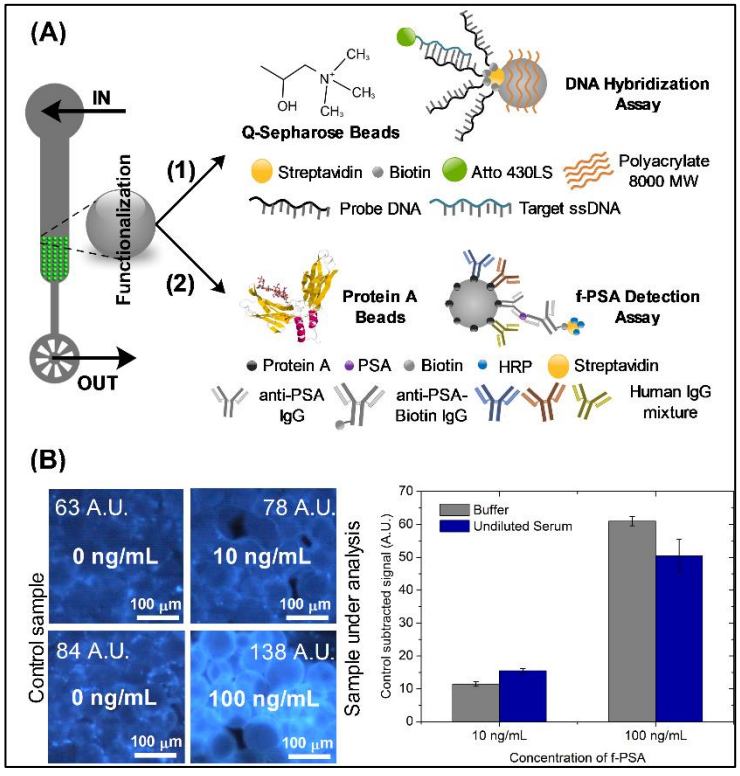
ANALYTICAL AFFINITY CHROMATOGRAPHY-ON-A-CHIP FOR SELECTIVE CAPTURE AND SENSITIVE DETECTION OF PROTEIN AND POLYNUCLEOTIDE BIOMARKERS

Ruben R.G. Soares, INESC-Microsystems and Nanotechnologies and iBB – Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Lisbon, Portugal
ruben.soares@tecnico.ulisboa.pt
Catarina R.F. Caneira, INESC-MN
Inês F. Pinto, INESC-MN and iBB
Narayanan Madaboosi, INESC-MN
Virginia Chu, INESC-MN
Ana M. Azevedo, iBB, Instituto Superior Técnico
João P. Conde, INESC-MN, Instituto Superior Técnico
M. Raquel Aires-Barros, iBB, Instituto Superior Técnico

Key Words: Chromatography, Affinity, Microfluidics, PSA, ssDNA

Affinity Chromatography is a powerful technique which has been applied to the highly selective purification of several biomolecules from complex mixtures. This technique is currently a core technology in the industrial purification of disruptive biopharmaceuticals such as monoclonal antibodies. The use of high affinity ligands, together with densely functionalized three-dimensional solid-phase supports, confers a remarkable analytical potential, making it a current standard for the quantification of several compounds in certified laboratories, ranging from health biomarkers to environmental contaminants. Aiming at extending the application of affinity chromatography to a portable setup, we report the miniaturization of this system down to nL-scale, by trapping Q-sepharose or protein-A agarose beads in microfluidic channels with total volumes ranging from 60 to 210 nL. This versatile and simple platform combined the high surface area and robust surface chemistry provided by the chromatographic media with the high degree of fluidic control, portability, improved reaction kinetics and low reagent expenditure inherent to microfluidics. Furthermore, the microfluidic structures are simple in terms of microfabrication and can be sequentially operated using standard pipette tips and a negative pressure source at the outlet (Figure A). This system was tested within the scope of prostate cancer diagnostics for the capture of protein and polynucleotide biomarkers. Along these lines, prostate specific antigen (PSA) was selectively captured from unprocessed human serum and a 23 bp polynucleotide (ssDNA analogous to micro RNA MIR145) in fetal bovine serum as model matrix, by coupling a monoclonal anti-PSA IgG2a with protein-A beads or a complementary ssDNA strand with Q-sepharose beads, respectively. The assay schematics are described in Figure A. Clinically relevant sensitivities below 10 ng/mL PSA (Figure B) and 10 pM polynucleotide were achieved using a horseradish peroxidase-labelled reporter and measuring chemiluminescence directly on the bead surface. The results demonstrate a high potential for the miniaturization of analytical affinity chromatography, providing good sensitivities in a portable setup, particularly considering the amenability of integrating miniaturized thin-film sensors for optical transduction, as previously demonstrated by our group.



References:
- P. Novo et al., Biosens Bioelectron 57 (2014) pp 284-291
- R.R.G. Soares et al., Biosens Bioelectron 87 (2016) pp 823-831
- I.F. Pinto et al., Anal. Chem. 88:16 (2016) pp 7959-7967