Structure, mechanism, and inhibition of the membrane motor of the ATP synthase inferred from quantitative integrative modeling

Vanessa Leone
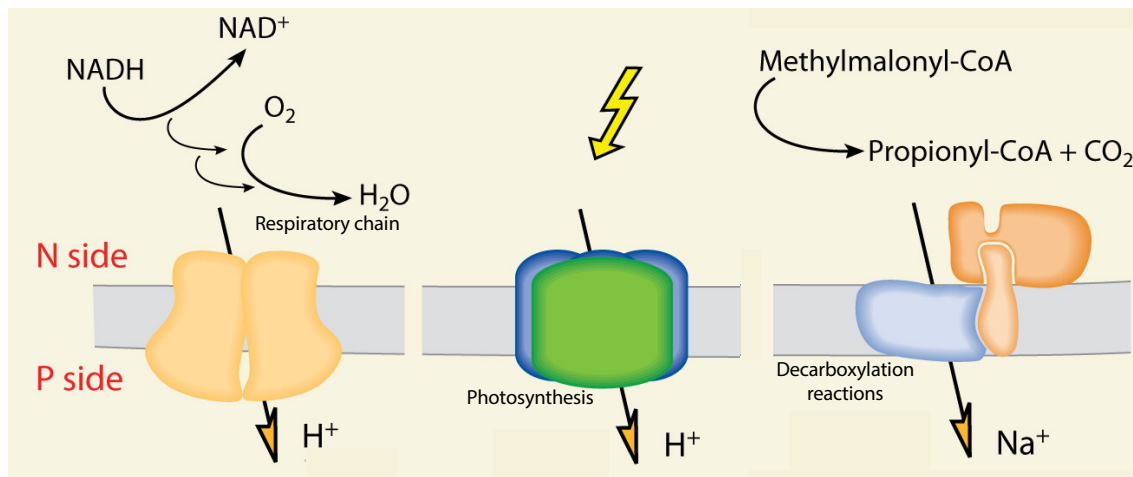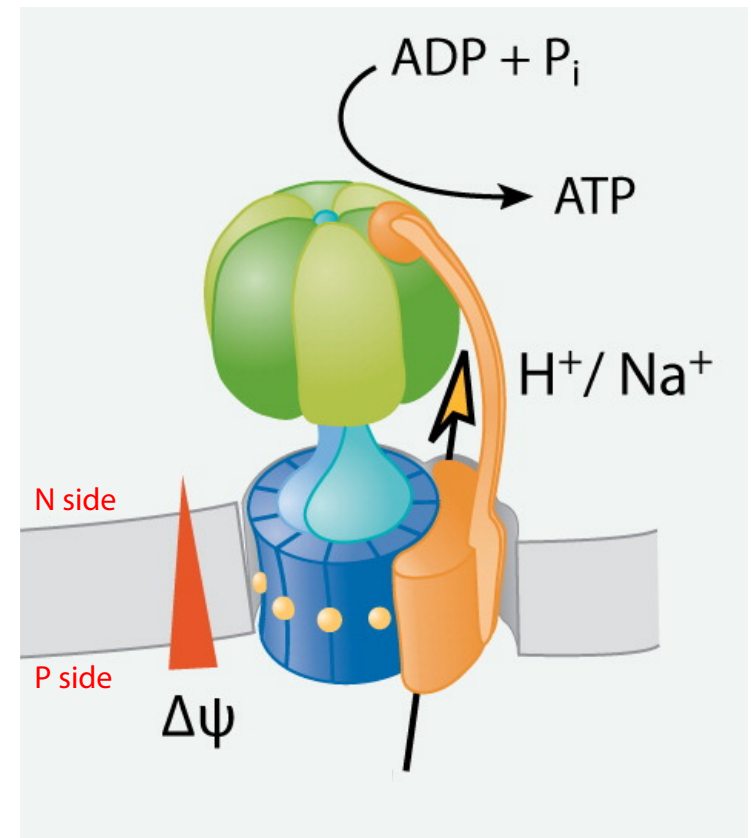
ATP synthases uses the electrochemical gradient to catalyze the production of ATP

During cell metabolism proton or sodium ions are exported across membranes, establishing an electrochemical potential gradient


NAD$^+$

NADH

O$_2$

H$_2$O

Respiratory chain

N side

P side

H$^+$

Photosynthesis

H$^+$

Methylmalonyl-CoA

Propionyl-CoA + CO$_2$

Decarboxylation reactions

Na$^+$

From von Ballmoos *et al.*, Annu Rev Biochem. '09

ADP + P$_i$

ATP

H$^+$/ Na$^+$

N side

P side

Δψ

How the ions translocation power the ATP production?

Architecture of the ATP synthase

During cell metabolism proton or sodium ions are exported across membranes, establishing an electrochemical potential gradient
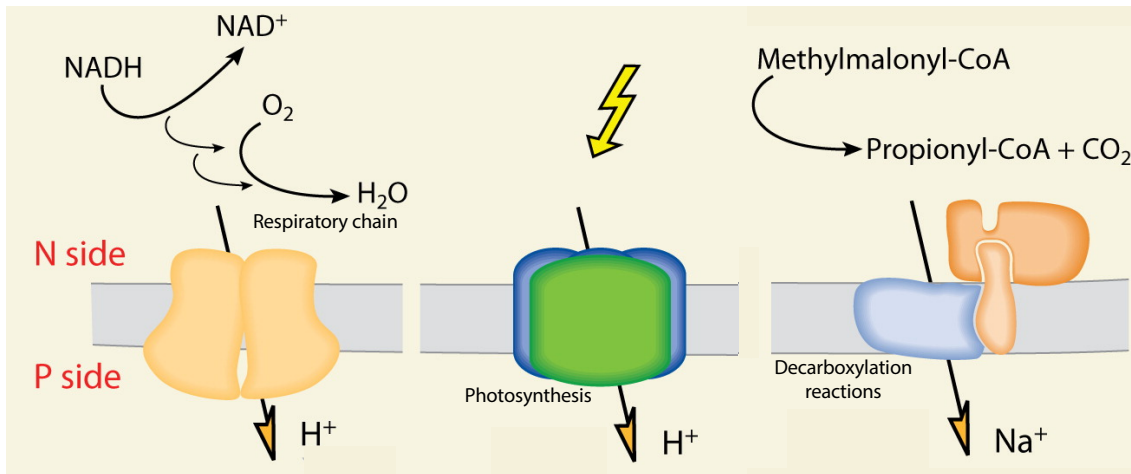
From von Ballmoos *et al.*, Annu Rev Biochem. '09

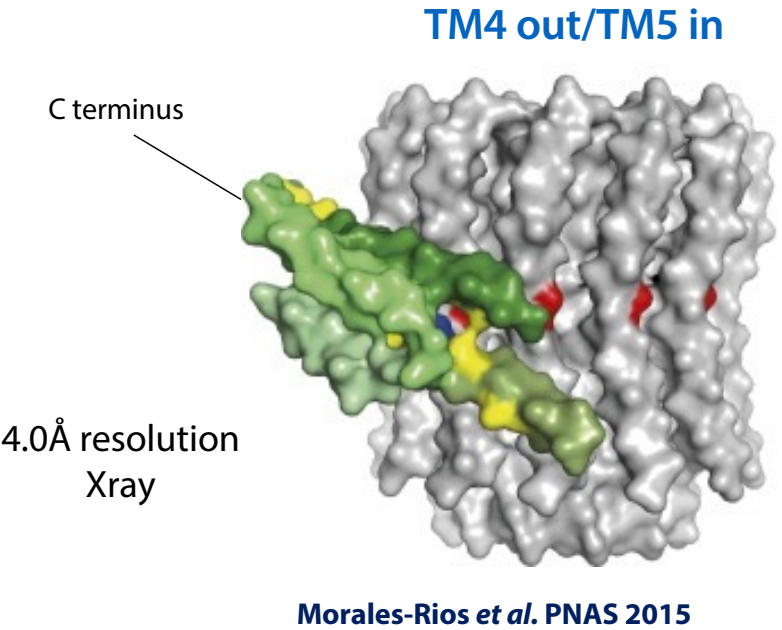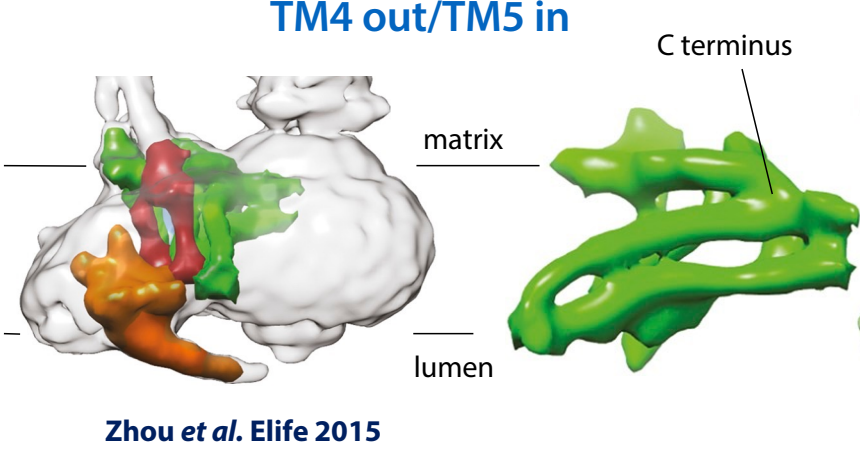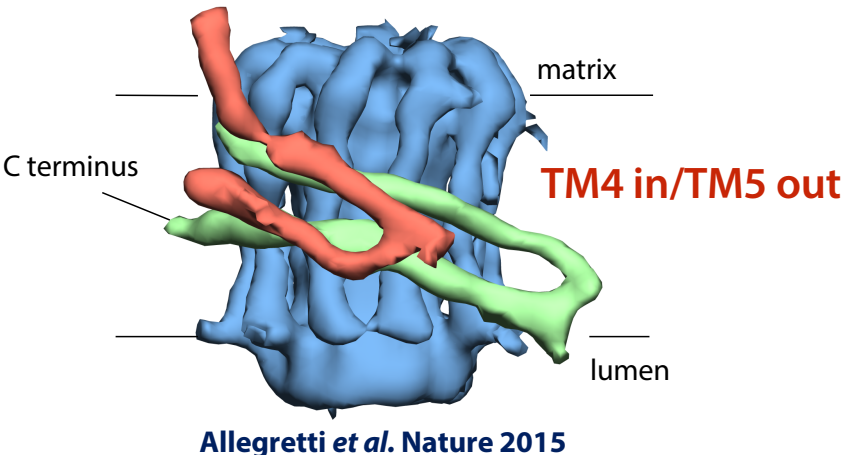Adapted from Meier et al. *Molecular Machines in Biology*. Cambridge University Press '11

Ion translocation and ATP production occur in two distinct domains
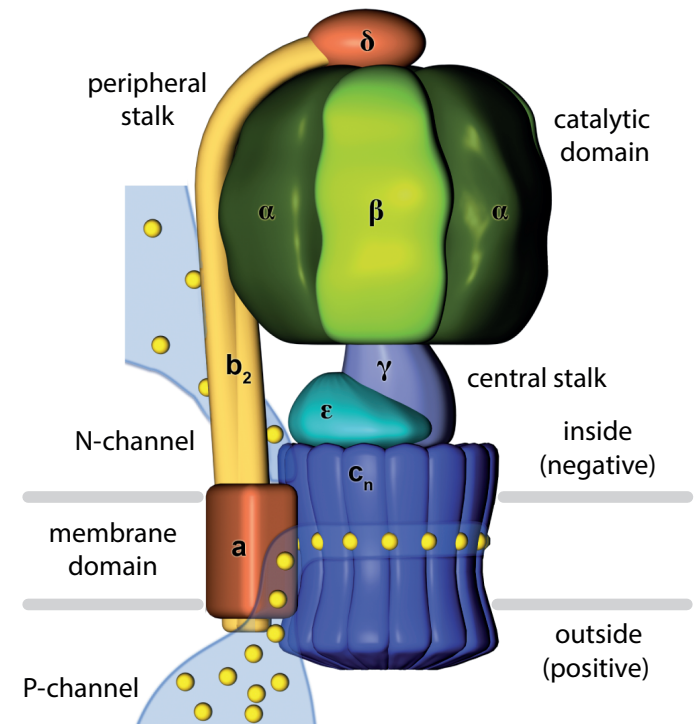
Ion is transported across c-ring and subunit-a interface
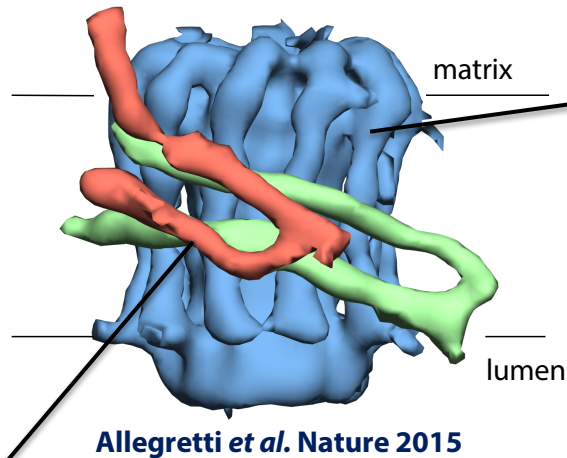
No atomistic detail of complex or isolated subunit-a

CryoEM maps and low resolution X-ray structure show a horizontal arrangement of subunit-a helices, but different topologies are proposed

TM4 in/TM5 out

C terminus

matrix

lumen

Allegretti *et al*. Nature 2015

TM4 out/TM5 in

C terminus

matrix

lumen

Zhou *et al*. Elife 2015

TM4 out/TM5 in

C terminus
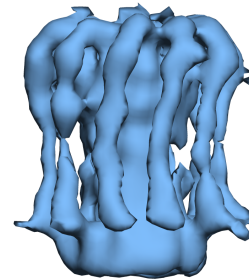
4.0Å resolution Xray

Morales-Rios *et al*. PNAS 2015

Goal: provide a quantitate interpretation of the low resolution (~7 Å) cryoEM structural data

Model c-ring/subunit-a complex of *Polytomella*

Allegretti *et al.* Nature 2015

matrix

lumen

1. Model the c₁₀-ring

- **8.0Å resolution** cryoEM map of *Polytomella* c-ring

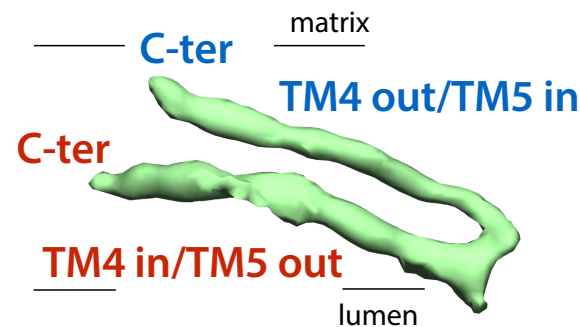- **2.0Å resolution** Xray of **yeast** c-ring

Symersky *et al.* NSMB 2012

Homology modelling & fitting this model into cryoEM map
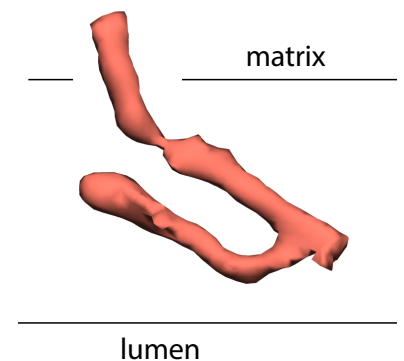
2. Model subunit-a

- **6.5Å resolution** cryoEM map of *Polytomella* c-ring
- **Cys crosslinks** and **residue accessibility**

- No atomistic structural information: **ab-initio approach?**

- **Hand-traced approach? How much it depend on the user?**

- **Co-variant** residue pairs & **Cys crosslink data** to **sort** among **models** fitted into **cryoEM map**

- **Uncertainty** on subunit-a **C-terminal position** and **topology**
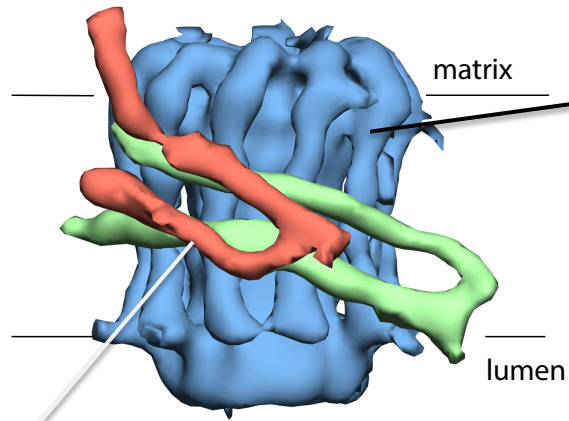
2a. Model TM4-TM5 hairpin (C-terminus)

matrix

C-ter

TM4 out/TM5 in

C-ter

TM4 in/TM5 out

lumen

- **Model both C-ter possibilites** & sort them by co-variant pairs and Cys Xlinks

2b. Model TM2-TM3 hairpin
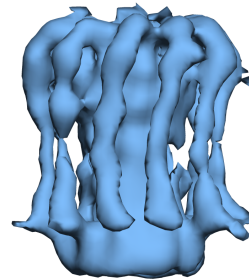

matrix

lumen

- **Define** TM bundle **topology** using **co-variant** residue pairs

Model c-ring/subunit-a complex of *Polytomella*

Allegretti *et al.* Nature 2015

matrix

lumen

1. Model the c₁₀-ring

- **8.0Å resolution** cryoEM map of *Polytomella* c-ring

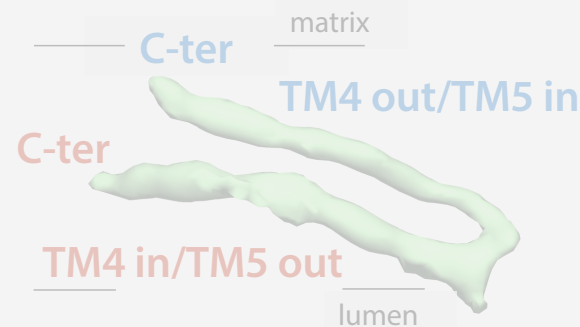- **2.0Å resolution** Xray of **yeast** c-ring

Symersky *et al.* NSMB 2012

Homology modelling & fitting this model into cryoEM map
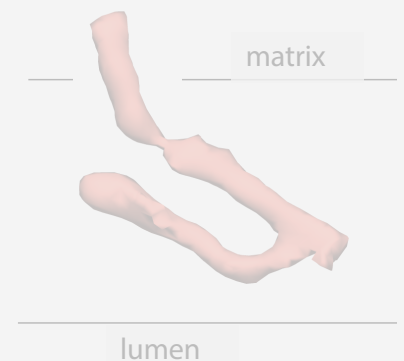
2. Model subunit-a

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- **Hand-traced approach? How much it depend on the user?**

- **Co-variant** residue pairs & **Cys crosslink data** to **sort** among **models** fitted into **cryoEM map**

- **Uncertainty** on subunit-a **C-terminal position** and **topology**
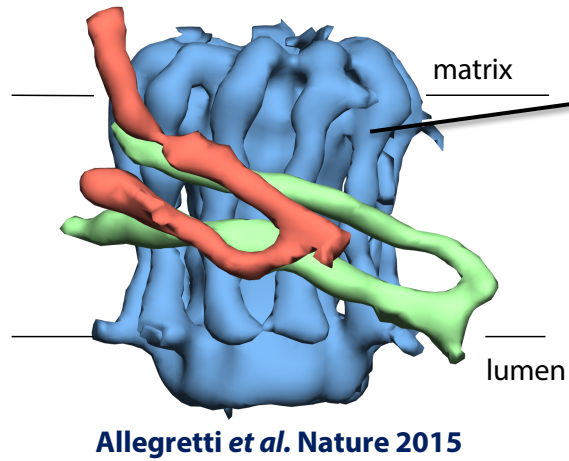
2a. Model TM4-TM5 hairpin (C-terminus)

C-ter

matrix

TM4 out/TM5 in

C-ter

TM4 in/TM5 out

lumen

- **Model both C-ter possibilites** & sort them by co-variant pairs and Cys Xlinks

2b. Model TM2-TM3 hairpin

matrix

lumen
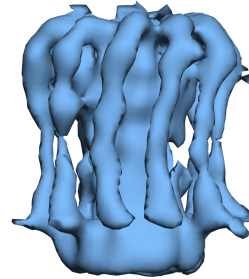
- **Define** TM bundle **topology** using **co-variant** residue pairs

Model c-ring/subunit-a complex of *Polytomella*

Allegretti *et al.* Nature 2015

matrix

lumen

1. Model the c$_{10}$-ring

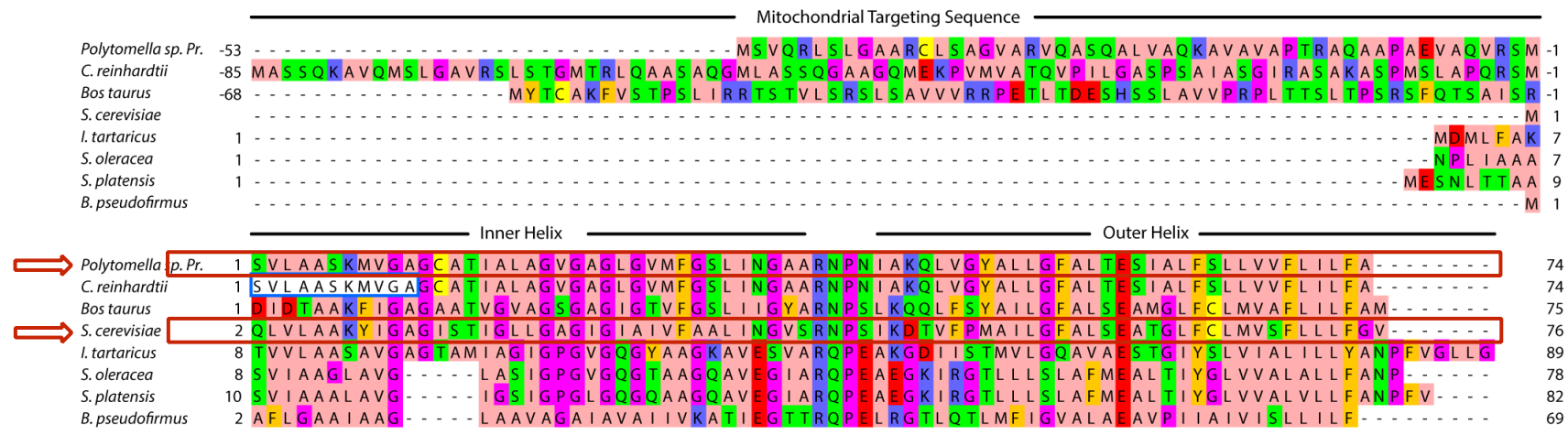- **8.0Å resolution** cryoEM map of *Polytomella* c-ring

- **2.0Å resolution** Xray of **yeast** c-ring

Symersky *et al.* NSMB 2012

Homology modelling & fitting **this model into cryoEM map**

Homology model c-ring of *Polytomella* based on yeast ring
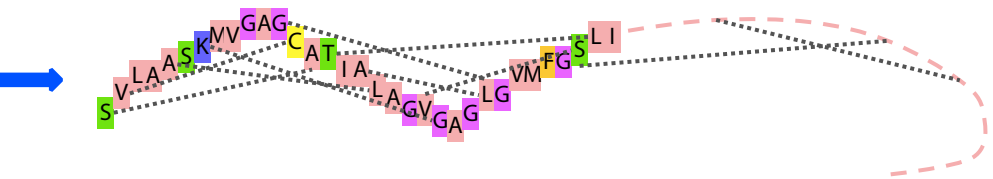

Alignment between *Polytomella* and yeast c-subunit (HHBLITS)

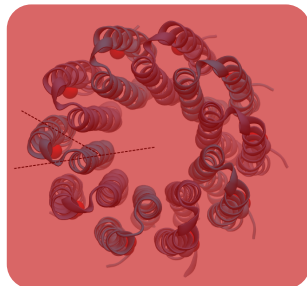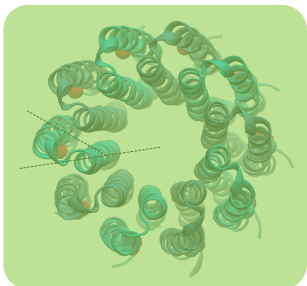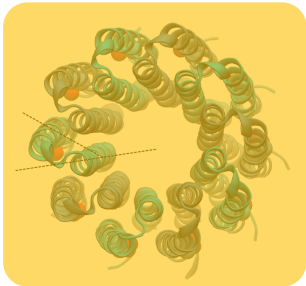**Extract spatial restrains** and transfer them to *Polytomella* c-ring

Cα-Cα distances; hydrogen bonds; main chain & side chain dihedrals

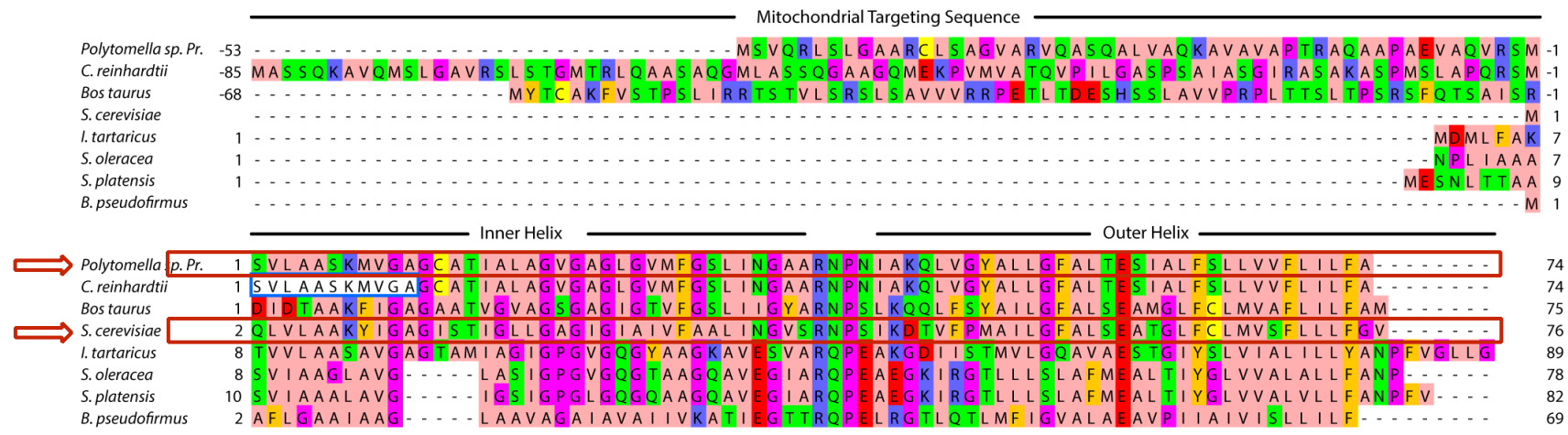**2.0Å resolution** Xray of **yeast** c-ring; 47% %identity with *Polytomella* c-subunit

Models of *Polytomella* c-ring **that satisfy all the restraints as well as possible**

2000 models

Homology model c-ring of *Polytomella* based on yeast ring

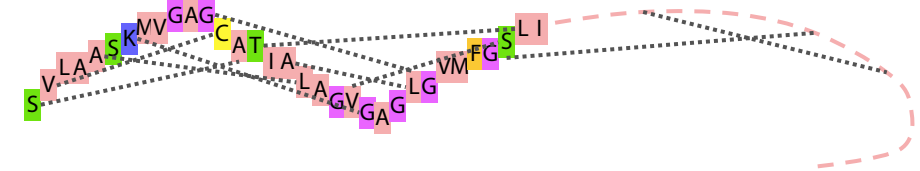# Model c-ring/subunit-a complex of *Polytomella*

Allegretti *et al.* Nature 2015

matrix

lumen
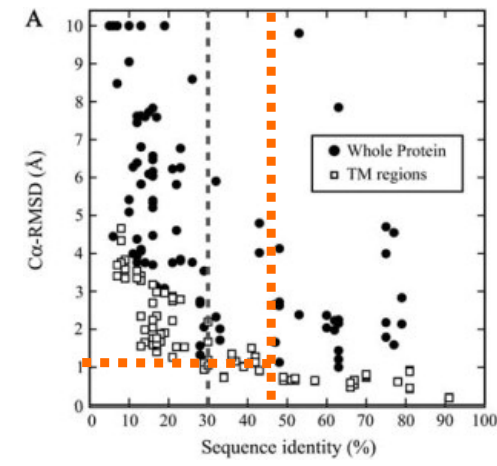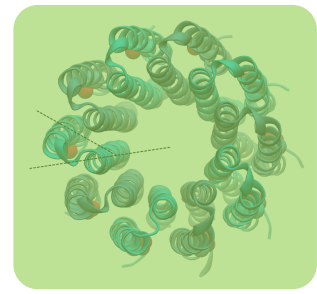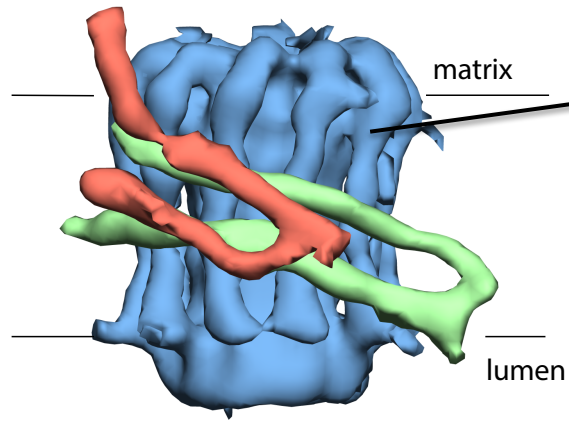
1. Model the c₁₀-ring

- **8.0Å resolution** cryoEM map of *Polytomella* c-ring

- **2.0Å resolution** Xray of **yeast** c-ring

Symersky *et al.* NSMB 2012

Homology modelling & fitting this model into cryoEM map

Refinement of the c-ring homology model into the cryoEM map

Compare computed map of refined models from homology model **against experimental map**

Selected homology model

Cα-restrained 10-fold symmetry imposed

Exp. cryoEM map

1200 models

Select model with lower Rosetta membrane score & fit-to-density score

selected model

Rosetta score (a.u.)

Fit-to-density score (a.u.)

RMSD (Å)

a

N-side (in)

Lipid membrane

P-side (out)

New model has improved geometrical parameters (i.e. less clashes, bad wrong length)
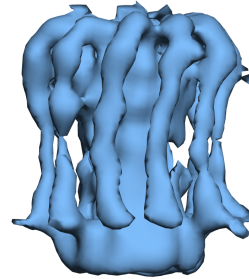
Model c-ring/subunit-a complex of *Polytomella*

Allegretti *et al.* Nature 2015

matrix

lumen

1. Model the c₁₀-ring
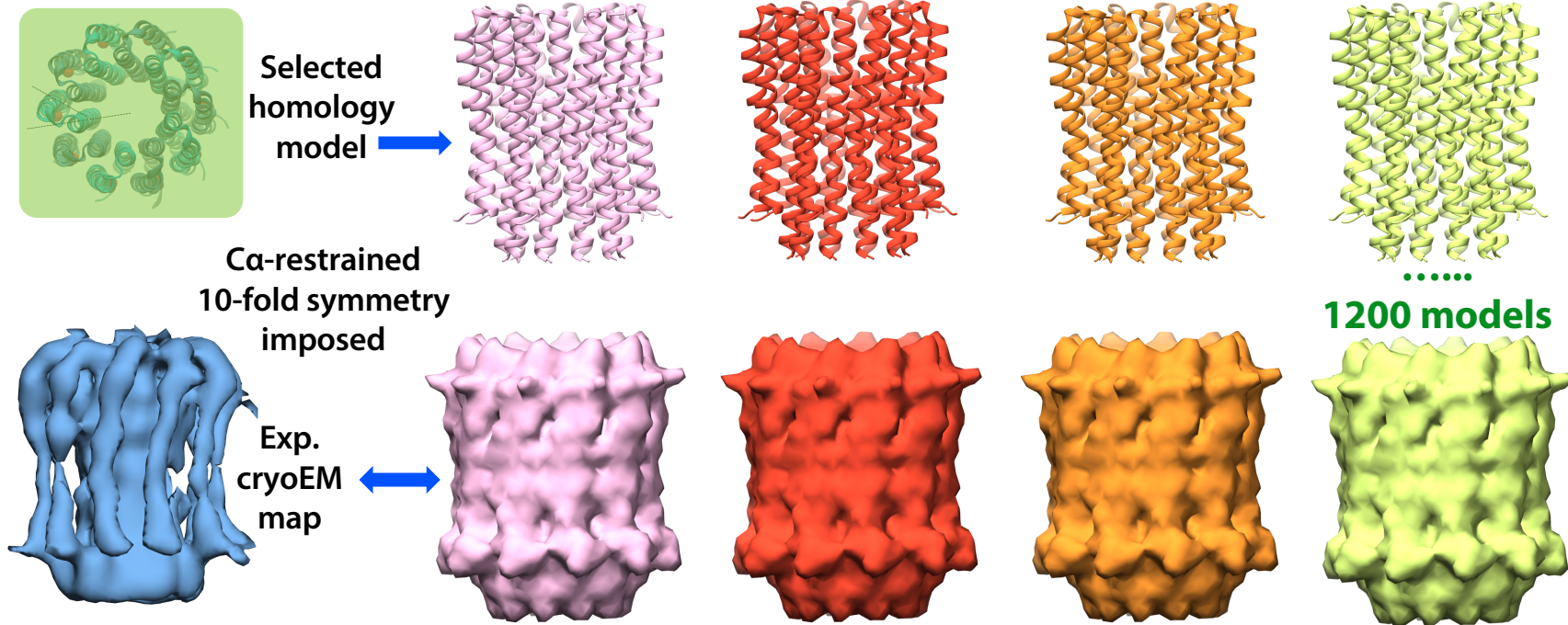

- **8.0Å resolution** cryoEM map of *Polytomella* c-ring

- **2.0Å resolution** Xray of **yeast** c-ring
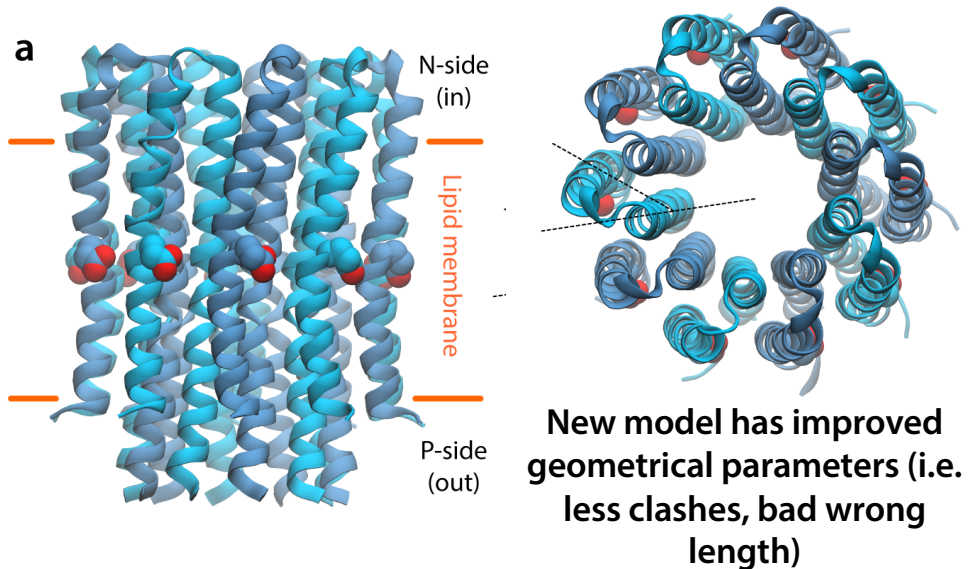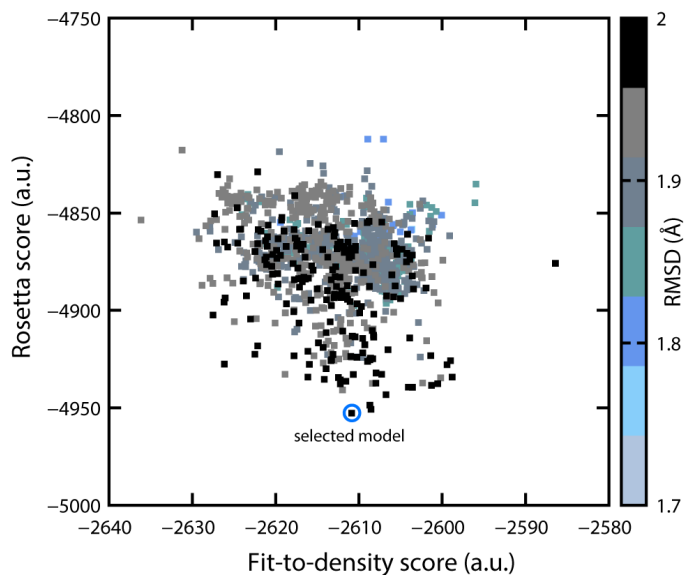
Symersky *et al.* NSMB 2012

Homology modelling & fitting this model into cryoEM map

2. Model subunit-a

- **6.5Å resolution** cryoEM map of *Polytomella* c-ring
- **Cys crosslinks** and **residue accessibility**

- No atomistic structural information: **ab-initio approach?**

- **Hand-traced approach? How much it depend on the user?**

- **Co-variant** residue pairs & **Cys crosslink data** to **sort** among **models** fitted into **cryoEM map**

- **Uncertainty** on subunit-a **C-terminal position** and **topology**

2a. Model TM4-TM5 hairpin (C-terminus)

matrix

C-ter

TM4 out/TM5 in

C-ter

TM4 in/TM5 out

lumen

- **Model both C-ter possibilites** & sort them by co-variant pairs and Cys Xlinks

2b. Model TM2-TM3 hairpin

matrix

lumen

- **Define** TM bundle **topology** using **co-variant** residue pairs

Model c-ring/subunit-a complex of *Polytomella*

Allegretti *et al.* Nature 2015

matrix

lumen

1. Model the c_{10}-ring

- **2.0Å resolution** Xray of **yeast** c-ring

  Symersky *et al.* NSMB 2012

- **8.0Å resolution** cryoEM map of *Polytomella* c-ring
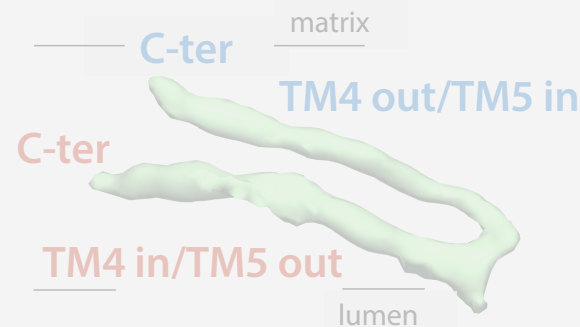
  Homology modelling & fitting this model into cryoEM map
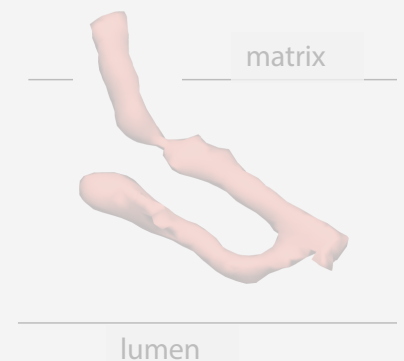
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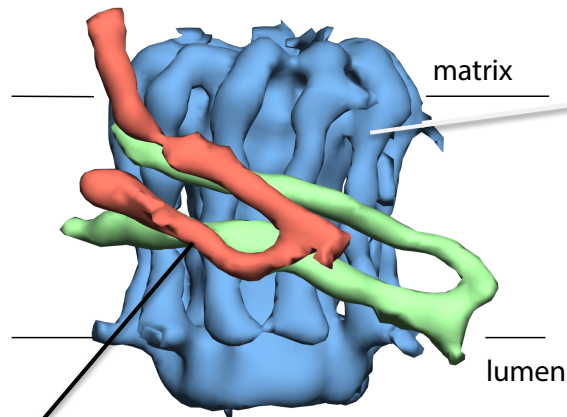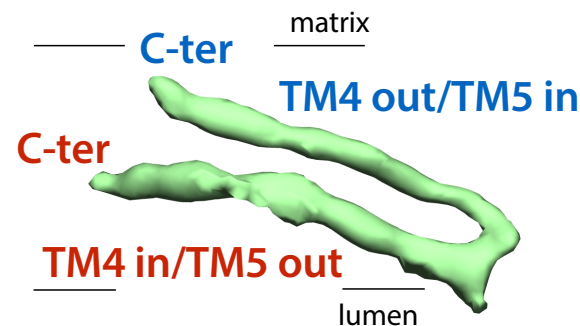
2a. Model TM4-TM5 hairpin (C-terminus)

matrix

C-ter

TM4 out/TM5 in

C-ter

TM4 in/TM5 out

lumen

- **Model both C-ter possibilites** & sort them by co-variant pairs and Cys Xlinks

2b. Model TM2-TM3 hairpin

matrix

lumen

- **Define** TM bundle **topology** using **co-variant** residue pairs

Model of TM4 in/TM5 out and TM4 out/TM5 in positions

Create different tracing of both C-terminal assignments

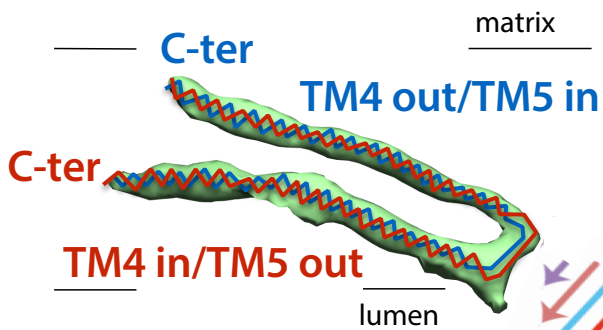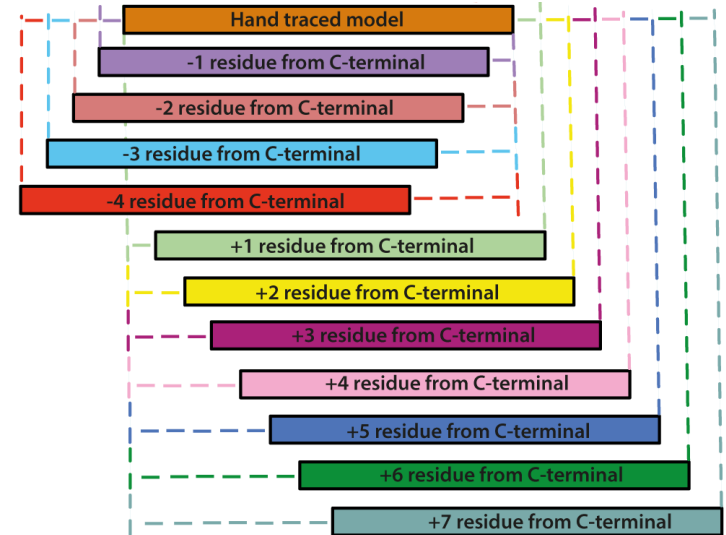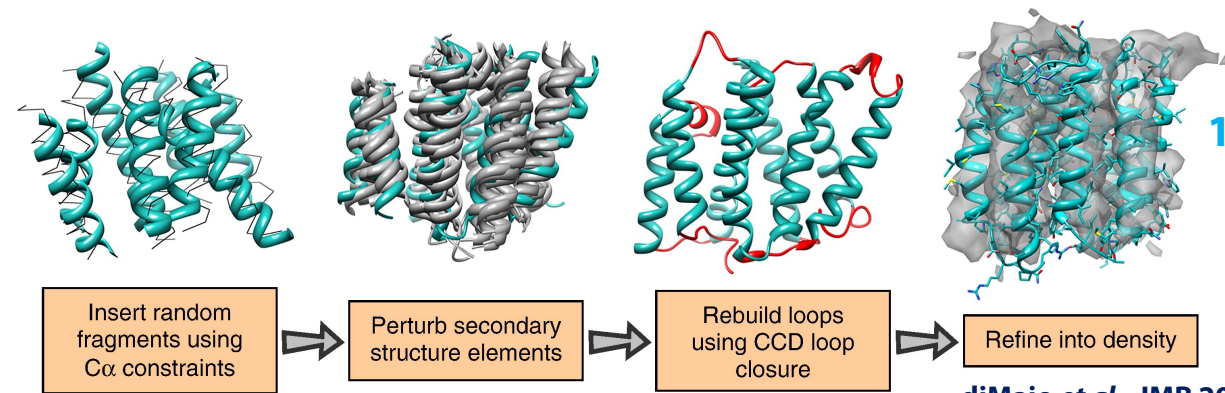### Thread the sequence into the map from the C-terminus

Model map rods as ideal helices, identification of rods on the map using SSEHunter

Baker et al. Nature Protoc 2010

Sequence threading shifted by increments of one residues to add variability to the sample

- Hand traced model
- -1 residue from C-terminal
- -2 residue from C-terminal
- -3 residue from C-terminal
- -4 residue from C-terminal
- +1 residue from C-terminal
- +2 residue from C-terminal
- +3 residue from C-terminal
- +4 residue from C-terminal
- +5 residue from C-terminal
- +6 residue from C-terminal
- +7 residue from C-terminal

Fragment approach used to sample better each threaded model

Insert random fragments using Cα constraints → Perturb secondary structure elements → Rebuild loops using CCD loop closure → Refine into density

diMaio et al., JMB 2009

......
1200 models*12 traces*2
C-ter positions=28'800

Sort the models by co-variant residues

EVC single protein

EVC complex

Marks et al., PLoS ONE 2011

Hopf et al., Elife 2014
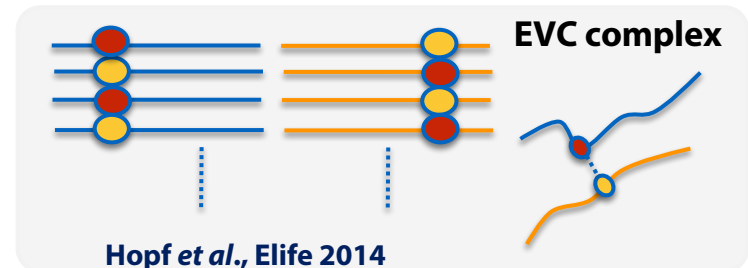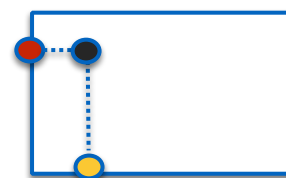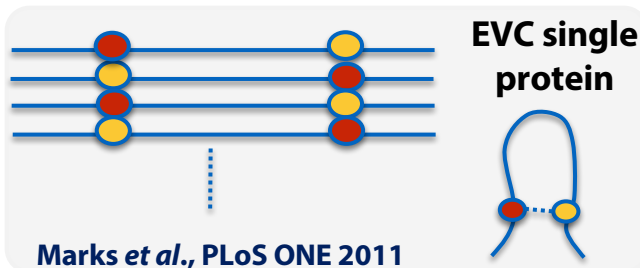
Model of TM4 in/TM5 out and TM4 out/TM5 in positions
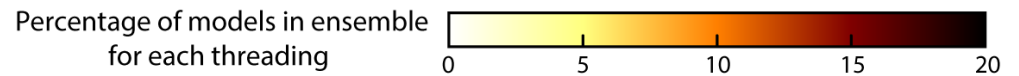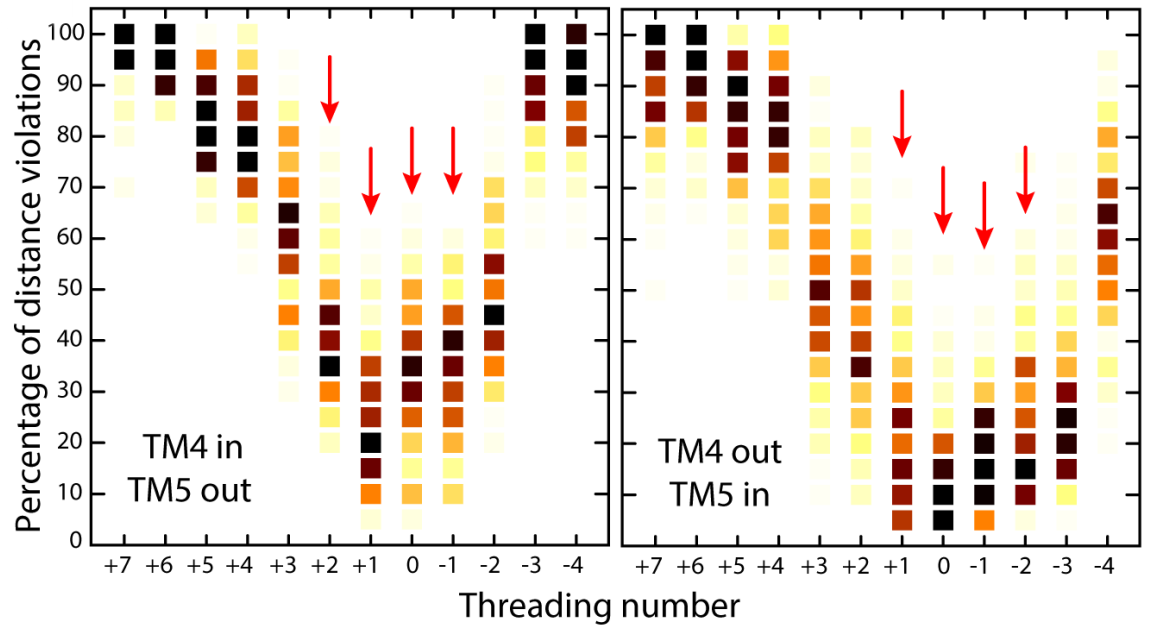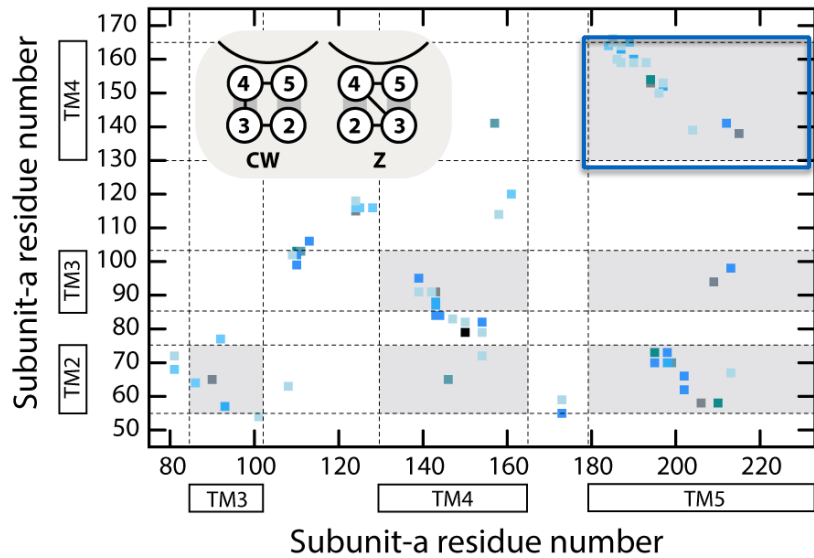
All models fit comparably into the cryoEM density (no details on side chains)

Best threading models selected based on **TM4-TM5 covariant residues**

TM4-TM5 covariant residues cannot distinguish the two C-ter positions

Data between subunit-a/c-ring is needed

Covariant residues between subunit-a and c-ring select TM4 out/TM5 in assignment

matrix

C-ter

TM4 in/TM5 out

lumen

matrix

C-ter **TM4 out/TM5 in**

lumen

TM4 in, TM5 out **TM4 out, TM5 in**

Threading +2   Threading +1
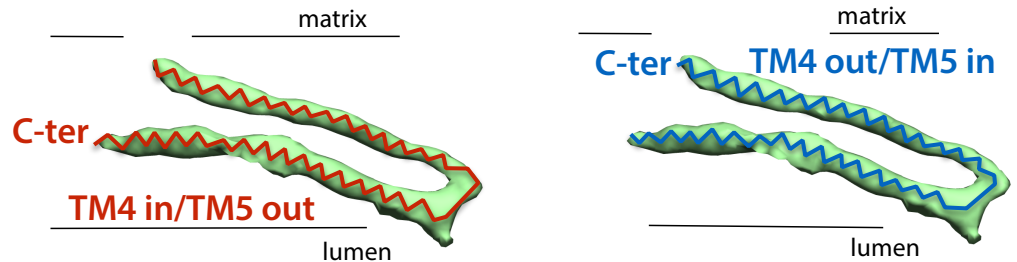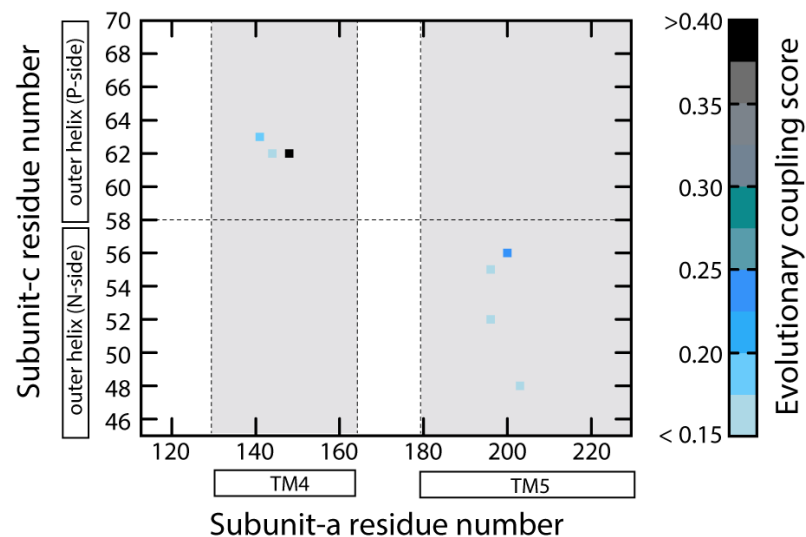
Worse Better   Threading 0

Threading +1

Threading 0   Threading -1

Threading -1   Threading -2

Percentage of models in ensemble

Global evolutionary-coupling score*

Subunit-c residue number

outer helix (P-side)   outer helix (N-side)

Subunit-a residue number

TM4   TM5

Evolutionary coupling score

>0.40

0.35

0.30

0.25

0.20

< 0.15

* Score that sums one for each coupling fulfilled
(covariant residues are within a threshold of 15 Å)

Cys crosslinked residues on *E.coli* F$_o$ select TM4 out/TM5 in assignment
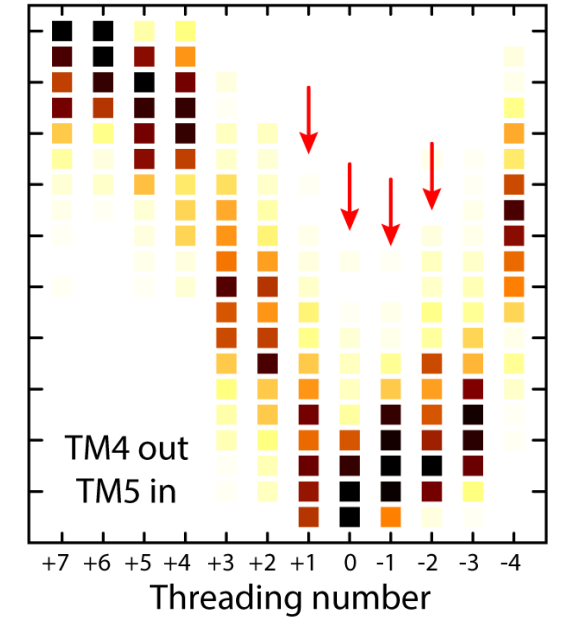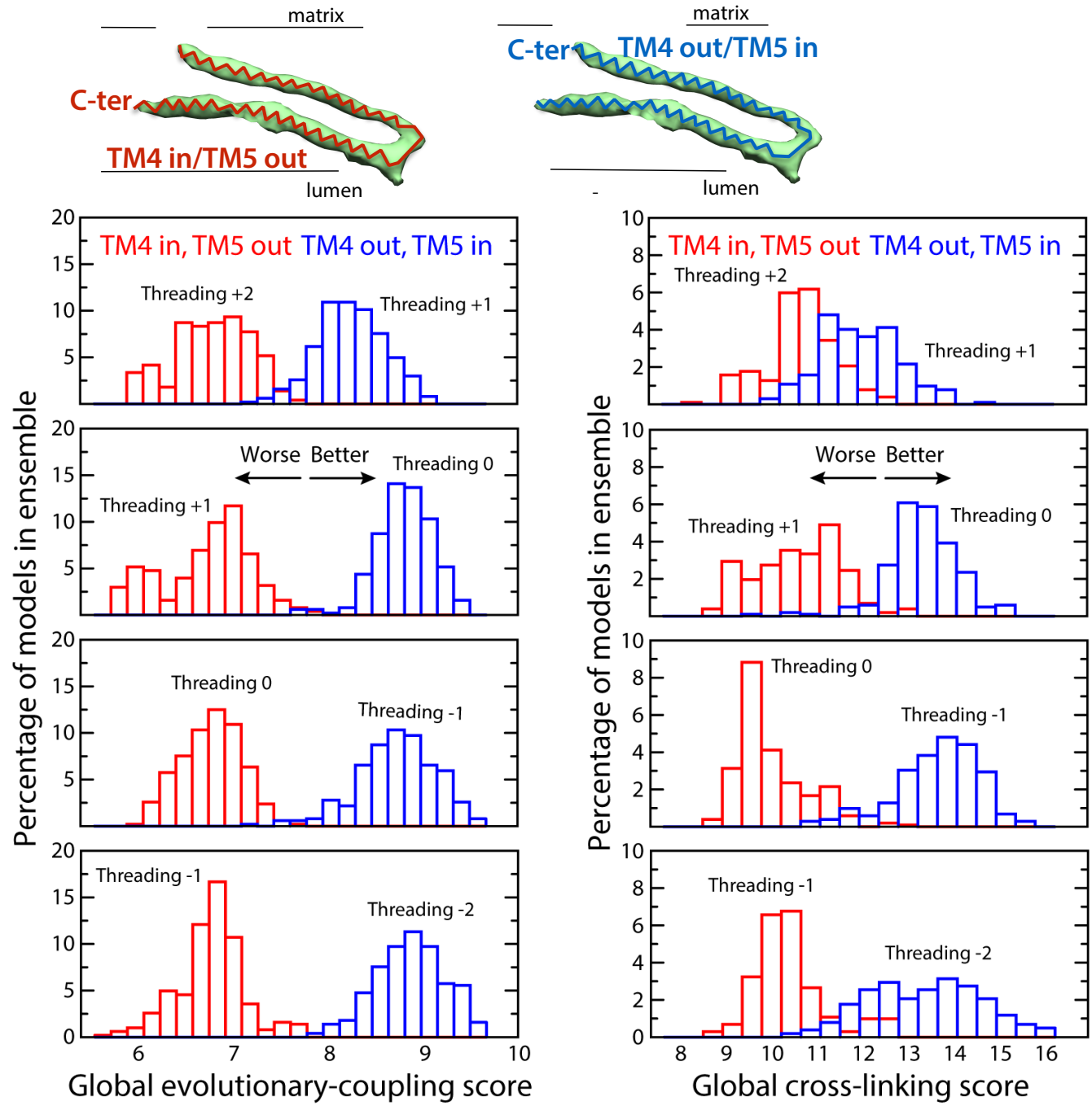
How to distinguish between TM4 out/TM5 in best traces?

Conserved **Arg on TM4** can be **translated** to a conserved **Gln on TM5** (252 in *E.coli*) **retaining** the **enzymatic function**

Ishmukhametov et al. BBA Bioener 2008
Bae & Vik et al. BBA Bioener 2009

Conserved Arg and Gln of subunit-a must be **proximal to conserved Glu** of c-ring

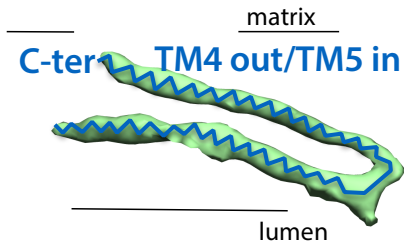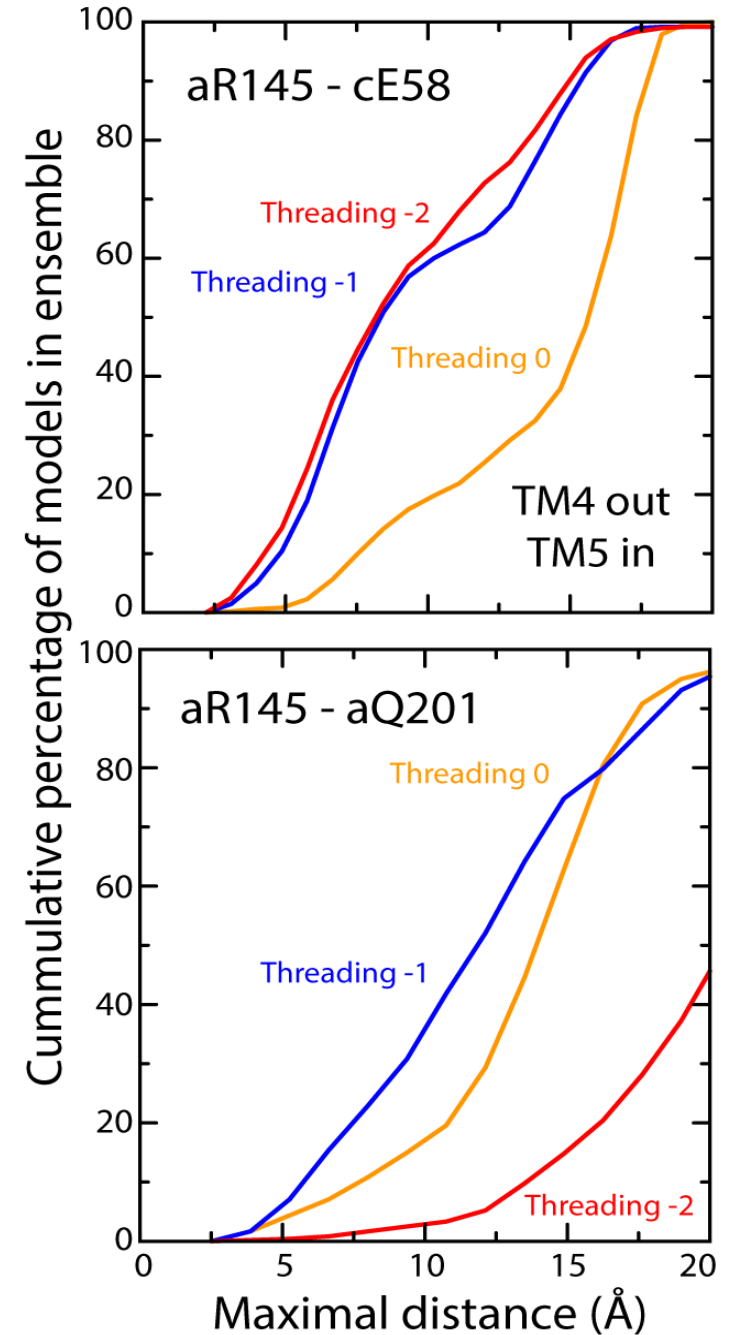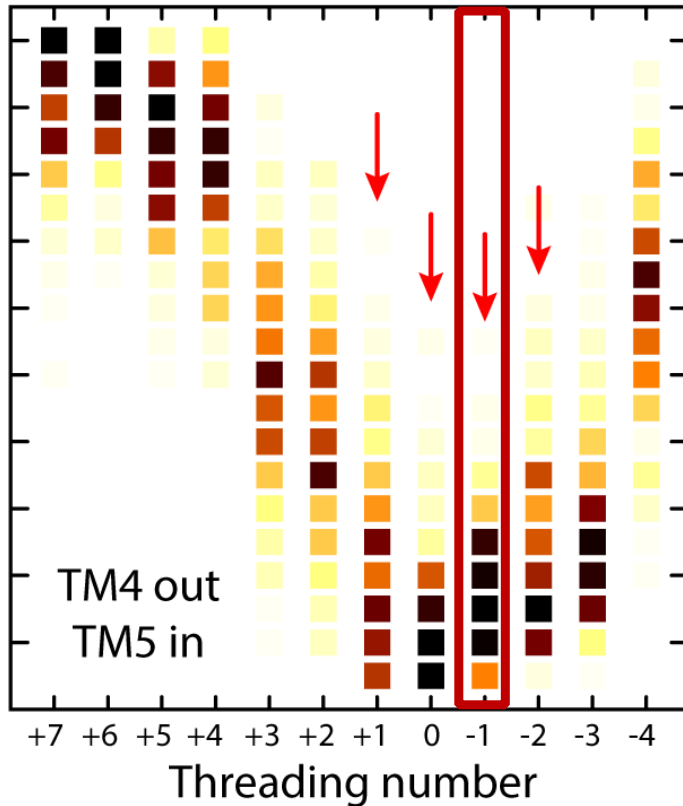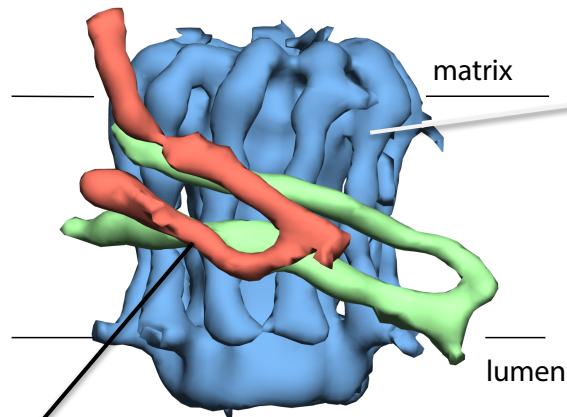# Threading-1 of is selected based on functional data

Model c-ring/subunit-a complex of *Polytomella*

Allegretti *et al.* Nature 2015

matrix

lumen

1. Model the c₁₀-ring

$1.$ Model the c_{10}-ring

* **8.0Å resolution** cryoEM map of *Polytomella* c-ring

* **2.0Å resolution** Xray of **yeast** c-ring

Symersky *et al.* NSMB 2012

Homology modelling & fitting this model into cryoEM map

2. Model subunit-a

* **6.5Å resolution** cryoEM map of *Polytomella* c-ring

* **Cys crosslinks** and **residue accessibility**

* No atomistic structural information: **ab-initio approach?**

* **Hand-traced approach? How much it depend on the user?**

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matrix

C-ter

TM4 out/TM5 in

C-ter

TM4 in/TM5 out

lumen

* **Model both C-ter possibilites** & sort them by co-variant pairs and Cys Xlinks

2b. Model TM2-TM3 hairpin

matrix

lumen

* **Define** TM bundle **topology** using **co-variant** residue pairs

Model c-ring/subunit-a complex of *Polytomella*

Allegretti *et al.* Nature 2015

matrix

lumen

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- **2.0Å resolution** Xray of **yeast** c-ring

Symersky *et al.* NSMB 2012

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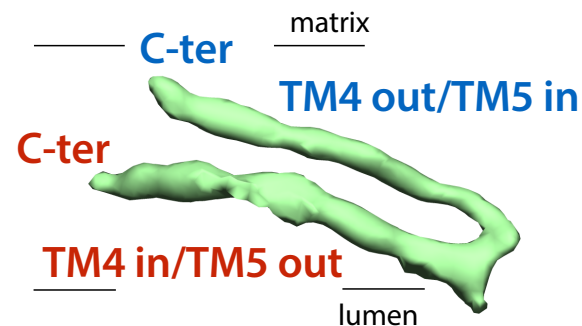
Homology modelling & fitting this model into cryoEM map
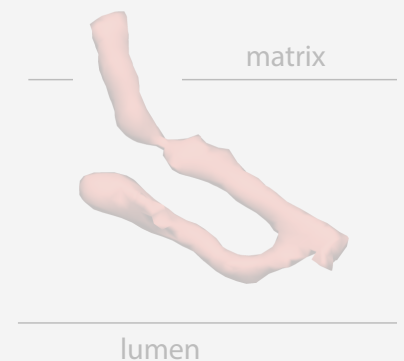
2. Model subunit-a

- **6.5Å resolution** cryoEM map of *Polytomella* c-ring
- **Cys crosslinks** and **residue accessibility**

- No atomistic structural information: **ab-initio approach?**

- **Hand-traced approach? How much it depend on the user?**

- **Co-variant** residue pairs & **Cys crosslink data** to **sort** among **models** fitted into **cryoEM map**

- **Uncertainty** on subunit-a **C-terminal position** and **topology**

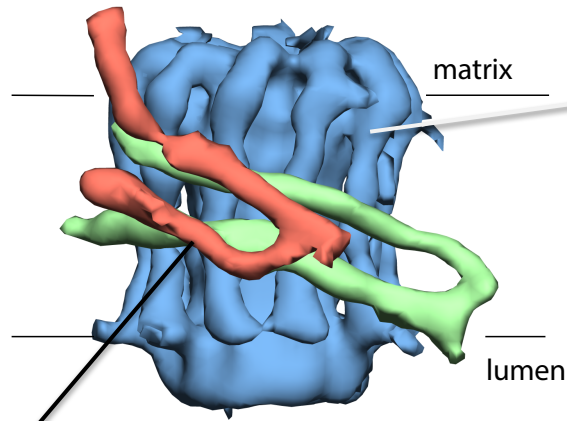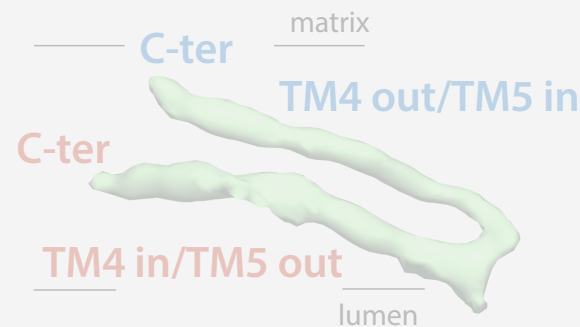## 2a. Model TM4-TM5 hairpin (C-terminus)

matrix

C-ter

TM4 out/TM5 in

C-ter

TM4 in/TM5 out

lumen

- **Model both C-ter possibilites** & sort them by co-variant pairs and Cys Xlinks

2b. Model TM2-TM3 hairpin

matrix

lumen

- **Define** TM bundle **topology** using **co-variant** residue pairs

Model of TM2-TM3 hairpin in a clockwise topology respect to TM4-TM5 as indicated by subunit-a residue covariance
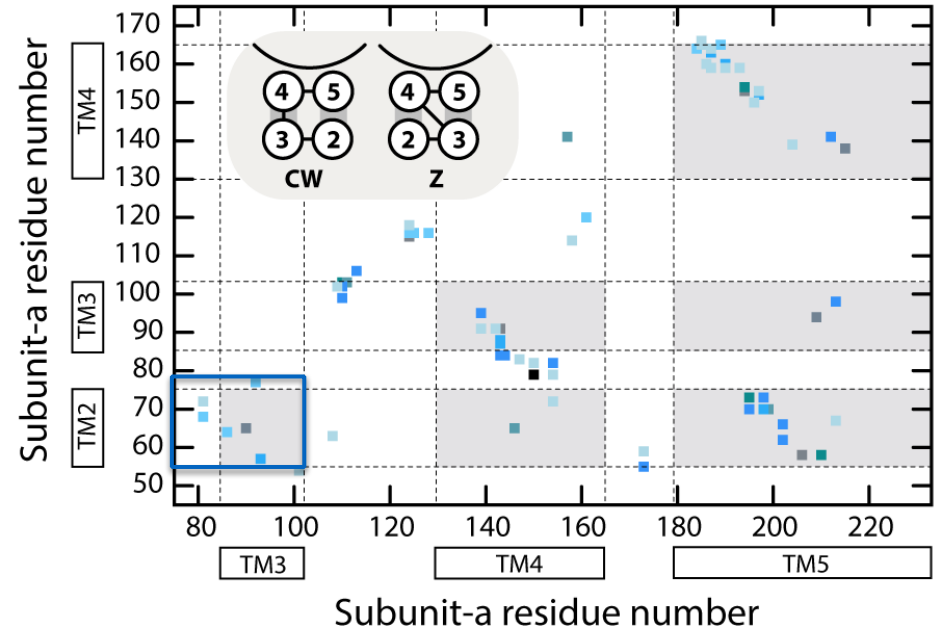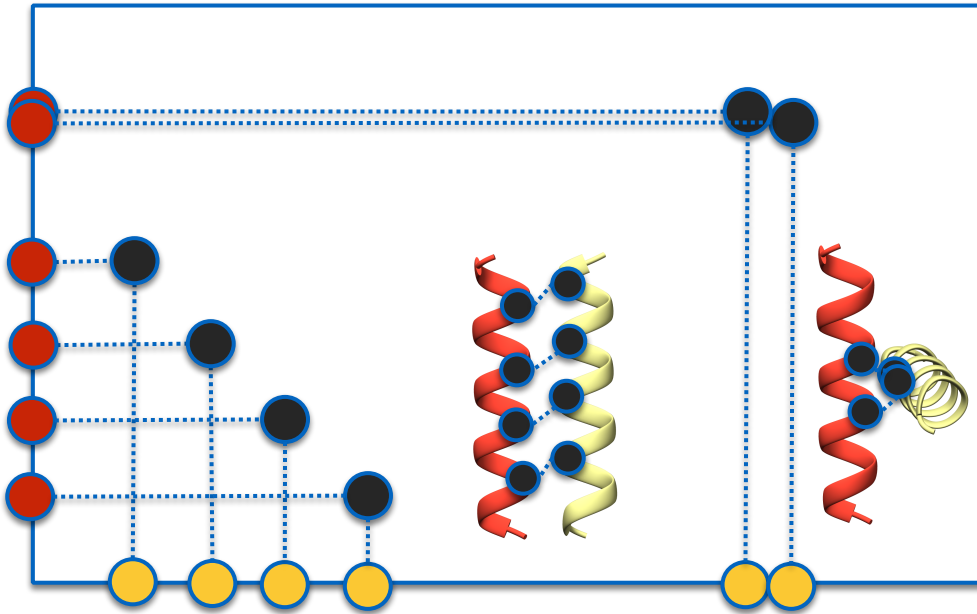
Different **threaded models** are **generated** from the **initial hand-traced one & variants** of each trace are **sampled**
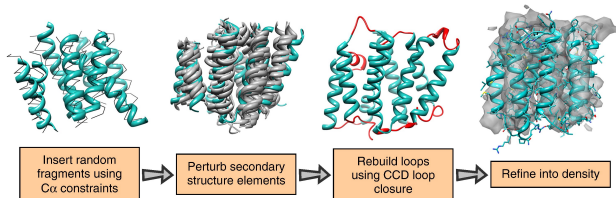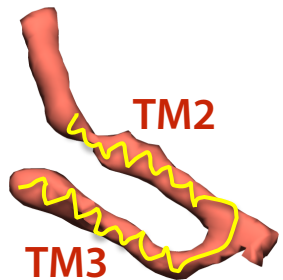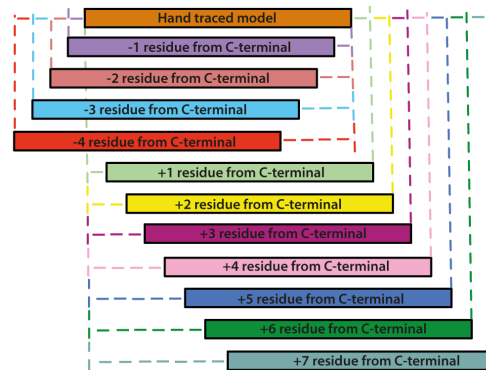
Models **sorted by residue co-variance**
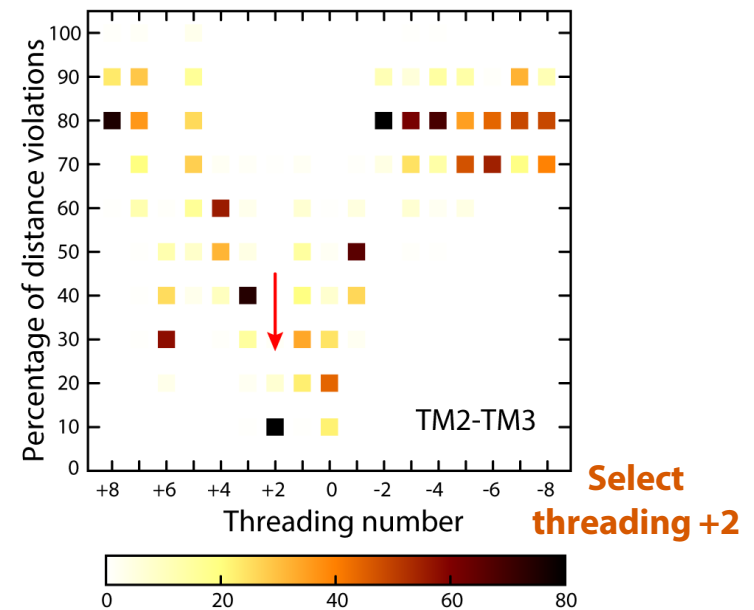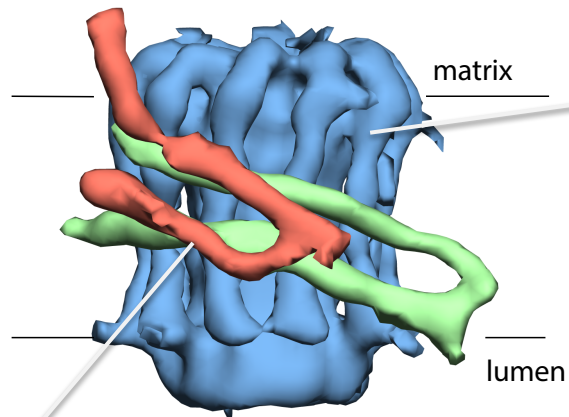
Select threading +2

Model c-ring/subunit-a complex of *Polytomella*

Allegretti *et al.* Nature 2015

matrix

lumen

1. Model the c₁₀-ring

- **8.0Å resolution** cryoEM map of *Polytomella* c-ring

- **2.0Å resolution** Xray of **yeast** c-ring

Symersky *et al.* NSMB 2012

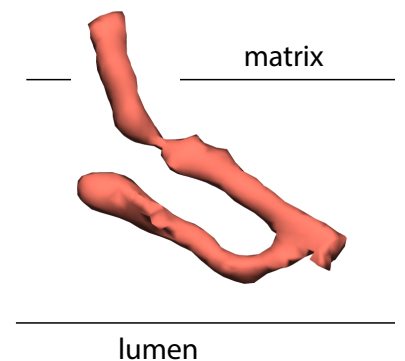**Homology modelling & fitting this model into cryoEM map**

2. Model subunit-a

- **6.5Å resolution** cryoEM map of *Polytomella* c-ring
- **Cys crosslinks** and **residue accessibility**
- No atomistic structural information: **ab-initio approach?**
- **Hand-traced approach? How much it depend on the user?**
- **Co-variant** residue pairs & **Cys crosslink data** to **sort** among traced **models** sampled within **cryoEM map**
- Add **variability** to each traced model with a **fragment approach**
- **Uncertainty** on subunit-a **C-terminal position** and **topology**

2a. Model TM4-TM5 hairpin (C-terminus)

matrix

C-ter

TM4 out/TM5 in

C-ter

TM4 in/TM5 out

lumen

- **Model both C-ter possibilites** & sort them by co-variant pairs and Cys Xlinks

2b. Model TM2-TM3 hairpin

matrix

lumen

- **Define** TM bundle **topology** using **co-variant** residue pairs

Model c-ring/subunit-a complex of *Polytomella*

Allegretti *et al.* Nature 2015

3. Model whole subunit-a bundle using the best threading of TM2-TM3 and TM4-TM5 and fit into the map

1. Model the c₁₀-ring
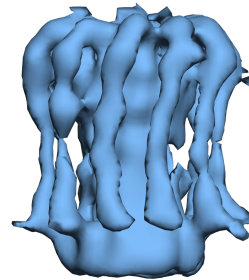
- **2.0Å resolution** Xray of **yeast** c-ring

Symersky *et al.* NSMB 2012

- **8.0Å resolution** cryoEM map of *Polytomella* c-ring

Homology modelling & fitting this model into cryoEM map

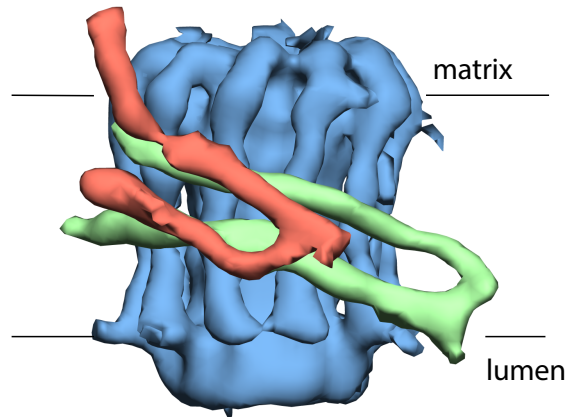## 2a. Model TM4-TM5 hairpin (C-terminus)
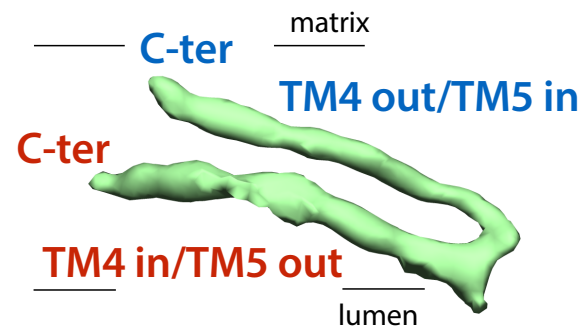
- **Model both C-ter possibilites** & sort them by co-variant pairs and Cys Xlinks

2b. Model TM2-TM3 hairpin
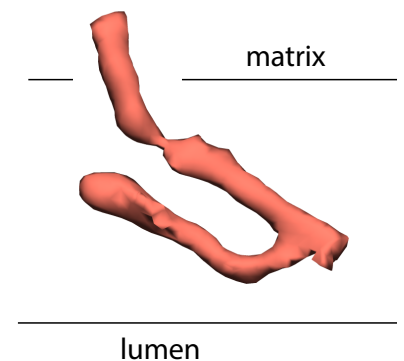
- **Define** TM bundle **topology** using **co-variant** residue pairs

Mapping Cd²⁺ accessible residues on c-ring/subunit-a structure
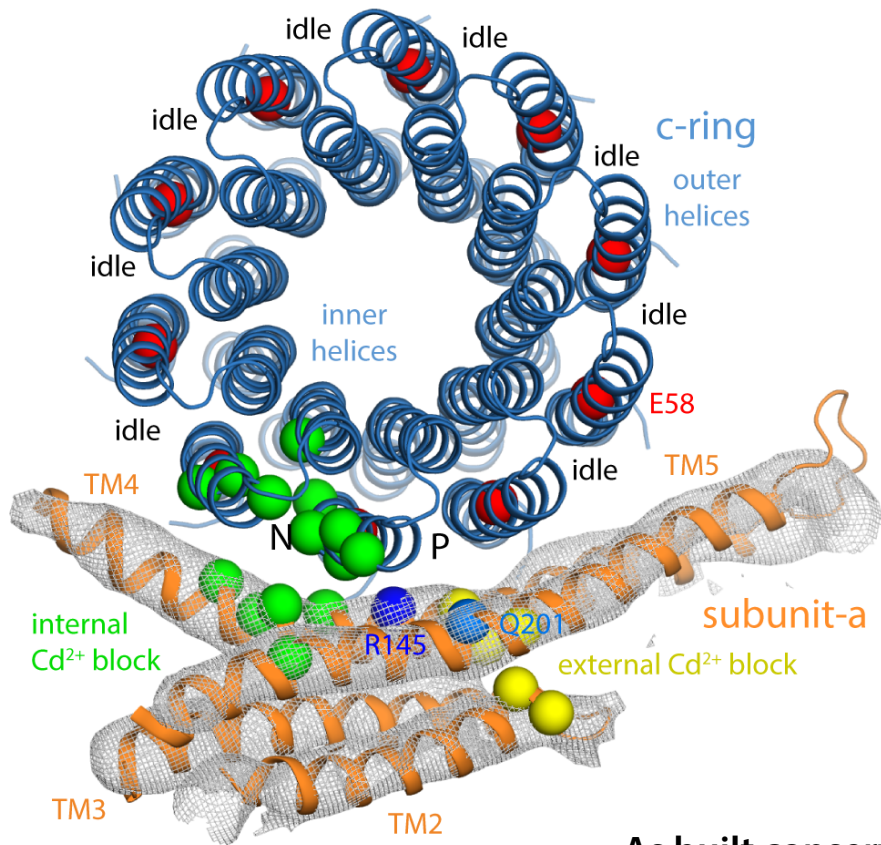
As built conserved **Arg** and **Gln** of subunit-a are proximal to conserved **Glu** of the c-ring
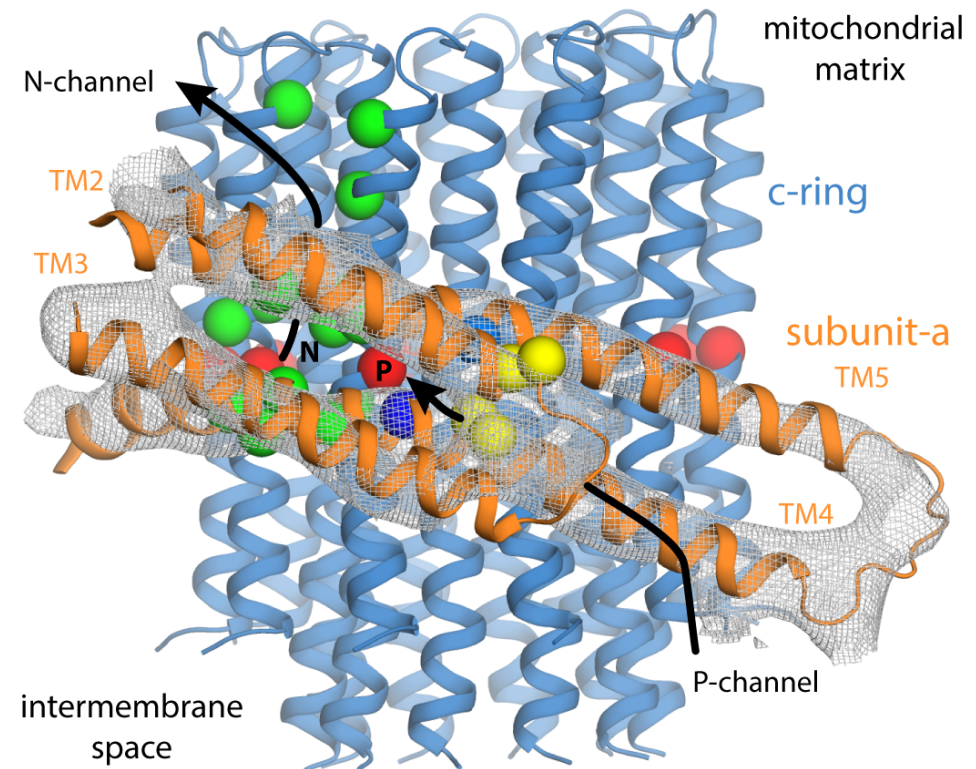
Residues proposed to belong to **N-** and **P-channels** are clustered together

Leone and Faraldo *J. Gen Phys*. 2016

Structure of the c-ring/subunit-a complex supports the two-half-channel hypothesis
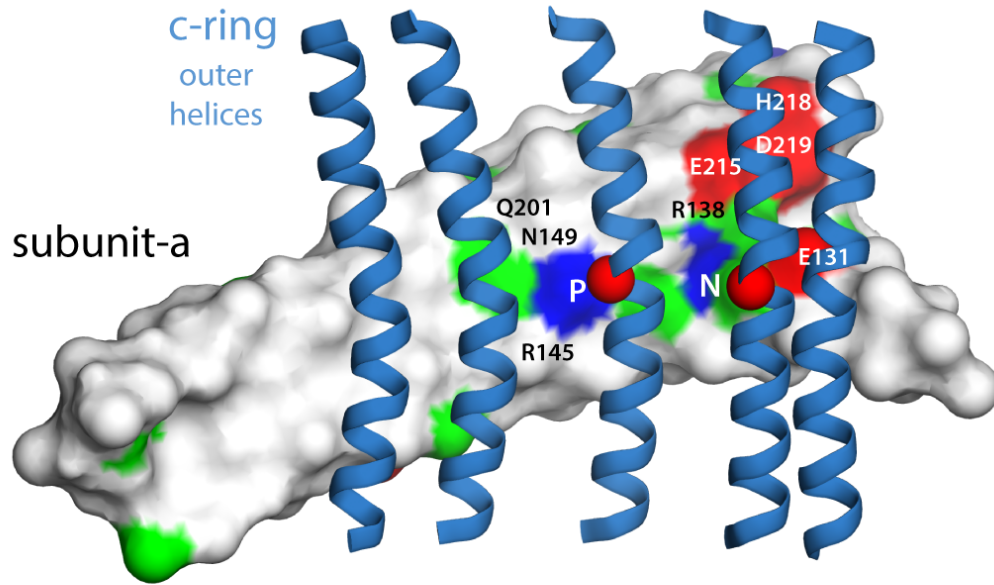
Ionizable residues lines on the proposed **P** and **N-channel** while **a/c-interface on P-side is sealed**
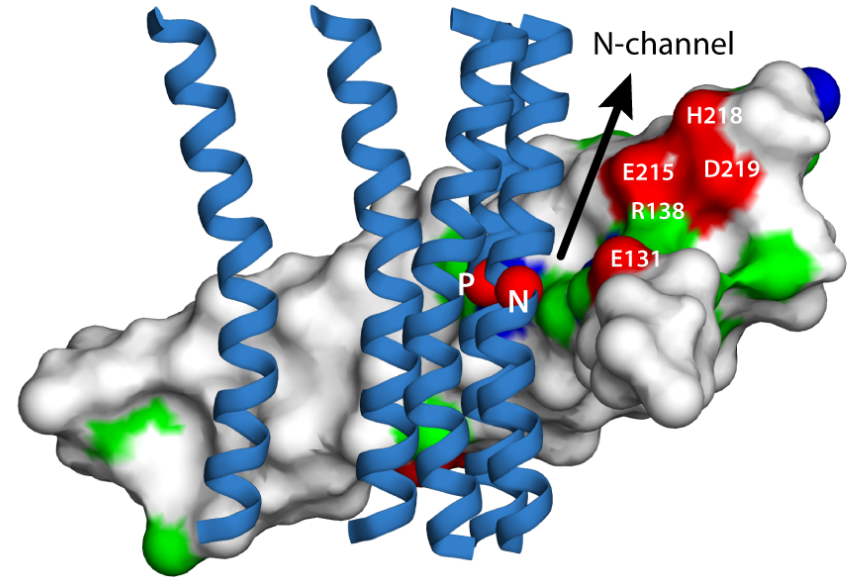
Solvent may be **stabilized** by interaction with **polar residues** at the **N-channel**

Conserved **Arg** between the two channels **shortcuts the proton leakage** across them

H154 and E194 are highly co-variant; proton buffer
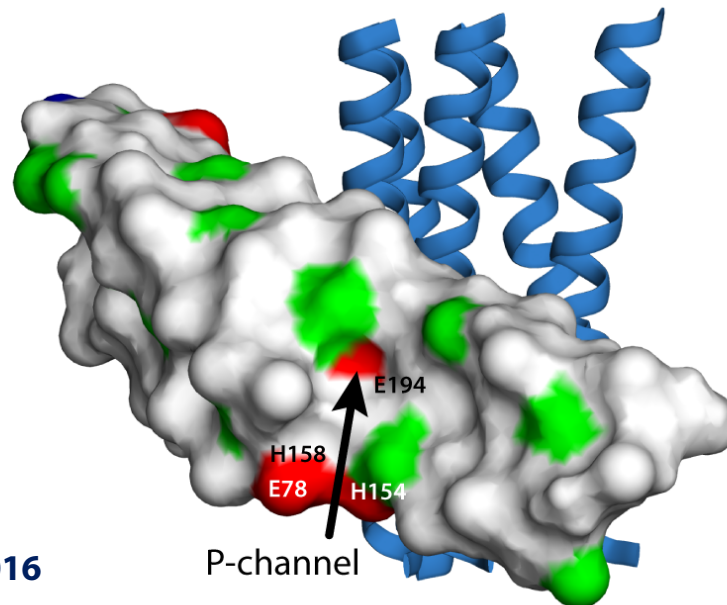
E131 affects kinetics but not H$^+$-binding

Leone and Faraldo *J. Gen Phys*. 2016

ATP synthase H⁺ transport mechanism derived from our model
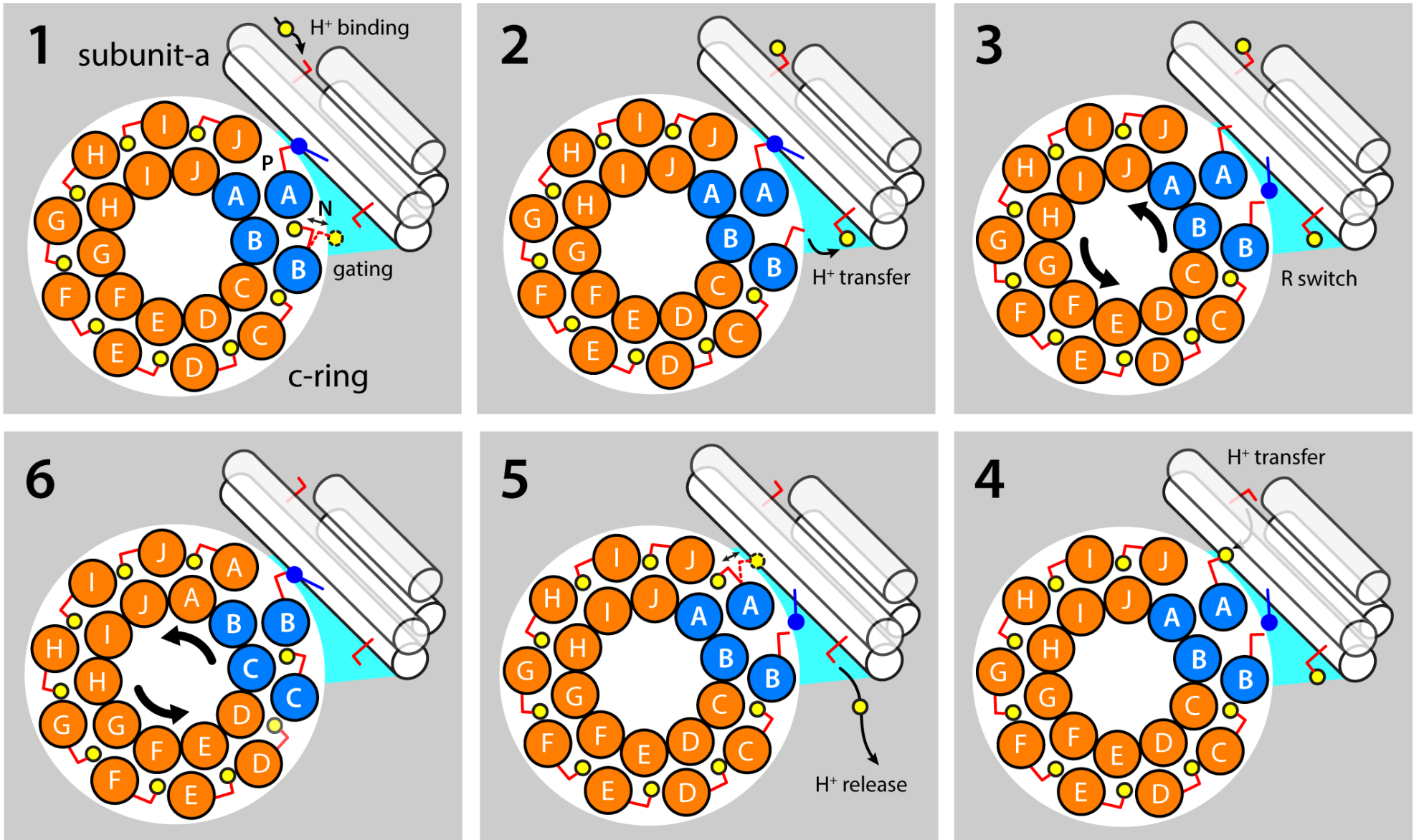
Model of c-ring/subunit-a complex is in agreement with previously published biochemical data

c-ring

disulfide
cross-links

subunit-a

outer helices

TM5

TM4

M2M
cross-links

TM2

TM2
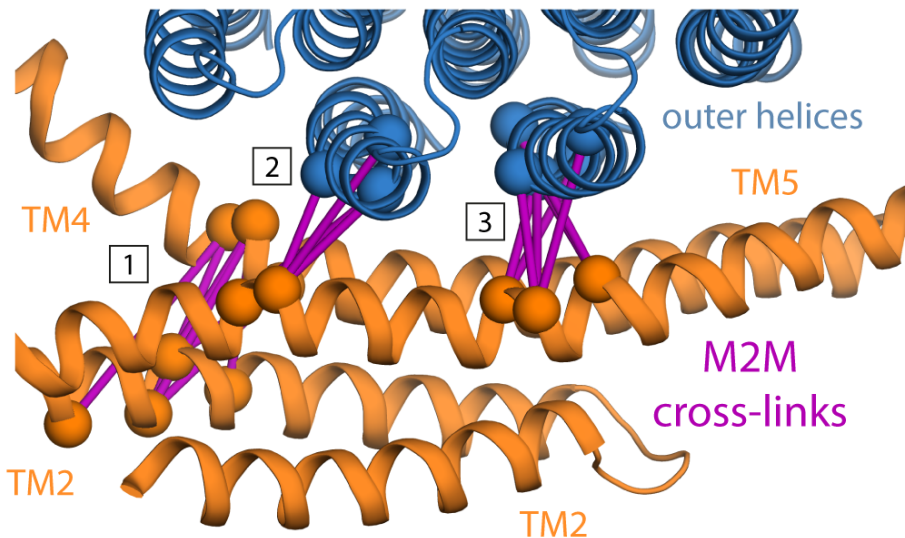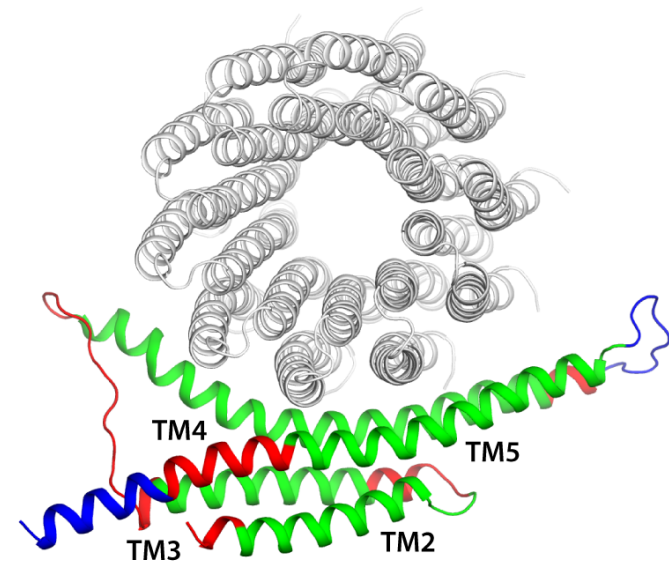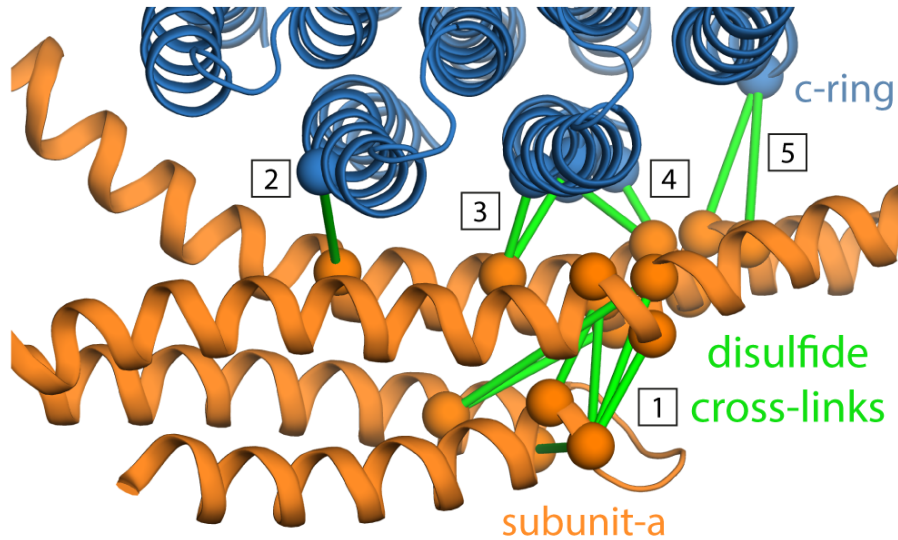
Water accessible regions

Insertions in *Polytomella*

Regions flanked by non reactive positions
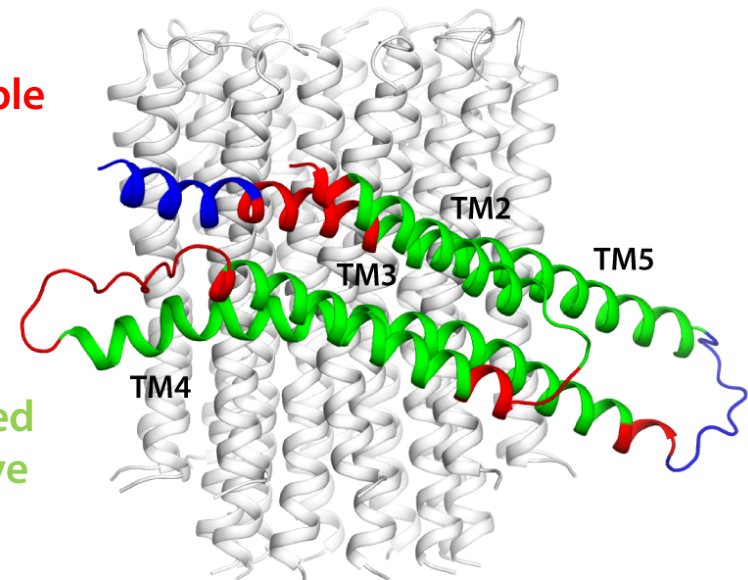
TM4

TM3

TM2

TM5

Leone and Faraldo *J. Gen Phys*. 2016

Our model of the c-ring/subunit-a complex explains why olygomycin is able to block both ATP synthesis and hydrolysis

ATP synthase as **potential pharmacological target** against dormant or resistant bacterial strains

Recent FDA-approved anti-tuberculosis **drug targets** ATP synthase **membrane domain**

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ATP synthase as **potential pharmacological target** against dormant or resistant bacterial strains

Recent FDA-approved anti-tuberculosis **drug targets** ATP synthase **membrane domain**

Oligomycin **antibiotic binds** to the **c-ring ion binding site** (Xray structure) and **resistant mutations** are located in both **subunit-c and –a**

Probably antibiotics **binds to the a/c-interface** blocking the rotation

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ATP hydrolysis

c-ring

subunit-a

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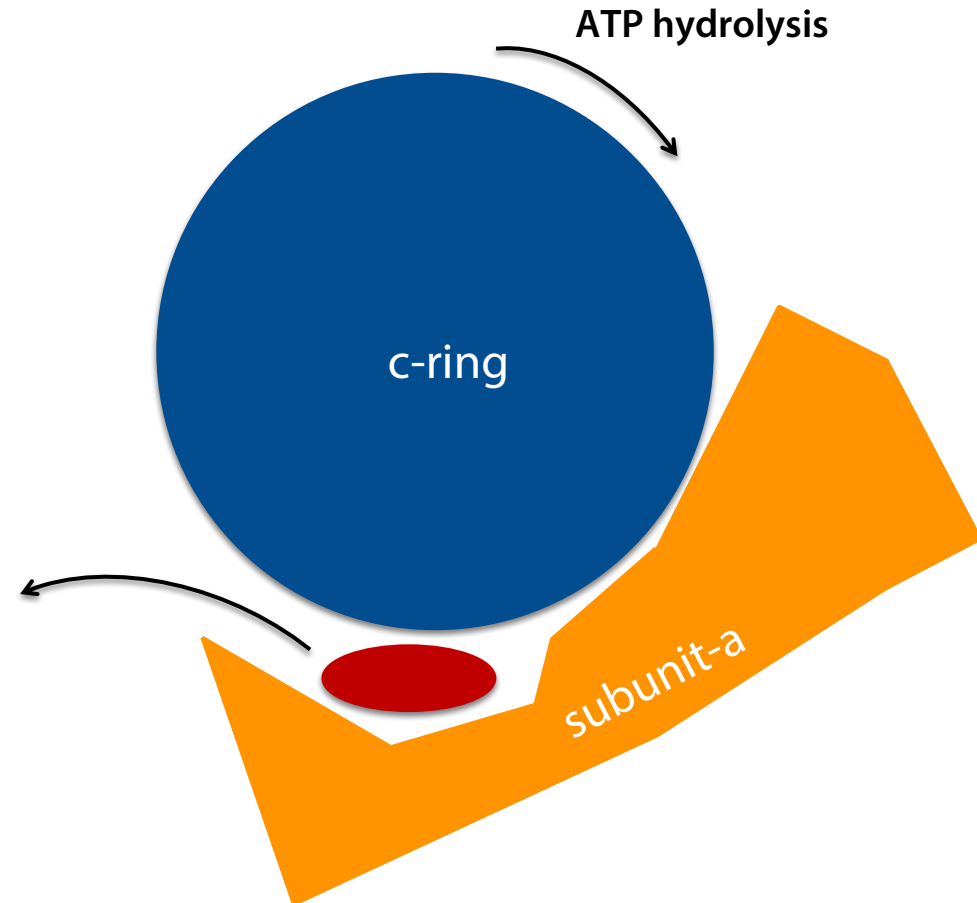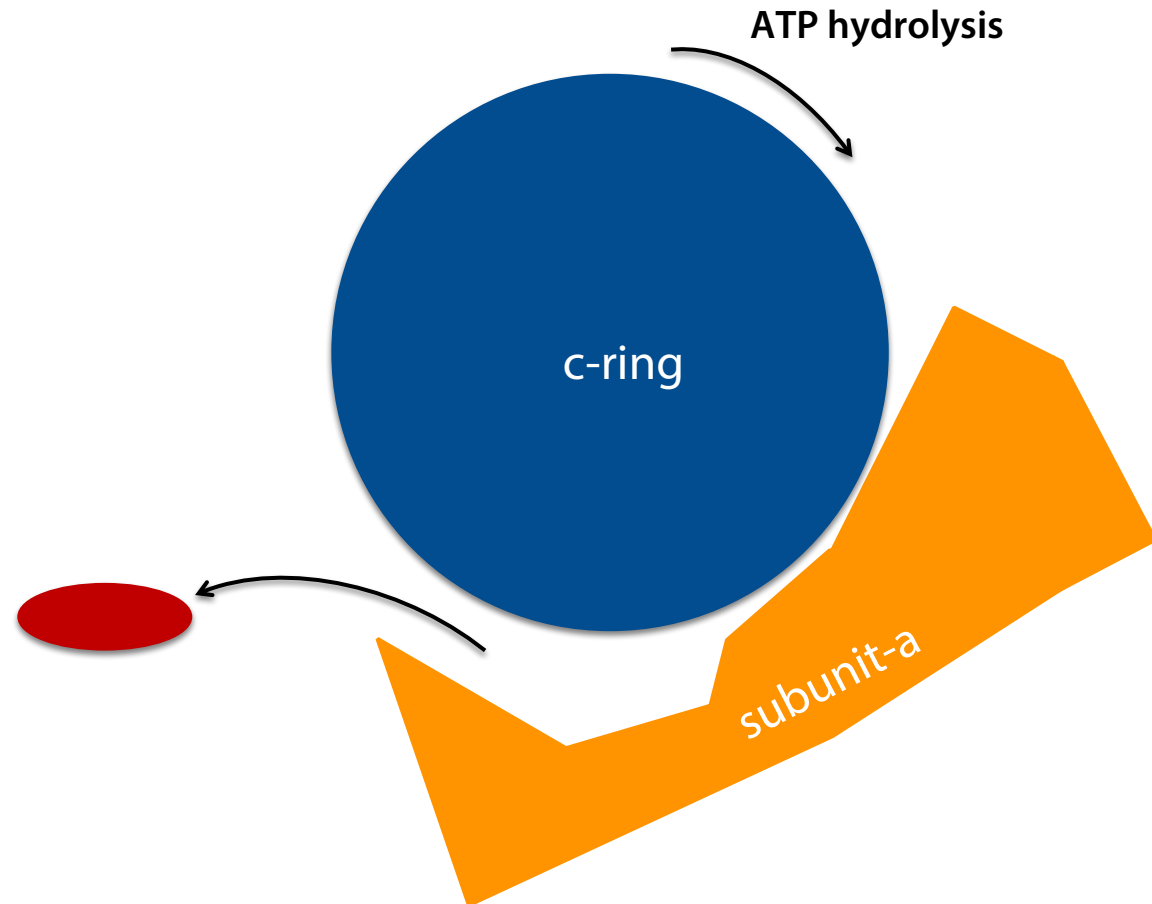
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c-ring

subunit-a

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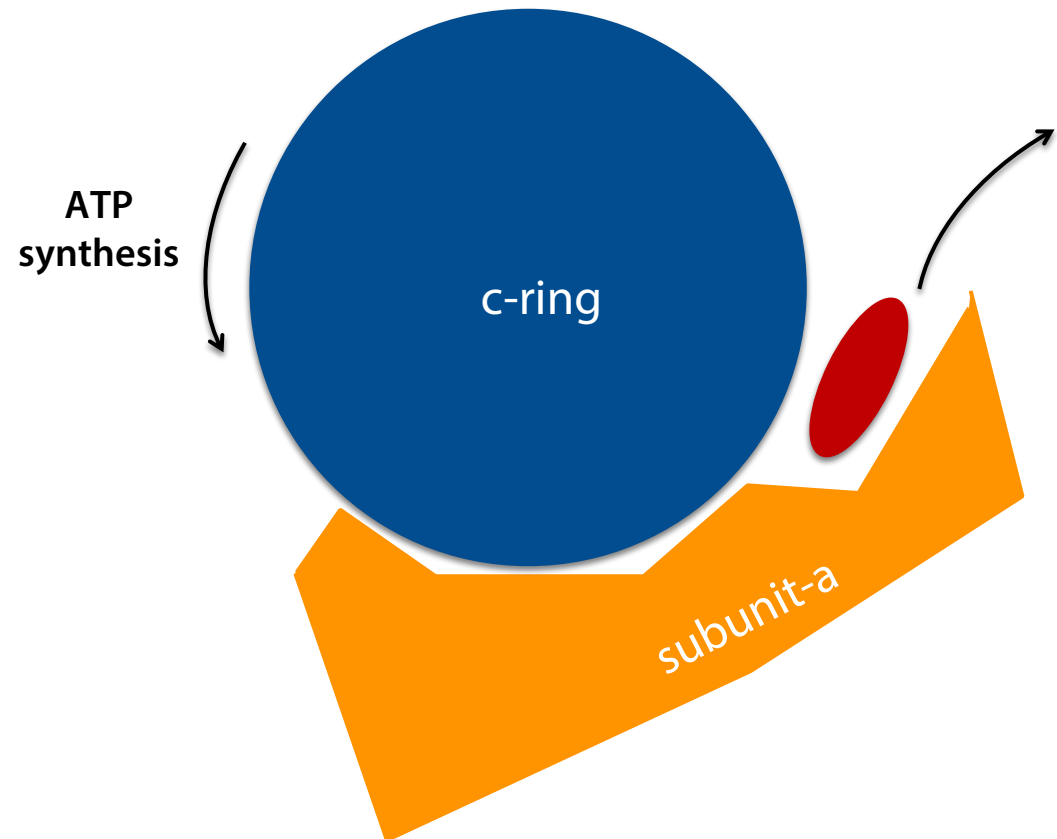
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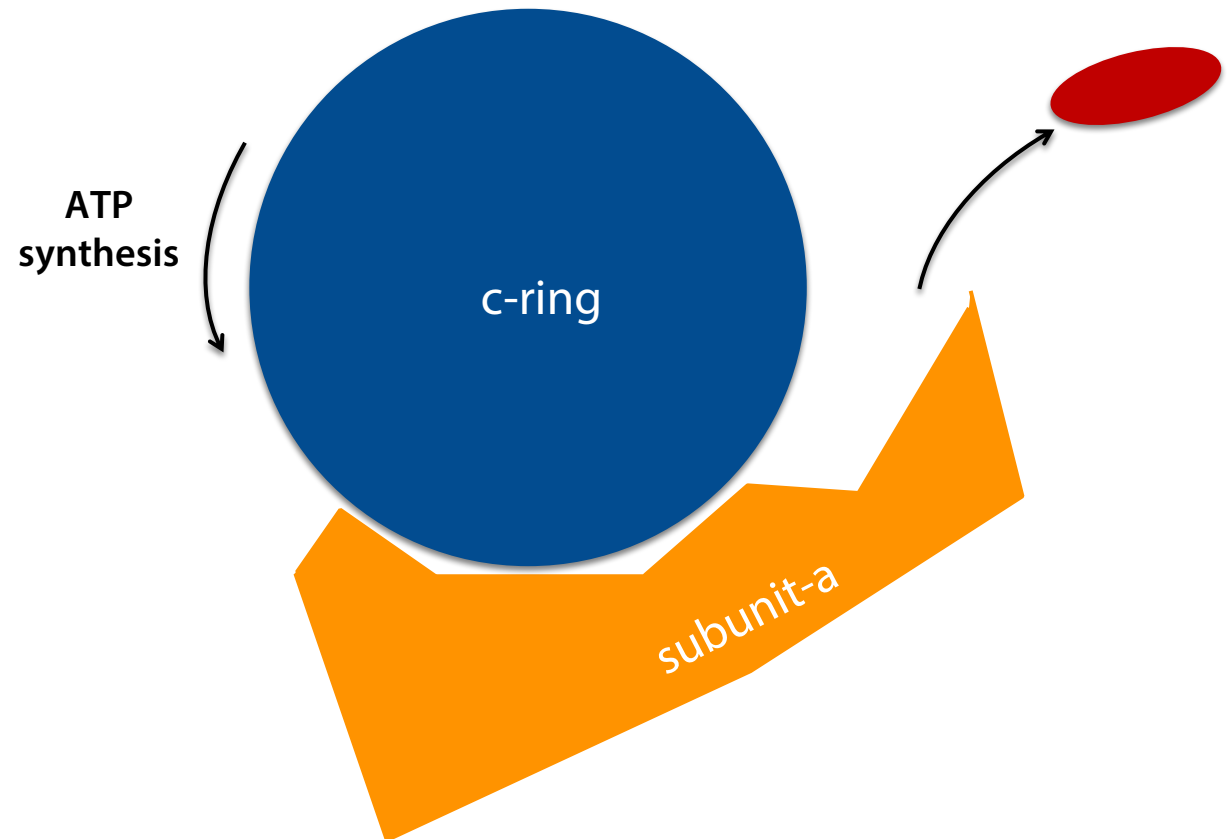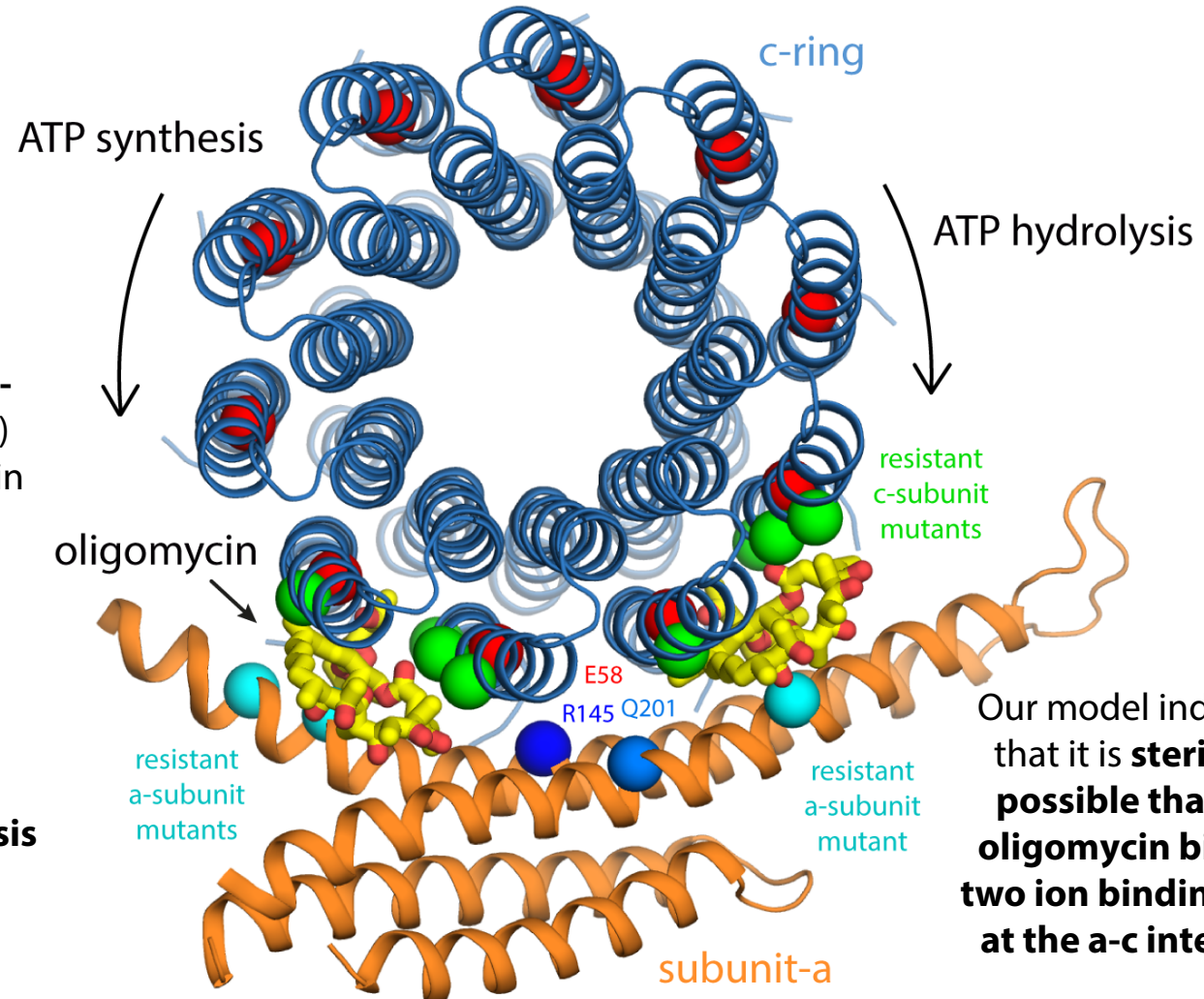
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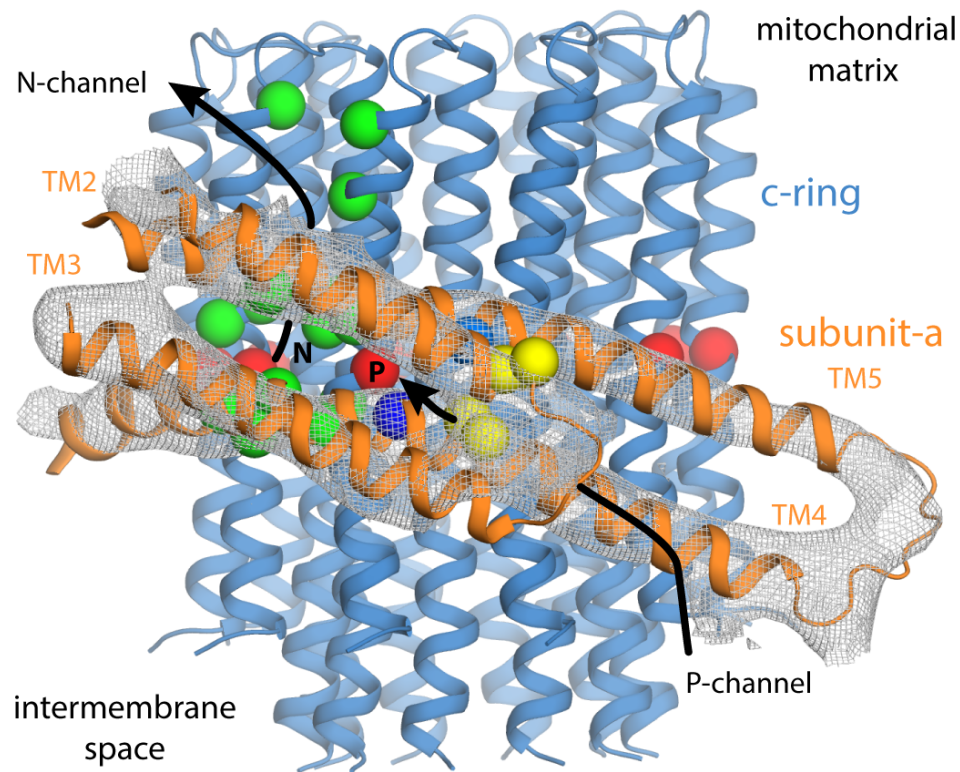Probably antibiotics **binds to the a/c-interface** blocking the rotation

Oligomycin **inhibit** both **ATP synthesis and hydrolisis**

c-ring

ATP synthesis

ATP hydrolysis

oligomycin

resistant c-subunit mutants

E58
R145 Q201

resistant a-subunit mutants

resistant a-subunit mutant

Our model indicates that it is **sterically possible that the oligomycin bind to two ion binding sites at the a-c interface**

subunit-a

Resistant mutations of **subunit-a coincide** with proposes **oligomycin binding sites**

Summary

mitochondrial matrix

N-channel

c-ring

TM2

TM3

subunit-a
TM5

N

P

TM4

P-channel

intermembrane space

We **have integrated different types of information to interpret the cryoEM structure** of an ATP synthase membrane rotor

Our c-ring/subunit-a complex model supports a **two-half-channel model**

Previous **biochemical data is in agreement** with the cryoEM structure

Our model provides **insights on how some antibiotics can inhibit both ATP synthesis and hydrolysis**

The strategy devised in this work can be **applied to interpret low resolution cryoEM structures** in other systems

Acknowledgments