Fabrication of Aptamer-Functionalized Gold Nanorod Biosensor for Colorimetric Detection of Lysozyme

T. Tohidi Moghadam[*] and B. Ranjbar✉[**]

[*]Department of Nanobiotechnology, Faculty of Biological Sciences, Tarbiat Modares University
Tehran, Iran, t.tohidi@modares.ac.ir
[**] Departments of Biophysics and Nanobiotechnology, Faculty of Biological Sciences
Tarbiat Modares University, Tehran, Iran, ranjbarb@modares.ac.ir

ABSTRACT

Anisotropic nanoparticles have evinced outstanding optical, catalytic and thermal properties in the recent decade, amongst which, gold nanorods (GNRs) have been nominated as good candidates for biosensing, diagnostic, and imaging technologies. Herein, aptamer functionalized GNRs have been used for calorimetric detection of lysozyme. Nanostructures were covalently conjugated with thiolated sequence of lysozyme aptamer, and the characteristic surface plasmon resonance bands were monitored for stability considerations. Upon introduction of analyte, the nanobiosensor started to develop distinct change of colour shade at various concentrations of biomolecule. The colorimetric detection occurred due to the strong sensitivity of GNRs' LSPR to minute changes of local refractive index; where presence of target biomolecule dictates specific aggregation of the nanostructures. This effort encourages fabrication of novel nanorod based aptasensors for the emerging *lab-in-a-vial* techniques.

Keywords: gold nanorods, surface plasmon resonance, aptamer

1 INTRODUCTION

Development in sensing technologies has received enormous attention of nanobiotechnologists, for which, there is an urgent demand arising from awareness of both environmental issues and healthcare concerns. Early detection of disease and its progression promises taking better therapeutic strategies to achieve precise and efficient solutions with considerable applicability [1]. Today, the ultimate goal in biosensor evolution is to produce nanoscale assemblies being capable of simple, reliable, sensitive, and specific monitoring of analyte in a continuous manner. This requires involving materials of novel characteristics at the nanoscale to design biosensors with outstanding improvement in the recognition capability and report of the binding events. Plasmonic nanoparticles such as gold and silver constitute one of the most researched branches of nanobiotechnology, evincing outstanding optical, catalytic and thermal properties. Amongst anisotropic nanoparticles with plasmonic characteristics, gold nanorods (GNRs) have been nominated as good candidates for biosensing, diagnostic, and imaging technologies. The wavelength dependence of the longitudinal surface plasmon resonance of gold nanorods on minute changes in the immediate vicinity makes them extremely sensitive reporters of molecular binding events with excellent multiplexing capability. Such property provides opportunity to design a powerful tool for biomolecular detection and identification of specific pathogens. So far, there have been a number of reports about optical detection of DNA [2], multiple pathogens [3], Hepatitis B [4], Human immunodeficiency virus (HIV) [5, 6], selective detection of amino acids [7], and multiplex recognition of biological targets [8] by GNRs. Herein, aptamer functionalized GNRs have been used for calorimetric detection of lysozyme. As an enzyme of antibacterial activity, lysozyme has been widely applied in pharmaceutical and food industries, with well-known structure, function, dynamics and physico-chemical properties [9-12]. Meanwhile, excretion of elevated levels of this protein in urine or blood could be a sign of kidney's tubular cell damage and Acute myelomonocytic leukemia. Taking the problem of availability, time consuming and costly analysis techniques into consideration, design of an independent, non-instrumental diagnostic technique with detection simplicity could be of great interest.

2 EXPERIMENTALS

2.1 Materials

Chicken egg white lysozyme, $HAuCl_4 \cdot 3H_2O$, $NaBH_4$, Ascorbic acid, Hexadecyltrimethylammonium bromide (CTAB), and $AgNO_3$ were procured from Sigma. Synthetic anti-lysozyme oligonucleotides (sequence 5'-(SH)-$(CH2)_6$-ATC TAC GAA TTC ATC AGG GCT AAA GAG TGC AGA GTT ACT TAG-3' , HPLC purified) was purchased from Eurofins (MWG operon, Germany). Dithiothreitol (DTT) was procured from Merck for reduction of disulfide bonds. Buffers were prepared using phosphate buffered saline tablet (PBS) and sodium acetate, from Merck. Glassware were thoroughly cleansed with sulfochromic

acid and detergent. Deionized water was used throughout the experiments.

2.2 Equipments for Characterization

Characteristic surface plasmon resonances of gold nanorods were recorded in the wavelength region of 400 to 900 nm, using a Cary-100 spectrophotometer. For TEM characterization, purified GNRs were deposited on carbon coated copper grids and imaged on a LEO 906 transmission electron microscopy (Germany). Prior to each experiment the nanoparticles were homogenoeusly dispersed by Wisd Wise Clean sonicator.

2.3 Synthesis and Purification of GNRs

Gold nanorods were synthesized using sequential seed mediated growth protocol [9, 10]. In a typical experiment, small spherical gold nanoparticles (seeds) were prepared by mixing aqueous solutions of $HAuCl_4 \cdot 3H_2O$ (250 µL, 0.01 M) and CTAB (7.5 mL, 0.095 M), followed by immediate addition of ice-cold $NaBH_4$ solution (600 µL, 0.01M). The reactants were mixed by rapid inversion for two minutes and kept undisturbed at room temperature, for a minimum of 2 hours. The growth solution was prepared by sequential addition of CTAB (9.5 mL, 0.095 M), $HAuCl_4 \cdot 3H_2O$ (400 µL, 0.01 M), $AgNO_3$ (60 µL, 0.01 M) and ascorbic acid (64 µL 0.10 M) solutions, followed by mixing with seed particles (40 µL). It will take several hours for termination of reaction and formation of rod-shaped nanostructures. Samples were centrifuged twice (14000 rpm, 7 min) to remove excess cationic surfactant (CTAB) and unreacted gold ions. The precipitate was diluted by PBS buffer and sonicated for several minutes to redisperse the nanorods. Prior to bioconjugation with lysozyme aptamer, concentration of stock GNRs was adjusted to 2 OD.

2.4 Nanoprobe Preparation

The sulfide functional group of the aptamer was activated by addition of 10 µL, 1.0 N Dithiothreitol (in 0.01 M Sodium acetate, pH 5.2) to oligonucleotides (5 OD based) [13]. The mixture was vortexed and incubated at ambient temperature for 15 minutes. Excess DTT and unwanted thiol fragments were removed by ethyl acetate extraction (three times). Freshly cleaved oligonucleotides (30 µL) was immediately added to 100 µL GNRs (dispersed in PBS, pH 7.4). The nanoprobe was allowed to incubate at ambient temperature for 30 minutes. To maximize DNA loading on the surface of GNRs, a stock solution of 2 M NaCl was used for gradual increment of concentration from 0.1 M to 1 M. Meanwhile, every 20 minutes the solution was sonicated for 10 seconds. The sample was incubated overnight. Prior to use, excess oligonucleotides were decanted after one round of centrifugation and pellets of GNRs were resuspended in PBS buffer (pH 7.4). To optimize the aptasensor condition, surface plasmon resonance of the nanoprobe was monitored at different concentrations of both nanostructure and aptamer. For target detection, working solutions of lysozyme were prepared with diverse range of concentrations. Specificity of the aptasensor was checked with equal concentration of BSA as the control experiment.

3 RESULTS & DISCUSSION

3.1 Characterization of Nanoprobe

Formation of gold nanostructures with rod morphology was monitored by appearance of two absoption bands in the visible and near infrared region. Figure 1 shows the characteristic surface plasmon resonance bands of GNRs, and its changes upon interaction with three different concentrations of aptamer. Depending on the polarization of the incident light, oscillation of the electrons in the conduction band occurs in two different directions [9, 10]. Excitation of surface plasmon oscillation along the short axis of naostructures induces an absorption band in the visible region. When the surface plasmon of the nanostructure is oscillated along the other axis, an absorption band of much stronger intensity appears at a longer wavelength. These absorption peaks could be referred to as the transverse and longitudinal plasmon resonance bands, respectively; being characteristic of the rod morphology of gold nanostructures. According to Figure 1, the transverse and longitudinal SPR bands of GNRs in this study appeared around 543 and 706 nm, respectively. Upon incubation of GNRs with different concentrations of aptamer (1, 10 and 20 nM), intensity of LSPR decreased to some extent; wherase the transverse SPR did not show any change. In case of using higher concentrations, apart from decrease of intensity the LSPR band experiences red shifts (data not shown). Overall, the LSPR of GNRs always develops much faster response compared to that of transvesre SPR; justifying its advantage over spherical gold nanoparticles.

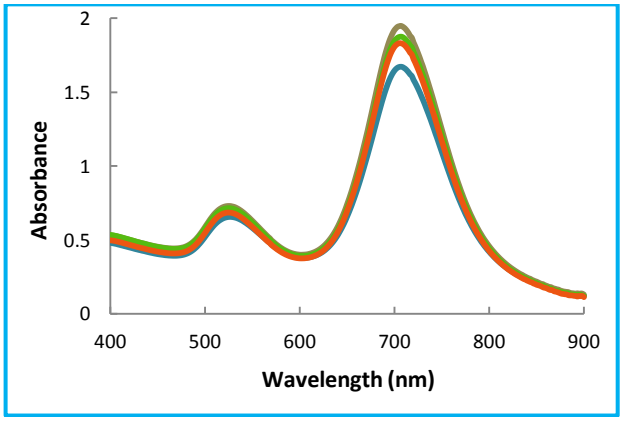

Figure 1: SPR bands of GNRs. From top to bottom: bare GNRs, and nanoprobe with 1, 10 and 20 nM of aptemer.

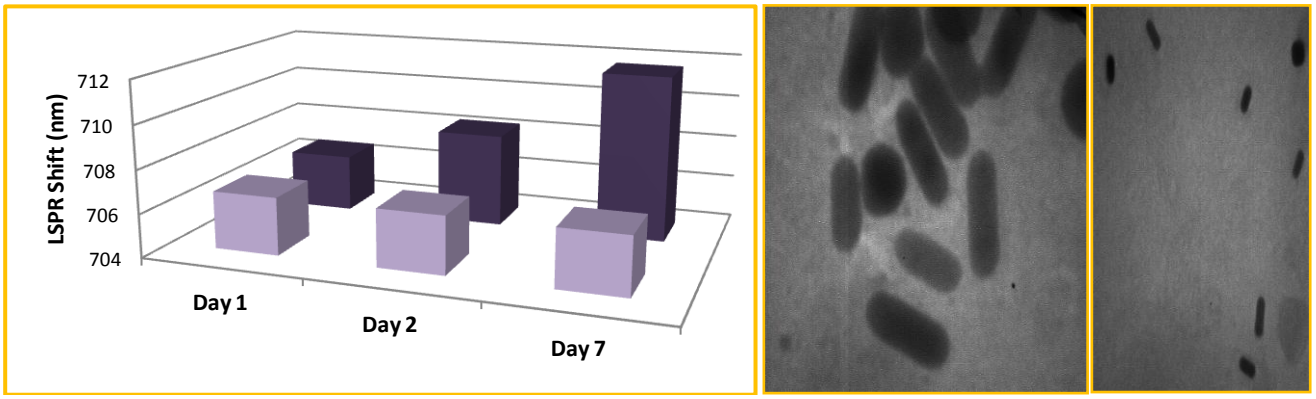

Figure 2: LSPR shifts of one week aged GNRs (light bars) and nanoprobe (dark bars), with characteristic TEM images of bare GNRs and nanoprobe (from left to right).

Such sensitivity of LSPR to trace changes in its vicinity has nominated the nanostructure as an appropriate candidate for biosensing applications. It is worth to mention that optimizing both nanostructure and aptamer concentartion is of high importance; since increment of aptamer concentartion range to micromolar level inevitably guides morphological perturbations and unspecific aggregation of the nanoprobe.

3.2 Optimization of Nanoprobe

Stability of nanoprobe was further studied by monitoring its characteristic SPR bands at three time intervals. Figure 2 (left) compares changes in the longitudinal surface plasmon resonance band of bare GNRs with its aptamer conjugated state (10 nM nanoprobe). It could be noticed that LSPR of GNRs has not undergone any change after aging for one week; whereas it shifted to longer wavelengths in case of nanoprobe. Such changes in the LSPR position and intensity is inevitable, but should not exceed a critical border. Since the LSPR shift of nanoprobe is negligible, 10 nM was considered as the optimized concentartion of aptamer to yield a stable nanoprobe.

To further characterize the samples, transmission electron microscopy was used to image the nanostructures. Figure 2 (right) depicts TEM images of one week aged GNRs and the nanoprobe, respectively. Considering maintanance of rod morphology and absence of unspecific aggregation, it could be concluded that nanoprobe shows good stability within nanomolar range of concentration. Since the optimized concentartion of aptamer in this study is found to be in the nanomolar range, further experiments were carried out within the same range. Further investigations on the stability of nanoprobe showed that the nannostructres are stable for more that one month and aging does not induce morphological perturbations.

3.3 Target Detection

Detection capability of the aptasensor was tested in the presence of target biomolecule. The Nanoprobe was incubated with various concentartions of the target at the ambient temperature. Figure 3 shows appearance of the nanoprobe solutions before and after intercation with varoius concentartions of lysozyme. Upon introduction of analyte, the nanobiosensor started to develop clear shifts in its characteristic longitudinal surface plasmon resonance (LSPR) (data not shown), accompanied by distinct change of colour shade The colorimetric detection occurred due to the strong sensitivity of GNRs' LSPR to minute changes of local refractive index; where presence of target biomolecule or pathogen dictates specific aggregation of the nanostructures. According to the Figure, there is a remarkable change from the typical purpule colour of nanoprobe into violet shade. It could be feasibly distinguished that upon increment of target biomlecule concentration (from nano to micro and millimolar), color changes more apparently. When lysozyme concentration reaches to higher value (10 mM), traces of black precipitates form within the solution; evincing the occurrence of aggregation phenomenon. Since the black precipitate forms in the presence of lysozyme, it could be concluded that target biomolecule is responssible for purturbation of nanostructure monodispersity, dictating it toward specific aggregation. Transmission electron microscopy confirmed the same, representing the aggregated naoprobe-protein complex. Sensitivity of target detection by the present aptasensor was estimated to be down to nanomolar level at the ambient temperature.

Specificity of target detection was also monitored in the presence of control protein (BSA), where the aptasensotr did not respond in the sense of color change (Figure 3). Therefore, the fabricated aptamer-functionalized gold nanorod biosensor could be considered as a new tool for

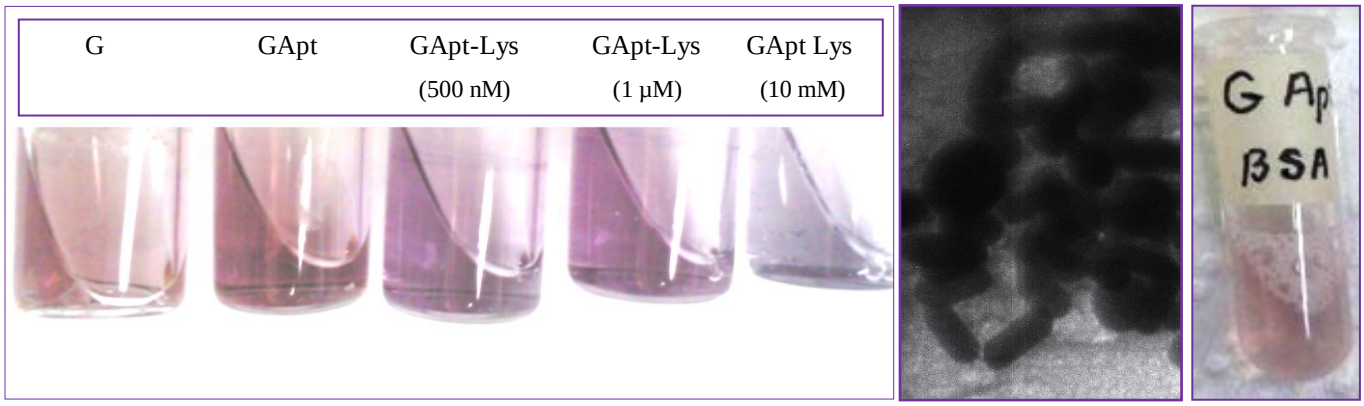

Figure 3: Colorimetric detection of target (left), TEM image of nanoprobe-target (middle), and specificity test with BSA (right). G, GApt and Lys represent gold nanorods, nanoprobe and lysozyme, respectively.

colorimetric detection of lysozyme. However, at this level of experiments the sensor responds more qulitatively, rather than a quantitative fashion. Hence, it is important to take proper strategies to increase the capability of colorimetric detection from quantitative point of view. It is worth to mention that optimization of the nanoprobe at the preliminary stage is of high significance. Although the aptamer is covalantly conjugated to the matrix of GNRs, electrostatic interaction also plays role in nanostructure-oligonuleotide interaction. Therefore, the concentartion of negatively charged apatmer should be conrolled in a fashion which does not lead the nanostructures to unspecific aggregation.

CONCLUSION

This effort reported design and fabrication of a rod-shaped aptasensor for colorimetric detection of lysozyme. Gold nanorods were synthesized according to seed mediated growth protocol and covalantly conjugated with aspecific sequence of lysozyme aptamer. Prior to detection investigations, stability of the naoprobe was studied with different concentrations of aptamer at specified time intervals, by monitorig its characteristic transverse and longitudinal surface plasmon resonance bands. Detection capability of the nanoprobe was tested in various concentrations of biomolecule, where a distinc change in its colour shade was observed. The nanoprobe showed sensitivity of target dection down to nanomolar level of lysozyme at the ambient temperature. Specificity of the aptasensor was tested in the presence of control protein, which did not respond colorimetrically. The specific dictated aggregation of gold nanorods by target biomolecule encourages utilization of these nanostructure for fabrication of a new generation of *lab-in-a-vial* technique based on gold nanotechnology.

REFERENCES

[1] R-D Jean, W-D Cheng, M-H Hsiao, F-H Chou, J-S Bow, D-M Liu, Biosensors and Bioelectronics, 52, 111-117, 2014.
[2] Z. Ma, L. Tian, T. Wang, C. Wang, Analytica Chimica Acta, 673, 179-184, 2010.
[3] C. Wang, J. Irudayaraj, Small, 4, 2204-2208, 2008.
[4] X. Wang, Y. Li, H. Wang, Q. Fu, J. Peng, Y. Wang, J. Du, Y. Zhou, L. Zhan, Biosensors and Bioelectronics, 26, 404-410, 2010.
[5] G. K. Darbha, U. S. Rai, A. K. Singh, P. Chandra, Chemistry, 14, 3896-3903, 2008.
[6] W. He, C. Z. Huang, Y. F. Li, J. P. Xie, R. G. Yang, P. F. Zhou, J. Wang, Analytical Chemistry, 80, 8428-8430, 2008.
[7] P. K. Sudeep, S. T. S. Joseph, K. G. Thomas, Journal of American Chemical society, 127, 6516-6517, 2005.
[8] C. Yu, J. Irudayaraj, Analytical Chemistry, 79, 572-579, 2007.
[9] T. Tohidi Moghadam, B. Ranjbar, K. Khajeh, International journal of Biological Macromolecules, 51, 91-96, 2012.
[10] T. Tohidi Moghadam, B. Ranjbar, K. Khajeh, S. M. Etezad, K. Khalifeh, M. R. Ganjalikhany, International journal of Biological Macromolecules, 49, 629-636, 2011.
[11] N. Alizadeh, B. Ranjbar, M. Mahmodian, Colloids and Surfaces A, 212, 211-218, 2003.
[12] B. Maroufi, B. Ranjbar, K. Khajeh, H. Naderi Manesh, H. Yaghoubi, BBA-Proteins and Proteomics, 1784, 1043-1049, 2008.
[13] S. J. Hurst, A. K. R. Lytton-jean, C. A. Mirkin, Analytical Chemistry,78, 8313-8318, 2006.