

Novel application of an FAD dependent glycoside oxidoreductase for biosensing and bioelectrocatalysis

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Roland Würschum² and Bernd Nidetzky¹

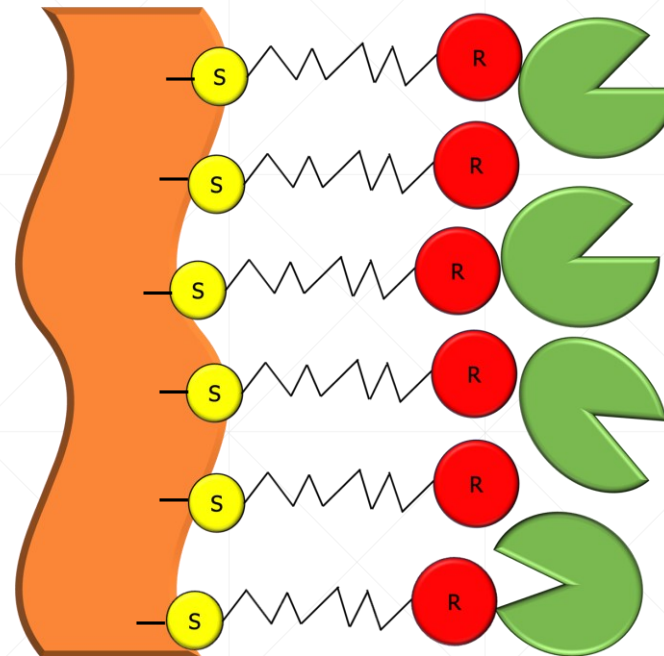
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What are enzyme electrodes?

Enzyme electrodes are miniature devices capable of transducing enzymatically catalyzed reactions into electrochemical signals

MATERIAL SCIENCE MEETS BIOTECHNOLOGY



Electrode

Linker

Bio-catalyst



Applications



Biosensors – electrical devices that measure biological signals and convert them into electrical signals¹



Bioelectrocatalysis – utilization of materials derived from biological systems as catalysts to catalyse the redox reactions occurring at the electrode²



Enzyme based biofuelled cells – subclass of fuel cells employing redox enzymes as catalysts³



¹Chadha, U., Bhardwaj, P., Agarwal, R., Rawat, P., Agarwal, R., Gupta, I., ... & Chakravorty, A. (2022). Recent progress and growth in biosensors technology: A critical review. *Journal of Industrial and Engineering Chemistry*, 109, 21-31.

²Chen, H., Simoska, O., Lim, K., Grattieri, M., Yuan, M., Dong, F., ... & Minteer, S. D. (2020). Fundamentals, applications, and future directions of bioelectrocatalysis. *Chemical Reviews*, 120(23), 12903-12993.

³Xiao, X., Xia, H. Q., Wu, R., Bai, L., Yan, L., Magner, E., ... & Liu, A. (2019). Tackling the challenges of enzymatic (bio) fuel cells. *Chemical reviews*, 119(16), 9509-9558.

Assembly of an enzyme electrode

Electrode material

- Metal based electrodes
- Carbon based electrodes

Electrode geometry

- Planar
- Nano-porous
- 3D printed



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Glassy Carbon Electrode
(GCE)

Planar Gold Electrode
(PAu)



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Glassy Carbon Electrode
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Planar Gold Electrode
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Enzyme

- Redox active enzyme



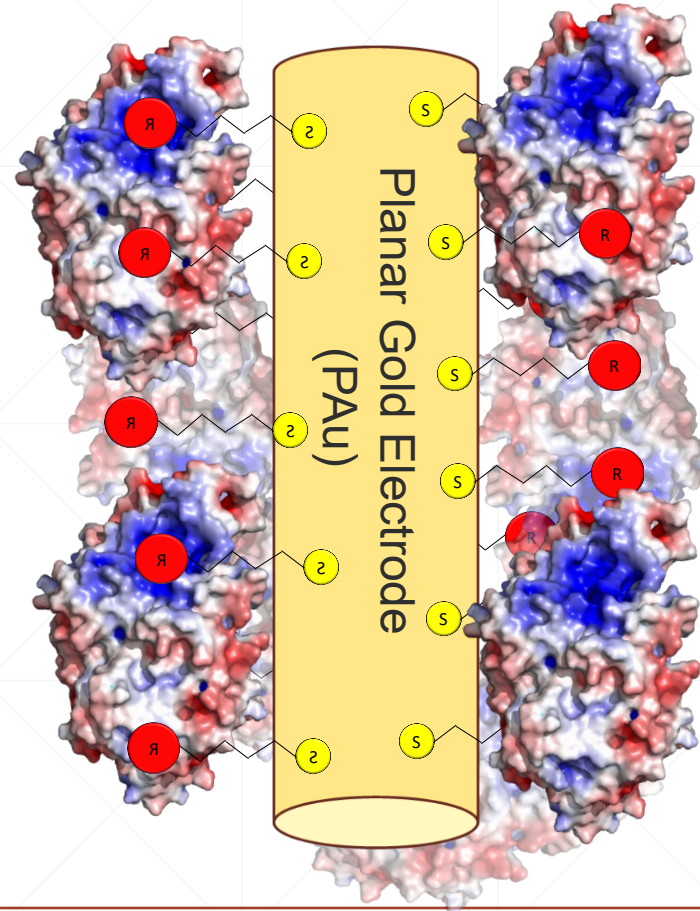
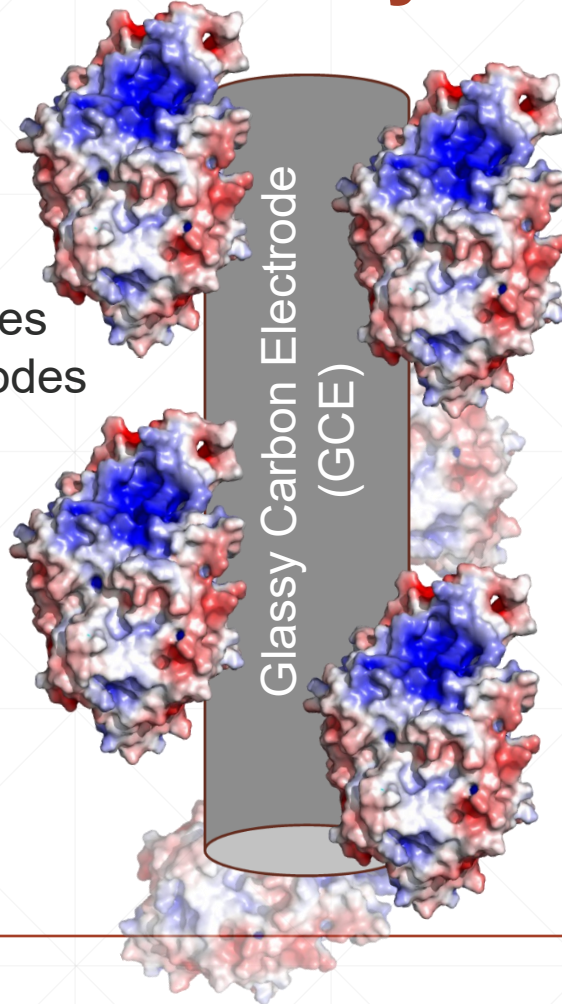
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Enzyme

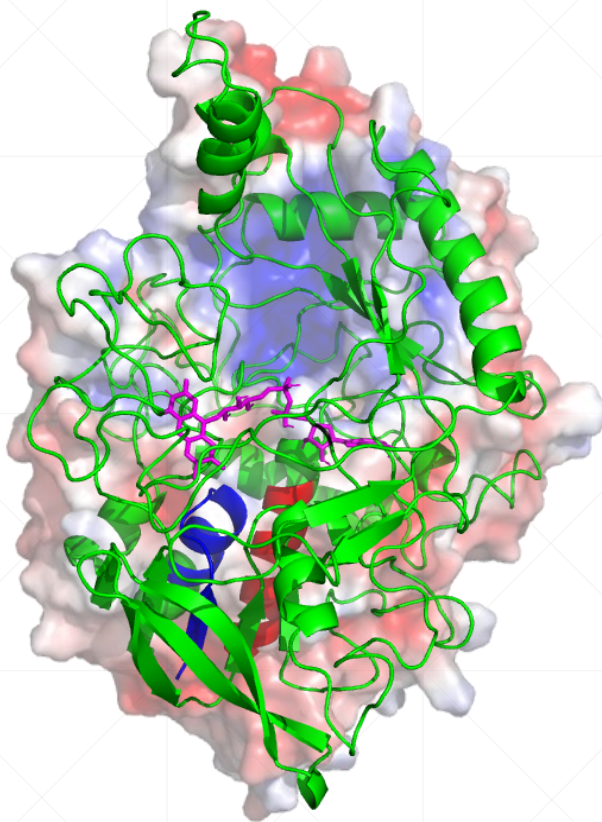
- Redox active enzyme

Surface functionalization

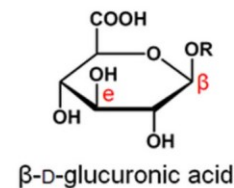
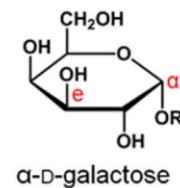
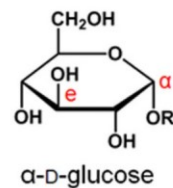
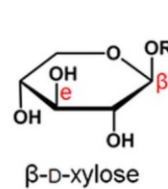
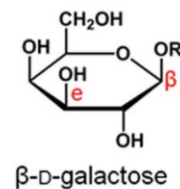
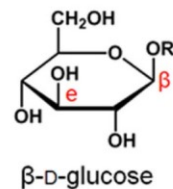
- Self-assembled monolayers (SAMs)
- Au-SH covalent bond



(FAD)-dependent glycoside oxidoreductase



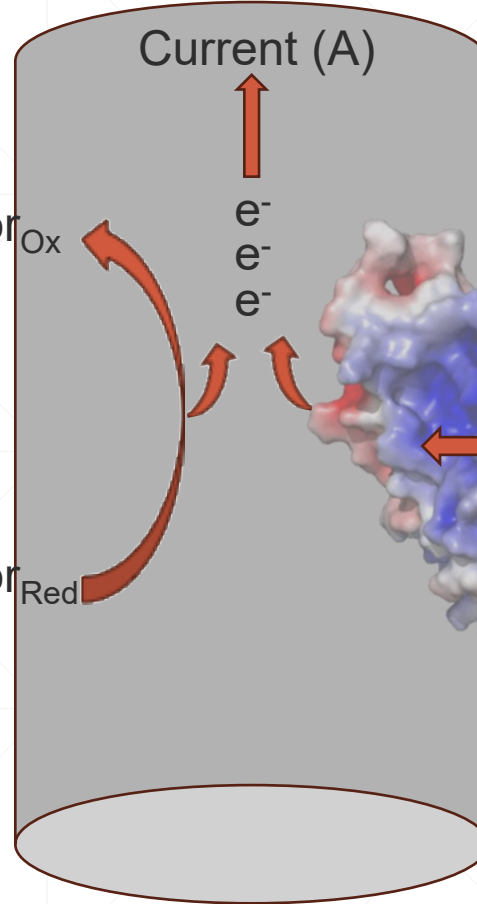
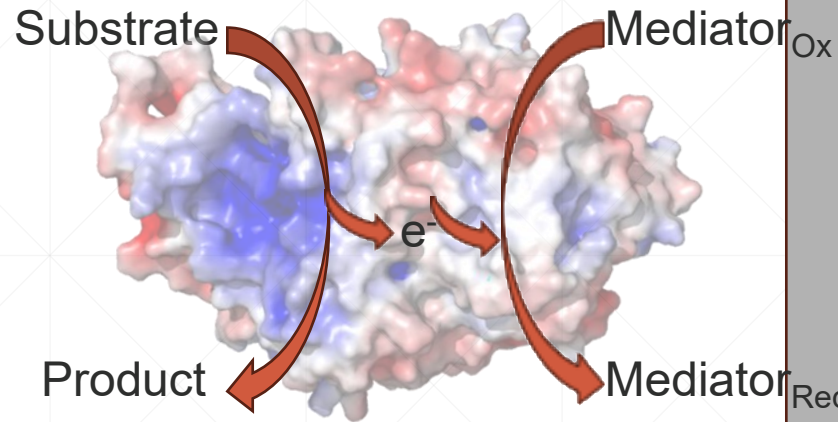
- Redox enzyme isolated from *Rhizobium* sp. GIN611
 - Gram-negative soil bacteria
- Large substrate spectrum
 - Accepts different glycosidic substrates
 - More specific for glycone part then aglycone
- Performs oxidation reaction of hydroxyl group at C3 position



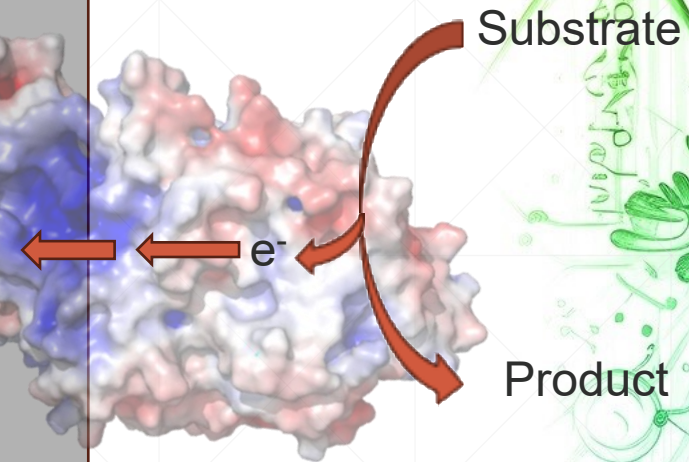
Spectrum of some of the potential glycone substrates⁴

Enzyme electrode work principle

Mediated Electron Transfer (MET)



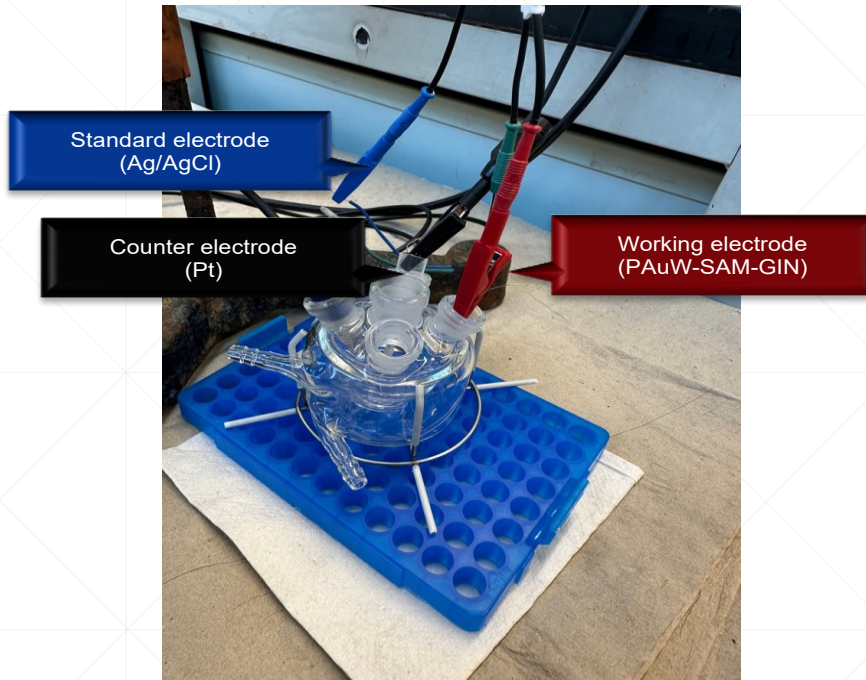
Direct Electron Transfer (DET)



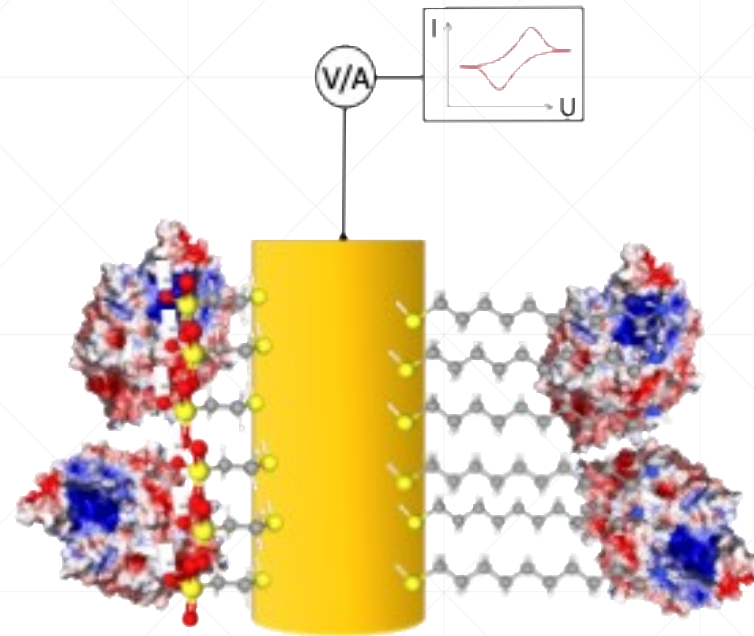
Electron tunnelling
distance 2 nm!!!



Biosensor based on planar gold electrode, modified with SAMs and immobilized GlycOXO



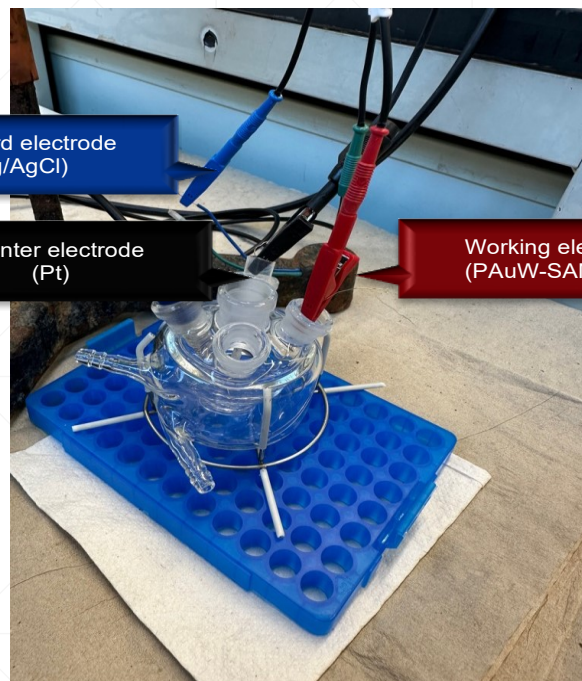
Three electrode set-up



Scheme of the working electrode



Biosensor based on planar gold electrode, modified with SAMs and immobilized GlycOXO

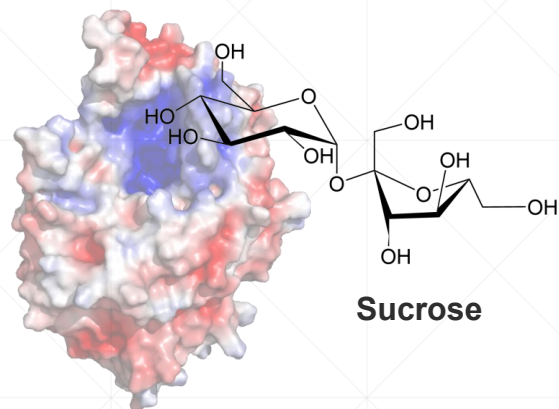


Standard electrode
(Ag/AgCl)

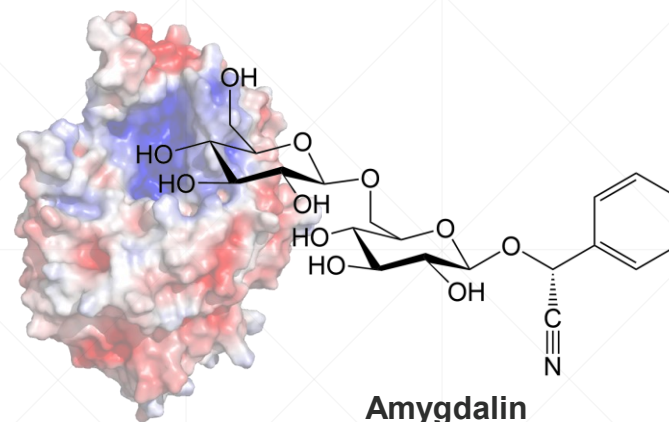
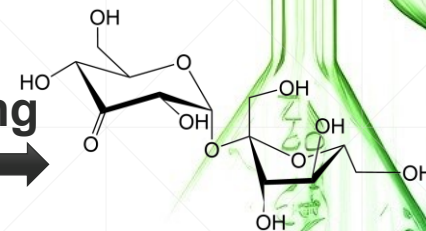
Counter electrode
(Pt)

Working electrode
(PAuW-SAM-GIN)

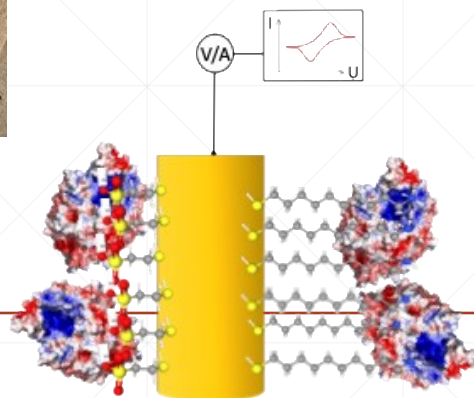
Three electrode set-up



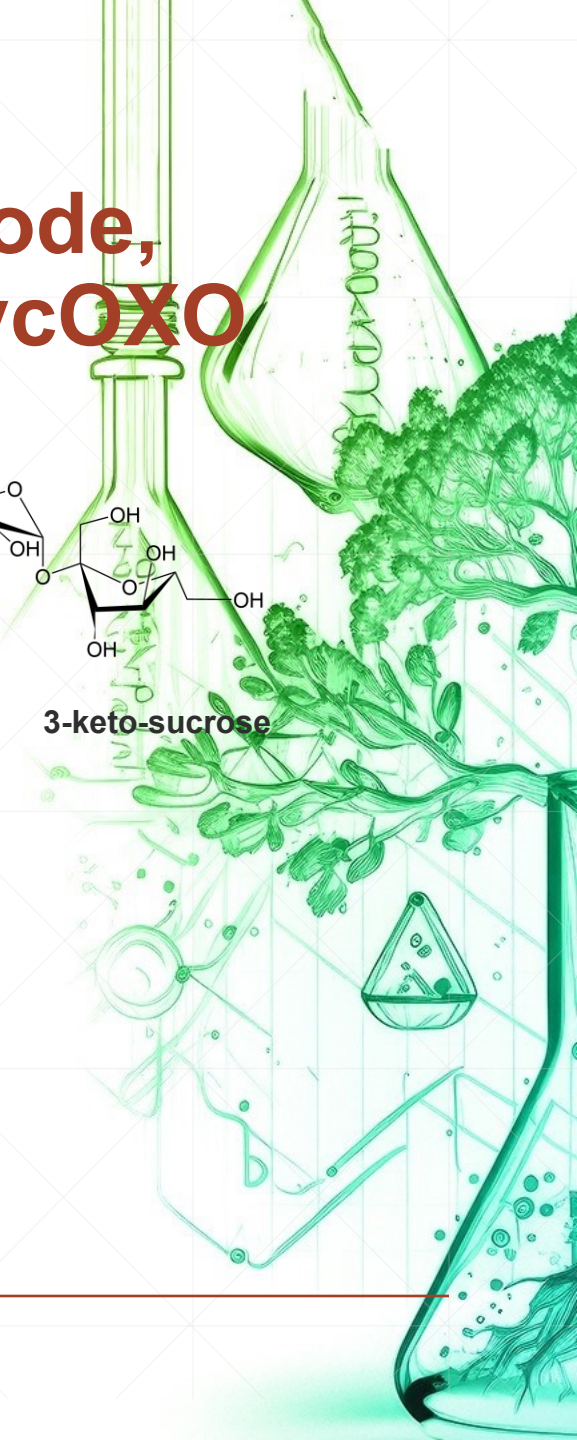
~25 U/mg



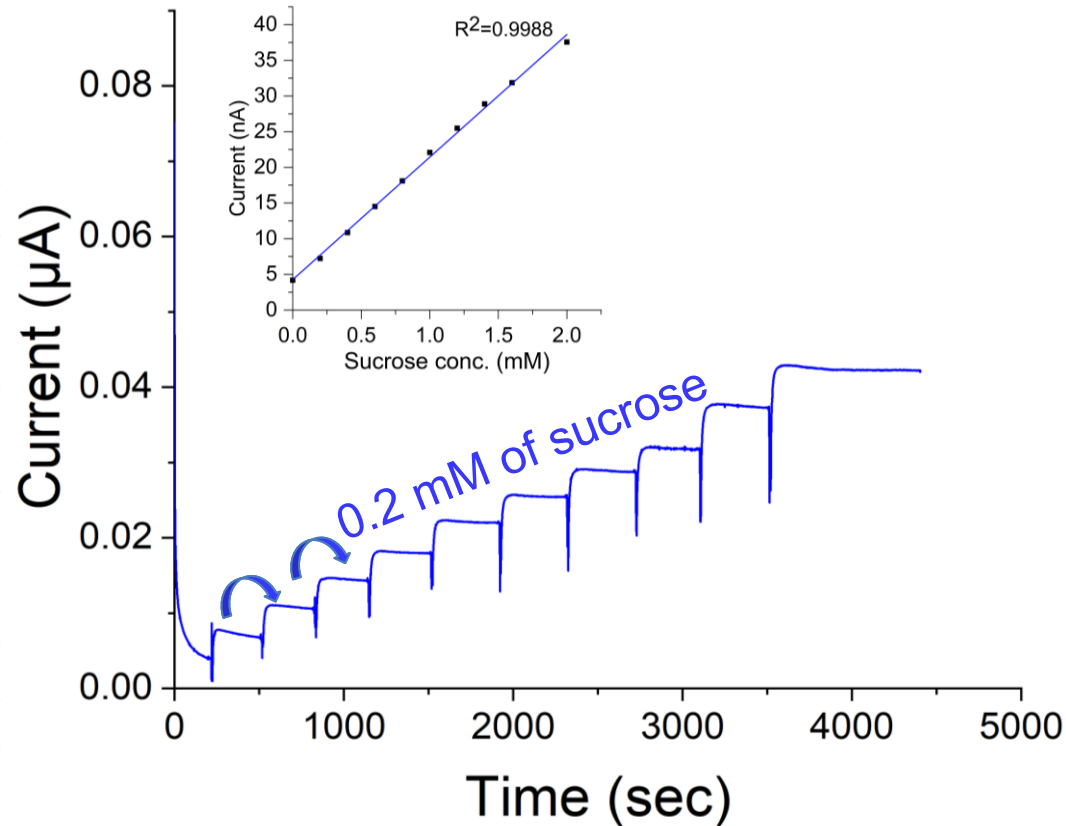
~50 U/mg



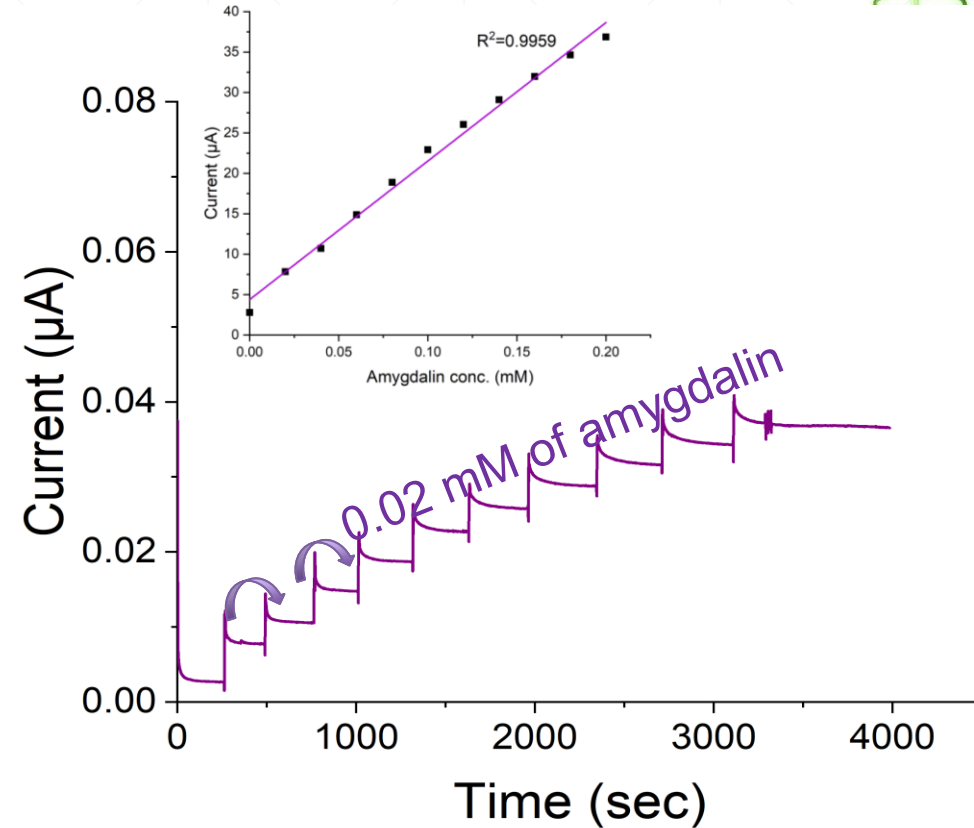
Scheme of the working electrode



Biosensor based on planar gold electrode, modified with SAMs and immobilized GlycOXO



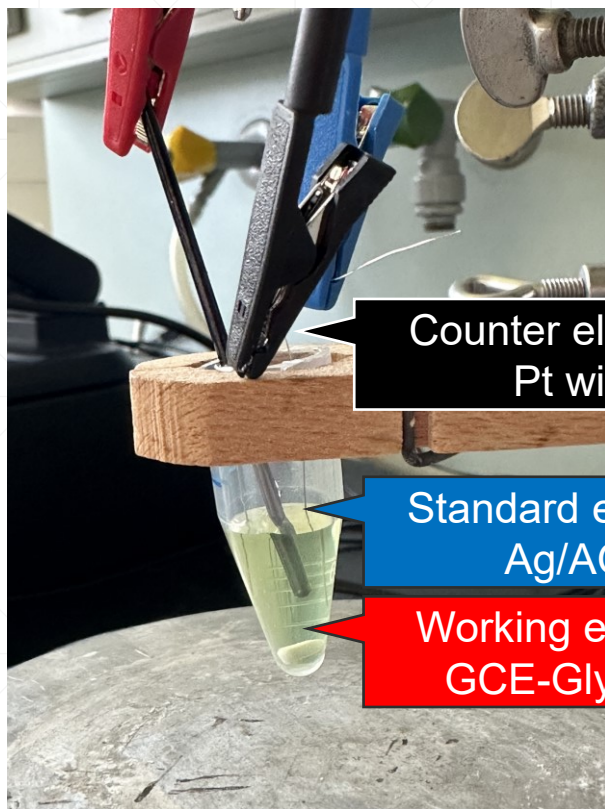
Chronoamperometry chart of PAuW-SAM-GlycOXO – sucrose biosensing; insert chart – linearity in the range of 0.2-2mM



Chronoamperometry chart of PAuW-SAM-GlycOXO – amygdalin biosensing; insert chart – linearity in the range of 0.02-0.2mM

Higher specific activity makes the system more sensitive!

Bioelectrocatalysis – sucrose conversion to 3-ketosucrose



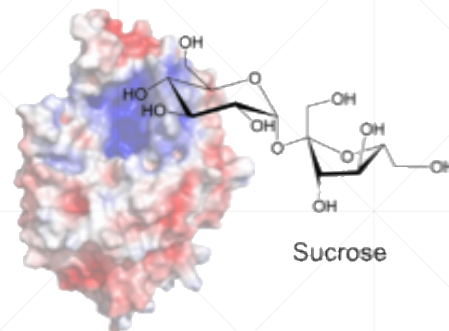
Counter electrode
Pt wire

Standard electrode
Ag/AGCl

Working electrode
GCE-GlycOXO



Enzyme drop casting on GCE

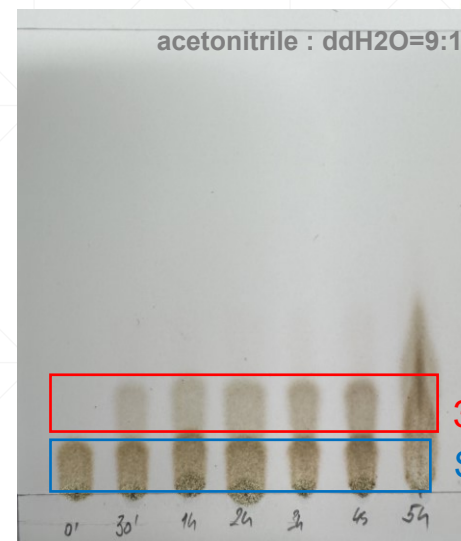


Reaction mixture:

- 40mM sucrose
- 10mM $K_3[Fe(CN)_6]$
- 100mM KPi buffer (pH 6.0)

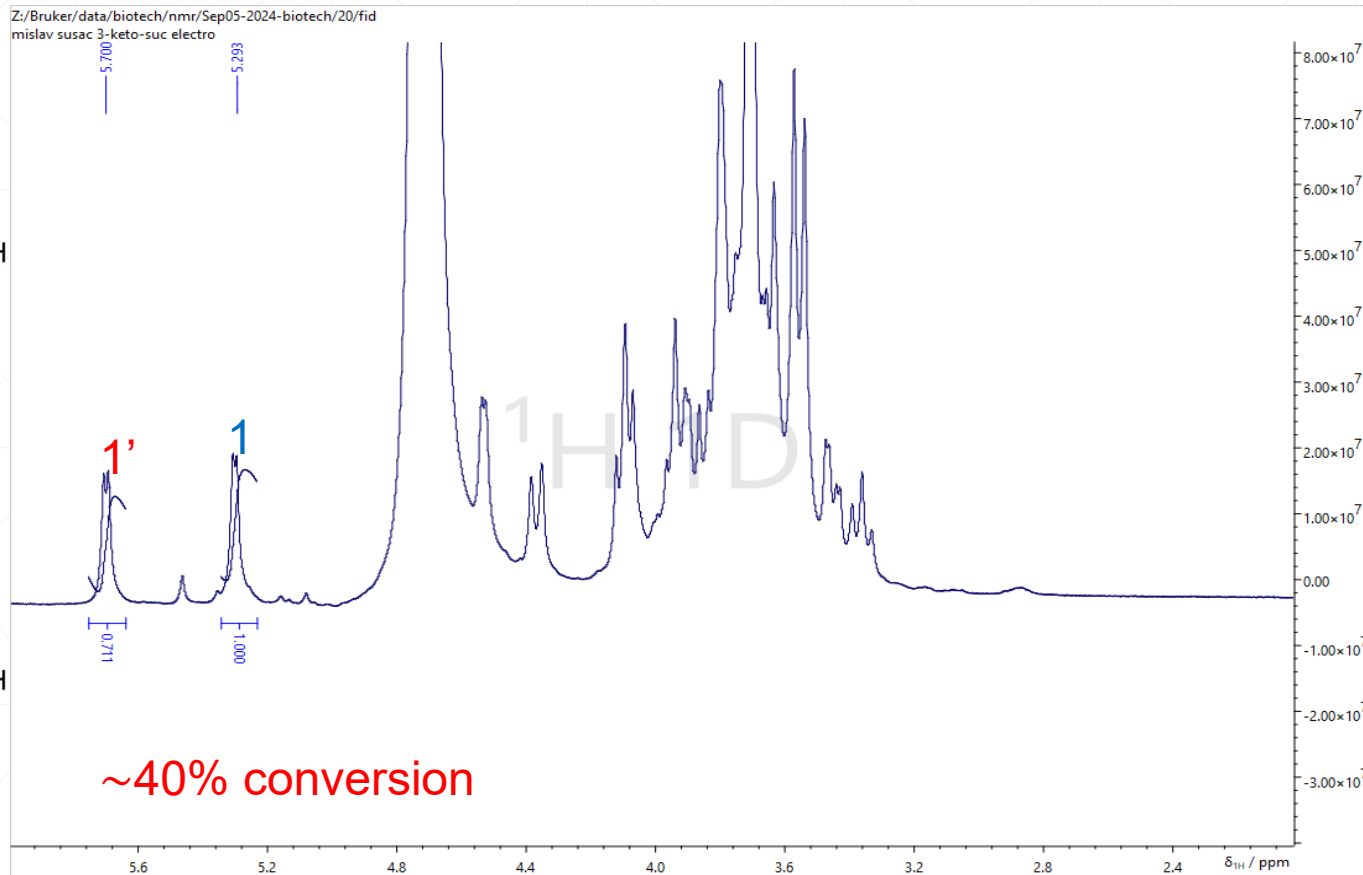
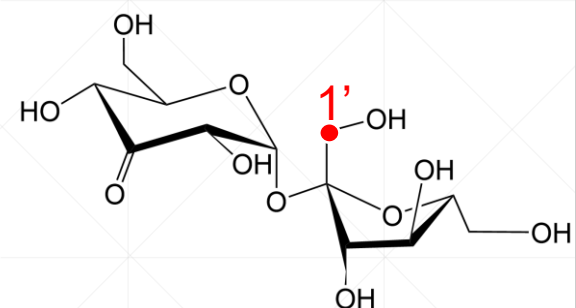
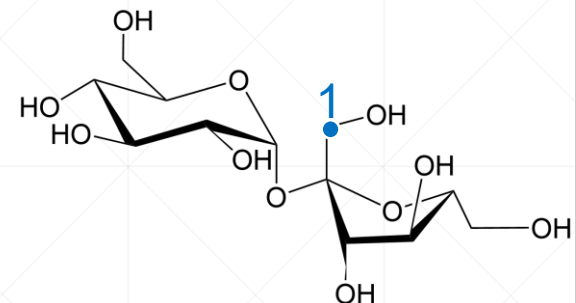
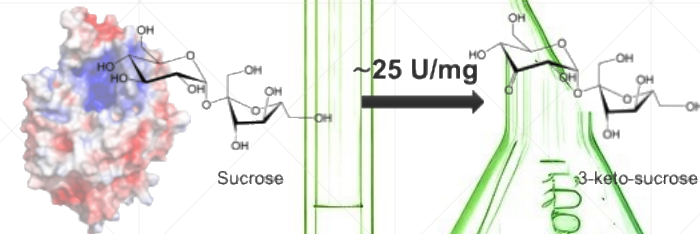
Conditions:

- $E_{dc} = 0.6V$
- $35^\circ C$
- 100rpm stirring
- 24h



TLC of reaction time points

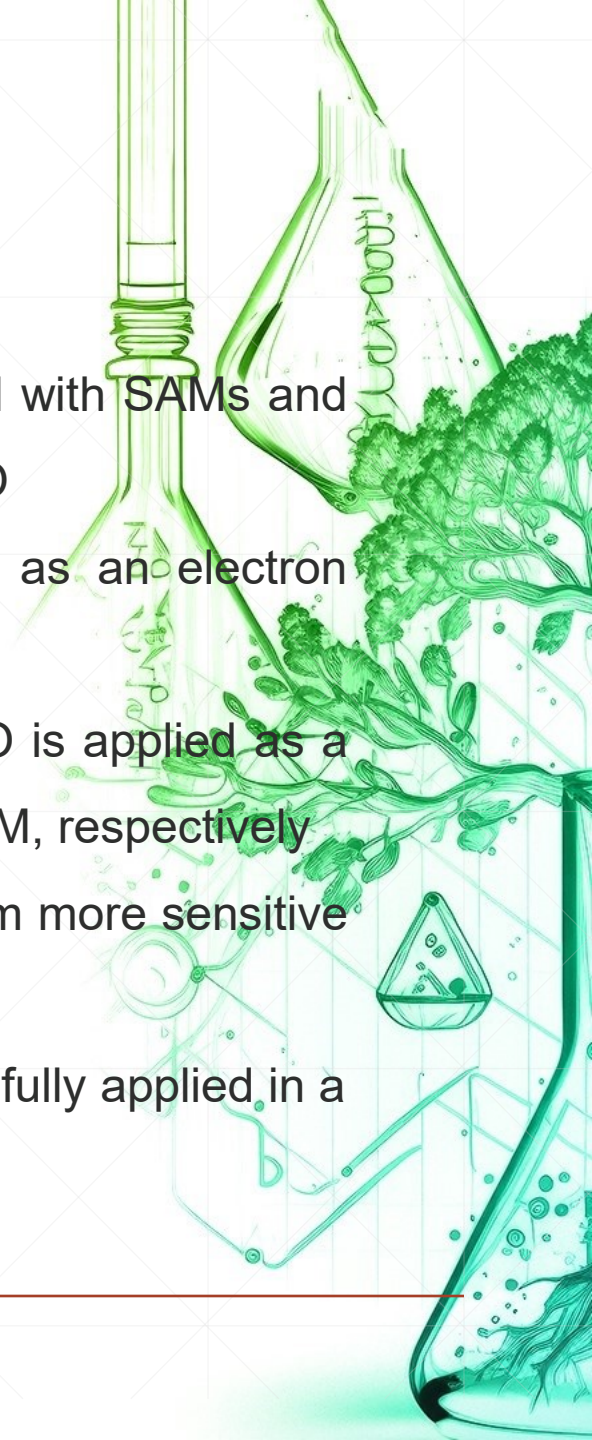
Bioelectrocatalysis – sucrose conversion to 3-ketosucrose



¹H NMR spectrum of reaction mixture after 24h

Conclusions

1. Successful assembly of two types of enzyme electrode; planar gold modified with SAMs and immobilized GlycOXO and glassy carbon electrode drop casted with GlycOXO
2. Mediated electron transfer is achieved by utilizing potassium ferricyanide as an electron mediator
3. System based on planar gold modified with SAMs and immobilized GlycOXO is applied as a biosensor for low sucrose and amygdalin detection, 0.2-2 mM and 0.02-0.2 mM, respectively
4. Higher specific activity for amygdalin, compared to sucrose, makes the system more sensitive
- allowing the detection of lower concentrations
5. System based on glassy carbon electrode with drop-cast GlycOXO is successfully applied in a bioelectrocatalytic sucrose conversion
6. Approximately 40% conversion yield was achieved



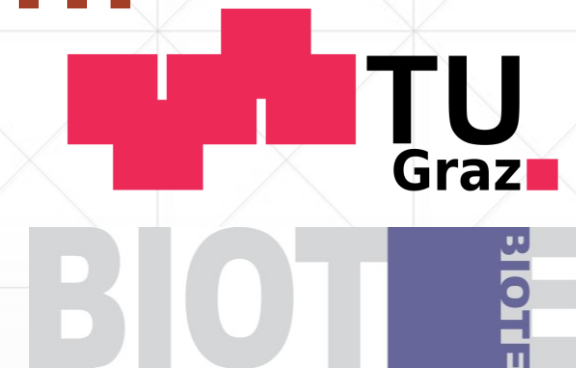
Thank you for your attention!

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Questions?

