

## Supplementary information

### Flow-electrochemical synthesis of Prussian Blue based nanozymes 'artificial peroxidase'

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### Materials

Experiments were carried out in Millipore Milli-Q water (resistivity 18.2 MΩ·cm). 30% H<sub>2</sub>O<sub>2</sub> solution, 3,3',5,5'-tetramethylbenzidine (TMB), FeCl<sub>3</sub>·6H<sub>2</sub>O and K<sub>3</sub>[Fe(CN)<sub>6</sub>] were obtained at the highest purity from Sigma Aldrich (USA). KCl, K<sub>2</sub>HPO<sub>4</sub>, citric acid and organic solvents were obtained at the highest purity from Reachim (Russia) and used as received. Solutions of hydrochloric acid and sulfuric acid were prepared from standard titrimetric solution Germen (Germany). Planar screen-printed three-electrode structures produced by Ltd. Rusens (Russia), carbon felt with 90 mg·mL<sup>-1</sup> density (Research Institute «Grafit», Russia) and carbon paste (Gwent Electronic Materials Ltd., UK) were used for the electrodes elaboration.

### Instrumentation

Electrochemical investigations were performed using PalmSens 4 potentiostat (PalmSens B.V., the Netherlands) in a three-electrode setup. The reaction mixture was supplied with the Cole Parmer (Vernon Hills, IL) peristaltic pump. Prussian Blue nanoparticles (PB NPs) separation was carried out in Eppendorf MiniSpin centrifuge (Germany). PSB-Gals (Russia) ultrasonic bath was used for dispergation of PB NPs suspensions. Nanoparticles sizing was performed with Malvern Zetasizer Nano ZS (Malvern Instruments Ltd, UK). Scanning electron microscope Supra 50 VP LEO (Carl Zeiss, Germany) equipped with InLens detector was used to examine carbon felt/graphite electrodes surface and to determine size and shape of nanoparticles at 5 kV accelerating voltage and 8-9 mm working distance. X-ray powder diffraction was performed with the use of a HUBER G670 Guinier camera (CoKα radiation, λ = 1.78965 Å). Nanozymes catalytic activity was studied using Lambda 35 UV/Vis Spectrophotometer (Perkin Elmer, USA) operated in transmission mode.

### Methods

To manufacture electrodes with increased electroactive surface area the mixture of carbon felt and carbon paste (1:2 by weight) was distributed onto the working electrode of the planar screen-printed structures. Then the electrodes were heated at 120°C for 45 minutes.

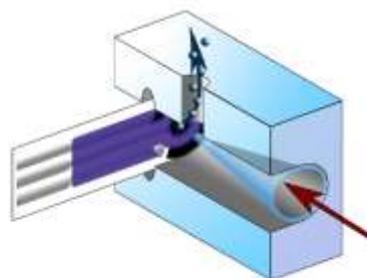
Electrochemical synthesis of nanoparticles was carried out in a self-made flow-through wall-jet cell with 0.5 mm nozzle positioned at 2 mm distance from the carbon felt/graphite working electrode surface (Fig. S1). The reaction mixture, containing 1:1 mixture of FeCl<sub>3</sub> and K<sub>3</sub>[Fe(CN)<sub>6</sub>] (25-150 mM) in 0.1 M KCl/0.1 M HCl solution was supplied to the wall-jet cell during 40 minutes by means of the peristaltic pump with 0.15 – 2.35 mL·min<sup>-1</sup> volume flow rate. The reducing potential (0.2 – 0.7 V vs. Ag/AgCl) was applied to the working electrode simultaneously. The resulting colloidal solutions of Prussian Blue nanoparticles in the reaction mixture were

centrifuged at 13000 rpm. during 3 min. Then the supernatant containing non-reacted salts was removed and the nanoparticles precipitate was redispersed into 0.1 M KCl/0.1 M HCl solution. The procedure of centrifugation and redispersion was repeated 7 times. Concentrations of Prussian Blue in suspensions were estimated spectrophotometrically ( $\epsilon_{700\text{ nm}}$ ,  $\text{KFe}[\text{Fe}(\text{CN})_6] = 12250\text{ M}^{-1}\text{ cm}^{-1}$ ). Mean diameters of PB nanoparticles were obtained from dynamic light scattering and confirmed with scanning electron microscopy. The nanozymes concentration was calculated considering not only mean values, but their size distribution and unit cell parameter (1.02 nm). Size distribution in number (DLS) was used for calculations due to it is well-correlated to electron microscopy data. The colloidal solutions of nanoparticles were stored as prepared and vigorously ultrasonicated before use.

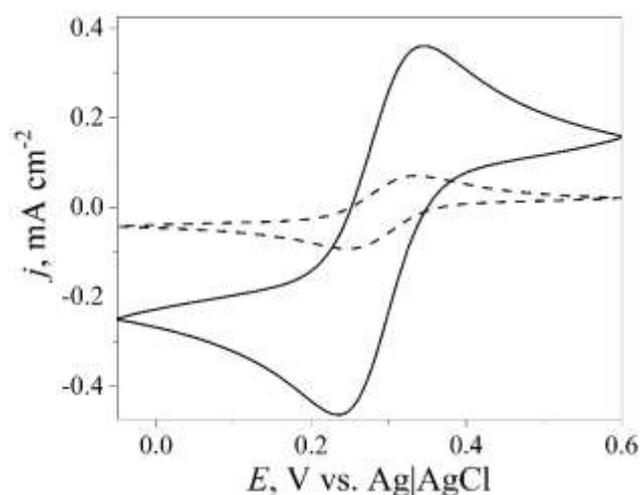
For X-ray powder diffraction experiment PB NPs were washed with 0.1 M HCl and dried at room temperature.

Catalytic activity of electrochemically synthesized Prussian Blue nanoparticles was studied at room temperature in 50 mM phosphate-citrate buffer (pH 5.0), containing 1 – 5 mM of  $\text{H}_2\text{O}_2$ , 1 – 20  $\mu\text{M}$  of TMB and 0.02 mM of Prussian Blue. TMB was dissolved in dimethyl sulfoxide (DMSO), only freshly prepared stock solutions (50 mM) were used. for every sample we measured the amount of Prussian Blue spectrophotometrically. Apparent catalytic rate constants  $k_{\text{cat}}^{\text{app}}$  for TMB were determined normalizing maximum reaction rate value  $v_{\text{max}}$  at the constant  $\text{H}_2\text{O}_2$  concentration (2 mM) to the number of nanozymes per liter.

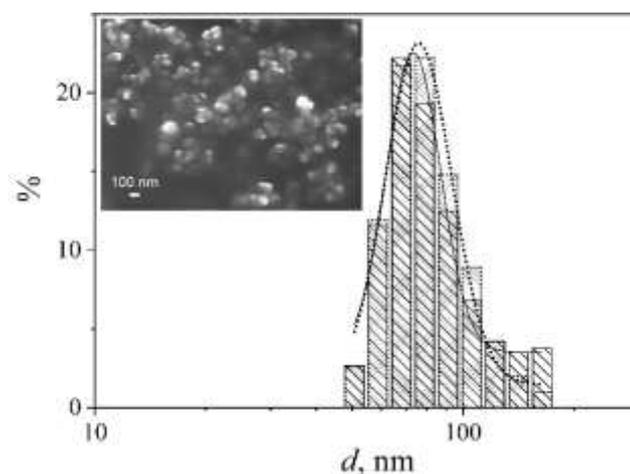
For electrochemical investigations 2  $\mu\text{l}$  of nanoparticles suspension (0.02–3 mM of  $\text{KFe}[\text{Fe}(\text{CN})_6]$ ) was drop-cast onto the working electrode surface and heated at 100 °C during 1 h. Analytical performance characteristics of the resulting  $\text{H}_2\text{O}_2$  electrochemical sensors were investigated in chronoamperometry regime ( $E_{\text{DC}} = 0.00\text{ V}$ ) in batch mode upon stirring.



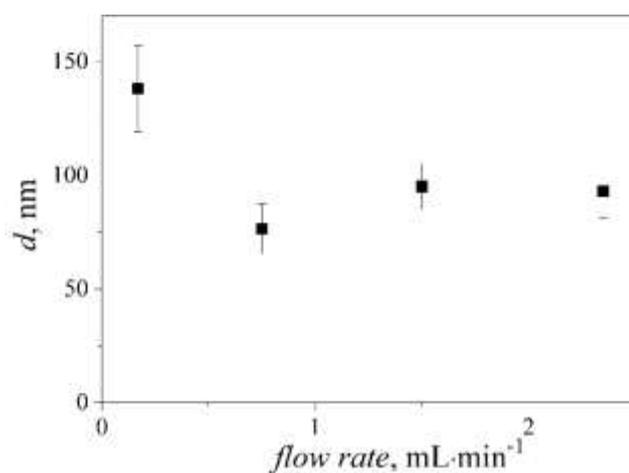
**Figure S1** | Wall-jet flow-through electrochemical cell with integrated screen-printed three-electrode structure for electrochemical synthesis of Prussian Blue nanoparticles.



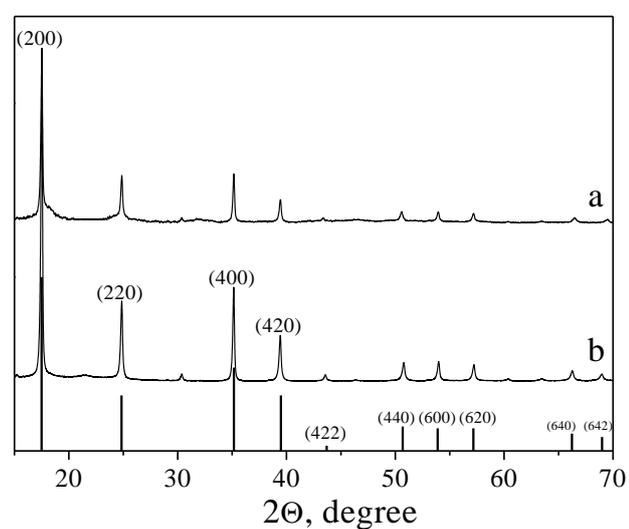
**Figure S2** | Cyclic voltammogram of 1 mM  $\text{K}_3[\text{Fe}(\text{CN})_6]$  registered at carbon felt/graphite (solid line) and conventional screen-printed graphite (dashed line) electrodes; 0.1 M KCl in 0.1 M HCl background electrolyte,  $0.04 \text{ V}\cdot\text{s}^{-1}$  scan rate.



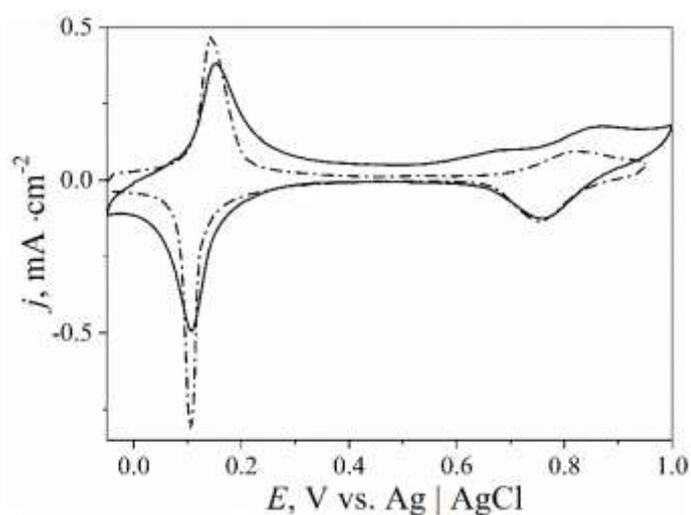
**Figure S3** | The hydrodynamic size distribution (based on number) for Prussian Blue nanoparticles, synthesized in 1:1 mixture of  $\text{FeCl}_3$  and  $\text{K}_3[\text{Fe}(\text{CN})_6]$  (75 mM): upon reduction with 75 mM  $\text{H}_2\text{O}_2$  (diagonal crosshatch fill pattern), electrochemically at 0.4 V potential in the wall-jet flow cell ( $0.75 \text{ mL}\cdot\text{min}^{-1}$  volume flow rate) (diagonal down fill pattern). Inset: SEM image of electrochemically synthesized nanoparticles.



**Figure S4** | The dependence of Prussian Blue nanoparticles size, electrochemically synthesized in 1:1 mixture of  $\text{FeCl}_3$  and  $\text{K}_3[\text{Fe}(\text{CN})_6]$  (75 mM) at 0.4 V potential on the reaction mixture flow rate.

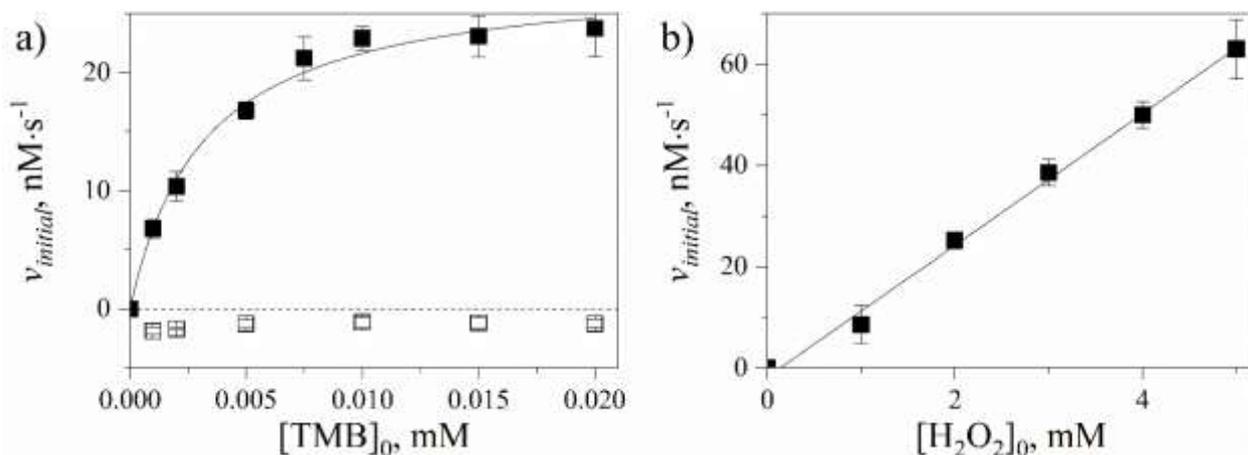


**Figure S5** | X-ray powder diffraction patterns, normalized to the intensity of the (200) reflection, for electrochemically synthesized Prussian Blue nanoparticles with average diameters  $\varnothing = 50$  nm (a) and  $\varnothing = 120$  nm (b); vertical lines record 73-687 (PDF-2).

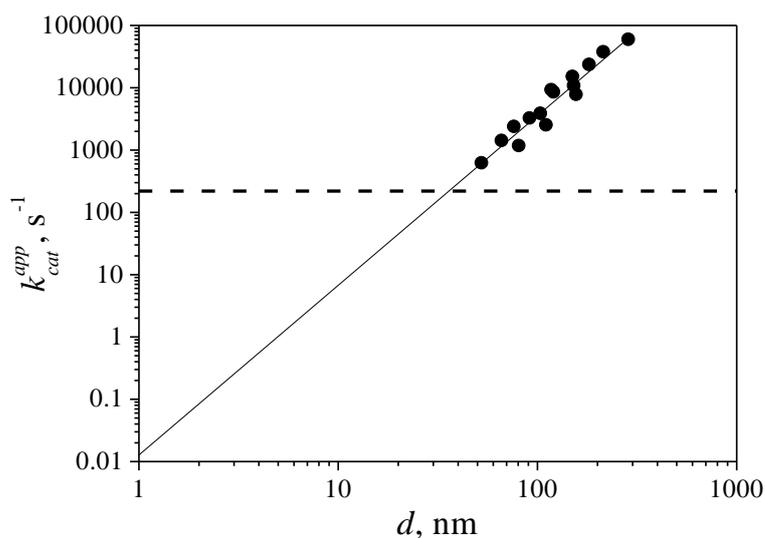


**Figure S6** | Cyclic voltammograms of graphite electrodes modified with Prussian Blue film through electrochemical deposition (dash line) and with electrochemically synthesized Prussian

Blue nanoparticles ( $\varnothing = 76$  nm) immobilized (solid line); 0.1 M KCl in 0.1 M HCl background electrolyte, potential sweep rate  $0.04 \text{ V}\cdot\text{s}^{-1}$ .



**Figure S7** | The dependences of the initial reaction rate on concentrations of: a) TMB,  $[\text{H}_2\text{O}_2]_0$ : ( $\square$ ) 0 mM, ( $\blacksquare$ ) 2 mM; b)  $\text{H}_2\text{O}_2$ ,  $[\text{TMB}]_0 = 0.02$  mM; 0.005 nM of PB nanoparticles ( $\varnothing = 76$  nm),  $\lambda = 450$  nm, phosphate-citrate buffer, pH 5.0.



**Figure S8** | The dependence of monomolecular apparent catalytic rate constant  $k_{cat}^{app}$  for TMB,  $[\text{H}_2\text{O}_2]_0 = 2$  mM,  $[\text{Prussian Blue}] = 0.02$  mM on the size of the electrochemically synthesized Prussian Blue based nanozymes. Dashed line:  $k_{cat}^{app}$  for horseradish peroxidase under similar conditions ( $\lambda = 450$  nm).