Improved drug delivery systems: Targeting specific organelles and suborganellar compartments

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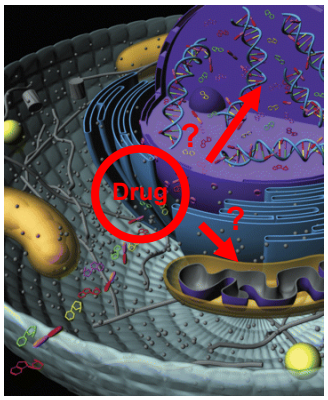
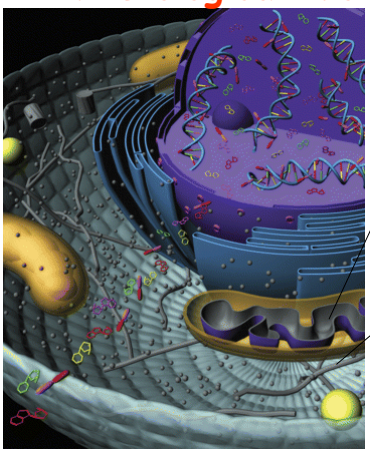
1900s Ehrlich , 'Magic bullet'

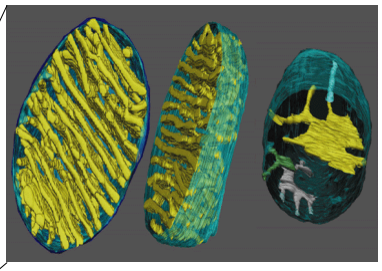
Goal: Tailored and efficient therapeutics



Critical need for drug delivery site-specifically at the subcellular Level

The Biological Problem





Mito facts:
1500 proteins
own mtDNA encoding only 13 proteins
>99% have to be imported

→Protein import is the crucial mechanism of mitochondria biogenesis

1. Components?
2. Mechanisms?
3. Relevance in health and disease?

90% of the cells energy is provided by mitochondria

-More than 300 mitochondrial diseases
-Involved in ageing, cancer, heart disease
-Key regulators of apoptosis
-United Mitochondrial Disease foundation: a child born every 15 min suffers or will develop a mito disease by the age of 5

Mitochondria-specific nanotechnology

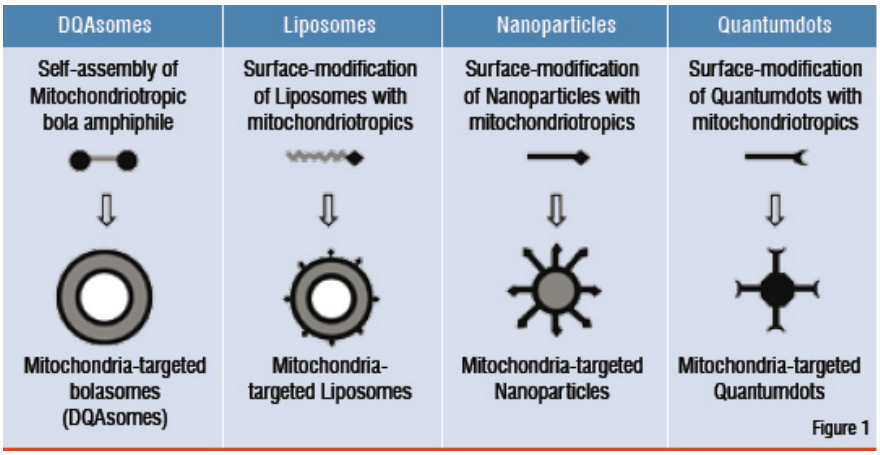
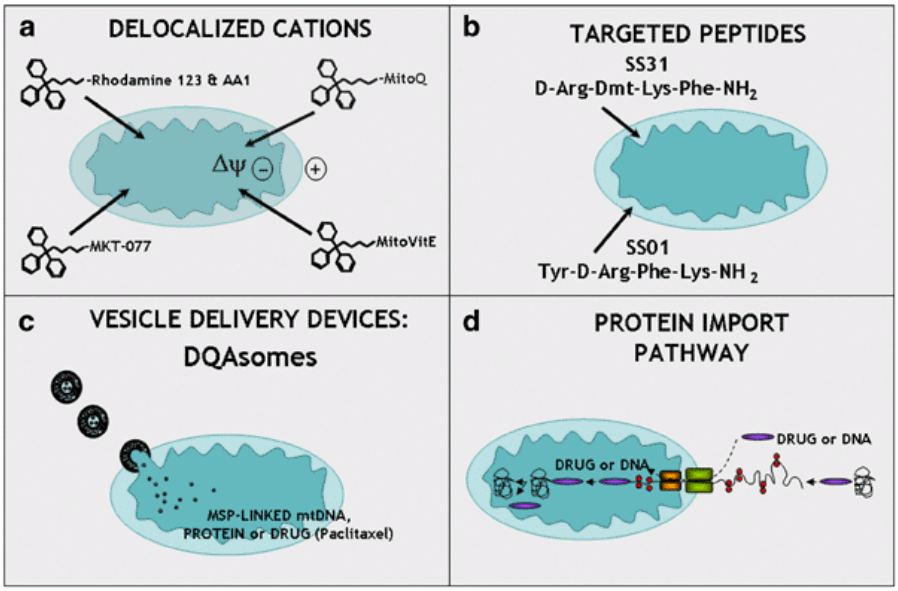
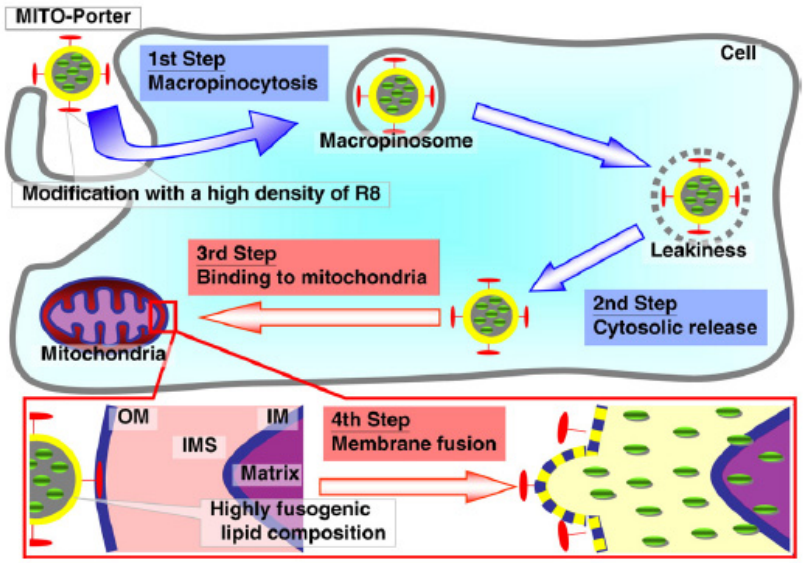


Figure 1

Pharmacological targeting of mitochondria in disease





Mitochondria-specific drug carrier systems are badly needed

Example: Ischemia Reperfusion

↓

cellular damage

↓

Necrotic cell death

↓

mitochondria MPTC

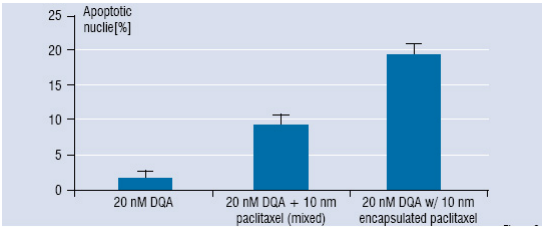
↓

mitochondria MPTC

Cyclosporin A (CsA)inhibits MPTC by binding to cyclophilin

Problem: There are at least 9 non-mitochondrial cyclophilins…

Effect on cell death of the encapsulation of paclitaxel in mitochondrially-targeted DQAsomes



Weissig et al. 2004

more than 30% of proteome
are membrane proteins

About 50% of drug targets
in Pharma Industry
are membrane proteins

Mitochondria are
<u>essential</u> for life

Functional Complexity

Respiration and ATP Synthesis

Synthesis of heme, lipids, amino acids and nucleotides

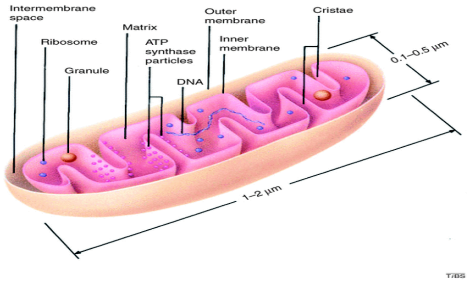
Intracellular homeostasis of inorganic ions

Structural Complexity

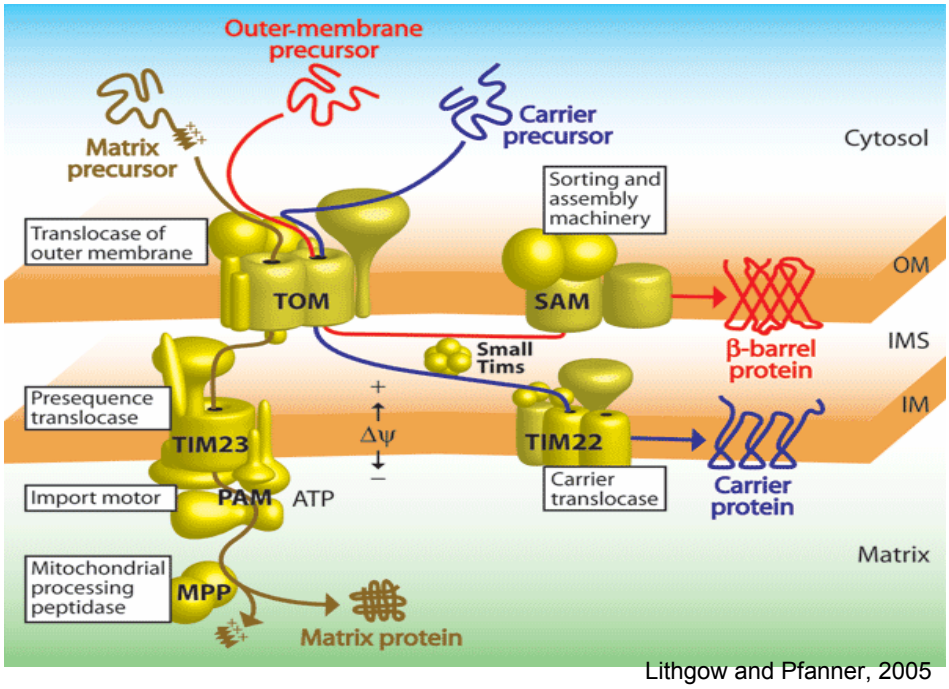
5-15% of total cell protein
20% volume of eukaryotic cell
IM is 1/3 of total cell membrane

About 1000 different polypeptides (900 in yeast)

Only a dozen encoded by mtDNA

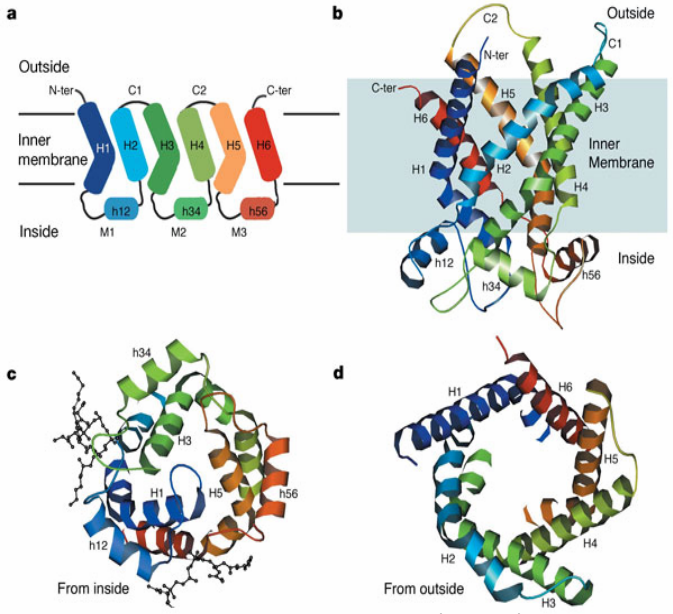


Protein import is the major mechanism of mitochondria biogenesis



Lithgow and Pfanner, 2005

The 3D structure of the AAC



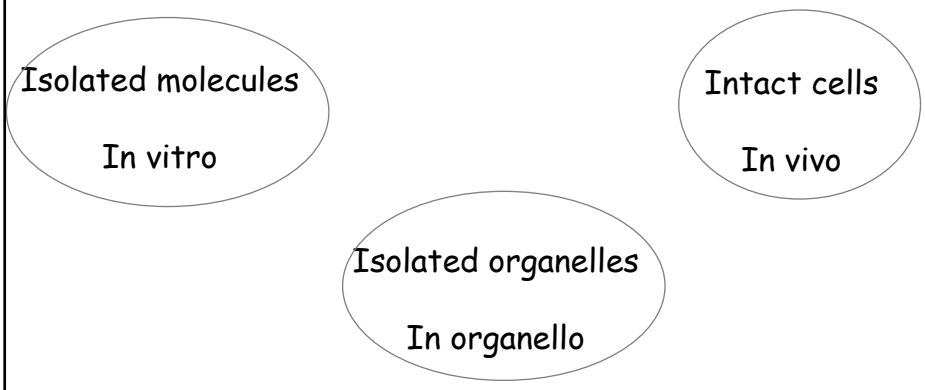
Pebay-Peyroula et al, Nature, 2003

How does the TIM10 complex assemble?

How do the subunits fold on their own?

How do the folded subunits assemble?

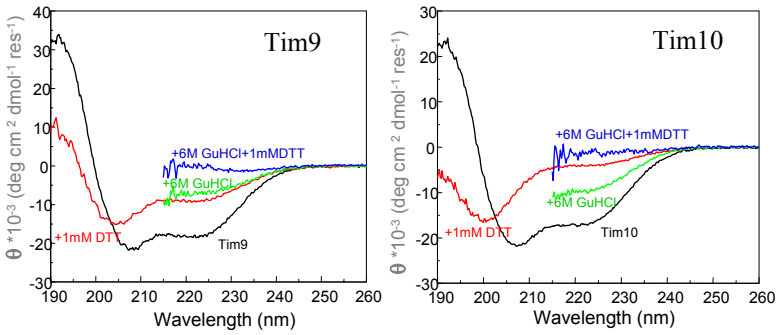
Approach



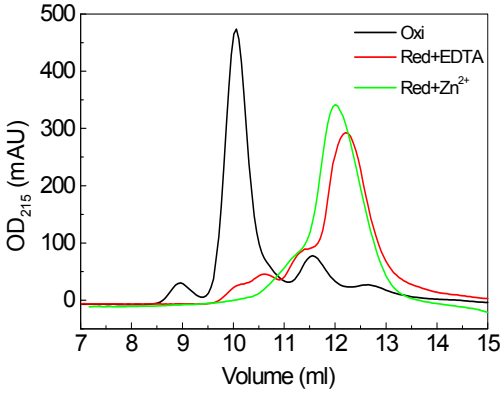
By using …

Protein purification
Mutagenesis
CD analysis
Limited proteolysis
Bioinformatics
Mass spectrometry
Chemical modification of thiol groups. in vivo thiol trapping
Gel filtration
Isothermal titration calorimetry ITC
Analytical centrifugation
Multi-angle light static scattering

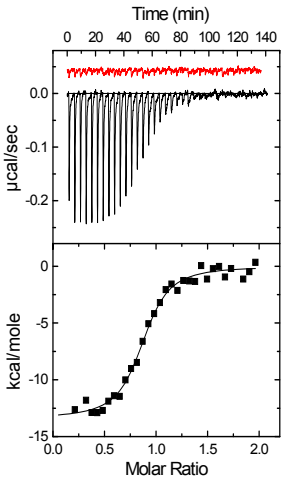
CD analysis of Tim9 and Tim10



Oxidation of Tim9 and Tim10 is required for complex formation



ITC study of the interaction between Tim9 and Tim10

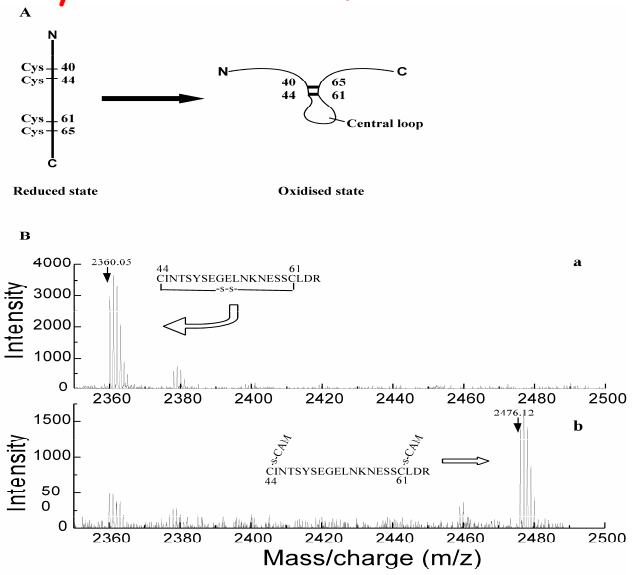


Reduced Tim9/Tim10

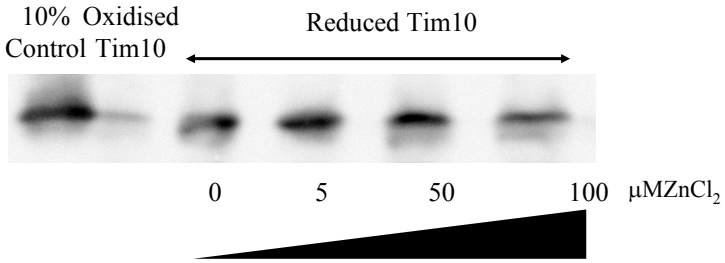
***Oxidised* Tim9/Tim10**

N= 0.9 (Tim10/Tim9)
$K_a = 5x10^6\ M^{-1}$
ΔH= -13kcal/mol

Mass spectrometry analysis of the Cys connectivities of Tim10



Prior Oxidation inhibits import

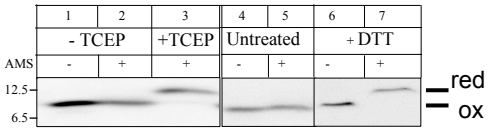


Additional data:

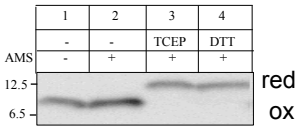
1. NEM alkylation
2. Cys mutants

Tim10 is oxidised *in vivo*

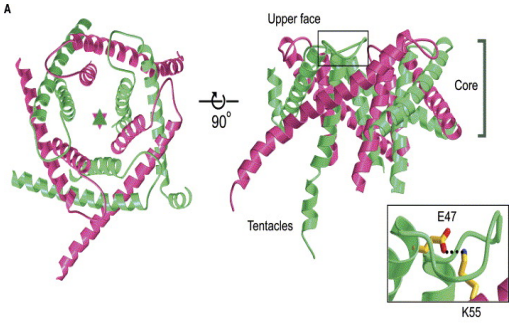
Intact cells

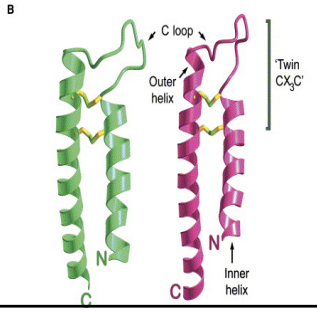


Intact mitochondria



Crystal structure of the human Tim9-Tim10 complex





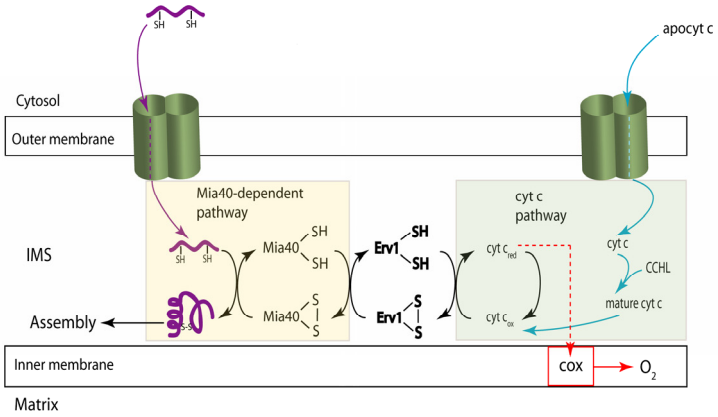
…in agreement with our biochemical analysis and SAXS studies

Webb et al, Mol Cell 2006

What does this mean?

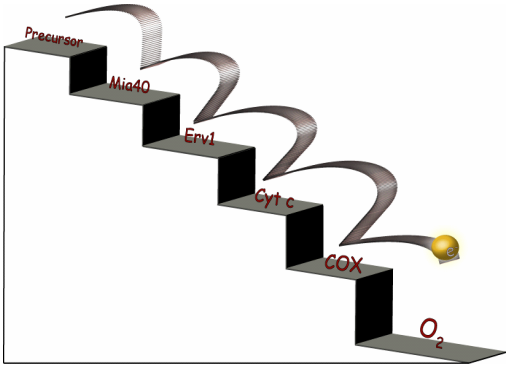
NOVEL oxidative folding pathway operating in mitochondria *in vivo*

…closing the loop:
CytC and the respiratory chain are the final acceptors of electrons from the imported precursor

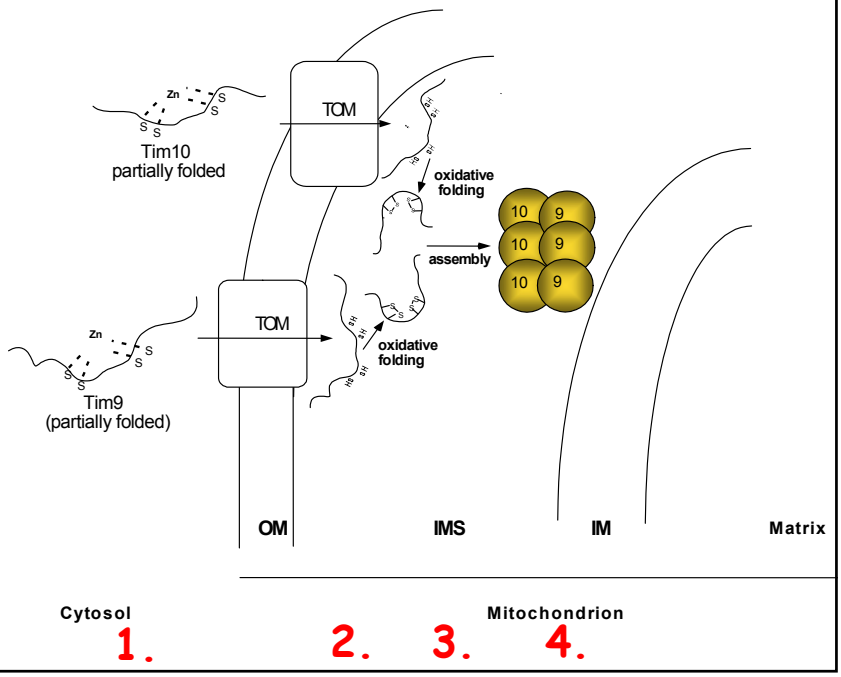


Allen et al., JMB, 2005; cover

A link between protein import and respiration: The Electron flow

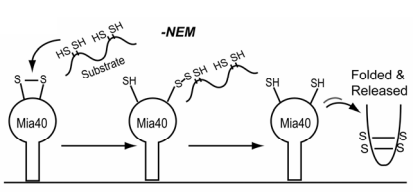


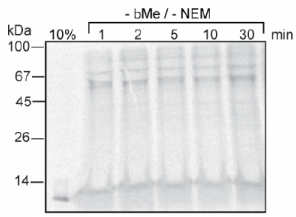
Multistep assembly pathway : Dissection of subreactions

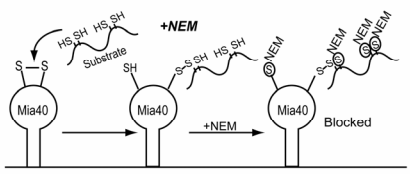


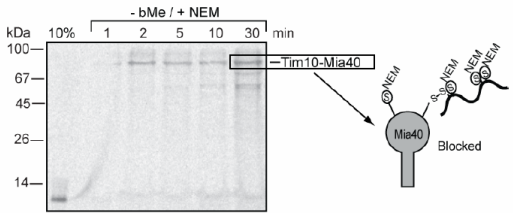
Functional and Structural analysis of Mia40

In vitro reconstitution of the interaction with the substrate

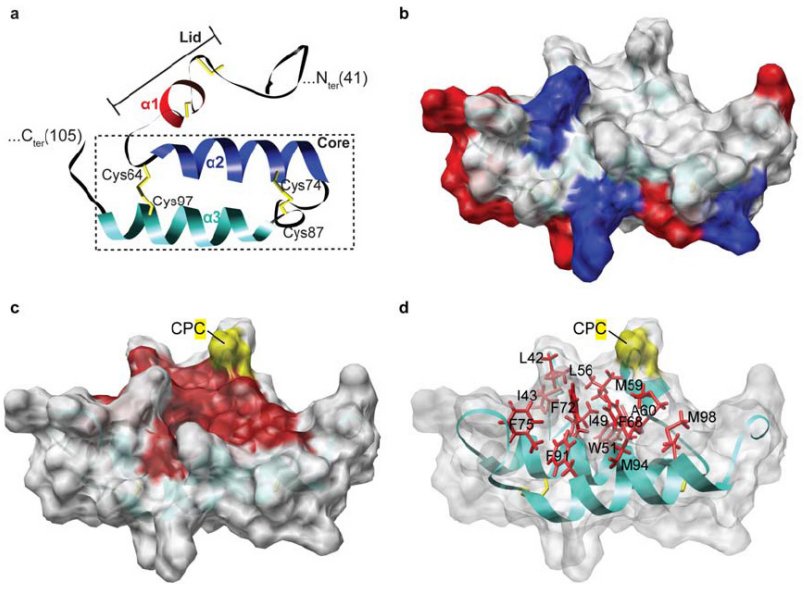




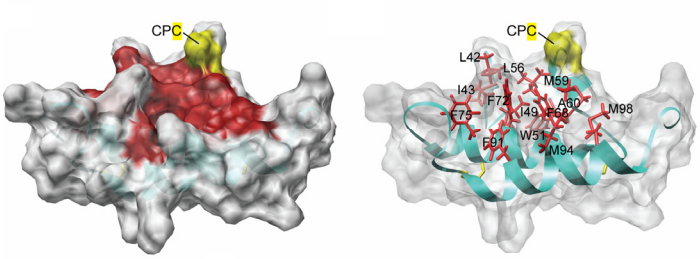




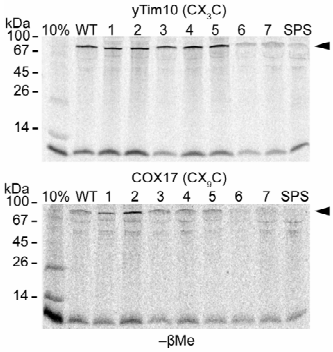
Solution structure of MIA40 by NMR

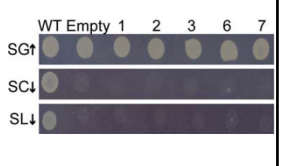


The hydrophobic cleft mediates non-covalent binding of the substrate



No.	yMia40	MIA40
1	I292A W294A F311A F315A	I49A W51A F72A F75A
2	L298A M302A F311A F315A	L56A M59A F72A F75A
3	F311A F315A F334A M337A	F72A F75A F91A M94
4	I292A W294A L298A M302A F311A F315A	I49A W51A L56A M59A F72A F75
5	I292A W294A F311A F315A F334A M337A	I49A W51A F72A F75A F91A M94
6	L298A M302A F311A F315A F334A M337A	L56A M59A F72A F75A F91A M94A
7	I292A W294A L298A M302A F311A F315A F334A M337A	I49A W51A L56A M59A F72A F75A F91A M94A





What is the mechanism of interaction between Mia40 and the substrate?

Biochemical dissection of the oxidation of small Tims by Mia40:

-Mia40 is a specific oxidase, distinguishing between Cys residues of the substrate

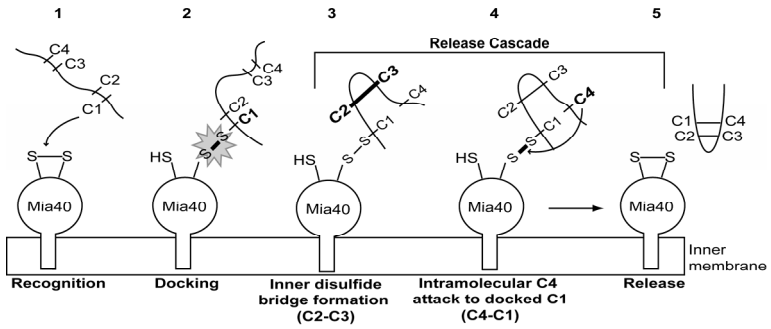
-The N-terminal first Cys serves as an essential docking point onto Mia40 upon import of the substrate

- The C-terminal cysteine is necessary for release

- Metal binding is not required

- We have established an efficient reconstitution system in vitro and in organello for the interaction of the substrates with Mia40

Coordinated Docking and release of the substrate onto Mia40



→ Mia40 functions in a site-specific and processive manner

Sideris and Tokatlidis Mol Microbiol 2007

What about other substrates for Mia40?

How are these recognised?

NMR of hMia40 together with the substrate hCox17

Mia40 completely oxidizes Cox17 in the presence of oxygen

Two disulfide pairs of Cox17 are formed to the detriment of one CPC of Mia40

Mia40 CPC is concomitantly reduced

The reaction proceeds with 1:1 stoichiometry

Only the CPC region undergoes structural changes upon interaction with the substrate

Banci et al., Nature SMB, 2009

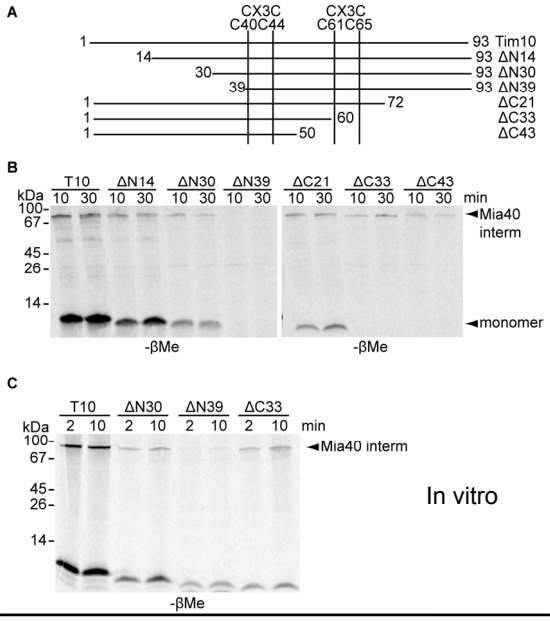
Cox17 is recognised on its 3rd Cys of the CX9C motifs …!!!

→CONUNDRUM?

How can Mia40 recognise site-specifically two completely unrelated substrates?

SOLUTION: Common targeting signal on both substrates, the ITS

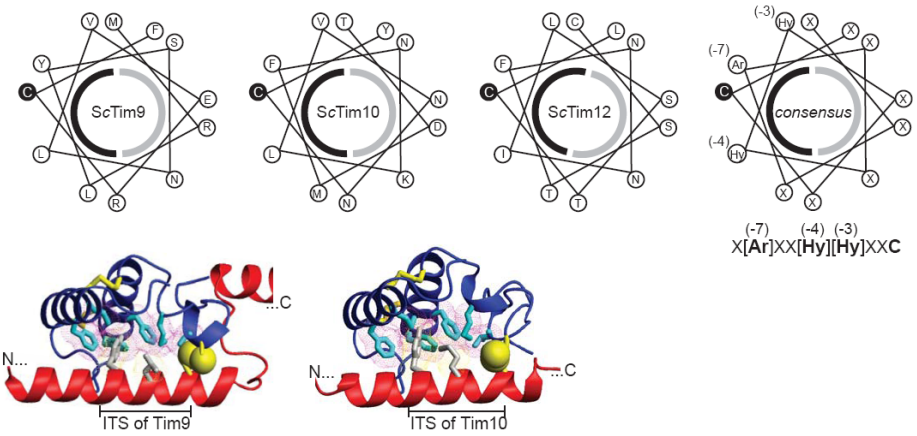
Identification of the targeting signal of Tim10 for Mia40
The Inetermembrane space Targeting Signal (ITS)



In organello

In vitro

Structural basis for the binding of the ITS onto the cleft of Mia40



Sideris et al. 2009 submitted

Generality of the ITS function

It is present in essentially all of the CX3C and CX9C substrates

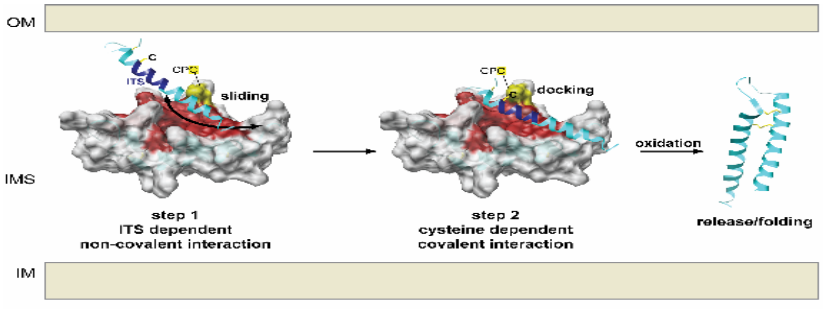
It functions independently and can be fused C-terminally to a protein

It can target non-mitochondrial proteins

Recognition by Mia40 is mainly through hydrophobic interactions

Isothermal titration calorimetry measurements give a Kd of 2 μM

Mechanism of substrate recognition by Mia40: The sliding – docking model



Conclusions

An oxidative folding pathway operates in mitochondria

Docking of the substrate to the Mia40 represents a site specific event that is crucial step for the oxidative folding process

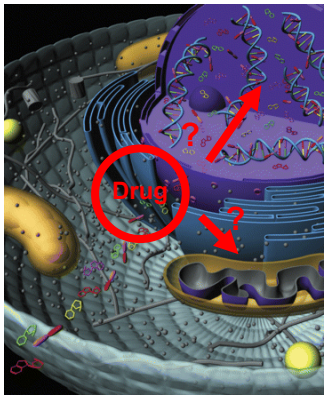
The process is guided by a novel ITS that directs the first step of noncovalent recognition by Mia40

Mia40 represents structurally, functionally and mechanistically a new type of cellular oxidoreductase

A new mechanism of peptide-based targeting to the intermembrane space of mitochondria

1900s Ehrlich , 'Magic bullet'

Goal: Tailored and efficient therapeutics



Critical need for drug delivery site-specifically at the subcellular and sub-organellar level

Acknowledgements

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