

Journée scientifique du Centre de Calcul et de la Maison de la Simulation

Sialic acids and diabetes : impact of desialylation on insulin receptor's functionality

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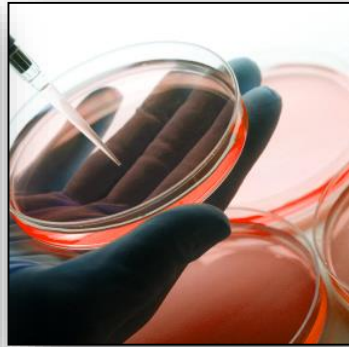
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CNRS UMR 7369 MEDyC
Team "matrix aging and vascular remodelling"



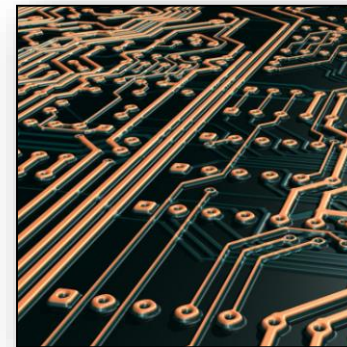
Supervisors

In vivo / In vitro



Dr Sébastien BLAISE
Pr Vincent DURLACH

In silico



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Pr Manuel DAUCHEZ

Diabetes



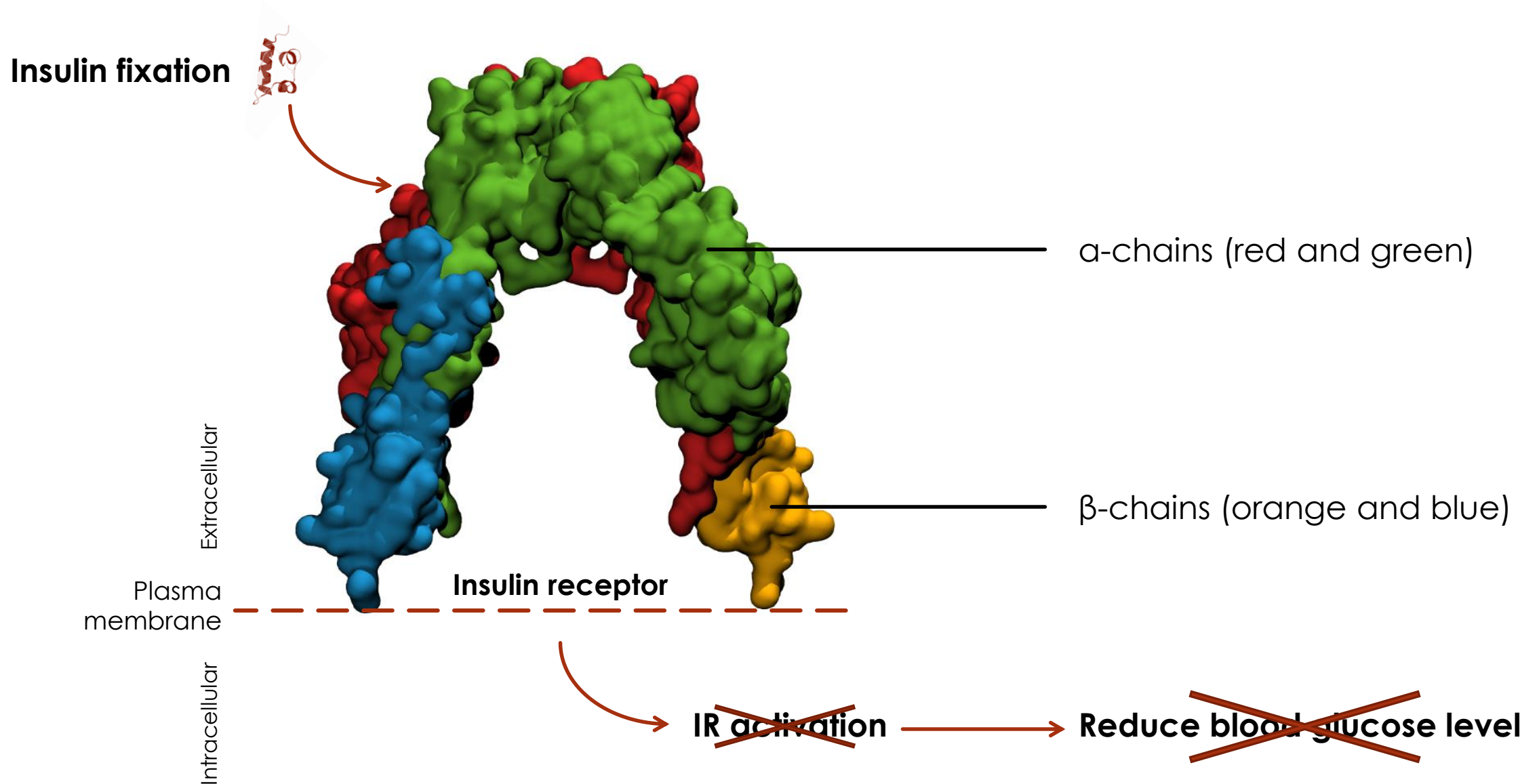
360 million diabetics

France : 3 million + 400 each day

- Abnormal and chronic increase of blood sugar level
- Type 1 diabetes
 - Low insulin production
 - Genetic factors
- Type 2 diabetes
 - Inefficient insulin
 - Aging, obesity...

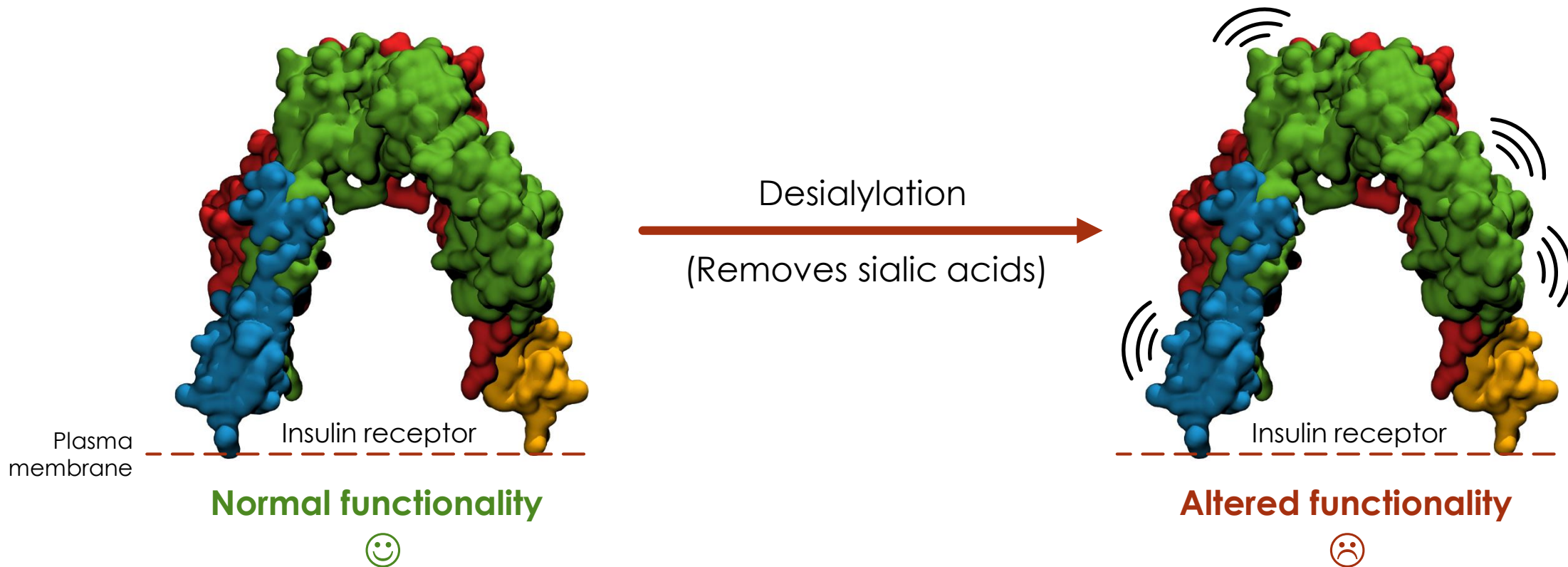
80 % of all diabetes

Insulin receptor

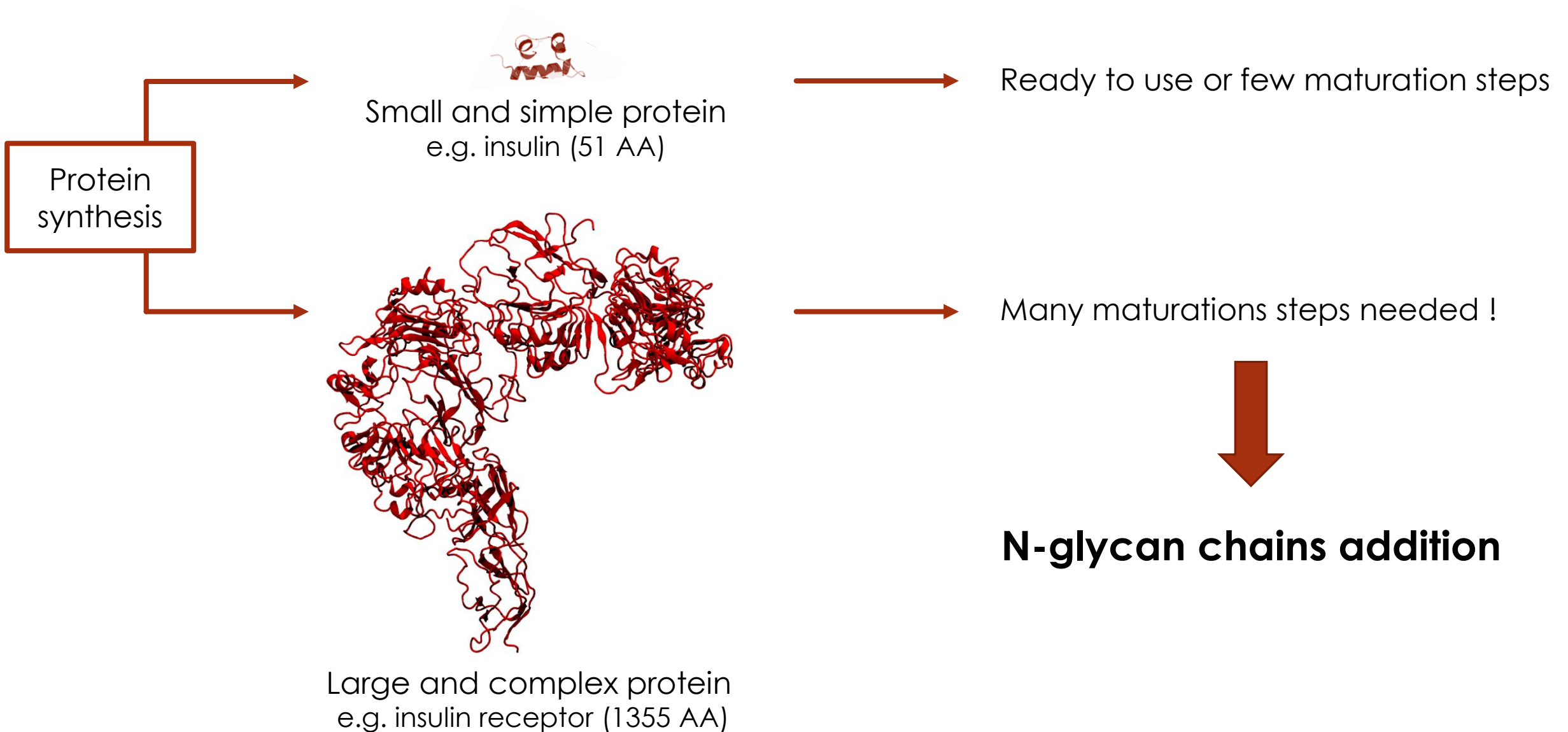


Aim of the study

Sialic acids and diabetes : impact of desialylation on insulin receptor's functionality

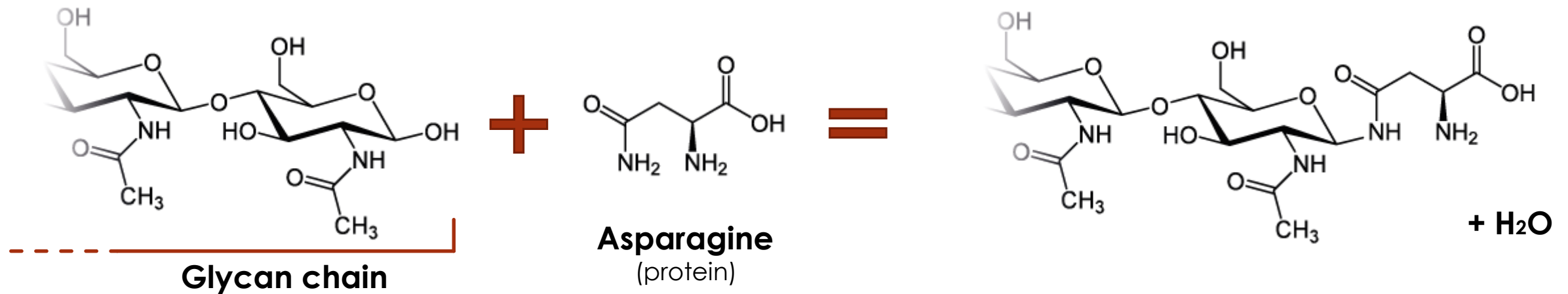


N-glycan chains



N-glycan chains

- Sugar or modified sugar linked to proteins' specific residues (asparagine)

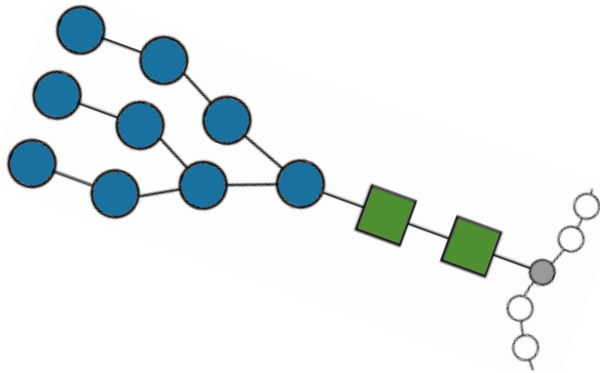


- Various purposes...
 - Protein folding
 - Reduce protein degradation
 - Protein-protein interactions
 - ...
- ... depending on :
 - Glycan chain localisation
 - Protein's partners
 - Glycan type
 - ...

N-glycan chains

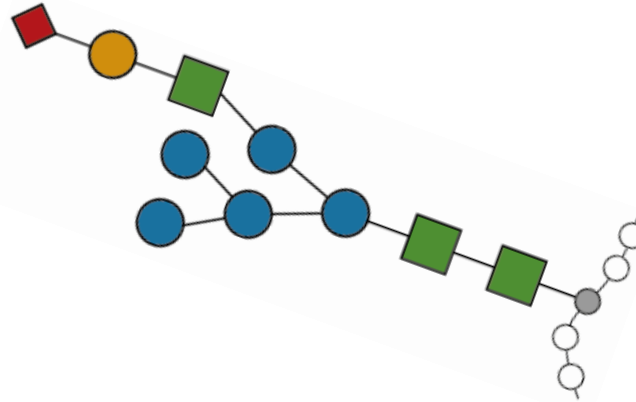
- 3 groups :

High mannose



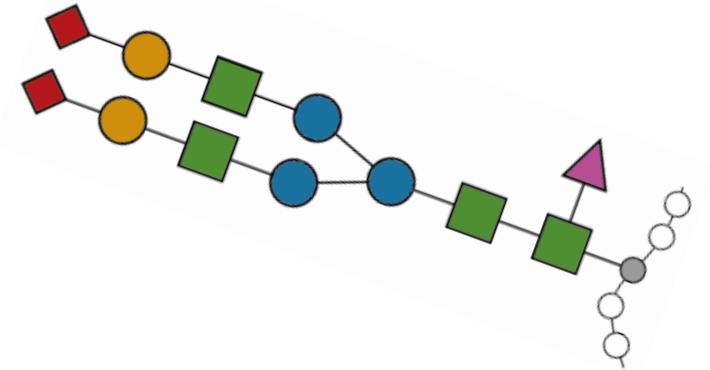
Many mannose, 2 N-acetylglucosamines

Hybrid



Mix between high mannose and complex types

Complex

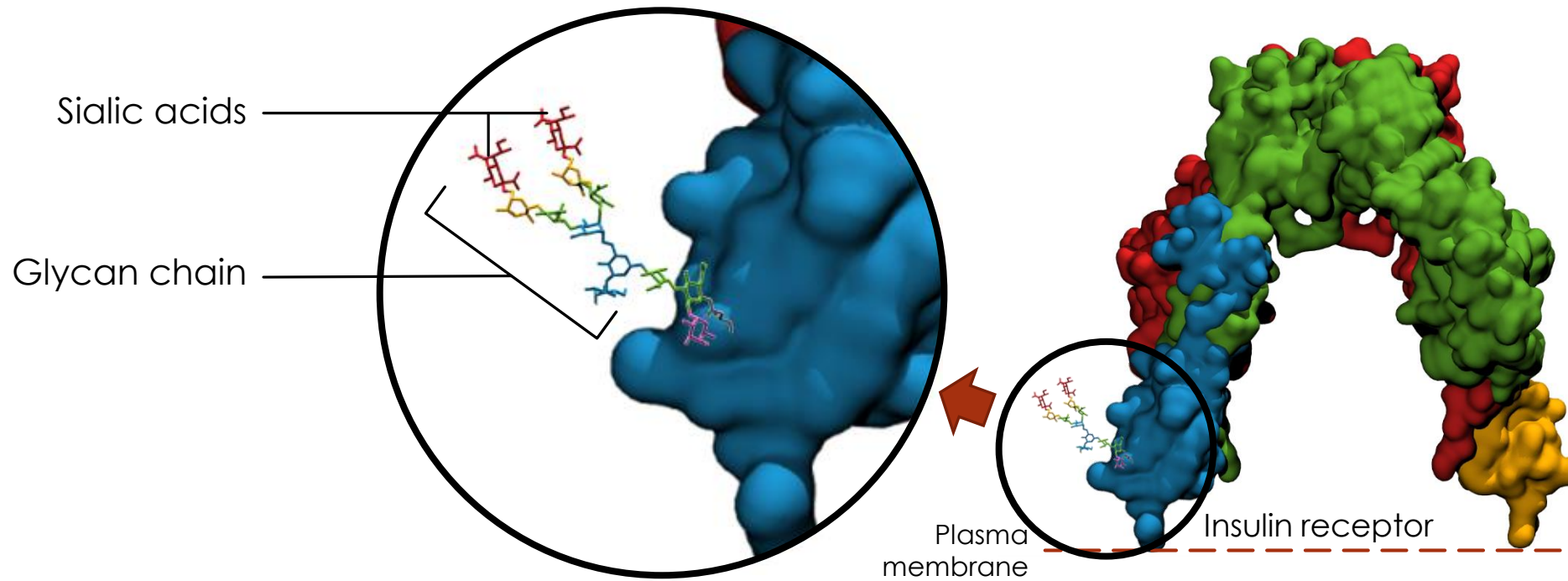


Various types of saccharides :

- N-acetylglucosamine
- Mannose
- Galactose
- Fucose
- **Sialic acids**

+ Various lengths and antenna number in each group

Insulin receptor and sialic acids



What are the consequences of removing sialic acids over the insulin receptor mechanisms ?

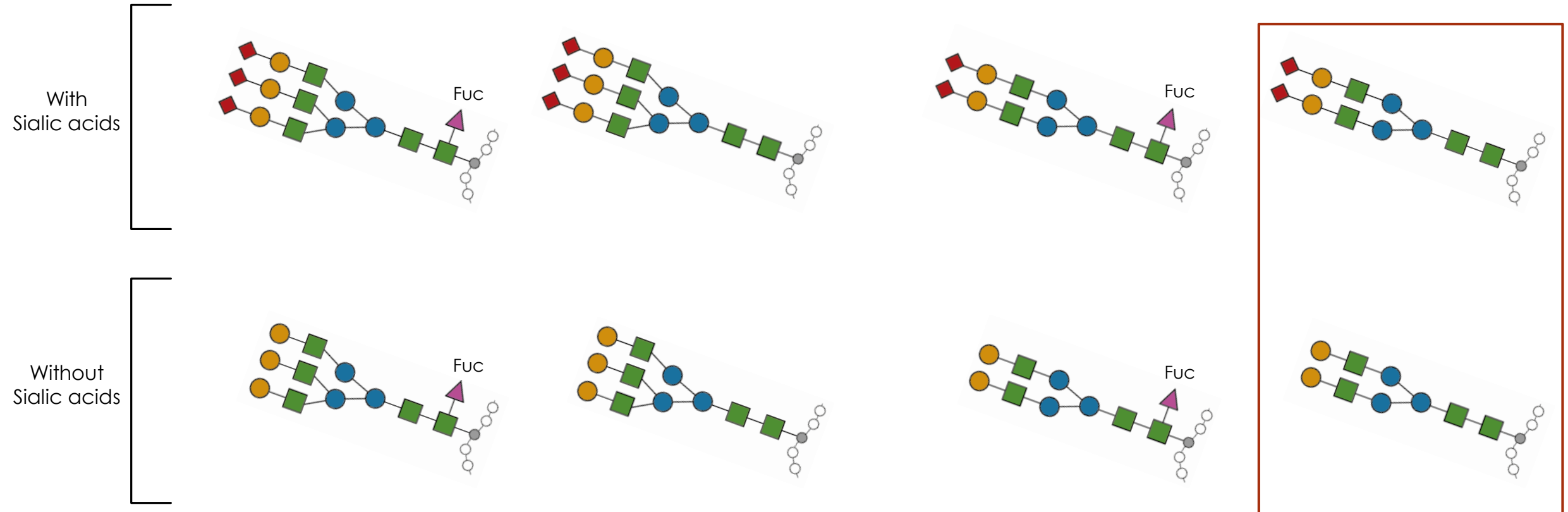
First step : will the removal of sialic acids change the glycan chain dynamics

Choice of the structure

- According to literature and the most common structures found : 4 chains have been selected

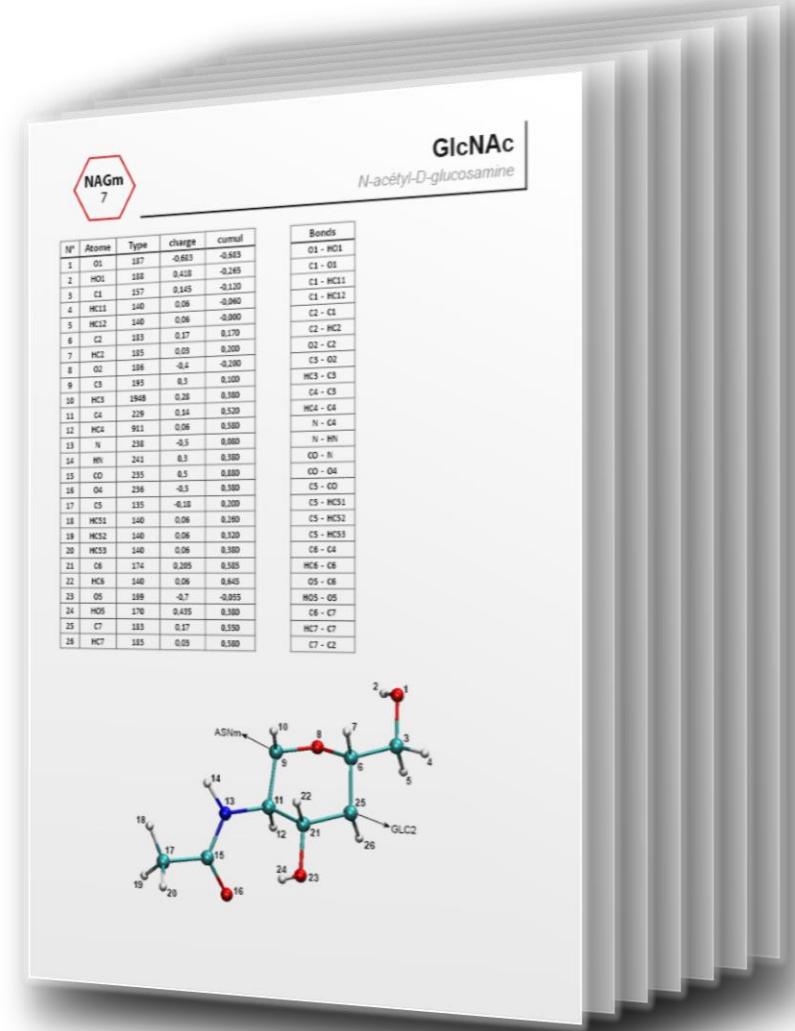
Tri-antennary

Bi-antennary




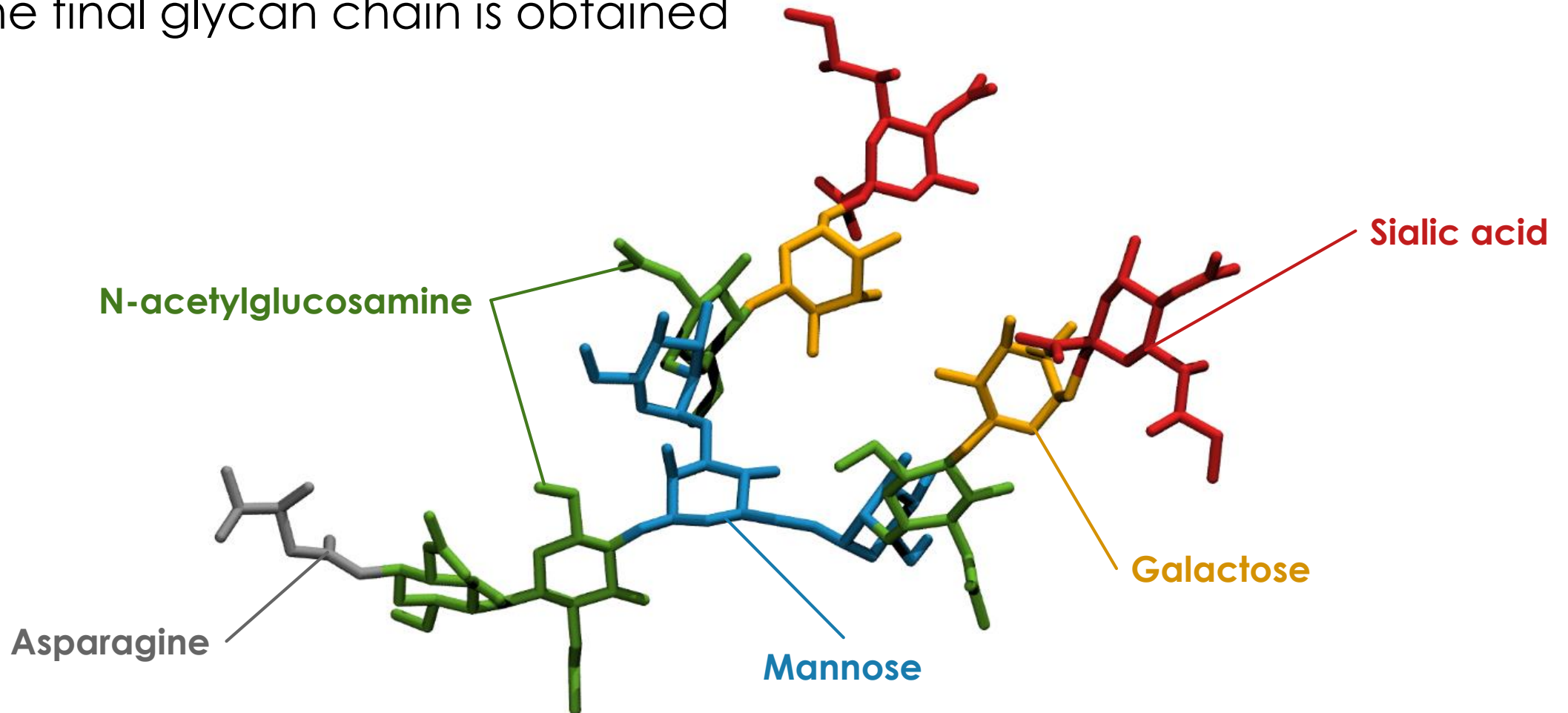
Force field

- GLYCAM, AMBER...
 - Already used for glycan chains
 - Rarely used for both glycan + protein
 - OPLS (all atoms)
 - Often used for proteins
 - Miss glycan atoms description
- ↓
- Missing atoms added by homology with already described atoms

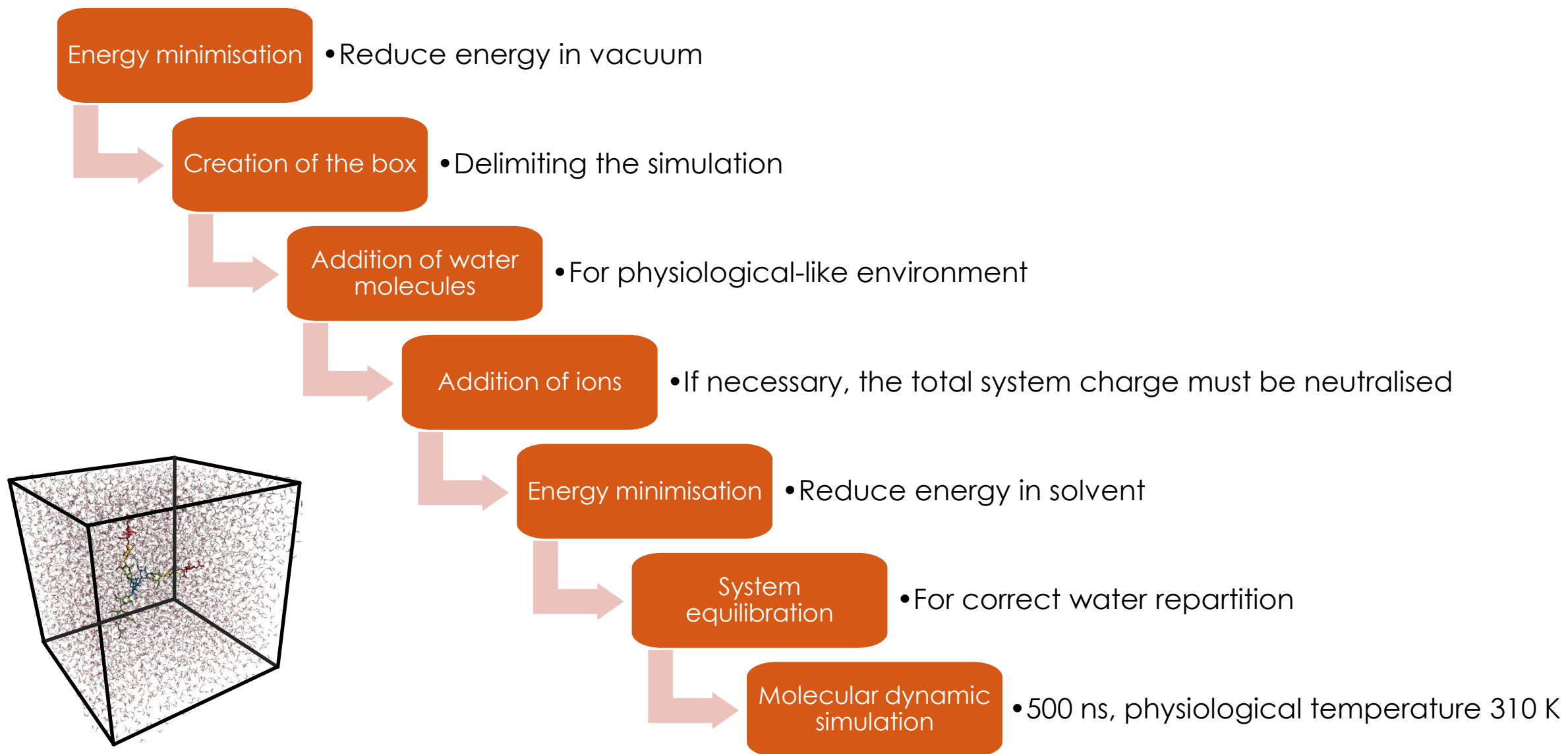


Construction

- Each chain link is separately built using Avogadro software 
- Residues are then linked 1 by 1 with energy minimisation at each step until the final glycan chain is obtained

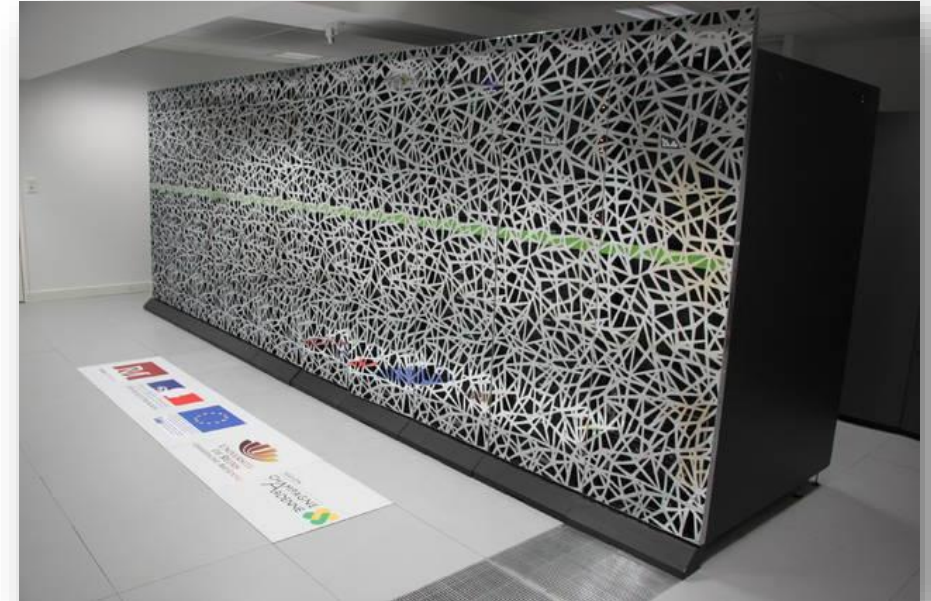


Molecular dynamic simulations (GROMACS)



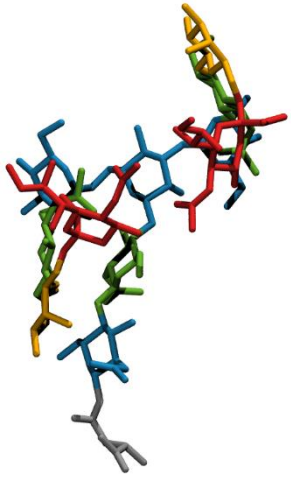
Molecular dynamic simulations

- About 13,000 atoms during simulation
- Computation time (500ns) :
 - 10 nodes
 - 160 cores
 - **60 h**
- Output files :
 - 12 GB
 - 500,000 positions saved for each atom (1/picosecond)
 - Light version : no more water and 50,000 positions

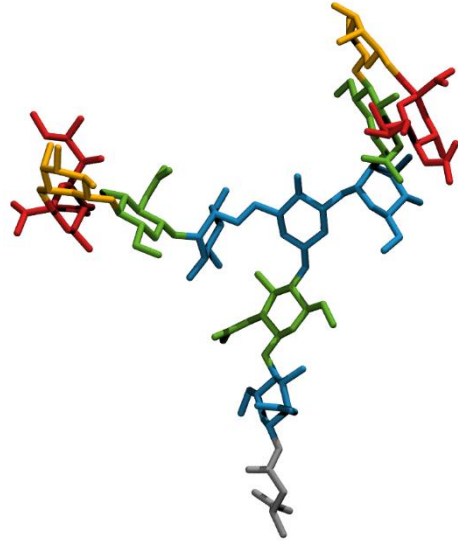


Results analysis : clustering

Cluster #1



Cluster #2



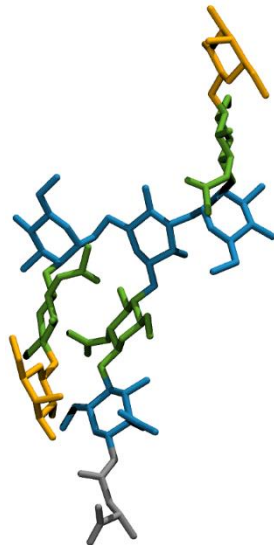
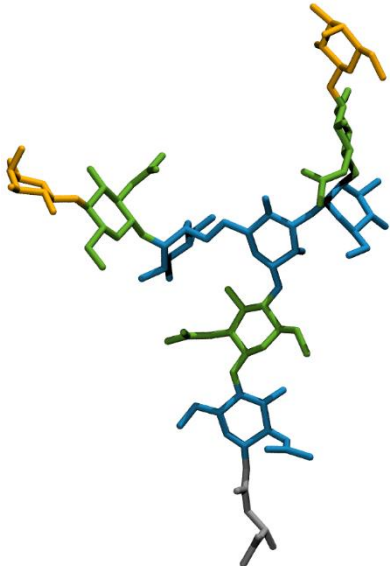
- Objective: identify mains conformations that the chain will adopt.

	Cluster #1	Cluster #2	Other
With SA	72%	14%	14% (18 clusters)
Without SA	28%	24%	48% (41 clusters)

- Cluster size :
 - Less and bigger clusters for chain with SA
 - More and smaller clusters for chain without SA

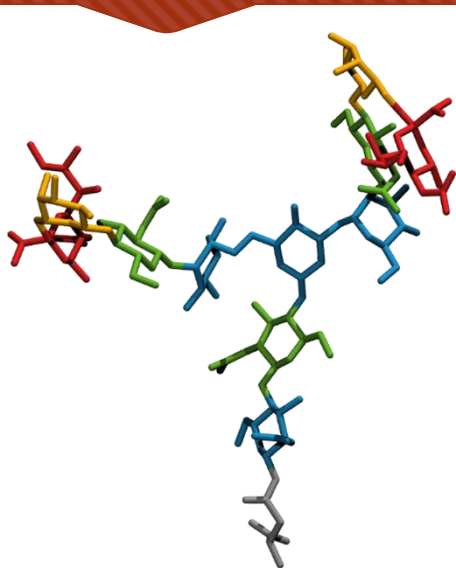
With SA

Without SA



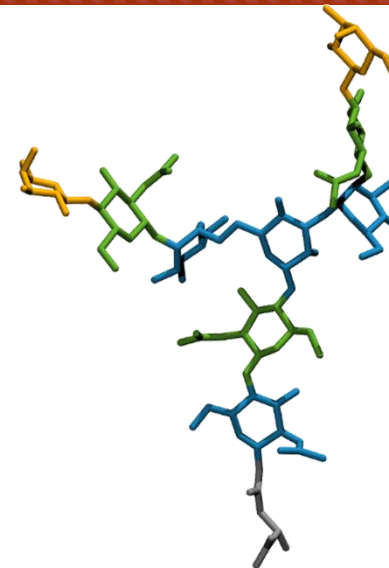
Results analysis : clustering

Cluster #1
With SA
72 %

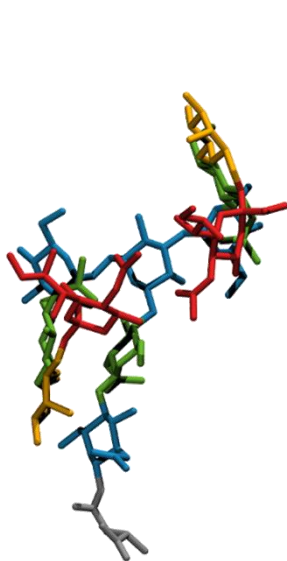


Cluster #1 with SA = cluster #2 without SA

Cluster #2
Without SA
24 %

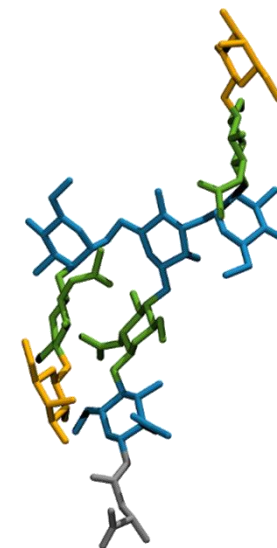


Cluster #2
With SA
14 %

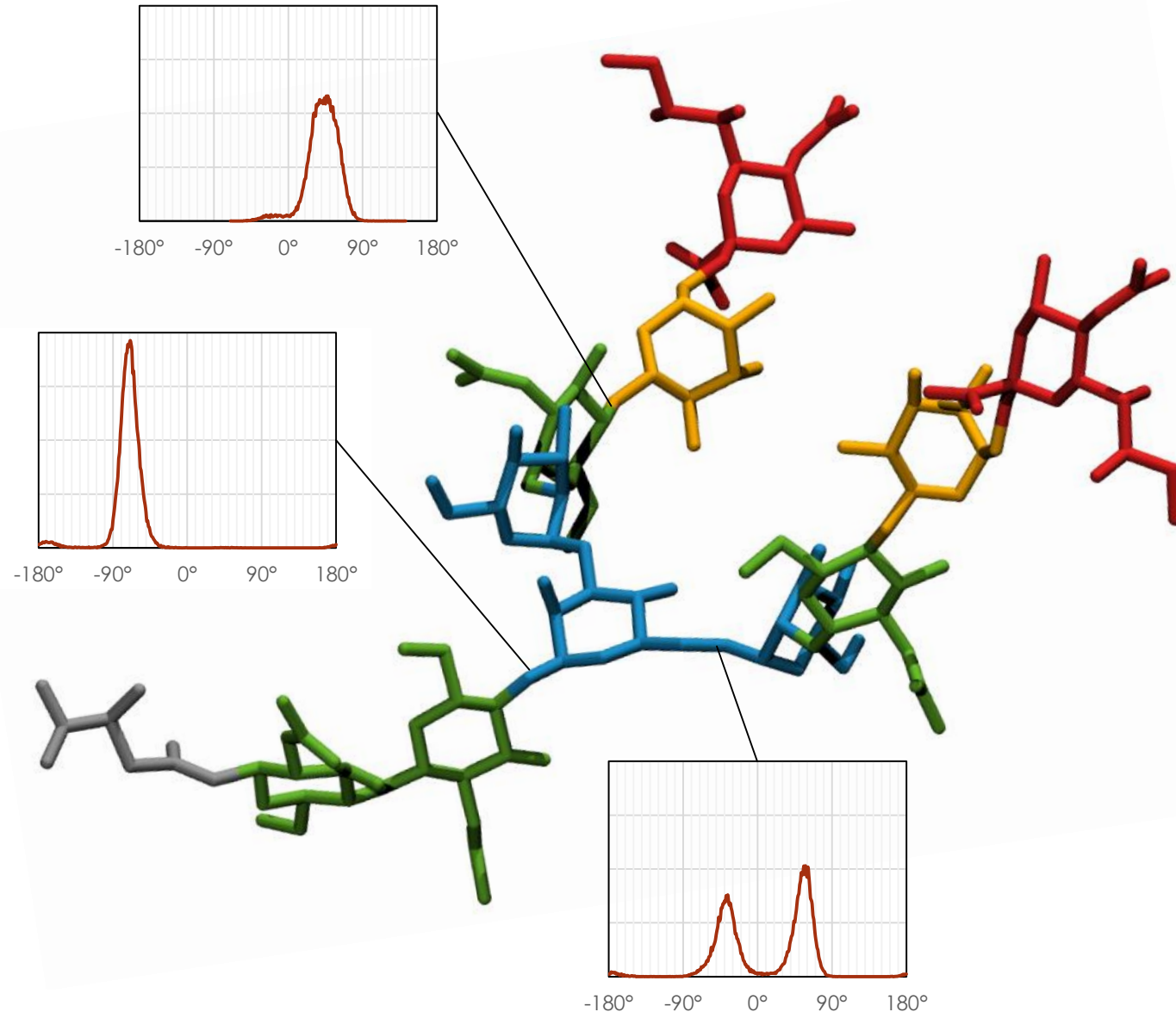


Cluster #2 with SA = cluster #1 without SA

Cluster #1
Without SA
28 %

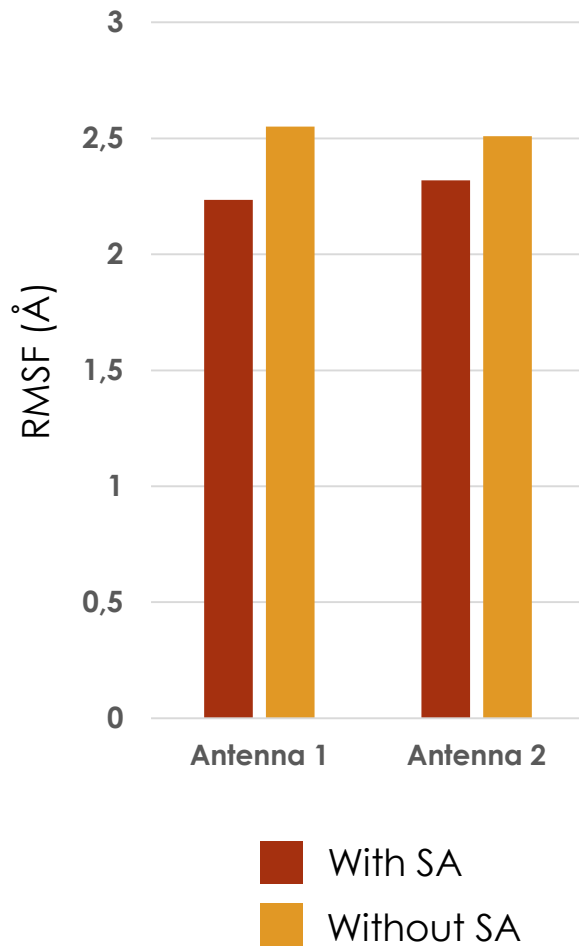


Results analysis : dihedral angle

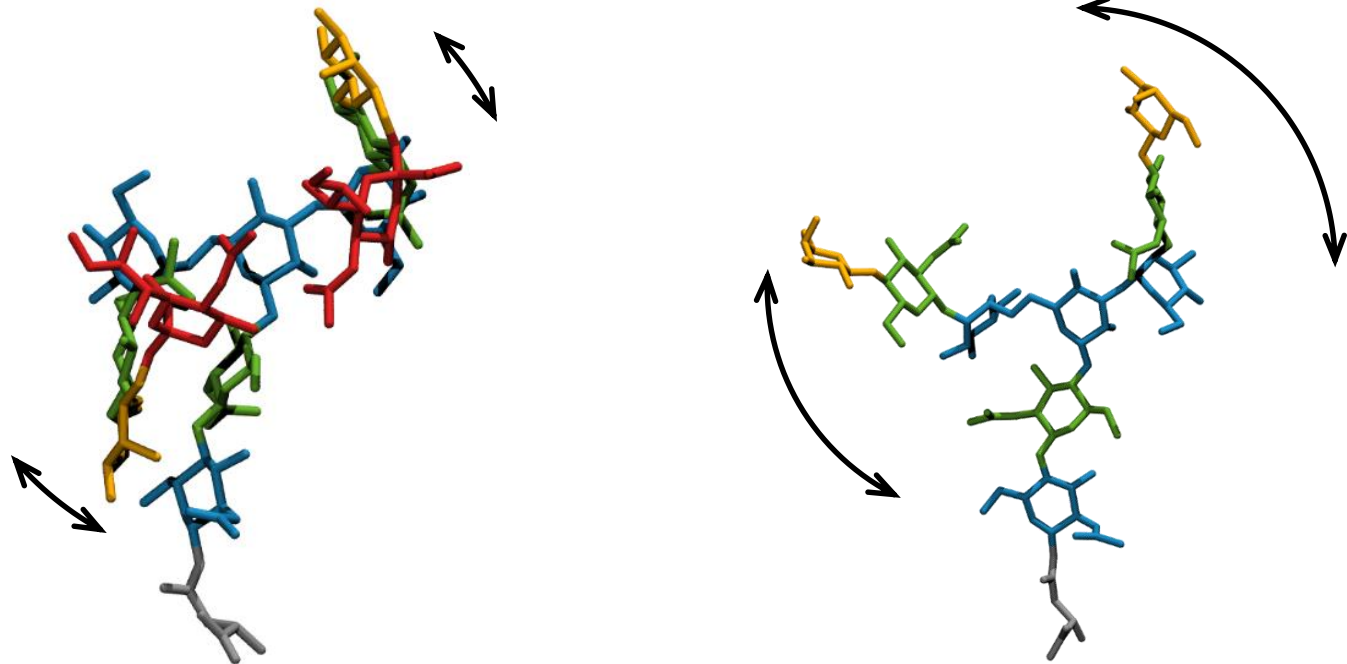


- Objective: identify flexibility points by measuring phi, psi and omega dihedral angles.
- 3 angle types:
 - 1 single thin pic: less flexibility
 - 1 single large pic: more flexibility
 - More than 1 pic: many conformations
- Antenna fixation sites are more flexible
- Removing sialic acid can change dihedral angle

Results analysis : antenna movements (RMSF)

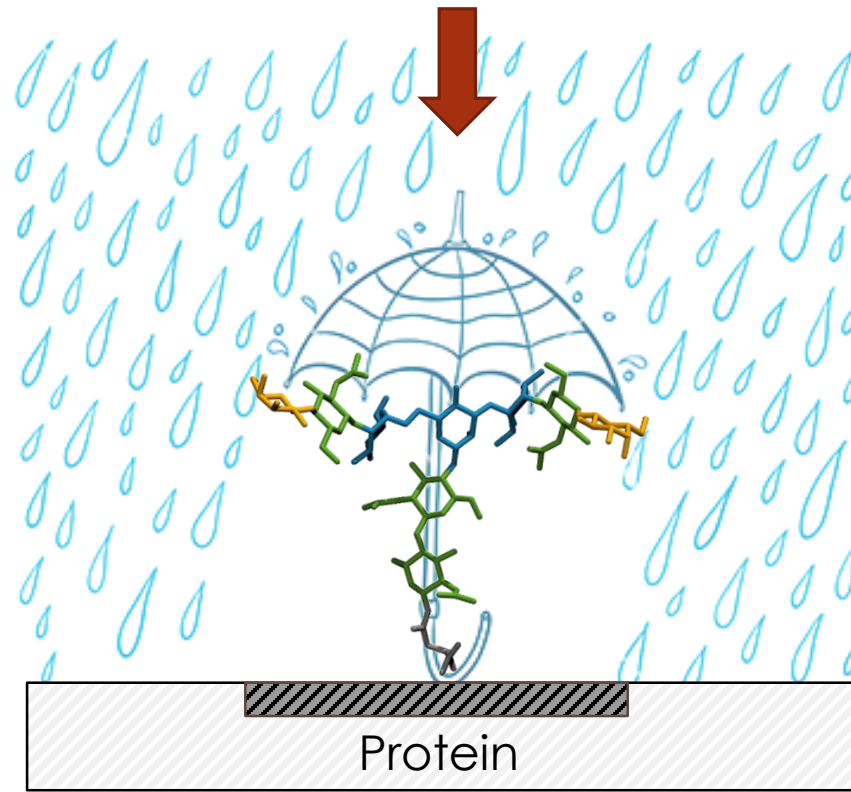


- Objective: measure the motion of each antenna
- RMSF : root mean square fluctuation
- Removing sialic acids increase antenna motions



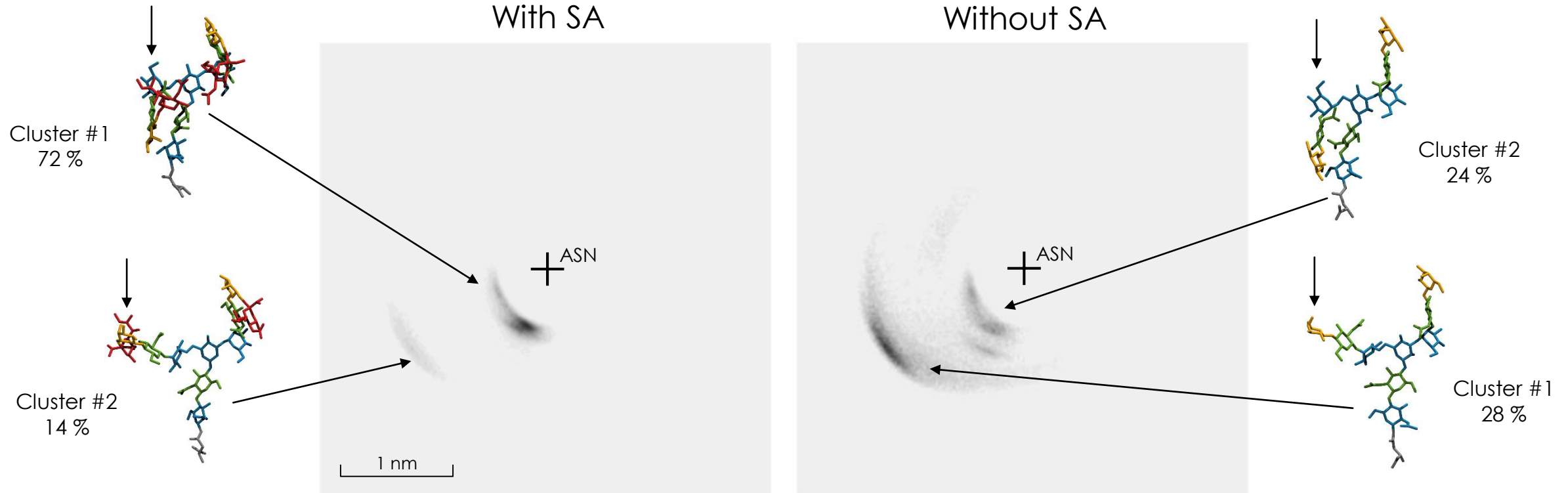
Results analysis : umbrella visualisation

- Objective: visualise the covered zone by each antenna



Results analysis : umbrella visualisation

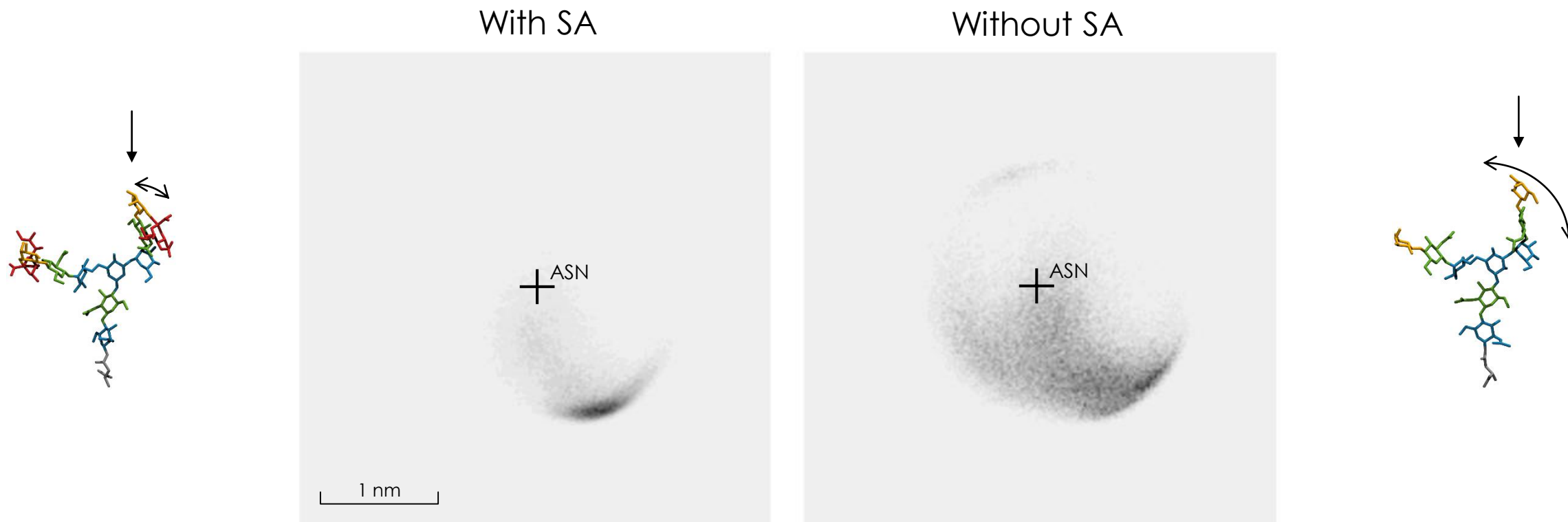
Antenna 1



Antenna 1 displays the 2 previously identified clusters

Results analysis : umbrella visualisation

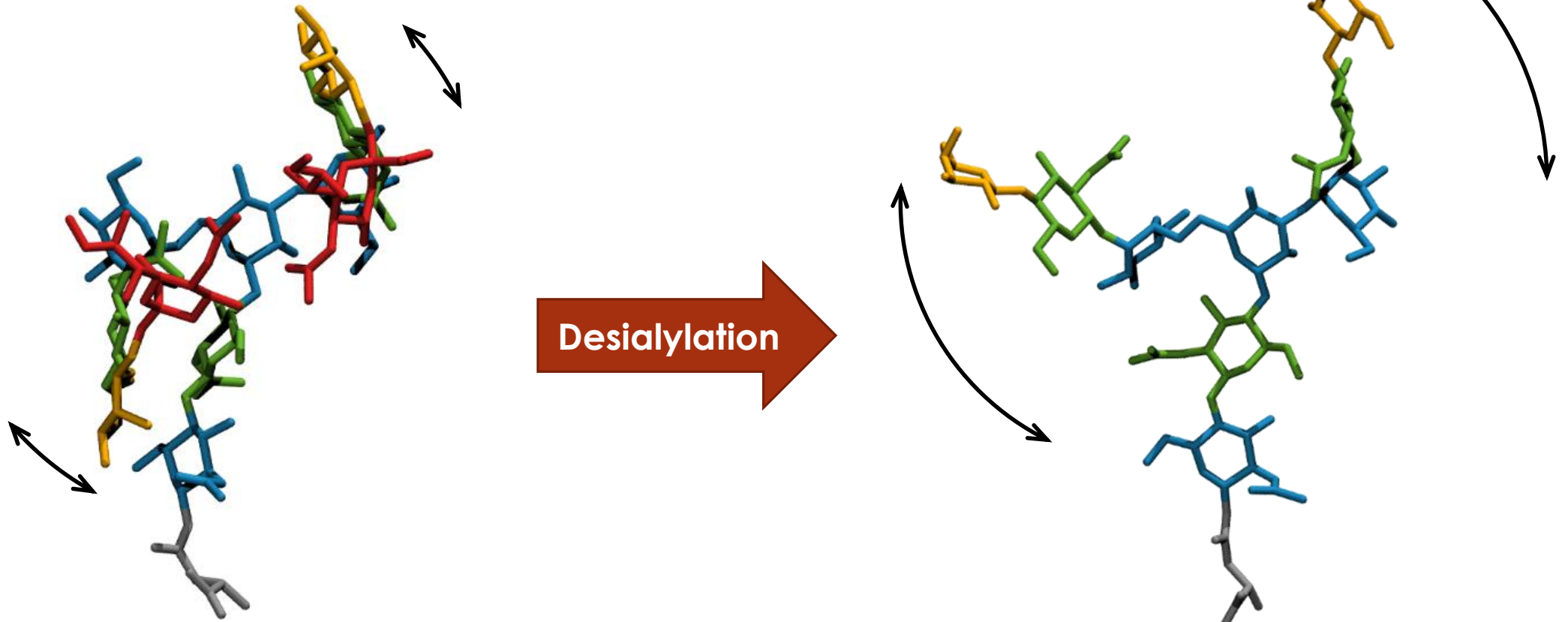
Antenna 2



Antenna 2 is more flexible when sialic acids are removed

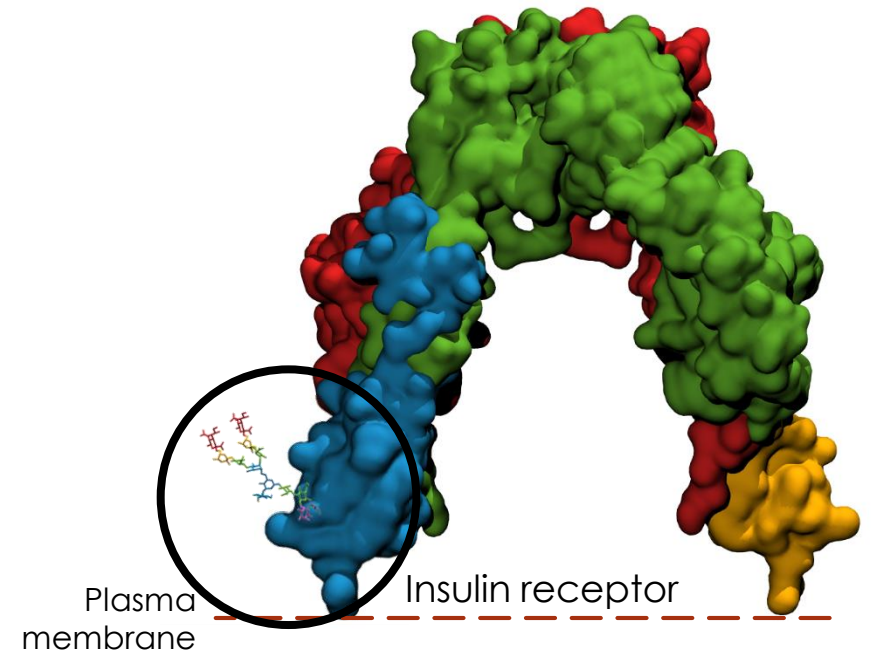
Conclusion

- Impact of desialylation on glycan chains
 - Changes average conformations
 - Increases antenna flexibility
 - Covered zone displays different profiles



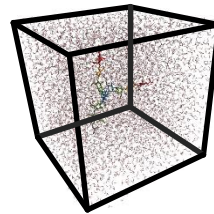
Further work

- Impact of desialylated N-glycan chains on insulin receptor
- Objective: show conformational modifications of IR when SA are removed
 - Structure
 - Insulin binding site
 - Dimerization
 - Insulin receptor activity
- N-glycan chain linked to the receptor
- New molecular dynamic simulations

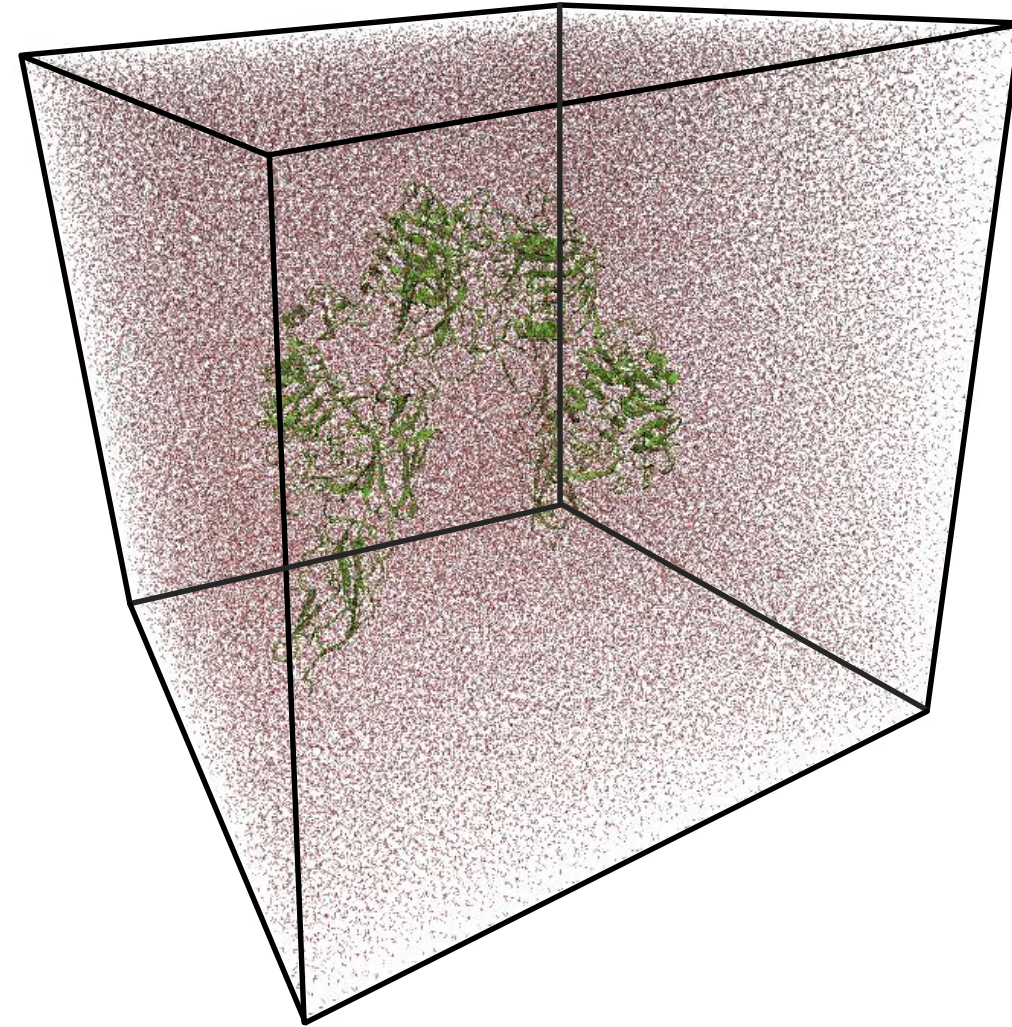


Further work

- A whole new challenge :
 - Insulin receptor: **18** potential glycan sites
 - Addition of plasma membrane
 - **HUGE** system
 - 620,000 atoms
 - 20 nodes
 - 320 cores
 - 130 h for a 500 ns simulation
 - Terabytes of data

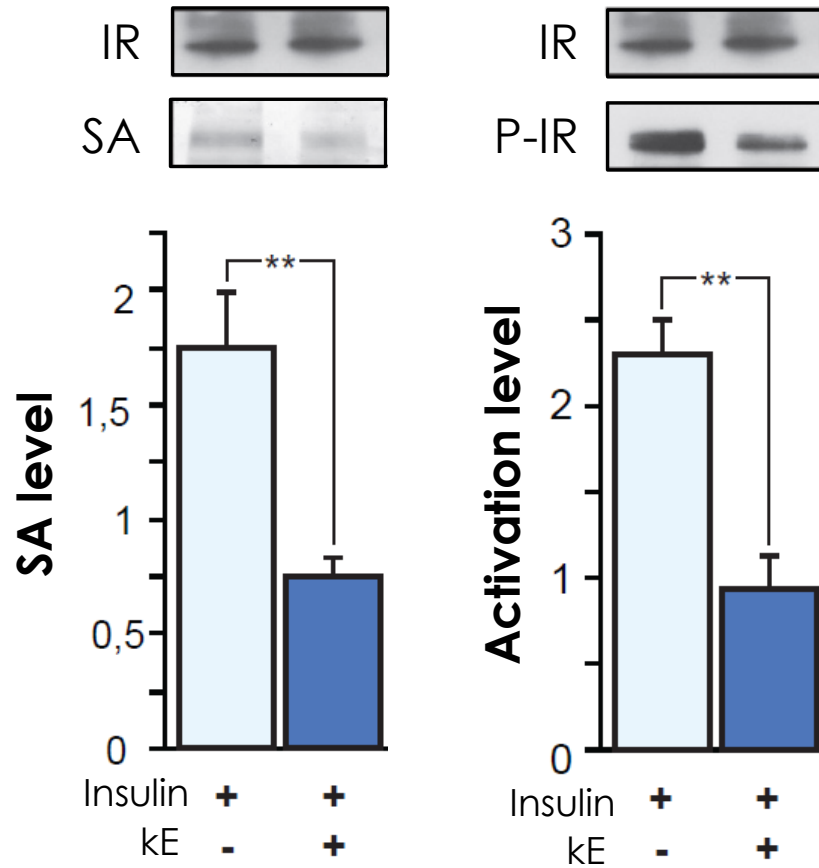


13,000 atoms



620,000 atoms

Further work



- kE : induce the removal of sialic acids from insulin receptor
- P-IR : phosphorylation \approx activation of the IR
- Correlation between insulin receptor sialylation and activity
- **We need *in silico* studies to understand this mechanism at a molecular level**

Acknowledgements

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Team 2 "Matrix aging and Vascular remodelling"
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