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Adhesion and stiffness of biotin-superavidin bonds

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ABSTRACT

A non-invasive vibrational spectroscopy technique is introduced and utilized to characterize the average spring constant of a single Superavidin (SAv)-Biotin (Bi).polyethylene glycol (PEG) ligand receptor complex as well as the effective Young's modulus and adhesion of a layer formed by the SAv-Bi.PEG ligand-receptors. In the reported experiments, SAv coated Polystyrene (PS) particles are deposited on a layer of Bi.PEG receptors, bound to a silicon (Si) substrate by silanization. The substrate and the bonded particles are subjected to a pulsed ultrasonic excitation field and their nanometer scale out-of-plane dynamic responses are acquired using a laser vibrometer. The acquired waveforms are processed to obtain the resonance frequencies of the particle motion. Employing a particle adhesion model, the average spring constant of the single ligand-receptor complex and the effective Young's modulus and work-of-adhesion of the SAv-Bi.PEG ligand-receptor layer are extracted from the resonance frequencies. The average spring constant of an individual SAv-Bi.PEG bond is approximated as 0.1-0.4 mN/m. The work-of-adhesion and effective Young's modulus of the SAv-Bi.PEG layer are determined to be 0.54–2.62 J/m^2 and 0.15–2.80 MPa, respectively. The compressive Young's modulus of the SAv-Bi.PEG layer is estimated as 31.0–58.0 MPa. The current approach provides a direct non-contact measurement technique for the stiffness of single ligand receptor complexes and the adhesion of their interfaces. SAv-Bi bonds and PEG polymers are among the most widely utilized complexes in the pharmaceutical and biological applications. Understanding the mechanical properties of PEG and SAv-Bi is an important step towards optimization of their utilization in practical applications such as biosensors and targeted drug delivery.

1. Introduction

The migration of cell populations through living organisms is dependent on the continuous formation and dissociation of specific bonds between adhesion molecules (ligand-receptors) borne by cells and surrounding tissues [1]. Molecular interactions between the ligands and receptors are generally derived from multiple weak bonds between geometrically complementary surfaces of recognition sites, are short-range, noncovalent and with varying levels of bond strength [2]. Ligand-receptor interactions are not only a crucial element of cellular mobility inside living organisms, but are also essential for the design of biosensors and immobilization of particles and macromolecules in targeted drug delivery.

One of the widely utilized ligand-receptor complexes in life science applications today is Avidin (Av)-Biotin (Bi) bonds. Av is a glycoprotein found in egg whites and Bi is a small molecule water-soluble vitamin (B_7). A modified version of Av molecules is Superavidin (SAv) which is with lower nonspecific binding than Av, but with the same binding affinity to biotinylated ligands. Av and Bi exhibit an extraordinary affinity towards each other with a dissociation constant of 10^{-15} M (mol/L), which is the highest among the known noncovalent bonds [3]. The high stability of the Av.Bi complex, compared to other noncovalent bonds and the wide range of commercially available reagents, explains its widespread utilization [4] in biology research and an extensive variety of medical and diagnosis applications such as bacteriophage inactivation, biosensors, gene and cellular mapping, selective absorption of cells, enzymatic synthesis and immobilization of macromolecules [5–7].

In the literature, the mechanical properties of individual Av-Bi bonds [2,6–10] and also PEG [11–14] are predominantly measured using Atomic Force Microscopy (AFM). AFM requires direct contact with the material under study. The direct contact of an AFM probe tip with the sample creates an extra bond with unknown properties, which substantially complicates the adhesion characterization task.

In the current work, a non-contact non-invasive method is introduced to characterize the mechanical properties of SAv.Bi.Polyethylene glycols (PEG) ligand receptors by acquiring the nanoscale dynamics of SAv coated on a substrate coated with Bi.PEG

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ligands employing laser vibrometery and ultrasonic excitation. PEG polymers are chosen as the anchor agents for Bi ligands, attaching the Bi ligands to a Silicon (Si) substrate by silanization. PEGs are non-branched polymers with high exclusion volumes, due to high conformational entropy, that resist the unwanted (bio-) polymers including proteins. Thus, surface-attached PEG layers substantially decrease non-specific binding of proteins and other macromolecules to artificial surfaces [15]. PEGs are widely used in a variety of biomedical applications, including matrices for controlled release of biomolecules and scaffolds for regenerative medicine [16].

2. Experimental setup and procedure

The key objective of current study is to determine the spring stiffness of a single SAv-Bi.PEG ligand receptor and the adhesion and the effective Young's modulus of a layer of these ligand receptors, using a non-contact non-destructive technique. In the reported experiments, SAv coated polystyrene (PS) particles are attached to Bi.PEG ligands on a Si substrate and are subjected to a high-frequency acoustic base excitation. The nanometer-scale out-of-plane transient responses of the particles and the substrate to the acoustic excitation field are acquired using laser vibrometery (Fig. 1a), and then analyzed to extract the mechanical and adhesion properties of SAv-Bi.PEG complex.

2.1. Materials and preparation

2.1.1. Si substrate preparation

Rectangular-cut polished Si pieces (2220 100 N, University Wafer, Inc., Massachusetts, USA) are cleaned in three successive cleaning stages by rinsing and sonicating them (for 5 min at each stage) with acetone (9006-33, Avantor, Maryland, USA), Hellmanex III (Z805939, Hellma Analytics, Baden-Württemberg, Germany) and DI water and then sonicated with Potassium Hydroxide (P250-500, Fisher Scientifics, Pennsylvania, USA) for 5 min to prepare them for silanization by increasing the density of the reactive hydroxyl groups on their surfaces. Each activated piece is placed into a glass petri dish to subsequently deposit the functionalizing solution on its surface.

2.1.2. Bi.PEG.Silane and mPEG.Silane solutions preparation

A stock solution of 95% ethyl alcohol 200 proof (111000200CSPP, Pharmco-Aaper, Connecticut, USA) is prepared and 1% acetic acid glacial (9006-33, Avantor, Maryland, USA) is added to the solution to increase the efficiency of the process. Bi.PEG.Silane (Biotin.PEG.SIL-3400, Laysan Bio, Alabama, USA) and mPEG.Silane (mPEG.SIL-2000, Laysan Bio, Alabama, USA) are added to this stock solution to a concentration of 1 mg/ml inside separate vials. Subsequently, 10 ml of the mixings of mPEG.Silane and Biotin-PEG.Silane solutions with mixing

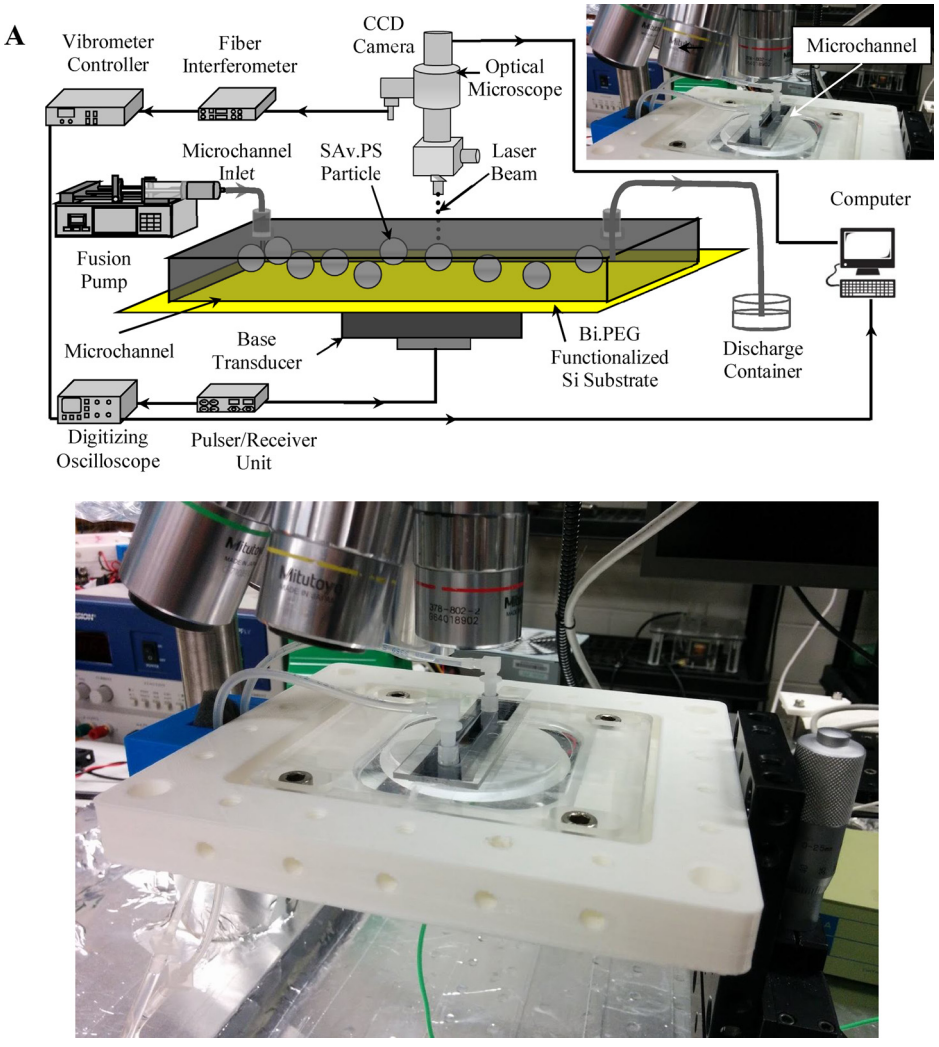

Fig. 1. (a) Instrumentation and connectivity diagram of the experimental set-up. Not to the scale, inset to Fig. 1a. A photograph of the monitoring zone of the experimental set-up. (b) Schematic of a SAv.PS particle on a Bi.PEG coated Si substrate, excited by an ultrasonic transducer. Inset to Fig. 1b. Optical microscopic image of SAv.PS particles on a Bi.PEG coated Si substrate inside a microchannel at 20× with the vibrometer laser beam focused on top of a single particle for acquiring its out-of-plane transient response to an acoustic base excitation. Scale: 50 μm.