Studies on albumins' interactions with gold-based nanozymes

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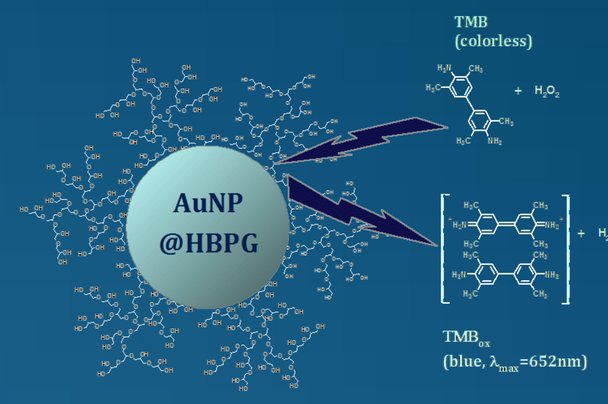
1 Introduction

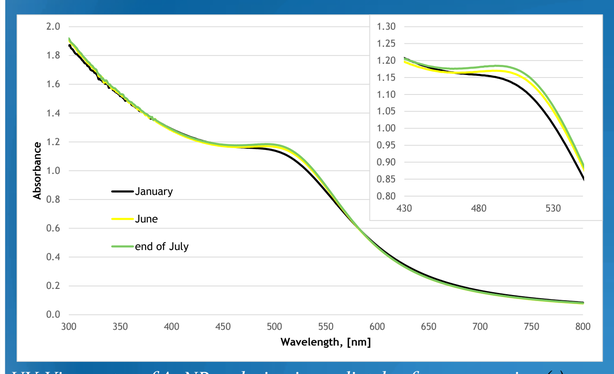
The nanoparticles (NPs) have gained tremendous attention in many branches of science and technology thanks to the unique properties arising from the nanometer size. The enzyme-like catalytic activity of gold nanostructures as well as facility of their functionalization make them interesting material in analytical tools development. Gold-based nanozymes are able to catalyze oxidation of wide range of chromogenic, fluorogenic and electroactive compounds like 3,3',5,5'-tetramethylobenzidine-TMB, 2,2'-azinobis(3-ethylobenzothiazoline-6-sulfonic acid)-ABTS or phenolate derivatives, which offers a great applicability in systems of various detection modes. The advantages of AuNPs, such as low cost, resistance to pH-dependent denaturation and lack of inhibition by substrates or heavy metal ions make them an interesting alternative in biotests or immunoassays to widely used enzyme - horseradish peroxidase (HRP). For this purpose, aspects like influence of reaction environment or adsorption processes, often overlooked in literature reports, should be taken into account.
The main aim of this work concerns studies on interactions between albumins and gold-based nanozymes. Comprehensive studies of protein influence on previously prepared gold nanoparticles coated with hyperbranched polyglycidol (HBPG) were presented. The mechanisms and kinetics of non-specific adsorption of bovine serum albumin (BSA) as well as interactions of proteins with various peroxidase substrates were examined according to Michaelis-Menten model. Activity studies included the influence of factors such as protein concentration, incubation time, pH of the reaction medium or substrate type. In addition, putative explanation of unusual mechanism of ABTS oxidation at low pH in the presence of the protein was proposed.



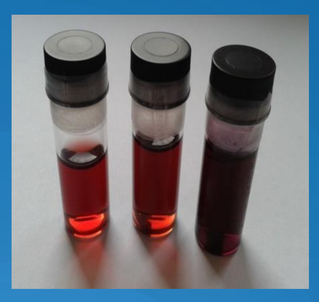
2 Preparation and characterization of HBPG@AuNPs

Gold nanoparticles were prepared in simple, one pot reaction. 10mM aqueous solution of tetrachloroauric acid (1mL) and hyperbranched polyglycidol solution (5mg/mL, 8mL) were mixed in the darkness for 10 minutes. In the next step, 50 mM, freshly prepared solution of $NaBH_4$ (1mL) was rapidly injected. After 5 minutes of stiring colloidal gold nanoparticles stabilized with HBPG were obtained, what was visually confirmed – yellowish gold(III) salt turned ruby red as a result of reduction to zerovalent gold.

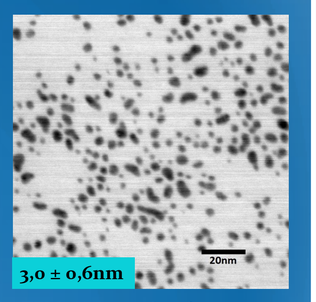
Characterization of morphology as well as long-term stability of prepared nanoparticles was made through UV-Vis absorption spectroscopy. Mean diameter was estimated by means of Transmission Electron Microscopy (TEM) micrographs analysis. Results show, that HBPG@AuNPs are very stable.



UV-Vis spectra of AuNPs solution immediately after preparation (•), after 5 months (•) and after 7 months (•). Insert shows magnification of AuNPs LSPR band (only slight shift was noticed, what confirms high stability)



Gold nanoparticles obtained with the use of different concentrations of hyperbranched polyglycidol

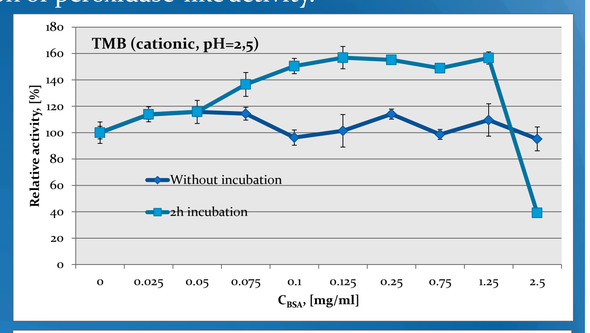


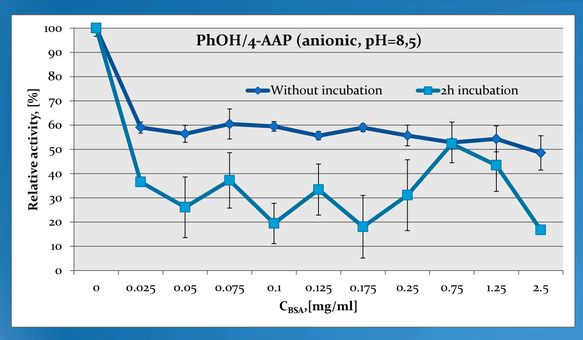
TEM micrographs of HBPG@AuNPs. Capturing this graphs allowed us to estimate AuNPs core diameter.

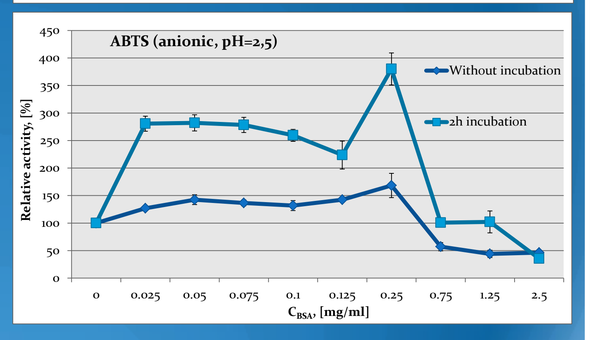
3 Albumins influence on AuNPs HRP-like activity – concentration and incubation time

Proteins concentration can have influence of gold nanoparticles enzyme-liked properties, because albumins tend to non-specifically adsorb onto metal surfaces including nanoparticles, what leads to blocking active sites on the surface of gold nanoparticles. As important as proteins concentration is time of albumins presence in the solution of gold nanoparticles. Prolonged contact of proteins with nanoparticles before reaction may cause total inhibition of peroxidase-like activity.

In performed experiments, activity of AuNPs (expressed as slope of absorbance in time) was examined in presence of a set of bovine serum albumin concentrations. Utilization of acidic and basic medium enable studies of protein in cationic (under pI, at pH 2,5) and anionic form (at pH 8,5). Application of substrates of various charges in solutions may allow for investigation of protein – substrate interactions at AuNPs catalyst's surface.





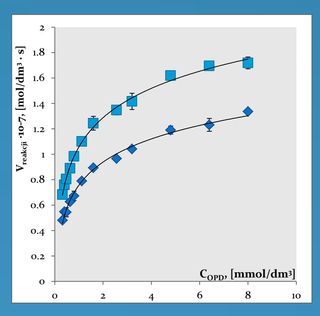


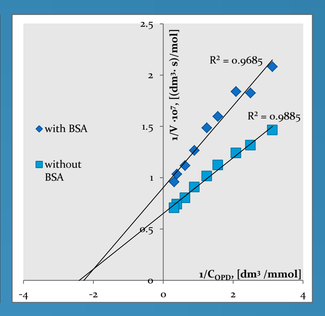
4 Mechanism of AuNPs – BSA interactions; Michaelis – Menten kinetic parameters

Relation of reaction rate and substrate concentration

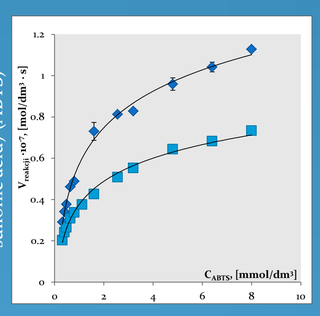
Lineweaver-Burk double reciprocal plot

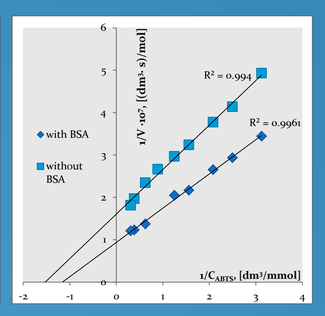
o-phenylenediamine (OPD)





2,2'-azinobis(3-ethylobenzothiazoline-6-sulfonic acid) (ABTS)





Plots of reaction rate versus substrate concentration and double reciprocal plots of HBPG@AuNPs activities according to M–M model. Steady state reaction rates were obtained from initial slopes of absorbance curves in time for varied OPD or ABTS concentrations and fixed H_2O_2.

The additional layer on the surface may adversely affect the diffusion of reactants to the active sites (diffusion barrier), as well as to physically block access to the active site, thereby reducing the nanozyme's affinity to substrate.

Comparative studies of the Michaelis - Menten kinetic parameters in absence and presence (C_{BSA}=100μg/mL) of the protein in pH 4.5 for OPD (near isoelectric point of BSA, what promotes adsorption) as well as in pH 2.5 for ABTS can become a convenient tool for studying nanoparticle - protein interactions .

$$\frac{1}{v_0} = \frac{K_M}{V_{max}} \cdot \frac{1}{[S]} + \frac{1}{V_{max}}$$

K_M –Michaelis constant (thermodynamical parameter)

v_{max} – maximum of reaction rate (kinetical parameter)

[S] – substrate concentration [μM]

	K_M [mM]		V_{max} [10^{-7}M·s^{-1}]	
	no protein	C_{BSA}=100μg/mL	no protein	C_{BSA}=100μg/mL
OPD	0,42	0,68 (increase-lower affinity)	1.53	1.28 (decrease-slower kinetics)
ABTS	0,65	0,87 (increase-lower affinity)	0.62	1.07 (increase-reaction is faster?!)

- ✓ Addition of protein results in lower affinity of both substrates to AuNP surface (protein acts as „inhibitor" blocking active sites)
- ✓ Peculiar behaviour of V_{max} value for ABTS may be explained by atypical reaction mechanism
- ✓ Utilization of ABTS in acidic pH is inadvisable due to occurence of side reactions resulting in false high activities

5 Role of protein in acidic media

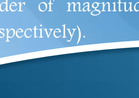
Observed increase of relative catalytic activity in presence of protein in acidic media (oxidation of TMB and ABTS) was totally unexpected phenomenon. It may suggest, that protein in pH 2,5 acts not only as steric hindrance at AuNPs surface, but also actively promotes catalysis or also interacts with substrates.

Putative role of BSA

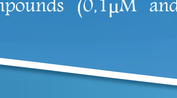
- catalytic activity of protein functional groups?
- oxidizing agent (besides H_2O_2), resulting in enhanced rate of product formation
- stabilizer/blocking agent

6 Influence of functional groups in BSA

-COOH group



–NH_2 group



-SH group

phenolic ring

Relative activities (absorbance slopes) of ABTS oxidation in presence of selected substances mimicking aminoacids' residues. Due to low solubility of tyrosine, its concentrations were one order of magnitude lower than in case of remaining compounds (0,1μM and 1μM respectively).

Structure of bovine serum albumin is very complicated, so many functional groups might be responsible for unusual activity. Our aim was to check, which functional groups of the most common aminoacids in BSA can enhance enzyme-like activity of gold nanoparticles. Two pH values were chosen for comparative experiments (pH 2,5, where observed effects are substantial and less acidic medium – pH 4,0, closer to the pI value of BSA).
In addition to finding the group responsible for atypical reaction mechanism, main goal of study was also to assess the significance of pH value on observed processes.
We concluded, that among selected structures only phenolic ring in tyrosine might be responsible for enhanced product formation.
Thiol residues in cysteine completely block activity due to chemisorption on Au surface.

7 Conclusions:

- ✓ Hyperbranched polyglycidol allows for preparation of highly stable and catalitically active nanoparticles, even in complex media of high ionic strength,
- ✓ Proteins substantially influent AuNPs peroxidase-like activity in case of HBPG as stabilizing agent,
- ✓ Prolongation of incubation time intensifies effects – positives and negatives,
- ✓ Protein adsorption may be described as a dynamic, equilibrium process,
- ✓ Between examined functional groups in BSA, only tyrosine residues might be responsible for its peculiar properties in acidic pH,
- ✓ Albumin can have multiple function – it can adsorb onto nanoparticles surface and blocked active sites or actively participate in catalysis (at low pH of catalysis medium),
- ✓ Exhaustive explanation of observed effects needs further investigations,
- ✓ Phenomena underlined in this work should be taken into account during development of analytical tools based on HRP-like nanozymes activity,

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