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Theoretical analysis of a high performance protein imprint on a nanosensor

Jianfa Chen [a], Cale Lewis [a], D. Balamurugan [a,c], Zhen Yang [b], Liqiang Ai [d], Dong Cai [a,*]

[a] Department of Physics, University of Houston, Houston, TX 77004, United States
[b] Department of Chemical and Biomolecular Engineering, University of Houston, Houston, TX 77004, United States
[c] Computation Institute, University of Chicago, 5735 S Ellis Ave, Chicago, IL 60637, United States
[d] School of Aerospace Engineering, Tsinghua University, Tsinghua University, Beijing 100084, China

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ABSTRACT

The structural details and flexibilities of protein impose significant challenges to develop protein imprint, especially for the selection of functional monomer. Using NAMD, AutoDock 4 and AutoDock Vina, we investigated the formation of a high performance protein imprint on a nanosensor that detected human papillomavirus (HPV) biomarker protein E7 with high sensitivity. According to molecular dynamics, the phenolic oligomers were shown to assemble with the E7 protein and form a complex at specific targeting areas on the protein. Docking analysis efficiently screened chemical compounds by evaluating the binding affinity. A new parameter, i.e., average binding energy (ΔG/contact), was used together with binding energy (ΔG) to screen compounds. The screening went through 189 compounds and identified a subpopulation of 22 compounds showing unique characteristics of binding, and could potentially be used to develop the specific and robust imprint. Accordingly, the study implicated a novel approach to screen functional compounds for rational design of the protein imprint.

1. Introduction

Biorecognition of proteins with molecular imprints (MI) attracts broad interests of applications in biodetection, biopurification and bioseparation. However, it is still a serious challenge due to the complexity of protein molecules [24]. Conventional approaches established for imprinting small molecules can't be directly applied to protein regarding the choices of monomers selection, temperature, pH, solvent, and ionic strength for polymer preparation. The massive amount residues on the protein surface can orchestrate a multitude of interactions with environmental molecules by hydrophobicity, electrical attraction, polarity, hydrogen bond, and van der Waals force. Traditionally, functional monomers (fMer) are used to pre-complex with the template based on the complementary interactions between two molecules. Then, the complex is fixed in the scaffold of polymerized cross-linking monomers [22]. The special requirements to design protein imprint reside in at least two folds: first, a fMer screening by computational chemoinformatics analysis is necessitated, yet the validated guideline and strategy are still unavailable. In line of such effort, GOLD package was used to study the docking of 7 fMers on human serum albumin [11]. It derived the characteristics of the fMer binding, as well as their probable interruption of the protein structures. Second, a complexation between multiple fMers and a protein could help to imprint recognition sites in different aspects of the protein molecule. In a pilot research, the statistical copolymerization of simple binding monomers in defined stoichiometric ratios had shown improved selectivity of protein imprints [10]. But as the number of functional monomer species increases, it becomes difficult to satisfy the reaction conditions simultaneously in a single polymerization system. To solve the problem, we should first have the binding details of the fMers for a given protein template, which could allow more freedom to choose compatible fMers to produce multiple recognition features in the imprint.

Electropolymerization has been exploited for surface imprinting in biosensors [6]. The reaction normally happens in aqueous electrolyte solution, which is favorable to retain the physiological conformation of proteins. Various polymers like pyrrole and thiophene derivatives have been used as the imprinting materials [25]. Although mostly no co-polymers are used as the fMer to fabricate imprints in this paradigm, the electropolymerization-based sensors still exhibit remarkable sensitivity and selectivity [14,15,20]. There may be unrevealed advantages of the process to facilitate the performance of the imprint. In our previous study, protein imprints were fabricated on the tips of a carbon nanotube

* Corresponding author.
E-mail address: dcai@uh.edu (D. Cai).

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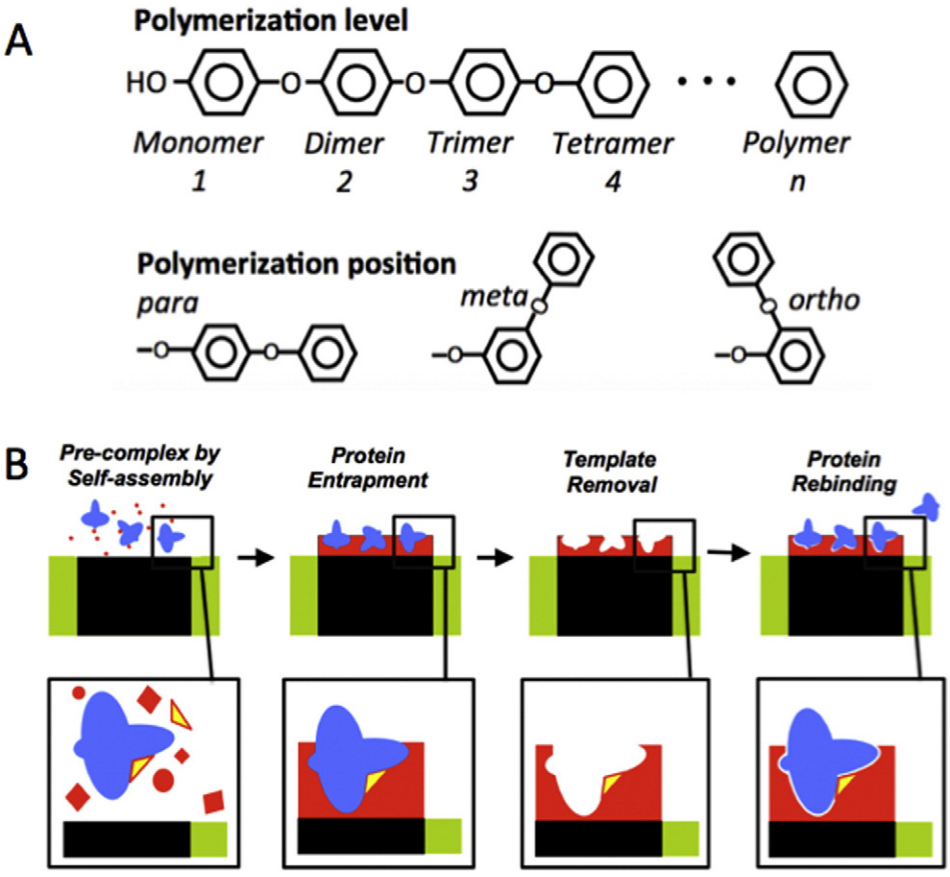
(CNT) array through electropolymerization of polyphenol (PPn), and were used to conduct ultrasensitive protein sensing [5]. The recognition of target by the imprint was demonstrated with the detections of various proteins including human ferritin E7 biomarker protein (E7) of human papillomavirus (HPV), and bovine calmodulin protein with responsive concentration < 1 ng/L. The sensor differentiated the hFtn under the competition of horse ferritin, apoferritin and whole protein constituents extracted from bovine muscle tissues in the spiked buffers. Using the same fabrication method, Viswanathan S, et al., also achieved exceptional detection of ovarian cancer biomarker CA125 in human serum samples [23].

Here, we are looking for the answer of the following question: how could imprint produced in the simple electropolymerization provide such high performance? It may help us to understand the unusual functionality of the electropolymerization products and uncover a new mechanism to efficiently and effectively develop imprints for other protein biomarkers. Our hypothesis is shown in Scheme 1. The polymerization of PPn randomly derives isomeric structures with ortho-, meta- and para-dialkoxy benzene units in various polymerization degrees [4,7,8]. Such a repertoire of oligomers provides an interaction system to facilitate the interaction with the protein template and preferentially self-assemble into a complex to form recognition interfaces. The complex can be entrapped in the PPn scaffold and transfer the unique recognition interface to the imprint after the removal of the template. In this process, the oligomers serve as the fMers used in conventional imprinting. The phenolic compounds actually fall in a category of fMer currently chosen for the protein imprint, which is selected very roughly by their property: either neutral or weak acidic or basic [24]. To explore the hypothetical process in this paper, we take a computational approach to analyze the interaction of the compounds on the E7 biomarker, to outline the interactions between the compounds and the template. The results could suggest the experimental approach to improve the imprint.

2. Results and discussion

2.1. PPn concentration

The strong binding at a protein interface with its receptor is formed by a group of weak interactions at multiple residues [1,9,12]. Accordingly, if the proposed interface of the E7 protein is formed by a weak interaction, the concentration of the oligomers should be high enough so that the assembly of PPn products on the E7 protein could happen. As shown in Fig. 1A, the self-limiting Faradic current during the electropolymerization was 146 μC. It was converted into the theoretical volume (denoted as V_{PPn}^{*}) of the PPn deposited on the sensor. The calculation is shown in the Supporting Information. In a transmission electron microscope (TEM) image, the thickness of PPn coating on CNT was 15 ± 3 nm (n = 20) (Fig. 1B). Based on that, the actual volume of PPn deposition (V_{PPn}) was estimated too. The comparison of the volumes suggested that only 10% of the oxidized phenol molecules were deposited on the CNT tips (Fig. 1C). The rest could be diffused into the solution at the vicinity of the CNT. Such a high molecular transfer rate was facilitated by the geometry of the nanosensor [2]. Assuming all products were phenolic dimers and applying the Fick's second law of diffusion, the concentration profiles of PPn at the nanoelectrode were estimated (see details in Supporting Information). During the first CV cycle, the concentration could reach as high as 60 mM within a spherical space 20 μm to the CNT (Fig. 1D).



Scheme 1. Hypothetical imprinting process. (A) Isomeric PPn produced in the electropolymerization. (B) Isomeric oligomers shown in different shapes are produced on the CNT nanoelectrode (black). Some of the products (yellow) have higher affinity with the template protein (blue) than others (red), and complex with the protein.