Computational investigation on Rap1 interaction with DNA ends through Umbrella Sampling simulations

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Rif2 along with Rap1 and Rif1 forms a protein complex at the telomers which inhibits telomerase-dependent telomere elongation. Rif2 along with Rap1 is also able to bind DNA at double strand breaks (DSBs) and interacts with Mre11-Rad50-Xrs2 complex (MRX), inhibiting such complex. In particular, Rap1 binds the DNA ends through two Myb-like domains and it recruits Rif2, which counteracts the MRX association to DSBs.

Different mutant variants of Rap1 and Rif2 have been isolated in professor Longhese's laboratory that are able to abolish or exacerbate the hypersensitivity to DNA damaging agents in a background slightly defective in MRX binding to DSBs. The selected mutations are substitution L341S in Rif2 and substitutions R381W and P520L in Rap1.

Aim of this work is to characterize the effect of such mutations using advanced molecular dynamics techniques as the Umbrella Sampling (US) protocol to test the interaction between Rap1 and Rif2 and between Rap1 and DNA. Thanks to US method, it is possible to calculate the potential of mean force (PMF), which represents the free energy change of a system, as a function of some inter/intramolecular coordinate. When the selected coordinate represents the approach/removal of two objects, PMF can be used as an estimate of free binding energy.

Our simulations have indicated that the Rif2-L341S substitution weakens the interaction between Rif2 and Rap1 RCT domain; in contrast, Rap1-R381W increases the affinity of Rap1 MybN domain with DNA, whereas Rap1-P520L decreases the association of Rap1 MybC domain with DNA. These data and the experimental ones indicate that Rap1 can interact with DNA in two different modes: high stoichiometry in which both Myb-like domains take contact with DNA and MRX is regulated primarily through Rif2; low stoichiometry in which single Myb-like domain interacts with DNA and MRX is modulated through Rif2-independent mechanism.