Supplementary materials

Towards one-step design of tailored enzymatic nanobiosensors

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Figure S1. Reversed-phase HPLC-trace of a droplet spotted for 30 min onto the hybrid GOx-Nafion-Pd-NPs nanobiosensor (*top*) and LbL-produced biosensor (*bottom*). Chromatographic conditions: column ZORBAX Eclipse Plus C 18 (2.1×50 mm, particles size 1.8 µm), operation temperature was maintained at 30 °C; solvent (A) – 0.3% formic acid in water, solvent (B) – ACN; gradient: 90% A was held for 2 min, then decreased to 10% in 9 min and held for 2 min, and returned back to the starting conditions in 0.5 min for 8 min equilibration; flow rate was set at 0.3 mL/min; injection volume was 5 µL.



Figure S2. FT-IR transmission spectra obtained from pure Nafion (*top*), GOx (*middle*) and Pd-electrolyte (*bottom*) solutions and their mixtures in a ratio of 1:1 *v/v*.



Figure S3. SEM images of the hybrid nanostructures prepared at different deposition time and current by co-deposition from multiple electrolytes having different pH, Nafion and GOx concentration and mixed at different ratios.



Figure S4. FT-IR transmission spectra recorded from the scratched deposits (Nafion+Pdelectrolyte) prepared at the ratios: (**red**) – 1:1 v/v; (**black**) – 1:2 v/v). *Note*: the characteristics lines corresponded to Nafion at the range 2900 cm⁻¹, 800-1500 cm⁻¹ are not visible for the deposits prepared at the ratio Nafion+Pd-electrolyte 1:2 v/v.



Figure S5. FT-IR transmission spectra recorded from the scratched deposits (GOx+Nafion+Pd-electrolyte) prepared at the ratios: (red) - 1:1:1 v/v/v; (black) - 2:1:1 v/v/v).



Figure S6. The score of applications of the novel mechanically stable nanobiosensors.