

1st Annual ISS Research and Development Conference

Discussion Panel #1

ISS – Top Science and Technology Results

**Drug Therapy of Duchenne Muscular
Dystrophy with inhibitors of
Hematopoietic Prostaglandin D Synthase**

Yoshihiro Urade

Osaka Bioscience Institute

June 26, 2012

OCTOBER 2006

www.asbmb.org

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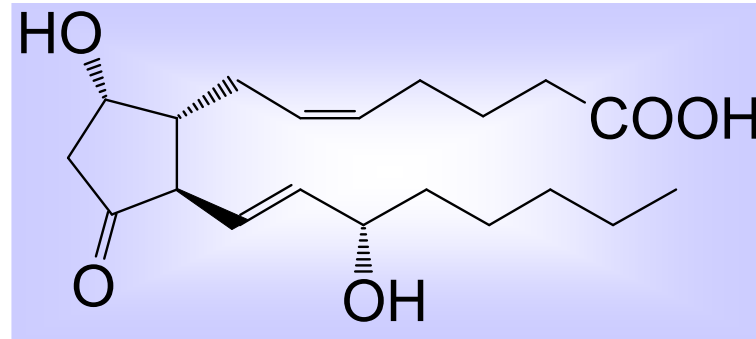
Osaka Bioscience Institute: A World Leader in Scientific Research

The Osaka Bioscience Institute (OBI) was established in 1987 as part of the centennial commemoration of the City of Osaka. OBI is a non-profit organization with support and cooperation

ing a strict advisory system. An advisory committee consisting of two foreign and three domestic scientists meets annually and is dedicated not only to evaluating each research project, but also the programs of the

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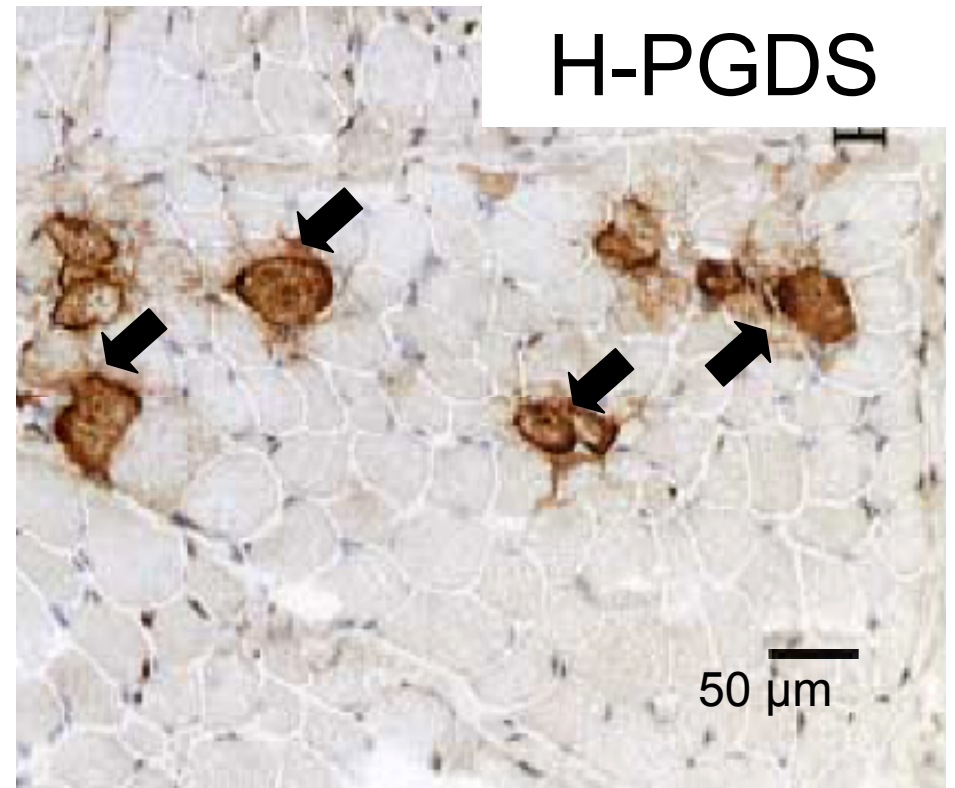
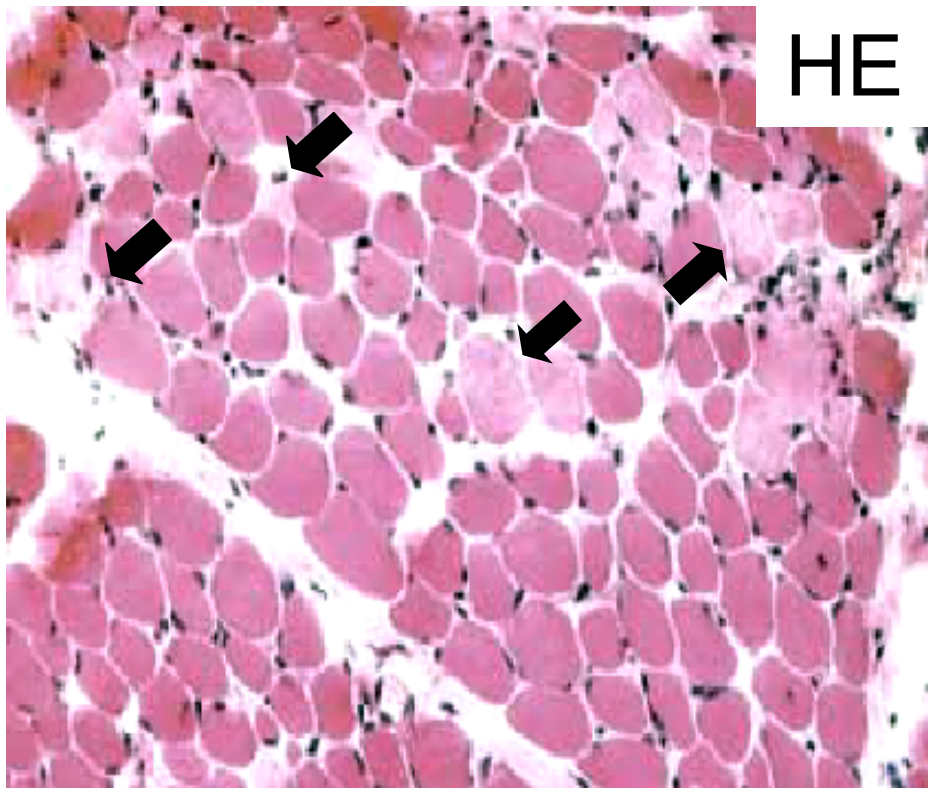
Prostaglandin (PG) D₂ and Inflammation



- ◆ PGD₂ is a major prostanoid produced in mast cells and a variety of inflammatory cells.
- ◆ Hematopoietic PGD synthase (HPGDS) is induced in activated microglial cells and involved in neuroinflammation.
- ◆ HPGDS is induced in the necrotic muscle fibers of patients with Duchenne muscular dystrophy.

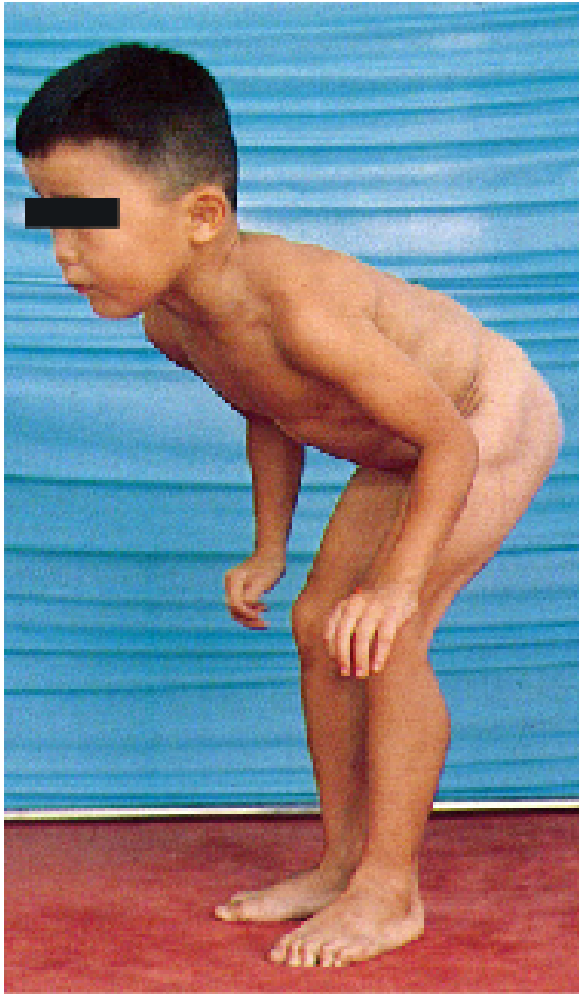
(Okinaga et al., Acta Neuropathol 2002)

Expression of hematopoietic PGD synthase (HPGDS) in DMD patients



Okinaga et al., *Acta Neuropathol*, 104:377 (2002)

Duchenne muscular dystrophy (DMD)



- The most common form of muscular dystrophy that occurs in 1 out of 3,500 boys.
- DMD is caused by mutations of the dystrophin gene leading to low or no production of the cytoskeletal protein “dystrophin”.

Courtesy of Dr. Ikuya Nonaka, National Center of Neurology and Psychiatry

X-ray crystallographic structures of HPGDS

Resolution

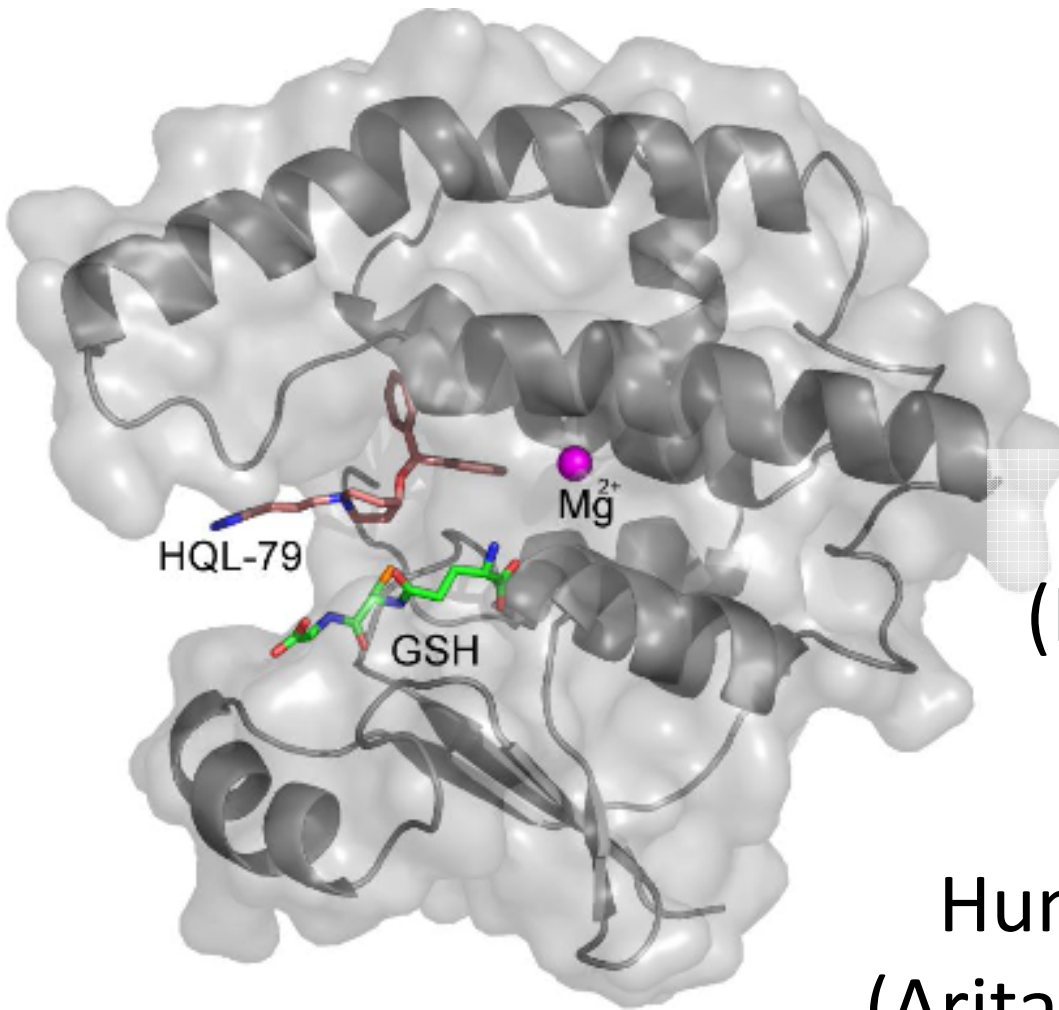
Rat HPGDS 2.3 Å
(Kanaoka et al., *Cell* 1997)



Human HPGDS 1.7 Å
(Inoue et al., *Nat Struct Biol.*
2003)



Human HPGDS/HQL-79 1.45 Å
(Aritake et al., *J Biol Chem.* 2006)



Protein crystallization under microgravity condition on the ISS

Space vessel
"Progress"

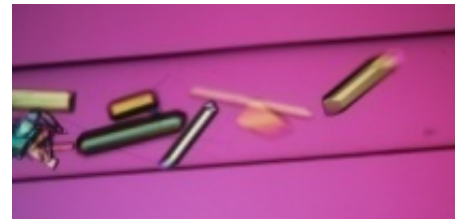


Japan Experimental Module
for 2.5 months

SPring-8



X-ray diffraction data



Crystals

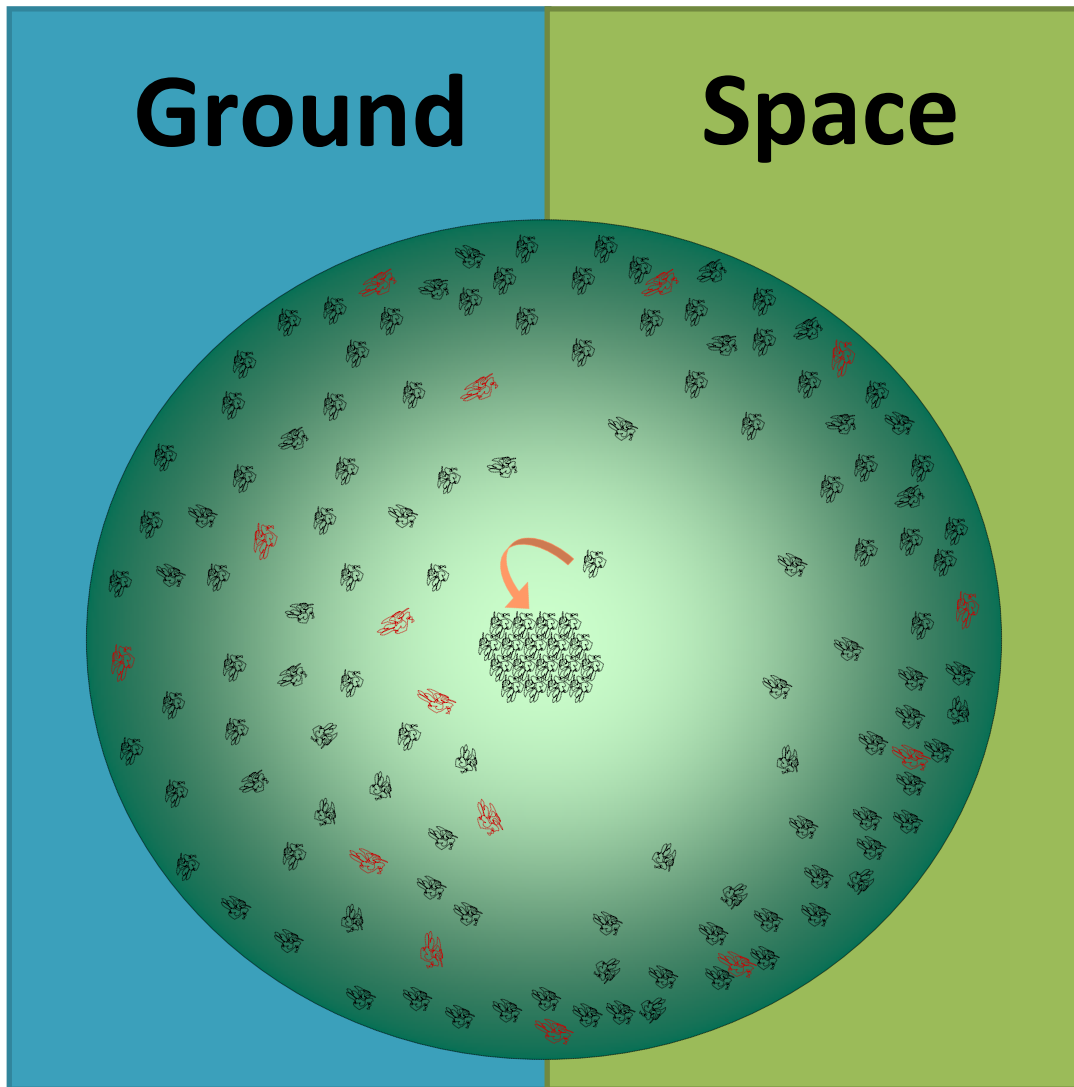


Protein Crystallization



Baikonur,
Kazakhstan

Effects of microgravity on crystal growth



Suppression of density-driven convective flow

Depletion zones of protein and impurity

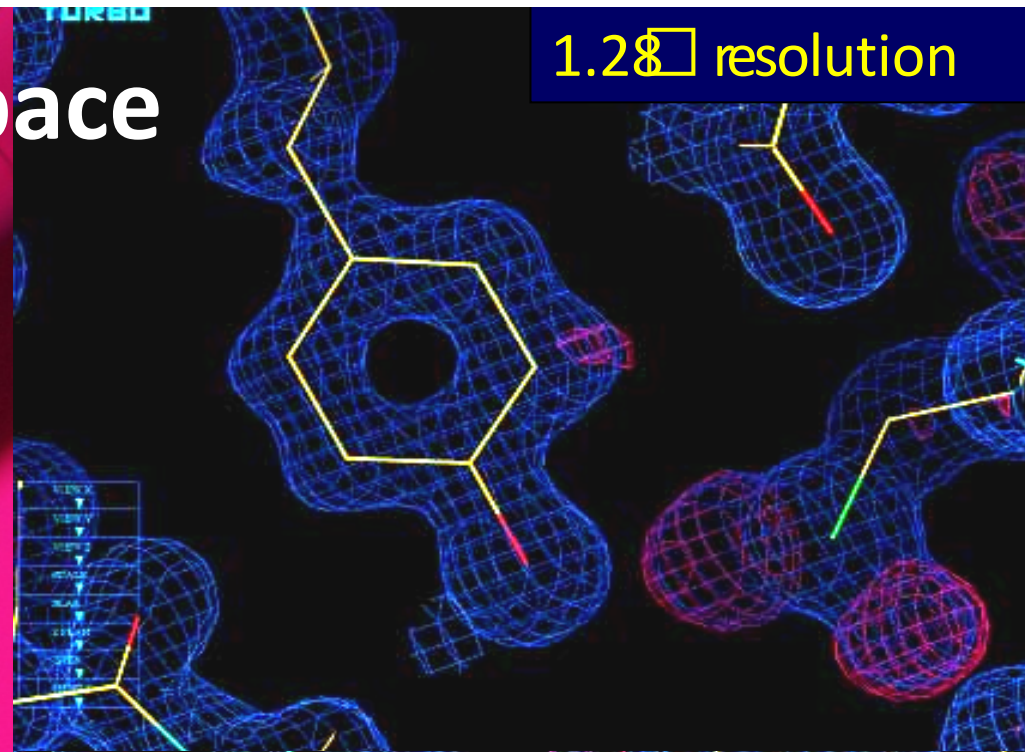
Lower super-saturation growth

Suppression of cluster formation

High-quality protein crystal growth

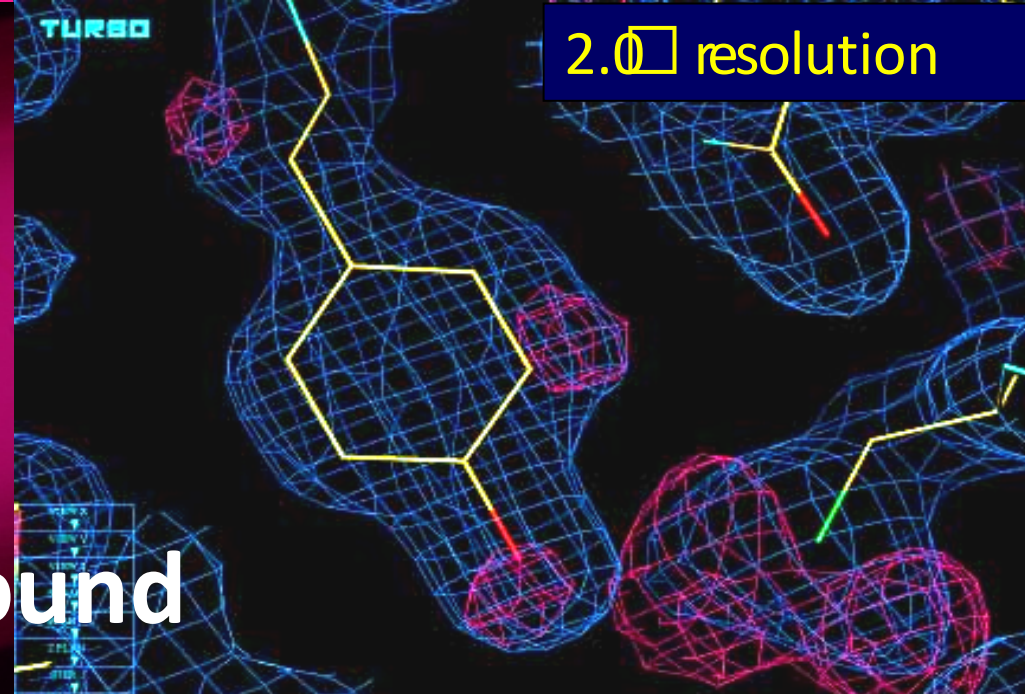
Space

1.28 Å resolution



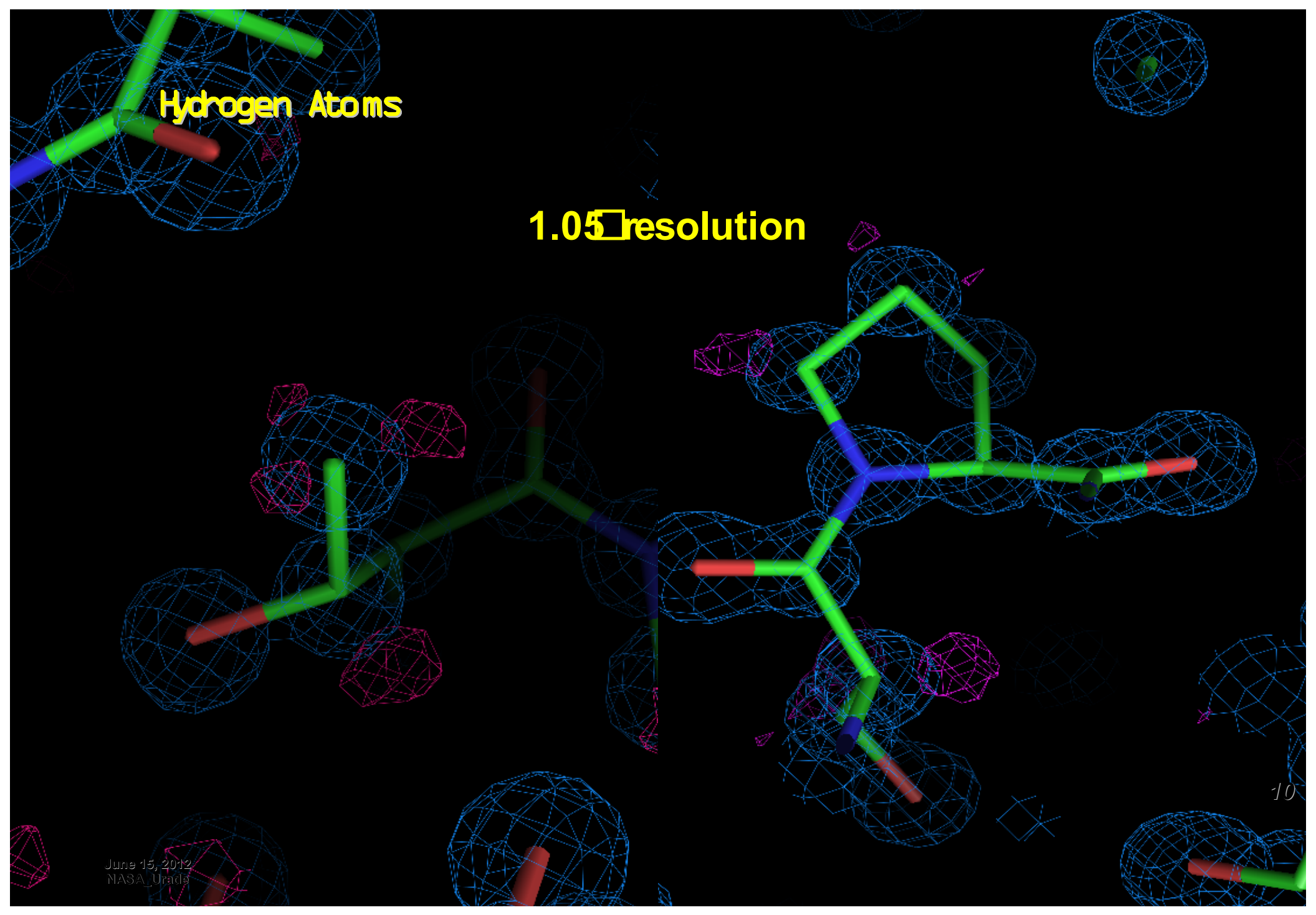
Ground

2.0 Å resolution



Hydrogen Atoms

1.05 Å resolution



Potentialiation of HPGDS inhibitor

		HQL-79	TC-1
Purified enzyme(IC_{50})		6 μ M	0.04 μ M
PGD ₂ inhibition	Cells(IC_{50})	100 μ M	0.03 μ M
	Rat (ED_{50})	100 mg/kg	1 mg/kg
Acute toxicity Chronic toxicity Genetic toxicity Reproductive toxicity		Not tested	Negative
Administration		Oral	Oral

Treatment of DMD dog with HPGDS inhibitor

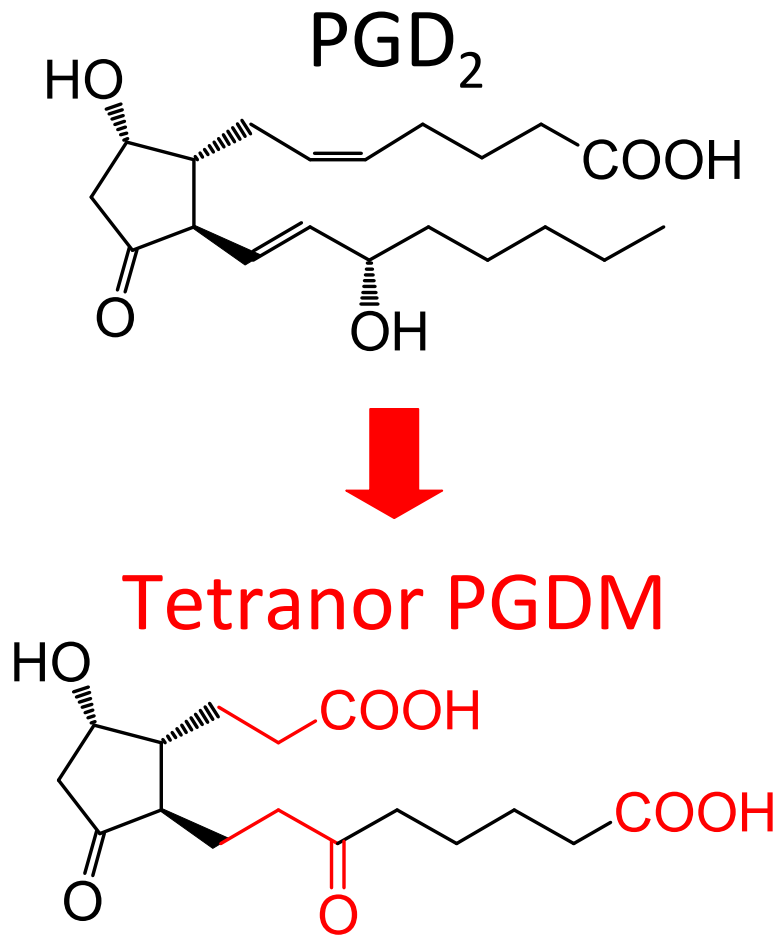


During therapy
(4 months)

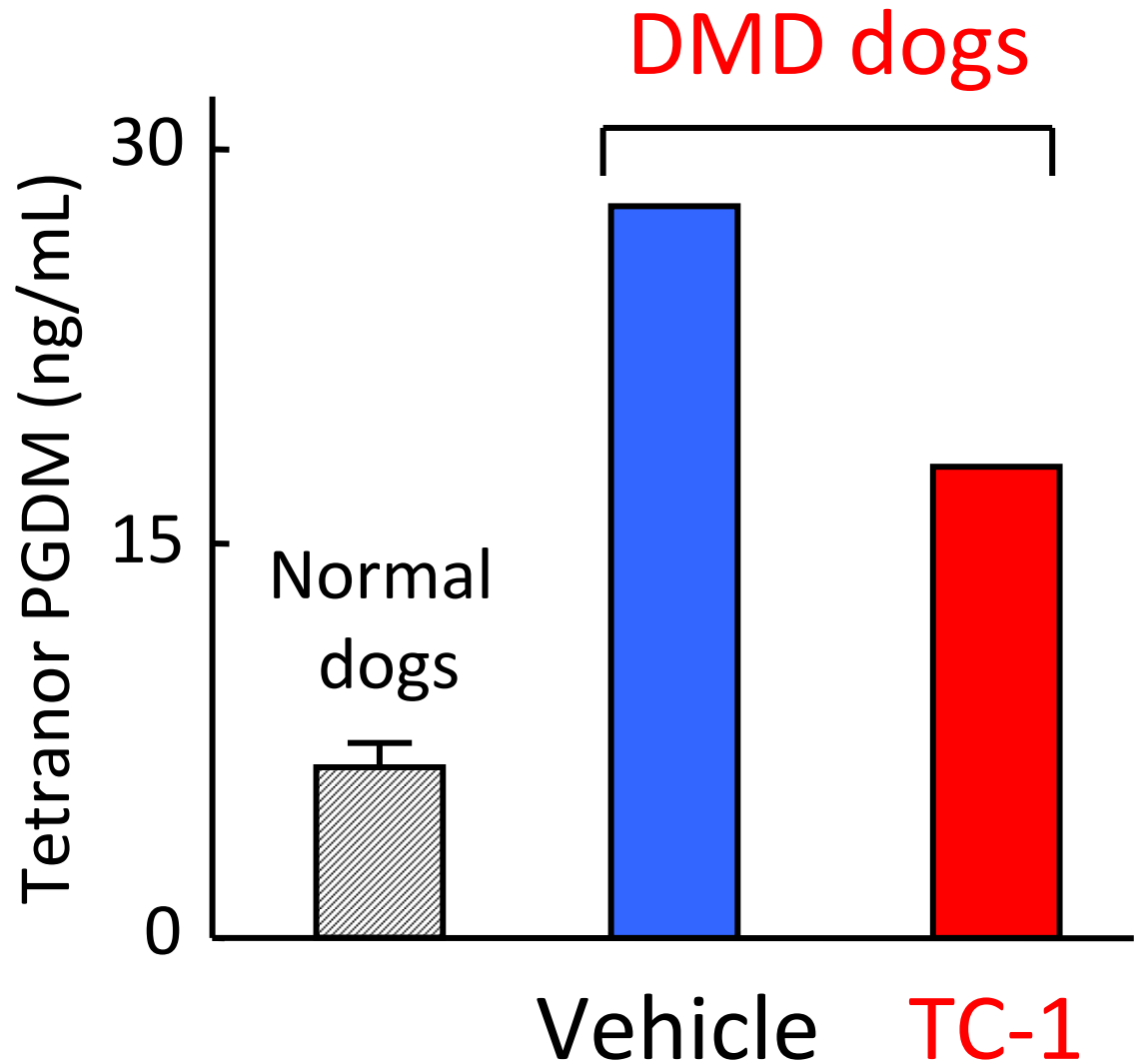
Post therapy
(2 months)



Reduction of PGD₂ metabolite in DMD dog



(Song WL, *et al.*, JBC 2008)

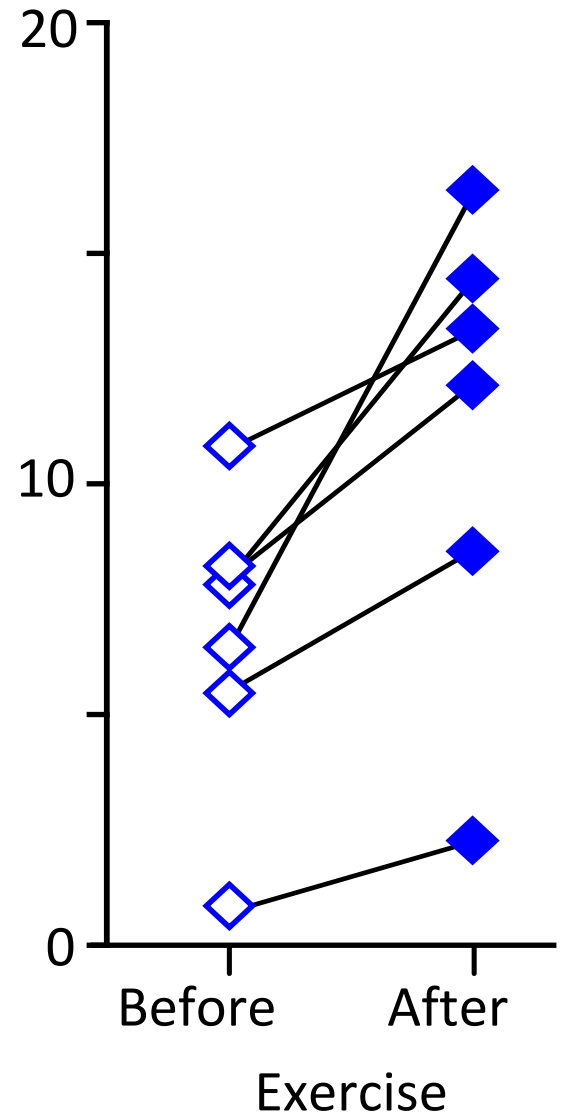
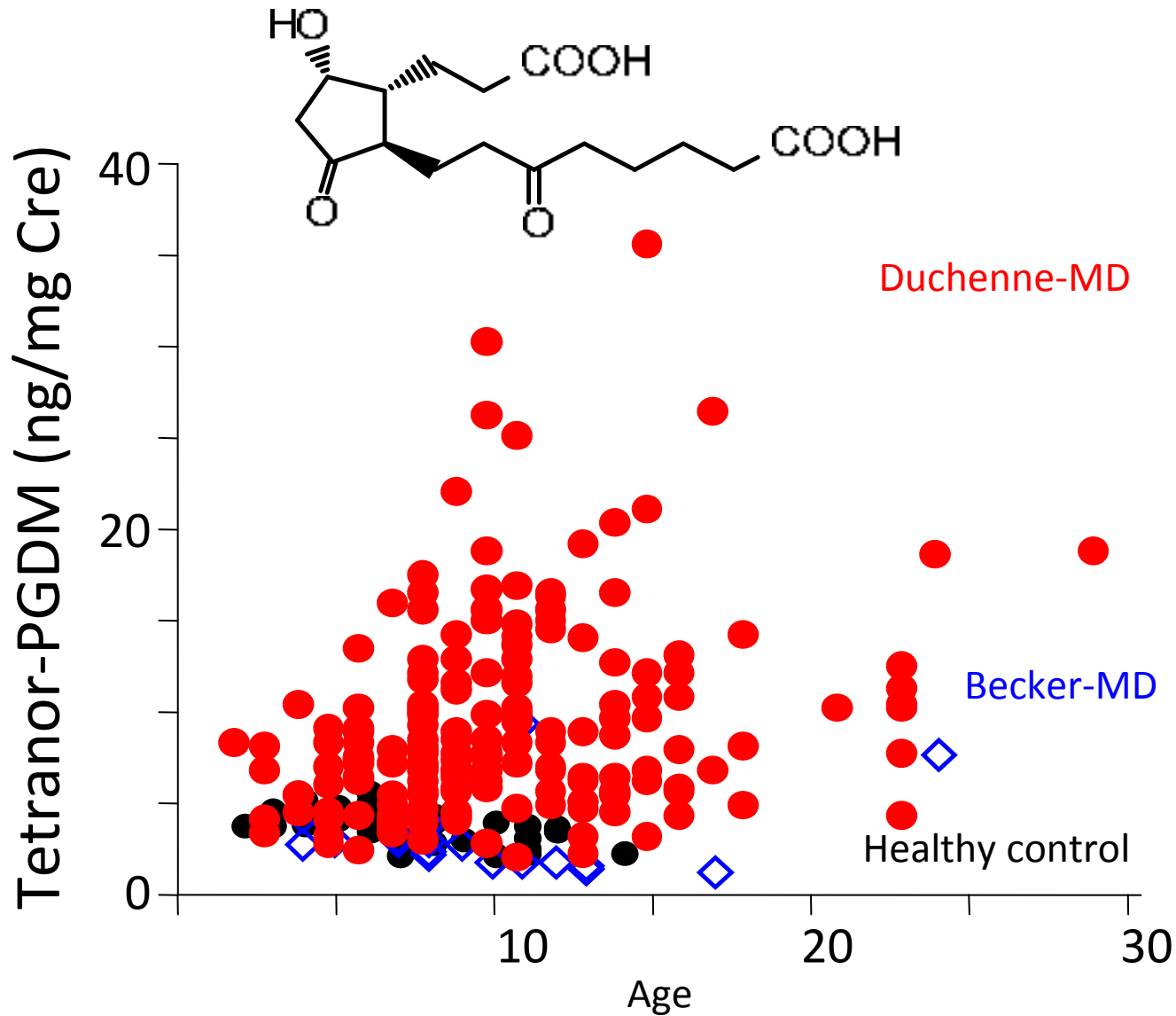


Development of HPGDS inhibitor

- ✓ Acute toxicity
- ✓ Chronic toxicity
- ✓ Synthesis of GLP grade

Phase I study

Tetranor-PGDM in muscular dystrophy patients



Summary

HPGDS inhibitors reduced muscular necrosis of DMD beagle.

HPGDS inhibitors are good candidates for drug therapy of DMD.

What's Next?

- Molecular mechanism of HPGDS induction in DMD muscle
- Clinical trial of HPGDS inhibitors
- New HPGDS inhibitors of different structures
- Other application of HPGDS inhibitors

Members of the 2nd Department



Acknowledgement

 Japan Aerospace Exploration Agency

 NASA

 Russian Federal Space Agency

 European Space Agency

 University of Granada

 SPring-8 (JASRI, RIKEN)

 Confocal Science Inc.

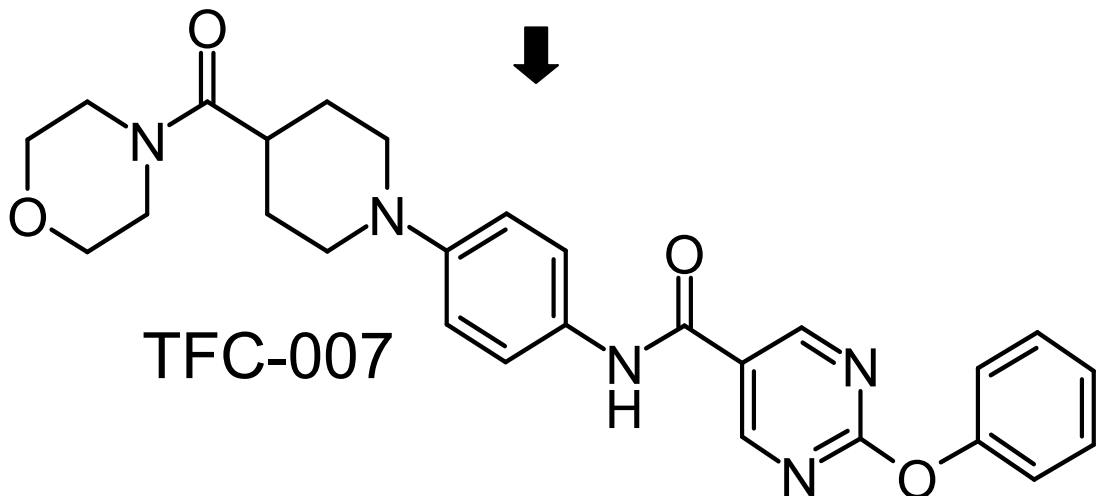
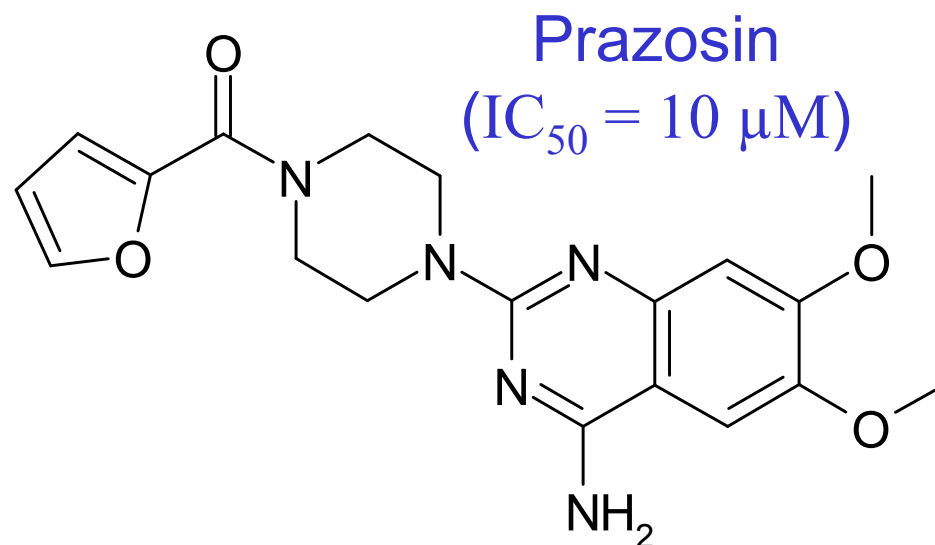
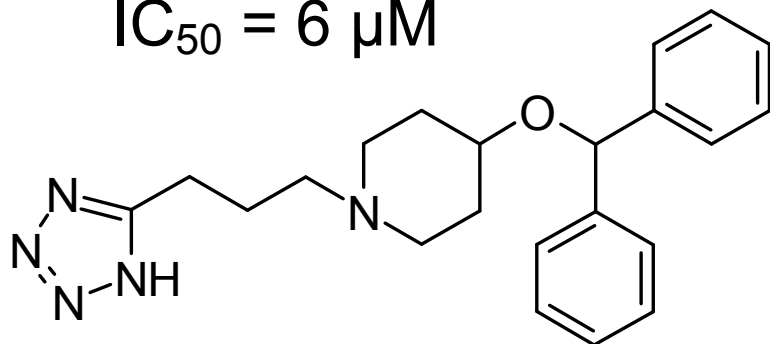
 Maruwa Foods Bioscience Inc.

 TAIHO Pharama Inc.

To be continued

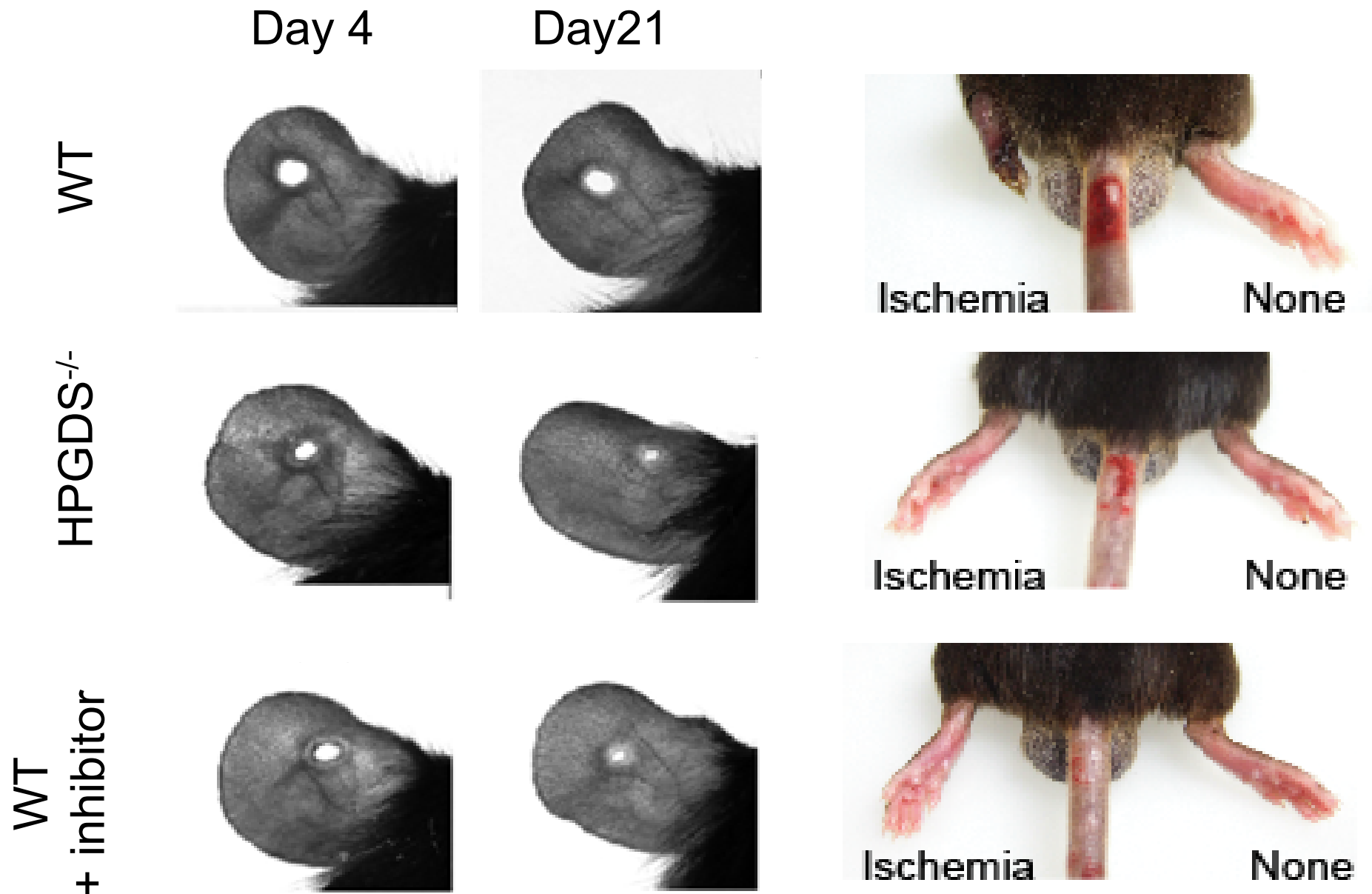
Development of HPGDS inhibitors

HQL-79
(early lead compound)
 $IC_{50} = 6 \mu\text{M}$



TC-2
 $IC_{50} = 0.04 \mu\text{M}$

Rapid recovery from injury by HPGDS inhibitor





This certificate of appreciation is presented to

Doshihiro Urade

In recognition of your efforts contributing to the success of the
**Protein Crystallization Research in Space
Experiment on STS-84**

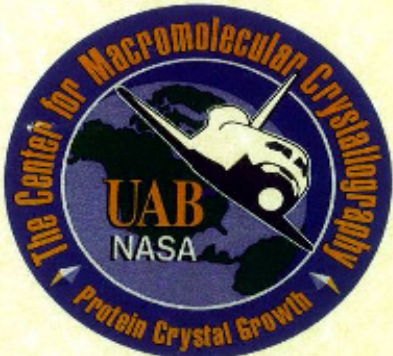
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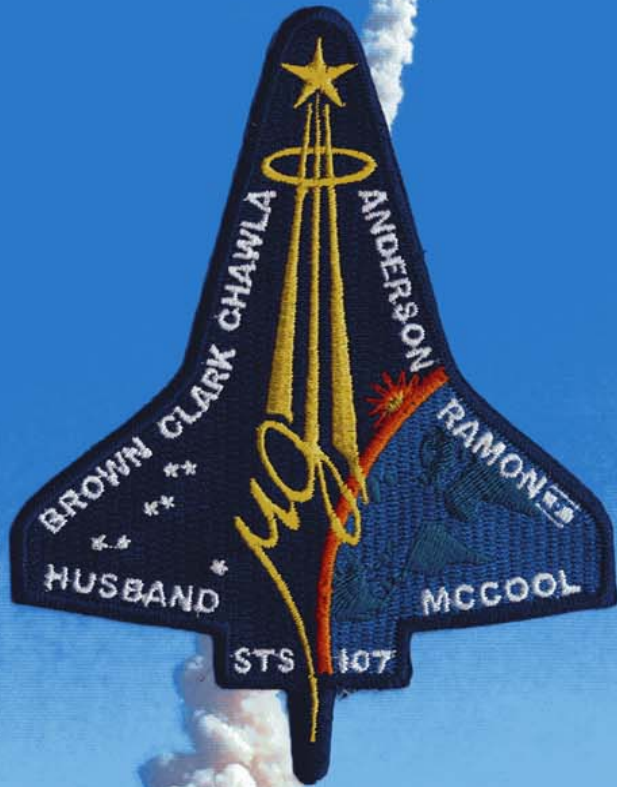
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SPACEHAB and Boeing
proudly present this certificate to

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in recognition of your significant contributions
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BOEING Program Manager

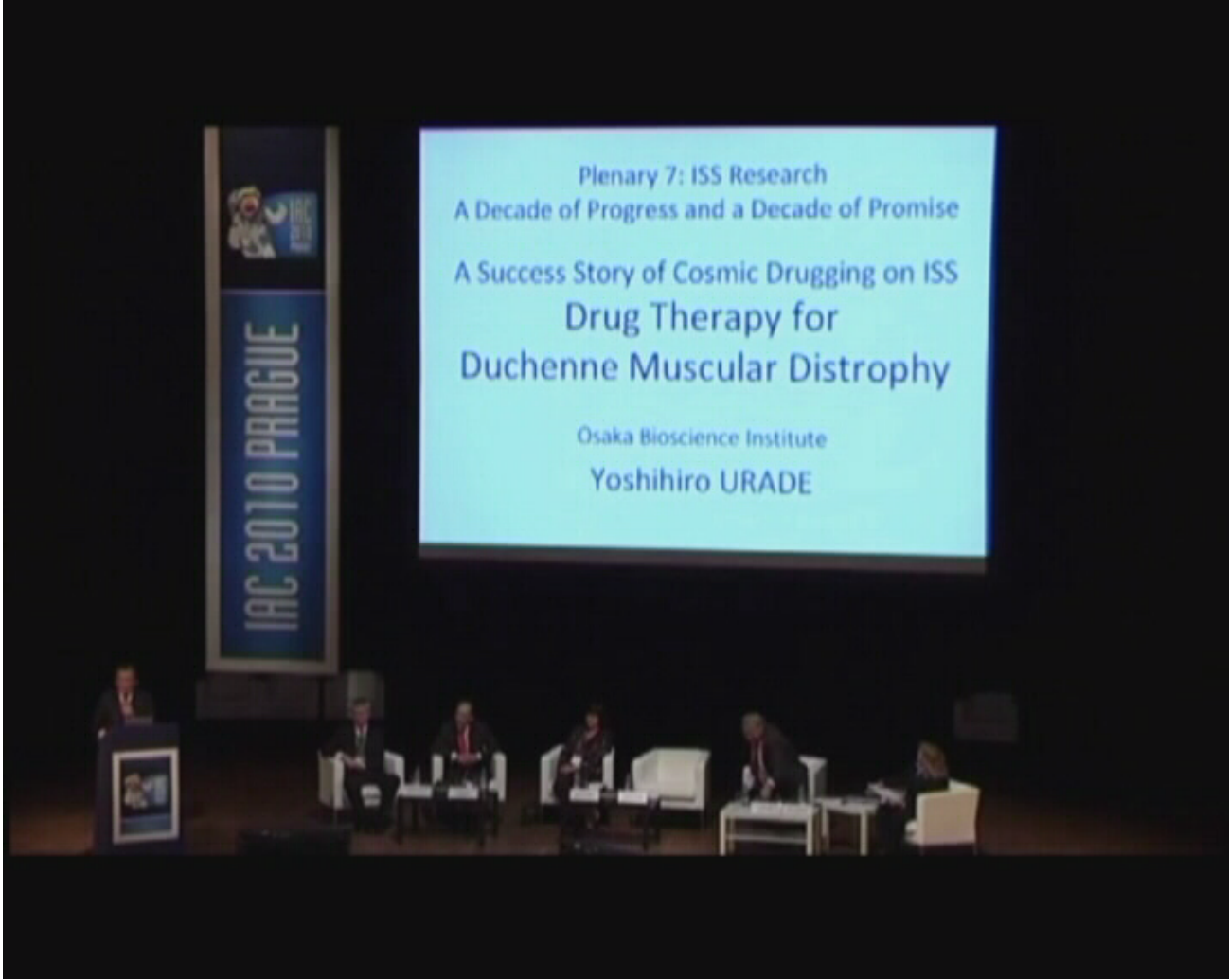


61st International Astronautical Congress

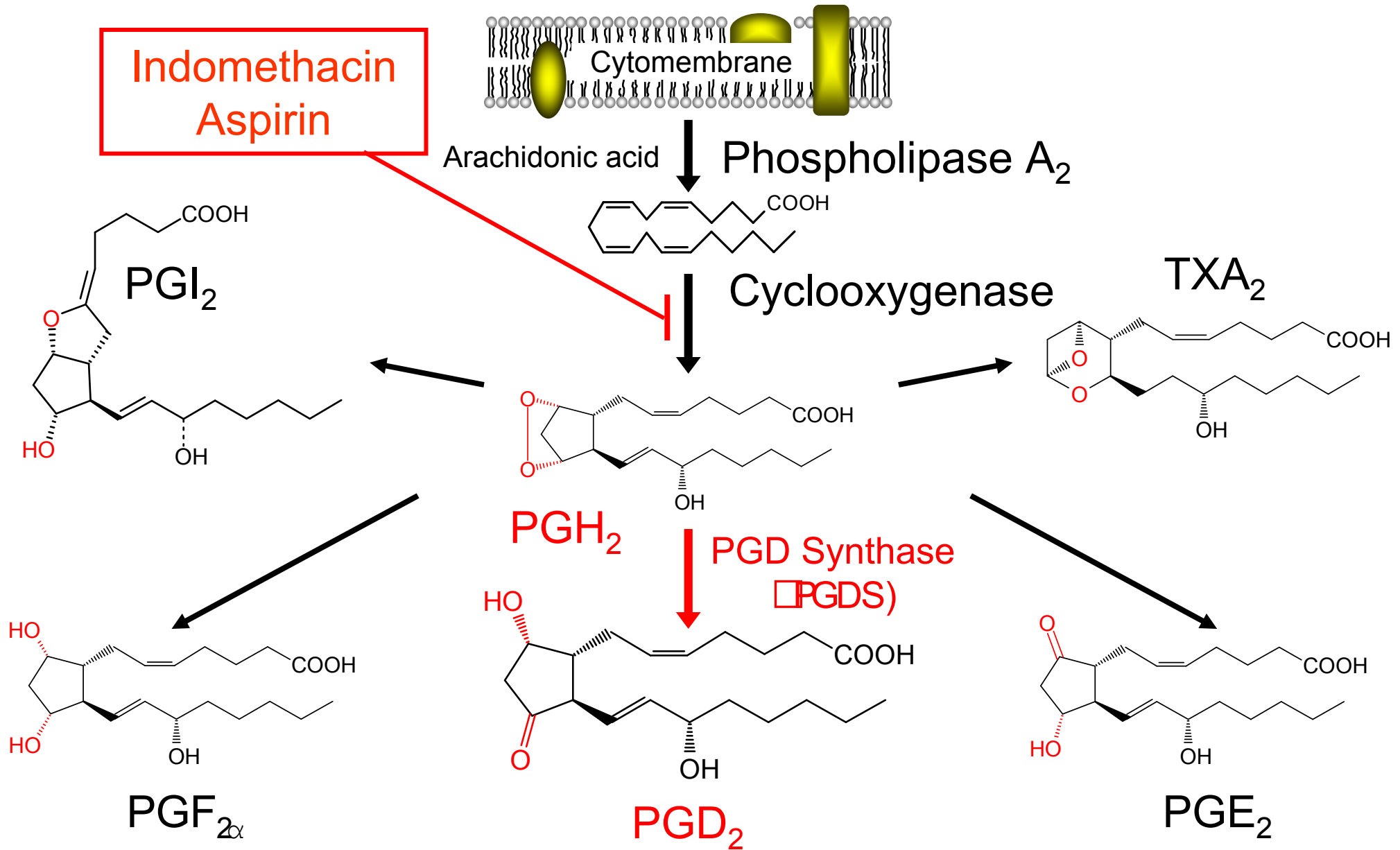
Prague, Czech Republic
27 September - 1 October 2010

"Space for Human benefit and exploration"

Plenary 7: **ISS Research – A Decade of Progress and a Decade of Promise**



Biosynthesis of prostaglandins (PGs)



Potentiation of H-PGDS inhibitors

		HQL-79 (2006)	TC-1 (2007)	TC-2 (2009)
H-PGDS (IC_{50})		6 μ M	0.04 μ M	0.023 μ M
PGD ₂ inhibition	Cells (IC_{50})	100 μ M	0.03 μ M	0.043 μ M
	Rat (ED_{50})	100 mg/kg	1 mg/kg	10 mg/kg
Toxicity tests		Not tested	Negative	Negative
Absorption		Not tested	good	excellent

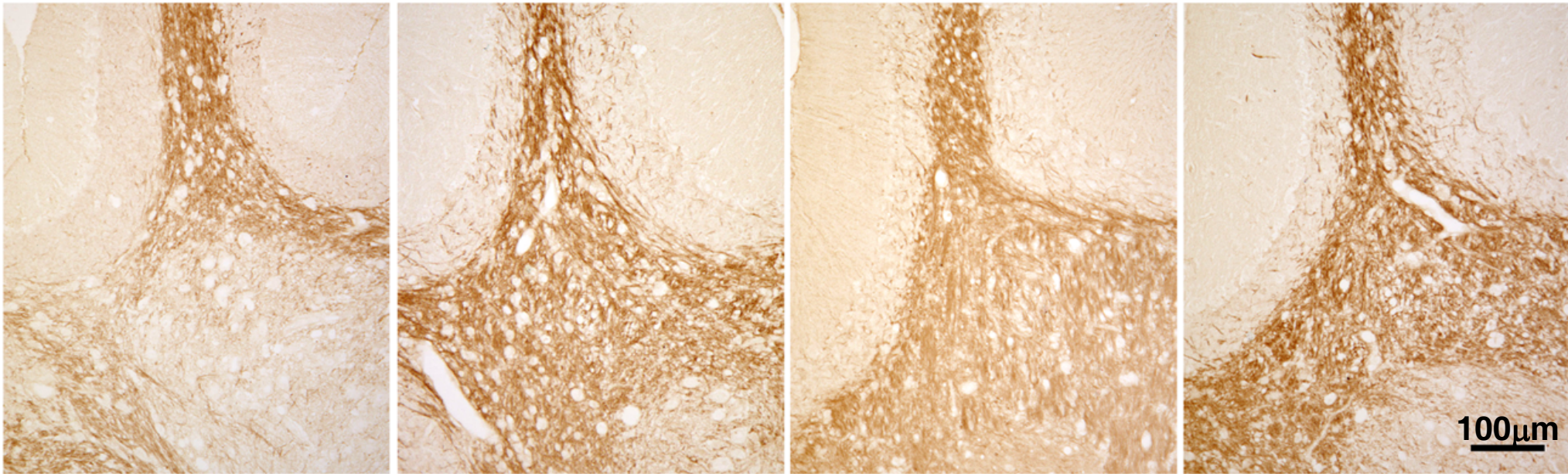
twi tcher HPGD

twi/twi

HPGDS^{-/-}
twi/twi

DPR^{-/-}
twi/twi

HQL-79-
treated
twi/twi



(Mohri et al., J Neurosci 2006)

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新ける舞台だ。
裏山 良博

第2章 「村間」を見つけた堀江貴文。
堀江 貴文

第3章 重力とケンカして、
上を目指そう！
柿松 悠

第4章 人間は、
たくましい生きものなんだ。
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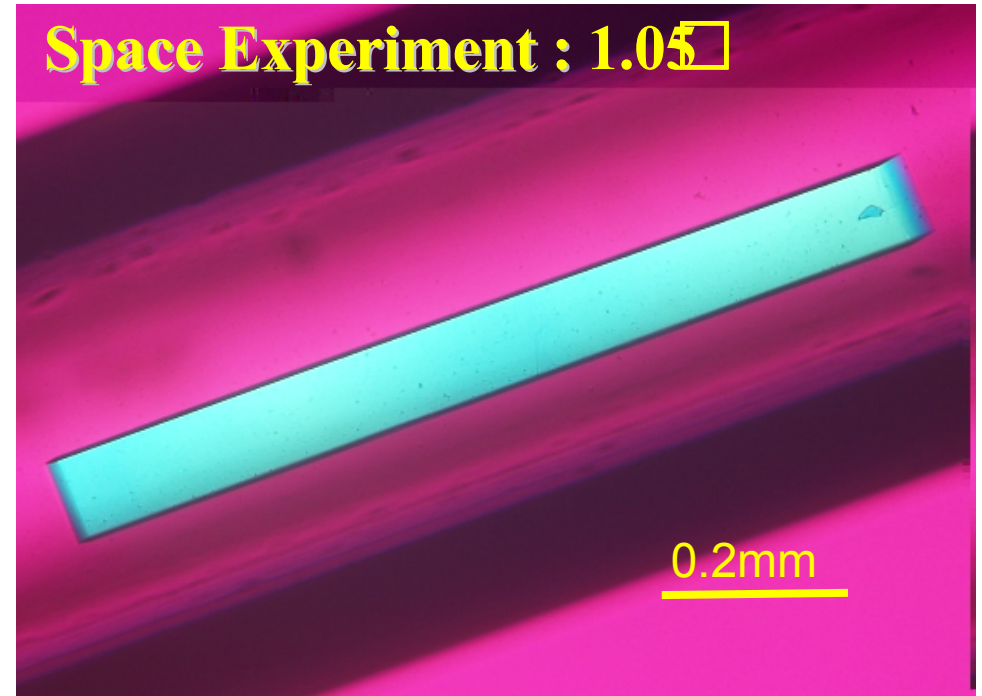
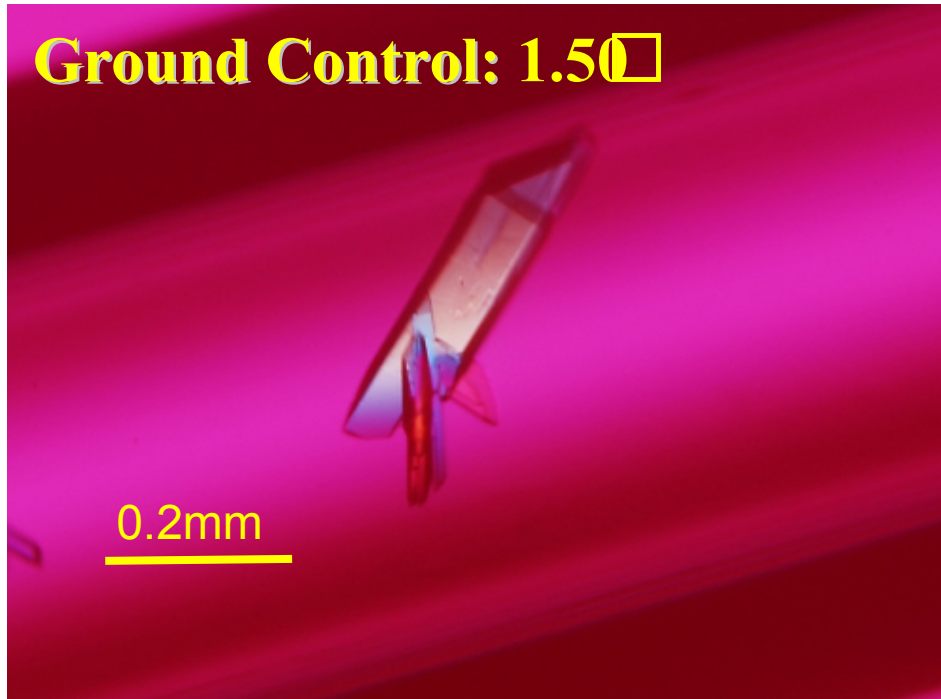
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High Quality Protein Crystallization Experiment : JAXA PCG in 'Kibo'



Protein solution

3mg/ml Protein, 12%(w/v) PEG6000
2mM GSH, 1mM MgCl₂, 50mM Tris-HCl pH8.5

Reservoir Solution

35%(w/v) PEG6000
2mM GSH, 2mM MgCl₂, 50mM Tris-HCl pH8.5

J. Synchrotron Radiation (2010), 18, 88-91.

31

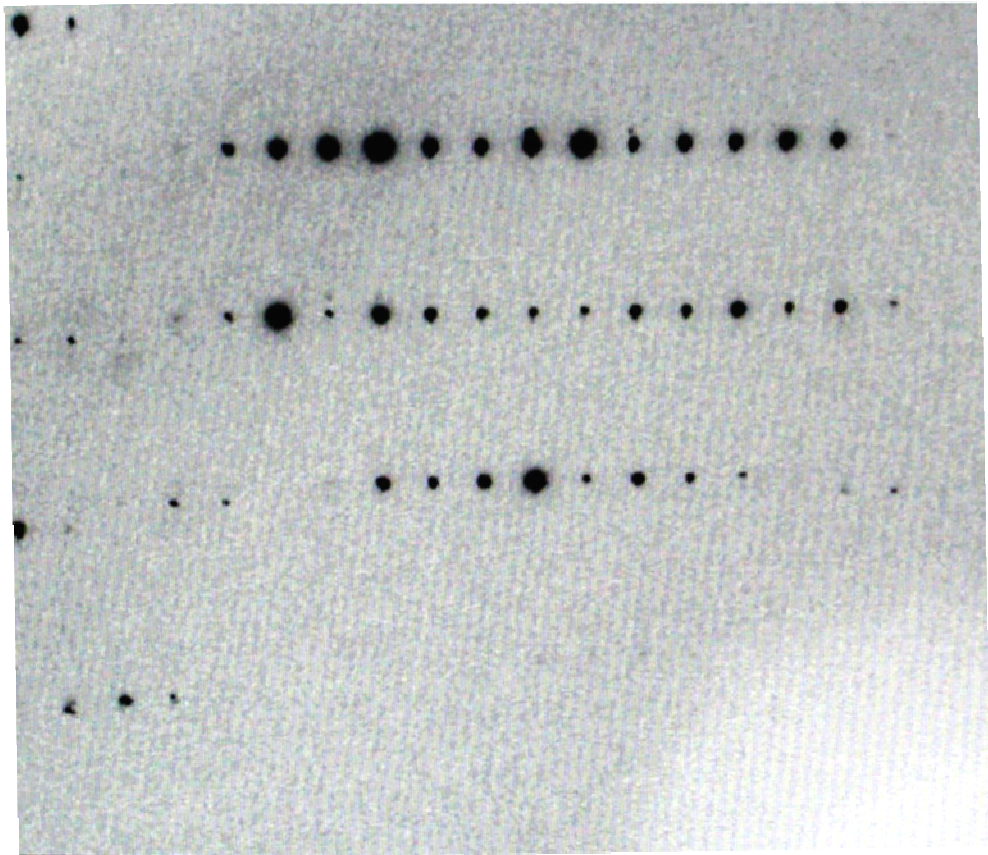
Improvement of Mosaicity



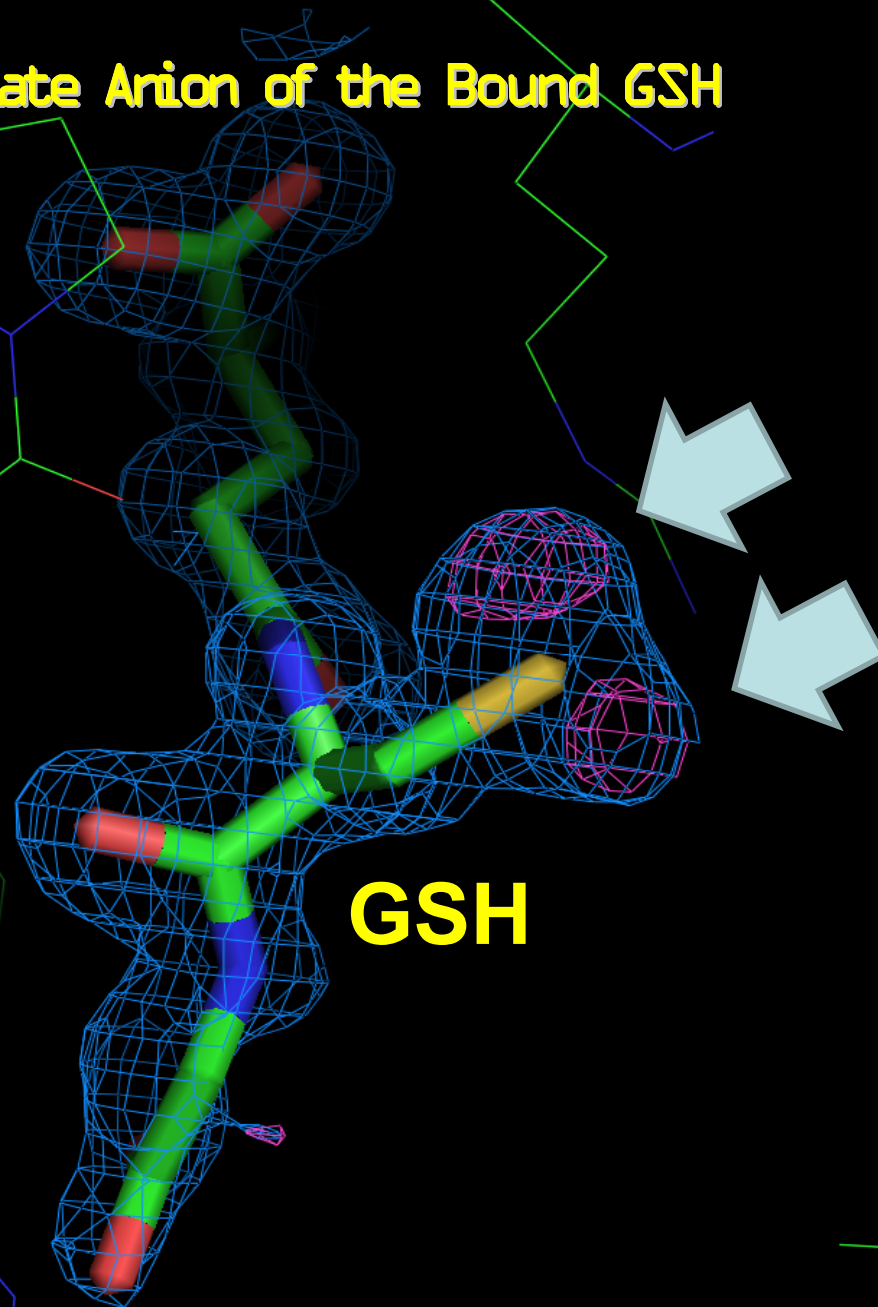
Ground Control: 0.53



Space Experiment: 0.21



Electron Density of Thiolate Anion of the Bound GSH



GSH

Electron Density of Thiolate Anion of the Bound GSH

