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# Paper analytical devices for dynamic evaluation of cell surface N-glycan expression via a bimodal biosensor based on multibranched hybridization chain reaction amplification

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ABSTRACT

A novel colorimetric/fluorescence bimodal lab-on-paper cyto-device was fabricated based on concanavalin A (Con A)-integrating multibranched hybridization chain reaction (mHCR). The product of mHCR was modified PtCu nanochains (colorimetric signal label) and graphene quantum dot (fluorescence signal label) for in situ and dynamically evaluating cell surface N-glycan expression. In this strategy, preliminary detection was carried out through colorimetric method, if needed, then the fluorescence method was applied for a precise determination. Au-Ag-paper devices increased the surface areas and active sites for immobilizing larger amount of aptamers, and then specifically and efficiently captured more cancer cells. Moreover, it could effectively reduce the paper background fluorescence. Due to the specific recognition of Con A with mannose and the effective signal amplification of mHCR, the proposed strategy exhibited excellent high sensitivity for the cytosensing of MCF-7 cells ranging from 100 to $1.0 \times 10^7$ and 80-$5.0 \times 10^7$ cells $mL^{-1}$ with the detection limit of 33 and 26 cells $mL^{-1}$ for colorimetric and fluorescence, respectively. More importantly, this strategy was successfully applied to dynamically monitor cell-surface multi-glycans expression on living cells under external stimuli of inhibitors as well as for N-glycan expression inhibitor screening. These results implied that this biosensor has potential in studying complex native glycan-related biological processes and elucidating the N-glycan-related diseases in biological and physiological processes.



## 1. Introduction

Cells are covered by a large number of glycans which are covalently attached to underlying proteins or lipids with high density and complex diversity (Pilobello et al., 2007). The carbohydrate on the surface of a eukaryotic cell is in a complex milieu and plays vital roles in a broad range of crucial biological and physiological processes, including growth, development, differentiation, cell adhesion, cell-cell communication, signaling, immune response, disease, and progression of cancer (Dennis et al., 2009; Gu et al., 2012). As a result, glycan epitopes are generally the surface markers to detect the different expression during a variety of cell biological processes (Arnold et al., 2011). It is critical both to understand their role in disease development as well as to provide effective diagnostic tools to help guide cancer therapeutics. Currently, various approaches, including high-performance liquid chromatography (Royle et al., 2008), mass spectrometry (Nishikaze et al., 2012), nuclear magnetic resonance (Carneiro et al., 2015), and capillary electrophoresis (Vanderschaeghe et al., 2010), have been applied for glycan analysis. However, these methods are not feasible for the in situ and dynamically evaluating N-glycan expression of living cells due to their destructive procedures. Furthermore, these approaches are time-consuming, with complicated sample preparation and sophisticated instrumentation.

Microfluidic paper-based analytical devices (μPADs) have appeared as a very potential platform for cell culture and cell-based assays (Meyvantsson et al., 2008; Yeo et al., 2011; Kovarik et al., 2012). In particular, the μPADs typically exhibit low sample and reagent consumption, low cost, small size, portable, easy modification and operation. The great number of advantages make the μPADs play a potential platform for the facile and fast analysis of the glycan profiling in a high-throughput manner (Primack et al., 2011). In clinic, two or more detection methods are usually applied

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together to reach more reliable diagnostic results, since each method has its specific advantages as well as limitations. For example, colorimetric is the most commonly method on μPADs (Li et al., 2011) because of the easy operation and straightforward signal readout, but relatively low sensitivity. The fluorescence (Deiss et al., 2013; Deiss et al., 2014; Liang et al., 2016) is another kind of optical method possessing inherently much higher sensitivity than colorimetry, While its relatively sophisticated and expensive. Thus, we combined the colorimetric and fluorescence on μPADs to achieve the determination of cells and cell surface N-glycan expression. Due to the previously discussed advantages, a dense interconnected Au-Ag nanoparticles (NPs) layer was grown on the surfaces of cellulose fibers in the paper sample zone, which were used as carriers for a large sum of tumor cells. Moreover, it could effectively reduce the paper background fluorescence signal.

To further perform high sensitivity visible colorimetric and fluorescent analysis, more attention has been paid on nanomaterials and popular strategies for signal amplification. Recently, zhu et al. have addressed green-photoluminescent graphene quantum dots for bioimaging applications (Zhu et al., 2011). As newcomers to the carbon nanomaterials family, graphene quantum dots (GQDs) (Loh et al., 2010) has attracted increasing attention, owing to their unique chemical and physical properties, such as low toxicity, easy preparation, broad excitation spectra, photobleaching resistance, a larger Stokes shift over conventional organic fluorophores (Wu et al., 2009; Gosso et al., 2011; Ratanatawanate et al., 2011; Wang et al., 2010) and narrow, symmetric, and tunable emission spectra. Because of these attractive merits, GQDs have been widely used in bioimaging, drug delivery, fluorescence sensors and optoelectronic devices. In this work, the prepared GQDs showed a relatively strong fluorescence and bright blue emission under excitation of 365 nm UV light. Recently, multicomponent nanostructures (Ge et al., 2014) have attracted tremendous attention. Their surface area, catalytic performance and biocompatibility could be enhanced by the synergistic and the electronic effects between different components. There have been several reports regarding enzymeless biosensors based on $Pt_xM_{1-x}$ alloy nanomaterials (Park et al., 2012; Ding et al., 2012). For example, Nanoporous PtCu alloys were also employed for glucose sensing (Xu et al., 2011). Compared with single metal PtNPs, the $Pt_xM_{1-x}$ alloys present distinctly synergetic characteristics, in which Pt shows the specific catalytic activity, and the other metal takes on a biocompatibility, an increasement of the active sites and the enhancement of the long-term stability. Therefore, $Pt_xM_{1-x}$ alloys provide rapid response, good stability and high catalytic efficiency. Due to the previously discussed advantages, PtCu nanochains as mimicking natural peroxidases was fabricated to assay cells using the colorimetric assay. Moreover, the multibranched hybridization chain reaction (mHCR) has not been investigated previously in the colorimetric and fluorescence cytosensing on μPADs. Different from the conventional HCR, the mHCR can produce long products with multiple branched arms (Huang et al., 2011; Choi et al., 2010; Lan et al., 2016). It could attach abundant PtCu nanochains and GQDs for efficient signal amplification, which significantly enhanced the sensitivity of the biosensors.

Therefore, in this work, we successfully designed a novel lab-on-paper cyto-device based on concanavalin A (Con A)-integrating mHCR. The product of mHCR was modified PtCu nanochains and GQDs (PtCu-mHCR-GQDs) for dynamically evaluating cell surface N-glycan expression. Compared with the previously reported fluorescence-based cell surface N-glycan expression detection strategies, our proposed method possessed some remarkable features: (1) Au-Ag-paper devices not only increased the surface areas and active sites to immobilize more cancer cells but also could effectively reduce the paper background fluorescence. (2) PtCu nanochains showed superior peroxidase-like catalytic performance. (3) The mHCR could produce long products with multiple branched arms for signal amplification (4) This biosensor combined the colorimetric and fluorescence method to visual detection of cell surface N-glycan. Therefore, the developed biosensor holds potential for ultrasensitive visual detection of cell surface N-glycan expression and supplies valuable information for diabetes mellitus research and clinical diagnosis.

## 2. Experimental section

### 2.1. Preparation of PtCu nanochains

The PtCu nanochains were synthesized according to the literature (Cao et al., 2013). The detailed preparation process for PtCu nanochains were provided in the Supplementary information.

### 2.2. Preparation of PtCu-mHCR-GQDs

The mHCR was carried out in a similar way to the reported method created by Pierce's group (Dirks et al., 2004) and a detailed procedure was described in the Supplementary information.

### 2.3. Preparation of the Ag-Au-μPADs

The fabrication of the 3D μPADs was similar to our previous work (Li et al., 2013; Ge et al., 2012) and a detailed procedure was described in the Supplementary information (Scheme S1, Figs. S1–S3). Prior to the mobilization, the novel Ag-Au-μPADs was fabricated on the surfaces of cellulose fibers in the fluorescence layer sample zone of μPADs to enlarge effectively surface area μPADs for the further immobilization of aptamer array. The fabrication procedures of the Ag-Au-μPADs were described in the Supplementary information.

### 2.4. Operation and assay procedures of this biosensor

The schematic diagram of the N-glycan biosensor format was shown in Scheme 1. MCF-7 cell-targeting aptamer was chosen as molecular probes to functionalize the Ag-Au-μPADs due to their salient properties, such as specificity and high affinity, good stability, ease of chemical modification and low immunogenicity (Bagalkot et al., 2006; Shangguan et al., 2006). Briefly, on the α-sheet, 10 μL of 4.0 μM aptamer solution was dropped into corresponding paper cell zone, and incubated at room temperature for 15 min. Then, physically absorbed excess aptamers were carefully rinsed. Subsequently, a drop of 10 μL 2% BSA solution was injected into each aptamer/Ag-Au-μPADs and allowed to react at room temperature for 5 min to ensure the blocking of nonspecific binding sites. Thereafter, 10 μL of homogeneous MCF-7 cell suspension at a certain concentration was dropped into each BSA/aptamer/Ag-Au-μPADs and incubated at room temperature for 15 min (Fig. S4A) to capture the cells though the specific binding between the immobilized aptamers and cells. Then, the obtained MCF-7/BSA/aptamer/Ag-Au-μPADs were carefully rinsed with incubation buffer to remove the noncaptured cells, and ready for subsequent assays.

The colorimetric and fluorescence detection of N-glycan could be achieved using PtCu-mHCR-GQDs. Firstly, the PtCu-mHCR-GQDs bioprobe was dropped into the prepared paper zone in the middle, and incubated for 5 min (Fig. S4B). For the binding of PtCu-mHCR-GQDs to mannose on captured MCF-7 cell membrane, 0.1 mM $Ca^{2+}$ and $Mn^{2+}$ should be added to PtCu-mHCR-GQDs solution. The fluorescence spectra were obtained on a LS-55