Supporting Information

Glucose Microsensor with Covalently Immobilized Glucose Oxidase for Probing Bacterial Glucose Uptake by Scanning Electrochemical Microscopy

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The supplementary information includes:

EXPERIMENTAL

Chemicals. GOD from *Aspergillus niger* (>250 U/mg) was purchased from EMD Millipore Corp. (MA, USA). Non-graphitized multiwalled carbon nanotubes (MWCNTs) were purchased from US Research Nanomaterials, Inc. (TX, USA) and 1-butyl-4-methylpyridinium hexafluorophosphate (ionic liquid [IL]) was purchased from Aldrich. 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide HCl (EDC) was purchased from Thermo Scientific. N-hydroxysuccinamide (NHS) and hydroxymethyl ferrocene were purchased from Alfa Aesar (USA) and D-glucose and potassium ferrocyanide from Macro Fine Chemicals. For pH sensor preparation, iridium (IV) chloride hydrate was purchased from TCI, oxalic acid from EMD, and hydrogen peroxide from Macron Fine Chemicals. Lactic acid was from EMD. Sucrose was purchased from J.T. Baker and alginic acid sodium salt from Aldrich. Artificial saliva (AS) solution was prepared by mixing 4.0 mM sodium phosphate dibasic heptahydrate, 0.7 mM calcium chloride dihydrate, 30.0 mM potassium chloride, and 30.0 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES). Calcium chloride dihydrate was purchased from Macron Fine Chemicals and all other chemicals used for AS preparation were purchased from EMD. The pH of the solution was adjusted to 7.2 with 1.0 M HCl. Deionized water (18 MΩ) was used to prepare each solution. Wild-type *S. mutans* UA159 was a kind donation from the Kreth lab at Oregon Health & Science University.

Instrumentation. All amperometric measurements were carried out with a standard three-electrode system. The working electrode was the newly developed enzymatic sensor and the counter electrode was a stainless-steel wire (purchased from Consolidated Electronic Wire & Cable (IL, USA). All potential values reported are with respect to the Ag/AgCl (1.0 M KCl) reference electrode. Enzyme sensor characterizations and biofilm studies were performed by using a bipotentiostat and scanning electrochemical microscopy (SECM) model 920D from CH

instruments, Inc. (TX, USA). The pH sensor calibrations and measurements above the biofilm were performed with a high impedance potentiometer (model 9083, Lawson Labs, Inc.).

Fabrication of dual SECM probe with a pH sensor. Dual-tip SECM probes with two 25 μm Pt electrodes were fabricated, and an iridium oxide layer was deposited on one of the Pt electrodes by cycling the potential between 0 V and 0.7 V in the deposition solution. The iridium oxide deposition solution was prepared as reported in the literature and aged for 2 days prior to use [1]. The unmodified Pt UME of the dual tip SECM probe was used to obtain the negative feedback approach curve and the iridium oxide-deposited electrode was used as a potentiometric pH sensor.

Preparation of *S. mutans* biofilms. Brain heart infusion liquid media was inoculated with the bacterial colonies from a streak plate. This liquid culture was incubated for 12 h at 37 ^{o}C in 5% CO_2 and the bacterial cells were separated from growth media via centrifugation. Bacteria were then washed with PBS to remove any residual growth media. Following this, the bacterial optical density at 600 nm was adjusted to two. A 0.5 mL portion of this prepared dispersion was then mixed with 0.5 mL of 4% alginate solution. A polydimethylsiloxane (PDMS) mold with a 1.2 mm × 2.0 mm cavity was made inside a glass-bottom Petri dish. The bacteria-alginate mixture (10.5 mL) was dropped into the cavity of the PDMS mold and left undisturbed for 15 min to settle down. The mold was then covered with 2% calcium chloride solution and the alginate-bacteria gel further cured at 4 ^{o}C for 1 h before the experiment.

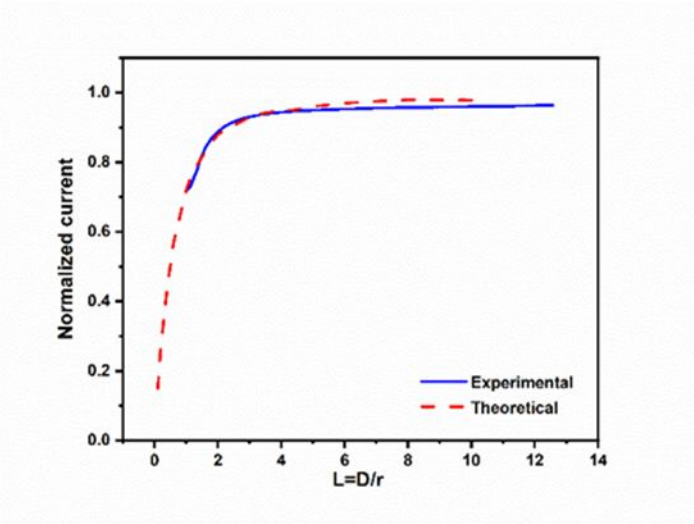

Figure S-1: Negative feedback approach curve on the alginate-*S. mutans* biofilm obtained with the new dual tip probe in 1.0 mM ferrocyanide solution fitted with the theoretical approach curve with a single tip SECM probe having the same dimensions

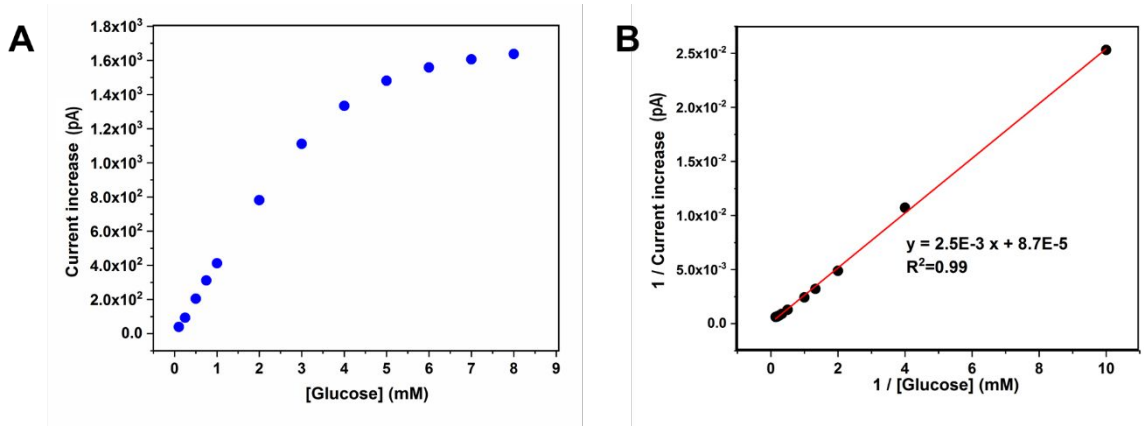

Figure S-2*:* (A) The plot of increase in current vs glucose concentration for a glucose sensor in AS with pH=7.2 and at 37 ^{o}C (E = +0.5 V vs Ag/AgCl) (B) The corresponding Line weaver–Burk plot for the same glucose sensor.

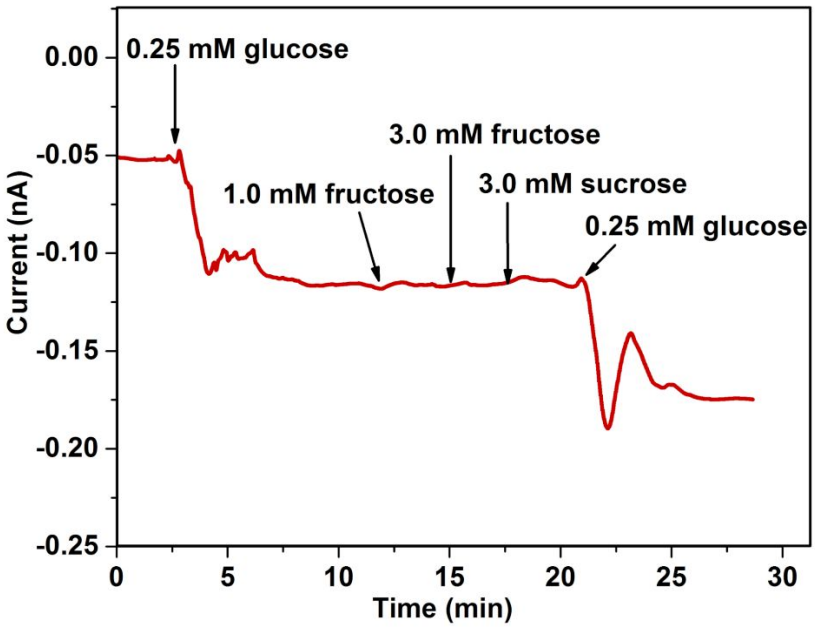

Figure S-3: Investigating the interference of fructose and glucose on glucose sensor in AS with pH=7.2 and at 37 ^{o}C

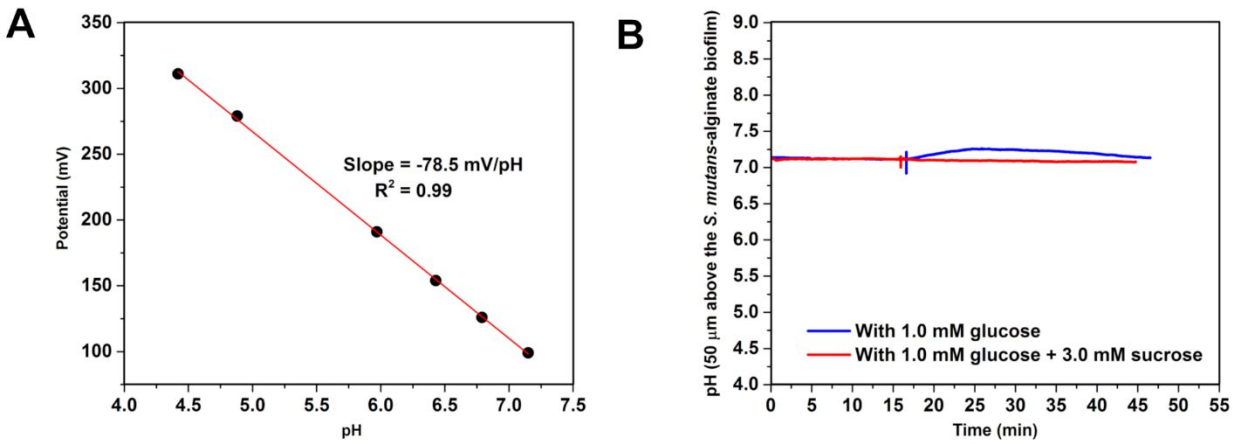

Figure S-4: (A) Calibration of the Pt/IrO_x pH sensor at 37 ^oC upon addition of 1.0 M lactic acid (B) Local pH change above *S. mutans-alginate* biofilm upon addition of 1.0 mM glucose and 1.0 mM glucose + 3.0 mM sucrose at 37^oC

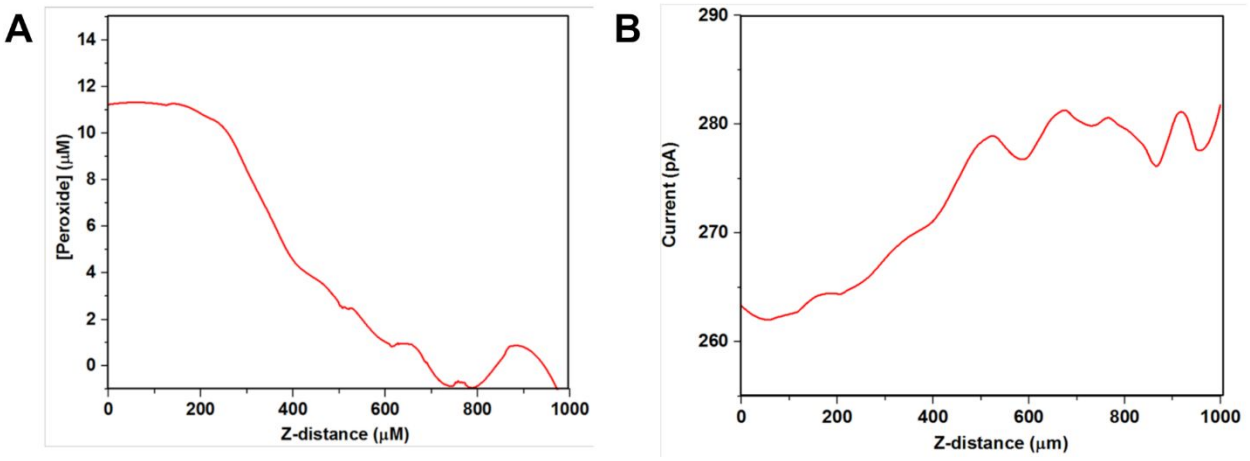

Figure S-5: (A) Z-direction peroxide concentration of *S.mutans-alginate* biofilm with addition of 1.0 mM glucose at 37°C measured with a platinized Pt UME of a dual tip SECM probe (When Z=0, distance to the biofilm = 25 μm) (B) Z-direction oxygen reduction current (at -0.2 V) above *S.mutans*-alginate biofilm with addition of 1.0 mM glucose at 37 °C measured with a Mercury deposited Pt UME of a dual tip SECM probe (When Z=0, distance to the biofilm = 25 μm)

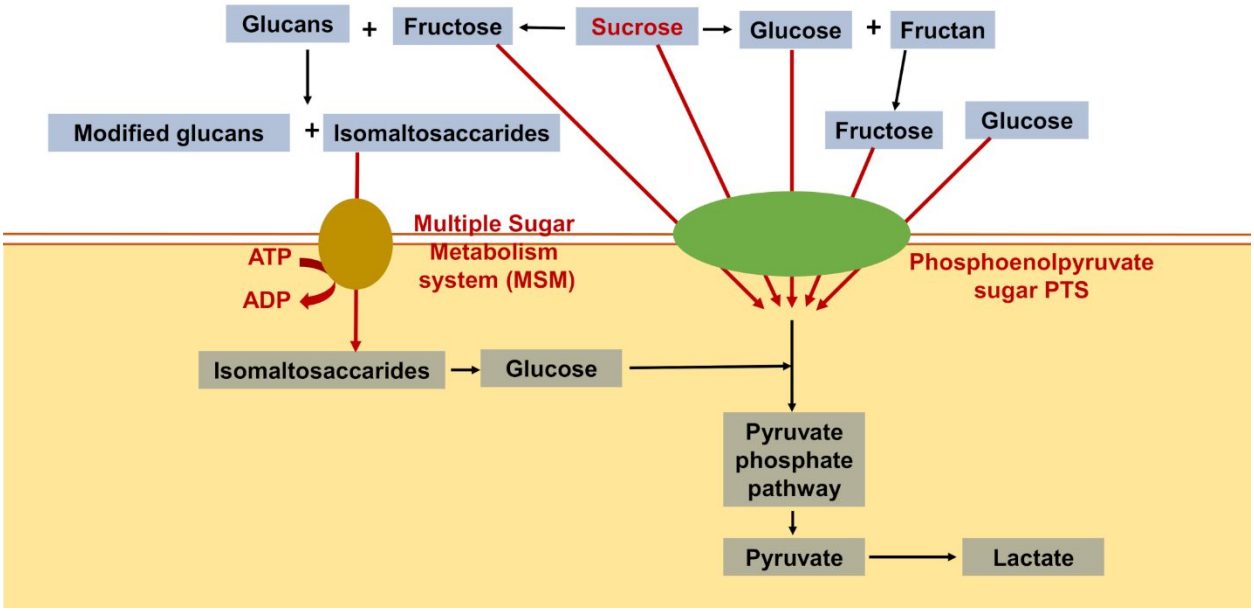

Figure S-6: Schematic diagram showing the glucose uptake pathways of *S.mutans* bacteria

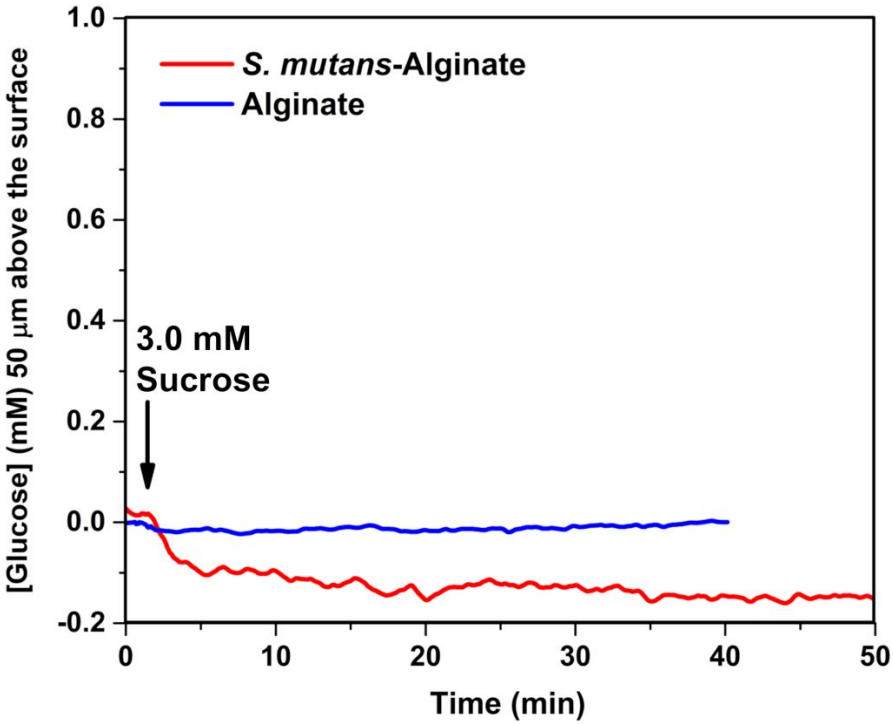

Figure S-7: Glucose sensor response recorded above *S.mutans*-alginate biofilm with addition of 3.0 mM sucrose at 37°C in AS pH=7.2

REFERENCE:

1. Marzouk, S. A. Improved Electrodeposited Iridium Oxide PH Sensor Fabricated on Etched Titanium Substrates. *Analytical chemistry* **2003**, *75* (6), 1258–1266.