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Employing gold nanoclusters for analysis of pectinase activity

Sophia Ya. Sakhina,[a] Nikolai P. Iakimov,[a] Evgenii O. Fomin,[a] Irina D. Grozdova,[a] Margarita V. Semenova,[a] Elena V. Kudryashova,[a] Victor N. Orlov[b] and Nikolay S. Melik-Nubarov*[a]

[a] *Department of Chemistry, M. V. Lomonosov Moscow State University, 119991 Moscow, Russian Federation. E-mail: melik.nubarov@belozersky.msu.ru*

[b] *A. N. Belozersky Research Institute of Physico-Chemical Biology, M. V. Lomonosov Moscow State University, 119992 Moscow, Russian Federation*

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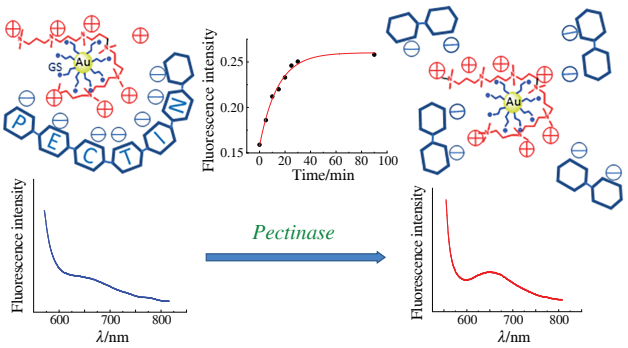
A method for sensing pectinase activity was proposed based on the formation of polyelectrolyte complexes of negatively charged, glutathione-coated, fluorescent gold nanoclusters $Au_{22}(SG)_{18}$ with the polycation 3,3-ionene and the anionic polysaccharide pectin. The formation of polyelectrolyte complexes of the nanoclusters with ionene leads to an increase in fluorescence due to the aggregation-induced emission enhancement effect, while after the addition of the natural polyanion pectin, the emission decreases. Enzymatic hydrolysis of pectin by pectinase resulted in the recovery of the fluorescence of nanoclusters, which allows sensing the activity of pectinase.



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Gold nanoclusters (AuNCs) are a class of complex compounds containing typically 10–100 metal atoms in oxidation states 0 and +1.[1] Formation of AuNCs commonly occurs under kinetic control. The synthesis is carried out either by the reaction of chloroaurate with small amounts of a reducing agent in the presence of equivalent amounts of the ligand[2,3] or by the reduction of preformed Au–thiolate complexes with carbon monoxide[4] or sodium borohydride.[5] A wide variety of atomically precise nanoclusters have been reported with the natural tripeptide glutathione (GSH, γ-Glu–Cys–Gly) as a ligand. When incorporated into a nanocluster, GSH loses a proton, converting to the thiolate form, which is usually denoted as SG in the nanocluster formula. Many SG-containing nanoclusters emit bright fluorescence in the blue,[6] red[7] and even near-infrared[8] spectral ranges. In particular, $Au_{22}(SG)_{18}$ nanoclusters, whose composition was studied by ESI-MS and EXAFS, exhibit extremely bright luminescence. According to DFT modeling, these nanoclusters have a core–shell structure with a core of eight Au^0 atoms coated with a hydrophilic shell consisting of four staples of Au^I thiolate complexes.[7]

Fluorescent AuNCs have been widely used for the analysis of various heavy metals, which can affect their fluorescence. In particular, multiply charged cations such as Ce^{3+},[9] Al^{3+},[10] Zn^{2+},[11] *etc.* can change the mobility of Au–thiolate stapples, thereby enhancing the fluorescence of AuNCs *via* Ligand-to-Metal-to-Metal Charge Transfer (LMMCT),[12] which explains the Aggregation-Induced Emission Enhancement (AIEE) effect of AuNCs.[13] This effect seems to be responsible for the polycation-induced enhancement of AuNCs fluorescence caused by the formation of electrostatic complexes with polycations.[14–17] It has been shown that interaction with polyarginine and Arg-rich polypeptide AG73 leads to the enhancement of AuNCs fluorescence.[18] This effect is suppressed in the presence of polyanions, which has been exploited to develop a sensitive assay for heparin in biological fluids.[14,17] Cleavage of cationic polypeptides with trypsin reduced the fluorescence of the complexes to the initial level.[14] However, to the best of our knowledge, there are no examples of using this approach to sense the activity of hydrolases specific towards polyanionic substrates. In this work, we investigated the polycation-induced enhancement of photoluminescence of nearly monodisperse $Au_{22}(SG)_{18}$ nanoclusters and applied this effect for sensing the catalytic activity of fungal pectinase.

The nanoclusters were synthesized according to the previously proposed[7] and later modified[5] method [Figure 1(*a*)]. Briefly, in the first step, linear gold(I) thiolate complexes were prepared, which in the second step were reduced with a small amount of sodium borohydride under alkaline conditions, using a $HAuCl_4$/GSH/$NaBH_4$ ratio of 1 : 1.5 : 0.07 (for details, see Online Supplementary Materials, Section S1).

It can be assumed that the terminal gold atoms in the thiolate chains are primarily subjected to reduction by borohydride [see Figure 1(*a*)]. Further maturation of the particles formed in this way was accompanied by the appearance of a pronounced absorption maximum at 520 nm and bright fluorescence at 665 nm [Figure 1(*b*)]. The resulting nanoclusters were isolated by fractional precipitation with methanol. Analysis of the resulting sample by UV and fluorescence spectroscopy and electrophoresis in polyacrylamide gel [Figure 1(*c*)] showed its identity to the $Au_{22}(SG)_{18}$ nanoclusters described earlier.

The interaction of $Au_{22}(SG)_{18}$ nanoclusters with 3,3-ionene **1** [Figure 2(*a*)] at pH 5.0 resulted in a considerable increase in their fluorescence [Figure 2(*b*), curves *1* and *2*]. This interaction was accompanied by a 1000-fold increase in the hydrodynamic diameter (D_H) of the particles due to the electrostatic interaction of $Au_{22}(SG)_{18}$ with 3,3-ionene [Figure 2(*c*), diagrams *1* and *2*].

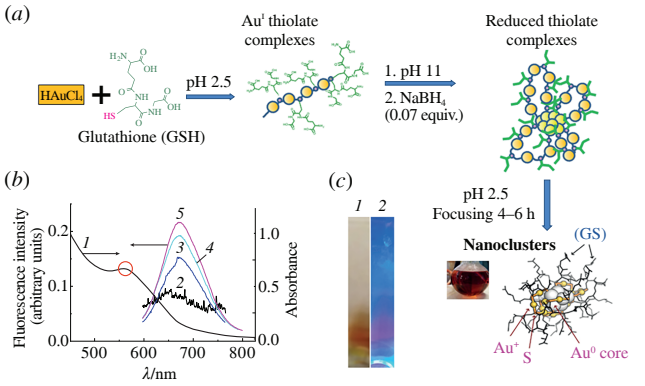

Figure 1 (*a*) Scheme of the synthesis of Au^{I} thiolate complex with GSH (green) and the reduction of Au^{I} (dark yellow circles) to Au^{0} (bright yellow circles) with sodium borohydride to form AuNCs. (*b*) Optical spectra of AuNCs obtained by electrophoretic focusing of the reduced thiolate complexes: (*1*) UV-VIS absorption spectrum and fluorescence spectra at (*2*) 3, (*3*) 170, (*4*) 270 and (*5*) 370 min. (*c*) Electrophoretic pattern of purified nanoclusters (*1*) in visible light and (*2*) under UV light excitation (365 nm).

This result is fully consistent with previous data suggesting the involvement of the LMMCT phenomenon in the AIEE effect for polyelectrolyte complexes of GSH-stabilized AuNCs with poly-L-lysine,[16] poly-L-arginine and arginine-rich polypeptide.[18]

However, the addition of an excess amount of the anionic polysaccharide pectin **2** [see Figure 2(*a*)] resulted in a sharp decrease in the fluorescence signal, accompanied by an increase in the scattered light intensity [Figure 2(*b*), curve *3*]. In this case, the fluorescence of AuNCs was negligible even after subtracting the contribution of scattered light (Figure S1, see Online Supplementary Materials). In the context of this work, the question of the reasons for the decrease in the fluorescence of nanoclusters in the presence of pectin is not fundamental.

The disappearance of the fluorescence signal could be the result of either the displacement of nanoclusters from their interpolyelectrolyte complex with ionene or the formation of 'AuNCs–ionene–pectin' ternary complexes.

In the first case, the decrease in fluorescence could be explained by a decrease in the AIEE effect due to the release of AuNCs from complexes with the polycation. The formation of ternary complexes agrees well with the significant increase in the turbidity of the system upon addition of pectin. In the latter case, the decrease in the fluorescence signal can be explained by

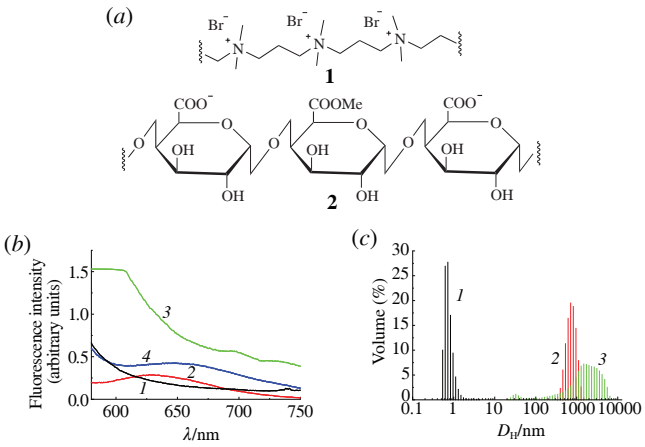

Figure 2 (*a*) Structures of 3,3-ionene **1** and pectin **2**. (*b*) Fluorescence spectra of (*1*) bare $Au_{22}(SG)_{18}$ nanoclusters (0.05 mg ml^{-1}, 0.91 mM COO^{-} groups), (*2*) their binary complexes with 3,3-ionene (9.1 mM ammonium groups), (*3*) ternary '$Au_{22}(SG)_{18}$–3,3-ionene–pectin' complexes (1 : 10 : 100 ratio) and (*4*) those treated with pectinase (0.1 mg ml^{-1}), all measured in 5 mM acetate/5 mM HEPES buffer (pH 5.0) at 25 °C and excitation wavelength of 520 nm. (*c*) Size distributions of (*1*) bare nanoclusters and their (*2*) binary and (*3*) ternary complexes measured by DLS.

the fact that strong scattering of the system reduces the excitation efficiency of nanoclusters, which obscures their fluorescence.

Addition of pectinase to the ternary system $Au_{22}(SG)_{18}$–3,3-ionene–pectin resulted in a sharp decrease in the light scattering intensity [Figure 2(*a*), curves *3* and *4*], thus increasing the fluorescence signal [Figure 2(*a*), curves *2* and *4*]. The recovery of fluorescence is apparently triggered by the enzymatic cleavage of pectin, leading to a decrease in the turbidity of the system and an increase in the efficiency of excitation of nanoclusters by the incident light beam.

These changes in the ternary system fluorescence were used to estimate the rate of pectinase-catalyzed pectin hydrolysis. For this purpose, ternary systems formed at pH 5.0 were incubated with pectinase (0.1 mg ml^{-1}) and the evolution of sample fluorescence was recorded [Figure 3(*a*)]. As the pectinase-catalyzed hydrolysis of pectin proceeded, a decrease in the signal caused by light scattering by aggregated ternary complexes was observed [see Figure 3(*a*)].

The fluorescence of the binary 'AuNC–ionene' complexes was almost completely restored within an hour. The degree of fluorescence signal enhancement was used to estimate the fraction of hydrolyzed pectin from Figure 3(*a*) as

$$F = (I_t - I_0)/(I_\infty - I_0), \quad (1)$$

where I_0 is the initial fluorescence intensity and I_∞ is the fluorescence corresponding to the plateau (for details, see Online Supplementary Materials, Figure S1).

The specific initial rate of pectinase-catalyzed pectin degradation was estimated from curve *1* in Figure 3(*b*) as 3.0 (% pectin) min^{-1} (mg enzyme)$^{-1}$ and an effective first-order rate constant of enzymatic hydrolysis of about 0.032 ± 0.003 min^{-1}. Taking into account that pectinase is characterized by a rather high Michaelis constant ranging from 0.5 to 11.3 mg ml^{-1},[19–21] the absolute rate of pectin hydrolysis should be proportional to the substrate concentration. However, the fraction of hydrolyzed pectin does not depend on its initial concentration. The specific initial rate of pectinase-catalyzed hydrolysis, determined by the conventional method using 3,5-dinitrosalicylic acid (DNS) reagent, was also about 3.0 (% pectin) min^{-1} (mg pectinase)$^{-1}$. The rate of pectin hydrolysis determined by the conventional method practically coincided with the rate of fluorescence enhancement of the ternary system with the observed first-order rate constant of 0.036 ± 0.007 min^{-1} [Figure 3(*b*), curve *2*]. Thus, the approach to sensing hydrolase activity using AuNCs allows for the correct detection of the hydrolase activity of fungal pectinase.

In this work, we have shown for the first time that complexes of GSH-stabilized AuNCs can be used for sensing the activity of polyanion-specific hydrolases. Unlike earlier studies, in our work, instead of a binary complex of nanoclusters with a biodegradable polycation, we used a ternary system

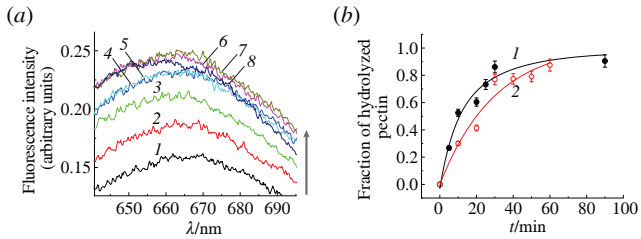

Figure 3 Sensing the catalytic activity of pectinase using AuNCs. (*a*) Time evolution of fluorescence spectra of the 'AuNC–ionene–pectin' ternary system (molar ratio 1 : 10 : 50) after addition of pectinase (0.1 mg ml^{-1}). Fluorescence was measured after (*1*) 0, (*2*) 5, (*3*) 10, (*4*) 15, (*5*) 20, (*6*) 25, (*7*) 30 and (*8*) 90 min, and baseline data were subtracted. (*b*) Comparison of kinetic profiles of pectin enzymatic hydrolysis measured (*1*) by AuNCs fluorescence and (*2*) using the conventional DNS reagent.

'AuNC–polycation–polyanion' as a hydrolysable biopolymer. The results obtained demonstrate the versatility of the proposed approach to the design of molecular sensors based on AuNC complexes with charged biopolymers.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.71267/mencom.7686.

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