Evolutionary Screening of Biomimetic Coatings for Selective Detection of Explosives

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Susceptibility of chemical sensors to false positive signals remains a common drawback due to insufficient sensor coating selectivity. By mimicking biology, we have demonstrated the use of sequence-specific biopolymers to generate highly selective receptors for trinitrotoluene and 2,4-dinitrotoluene. Using mutational analysis, we show that the identified binding peptides recognize the target substrate through multivalent binding with key side chain amino acid elements. Additionally, our peptide-based receptors embedded in a hydrogel show selective binding to target molecules in the gas phase. These experiments demonstrate the technique of receptor screening in liquid to be translated to selective gas-phase target binding, potentially impacting the design of a new class of sensor coatings.

1. Introduction

The ability to detect and analyze volatile organic compounds (VOCs) such as explosives,[1] pesticides,[2] disease markers,[3] and food aromas[4] has significant bearing on our security, health, and general well being. As opposed to mobility-based[5] and optical[6] sensors, those based on ligand−receptor binding that emulate the olfactory system enable miniaturization. While there are several highly sensitive ways to convert ligand−receptor binding to electrical or optical signals,[7−13] the lack of selectivity has remained a key challenge, making such sensors inadequate in many applications and preventing their widespread use. At issue are functional coatings, which in the past have relied on arrays of oxide layers,[14] readily available polymers,[15] or even de novo designed receptors.[9,16−18] Although such arrays provide a pattern of binding that can be mathematically processed to distinguish molecular species, the affinity differences are often insufficient for highly selective and sensitive chemical analysis in realistic conditions. Here we exploit an evolutionary screening process with phage libraries to identify highly selective peptide-based recognition motifs for trinitrotoluene (TNT) and 2,4-dinitrotoluene (DNT) and develop gas-phase binding assays which can be selective prototypes of VOC sensors. By mutating the peptide sequences, we demonstrate that increased selectivity results from multivalent binding. Furthermore, we show that by immobilizing the DNT binding peptide with hygroscopic ethylene glycol oligomers we can create biomimetic coatings that selectively detect gas-phase DNT molecules in mixtures containing TNT.

Molecular biology is replete with examples where weak interactions based on hydrogen bonding or van der Waals interactions can lead to highly specific molecular recognition through multivalent or cooperative binding. The diversity of chemistry and structure produced by sequence-specific biopolymers such as nucleic acids and peptides allows specific multivalent receptors to be created for a wide range of target ligands. The immune system, for example, utilizes this to create specific protein-based receptors against a large variety of foreign antigens. The use of sequence-specific heteropolymers and multivalent binding has, however, not been thoroughly explored for the detection of VOCs. While oligomers of DNA and RNA have been used recently,[19] the multivalency of their binding against VOCs has remained undetermined. Moreover, the diverse

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(1) Yang, Y.-S.; Swager, T. M. *J. Am. Chem. Soc.* **1998**, *120*, 11864.

(2) Mulchandani, A.; Chen, W.; Mulchandani, P.; Wang, J.; Rogers, K. R. *Biosens. Bioelectron.* **2001**, *16*, 225.

(3) Turner, A. P. F.; Magan, N. *Nat. Rev. Microbiol.* **2004**, *2*, 161.

(4) Venugopal, V. *Biosens. Bioelectron.* **2002**, *17*, 147.

(5) Berezkin, V. B.; Drugov, Y. S.; Drugov, Y. *Gas Chromatography in Air Pollution Analysis*; Elsevier Science Ltd.: New York, 1991.

(6) Baldini, F.; Martellucci, S.; Chester, A. N.; Homola, J. *Optical Chemical Sensors*; Springer-Verlag: New York, 2006.

(7) Burg, T. P.; Godin, M.; Knudsen, S. M.; Shen, W.; Carlson, G.; Foster, J. S.; Babcock, K.; Manalis, S. R. *Nature* **2007**, *446*, 1066.

(8) Thundat, T.; Majumdar, A. In *Sensors and Sensing in Biology and Engineering*; Barth, F. G., Humphrey, J. A. C., Seecomb, T. W., Eds.; Springer-Verlag: New York, 2003.

(9) Grate, J. W.; Patrash, S. J.; Abraham, M. H.; Du, C. M. *Anal. Chem.* **1996**, *68*, 913.

(10) Patel, S. V.; Mlsna, T. E.; Fruhberger, B.; Klaassen, E.; Cemalovic, S.; Baselt, D. R. *Sens. Actuators, B* **2003**, *96*, 541.

(11) Hagleitner, C.; Hierlemann, A.; Lange, D.; Kummer, A.; Kerness, N.; Brand, O.; Baltes, H. *Nature* **2001**, *414*, 293.

(12) Freund, M. S.; Lewis, N. S. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 2652.

(13) Shekhawat, G.; Tark, S. H.; Dravid, V. P. *Science* **2006**, *311*, 1592.

(14) Suehle, J. S.; Cavicchi, R. E.; Gaitan, M.; Semancik, S. *IEEE Electron Device Lett.* **1993**, *14*, 118.

(15) Hierlemann, A.; Ricco, A. J.; Bodenhofer, K.; Dominik, A.; Gopel, W. *Anal. Chem.* **2000**, *72*, 3696.

(16) Rakow, N. A.; Suslick, K. S. *Nature* **2000**, *406*, 710.

(17) Looger, L. L.; Dwyer, M. A.; Smith, J. J.; Hellinga, H. W. *Nature* **2003**, *423*, 185.

(18) Suman, M.; Freddi, M.; Massera, C.; Ugozzoli, F.; Dalcanale, E. *J. Am. Chem. Soc.* **2003**, *125*, 12068.

(19) Staii, C.; Johnson, A. T. *Nano Lett.* **2005**, *5*, 1774.

10.1021/la7035289 CCC: $40.75
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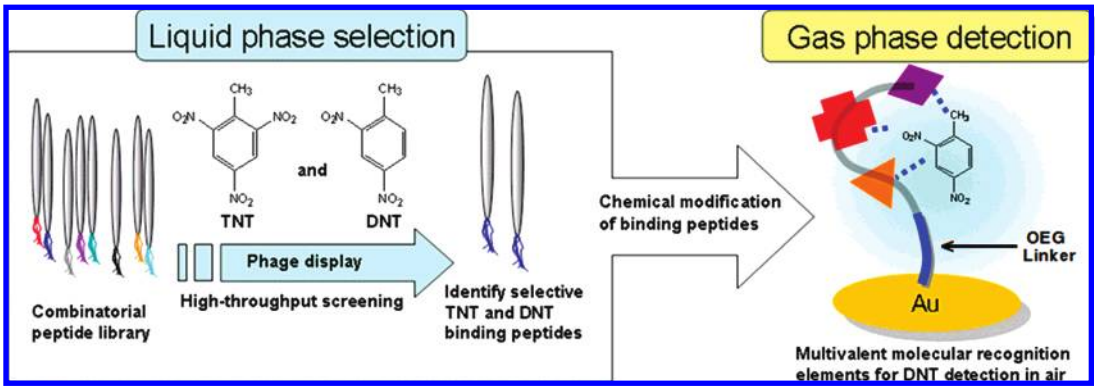

Figure 1. Schematic diagram showing our biomimetic approach to develop selective coatings for gas-phase explosive molecules. Identified molecular recognition elements from the directed evolution process of phage display are used for their multivalent recognition of explosive targets in liquid-phase screening. Through chemical modification, the peptide receptors are linked with oligo(ethylene glycol) and immobilized as coatings capable of binding explosive targets in air.

chemistry of amino acids suggests that peptides are better suited as receptors against a wide range of target molecules. The challenge, however, is to identify the specific *N*-mer amino acid sequence that provides the strongest multivalent binding among 20^N possible sequences. Herein, we utilized the combinatorial screening power of phage display, an information mining tool, for the identification of peptides that can specifically recognize a desired target material at the molecular level (Figure 1). Evolutionary screening processes have been previously used in liquid environments for diverse material targets including semiconductors,[20,21] metals,[22−24] and proteins.[25−28] We utilized this approach to discover molecular recognition motifs capable of selectively binding explosive molecules TNT and DNT. Furthermore, to make these relevant for VOC detection, we have translated liquid-phase screening into gas-phase selective binding through the formation of receptor-laden hydrogels that attempts to emulate the olfactory system.

2. Experimental Section

2.1. Materials. Ph.D.-12 and -C7C phage display peptide library kits were purchased from New England Biolabs (Ipswich, MA). Tris−HCl, glycine−HCl, NaCl, BSA, Tween-20, Atto 425−streptavidin, and IPTG/Xgal were purchased from Sigma-Aldrich (St. Louis, MO). TNT and DNT crystals were obtained from Chem Service, Inc. (West Chester, PA). For peptide synthesis, preloaded (cysteine and biotinylated lysine) Wang resins were purchased from Novabiochem (San Diego, CA). Fmoc-aminodiethoxyacetic acid and cleavage reagents trifluoroacetic acid, thioanisole, water, phenol, ethanedithiol, and triisopropylsilane were also obtained from Sigma-Aldrich.

2.2. Phage Display for Selection of TNT and DNT Binding Peptide Motifs. Receptor screening utilized a phage library which possessed 3.9×10^9 different peptides, composed of both linear 12-mer (Ph.D.-12) and constrained 7-mer (Ph.D.-C7C) random amino acids, fused to the pIII coat proteins of M13 phages. The input phage library solution was prepared by adding 10 μL of each library to 1 mL of 0.1% TBST buffer (50 mM Tris−HCl (pH 7.5), 150 mM NaCl, 0.1% (v/v) Tween-20). To eliminate substrate heterogeneity, we screened the library against the molecular crystal form of TNT and DNT using standard phage display for peptide selection.[20,21] Phage display was performed with the initial binding condition of 0.1% TBST for 30 min at room temperature on a rocking platform with 5 mg of target TNT or DNT crystals. Following binding, a series of 10 wash steps were performed using the same binding buffer to remove nonspecific binders. Specific binding phages were eluted with 1 mL of 0.2 M glycine−HCl (pH 2.2), 1 mg/mL BSA. The progressive screening rounds utilized increasing surfactant concentration to increase stringency of binding to TNT or DNT targets. Screening results were obtained through sequence analysis of the receptor region following each screening round.

To determine the strongest TNT binding receptors from the large subset of phage display results, the phages bearing TNT binding peptides were collected. These 33 individual phage samples from TNT phage display screening results were picked and separately amplified and diluted into a single minilibrary of TNT binding phages having a 10^6 pfu/μL concentration of each phage. A single round of phage screening was performed against 5 mg of TNT in 0.2% TBST. Here, the TNT binding phages were simultaneously exposed to the TNT target substrate in solution. After this competitive binding, the weak binding phages were washed from the target, while the remaining strong binding phages were captured. These remaining phages were titrated on LB Xgal/IPTG agar plates.[20] Phage titration was used to select phage plaques with receptor inserts.[20] Twenty plaques, which appeared blue, were picked and sequenced to reveal the strongest binding sequence. Similarly, we identified the strongest DNT binding sequences from DNT target screening.

To determine the most selective receptors from phage display screening results, TNT binding phages and DNT binding phages were separately screened against both TNT and DNT substrates. To evaluate the extent of receptor binding activity to these target molecules, we measured the ratio of the amount of phages present after one round of screening with stringent washing steps to the amount of phages initially introduced (output/input). Specifically, the phages were amplified to a concentration of 10^6 pfu/μL (input), and each phage sample underwent one round of the screening process with 0.2% TBST against 5 mg of TNT and DNT separately. The amount of phages was identified via UV spectroscopy as well as titration. After titration, blue plaques were counted to determine the concentration of bound phages (output). This ratio of output to input was calculated for each phage sample and then related to that of a polystyrene (PS-BP) specific phage which has no particular binding preference to either TNT or DNT. PS-BP was, therefore, used as a negative "nonspecific" control. The receptor with the largest ratio difference between TNT and DNT substrate binding was designated most specific for its target substrate.

2.3. Peptide-Based Receptor Synthesis. All receptors were synthesized using standard Fmoc chemistry based solid-phase peptide

(20) Whaley, S. R.; English, D. S.; Hu, E. L.; Barbara, P. F.; Belcher, A. M. *Nature* **2000**, *405*, 665.
(21) Lee, S.-W.; Mao, C.; Flynn, C. E.; Belcher, A. M. *Science* **2002**, 892.
(22) Naik, R.; Stringer, S. J.; Agarwal, G.; Jones, S. E.; Stone, M. O. *Nat. Mater.* **2002**, *1*, 169.
(23) Sarikaya, M.; Tamerler, C.; Jen, A.; Schulten, K.; Baneyx, F. *Nat. Mater.* **2003**, *2*, 577.
(24) Sano, K.; Shiba, K. *J. Am. Chem. Soc.* **2003**, *125*, 14234.
(25) McCafferty, J.; Griffiths, A. D.; Winter, G.; Chiswell, D. J. *Nature* **1990**, *348*, 552.
(26) Smith, G. P.; Petrenko, V. A. *Chem. Rev.* **1997**, *97*, 391.
(27) Smith, G. P. *Science* **1985**, *228*, 1315.
(28) Belcher, A. M.; Lee, S. K.; Lee, S. W. *Adv. Mater.* **2003**, *15*, 689.

synthesis[29] with amino acids and preloaded (cysteine or biotinylated lysine) Wang resins. OEG-conjugated DNT receptors were synthesized by coupling three subunits of Fmoc-aminodiethoxyacetic acid to cysteine resin prior to addition of the DNT-BP sequence. Cleavage reactions were performed for 2 h with a cocktail of 82.5% trifluoroacetic acid, 5% thioanisole, 2.5% water, 5% phenol, 2.5% ethanedithiol, and 2.5% triisopropylsilane. Samples were purified by HPLC to >95% purity.

2.4. Mutational Assay. Utilizing alanine-substituted control peptides, we could identify the influence of individual substitutions on the receptor's binding capability and substrate specificity and thus identify the role of multivalent binding. By using a tetrapeptide biotinylated linker, the receptors could be functionalized with Atto 425−streptavidin probes for fluorescence binding assays. In this assay, 10 μL of 100 μg/mL TNT or DNT in acetonitrile was placed in 96-well TCPS plates. The TNT or DNT target was coated on the surface by introduction of 300 μL of TBS (50 mM Tris−HCl (pH 7.5), 150 mM NaCl) and allowed to set overnight at room temperature. The solutions were removed and rinsed with TBS to remove nonadsorbed TNT or DNT target. A 1 mM concentration of peptides was introduced to the TNT- or DNT-coated wells for 30 min. After binding, the substrate was washed with 0.1% TBST. A bovine serum albumin (BSA) blocking buffer was then used for 30 min to prevent nonspecific adsorption of the fluorophore. An avidin-labeled fluorophore, Atto 425−streptavidin, was then introduced while 20 min was allowed for binding to the attached biotinylated peptides. The substrate was washed with 0.1% TBST to remove any nonspecifically bound fluorophore. Finally, the bound receptor peptides, now conjugated with the fluorophore, were eluted from the TNT substrate with vigorous washing with 0.5% TBST. An Electromax Gemini EM plate reader was used to obtain the emission intensity at $\lambda = 476$ nm with excitation at $\lambda = 436$ nm to characterize the amount of eluted peptide, and the relative fluorescence signal was compared to the BSA background.

2.5. Isothermal Titration Calorimetry. Isothermal titration calorimetry (ITC) was used to obtain binding isotherms of the complex between TNT and the peptide TNT-BP, from which the dissociation constant was determined. The ITC reservoir cell (kept at 25 °C under constant mixing) contained a 10 μM solution of TNT-BP in acetonitrile to which a stock solution of TNT (100 μM in acetonitrile) was added with an initial 5.0 μL equilibration injection volume followed by 15 μL injections every 5 min. The heat released per ITC injection of TNT was measured, and the integrated heat plotted against the molar ratio of TNT added to TNT-BP was obtained to give a complete binding isotherm for the interaction. To determine the dissociation constant, the data were fit to a one-site binding model.

2.6. Gold Chip Preparation. A (100) silicon wafer was cleaned with heated piranha solution. A 5 nm chrome layer was thermally evaporated onto the wafer as an adhesion layer between the gold and silicon. A 25 nm thick layer of gold was then thermally evaporated onto the wafer. The wafer was then protected using 2 μm of G-line photoresist prior to dicing into 3 mm by 3 mm chips. The photoresist was then stripped using heated PRS-3000 solution, and the chips were cleaned and dried.

2.7. Gas-Phase Binding Assays. As a novel extension to standard liquid-phase receptor identification and to make it relevant for gas-phase chemical sensing, we embedded the selective DNT receptors in a hygroscopic oligo(ethylene glycol), OEG, coating (described in section 2.3) to test gas-phase binding. Given DNT's higher vapor pressure compared to that of TNT, we found it more applicable to focus our gas-phase experiments solely on the identified DNT binding peptide. Vapor pressures of TNT and DNT are 0.7×10^{-3} and 1.6×10^{-3} mmHg, respectively, under experimental conditions of 60 °C.[30,31] Multiple coating conditions were analyzed including (i) DNT receptor−OEG−Cys on gold chips, (ii) OEG−Cys on gold chips, (iii) a blank gold surface, (iv) DNT receptor−OEG−Cys on a blank silicon surface, (v) OEG−Cys on a blank silicon surface, and (vi) a blank silicon surface. Immobilization of the coating layers was carried out by immersing the different chips in a 1 mM solution of either DNT−OEG−Cys or OEG−Cys for 24 h utilizing the available gold−thiol bond chemistry. The coatings were then exposed to target gas in ambient air by placing the chips inside a scintillation tube containing crystalline DNT or TNT which was heated uniformly to 60 °C for 2 h using a custom-designed aluminum heat block with an NIST-certified temperature controller (VWR Inc.) to generate DNT or TNT gas. All experiments were performed with chips exposed to 18 ppm DNT gas.[30,31] The chips were immediately analyzed for the amount of bound DNT and TNT by placement in the thermal desorption tube of a Unity thermal desorption system, which heated the chips to 300 °C and passed the desorbed particles directly to an Agilent GC−MS system (Santa Clara, CA). Partition coefficients are identified as the ratio of the concentration of analyte bound to the coating to the concentration of analyte in the exposed gas headspace and normalized to the appropriate control conditions (i.e., blank Si and OEG−Cys coatings).

2.8. Statistical Analysis. To determine the statistical significance of the fluorescence experiments, normalization was performed from the corresponding BSA control samples to account for baseline variations between different substrates. After normalization, the ratio values (peptide/BSA) were analyzed for statistical significance using one-tailed ANOVA within substrate groups ($P < 0.0001$, $n = 4$) with STATA software (Stata Corp., College Station, TX). To determine the statistical significance between TNT/DNT gas experiments, the values were compared using a two-tailed Student's t test ($P < 0.001$, $n = 4$). All data are presented as the mean ± SD as indicated.

3. Results and Discussion

3.1. Identification of TNT and DNT Binding Peptides. The phage display results from third and fourth round screenings against molecular TNT crystals are represented in Supporting Information Figure 1. After four rounds of TNT screening, we identified TNT binding sequences with the consensus motif of Trp-His-Trp-X (X = hydroxylated, amine, or positively charged side chain) at the N terminus of the receptor. Similarly, the resulting phage display sequences from third, fourth, and fifth round screenings against the molecular DNT crystal target can be seen in Supporting Information Figure 2. Through these experiments, we arrived at the consensus DNT binding motifs and TNT binding motifs depicted in Figure 2a.

Within the competitive screening experiments, 33 phage samples were screened against TNT, and their corresponding pIII receptor sequences can be seen in Supporting Information Figure 3. The remaining bound phages were captured, and 20 of the phages were randomly picked for sequence analysis. The last column of Supporting Information Figure 3 represents the number of appearances of a given receptor amino acid sequence from the 20 random phages. The most abundant of these receptor sequences indicates the highest level of TNT binding. Importantly, 95% of these strongest TNT binding phages exhibited the same N terminal tetrapeptide motif: Trp-His-Trp-X. Through this rigorous competitive screening, we assigned the most abundant binding sequence as the strongest TNT binding peptide candidate (Trp-His-Trp-Gln-Arg-Pro-Leu-Met-Pro-Val-Ser-Ile, TNT-BP). Similarly from the identified DNT binding sequences, His-Pro-Asn-Phe-Ser-Lys-Tyr-Ile-Leu-His-Gln-Arg was found to be the strongest DNT binding sequence and was denoted DNT-BP.

From the phage display results, we assessed the best binding phage by greatest target selectivity. This target selectivity was determined on the basis of the relative level of phage binding to TNT and DNT target substrates. Among the subset of TNT binding sequences, several exhibited varying affinity for DNT

(29) Grun, J.; Revell, J. D.; Conza, M.; Wennemers, H. *Bioorg. Med. Chem.* **2006**, *14*, 6197.
(30) Rittfeldt, L. *Anal. Chem.* **2001**, *73*, 2405.
(31) Lenchitz, C.; Velicky, R. W. *J. Chem. Eng. Data* **1970**, *15*, 401.

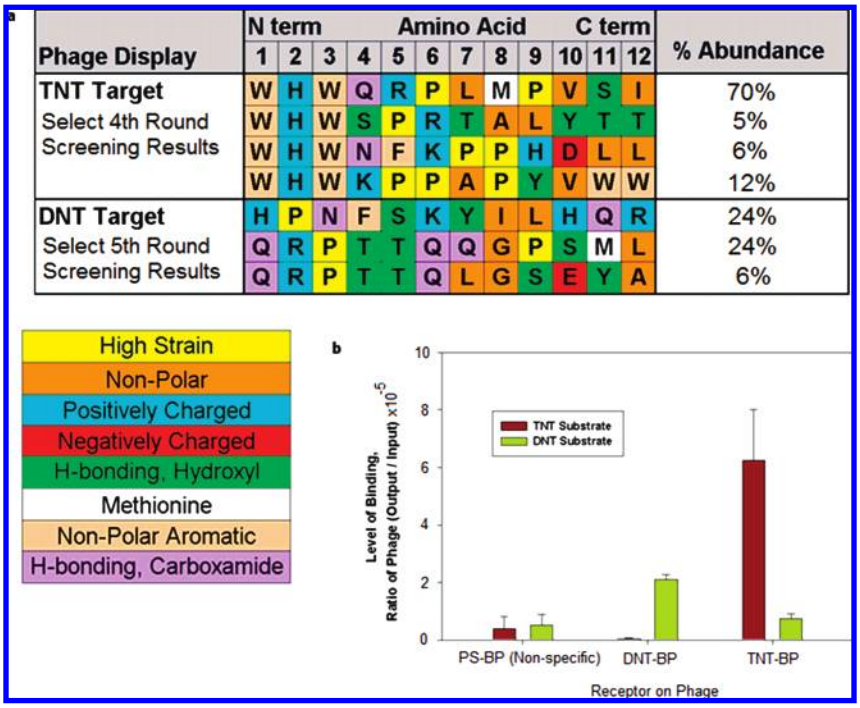

Figure 2. Phage display screening results: **(**a**)** converged amino acid sequences from the fourth round of phage display screening with a noted percentage abundance obtained from sequencing results, **(**b**)** selectivity screening of the DNT receptor and TNT receptor against DNT substrates and TNT substrates with the level of binding quantified from phage titration. Nonspecific binding levels are identified by PS binding phages against TNT and DNT substrates. All data are presented as the mean ± standard deviation.

(Supporting Information Figure 4). We selected the phage with the largest binding difference between TNT and DNT targets (those with strong binding to TNT and low affinity for DNT), which was determined to have the TNT binding peptide, TNT-BP. Similarly from DNT phage display results, we screened various DNT binding phages against TNT and DNT and identified the most selective phage to contain the sequence from DNT-BP (Supporting Information Figure 5). From our selectivity screening, the peptide TNT-BP was found to have only nonspecific interactions with DNT as demonstrated by comparison to the negative control phage (Figure 2b). Interestingly, DNT-BP binding to TNT also remained on the order of nonspecificity (Figure 2b). This important result demonstrates that M13-linked receptor sequences identified from phage display can selectively bind a predetermined target.

3.2. Binding Studies of Synthetic TNT Binding Peptides. While target-specific phage binding was a critical first step, the fluorescence binding studies indicated that the receptors retained high substrate specificity despite no longer being attached to the M13 phage (Figure 3). The selectivity of the TNT receptor is demonstrated by the significantly higher fluorescence levels of TNT-BP to the TNT substrate as compared to the low levels of binding against the DNT substrate. Substitutions in the N terminal region with alanine residues demonstrate the importance of the conserved tryptophan and histidine residues in the binding event. This supports the importance of multisite interactions, as the tryptophan replacement resulted in a 58% decrease in binding while the histidine replacement decreased binding by 48%. By comparing the TNT binding sequence, scrambled TNT binding sequence, histidine substitute, and tryptophan substitute for different substrates, we provide evidence of substrate selectivity through multivalent binding. Direct comparison of the TNT binding sequence for TNT and DNT substrates demonstrates the ability of the isolated TNT receptor to bind selectively (over 3-fold increase) to the TNT substrate while remaining relatively inert to the DNT substrate (on the order of the nonspecific scrambled sequence and background (Figure 3b)). Similarly, our isolated DNT receptor preferentially bound the DNT substrate (over 2-fold increase compared to the response against the TNT substrate (Figure 3b)).

The binding isotherm obtained from the titration of TNT into a solution of TNT-BP is provided in Figure 4. This isotherm, calculated from the integrated heat change per mole of TNT injected and fit to a one-site binding model, provided the best-fit parameter values of the binding constant and the enthalpy of $K = 1.4 \times 10^7 \pm 3 \times 10^6\ M^{-1}$ and $\Delta H = -49 \pm 1$ kcal/mol, respectively. The inverse of the calculated K value gives us the dissociation constant of 71 nM for TNT-BP interaction with TNT. These results support our ability to use evolutionary screening to identify a receptor for a predetermined target.

3.3. Gas-Phase Binding Assays. The results of Figure 5 represent the various control experiments performed to identify the extent to which DNT would interact with the various components of the Au−DNT-BP coating. Silicon chips were exposed to DNT gas and used as the background signal for DNT partition coefficient measurements for the various coatings of the Si chips. Six chip conditions were utilized for DNT gas experiments: (i) DNT receptor−OEG−Cys on gold chips, (ii) OEG−Cys on gold chips, (iii) a blank gold surface, (iv) DNT receptor−OEG−Cys on a blank silicon surface, (v) OEG−Cys on a blank silicon surface, and (vi) a blank silicon surface (control). Importantly, the amounts of DNT bound to conditions iv and v were relatively the same as that for the blank Au control (iii). This indicates the OEG−Cys or DNT receptor−OEG−Cys coating attachment is inhibited on the Si substrate as compared to their attachment to Au-coated substrates under the same conditions. Furthermore, Figure 5 identifies the highest DNT partition coefficient for condition i in which DNT receptor−OEG−Cys was used as the coating for the Au chip. By displaying this comparatively large DNT partition coefficient using the DNT receptor, we demonstrate the ability to translate from liquid-phase screened receptors into gas-phase target binding.

a

Peptide Name	N term 1	2	3	4	5	6	7	Amino Acid 8	9	10	11	12	13	14	15	C term 16	Sequence Description
TNT-BP	W	H	W	Q	R	P	L	M	P	V	S	I	G	G	G	K-Biotin	Trinitrotoluene binder
W-Sub	*A*	H	W	Q	R	P	L	M	P	V	S	I	G	G	G	K-Biotin	1st Tryptophan replaced TNT-BP
H-Sub	W	*A*	W	Q	R	P	L	M	P	V	S	I	G	G	G	K-Biotin	Histidine replaced TNT-BP
Scram-Ctrl	L	P	S	M	R	V	W	P	Q	W	I	H	G	G	G	K-Biotin	Scrambled TNT-BP, - control
DNT-BP	H	P	N	F	S	K	Y	I	L	H	Q	R	G	G	G	K-Biotin	2,4 Dinitrotoluene binder

b

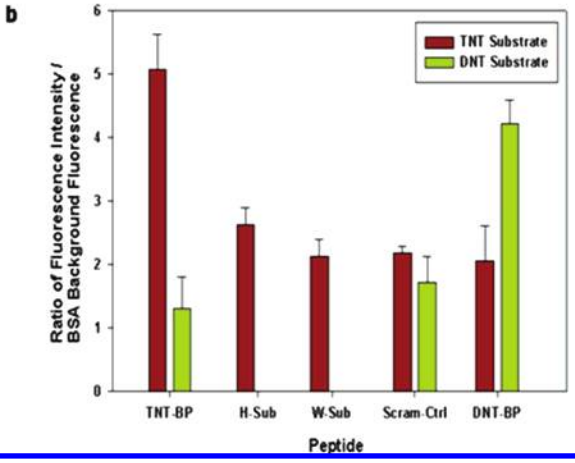

Figure 3. Mutational analysis for identified TNT and DNT binding peptides. **(**a**)** Sequence of receptors synthesized with C terminal biotinylated lysine to allow functionalization with the fluorescent probe Atto-425. TNT-BP and DNT-BP represent the identified TNT and DNT binding receptor sequences, respectively, while H-Sub (histidine substituted by alanine) and W-Sub (tryptophan substituted by alanine) are mutations at amino acids 1 and 2 of TNT-BP. Scram-Ctrl is a nonspecific sequence derived from scrambling the TNT-BP sequence to demonstrate the sequence importance for encoding target selectivity and hence a negative control. **(**b**)** Fluorescence binding assay against TNT substrate and DNT substrate, revealing the importance of tryptophan and histidine residues in TNT-BP as well as demonstrating the ability of the lone peptides TNT-BP and DNT-BP to bind selectively to their targets in liquid when not associated with the other phage body proteins. Fluorescence levels are normalized to the BSA fluorescence background signal ($P < 0.0001$, $n = 4$). All data are presented as the mean ± standard deviation.

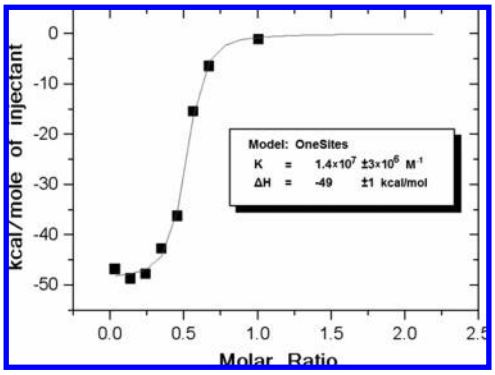

Figure 4. Measurement of the dissociation constant of the complex between TNT and the peptide TNT-BP determined from isothermal titration calorimetry. Data points (values for the integrated heat change during each injection, normalized per mole of TNT) are represented by filled squares. A one-site model was used to fit the data. The solid red line is the calculated curve using the best-fit parameters.

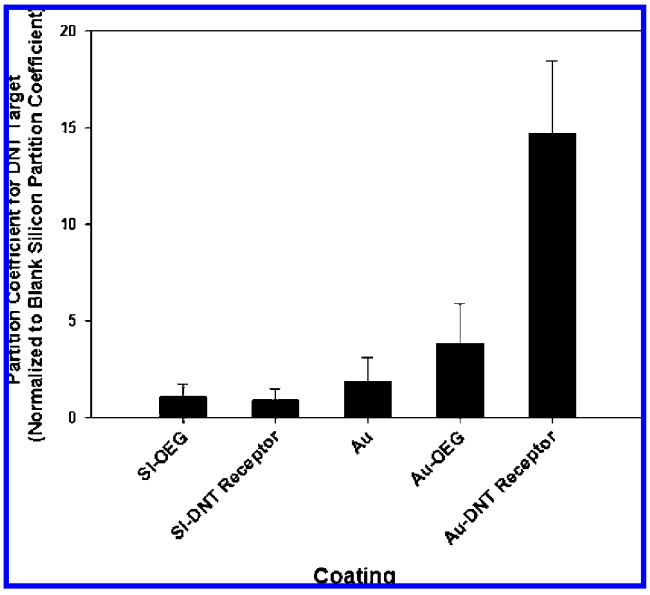

Figure 5. Gas-phase screening for partition coefficients of various coatings on Si exposed to DNT gas. The values are normalized to the DNT gas partition coefficient of blank Si substrates to observe the contribution attributed solely to the coating layer. Partition coefficients are calculated as the ratio of the concentration of analyte bound to the coating (identified through thermal desporption GC−MS) to the concentration of analyte in the exposed gas headspace.

The gas-phase binding results for the DNT binding peptide (Figure 6b) show a 4-fold increase in the partition coefficient for DNT over TNT as a result of the DNT receptor. The preferred coating condition for selective binding of DNT gas was that of the identified DNT-BP. Additionally, we found the DNT-BP partition coefficient for TNT gas is in the same range as that of the OEG-coated chip without a receptor, indicating that the selectivity of the DNT receptor remains when implemented in the gas phase.

While successful biosensors have previously been developed for DNT detection in aqueous solution,[19,32,33] this demonstration of a selective coating for DNT in the gas phase is of particular importance as these short OEG-embedded receptors are capable of retaining efficacy outside the liquid environment. The success of gas-phase binding may be attributed to the properties of OEG indicated by various research groups including (i) the ability of

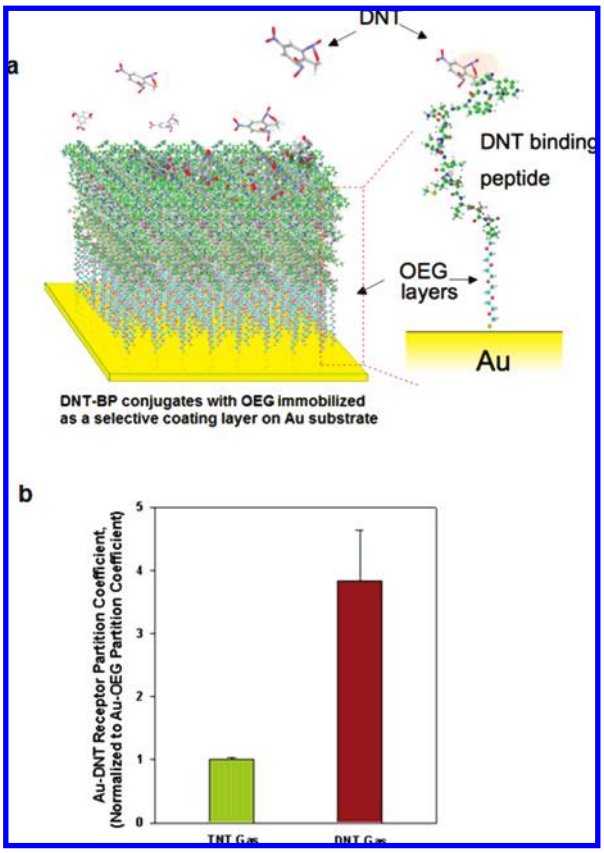

Figure 6. Selective gas-phase binding assay for DNT-specific coating: **(**a**)** schematic diagram of the DNT binding peptide conjugated to oligo(ethylene glycol) and their coating onto a gold surface for gas-phase selective binding; **(**b**)** partition coefficient of DNT receptor coatings exposed to TNT gas and DNT gas. The values are normalized to the target gas partition coefficient of OEG coating on a Au substrate to isolate the contribution attributed to the DNT receptor element. Partition coefficients are identified as the ratio of the concentration of analyte bound to the coating to the concentration of analyte in the exposed gas headspace. The results are obtained through thermal desorption GC−MS experiments on exposed coating surfaces ($P < 0.001$, $n = 4$). All data are presented as the mean ± standard deviation.

OEG to retain the conformation of biomolecules[34] and (ii) the selectivity of peptides remaining unaffected by OEG conjugation.[29] Additionally, PEG is often used for its nonfouling properties, which may be beneficial in terms of minimizing false positives in sensing applications.[35]

4. Conclusion

Using directed evolutionary screening processes, we identified specific TNT and DNT binding peptides. Compared with the resulting TNT binding peptide, (Trp-His-Trp-X, where X represents Gln, Ser, Asn, or Lys), the active site of nature's known TNT binding protein *Enterobacter cloacae*'s pentaerythritol tetranitrate (PETN) reductase (Trp_{102}, His_{181}, Tyr_{186}, Thr_{26}) contains many compositional similarities.[36] PETN reductase and other TNT binding proteins display a highly conserved tryptophan residue involved in the binding event.[36,37] Furthermore, these previous studies show that changes in the tryptophan's neighboring amino acid, histidine, can drastically modulate the TNT binding ability of these proteins. Similarly, through mutational analysis of our TNT binding motif (Figure 3b), we have demonstrated the role of multivalent binding involved with neighboring tryptophan and histidine residues. Various mechanisms exist by which tryptophan may attribute its strong role in the binding motif for TNT. The PETN reductase utilizes the aromatic stacking between tryptophan and the ring structures of nitroaromatics.[38] Through computational approaches, researchers have identified similar dual aromatic residues as part of a high-affinity TNT binding motif which parallels the tryptophan arrangement of our own TNT binding sequence.[17] Tryptophan's interaction with TNT may take on a donor−acceptor character due to the electron deficiency of the ring in TNT, while those of tryptophan are electron rich.[39] Histidine can also contribute to $\pi-\pi$ interactions.[40] In addition, imidazole side chains can coordinate with the nitro group in the TNT molecules through partial charge−charge interactions or hydrogen bonding.[36,41]

In summary, we have shown the successful evolutionary screening of highly selective peptide receptors for explosive targets, such as TNT and DNT. We discovered a peptide motif which coincides with the TNT binding site in PETN reductase that has evolved in nature. Using mutational analysis, we demonstrated that multivalent binding is the key to selectivity of the TNT binding motif. To make the discoveries relevant for gas-phase chemical sensing, we created a biomimetic coating for highly selective detection of DNT in ambient conditions. We believe that this approach of evolutionary peptide screening followed by the creation of biomimetic coatings, when generalized for other target molecules, reflects a significant advance to enable highly selective and sensitive miniaturized chemical sensors.

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Supporting Information Available: Complete phage display screening results and further fluorescence binding assay results. This material is available free of charge via the Internet at http://pubs.acs.org.

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(32) Naal, Z.; Park, J. H.; Bernhard, S.; Shapleigh, J. P.; Batt, C. A.; Abruna, H. D. *Anal. Chem.* **2002**, *74*, 140.
(33) Radhika, V.; Proikas-Cezanne, T.; Jayaraman, M.; Onesime, D.; Ha, J. H.; and Dhanasekaran, D. N. *Nat. Chem. Biol.* **2007**, *3*, 325.
(34) Keeling-Tucker, T.; Rakic, M.; Spong, C.; Brennan, J. D. *Chem. Mater.* **2000**, *12*, 3695.
(35) Otsuka, H.; Nagasaki, Y.; Kataoka, K. *Curr. Opin. Colloid Interface Sci.* **2001**, *6*, 3.
(36) Khan, H.; Barna, T.; Harris, R. J.; Bruce, N. C.; Barsukov, I.; Munro, A. W.; Moody, P. C. E.; Scrutton, N. S. *J. Biol. Chem.* **2004**, *279*, 30563.
(37) Little, J. R.; Eisen, H. N. *Biochemistry* **1967**, *6*, 3119.
(38) Dower, S. K.; Gettins, P.; Jackson, R.; Dwek, R. A.; Givol, D. *Biochem. J.* **1978**, *169*, 179.
(39) Guan, X. Y.; Gu, L. Q.; Cheley, S.; Braha, O.; Bayley, H. *ChemBioChem* **2005**, *6*, 1875.
(40) McGaughey, G. B.; Gagne, M.; Rappe, A. K. *J. Biol. Chem.* **1998**, *273*, 15458.
(41) Khan, H.; Harris, R. J.; Barna, T.; Craig, D. H.; Bruce, N. C.; Munro, A. W.; Moody, P. C. E.; Scrutton, N. S. *J. Biol. Chem.* **2002**, *277*, 21906.