**Electroactive Peptides via Phage Display for Biosensor Applications**

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**Statement of Purpose:** In biosensor devices, functionalization of the surface with covalent linkers is usually employed. However, it is difficult to avoid the loss of activity following the bioreceptor-analyte binding event, which limits the lifetime of the device. The goal of our group is to use phage display to biopan for inorganic binding peptides that are reversible upon application of an electric field. This can provide dynamic functionalization of surfaces with applications such as self cleaning devices. For example, when the bioreceptor becomes clogged, the peptides may be released by triggering an electric field to generate a non-binding state. A fresh surface of bioreceptors can then be applied via a flow-through setup (Figure 1).

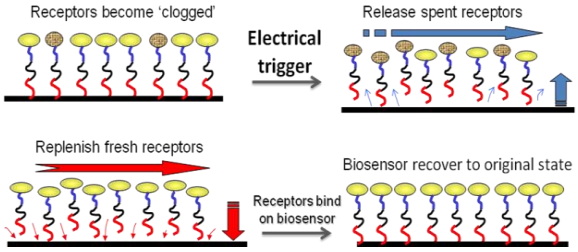


**Fig. 1.** An example for self cleaning device. The surface could be patterned for multi-components as well.

Our group has been panning for peptides that bind strongly to indium zinc oxide (IZO), a transparent semiconducting oxide which makes it an attractive electrode for biochemical sensors. An electro-releasing method is being used to select for strong binding peptides that can be released from a device surface upon application of an electric field. In an alternative method, because a reversible peptide may not necessarily be a strong binding peptide, our group has also developed a novel phage display biopanning protocol with an electro-elution process instead of the regular chemical elution

**Methods:**

**Phage display:** Phage display was performed by incubating a phage library kit with an IZO substrate, washing away unbound phage, eluting and amplifying the bound phages. This was repeated for three rounds to evolve peptides with strong binding affinity to IZO. After each round, single phage colonies were selected for DNA sequencing to determine the 12-mer amino acid sequences. **IZO thin film:** The substrate consists of an amorphous IZO thin film which was sputter-coated onto the top and bottom surface of a sapphire plate. **IZO coated device:** $SiO_2$/Si wafers were used as substrates for photolithography. A sputtering system was used to make metal electrodes, and IZO was then sputter-coated onto the top, followed by gold wires connecting to the metal electrodes. **Binding affinity:** The binding affinity of several phage clones to IZO was evaluated with Immunofluorescence (IF) analysis. **Electro-releasing method:** Strong-binding phage clones were bound to the IZO device surface, an electric field was applied to release phage, as determined IF microscopy. **Electro-elution method:** This phage display method was performed with the same protocol described above but with an electro-elution step applied by the device, in place of chemical elution.

**Results:** The specificity of various clones to the IZO surface as well as three inorganic substrates was compared, where Sapphire (0001), Si (100), and $SiO_2$ were examined as negative controls. Sapphire was chosen because it is present on the side edges of the panning substrates; Si and $SiO_2$ are common materials in electronics devices. An example of phage clones with good binding affinity to IZO is shown in Figure 2. This clone has a 12-mer amino acid sequence of SHAPDSTWFALF, and showed a preferential binding affinity to IZO as well as sapphire, but not to Si or $SiO_2$. Although affinity to sapphire was not targeted, it should not be a problem since sapphire is not used in such electronic devices.

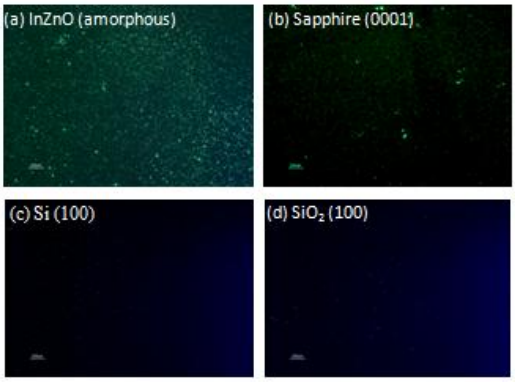


**Fig. 2.** Immunofluorescence images of phage clone: SHAPDSTWFALF binding to difference surfaces

For the electro-elution protocol, the strongly bound phages were eluted by applying a voltage. In the first round of biopanning, one phage clone with amino acid sequence YAEKTVDITMIP was eluted. The IF analysis shows that this phage clone had a high binding affinity on IZO, sapphire, and Si, but had a low binding affinity on $SiO_2$.

**Conclusions:** The results in this study show that the combinatorial approach of phage display can find peptides with strong binding affinity to IZO, as well as peptides that can be electro-eluted. Although the IZO binding peptides also had affinity to sapphire, this lack of specificity might be improved by using a bioinformatics approach in the future. On-going work examines and compares these two approaches for the goal for developing self-cleaning devices and other potential applications of electroactive peptides.