

Programme

8.00-8.20	Quiz results
8.20-9.00	Case story: TMX3
9.00-9.15	Break
9.15-9.45	PyMOL scripting
9.45-12.00	PyMOL hand-in exercise I

Feedback Person(s)



<http://www.bio-evaluering.dk/>

Programme

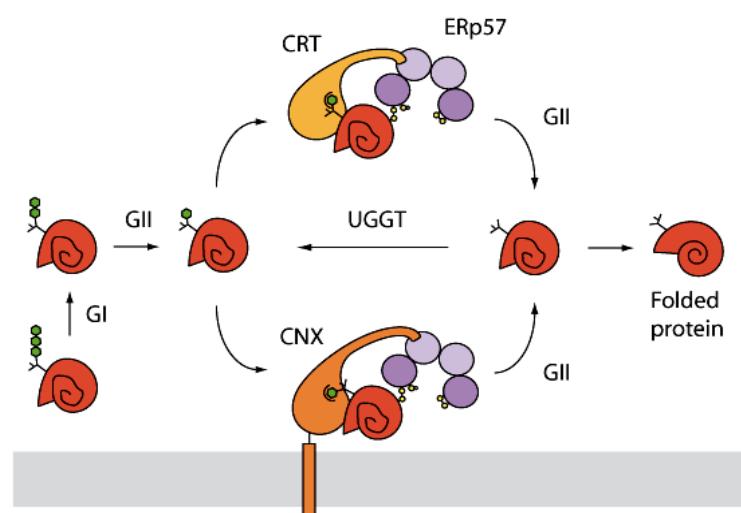
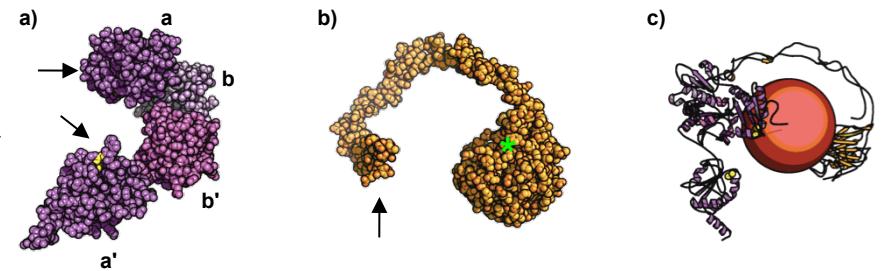
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TMX3

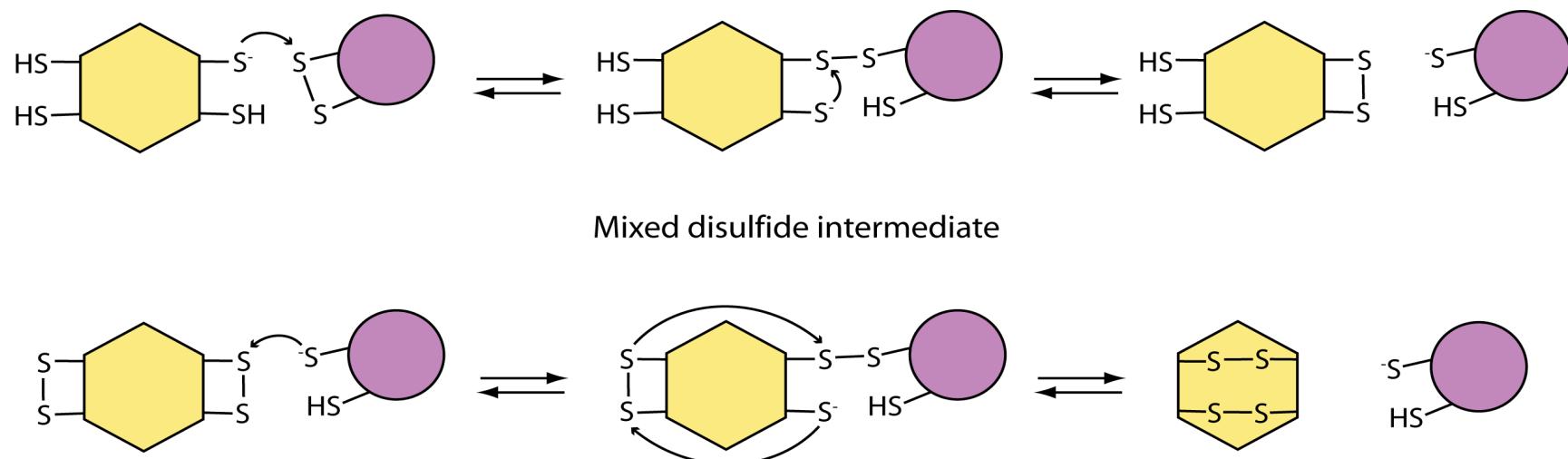
A Novel Thioredoxin-Like Protein
of the
Endoplasmic Reticulum

Background

- Disulfide bond formation
 - Mostly by protein disulfide isomerase (PDI) in the ER
 - Also by ERp57 (a PDI homologue), calreticulin and calnexin
 - Many other PDI relatives with unknown function

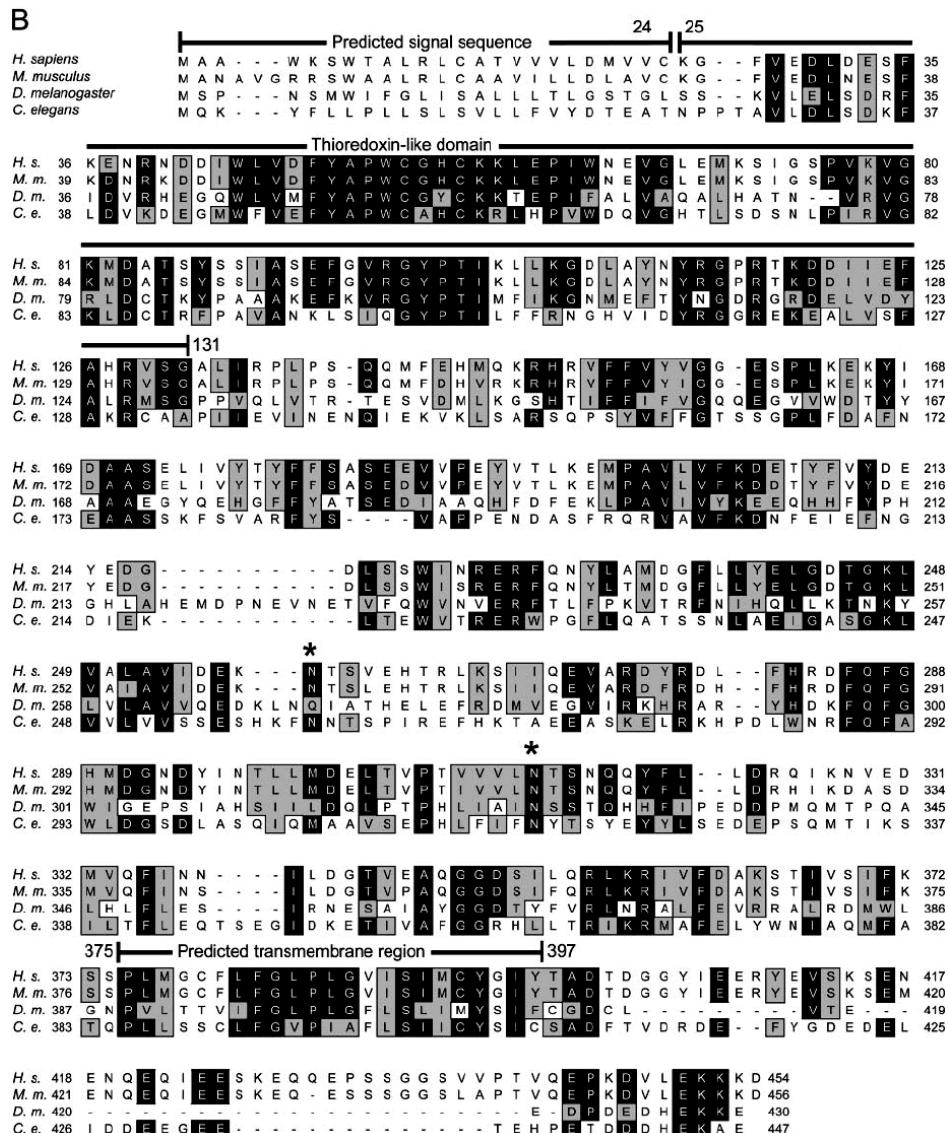


Disulfide Metabolism

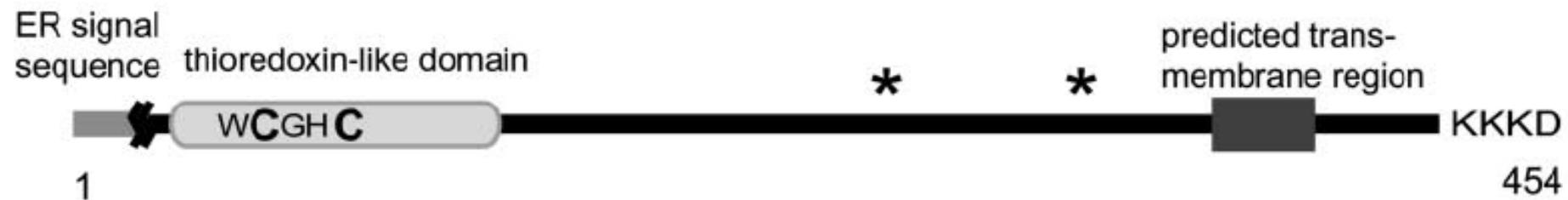


Discovery of TMX3

- Search for Trx-like proteins
- Found several conserved proteins
- TMX3
 - Conserved in all higher eukaryotes
 - Signal peptide
 - Trx domain
 - Transmembrane helix
 - Glycosylation sites
 - ER retention signal

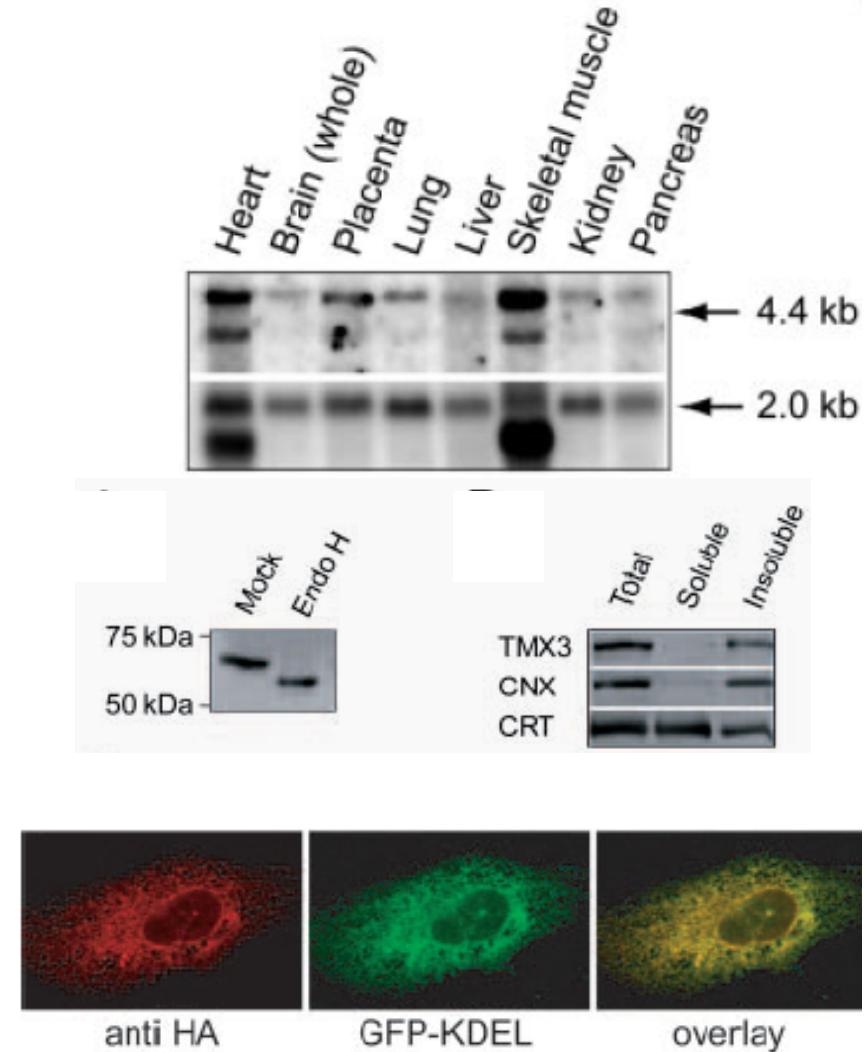


Overview of TMX3



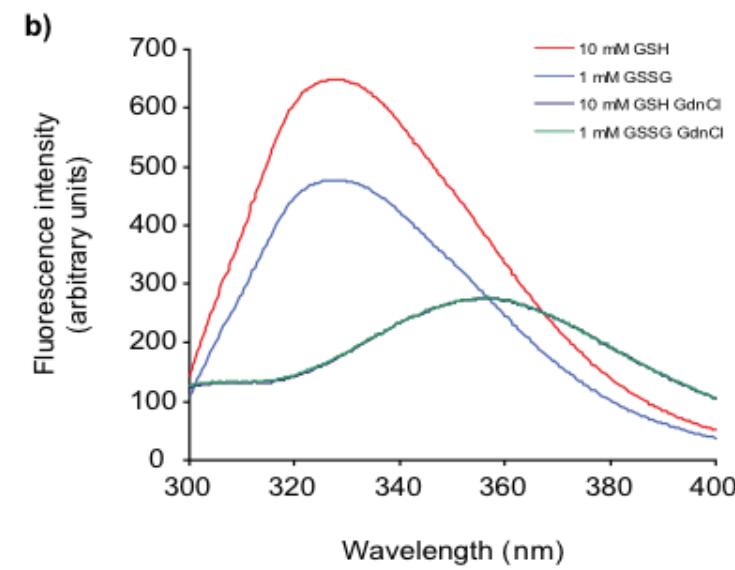
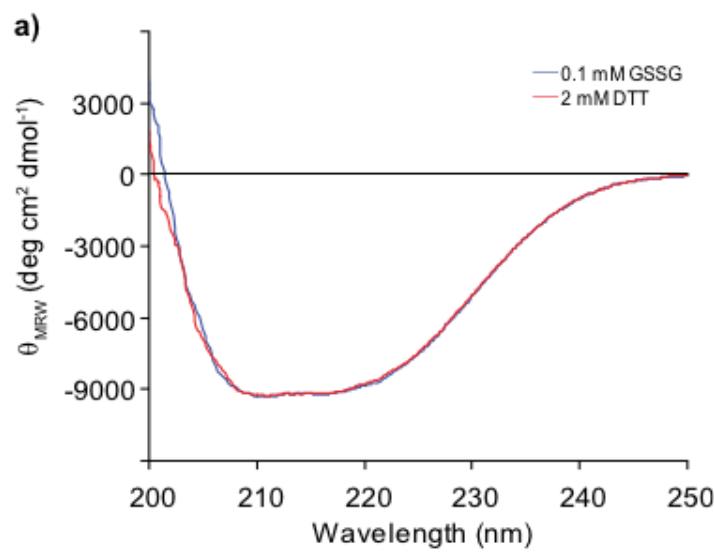
Experimental Data – I

- Expression in most tissues – highest in muscle tissues.
- Inside ER
- Transmembrane protein
- Glycosylated



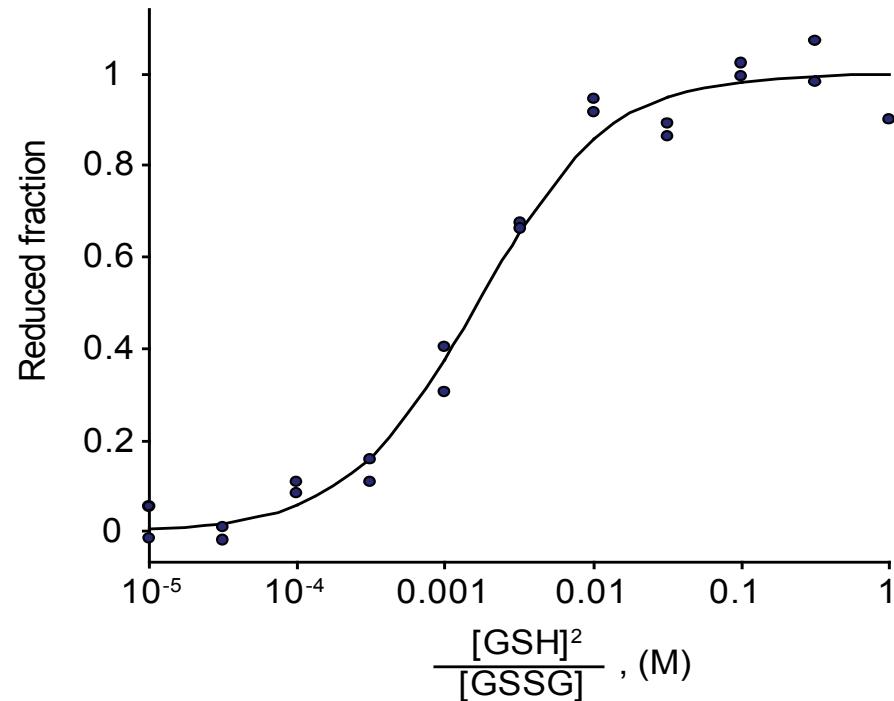
Experimental Data – II

- CD spectroscopy
 - Secondary structure content
- Fluorescence spectroscopy
 - Functional implications



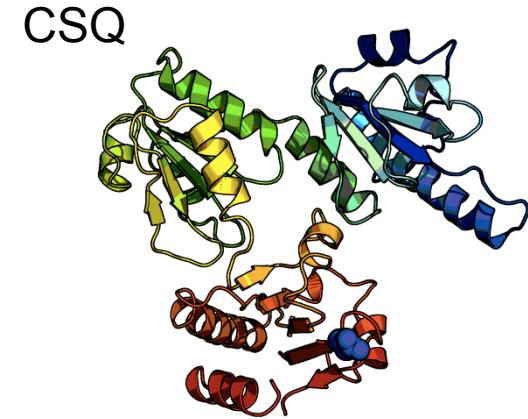
Experimental Data – III

- Redox titration
 - Redox potential:
-157 mV
 - Similar to PDI
- ↓
- Similar function to PDI?

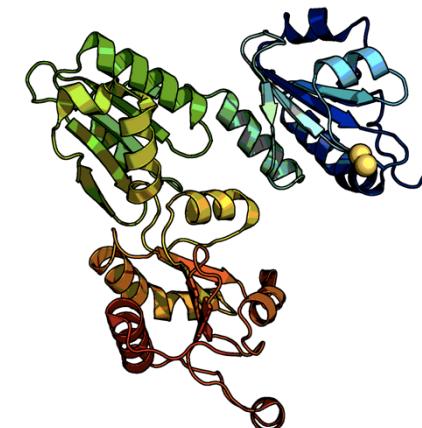


Building a Model

- Alignments showed similarity to
 - PDI domains
 - 40% sequence ID to a domain
 - ERp57 domains
 - Calsequestrin
 - An acidic Trx-domain protein involved in calcium storage in muscle cells.
 - 25% sequence ID to Trx2+3



PDI abb'



Modelling Alignment

TMX3	1	KGFVEDLDESFKEENRNDDIWLVDFYAPWCGHCKKLEPIWNEVGLEEMKSIGSPVKVGKM	DATSYSSIASEF
PDIa	8	HVLVLRKSNFAEAALAAHKYLTVFYAPWCGHCKALAPEYAKAAGKLKAEGSEIRLAKV	DATEESSDLAQOY
CSQrs	65	EDKGVG....FGLVDSEKDAAVAKKL

★

TMX3	71	GVRGYPTIKLLK.GDLA...NYRGPRTKDDIIIEFAHRVSGALIRPLPSQ...	QMFEHMQKRHRVFFVYVG
PDIa	78	GVRGYPTIKFFRNGDTASPKETYTAGREADDIVNWLKTRTG...	PAA.....
CSQrs	88	GLTEEDSIVYFKE.DEV..IEYDGEFSADTLVEFLLDVLEDPVELIEGERELQAFENIEDEIKLIGYFKN	

TMX3	135	GESPLKEKYIDAASELIVTYFFSASEEVPEYVTLK..EMPAVLVFKDETETYFVYDE.YEDGD	LSSWINR
CSQrs	155	KDSEHYKAFAKEAAEEFHPIPFATFDISKVAKKLTLKLNEIDFYEAFMEEPVTI	PDKPNSEEEIVNFVVE

★

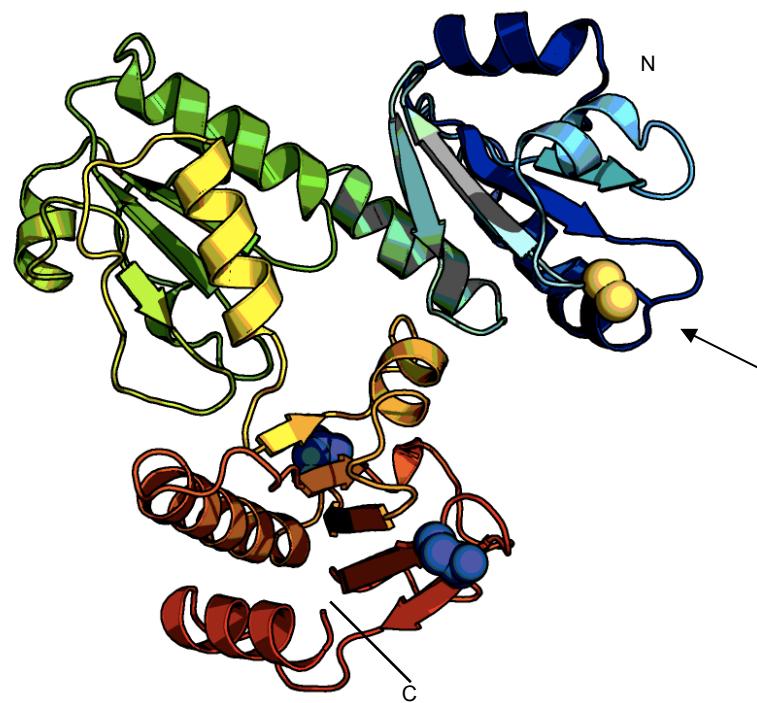
TMX3	202	ERFQONYLAMDGFLLYELGDTGKLVALAVIDEKNTSVEHTRLKSIIQEVARDYRDLFHRDFQFGHMDGND.	
CSQrs	225	HRRSSTLRKLKPESMYETWEDDMGITHIVAFEEADPDGYEFL	EILKSVAQDNTD..NPDL

TMX3	271YINTLLMDELTVPTVVLNTSNQ	QYFLIDRQIKNVEDMVQFINNL
CSQrs	293	PLLVPYWEKTFDIDL	SAPQIGVVNTDA

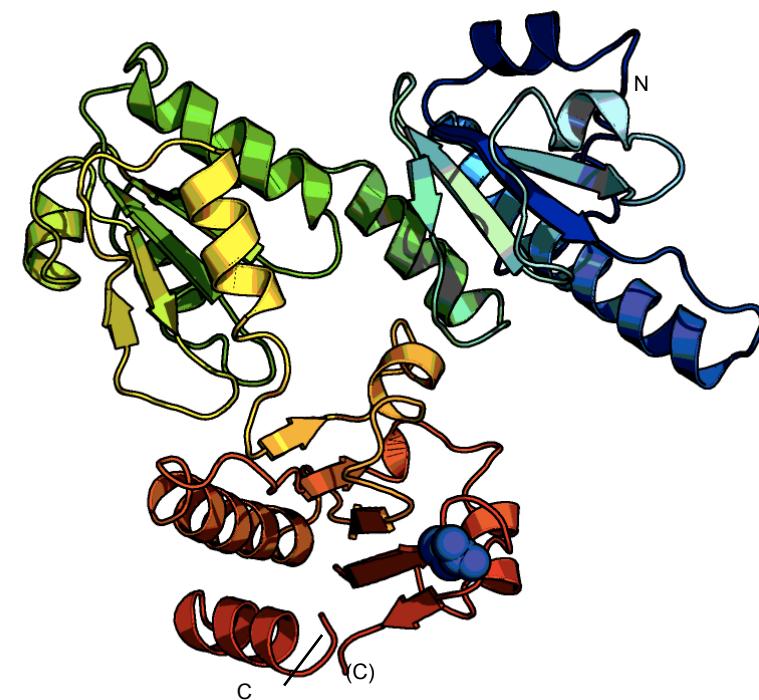
- Model built with MODELLER

Model vs. Template

a)



b)



ProQ

ProQ - Results

Prediction using secondary structure

Predicted LGscore : **4.109**

Predicted MaxSub : **0.487**

Different ranges of quality:

LGscore>1.5 fairly good model

LGscore>2.5 very good model

LGscore>4 extremly good model

MaxSub>0.1 fairly good model

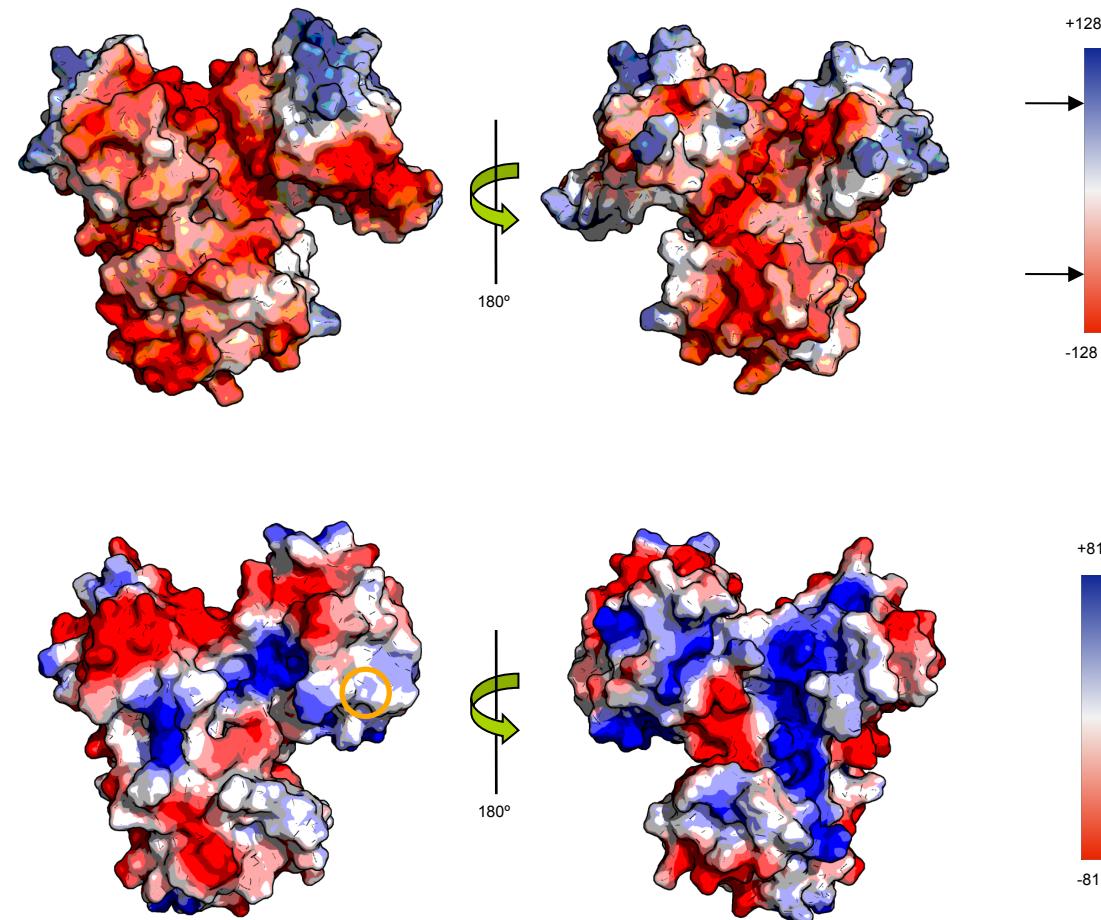
MaxSub>0.5 very good model

MaxSub>0.8 extremly good model

<http://www.sbc.su.se/~bjornw/ProQ/ProQ.cgi>

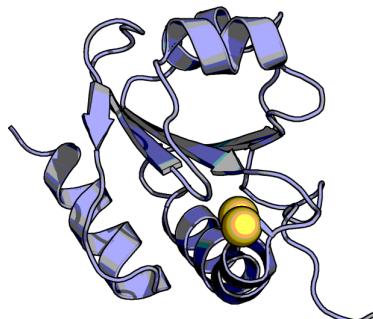
Surface Potential

- CSQ is an acidic protein
- TMX3 is not...
- ...and is probably not regulated by calcium ions.

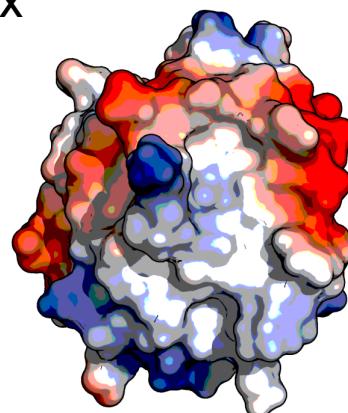


The Catalytic Domain

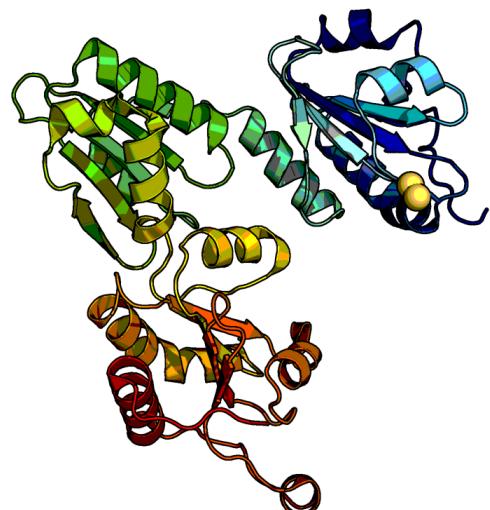
PDI a



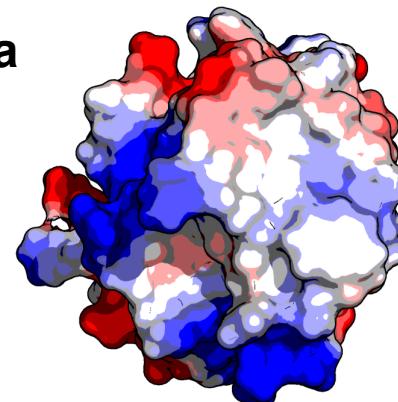
Trx



PDI abb'

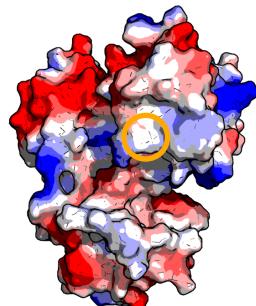


TMX3 a



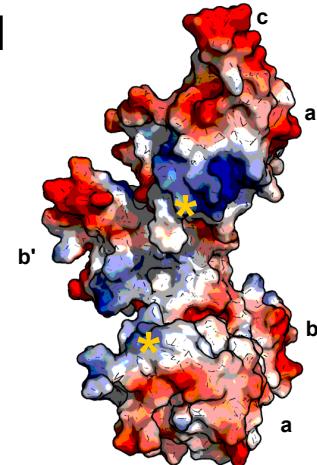
A Hydrophobic Pocket?

TMX3

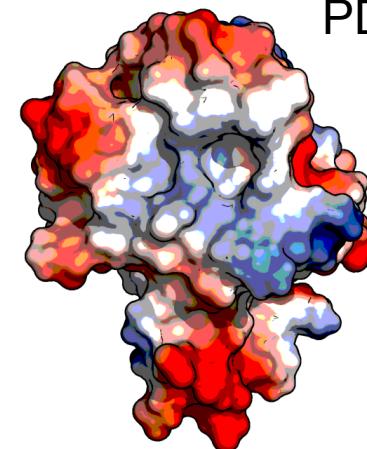


→ 25°

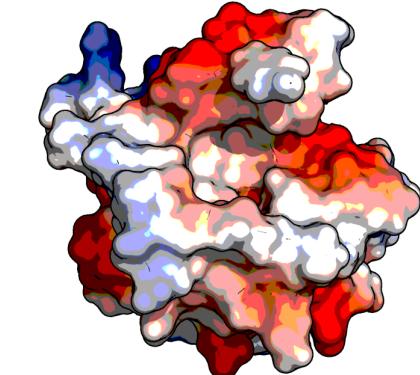
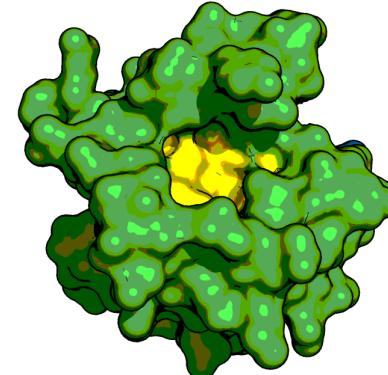
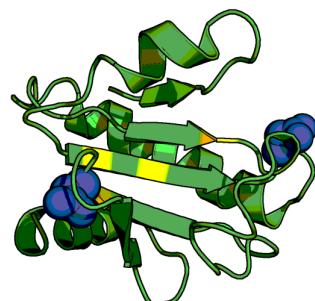
PDI



PDI b'



TMX3 b'



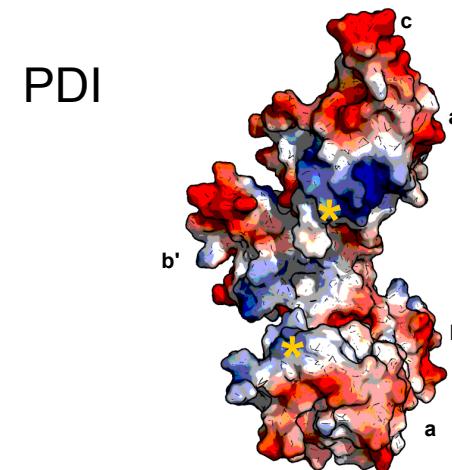
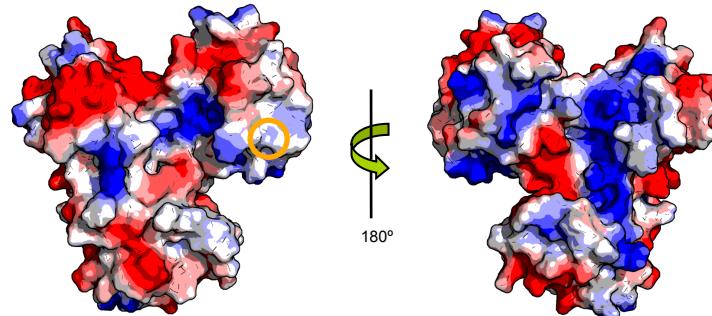
Missing Parts

- Transmembrane helix

375 ━━━━ Predicted transmembrane region ━━━━ 397

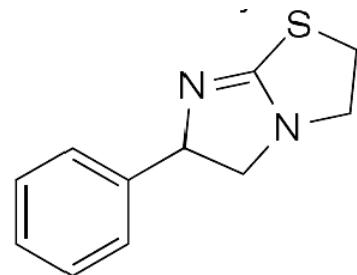
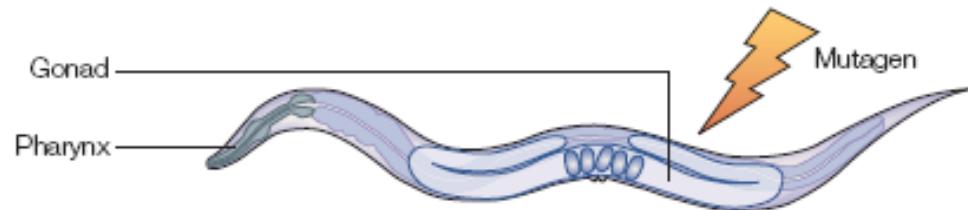
H. s. 373	S	S	P	L	M	G	C	F	L	F	G	L	P	L	G	V	I	S	I	M	C	Y	G	I	Y	T	A	D	T
M. m. 376	S	S	P	L	M	G	C	F	L	F	G	L	P	L	G	V	I	S	I	M	C	Y	G	I	Y	T	A	D	T
D. m. 387	G	N	P	V	L	T	T	V	I	F	G	L	P	L	G	F	L	S	L	I	M	Y	S	I	F	C	G	D	C
C. e. 383	T	Q	P	L	L	S	S	C	L	F	G	V	P	I	A	F	L	S	I	I	C	Y	S	I	C	S	A	D	F

- Dimerisation?

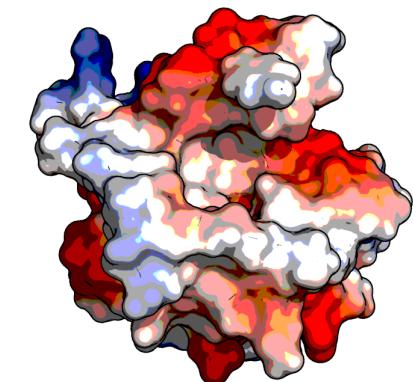
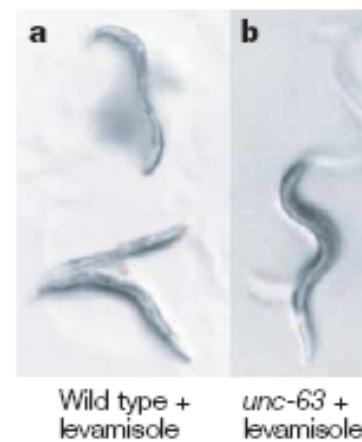


Levamisole

- Unc-74 and C. elegans
- Cysteine-loop ligand-gated ion channels



Levamisole



Conclusions

- Suggestions from model & experiments
 - TMX3 is a thioredoxin-like molecule of the ER
 - TMX3 is responsible for formation of cysteine-loop-gated ion channels involved in voluntary muscle contraction.
- Future experiments
 - Structure(!)
 - Test suggestions from model
 - Hydrophobic pocket/target?
 - Identify interaction partner(s)

Acknowledgements

- Johannes Haugstetter (ETH, Zürich)
- Lars Ellgaard (University of Copenhagen)
- Further reading:

Haugstetter J, Blicher T, Ellgaard.L.

Identification and characterization of a novel thioredoxin-related transmembrane protein of the endoplasmic reticulum.

J Biol Chem. 2005 Mar 4;280(9):
8371-80.

Haugstetter J, Maurer MA, Blicher T,
Pagac M, Wider G, Ellgaard.L.

Structure-function analysis of the endoplasmic reticulum oxidoreductase TMX3 reveals interdomain stabilization of the N-terminal redox-active domain.

J Biol Chem. 2007 Nov 16;282(46):
33859-67.

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PyMOL scripting

A demonstration

Scripts

- PyMOL is based on the programming language Python.
- PyMOL will read python commands.
- PyMOL also has a set of native commands.
- See PyMOLWiki and elsewhere for examples.
 - <http://pldserver1.biochem.queensu.ca/~rlc/work/pymol/>

Automating Tasks

- Scripts are useful for automating tasks
 - Reading in large numbers of structures
 - Repeated analyses
 - Making illustrations!
 - get_view
 - set_view
 - viewport
 - Saves space!
 - Relative to PyMOL session files (.pse)
-

A Useful Example

Using PyMOL commands:

```
list=[]
iterate (name ca),list.append((resn,resi))
print list

[ ('ASP', '1'), ('CYS', '2'), ('ALA', '3'), ('TRP', '4'), ('HIS', '5'), ('LEU',
 '6'), ('GLY', '7'), ('GLU', '8'), ('LEU', '9'), ('VAL', '10'), ('TRP', '11'),
 ('CYS', '12'), ('THR', '13')]
```

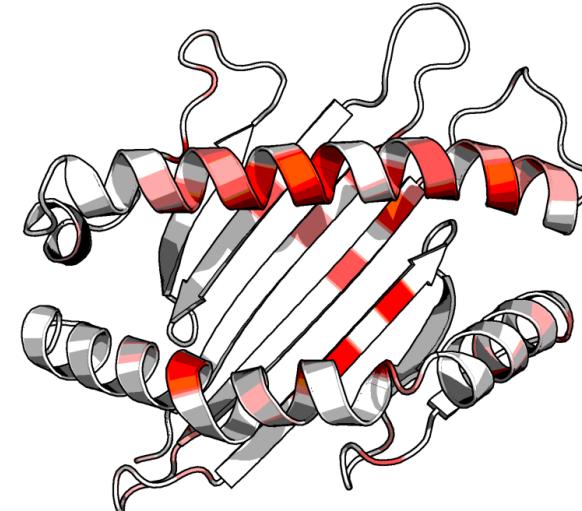
or using a Python script (in PyMOL):

```
from pymol import cmd,stored
stored.list=[]
cmd.iterate("(name ca)","stored.list.append((resi,resn))")
print stored.list

[ ('1', 'ASP'), ('2', 'CYS'), ('3', 'ALA'), ('4', 'TRP'), ('5', 'HIS'), ('6',
 'LEU'), ('7', 'GLY'), ('8', 'GLU'), ('9', 'LEU'), ('10', 'VAL'), ('11',
 'TRP'), ('12', 'CYS'), ('13', 'THR')]
```

color_b.py

- To colour proteins according to the values of the B-factor column (can be replaced with any desired value).



<http://www.mcgnmr.ca/ProtSkin/>

- Syntax: (see script header)

color_b (c;a or c;b), mode=ramp, gradient=bwr, nbins=30, sat=0.5, value=1

to color chains A and B with the Blue-White-Red gradient in 30 colors of equal numbers of atoms in each color.

PyMOL Log Function

- Records all actions as commands in a PyMOL session
 - GUI clicks
 - Written commands
 - Needs to be opened at start of session.
 - Is only written when closed (File→Close log).
-

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