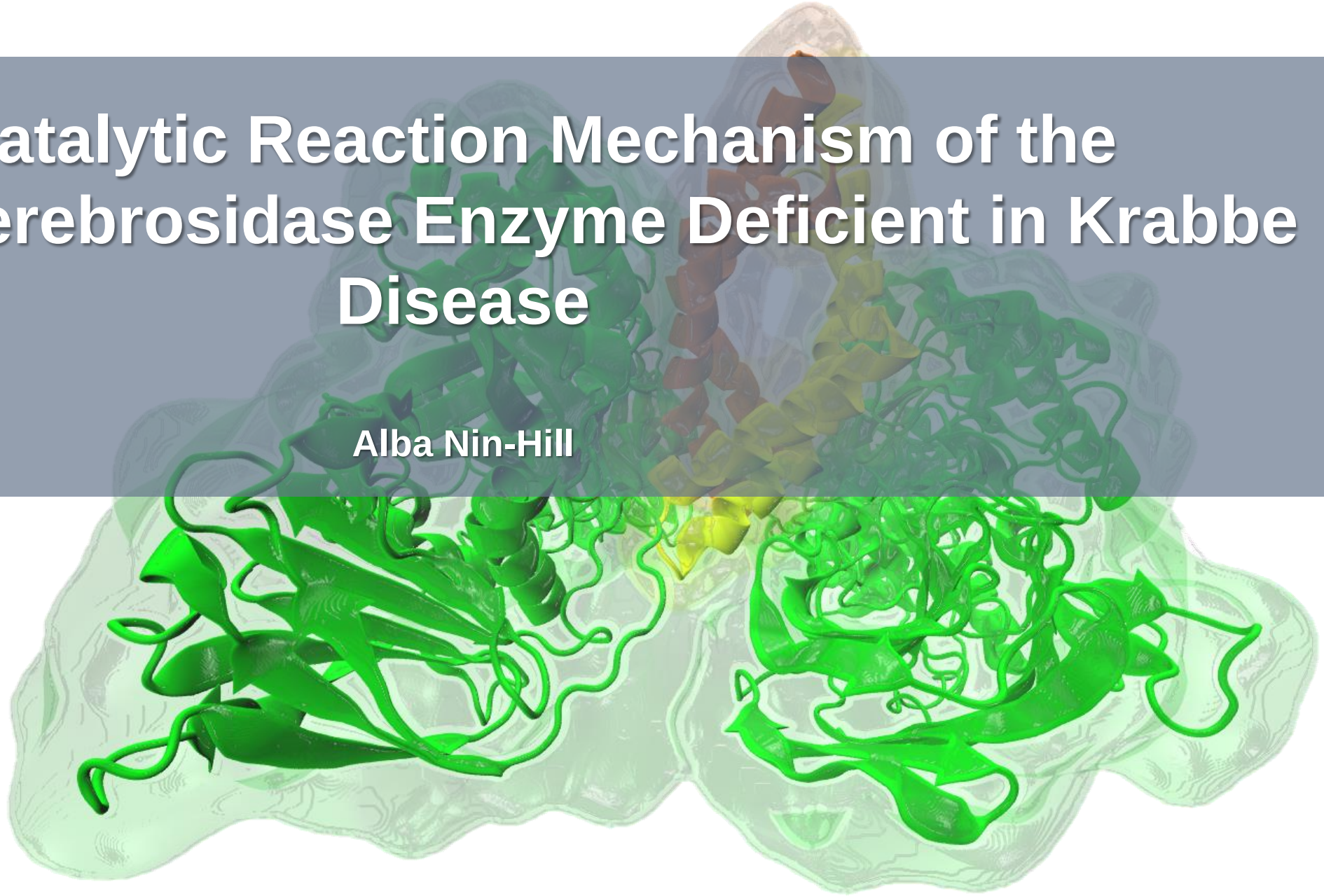
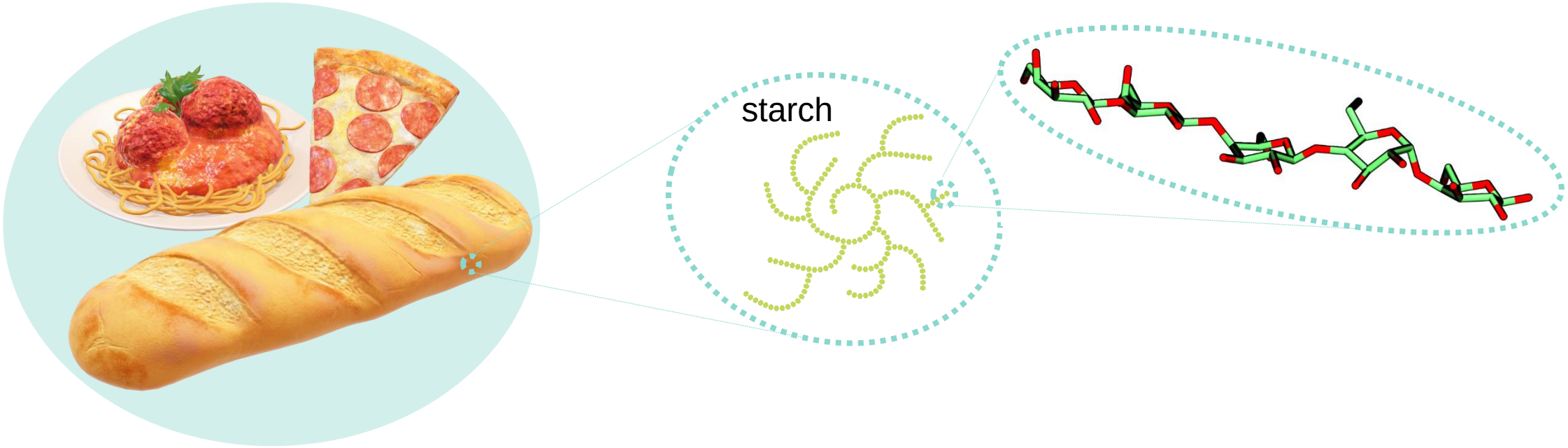
The Catalytic Reaction Mechanism of the β-Galactocerebrosidase Enzyme Deficient in Krabbe Disease

Alba Nin-Hill



16th RES USERS CONFERENCE | 15th September 2022

CARBOHYDRATES



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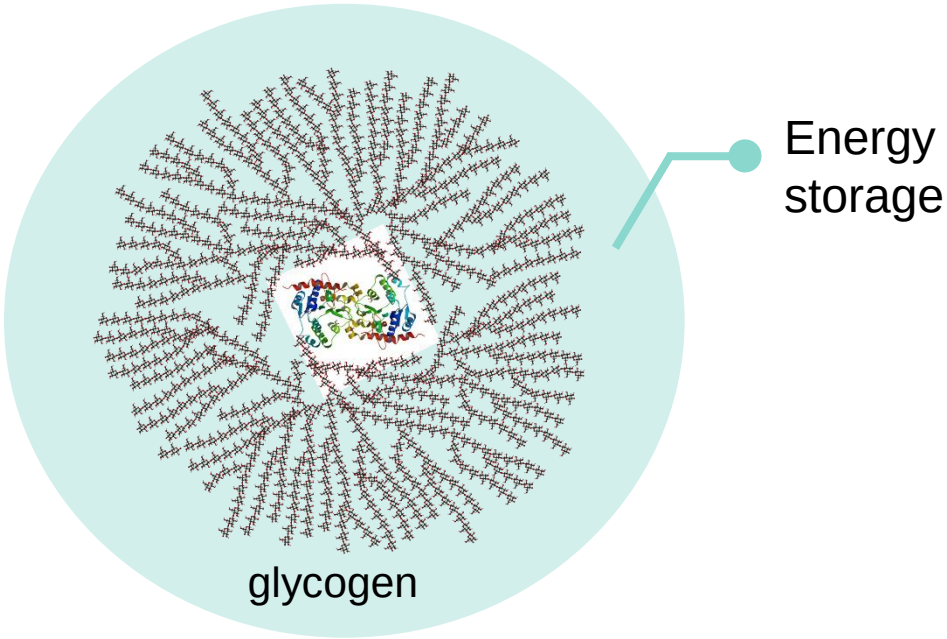
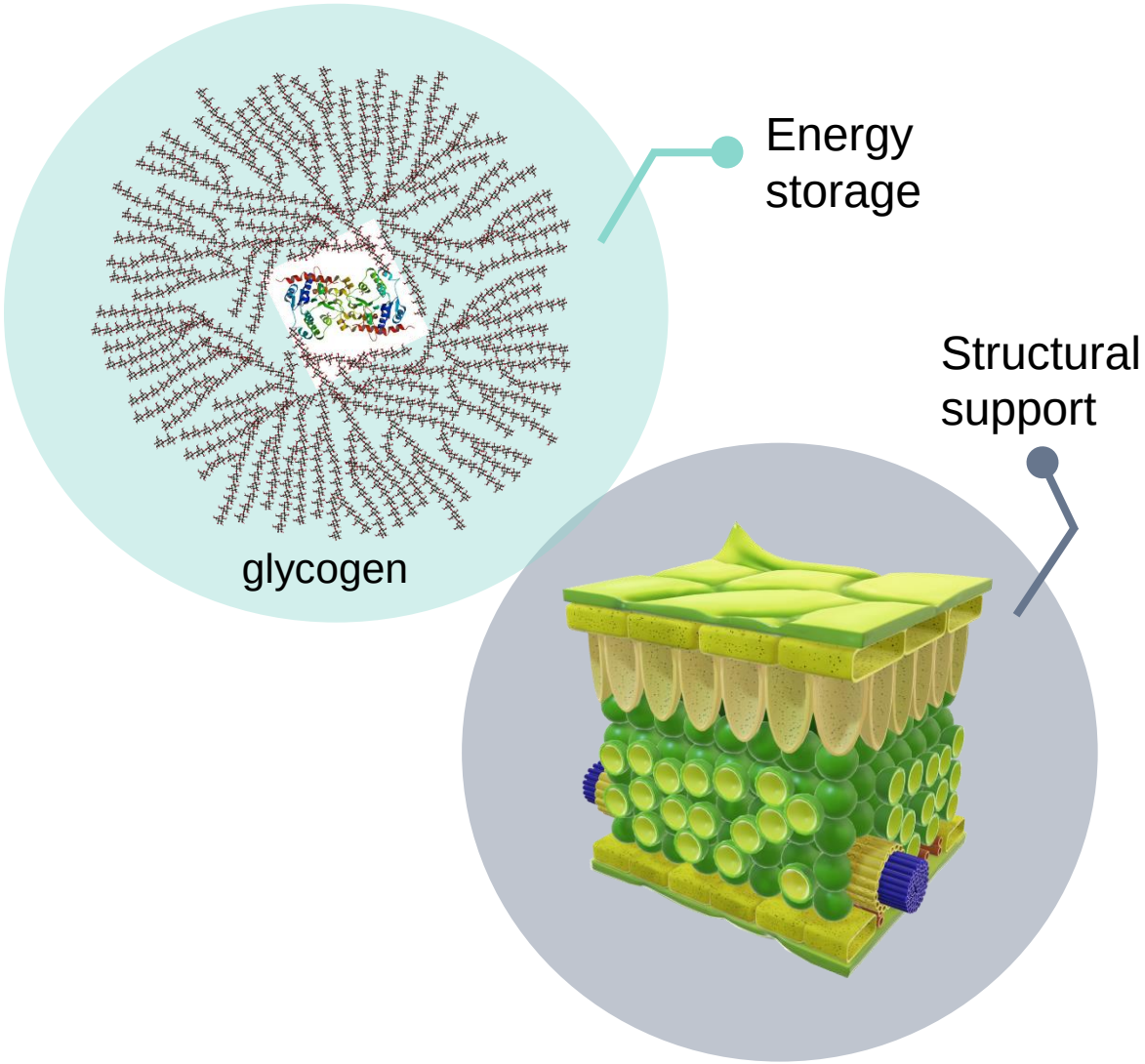


Energy storage

CARBOHYDRATES



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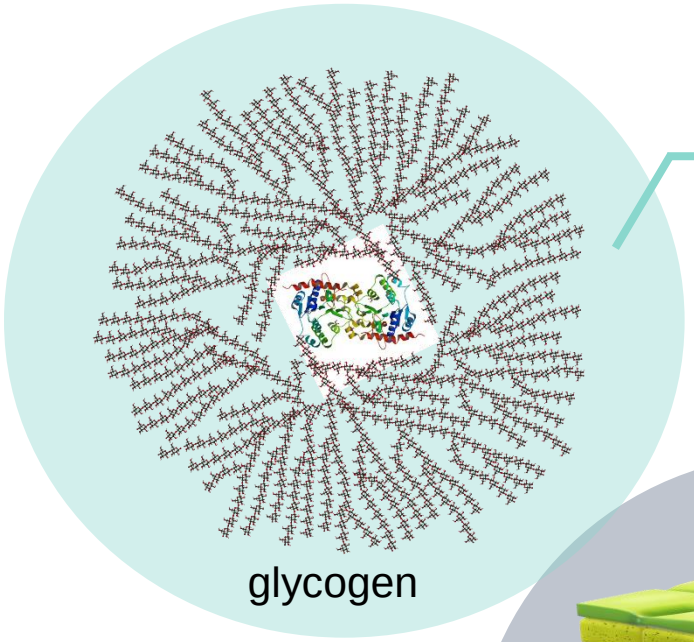
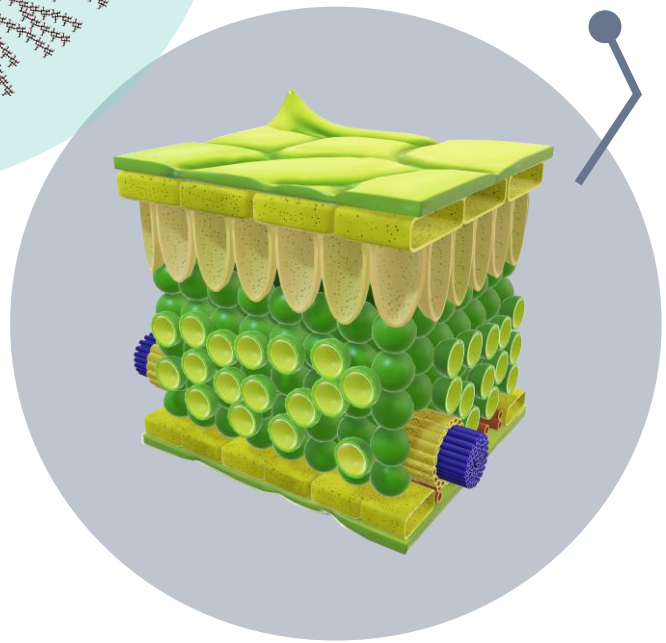


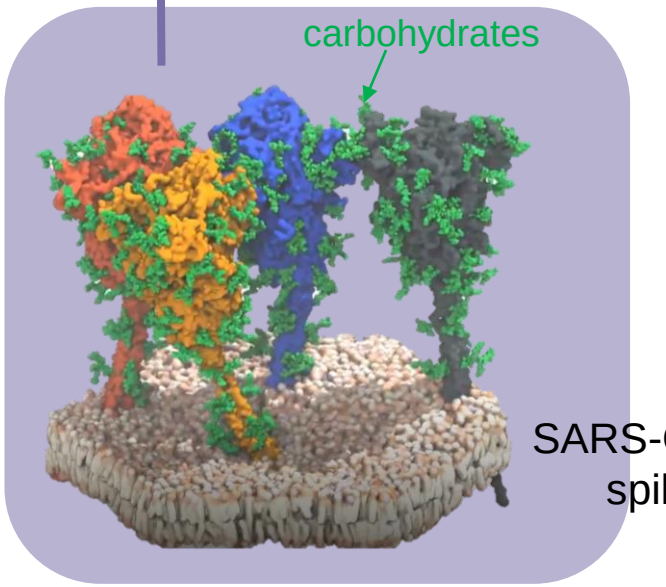
glycogen

Energy storage



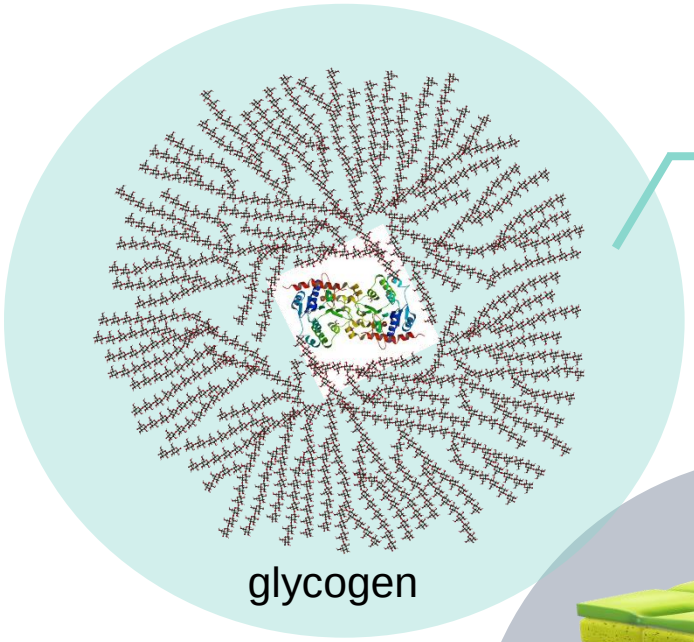
Structural support

Virus infections



Turoňová, et. al. Science 2020, 370, 203-208

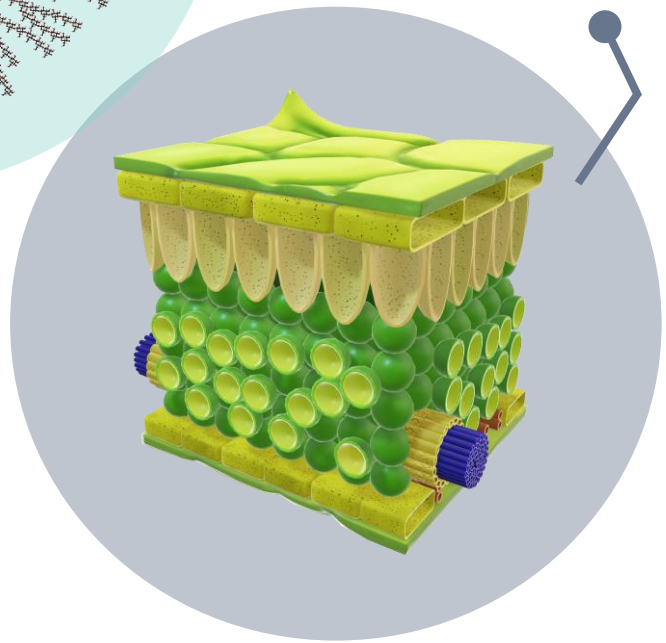
CARBOHYDRATES



glycogen

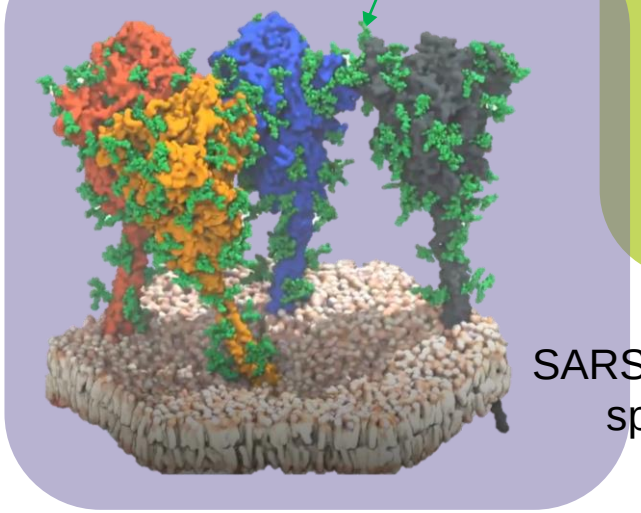
Energy storage

Structural support



Virus infections

carbohydrates

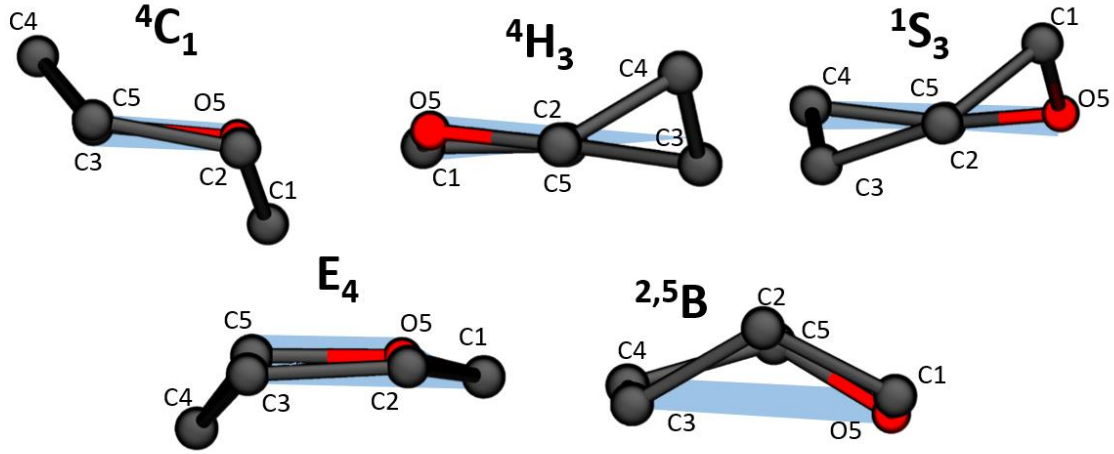


SARS-CoV-2 spike

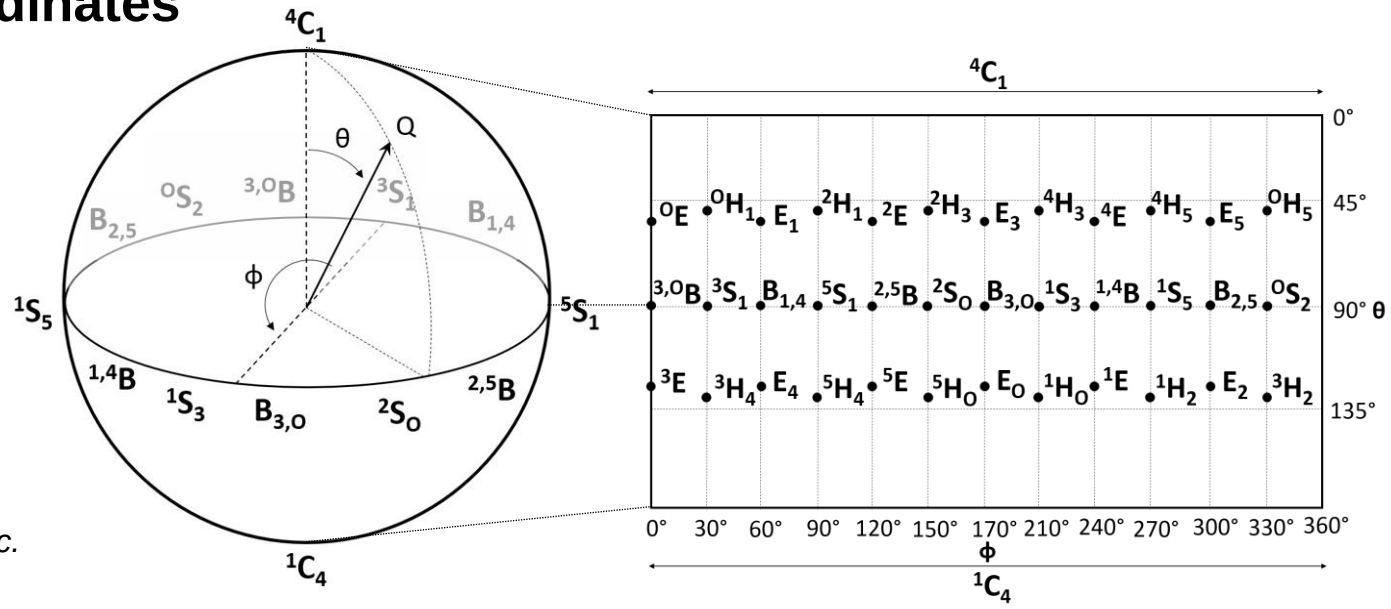
Drug delivery strategies

Turoňová, et. al. Science 2020, 370, 203-208

PYRANOSE CONFORMATIONS

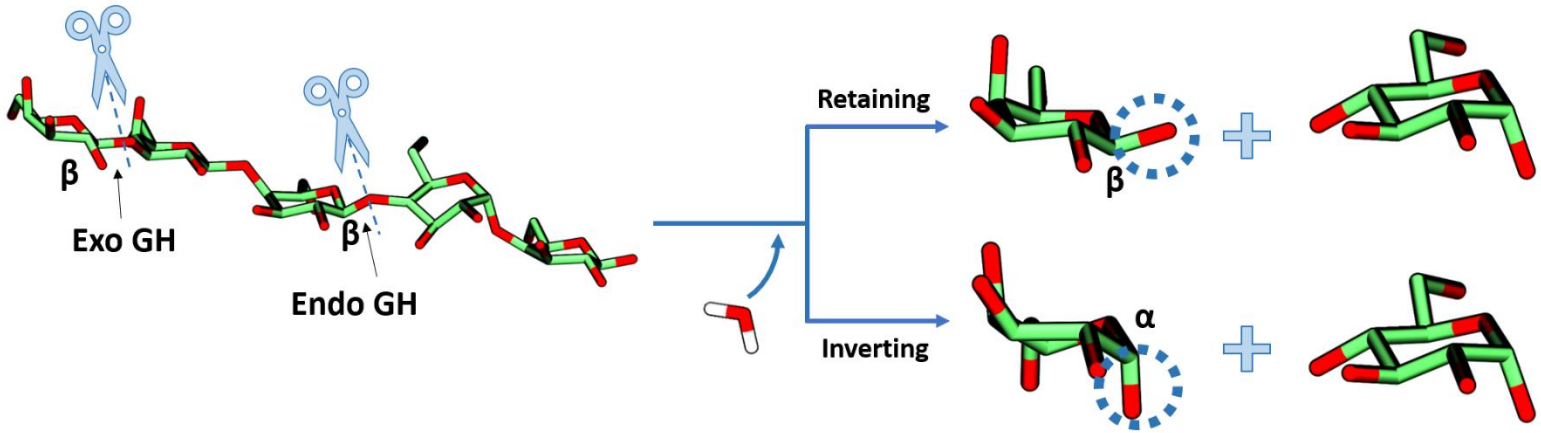


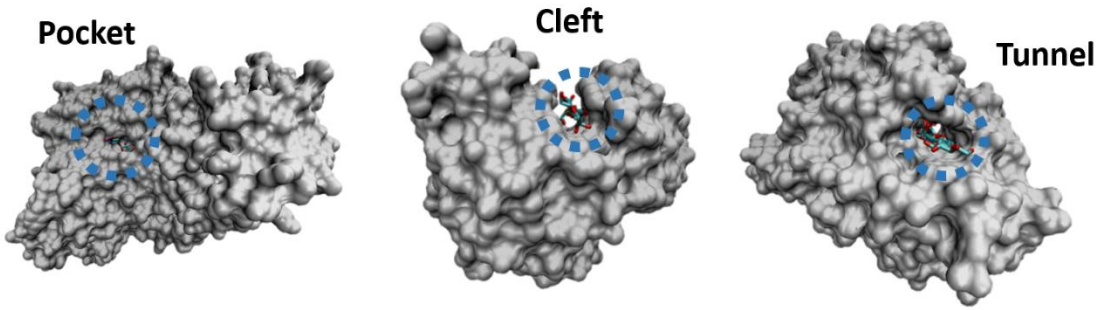
Puckering coordinates



Cremer and Pople *J. Am. Chem. Soc.* 1975, 97, 1354-1358

GLYCOSIDE HYDROLASE (GHs)

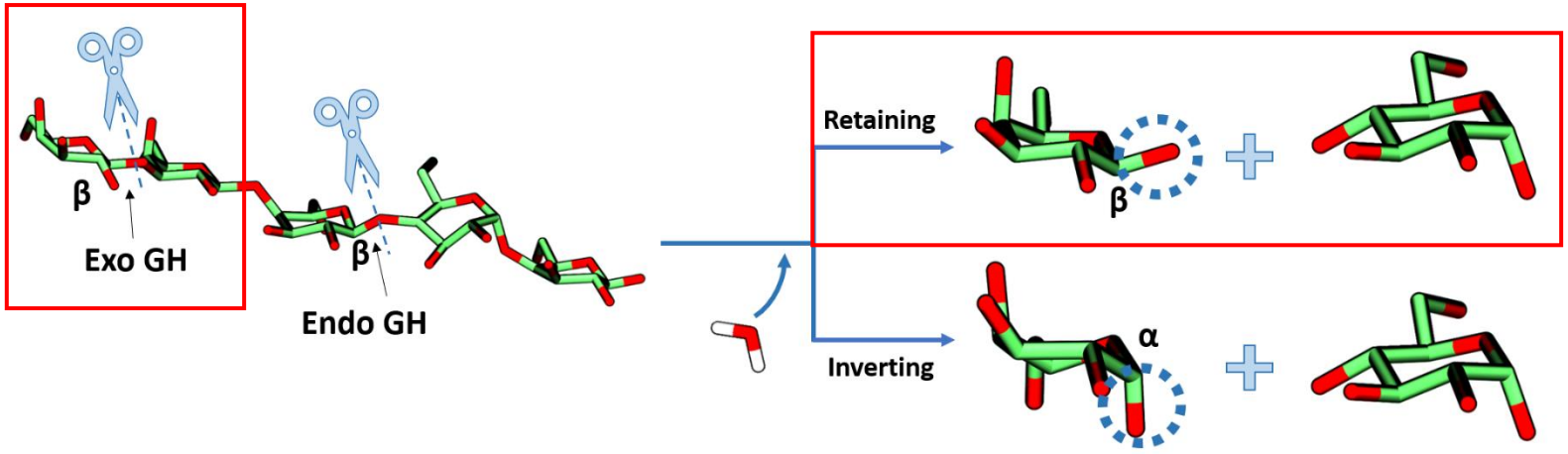


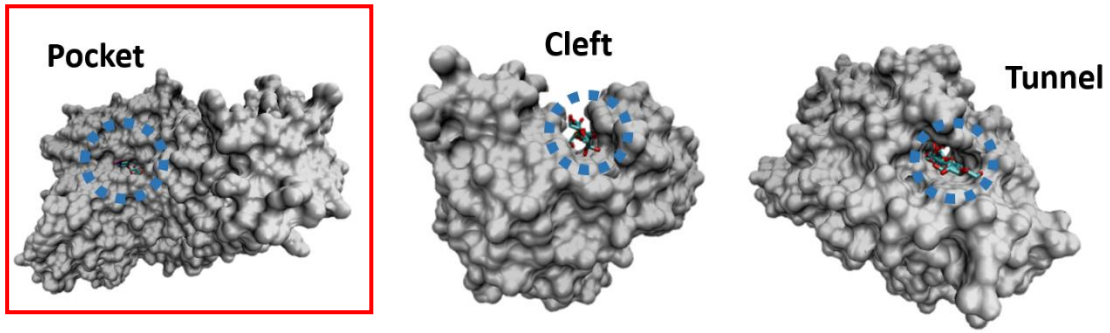


1. Stereochemistry of the product
   Retaining or inverting
2. Location of the glycosidic bond to be cleaved
   Exo- or Endo-GHs
3. Similarity in amino acid sequence
   162 families

GLYCOSIDE HYDROLASE (GHs)

β-galactocerebrosidase



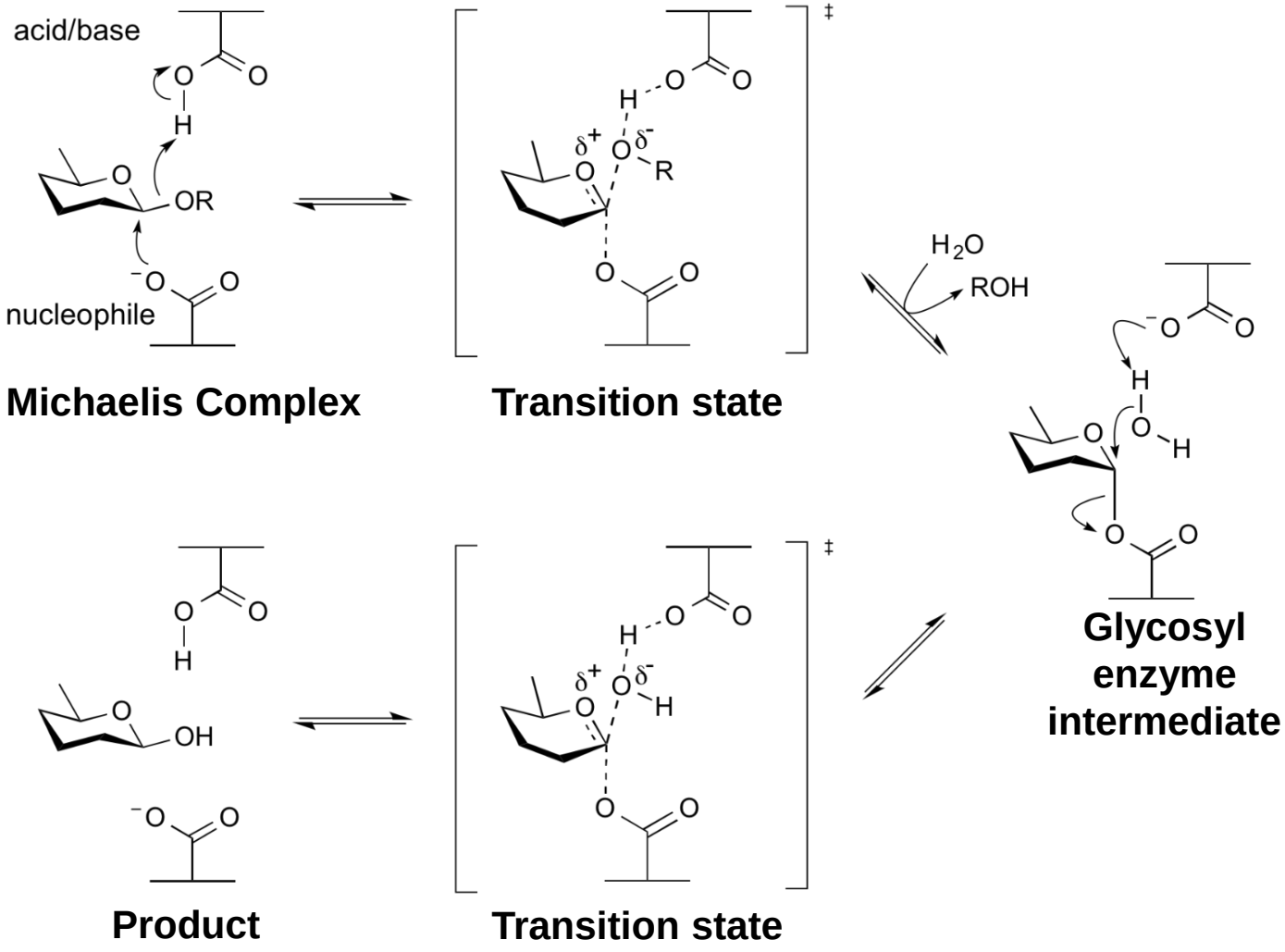


1. Stereochemistry of the product
   Retaining or inverting

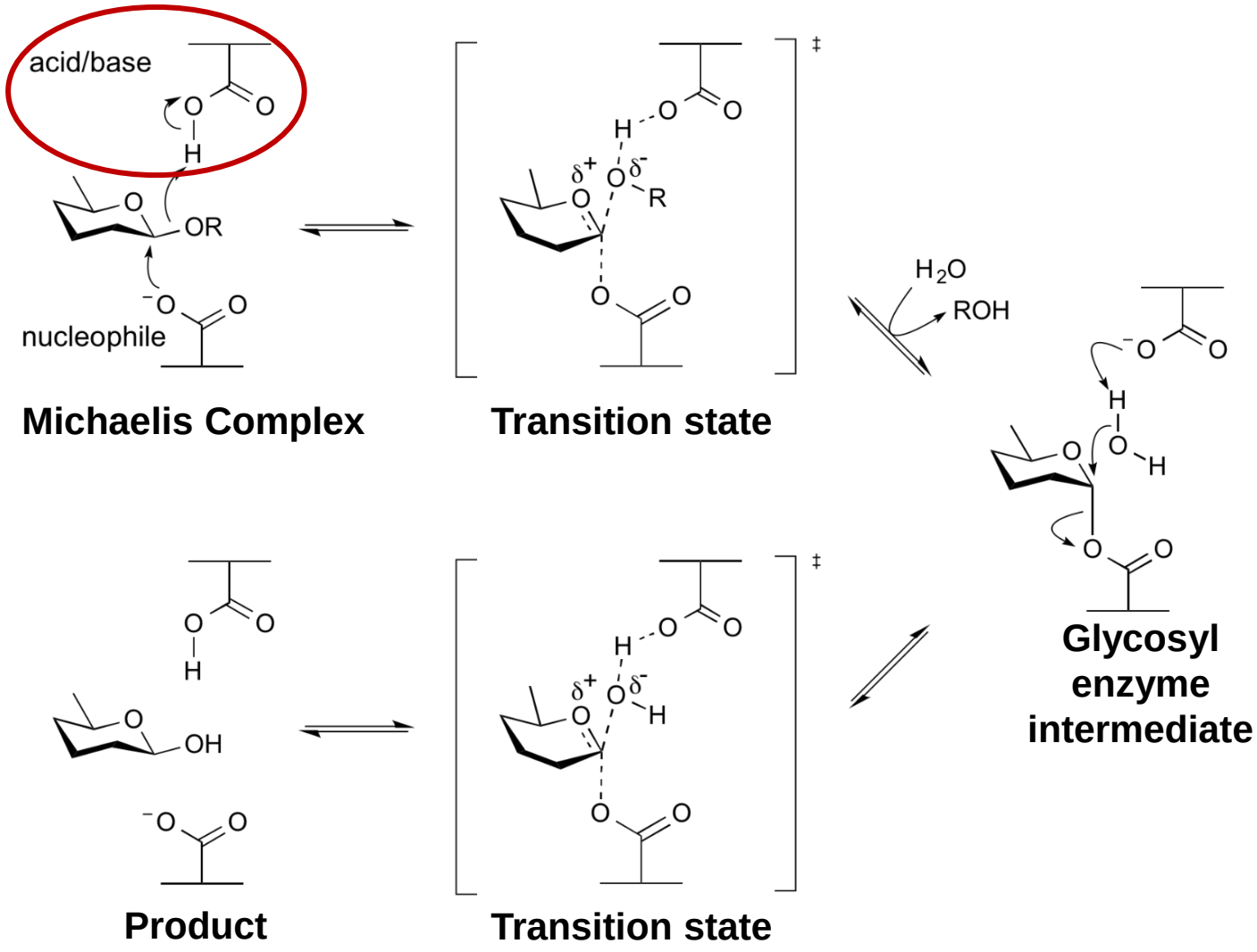
2. Location of the glycosidic bond to be cleaved
   Exo- or Endo-GHs

3. Similarity in amino acid sequence
   162 families
   → GH59

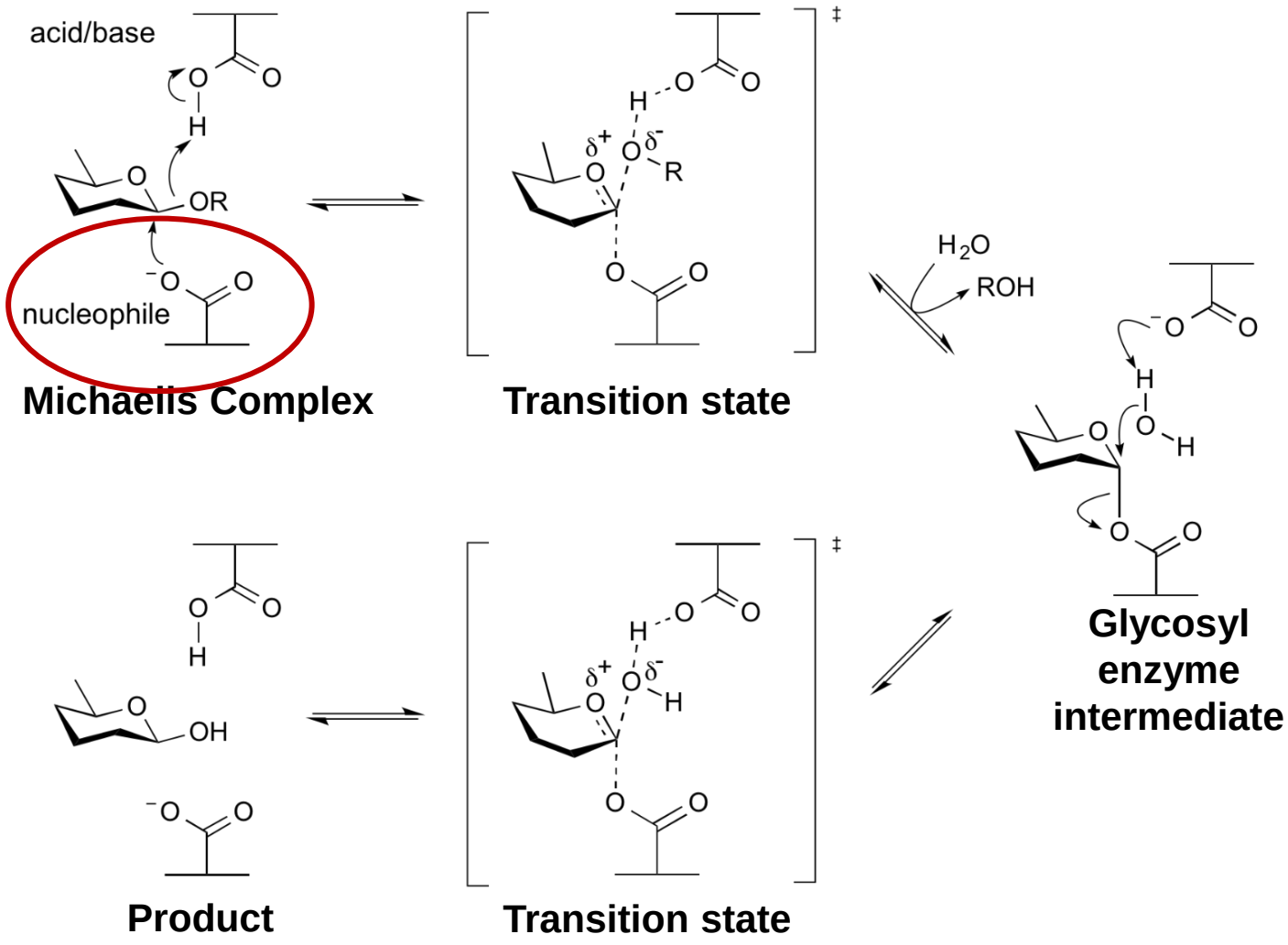
RETAINING GHs REACTION MECHANISM



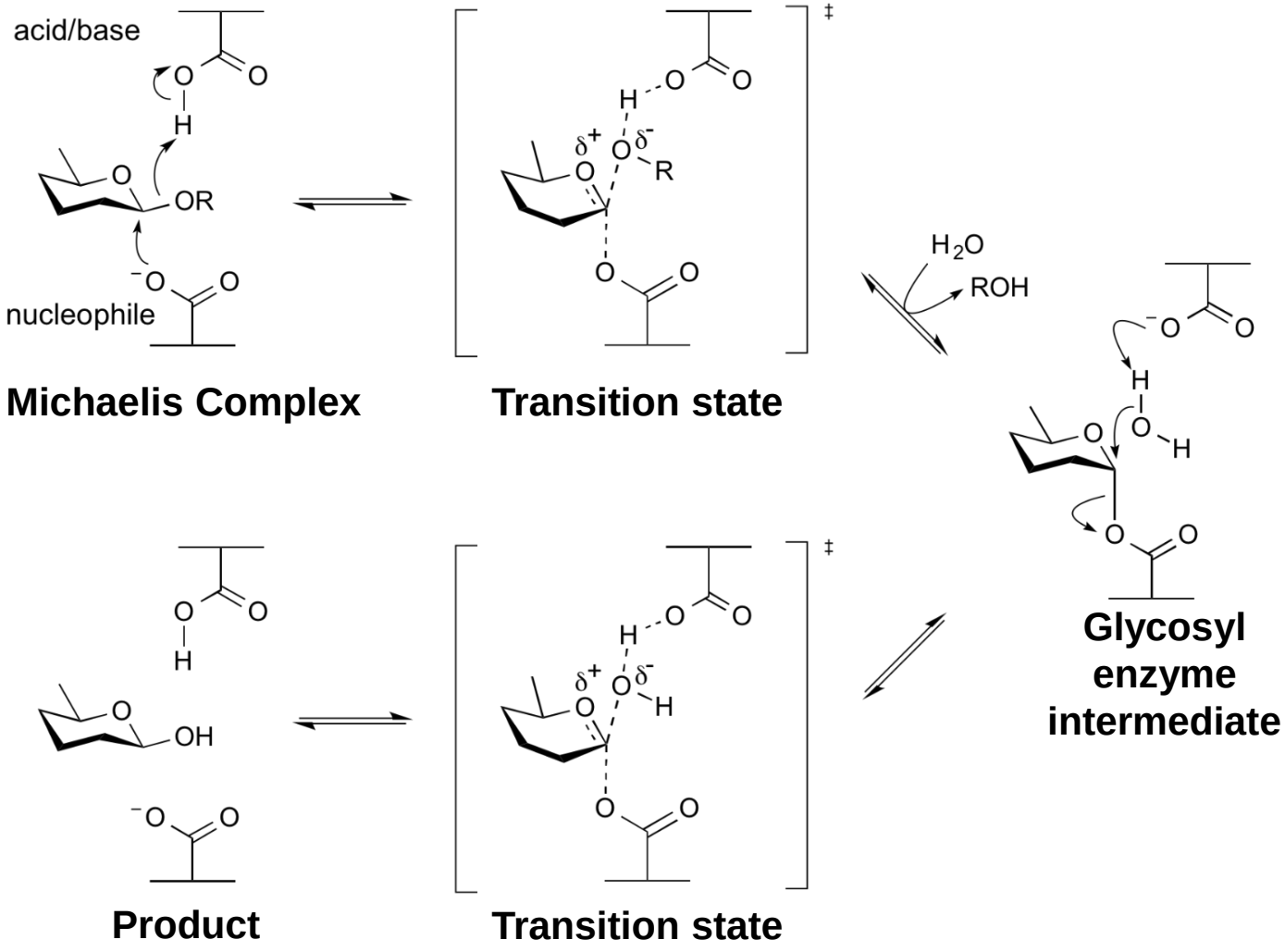
RETAINING GHs REACTION MECHANISM



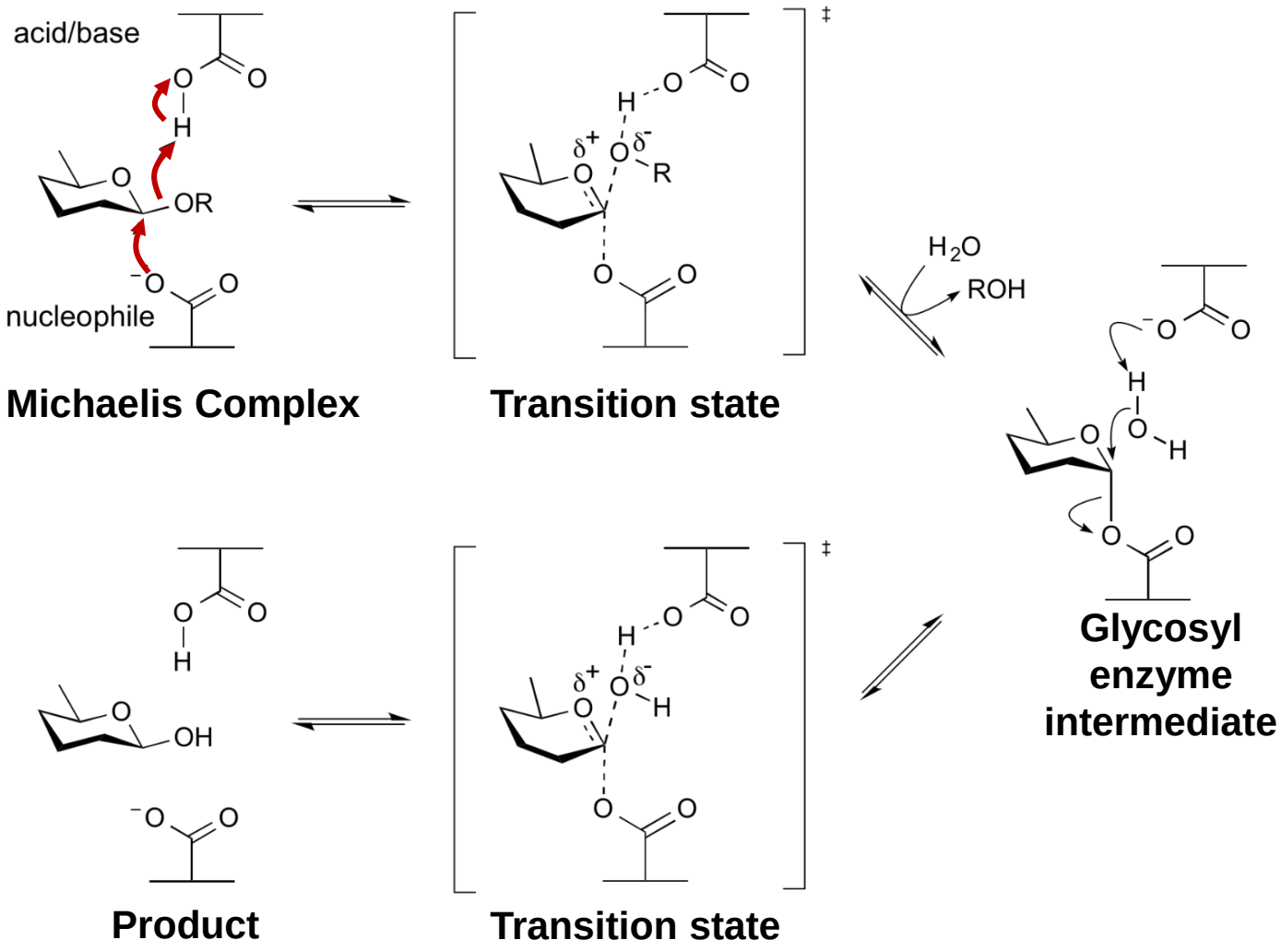
RETAINING GHs REACTION MECHANISM



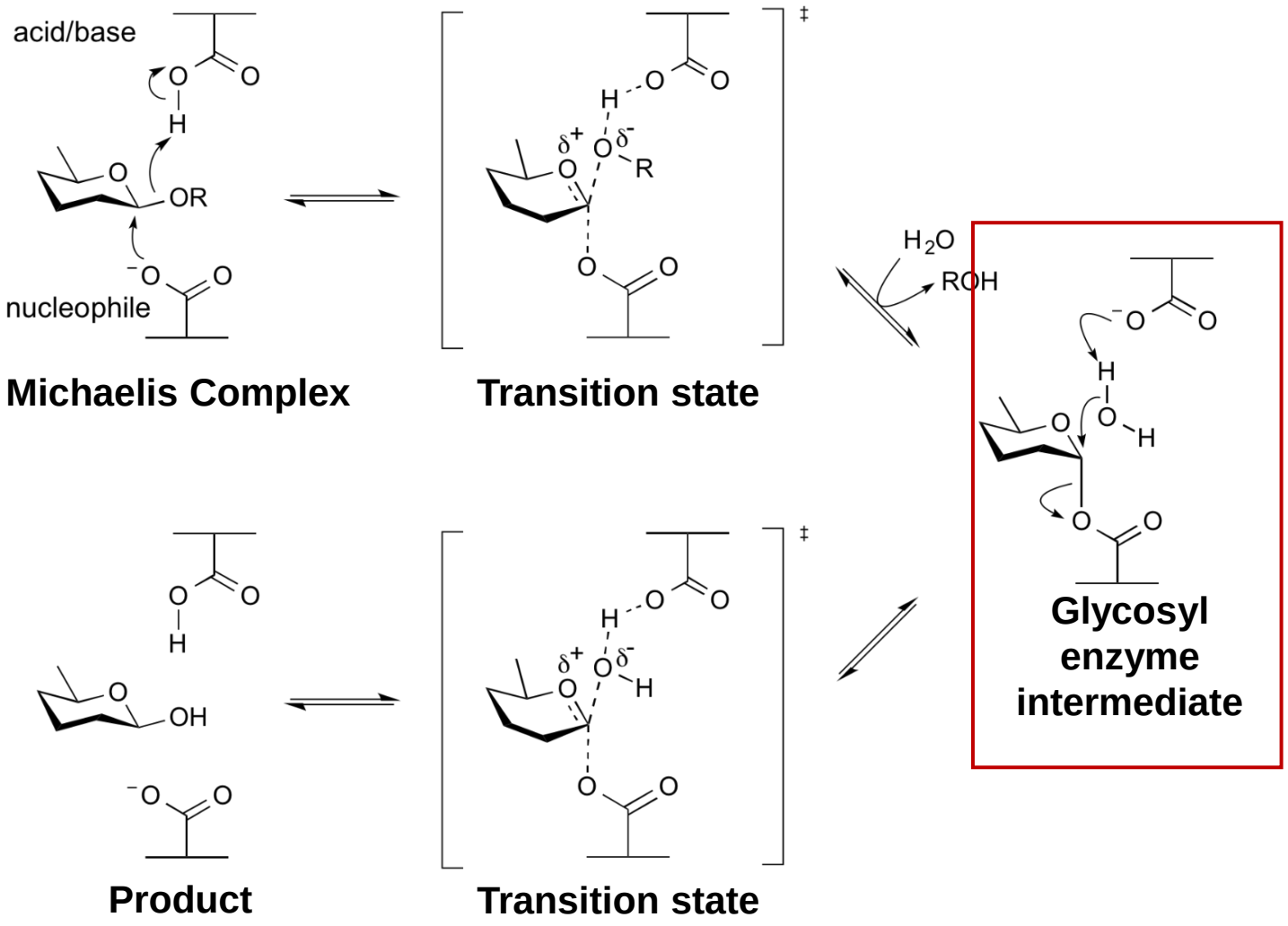
RETAINING GHs REACTION MECHANISM



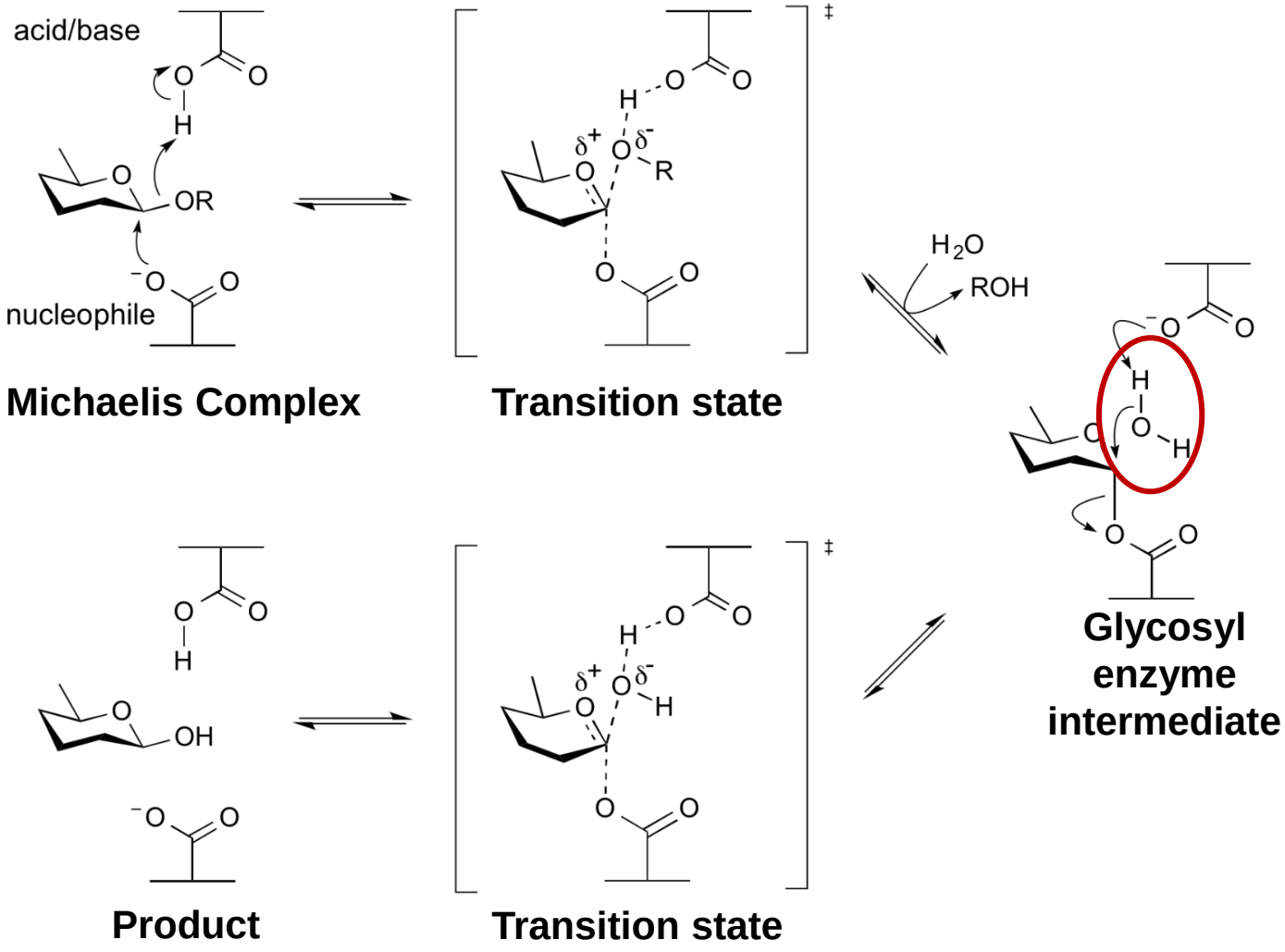
RETAINING GHs REACTION MECHANISM



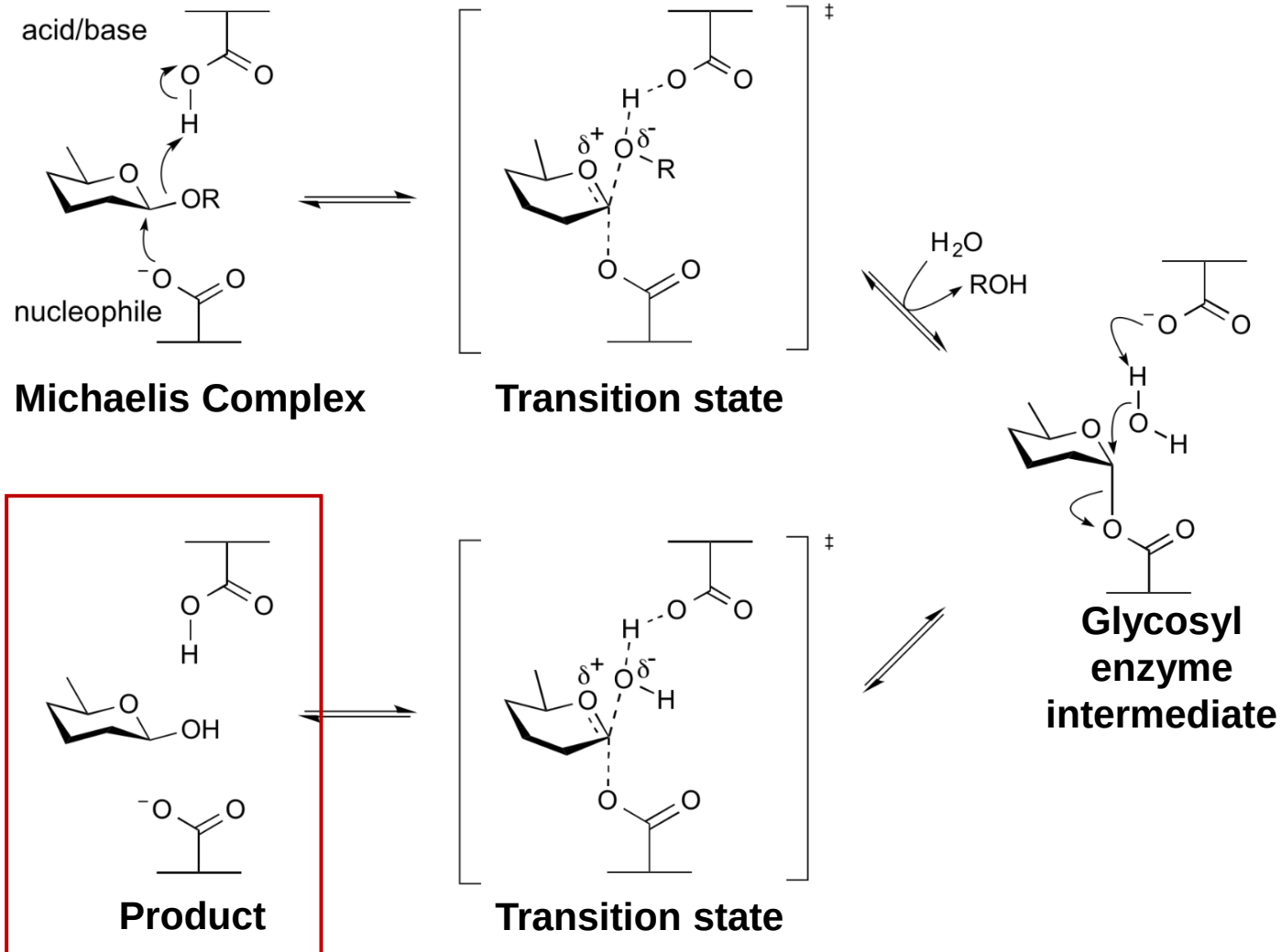
RETAINING GHs REACTION MECHANISM



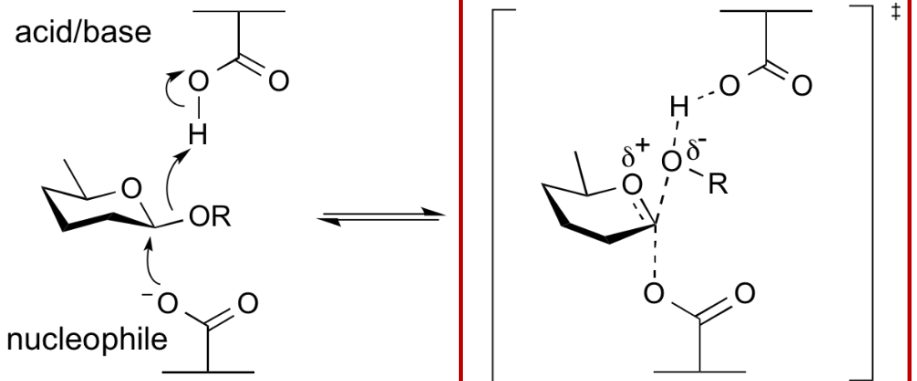
RETAINING GHs REACTION MECHANISM



RETAINING GHs REACTION MECHANISM



RETAINING GHs REACTION MECHANISM

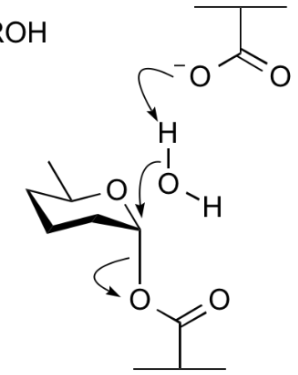


Michaelis Complex

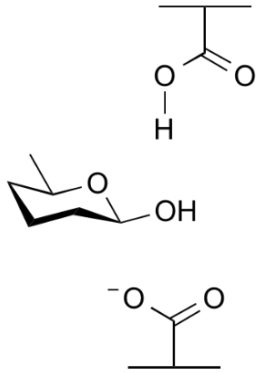
Transition state

H_2O

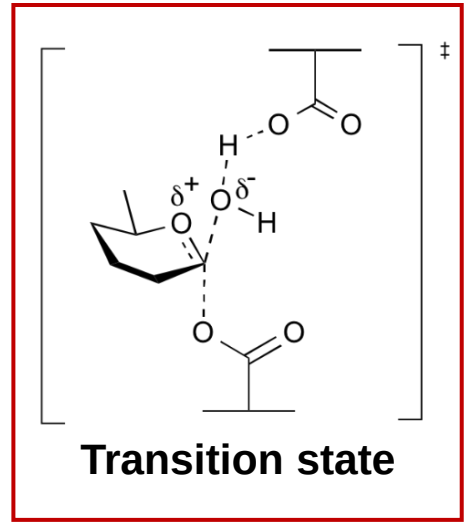
ROH



Glycosyl enzyme intermediate

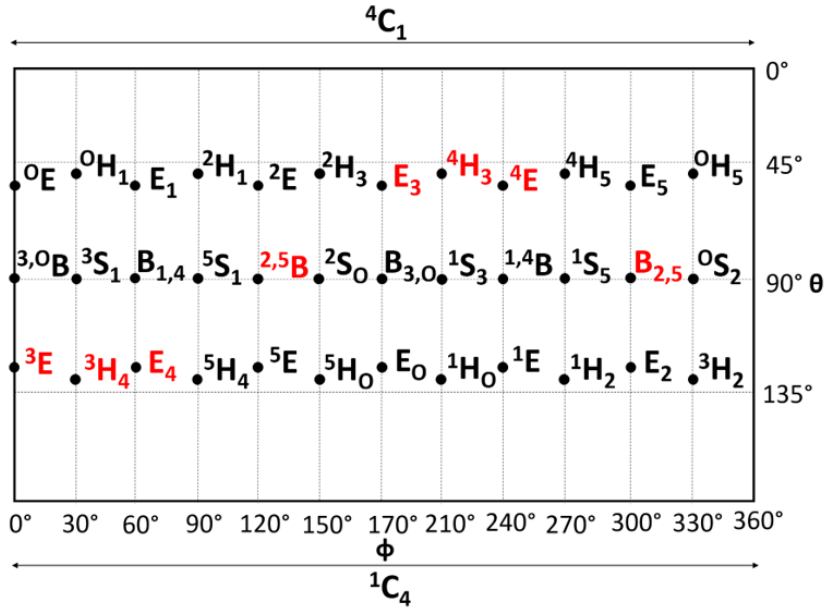


Product

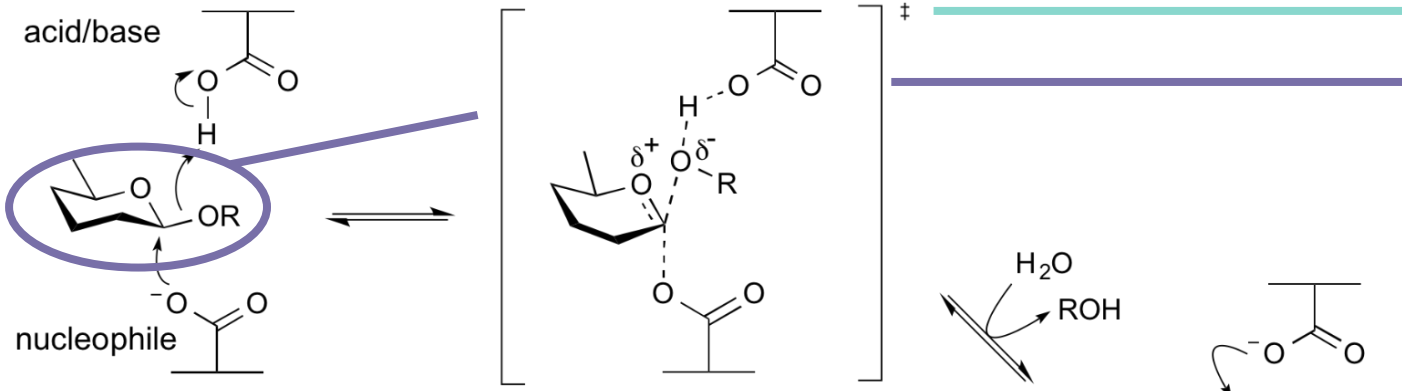


Transition state

Oxocarbenium ion-like character

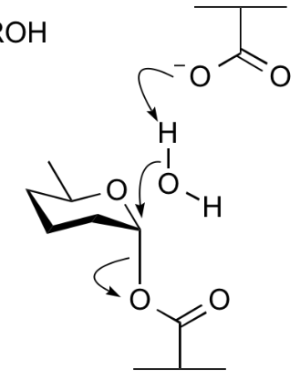


RETAINING GHs REACTION MECHANISM

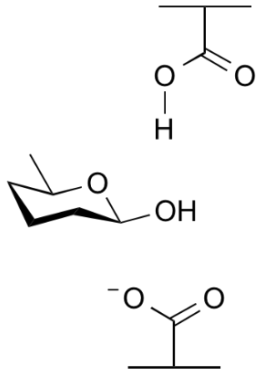


Michaelis Complex

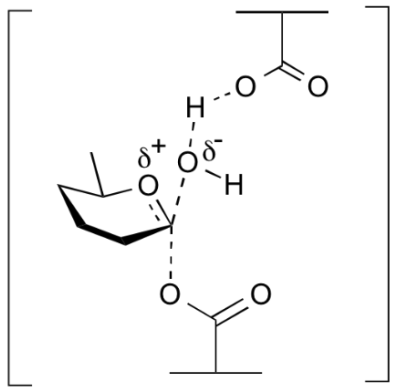
Transition state



Glycosyl enzyme intermediate



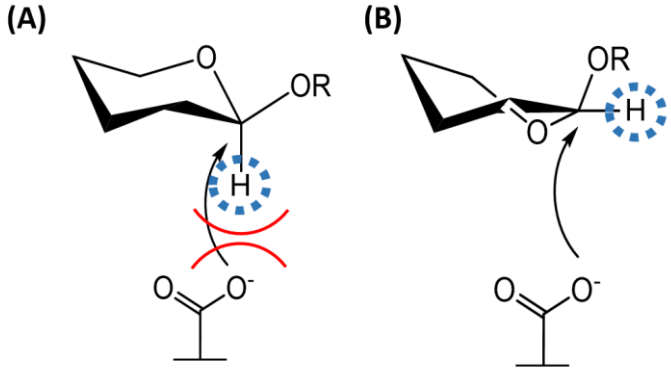
Product



Transition state

Oxocarbenium ion-like character

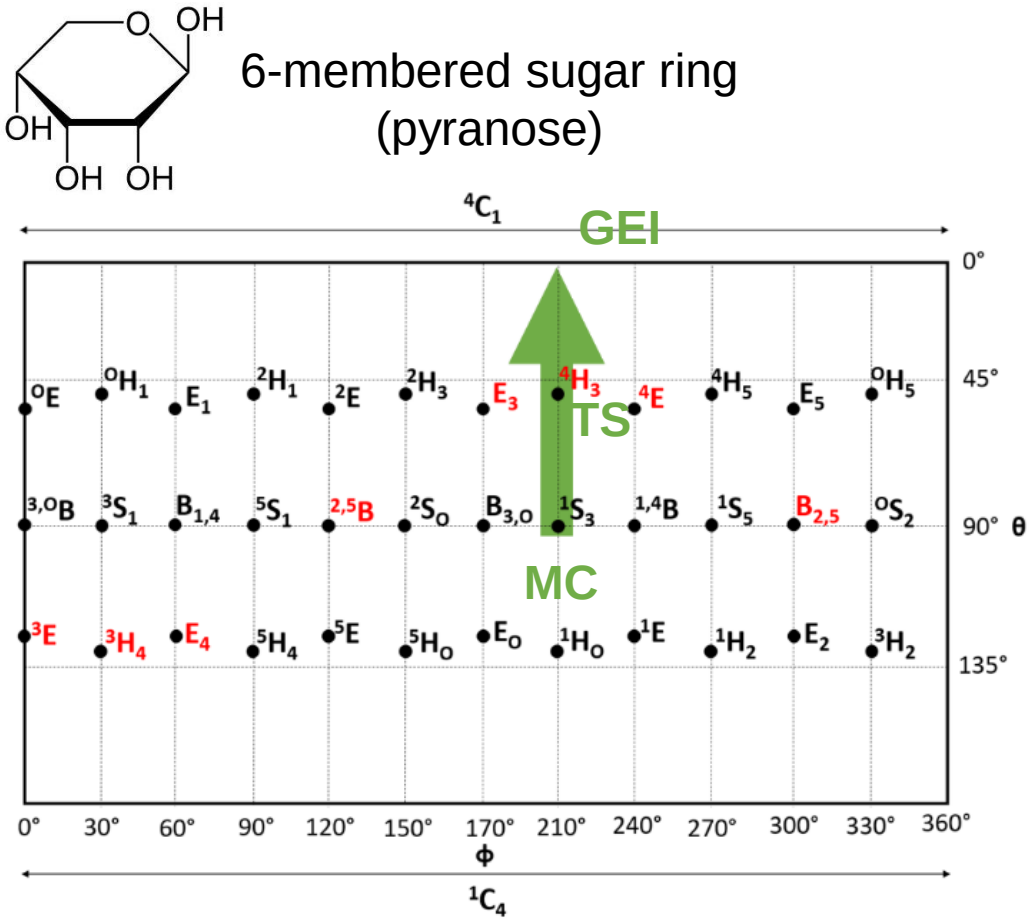
Most β-GHs exhibit a **distorted** conformation



$^4C_1$

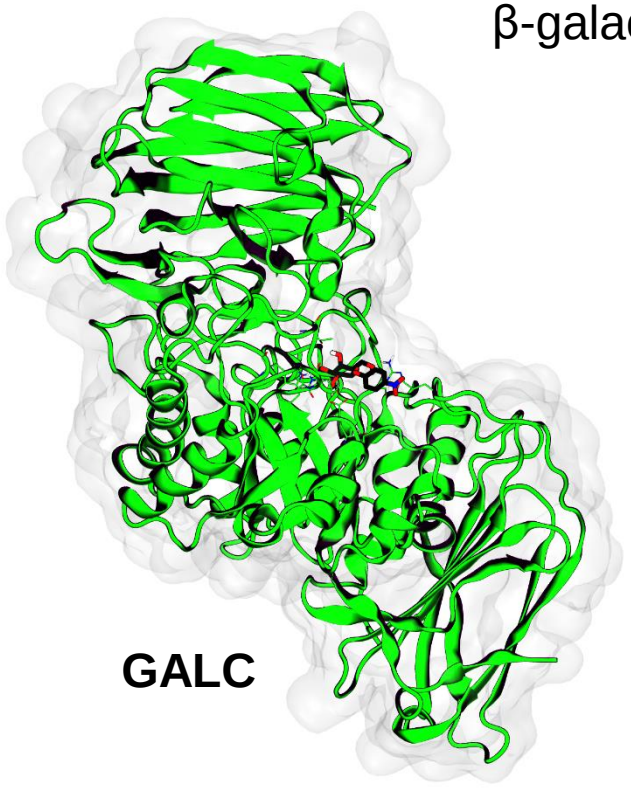
e.g. $^1S_3$

CONFORMATIONAL CATALYTIC ITINERARIES



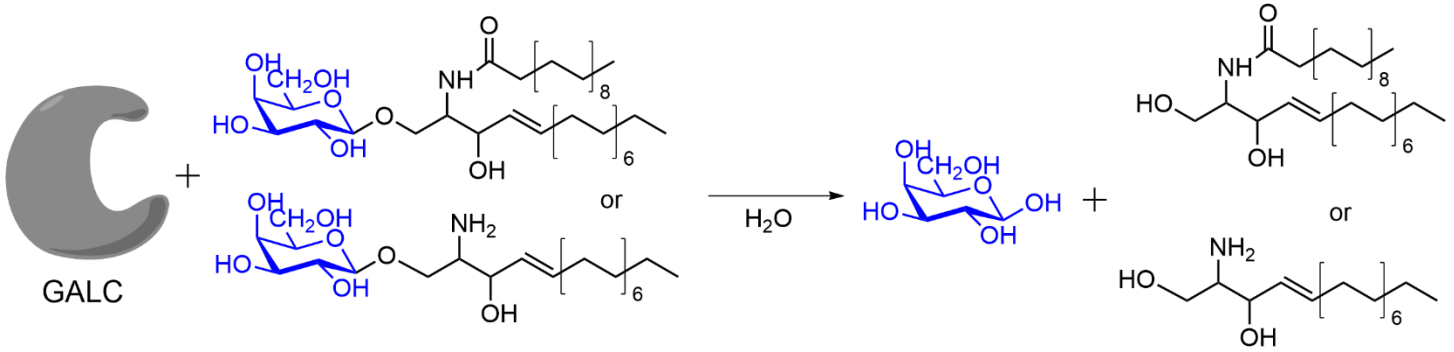
MC = Michaelis complex
TS = transition state
GEI = glycosyl-enzyme intermediate

β-GALACTOCEREBROSIDASE

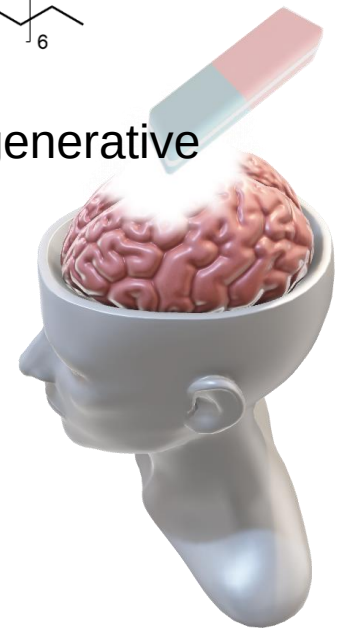


GALC

β-galactocerebrosidase (GALC) catalyzes the hydrolysis of β-galactocerebroside (GalCer) to β-galactose and ceramide

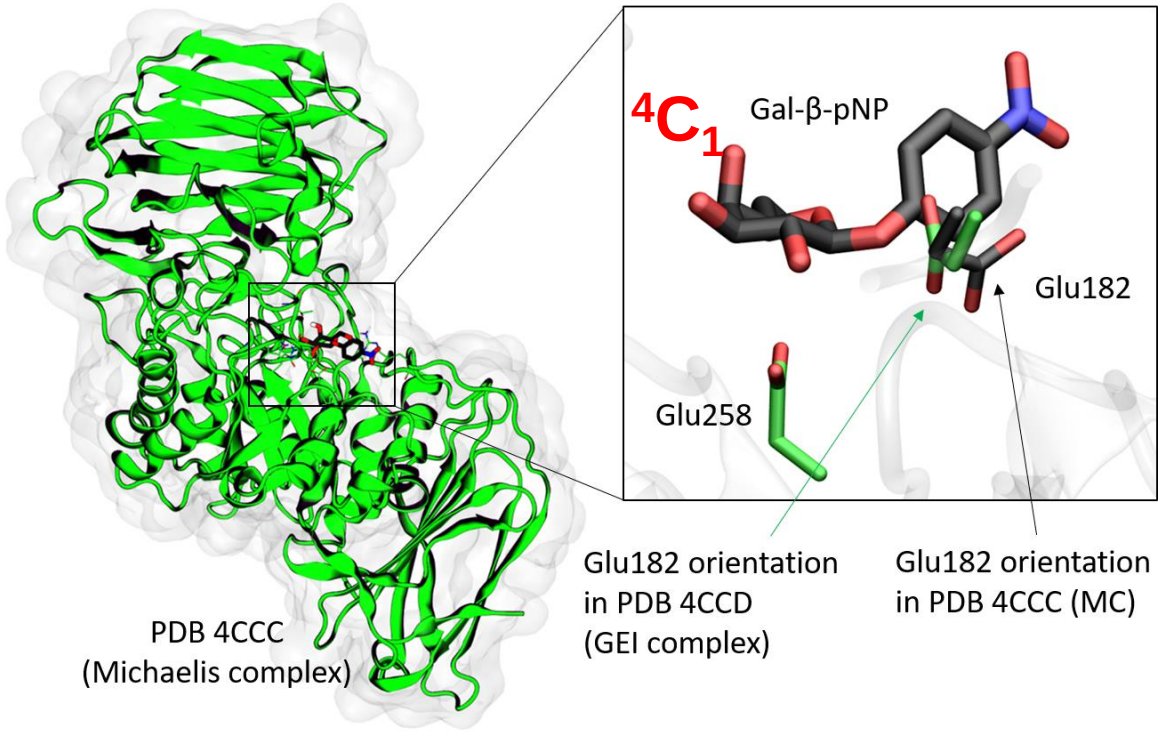


Defects on GALC causes **Krabbe disease**, an incurable neurodegenerative disorder



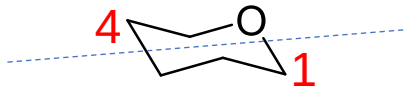
Understanding the catalytic mechanism of GALC at atomic detail is important to boost the development of efficient conformational chaperones for this enzyme

UNUSUAL SUGAR CONFORMATION IN A MC STRUCTURE



First Michaelis complex (MC) structure of GALC in complex with an hydrolyzable substrate analogue, Gal-β-pNP.

The MC structure exhibits an **unusual** substrate conformation, $^4C_1$.

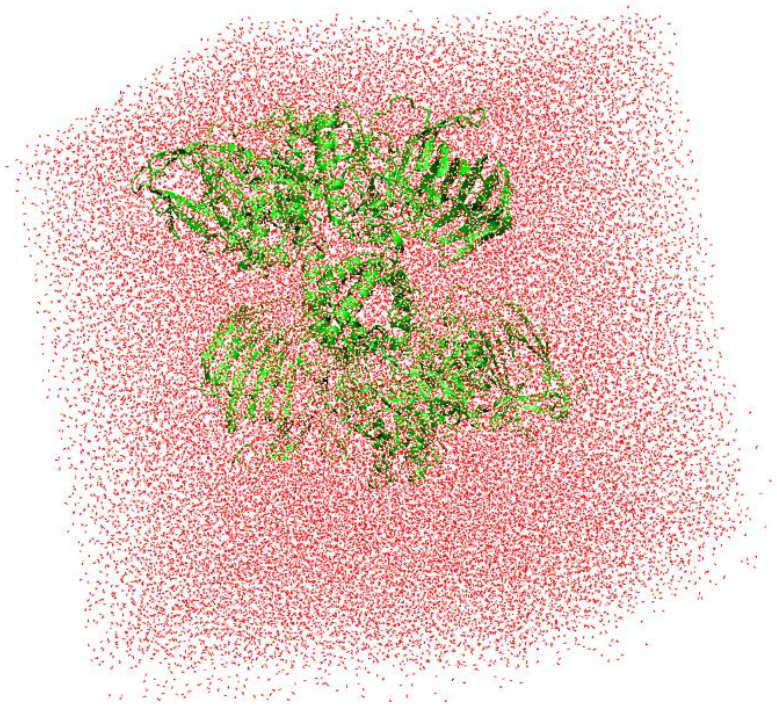


Is this a non-catalytic structure?
Can this unusual conformation be functional?

Hill et. al.; *Proc. Natl. Acad. Sci*. 2013, 110, 20479-20484

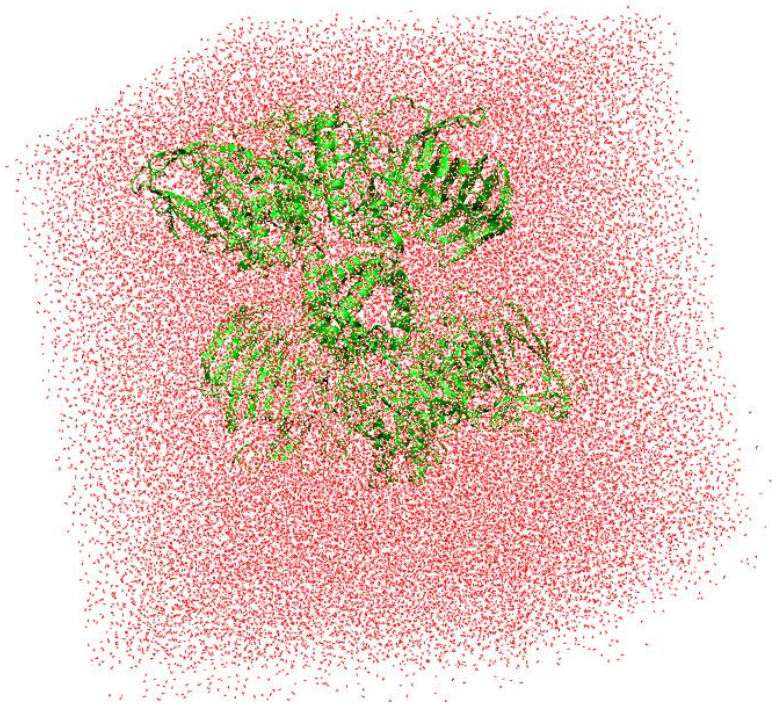
THEORETICAL METHODS

Model building



THEORETICAL METHODS

Model building

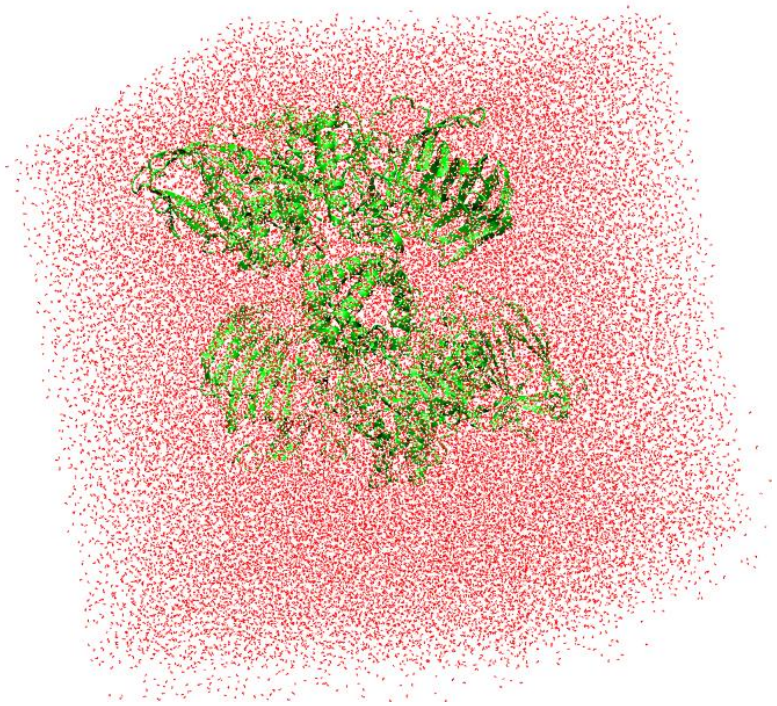


Simulations

1. Classical MD
   - Amber force field

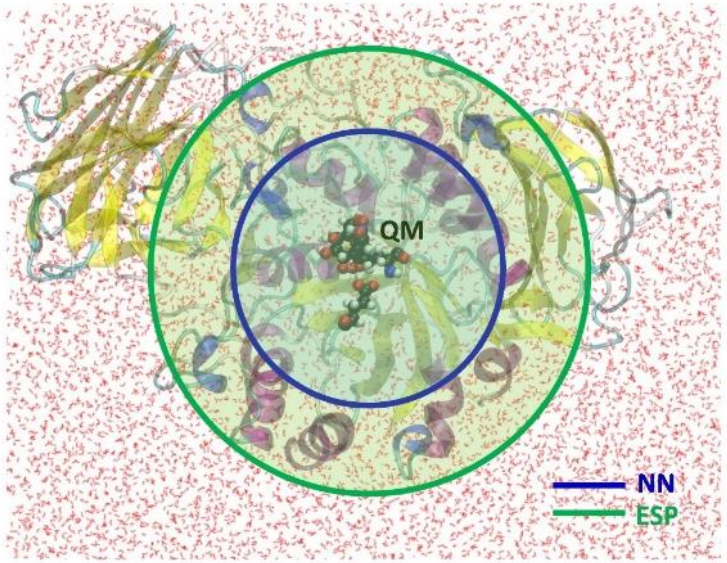
THEORETICAL METHODS

Model building



Simulations

1. Classical MD
2. QM/MM MD

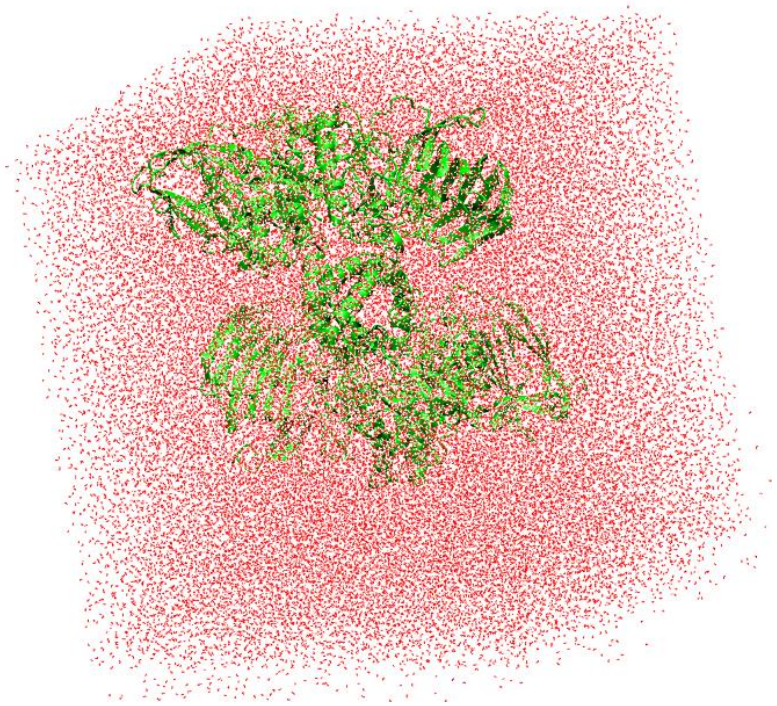


Car-Parrinello MD
DFT (PBE)

Marianski, Mateusz, et al *JCTC* 2016, 12(12), 6157-6168.

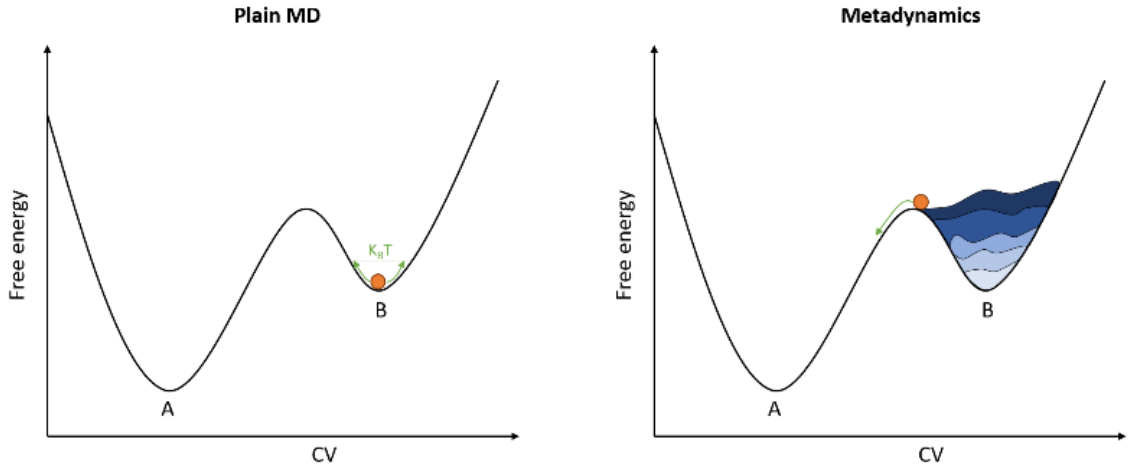
THEORETICAL METHODS

Model building



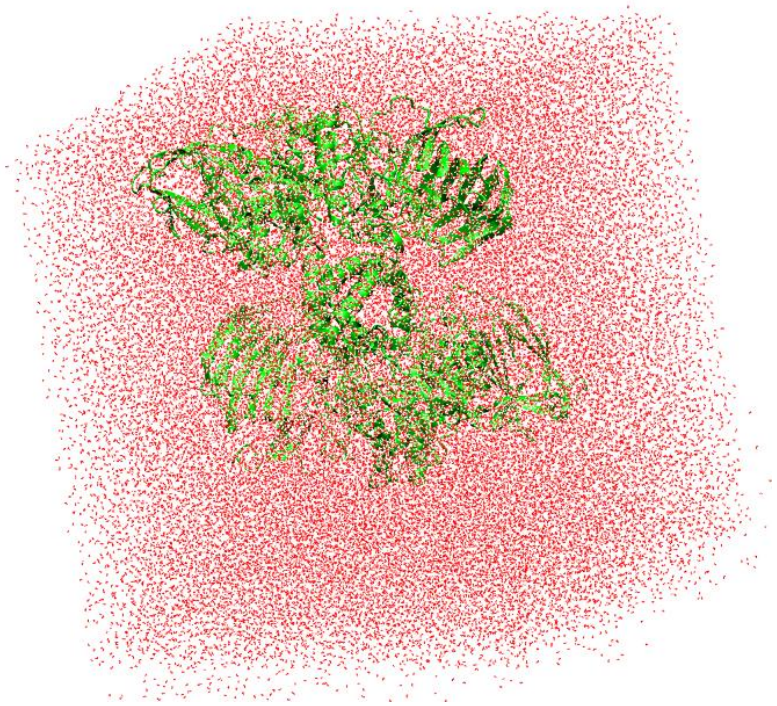
Simulations

1. Classical MD
2. QM/MM MD
3. QM/MM metadynamics



THEORETICAL METHODS

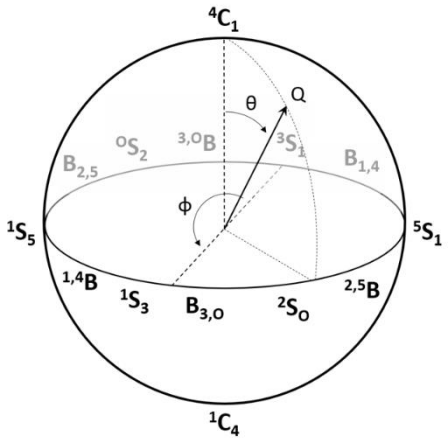
Model building



Simulations

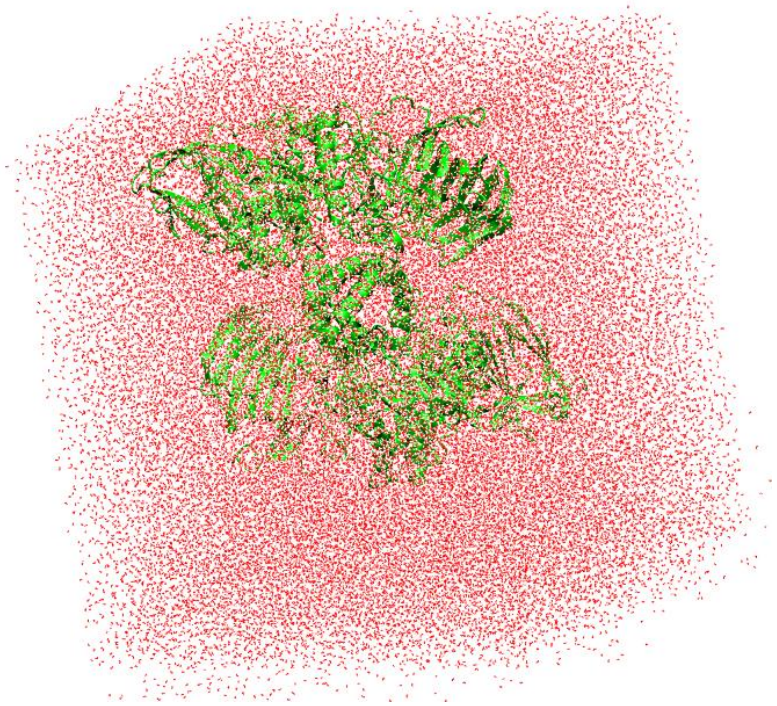
1. Classical MD
2. QM/MM MD
3. QM/MM metadynamics

θ and φ puckering coordinates as CVs



THEORETICAL METHODS

Model building



Simulations

1. Classical MD
2. QM/MM MD
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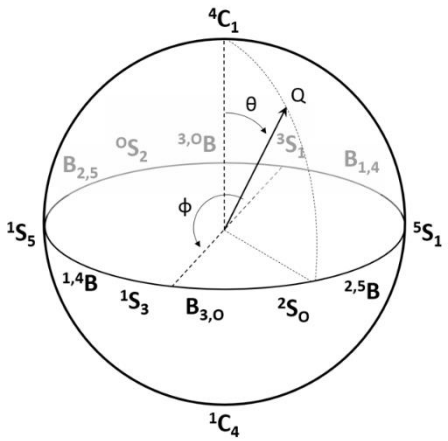
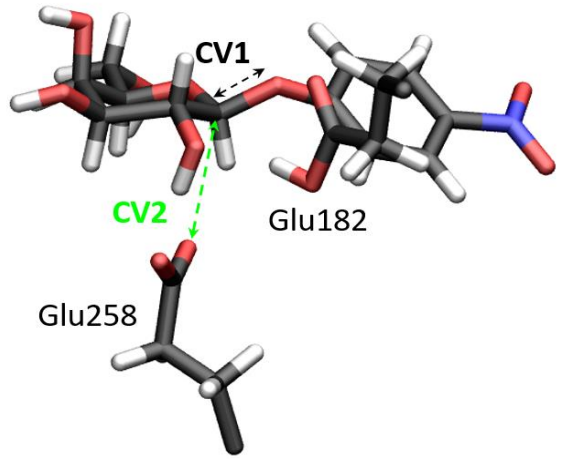
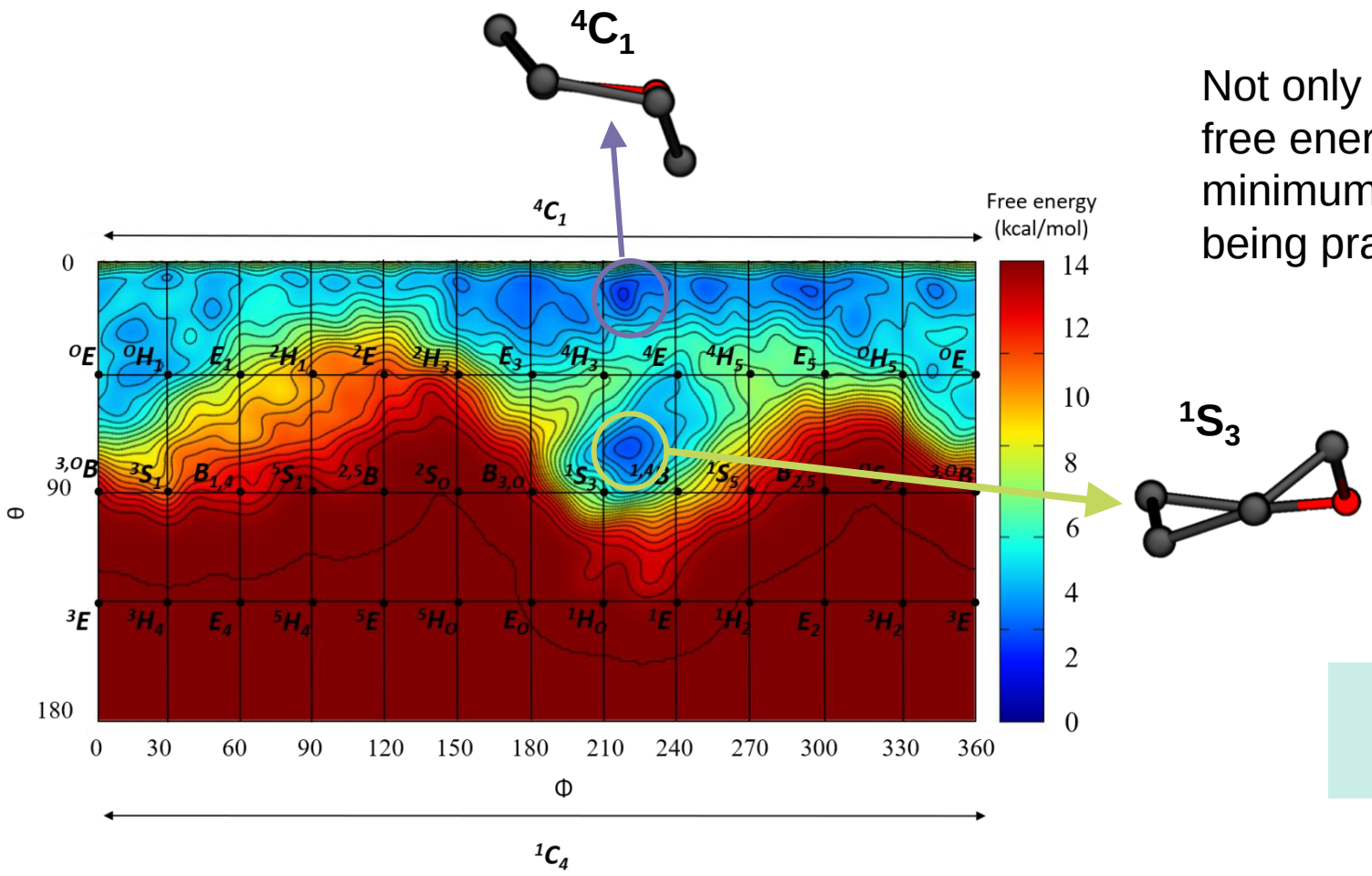
θ and φ puckering coordinates as CVs



Cleaved and formed bonds as CVs



2 CONFORMATIONS OF Gal-β-pNP IN THE ACTIVE SITE OF GALC

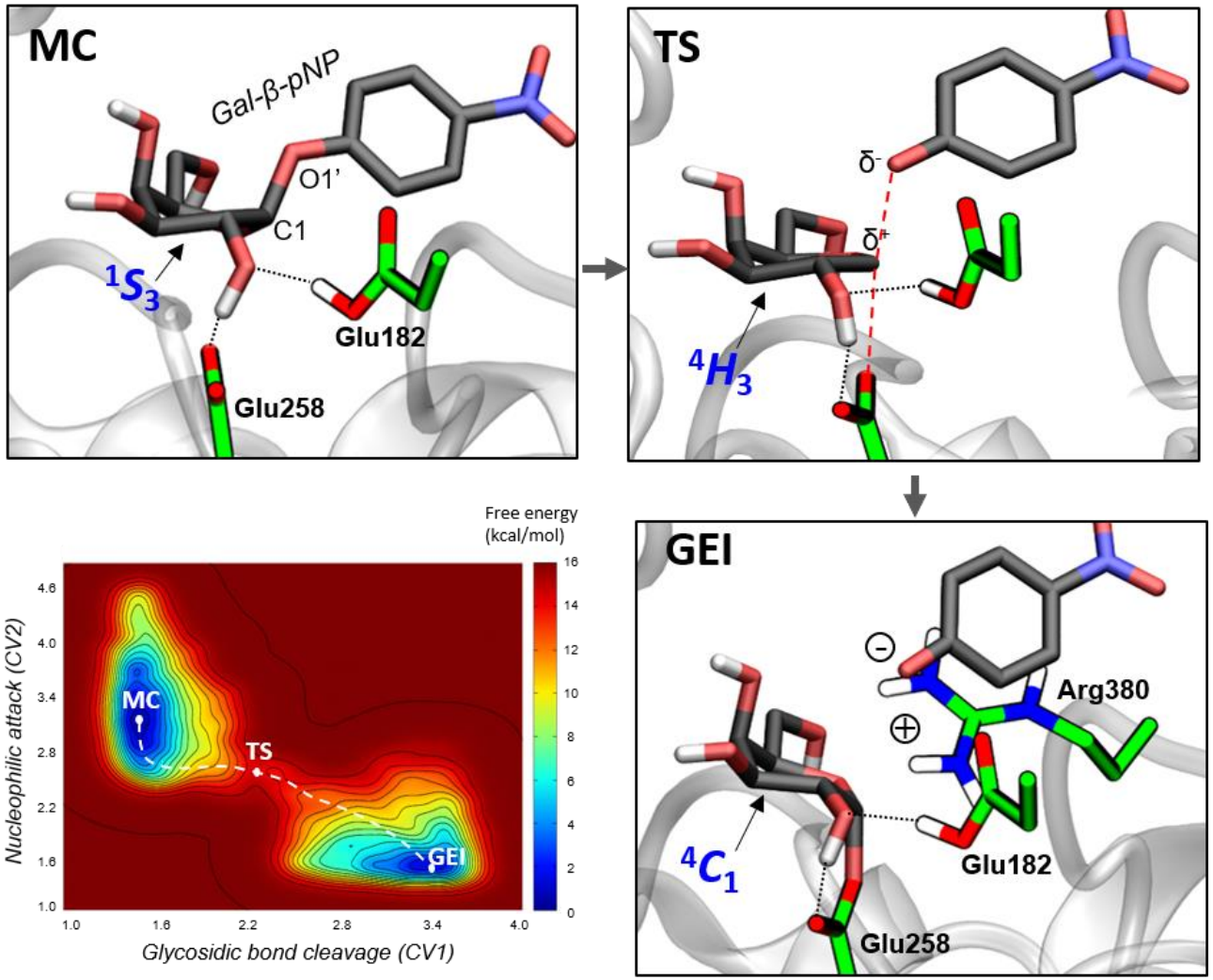


Not only the $^4C_1$ conformation corresponds to a free energy minimum; there is another relevant minimum close to the $^1S_3$ conformation, both being practically isoenergetic.

Are the two conformations catalytically competent?

A. Nin-Hill, C. Rovira; *ACS Catal.* 2020, 10, 12091-12097

REACTIVITY OF THE GAL-β-pNP IN THE $^{1}S_{3}$ CONFORMATION



$\Delta G^{\ddagger}$ = 13.5 kcal/mol

(experimentally ≈ 15 kcal/mol)

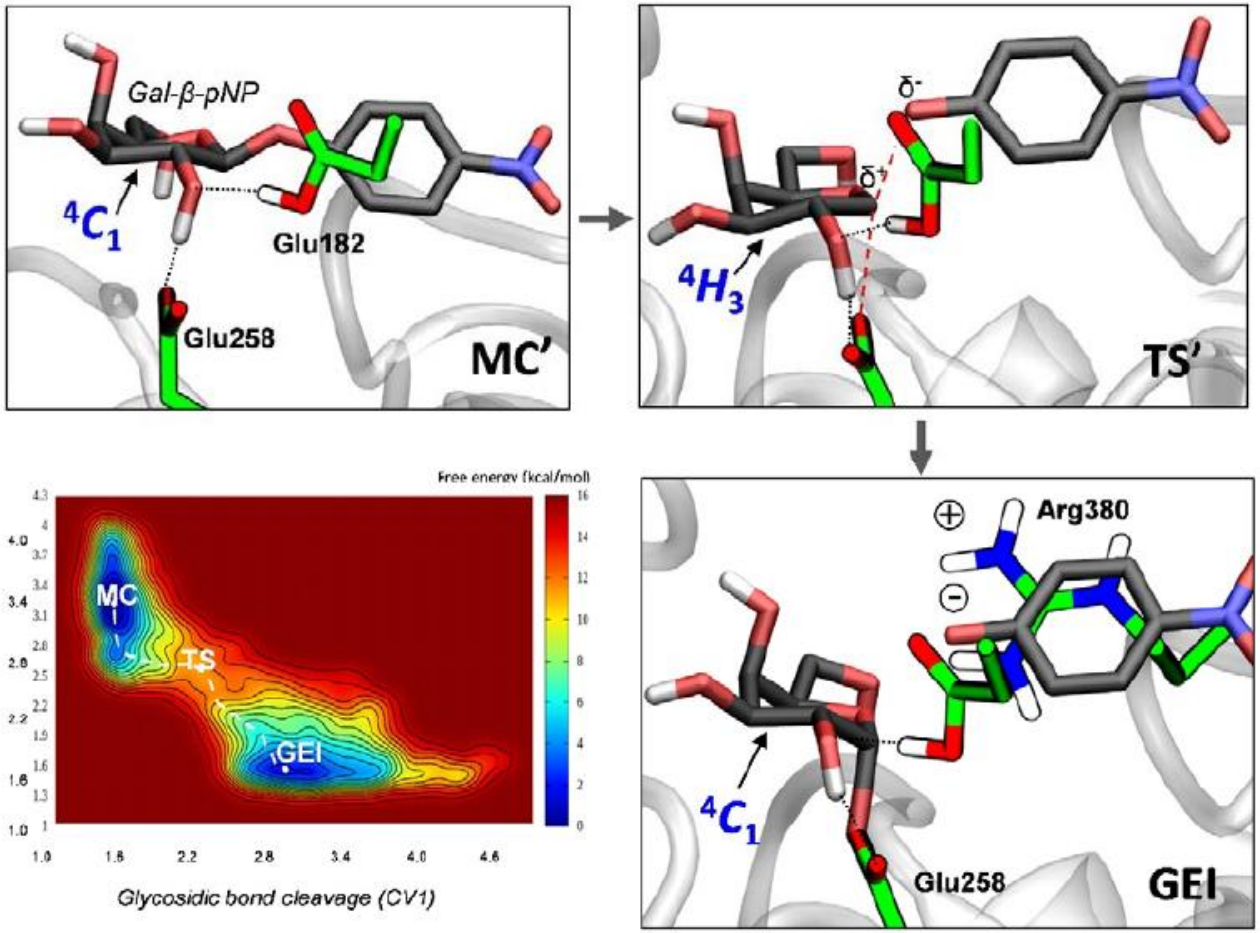
Hill et. al.; *Proc. Natl. Acad. Sci*. 2013, 110, 20479-20484

It is a feasible reaction

$^{1}S_{3} \rightarrow [^{4}H_{3}]^{\ddagger} \rightarrow {}^{4}C_{1}$ itinerary

A. Nin-Hill, C. Rovira; *ACS Catal.* 2020, 10, 12091-12097

REACTIVITY OF THE GAL-β-pNP IN THE $^4C_1$ CONFORMATION



$\Delta G^{\ddagger}$ = 15.4 kcal/mol

(experimentally ≈ 15 kcal/mol)

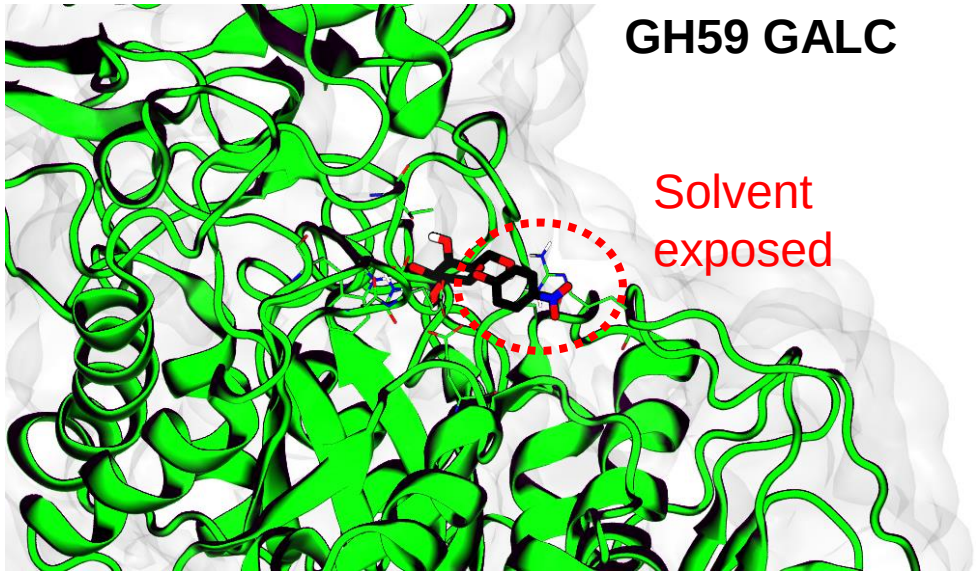
Hill et. al.; *Proc. Natl. Acad. Sci*. 2013, 110, 20479-20484

It is also a feasible reaction

$^4C_1 \rightarrow [^4H_3]^{\ddagger} \rightarrow {}^4C_1$ itinerary

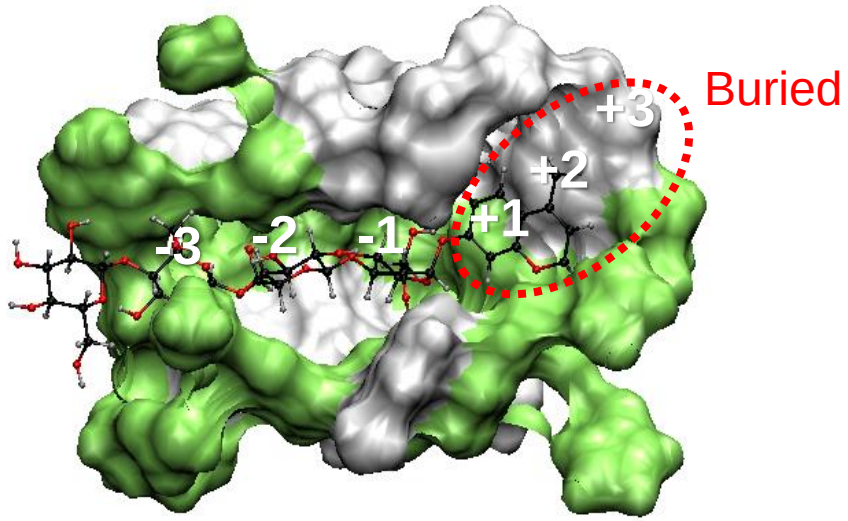
A. Nin-Hill, C. Rovira; *ACS Catal.* 2020, 10, 12091-12097

REACTIVITY OF GAL-β-pNP FROM 2 CONFORMATIONS



$^{1}S_{3} \sim {}^{4}C_{1}$

GH16 endo-glucanase



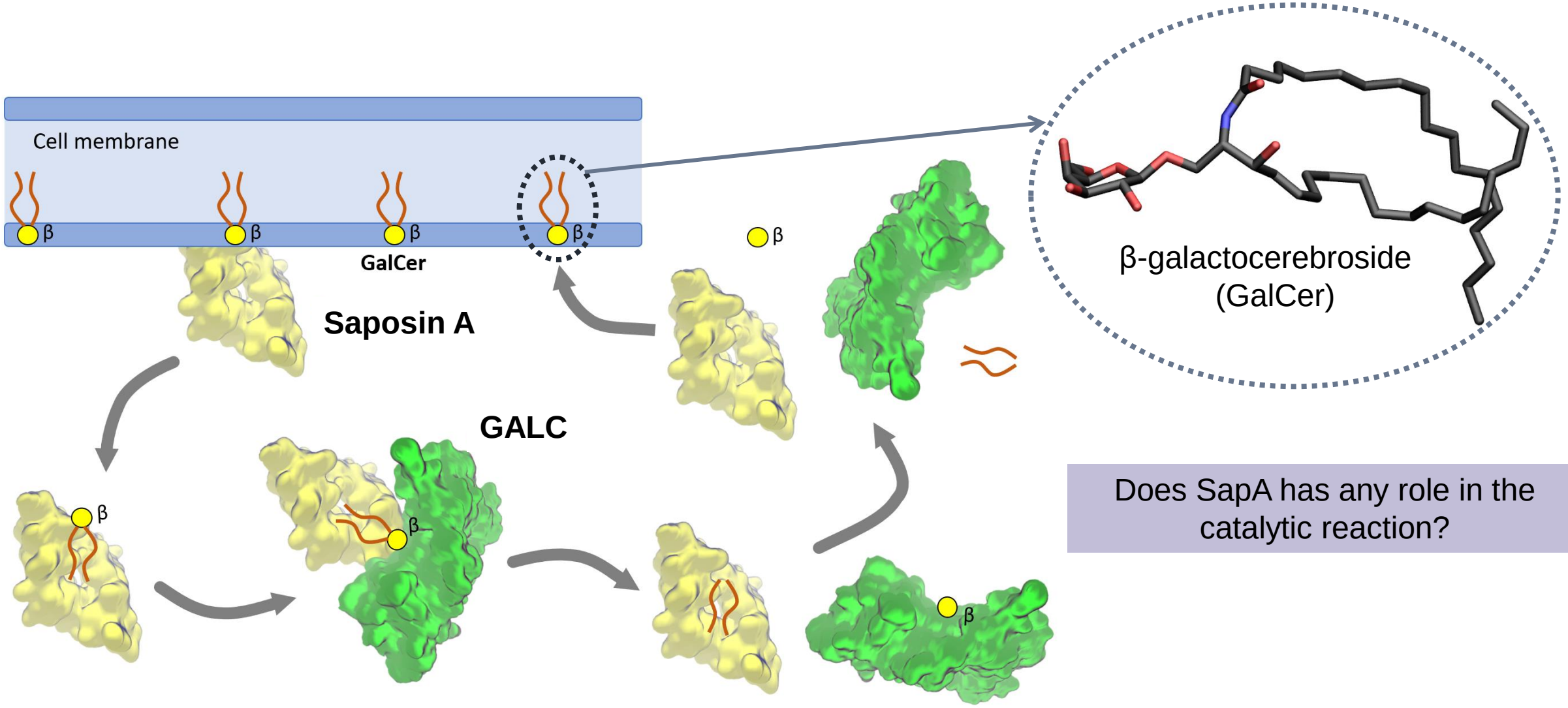
$^{1}S_{3} < {}^{4}C_{1}$ (by 11 kcal/mol)

Biarnés, et. al. *J. Biol. Chem*. 2006, 281, 1432-1441

The **lack of steric determinants** is probably the reason why the β-galactose moiety can switch between the distorted ($^{1}S_{3}$) and undistorted ($^{4}C_{1}$) conformers, both of which are catalytically relevant.

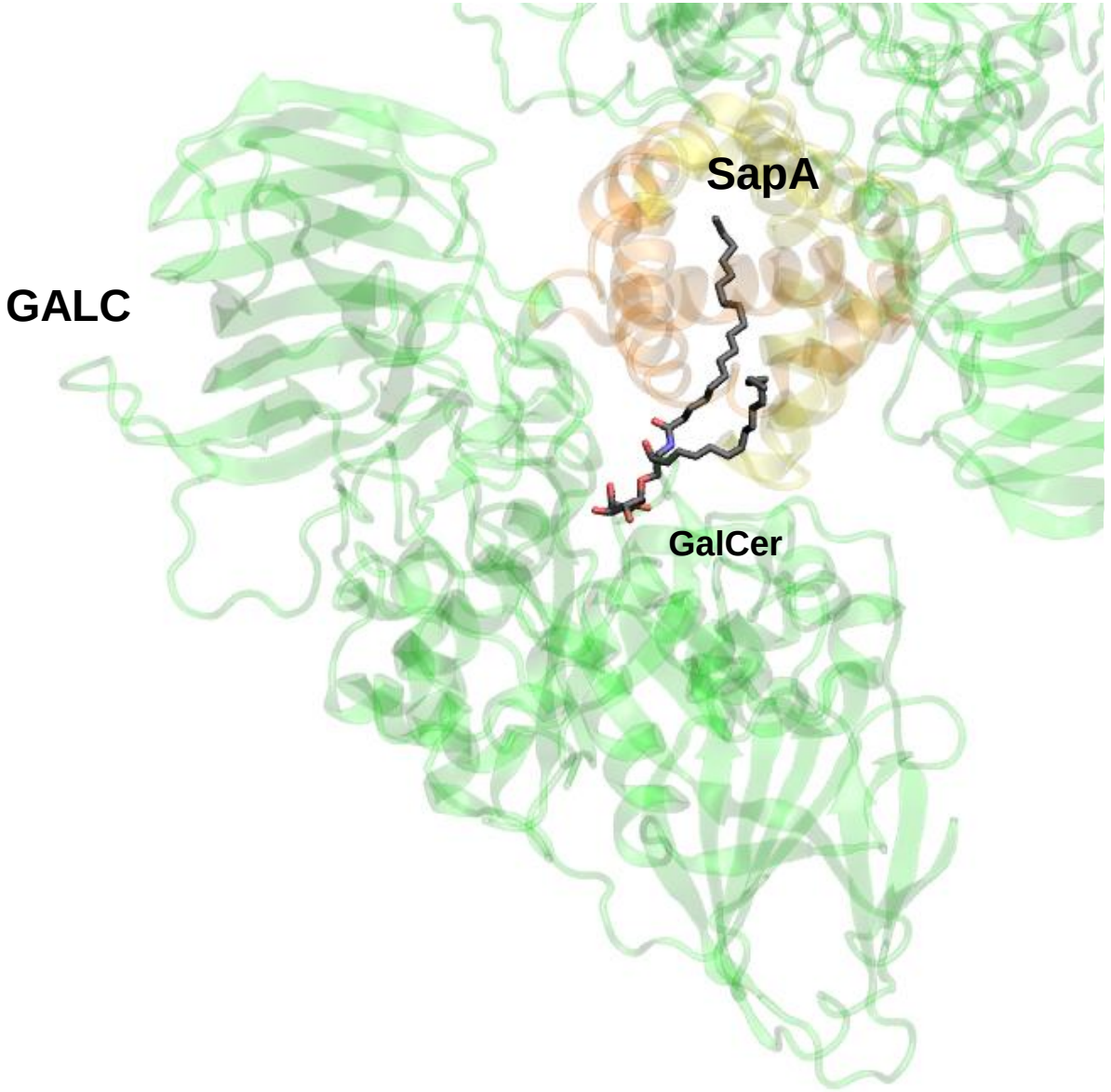
A. Nin-Hill, C. Rovira; *ACS Catal.* 2020, 10, 12091-12097

IN VIVO MODEL: CONSIDERING THE GalCer SUBSTRATE AND THE SAPOSIN LIPID TRANSFER PROTEIN



Does SapA has any role in the catalytic reaction?

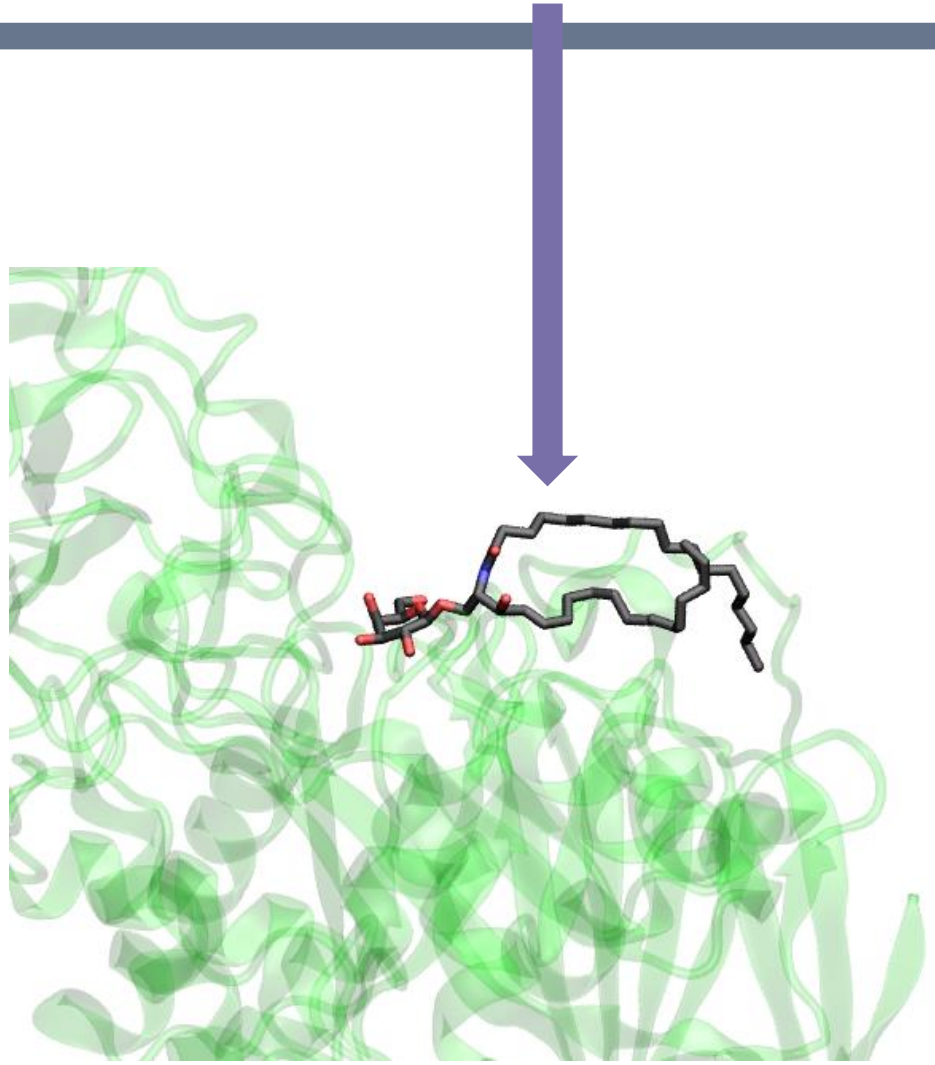
BUILDING THE GALC-SapA DIMER IN COMPLEX WITH GalCer



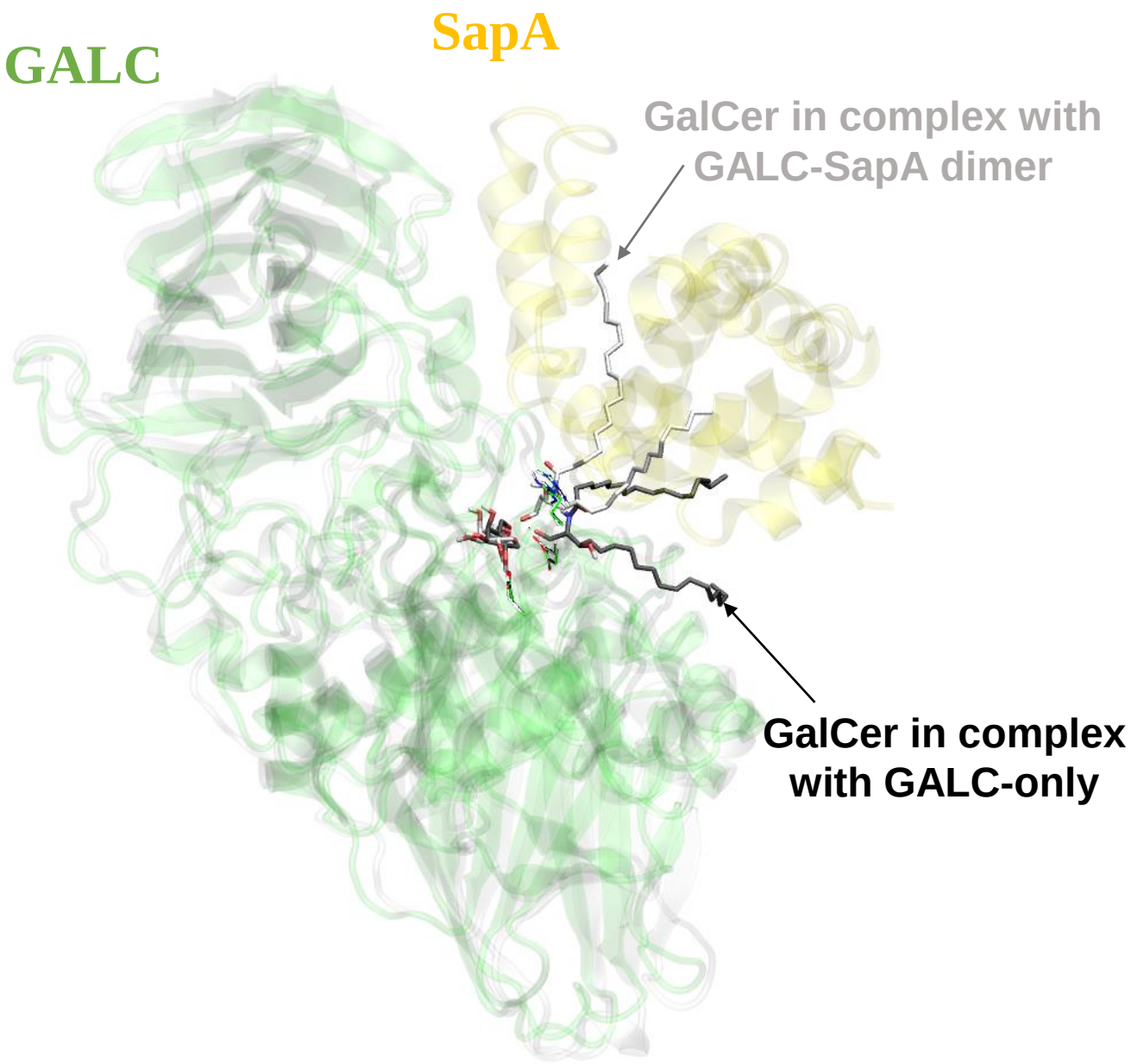
We tried several poses until the lipid fits optimally.

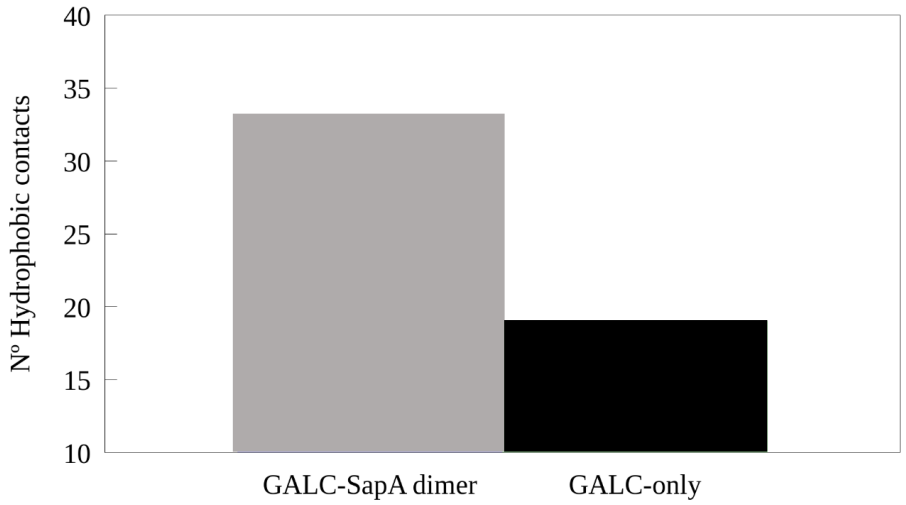
MD simulations were performed (~150 ns) to equilibrate the model.

GALC IN COMPLEX WITH β-GALACTOCEREBROSIDE



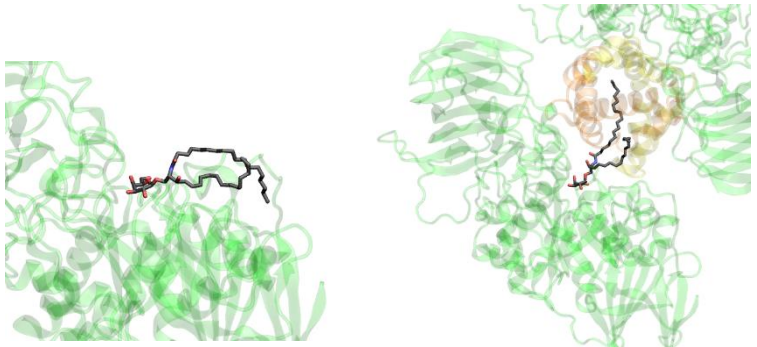
GalCer IN COMPLEX WITH GALC and GALC-SapA

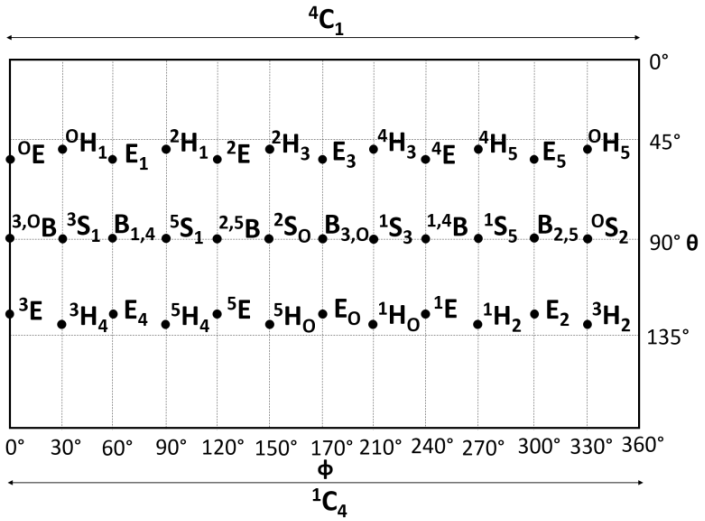




FUTURE PROSPECTS

1. Obtain the conformational FEL of GalCer in complex with GALC-only and GALC-SapA dimer





FUTURE PROSPECTS

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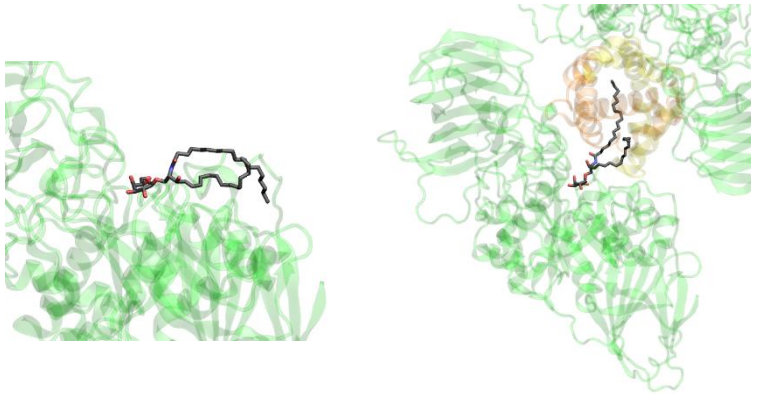
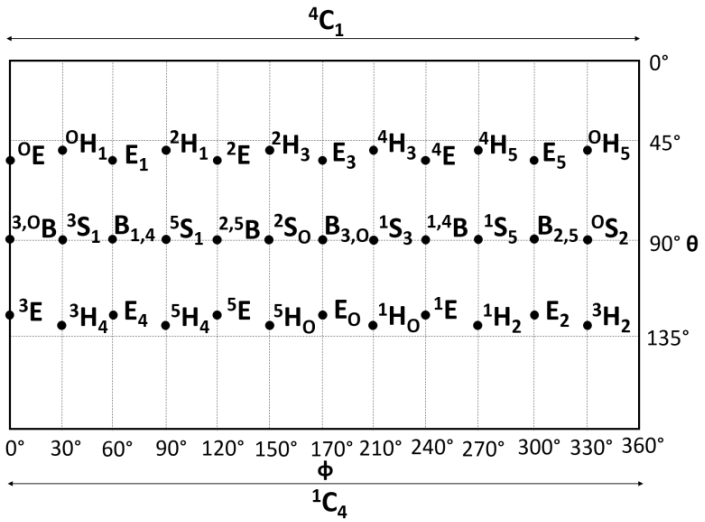
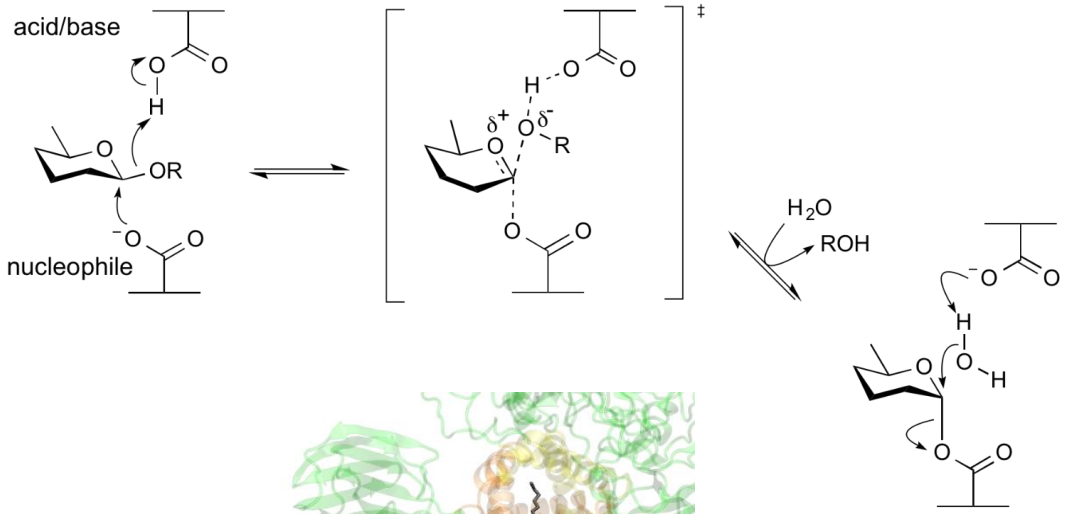
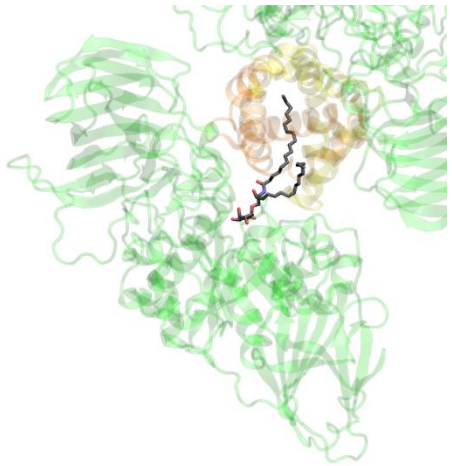




2. Calculate the reaction mechanism of GALC-SapA with the GalCer natural substrate.



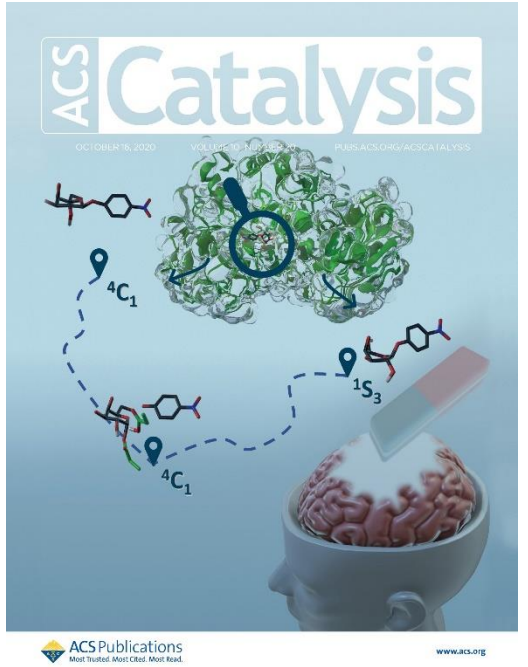


CONCLUSIONS

Two itineraries, $^{1}S_{3} \rightarrow [^{4}H_{3}]‡ \rightarrow {}^{4}C_{1}$ (classical) and $^{4}C_{1} \rightarrow [^{4}H_{3}]‡ \rightarrow {}^{4}C_{1}$ (non-classical), contribute to the hydrolysis of the Gal-β-pNP substrate by GALC.

The SapA protein stabilizes the natural GalCer substrate with a high number of hydrophobic contacts.

In the future we would like to find out whether the SapA protein has a role in the catalytic reaction.



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