Electronic Supplementary Material (ESI) for Chemical Communications.

Supplementary Information

Light-controlled imaging of biocatalytic reactions *via* scanning photoelectrochemical microscopy for multiplexed sensing

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METHODS

Materials: N-(2-hydroxyethyl)piperazine-N'-2-ethansulfonic acid (99.5%; HEPES), D-glucose, sodium L-lactate, fluorine-doped tin oxide-coated glass slides (7 Ω cm^{-1}; FTO), lead(II) nitrate (99.999%; $Pb(NO_3)_2$), ammonium sulfide solution (20% in H_2O; $(NH_4)_2S$), polystyrene latex beads (LBs, diameter 0.8 µm) and titaniumtetraisopropoxide (97%; TTIP) were purchased from Sigma-Aldrich (Steinheim, Germany). Poly(ethylene glycol)diglycidyl ether (PEGDGE, M_n = 400 Da) was acquired from Polysciences (Hirschberg, Germany). The poly(1-vinylimidazole-*co*-allylamine)-[Os(2,2'-bipyridine)$_2$Cl]Cl redox polymer (P_{Os}) has been synthesized and purified as reported before.[1] However, ethylene glycol was used as reaction medium instead of more volatile ethanol. This circumvents refilling of evaporated solvent during the reaction. FAD-dependent glucose dehydrogenase (FAD-GDH) from *Aspergillus sp.* has been obtained from SEKISUI CHEMICAL. Lactate oxidase (LOX) from *Pediococcus sp.* was purchased from Fluka (Seelze, Germany).

Fabrication of IO-TiO_2|PbS electrodes: The IO-TiO_2|PbS electrodes have been fabricated as reported previously.[2,3] FTO-coated glass slides have been cleaned by successive ultrasonication in deionized water, isopropanol, and acetone for 15 min each and afterwards dried. A mixture of 100 mg mL^{-1} latex beads (LB; 0.8 µm diameter) and 100 mg mL^{-1} titaniumtetraisopropoxide (TTIP) in isopropanol has been prepared. Therefore, 200 µL of aqueous 100 mg mL^{-1} LB dispersion was mixed with 800 µL ethanol and centrifuged at 25000 g for 8 min in order to remove the water. The supernatant was discarded and the LB pellet has been suspended in 1 mL ethanol followed by a second centrifugation at 25000 g for 8 min. After removing the supernatant, the pellet has been suspended in 180 µL of isopropanol in an ultrasonication bath for 5 min. Finally, 20 µL TTIP has been added fast to the LB suspension, which has been again ultrasonicated for 5 min. Afterwards 4 × 6 µL LB/TTIP-mixture has been dropped onto the cleaned FTO slides and spin coated at 80 rps with a waiting time of 15 s between the deposition steps. The prepared electrodes have been sintered at 450 °C under air for 2 h, resulting in the final 4-layered IO-TiO_2 electrodes. To define the area of the IO-film, rounded or rectangular shape structures have been built up by scratching away the superfluous material. While for the basic characterization of the individual enzyme electrodes and the gradient loading a round shaped structure was used, for the multiplex approach rectangular structures have been used for a better visualization. Except for the different shape, there are no differences between the IO-films. For PbS QD deposition the electrodes have been immersed alternately four times in aqueous 0.02 M $Pb(NO_3)_2$ and aqueous 0.02 M $(NH_4)_2S$ for 1 min each, starting the SILAR process with the Pb^{2+} precursor, following a SILAR approach.[4–6] Between the deposition steps the electrode has been carefully rinsed with deionized water and ethanol in order to remove unbound precursors.

Assembly of redox polymer and enzyme: 1.0 µL of a mixture containing 0.22 mg mL^{-1} PEGDGE, 5.0 mg mL^{-1} P_{Os}, and 5.0 mg mL^{-1} enzyme (FAD-GDH or LOX) in 5 mM HEPES pH 7.0 has been directly drop casted onto the IO-TiO_2|PbS electrode and allowed to incubate for 60 min at room temperature in the dark. Subsequently, the modified electrode has been extensively rinsed with buffer in order to remove any unbound material.

Fabrication of a sample with gradient POs/FAD-GDH loading: an electrode presenting a large circular IO-TiO_2|PbS modified region was used for this purpose. A mixture of 0.75 µL PEGDGE (2 mg mL^{-1}), 2 µL P_{Os} (7.5 mg mL^{-1}), and 2 µL FAD-GDH (5 mg mL^{-1}) was prepared. An aliquot of 1.5 µL of this solution was drop cast over one of the edges of the IO-TiO_2|PbS circular spot. The solution was thus allowed to diffuse by capillary action through the three-dimensional structure, creating a gradient in concentration of immobilized P_{Os}/FAD-GDH. The sample was incubated for 60 min at room temperature in the dark. Finally, the modified electrode was extensively rinsed with buffer in order to remove any unbound material.

SEM experiments: Scanning electron microscopy (SEM) measurements have been performed with a JSM-6510 from JEOL at an acceleration voltage of 30 kV with a 20.000-fold magnification.

Photoelectrochemical experiments: For the localized readout of the samples, scanning photoelectrochemical microscopy (SPECM) was used.[7] Briefly, the setup consisted of a positioning system making use of step-motor driven micrometer screws (Owis, Germany), a potentiostat (PGU-BI 100; IPS-Jaissle, Germany), a Hg-Xe lamp (LC8 type 03; Hamamatsu Photonics, Japan), and an in-house written control software. A Pt microelectrode (25 µm diameter) was coupled with the light source by means of an optical fiber (HITRONIC POF Simplex PE; Lapp Kabel, Germany) and used for local illumination of the samples (glass tip diameter: 530 µm). Prior to each experiment, the microelectrode was precisely positioned at a distance of 30 µm above the surface. For this, an approach curve was recorded following O_2 reduction at the Pt microelectrode polarized at −600 mV *vs.* Ag/AgCl/3 M KCl. Before scan experiments, the same procedure was repeated at three different locations of the sample, enabling to account for any possible substrate tilt and maintaining a constant tip-to-sample distance during the scan. The samples were illuminated using white light (380 nm to 700 nm, see Fig. S5) with an effective light intensity reaching the sample surface of 20 mW cm^{-2}.

SUPPORTING FIGURES

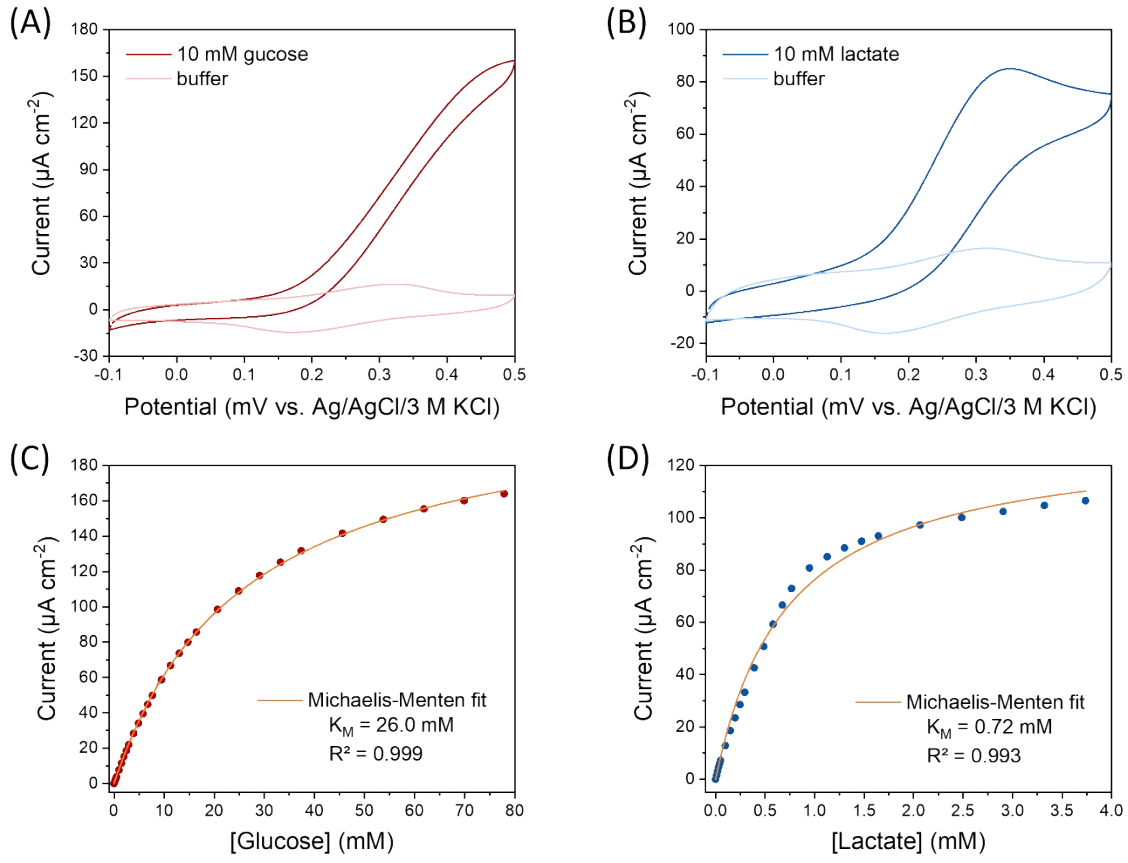

Fig. S1. Cyclic voltammograms (5 mV s^{-1}) in the absence and presence of enzymatic substrates for graphite electrodes modified with P_{Os}/FAD-GDH (A) and P_{Os}/LOX (B). Calibration curves obtained with the P_{Os}/FAD-GDH electrode for additions of glucose (C) and with the P_{Os}/LOX electrode for additions of lactate (D); E_{app} = 500 mV *vs.* Ag/AgCl/3 M KCl. Electrolyte: 5 mM HEPES buffer, pH 7.0. The data shown in panels C and D has been fitted to the Michaelis-Menten model.

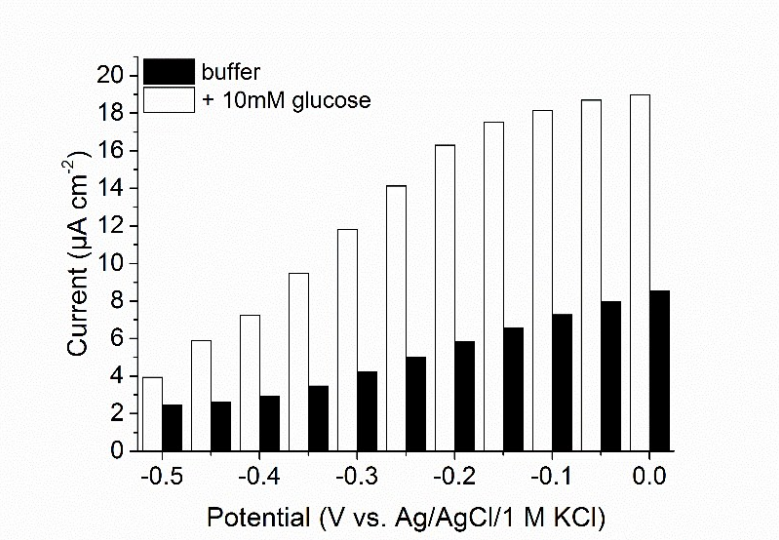

Fig. S2. Photocurrent response obtained with IO-TiO_2/PbS/P_{Os}/FAD-GDH substrates in the absence and presence of glucose at different applied potentials. Electrolyte: 5 mM HEPES buffer, pH 7.0.

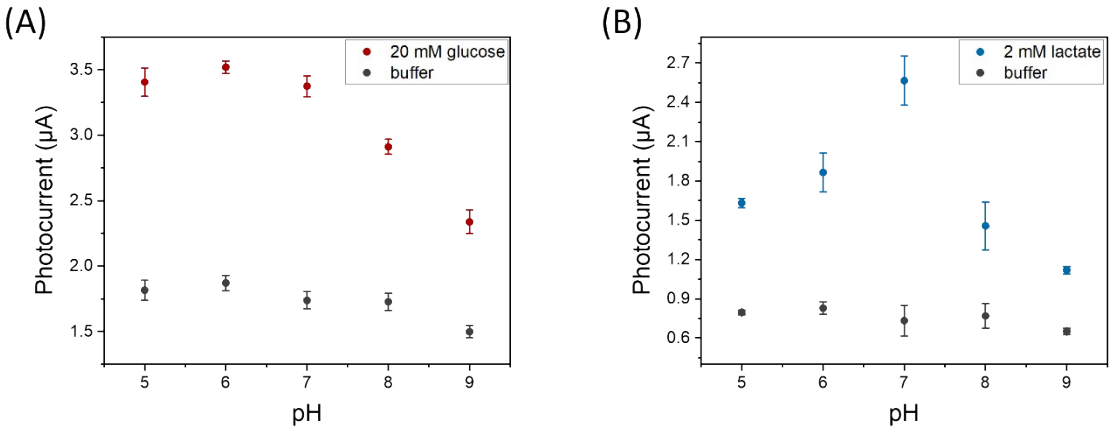

Fig. S3. Influence of the pH value on the photoelectrochemical response obtained for IO-TiO_2/PbS substrates modified with P_{Os}/FAD-GDH (A) or P_{Os}/LOX (B). Photocurrents recorded in the absence and presence of enzymatic substrates at each pH value (*N* = 2). Global illumination using white light.

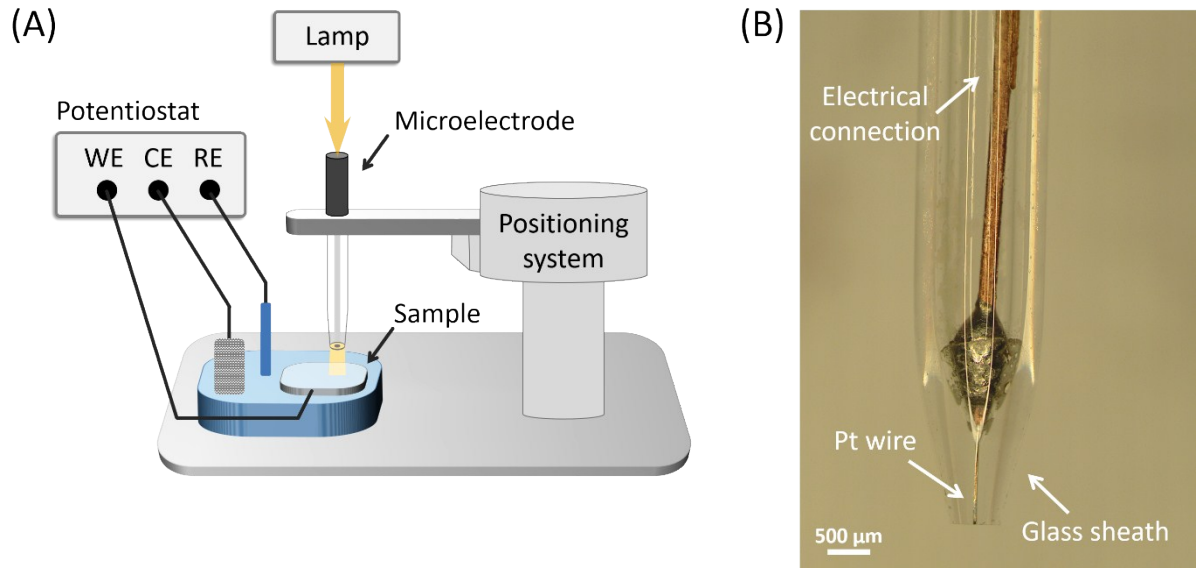

Fig. S4. (A) Schematic representation of the SPECM setup used for the analysis of the fabricated samples enabling local illumination and precise distance control. CE: counter electrode, RE: reference electrode, WE: working electrode. (B) Micrograph of the Pt microelectrode used for sample analysis.

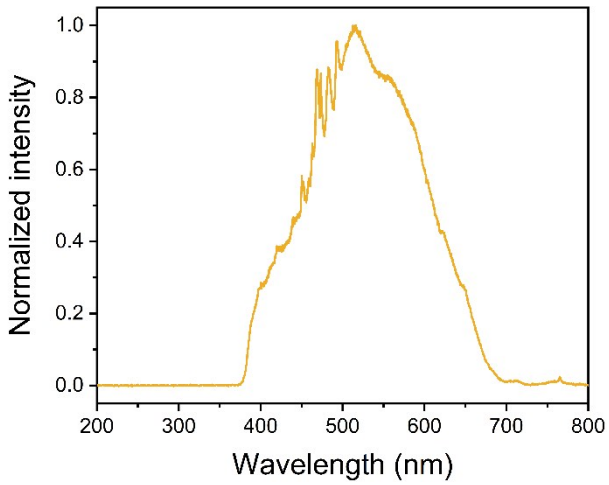

Fig. S5. Spectrum of the light used for sample illumination. The intensities have been normalized with respect to the wavelength exhibiting the maximum intensity.

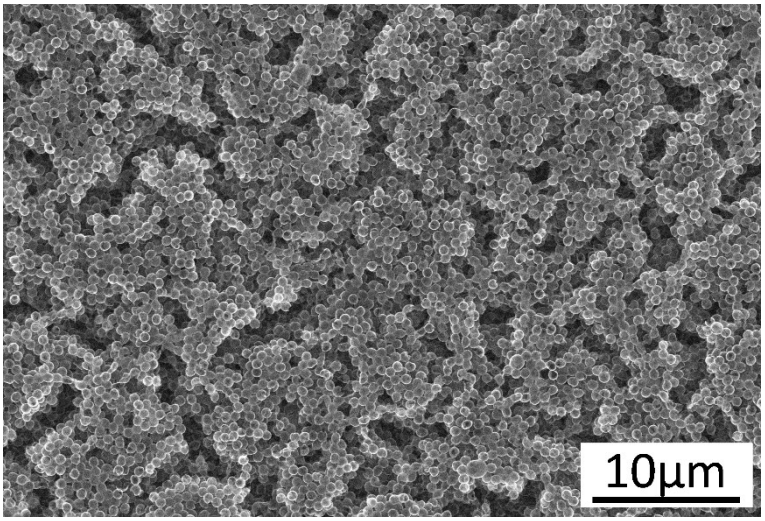

Fig. S6. SEM image of an IO-TiO_2 electrode with 2500-fold magnification.

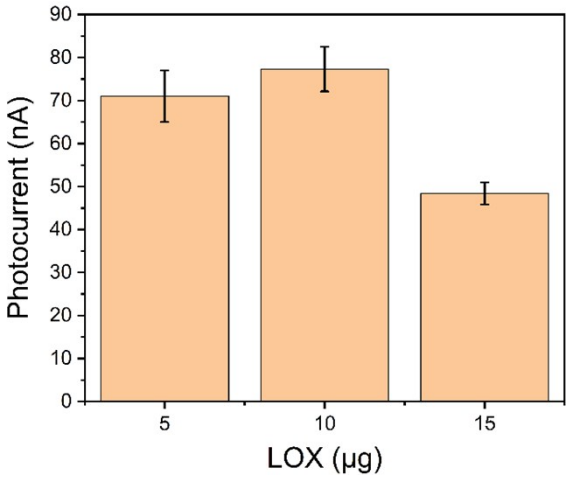

Fig. S7. Optimization of the amount of LOX used for substrate modification. Photocurrent responses obtained for IO-TiO_2/PbS/P_{Os}/LOX electrodes (N = 3), using the specified amounts of enzyme in the solutions used for substrate modification. Local illumination. E_{app} = 0.0 mV *vs.* Ag/AgCl/3 M KCl. Electrolyte: 20 mM lactate in 5 mM HEPES buffer, pH 7.0.

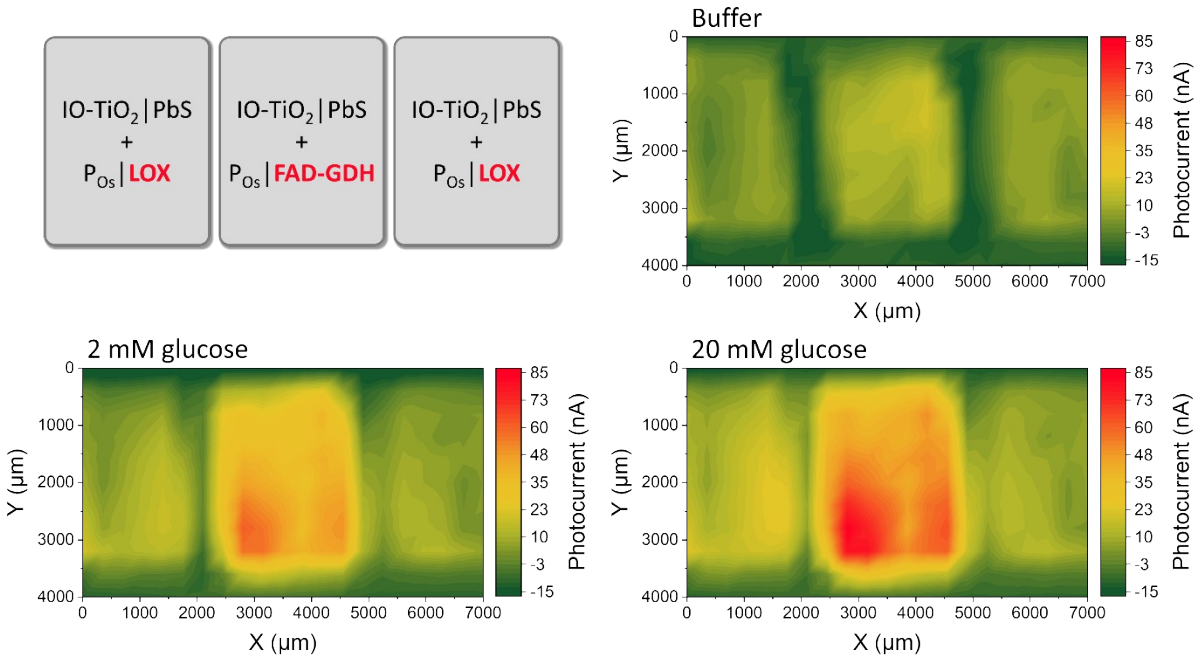

Fig. S8. Photocurrent response obtained over an IO-TiO_2/PbS substrate modified locally with P_{Os}/LOX and P_{Os}/FAD-GDH as indicated in the scheme (top-left corner). Successive scan experiments in buffer only and with increasing glucose concentrations. Local illumination. Scan increments: X = 350 µm, Y = 400 µm. E_{app} = 0.0 mV *vs.* Ag/AgCl/3 M KCl. Electrolyte: 5 mM HEPES buffer, pH 7.0.

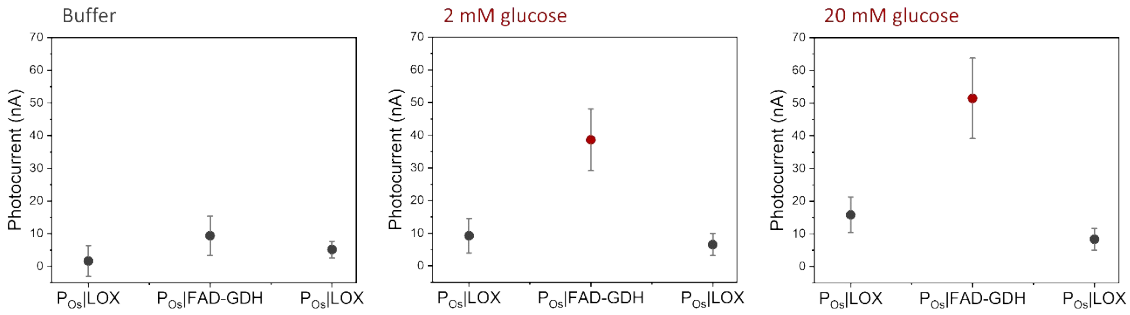

Fig. S9. Comparison of the responses obtained over the differently modified regions for the scan experiments presented in Fig. S8. The average and standard deviation for the data points recorded over each of the three modified regions is presented for scans performed in buffer only and with increasing glucose concentrations.

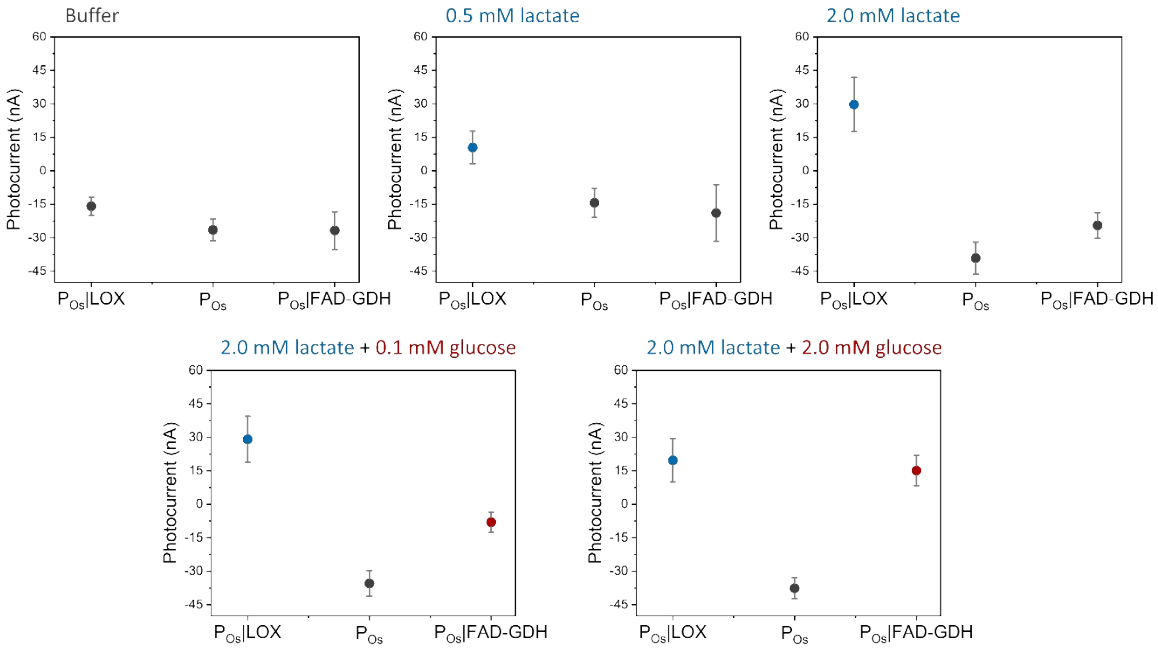

Fig. S10. Comparison of the responses obtained over the differently modified regions for the scan experiment presented in Fig. 4 (main text). The average and standard deviation for the data points (N = 42) recorded over each of the three modified regions is presented for scans performed in buffer only and with increasing lactate and glucose concentrations.

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