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Single-molecule force spectroscopy: A facile technique for studying the interactions between biomolecules and materials interfaces

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Abstract: The quantification of the interactions between biomolecules and materials interfaces is crucial for design and synthesis functional hybrid bionanomaterials for materials science, nanotechnology, biosensor, biomedicine, tissue engineering, and other applications. Atomic force spectroscopy (AFM)-based single-molecule force spectroscopy (SMFS) provides a direct way for measuring the binding and unbinding forces between various biomolecules (such as DNA, protein, peptide, antibody, antigen, and others) and different materials interfaces. Therefore, in this review, we summarize the advance of SMFS technique for studying the interactions between biomolecules and materials interfaces. To achieve this aim, firstly we introduce the methods for the functionalization of AFM tip and the preparation of functional materials interfaces, as well as typical operation modes of SMFS including dynamic force spectroscopy, force mapping, and force clamping. Then, typical cases of SMFS for studying the interactions of various biomolecules with materials interfaces are presented in detail. In addition, potential applications of the SMFS-based determination of the biomolecule-materials interactions for biosensors, DNA based mis-match, and calculation of binding free energies are also demonstrated and discussed. We believe this work will provide preliminary but important information for readers to understand the principles of SMFS experiments, and at the same time, inspire the utilization of SMFS technique for studying the intermolecular, intramolecular, and molecule-material interactions, which will be valuable to promote the reasonable design of biomolecule-based hybrid nanomaterials.

Keywords: single-molecule force spectroscopy, analytical method, biomolecules, materials interfaces, interactions

1 Introduction

Design and functional tailoring of nanomaterials have attracted more and more attentions as the structure and function of applied materials are highly related to their final properties and specific applications [1-3]. For this purpose, a lot of methods, such as design of various dimensions [4,5], surface modification [6,7], composites with other nanoscale building blocks [8], hybridization with biomolecules [9,10], and other techniques have been utilized to enhance the functions of nanomaterials, in which the functionalization of nanomaterials with biomolecules exhibited potential advantages due to the unique characteristics of biomolecules [11]. For instance, functionalization of nanomaterials with biomolecules could provide more bioactive groups, better biocompatibility, higher aqueous solubility and stability, as well as wider bio-related applications compared to the pure nanomaterials without bio-functionalization.

To promote the bio-functionalization and the applications of materials, it is necessary to understand the interactions between biomolecules and materials interfaces, which is crucial for the selection of biomolecules with suitable functional groups and the preliminary design and adjustment of the properties of materials interfaces [12-14]. Previously, various famous nanomaterials including metallic nanoparticles [15], carbon nanotubes [16], graphene [17], and other two-dimensional nanomaterials [18], have been conjugated with proteins, peptides, DNA/RNA, enzymes and others for realizing the bio-applications in bionanotechnology, biomedicine and tissue engineering. It is well known that the formation of stable bio-hybrids

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is based on the interaction forces between biomolecules and the materials interfaces, which could be measured experimentally and theoretically by using some techniques such as optical tweezers and fluorescent spectroscopy [19], calorimetry titration [20], polymerase chain reaction [21], force spectroscopy (FD) [22], and molecular dynamic simulations [23].

Atomic force microscopy (AFM)-based single-molecule force spectroscopy (SMFS) has been a powerful and direct method for measuring the interaction forces between various biomolecules and different materials interfaces quantitively [24-27]. In the measurement process, biomolecules are covalent or non-covalent bound onto the surface of an AFM tip, which is approached to interact with materials interface to form material-biomolecule bonds and then retracted from the materials interfaces to interrupt the bonds. By this approach and retract steps, the interaction forces between biomolecules and materials interfaces are obtained [28]. In addition, the measurement can be performed in the liquid phase, which is ideal for determining the forces of single molecule and materials interfaces [25].

In this work, we present advancement in the using of SMFS for measuring the interactions between biomolecules and materials interfaces. For helping readers to understand this analytical technique, we first introduce briefly the functionalization of AFM tip with biomolecules, the preparation of materials interfaces, and the operation modes of SMFS. Then, typical cases on the detection of the forces between DNA/RNA, protein, peptide, antibody, and antigen with various materials interfaces are demonstrated and discussed. After that, the material-biomolecule interaction-based applications are introduced. We believe this work will be valuable for readers to understand this analytical method and inspire more novel functional hybrid bionanomaterials for advanced applications.

2 Single-molecule force spectroscopy

In this section, we introduce the functionalization of AFM tips, the preparation of materials interfaces, and the operation modes of SMFS in order to assist the readers to understand this analytical method.

2.1 Tip functionalization

To obtain the interaction force between biomolecules and materials interfaces, it is crucial to conjugate biomolecules onto the surface of an AFM tip by noncovalent or covalent binding. If the biomolecules cannot be bound onto AFM tip with appropriate strength, for instance with a noncovalent adsorption, when the tip is approached to the materials interfaces, the interactions between biomolecules and materials interfaces are too large to cause the detaching of biomolecules from the AFM tip, which will affect the measurement of the interaction force. Currently, covalent binding of biomolecules onto the AFM tip for achieving SMFS measurement has been widely developed [29,30]. Various surface modification methods and functional linkers have been utilized for the tip functionalization based on the use of both Si_3N_4 and Au-coated Si_3N_4 tips [31].

For instance, Wei *et al.* demonstrated the surface functionalization of AFM Si_3N_4 tips with a facile method for binding DNA, peptide and proteins for the SMFS determination of the interactions between biomolecules and various materials [22,23,32]. As shown in Figure 1a, the Si_3N_4 tip was first modified with 3-aminopropyl triethoxysilane (APTES) and thiethoxychorosilane (TTCS) through the silanization to form -NH_2 group on the surface of the tip, which could further react with the bifunctional N-hydroxysuccimidyl (NHS)-PEG-NHS ester disulfide to provide the –NH_2-binding groups on the tip. Therefore, the biomolecules containing –NH_2 groups can be conjugated onto the tip surface easily. In this case, to keep the biomolecules bound on the tip surface with suitable density, block chemical, such as TTCS, was used with a volume ratio to APTES of 4:1 to get the force spectrum with single-molecule characteristics [22]. This method is universal and effective for binding various biomolecules onto the Si_3N_4 tip with adjustable density for high-performance SMFS measurement.

Besides the bifunctional NHS-PEG-NHS linker, another widely used bifunctional linker, NHS-PEG-maleimide (NHS-PEG-MAL), is also effective for covalent binding biomolecules onto AFM tip. For instance, King and co-workers reported the immobilization of proteins on AFM tip by using this NHS-PEG-MAL linker [33]. As indicated in Figure 1b, the Si_3N_4 tip was first etched with HF to form H-based Si_3N_4 surface, which was then alkylated by UV light to assist the hydrosilylation of the tert-butyl 3,6,9,12,15-pentahexacos-25-enylcarbamate linker to form the N-*Boc*-terminated monolayer. After that, the N-*Boc* groups were cleaved with trifluoroacetic acid and the created free amine could be coupled with the -MAL group to form -NHS groups on the tip surface (Step 1-5). Here, it is possible to bind various biomolecules with -NH_2 group by the NHS-PEG-MAL modification. In their study, in order to bind protein with specific binding site (Ni^{2+}-binding His_6 tag), further modification with a synthetic sulfhydryl-linked NTA molecule was used to react with the

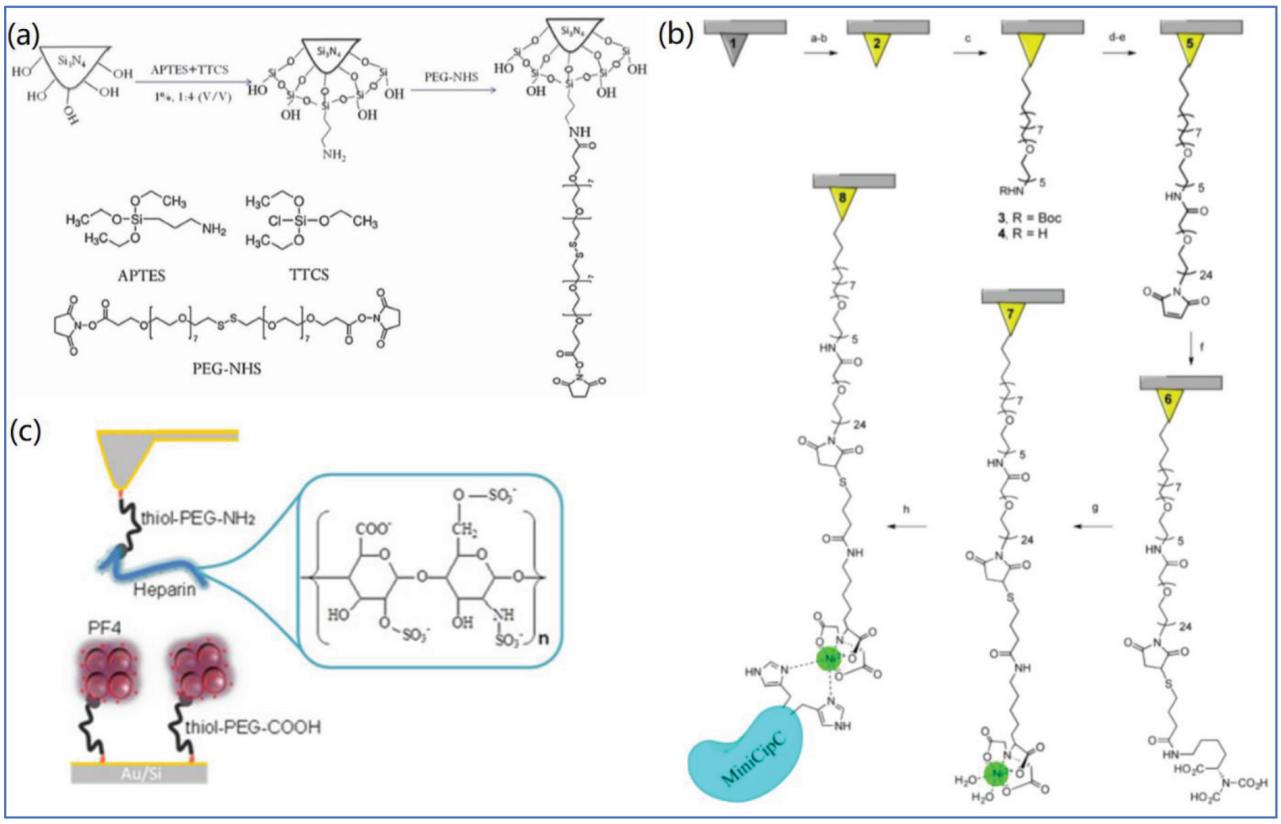

Figure 1: Typical methods of AFM tip functionalization for SMFS: (a) Functionalization of tip with APTES and then bifunctional NHS-PEG-NHS linker for binding NH_2-containing biomolecules. Reprinted image with permission from Ref. [22], Copyright 2014, Royal Society of Chemistry. (b) Functionalization of Si_3N_4 tip with NHS-PEG-MAL linker. Reprinted image with permission from Ref. [32], Copyright 2015, American Chemical Society. (c) Functionalization of Au-coated AFM tip with thiol-containing linker. Reprinted image with permission from Ref. [33], Copyright 2017, Wiley VCH.

NTA Ni^{2+} chelator (Step 6-8). It is possible to measure the interactions between biomolecules and materials at the specific binding sites through this method. To minimize undesired bivalent reaction of bifunctional linkers with -NH_2 groups on the AFM tip surface, Wildling et al. have reported another bifunctional linker, acetal-PEG-NHS, to link sensor molecules onto the amino-functionalized AFM tips [34]. In addition to the Si_3N_4 tip, in some cases Au-coated Si_3N_4 tips have been utilized. Through the formation of S–Au bonds by the thiol-Au reactions, it is facile to create active functional groups by using the thiol-based linkers (Figure 1c) [35]. Meanwhile, by using the bifunctional thiol-PEG-NHS linkers, it is also very simple to bind various biomolecules onto this kind of Au-coated AFM tips.

2.2 Preparation of materials interfaces

After the functionalization of AFM tips with biomolecules, it is also necessary to prepare uniform materials interfaces to further study the interactions between biomolecules and materials interfaces. As shown in Figure 2, typical surface chemistry and bioconjugation methods for the modification of both gold and mica surface are presented [36], which are in some way similar to the surface chemistry of tip functionalization. Usually, to measure the biomolecule-biomolecules/biomaterials interactions, the binding of various biomolecules onto gold and mica surface should be carried out. For the modification of gold substrate, bifunctional PEG linker with carboxy and –SH groups can be utilized by the formation of Au–S bonds; however, for mica and glass substrates, the amino and carboxy groups should be firstly created on the materials surface, and then corresponding bifunctional PEG linkers could be utilized for further functionalization and conjugation with biomolecules to form functional materials interfaces.

Besides, SMFS can also be utilized for studying the interactions between biomolecules and inorganic materials interfaces [37,38]. For instance, Iliafar and co-workers prepared materials interface of carbon nanotubes (CNTs) by depositing CNTs onto hydrophobic methyl-modified self-assembled monolayers (SAMs) of polymers, in order to study the interactions between single-stranded DNA (ssDNA) and

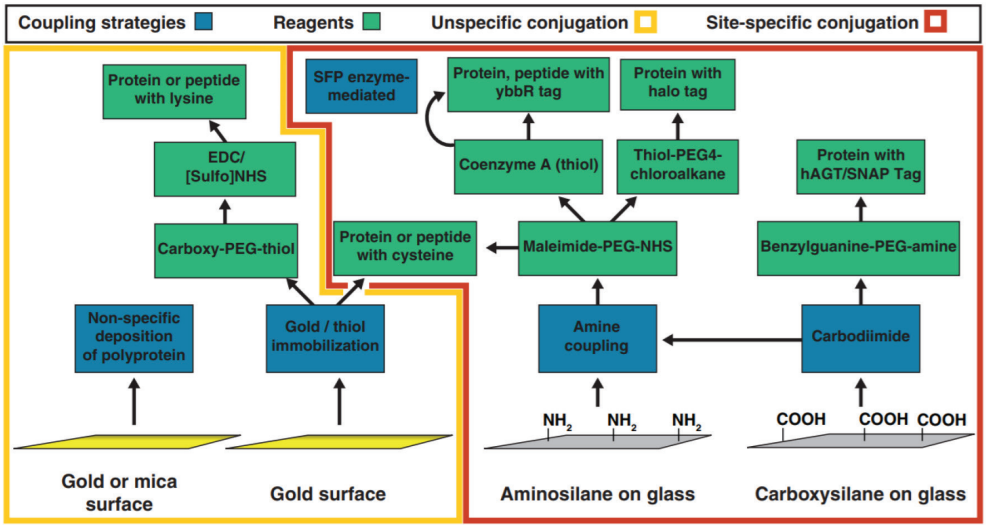

Figure 2: Typical surface chemistry and bioconjugation methods for the preparation of materials interfaces towards SMFS study. Reprinted image with permission from Ref. [36], Copyright 2017, Elsevier BV.

CNTs [39]. To further compare the forces between ssDNA and CNTs or graphene surface, a mechanical exfoliation of highly oriented pyrolytic graphite (HOPG) has been used to obtain graphene-like materials interface [39-41]. In another study, Lulevich et al. prepared CNT nanopore array on a silica substrate by using a commercial chemical vapor deposition reactor, and then measured the frictionless interaction between ssDNA and the inner wall of CNTs [42]. These carbon-based materials interfaces were useful for understanding the biomolecule-materials interactions, which play important roles in the design and synthesis of carbon material-biomolecule hybrid nanomaterials for various applications.

In addition, other kind of materials interfaces, such as silica wafer [32], single-crystalized fused silica [23], graphite [22,43], and single-crystalized Au(111) [44] surface have also been prepared for studying the interactions between materials interfaces and protein, peptide, as well as ssDNA molecules.

2.3 Operation modes

After the completement of the tip functionalization and materials interface, now it is ready to perform SMFS through approaching and retracting the AFM tip onto and from the materials surface. The operation modes of the SMFS experiments could be classified into dynamic force spectroscopy (DFS) [45-47], force mapping (FM) [48,49], and force clamping (FC) [50,51].

Early SMFS experiments have been done to measure the forces at a single point with a fixed pulling force, however, Evans et al. have found that the strength of the receptor-ligand bonds was governed by the prominent barriers in the energy landscape [45]. By changing the pulling force, they explored a series of loading rates (related to the pulling force and tip spring constant), and found that the bond strength was increasing with the enhancement of loading rates. As shown in Figure 3a, Sluysmans and co-workers investigated the folding behavior of the synthetic oligorotaxane foldamers by DFS [52]. With the retracting of AFM tip from the material surface, the oligorotaxane molecule was stretched, and a single force spectrum exhibited continuous unfolding of this biomolecule. Through the DFS measurements with adjusting loading rate, a lot of data points with various forces were obtained, which could be fitted to the Friddle-Noy-De Yoreo model [53].

In the FM mode, force-distance curves are recorded at the points of a scanned area, which is similar to the contact imaging mode of AFM. However, if a biological sample such as cell membrane or bacteria are used, the contact mode will destroy the surface of biological samples. Therefore, the AFM-based force-volume mode, called as FM, could be utilized for measuring the forces between tip and samples, and meanwhile getting the images of biological samples. For instance, Gaboriaud et al. performed the imaging of bacterial surfaces by using the FM mode [54]. As shown in Figure 3b, the 10 × 10 grid indicates the morphology of the bacterium *Shewanella putrefaciens*, and the corresponding approach and retraction curves show the interactions between the AFM tip and bacterial surface.

In the normal force spectroscopy, stretching force for bond rupture is changing. However, if the stretching

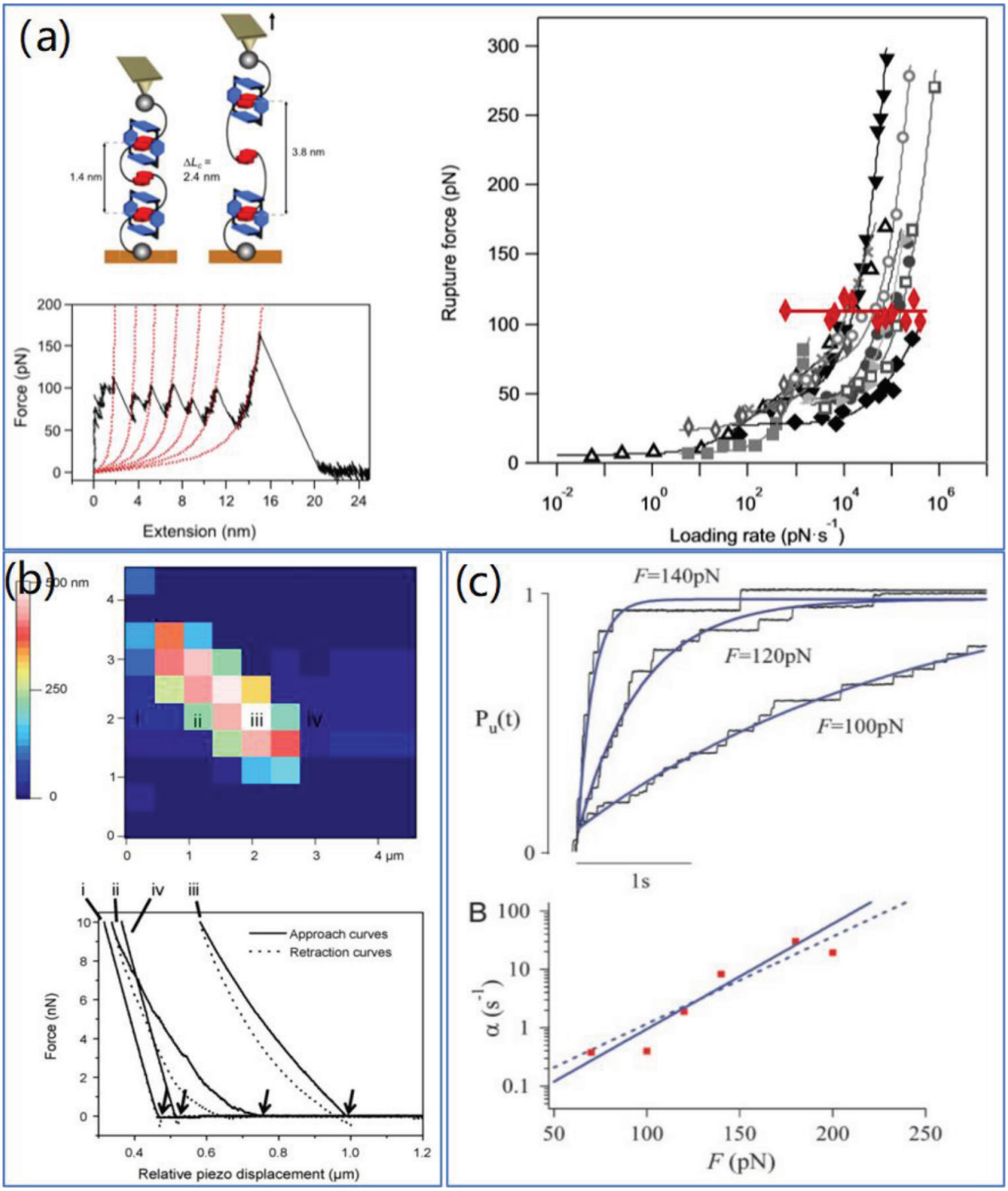

Figure 3: Operating modes of SMFS measurement: (a) DFS mode, Reprinted image with permission from Ref. [52], Copyright 2018. PNAS. (b) FM mode, Reprinted image with permission from Ref. [54], Copyright 2008, Elsevier BV. (c) FC mode. Reprinted image with permission from Ref. [55], Copyright 2004, PNAS.

force is fixed at a constant value, a FC mode is then carried out, which is useful for understanding the relationships between the force-driven unfolding of RNAs and proteins. In a typical study, Schlierf and co-workers investigated the unfolding kinetics of ubiquitin molecules by using the single-molecule FC technique [55]. By adjusting the stretching force, the unfolding rates of ubiquitin were obtained, and it was found that the applied stretching force linearly corresponded to the logarithmic plot of the unfolding rate, as shown in Figure 3c.

Besides the above-mentioned modes, some other techniques, including the single-cell force

spectroscopy, optical tweezers, magnetic tweezers, and others have also high potential for studying the interactions between molecules and various materials interfaces [56-59]. Here we would like to provide short introduction to a versatile nanopore-based SMFS technique, which does not require chemical functionalization of the molecules under studying or the used nanopores [60]. SMFS experiments could be utilized to analyze the captured biomolecules inside the nanopore, and therefore are useful to study the interactions between various biomolecules such as nucleic acids duplexes [61], DNA-PNA [62], and DNA-protein conjugates [63,64]. The solid-state or protein-based nanopore platforms have the advantages of low cost, real time monitoring, label-free probing, and single-molecule sensitivity, exhibiting the potential of biosensing, DNA sequencing, and energy estimating of DNA unzipping or duplex hybridization.

3 Studying the interactions between biomolecules and materials interfaces

In this section, some typical studies on the measurement of the interactions between biomolecules and materials interfaces by SMFS are introduced and discussed.

3.1 Interactions between DNA and materials interfaces

Studying the interactions between DNA molecules and various materials interfaces plays crucial roles in the design and synthesis of DNA-based hybrid nanomaterials for advanced applications [9,65]. SMFS is a direct technique to achieve this aim.

Tan et al. studied the formation of an artificial DNA duplex structure via Ag^+-induced formation of base pair by using SMFS [66]. As show in Figure 4a, the SiO_2 substrate was firstly functionalized with aminopropyldimethylethoxysilane (APDES) to create $-NH_2$ groups on the surface, which were then reacted with NHS–PEG4000–N_3 to form the azide-functionalized surface. After that, the ssDNA oligonucleotides with alkyne moiety at the 5'-end were bound onto the azide-functionalized surface by click chemistry. Meanwhile, complimentary ssDNA oligonucleotides were conjugated onto the Au-coated AFM tip via the formation of Au-S bond with a bifunctional PEG linker. Therefore, the addition of Ag^+ into this system resulted in the formation of a polymer bridge between the AFM tip and the functionalized materials interface. With the retraction of AFM tip from the materials surface, the polymer bridge was stretched and the cantilever deflected by the external force to create the typical force-distance curves shown in Figure 4b. When a loading rate of 10 nN/s was used for the experiment, the rupture force of the DNA duplex in the presence of Ag^+ was measured to be about 81 ± 31 pN. This work proved the formation of highly stable metal ion-mediated DNA duplex, which would be as ideal nanoscale building blocks for the design and synthesis of functional DNA nanomaterials.

Due to the interactions between DNA bases and the surface of carbon nanomaterials (such as CNTs, graphite, graphene, and others), the synthesis of DNA-carbon complexes has exhibited great potential in the applications of drug delivery, DNA nanotechnology, biosensors, cancer therapy, and others [67-69]. However, the rational design and synthesis of the DNA-carbon hybrid materials are highly relative to the quantitative measuring of the interactions between DNA molecules and carbon materials interfaces. Vezenov and co-workers performed the pioneered studies on the SMFS determination of the interaction forces between ssDNA oligomers and CNTs as well as graphite surface [39-41]. For instance, they quantified the interactions between ssDNA and mechanically cleaved graphite surface by using SMFS technique [40]. As indicated in Figure 4c, ssDNA oligomers were bound chemically onto the AFM tip and then the tip was approached and retracted to form the graphite surface to obtain the force-distance curves. It is clear that there are force jumps with the stable platforms when the tip was retracted from the graphite surface, indicating that the binding forces of different DNA bases with graphite surface can be measured precisely.

The interactions of ssDNA and enzyme can also be measured by SMFS technique. The Lyubchenko group studied the formation and rupture of the cytidine deaminase APOBEC3A (A3A)-ssDNA complexes by SMFS [70]. In their study, the enzyme A3A was conjugated onto the surface of the AFM tip, and the substrate was prepared by modifying the Au surface with thiol-ssDNA via the formation of S–Au bond (Figure 4d). When the tip was approached onto the surface, the A3A bound with ssDNA to form complexes, however, the retraction of tip interrupted the complexes and resulted in a sharp rupture force, as shown in Figure 4e. Control SMFS experiments proved that the interactions between A3A and ssDNA were sequence-dependent. In addition, this designed system is helpful for measuring the deaminase activity of A3A by analyzing the binding strength of the complexes.

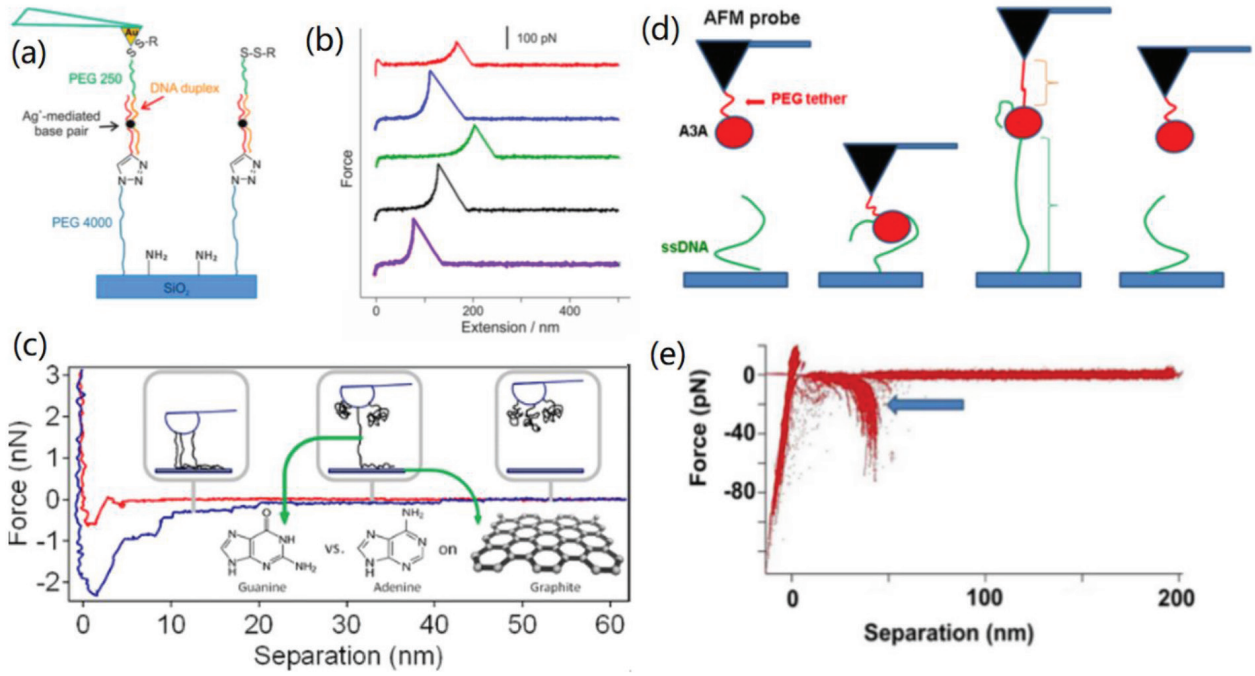

Figure 4: SMFS studying the interactions between DNA molecules and materials interfaces: (a,b) ssDNA-ssDNA duplex formation. (a) tip functionalization and materials interface preparation. (b) typical force-distance curves. Reprinted images with permission from Ref. [66], Copyright 2015. American Chemical Society. (c) interaction between ssDNA oligomers and graphite surface. Reprinted image with permission from Ref. [40], Copyright 2012. American Chemical Society. (d,e) interactions between A3A enzyme and ssDNA. (d) detection mechanism and (e) typical force-distance curves. Reprinted images with permission from Ref. [70], Copyright 2016. American Chemical Society.

3.2 Interactions between protein/peptide and materials interfaces

Similar to the DNA-material interactions, the understanding of the interactions between proteins/peptides and various materials interfaces are significant for developing nanomaterials for biosensors, biocatalysis, biomedicine, and tissue engineering [14,71-73]. Here we would like to present several typical cases of SMFS analysis on the interactions between protein/peptide and materials.

Srinivasan et al. investigated the face-specific binding of proteins with the Capto MMC multimodal ligands by SMFS (Figure 5a) [74]. For this aim, they utilized the Capto ligands to modify the Au-coated AFM tip to form a SAM on tip, and used two proteins with exposed "preferred" (UBQ S20C) and without (UBQ A46C) binding faces to modify the Au surface. It was found from the SMFS that the binding between proteins and ligands was face-specific. The binding force between the protein with preferred binding face and ligands was significantly higher than that between protein without binding face and ligands. This work is helpful for understanding the specific interactions between proteins and materials surfaces by tailoring the surface functionalities and the intermolecular forces. Vera and co-workers demonstrated the direct identification of protein-protein interactions by SMFS [75]. As shown in Figure 5b, the polyprotein was firstly modified by a single-molecule marker (three I27 module) and another parallel marker at both ends with S–S binding. Through stretching in SMFS tests, it was found that a series of force peaks were obtained, and finally the protein-protein interactions (labelled with blue force peaks) were also achieved. In addition, this method provided measurement of the cohesion-dockerin interaction (labelled with red force peaks). In other cases, Wei and co-workers measured the binding forces between lysozyme [76] and GCRL tetrapeptide [23] with SiO_2 and amorphous SiO_2 surface by SMFS, respectively.

In another study, Ma et al. reported SMFS determination of the binding of protein with membrane [77]. In their study, single proteins were firstly attached onto the membranes that supported by silica beads, and then the bound proteins were pulled from the membranes by optical tweezers, as shown in Figure 5c. Through the membrane binding and unbinding transition, the corresponding force-distance (extension) curves were obtained, which could be utilized to analyze the protein-membrane interactions with unprecedented spatiotemporal resolution. This proposed strategy is universal and could be widely applied for

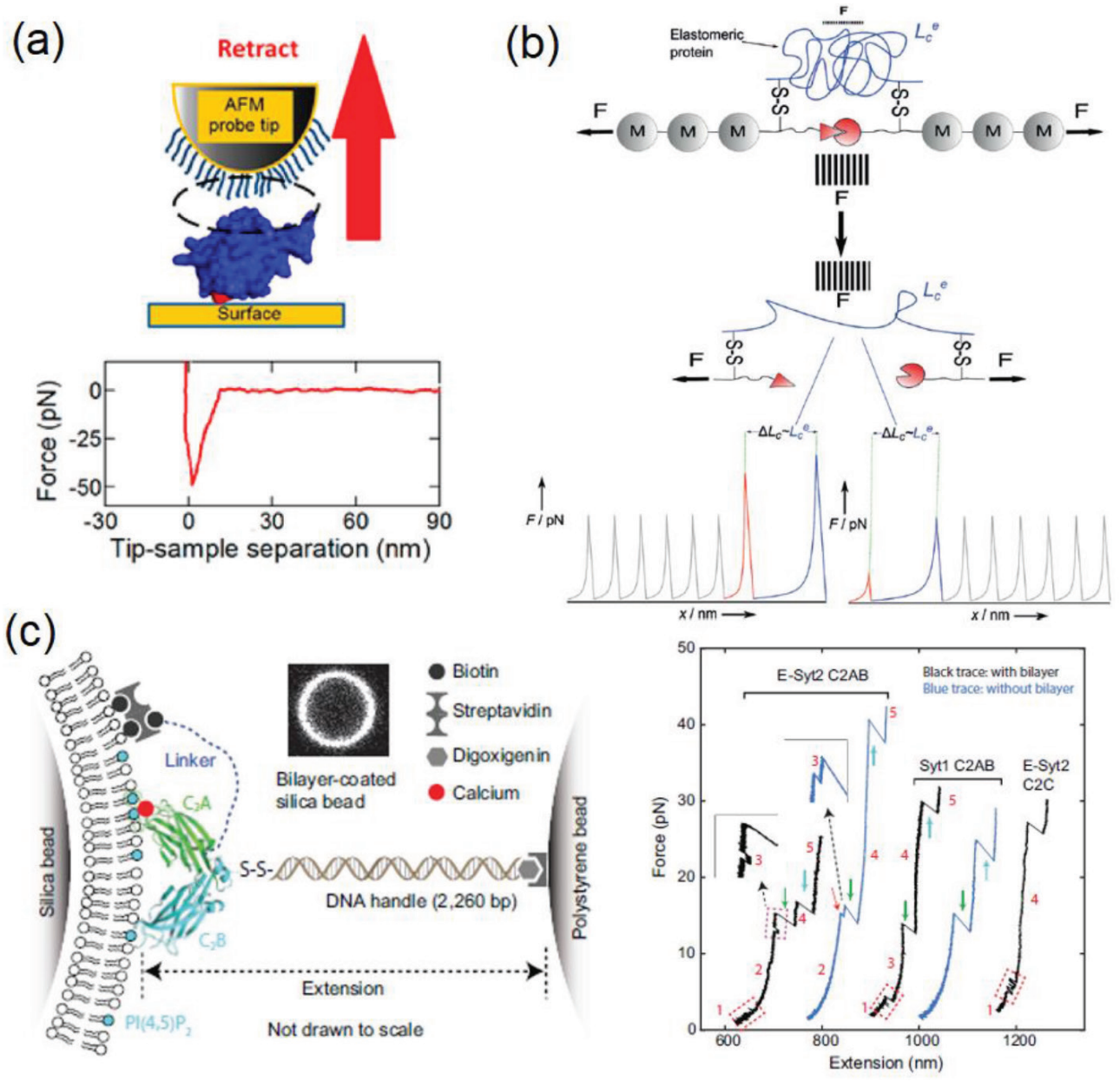

Figure 5: SMFS studying of protein/peptide-materials interactions: (a) Protein-ligand interaction. Reprinted image with permission from Ref. [74], Copyright 2017. American Chemical Society. (b) Protein-protein interaction. Reprinted image with permission from Ref. [75], Copyright 2016. Wiley VCH. (c) Protein-membrane interactions. Reprinted image with permission from Ref. [77], Copyright Ma et al.

measuring the interactions between different proteins and membranes easily.

3.3 Interactions between antibody/antigen and materials interfaces

Biomolecular recognition has been widely utilized for the synthesis of multifunctional nanostructures and nanomaterials for chemical, biological, and biomedical applications, in which the antibody-antigen interactions are especially crucial for the materials design. Previously, SMFS technique has been applied for understanding the affinity and binding forces between antibody/antigen and materials interfaces [78-82].

For instance, Casalini and co-workers reported detection of the antibody-antigen interactions by SMFS and organic transistors previously [83]. In this case, AFM tip was amino-functionalized with APTES, and NHS-PEG-MAL bifunctional linker was added to react with the formed $-NH_2$ groups to create –MAL groups on the AFM tip. Therefore, antigen could be conjugated onto the AFM tip

with covalent binding. Meanwhile, the flat mica substrate was coated with a 50 nm thick Au layer, which was then modified with 6-aminohexanethiol to form $-NH_2$ groups on the Au substrate. After that, glutaraldehyde was added to active the formed $-NH_2$ groups for assisting the binding with antibody via covalent conjugation. As indicated in Figure 6a, the approaching and retracting of the tip from the substrate caused a single force peak at the force-distance curve in the process of SMFS test. The obtained typical force-distance curve shown in Figure 6b exhibited an unbinding force of about 150 pN and an unbinding length of 25 nm. With the collected SMFS information, it is possible for us to quantify the binding force, length scale, binding free energy, and time scale within the specific antibody-antigen interactions. In another case, Li et al. designed a novel method by combining enzymatic cleavage and biotin-streptavidin interaction to achieve the determination of enzymatic reactivity [84]. The biotin-modified oligopeptides were conjugated onto the AFM tip by the bifunctional linker and the substrate was then functionalized with a monolayer of streptavidin. In addition, since the oligopeptide has a special motif that can be recognized and cleaved by thrombin, the typical force-distance curve with single force peak was obtained in the absence of thrombin; however, in the presence of thrombin, the peptide chain was cut, and the length scale and force obtained from the force-distance curve were changed clearly.

3.4 Interactions between biomolecules and biomaterial interfaces

In the above three sections, we have introduced and discussed various cases on analyzing the interactions between DNA, protein/peptide, and antibody/antigen with various materials interfaces. Still, many other cases on studying the interactions of biomolecules (such as biopolymers, enzyme, antigen, bacterial, and virus) and materials interfaces have also been reported [33,85-88].

For instance, Delguste et al. developed a FD curve-based AFM method for studying the interactions between single virus particles and living cell membranes [89]. In fact, it is a new AFM technique related to the peak-force tapping mode spectroscopy, which allows quantitative three-dimensional imaging of biological materials surface such as cell membranes with high resolution (about 50 nm) through analyzing the adhesion, elasticity, and deformation in the FD measurement. For the first time, they have obtained direct evidence that a herpesvirus surface glycoprotein played significant role in regulating the binding of herpesvirus in the first step. In another similar study, Alsteens and co-workers developed an atomic force and confocal microscopy set-up to observe the first binding steps of virus to animal cells, and the nanomechanical peak-force mapping with high resolution (< 50 nm) was achieved successfully [90]. Very recently, Kang and co-workers demonstrated the force imaging of CD-146 antibody across entire cell surfaces in the fast peak-force tapping mode [91], which exhibited high advantages to image the soft biomaterial surfaces with quicker scanning and higher resolution than the traditional force-volume mode.

4 Potential applications

By analyzing the change of the binding force and the distribution of different force, it is possible to apply SMFS-based methods for some applications such as biosensing, DNA sequencing, and the measurement of binding free energies of biomolecules with materials. In this part, the SMFS-based advanced applications are presented in brief.

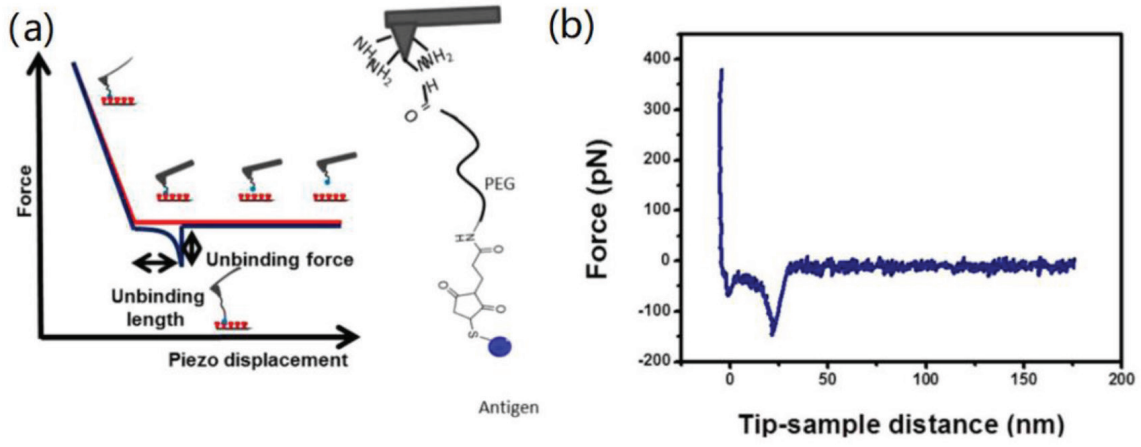

Figure 6: Antibody-antigen interactions measured by SMFS: (a) tip approaching and retracting models, and (b) typical force-distance curve. Reprinted image with permission from Ref. [83], Copyright 2015. American Chemical Society.

Firstly, SMFS technique can be utilized to fabricate highly sensitive sensor platforms for detecting DNA, RNA, protein, enzyme, drug molecules, and metallic ions. Readers are suggested to study our previous review on the SMFS-based bioimaging and biosensing [25,92,93]. Here several recent studies on SMFS-based biosensing are introduced. For instance, Wei et al. developed a label-free method for the determination of adenosine and mercury ions by using the FC-based force-to-color variety [94]. As shown in Figure 7a, the ssDNA adenosine aptamer (Ad-apta) was conjugated onto the AFM tip and the complimentary ssDNA (cDNA) was then bound onto Au surface to form cDNA SAM. In the absence of adenosine, DNA hybridization created dsDNA bond, which was disrupted in the process of tip retraction to form a force peak of about 300 pN. In the presence of adenosine, specific interactions between Ad-apta and adenosine promoted the formation of a loop DNA structure, which inhibited the formation of dsDNA structure and showed no rupturing force in the force-distance spectrum. Based on this mechanism, the FC mode was further utilized to achieve in naked eye observable determination of adenosine, as shown in Figure 7b. It was found that the addition of adenosine with different concentrations resulted in color change in the obtained force maps. Meanwhile, this method could be used for the fabrication of another mercury ion sensor by replacing the aptamer and cDNA with corresponding ones. Fabricated FC-based visual biosensors exhibited high sensitivity, low detection limits (0.1 nM for adenosine and 10 pM for mercury ions), quick detection, and simple readout. In another recent study, Li and co-workers utilized SMFS to detect the peptide cleavage by thrombin by measuring the biotin-streptavidin interactions [84]. Their results indicated that it was possible to engineer a highly sensitive sensor platform for detecting thrombin (detection limit of 0.2 μM) and its enzymatic activity through analyzing the antibody-antigen interactions with SMFS.

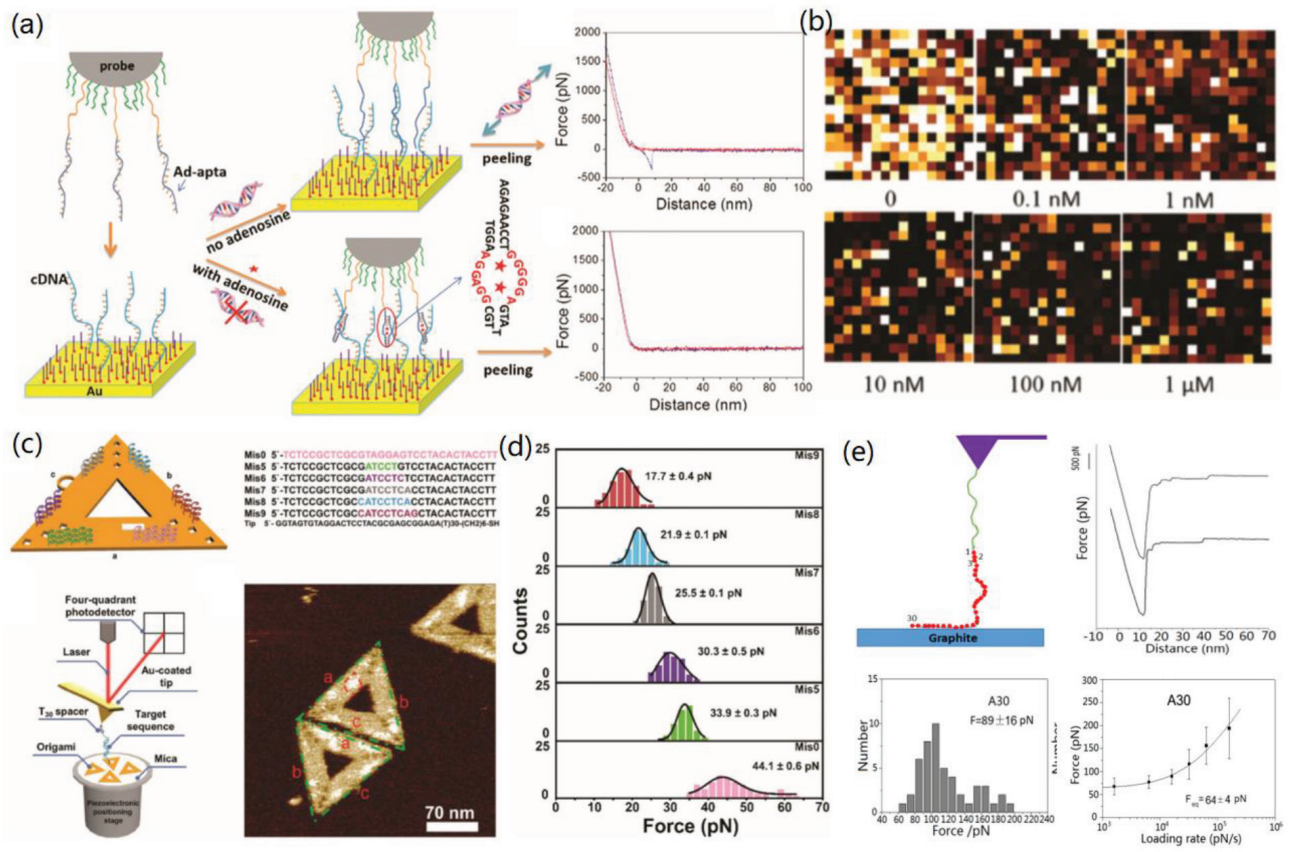

Figure 7: SMFS quantified biomolecule-material interaction for potential applications: (a,b) SMFS-based force mapping for color-variable biosensors with (a) sensing mechanism and (b) force maps with different analyte concentrations. Reprinted images with permission from Ref. [94], Copyright 2018. Royal Society of Chemistry. (c,d) Detection of DNA mismatch: (c) detection mechanism, DNA sequence, and self-assembled DNA nanostructure. (d) distribution of force with various DNA mismatches. Reprinted images with permission from Ref. [95], Copyright 2019. Royal Society of Chemistry. (e) SMFS-based experimental measurement of binding free energy of biomolecules and materials interfaces. Reprinted image with permission from Ref. [44], Copyright 2017. American Chemical Society.

AFM-based SMFS has also been applied for detecting DNA base mismatches previously. For instance, Liu and co-workers reported DNA origami-mediated detection of single base-pair mismatch by using SMFS [95]. As shown in Figure 7c, triangular DNA origami was first labelled with different nucleic acid sequences in the a, b, and c side of the origami structure. In the fabrication of the SMFS system, ssDNA was tethered onto the Au-coated AFM tip and the targeted ssDNA was anchored on the DNA origami. With this method, the rupture force between the two DNA strands was recorded by analyzing the force-distance spectroscopy. It was found that the force strength and the peak force distributions were highly relative to the mismatch point of the base pairs (Figure 7d). A clear, statistically significance variation of about 4 pN/addition mismatched base-pair was found. In addition, this SMFS and DNA origami-based method showed higher throughput compared to normal SMFS-based biosensors due to the use of two-dimensional DNA origami. Furthermore, this technique could be used to determine other biomolecules including protein, carbohydrate, and metallic ions.

Beside the fabrication of biosensor platforms, SMFS study on the biomolecule-material interactions can be used for helping the design and synthesis of novel hybrid bionanomaterials [96,97]. Previously, various biomolecules such as DNA, protein, peptide, and others have been used to modify CNTs, graphene, and nanoparticles [9,39]. SMFS provides a simple way to study the binding behavior and binding energy of biomolecules with different materials interfaces [76]. For instance, Meissner et al. made the estimation of the binding free energy of polypeptide on amorphous SiO_2 with SMFS and molecular dynamics simulations (MDS) [23]. Hughes et al. studied the adsorption of DNA fragments (A, T, G, and C) at graphite and Au(111) surfaces by the integration of SMFS and MDS [44]. As indicated in Figure 7e, a ssDNA oligomer was bound onto the AFM tip and the binding free energy between ssDNA and graphite surface was measured by SMFS. Through the DFS test and fitting with the Friddle-Noy-De Yoreo model, the force at the equilibrium state was obtained and then the binding free energy was calculated. Therefore, SMFS provided valuable reference for the design of DNA molecules with optimal sequence for adjustable binding ability with graphite and graphene materials. In addition, with this method it is possible to select suitable peptide sequences for binding with various nanomaterials towards the design and synthesis of functional hybrid materials. In a typical case, Li and co-workers studied the interactions between self-assembled sequence-designed peptide nanofibers and graphene oxide nanosheets as well as graphene quantum dots by using SMFS experiments [26]. It was clear that SMFS technique will benefit the selection of material-specific peptide sequences, the rational design of functional hybrid nanomaterials, and the understanding of the formation mechanisms and functions of materials.

5 Conclusions and outlooks

In summary, in this review we have presented preliminary knowledge on the use of SMFS for determining the interactions between biomolecules and materials interfaces through introducing AFM tip functionalization, substrate modification, and typical operation modes of SMFS. In the case studies, we demonstrated the applications of SMFS for studying the interactions between materials interfaces and the biomolecules such as DNA, protein, peptides, antigen, antibody, as well as other biomolecules. In addition, the analysis of the interaction information obtained from the SMFS experiments promoted the potential applications of SMFS technique for biosensing, DNA sequencing, estimation of binding free energy, and the design of various biomolecule-based hybrid nanomaterials. As a highly sensitive analytical technique, SMFS provides direct method for measuring the interactions of molecule- molecule and molecule-material. Besides the contents shown in this work, we suggest that SMFS can also be utilized for other studies. For instance, SMFS can be used to study the interactions between biomolecules and nanoparticles, as well as the interactions between biomolecular assembly and materials interfaces. In addition, the interactions between cell membranes and nanoparticles, drug molecules, or biomolecules can also be detected by SMFS methods.

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