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Supplemental Information

Diurnal *in vivo* xylem sap glucose and sucrose monitoring using implantable organic electrochemical transistor sensors

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Supplemental Information

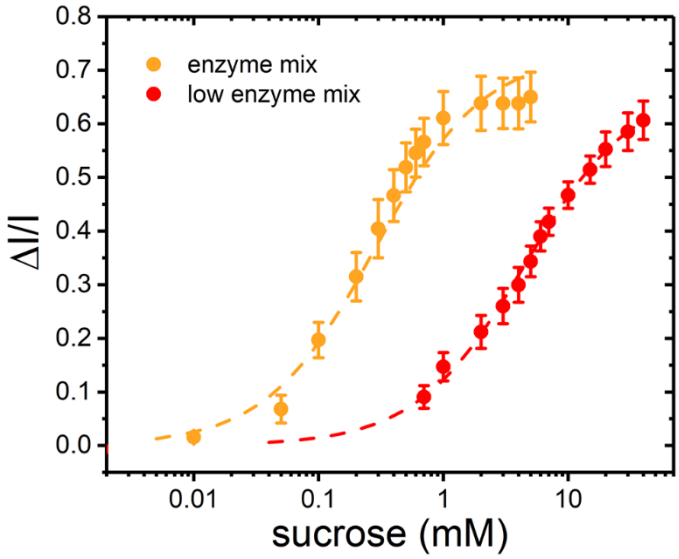

Figure S1: Calibration curves of sucrose sensors with different dynamic range in PBS buffer (Related to Figure 2)**.** Normalized response of sucrose sensors optimized for sucrose detection from 10µM to 1mM (orange) and from 1mM to 40mM (red). Dashed lines represent the sigmoid fit function. Error bars represent the standard error, n=8 for orange curve and n=5 for red curve.

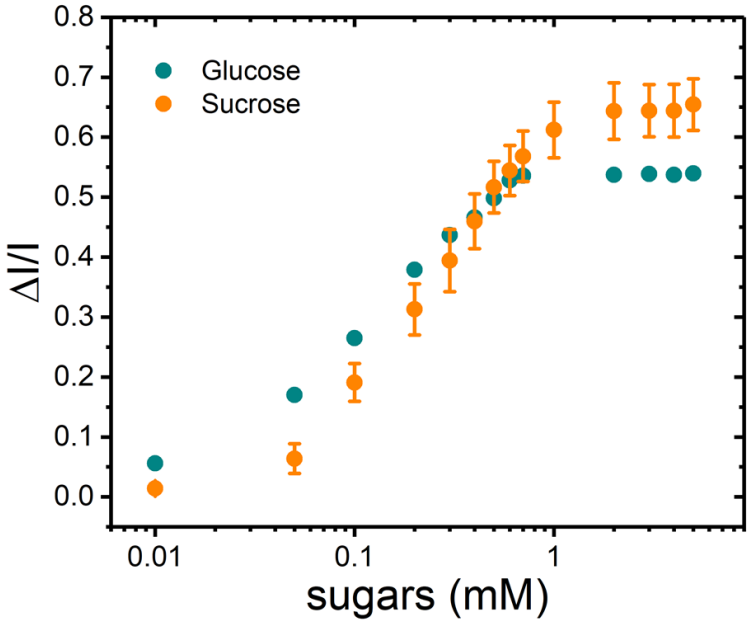

Figure S2: **Calibration curves of sucrose sensor for sucrose (orange) and glucose (cyan) solutions** (Related to Figure 2)**.** Device responses are the same for glucose solution and sucrose solution due to the presence of same amount of glucose oxidase.

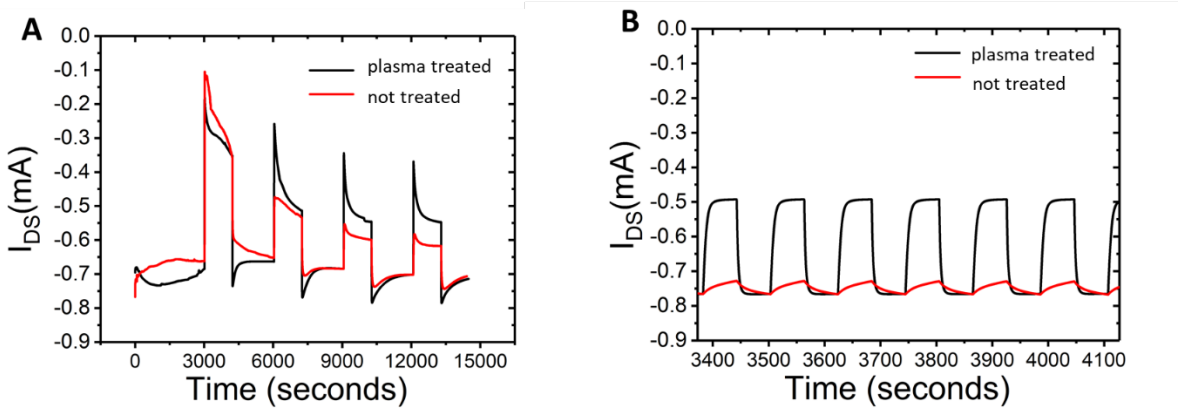

Figure S3: OECT sucrose sensors drain current I_{DS} response in the xylem tissue and effect of hydrophilicity (Related to Figure 5)**.** (A) A pulse gate voltage was applied (V_{GS} = (0V, +0.5V), V_{SD} = −0.4 V) right after insertion in the xylem tissue in device that was treated with oxygen plasma (black) and non-treated device (red). (B) Same characterization as in (a) performed after 24h from the insertion time. The non-treated device shows poor modulation.

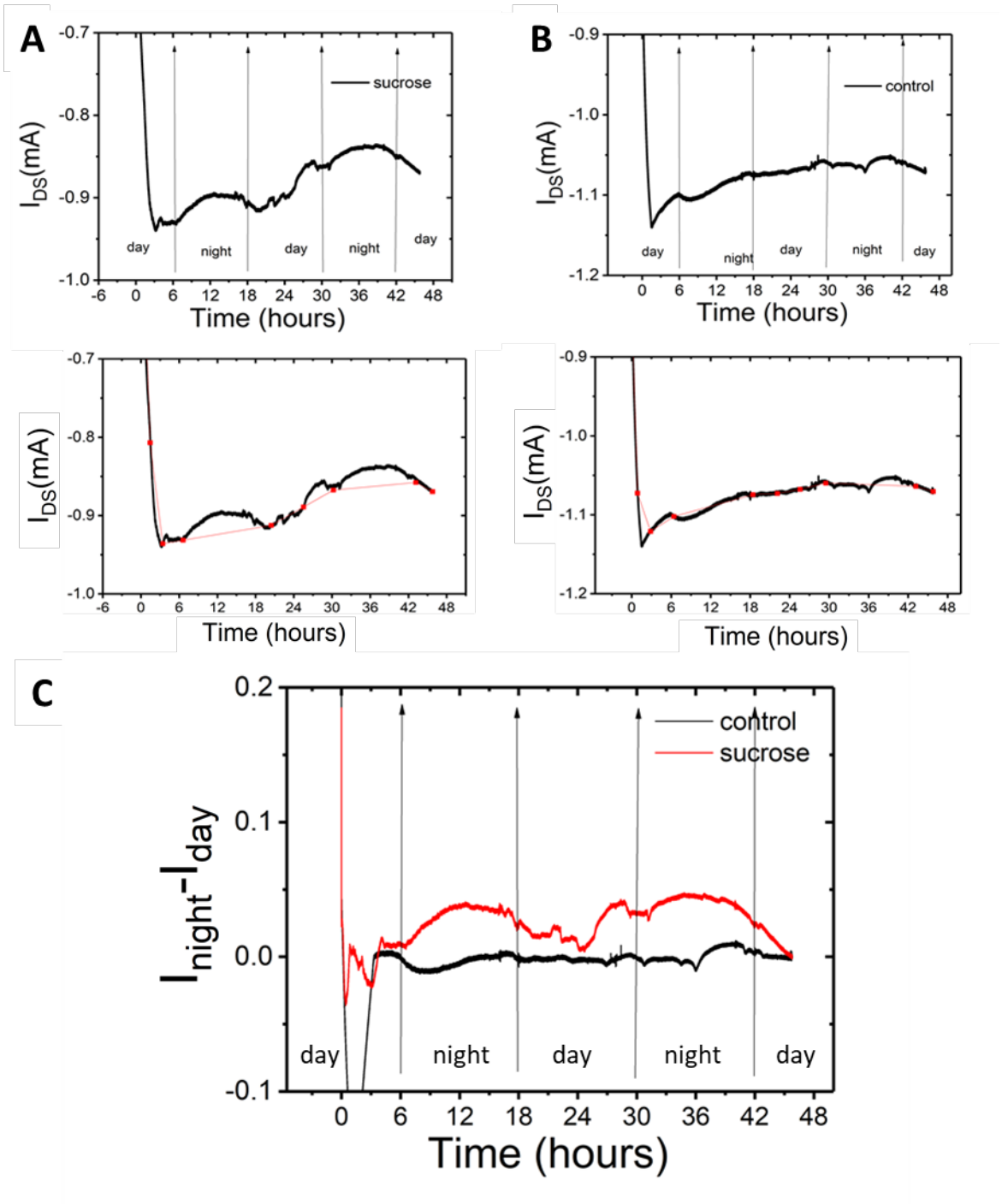

Figure S4: Baseline correction for in-vivo sensing measurements (Related to Figure 5)**.** The drain current of sucrose sensor (A) and control device (B) was recorded for 48 hours in the xylem tissue. The baseline for both measurements was defined as the drain current values during day-time, I_{day}. (C) Corrected current temporal response (I_{night}-I_{day}) for 48 hours for sucrose sensor and control device.

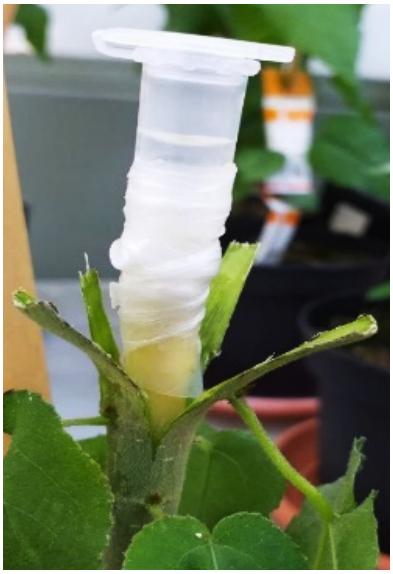

Figure S5: Xylem sap root pressure exudate collection from aspen stem (Related to Figure 5)**.**

Table S1: Soluble sugar concentrations during day as determined from ex-vivo xylem sap analysis (Related to Figure 5)

Day	**mM Glucose ± SD**	**mM Fructose ±SD**	**mM Sucrose ± SD**
Sample 1	0.30± 0.01	0.34± 0.0	0.43± 0.01
Sample 2	0.05± 0.01	0.06± 0.0	0.02± 0.01
Sample 3	0.1± 0.01	0.1± 0.0	0.16± 0.0
Sample 4	0.16± 0.01	0.21± 0.0	0.34± 0.0
Sample 5	0.22± 0.01	0.25± 0.0	0.27± 0.0
Average	**0.17±0.1**	**0.19±0.11**	**0.24 ± 0.16**

Table S2: Soluble sugar concentrations during night as determined from ex-vivo xylem sap analysis (Related to Figure 5)

Night	**mM Glucose ± SD**	**mM Fructose ±SD**	**mM Sucrose ± SD**
Sample 1	3,91± 0,02	2,92± 0,03	3,86± 0,05
Sample 2	2,95± 0,04	2,01± 0,05	5,79± 0,25
Sample 3	2,76± 0,04	2,3± 0,03	3,54± 0,09
Sample 4	2,66± 0,01	1,93± 0,01	2,66± 0,08
Sample 5	1,63± 0,02	1,35± 0,01	3,18± 0,25
Average	**2.78± 0.81**	**2.1±0.57**	**3.81±1.20**

Transparent Methods

Device fabrication

For the device fabrication a polyethylene naphtalate foil (Teonex Q65HA, 125 µm, Peutz Folien GMBH) was cut in a circular 4" substrate. The substrate was cleaned with water and acetone, then vacuum backed for 90 s at 120°C. Metal films of 2nm titanium (Ti) and 50 nm gold (Au) were evaporated onto the clean surface. Photolithography (Karl Suss MA/BM 6 mask aligner) and a Shipley 1805 positive resist were used to pattern contacts, wiring, channel and gate. The substrate was then wet etched in I_2/KI solution for Au, and $H_2O_2/NH_4Cl/H_2O$ for Ti. The remaining resist was stripped with acetone. A PEDOT:PSS (Clevios PH1000) mixture with 5% v/v EG (ethylene glycol) and 1% v/v GOPS (3-Glycidyloxypropyl)trimethoxysilane) and dodecylbenzenesulfonic acid (50 µl drop per 5 ml) was spin-coated and patterned using a Shipley 1813 positive resist, then dry etched with CF_4/O_2 reactive ions, in order to create channels and gates. The remaining resist was stripped again with acetone. In the end, the substrate was encapsulated with SU-8 2010 (MicroChem) and openings on the active areas are defined by wet etching with developer mr-Dev 600 (Microresist Techonology). Chemicals were used as received from Sigma-Aldrich unless stated otherwise.

Device functionalization

Pt nanoparticles were deposited onto the PEDOT:PSS gate electrode using a solution of 5 mM H_2PtCl_6 in aqueous 50 mM H_2SO_4, through electrochemical deposition (potentiostat, BioLogic SP-200). Deposition was performed using gate as working electrode and applying a first fixed potential of +0.7 V for 10 seconds and a second fixed potential of -0.2 V for 15 seconds. Two different enzyme mixtures were prepared to functionalize respectively sucrose and glucose sensor. The sucrose sensor mixture was prepared by adding 3 mg/ml Glucose oxidase, 5mg/ml Invertase and 2.5% v/v of Mutarotase Suspension (Wako) in in Phosphate Buffer Saline (PBS,

Thermo Fisher). The glucose sensor mixture was prepared with 3mg/ml Glucose Oxidase only. Enzyme solutions were mixed with a solution of filtered chitosan 5 mg/ml in 50 mM CH_3COOH in a proportion 1:2 and 2,5% v/v of glutaraldehyde 2.5 wt% (Sigma Aldrich). Immobilization was performed by drop-casting 1.5 µl of enzyme/chitosan mixture on the gate electrode. After 30 minutes, the electrode was rinsed with deionized water to remove the remaining CH_3COOH. Chemicals were used as received from Sigma-Aldrich unless stated otherwise.

Arduino measurement units

Each unit was built using an Adafruit Feather M4 Express microcontroller (Adafruit Industries). Each OECT channel (gate and source) circuit used one analog-digital converter (ADC), one digital-analog converter (DAC), and one precision resistor (1 kOhm for source and 1 MOhm for gate). The ADCs and DACs were configured for 12-bit operation, and the sampling times for the ADCs optimized for the expected gate/source impedances (60 µs for source, 340 µs for gate). Two PIDs running on the microcontroller regulated the channel voltages (measured at the corresponding input using the ADCs) to match the setpoints by changing the corresponding output voltage (using the DACs). The PIDs iterated at about 1700 Hz, and for each iteration the resistor voltages, calculated as the difference between channel output and input voltages, were inserted into a digital 10Hz low-pass filters. Measurements were produced at 10 Hz, by transferring the mean resistor voltages over the measurement period by serial communication through a USB cable. A LabView interface was used to send commands and collect data from the three Arduino units, and to convert the resistor voltages to gate and drain currents using Ohm's law and Kirchhoff's current law.

Devices characterization and experimental set-up

Sucrose and glucose dose-curve responses were perfomed in Phosphate Buffer Saline (50 mM pH 7.4) with a Keithley 2600 series Source Meter. All measurements were carried out at room temperature and devices were operated at constant bias mode with V_{DS}= -0.4 V and the V_{GS}= +0.5 V. Devices designated for in vivo measurements were treated with oxygen plasma (Zepto W6) at 50W for 2 minutes, in order to increase surface wettability. In vivo measurements were performed with Arduino units, in the greenhouse environment for period of 24 or 48 hours in four different trees. Devices were connected to the Arduino system through ZIF connectors and inserted subsequently a 3-4 mm scalpel incision into the tree stem. A drop of sodium alginate gel (2% in PBS) was added at the epidermis exactly at the insertion site to prevent drying of the tissue. When the gel dried it formed a seal ensuring that the insertion site was no longer exposed to air.

Plant material and growing conditions

Hybrid aspens (*Populus tremula x tremuloides*) were grown in the greenhouse in a commercial soil/sand/fertilizer mixture (Yrkes Plantjord; Weibulls Horto, http://www.weibullshorto.se) at 20/15 °C (light/dark) with a 18 h light/6 h dark photoperiod and 60% relative humidity. Trees were watered every other day and they were fertilized using approximately 150 ml 1% Rika-S (N/P/K 7:1:5; Weibulls Horto) once a week after planting. 4 hybrid aspen trees (*populus tremula x tremuloide*), 8 weeks old, were then cultivated in day-neutral photoperiodic condition (12 hour light/12 hour dark) under white fluorescent light (250 μmole m-2 s-1) at room temperature with 50-60 % relative humidity. In the sensing experiments 8-16 weeks old trees were used.

Optical microscopy images

For the stem tissue analysis after sensor insertion, cross sections were prepared from OECT insertion sites located at 3mm depth from the surface of the stem as well as control trees without sensor insertion. Then the effect of was followed for 1, 2 and 5 days. A total of 12 trees were assessed, three for each time course and three for control. For each stem five stem cross-sections at 60 µm thickness were prepared using a vibratome apparatus (Leica VT 1000S). The stem sections were then stained in 0.02% (W/V) Toluidine Blue O solution. Samples were observed under a Leica DMi8 inverted optical microscope.

Data analysis

Sucrose and glucose calibration curves (Fig.2C) were calculated by normalizing the drain current I using the equation (1):

$$\Delta I/I = \frac{I_{[M]} - I_0}{I_{[M]}} \qquad (1)$$

Where $I_{[M]}$ is the drain current (I_D) at the *[M]* concentration for sucrose and glucose and I_0 is the drain current at the baseline. The calibration curve s were fitted with a sigmoid function $y = ax/(b + x)$, were a and b are constants.

The in vivo measurements (Figure 5) recorded for 24 and 48 hours were corrected for a baseline obtained using the drain current during day-time with equation (2):

$$I_{corrected} = I_{night} - I_{day} \qquad (2)$$

Where I_{night} is the current for night-time and I_{day} is the current for day-time as described in the SI (Fig. S4). The sucrose sensor trace in Fig.5A is the average of four different experiments. Normalized average variations, Fig.5B $(I_{night} - I_{day})/I_{day}$ for sucrose (n=11), glucose (n=3) and control devices (n=9) were calculated considering current values at the halftime of the measurement for day-time and night-time. For each experiment we always used a new device. For the experiments with a duration of 24 hours we get one set of data for day/night cycle. For the experiments with duration of 48hours we get 2 set of data of day/night cycle from the same device. The bar chart corresponds to the average of the normalized signal $(I_{night} - I_{day})/I_{day}$ both for the 24hours duration experiments and 48hour duration experiments.

Ex-vivo xylem sap collection and analysis

For collecting xylem sap, root pressure exudate method was used (Alexou *et al.*, 2013). Plants were cut at the bottom of trees about 10 cm from soil surface and then 1–2 cm of the bark below the cut site was removed. Then root pressure sap was collected in individual plant at midday and midnight for 1 hour (Figure S5 and Tables S1, S2).

Soluble sugars; Glucose (Glc), fructose (Fru) and sucrose (Suc), in xylem sap were assayed enzymatically (Stitt *et al*., 1989). Briefly, 50 µl of diluted xylem sap was bolide for 10 min and then was used for soluble sugar measurement. Glc, Fru and Suc contents were sequentially quantified in each sample by enzyme-based spectrophotometric assay of $NADP^+$ reduction at 340 nm.

Alexou, M. and Peuke, A. D. (2013) 'chapter 13 Methods for Xylem Sap Collection', *Plant Mineral Nutrients*, 953(July), pp. 195–207.

Stitt, M. *et al.* (1989) '[32] Metabolite levels in specific cells and subcellular compartments of plant leaves', in *Biomembranes Part U: Cellular and Subcellular Transport: Eukaryotic (Nonepithelial) Cells*. Academic Press, pp. 518–552.