Experiments Report

Proposal Code MX- 1011
Proposal Title Structural Studies of Chromatin Remodelling Proteins

This experiment aimed at obtaining a structural characterization for CHD4, a human chromatin remodelling ATPase. Chromatin remodelling ATPases are evolutionary conserved proteins belonging to the SNF2 subfamily of DEAD/H helicases, containing an ATPase domain and one or more histone binding domains [1]. In order to obtain a full structural and functional characterization of CHD4, we cloned and purified the full length protein, as well as its isolated domains and their combinations: the ATPase domain and the histone binding domains [double chromodomains (dCHD) and double PHD (dPHD)]. We were then able to characterize how histone binding domains direct ATPases to specific chromatin structures and how these domains interact with and regulate the ATPase domain by a combination of DNA/nuclesome binding assays, ATPase assays and Surface Plasmon Resonance histone binding studies.
In October 2009, we were able to collect solution small angle X-ray scattering (SAXS) data using synchrotron radiation at the ESRF beamline ID14-3 (Experiment MX1011), which allowed us to obtain individual models for the following CHD4 constructs: the double chromodomains (dCHD), the double PHD (dPHD), the dPHDdCHD and dCHD/ATPase. Comparison of the dCHD and dPHD models with that of the dPHDdCHD model suggested a plausible way by which the two double domains might be placed within the latter. We also obtained a model for the domain disposition within the dCHD/ATPase. This was done by manually fitting a model for the ATPase domain (based on threading the CHD4 ATPase sequence onto the structure of the related RAD54 ATPase PDB:1z63) together with the individual dCHD model into the dCHD/ATPase model. Based on these results we proceeded to build a model for the domain arrangement in dPHDdCHDATPase. The SAXS derived model suggests extensive domain-domain interactions which strongly supports our biochemical and biophysical data and mechanistically explains the modulation of CHD4 by intra molecular. The results of our studies allowed us to prepare and submit a manuscript for publication.

References

1. Marfella CG, Imbalzano AN. (2007).The Chd family of chromatin remodelers. Mutat Res. **618**, 30-40.