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Binding-regulated click ligation for selective detection of proteins

Ya Cao [a], Peng Han [a,b], Zhuxin Wang [a], Weiwei Chen [a], Yongqian Shu [c,*], Yang Xiang [d,*]

[a] Laboratory of Biosensing Technology, School of Life Sciences, Shanghai University, Shanghai 200444, China
[b] Shanghai Key Laboratory of Bio-Energy Crops, Shanghai University, Shanghai 200444, China
[c] Department of Oncology, The First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, China
[d] School of Life Sciences, Nanjing University, Nanjing 210093, China

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ABSTRACT

Herein, a binding-regulated click ligation (BRCL) strategy for endowing selective detection of proteins is developed with the incorporation of small-molecule ligand and clickable DNA probes. The fundamental principle underlying the strategy is the regulating capability of specific protein-ligand binding against the ligation between clickable DNA probes, which could efficiently combine the detection of particular protein with enormous DNA-based sensing technologies. In this work, the feasibly of the BRCL strategy is first verified through agarose gel electrophoresis and electrochemical impedance spectroscopy measurements, and then confirmed by transferring it to a nanomaterial-assisted fluorescence assay. Significantly, the BRCL strategy-based assay is able to respond to target protein with desirable selectivity, attributing to the specific recognition between small-molecule ligand and its target. Further experiments validate the general applicability of the sensing method by tailoring the ligand toward different proteins (i.e., avidin and folate receptor), and demonstrate its usability in complex biological samples. To our knowledge, this work pioneers the practice of click chemistry in probing specific small-molecule ligand-protein binding, and therefore may pave a new way for selective detection of proteins.

1. Introduction

The requirement of selective assay for proteins is steadily growing in areas ranging from basic biological research to clinical diagnosis (Wu and Qu, 2015). In the past few years, different types of specific recognition elements have been intensely investigated as solutions to the increasing demand, and have found widespread applications (Chikkaveeraiah et al., 2012; Iliuk et al., 2011; Li et al., 2014; Mascini et al., 2012). Amongst these elements, small-molecule ligands are receiving considerable attention, in view of their mature screening procedures and ultra-high binding affinity toward proteins of interest (Kubota and Hamachi, 2015; Stockwell, 2004). Up to now, the widely-used strategy for fabricating small-molecule ligand-based assays depends on the conjugation of the ligand to a fluorophore, which could produce a detectable response upon binding of target protein (Hou et al., 2015; Mizusawa et al., 2010; Sun et al., 2014). Notably, such strategy allows detection of target protein in a selective and timely manner; however, may be restricted for further developments due to elaborated synthetic steps and limited kinds of usable fluorophores.

Engineering small-molecule ligands to attach along DNA strands via facile conjugation may tackle the aforementioned issues and open up new opportunities for the construction of selective protein assays, as the employed DNA strands can easily integrate varied kinds of signal transduction and readout modalities (Li et al., 2013; Wu et al., 2009; Zhang et al., 2014; Zhen et al., 2012). As a pioneering work, Wu et al. (2009) proposed a terminal protection strategy, relying on their finding that specific small-molecule ligand-protein binding could protect the linked DNA probe from exonuclease-catalyzed degradation. This strategy was demonstrated to be effective for protein detection, remarkably promoting the development of the area (Cao et al., 2012; He et al., 2013; Wang et al., 2014). Nevertheless, this strategy may suffer from the use of enzymes that require special enzymatic conditions (Heuer-Jungemann et al., 2013), which makes the operations for signal transduction complicated. In this context, developing a new strategy with the capability of facilitating signal transduction of protein-ligand binding events will be remarkably attractive.

Click chemistry is a newly-emerging technique to realize facile bio-conjugation at the molecule level (Kolb et al., 2001) and has recently been explored for potential functions in biosensing systems (Shen et al., 2014; Zhou et al., 2014; Zhu et al., 2012). By means of enzyme-free ligation between clickable DNA probes, we herein propose a binding-regulated click ligation (BRCL) strategy for selective detection of proteins. From the model experiments

* Corresponding authors.
E-mail addresses: yqshu_njmu@foxmail.com (Y. Shu), xiangy@nju.edu.cn (Y. Xiang).

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using avidin and folate receptor (FR), it is clear that the signal transduction of specific protein-ligand binding events can be significantly simplified, highlighting the advantage of incorporating click chemistry. To our knowledge, this work is the first example for the application of click chemistry in small-molecule ligand-based sensing methods, and may hold broad potential to facilitate more efficient design for selective detection of proteins.

2. Materials and methods

2.1. Chemicals and materials

DNA probes (P1, S-P2 and P3) used in this work were synthesized by Takara Biotechnology Co., Ltd. (Dalian, China), and their sequences are listed in Table S.1 in Supplementary data. The probes were used as supplied by preparing stock solutions (10 μM) in 10 mM TE buffer (10 mM Tris–HCl containing 0.1 mM EDTA, pH 7.4) and stored in the dark at −20 °C. Recombinant FR was purchased from Sino Biological Inc. (Beijing, China). Avidin, bovine serum albumin (BSA), hemoglobin (Hb), sodium ascorbate, copper sulfate ($CuSO_4$), 3-(N-morpholino)-propane sulfonic acid (MOPS) and mercaptohexanol (MCH) were obtained from Sigma Aldrich. Graphene oxide was bought from Nanjing XFNANO Materials Tech Co. Ltd. (Nanjing, China). Other chemicals were of analytical grade and used without additional purification. All buffer solutions were prepared with double-distilled water (18.2 MΩ cm), which was from a Milli-Q system (Branstead, USA).

2.2. Experimental confirmation of BRCL strategy using gel electrophoresis analysis

8 μL of DNA samples (the hybridized DNA probe P1/S-P2 (Biotin-P2), the alkyne-labeled DNA probe P3, and the BRCL mixture of P1/S-P2 and P3 in the absence or presence of target avidin) were first prepared, followed by incubating with 2 μL of 5 × loading buffer that contained fluorescence stain GelRed. Afterward, the samples were subjected to a 4% agarose gel in 1 × Tris-acetate-EDTA (TAE) buffer, and electrophoresis separation was then running at a constant voltage of 90 V. After being separated for 30 min, the resulting gel was photographed with a GelDoc XR$^+$ System (Bio-Rad, USA).

2.3. Electrochemical validation of BRCL strategy

Electrochemical experiments were performed on a model 660c electrochemical analyzer (CH Instruments) with a conventional three-electrode system, in which a P3-modified gold electrode was employed as the working electrode. Specifically, the P3-modified gold electrode was fabricated as described in the literature (Miao et al., 2013). In brief, the substrate gold electrode was treated with piranha solution, and then polished to be a mirror surface with alumina slurry. Afterwards, the electrode was soaked in nitric acid (50%) for 30 min and then electrochemically cleaned in 0.5 M H_2SO_4 by scanning the potential from 0 to 1.6 V for 30 cycles. After being dried with nitrogen, the electrode was immersed into 100 μL of DNA immobilization buffer (10 mM Tris–HCl, 1 mM EDTA, 10 mM TCEP, 0.1 M NaCl, pH 7.4) that contained 0.5 μM Thiolated (SH-) P3 for 16 h, followed by 1 h treatment with 1 mM MCH to displace any unbounded DNA probes. The obtained P3-modified electrode was thoroughly rinsed with double-distilled water and then suspended over PBS (pH 7.4) at 4 °C prior to use.

A typical experiment for electrochemical validation of BRCL strategy was prepared by simply mixing 0.5 μM P1 and S-P2 (Biotin-P2) in 50 μL of DNA hybridization buffer (10 mM PBS containing 1 M NaCl, pH 7.4) for 2.5 h. Thereafter, the mixture was treated with 50 μL of 0 or 100 nM target avidin to sustain the protein binding. After 1 h duration, the resulting solution was further mixed with 100 μL of 10 mM PBS buffer containing 10 μM $CuSO_4$ and 1 mM sodium ascorbate, and then incubated with the P3-modified electrode for 30 min. Finally, the electrode was thoroughly rinsed with washing buffer (20 mM Tris–HCl, 5 mM $MgCl_2$, 0.1 M NaCl and 1.0% Tween-20, pH 7.4), and used for electrochemical impedance spectroscopy (EIS) measurements by applying a bias potential of 0.224 V and 5-mV amplitude in the frequency range from 0.01 Hz to 10 kHz.

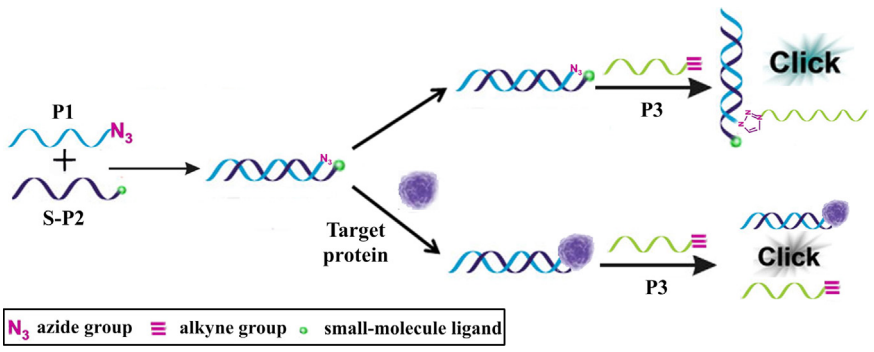
2.4. BRCL strategy-based fluorescence detection of proteins

BRCL strategy-based fluorescence detection was conducted in a 500 μL reaction mixture. First, 36 μL of P1 and S-P2 (0.5 μM) were mixed in 81 μL of M-buffer (20 mM MOPS, 300 mM NaCl, 2 mM $MgCl_2$, pH 7.5) for 2.5 h. Then, 90 μL of protein-containing solution (either the target or nonspecific proteins) was added into the mixture, allowing the protein binding to proceed for 1 h. Afterward, the mixture was brought to a volume of 460 μL by adding 18 μL of 1 μM P3, 5 μL of 100 mM sodium ascorbate and 230 μL of 200 μM $CuSO_4$. After incubation for 30 min, 40 μL of 200 μg/mL graphene oxide was added into the above solution and mixed vigorously. The fluorescence emission spectrum over 500–670 nm of the resulting reaction mixture was collected 10 min later using a Hitachi F-7000 fluorometer (Hitachi, Japan) under 340 nm excitation. The slit width for both excitation and emission was fixed at 5 nm.

3. Results and discussion

3.1. The principle of BRCL strategy

The working principle of BRCL strategy is illustrated in Scheme 1. In general, three modular DNA probes are involved in



Scheme 1. Representation of the principle of BRCL strategy.