
JVK
2004

SBS-922 Membrane Proteins

Mitochondria and respiratory chains

John F. Allen

School of Biological and Chemical Sciences,
Queen Mary, University of London

Presentations
and
supplementary information

jfallen.org/lectures/

Queen Mary
University of London

Lectures in Membrane Proteins

- Lecture 1. Mitochondrial membranes and chemiosmotic coupling. (Acrobat - .pdf)
- Lecture 2. Redox carriers. (Acrobat - .pdf)
- Lecture 3. Complex I. Structure and Function. Part 1 (Acrobat - .pdf)
- Lecture 4. Complex I. Part 2. Complete structure, including the hydrophobic domain. (Acrobat - .pdf)
- Lecture 5. Complex II and complex III Part 1. Structure and Function. (Acrobat - .pdf)
- Lecture 6. Complex III. The Q-cycle. (Acrobat - .pdf)
- Lecture 7. ATP Synthase. (Acrobat - .pdf)

Membrane Proteins

- Membrane Proteins course web page

References

- Nicholls DJ, Ferguson, SJ. Bioenergetics3. Academic Press/Elsevier Science 2002
- Molecular Biology of the Cell. Bruce Alberts, Alexander Johnson, Julian Lewis, Martin Raff, Keith Roberts, and Peter Walter. 5th edition. 2007. Garland Science. 4th Edition online at NCBI...

Specialist web sites

- Complex I Home Page
- News on the 3D structure from the Complex I Home Page
- Complex II Home Page
- Complex III Home Page
- Cytochrome oxidase home page
- ATP Synthase

Broader interest

- Introduction to Protein Structure. 2nd edition. Carl Branden, John Tooze
- Lane N. Power, Sex, Suicide. Mitochondria and the Meaning of Life. Oxford University Press. October 2005
- Nick Lane home page. Author of *Oxygen: The Molecule that Made the World* (OUP 2002); *Power, Sex, Suicide: Mitochondria and the Meaning of Life* (OUP 2005); *Life Ascending: The Ten Great Inventions of Evolution* (Profile/Norton 2009)
- Ina Schuppe Koistinen
- Odra Noel
- Mitochondrial Proteome. Database for mitochondria-related genes, proteins and diseases
- Proteopedia. Including membrane proteins, for example, 1prc and 1jb0
- queenmaryphotosynthesis.org

Qo. The movie

- Qo.mov

Broader interest

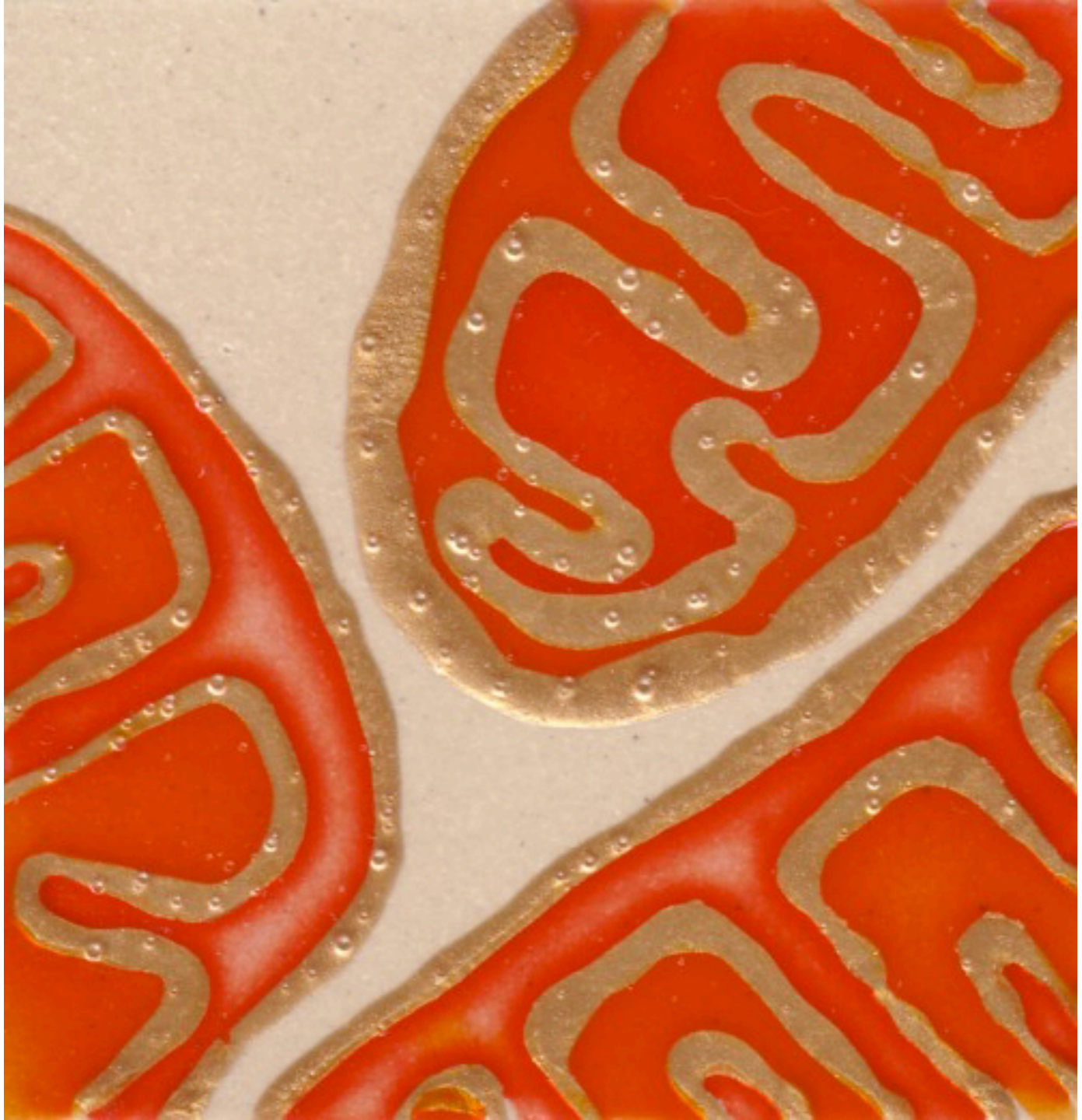
- Introduction to Protein Structure. 2nd edition. Carl Branden, John Tooze
- Lane N. Power, Sex, Suicide. Mitochondria and the Meaning of Life. Oxford University Press. October 2005
- Nick Lane home page. Author of *Oxygen: The Molecule that Made the World* (OUP 2002); *Power, Sex, Suicide: Mitochondria and the Meaning of Life* (OUP 2005); *Life Ascending: The Ten Great Inventions of Evolution* (Profile/Norton 2009)
- Ina Schuppe Koistinen
- Odra Noel
- Mitochondrial Proteome. Database for mitochondria-related genes, proteins and diseases
- Proteopedia. Including membrane proteins, for example, 1prc and 1jb0
- queenmaryphotosynthesis.org

Qo. The movie

- Qo.mov

Molecule of the Month

- ATP Synthase
- Bacteriorhodopsin
- Cytochrome bc1
- Cytochrome c
- Cytochrome c Oxidase
- Hydrogenase
- Mechanosensitive Channels
- Photosystem I
- Photosystem II
- Potassium Channels
- Sodium-Potassium Pump



Complex I. Part 2. Complete structure, including the hydrophobic domain.

jfallen.org/lectures/

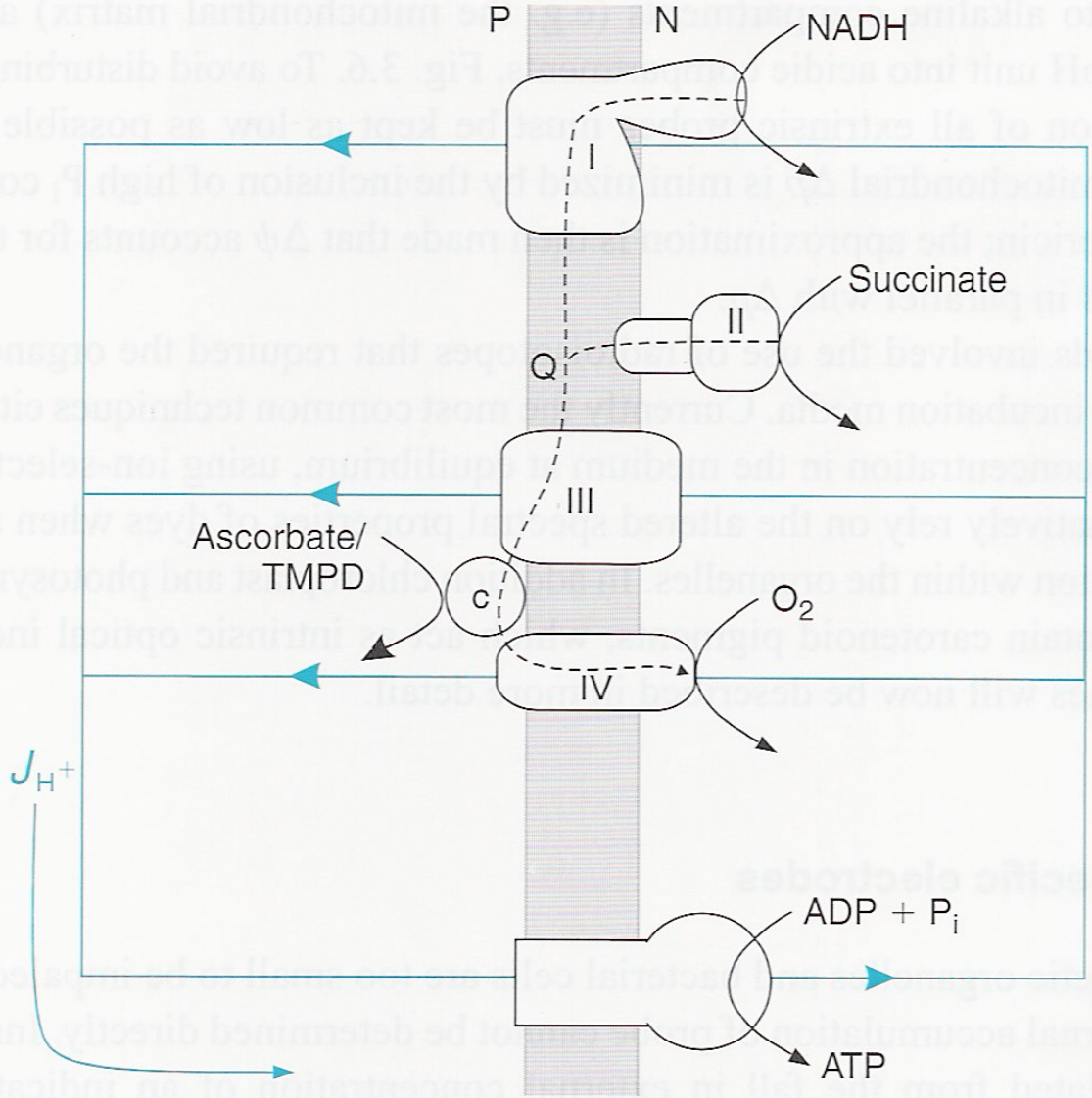
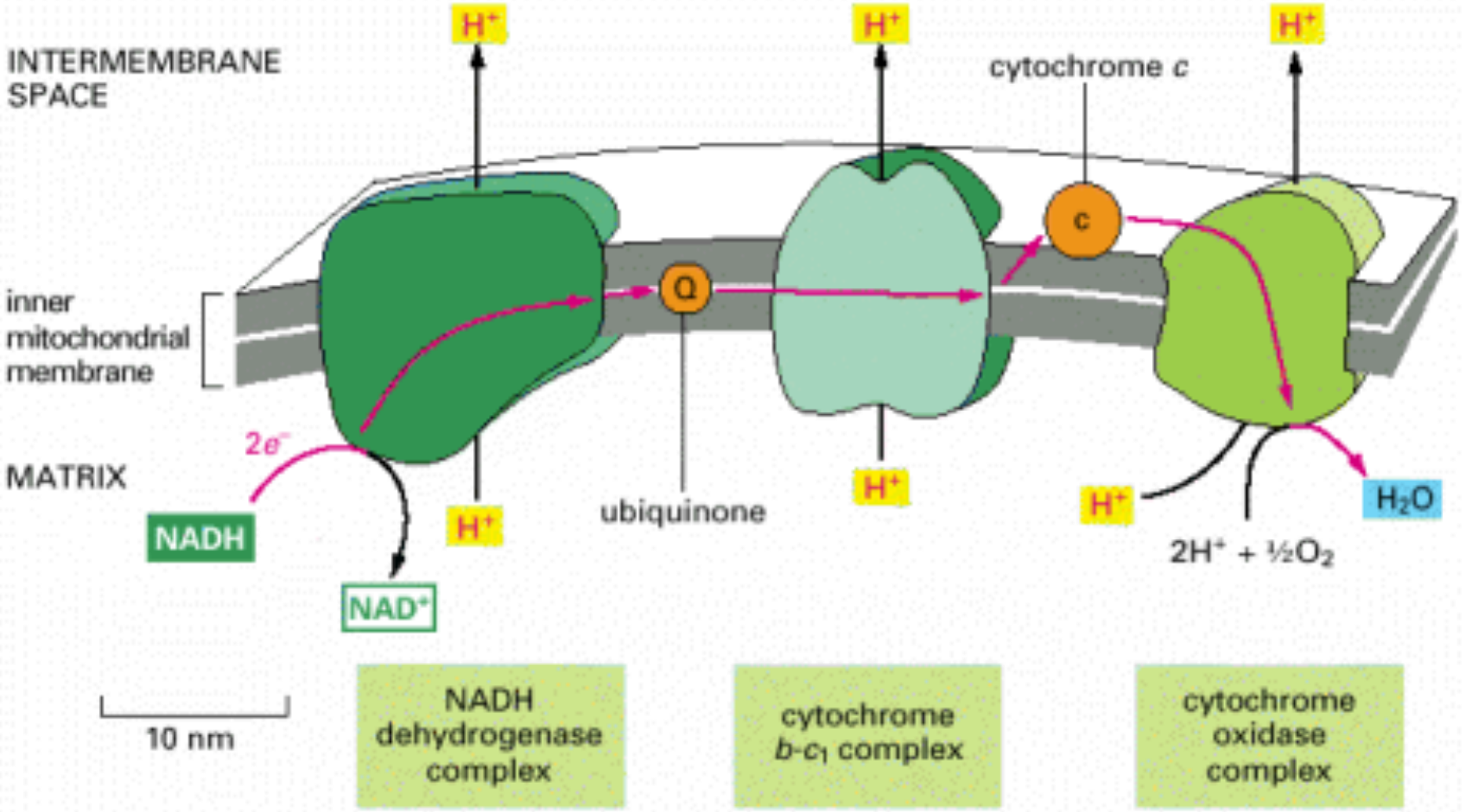


Figure 4.2 The mitochondrial respiratory chain consists of three proton pumps (complexes I, III and IV), which act in parallel with respect to the proton circuit and in series with respect to the electron flow.
Solid lines: pathway of proton flux; dotted line: pathway of electron transfer. For redox couples, see Table 3.2.



1. The NADH dehydrogenase complex (generally known as complex I) is the largest of the respiratory enzyme complexes, containing more than 40 polypeptide chains. It accepts electrons from NADH and passes them through a flavin and at least seven iron-sulfur centers to ubiquinone. Ubiquinone then transfers its electrons to a second respiratory enzyme complex, the cytochrome b-c_1 complex.

Molecular Biology of the Cell Bruce Alberts, Alexander Johnson, Julian Lewis, Martin Raff, Keith Roberts, and Peter Walter. 2002

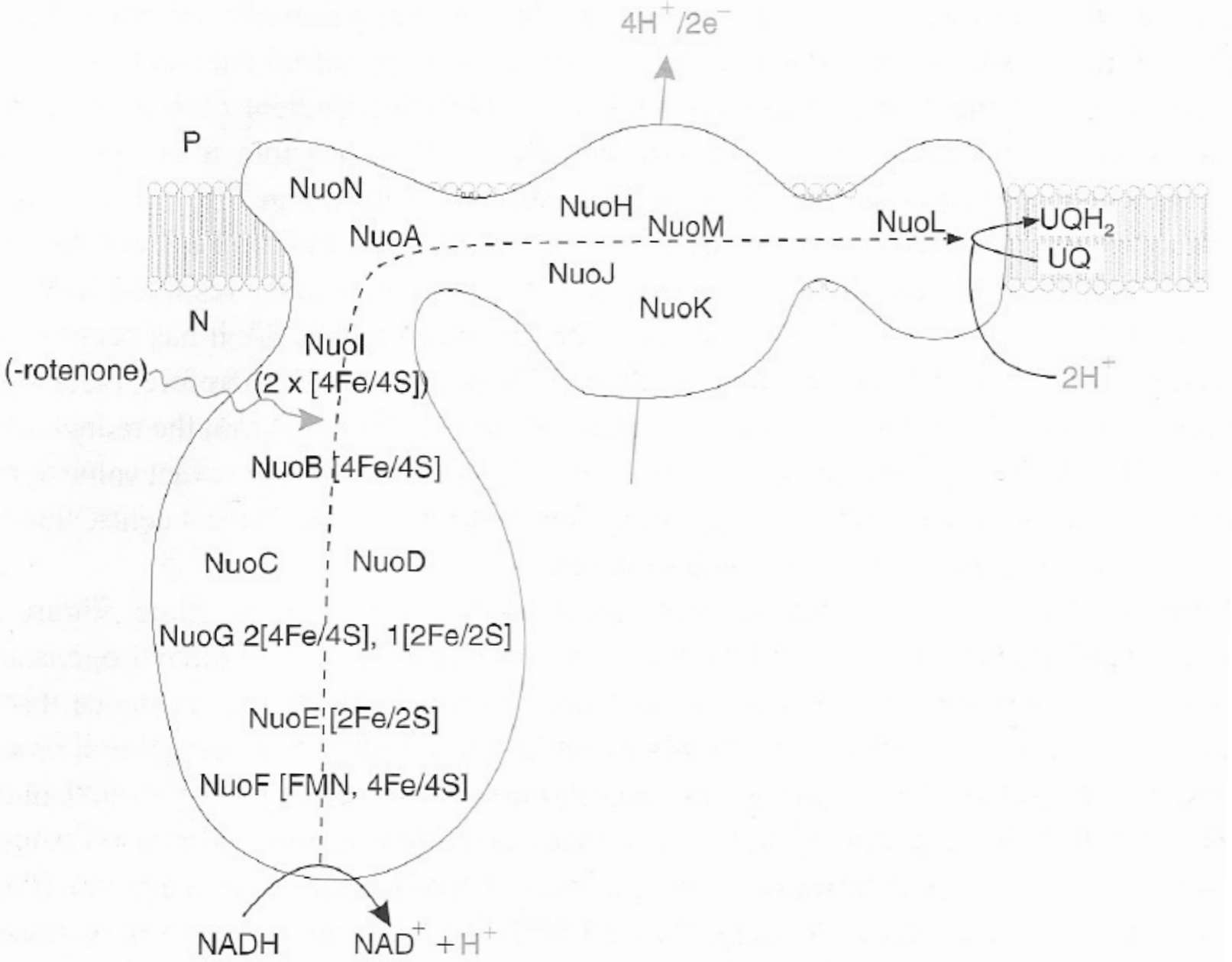
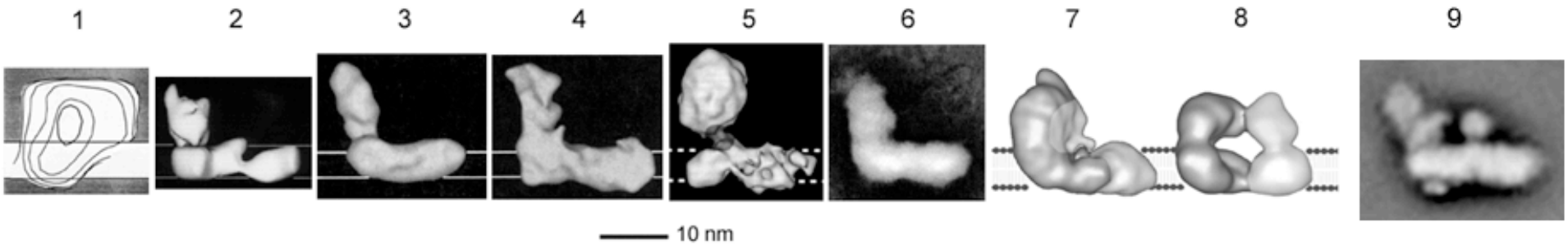


Figure 5.12 An outline structure for the mitochondrial (and bacterial) proton-translocating NADH–ubiquinone oxidoreductase (complex I).

EM images and 3D models of complex I



1. *N. crassa*. (Leonard et al., 1987)
2. *N. crassa*. (Hofhaus et al., 1991)
3. *N. crassa* (Guénebaut et al., 1997)
4. *E. coli NDH-1* (Guénebaut et al., 1998)
5. Bovine heart (Grigorieff, 1998)
6. *Y. lipolytica* (Djafarzadeha et al., 2000)

7 and 8. *E. coli* (Böttcher et al., 2002); 7 is "inactive" form, 8 is "active" form

9. *Arabidopsis* (Dudkina et al., 2005)

Relevant papers as pdf files. Please observe copyright

- Structure of the Hydrophilic Domain of Respiratory Complex I from Thermus thermophilus
- Structure of the Hydrophilic Domain of Respiratory Complex I from Thermus thermophilus pdf file
- The architecture of respiratory complex I
- Structure of the membrane domain of respiratory complex I
- Respiratory complex I: 'steam engine' of the cell?
- News and Views. Structural biology: Piston drives a proton pump
- Functional Modules and Structural Basis of Conformational Coupling in Mitochondrial Complex I
- An atypical haem in the cytochrome b_6f complex
- Structure of the Cytochrome b_6f Complex of Oxygenic Photosynthesis: Tuning the Cavity
- An Electronic Bus Bar Lies in the Core of Cytochrome bc_1
- Cytochrome b_6f: Structure for signalling and vectorial metabolism
- The Q cycle of cytochrome bc complexes: A structure perspective
- Protein synthesis by isolated pea mitochondria is dependent on the activity of respiratory complex II
- Energy transduction anchors genes in organelles
- The function of genomes in bioenergetic organelles
- Why do we still have a maternally inherited mitochondrial DNA? Insights from evolutionary medicine
- Power for life
- Chemiosmotic coupling: The Cost of Living Professor Peter Rich
- How did LUCA make a living? Chemiosmosis in the origin of life By Nick Lane, John F. Allen, William Martin
- Power Games By Nick Lane
- Photosynthesis - telling it like it is Review of Blankenship's Molecular Mechanisms of Photosynthesis

---

Nobel prizes

- 1978 Chemistry Nobel Prize to Peter Mitchell
- 1988 Chemistry Nobel Prize to Johann Deisenhofer, Robert Huber and Hartmut Michel
- 1997 Chemistry Nobel Prize to Paul D. Boyer, John E. Walker and Jens C. Skou

RESEARCH ARTICLE

Structure of the Hydrophilic Domain of Respiratory Complex I from *Thermus thermophilus*

Leonid A. Sazanov* and Philip Hinchliffe

Respiratory complex I plays a central role in cellular energy production in bacteria and mitochondria. Its dysfunction is implicated in many human neurodegenerative diseases, as well as in aging. The crystal structure of the hydrophilic domain (peripheral arm) of complex I from *Thermus thermophilus* has been solved at 3.3 angstrom resolution. This subcomplex consists of eight subunits and contains all the redox centers of the enzyme, including nine iron-sulfur clusters. The primary electron acceptor, flavin-mononucleotide, is within electron transfer distance of cluster N3, leading to the main redox pathway, and of the distal cluster N1a, a possible antioxidant. The structure reveals new aspects of the mechanism and evolution of the enzyme. The terminal cluster N2 is coordinated, uniquely, by two consecutive cysteines. The novel subunit Nqo15 has a similar fold to the mitochondrial iron chaperone frataxin, and it may be involved in iron-sulfur cluster regeneration in the complex.

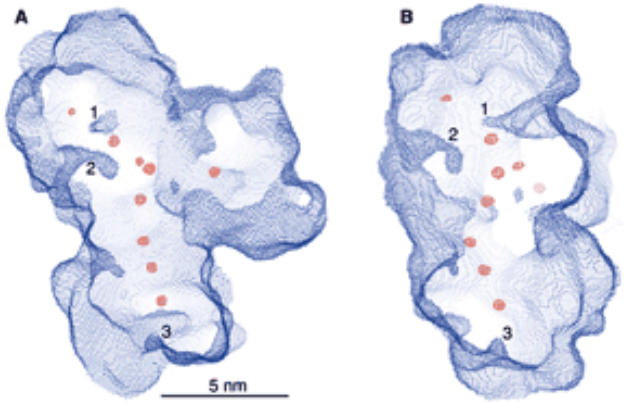
Structure of the Hydrophilic Domain of Respiratory Complex I from *Thermus thermophilus*

Leonid A. Sazanov* and Philip Hinchliffe

Respiratory complex I plays a central role in cellular energy production in bacteria and mitochondria. Its dysfunction is implicated in many human neurodegenerative diseases, as well as in aging. The crystal structure of the hydrophilic domain (peripheral arm) of complex I from *Thermus thermophilus* has been solved at 3.3 angstrom resolution. This subcomplex consists of eight subunits and contains all the redox centers of the enzyme, including nine iron-sulfur clusters. The primary electron acceptor, flavin-mononucleotide, is within electron transfer distance of cluster N3, leading to the main redox pathway, and of the distal cluster N1a, a possible antioxidant. The structure reveals new aspects of the mechanism and evolution of the enzyme. The terminal cluster N2 is coordinated, uniquely, by two consecutive cysteines. The novel subunit Nqo15 has a similar fold to the mitochondrial iron chaperone frataxin, and it may be involved in iron-sulfur cluster regeneration in the complex.

Medical Research Council Dunn Human Nutrition Unit, Wellcome Trust/MRC Building, Hills Road, Cambridge CB2 2XY, U.K.

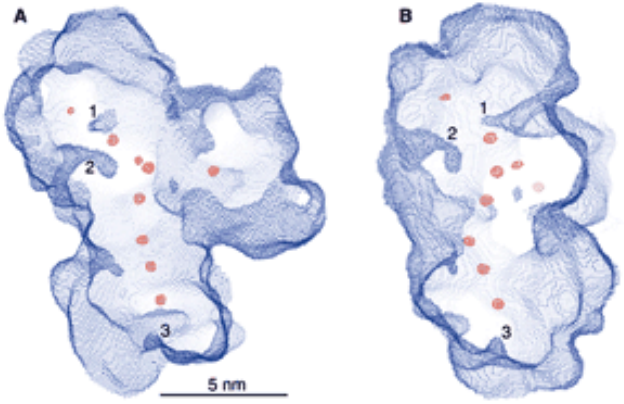
Hot news on the 3D structure: Hinchliffe and Sazanov recently reported the arrangement of iron-sulfur clusters in the hydrophilic domain of complex I from *Thermus thermophilus* as determined by x-ray crystallography.



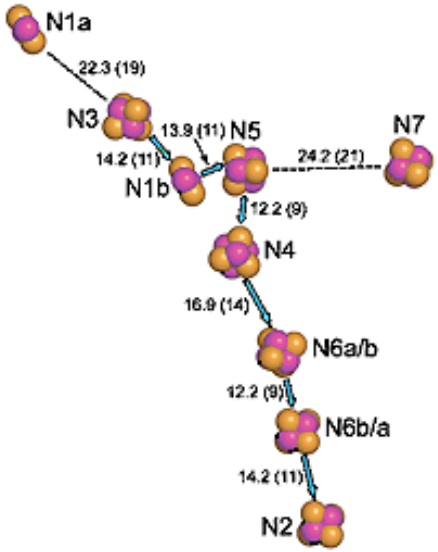
The electron density of Fe-S clusters shown within the molecular surface of the peripheral arm

Reference: Hinchliffe, P. and Sazanov, L. A. (2005) **Organization of iron-sulfur clusters in respiratory complex I**. *Science* **309**:771-774.

Hot news on the 3D structure: Hinchliffe and Sazanov recently reported the arrangement of iron-sulfur clusters in the hydrophilic domain of complex I from *Thermus thermophilus* as determined by x-ray crystallography.

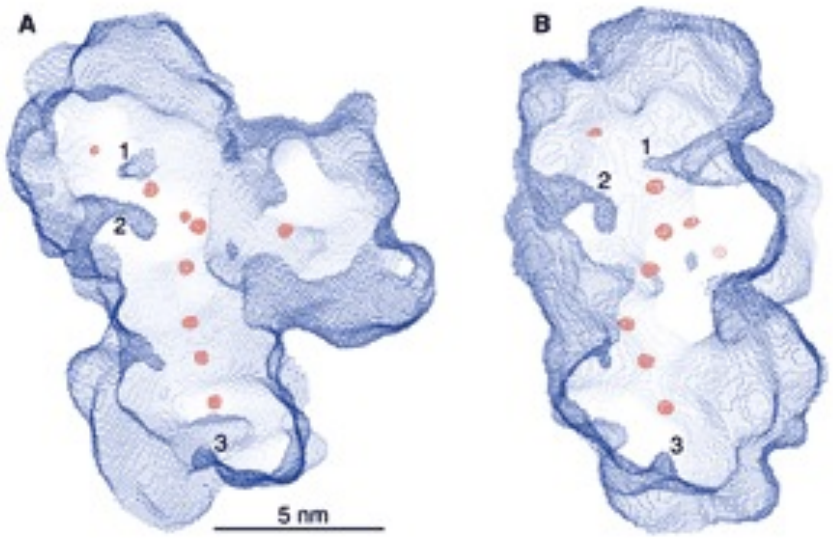


The electron density of Fe-S clusters shown within the molecular surface of the peripheral arm

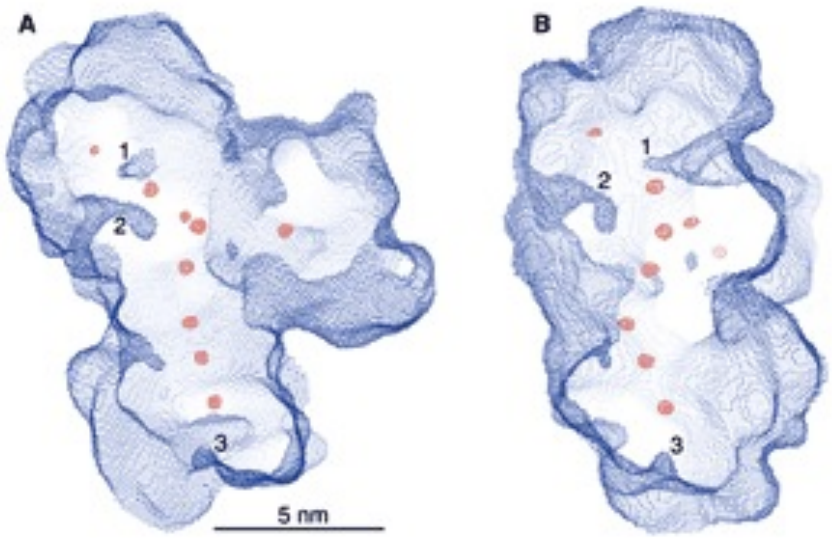


Arrangement of Fe-S clusters in complex I

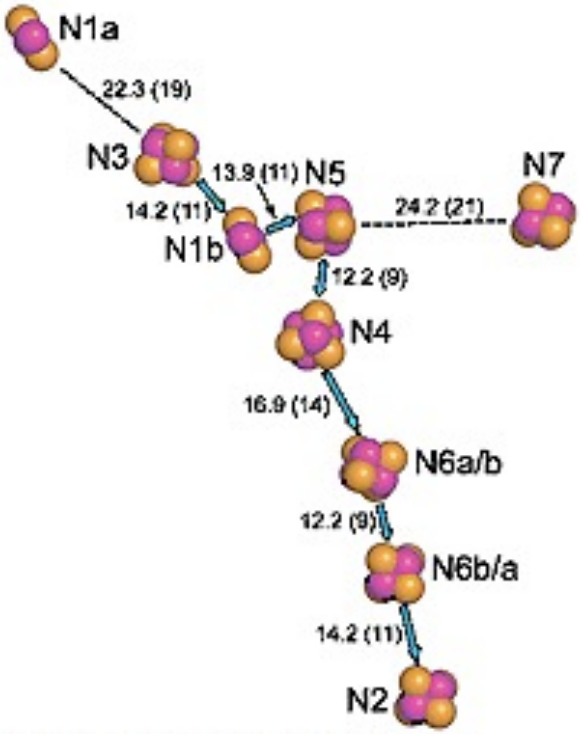
Reference: Hinchliffe, P. and Sazanov, L. A. (2005) **Organization of iron-sulfur clusters in respiratory complex I**. *Science* **309**:771-774. Reprinted with permissions from AAAS. Permission from AAAS is required for all other uses.



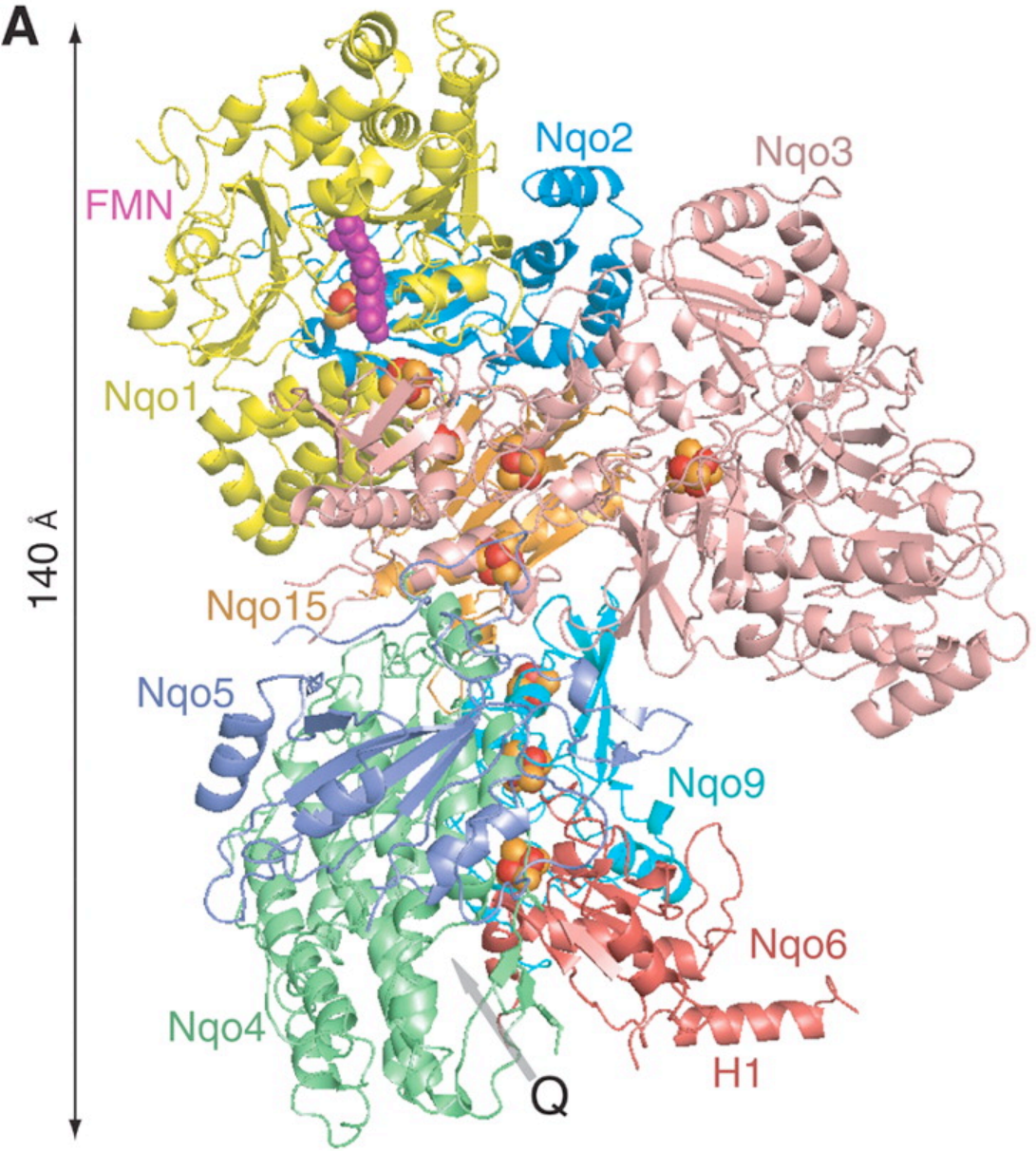
The electron density of Fe-S clusters shown within the molecular surface of the peripheral arm

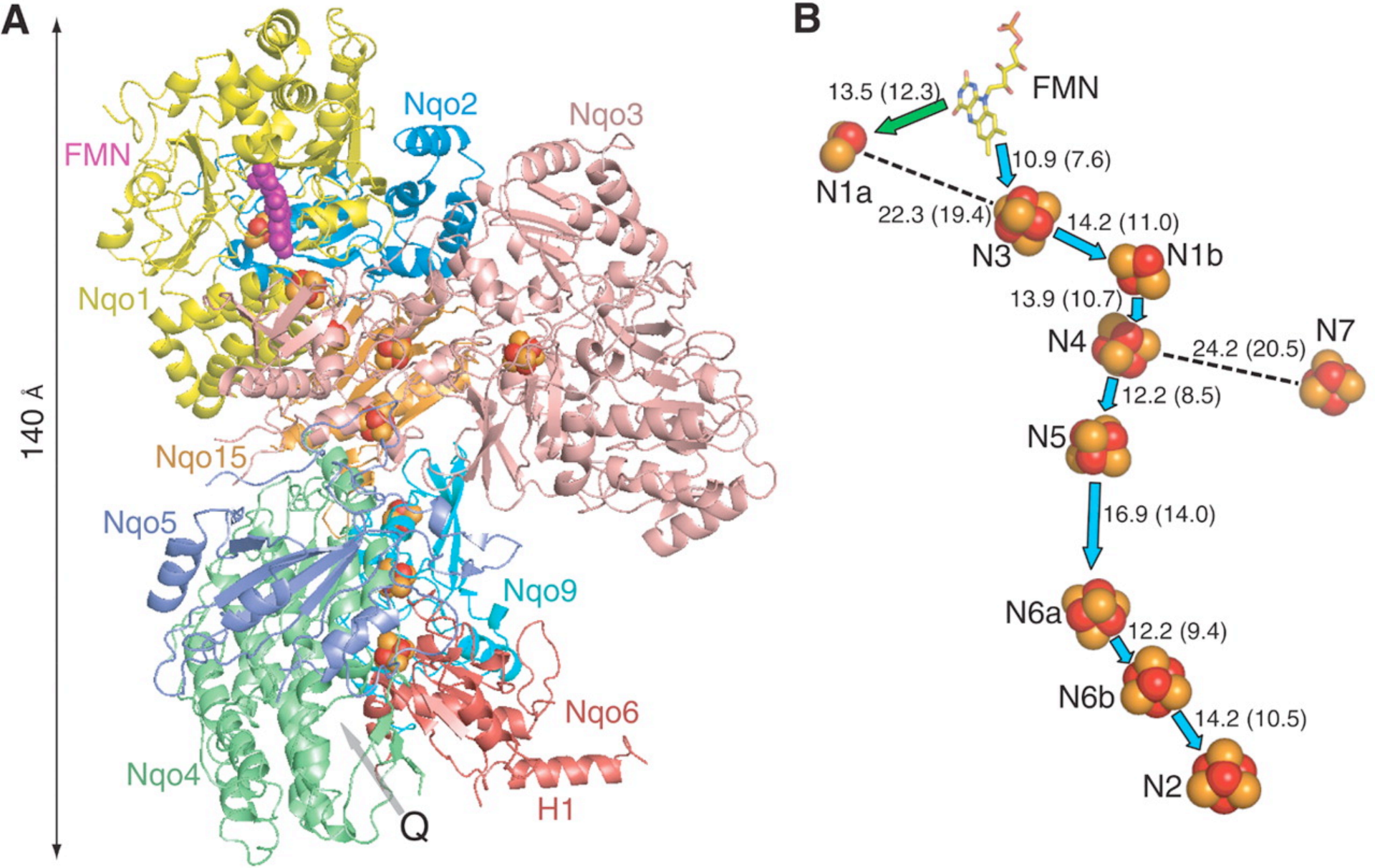


The electron density of Fe-S clusters shown within the molecular surface of the peripheral arm



Arrangement of Fe-S clusters in complex I


A
140 Å
FMN
Nqo2
Nqo3
Nqo1
Nqo15
Nqo5
Nqo9
Nqo6
Nqo4
H1
Q


A
140 Å
FMN
Nqo2
Nqo3
Nqo1
Nqo15
Nqo5
Nqo9
Nqo6
Nqo4
H1
Q
B
FMN
13.5 (12.3)
N1a
10.9 (7.6)
22.3 (19.4)
N3
14.2 (11.0)
N1b
13.9 (10.7)
N4
24.2 (20.5)
N7
12.2 (8.5)
N5
16.9 (14.0)
N6a
12.2 (9.4)
N6b
14.2 (10.5)
N2

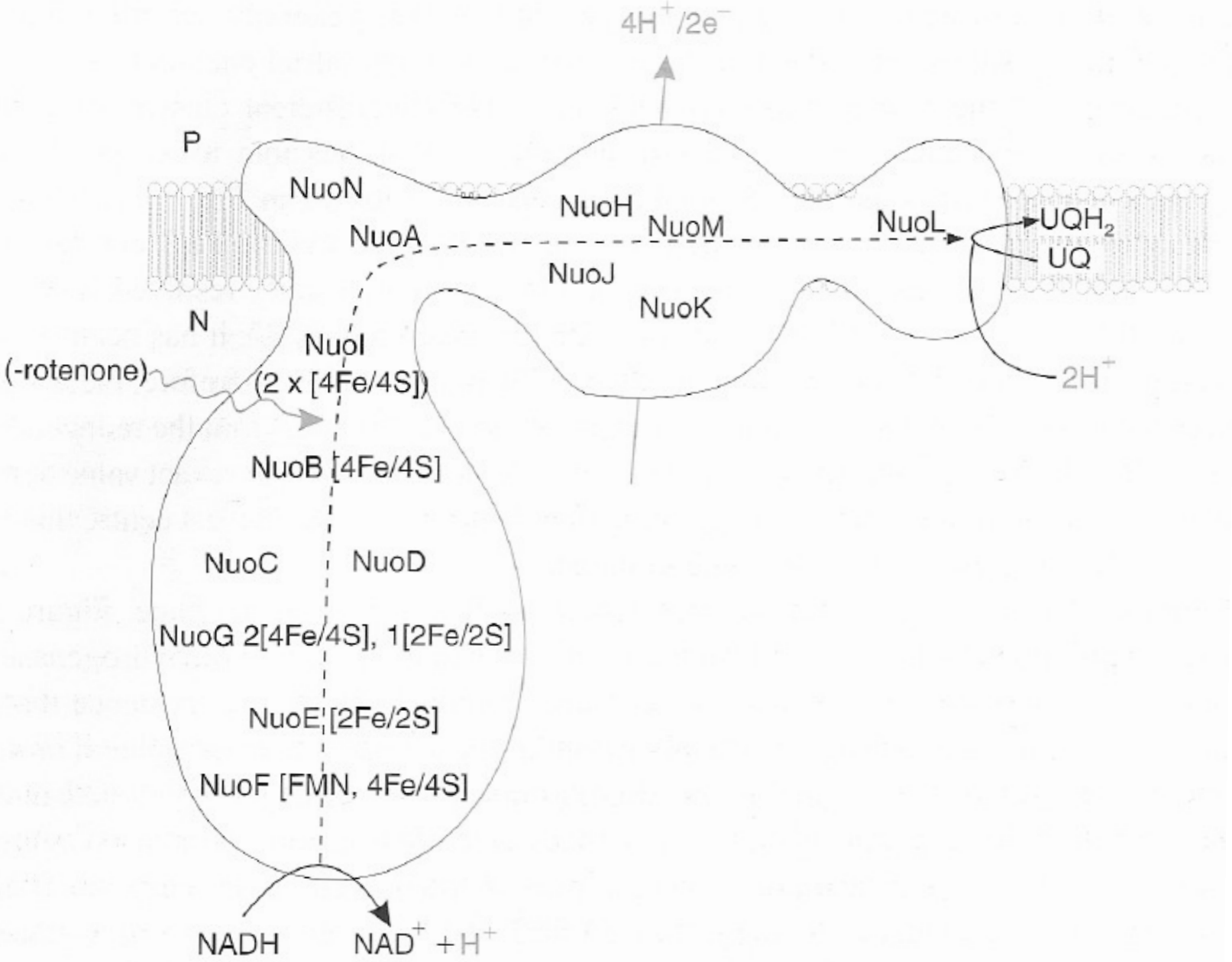
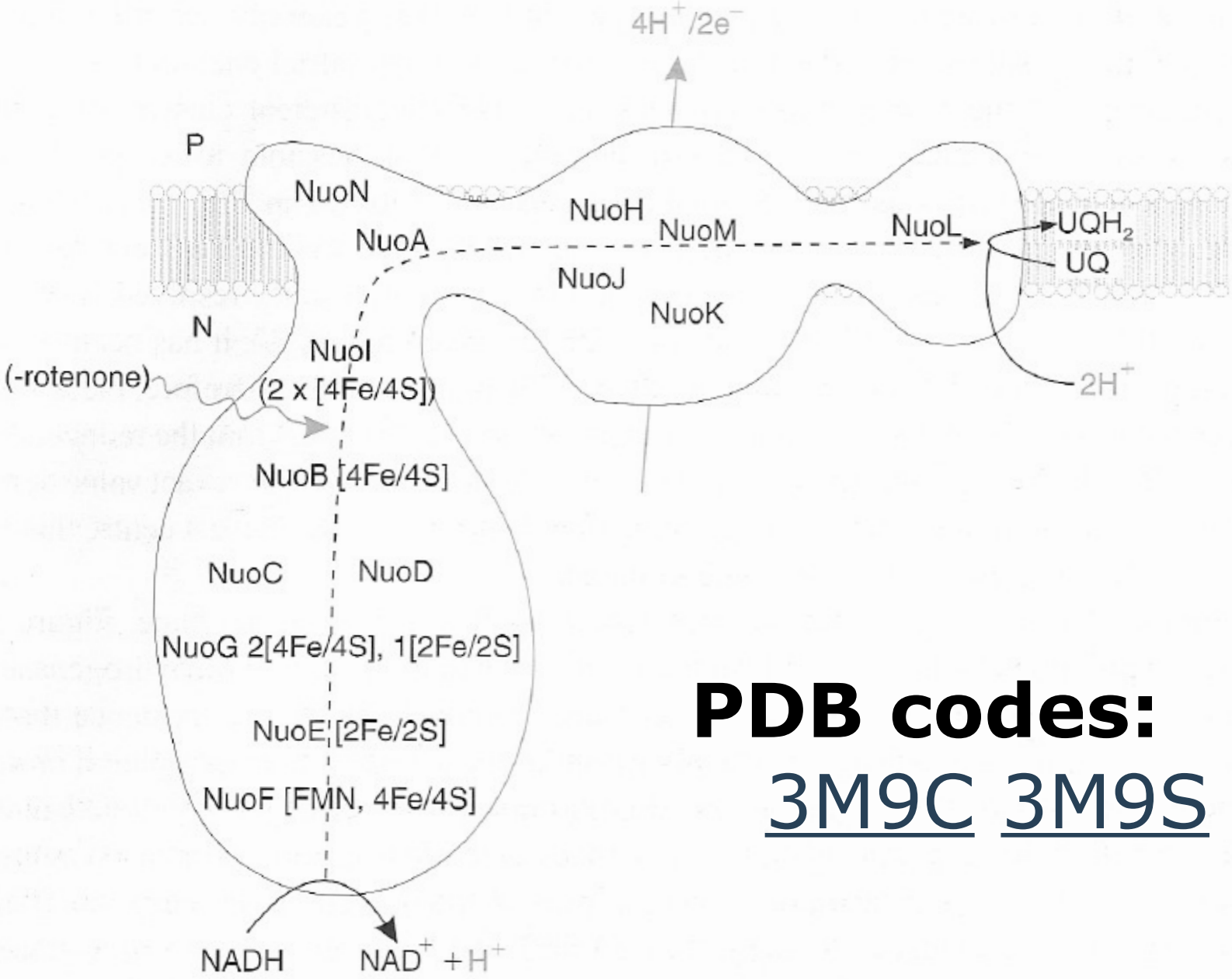


Figure 5.12 An outline structure for the mitochondrial (and bacterial) proton-translocating NADH–ubiquinone oxidoreductase (complex I).



PDB codes:

3M9C 3M9S

Figure 5.12 An outline structure for the mitochondrial (and bacterial) proton-translocating NADH–ubiquinone oxidoreductase (complex I).

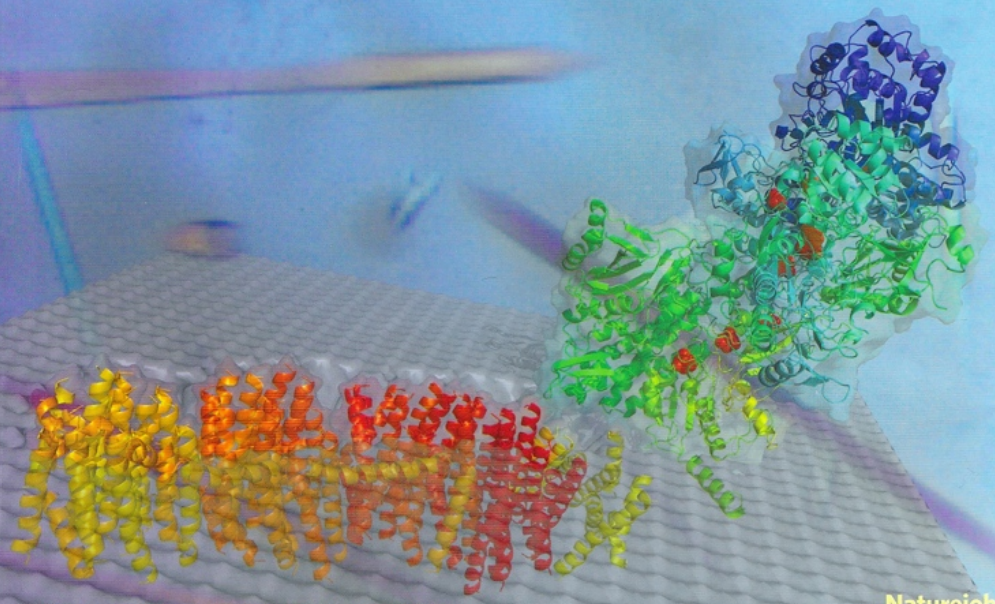
27 May 2010 | www.nature.com/nature | £10

THE INTERNATIONAL WEEKLY JOURNAL OF SCIENCE

nature

POWER BASE

Architecture of respiratory complex I



GULF OF MEXICO OIL SPILL
Testing the waters

AIRPORT SECURITY
Science and pseudoscience

THE SIMPLE LIFE
That 'synthetic cell' in context

Naturejobs
Bioscience in Denmark

9 770028 083095 21

Vol 465|27 May 2010|doi:10.1038/nature09066 nature

ARTICLES

The architecture of respiratory complex I

Rouslan G. Efremov*, Rozbeh Baradaran* & Leonid A. Sazanov

Complex I is the first enzyme of the respiratory chain and has a central role in cellular energy production, coupling electron transfer between NADH and quinone to proton translocation by an unknown mechanism. Dysfunction of complex I has been implicated in many human neurodegenerative diseases. We have determined the structure of its hydrophilic domain previously. Here, we report the α-helical structure of the membrane domain of complex I from *Escherichia coli* at 3.9 Å resolution. The antiporter-like subunits NuoL/M/N each contain 14 conserved transmembrane (TM) helices. Two of them are discontinuous, as in some transporters. Unexpectedly, subunit NuoL also contains a 110-Å long amphipathic α-helix, spanning almost the entire length of the domain. Furthermore, we have determined the structure of the entire complex I from *Thermus thermophilus* at 4.5 Å resolution. The L-shaped assembly consists of the α-helical model for the membrane domain, with 63 TM helices, and the known structure of the hydrophilic domain. The architecture of the complex provides strong clues about the coupling mechanism: the conformational changes at the interface of the two main domains may drive the long amphipathic α-helix of NuoL in a piston-like motion, tilting nearby discontinuous TM helices, resulting in proton translocation.

Medical Research Council Mitochondrial Biology Unit, Wellcome Trust/MRC Building, Hills Road, Cambridge CB2 0XY, UK.

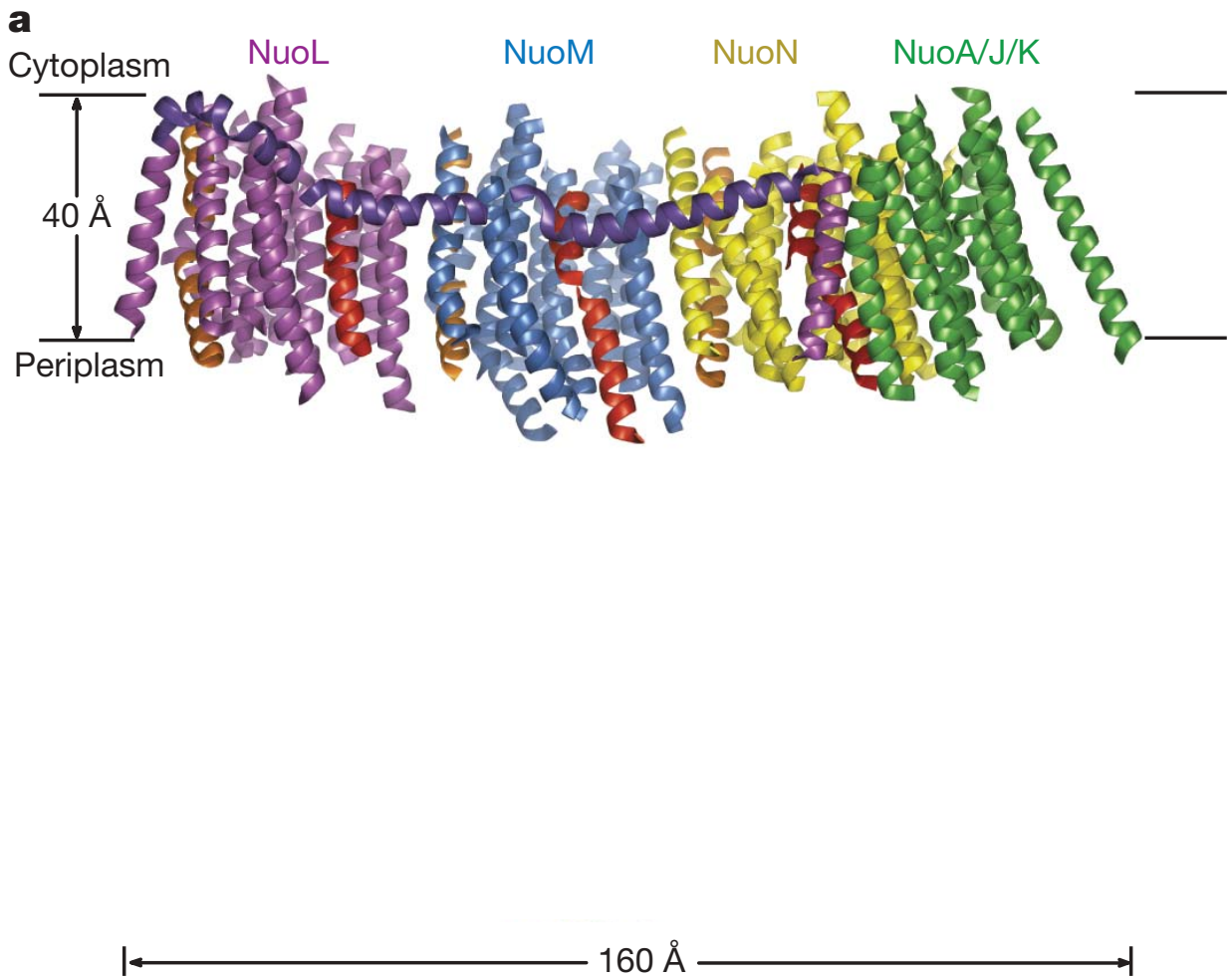


Figure 1 | The α-helical model of the membrane domain of *E. coli* complex I. **a**, Side view, in the membrane plane. **b**, View from the periplasm into the membrane. Subunit NuoL is magenta, NuoM blue, NuoN yellow and subunits NuoA/J/K green. Helix HL from NuoL is in a darker colour as indicated. The expected position of the lipid bilayer is shown. The cytoplasmic and periplasmic sides are identified in accordance with the structure of the entire complex (Fig. 3). Two discontinuous helices present in each of subunits NuoL, M and N are shown in red for those in contact with helix HL, and in orange for those farther away.

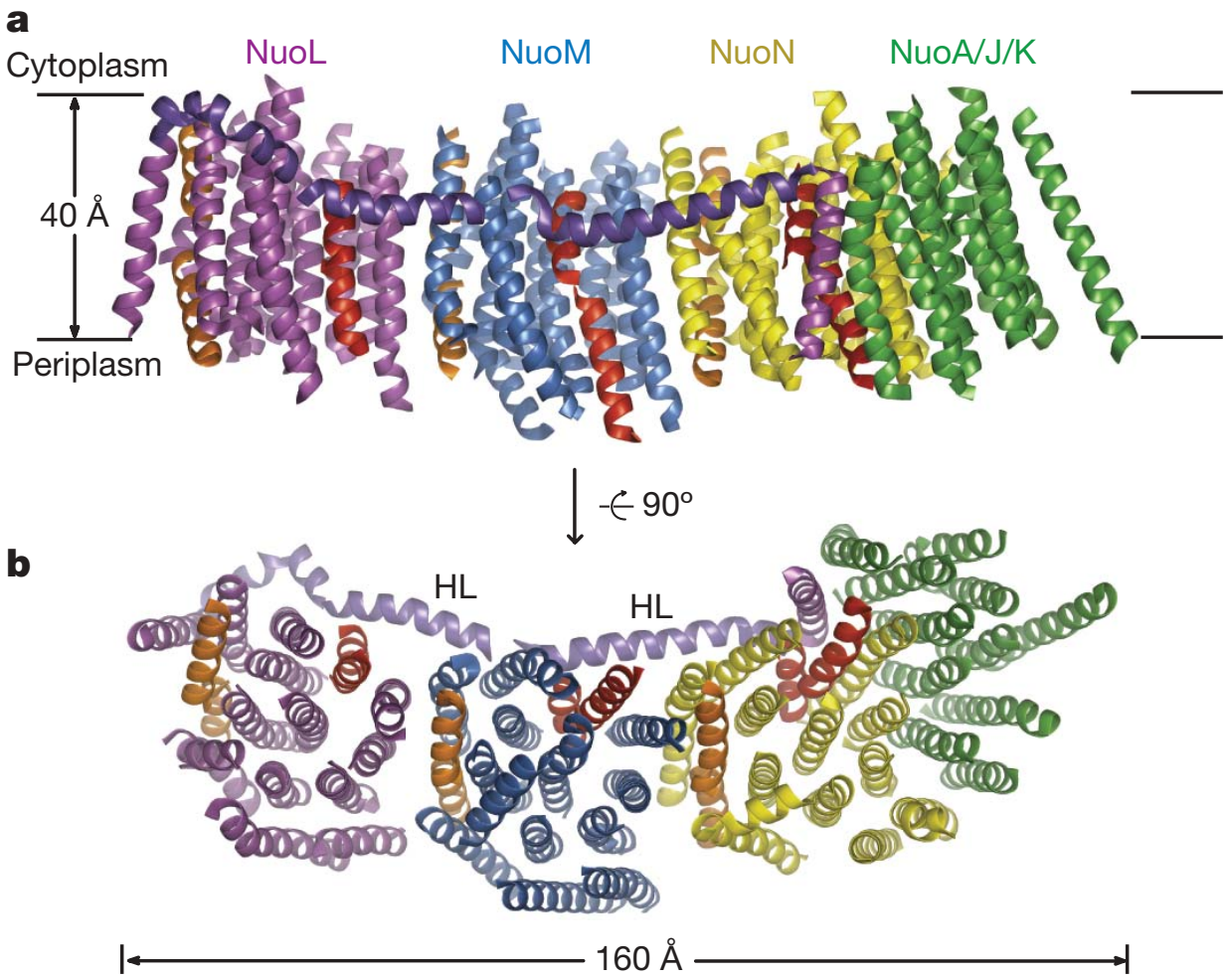


Figure 1 | The α-helical model of the membrane domain of *E. coli* complex I. **a**, Side view, in the membrane plane. **b**, View from the periplasm into the membrane. Subunit NuoL is magenta, NuoM blue, NuoN yellow and subunits NuoA/J/K green. Helix HL from NuoL is in a darker colour as indicated. The expected position of the lipid bilayer is shown. The cytoplasmic and periplasmic sides are identified in accordance with the structure of the entire complex (Fig. 3). Two discontinuous helices present in each of subunits NuoL, M and N are shown in red for those in contact with helix HL, and in orange for those farther away.

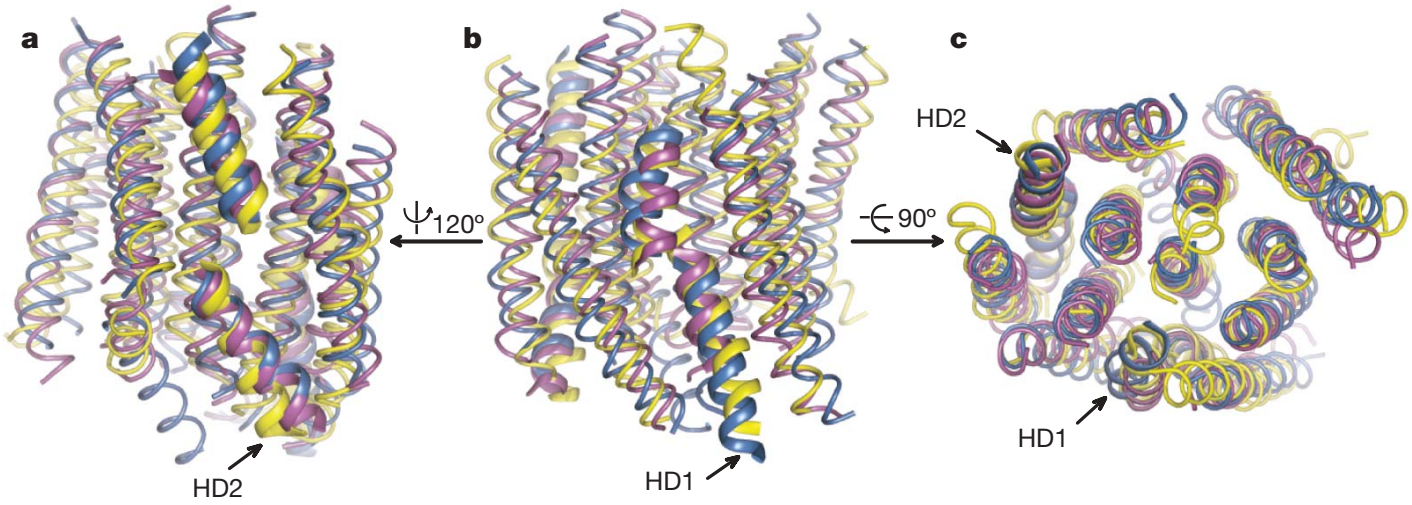
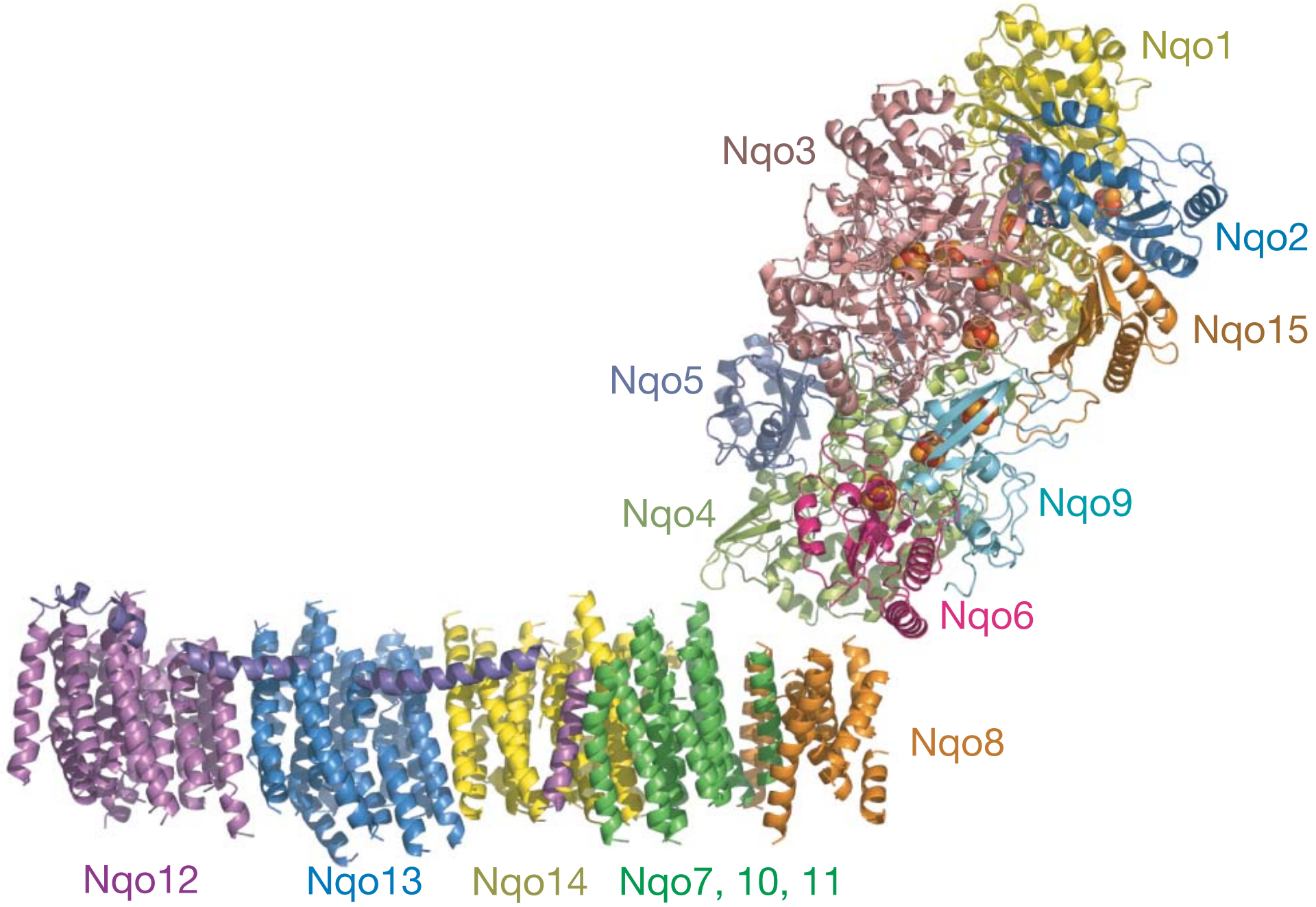


Figure 2 | Structural alignment of the antiporter-like subunits NuoL, NuoM and NuoN of *E. coli* complex I. **a**, **b**, Side views in the membrane plane, with the cytoplasmic side top. **c**, View from the cytoplasm into the membrane. The discontinuous helices are highlighted and indicated as HD1 for those in contact with helix HL and as HD2 for those farther away. Lipid-exposed helix next to HD2 (seen to the left of it in **a**) resembles the discontinuous one, but its main part approaches normal TM length and the nearby short helix is nearly parallel to the bilayer. Helices HD1/HD2 do not form an interacting pair, as is usual[24], but are separated by a layer of helices (**c**). Helices from the C-terminal extension of subunit NuoL, absent in NuoM and NuoN, are omitted. The colour scheme is the same as in Fig. 1.


Nqo1
Nqo3
Nqo2
Nqo15
Nqo5
Nqo4
Nqo9
Nqo6
Nqo8
Nqo12
Nqo13
Nqo14
Nqo7, 10, 11

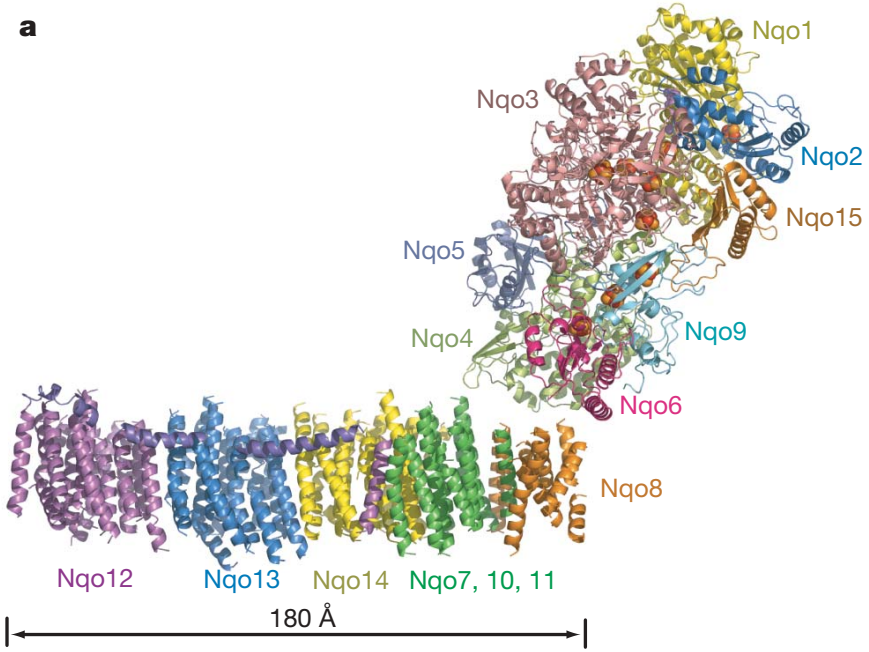


Figure 3 | The structure of the entire complex I from *T. thermophilus*. The structure consists of the atomic model for the hydrophilic domain, determined previously[12,13] (PDB 3I9V), and the α-helical model for the membrane domain. **a**, **b**, Side views, in the membrane plane. **c**, Top view, from the cytoplasm into the membrane. **d**, The likely quinone-binding cavity (Q) at the interface of the two main domains. Helix H1 from subunit Nqo6, the 4-helix bundle from subunit Nqo4 (4HB, in darker colour), helix TM1 from subunit Nqo8 and cluster N2 are indicated. Fe-S clusters are shown as red and yellow spheres, and FMN as magenta spheres. Each subunit is coloured differently and indicated.

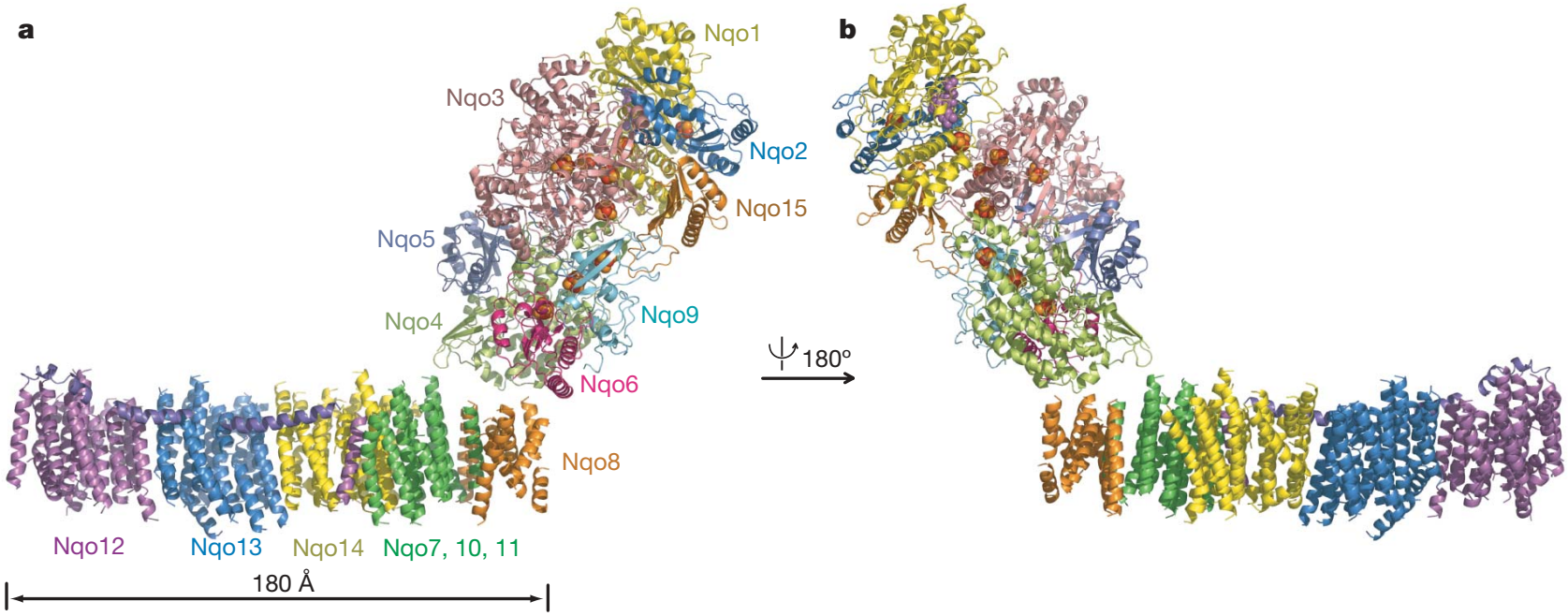


Figure 3 | The structure of the entire complex I from *T. thermophilus*. The structure consists of the atomic model for the hydrophilic domain, determined previously[12,13] (PDB 3I9V), and the α-helical model for the membrane domain. **a**, **b**, Side views, in the membrane plane. **c**, Top view, from the cytoplasm into the membrane. **d**, The likely quinone-binding cavity (Q) at the interface of the two main domains. Helix H1 from subunit Nqo6, the 4-helix bundle from subunit Nqo4 (4HB, in darker colour), helix TM1 from subunit Nqo8 and cluster N2 are indicated. Fe-S clusters are shown as red and yellow spheres, and FMN as magenta spheres. Each subunit is coloured differently and indicated.

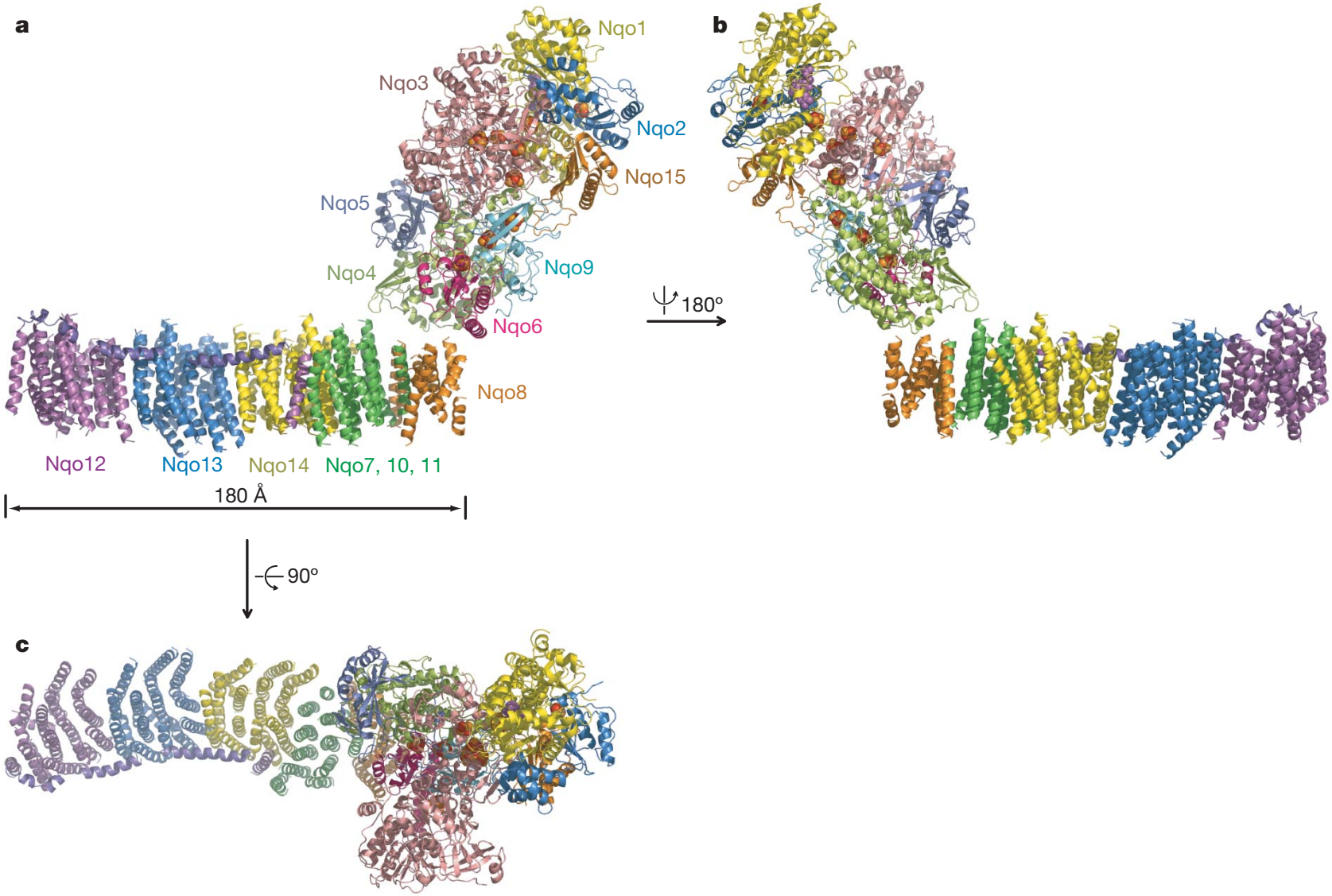


Figure 3 | The structure of the entire complex I from *T. thermophilus*. The structure consists of the atomic model for the hydrophilic domain, determined previously[12,13] (PDB 3I9V), and the α-helical model for the membrane domain. **a**, **b**, Side views, in the membrane plane. **c**, Top view, from the cytoplasm into the membrane. **d**, The likely quinone-binding cavity (Q) at the interface of the two main domains. Helix H1 from subunit Nqo6, the 4-helix bundle from subunit Nqo4 (4HB, in darker colour), helix TM1 from subunit Nqo8 and cluster N2 are indicated. Fe-S clusters are shown as red and yellow spheres, and FMN as magenta spheres. Each subunit is coloured differently and indicated.

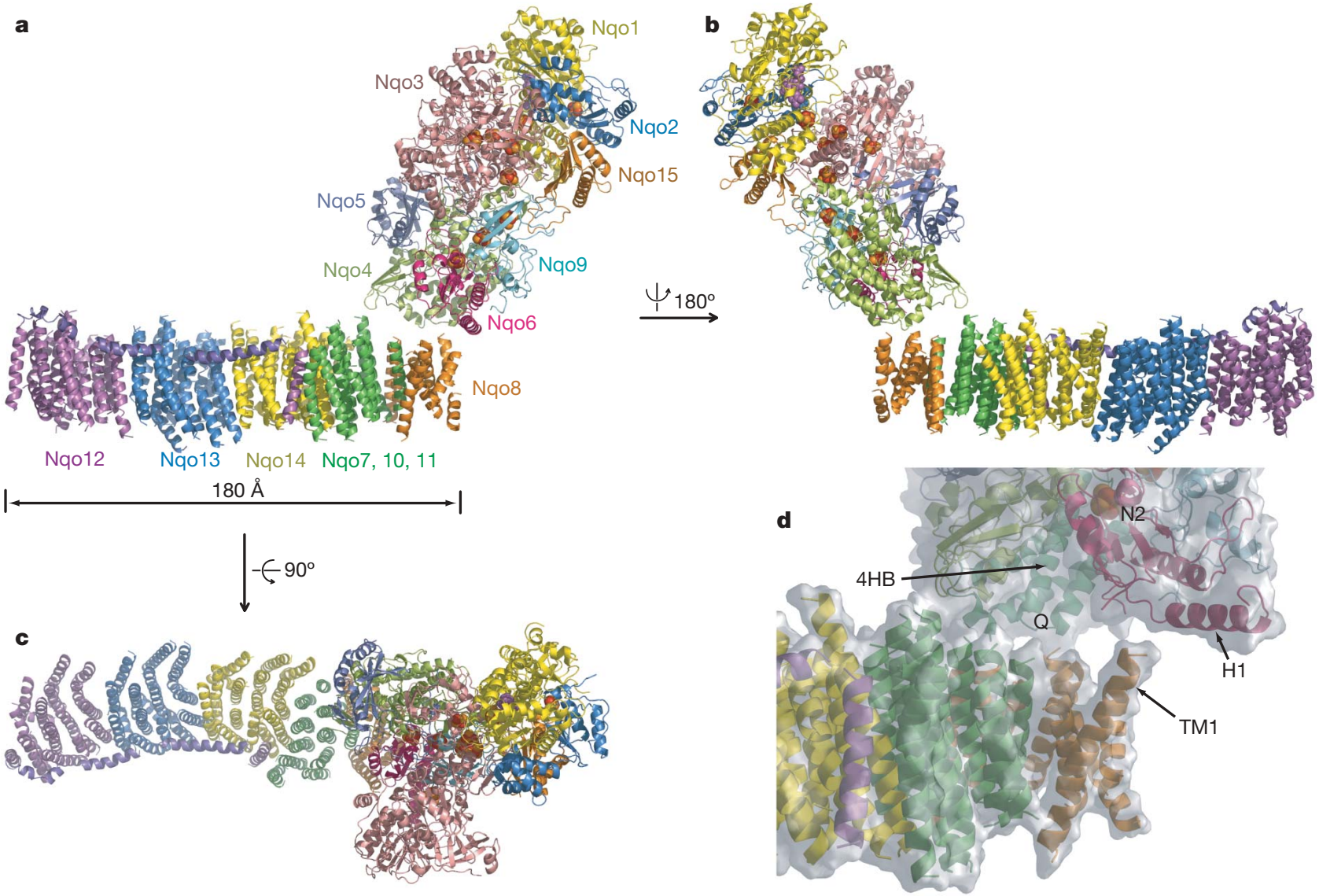


Figure 3 | The structure of the entire complex I from *T. thermophilus*. The structure consists of the atomic model for the hydrophilic domain, determined previously[12,13] (PDB 3I9V), and the α-helical model for the membrane domain. **a**, **b**, Side views, in the membrane plane. **c**, Top view, from the cytoplasm into the membrane. **d**, The likely quinone-binding cavity (Q) at the interface of the two main domains. Helix H1 from subunit Nqo6, the 4-helix bundle from subunit Nqo4 (4HB, in darker colour), helix TM1 from subunit Nqo8 and cluster N2 are indicated. Fe-S clusters are shown as red and yellow spheres, and FMN as magenta spheres. Each subunit is coloured differently and indicated.

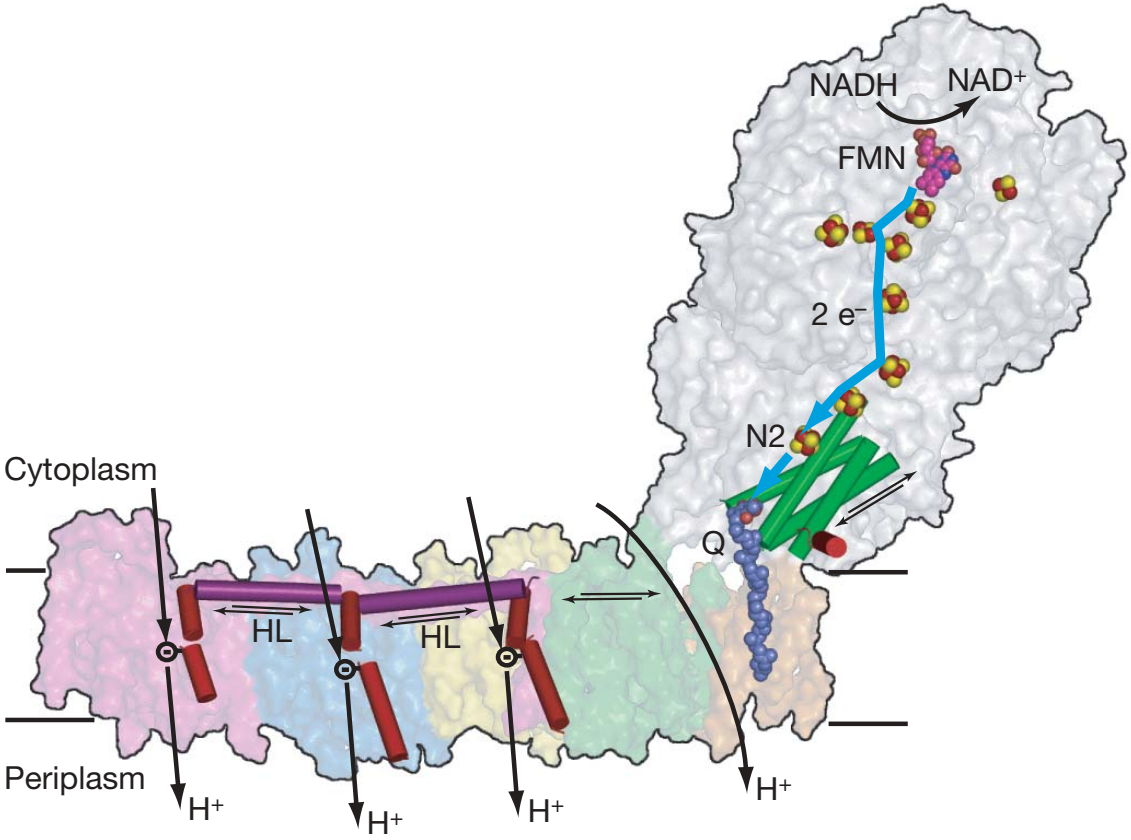


Figure 4 | Proposed model of proton translocation by complex I. NADH, via FMN (magenta), donates two electrons to the chain of Fe-S clusters (red and yellow spheres), which are passed on (blue line), via terminal cluster N2, to the quinone (dark blue, moved out of the membrane by about 10 Å). Electron transfer is coupled to conformational changes (indicated by arrows) in the hydrophilic domain, observed[13] for Nqo4 four-helix bundle (green cylinders) and Nqo6 helix H1 (red). These changes are transmitted to the amphipathic helix HL (magenta), which tilts three discontinuous helices (red) in antiporter-like subunits, changing the conformation of ionizable residue inside respective proton channels, resulting in translocation of three protons. The fourth proton is translocated at the interface of the two main domains. The hydrophilic domain surface is shown in grey, whereas the membrane domain surface is coloured as in Fig. 3.

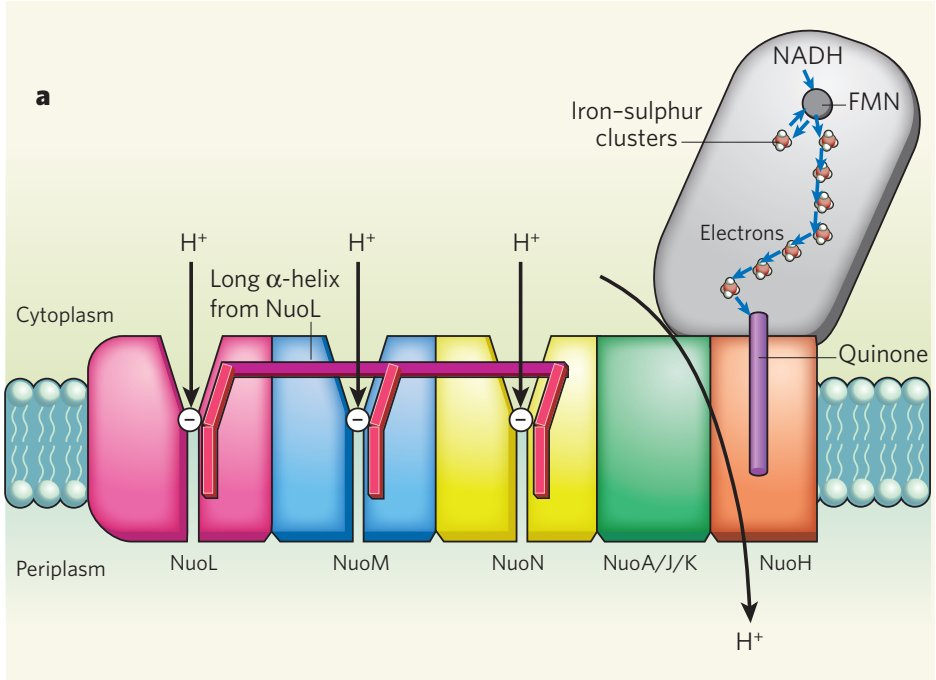


Figure 1 | Indirect coupling of electron transfer to proton pumping in complex I. Complex I, an enzyme found in mitochondrial and bacterial membranes, converts energy by coupling electron transfer to proton pumping. Sazanov and colleagues' crystal structures[1] of bacterial complex I reveal that the transmembrane NuoL subunit of the enzyme projects a long α-helix through the adjacent NuoM and NuoN subunits. They suggest the following mechanism to explain how electron transfer drives proton pumping. **a**, Pairs of electrons from the metabolic intermediate NADH are transferred to a cofactor (flavin mononucleotide, FMN) and then passed along a chain of iron–sulphur clusters in the extramembrane region of complex I, eventually reaching a quinone cofactor; blue arrows indicate the electron-transfer pathway. This allows a proton (H^+) to pass through complex I at the interface of the extra- and intramembrane regions. Protons can also enter channels in NuoL, NuoM and NuoN from the cytoplasm, but cannot pass through. White circles with minus signs represent negatively charged amino acids, which are key to proton transport. **b**, Conformational changes in the NuoA/J/K/H subunits push the long α-helix towards the other transmembrane subunits. This tilts three other helices in NuoL, NuoM and NuoN, causing the reorientation of certain residues in the subunits' channels. These local conformational changes allow protons in the channels to pass through the channels and enter the periplasm (the space between the inner and outer bacterial membranes).

Ohnishi, T. (2010) Structural Biology. Piston drives and proton pump. Nature 465, 428-429

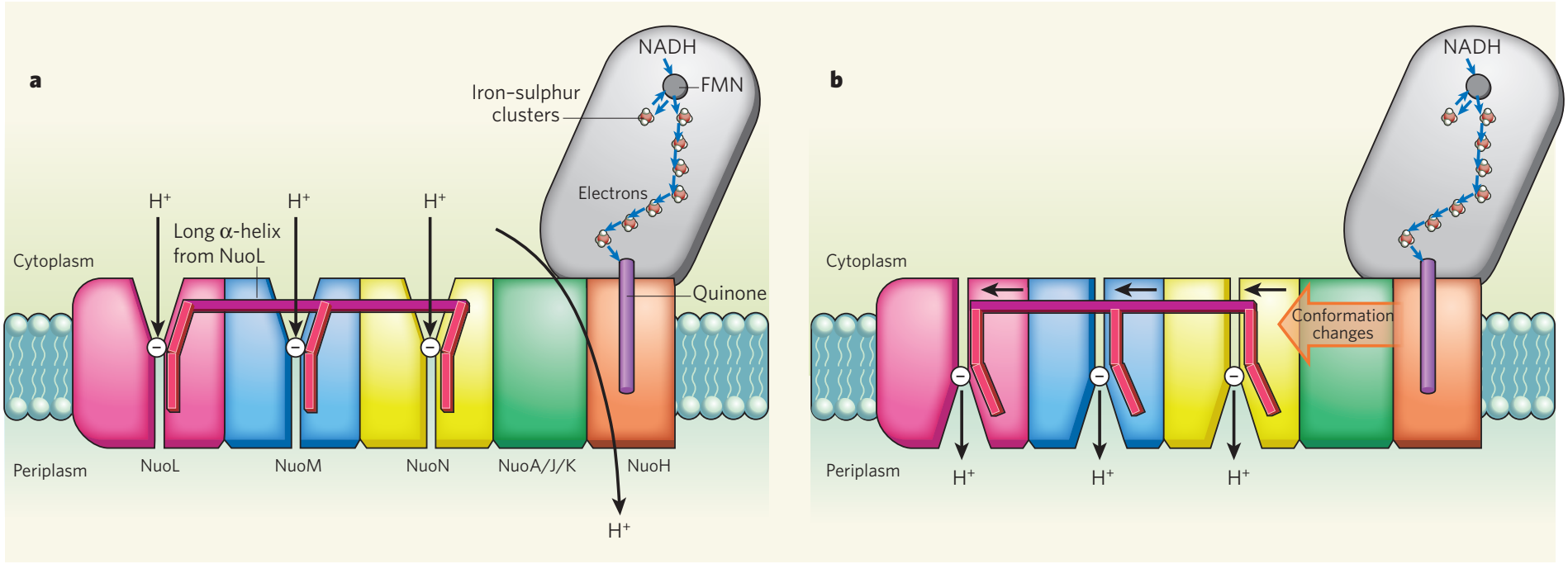


Figure 1 | Indirect coupling of electron transfer to proton pumping in complex I. Complex I, an enzyme found in mitochondrial and bacterial membranes, converts energy by coupling electron transfer to proton pumping. Sazanov and colleagues' crystal structures[1] of bacterial complex I reveal that the transmembrane NuoL subunit of the enzyme projects a long α-helix through the adjacent NuoM and NuoN subunits. They suggest the following mechanism to explain how electron transfer drives proton pumping. **a**, Pairs of electrons from the metabolic intermediate NADH are transferred to a cofactor (flavin mononucleotide, FMN) and then passed along a chain of iron–sulphur clusters in the extramembrane region of complex I, eventually reaching a quinone cofactor; blue arrows indicate the electron-transfer pathway. This allows a proton (H^+) to pass through complex I at the interface of the extra- and intramembrane regions. Protons can also enter channels in NuoL, NuoM and NuoN from the cytoplasm, but cannot pass through. White circles with minus signs represent negatively charged amino acids, which are key to proton transport. **b**, Conformational changes in the NuoA/J/K/H subunits push the long α-helix towards the other transmembrane subunits. This tilts three other helices in NuoL, NuoM and NuoN, causing the reorientation of certain residues in the subunits' channels. These local conformational changes allow protons in the channels to pass through the channels and enter the periplasm (the space between the inner and outer bacterial membranes).

Ohnishi, T. (2010) Structural Biology. Piston drives and proton pump. Nature 465, 428-429

Functional Modules and Structural Basis of Conformational Coupling in Mitochondrial Complex I

Carola Hunte,[1,2,3]* Volker Zickermann,[4]* Ulrich Brandt[4]†

Proton-pumping respiratory complex I is one of the largest and most complicated membrane protein complexes. Its function is critical for efficient energy supply in aerobic cells, and malfunctions are implicated in many neurodegenerative disorders. Here, we report an x-ray crystallographic analysis of mitochondrial complex I. The positions of all iron-sulfur clusters relative to the membrane arm were determined in the complete enzyme complex. The ubiquinone reduction site resides close to 30 angstroms above the membrane domain. The arrangement of functional modules suggests conformational coupling of redox chemistry with proton pumping and essentially excludes direct mechanisms. We suggest that a ~60-angstrom-long helical transmission element is critical for transducing conformational energy to proton-pumping elements in the distal module of the membrane arm.

[1]Institute for Biochemistry and Molecular Biology, Centre for Biological Signalling Studies (BIOSS), University of Freiburg, D-79104 Freiburg, Germany. [2]Institute of Membrane and Systems Biology, University of Leeds, Leeds LS2 9JT, UK. [3]Department of Molecular Membrane Biology, Max Planck Institute of Biophysics, D-60438 Frankfurt am Main, Germany. [4]Molecular Bioenergetics Group, Medical School, Cluster of Excellence Frankfurt "Macromolecular Complexes," Center for Membrane Proteomics, Goethe-University, D-60596 Frankfurt am Main, Germany.

23 JULY 2010 VOL 329 SCIENCE www.sciencemag.org 448-451

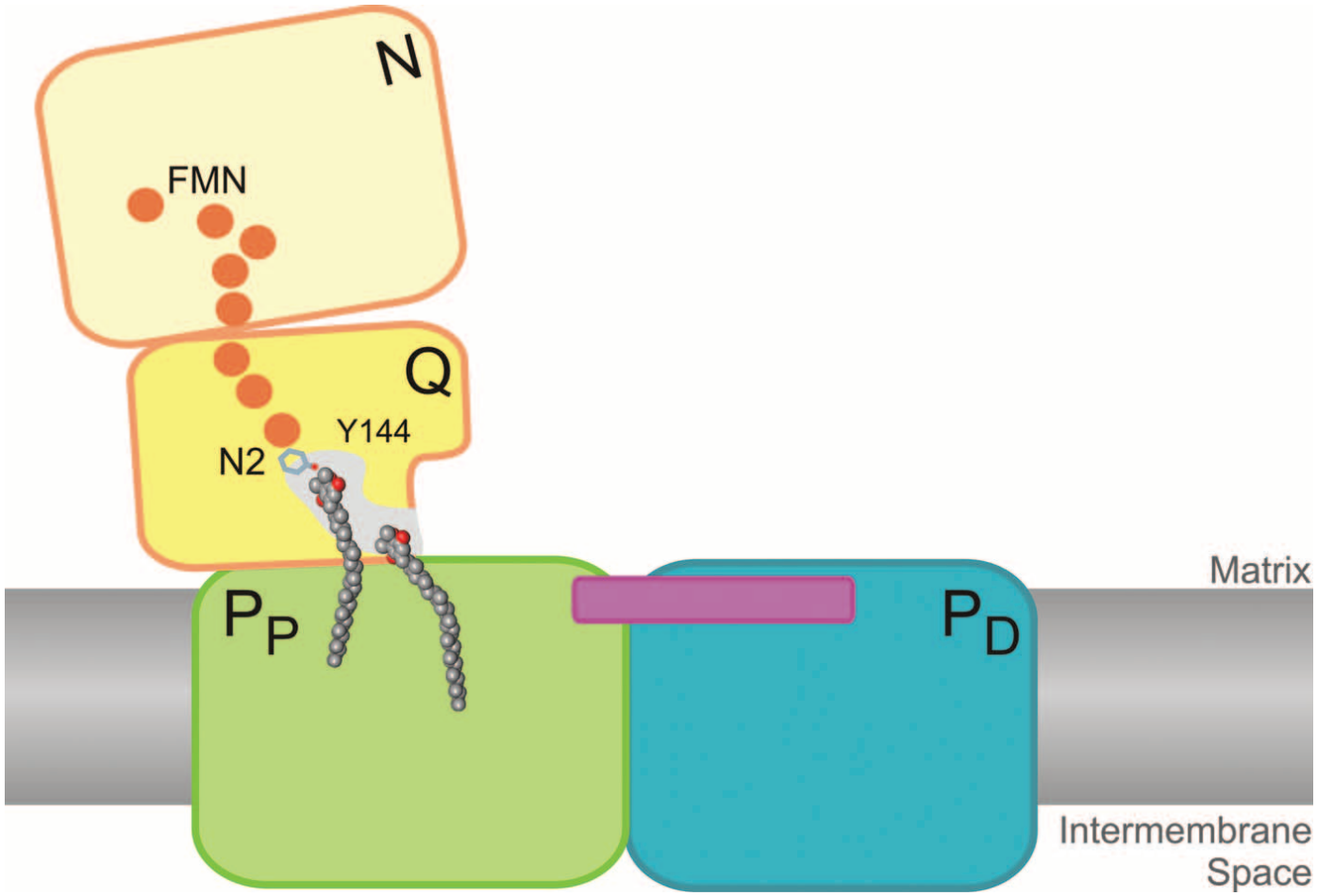


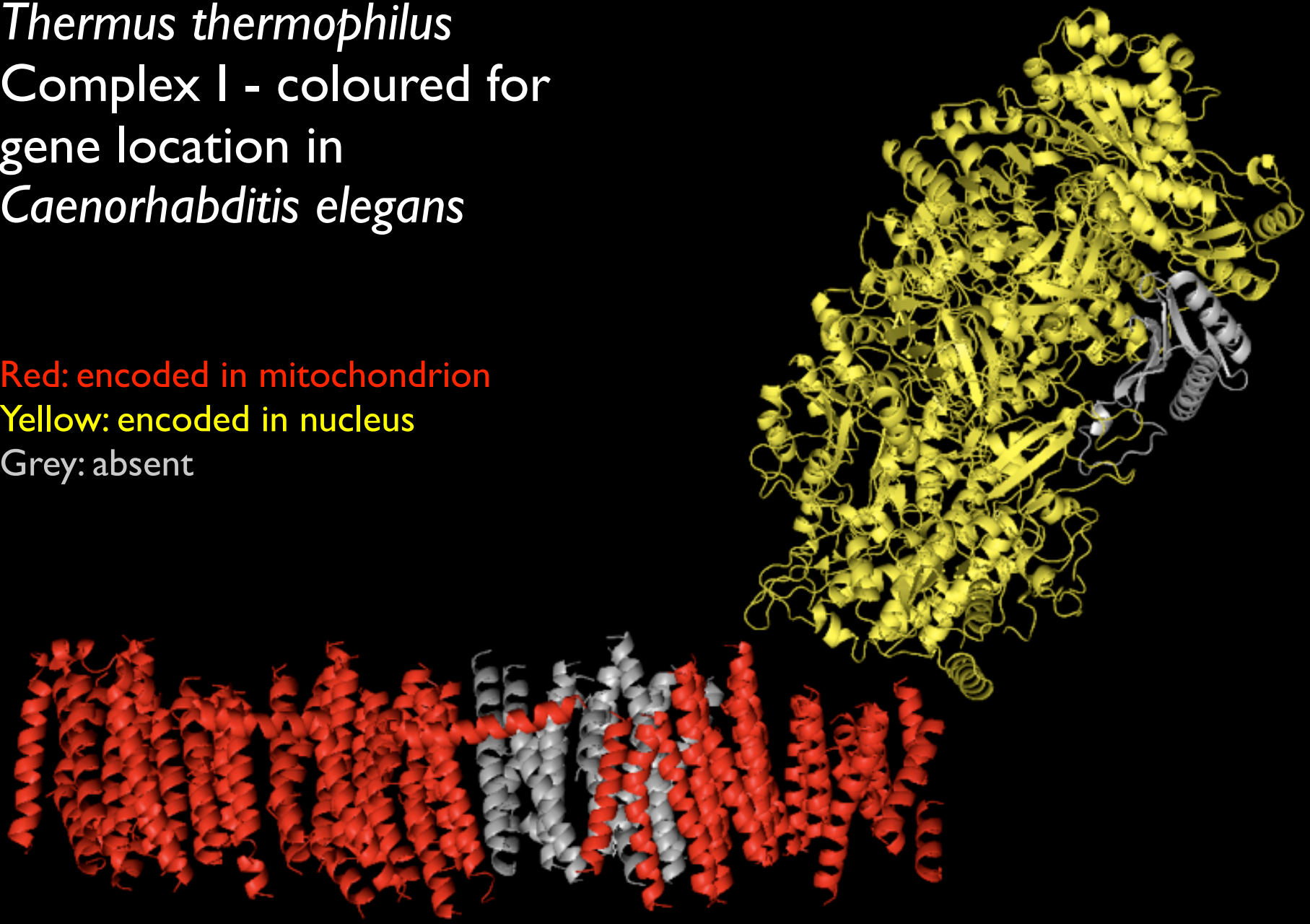
Fig. 4. Schematic model of the four functional modules of complex I. A chain of seven iron-sulfur clusters (orange spheres) leads from the N module (light yellow) with FMN as the primary electron acceptor to the ubiquinone reduction site in the Q module (yellow) involving cluster N2 and tyrosine-144. The position of N2 is close to 30 Å above the membrane surface. With the ubiquinone headgroup (space-fill representation) in functional distance to the electron donor at the end of the extended binding cavity (light gray), the hydrophobic isoprenoid side chain will be about half-way out of the core membrane region. This suggests the presence of a hydrophobic access path and excludes direct coupling between redox chemistry in the N and Q modules and vectorial proton translocation by subunits of the proximal (P_P, green) and distal (P_D, cyan) domains of the P module. An extended transmission element (magenta) forms a bridge across the two domains.

Thermus thermophilus
Complex I - coloured to distinguish subunits

Graphics by Wilson de Paula
from 3m9s.pdb

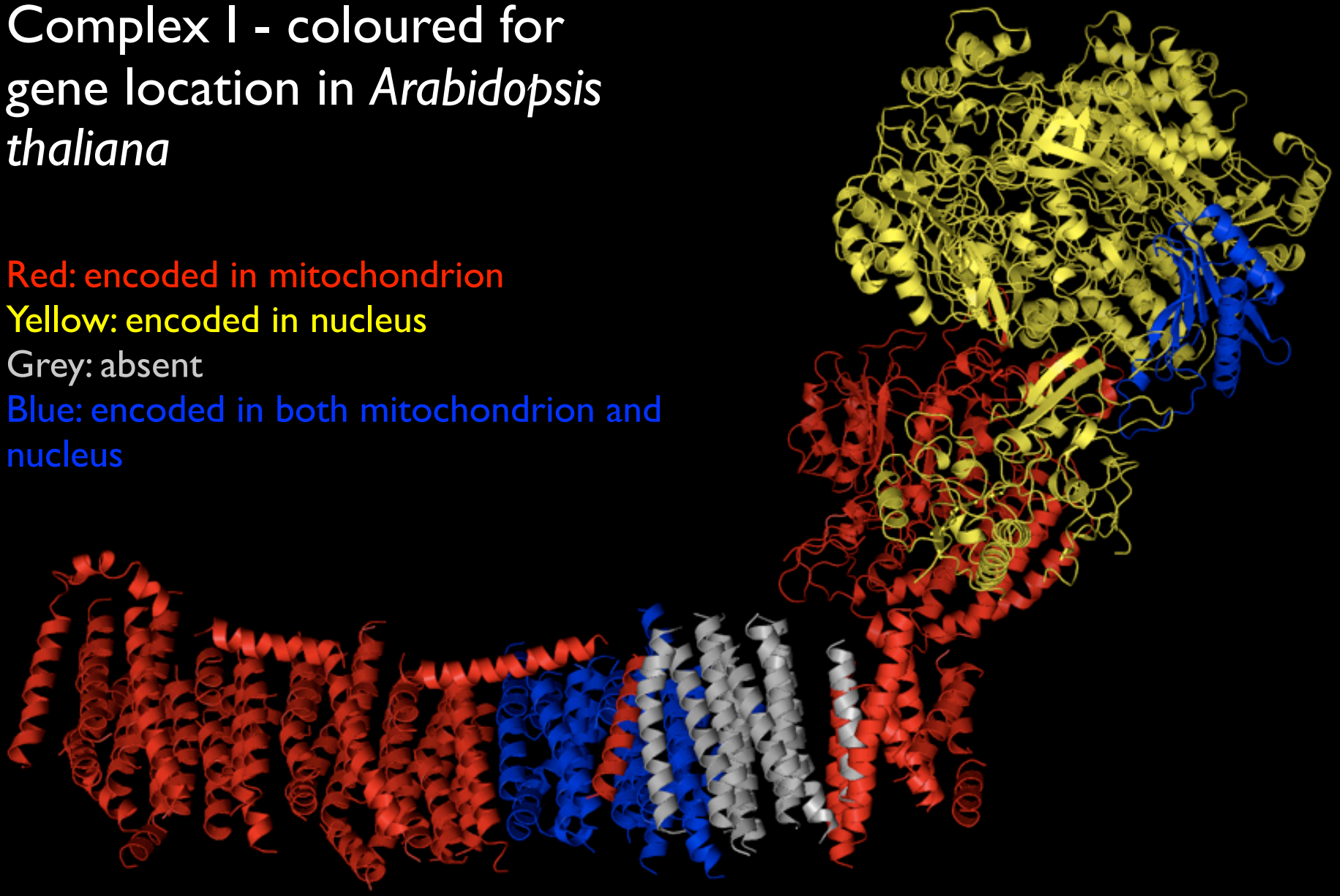
Thermus thermophilus
Complex I - coloured for gene location in *Caenorhabditis elegans*

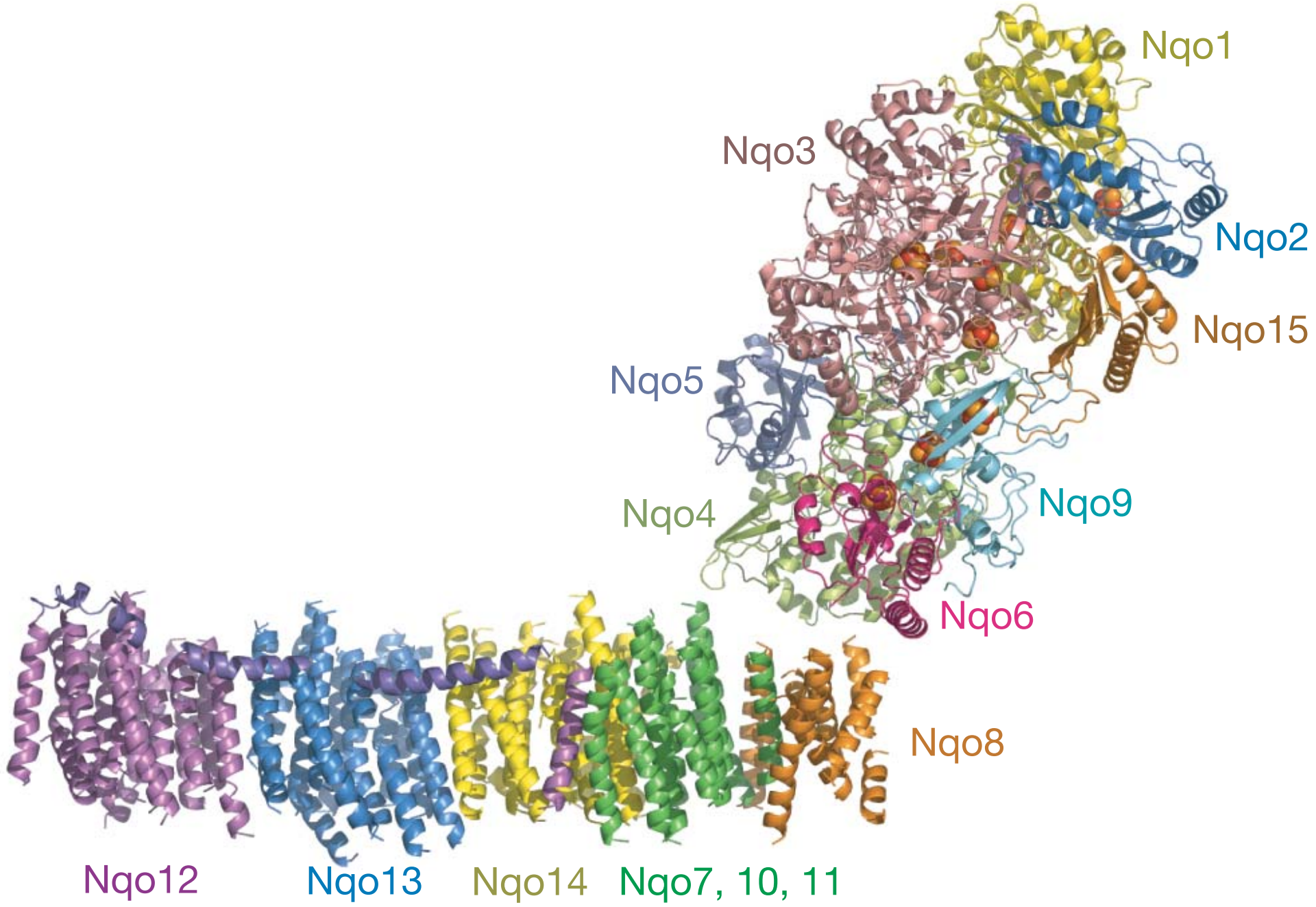
Red: encoded in mitochondrion
Yellow: encoded in nucleus
Grey: absent



Thermus thermophilus
Complex I - coloured for gene location in *Arabidopsis thaliana*

Red: encoded in mitochondrion
Yellow: encoded in nucleus
Grey: absent
Blue: encoded in both mitochondrion and nucleus




Nqo1
Nqo3
Nqo2
Nqo15
Nqo5
Nqo4
Nqo9
Nqo6
Nqo8
Nqo12
Nqo13
Nqo14
Nqo7, 10, 11

ARTICLE

doi:10.1038/nature10330

Structure of the membrane domain of respiratory complex I

Rouslan G. Efremov[1]† & Leonid A. Sazanov[1]

Complex I is the first and largest enzyme of the respiratory chain, coupling electron transfer between NADH and ubiquinone to the translocation of four protons across the membrane. It has a central role in cellular energy production and has been implicated in many human neurodegenerative diseases. The L-shaped enzyme consists of hydrophilic and membrane domains. Previously, we determined the structure of the hydrophilic domain. Here we report the crystal structure of the *Escherichia coli* complex I membrane domain at 3.0 Å resolution. It includes six subunits, NuoL, NuoM, NuoN, NuoA, NuoJ and NuoK, with 55 transmembrane helices. The fold of the homologous antiporter-like subunits L, M and N is novel, with two inverted structural repeats of five transmembrane helices arranged, unusually, face-to-back. Each repeat includes a discontinuous transmembrane helix and forms half of a channel across the membrane. A network of conserved polar residues connects the two half-channels, completing the proton translocation pathway. Unexpectedly, lysines rather than carboxylate residues act as the main elements of the proton pump in these subunits. The fourth probable proton-translocation channel is at the interface of subunits N, K, J and A. The structure indicates that proton translocation in complex I, uniquely, involves coordinated conformational changes in six symmetrical structural elements.

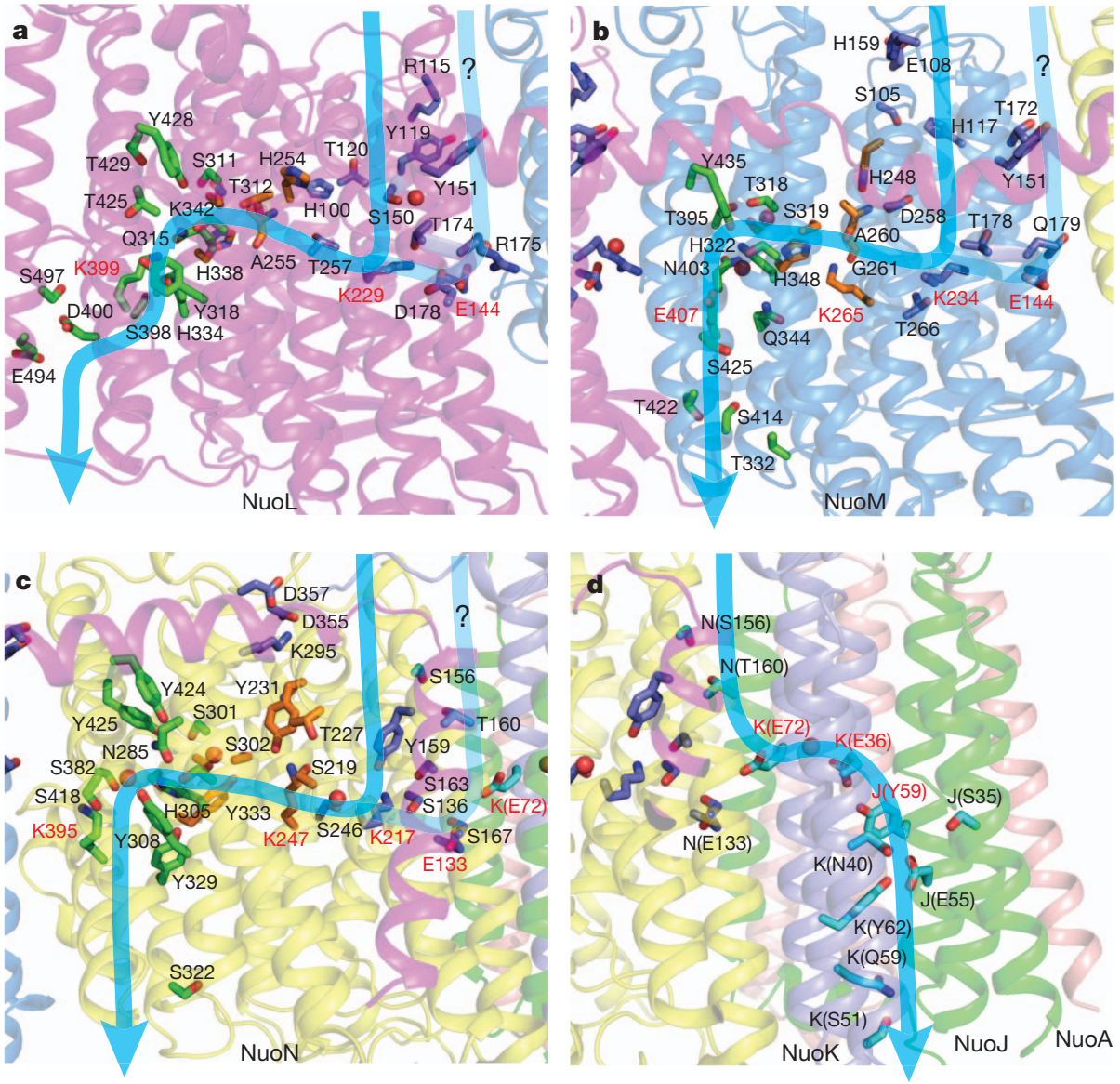


Figure 4 | Proton translocation channels. **a–c**, Putative channels in the antiporter-like subunits. Polar residues lining the channels are shown as sticks, with carbon in dark blue for the first channel, in green for the second channel and in orange for connecting residues. **d**, Channel at the interface of subunits NuoN, K and J, with polar residues in cyan. All views are in a similar orientation to Fig. 1a. Approximate proton translocation paths are indicated by blue arrows. Additional, less likely, input channels at the interfaces of subunits are paler in colour and labelled '?'. Essential charged residues are labelled in red. Water molecules resolved in the structure are shown as red spheres. Alanine and glycine from the π-bulge in TM8 may coordinate water molecules via exposed backbone carbonyls.

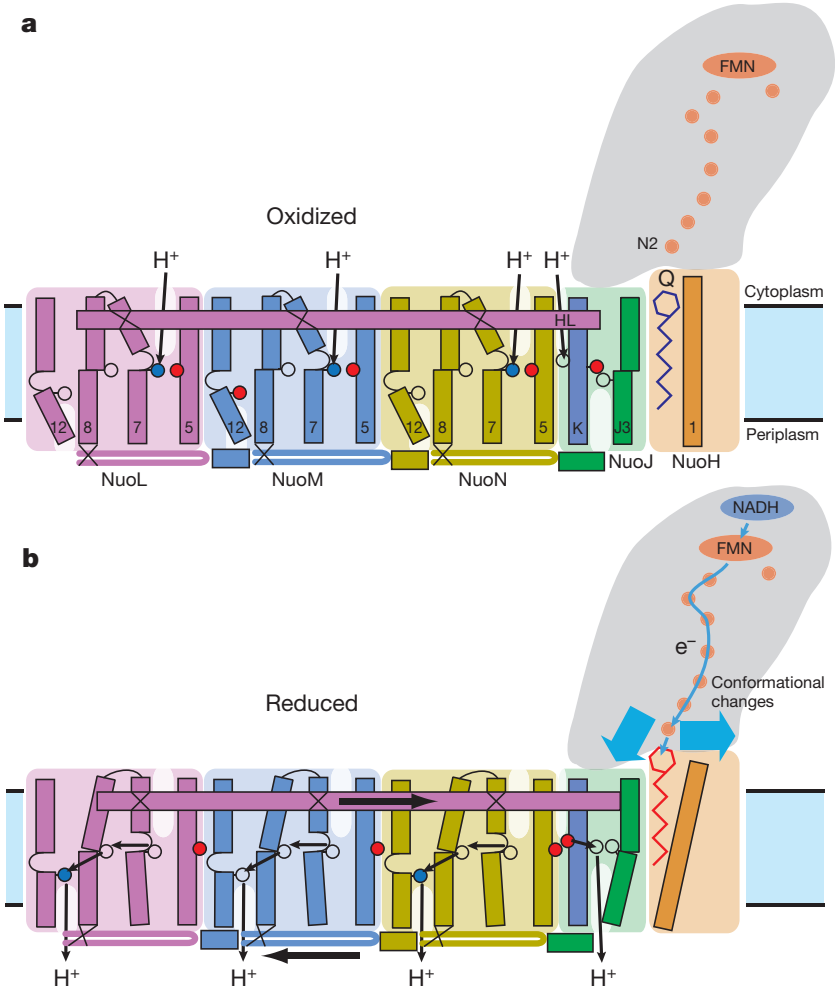


Figure 5 | Proposed mechanism of complex I. **a**, Oxidised state. **b**, Reduced state. Conformational coupling between electron transfer and proton translocation is mediated by helix HL (cytoplasmic side) and the βH element (periplasmic side). TM helices are numbered. Crucial charged residues (Glu TM5, Lys TM7, Lys/Glu TM12 and Lys/His TM8 from NuoL, M and N, as well as K(Glu 72) and K(Glu 36), interacting with J(Tyr 59)) are indicated by red or empty circles (Glu) and empty or blue circles (Lys) for unprotonated or protonated residues, respectively. In NuoL, M and N, Lys TM7 from the first half-channel is assumed to be protonated in the oxidized state. Upon reduction, it donates its proton to the connecting Lys/His TM8 and then on to Lys/Glu TM12 from the second half-channel. Lys/Glu TM12 ejects its proton into periplasm upon return from the reduced to the oxidized state. A fourth proton per cycle is translocated at the interface of NuoN, K and J.

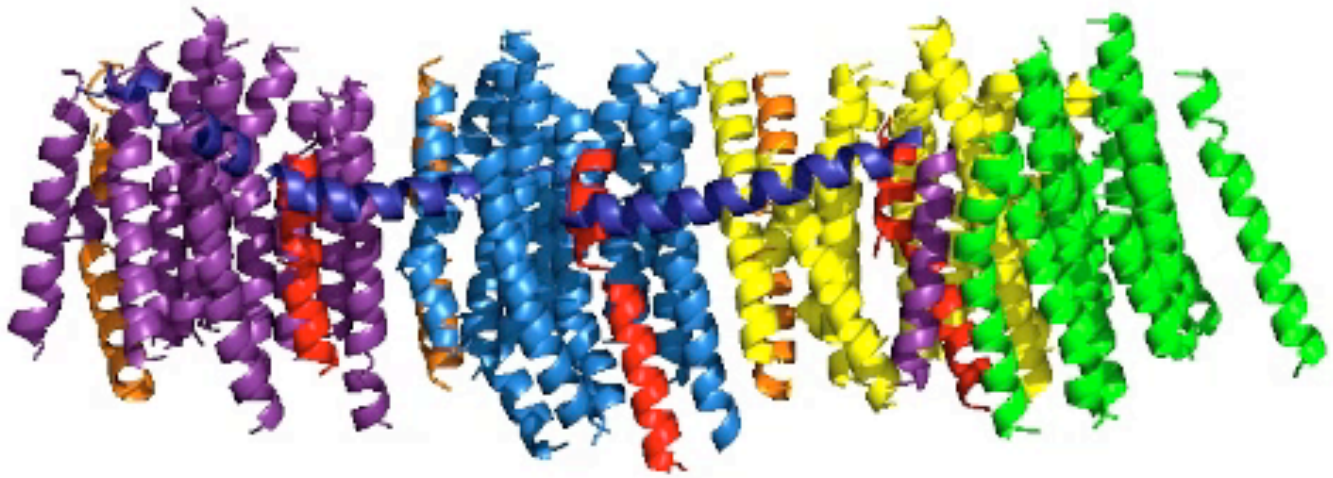
Available online at www.sciencedirect.com
ScienceDirect
Current Opinion in Structural Biology

Respiratory complex I: 'steam engine' of the cell?

Rouslan G Efremov[1] and Leonid A Sazanov

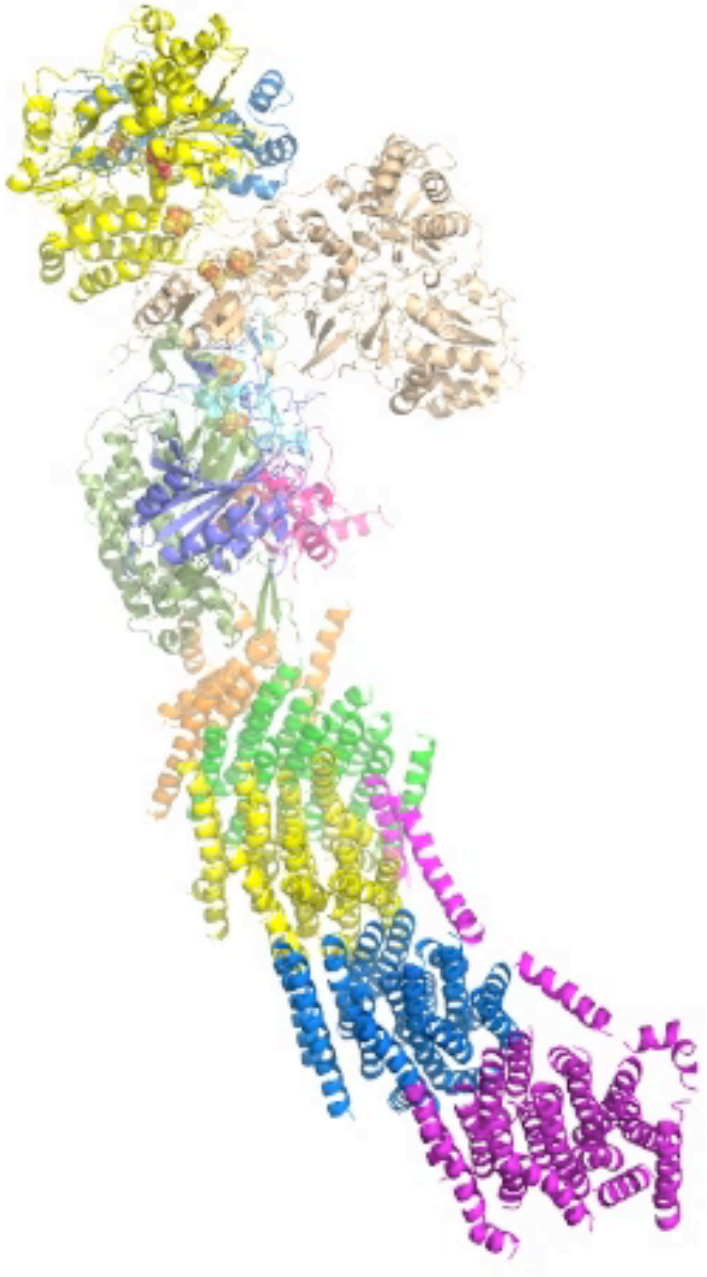
Complex I is the first enzyme of the respiratory chain and plays a central role in cellular energy production. It has been implicated in many human neurodegenerative diseases, as well as in ageing. One of the biggest membrane protein complexes, it is an L-shaped assembly consisting of hydrophilic and membrane domains. Previously, we have determined structures of the hydrophilic domain in several redox states. Last year was marked by fascinating breakthroughs in the understanding of the complete structure. We described the architecture of the membrane domain and of the entire bacterial complex I. X-ray analysis of the larger mitochondrial enzyme has also been published. The core subunits of the bacterial and mitochondrial enzymes have remarkably similar structures. The proposed mechanism of coupling between electron transfer and proton translocation involves long-range conformational changes, coordinated in part by a long α-helix, akin to the coupling rod of a steam engine.

Supplementary Movie 1. An overview of the *E. coli* complex I membrane domain structure, with HL and discontinuous helices highlighted, followed by an overview of the entire *T. thermophilus* complex.

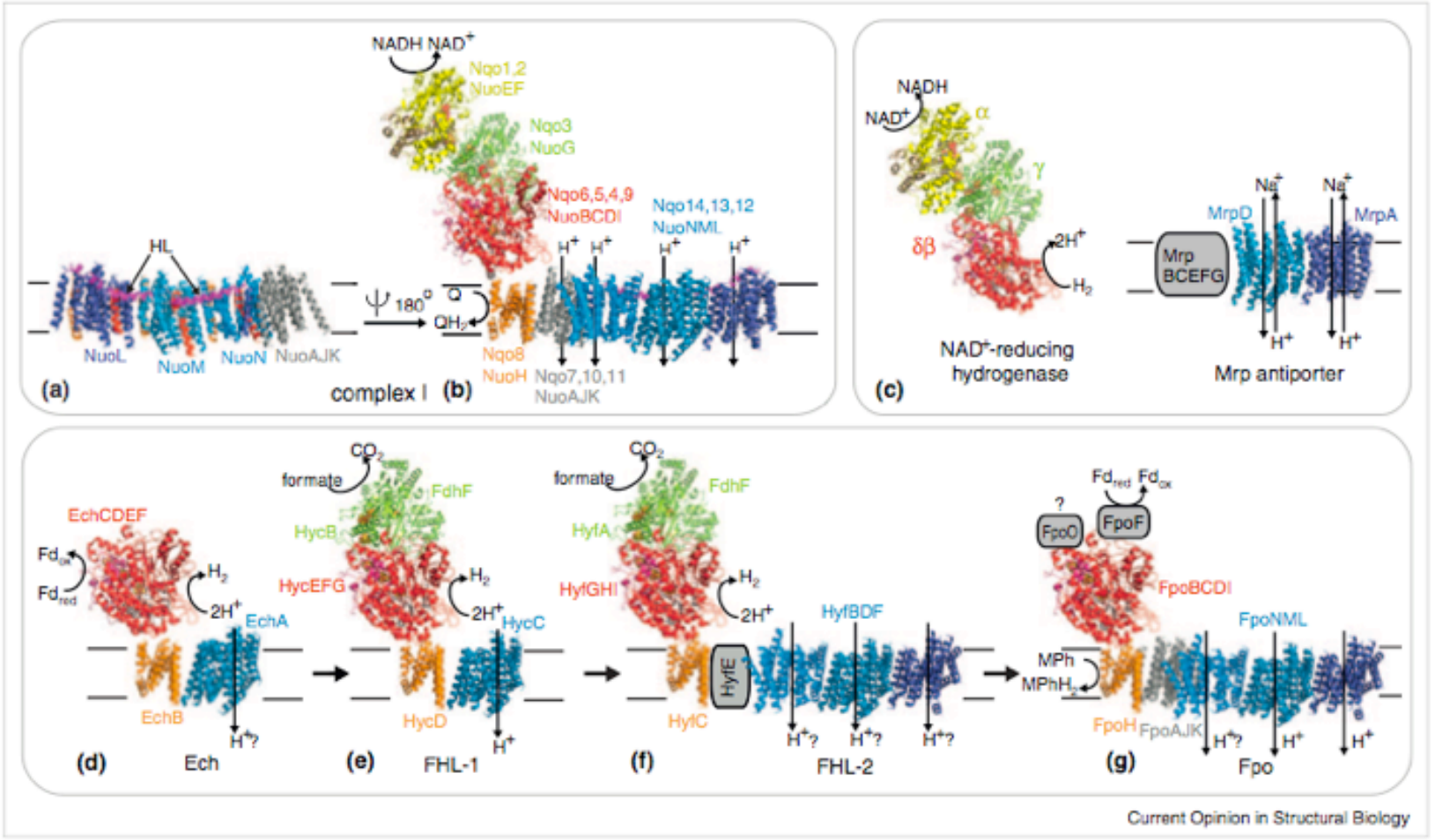


Supplementary Movie 1. An overview of the *E. coli* complex I membrane domain structure, with HL and discontinuous helices highlighted, followed by an overview of the entire *T. thermophilus* complex.

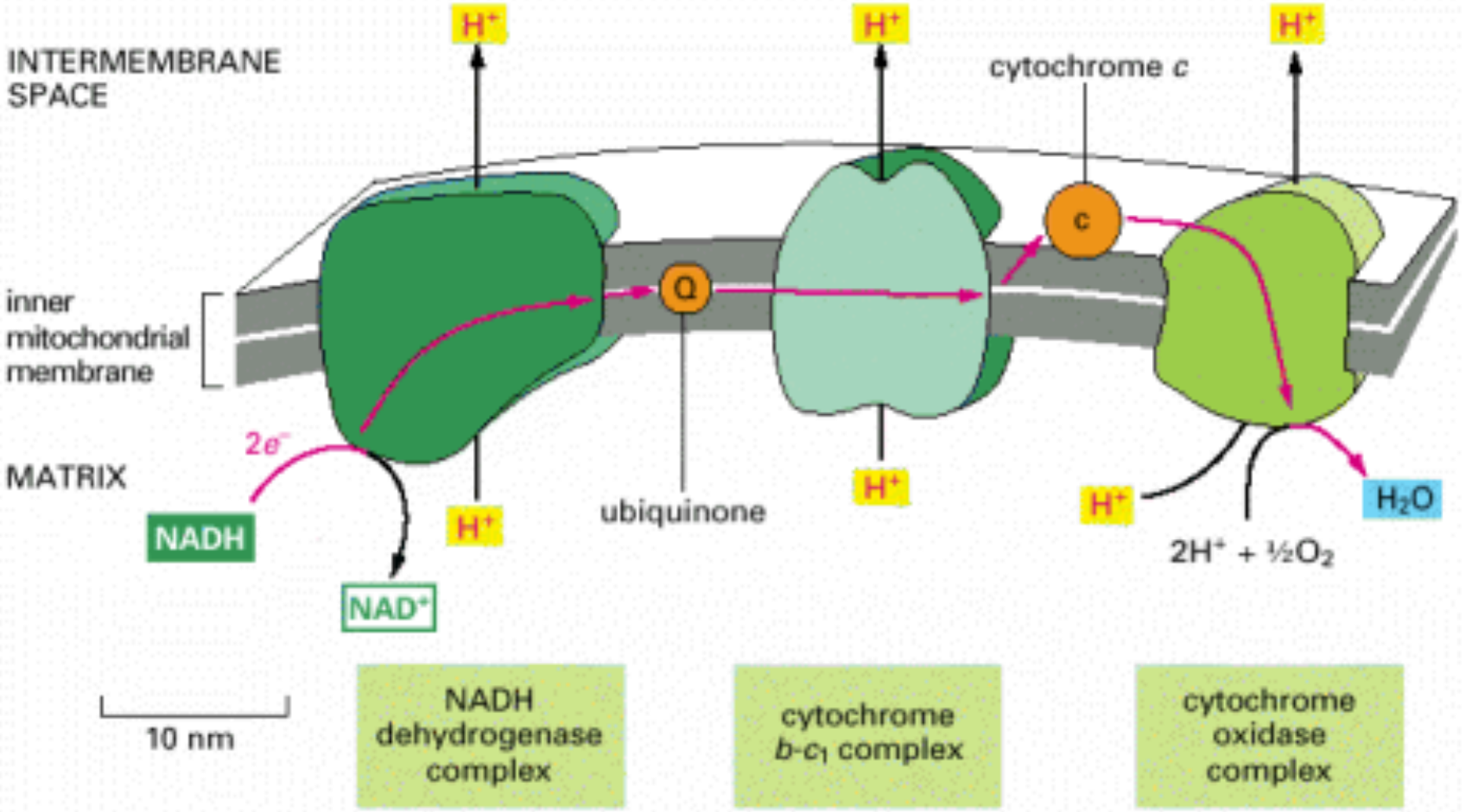
Supplementary Movie 2. Morph between core subunits of *T. thermophilus* and *Y. lipolytica* complexes I, followed by an overview of *Y. lipolytica* enzyme with helices from accessory subunits shown in red for TM and the rest in grey.



Supplementary Movie 2. Morph between core subunits of *T. thermophilus* and *Y. lipolytica* complexes I, followed by an overview of *Y. lipolytica* enzyme with helices from accessory subunits shown in red for TM and the rest in grey.



Structure and evolution of complex I. **(a)** Structure of the membrane domain of *E. coli* complex I [14••]. Helix HL is in magenta and discontinuous helices are in red (those contacting HL) or yellow. **(b)** Structure of the entire *T. thermophilus* complex I [14••], with evolutionary modules colour-coded (the same coding is used throughout): yellow and green, N module (NuoEFG) [6]; red, Q module (NuoBCDI); orange, NuoH-like subunits; blue, antiporter-like subunits of P module and grey, adapter subunits connecting Q and P modules. **(c)** Two separate origins of complex I: closely related protein complexes that have either only oxidoreductase activity, bidirectional NAD^+-reducing NiFe hydrogenase (homologous to subunits NuoEFGBD) [60], or transport activity, Mrp antiporters (NuoLMNK; MrpC is homologous to NuoK) [42]. **(d)–(g)** Evolution of complexity, from simplest known complex I-related proton-pumping oxidoreductase, Ech hydrogenase from *Methanosarcina barkeri* (NuoBCDIHM) (d), to formate hydrogen lyase 1 (FHL-1) of *E. coli* (NuoGBCDIHM) (e), to FHL-2 of *E. coli* (NuoGBCDIHKLMN; C-terminus of HyfE is homologous to NuoK) (f) [60] and to 11 subunit archeal homologue, Fpo complex from *Methanosarcina mazei* (NuoBCDIHAJKLMN) (g) [61]. NDH-1 complex from chloroplasts and cyanobacteria also contains 11 core subunits, lacking N module but with many additional subunits present, although the identity of electron donor is unclear [62,63]. Names of subunits constituting the complexes are indicated. Subunits unrelated to complex I are shown as grey rectangles. Question marks indicate characteristics that have not been unambiguously established experimentally, such as proton-pumping of Ech and FHL-2, H^+/e^- stoichiometry of Fpo, as well as association of subunit FpoO with the complex. *Abbreviations*: MPh: methanophenazine; Fd_{red} and Fd_{ox}: reduced and oxidized ferredoxin; Q: ubiquinone or menaquinone.



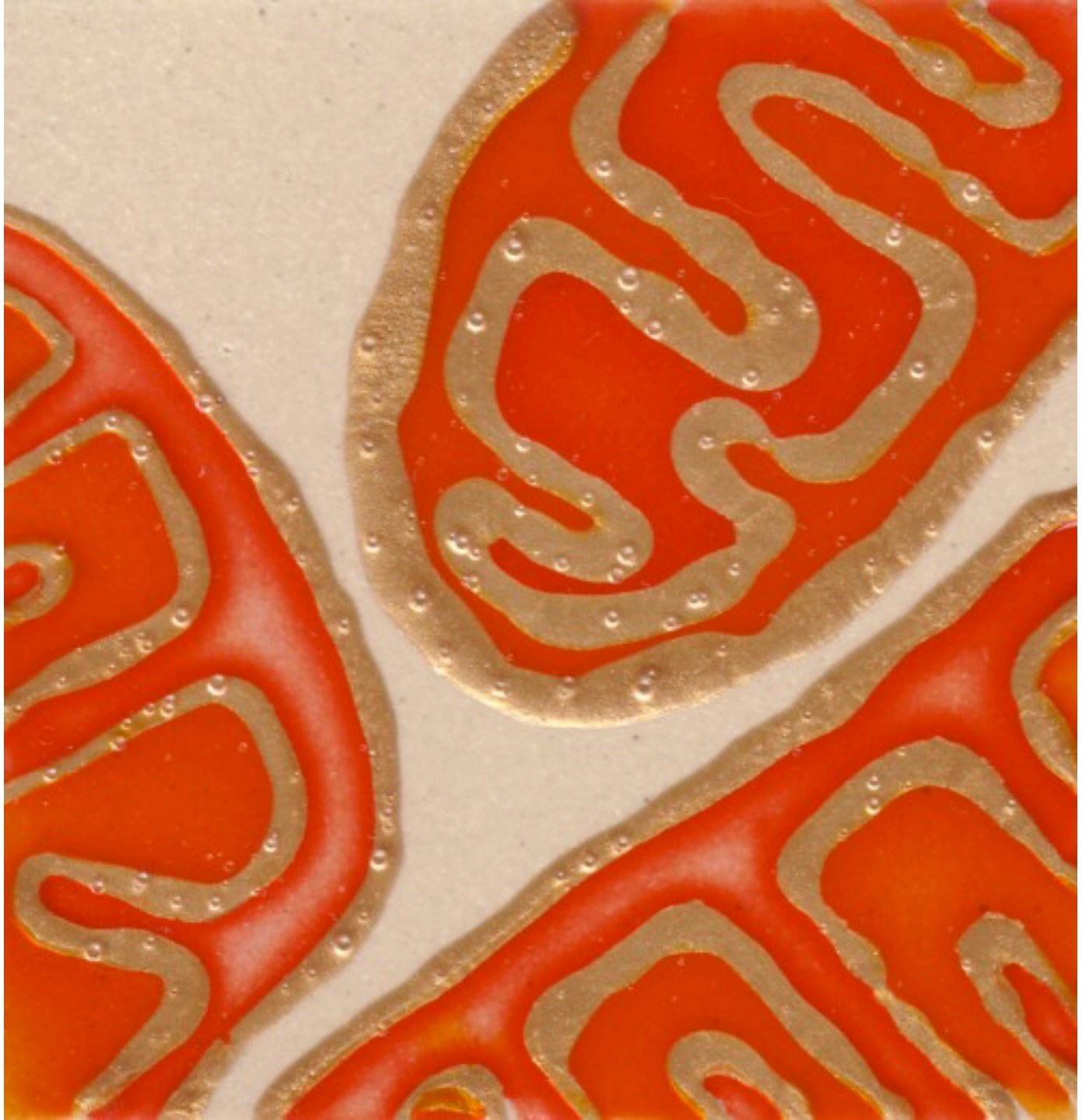
The Respiratory Chain Includes Three Large Enzyme Complexes Embedded in the Inner Membrane

Molecular Biology of the Cell Bruce Alberts, Alexander Johnson, Julian Lewis, Martin Raff, Keith Roberts, and Peter Walter. 2002

Next lecture

Complex II and complex III.
Structure and Function.

jfallen.org/lectures/




Thank you for listening


JVK
2004