Access to Atomic Resolution Structural Information of Homo-Repeats by Combining Chemical Biology and NMR: The Huntingtin Case

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Homorepeats (HRs), protein fragments composed by the same amino repeated multiple times, are very common in eukaryotes and are involved in key biological processes and multiple pathologies. HRs are enriched in particular biophysical properties that enables them to perform very specialized functions but that can also trigger disease. Despite their interesting properties, the high-resolution structural characterization of HRs has been impaired due to their inherent flexibility and polymeric nature, which give poorly dispersed NMR spectra. Huntingtin (Htt), the causative agent of Huntington's disease (HD), is the prototypical example of a HR hosting protein. Htt has a poly-Glutamine tract of variable length that becomes toxic when the number of consecutive glutamines exceeds 35 (pathological threshold). Moreover, Htt contains two Poly-Proline tracts with 11 and 10 consecutive prolines. The long-term aim of our study is to decipher the structural perturbations exerted by the extension of Poly-Glutamine tract beyond the pathological threshold, and the role that flanking regions, including the Poly-Proline tracts, have in the pathology.

To overcome challenges posed by HRs, we have developed a chemical biology strategy to isotopically label individual glutamines and prolines within HRs by combining nonsense tRNA suppression and cell-free expression. Our method disentangles the spectroscopic complexity of the HR and has enabled the NMR investigation of two huntingtin exon1 versions with 16 and 46 consecutive glutamines. In addition, the application to poly-Proline has allowed us to precisely explore the proline cis/trans isomerization in these HR regions. Implications of these observations to understand the structural bases of HDs, and the future perspectives of the site-specific isotopic labelling will be discussed.