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Soutenance de thèse

Vendredi 14 Dec. 2012

A 14h - Salle des séminaires de l'IBS

Par Thomas Cutuil

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Groupe de RMN biomoléculaire

Folding of proteins studied by real-time NMR and other biophysical methods: the example of beta2-microglogulin

Thèse de Doctorat de l'Université Joseph Fourier

High-energy structural states that are sparsely populated in equilibrium or that transiently accumulate during the folding of a protein, may play important roles for the protein's cellular function, or they are responsible for the formation of toxic aggregates such as large oligomers or amyloid fibrils. In this thesis work, we investigated the folding of beta-2-microglobulin (B2M) using NMR spectroscopy combined with other biophysical methods (SAXS, UV-fluorescence, EM, …). B2M is a 12 kDa protein, involved in a misfolding disease: dialysis related amyloidosis. Our study revealed essential features of the B2M folding mechanism, and the occurrence of transiently populated high-energy states. A major finding is the existence of a monomer-oligomer equilibrium involving several folding intermediate states. The existence of oligomeric species that accumulate during the initial folding phase supports the idea that B2M folding intermediates are responsible for amyloid fibril formation. This conclusion is further supported by the observation that a single-point mutation (W60G) that has previously been shown to slow down amyloid fibril formation also reduces the population of oligomeric folding intermediates. Our results underline that protein folding and oligomerisation are coupled processes. Finally, we studied at atomic resolution the monomeric folding intermediate state that is populated at a level of ~60% at the beginning of the folding, and that has a half-lifetime of a few tens of minutes. The development of innovative real time 3D NMR experiments allowed us to assign the NMR spectra and to characterize this state in terms of local structure and dynamics. The NMR methods developed in this work may also find application in other contexts where the protein of interest has a short lifetime, e. g. in-cell NMR studies.