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Poly-Alanines: Structural features and their importance in developmental diseases

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Compositionally biased sequences endow special structural and functional features in proteins. Particularly interesting is the case of homorepeats, stretches of a single amino acid, among which poly-alanines (poly-A) stand out for their abundance. Indeed, some protein families, such as transcription factors, are specially enriched in poly-As, although their specific functional role remains to be deciphered. Moreover, nine rare developmental diseases have been linked to aberrant poly-A expansions, suggesting unknown roles for these repeats.

The disorder and the compositional bias of Poly-A hamper the high-resolution investigation of the structure-function relationships. Here, we present our attempts to address this challenge using theoretical and experimental approaches. Bioinformatic analyses of Alanine-rich sequences from 18 eukaryotic proteomes suggest that flanking regions modulate their structural properties of these regions, and that their role has changed along evolution. Moreover, our analyses reveal new functions of these homorepeats as signal and mitochondrial transit peptides.

From an experimental perspective, we have focused our structural study on Phox2B, a transcription factor essential for the autonomic nervous system development that contains two poly-A tracts of 9 and 20 consecutive Alanines. The aberrant expansion of the largest one with +5, +7 or +13 additional Alanines triggers the congenital central hypoventilation syndrome (CCHS), a rare autosomal dominant syndrome that hinders autonomous respiration. The site-specific isotopic labeling (SSIL) approach developed in our group allowed to study single alanines within both poly-As present in Phox2B by nuclear magnetic resonance (NMR), indicating an enrichment in α-helical conformations that is modulated by the length of the homorepeat. Moreover, this residue-specific investigation reveals the presence of different conformations in slow exchange, whose role is still unknown. Importantly, our experimental chemical shifts are in excellent agreement with these computed from long molecular dynamics simulations, validating this theoretical approach as a tool to understand the structural details in this family of homorepeats. Molecular dynamics on Phox2B revealed the formation of flexible α-helix conformations on this poly-A, despite the general disorder. Then, all together, our studies pave the way to a structural understanding of the functional role of PolyA and the mechanisms driving to poly-A expansion diseases.