Assembling multi-protein complexes by SAXS, FRET, EPR and molecular simulations

Bartosz Rozycki, Institute of Physics, Polish Academy of Sciences

Many biological functions are carried out by large and dynamic multi-protein complexes. A common architecture of these protein complexes is several functional domains that are connected with intrinsically disordered linkers. Despite their importance in molecular biology, there is currently no single method which can provide information on the overall structure of such protein systems [1]: They are not directly accessible to X-ray crystallography due to the presence of the disordered regions (although their individual domains can be crystallized); they are also not accessible to NMR techniques due to their large molecular weights; and their inherent flexibility and the lack of symmetries make them practically inaccessible to cryoEM. This gap in the market has recently led to the advancement of hybrid methods that use state-of-the-art computational tools to combine complementary data from various experiments and, in this way, to determine the conformational ensembles of the large, dynamic, multi-protein complexes. We have developed an ensemble refinement method that enables to combine molecular simulations with small-angle X-ray scattering (SAXS), single-molecule fluorescence energy transfer (FRET) and spin-label distance measurements (EPR). This development allowed us to obtain detailed representations of the structures and motions in systems ranging from the ESCRT membrane-protein trafficking system [2-4] to multi-domain kinases and kinases in dynamic complexes with phosphatases [5-7]. Our results help explain the molecular mechanisms that underlie the biological functions performed by these protein systems.

[1] “Large, dynamic, multi-protein complexes: a challenge for structural biology”.
B. Rozycki and E. Boura. J. Phys.: Condens. Matter **26**: 463103 (2014).
[2] “SAXS ensemble refinement of ESCRT-III CHMP3 conformational transitions”.
B. Rozycki, Y.C. Kim and G. Hummer. Structure **19**: 109-116 (2011).
[3] “Solution structure of the ESCRT-I complex by small angle X-ray scattering, EPR, and FRET spectroscopy”. E. Boura, B. Rozycki, D.Z. Herrick, H.S. Chung, J. Vecer, W.A. Eaton, D.S. Cafiso, G. Hummer and J.H. Hurley. Proc. Natl. Acad. Sci. USA **108**: 9437-9442 (2011).
[4] “Solution structure of the ESCRT-I and -II supercomplex: implications for membrane budding and scission”. E. Boura*, B. Rozycki*, H.S. Chung, D.Z. Herrick, B. Canagarajah, D.S. Cafiso, W.A. Eaton, G. Hummer and J.H. Hurley. Structure **20**: 874-886 (2012).
[5] “Crystal structure and allosteric activation of protein kinase C beta-II”.
T. A. Leonard, B. Rozycki, L.F. Saidi, G. Hummer and J.H. Hurley. Cell **144**: 55-66 (2011).
[6] “Resting and active states of the ERK2:HePTP complex”.
D.M. Francis*, B. Rozycki*, A. Tortajada, G. Hummer, W. Peti and R. Page.
J. Am. Chem. Soc. **133**: 17138-17141 (2011).
[7] “Structural basis of p38-alpha regulation and specificity by hematopoietic tyrosine phosphatase”. D.M. Francis, B. Rozycki, D. Koveal, G. Hummer, W. Peti and R. Page.
Nature Chem. Biol. **7**: 916-924 (2011).