A structural bioinformatics approach to study neurofibromin

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Keywords: neurofibromin, neurofibromatosis, cancer, computational biology, molecular modelling

Neurofibromin (NF1) is a multidomain protein acting as a Ras-GAP, mainly in the nervous system. Mutations in its gene give rise to Neurofibromatosis type 1, an autosomal dominant genetic disease that leads affected patients to develop, among other serious developmental and neuronal symptoms, cancers in the peripheral nervous system. So far, only the structure of the central GAP domain and the next Sec14-PH module have been solved by X-ray. Most biochemical features of the former have been extensively investigated. Two isoforms of the GAP domain are elicited by alternative splicing, resulting in substitution of a loop (in the so-called isoform I), mainly expressed in the central nervous system, with a longer one (isoform II), with still unknown consequences on the GAP domain function, but suggested to reduce the GAP activity of NF1. Instead, the role of the Sec14-PH domain is still elusive, with the exception of a putative lipid exchange function and a role in protein-protein interaction. In particular, the Sec14 domain and the PH appear to be strictly structurally coupled. It has been suggested indeed that an intrinsic motion of the PH domain would regulate the opening of the lipid cage in the Sec14 moiety.

Molecular dynamics (MD) simulations were performed to address the questions about the structural properties of GAP and Sec14-PH domains of NF1. In detail, an umbrella sampling approach has been exploited to calculate the Ras binding affinity of the two isoforms of NF1 GAP domain. According to our simulations, isoform II would have a weaker affinity for Ras, thus explaining the reduced GAP activity.

To study the dynamical features of the Sec14-PH domain of NF1, high temperature all-atom (MD) simulations have been performed for both the wild type domain and a pathological mutant (K1475ΔΔ) whose structure has been solved, but did not reveal significant differences from the WT domain structure. Our simulations results agree with literature reported circular dichroism data on thermal denaturation experiments, revealing a lower stability of the mutant domain at higher temperatures, allowing us to interpret those data at the molecular level. Highly dynamical regions of the two coupled domains have been identified, which are affected by pathogenic mutations in neurofibromatosis type 1 patients described in literature, confirming the importance of the stability of the PH moiety to control not merely the hypothetical Sec14 lipid-cage opening but primarily to guarantee the integrity of the entire module.