Friday, March 21, Session IV

L14

STRUCTURAL MECHANISMS OF REGULATION OF HUMAN Nedd4-2 BY 14-3-3 DIMER AND CALCIUM IONS

M. Janosev[1], T. Obsil[1,2], V. Obsilova[1]

[1]Laboratory of Structural Biology of Signalling Proteins, Detached workplace BIOCEV, Institute of Physiology of the Czech Academy of Sciences, Vestec, Czech Republic
[2]Dept. of Physical and Macromolecular Chemistry, Faculty of Science, Charles University in Prague, Czech Republic
masa.janosev@fgu.cas.cz

Nedd4-2 (NEuronal precursor cell-expressed Developmentally Down-regulated 4-2) ubiquitin ligase is a member of the Nedd4 HECT E3 family with whom it shares the same domain architecture: C2 domain (membrane- and calcium-binding), four WW domains (substrate recognition) and bilobed HECT domain (catalytic activity). It is the last enzyme of the ubiquitination cascade, responsible for altering the function and activity of its targets by attaching variable ubiquitin chains to them. Nedd4-2 is involved in numerous signalling pathways and its dysfunction is linked to different pathophysiological conditions (Liddle syndrome – form of hypertension, respiratory distress, heart and kidney diseases, epilepsy and so on), which highlights the importance of understanding its regulation. Proposed mechanisms so far include autoinhibition caused by intramolecular binding [1], activation by calcium ions [2] and intermolecular interactions [3,4].

We described how 14-3-3 homodimer (binds to pSer residues surrounding the WW2 domain) and calcium ions affect Nedd4-2 using following methods: liposome-binding and ubiquitination assays, analytical ultracentrifugation, X-ray Crystallography, SAXS, H/D exchange coupled to Mass Spectrometry and CryoEM. Our results show that calcium is necessary for membrane binding but not for activating Nedd4-2. We found which amino acids interact with these ions and that they don't cause significant structural reorganization in contrast to membrane binding. Our CryoEM model described the specific way Nedd4-2 domains interact to keep it inactive. The complex of Nedd4-2:14-3-3 wasn't influenced by calcium but was inhibitory of the enzyme's ability to bind to membranes and perform ubiquitination.

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SERVALCAT AND *METALCOORD*: NEW STRUCTURE REFINEMENT STRATEGIES IN *CCP4* AND *CCP-EM*

M. Malý[1], K. Yamashita[2], K. H. Babai[3], F. Long[1], M. Wojdyr[4], S. McNicholas[5], P. Bond[5], T. Burnley[6], G. N. Murshudov[1,3]

[1]*MRC Laboratory of Molecular Biology, Francis Crick Avenue, Cambridge CB2 0QH, United Kingdom*
[2]*Structural Biology Division, Research Center for Advanced Science and Technology, The University of Tokyo, 4-6-1 Komaba, Meguro-ku, Tokyo 153-8904, Japan*
[3]*Institute of Molecular Biology and Biotechnology, Ministry of Science and Education, 11 Izzat Nabiyev, Baku, Azerbaijan*
[4]*Global Phasing Ltd, Cambridge, United Kingdom*
[5]*York Structural Biology Laboratory, Department of Chemistry, University of York, York YO10 5DD, United Kingdom*
[6]*Scientific Computing Department, UKRI Science and Technology Facilities Council, Rutherford Appleton Laboratory, Harwell Campus, Didcot OX11 0FA, United Kingdom*
martin.maly@mrc-lmb.cam.ac.uk

In recent years, the field of structural biology has seen rapid advancements, which in turn has driven the scientific software development. Here, we present new features for atomic model refinement within the *CCP4* [1] and the *CCP-EM* [2] software suites.

Servalcat [3] is a comprehensive atomic model refinement program drawing inspiration from *Refmac5*/ *Refmacat* [4] software tools. Recently, it has been integrated into the *CCP4i2* and *CCP-EM Doppio* graphical user interfaces making it accessible to users.

For crystal structures, *Servalcat* allows direct refinement against reflection intensities, eliminating the need for French-Wilson conversion to structure factor amplitudes. This approach enhances the quality of the resulting density maps, such as producing more detailed omit maps for partially occupied ligands. Additionally, the program is also now able to do refinement against twinned data addressing some of the limitations previously encountered in the *Refmac5* program.

Moreover, for cryoEM structures, *Servalcat* offers several advanced features, such as refinement under point group or helical symmetry constraints, a weighted and sharpened $F_o - F_c$ difference map for validation, and half-map cross-validation.

MetalCoord [5] is a program designed to tackle one of the longstanding problems that is the modelling and refinement of metal coordination environments in macromolecular structures (Figure 1). It performs a thorough analysis of metal-coordination geometries based on reference data extracted from the Crystallography Open Database (COD) [6]. As a result, ideal stereochemical information is provided, given as external distance and angle restraints, which can then be used in subsequent structure refinement by *Servalcat*. *MetalCoord* is currently available from GitHub (*https://github.com/Lekaveh/MetalCoordAnalysis*) and is planned for inclusion in the *CCP4* Suite.

The *MetalCoord* program has also been incorporated within the *AceDRG* program [7] to provide improved stereochemical dictionaries for metal-containing monomers. The enhanced tools have been used to update all metal containing ligands available from the latest version

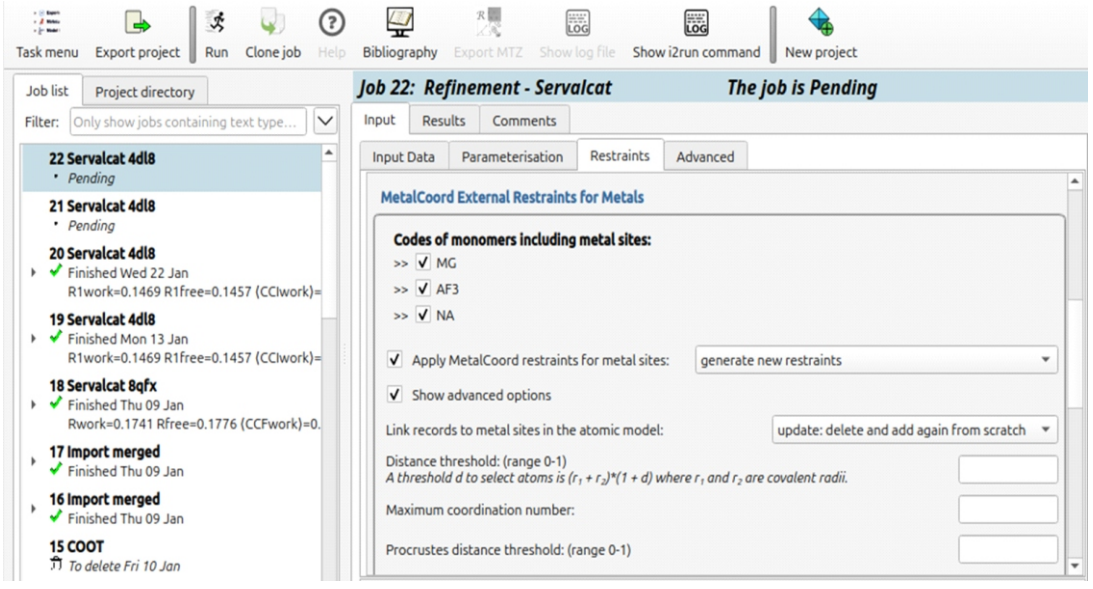

Figure 1. Implementation of *MetalCoord* [5] restraint generation within the *Servalcat* [3] structure refinement pipeline in *CCP4i2* [1].

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FROM CHARACTERIZATION OF PROTEINS AND THEIR INTERACTIONS TO THEIR DETERMINATION IN NANOCARRIERS

H. Mácha, M. Dudák

ANAMET s.r.o., Kováku 3210/26, Praha, Czech Republic
macha@anamet.cz

The study of proteins using complementary analytical techniques is crucial for successful basic research or its application in many areas of science. ANAMET s.r.o. offers leading analytical instrumentation from basic protein characterization, and determining their interactions, to determining protein presence in extracellular vesicles or other nanocarriers.

Differential scanning calorimetry (DSC) is capable of monitoring heat changes associated with protein structural transitions, determining the melting temperature and thereby studying the effects of mutations, chemical modifications or ligand binding on protein stability. Gel permeation chromatography (GPC) is essential for determining the molecular weight and size distribution of proteins. It is particularly useful for assessing the purity of protein samples by detecting oligomerization or aggregation states. Isothermal titration calorimetry (ITC) monitors the thermodynamics of protein-ligand interactions to quantify binding affinity, kinetics, stoichiometry, and thermodynamic parameters such as enthalpy and entropy. ITC provides real-time, label-free data on interactions in solution and offers a direct understanding of how proteins interact with ligands such as small molecules, DNA or partner proteins. Grating-coupled interferometry (GCI) is a highly advanced on-chip technique for assessing molecular interactions technologically close to SPR. By measuring phase shift in the optical guided wave by the chip surface caused by the binding between analyte and ligand, high sensitivity is achieved even for very weak bonds whose values could not be measured before. GCI provides information on binding kinetics, equilibrium dissociation constant (KD) and association and dissociation rates. Flow cytometry in nanoliter volumes nanoFCM is the only analyzer of its type developed specifically for nanoparticle analysis and you are able to determine the presence of proteins on the surface of extracellular vesicles, determine the protein content of liposomes, or determine protein expression by viral particles. In addition to detecting fluorescent labels, you are also able to determine the size and concentration of particles in the sample.

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