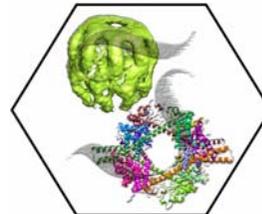


# Crystal structure of mitochondrial respiratory Complex II

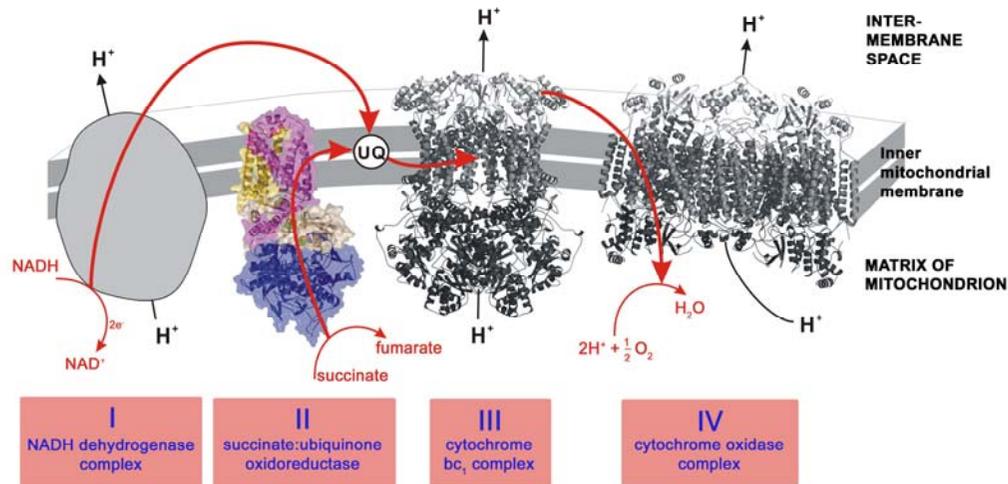
*Fei Sun*

*The Institute of Biophysics, CAS*

*2006/10/25*

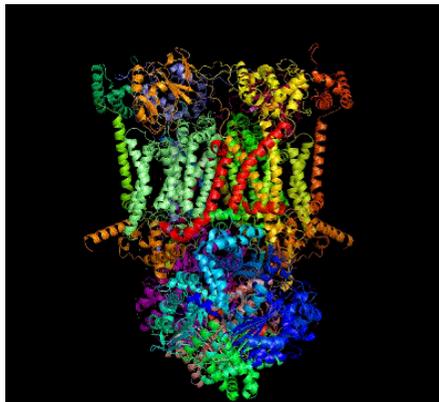


# Four membrane complexes in respiratory chain



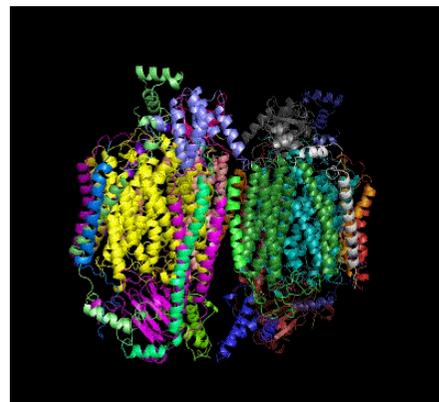
Mitochondria are cellular organelles of prokaryotic origin that are found in almost all eukaryotic cells. The mitochondrial respiratory system, consisting of five membrane protein complexes (I to V), produces most of the energy in eukaryotic cells (Saraste, 1999).

**Complex III  
(bc<sub>1</sub> complex)  
from Bovine heart**



Xia et al., 1997; Zhang et al., 1998 ;Iwata et al., 1998; Lange and Hunte, 2002;

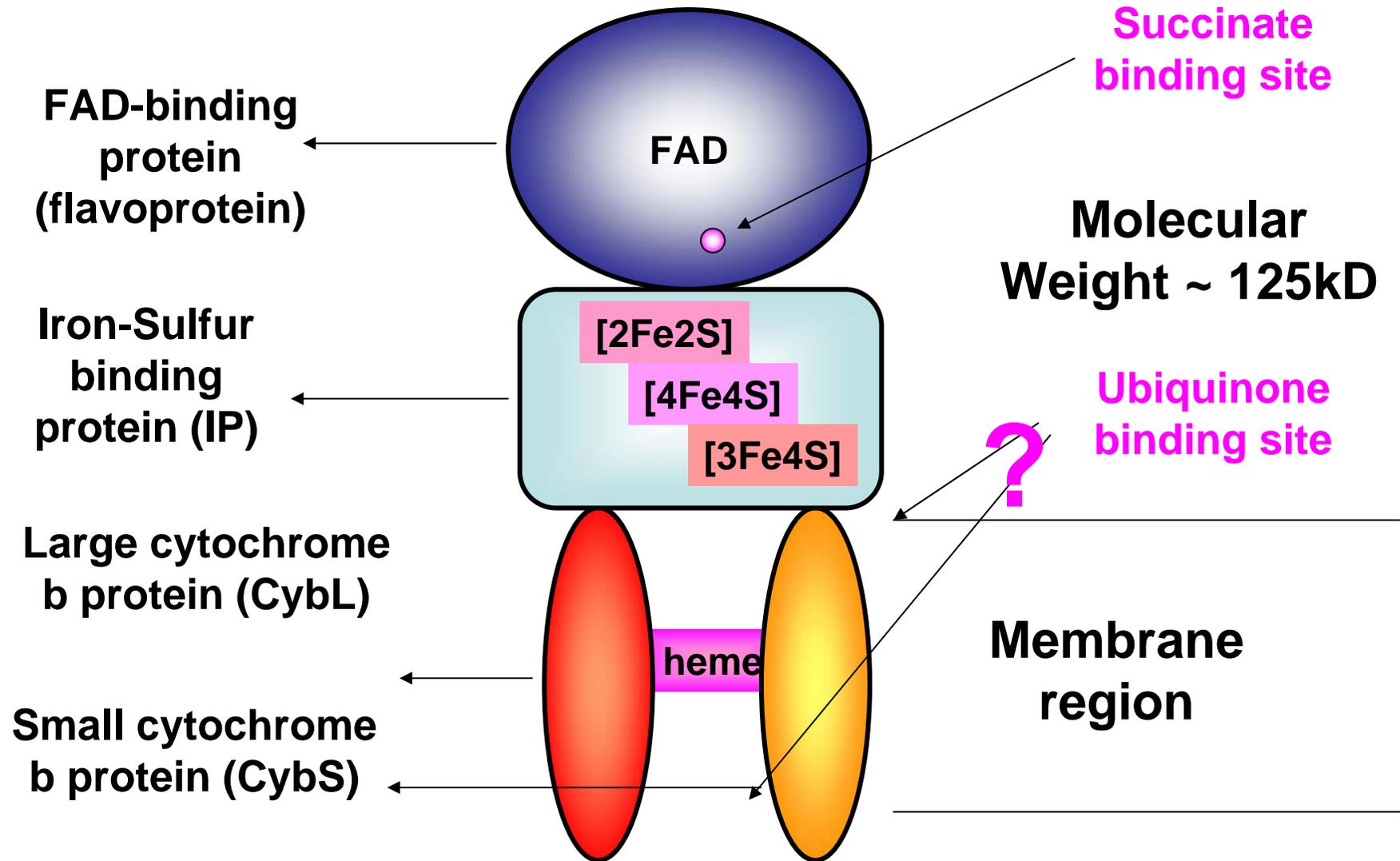
**Complex IV  
(cytochrome c oxidase )  
from Bovine heart**

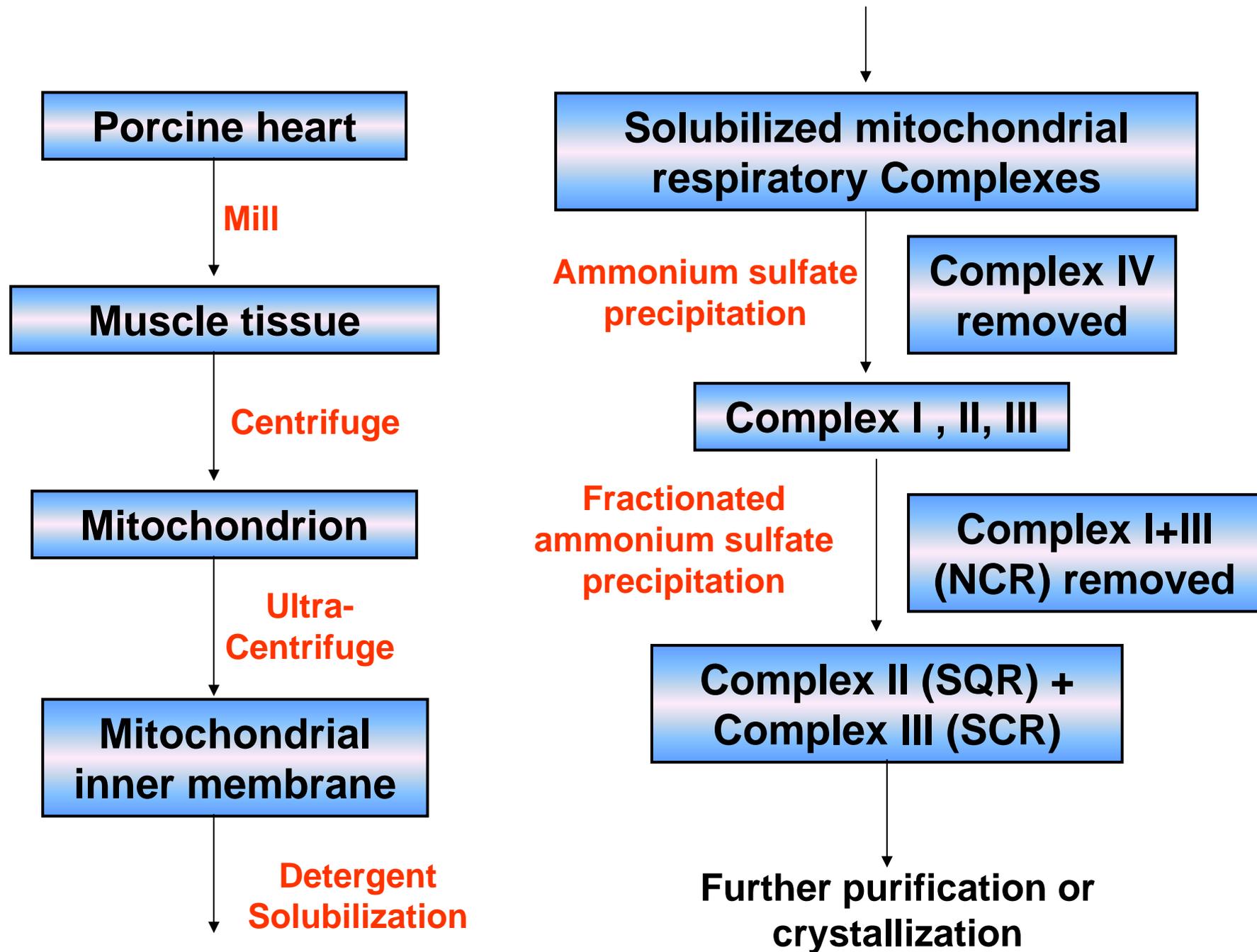


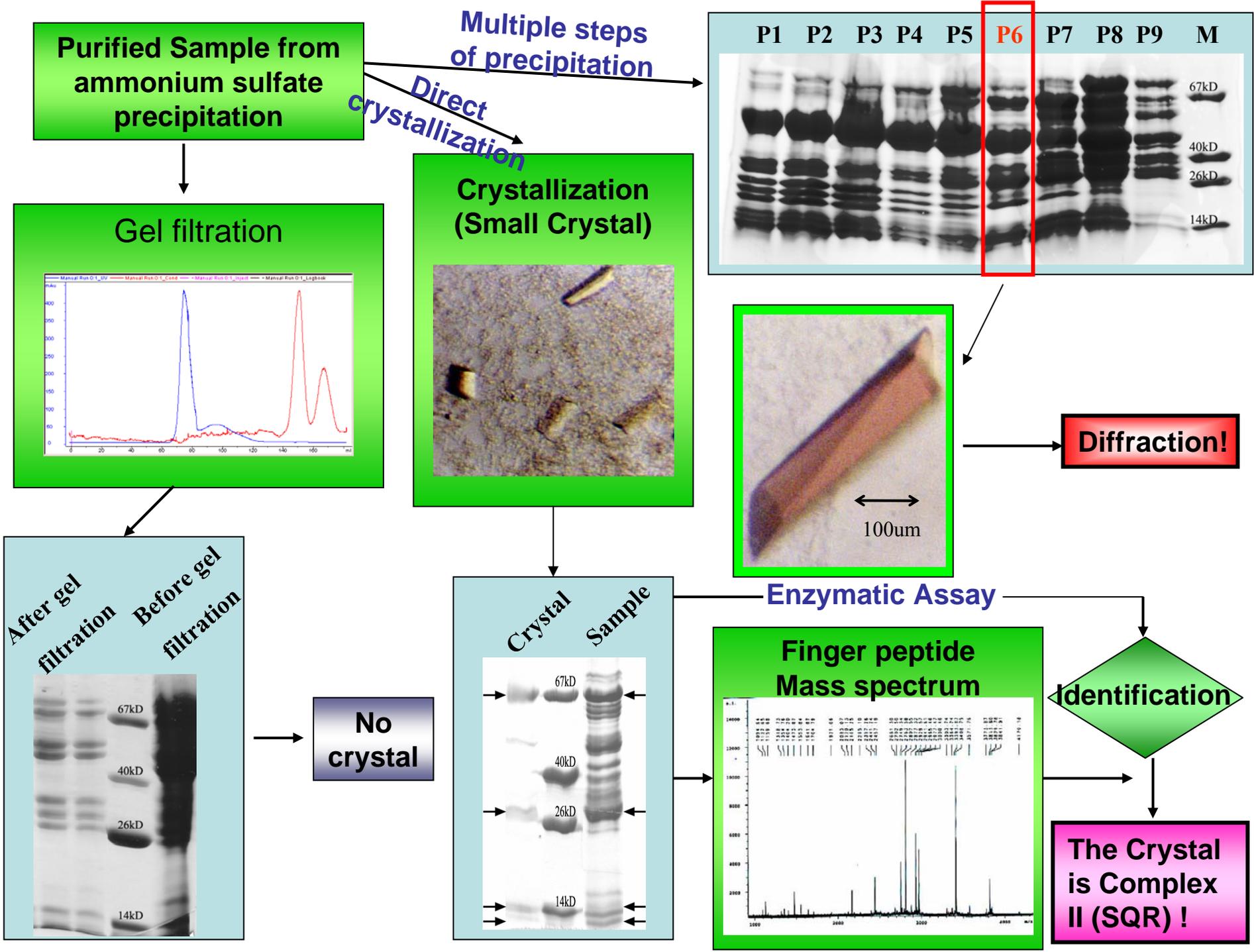
Tsukihara et al., 1996

To date, the structures of Complex III (cytochrome bc<sub>1</sub> complex), Complex IV and Complex V (ATPase) have been determined. However, no breakthroughs have been made on the structures of Complex I and II.

# Mitochondrial respiratory chain Complex II

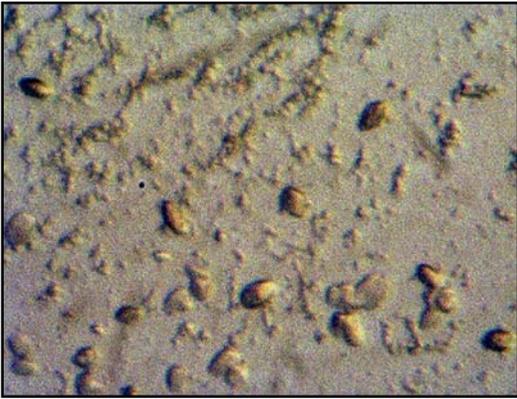




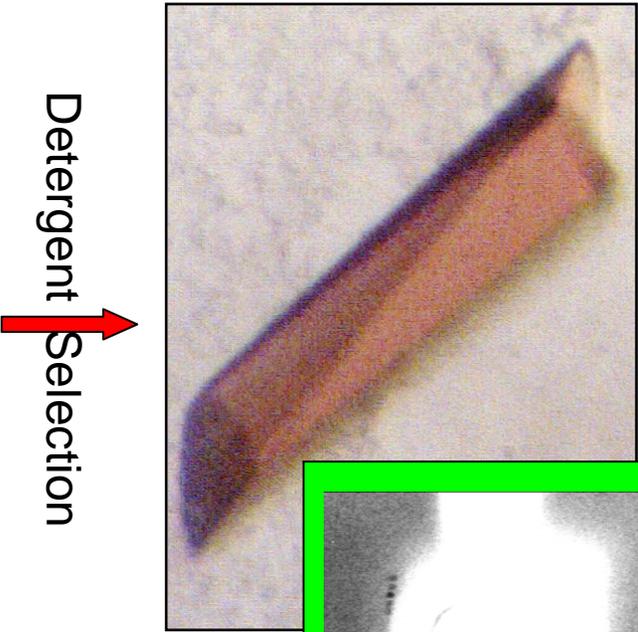
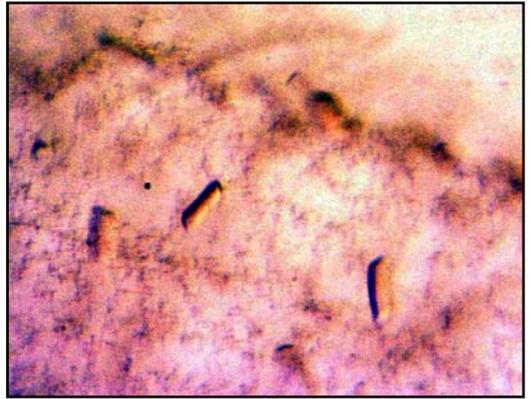




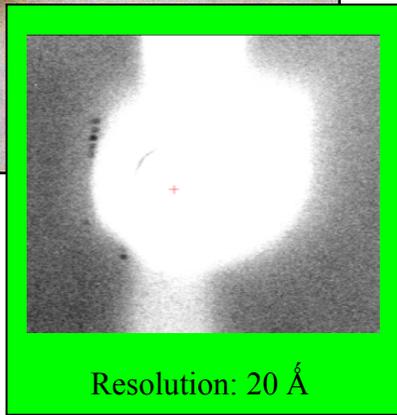
Sucrose  
Additive



Further  
Optimization



Detergent  
Selection

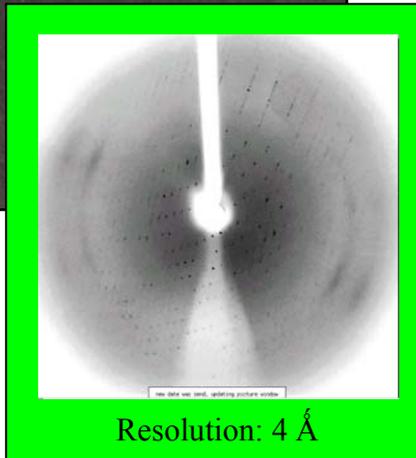
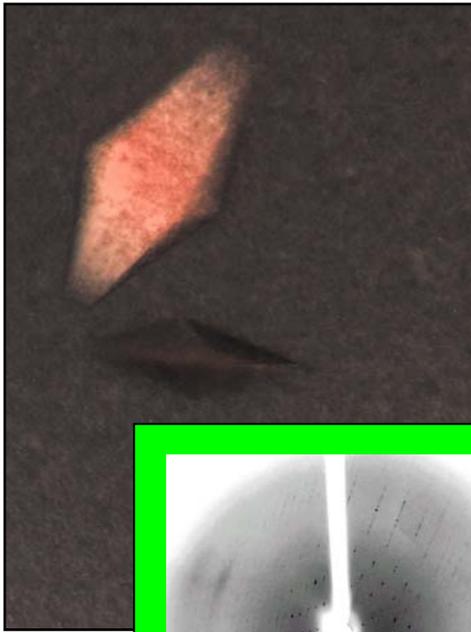


Resolution: 20 Å

More Careful  
Purification Strategy



Using Detergent  
Mixture



Resolution: 4 Å

2.3 Å Diffraction  
at APS

# Data Collection in synchrotron

Anomalous

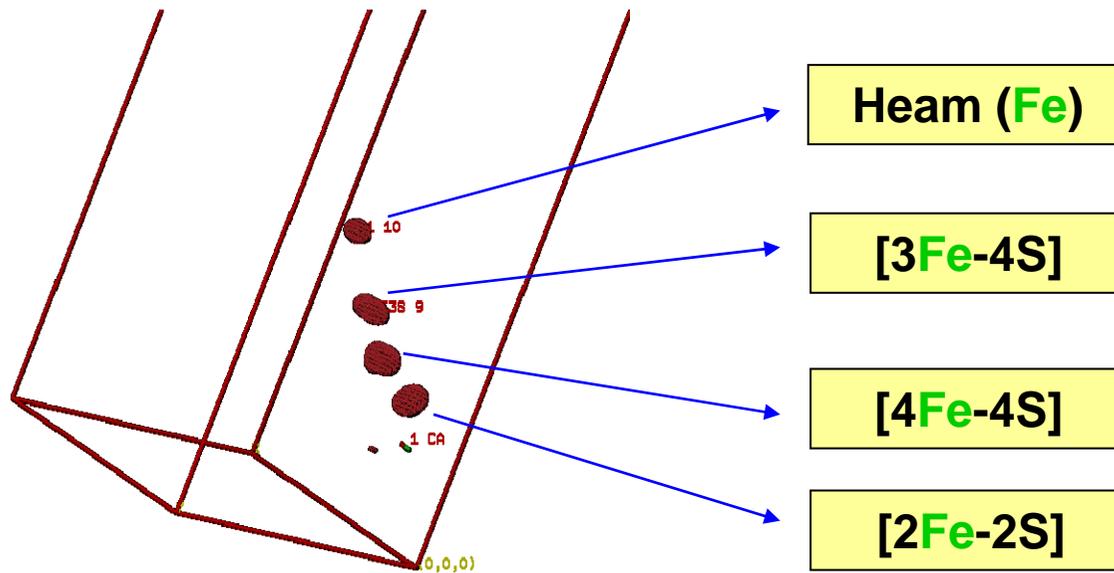
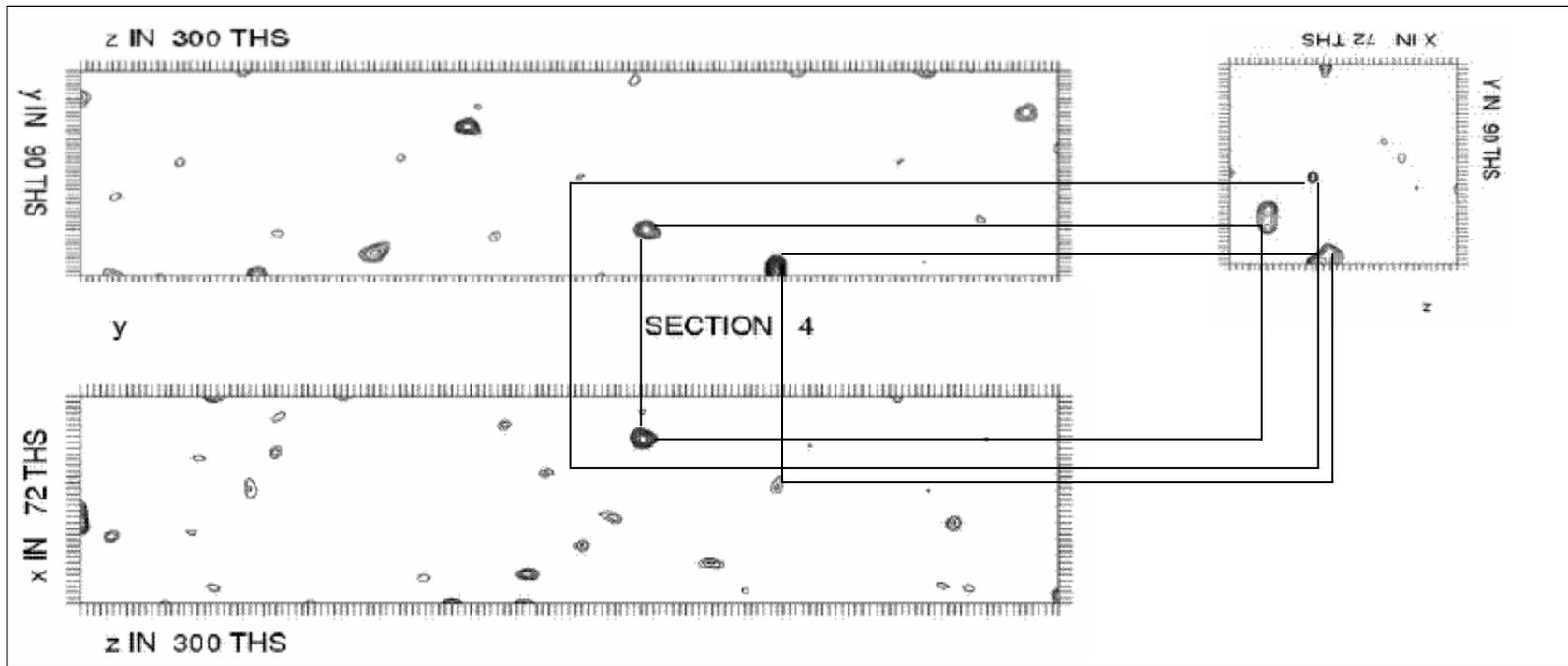
Data Set

Shell limit	Lower Angstrom	Upper Angstrom	Average I	Average error	Average stat.	Norm. Chi**2	Linear R-fac	Square R-fac
50.00	6.46		2422.3	58.9	45.8	3.094	0.058	0.058
.....								
3.23	3.11		118.9	51.8	51.7	0.560	0.353	0.240
3.11	3.00		102.2	52.8	52.7	0.539	0.417	0.304
<b>All reflections</b>			<b>687.6</b>	<b>52.4</b>	<b>49.7</b>	<b>1.164</b>	<b>0.103</b>	<b>0.073</b>

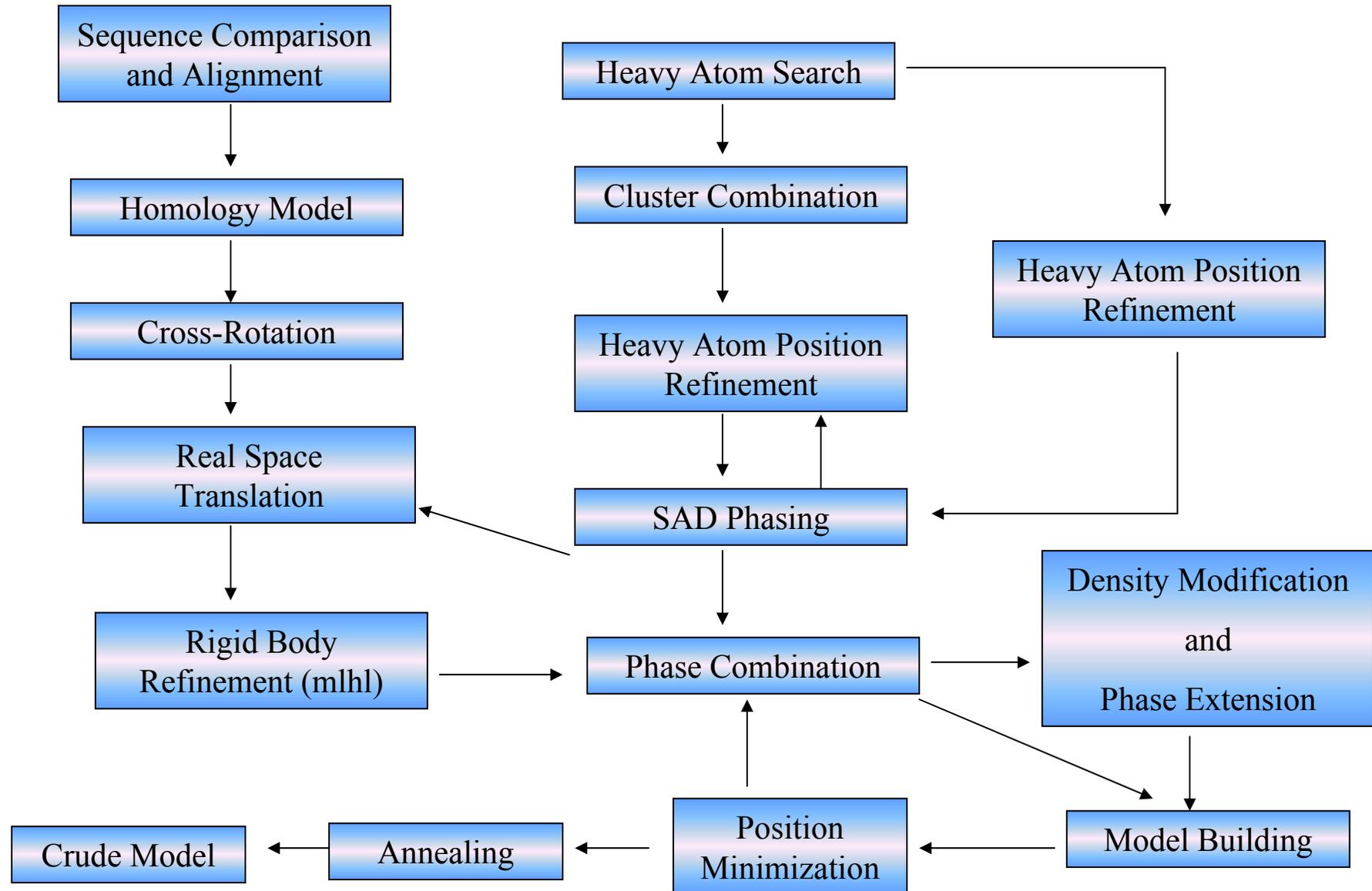
High Resolution

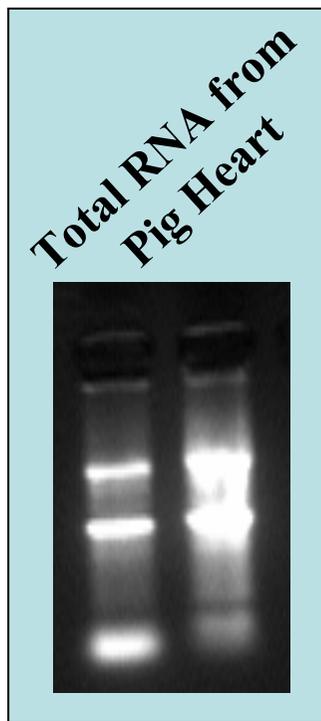
Data Set

Shell limit	Lower Angstrom	Upper Angstrom	Average I	Average error	Average stat.	Norm. Chi**2	Linear R-fac	Square R-fac
50.00	5.17		3746.0	146.1	50.4	1.250	0.070	0.076
.....								
2.59	2.49		130.1	37.4	37.3	0.615	0.442	0.428
2.49	2.40		124.7	44.0	44.0	0.615	0.491	0.448
<b>All reflections</b>			<b>1213.2</b>	<b>61.6</b>	<b>37.8</b>	<b>0.948</b>	<b>0.113</b>	<b>0.101</b>

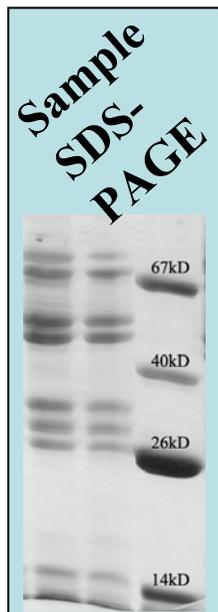
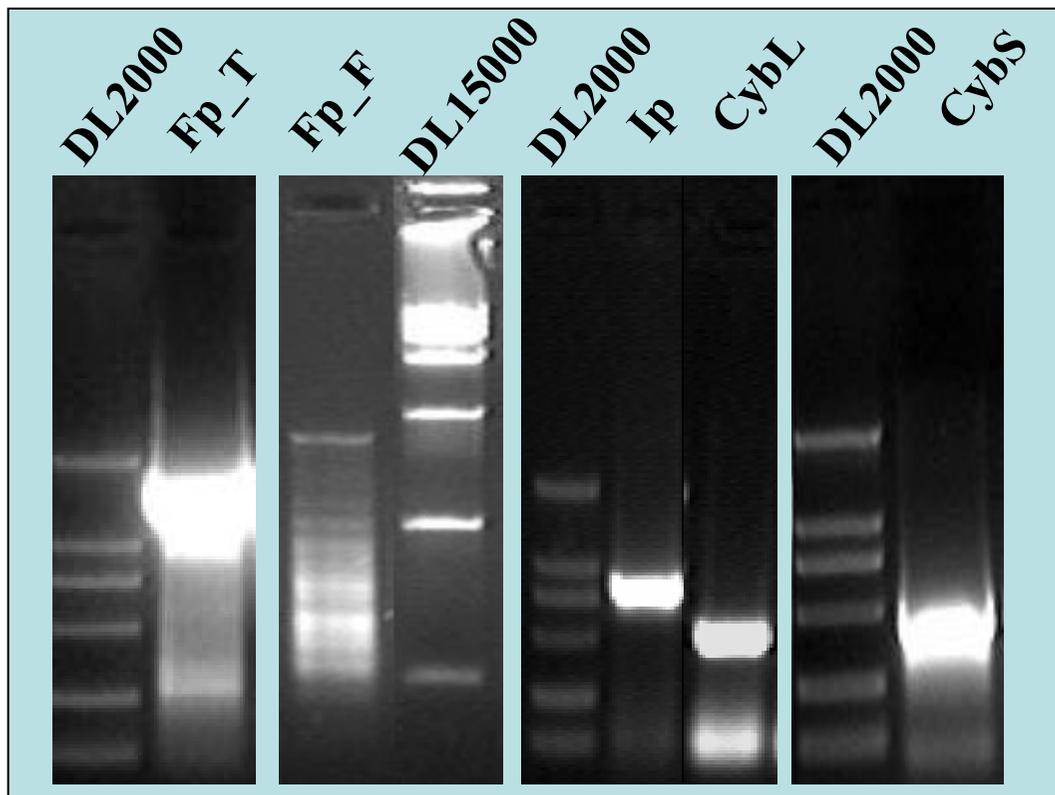


# Structure Determination Flow Chart





RT-PCR



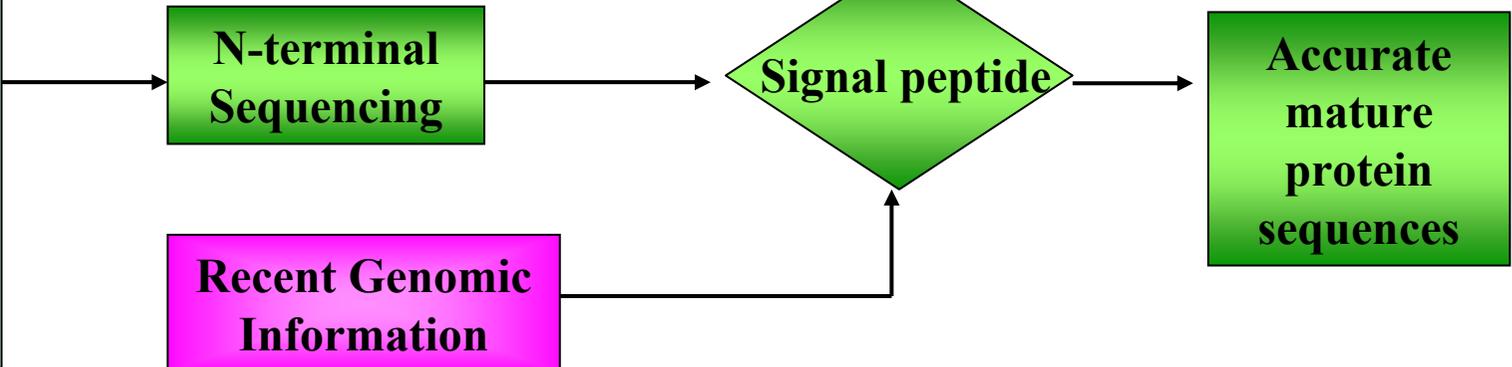
**N-terminal Sequencing**

**Recent Genomic Information**

**Signal peptide**

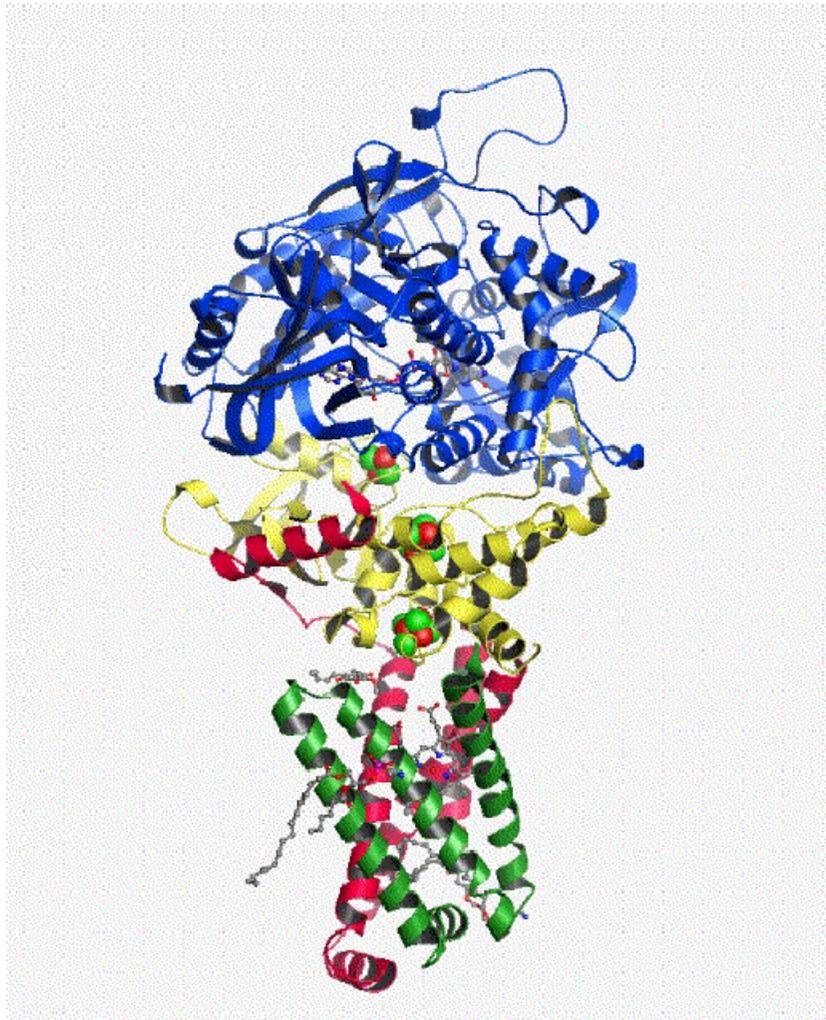
**Full Sequences**

**Accurate mature protein sequences**



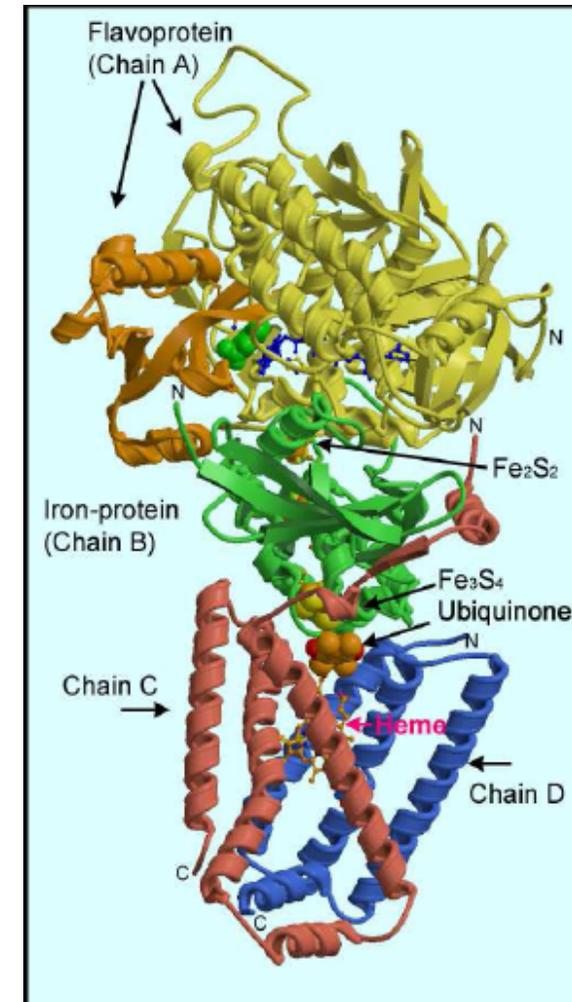
# Overview of mitochondrial respiratory Complex II

Porcine, 2.4Å



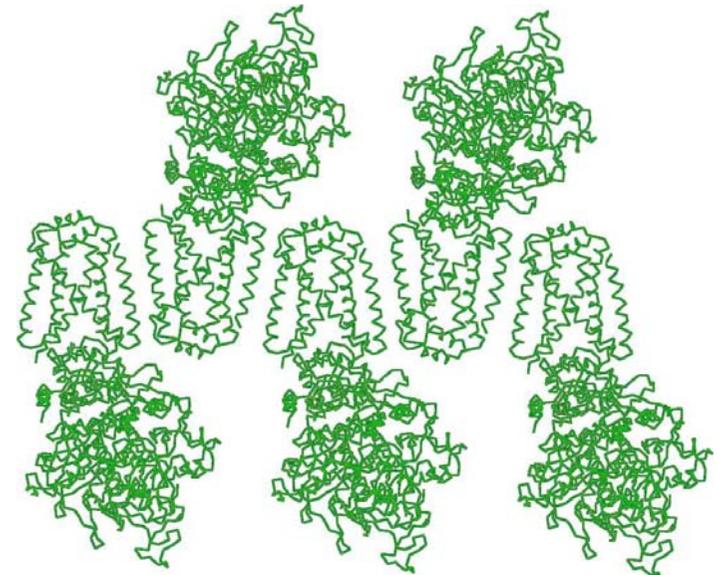
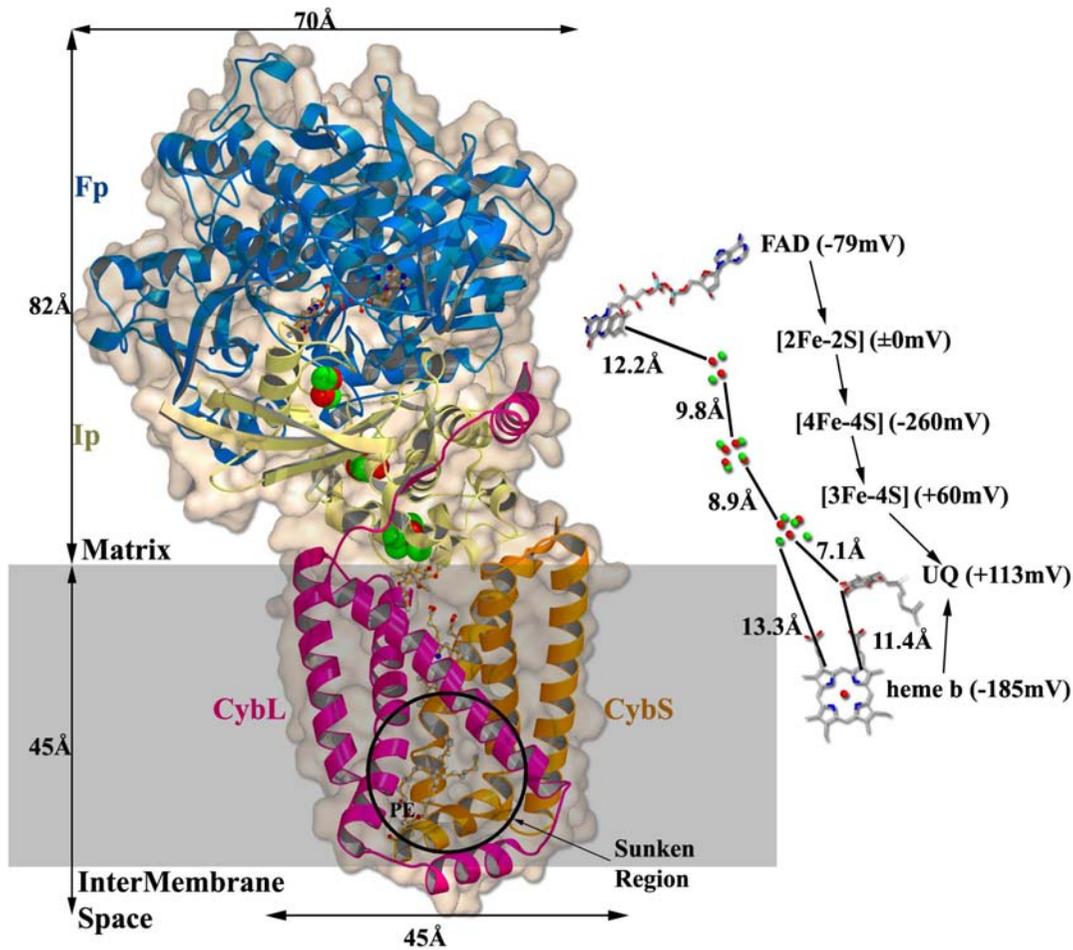
Sun et.al, **Cell**,  
121(7):1043-57

Chicken, 2.1Å



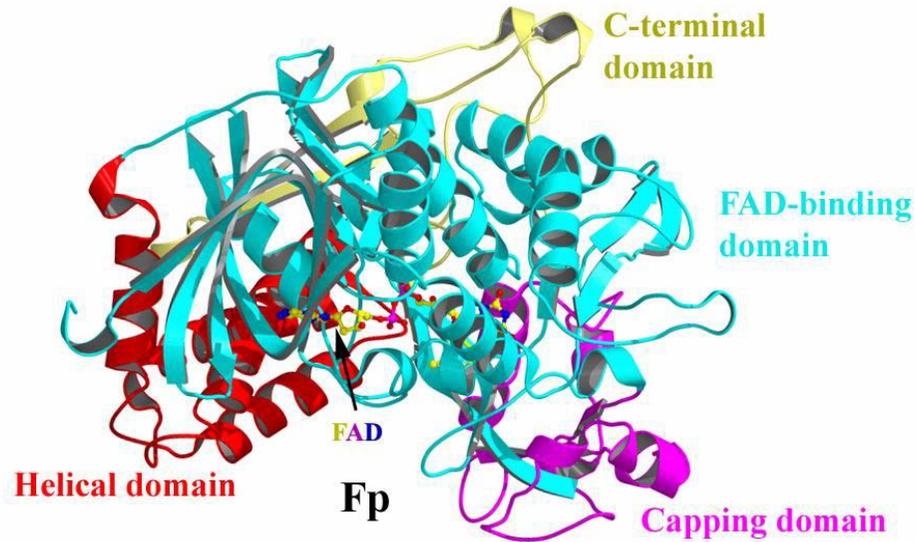
Huang et.al, **J.Bio.Chem.**,  
281(9):5965-72

# Complex II is a trans-cross-membrane biological monomer protein complex



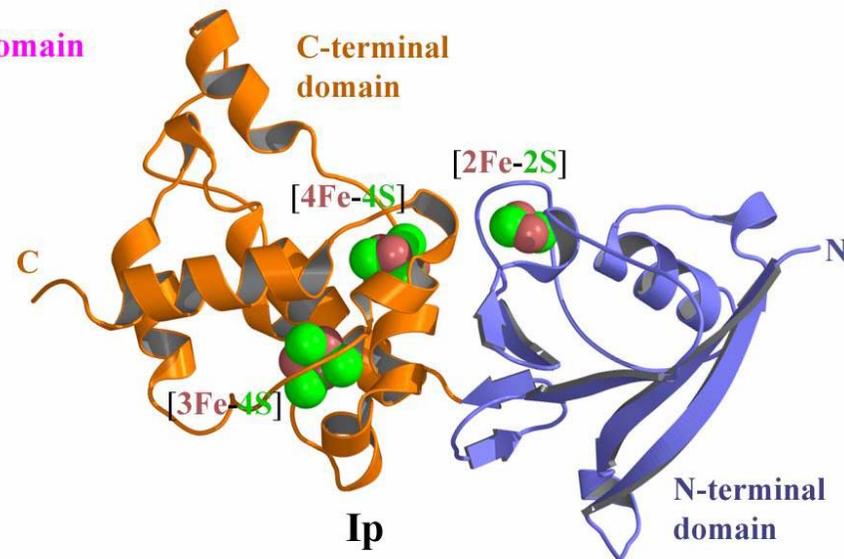
Mitochondrial Complex II packing in crystal, partially mediated by trans-membrane region hydrophobic interactions.

# Hydrophilic subunits

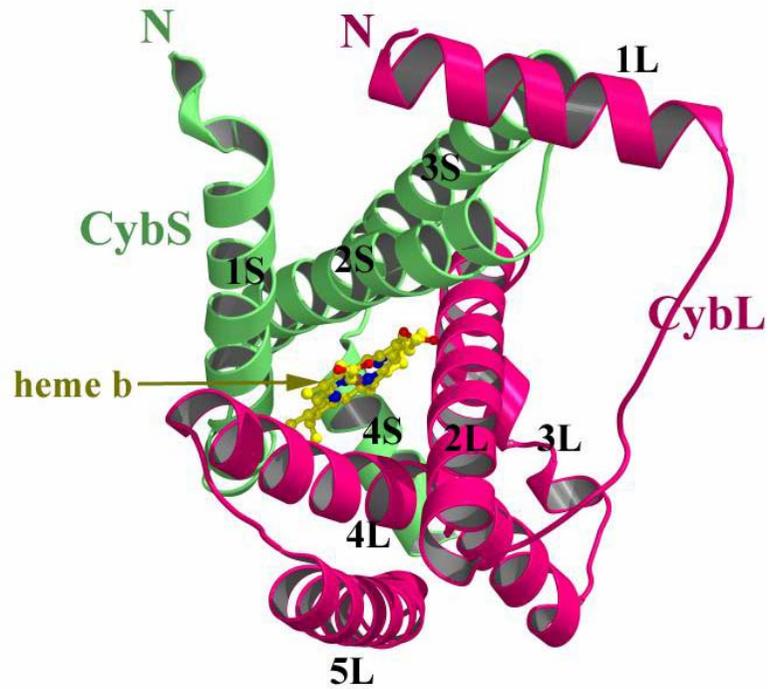


Flavoprotein

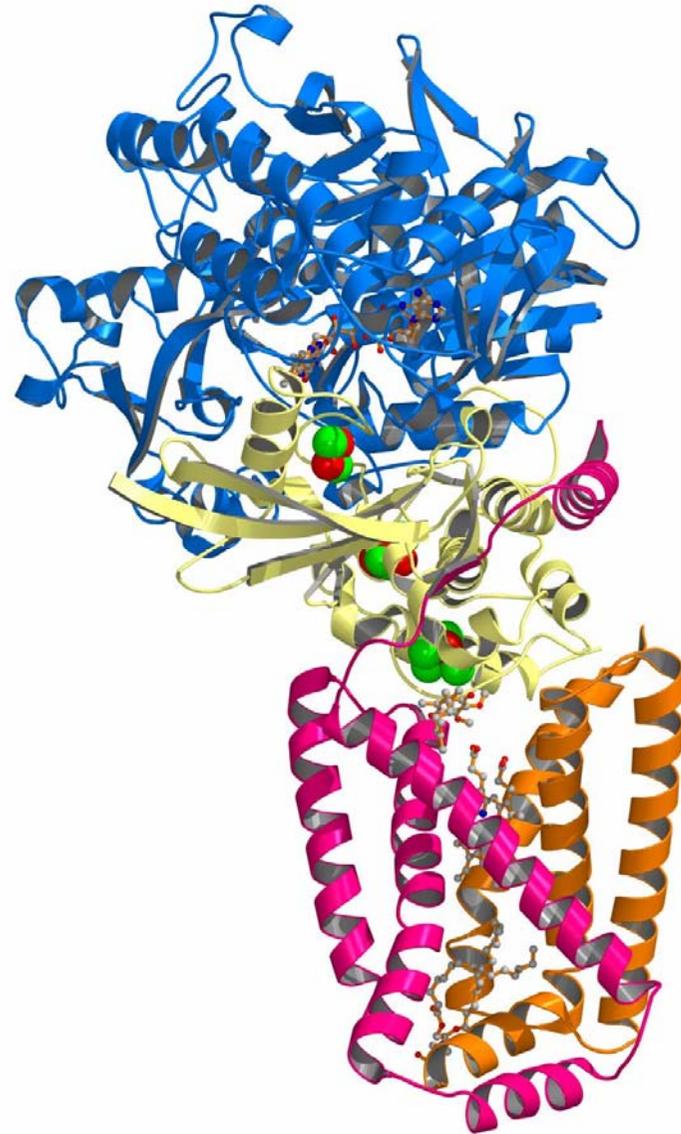
## Iron-sulfur protein



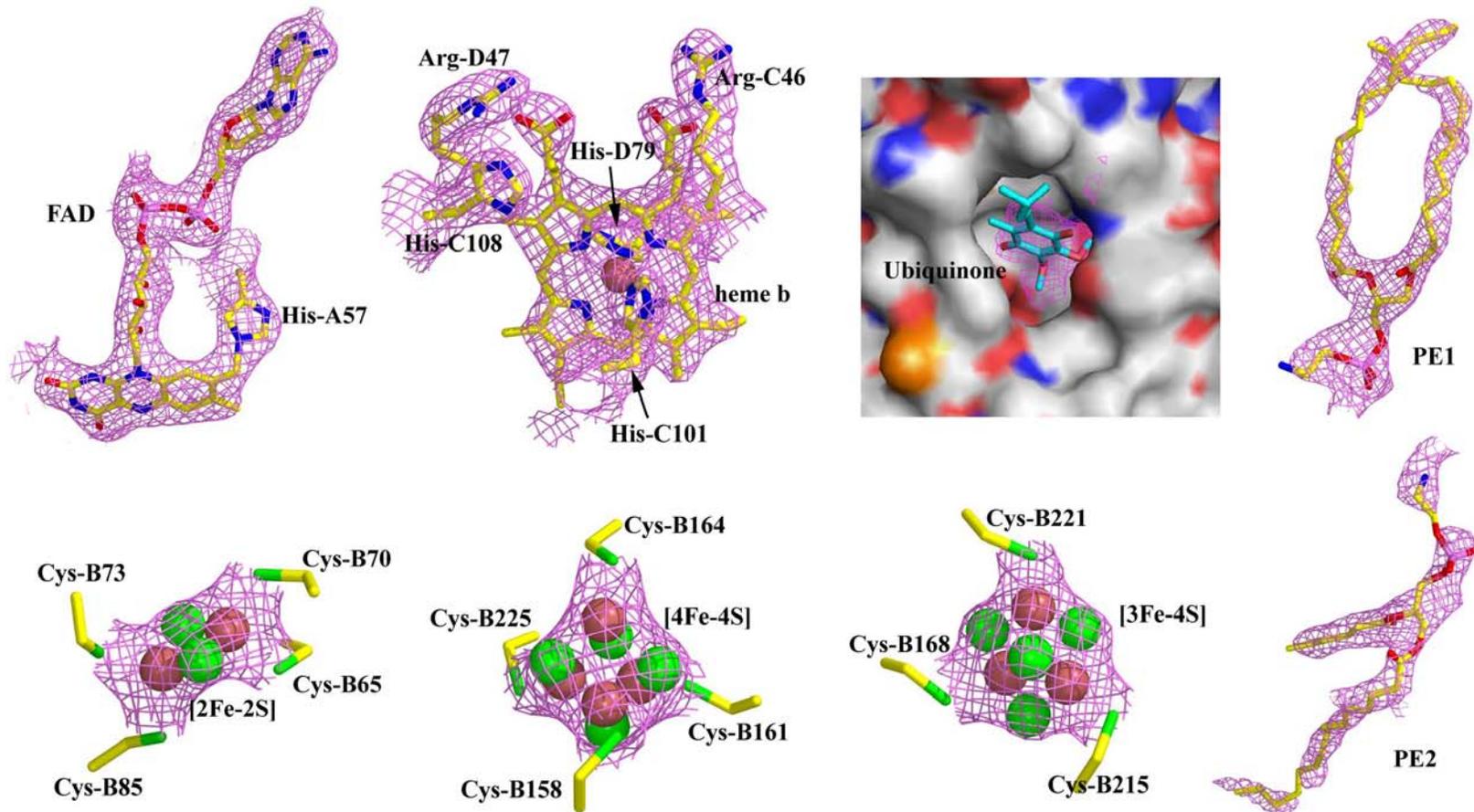
# Hydrophobic subunits



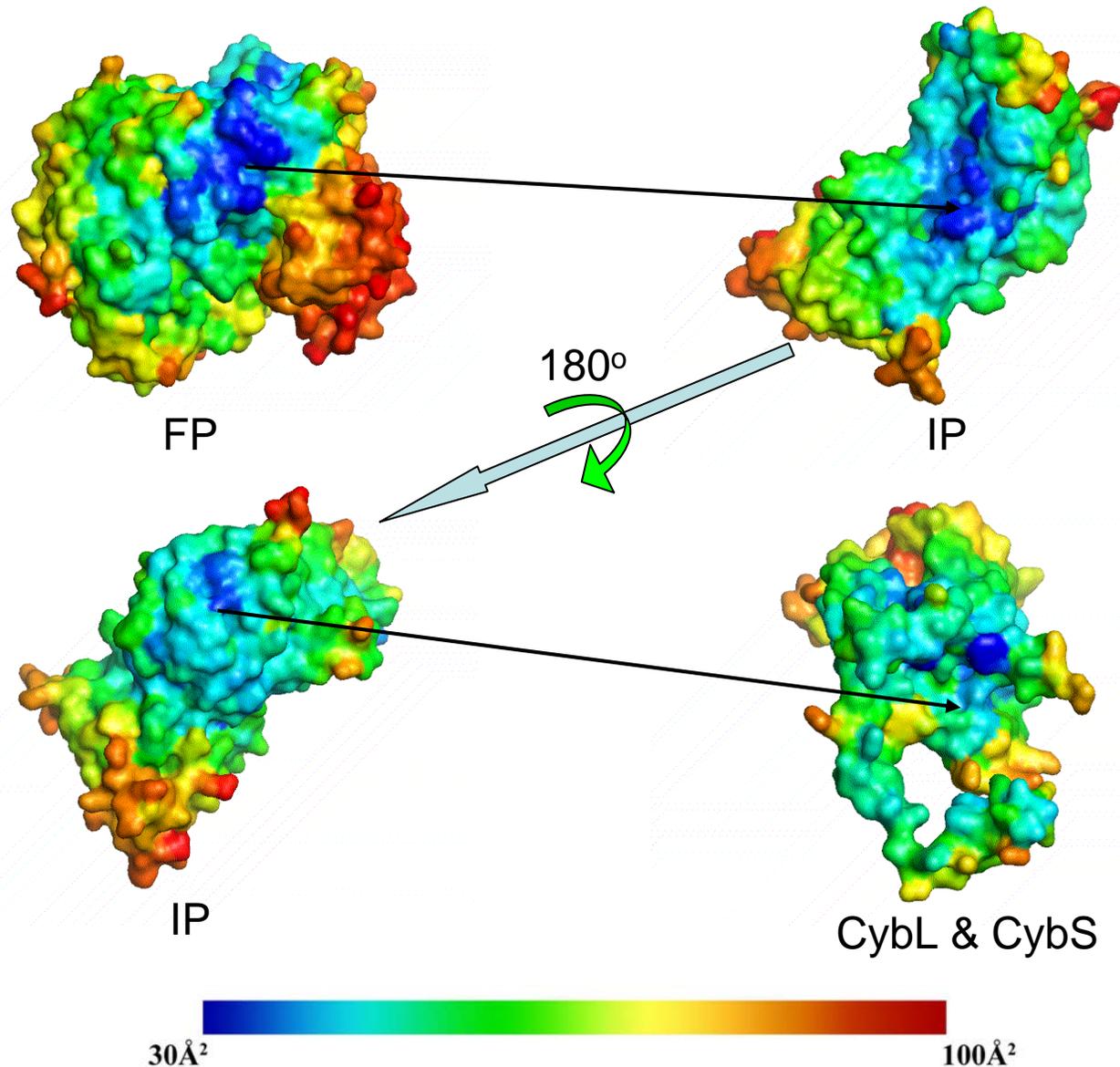
CybL and CybS



# Prosthetic groups are well buried in Complex II

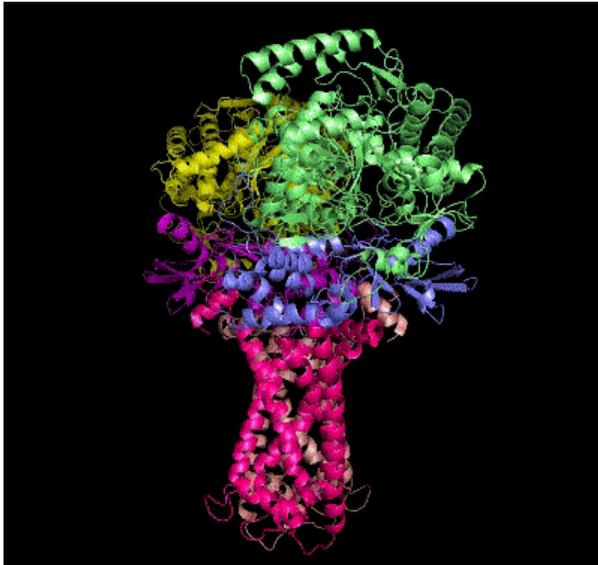


# Interaction between subunits, thermal factor distribution representation



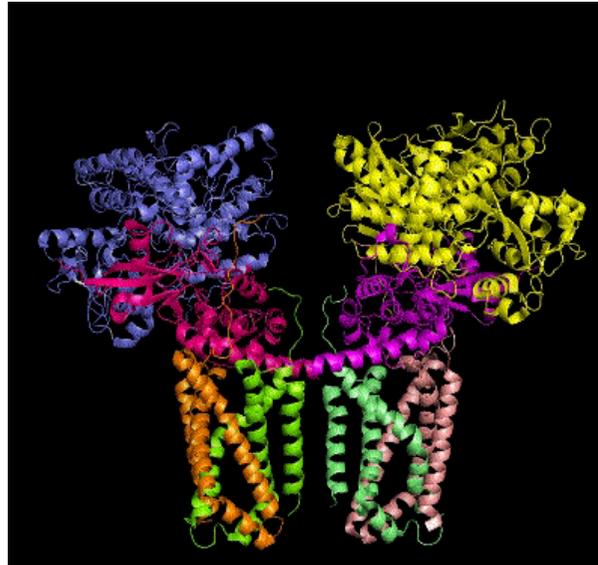
**Fumarate reductase from  
*Wolinella succinogenes***

Lancaster et al., 1999



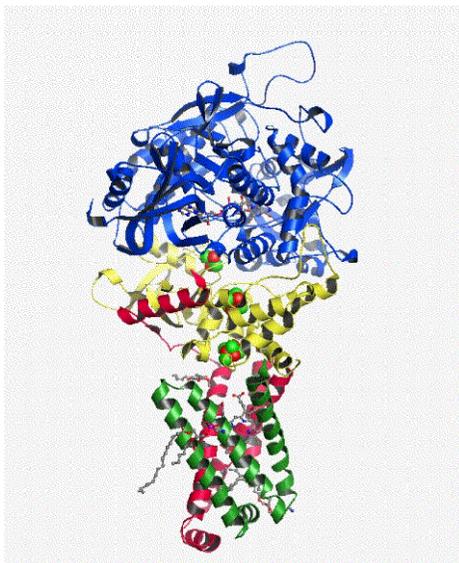
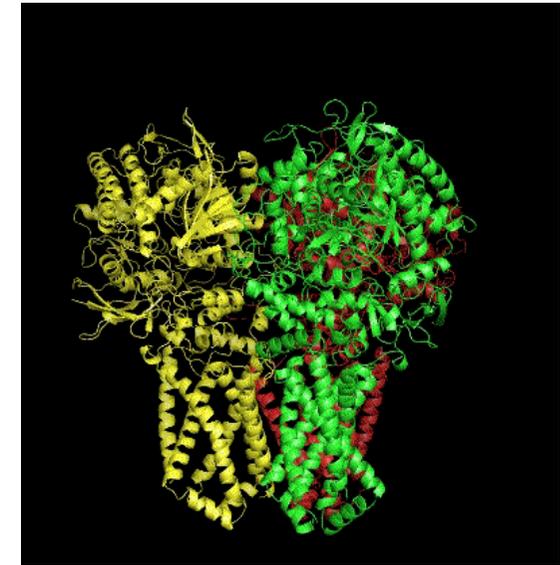
**Fumarate reductase  
from *E.coli***

Iverson et al., 1999



**Succinate dehydrogenase  
from *E.coli***

Yankovskaya et al., 2003



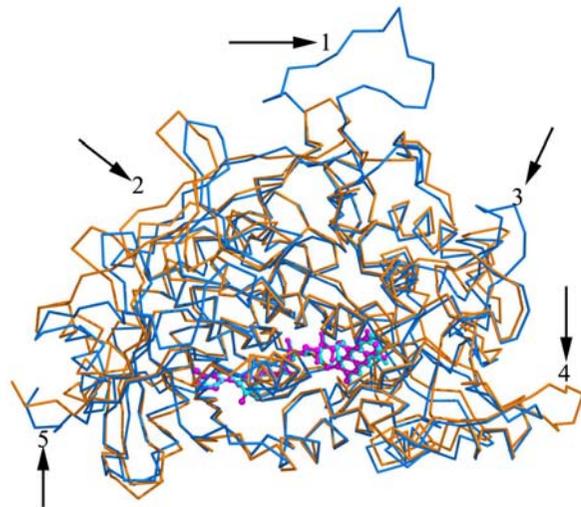
**QFR from *Wolinella succinogenes* :**  
**dimer, one peptide, five trans-membrane helices, two hemes**

**QFR from *E.coli* :**  
**dimer, two peptides, six trans-membrane helices, no heme**

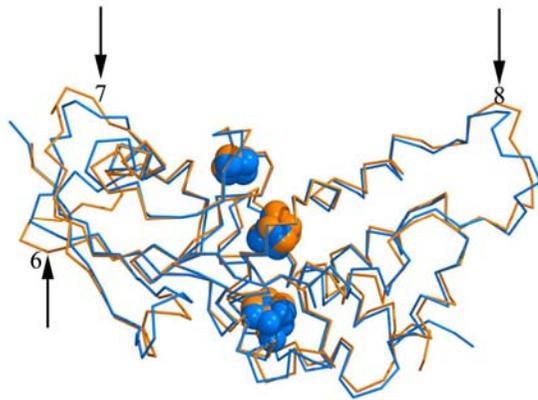
**SQR from *E.coli* :**  
**trimer, two peptides, six trans-membrane helices, one heme**

**SQR from Mitochondria:**  
**monomer, two peptides, six trans-membrane helices, one heme**

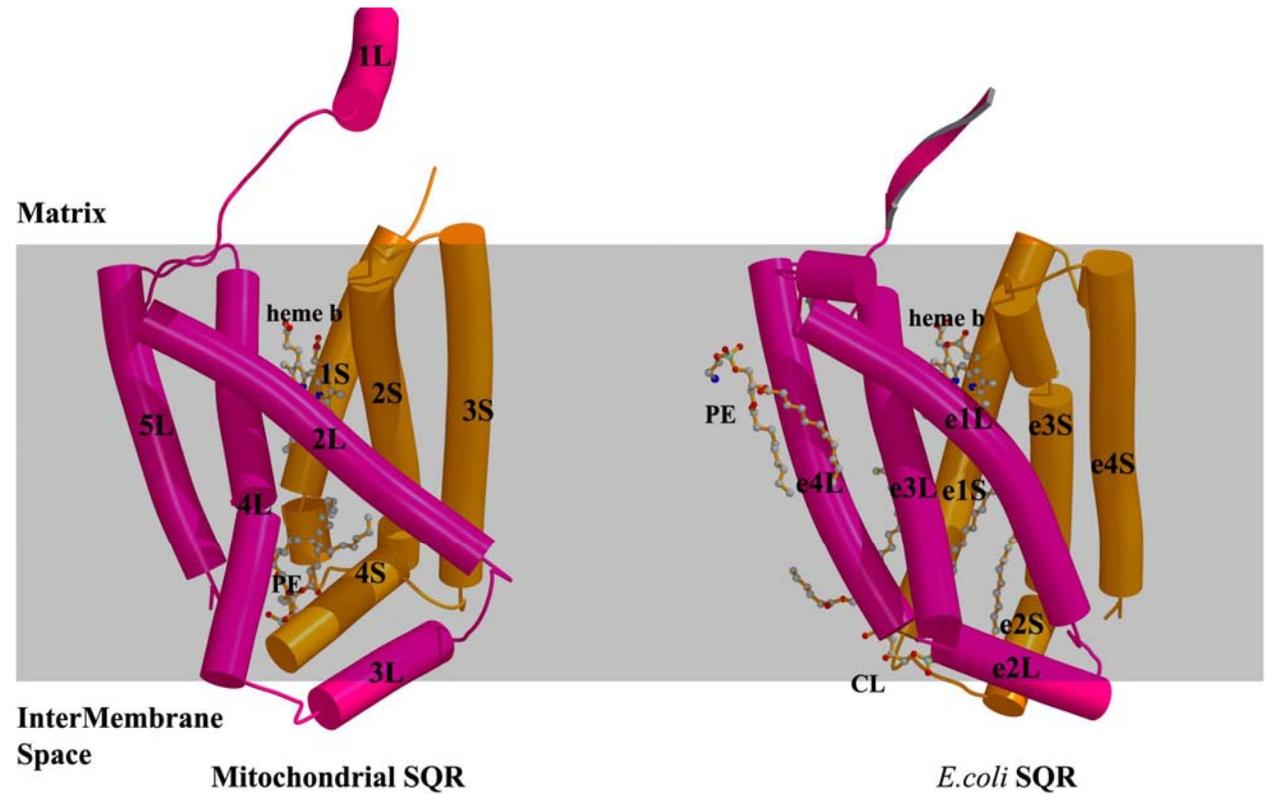
# Diversity of trans-membrane region in SQR family.



FAD binding protein



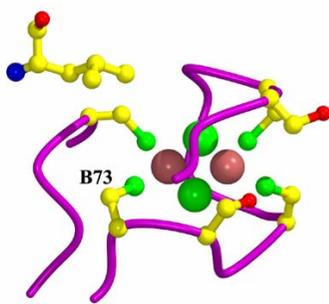
Iron-sulfur protein



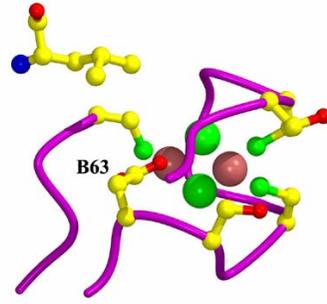
- Different CybS fold
- Hydrophilic helix
- Large structure diversity
- Low faithfulness for model representation

# Differences in environments of the iron-sulfur clusters between mitochondrial and *E. coli* SQR structures

[2Fe-2S]

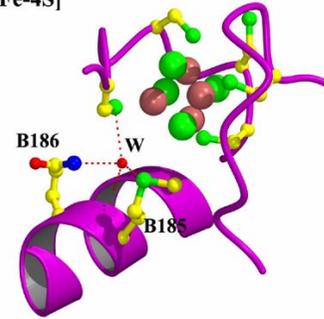


0mV

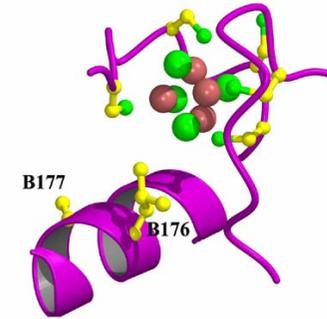


10mV

[4Fe-4S]

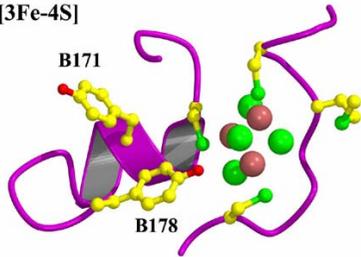


-260mV



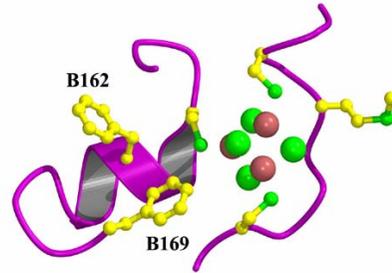
-175mV

[3Fe-4S]



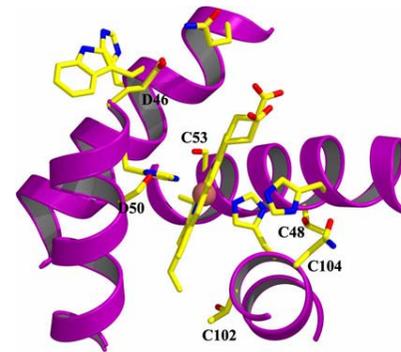
Mitochondrial SQR

60mV



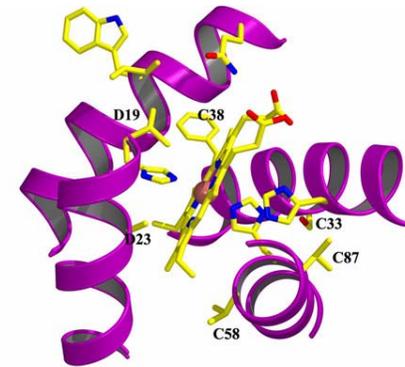
*E. coli* SQR

65mV



Mitochondrial SQR

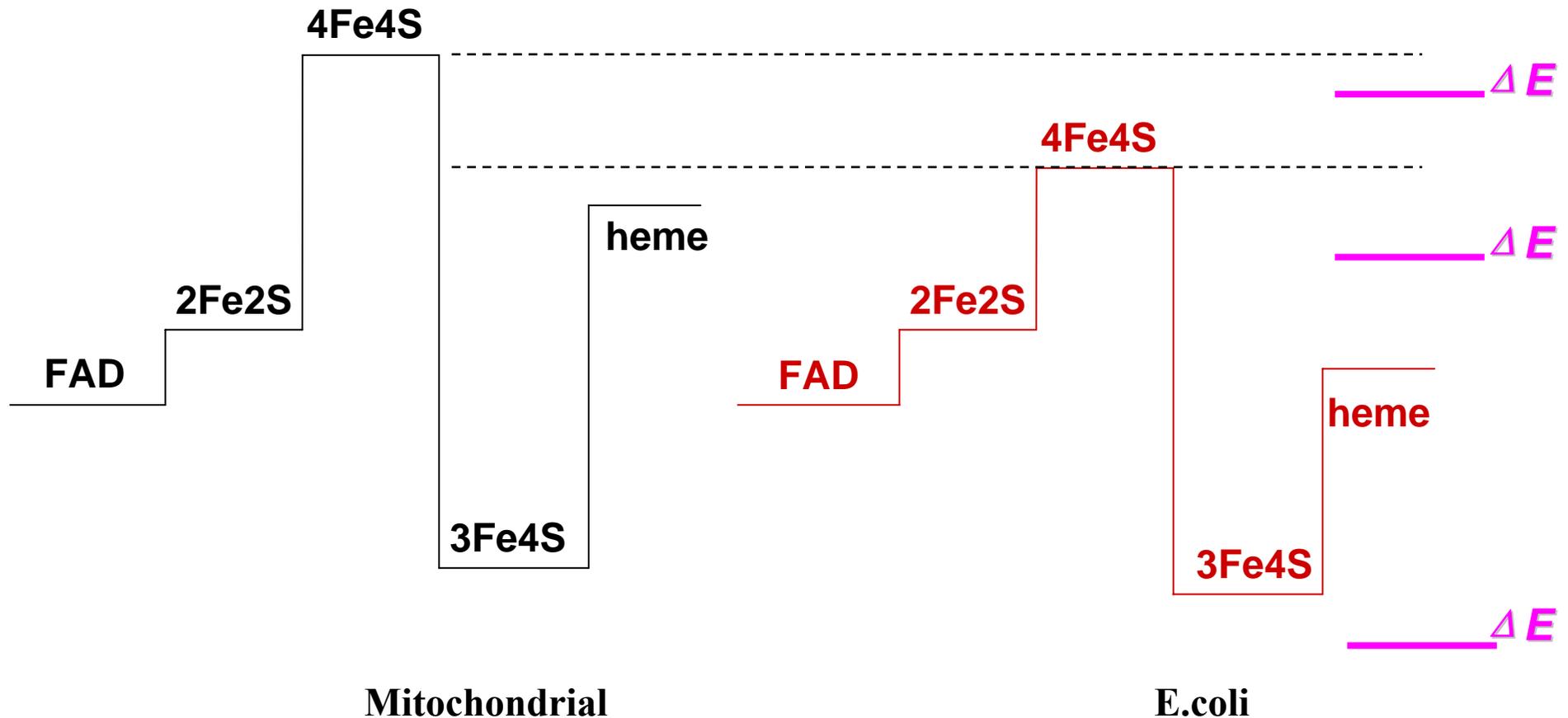
-185mV



*E. coli* SQR

36mV

# Differences in environments of the iron-sulfur clusters between mitochondrial and E. coli SQR structures



In particular, the lower redox potential of [4Fe-4S] increases the energy barrier for electron transfer and, more importantly, might avoid electron flow in the reverse direction in mitochondrial SQR.

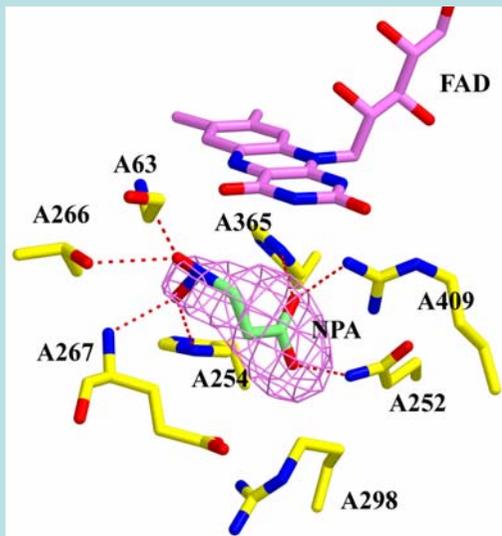
# Mitochondrial respiratory chain Complex II

— complex structure bound with two inhibitors

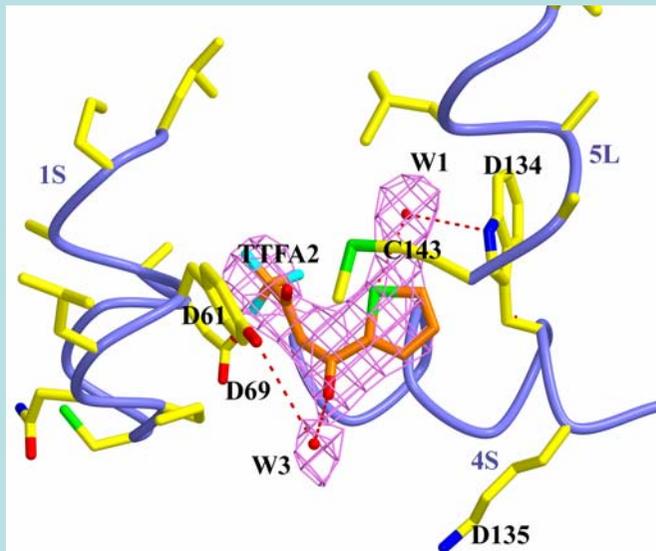
3-nitropropionic acid (NPA) is a succinate analog and a strong inhibitor for the succinate-oxidation enzymatic activity of Complex II.

2-thenoyltrifluoroacetone (TTFA) is a classical inhibitor for the ubiquinone reduction of Complex II by holding its ubiquinone binding sites.

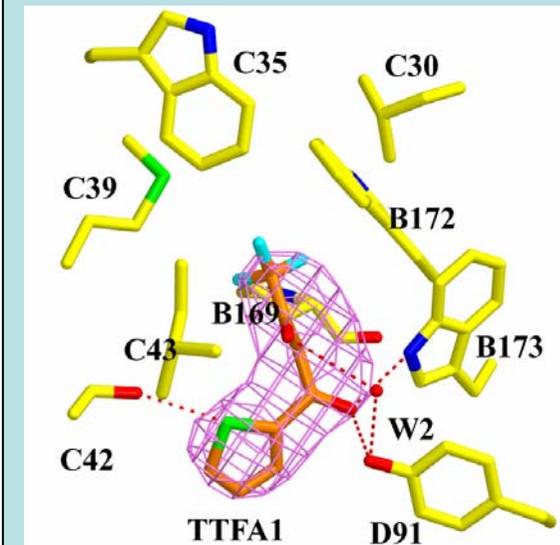
At succinate binding site  
3-nitropropionic acid (NPA)



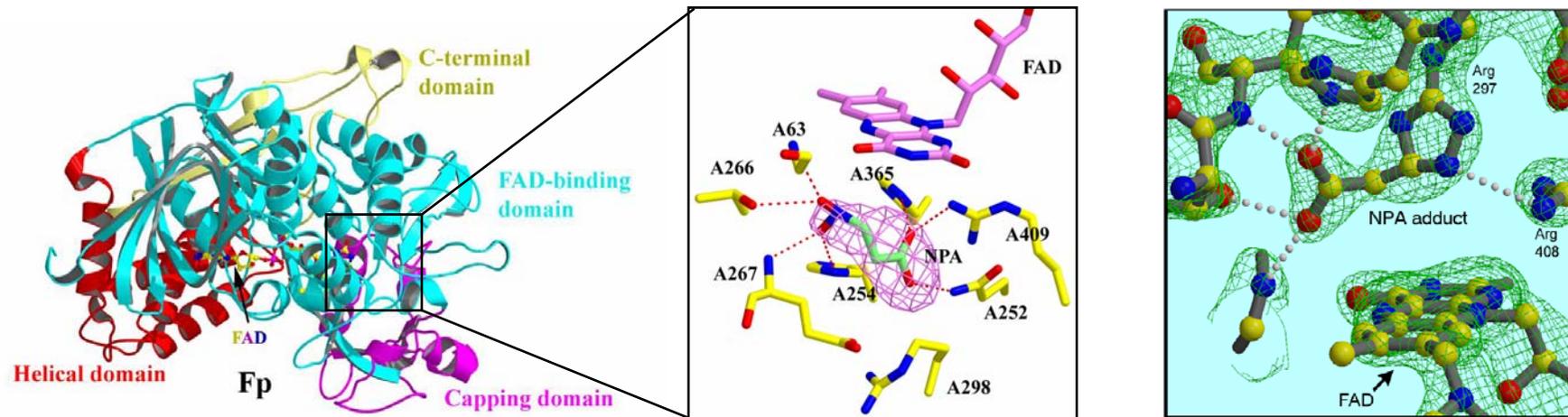
At ubiquinone binding site (Qd)  
2-thenoyltrifluoroacetone (TTFA2)



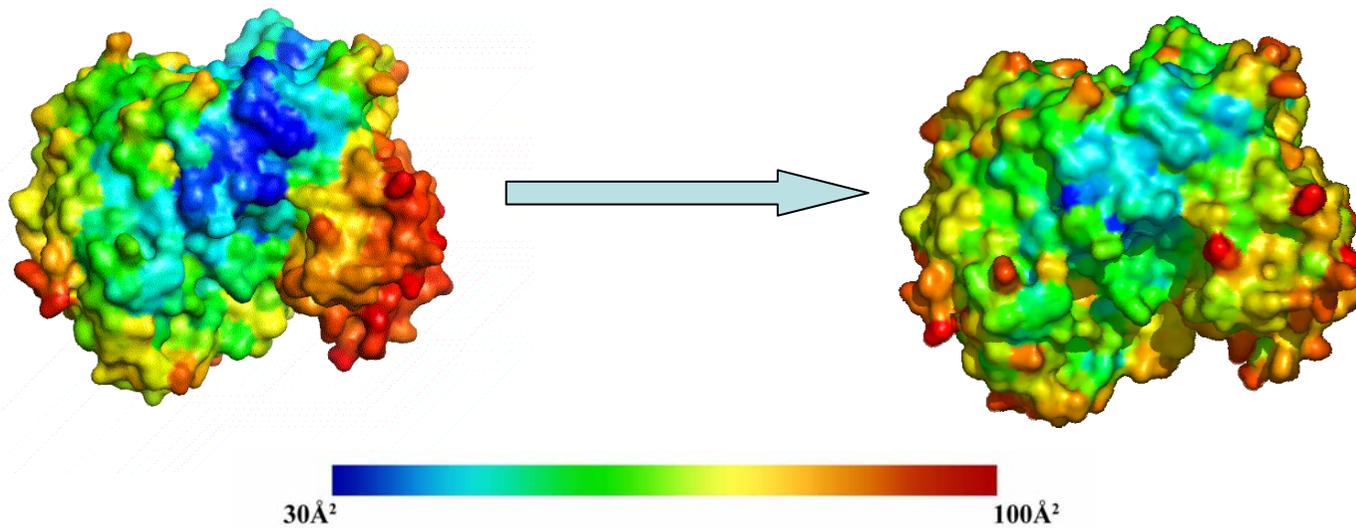
At ubiquinone binding site  
(Qp)  
2-thenoyltrifluoroacetone  
(TTFA1)



# Flexibility of Fp Capping domain reduced by inhibitor



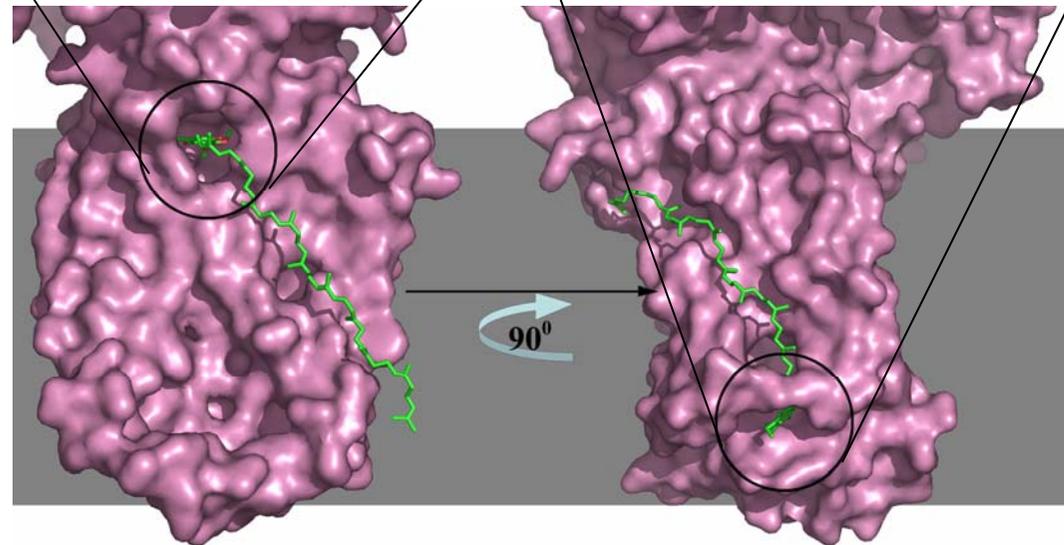
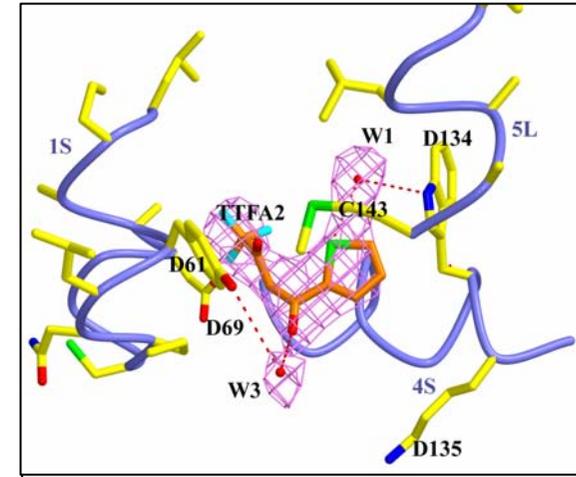
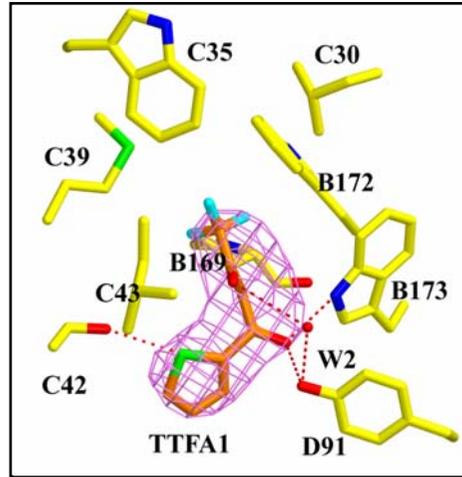
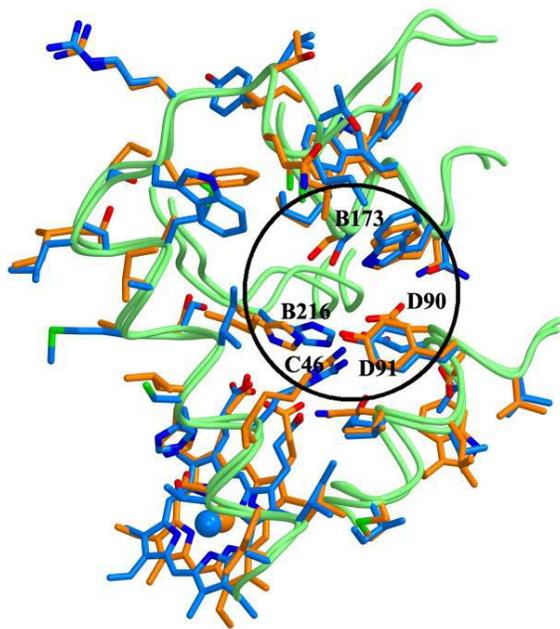
Huang et.al, *J.Bio.Chem.*  
281(9):5965-72



# Mitochondrial respiratory chain Complex II

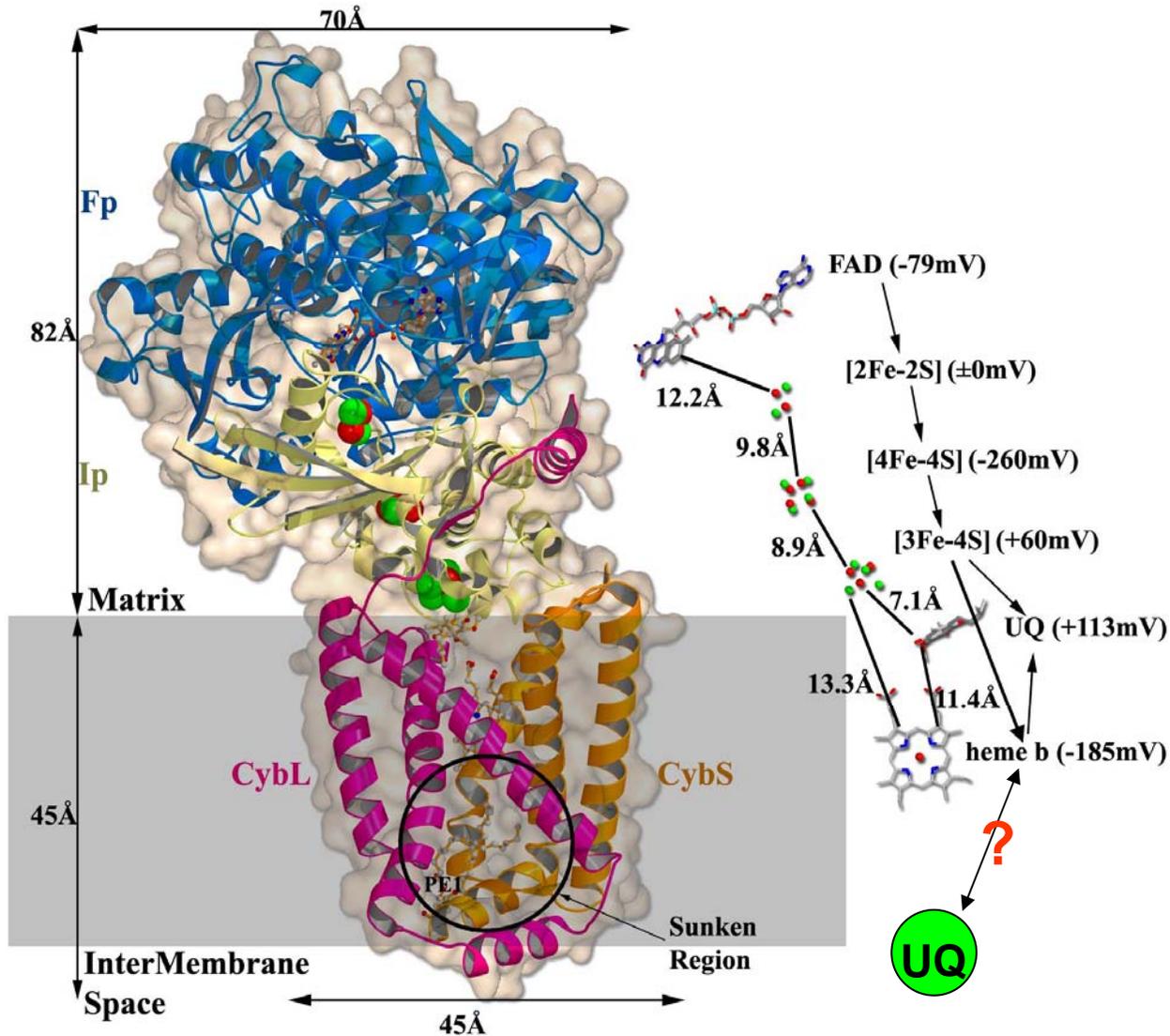
## — Ubiquinone binding sites

One conserved ubiquinone binding site (Qp)



# Mitochondrial respiratory Complex II

## — Electron transferring chain



Low temperature  
electron parallel  
magnetic  
resonance to  
identify the electron  
transfer pathway

# Mitochondrial respiratory chain Complex II

## — Sequence comparison

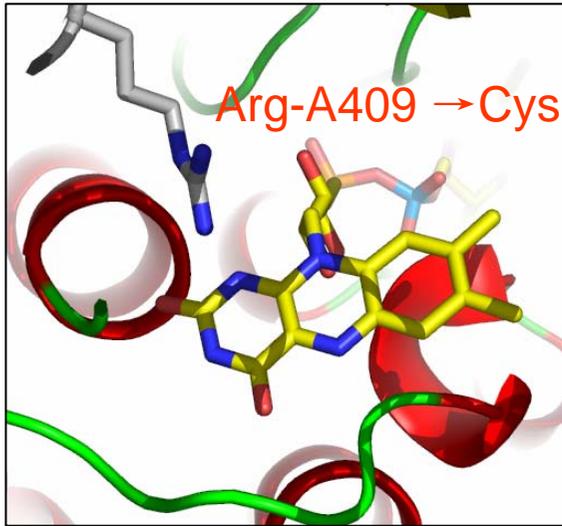
1. The respective sequence homologies of subunits A, B, C and D between **porcine** and *E.coli* are 51%, 50%, 19% and 18%, respectively.
2. SQRs from mammalian mitochondrial membranes share very high sequence similarity and, in particular, the respective sequence homologies of subunits A, B, C and D between **human** and **porcine** are 95%, 98%, 92% and 90%, respectively.
3. The very high sequence and structural homology between swine and human SQRs allows us to use this structure model to explain the relationship between the dysfunction of mitochondrial SQR and various mutations associated with the diseases.

# Diseases related with defects of mitochondrial Complex II

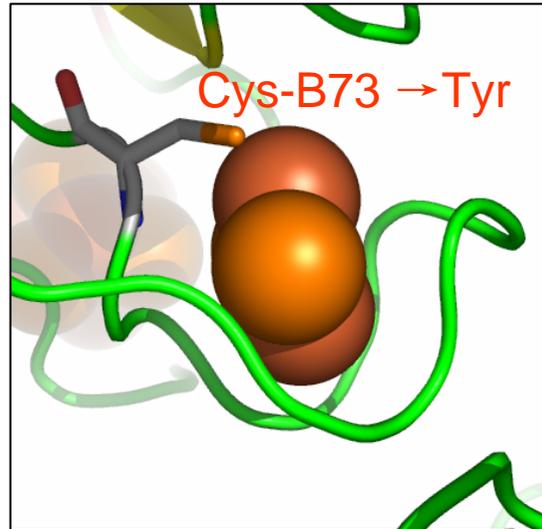
Subunit	Point Mutation	Disease and Phenotype	Explanation
A	Arg-A409 → Cys (Birch-Machin et al., 2000)	Optic atrophy, ataxia, myopathy	Altering succinate binding specificity
	Ala-A482 → Val (Parfait et al., 2000)	Leigh syndrome	?
	Arg-A512 → Trp (Bourgeron et al., 1995)		Unstable Fp helix domain
	Gly-A513 → Glu (Van Coster et al., 2003)		
B	Cys-B73 → Tyr (Neumann et al., 2002)	Phaeochromocytoma	Destroys the ligation of [2Fe-2S]
	Pro-B103 → Arg (Baysal et al., 2002)	Head and neck paraganglioma	No room for this large residue
	Cys-B164 → Arg (Neumann et al., 2002)	Phaeochromocytoma	Destroys the ligation of [4Fe-4S]
	Cys-B168 → Tyr (Neumann et al., 2002)		Destroys the ligation of [3Fe-4S]
	Pro-B169 → Arg (Astuti et al., 2001b)		Changes the [3Fe-4S] ligation conformation and destroy ubiquinone binding site (Qp)
	Arg-B214 → His (Neumann et al., 2002)		Might destroy ubiquinone binding site (Qs)
	Cys-B221 → X (Neumann et al., 2002)		Destroys the ligation of [3Fe-4S]

# Diseases related with defects of mitochondrial Complex II

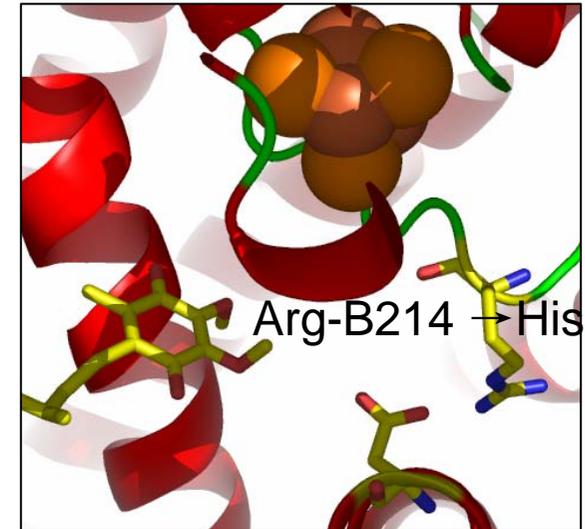
Subunit	Point Mutation	Disease and Phenotype	Explanation
C	Leu-C132 → Pro (Bauters et al., 2003)	Paraganglioma	Destroys the stability of helix 5L, then might destroy the ubiquinone tail binding (Qd)
D	Arg-D47 → Gly (Taschner et al., 2001)	Head and neck paraganglioma	Destroys the interaction with heme b carboxylate group
	Pro-D58 → Leu (Baysal et al., 2000)		Changes the folding of helix 1S, then destroys the ubiquinone binding site (Qd)
	Asp- D69 → Tyr (Baysal et al., 2000)	Head and neck paraganglioma / pheochromocytoma	Might lose the catalytic activity for ubiquinone reduction
	Leu-D72 → Pro (Taschner et al., 2001)	Head and neck paraganglioma	Changes the folding of helix 2S, then the position of one heme b ligand His-D79
	His-D79 → Leu (Baysal et al., 2000)		Destroys the ligation of heme b
	Tyr-D91 → Cys (Milunsky et al., 2001)		Destroys the binding of ubiquinone (Qp)
	Gln-D98 → X (Neumann et al., 2002)	Phaeochromocytoma	helix 3S and 4S missing
Leu-D116 → Pro (Taschner et al., 2001)	Head and neck paraganglioma	Changes the folding of helix 3S, then destroy the Qd site	



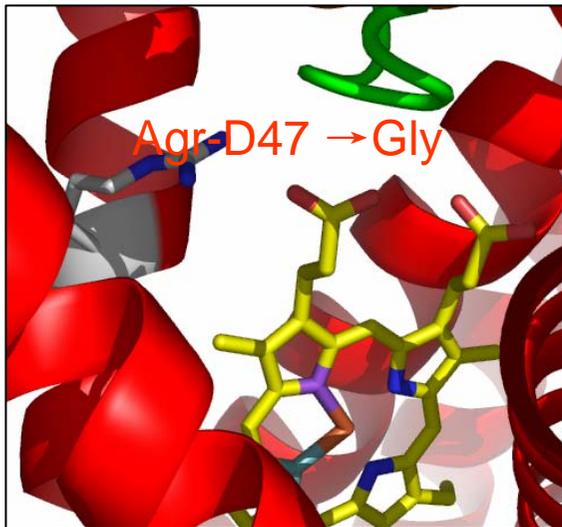
Optic atrophy, ataxia,  
myopathy



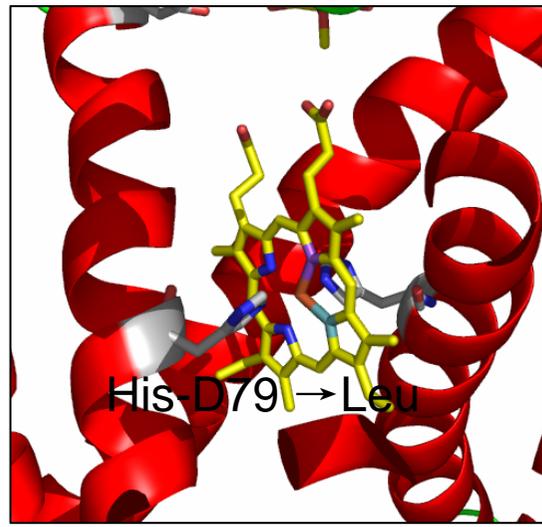
Phaeochromocytoma



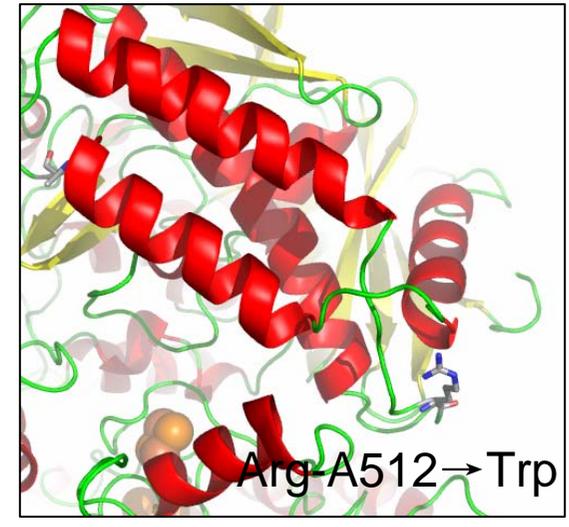
Phaeochromocytoma



Head-and-neck  
paraganglioma



Head-and-neck  
paraganglioma



Leigh syndrome

# Structure Analysis and Implication

- First structure at 2.4 Å resolution, provides a *bona fide* model for understanding the mitochondrial respiratory system.
- Complex II is a trans-cross-membrane biological monomer protein complex.
- Prosthetic groups are well buried in Complex II.
- N-terminal hydrophilic helix of CybL is crucial for the assembly and stability of Complex II.
- Capping domain of flavoprotein is flexible.
- Diversity of trans-membrane region in SQR family.
- Middle point redox electron potential of prosthetic groups are mostly modulated by residues surrounded.
- Two ubiquinone binding pockets are revealed.
- Explanation of diseases related with dysfunction of Complex II

The Laboratory of Structure Biology,

Tsinghua University

**Zihe Rao**

Fei Sun, Yujia Zhai, Dan Su,  
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Dr. Rongguang Zhang

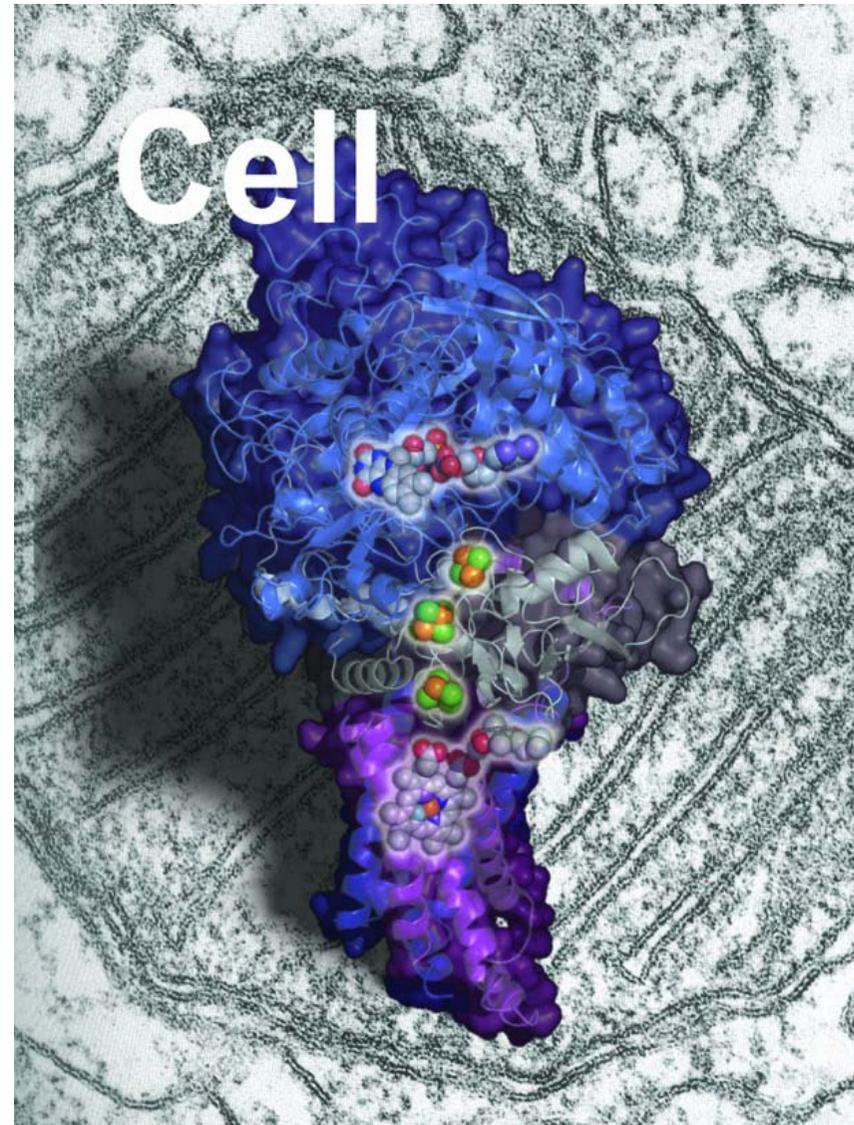
Dr. Andrzej Joachimiak

**Critical Comments:**

Dr. Luet Wong, X.

Dr. Cai Zhang

Dr. Di Xia

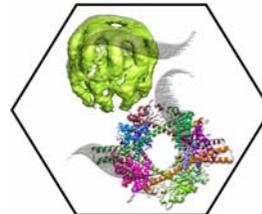


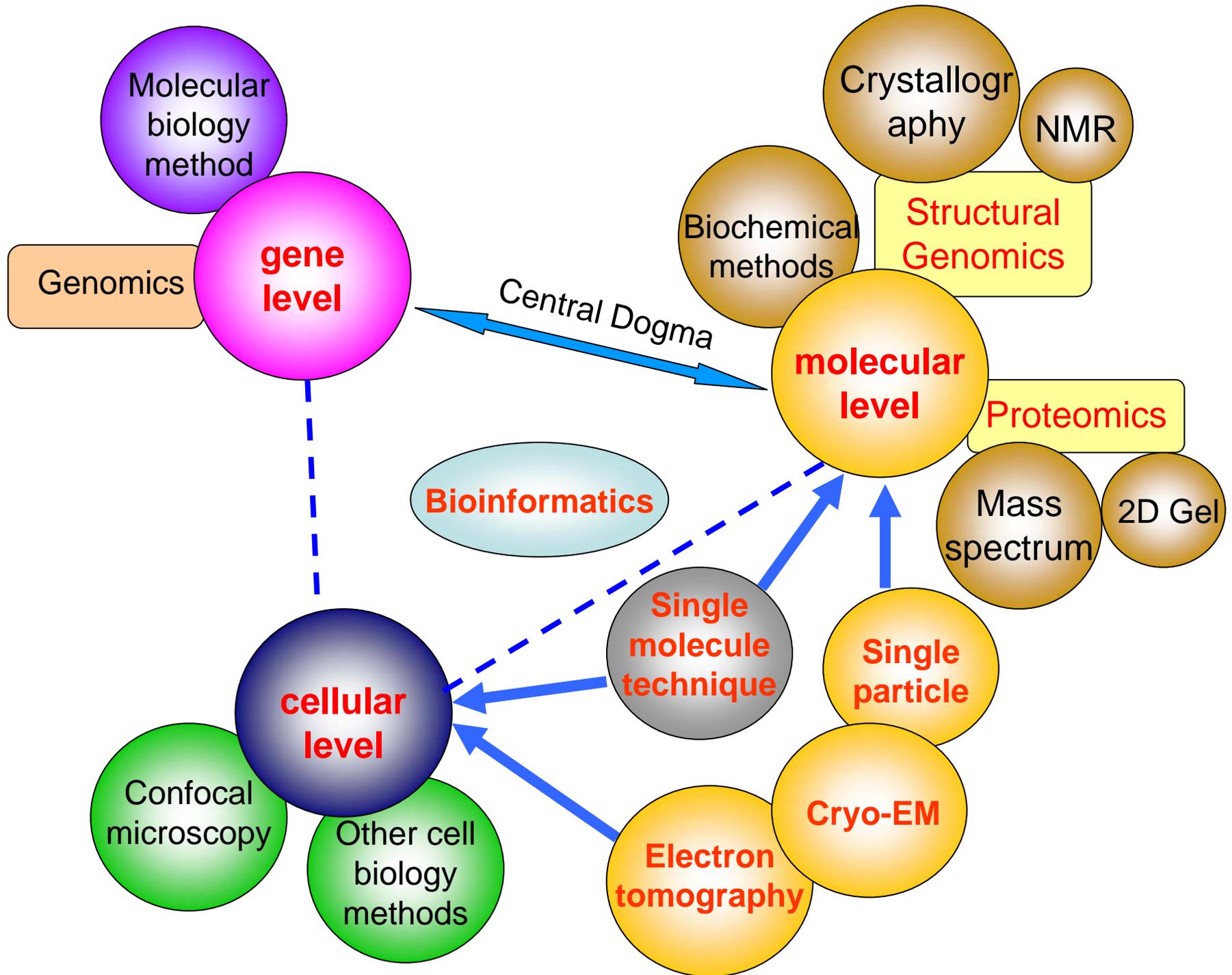
Sun F, Huo X, Zhai Y, Wang A, Xu J, Su D,  
Bartlam M & Rao Z., **Cell**, **121**: 1043-1057, (2005).

# 3D research on biological nano-machinery

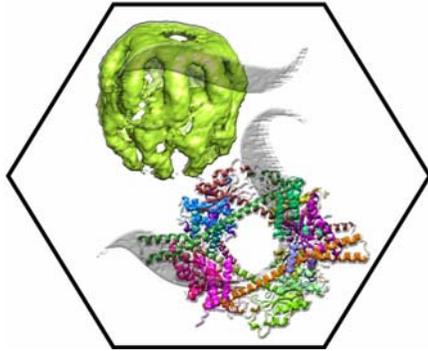
Fei Sun

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The Institute of Biophysics, CAS



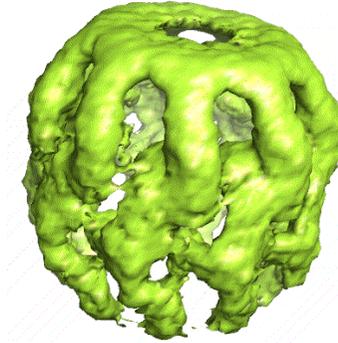
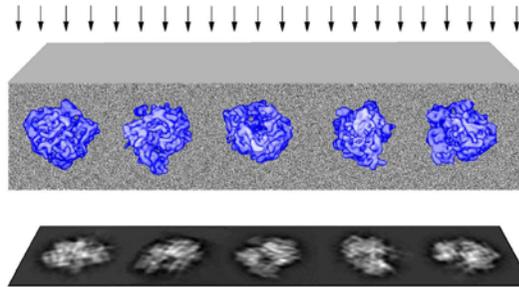


# Scientific Program

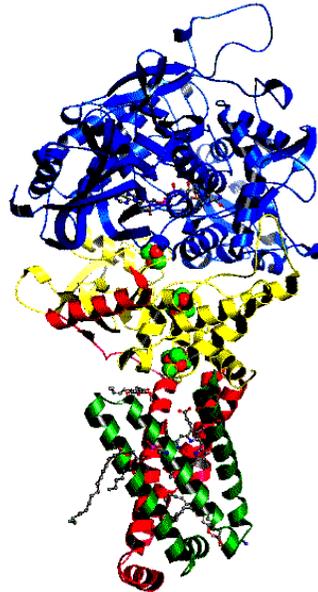
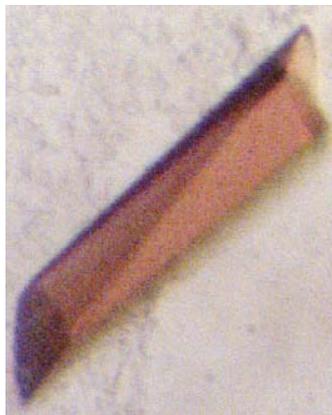


The Center for  
Electron Microscopy

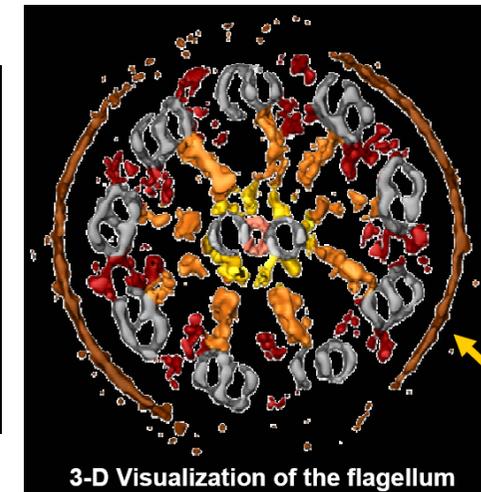
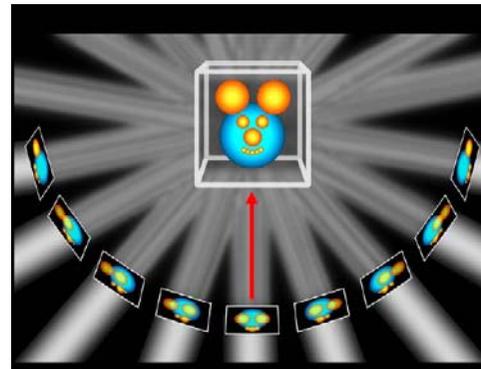
## Structural Biology by cryo-Electron Microscopy



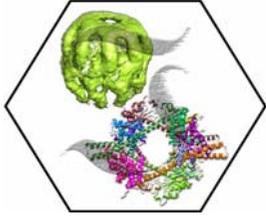
## Structural Biology by X-ray Crystallography



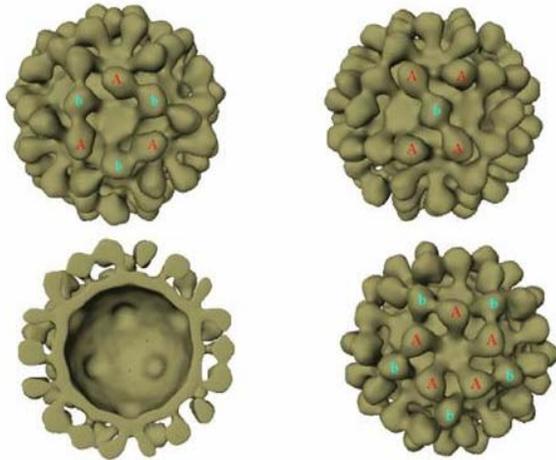
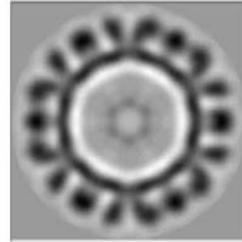
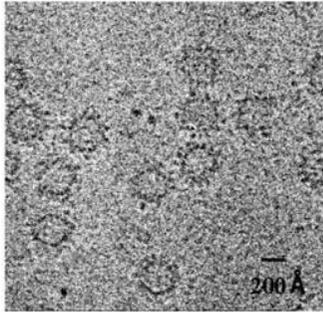
## Cell Biology by Electron Microscopy Tomography



3-D Visualization of the flagellum

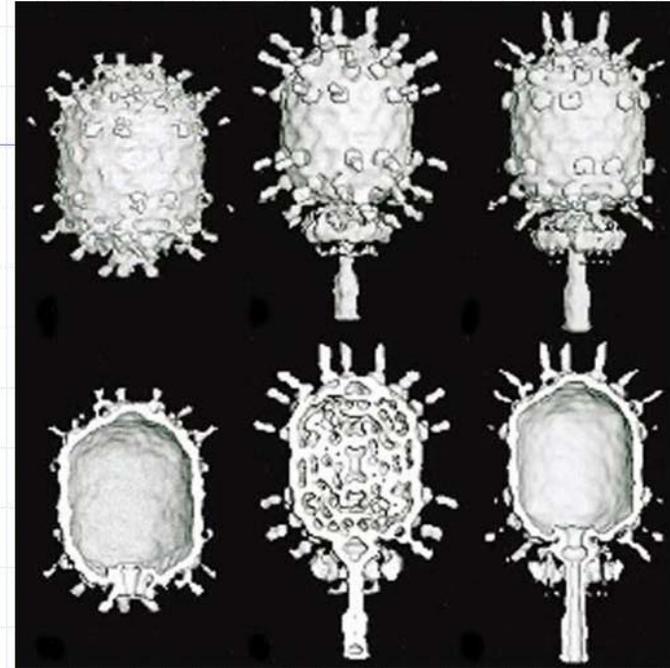


# Present Research Experiences



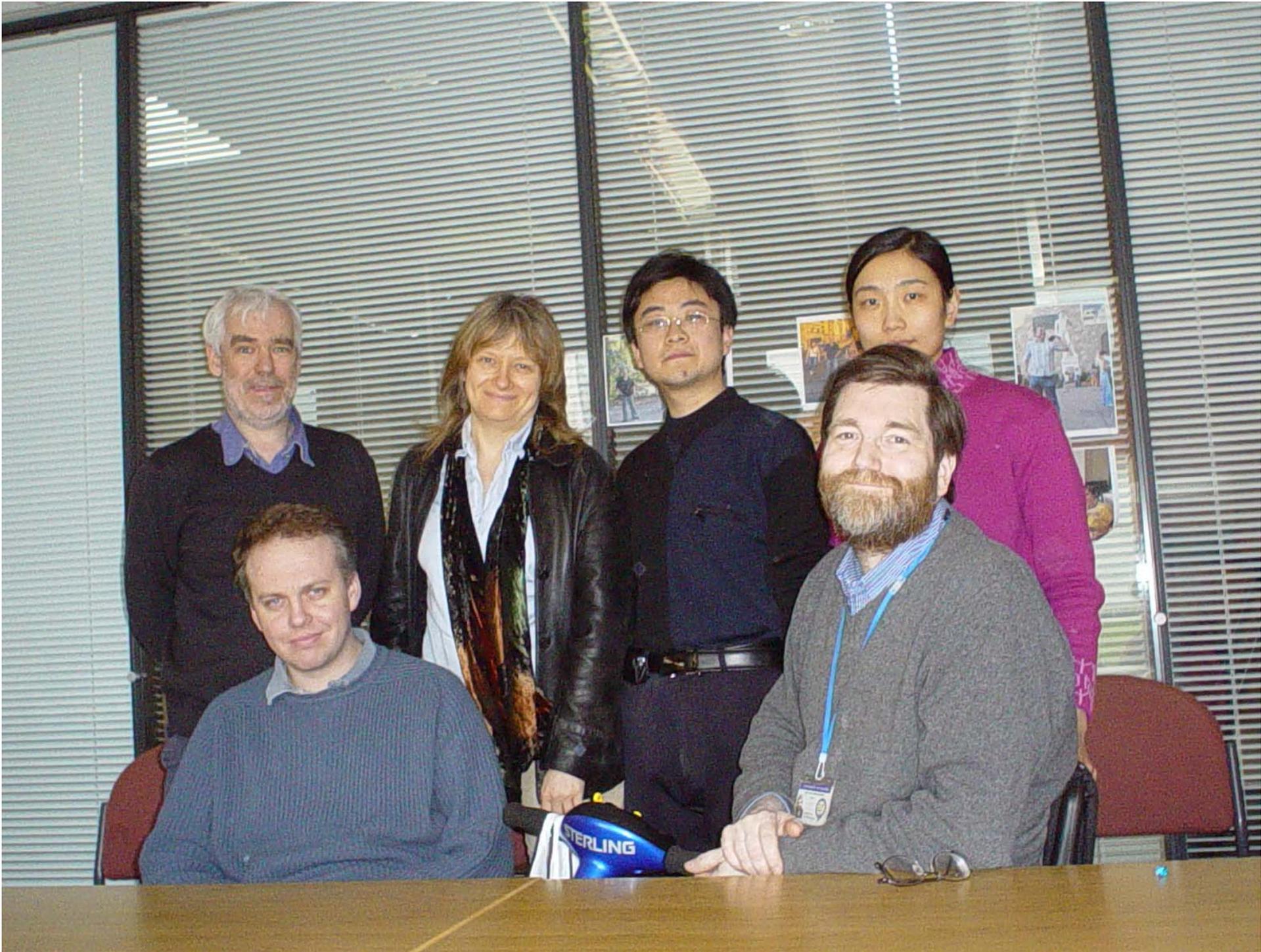
**RHDV**

ZHENG Dong and XU Wei, et al. (2001) Three-dimensional structure of the wild-type RHDV. *Chinese Science Bulletin*. 46(12):1004-9.

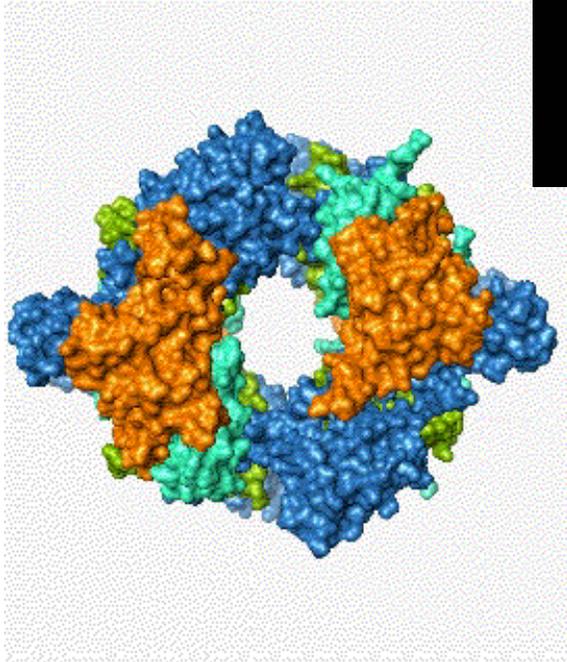


**TBV**

TAO Yizhi, Olson Norman H and XU Wei, et al. (1998). Assembly of a Tailed Bacterial Virus and Its Genome Release Studied in Three Dimensions *Cell*. 95(3):431-7.

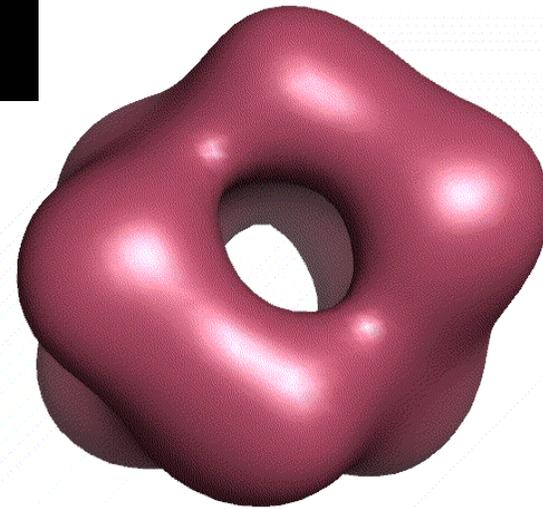
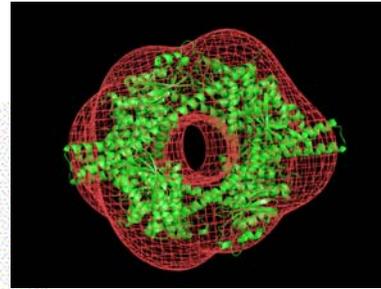


# X-Ray Structure vs. EM structure



2.4 Å resolution

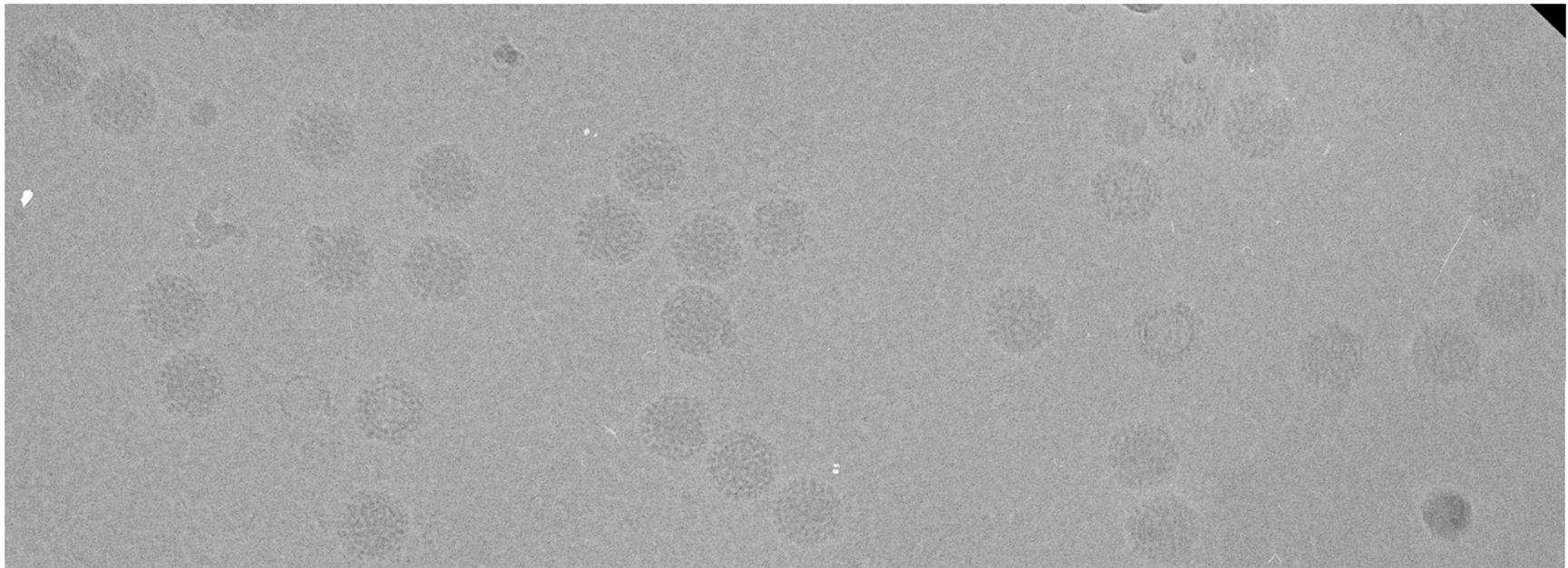
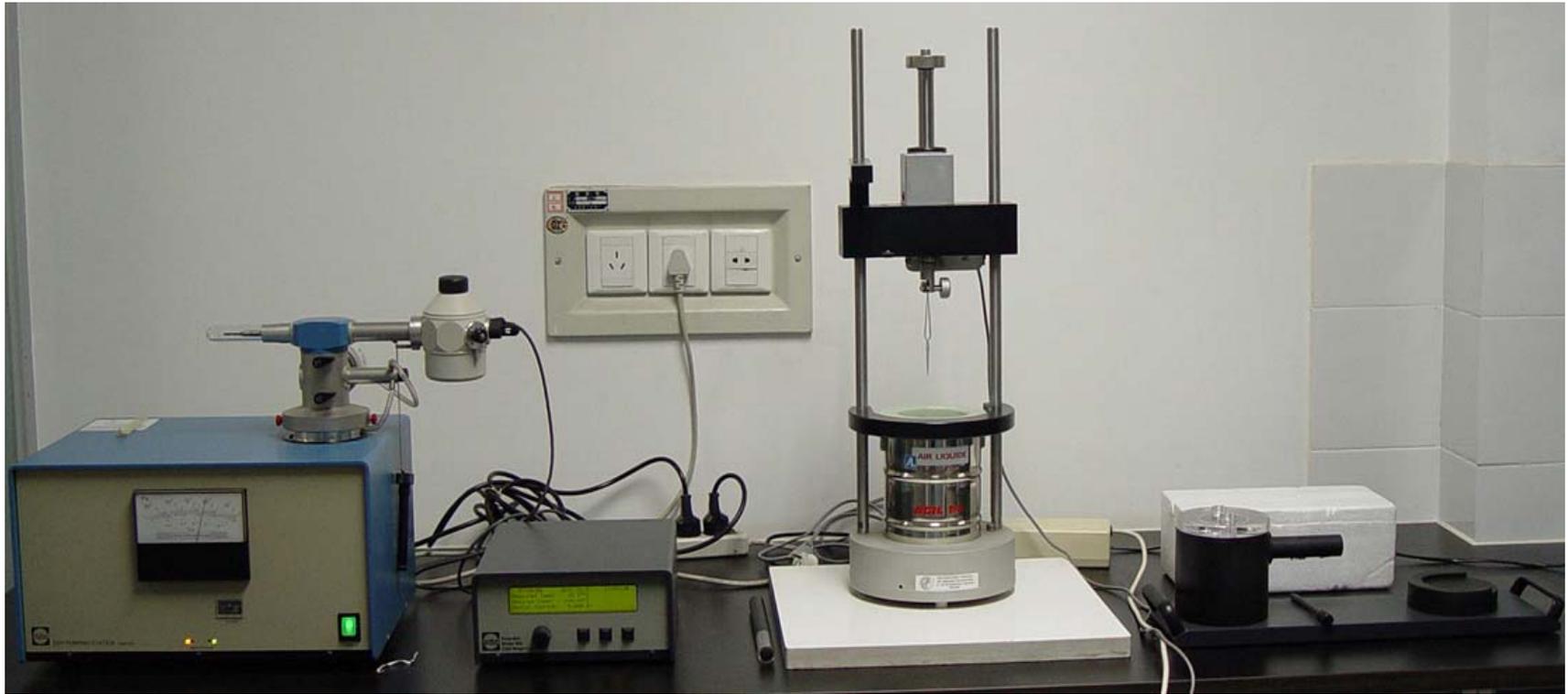
Crystal Structure



35 Å resolution

Reconstruction from  
negative stained sample







大学博士学位论文答辩会



of. Wei Xu



Thank you very much for your  
careful attention, your help and  
your future collaboration.

Have a good time in the  
following conference banquet!

