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Bacterial Display Peptides for Use in Biosensing Applications

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1. Introduction

Peptide affinity reagent technology including bacterial display encapsulates an in vitro analog of classical Darwinian evolution through a physical linkage between polypeptide sequence and the encoding of genetic information. Similar to the inate human imune system a unique binder is isolated, however, in this directed evolution method a large library displaying billions of diverse peptide sequences is created and the sythetic binder to the target of interest is determined. Subsequently the synthetic reagent is mass-produced for use in the specific biosensing application (Park & Cochran, 2010; Stratis-Cullum & Sumner 2008).

Currently, a number of systems, including messenger ribonucleic acid (mRNA) and ribosome display (Wilson, Keefe, & Szostak, 2001), eukaryotic virus display (Bupp and Roth, 2002; Muller, 2003), and bacterial and yeast surface display (Georgiou et al., 1997, Boder & Wittrup, 1997), are used to rapidly generate affinity reagents that can be used for diagnostics, proteomics, and therapeutic applications (Kodadek, 2001; Nixon, 2002). Bacterial cell surface display is advantageous because the use of bacterial cells simplifies the polypeptide selection method and enables fast screening of potential recognition elements typically using fluorescence activated cell-sorting (FACS) and/or magnetic activated cell-sorting (MACS). Polypeptide affinity reagents can offer more stable alternatives to antibody technology, enabling more rugged application in the field (e.g., enhanced thermal stability). A key advantage of bacterial display technology over traditional antibody creation as well as other peptide display alternatives is that it offers an strategy for generating tailor-made affinity ligands in a very short time period of several days (Georgiou et al., 1997; Bessette, Rice and Daugherty 2004), see Figure 1.

The development of a bacterial display system suitable for robust reagent discovery has proven challenging (Lee, Choi & Xu, 2003). There are three main challenges in creating a high speed affinity ligand isolation technology against unknown/un-catalogued pathogens: (1) creation of a large (high diversity) and robust cell based library, (2) creation of an ultrahigh throughput, disposable screening system, and (3) gaining a fundamental understanding of the factors which influence binder performance along with this understanding the development of methodologies to enable universal isolation and optimization of ligand binder performance.
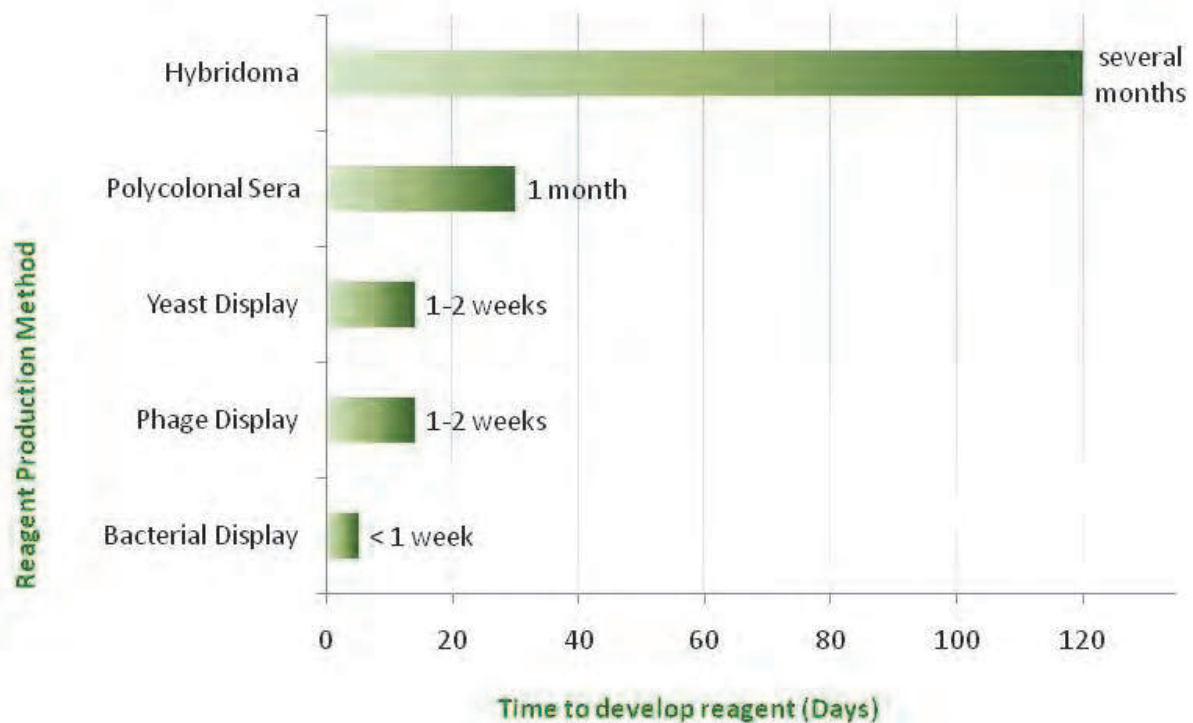
Fig. 1. Comparison of time to develop reagents using various reagent methodologies.

The first challenge is critical to any synthetic library. In general, the larger the pool of mutants and the longer the polypeptide sequence, the greater the potential pool of binders from which the sequences with the desired properties are isolated. However, the reproducible control over the expression and ease, speed, and robustness of associated isolation protocols are also key to practical implementation across varied laboratory settings. The second challenge is also critical to practical and routine reagent isolation, as the high throughput processing and removal of human bias and error throughout the selection process is critical. Coupled with this, the ability to perform the isolation in a contained area or disposable unit is ideal for many biohazard applications, to minimize human exposure.

Sorting, or screening of peptide libraries for suitable reagent candidates is typically performed by a combination of several rounds of manual MACS for pre-enrichment and several rounds of FACS. Although effective, the approach is quite labor intensive, and most importantly, the performance characteristics of the resulting reagents are highly operator dependent. Furthermore, the cost of an instrument equipped with full FACS sorting and isolation is often prohibitively expensive (~$350-500K) for many laboratories. Consequently, the ability to automate the process on an inexpensive platfrom is highly desired.

There are several key capabilities that must be considered in the evaluation of any potential platform for library cell-sorting. For example, high throughput screening is desired to handle large libraries (several mL of $> 10^{10}$ member libraries) in a reasonable time frame (several minutes). Typically with the currently employed sorting technologies relying on a combination of MACS and FACS sorting, the throughput is determined by the MACS sample pre-enrichment prior to FACS sorting since fluorescence cell sorting methods using ultra high-speed sorting only approach 100,000 cells/sec (Leary, 2005). The recovery (fraction of binders collected relative to the total number of binders in the naïve library) is

also critical to affinity ligand development and to applications in medicine for cell identification, such as cancer cell isolation and population enrichment (Lara et al., 2004; Xu et al., 2009; & Krivacic et al., 2004). The purity of the isolated fraction is another consideration since the goal is to isolate the rare population of binder clones, without the other library clones present in order to enrich the binding pool in subsequent growth cycles. We reported recently on our approach to address both the challenges in diversity and reproducible isolation through the combination of two innovative technologies, microfluidic cell sorting, and bacterial display technology (Stratis-Cullum, Kogot, & Pellegrino, 2008; Sooter et al., 2009; Zhang et al., submitted for publication). Previous work by the Daugherty lab, details the development and early screening results with the eCPX platform (Rice & Daugherty, 2008). Briefly, this peptide library employs an *E. coli* bacterial display platform, generated from the extracellular loop of OmpX outer-membrane protein (see Figure 2). The randomizedportion of the library is a 15-mer, yielding a greater than 10^{10} member library. In the library utilized throughout these studies is an enhanced form, termed eCPX. The OmpX outer membrane protein has been engineered into a circularly permuted scaffold to allow both C-terminal and N-terminal display of proteins.
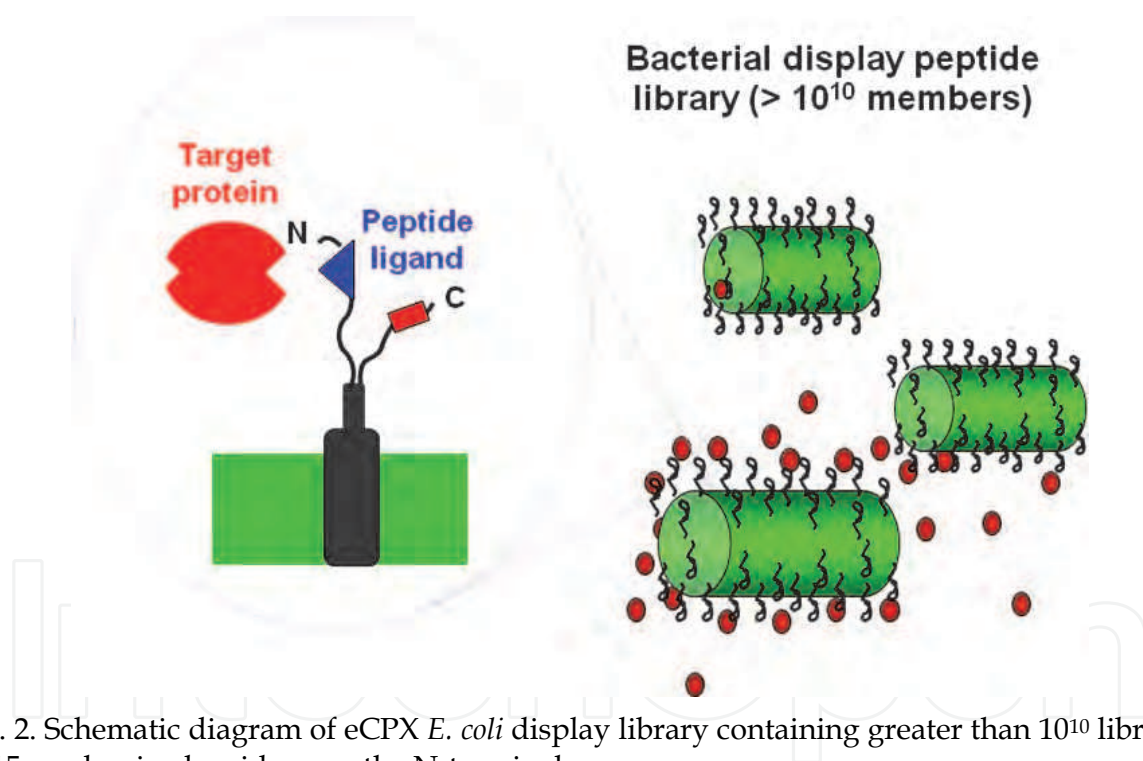

Fig. 2. Schematic diagram of eCPX *E. coli* display library containing greater than 10^{10} library of 15 randomized residues on the N-terminal.

To address the need for a rapid, safe, efficient, cost effective, and reproducible method for peptide affinity ligand isolation, we previously developed an automated magnetic bacterial cell-sorting system, the micromagnetic cell sorter (MMS) shown in Figure 3 (Stratis-Cullum, Kogot & Pellegrino, 2010). The system is equipped with a disposable polypropylene microfluidic cartridge and is capable of autoclave sterilization. The aim is to contain the mixing, incubation, high performance magnetic trapping and isolation of library binders within the disposable cartridge to minimize human error, human exposure, and automate the sorting process for reproducible sorting.
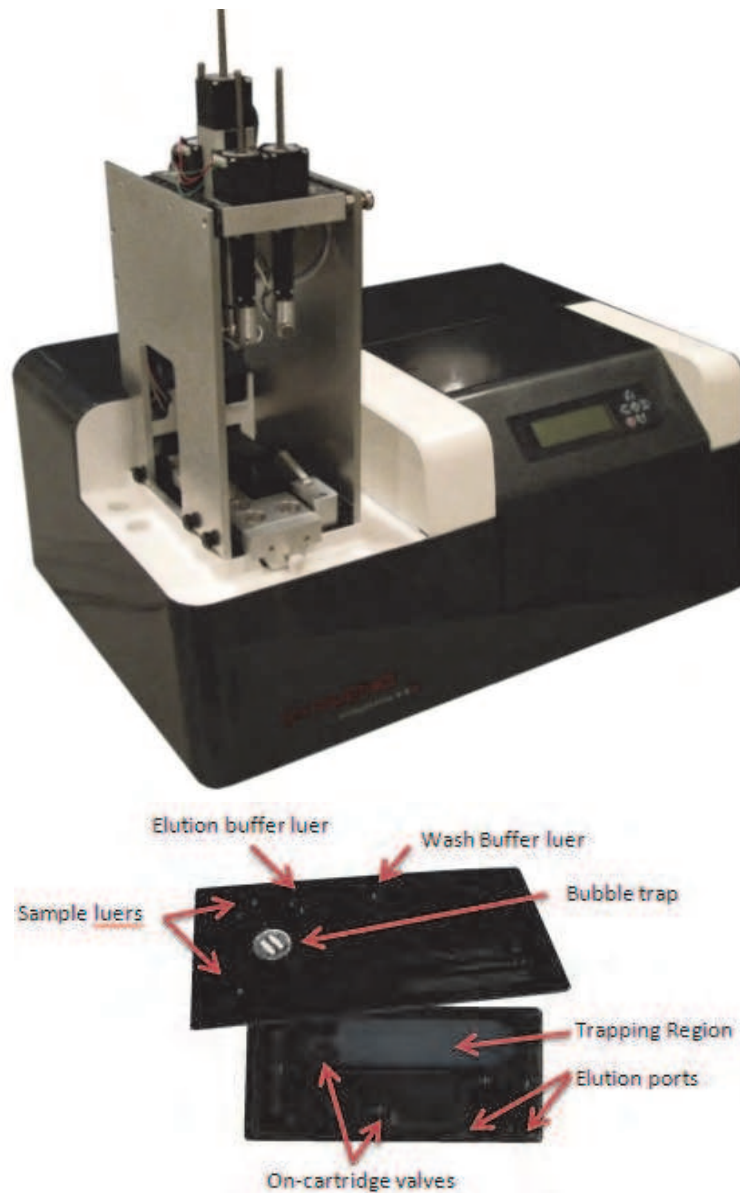
Fig. 3. Photograph of the semi-automated Micro Magnetic Sorter (MMS) platform and disposable polypropylene cartridge for fluid handling and peptide ligand isolation.

To demonstrate the sorting ability of the MMS system to isolate peptide binders, protective antigen (PA) protein of *Bacillus anthracis* was chosen for evaluation. The eCPX (CytomX Therapeutics) bacterial display library (Rice, 2008), expressing ~3 x 10^{10} discreet random peptides, was screened for affinity reagents capable of binding to protective antigen. Of note, comparable results to manual MACS and FACS screening were obtained (Stratis-Cullum, Kogot & Pellegrino, 2010). In addition, excellent recovery performance through MMS selection yielded a consensus sequence among 24 unique binders and directly correlates to a MACS/FACS binder sequence.

Despite the promise of rapid selection of peptides for detection, initial selections do not typically yield the most specific or highest affinity binders and often require continued experimental optimization and selection stringency with variable results (Park & Cochran, 2010). Currently, soft randomization of peptide display libraries provides the most

convenient method to achieve the highest binding affinity for isolated peptides. The third major challenge in affinity reagent isolation identified above stems from these inherent limitations from any directed evolution/biopanning library methodology. The selection of affinity ligands is performed typically without prior knowledge of the target. Depending on the stringency of selection, and criteria of selection many clones will be excluded from propagation early in the process. Moreover, there are fundamental biases in starting library composition as well as statistical losses throughout the emperical sorting methods. Often the highest affinity binder is selected, while the specificity of the binding element (equally critical to successful implementation in a biosensor device) is not optimized. Alternatively, specificity can be a criteria of selection, particularly through controlled negative sorting rounds. In either case, there is an affinity maturation process that must be employed to optimize back the best binders which may not have propagated during the selection process. There are also performance issues with moving in an on-cell format during the selection process, to off-cell in a solubilized peptide reagent format that must be considered when characterizing potential reagent performance.

In this paper, we have chosen protective antigen (PA) of an anthrax toxin (*Bacillus anthracis*) as a target and performed peptide reagent isolation studies using the eCPX library and the high performance sorting technology we previously developed known as the Micro Magnetic Sorter (MMS). In these studies we compare the binding performance of the isolated clones on scaffold (cell) to both the PA target and a streptavidin negative control to evaluate relative cross-reactivity. Finally, we compare the best on-scaffold peptide candidate to the off-scaffold performance.

2. Materials and methods

2.1.1 MMS disposable microfluidic card

The Micro-Magnetic Sorter (MMS) is an automated magnetic separation system consisting of a disposable microfluidic cartridge and a companion instrument (Figure 3). The disposable cartridges are made of injection-molded polypropylene (Pinnacle Polymers PP 5135C). The 200 µm deep fluidic channels are defined by two injected parts, which are laser-welded (California Lasers, Simi Valley, CA) and a portion is heat staked with a hydrophobic membrane for bubble removal (Pall Co, Ann Arbor, MI). The trapping region was designed to accommodate up to 1×10^9 of 1 µm trapped magnetic beads and process up to 1×10^{11} bacterial cells. Female luer fittings on the top of the cartridge allow for a leak-proof interface between the cartridge and disposable syringes (Becton Dickinson, San José, CA). The luer fittings on the cartridge are designed to hold a reservoir array, for pneumatically driven applications as well as the injector inputs. There are a total of four luer ports required for two sample injectors (1 or 5 mL volume), one running/wash buffer injector (up to 10 mL) and one elution buffer injector (up to 3 mL volume). Strategically designed micro-channels allow for full automation of magnetic separation on the cartridge. To accomplish this, five pneumatically actuated pinch valves are located on the underside of the cartridge, which allow for the redirection of flow. These valve membranes require a force of ~15 lb/in² to seal and are robust enough to be actuated multiple times.

2.1.2 MMS Instrumentation

The instrument utilizes a cRIO controller with LabVIEW script (National Instruments, Austin, TX) outfitted with standard digital and analog in/out modules for control of the

internal components. Flow rates within the cartridge are controlled by four stepper motors (Figure 7c) and controller boards (Haydon and Anaheim Automation, respectively), which physically push on the injectors (Figure 7d). These motors are fitted with micro-switches (Panasonic ECG, Secaucus, NJ) that allow for the automatic calculation of input volume. Valves on the cartridge are actuated using pneumatically controlled air cylinders (SMC Corp, Noblesville, IN) and a DC diaphragm pump (Thomas provided by Nor Cal Controls, San Jose, CA). There are seventy custom neodymium-iron boron magnets, which are position-controlled by another Haydon stepper motor. The magnets are distributed equally among top and bottom portions of a magnetic rack, which sandwich the disposable cartridge. A single motor, in conjunction with a spring, allows for both horizontal and vertical movement of magnets. This facilitates horizontal movement required for trapping and elution, and vertical movement capable of agitating the sample within the cartridge. Software control is provided using a LabVIEW interface. Push button applications have been created for bacterial library sorting. Advanced users can generate custom sorting routines, which allow full access to all the operation parameters with minimal training. Direct control of flow rates, wash stringencies, and positive/negative selection criteria enables the end user to specify the magnetic bead and protocol of choice, and optimize it for applications beyond bacterial library sorting (i.e. cell culture, flow cytometry, toxicology studies, etc.).

2.2 Sorting procedures and sample preparation

Figure 4 shows a general schematic of the library sorting scheme. A bacterial display library (Cytomx Therapeutics; San Francisco, CA: eCPX library) which contains approximately 3×10^{10} members was screened for clones that display PA binding peptides. The random library is first grown in 500 mL LB media containing 25 μg/mL chloramphenicol (LB-Cm[25]) to an OD_{600nm} of approximately 0.6 (Eppendorf Biophotometer; Eppendorf, Hamburg, Germany). At this point in exponential growth phase the cells were induced by the addition of arabinose to a final concentration of 0.04% (w/v); the enhanced circularly permuted OmpX (eCPX) gene expressing the library peptides is under the control of an arabinose inducible promoter (Rice, 2008). The cells were shaken at 37 °C for an additional 45 mins, after which the OD_{600nm} was again measured and, using the assumption that an OD_{600nm} of 1.0 relates to a bacterial concentration of 1×10^9 cfu/mL, approximately 2×10^{11} cells were pelleted by centrifugation at 3000g for 20 mins.

2.3 Streptavidin-binder depletion

The bacterial pellet was re-suspended in 1.5mL of PBSB (PBS buffer plus 0.5% BSA) containing 1×10^9 paramagnetic beads (Invitrogen DynabeadsMyOneStreptavidin C-1; Invitrogen, Carlsbad, CA). The cell suspension was incubated at 4 °C for 45 mins with rotation to allow depletion of streptavidin binders from the library prior to selections. To remove these beads and any cells bound to them the sample was loaded onto an MMS cartridge and separated at a sample flow rate of 50 mL/hr and buffer flow rate of 10 mL/hr. The MMS cartridge captured the unwanted bead bound cells and allowed collection of the depleted library ready for enrichment. SA binder depletion was performed using using a benchtop magnetic bead separator (manual MACS). The bacterial cell pellet with 1×10^9 paramagnetic beads was pelleted using a magnet next to the tube. The magnetic separation was performed for 5 mins to allow the bead pellet to form, the sample was washed and aspirated with 5 x 1 mL PBS washes, and resuspended in 1 mL PBSB for PA binder enrichment.
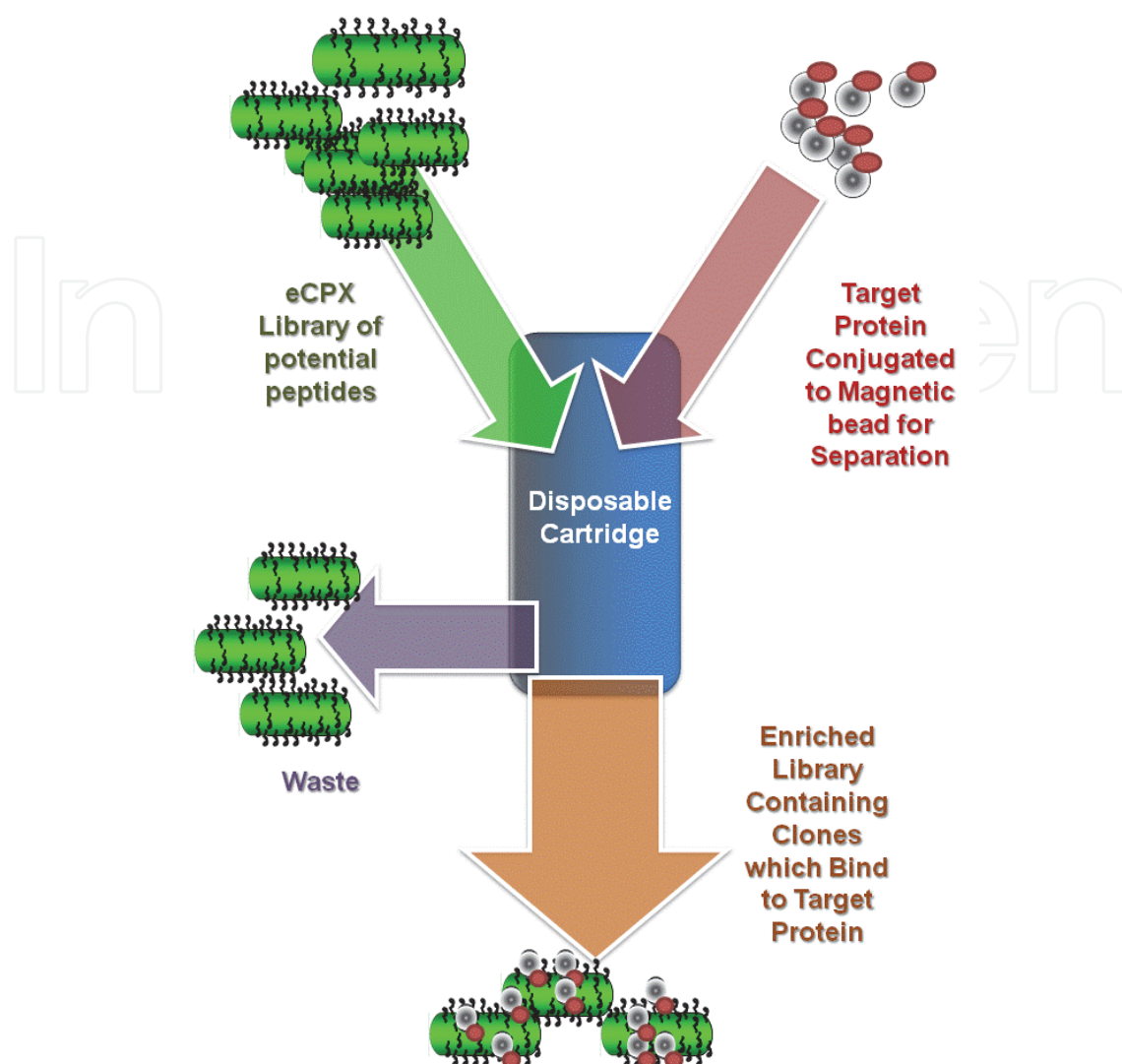
Fig. 4. Schematic diagram of library sorting inputs, and outputs using eCPX technology and target conjugation to magnetic bead for trapping within disposable cartridge.

2.4 PA-binder enrichment

The SA-binder depleted library was centrifuged at 3000 g for 20 mins, resuspended in 1mL PBSB buffer containing 600 nM biotinylated protective antigen (List Biological Laboratories, Inc; Campbell, CA), and incubated at 4 °C for 45 mins. Cells were centrifuged as above and re-suspended in 1 mL PBSB buffer with 1×10^9 pre-washed magnetic beads. After 45 mins at 4 °C with rotation, the cell-beads suspension was loaded into an MMS cartridge (or separated by manual MACS using the same methods as SA binder depletion). Bacterial cells bound to PA were trapped on cartridge, and then eluted into a collection vessel. A second round of sorting was performed following the same protocol as the first; however, the assay parameters were adjusted to account for the smaller starting population and to increase the selection pressure in the second round, therefore 1×10^8 cells in 50 μL of 300 nM PA and 1×10^8 magnetic beads were used. Cells were incubated static on ice for all labeling steps. Also, 1 μM biotin was added in the washing buffer to compete with any remaining streptavidin binders (peptides which bind to streptavidin typically have a much lower affinity than

biotin). In the third round of MMS sorting, cells were labeled with 150 nM biotinylated PA, and then labeled with 1×10^6 magnetic beads in 50 μL of PBSB. After each round of magnetic separation, the bead-bound enriched library was added to LB-Cm[25] media supplemented with 0.2% glucose to inhibit expression of the eCPX gene and therefore prevent growth bias. The cultures were then grown overnight at 37 °C with shaking.

2.5 Flow cytometry analysis of binder enrichment

To quantify the library enrichment of potential PA binders, flow cytometry analysis (BD FACSAria; BD Biosciences, Franklin Lakes, NJ) was performed using biotinylated PA (EZ-Link Sulfo-NHS biotinylation kit; Thermo Scientific, Rockford, IL) labeled with alternating fluorescent secondary labels: streptavidin, R-phycoerythrin conjugate (SAPE; Invitrogen, Carlsbad, CA), anti-biotin-phycoerythrin (Miltenyi Biotec; Bergisch Gladbach, Germany), and Neutravidin, R-phycoerythrin conjugate (NAPE; Invitrogen, Carlsbad, CA), similar to previously published procedures (Georgiou et al., 1997; Daugherty, Iverson, & Geogiou, 2000). Following each round of PA selection, the arabinose induced cell population was incubated with 100 nM biotin-PA solution for 45 mins. The sample was centrifuged at 3000 g for 10 mins to remove unbound biotin-PA and was resuspended in a 25 μL solution of PBSB with secondary label concentration of 5 μg/mL and incubated for 45 mins at 4 °C. The sample was centrifuged and resuspended in 1 mL ice-cold BD FACSFlow (BD Biosciences, Franlin Lakes, NJ) sheath immediately prior to flow cytometry. Cells labeled with SAPE exhibit increased red fluorescence and are easily distinguishable by flow cytometry.

2.6 Immunoassay characterization

ELISA analysis for each of the peptides was completed using a Maxisorp (Nalge Nunc; Rochester, NY) 96-well plate by initially dissolving each peptide at 10 μg/ml in 0.2 M sodium bicarbonate buffer (pH=9.5). The peptides were diluted serially across the row of the plate beginning with the 10 mg/ml stock peptide solution (typically 10, 5, 2.5, 1.25, 0.675, 0.338, 0.169, 0.084, 0.042, and 0.021 μg/ml). A single row of buffer was used as a negative control. Following a 2-hour incubation for peptide binding to the plate surface, each well was blocked for 1 hour using PBS (pH=7.4) + 0.1% Tween (PBST). Protective Antigen (PA) was labeled with a horseradish peroxidase enzyme using EZ-Link Plus Activated Peroxidase (Thermo Fisher Scientific; Rockford, IL) and was used at 0.2 μg/ml in PBST to determine the total PA binding to each peptide at varying peptide concentrations. After a 45 min incubation period of the PA with each peptide, the wells were washed with PBS and detected using 1-Step Ultra TMB ELISA substrate (Thermo Fisher Scientific; Rockford, IL) according to the manufacturers recommended procedure. The data was recorded as total absorbance at 450 nm using a Synergy HT Microplate reader (Biotek; Winooski, VT). The binding dissociation constant (K_d) was determined by plotting the total absorbance versus the concentration of peptide and fit using a sigmoid function with IGOR Pro (WaveMetrics Inc.; Lake Oswego, OR).

3. Results and discussion

Previously, we demonstrated the effectiveness eCPX technology for bacterial library sorting and the capability of automating the selection using the MMS platform (Stratis-Cullum, Kogot, & Pellegrino, 2008; Sooter et al., 2009; Zhang et al., submitted for publication).

Advantages to this approach include reproducible reagent isololation in disposable cartridge format to avoid exposure to potentially harmful threat materials which may be the target system under investigation. For a typical 1 mL sample volume, MMS requires only 5 mins of user interaction, while manual selection requires more than 20 mins. With reagent sorting, there are three key parameters that are critical to evaluate with the first being *throughput*, i.e. how many cells can be sorted per second. The MMS platform achieves high throughput screening since it is capable of screening a bacterial library containing 3×10^{10} members in 15 mins. With regard to gross throughput per hour, MMS is able to process 5×10^{12} cells/hr (50 mL/hr at a cell concentration of 1×10^{11} cells/mL), which is four orders of magnitude higher than that achieved using state-of-art FACS instrumentation or a previously reported dielectrophoretic cell sorter (Hu et al., 2005). In this work, we utilize the MMS sorter platform to rapidly isolate peptide binders from the eCPX platform to the target Protective Antigen (PA) from *Bacillus anthracis.* Three rounds of reagent isolation were performed by two separate users and isolated clones from all three sorting populations were evaluated using flow cytometry analysis.

Flow cytometry is a powerful tool for determining the relative binding of microscopic particles, such as cells to a target of interest by separating them in a stream of fluid and performing optical analysis of the light scattering and fluorescence observed from an incorporated dye label or tag. (Daugherty, Iverson & Georgiou, 2000) In this work, our target PA material is tagged with a red fluorescent label (SAPE) which is easily distinguished by flow cytometry analysis.

Figure 5 compares the binding results for two isolated clones using fluorescence activated cell sorting (flow cytometry) analysis. In all cases the binding population is indicated in red and the overall percentage of the population determined as binding to the target or interferent is given. It is clear that there is very little binding to the cell or library scaffold itself, designated as the negative control panels a) and d) for the DS-28 and SM545 binders, respectively. It is clear upon examination of panels 5b and 5e, that both DS-28 and SM545 exhibit marked binding to the protective antigen target, with the SM545 binder exhibiting superior performance (95.4 % binding). Negative sorting is performed during the selection process to remove potentially cross-reactivity with the bead chemistry used for reagent isolation. However, it is necessary to evaluate the degree of cross-reactivity for any isolated reagent to have practical relevance. Figure 5 c and f show the cross-reactivity to streptavidin for the clones DS-28 and SM545, respectively. From these data it is clear that both clones exhibit cross-reactivity to streptavidin and in the case of DS-28, the binding population to streptavidin is even greater than that to the target. The binding to the target PA over the streptavidin is significantly greater, however, for the SM545 clone at 95.4% (PA) and 24% (Streptavidin).

A summary of the top 10 binders arranged in order of highest percentage of the population binding to PA is provided in Table 1 along with associated streptavidin binding percentages. All analysis was performed on-cell (i.e., on-scaffold) using fluorescence activated cell sorting. It is not surprising that clones isolated from the eCPX library exhibit activity to streptavidin, as streptavidin was used in the coupling chemistry of the PA to the magnetic bead. What is surprising is that despite classical negative sorting against the streptavidin beads used in the reagent clone isolation, significant activity to streptavidin is still evident. Future sorting experiments have been adjusted to remove streptavidin from the sorting, and instead employ direct coupling of the magnetic bead and the target protein.
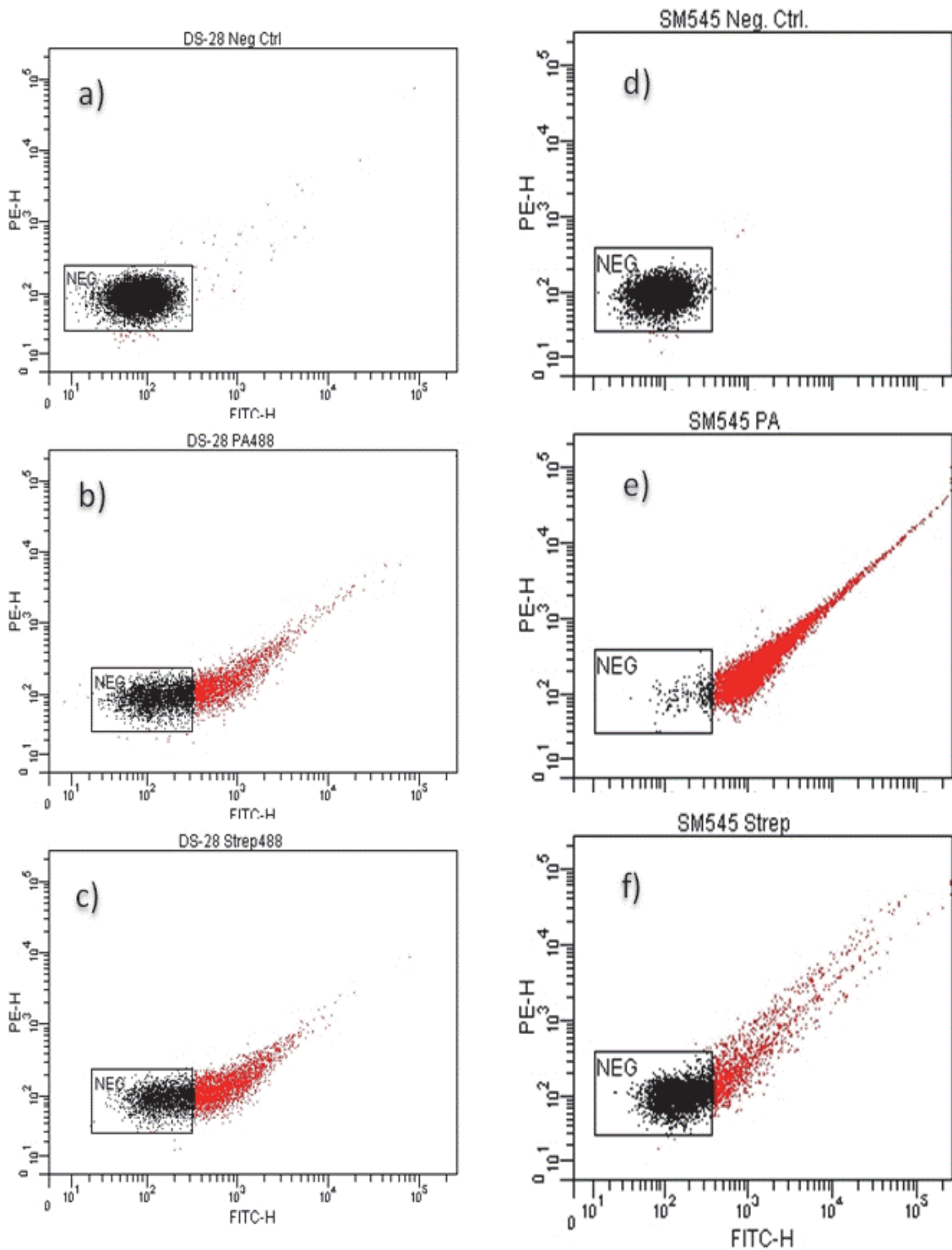
Fig. 5. Flow cytometry analysis of two peptide reagent candidates designated DS-28 (a,b, c) and SM545, designated d, e, f. The binding to the cell scaffold alone is illustrated in a) for DS-28 and d) for SM545. The binding to the PA target is illustrated in b and c and c) and f) represents the fraction which binds to streptavidin. In all panels, the binding population is indicated in red.

Name	Sequence	PA (%)	Strep (%)
SM545	GSFYDSILFYCMTCR	95.4	24.0
SM589	FYCHYVFCDFYRPFG	93.3	77.1
SM574	YLHTTYAQYLTWYSP	82.9	59.4
DS28	VNHVRPHASPRWLLY	39.9	50.9
SM583	TPRDILSPYFRNWWL	42.4	25.0
SM575	IKTLLEMMFFQRNSG	23.7	26.9
SM579	YYYHLIEDWYHGNP	15.7	17.1
DS25	GSNLRSTRTETHCTN	15.4	13.9
DS21	RHNHCGSAHATPYRT	12.2	13.0
SM541	IIHFIHHHAKETHSH	11.4	4.4

Table 1. Sample table of the top binders to protective antigen (PA) sorted by the % of the population which binds to PA. The % binding to streptavidin is also tabulated.

In order for a peptide reagent to have practical use ultimately in a variety of potential assay formats, it is critical that the peptide performance is characterized off-cell or off-scaffold. Despite being such a critical barrier to success, this off-scaffold characterization is typically not investigated throughout the peptide library sorting literature. To investigate this further in our studies, the randomized portion of the best candidate clone SM545 was synthesized (GSFYDSILFYCMTCR).
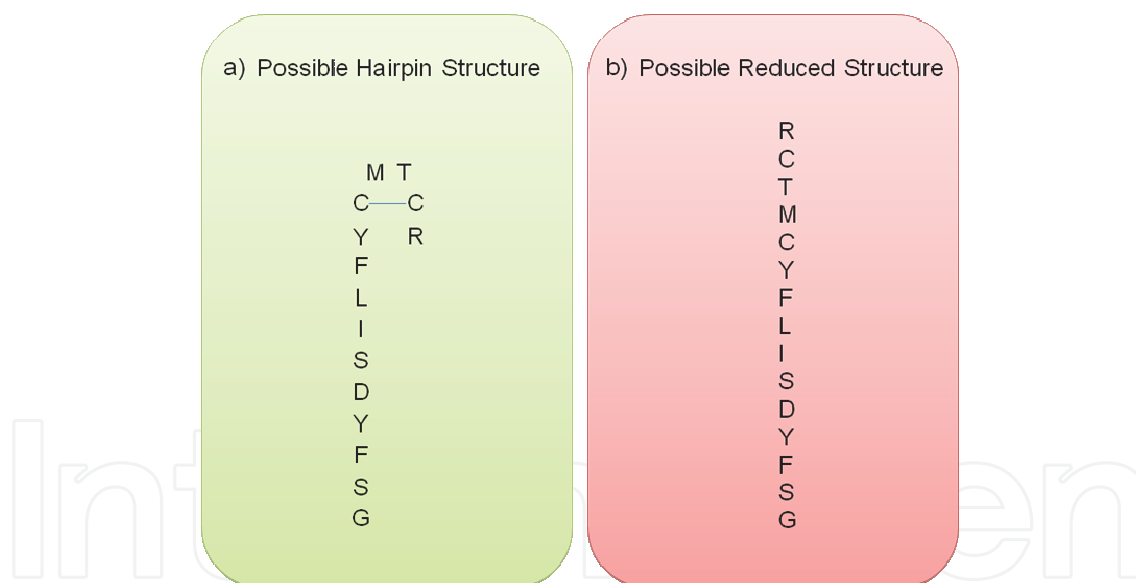
Fig. 6. Schematic diagram illustrating two possible peptide configurations including a disulfide bridge (a) and reduced form (b).

One challenge when dealing with affinity reagent development using bacterial or other library display technology is the potential for differences is binding behavior on-scaffold and off-scaffold. Furthermore, the actual structure of the displayed peptide on-scaffold is not known. For example, the SM545 peptide above could be displayed in at least two different presentations shown in Figure 6. Figure 6a illustrates a possible hairpin structure formed from between the thiol groups of two cysteine residues creating a disulfide bridge. Figure 6b illustrates a simpler, linear peptide where this bond has been reduced.

Consequently, the SM545 was synthesized for testing with the disulfide bridge intact and also in a reduced format.

Flow cytometry analysis using FACS gives a relative estimate of binding performance with a general trend that the greater % binding to the target, the greater the affinity should be for that target should be. To obtain a more quantitative assessment of binding performance of the synthetic peptides, a peptide-based enzyme-linked immunosorbent assay (ELISA) was developed utlilizing the peptide as a capture step. There are advantages and disadvantages to the incorporation of the peptide reagent as the capture vs. the detector reagent in the immunossay. These considerations include steric issues and interferences from label modifications amoung others. To avoid the complexity and cost of a synthetic dye label and the associated interactions of the label with the target analyte, we chose to integrate the peptide into a capture reagent for the purpose of these studies.

To determine the binding constant, the peptide binding to PA was investigated as a function of peptide concentration. In these experiments the PA was HRP labeled and an absorbance endpoint was measured. The binding dissociation constants (K_d) were obtained by fitting the data using a sigmoid function and were determined to be 770.19 nm ± 26.4 for the SM545 sequence containing the disulfide bridge (Figure 7a) and 616.93 nm ± 7.48 for the reduced form (Figure 7b). The binding constants are virtually identical (considering experimental error) and consequently the hairpin structure does not appear to play a critical role in the binding interaction. However, the binding constants for both forms are impressive for synthetic peptide performance off-scaffold without optimization of binder sequence using affinity maturation techniques.

These results not only demonstrate the potential of bacterial display technology and automated reagent discovery but could lead to a much broader extension to a variety of applications requiring rare-cell recovery. For example, the ability to consistently recover and isolate a rare cell population from a large negative control population provides a useful method for pathogen detection in food and water using this low cost, disposable cartridge system. The use of a disposable cartridge permits the analysis of potentially hazardous materials with minimal user exposure and eliminates any concerns for cross-contamination of samples. Above all, the MMS performs with consistency and can be coupled with display libraries to rapidly isolate peptide affinity binders for sensing, diagnosis, or detection of potential biohazard threats, such as protective antigen of *Bacillus anthracis*.

4. Conclusion

In this chapter, we describe the challenges of bacterial display isolation of peptide ligands for biosensing applications, report on semi-automated isolation of binders to Protective Antigen from *Bacillus anthracis* using the MMS approach, and investigate binding performance of isolated clones to include affinity and specificity studies. Several clones exhibited significant affinity to the target species, with many exhibiting significant cross-reactivity to streptavidin despite negative sorting used conventionally in peptide selection. This emphasizes the importance of utilizes both affinity and specificity studies to evaluate best candidate reagents prior to affinity maturation. We characterized the best candidate peptide from these studies off-scaffold through an ELISA-type assay and found impressive binding affinity for the free-solubized format which is also a critical consideration for practical assay integration of synthetic reagent alternatives. The ease and speed at which
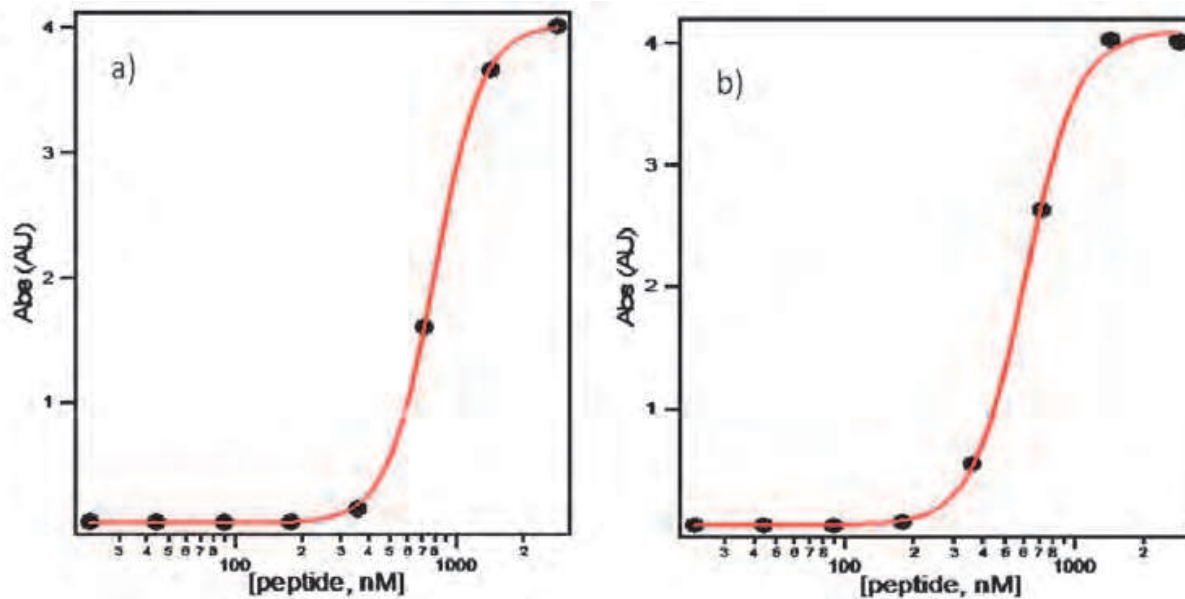
Fig. 7. Binding affinity study using ELISA of SM545 peptide (a) with disulfide bridge and (b) reduced form.

new reagents can be developed makes the bacterial display technology an attractive alternative to antibody technology. Future directions include development of a modeling toolkit to address the optimization of binder performance in terms of both affinity and specificity to be performed *in-silico*. This will further reduce the time-to-reagent.

5. Acknowledgment

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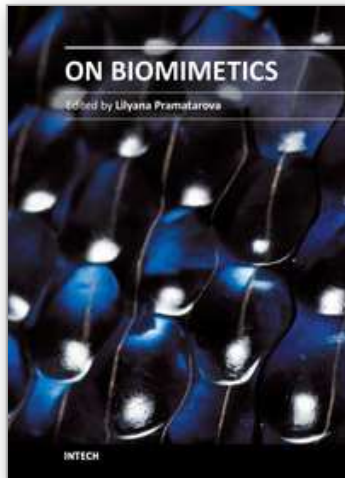
On Biomimetics

Edited by Dr. Lilyana Pramatarova

Bio-mimicry is fundamental idea â€˜How to mimic the Natureâ€™ by various methodologies as well as new ideas or suggestions on the creation of novel materials and functions. This book comprises seven sections on various perspectives of bio-mimicry in our life; Section 1 gives an overview of modeling of biomimetic materials; Section 2 presents a processing and design of biomaterials; Section 3 presents various aspects of design and application of biomimetic polymers and composites are discussed; Section 4 presents a general characterization of biomaterials; Section 5 proposes new examples for biomimetic systems; Section 6 summarizes chapters, concerning cells behavior through mimicry; Section 7 presents various applications of biomimetic materials are presented. Aimed at physicists, chemists and biologists interested in biomineralization, biochemistry, kinetics, solution chemistry. This book is also relevant to engineers and doctors interested in research and construction of biomimetic systems.

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