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Multiplex Binding of Amyloid-like Protein Nanofilm to Different Material Surfaces

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ABSTRACT

The development of a reliable universal way to form strongly bonded coating with underlying substrate independent on material chemistry is ideal surface modification strategy and remains great challenge. Recently developed polydopamine and amyloid-based adhesion systems are two representative examples reported so far to fulfill this aim, and nonetheless the universal surface adhesion mechanism in these two cases is still unclear up to now. Herein the systematical studies on the adhesive strength of the amyloid-like coating on metal, organic and inorganic material surfaces reveal a novel multiplex bonding model on polar and non-polar abiotic surfaces, and different binding mode for respective material chemical structure including metal-sulfur coordination bonding, hydrogen bonding, electrostatic and hydrophobic interaction is elucidated with affirmative bonding strength. Our findings lend insight into amyloid adhesion mechanisms and reveal strategies for theory-driven design of engineered adhesives that harness great promise for advanced materials and devices.

Material adhesion on solid surface is not only a widespread phenomenon in nature [1], but also harnessed for significant applications, such as engineered coating [2], biomedicine [3], membrane separation [4], energy/electronics and environmental-related devices [5,6]. Such feature has inspired the successful development of a range of surface engineering strategies for surface modification and coating techniques [7,8]. Since chemical properties of organic, inorganic and metal surfaces are quite distinct, current approaches are typically designed based on monovalent surface bonding chemistry so that these methods are highly material-dependent and respectively confined to their local material type. Such situation often results in undesirable adhesion failure and disability of surface modification. For instance, as one of the most successful surface monolayer examples, the strong adhesion of thiol-terminated material on Au surface based on Au−S bond could not be transferrable to organic polymeric substrates [9]. In another case, silanization coating widely applied on silicon surface based on *Si*-O-Si bond could not be directly constructed onto metal surface [10]. Undoubtedly, solving the issues above would give impetus to the progress of highly reliable material and devices, and the fundamental question is can we find a universal adhesion way independent on material type? [11] This question is addressed by protein-inspired polydopamine [10] and amyloid adhesion [12–17]. Polydopamine is a synthetic mimic of mussel foot protein that is adopted widely by mussel to adhere itself on solid surfaces, and recently-developed amyloid is based on spontaneous assembly of protein to form cross-β sheet stacking. Both of these two strategies have been finely manipulated by natural entities such as mussels, bacteria and cells, generally utilizing complex binding chemistry with underlying substrate to construct stable bonding and biofilms with various surfaces irrespective of material chemical structure [18–27].

In spite of increasingly great achievements based on these strategies, the underlying mechanism of polydopamine or amyloid-surface interactions remains unclear and still being extensively investigated [4,28]. Although the origin of such strong adhesion has been attributed to multiplexed co-contributions from hydrogen bonding, covalent cross-linking, coordinate bonding with metal/metal oxide, some important questions must be addressed before dictating such nature-inspired adhesion principle into new generation adhesive material design in synthetic system: do these interfacial materials utilize different binding mechanism for such diverse types of surfaces? And what is the corresponding binding strength? [29] In recent years, such molecular mechanism for polydopamine system has been actively addressed [29,30], and in sharp contrast, for the very recently developed amyloid system, the studies to address the above questions are in great infancy and challenging due to much more complex protein molecular structure and sequence than simple polydopamine system [28]. With this respect,

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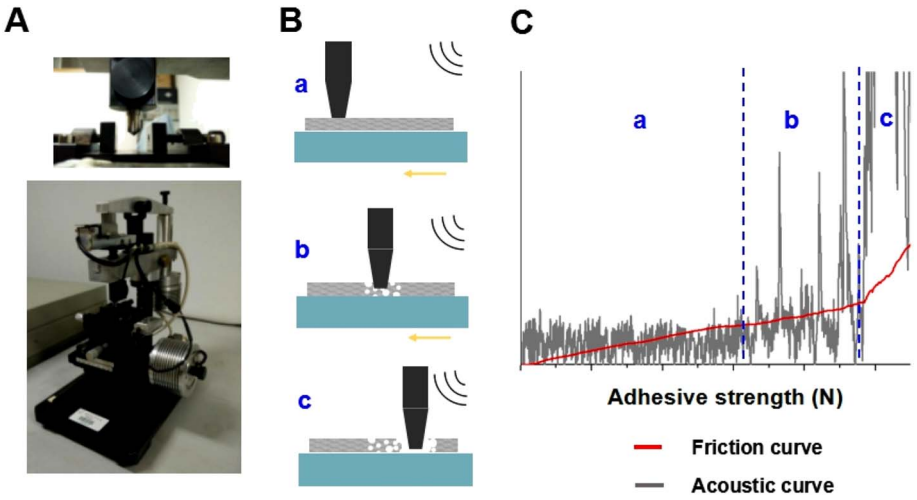
herein we for the first time demonstrate the multiplex bonding mechanism of amyloid-like lysozyme assemblies on polar and non-polar abiotic surfaces, and different binding mode for respective material chemical structure is elucidated with affirmative bonding strength. Our findings lend insight into amyloid adhesion mechanisms and reveal strategies for theory-driven design of engineered adhesives that harness great promise for advanced materials and devices [31].

The model we used to mimic the amyloid adhesion onto a solid surface is developed from our recent report on novel lysozyme derived amyloid-like nanofilm [12–17]. Lysozyme is a class of important functional protein in nature and a model of amyloid-type protein aggregation [32]. The flexible change of its conformation can induce rich phase behavior in different environments, which typically form crystals, viscous fluid, gel and other types of amyloid fibril assemblies [33]. Very recently, our group discovered that in a lysozyme buffer solution containing tris(2-carboxyethyl)phosphine (TCEP) at neutral pH, lysozyme through self-assembly process could form amyloid-like assemblies so-called phase transition lysozyme (PTL) [12–17]. In sharp contrast to black or brown polydopamine coating, the PTL could easily form a robust nanofilm coating with controllable thickness and morphology as well as strong adhesion strength onto virtually arbitrary material surfaces. Such nanofilm therefore provides a good model to study the interfacial adhesion mechanism. In order to investigate systematically adhesion mechanism of the PTL coating, herein we mainly make use of acoustic emission analysis to directly obtain the peeling strength (N) of a coating from underlying substrate [33–37]. For this purpose, a scratch tester operated in a progressive mode is employed to measure the adhesion of a coating formed on a solid surface. The scratch tracks are characterized with an optical microscope and scanning electron microscope (SEM). The adhesion at the interface between the amyloid-like coating and the substrate are then evaluated by combining the acoustic emission analysis, frictional force, the optical images and SEM micrographs of the scratches.

Acoustic emission, frictional force and coefficient of friction curves were recorded online in a computer-based data acquisition system, during the scratch test. The adhesion testing instrument consisted of a piezoelectric transducer mounted near base of the indenter, for recording the emitted acoustic signals. Through the automatic loading sensor, certain load was continuously added to the diamond indenter stylus, at the same time, the specimen was mechanically moving to make the stylus to scratch the sample surface. Consequently, by detecting the resultant change of acoustic curve reflected from the specimen, the critical load of the coating just peeled from the underlying substrate could be in situ monitored. As shown in Scheme 1, this detection could be divided into three stages: (a) when the stylus touched and moved laterally on the coating, the acoustic curve was a weak signal and the friction curve to represent the friction coefficient between the coating and the substrate showed an almost straight line; (b) with the increase of the load, the coating material was scratched out to form a few pieces without detachment from the substrate, and at this time, the acoustic curve produced a weak sound signal and the resultant friction coefficient of the friction curve still did not change obviously; (c) with further applying the load, the stylus completely delaminated the coating material to induce the peeling, and consequently, the reflected sound was issued as a strong signal curve and the friction coefficient changed greatly. At the stage (c), the inflection point would be appeared in the reflected acoustic wave, and the critical load at this infection point was then defined as the adhesion strength (critical peeling strength) of the coating material on the substrate.

The above three stages in the acoustic wave measurement were then confirmed by performing the peeling test of the PTL nanofilm coated on Au (Fig. 1) and silicon (**Fig. S1**) surfaces. The stages a, b, c shown in Scheme 1 were correspondingly indicated in the SEM images of the scratched nanofilm to demonstrate the proposed morphology change on the coating surface. For instance, on Au surface, the stage (a) that was supposed as the weak scratch on the nanofilm showed nearly 100% remaining of protein material on the surface, either judged by the surface topography observation or EDX measurement to reflect a high content of N concentration for proteins; the stage (b) as the fragmentation of the protein nanofilm without detachment from the substrate showed the coating species broken by the stylus with a decreased content of N concentration; the stage (c) finally reflected a complete peeling of the nanofilm from the substrate with the expose of underlying bare Au surface and nearly absence of N content (Fig. 1). Similar evidences to demonstrate such three stages were also confirmed in the peeling test of the PTL nanofilm coated on a silicon surface (**Fig. S1**).

In order to design target surfaces to probe possible interaction patterns with the coating, the functional groups on the PTL film must be accurately evaluated. Laser confocal micro-Raman spectra of the PTL nanofilm presented a series of functional groups on the film surface, which typically included alkyl, aromatic ring, carboxyl (COOH), hydroxyl (OH), amine (NH_2), nitrogen and thiol groups (Fig. 2A, B). These functional groups were also consistently revealed by ATR-FTIR spectra (Fig. 2C), and the representative elemental signals for these groups were further confirmed by X-ray Photoelectron Spectroscopy (XPS) analysis on the coating surface (Fig. 2D–F). It was shown by XPS that the relative percentage for predominant groups on the PTL film surface (Fig. 2B) were alkyl groups (20%), aromatic ring (9%), hydroxyl (23%), carboxyl (12%), amine (13%), quaternized nitrogen (2%) and thiol groups (15%). By using the ninhydrin reaction to quantify the group density of amines across the surface, the distribution density for all of the functional groups on the PTL nanofilm surface was further



Scheme 1. The cartoon to show the peel measurement by acoustic emission analysis. (A) The picture to show the instrumentation for acoustic emission analysis; (B) the cartoon to show the schematic measurement by acoustic wave (different stages as a, b, c); (C) a typical curve from reflected acoustic wave to detect the adhesion strength of the coating on a material surface (different stages as a, b, c corresponding to the stages a–c in (B) and next Fig. 1).