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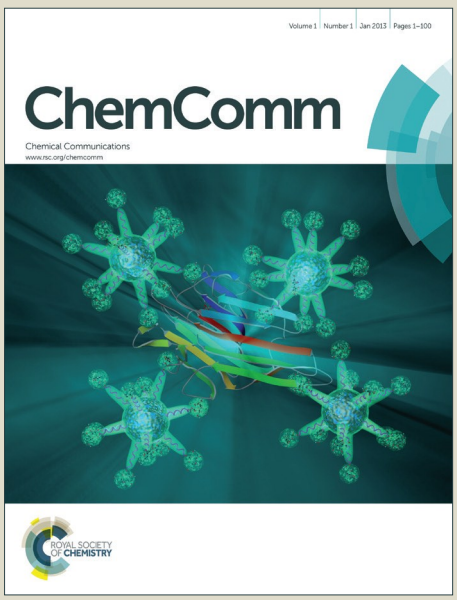

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A simple aptamer-functionalized gold nanorods based biosensor for sensitive detection of MCF-7 breast cancer cells

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Yuan Li,[1,a,b] Yulong Zhang,[1,a] Man Zhao,[1,a] Qianqian Zhou,[a] Lili Wang,[b,a] Huizhong Wang,[c] Xiaohui Wang,*[a,d] and Linsheng Zhan*[a]

Herein, we describe a novel approach for the rapid diagnosis of human breast carcinoma MCF-7 cells with a detection limit of 100 cells mL^{-1}. In our strategy, MCF-7 cells are specially recognized by Mucin 1 protein (MUC-1) aptamer-functionalized gold nanorods (GNRs) through specific interactions, whose signals are simply read out by its unique localized surface plasmon resonance (LSPR) spectra.

By far, breast cancer remains the most frequent cancer among women in both developing and developed regions.[1] Many researchers are ongoing to develop more sensitive methods for early diagnosis and therapy of breast cancer.[2] In 2003, the National Comprehensive Cancer Network identified needle biopsy (NB) as "preferred" over surgical excision for breast diagnosis.[3,4] Although NB possesses the advantages of painless, inexpensive and simple, what severely limits its application is that the analysis of extracted samples requires a substantial amount of experience for the operators.[5,6]

Aptamers are identified by an in vitro selection process termed system evolution of ligands by exponential enrichment (SELEX) against various targets, including small molecules, ions, proteins, and whole cells.[7, 8, 9] As targeted molecular probes, aptamers have been widely used in biomedical research because of their high binding affinity, high specificity, facile synthesis and flexible modification.[10] Therefore, compared to antibodies, aptamers are preferred to be used as potential candidates for diagnosis,[11] owing to their superiorities of custom-design and low-cost.

Herein, aiming to develop a simple and fast diagnosis method, we established a kind of aptamer-functionalized gold nanorods (GNRs) based biosensor to detect human breast carcinoma MCF-7 cells (Scheme 1). GNRs have unique optical property, namely their longitudinal plasmonic peaks can be readily regulated from the visible to near infrared region by controlling their morphology. Because of the existence of near-infrared (NIR) window, GNRs are considered as potential candidates for imaging or therapy in vivo to achieve deep penetration into soft tissue, such as skin, let alone cells, which underlies the strategy of our current design. Previous work reported by Xia's group provided further evidence that the LSPR properties of the Au-based nanostructure were not interfered by the presence of the cells.[12] Naturally, we hypothesize that combining the advantages of aptamers and gold nanorods may hold great promise for the detection of MCF-7 cells. The mucin 1 protein (MUC-1) is reported as a cancer biomarker in human beings, which is over-expressed on the surface of cancer cells such as breast, overian, lung and pancreatic cancers, in particular, on the primary and metastatic breast cancers.[13] In the current work, we detected MUC-1-positive human breast carcinoma MCF-7 cells using the specific interaction between MUC-1 and its oligonucleotide aptamer[14, 15, 16] (5'-GCA GTT GAT CCT TTG GAT ACC CTG G-3') which was covalently conjugated onto the surface of GNRs through Au-thiolate chemistry, and the detection results could be read directly through localized surface plasmon resonance (LSPR) of GNRs. We believe that along with the development of medicinal technology, our work has potential applications in primary diagnosis and thermal therapy of breast cancer in clinic, especially in NB.

As shown in Scheme 1, after the modification by MUC-1 aptamers, the special reaction between functionalized GNRs and target cells were detected by LSPR spectra. Because of the anisotropy of GNRs, the longitudinal LSPR band was demonstrated to be more sensitive in reflecting the dispersion state of nanoparticles than the transverse one both experimentally and theoretically.

[a] Beijing Institute of Transfusion Medicine, Beijing Key Laboratory of Blood Safety and Supply Technologies, Beijing 100850, China. Email: lszhan91@yahoo.com; lovechina1980@163.com.
[b] State Key Laboratory of Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, Hunan University, Changsha, 410082, China.
[c] The 305th Hospital of Chinese PLA. Beijing 100017, China.
[d] Key Laboratory of Advanced Energy Materials Chemistry (Ministry of Education), Nankai University, Tianjin 300071, China.
[1] These authors contributed equally to this work.
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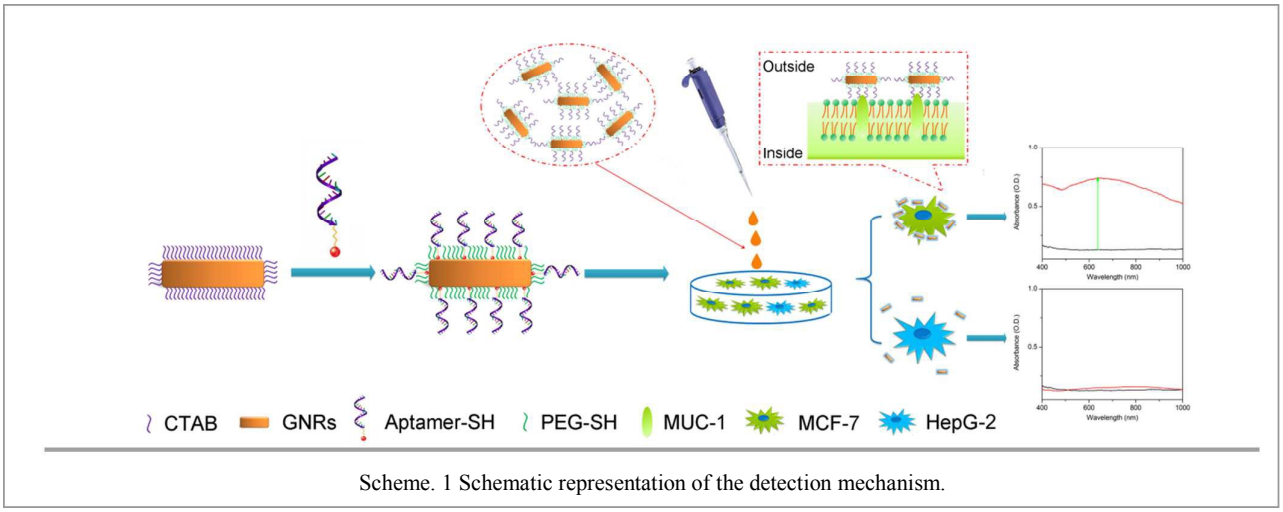

Scheme. 1 Schematic representation of the detection mechanism.

The as-prepared GNRs in this work were positively charged with the zeta potential of 45.17 ± 1.27 mV. The LSPR spectrum of the GNRs before and after the modification and blocking were shown in Fig. S1 (ESI†). We observed a shift of the longitudinal LSPR peak after the aptamers binding at concentrations of 1 μM, 2 μM or 4 μM. As has been demonstrated that the location of longitudinal plasmon wavelength reflect the changes in the dielectric properties of the surroundings and external environment of the GNRs, the pronounced red shift probably resulted from the changes of the effective thickness of the adsorbate layer. Meanwhile, the zeta potentials of thiolated MUC-1 aptamers modified GNRs at all concentrations examined were measured to be -30.53 ± 1.43, -29.43 ± 0.45 and -31.87 ± 1.42 mV, respectively, further indicating the replacement of surfactant CTAB by negatively charged aptamers. In addition, the agarose gel electrophoresis (AGE) assay (Fig. S1 inset, ESI†) of the MUC-1 aptamers in supernatants of the modification stage verified that the majority of aptamers were successfully conjugated onto the surface of GNRs. TEM observations (Fig. S2, ESI†) indicated that the functionalized GNRs probes maintained a good dispersion instead of mutually related to each other before detection.

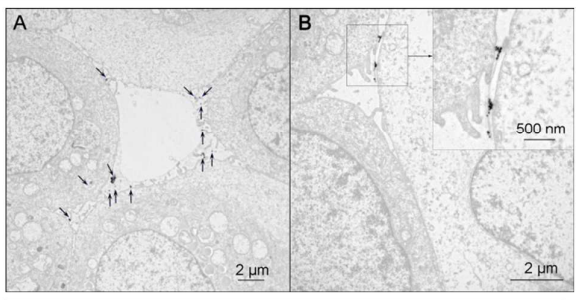

Fig. 1 TEM microimages of the 1 μM aptemer-functionalized GNRs after incubating with MCF-7 cells, GNRs probes conjugated onto the cell surface. The magnification of Picture A and B were 6000 and 12000, respectively. Several arrows were added to clarify the existence of nanoparticles and nano-clusters.

According to the target-induced combination strategy, we firstly compared the increased intensity of the longitudinal LSPR peak of aptamer-GNRs modified at the concentrations of 1 μM, 2 μM or 4 μM aptamers (Fig. S3, ESI†), and it was obvious that the modification concentration of 1 μM was more sensitive for the detection of target cells. This phenomenon probably suggests that low surface density of the surface aptamers offeres advantages for the formation of favorable secondary structures, which benefits the interactions between synthetic nucleic acid aptamers and the target protein and in turn increases the recognition efficiency of the target cells.

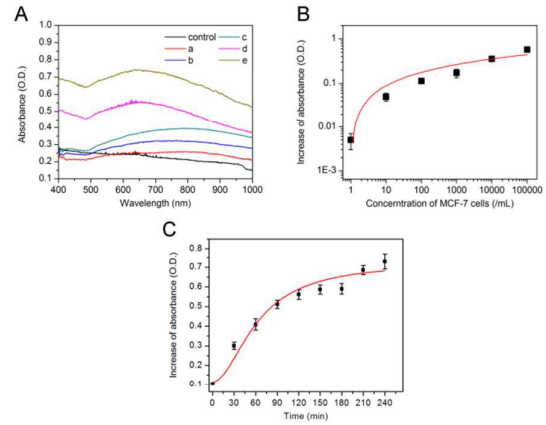

Fig. 2 (A) The LSPR spectra of cells after the incubation with aptamer-GNRs modified at 1 μM aptamers with incubation time of 30 minutes (a-e. $1 \times 10^1 - 1 \times 10^5$ cells mL^{-1} MCF-7 cells and HepG-2 cell was set as a control with the concentration of 1×10^5 cells mL^{-1}); (B) The investigation of the dynamic range together with the sensitivity for aptamer-GNRs. The intensity was tracked at a fixed wavelength of 652 nm. (C) The dynamic curve of detections by incubation aptamer-GNRs with 1×10^4 cells mL^{-1} MCF-7 cells. Values are expressed as mean ± SD.

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As shown in Fig. 1, in the presence of target cells, the strong driving force brought about by specific interactions between overexpressed MUC-1 and its aptamers caused the functionalized GNRs conjugation onto the surface of MCF-7 cells, which resulted in a sharp increase of the LSPR intensity (Scheme 1 and Fig. S3, ESI†). Simultaneously, it was worth noting that some aptamer-GNRs could also be internalized by cells through endocytosis[17, 18] according to Fig. S4 (ESI†), which probably contributed to the background value in sensing.

We then proceeded to investigate the linear range, LOD and detection dynamics of this biosensor assay (Fig. 2). MCF-7 cells at series concentrations were incubated with the aptamer-GNRs, and the result showed that the LOD could be determined as 100 cells mL^{-1} at least in 30 minutes, and the dose-dependent response was ranging from 10^2 to 10^5 cells mL^{-1} (Fig. 2A and 2B). The detection dynamics curve displayed in Fig. 2C indicated that the LSPR instensity ascended along with the incubation time. However, the incubation time of 30 minutes was basically enough to discriminate the interactions between the aptamer-GNRs and MCF-7. The current study not simply serves as a proof of concept for the diagnosis of MCF-7 cells based on the LSPR property of GNRs, furthermore, if its generality taken into consideration,[19, 20] we believe that the function of the nanoprobes will be preserved in harsh conditions, namely in other biological fluids, which broadens its range of application.

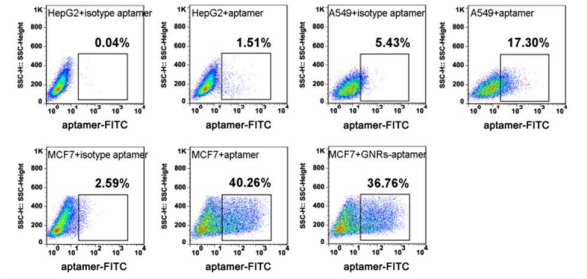

Fig. 3 Flow cytometry analysis of MCF-7 cells treated with FITC-labelled aptamer or FITC-labelled aptamer functionalized GNRs. HepG-2 cells, A549 cells or isotype aptamer were used as the control. The sequences of isotype aptamer was presented in Table S1 as the non-complementary sequences.

Simultaneously, flow cytometry analysis was carried out to validate whether the LSPR intensity increase was caused by the specific recognitions between the overexpressed membrane protein MUC-1 and its unique aptamer or not. Although MUC-1 was also overexpressed on A549 cell, Fig. S5 (ESI†) revealed that the surface abundance of it was much lower than that of MCF7 in the current study. As a result, Fitc-labelled aptamer tagged only 17.30% A549 cell in contrast to 40.26% in the case of MCF7 (Fig. 3). And the aptamer showed quite low cross-reaction with other cells in spite of the fact the FITC-labelled isotype aptamer tagged 2.59% MCF-7 cells and 5.43% A549 cells. Furthermore, it was obvious that binding with the GNRs brought about little influence on the recognition behaviours by inspecting the decrease of 40.26% to 36.76%. Collectively, although MCF7 cell was not the unique one that our biosensor assay was able to target, which partly affected the specificity of the current method, however, when the fact that the especial sampling location of NB was breast was regarded, the as-prepared assay was still believed to be a useful tool serving as a complement to pathologic examination in NB. Subsequently, we turned our attention to investigate the specificity of as-prepared biosensor assay in differentiating MCF7 cells and carcinoma adjacent tissue in transplanted breast cancer in nu/nu mice (Fig. S6, ESI†). And the LSPR spectra showed that assay was credible with satisfactory specificity.

In conclusion, a kind of MUC-1 aptamer functionalized-GNRs was developed and applied for the detection of human breast carcinoma MCF-7 cells. This method gives an excellent dynamic range from 10^2 to 10^5 cells mL^{-1} for MCF-7 cell with a detection limit of at least 100 cells mL^{-1} in only 30 minutes. With high sensitivity, good stability, simple operation, low cost and convenient readout manner, this biosensor assay may shed light on the applications in early clinical diagnosis of human breast carcinoma, especially in NB.

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Notes and references

Student's t test was used for statistical analysis of the results in this work.

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