New Developments of Direct Methods in Protein Crystallography

Professor Hai-fu Fan and colleagues at the Institute of Physics have been long engaged in the study of direct methods in crystallography. Recent progresses were made in the iterative dual-space direct-method SAD (Single Anomalous Diffraction) phasing proposed in 2004[1] and the iterative dual-space direct-method MR (Molecular Replacement) model completion proposed in 2006[2]. These significantly improved conventional SAD phasing and MR-model completion (see Figures 1 and 2 respectively).

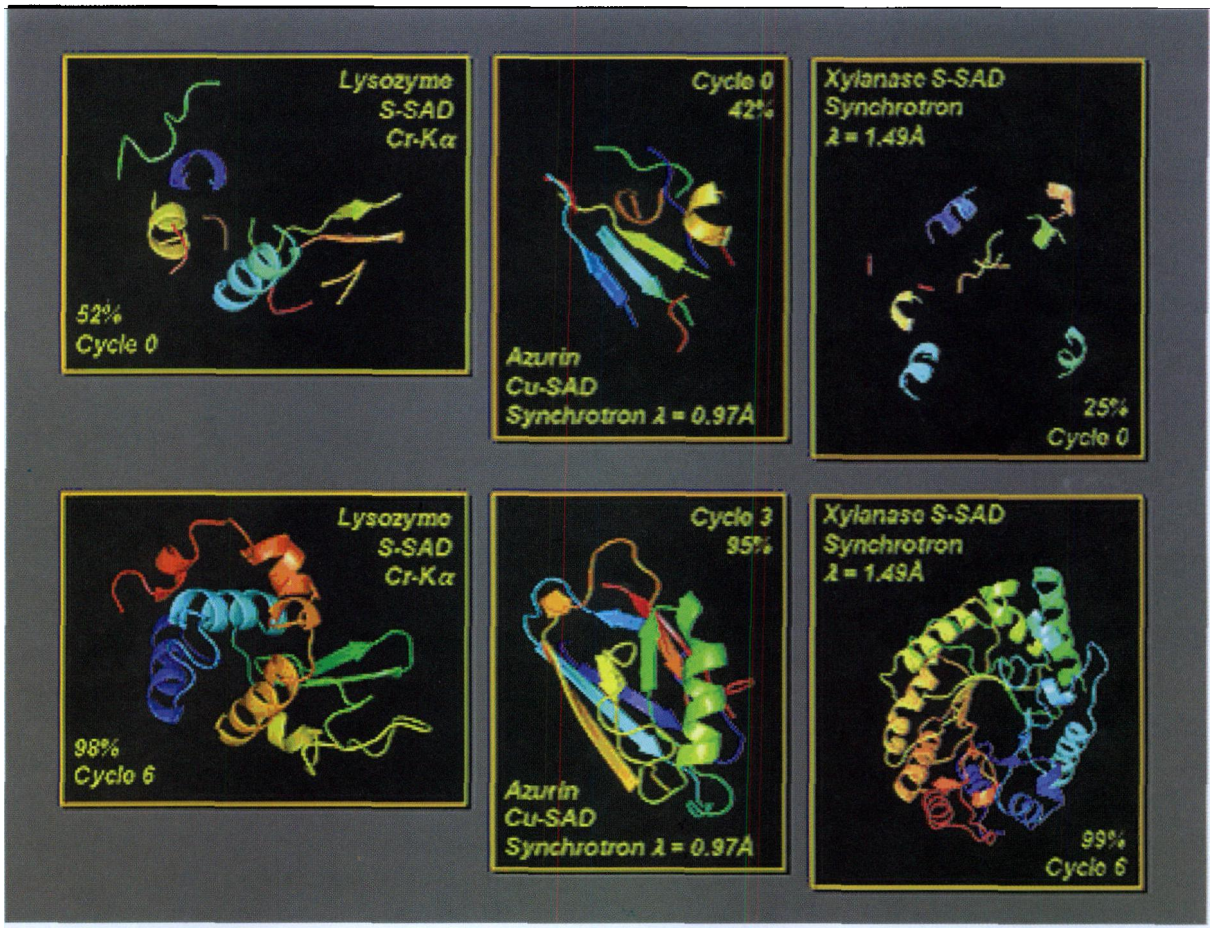


Figure 1 ***Iterative dual-space direct-method SAD phasing and fragment extension Comparison of results from OASIS-2000 (upper row, without dual-space iteration) and that from OASIS-2004 (lower row, with dual-space iteration). Samples from left to right are lysozyme, azurin and xylanase.***

Solving the crystal structure of proteins forms an indispensable part of the experimental basic for understanding the relationship between structure and function of biological macromolecules. MR method is most frequently used in solving protein structures when there is a previously solved protein which is homologous to the structure to be solved; SAD method is the first choice in solving de novo protein structures. In recent years, about 80% protein structures newly deposited in the Protein Data Bank (http://www.rcsb.org) were solved by either MR or SAD method.

The study of combining direct methods with SAD/SIR (Single Isomorphous Replacement) data was started in the Institute of Physics in the first half of 1960's. During the early 1980's to the late 1990's simi-

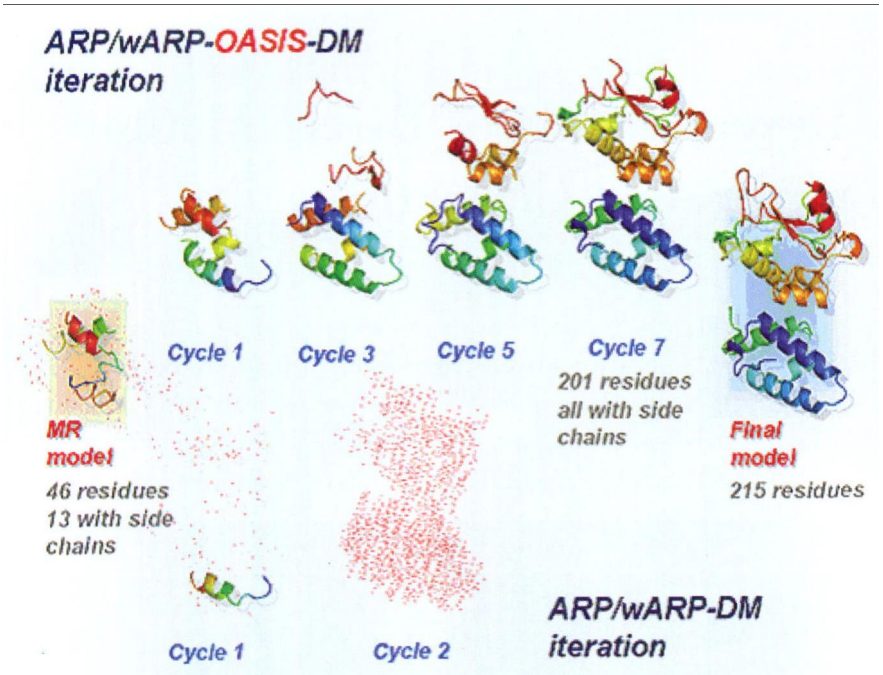


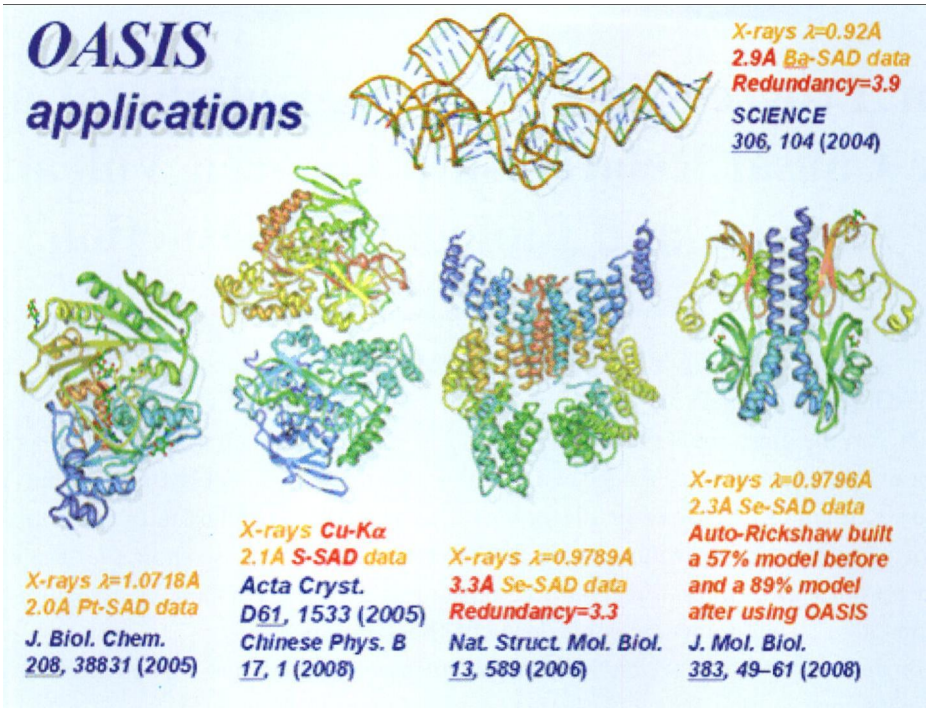
Figure 2 ***Iterative dual-space direct-method MR-model completion Comparison of results from the program combination of ARP/wARP-OASIS-DM (upper row) and that of ARP/wARP-DM (lower row) with the starting and the final model shown on the left and right respective.***

lar investigation had been the focus of most direct-methods centers around the world. The method proposed by Fan in 1965[3,4] was significantly improved in 1984[5] and 1985[6] by the research group on Methods of Solving Crystal Structures (http://cryst.iphy.ac.cn) at the Institute of Physics. Since then, their work becomes an important part of direct-methods research in the world crystallographic community.

At the moment, applications of direct methods in protein crystallography can be divided into three categories:

1. Locating heavy atoms in protein structures;
2. Ab initio determination of protein structures;
3. Resolving the phase ambiguity intrinsic to some traditional protein crystallographic techniques.

Methods of the first category do not solve the entire structure of proteins; methods of the second category require diffraction data at resolution higher than ~1.2Å and, only ~5% of the protein diffraction data that have been deposited in the Protein Data Bank satisfy this requirement; in contrast, methods of the third category are applicable in most cases. Direct methods developed in the Institute of Physics belong to the third category. The program OASIS written by Fan et al. based on their own methods is the most important program for the implementation of direct methods of the third category. The first edition of OASIS was the only program that was included in the CCP4 (http://www.ccp4.ac.uk) suite during 2000 to 2008 for breaking SAD/SIR phase ambiguities by direct methods. The latest edition of OASIS released in 2006 incorporates methods proposed by Fan and colleagues in 2004 and 2006 and has been included in the latest version of CCP4 in 2008. Besides, OASIS-2006 has also been incorporated since 2006 into the EMBL-HH Automated Crystal Structure Determination Platform (http://www.embl-hamburg.de/Auto-Rickshaw) enabling iterative dual-space phasing and model completion. OASIS has been applied by researchers in the world community of protein crystallography for solving protein structures with SAD data that were difficult in phasing with other methods (see Figure 3). A new and significantly improved version of OASIS will be released in 2009.



References

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[2] Acta Cryst. (2007). D63, 793—799.

[3] Acta Phys. Sin. (1965). 21, 1114—1118 (in Chinese).

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[5] Acta Cryst. (1984). A40, 489—495.

[6] Acta Cryst. (1985). A41, 280—284.