Supplementary Information

Bioengineering a glucose oxidase nanosensor for near-infrared continuous glucose monitoring

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Table S1: *A. niger* wild type GOx genes from GenBank. The signalling sequence is indicated in bold and was replaced by a deletion variant of the *S. cerevisiae* mating factor alpha signal sequence for expression by *K. phaffii.*

>gi|2357|emb|CAA34197.1| Glucose oxidase protein form *Aspergillus niger*

MQTLLVSSLVVSLAAALPHYIRSNGIEASLLTDPKDVSGRTVDYIIAGGGLTGLTTAARLTENPNISVLVIESGSY
ESDRGPIIEDLNAYGDIFGSSVDHAYETVELATNNQTALIRSGNGLGGSTLVNGGTWTRPHKAQVDSWETVFG
NEGWNWDNVAAYSLQAERARAPNAKQIAAGHYFNASCHGVNGTVHAGPRDTGDDYSPIVKALMSAVEDRG
VPTKKDFGCGDPHGVSMFPNTLHEDQVRSDAAREWLLPNYQRPNLQVLTGQYVGKVLLSQNGTTPRAVGVE
FGTHKGNTHNVYAKHEVLLAAGSAVSPTILEYSGIGMKSILEPLGIDTVVDLPVGLNLQDQTTATVRSRITSAGA
GQGQAAWFATFNETFGDYSEKAHELLNTKLEQWAEEAVARGGFHNTTALLIQYENYRDWIVNHNVAYSELFL
DTAGVASFDVWDLLPFTRGYVHILDKDPYLHHFAYDPQYFLNELDLLGQAAATQLARNISNSGAMQTYFAGET
IPGDNLAYDADLSAWTEYIPYHFRPNYHGVGTCSMMPKEMGGVVDNAARVYGVQGLRVIDGSIPPTQMSSHV
MTVFYAMALKISDAILEDYASMQ

Table S2: Synthetic double stranded DNA primers used for preparation of mutated GOx expression plasmids

Primer	Primer sequence (5' to 3')	Size (bp)
K13C-F	GGA CTG ACC CTT GTG ACG TCT CAG GTC	27
K13C-R	GAC CTG AGA CGT CAC AAG GGT CAG TCC	27
D70C-F	GAA TGC TTA TGG TTG TAT CTT CGG ATC TTC	30
D70C-R	GAA GAT CCG AAG ATA CAA CCA TAA GCA TTC	30
A418C-F	GAC CTG AGA CGT CAC AAG GGT CAG TCC	27
A418C-R	GTT CCT TGA CAC TTG TGG TGT CGC TTC	27
H446C-F	GGA CCC ATA CCT TTG TCA CTT CGC TTA CG	29
H446C-R	CGT AAG CGA AGT GAC AAA GGT ATG GGT CC	29

Table S3: Cell culture media used for *E. coli* and *P. pastoris* culturing

Medium	**Preparation protocols**
LB growth medium	10 g of tryptone (Sigma), 5 g of yeast extract (Becton Dickinson and Company), and 10 g of NaCl (Sigma) dissolved in dH_2O in a 1 L bottle. Next, adjusted pH to 7.0 and sterilize in an autoclave.
LB growth medium and agar	Agar added to the LB growth medium at concentration 15 g L^{-1}.
YPD growth medium	10 g of yeast extract, 20 g of peptone (Becton Dickinson and Company) were dissolved in 900 mL dH20 and autoclaved. 100 mL of sterile 220 g L^{-1} dextrose (Sigma) were added to the solution.
YPD growth medium and agar	15 g L^{-1} of agar were dissolved in the YPD growth medium.
BMD 1% growth medium	In a 1 L bottle was mixed sterile solutions of 50 mL of 220 g L^{-1} dextrose (Sigma), 200 mL of 1M K_2PO_4 buffer at pH 6, 100 ml of 134 g L^{-1} of yeast nitrogen base w/o amino acids (Becton Dickinson and Company), and 2 mL of 200 µg mL^{-1} Biotin (Fluka Chemia AG) filtered through a sterile 0.2 µm porous filter. The bottle filled with sterile dH_2O up to the 1 L mark. Zeocin (InvivoGen-Eubio) added at concentration 100 µg mL^{-1}.
BMM2 (1% methanol) medium	In a 1 L bottle was mixed 10 mL of methanol (Carl Roth GmbH), 200 mL of 1M K_2PO_4 buffer at pH 6, 100 mL of 134 g L^{-1} of yeast nitrogen base yeast nitrogen base w/o amino acids, 2 mL of 200 µg mL^{-1} Biotin filtered through a sterile 0.2 µm porous filter. The bottle filled with sterile dH_2O up to the 1 L mark. Zeocin added at concentration 100 µg mL^{-1}.
BMM10 (5% methanol) medium	In a 1 L bottle was mixed 50 mL of methanol, 200 mL of 1M K_2PO_4 buffer at pH 6, 200 mL of 134 g L^{-1} of yeast nitrogen base yeast nitrogen base w/o amino acids, 2 mL of 200 µg mL^{-1} Biotin filtered through a sterile 0.2 µm porous filter. The bottle filled with sterile dH_2O up to the 1 L mark. Zeocin added at concentration 100 µg mL^{-1}.

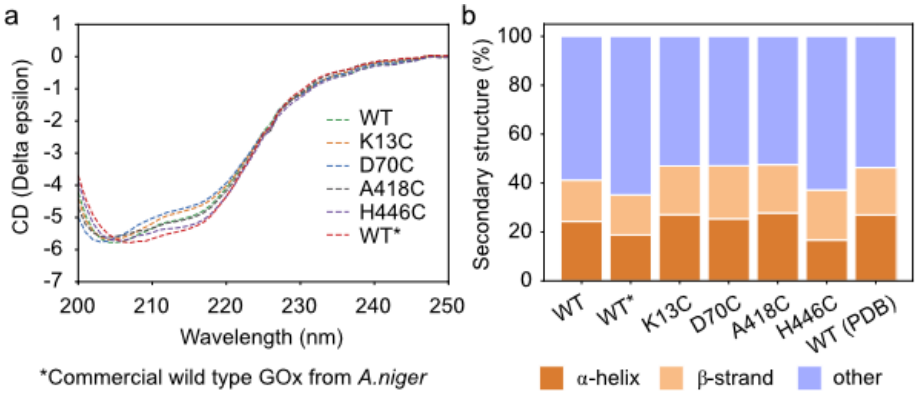

Fig. S1: (a) Fitted CD spectra of GOx variants using a secondary structure prediction software BeStSel. **(b)** Distribution of BeStSel-predicted percentage of structure features (α-helixes and β-strands) in the GOx variants. The variants are compared to the crustal structure from the PDB 1CF3 code. A star symbol indicate the sample which was made of commercial GOx obtained from *A. niger*.

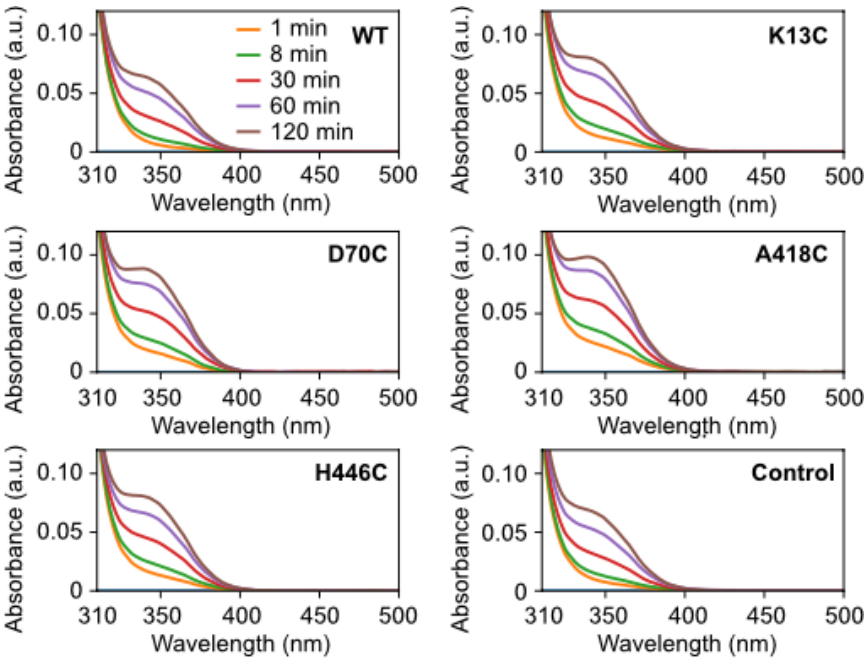

Fig. S2: UV-Vis absorption spectra after addition of SPDP to the GOx solutions measured at 1, 8, 30, 60, and 120 minutes time points. Spontaneous degradation of SPDP was measured in PBS (control). The absorbance spectra measured before addition of SPDP were subtracted for each measurement.

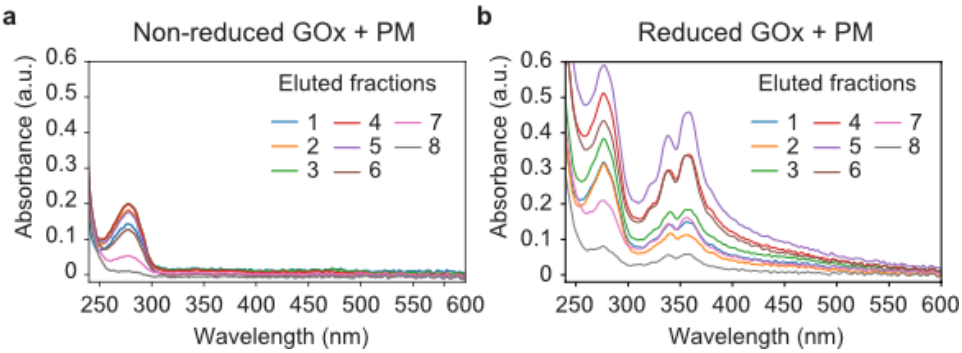

Fig. S3: Absorbance of the elution fractions (150 µl each fraction) collected from a desalting column after conjugation of non-reduced (a) and reduced (b) GOx to the PM crosslinker.

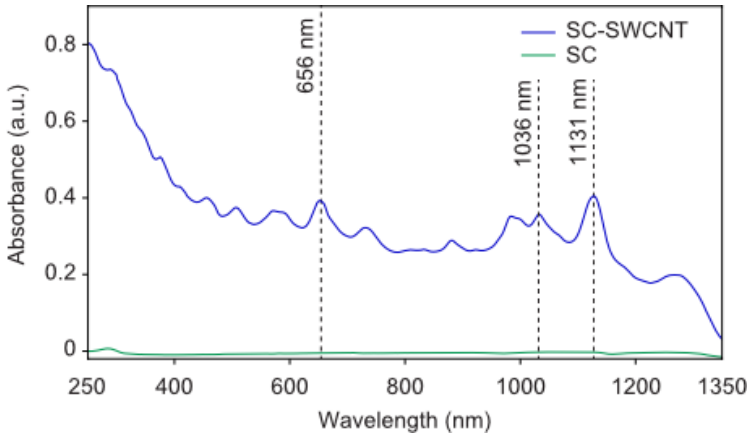

Fig. S4: Absorbance spectra of SC-SWCNTs (1% SC) and 1% SC.

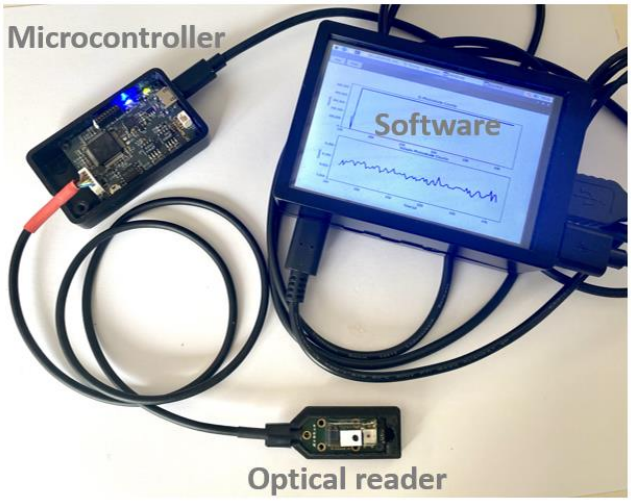

Fig. S5: Photo of the miniaturized device for continuous glucose monitoring.