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Temperature behavior of glucose oxidase immobilized into surface-attached stimuli-sensitive copolymer microgel

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Experimental Section

Materials: Glucose oxidase (GO) from *Aspergillus niger*, E.C. 1.1.3.4, activity 168100 U/g solid was purchased from Sigma-Aldrich (UK), *β*-D-glucose was received from ICN Biomedicals, Inc. Tris(hydroxymethyl)aminomethane (TRIS) and its hydrochloride (TRIS-HCl) were obtained from Sigma-Aldrich (Steinheim, Germany). The microgel was synthesized via precipitation polymerization of *N*-isopropylacrylamide (NIPAM) and a cationic comonomer *N*-(3-aminopropyl)methacrylamide (APMA) in the presence of a cross-linker *N*,*N*′-methylene bisacrylamide [S1] and contains about 16% (mol.) of the cationic comonomer (APMA) units. All other chemicals were of analytical grade and used without further purification. Deionized water (18.2 MΩ cm) purified with a Milli-Q purification system from Millipore was used as a solvent for preparation of all solutions.

Turbidimetry; The transmittance of 0.5 g/L aqueous P(NIPAM-*co*-APMA) microgel solutions prepared in 10 mM TRIS at the specified pH values was measured on a Shimadzu UV-1800 double-beam spectrophotometer (Shimadzu, Japan). The temperature-controlled sample holders were connected to a temperature controller allowing 6 simultaneous measurements. The solutions were heated-cooled with a rate of 0.4°C/min with a step of 2°C and transmittance was scanned with a temperature trend at a fixed wavelength of 500 nm. The cloud points were determined by the lowest point extracted from the derivative of the transmittance versus temperature curve.

Electrochemical Microgel-Enzyme Constructs: The screen-printed electrodes (SPEs) were fabricated on poly(vinyl chloride) substrates of 0.2 mm thickness by means of conductive graphite paste (Gwent, UK) screen-printed by a semi-automated Winon machine (model WSC-160B, China) with a 200 mesh screen stencil. Each SPE consisted of a round-shaped working area (2.5 mm diameter), a conductive track (30 mm × 1.5 mm), and a square extremity (3 mm × 7 mm) for electrical contact. The SPEs were pre-modified with a peroxide-sensitive layer of manganese dioxide nanoparticles according to procedure described elsewhere [S2]. The SPE/MnO_2 electrodes were stored dry at an ambient temperature until further use. Microgel particles were adsorbed onto SPE/MnO_2 at a temperature of 50 °C via the dip coating method by dipping the electrodes into preheated 1 g/L dispersion of P(NIPAM-*co*-APMA) microgel in 10 mM TRIS of pH 9,5 for 60 min adsorption. After that time, the electrode surface was rinsed with Milli-Q water and was very shortly (for 1−2 s) blown by a stream of air. Directly after this, GO was adsorbed in a similar way from 9×10^{-5} M solution in 10 mM TRIS of pH 7 at 20 °C for 40 min, followed by rinsing with Milli-Q water and shortly drying with a stream of air. To prevent the loss of enzymatic activity, the SPE covered by microgel-enzyme films were stored at +4 °C until further use.

Electrochemical Assay: Electrochemical experiments were performed in a water-jacket one-compartment electrochemical cell with stirring (volume of 1 mL), using a three-electrode configuration. The SPE/MnO_2/Microgel/GO constructs were used as the working electrode, while an Ag/AgCl and a platinum wire were served as a reference and a counter electrode,

respectively. A potentiostat IPC Compact (Kronas Ltd., Moscow, Russia) used for electrochemical measurements was interfaced with a PC and electrochemical parameters were controlled by the potentiostat software. The necessary temperature of the water-jacket electrochemical cell was maintained by a thermostat Huber CC-K6 (Huber, Offenburg, Germany). Electrochemical responses were assayed in a 50 mM HEPES/30 mM KCl buffer (pH 7.5, 8.5 or 9) by recording the current arising after the addition of a solution of *β*-D-glucose with a standard concentration (0.2 mM in the cell). The oxidative current is generated in response to the addition of a substrate (*β*-D-glucose) solution at an applied potential of +450 mV *vs* Ag/AgCl. Each electrochemical response was determined as a value of steady-state baseline current change (the difference between an average value of steady-state baseline current before and after the analyte addition).

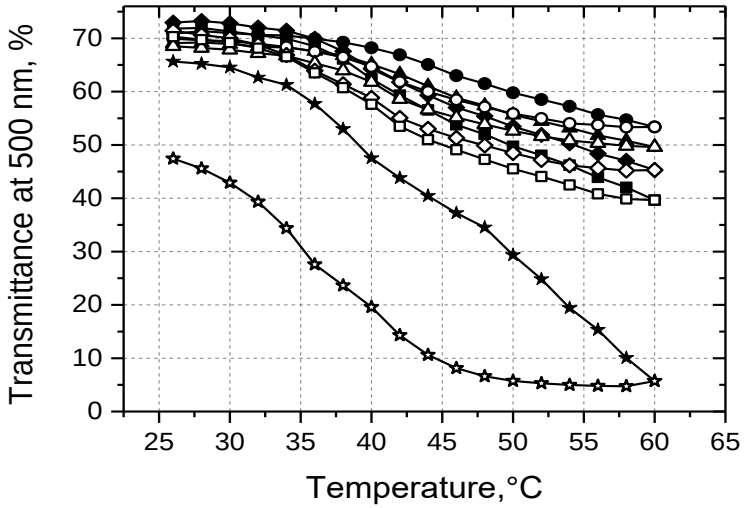

Figure S1 Temperature-induced changes of transmittance of a 0.5 g/L P(NIPAM-*co*-APMA) microgel dispersion in 10 mM TRIS at different pH values: pH 7.5, T_{CP}= 44ºC, α = 0.89 (circles); pH 8, T_{CP}= 43ºC, α = 0.84 (triangles); pH 8.5, T_{CP}= 40ºC, α = 0.76 (diamonds); pH 9, T_{CP}= 39ºC, α = 0.62 (squares); pH 9.5, T_{CP}= 38ºC, α=0.45 (stars). Solid and open symbols correspond to heating and cooling, respectively. The cloud point (T_{CP}) for each pH was determined as a minimum of the derivative of the heating part of the corresponding transmittance-temperature curve. The values of the degree of protonation (α) were extracted from the potentiometric titration data [S1].

References

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