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# Contending With Target Unrelated Peptides from Phage Display

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Bacteriophage (phage) display is a popular technique employed to generate peptides, antibody fragments, or proteins with specificity for any number of desired targets. In phage display, foreign polypeptides are genetically fused to a phage coat protein so that the random polypeptide sequence is exposed on the surface of the virion. Surface exposure of the polypeptide allows for affinity selection in a high-throughput manner to isolate clones that bind the target. The target may be a purified protein, receptor, nucleic acid, carbohydrate, cell, organ, tumor, etc [1-10]. The genotype-phenotype link in phage display technology generates an easy and efficient means of ligand identification. Phage display technology is a deceptively complex procedure, however, with numerous variables that, if not taken into consideration, can lead to the selection of targeting sequences with unintended and/or undesired properties. While it is important to design a rigorous selection protocol aimed at experimental success, it is equally important to share both positive and negative selection results with the scientific community. If results are not shared, each investigator utilizing phage display technology runs the risk of re-selecting amino acid sequences selected by others and/or wasting time characterizing unwanted amino acid sequence. Phage display can be described as "ignorance based discovery" or a "blind" process due to the selection method relying upon the affinity of phage possessing the necessary characteristics to bind to the presented target in order to purify/select individual phage clones from a vast library of unknown phage. As a result, when utilizing phage display it is imperative to recognize the intrinsic bias contained in the libraries and inherent in the selection protocol. For example it is known that phage displaying peptides composed of amino acid residues that are incompatible with virion assembly, secretion and/or infection processes are censored [11]. A short list of these biases is presented in (Table1) with corresponding references which describe each issue.

A different but equally important pitfall of phage display is the existence of false positive peptides generally referred to as Target Unrelated Peptides (TUPs) that can potentially predominate a selection [23]. Some examples of TUPs are plastic binding or albumin binding sequences, or sequences that bind to the capture moiety (i.e. streptavidin, FLAG, His, c-myc, etc). These types of unwanted sequence are mostly avoided through the use of appropriate negative selections and are consequently categorized as a result of selection protocol bias. However, because phage display is based upon a biological system the libraries of phage will always contain genetic differences between the various phage clone genomes. This heterogeneity can lead to a small number of phage clones being selected because of biological advantage instead of the desired high affinity for the presented target [14,15]. Propagation advantages might include individual phage clones with greater infection efficiency, assembly advantages, and/or faster replication rates. These propagation advantages are difficult to predict and/or prevent, thus it is important to contribute these data to the scientific community and deposit sequence information into public domain databases.

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|---|---|---|
| Insert Sequence Bias | Within the naïve library | • Partially due to the construction of the libraries [11,12]<br>• Partially due to the propagation of the libraries [13-15] |
| Selection Protocol Bias | Must take into account the final intended use of the selected targeting motif | • Appropriate negative selections [13]<br>• Stringency vs. yield[13]<br>• *In Vitro* - capture method/moiety [16]<br>• *In Vivo* - intra vs. extra-vascular [5] |
| Elution Protocol Bias | Elution method utilized | • Hydrophobic vs. hydrophilic elution [5,17,18]<br>• Competitive vs. non-competitive elution [19,20]<br>• Elution vs. enzymatic cleavage [21,22] |

**Table 1:**

Identification of TUP sequences is an important step in a successful phage display selection protocol. All sequences from the various rounds of selection should first be screened against databases for previous selection and then examined for affinity and specificity against the putative binding partner. Two examples of TUPs with growth advantages are the HAIYPRH phage clone (NEB, PhD-7 library) [15,24-35] and PFARAPVEHHDVVGL phage clone (University of Missouri, fUSE5 library) [14,36-38]. Both of these sequences have been "identified" multiple times in various selections against different targets, primarily due to a growth advantage conferred upon the phage by rearrangements and mutations within their respective genomes. The HAIYPRH peptide has been reported by researchers utilizing phage display in selections against Arabidopsis polyadenylation complex, various human cell lines including many cancerous cell lines, Clostridium difficile toxins A and B, and multiple types of hepatitus virus [15,24-35]. In actuality the HAIYPRH phage clone was found by Brammer and co-workers in 2008 to possess a mutation in the Shine-Dalgarno sequence for gIIp, a protein involved in phage replication, imparting to the Shine-Dalgarno sequence better complementarity to the 16S ribosomal RNA [15]. Similarly, the PFARAPVEHHDVVGL peptide was reported to bind to human breast carcinoma and

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melanoma, the human blood-brain barrier, and the malarial protein apical membrane antigen-1[14,36-38]. However, the phage displaying PFARAPVEHHDVVGL was recently found, by Thomas et al. [14] to possess complex rearrangement of its genome that restored the minus-strand origin while retaining tetracycline resistance.

Efficient distinction of TUPs can only be achieved if there is a shared-public database of TUP sequences in which many researchers participate and add sequences. There are a handful of established websites designed to aid phage display researchers in sequence analysis. Most are designed with the selection and characterization of mimotopes in mind. However, two websites, PepBank and SAROTUP (Scanner And Reporter Of Target-Unrelated Peptides), contain databases and software to aid in the identification of unwanted TUPs and/or previously selected peptides [39,40]. SAROTUP is a website with multiple tools to aid in the identification of possible TUPs [39,41]. In the SAROTUP suite, the TUPScan tool compares each peptide against 23 known TUP motifs, while the MimoSearch and MimoBlast tools are utilized to identify peptides already in the MimoDB database. In comparison, PepBank is a web-based software that mines the text of MEDLINE abstracts for of peptide sequences. These data are then combined with both Artificially Selected Proteins/Peptides Database (ASPD) and UniProt public peptide sequence data, as well as with peptide data culled from abstracts and full text articles [40,42,43].

No amount of shared negative data will alleviate the problems arising from a sub-optimal selection protocol. Conversely, no amount of preparation and consideration will eliminate phage possessing growth advantages. Thus, communication between phage display researchers is indispensable for continued progress in this exciting field of peptide discovery.

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