POLYMER FILMS MOLECULARLY IMPRINTED WITH PROTEINS AS SYNTHETIC RECEPTORS CHARACTERIZED BY MOLECULAR FORCE SPECTROSCOPY

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Molecularly imprinted polymers (MIPs) are synthetic receptors that specifically recognize target molecules. MIPs have found applications for example as antibody mimics in immunoassays or as recognition elements in biosensors. They are produced by polymerizing functional and cross-linking monomers in the presence of the target molecule that acts as a molecular template. Although the majority of MIPs have been imprinted with small molecules, proteins can also serve as template. However, due to their size and their possible denaturation under the conditions normally used in molecular imprinting, specifically adapted protocols have to be used. Here we describe the preparation of a MIP for cytochrome c (cyt c), a 12 kDa protein from the mitochondrial intermembrane space that is involved in the electron transfer chain and that is released into the medium during cell death. We use, for the first time, molecular force spectroscopy for the direct detection of molecularly imprinted binding sites.

To obtain accessible surface binding sites, we chose to use an approach based on an immobilized template. A monolayer of cyt c was grafted onto a mica surface before imprinting, via an amino-terminated silane and a bifunctional coupling agent. Each step of the grafting procedure was verified by atomic force microscopy (AFM) in terms of topography and resistance to scratching. The MIP was then cast on that surface using polyacrylamide with different cross-linkers as the imprinting matrix. Methacrylic acid as an additional monomer allowing for ionic interactions with the basic protein was also used.

After removal of the template protein, the MIP was tested for selective binding of cyt c by fluorescence measurements in competitive mode with fluorescein-labeled cyt c. Other proteins and a non-imprinted polymer surface were used as controls. At the nanometric scale, force spectroscopy was employed to directly reveal molecularly imprinted binding sites. For this purpose, AFM tips were modified with cyt c and used to evaluate the affinity of the MIP for the target protein. Competition experiments with free cyt c and other proteins were performed to confirm the existence of specific binding sites.

Further work will use surface plasmon resonance measurements to assess the MIP's specificity for cyt c. This MIP based synthetic receptor specific for cyt c will be integrated into a microfluidic biochip for the detection of cell death in real time.