Computational Design of a *de novo* transmembrane cytochrome

EPSRC & BBSRC Centre for Doctoral Training in Synthetic Biology
SynBio
University of Bristol

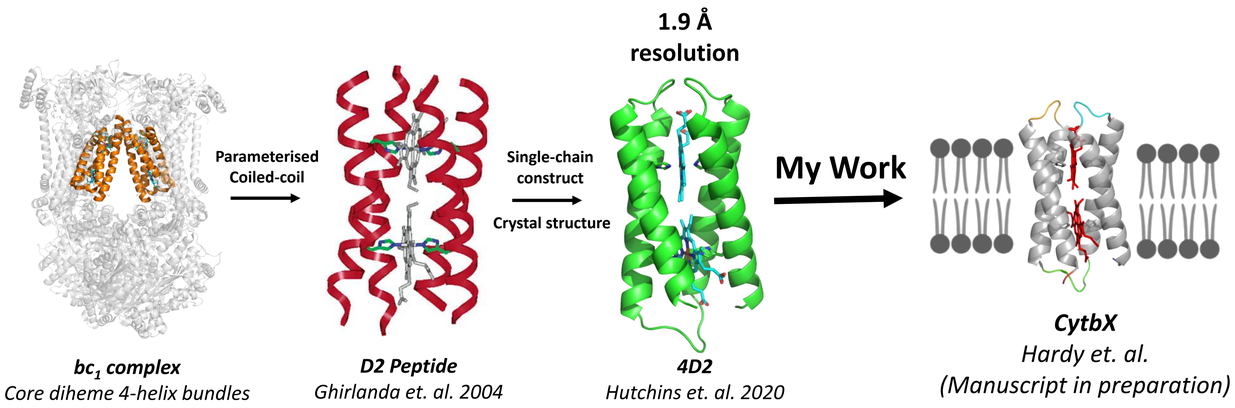
Ben Hardy, Ross Anderson, Paul Curnow corresponding email: ben.hardy@bristol.ac.uk
School of Biochemistry, University of Bristol, BS8 1TD

@benhardybristol

BrisSynBio
biomolecules to biosystems
from understanding to design

Background and Aims

- The aim of this work is to **convert** the water-soluble *de novo* design 4D2, a **parameterised maquette** of the bc_1 core **di-heme** four-helix bundle[1], **to a membrane protein** via computational design
- The purpose of the design is to be a **minimal module for transmembrane electron transfer**

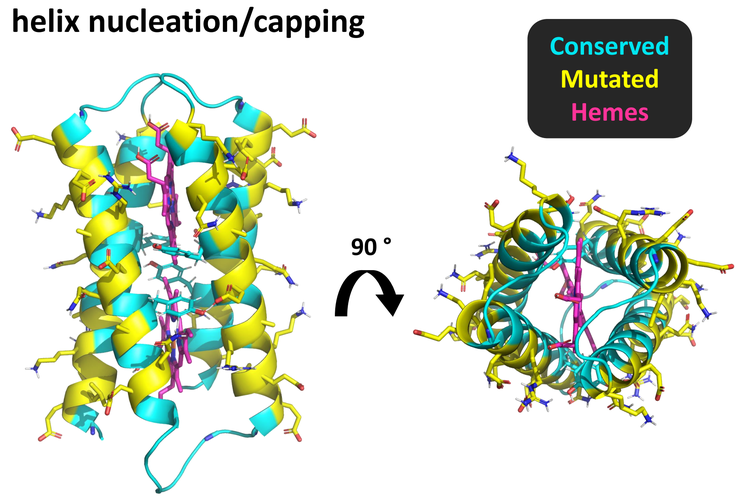


Computational Protein Design

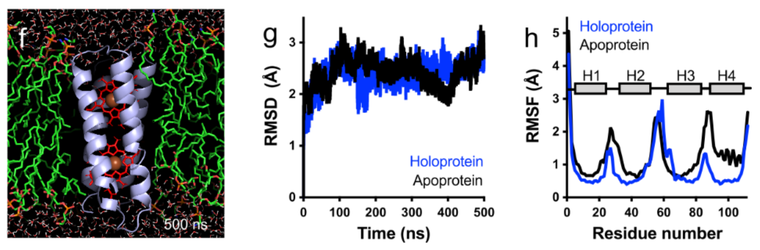
Rosetta was used to **mutate surface residues** of 4D2 to become lipophilic, whilst **preserving residues** responsible for **coiled-coil interactions**, **heme binding**, and **helix nucleation/capping**

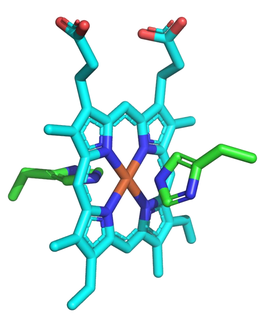
Mutations were evaluated using the ***franklin2019*[2] scorefunction**

Designs were **ranked** based on total **Rosetta score**, quality of **core sidechain packing**, and number of **hydrogen bonds** to hemes



500 ns MD simulations showed CytbX is stable in a **3:1 DOPE:DOPG bilayer** in both the **apo** and **holo** states





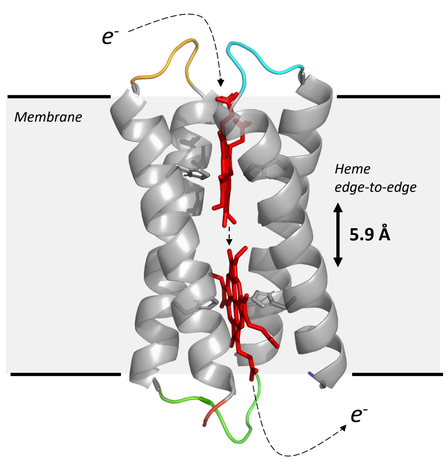
Two b-type hemes bound by **bis-histidine coordination,** supplied by the **endogenous heme biosynthesis pathway of *E. coli***

Anti-parallel four-helix bundle with **N_{in}-C_{in} topology** enforced by the **positive-inside rule** (charged loops)

Interfacial aromatic residues for anchoring at the **lipid head-group water boundary**

Leucine/isoleucine-rich hydrophobic surface

Knobs-into-holes packing motifs to specify **helix-helix interactions** in the membrane

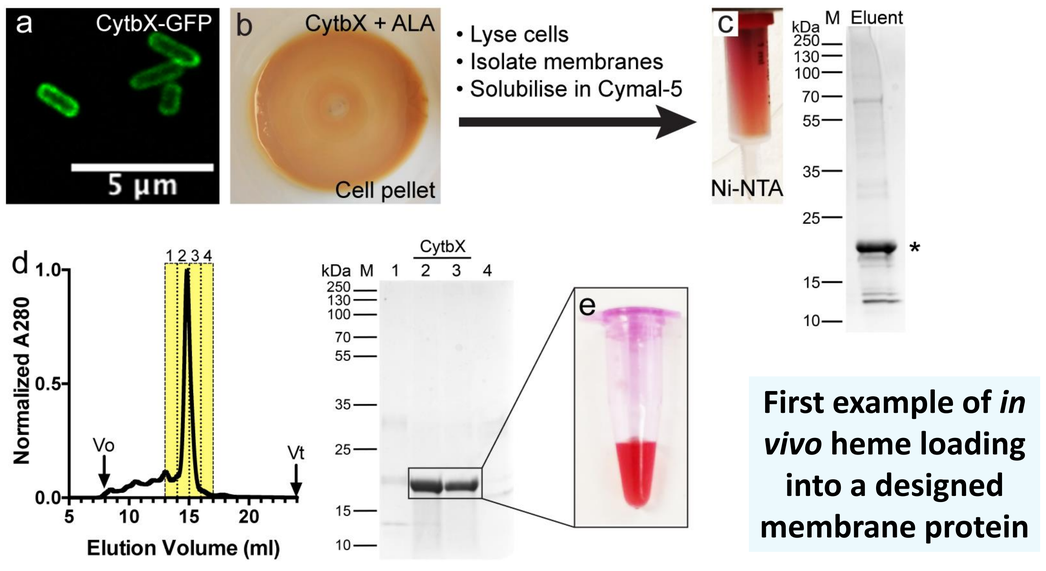


Protein Orientation: Specified
Electron transfer directionality: Unspecified

Recombinant Expression and Purification

CytbX is **recombinantly expressed** in **C43** (DE3) *E. coli* and inserted into the plasma membrane by the natural translocation machinery.

Monodisperse, red-coloured CytbX can be **purified from** *E. coli* **membranes** using maltoside detergents
For purification methods see ref.[3]



First example of *in vivo* heme loading into a designed membrane protein

Biophysical and Electrochemical Characterisation

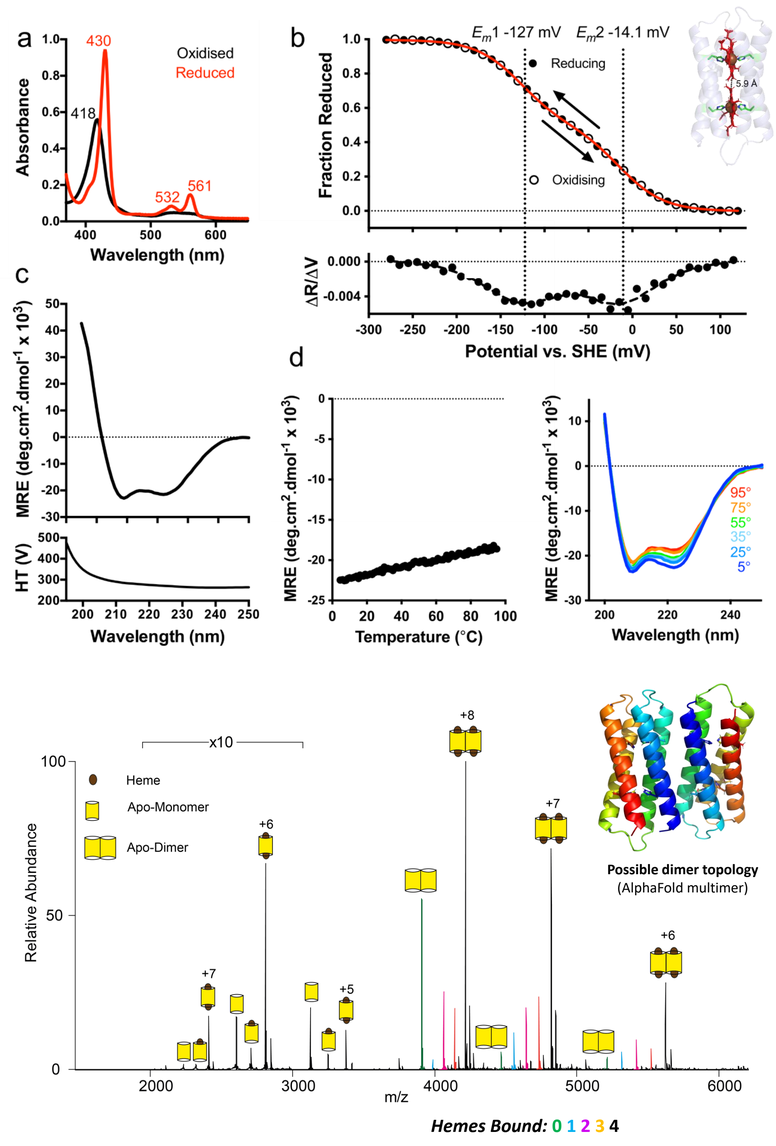
UV-vis absorbance spectroscopy confirms binding of **heme *b*** by **bis-histidine** coordination

Two distinct **midpoint potentials** are observed, **split** by about **110 mV**, indicating **electronic coupling** of **two hemes in close proximity**, as designed.
Resembles b_L and b_H of the bc_1 complex

Circular dichroism (CD) spectroscopy confirms that CytbX contains **alpha-helical** secondary structure, and reveals **exceptional thermal stability**

Native mass spectrometry (MS) of purified CytbX confirms binding of **two hemes per protein**
(collaboration with Robinson Group, Oxford UK)

MS reveals that CytbX is purified as a **stable dimer,** reminiscent of the **dimeric** interface of ***b subunits*** in the **bc_1/b_6f complexes**

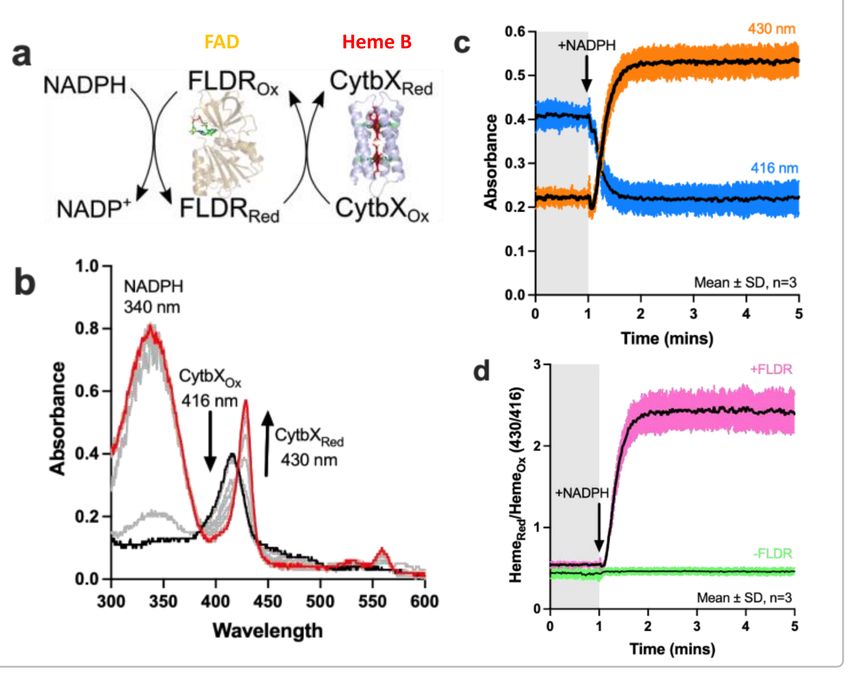


Minimal Electron Transport Chain

E. Coli ***f*lavodoxin reductase** (FLDR) can rapidly transfer electrons **from NADPH to the hemes** of CytbX (in micelles)

Oxidation state of the hemes is monitored **spectroscopically**

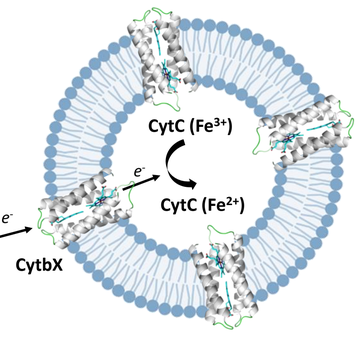
This demonstrates that the **membrane embedded hemes** are **accessible** to small diffusive redox partners



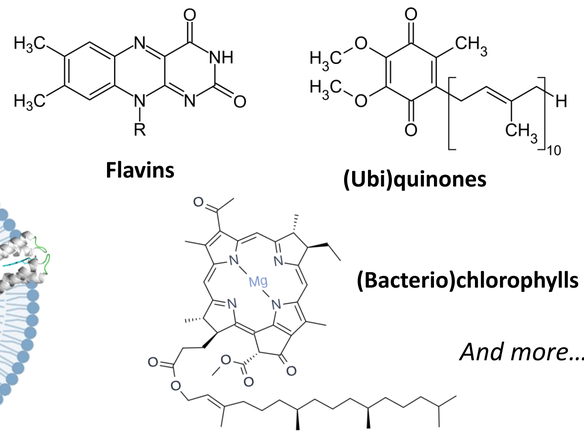
Conclusions and Future Work

Conversion of 4D2 to a transmembrane protein was **successful**, and CytbX efficiently folds and binds the desired **cofactors *in vivo***.

Demonstrate transmembrane electron transfer in **proteoliposomes**



Alternative Cofactors



1. Hutchins, G. H et al. *bioRxiv* **2020**, 2020.09.24.311514. https://doi.org/10.1101/2020.09.24.311514
2. Alford, R. F, et al. *Biophys. J.* **2020**, *118* (8), 2042–2055. https://doi.org/10.1016/J.BPJ.2020.03.006
3. Curnow, P., Hardy, B.J., *et al. Sci Rep* **10,** 15203 (2020). https://doi.org/10.1038/s41598-020-71585-8