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

Séverine Clavier1,2, Sandrine Sagan1, Rodrigue Marquant1, Xiuxia Du2, Gérard Chassaing1, Gérard Bolbach1,2, Emmanuelle Sachon1,2

1 - Université P. et M. Curie Paris 6, UMR 7203 CNRS-UPMC-ENS , 4, place Jussieu, 75005 Paris, École Normale Supérieure, Département de Chimie, 24, rue Lhomond, 75005 Paris, France
2 - Plateforme de Spectrométrie de Masse et Protéomique, Université P. et M. Curie, IFR 83, 7-9 Quai St Bernard, 75005 Paris, France
3 - Department of Bioinformatics and Genomics, University of North Carolina at Charlotte, Charlotte, North Carolina 28223, United States

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 phenotye[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 pl 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)G4KRRWRRWRR or Biot(O2)G4CRWWRRWRR) 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.