteins produced through trans infection by KO7$^+$. Complete display of the P8 AKAS insert upon the KO7$^+$ surface was confirmed by LCMS and DNA sequencing (Supporting Information, Figure S1).

In phage-based ELISAs, KO7$^+$ 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 latter example includes phage display of CSD for assay of CSD dimers, which were previously observed by NMR.[13] Against the four targets, the exceptionally strong binding to conventional M13KO7 helper phage precludes such binding assays. Despite these results, selections against multiple targets using KO7$^+$ as a helper phage resulted in selectants harboring an empty phagemid. In addition, the stability of KO7$^+$ phage was significantly reduced, compared to wild-type M13KO7. Refrigeration of KO7$^+$ 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$^+$ phage were diminished as well, as demonstrated by a two- to three-fold reduction 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 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 via 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 that have a DNA monolayer without pLys, or onto the five array elements modified with a polyethylene glycol monolayer instead of DNA. However, in ELISA experiments, pLys blocked non-specific interactions only poorly, and appeared to actually cross-link phage with target proteins and blocking agents non-specifically (e.g., 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

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phage with Lys_n peptide variants, followed by assay for binding to DNase (Figure 2B). Oligolysines between 12 and 24 residues could wrap the phage completely, and prevent non-specific binding. As described above, pLys, a heterogenous mixture of different lengths and attachments (isopeptidic and peptidic), failed to completely block non-specific binding to DNase. 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_n variant (25 μ/mL), only peptides with 12 or more Lys residues could bind to the phage to block background binding. Furthermore, similar results were observed for wrapping of $KO7^+$ and M13KO7 phage to both DNase and lysozyme (pI 9.5), respectively (Supporting Information, 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_{18} diminished phage infectivity by ~40% at high Lys_{18} concentrations (100 μM) (Supporting Information, Table S2), which is much less than the 2–3 fold reduction in infectivity associated with $KO7^+$. 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.[14–16] Wrapping M13KO7 phage with oligolysine or adding a lysine residue to the $KO7^+$ coat alters the negatively charged surface of the phage, thus diminishing its infectivity. Other viruses employ charged coat proteins to infect their hosts; for example, the cucumber mosaic virus requires an aspartate rich domain in one of its viral capsid proteins to infect its host[17], and aspartate residues in the DAG motif of the coat protein of tobacco etch virus are necessary for viral infectivity.[18]

In ELISA experiments using lysozyme and DNase as targets, wrapping with Lys_{20} and Lys_{18} peptides, respectively, abolished non-specific binding to these high pI proteins (Figures 3A and 3B). As expected, $KO7^+$ phage showed less non-specific binding than wild-type M13KO7 in the absence of lysine peptide. However, when wrapped by Lys_{18} or Lys_{20}, background binding of both M13KO7 and $KO7^+$ phage to lysozyme and DNase was reduced about 10-fold, for phage concentrations of 5 nM or less. A 200-fold molar excess of oligolysine is required to completely wrap the phage, as spurious binding returns at higher phage concentrations (>5 nM).

Lys_{18} wrapping of a phage-displayed peptide library was used to select for ligands to DNase, which has therapeutic potential in ADEPT systems.[19] A naïve library of 10^9 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_{18}. Low concentrations of phage (5 nM) and excess Lys_{18} (1 μM) ensured complete wrapping. These otherwise conventional phage selections yielded five high affinity DNase ligands, with 4 out of 5 having the sequence $X_2CX_8CX_2$ (Table 1). Several sequences had one or more glutamic acid residues at the N-terminus, which suggests the conditions also selected for interaction with the added Lys_{18} peptide.

The ligand DNase-L demonstrated the best display levels and highest affinity for DNase in both the presence and absence of Lys_{18} (Figure 4). Without the addition of Lys_{18}, binding of phage-displayed DNase-L to DNase is stronger than 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-displayed DNase-L retains significant affinity, despite the oligolysine wrapper. Experiments with chemically synthesized, non-phage-displayed DNase-L further demonstrated the strong binding of DNase-L to the target DNase (Supporting Information, Figure S3).

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Thus, the addition of Lys_{18} drops M13KO7 background binding to a minimum at phage concentrations <5 nM. As described above for $KO7^+$ phage display of CSD, the wrapper does not interfere with the successful display and binding of a ligand on the phage surface. Though successful selections for DNase ligands from M13KO7-displayed libraries have been reported, the exceedingly stringent washing conditions (40 washes)[20] could potentially denature the target protein or the displayed proteins and typically lead to low efficiency selections. Wrapping phage with oligolysine 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 oligolysine 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 oligolysines (Lys_8) than the phage wrappers blocked non-specific binding more effectively (data not shown). Analogous to the phage wrapping experiments, excess Lys_8 was required to block RNA binding to Vif (480 μM Lys_8), as demonstrated by ELISA (Figure 5). RNA showed exceptionally strong binding at low Lys_8 concentrations (e.g., 7.5 μM Lys_8), likely due to simultaneous interactions of Lys_8 to RNA and Vif. Low levels of Lys_8-dependent binding to Vif and streptavidin-HRP were subtracted as background.

Since mRNA is not readily amenable to backbone modifications to remove the negative charge (i.e., the strategy for ameliorating background binding demonstrated with $KO7^+$), the addition of oligolysine to RNA could provide a viable approach 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 exctracts were used. The efficacy of oligolysine wrapping in such RNA-based display systems remains to be determined.

Conclusion

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. Applying molecular display to positively charged proteins is especially important for structural proteomics and in vivo selections[21,22], as proteins with pI > 9 represent approximately 35% of the human proteome (estimated from reference [23]). In addition to the identification and optimization of ligands, molecular display systems find an increasing number of uses directly in biosensor applications.[24–31] The chemical and genetic approaches described here provide advantages to such applications, through both evasion of background binding and device construction by simple adherence of the display system to oligolysine-coated surfaces. Furthermore, the straight-forward solution described here could find broad applicability for many challenging selections stymied by false positives from background binding.

Experimental Section

Oligonucleotides Used for M13KO7 Helper Phage Genome Mutagenesis

KO7-P8-AKAS-fwd: 5’CTTATTC<u>GCTAGC</u>GACGATCCCGCAAAAGCG3’

KO7-P8-AKAS-rev: 5’TAAAGAGT<u>GCTAGC</u>TTTTGCACCCTCAGCAGCGAAAGAC3’

KO7-F1: 5’TGTAAAACGACGGCCAGTGCCTTCGTAGTGGCATTA3’

KO7-R1: 5’CAGGAAACAGCTATGACACAGTTTCAGCGGAGTGA3’

M13-F1: 5’TGTAAAACGACGGCCAGT 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) using 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 with 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[32] and plated on Luria Broth (LB)/kanamycin (10 μg/mL) agar plates. Individual transformants were used to inoculate cultures (2 mL LB containing 10 μg/mL kanamycin), and shaken overnight at 37 °C. Double-stranded ccc-DNA was recovered from cultures by alkaline lysis miniprep (Zyppy Plasmid Miniprep kit), and tested for inclusion of the insert by digestion with NheI before DNA sequencing, using an initial PCR reaction with the KO7-F1/R1 primer pair, followed with a sequencing reaction using the M13-F1 sequencing primer. The resultant $KO7^+$ dsDNA was transformed into chemically competent *E. coli* XL1-Blue.[32] Individual transformants were used to inoculate cultures (1 mL 2YT containing 25 μg/mL kanamycin) and shaken for 9 h at 37 °C. Starter cultures (250 μL) were transferred to 2YT media (5 mL) as above. Following 15.5 h incubation at 37 °C, cells were removed by centrifugation for 10 min at 10,000×g, the supernatant was transferred to a second centrifuge tube containing PEG/NaCl (20% polyethylene glycol 8000, 2.5 M NaCl; 1 mL) and incubated at 4 °C. Phage were harvested from the supernatant by centrifugation (10 min at 10,000×g) and resuspended 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 (Supporting Information, 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 (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) using 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 (MW 34,300; Sigma-Aldrich) as described elsewhere.[33,34] 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 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 acetylated N-terminus. Conventional peptide synthesis and purification protocols are provided in the Supporting Information. Identity and purity of oligolysine and DNase-L peptides were determined by MALDI-MS. Lys_4: expected m/z = 529.72, observed m/z = 553.01 ($M+Na^+$). Lys_8, expected m/z = 1042.43, observed m/z = 1043.41 ($M+H^+$) and 1065.04 ($M+Na^+$). Lys_{12}, expected m/z = 1555.12, observed m/z = 1555.79 ($M+H^+$), 1578.31 (M

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$+Na^+$) and 1593.56 ($M+K^+$). Lys_{16}, expected m/z = 2067.82, observed m/z = 2090.90 ($M+Na^+$). Lys_{20}, expected m/z = 2580.26, observed m/z = 2580.76 ($M+H^+$) and 2603.47 ($M+Na^+$). Lys_{24}, 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 μg/mL) in sodium carbonate buffer (50 mM Na_2CO_3, pH 9.6) before coating on a Nunc Maxisorp plate (100 μL/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 μL) for 30 minutes and later rinsed three times with wash buffer (0.05% Tween-20 (v/v, all others w/v) in PBS, 200 μL). For oligolysine-wrapping experiments, phage samples were incubated with the oligolysine peptides (2.5–25 μg/mL) or commercial poly-lysine (Sigma, M.W. > 30,000, 2.5–25 μg/mL) in PBT buffer (0.2% BSA in wash buffer) for 1 h. Control phage lacking the oligolysine or poly-lysine wrapping were incubated in PBT buffer instead. Phage samples were then transferred to the target protein-coated wells (100 μL/well), and incubated for 1 h, followed by removal of the phage solutions and rinsing with wash buffer (five times, 200 μL/well). To each well, a 1:4000 dilution of HRP-conjugated anti-M13 phage antibody (Amersham Biosciences) in PBT buffer (100 μL) was added for 30 minutes. 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*-phenylene diamine dihydrochloride/H_2O_2 solution (1 mg/mL OPD and 0.02% H_2O_2, 100 μL) in citric acid buffer (50 mM citric acid; 50 mM Na_2HPO_4, 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 using an optimized[35] oligonucleotide-directed mutagenesis protocol.[36] The oligonucleotide GCTACAAATGCCTATGCANNSNNSTGCNNSNNSNNSNNSNNSNN SNNSNNSTGCNNSNNSGGTGGAGGATCCGGCGGA (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 10^9 peptide variants fused to wild-type P8 was diluted (to 5 nM) in PBT and incubated in the presence of Lys_{18} (2.5 μg/mL) for 1 h. The phage-Lys_{18} mixture was transferred to the DNase-coated wells (100 μL/well), and incubated for 1 h. The wells were rinsed four times (200 μL of wash buffer), and the selected phage eluted with HCl (0.1 M, 100 μL/well) and vigorous shaking (250 rpm), followed by neutralization with Tris, pH 8.0 (1 M, 33 μL/well). In each subsequent round, the number of washes after the binding step was increased by two, and the blocking solution 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^{10} phage/mL) for 20 min. The infected culture was transferred to 2YT media (100 mL) supplemented with carbenicillin (50 μg/mL) and

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kanamycin (20 μg/mL) 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 μg/mL in TAE) were mixed with psoralen-PEO_3-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 μg/mL) for incubation with Lys_8 in wash buffer for 1 h.

Maxisorp plates were coated with HIV-1 Vif (20 μg/mL), blocked and washed as described above. The RNA-Lys_8 mixture was added to the Vif-coated wells (100 μL/well), and incubated for 2 h before rinsing once with wash buffer. HRP-conjugated streptavidin (0.25 μg/mL 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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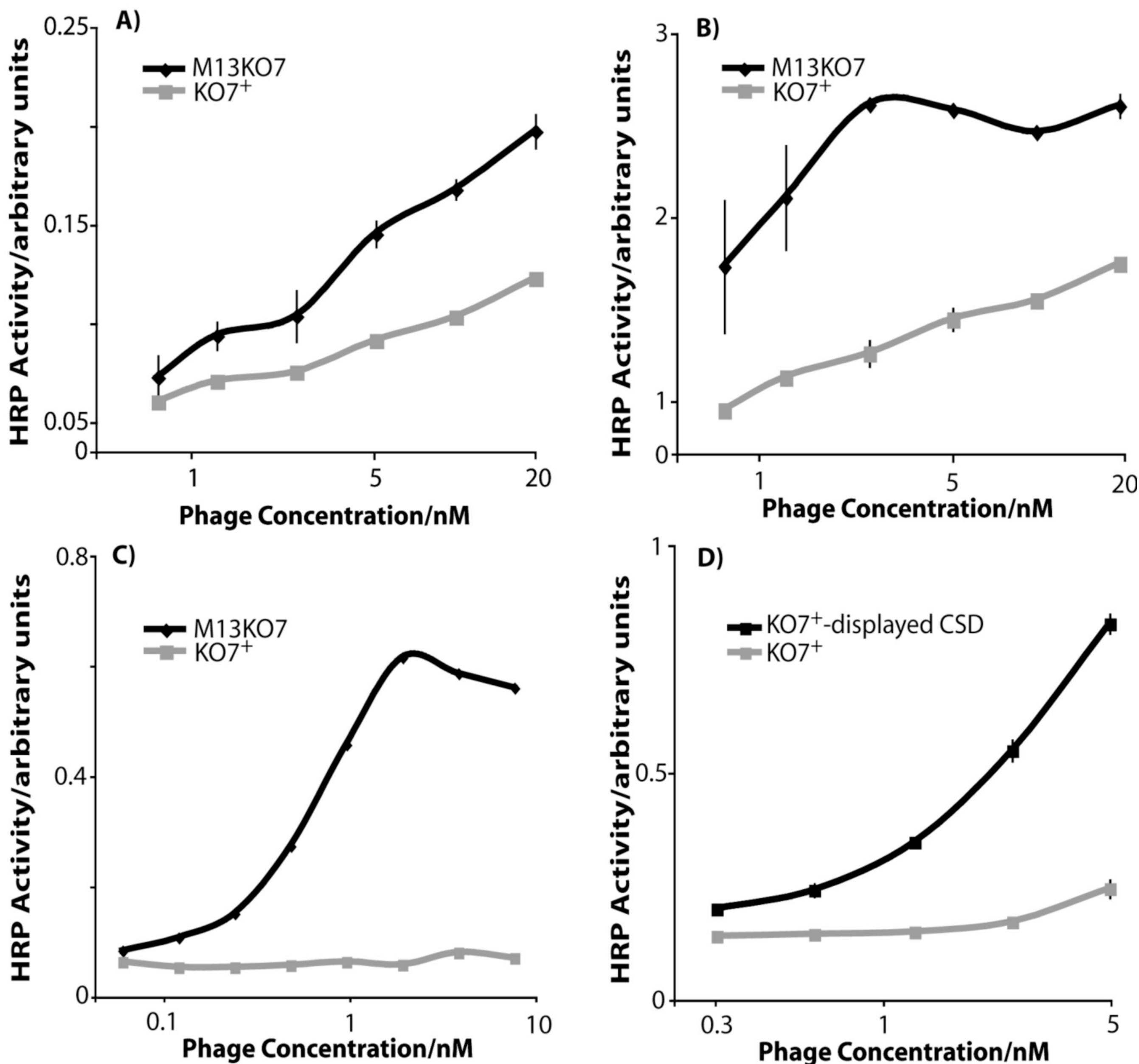

Figure 1.

Phage-based ELISAs demonstrating spurious binding by M13KO7 phage and avoidance of background binding by the genetically modified KO7^{+} 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 non-starters for phage display experiments. The problem persists, but can be solved by KO7^{+} helper phage for phage-displayed ligands, such as (**D**) CSD, pI 9.5. As shown, CSD displayed on the surface of KO7^{+} dimerizes with surface-immobilized synthetic CSD, and KO7^{+} lacking displayed CSD completely eliminates the non-specific binding observed previously with M13KO7.[10] Error bars indicate standard error in all experiments.

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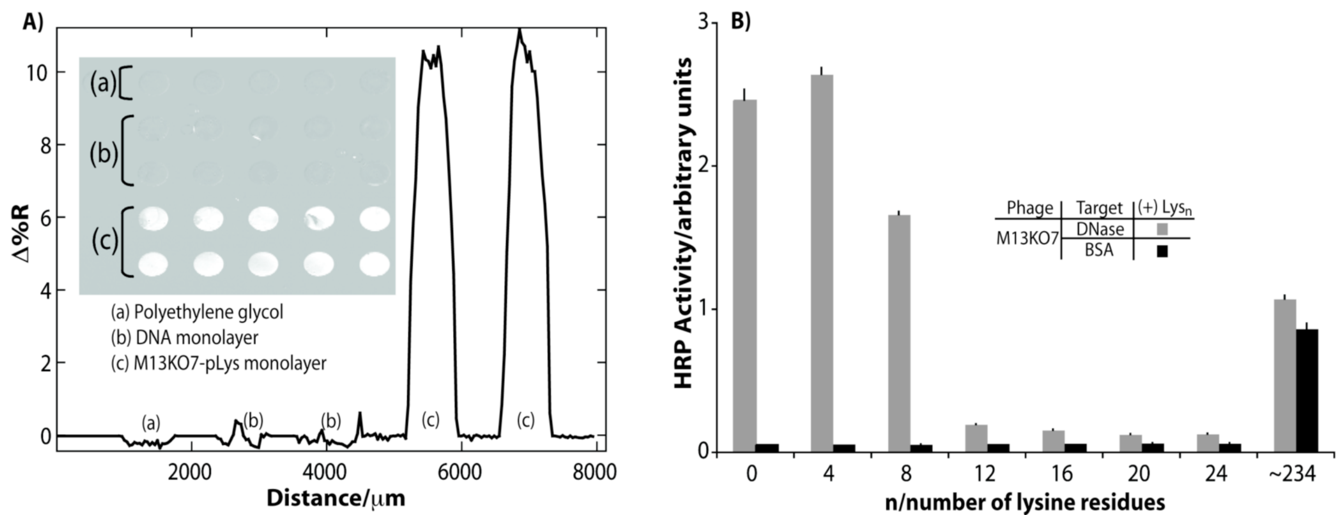

Figure 2.

(**A**) SPR imaging measurement of M13KO7 adsorption onto a pLys monolayer. The graph shows experiments without (a and b) and with pLys on a surface (c). (**B**) Oligolysine wrapping of phage shown by ELISA. Different length oligolysines (Lys_n) were synthesized to determine optimal lengths for wrapping to suppress non-specific phage binding to DNase. Note the unacceptably high background resulting from commercial poly-lysine (~234 lysines).

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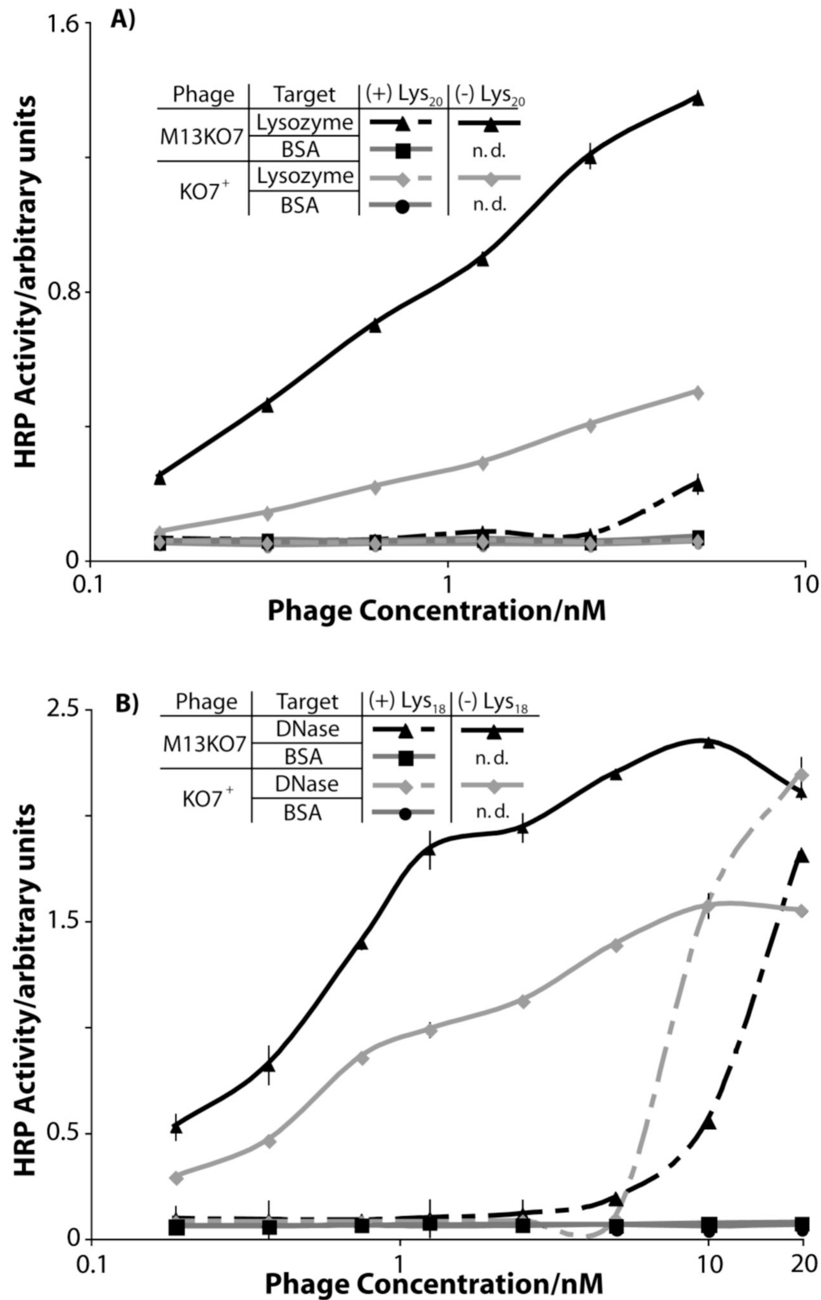

Figure 3.

Wrapping eliminates M13KO7 and KO7$^+$ phage background binding to (**A**) lysozyme (wrapping with Lys_{20}) and (**B**) DNase (Lys_{18}). In Figure 3 and Figure 4, n.d. indicates "not determined in this experiment." However, in experiments run with identical or similar conditions (not shown), binding between BSA and the phage partner was undetectable.

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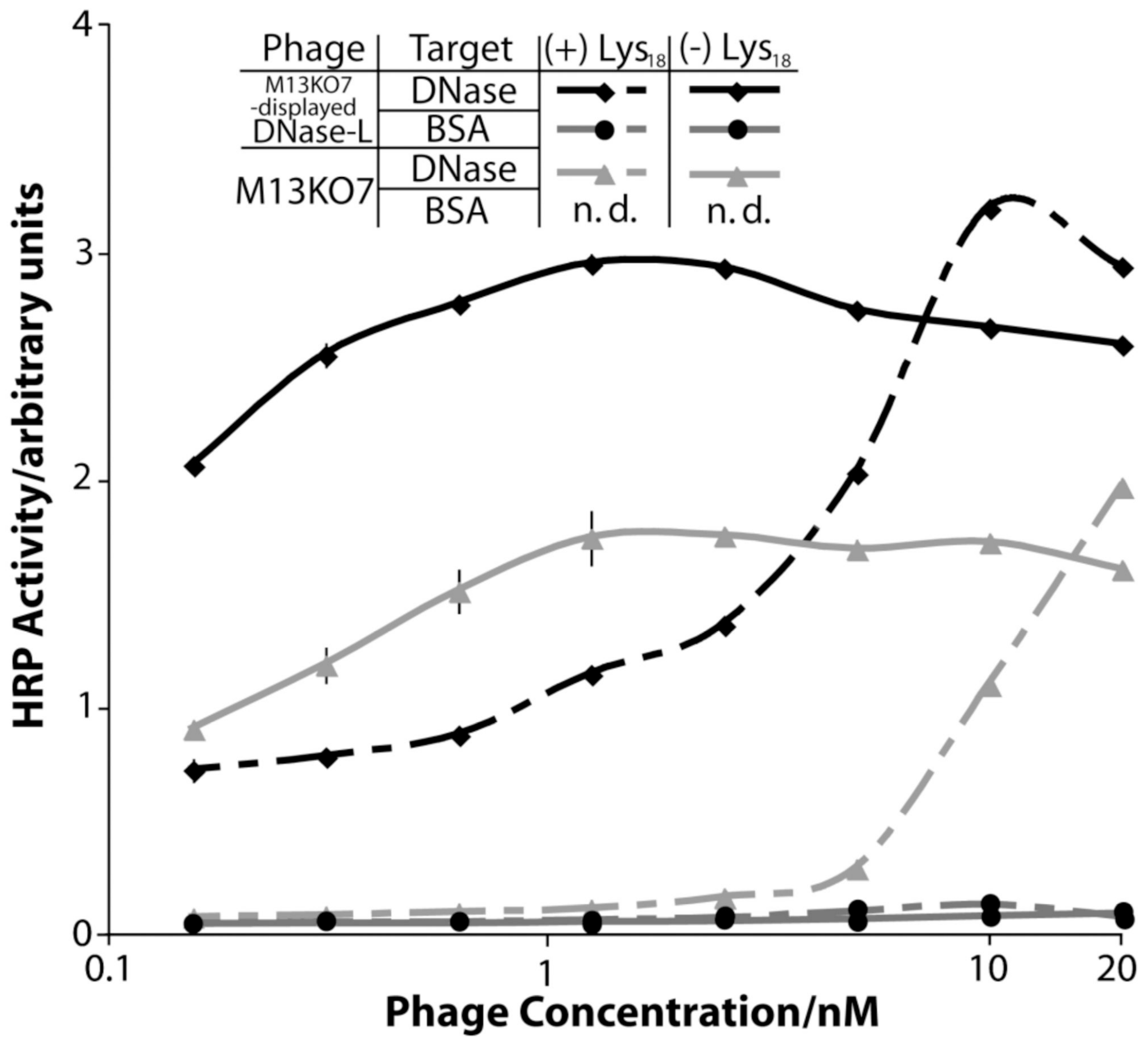

Figure 4.
Binding to DNase by the M13KO7-displayed DNase-L peptide in the presence or absence of Lys_{18} demonstrates successful selection against a previously challenging and marginal target.

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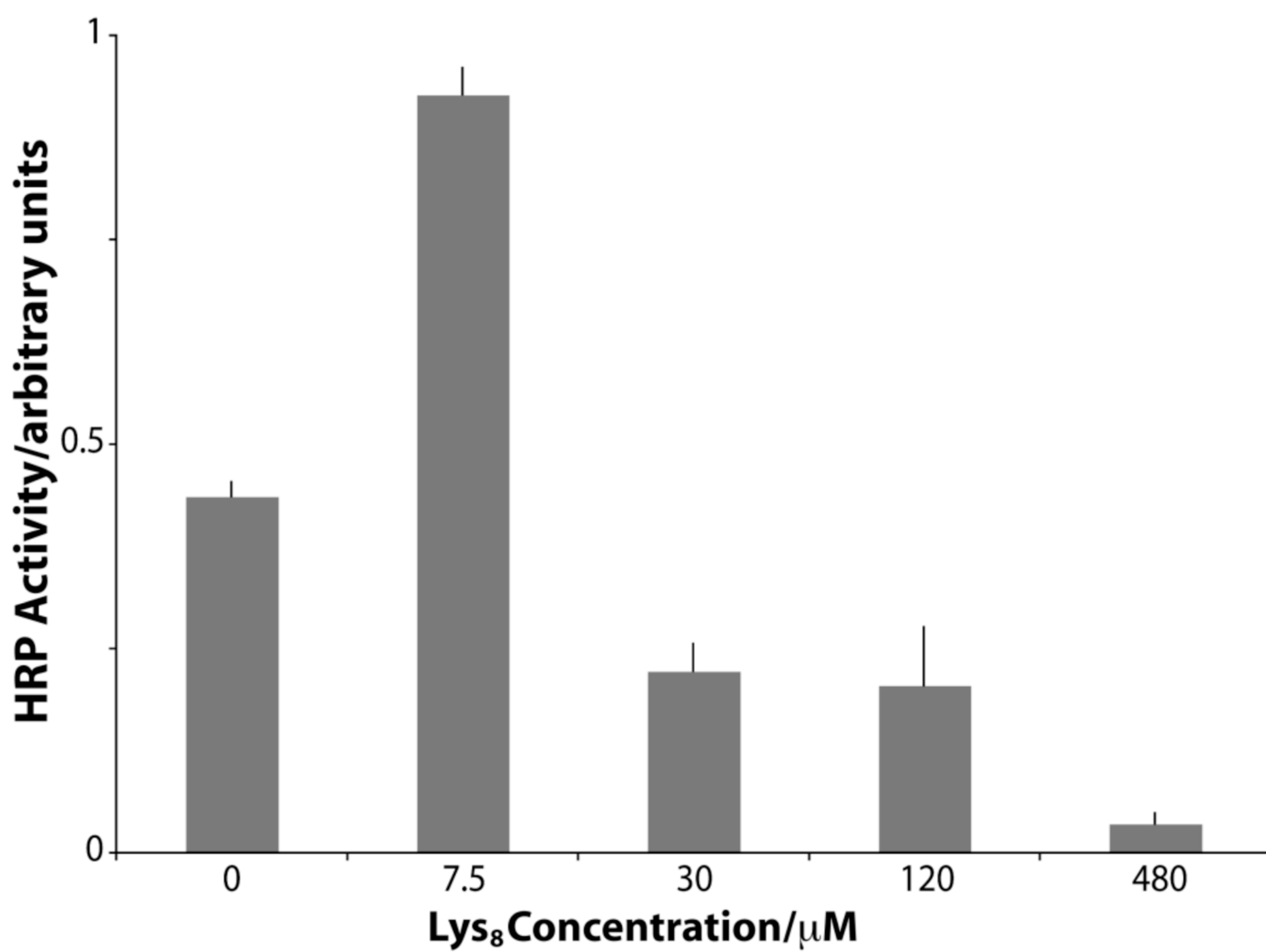

Figure 5.
The strategy of wrapping with Lys_8 also blocked non-specific binding by total RNA to the RNA-binding protein Vif.

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Table 1

Sequences of the high-affinity DNase ligands obtained from biopanning M13KO7-displayed libraries wrapped with Lys_{18}.

Peptide Name	Sequence	Abundance after 4 Rounds(%)[a]
DNase-A	AQCVSFESAFYCWV	73
DNase-G	EVCVTLDFGVWCLQ	2.4
DNase-J	EFCVTMDQWMVCVP	2.4
DNase-L	EEWYCLRQGTFETLYCFW	7.3
DNase-M	FPCGECVIARLCPA	2.4

[a]Non-readable and stop codon containing sequences comprised about 10% of the selectants.