Detection of proteins—we know how to build better locks for chemical keys

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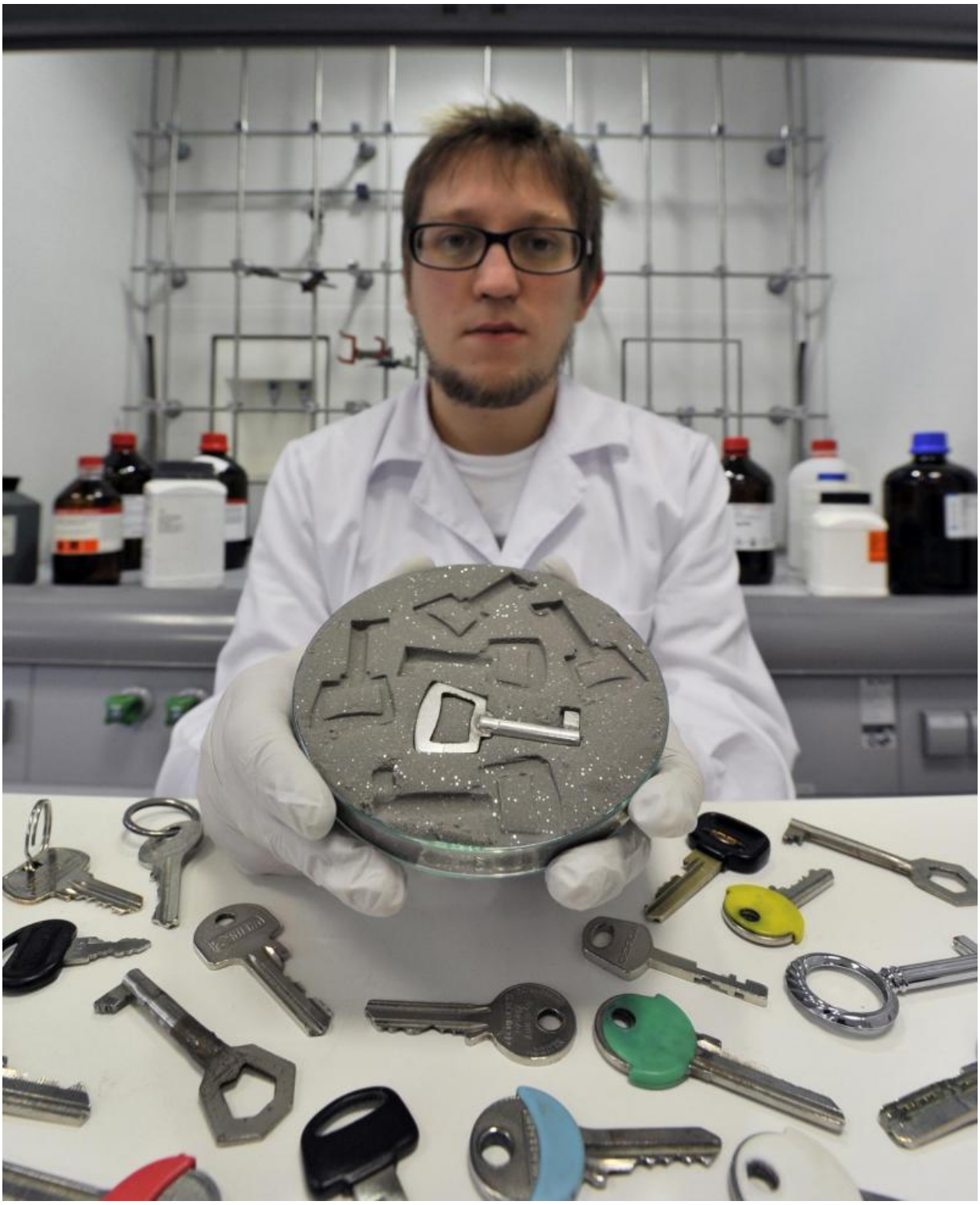

Molecularly imprinted polymers with molecular cavities matched to the molecules of specific proteins, produced using a method developed at the Institute of Physical Chemistry of the Polish Academy of Sciences in Warsaw,

Poland, opening the door to the construction of affordable detectors of the protein markers of many serious diseases. Credit: IPC PAS, Grzegorz Krzyżewski

It will be increasingly difficult for protein molecules to remain anonymous, and increasingly easy for doctors and patients to detect the early stages of latent diseases. Researchers at the Institute of Physical Chemistry of the Polish Academy of Sciences in Warsaw and the University of North Texas in Denton have perfected a method of producing thin detecting films that are able to recognize specific proteins. This is an important step towards the construction of low-cost chemical sensors, identifying even small concentrations of protein disease markers in body fluids.

Molecules of a selected chemical compound can be detected, even at very low concentrations, by molecularly imprinted polymer (MIP) detectors with appropriately constructed pits. These pits, called molecular cavities, only match molecules which have a shape and size that is the same as that of the original molecule that previously 'imprinted' it - and are like a lock chosen for the key that opens it. Preparation of molecularly imprinted polymers with cavities having a shape that corresponds to simple molecules is not a major problem today. Problems arise, however, in the case of very large molecules such as proteins. The cavities are then so large that molecules other than those used to build the MIP can also get stuck in them.

"At the Institute of Physical Chemistry of the PAS we have perfected a method for producing molecular cavities in molecularly imprinted polymers in such a way that it can successfully be used for imprinting various proteins. Not only that, our 'chemical locks' are now much better! Not only do they have shapes precisely corresponding to the

imprinted molecules, they are actually active: particular points of the cavity 'stick' electrostatically to corresponding parts of the imprinted molecule," says Dr. Eng. Maciej Cieplak (IPC PAS).

The hitherto method of producing molecularly imprinted polymers consisted of several stages. First, the proteins to be imprinted were placed in a solution with carefully selected monomers - that is, the basic 'building blocks' that could form the polymer - were allowed to spontaneously arrange themselves around the protein molecules. Subsequently, the mixture was then subjected to polymerization. At the final stage, the molecule-keys were removed from the thus created, hardened structure.

"The main drawback of the traditional approach is that the functional monomers are quite loosely bound to the surface of the protein. So a significant number of them are arranged quite randomly throughout the polymer. The cavities thus match the molecule-key practically only in shape. For this reason, a sensor made using such an MIP could react to the presence of compounds whose molecules showed a tendency to engage in the cavities for completely random reasons," explains Dr. Cieplak.

The researchers from the IPC PAS have developed a more accurate method of imprinting proteins, which they illustrated using albumin as an example. Albumin is the basic protein component of plasma in the blood, where it is responsible for, among others, the transport of certain substances. The presence of albumin in the urine indicates renal failure, most often associated with diabetes or hypertension.

In the new method, researchers from the IPC PAS first joined functional monomers, prepared by the group of Prof. Francis D'Souza of the University of North Texas, with molecules of albumin by chemical bonds, after which they removed the excess monomers. Only after this

step were crosslinking monomers added, the solution was polymerized, and the molecule-keys were removed from the resulting polymer. The molecular cavities that resulted matched the original molecules not only in shape but also in the arrangement of sites that bound electrostatically to the template molecule.

The polymer layers with molecular cavities corresponding to albumin were produced at the IPC PAS on gold electrodes. The coatings had a thickness of approx. 200 nanometres with cavity dimensions of approx. 5 nanometres. If an electrode which had been prepared in this manner was immersed in a solution in which albumin was present, over time, molecules of it were deposited in the MIP cavities. This led to the relatively easy detection of a change in the flow of current. The selectivity of this measurement was very high. If albumin was present, the MIP matched to albumin generated a signal at least several times stronger than in the case of interaction with other similar proteins.

An important advantage of MIPs prepared by the new method is their durability. After one measurement the MIP cavities can be emptied and another measurement performed. Tests at the IPC PAS have shown that the MIP can be used even dozens of times before the structure of the polymer is damaged.

"The detection of albumin, although important, is only a demonstration of the method. What is most important is that we now have a tool that lets us build molecularly imprinted polymers with cavities matching virtually any protein. From here it's only one step to the construction of easily accessible, affordable and small reusable sensors, capable of responding to even minimal concentrations of the protein markers of many diseases that we are nowadays detecting too late," Prof. Wlodzimierz Kutner (IPC PAS) concludes.

More information: Maciej Cieplak et al. Selective electrochemical

sensing of human serum albumin by semi-covalent molecular imprinting, *Biosensors and Bioelectronics* (2015). DOI: 10.1016/j.bios.2015.07.061