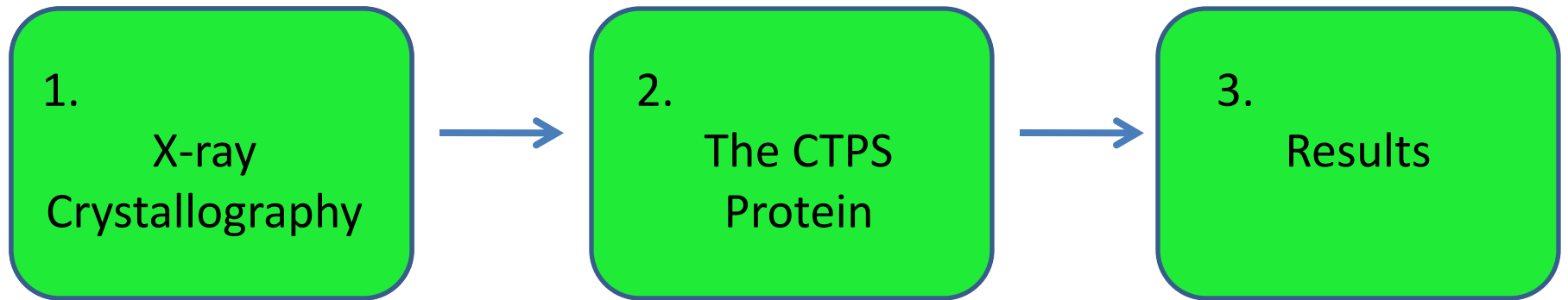


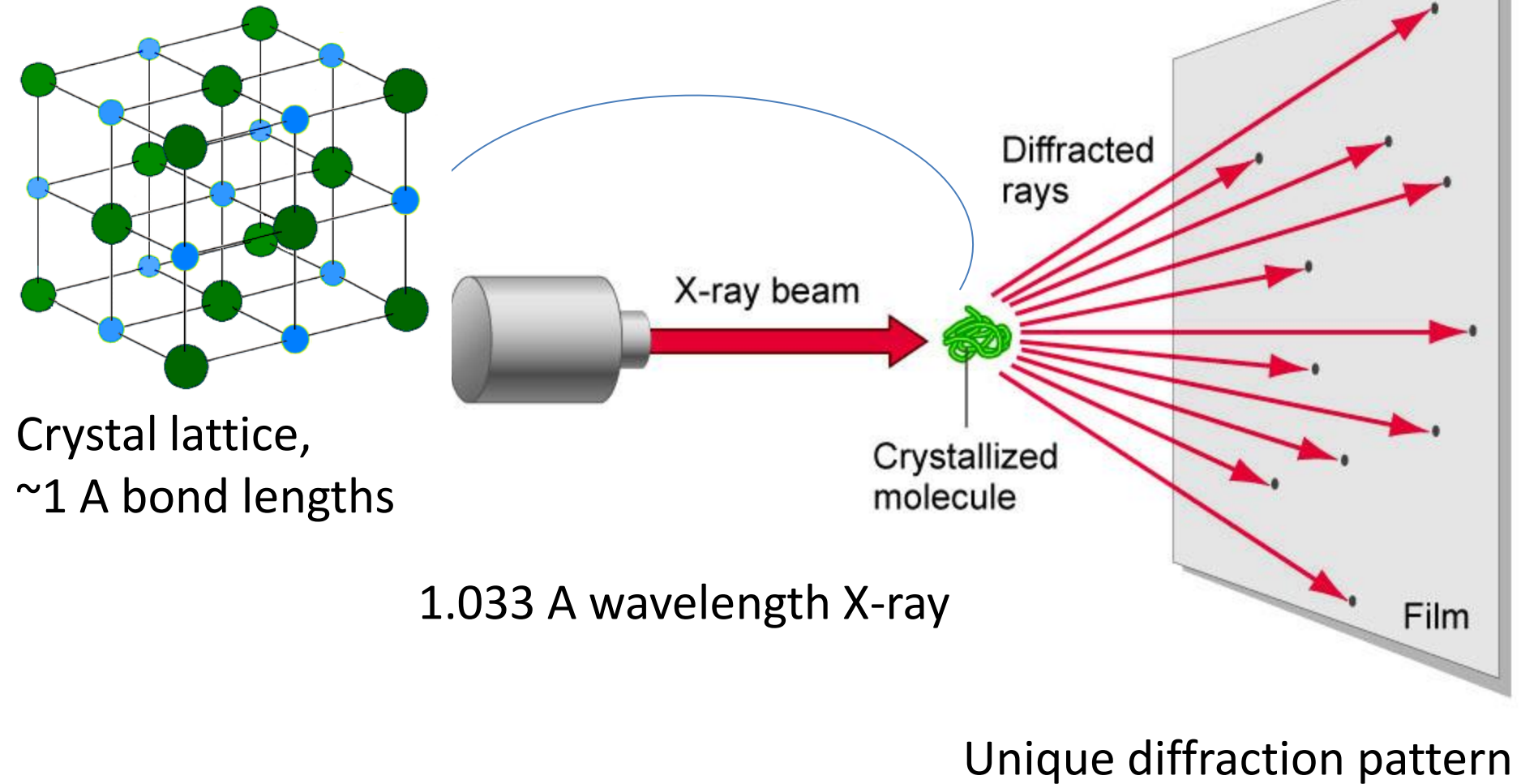
Crystals, X-Ray Beams, & the Search for the CTPS-GTP Active Site

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Smith Lab
Summer 2011

Agenda

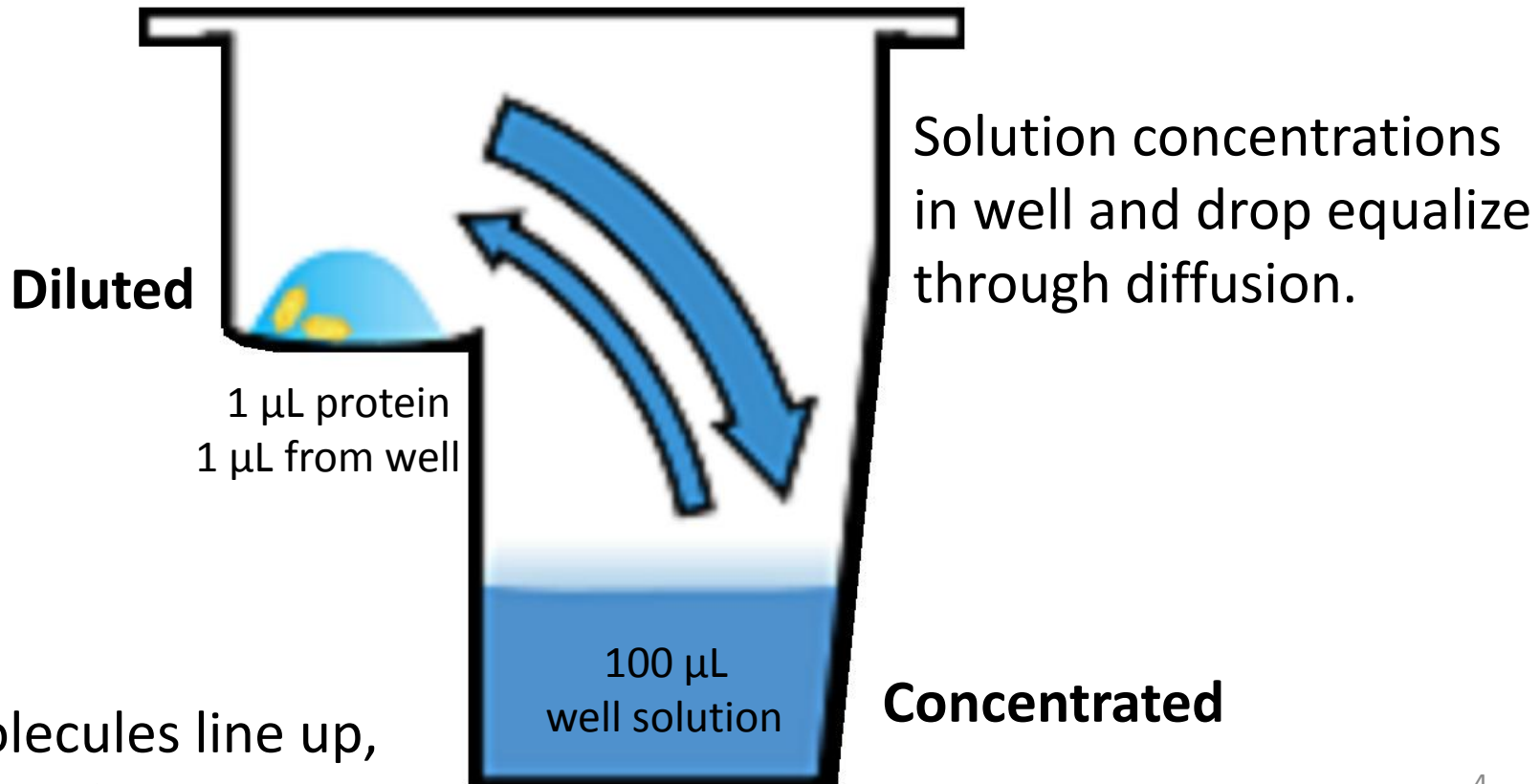


What is X-ray Crystallography?



Growing Crystals

768 combinations of temperatures, buffers, salts & precipitants



Protein molecules line up,
forming crystals.

Choosing Crystals

Before Optimization

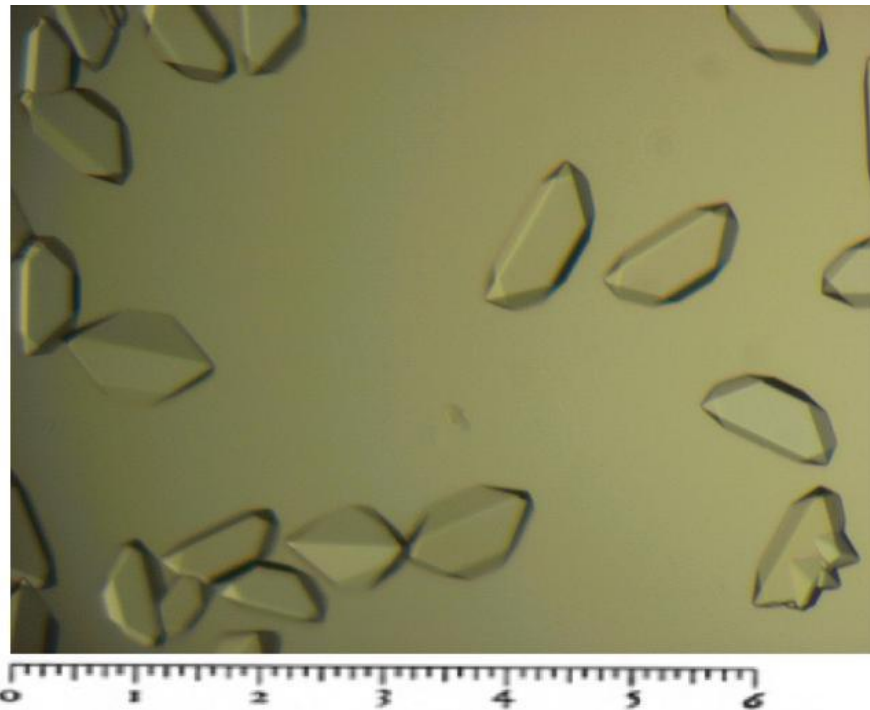


X 100 microns

**We collected, cryoprotected
& froze 64 crystals.**

Good crystals:

- Smooth
- Larger than beam
- Single



The X-ray Beam

Magnets accelerate electrons around a kilometer-circumference circular ring.

Electrons accelerate, emitting synchrotron radiation.



The Advanced Photon Source at Argonne lab

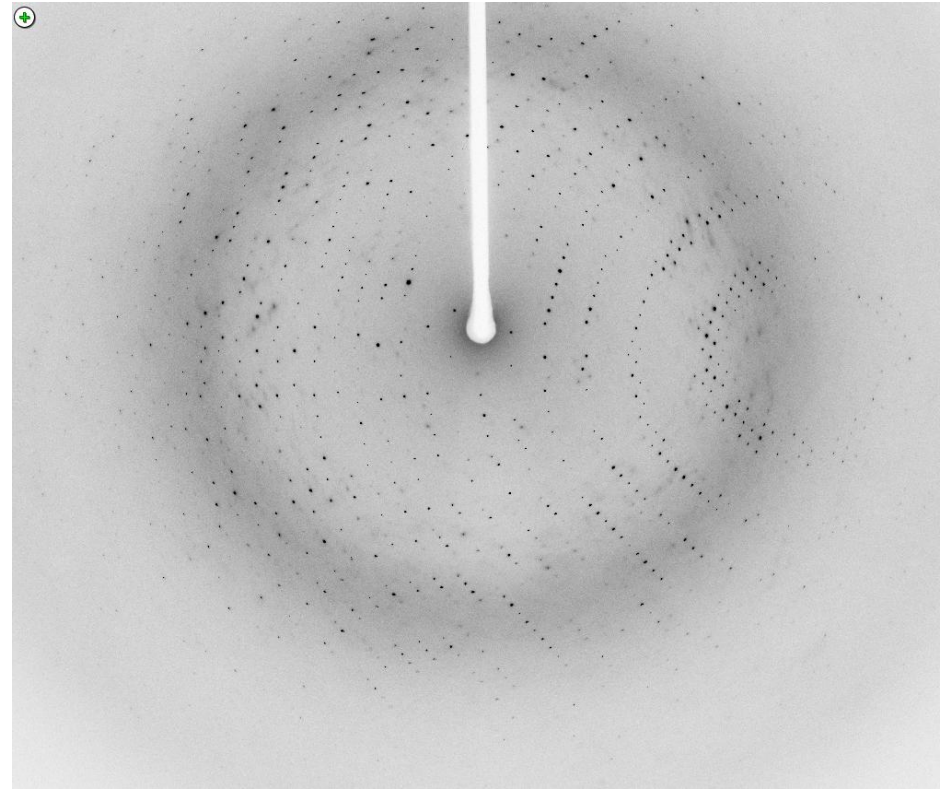
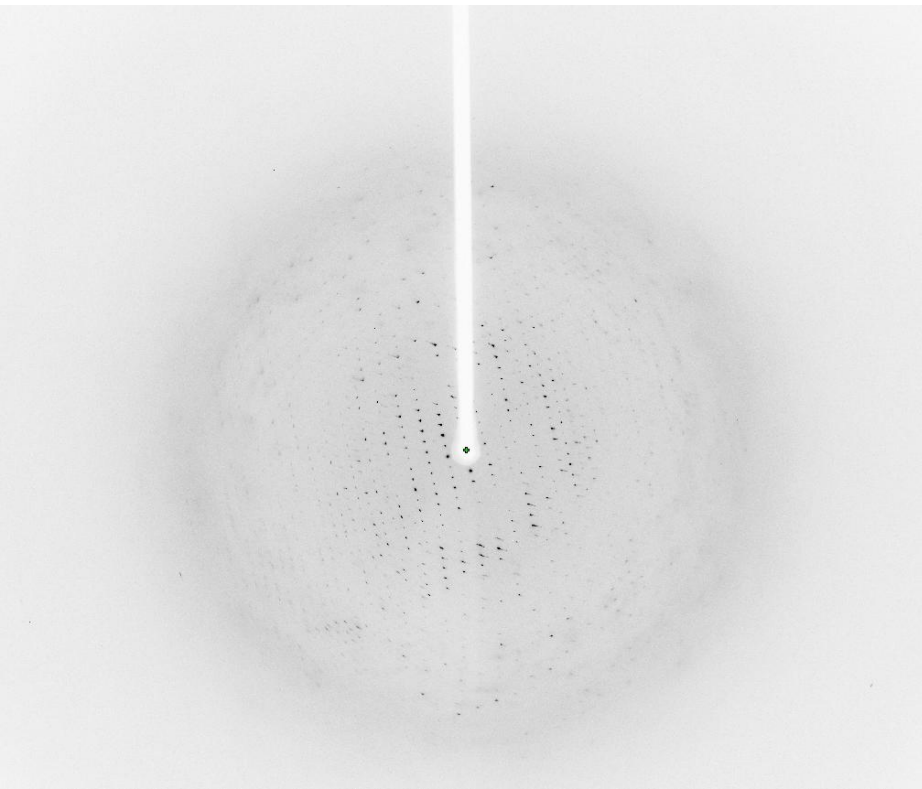


The radiation is filtered and focused into a monochromatic, high-powered X-ray beam.

Diffraction Patterns

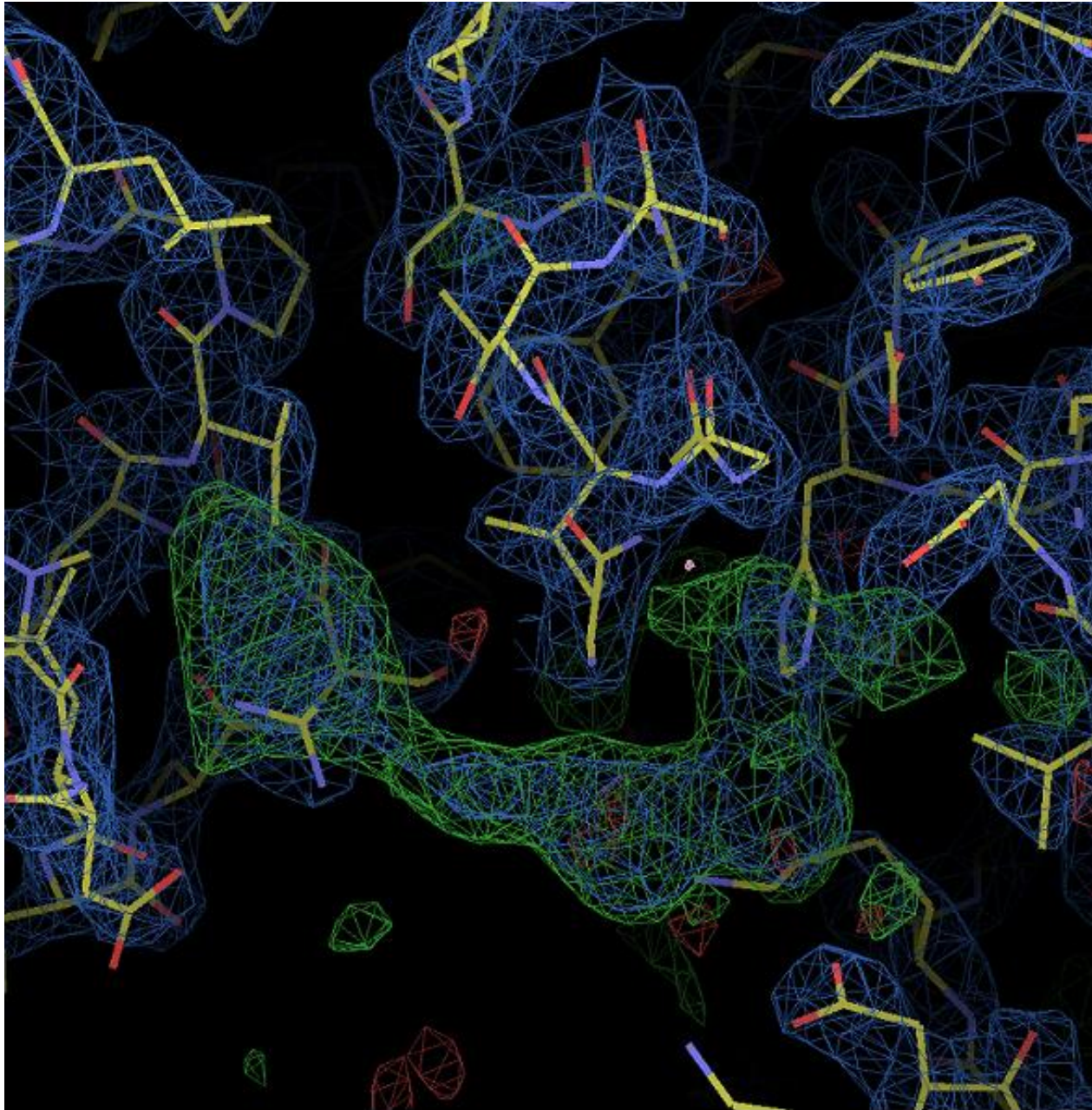
Factors to look for:

- Round spots
- A complete pattern
- Spots far from the center



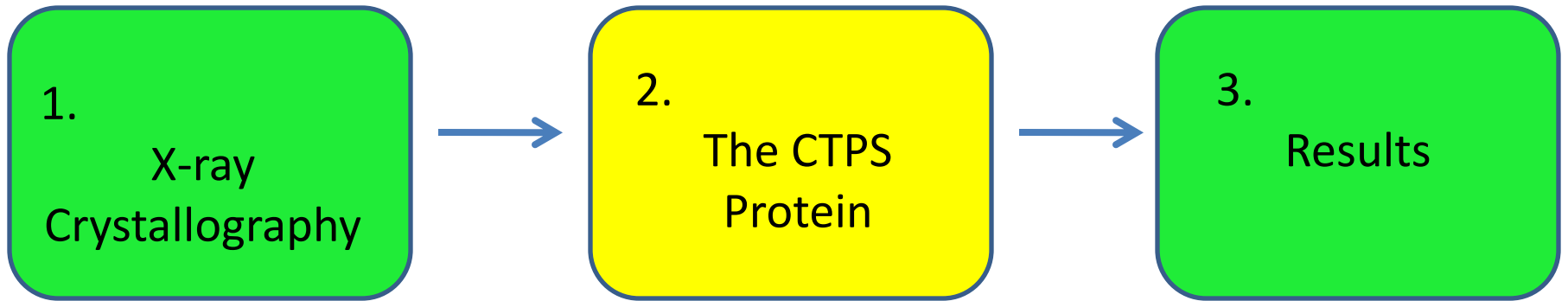
- Test shots of 64 crystals
- Collected from 10 crystals:
 - Rotate crystal
 - 180 pictures, 1 degree apart

Electron Density



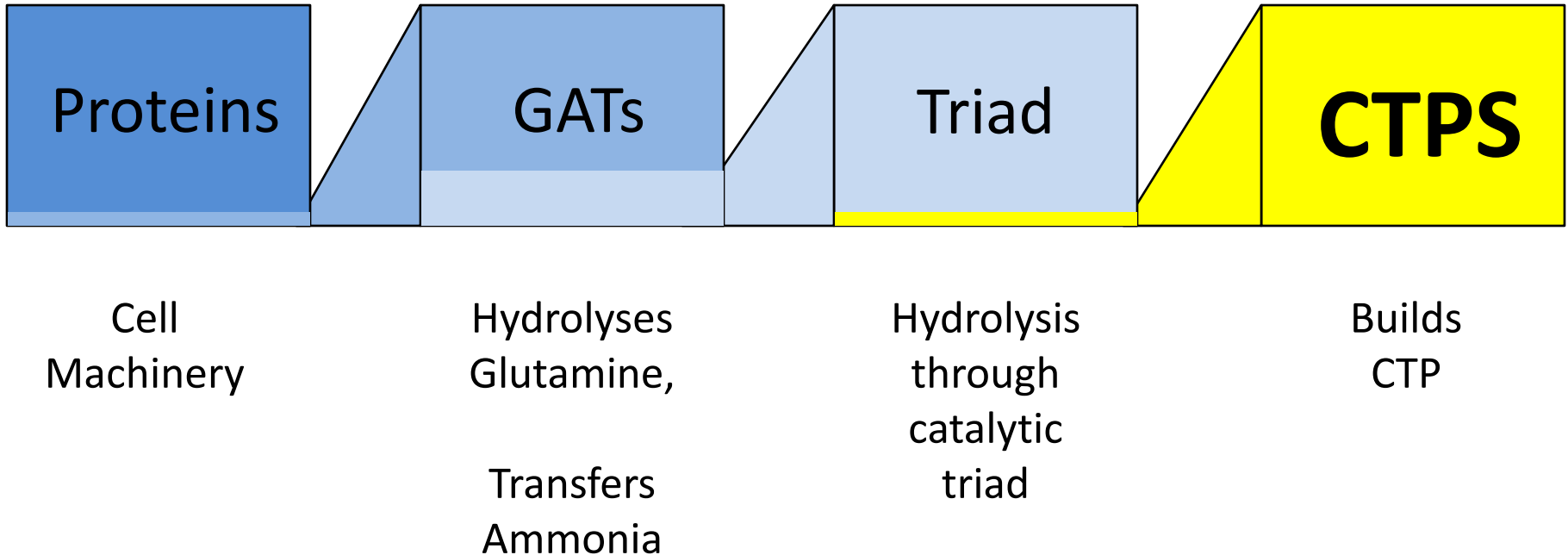
- Fourier transform
- Phases from past CTPS structure
- Amplitudes from diffracted x-ray intensities

1.9 Å resolution



- Make crystals
- Shoot with x-rays
- Analyze diffraction

Context

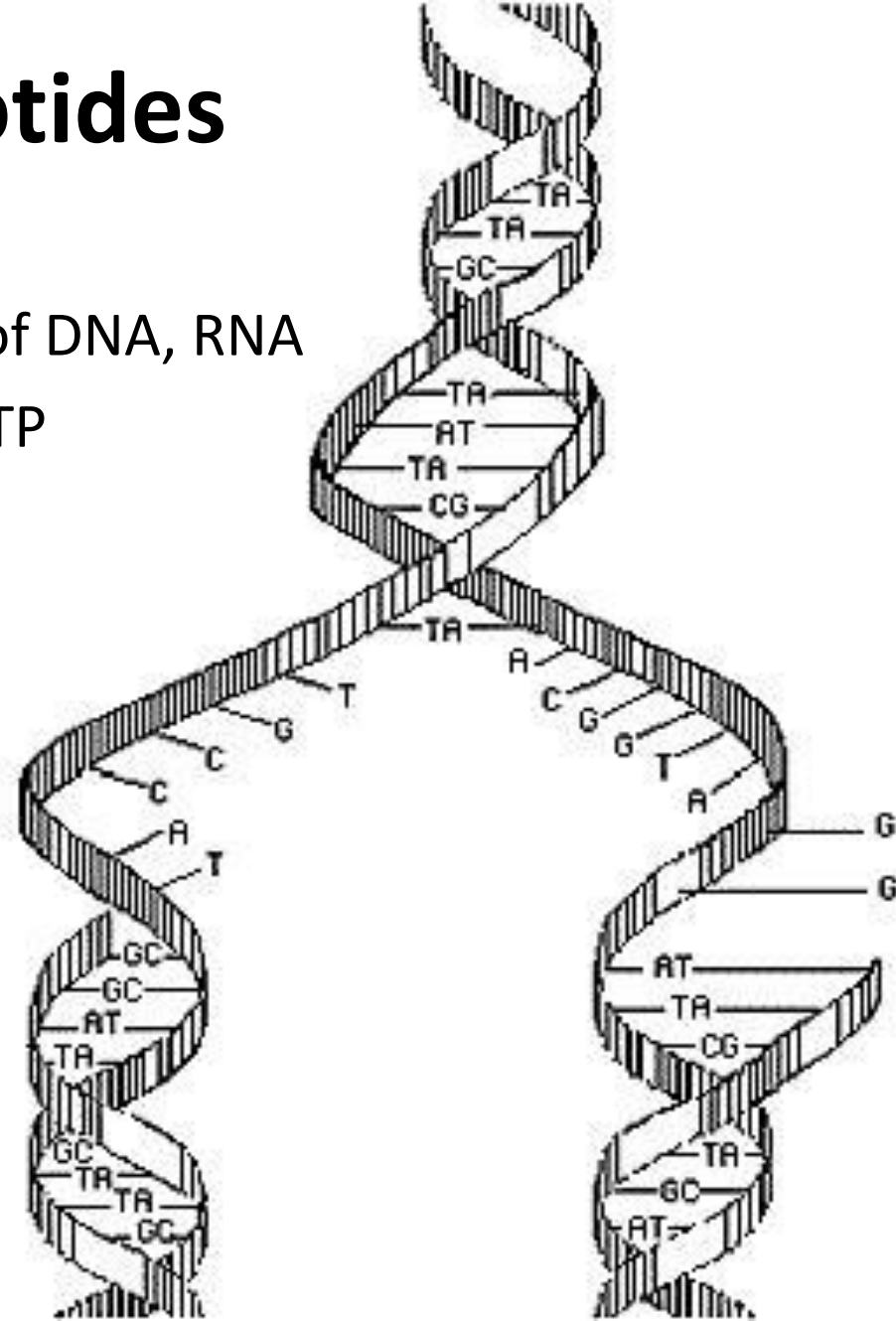


Nucleotides

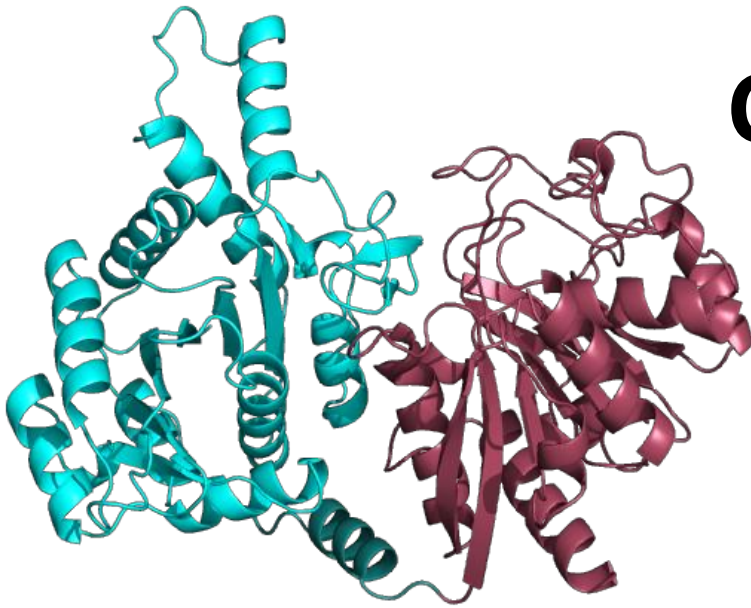
- Building blocks of DNA, RNA
- CTP, ATP, UTP, GTP

Nucleotides & CTPS

- CTPS synthesizes **CTP**
- Process requires **ATP** & **UTP**
- **GTP** accelerates hydrolysis

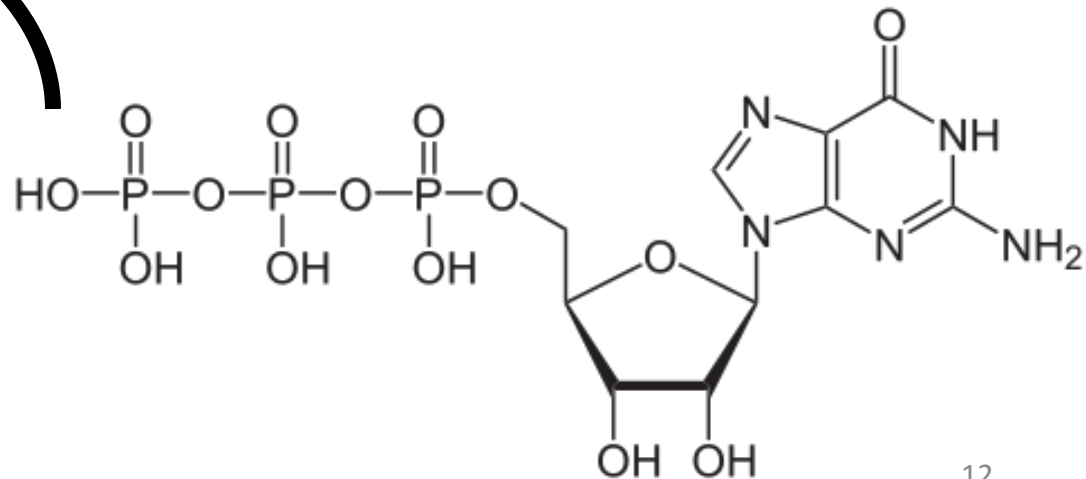


CTPS Regulation

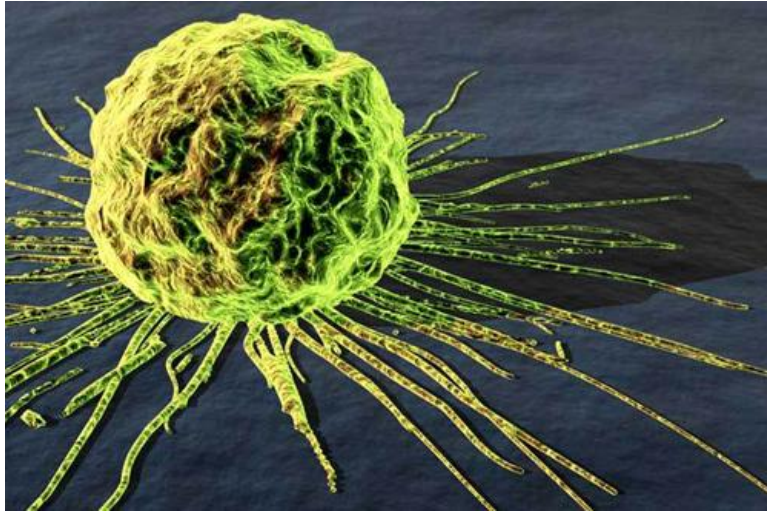


- Gln + ATP + UTP + Mg: CTPS **active**
- High ATP, CTP, GTP: CTPS **inactive**
- CTPS active + GTP bound:
glutamine hydrolyzed
3 to 4 times **faster**

Where does GTP bind?



Why Does This Matter?



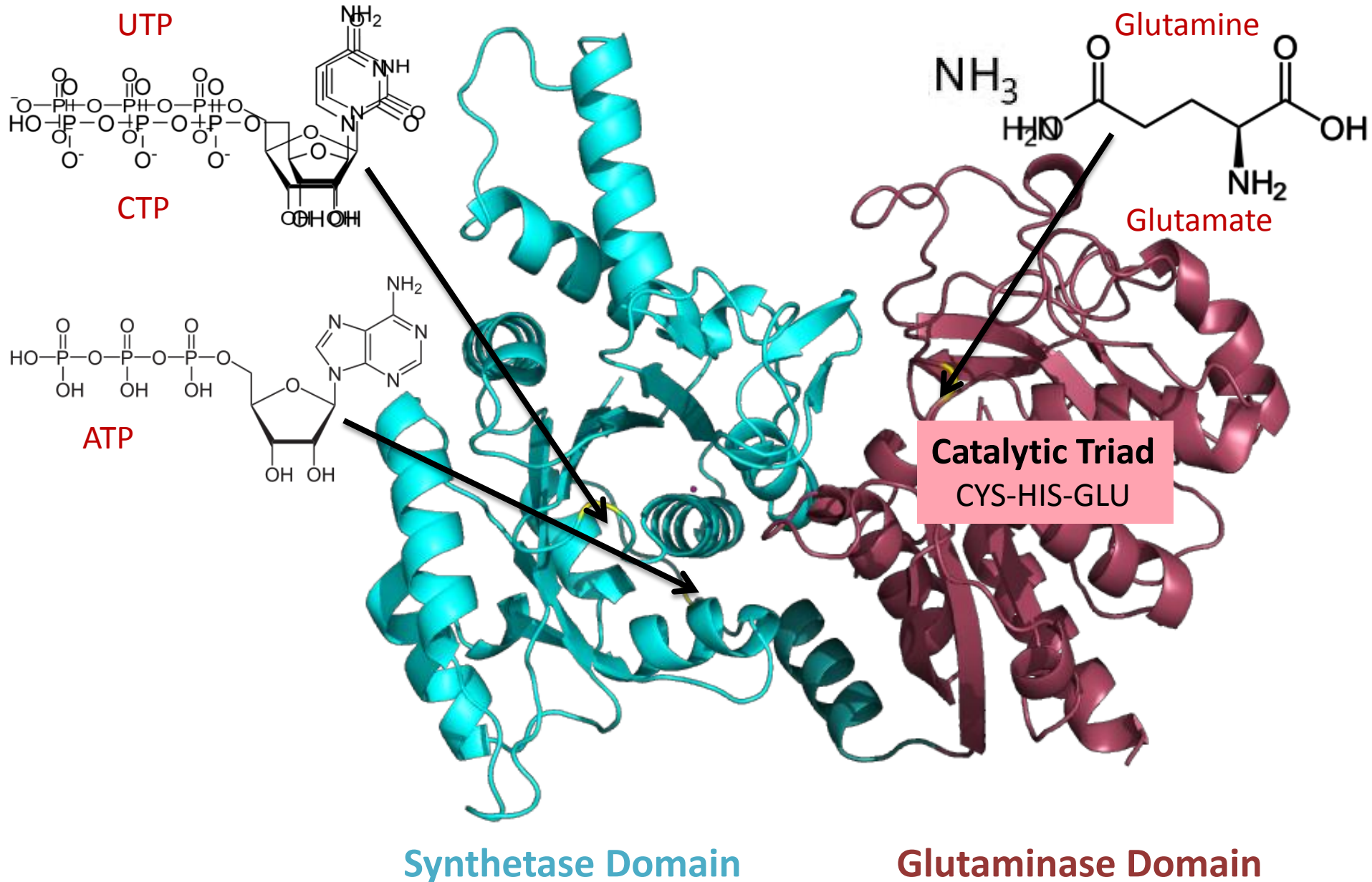
CTPS & Cancer

- Active, insensitive CTPS in cancer cells
- GTP accelerates CTP production
- More CTP -> more DNA replication
- Cancer spreads

Potential Drug

- Displace GTP
- Inhibit CTPS
- Slow tumor growth.

CTP Production



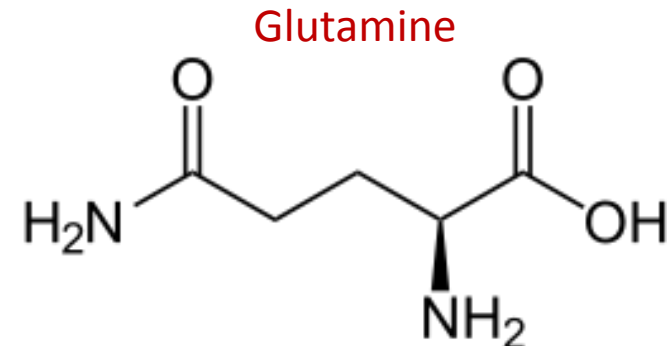
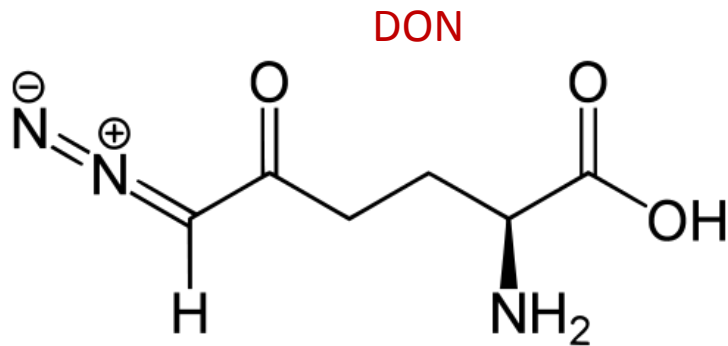
Getting CTPS and GTP to Bind

We combined:
CTPS & GTP with UTP & DON

Why UTP?

May activate CTPS

Why DON?

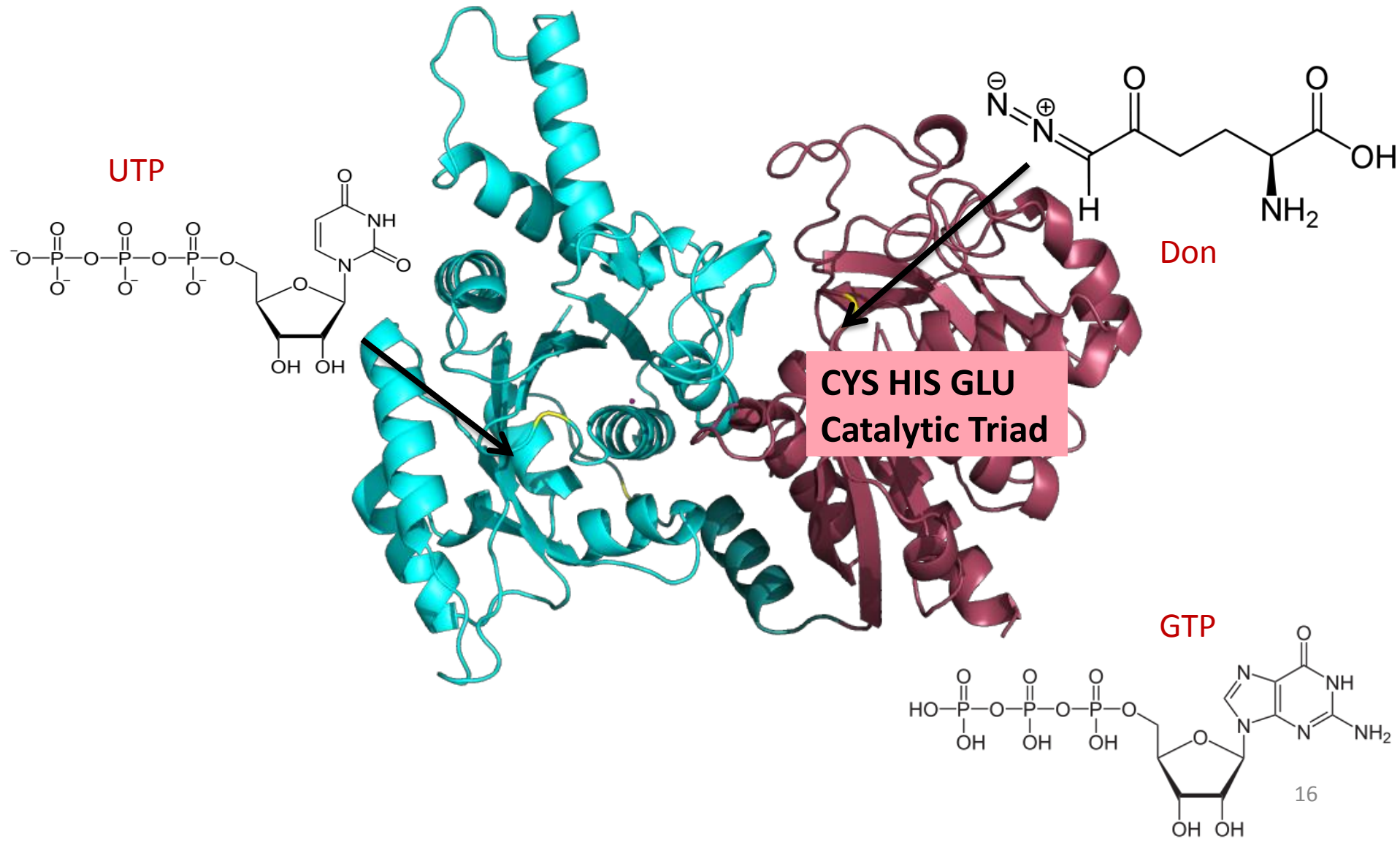


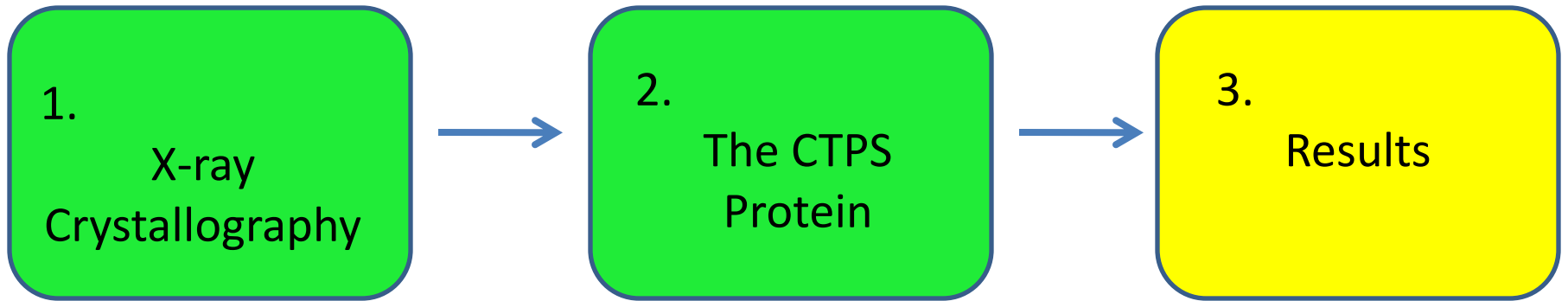
- DON = glutamine analogue
- Same binding site
- DON binds permanently, inactivating CTPS

New Reaction

Synthetase Domain

Glutaminase Domain



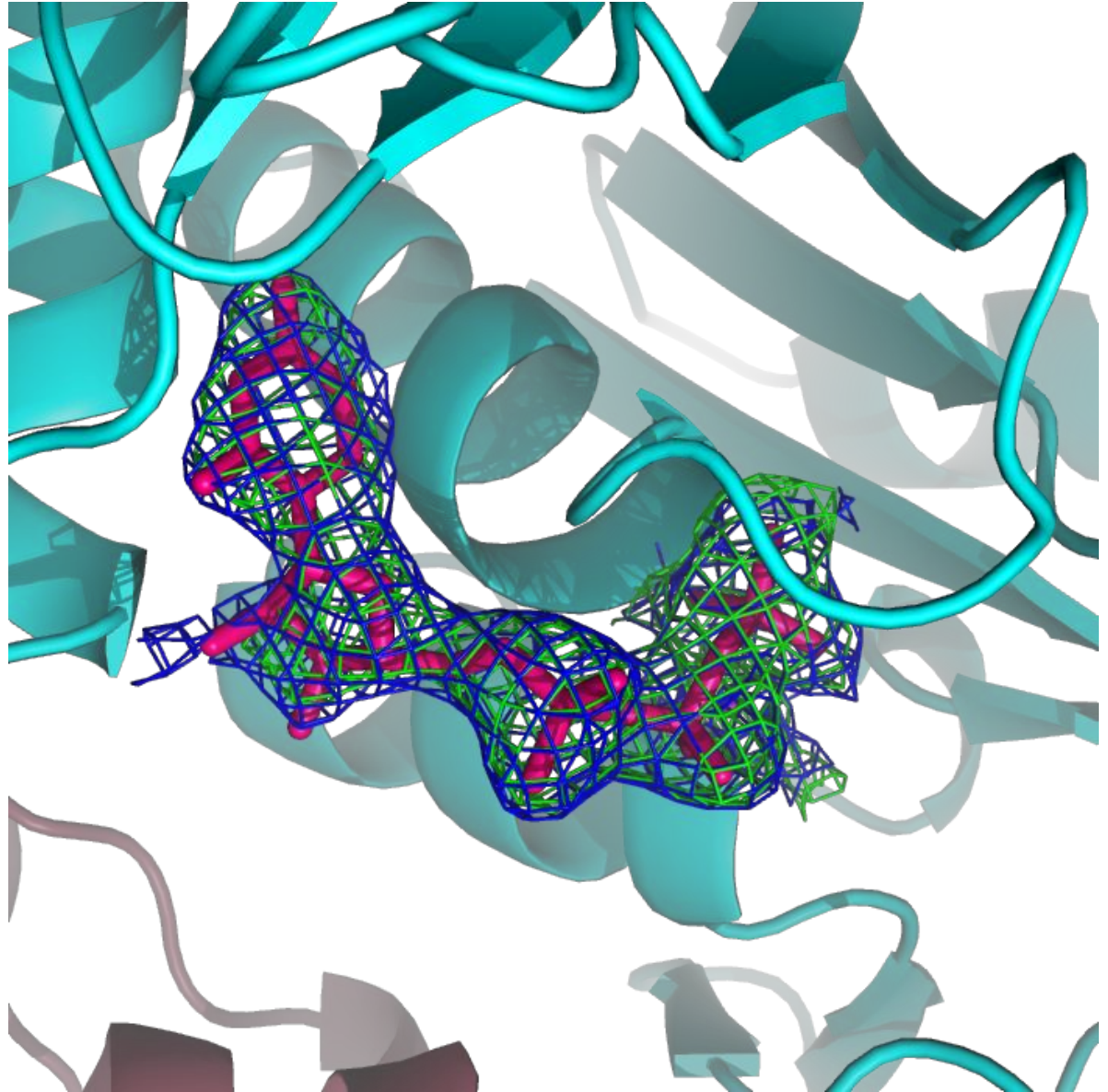


Where is the GTP
active site?

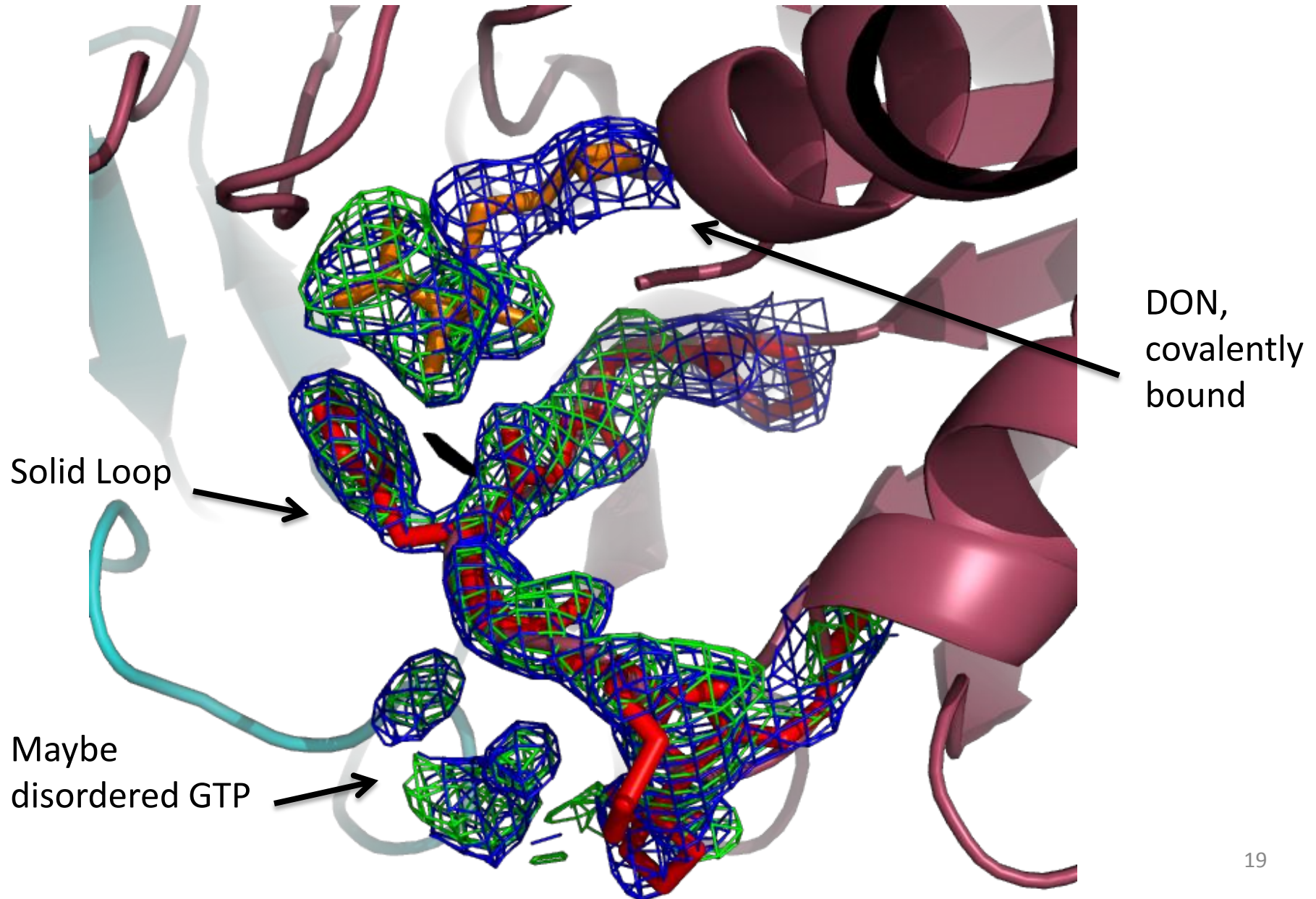
UTP

UTP bound
in ATP binding site

CTPS preparing for
activity?

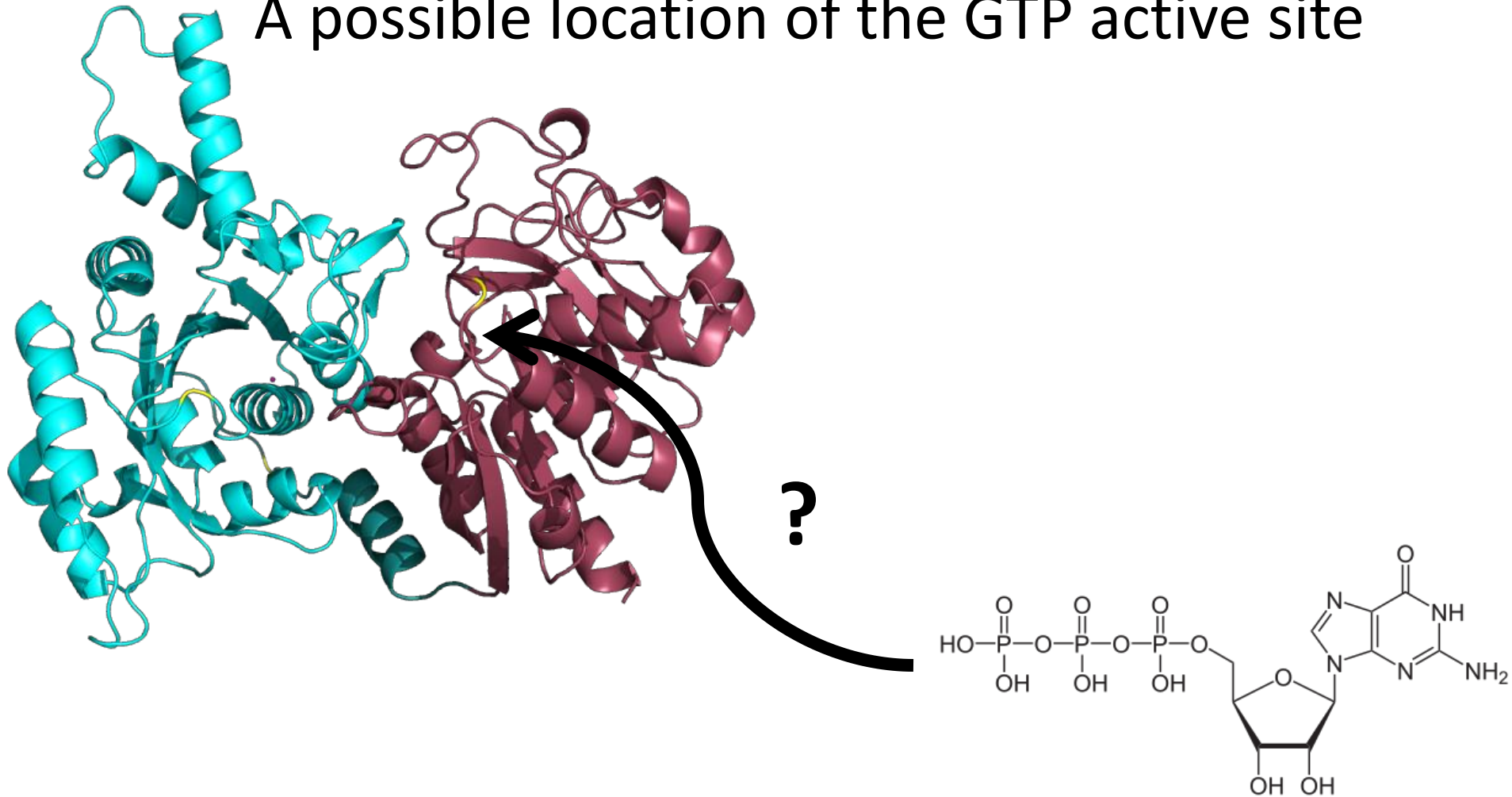


Where Does GTP Bind?



What Did We Learn?

A possible location of the GTP active site



What Next?

- Same condition, no GTP
Is density still there?



- GTP, UTP, and CTPS (no DON)
DON alters protein.
Stronger bond without DON?
- Catalytic triad mutation
No glutamine hydrolysis

Thank You!



Smith Lab

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