In Silico Design of Ligands for the Detection of Sucrose at Ultra Lower Concentrations in Physiological Condition

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Ómicas - Project 2 - Nanosensors

Faculty of Engineering and Sciences

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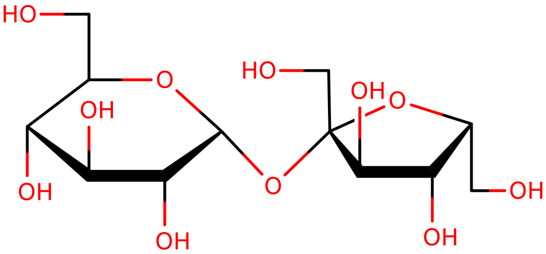
Sucrose



α-D-glucopyranoside-(1→2)-β-D-Fructofuranosyl
Sucrose
Table sugar

- Mechanism for carbon fixation in the trophic chain

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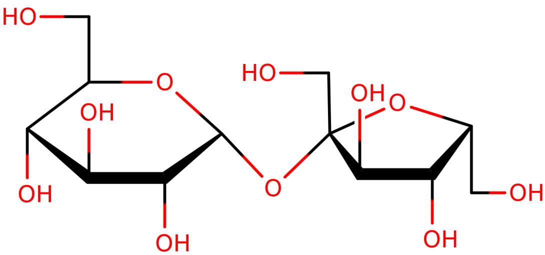

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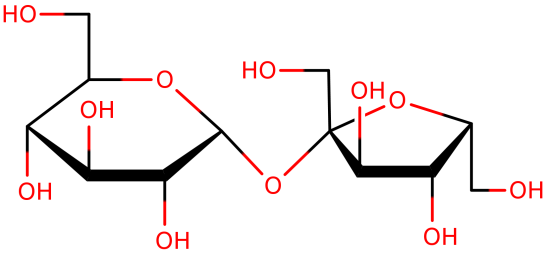

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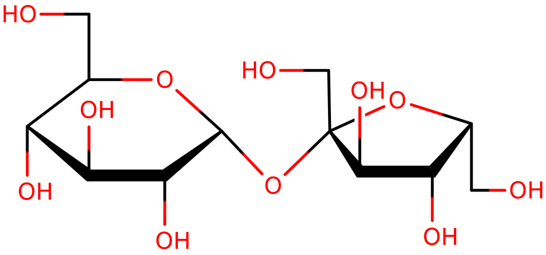

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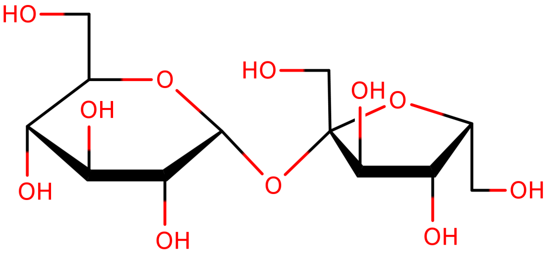

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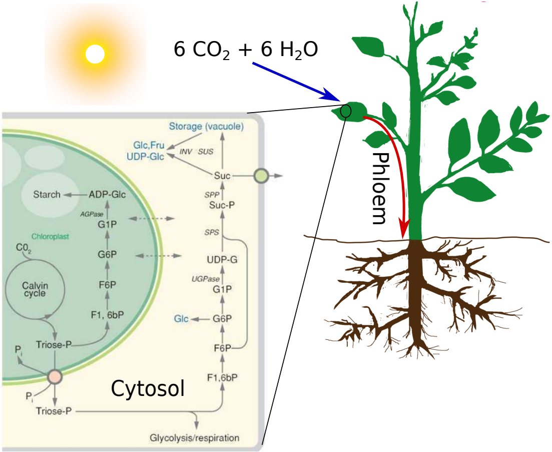
- Mechanism for carbon fixation in the trophic chain
- Stable molecule for energy storage (non-reducing sugar)
- Plant growth and development regulator (Phytohormone)
- Precursor for the synthesis of structural molecules, phytohormones, among others
- Principal agroindustrial commodity in the geographic valley of Cauca river

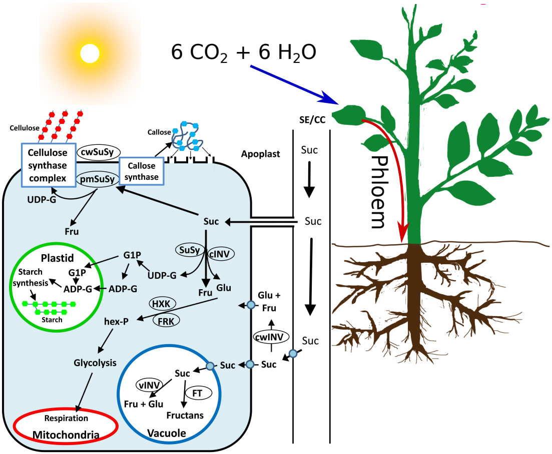
Sucrose biosynthesis



Simplified model of carbon flux and signaling for photosynthesis, transport and hydrolysis of sugars in photosynthetic cells during the day.

Rolland, F. et al. *Annu. Rev. Plant. Biol.* **2006**, 57, 675–709.

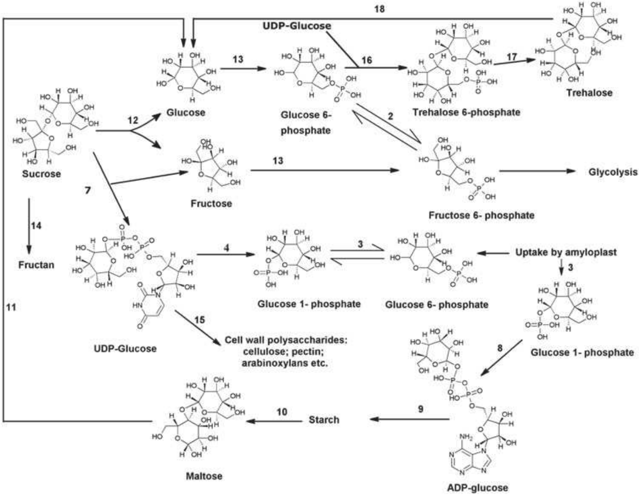
Sucrose metabolism



Simplified representation of sugar metabolism in non-photosynthetic tissue cells.

Stein, O. and Granot, D. *Front. Plant. Sci.* **2019**, 10, 95.

Sucrose metabolism



Routes for the breakdown of sucrose and its uses.

Hallford, N. G. et al. *Annals of Applied Biology.* **2011**, 158, 1-25.

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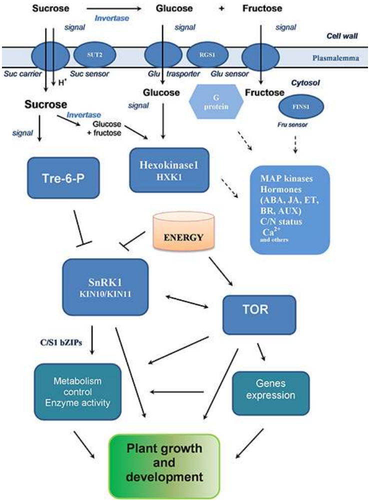
Some functions of sugars in plants



- Glucose > 6% reduces the germination and development in *arabidopsis thaliana*

Ciereszko, I. *Acta Soc Bot. Pol.* **2018**, 87, 3583.

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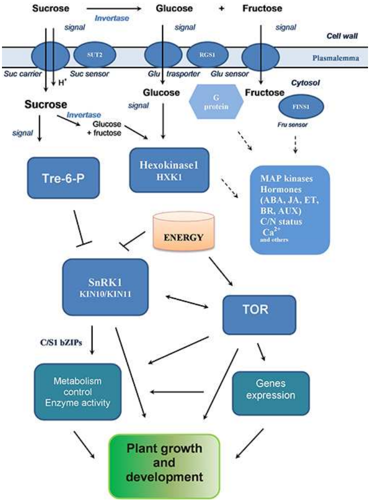

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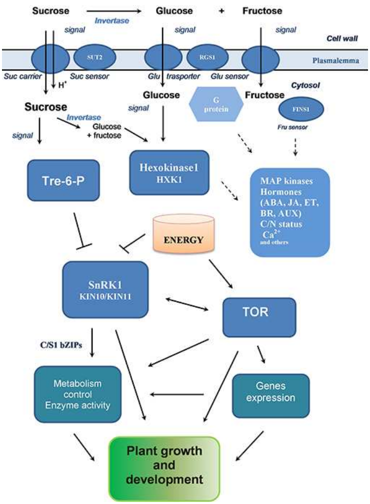

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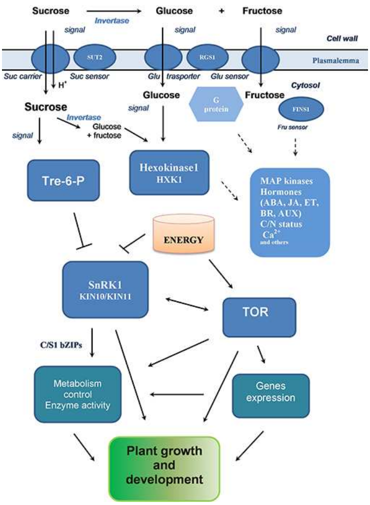

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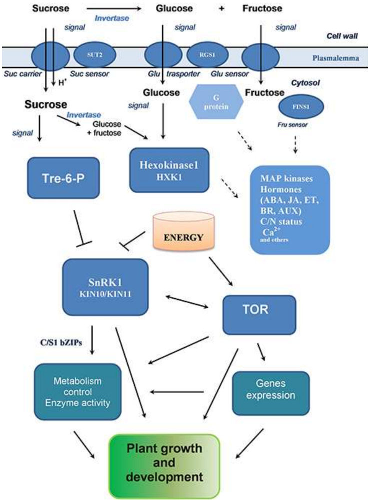

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- Depending on their structure, sugars can differentially regulate pollen germination in *arabidopsis*
- Trehalose 25 mM inhibits root elongation in *a. thaliana* seedlings; while sucrose does not affect this process
- Genes that encode enzymes in the metabolism of sucrose, are involved in the production of sugar signaling factors, and in turn are controlled according to the levels of sugars

Ciereszko, I. *Acta Soc Bot. Pol.* **2018**, 87, 3583.

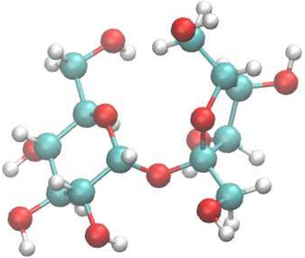
Chemical composition inside a cell

Table 2–2 The Approximate Chemical Composition of a Bacterial Cell

	PERCENT OF TOTAL CELL WEIGHT	NUMBER OF TYPES OF EACH MOLECULE
Water	70	1
Inorganic ions	1	20
Sugars and precursors	1	250
Amino acids and precursors	0.4	100
Nucleotides and precursors	0.4	100
Fatty acids and precursors	1	50
Other small molecules	0.2	~300
Macromolecules (proteins, nucleic acids, and polysaccharides)	26	~3000

Cooper, G., The Cell: A Molecular Approach; *Sinauer Associates Inc* **2000**; 2nd Edición.

Sucrose detection



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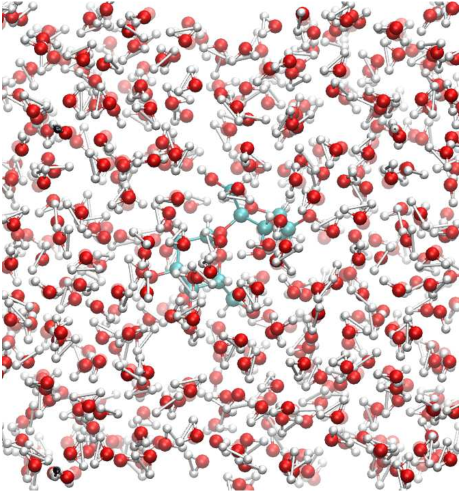
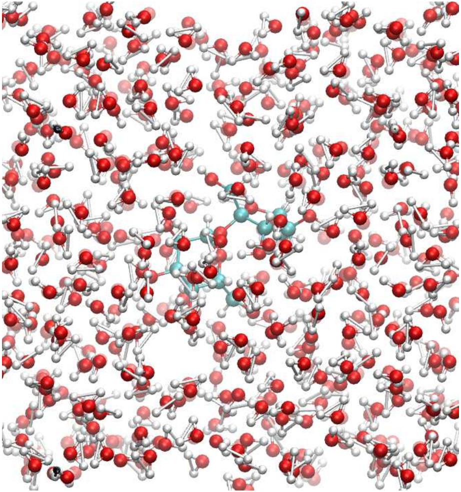


Highly solvated system

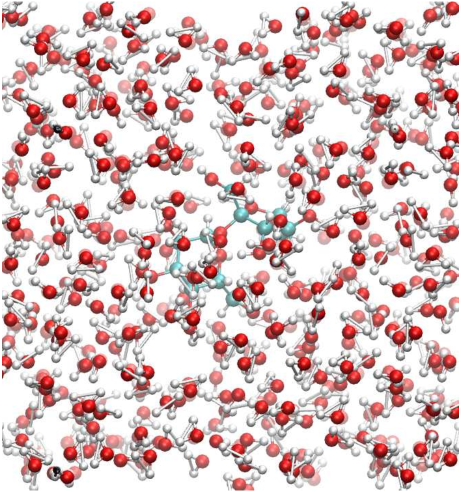
Sucrose detection



Highly solvated system

Conventional detection techniques drawbacks:

Sucrose detection



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Conventional detection techniques drawbacks:

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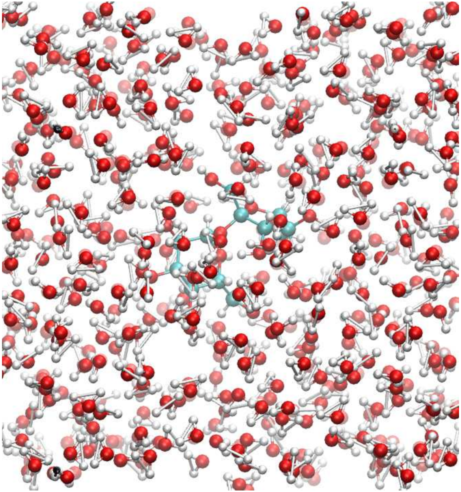

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Conventional detection techniques drawbacks:

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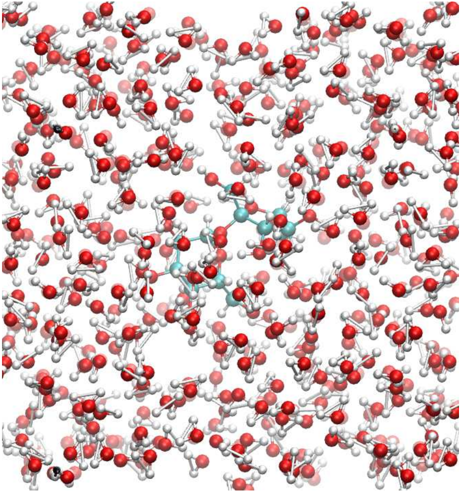

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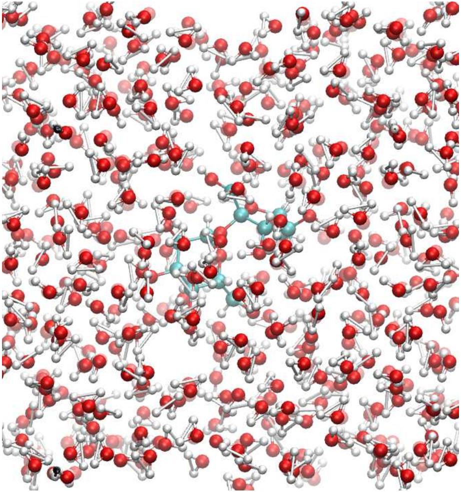

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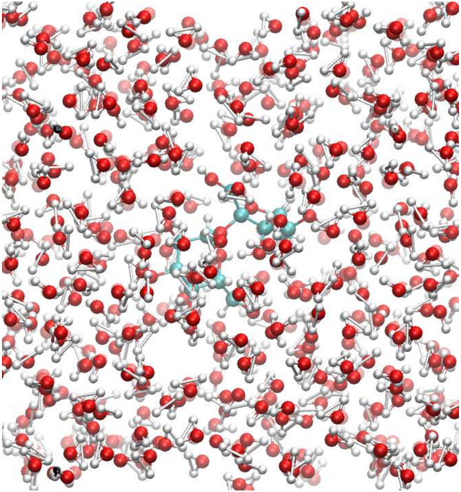

Highly solvated system

Conventional detection techniques drawbacks:

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- Loss of other analytes of interest
- Destruction of the plant
- Sample pretreatment and processing
- Expensive (robust) detection equipment

Analyte *in vivo* detection

Sensors for *in vivo* detection

Characteristics:

- Easily implantable and removable

Rajpoot K., *Biosens. J.* **2017**, 2, 145.

Sensors for *in vivo* detection

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Characteristics:

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- Low molecular weight

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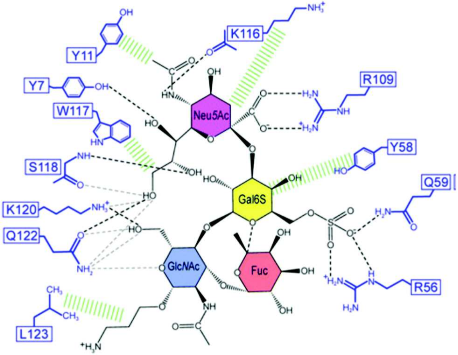
Lectins

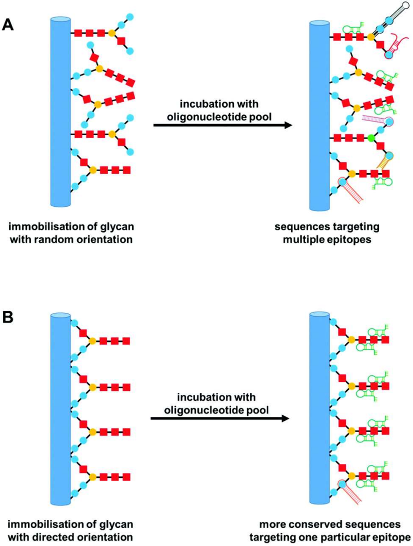


Characteristics:

- Interacts with carbohydrates through hydrogen bonds, CH-π bonds, and electrostatic interactions
- Multiple interactions with sugars, especially polysaccharides ($\sim$7 kcal/mol)
- High biocompatibility
- Low binding energies, selectivity and affinity ($K_D \sim$ mM) for mono and dissaccharides
- Template selection

Tomassone, S. et al. *Chem. Soc Rev.* **2019**, 48, 5488-5505.

Aptamers

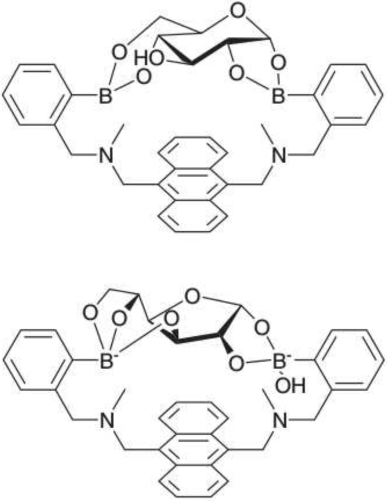


Characteristics:

- Non-covalent interactions; primarily by hydrogen bonding
- Single strand synthetic oligonucleotides < 100 bases
- In carbohydrates with electric charge, $K_D \sim 1.35$ nM have been reported
- The absence of aromatic rings and groups with a net charge limits non-covalent interactions
- Modification with boronic acids improves selectivity to simple sugars

Tomassone, S. et al. *Chem. Soc Rev.* **2019**, 48, 5488-5505.

Phenylboronic acid



Characteristics:

- Covalent bonds to diols groups at the 1,2 and 1,3 positions.
- Reversible reaction and binding energy pH-dependent
- Versatility for integration into different sensing platforms (polymers, nanotubes, aptamers, nanoparticles)
- Controlling the orientation of the boronic groups improves the selectivity of the saccharide; low molecular weight
- Binding constants in the range of mM-μM
- Binding energy improves at pH $>$ 7.0

Tomassone, S. et al. *Chem. Soc Rev.* **2019**, 48, 5488-5505.

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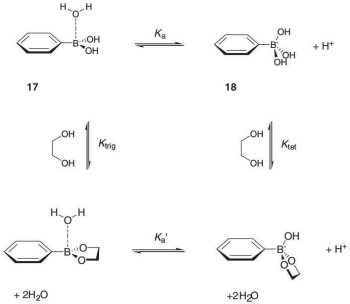
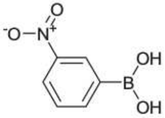
Phenylboronic acid in aqueous solvent



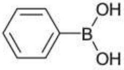
- The equilibria are shifted towards the anionic form
- The acidity of boronic acid increases after the formation of the boron diester cycle (Ka’ > Ka)
- The reaction kinetics of the borate anion is greater than the kinetics of neutral boronic acid ($K_{tet} > K_{trig}$)
- sp^3 hybridization reduces cyclic diester strain relative to sp^2 hybridization

Rowan, A. et al. Boronic Acids in Saccharide Recognition, *RSC* **2006**.

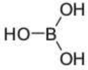
Phenylboronic acid in aqueous media



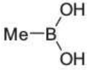
31 *m*-nitrophenylboronic acid
pK_a 6.96



16 phenylboronic acid
pK_a 8.72



15 boric acid
pK_a 8.98



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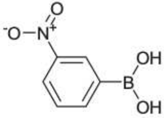
Phenylboronic acid in aqueous media



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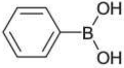

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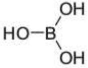

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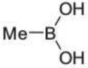

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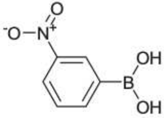

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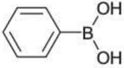

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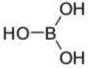

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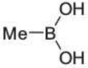

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- If pH > pKa, the binding constant of phenylboronic acid increases by fivefold.
- The stability of the boronic diester complex increases with the acidity of the ligand and boronic acid.

Rowan, A. et al. Boronic Acids in Saccharide Recognition, *RSC* **2006**.

Sucrose conformers

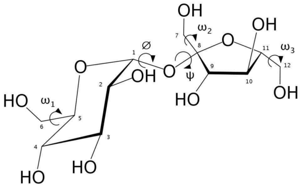
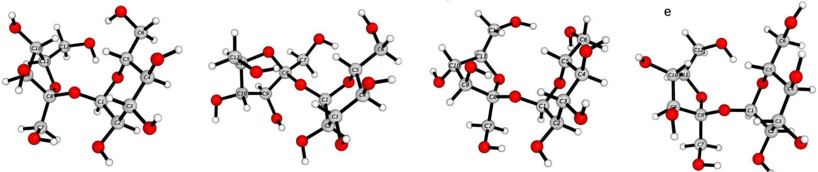


Table 2 Relative energy (kcal mol^{-1}) for the main conformations of D-Sucrose and dif erent conformations of S1 regarding the dihedral angle ω_1 at dif erent theory levels

Entry	Conformer	M06-2X[a]		MP2[b]	
		$\Delta E_{vac.}$	$\Delta E_{wat.}$	$\Delta E_{vac.}$	$\Delta E_{wat.}$
1	S1-gt-tg-gg	0.58	0.00	0.25	0.00
2	S2-gt-gt-gg	7.16	1.79	8.14	2.29
3	S3-gt-tg-gt	7.73	3.21	9.32	3.21
4	S1-gg-tg-gg	0.38	0.08	0.00	0.44
5	S1-tg-tg-gg	0.00	1.01	0.31	1.44
6	S1-gt-tg-gg^{ccw}	2.27	0.75	2.45	0.66

[a] optimization with 6-31++G(d,p) basis set function, ZPE correction and IEF-PCM/Bondi solvation model when in water.
[b] energy calculation with 6-311++G(2df,2pd) basis set function and IEF-PCM/Bondi solvation model when in water.

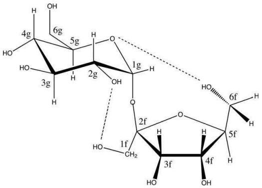


S1-gt-tg-gg S2-gt-gt-gg S3-gt-tg-gt S1-tg-tg-gg

Ab initio calculations, at the theory level M06-2X/6-31++G(d,p), for the geometry and energy of the conformers of sucrose in the gas phase and implicit solvent (water).

Thiago de Castro Rozada et al.; *RSC Advances*, 2016, 6, 112806-112812.

Dynamics of solvated sucrose



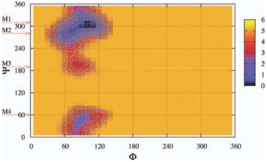
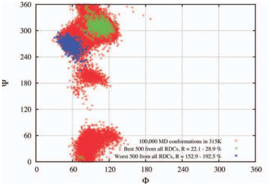


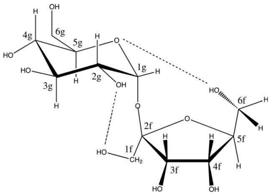
FIGURE 2 Free energy landscapes in / -w glycosidic space for sucrose in the TIP4P/Ew model. Contour lines are shown from 0.0 to 5.0 kcal/mol in steps of 0.1. The local minima are identi ed as M1, M2, M3, and M4; see also Table I.

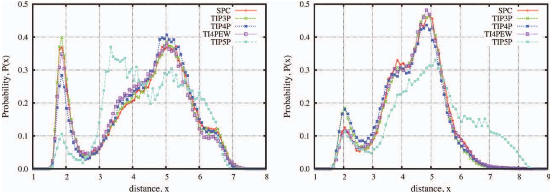


Free energy and dipolar residual coupling calculations for the conformational changes of sucrose in water. Classical trajectory simulation performed with the GLYCAM-06 force field and explicit solvent for water.

Xia J., et al.; *Biopolymers*, 2011, 97, 276.

Dynamics of solvated sucrose





Hydrogen bond distributions of sucrose with several models of explicit solvent for water. Classical trajectory simulation performed with the GLYCAM-06 force field.

Xia J., et al.; *Biopolymers*, 2011, 97, 276.

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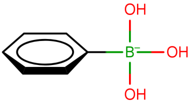
Phenylboronic acid-Sucrose binding energies



pKa=8.7

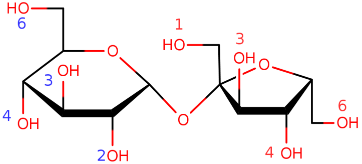
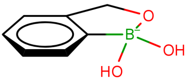


Table 1: Binding energies (kcal/mol) for the PBAOH-Sucrose-PBAOH compounds calculated with the PBEh-3c theory level with the implicit solvent model CPCM (H2O).

		2-1		1-3	
		R	S	R	S
2-3	R			3.4	3.1
	S			4.1	5.1
4-6	R	3.9	-6.8	-5.1	-9.8
	S	-2.3	-3.0	-7.9	-6.9

Benzoxaborole-Sucrose binding energies



pKa=7.3

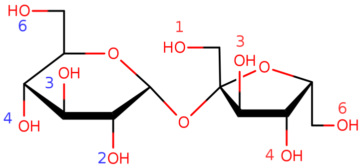
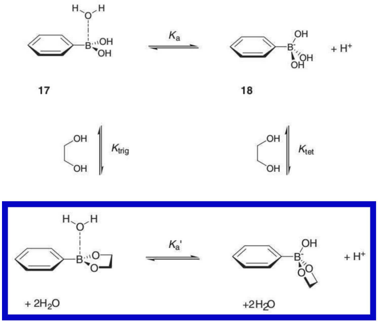
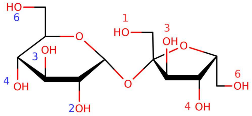


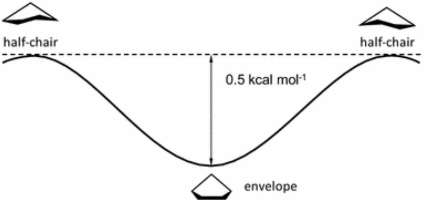
Table 2: Binding energies (kcal/mol) for the BOBOH-Sucrose-BOBOH compounds calculated with the PBEh-3c theory level with the implicit solvent model CPCM (H2O).

		2-1		1-3	
		R	S	R	S
2-3	R			9.7	7.4
	S			6.8	7.5
4-6	R	-4.1	-4.9	-1.0	0.2
	S	-1.4	-3.1	1.6	1.4

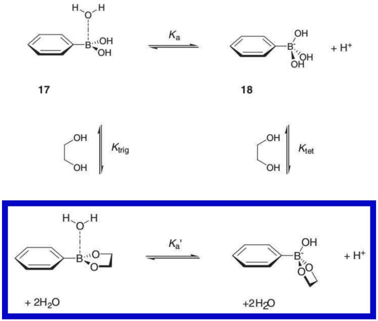
Conformational energies

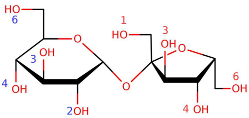


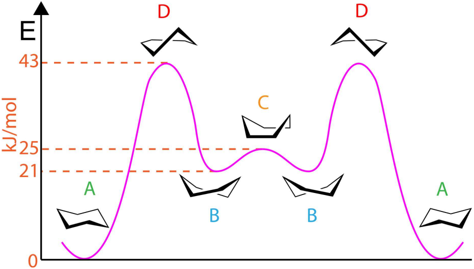




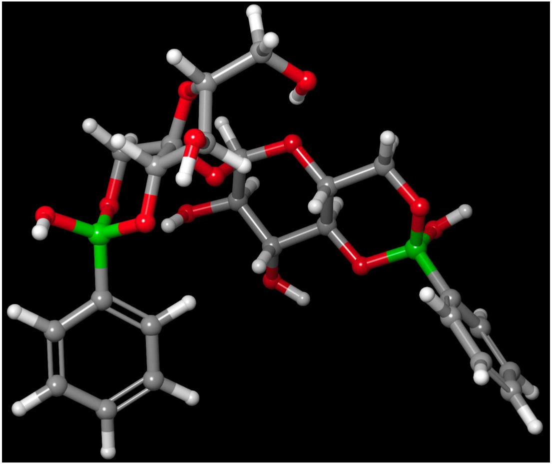
Conformational energies





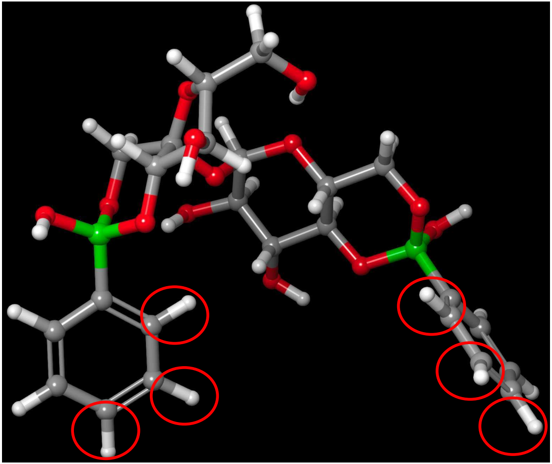


Bidentate ligands for sucrose



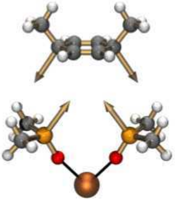
PBAOH(S)-1-3-Fructose-Glucose-4-6-PBAOH(S)

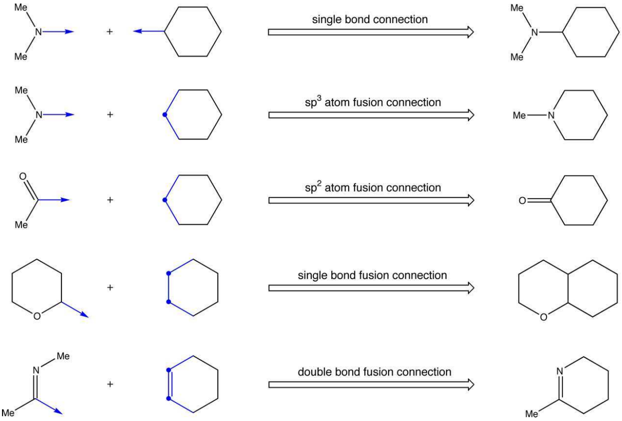
Bidentate ligands for sucrose



PBAOH(S)-1-3-Fructose-Glucose-4-6-PBAOH(S)

Ligand desing

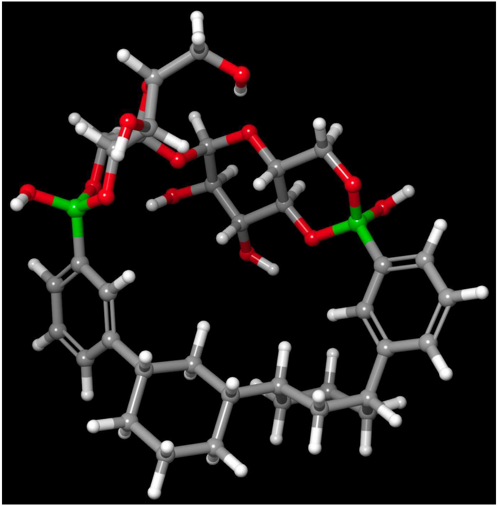




Organic ligands design with the OVERLAY program of the HostDesigner suit.

Hay, B. P.; Firman, T. K.; *Inorg. Chem.*, 2002, 41, 5502-5512.
Hay, B. P., Jia, C.; Nadas, J. *Comp. Theor. Chem.* 2014, 1028, 72-80.

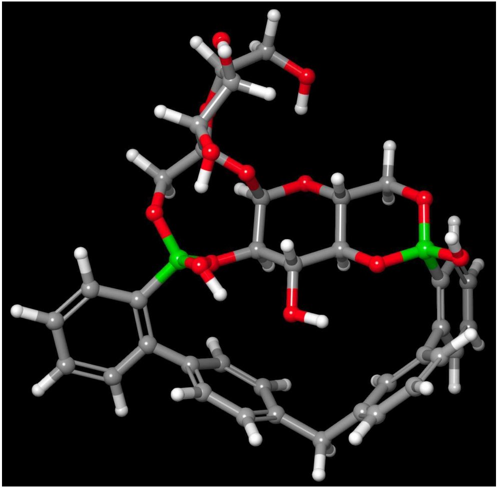
Bidentate ligands for sucrose



PBAOH(S)-1-3-Fructose-Glucose-4-6-PBAOH(S)

- Binding energy:-9.8 kcal/mol
- 1 ligand structure
- RMS < 0.1 Å

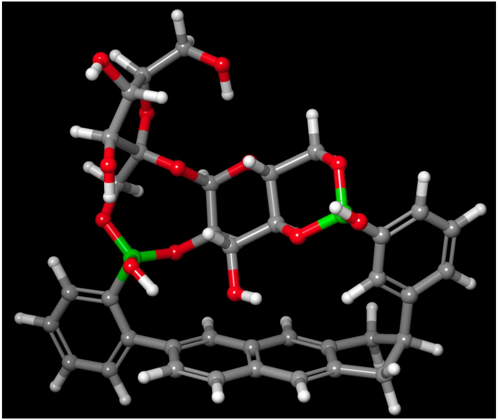
Bidentate ligands for sucrose



Fructose-1-PBAOH(S)-2-Glucose-4-6-PBAOH(R)

- Binding energy:-6.8 kcal/mol
- 467 ligand structures
- RMS < 0.5 Å

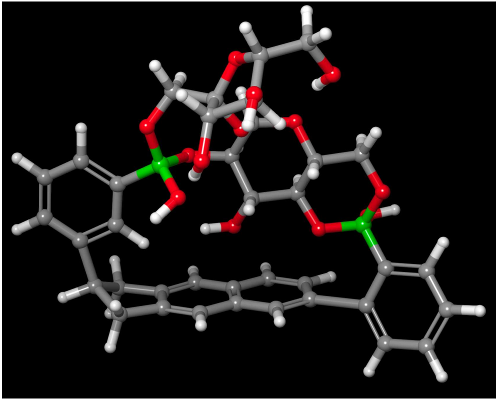
Bidentate ligands for sucrose



Fructose-1-PBAOH(S)-2-Glucose-4-6-PBAOH(S)

- Binding energy:-3.0 kcal/mol
- 7 ligand structures
- RMS < 0.2 Å

Bidentate ligands for sucrose



Fructose-1-PBAOH(R)-2-Glucose-4-6-PBAOH(S)

- Binding energy:-2.3 kcal/mol
- 2 ligand structures
- RMS < 0.2 Å

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Conclusions and perspectives

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Conclusions and perspectives

- We have designed ligands specific for the detection of sucrose, with potential application for *in vivo* detection.
- The ligands designed has binding energies of $\sim$ 8 kcal/mol; similar to the binding energies of lectins to polysaccharides.
- The binding energies of the designed ligands can be increased with the addition of functional groups, to form new hydrogen bonds with the free -OH groups in sucrose.
- The addition of Electron withdrawing groups to the ligand will decrease the pKa, increasing the binding energy. Also, can improve the signal detection for the analyte.

Acknowledgments

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Thanks!