

Dynamics of the Mineral–Microbe Interface: Use of Biological Force Microscopy in Biogeochemistry and Geomicrobiology

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At the most fundamental level, inter- and intramolecular forces delineate the interface between a microorganism and a mineral surface. A new technique, termed biological force microscopy (BFM), is described that can be used to directly probe the dynamics of the mineral–microbe interface. BFM quantifies attractive and repulsive forces in the nano-Newton range between living microbial cells and mineral surfaces in aqueous solution. Native bacterial cells are linked to a force-sensor that is used in a force microscope to measure bacteria–mineral interactions as a function of the distance between the mineral surface and the cells on the sensor. The magnitudes and ranges of the measured forces reflect the chemical and structural intricacies of the mineral–microbe interface. BFM is presented with potential applications to studies assessing the role that microbes or biomolecules play in geochemical and mineralogical processes.

Keywords adhesion, atomic force microscopy, bacteria, force, geomicrobiology, interface, intermolecular, intramolecular, microorganism, mineral surface, nanotechnology

Mineral–microbe interactions have been occurring for at least 3 billion years on and within the earth. Thousands of mineral species with enormous variability in surface chemistry and structure may interact with any of millions of bacterial species that display diverse surface mosaics and physiologies. Minerals and microorganisms are intimately linked such that one often cannot exist without the other in nature. Microbial processes play roles in the cycling of elements and sorption of metals (Stotzky 1986; Ehrlich 1990; Marshall 1996; Fein, Daughney, Yee, and Davis 1997; Langley and Beveridge 1999), the dissolution of minerals (Lovley and Phillips 1986; Robert and Berthelin 1986; Lovley and Phillips 1987; Myers and Nealson 1988; Krumbein, Urzi, and Gehrman 1991; Welch and Ullman 1993; Welch and Vandevivere 1994; Hersman, Lloyd, and Sposito 1995; Hersman, Maurice, and Sposito 1996; Roden and Zachara 1996; Banfield and Hamers 1997; Grantham, Dove,

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and DiChristina 1997; Stone 1997; Barker, Welch, Chu, and Banfield 1998; Edwards, Goebel, Rodgers, et al. 1998; Edwards, Schrenk, Hamers, et al. 1998; Forsythe, Maurice, and Hersman 1998; Schrenk, Edwards, Goodman, Hamers, and Banfield 1998), and mineral crystallization (Pentecost and Bauld 1988; Beveridge and Doyle 1989; Schultze-Lam, Harauz, and Beveridge 1992; Schultze-Lam, Fortin, Davis, and Beveridge 1996; Fortin and Beveridge 1997; Fortin, Ferris, and Beveridge 1997; Warren and Ferris 1998). Conversely, mineralogical processes influence the distribution, activity, and diversity of microbes (Stotzky 1986; Fredrickson, McKinley, Nierzwicki-Bauer, et al. 1995; Bennett, Hiebert, and Choi 1996; Fletcher 1996a; Marshall 1996; Barker, Welch, and Banfield 1997; Rogers, Bennett, and Choi 1998; Schrenk, Edwards, Goodman, Hamers, and Banfield 1998), the expression of genes (Arredondo, Garcia, and Jerez 1994; Fletcher 1996a; Dziurla, Achouak, Lam, Heulin, and Berthelin 1998; Gehrke, Telegdi, Dominigue, and Sand 1998), community structure and development (Lawrence, Korber, Hoyle, Costerton, and Caldwell 1991; Wolfaardt, Lawrence, Roberts, Caldwell, and Caldwell 1994; Thorseth, Torsvik, Furnes, and Muehlenbachs 1995; Brown, Sherriff, and Sawicki 1997; Kennedy and Gewin 1997), and transfer of genetic material (Holben 1997; Trevors and van Elsas 1997).

The thread linking these unimaginably complex interactions is the fact that mineral-microbe processes are dependent on the intimate juxtaposition of a living and nonliving entity, that is, the *interface* between a microbial cell and a mineral surface. A fundamental appreciation of this interface is dependent on our understanding and characterization of the symphony of inter- and intramolecular forces between microbes and mineral surfaces in nature. Despite the vast amount of work on microbial affects on mineralogical processes and vice versa, the interface remains largely unexplored, primarily because it is difficult to directly probe this minute and dynamic space.

Atomic force microscopy—and variations thereof—is an elegant tool for measuring inter- and intramolecular forces between organic and inorganic surfaces (see Figure 1). For review, see Butt, Jaschke, and Ducker 1995; Cappella and Dietler 1999; Lower and Maurice, in preparation. Recently, we created biological force microscopy (BFM) as a method to *directly* probe interfacial and adhesion forces between bacteria and mineral surfaces in aqueous solution (Lower, Tadanier, and Hochella 2000). Living cells are linked to a sensor that is used to quantitatively measure attractive and repulsive forces in the nano-Newton range between bacteria and mineral surfaces at distances between 0 μm (i.e., contact) and 2 μm . Measured forces reflected the complex interactions of structural and chemical functionalities on the bacteria and mineral surfaces. Here, we demonstrate and discuss this technique and suggest potential applications of BFM to studies assessing the role that microbes or biomolecules play in geochemical and mineralogical processes.

Materials and Methods

Mineral and Bacteria

Freshly cleaved muscovite ($\text{KAl}_2(\text{AlSi}_3\text{O}_{10})(\text{OH})_2$) and a gram-negative soil bacteria were used for all experiments. The bacteria strain was obtained from a chemostat inoculated with an iron-oxide rich soil from Pandapas Pond, Jefferson National Forest, Virginia (Tadanier, Little, Berry, and Hochella, in press). Through comparison of 500 bp of 16S rRNA sequence, this bacterium has been aligned at the species level (0.67% difference) with the pseudomonad *Burkholderia cepacia* (MIDI Labs, Newark, DE). *Burkholderia* sp. were cultured on agar plates under oligotrophic (glucose concentration = 0.6 mM) or eutrophic conditions (glucose concentration = 2.8 mM) and used in BFM experiments as described next (see Tadanier et al., in press, for complete description of growth media).

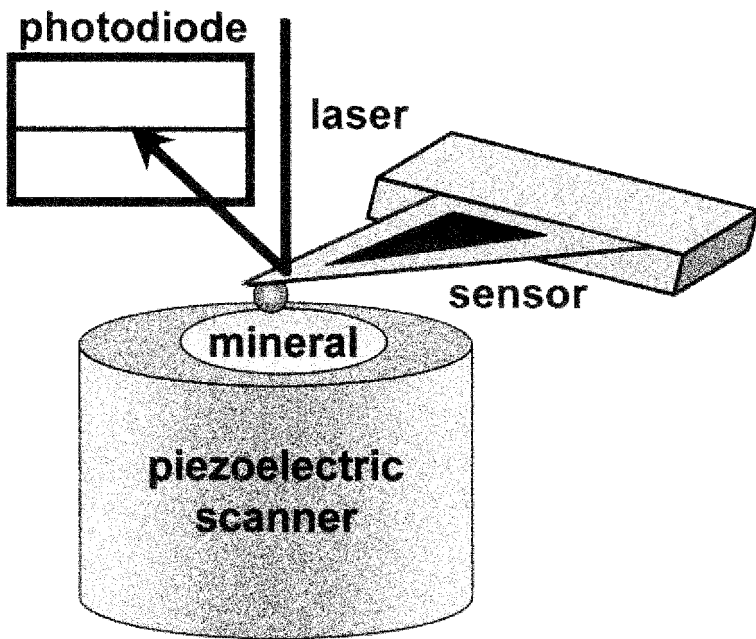


FIGURE 1 Schematic diagram showing the key components of a force microscope. The mineral and force sensor are within a fluid cell (not shown) containing aqueous solution. Force measurements are made by recording the deflection of a sensor (i.e., cantilever) in response to attractive or repulsive forces between itself and the sample (a mineral in this case). The sample, mounted on a piezoelectric scanner, indexes toward, makes contact with, and retracts from the sensor. Deflection of the sensor is detected by reflecting a laser off the top of the sensor and into a split segment photodiode. In this study, bacteria (shown as a sphere) have been linked to the sensor thereby creating a biologically active force probe (BAFP) used to measure interfacial and adhesive forces between bacteria and mineral surfaces, in situ.

Force Sensor Preparation and Characterization

Biologically active force probes (BAFPs) can be created by linking bacteria either directly to a silicon or silicon nitride force sensor (i.e., cantilever), or indirectly by linking a monolayer of bacteria to a small glass bead that is then fixed to the end of a cantilever. Direct linkage of bacteria to the cantilever was accomplished by first placing a cantilever in a 1–5% solution of the polycationic molecule, poly-D-lysine (135 to 150 kDa; pH near neutral). Bacteria were linked to the polylysine functionalized cantilever by lowering it into a colony of live bacteria with the aid of a microscope (Nikon, 200x magnification) and a micromanipulator to translate the cantilever. For the indirect-linkage method, small glass beads (Polysciences or Duke Scientific, radii 3–7 μm , cleaned with a solution of 1% hydrofluoric acid or 10% sodium hydroxide) were activated with a 1–5% solution of polylysine. The activated beads were placed in a suspension of *Burkholderia* sp. and centrifuged at $8000 \times g$ for 5 min. A single bacteria-coated bead was attached to a cantilever using a small quantity of epoxy resin, which has previously been found to be inert in aqueous solutions (Pincet 1995; Yoon, Flinn, and Rabinovich 1997). This attachment procedure was conducted in solution using a micromanipulator.

Prior to BFM measurements, scanning laser confocal microscopy (Zeiss LSM 510) was used to characterize the three dimensional nature of a BAFP. A probe was placed in the fluid cell used for force measurements and imaged with a 100x, 1.4 N.A. objective.

This imaging procedure was facilitated by transforming bacteria with a plasmid (pGLO, Bio-Rad or pSMC2, provided by G. A. O'Toole, Dartmouth University) that encoded an intracellular green fluorescent protein. The bacteria fluoresced when excited by light at 458 nm or 488 nm. Fluorescence emitted by the epoxy resin revealed that it was confined to the region between the cantilever and glass bead (i.e., resin was not in a position that would alter the interaction between the bacteria and mineral during BFM experiments).

Cantilever spring constants (N m^{-1}), essential for measuring force magnitudes with a force microscope, can vary substantially from the nominal value listed by the manufacturer (Cleveland, Manne, Bocek, and Hansma 1993; Senden and Ducker 1994). Spring constants were determined by attaching known masses to the end of a cantilever and recording the change in cantilever resonant frequency (Cleveland et al. 1993). A linear relationship was observed between added mass and resonant frequency with the slope being the spring constant (0.17 N m^{-1}). These measurements were accomplished using 10 cantilevers from the same wafer as that used to create BAFPs. The variability of spring constants from cantilever to cantilever within the same wafer has been determined to be very small (Senden and Ducker 1994). This was confirmed by the linear fit of our data and the reproducibility of the resonant frequency of unloaded cantilevers ($13.7 \text{ kHz} \pm 0.2$).

Force Measurements

BFM measurements were performed using a NanoScope IIIa Multimode SPM (Digital Instruments, Santa Barbara, CA) in aqueous solution (pH 6, ionic strength 10^{-5} M , 25°C). A piezoelectric scanner was used to translate the mineral toward and away from bacteria on a BAFP at rates of 0.1 to $3 \mu\text{m sec}^{-1}$. For comparison, this is within the range of velocities of motile bacteria (Marshall 1976). Interfacial forces were measured as the mineral approached the bacteria on the probe; whereas adhesion forces were measured upon contact and subsequent retraction of the mineral from the bacteria. Mineral samples were driven to the same contact force to normalize the effect that loading can have on adhesion forces during retraction (Weisenhorn, Maivald, Butt, and Hansma 1992).

Force measurement data are collected as cantilever deflection (diode voltage) and corresponding piezo displacement, typically termed a force curve (Figure 2A). This data must be manipulated to produce the familiar force–distance curve, which describes interfacial and adhesion forces (Figure 2B). Distance (i.e., separation between bacteria and mineral) is calculated by correcting the recorded piezo position (i.e., displacement) by the measured deflection of the cantilever. For example, if the mineral attached to the piezo scanner moves 10 nm toward bacteria attached to the cantilever, and the bacteria are repelled 2 nm due to repulsive forces, then the actual mineral–bacteria distance (or separation) changes by only 8 nm . The distance axis origin is chosen as the point on the force curve where sensor deflection becomes a linear function of piezo displacement (the sensor is in contact with the sample).

Force (F) is determined using Hooke's Law, $F = k_{sp}d$, where d is cantilever deflection (meters) and k_{sp} (N m^{-1}) is the cantilever spring constant. In order to use Hooke's Law, cantilever deflection measured by the photodiode in volts must be converted to meters. A diode/displacement conversion factor (also called "optical lever sensitivity") is defined from the slope of the force curve region where the cantilever is in contact with the sample on the piezo (Figure 2Aiii, called the "region of constant compliance"). The reciprocal of this conversion factor (in nm V^{-1}) can be used to convert measured cantilever deflection in volts to meters. A zero-force reference value is determined as the force curve region where sensor deflection is independent of piezo displacement (Figure 2Ai, the sensor and sample are not interacting because they are far apart).

It is important to note that using the constant compliance region of the force curve to convert photodiode response into force will overestimate the force of interaction if the bacteria are more compliant than the cantilever. Recent measurements of the elasticity

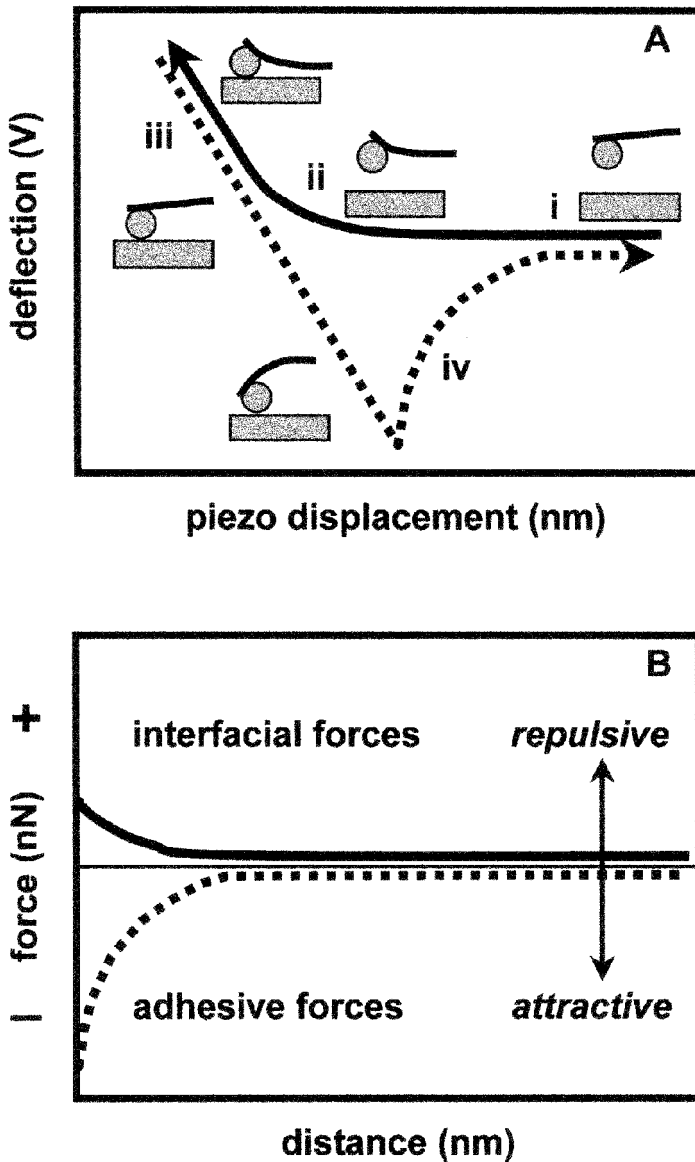


FIGURE 2 Schematic diagrams of a force curve (A) and force-distance curve (B). When the sensor and sample are far apart (i) they exhibit no interaction (region of zero force). As the sample approaches the sensor, intermolecular forces between the bacteria and mineral cause the sensor to deflect upward (ii) due to repulsive forces shown here. Eventually the probe makes contact with the sample (iii) and their movement becomes coupled (region of constant compliance). The sample is then retracted from the probe (iv) until the sensor and sample return to their original positions thereby completing one cycle (an entire cycle requires nano- to milliseconds). Hysteresis, shown here, may occur upon retraction due to adhesion forces. See text for discussion on converting cantilever deflection and piezo displacement into force and distance, respectively. Interfacial forces are measured on approach and adhesion forces are measured upon retraction; repulsive forces are positive and attractive forces are negative.

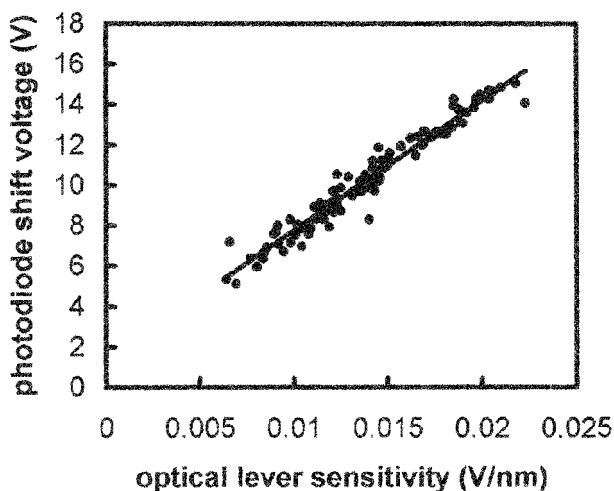


FIGURE 3 Variation of photodiode shift voltage as a function of optical lever sensitivity using a J-type piezoelectric scanner and eight silicon nitride cantilevers ($200\ \mu\text{m}$ in length). All measurements were performed on muscovite and hematite in a fluid cell containing aqueous solutions ranging in ionic strength from 0 (i.e., Milli-Q water) to 10^{-2}M . The slope of the regression line is $642\ \text{nm}$ and the intercept is $1.3\ \text{V}$ ($R^2 = 0.95$). With this correlation, the optical lever sensitivity can be determined in a matter of seconds by simply using the photodiode shift voltage. Furthermore, this method is valid regardless of whether the cantilever is the most compliant component of the system.

of bacterial surface macromolecules suggest that bacteria are less compliant (i.e., stiffer) than cantilevers having spring constants smaller than $10\ \text{N m}^{-1}$ (Xu, Mulhern, Blackford, Jericho, Firtel, and Beveridge, 1996; Yao, Jericho, Pink, and Beveridge 1999).

In instances where bacteria (or biomolecules) linked to the cantilever are more compliant than the cantilever or for cells with fragile appendages, other methods must be used to accurately convert the measured deflection of the cantilever (in volts) into a force (in Newtons) of interaction (D'Costa and Hoh 1995; Sader, Chon, and Mulvaney 1999). For example, because the optical lever sensitivity is strongly dependent on the shape of the laser spot on the photodiode detector, the "photodiode shift voltage" can be used to convert volts of cantilever deflection into meters of deflection (D'Costa and Hoh 1995). Photodiode shift voltage is measured as the difference in output voltage when the photodiode detector is shifted approximately $318\ \mu\text{m}$ (one full turn of the positioning screw) on either side of the zero setting. Figure 3 illustrates the strong correlation between photodiode shift voltages and optical lever sensitivities measured by directing the laser to different positions on the cantilever. Once this correlation is established for a given instrument, piezoelectric scanner, fluid cell, and cantilever (e.g., $200\text{-}\mu\text{m}$ long, V-shaped, silicon nitride cantilevers), the optical lever sensitivity can be accurately determined without pressing the cantilever against any other surface. This method ensures that forces can be determined regardless of the compliance of the cantilever relative to any microorganism attached to it, and also ensures the preservation of fragile macromolecules on microorganisms attached to the cantilever.

Results and Discussion

Characterization of Biologically Active Force Probes

BAFPs were created by either attaching a single bacterium to a force sensor (Figure 4A) or by attaching a single bacteria-coated-bead (Figure 4B) to a force sensor. Fluorescent

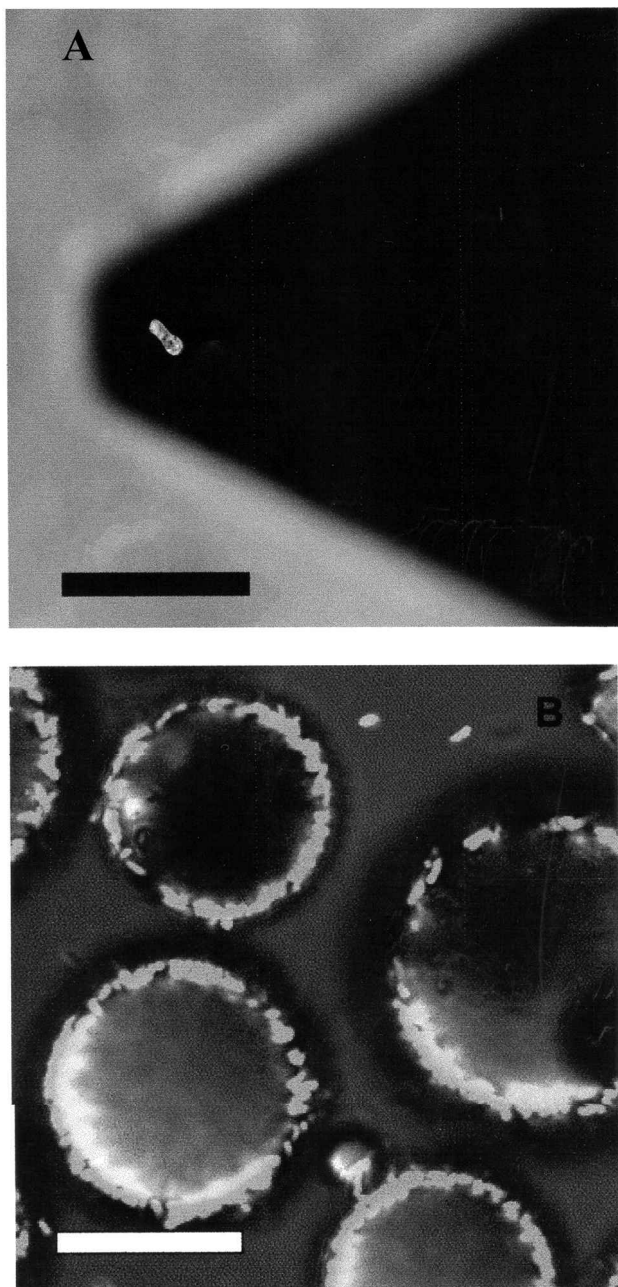


FIGURE 4 (A) Scanning laser micrograph of a BAFP created by directly linking a single cell of *Burkholderia* sp. to a force-sensing cantilever. (B) Confocal micrograph focused on the midplane of bacteria-coated beads, revealing a layer of cells. Focusing up and down on the beads reveal that many are evenly coated with cells. Beads showing the most uniform coverage are attached to cantilevers, thereby creating BAFPs. These cells have been transformed with a plasmid encoding a green fluorescent protein and are emitting “natural” fluorescence. This fluorescent protein, being intracellular, together with the confocal abilities of the laser scanning microscope allow noninvasive characterization of the orientation and distribution of cells on the force sensor, in situ, without affecting the surface chemistry of the microbes. Plating experiments in which BAFPs were used as the inoculum revealed that the bacteria on the sensors are viable and presumably active during force measurements. Scale bars = 10 μm .

dyes (ViaGram, Molecular Probes, Eugene, OR) and plating techniques revealed that cell membranes on these bacteria are intact and the cells are viable. Both the direct and indirect method of attaching bacteria to a cantilever preserves the orientation and structural integrity of macromolecules on the cell surface. This is important because the native conformation of macromolecules on a bacteria surface is critical to their function, activity, and role in nature. By using whole bacteria expressing macromolecules in their natural state rather than individual biomolecules (e.g., exopolysaccharides, proteins) purified from bacterial surfaces, we avoid situations in which the linkage procedure modifies the conformation of biomolecules such that they are no longer in a natural state (Stotzky 1986; Ellen and Burne 1996; Turner Peek, Wertz, Archibald, Geer, and Gaber 1996; Ingersoll and Bright 1997; Turner, Testoff, Conrad, and Gaber 1997).

As both gram-negative and gram-positive bacteria are typically negatively charged at most pH, polylysine with its high pK_a is a more-or-less universal linker molecule. However, due to the large diversity in bacterial surfaces, situations will likely arise in which other linker molecules are necessary. The versatility of our linkage protocol allows for modification on a case-by-case basis. For example, we have also fabricated BAFPs using aminopropyltriethoxysilane and polyethyleneimine with species of *Shewanella* and *Pseudomonas*. Additionally, methods used to attach proteins or antibodies to small glass spheres or sensors (e.g., Florin, Moy, and Gaub 1994; Frey and Corn 1996; Turner et al. 1997; Caruso, Caruso, and Mohwald 1998; Rezanian, Johnson, Lefkow, and Healy 1999) could be applied to our protocol. For example, lysozyme, which has been genetically altered such that it possesses an active binding site but an inactive catalytic site (Voet and Voet 1995), is an attractive choice because of its high affinity for bacterial cell walls. Antibodies specific for cell surface receptors may also be effective linkers.

A critical component to consider when designing BAFPs is the relative difference between those forces binding the cells to the sensor, and interfacial and adhesive forces that will be probed by the cells on the sensor. Forces holding bacteria to the sensor must be greater than the forces that are actually probed, otherwise the bacteria will be stripped from the cantilever during force measurements. Electrostatic attachment of bacteria to sensors via polylysine is very strong. Approximations using Coulomb's law and molecular dynamic calculations show that the energy associated with amine-silanol (i.e., the polylysine glass bead linkage or polylysine cantilever linkage) and amine-carboxylic pairs (i.e., the polylysine bacteria linkage) (Voet and Voet 1995; West, Latour, and Hench 1997; Pagac, Prieve, and Tilton 1998; Pagac, Tilton, and Prieve 1998) are at least one to four orders of magnitude stronger than potential intermolecular forces between a bacteria and mineral (Israelachvili 1992). Organosilane linkages such as aminopropyltriethoxysilane form covalent bonds with silanol groups (Plueddemann 1991) and should therefore be even stronger than polylysine linkages.

Comparison of the direct and indirect linkage protocols suggests that the latter is more desirable. Direct linkage of a single cell to a cantilever (Figure 4A) is very difficult. Techniques such as optical tweezers (Svoboda and Block 1994) or nanotweezers (Kim and Lieber 1999) could significantly enhance the linkage of a bacterium to a cantilever, but such techniques are not at present easily applicable to such procedures. Another practical reason for selecting the indirect protocol is the need to use cantilevers with appropriate spring constants in order to probe the entire range of potential forces between microbes and minerals. Cantilevers of different composition (e.g., glass, silicon, silicon nitride, and gold-coated cantilevers) are often used to vary the spring constant. Gold-coated and uncoated silicon nitride cantilevers, for example, have very different surface properties, and silicon nitride itself can vary from Si_3N_4 to $Si_{15}N_4$ (Weisenhorn, Maivald, Butt, and Hansma 1992).

These compositional differences would require use of more than one linker molecule to directly attach one particular strain of bacteria to different cantilevers. Conversely, indirect linkage allows the use of one protocol to attach bacteria to glass (or latex) beads of uniform composition, which can then be attached to cantilevers of different compositions.

Forces of Interaction Between Bacteria and Mineral Surfaces in situ

The interactions between muscovite and polylysine (Figure 5A) and muscovite and the soil *Burkholderia* sp. (Figures 5B and 5C) were studied with BFM at pH 6, ionic strength 10^{-5} M, 25°C. As expected, the positively charged polylysine exhibited a strong attraction towards the (001) surface of muscovite, which is negatively charged (Figure 5A). Hysteresis was not observed for the interaction between muscovite and polylysine. In contrast, the *Burkholderia* sp. exhibited a repulsive interaction with muscovite beginning at ~80 nm (Figure 5B, approach). However, once contact was made, this repulsive force was overcome, and the bacteria exhibited an attractive adhesion toward the mineral (Figure 5B, retraction).

The repulsive interfacial forces between the *Burkholderia* sp. and muscovite illustrated in Figure 5B (approach) are consistent with the negative surface charges of both the bacteria and mineral at circum-neutral pH. The distance at which repulsion occurs is slightly smaller than that expected based solely on electrostatic interactions (Debye length thickness is ~100 nm at this ionic strength). Other forces associated with hydrophobic, steric, and entropic effects likely play a key role in bacteria–mineral interactions due to the relatively long polymers on bacterial surfaces (Israelachvili and McGuiggan 1988; Israelachvili 1992).

The adhesive behavior between *Burkholderia* sp. and muscovite is drawn out for hundreds of nanometers (Figure 5B, retraction). This is likely due to stretching and fibrillation of biomolecules (e.g., lipopolysaccharides or flagella) as a result of attractive forces such as hydrogen bonds between polymers on the bacteria and surface hydroxyls or structured water molecules on muscovite (Jucker, Harms, Hug, and Zehnder 1997).

When the same *Burkholderia* sp. was grown in nutrient poor media it exhibited very strong affinity for muscovite (Figure 5C). Interfacial repulsion was not detected (Figure 5C, approach) and attractive adhesion forces were very large and long range (Figure 5C, retraction). This phenomena is consistent with the observation that many bacterial species alter their surface properties under oligotrophic versus eutropic environments (e.g., Bengtsson 1991). In fact, many bacteria show greater affinity for mineral surfaces under oligotrophic rather than eutropic conditions (Fletcher 1996b; Marshall 1996). This cycling between attached and planktonic states is believed to be a strategy bacteria use to proliferate in nature where many environments have eutropic/oligotrophic sequences.

Potential Applications of Biological Force Microscopy to Geomicrobiology Studies

BFM could provide a unique perspective into several aspects of geomicrobiology (e.g., mineral dissolution, crystal growth, and biofilm formation), particularly if used to complement other techniques. Next is a list of relevant questions considered as possible applications of BFM to the field of geomicrobiology and their answers.

1. Do bacteria “recognize” particular minerals or crystallographic orientations? Probe different minerals or different faces on the same mineral with one BAFFP.

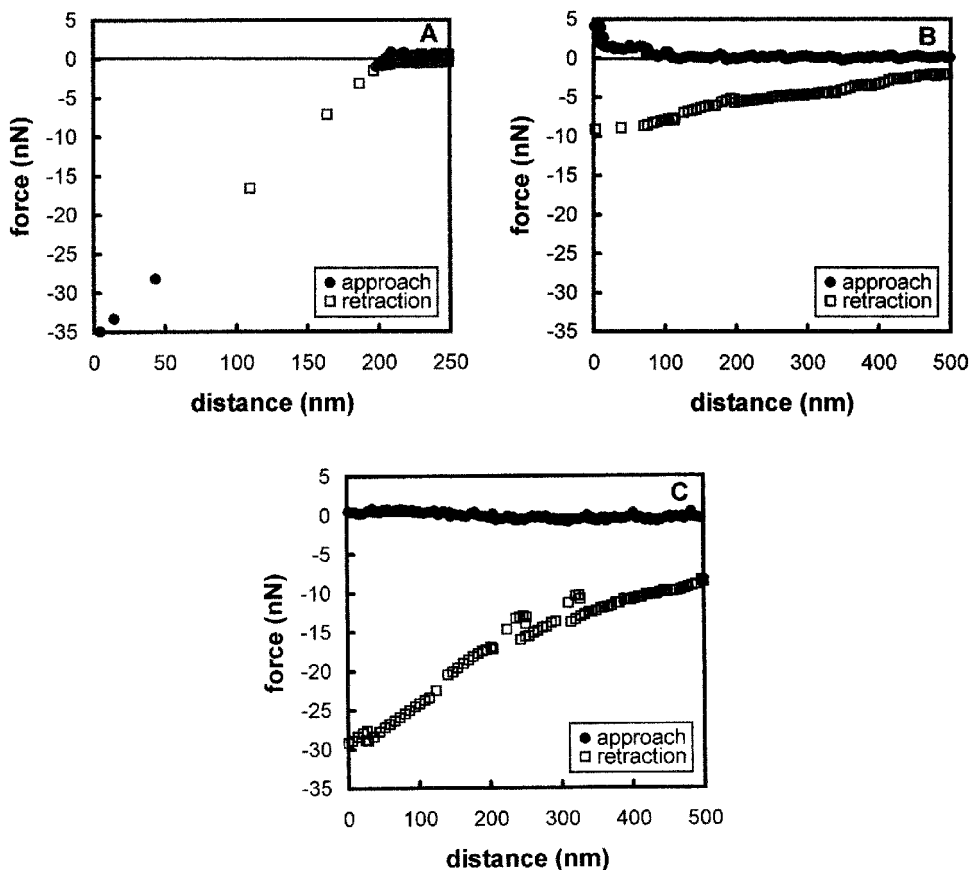


FIGURE 5 Biological force microscopy (BFM) force-distance curves between the (001) surface of muscovite, and a polylysine functionalized cantilever (A), versus *Burkholderia* sp. grown in a eutropic environment (B), versus *Burkholderia* sp. grown in an oligotrophic environment (C). Solution conditions were pH 6, ionic strength 10^{-5} M, 25°C . The force of interaction is plotted as a function of the distance between the two surfaces (i.e., separation between polylysine or bacteria on the sensor and the mineral surface). Note difference in x-axis scale. Curves begin on the right and proceed toward the left as the mineral is brought toward the sensor (solid circles, approach curve). The sensor makes contact with the mineral surface and subsequently withdraws from the muscovite (open squares, retraction curve) to complete one cycle.

2. Do bacteria show an increased affinity for particular minerals under oligotrophic or anaerobic conditions? Change solution conditions within fluid cell while collecting BFM measurements.
3. How does inter- and intraspecies communication affect biofilm development? Use a BAFP to measure interactions with various species of bacteria that are immobilized on a substrate.
4. What intermolecular forces (e.g., van der Waals, electrostatics, hydration, hydrophobic, entropic) are involved in bacterial attachment and detachment processes? Compare forces measured with BFM to those calculated from theoretical models (e.g., DLVO theory).
5. What cell surface macromolecules mediate bacterial attachment to mineral surfaces? Are conditioning films required for attachment? Create BAFFPs using various mutants that

- differ in surface macromolecules and use these in BFM on minerals with and without sorbed organic molecules.
6. How do bacteria alter their cell surface in response to changing environmental conditions? Do mineral surfaces induce genetic expression of particular macromolecules on a cell surface? If so, how much time is necessary for production of a protein or extracellular polysaccharide? What is the distribution of a particular macromolecule on the cell surface? Combine BFM measurements with biomolecule sensitive fluorophores, scanning laser confocal microscopy, and/or scanning near-field optical microscopy.
 7. Is direct bacteria–mineral contact necessary for oxidative or reductive dissolution of sulfides, iron oxides, or manganese oxides? Use BFM to measure bacterial affinity for various minerals under aerobic versus anaerobic conditions. Combine BFM with surface sensitive spectroscopies.
 8. How does the conformation of a protein change when electrons are shuttled to/from a mineral surface (e.g., in the case of *Shewanella*–iron oxide interactions)? Combine BFM measurements with fluorescence resonance energy transfer, scanning laser confocal microscopy, and/or proteins labeled with fluorescent tags.
 9. Do organic acids exhibit an increased affinity for particular mineral faces? How strong are the bonds between a siderophore or organic ligand and mineral surface? A force sensor could be functionalized with specific biomolecules and used to probe different faces on the same mineral.

Tools such as BFM offer biochemists, biophysicists, geochemists, environmental engineers, microbiologists, and mineralogists a unique insight into the fundamental intricacies of the ubiquitous interfaces between microbes and minerals.

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