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Plasmonic nanosensors with inverse sensitivity by means of enzyme-guided crystal growth

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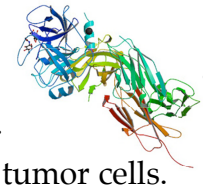
1. Introduction



PSA
a protease having
multi binding
site to antibody

1.1. Target molecule: prostate-specific antigen (PSA)

• PSA is a biomarker of prostate cancer (前立腺がん).

• Amount of a biomarker increases due to growth of tumor cells.

=> Detection of a biomarker at low concentration is needed for the early treatment of cancer.

1.2. Problem of conventional nanosensors

• Conventional nanosensors generate a signal due to directly proportional to the concentration of the target molecule.

=> Difficult to detect with confidence at ultralow concentrations of the molecule

1.3. This work

"Nanosensors made of enzyme-modified gold nanostars (AuNSs) as a plasmonic transducer with inverse sensitivity"

• Key point: Inverse sensitivity for the concentration of target molecule

– Strong signal is generated at lower concentration of PSA.

• Information of the concentration of PSA is converted to change of absorption of the AuNSs based nanosensors through enzyme-guided crystallization of silver nanocrystal on the surface of nanosensors (Figure 1).

• Detection of PSA at ultralow concentration

– **10^{-18} g mL^{-1} (= 4 x 10^{-20} M)** as a lowest concentration (Previous record: Digital ELISA[1]: 10^{-17} g mL^{-1}) => One order magnitude lower!

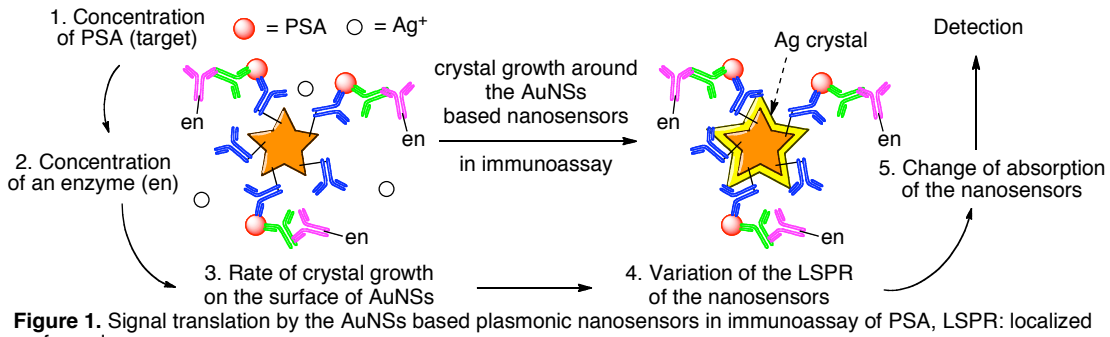


Figure 1. Signal translation by the AuNSs based plasmonic nanosensors in immunoassay of PSA, LSPR: localized surface plasmon resonance.

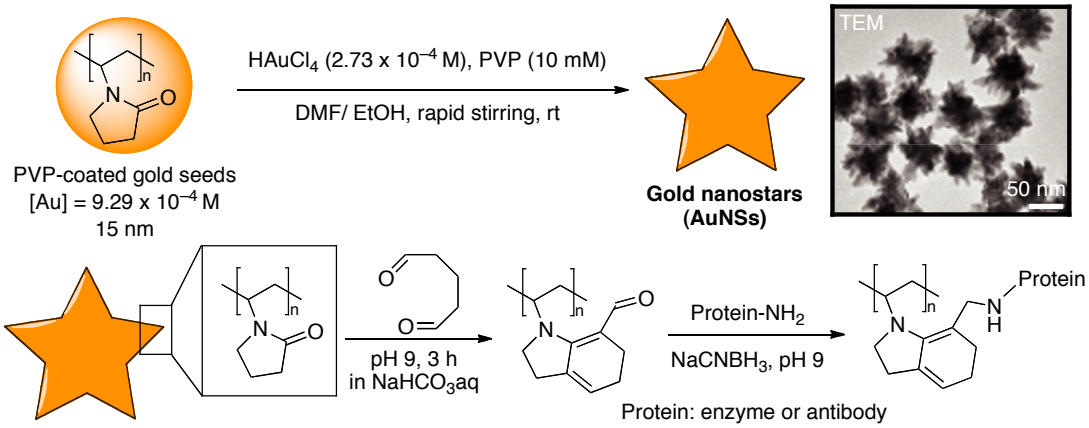
2. Results and Discussion

2.1. Preparation of protein-modified AuNSs (Scheme 1)

• AuNSs were prepared from poly(vinylpyrolidone) (PVP)-coated gold seeds.[2]

• Proteins such as enzyme or antibody were combined at the PVP moiety.

Scheme 1. Preparation of AuNSs and covalent attachment of proteins to PVP-stabilized AuNSs.



2.2. Signal-generation mechanism by means of enzyme-guided crystal growth (Proof of principle experiment) (Scheme 2)

• AuNSs were modified with glucose oxidase (GOx).

• Hydrogen peroxide is generated due to reduction of oxygen in biocatalytic cycle of GOx.

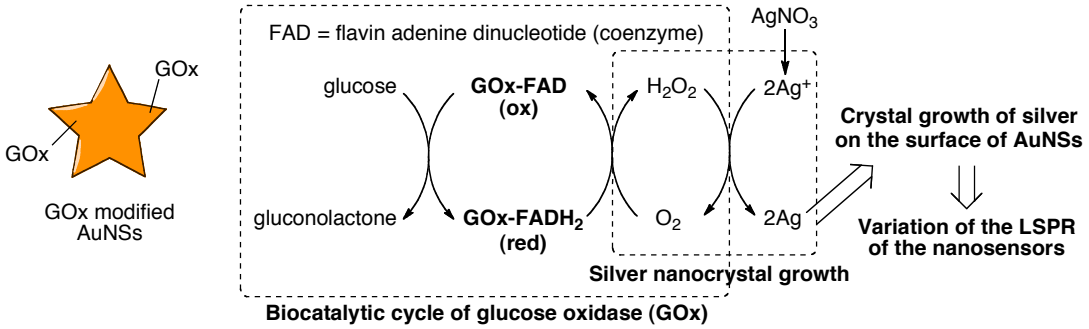
• Silver crystals grow on the surface of AuNSs due to reduction of Ag^+ by hydrogen peroxide.

=> Information of the concentration of GOx is converted to the rate of crystal growth.

• Localized surface plasmon resonance (LSPR) of the AuNSs changes in proportion to the signal from crystal growth because condition of LSPR changes when silver coates the surface of AuNSs.[3]

• The signal is amplified in the biocatalytic cycle.

Scheme 2. The proposed signal-generation mechanism of GOx-attached AuNSs.



2.3. Inverse sensitivity in the plasmonic nanosensors (Figure 2)

• The magnitude of the signal registered by plasmonic nanosensors depends on the rate of crystallization of silver nanocrystal.

(i) When silver crystals grew **slowly** at low concentration of GOx, the growth of a silver coating on the existing nanocrystal (AuNSs) was favored (Figure 2a).[4]

=> Visible/near-infrared spectra of the nanosensors blueshifted due to variation of the LSPR of the nanosensors (When the [Ag]/[Au] ratio of the plasmonic transducer is higher, the blueshift in the spectra becomes larger.)[3]

(ii) When silver crystals grew **fast** at high concentration of GOx, the nucleation of free-standing small particles was favored (Figure 2b).[4]

=> No change in the visible/near-infrared spectra

• The blueshift of λ_{max} in the spectra was larger at lower concentration of GOx due to the slow crystallization (Figure 2c and 2d).

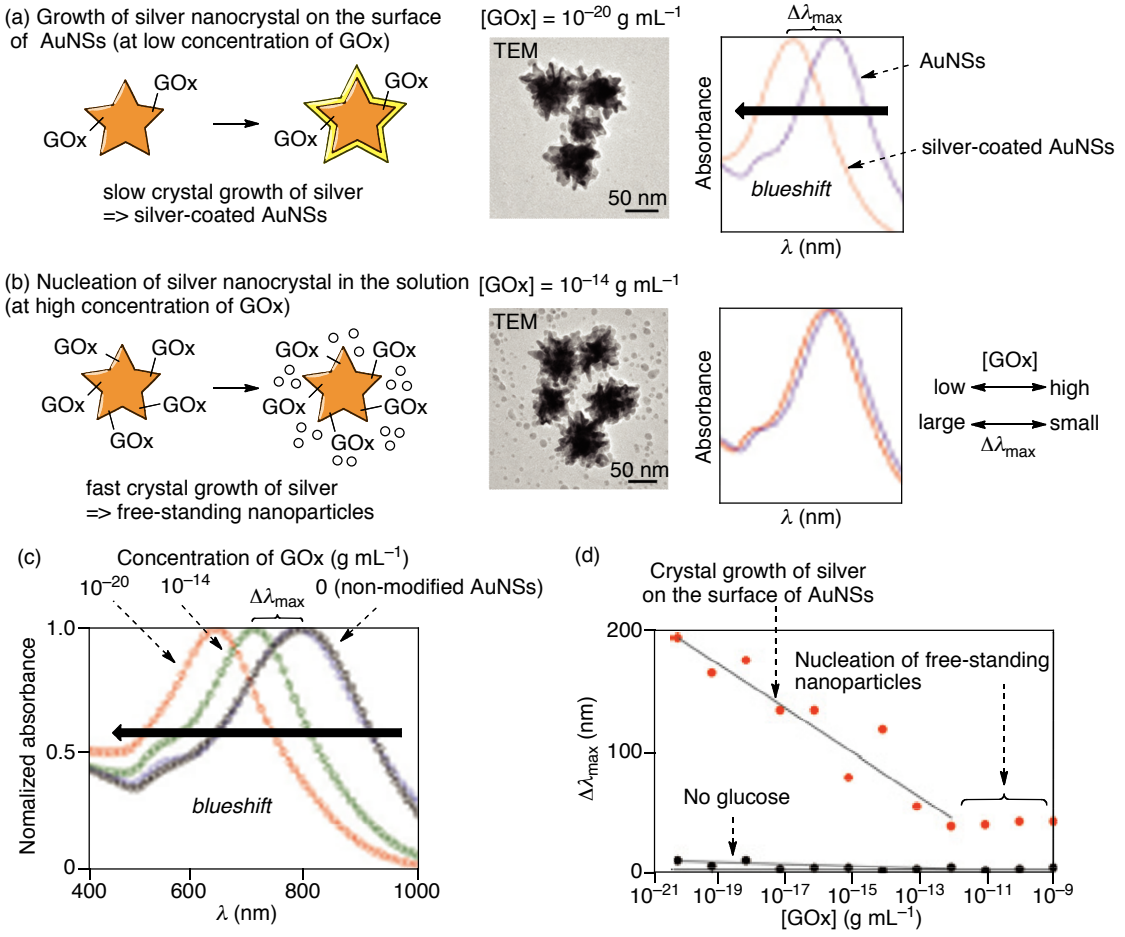


Figure 2. Inverse sensitivity in plasmonic nanosensors. (a) Silver nanocrystal growth on the surface of AuNSs at low concentration of GOx, (b) Nucleation of free-standing nanoparticles at high concentration of GOx, (c) Visible/near-infrared spectra of the nanosensors, (d) Relationship between the blueshift of λ_{max} and the concentration of GOx.

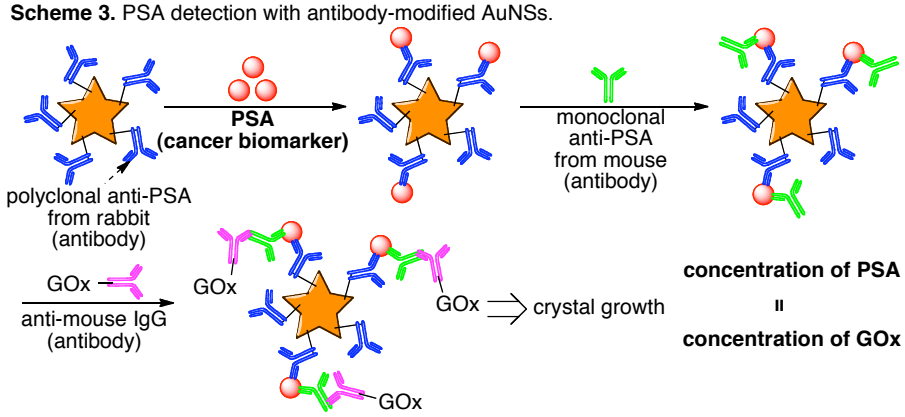
2.4. Immunoassay for the detection of PSA with AuNSs based nanosensors

• AuNSs were modified with anti-PSA antibody.

• PSA was captured between the AuNSs and GOx via antibodies (Scheme 3).

=> The concentration of PSA is the same as the concentration of GOx.

Scheme 3. PSA detection with antibody-modified AuNSs.



• Ranges of the concentration of PSA detection

– *in vitro*: from 10^{-13} g mL^{-1} to 10^{-18} g mL^{-1} (Figure 3a)

– *ex vivo* (whole serum): from 10^{-14} g mL^{-1} to 10^{-18} g mL^{-1} (Figure 3b).

=> Lower limit of PSA detection was 10^{-18} g mL^{-1} (= 4 x 10^{-20} M = 24000 molecules L^{-1}).

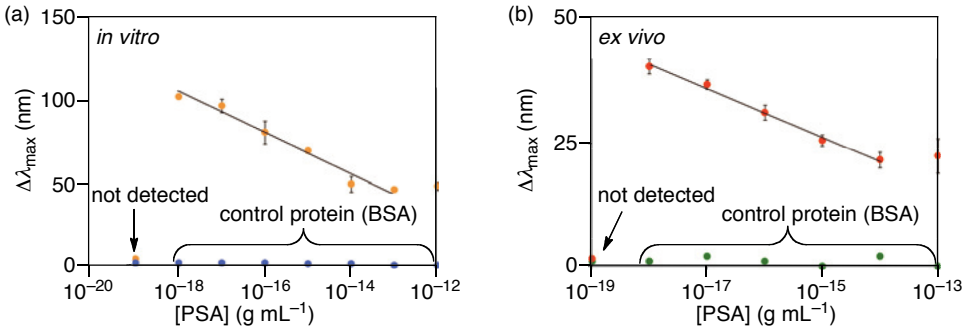


Figure 3. Relationship between the blueshift of λ_{max} Visible/near-infrared spectra of the AuNSs nanosensors and the concentration of PSA (a) *in vitro* and (b) *ex vivo*, BSA: bovine serum albumin.

3. Summary

• The inverse sensitivity phenomenon reported here was possible by controlling the kinetics of crystal growth with enzyme, which in turn determined the signal registered by the plasmonic transducer.

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[1] Rissin D. M. et al. *Nature Biotechnol.* **2010**, *28*, 596–599.
[2] Kumar, P. S.; Pastoriza-Santos, I.; Rodriguez-Gonzalez, B; Garcia de Abajo, F. J.; Liz-Marzan, L. M. *Nanotechnology*, **2007**, *19*, 015606.
[3] Cardinal, M. F.; Rodriguez-Gonzalez, B.; Alvarez-Puebla, R. A.; Perez-Juste, J.; Liz-Marzan, L. M. *J. Phys. Chem. C*, **2010**, *114*, 10417–10423.
[4] Jana, N. R.; Gearheart, L.; Murphy, C. J. *Chem. Mater.* **2001**, *13*, 2313–2322.