**Poster**

# Application of advanced multidimensional analysis methods to study modulated structures of the Hyp-1/ANS protein complex

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The phenomenon of structure modulation is relatively well understood in small-molecule crystallography, but its occurrence in macromolecular protein crystals has been surprising. As a result of modulation, the translational symmetry of the crystal is disrupted in three-dimensional space, and the periodicity of the structure is restored only in higher dimensions. This requires specialized methods of multidimensional analysis to correctly index diffraction images and describe the structure.

Existing methods of solving and refining structures routinely used in protein crystallography are inadequate for comprehensive analysis of modulated structures. The lack of appropriate tools for the analysis of macromolecular modulated structures leads to serious problems in the proper indexing and processing of diffraction data, and then constructing a complete model of the structure with satisfactory refinement indices. So far, it has been possible to carry out a complete structural analysis only for a few modulated protein crystals. These include complexes of the Hyp-1 protein from St. John's wort (*Hypericum perforatum*) with the fluorescent ligand ANS (8-anilinonaphthalene-1-sulfonate). Depending on crystallization conditions, the Hyp-1/ANS protein complexes can form crystals with seven- (7Hyp/ANS) or nine-fold structure modulation (9Hyp/ANS) along the c direction of the *C*2 space group.

As part of my research, I compared two modulated crystal structures of the Hyp-1/ANS protein complex. The first one, designated 7Hyp/ANS, had seven-fold modulation along the **c**-axis and contained 28 independent protein molecules in an extended supercell. The presence of modulation and tNCS (Translational Non-Crystallographic Symmetry) elements was coupled with tetartohedral twinning, which was an additional difficulty when solving and refining the structure. Shortly thereafter, during the change of crystallization and co-crystallization conditions in the presence of the plant hormone melatonin, another modulated structure of the Hyp-1/ANS complex was obtained. The discussed model of the 9Hyp/ANS complex with nine-fold modulation along the **c**-axis finally consisted of 36 Hyp-1 protein molecules arranged according to a motif containing 4 molecules (2 dimers) of Hyp-1 repeated 9 times along **c** (4 × 9 = 36).

The first part of my work involved the refinement of the crystal structure model of 9Hyp/ANS in supercell terms using conventional software and the related analysis of the structural elements of the crystal, especially issues related to the packing of atoms within the elementary cell, perturbations of the periodicity of the structure, comparison with the structure of 7Hyp/ANS and ligand distribution.

In the process of refinement of the 7Hyp/ANS structure, proprietary specialized software developed in the Matlab environment was used. Dropping the simplified assumption of commensurate modulation and introducing additional corrections to account for the disorder in the structure made it possible to obtain new models and improve their refinement parameters. The developed package was then extended with further modules that allowed visualization of the data and introduction of corrections related to thermal vibrations of the crystal lattice (phonons).

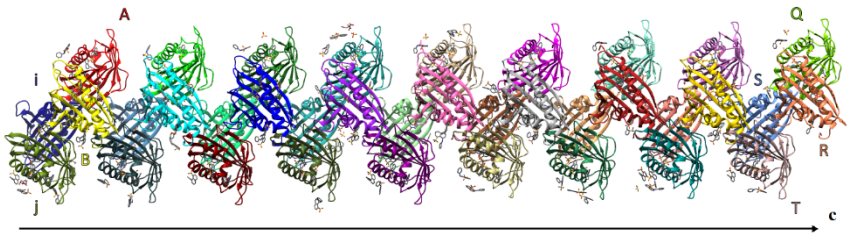


**Figure 1**. Crystal packing of 9Hyp/ANS molecules within the elemental cell along the **c** direction.

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