What did we learn from the structures of Membrane Associated Proteins in Eicosanoid and Glutathione Metabolism, MAPEG?

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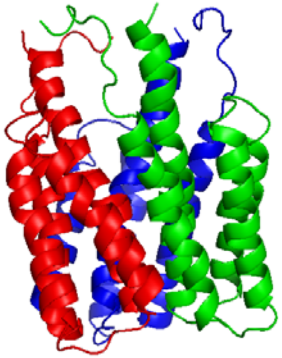
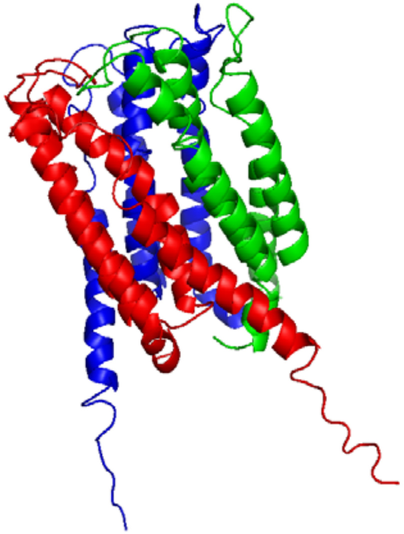
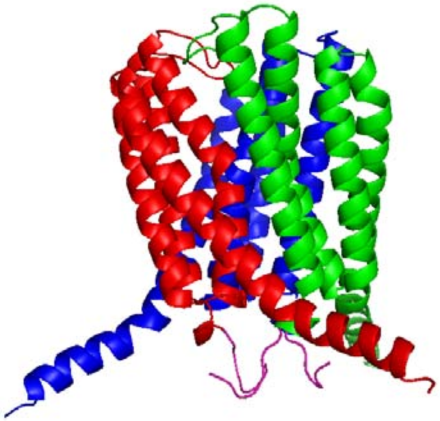
Caroline Jegerschöld, Qie Kuang, Pasi Purhonen


MGST1

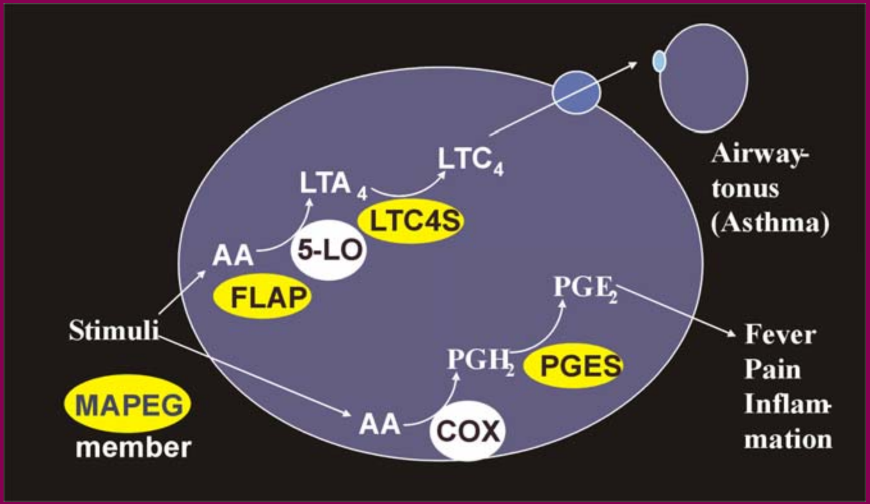

FLAP


LTC4S

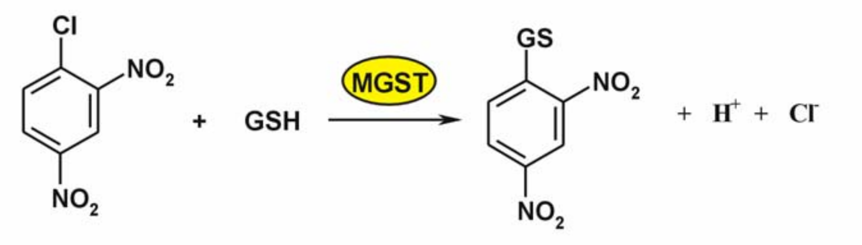

MPGES1

Eicosanoid and glutathione metabolism and MAPEG

- **Synthesis of arachidonic acid derived substances**



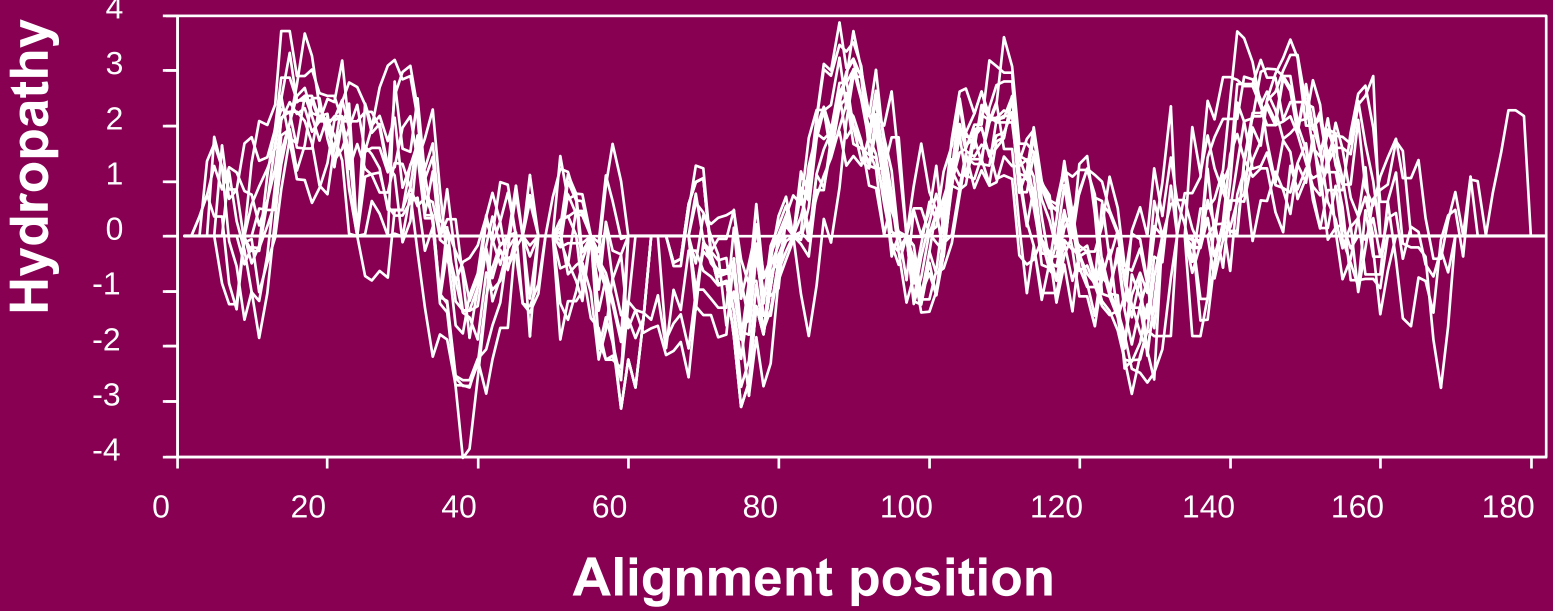
- **Detoxification**



MAPEG superfamily

Membrane Associated Proteins in Eicosanoid and Glutathione Metabolism

- **MGST1**
  Microsomal glutathione transferase 1
- **MPGES1**
  Microsomal prostaglandin E synthase 1
- **MGST2**
- **MGST3**
- **LTC4S, Leukotriene C_4 synthase**
- **FLAP, 5-lipoxygenase activating protein**



2D crystals of MGST1

Purification

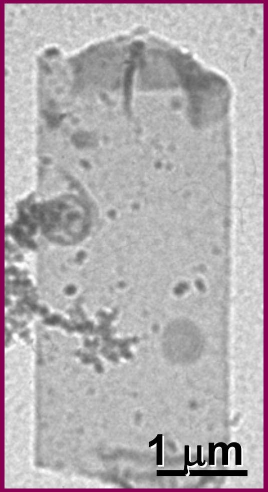
- Rat liver (3.1% of ER)
- Protocol:
  - Liver
  - Microsomes
  - Triton X100 solubilisation
  - Hydroxyapatite to bind MGST1
  - Cation exchange chromatography

2D crystallization

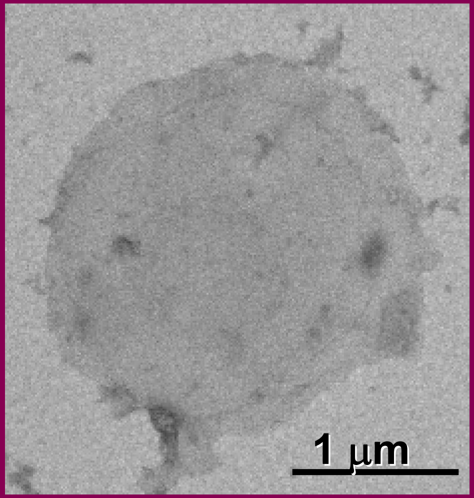
Protein-detergent-micelles
+
Lipid-detergent-micelles


Dialysis
Detergent

2D crystals



$P22_12_1$
a=91.9 Å
b=90.8
γ=90.0º

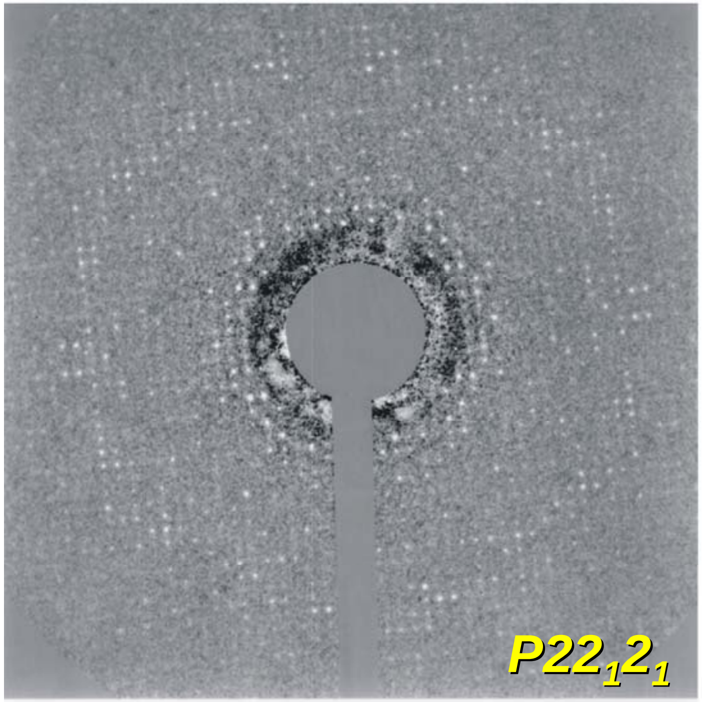


P6
a=81.8 Å
b=81.8
γ=120.0º

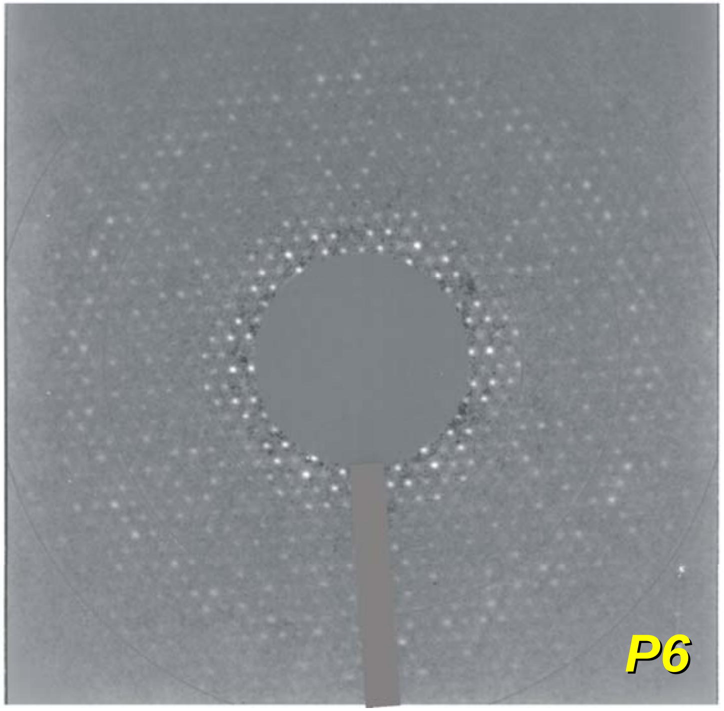
What did we learn from the MAPEG structures?

- MGST1 is a homotrimer with 12 TM helices

Electron diffraction intensities from the two crystal forms of MGST1

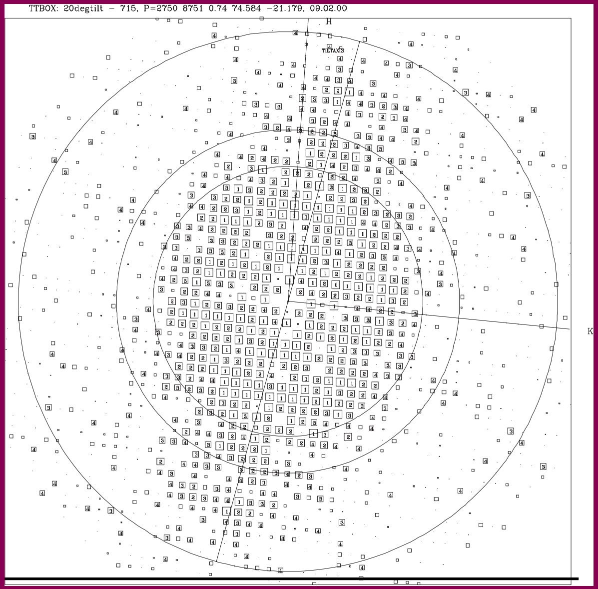


- 44 ED patterns, max tilt 60.6º
- I/σ (overall/(4.0-3.5 Å) 6.0/2.5
- $R_{Friedel}/R_{merge}$ 24.9/34.7
- Observed/used amplitudes to 3.5 Å 29211/11073



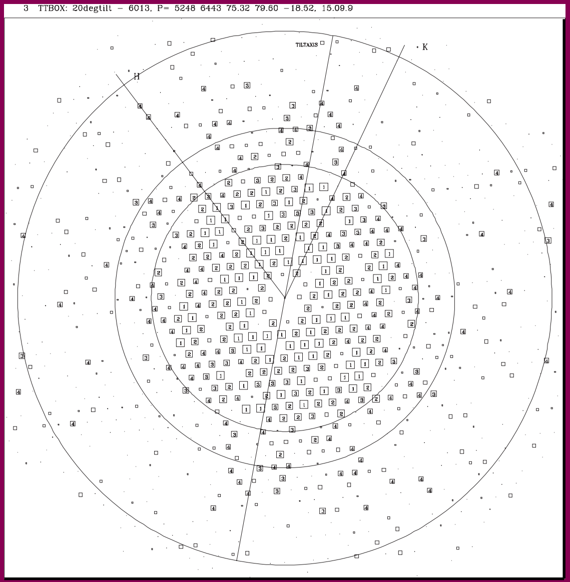
- 120 patterns, max tilt 64.4º
- I/σ (overall/(4.0-3.5 Å) 12.1/6.0
- $R_{Friedel}/R_{merge}$ 12.7/28.8
- Observed/used amplitudes to 3.0 Å 51754/5154

Phase data from images of MGST1



$P22_12_1$, 20°

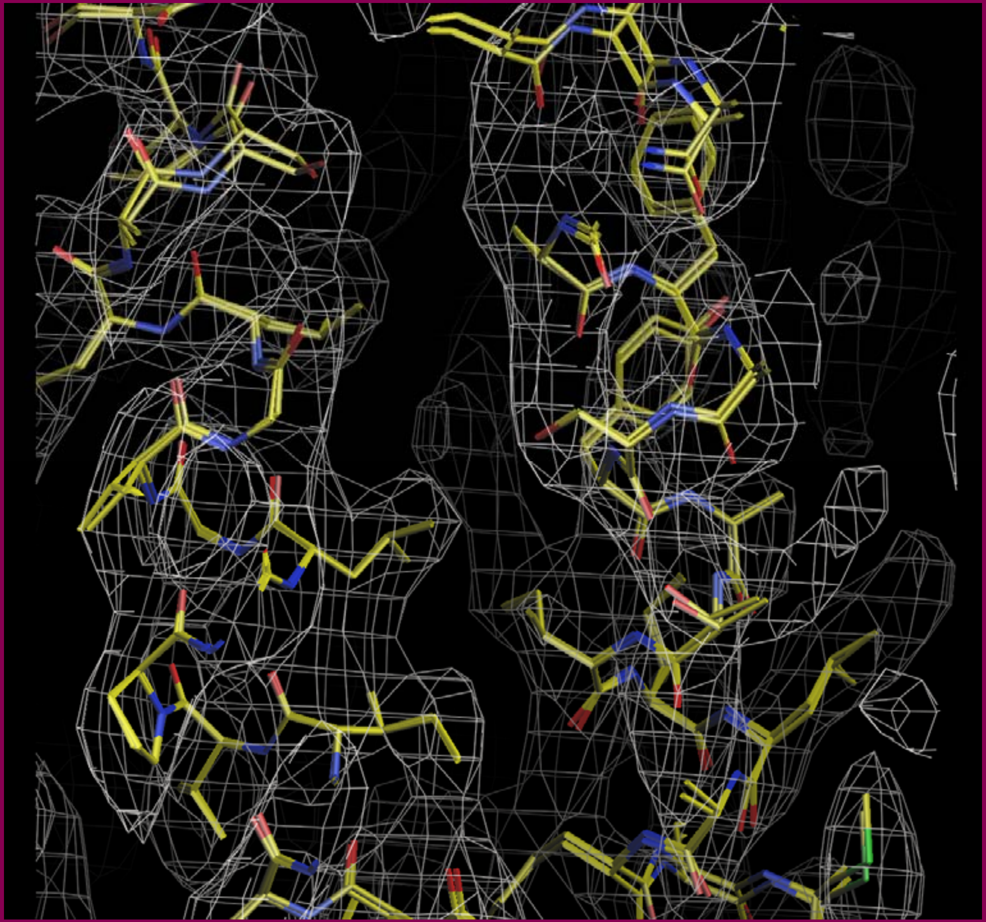
- 77 images, max tilt 62.8°
- Phase residual overall/(4.0-3.5 Å) 21.6º/54.6°
- Observed/used phases to 3.5 Å 41132/9561



P6, 20°

- 53 images, max tilt 62.9°
- Phase residual overall/(4.5-3.5 Å) 30.8º/42.4º
- Observed/used phases to 3.5 Å 17915/5300

Model building and crystallographic refinement



2Fo-Fc map, 1.2σ

- Model building in O
- Refmac5, P6 data
- Tight geometry constraints and restraints
- Observations/refinement parameters ~5.1
- R (10–3.2 Å) 33.9
- R_{free}(10–3.2 Å) 37.6
- R_{ort}(10–3.5 Å) 49.1

What did we learn from the MAPEG structures?

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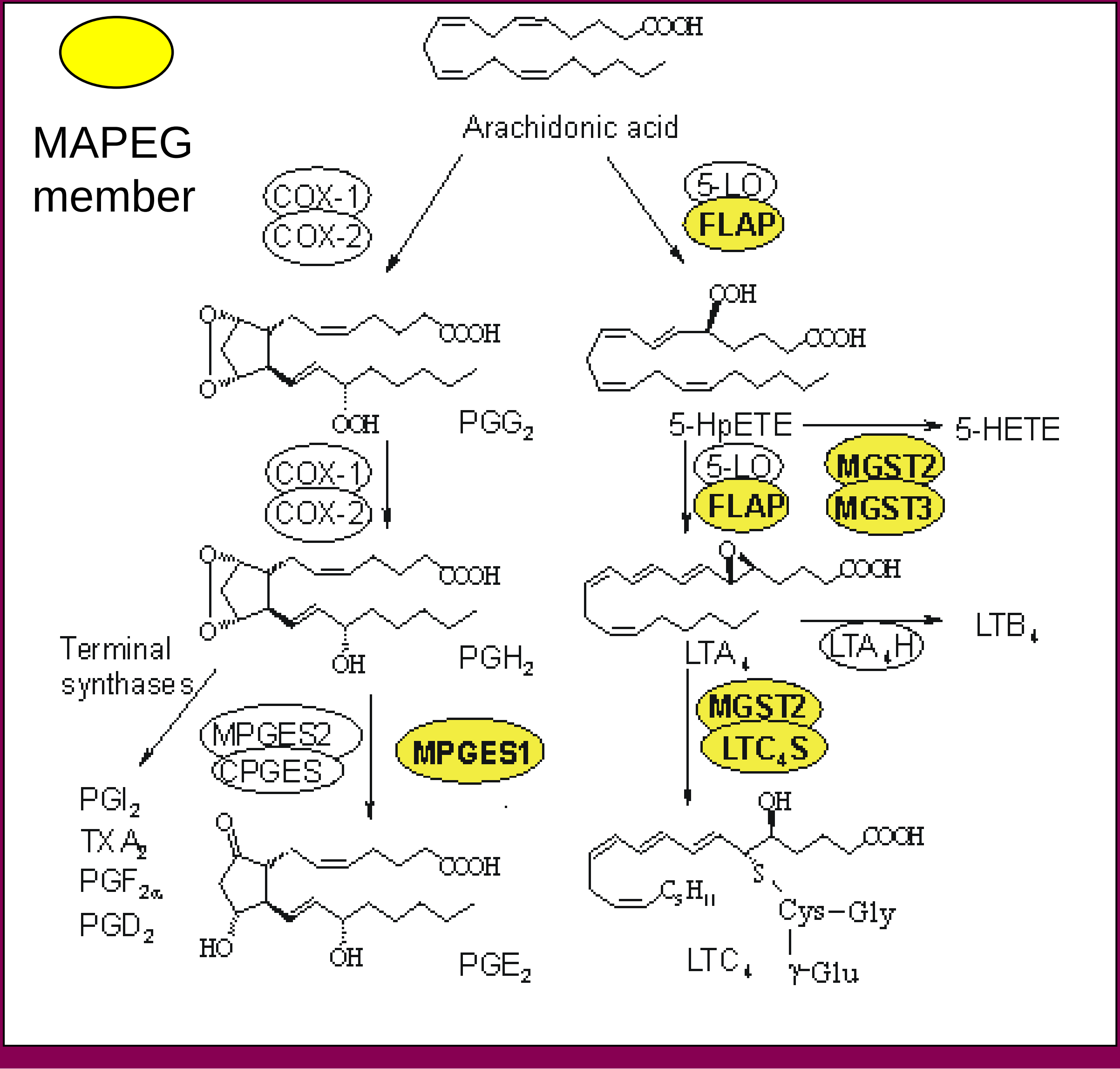
- Tracing of TM helices identified the common MAPEG fold
- Active site of MGST1 at the interface between monomers, TM1 and TM4 from neighbouring subunits

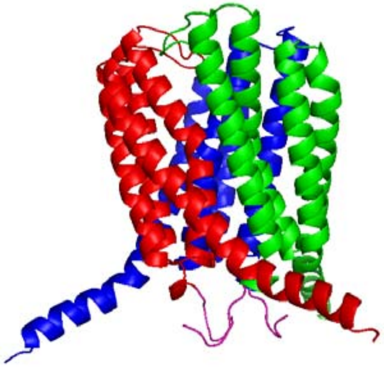
Cyclooxygenase pathways involving MAPEG members

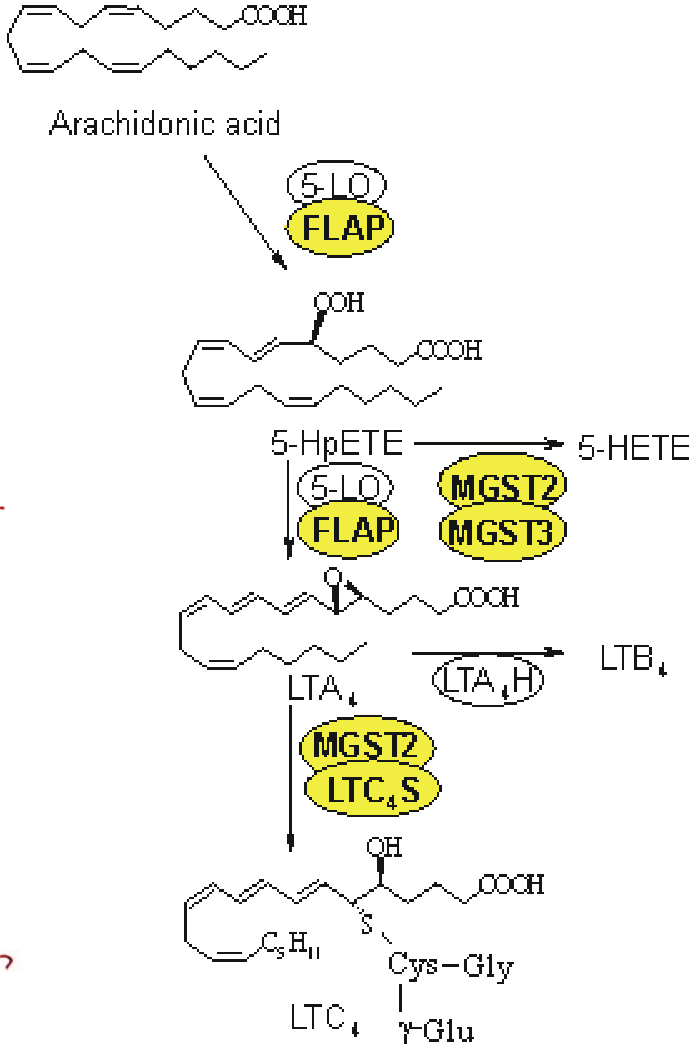


Cyclooxygenase pathways involving MAPEG members

FLAP
Ferguson
et al. 2007

LTC4S
Molina
et al. 2007
Ago
et al. 2007

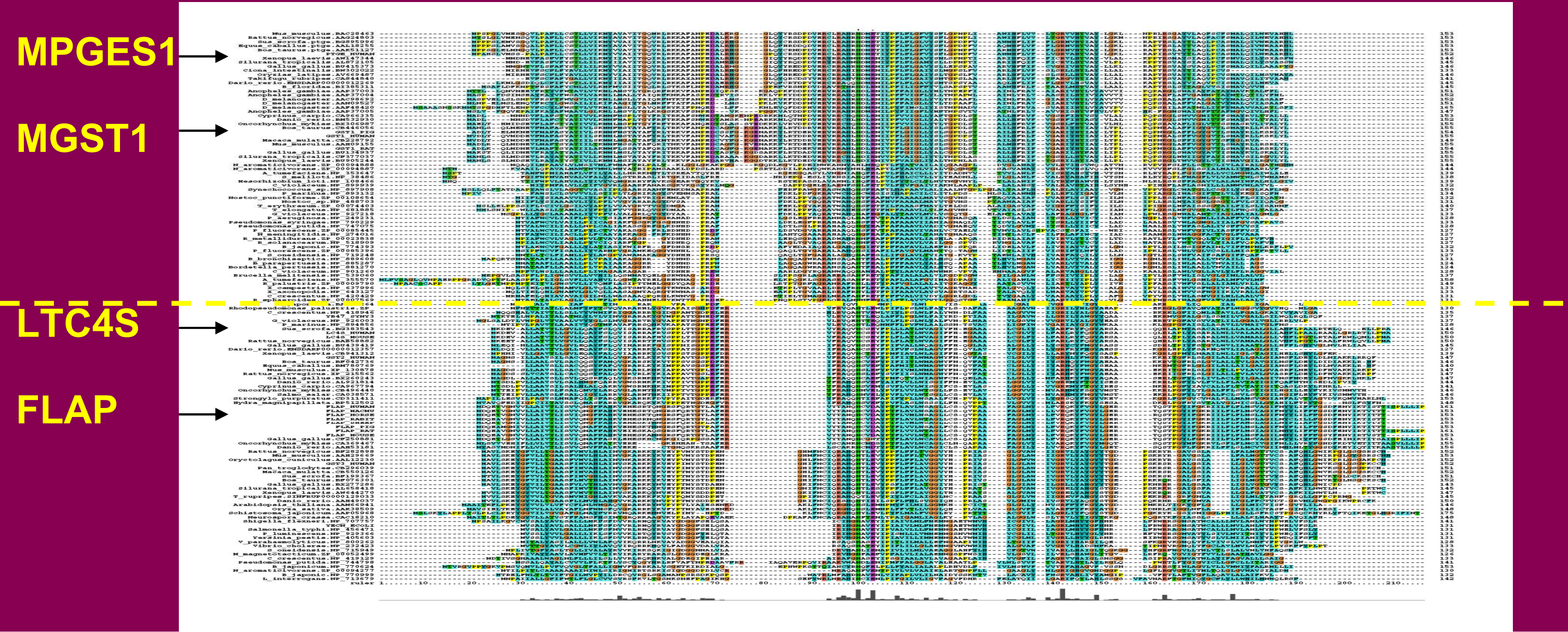




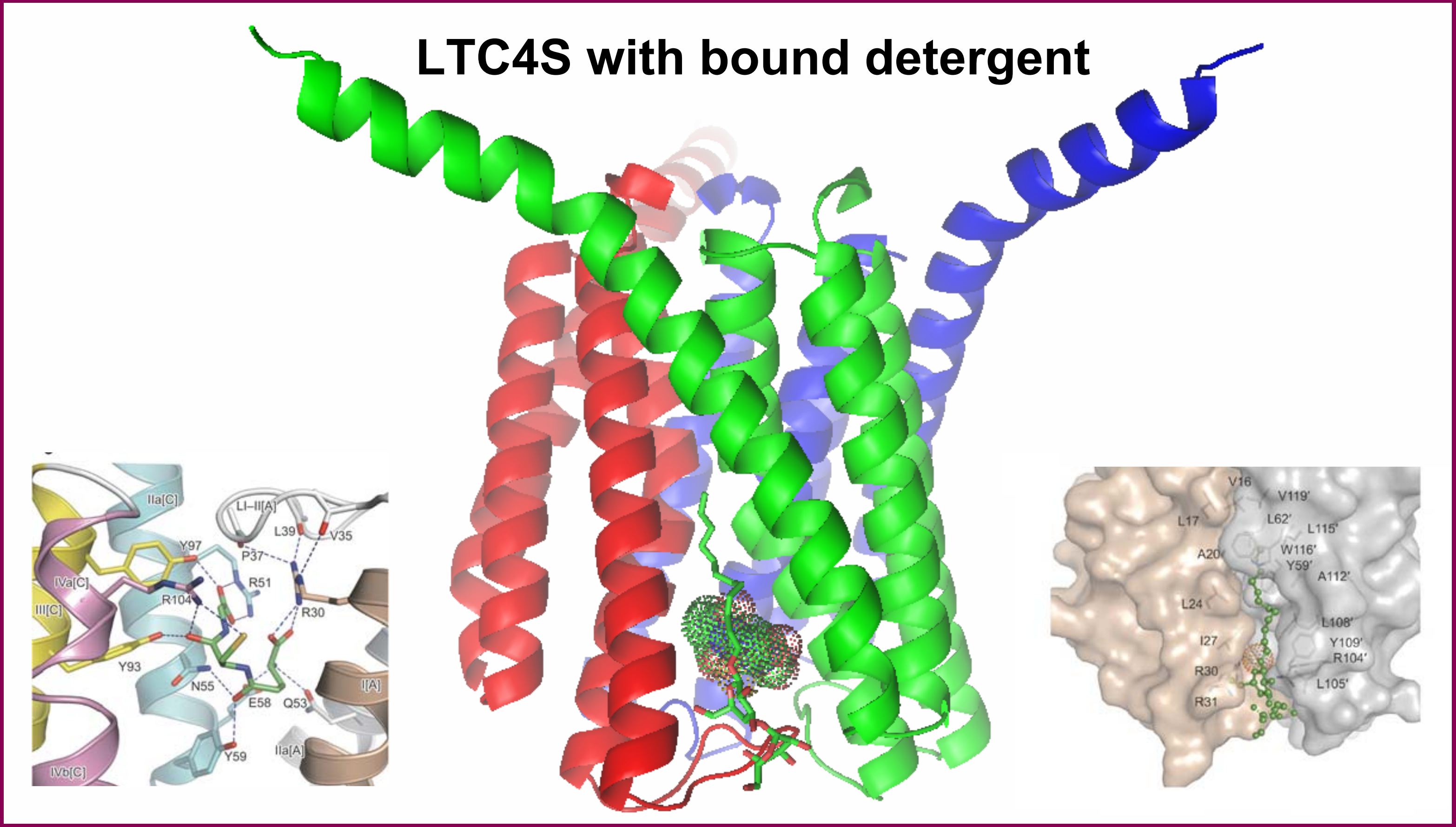
Two MAPEG subfamilies

- **MGST1**
- **Microsomal glutathione transferase 1**
- **MPGES1**
- **Microsomal prostaglandin E synthase 1**

- **MGST2**
- **MGST3**
- **LTC4S, Leukotriene C_4 synthase**
- **FLAP, 5-lipoxygenase activating protein**



X-ray structures of FLAP and LTC4S



LTC4S with bound detergent

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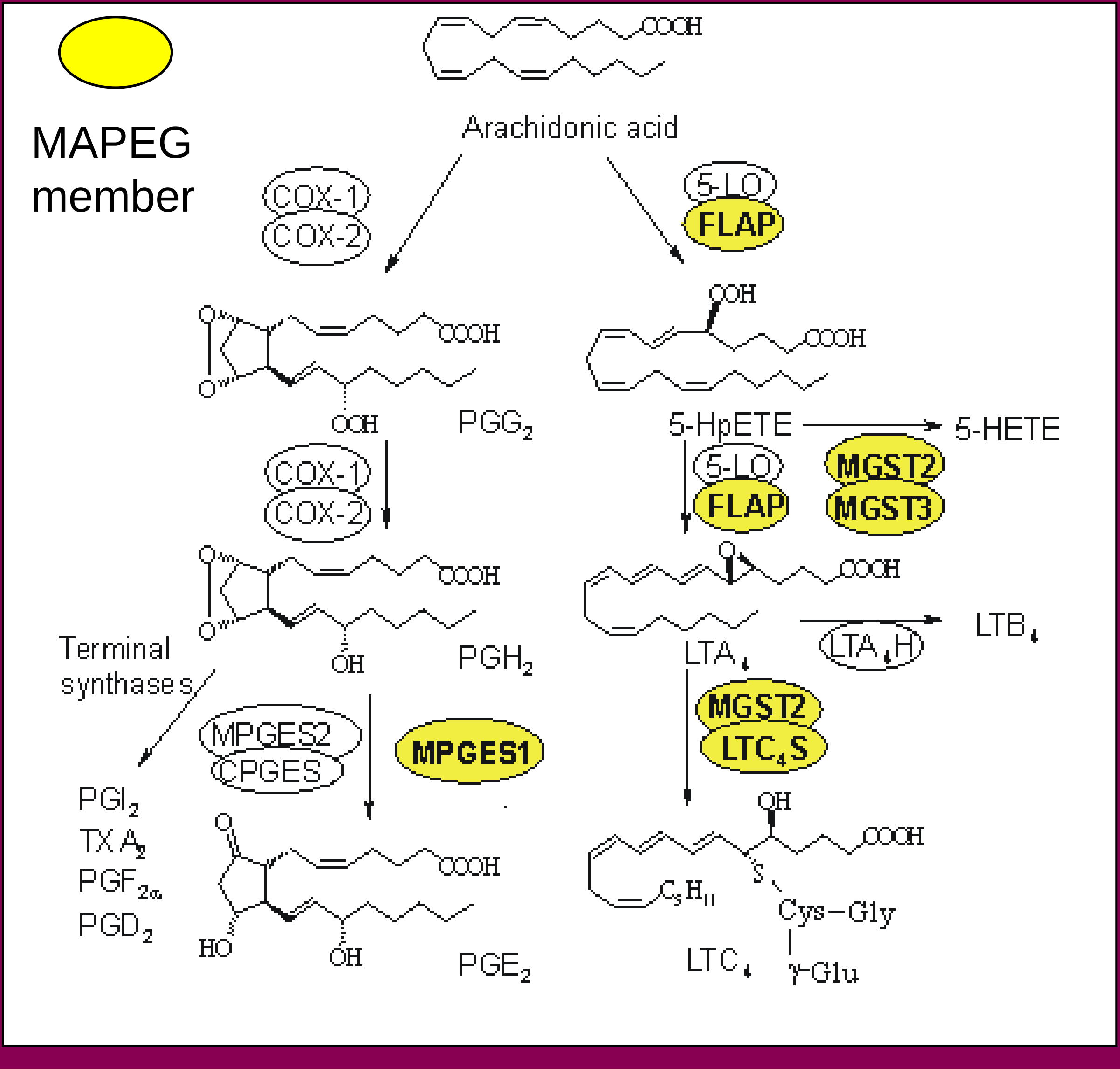
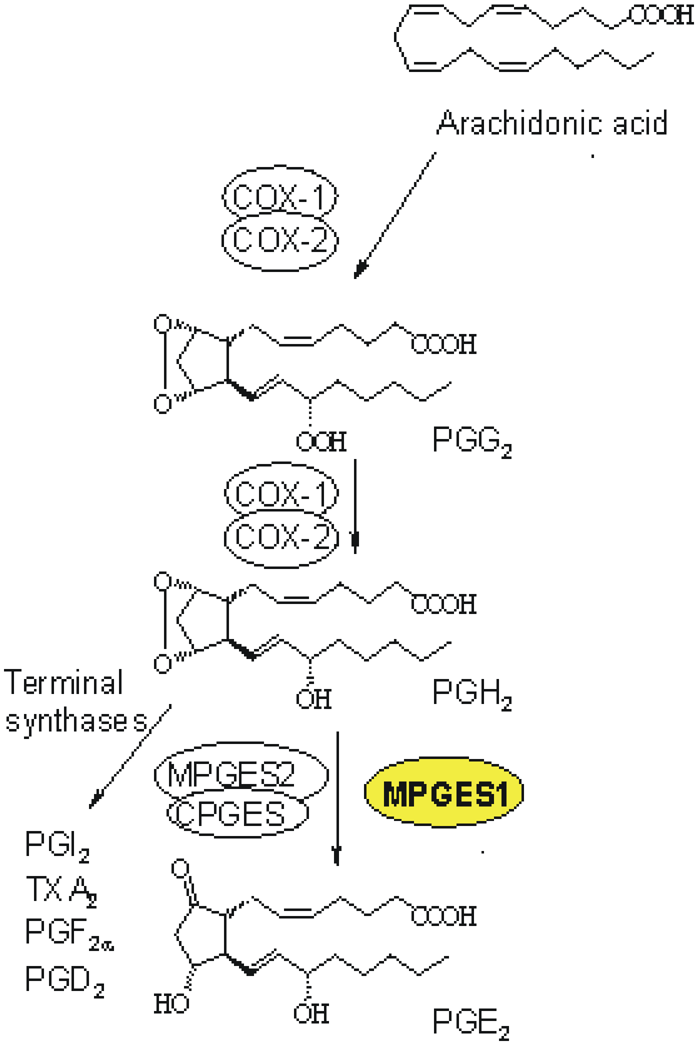
- GSH binds in a U-shaped conformation
- Thiol group of GSH positioned for activation by a nearby arginine (R104) at the membrane-enzyme interface
- Ligand/inhibitor binding at the site suggested from the MGST1 structure

Cyclooxygenase pathways involving MAPEG members



Cyclooxygenase pathways involving MAPEG members

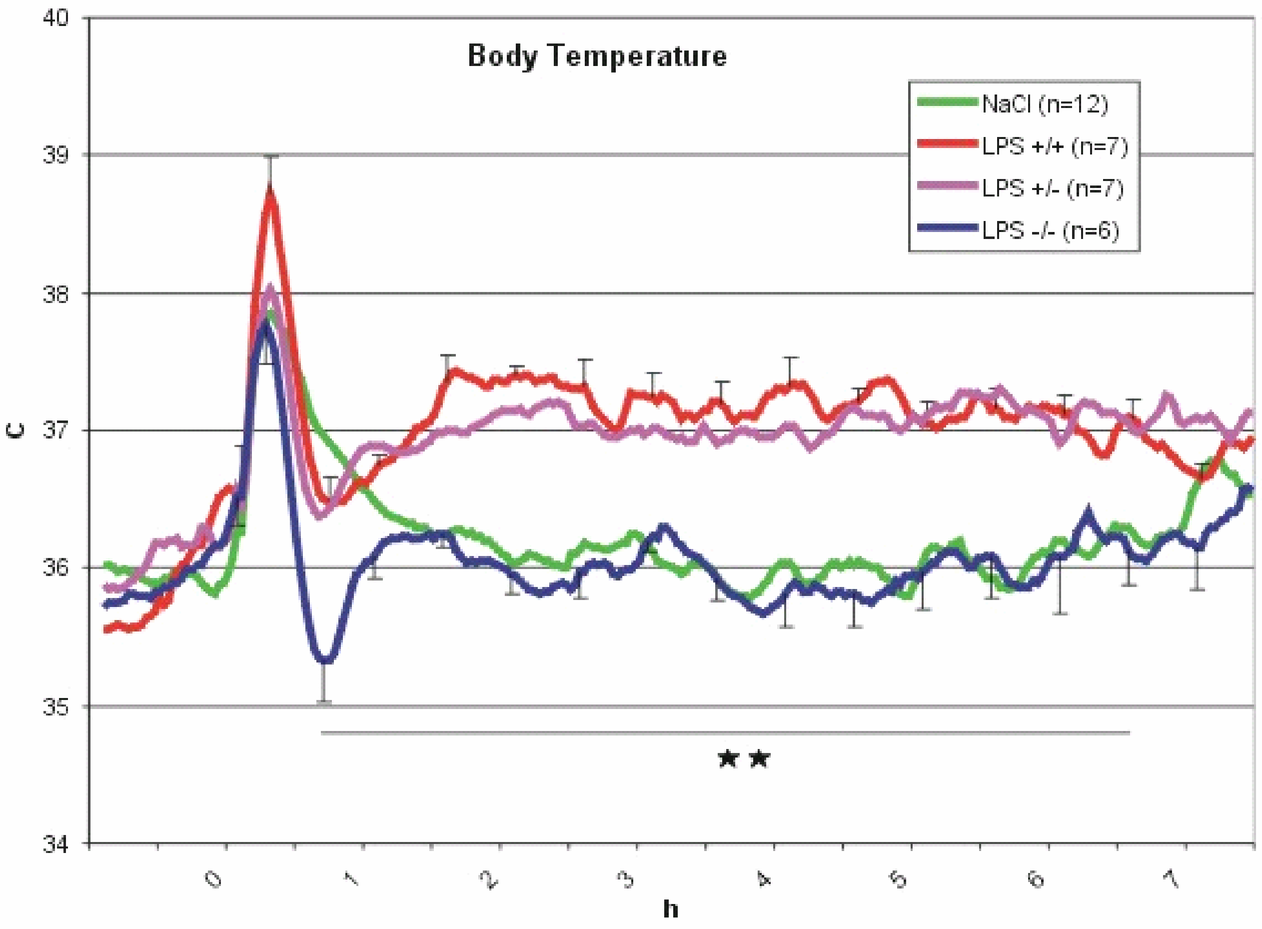


MPGES1…

- **catalyzes the oxidoreduction of prostaglandin endoperoxide H_2 into PGE_2 with an apparent k_{cat}/K_m of $310 mM^{-1}s^{-1}$.**

- **is mainly an induced isomerase, whereas cPGES and MPGES2 are constitutive enzymes.**
- **is the terminal enzyme in the catalytic pathway producing PGE_2.**
- **is a potential target for the development of therapeutic agents for treatment of several diseases.**

MPGES1 k.o. mice fail to develop fever after LPS treatment



Engblom, Saha et al. (2003) Nature Neurosci. 6, 1137.

2D crystals of MPGES1

Purification

- N-terminal His$_6$-tag
- pSP19T7LT vector
- *E. coli* BL21(DE3) cells
- HA/IMAC chromatography
- Desalting/gel electrophoresis
- 0.5 - 1 mg/ml protein in 1 % Triton X-100, 100 mM NaPi, 50 mM NaCl, 10% glycerol, 1 mM GSH, and 0.1% EDTA.

2D crystallization

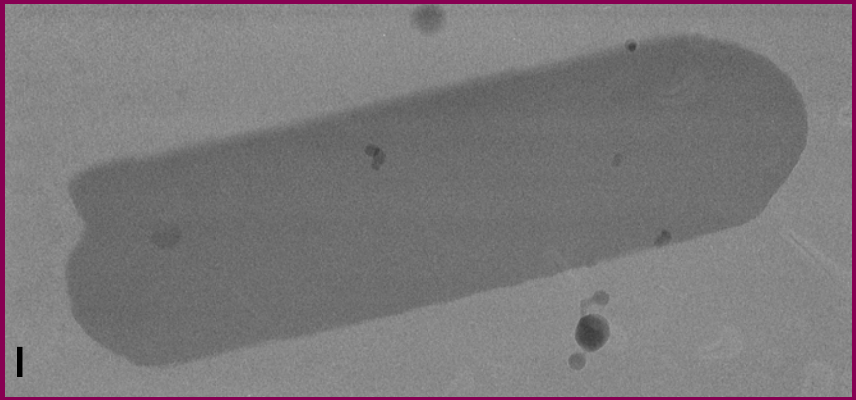
Protein-detergent-micelles

\+

Lipid-detergent-micelles

Dialysis ↓ → *Detergent*

2D crystals



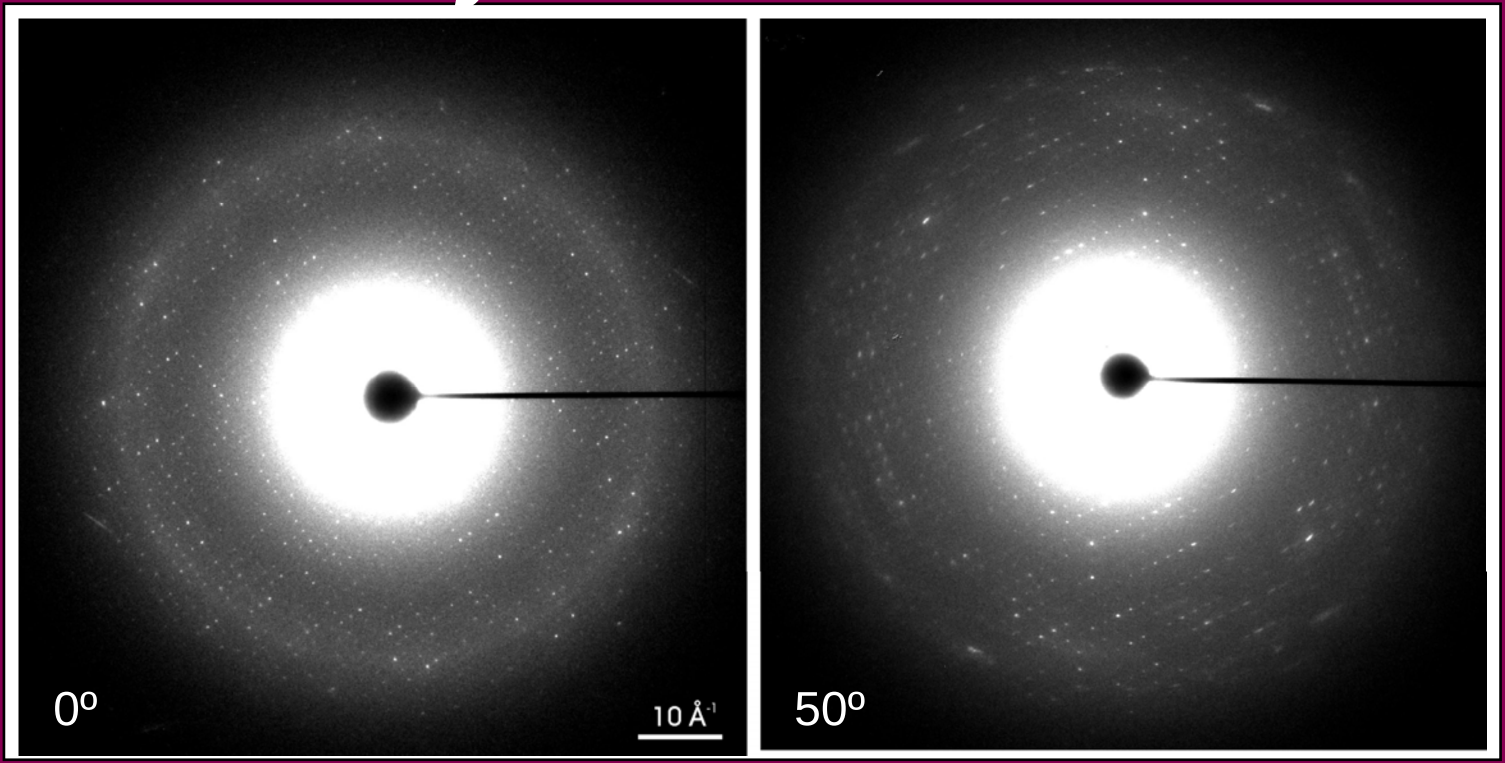
P22_12_1

a=93.2 Å

b=84.6

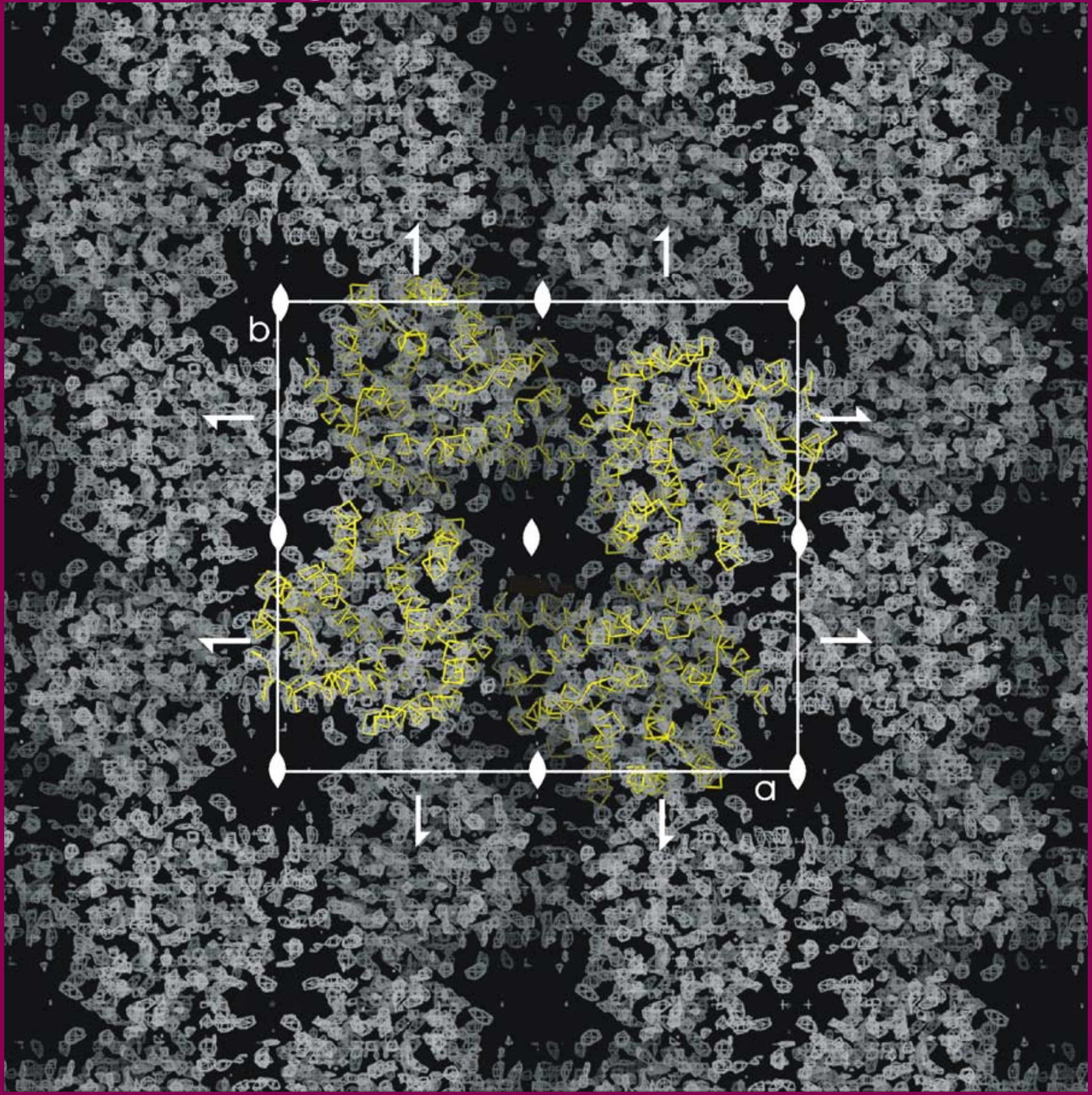
γ=90.0º

Electron diffraction intensities from 2D crystals of MPGES1

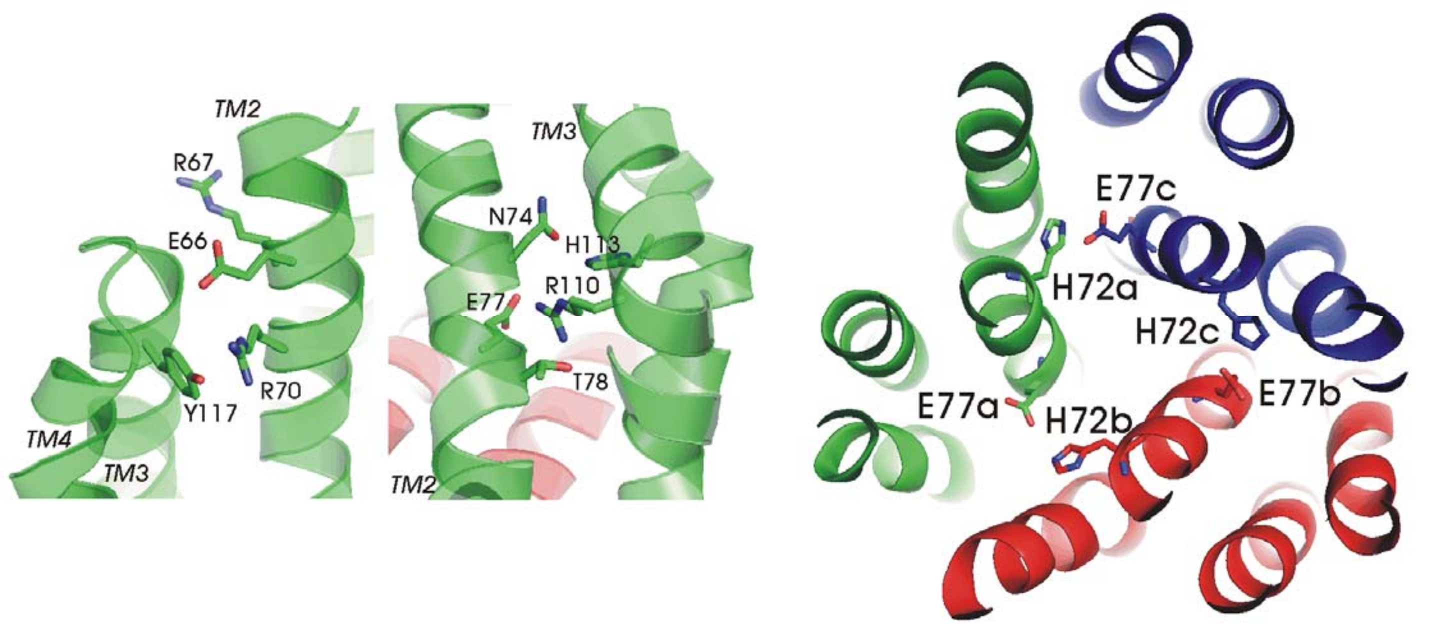


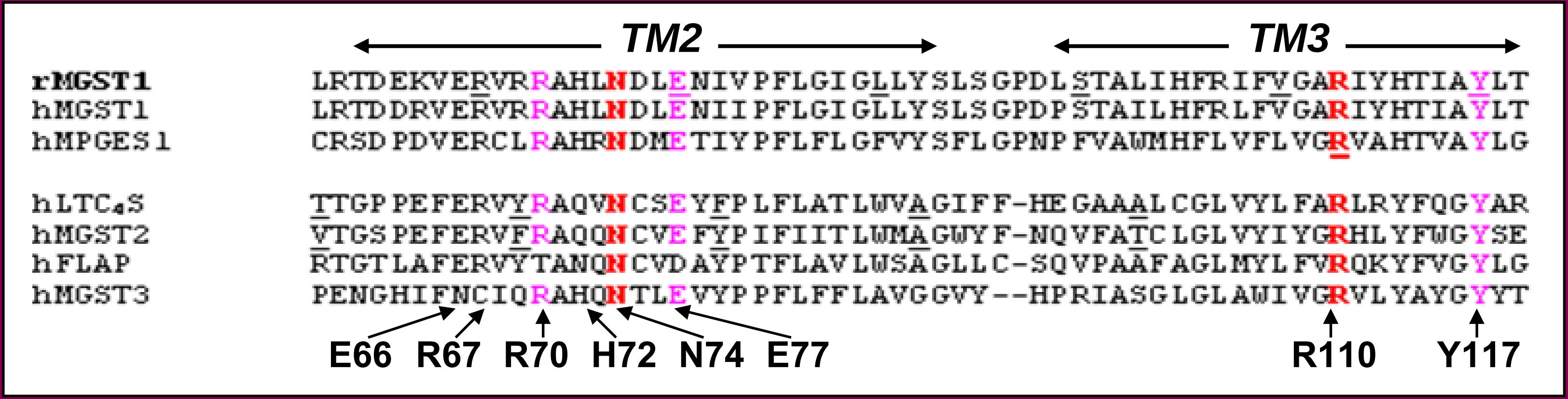
- 100 ED patterns, max tilt 62.0º
- F/σ (overall/(4.0-3.5 Å)) 4.5/3.5
- $R_{Friedel}/R_{merge}$ 14.4/46.1
- Observed/used amplitudes 45035/6185
- Resolution in an normal to membrane plane 3.5/4.2 Å.
- Tilt pairs to resolve heterogeneity

Molecular packing of MPGES1 as determined by molecular replacement



MPGES1 structure

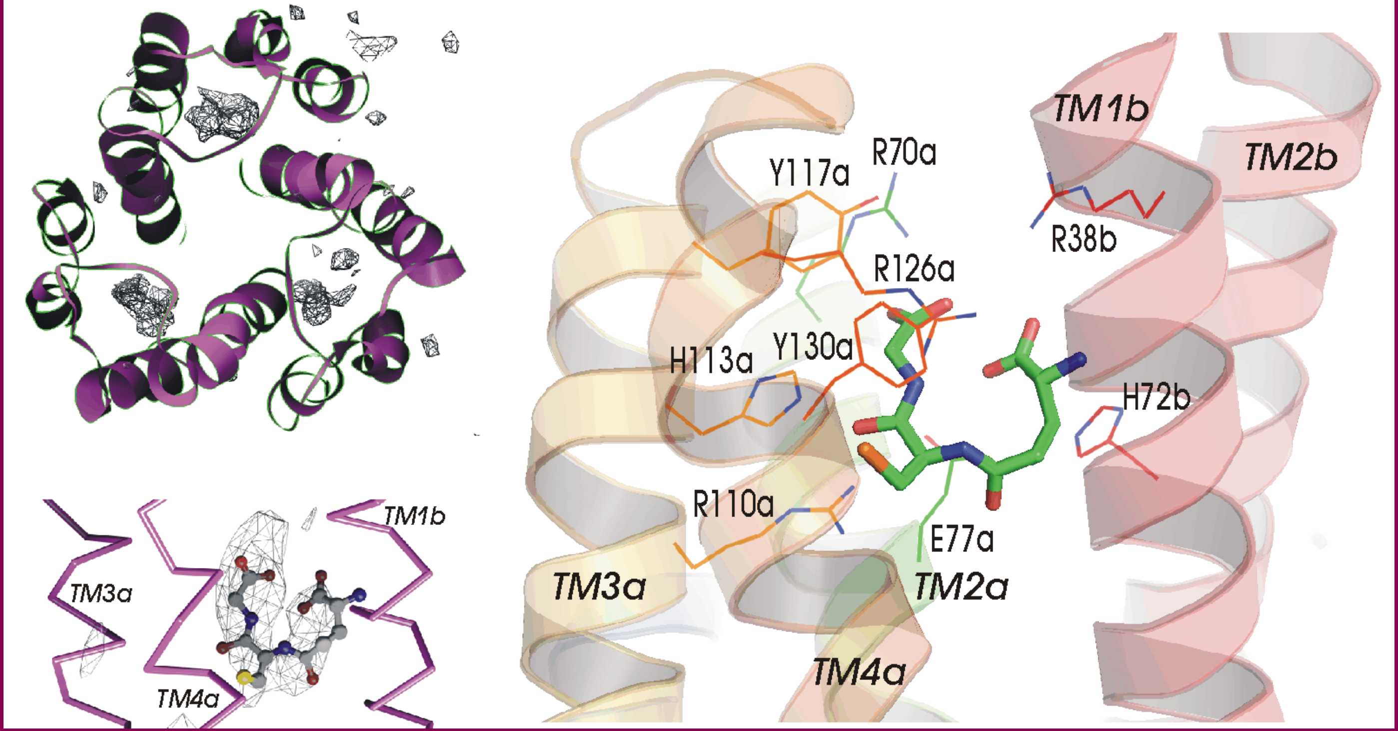




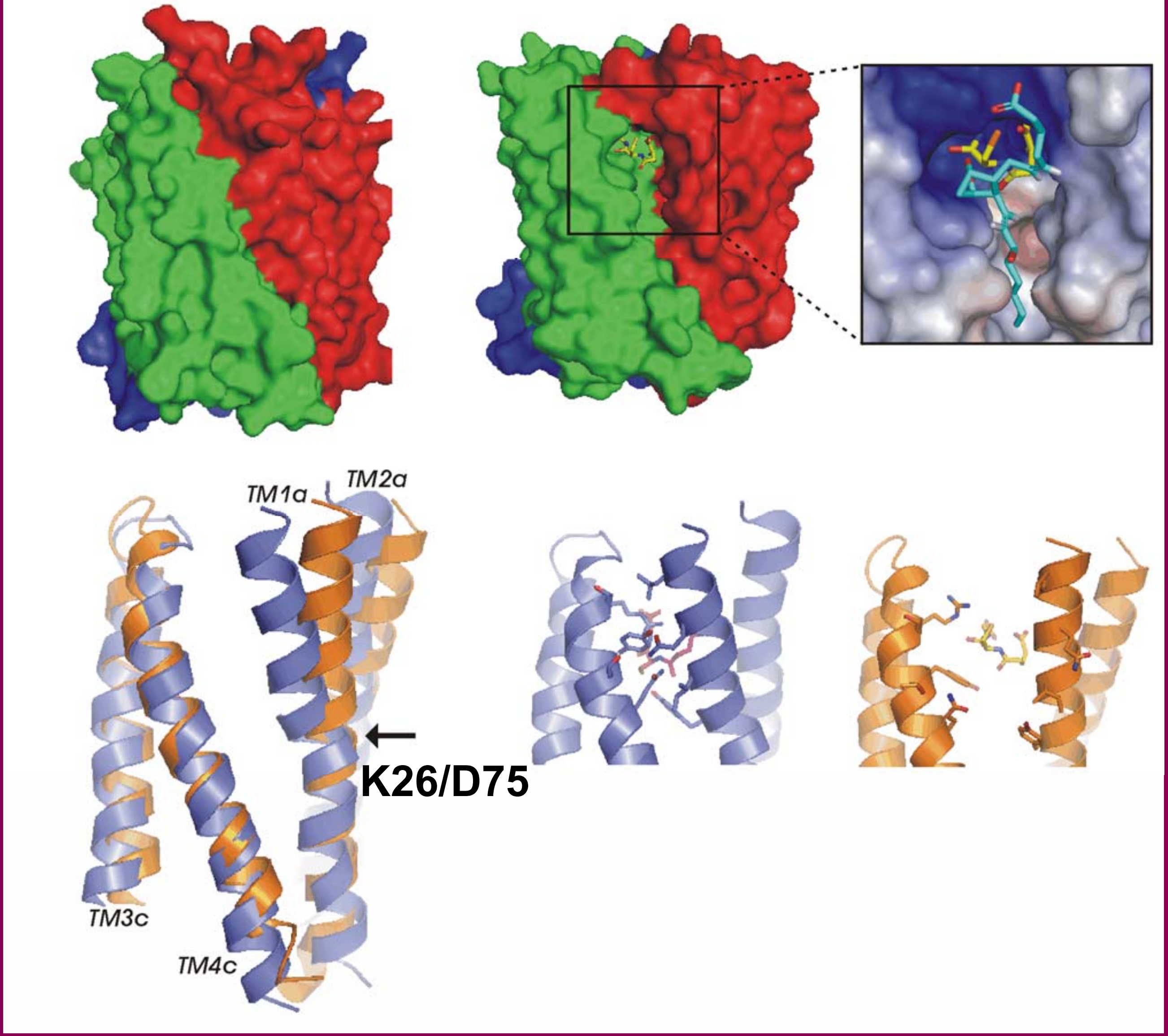
```
rMGST1    LRTDEKVERVRRAHLNDLENIVPFLGIGLLYSLSGPDLSTALIHFRIFVGARIYHTIAYLT
hMGST1    LRTDDRVERVRRAHLNDLENIIPFLGIGLLYSLSGPDPSTAILHFRLFVGARIYHTIAYLT
hMPGES1   CRSDPDVERCLRAHRNDMETIYPFLFLGFVYSFLGPNPFVAWMHFLVFLVGRVAHTVAYLG

hLTC4S    TTGPPEFERVYRAQVNCSEYFPLFLATLWVAGIFF-HEGAAALCGLVYLFARLRYFQGYAR
hMGST2    VTGSPEFERVFRAQQNCVEFYPIFIITLWMAGWYF-NQVFATCLGLVYIYGRHLYFWGYSE
hFLAP     RTGTLAFERVYTANQNCVDAYPTFLAVLWSAGLLC-SQVPAAFAGLMYLFVRQKYFVGYLG
hMGST3    PENGHIFNCIQRAHQNTLEVYPPFLFFLAVGGVY--HPRIASGLGLAWIVGRVLYAYGYYT
```

Jegerschöld et al. 2008

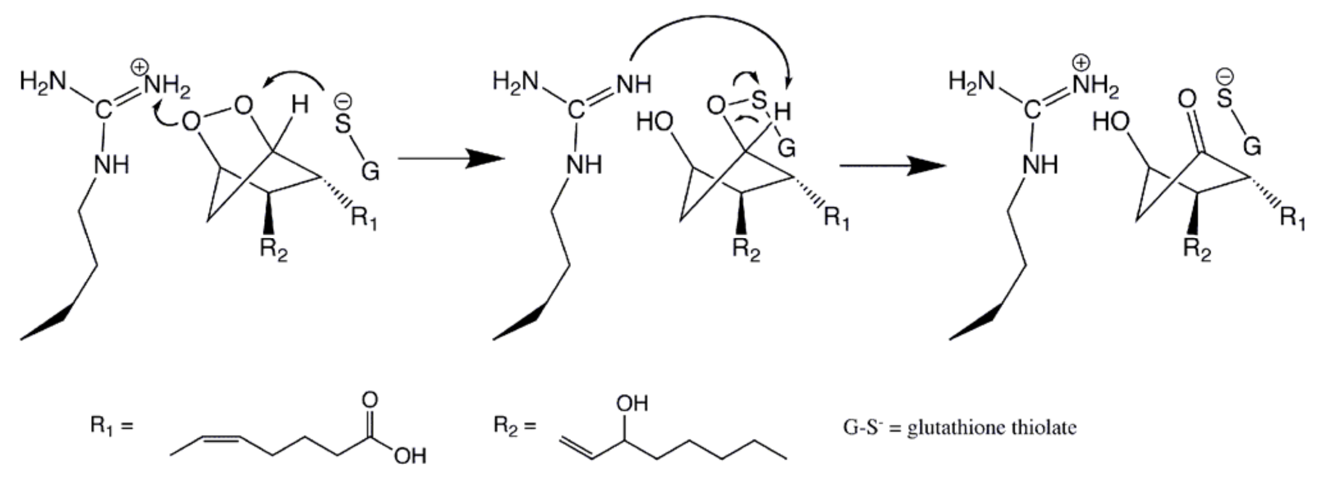
MPGES1 structure



Open/closed conformation



Suggested chemical mechanism



Attack of the GSH thiolate on O_9 of the endoperoxide bridge

Poton donation to O_{11} via R126

R126 abstracts a proton from C_9, a carbonyl is formed

What did we learn from the MAPEG structures?

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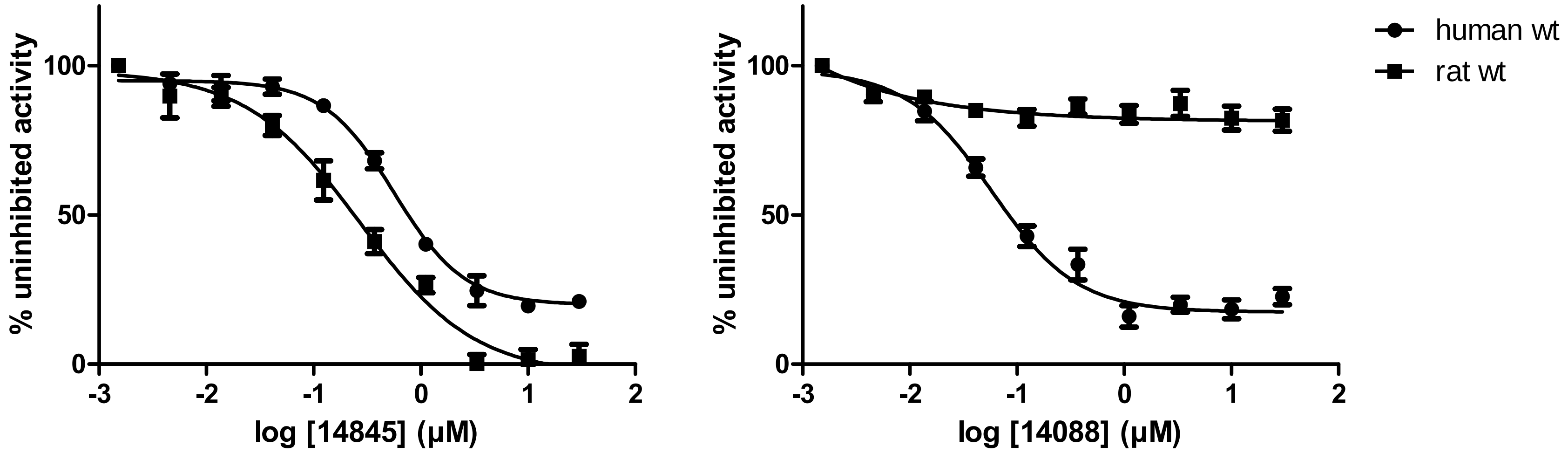
- Active site of MGST1 at the interface between monomers, TM1 and TM4 from neighbouring subunits
- GSH binds in a U-shaped conformation
- Thiol group of GSH positioned for activation by a nearby arginine (R104) at the membrane-enzyme interface
- Ligand binding at the site suggested from the MGST1 structure
- U-shaped GSH also in MPGES1
- Structural comparisons suggest conformational change during catalysis at a hinge in TM1
- Suggested chemical mechanism

Inhibition of rat and human recombinant mPGES-1

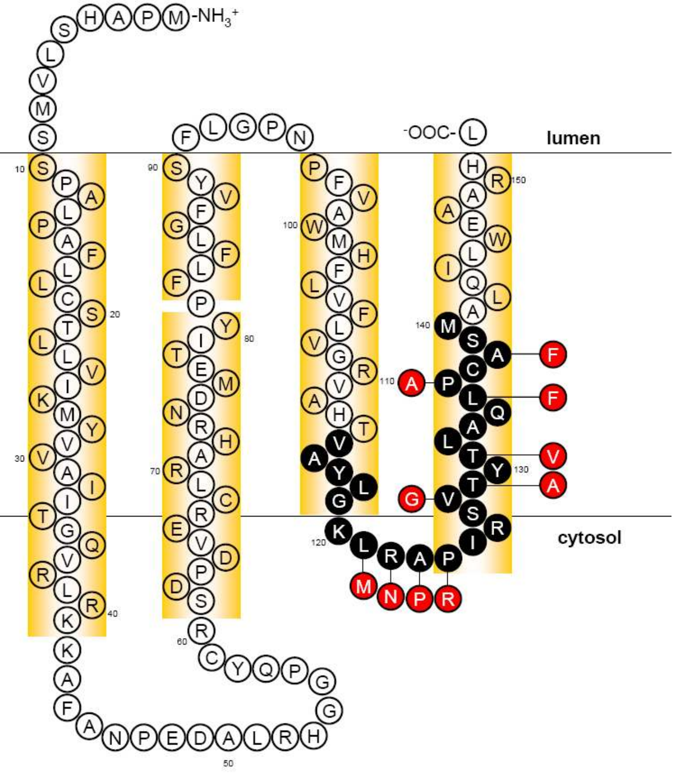


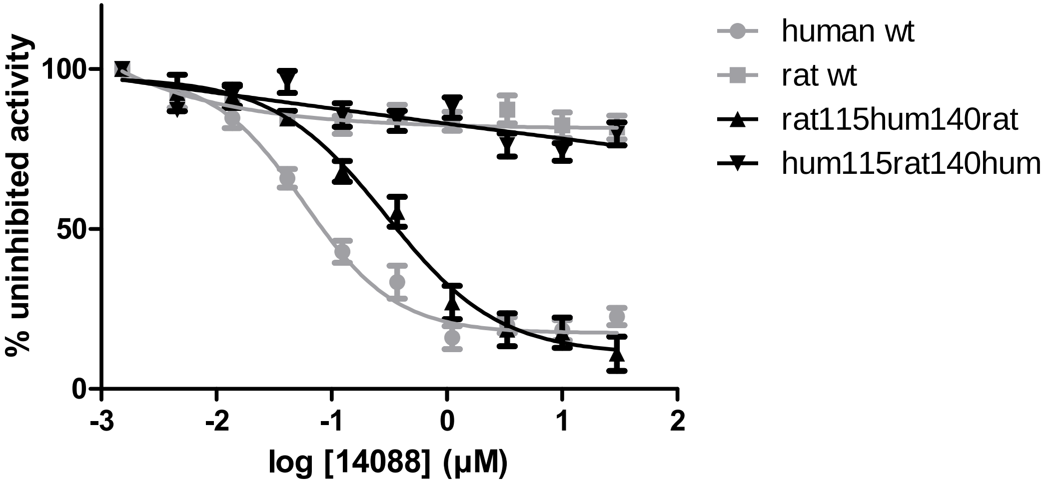
Sequences

```
human    MPAHSLAMSS PA LPAFLLC STLLVIKMYV VAIITGQVRL RKKAFANPED ALRHGGPQYC
rat      MTSLGLVMEN SQVLPAFLLC STLLVIKMYA VAVITGQVRL RKKAFANPED ALKRGGLQYC
mouse    MPSPGLVMES GQVLPAFLLC STLLVIKMYA VAVITGQMRL RKKAFANPED ALKRGGLQYY
                    +++++++++++TM1++++++++++++                           +
                     ↑                  ↑   ↑                     ↑↑  ↑

         RSDPDVERCL RAHRNDMETI YPFLFLGFVY SFLGPNPFVA WMHFLVFLLG RVVHTVAYLG
         RSDPDVERCL RAHRNDMETI YPFLFLGFVY SFLGPNPLIA WIHFLVVLTG RVVHTVAYLG
         RSDPDVERCL RAHRNDMETI YPFLFLGFVY SFLGPNPLIA WIHFLVVLTG RVVHTVAYLG
         +++++++++++++TM2+++++++++++++++++++      +++++++++++TM3++++++++++
                                                 ↑↑   ↑    ↑ ↑

         KLRAPIRSVT YTLAQLPCAS MALQILWEAA RHL
         KMNPRIRSGA YVLAQFACFS MALQILWEVA HHL
         KLNPRLRSGA YVLAQFSCFS MALQILWEVA HHL
              ++++++++TM4++++++++++
           ↑↑↑↑  ↑↑  ↑   ↑↑ ↑          ↑  ↑
```

Chimeric enzymes

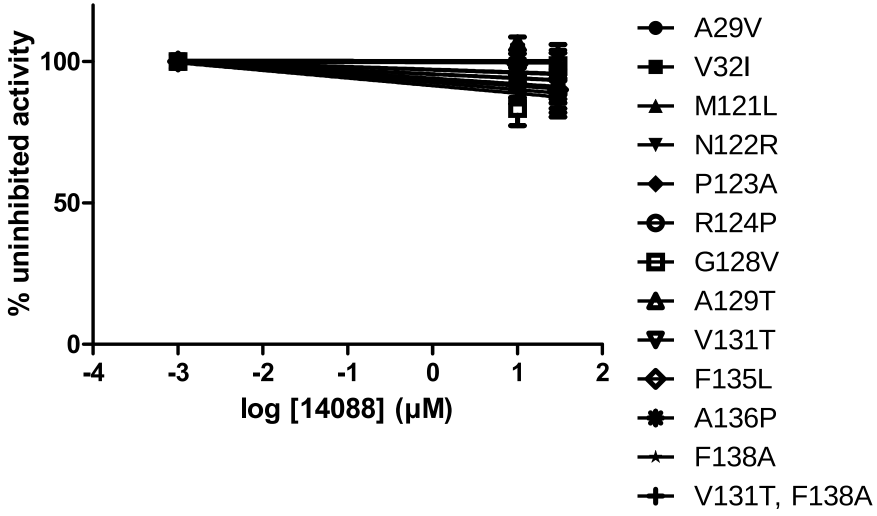
rat115hum140rat, hum115rat140hum

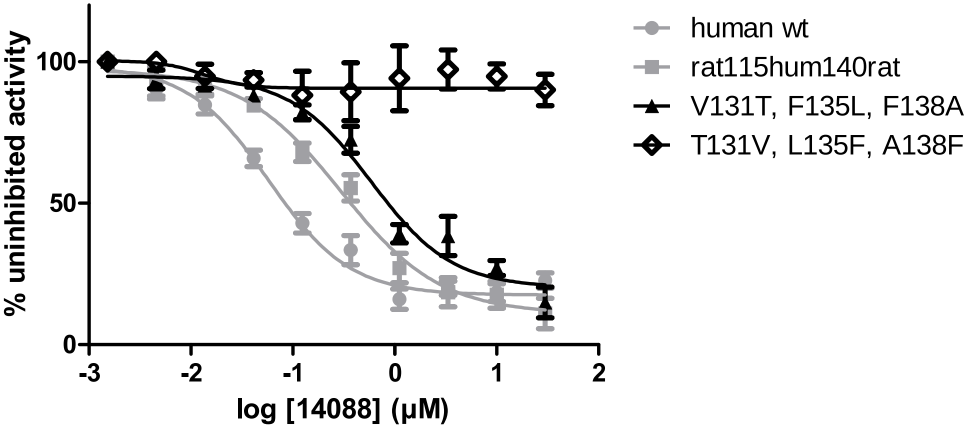




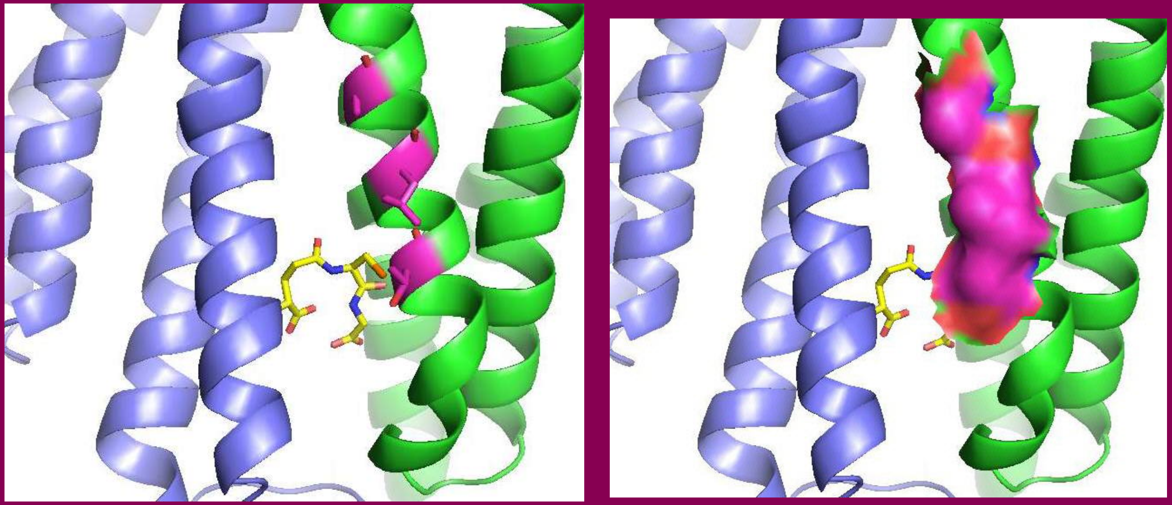
Pawelzik et al. 2010

Triple mutant
V131T, F135L, F138A

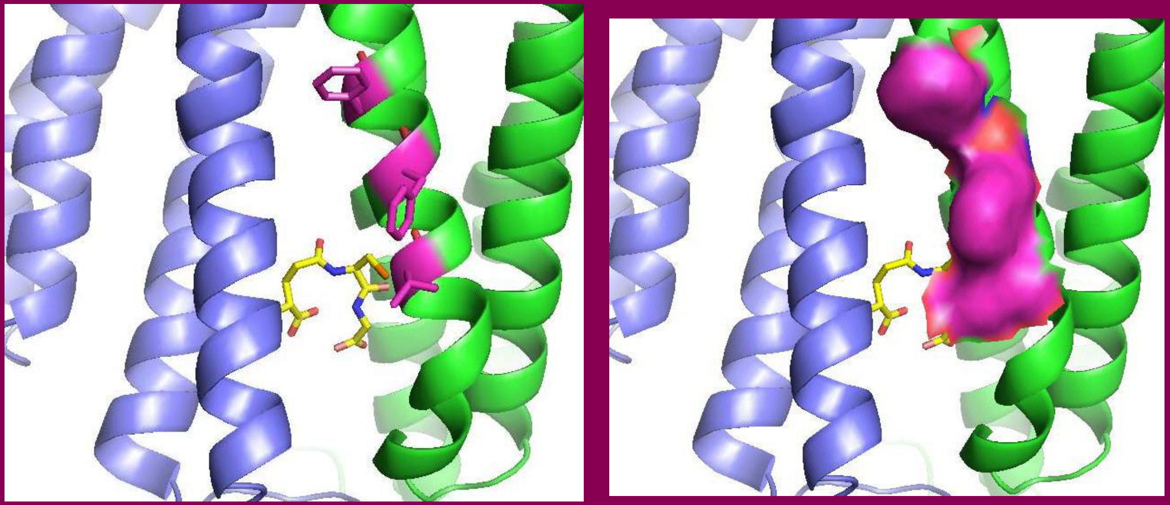




Triple mutant V131T, F135L, F138A



Human MPGES1



Rat MPGES1

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- Thiol group of GSH positioned for activation by a nearby arginine (R104) at the membrane-enzyme interface
- Ligand binding at the site suggested from the MGST1 structure
- U-shaped GSH also in MPGES1
- Structural comparisons suggest conformational change during catalysis at a hinge in TM1
- Suggested chemical mechanism
- Mutations at specific postions in the MPGES1 structure alters inhibitor binding properties.

Acknowledgments

Structural studies:
Caroline Jegerschöld
Pasi Purhonen
Qie Kuang

Role of MPGES1 in pain, fever, RA….:
Sipra Saha
Karina Gheorghe

MGST1/MPGES1 collaborators:
Ralf Morgenstern
Per-Johan Jakobsson
Sven-Christian Pawelzik

AIST, Tokyo/Kyoto Univ:
Kaoru Mitsuoka
Nobuhiko Gyobu
Yoshinori Fujiyoshi

From one of our MAPEG meetings

Vanderbilt University:
Richard Armstrong, Laura Busenlehner

MGST1

Data collection and refinement statistics

Two-dimensional crystal parameters		
Two-sided plane-group	p22121	p6
Unit cell (Å)	a=91.9	a= 81.8
	b=90.8	b= 81.8
	c=100.0*	c=100.0*
	γ=90.0°	γ=120.0°
Phase determination from images		
Number of images used	77	53
Maximum tilt angle (°)	62.8	62.9
Resolution in and normal to membrane plane (Å)**	3.5/7.0	3.5/7.0
No. of observed/used phases	41132/9561	17915/5300
Fourier space sampled (%)	73.4	74.4
Phase residual, overall/4.0-3.5 Å resolution shell (°)	21.6/54.6	30.8/42.4
Amplitude determination from electron diffraction		
No. of diffraction patterns	44	120
Maximum tilt angle (°)	60.6	62.6
Resolution in and normal to membrane plane (Å)**	3.5/4.5	3.0/4.0
No. of observed/used amplitudes	29211/11073	51754/5154
Fourier space sampled, overall/3.35-3.16 Å (p6) (%)	58.3	78.3/64.3
I/s, overall/4.0-3.5 Å	6.0/2.5	12.1/6.0
RFriedel (%)	24.9	12.7
Rmerge (%)	34.7	28.8
Crystallographic refinement		
Resolution (Å)		10.0 –3.2
No. of reflections		4409
No. of atoms, protein/substrate		964/20
Rwork (%)		34.8
RFree (%)		36.9
Rort ***(%)		49.1
Overall B-factor		34.5
R.m.s. deviations		
Bond lengths (Å)		0.014
Bond angles (°)		1.866
Ramachandran plot distribution (%)		60.0/38.1/1.9/0.0

MPGES1
Data collection and refinement statistics

Two-dimensional crystal parameters

Two-sided plane-group	p22121
Cell dimensions	
a, b, c (Å)	93.2, 84.6, 100.0*
γ (°)	90.0

Amplitude determination from electron diffraction

No. of diffraction pattern	100
Maximum tilt angle (°)	62.0
Resolution in and normal to membrane plane (Å)†	3.5/4.2
No. of observed/used amplitudes	43035/6185
Fourier space sampled (%)	58.8
F/σ, overall/4.0-3.5 Å	4.51/3.51
$R_{Friedel}$ (%)	14.4
R_{merge} (%)	46.1

Crystallographic refinement

Resolution (Å)	10.0 – 3.5
No. of reflections	6185
No. of atoms, protein/substrate	3396/60
Rwork (%)	35.2
RFree (%)	39.4
R.m.s. deviations	
Bond lengths (Å)	0.012
Bond angles (□)	1.612
Ramachandran plot distribution (%)	79.6/17.5/2.7/0.3

- *Assumed for sampling along z*. For lattice line adaption a thickness of 65 Å was used.
- †From calculation of point spread function