PERSONA CIÈNCIA EMPRESA
UNIVERSITAT RAMON LLULL

LABORATORY OF BIOCHEMISTRY

Conformational modulation in a glycosyltransferase: evidence of mixed conformational selection and induced fit mechanisms by Bias-exchange metadynamics.

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GOBIERNO DE ESPAÑA
MINISTERIO DE ECONOMÍA Y COMPETITIVIDAD
GLYCO ZYMES

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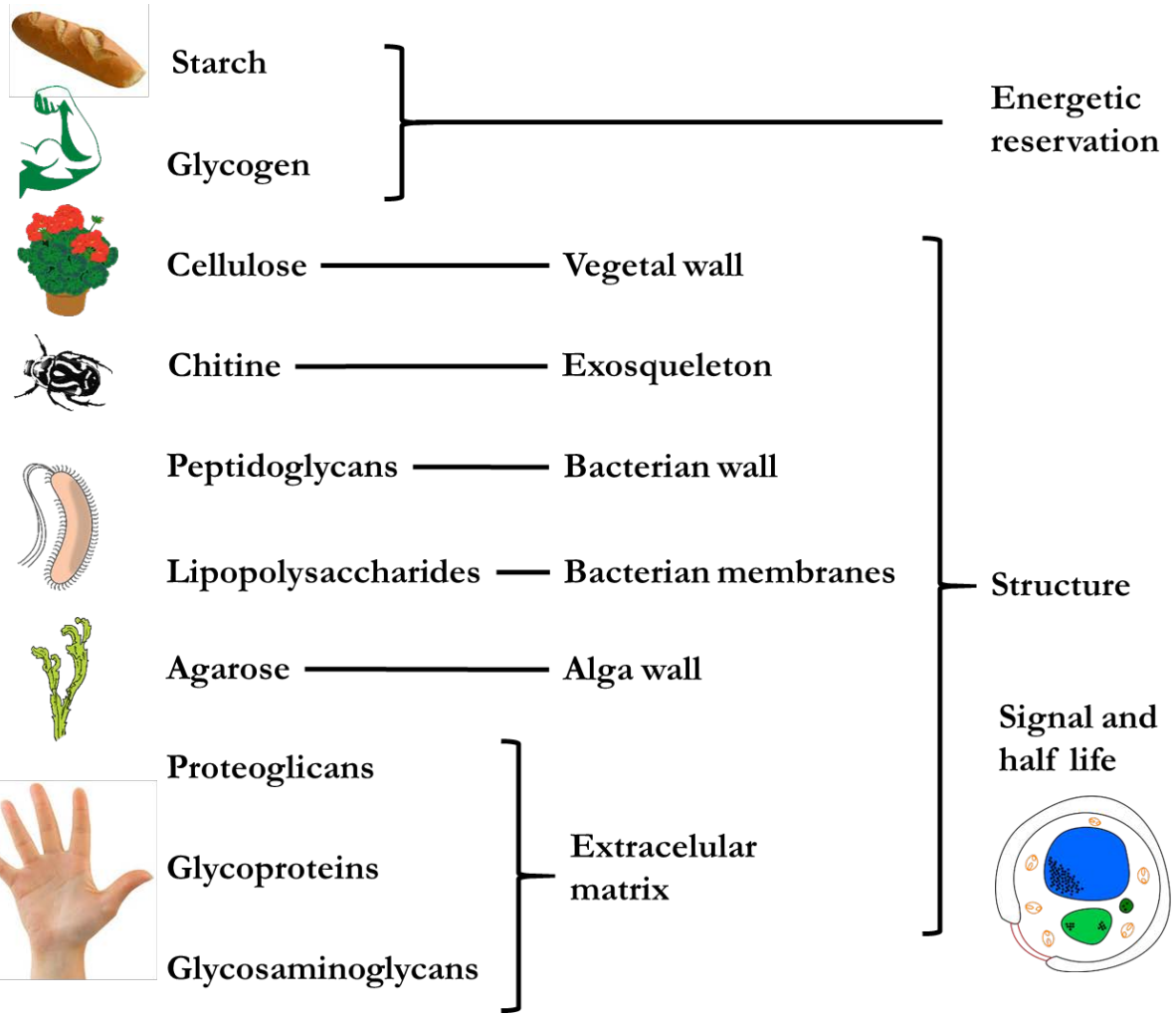
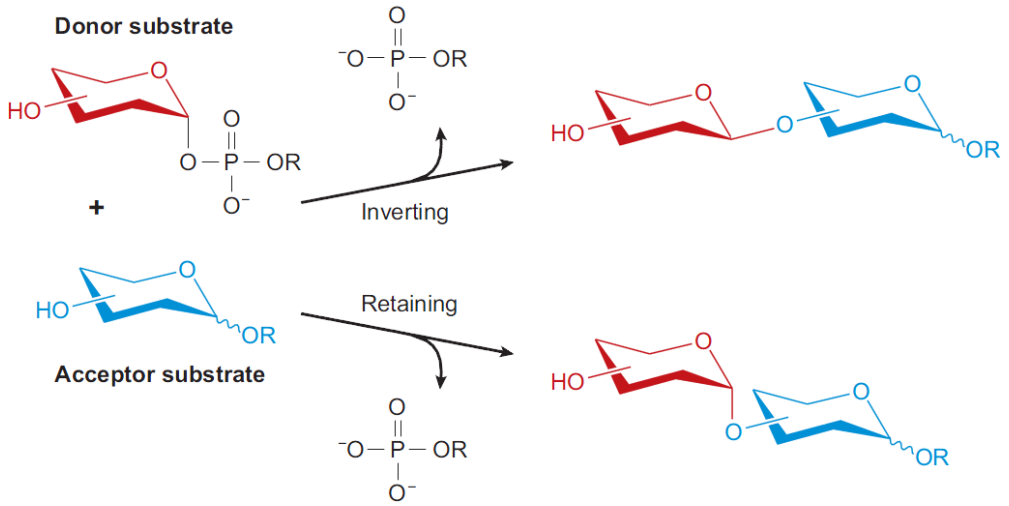
Glycosyltransferases catalyze the synthesis of glycoconjugates



- They catalyze the formation of the glycosidic bond between a sugar and an acceptor molecule.

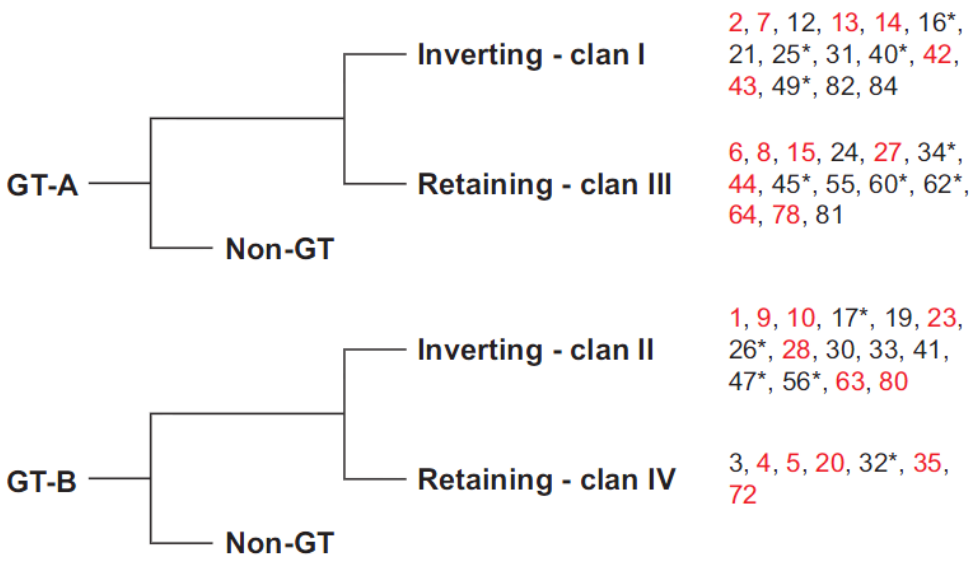


- **Donor substrates:**
  - UDP-Glc
  - UDP-Gal
  - UDP-GlcNAc
  - UDP-GalNAc
  - UPD-GlcUA
  - UDP-GalUA
  - UDP-Xyl
  - GDP-Man
  - GDP-Fuc
  - CMP-NeuAc
- **Acceptor substrates:**
  - Carbohydrates
  - Glycans
  - Lipids
  - Aminoacids
  - Proteins

Lairson et al. Annu Rev Biochem, 2008

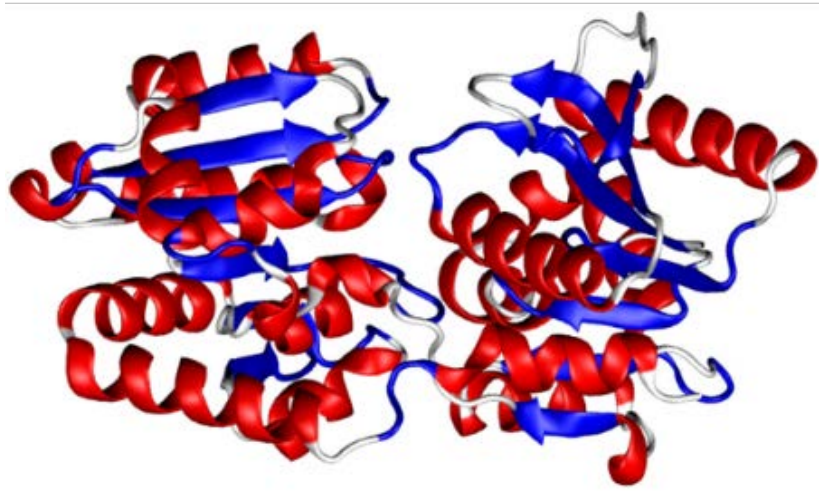
Glycosyltransferases are a diverse and widespread family of enzymes, that surprisingly share common structural folds

- 1% of the genome content (archaea, bacteria and eukariota).
- 97 GT families (CAZY).
- Common Rossmann-like folds.



GT-A fold

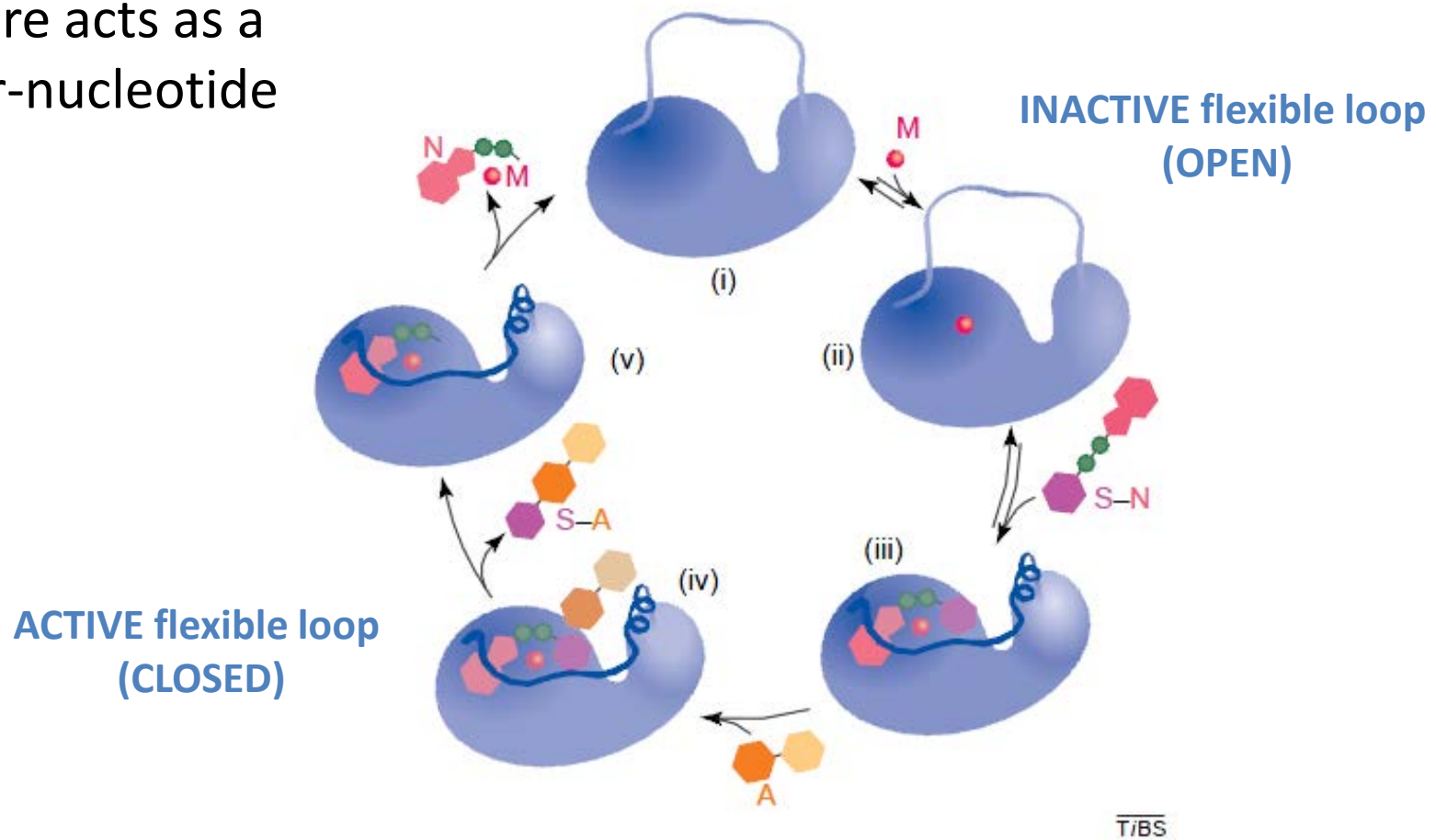
GT-B fold



Lairson et al. Annu Rev Biochem, 2008

Substrate induced conformational changes are a relevant characteristic of the catalytic cycle in many glycosyltransferases

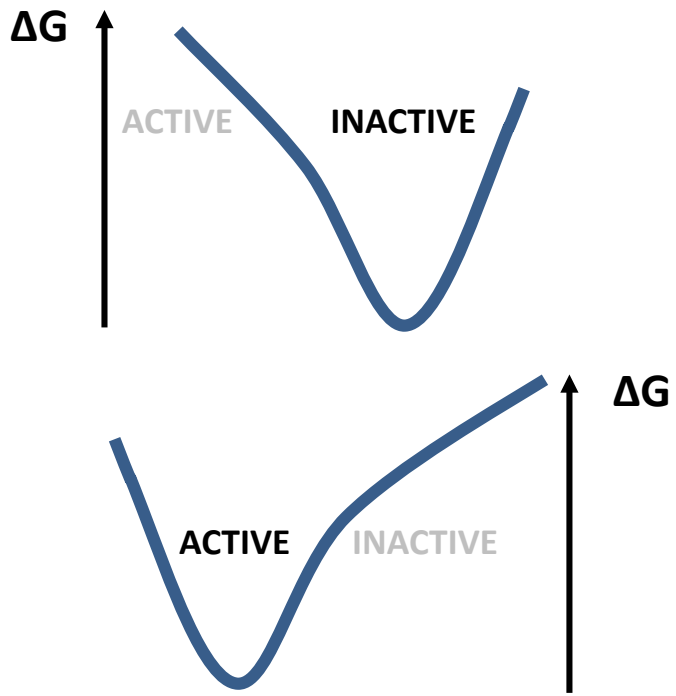
A flexible loop in the structure acts as a lid covering the bound sugar-nucleotide

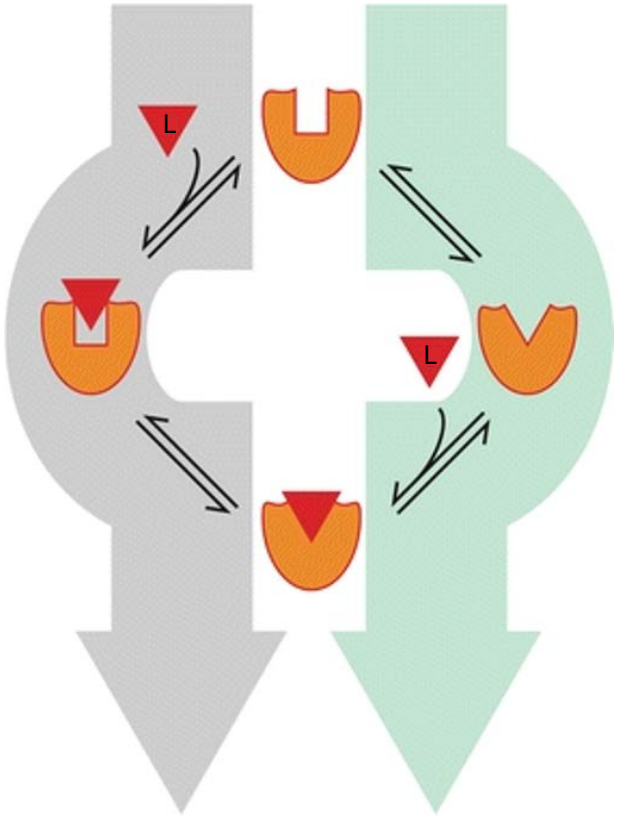


adapted from *Qasba et al.* TRENDS in Biochem Sci, 2005

The induced fit mechanism vs. conformational selection

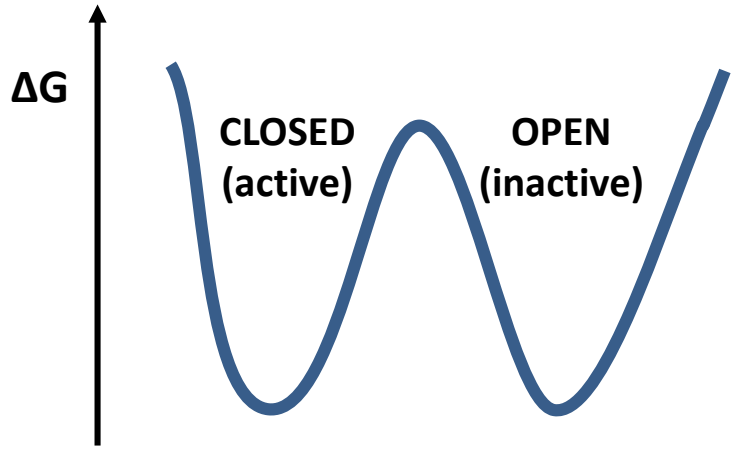
Induced fit





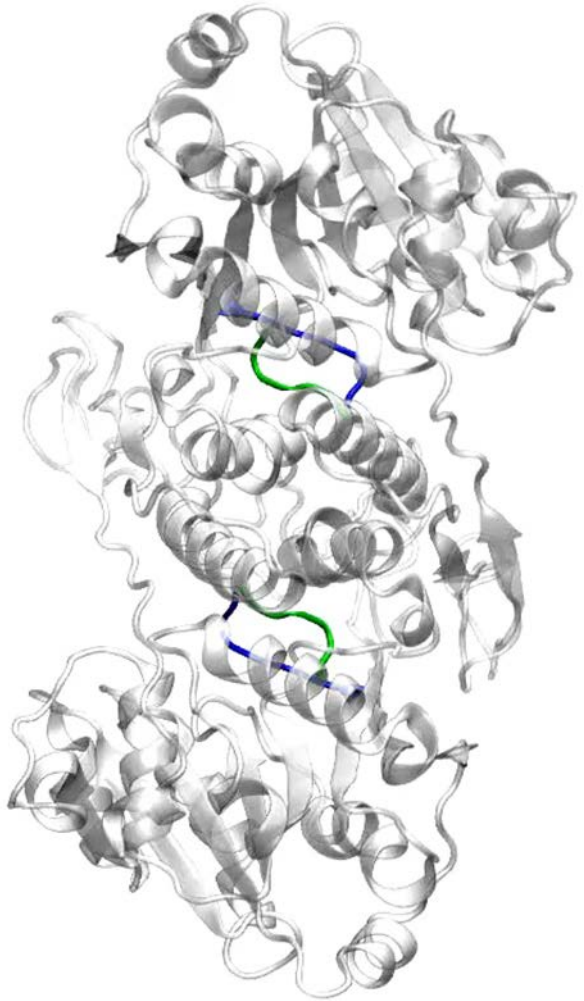
The ligand induces the conformational change of the protein

Conformational selection



The ligand binds to only one protein conformation

X-Ray structure reveals the co-existence of two different conformations of the loop in a family 81 glycosyltransferase



GpgS dimer.
LC/LO conformations.

Urresti et al. J Biol Chem 2012

Mycobacterium tuberculosis

- Glucosyl-3-phosphoglycerate Synthase (*Mt*GpgS)
- Biosynthesis of lipopolisacharides in mycobacteria

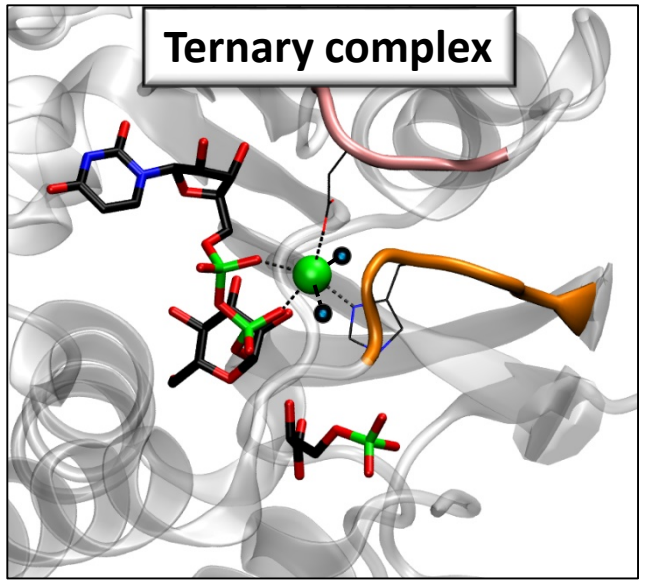
- **Loop $R_{256}AHRN_{260}$ in the active site.**
  - LC (Loop closed) conformation.
  - LO (Loop open) conformation.

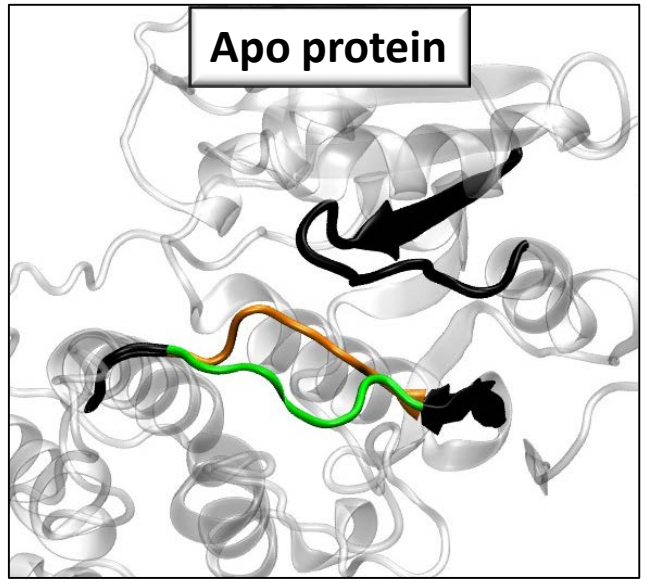
Ternary complex:

- Donor: UDPGlc+metal.
- Acceptor: PGA.
- **LC conformation**.

Apo protein LO (Loop inactive) conformation.

- **Both conformations (LC/LO)** in the same crystal (Occupation 56:44).

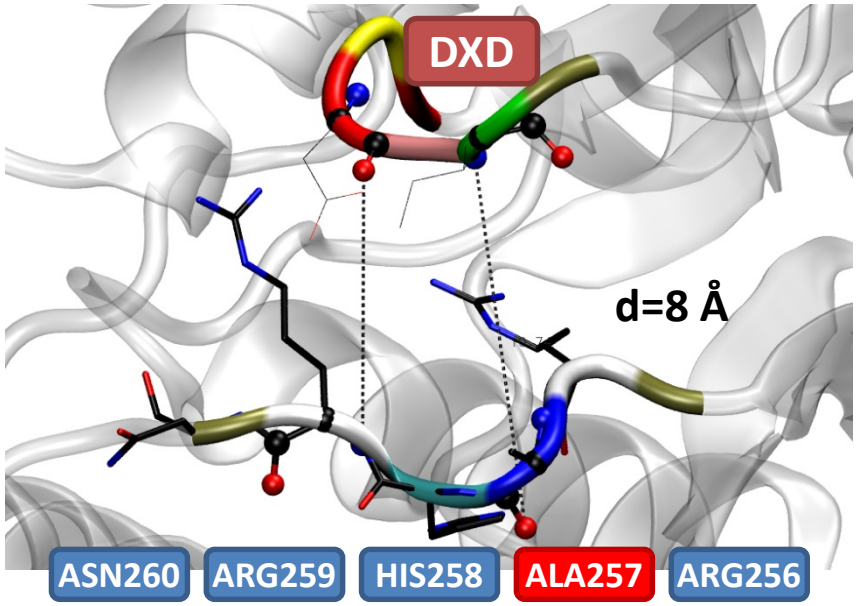




X-Ray structure reveals the co-existence of two different conformations of the loop in a family 81 glycosyltransferase

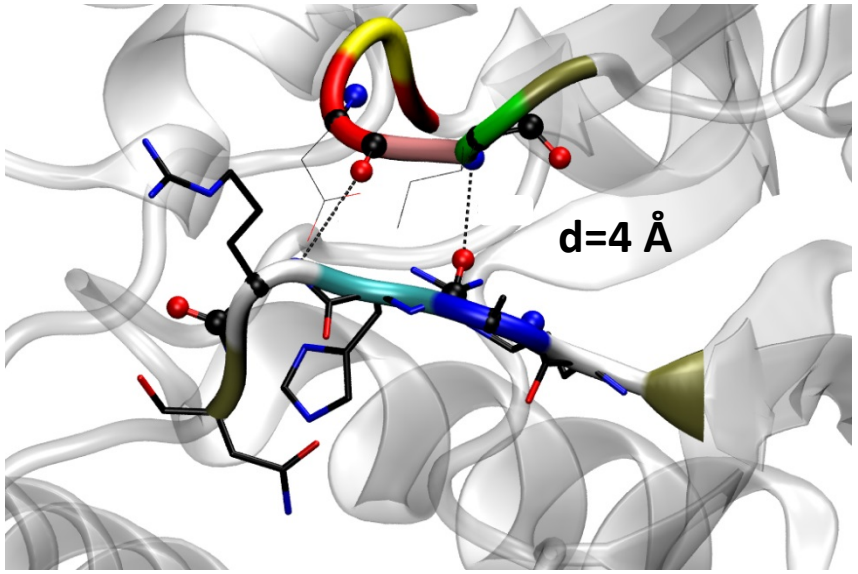
- The protein was crystallized in the absence of ligands! (apo form)

OPEN LOOP CONFORMATION (INACTIVE)



relative occupancy: **44 %**

CLOSED LOOP CONFORMATION (ACTIVE)

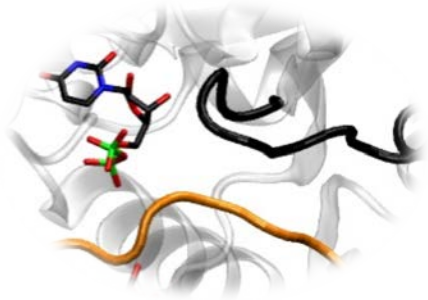


relative occupancy: **56 %**

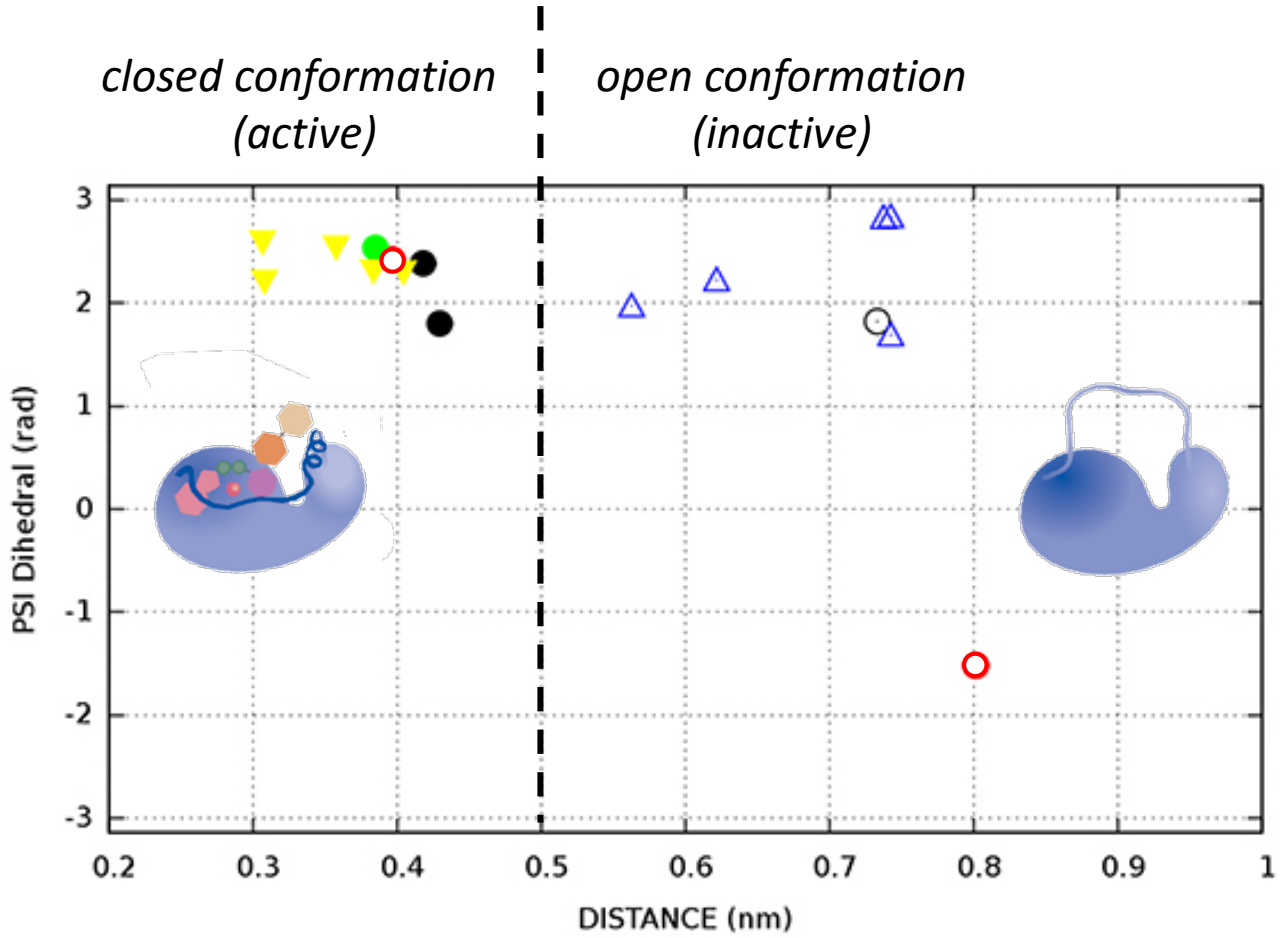
PDB: 4DDZ

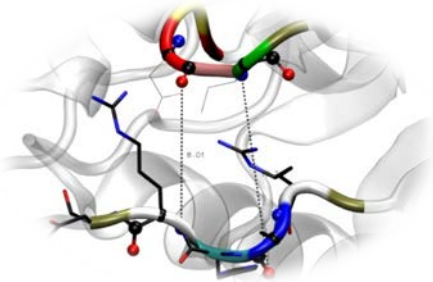
Urresti et al. J Biol Chem 2012

There is a variety of loop conformations in all GT81 X-Ray structures



UDP+PGA complex
● *Mt*GpgS
(PDB: 4DEC)



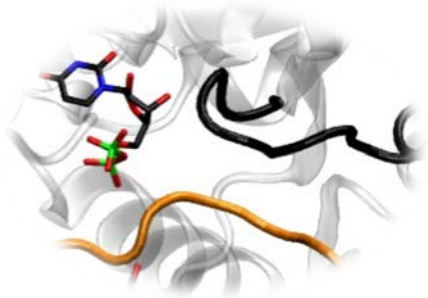


apo *Mt*GpgS
(PDB: 4DDZ) ○

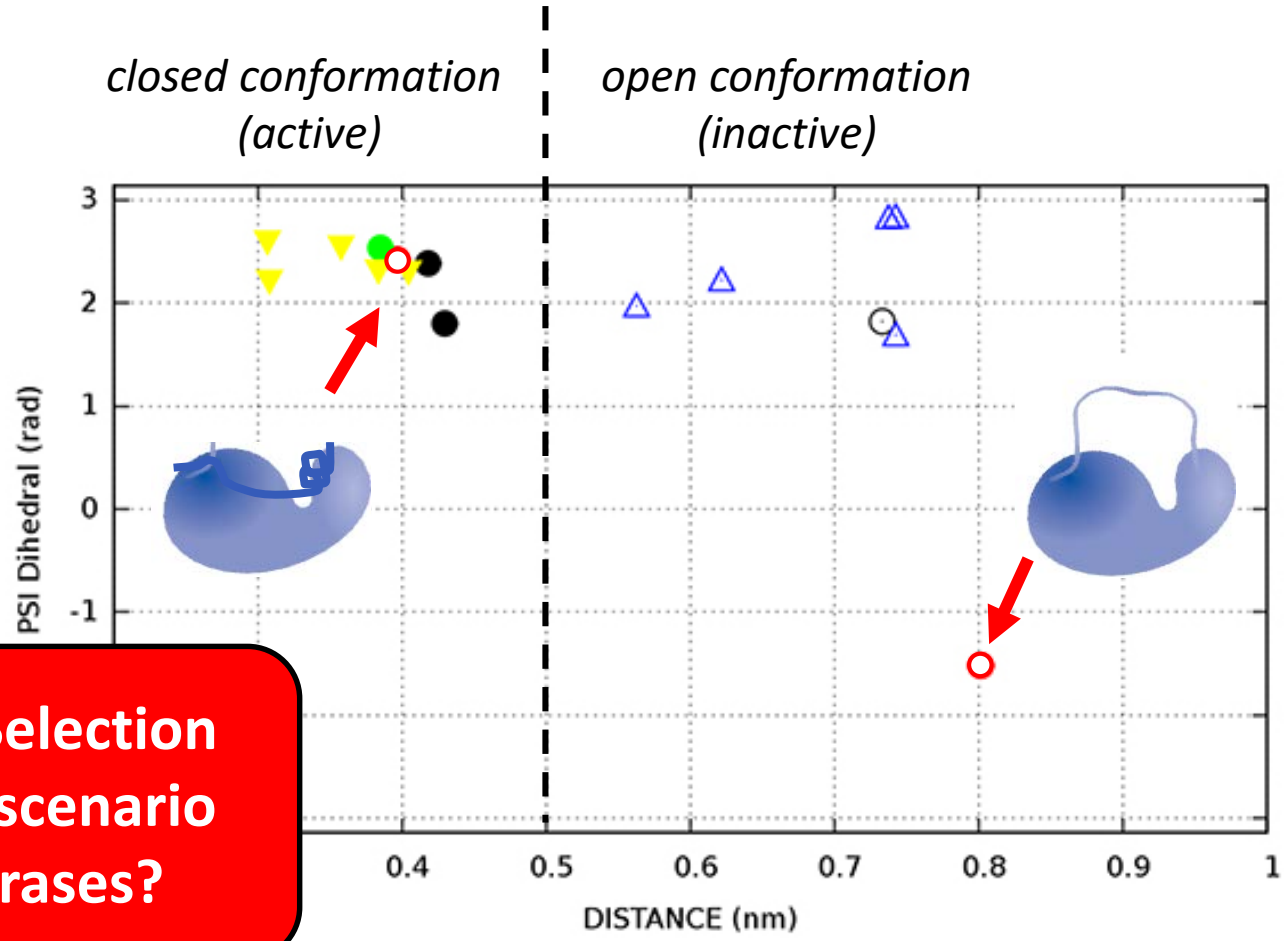
●●● *M. tuberculosis* GpgS (PDB: 4DDZ, 3E26, 4DE7, 4DEC, 4Y6N)
▼ *M. paratuberculosis* GpgS (PDB: 3CKJ, 3CKN, 3CKO, 3CKQ, 3CKV)
▲ *R. xylanophilus MpgS* (PDB: 3F1Y, 3KIA, 3O3P)

Filled symbols: enzyme-substrate complexes
Empty symbols: un-liganded proteins

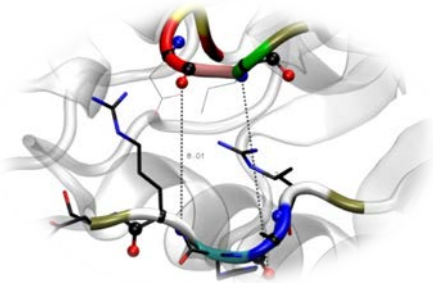
There is a variety of loop conformations in all GT81 X-Ray structures



UDP+PGA complex
● *Mt*GpgS
(PDB: 4DEC)



Is Conformational Selection a new mechanistic scenario in glycosyltransferases?



apo *Mt*GpgS
(PDB: 4DDZ) ○

●●● *M. tuberculosis* GpgS (PDB: 4DDZ, 3E26, 4DE7, 4DEC, 4Y6N)
▼ *M. paratuberculosis* GpgS (PDB: 3CKJ, 3CKN, 3CKO, 3CKQ, 3CKV)
▲ *R. xylanophilus MpgS* (PDB: 3F1Y, 3KIA, 3O3P)

Filled symbols: enzyme-substrate complexes
Empty symbols: un-liganded proteins

The project

PDB	Ligands	Simulation method
4DDZ	Apo	Molecular Dynamics
		Molecular Dynamics
		Metadynamics
4Y6N	Apo	Molecular Dynamics
		Molecular Dynamics
		Metadynamics
		Metadynamics
		Metadynamics
		BIAS-Exchange
	Ternary complex	Molecular Dynamics
		Metadynamics
	UDPGlc·metal	Metadynamics
		Metadynamics
		Metadynamics
		BIAS-Exchange
		BIAS-Exchange
	PGA	Metadynamics

- Long Molecular Dynamics.
- GROMACS v.4.5.3
- All atoms (~ 50,000).
- Explicit solvent.
- Amber forcefield.
- Triclinic box(-d 0.9).
- Na+, Cl- ions, 0,15 mM.
- pH 7.

~ 50 simulations ~ 40 µs

Magerit (CeSViMa) → 11 Activities → 3 years

Intel core I7 4 CPUs (3 ns/day) → 30 years.

Molecular Dynamics

Based on
motion equations

Metadynamics

Enhanced MD
Free energy calculations

BIAS-Exchange

Enhanced MetaD
Free energy calculations

Laio and Parinello, PNAS 2002
Laio and Piana, J Phys Chem B 2007
Marinelli et al. PLoS Comput Biol 2009
D'Abramo et al. Angew Chem Intl Ed 2012
Beneti, Biarnés et al. J Mol Biol 2014

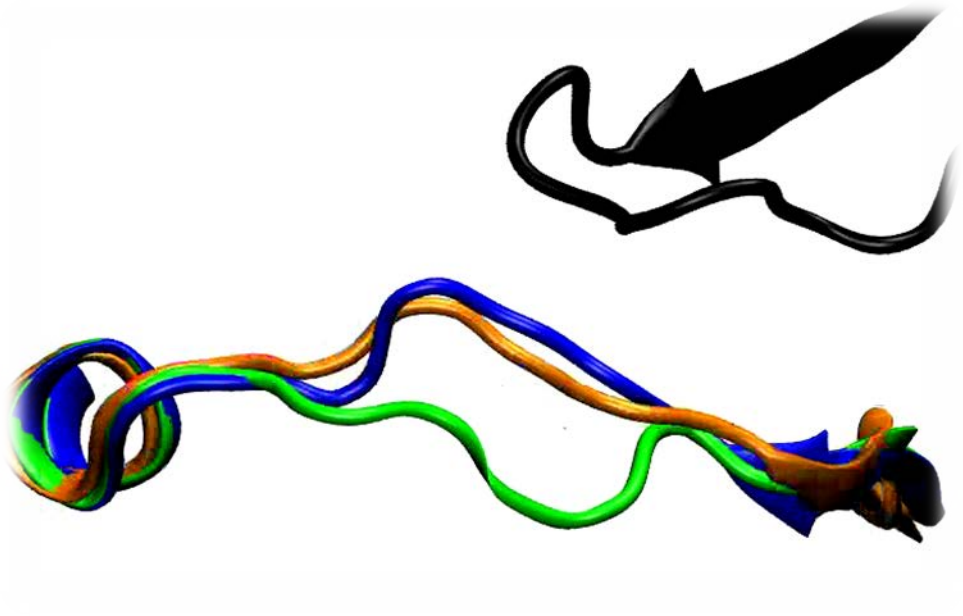
Classical Molecular Dynamics (MD) simulations allow exploring protein conformations in solution at room temperature

Simulations starting from different structures of *Mt*GpgS

- **Open** loop conformation
- **Closed** loop conformation

Simulations Details:
Initial structure: PDB 4DDZ
Simulation time: 500ns – 1μs
Time step: 2 fs
ForceField: amber03
Explicit Solvent: TIP3P

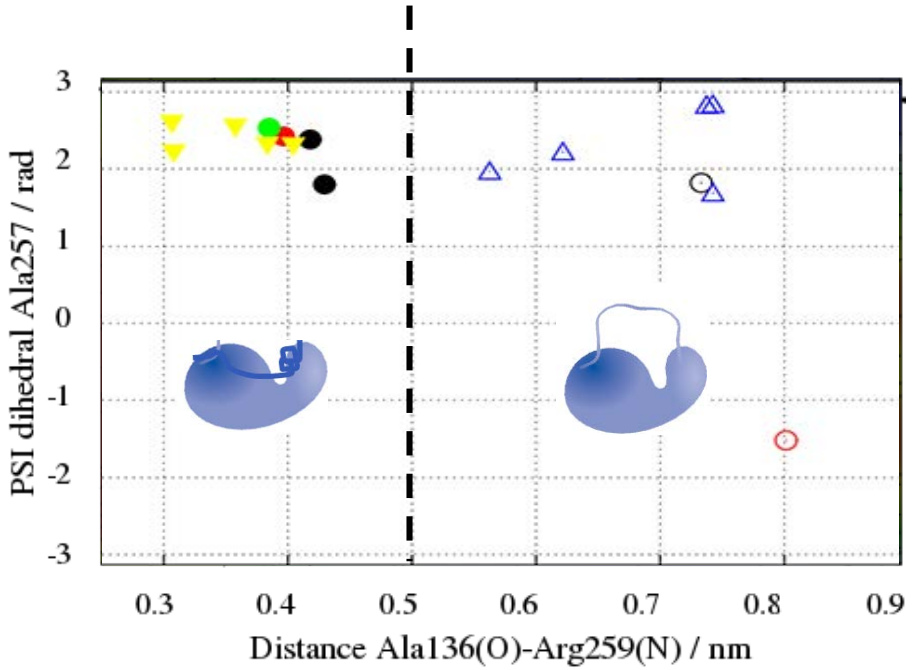
X-Ray Closed
MD simulation
X-Ray Open

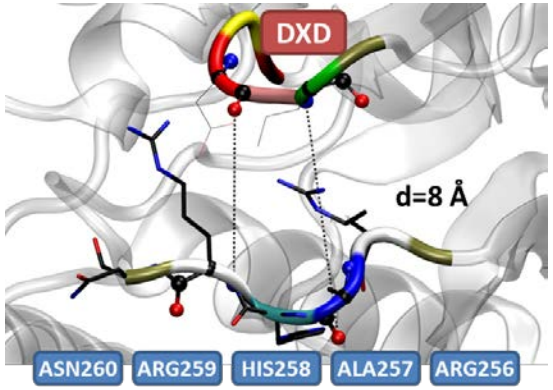


The flexible loop tends to adopt an open conformation

Examples of MD simulations on GTs:
Snajdrova et al. Carb Res 2004
Romero-García et al. PLoS ONE 2013

What's the conformational free energy landscape of the flexible loop in solution?





Collective variables:

Distance

Torsion

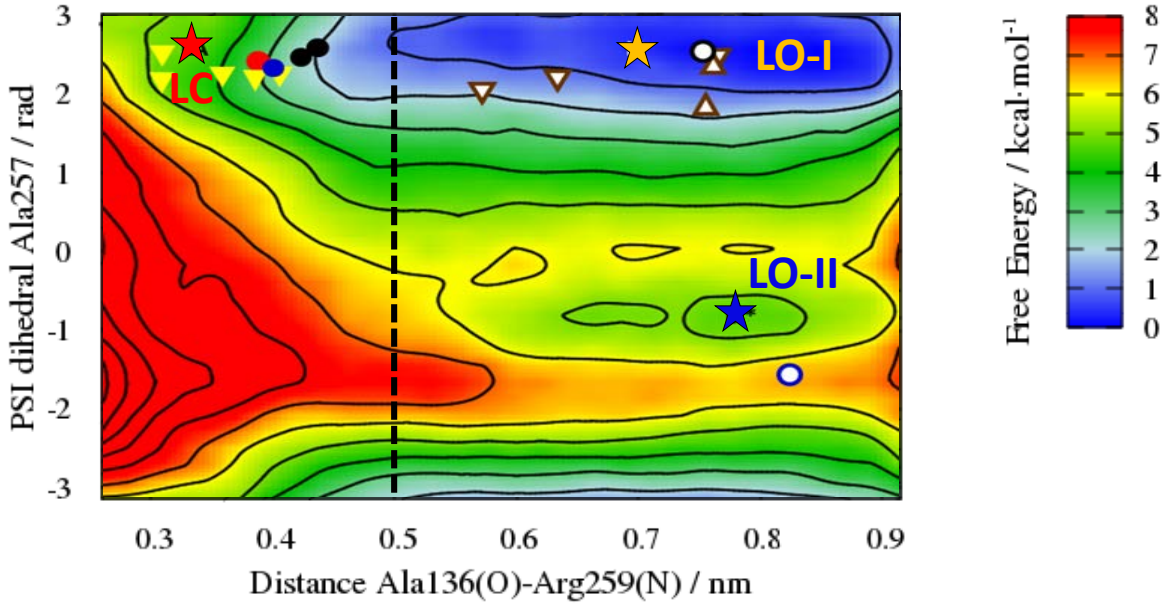
There are three thermodynamic stable states of the flexible loop in *Micobacterium tuberculosis* GpgS (GT81)

★ *closed*

★ *open-I*

★ *open-II*

unliganded *Mt*GpgS



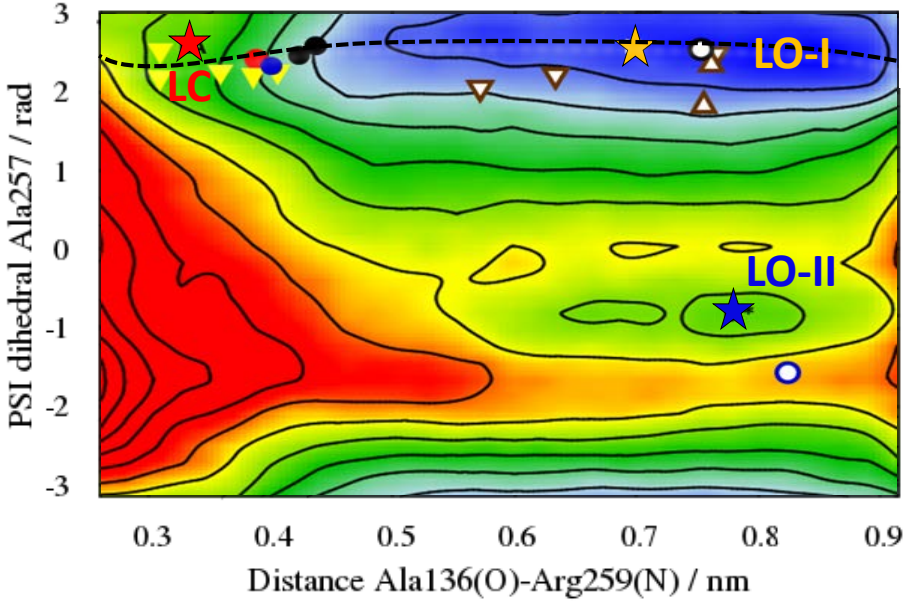
- Wide and flat free energy when the loop is in the open state (LO-I)
  (difficulties in solving this structure by x-ray cristallography)

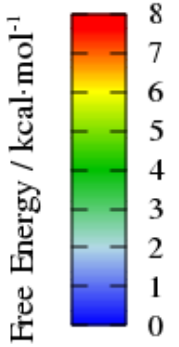
There are three thermodynamic stable states of the flexible loop in *Micobacterium tuberculosis* GpgS (GT81)

★ *closed*

★ *open-I*

★ *open-II*

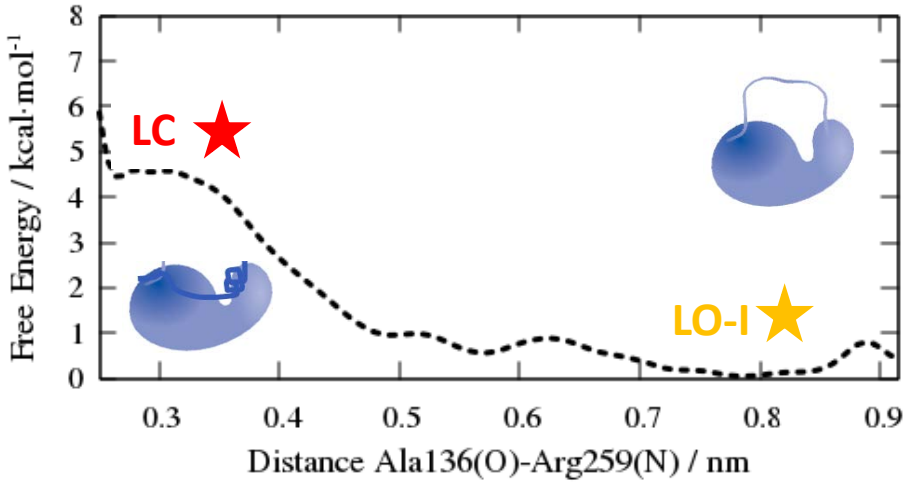


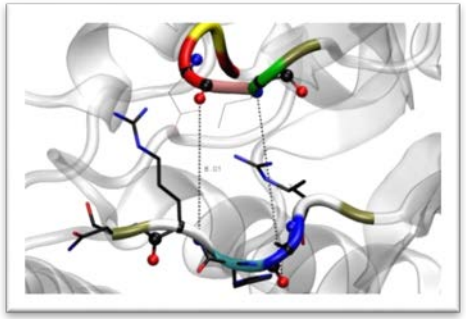


unliganded *Mt*GpgS

What is the effect of ligands in this conformational equilibrium?

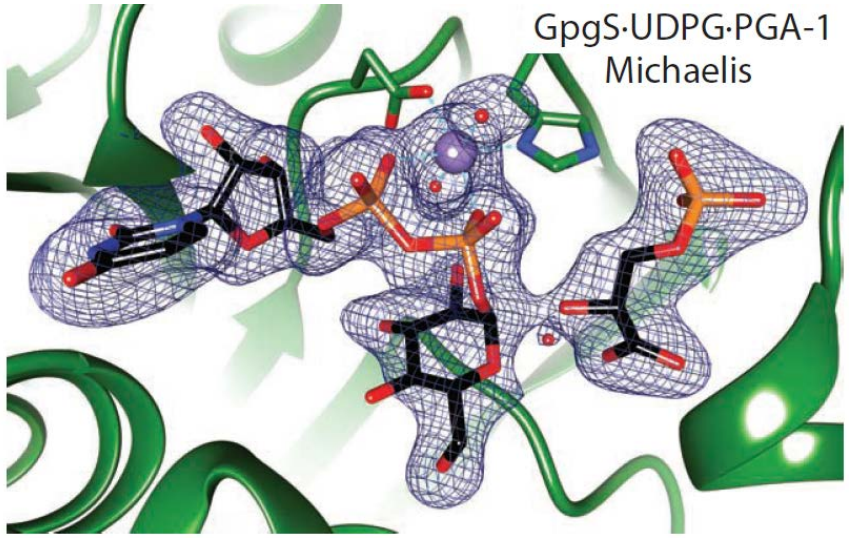
Closed state of the loop is disfavored by 4.5 kcal·mol^{-1}





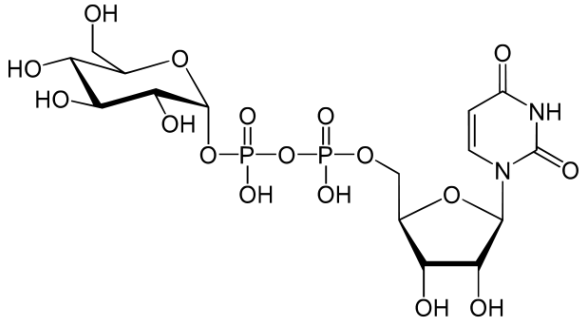
The first crystal structure of a native Michaelis complex in a glycosyltransferase

- *Mt*GpgS + Mn^{2+} + UDP-Glc + PGA
- Octahedral coordination of the metal
- Productive binding mode for catalysis
- Elucidation of the catalytic mechanism
  - support for front-side S_Ni-like
- Flexible loop in closed conformation



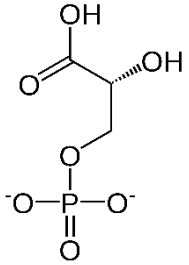
PDB: 4Y6N

D. Albesa-Jové et al
Angew Chem Int Ed Engl (2015)



UDP-glucose (UPD-Glc)

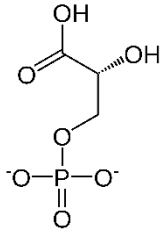
Mn^{2+}

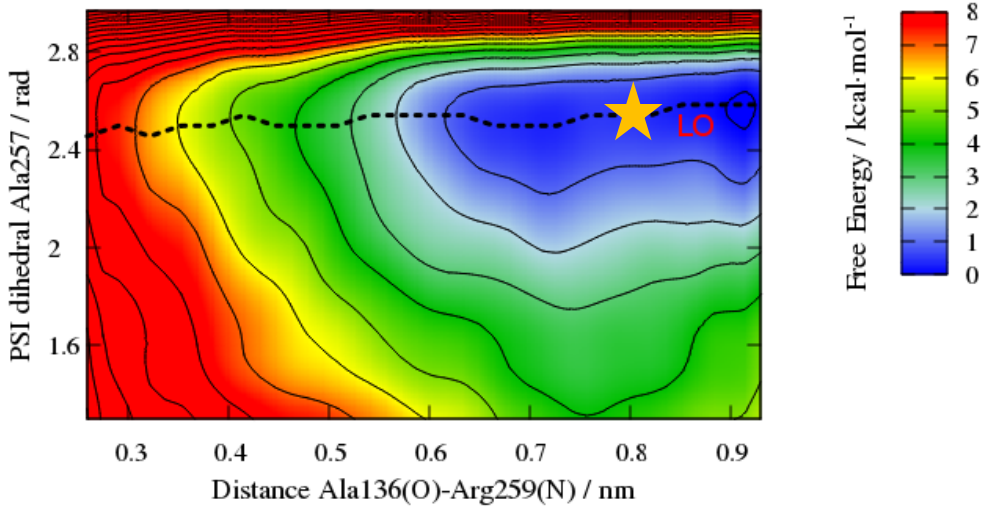


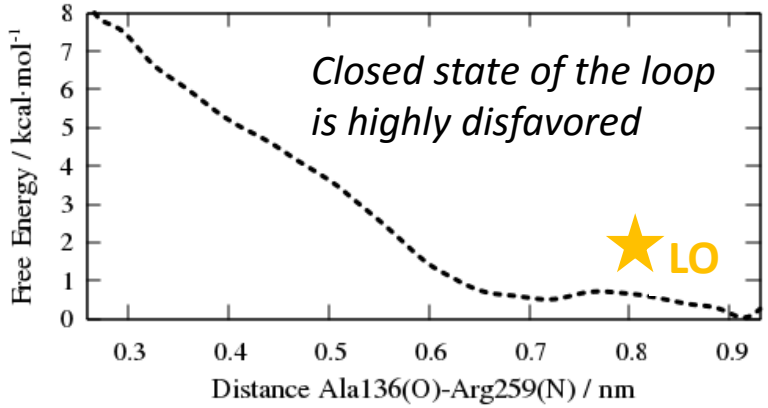
3-phosphoglycerate (PGA)

The effect of each separate ligand on the conformation of the flexible loop in *Mt*GpgS (GT81) is different

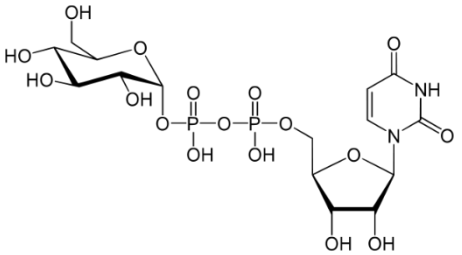
Free Energy of *Mt*GPGS in complex with **PGA**

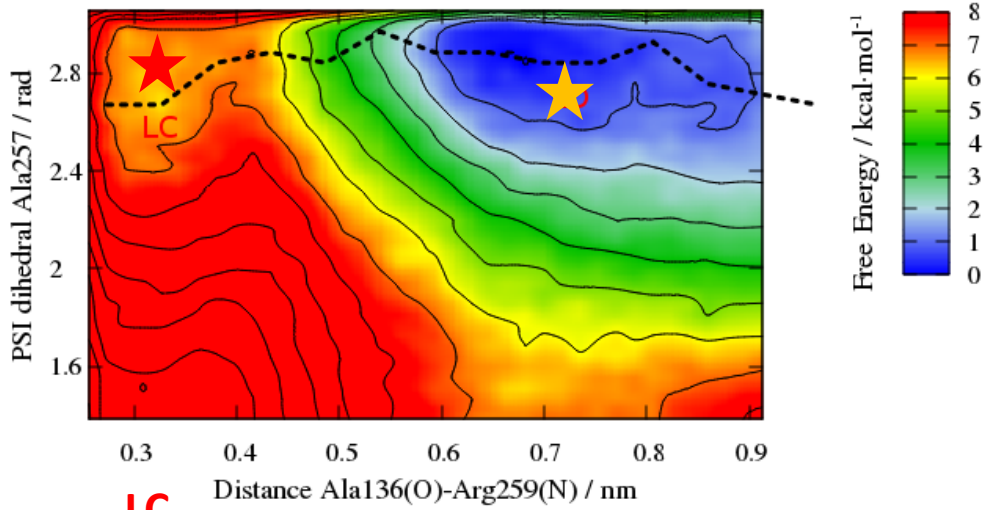


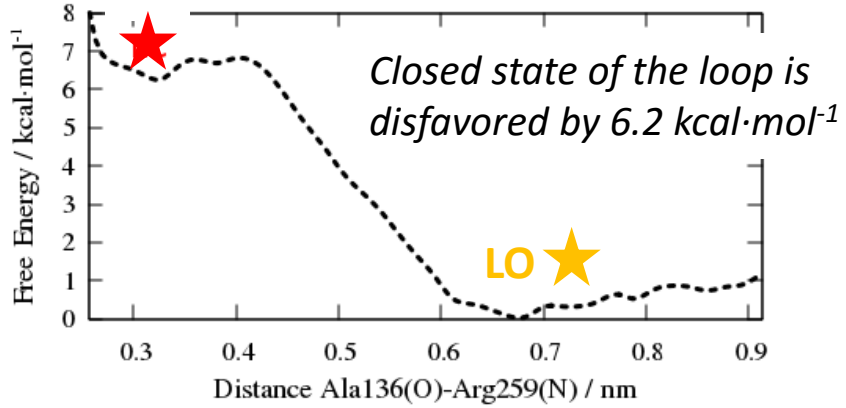




Free Energy of *Mt*GPGS in complex with **UDP-Glc**

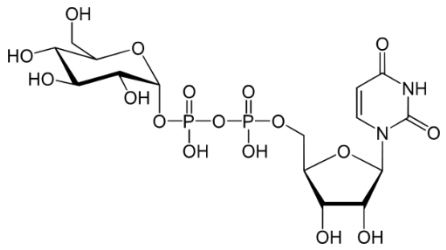


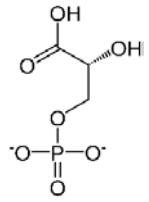


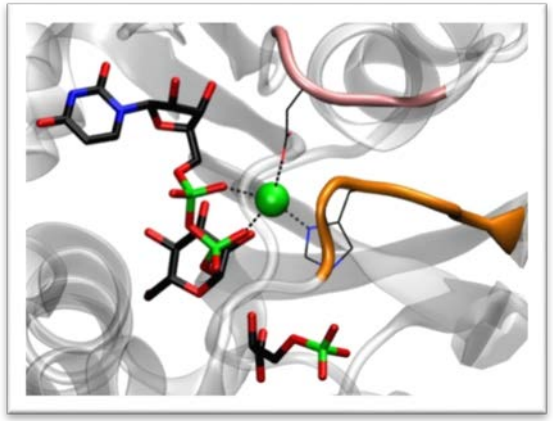


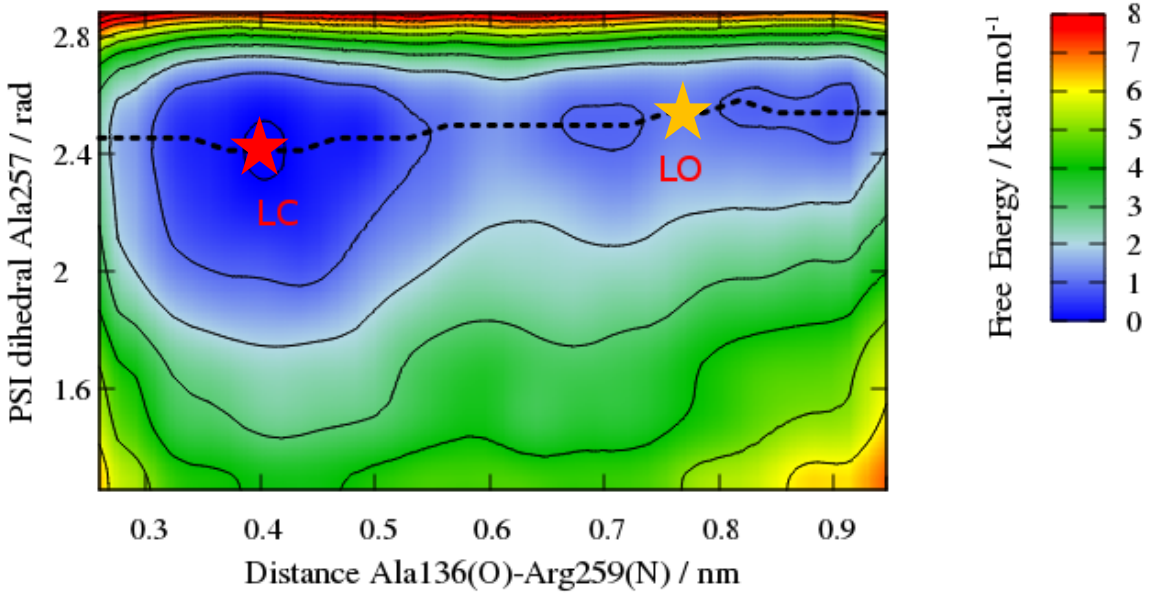
The presence of both ligands reverts the conformational equilibrium of the flexible loop to the closed conformation

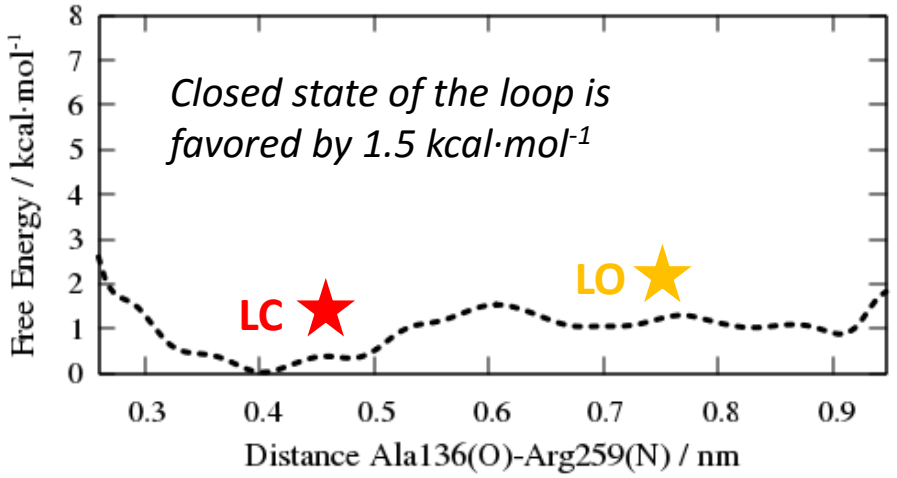
Free Energy of *Mt*GPGS in complex with **UPD-Glc + PGA**







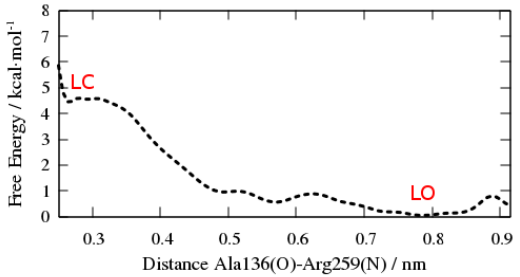




There is an equilbirium between protein conformations of this glycosyltrasnferase, modulated by the presence of ligands

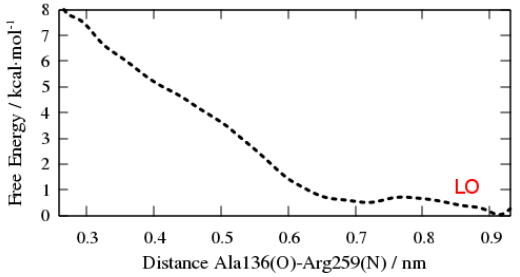
a)

apo



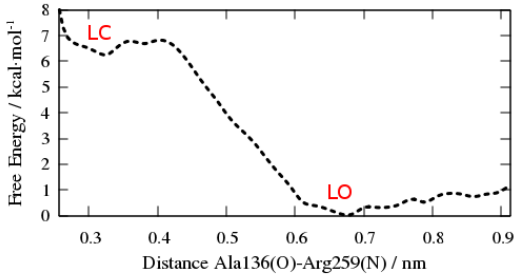
b)

PGA



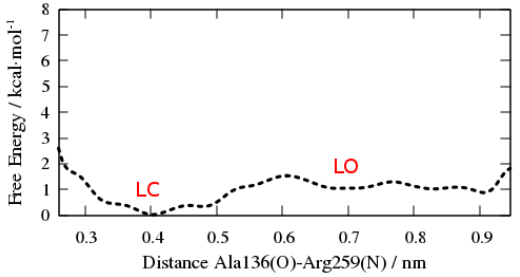
c)

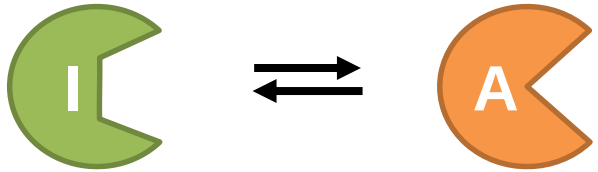
UDP-Glc



d)

UDP-Glc
+
PGA





INACTIVE
(open loop)

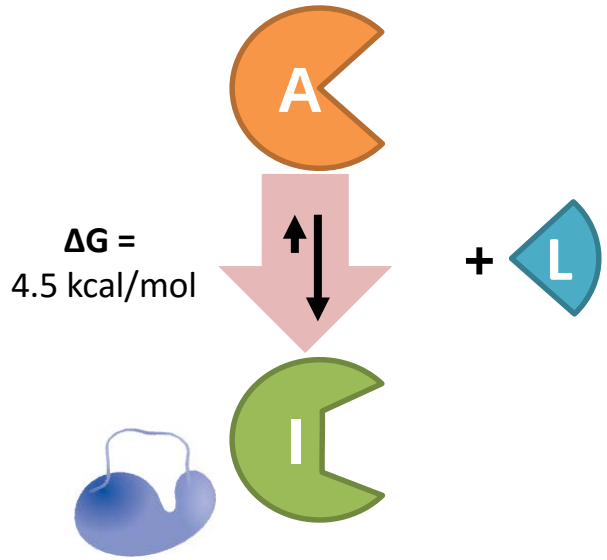
ACTIVE
(closed loop)

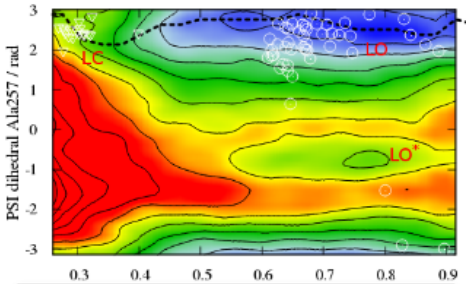
Conformational Free Energies of *Mycobacterium tuberculosis* GpgS, computed with Metadynamics

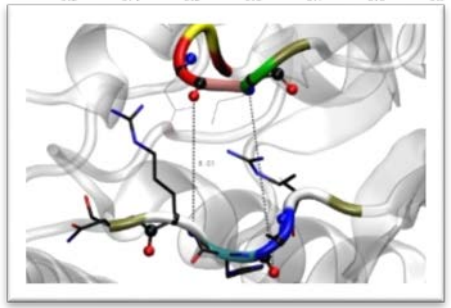
	Ligand	ΔG open→close
a)	-	+4.5 kcal·mol^{-1}
b)	3-phosphoglycerate	> 8.0 kcal·mol^{-1}
c)	UDP-glucose	+6.2 kcal·mol^{-1}
d)	3-phosphoglycerate and UDP-glucose	-1.5 kcal·mol^{-1}

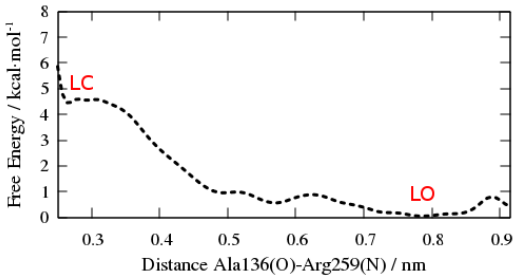
Conformational Selection vs. Induced Fit in *Mt*GpgS (GT81). A combination of both worlds.

CONFORMATIONAL SELECTION

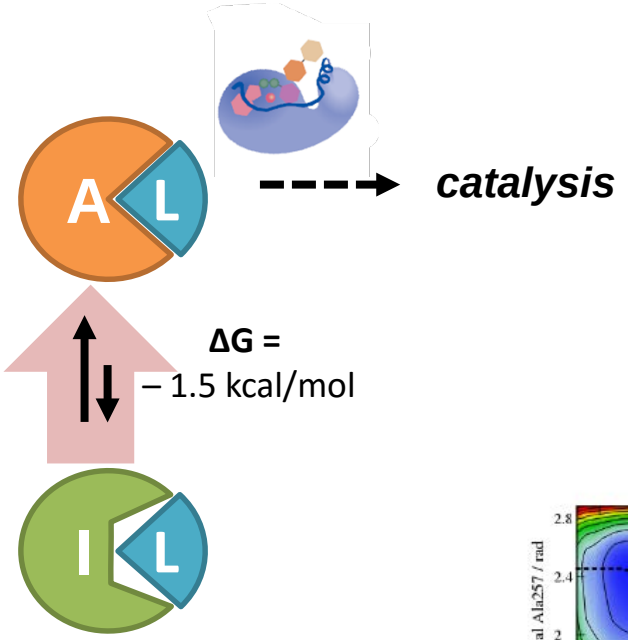


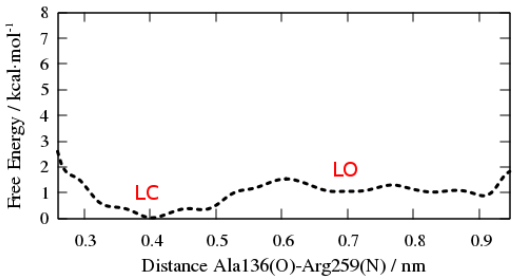


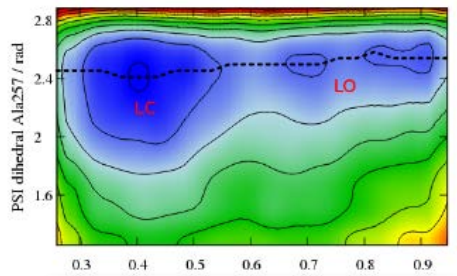


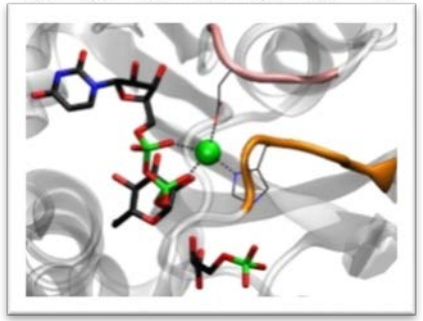


INDUCED FIT





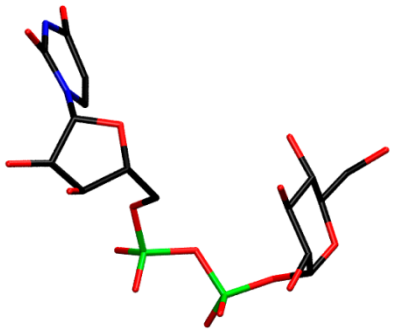




There are also conformational changes at the level of substrates taking place in the active site of *Mt*GpgS

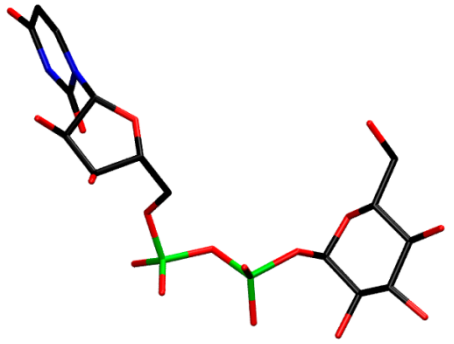
Docking ligands:

Same affinity between the loop conformations and ligands.



UDP-Glc in compact conformation

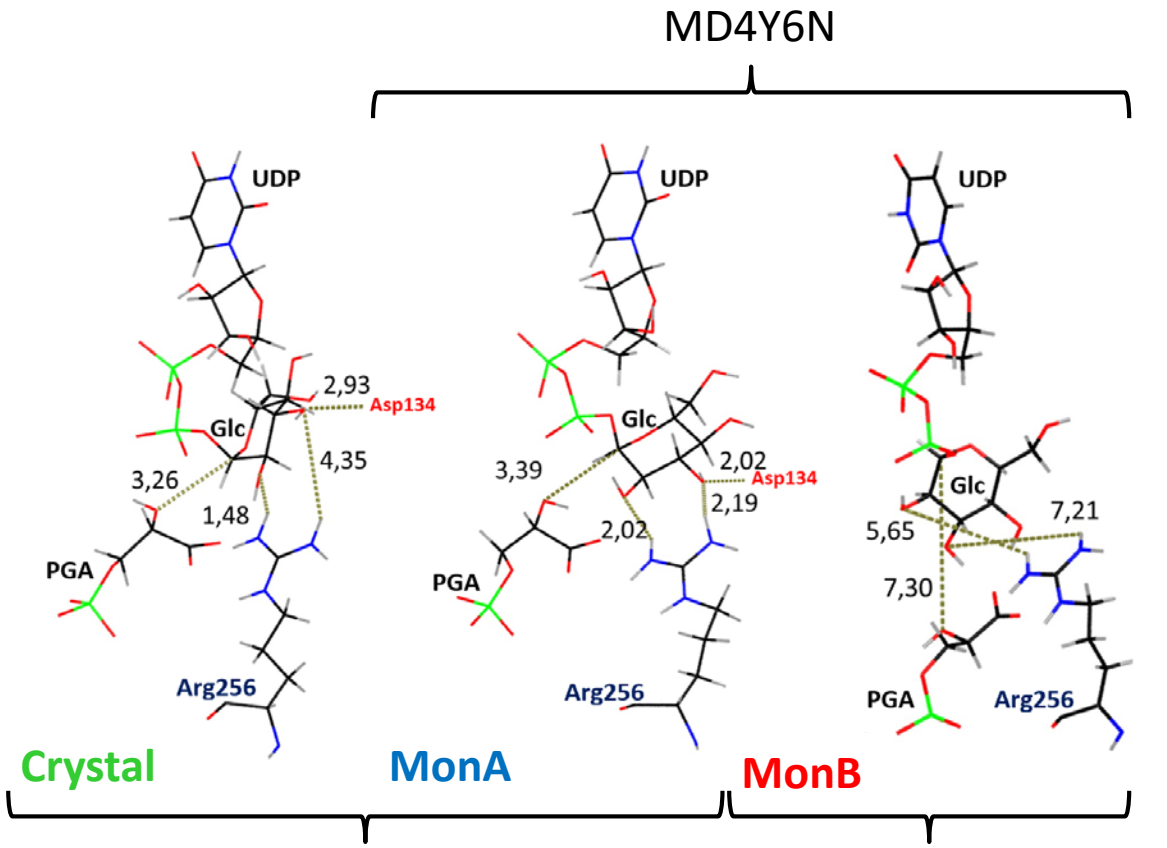
UDP-Glc espontaneously adopts an extended conformation in the active site of *Mt*GpgS **in the absence of PGA**



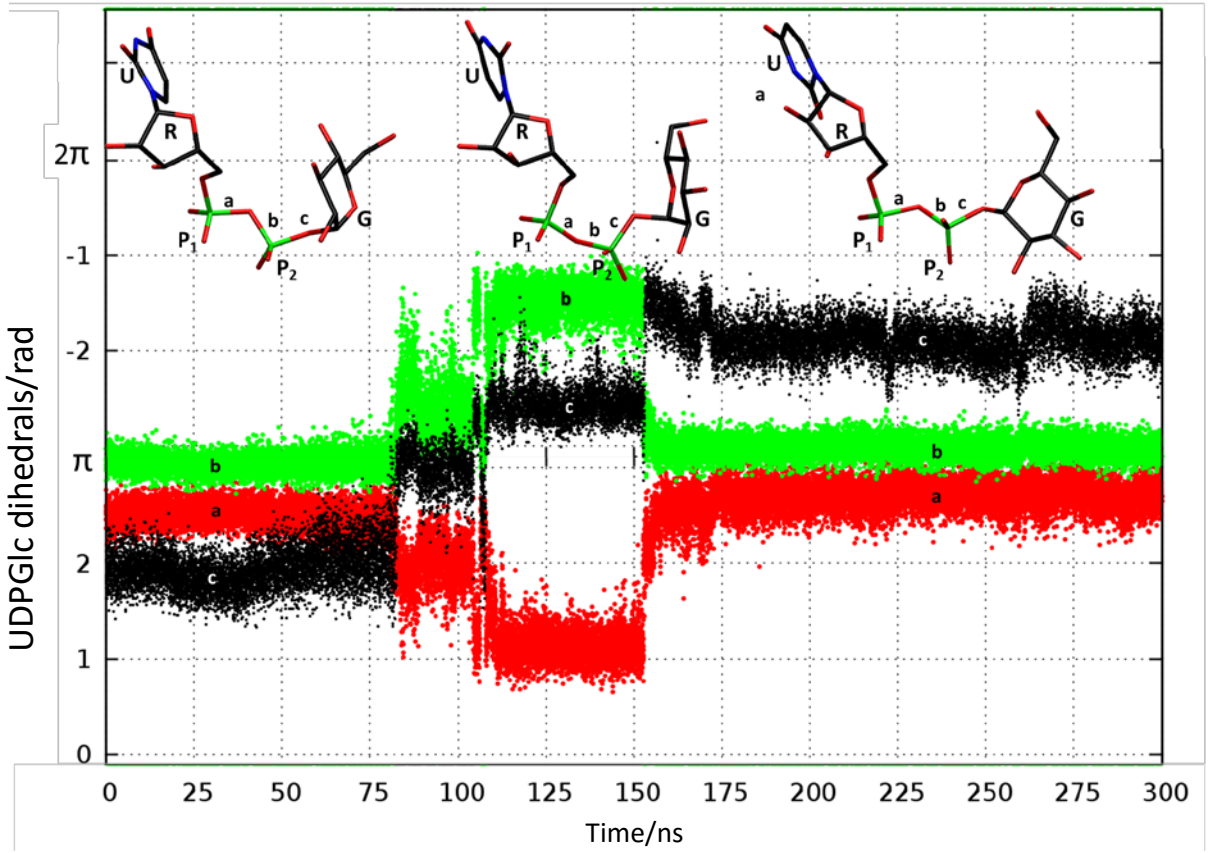
UDP-Glc in extended conformation

Donor conformational changes in ternary complex Molecular Dynamics simulations

- UDPGlc conformations.



MD4Y6N: Monomer B (LA conformation)

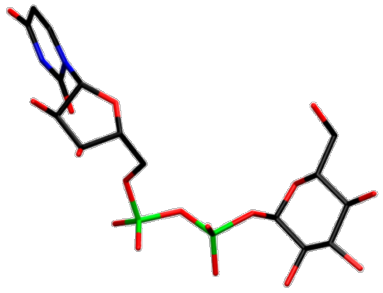


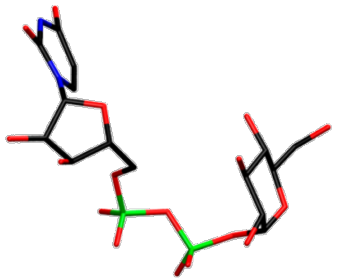
c dihedral Crystal → c dihedral monA → c dihedral monB

Compact — Extended

The affinity of each UDP-glucose conformation to *Mt*GpgS active site is different

UDP-Glc in extended conformation

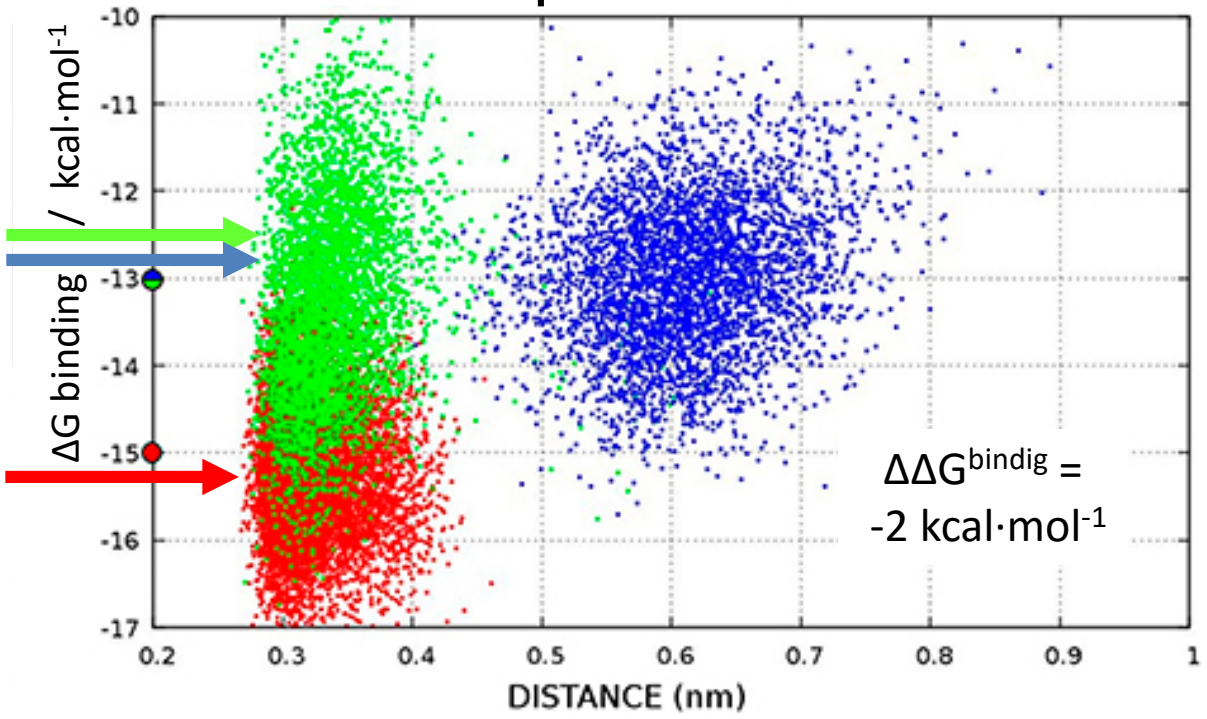




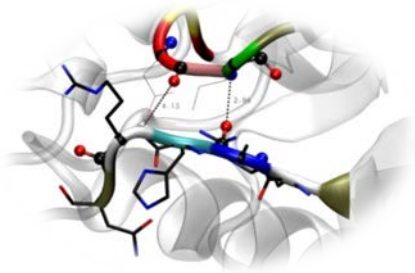
UDP-Glc in compact conformation

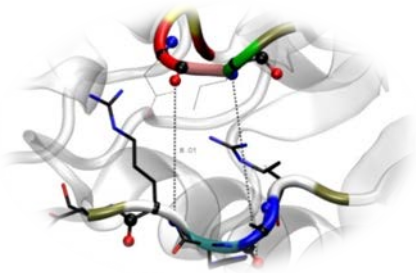
closed loop conformation (active)

open loop conformation (inactive)

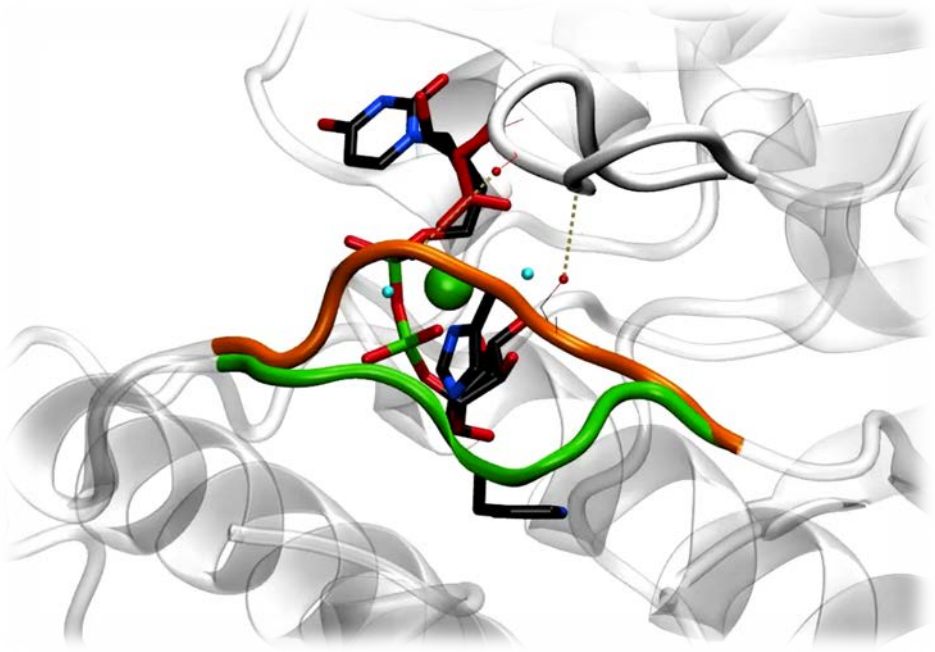


Free Energies of binding calculated with VINA score

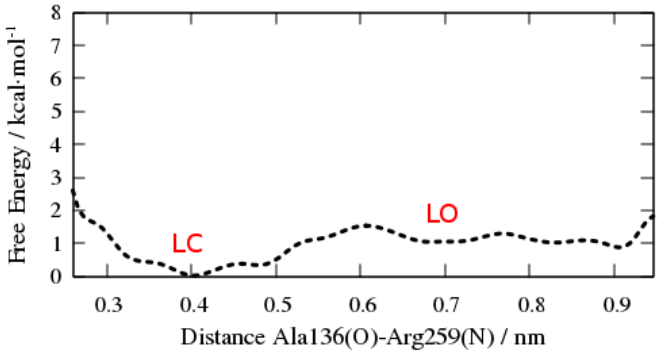




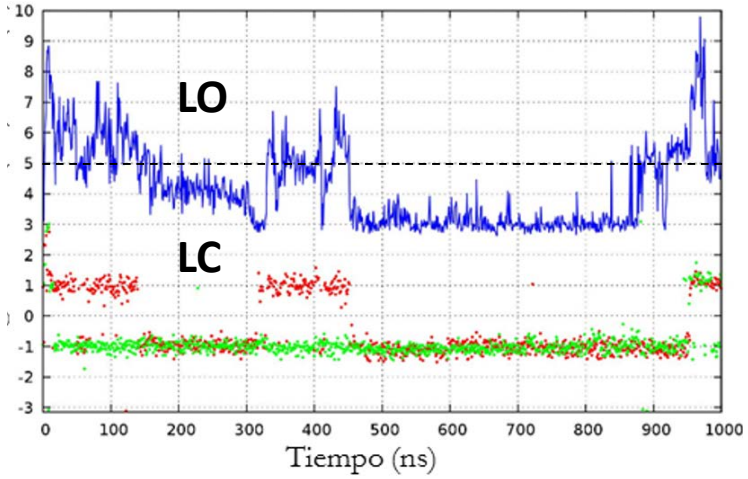
Ligand – Loop conformations relationships

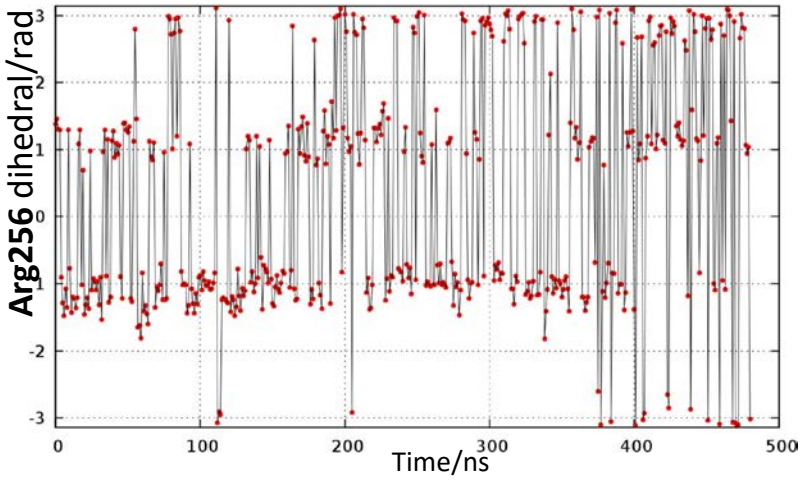


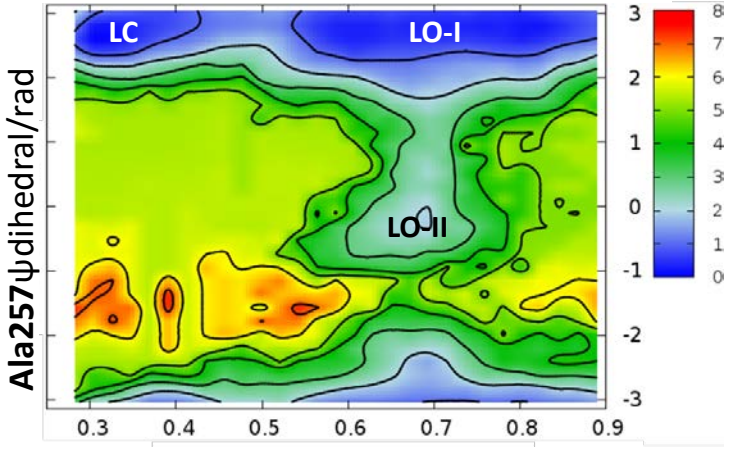
- **BIAS-Exchange (UDPGlc·metal)**
  - Distance: Asp136(O)-Arg259(N).
  - Torsion: Ala257 dihedral.
  - Torsion: **His258 dihedral**.
  - Distance: His258-Metal.
  - Torsion: **UDPGlc dihedral**.

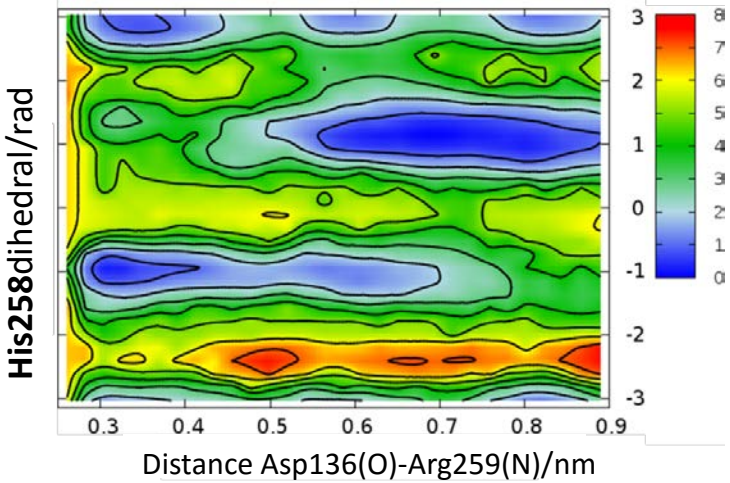


Apo MD simulation

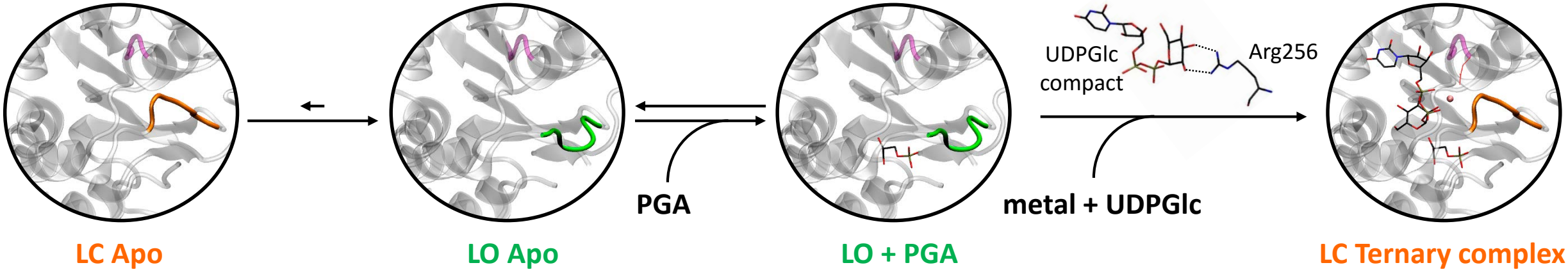








Conformational Selection versus Induced Fit in *Mt*GpgS (GT81).
A Combination of both worlds: Ligands modulate the actual LA/LI equilibrium in GpgS.



The Michaelis complex gets stabilized by a double contribution:

- ❑ The ligand preferentially binds to the active conformation of the loop
- ❑ The ligand itself induces a stability of the active conformation of the loop

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