Electroactive Peptides via Phage Display for Biosensor Applications

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Introduction: In general, functionalizing the surface of biosensor devices with covalent linkers is usually considered necessary. Thus, if there is a loss of activity during the bioreceptor-analyte binding event, the lifetime of the device will be limited. With a combinatorial approach like phage display for biopanning for inorganic binding peptides **[1, 2]**, one way to achieve this might be to use peptides which display reversible binding characteristics to the inorganic surface. Then, when the bioreceptor becomes clogged, the peptide may be released by triggering an electric field that generates a non-binding state (e.g., electrostatic repulsion) [**3**]. Subsequently, a new batch of fresh receptors attached to the linker peptides can be replenished with flow through the system when the surface is triggered back to the original binding configuration (See figure1).

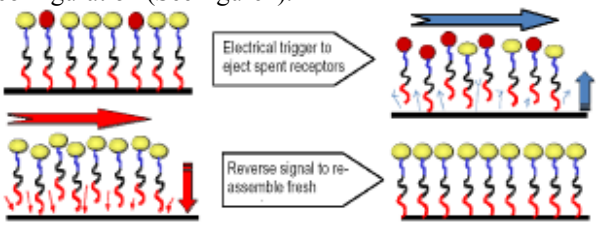

Figure 1. When the receptors become clogged (maroon) the electroactive peptides linkers (red) can be triggered to release from the surface. The device is then refurbished with inflow of fresh receptors whose linkers bind to the substrate upon return to its original state. The surface could be patterned for multi-components as well.

In this study, thin film coatings of indium zinc oxide (IZO) were examined as an activating substrate because IZO is a transparent conducting oxide, which makes it an attractive electrode for biochemical sensors. In addition, an amorphous structure is anticipated to provide a more homogenous surface which could evolve a consensus sequence more readily than a crystalline structure containing crystal defects and grain boundaries. The initial experiments were focused on evolving peptides with affinity to IZO, which could then be tested for release via an electric field. An alternative approach is to include electrodesorption in the panning protocol.

Methods: The substrate consisted of amorphous IZO thin films which were sputter coated onto the top and bottom surface of a sapphire plate at room temperature. A phage library kit (Ph. D. – C12C^{TM}; New England Biolab, Inc) was used for this study. Phage display was performed by incubating phage with IZO, washing away unbound phage, and then eluting the strongly bound phage. The eluted phages were amplified by infecting E. coli strain ER2738. This sequence of steps was repeated twice (or more) to enrich bound phage with affinity to the target material. After each round, single phage colonies were selected for DNA sequencing to determine the 12-mer amino acid sequences that were inserted into the minor PIII coat protein. The binding affinity of several phage clones to IZO was evaluated with Immunofluorescence analysis (IF). In IF analysis, anti-M13 PIII monoclonal antibody (Amersham Bioscience) and anti-mouse IgG-FITC (Sigma-Aldrich) were used to determine the affinity of M13 phages to the IZO substrate under fluoresce light.

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 (Amorphous) 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 FNGRHGTTDHPT, which basically consists of a hydrophobic and a hydrophilic block. This phage clone 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.

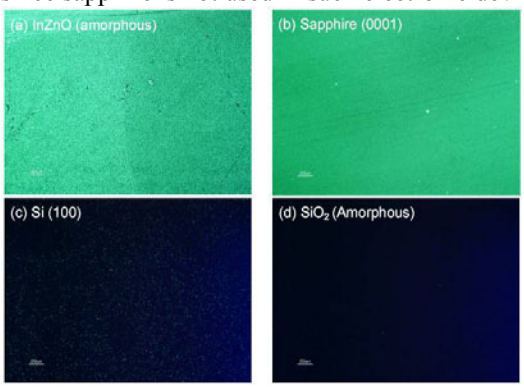

Figure 2. Immunofluorescence images of phage clone:FNGRHGTTDHPT (selected from a third biopanning round) bound to substrates of (a) InZnO (Amorphous), (b) Sapphire (0001), (c) Si (100), (d) SiO_2 (Amorphous).

Conclusions: The results in this study show that the combinatorial approach of phage display can find peptides with strong binding affinity to IZO. Although the IZO binding peptides also had affinity to sapphire, this lack of specificity might be improved using bioinformatics approach in the future. Current efforts are directed at developing an electrodesorption panning protocol that can screen for reversibly electroactive peptides via application of an electric field, a primary goal towards development of self-cleaning devices.

References:

1. Sarikaya, M.; Tamerler, C.; Jen, A. K.-Y.; Schulten, K.; Baneyx, F., *Nature Materials* 2003, 2, 577-585.
2. Whaley SR, English DS, Hu EL, Barbara PF, Belcher A., *Nature* 2000, 405, 665-8.
3. Yeh, P.-Y. J.; Kizhakkedathu, J. N.; Madden, J. D.; Chiao, M., *Colloids and Surfaces B: Biointerfaces 59* **2007,** 59, 67–73.