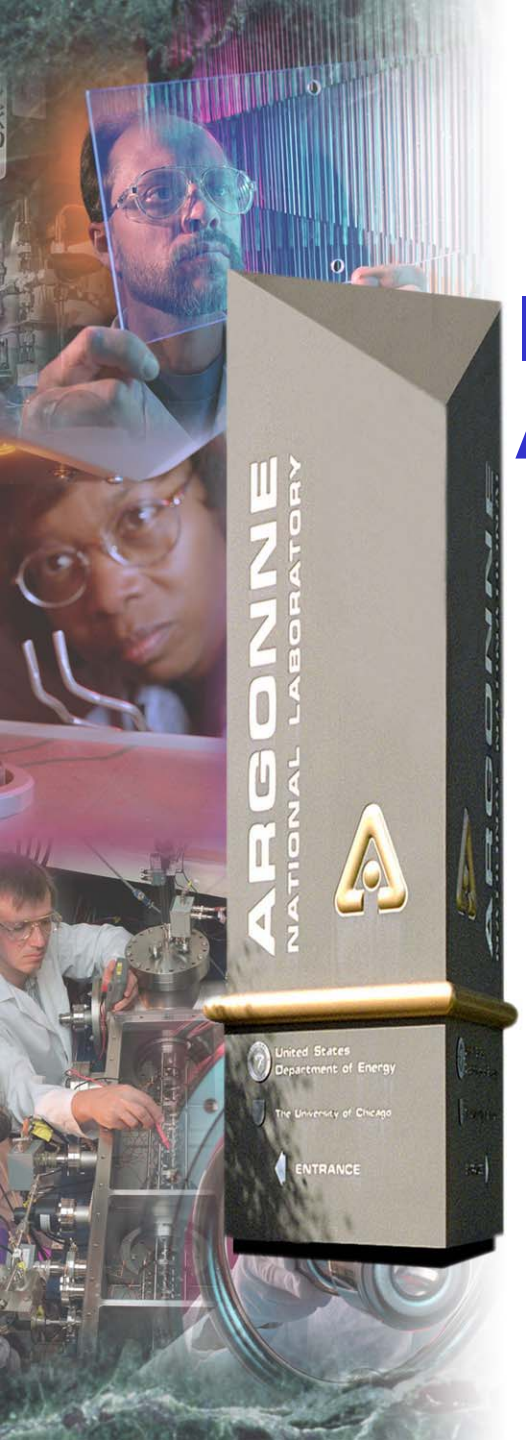


Biology at the Advanced Photon Source

Lee Makowski

The University of Chicago Review
for the Advanced Photon Source
at Argonne National Laboratory

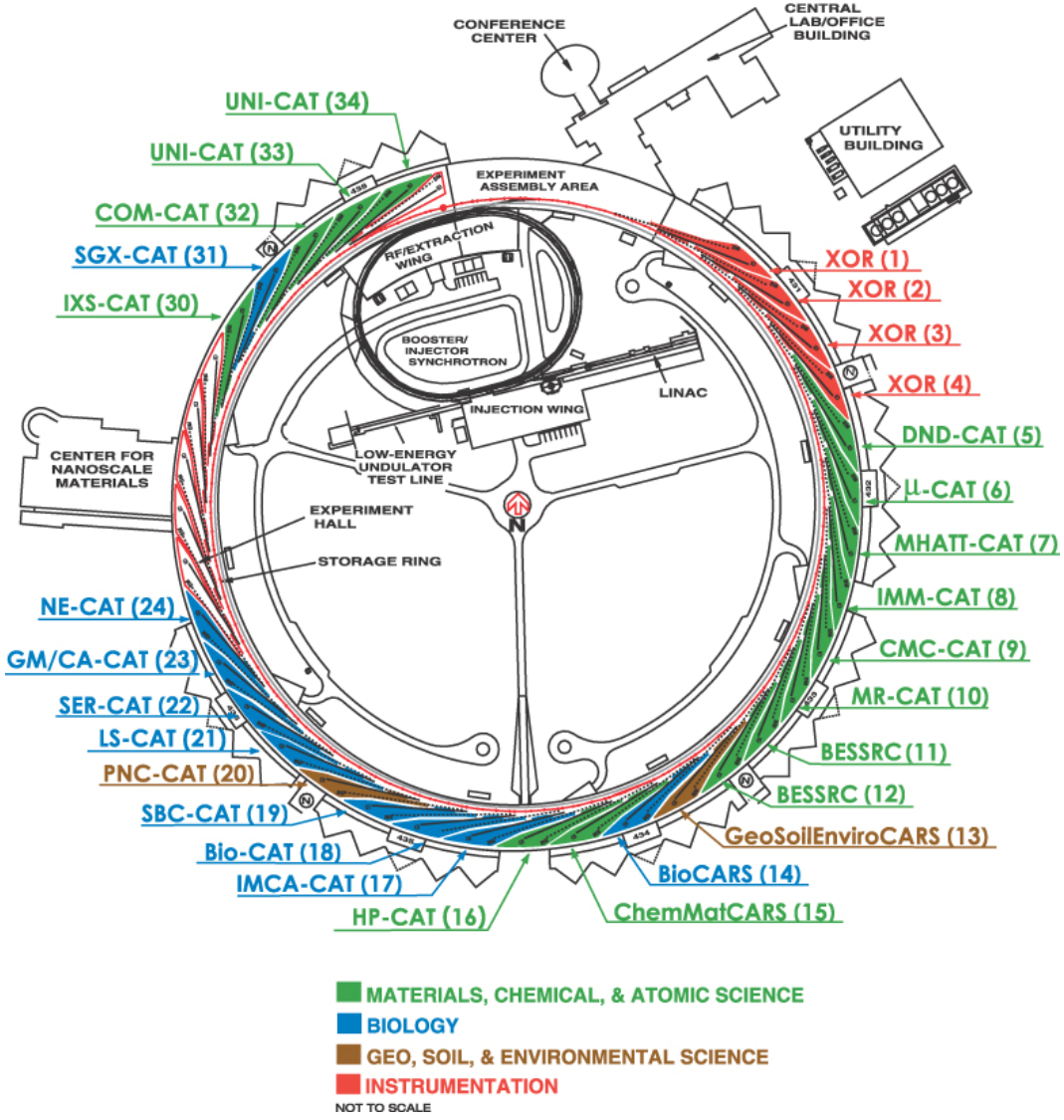
September 17-19, 2003

Office of Science
U.S. Department of Energy

A U.S. Department of Energy
Office of Science Laboratory
Operated by The University of Chicago

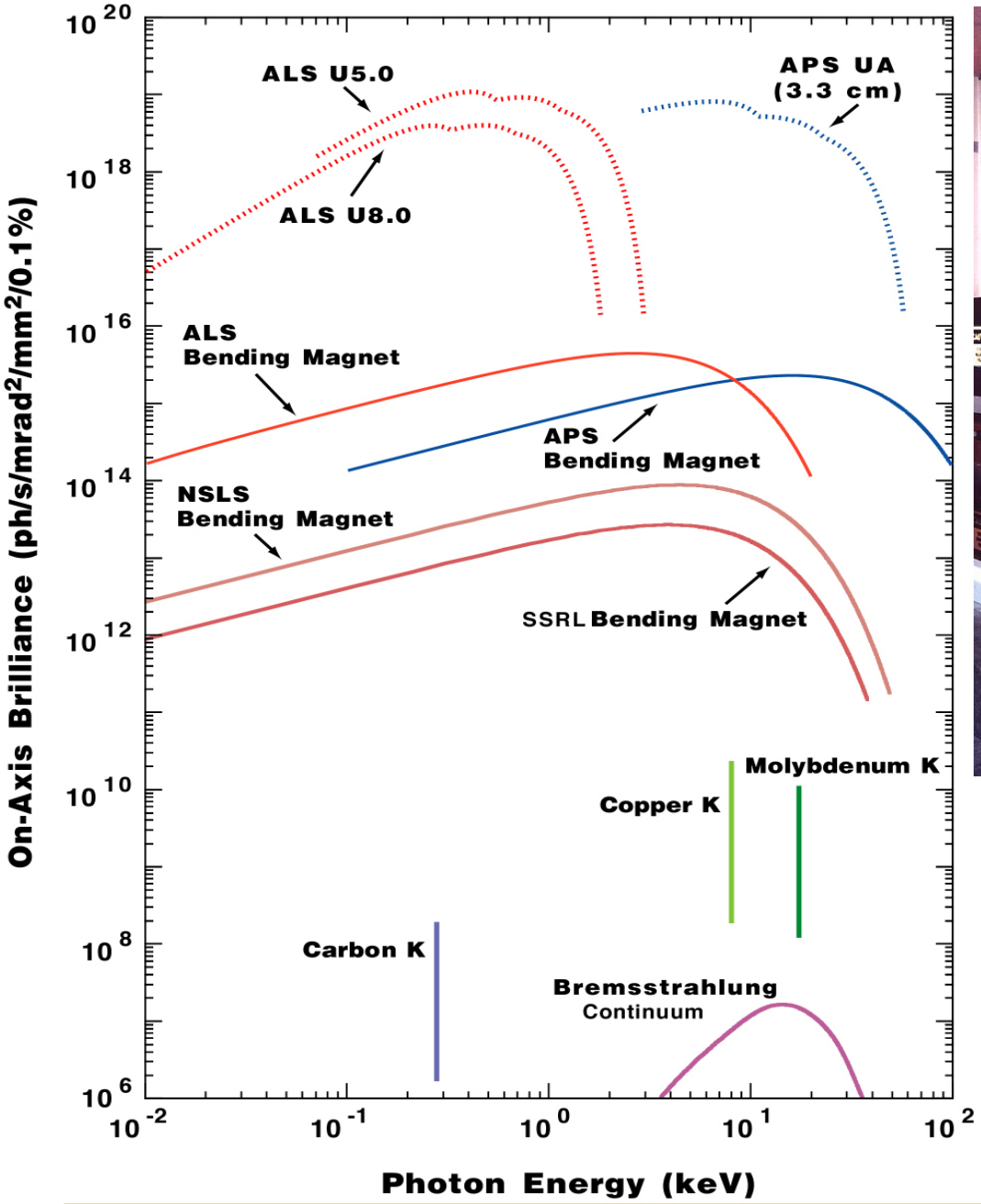
ADVANCED PHOTON SOURCE

Sector Allocations & Disciplines



APS Biology Facilities (APS-BIOs)

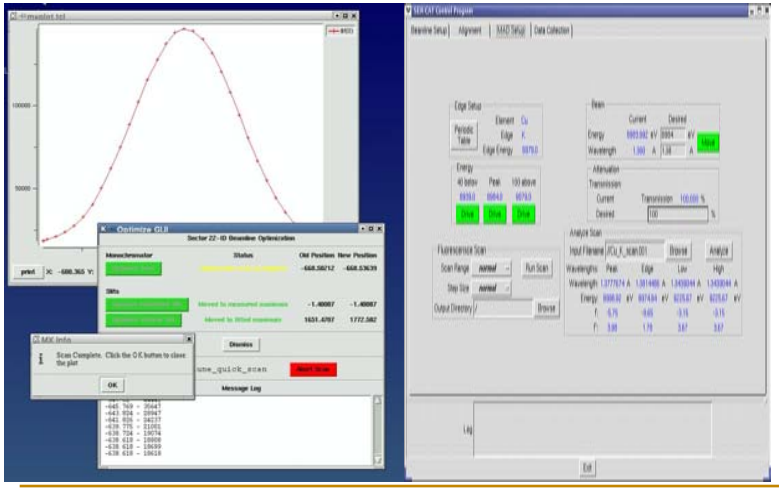
- Biology-oriented facilities at the APS
  - *DND (S5), BESSRC (S12), BioCARS (S14), IMCA (S17), Bio (S18), SBC (S19), SER (S22) LS (S21), NE (S24), GM/CA (S23), SGX (S31) and COM (S32)*
- These facilities offer, to the biology community, ~20 beamlines with bending magnets (BM) and insertion devices (ID, undulators and wigglers) and a variety of supplemental equipment
- Construction of these facilities was funded by government and private sources and are operated by consortia that include scientists from academia, research institutes, national laboratories, and industry



APS Undulator A

Southeast Regional Collaborative AccessTeam

- **SER-CAT is an organization consisting of 22 member institutions, initially formed in 1997 to provide third-generation x-ray capabilities to macro-molecular crystallographers and structural biologists in the southeastern region of this country.**
- **Emphasis is placed on new structure determinations, high-resolution structural analyses, drug design, protein engineering, site-directed mutagenesis projects, and support of the genome program. SER-CAT is unique from most other APS CATs in terms of its large diverse membership and its multiple sources of funding. SER-CAT does not have a single agency sponsor but is funded mainly through state legislative funds, agencies, and the individual universities at the university, department, or individual research group levels. SER-CAT is operated by the University of Georgia, with Professor Bi-Cheng Wang as Director.**



The beamline control software used at SER-CAT is the MX package developed at the Illinois Institute of Technology and IMCA-CAT by Bill Lavender.
The protein crystallography graphical user interfaces (GUIs) are going through an upgrade from a Tcl/Tk to a Python/TK tab-notebook format. The new GUI formats are a byproduct from the experience gained during the commissioning phase and will provide the users with an environment that will be easier to navigate and use.

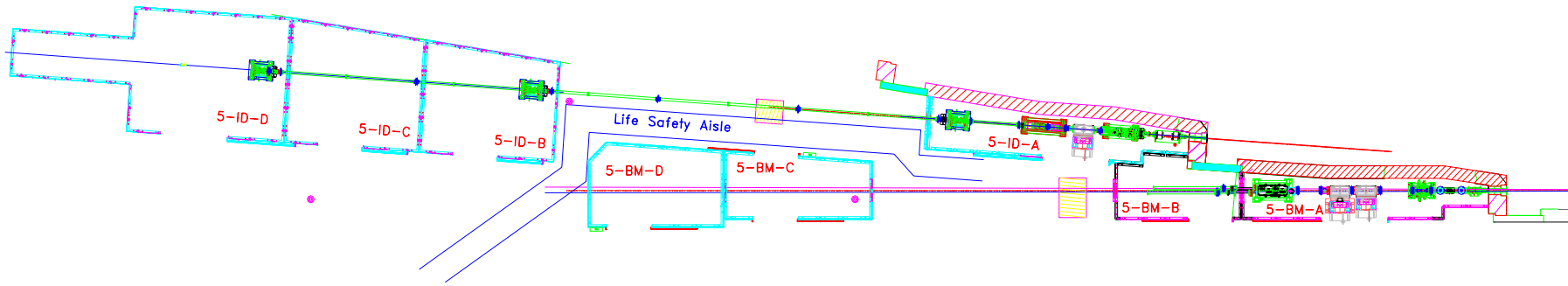
Structural Biology Center

- **SBC is a national user facility for macromolecular crystallography funded by DOE/OBER, constructed and operated by ANL/Biosciences Division**
- **SBC operates two beamlines at APS sector 19: 19-ID and 19-BM; both beamlines are fully operational**
- **User program is in place on both beamlines:**
  - 75% of beam time is allocated for the user program
  - 25% of beam time is used for internal projects
- **SBC staff supports:**
  - Active, successful, and productive user program
  - Continuous facility improvement
  - Research to enhance capabilities of the facility

APS-BIOs

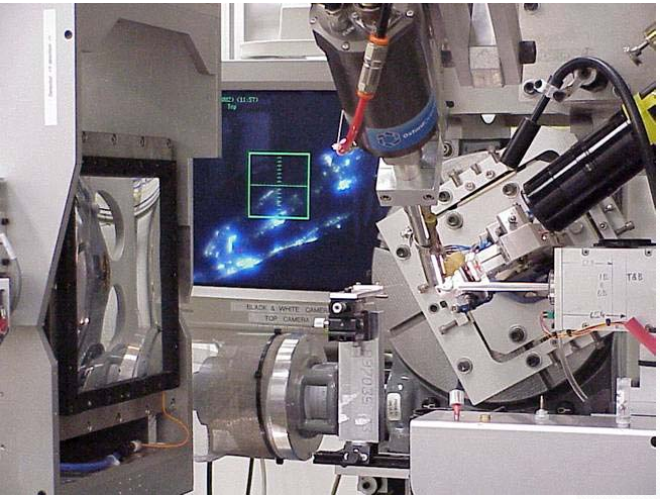
- Broad range of experiments are being offered that exploit brilliance, high flux, flexible bunch structure, coherence, and wide x-ray energy range of the APS:
  - Macromolecular crystallography
    - Single crystal diffraction
    - Macromolecular assemblies
    - Membrane protein crystallography
    - Time-resolved crystallography
    - Atomic resolution crystallography
    - Laue diffraction
    - Powder diffraction
  - Solution scattering
  - Fiber diffraction
  - X-ray fluorescence
  - Resonant inelastic x-ray scattering
  - Radiation damage is being investigated

DND-CAT Sector Layout

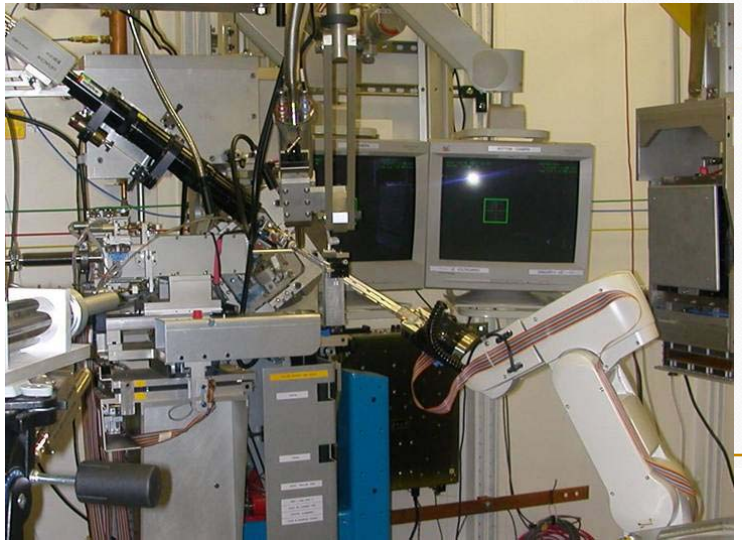


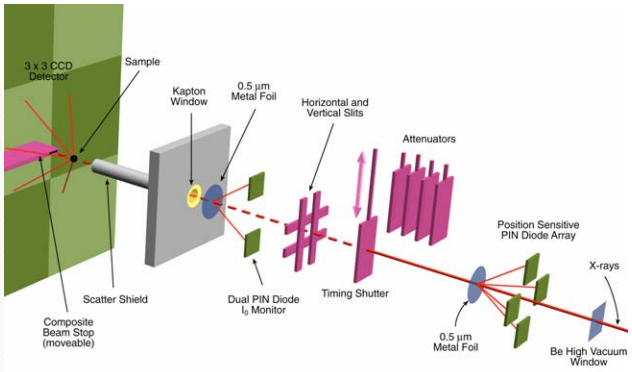
Protein Crystallography is performed in the 5-ID-B undulator station using a commercial MAR 225 detector on a MAR DTB Base

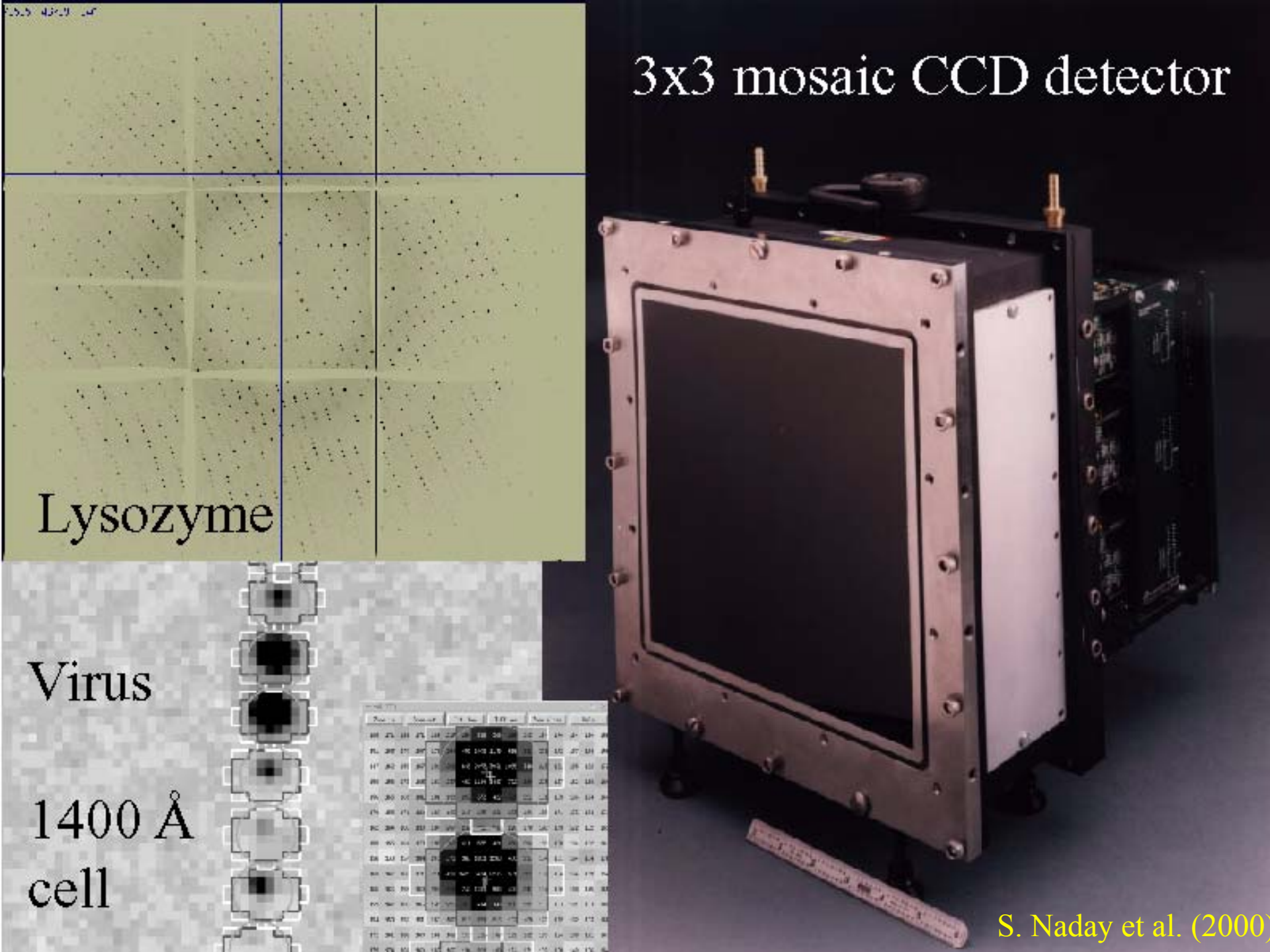
Dedicated X-ray Beamlines for Macromolecular Crystallography - SBC 19-ID and 19-BM Beamlines



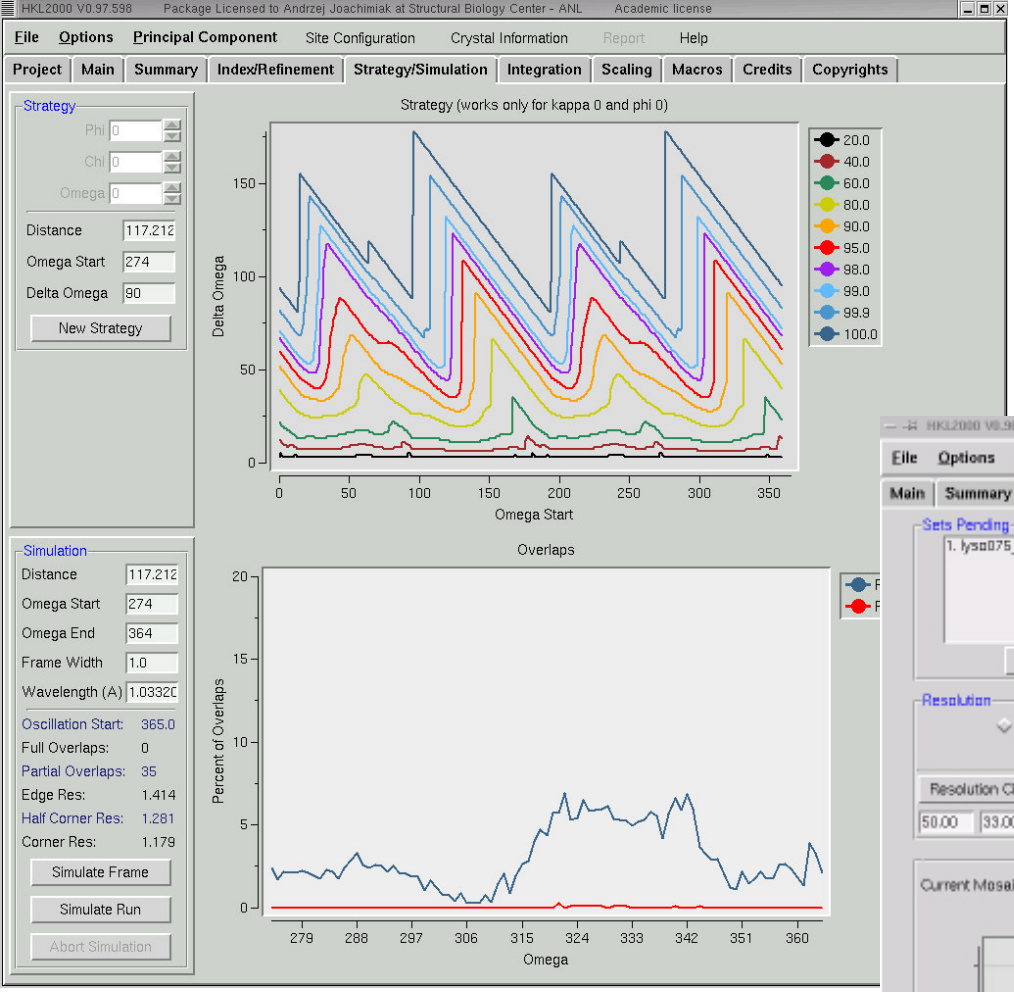
19BM Crystal Mounting Robot

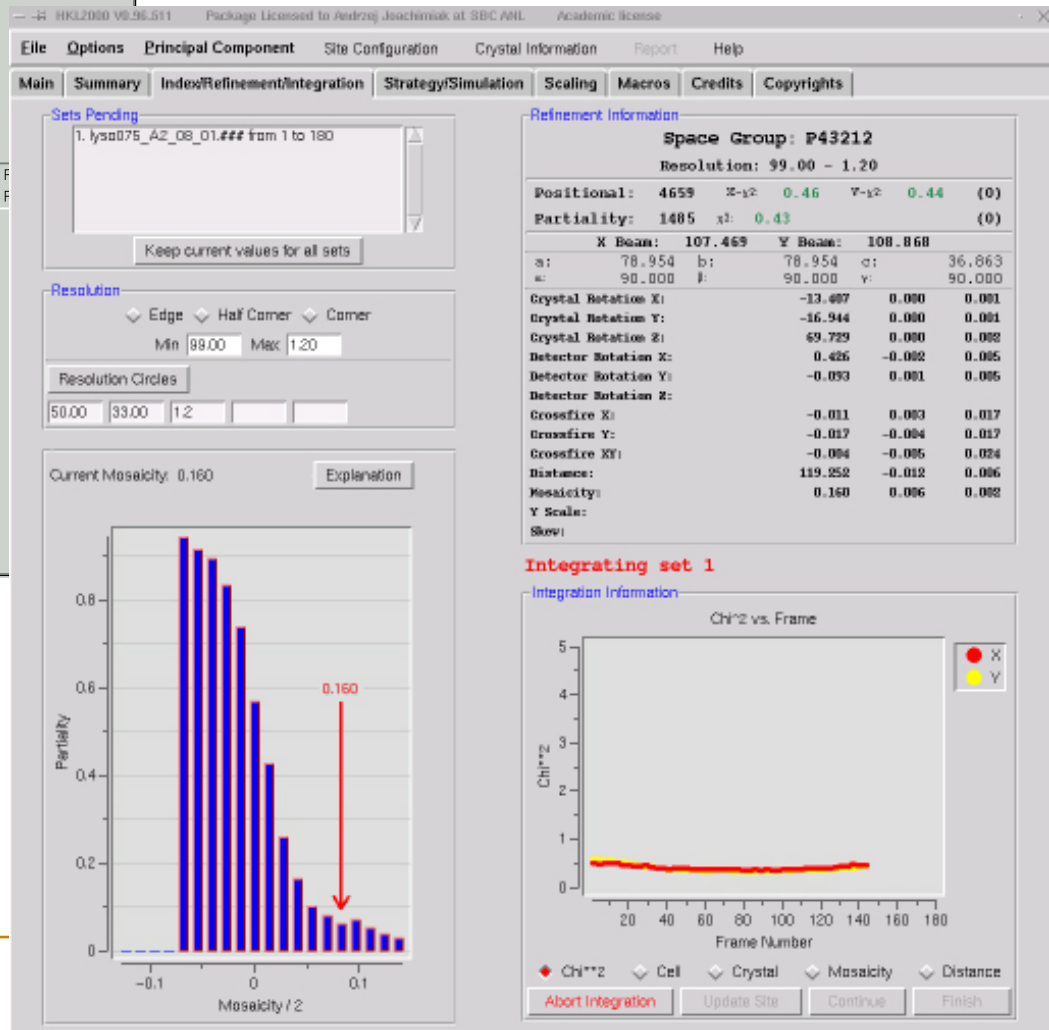





3x3 mosaic CCD detector
Lysozyme
Virus
1400 Å
cell
S. Naday et al. (2000)

HKL2000 - an Example of an Advanced Software for Data Analysis and Processing



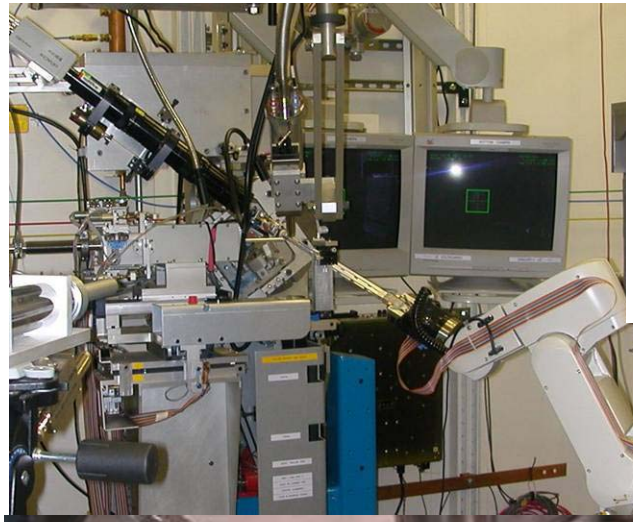


Minor and Otwinowski

Crystal Mounting Robot

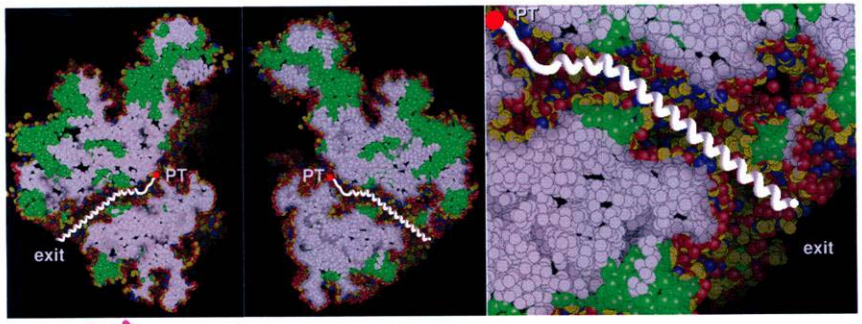
- **The goal of automating the crystal change is to reduce the frequency of experimental station access and the time it takes to align each sample. The challenge is to maintain sample cryogenic temperature and integrity throughout the process of data collection, from crystal mounting to alignment, and to the actual exposure to x-rays. The system includes:**
  - An automatic mounting tool for placement of the crystal from liquid nitrogen storage onto the goniostat and its retrieval
  - A micron-precision crystal mounting goniometer
  - A liquid nitrogen compatible sample transport system
  - A multi-sample mounting system for automatic sample change
  - New alignment tools and procedures

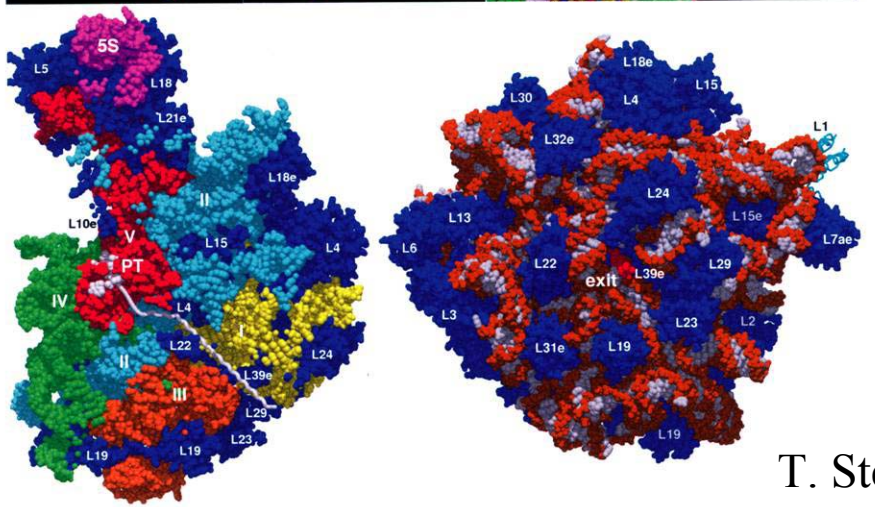
D. Shu et al. (2003)



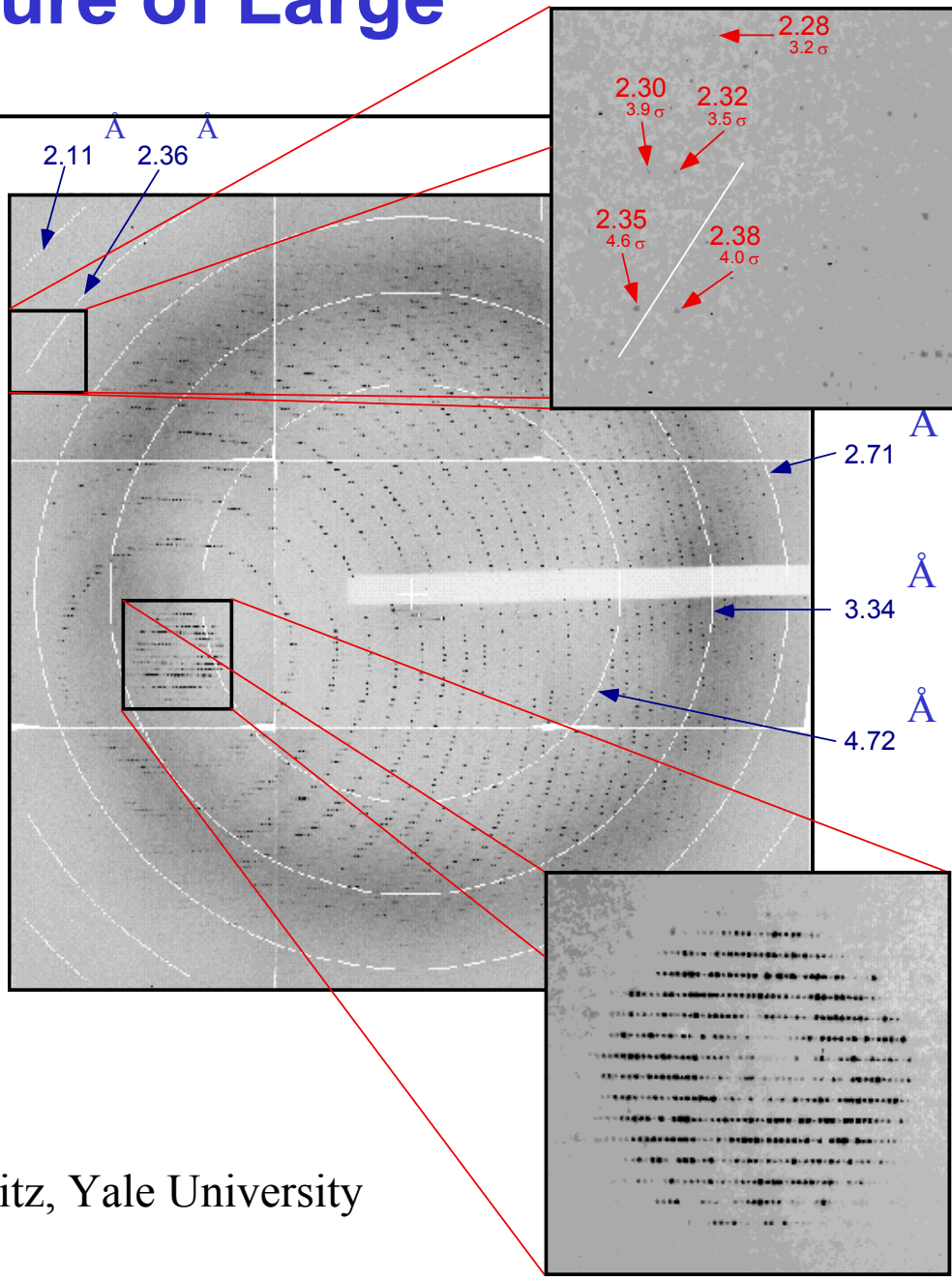
High-Resolution Structure of Large Ribosomal Subunit

- For the first time it was possible to show details of this large assembly – a key part of a universal molecular machine that synthesizes proteins in living cells

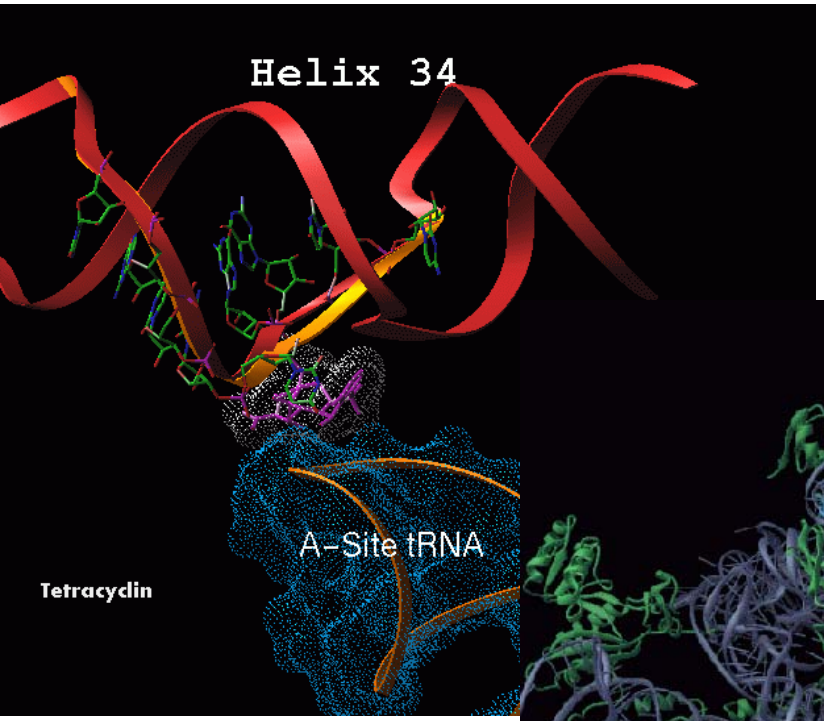


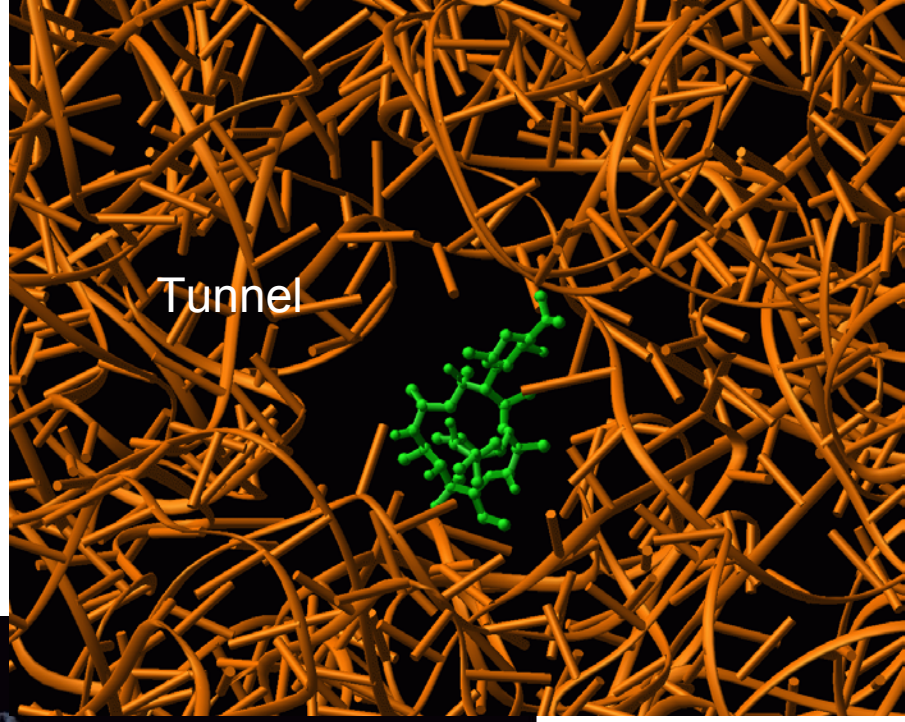


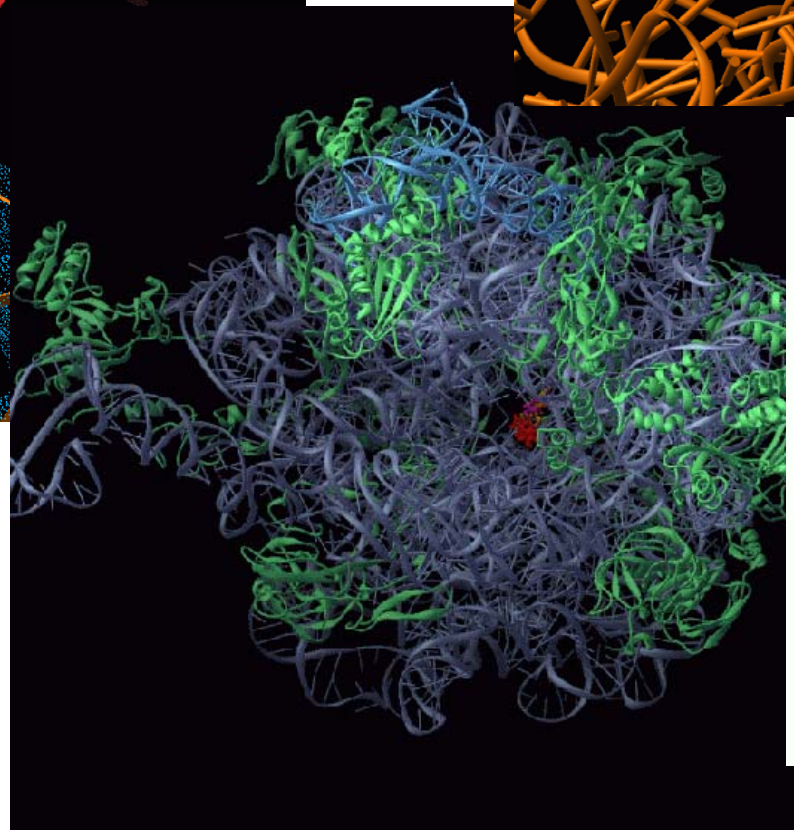
T. Steitz, Yale University

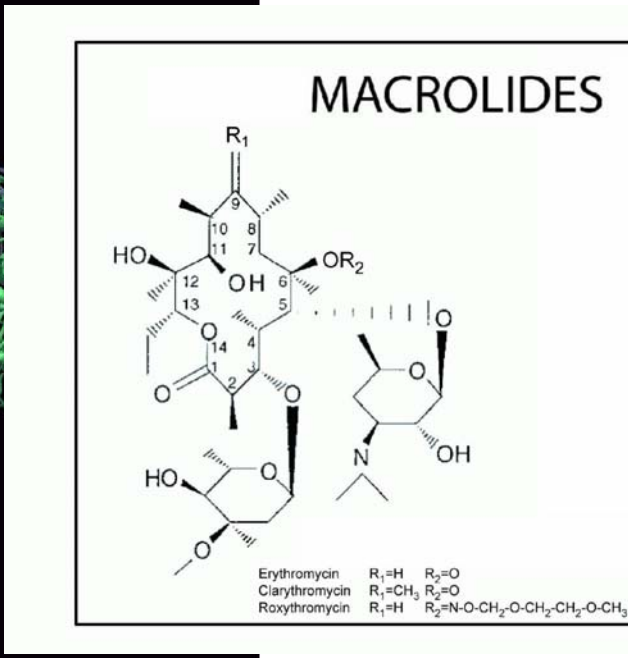


Mapping Binding of Antibiotics to Ribosomal Subunits





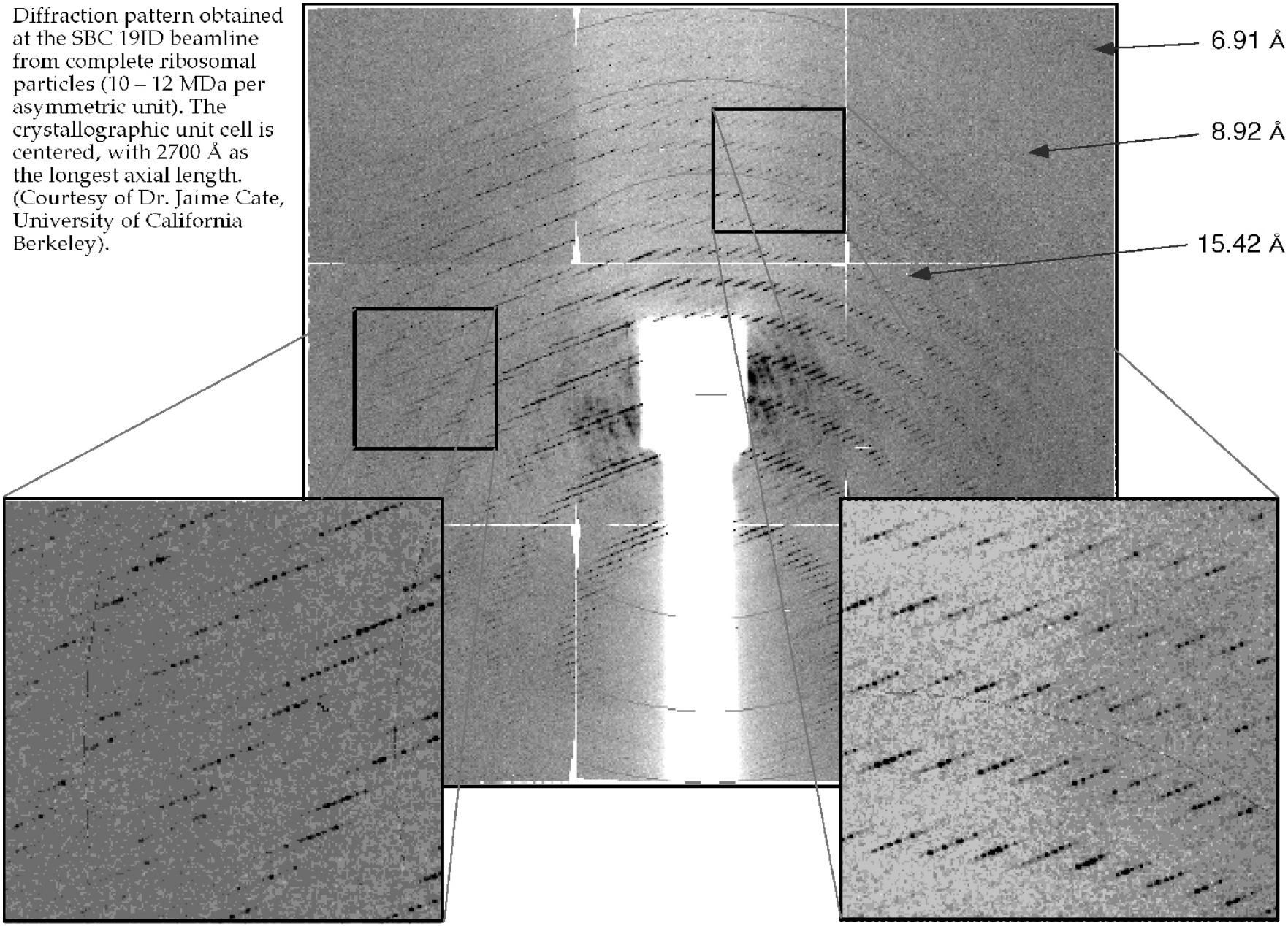




Yonath et al.

Resolving Very Large Unit Cell

Diffraction pattern obtained at the SBC 19ID beamline from complete ribosomal particles (10 – 12 MDa per asymmetric unit). The crystallographic unit cell is centered, with 2700 Å as the longest axial length. (Courtesy of Dr. Jaime Cate, University of California Berkeley).

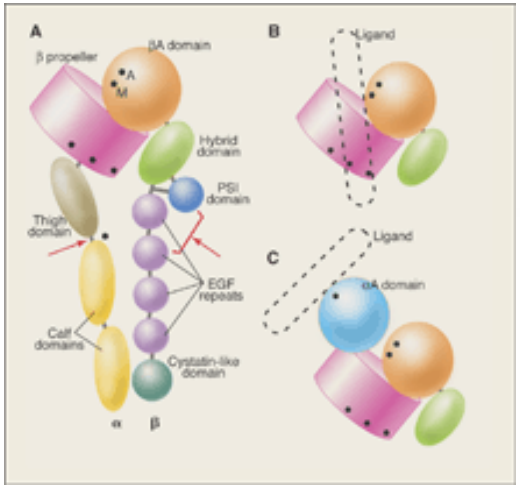


Integral Membrane and Membrane Associated Proteins

- **Structural Basis of Gating by the Outer Membrane Transporter FecA**
- **The E. coli BtuCD Structure: A Framework for ABC Transporter Architecture and Mechanism**
- **Crystal Structure of the Extracellular Segment of Integrin [alpha]V[beta]3**
- **Crystal Structure of the Extracellular Segment of Integrin [alpha]V[beta]3 in Complex with an Arg-Gly-Asp Ligand**
- **Structures of the [alpha] L I Domain and its Complex with ICAM-1 Reveal a Shape-Shifting Pathway for Integrin Regulation**
- **Structure and Mechanism of the Glycerol-3-Phosphate Transporter**

3.1-Å Structure of Anthropomorphic Integrin αVβ3– a Member of Adhesion Receptor Family

- Integrins are large (~2,000 residues), heterodimeric, multidomain, highly flexible, membrane associated cell surface receptors. Eighteen α and eight β subunits are known
- Integrins control key cellular activities including proliferation, migration, and survival by propagating bi-directional signals across the cell membrane
- Integrins contribute to the initiation and/or progression of many common diseases including neoplasm, tumor metastasis, immune dysfunction, viral entry into cells, osteoporosis
- The integrin αVβ3 is a pro-angiogenic, pro-inflammatory, and bone remodeling receptor

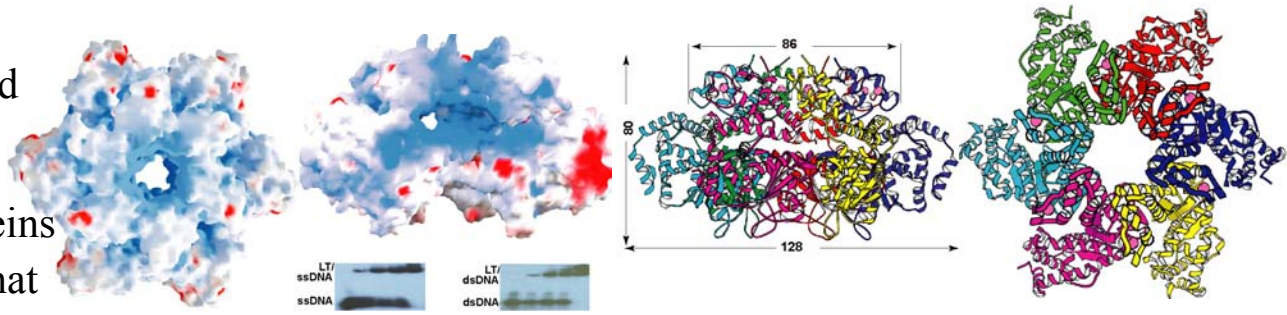


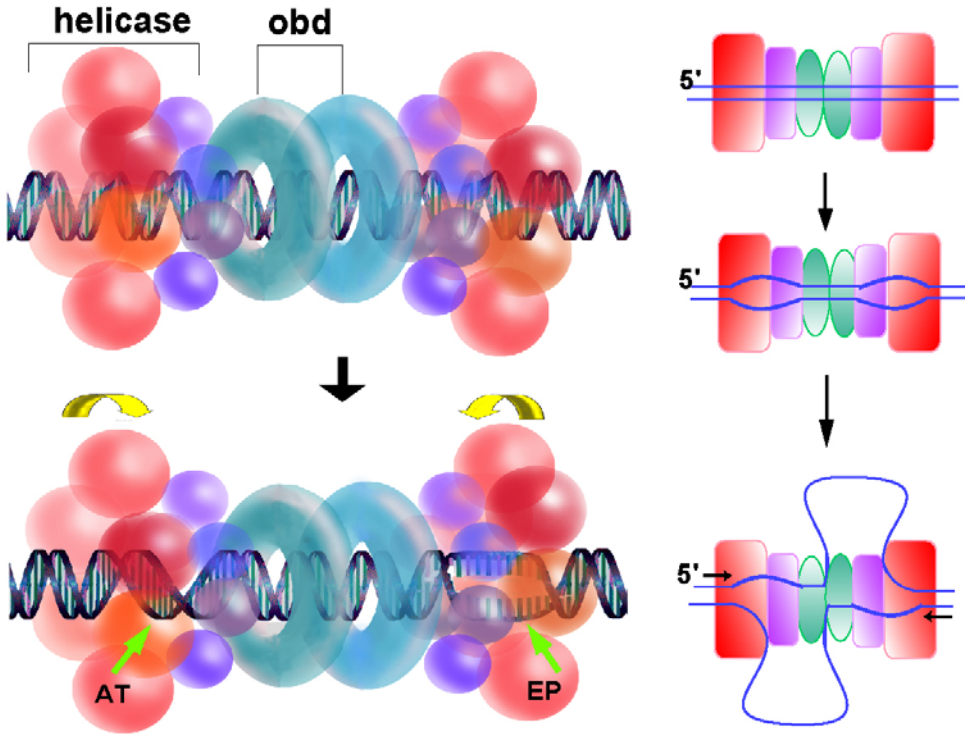
Receptor Proteins

- Structure of the inositol 1,4,5-trisphosphate receptor binding core in complex with its ligand
- Structural determinants for regulation of phosphodiesterase by a G protein
- Structure of a bacterial quorum-sensing transcription factor complexed with pheromone and DNA
- Ligand-receptor binding revealed by the TNF family member TALL-1
- Structural basis for the activation of anthrax adenylyl cyclase exotoxin by calmodulin
- The human nuclear xenobiotic receptor PXR: structural determinants of directed promiscuity
- Crystal structure of the glucocorticoid receptor ligand binding domain reveals a novel mode of receptor dimerization and coactivator recognition
- Mechanism of ubiquitin recognition by the CUE domain of Vps9p
- Structural basis for autoinhibition of the EphB2 receptor tyrosine kinase by the unphosphorylated juxtamembrane region
- Structure of the LDL receptor extracellular domain at endosomal pH
- Structural basis for phospho-dependent substrate selection and orientation by the SCFCdc4 ubiquitin ligase
- Prevention of chemotherapy-induced alopecia in rats by CDK inhibitors
- Complex between nidogen and laminin fragments reveals a paradigmatic [beta]-propeller interface

Structure of SV40 Large T Antigen

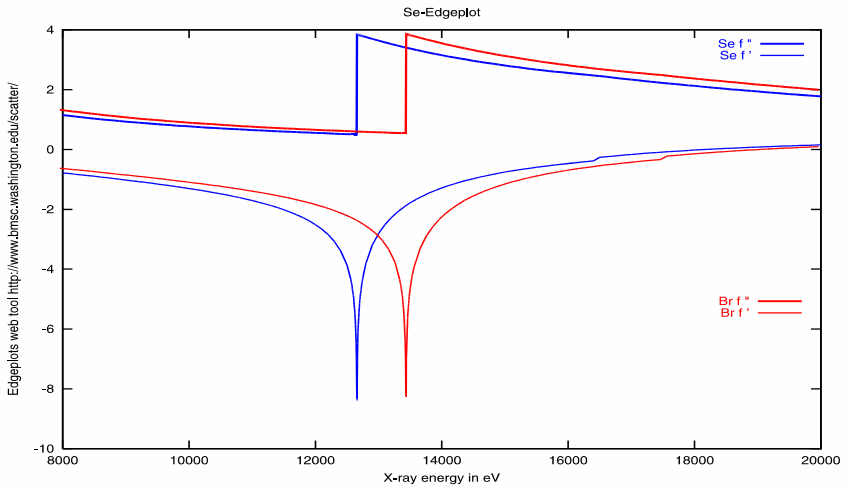
- The oncoprotein large tumor antigen (LTag) is encoded by the DNA SV40
- The protein transforms cells and induces tumors in animals by altering the functions of tumor suppressors and other key proteins
- LTag is a molecular machine that distorts/melts the DNA
- The structure of hexameric LTag with DNA helicase activity LTag identifies the DNA- and protein-binding surfaces
- The hexamer contains a long, positively charged channel with a large central chamber that binds both ssDNA and dsDNA
- The hexamer produces an 'iris' effect that could be used for distorting or melting the DNA
- LTag seems to be a functional homologue of the eukaryotic mini-chromosome maintenance complex





Multiwavelength Anomalous Diffraction (MAD) Using Synchrotron Sources Creates an Opportunity for Automation of Protein Structure Determination

- All "heavy – N>50" and "light – 20<N<50" atoms show good anomalous signal associated with K, L and M absorption edges
- "Heavy" atoms can be readily introduced into proteins (SeMet, Br, I, Xe, Ar, As, metal ions (Rb etc)) and DNA/RNA (Br)
- MAD/SAD does not require a native crystal
- Anomalous signal does not decay with resolution
- Use of anomalous signal simplifies approach to structure determination and improves isomorphism
- The anomalous signal is weak (1-6%)
- Optimal data collection requires a synchrotron facility



- **MAD for PROTEINS**
- *In vivo* protein labeling with SeMet
- Standard protocol for data collection and structure determination
- High resolution and high quality allows auto-tracing

Pushing the Limits

Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Taking MAD to the extreme: ultrafast protein structure determination

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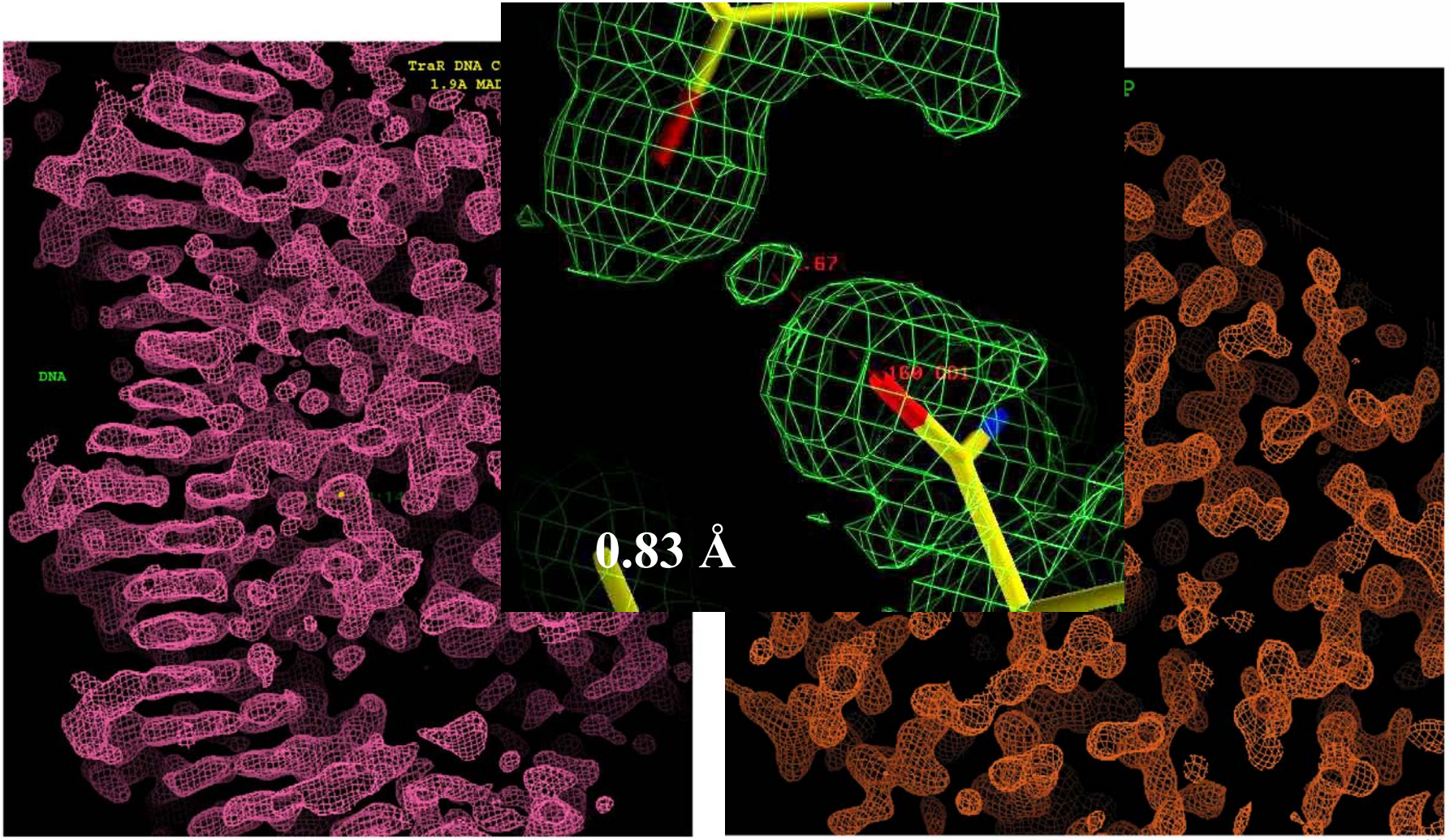
[a]Building 202, Argonne National Laboratory, 9700 South Cass Avenue, Argonne IL 60439, USA, and [b]Northwestern University, Department of Biochemistry, Molecular Biology and Cell Biology, Evanston IL 60208, USA

Multiwavelength anomalous diffraction data were measured in 23 min from a 16 kDa selenomethionyl-substituted protein, producing experimental phases to 2.25 Å resolution. The data were collected on a mosaic 3 × 3 charge-coupled device using undulator radiation from the Structural Biology Center 19ID beamline at the Argonne National Laboratory's Advanced Photon Source. The phases were independently obtained semiautomatically by two crystallographic program suites, *CCP*4 and *CNS*. The quality and speed of this data acquisition exemplify the opportunities at third-generation synchrotron sources for high-throughput protein crystal structure determination.

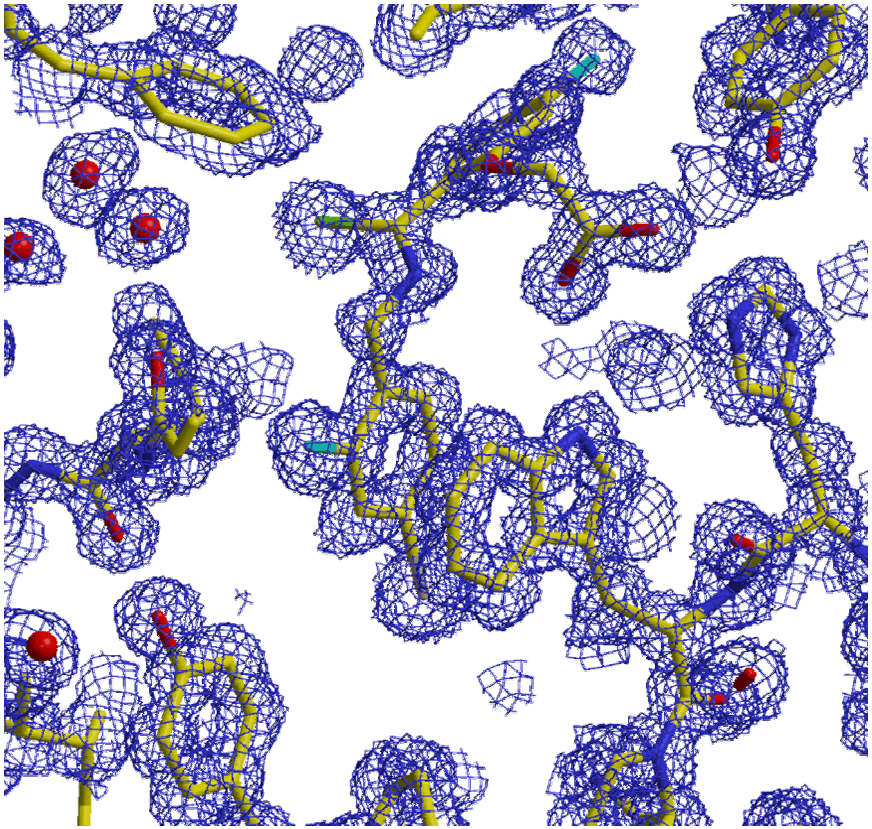
Received 25 September 1998
Accepted 3 March 1999

PDB Reference: chaperonin apical domain, 1srv.

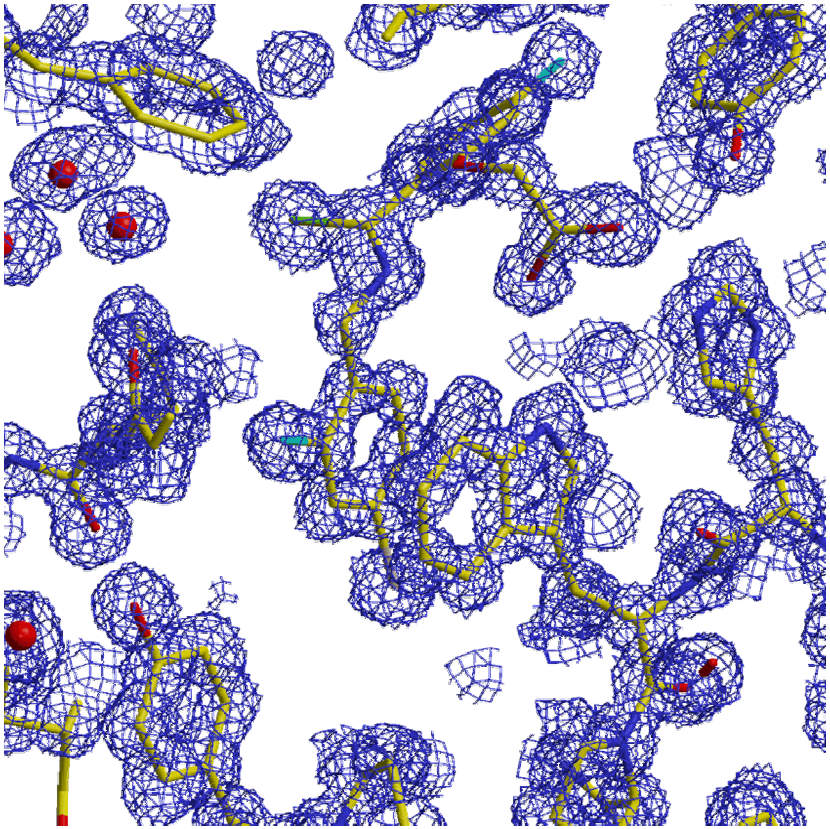
MAD Phasing Provides Higher Quality Electron Density Maps and Improves Structure Quality



Human Aldose Reductase – SeMet MAD at 0.9Å Comparison – Experimental vs. Refined Map



Refined map @ 0.9 Å, sigmaA ($2mF_o$-DF_c), contour level: 1 sigma

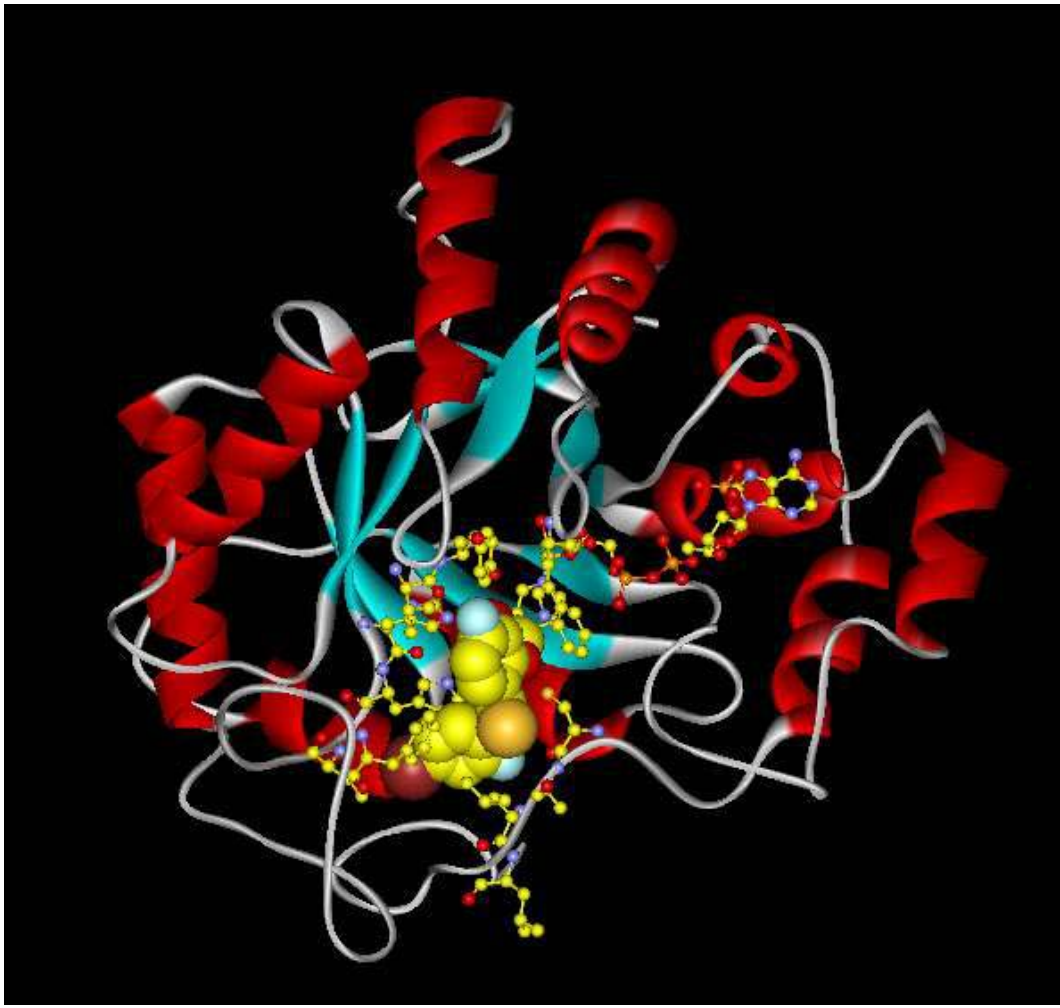


Experimental map @ 0.9 Å, F_o, contour level: 1 sigma

Podjarny *et al.* 2001

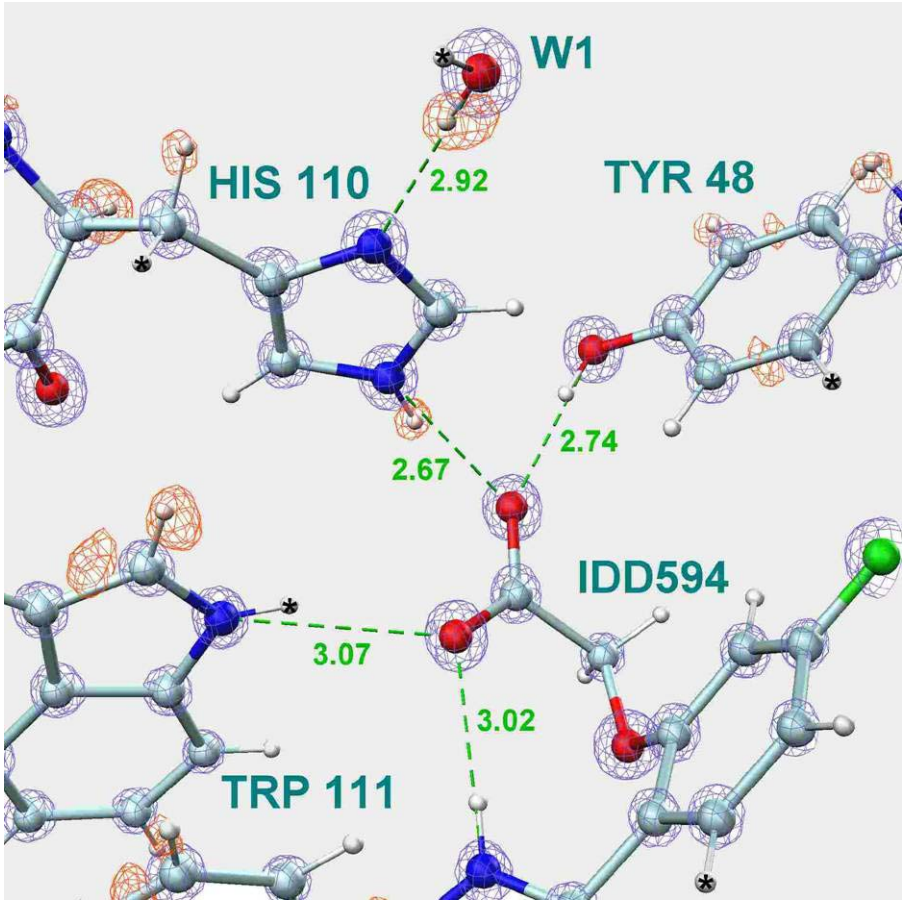
Data Collection at Subatomic Resolution

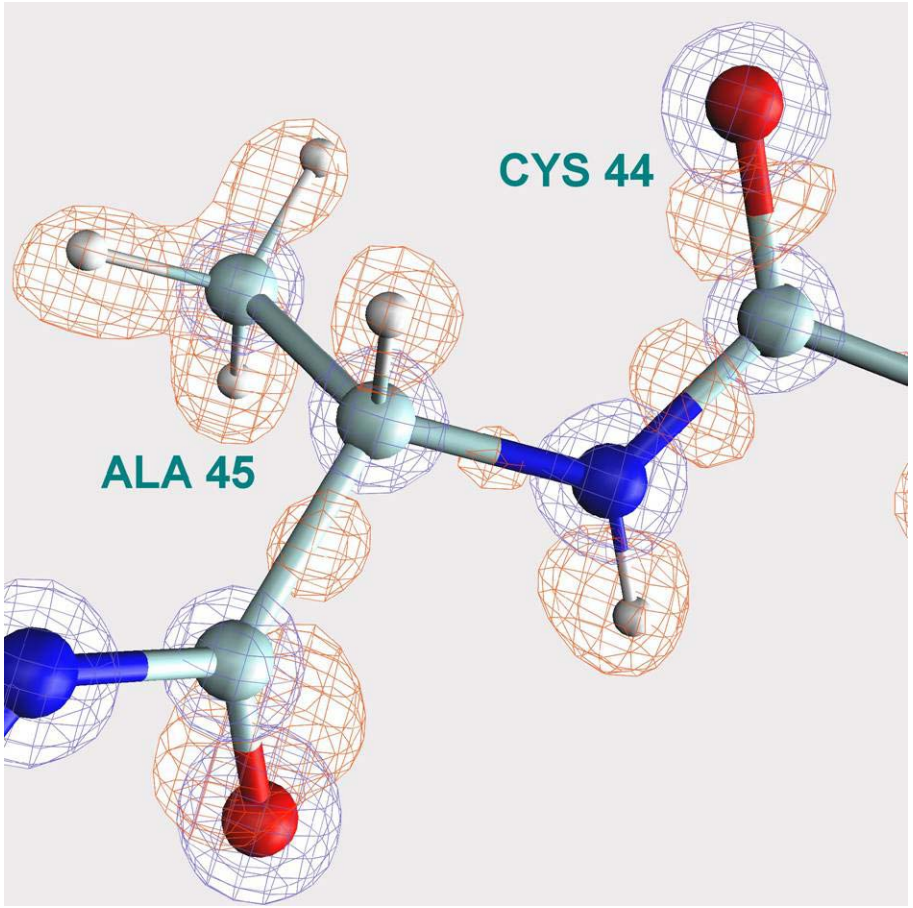
- Aldose reductase - a NADPH-dependent enzyme reduces D-glucose into D-sorbitol, which is believed to cause severe degenerative complications of diabetes
  - **315 amino acid residues**
  - **$(\alpha/\beta)_8$ barrel fold**
  - **Coenzyme $NADP^+$**
  - **One inhibitor molecule**
- The crystal structure of human aldose reductase complexed with IDD594 inhibitor and NADP+ was determined to 0.66 Å



Podjarny *et al.*

Structure of Human Aldose Reductase at 0.66 Å: His110 Protonation, Hydrogen Atoms (Ala45) and Bond Densities

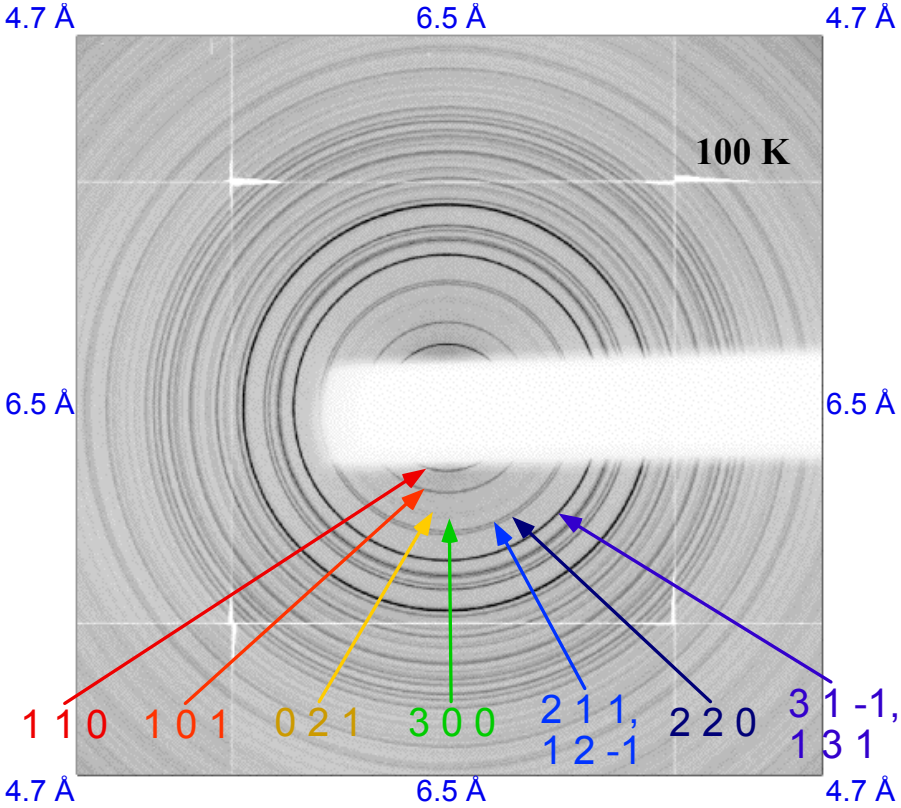


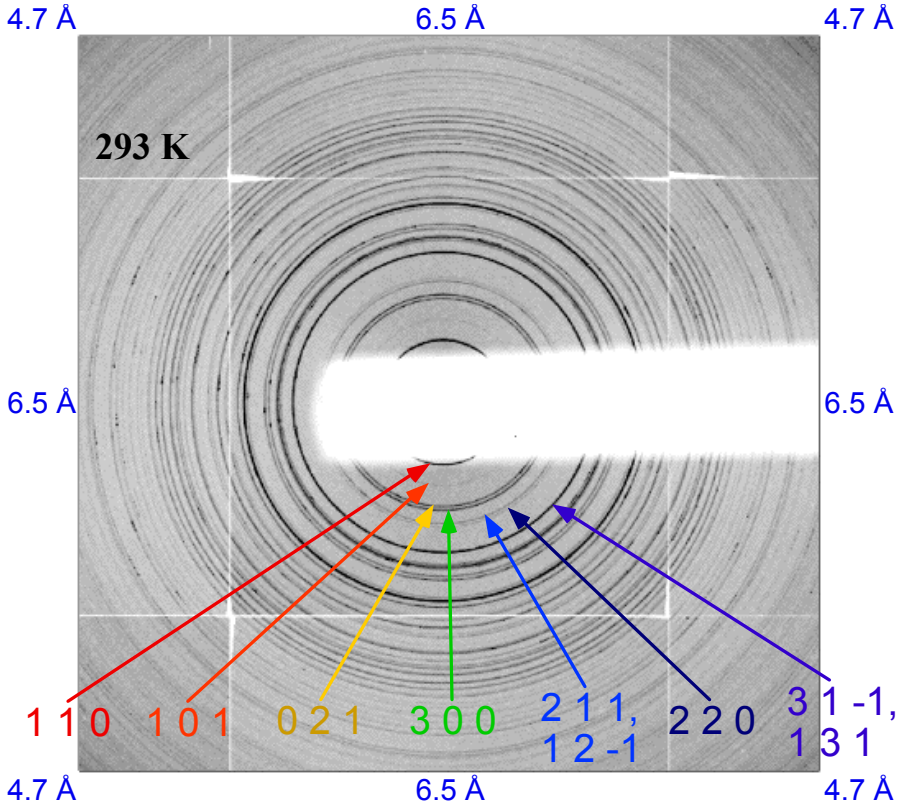


Podjarny *et al.* 2001

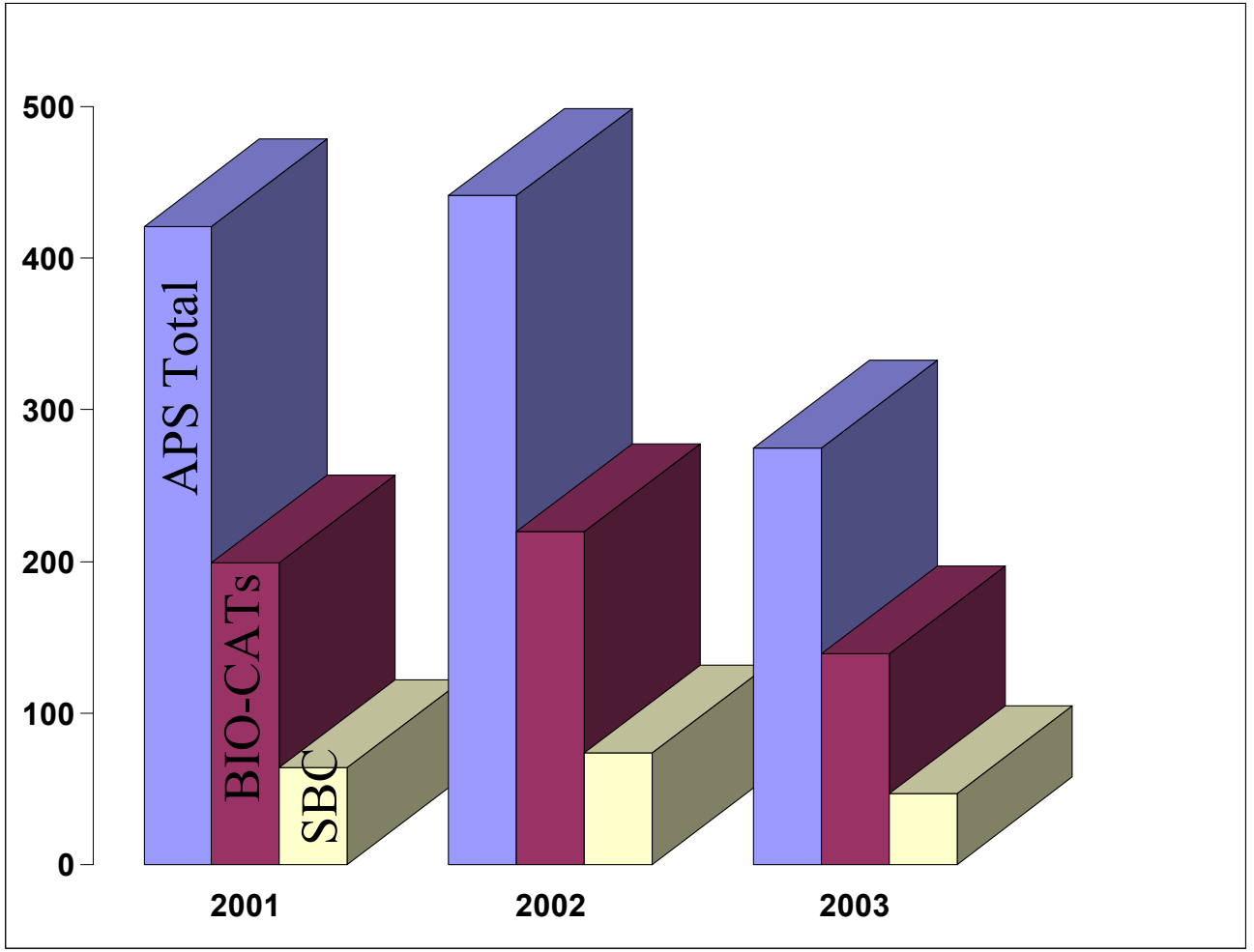
Powder Diffraction from Microcrystals of Porcine Insulin at Ambient Temperature and Flash Frozen at 100 K

- λ = 1.03321 Å; exposure = 180 s; diffraction is observed to 3.5 Å
- Beam size = 0.2 x 0.2 mm^2; sample oscillation = 360°; detector distance = 400 mm
- The patterns are indexed assuming a trigonal R3 lattice with a = 82.5 Å and c = 34.0 Å



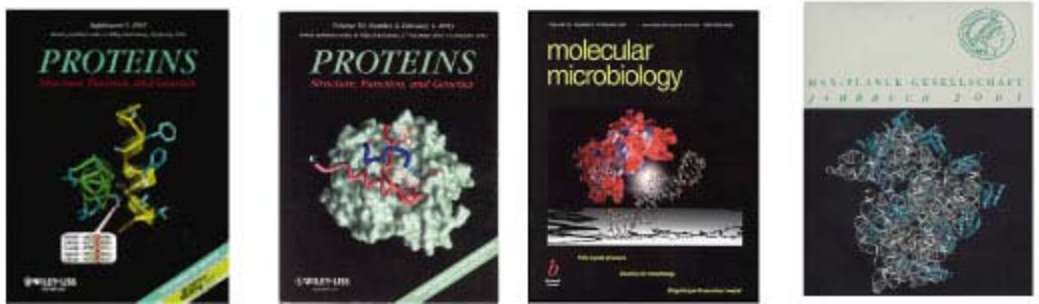


APS Users and Staff Journal Publications

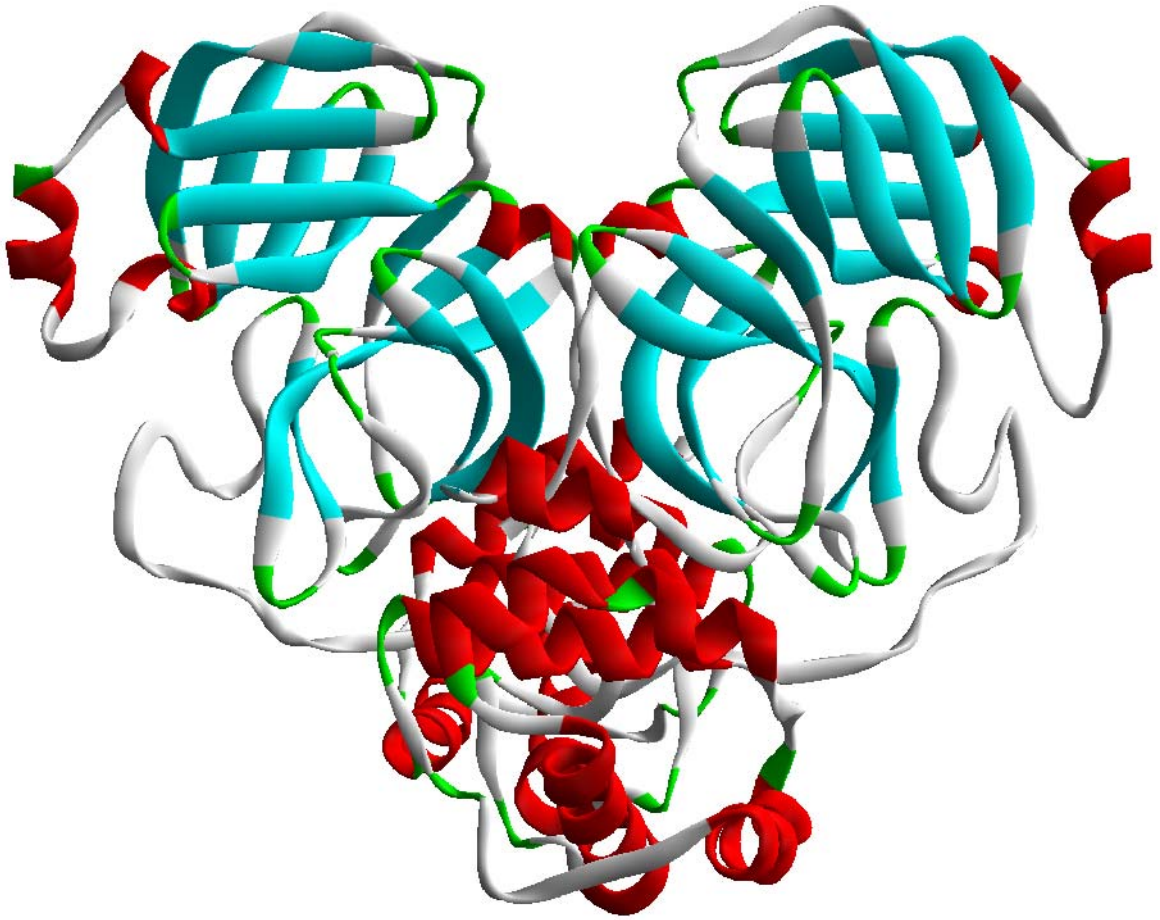


BIO-CATs – total 594 publications, 50 in NCS (8.4%)

APS-BIOs Journal Covers (Selected)



SARS Coronavirus Main Protease from SGX-CAT – an Example of Rapid Structure Determination in Response to New Emerging Disease



X-ray Radiation Damage Studies

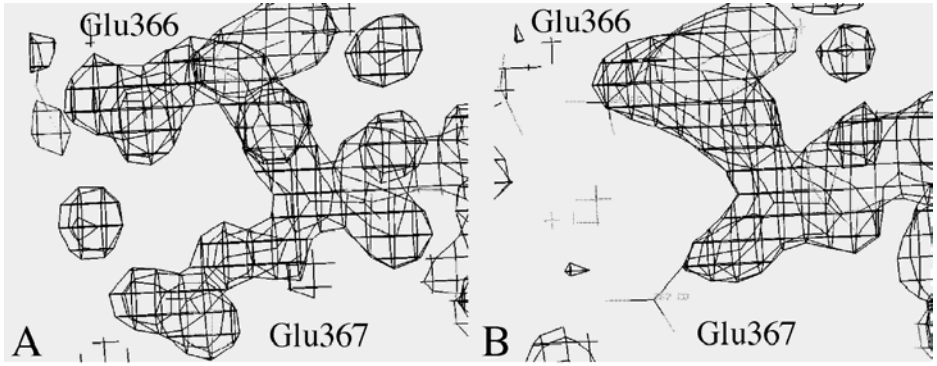
- Tsu-Yi Teng, Keith Moffat, "Radiation damage of protein crystals at cryogenic temperatures between 40 K and 150 K," J. Synchrotron Rad. **9**, July, 198-201, (2002)
- Piotr Sliz, Stephen C. Harrison, Gerd Rosenbaum, "How Does Radiation Damage in Protein Crystals Depend on X-Ray Dose?," Structure **11**, January, 13-19, (2003)
- R. Fischetti, A. Mirza, D. J. Rodi, T. C. Irving, E. Kondrashkina, L. Makowski, "Effect of beam dose on protein integrity," J. Synchrotron Rad. **10**, 398-404, (2003)

Molecular Effects

- Definition of disulfite bridges and carboxylates lost
- Occupancy of metal ions decrease rapidly

Crystallographic Effects

- Increase in unit cell dimensions
- Increased mosaicity
- Higher temperature factor
- Lower resolution



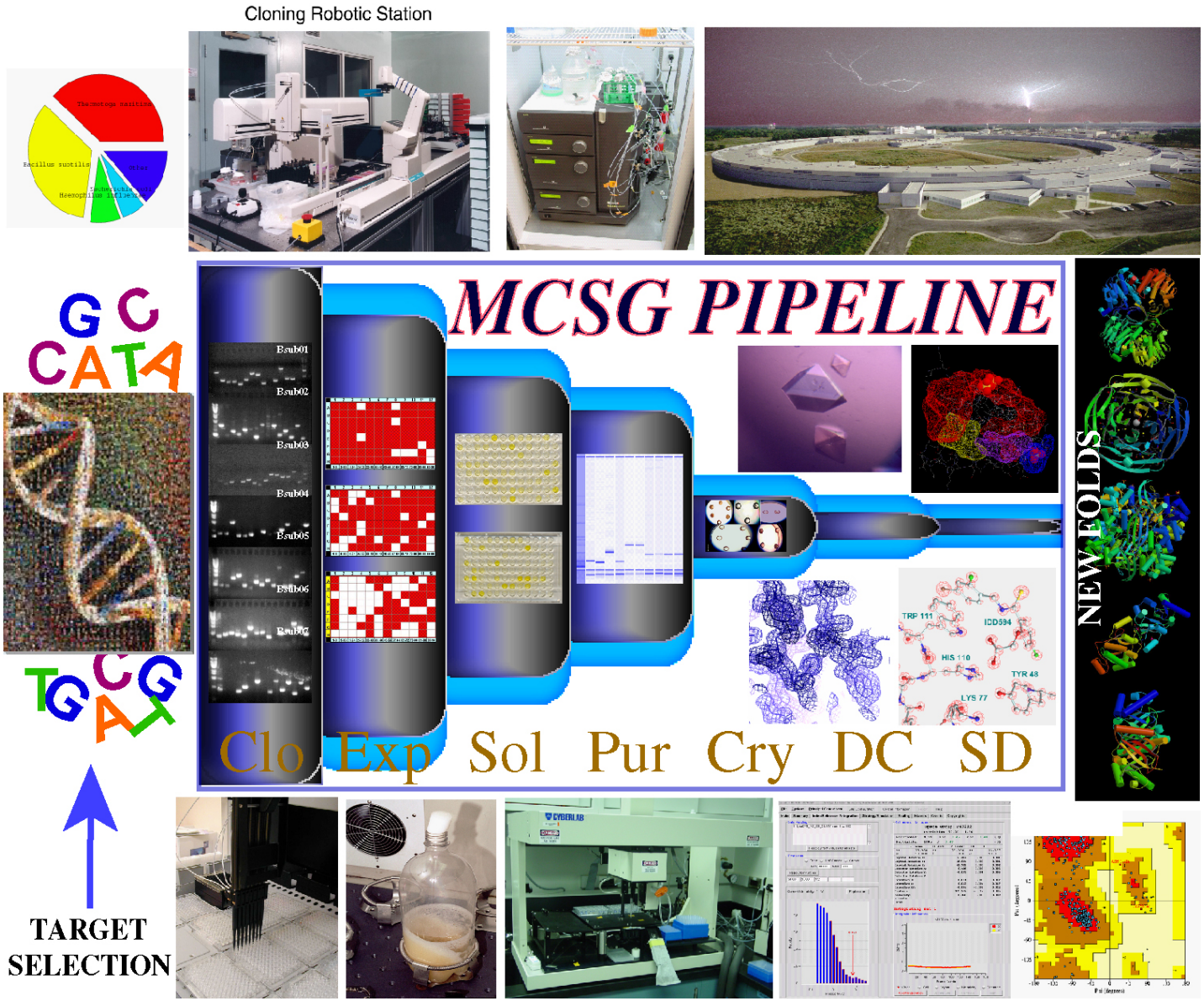
Structural Genomics (SG)

- The Protein Structure Initiative aims at providing one or more representative structures from each of the several thousands of protein domain families found in living organisms
- SG benefits to scientific community, biotechnology and pharmaceutical industries include:
  - New technology for cost-effective molecular biology and protein purification will evolve from the project - bottlenecks will be identified and practical solutions will be established
  - SG will enhance crystallographic capabilities by significantly reducing the time and cost required to determine protein 3D structures and will benefit structural biology
  - The HTP technologies will be developed to handle challenging biological systems and will benefit biology and biotechnology
  - Libraries of genes, expression clones and proteins will be produced and will be available to public

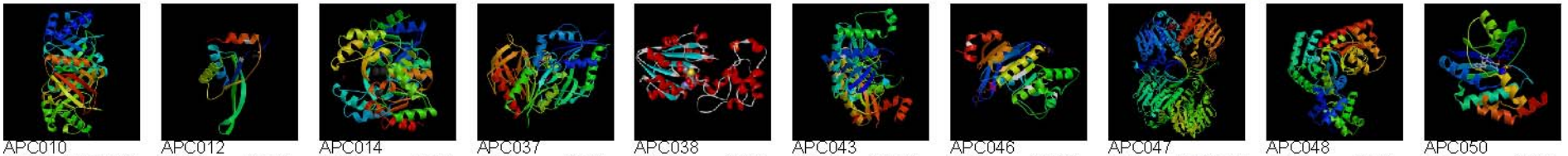
Structural Genomics at APS

- The Midwest Center for Structural Genomics – SBC and DND-CAT
- The Southeast Collaboratory for Structural Genomics– SER-CAT
- Function-to-Structure – IMCA-CAT
- Several other PSI pilot projects use APS-BIOs:
  - The Northeast Structural Genomics Consortium
  - The New York Structural Genomics Research Consortium
  - The Joint Center for Structural Genomics
  - The Berkeley Structural Genomics Center
  - The TB Structural Genomics Consortium
  - Structural Genomics of Pathogenic Protozoa Consortium
  - Center for Eukaryotic Structural Genomics

Protein Structure Determination Pipeline



MCSG Structures Deposited in the PDB, 01/2003

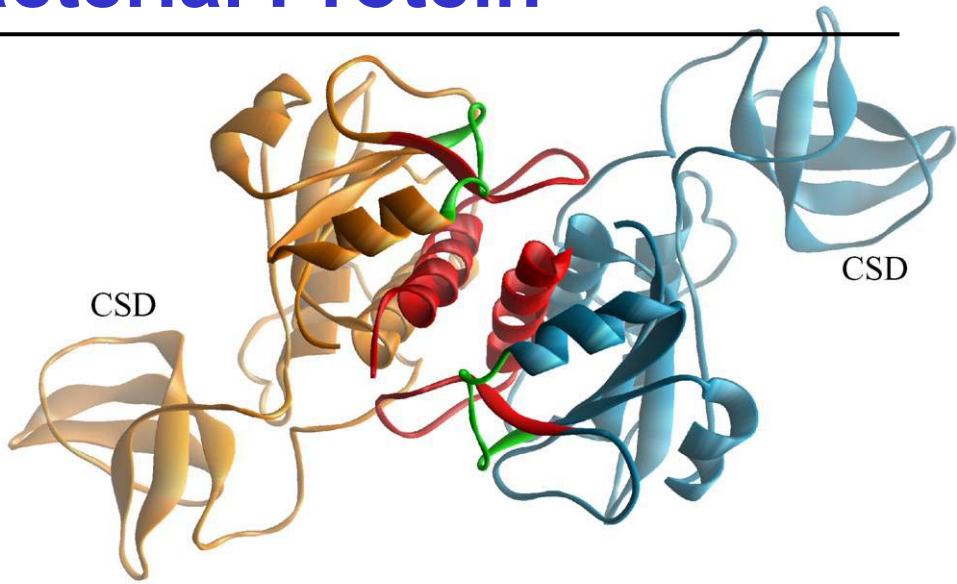


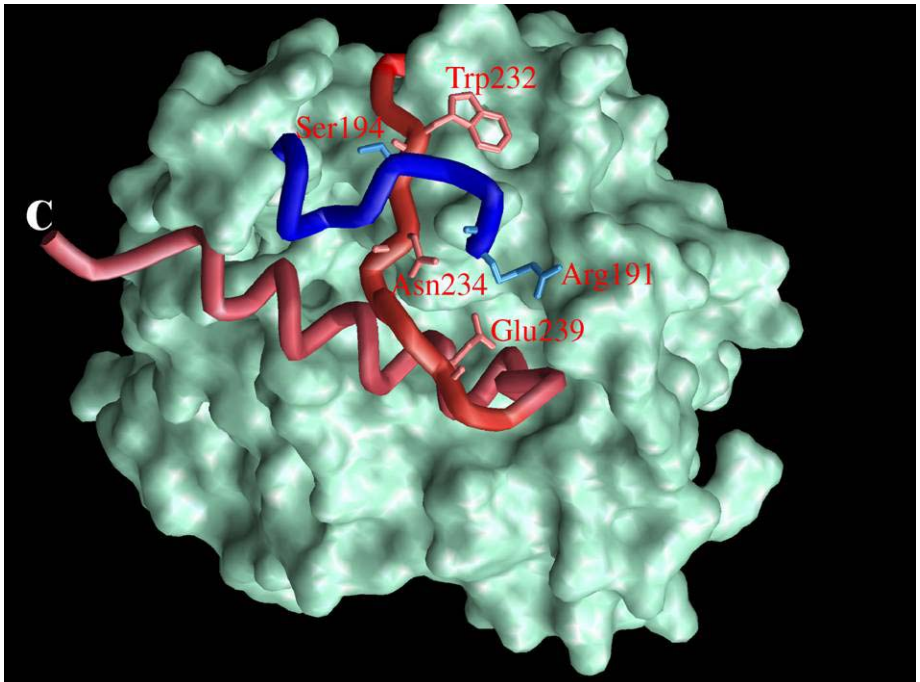
APC010 1NG5 New Fold	APC012 1KR4 25.7%	APC014 1KYT <20%	APC037 1KXJ 100%	APC038 1M6Y <20%	APC043 1KUT 24.7%	APC046 1JI0 33.2%	APC047 1INL New Fold	APC048 1JMR <20%	APC050 1EJ2 <20%
APC052 1DV7 22.9%	APC063 1MKZ 26.4%	APC064 1M33 28.2%	APC065 1KS2 <20%	APC066 1K77 20.6%	APC070 1K4N <20%	APC072 1NN4 <20%	APC074 1K7K New Fold	APC077 1KTN 100%	APC078 1KJN <20%
APC079 1L1S <20%	APC085 1G2R New Fold	APC100 1LJ9 <20%	APC115 1K7J New Fold	APC116 1EG2 23.3%	APC117 1HRU New Fold	APC121 1EX2 New Fold	APC127 1DWK New Fold	APC128 1G60 31.2%	APC131 1K3R New Fold
APC132 1I36 <20%	APC175 1ILV New Fold	APC234 1KYH <20%	APC236 1I6N <20%	APC250 1K6D 28%	APC409 1NPY <20%	APC446 1NRI <20%	APC784 1G6O <20%	APC1040 1MKI <20%	APC1052 1L7A <20%
APC1056 1M3S 33.1%	APC1068 1NSL <20%	APC1138 1NRH <20%	APC1167 1NNI <20%	APC1392 1NJH <20%	APC1490 1MK4 <20%	APC1524 1NG6 <20%	APC1644 1NC5 <20%	APC1773 1NRW <20%	APC3005 1J8R <20%
APC3503 1NJK <20%	APC4568 1NC7 New Fold	APC5002 1NQK 100%	APC5006 1ML8 <20%	APC5007 1NI9 <20%	APC5008 1NR9 36.7%	APC5513 1NE2 <20%	APC10009 1NIG <20%	APC20011 1NOG <20%	APC35040 1MKF <20%

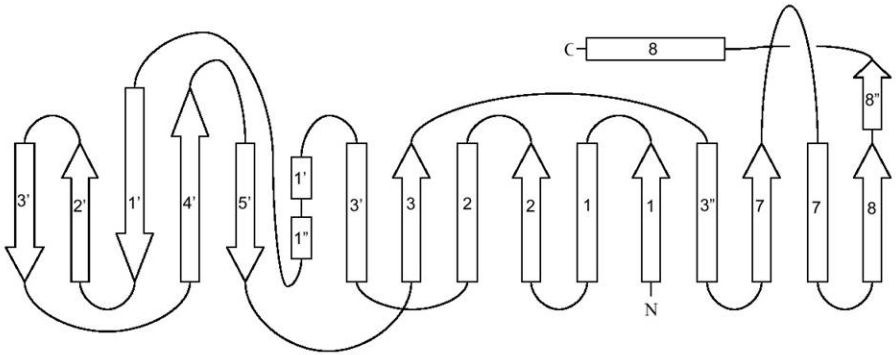
New Fold indicates no structural similarity to the PDB as determined by combinatorial extension. Percentages indicate global sequence identity to prior deposits in the PDB.

Deep Trefoil Knot Implicated in RNA Binding Found in an Archaebacterial Protein

- The structure contains a novel topological unit – a deep C-terminal trefoil knot
- MT1 has only five (β/α) units and the arrangement of its hydrophobic and hydrophilic surfaces is opposite to that found in classical barrel proteins
- Functionally it has strongly conserved residues clustered on the surface that form a potential catalytic site
- The structure provides a first example of a barrel-like fold linked to RNA-binding domain







APS-BIO's Future

- Developing instruments that take full advantage of the APS capabilities:
  - Canted undulator design
  - Fast, large surface, and dynamic range detectors for crystallography
- Developing stations that combine simultaneous measurements from different biophysical techniques
- Developing nanoscale imaging techniques – whole cell imaging and nanostructures
- Robotics, automation, and remote data collection