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Multiple-targeted graphene-based nanocarrier for intracellular imaging of mRNAs

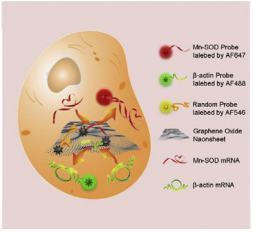
Ying Wang [a, c, d], Zhaohui Li [b, c], Misha Liu [a], Jinjin Xu [a], Dehong Hu [c], Yuehe Lin [c, e, *], Jinghong Li [d, **]

[a] Shanghai Key Lab of Chemical Assessment and Sustainability, School of Chemical Science and Engineering, College of Environmental Science and Technology, Tongji University, Shanghai, 200092, China
[b] College of Chemistry and Molecular Engineering, Zhengzhou University, Zhengzhou, 450001, PR China
[c] Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory, Richland, WA 99352, USA
[d] Department of Chemistry, Tsinghua University, Beijing, 100084, PR China
[e] School of Mechanical and Materials Eng., Paul G. Allen School for Global Animal Health, Washington State University, Pullman, WA 99164-2920, USA

HIGHLIGHTS

- A multiple-targeted GO nanocarrier was used for mRNAs imaging and expression changes after drug treatment can be monitored successfully.
- Sensitive detection limit of 1.84 nM for manganese superoxide dismutase (Mn-SOD) mRNA and 2.45 nM for β-actin mRNA was accomplished.
- Changes of the expression levels of mRNA in living cells before or after the drug treatment can be monitored successfully.

GRAPHICAL ABSTRACT



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ABSTRACT

Simultaneous detection and imaging of multiple intracellular messenger RNA (mRNAs) hold great significant for early cancer diagnostics and preventive medicine development. Herein, we propose a multiple-targeted graphene oxide (GO) nanocarrier that can simultaneously detect and image different type mRNAs in living cells. First of all, in vitro detection of multiple targets have been realized successfully based on the multiple-targeted GO nanocarrier with linear relationship ranging from 3 nM to 200 nM, as well as sensitive detection limit of 1.84 nM for manganese superoxide dismutase (Mn-SOD) mRNA and 2.45 nM for β-actin mRNA. Additionally, this nanosensing platform composed of fluorescent labelled single strand DNA probes and GO nanocarrier can identify Mn-SOD mRNA and endogenous mRNA of β-actin in living cancer cells, showing rapid response, high specificity, nuclease stability, and good biocompatibility during the cell imaging. Thirdly, changes of the expression levels of mRNA in living cells before or after the drug treatment can be monitored successfully. By using multiple ssDNA as probes and GO nanocarrier as the cellular delivery cargo, the proposed simultaneous multiple-targeted sensing platform will be of great potential as a powerful tool for intracellular trafficking process from basic research to clinical diagnosis.

* Corresponding author. Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory, Richland, WA 99352, USA.
** Corresponding author. Department of Chemistry, Tsinghua University, Beijing, 100084, PR China.
E-mail addresses: yuehe.lin@wsu.edu (Y. Lin), jhli@mail.tsinghua.edu.cn (J. Li).

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1. Introduction

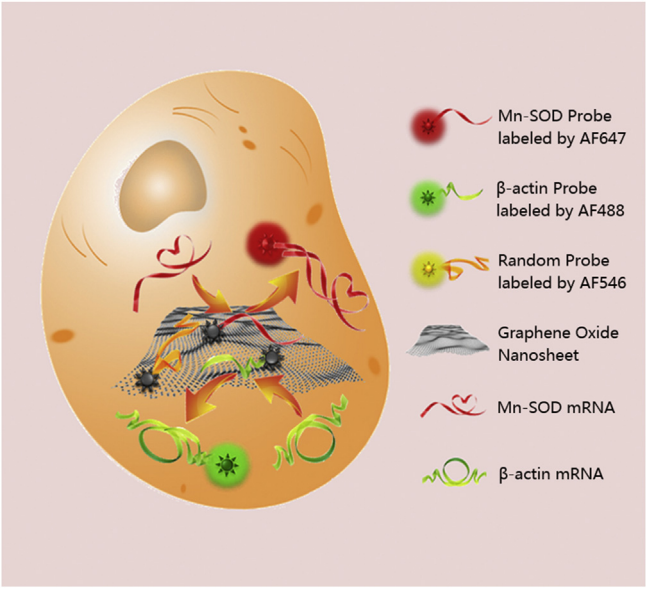
As a specific biomarker, mRNA is considered as the blueprint for the cellular production of proteins and is involved in a number of sorting and trafficking-associated processes thought to enable specific spatial regulatory functions within the cell, such as elongation of axonal growth cones and guidance of fibroblast migration [1,2]. Therefore, detection or monitoring of intracellular mRNA could provide valuable information for biological study, medical diagnosis, and drug discovery [3,4].

To date, numerous approaches have been developed for mRNA analysis, such as polymerase chain reaction (PCR), nanosensors, fluorescent molecular probes, *etc* [5–9]. However, these methods usually cannot reveal the spatial and temporal variations of RNA within a single cell. In contrast, live-cell imaging allows examination of both distribution and dynamics of mRNAs in cells, and provides new perspectives into the cell biology of mRNA [10,11]. Hence, there is an increasing need for methods that can detect the given mRNA expression at the single-cell level and realize the simultaneous detection of multiple target mRNAs to improve the specificity of the imaging results.

Recently, remarkable efforts have been made to develop the intracellular mRNA imaging by using various materials or biotechnologies [12–19]. Fore examples, Chen et al. reported a DNA micelle flares for intracellular mRNA imaging, presenting easy probe synthesis, efficient cellular uptake and excellent target selectivity [13]. Pan et al. have introduced a four-colour nanoprobe that can detect and image four types of mRNAs in living cells [15]. Jayagopal et al. designed hairpin DNA-functionalized gold colloids for the imaging of mRNA in live cells [16]. Wang and co-workers have reported an imaging method for mRNA expression levels in living cells with PNA·DNA binary Förster resonance energy transfer (FRET) probes delivered by cationic shell-cross-linked nanoparticles [19]. Luan et al. have developed a multicolour fluorescent nanoprobe for assessing cellular migration and invasion by simultaneously imaging miRNA-221, PTEN mRNA and MMP-9 involved in the PI3K/AKT pathway which can regulate cellular mobility and invasiveness [20]. The existed methods demonstrate the introduction of novel functional materials or carriers is of great significant and will promote the sensitivity and selectivity for cellular mRNA imaging.

In recent years, graphene oxide (GO) has been emerging as a novel two dimensional nanomaterial with several unique properties including planar sheet structure, high fluorescence quenching ability, easy functionalization, and good biocompatibility [21–26]. Most importantly, it has been proved that GO has great ability to protect DNA/RNA from enzymatic digestion [27]. Because of the robust fluorescence quenching efficiency, GO could also lead to a high signal-to-background when combined with dye-labelled single-strand DNA (ssDNA) probes based on FRET effect [28,29].

To overcome the limitation of intracellular mRNA monitoring, simultaneous detection and imaging of intracellular mRNAs in single breast cancer cell has been conducted in this work. By using the unique loading ability of GO nanocarrier for multiple probes and its high efficient fluorescence quenching capacity of labelled probes, cellular imaging of multiple mRNA has been realized successfully. To validate the sensing ability of this proposed technology, manganese superoxide dismutase (Mn-SOD) probe, β-actin probe as well as a random probe were employed to construct the ssDNA/GO nanocarrier (Scheme 1). As we know, β-actin is one of six different actin isoforms which have been identified and are involved in cell motility, structure and integrity [30]. Since the expression level of β-actin in cells is usually at constant level, β-actin is always selected as the internal control for various biological experiments like PCR, western blot or imaging [31]. Another demo probe utilized in the present work is Mn-SOD probe. Mn-SOD is localized at the mitochondria matrix and is known to be induced by lipopolysaccharide (LPS) [32,33]. Hence, Mn-SOD is considered as a typical cellular mRNA indicator and the Mn-SOD probe for cellular Mn-SOD mRNA expression is designed for constructing the multiple-targeted GO nanocarrier. Finally, a control DNA probe with random sequence is applied for the control experiments for non-specific response test. Briefly, an easily performed and multiple targeted strategy is proposed for simultaneously imaging of multiple mRNAs including Mn-SOD and β-actin inside single living breast cancer cell. The proposed simultaneous multiple-targeted sensing platform will be of promising potential as a powerful tool for intracellular trafficking process from basic research to clinical diagnosis.



Scheme 1. Schematic illustration of simultaneously multiple mRNAs monitoring inside single living breast cancer cell based on GO nanocarrier. In particular, the fluorescent signals could be monitored when Mn-SOD probe (red) and β-actin probe (green) hybridizes with their mRNA targets inside the living cells. Random probe (orange) was regarded as control probe for the sensing strategy.

2. Experimental methods

2.1. In vitro target detection

A mixture solution consisting of 100 nM AF488-β-actin probe, 100 nM AF546-random probe, and 100 nM AF647-Mn-SOD probe were firstly incubated with 2.5 μg/mL GO nanocarrier solution in a total volume of 100 μL, and then incubated with complementary targets for the three probes (from 0 nM to 200 nM) separately in × 5 PBS buffer at 25 °C for 4 h. For the simultaneous detection of β-actin mRNA and Mn-SOD mRNA, a mixture solution consisting of 100 nM AF488-β-actin probe, 100 nM AF546-random probe, and 100 nM AF647-Mn-SOD probe were firstly incubated with 2.5 μg mL^{-1} GO nanocarrier solution in a total volume of 100 μL, and then incubated with the complementary sequences with different concentrations of 3 nM, 6 nM, 13 nM, 25 nM, 50 nM, 100 nM and 200 nM. Fluorescence intensity of AF488, AF546 and AF647 are acquired with Ex/Em at 495/519 nm, 556/573 nm, and 650/668 nm, respectively.

2.2. In situ live cell imaging

MDA-MB-231 cells were incubated with a mixture solution