OPTIMIZATION OF A CROSSLINKING AND MASS SPECTROMETRY WORKFLOW (MALDI-TOF/TOF, ESI-MS/MS) FOR THE IDENTIFICATION AND CHARACTERIZATION OF THE (R/W)9 CELL PENETRATING PEPTIDE INTERACTION PARTNERS

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We aim at studying the intracellular partners of a Cell-Penetrating Peptide (R/W)9 RRWWRRWRR using Chemical Crosslinking and Mass Spectrometry. It was found that this amphipathic and polycationic peptide has an actin-remodeling activity in malignant fibroblasts leading to a reversion of the tumoral phenotype[1]. Identifying the interacting partners of this peptide could allow a better understanding of its mode of action.

However before performing *in vivo* chemical crosslinking and especially before being able to interpret the mass spectrometry data for these complex systems, we needed to set up, evaluate and optimize a Crosslinking/Mass Spectrometry/Data analysis workflow based on *in vitro* experiments. The influence of several parameters was tested : nature of the crosslinker reactive group, arm length, concentrations and *ratio* between the two interacting biomolecules.

The first model system used was the Glutathione-S-Transferase protein that is well known for its ability to form homodimers. Then we studied a peptide/protein interaction between (R/W)9 and bovine serum albumin or actin. This basic peptide was expected to electrostatically interact with these proteins with an acidic pI and known to have a submicromolar affinity with actin (ITC measurements).

For the crosslinking reactions we tested four different crosslinkers with a reactivity towards K or C residues (SBAT, GBAT, BS3 and EMCA), different concentrations and peptide: protein ratios (1:1 10 µM:10 µM, 10:1 40 µM:4 µM, 1:10 4 µM:40 µM). In order to selectively enrich the crosslinked species involving the CPP, we used (R/W)9 labeled with a biotin tag (Biot(O2)G4KRRWWRRWRR or Biot(O2)G4CRRWWRRWRR) and performed affinity purification on streptavidin beads. We used both a MALDI-TOF/TOF and a NanoLC-LTQ-Orbitrap systems for the identification and characterization of the interaction sites. In collaboration with the Department of Bioinformatics and Genomics of North Carolina (Dr. X. Du *et al.*) we are currently working to adapt the Xlink-Identifier software[2] for the determination of the biotinylated CPP (R/W)9 interacting partners in a complex mixture such as a cell lysate. In parallel we started the study of (R/W)9-protein interactions by photocrosslinking using a photoactivable version of the CPP.

Thus we wished to evaluate the relevance of crosslinking techniques for interaction networks studies and to develop a robust workflow for the understanding of CPPs intracellular action mechanisms.

[1] *Delaroche, D. Cell penetrating Peptides with Intracellular Actin-Remodeling Activity in Malignant Fibroblasts. J. Biol. Chem. 2010, pp. 7712-7721.*

[2] *Du, X. Xlink-Identifier : An Automated Data Analysis Platform for Confident Identifications of Chemically Cross-linked Peptides Using Tandem Mass Spectrometry. J. Proteome Res. 2011, pp. 923-931.*