Application of protein X-ray crystallography to molecular glycobiochemistry

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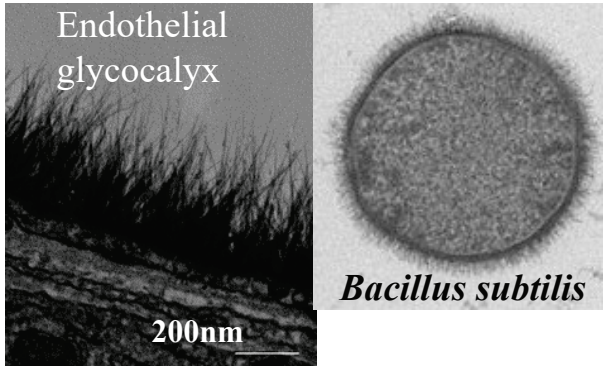
« Structural Glycosciences » Summer School

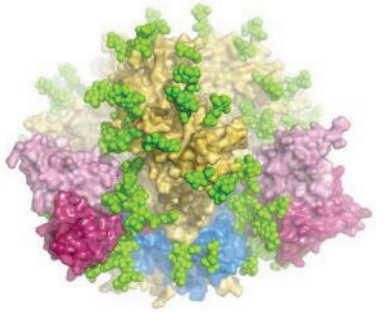
CERMAV, Grenoble 3rd July 2018

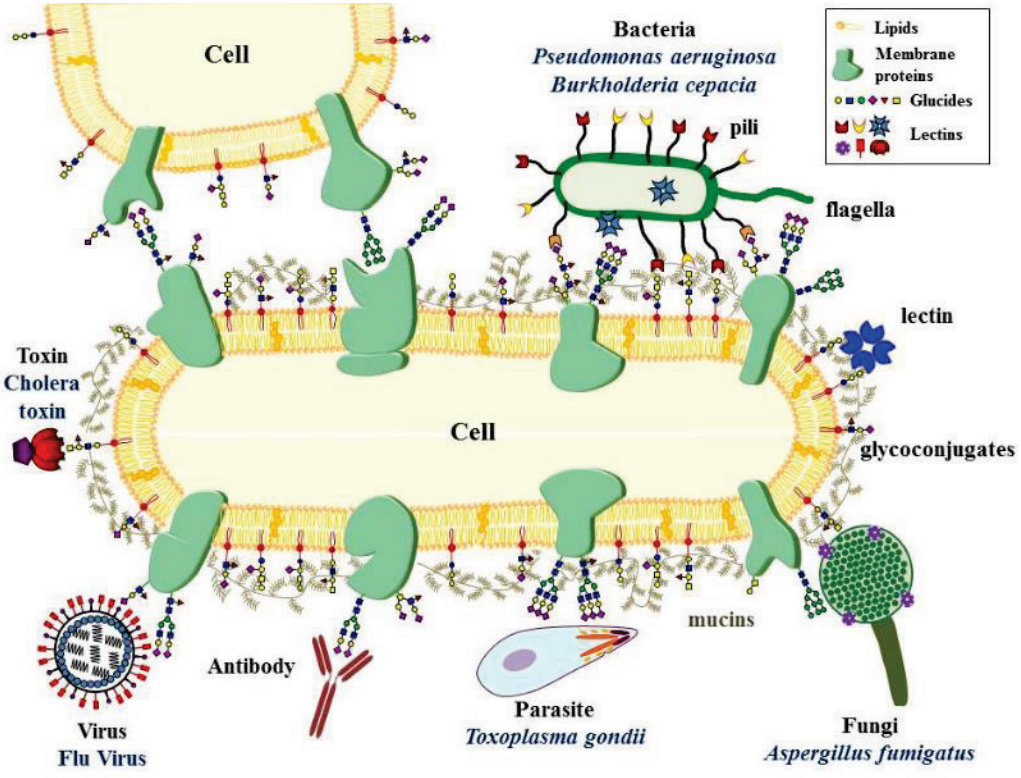
The “sweet side” of the cell surface

- Every cell surface is covered by **glycoconjugates**
- 1st cell information received and sent: **glycocode**
  - Essentials in cell identity, recognition and signaling



CryoEM, HIV GP120 glycan shield





The Scripps Research Institute, Wikipedia, Varrot A & Imberty A Curr Op Struct Biol 2008 18:567

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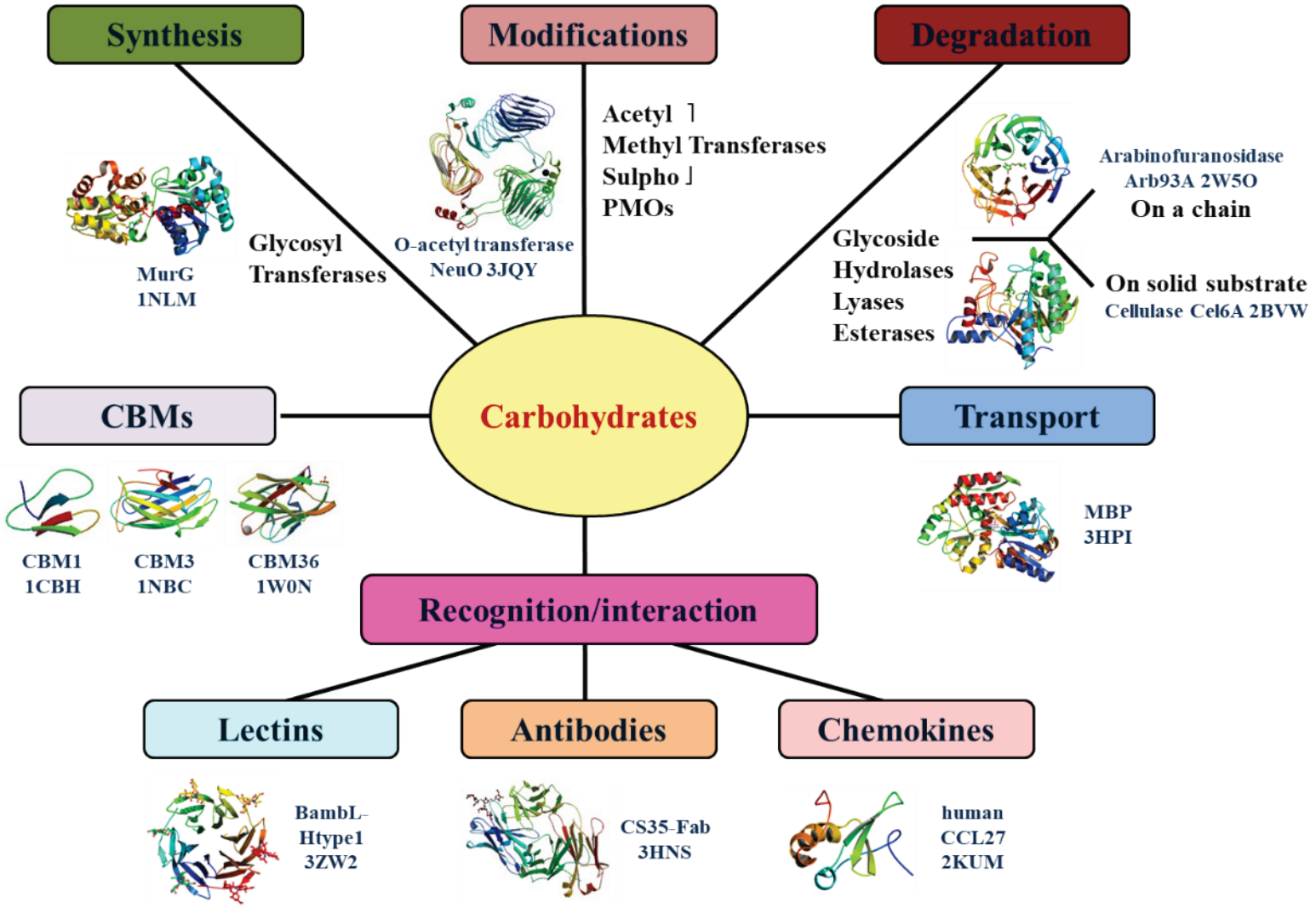
Protein-sugar interactions



Sarkar A & Pérez S Structural Glycobiology CRC Press 2012 71

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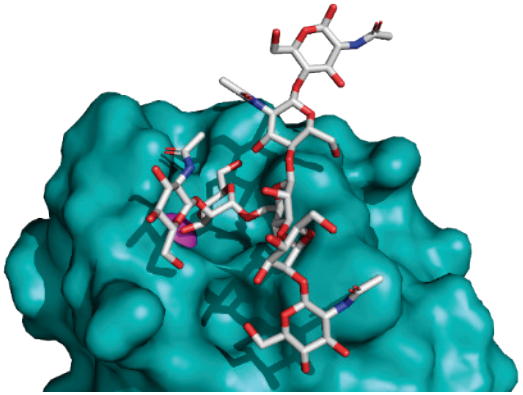
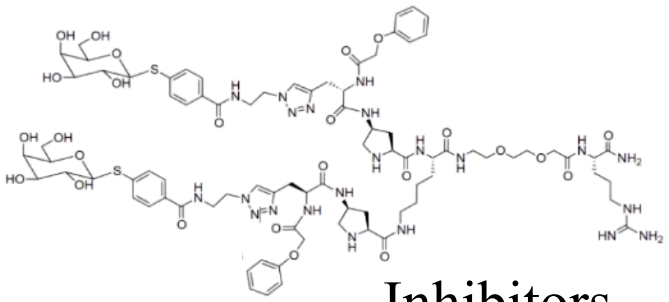
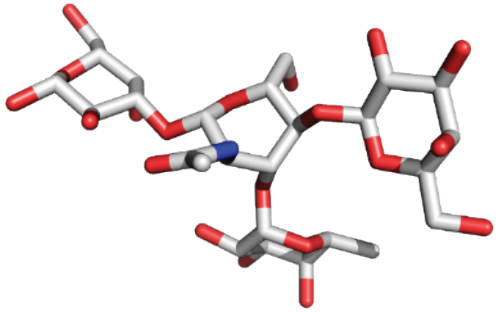
Which info do you get from crystallography?



Binding site architecture



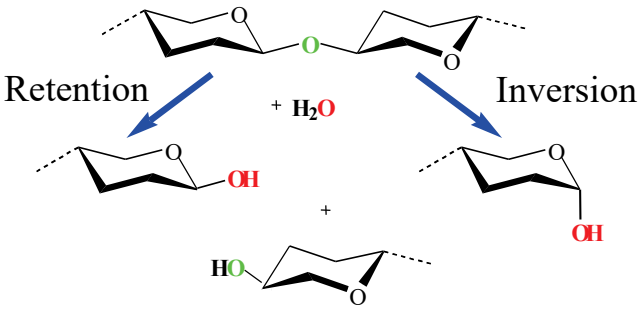
Inhibitors



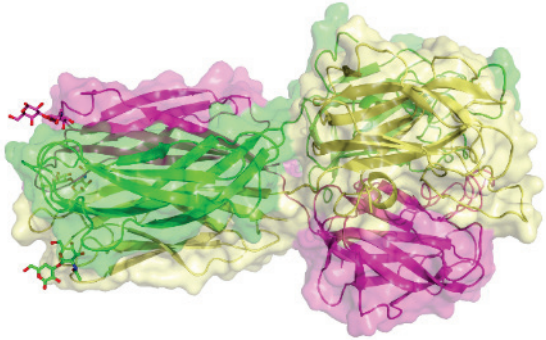
Ring conformation

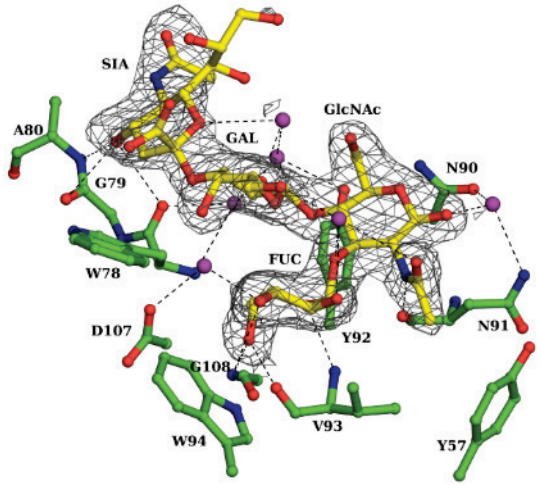
structure

Mechanism



Multivalency





Interactions

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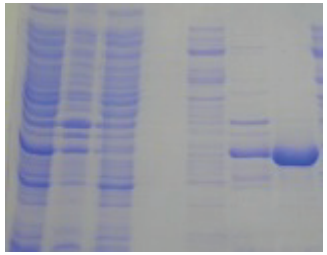
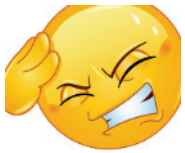
Protein X-ray crystallography

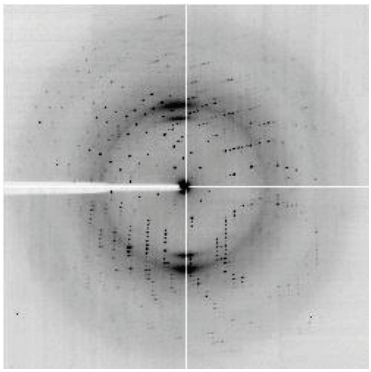
Expression
Purification



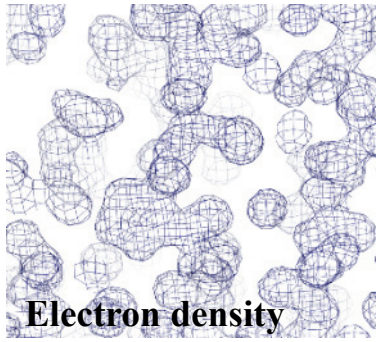


Crystallization

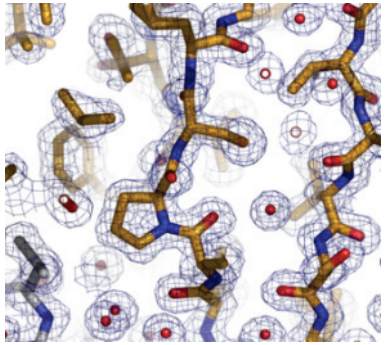
X-ray
diffraction



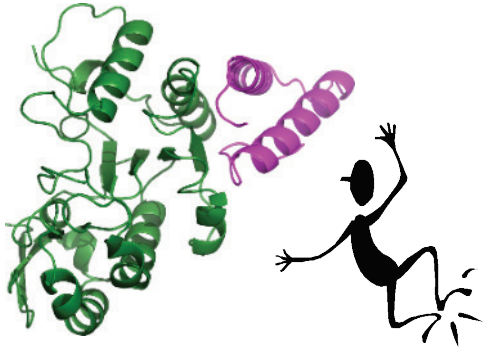
Phasing



Modelling



Refinement



➢Advantages

- High resolution structure
- Study of complex at the atomic level

➢Disadvantages

- Molecules in solid-state environment
- Require crystals

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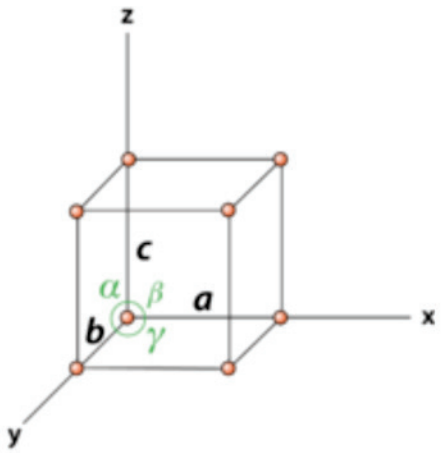
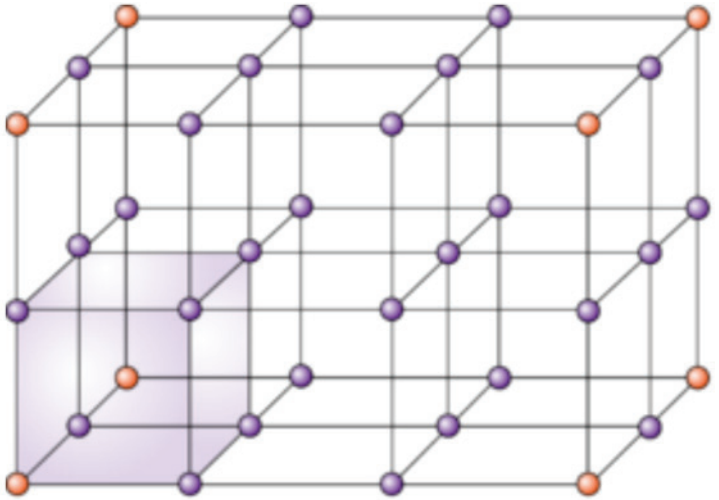
What is a crystal?



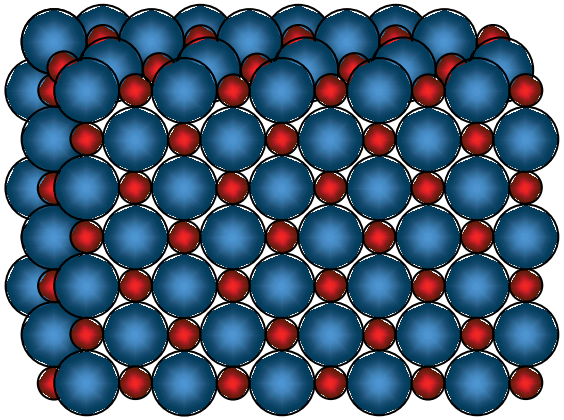
translation



Unit cell

Crystal lattice

NaCl



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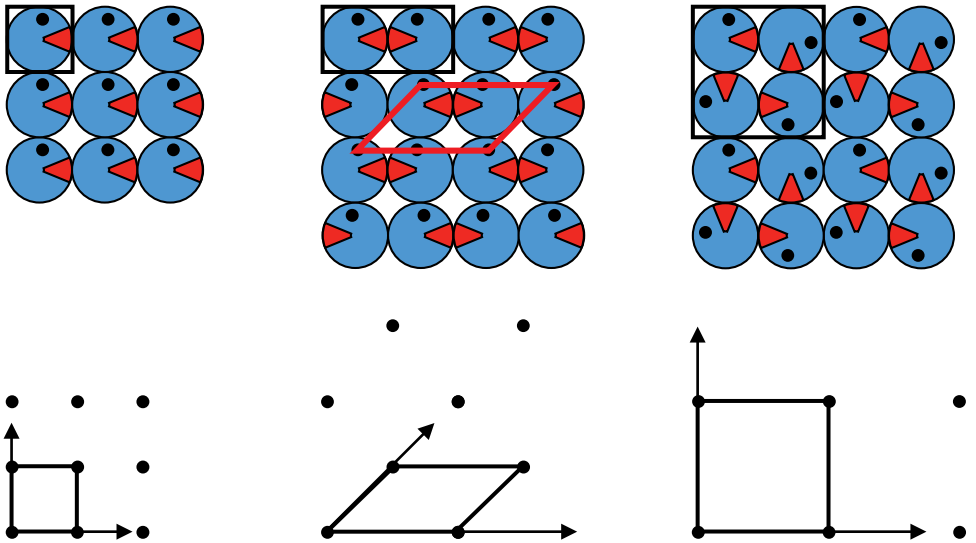
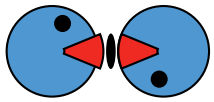
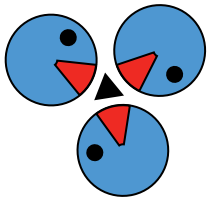
Crystal packing





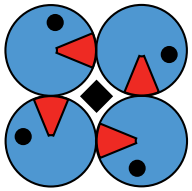
2 fold axis

2_1



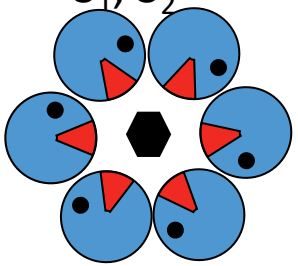
3 fold axis

3_1, 3_2



4 fold Axis

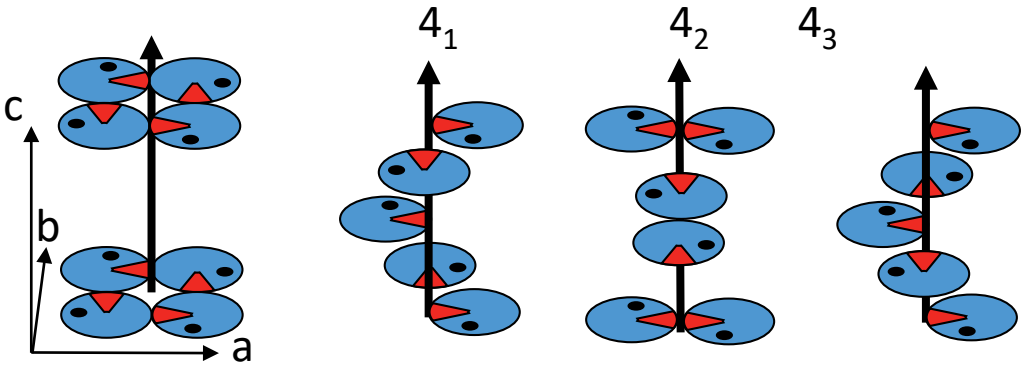
4_1, 4_2, 4_3



6 fold axis

6_1, 6_2, 6_3, 6_4, 6_5

Screw axis



65 spacegroups for protein crystals

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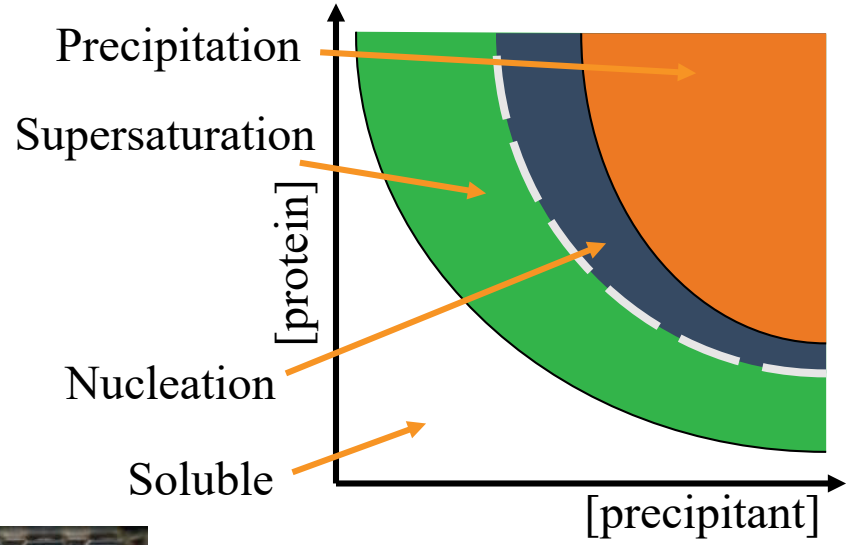
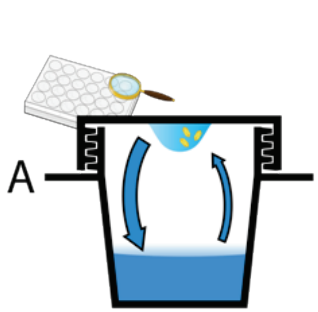
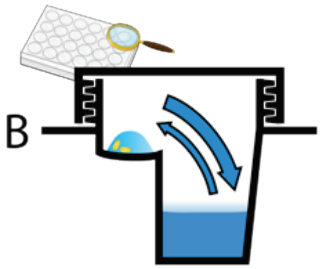
Protein crystallogenesis

- Need pure and homogenous protein
- Empirical
  - pH, buffer, temperature, [salt], [precipitant] dependant
- Precipitants
  - High salt concentration
  - Alcools or volatil compounds
  - Organic polymers
- Manual or robotized
- Vapor diffusion mostly used

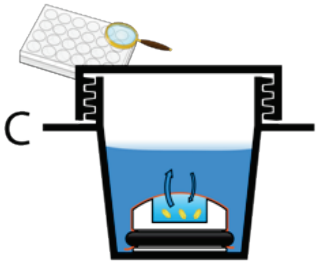




Hanging drop



Sitting drop



microdialysis

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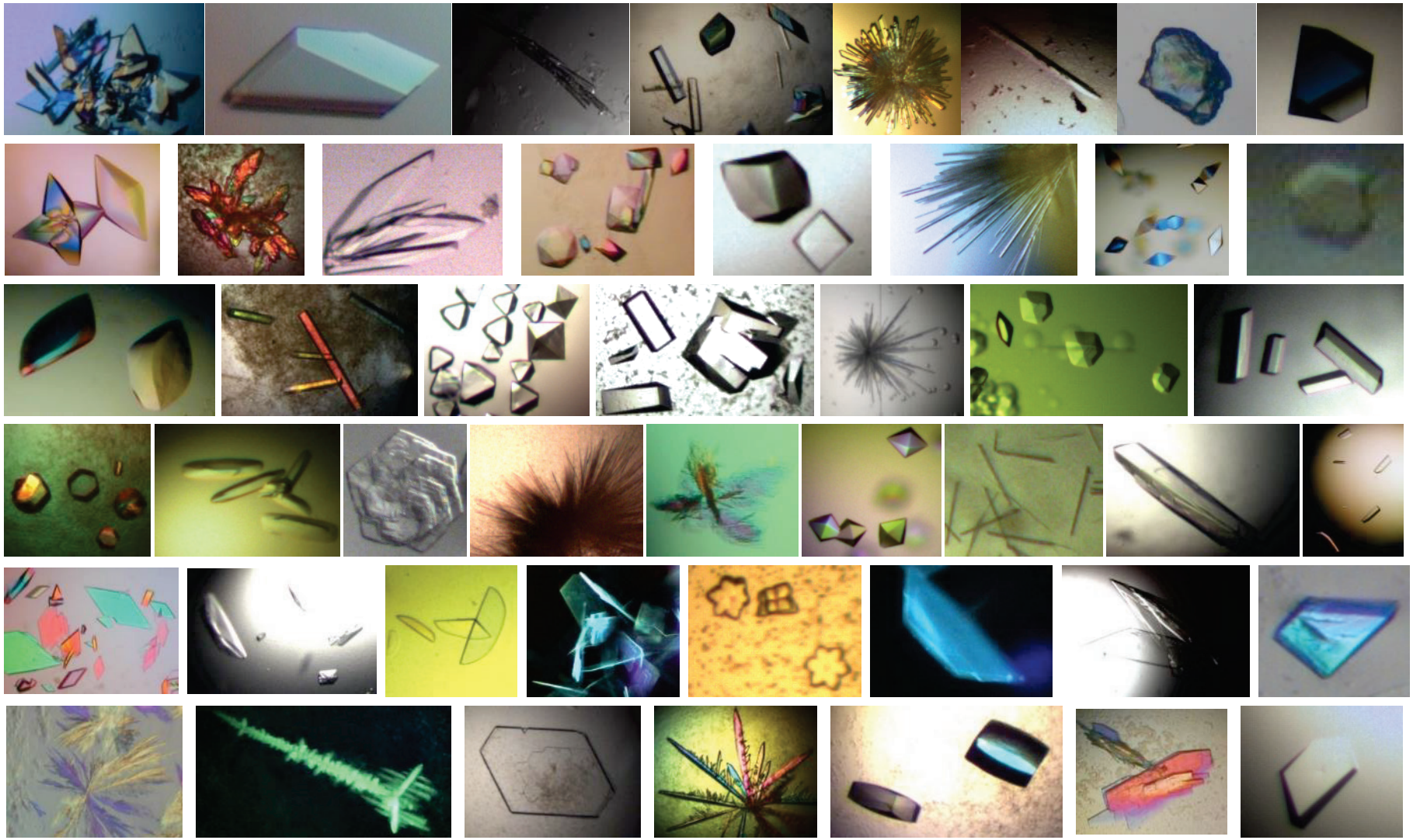
CERMAV protein crystals



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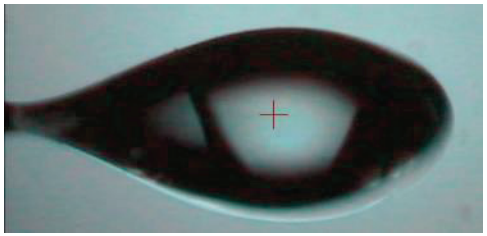
How to obtain crystals of protein complexes with ligand

Soaking

- Add the ligand to the reservoir solution
  - Add concentrated solution to limit dilution
  - Transfer the crystal in solution with ligand
  - Add bit of powder

- Try different concentrations and soaking time
- Can soak the crystal after data collection
  - For resistant crystals diffracting well
  - BambL: lectin from *Burkholderia ambifaria*
    - Soaking with 2 mM methyl-α-L-fucopyranoside (MFU)
      - Data collected at 1.3 Å BM14, ESRF: empty
    - Resoaked with 20mM
      - Data collected at 1.3 Å BM14: still empty



Audfray A JBC 2012 287:4335

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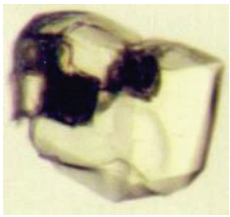
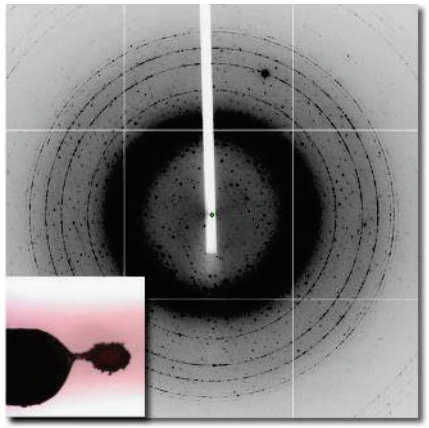
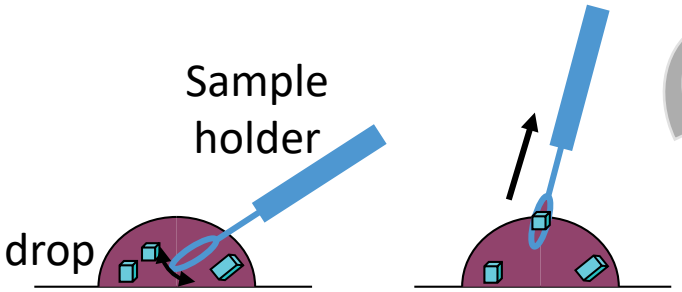
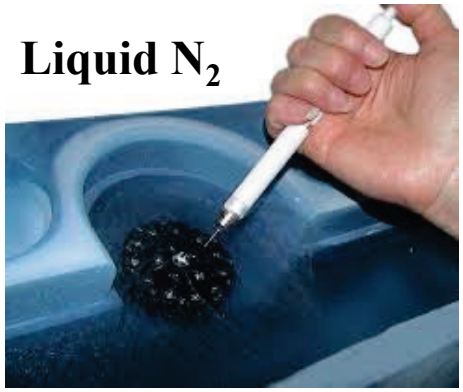
Soaking while freezing

- Freezing limits radiation damages
- Add ligand to the cryoprotectant solution
  - Less chance of replacement by glycerol, ethylene glycol and MPD

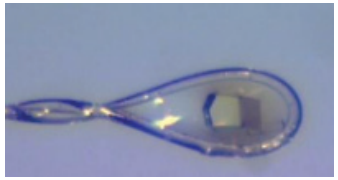


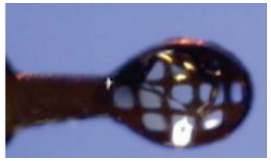






Hampton **Litholoop Molecular Dimensions** **Mitigen**





https://www.youtube.com/watch?v=FENUWRYXMOM

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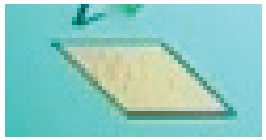
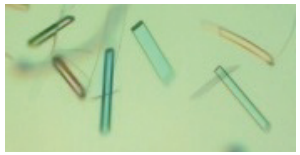
Cocrystallization

- Excess of pure ligand > 2X K_D
- Put the ligand in reservoir
- Or preincubation with the protein
  - Limit protein dilution: >1/10
  - Set duration and temperature
  - Easily reproducible
  - Require little ligand
- Can have to screen for each complex: LecA

Gal	**Galα1-2Galβ-O-Me**	**Galα1-3Galβ1-4Glc**	**Galα1-6Glc**	**Inhibitor**
1.5 M $(NH_4)_2SO_4$ pH 4.7, 5% MPD, 2% glycerol	20% PEG6K 1 M LiCl 0.1 M NaAc pH 4	10% PEG 5Kmme 25 mM KSCN 0.1 M NaAc pH 4.6	20% PEG 2Kmme, 0.2 M KBr 0.1 M NaAc pH 4.6	0.8 M Li_2SO4 pH 4.5
				

Blanchard B JMB 2008 383:837, Blanchard B Proteins 2014 82:1060; Novoa A Angewandte Chemie 2014 53:8885

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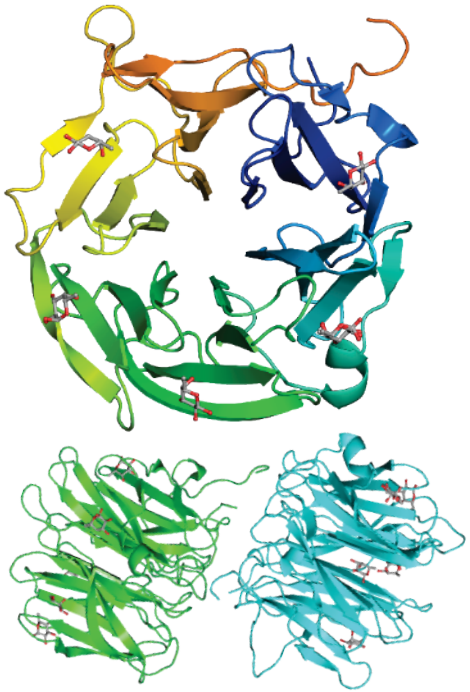
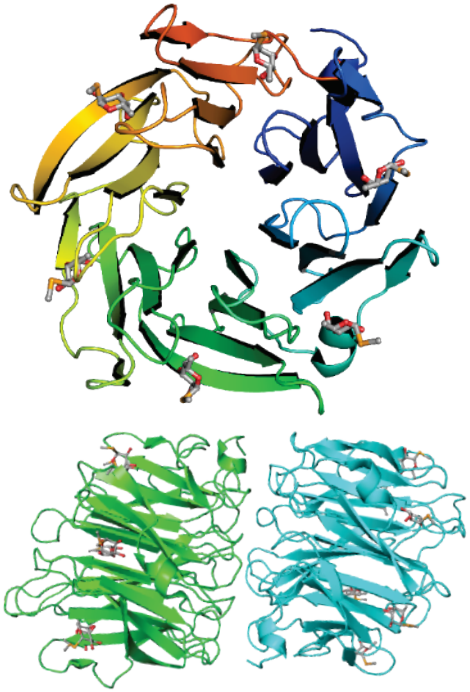
Phasing: molecular replacement

- Have a good model from homologous protein
  - Sequence identity > 25%
  - Good conservation secondary structure: difficult for β-sheets

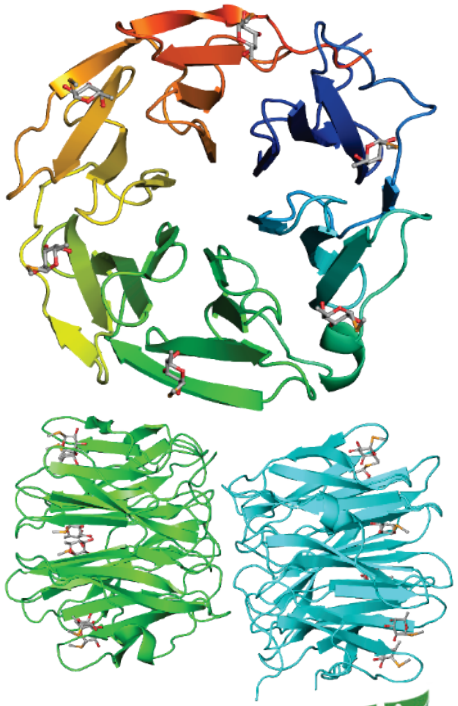
AAL/1OFZ/Hg
Aleuria aurantia



AFL/4AGI/SFU (35%)
Aspergillus fumigatus



Trfbl1/not deposited/SFU (32/43%)
Terfezia boudieri



Wimmerova M, JBC 2003 278:27059; Houser J Plos One 2013 8:e83077; Basheer S, unpublished

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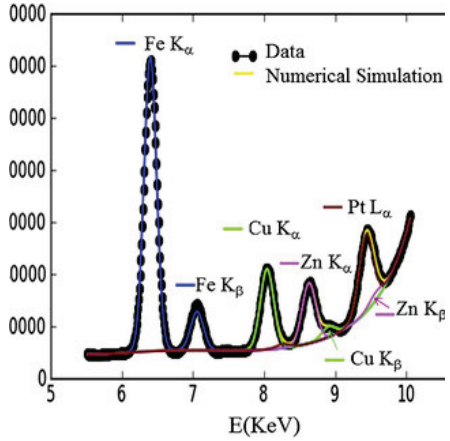
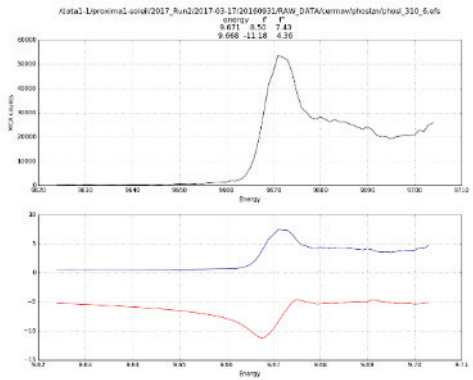
Phasing: isomorphous or anomalous

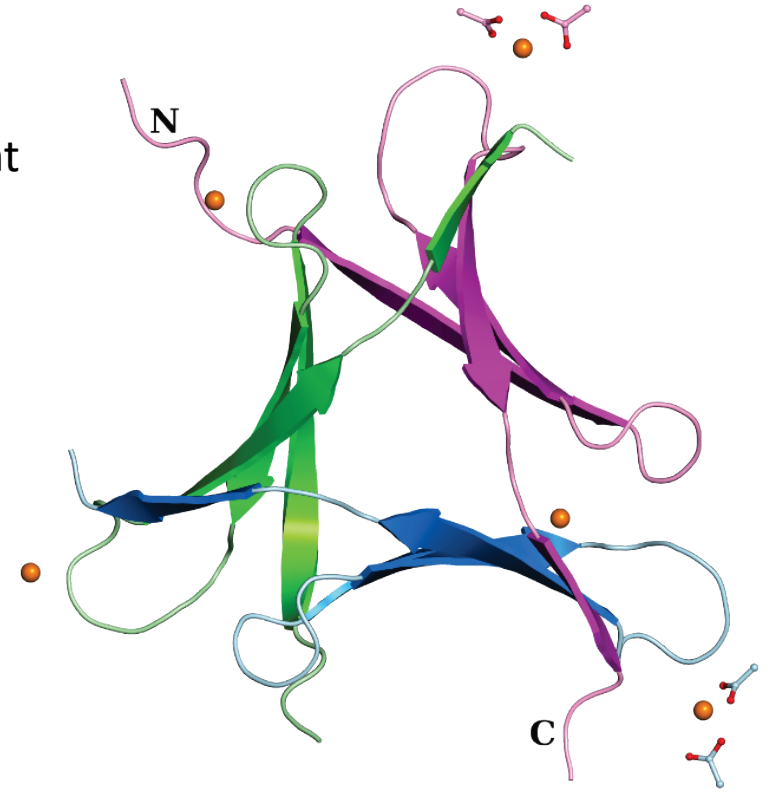
- Look in your crystallization conditions
  - PhoSL from *Pholiota squarrosa*
    - 300 mM Zinc acetate, 0.1 M Imidazole-HCl pH 6-7



X-ray fluorescent
XRF scan



Absorption
scan



Cabanettes A Angewandte Chemie 2018 in press

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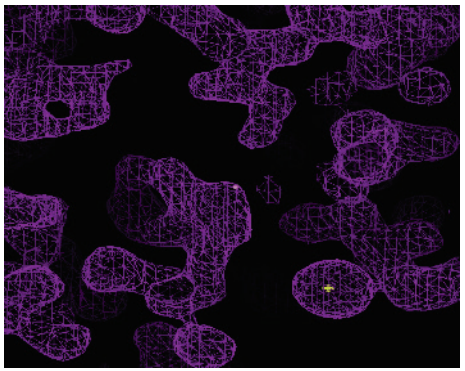
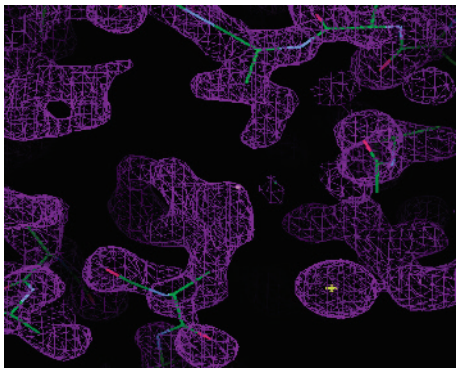
Phasing: isomorphous or anomalous-2

- Use selenomethionin protein, heavy metals
- Use ligand with heavy atoms (Se, Br, S, F)

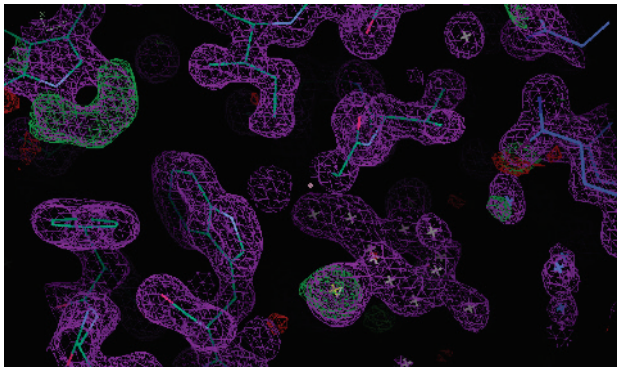
Se peaks



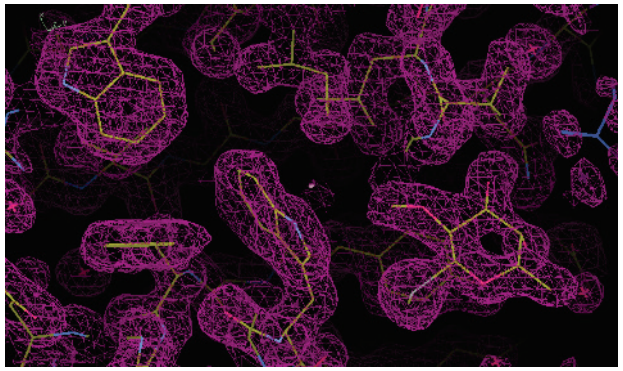
PolyAla building

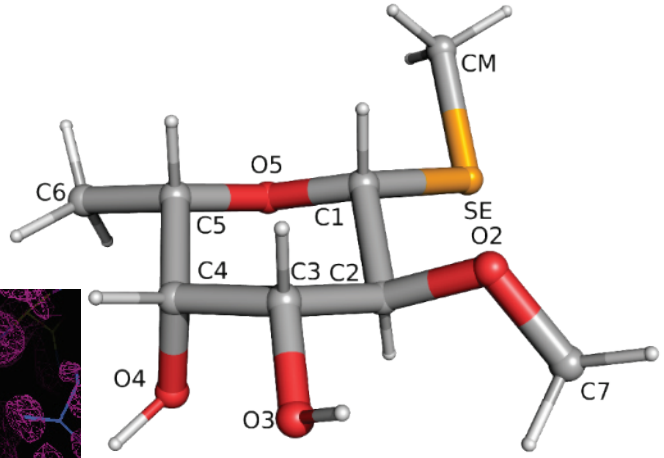


Sequence assigned



Final





2-O-Methyl-β-methyl-L-selenofucoside
2MeSeFuc

Buts L Acta Cryst D 2003 59:1012-1015, Sulak O Structure 2010 18:59-72; Sommer R Beilstein J Org Chem 2016 12:2828

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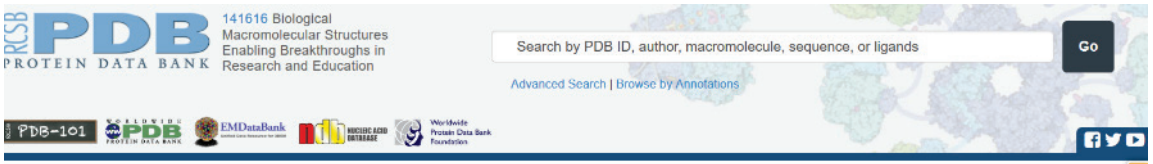
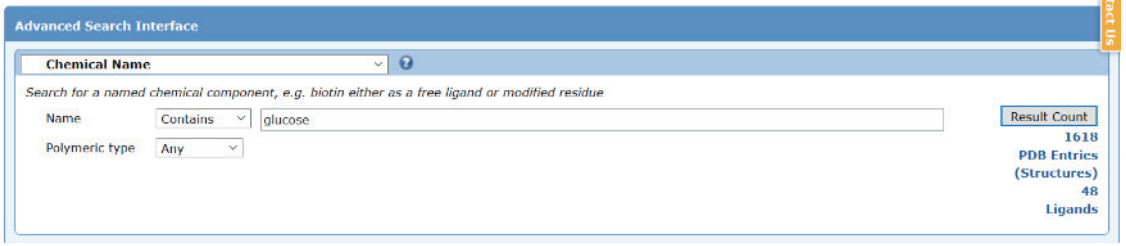
Refinement

- 3-letter code for each monosaccharide and protein residues
  - Check ligand database
    - HIC-Up: http://xray.bmc.uu.se/hicup/
    - PDB: http://www.pdb.org/pdb/home/home.do





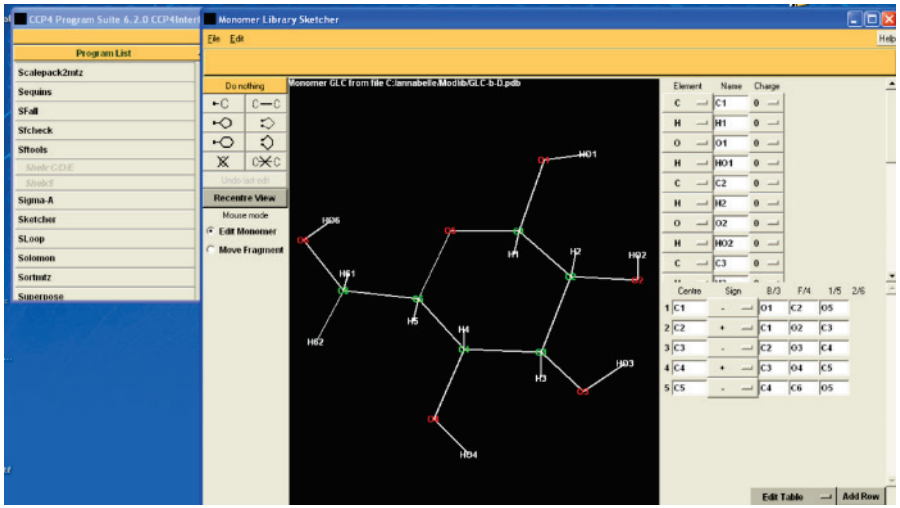
Ligand Structure	ID / Formula / Name	Structures with Specific Ligands
	GLC C6 H12 O6 ALPHA-D-GLUCOSE	686 PDB Structures contains GLC (1A47,1AC0,1ACZ ...)
	GLD C6 H12 O4 4,6-DIDEOXYGLUCOSE	9 PDB Structures contains GLD (1E3Z,1UA7,1UH3 ...)
	GLF C6 H11 F O5 1-FLUORO-ALPHA-1-DEOXY-D-GLUCOSE	6 PDB Structures contains GLF (1CXL,3IJ7,3SCO ...)
	GLO C6 H12 O6 D-GLUCOSE IN LINEAR FORM	16 PDB Structures contains GLO (1AC0,1EZ9,1FBO ...)

- Main monosaccharide have same code than common name
- 1 code for each anomer
  - α-D-Glucose: GLC
  - β-D-Glucose: BGC

Cermav

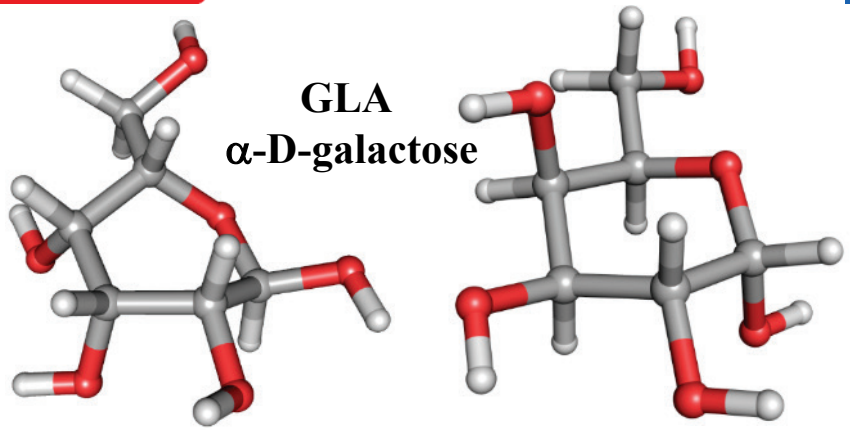
Refinement : ligand library

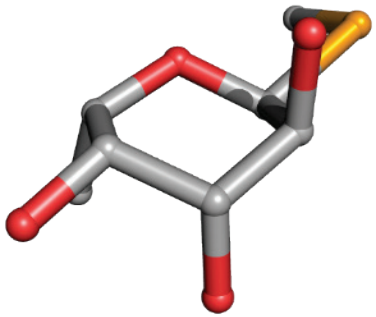
- Contains atom type and name, tree, distances, angles, torsions, chiral center, planes
- Creating new ligand
  - Old: Jligand or Sketcher
  - New: Acedrg



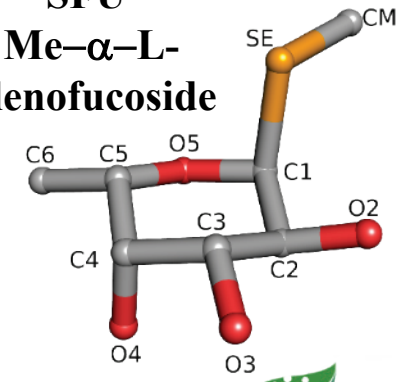
Check library is correct: no distorsion

GLA
α-D-galactose





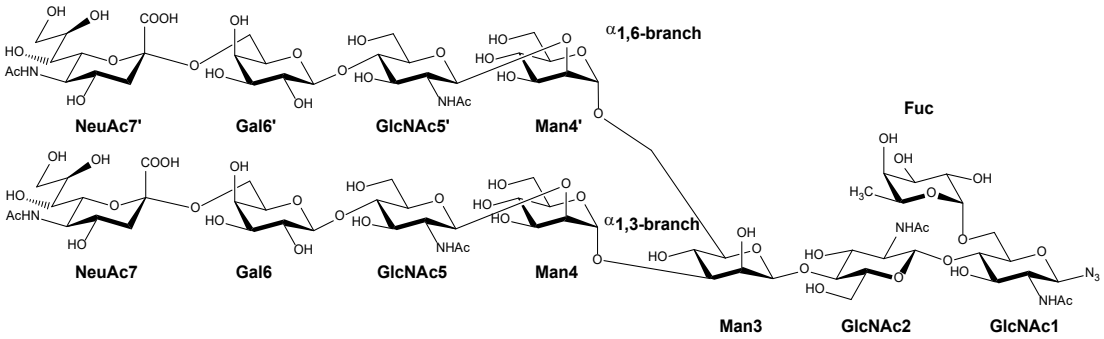
SFU
Me–α–L-selenofucoside

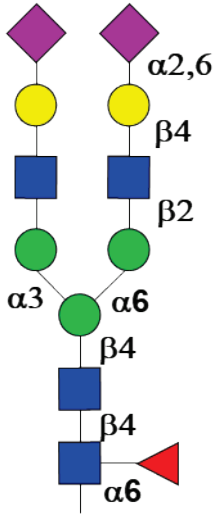


Cermav

Refinement

- Add description of the linkages
  - code could depend on program






```
LINKR        O6   LOG A 102      C1   FUC A 101      BETA1-6
LINKR        O4   LOG A 102      C1   NAG A 103      BETA1-4
LINKR        O4   NAG A 103      C1   BMA A 104      BETA1-4
LINKR        O6   BMA A 104      C1   MAN A 105      ALPHA1-6
LINKR        O2   MAN A 105      C1   NAG A 106      BETA1-2
LINKR        O4   NAG A 106      C1   GAL A 107      BETA1-4
LINKR        C2   SIA A 108      O6   GAL A 107      SIA-GAL
LINKR        O3   BMA A 104      C1   MAN A 109      ALPHA1-3
LINKR        O2   MAN A 109      C1   NAG A 110      BETA1-2
```

- Programmers do not know how to deal with L-sugars

Cabanettes A Angewandte Chemie 2018 in press

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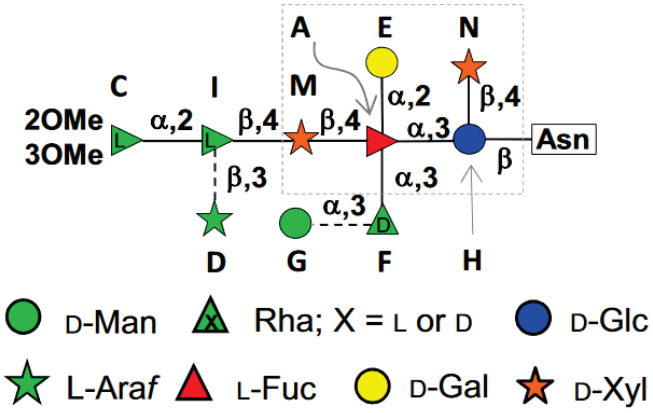
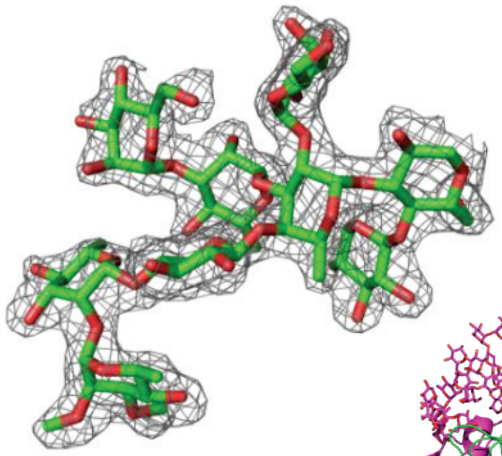
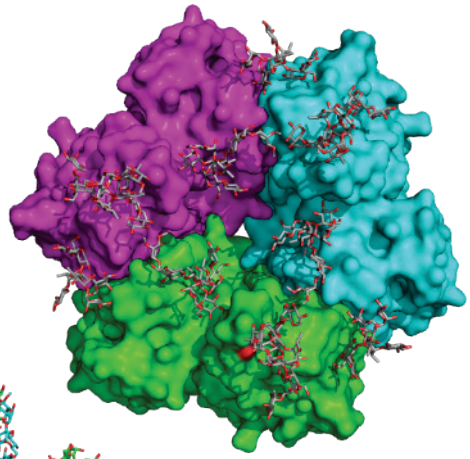
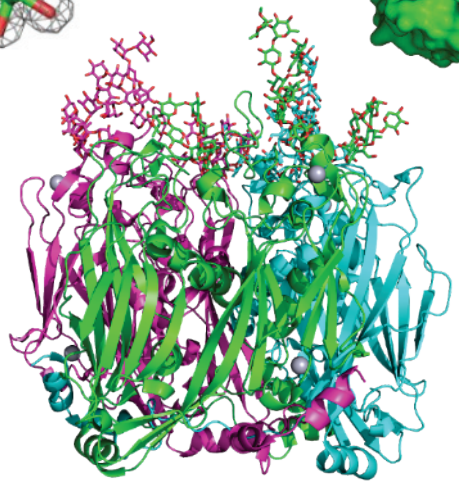
Can be tricky for non glycobiologist

- Major capsid protein (Vp54) of chlorovirus PBCV-1
  - X-ray 2002: try to fit classical N-glycan
  - Sugar NMR 2013: highly complex N-glycosylation
  - X-ray revised in 2017 + modelling









4 glycosylation sites

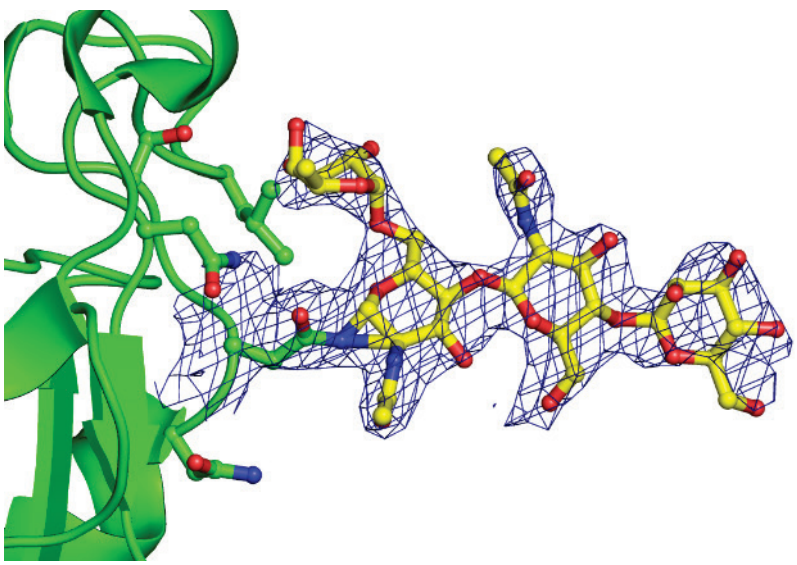
H = BGC
N/M = XYP
F = XXR
I = RM4
A = FUC
E = GLA
G = MAN
C = 7CV

Nandhagopal N PNAS 2002 99:14758, De Castro C PNAS 2013 90:2840; De Castro C PNAS 2017 115:E44

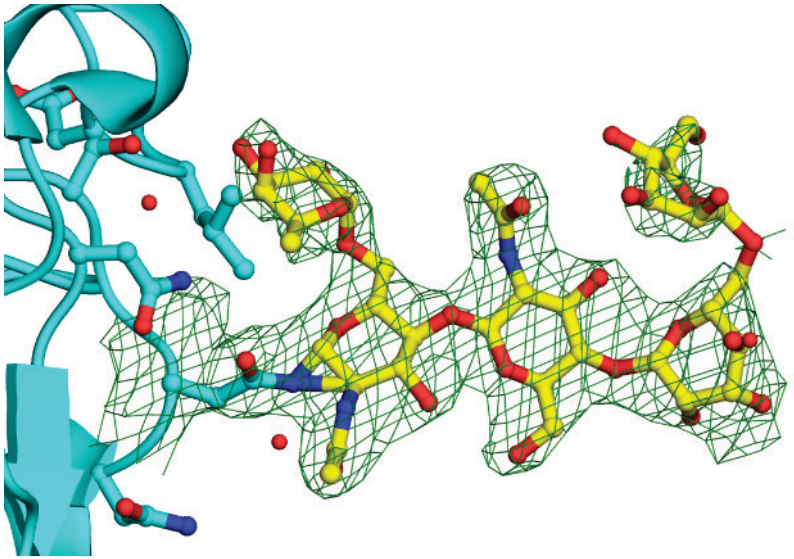
Cermav

Carbohydrate validation

- Do not overfit at low resolution



2.8 Å

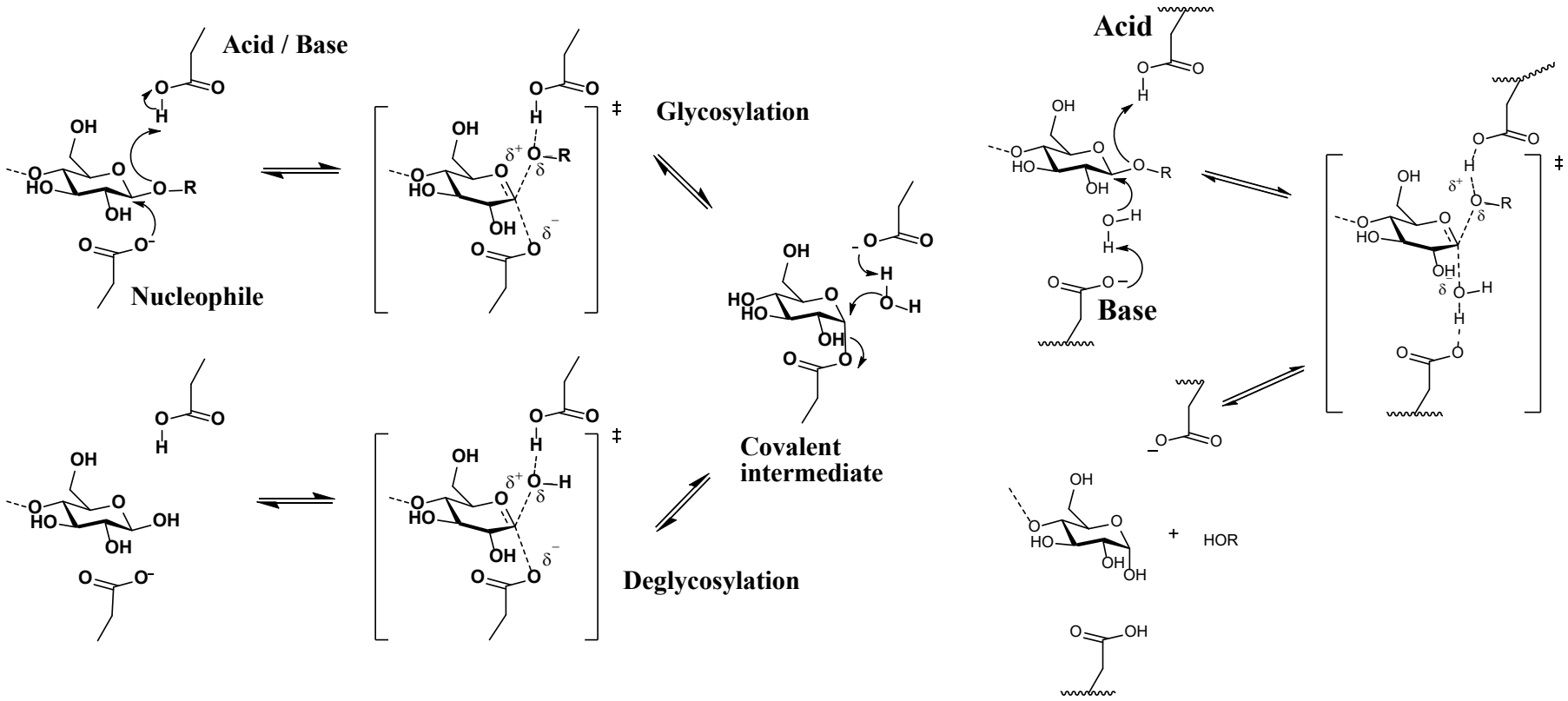


- Check nomenclature and ring conformation
  - PDB-care
  - Privateer: ccp4

Lütteke T BMC Bioinformatics 2004 5:69; Agirre NSMB 2015 22:833; Agirre J Acta Cryst D 2017 D73:171

Cermav

Glycoside hydrolases mechanism



- Try to trap each step by X-ray crystallography
  - Use specific ligand, mutated protein, inactive pH

Koshland Biol Rev 1953 28:416

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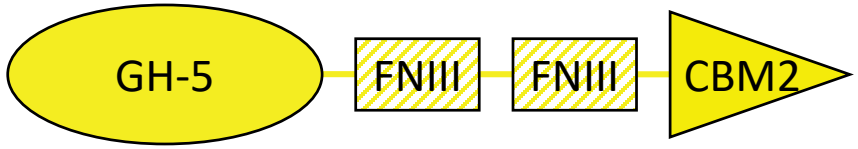
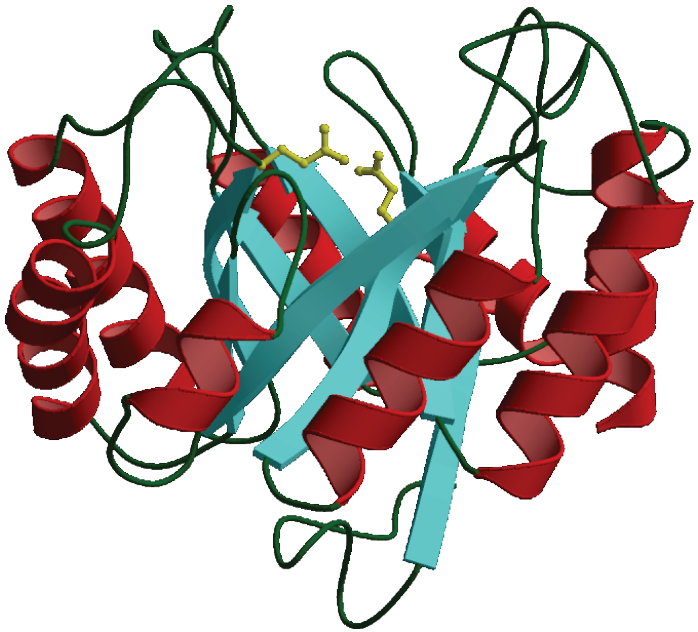
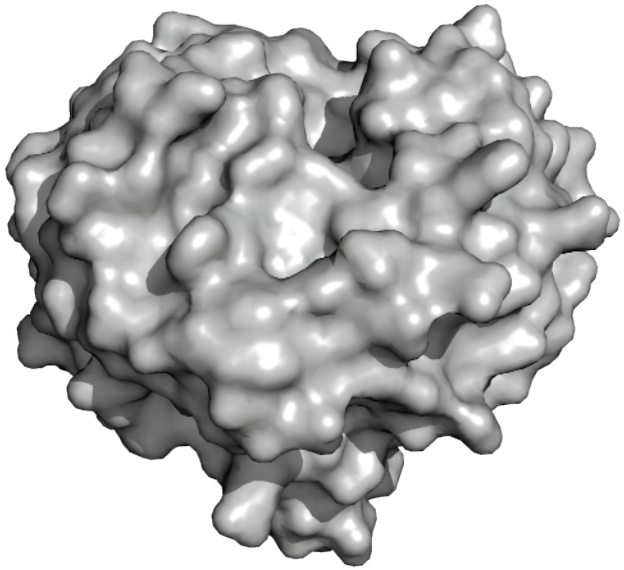
Retaining mechanism

- Cellulase Cel5A from *Bacillus agaradhaerens*
  - Modular endoglucanase
  - Active pH range 5.0-13.0
  - Glu139 (acid/base) and Glu228 (nuc)
  - Shallow active site cleft with 5 subsites







Davies GJ Biochemistry 1998 37:1926

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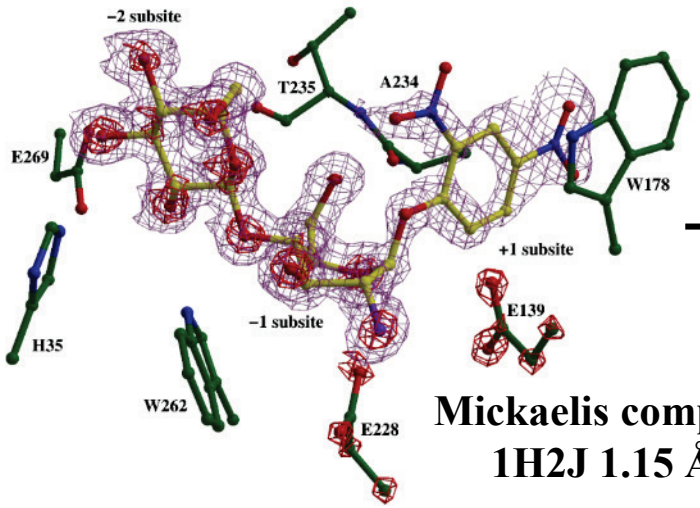
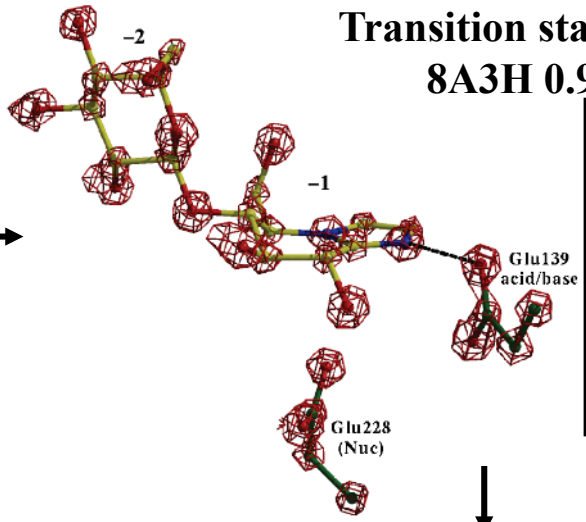
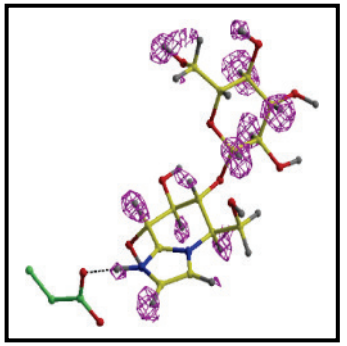
Cel5A snapshot at atomic resolution

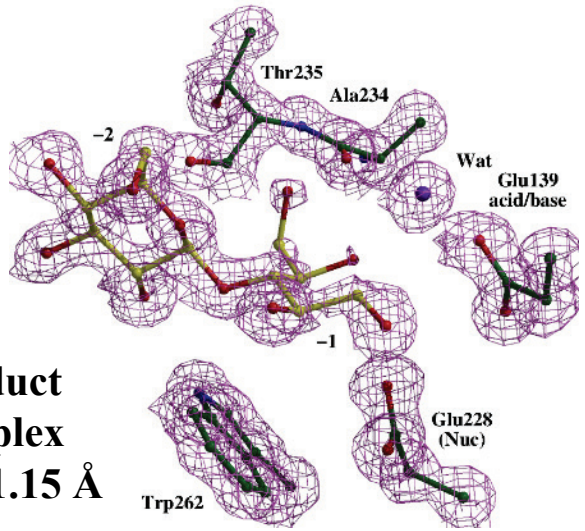


Mickaelis complex
1H2J 1.15 Å

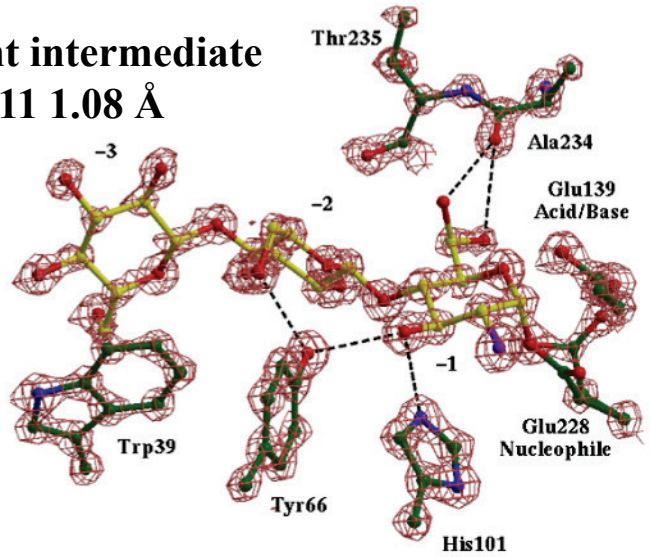


Transition state mimic
8A3H 0.97 Å





Product complex
1HF6 1.15 Å



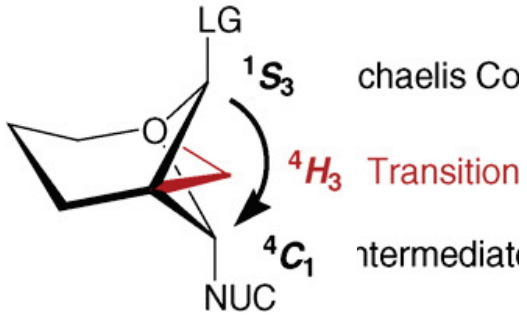
Covalent intermediate
1h11 1.08 Å

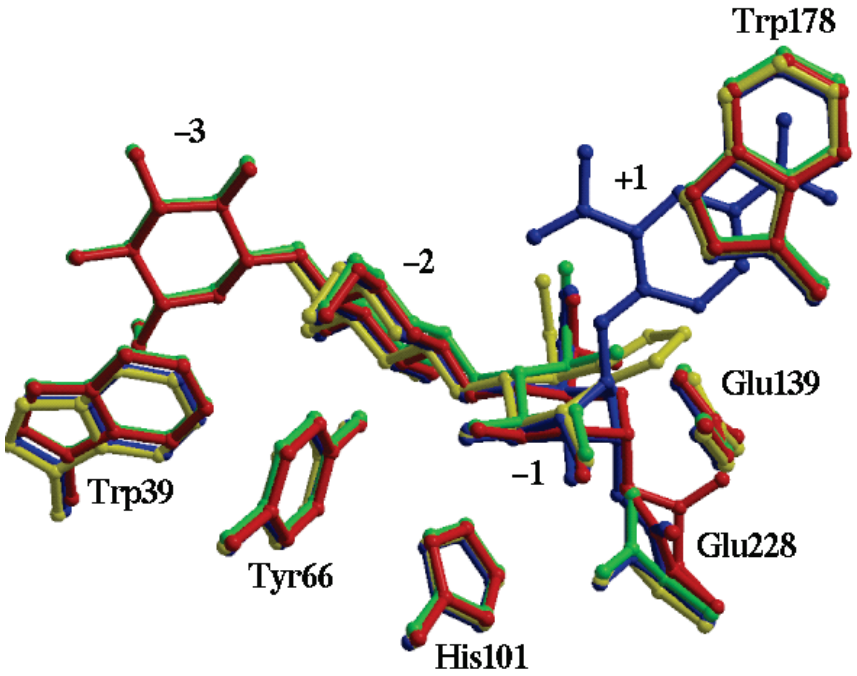
Varrot A JACS 1999 121: 2621; Varrot A Acta Cryst D 2001 D57: 1739, Vocadlo D, Nature 2001

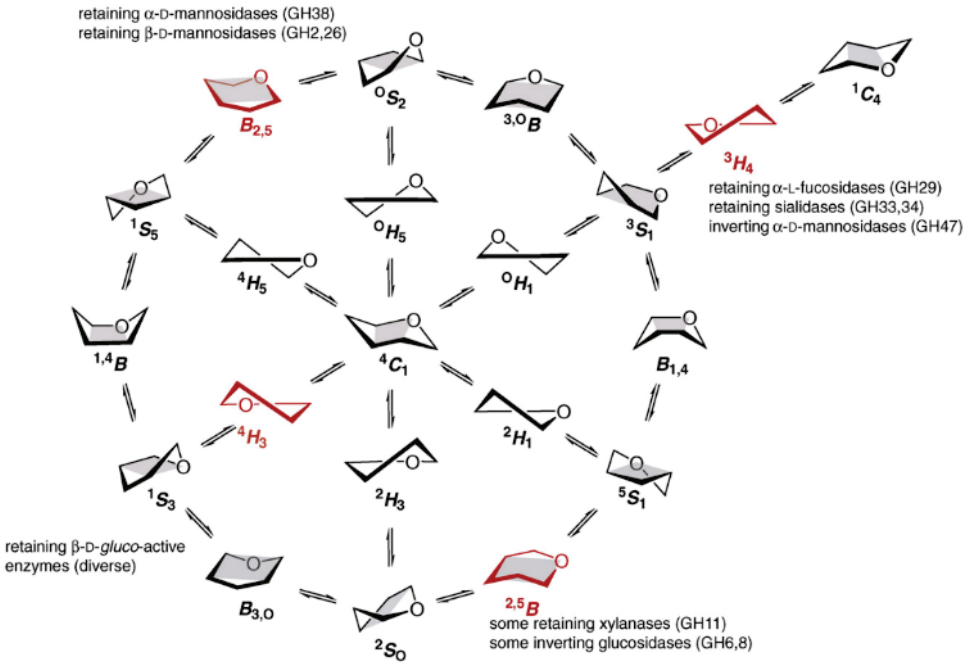
Cermav

Importance of ring distorsion

- Protein quite static
- Nucleophilic migration





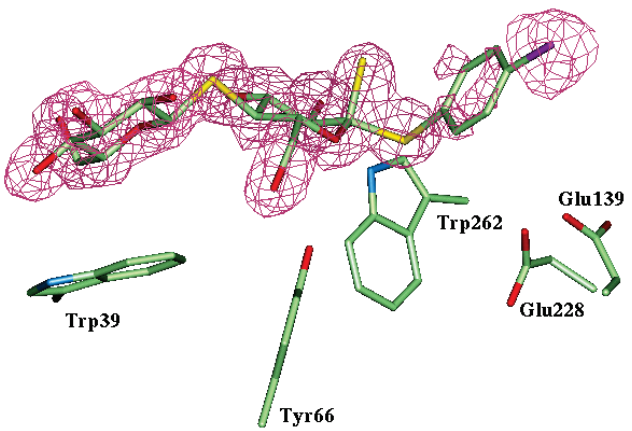


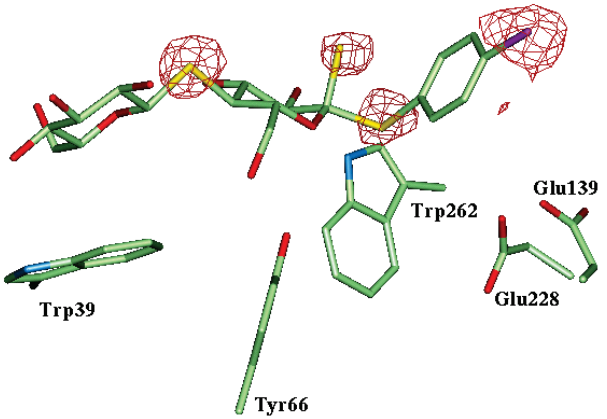
Varrot A Acta Cryst D 2001 D57: 1739; Vocadlo and Davies COSB 2008 12:539

Cermav

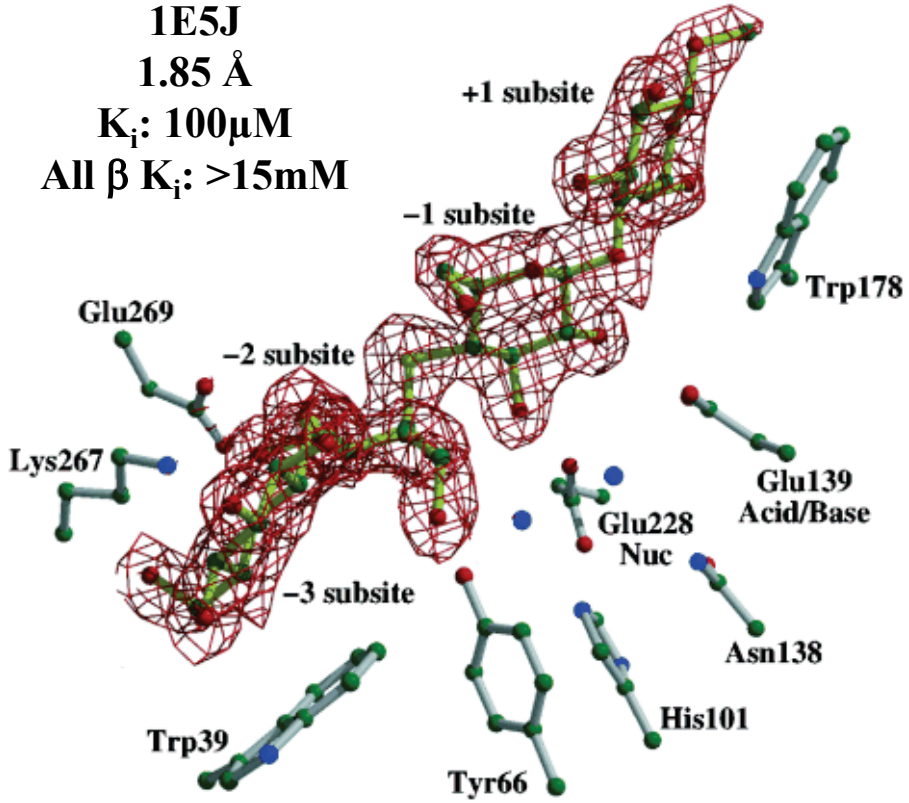
Serendipiry

- Strange density with TBR 1.68 Å on home source
  - New inhibitor class





1E5J
1.85 Å
K_i: 100µM
All β K_i: >15mM

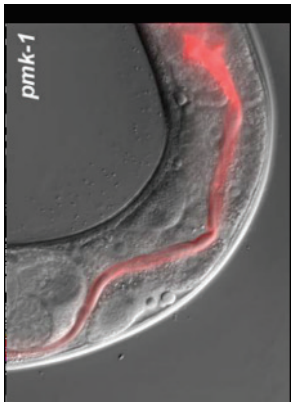


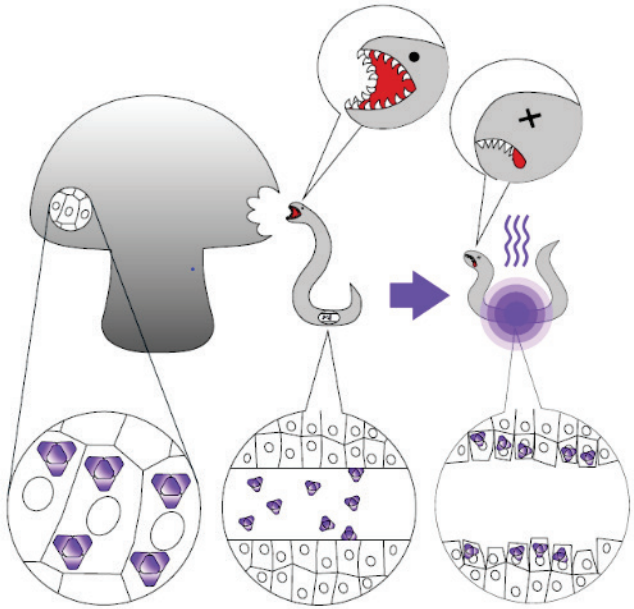
Unpublished and Fort S ChemBiochem 2001 2: 319

Cermav

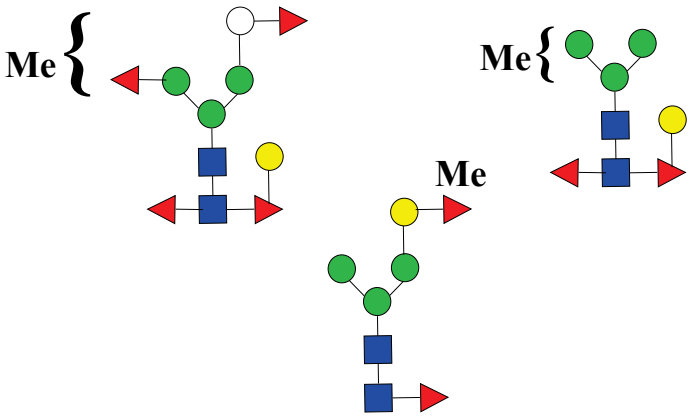
Lb-Tec2 from *Laccaria bicolor*

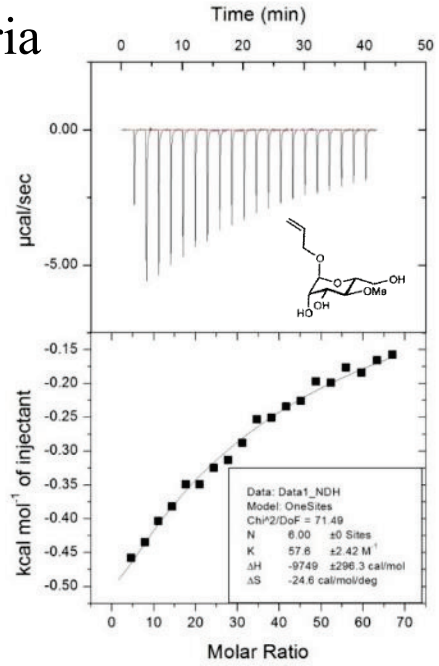
- Defense protein
  - Agglutinates Gram- bacteria
  - Nematotoxic
    - Binds to the worm gut





- Recognize O-methylated sugars
  - On worms, LPS bacteria
  - None in mammals





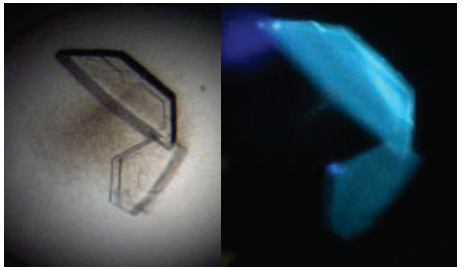
Sugar	K_d (mM)
Fuc	75
Allyl-2MeFuc	4
Allyl-3MeMan	20
Allyl-4MeMan	16

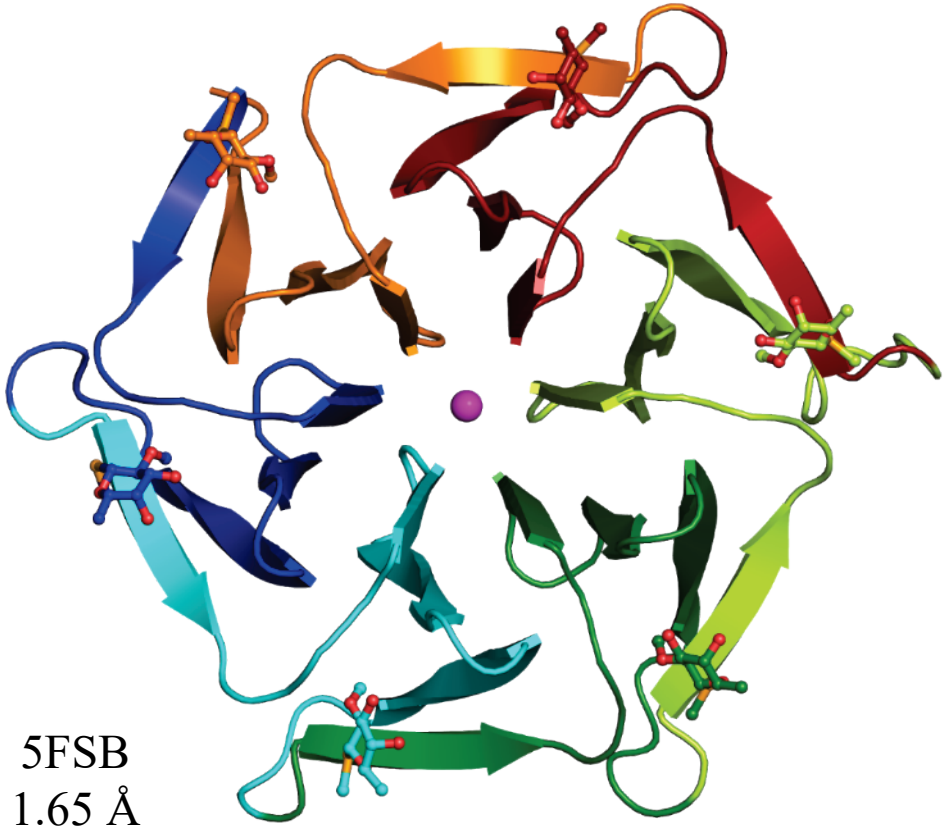
Wohlschlager T PNAS 2014 E2787, Staudacher E Biol Chem 2012 393:675; Yan S Mol Cell Proteomics. 2015 14:2111; Cordara G Structure 2018 26:369

Cermav

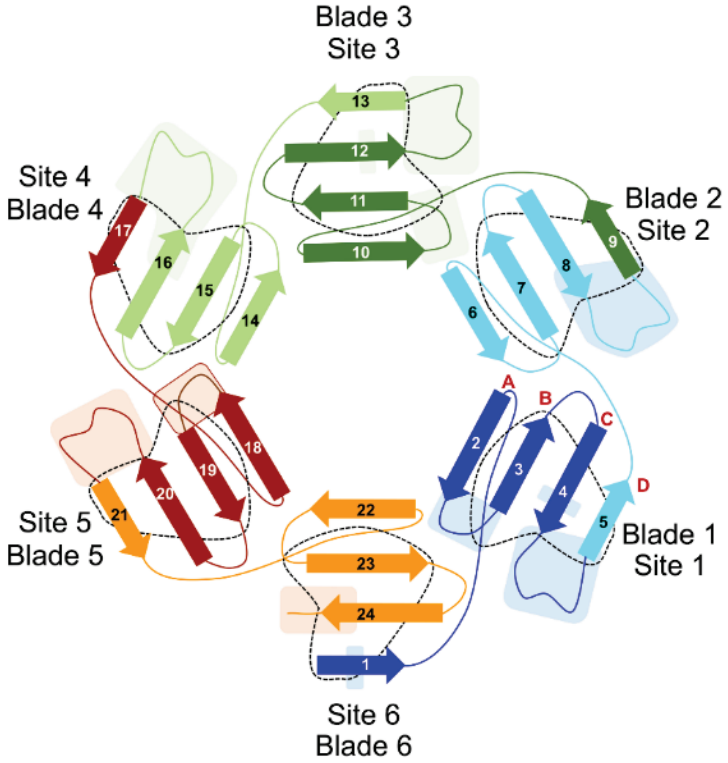
Lb-Tec2 structure

- 4-6 months to grow crystals
- Solved by MAD with soaked 2MeSeFuc





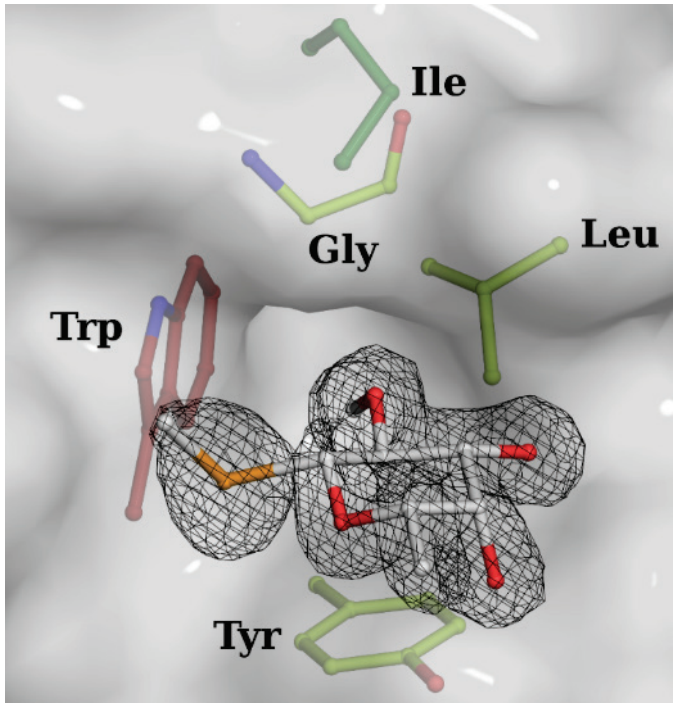
5FSB
1.65 Å

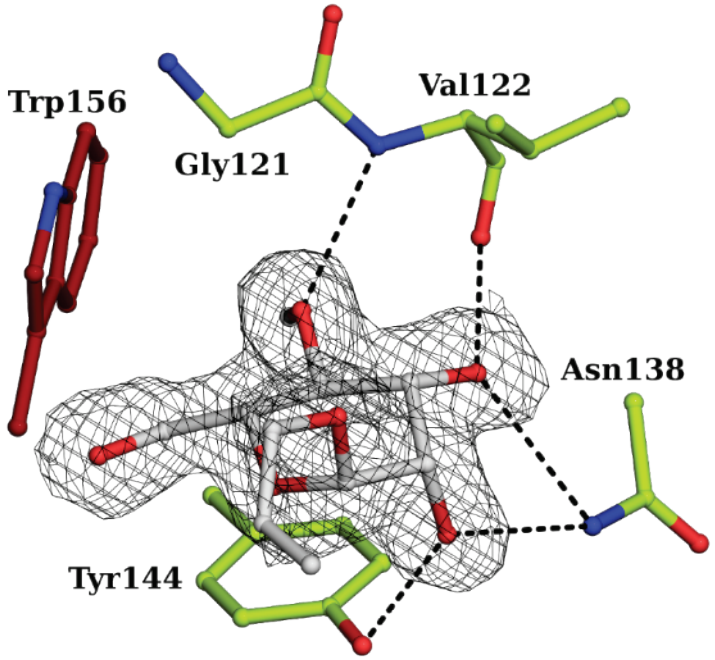


Sommer R Beilstein J Org Chem 2016 12:2828; Sommer R Structure 2018 26:391

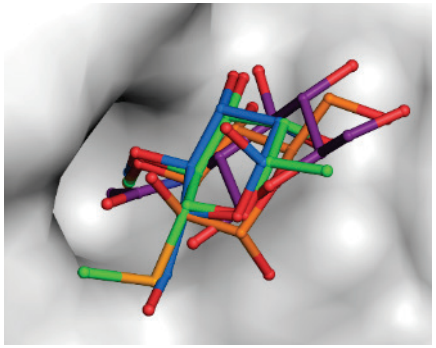
Cermav

Methyl recognition pocket

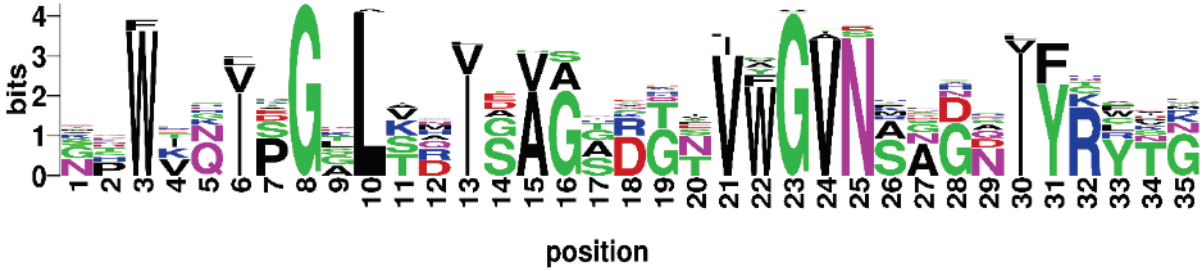




5FSC
1.95 Å



Highly conserved residues

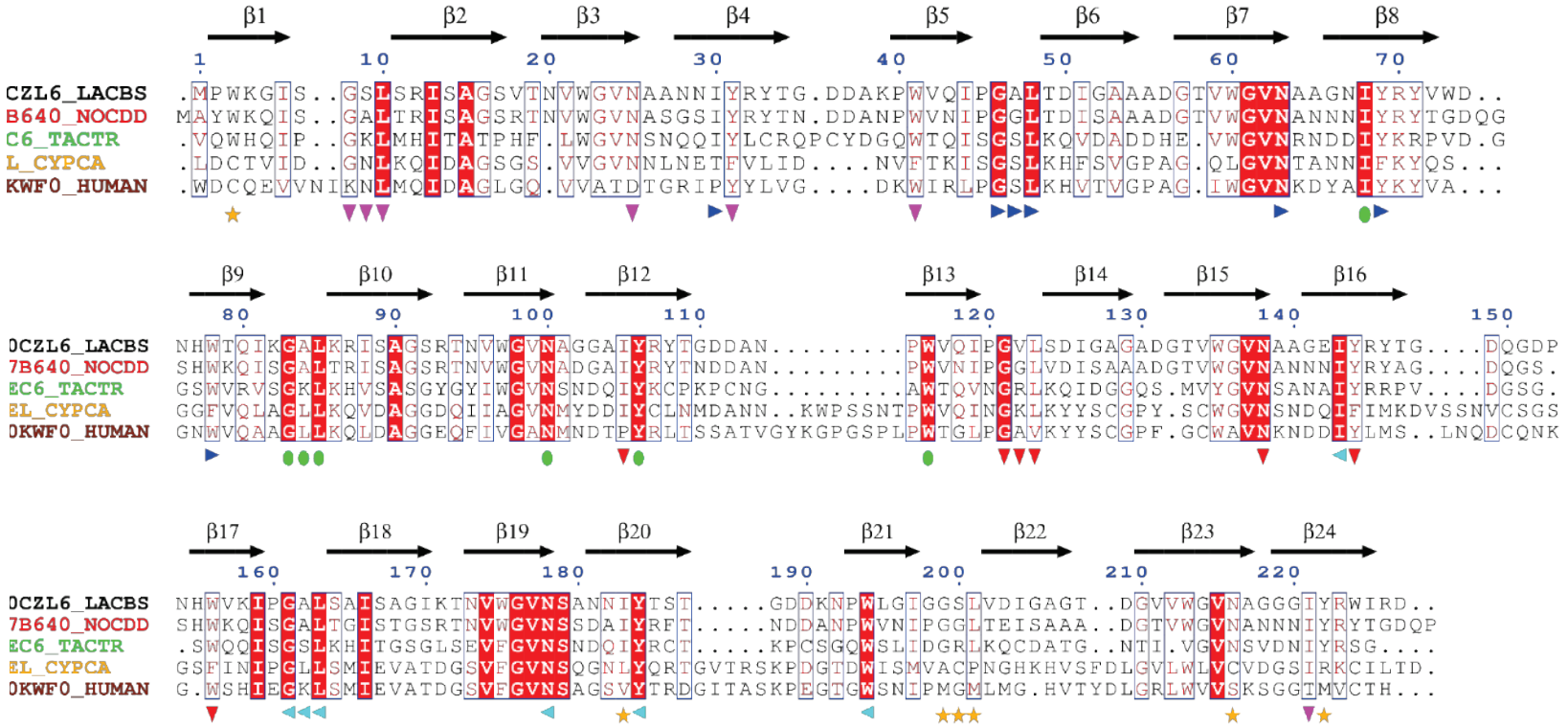


Sommer R Structure 2018 26:391

Cermav

Binding site formation

- Requires 3 TECPR repeats



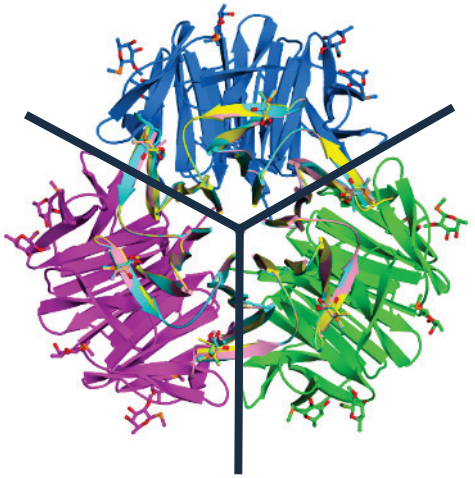
- Specificity conserved through evolution
  - Importance in innate immunity

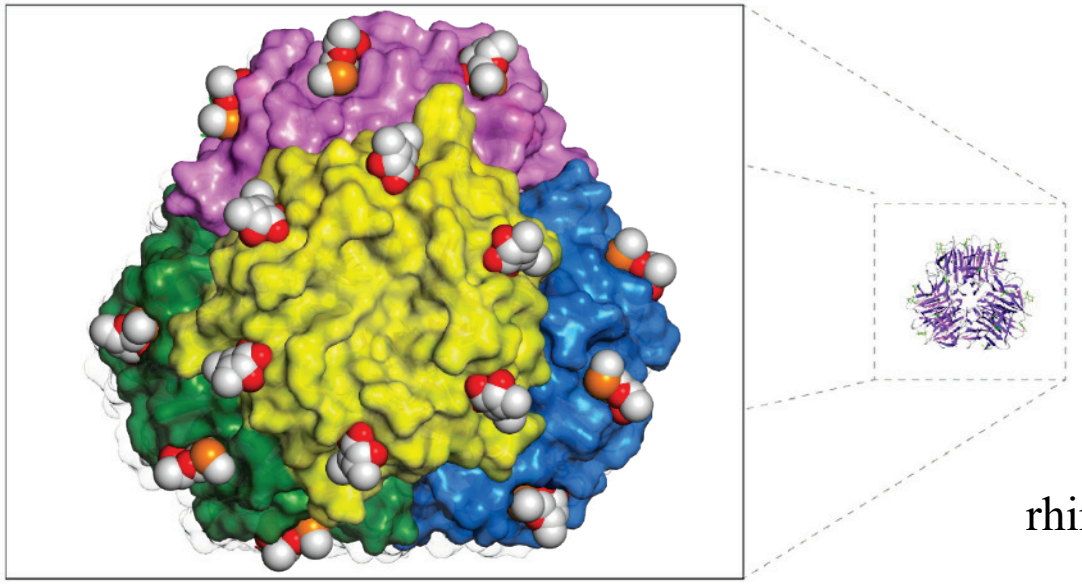
Sommer R Structure 2018 26:391

Cermav

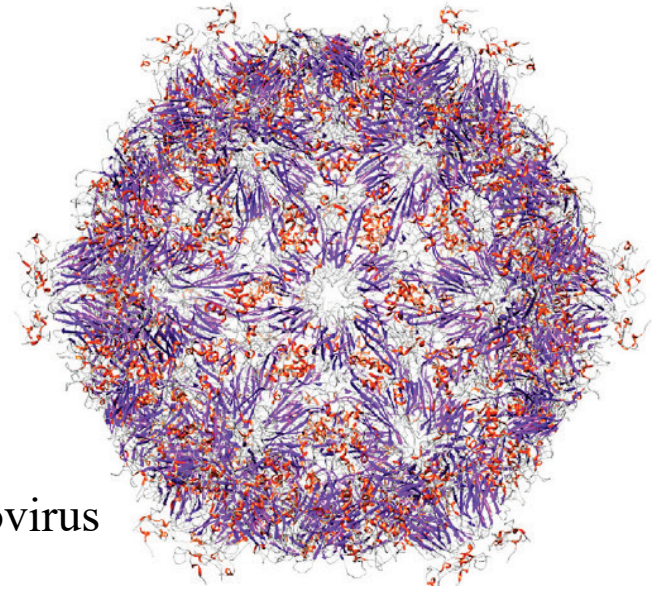
Unique quaternary structure

- Ball-shaped tetramer:
  - Unique with three fold axis in the PDB
  - Confirmed by SAXS
- 24 sites distributed on the surface
- Ressembles mini viral capsid





rhinovirus



Cordara G Structure 2018 26:369; Sommer R Structure 2018 26:391

Cermav

Mini lectin rPhoSL

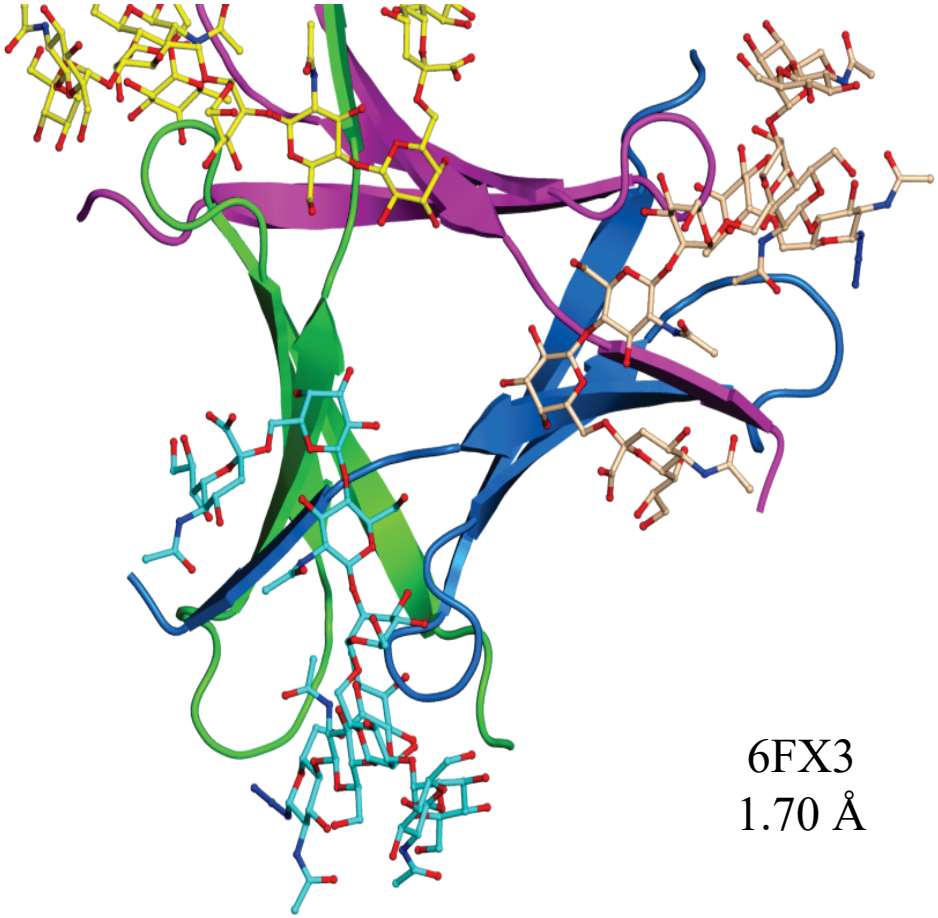
- 40 AA long
- Described as strictly specific for α1,6 core fucosylation
- Great potential as biomarker
  - Core fucose associated with cancer: hepatocarcinoma,
  - Diminish bioactivity of mAbs
    - See Tony's talk tomorrow
- Unknown fold
- Unknown recognition motif
- Produced recombinantly

Kobayashi Y JBC 2012 287:41; Yamasaki Y Scien Rep 2018 8:7740; Cabanettes A Angewandte Chemie 2018 in press

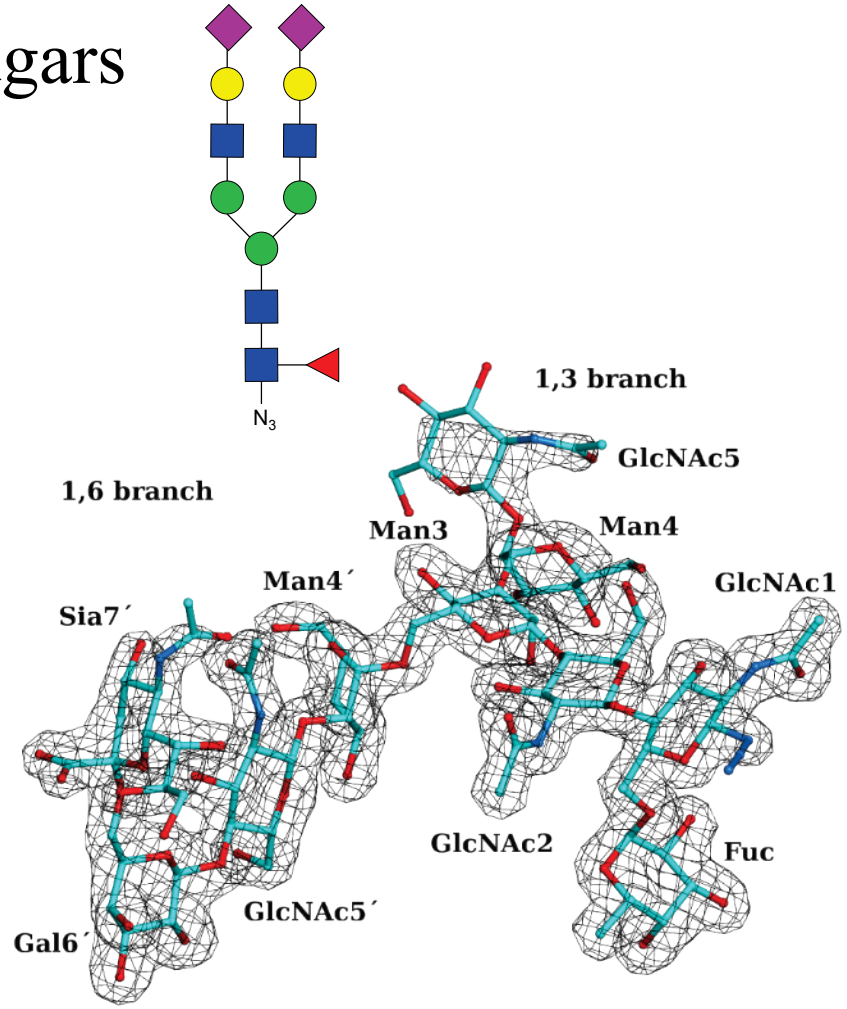
Cermav

Novel fold: β-prism III

- 1/3 of the ASU occupy by sugars



6FX3
1.70 Å



Cabanettes A Angewandte Chemie 2018 in press

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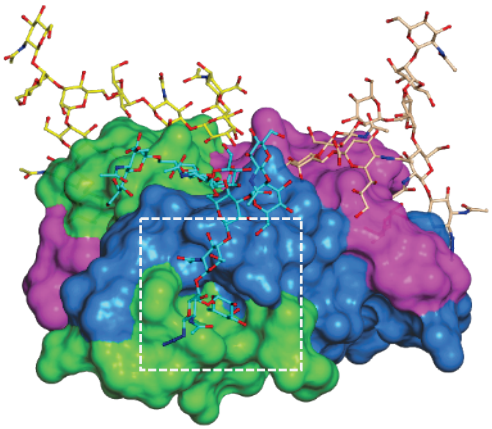
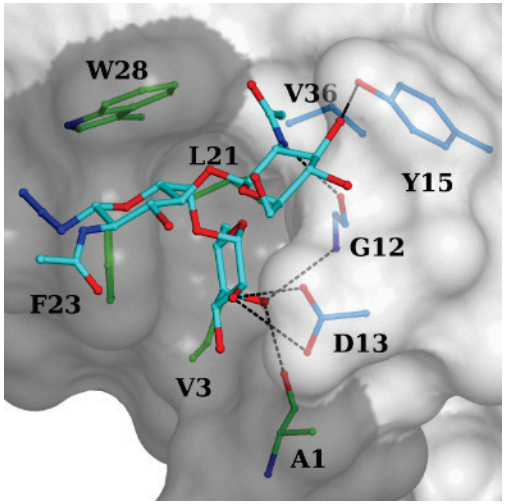
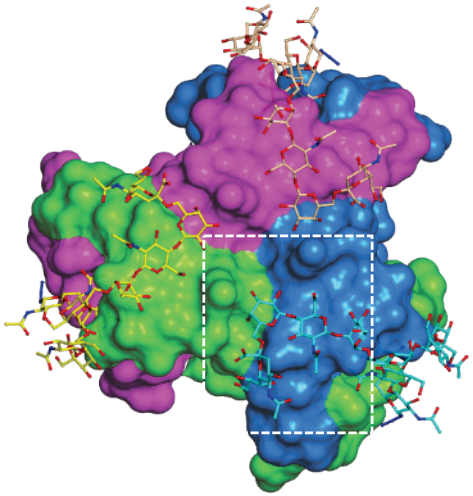
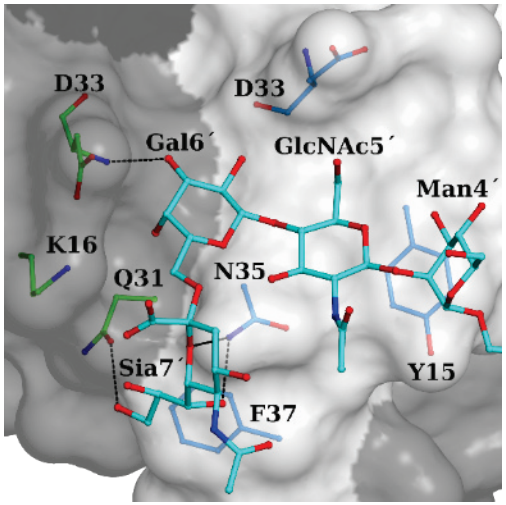
Binding sites

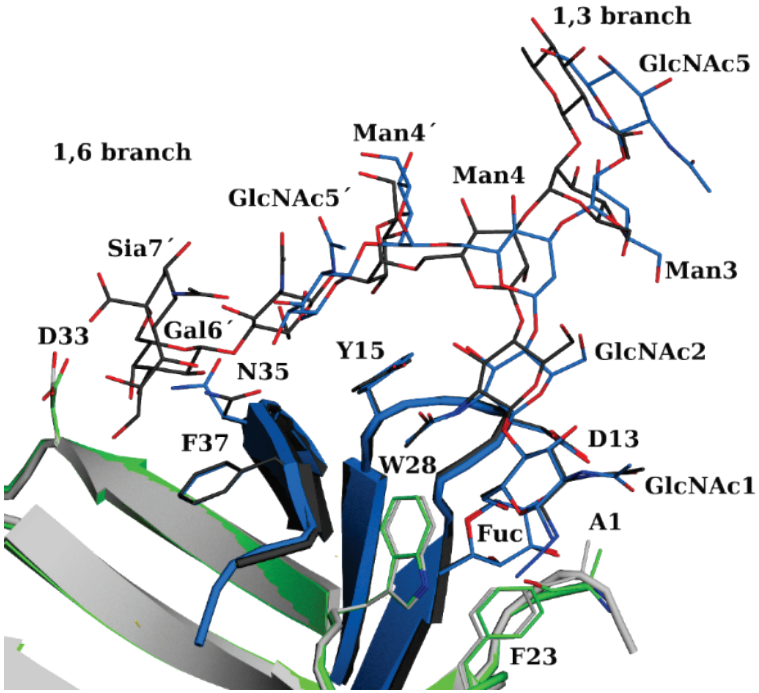
- 2 binding pockets: Fuc: conserved / Gal: can vary











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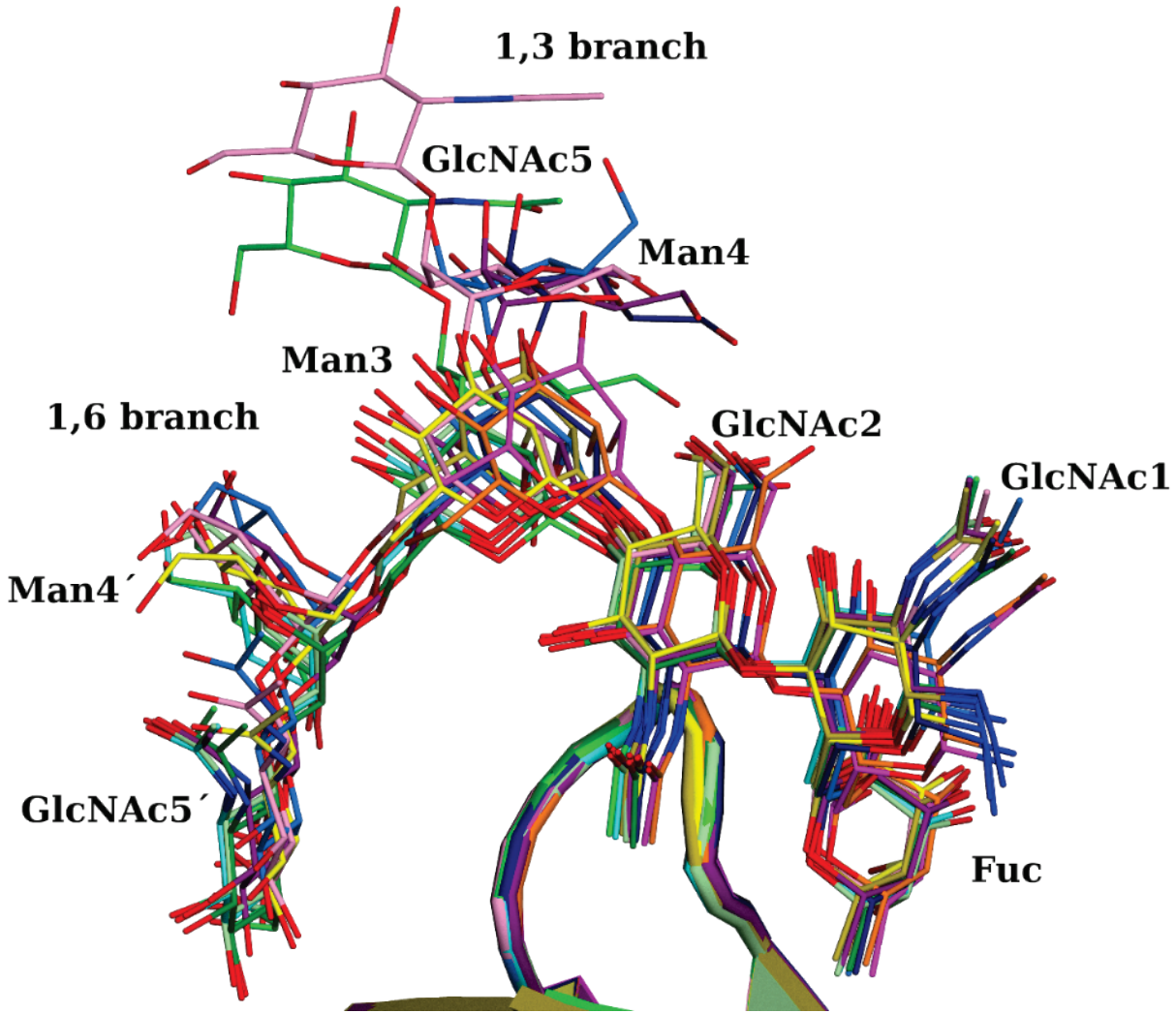
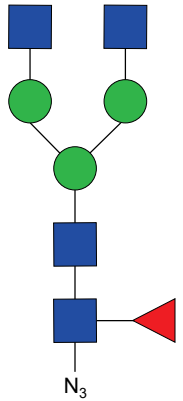
Flexibility in crystal structures





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Conclusions

- X-ray crystallography gave access to protein sugar interaction at the atomic level
  - Highest the resolution the better
- Could be tricky for non glycobiologists
- Distorsion
  - Real or artefact from user/program errors
- No « Structural glycobiology for dummies »
  - Do not hesitate to contact expert of CCP4bb

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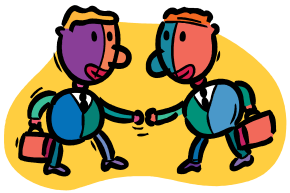

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