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Plasmonic ruler on field-effect devices for kinase drug discovery applications

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ABSTRACT

Protein kinases are cellular switches that mediate phosphorylation of proteins. Abnormal phosphorylation of proteins is associated with lethal diseases such as cancer. In the pharmaceutical industry, protein kinases have become an important class of drug targets. This study reports a versatile approach for the detection of protein phosphorylation. The change in charge of the myelin basic protein upon phosphorylation by the protein kinase C-alpha (PKC-α) in the presence of adenosine 5′-[γ-thio] triphosphate (ATP-S) was detected on gold metal–insulator–semiconductor (Au-MIS) capacitor structures. Gold nanoparticles (AuNPs) can then be attached to the thio-phosphorylated proteins, forming a Au-film/AuNP plasmonic couple. This was detected by a localized surface plasmon resonance (LSPR) technique alongside MIS capacitance. All reactions were validated using surface plasmon resonance technique and the interaction of AuNPs with the thio-phosphorylated proteins quantified by quartz crystal microbalance. The plasmonic coupling was also visualized by simulations using finite element analysis. The use of this approach in drug discovery applications was demonstrated by evaluating the response in the presence of a known inhibitor of PKC-α kinase. LSPR and MIS on a single platform act as a cross check mechanism for validating kinase activity and make the system robust to test novel inhibitors.

1. Introduction

Kinase mediated phosphorylation is one of the major post-translational modifications of the proteins essential for the regulation of various cellular functions, such as cell cycle, motility, metabolism or genetic expression (Cohen, 2002; Noble et al., 2004; Overington et al., 2006; Weller 1979). Abnormal phosphorylation of proteins is associated with etiology of fatal diseases such as cancer (Chakraborty et al., 2014; Viatour et al., 2005), cardiovascular (Honda et al., 1998; Liu and Molkentin, 2011), spinal (De Laat et al., 2014; Kyriakis, 2014) and neural disorders (Abeliovich, 2014; Buee et al., 2000). In this perspective the development of inhibitors for kinases could serve as powerful drugs for a specific disease associated with phosphorylation. Kinases catalyse the addition of phosphoryl group to the serine, threonine and tyrosine amino acids in the presence of a phosphate source, like ATP, where the γ-phosphoryl group is transferred to the amino acid (Cohen, 1988; Kerman and Kraatz, 2009). Upon phosphorylation, there is an addition of negative charge on the protein and a release of proton, while ATP is converted into ADP (Lindsay, 2012). Electrochemical (Kerman et al., 2007, 2008; Kerman and Kraatz, 2009) and optical techniques (Li et al., 2010) to detect protein phosphorylation have proved to be highly sensitive, selective, less time consuming and more cost effective than the conventional techniques such as mass spectroscopy (Kruger et al., 2006; Rusinova et al., 2009), radioactive isotope (Steen et al., 2005) and antibody labelling assay (Morgan et al., 2004). Recently, we detected the release of proton associated with phosphorylation reaction on electrolyte–insulator–semiconductor (EIS) structures (Bhalla et al., 2014) and EIS structures couple with plasmonic effects (Bhalla et al., 2015). In the current study, on the other hand, we detect the phosphorylation of proteins by identifying the change in the charge of the myelin basic protein (MBP) with the use of metal (gold)–insulator–semiconductor (MIS) capacitor structures (Fig. 1). We employ the use of 5′-[γ-thio] triphosphate (ATP-S) where the γ-phosphoryl group linked with the sulfide

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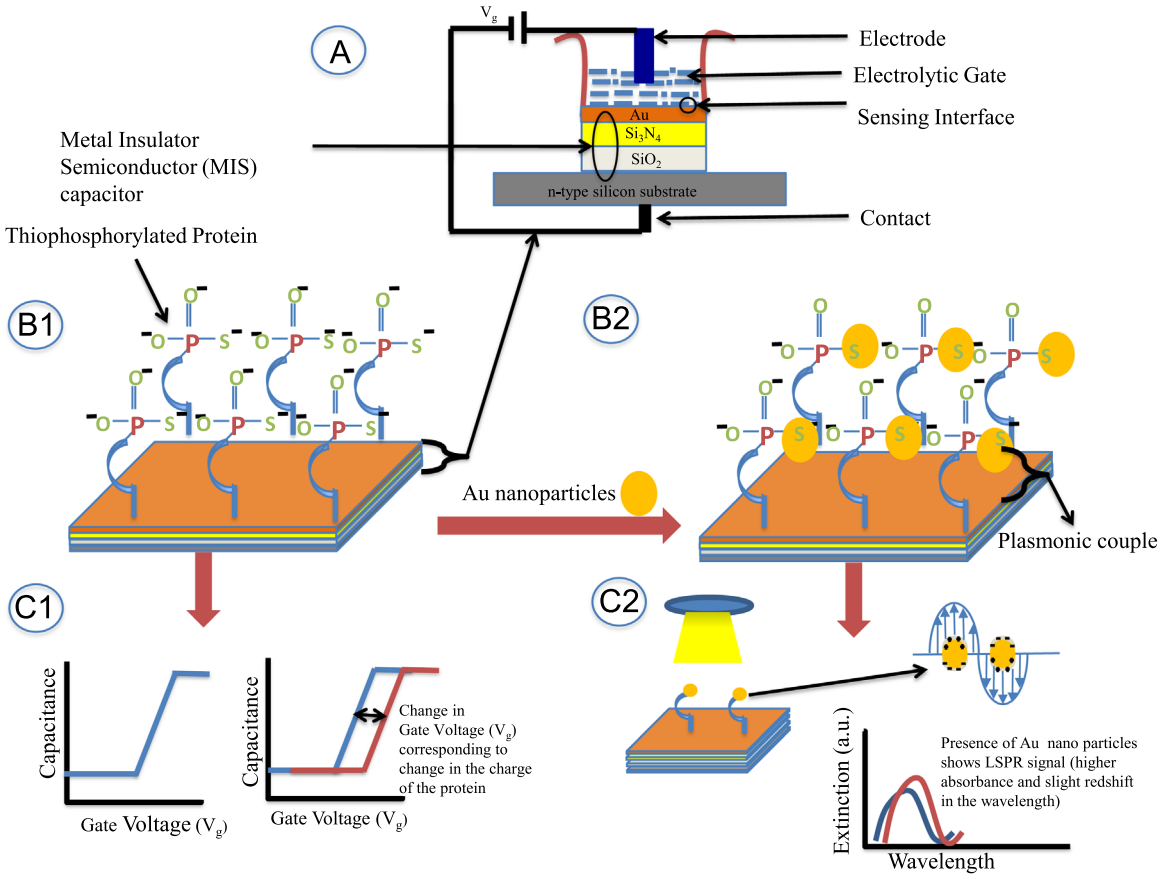

Fig. 1. Scheme for protein phosphorylation analysis. (A) Field effect device well for protein phosphorylation measurement; (B1) metal insulator semiconductor (MIS) capacitor structure with immobilized proteins after thio-phosphorylation; (B2) AuNPs attached to the thiol groups of thio-phosphorylated protein in plasmonic ruler configuration; (C1) capacitance vs. gate voltage characteristics curve of MIS and change in gate voltage corresponding to the charge on the phosphorylated proteins; and (C2) LSPR measurement of plasmonic ruler.

group enables the attachment of gold nanoparticles (AuNPs) to the thio-phosphorylated substrate and forms Au-film and AuNP plasmonic couple. This plasmonic coupling results in the shift of resonance wavelength of gold. It also precisely quantifies the distance between the sensor film and the NP by slightly modifying the decay constant of plasmonic ruler equation demonstrated for AuNP–AuNP pair (Jain et al., 2007a). A practical application of plasmonic rulers as a complementary validation on the MIS sensing platform that distinguishes phosphorylated proteins from the un-phosphorylated ones was therefore investigated. For validation purposes, all the reaction steps were followed using automated surface plasmon resonance (SPR) in real time on gold chips. This allowed: (1) quantification of protein surface coverage; (2) rough estimation of the percentage of total sites that get phosphorylated; and (3) to determine the effect of NP binding on the thio-phosphorylated gold substrates. Quartz crystal microbalance (QCM) measurements were also conducted to quantify the nanoparticles and co-relate with the thio-phosphorylated sites estimated by SPR on the gold surface. Moreover this work emphasizes the need to converge multiple sensing technologies in a single platform to analyse multiple parameters of a chemical reaction in real time for robust verification of biochemical processes. Multiple techniques incorporated on a single platform could potentially save time and cost of analysis for authenticating a chemical reaction. For instance, in this study we unveil the capabilities of AuNPs to combine multiple sensing techniques that enable simultaneous localized surface plasmon resonance (LSPR) and MIS validation of protein phosphorylation. In addition, the use of AuNPs massively amplifies the angle resolved sensitivity of SPR setup. Most significant advantage of this methodology is that the advances in the microelectronics can easily replicate these techniques in an array format for high throughput analysis of kinase assays (Liu et al., 2014). This would ultimately speed up the development of novel kinase inhibitors and activators that are currently being explored in pharmaceutical research and drug discovery. In addition, it may have important applications for the future use of nanoparticle-based technologies in drug discovery.

2. Materials and methods

2.1. Reagents

All chemicals were of analytical grade and used as received, unless otherwise specified. All aqueous solutions were made with deionized water, 18.2 MΩ cm, with a Pyrogard filter (Millipore, USA). Tris base, magnesium chloride, sodium chloride, acetone, ammonium hydroxide, hydrochloric acid, hydrogen peroxide, mercaptoundecanoic acid (MUA), mercaptohexanol (MCH), ethanolamine, ethanol, N-hydroxysuccinimide (NHS), ethyl-dimethylaminopropyl carbodiimide (EDC), PKC-α kinase inhibitor GF 109203X, adenosine tri-phosphate (ATP), gold nanoparticles, 20 nm average spherical diameter, in 0.1 mM PBS were purchased from Sigma-Aldrich. Dephosphorylated myelin basic protein (MBP), purified from bovine brain using fast protein liquid chromatography (FPLC), was purchased from Millipore. PKC-α human recombinant kinase produced in Sf9, was procured from ProSec-Tray TechnoGene Ltd. PKC lipid activator cocktail was obtained