

The strange structures of special cytochromes c from methylotrophic bacteria

The molecular structure of an unusual cytochrome c_2 determined at 2.0 Å; the cytochrome c_H from *Methylobacterium extorquens*

Jon Read, Raj Gill, Simon L. Dales, Jon B. Cooper, Steve P. Wood, and Chris Anthony *Protein Sci.* 1999 8: 1232-1240

The 1.6 Å X-ray structure of the unusual c-type cytochrome, cytochrome c_L , from the methylotrophic bacterium *Methylobacterium extorquens*

Paul Williams, Leighton Coates, Fiyaz Mohammed, Raj Gill Peter Erskine, Dominique Bourgeois, Steve P. Wood, Chris Anthony and Jonathan B. Cooper*
J. Mol. Biol. (2006) 357, 151–162

INTRODUCTION:

cytochrome c: methylotrophs: methanol oxidation: electron transport chains
methylotroph cytochromes c (earlier work)

Cytochrome c (Class I)

Mitochondrial cytochrome c and the equivalent bacterial cytochrome c_2 .

Small water soluble protein; about 100 amino acids

Hundreds of sequences and many structures are known.

Haem prosthetic group covalently bonded by thioether bridges to protein

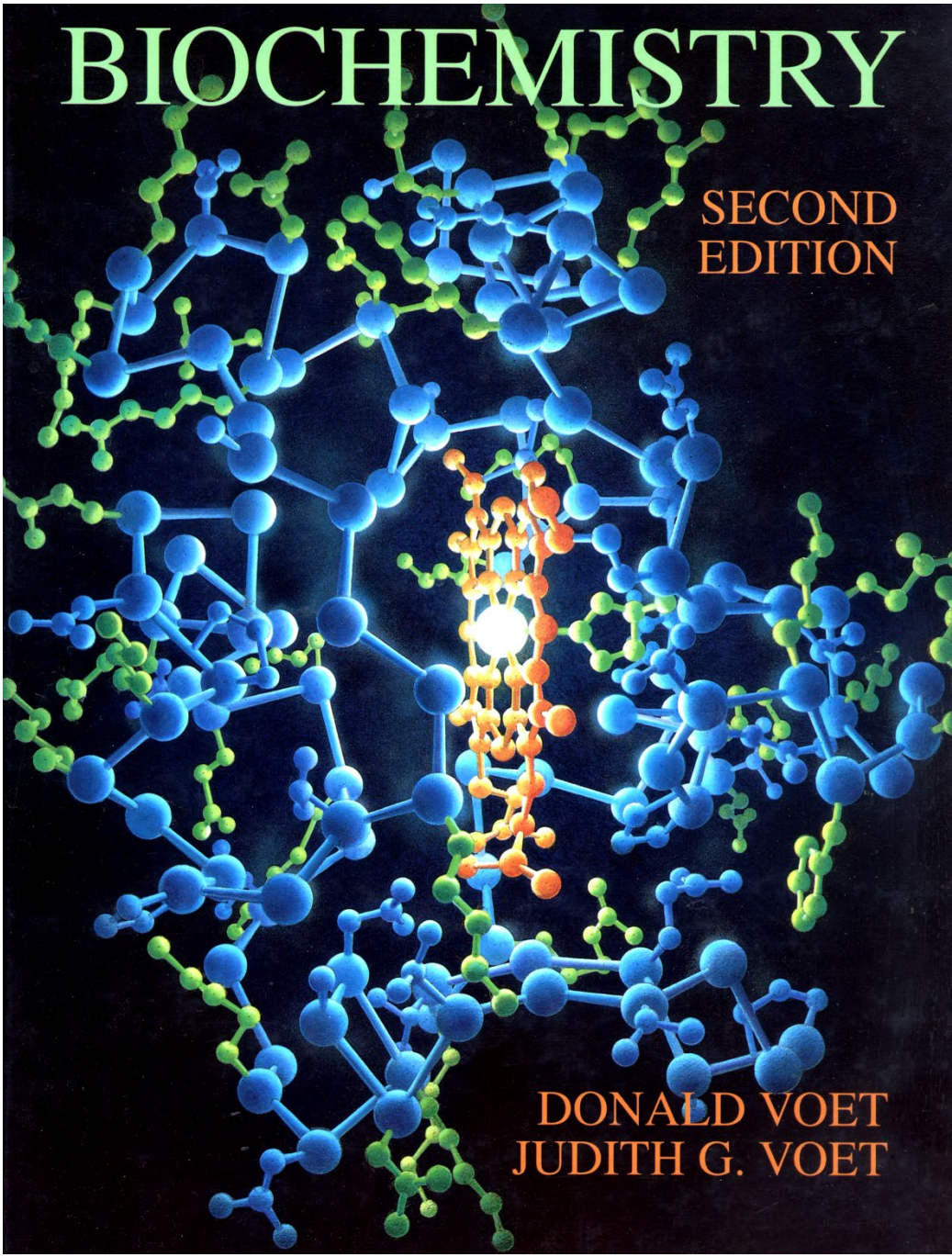
The iron atom forms ligands with the 4 pyrrole N of the haem; the 5th and 6th ligands are histidine and METHIONINE

Electron carriers $\text{Fe}^{+++} + e^- \longleftrightarrow \text{Fe}^{++}$

They function in electron transport chains, mediating electron transfer between membrane cytochrome bc1 complex and the oxidase [or between membrane proteins in photosynthetic electron transport in bacteria]

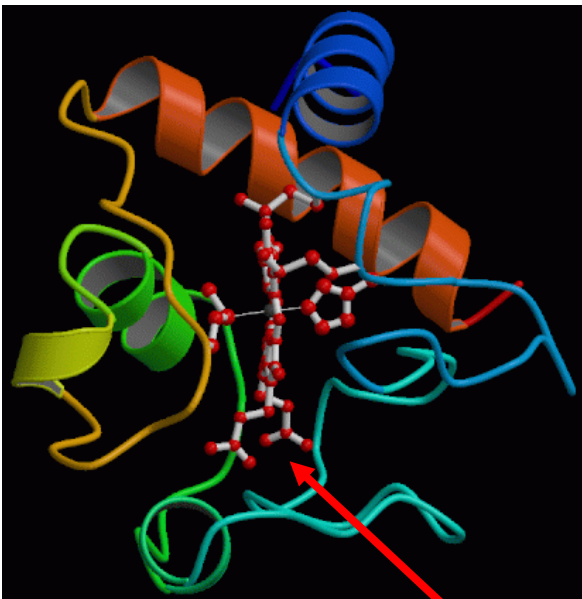
They are located between the inner and outer membranes of mitochondria and in the equivalent periplasmic space in bacteria

BIOCHEMISTRY

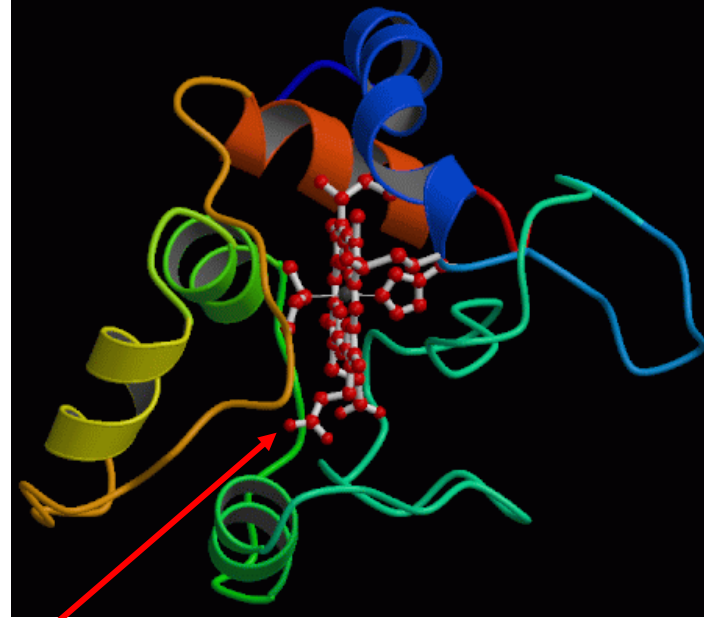


SECOND
EDITION

DONALD VOET
JUDITH G. VOET

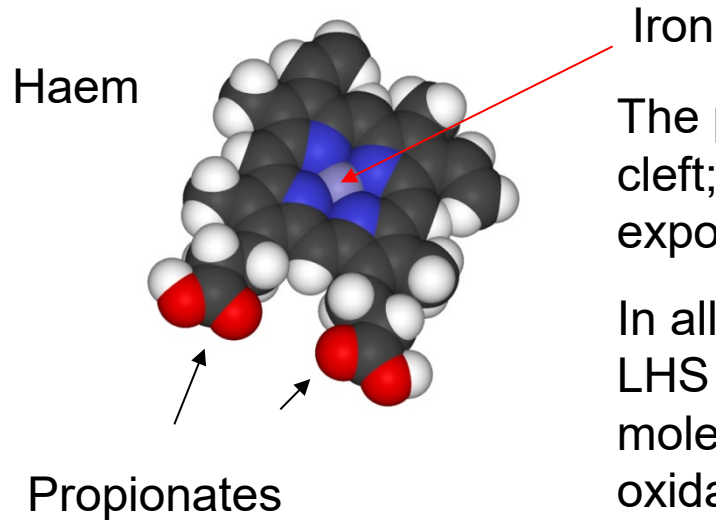


Cytochrome c



Bacterial cytochrome c₂

Propionates

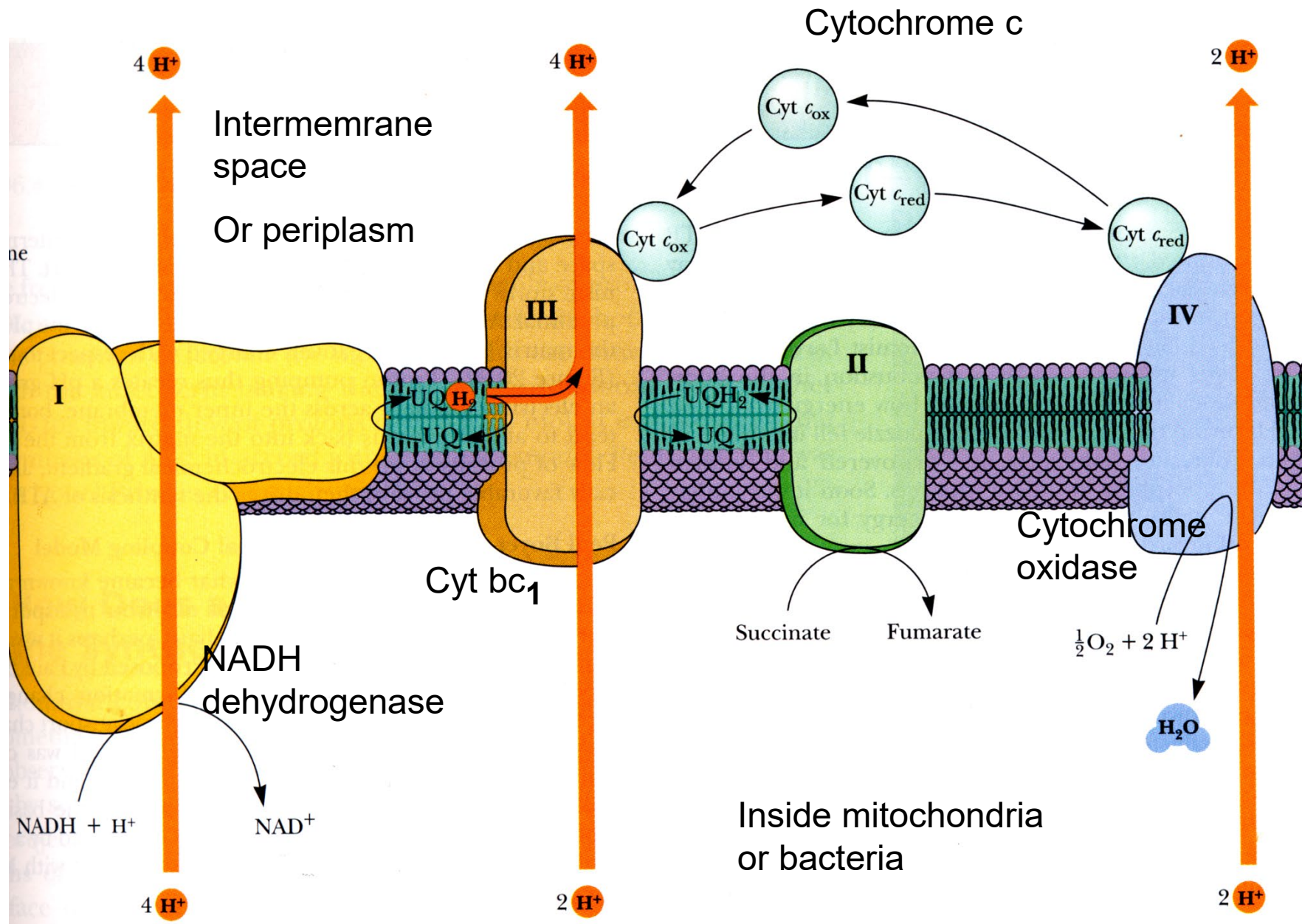


Iron

The planar haem lies enclosed in the haem cleft; electrons enter and leave the haem at its exposed edge

In all structures there is a water molecule in the LHS of the haem cleft; the movement of this molecule is said to be essential during the oxidation and reduction processes

Electron transport chain [Garrett & Grisham]



Electron transport chain in mitochondria

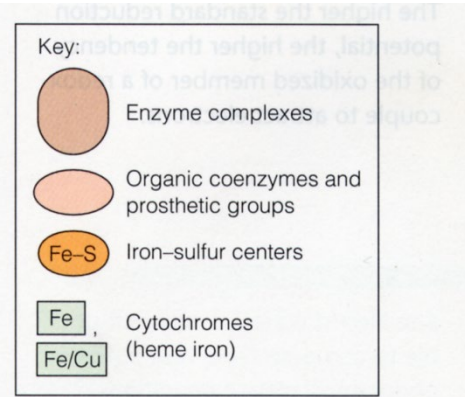
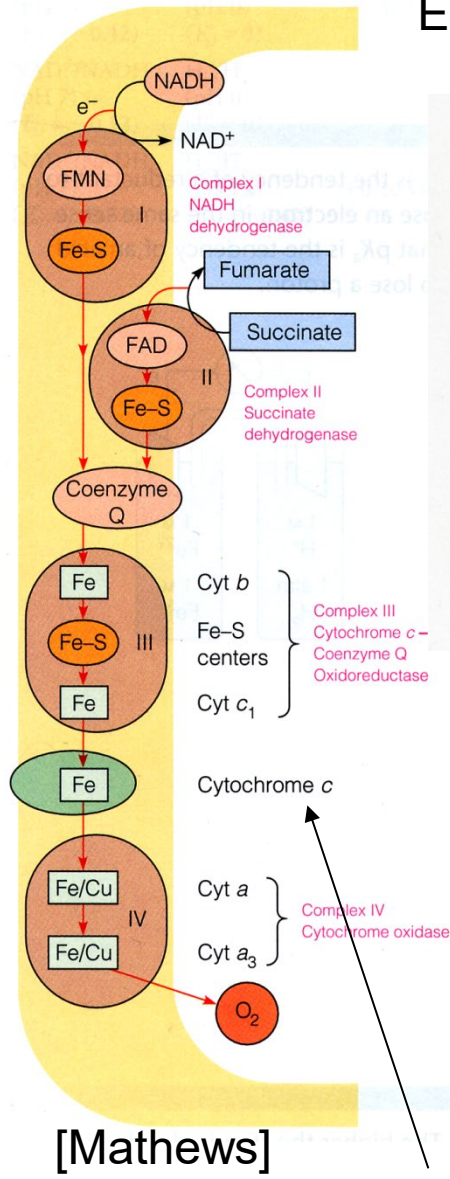
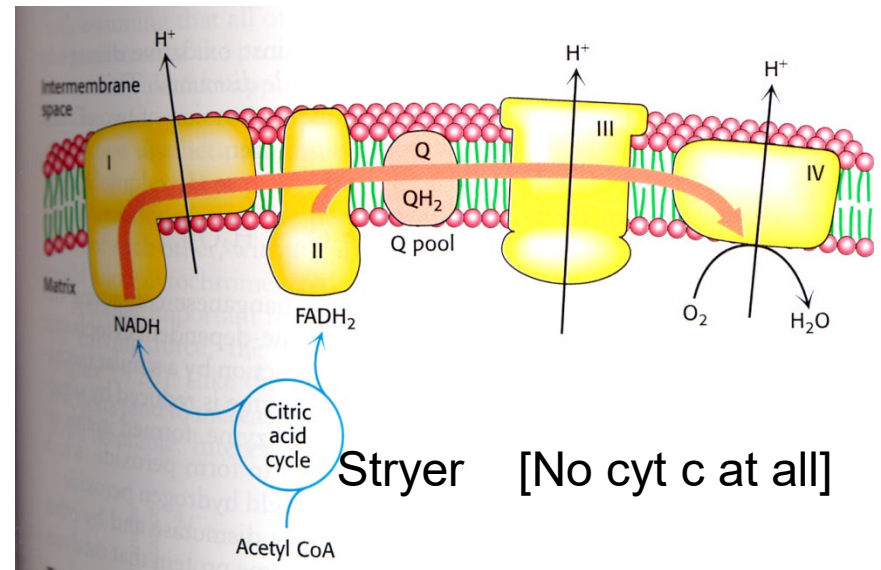


FIGURE 15.3

Respiratory electron carriers in the mitochondrion. This figure shows the sequence of electron carriers that oxidize succinate and NAD⁺-linked substrates in the inner membrane.

Text books are nearly all wrong

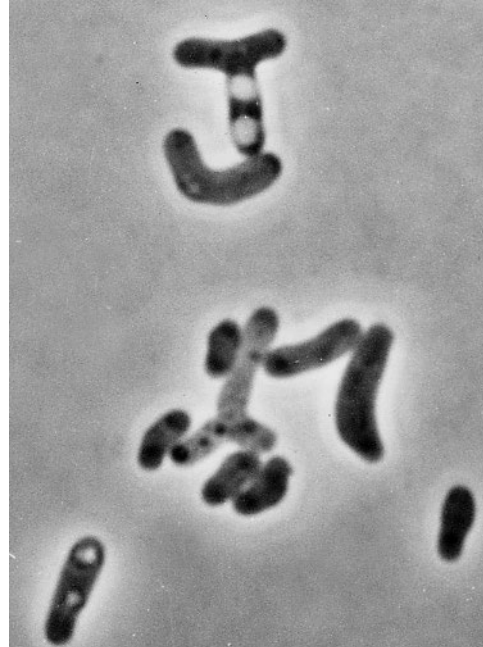
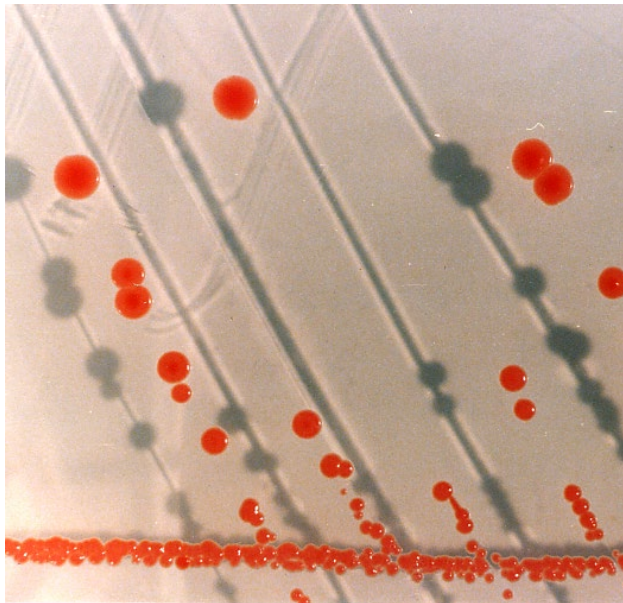
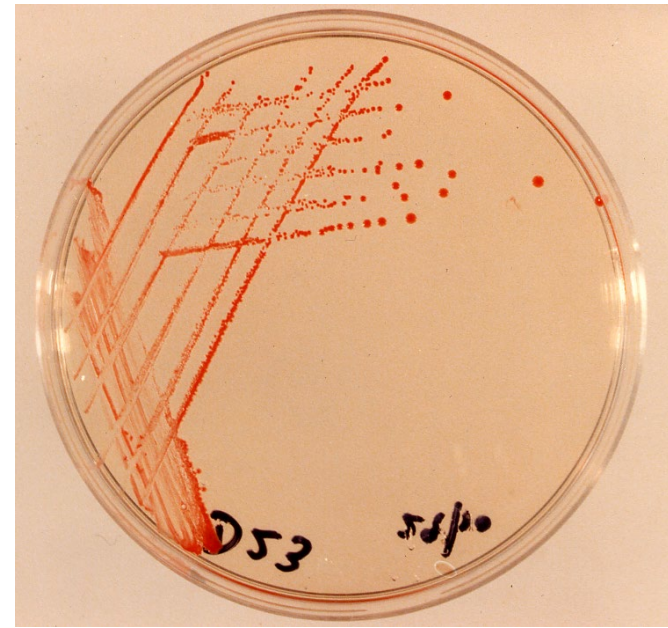
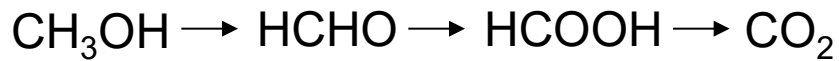


[Mathews]
Cyt c is a membrane protein!

Stryer [No cyt c at all]

Methylobacterium extorquens

- *Pseudomonas* AM1 (Peel & Quayle, 1961)
- *Pseudomonas* sp. M27 (Anthony & Zatman, 1964)



The oxidation of methanol

Catalysed by soluble Methanol dehydrogenase [MDH] in the periplasm.
NAD is not involved.

MDH has a 'novel' prosthetic group called PQQ

MDH is equivalent to a soluble flavoprotein but with PQQ instead of FMN or FAD

The question: how is energy obtained from this oxidation of methanol; how does MDH interact with the electron transport chain?

Cytochrome c is particularly important in methanol oxidation [kinetic and mutant studies].

The two cytochromes *c* in the facultative methylotroph *Pseudomonas* AM1

David T. O'KEEFFE and Christopher ANTHONY

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Cytochrome c_H High isoelectric point [basic, +ve charge at pH 7; $M_r = c$ 10,000
Does not react with MDH; **rapidly oxidised by the oxidase.**
It is also involved in oxidation of NADH
This cytochrome appears to be a typical bacterial cytochrome c_2

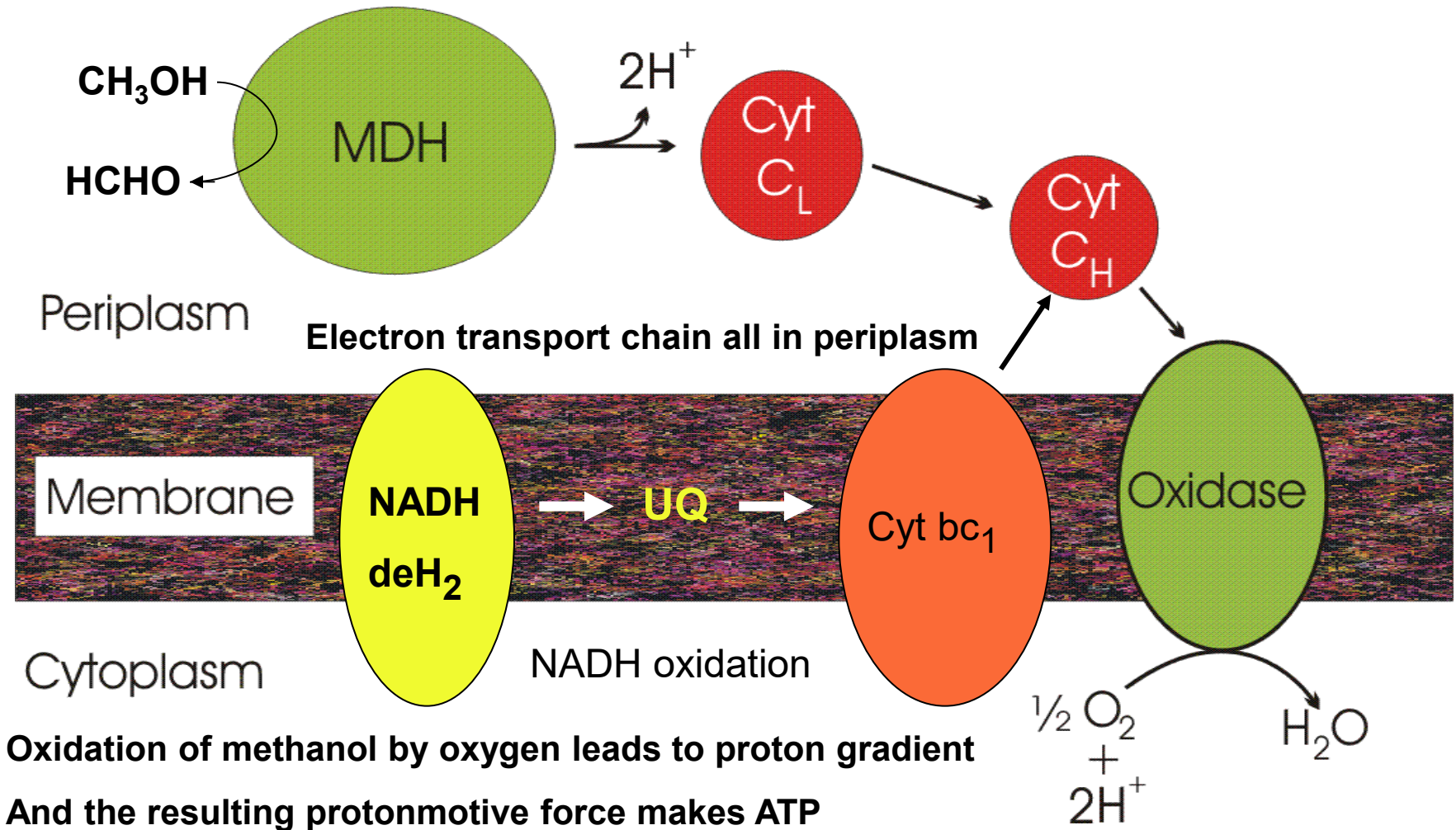
Cytochrome c_L Low isoelectric point [acidic, –ve charge at pH 7; $M_r = c$ 20,000
Reacts rapidly with MDH; not oxidised by the oxidase.
Not involved in NADH oxidation

Reacts slowly with CO. This displaces the 6th Methionine ligand.
This is very rare in cytochromes *c* and indicates an exceptionally flexible structure in the LHS of the haem cleft

The two cytochromes *c* are able to react very rapidly with each other



The oxidation of methanol in methylotrophs



Project: Solve structure of all the soluble proteins

Study electron transport within the proteins and between them

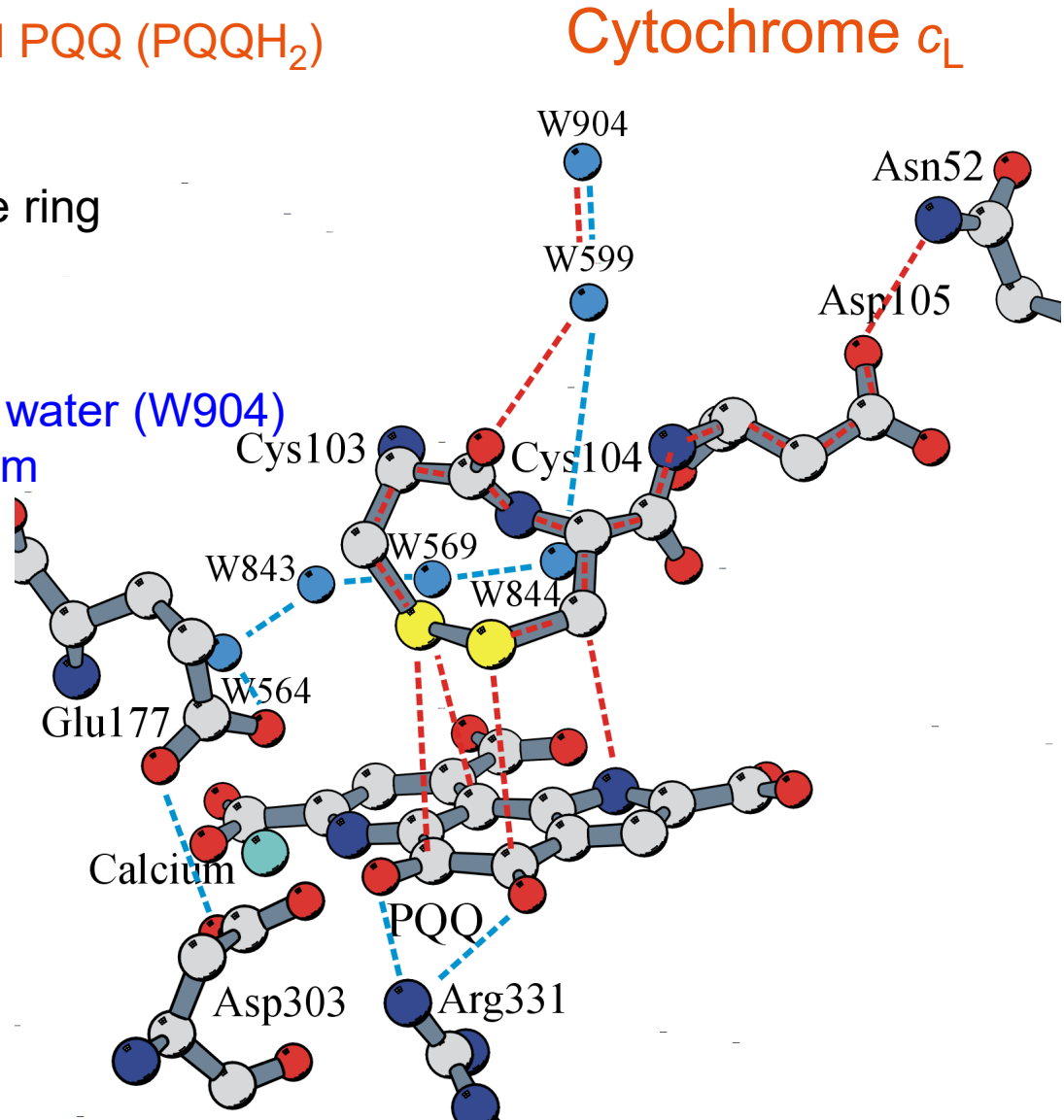
The path of electrons and protons during the oxidative part of the reaction mechanism of MDH

Electrons must pass from reduced PQQ (PQQH₂)
to cytochrome c_L

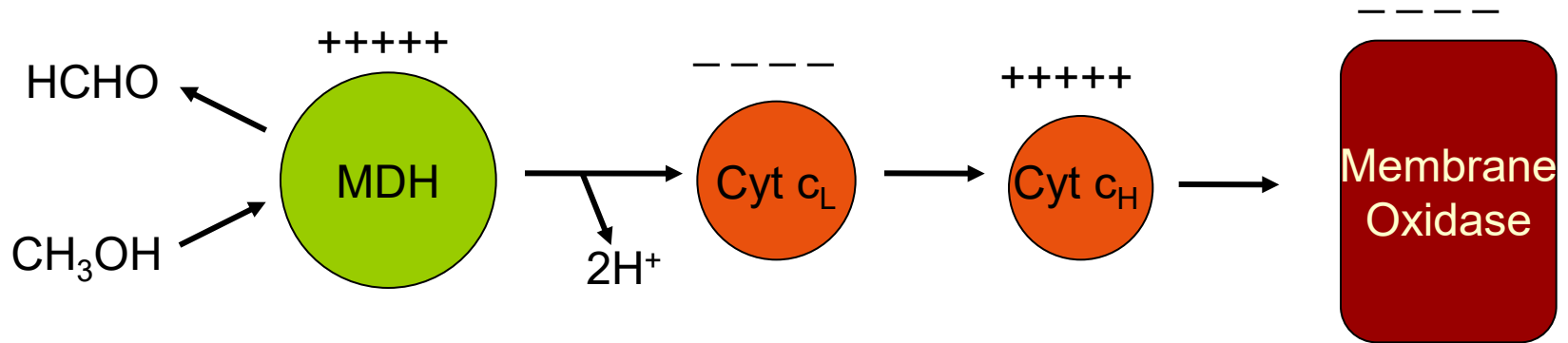
Note involvement of the disulphide ring

Protons must pass from PQQH₂
by way of its hydroxyls, and the
hydrogen-bonding network, to the water (W904)
which is accessible to the periplasm

The pathways are based on those
proposed for the quinohaemoprotein
alcohol dehydrogenase by *Oubrie,
Dijkstra et al, and Chen, Matsushita,
Toyama, Scott Mathews et al.*



Interaction of the electron transport proteins

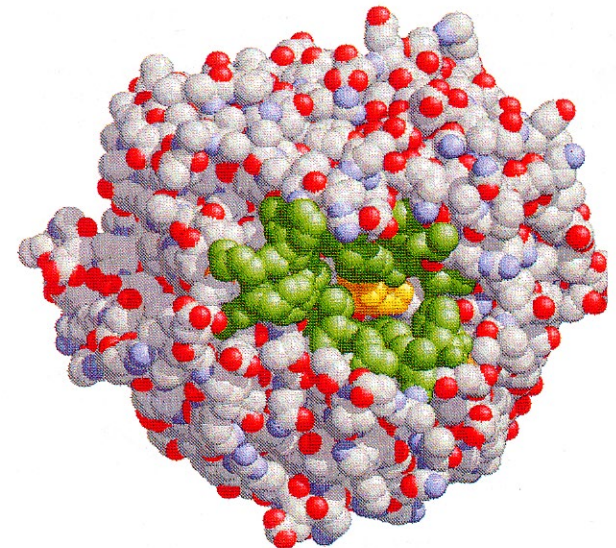


MDH and cytochrome c_L 'dock' together by ionic interactions between lysines on MDH and carboxylates on cytochrome c_L.

The electron transfer complex may involve interaction of the cytochrome with the hydrophobic funnel on the surface of MDH.

Cytochrome c_L dissociates, and then passes electrons to cytochrome c_H.

Cytochrome c_H is almost a typical cytochrome c, passing its electrons to the oxidase



Claude Chan, Darren Day, Jon Cox, Jim Elliott, Simon Dales

Research students (Graduated 1990)



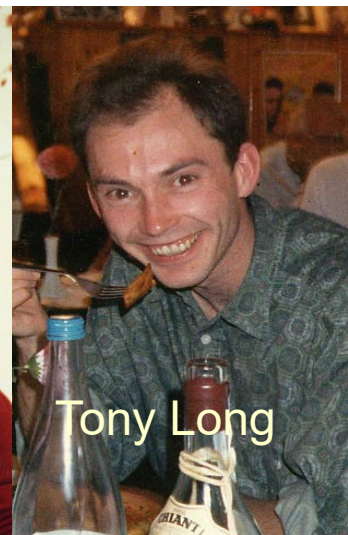
Darren Day



Claude Chan



Ian Richardson



Tony Long



Darren

Jon Cox



The two cytochromes *c* in the facultative methylotroph *Pseudomonas* AM1

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Department of Biochemistry, University of Southampton, Southampton SO9 3TU, U.K.

Cytochrome c_H High isoelectric point [basic, +ve charge at pH 7; $M_r = c$ 10,000

Cytochrome c_L Low isoelectric point [acidic –ve charge at pH 7; $M_r = c$ 20,000

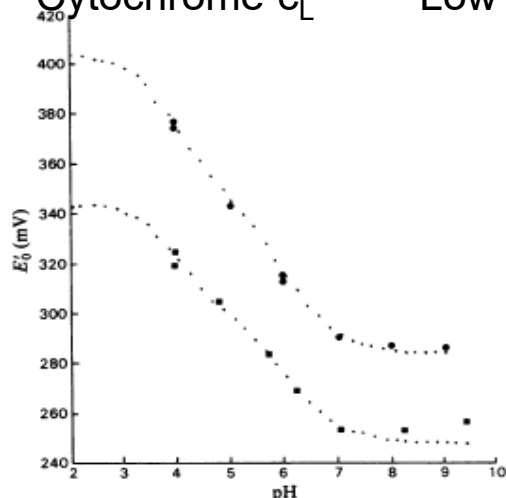


Fig. 5. pH-dependence of the midpoint redox potentials of cytochromes c_H and c_L .

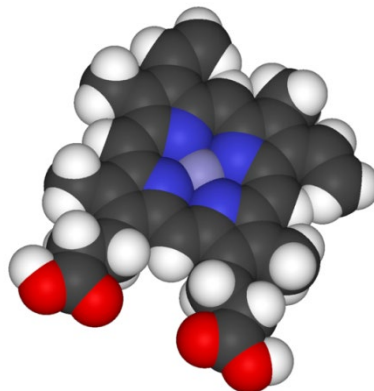
Details of the methods are given in the Materials and methods section. Oxidative and reductive titrations gave identical results. ●, Midpoint potentials of cytochrome c_H ; ■, midpoint potentials of cytochrome c_L . The dotted lines are theoretical curves calculated from the equation:

$$E'_0 = E_0 + 59 \log \left(\frac{[H^+]^2 + K_{r1}[H^+] + K_{r1}K_{r2}}{[H^+]^2 + K_{o1}[H^+] + K_{o1}K_{o2}} \right)$$

where K_{o1} and K_{o2} are the dissociation constants for the first and second ionizations in the oxidized species and K_{r1} and K_{r2} are the equivalent dissociation constants for the reduced species. The pK ($-\log K$) and E_0 values assumed for calculation of the theoretical curves are given in Table 3.

There were four ionizations affecting the redox potentials in the pH range studied (pH 4.0–9.5), two in the oxidized form (pK values about 3.5 and 5.5) and two in the reduced form (pK values about 4.5 and 6.5),

suggesting that the ionizing groups involved may be the two propionate side chains of the haem.



This suggests the propionates are very unusual in being exposed to solvent and not buried within the cytochrome structure as with other cytochromes

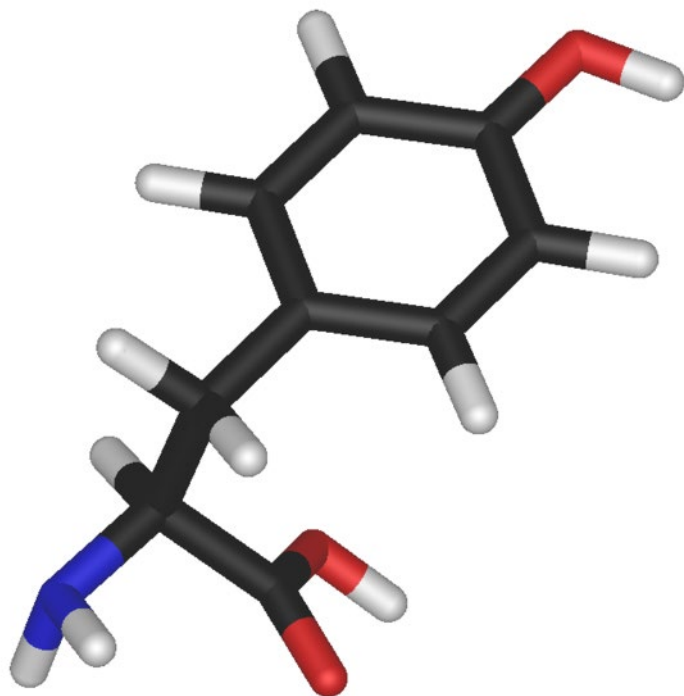
Fig. 1. The sequence of cytochrome c_H of *M. extorquens*.

	-5	1	10	20	30	40	50
Yeast		TEFKAGSAKKGATLFKTRCLQCHTVEKGGPHKVGPNLHGIFGRH	SGQAE-GYST	YTDA			
Horse		GDVEKGKKIFVQKCAQCHTVEKGGKHKTGPNLHGLFGRKT	GTQAP-GF-T	YTDA			
Tuna		GDVAKGKKTFVQKCAQCHTVENGKHKVGPNLWGLFGRKT	GTQAE-GY-S	YTDA			
		1	10	20	30	40	
Methylobacterium		EGDAAAGEKAFA-PCKACHNFEKNG---	VGPTLKGVVGA	KA	AGEGADGYA-F	SDA	
		60	70	80	90	112	
Yeast		NIK---KNVLWDENNMSE	YLTNP-KYIPG-----	TKMAFAGLKKEKDRNDITYLKKACE			
Horse		NKN---KGITWKEETLME	YLENPKKYIPG-----	TKMIFAGIKKKTEREDLIAYLKKATNE			
Tuna		NKS---KGIVWNENTLME	YLENPKKYIPG-----	TKMIFAGIKKKGERQDLVAYLKSATS			
	50	60	70	80	90	100	
Methylobacterium		LKK---SGLTWDQADLK	WLADPKKKVPG-----	TKMVFPGISDPKKVDDIIAYLKT	KS		

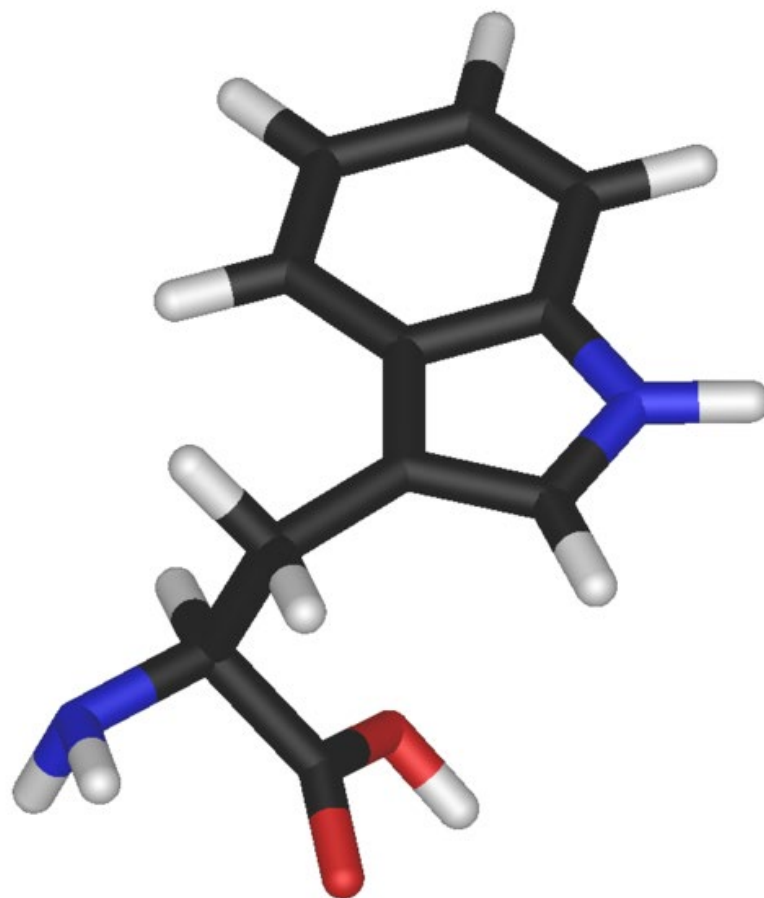
About 50% identity with sequence of typical cytochromes c

Almost all 'conserved residues' are present

Residues in red box are always Tyrosine
(100% conservation in 160 sequences).
But replaced by tryptophan in cytochrome c_H



Tyrosine



Tryptophan

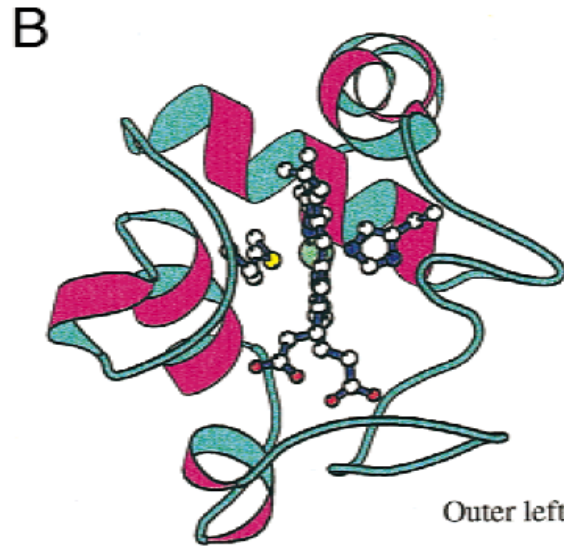
The molecular structure of an unusual cytochrome c_2
determined at 2.0 Å; the cytochrome c_H from
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JON READ, RAJ GILL, SIMON L. DALES, JON B. COOPER, STEVE P. WOOD,
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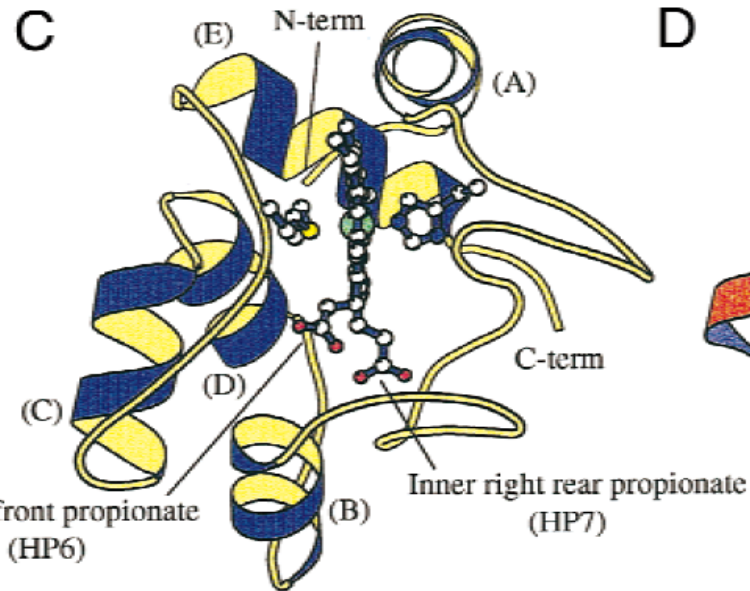
Division of Biochemistry and Molecular Biology, School of Biological Sciences, University of Southampton,
Southampton SO16 7PX, United Kingdom

Protein Science (1999), 8:1232–1240.

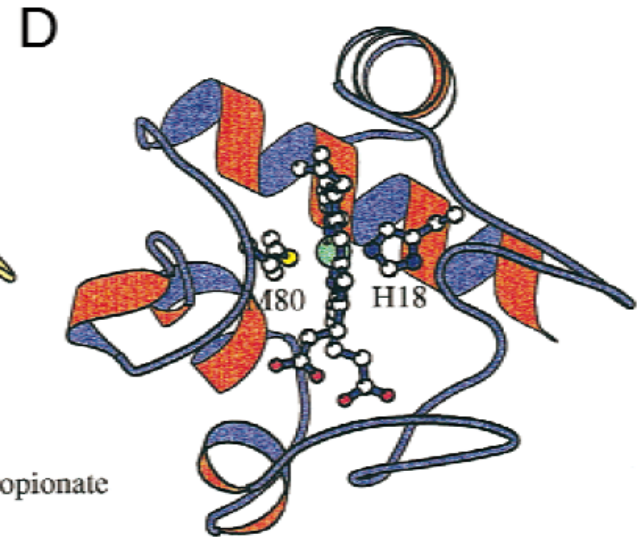
The overall folds of cytochrome c_H , cytochrome c , and cytochrome c_2 .



cytochrome c_H



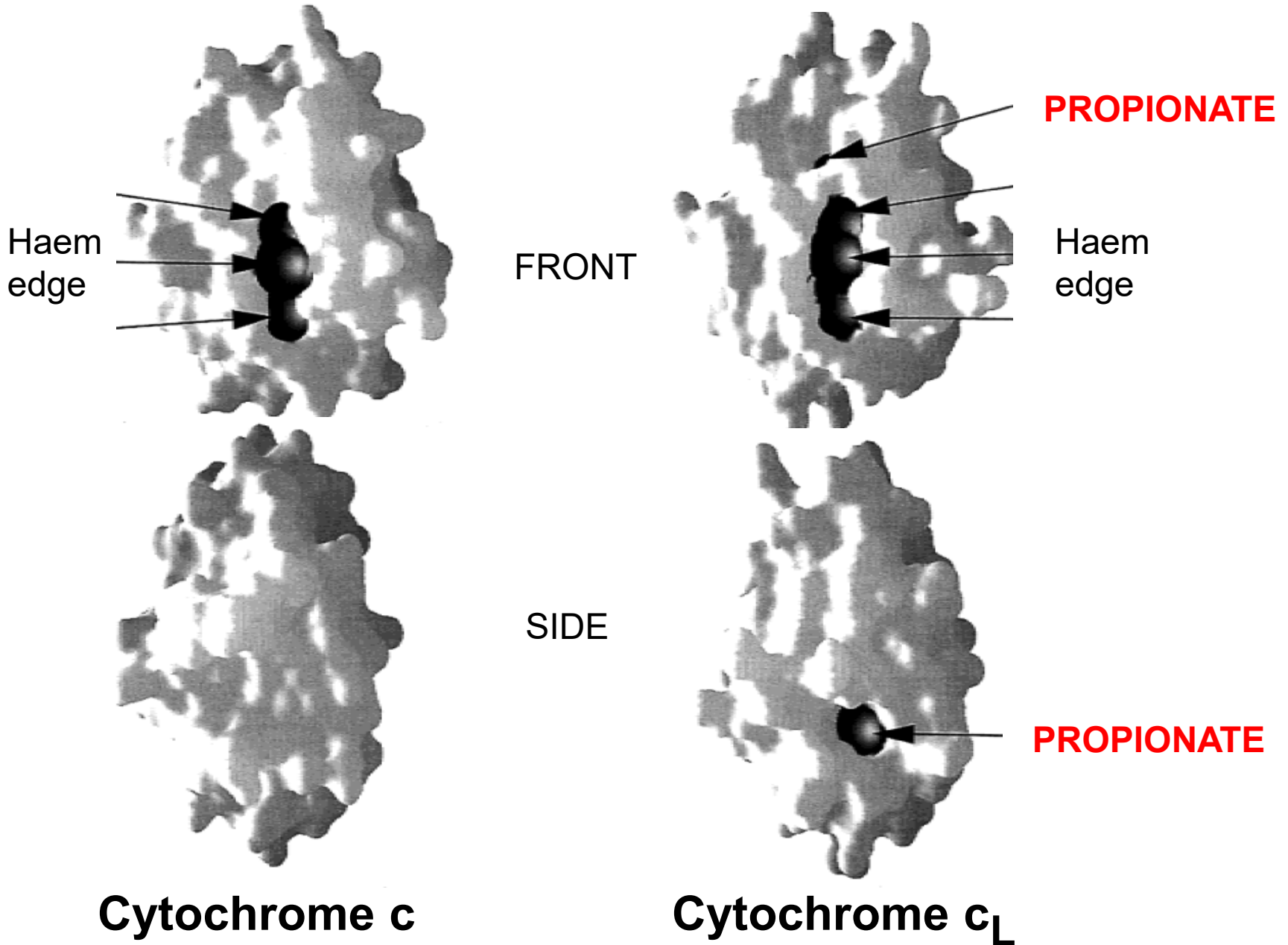
cytochrome c_2



cytochrome c

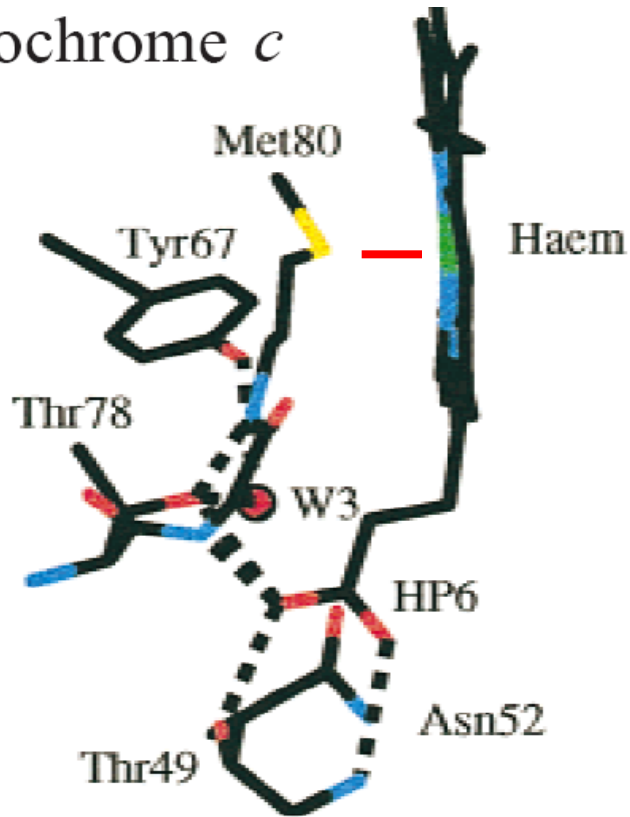
Cytochrome c_H is unique in being a bacterial cytochrome c that is folded into a mitochondrial cytochrome c structure

The exposed propionates in cytochrome c_H

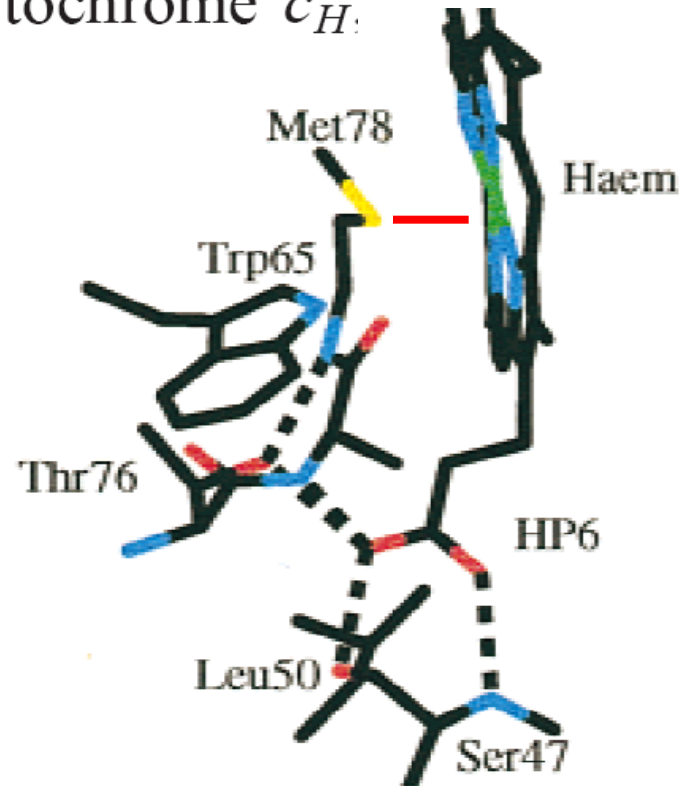


The LHS of the haem cleft in the cytochromes c

cytochrome *c*



cytochrome *c_H*



The 'absolutely conserved' Tyr67 is replaced by tryptophan

The 'conserved' water is absent; the LHS of haem cleft is hydrophobic

This shows that the previously proposed mechanism of cytochrome *c*, involving the 'essential water' is no longer valid.

The 1.6 Å X-ray Structure of the Unusual c-type Cytochrome, Cytochrome c_L , from the Methylophilic Bacterium *Methylobacterium extorquens*

Paul Williams¹, Leighton Coates¹, Fiyaz Mohammed¹, Raj Gill¹
Peter Erskine¹, Dominique Bourgeois², Steve P. Wood¹
Chris Anthony¹ and Jonathan B. Cooper^{1*}

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J. Mol. Biol. (2006) **357**, 151–162

172 residues instead of usual 100. Has 40-50 extra residues at N terminus
and a C-terminal extension

Negligible sequence identity to other cytochromes c.

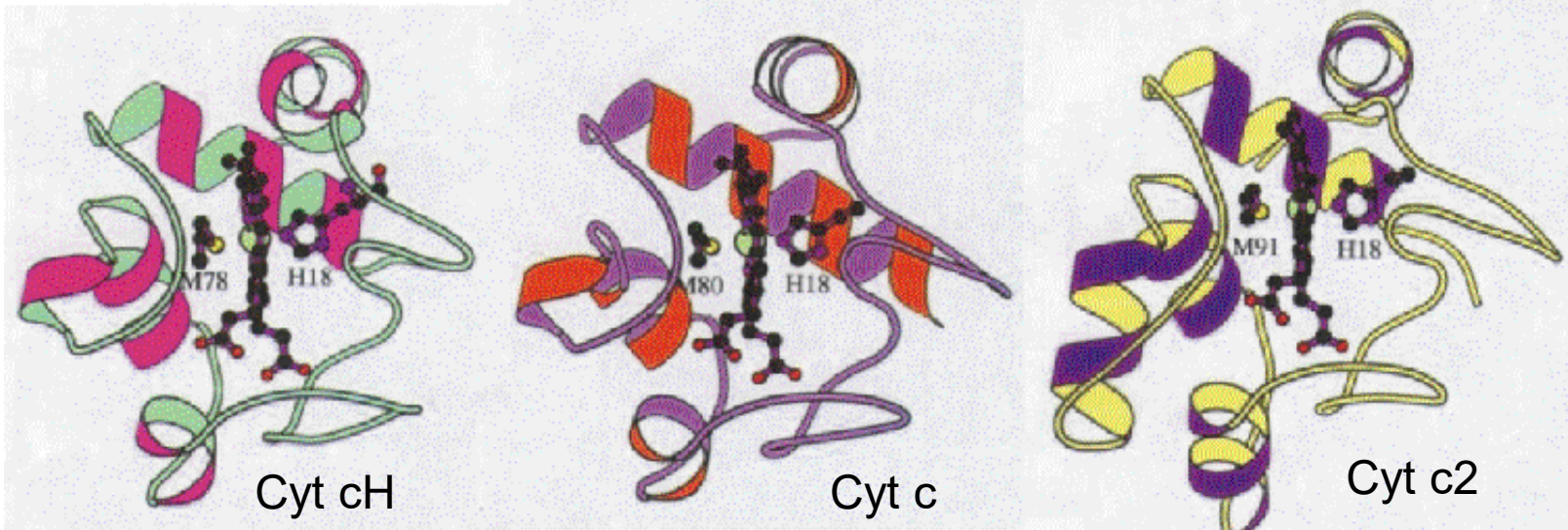
Predictions:

The haem propionates would be exposed to solvent

The loop bearing the 6th ligand methionine might be unusually flexible

The two very unusual cysteines would be involved in a disulphide bridge to hold the long loops together in the structure

The structure of cytochrome c_L



172 amino acids

No sequence similarity

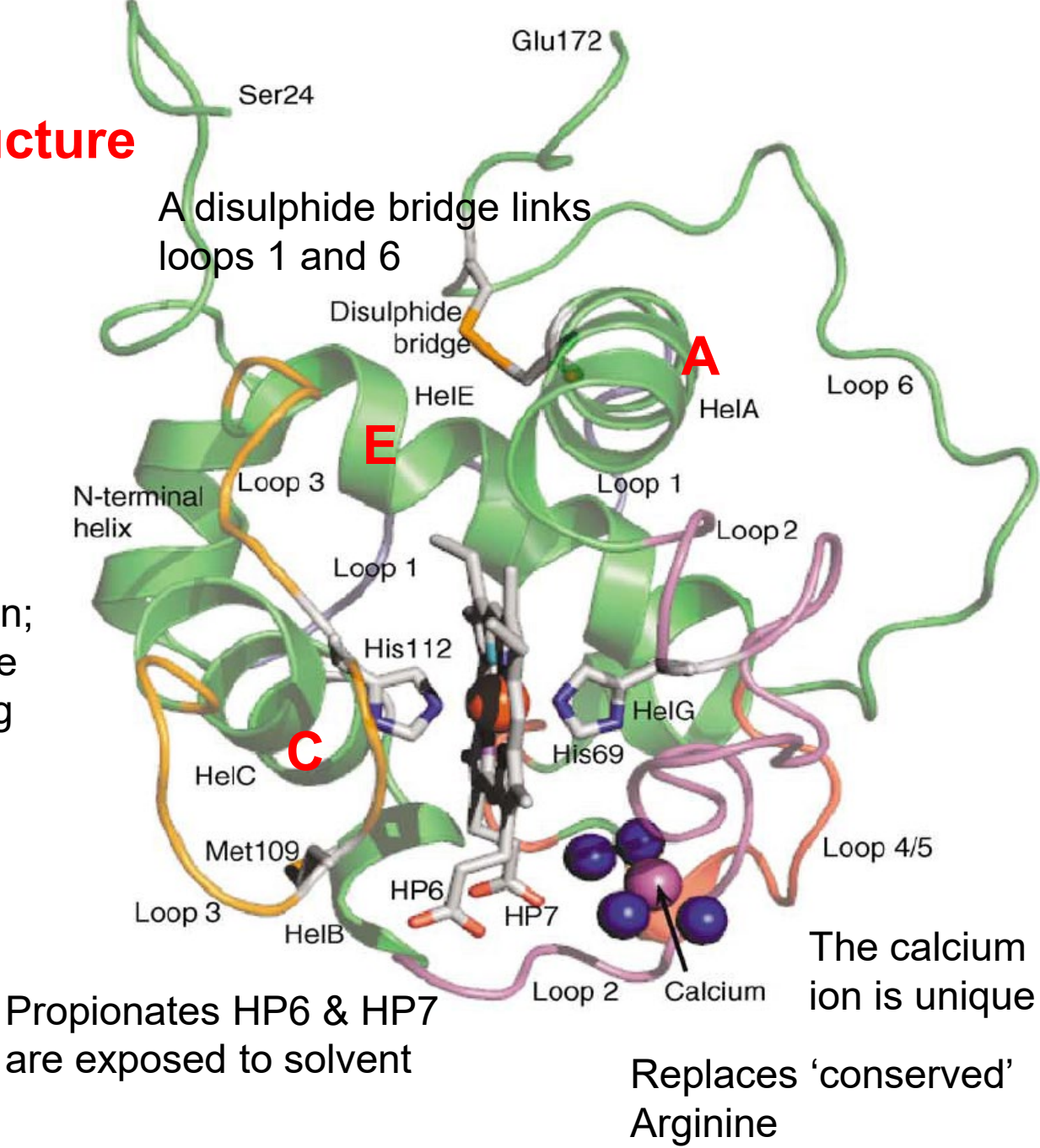
Similar basic structure
with additional features

Paul Williams

Cytochrome c_L structure

Helices A, C and E are usual haem-enclosing helices

His112 has replaced Met 109 as ligand to haem iron; Loop 3 is relatively flexible in this cytochrome leading to reaction with CO



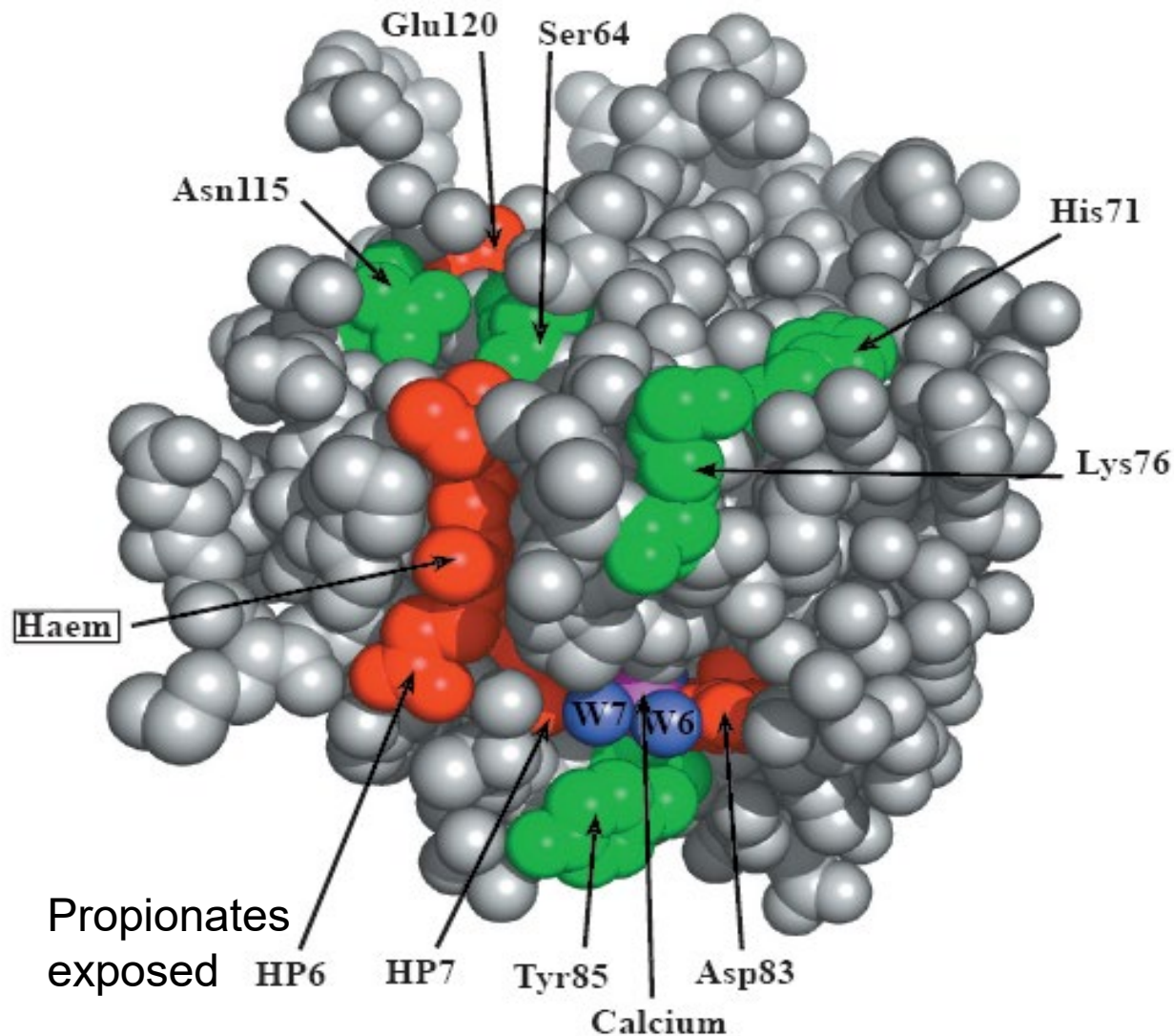
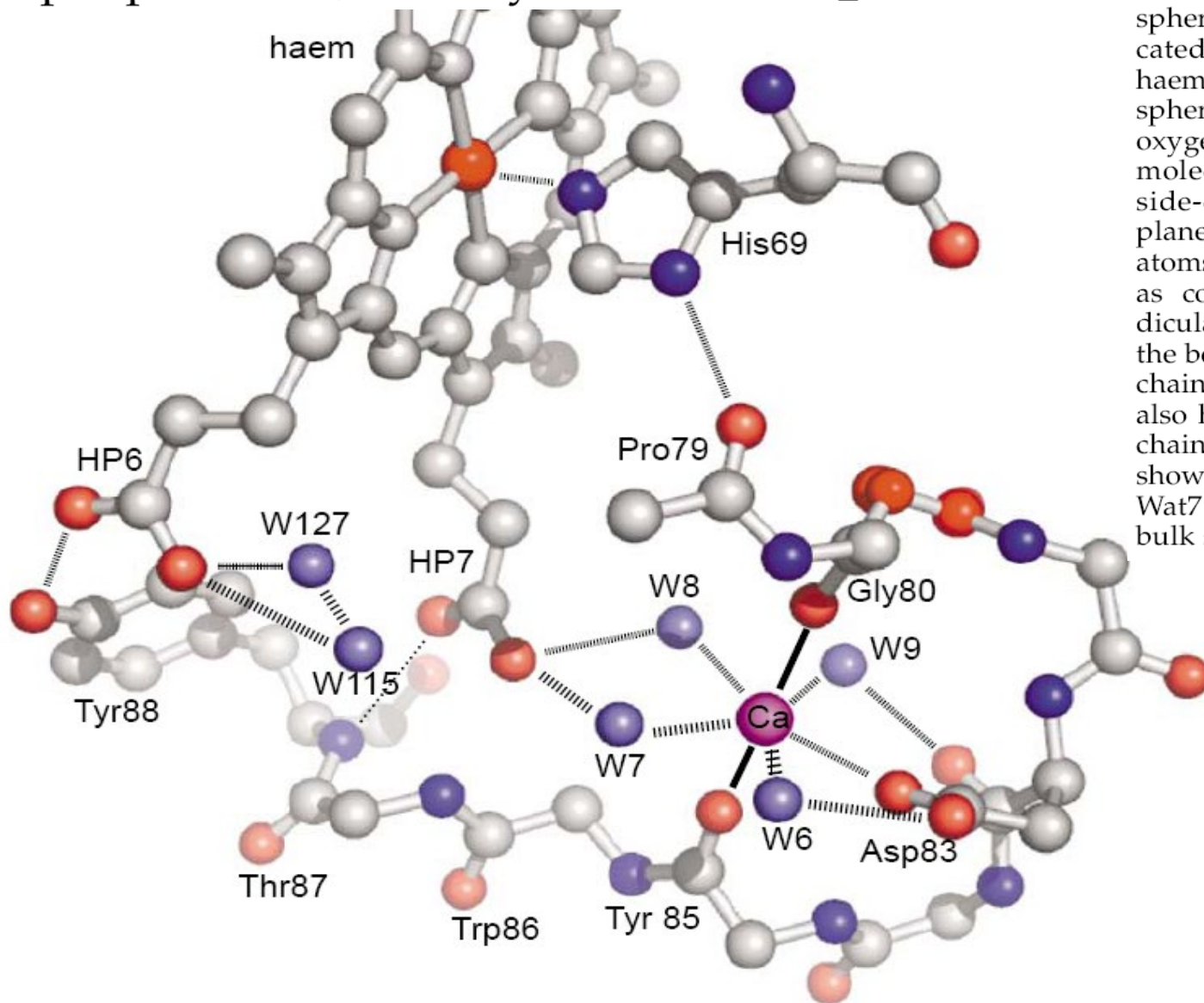


Figure 4. Surface view of cytochrome cL. The outer haem propionate (HP6) is clearly exposed to solvent as are the two waters (W6 and W7). W7 is bonded to the inner haem propionate (HP7). Asp83, one of the ligands to the Ca^{2+} ion, has been proposed to be involved in interaction with methanol dehydrogenase together with Glu120, the outer haem propionate (HP6) and those residues coloured green²⁰.

Figure 4. The calcium ion and the interactions of the two haem propionates, in cytochrome c_L .



HP6 is the outer haem propionate and HP7 is the inner haem propionate. The lines indicate hydrogen bonds or coordinations. Water molecules (Wat6–Wat9, Wat115, and Wat127) are indicated by blue spheres; the calcium ion is indicated by a magenta sphere; the haem iron is indicated by a red sphere. The calcium ion and the oxygen atoms of the four water molecules (Wat6–Wat9) and the side-chain of Asp83 lie in one plane; the bonds to the oxygen atoms of Gly80 and Tyr85 (shown as continuous lines) are perpendicular to this plane. In addition to the bonds indicated here, the main-chain carbonyl group of Asp83 is also hydrogen bonded to the side-chain nitrogen atom of Trp86, (not shown). The water molecules Wat6, Wat7 and Wat115 are exposed to bulk solvent (Figure 7).

Two unusual c-type cytochromes are involved in methanol oxidation pathway

Cytochrome c_H

Electron donor to the oxidase; Same sequence and function as typical cytochrome c

Typical cytochrome c structure except the folds are like mitochondrial (not bacterial) cytochrome

The 'conserved' tyrosine and water are absent; the hydrophobic haem cleft shows the water is not important in cytochrome mechanism

The propionates are exposed to solvent

Cytochrome c_L

Completely novel cytochrome. Although the 3-helix core is same as other cytochromes, there is negligible sequence identity and it is unique in lacking one of the 7 conserved positions found in all families of cytochromes c [haem binding and residues that hold 3 helices together in position]

A calcium ion replaces the 'conserved' arginine in balancing propionate charge

As predicted the haem propionates are exposed and there is a novel disulphide bridge

The two cytochromes, in the same organism and the same electron transport chain seem to have evolved completely independently

Acknowledgements:

David O’Keeffe, Mathew Beardmore-Gray, Sandy Cross, Jim Elliott, Bill Keevil, Sasha Netrusov, Claude Chan, Darren Day, Jon Cox, Simon Dales, Karen Amaratunga, Paul Skipp, Tosin Majekodunmi, Remi Afolabi,

Jon Read, Raj Gill, Jon B. Cooper, Steve P. Wood, Paul Williams, Leighton Coates, Fiyaz Mohammed, Raj Gill, Peter Erskine, Dominique Bourgeois,

Prof Akhtar for encouragement (“We are not interested in cytochromes, are we?”)