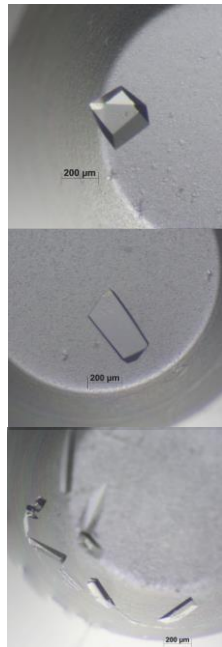




วิเคราะห์ปฏิสัมพันธ์ของโปรตีน-สารยับยั้ง และโปรตีน-โปรตีน ในสภาวะแรงโน้มถ่วงต่ำ สำหรับการพัฒนายาต้านมาลาเรีย

จารุณี วานิชชนันกุล, ดนุ วิทย์สุภากร, ธนญา แซ่อย่าง,
ชัยรัตน์ อุทัยพิบูลย์, สุมาลี กำจรวงศ์ไพศาล

ทีมวิจัยวิศวกรรมโปรตีน-ลิแกนด์และชีววิทยาโมเลกุล (IPMT)
กลุ่มวิจัยเทคโนโลยีชีวภาพระดับโมเลกุลทางการแพทย์ (IMBG)
BIOTEC, NSTDA



20 February 2019



MATCHON ONLINE

จิสต้า จับมือ ไบโอเทค

เตรียมส่งโปรตีนไปทดลองบนสถานีอวกาศ

พัฒนายาต้านมาลาเรีย

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เมื่อวันที่ 20 กุมภาพันธ์ จิสต้า ร่วมกับ ไบโอเทค ส่งมอบโปรตีนให้กับ**องค์การสำรวจอวกาศญี่ปุ่น หรือ JAXA** ณ เมืองสึกุบะ ประเทศญี่ปุ่น เพื่อตรวจสอบคุณภาพของสาร และเตรียมส่งขึ้นไปทดลองบนสถานีอวกาศนานาชาติ หรือ International Space Station : ISS ภายใต้**โครงการวิจัย วิทยาศาสตร์อวกาศและการทดลองในอวกาศ National Space Exploration : NSE** ของจิสต้า เพื่อให้ได้ผลึกโปรตีนที่มีความสมบูรณ์มากกว่าการทดลองบนพื้นโลกสู่การพัฒนาต้านมาลาเรียที่มีประสิทธิภาพมากขึ้น

GISTDA

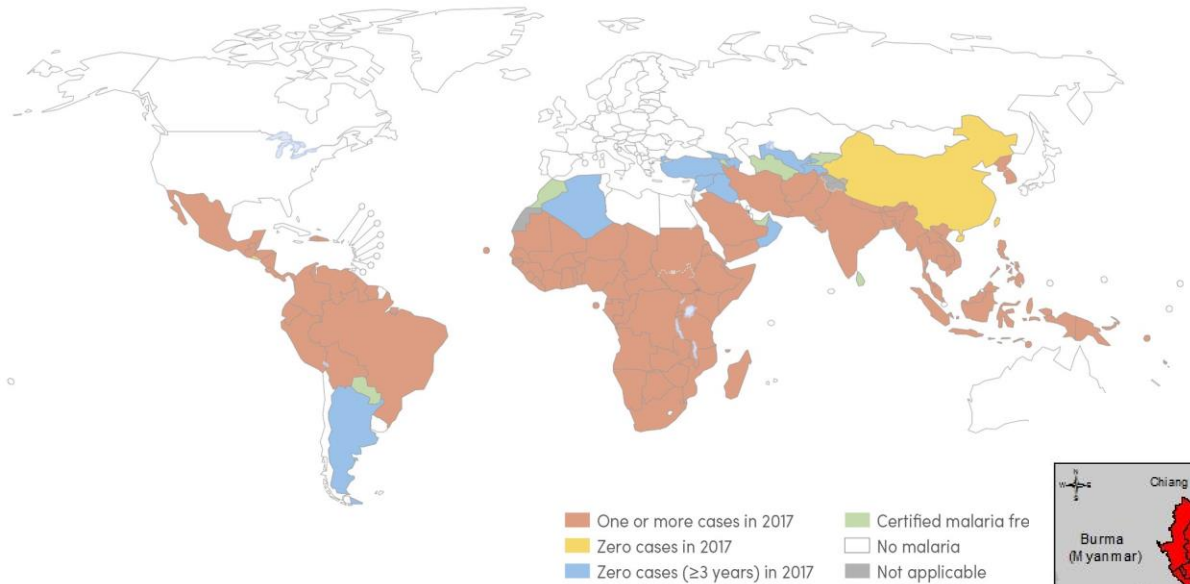
JAXA
Explore to Realize

สวทช.
NSTDA

World Malaria Report 2018

Countries with indigenous cases in 2000 and their status by 2017. Countries with zero indigenous cases over at least the past 3 consecutive years are considered to be malaria free. All countries in the WHO European Region reported zero indigenous cases in 2016 and again in 2017. In 2017, both China and El Salvador reported zero indigenous cases. Source: WHO database.

219 million cases with 435,000 death worldwide in year 2017



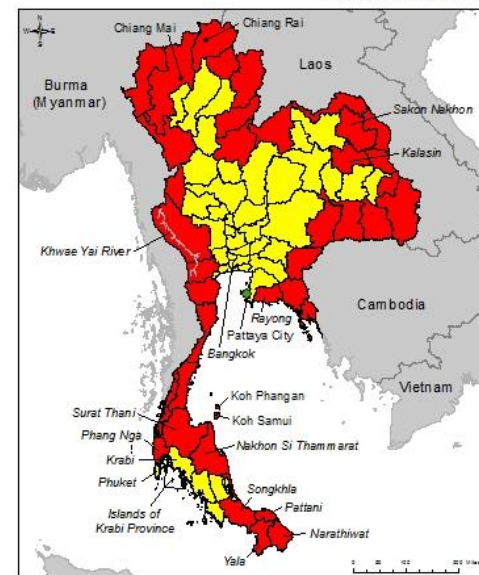
WHO: World Health Organization.

Plasmodium falciparum is the most deadly species of 5 human malaria parasites

- *P. falciparum* 50% (up to 75% some areas)
- *P. vivax* 50% (up to 60% some areas)
- *P. ovale*, *P. malariae* and *P. knowlesi* rare

<https://www.who.int/gho/malaria/en/>

Thailand Areas of Malaria Transmission

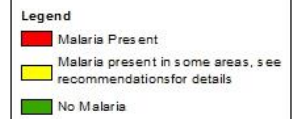


Malaria is present primarily in provinces that border Burma (Myanmar), Cambodia, and Laos and the provinces of Kalasin, Krabi (Plai Phraya district), Nakhon Si Thammarat, Narathiwat, Pattani, Phang Nga (including Phang Nga City), Rayong, Sakon Nakhon, Songkhla, Surat Thani, and Yala, especially the forest and forest fringe areas of these provinces. Rare to few cases in other parts of Thailand, including other parts of Krabi Province and the cities of Bangkok, Chiang Mai, Chiang Rai, Koh Phangan, Koh Samui, and Phuket. None in the islands of Krabi Province (Koh Phi Phi, Koh Yao Noi, Koh Yao Yai, and Koh Lanta) and Pattaya City.

Recommended chemoprophylaxis:

Provinces that border Burma (Myanmar), Cambodia, and Laos, the provinces of Kalasin, Plai Phraya district of Krabi, Nakhon Si Thammarat, Narathiwat, Pattani, Phang Nga (including Phang Nga City), Rayong, Sakon Nakhon, Songkhla, Surat Thani, and Yala: Atovaquone-proguanil or doxycycline.

All other areas of Thailand with malaria including the cities of Bangkok, Chiang Mai, Chiang Rai, Koh Phangan, Koh Samui, and Phuket: Mosquito avoidance only.



Antimalarials

Malaria

A preventable and treatable mosquito-borne illness

Main drugs

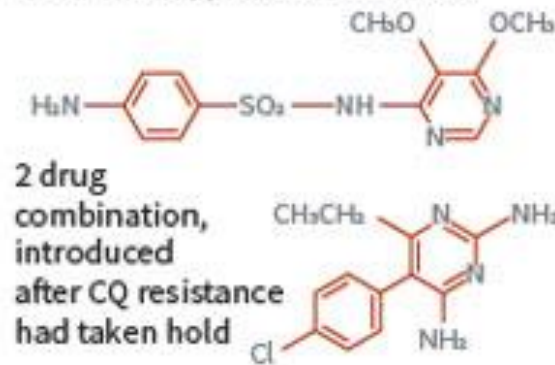
Chloroquine (CQ)



Resistance

Started independently in Colombia and Thailand

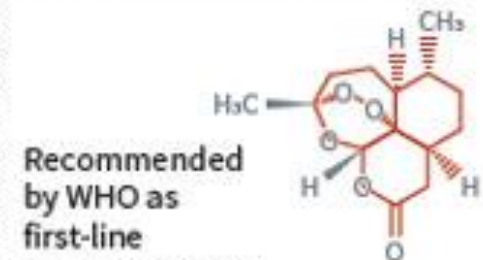
Sulfadoxine-pyrimethamine (SP)



Resistance

Evolved rapidly and now occurs in high frequency in major malaria regions

Artemisinin combination (ACT)

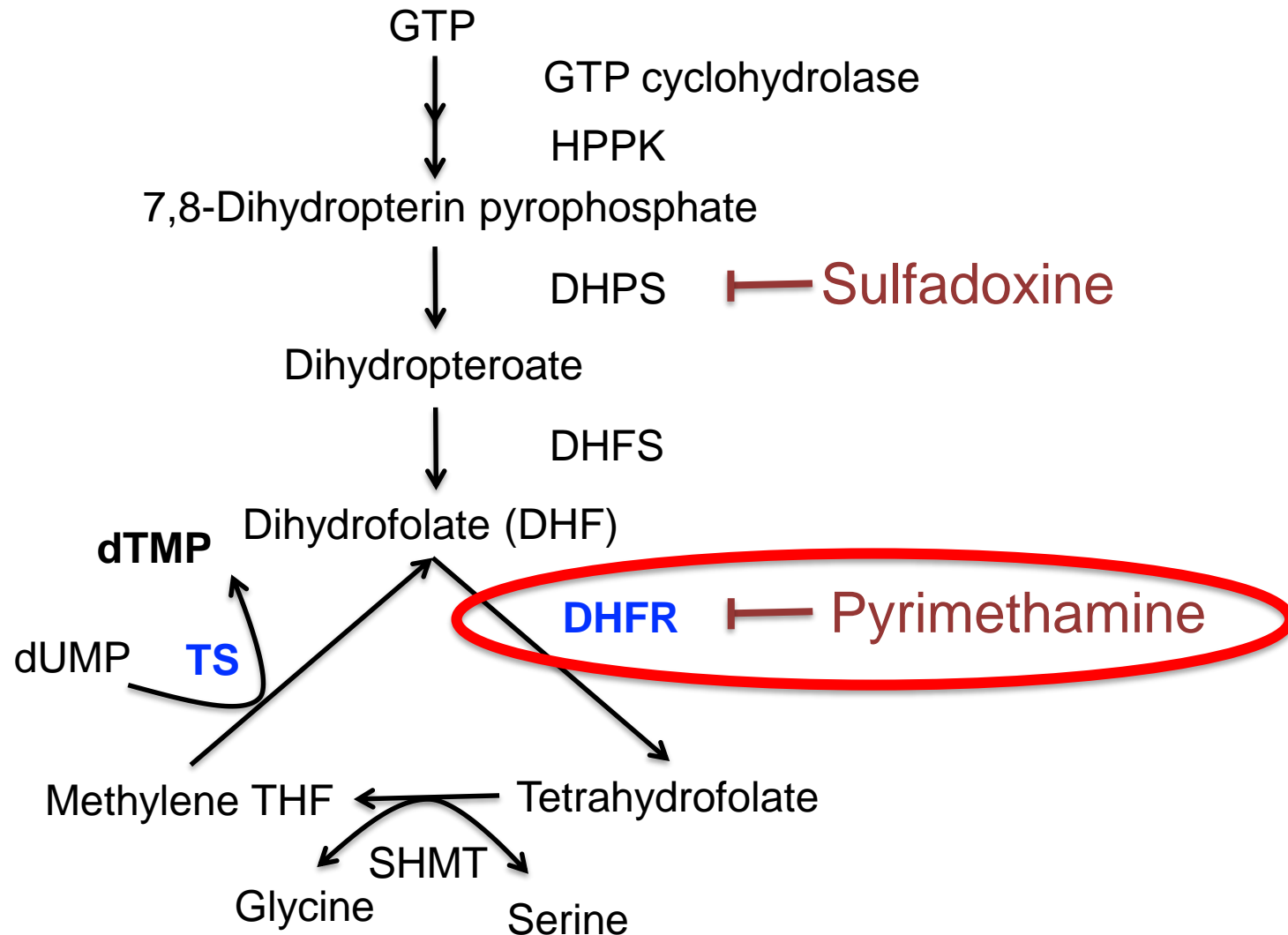


Recommended by WHO as first-line treatment since 2001

Resistance

Now established in parts of Cambodia, Myanmar, Thailand and Vietnam

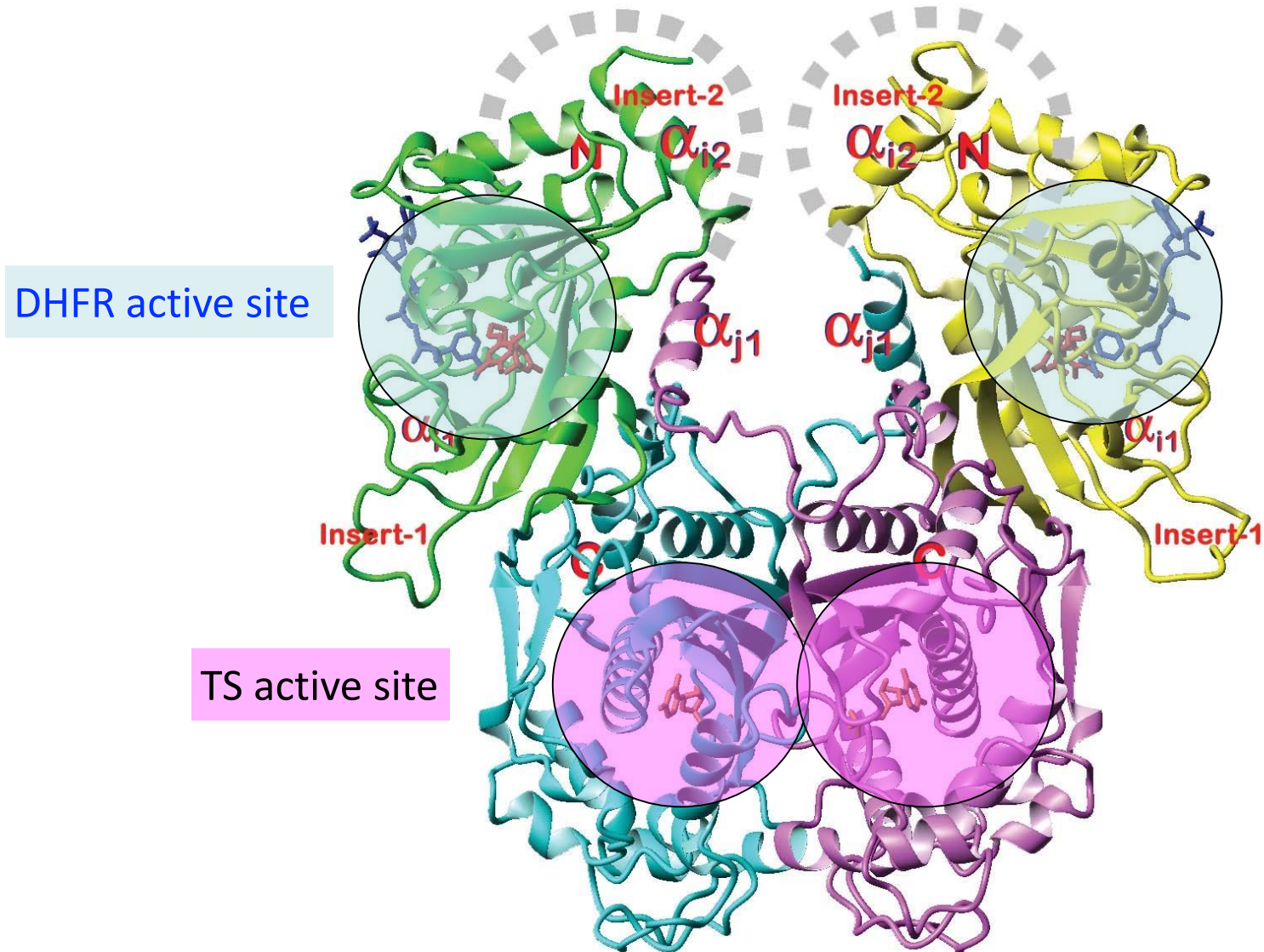
De novo pyrimidine synthesis in malaria



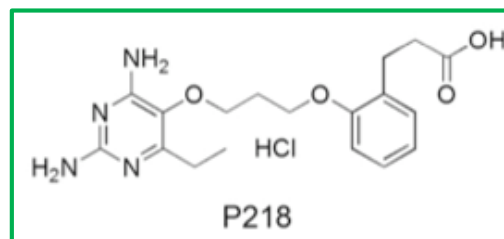
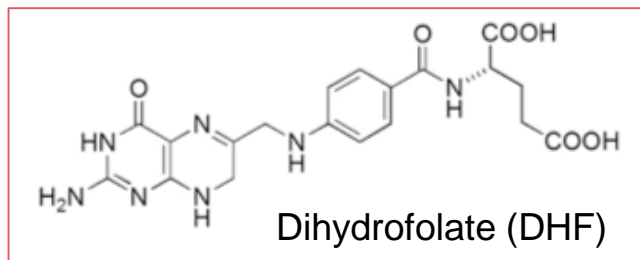
**Dihydrofolate Reductase-
Thymidylate Synthase (DHFR-TS)**

Mutations of DHFR cause drug resistance!!!

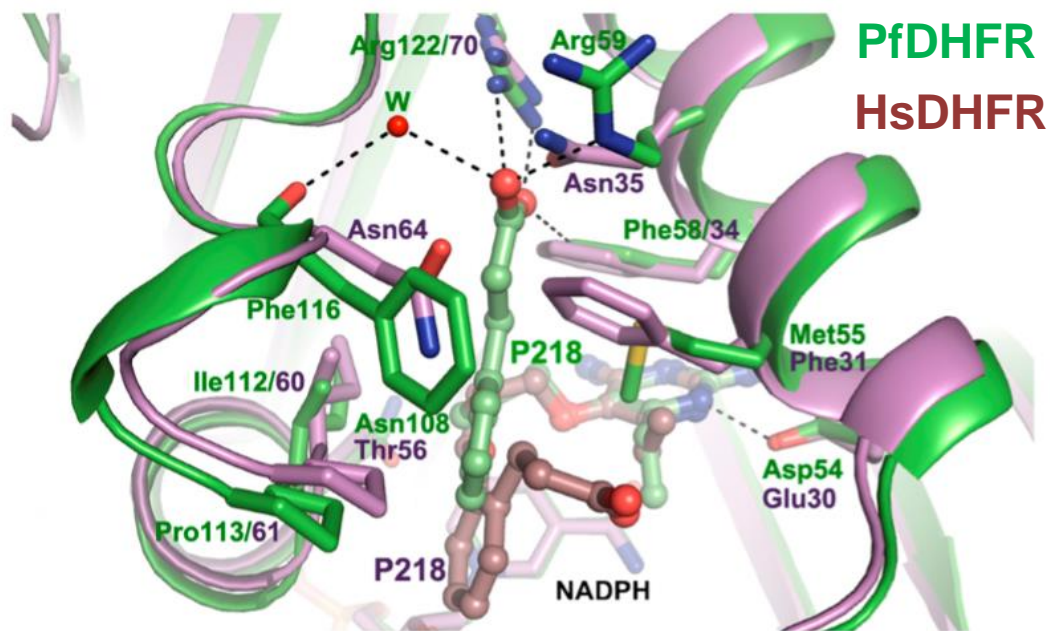
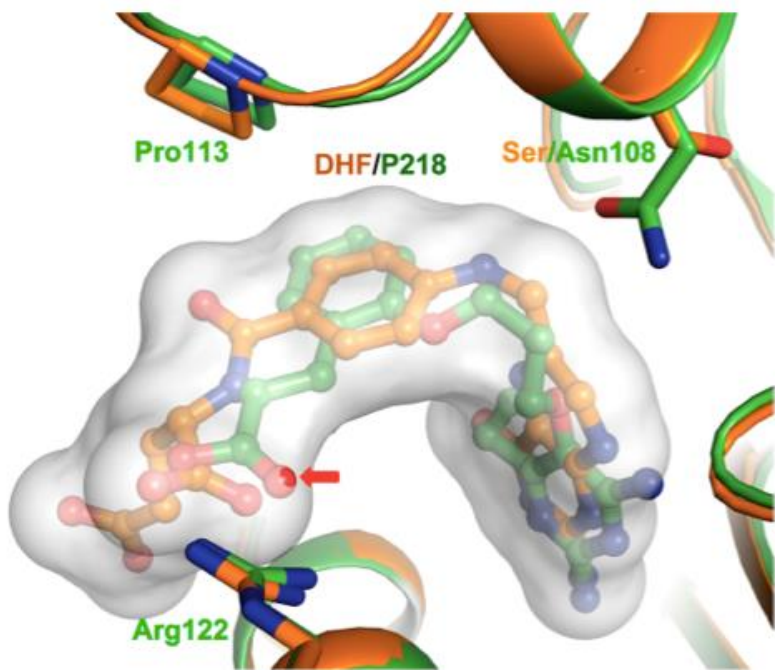
P. falciparum Dihydrofolate Reductase-Thymidylate Synthase (DHFR-TS)



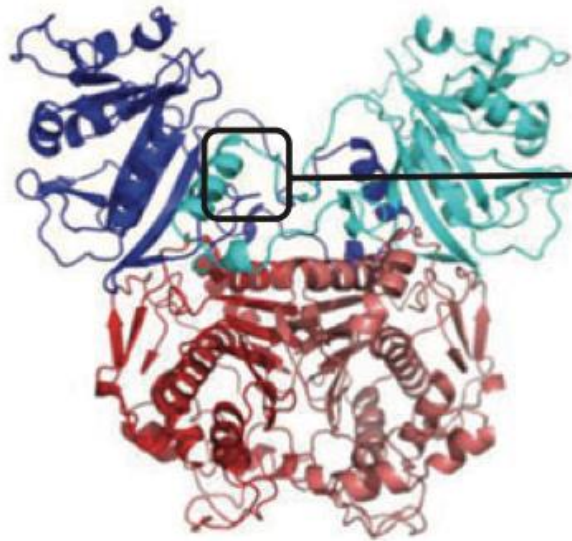
Substrate/P218 binding to PfDHFR



P218 has currently completed Phase I trials (NCT02885506).

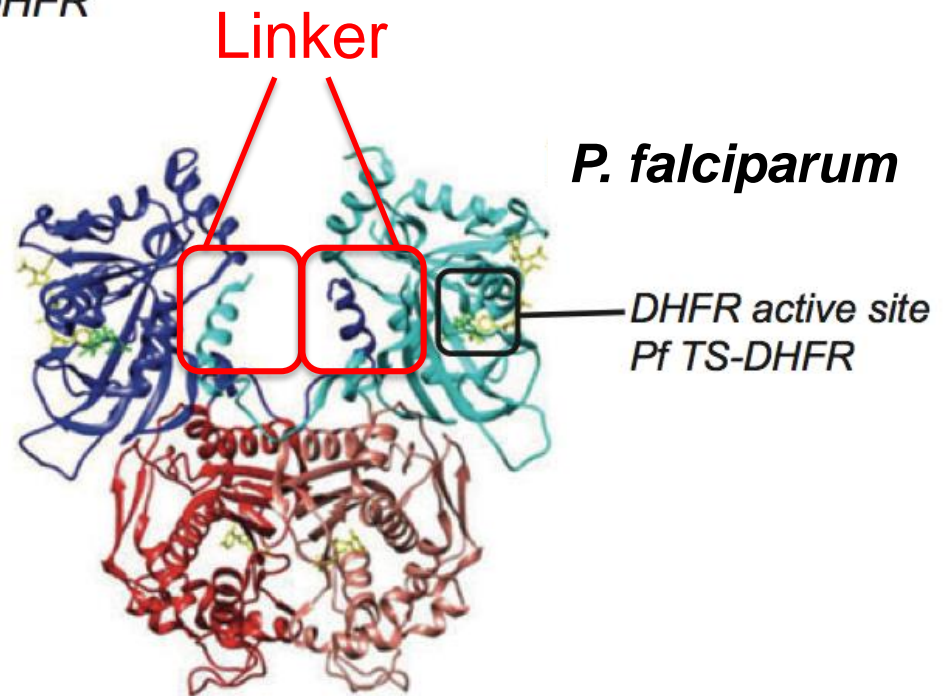


Non-active sites



Cryptosporidium hominis

allosteric pocket near
Crossover helix
Ch TS-DHFR



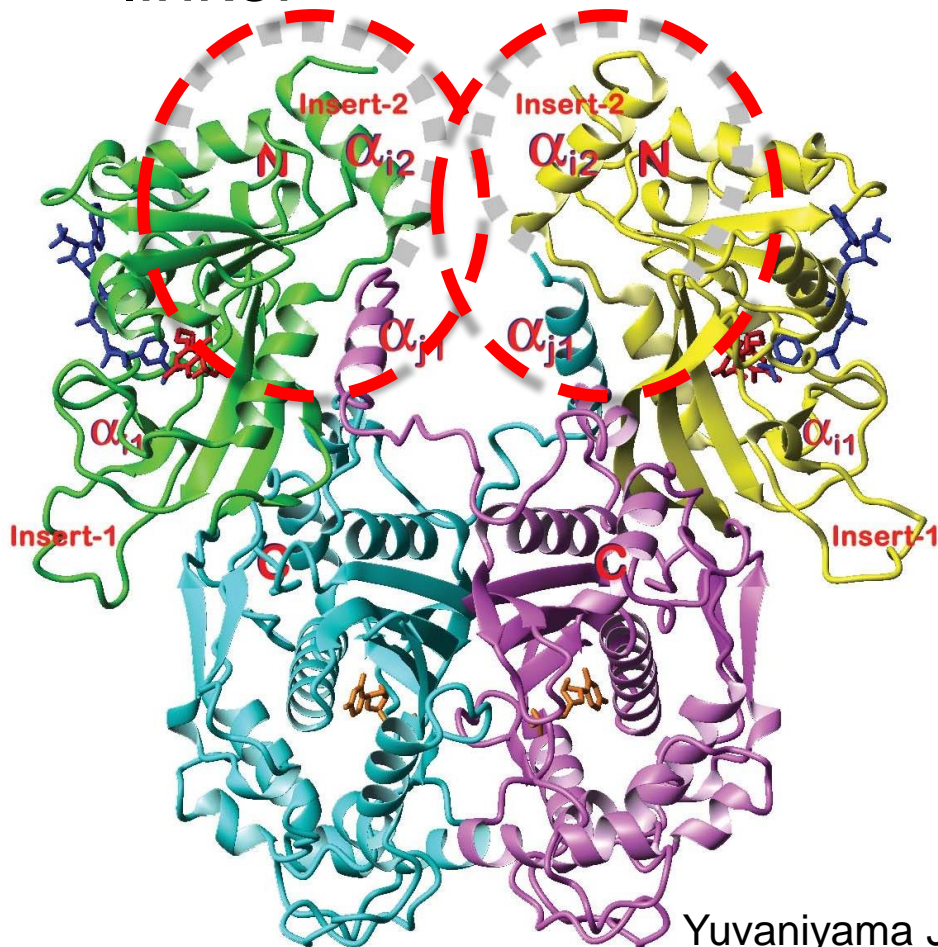
P. falciparum

DHFR active site
Pf TS-DHFR

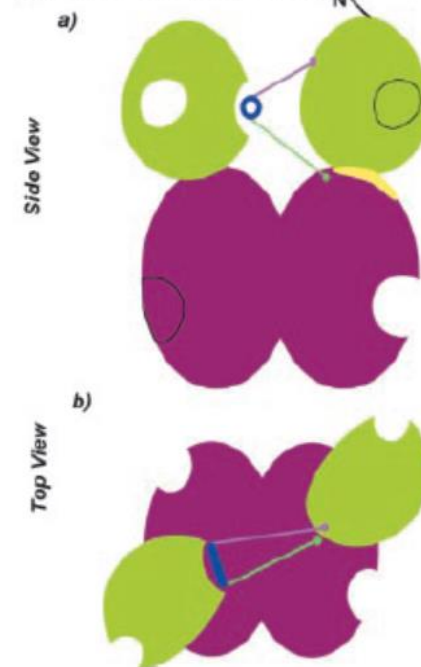
DHFR	linker	TS	<i>Plasmodium falciparum</i>
231 aa	91 aa	286 aa	
DHFR	linker	TS	<i>Toxoplasma gondii</i>
252 aa	69 aa	289 aa	
DHFR	linker	TS	<i>Cryptosporidium hominis</i>
179 aa	55 aa	287 aa	

Problem

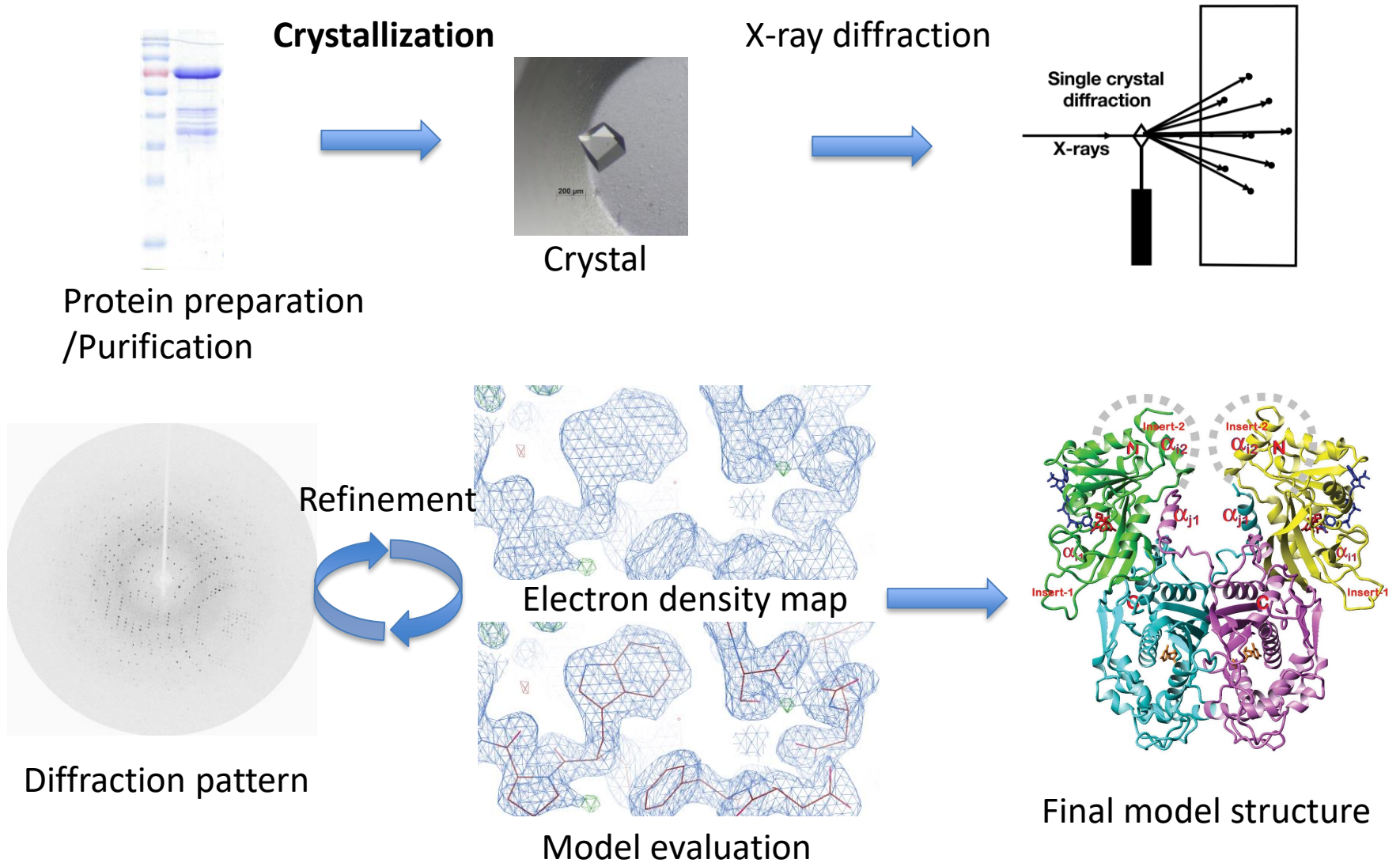
- Un-dissolved region of loop 2 and junctional linker



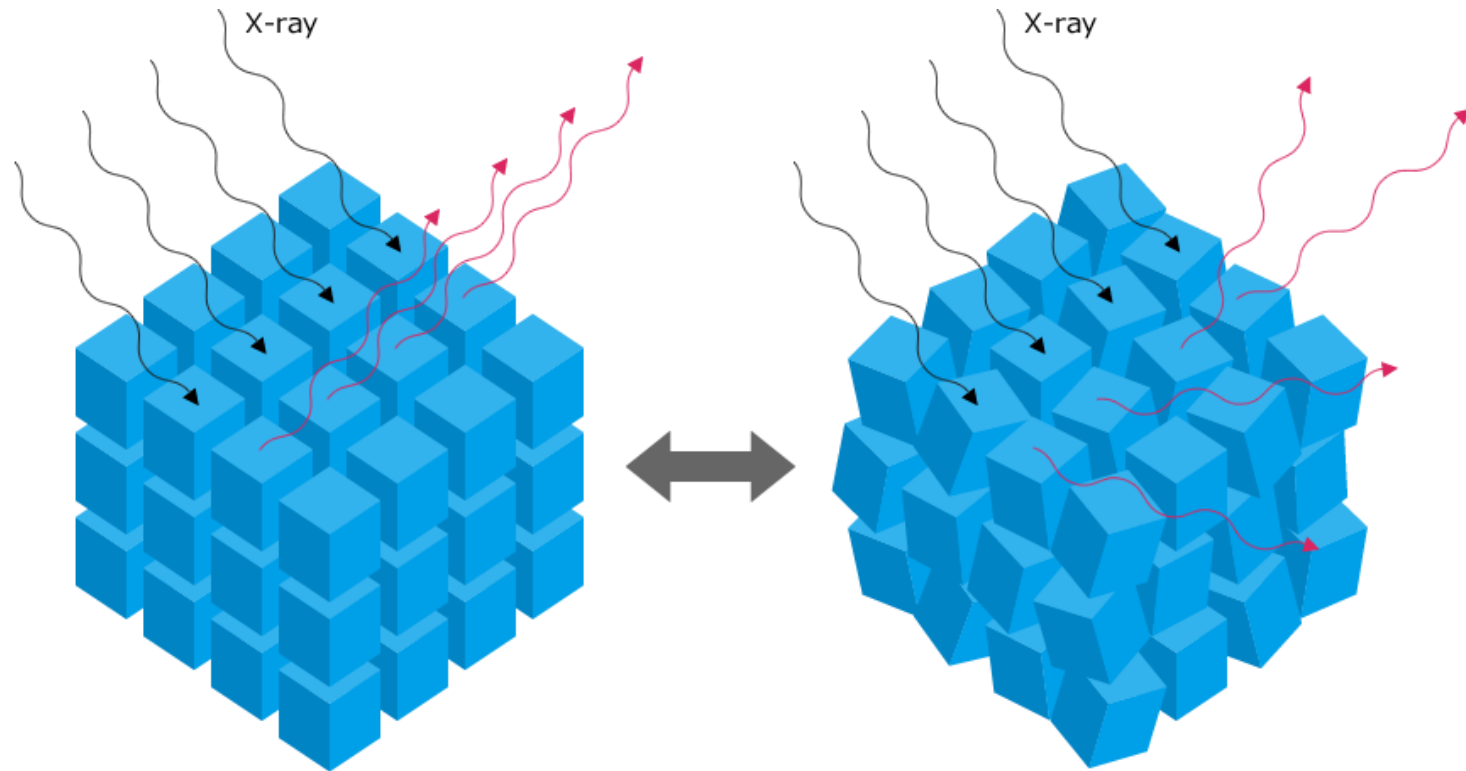
Long Linker Family
(eg. ChDHFR-TS or PfDHFR-TS)



X-ray crystallographic workflow



Crystal quality affecting diffraction spots



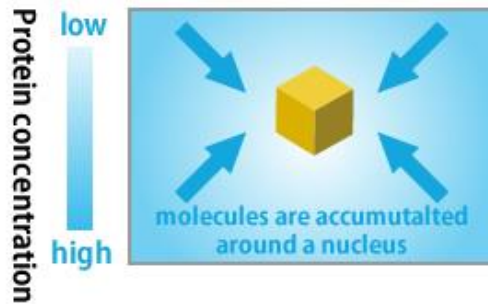
A well ordered crystal

The incoming X-ray is diffracted in a well ordered manner.

A disordered crystal

The incoming X-ray is diffracted in a disordered manner.

Crystal growth in space

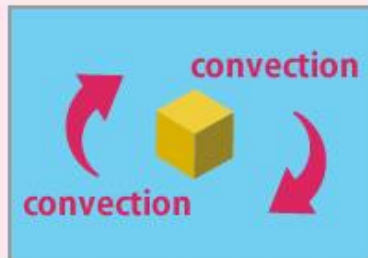


Characteristics of protein crystal growth

A Crystal grows by using the molecules around it, thus its concentration around the crystal momentarily decreased.

Low quality < High quality

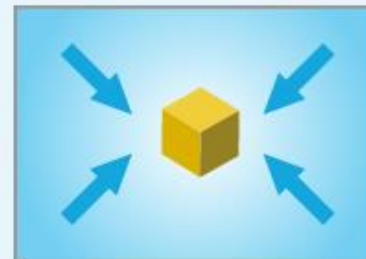
On the earth



Density-driven convection disturbs the concentration gradient in the solution.

- Disorder in molecular alignment
- Clustering

In space (under microgravity)



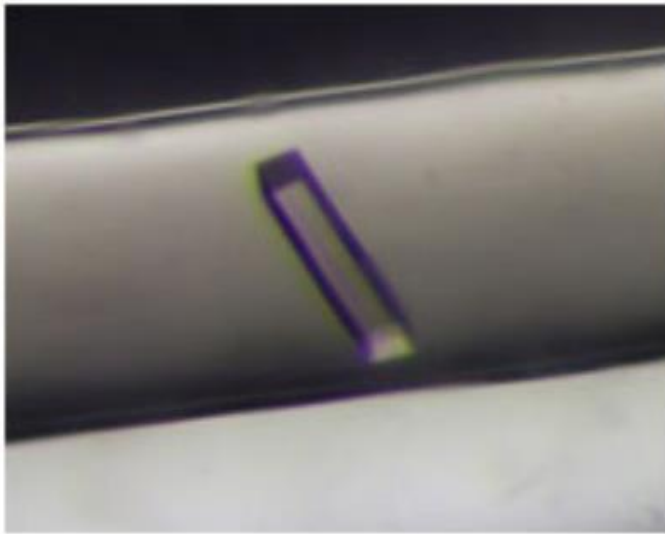
The concentration gradient is maintained without density-driven convection.

- Improved crystal quality (less impurity)
- Single crystal

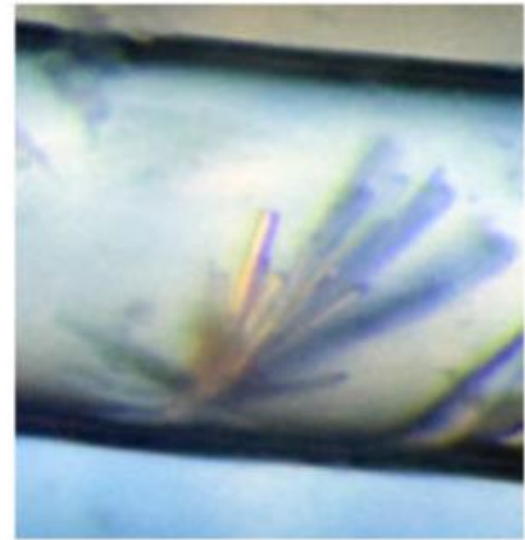
High quality crystal (high purity and organization) can be grown in space.

Crystals of protein kinase

Crystals of protein kinase (© Osaka Prefecture University/JAXA)



A crystal grown in space



Clustered crystal grown on the ground (comparative experiment)

http://iss.jaxa.jp/en/kiboexp/news/160816_pcg4deg.html

Hypothesis and Theory

- Protein crystallization in the Space would be able to stabilize the flexible regions of *Pf*DHFR-TS protein, especially in the un-structured region of loop 2 and junctional linker region.
- The results will provide clearer protein structure that would aid the design of specific inhibitors/drugs.

Objectives

- To crystallize apo-protein and protein-inhibitor in the Space.
- To solve the structure of protein and protein-inhibitor complex, in order to get information on apo-enzyme structure/protein-inhibitor binding interaction, that will aid the design of new drugs.

Expected Result

- Crystals of apo-enzyme and protein-inhibitor crystallized in the Space experiment will be obtained.
- Protein crystals would be able to grow better in the Space than those grown on earth.
- The results will provide us with the information on apo-enzyme structure/protein-inhibitor binding that could not be solved by crystallization experiments on earth.

Innovation idea/Future directions

- The un-structured region of loop 2 and junctional linker of PfDHFR-TS *can be target for non-active-site inhibitors*, that could aid antimalarial drug discovery and development.
- The knowledge will contribute to the development of effective new drugs for (malaria), and other (tropical) diseases.

Acknowledgements

- Prof. Yongyuth Yuthavong

