Synthesis and application of albumin nanoparticles loaded with prussian blue nanozymes

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Supporting information

Additional methods

Size tuning of nanozyme-loaded BSA nanoparticles

Four milliliters of an aqueous solution containing 20 mg/ml BSA+2 mg/ml PB nanoparticles or 30 mg/ml BSA+3 mg/ml PB nanoparticles or 40 mg/ml BSA+4 mg/ml PB nanoparticles were added to glass vial. Sixteen milliliters of 95% ethanol was added dropwise (4 ml/min) while solution was kept under magnetic stirring (1000 RPM). Then, the thermal sensor was immersed in the suspension and temperature was gradually increased to +70 °C. Heated suspension was stirred for 2 h, then cooled to room temperature. Nanoparticles were collected into 2 ml centrifuge tubes and washed with deionized water three times by centrifugation at 20000 g for 20 min. After each centrifugation pellet was redispersed by sonication (probe diameter - 3 mm; duration - 15 s; power - approx. 8-9 W). Resulting nanoparticles were combined and stored at +4 °C.

Substrate optimization

pH. 96-well plates were filled with 100 µl of 100 ng/ml Bi-BSA in 0.1 M carbonate buffer, pH 9.5. Plates were kept at +37 °C for 2 h, then three times washed with 300 µl of PBT (10 mM phosphate buffer, pH 7 containing 0.1% Tween-20). Blocking buffer (250 µl; PBT with 1% casein and 1% BSA) was added to the wells. After that, plates were incubated on a shaker (300 rpm) at +37 °C for 60 min and triply washed. In 48 of 96 wells, 100 µl of PB@BSA/Str diluted in the blocking buffer to 0.1 mg/ml was added. Remaining 48 wells were filled with blocking buffer instead of conjugate. After 60 min of incubation and triple washing, 100 µl of substrate solution was added. Substrates were prepared by mixing 900 µl of 0.1 M citrate/0.2 M phosphate (McIlvaine) buffer with 100 µl of 1 mg/ml TMB in DMSO and 10 µl of 30% H_2O_2. pH of the McIlvaine buffer was from 2.5 to 8.0. Each type of substrate was added to 8 wells (4 treated and 4 non-treated with conjugate). Color development was performed for 30 min, then 100 µl of 2 M H_2SO_4 was added in each well. Absorbance at 450 nm was immediately measured. All the assay steps except for washing were performed at +37 °C on a shaker (300 RPM). Plates were not shaken in the course of coating.

Buffer composition. Forty eight substrate buffers were prepared from the set of 6 acids (0.1 M acetic, 0.1 M oxalic, 0.1 M citric, 0.1 M propionic, 0.1 M butyric, and 0.1 M formic) and 8 bases (1 M KOH, 1 M NaOH, 1 M NH_4OH, 1 M imidazole, 1 M TRIS, 1 M HEPES, 0.1 M Na_2HPO_4, and 1 M glycine). pH of all buffers was 4.00±0.02. Analysis was performed as described in the previous section, except that two 96-well plates were used instead of one. Each buffer was added in 4 wells: 2 treated with PB@BSA/Str and 2 filled with blocking solution. Five buffers with the highest enhancing effect (0.1 M citrate/1 M KOH, 0.1 M citrate/1 M NH_4OH, 0.1 M

formate/1 M KOH, 0.1 M formate/1 M NH_4OH, 0.1 M butyrate/1 M NH_4OH) along with the McIlvaine buffer, pH 4 were retested in the additional experiment in the following way. 96-well plate was coated with 1, 10, 100, and 1000 ng/ml of Bi-BSA in 0.1 M carbonate buffer, pH 9.5. After blocking, 100 µl of PB@BSA/Str diluted in the blocking buffer to 0.1 mg/ml was added for 60 min. Substrates prepared using aforementioned buffers were then added (four technical replicates for each substrate). Reaction was stopped with 100 µl of 2 M sulphuric acid.

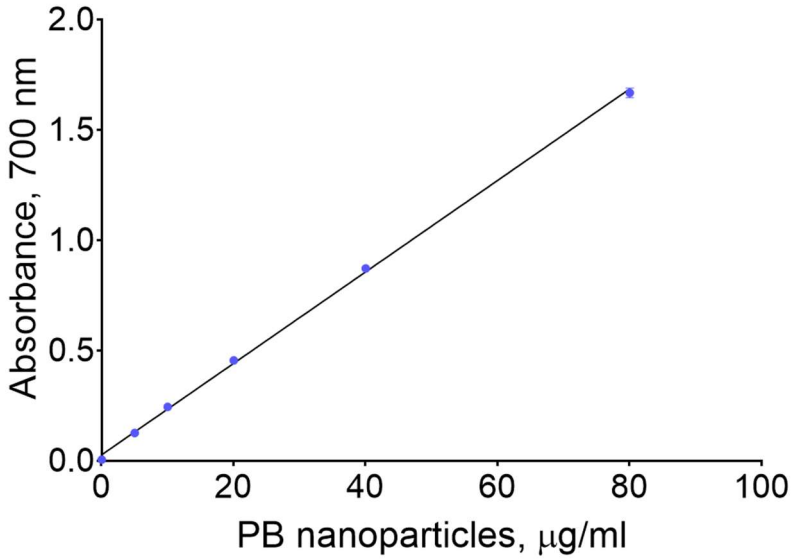


Figure S1. Calibration curve of prussian blue nanozymes (PB)

			Size (d.nm):	% Intensity	Width (d.nm):
Z-Average (d.nm):	40,94	Peak 1:	49,52	100,0	22,65
PdI:	0,163	Peak 2:	0,000	0,0	0,000
Intercept:	0,927	Peak 3:	0,000	0,0	0,000
Result quality :	Good				

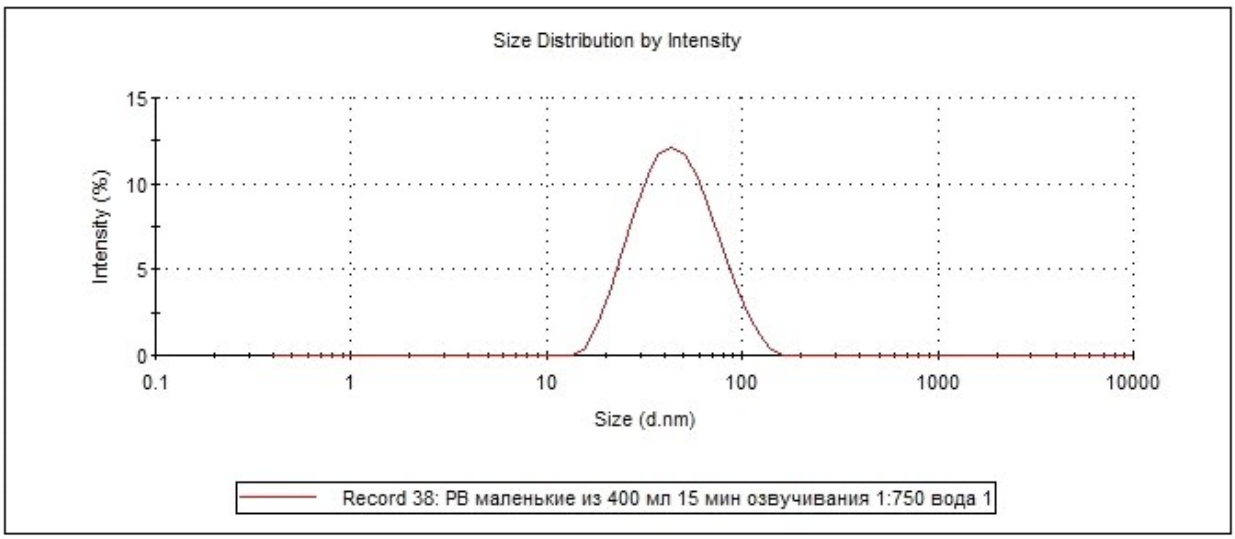


Figure S2. Intensity-weighted size distribution plot of prussian blue nanozymes

Figure S3. PB@BSA5 nanoparticles after thermal cross-linking

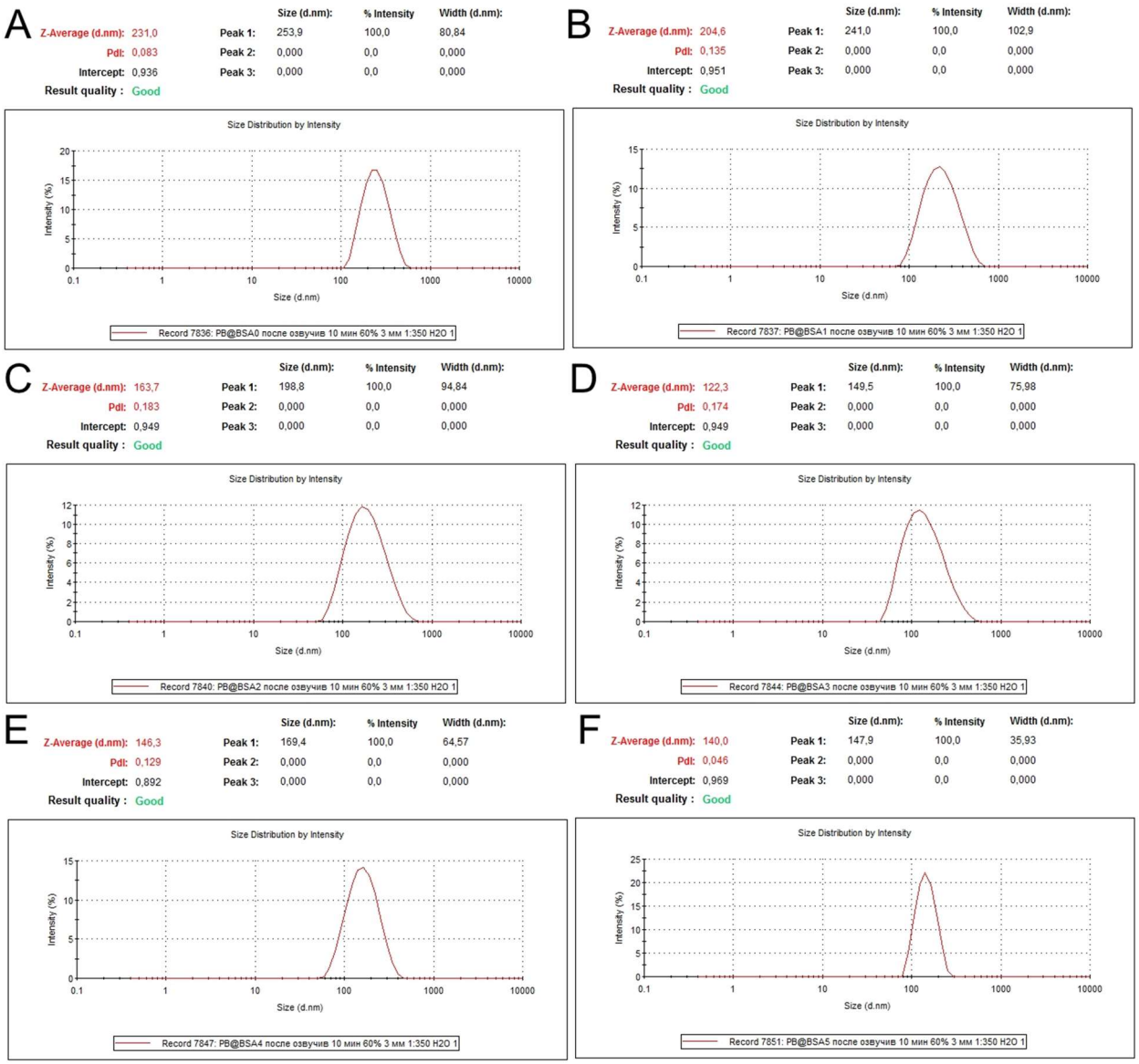
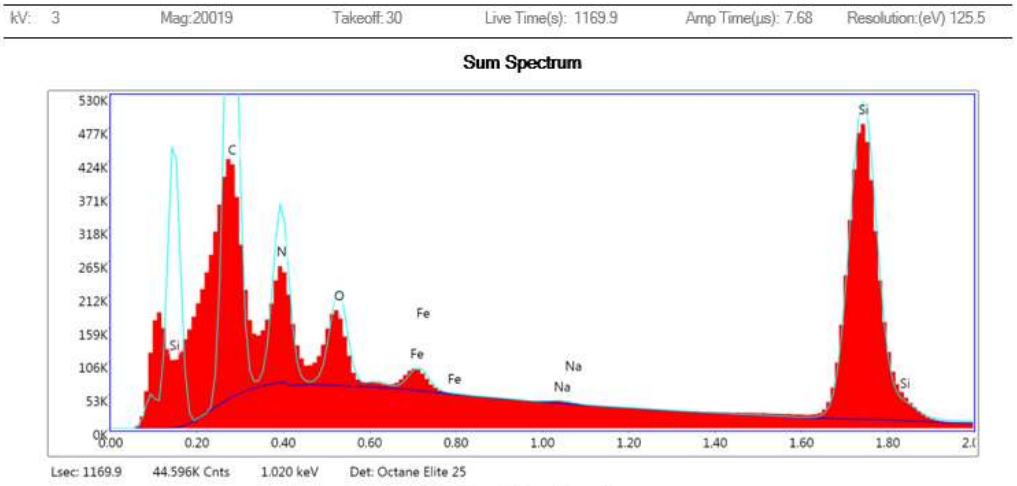


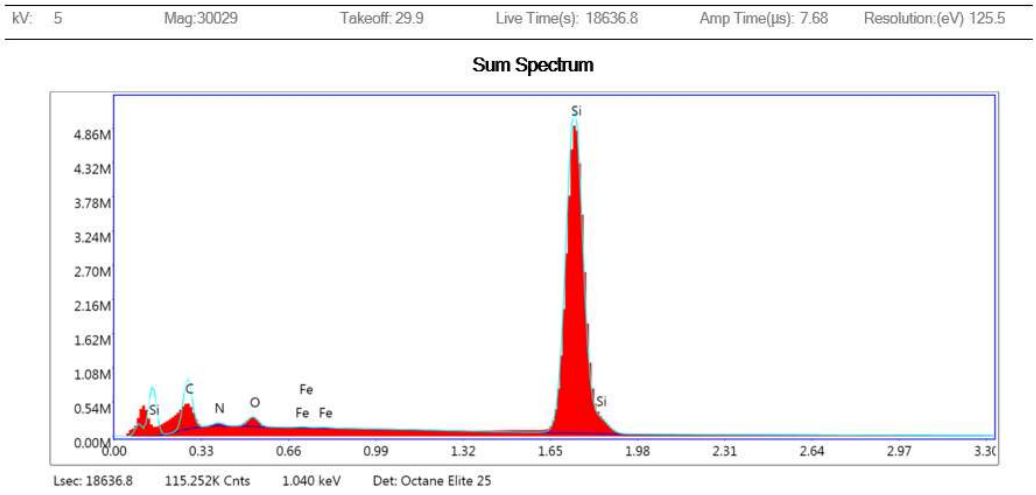
Figure S4. Intensity-weighted size distribution plots of A – BSA NP, B – PB@BSA1, C – PB@BSA2, D – PB@BSA3, E – PB@BSA4, F – PB@BSA5



eZAF Smart Quant Results

Element	Weight %	Atomic %	Net Int.	Error %	Kratio	Z	A	F
C K	15.47	28.37	2848.70	10.48	0.1082	1.2006	0.5823	1.0000
N K	5.53	8.70	1096.10	9.63	0.0460	1.1530	0.7213	1.0000
O K	2.45	3.37	634.20	8.98	0.0229	1.1127	0.8399	1.0000
FeL	1.22	0.48	111.10	8.36	0.0093	0.7600	0.9994	1.0000
NaK	0.18	0.17	22.00	14.29	0.0017	0.9758	0.9761	1.0032
SiK	75.15	58.91	3422.80	14.56	0.7113	0.9473	0.9991	1.0002

Figure S5. Elemental analysis (EDS) of PB@BSA5



eZAF Smart Quant Results

Element	Weight %	Atomic %	Net Int.	Error %	Kratio	Z	A	F
C K	10.82	21.90	177.60	10.67	0.0389	1.1992	0.2997	1.0000
N K	0.44	0.76	11.10	9.75	0.0024	1.1571	0.4816	1.0000
O K	0.92	1.39	40.40	7.12	0.0071	1.1212	0.6883	1.0000
FeL	0.09	0.04	1.80	7.40	0.0007	0.7945	0.9945	1.0000
SiK	87.73	75.91	2115.80	5.39	0.8534	0.9734	0.9992	1.0001

Figure S6. Elemental analysis (EDS) of BSA NP

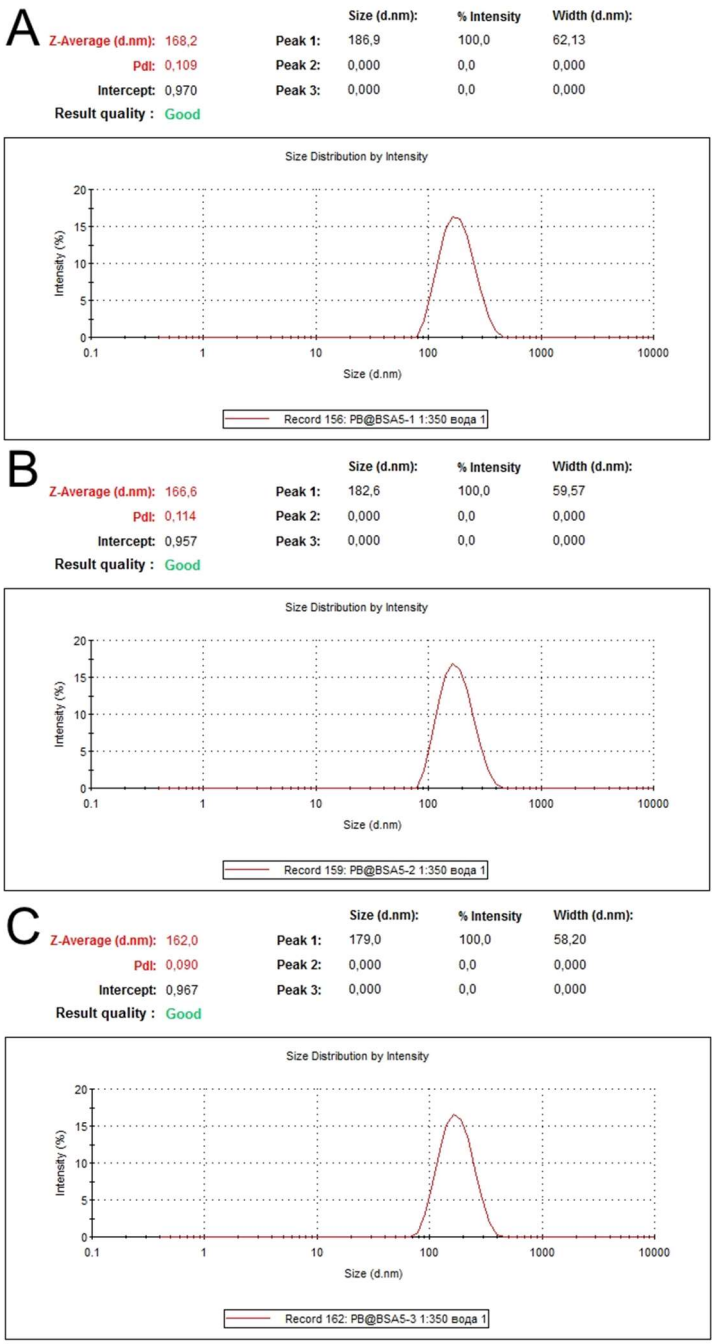


Figure S7. Intensity-weighted size distribution plots of A – PB@BSA5-1, B – PB@BSA5-2, C – PB@BSA5-3

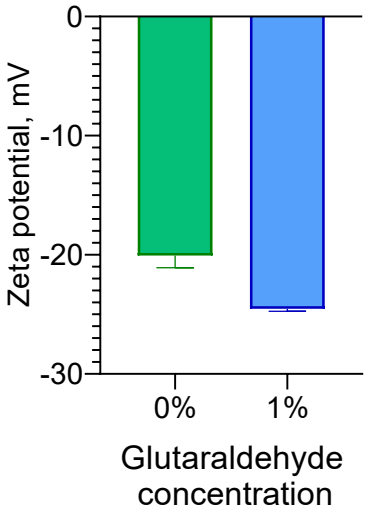


Figure S8. Decrease of zeta potential of PB@BSA5 after the glutaraldehyde treatment

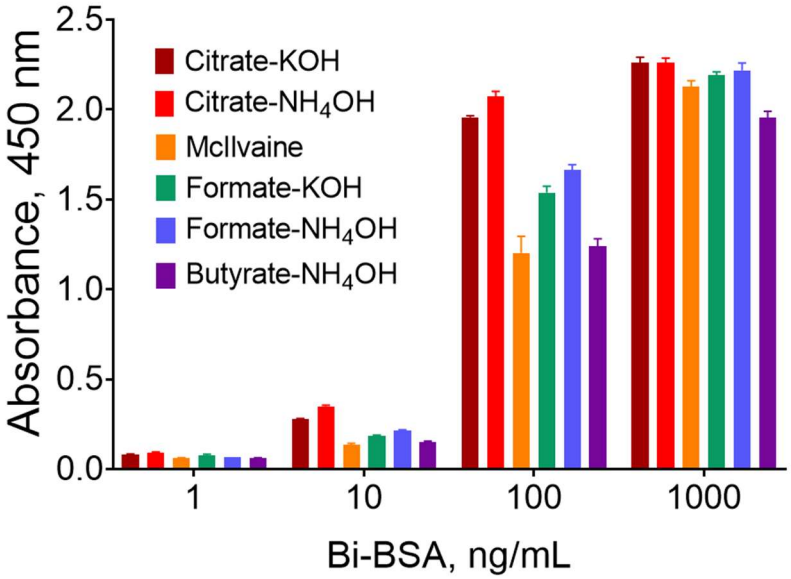


Figure S9. Re-testing of substrates provided the highest signal. Molarity of buffers (except for McIlvaine buffer) is 0.1 M. H_2O_2 concentration is 0.3%. n=4, mean±SD

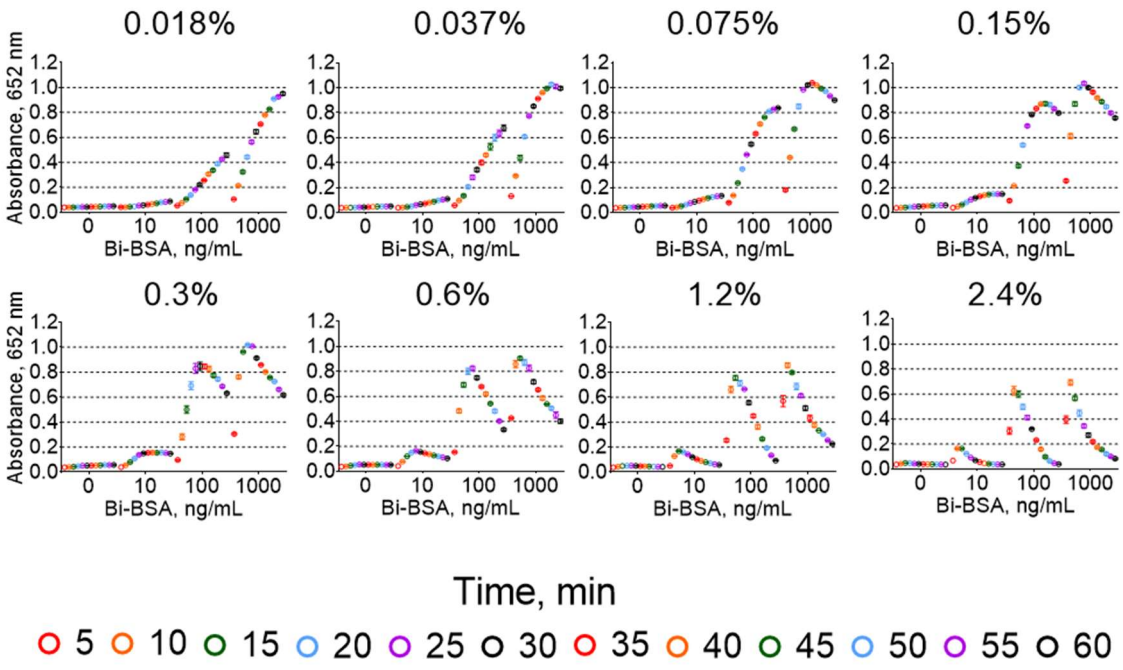


Figure S10. Dynamics of color intensity change at different H_2O_2 concentration in substrate solution. Assay format: direct detection of Bi-BSA by PB@BSA5/Str. Concentration of H_2O_2 is given above the graphs. n=3, mean±SD

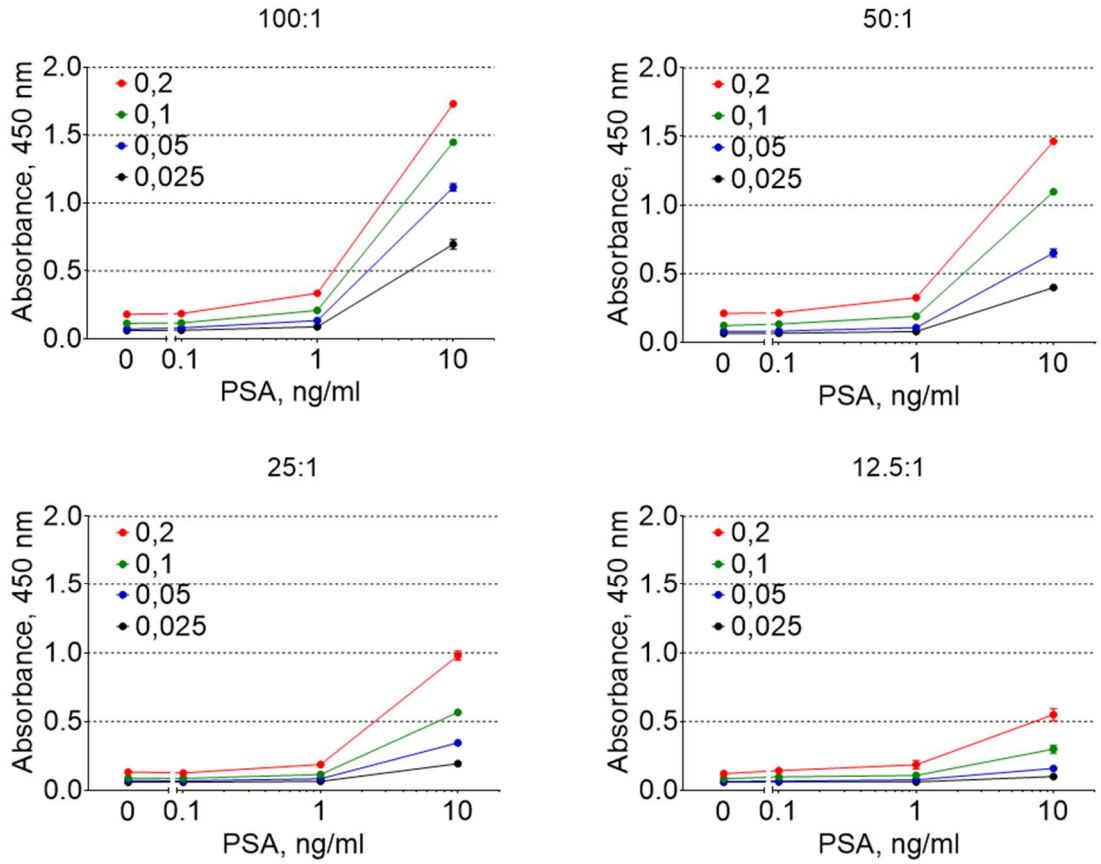


Figure S11. Optimization of PSA assay conditions: MAbs to PB@BSA5 ratio (is given above the graphs) and PB@BSA5/MAb concentration. n=3, mean±SD

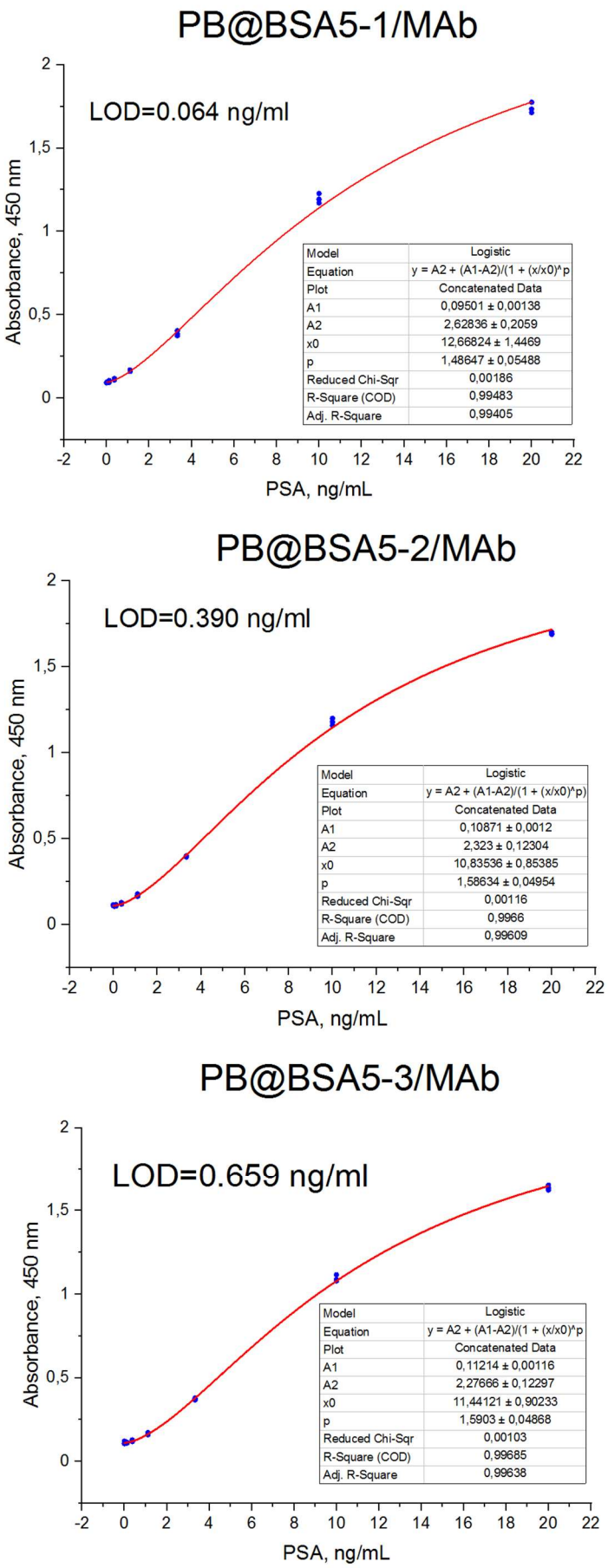


Figure S12. PSA assay: results of curve fitting