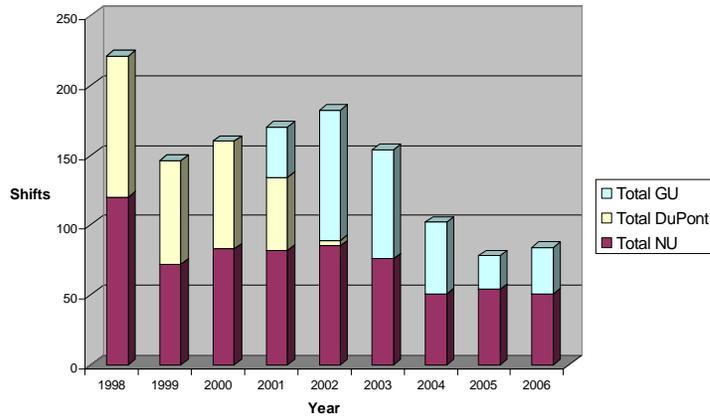


# DND-CAT and LS-CAT

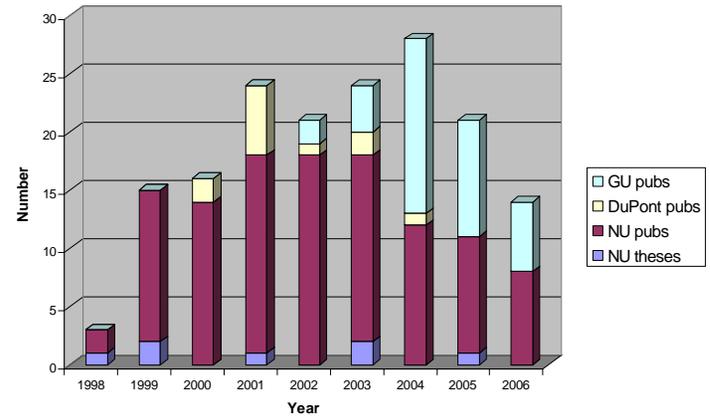
- DND-CAT
  - Director Denis Keane
  - Sector 5
- LS-CAT
  - Beamline Manager - Keith Brister
  - Sector 21

# DND-CAT MC History

DND Macromolecular Shifts

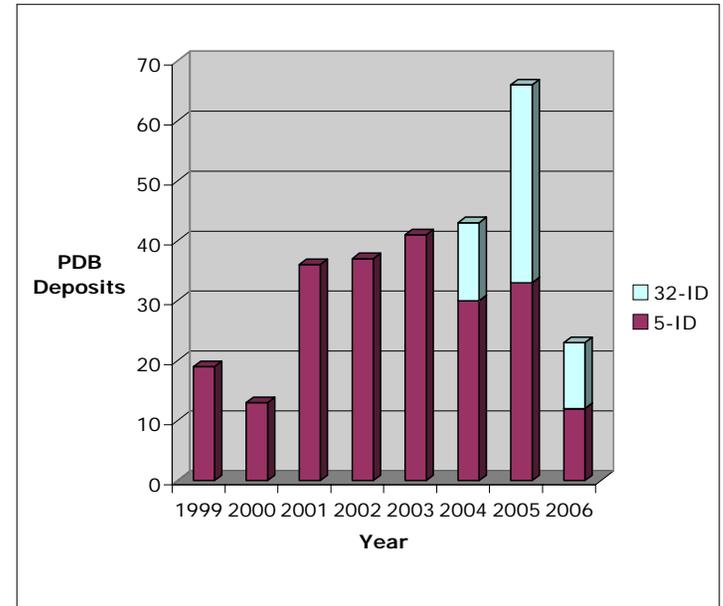


DND Macromolecular Publications



- MC Equipment

- 1997 RAXIS-IV
- 1998 MAR CCD + MAR Base
- 1999 MAR CCD + MAR Base
- 2000 MAR 165 + MAR Base
- 2001 MAR 165 + MAR dtb
- 2002 MAR 165 + MAR dtb
- 2003 MAR Mosaic 225 + MAR dtb
- 2004 MAR Mosaic 225 + MAR dtb + sample changer
- 2005 MAR Mosaic 225 + MAR dtb + sample changer
- 2006 MAR Mosaic 225 + MAR dtb + sample changer



# DND-CAT and LS-CAT MC

## Highlights

- **Nature**

- S.C. Garman, B.A. Wurzburg, S.S. Tarchevskaya, J.P. Kinet, T.S. Jardetzky, "Structure of the Fc fragment of human IgE bound to its high-affinity receptor Fc[<sub>epsilon</sub>]RI[ $\alpha$ ]," *Nature* **406**, July, 259-266 (2000)
- A.S. Krasilnikov, X. Yang, T. Pan, A. Mondragon, "Crystal structure of the specificity domain of ribonuclease," *Nature* **421**, 760-764 (2003)
- R.L. Lieberman, A.C. Rosenzweig, "Crystal structure of a membrane-bound metalloenzyme that catalyses the biological oxidation of methane," *Nature* **434**, March, 177-182 (2005)
- Alfredo Torres-Larios, Kerren K. Swinger, Andrey S. Krasilnikov, Tao Pan, Alfonso Mondragon, "Crystal structure of the RNA component of bacterial ribonuclease P," *Nature* **437**, September, 584-587 (2005)
- Hsien-Sheng Yin, Xiaolin Wen, Reay G. Paterson, Robert A. Lamb, Theodore S. Jardetzky, "Structure of the parainfluenza virus 5 F protein in its metastable, prefusion conformation," *Nature* **439** (5), January, 38-44 (2006). DOI: 10.1038/nature04322

- **Science**

- A. Changela, K. Chen, Y. Xue, J. Holschen, C.E. Outten, T.V. O'Halloran, A. Mondragon, "Molecular basis of metal-ion selectivity and zeptomolar sensitivity by CueR," *Science* **301**, 1383-1387 (2003).
- Pamela Focia, Irina Shepotinovskaya, James Seidler, Douglas Freymann, "Heterodimeric GTPase core of the SRP targeting complex," *Science* **303**, January, 373-377 (2004).
- Andrey S. Krasilnikov, Yinghua Xiao, Tao Pan, Alfonso Mondragon, "Basis for Structural Diversity in Homologous RNAs," *Science* **306**, October, 104-107 (2004).
- Bo Li, John Paul J. Yu, Joseph S. Brunzelle, Gert N. Moll, Wilfred A. van der Donk, Satish K. Nair, "Structure and Mechanism of the Lantibiotic Cyclase Involved in Nisin Biosynthesis," *Science* **311**, 1464-1467 (2006).

# LS-CAT Consortium

University of Michigan

Michigan State University

Wayne State University

VanAndel Research Institute

Northwestern University

University of Wisconsin

Vanderbilt University

University of Illinois, Urbana/Champaign



# LS-CAT Scientific Focus

- Facilitating Advances in Structural Biology
  - Technically demanding problems
    - Membrane proteins and complexes
    - Multicomponent assemblies in signaling, transport and pathogenesis
    - Protein-nucleic acid interactions
  - Application of macromolecular crystallography to biomedical problems requiring high throughput
    - Enzyme action and design
    - Drug discovery
    - Structural genomics approaches





# LS-CAT Status

- 21-ID-D Up and Running with Mar DTB
  - MD-2 diffractometer and K-B adaptive optics on order
  - First run December 2006 successfully delivered beam to LS-CAT members.
  - New structures have been determined using SAD phasing and molecular replacement.
- 21-ID-F/G To be commissioned June 2007
  - MD-2 diffractometers and Be refractive lenses arriving Q2 2007
- 21-ID-E To be commissioned early 2008



# Microdiffractometer MD2



- Designed for micro crystals and micro beams
- Fast & high precision phi axis --  $2\mu\text{m}$  sphere of confusion
- Beam and sample view video-microscope
- Optional mini-kappa



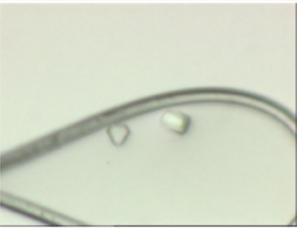
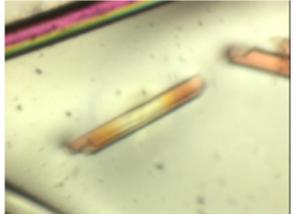
# Phi axis

Single horizontal air bearing axis, driven by a torque motor (no gearbox), coupled to a motorised sample-centring table and mounted on a motorised XYZ table.

- ◆ Free rotation
- ◆ Max rotation speed 120Deg/s
- ◆ Positioning error +/-2 mDeg (HP option +/-1 mDeg)
- ◆ Following error < +/- 1 mDeg RMS@ 10 Deg/s
- ◆ 2  $\mu\text{m}$  sphere of confusion radius  
(sample off centring < +/- 1 mm)
- ◆ Sample centring range +/- 2.5mm
- ◆ Sample length adjustment (Y) +/-6 mm.
- ◆ Fast multi pass.
- ◆ Minimum sample to X-ray detector distance 60 mm



# Video-Microscope

	10 µm sample	<b>Camera</b> Sight coaxial to the beam to see the sample or the beam (scintillator) without parallax error: High resolution (0.28 N.A. objective lens); 12X motorised zoom; 576 x 768 pixels display; condenser lighting with polarizer and motorised analyser. Field: About 1.8x1.5 mm @ zoom min, 0.18x0.12 mm @ zoom max.
	12µm needle (zoom 10)	<b>Beam viewing scintillator</b> Fluorescent single crystal mounted on a motorised support. Allows to control the beam shape and position; exploits the full video-microscope resolution; can be set remotely at any time, even when a sample is mounted on the PHI axis (sample is shifted); set in the sample plane to visualise the beam exactly as hits the sample (with the aperture and beam cleaning aperture set).
	30µm beam (zoom 6)	



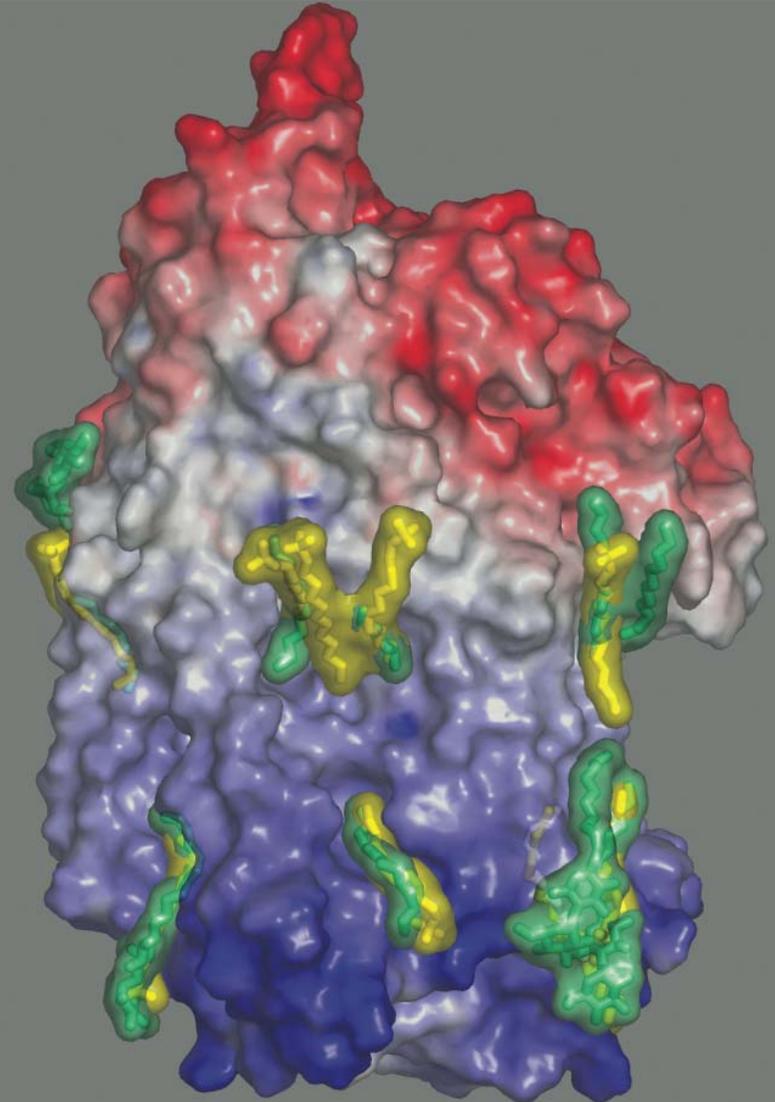
# Membrane Proteins

More than a dozen membrane protein projects are underway and data collection on some will occur during the coming run.



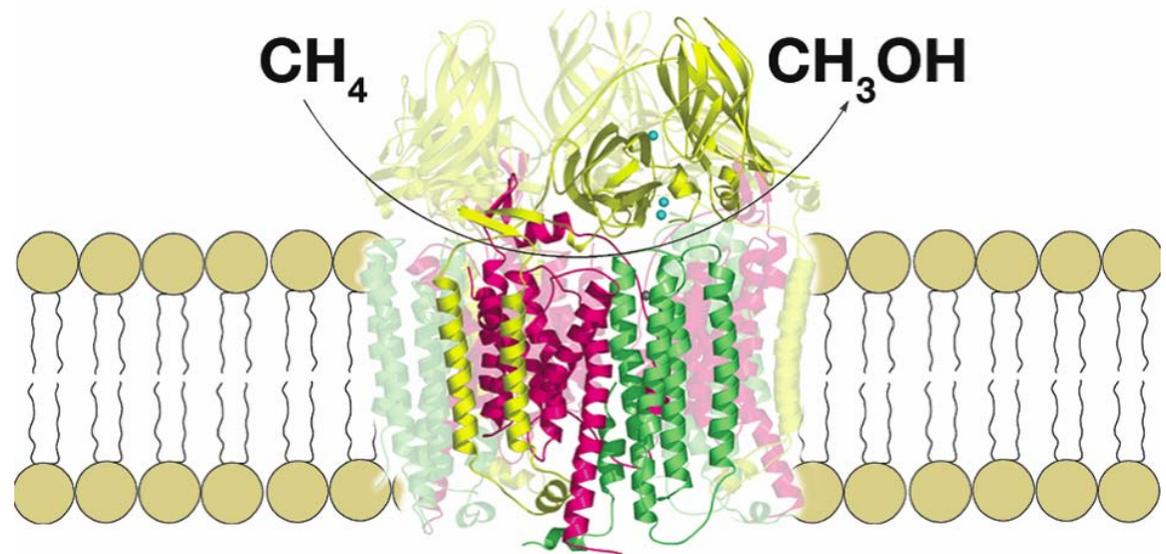
# Cytochrome C Oxidase

- **Conserved Lipid/Detergent Binding Sites in Cytochrome c Oxidase**
- Comparison of cytochrome c oxidase structures reveals conserved positions of membrane lipids and detergent substitutes, suggesting a more prevalent and specific role of lipid in membrane protein structure than usually envisioned. Alkyl chains shown in green (dodecyl maltoside and lipid, in *R. sphaeroides*) and yellow (lauryldimethylamineoxide in *P. denitrificans*) on a surface rendition of cytochrome oxidase, colored by charge (red= negative, blue = positive).
- picture was created by Ling Qin and S.F-M, and is the structure reported in the PNAS article: L.Qin, C.Hiser, A. Mulichak, M. Garavito, S. Ferguson-Miller / Identification of conserved lipid/detergent-binding sites in a high-resolution structure of the membrane protein cytochrome c oxidase. **Proc Natl Acad Sci U S A.** (Oct 31)103,16117-22. (2006)



# Particulate Methane Monooxygenase

- Oxidizes methane to methanol
- Allows organism to use methane for its energy and carbon source



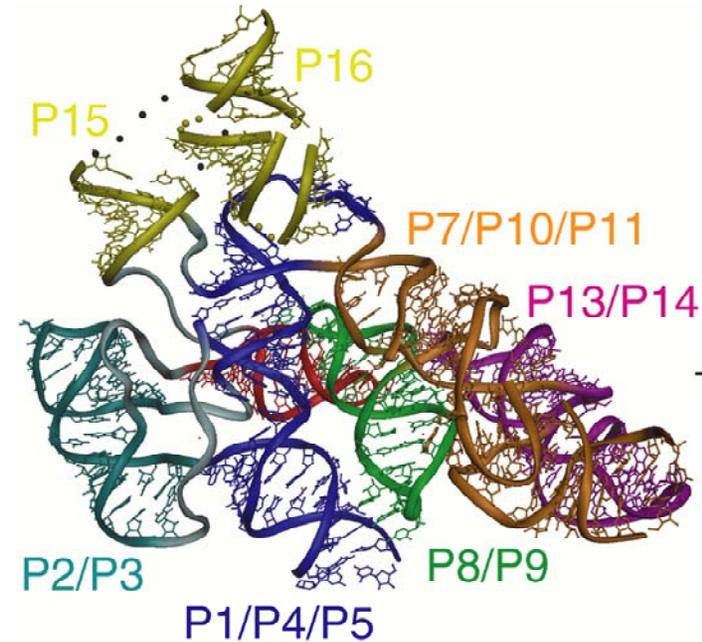
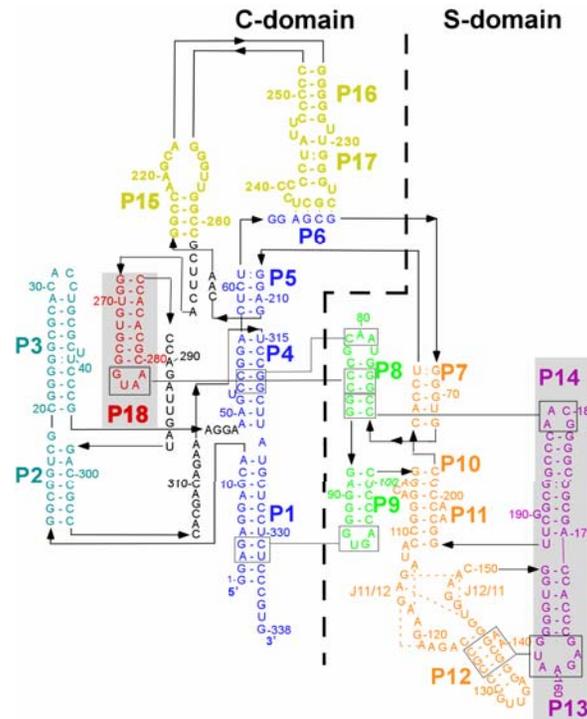
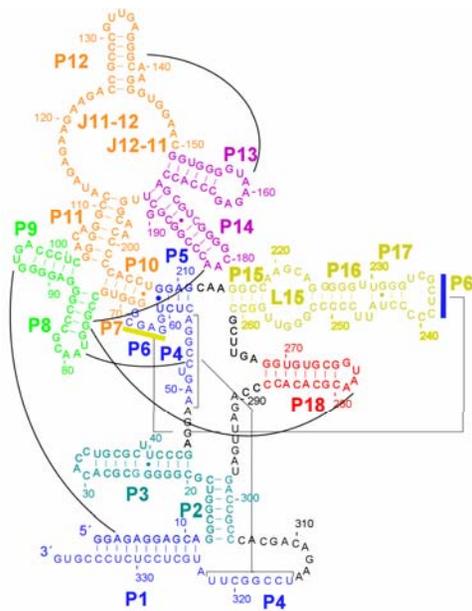
- R.L. Lieberman, A.C. Rosenzweig, "Crystal structure of a membrane-bound metalloenzyme that catalyses the biological oxidation of methane," *Nature* \*434\*, March, 177-182 (2005)

# Complex Macromolecular Systems

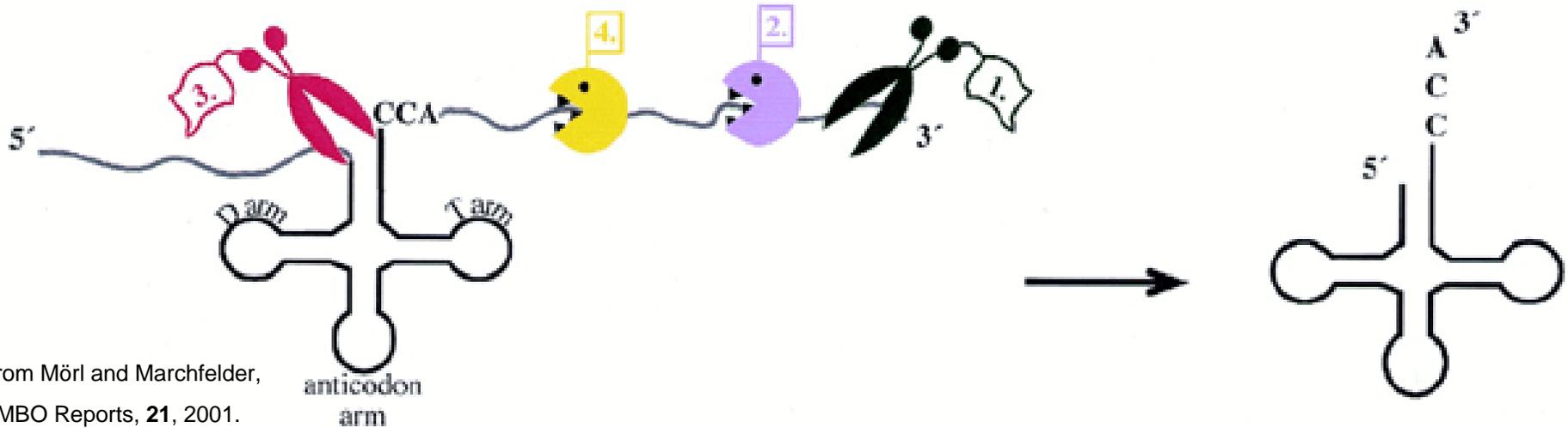
- Protein-Nucleic acid interactions
- Protein complexes
- Multicomponent complexes in signaling



# Understanding RNase P, a universal ribozyme. A structural perspective



# RNase P is the ribozyme responsible for processing the 5' end of tRNA



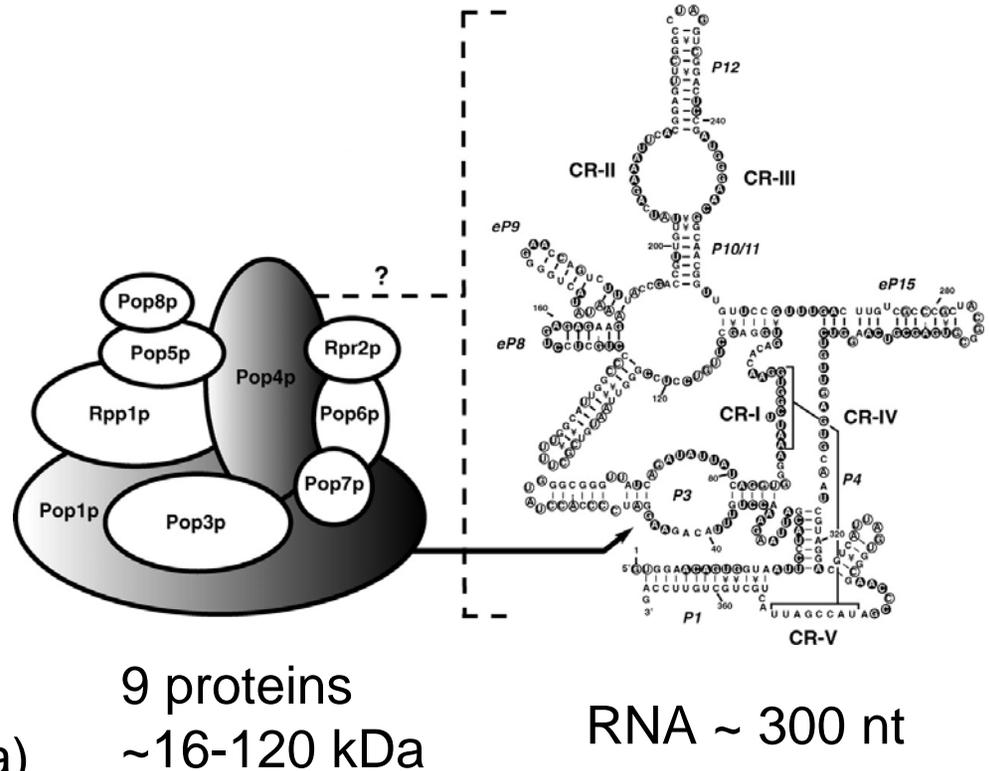
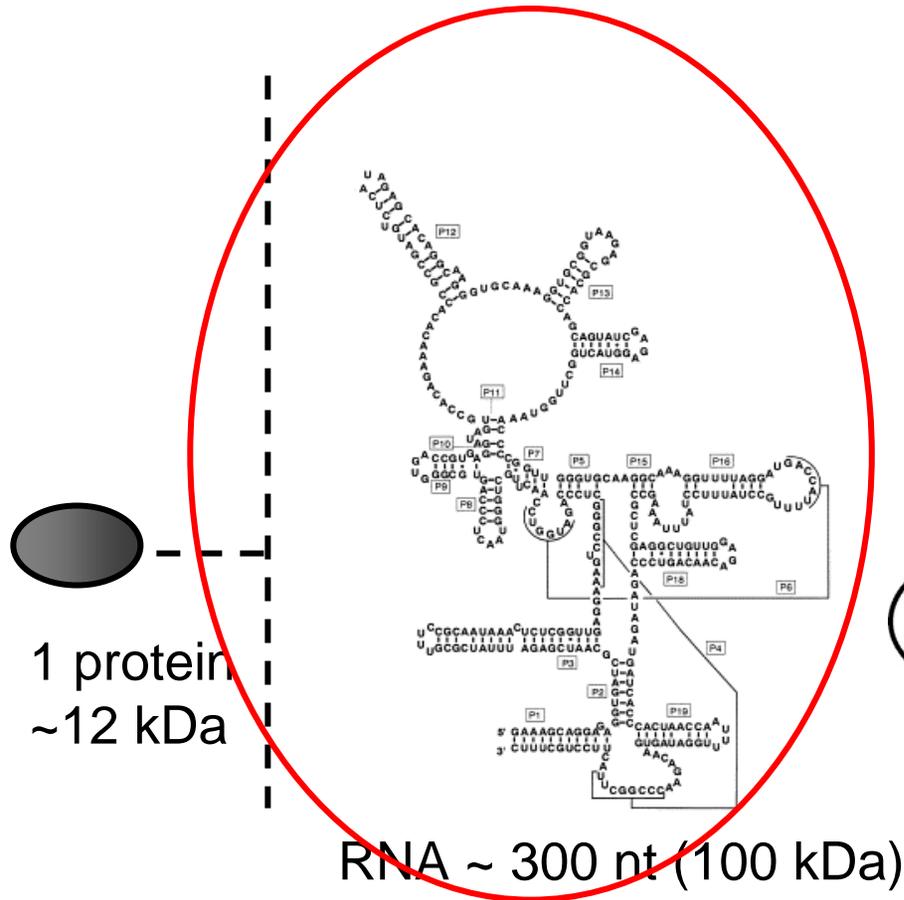
- Ribonuclease P (RNase P) is the endoribonuclease which generates the mature 5'-end of tRNA
- It is present in all three domains of life (*Bacteria, archaea, and eukarya*) and consists of an RNA and protein components\*
- The ribosome and RNase P are the only two universal ribozymes
- Is ubiquitous and probably a molecular fossil from the RNA world
- In bacterial and some archaeal RNase Ps, the RNA component (P RNA) alone is catalytically active *in vitro*
- Catalyses a multiple turnover reaction
- Recognizes its substrates in *trans* and not through base pairing

\*only the protein in chloroplasts

# RNase P composition

## Bacteria

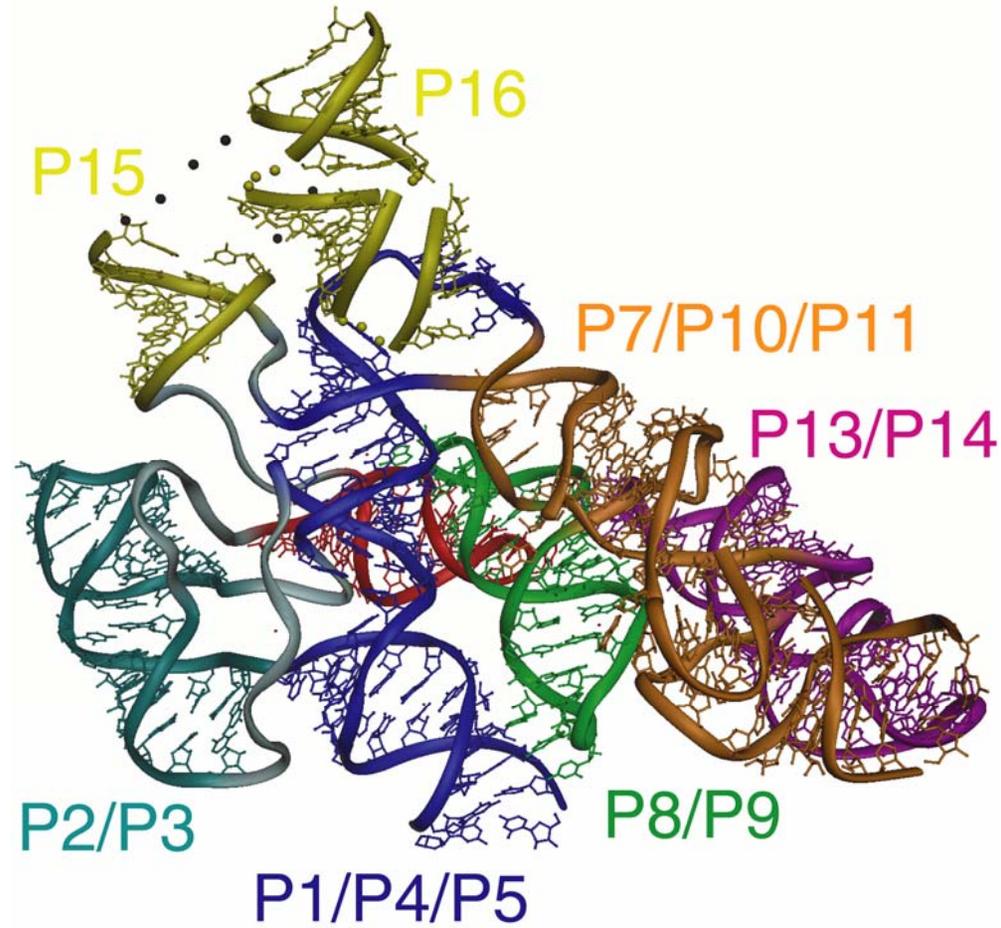
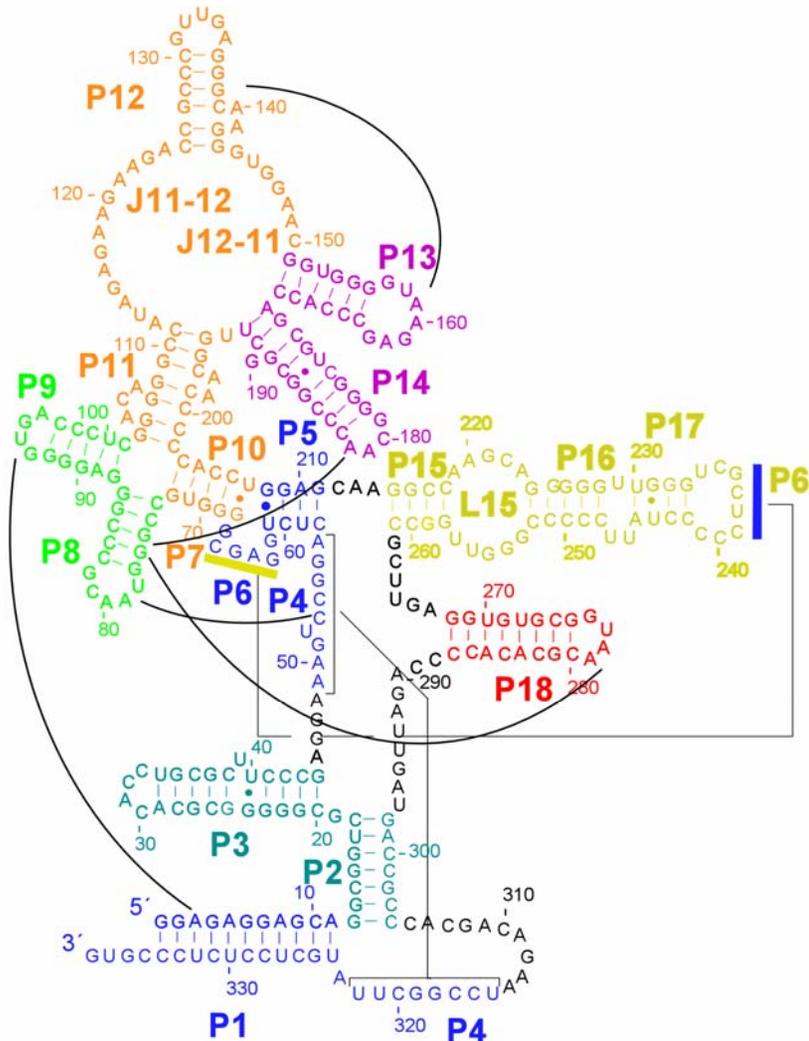
## Archaea, eukarya



The RNA component can work alone *in vitro*

The RNA component needs at least 4 protein partners

# 3.85 Å structure of *Thermotoga maritima* RNase P

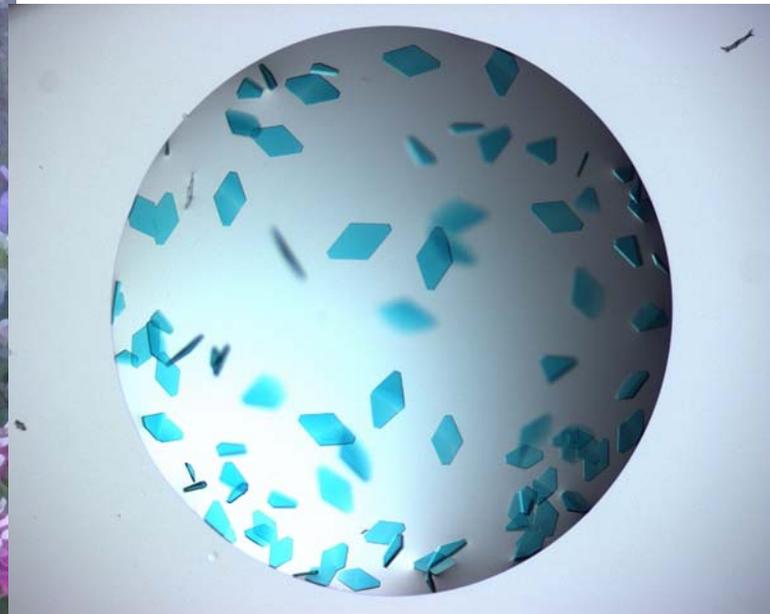


338 nucleotides

~110 kDa MW

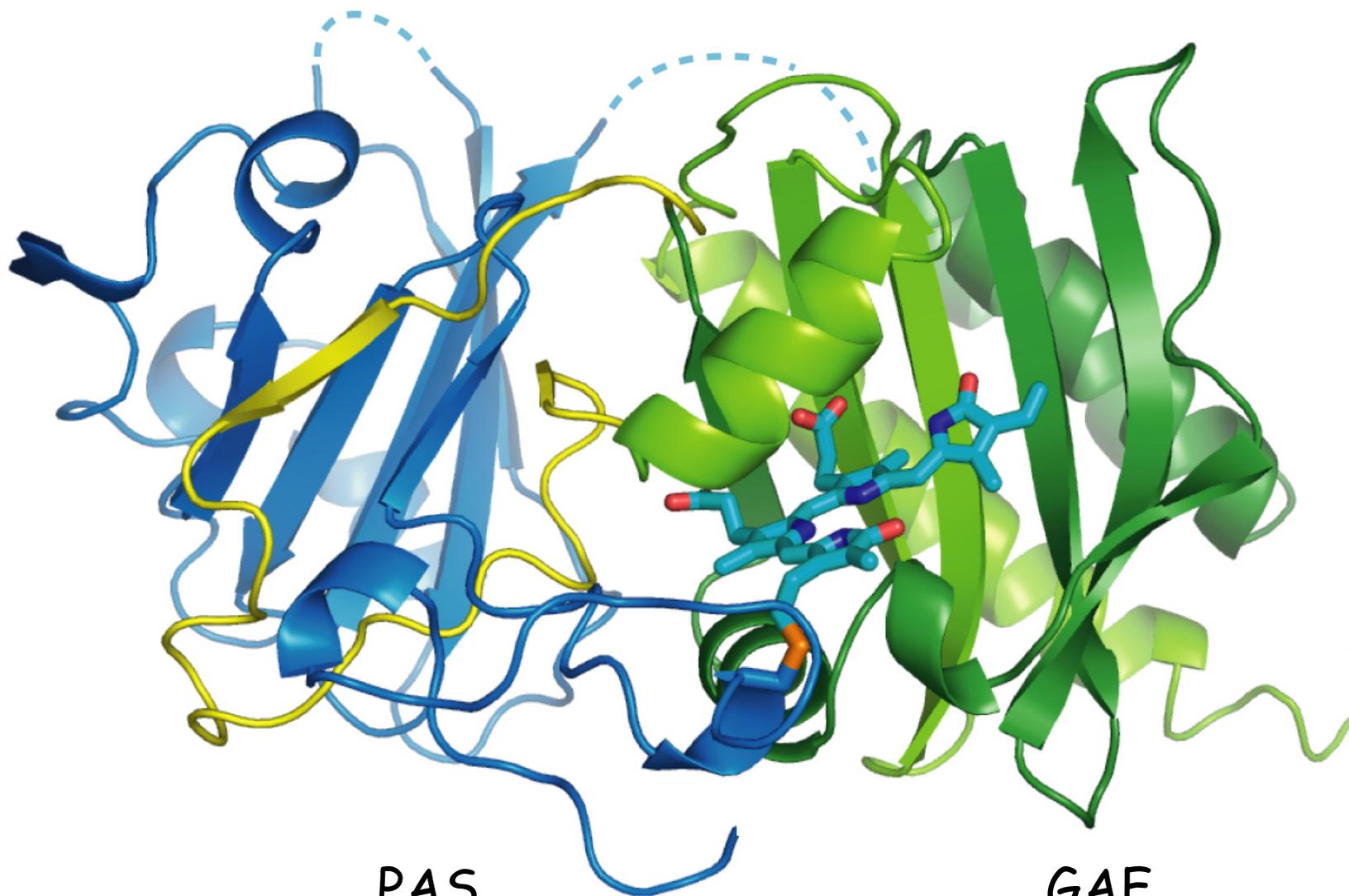
Torres-Larios et al. Nature. Doi:10.1038/nature04074 (2005).

# The structure of phytochrome: a knotted photoreceptor



Images provided by Katrina Forest,  
University of Wisconsin  
Wagner et al, Nature 438, 325-331 (2005)

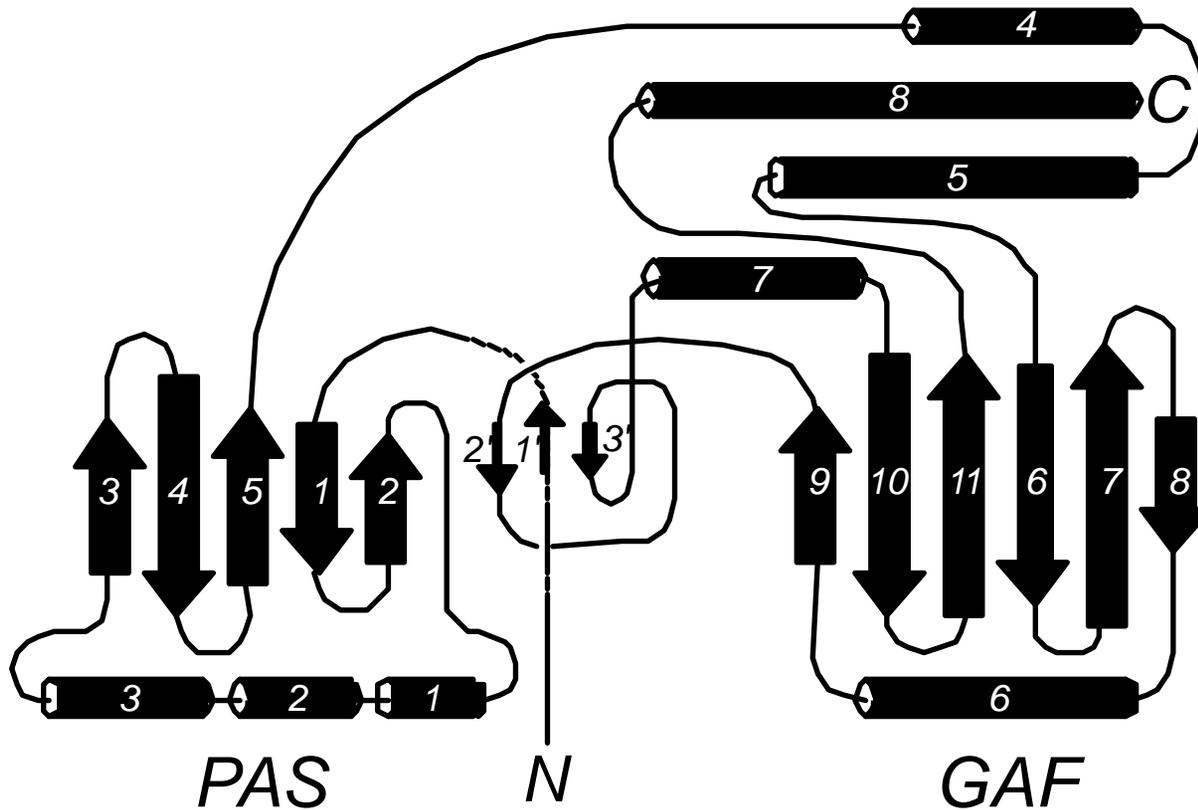
# Overall Fold



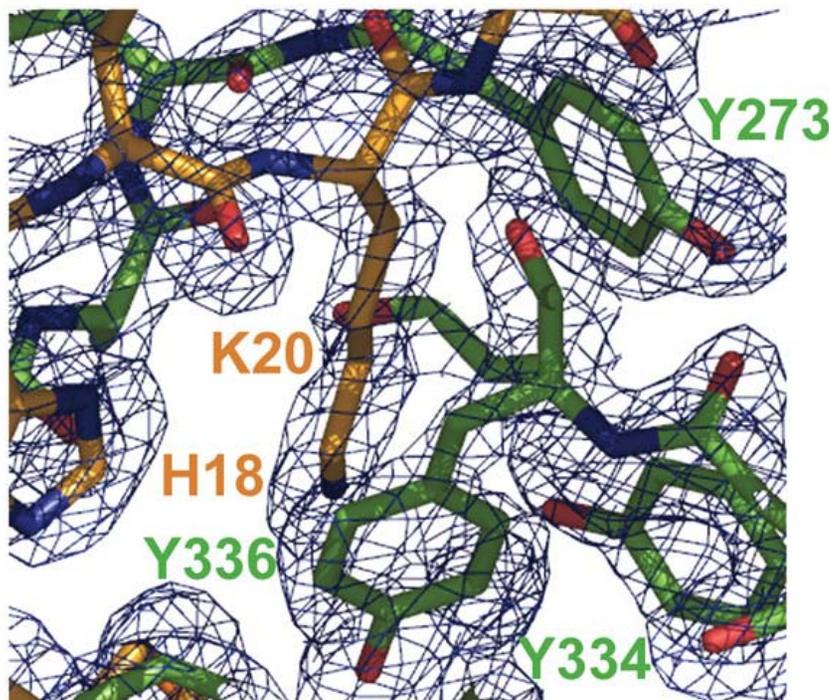
PAS

GAF

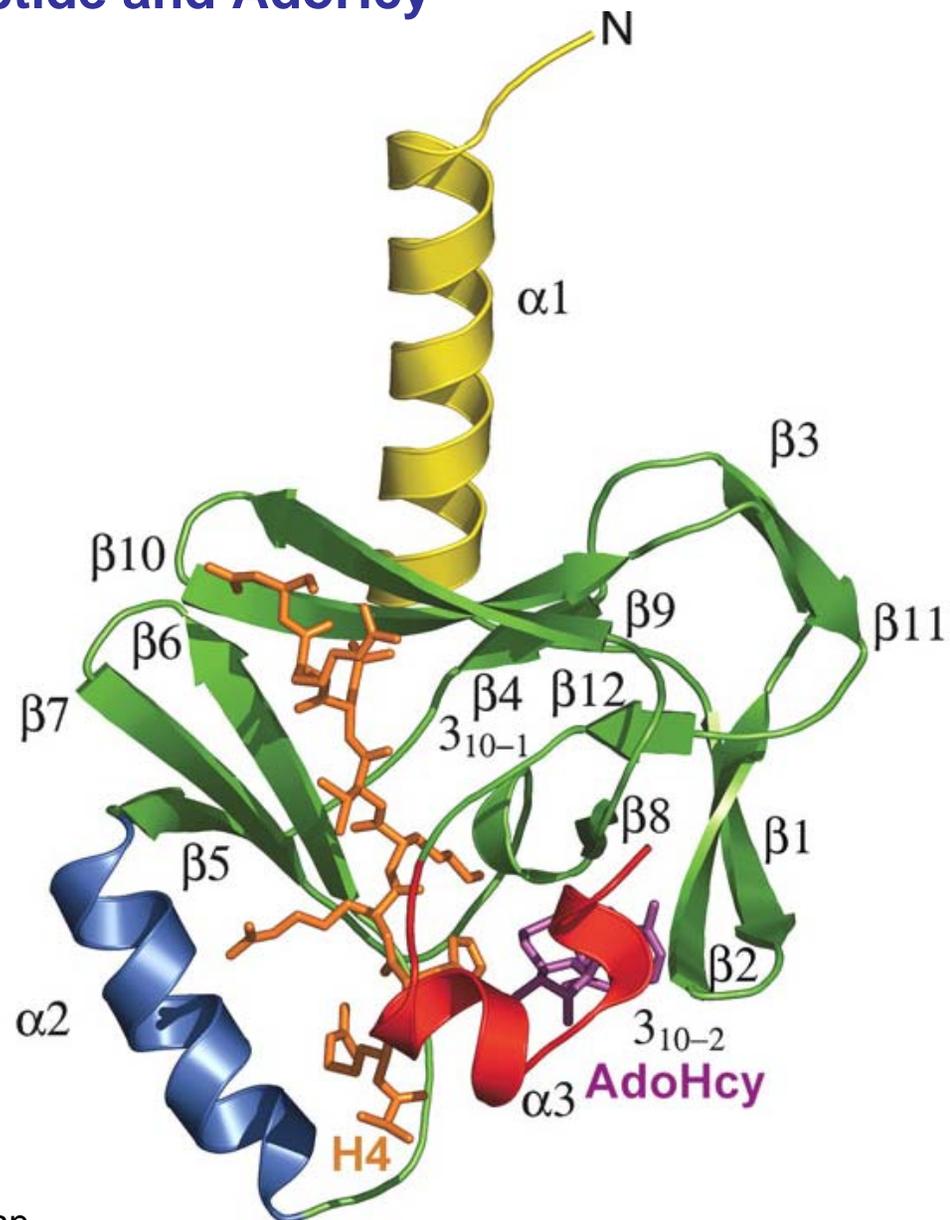
# Folding Mechanism?



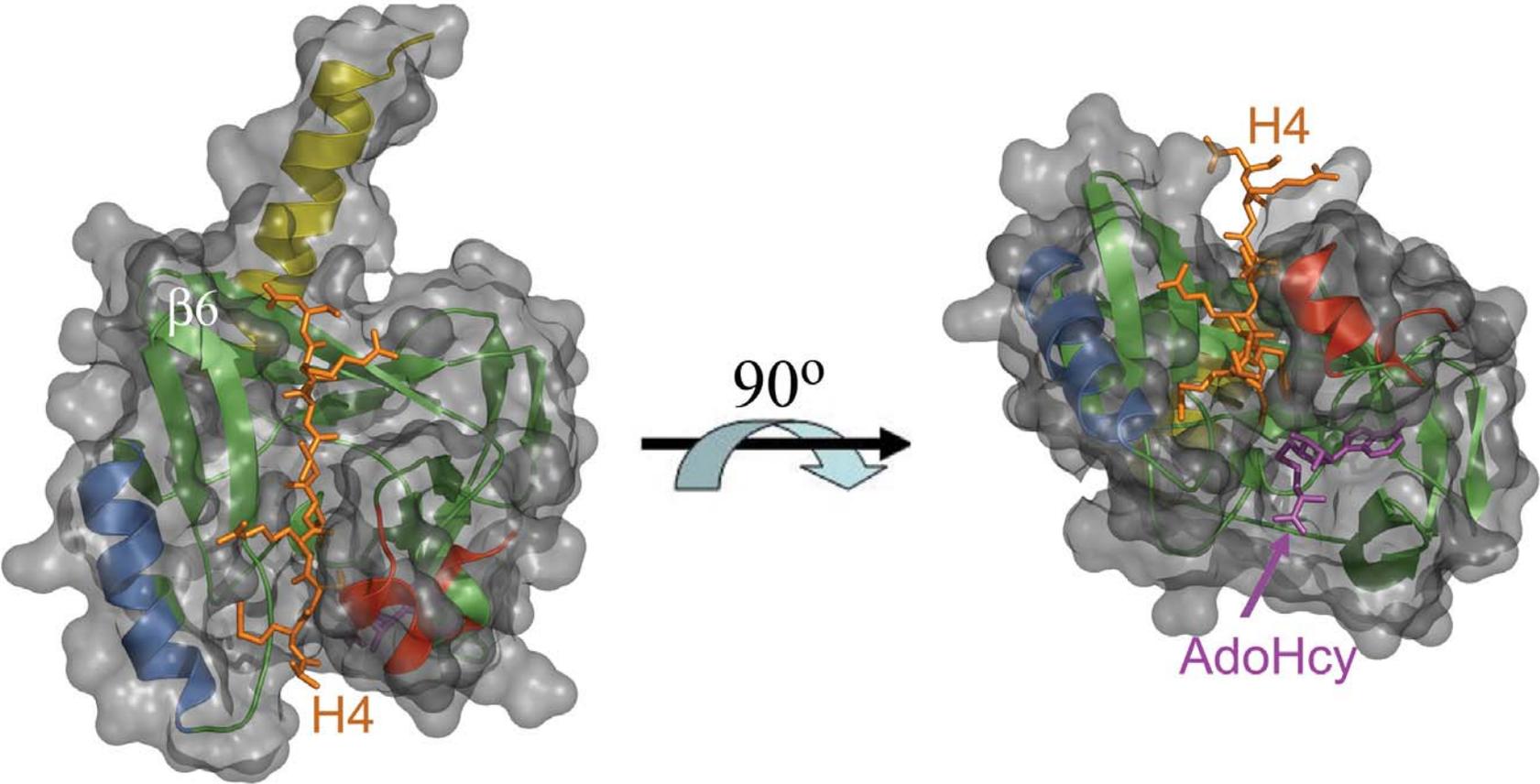
# Crystal Structure of Human SET8<sub>(191-352)</sub> bound to a histone H4 peptide and AdoHcy



SeMet MAD phased experimental map at 2.0 Å resolution

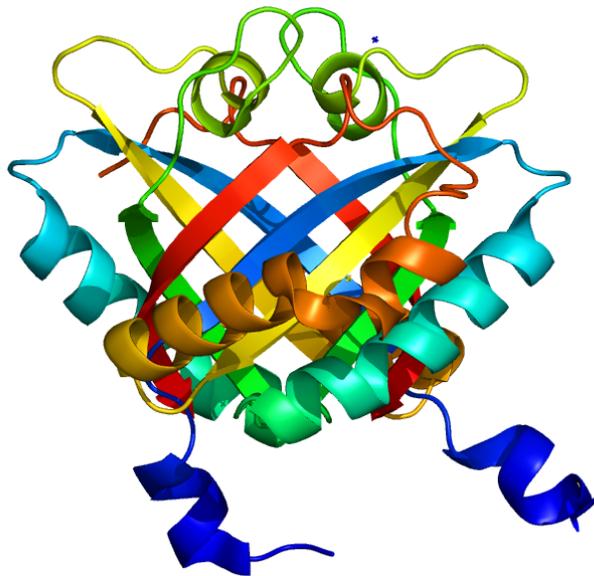


# Structure of the SET8/AdoHcy/Histone H4 Ternary Complex

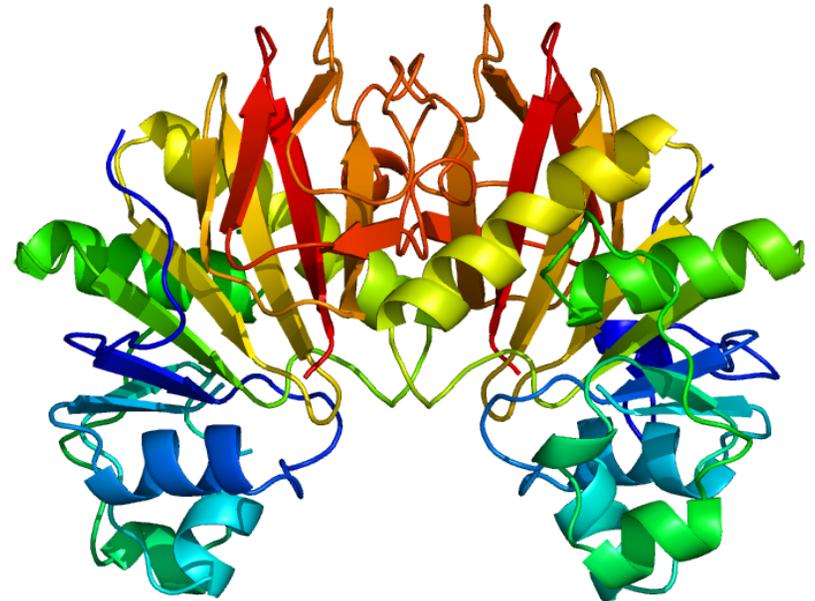


# First PDB Deposits from 21-ID-D

- The first structures determined using data from 21-ID-D collected in December 2006 have been deposited in the PDB



**2OKQ**      **APC27291**  
SeMet SAD Phasing  
1.8Å Resolution  
R = 18.3      R<sub>free</sub> = 22.6  
YbaA, Unknown Function  
from *Shigella flexneri*



**2OMK**      **APC81767**  
SeMet SAD Phasing  
1.8Å Resolution  
R = 16.5      R<sub>free</sub> = 20.1  
Thiamin Pyrophosphokinase  
From *Bacteroides thetaiotaomicron*

