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Optical and impedimetric study of genetically modified cells for diclofenac sensing

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Supplementary material

S1. Verification of the photonic transducer

In order to verify the sensitivity of a photonic transducer, the photodiode current was measured in dependence on the concentration of the isolated enhanced green fluorescent protein (eGFP) as well as of *S. cerevisiae* BY4741-p426GPD-eGFP cells that constitutively express eGFP. Additionally, the corresponding fluorescence spectra were recorded.

Protein purification

The recombinant proteins carry a hexa-histidine tag at their carboxylterminus and were purified by immobilized metal ion affinity chromatography (IMAC) on a Ni-NTA column.

Plasmid pET23b-eGFP was transformed into *Escherichia coli* (*E. coli)* expression strain BL21(DE3) pLysS (NEB) for expression of the enhanced green fluorescent protein (eGFP). Purification was carried out applying Ni^{2+} affinity chromatography. Transformed cells were grown in LB medium (1.0% (w/v) peptone, 0.5% (w/v) yeast extract, 0.5% (w/v) sodium chloride, pH 7.4) at 30 °C for 2 h and expression of the fluorescent proteins was induced by addition of 0.4 mM isopropyl-β- D -thiogalactoside. After 4 h, cells were harvested and washed twice using 50 mM Tris/HCl (pH 7.5). Cells were resuspended in 30 ml Tris/imidazole (20 mM Tris, 500 mM NaCl, 5 mM imidazole, pH 7.9), containing 60 mg lysozyme, 1 x protease inhibitor (cOmplete, Roche) 2,500 units Benzonase (Sigma-Aldrich) and incubated for 30 min at 7 °C. The suspension was chilled on ice for 15 min and sonicated (power 70 %, UW-70, Bandelin, Germany). Upon centrifugation (15,000 x g, 30 min, 4 °C) the supernatant was placed onto a His-bind Resin column (Novagen) and purified according to the manufacturer's instruction. Purified proteins were dialysed against PBS at 4 °C for at least 48 h.

Protein determination was carried out with Lowry's test (DC, BioRad) according to manufacturer's instructions utilising VWR V-1200 Spectrophotometer.

Preparation of S. cerevisiae BY4741 p426GPD-eGFP cells

Cells of the *S. cerevisiae* strain BY4741 were grown in YPD media, harvested after 4 h and transformed with 1 µg plasmid p426GPD-eGFP utilising Zymo Research Frozen-Yeast Transformation kit II according to manufacturer's instructions. In order to obtain inactivated yeasts, cells were pelleted by centrifugation and resuspended in the medium-ethanol mixture (50 % (v/v) medium, 35 % (v/v) ethanol, 15 % (v/v) distilled water).

Optical module

The LED light source and the corresponding excitation/emission filter set were chosen in accordance with the excitation/emission spectral ranges of the green fluorescent proteins. The fluorescence of GFP (absorption maximum at 475-

489 nm, emission maximum at 504-510 nm (Patterson et al., 1997; Tsien, 1998; Mujiono et al., 2015)) was excited by a blue LED light source (470 nm, Fig. S1) and detected by an OC808 photodiode with an OG515 long-pass emission filter.

The LED light source was supplied with a constant current of 8 mA at 26 mV. The temperature near the sample was measured using a temperature sensor LM61CIZ (Texas Instruments Inc.). During the operation of the LED light source for 0.5 min, the temperature increase in the sample plane was less than 0.5 K. The experiments were carried out under clean-room conditions at a constant temperature of 21 °C.

Spectroscopy

The LED-excited fluorescence spectra were recorded with a USB2000 spectrometer (Ocean Optics Inc., USA), operating in transmission mode, with the corresponding *SpectraSuite* software. The spectrometer has the following specifications: optical resolution ~0.3 to 10.0 nm FWHM (full width at half maximum) (depending on grating and size of entrance aperture), detector range 200 to 1.100 nm, integration time 3 ms to 65 s. The dark and reference spectra were recorded in darkness using an integration time of 100 ms and 10 signal repetitions. The background correction was performed by means of the *SpectraSuite* software.

The integration time of 100 ms was chosen to achieve a sufficient peak intensity. The integrated area of the emission band were calculated with OriginPro (Version 9.1G) using the *Peaks and Baseline* analysis. All experiments were performed as biological triplicates. Afterwards, the mean values and standard deviation (SD) were calculated. The concentration dependence of the integrated band area was fitted using the *Fitting* analysis.

The fluorescence spectra of the isolated eGFP with an absorption maximum at 485 nm and an emission maximum at 510 nm are shown in Fig. S2a. The corresponding concentration-dependent values of the integrated peak area are given in Figs. 2b. Likewise, the fluorescence of *S. cerevisiae* BY4741-p426GPD-eGFP cells that constitutively express eGFP, increased with increasing cell number (Fig. S3). Figure S4 illustrates the corresponding values of the photodiode current, obtained for the isolated eGFP (Fig. S4a) and for genetically modified yeast cells expressing eGFP (Fig. S4b) by means of the optical module.

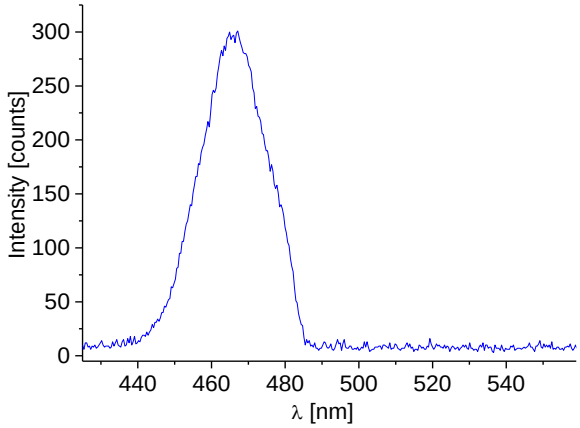

Fig. S1: Spectrum of the used LED light source

a) b)

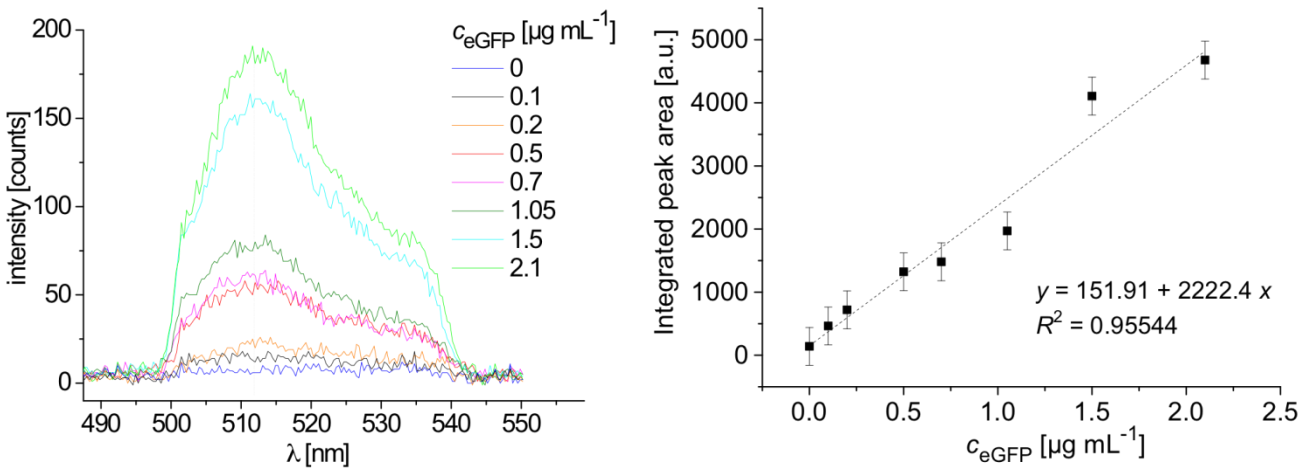

Fig. S2: Fluorescence spectra of isolated eGFP proteins (a) and the corresponding dependence of the integrated peak area on the protein concentration c_{eGFP} in PBS (b). The spectra were recorded by using a spectrometer USB2000, operating in the transmission mode.

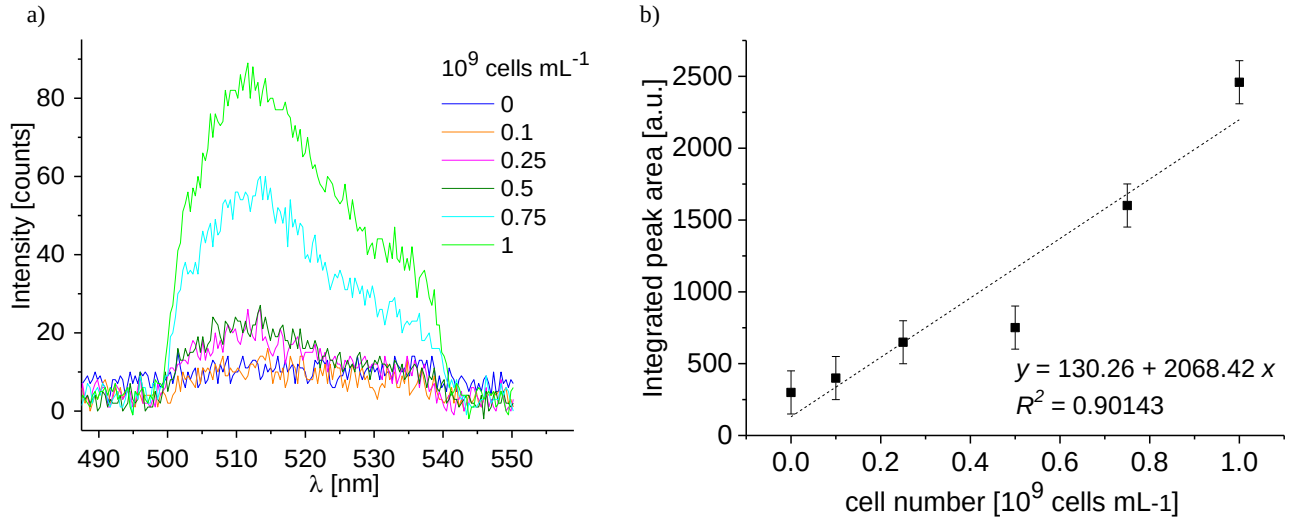

Fig. S3: Fluorescence spectra of inactivated *S. cerevisiae* BY4741 p426GPD eGFP cells, immobilized in gel (a), and the corresponding dependence of the integrated peak area on the cell number in PBS (b). The spectra were recorded by using a spectrometer USB2000, operating in the transmission mode.

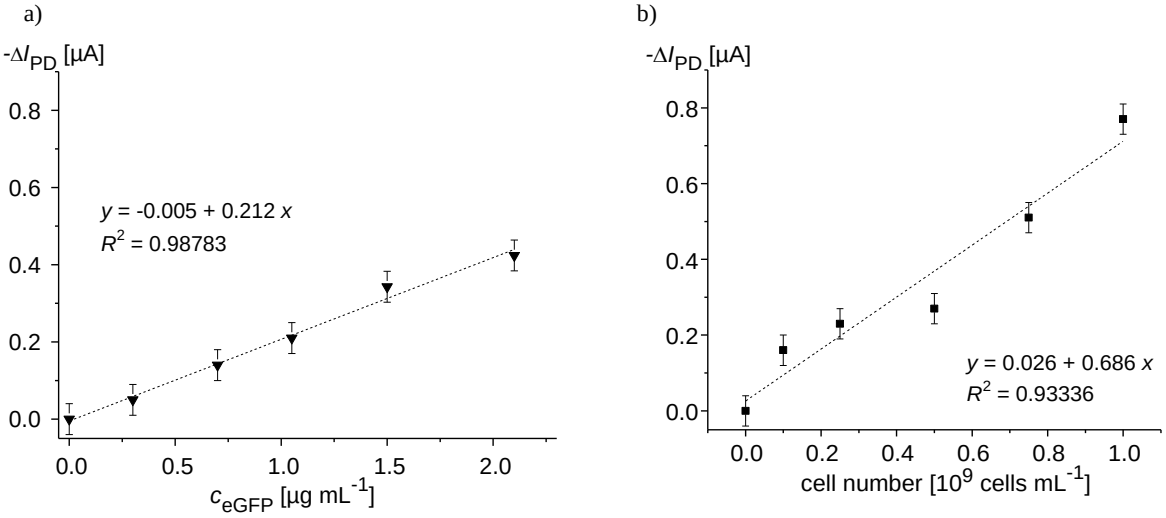

Fig. S4: Change of the photodiode current I_{PD} in dependence on the concentration of eGFP (a) and on the cell number for inactivated *S. cerevisiae* BY4741 p426GPD-eGFP cells, immobilized in gel (b).

S2. ITO interdigitated electrodes

In order to enable a combination of optical and electrical measurements, transparent ITO (indium tin oxide, 0.5 µm thick) interdigital structures for impedance spectroscopy were coated on the B270i glass wafers (wafer thickness (1.0 ± 0.1) mm) (Fig. S5). The prepared structures were optically transparent and conductive. The living cells were immobilized on the glass substrate with two coated ITO-electrode arrays in a four-chamber microfluidic cell.

Two ITO-electrode arrays provided the independent impedance measurements for the measuring and reference channels. Each array consists of 24 fingers (with a finger length L of 11 mm).The distance S between the fingers varies between 2.5 µm and 133.5 µm and the width W between 5 µm and 186 µm (Fig. S5 and Table S1). Using a multiplexer, it was possible to connect the finger structures arbitrarily to form arrays or to operate them individually. The multiplexer board (CiS, Erfurt, Germany) consists of a main board and a needle adapter board. The latter was detachably connected to the main board by two ZIF flex connections. It consists of a 2x12 dual analog multiplexer, 2x24 spring contact pins and 4 micro coax connectors. The main board was connected to an impedance analyzer ISX-3v2 (Sciospec GmbH, Germany). The recorded impedance spectrum of the genetically modified yeast cells at t = 0 h, shown in Fig. S6, were used as a reference.

In order to determine the experimental value of the cell constant K_{cell}, a calibration medium of 0.1 M KCl solution was used, which has the tabled conductivity value of 11.91 mS cm^{-1} at 21 °C. The impedance spectrum of 0.1 M KCl solution at 21 °C is shown in Fig. S6. The cell constant could be calculated as

$$K_{Cell} = R_s \sigma \quad \text{(S1)}$$

using the value of the real part of the impedance at 100 kHz, which corresponds to the solution resistance R_s. The value of the cell constant K_{cell} was determined as 5.4 cm^{-1}.

Table S1: Parameters of the used interdigitated electrode configuration with 24 fingers of width *W* and length *L* = 11 mm, spaced *S* apart.

Finger Nr.	*W*, µm	*S*, µm
1	5	2.5
2	10	7.5
3	18	13.5
4	26	19.5
5	34	25.5
6	42	31.5
7	50	37.5
8	58	43.5
9	66	49.5
10	74	55.5
11	82	61.5
12	90	67.5
13	98	73.5
14	106	79.5
15	114	85.5
16	122	91.5
17	130	97.5
18	138	103.5
19	146	109.5
20	154	115.5
21	162	121.5
22	170	127.5
23	178	133.5
24	186	

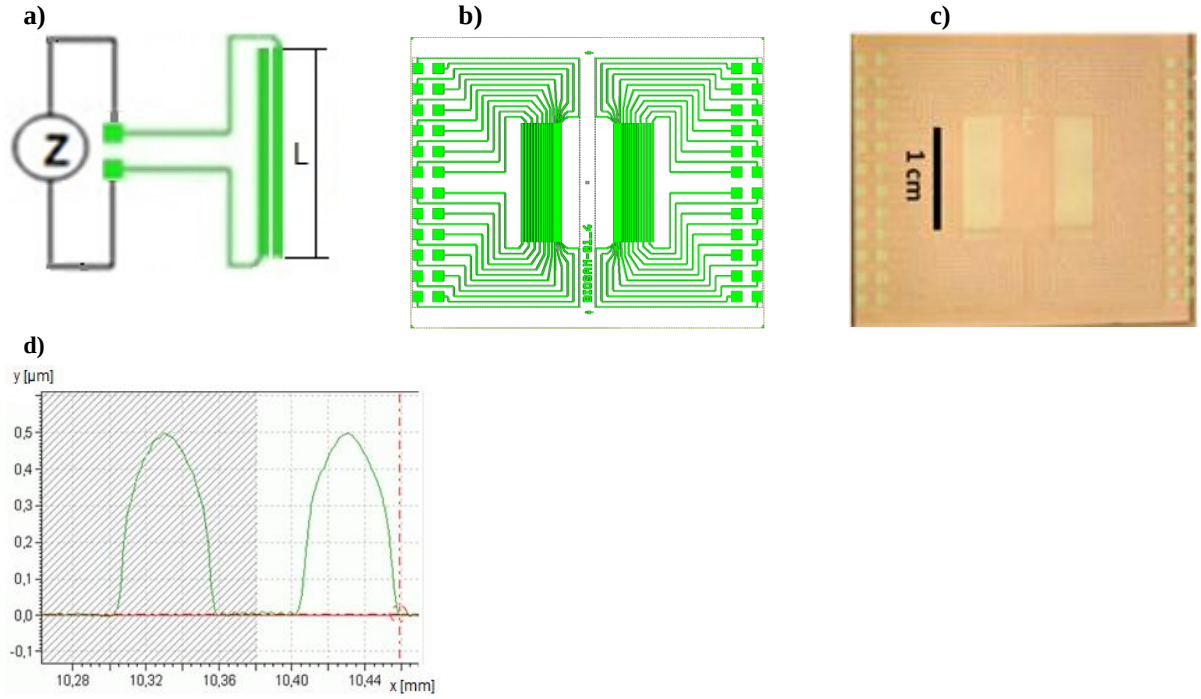

Figure S5: ITO electrode structure: two individually operated electrode fingers (length 1.1 cm, thickness 0.5 µm) (a) on the glass substrate with two electrode arrays (b, c) and their height profile after wet-chemical patterning (d).

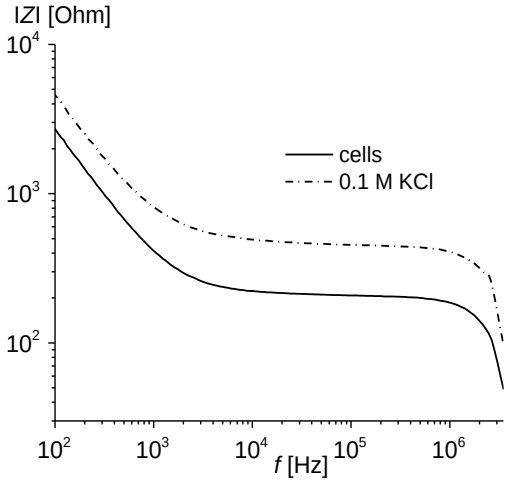

Fig. S6: Impedance spectra of yeast cells *S. cerevisiae* BY4741 p426PDR5-tGFP, immobilized in 0.75 % low-melting agarose, and 0.1 M KCl solution at 21 °C.

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