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Employing gold nanoclusters for analysis of pectinase activity

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S1. Materials and Methods

Materials. The materials used were hydrochloric acid, glutathione, sodium borohydride, sodium hydroxide, ammonium persulfate, HEPES and TEMED manufactured by Sigma-Aldrich (USA). Hydrochloric acid was from Komponent-Reactiv (Russia) and agarose was from Difco (USA). 3,3-ionene was kindly provided by Prof. V.A. Izumrudov. 5 mM HEPES-acetate buffer with pH 5.0 was used for all measurements. Pectin sample with low degree of methyl esterification was purchased from CP Kelco, Lille Skensved, Denmark and used as received. The pectinase produced by "Agroferment Ltd." was used in this work. Its activity is determined by two enzymes - pectinlyase from *Penicillium canescens* and polygalacturonase from *Aspergillus niger*.

$Au_{22}(SG)_{18}$ nanoclusters synthesis. Glutathione stabilized Au nanosclusters were synthesized according to[S1]. Initially, thiolate complexes of Au(I) and glutathione were prepared (Figure 1A in the). For this purpose, 20 mM tetrachloroaurate solution was added to a round bottom flask with 94 mL of deionized water to a final concentration of 1 mM and a 1.5-fold molar excess of 50 mM glutathione was added. Thus, thiolate complexes of Au(I) and glutathione were formed, which are poorly soluble in water under acidic conditions, so the solution appeared opalescent and the yellowish coloration of chloroaurate gradually faded. After the mixture became colorless, pH was adjusted to 11.0 by addition of about 1 mL of 1 M NaOH and 0.096 mL of 0.125 mM $NaBH_4$ solution in 10 mM NaOH was added by portions of 16 μL and the mixture was allowed to react for 30 min. Final $HAuCl_4$/GSH/$NaBH_4$ molar ratio was 1:1.5:0.07. Then the reduction was terminated by acidification with about 1 mL 1 M HCl to pH 2.5, and the clusters were allowed to maturate for additional 6.5 h. (Figure 1A) The solution was concentrated and subjected to fractional precipitation with isopropyl alcohol to obtain a pellet containing nearly monodisperse AuNCs as judged by polyacrylamide gel electrophoresis (Figure 1B). Finally, a fluorescent fraction with about 90% purity was isolated using preparative gel electrophoresis as described elsewhere[S1].

Fluorescence of AuNCs (0.05 mg/mL, 5.4 μM in 5 mM HEPES-acetate, 5 mM acetate buffer, pH 5.0) was measured using Hitachi HR-3010 fluorimeter. All measurements were performed in triplicates.

The mean hydrodynamic diameter of AuNC was measured by dynamic light scattering using the Malvern ZetaSizer with a thermostatted cuvette compartment at a fixed scattering angle of 175°. Software provided by the manufacturer was employed to calculate hydrodynamic diameter distribution.

The activity of pectinase in solution was determined using dinitrosalicylic acid (DNS reagent). The method is based on the reduction of the nitro group of the DNS reagent by an aldehyde group located on the terminal carbohydrate of the polysaccharide. 2.4 mg/ml of pectin was mixed with 0.5 mg/mL of pectinase and incubated for various time intervals. The reaction was stopped by mixing with 1 mL of 0.1 M 3,5-dinitrosalicylic acid neutralized with equivalent amounts of NaOH and incubated in a water bath for 5 minutes. After cooling the samples to room temperature, the optical density was measured at a wavelength of 540 nm [S2].

Calculation of the degree of pectinase hydrolysis. In addition to the direct determination of the degree of pectin hydrolysis by the formation of reducing end saccharides with DNS-reagent, the degree of pectin hydrolysis was also calculated from the fluorescence data. For this purpose, we used formula 1, assuming that the degree of fluorescence unfolding of the nanoclusters is determined by the perctin

content. The coincidence of the kinetics of the accumulation of terminal saccharide groups and the degree of fluorescence increase of nanoclusters allows us to assume that it is the decomposition of pectin that determines the fluorescence increase. Consequently, the value calculated by formula (1) shows the fraction of hydrolyzed pectin.

S2. Subtraction of the background caused by the light scattering of the exciting beam from fluorescent spectra of turbid samples.

The background spectrum was calculated for each spectrum. To this end, the parts of the spectrum corresponding to the fluorescence signal of nanoclusters (600-750 nm region) were masked, after which the remaining areas before and after the fluorescence peak were approximated by an empirically fitted function:

$$I = \frac{A_1}{(\lambda - \lambda_0)^p} + A_2,$$

where A_1 sets the scale of changes in the fluorescence signal, A_2 - allows to shift the function along the ordinate axis, and the exponent p - shows the sharpness of the signal change and is determined by the size and concentration of scattering particles. The resulting curve Figure S2 depicts the example of such treatment.

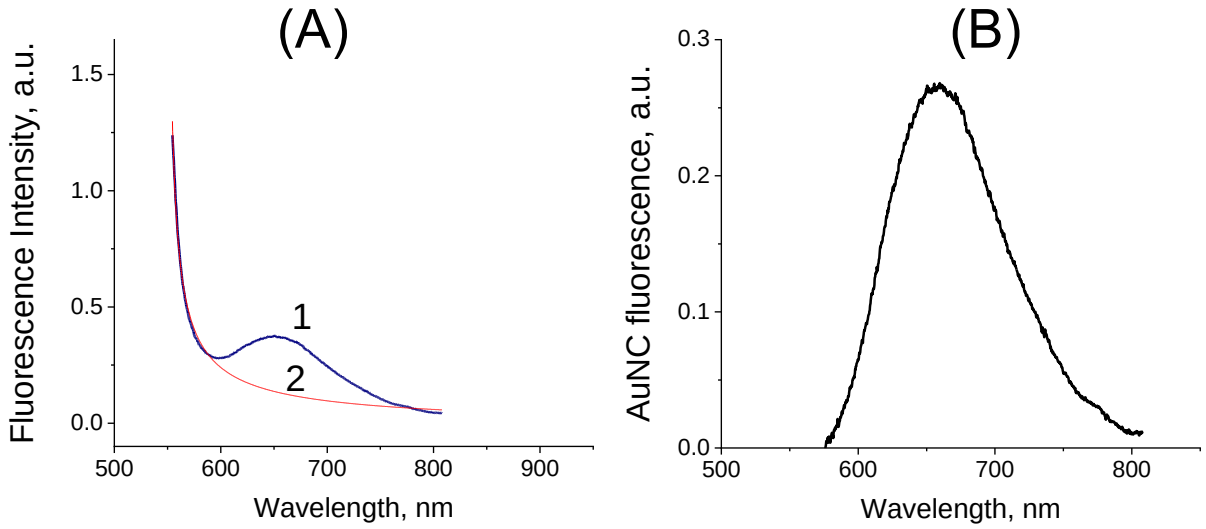

Figure S1 Treatment of fluorescence spectra of turbid samples. (A) Spectrum of the ternary complex "Au22(SG)18-ionene-3,3-pectin" incubated with 0.5 mg/mL of pectinase for 90 min (1) and the background curve (2) in 5 mM HEPES-acetate buffer, pH 5,0 at concentration of Au22(SG)18 0.05 mg/mL, and Au22(SG)18/ionene-3,3/pectin molar charge ratio of 1:10:100, ambient temperature. (B) The spectrum of Au22(SG)18 fluorescence as a result of subtraction of the background spectrum.

The resulting curve represented the law according to which the fluorescence signal changes due to the variation of the intensity of the excitation beam with varying wavelength (Fig. S2A, red curve). Subsequent subtraction of the background spectrum from the fluorescence spectrum of the sample gave the fluorescence spectrum of the nanoclusters free from the contribution of scattered light (Fig. S2B).

References

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