INCREASING THE SENSITIVITY OF PROTEOMIC IDENTIFICATION OF S-NITROSYLATED PROTEINS: TOWARDS THE S-NITROSOPROTEOMES IN (PATHO)PHYSIOLOGICAL SETTINGS

Daniel Tello[1], Carlos Tarín[2], Pablo Martínez-Acedo[3], Ana M. Maldonado[4], Samuel Ogueta[5], Jesús V. Jorrín[4], Jesús Vázquez[3], Santiago Lamas[6], Antonio Martínez-Ruiz[1]

[1]Servicio de Inmunología, Hospital Universitario de La Princesa, Madrid (Spain);
[2]Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), Madrid (Spain);
[3]Centro de Biología Molecular "Severo Ochoa", CSIC-UAM, Madrid (Spain);
[4]Departamento de Bioquímica y Biología Molecular, Universidad de Córdoba (Spain);
[5]Unidad de Proteómica, Universidad de Córdoba (Spain);
[6]Laboratorio Mixto CSIC-FRIAT de Fisiopatología Vascular y Renal,
Centro de Investigaciones Biológicas (CSIC), Madrid (Spain)

S-nitrosylation (formation of a thionitrite group in cysteines, R-S-N=O) has emerged as an important reversible post-translational modification involved in many cell signalling processes related with nitric oxide production, and can even represent a new paradigm in signal transduction due to some of its particular features.

The "biotin switch" technique allows the replacement of S-nitrosylation with a more stable biotinylation that allows easy purification of the modified proteins and has been used to study several S-nitrosoproteomes, as well as to detect the modification in individual proteins. However, this technique still bears limitations, especially in physiological or pathophysiological settings, due to its reduced sensitivity.

We have developed a "fluorescent switch" technique that replaces the S-nitrosylation by a fluorescent label. This approach coupled with 2-DE has allowed us to greatly reduce the amount of starting protein extract while maintaining the number of identified proteins. We have applied it to endothelial cells treated with a nitrosylating agent, as well as to identify proteins that are endogenously S-nitrosylated in activated macrophages when the denitrosylating activity of thioredoxin is inhibited. This approach can be an advantage to study *in vivo* samples where starting material is limited.

In a different approach, we have greatly increased the number of identified proteins and located S-nitrosylation sites by applying a "second-generation proteomics" approach to the biotin switch: biotinylated proteins were digested, and peptides were purified using an avidin column and identified by LC-ESI-MS/MS. We have created a collaborative team ("nitrosoteam") to implement these methodologies and compare the S-nitrosoproteomes in different biological systems, human and plants, in order to get a deeper knowledge in the appearance and functional relevance of this modification.