Proteins are widely regarded as insulators, despite reports of electrical conductivity. Here we use measurements of single proteins between electrodes, in their natural aqueous environment, to show that the factor controlling measured conductance is the nature of the electrical contact to the protein, and that specific ligands make highly selective electrical contacts. Using six proteins that lack known electrochemical activity, and measuring in a potential region where no ion current flows, we find characteristic peaks in the distributions of measured single-molecule conductances. These peaks depend on the contact chemistry, and hence, on the path through the protein. In consequence, the measured conductance distribution is sensitive to changes in this path caused by ligand binding, as shown with streptavidin–biotin complexes. Measured conductances are on the order of nanosiemens over distances of many nanometers, orders of magnitude more than could be accounted for by electron tunneling. The current is dominated by contact resistance, so the conductance for a given path is independent of the distance between electrodes, as long as the contact points on the protein can span the gap between electrodes. While there is no currently known biological role for high electronic conductance, its dependence on specific contacts has important technological implications, because no current is observed at all without at least one strongly bonded contact, so direct electrical detection is a highly selective and label-free single-molecule detection method. We demonstrate single-molecule, highly specific, label- and background-free electronic detection of IgG antibodies to HIV and Ebola viruses.

Proteins lack electronic conduction bands because the interactions that facilitate hopping are weak compared with the vibronic coupling (1) and also because they are not highly ordered (but see Vattay et al. (2)). Nonetheless, long-range electron transport can occur when energetic carriers are injected at potentials that exceed the redox potentials of amino acid residues in the protein. The use of chromophores to allow optical injection of carriers at well-defined energies has enabled a detailed elaboration of charge-transfer pathways in many cases (3, 4). When proteins are contacted by metal electrodes, the situation is more complicated (5, 6). Single-protein conductances of nanosiemens (nS) over nanometer (nm) distances have been reported (7), with essentially the same conductance measured across a 2-nm protein (8) as across a 5.4-nm protein (9); temperature-independent transport has been observed (10) and siemens per meter conductivities over micrometers have been reported in bacterial pili (11, 12). Carrier injection via a contact is extremely sensitive to surface charge at the interface, with different preparations of oxide barrier at a semiconductor interface being the determining factor in whether electron transport in bacteriorhodopsin is temperature dependent or not (13). Accordingly, a reproducible method for forming electrical contacts is highly desirable. In addition, these prior studies are subject to uncertainties about the number of molecules contacted, the size of the gap, the nature of the contacts, and possible ionic contributions to current. Here, we report single-molecule measurements made using ligand-functionalized electrodes in solution under potential control, with electrode potentials set such that no significant ionic current flows. We find that the binding of a ligand specific to a particular protein forms an excellent electrical contact: The conditions for specific binding are also the conditions for charge injection. The specificity of ligands on electrode surfaces also serves to indicate that proteins are still functionally selective on these electrode surfaces.

Single-Molecule Conductance Measurements

Reproducible two-point measurements of the conductance of molecules require reproducible contacts (14), so the reproducible observation of large (nS-scale) conductance fluctuations in single integrin molecules (bound to just one of two electrodes by their cognate ligands) was a surprising finding (15). This prior work did not probe the low bias region (where fluctuations were absent) owing to leakage currents that obscured any dc current through the protein. In the present study, we used a scanning tunneling microscope (STM) to make single-molecule measurements in solution (Fig. L4), systematically exploring the role of contacts, both specific and nonspecific (Fig. 1B). With suitably insulated STM probes (16) and potential control of the electrodes (Methods and SI Appendix, Fig. S1), the background leakage current was reduced to less than 1 pA over the entire bias range. With adequate stabilization, the STM gap remained constant over periods of a minute (Methods and SI Appendix, Fig. S2) so we were able to disable the gap control servo, retract the tip, and record current–voltage (IV) curves. Up to 60 such curves (sweeping both up and down) were recorded before reengaging the servo and repeating...
the process on another area of the substrate. To make two specific contacts, we have used bivalent antibodies (an IgE and two IgGs), each of which presents two binding sites, as well as streptavidin which binds up to four biotin molecules, so that epitope- or biotin-functionalyzed electrodes could be bridged by specific bonds. In the cases where bare metal electrodes were used, contacts were made to surface thiol on streptavidin modified with an average of 2.5 surface thiols per molecule. In all cases. We originally observed these fluctuations for a protein captured in a fixed-junction chip (15) and although the present work uses an STM, we have replicated measurements of TN for one of the proteins studied here (anti-DNP) in a chip as well as in the STM to show that these are not some artifact of the measurement method. Examples of this TN are given in SI Appendix, Fig. S7. The voltage threshold for TN does not depend on gap until the contact is almost broken (see Fig. 3E and SI Appendix, Figs. S5 and S6), implying that it is associated with fluctuations of the contacts driven by a potential drop that occurs mostly at the contacts, as previously proposed (15) and discussed in more detail below.

With the exception of the TN, the response is linear, so that each IV trace can be characterized by a single conductance value. G. Measured distributions of G are shown in Fig. 2, and they follow the log-normal distribution usually observed in single-molecule measurements (18). The distributions are similar to distributions of current values obtained by recording current vs. time at a fixed gap and bias (SI Appendix, Fig. S4 C and D) so we ascribe the distribution to different kinds of contact between the electrodes and the molecule. The distributions for integrin (gap = 4.5 nm) and thio-streptavidin (gap = 2.5 nm) have a single peak at about 0.3 nS (Fig. 24). Bare metal electrodes were used to capture the thiolated streptavidin, where the thiol-mediated binding site (S-S) is observed (15) for integrin is also observed here for anti-DNP (Fig. 1 C and D), and all of the other proteins studied (SI Appendix, Fig. S3) above 0.1 V. It is a ubiquitous signal of protein capture, showing the same two-level switching in all cases. We originally observed these fluctuations for a protein captured in a fixed-junction chip (15) and although the present work uses an STM, we have replicated measurements of TN for one of the proteins studied here (anti-DNP) in a chip as well as in the STM to show that these are not some artifact of the measurement method. Examples of this TN are given in SI Appendix, Fig. S7. The voltage threshold for TN does not depend on gap until the contact is almost broken (see Fig. 3E and SI Appendix, Figs. S5 and S6), implying that it is associated with fluctuations of the contacts driven by a potential drop that occurs mostly at the contacts, as previously proposed (15) and discussed in more detail below.

Measured Conductances Depend on Contacts

Currents were only recorded when the protein was bound specifically to at least one of the two electrodes and a representative IV curve for antidinitrophenol (anti-DNP) binding DNP-coated electrodes is shown in Fig. 1C (examples are given in SI Appendix, Fig. S3 for the other proteins). The trace shown here is for a tip retraction of 2 nm for an overall gap of ∼4.5 nm, given that, at the 20-picosiemens (pS) set-point (4 pA at 0.2 V), the gap is ∼2.5 nm (17). Typically, no current was recorded for several seconds after retraction, after which the current jumped to a large (and variable) value in the presence of bound protein. Examples of current vs. time recordings at a constant 50-mV bias are shown in SI Appendix, Fig. S4 A and B. Although the current fluctuates over minute timescales, it is usually stable over a few seconds, so that 80% of the recorded curves on the sweep up (black trace) are reproduced on the sweep down (red trace). Controls (buffer alone or noncognate proteins in solution) gave no signals. The rapidly fluctuating (millisecond-timescales) telegraph noise (TN) reported (15) for integrin is also observed here for anti-DNP (Fig. 1 C and D), and all of the other proteins studied (SI Appendix, Fig. S3) above 0.1 V. It is a ubiquitous signal of protein capture, showing the same two-level switching in all cases. We originally observed these fluctuations for a protein captured in a fixed-junction chip (15) and although the present work uses an STM, we have replicated measurements of TN for one of the proteins studied here (anti-DNP) in a chip as well as in the STM to show that these are not some artifact of the measurement method. Examples of this TN are given in SI Appendix, Fig. S7. The voltage threshold for TN does not depend on gap until the contact is almost broken (see Fig. 3E and SI Appendix, Figs. S5 and S6), implying that it is associated with fluctuations of the contacts driven by a potential drop that occurs mostly at the contacts, as previously proposed (15) and discussed in more detail below.

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Conductances Do Not Depend on Gap Size

The existence of an internal [through-molecule (20)] high-conductance path is illustrated by a series of measurements taken at different gap sizes, using the technique described above, but increasing the amount of the initial tip retraction (Fig. 3). Strikingly, the peak conductance values do not change with the gap size (Fig. 3E and SI Appendix, Table S2) although the frequency with which data are accumulated falls (SI Appendix, Table S1). This effect reflects the area of the probe available for contacts at a given height, as illustrated in Fig. 3 (Left). Very few sites are available when the gap is comparable to the protein height (listed for similar structures found in the protein database in Table 1). Gap-independent conductance has been reported before for azurin [see the SI of Ruiz et al. (21)] and a rod-like molecule trapped between a probe and a substrate (20). As pointed out above, the contact point changes over the (~minute) course of a measurement, a reflection of the angstrom-scale change in the position of the STM probe. It is these various contact geometries that generate the overall shape of the conductance distributions (SI Appendix, Fig. S4). Since the distributions retain the same peak positions and shapes at the different gap sizes, the data show no indications of proteins being “squeezed” at the smaller gap sizes.

Fig. 3E also plots the voltage thresholds for the turn-on of TN as a function of gap size. They also do not change significantly with the gap size. Thus, TN fluctuations must be driven by the local field at the metal–molecule interface, with relatively little potential dropped across the interior of the protein. This is also consistent with our finding that the lifetime of the TN is exponentially related to the peak current value, an observation that...
can be accounted for by a single “weak-link” tunneling junction in the circuit that dominates the conductance (15).

Conductance Is Sensitive to Changes of Protein Structure

Since the conductance path follows the protein geometry, either internally or along a surface contour, changes in protein geometry, and thus in the conduction path, could affect which contact points control the conductance. This would enable direct electrical sensing of structural changes of the protein. We demonstrate this effect in Fig. 4A and B. Fig. 4A shows the conductance distribution (replicating the data in Fig. 2A) for a thiolated-streptavidin sample that was subsequently incubated with biotin (1 mM for 1 h), a process that alters the structure of the streptavidin tetramer substantially (22). Biotin complexation changes the conductance distribution significantly (Fig. 4B). A streptavidin molecule has four biotin binding sites (23), so that the unthiolated apo-protein can be cross-linked by two biotins (S-S in Fig. 1 B, vi). We synthesized a thiolated biotin (Methods) and functionalized both the probe and substrate with it (Fig. 4C), subsequently flowing apo-streptavidin into the sample cell. The consequent G distribution had three peaks, with the highest at nearly 7 nS. Thus, the measured conductance is sensitive to both local changes in protein structure and the chemical nature of the contact, showing how contacts are affected by structural changes of the protein. We also attached thiolated streptavidin to the substrate and probed it with a biotinylated probe with similar results (Fig. 4D) demonstrating that a single biotin-mediated contact is sufficient to generate the high-conductance state at ~7 nS.

Possible Mechanisms

Electron tunneling decays far too rapidly to account for long-range transport. A tunneling conductance can be estimated from $G \sim G_0 \exp(-\beta x)$ where $G_0$ is 77 $\mu$S and $\beta \sim 1$ Å$^{-1}$ (24). For a small protein with $x \sim 4$ nm, this yields $G < 10^{-21}$ S, 12 orders of magnitude smaller than observed. To account for the observation of nS conductance over 10-nm distances would require a $\beta < 0.1$ Å$^{-1}$. In the well-studied case of DNA, thermally activated hopping (25) leads to nearly distance-independent transport when the distance between the readily ionized guanines exceeds three nucleotides (26). Similar transport (via readily oxidized amino acids) has been observed in peptides (4). In these cases, transport was limited by charge injection, and the $\sim 1.5$-eV barrier to charge injection was overcome using a chromophore excited with 630-nm (~2-eV) light. If a similar transport mechanism operates in the case of charge injection from electrodes, the barrier would be determined by the energy gap between the Fermi energy of Pd (work function 5.2 eV) and the absolute redox potential of the readily oxidized residues tyrosine and tryptophan. These potentials are $\sim +1$ to $+1.2$ V vs. the normal hydrogen electrode (NHE) (27, 28) so using 4.4 eV for the work function of the NHE (29) gives absolute potentials around 5.4–5.6 eV below the vacuum or a barrier of $+0.2–0.4$ eV with respect to the Fermi energy of Pd. Thus, a barrier of this magnitude must be overcome by the bond polarization associated with the binding of the protein to the electrode. This is well within the range of work-function changes observed for small molecules attached to a noble-metal surface by a thiol linkage (30). We obtain significant currents in three situations: (i) when contacts...
of some special geometry (32) or arrangement of hydrogen bonds (33).

We turn finally to the fluctuations that set in above ±100-mV applied bias. The small dependence of this threshold voltage on gap size (Fig. 3E and SI Appendix, Fig. S6) is consistent with the hypothesis that the internal conductance of the proteins is much higher than the conductance at the contacts, implying that these signals arise from voltage-driven fluctuations of the contacts themselves. We proposed such a mechanism in our earlier study of integrin (15), where we showed that the lifetime of the “on” states, $\tau$, was related to the peak current, $I_p$, of the telegraph noise peaks via $\tau \approx e^{V_0 / kT}$, a relationship that can be explained by means of a single barrier determining both current and bonding strength. SI Appendix, Fig. S5 shows that, once turned on, the current grows linearly with voltage, indicating that an Ohmic conductance channel opens. The turn-on process is described by an exponential of the form $\exp \left( \frac{V - V_c}{kT/\tau} \right)$, where $V_c$ is an activation voltage. Fits yield $V_c \approx 0.25$ V, a value characteristic of hydrogen bond strengths in water (34) suggesting that a hydrogen bond may be the weak link in the circuit.

It is interesting to note that this 0.25-V barrier is similar to the charge-injection barrier deduced from the redox potentials of the amino acids, as discussed above. If the charge-injection rate was limited by thermally activated hopping over a 0.22-0.47-V barrier, and it is this rate that determines the conductance, then we would expect to observe a conductance of $\approx G_0 \exp \left( -\frac{V}{kT/\tau} \right)$, where $0.22 < V < 0.47$ V, yielding from 12 nS to 0.5 pS, a range which encompasses the values reported here.

**Role of Specific Binding in Electronic Conductance**

We conclude that specific ligand–receptor interactions form good electrical connections to proteins. This is illustrated by the data shown in Fig. 4 B–D. Connections made via covalent (thiol) modification of surface lysines directly bonded to the metal electrodes yield a lower maximum conductance (0.56 nS) than the noncovalent streptavidin–biotin coupling linked to the electrodes via a thiol-terminated ethane linkage (6.8 nS). This conductance is barely altered when only one biotinylated linker is used (Fig. 4D). Thus, a weaker coupling to the hydrophilic interior of a protein is more effective than a stronger coupling to the hydrophilic exterior, even if only one such coupling is made. If, once injected, electrons move readily in the interior of the protein, then a second (nonspecific) contact will act only as a barrier at the hydrophilic surface of the protein. Such a mechanism would account for the high conductance of integrin when bound by a ligand at only one site, and also for the complete lack of conductance when both contacts are noncovalent and nonspecific.

**Future Applications**

The fact that specific ligands make excellent electrical connections clearly has technological implications. The requirement of at least one specific bond for conduction means that there is no background signal at all in the presence of proteins that do not bind the electrode-tethered capture probes or react directly with electrodes, in contrast to fluorescent tags for which background signal is always present. Thus, direct, label-free, sensitive, and very selective (background-free) single-molecule detection may be possible. Another application may lie in dynamic recording of conductance changes. Electrostatic sensing has been used to record enzyme motions on sub-ms timescales (35), and the sensitivity of conductance to structural changes in a protein raises the possibility of direct electrical sensing of these motions, possibly enabling real-time recording of enzyme motions.

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**Fig. 4.** Conductance changes on ligand binding and also with the chemistry of the contact. Distribution for thiolated-streptavidin (A) and for the same sample after incubation with biotin (B). The single conductance peak changes to two peaks with significantly different values. Streptavidin captured by biotin probes on the electrodes (C) has an additional feature of much higher conductance. These features are retained with one thiol bond and one biotin bond (D). Biotin size is exaggerated for clarity.
overnight. Substrate functionalization with small ligands was characterized by Fourier transform infrared spectroscopy (SI Appendix, Fig. 59) and ellipsometry. Coverage of the substrate was monitored by STM and atomic force microscopy photoactivation of DNA (SiPhoCheck, Figs. 510 and 511).

STM probes were etched from a 0.25-mm Pd wire (California Fine Wires) by an ac electrochemical method. To avoid current leakage, probes were insulated with high-density polyethylene following the method described previously for gold probes (16). Each probe was tested by STM in 1 mM PB buffer insulated with high-density polyethylene following the method described in SI Appendix. Binding affinities of all of the three antibodies were measured by surface plasmon resonance. Thiolated streptavidin with an average of 2.5 thiols per tetramer was from ProteinMods. Ag/AgCl reference electrodes salt-bridged by 3 M KCl or 10 mM KCl were prepared as described previously (15). Full details of the cyclic voltammetry are provided in SI Appendix. The anti-Ebola antibody and the corresponding monomeric Fab fragment were prepared and purified as described in SI Appendix.

**Sources of Materials.** RGD peptide was purchased from Peptides International. Peptide ligands for the anti-HIV antibody and the anti-Ebola antibody were synthesized by CPC Scientific with a purity >95%. DNP and biotin disulfides were synthesized in our laboratory (SI Appendix, Figs. 512 and 513) and reduced for 2 h before use by an immobilized (Tris[2-carboxyethyl]phosphine hydrochloride) disulfide reducing gel from Thermo Scientific and DNP antibody (mouse monoclonal IgE antibody), wild-type streptavidin, and Fab fragment were prepared and purified as described in SI Appendix.

**Methods**

Functionalizing Substrates and STM Probes. Palladium substrates for STM measurement were prepared by evaporating a 200-nm palladium film onto a silicon wafer using an electron-beam evaporator (Lesker PVD 75), with a 10-nm titanium adhesion layer. The substrates were treated with a hydrogen flame immediately before functionalizing and then immersed in solutions of thiolated DNP, biotin, streptavidin, or peptides containing a cysteine residue, overnight. Substrate functionalization with small ligands was characterized by Fourier transform infrared spectroscopy (SI Appendix, Fig. 59) and ellipsometry. Coverage of the substrate was monitored by STM and atomic force microscopy photoactivation of DNA (SiPhoCheck, Figs. 510 and 511).

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