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„Biophysical characterization of metallothionein Zn(II)-loaded states and their role in cellular zinc buffering”

Abstract

The structural characterization of proteins and post-translational modifications as well as proteins complexes and protein interactions is of utmost importance in order to understand biological processes. Biophysical approaches such us X-ray, NMR or electron microscopy excel in obtaining atomic details. However, many systems are not amenable for analysis by using these methods. Mass spectrometry (MS) provides an underpinning resource for structural and functional proteomics, and thus it has been established as a complementary method in structural biology. In my PhD, I attempted to use native MS and MS-based proteomics approaches together with computer simulations to elucidate the structure-to-function relationship of a metalloprotein named metallothionein (MT). Metallothioneins are a family of low-molecular-weight cysteine-rich proteins (~ 6 kDa) that binds up to seven Zn(II) ions and represent one of the main cellular Zn(II) buffer system in the cell. A decade ago, it was demonstrated that under cellular free Zn(II) concentration (10^{-11} to 10^{-9} M), MTs must exist as partially Zn(II)-load species. Based on this, MTs gained a new biological function as a molecular sponge; MTs captures excess Zn(II) or donate Zn(II) to apoproteins. However, their mode of action has remained unknown due to the experimental challenges to study these proteins. During my studies, we have designed an analytical methodology based on chemical labeling and mass spectrometry that allowed us to map the Zn(II) binding sites in these structures. Complementary, we developed a comprehensive data analysis software based on R language for the analysis of mass spectrometry data. Combining a set of MS and chemometric approaches with advanced molecular dynamics simulations we have also unveiled the molecular bases for the origin of the different Zn(II) binding affinities to MTs. Altogether permitted us to throw light on the mode of functioning of these small albeit important cellular proteins.