A Single Particle LSPR Biosensor for Thrombin Activity Determination in Real-time

Rui Oliveira-Silva [1,2,3]
Y Wang[1], Duarte M.F Prazeres[2,3], Pedro M. R. Paulo[4], Peter Zijlstra[1]
[1]MBx Molecular Biosensing for Medical Diagnostics, Faculty of Applied Physics and Faculty of Biomedical Engineering and Institute for Complex Molecular Systems, Eindhoven University of Technology, P.O. Box 513, 5600 MB, Eindhoven, The Netherlands; [2]iBB – Institute for Biotechnology and Bioengineering, [3]Associate Laboratory i4HB—Institute for Health and Bioeconomy, and [4]CQE - Centro de Química Estrutural Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais 1, 1049-001 Lisboa, Portugal
Ruipsilva@tecnico.ulisboa.pt (Arial 10)

Belonging to the Protease's family, a key example and probably the most clinically relevant nowadays is Thrombin that despite being involved in other cellular processes, as angiogenesis and inflammation, it is mostly known for its pivotal role in the coagulation of blood. Known by being involved in several diseases such as cancer, chronic inflammatory diseases, atherosclerosis and others,[1] its activity is strictly regulated by a cascade mechanism involving activators and inhibitors in a feedback loop. Unfortunately, this implies that the correlation between the quantity/concentration of Thrombin present within a sample and its activity – which detrimental to understand the condition and often even its extent - is unreliable, mitigating its potential as biomarker. Additionally, and besides diagnosis and prognosis purposes a reliable proteolytic activity sensor can be crucial in developing protease-target therapies, or even in biochemical studies to understand the role of such enzymes.[2]
Acknowledging this, and based on previous work,[3] we resort to a dark-field microscopy setup with the ability to map single particle gold nanorods with nearly background free resolution to determine thrombinolytic activity in real-time. Using this construction, concentrations ranging from 1 – 300nM of active thrombin were detected in real-time. Besides the advantages aforementioned, this system has the potential for multiplexing while enabling monitoring proteolytic processes for long periods of time with sub-second resolution, since spurious effects such as photobleaching and photoblinking that are common in optical biosensors do not occur.

REFERENCES

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FIGURES

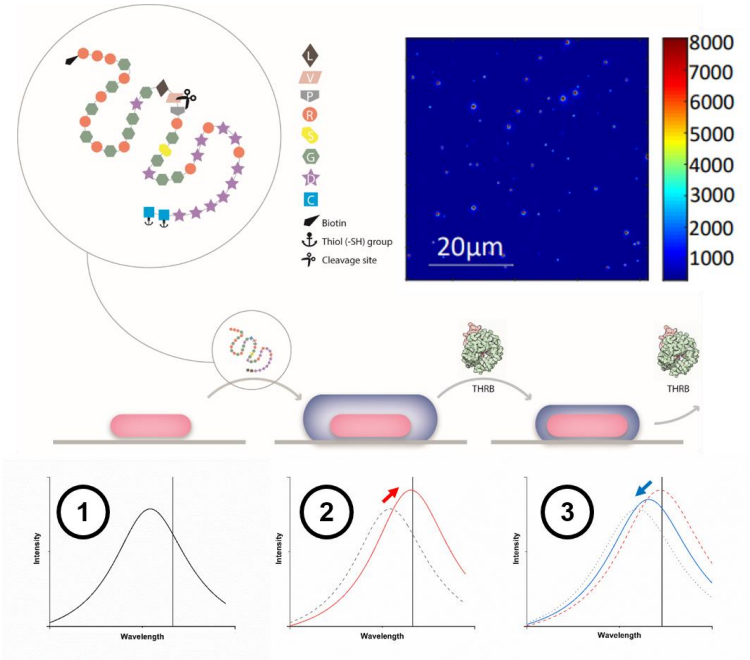

Figure 1: Schematics of the experimental design and principle.