Investigation of biomolecular recognition of C-reactive protein by phage display-derived biosensing elements

Katarzyna Szot-Karpińska[1,†]

[1]Institute of Physical Chemistry, Polish Academy of Sciences, Kasprzaka 44/52, 01-224, Warszawa, Poland
† corresponding author's email: ksztot@ichf.edu.pl

The phage display technique from molecular biology was used to isolate new binding molecules/receptors (bacteriophages, peptides and nanobodies) that specifically recognise CRP, a marker of inflammatory processes in the human body. The interactions between CRP and the new receptors were evaluated using experimental (biological and physicochemical) methods, with the support of theoretical simulations (computational modelling analysis), in the case of peptide receptors (designated as P2, P3, and P9). The new CRP binding molecules were successfully applied a sensing elements for the development of CRP-sensing platforms [1-5].

Firstly, CRP-binding bacteriophages (phages, viruses of bacteria) were identified. The performed studies showed that phage P2 exhibits the highest affinity to CRP. Therefore, this phage was used to modify the electrode as a new CRP receptor. It was deposited together with CNFs using a layer-by-layer approach on a glassy carbon electrode. This was possible due to the electrostatic interactions that occur between the P2 phage (negatively charged) and the CNFs (positively charged). The obtained phage-based electrode was applied for CRP detection [1].

In the case of peptides derived from phages, numerical and experimental studies have consistently shown that the P3 peptide is the most effective CRP binder. Therefore, this peptide was used as a recognition element. Firstly, P3 was used for CRP recognition on silicate-modified indium tin oxide-coated glass electrodes [2]. Then, the P3 peptide was successfully incorporated into a point-of-care testing sequential microfluidic device and used for CRP detection. The device was tested with serum, plasma, and whole blood samples to validate its applicability, yielding satisfactory results and a very low limit of detection compared to an antibody-based device on the same platform [3].

Nanobodies are small protein fragments of approximately 15 kDa, derived from the VHH domain of camelid antibodies, and are capable of binding specific antigens. In our studies, we utilised the E12 anti-CRP nanobody, which exhibited the highest affinity for CRP among those selected via phage display [4]. The selected nanobodies were successfully applied in an NFC smartphone-based electrochemical microfluidic device for the detection of CRP [5].

The presented new receptors are more resistant towards external factors. They recognise CRP in a concentration range similar to monoclonal antibodies. Therefore, new CRP binding receptors could become the sought-after solution as an alternative to antibodies and can be used for differentiating between viral and bacterial infections

References

[1] K. Szot-Karpińska, P. Kudła, A. Szarota, M. Narajczyk, F. Marken, J. Niedziółka-Jonsson. *Bioelectrochemistry* 136 (2020) 107629.
[2] K. Szot-Karpińska, P. Kudła, U. Orzeł, M. Narajczyk, M. Jönsson-Niedziółka, B. Pałys, S. Filipek, A. Ebner, J. Niedziółka-Jönsson, *Analytical Chemistry*, 95 (2023) 14475.
[3] S. Boonkaew, K. Szot-Karpińska, J. Niedziółka-Jönsson, B. Pałys, M. Jönsson-Niedziółka, *Sensors and Actuators B: Chemical* 397 (2023) 134659.
[4] S. Oloketuyi, R. Bernedo, A. Christmann, J. Borkowska, G. Cazzaniga, H. W. Schuchmann, J. Niedziółka-Jönsson, K. Szot-Karpińska, H. Kolmar, A. de Marco, *Biosensors* 11 (2021) 496.
[5] S. Boonkaew, K. Szot-Karpińska, J. Niedziółka-Jönsson, A. de Marco, M. Jönsson-Niedziółka, *ACS Sensors* 9 (2024) 3066.