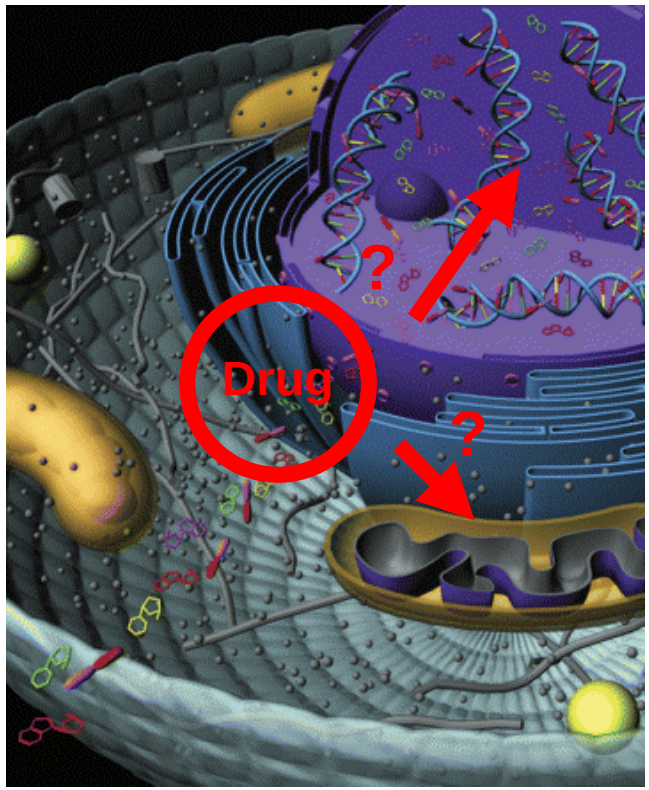
# Nanomedicine and Innovation in drug delivery: Mitochondrial nanotechnology

Kostas Tokatlidis
Institute of Molecular Biology and Biotechnology
(IMBB-FORTH)
and
University of Crete

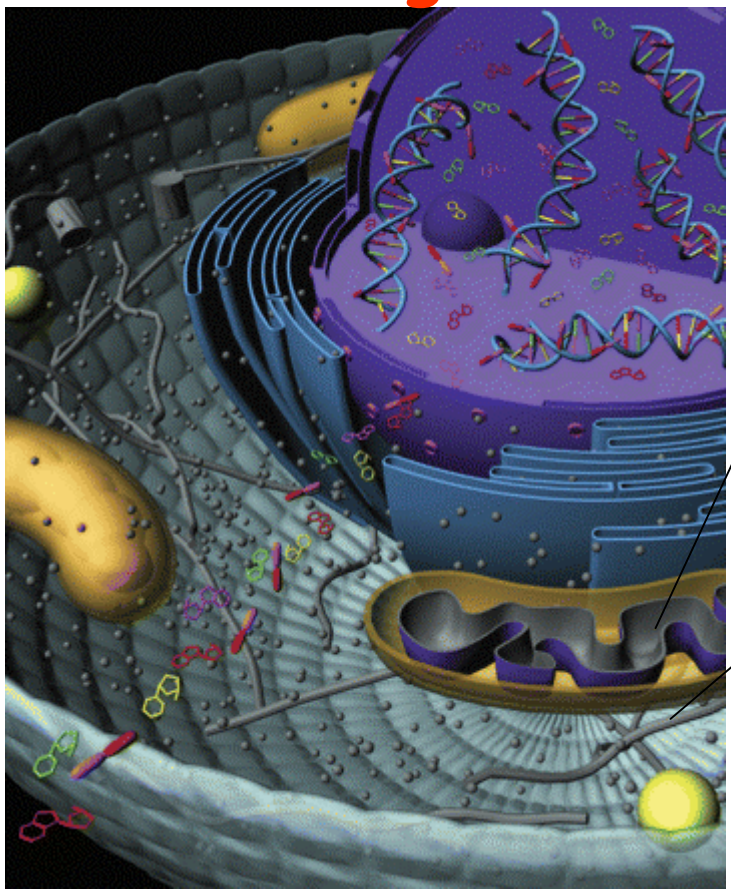
# 1900s Ehrlich , 'Magic bullet'

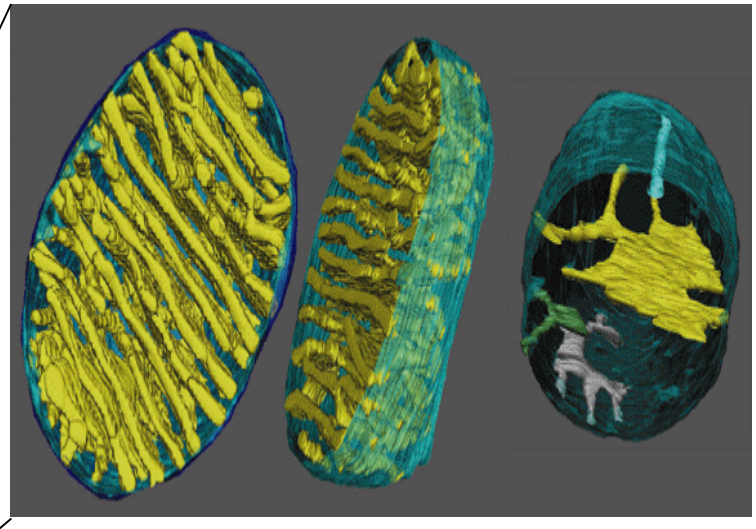
## Goal: Tailored and efficient therapeutics




**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

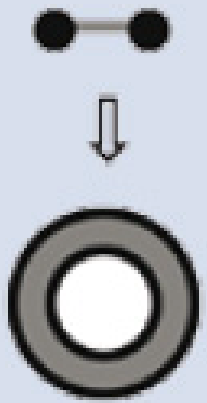
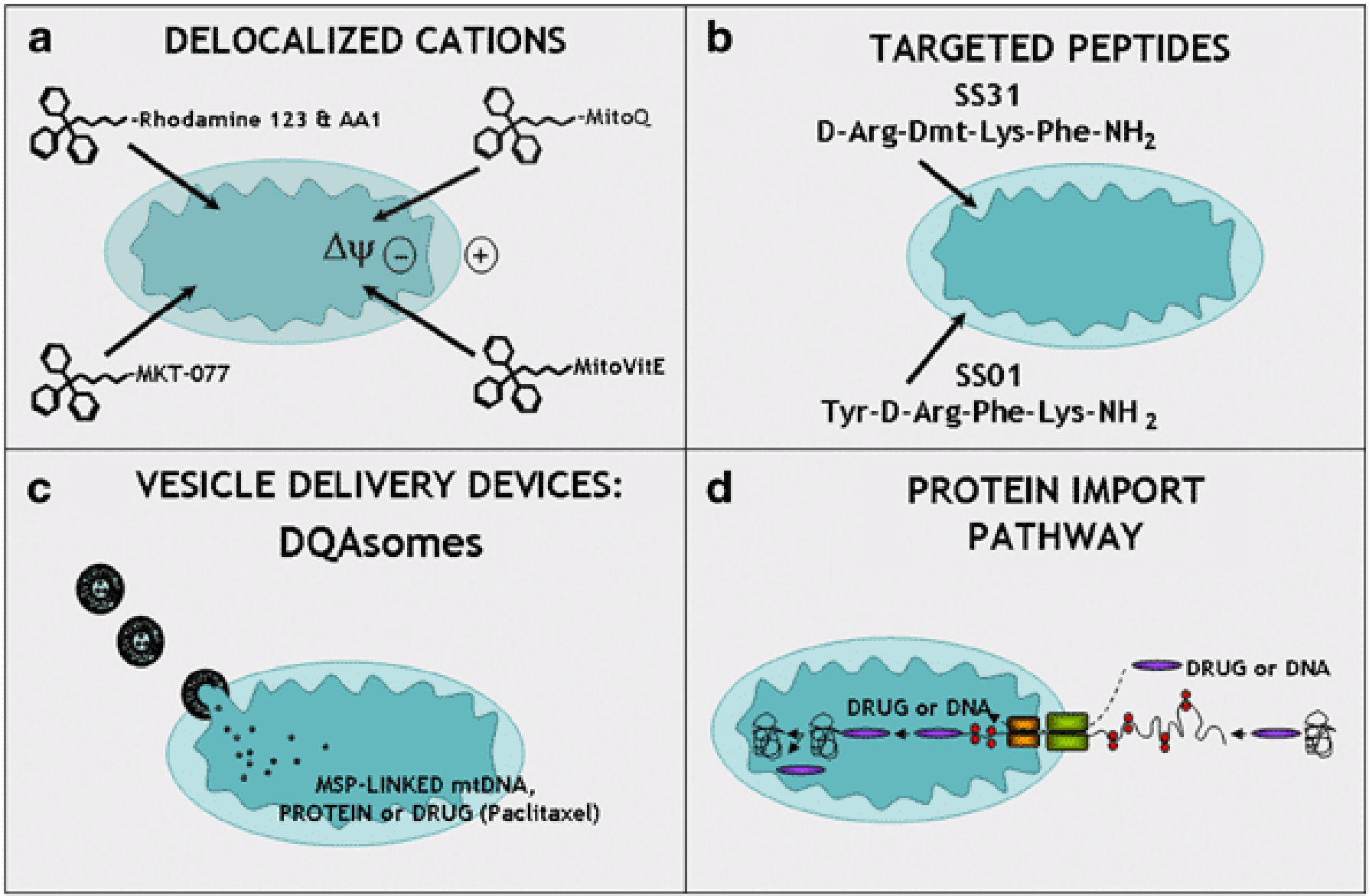
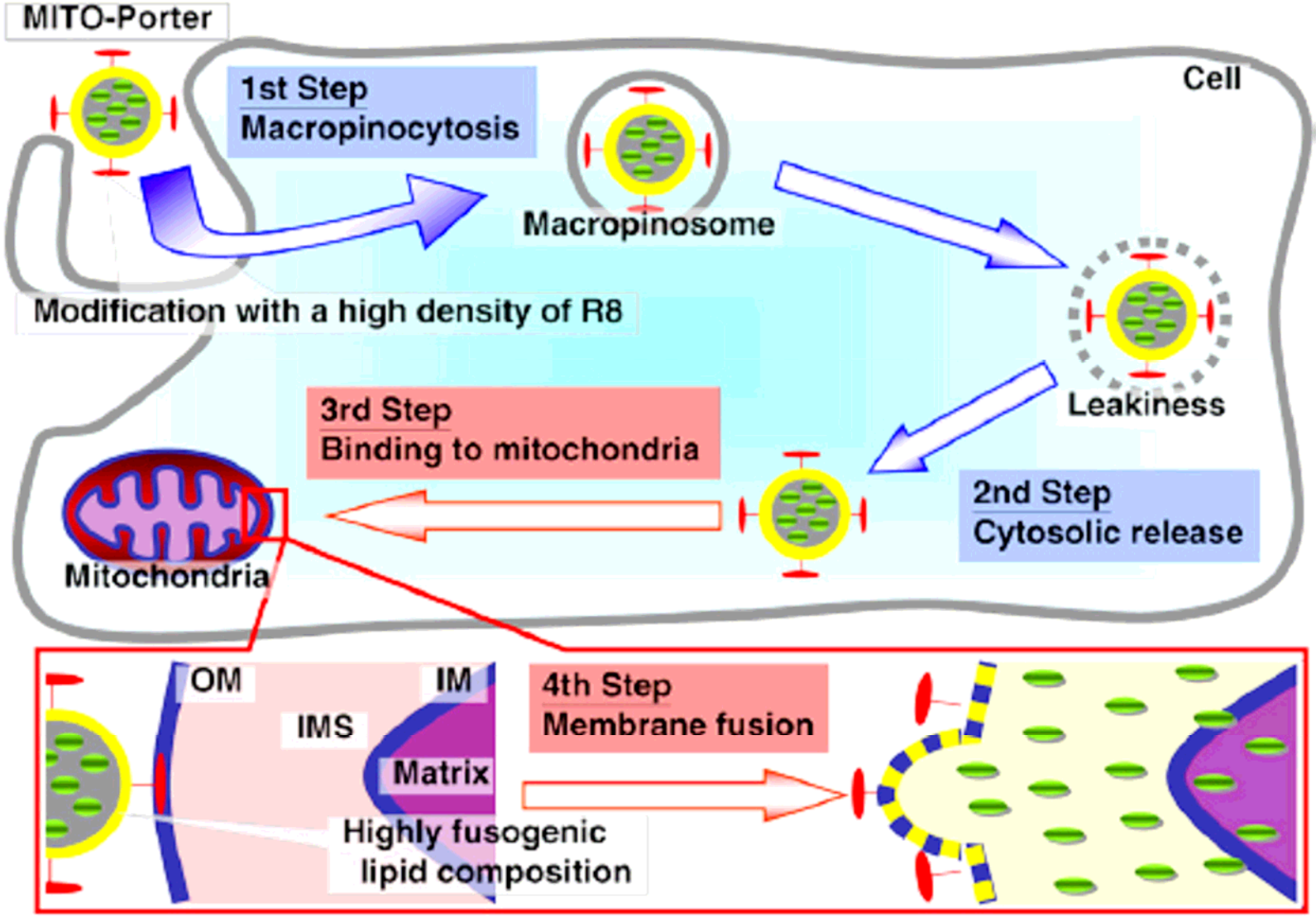
| DQAsomes | Liposomes | Nanoparticles | Quantumdots |
|---|---|---|---|
| Self-assembly of Mitochondriotropic bola amphiphile | Surface-modification of Liposomes with mitochondriotropics | Surface-modification of Nanoparticles with mitochondriotropics | Surface-modification of Quantumdots with mitochondriotropics |
|  | | | |
| Mitochondria-targeted bolasomes (DQAsomes) | Mitochondria-targeted Liposomes | Mitochondria-targeted Nanoparticles | Mitochondria-targeted Quantumdots |

Figure 1

# Pharmacological targeting of mitochondria in disease




MITO-Porter
Cell
1st Step
Macropinocytosis
Macropinosome
Modification with a high density of R8
Leakiness
3rd Step
Binding to mitochondria
2nd Step
Cytosolic release
Mitochondria
OM
IM
IMS
Matrix
4th Step
Membrane fusion
Highly fusogenic
lipid composition

Mitochondria-specific drug carrier systems are badly needed

Example: Ischemia Reperfusion

↓

cellular damage

↓

Necrotic cell death

↓

mitochondria MPTC

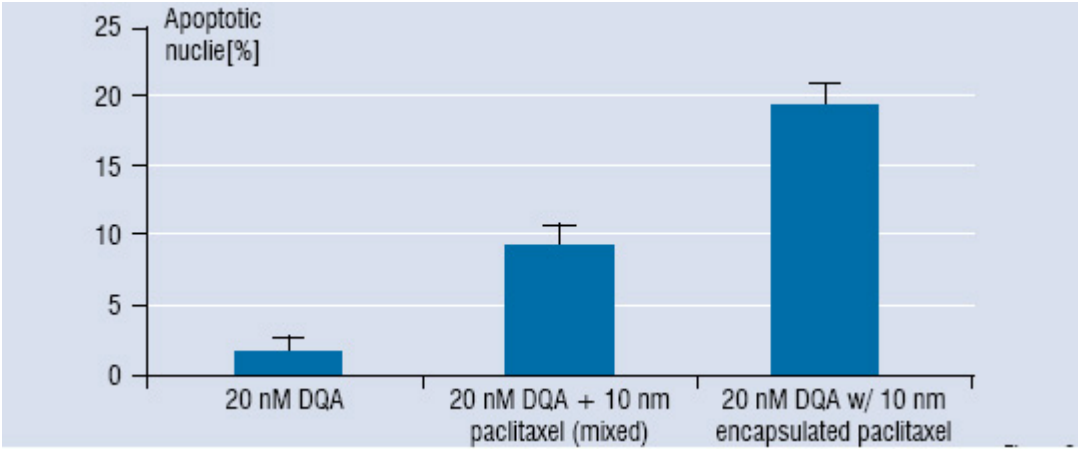
↓

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 essential for life

# Functional Complexity

Respiration and ATP Synthesis

Synthesis of heme, lipids, amino acids and nucleotides

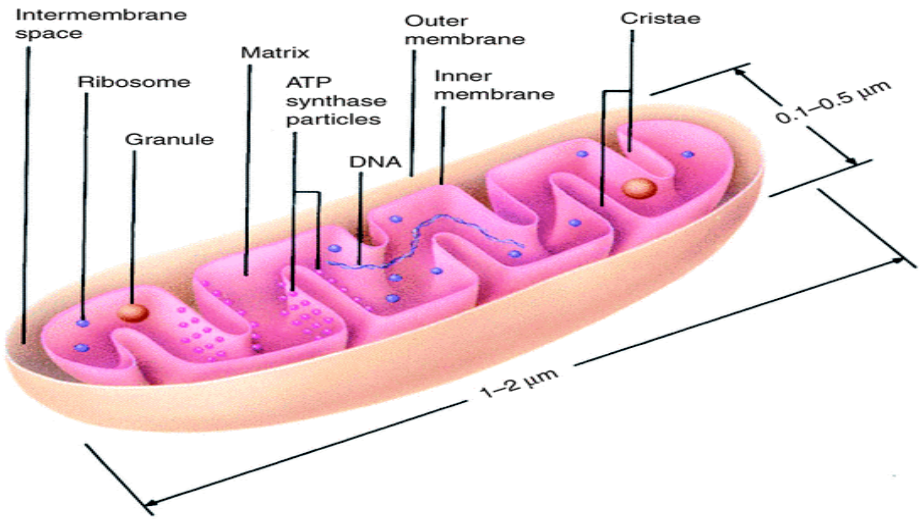
Intracellular homeostasis of inorganic ions

# Structural Complexity

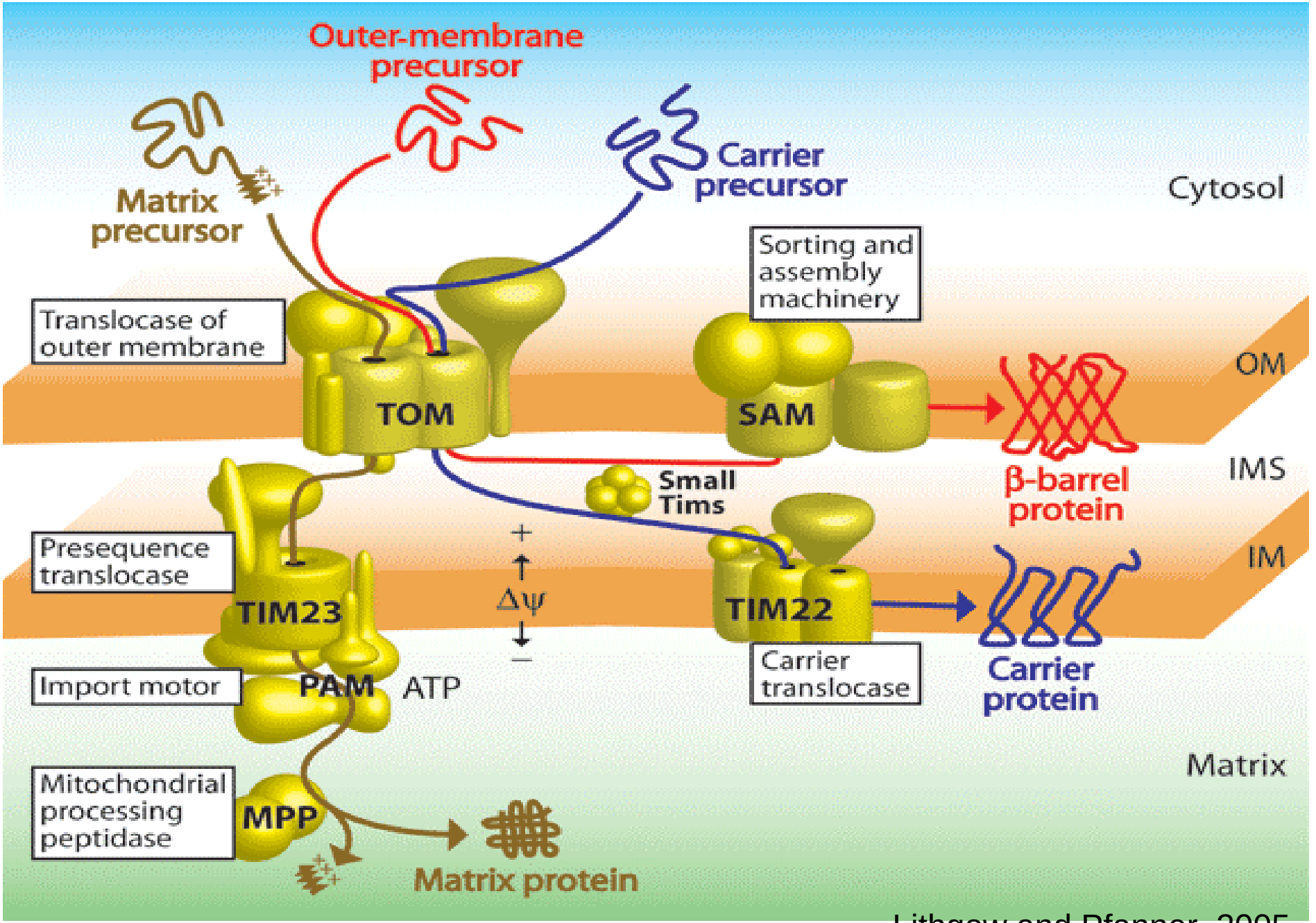
5-15% of total cell protein
20% volume of eukaryotic cell
IM is 1/3 of total cell membrane

About 1000 different polypeptides (900 in yeast)

Only a dozen encoded by mtDNA

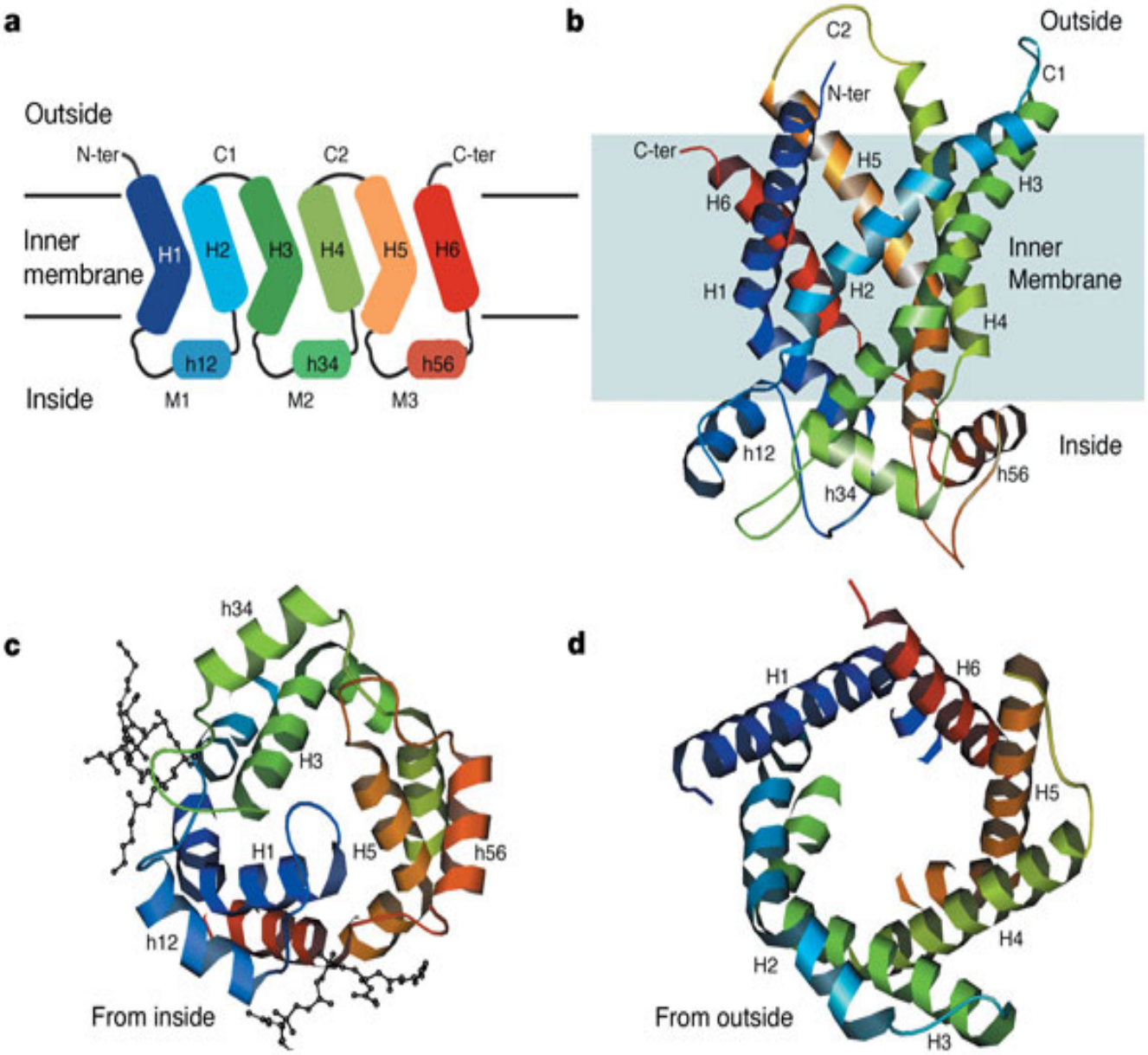


# Protein import is the major mechanism of mitochondria biogenesis




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

Isolated molecules

In vitro

Intact cells

In vivo

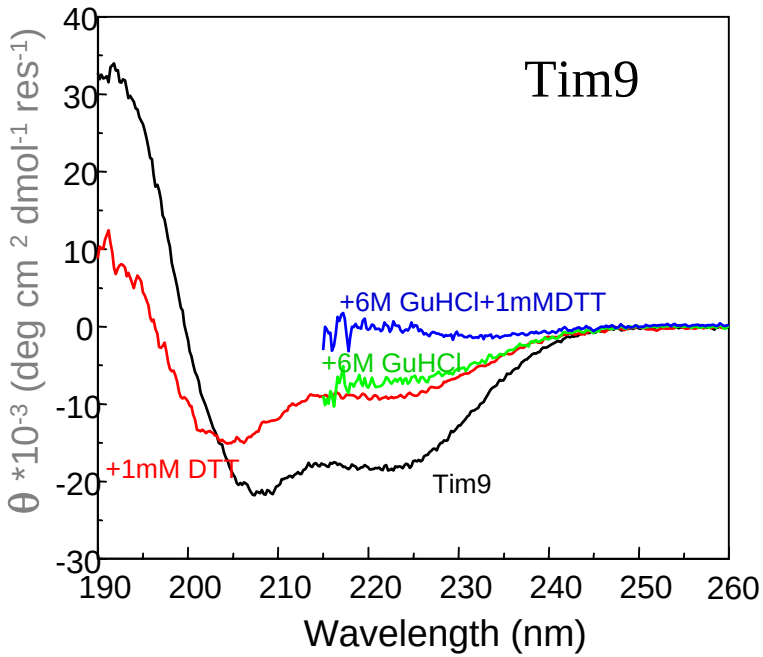
Isolated organelles

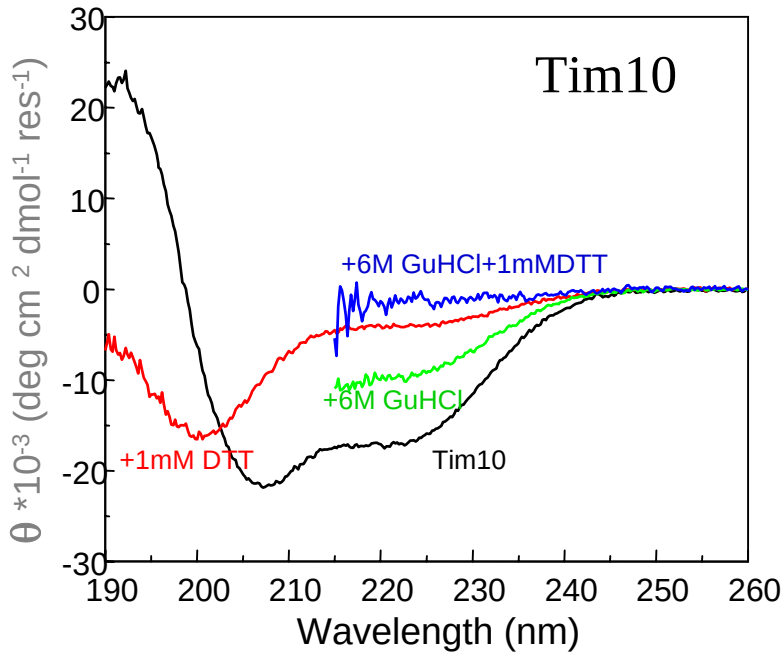
In organello

By using …

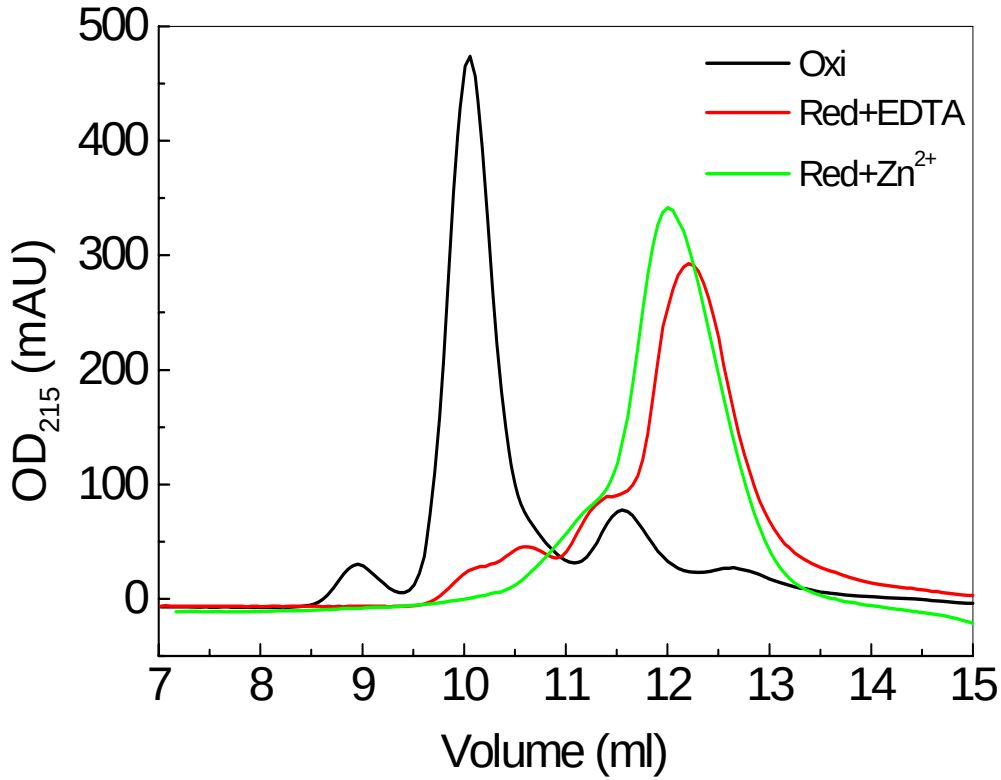
Protein purification
Mutagenesis
CD analysis
Limited proteolysis
Bioinformatics
Mass spectrometry
Chemical modification of thiol groups. in vivo thiol trapping
Gel filtration
Isothermal titration calorimetry ITC
Analytical centrifugation
Multi-angle light static scattering

# CD analysis of Tim9 and Tim10

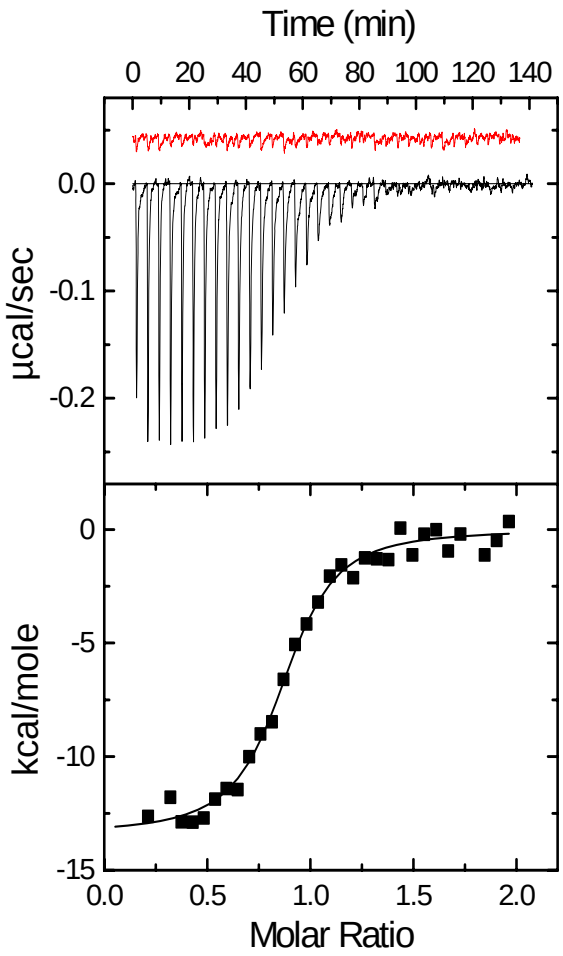


# Oxidation of Tim9 and Tim10 is required for complex formation



# ITC study of the interaction between Tim9 and Tim10




**Reduced Tim9/Tim10**

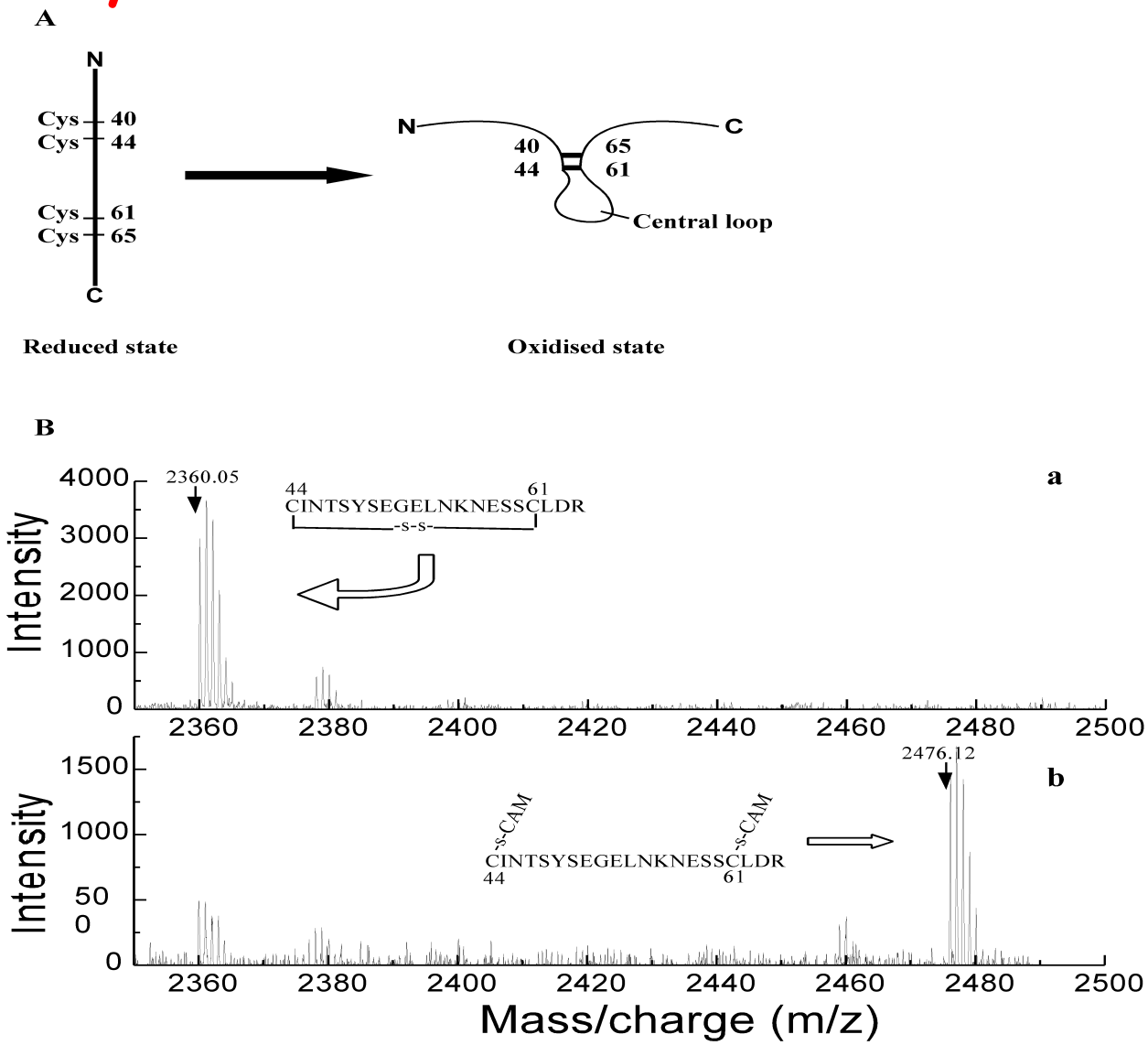
**Oxidised Tim9/Tim10**

N= 0.9 (Tim10/Tim9)

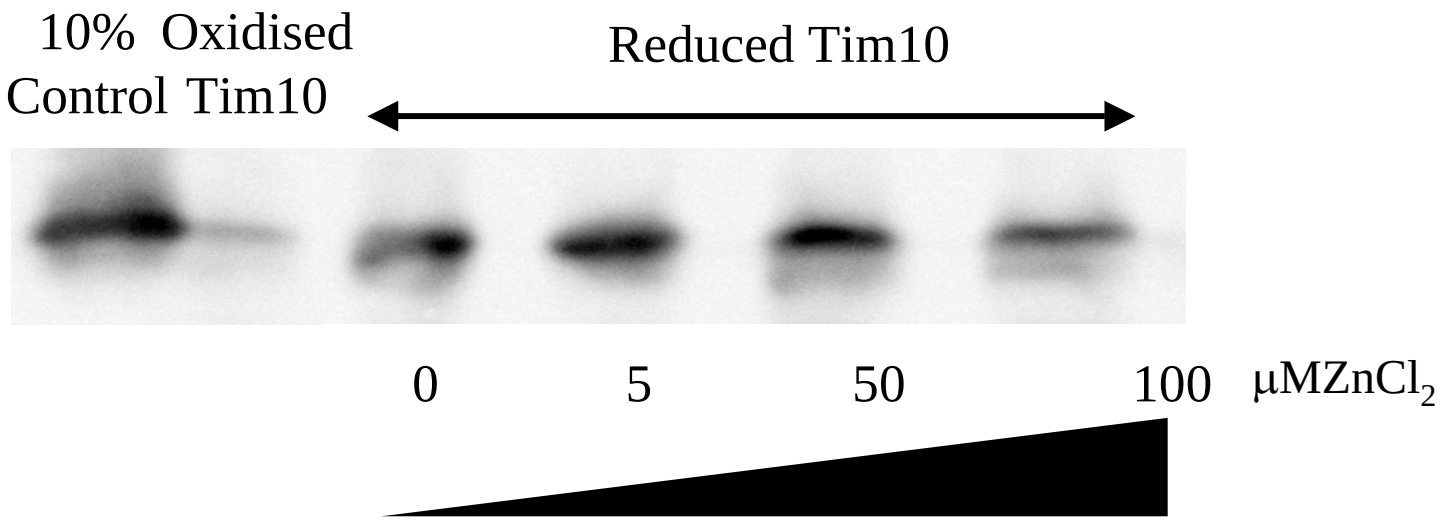
$K_a = 5 \times 10^6\ M^{-1}$

$\Delta H$= -13kcal/mol

# Mass spectrometry analysis of the Cys connectivities of Tim10



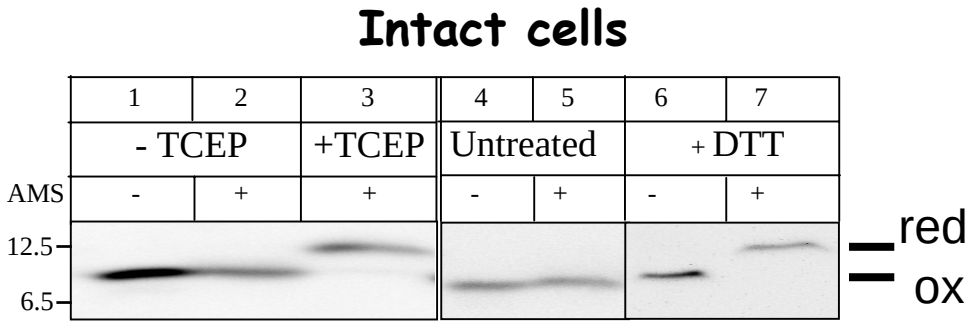
# Prior Oxidation inhibits import

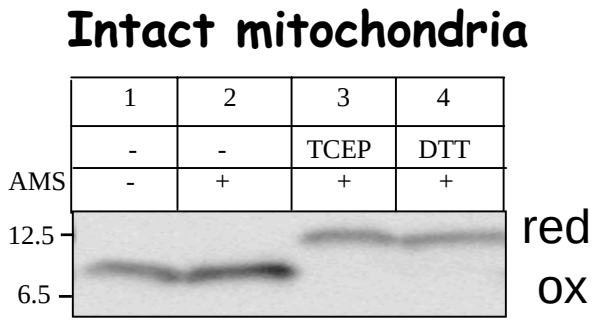



Additional data:

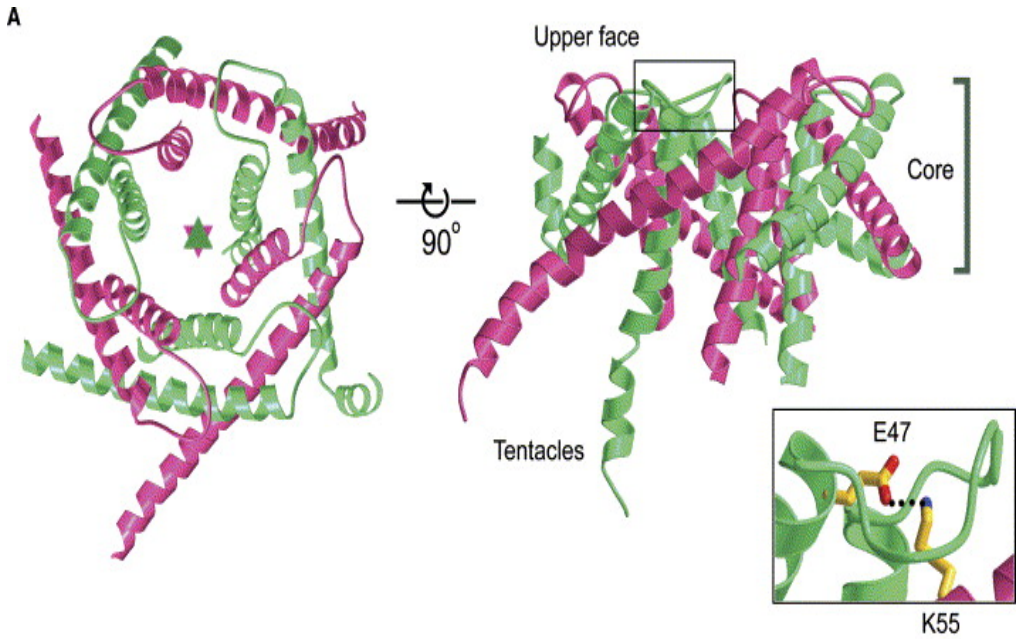
1. NEM alkylation
2. Cys mutants

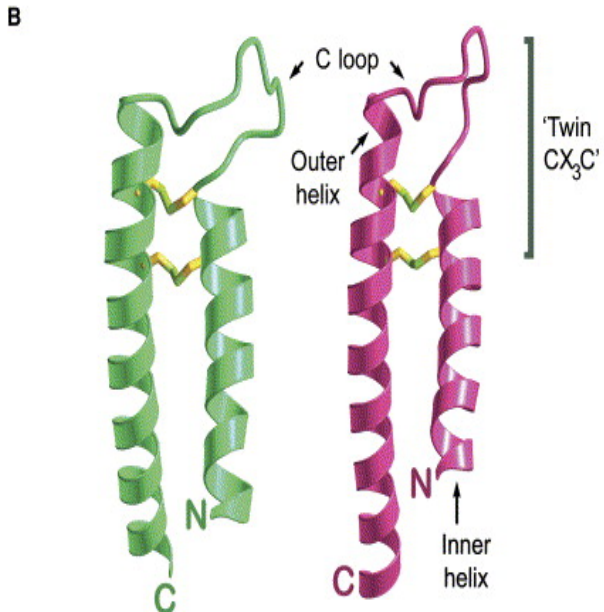
# Tim10 is oxidised *in vivo*



# Crystal structure of the human Tim9-Tim10 complex



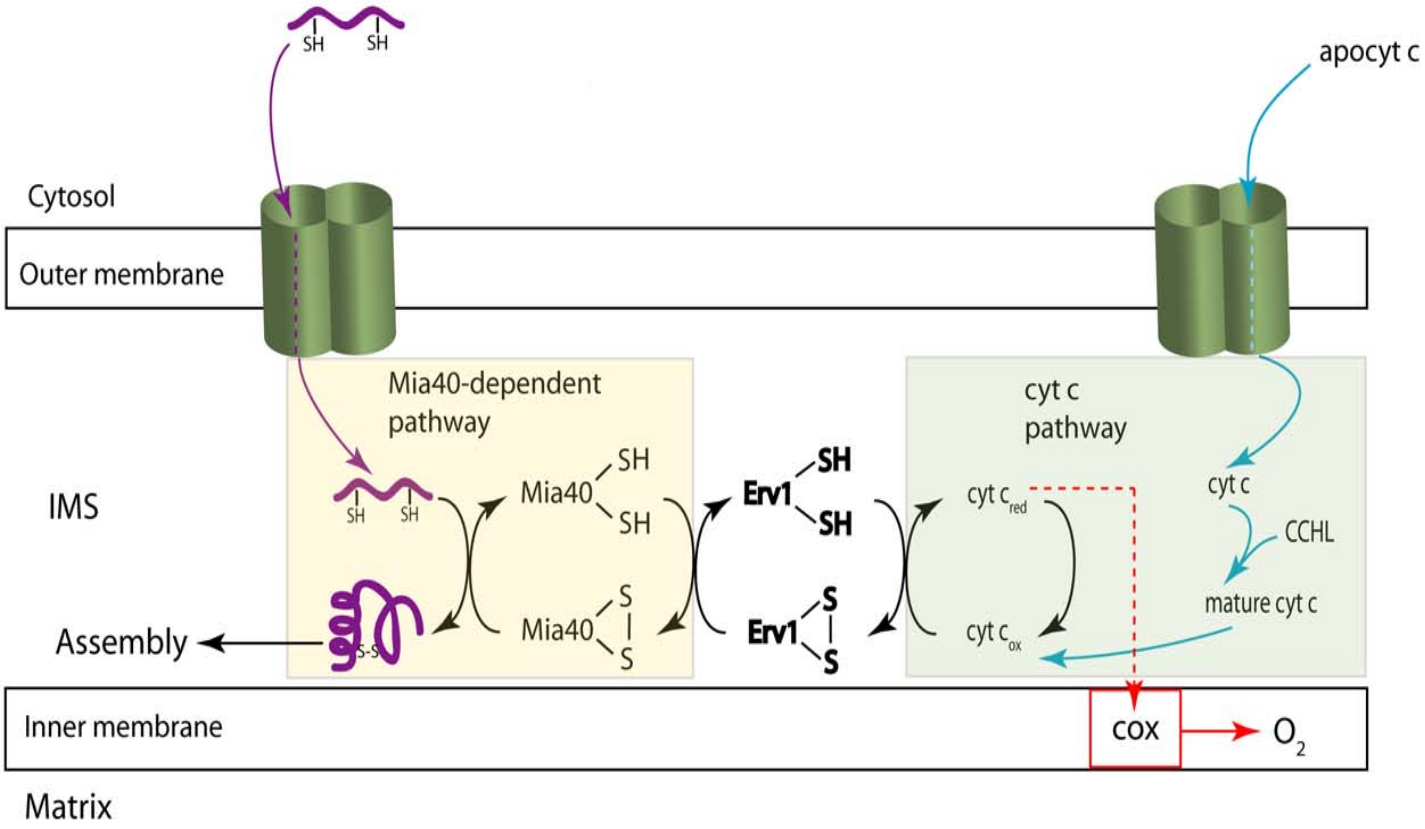

…in agreement with our biochemical analysis and SAXS studies

Webb et al, Mol Cell 2006

**What does this mean?**

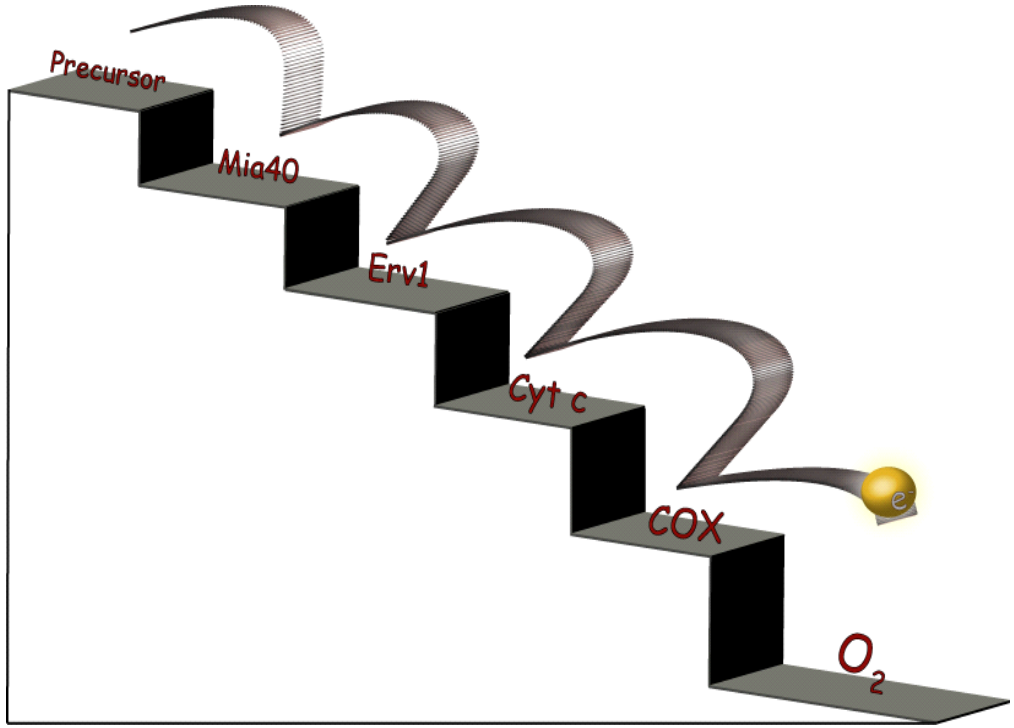
**NOVEL oxidative folding pathway operating in mitochondria *in vivo***

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

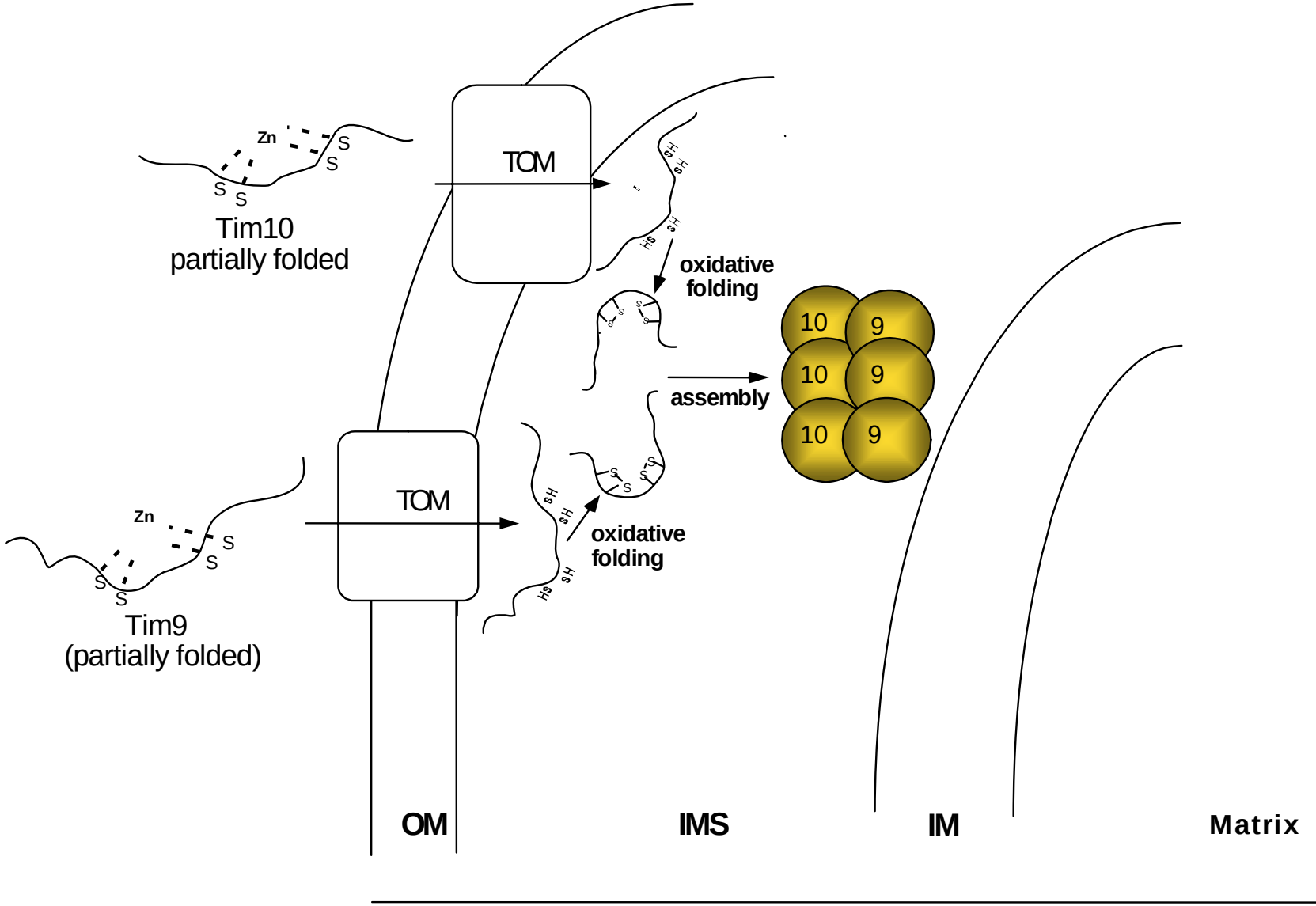



Allen et al., JMB, 2005; cover

# A link between protein import and respiration: The Electron flow

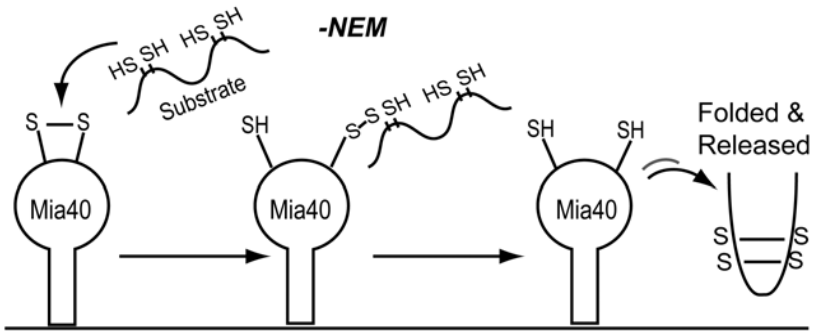


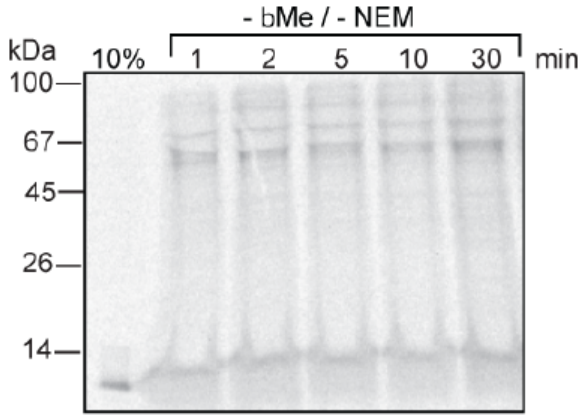
# Multistep assembly pathway : Dissection of subreactions

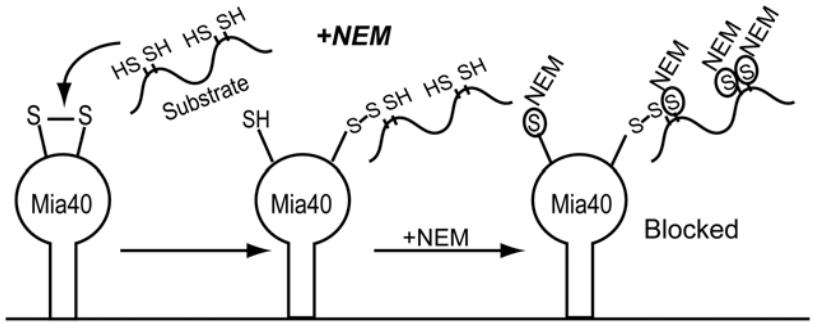


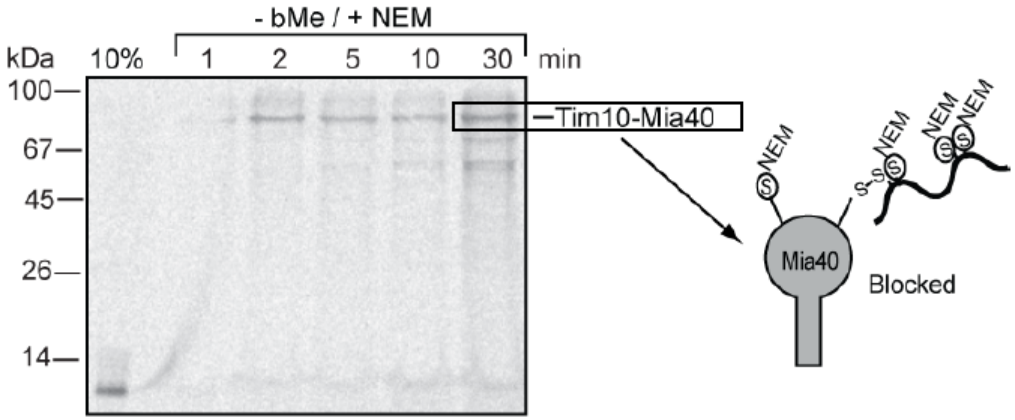
# Functional and Structural analysis of Mia40

# *In vitro* reconstitution of the interaction with the substrate

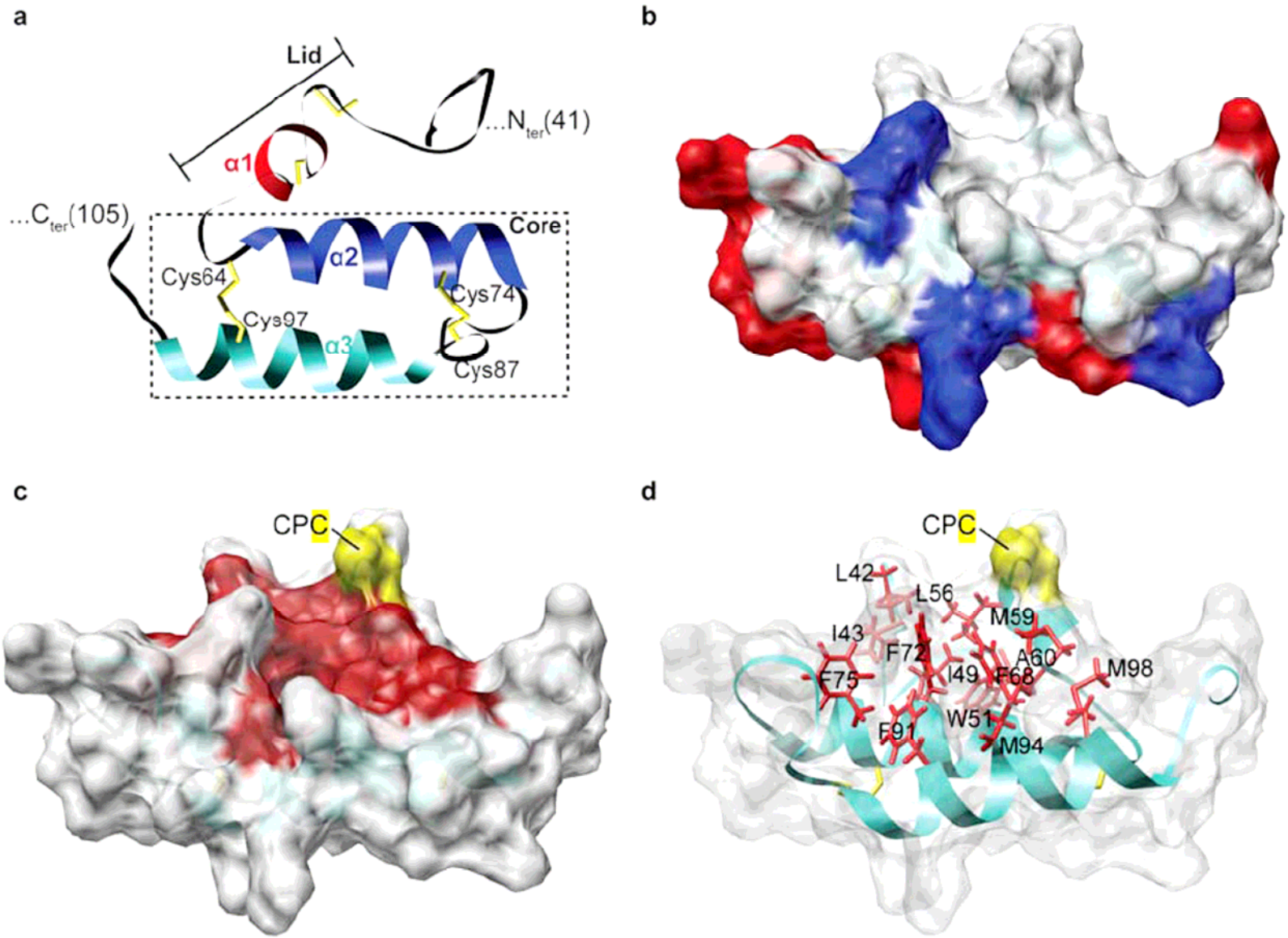


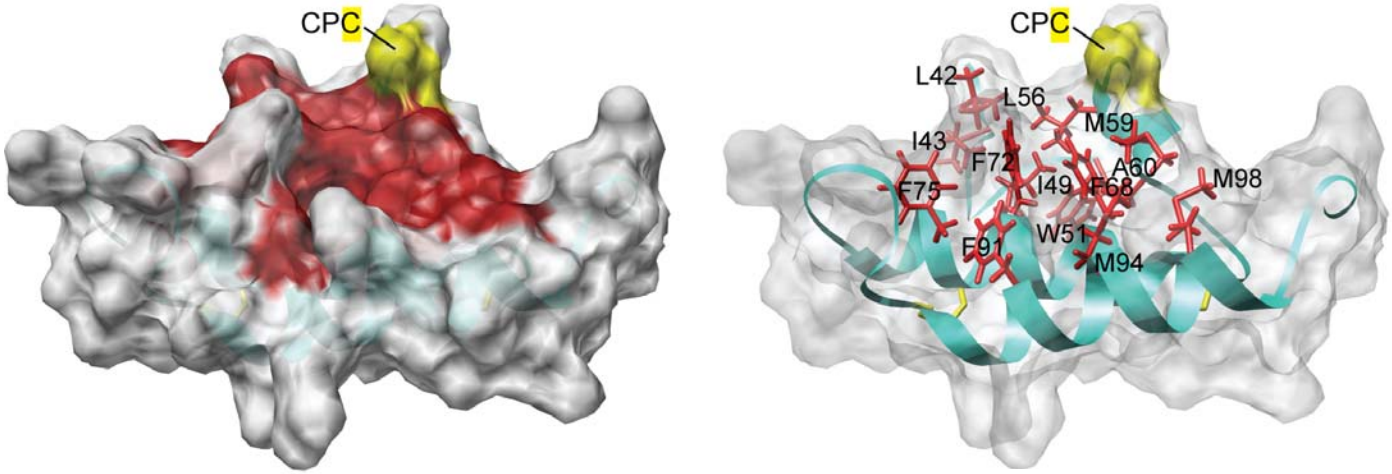



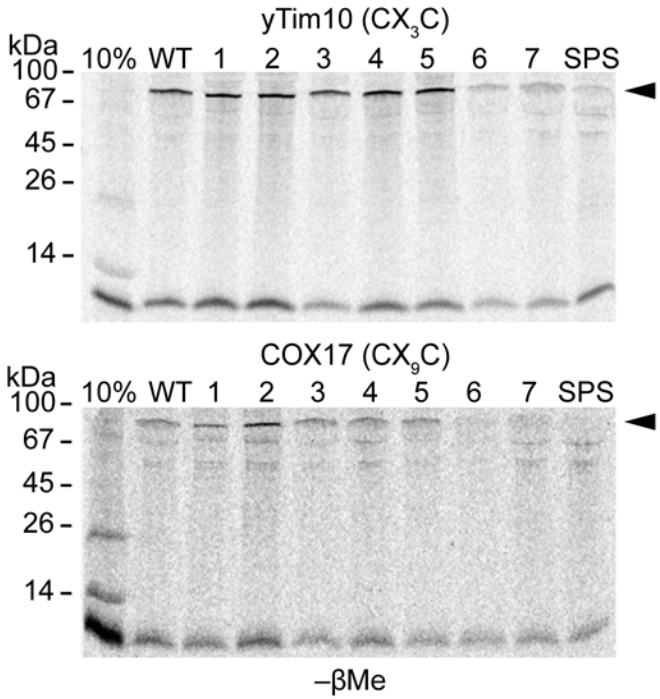
# Solution structure of MIA40 by NMR

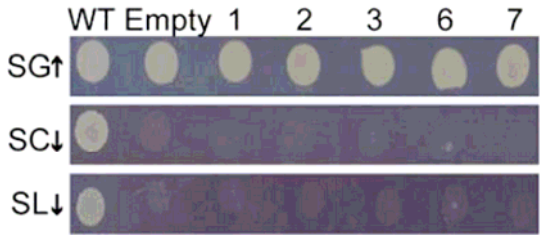


# The hydrophobic cleft mediates non-covalent binding of the substrate




| No | yMia40 | MIA40 |
|---|---|---|
| 1 | I292A W294A F311A F315A | I49A W51A F72A F75A |
| 2 | L299A M302A F311A F315A | L56A M59A F72A F75A |
| 3 | F311A F315A F334A M337A | F72A F75A F91A M94 |
| 4 | I292A W294A L299A M302A F311A F315A | I49A W51A L56A M59A F72A F75 |
| 5 | I292A W294A F311A F315A F334A M337A | I49A W51A F72A F75A F91A M94 |
| 6 | L299A M302A F311A F315A F334A M337A | L56A M59A F72A F75A F91A M94A |
| 7 | I292A W294A L299A 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

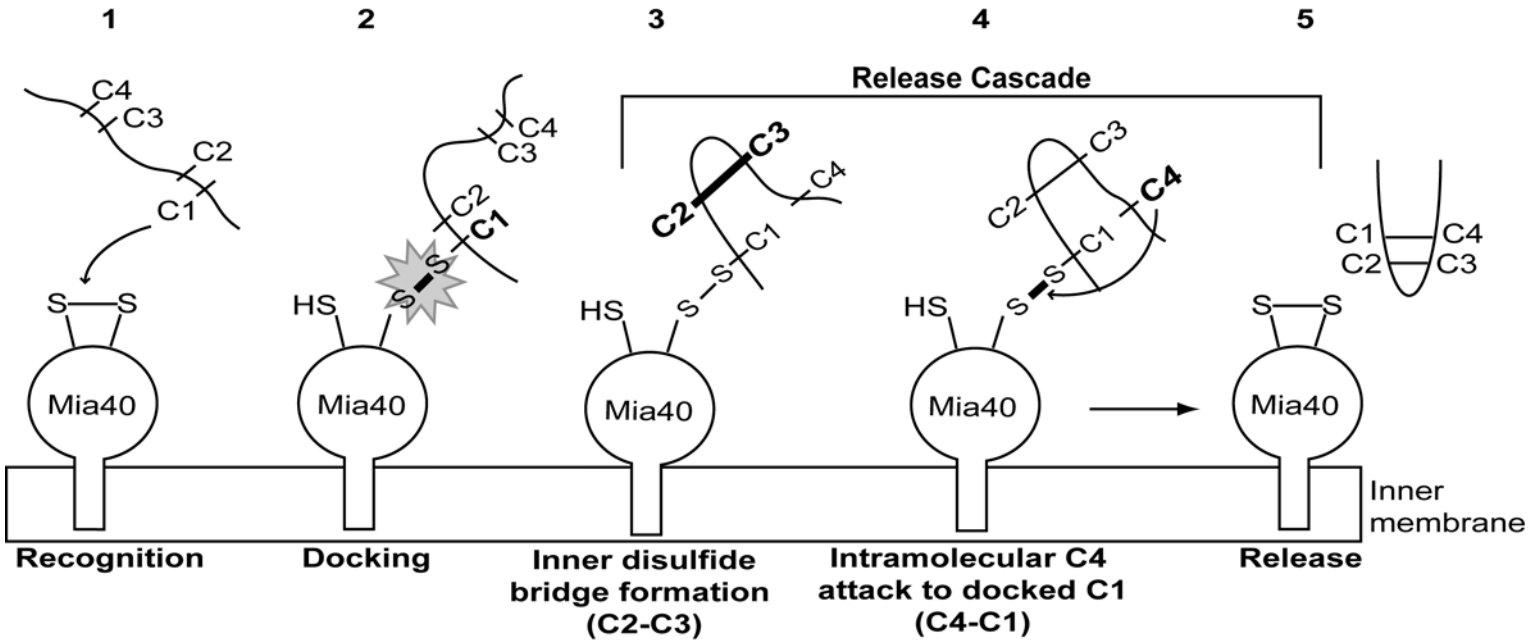
-The N-terminal first Cys serves as an essential docking point onto Mia40 upon import of the substrate

- 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

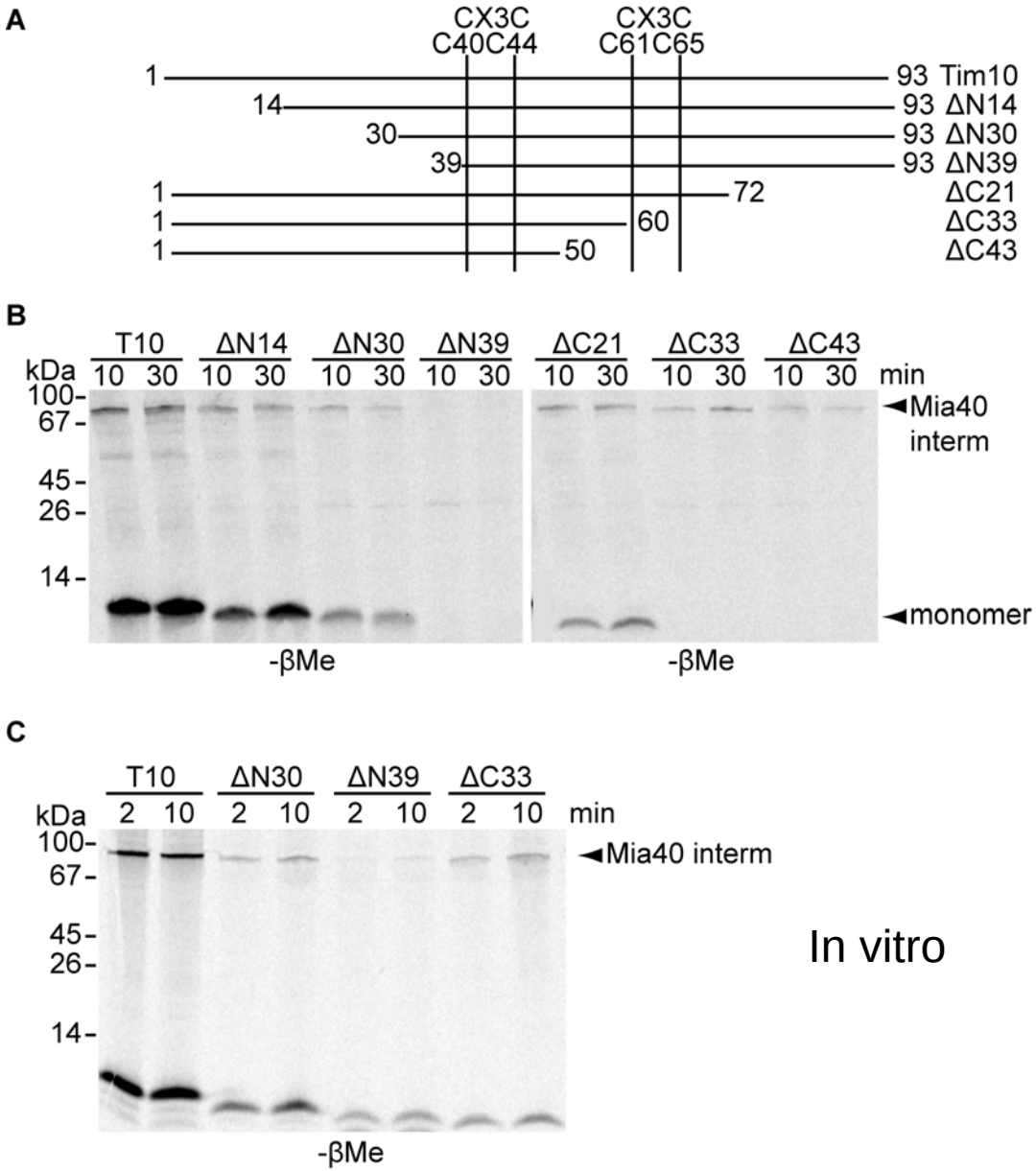
Cox17 is recognised on its 3rd Cys of the CX9C motifs …!!!

→CONUNDRUM?

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

SOLUTION: Common targeting signal on both substrates, the ITS

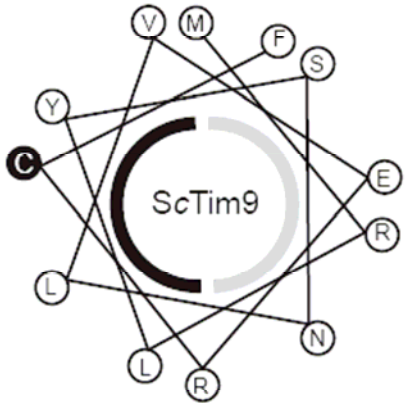
# Identification of the targeting signal of Tim10 for Mia40
# The Inetermembrane space Targeting Signal (ITS)

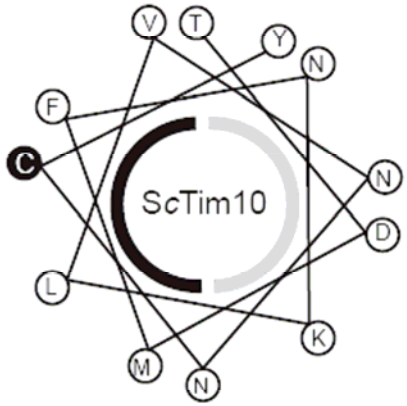


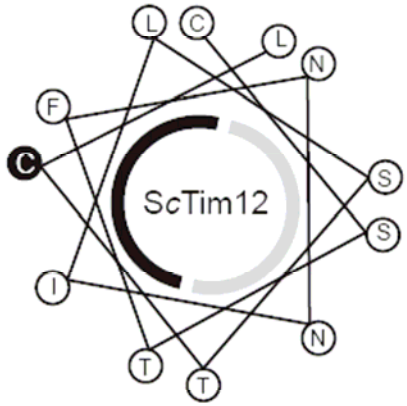

In organello

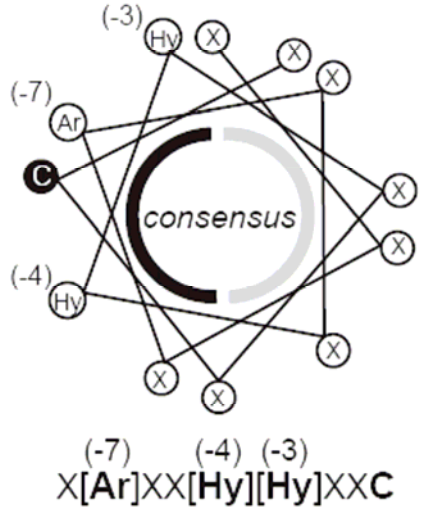
In vitro

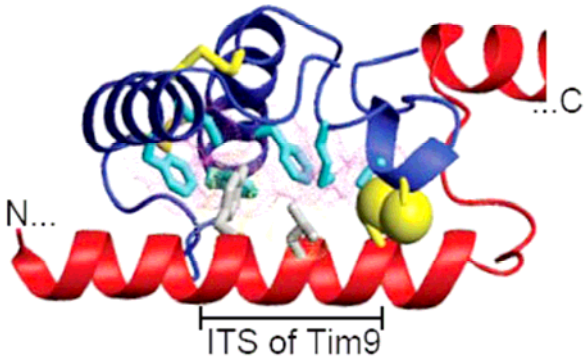
# Structural basis for the binding of the ITS onto the cleft of Mia40

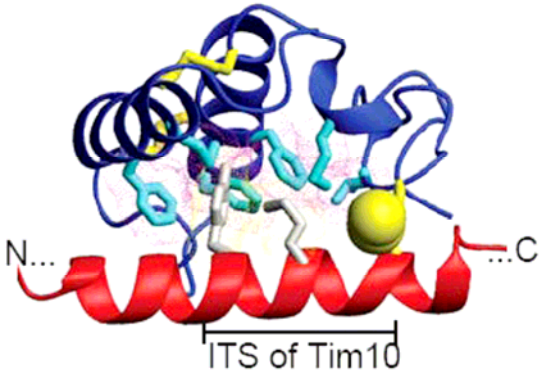



Sideris et al. 2009 submitted

# Generality of the ITS function

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

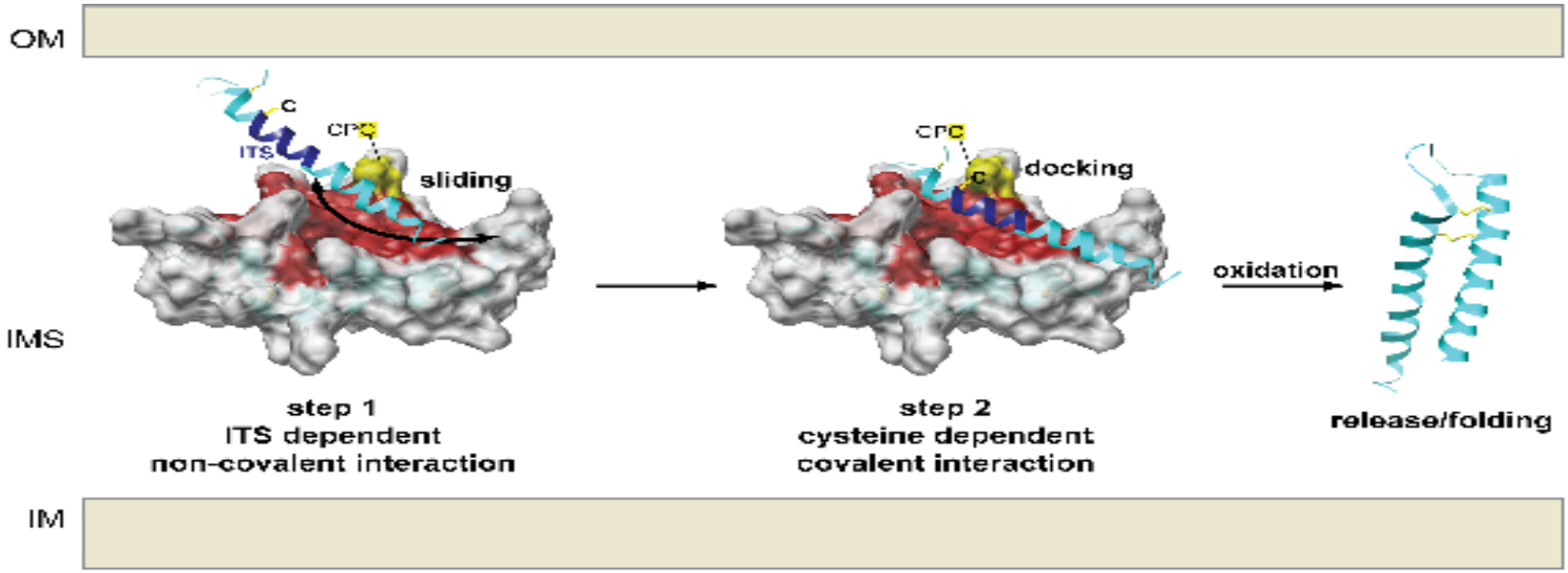
It functions independently and can be fused C-terminally to a protein

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

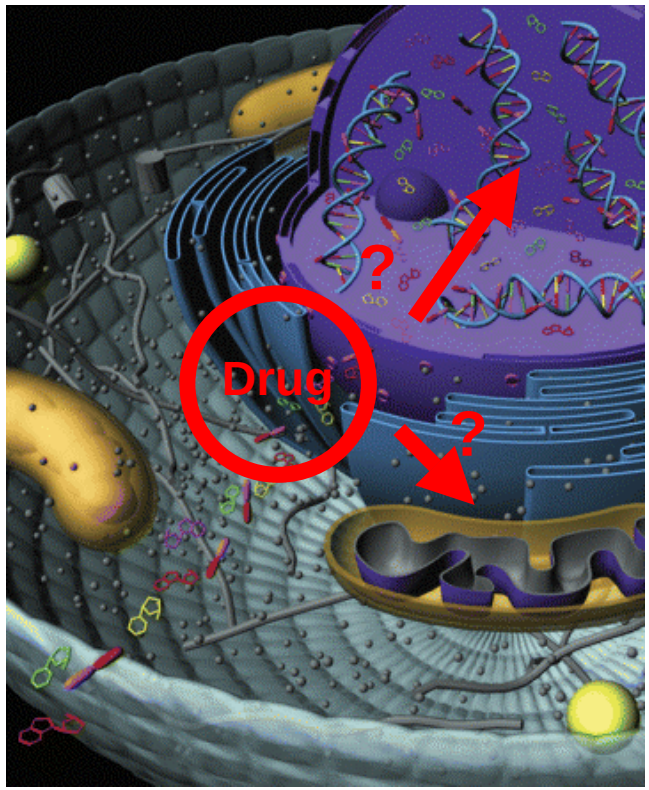
The process is guided by a novel ITS that
directs the first step of noncovalent recognition by Mia40

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

IMBB/Crete:

Eirini Lionaki
Afroditi Hatzi
Emanouela Kalergi
Nitsa Katrakili
Babis Pozidis

Dionisia(Jenny) Sideris (Biology, MIT)
Paraskevi Kritsiligkou (Biochemistry, Oxford)

Collaborators
CERM-Florence (NMR): Ivano Bertini
**Simone Cioffi**
Lucia Banci
Chiara Cefaro
Angelo Gallo
Manuele Martinelli

Funding:
GSRT, IMBB, UoC
EU NMR