at different temperatures which gives information on the biological relevance of the protein structures solved at cryogenic conditions.

Keywords: serine protease, reaction mechanisms, high-resolution protein structures

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Electrochemically assisted protein crystallization. Applications to protein crystallography

Abel Moreno[1], Jean Jakoncic[2], Bernardo A. Frontana-Uribe[1], Vivian Stojanoff[2]
[1]Universidad Nacional Autonoma de Mexico (UNAM), Institute of Chemistry, Circuito Exterior, C.U., Mexico, D.F., 04510, Mexico, [2]Brookhaven National Laboratory, NSLS Upton New York 11973-5000 USA., E-mail：abel.moreno@mac.com

The electrochemically-assisted crystallization of non-purified (commercial purity) cytochrome c was successfully achieved inside of small cell of a dynamic light scattering (DLS) apparatus. The method combined batch crystallization conditions and an internal electrical field to favor the nucleation stage. This methodology crystallizes commercial cytochrome c without previous isoforms separation, decreasing costs and experimental time to obtain crystals. The effect of the electric field on the aggregation time and on the protein nucleation was observed in real time by means of dynamic light scattering methods. The results showed a marked decrease of the crystallization time (from 45 days to 5 days) highly improving the previous reported method of crystallization. The HPLC signal of re-dissolved crystals of these protein crystals showed that the protein corresponds to the same isoform previously crystallized by micro-seeding methods. The excellent crystal quality of the cytochrome c crystals obtained in the presence of electrical current was confirmed by protein X-ray crystallography reaching 1 angstrom of resolution.

Keywords: cytochrome c, protein electrocrystallization, high-resolution protein crystallography

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Invariom refinement of 5 K 0.66 Å data of the ethanol solvate of gramicidin A

Birger Dittrich[1], Alke Meents[2]
[1]Georg August Universitaet Goettingen, Institut fuer Anorgansiche Chemie, Tammannstr. 4, Goettingen, Niedersachsen, 37077, Germany, [2]HASYLAB at DESY, Notkestr. 85, D-22603 Hamburg, Germany, E-mail：bdittri@gwdg.de

It is now 20 years since the first structure of Gramicidin D has been reported [1]. Wild type Gramicidin D from bacillus brevis is a mixture of three peptides (Gramicidin A, B and C) each consisting of 15 residues that differ only at position 1 and 11. Gramicidin exhibits antibiotic activity against Gram-positive species by forming ion channels through cell membranes that preferably transport Na^+/K^+. Various solvate structures and ion-complexes of Gramicidin are known to date, as summarized recently [2]. We have re-examined the original ethanol solvate to illustrate the benefits of ultra-high resolution in protein crystallography. To minimize rotational disorder and to maximize resolution we collected data on purified Gramicidin A at a temperature of 5 K at the 3rd generation synchrotron SLS in Switzerland. The resulting Bragg data to 0.66 Angstrom resolution were evaluated with the non-spherical scattering factors of the invariom database [3,4] which is based on the Hansen-Coppens variation of the rigid pseudoatom formalism. Single crystal diffraction data evaluated this way provide a wealth of detailed and accurate information on structure, geometry and electron-density derived properties. For Gramicidin and its biological function an analysis of the electrostatic potential [5] is especially relevant in this respect.

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Keywords: charge density, X-ray structure of membrane proteins, synchrotron radiation

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Ultra-high resolution and very cold structure of lysozyme

Zbigniew Dauter[1], Elzbieta Nowak[1], Anna Brzuszkiewicz[1], Miroslawa Dauter[2], Stephen Ginell[3]
[1]National Cancer Institute, Argonne Natl. Lab., Bldg. 202, Rm. Q142, 9700 S. Cass Ave., Argonne, IL, 60439, USA, [2]SAIC-Frederick Inc., Argonne National Laboratory, Argonne, USA, [3]Structural Biology Center, Biosciences Division, Argonne National Laboratory, Argonne, USA, E-mail：dauter@anl.gov

Extension of data resolution from high to atomic level reveals a wealth of information about the intricate details of protein structures, important for discussing their chemical and biological behavior or their potential to interact with other molecules. The triclinic form of hen egg-white lysozyme is an example of protein crystals able to diffract beyond the ultra-high resolution limit of 0.8 Å. The ultra-high resolution data measured from these crystals at extremely low temperature of 16 K allowed us to obtain a very accurate model of the molecule. About half of the whole structure displays multiple conformations of the main and side chains. Electron densities for hydrogen atoms and bonding electrons are apparent in many fragments as well as strong indications about protonation states of potentially charged groups. Several discrepancies from the library of geometrical parameters are suggestive for reevaluation of some of such library targets. The structural model will be compared in detail with several available structures of lysozyme obtained with different data resolution limits and temperatures.

Keywords: lysozyme, ultra-high resolution, ultra-low temperature

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Crystal structure of fully oxidized human thioredoxin1 containing disulfide between Cys62 and Cys69

Jung Won Hwang[1,2], Jie-Oh Lee[2], Tae-Kwang Oh[1], Myung Hee Kim[1]
[1]Korea Research Institute of Bioscience and Biotechnology (KRIBB), Systems Microbiology Research Center, KRIBB, 52 Eoeun-Dong,