OPTIMIZING THE CAPTURE OF PROTEIN INTERACTION PARTNERS IN THE BIACORE T100 FOR MAPPING SIGNALING PATHWAYS WITH LOW CELL AMOUNTS

J.A. Lopez [(1)], ***E. Calvo*** [(1)], ***N. Blanco*** [(1)], ***S. Pileri*** [(2)], ***E. Camafeita*** [(1)], ***D. Sancho*** [(1)].

[(1)] Fundación CNIC, [(2)] GE Healthcare.

The characterization of protein interactions is a crucial step in the understanding of protein function within regulatory networks. For the in vitro characterization of protein binding partners, typically proteins eluted from affinity pull-down experiments are identified by mass spectrometry (MS). A promising tool for protein interaction analysis is the specific capture of proteins on biosensors like the Biacore's surface plasmon resonance (SPR)-based biosensors, which serve as an affinity-chromatography matrix for selected bait molecules. This biomolecular interaction analysis (BIA) can be coupled to MS for the identification of specific protein partners to complement real-time BIA information on the interaction specificity, affinity and kinetics.

Here we describe the optimization and in-depth investigation of the fishing capabilities of the Biacore T100 using two biotinylated peptides, the unphosphorylated and tyrosine-phosphorylated forms of a transmembrane protein receptor, immobilized on the biosensor surface. After incubation with a complex protein extract from stimulated mouse cells, specifically captured proteins were eluted from the sensor surface, digested with trypsin and analyzed by Orbitrap MS for protein identification and characterization.

Although further work is needed to improve the whole SPR-MS process, as compared with parallel pull-down experiments, SPR-MS interfacing shows several potential advantages: i) diminished sample requirements, ii) reduced number of unspecific protein partners, iii) diminished analysis time, iv) real-time monitoring of the interaction process, and v) Biacore T100 can validate some of the potential interactions detected. Work is in progress to examine the role of the protein partners identified in the cellular events taking place under activation.