ROLE OF INTRINSIC DISORDER IN THE PROTEIN NETWORK FOR ACTIVATION OF NICKEL-DEPENDENT UREASE

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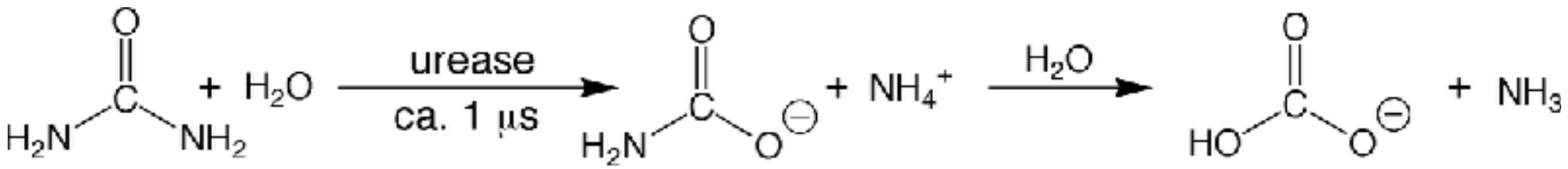
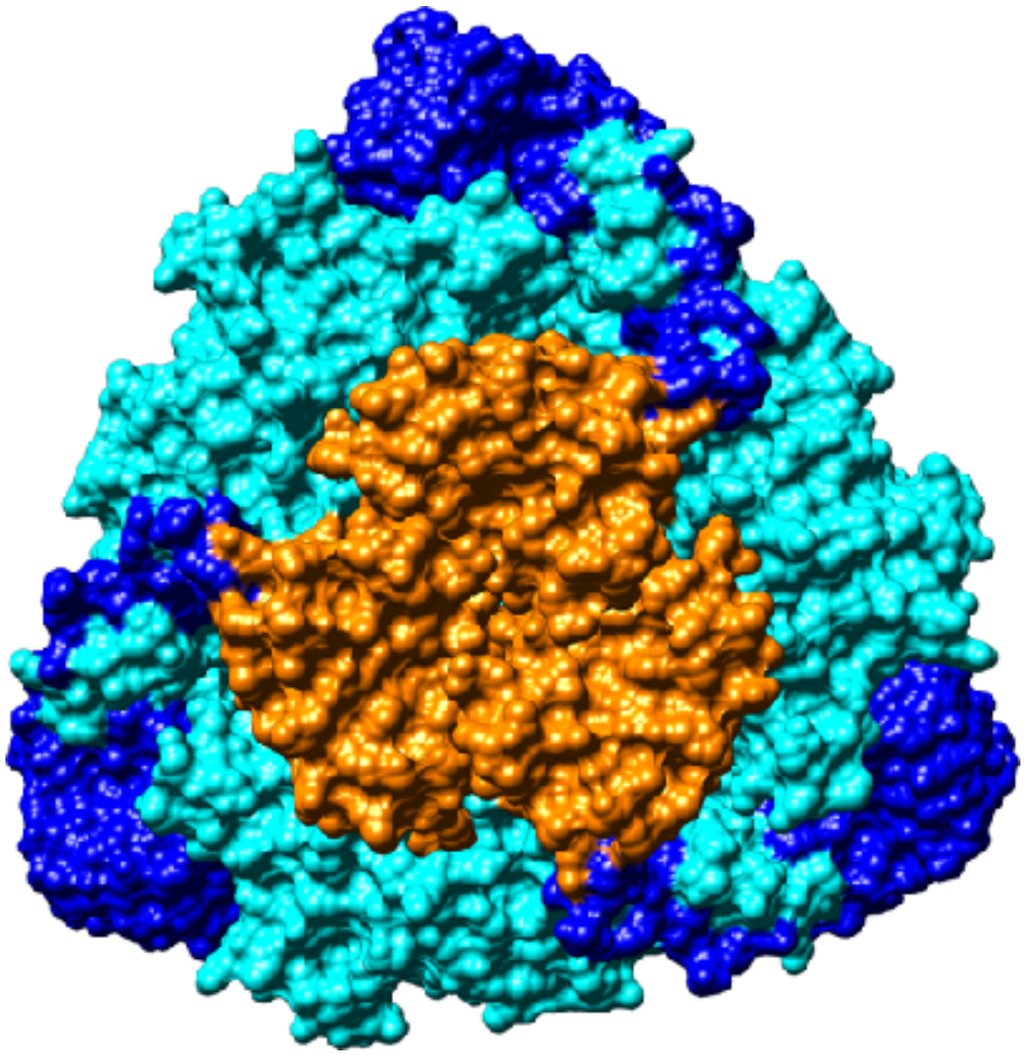
Arbre-Mobieu plenary meeting, Warsaw, March 20th, 2018

Intrinsically disordered enzymes

Structure and active site

Sporosarcina pasteurii urease





S. Benini, S. Ciurli, W.R. Rypniewski, K.S. Wilson, S. Miletti, S. Ciurli, S. Mangani, *Structure* **1999**

Intrinsically disordered enzymes

Structure and active site

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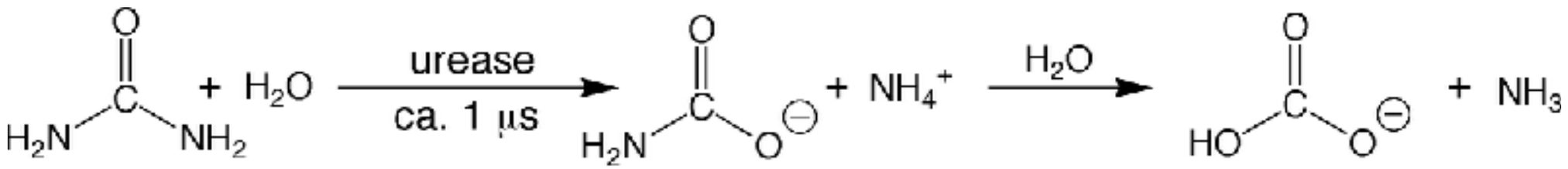

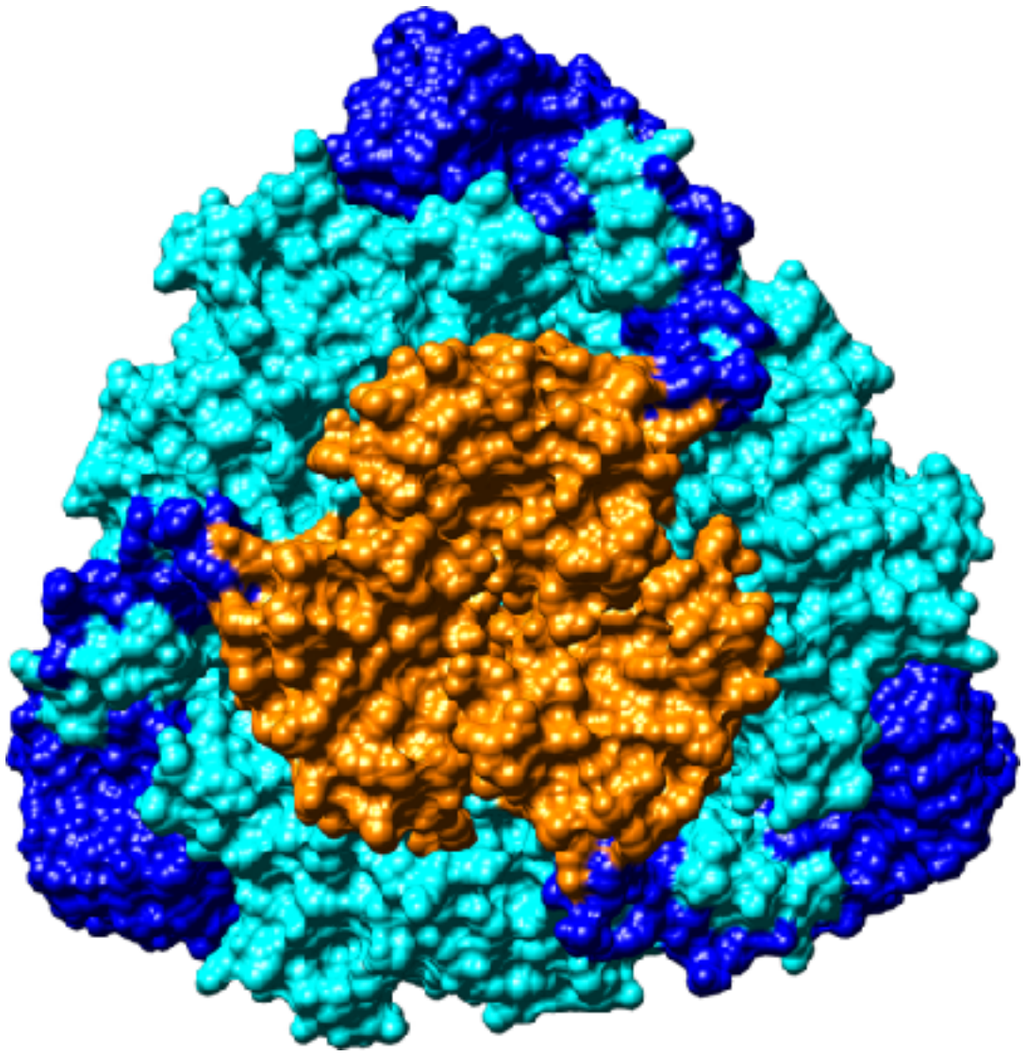

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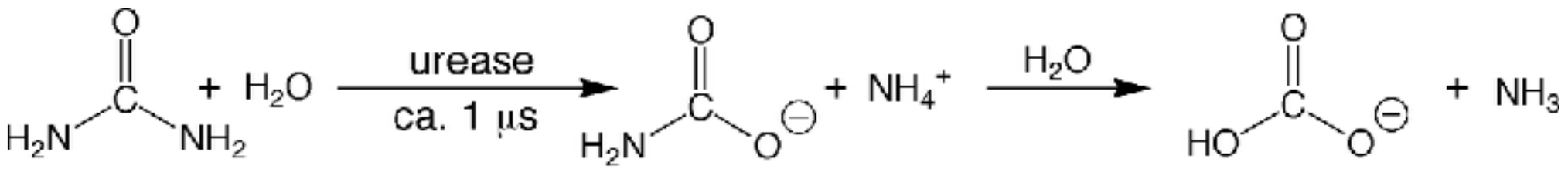

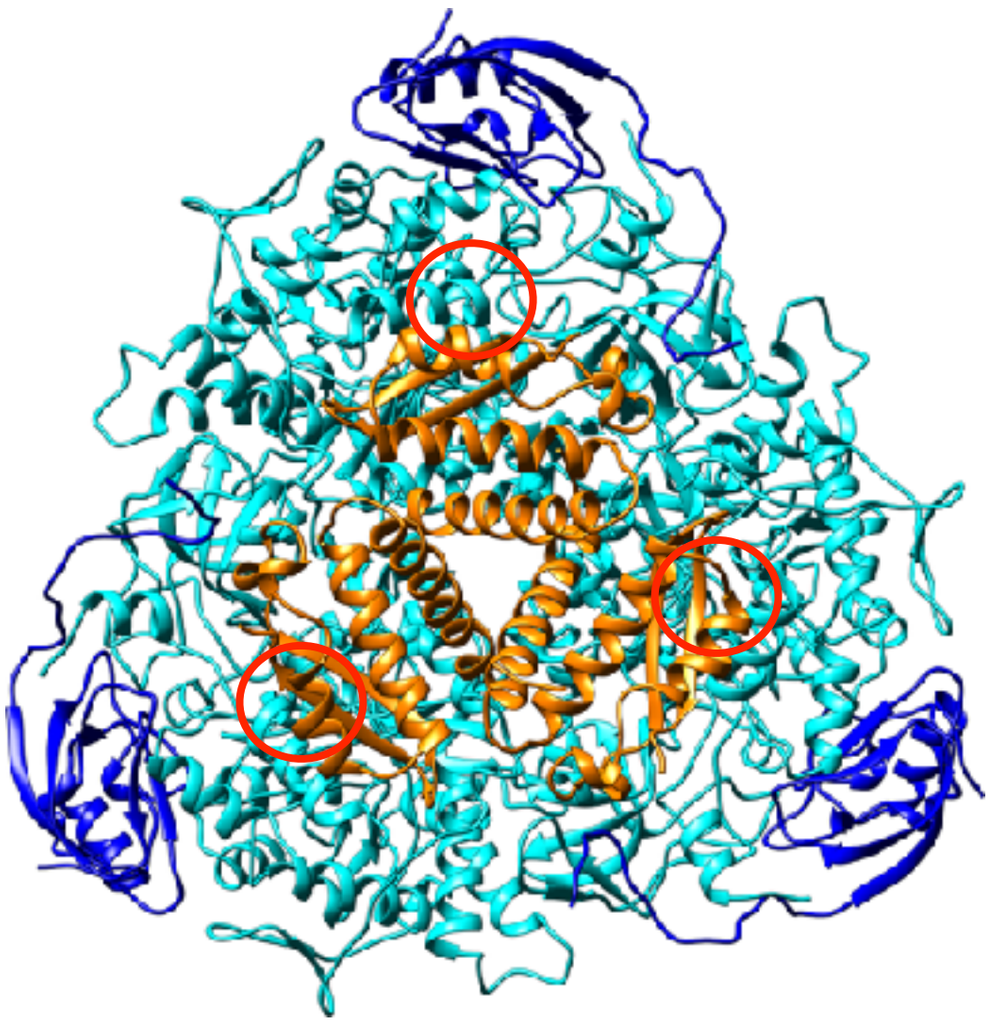

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Intrinsically disordered enzymes

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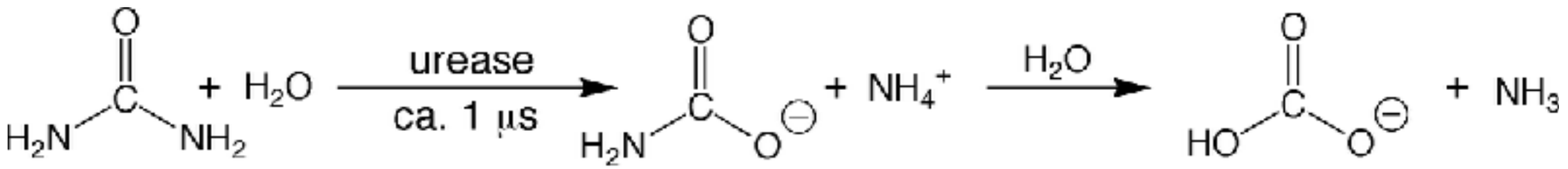
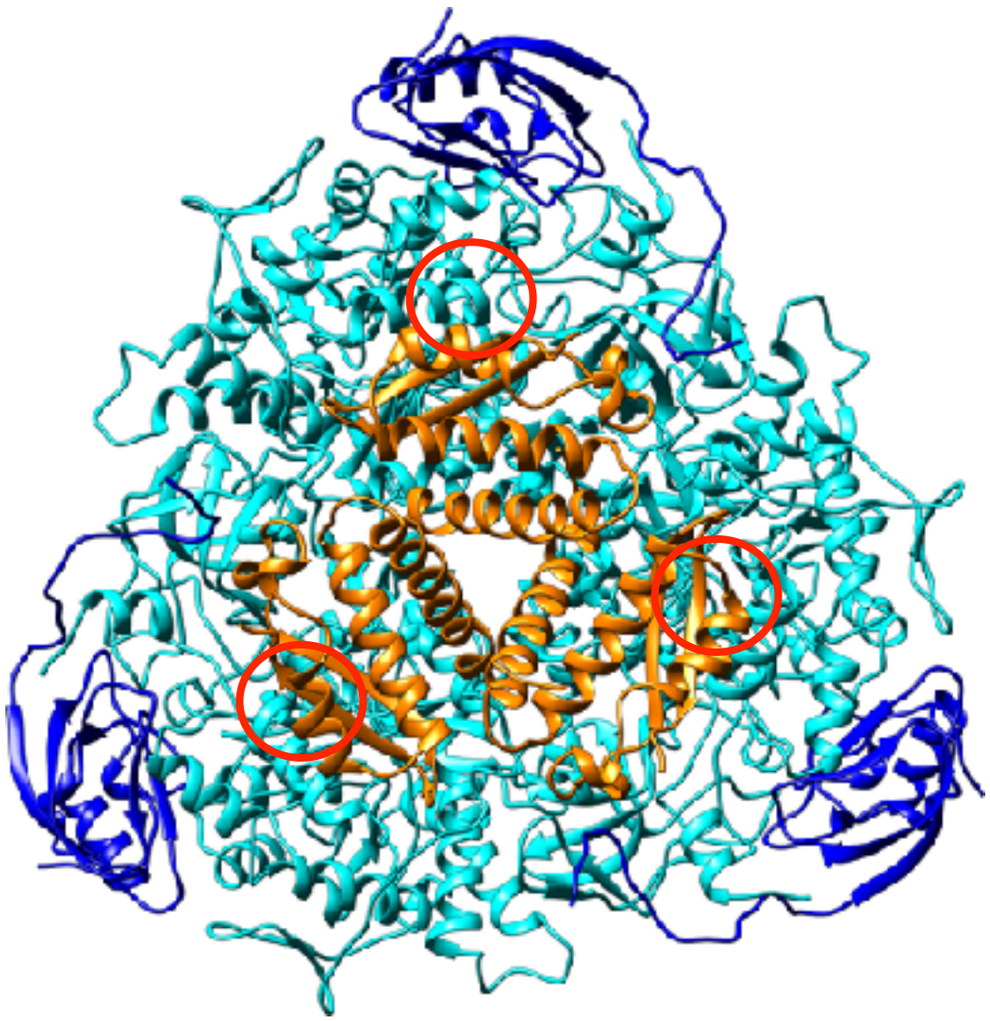
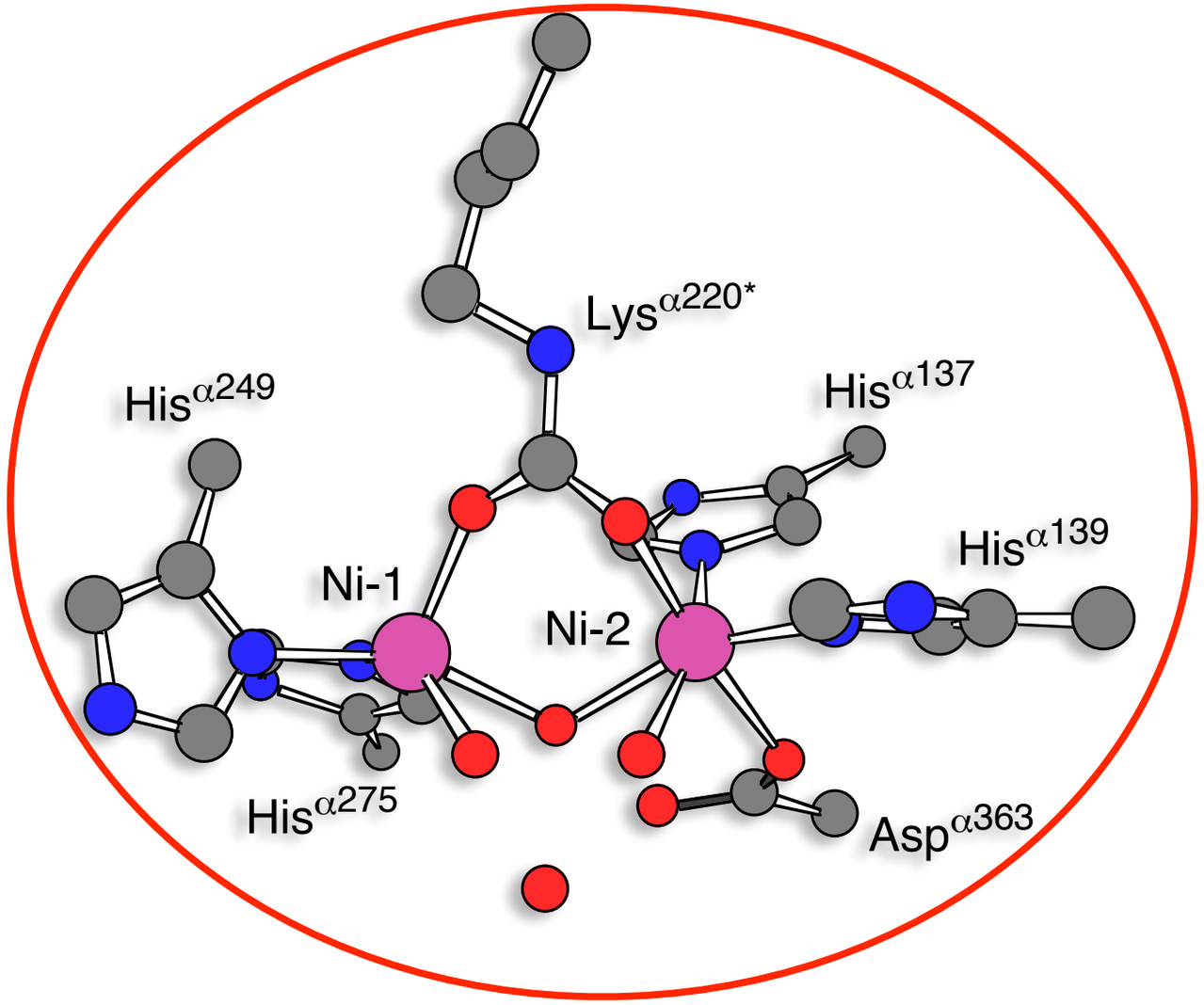
Sporosarcina pasteurii urease







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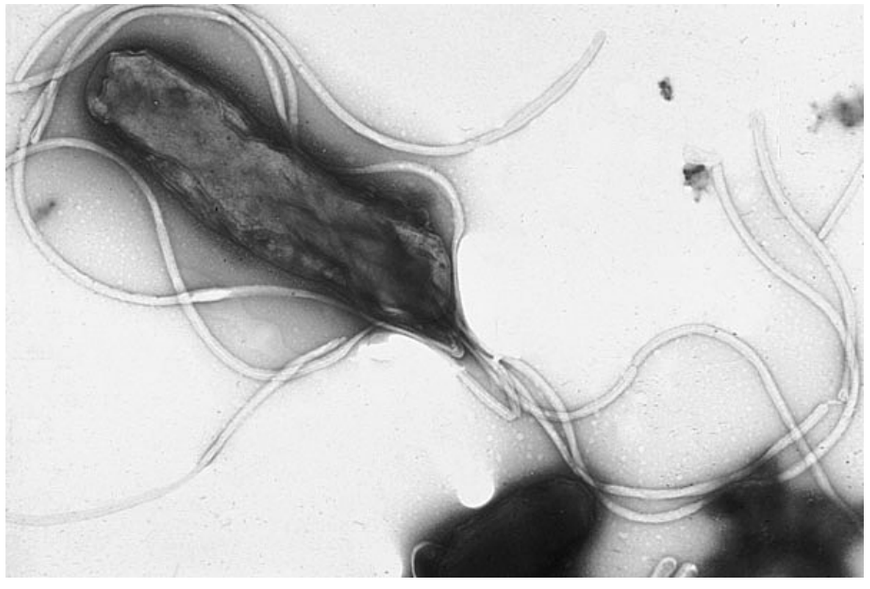
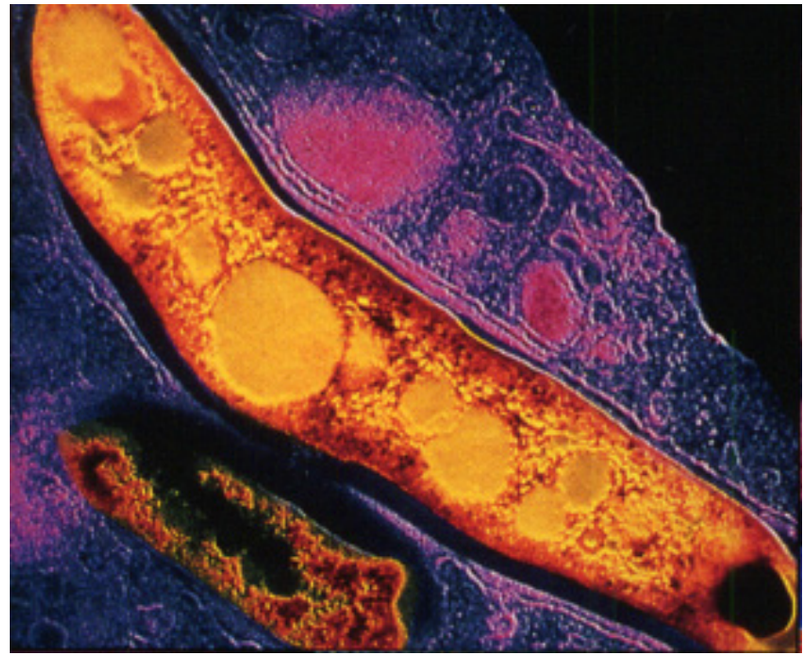
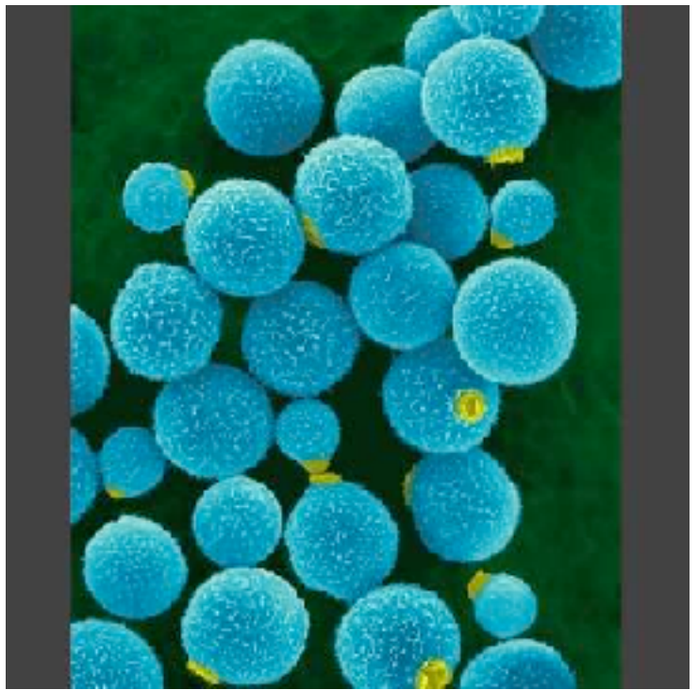
Urease as a virulence factor



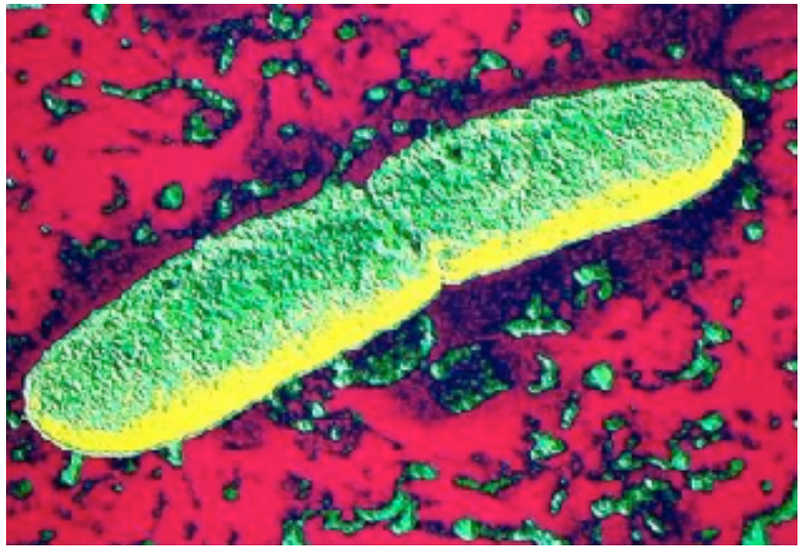
- *Helicobacter pylori* infects ca. 50% of the world population and is the main cause of cancer in gastrointestinal tract (class I carcinogen by WHO)



- *Mycobacterium tubercolosis* causes 2 million deaths per year

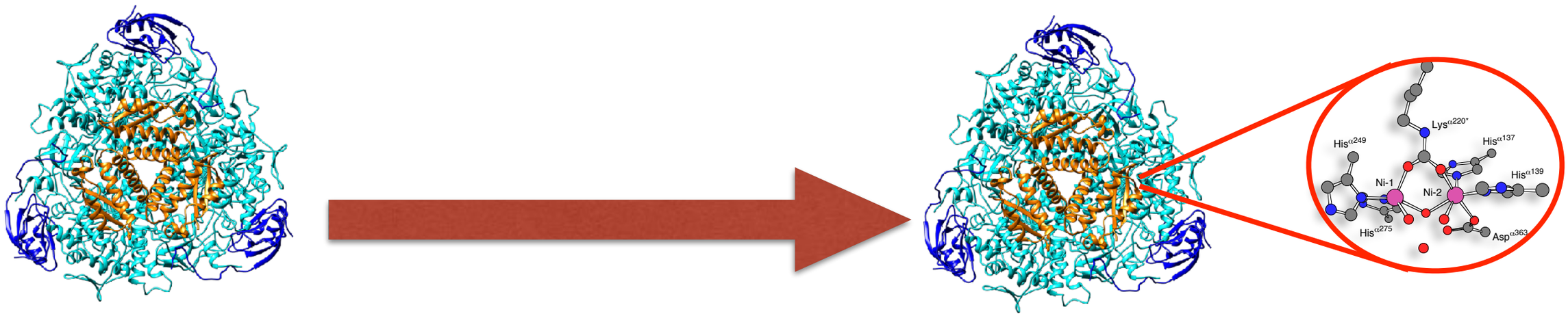


- *Yersinia pestis* causes pneumonic, septicemic and bubonic plagues



- *Cryptococcus neoformans* causes meningitis and related brain damage

Urease activation pathway

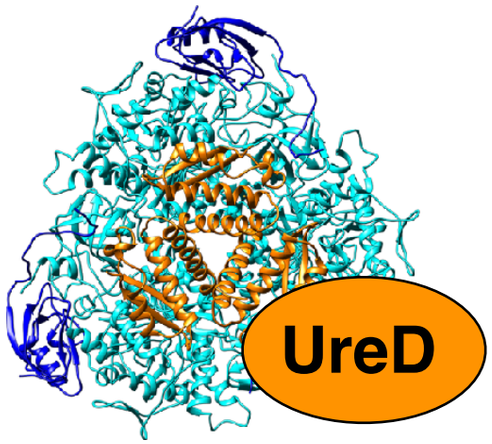


Apo-$(UreABC)_3$

Holo-$(UreABC)_3$

Zambelli B., Musiani, F., Benini S., Ciurli S. - *Acc. Chem. Res.* **2011**

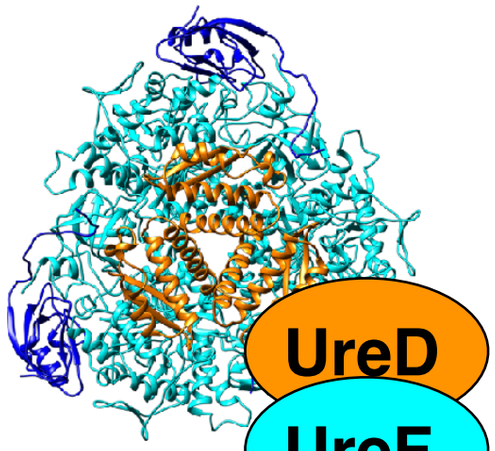
Urease activation pathway



UreF

UreD

Regulation
of GTPase



UreD

UreF

UreG

GTP hydrolysis,
Lys carbamylation

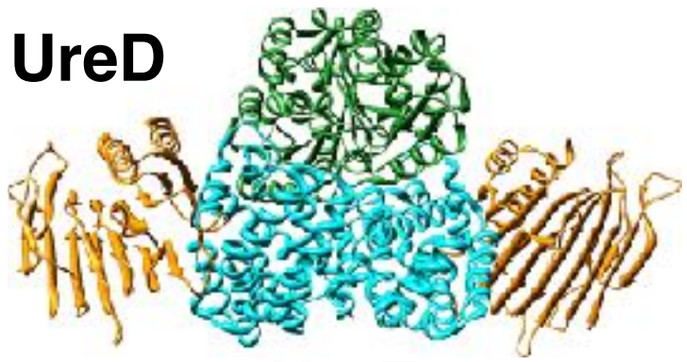
GTP

Pi +
GDP

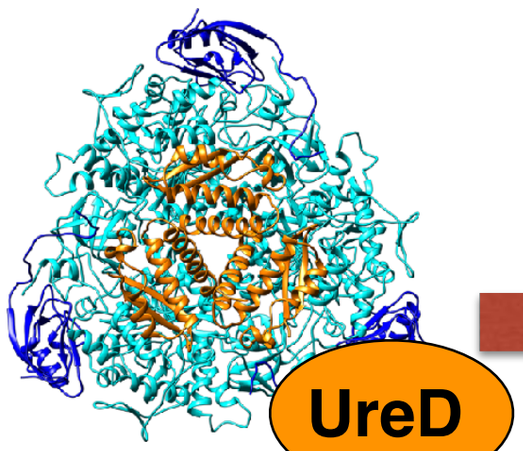
Recognition
platform

UreG

UreD

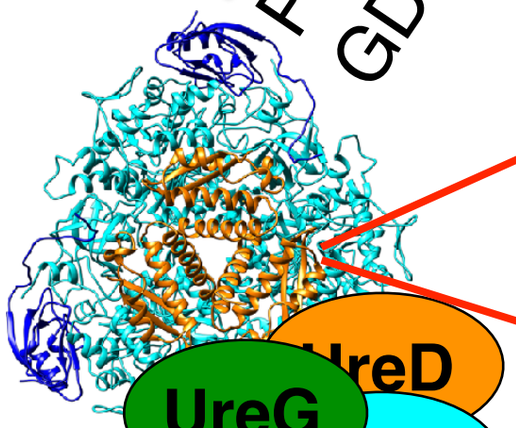


UreF



UreD

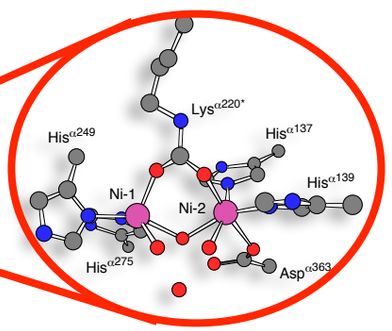
Apo-$(UreABC)_3$



UreD

UreG

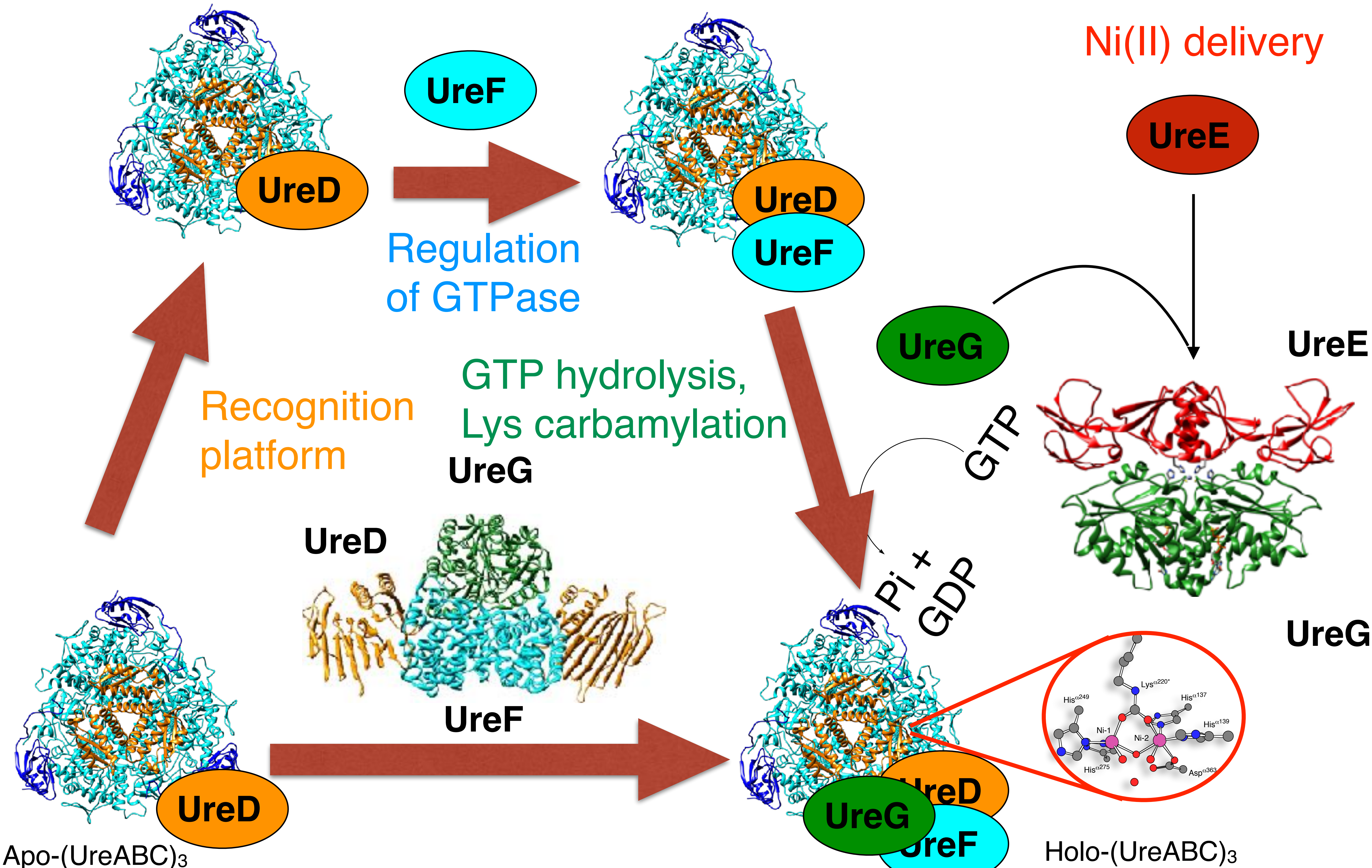
UreF



Holo-$(UreABC)_3$

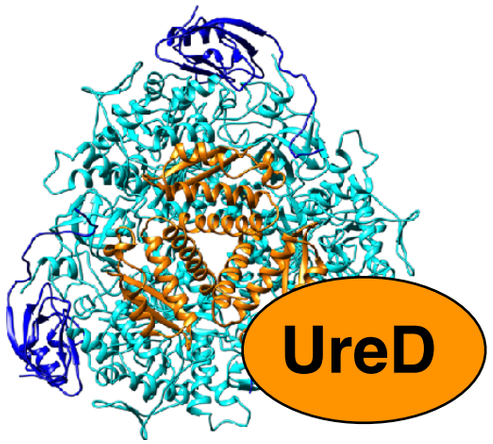
Zambelli B., Musiani, F., Benini S., Ciurli S. - *Acc. Chem. Res.* **2011**

Urease activation pathway



Zambelli B., Musiani, F., Benini S., Ciurli S. - *Acc. Chem. Res.* **2011**

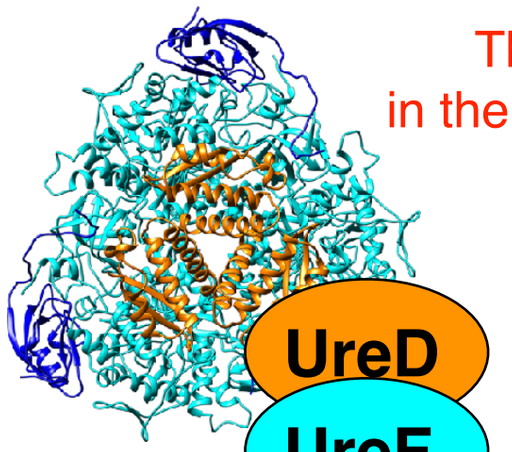
Urease activation pathway



UreD

UreF

Regulation
of GTPase



UreD

UreF

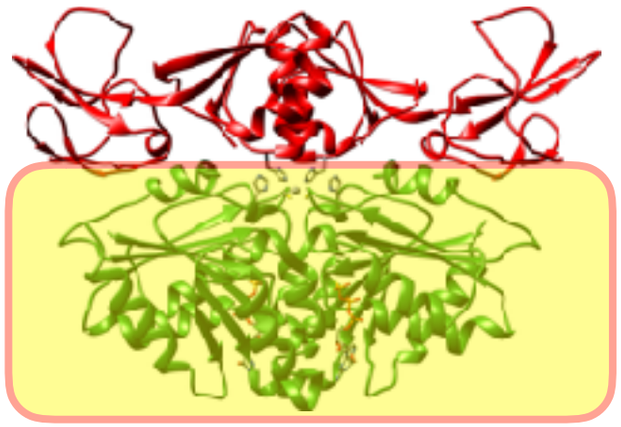
The GTPase
in the urease system

Ni(II) delivery

UreE

UreG

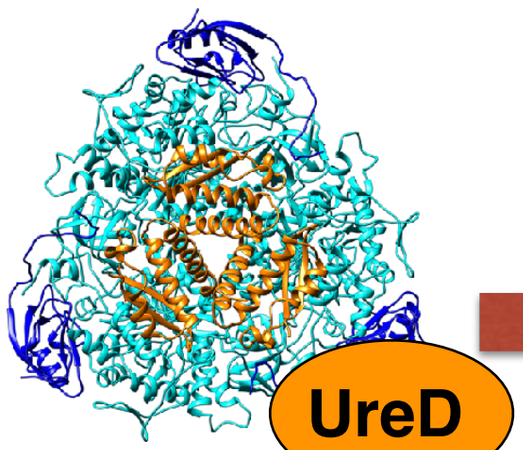
UreE



GTP

Pi +
GDP

UreG



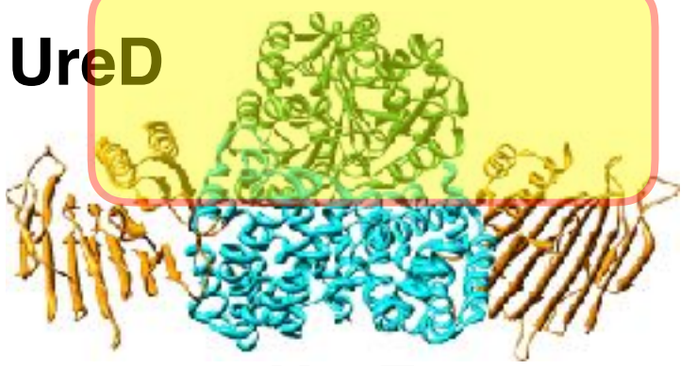
UreD

Apo-(UreABC)$_3$

Recognition
platform

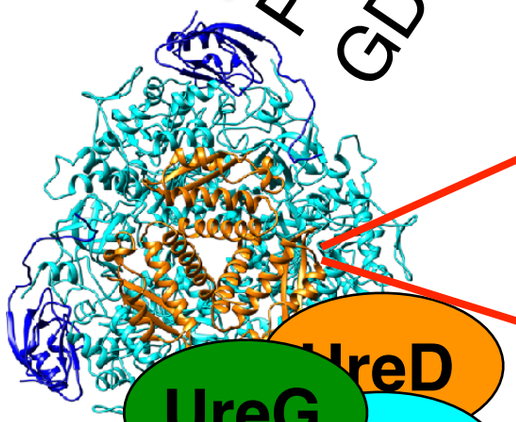
GTP hydrolysis,
Lys carbamylation

UreG



UreD

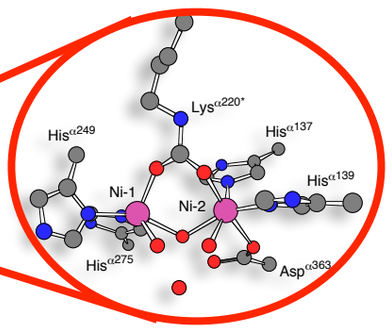
UreF



UreD

UreG

UreF



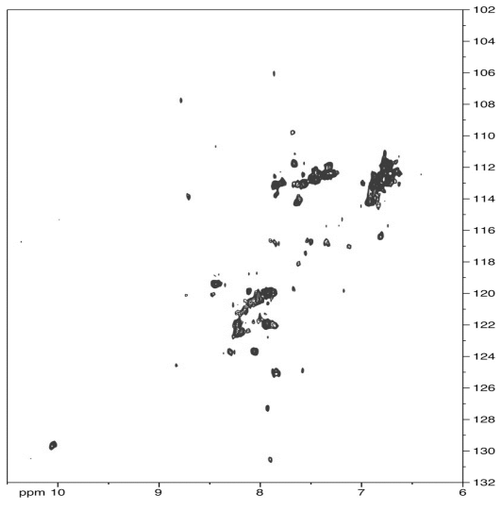
Holo-(UreABC)$_3$

Zambelli B., Musiani, F., Benini S., Ciurli S. - *Acc. Chem. Res.* **2011**

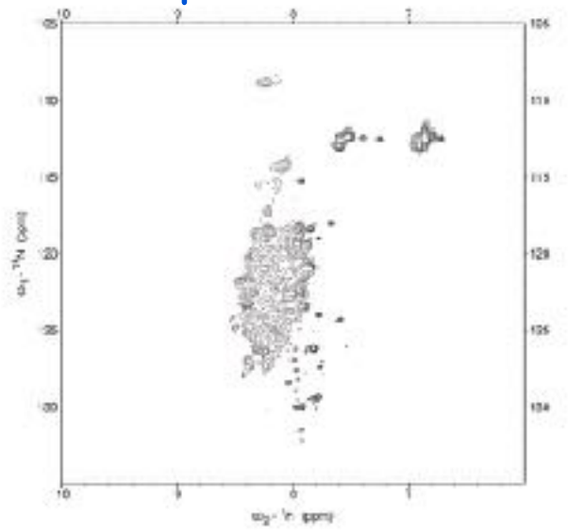
UreG proteins are all intrinsically disordered

Protein rigidity

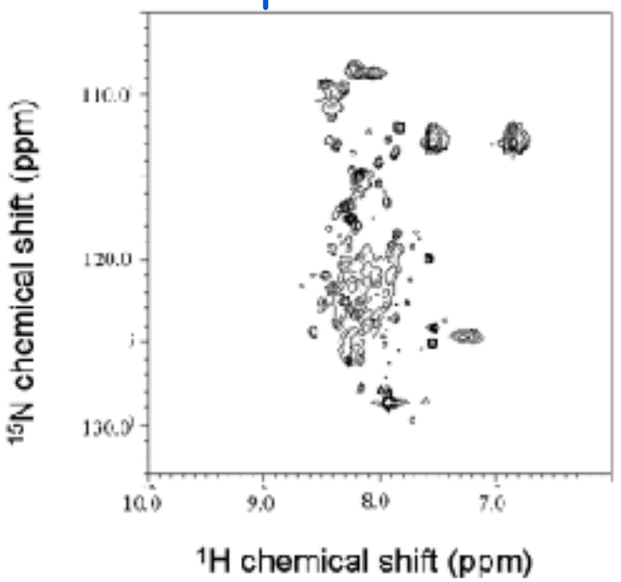
M. jannaschii UreG
Thermophilic archea



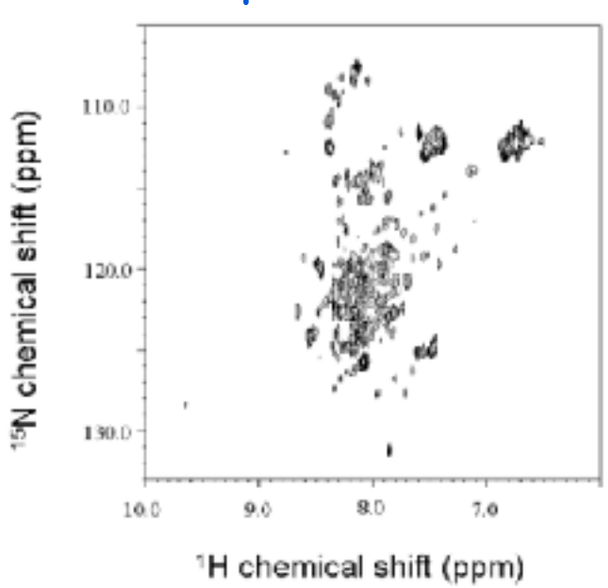
M. tuberculosis UreG
Mesophile Bacterium



S. pasteurii UreG
Mesophile Bacterium

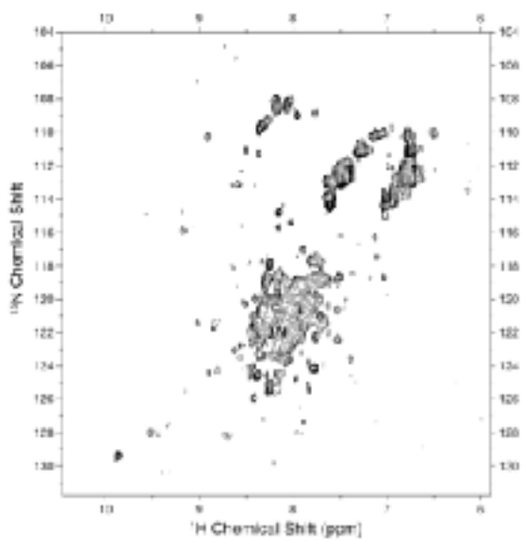


K. aerogenes UreG
Mesophile Bacterium

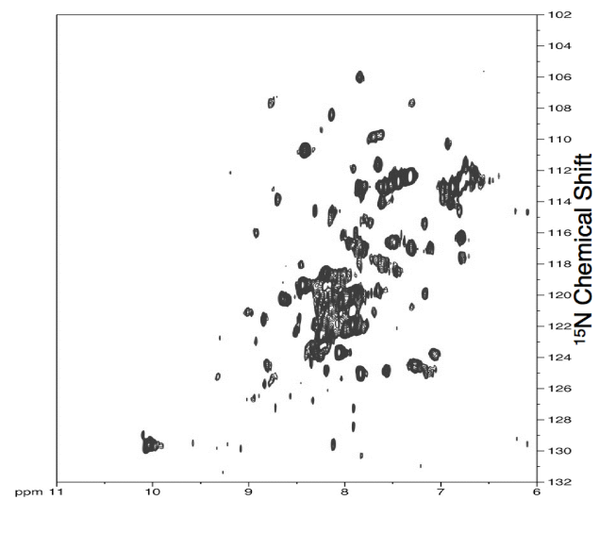


Protein rigidity

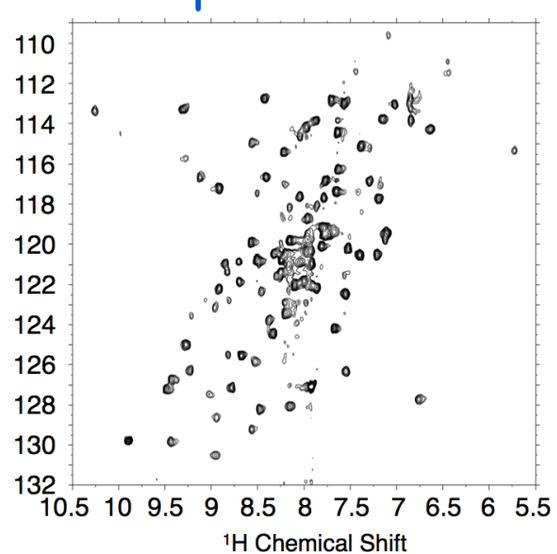
G. max UreG
Plant



M. saedula UreG
Thermophilic Archea



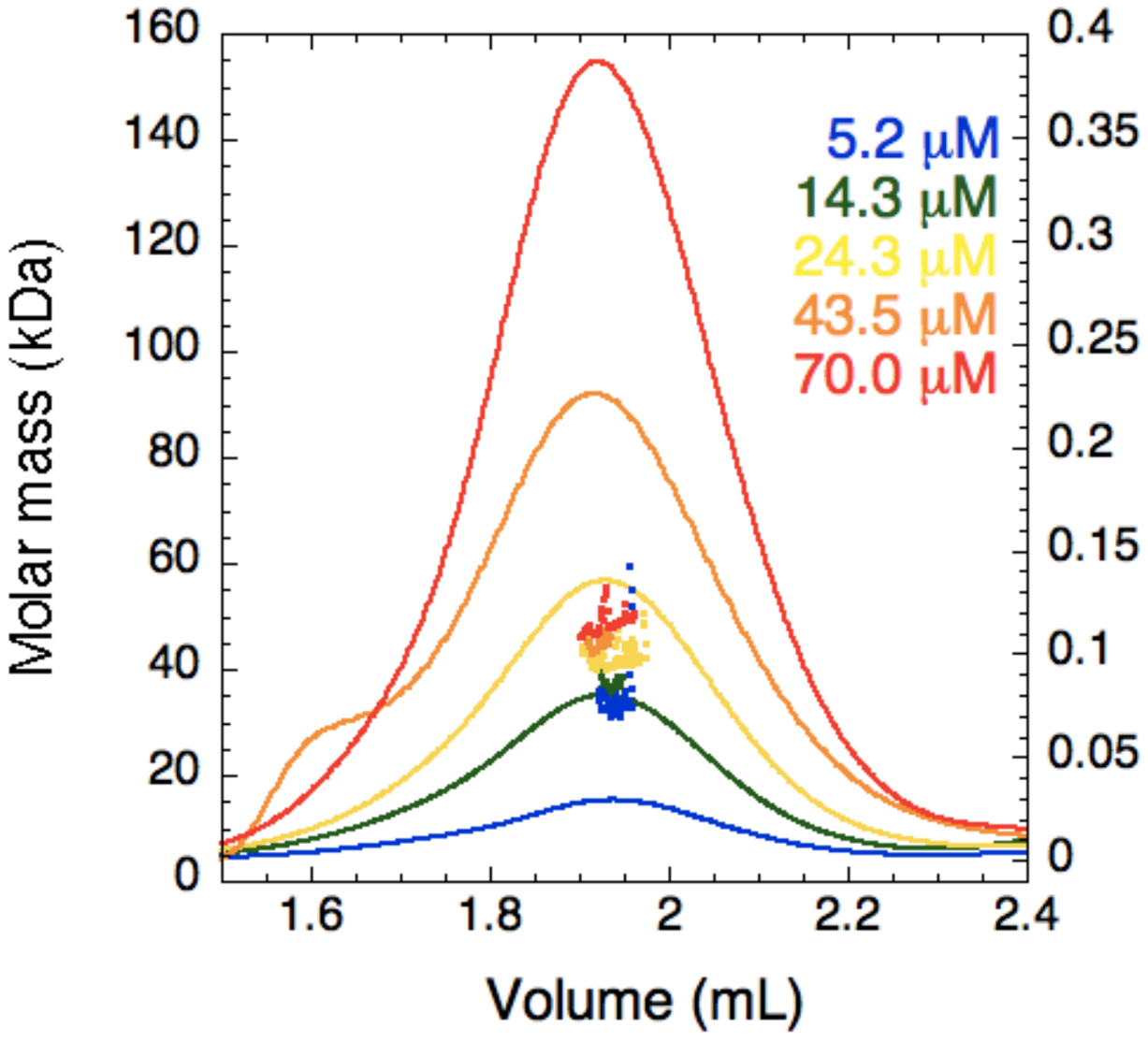
H. pylori UreG
Mesophile Bacterium

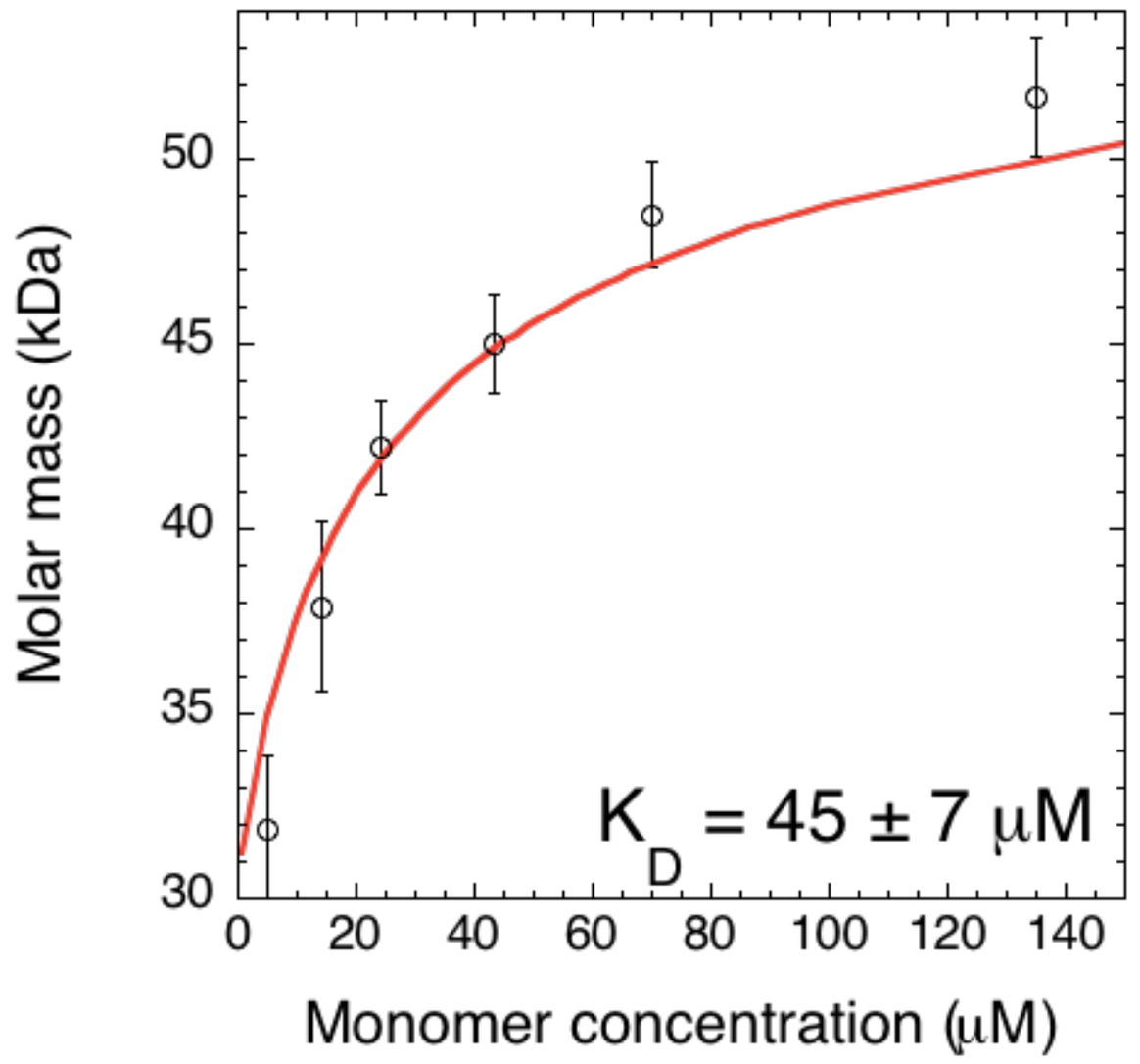


Zambelli et al. - *J. Biol. Chem.* **2005**
Zambelli et al. - *Biochemistry* **2006**
Zambelli et al. - *Proteins* **2005**
Guerra et al. - *Plant J.* **2012**
Miralula et al - *J. Biol. Inorg. Chem.* ***2014***

UreG quaternary structure

Size exclusion chromatography + light scattering of SpUreG



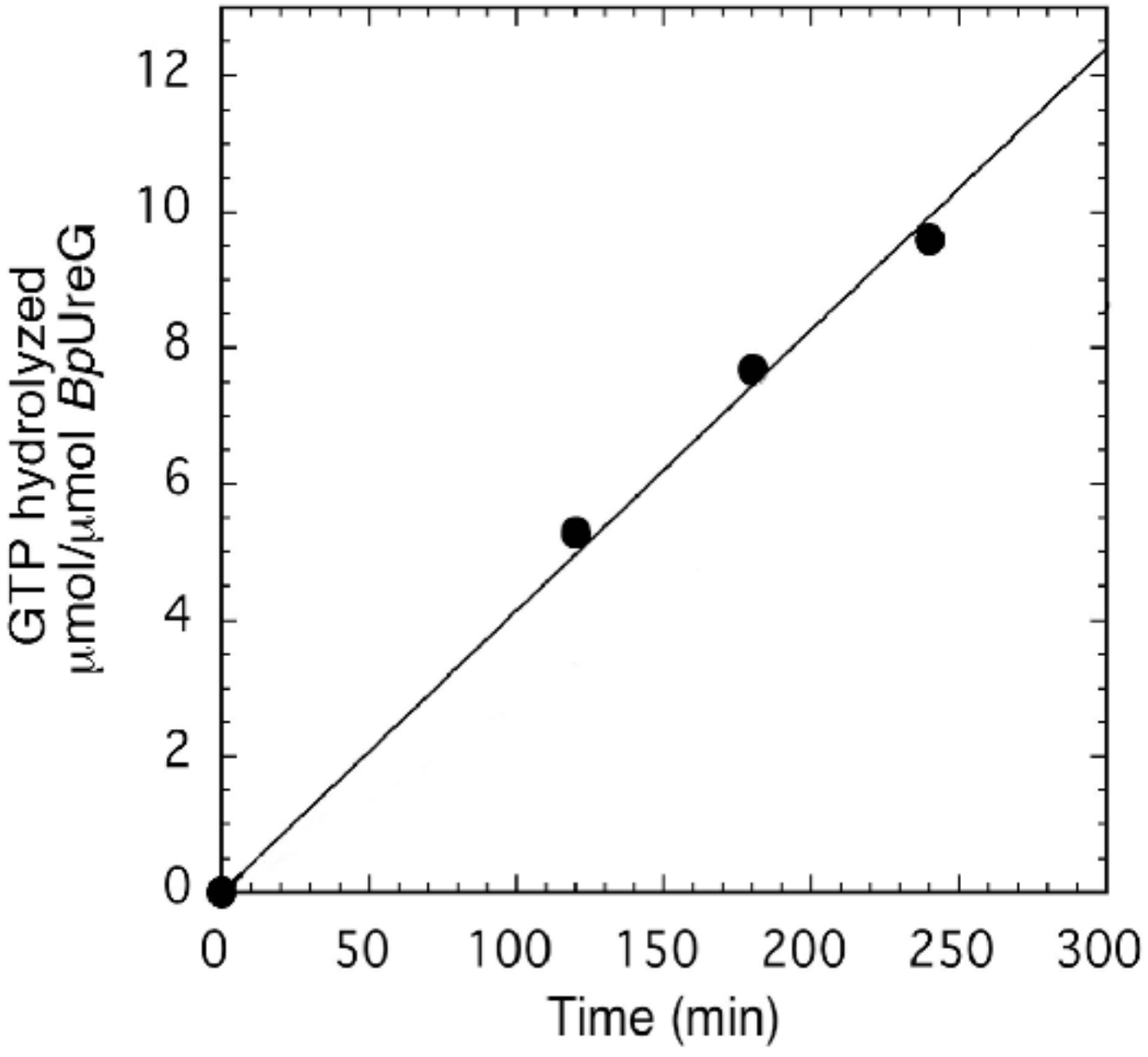


UreG exists in concentration-dependent monomer-dimer equilibrium

D'Urzo, A.; Santambrogio, C; Grandori, R; Ciurli, S.; Zambelli, B.: J. Biol. Inorg. Chem. **2014**

Some isolated UreG proteins are active

Functional studies: GTP hydrolysis



***Sp*UreG: k_{cat}= 0.04 min^{-1}**
*Mt*UreG: k_{cat}= 0.01 min^{-1}
*Ms*UreG: k_{cat}= 0.02 min^{-1}
*Gm*UreG: k_{cat}= 0.01 min^{-1}
*Ka*UreG: no activity
*Hp*UreG: no activity
*Mj*UreG: no activity

B. Zambelli, M. Stola, F. Musiani, S. Ciurli, *et al.* - *J. Biol. Chem.* **2005**

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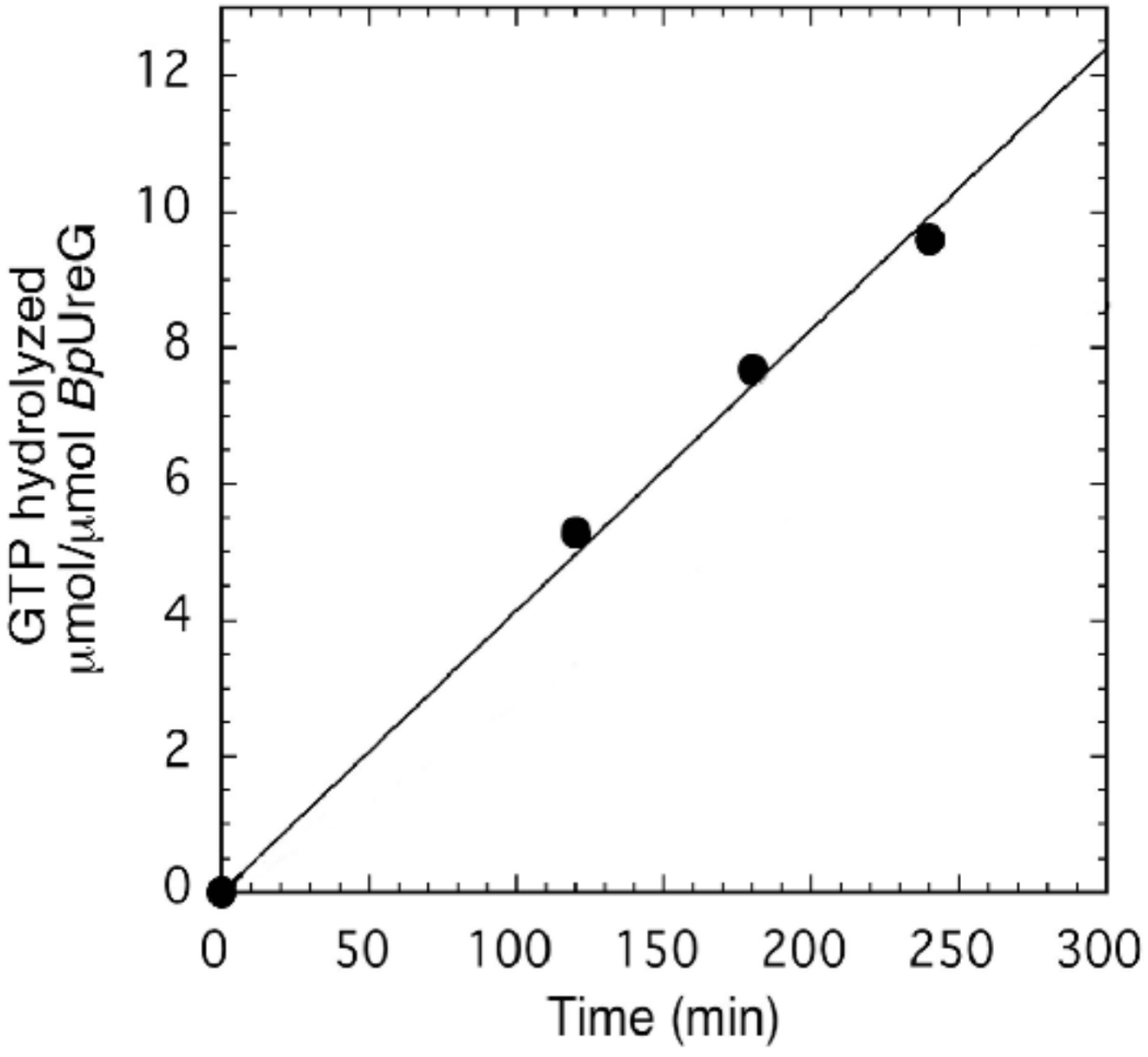

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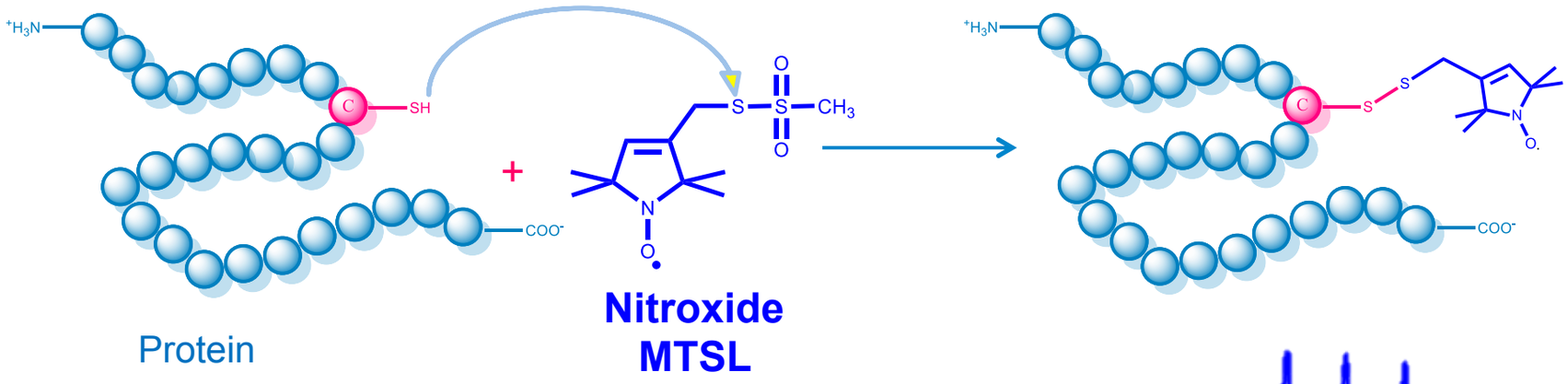
*Gm*UreG: k_{cat}= 0.01 min^{-1}
*Ka*UreG: no activity
*Hp*UreG: no activity
*Mj*UreG: no activity

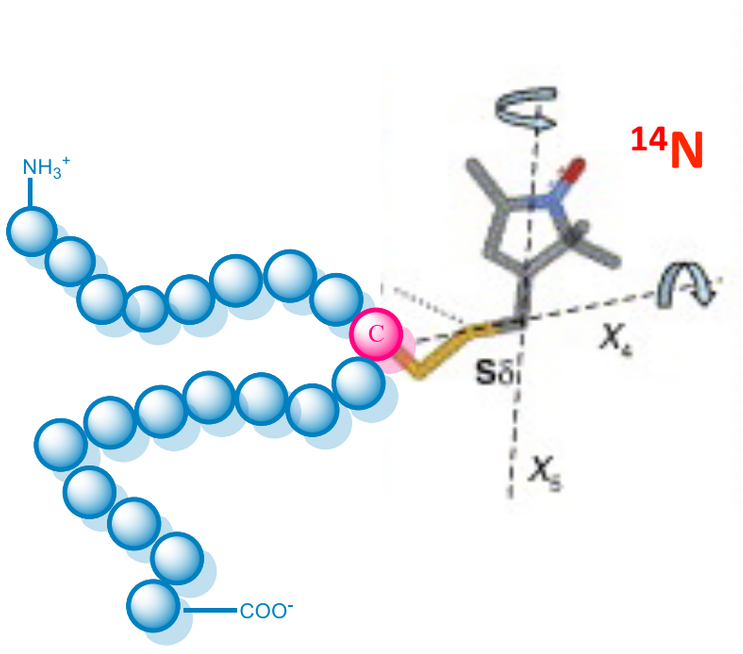
*Ec*HypB*:* k_{cat}= 0.17 min^{-1}
*Hp*HypB: k_{cat}= 0.36 min^{-1}
*Bj*HypB: k_{cat}= 0.18 min^{-1}

B. Zambelli, M. Stola, F. Musiani, S. Ciurli, *et al.* - *J. Biol. Chem.* **2005**

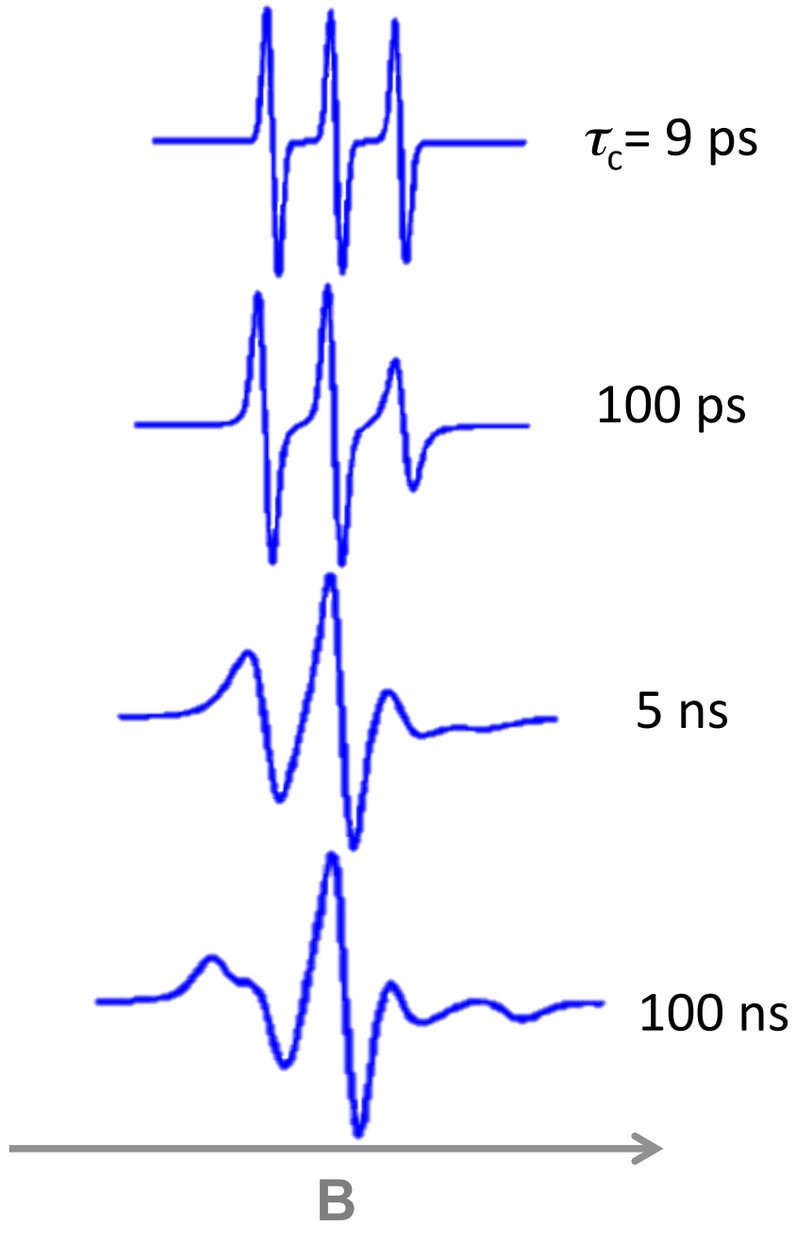
Understanding the **conformational** and **functional** landscape sampled by UreG as a function of **temperature**, **denaturants** and **additives** provides insight into the structural organization and stability of UreG **in solution**

Site-directed spin labelled EPR



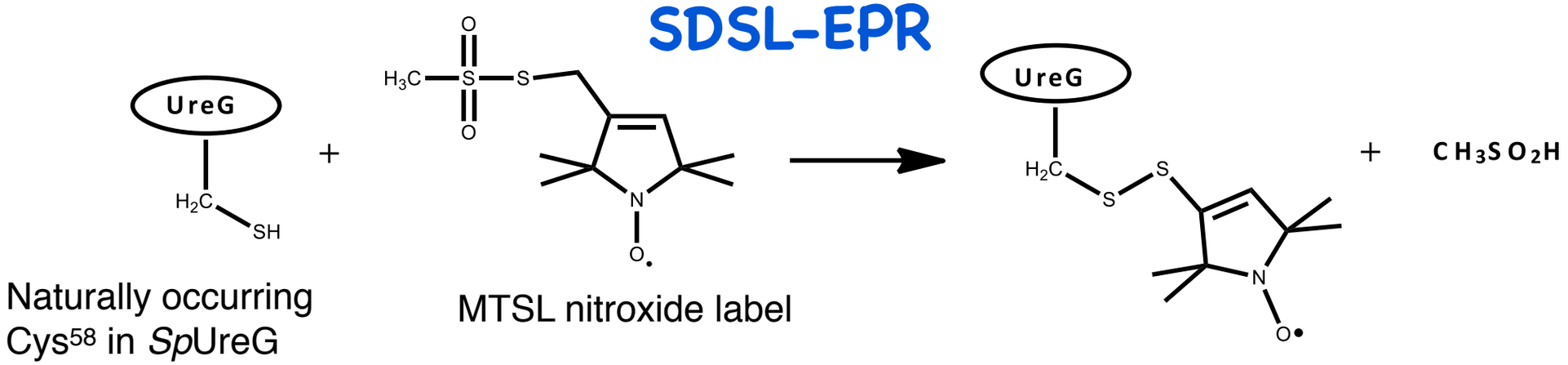


A nitroxide label is specifically grafted on a cysteine residue. The paramagnetic label bound to the protein is a reporter of the local dynamics, reflected in the EPR spectral line shape.



Site-directed spin labelled EPR on UreG

SDSL-EPR



Naturally occurring Cys[58] in *Sp*UreG

MTSL nitroxide label

Labelled *Sp*UreG

M. Palombo , S. Ciurli , V. Uversky , E. Mileo, B. Zambelli et al. *Scientific Reports,* **2017**

Site-directed spin labelled EPR on UreG

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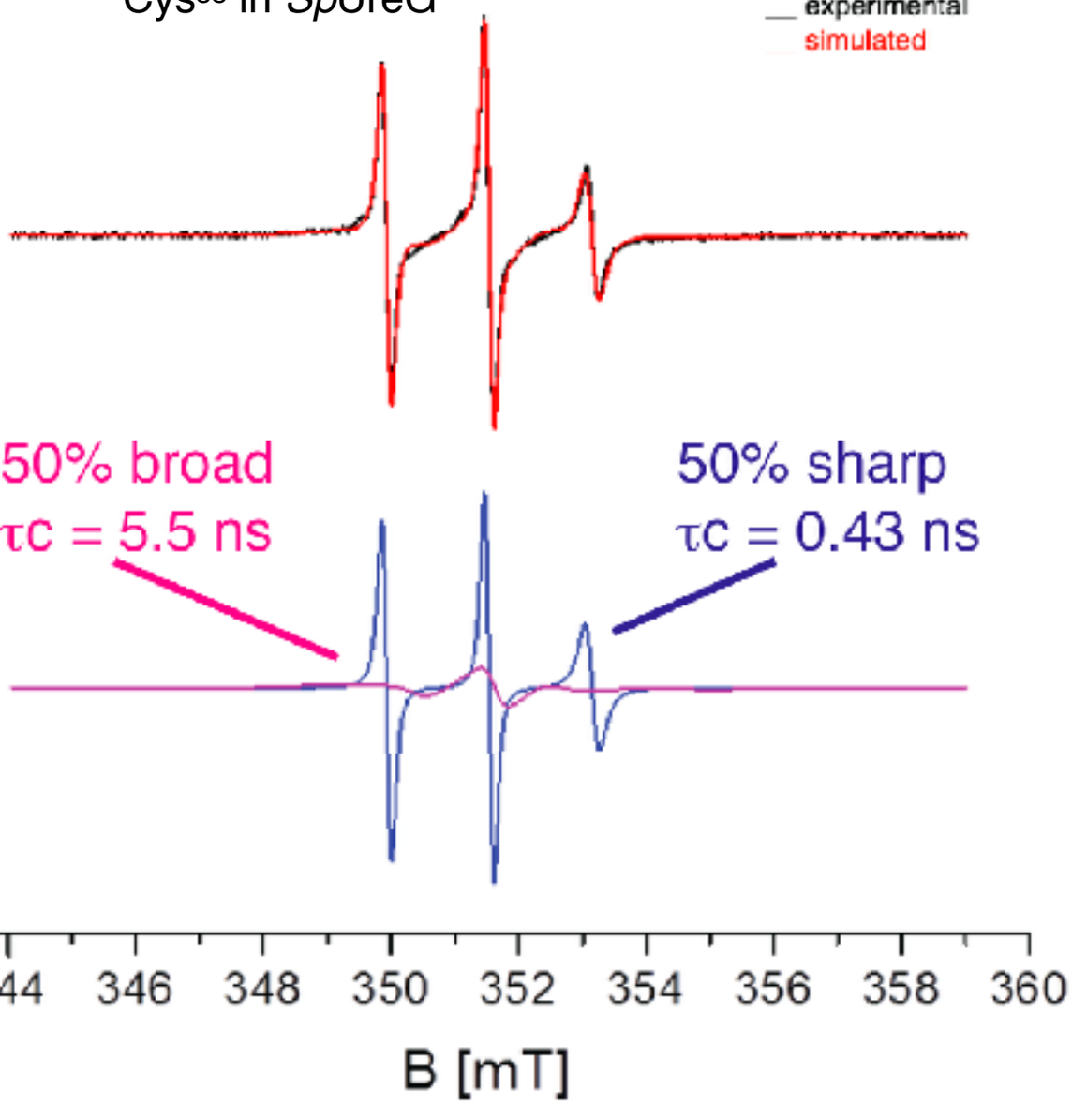

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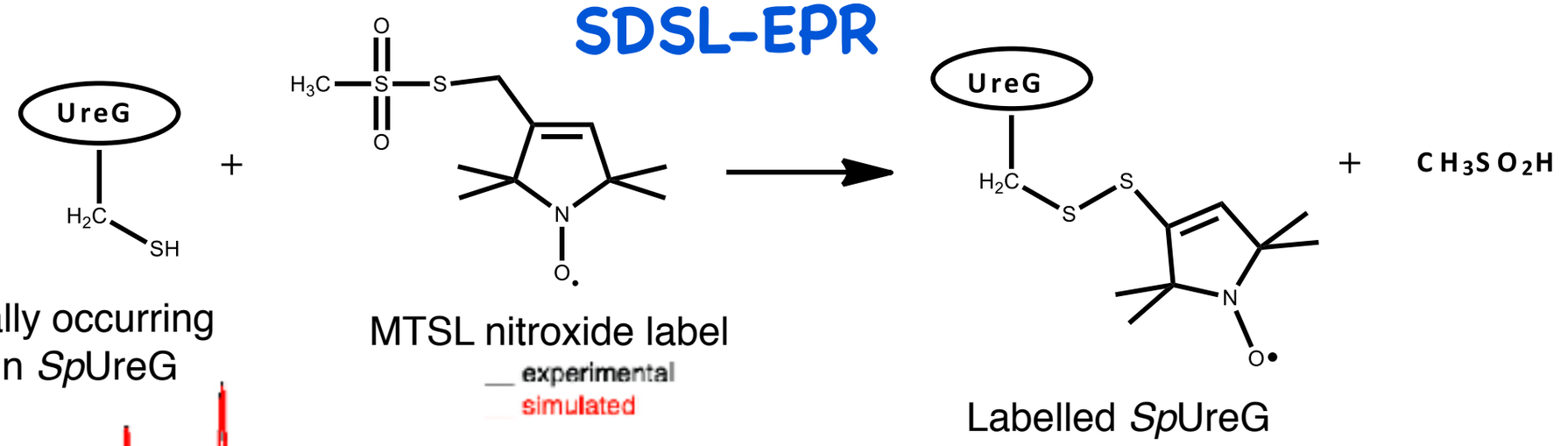

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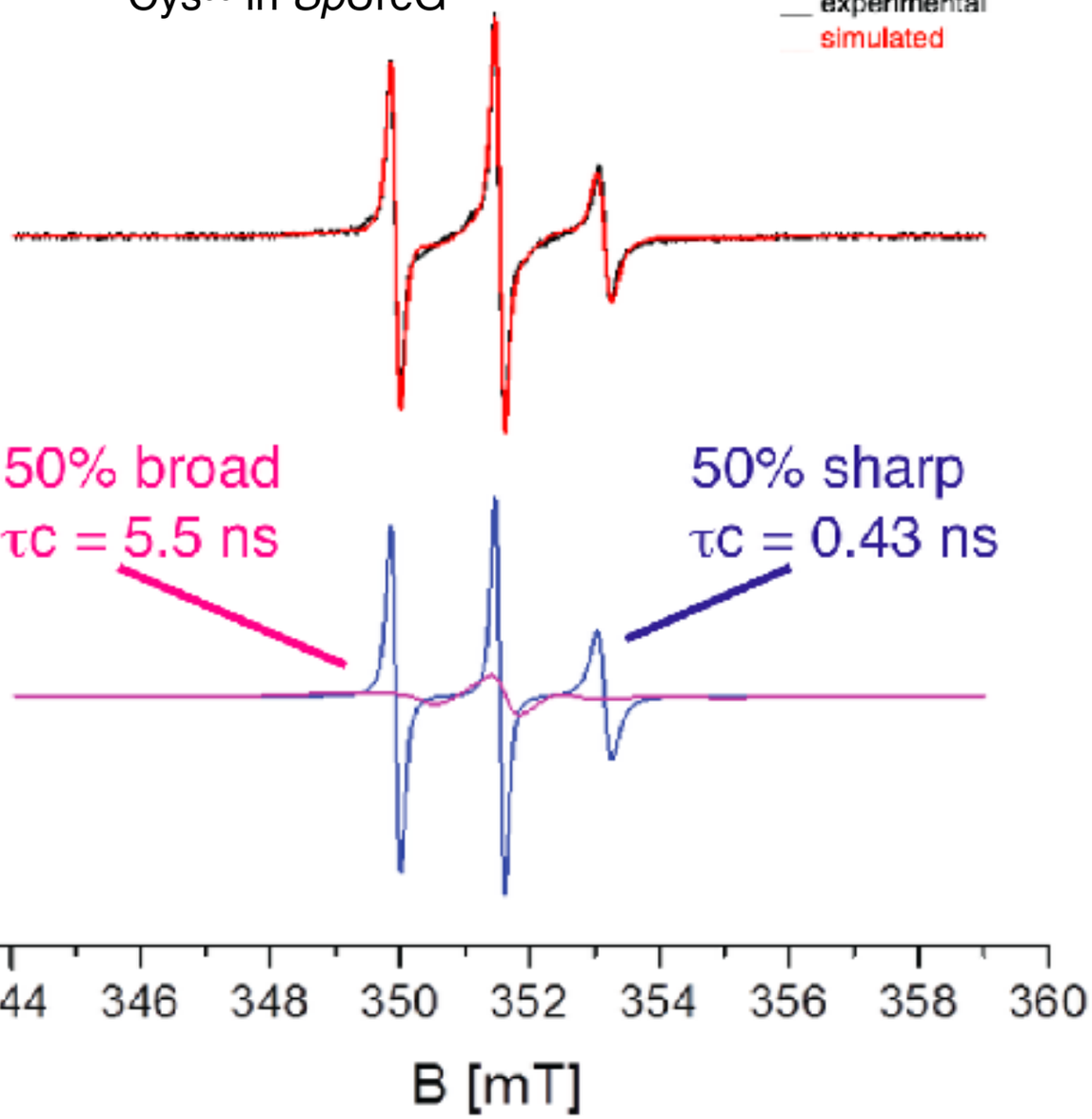
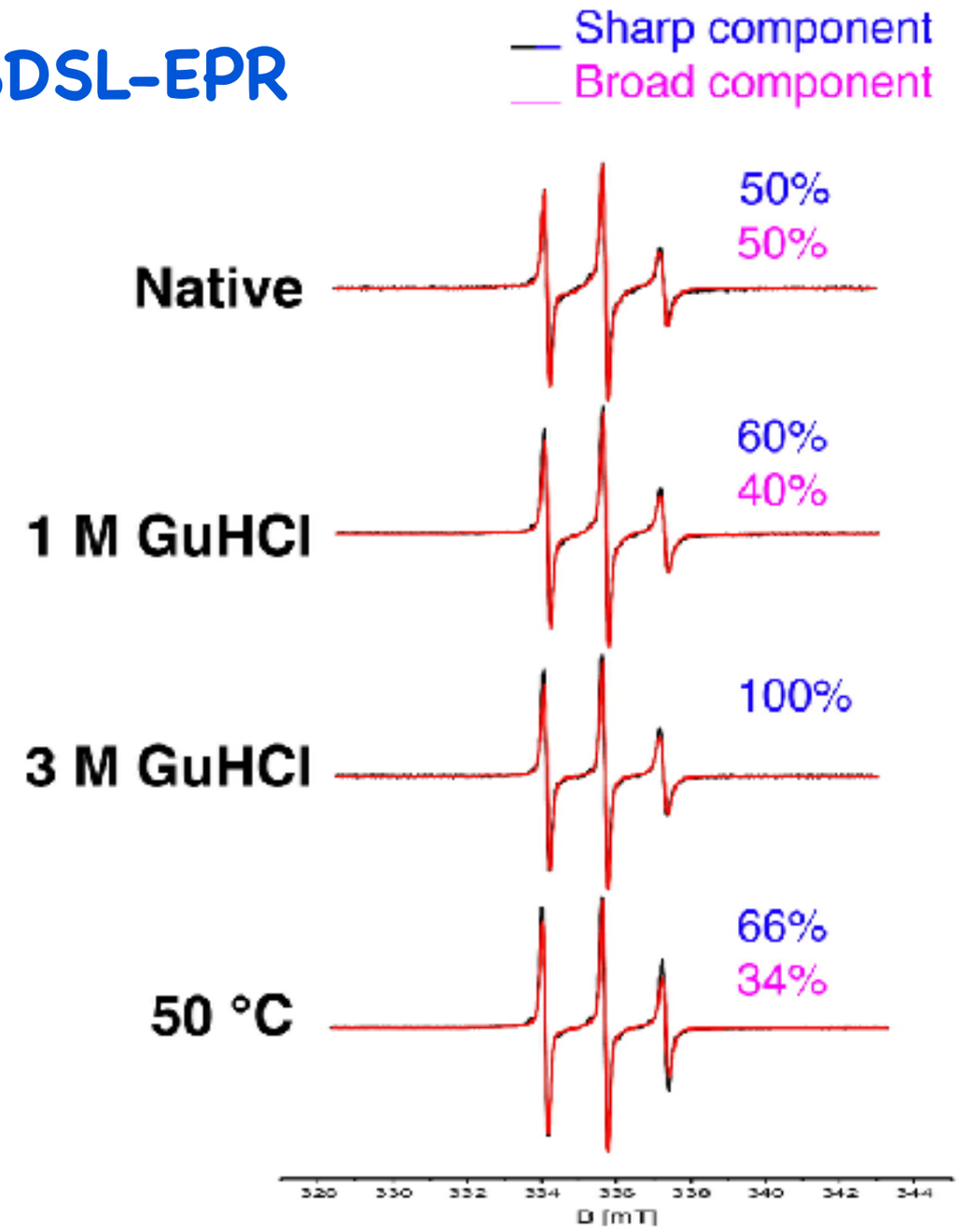


UreG exists in at least two major conformations of equal representation : a «sharp» component and a « broad » component.

M. Palombo , S. Ciurli , V. Uversky , E. Mileo, B. Zambelli et al. *Scientific Reports,* **2017**

Dependence of UreG dynamics by denaturants and temperature

SDSL-EPR



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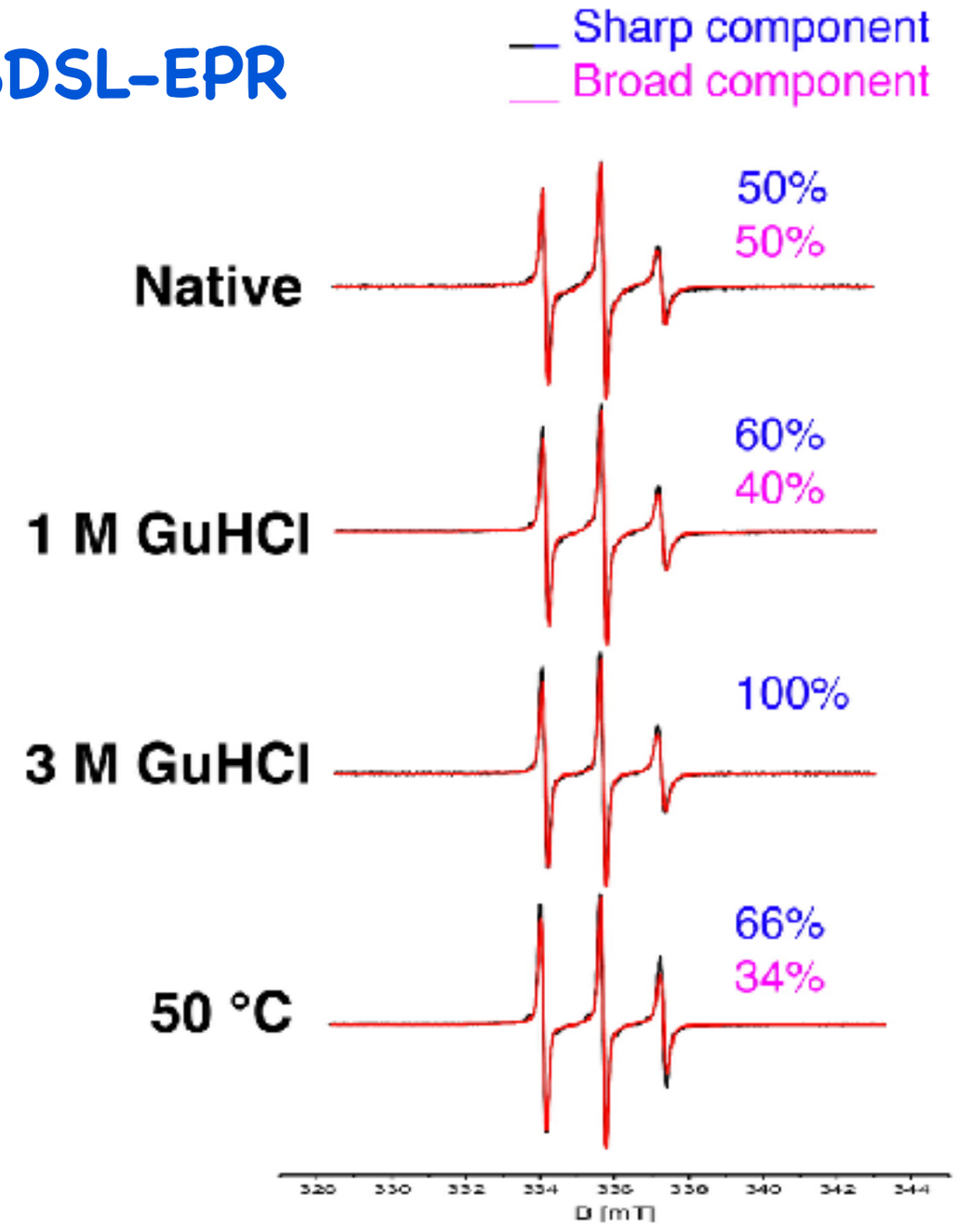
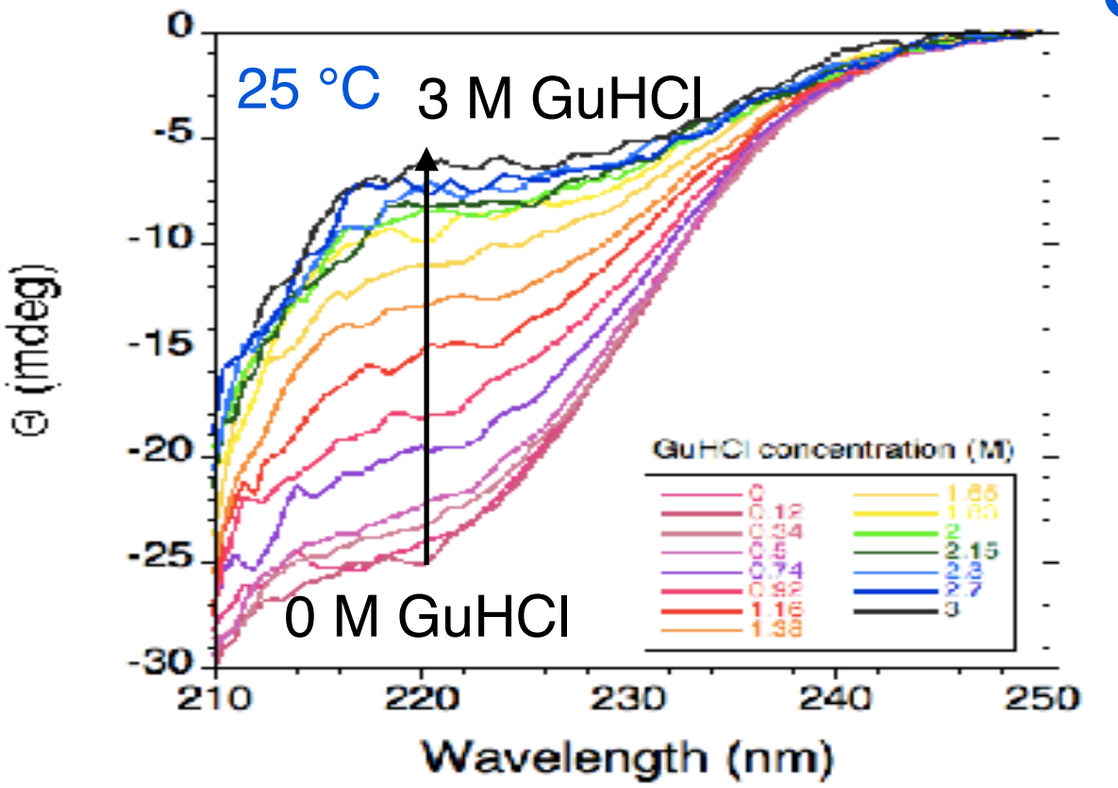
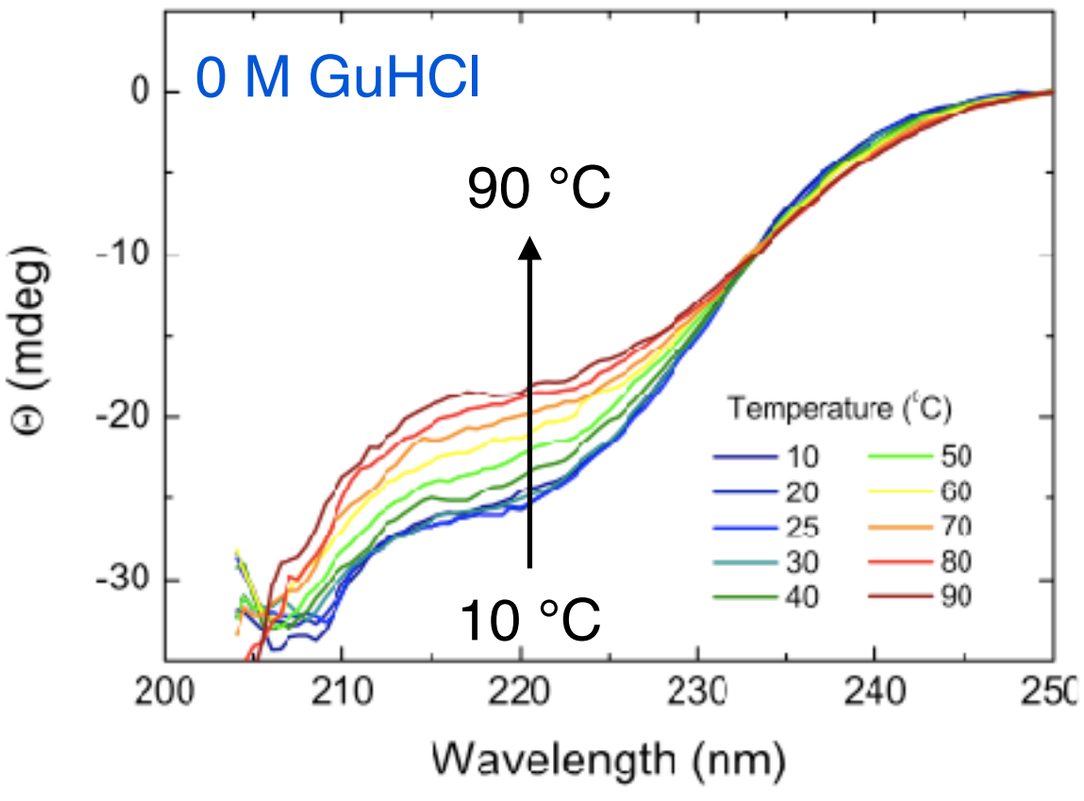


CD





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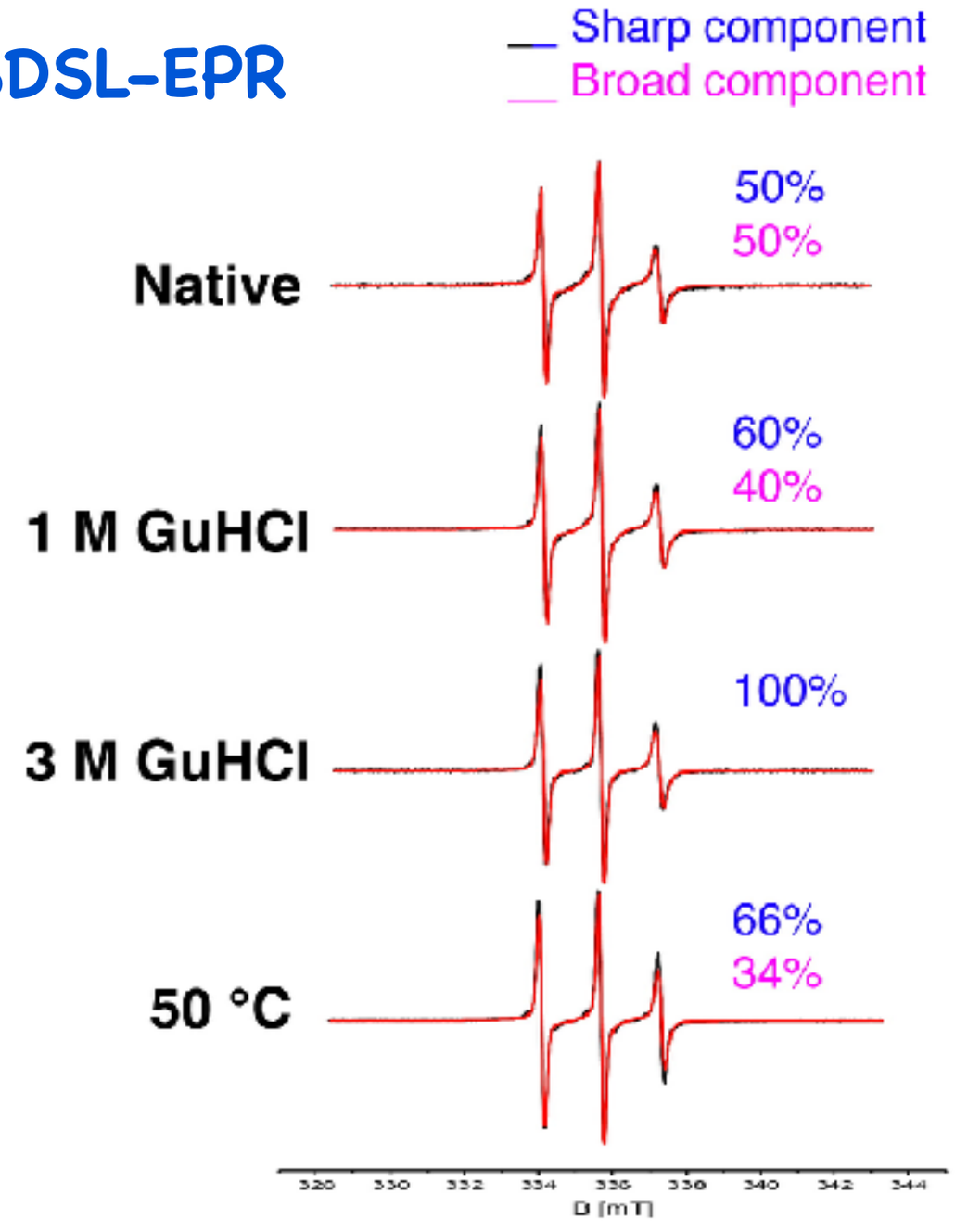
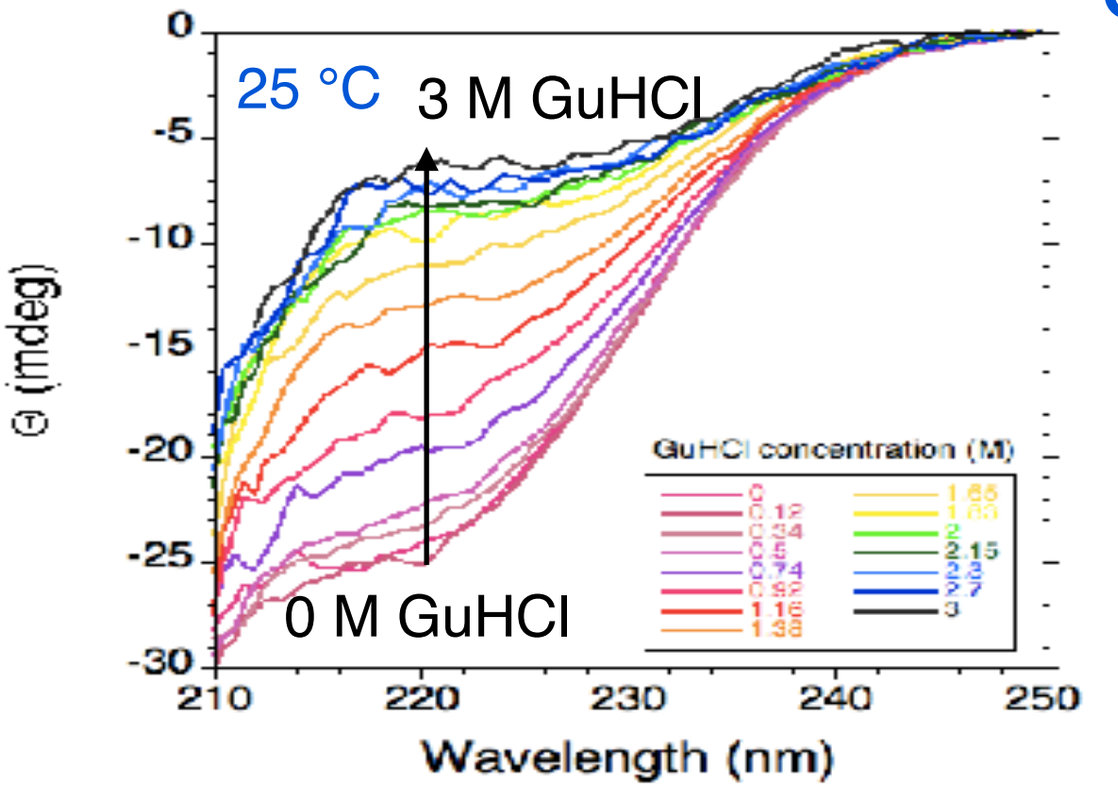
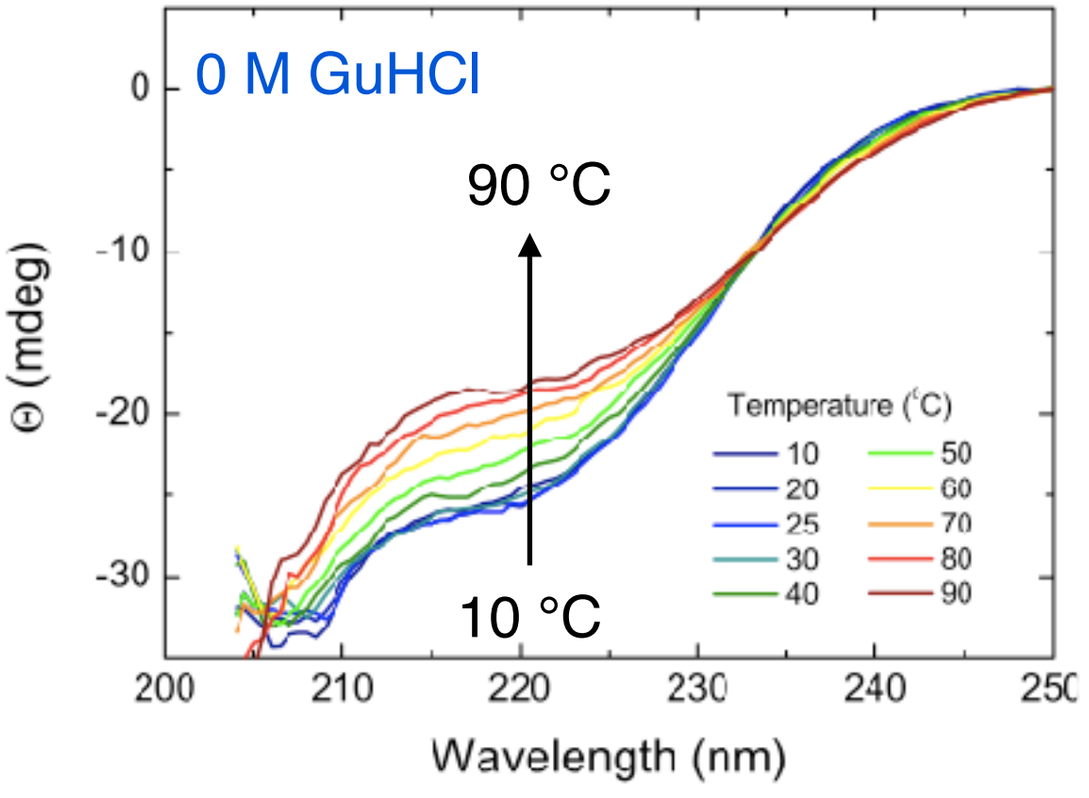


Temperature and denaturants decrease UreG rigidity and secondary structure

CD





Dependence of UreG dynamics by folding inducers

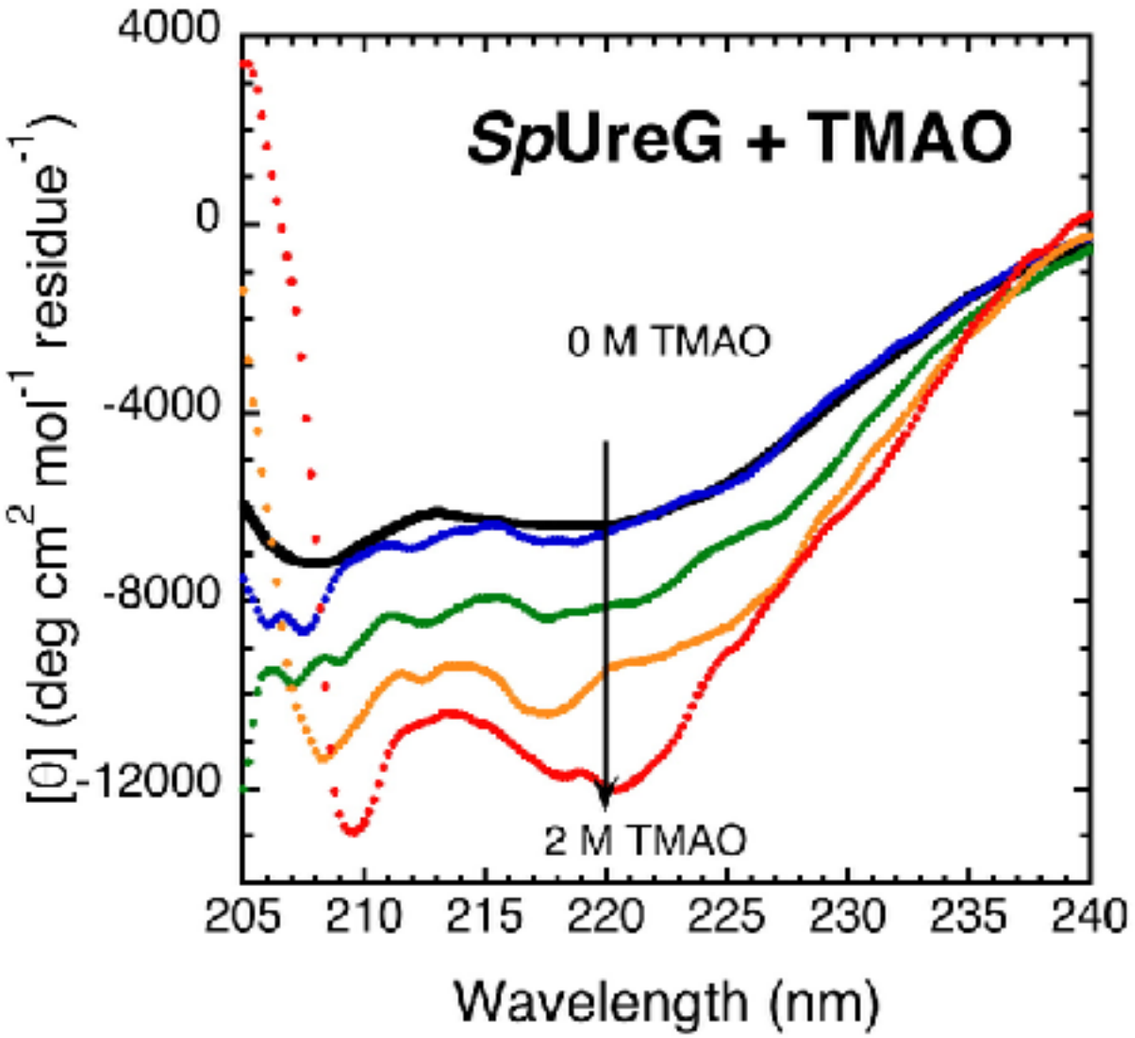
Sharp component
Broad component

Native 50% 50%

1 M TMAO 23% 77%

2 M TMAO 11% 89%

Circular dichroism



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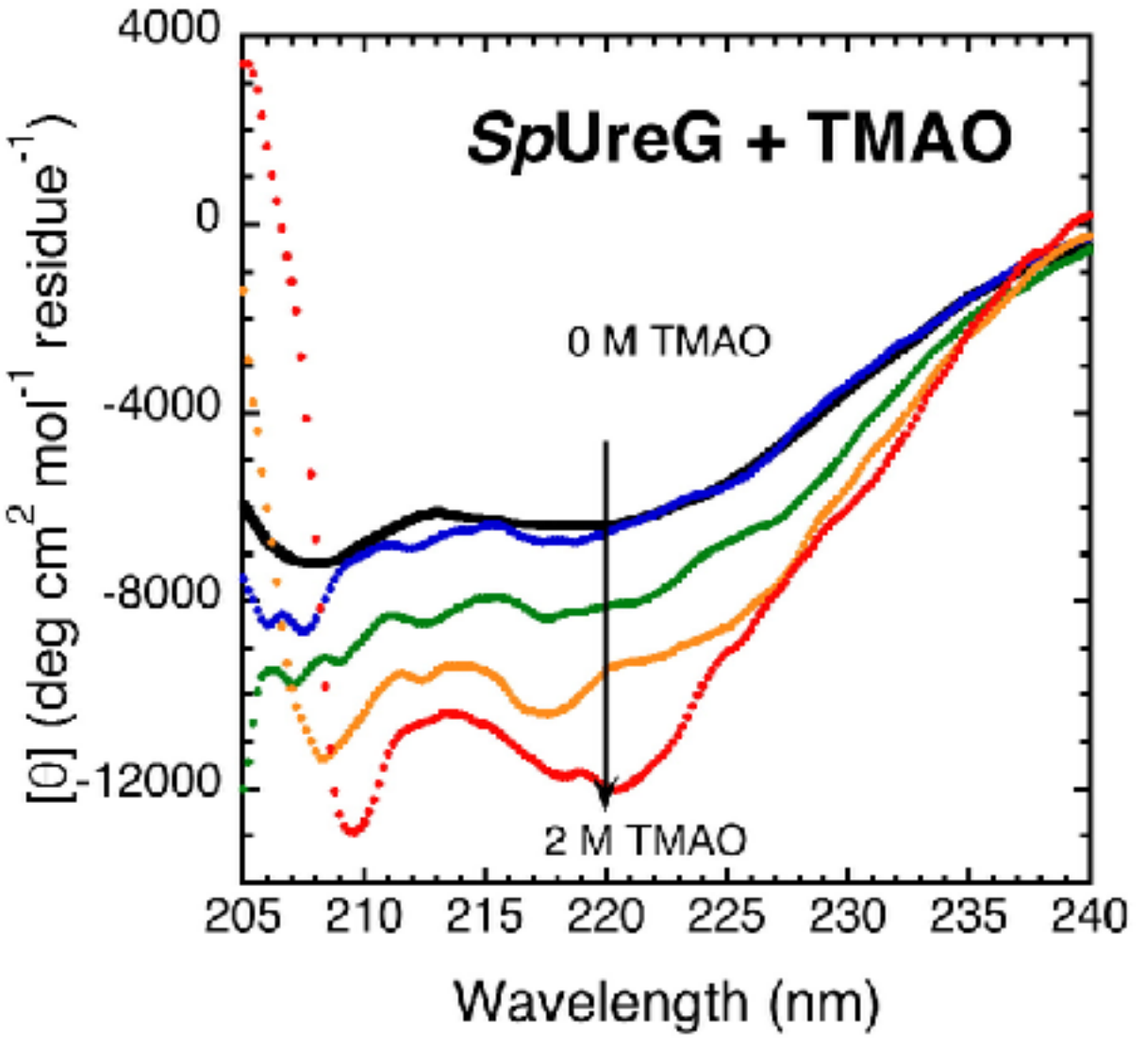


Trimethylamine N-oxide (TMAO) increases UreG rigidity and secondary structure

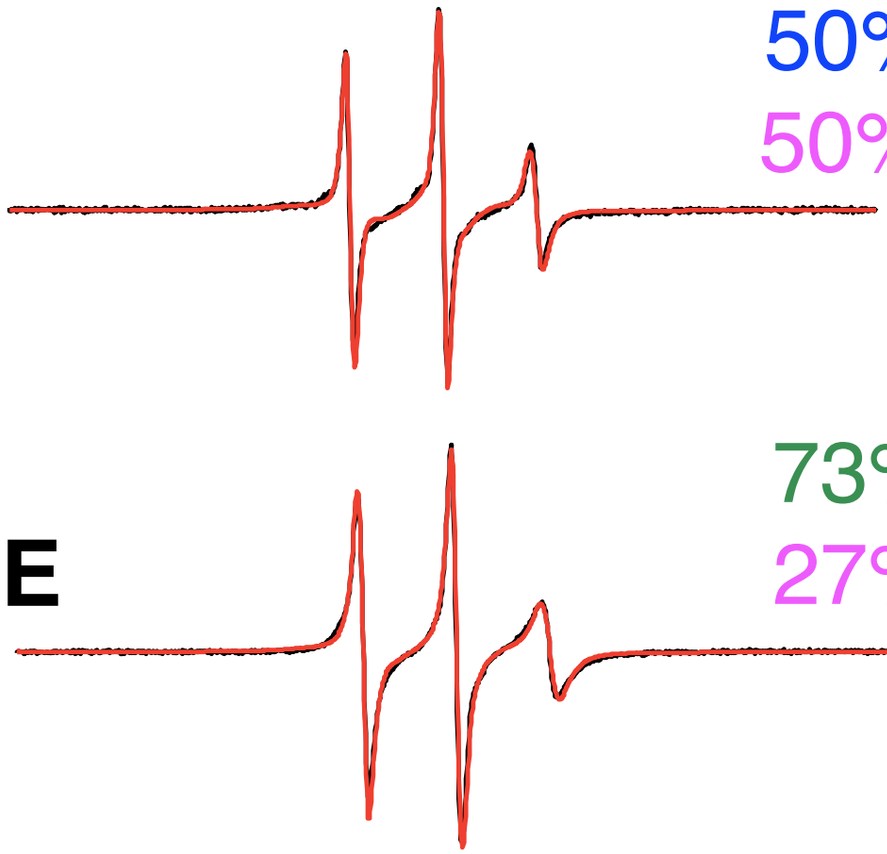
Dependence of UreG dynamics by folding inducers

SDSL-EPR

Sharp component: τc = 0.44 ns TFE component: τc = 0.73 ns

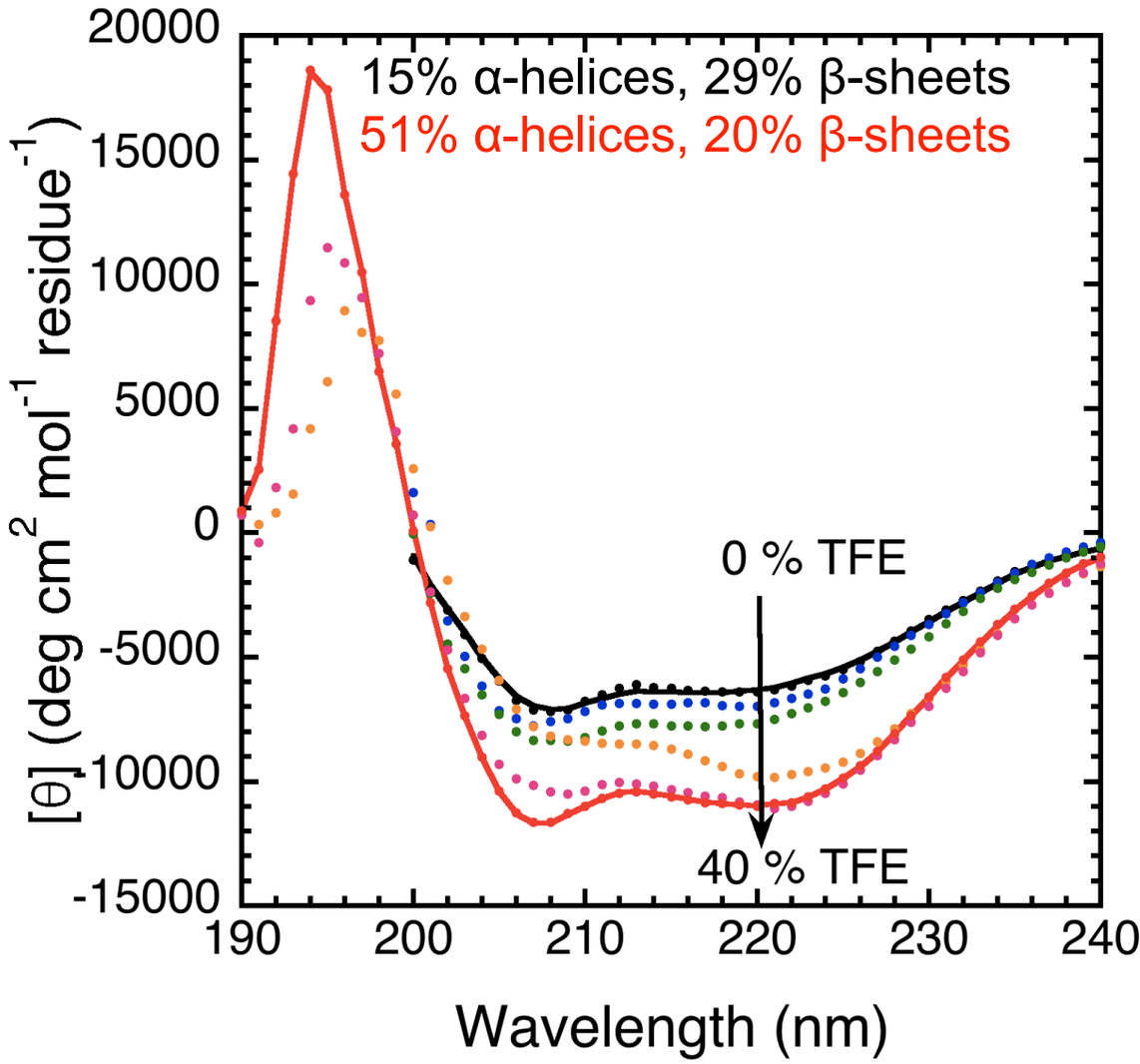
Broad component: τc = 5.5 ns

Native



40% TFE

Circular dichroism



M. Palombo , S. Ciurli , V. Uversky , E. Mileo, B. Zambelli et al. *Scientific Reports,* **2017**

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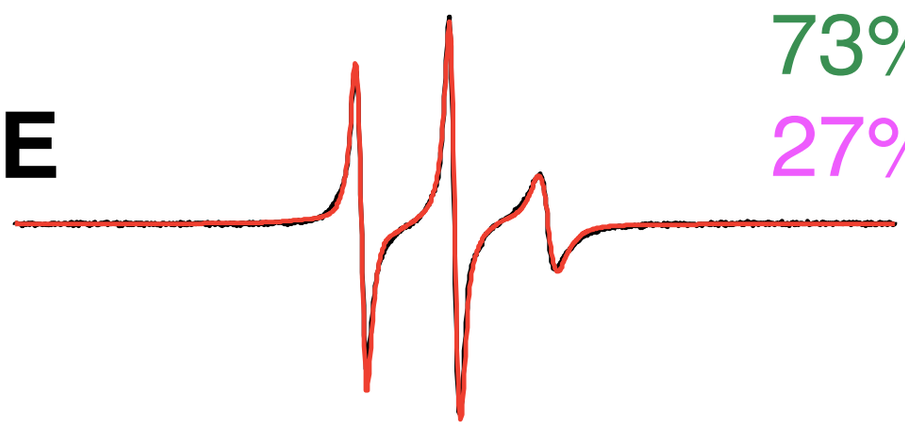
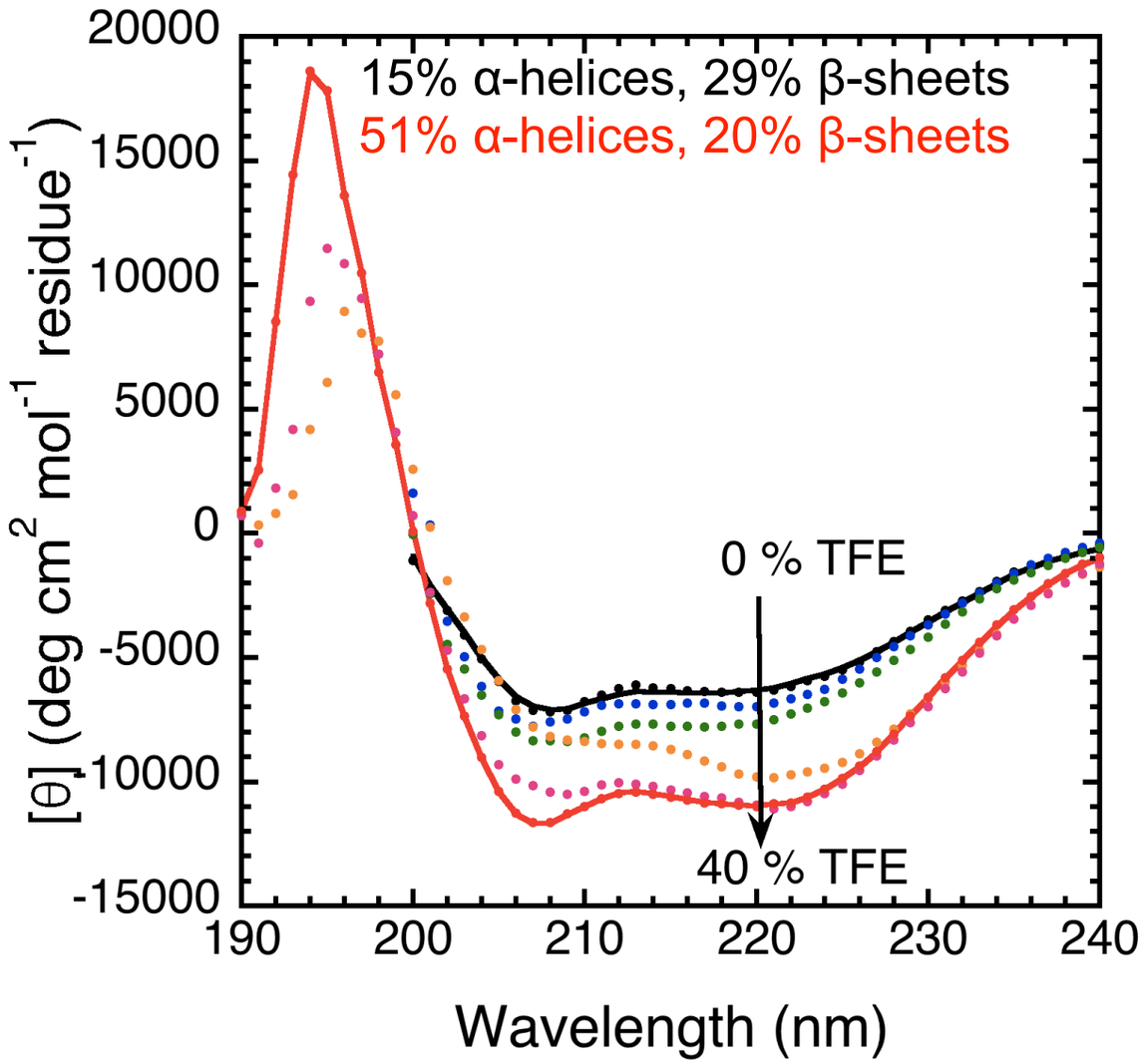


Circular dichroism



Trifluoroethanol (TFE) changes the "sharp" component, while increasing secondary structure

M. Palombo , S. Ciurli , V. Uversky , E. Mileo, B. Zambelli et al. *Scientific Reports,* **2017**

Dependence of UreG dynamics by folding inducers

SDSL-EPR

Sharp component: τc = 0.44 ns SDS component: τc = 1.7 ns

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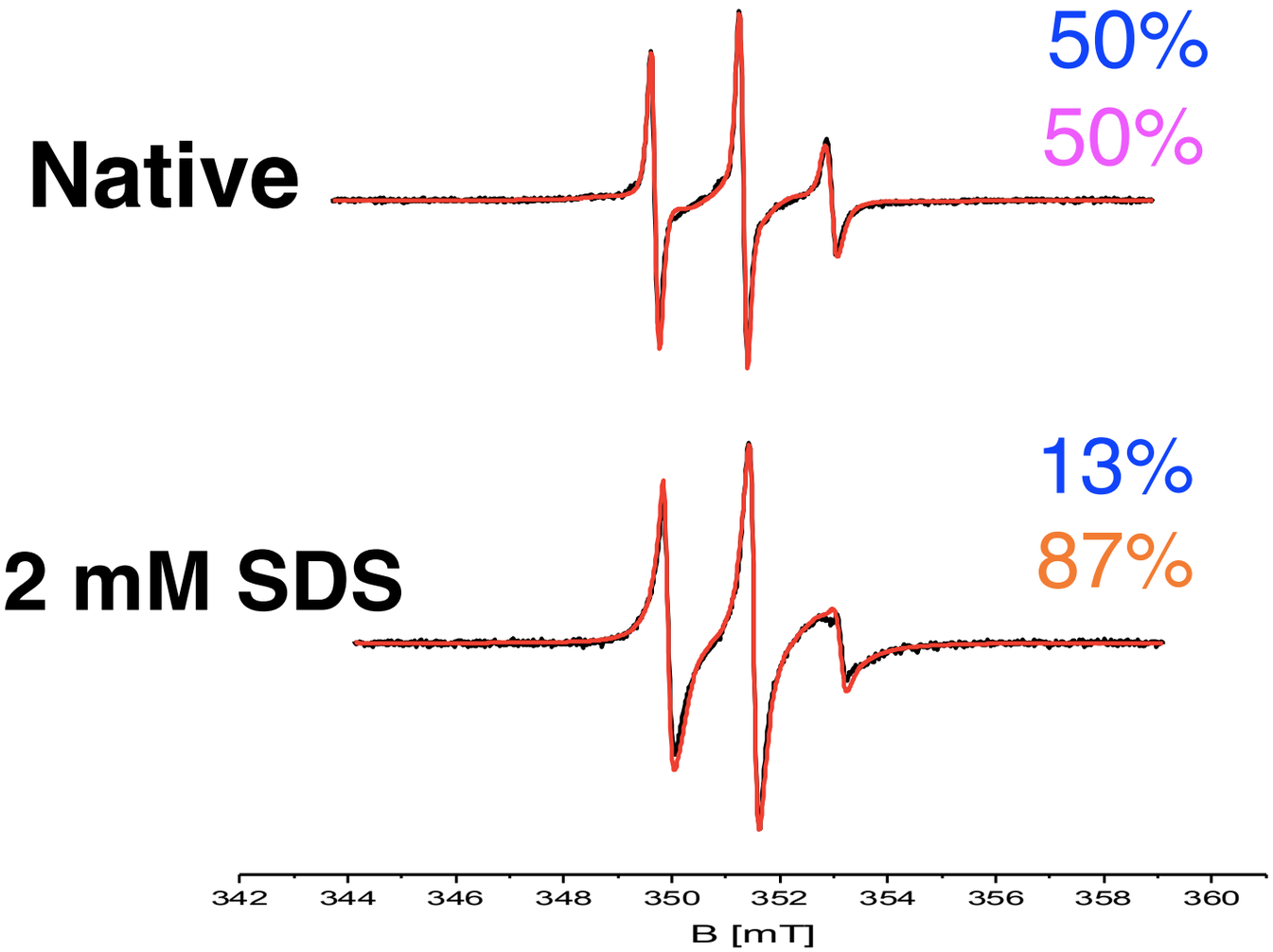
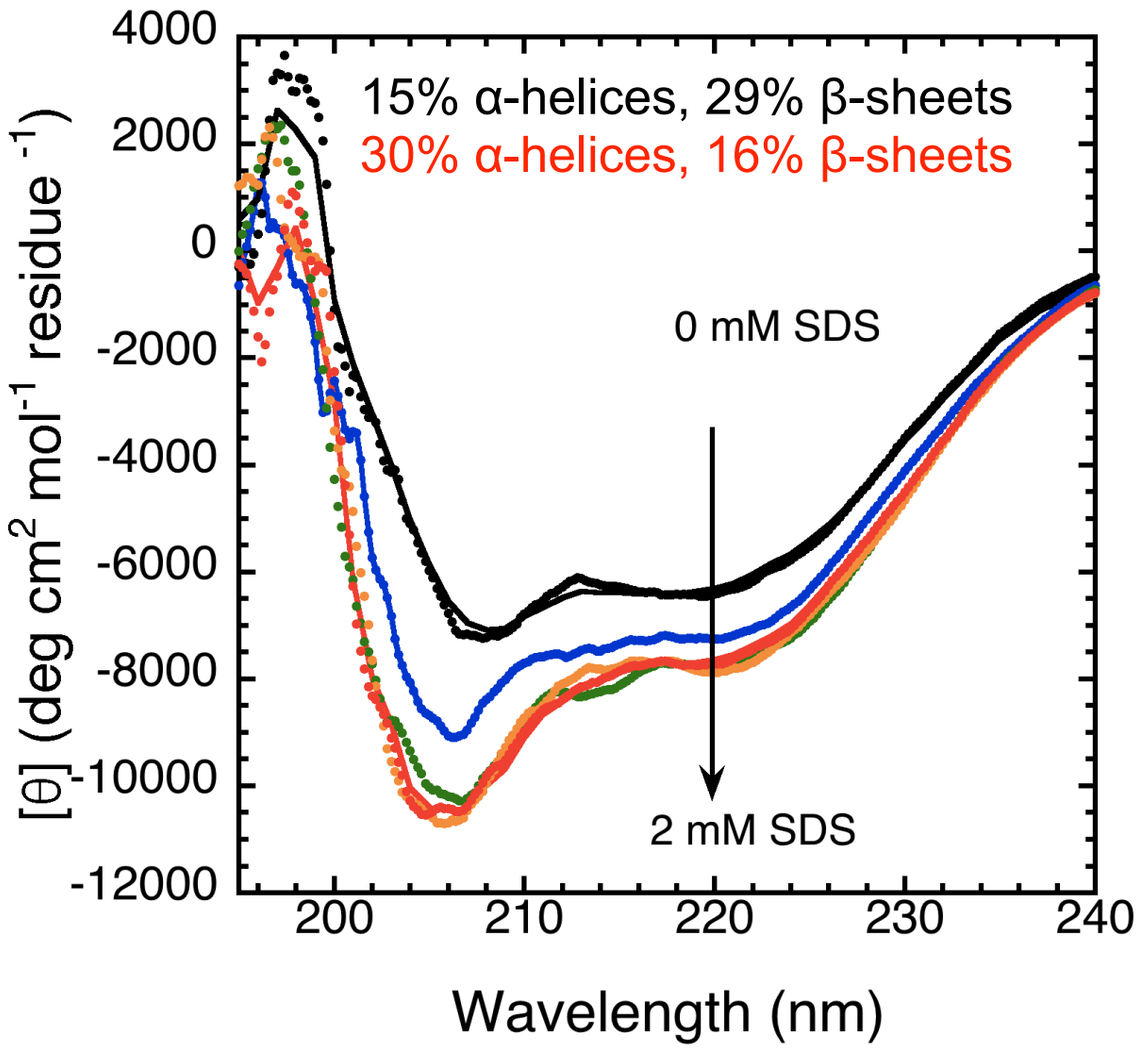


Circular dichroism



M. Palombo , S. Ciurli , V. Uversky , E. Mileo, B. Zambelli et al. *Scientific Reports,* **2017**

Dependence of UreG dynamics by folding inducers

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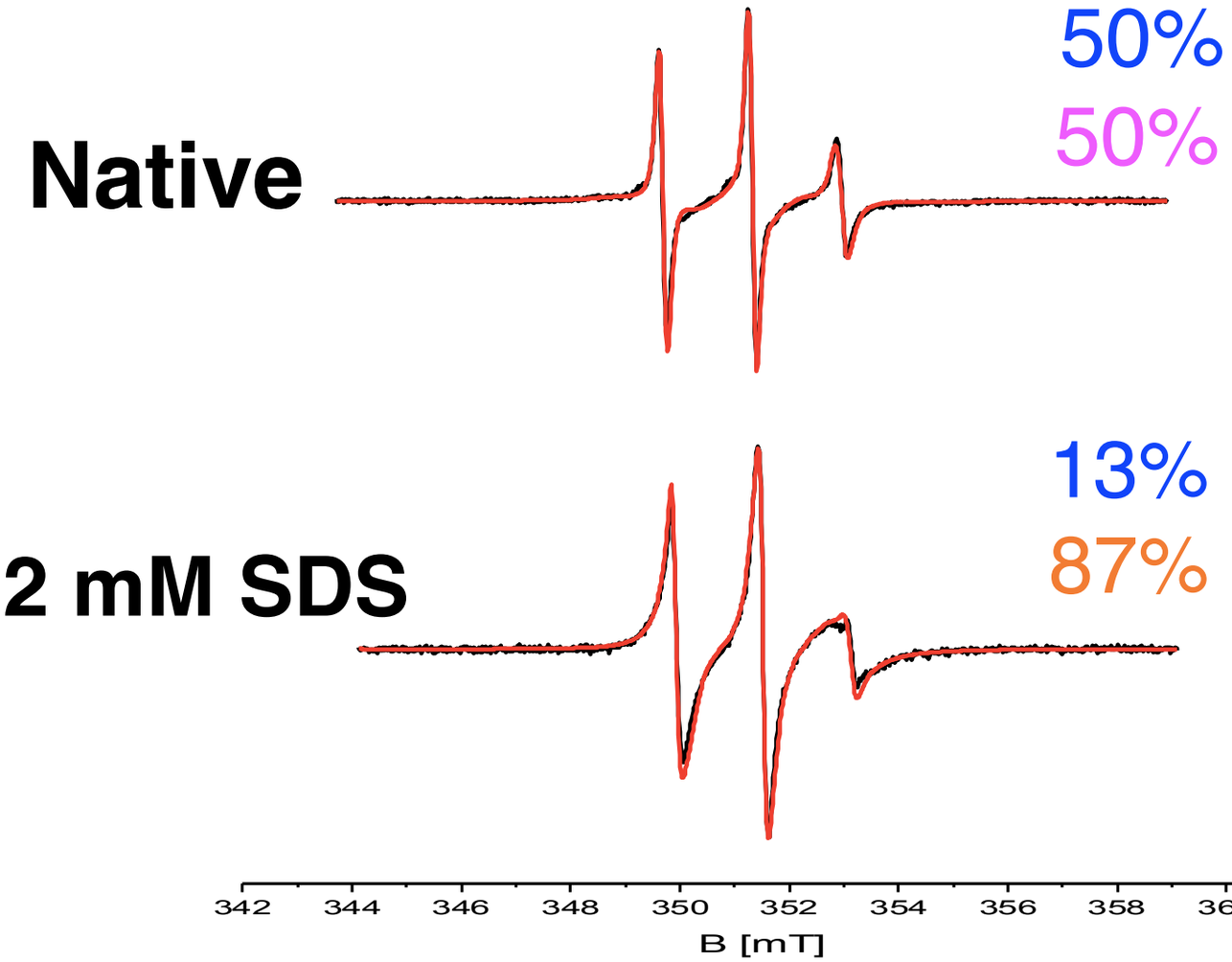
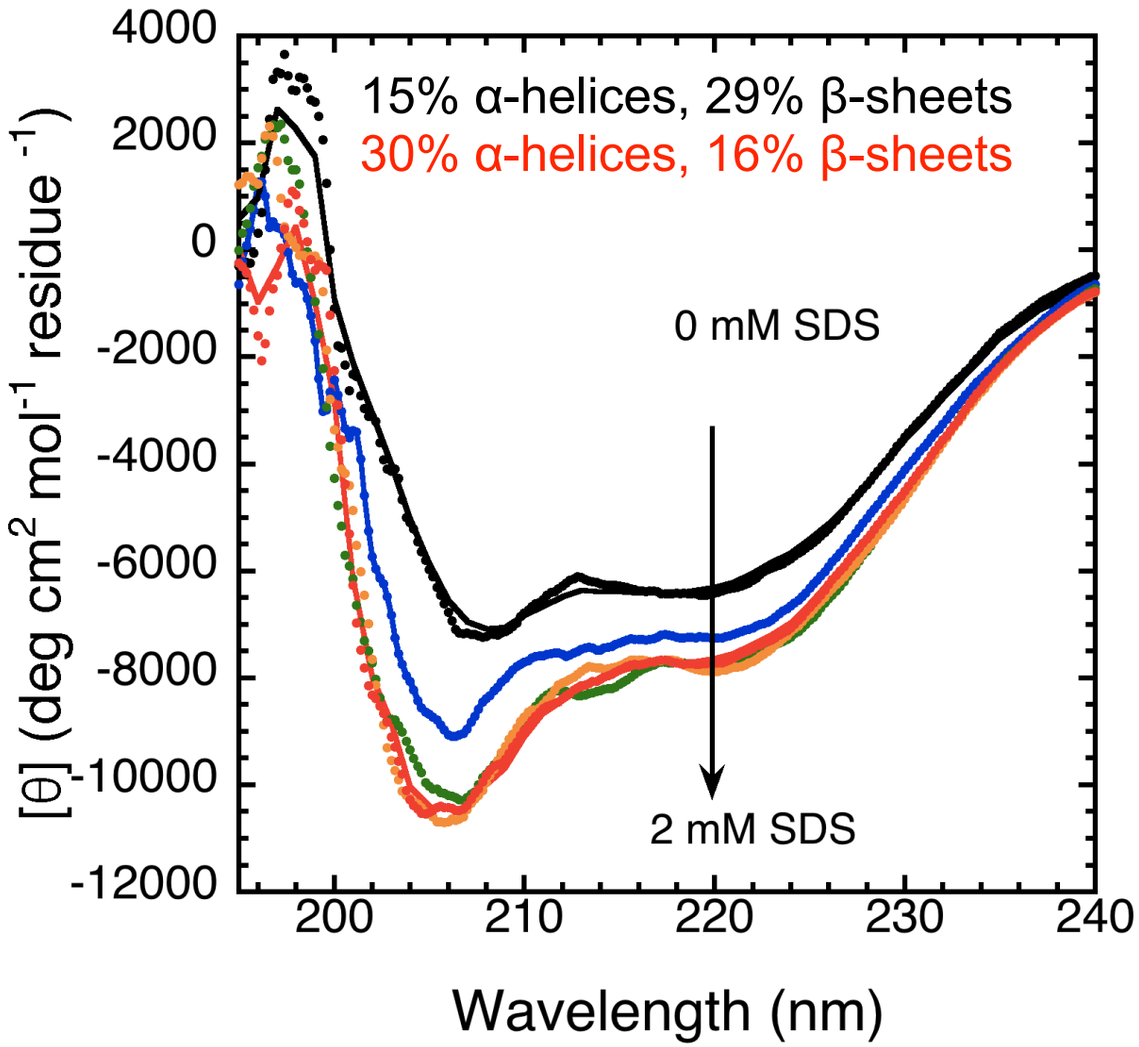


Circular dichroism



SDS changes the "broad" component, while increasing secondary structure

M. Palombo , S. Ciurli , V. Uversky , E. Mileo, B. Zambelli et al. *Scientific Reports,* **2017**

The relationship between folding and activity of UreG

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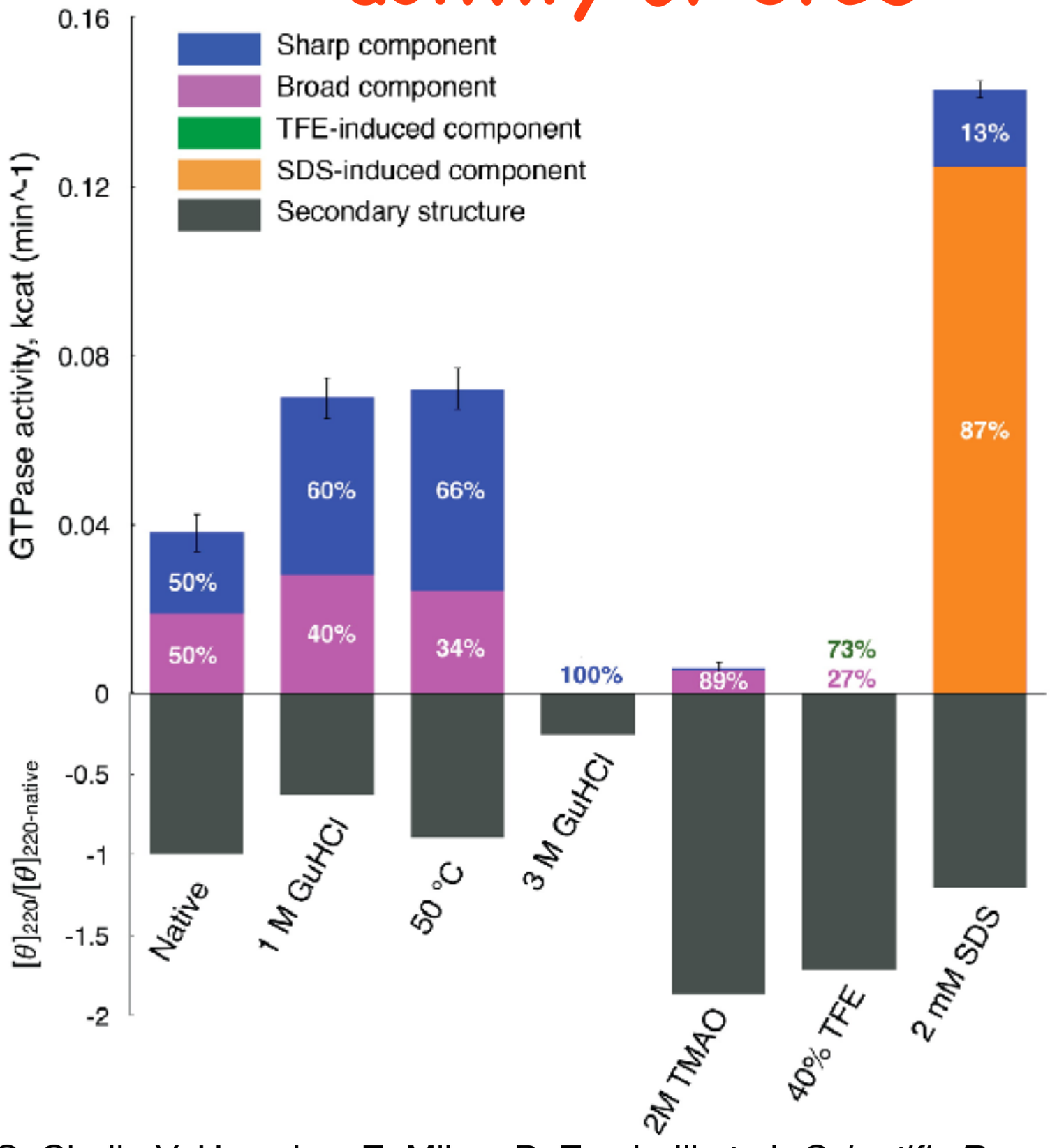
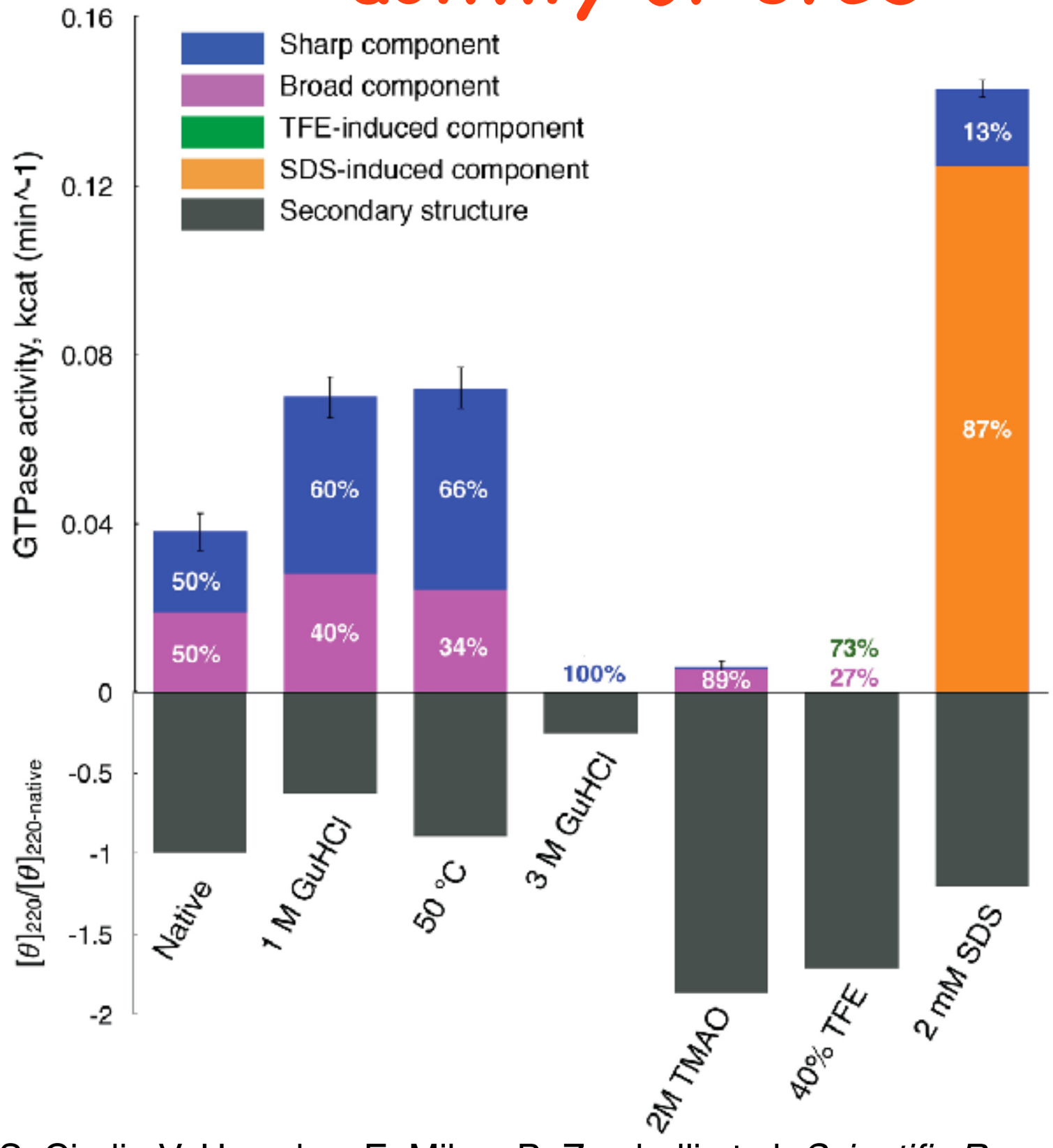


Activity assay

M. Palombo , S. Ciurli , V. Uversky , E. Mileo, B. Zambelli et al. *Scientific Reports,* **2017**

The relationship between folding and activity of UreG



Activity assay

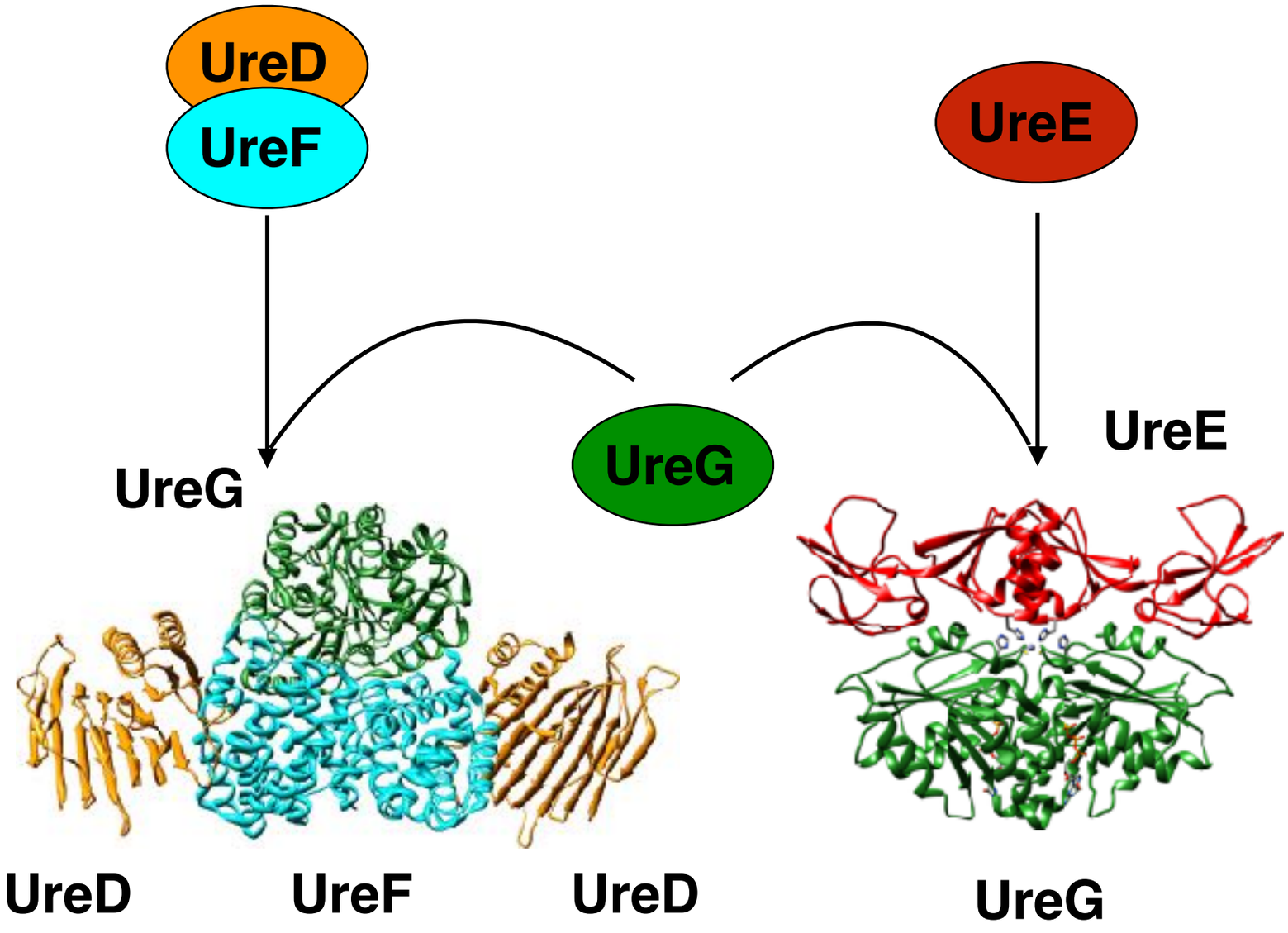
Activity of UreG is not correlated with protein rigidity

M. Palombo , S. Ciurli , V. Uversky , E. Mileo, B. Zambelli et al. *Scientific Reports,* **2017**

Conclusions

- UreG exists in solution as a conformational ensemble of different interconverting folding states.
- The degree of folding of the different conformers depends on temperature and additive concentration
- The enzymatic activity does not correlate to the protein rigid behaviour
- Intrinsic disorder likely allows the enzyme to interact with different partners that regulate its enzymatic activity

Why does UreG need ID?



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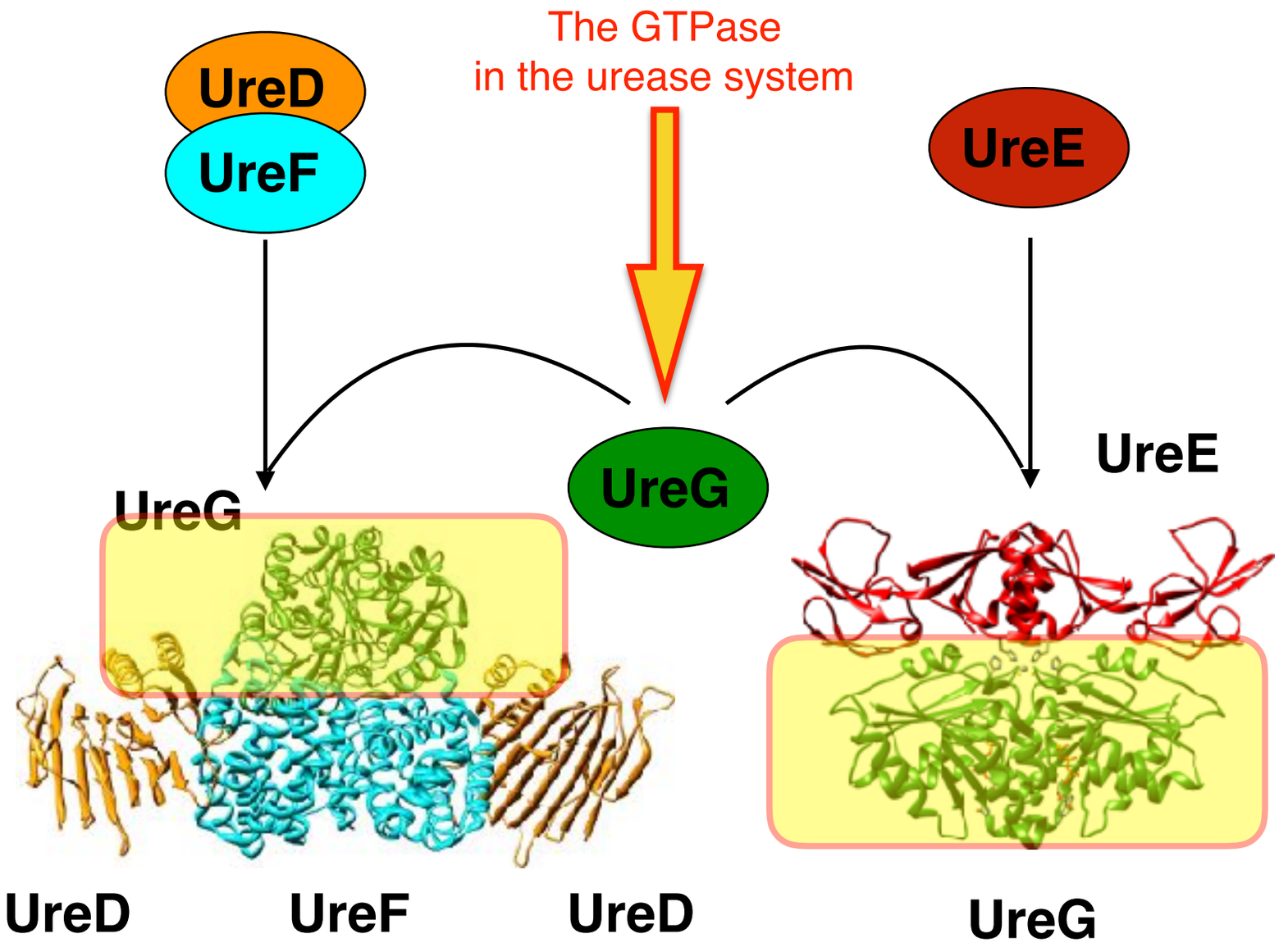

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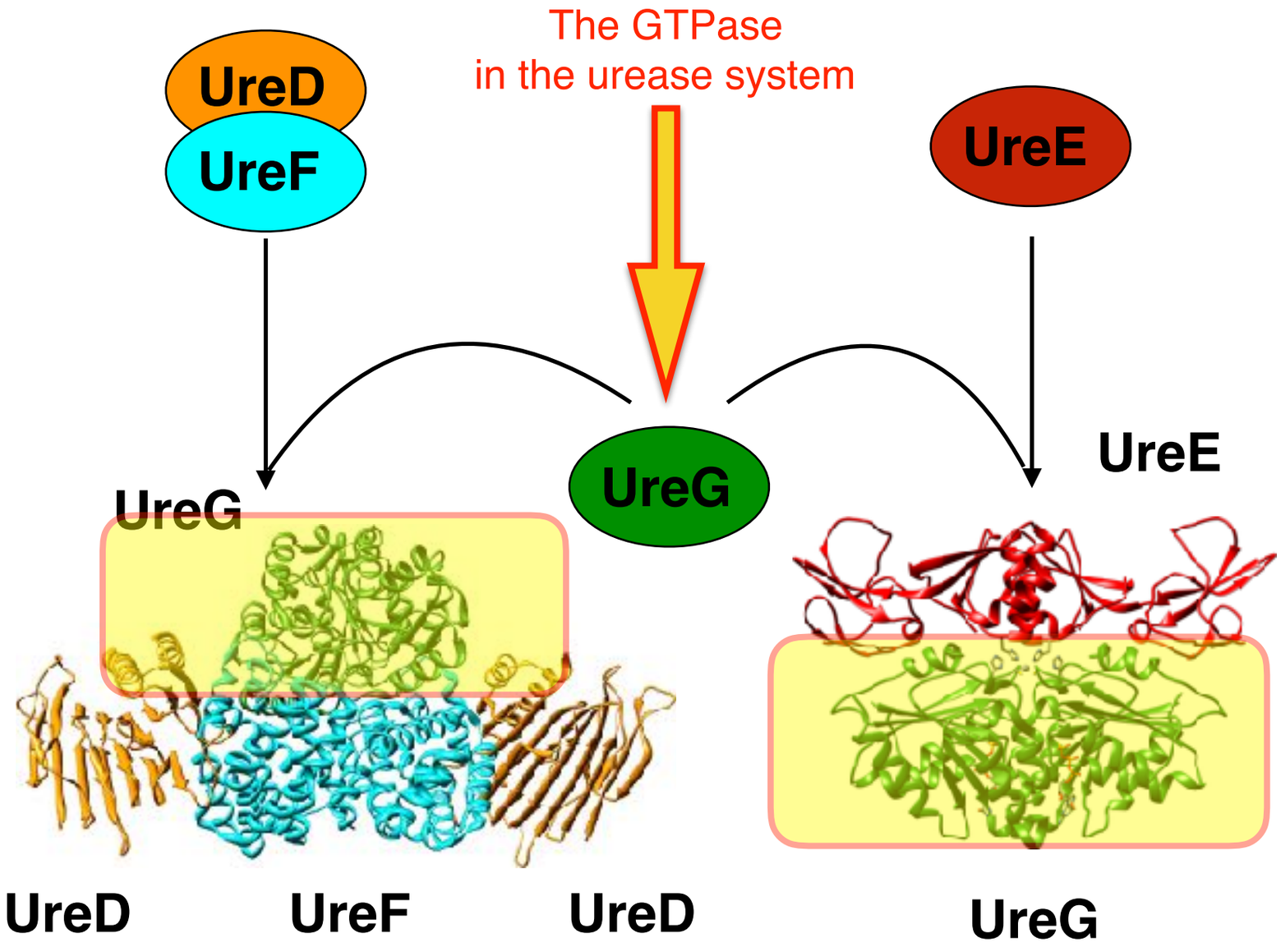
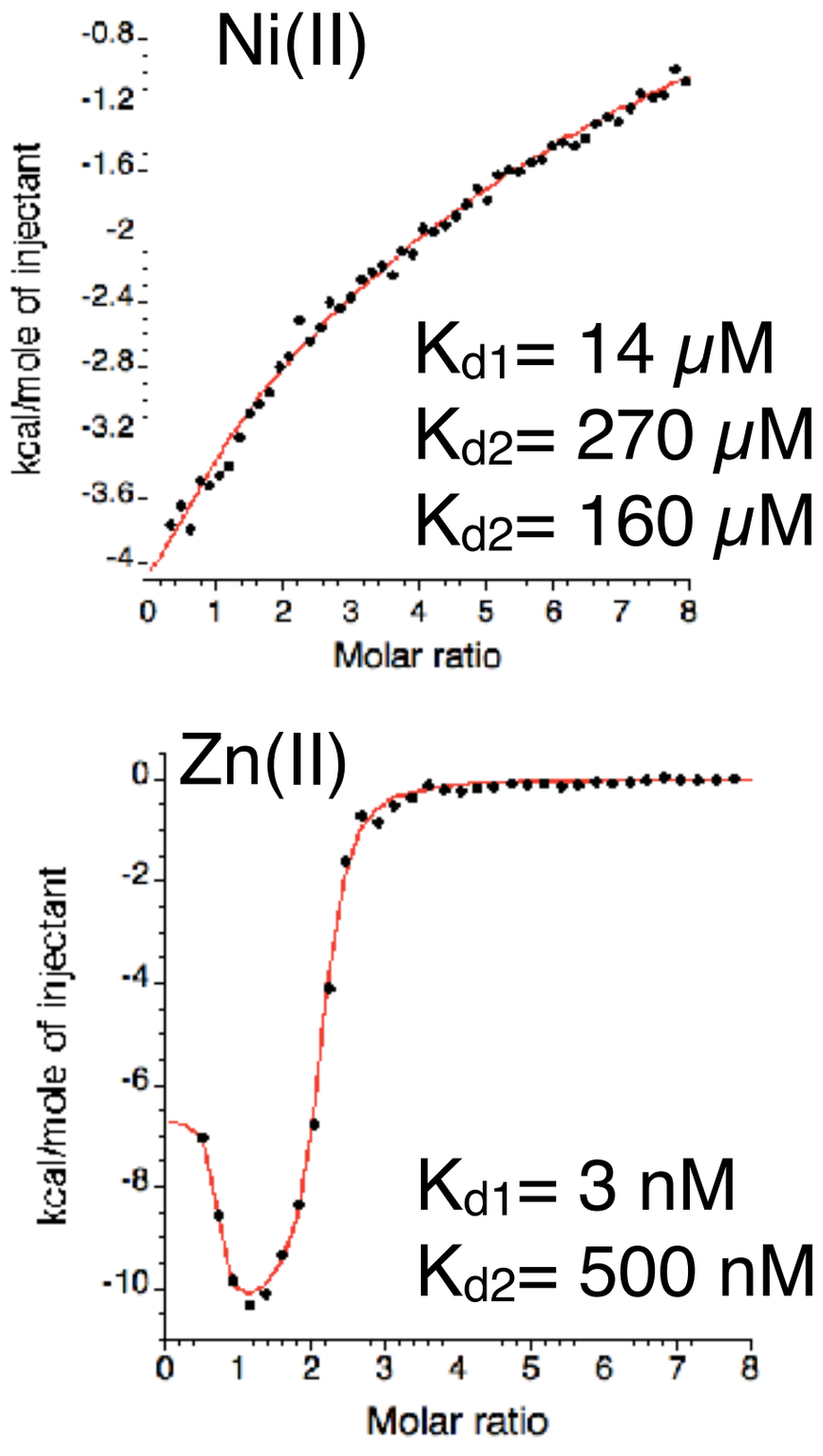




Why does UreG need ID?

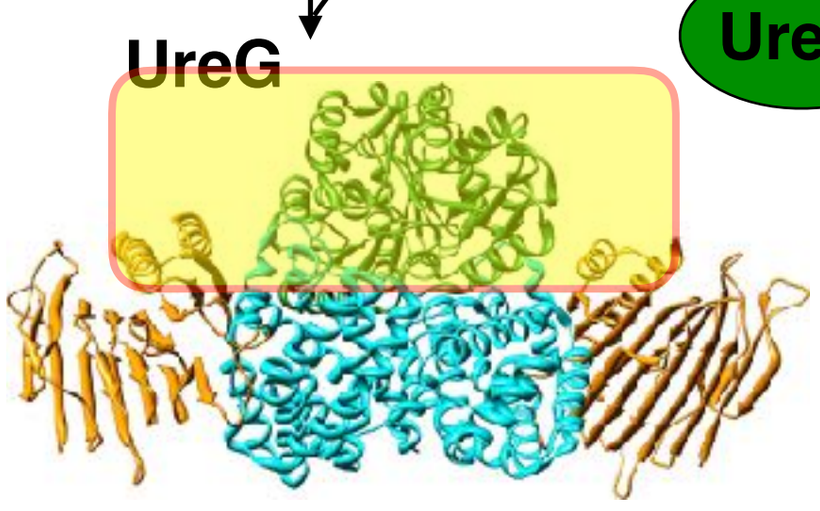
The GTPase
in the urease system

UreD

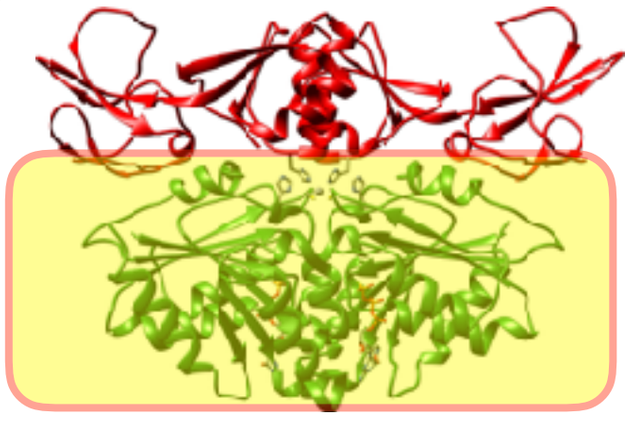
UreF

UreE

UreG



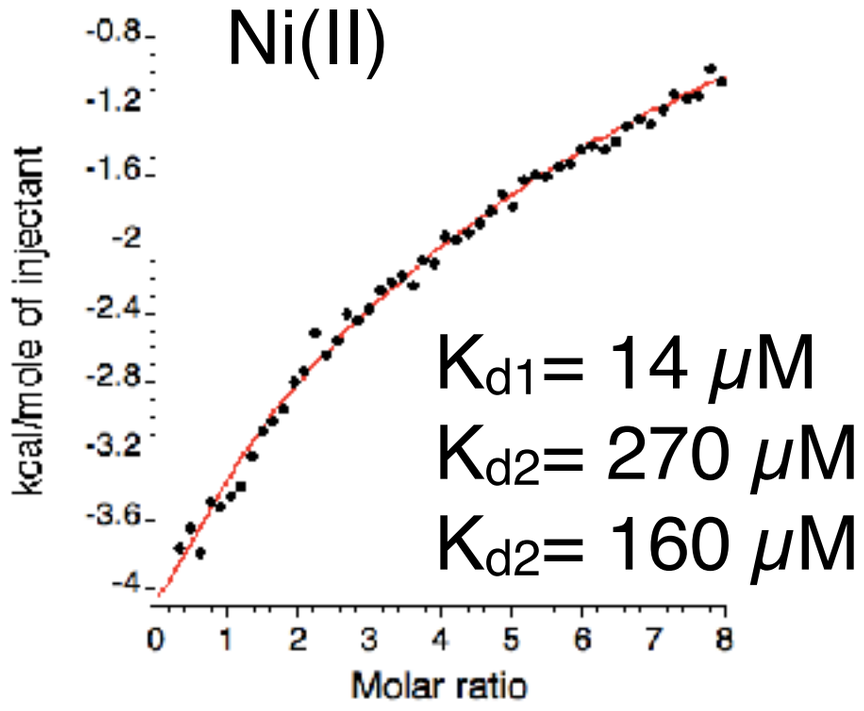
UreG

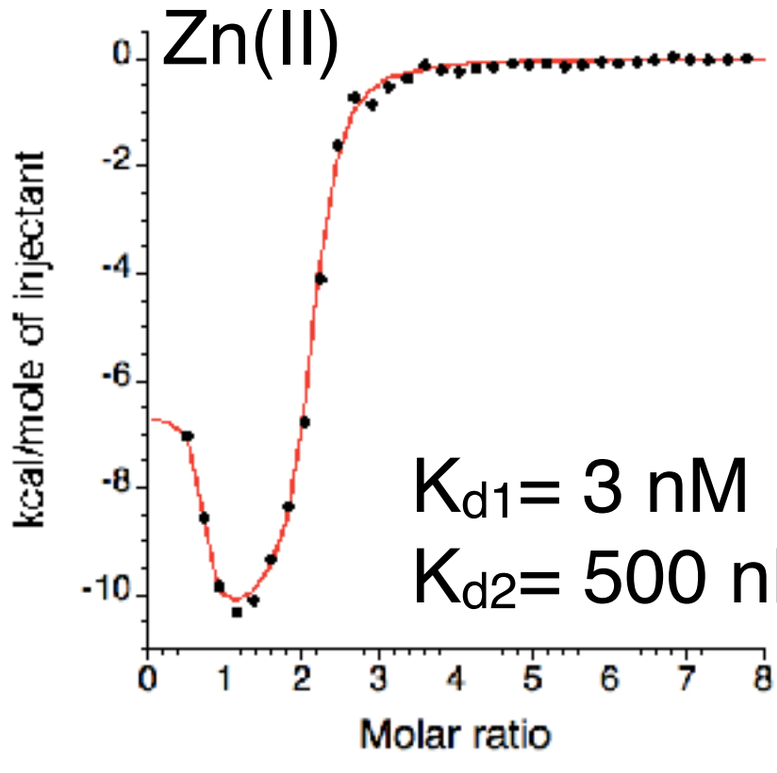


UreD UreF UreD

UreE

UreG

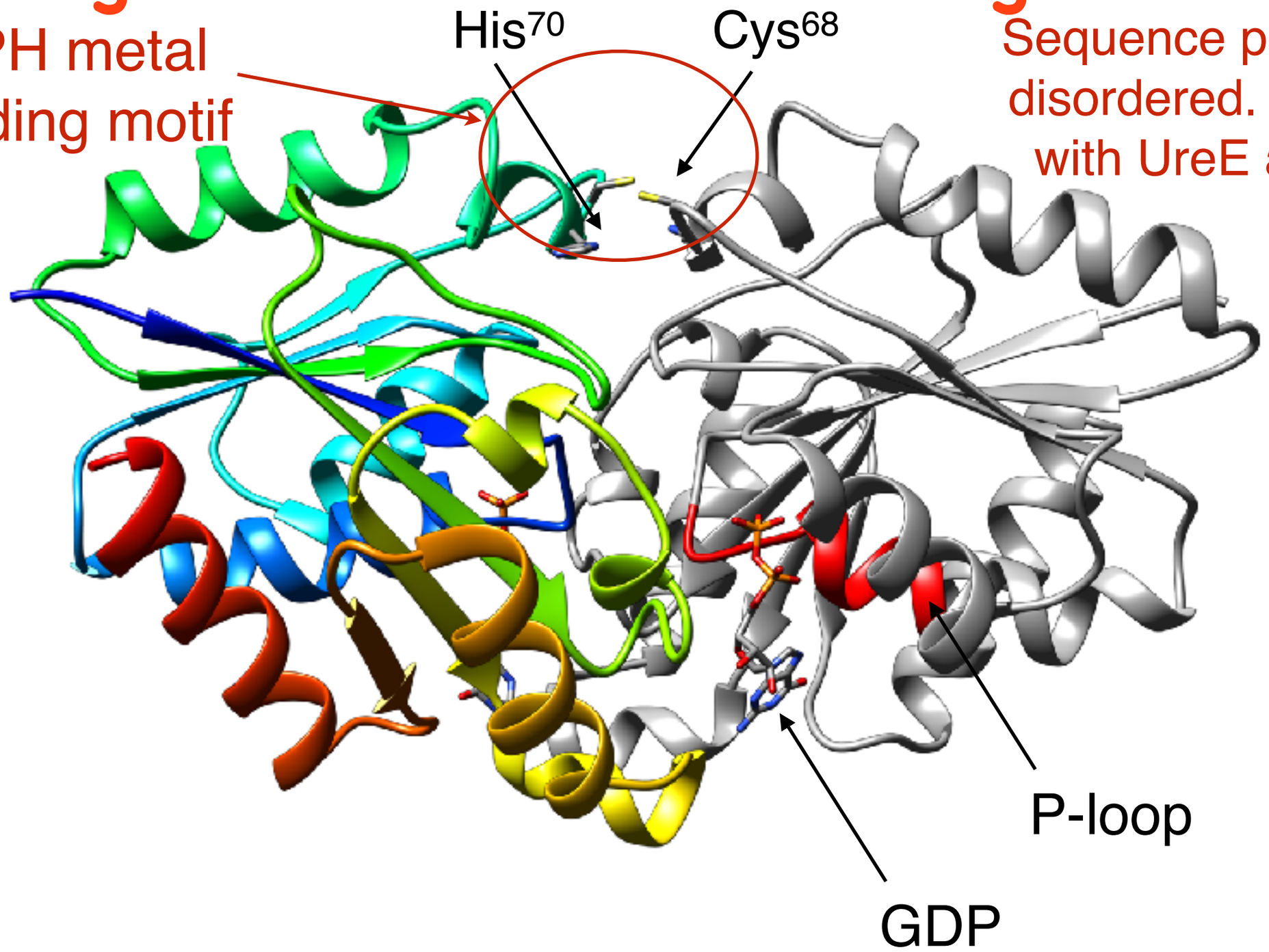




UreG interacts with multiple partners and ligands

Sampling disorder in different regions of UreG

CPH metal binding motif



Sequence predicted as disordered. Interaction with UreE and UreF.

A. Merloni, B. Zambelli, F. Musiani, S. Ciurli, et al. - *BBA Proteins and proteomics,* **2014**
F. Musiani, E. Ippoliti, C. Micheletti, S. Ciurli, P. Carloni, *Biochemistry*, **2013**, *52*, 2949-2954

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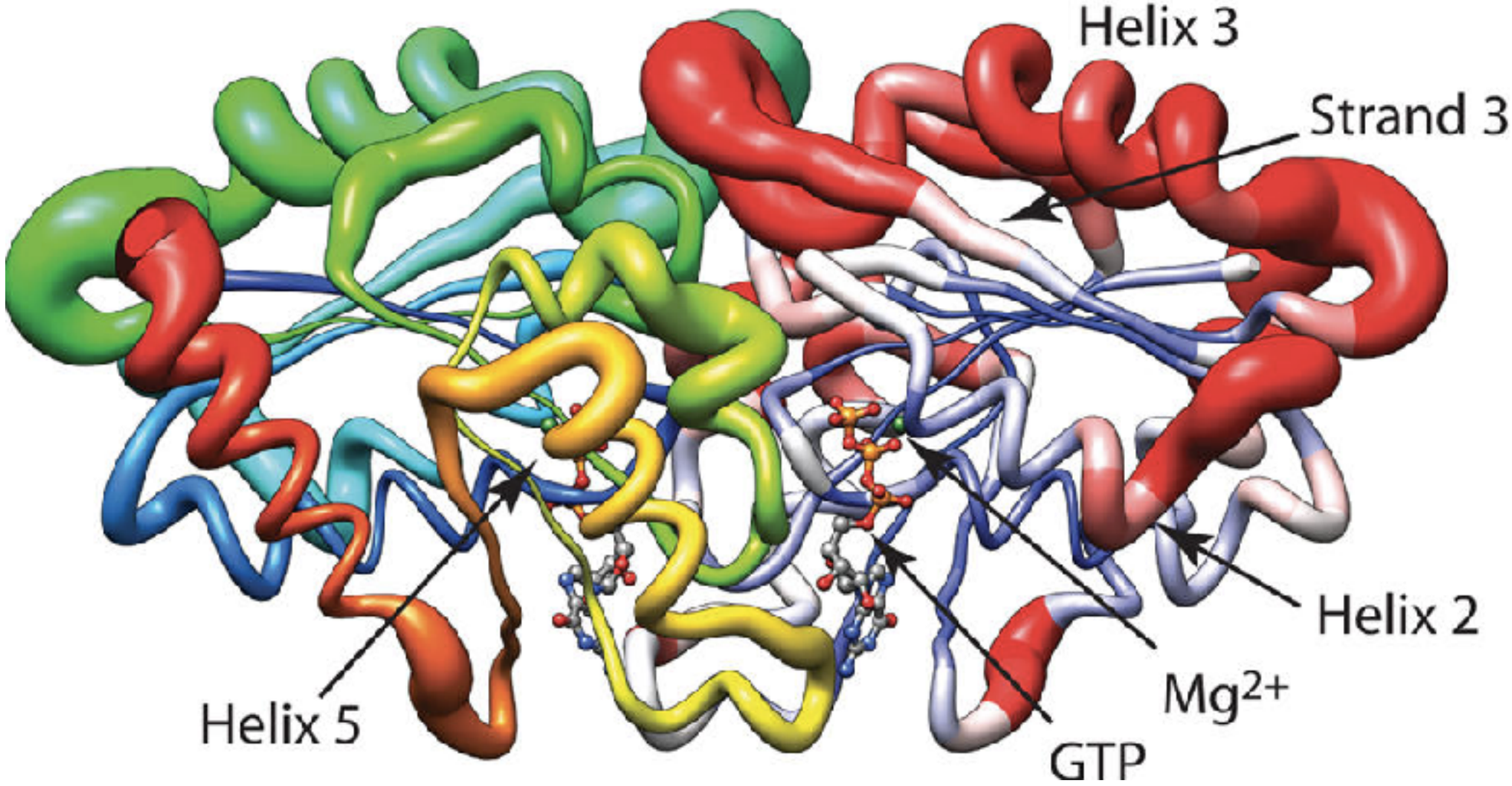
Sampling disorder in different regions of UreG

Molecular Dynamics simulations



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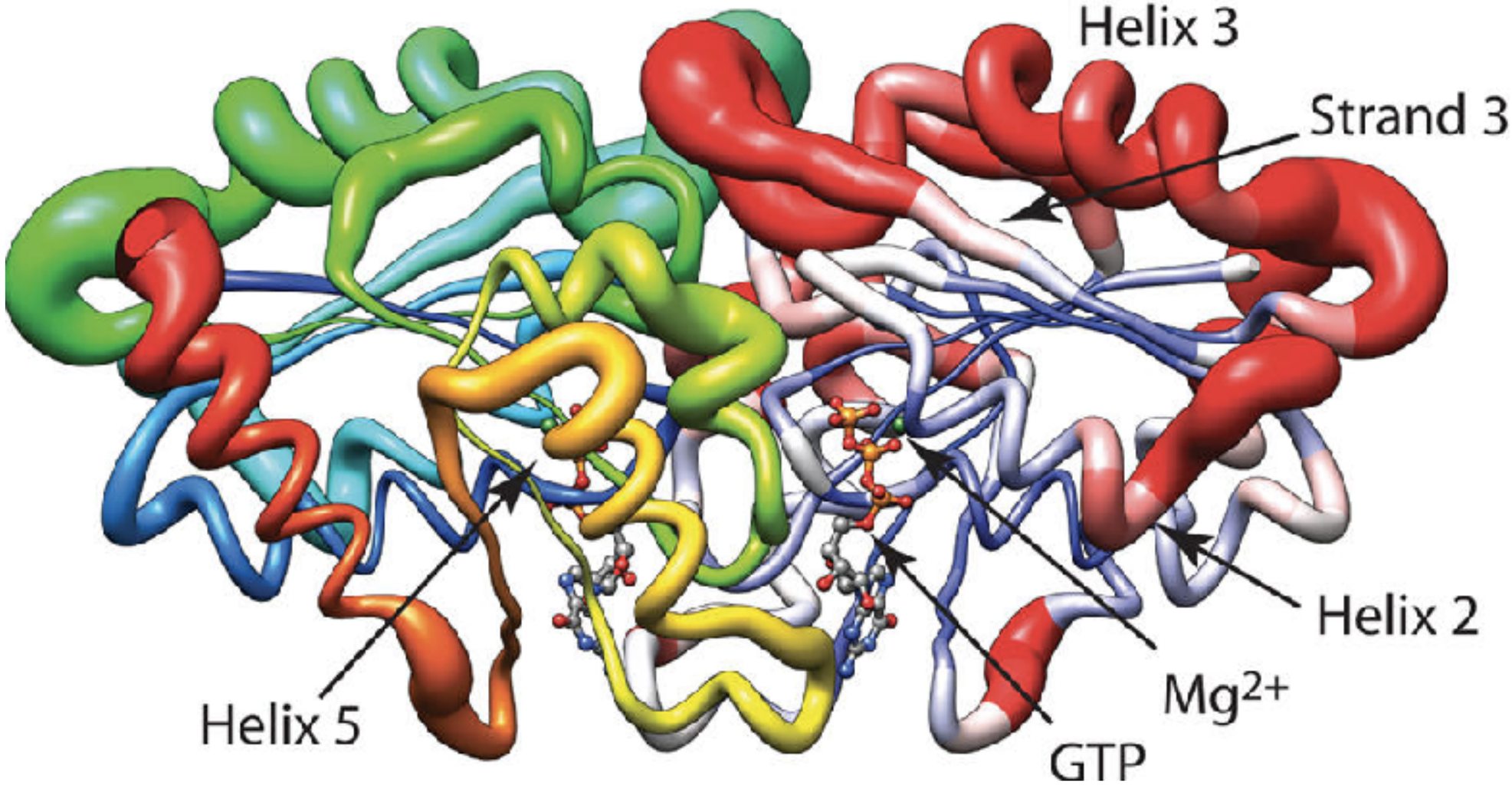

UreG shows substantial rigidity of the protein regions involved in catalysis, while it tends to unfold in regions involved in protein interactions necessary for the formation of multiprotein complexes.

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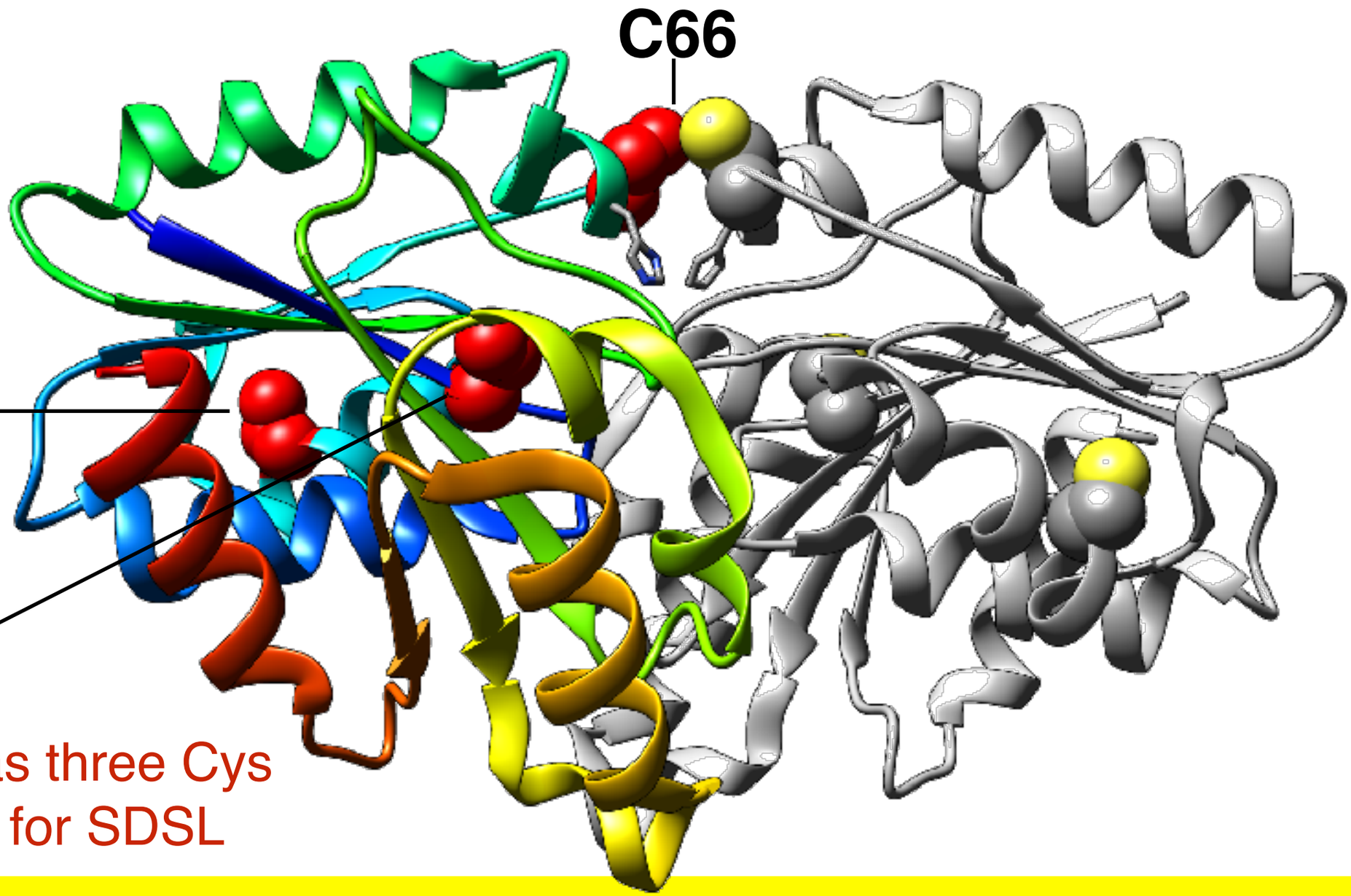

*Hp*UreG has three Cys available for SDSL

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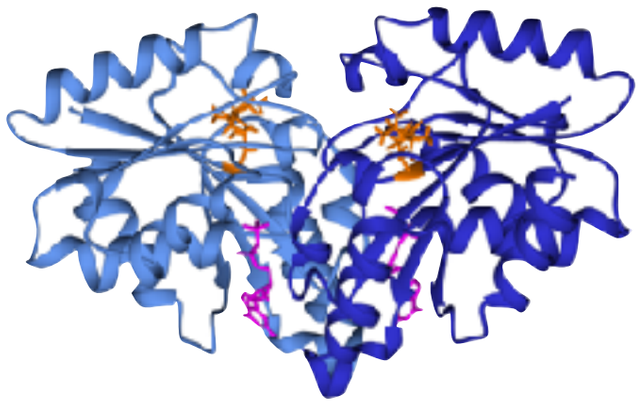
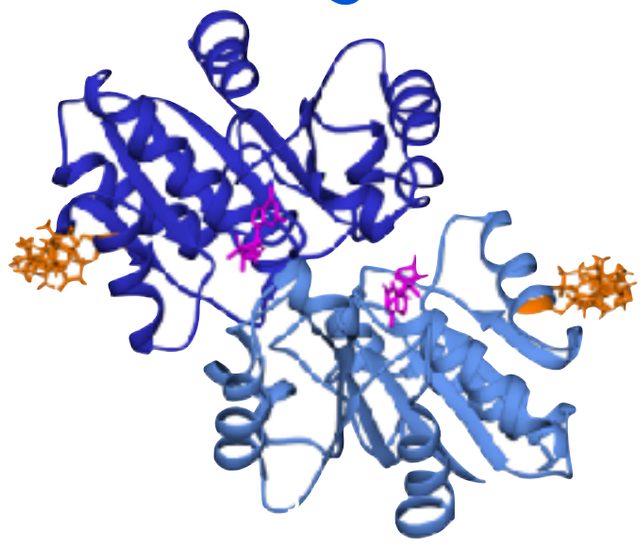
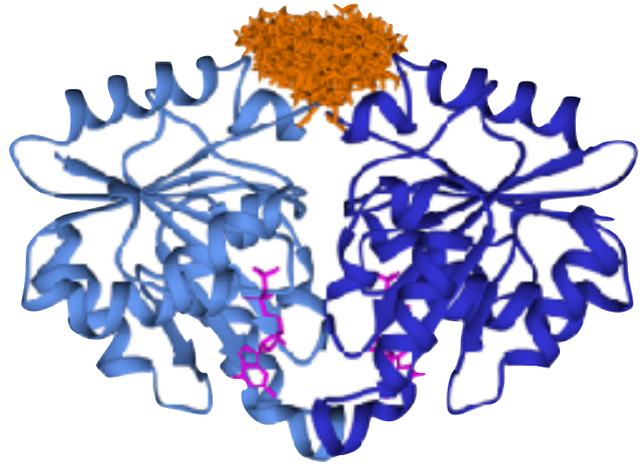
Sampling different regions of UreG with EPR

Site directed mutagenesis + SDSL-EPR







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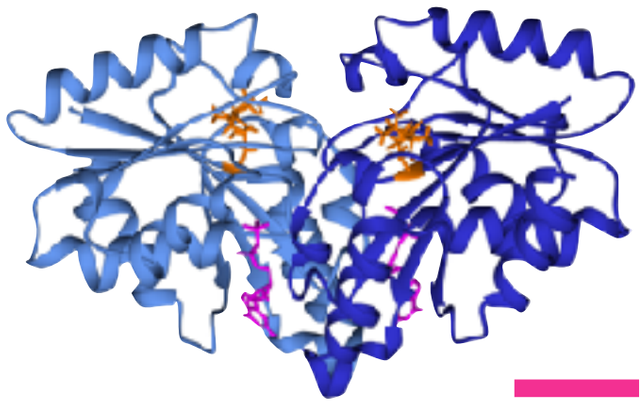
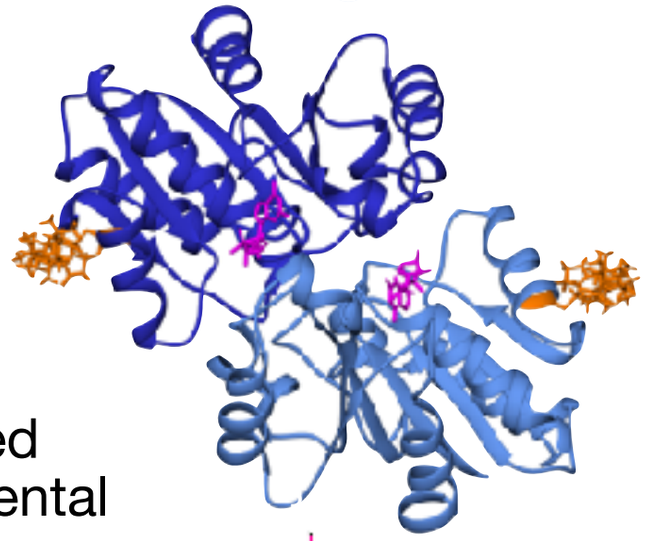
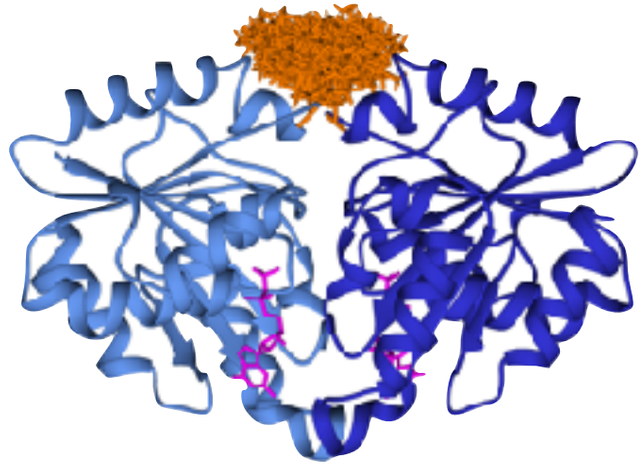
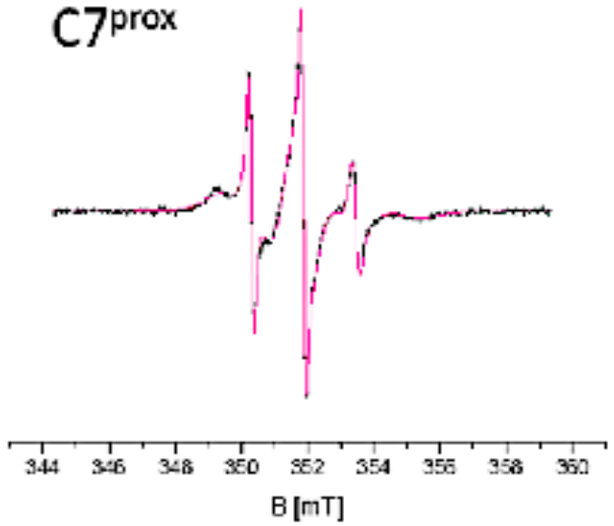




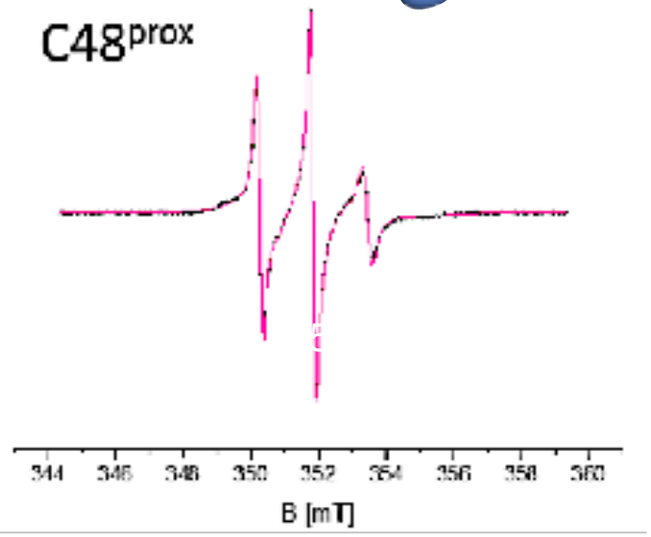


Simulated
Experimental

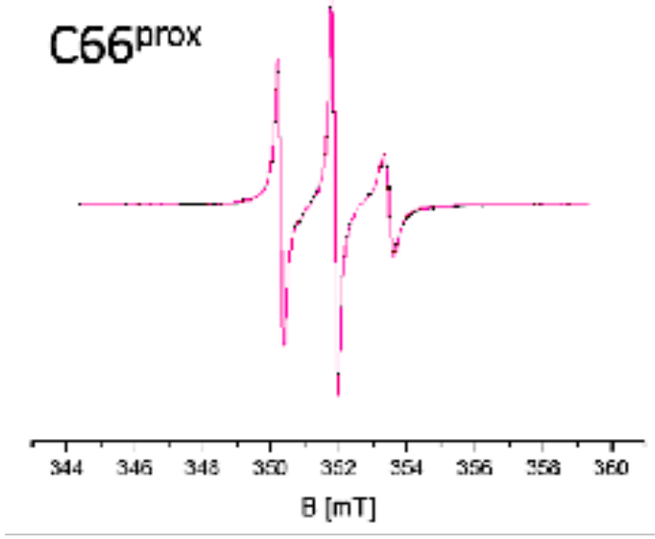
C7^{prox}



C48^{prox}

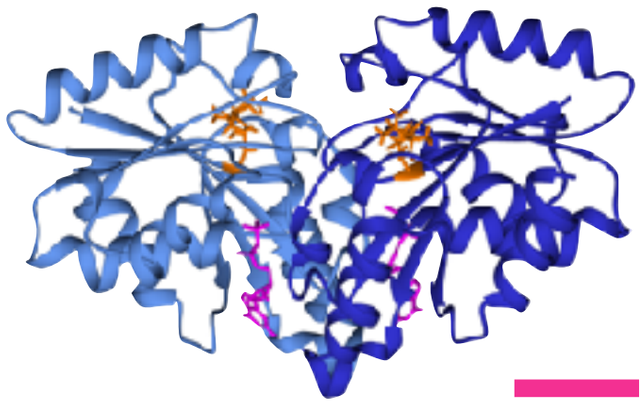


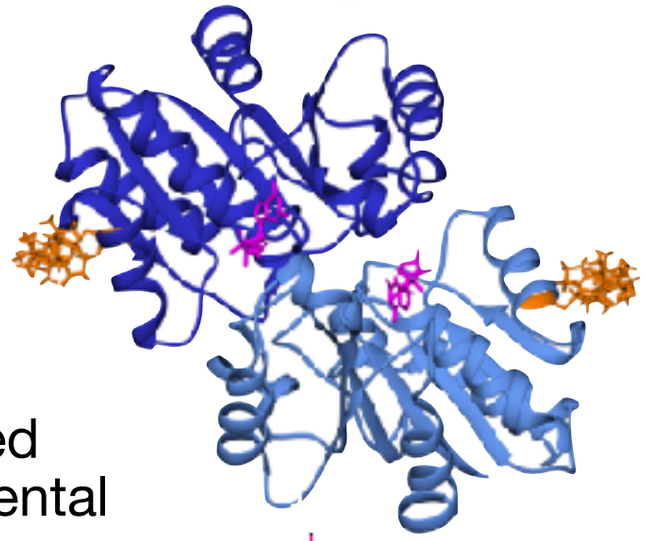
C66^{prox}

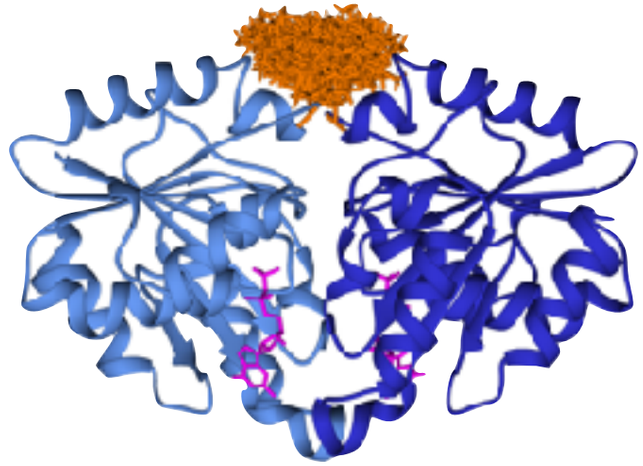


Sampling different regions of UreG with EPR

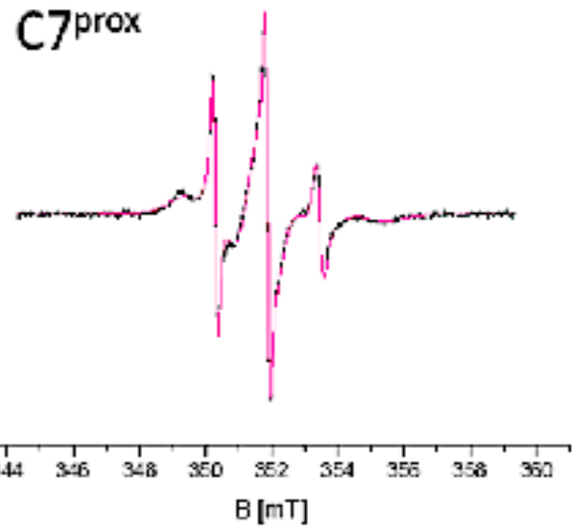
Site directed mutagenesis + SDSL-EPR

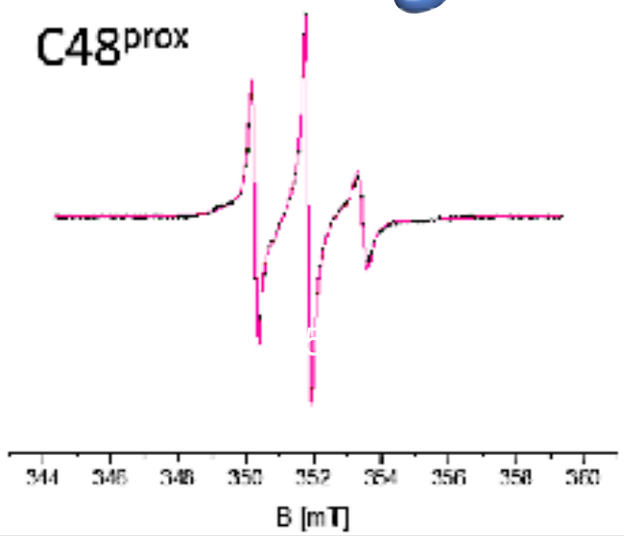


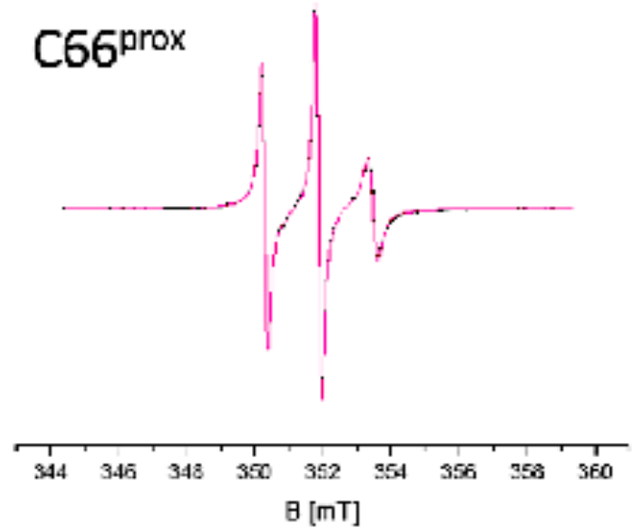


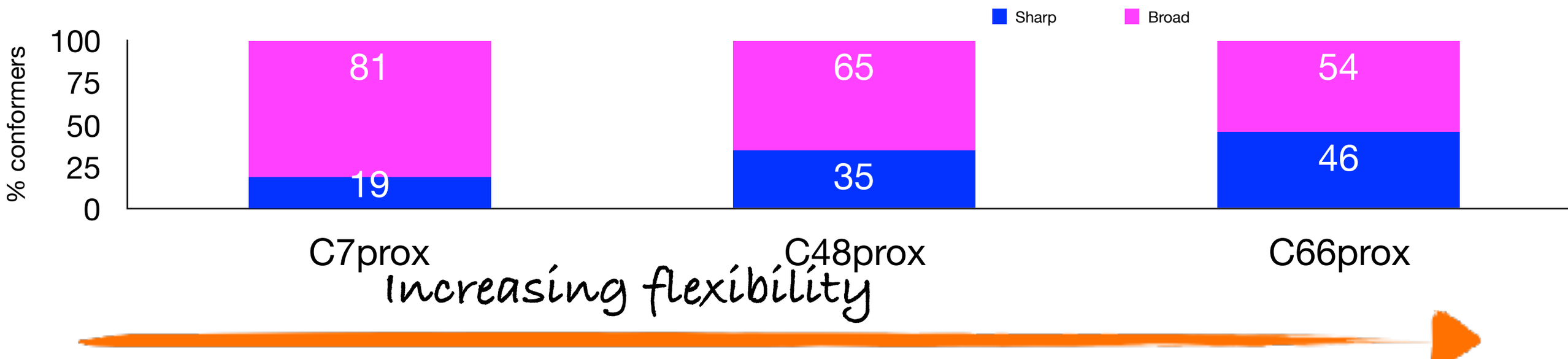


Simulated
Experimental









Increasing flexibility

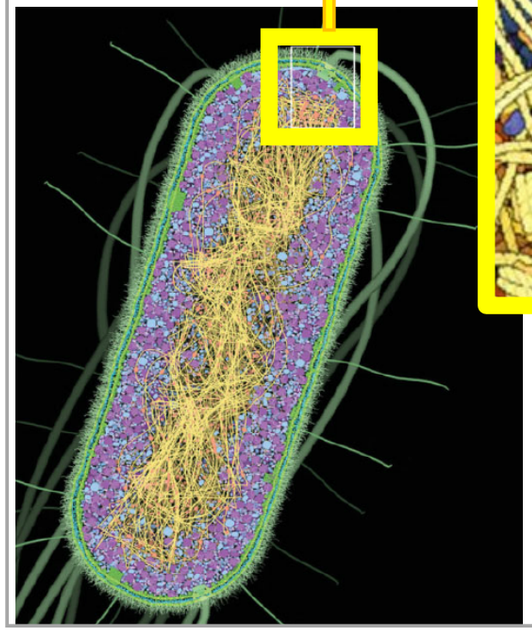
What is the **conformational** and **functional** landscape sampled by UreG **in cellular environment?**

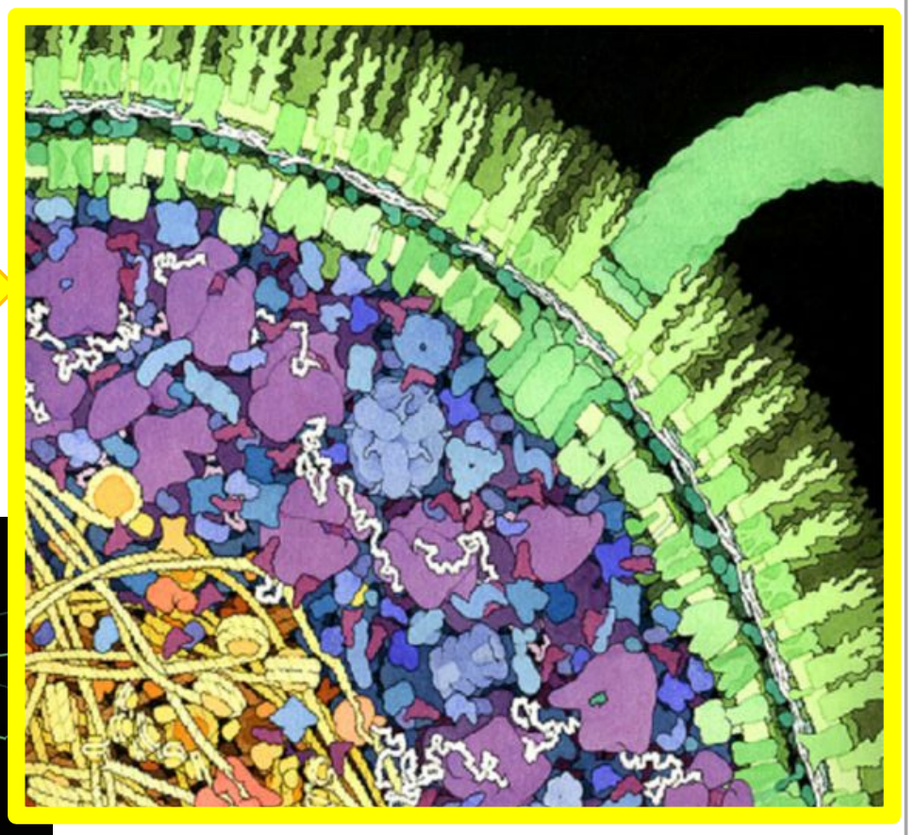
What is the **conformational** and **functional** landscape sampled by UreG **in cellular environment?**

Cellular crowding, aspecific interactions, intracellular viscosity, interaction with partnes…. : what is the effect on protein structure-dynamics-function?

in vitro

versus

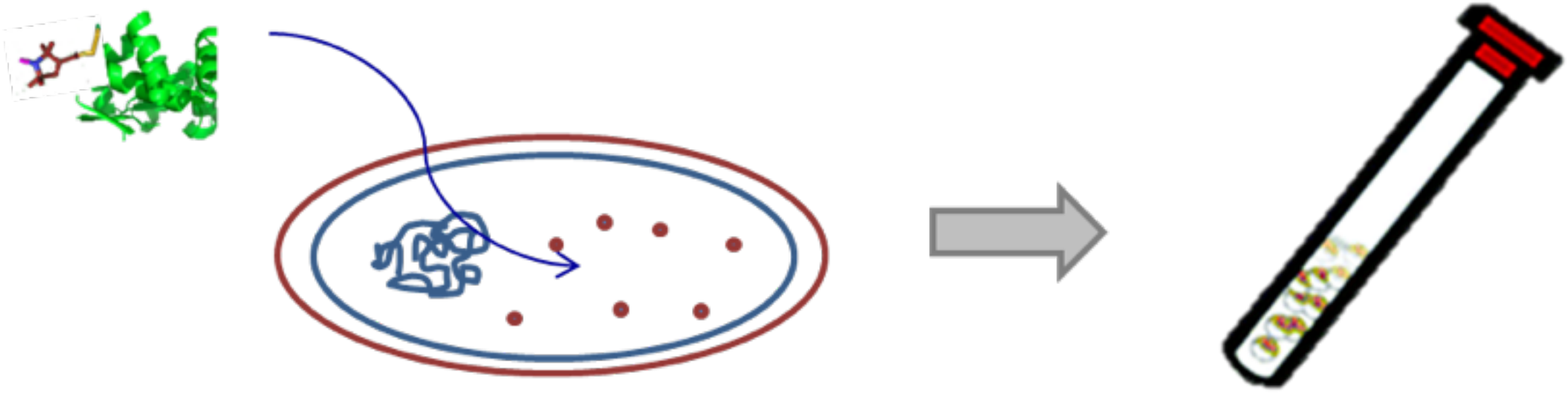




How it looks like inside the cell

Delivery of labelled proteins inside the cell

In cell
SDSL-EPR



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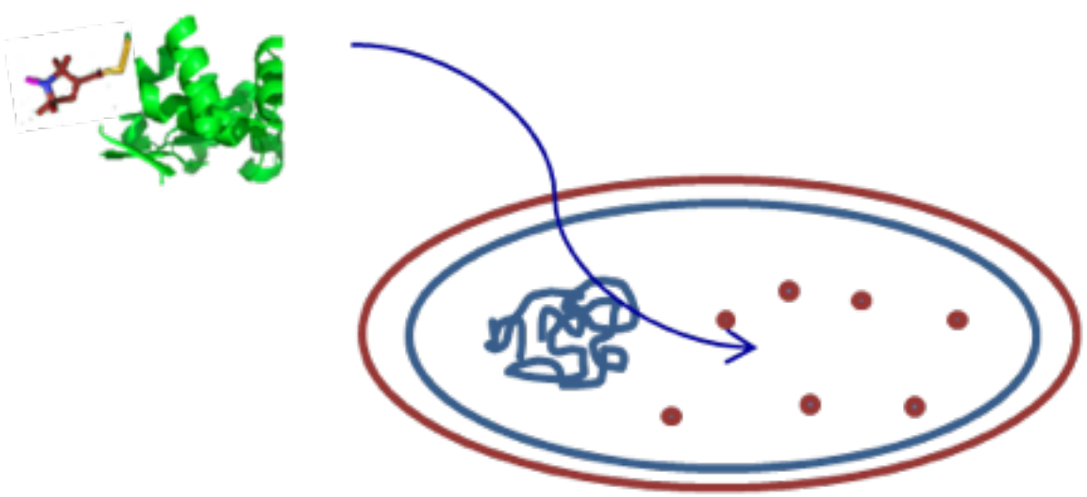
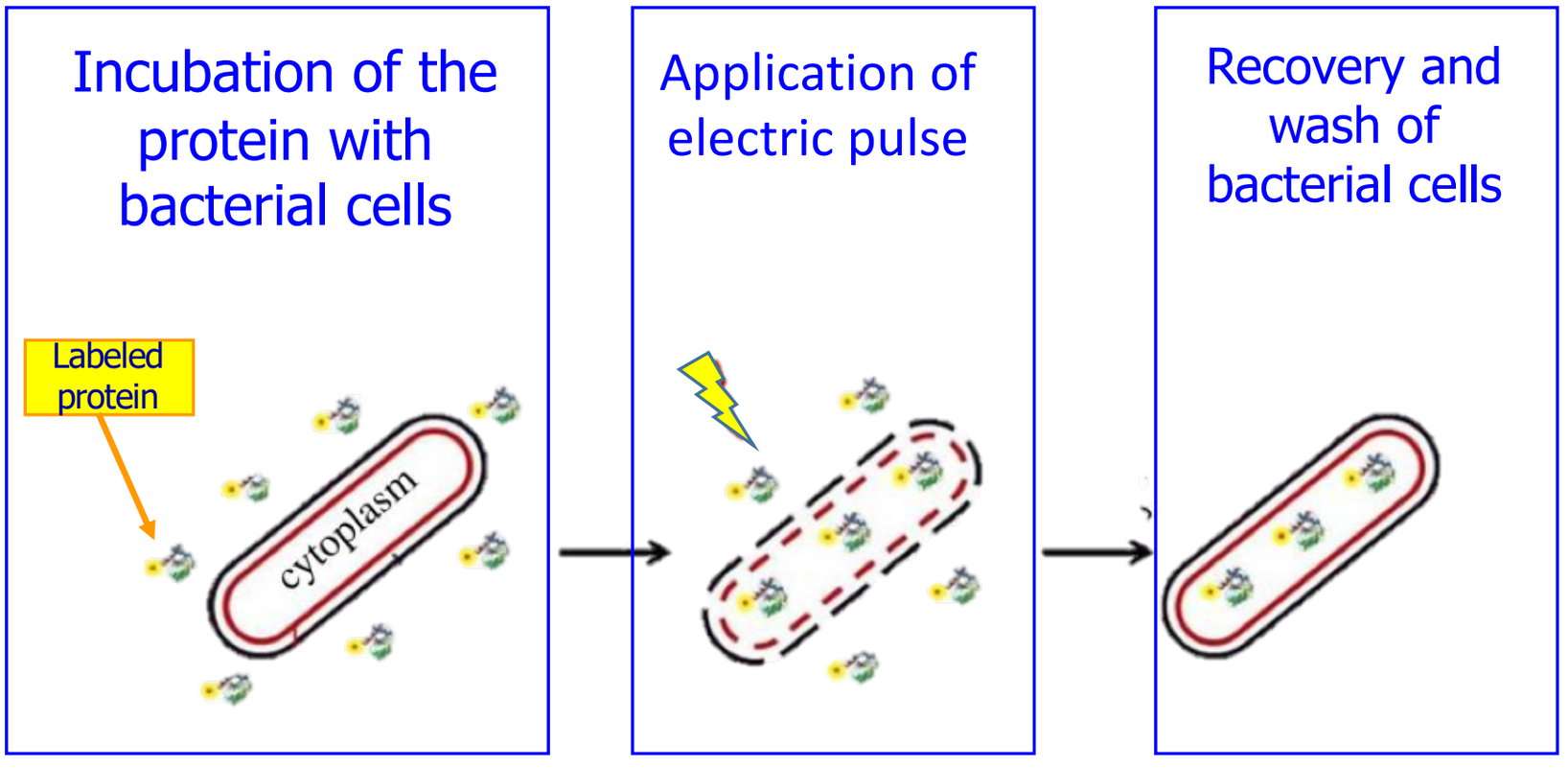


In cell
SDSL-EPR

Electroporation of labeled protein



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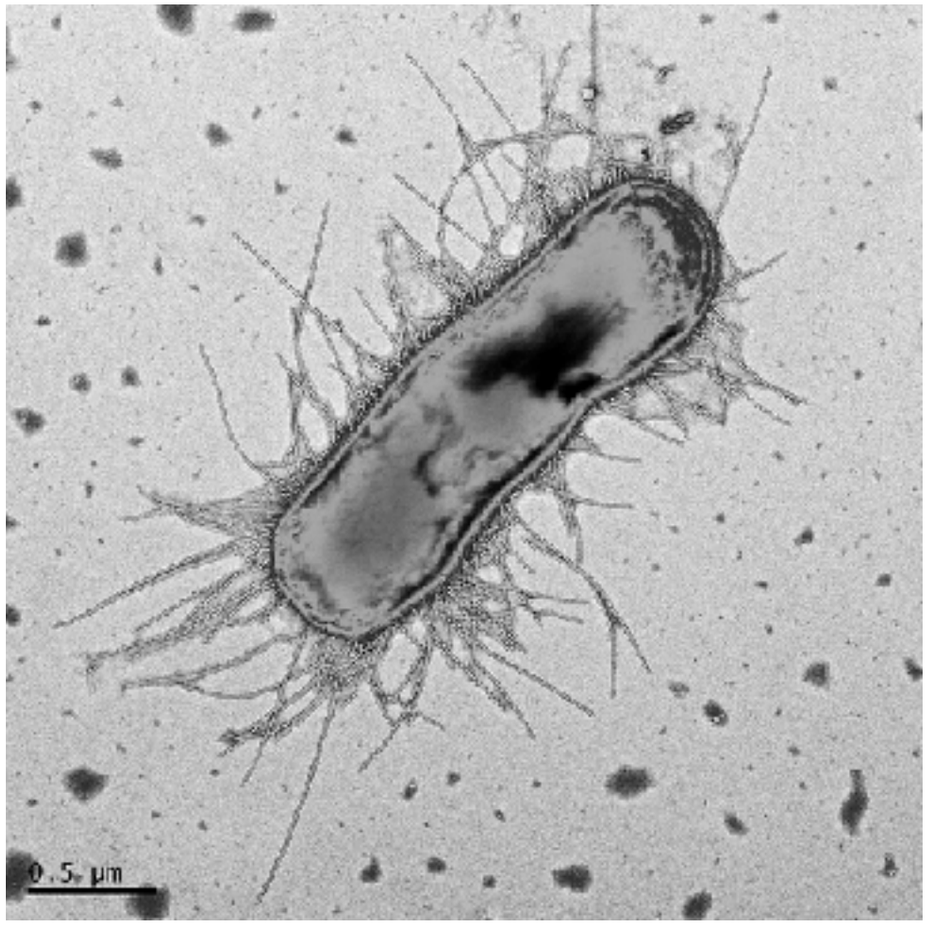

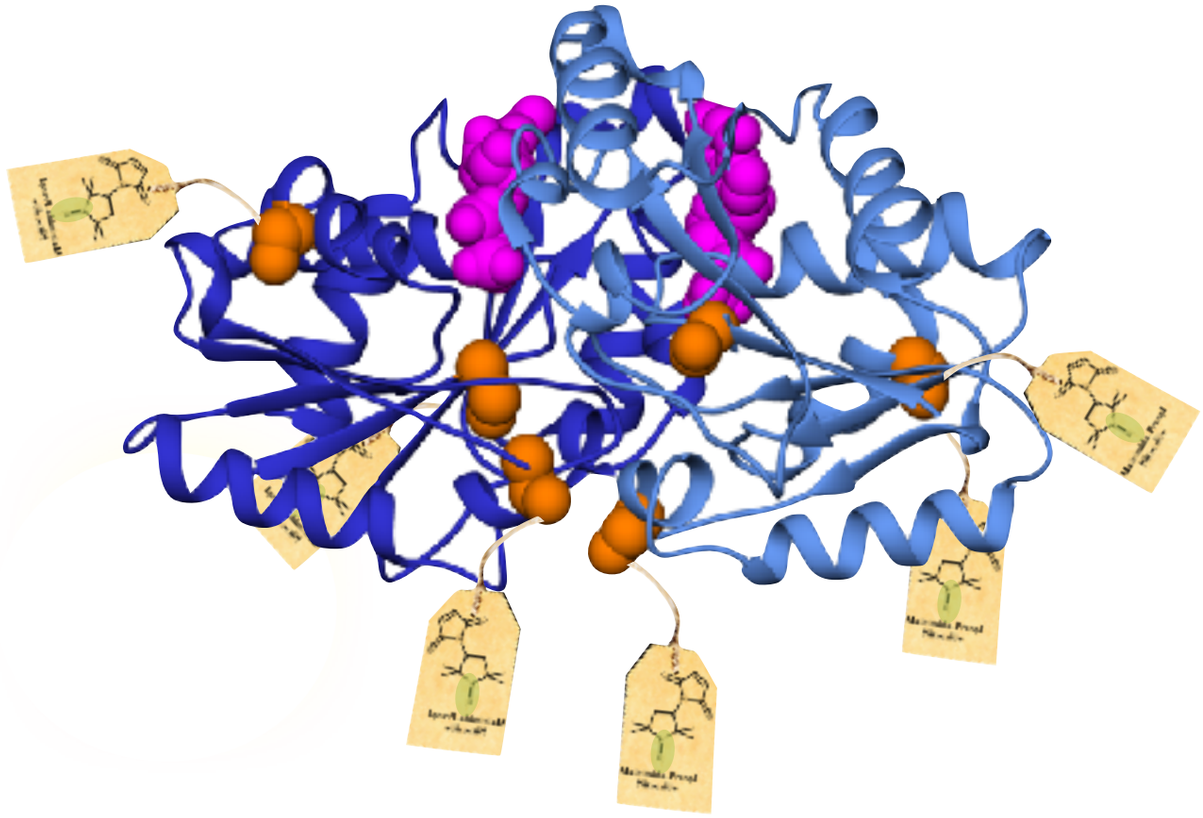

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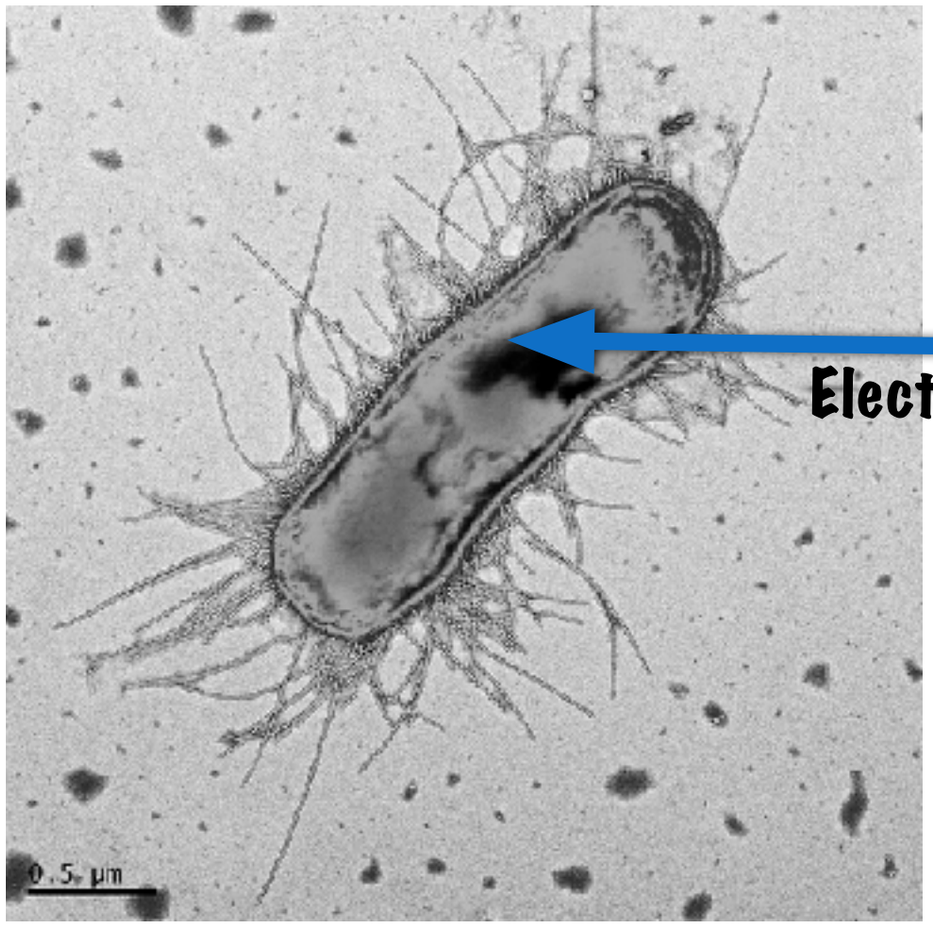
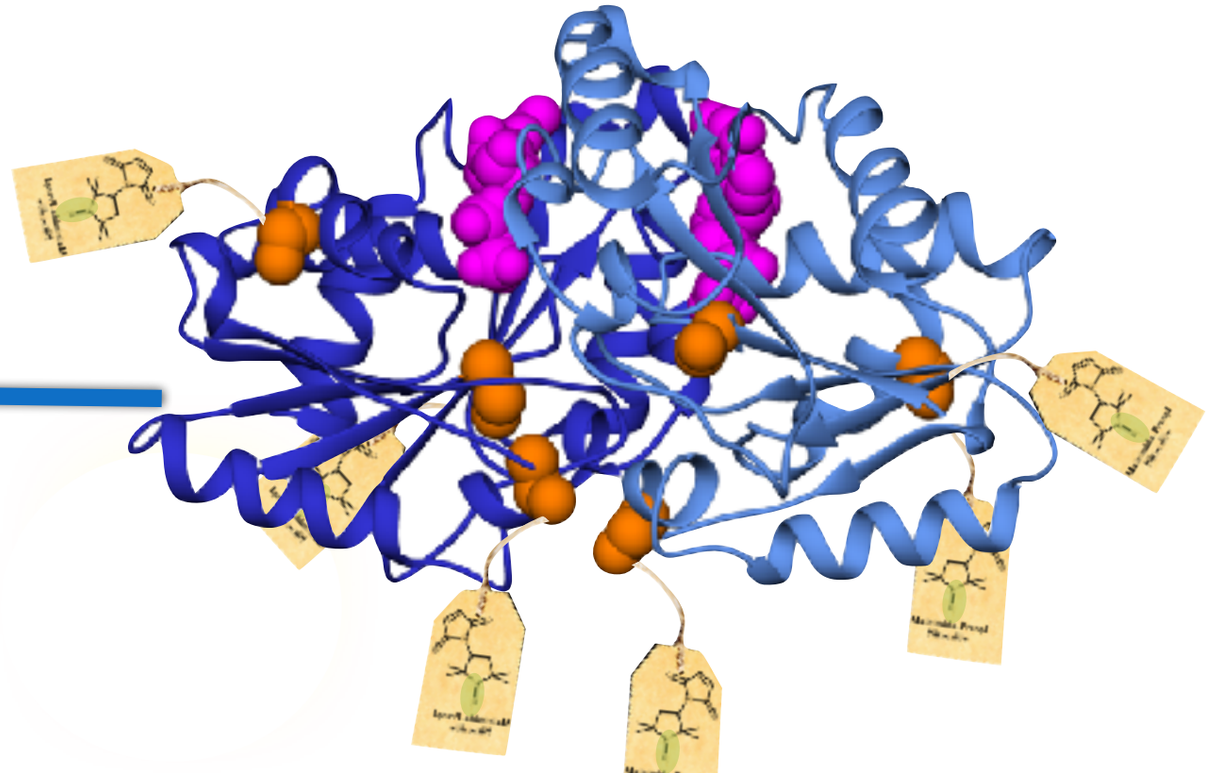


Electroporation



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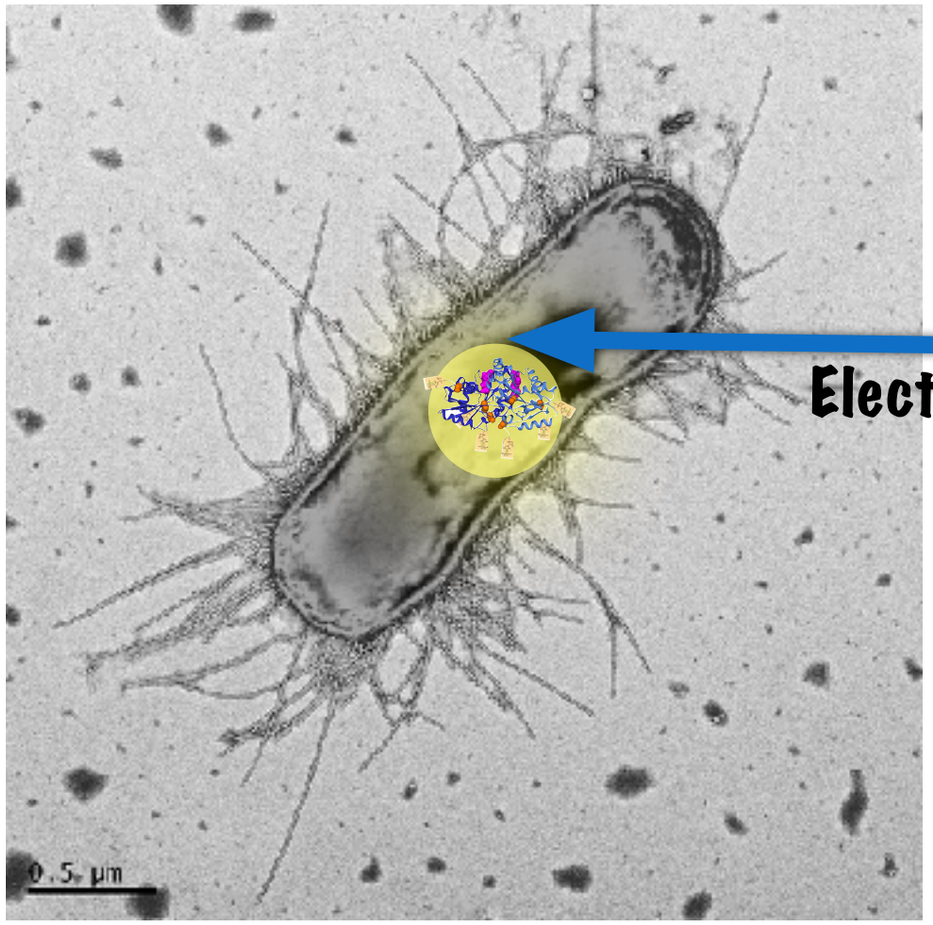

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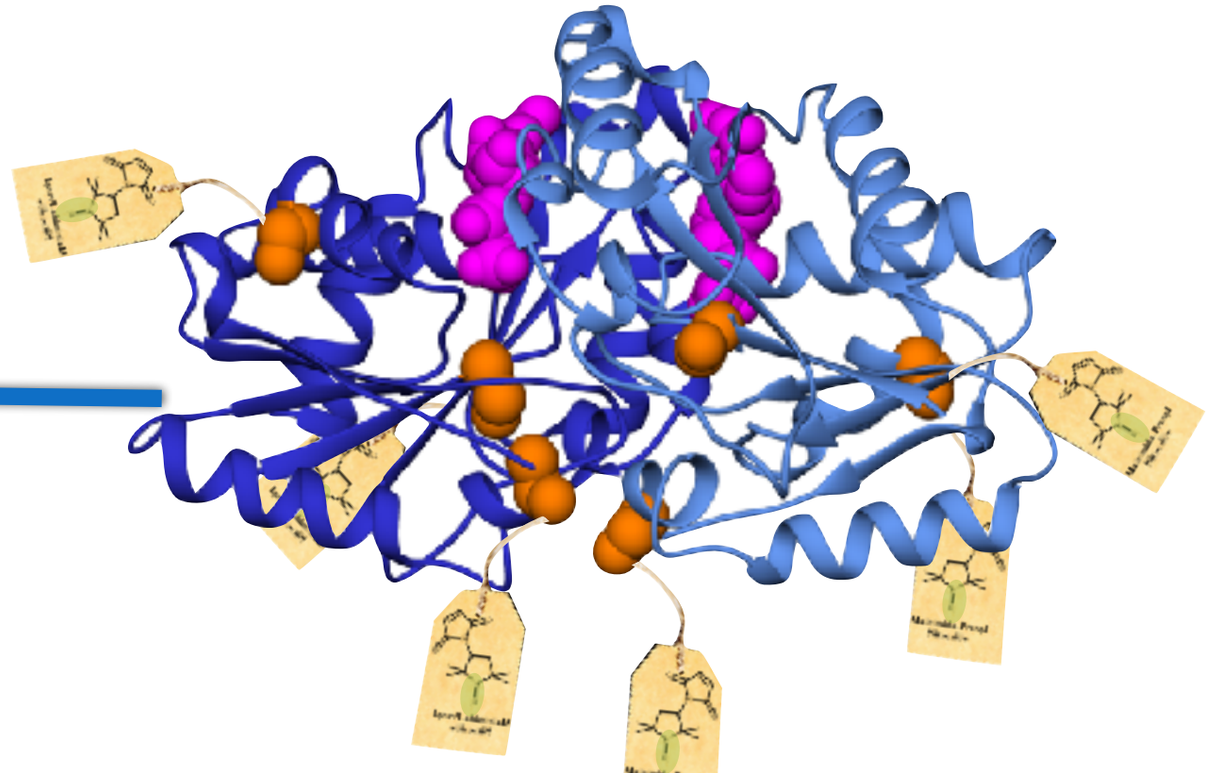

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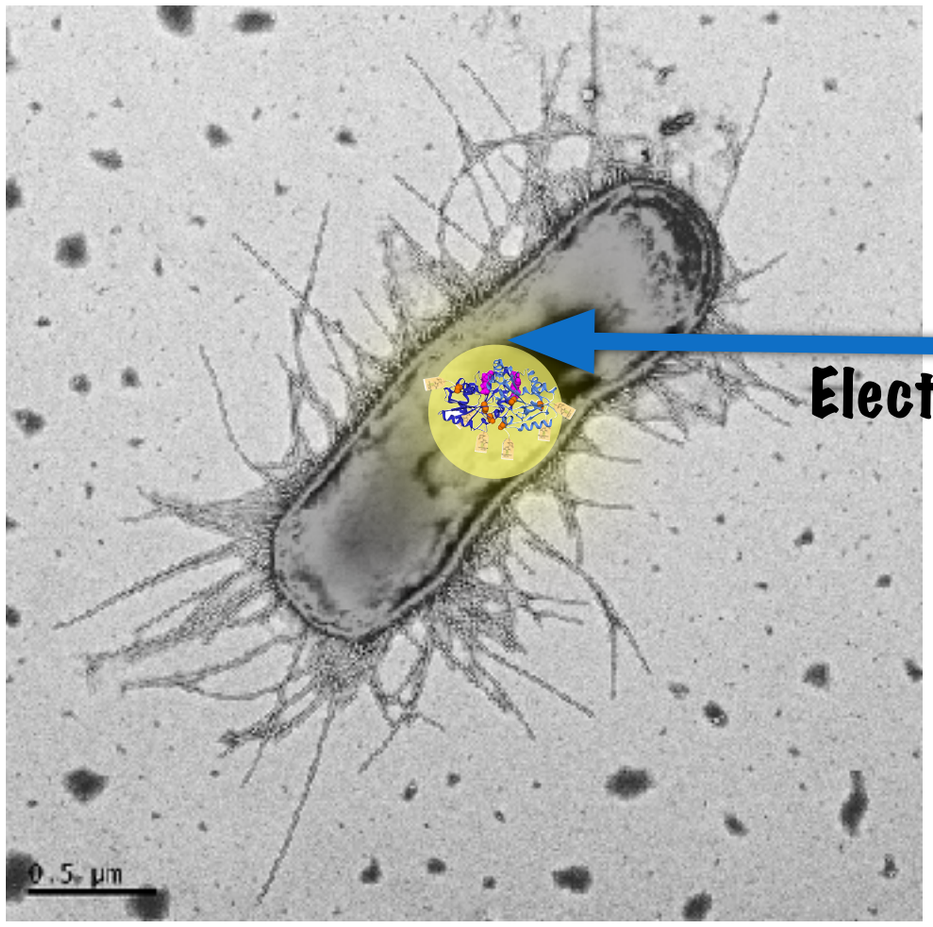

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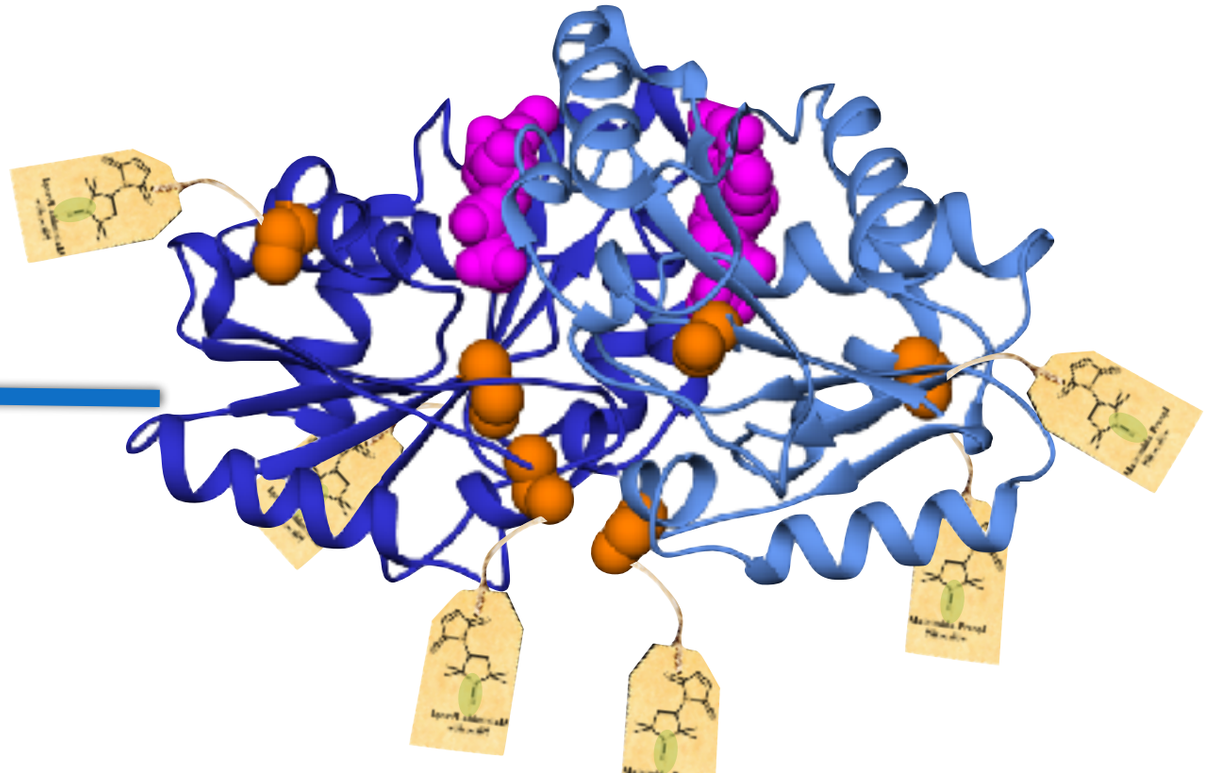
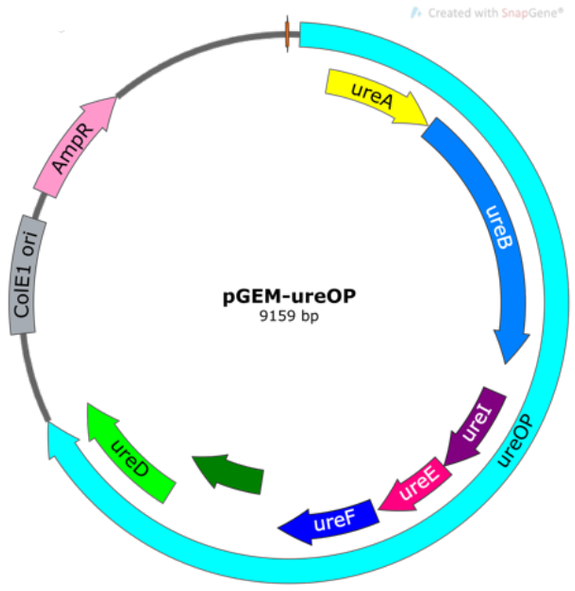
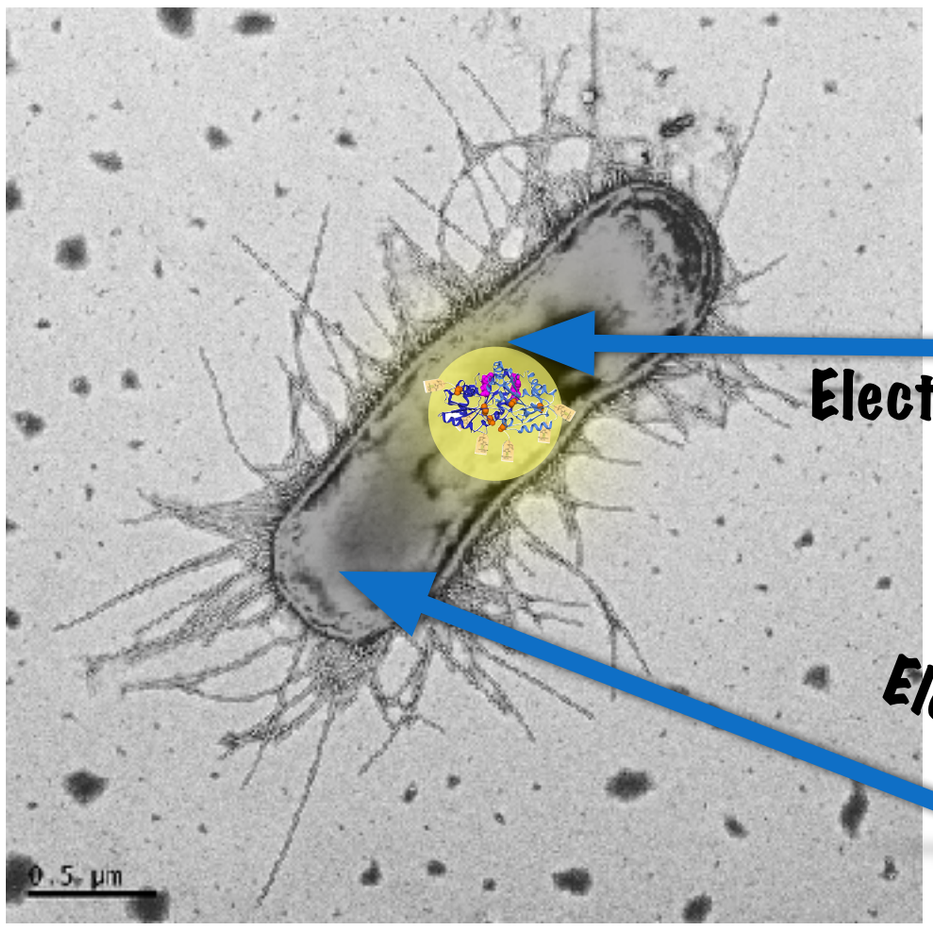




H. pylori genomic urease operon cloned into a plasmid vector and transformed into *E. coli*.

How it looks like inside the cell



Electroporation

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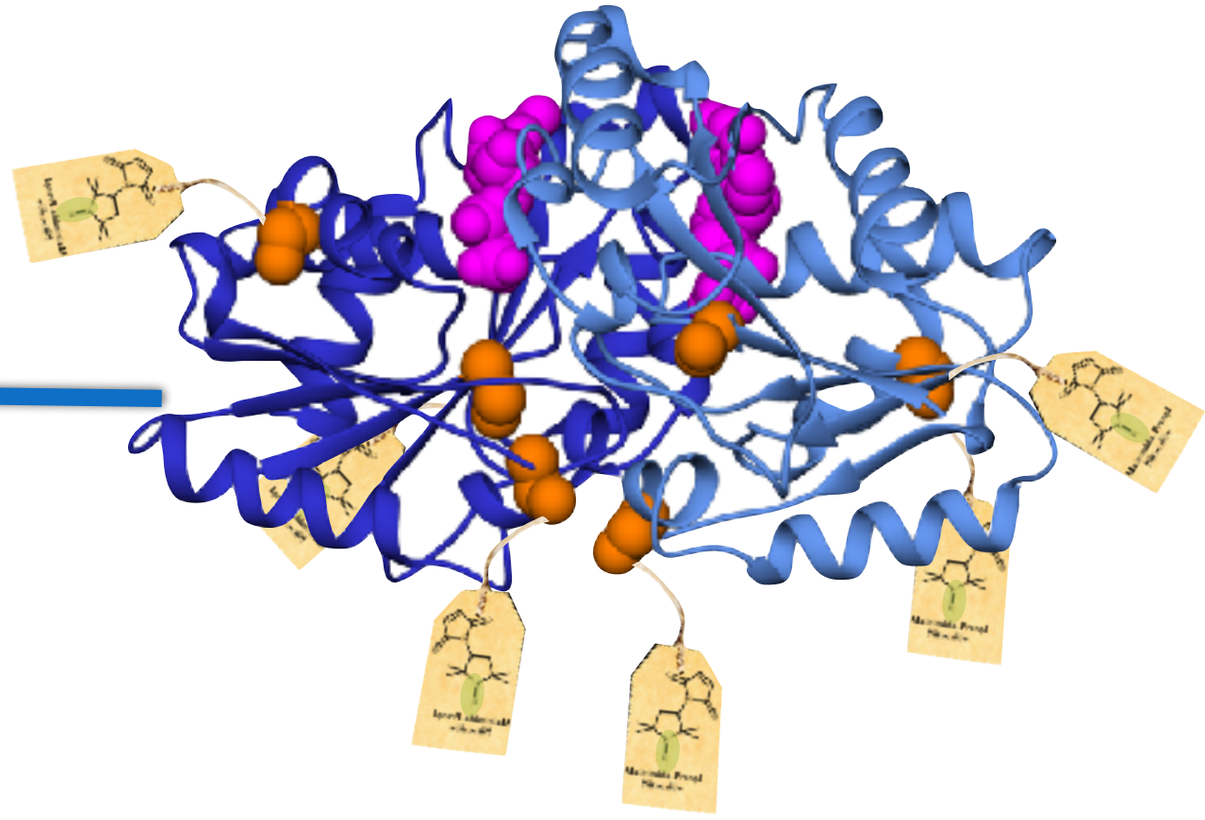

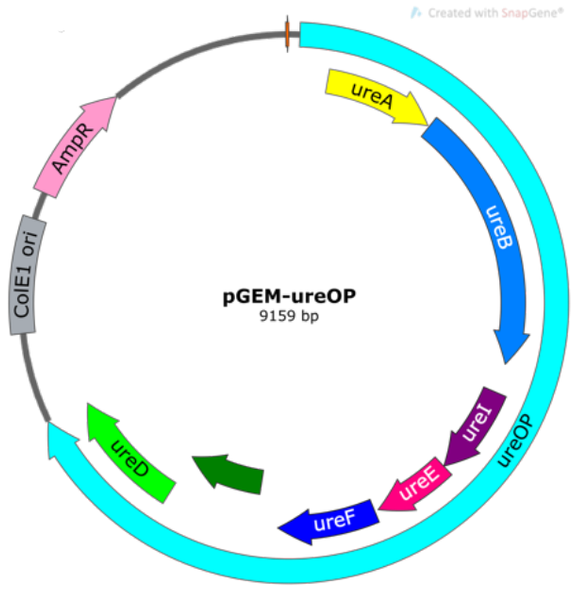

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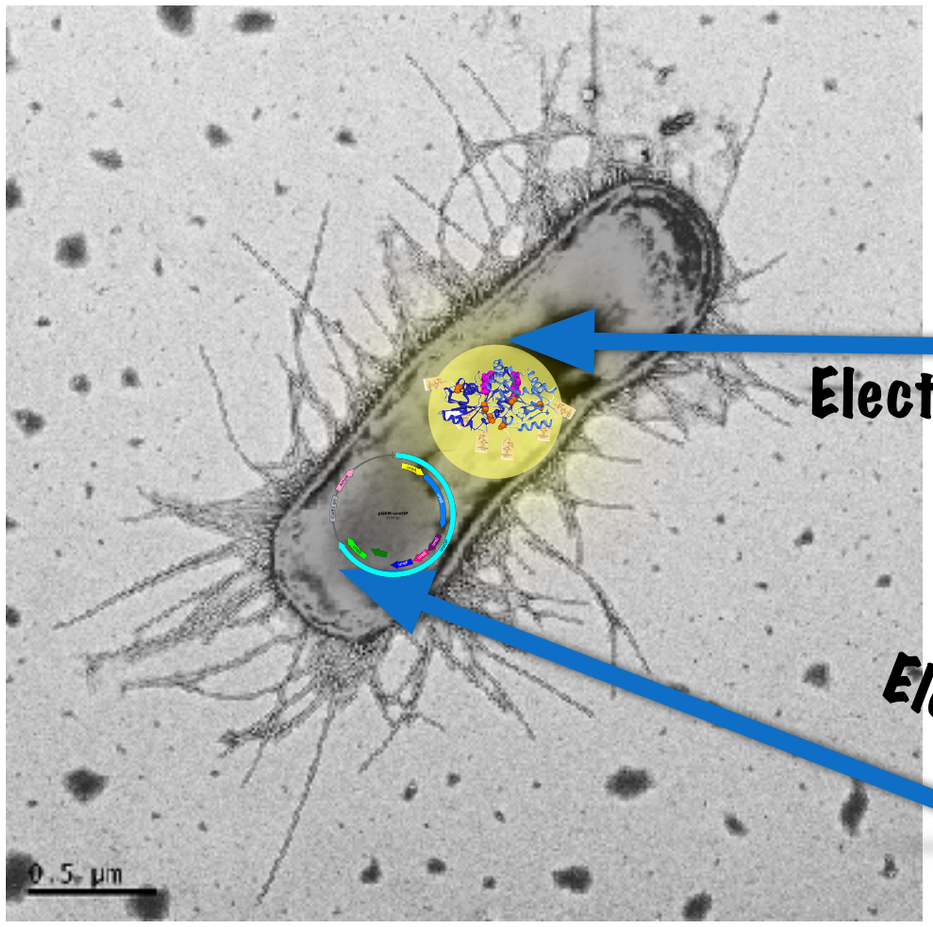

Electroporation

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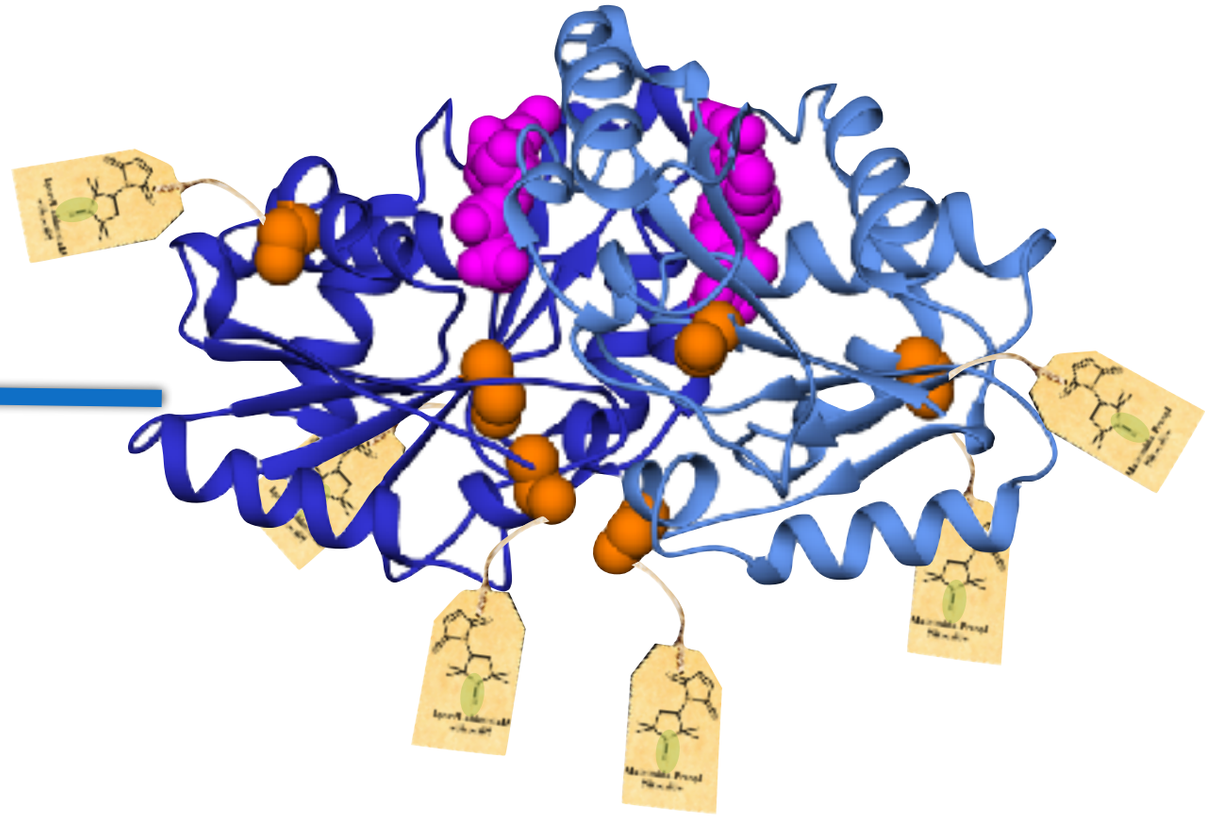

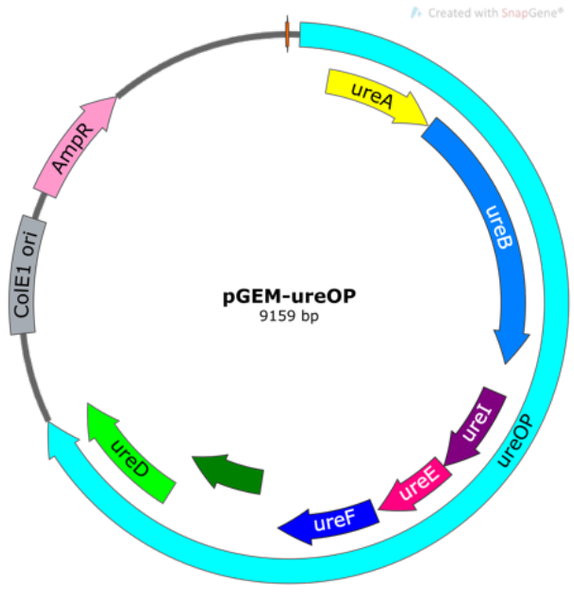

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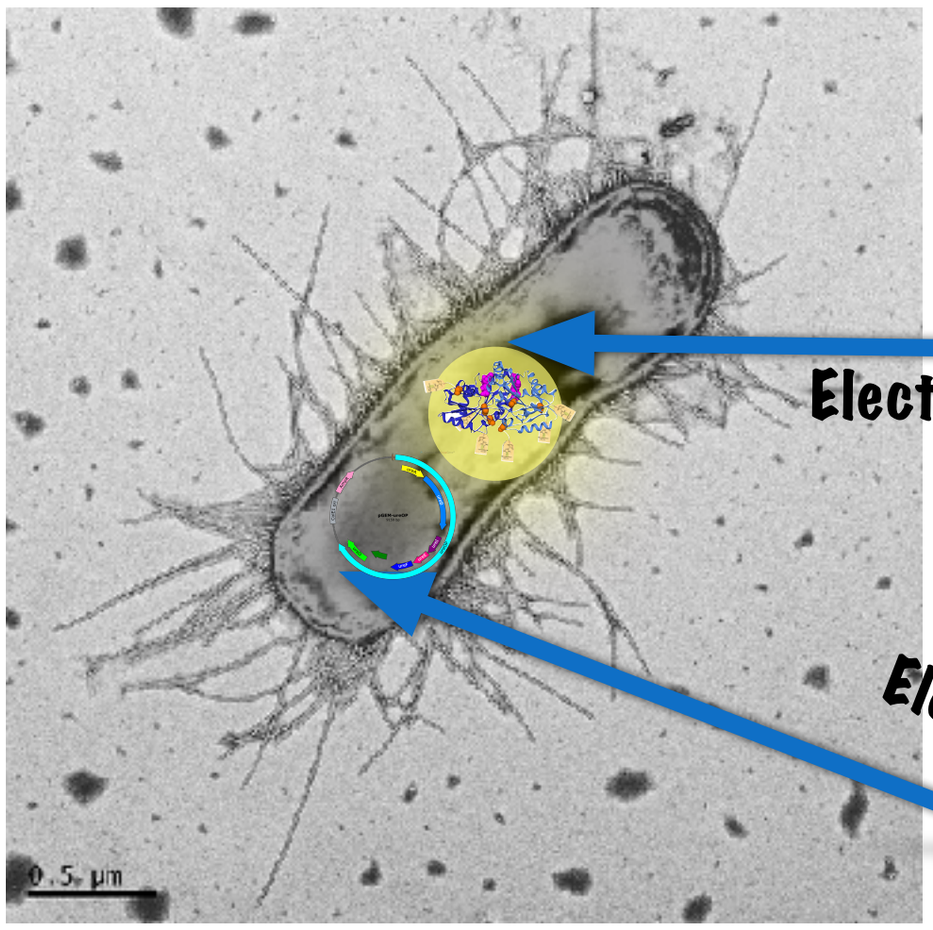

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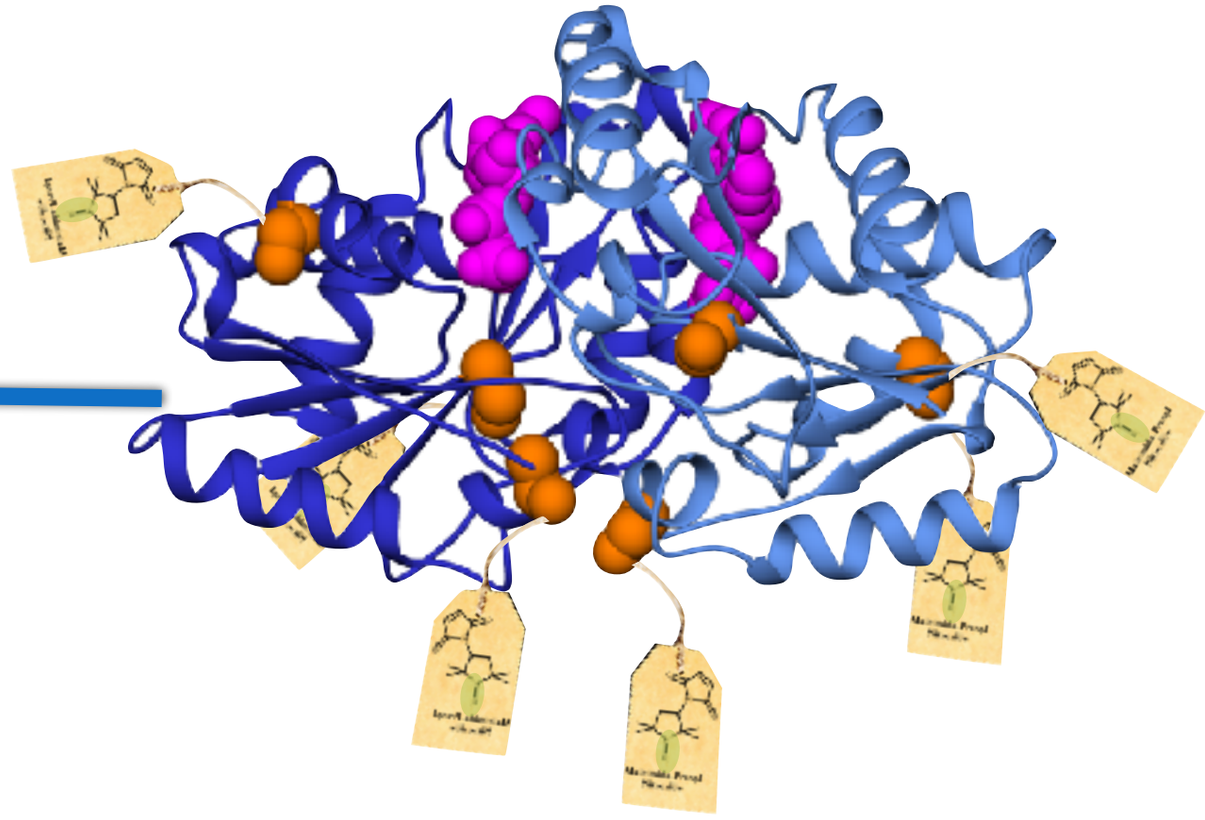
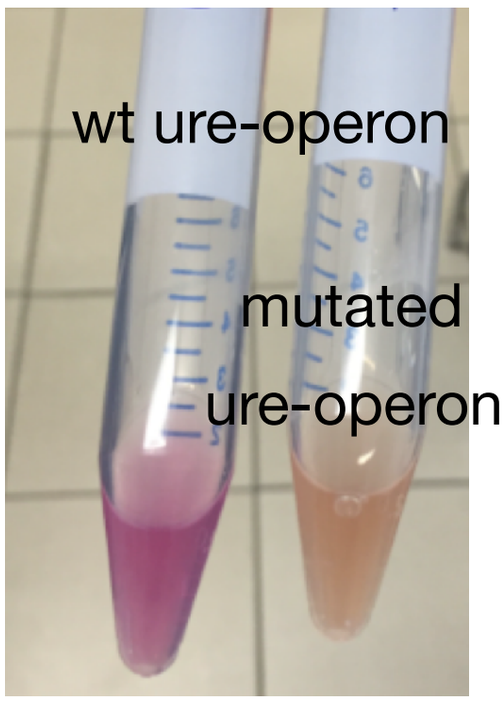
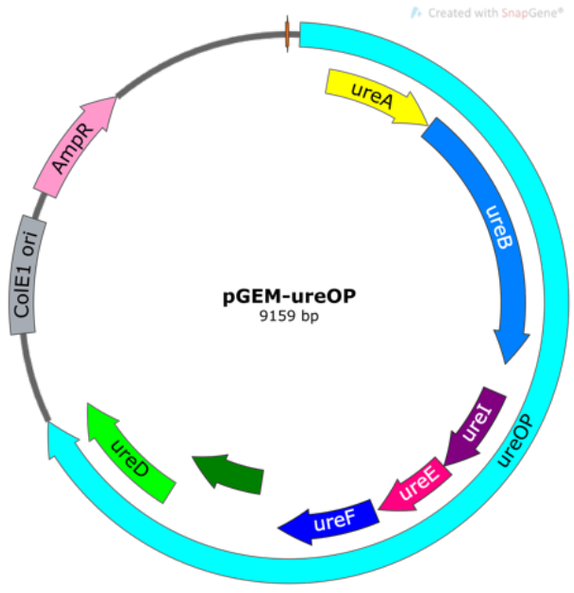




E. coli transformed with ureOP construct grown LB medium with urea, Ni and cresol red.



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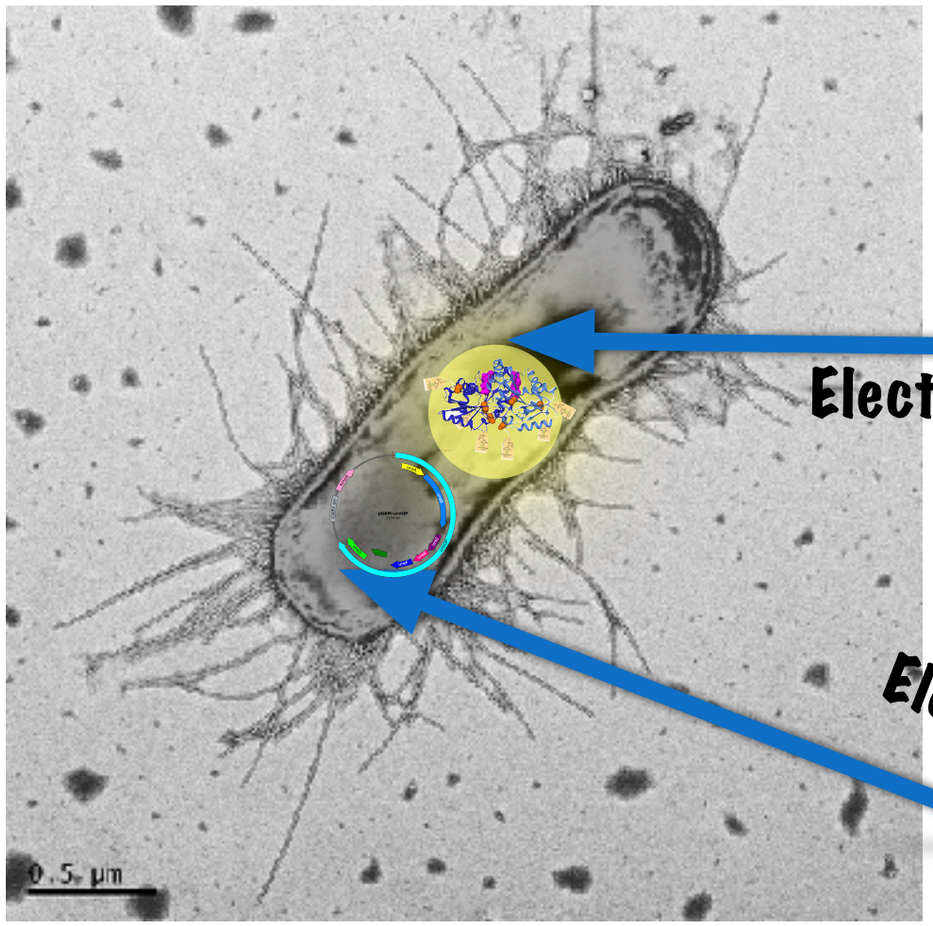

Electroporation

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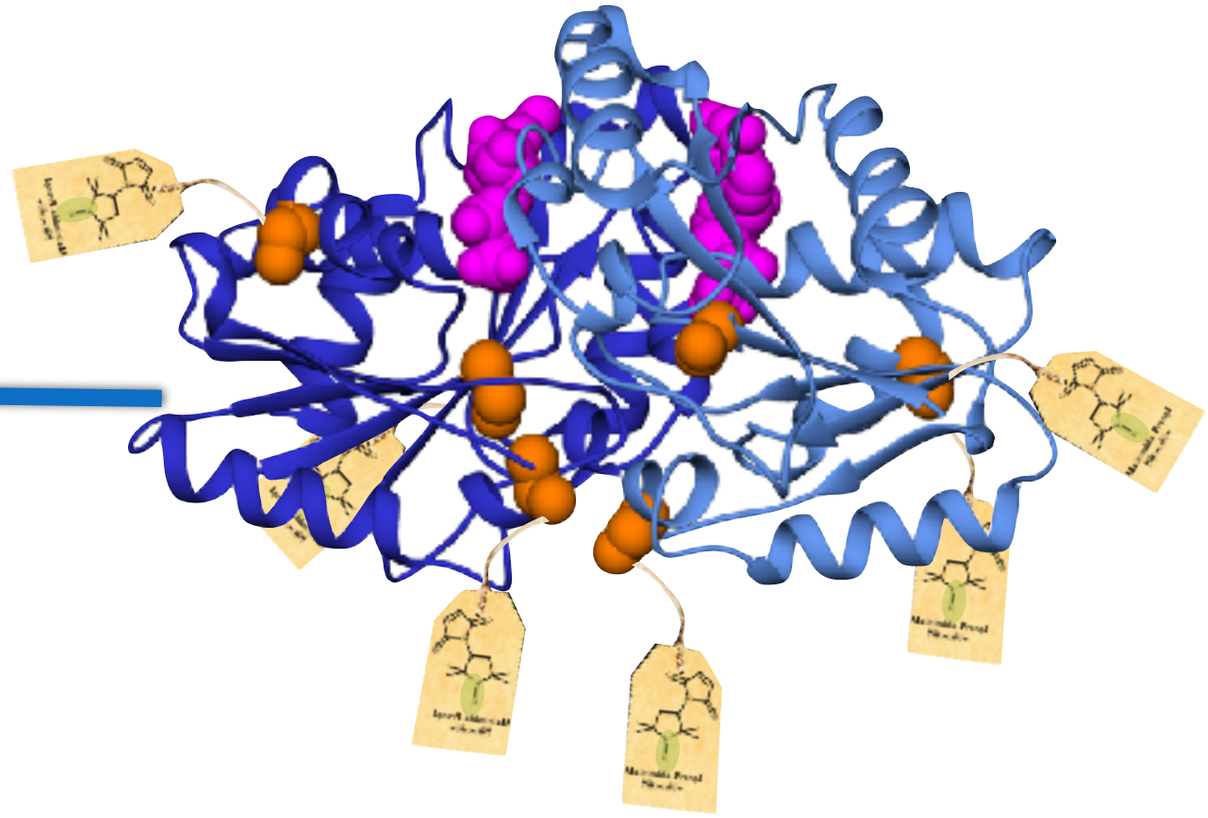

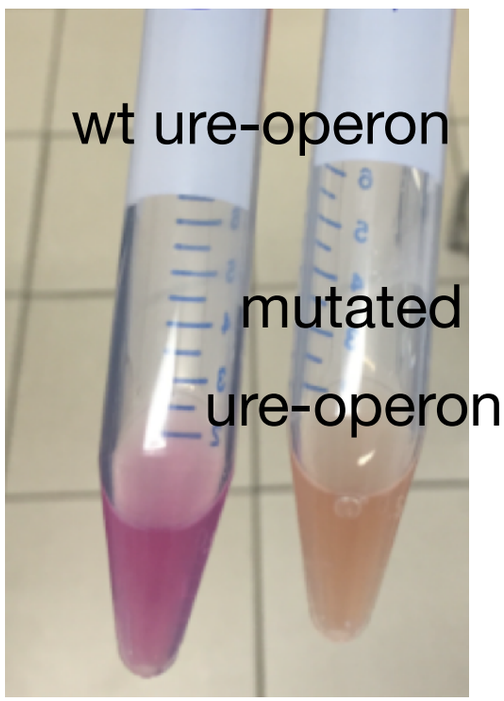

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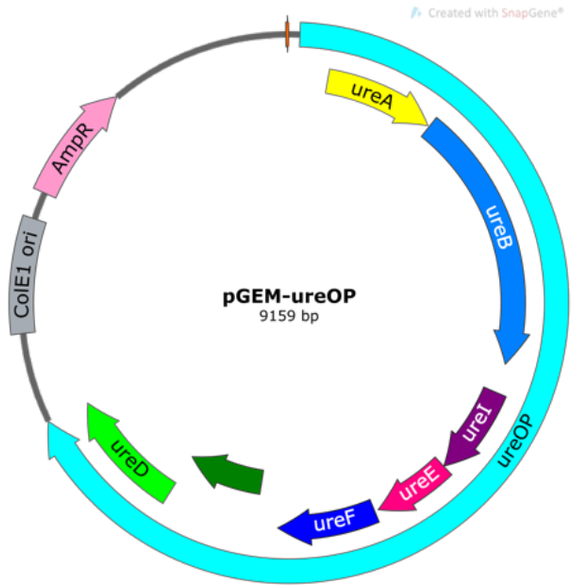

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Acknowledgments

My group @UniBO

Stefano Ciurli
Francesco Musiani
Luca Mazzei, Post-doc
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Abre-Mobieu cost action