

## Aptamer displacement Lateral Flow assay for Phenylketonuria monitoring.

**Celia Fuentes-Chust**<sup>1</sup>,

Andrea Idili<sup>2</sup>, Claudio Parolo<sup>3</sup>, Andrew Piper<sup>1</sup>, Giulio Rosati<sup>1</sup>, Arben Merkoçi<sup>1,4</sup>.

<sup>1</sup>Institut Català de Nanociència i Nanotecnologia (ICN2), Campus UAB, 08193 Bellaterra, Barcelona, Spain

<sup>2</sup>Department of Chemical Science and Technologies, University of Rome Tor Vergata, Rome, Italy

<sup>3</sup>ISGlobal, Barcelona Centre for International Health Research (CRESIB), Hospital Clínic (Department of International Health), Universitat de Barcelona, Spain

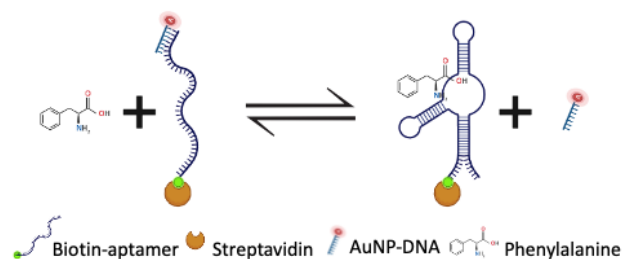
<sup>4</sup>Catalan Institution for research and Advanced Studies (ICREA), Barcelona, Spain  
arben.merkoci@icn2.cat

Phenylketonuria (PKU) is an inherited autosomal-recessive metabolic disorder that inhibits the metabolism of the amino acid phenylalanine[1]. Since 1963, it has been identified in newborns using bacterial inhibition test, a well-established screening approach. Its buildup in blood, urine, and other tissues may cause seizures, intellectual impairments, and other mental illnesses if not properly checked via diet and occasional visits to a PKU clinic. [2] Fast, robust, sensitive, and user-friendly assays are preferred for better monitoring of phenylalanine levels in PKU patients in order to enhance their quality of life. We describe a Point-of-Care aptamer lateral flow biosensor in a strand displacement format with gold nanoparticles (AuNPs) as an optical label for phenylalanine measurement in a buffer sample, which enables the identification of mild hyperphenylalaninemia, mild PKU, and classic PKU. In this work, we conjugated AuNPs to a short nucleic acid sequence that matches a segment of a previously reported aptamer that recognizes phenylalanine and printed the complex as the first line. [3] When a phenylalanine-containing buffer sample reaches the aptamer, the competition displaces the AuNP conjugate, causing the optical signal in the first line to decrease. As the AuNP conjugate flows across the rest of the lateral flow strip, it is captured by a complementary sequence in a second line, resulting in the development of a second quantifiable signal. The test is performed in 20 minutes and has a detection limit of 50  $\mu$ M, which corresponds to the normal concentration of phenylalanine in the blood of healthy individuals.

## References

- [1] N. Blau, F.J. Van Spronsen, H.L. Levy, *Lancet*, 376 (2010) 1417–1427.
- [2] J. Miné, Manuèle; Chen, I. Desguerre, D. Marchant, M. Abitbol, D. Ricquier, P. De Lonlay, A. Bernard, C. Fe, *Hum. Mutat.* 28 (2007) 831–845.
- [3] A. Idili, J. Gerson, C. Parolo, T. Kippin, K.W. Plaxco, *Anal. Bioanal. Chem.* 411 (2019) 4629–4635.

## Figures



**Figure 1.** Schematic representation of the designed displacement assay.