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Phage Wrapping with Cationic Polymers Eliminates Nonspecific Binding between M13 Phage and High p*I* Target Proteins

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Abstract: M13 phage have provided scaffolds for nanostructure synthesis based upon self-assembled inorganic and hard materials interacting with phage-displayed peptides. Additionally, phage display has been used to identify binders to plastic, TiO_2, and other surfaces. However, synthesis of phage-based materials through the hybridization of soft materials with the phage surface remains unexplored. Here, we present an efficient "phage wrapping" strategy for the facile synthesis of phage coated with soluble, cationic polymers. Polymers bearing high positive charge densities demonstrated the most effective phage wrapping, as shown by assays for blocking nonspecific binding of the anionic phage coat to a high p*I* target protein. The results establish the functional group requirements for hybridizing phage with soft materials and solve a major problem in phage display—nonspecific binding by the phage to high p*I* target proteins.

Introduction

Phage display provides a powerful tool for the dissection of protein binding interactions, isolation of ligands, and affinity maturation.[1−5] In addition, the technique has found diverse applications in materials science; for example, peptides from phage display libraries capable of recognizing stereoregularity in polymeric materials have been identified.[6] In addition, peptide-directed deposition of hard materials on the phage coat has been used to fabricate nanoscale magnetic and semiconducting materials.[7−12] However, hybrids of phage and soluble soft materials remain unexplored. This paper defines the functional requirements for forming self-assembled coats of organic polymers on the phage surface and applies the information to solve a major problem in phage display—nonspecific binding to high p*I* target proteins.

The surface of M13 bacteriophage largely consists of ∼2700 copies of the major coat protein, P8, an α-helical protein with an unstructured N-terminus.[13] Three negatively charged P8 residues (Glu2, Asp4, and Asp5) contribute to the overall large negative charge of the phage particle. Thus, phage bind essentially irreversibly and nonspecifically to high p*I* proteins. Such deleterious binding typically derails phage-based assays and selections, as every member of the library binds with strong affinity to the high p*I* target.

A survey of proteins successfully targeted by phage, ribosome, and mRNA display reveals an abundance of target proteins with p*I* values below 9.5, and only one example with a p*I* above 9.5 (Table S1, Supporting Information). Target proteins with p*I*s above 9.5 correspond to an estimated 35% of the human proteome[14] and could present attractive targets for many applications. However, such high p*I* proteins are off-limits

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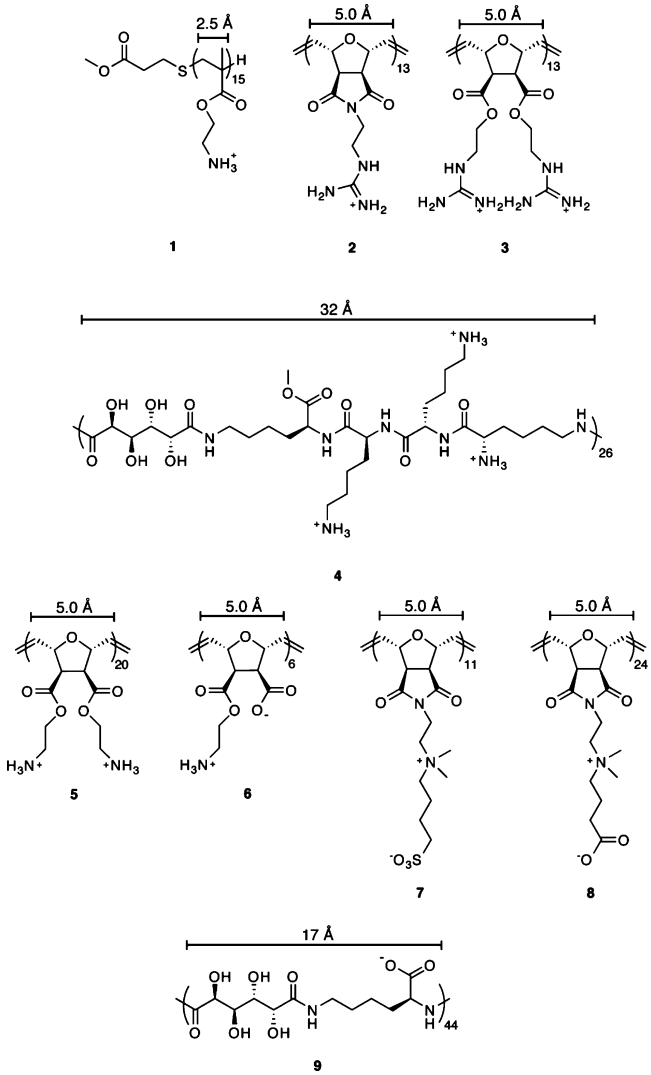
Chart 1. Phage-Wrapping Polymers in pH 7 Buffer



to phage display due to the charge complementarity described above. Recently, we described a strategy to block nonspecific binding by the phage coat to high p*I* target proteins using short oligolysine peptides. The approach enabled phage-based experiments with otherwise inaccessible targets.[15]

Though effective, oligolysine phage wrappers require multistep syntheses and extensive purification efforts. Thus, we sought a less expensive, synthetic analogue to oligolysine wrappers. Initial efforts with the commercially available, cationic polymer polyethyleneimine (PEI) failed to block nonspecific interactions between the phage and high p*I* targets (data not shown). The unstructured N-terminus of the phage coat protein P8 and flexible PEI likely present a nonoptimal arrangement of oppositely charged side chains sweeping through a wide swath of structural space. Improved phage-wrapper binding could result from a polymer having an appropriately rigidified polymeric backbone and optimal charge density. To define such requirements, we collected cationic polymers from three laboratories (Chart 1) and compared the phage wrapping abilities of each polymer through assay of phage binding to high p*I* targets.

Table 1. Infectivity of Phage Particles after Polymer Wrapping[a]

	colony-forming units			
polymer concn (μM)	polymer **1**	polymer **2**	polymer **3**	polymer **4**[b]
100	>200	0	1.0 ± 1.4	>200
10	>200	>200	7.0 ± 5.7	>200
1	>200	>200	>200	>200
0.1	>200	>200	>200	>200

[a] This summary of the titer plates shown in Table S2 quantifies the number of viable phage-infected bacterial colonies spotted at a fixed concentration on LB-kanamycin plates, as described in the Supporting Information. ">200" indicates a solid lawn of bacterial colonies. [b] As described below, polymer **4** wraps the phage less efficiently, and therefore 10-fold dilutions of the polymer started at a polymer concentration of 260 μM.

Results and Discussion

The synthetic, cationic polymer wrappers presented one to two charged functionalities per 2.5, 5.0, and 32 Å of extended polymer backbone. As expected for highly charged molecules, the polymers dissolved readily in aqueous solution (e.g., phosphate-buffered saline at pH 7.4). In ELISAs targeting the high p*I* colicin E9 DNase (p*I* 9.5), polymer **5** and the zwitterionic polymers **6**−**8** failed to wrap phage (Figure S1, Supporting Information). The discussion here, thus, focuses on the following effective phage wrappers: polymethacrylate derivative **1**,[16] polyguanidino-oxanorbornene (**2**),[17] polydiguanidinium (**3**), and galactaro-oligolysine (**4**).[18]

As required for effective phage wrapping, polymers **1**−**4** do not impinge upon phage or bacterial viability at the concentrations required (1−10 μM). However, at higher concentrations (10−100 μM), polymers **2** and **3**, but not **1** and **4**, eliminated or reduced phage infectivity without affecting bacterial viability (Tables 1 and S2, Supporting Information). Fixed concentrations of M13-KO7 phage, which harbors a gene conferring kanamycin resistance, were incubated in the presence of **1**, **2**, **3**, or **4** at the concentrations indicated in Tables 1 and S2. The polymer-wrapped phage was allowed to infect a tetracycline-resistant *Escherichia coli* strain; to assess either phage or bacterial viability, the infected bacteria were then spotted on LB plates supplemented with either kanamycin (Tables 1 and S2A−S2C) or tetracycline (Table S2D−F), respectively. Unlike polymers **1** and **4**, which were tolerated at all concentrations, polymers **2** and **3** at concentrations above 10 μM induced formation of white insoluble precipitates of the phage. The precipitated phage had dramatically lower phage infectivity. However, the bacteria were unaffected at all tested concentrations of polymers (Table S2D−F). Importantly, the optimal polymer concentrations for wrapping the phage (described below) required 10- to 100-fold less material (Figure 1) than the concentrations resulting in phage precipitation.

Having established that the phage can tolerate the synthetic polymers, we next compared the relative efficiencies of phage wrapping by the cationic polymers. In these experiments, the M13-KO7 phage (1 nM) were incubated with serial dilutions of each polymer for 1 h. The charge shielding abilities of each polymer were then assessed by a phage-based ELISA, which challenges the negatively charged phage to bind to DNase (p*I*

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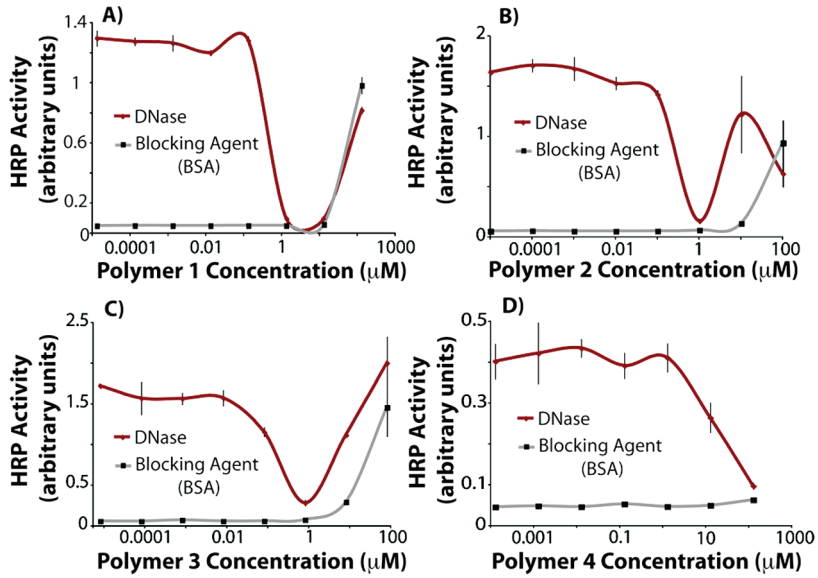

Figure 1. Phage-based ELISAs of M13-KO7 phage wrapped with polymers (A) **1**, (B) **2**, (C) **3**, and (D) **4** binding to either DNase or BSA (negative control). Wrapping the phage (1 nM) with a 1000-fold molar excess (1 μM) of polymer **1**, **2**, or **3** was sufficient to reduce or eliminate nonspecific binding to target DNase. Polymer **4**, however, required a 10^5-fold molar excess ($\sim$100 μM) to wrap the phage effectively. Throughout this report, error bars indicate standard error ($n = 2-3$).

9.5) (Figure 1). Polymers **1**−**4** effectively eliminated the undesirable phage binding to DNase at different concentrations, which reflects their relative effectiveness at wrapping and charge shielding the phage surface. Polymers **1**, **2**, and **3** wrapped phage most effectively when applied in 1000-fold molar excess to the phage (Figure 1A−C, respectively), whereas the less efficient wrapper **4** required a 10^5-fold molar excess to eliminate nonspecific binding (Figure 1D). Thus, despite the large number of cationic functionalities in polymer **4** (78 primary amines per molecule), its dispersed charge density delivers less efficient phage wrapping than the other polymers.

The synthetic polymers wrapped the phage as well as or better than the previously reported oligolysine wrappers.[15] Also analogous to oligolysine wrapping, polymers **1**, **2**, and **3** cause nonspecific binding to many targets, including the blocking agent (bovine serum albumin, BSA), at very high polymer concentrations ($\geq$100 μM). Polymer **4** also demonstrated this cross-linking effect at a high concentration ($\sim$1 mM); these data are not shown in Figure 1D, as the resultant high HRP activity saturated the ELISA absorbance measurement. Excess polymer could cross-link the phage to the blocking agent by saturating charged sites on the phage surface and allowing cationic functionalities of the polymer to trail off the phage surface. Such high wrapper concentrations far exceed the requirements for blocking nonspecific interactions but demonstrate the importance of determining optimal wrapper concentrations for new polymers before phage-based assays or selections.

The observed cross-linking at higher polymer concentrations also correlated with increased polymer length, as demonstrated by comparing two variants of polymer **1**. In this experiment, the extended length variant of polymer **1** (**1-ext**) included 23 primary amine functionalities per molecule, and the truncated length variant of polymer **1** (**1-trunc**) included 9 primary amines per molecule (Figure 2). When used in excess concentrations, the longer, cationic polymer **1-ext** results in more deleterious cross-linking, presumably due to the greater propensity of the longer polymer to extend off the phage surface. However, the structure of the polymer and not just the length determines cross-linking ability. For example, the long polymer **4** fails to cross-link the phage to the target at the concentration ($\sim$100 μM) required for cross-linking by the other polymers (Figure 1); thus, a higher concentration of the less efficient wrapper, polymer **4**, is required to saturate charged sites on the phage surface before cross-linking to the target or blocking agent.

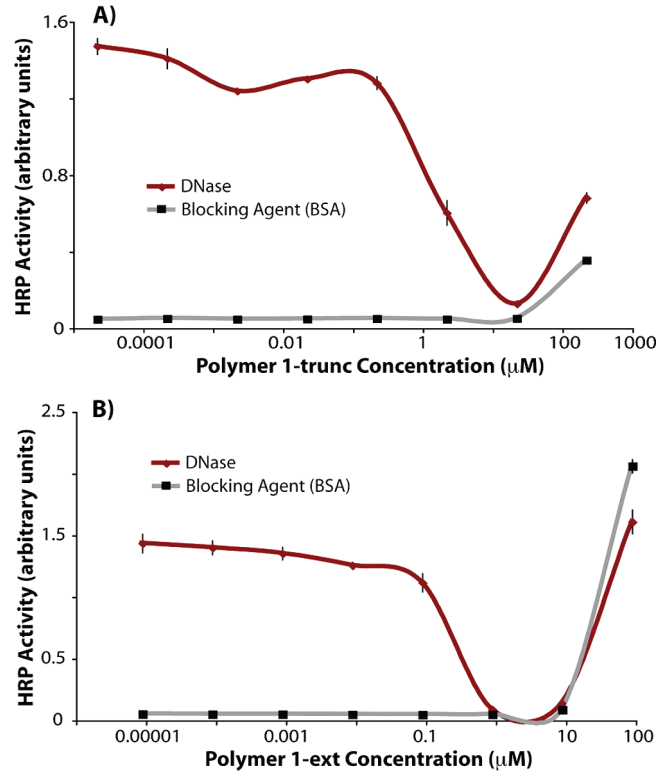

Figure 2. Phage-based ELISAs of M13-KO7 phage wrapped by polymers (A) **1-trunc** and (B) **1-ext**. A fixed concentration of M13-KO7 phage (0.5 nM) was incubated with the indicated concentrations of each polymer, and exposed to either DNase or the blocking agent BSA for assay. Polymer **1-ext** (23 amine functionalities) at high concentrations ($\sim$100 μM) resulted in high levels of cross-linked phage to DNase, but polymer **1-trunc** exhibited only moderate cross-linking at the same polymer concentrations.

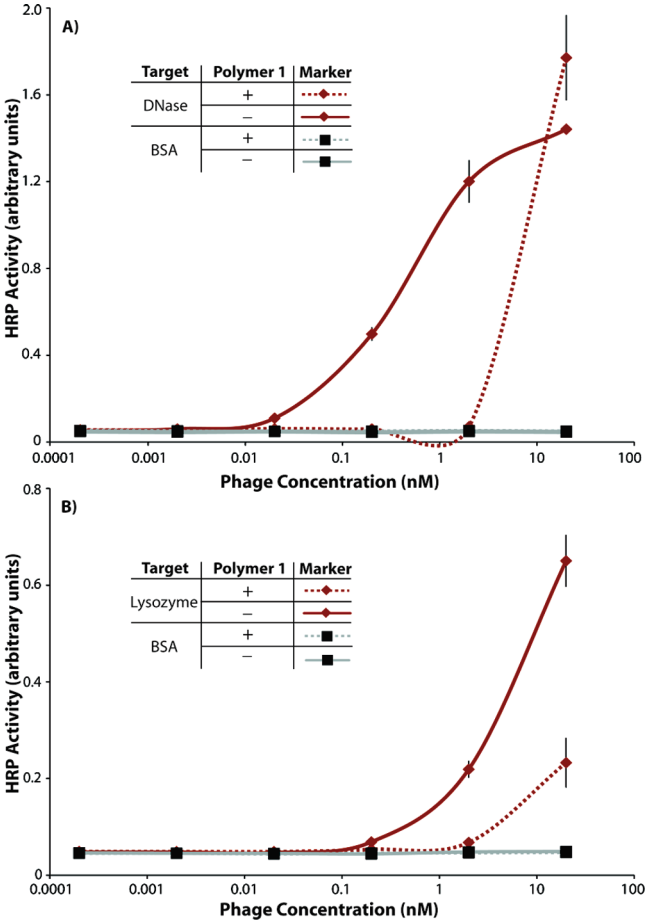

Figure 3. Phage wrapping ELISAs of M13-KO7 phage mixed with polymer **1** (1 μM). (A) Wrapped M13-KO7 phage abrogated nonspecific binding to target DNase at phage concentrations ≤2 nM. However, binding between phage and DNase was observed in the absence of polymer **1** and at high phage concentrations. (B) Nonspecific binding of M13-KO7 phage to lysozyme was also abolished by wrapping with polymer **1**.

In addition to the potential for novel materials, phage wrapping by cationic polymers could find important applications in preventing nonspecific binding between phage and high p*I* target proteins. For such applications, the cationic wrapper must remain attached to the phage during both phage display selections and screens. To demonstrate the utility of synthetic wrappers in screens, a fixed concentration of wrapper was used to wrap phage at a range of concentrations. At the optimal polymer concentration determined by the Figure 1 experiment (1 μM), polymer **1** successfully blocked nonspecific binding of phage to both DNase and lysozyme (p*I* 9.5) (Figure 3). The experiment demonstrated the absence of spurious binding at phage concentrations ≤2 nM, while higher phage concentrations elicited nonspecific binding to both targets, presumably due to incomplete wrapping of the phage by an inadequate quantity of wrapping polymer. The experiment also demonstrated that wrapping the phage with synthetic polymers also blocks nonspecific binding to other high p*I* targets, such as lysozyme.

To determine the most effective phage wrapper among the polymers described here and previously,[15] an ELISA compared phage wrapping and cancellation of nonspecific binding by different polymers (Figure 4). In this experiment, 10-fold serial dilutions of 20 nM M13-KO7 phage were incubated with the phage wrappers Lys_{20}, **1-ext**, or **2**. Polymer **1-ext** demonstrated essentially perfect wrapping, as nonspecific binding to the target

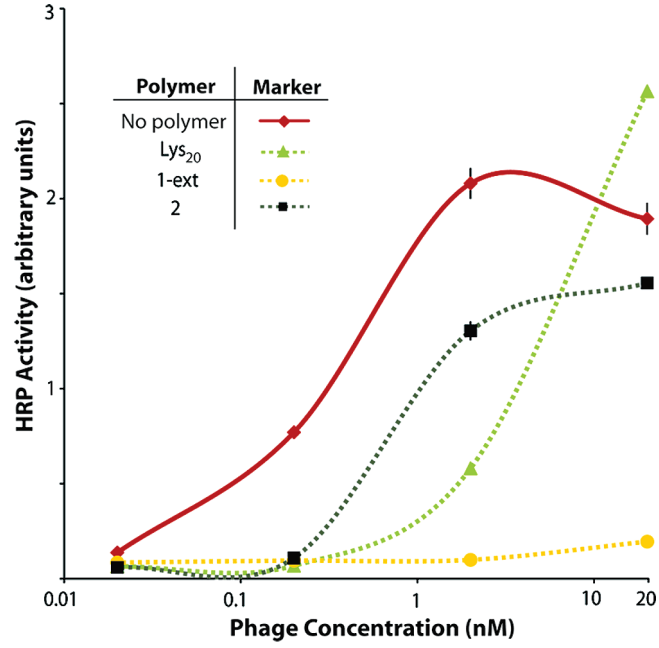

Figure 4. Phage-based ELISA comparing the phage wrapping efficiencies of peptide Lys_{20} with polymers **1-ext** and **2**. The indicated phage concentrations were incubated with 10 μM Lys_{20} and polymer **1-ext** and 3 μM polymer **2** before testing for binding to DNase. Polymer **1-ext** resulted in the most efficient wrapping, as this wrapper eliminated nonspecific binding to the target at typically problematic phage concentrations (≥5 nM).

DNase was absent at phage concentrations up to the limits of phage solubility.

Polymer **2** and peptide Lys_{20} revealed similar levels of moderate efficiency for phage wrapping. However, Lys_{20} exhibited poor efficiency at a high phage concentration (20 nM), where **1-ext** demonstrated ~12.5 times more efficient wrapping than Lys_{20}. Polymer **1-ext** presents a denser collection of cationic amines (one amine side chain per 2.5 Å of polymer backbone) than Lys_{20} (one amine per 5.0 Å) and polymer **4**, which exposes two amine side chains per galactaro-oligolysine subunit (32 Å backbone length). As phage wrapping is mediated by an avidity effect,[15] polymers presenting higher positive charge densities demonstrate superior phage wrapping than polymers with a lower density of cationic side chains.

In addition to its effectiveness in phage-based screening assays, the best phage wrapper, **1-ext**, works exceptionally well to block nonspecific interactions during selections with phage-displayed libraries. In the absence of added wrapper, phage display selections targeting high p*I* proteins generally fail; phage without a displayed protein are amplified preferentially, as such phage place less demand on the bacteria during phage proliferation. During phage display selections and screens, **1-ext** was added in 1000-fold molar excess, a concentration suggested by the previously described polymer optimization experiments (Figure 2). The phage-displayed library consisted of 24 different configurations of cystine disulfide-linked peptides displayed as fusions to the N-terminus of P8. With the exception of the invariant cysteine residues, each peptide included 5−18 residues encoded by the NNS codon (where N designates any nucleotide and S designates either G or C). Thus, each degenerate position encodes all 20 naturally occurring amino acids but prevents the occurrence of the nonsuppressible stop codons TGA and TAA, which could result in nondisplayed peptides. From a combined peptide diversity of 2.5×10^{10} different peptides, two new peptide ligands to DNase, DNase-1 (amino acid sequence

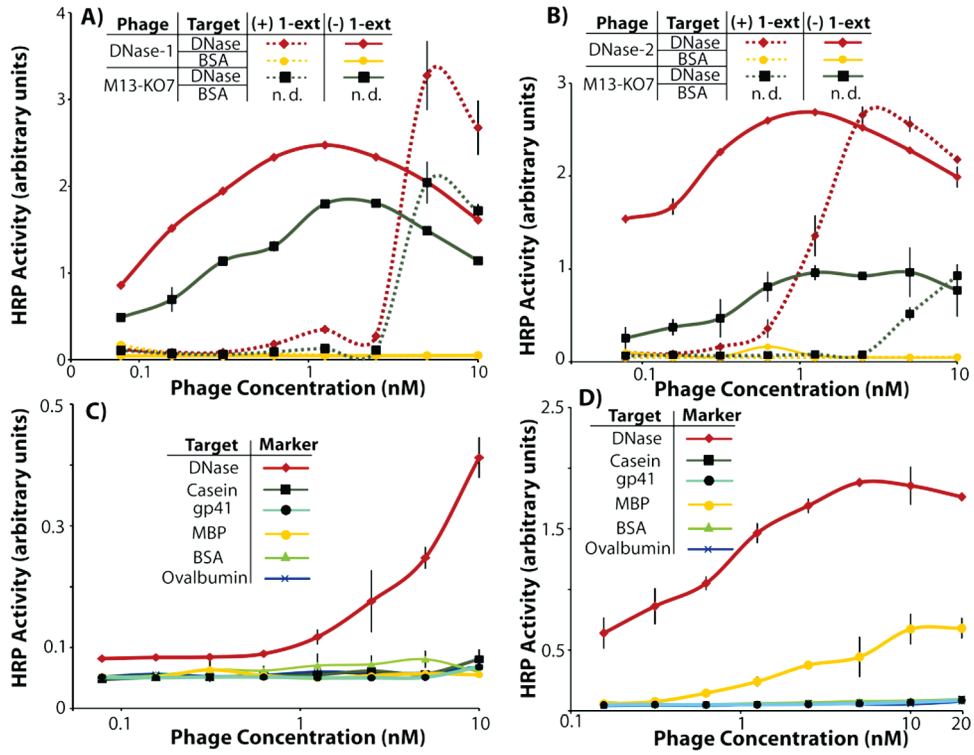

Figure 5. Binding affinity for DNase by phage-displayed (A) DNase-1 and (B) DNase-2, and specificity for other proteins by phage-displayed (C) DNase-1 and (D) DNase-2 wrapped with **1-ext**. In these phage-based ELISAs, the unwrapped phage bound strongly and nonspecifically to DNase. However, wrapping the phage with **1-ext** (2.5 μM) dramatically lowered nonspecific binding, and, particularly for DNase-2, strong binding to DNase was retained. In a phage-based ELISA examining specificity of binding by phage-displayed ligands, both DNase-1 and DNase-2 preferentially bound DNase. In this experiment, n.d. indicates "not determined". However, other experiments performed under identical conditions (not shown) revealed no interaction between M13-KO7 phage (both wrapped and unwrapped) and BSA.

RWCLSELSG) and DNase-2 (EEWYCLRQGTFETLFF), were identified as DNase binders after only two rounds of selection.

ELISAs with phage-displayed ligands confirmed the moderate to high affinity binding and specificity for DNase by peptides DNase-1 and -2 (Figure 5). In the absence of the **1-ext** wrapper, phage displaying either DNase-1 or -2 demonstrated stronger binding to DNase than the negative control M13-KO7 phage, which lacks a DNase binding peptide on its surface (Figure 5A,B, respectively). Such strong binding of phage-displayed DNase-1 and -2 suggests that the peptide ligands displayed on the phage surface reinforce the already strong nonspecific binding of the phage coat to DNase. After wrapping by the **1-ext** polymer, the phage-displayed DNase ligands bound with higher affinity to DNase than the wrapped M13-KO7 phage control, with DNase-2 having much greater apparent affinity.

To examine specificity, a panel of target proteins, including DNase, casein, HIV-1 gp41, maltose binding protein (MBP), BSA, and ovalbumin, was assayed for binding to DNase-1 or -2 wrapped with **1-ext** (Figure 5C,D, respectively). Both DNase-1 and -2 exhibited strong specificity to DNase. Surface plasmon resonance (SPR) imaging also confirmed that phage-displayed DNase-1 wrapped with polymer **1** binds well to the target DNase (Figure S2, Supporting Information). Additional control experiments with the two phage-displayed DNase ligands demonstrated that a negatively charged polymer **9** has no effect on blocking nonspecific interactions between the phage coat and DNase (Figure S3, Supporting Information). These results confirmed that the polymers bind phage primarily through complementary charge−charge interactions.

Interestingly, ligand DNase-2 (EEWYCLRQGTFETLFF) demonstrated a very high sequence similarity to the DNase ligand denominated DNase-L (EEWYCLRQGTFETLYCFW) described in our previous report.[15] The selection of DNase-L required wrapping of the phage library with a related peptide wrapper (Lys_{18}). Importantly, both DNase-2 and DNase-L were selected from two distinct phage-displayed libraries within the pool of peptide libraries, as shown by the longer sequence of DNase-L. Isolation of two similar ligands from two independent experiments validates the use of synthetic polymers for phage wrapping and establishes the robustness of affinity selections using wrapped phage libraries.

Since the ligand DNase-1 belongs to a new class of DNase ligands, the peptide was synthesized for off-phage binding experiments (Figure 6). Analogous experiments have already been reported with DNase-L,[15] which is again closely related to DNase-2. As expected, the chemically synthesized DNase-1 retained binding to DNase off the phage. Addition of polymer **1** had no effect on DNase-1 binding to DNase. The result demonstrated that wrapping with a synthetic polymer had no effect on the outcome of the phage-based selections beyond blocking nonspecific interactions between the scaffold for molecular display and the target. Though the reported experiments apply peptides fused to the major coat protein, P8, we expect similar outcomes for proteins displayed on P3, as phage infectivity, which relies on P3, remained unchanged in the presence of polymer wrapper. Such benign suppression of deleterious, nonspecific binding fullfills the requirements for the perfect phage wrapper.

Conclusion

Synthetic polymers bearing cationic amine and guanidinium functionalities prove highly effective in wrapping phage and

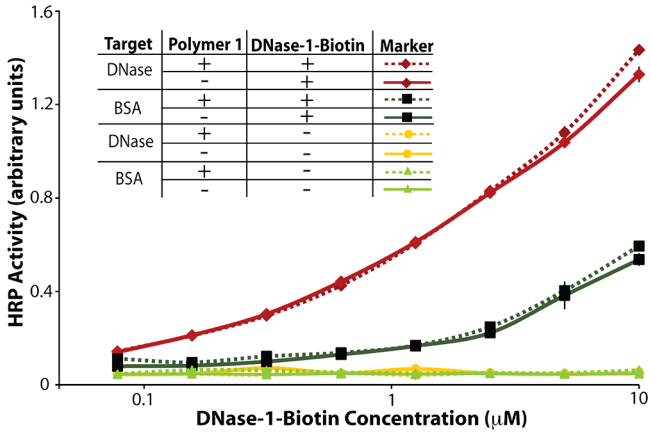

Figure 6. ELISA demonstrating high-affinity binding of synthetic peptide DNase-1 to DNase. The DNase-1 peptide was N-terminally biotinylated for detection with HRP-conjugated streptavidin. Addition of 1 μM polymer **1** to the peptide solution resulted in a marginal effect on DNase binding.

eliminating nonspecific binding to high p*I* proteins. Each polymer demonstrates a unique optimal concentration range for wrapping the phage particles; too low a polymer concentration results in inefficient wrapping, and too high a polymer concentration cross-links the phage to the target and blocking agent. As shown for the primary amine containing polymethacrylates (polymers **1**, **1-trunc**, and **1-ext**), the wrappers demonstrate a direct correspondence between longer polymer length and higher levels of phage cross-linking to the target. In addition, the density of positive charge presented by the polymers influences the efficiency of the wrapping. Polymers bearing more densely clustered cationic side chains demonstrate superior wrapping than a polymer presenting dispersed cationic functionalities (e.g., polymer **4**). As demonstrated by successful selections and screens against the otherwise inaccessible target DNase, polymer **1-ext** solves the problem of deleterious background binding between the phage display scaffold and high p*I* target proteins. The solution, synthetic polymers, provides superior wrapping abilities to synthetic peptides and offers easier synthesis and purification. The functional determinants for phage wrapped with soft materials could provide libraries with greater diversities, including non-natural functionalities, and materials with new properties.

Materials and Methods

Titering of Polymer-Wrapped Phage. Polymers **1**, **2**, **3** and **4** were each diluted (10-fold, 50 μL volume) in PT buffer (0.05% Tween-20 in PBS) and added to the designated wells in a microtiter plate. A fixed concentration of M13-KO7 phage (1×10^8 phage/μL) was then added to each diluted polymer. After mixing for 1 h at room temperature, log phase XL1 Blue *E. coli* (100 μL/well) was added to the wrapped phage, and the solutions were incubated for 30 min at 37 °C to allow infection to take place. A 10-μL aliquot of the infected cells was then spotted on LB agar plates supplemented with kanamycin (40 μg/mL) or tetracycline (5 μg/mL). The plates were incubated at 37 °C for 14 h.

Phage Wrapping ELISAs. All binding steps were carried out on an orbital shaker (150 rpm) at room temperature. A 96-well Maxisorp plate (Nunc, Rochester, NY) was coated with 100 μL/well of protein target (10 μg/mL in PBS) and incubated for 2 h at room temperature. The coating solution was removed, and the wells were then blocked with 400 μL of blocking buffer (0.2% BSA in PBS) for 30 min, followed by removal of the blocking buffer and three washes with 200 μL of PT buffer per well. In separate tubes, the phage were wrapped with polymer by incubating phage solutions in PT buffer with the corresponding polymers for 1 h. Control phage lacking a polymer wrapper were incubated in the presence of PT buffer instead. The phage solution was transferred to the target-coated plate (100 μL/well) and incubated for 1 h. After the wrapped phage solution was removed, the wells were washed five times as described above and then incubated with 100 μL of HRP-conjugated anti-M13 phage antibody (1:4000 dilution in PT buffer; Amersham Biosciences) for 30 min. The wells were washed five times with PT buffer and then rinsed once with PBS, followed by addition of 100 μL of OPD$-H_2O_2$ solution (1 mg/mL *o*-phenylene diamine dihydrochloride, 0.02% H_2O_2 in 50 mM citric acid, 50 mM Na_2HPO_4, pH 5.0). The HRP activity was measured as absorbance at 450 nm in a microtiter plate reader (Bio-Tek Instruments, Inc.).

Affinity Selections Using a Polymer-Wrapped Phage Library. Phage (17.8 nM phage-displayed peptide library in the first round of selection and 83 pM in the second round) were incubated with 1000-fold molar excess of **1-ext** in PBT buffer (0.2% BSA and 0.05% Tween-20 in PBS) for 1 h with rocking at room temperature. A 96-well Maxisorp plate was coated with DNase as described for the ELISA protocol. The wells were blocked for 1 h with either BSA or casein (0.2% in PBS). Wells were washed three times with 150 μL of PT. The phage library was suspended in PBT, and 100 μL of the library was added to each well and incubated for 1 h. Wells were washed five times as described above. Phage were eluted with 100 μL of 100 mM HCl and vigorous shaking for 5 min. The HCl solution was neutralized by addition of 1 M Tris-HCl (33 μL, pH 8). Half the phage solution was incubated with 15 mL of XL-1 Blue *E. coli* cells (OD_{600} 0.5−1.0) for 20 min while shaking at 37 °C. The infected cells were transferred to 400 mL of 2YT containing M13-KO7 helper phage and incubated overnight at 37 °C with shaking. Individual selectants were analyzed by the phage ELISA described above. The two DNase ligands, DNase-1 and DNase-2, were selectants from the second round of selections.

SPR Imaging Measurements. A SPR imager (GWC Technologies) was used for investigating adsorption of phage-displayed DNase-1 binding to either DNase or PEG covalently attached to 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. The DNase-modified surfaces were created by spotting carboxy-terminated undecanethiol on the gold for 12 h, followed by addition of *N*-hydroxysuccinimide (NHS) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) on the surface for 2 h, followed by a 1 h incubation with 0.5 μL of a 10 μg/mL DNase stock solution. The poly(ethylene glycol)-modified surfaces were created by spotting thiol-PEG on the surface of the gold for 12 h. The surface was exposed to a solution of 2.5 nM phage wrapped in 2.5 μM polymer **1** in PBS. The surface was then exposed to PBS to wash away any nonspecific binding of the phage. The difference images were obtained by subtracting the images acquired before and after phage binding.

Synthesis of the DNase-1-Biotin Peptide. DNase-1 was synthesized at 0.1 mmol scale using conventional solid-phase peptide synthesis protocols on rink amide resin (Novabiochem), which yields a carboxamide C-terminus. At the N-terminus, two additional glycine residues were added as a linker, and the peptide was biotinylated (Novabiochem) using conventional coupling protocols. The peptide was cleaved from the resin using a mixture of 9.5 mL of trifluoroacetic acid, 0.25 mL of triisopropylsilane, and 0.2 mL of H_2O for 4 h under N_2. DNase-1-biotin was purified by reverse-phase HPLC, and the identity of the peptide (biotin-GGRW-CLSELSG-NH_2) was confirmed by MALDI-TOF analysis. Expected *m/z* 1389.60; observed *m/z* 1389.78.

Peptide-Wrapping ELISA. The ELISA using chemically synthesized DNase-1 peptide followed the phage-wrapping ELISA protocol described above. For samples with polymer **1** added, the

DNase-1 peptide was incubated in the presence of polymer **1** (1 μM) in PT buffer for 1 h before being transferred to the corresponding DNase-coated wells.

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Supporting Information Available: Additional experiments and experimental protocols including stability studies of polymer-wrapped phage, SPR imaging experiments using phage-displayed DNase-1, and wrapping experiments using polymers **5**−**9**; updated table of proteins successfully targeted by molecular display methods and their estimated p*I* values. This material is available free of charge via the Internet at http://pubs.acs.org.

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