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Plasmonic imaging of protein interactions with single bacterial cells

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ABSTRACT

Quantifying the interactions of bacteria with external ligands is fundamental to the understanding of pathogenesis, antibiotic resistance, immune evasion, and mechanism of antimicrobial action. Due to inherent cell-to-cell heterogeneity in a microbial population, each bacterium interacts differently with its environment. This large variability is washed out in bulk assays, and there is a need of techniques that can quantify interactions of bacteria with ligands at the single bacterium level. In this work, we present a label-free and real-time plasmonic imaging technique to measure the binding kinetics of ligand interactions with single bacteria, and perform statistical analysis of the heterogeneity. Using the technique, we have studied interactions of antibodies with single *Escherichia coli O157:H7* cells and demonstrated a capability of determining the binding kinetic constants of single live bacteria with ligands, and quantify heterogeneity in a microbial population.

1. Introduction

Bacteria interact with environment through their surface constituents, such as lipid bilayers, peptidoglycan layers, lipopolysaccharides (LPS), pilli, flagella and outer membrane proteins. The surfaces of bacteria act as the first line of defense against harmful external stimuli, including antibiotics (Delcour, 2009) and antimicrobial peptides (Fantner et al., 2010; Sochacki et al., 2011), and also play crucial roles in interacting with other surfaces, including host tissues (Van Houdt and Michiels, 2005) and medical plastics (Lower et al., 2011), to help bacterial cells attach and colonize. In order to survive in a changing environment, bacteria replicate and evolve quickly (Carnes et al., 2010; Van der Mei and Busscher, 2012), leading to diversity of different bacteria species, and variability within the same species (Van der Mei and Busscher, 2012; van der Woude and Bäumler, 2004). It is thus important to study and quantify the interactions of bacteria with external ligands at the single bacterium level.

The interactions of external ligands and bacteria have been studied using ex situ and in situ approaches, such as fluorescence assay (Cywes-Bentley et al., 2013), quartz crystal microbalance (QCM) (Shen et al., 2007), surface plasmon resonance (SPR) (Medina et al., 1997; Subramanian et al., 2006), microcantilevers (Longo et al., 2013) and atomic force microscope (AFM) (Fantner et al., 2010; Lower et al., 2011). The ex situ approaches include the study of reconstituted artificial membranes (Früh et al., 2011; Hirst et al., 2013), membrane protein embedded liposomes (Liu and Boyd, 2013), and extracted surface constituents (e.g., membrane proteins (Holden et al., 2006) and sugars (Grant et al., 2008) from bacteria. Given the complexity of the bacteria, in situ study of intact bacterial cells in their native environments are more attractive (Lee, 2004).

Traditional studies of intact bacteria cells are largely based on bulk assays and susceptibility testing assays, using techniques such as SPR (Chiang et al., 2009; Medina et al., 1997; Subramanian et al., 2006) and disk-diffusion (Jorgensen and Ferraro, 2009). The data generated with these bulk assays are averaged over many bacteria, which wash out important variability or heterogeneity of different bacterial cells. Various imaging techniques, such as fluorescence (Cywes-Bentley et al., 2013; Sochacki et al., 2011), AFM (Fantner et al., 2010; Lower et al., 2011) and transmission electron microscopy (TEM) (Cywes-Bentley et al., 2013), and non-imaging microfluidics techniques, such as flow cytometry (Tracy et al., 2010) and micro-electrophoresis (Van der Mei and Busscher, 2012), have been used to study bacterial surfaces. These techniques have contributed to the understanding of bacteria, but each has disadvantages. For example, the fluorescence method requires labeling, which limits its application to only certain probe molecules and cultivable strains, and gram negative bacteria with sugars cannot be easily labeled by

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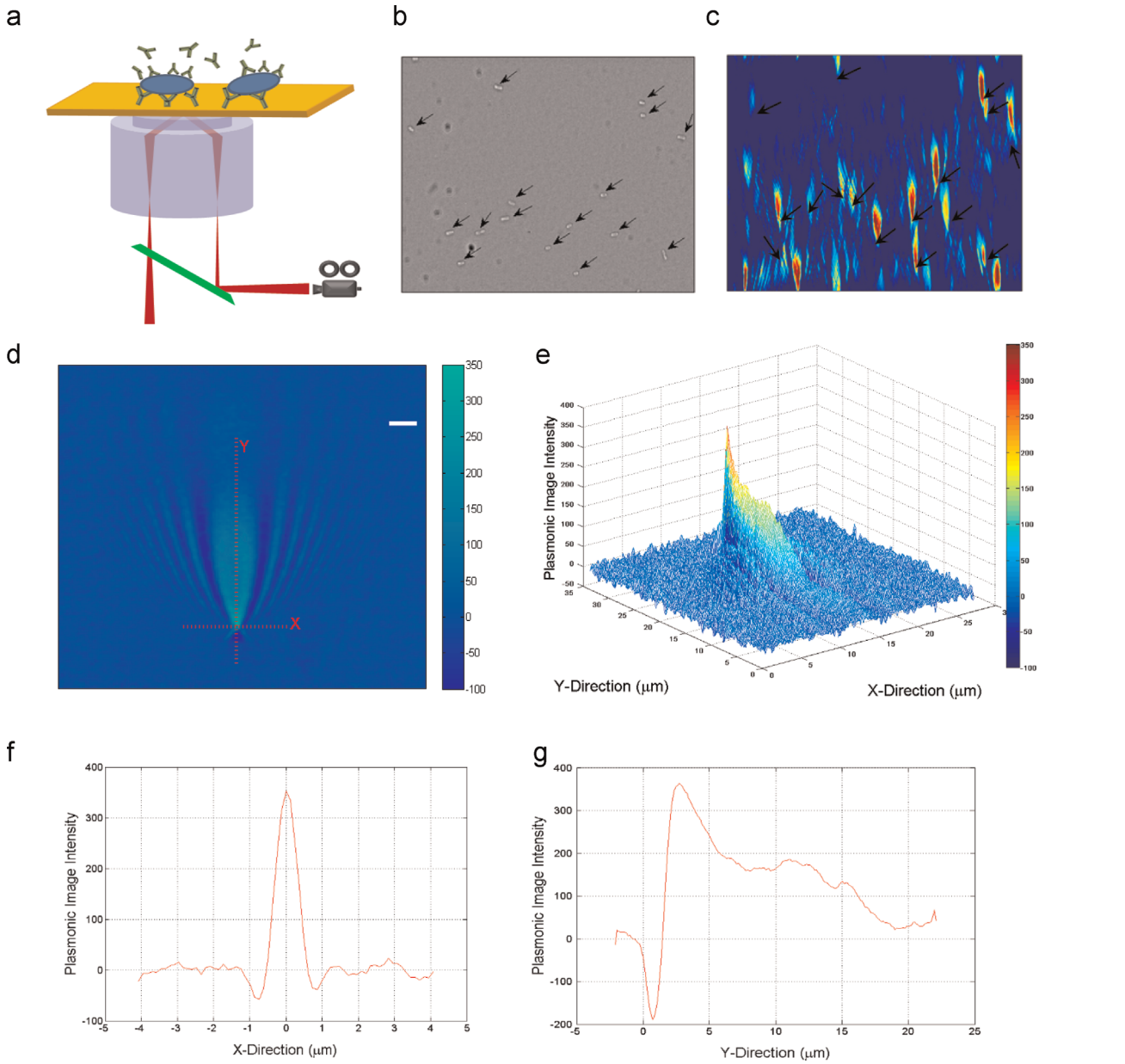

Fig. 1. (a) Schematic of the plasmonic imaging setup using a high numerical objective and the immobilization of bacterial cells on top of gold chip by covalently attached antibodies. (b) Bright-field optical image of immobilized bacteria. (c) Plasmonic image of bacteria shown as V-shaped diffraction patterns at positions of bacteria on bright field image. (d) Magnified plasmonic image of a single bacterium showing clearly the V-shape diffraction pattern. (e) 3D histogram of the bacteria in (d). (f) Profile of the V-shaped pattern along the basin of V in Fig. 4d. (g) Profile of the V-shaped pattern along the middle axis in Fig. 4d. Scale bar: 2 μm.

engineering cells (Chang and Bertozzi, 2012). In addition, the fluorescence method is an end-point assay, which is not suitable for quantifying the kinetics of molecular binding to bacteria. TEM requires extensive sample preparations and is unsuitable for live cell analysis in aqueous solutions. AFM can operate in aqueous solutions, but it is usually too slow to follow fast binding of ligands with bacteria, and the scanning AFM probe may perturb the binding process. In this study, we present a plasmonic imaging technique (Huang et al., 2007; Wang et al., 2010, 2012) (Fig. 1a) to study and quantify the interactions of a single *Escherichia coli O157:H7* cell with an antibody, and perform statistical analysis of the bacterial heterogeneity.

E. coli O157:H7 is a highly virulent food borne pathogen that causes diseases, such as diarrhea, hemorrhagic colitis and hemolytic uremic syndrome (Besser et al., 1999). Many groups have tried detecting this pathogen by several culture assays as well biosensing approaches. Several groups have used conventional SPR to detect *E. coli O157:H7* by direct detection of bacterial cells binding to surface (Tawil et al., 2012; Torun et al., 2012) , indirect detection of surface immobilized cells using complementary probes (Medina et al., 1997; Subramanian et al., 2006) or coupling SPR with other techniques (Zordan et al., 2009). In this study, we focus on the binding kinetics of goat *anti-E. coli O157:H7* IgG polyclonal antibody (Ab157) (Medina et al., 1997; Subramanian et al., 2006) onto single *E. coli O157:H7* cells. Commercial humanized antibodies are increasingly used as an alternate therapy for immune clearance of pathogens (Casadevall et al., 2004; Cywes-Bentley et al., 2013); hence the study of antibody binding kinetics with single bacterial cells is important to elucidate their efficacy and potential as future drugs.