

# SMALP Conference

October 1, 2021

Robert Gennis

Department of Biochemistry  
University of Illinois at Urbana-Champaign

***High resolution CryoEM structures of the E. coli respiratory O<sub>2</sub> reductase cytochrome c<sub>1</sub> in SMALPS and in MSP nanodiscs.***

See Li et al PNAS(2021)118 No. 34

# Thanks to ...

Nanjing University of Chinese Medicine, Nanjing, China

cryoEM  
SMALPS

Jiao Li, Yanmei Luo, Bin Liu, **Jiapeng Zhu**

Yale University, New Haven

cryoEM  
SMALPS

Long Han, **Kai Zhang**

Columbia University, New York

cryoEM  
MSP  
nanodiscs

Francesca Vallese, **Oliver Clarke**

University of Illinois at Urbana–Champaign

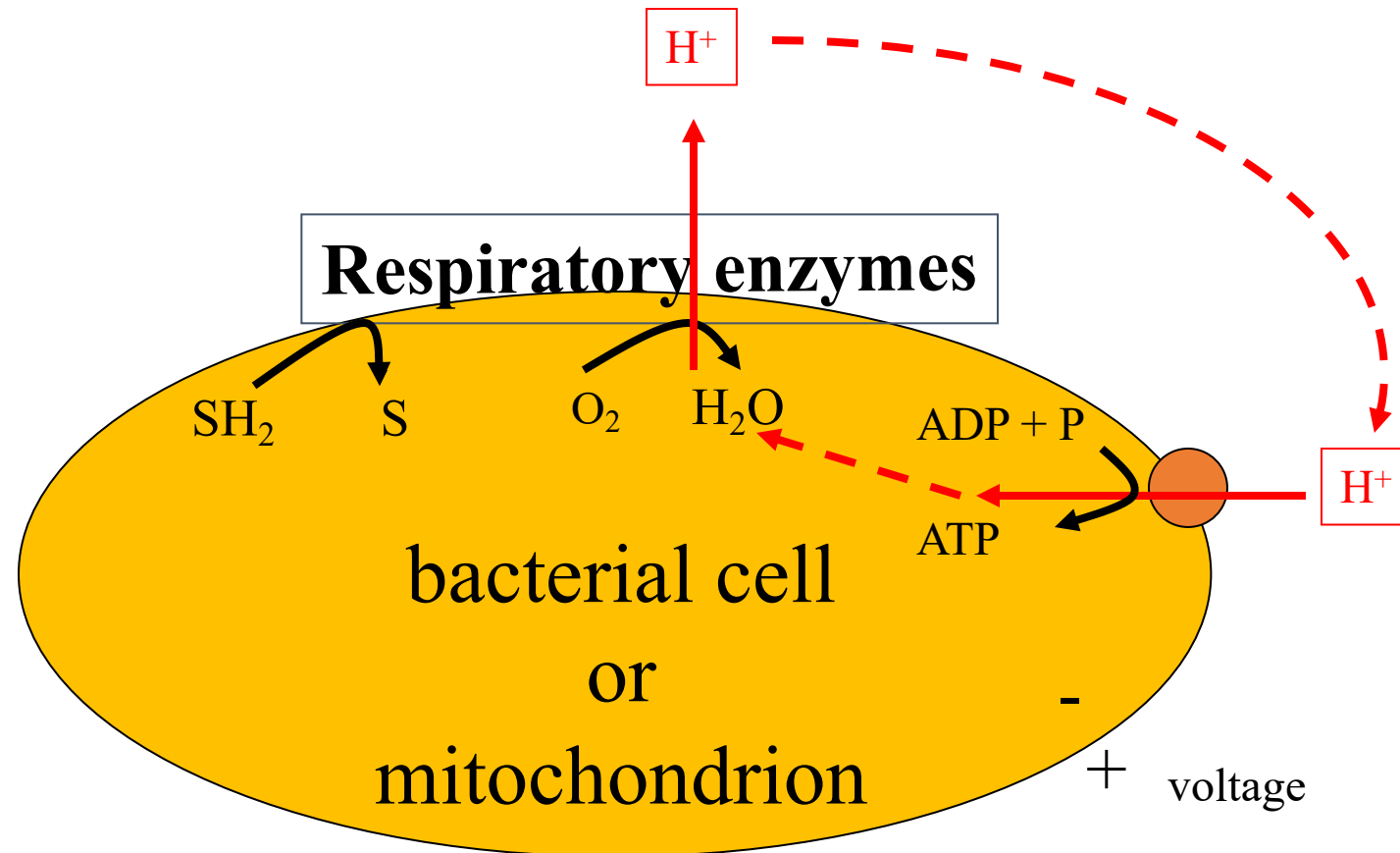
Mol. Dynamics

Chun Kit Chan, **Emad Tajkhorshid**

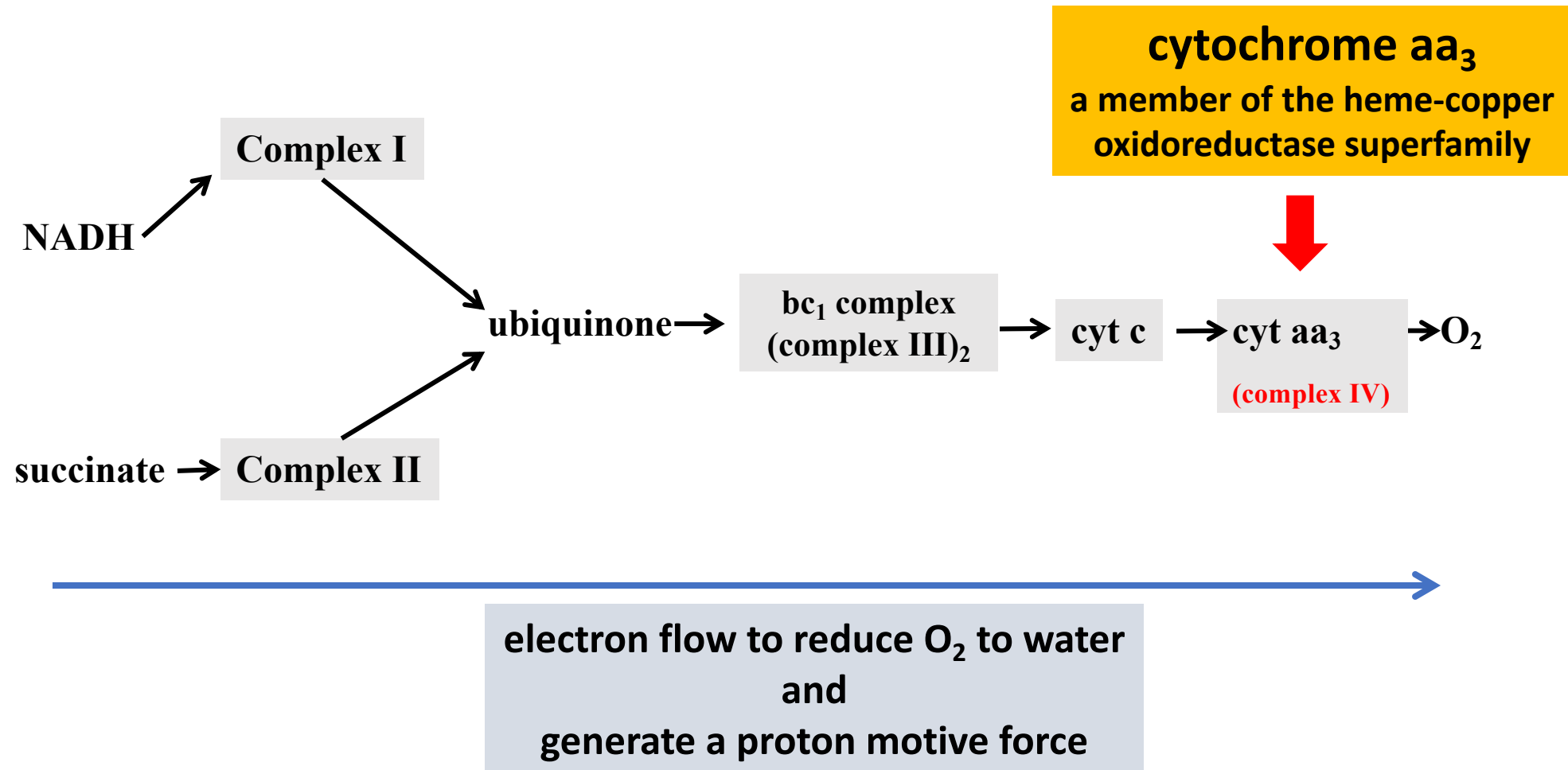
Protein characterization

Sangjin Hong, Ziqiao Ding, Sylvia K. Choi

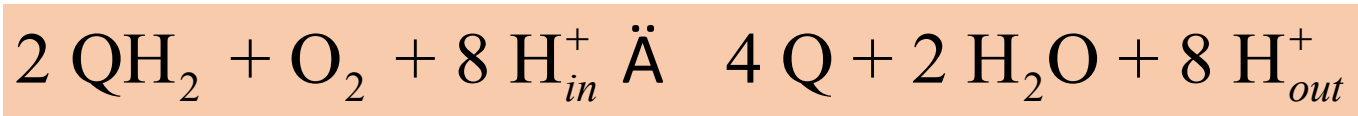
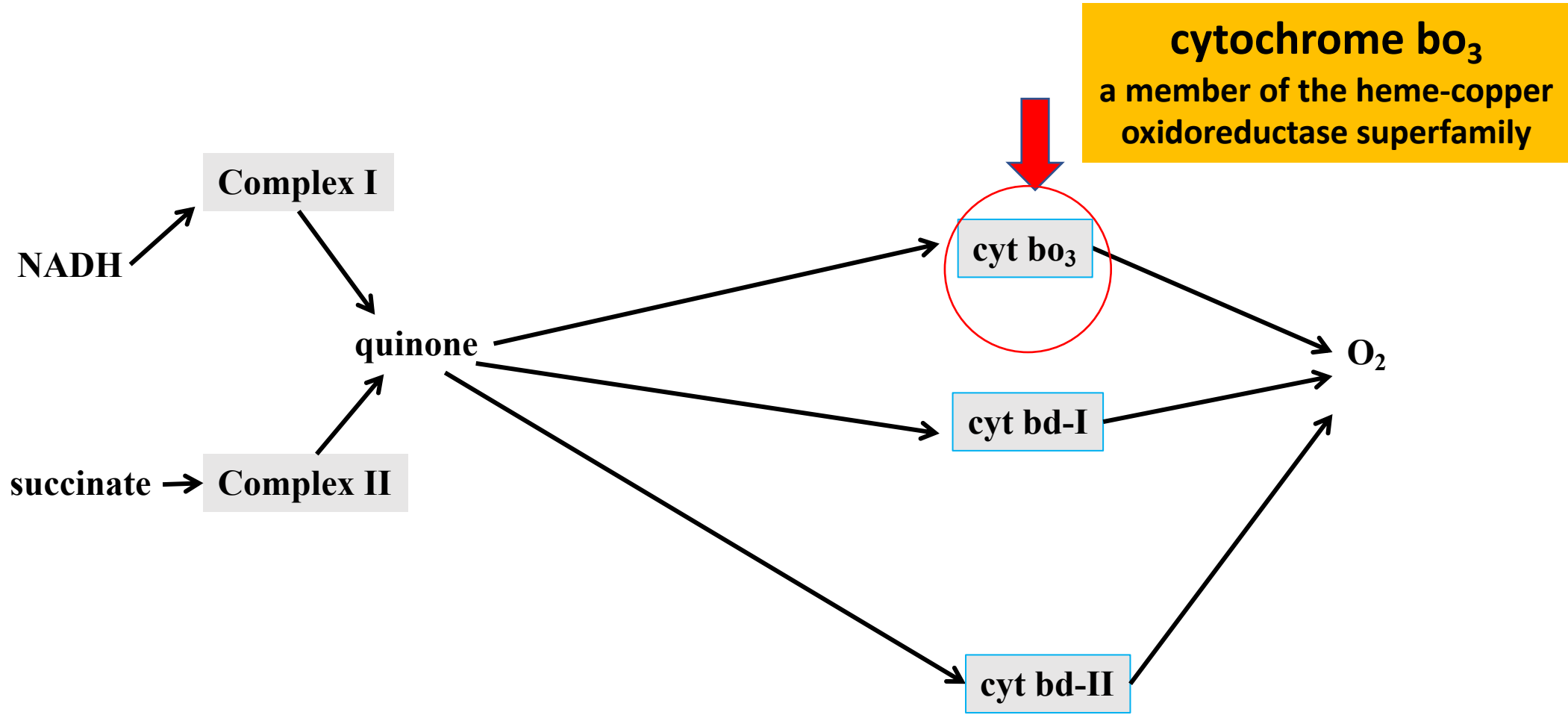
An essential role of the **aerobic respiratory chain** is to generate a proton electrochemical gradient ( $\Delta\Psi + \Delta\text{pH}$ ) across the membrane to drive ATP synthesis and active transport processes



# The mitochondrial respiratory chain has one oxygen reductase

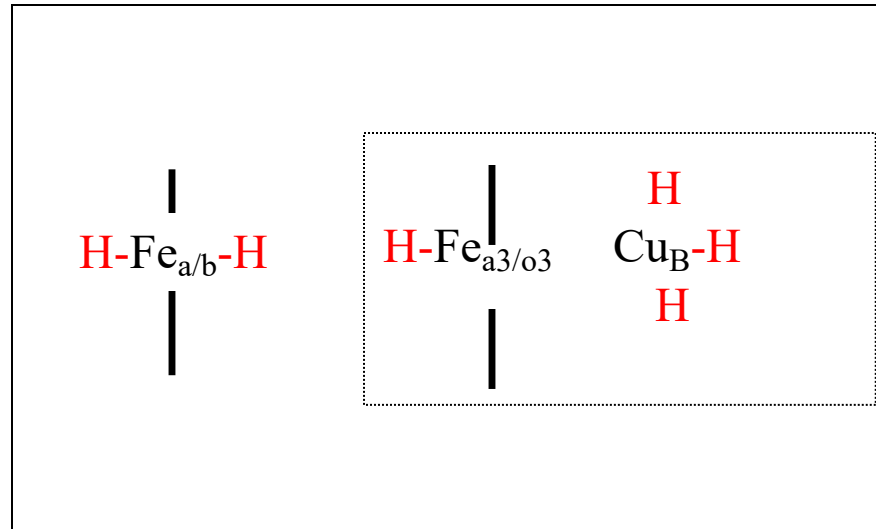


**The *E. coli* respiratory chain has 3 different oxygen reductases and no cytochrome c**

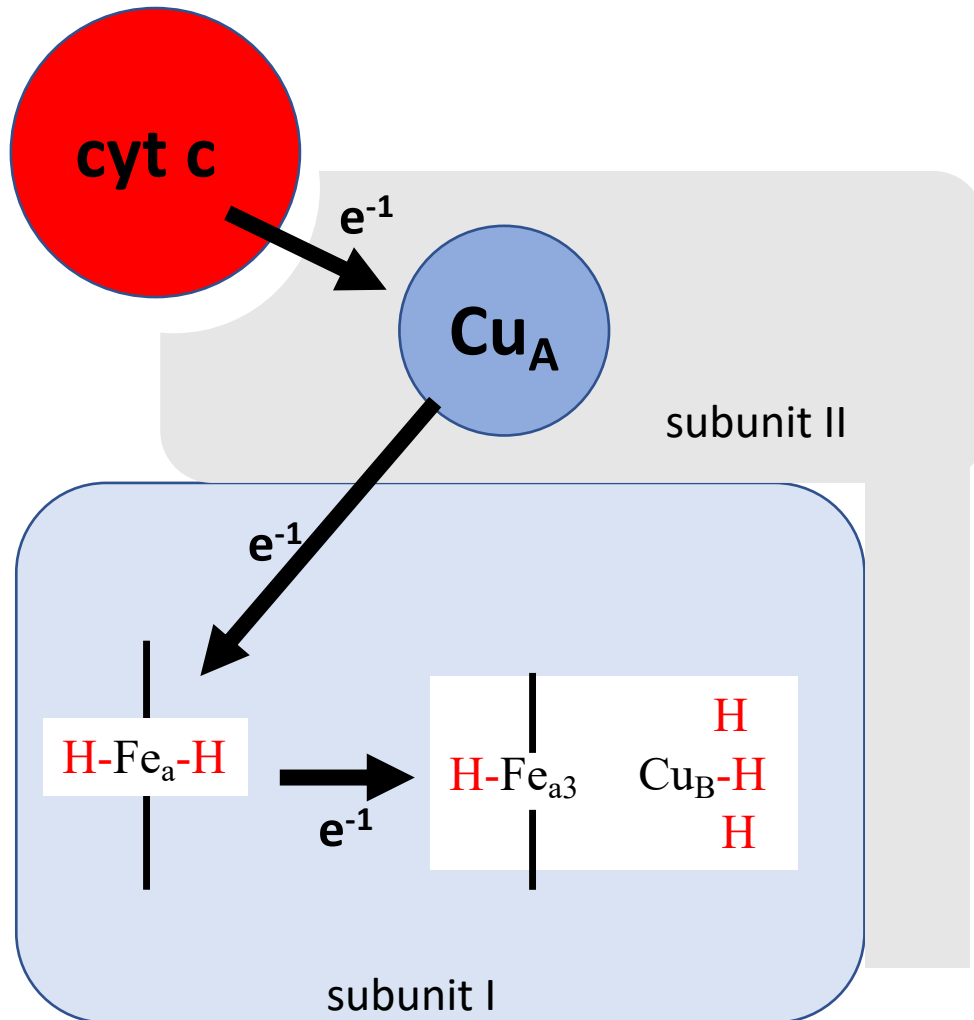


# What is common to all heme-copper oxygen reductases?

1. Homologous subunit I.
2. One low spin heme
3. High spin heme-Cu binuclear site
4. Six histidine ligands for metals

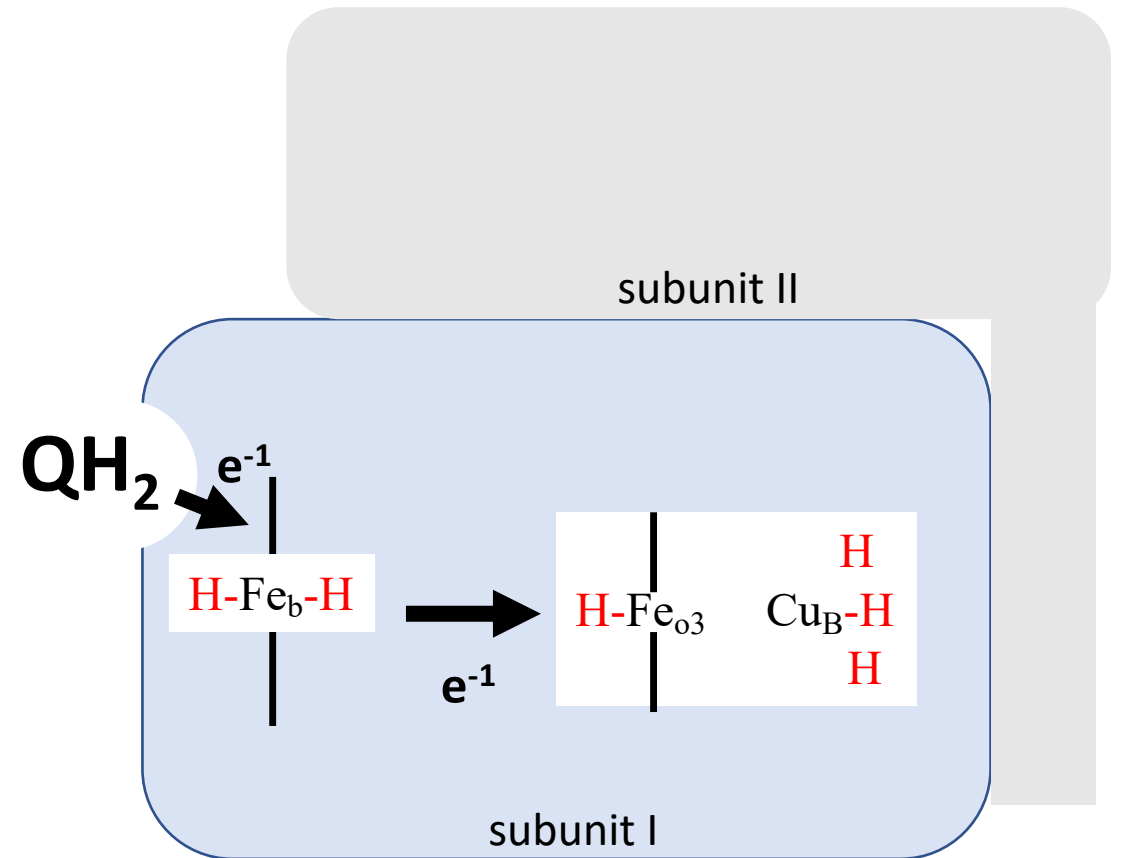


# Cytochrome aa<sub>3</sub>



# Cytochrome bo<sub>3</sub>

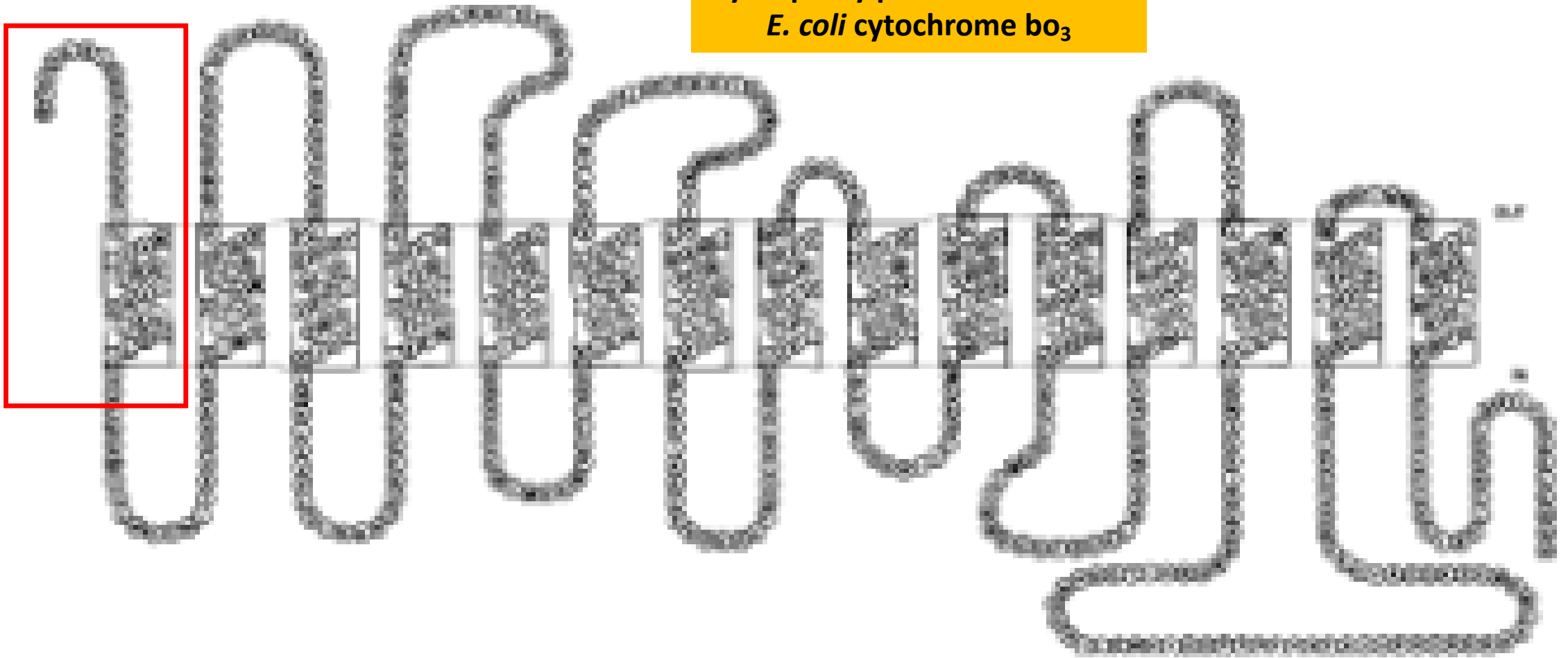
Lost cyt c docking site  
Lost  $Cu_A$  redox center  
Gain Quinol binding site



All quinol oxidases have a unique “extra” transmembrane helix at the N-terminus of subunit I compared to the cytochrome c oxidases.

**TM0**

Hydropathy profile of subunit I  
*E. coli* cytochrome  $bo_3$





## Preparation of cytochrome $bo_3$

Cyt  $bo_3$  has a natural affinity for the “his-tag” Ni-NTA column and has been purified in many laboratories by accident. This was the case in the current work.

1. **Laboratory of Jiapeng Zhu:** solubilized *E. coli* membranes with **1% SMA 3:1 styrene/maleic acid** 3000HNA free sample from Cray Valley/Total Petroleum Chemicals and Refinery

2. **Laboratory of Oliver Clarke:** solubilized *E. coli* membranes with **1% DDM (dodecylmaltoside)**

Reconstituted into Membrane Scaffold Protein (1D1) nanodiscs in POPG

POPG: (C16)(C18:1)-phosphatidylglycerol

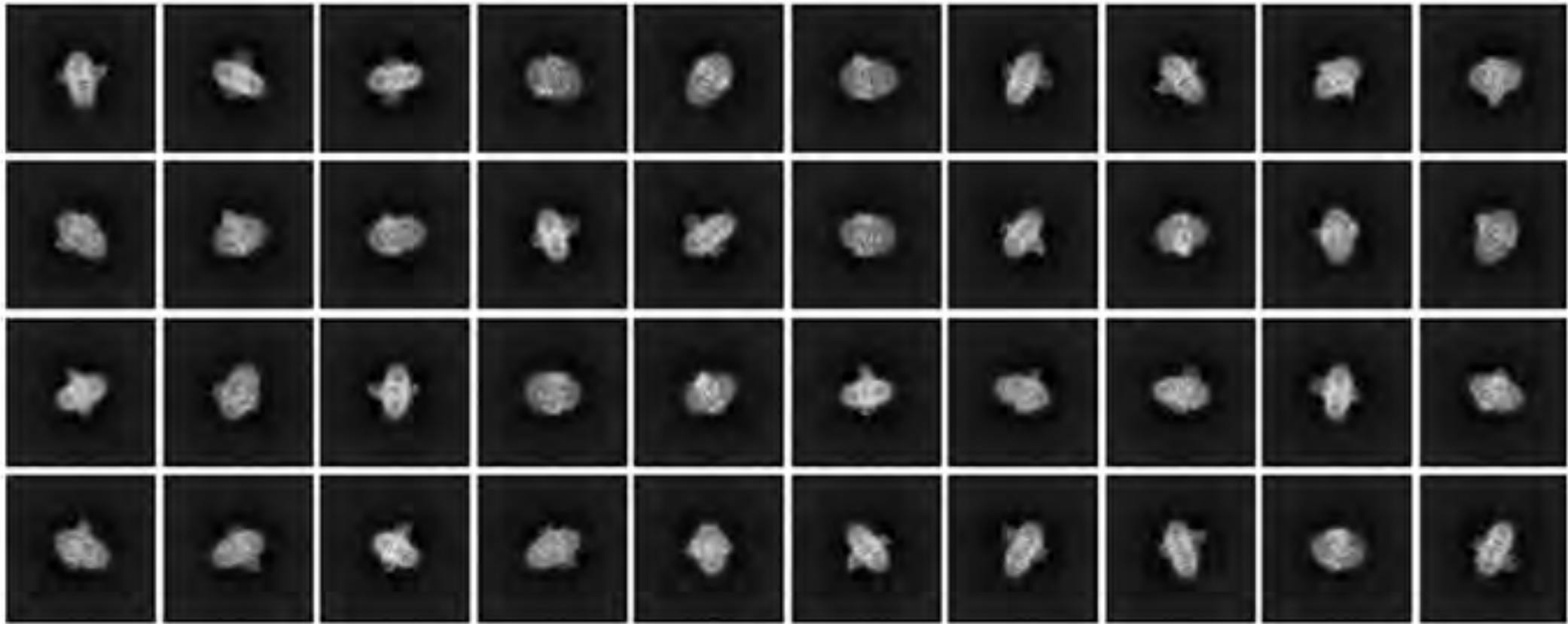
**The two protein structures are virtually identical**

**cyt  $b_0_3$ -SMALP: 2.55 Å**

**cyt  $b_0_3$ -MSP/POPG nanodiscs: 2.19 Å**

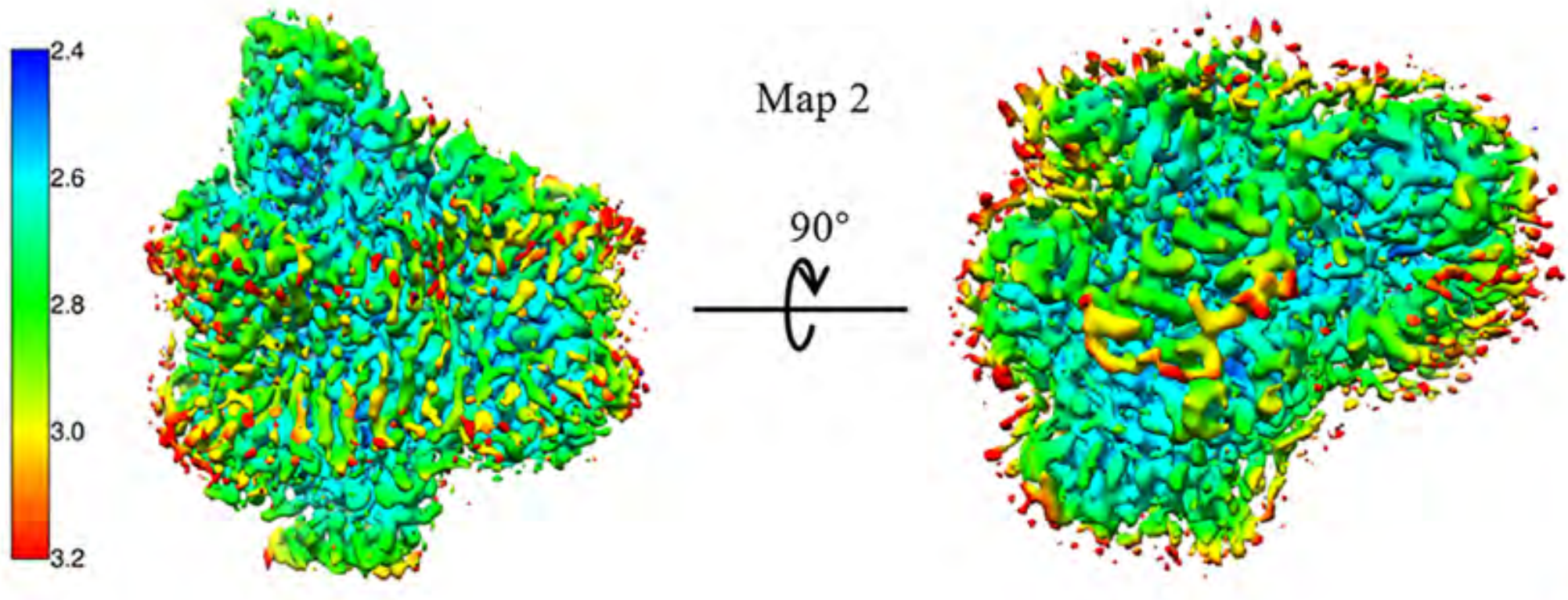
# cryoEM of cyt $b_3$ -SMALP

563,000 particles were used to obtain the class averages shown below



# Local resolution map of cyt $b_0_3$ -SMALP

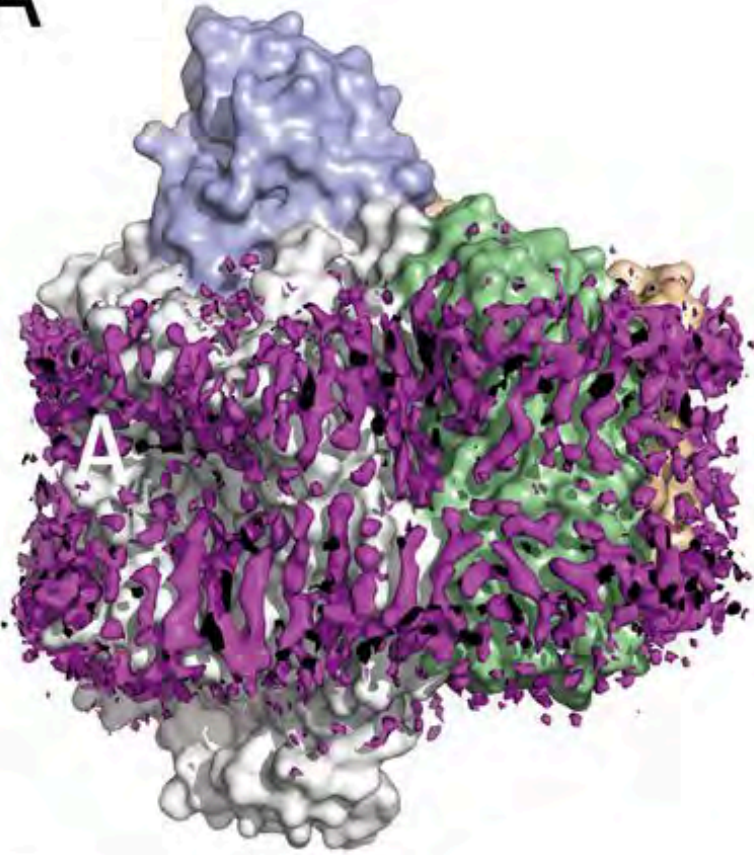
average resolution 2.55 Å



# cyt $b_0_3$ -SMALP

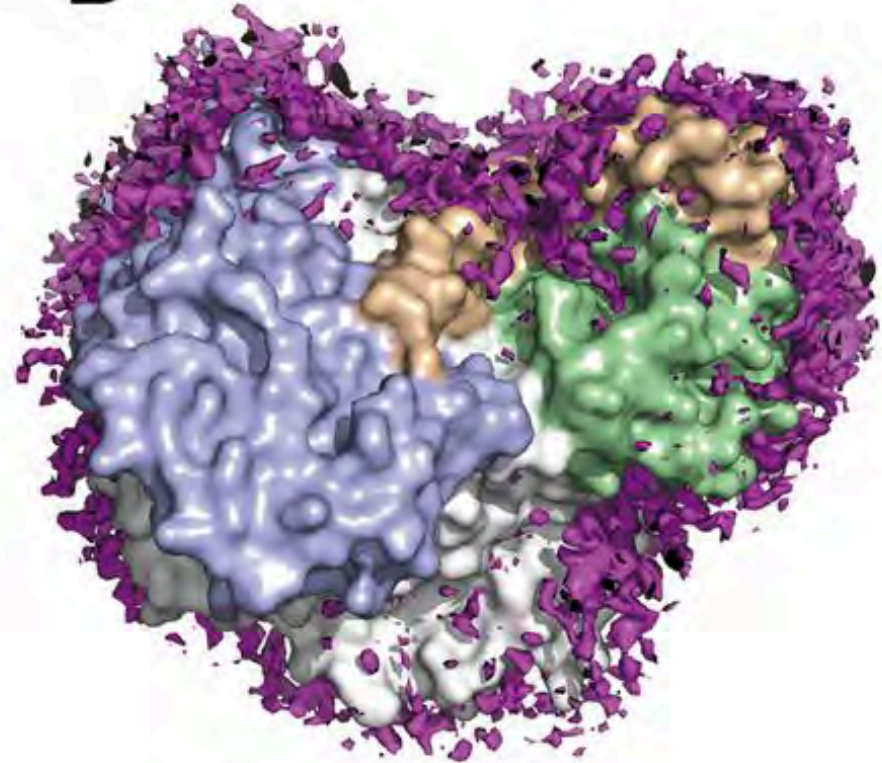
Four subunits surrounded by native phospholipids

A



90°  
↻

B



# The four subunits of cyt $b_0_3$



**Subunit I**

**15 TM helices**



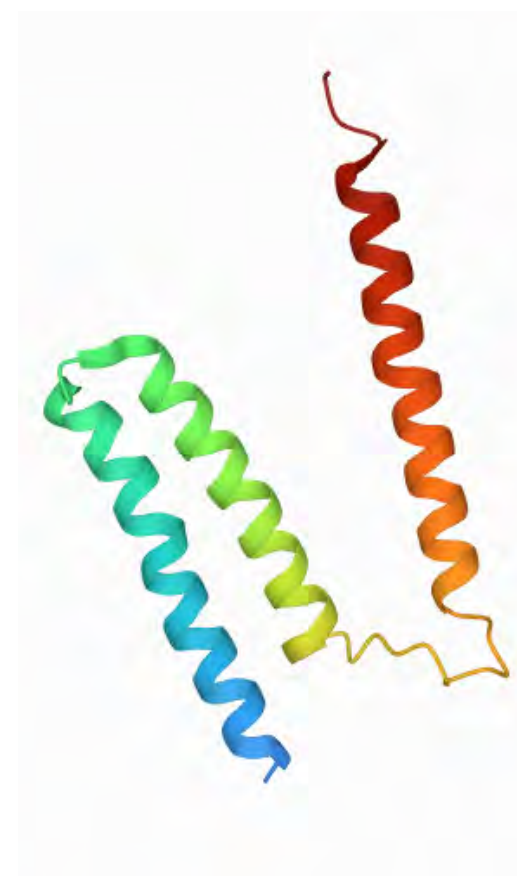
**Subunit II**

**2 TM helices**



**Subunit III**

**5 TM helices**



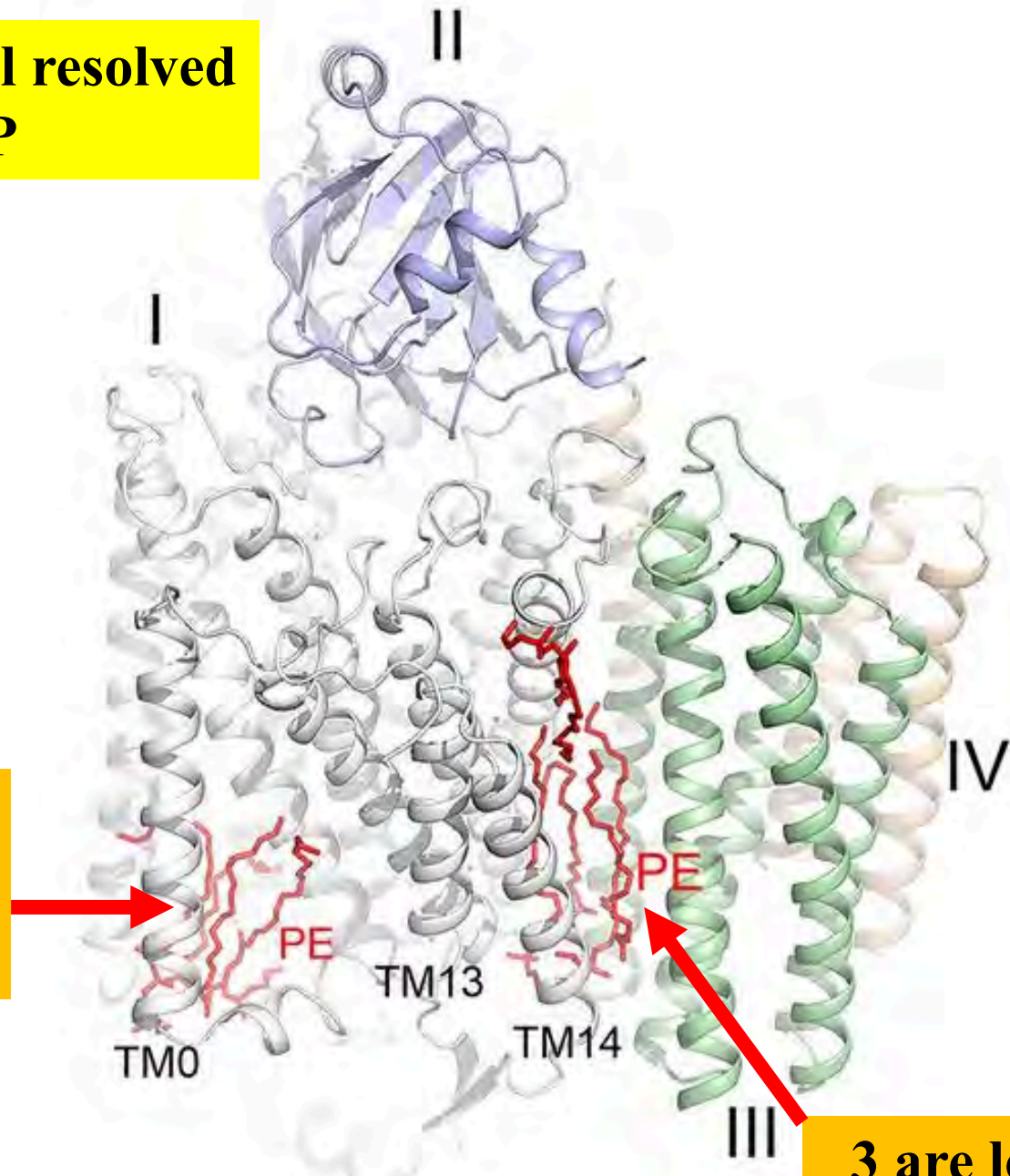
**Subunit IV**

**3 TM helices**

**5 native phospholipids are well resolved  
in the cyt  $b_0_3$ -SMALP**

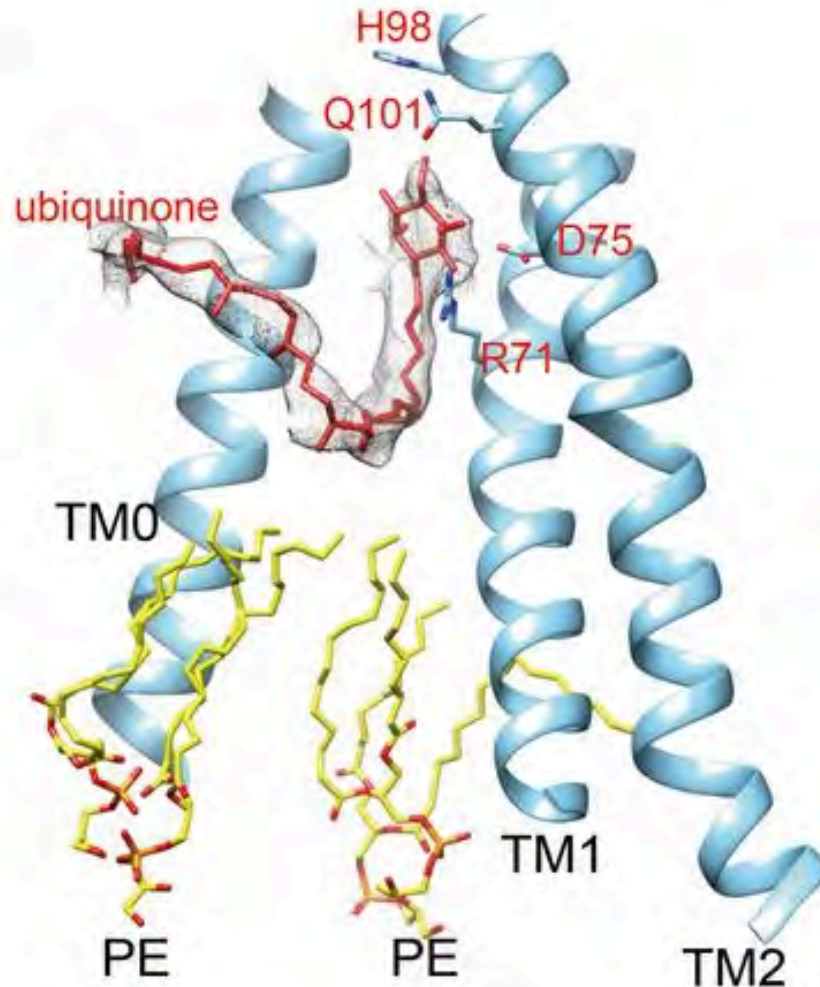
**all are di-stearoyl-PE**

**2 are located in a groove  
in subunit I  
between TM0, TM1 and TM2**



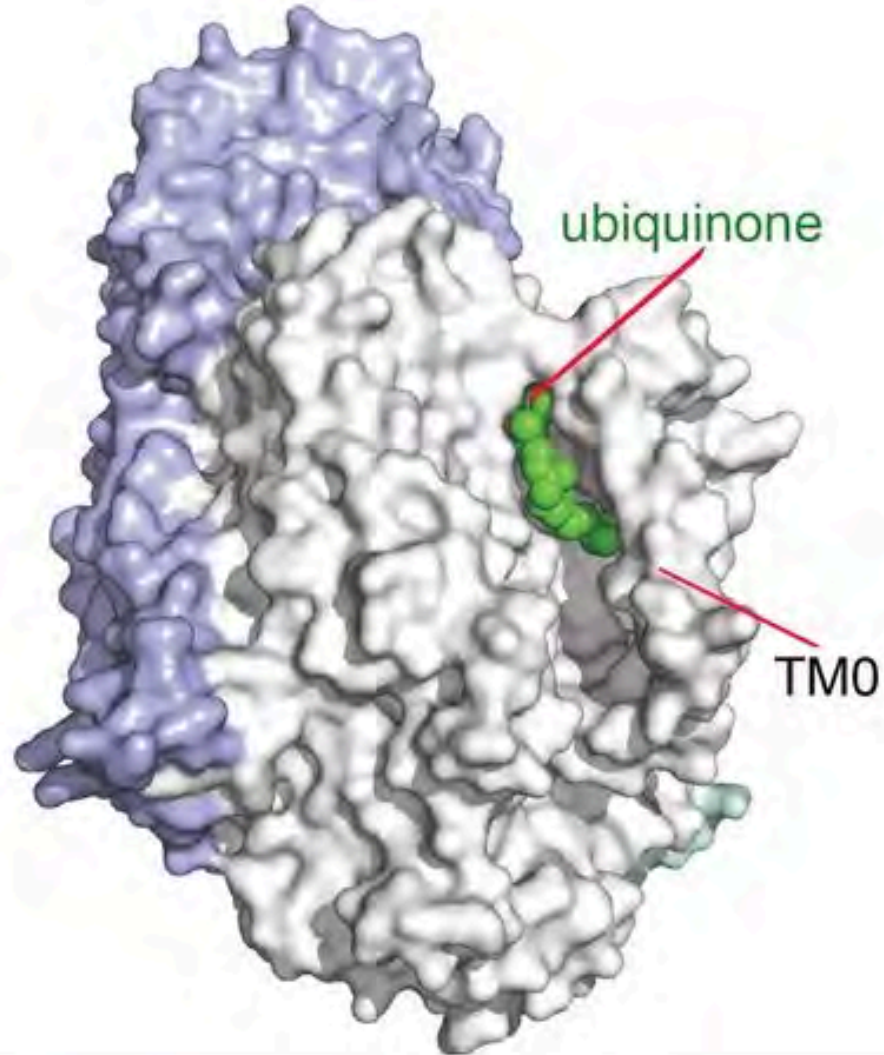
**3 are located in a crevice  
between subunits I and III**

**Cyt  $bo_3$ -SMALP has one ubiquinone-8 molecule sandwiched between TM0, TM1 and TM2 in subunit I**

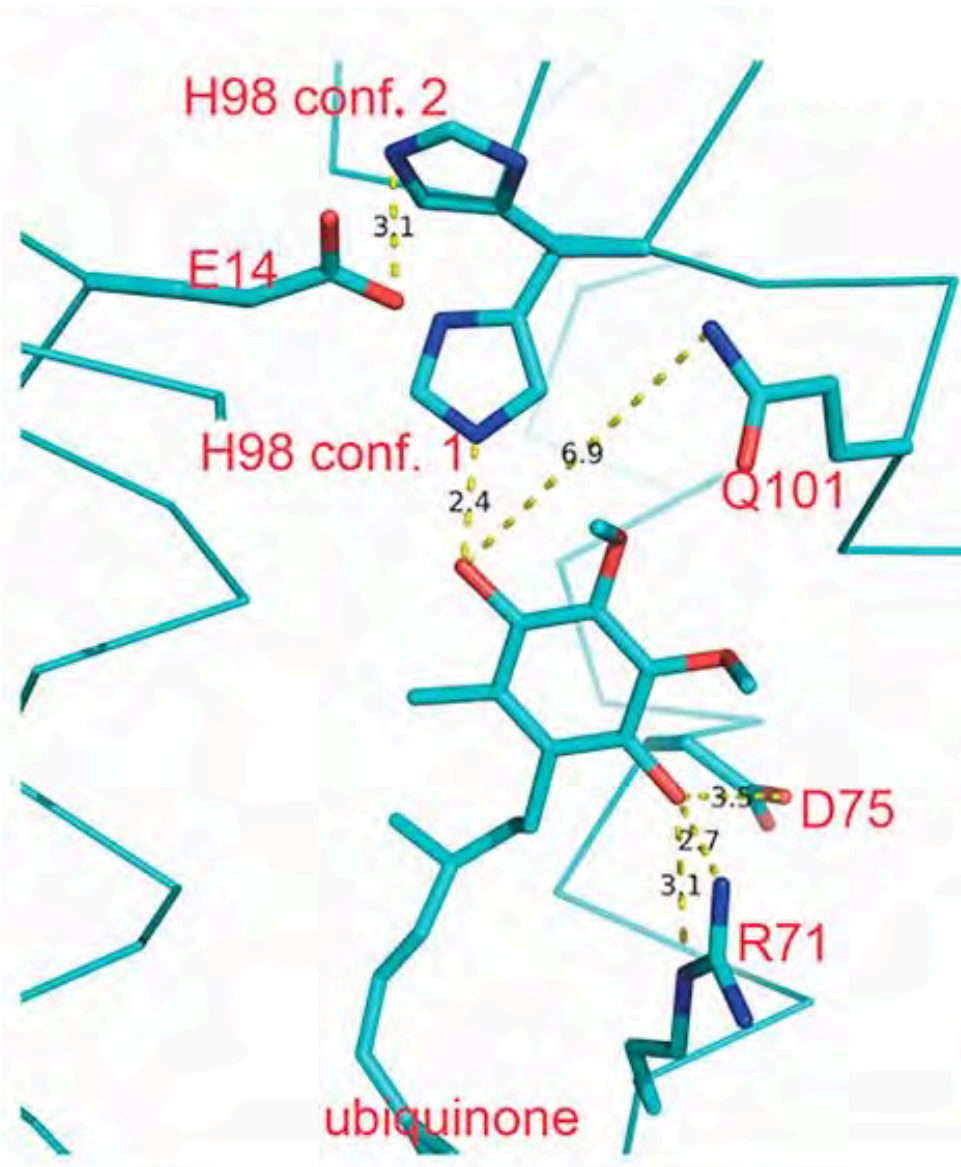




**Surface representation of cyt  $b_0_3$ -SMALP shows the hydrophobic tail of ubiquinone-8 held against TM1 and TM2 by the “extra” TM0**

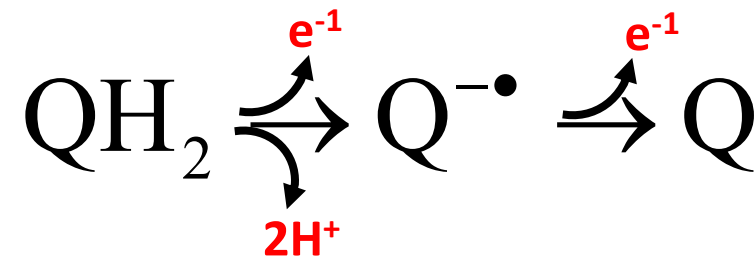


# Ubiquinone-8 headgroup is hydrogen bonded to Asp75, Arg71 and His98



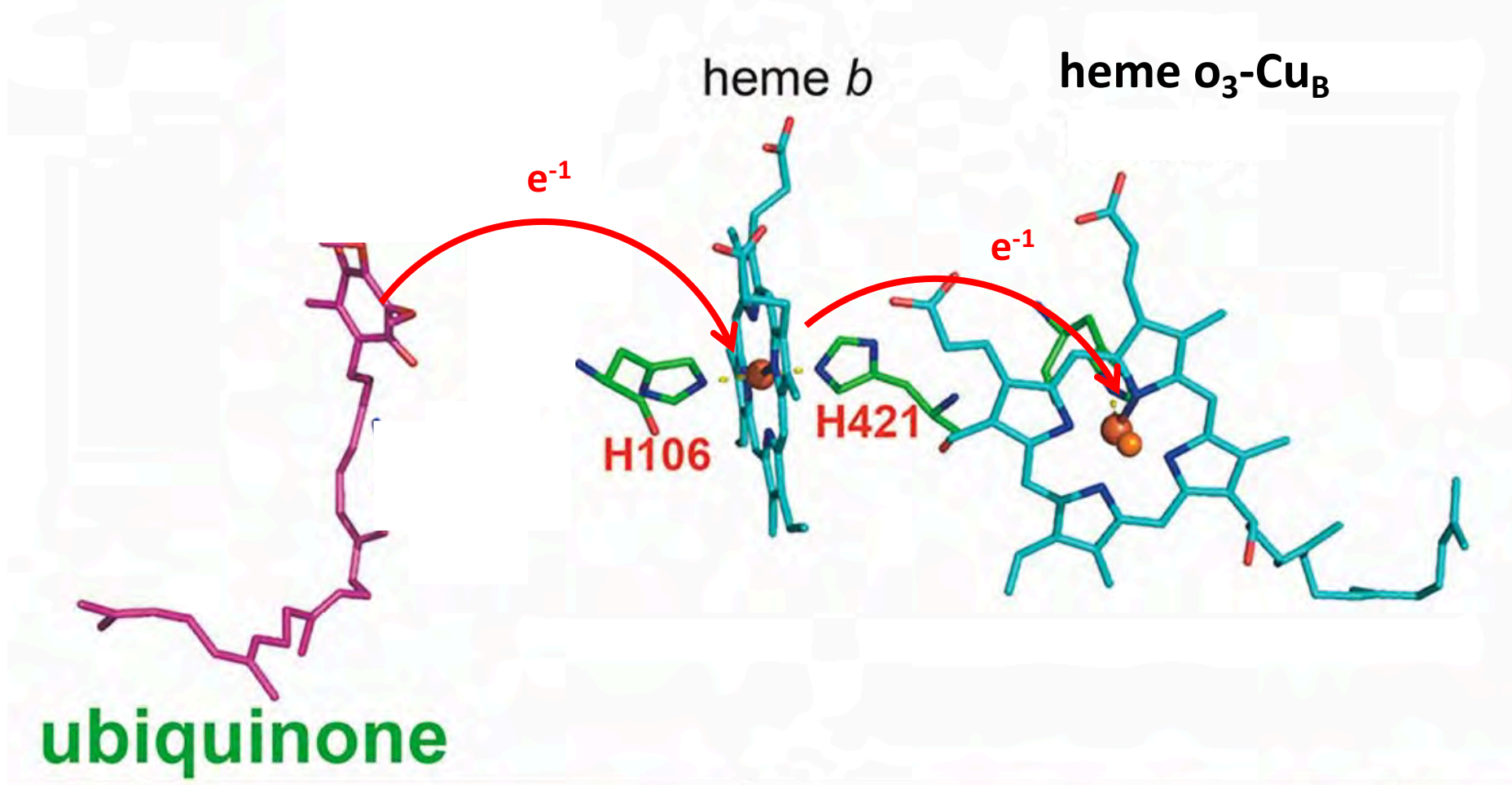
His98 has two conformations  
conf. 1: hydrogen bonded to ubiquinone  
conf. 2: hydrogen bonded to Glu14

Likely functional role of His98 is to help shuttle protons from quinol to the periplasm upon oxidation

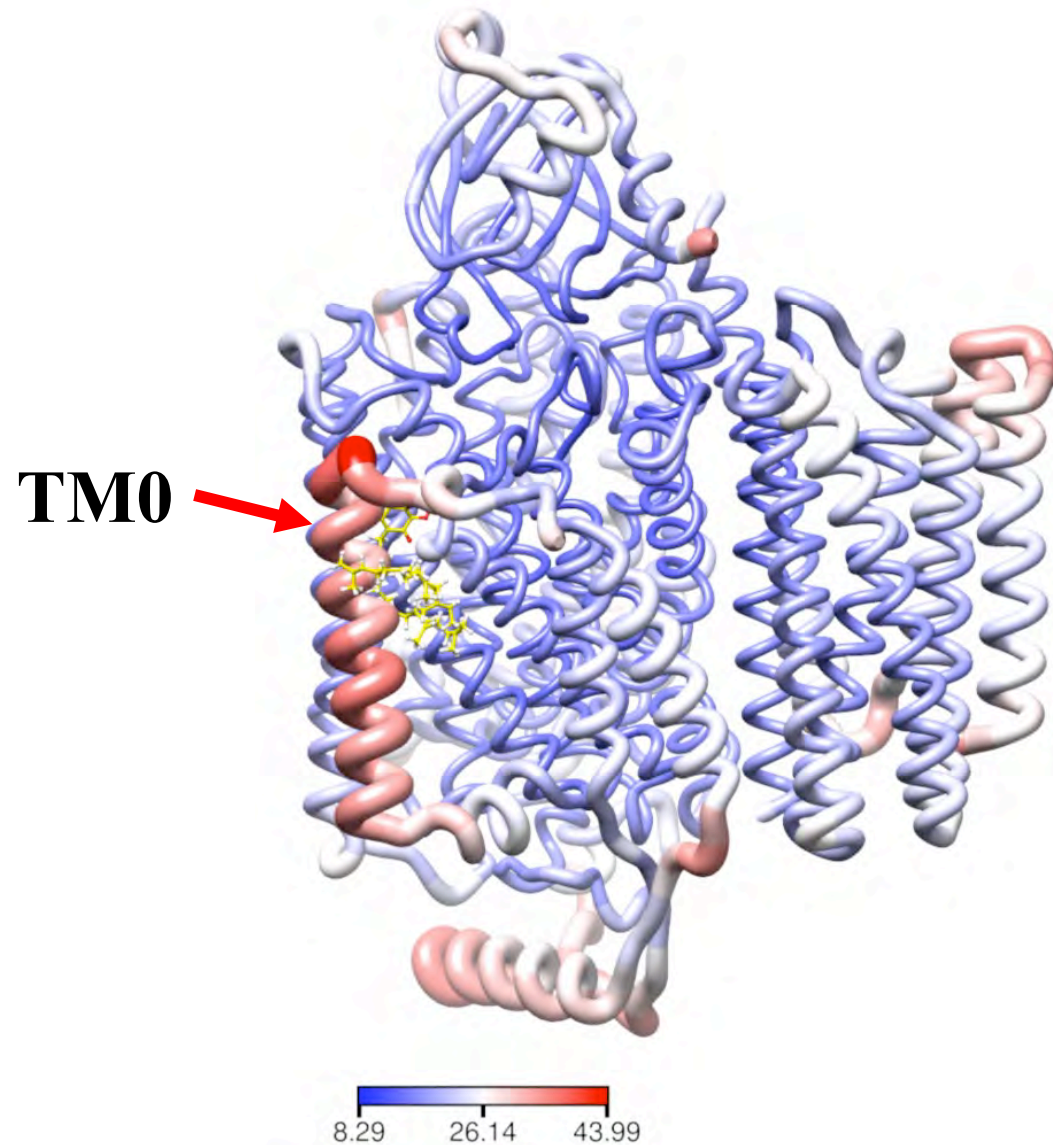


Headgroup of ubiquinone-8 is 14 Å from heme b

Two 1-electron transfers: ubiquinol-8 → heme b → heme o<sub>3</sub>-Cu<sub>B</sub> (O<sub>2</sub>)



**B-factor distribution of cyt  $b_0_3$  in the MSP nanodiscs indicates TM0 is dynamic**



**Consistent with the need for turnover of ubiquinol-8 every few milliseconds.**

**Thanks**

**Questions**