

# Unfolding Individual Als5p Adhesion Proteins on Live Cells

David Alsteens,<sup>†,‡</sup> Vincent Dupres,<sup>†,‡</sup> Stephen A. Klotz,<sup>\*</sup> Nand K. Gaur,<sup>§</sup> Peter N. Lipke,<sup>||</sup> and Yves F. Dufrêne<sup>†,\*</sup>

<sup>†</sup>Unité de Chimie des Interfaces, Université Catholique de Louvain, Croix du Sud 2/18, B-1348 Louvain-la-Neuve, Belgium, <sup>‡</sup>University of Arizona Health Sciences Center, Tucson, Arizona, <sup>§</sup>Southern Arizona Veterans Administration Health Care System, Tucson, Arizona, and <sup>||</sup>Brooklyn College, Brooklyn, New York. <sup>‡</sup>These authors contributed equally to this work.

Cell adhesion mediated by specific cell surface molecules plays a pivotal role in life sciences.<sup>1–7</sup> Neuronal interactions,<sup>1</sup> cellular communication,<sup>2</sup> tissue development,<sup>3</sup> inflammation,<sup>4</sup> cancer,<sup>5</sup> and microbial infection<sup>6,7</sup> are just a few examples of cellular processes regulated by cell adhesion molecules. Microbial infection is generally initiated by the specific adhesion of the pathogens to host tissues.<sup>6,7</sup> A prominent example is found in *Candida albicans*, the most common agent causing opportunistic infections in immunocompromised patients. Adhesion of *C. albicans* yeast cells to host tissues is a key factor in the maintenance of commensal and pathogenic states, which is mediated by a family of cell surface proteins known as the agglutinin-like sequence (Als) proteins.<sup>6,8–11</sup> Of the eight different Als proteins, Als5p is one of the most extensively characterized.<sup>8</sup> Als proteins possess four functional regions (Figure 1a), that is, an N-terminal immunoglobulin (Ig)-like region, which initiates cell adhesion, followed by a threonine-rich region (T), a tandem repeat (TR) region that participates in cell–cell aggregation, and a stalk region projecting the molecule away from the cell surface. Microscopic assays using *Saccharomyces cerevisiae* surface display models have revealed that Als-mediated adherence involves two steps: initial adhesion *via* the specific binding of the Ig region to peptide ligands, followed by cell–cell aggregation mediated by the TR regions.<sup>10,12–14</sup> Despite the vast amount of data available on the molecular biology of Als proteins, the molecular details underlying their strength and mechanics have not been elucidated yet.

**ABSTRACT** Elucidating the molecular mechanisms behind the strength and mechanics of cell adhesion proteins is of central importance in cell biology and offers exciting avenues for the identification of potential drug targets. Here we use single-molecule force spectroscopy to investigate the adhesive and mechanical properties of the widely expressed Als5p cell adhesion protein from the opportunistic pathogen *Candida albicans*. We show that the forces required to unfold individual tandem repeats of the protein are in the 150–250 pN range, both on isolated molecules and on live cells. We also find that the unfolding probability increases with the number of tandem repeats and correlates with the level of cell adherence. We suggest that the modular and flexible nature of Als5p conveys both strength and toughness to the protein, making it ideally suited for cell adhesion. The single-molecule measurements presented here open new avenues for understanding the mechanical properties of adhesion molecules from mammalian and microbial cells and may help us to elucidate their potential implications in diseases such as inflammation, cancer, and infection.

**KEYWORDS:** AFM · *Candida albicans* · cell adhesion proteins · pathogens · protein unfolding · single-molecule force spectroscopy

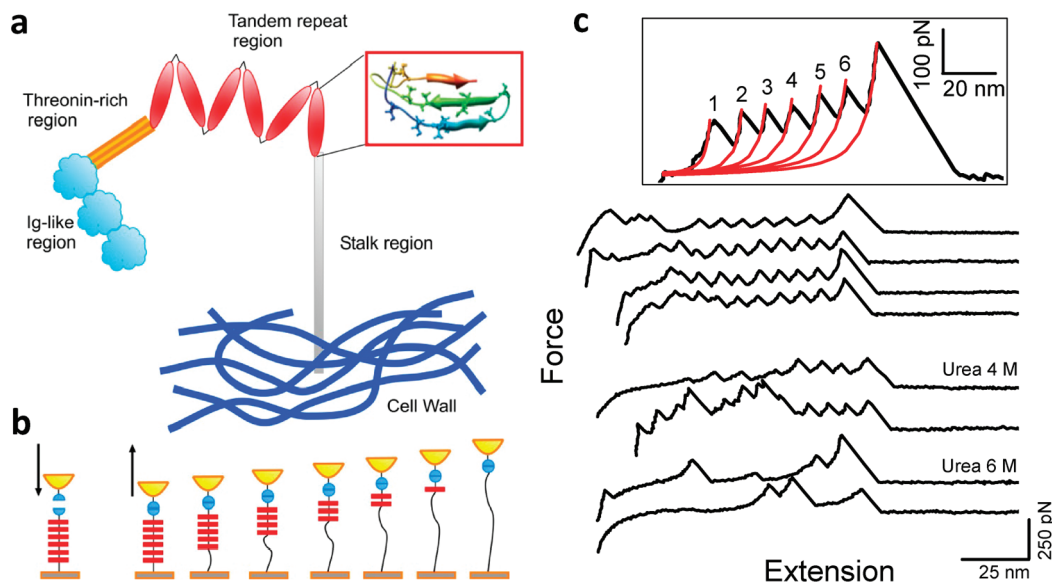
With its ability to observe and manipulate single molecules at work, atomic force microscopy (AFM)<sup>15–18</sup> has provided a wealth of novel opportunities in life sciences. Notably, progress in single-molecule force spectroscopy (SMFS) has allowed researchers to measure the specific binding strength of adhesion molecules<sup>19,20</sup> and to elucidate the folding/unfolding mechanisms of water-soluble or membrane proteins.<sup>21–24</sup> However, studying the molecular elasticity of proteins on live cells (*i.e.*, in their fully functional state) remains a challenging task. Here, we demonstrate the ability of SMFS to study the adhesive and mechanical properties of Als5p proteins, both on isolated molecules and on cell surfaces. Single Als5p proteins are localized on the surface of living yeast cells, and their individual TR domains unraveled. The unfolding probability is found to correlate with cell adherence, suggesting that protein mechanics may play an important role in fungal adhesion.

\*Address correspondence to yves.dufrene@uclouvain.be.

Received for review January 27, 2009 and accepted June 10, 2009.

Published online June 17, 2009.  
10.1021/nn900078p CCC: \$40.75

© 2009 American Chemical Society



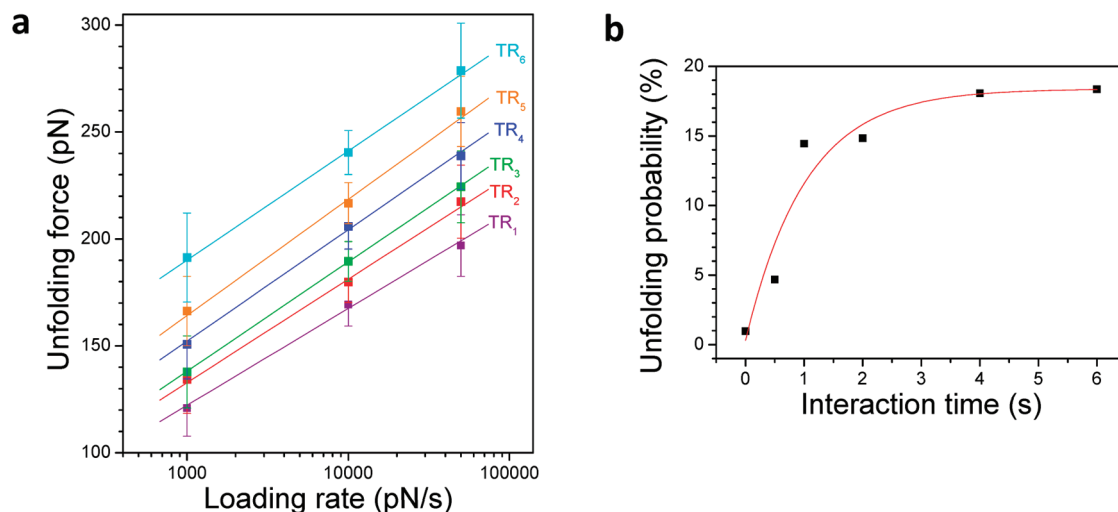
**Figure 1.** Unfolding isolated Als5p proteins. (a) Representation of an Als molecule projecting outward from the *C. albicans* cell wall by means of the stalk region. The tandem repeat (TR) region comprises multiple glycosylated 36 amino acid repeats that are arranged in antiparallel  $\beta$ -sheets. As shown in the inset, modeling with ROSETTA and LINUS consistently predicted independently folded three-stranded antiparallel  $\beta$ -sheet domains for each repeat. Thus, the TR region consists of six such repeats, which would unfold independently. The amyloidogenic threonine-rich region (T) is the most conserved sequence in Als proteins and connects to the ligand-binding Ig-like region which possesses three equal-sized  $\beta$ -sheet-rich domains. (b) Principle of the SMFS experiments. Ig-T-TR<sub>6</sub> fragments were attached on a gold surface and stretched *via* their Ig domains using an Ig-T-tip. (c) Force-extension curves obtained by stretching single Ig-T-TR<sub>6</sub> showed periodic features reflecting the sequential unfolding of the TR domains (upper traces). Force peaks were well-described by the worm-like-chain model (inset; red line), using a persistence length of 0.4 nm:  $F(x) = k_B T/l_p [0.25(1 - x/L_c)^{-2} + x/L_c - 0.25]$ , where  $L_c$  and  $l_p$  are the contour length and persistence length of the molecule,  $k_B$  is the Boltzmann constant, and  $T$  is the absolute temperature. Addition of urea dramatically altered the unfolding peaks due to hydrogen bond disruption (lower traces). All curves were recorded using a loading rate of 10 000 pN/s and an interaction time of 500 ms. Similar data were obtained using more than 10 different tips and four independent samples.

## RESULTS AND DISCUSSION

We first investigated the mechanical unfolding of isolated Als5p proteins. To this end, soluble Ig-T-TR<sub>6</sub> fragments terminated with a His-tag were attached at low density on gold surfaces modified with nitrilotriacetate groups (Figure 1b). Ig-T-TR<sub>6</sub> proteins were picked up and stretched by their terminal Ig domain using AFM tips modified with Ig-T fragments (Figure 1b). Force extension curves for Ig-T-TR<sub>6</sub> proteins showed a sawtooth pattern with well-defined force peaks (Figure 1c). These periodic features represent the characteristic fingerprint of modular proteins,<sup>21,22</sup> in which each peak corresponds to the force-induced unfolding of an individual domain. In unfolding experiments, proteins are generally picked up anywhere along their length using bare tips, meaning the number of unfolding peaks per curve varies. Here, the use of an Als5p Ig-T-tip allowed us to reproducibly observe sawtooth patterns with seven well-defined force peaks. It is interesting to note that force peaks were never (or hardly) seen using a bare tip, indicating that the specific Ig-Ig interaction is required in order to unfold the protein domains.

The first six peaks were equally spaced and showed ascending forces (Figure 1c), ranging from  $160 \pm 10$  to  $240 \pm 11$  pN (mean  $\pm$  sem;  $n = 100$  curves), that are consistent with the sequential unfolding of the six TR

repeats of Als5p. The seventh peak showed a much higher force,  $350 \pm 25$  pN, and larger spacing. Although the origin of this last peak is not fully understood, it may be attributed to the stretching of Ig domains, leading eventually to the rupture of the Ig-Ig complex. We note that most sawtooth patterns were preceded by a few poorly defined peaks, varying widely in magnitude and spacing. We suggest these events reflect the unfolding of the T regions for two reasons. First, molecular modeling has revealed that these regions may fold into an ensemble of structures of different sizes. Second, T regions are known to partly unfold under native (unstressed) conditions to allow amyloid formation.<sup>25</sup> In agreement with molecular modeling and CD studies revealing that Ig, T, and TR regions are antiparallel  $\beta$ -sheet-rich structures, our measured forces are in the range of unfolding forces reported for  $\beta$ -fold domains, such as Ig and fibronectin domains in titin<sup>21</sup> and tenascin,<sup>22</sup> and larger than the forces needed to unfold  $\alpha$ -helical domains.<sup>26</sup> In the muscle protein titin, it is well-known that the mechanical properties of the Ig domains are essential for the biological function of the protein. Similarly, the mechanical response of Als5p may be function-related. We suggest that the modular and flexible nature of Als5p conveys both strength and toughness to this cell adhesion molecule.<sup>27</sup>

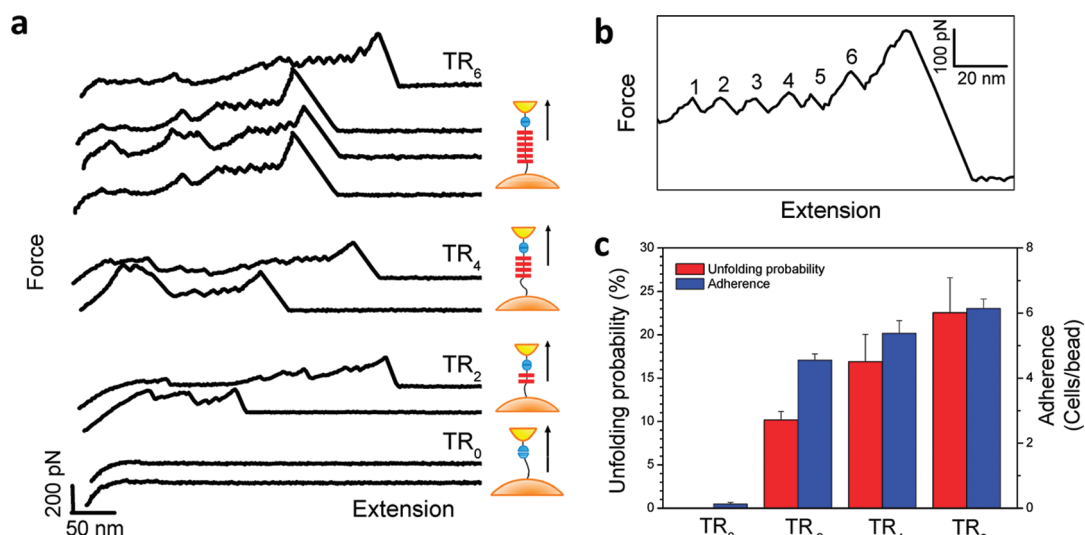


**Figure 2.** Unfolding forces depend on pulling speed and interaction time. (a) Unfolding forces of individual TR domains as a function of the loading rate (constant interaction time of 500 ms). The unfolding force  $F$  is related to the loading rate ( $k_c v$ ) by  $F = (k_B T) / (x_u) \ln((k_c v x_u) / (k_B T k_u^0))$ , where  $k_c$  is the spring constant,  $v$  is the pulling speed,  $x_u$  is the width of the potential barrier, and  $k_u^0$  is the mechanical unfolding rate constant under zero force. Thus,  $x_u$  can be determined from the slope of this plot and  $k_u^0$  from the intercept. Data represent the mean  $\pm$  sem ( $n = 50$ ). (b) Dependence of the unfolding probability on the interaction time (constant loading rate of 10 000 pN/s). The data can be fitted with an exponential function (red line).

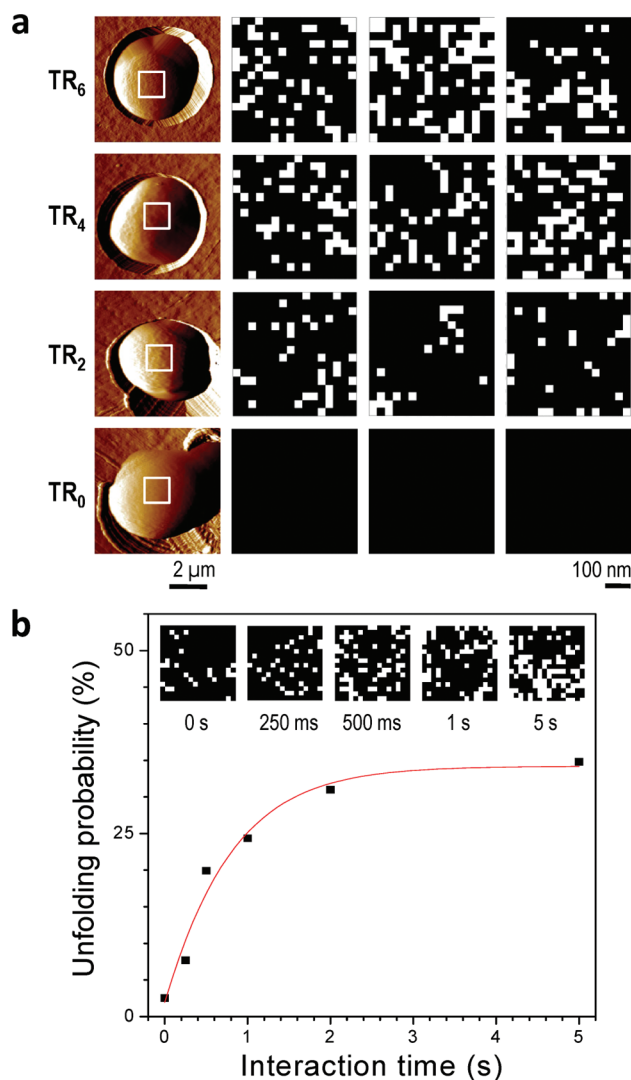
The force-extension curves obtained by stretching Ig-T-TR<sub>6</sub> proteins were well-described by the worm-like chain model (Figure 1c, inset), supporting further our interpretation of the sawtooth pattern. For peaks 1–6, the change in contour length between consecutive peaks was constant,  $\Delta L_c = 8.4 \pm 1.6$  nm ( $n = 100$ ). Assuming that each amino acid contributes 0.36 nm to the contour length of a fully extended polypeptide chain and that the folded length of a TR repeat is 4.3 nm, we found that the measured 8.4 nm increment corresponds to the expected 36 amino acids of a single TR repeat. We also observed that urea strongly alters the shape of the unfolding peaks (Figure 1c), confirming

that disruption of the protein hydrogen bonds leads to a loss of mechanical stability. This observation correlates with cellular behavior since Als5p-mediated adhesion is known to be reversibly inhibited by urea and formamide.<sup>12</sup>

We then explored the dependence of the unfolding forces on the loading rate, that is, the rate at which the load is applied to the molecules.<sup>21,22,28</sup> We found that the average unfolding forces of each TR domain increases linearly with the logarithm of the loading rate (Figure 2a). From these dynamic force spectroscopy data, the width of the unfolding potential ( $x_u$ ) and the unfolding rate constant ( $k_u$ ) were estimated and found



**Figure 3.** Unfolding Als5p proteins on live cells. (a) Force-extension curves recorded between an Ig-T-tip and the surface of *S. cerevisiae* cells expressing Als5p with six, four, two, and no TR repeats. All curves were recorded using a loading rate of 10 000 pN/s and an interaction time of 500 ms. Similar data were obtained using more than 10 different tips and 10 cells from independent cultures. (b) Higher magnification curve documenting the sequential unfolding of six TR domains. (c) Both the unfolding probability and the level of yeast adherence increased with the number of TR repeats, indicating there was a good agreement between single-molecule and microscale assays.



**Figure 4.** Mapping unfolding forces on cell surfaces. (a) Left column: AFM topographic images recorded in buffer with an Ig-T-tip showing single *S. cerevisiae* cells expressing different numbers of TR repeats. Cells were trapped into the pores of a porous polymer membrane. Columns 2–4: representative unfolding maps (256 force curves;  $n = 256$ ) recorded on  $500 \text{ nm} \times 500 \text{ nm}$  areas on top of the cells (loading rate of  $10\,000 \text{ pN/s}$ , interaction time of  $500 \text{ ms}$ ). Each bright pixel reflects the detection and unfolding of single Als5p proteins. The unfolding probability is clearly proportional to the number of TR repeats. (b) Probability of unfolding increased with interaction time (inset: unfolding maps obtained at increasing times).

to be  $x_u \approx 0.2 \text{ nm}$  and  $k_u \approx 6 \times 10^{-2} \text{ s}^{-1}$ . The width of the unfolding potential obtained here compares well with that reported for other proteins, usually in the range of  $0.17\text{--}0.25 \text{ nm}$ . Interestingly, we also found that the unfolding probability (number of curves showing sawtooth patterns) increases exponentially with interaction time to reach a plateau after  $4 \text{ s}$  (Figure 2b). This behavior is reminiscent of that of cadherins (*i.e.*, transmembrane glycoproteins known to play an important role in cell-to-cell adhesion), except that bond formation is much faster for cadherin.<sup>29</sup> Our results are consistent with the notion that Als5p-mediated adhesion is a time-dependent process.<sup>14</sup> They are also in line

with the slow kinetics of bonding of the Als homologue  $\alpha$ -agglutinin.<sup>30</sup>

Next, we could localize and stretch single Als5p proteins directly on live cells. Using an Ig-T-tip, force-extension curves were recorded across the surface of yeast cells expressing Als5p with different numbers of TR repeats (Figure 3a). The curves obtained on cells expressing six repeats (TR<sub>6</sub>) displayed sawtooth patterns similar to those found on isolated Ig-TR<sub>6</sub>; that is, they generally showed six force peaks varying from  $150$  to  $250 \text{ pN}$  and spaced by  $9.1 \pm 1.2 \text{ nm}$  ( $n = 25$ ), followed by a last peak of  $350\text{--}400 \text{ pN}$  magnitude (Figure 3b). Cells expressing a smaller number of repeats showed patterns with fewer force peaks (Figure 3a) and lower unfolding probability (Figure 3c), the unfolding frequency decreasing in the order  $\text{TR}_6 > \text{TR}_4 > \text{TR}_2 > \text{TR}_0$ . Further work is needed to understand the molecular origin of this behavior. One may argue that a lower unfolding frequency may simply reflect lower expression of the proteins. However, this interpretation is unlikely since the intensity of cell surface immunofluorescence with anti-Als antibody was shown to be similar for each of these proteins, thus implying similar cell surface concentrations.<sup>12</sup> Alternatively, lower unfolding frequencies may indicate that the shorter proteins are less accessible for surface interaction.

To demonstrate that these nanoscale measurements correlate with microscale cellular behavior, yeast adherence was measured using bead adherence assays (Figure 3c).<sup>12</sup> Fibronectin-coated beads and yeast cells expressing different numbers of TR repeats were agitated in buffer resulting in adhesion of single yeast cells to the beads, followed by cell-to-cell aggregation, eventually forming small microcolonies of aggregated cells. Figure 3c shows that the level of yeast adherence (number of cells per bead) was correlated with the unfolding probability, suggesting that the mechanical properties of the TR region are important for yeast adherence.

We also explored the distribution of unfolding forces over the cell surface (Figure 4a). For cells expressing Als5p with six TR repeats, unfolding patterns were detected in about 25% of the locations (bright pixels), revealing that the proteins were exposed at the surface. Although the distribution of unfolding events was generally homogeneous, in some localized regions, they seemed to be organized into nanoscale domains, possibly reflecting clustered Als molecules. Whether these clusters are indeed due to multiple proteins or to one protein interacting with the tip multiple times remains to be clarified. As shown before, the probability of unfolding decreased when decreasing the number of repeats (Figure 4a). In addition, we found that the unfolding probability increases with interaction time (Figure 4b), consistent with the notion that Als5p-mediated adhesion is a time-dependent process. Thus, we could localize single Als5p proteins on the surface of yeast cells,



showing they are surface-exposed, and we were able to unfold their individual TR domains, revealing that their mechanical properties may be essential for yeast adherence.

## CONCLUSIONS

Our experiments demonstrate that SMFS provides new insights into the mechanics of cell adhesion proteins, both on isolated molecules and on cell surfaces. Stretching of single Als5p proteins leads to the unfolding of their individual TR domains. Owing to its modu-

lar and flexible nature, Als5p is able to resist high mechanical force. We believe that this modular elongation mechanism conveys both strength and toughness to the protein, making it ideally suited to function as an adhesion molecule. The unfolding probability increases with the number of TR repeats and is correlated with yeast adherence, suggesting that this process is relying on the mechanical properties of Als5p proteins. This finding may have broad implications since modular TR structures are found in cell adhesion molecules from other pathogens.<sup>6,8</sup>

## METHODS

**Expression and Purification of Als5p.** Ig-T and Ig-T-TR<sub>6</sub> fragments were expressed and purified as described elsewhere.<sup>12</sup>

**Microorganisms, Cultures, and Aggregation Assays.** *S. cerevisiae* YPH499 harboring different plasmids (pGK114 (6TR), pDG100 (2TR), pDG101 (0TR), pDG102 (4TR)) were grown as previously described.<sup>17</sup> Briefly, two or three colonies from the SC-trp plate used as inoculum were transferred into YPRG medium (10 g/L yeast extract, 20 g/L peptone, 10 g/L raffinose, and 10 g/L galactose). Cells were agitated at 30 °C, grown up to the late logarithmic phase, and harvested by centrifugation. They were washed three times with sodium acetate buffer and resuspended in 10 mL buffer to a concentration of  $\sim 10^6$  cells/mL. The protocol that we used for bead adherence assays is similar to that described elsewhere.<sup>10,12</sup> Briefly, Als5p-expressing cells were mixed with fibronectin-coated magnetic beads at a cell-to-bead ratio of 100:1 in Tris-EDTA buffer, vortexed, and incubated at room temperature with gentle shaking for 30 to 45 min. Each tube was vortexed briefly and immediately placed into a magnetic separator (Dyna). Adherent and aggregated cells were gently washed three times with buffer, while the tube remained within the magnet. The cells were resuspended in buffer, and a sample was placed onto a microscope slide for examination.

**Atomic Force Microscopy.** AFM measurements were performed at room temperature (20 °C) in buffered solutions (sodium acetate; pH 4.75), using a Nanoscope IV Multimode AFM (Veeco Metrology Group, Santa Barbara, CA) and oxide-sharpened microfabricated Si<sub>3</sub>N<sub>4</sub> cantilevers (Microlevers, Veeco Metrology Group). Cells were immobilized by mechanical trapping into porous polycarbonate membranes (Millipore), with a pore size similar to the cell size. After filtering a concentrated cell suspension, the filter was gently rinsed with buffer, carefully cut (1 cm × 1 cm), attached to a steel sample puck (Veeco Metrology Group) using a small piece of double face adhesive tape, and the mounted sample was transferred into the AFM liquid cell while avoiding dewetting. The spring constants of the cantilevers were measured using the thermal noise method (Picoforce, Veeco Metrology Group), yielding values ranging from 0.01 to 0.025 N/m. Unless otherwise specified, all force measurements were recorded with a maximum applied force of 250 pN and a loading rate of 10 000 pN/s, calculated by multiplying the tip pulling velocity (nm/s) by the slope of the unfolding peaks (pN/nm). For interaction time experiments, delays of 500 ms, 1 s, 2 s, 4s, or 6 s were applied between tip approach and tip retraction, while keeping constant the maximum applied force.

To attach purified Ig-T and Ig-T-TR<sub>6</sub> fragments onto AFM tips and supports, AFM cantilevers and silicon wafers (Siltronix) were coated using electron beam thermal evaporation with a 5 nm thick chromium layer followed by a 30 nm thick gold layer. The gold-coated surfaces were cleaned for 15 min by UV and ozone treatment, rinsed with ethanol, dried with a gentle nitrogen flow, and immersed overnight in ethanol containing 0.05 mM of nitrilotriacetate-terminated (5%) and triethylene-glycol-terminated (95%) alkanethiols. After rinsing with ethanol, the tips and supports were immersed in a 40 mM aqueous solution of

NiSO<sub>4</sub> (pH 7.2) for 1 h, rinsed with water, incubated in acetate buffer containing 10 μg/mL Ig-T or Ig-T-TR<sub>6</sub> for 2 h, and finally rinsed with buffer.

**Acknowledgment.** This work was supported by the National Foundation for Scientific Research (FNRS), the Université Catholique de Louvain (Fonds Spéciaux de Recherche), the Région wallonne, the Federal Office for Scientific, Technical and Cultural Affairs (Interuniversity Poles of Attraction Programme), and the Research Department of the Communauté française de Belgique (Concerted Research Action). Y.F.D. and D.A. are Senior Research Associate and Research Fellow of the FRS-FNRS. N.K.G. was a recipient of the VA merit review grant. We thank Raymond Fung and Greg Soybelman for technical assistance. Work at Brooklyn College was supported by NIH Grants S06 GM076168 and SC1 GM083756.

## REFERENCES AND NOTES

1. Takeichi, M. The Cadherin Superfamily in Neuronal Connections and Interactions. *Nat. Rev. Neurosci.* **2007**, *8*, 11–20.
2. Dalva, M. B.; McClelland, A. C.; Kayser, M. S. Cell Adhesion Molecules: Signalling Functions at the Synapse. *Nat. Rev. Neurosci.* **2007**, *8*, 206–220.
3. Morgan, M. R.; Humphries, M. J.; Bass, M. D. Synergistic Control of Cell Adhesion by Integrins and Syndecans. *Nat. Rev. Mol. Cell Biol.* **2007**, *8*, 957–969.
4. Weber, C.; Fraemohs, L.; Dejana, E. The Role of Junctional Adhesion Molecules in Vascular Inflammation. *Nat. Rev. Immunol.* **2007**, *7*, 467–477.
5. Gray-Owen, S. D.; Blumberg, R. S. CEACAM1: Contact-Dependent Control of Immunity. *Nat. Rev. Immunol.* **2006**, *6*, 433–446.
6. Verstrepen, K. J.; Reynolds, T. B.; Fink, G. R. Origins of Variation in the Fungal Cell Surface. *Nat. Rev. Microbiol.* **2004**, *2*, 533–540.
7. Telford, J. L.; Barocchi, M. A.; Margarit, I.; Rappuoli, R.; Grandi, G. Pili in Gram-Positive Pathogens. *Nat. Rev. Microbiol.* **2006**, *4*, 509–519.
8. Dranginis, A. M.; Rauceo, J. M.; Coronado, J. E.; Lipke, P. N. A Biochemical Guide to Yeast Adhesins: Glycoproteins for Social and Antisocial Occasions. *Microbiol. Mol. Biol. Rev.* **2007**, *71*, 282–294.
9. Hoyer, L. L. The Als Gene Family of *Candida albicans*. *Trends Microbiol.* **2001**, *9*, 176–180.
10. Gaur, N. K.; Klotz, S. A. Expression, Cloning, and Characterization of a *Candida albicans* Gene, ALA1, that Confers Adherence Properties upon *Saccharomyces cerevisiae* for Extracellular Matrix Proteins. *Infect. Immun.* **1997**, *65*, 5289–5294.
11. Fu, Y.; Rieg, G.; Fonzi, W. A.; Belanger, P. H.; Edwards, J. E.; Filler, S. G. Expression of the *Candida albicans* Gene Als1 in *Saccharomyces cerevisiae* Induces Adherence to Endothelial and Epithelial Cells. *Infect. Immun.* **1998**, *66*, 1783–1786.

12. Rauceo, J. M.; De Armond, R.; Otoo, H.; Kahn, P. C.; Klotz, S. A.; Gaur, N. K.; Lipke, P. N. Threonine-Rich Repeats Increase Fibronectin Binding in the *Candida albicans* Adhesin Als5p. *Eukaryot. Cell* **2006**, *5*, 1664–1673.
13. Klotz, S. A.; Gaur, N. K.; Lake, D. F.; Chan, V.; Rauceo, J.; Lipke, P. N. Degenerate Peptide Recognition by *Candida albicans* Adhesins Als5p and Als1p. *Infect. Immun.* **2004**, *72*, 2029–2034.
14. Gaur, N. K.; Klotz, S. A.; Henderson, R. L. Overexpression of the *Candida albicans* ALA1 Gene in *Saccharomyces cerevisiae* Results in Aggregation Following Attachment of Yeast Cells to Extracellular Matrix Proteins, Adherence Properties Similar to those of *Candida albicans*. *Infect. Immun.* **1999**, *67*, 6040–6047.
15. Engel, A.; Müller, D. J. Observing Single Biomolecules at Work with the Atomic Force Microscope. *Nat. Struct. Biol.* **2000**, *7*, 715–718.
16. Hinterdorfer, P.; Dufrêne, Y. F. Detection and Localization of Single Molecular Recognition Events Using Atomic Force Microscopy. *Nat. Methods* **2006**, *3*, 347–355.
17. Helenius, J.; Heisenberg, C. P.; Gaub, H. E.; Müller, D. J. Single-Cell Force Spectroscopy. *J. Cell Sci.* **2008**, *121*, 1785–1791.
18. Müller, D. J.; Dufrêne, Y. F. Atomic Force Microscopy as a Multifunctional Molecular Toolbox in Nanobiotechnology. *Nat. Nanotechnol.* **2008**, *3*, 261–269.
19. Dupres, V.; Menozzi, F. D.; Loch, C.; Clare, B. H.; Abbott, N. L.; Cuenot, S.; Bompard, C.; Raze, D.; Dufrêne, Y. F. Nanoscale Mapping and Functional Analysis of Individual Adhesins on Living Bacteria. *Nat. Methods* **2005**, *2*, 515–520.
20. Benoit, M.; Gabriel, D.; Gerisch, G.; Gaub, H. E. Discrete Interactions in Cell Adhesion Measured by Single-Molecule Force Spectroscopy. *Nat. Cell Biol.* **2000**, *2*, 313–317.
21. Rief, M.; Gautel, M.; Oesterhelt, F.; Fernandez, J. M.; Gaub, H. E. Reversible Unfolding of Individual Titin Immunoglobulin Domains by AFM. *Science* **1997**, *276*, 1109–1112.
22. Oberhauser, A. F.; Marszalek, P. E.; Erickson, H. P.; Fernandez, J. M. The Molecular Elasticity of the Extracellular Matrix Protein Tenascin. *Nature* **1998**, *393*, 181–185.
23. Oesterhelt, F.; Oesterhelt, D.; Pfeiffer, M.; Engel, A.; Gaub, H. E.; Müller, D. J. Unfolding Pathways of Individual Bacteriorhodopsins. *Science* **2000**, *288*, 143–146.
24. Kedrov, A.; Krieg, M.; Ziegler, C.; Kuhlbrandt, W.; Müller, D. J. Locating Ligand Binding and Activation of a Single Antipporter. *EMBO Rep.* **2005**, *6*, 668–674.
25. Otoo, H. N.; Lee, K. G.; Qiu, W. G.; Lipke, P. N. *Candida albicans* Als Adhesins Have Conserved Amyloid-Forming Sequences. *Eukaryot. Cell* **2008**, *7*, 768–782.
26. Rief, M.; Pascual, J.; Saraste, M.; Gaub, H. E. Single Molecule Force Spectroscopy of Spectrin Repeats: Low Unfolding Forces in Helix Bundles. *J. Mol. Biol.* **1999**, *286*, 553–561.
27. Smith, B. L.; Schaffer, T. E.; Viani, M.; Thompson, J. B.; Frederick, N. A.; Kindt, J.; Belcher, A.; Stucky, G. D.; Morse, D. E.; Hansma, P. K. Molecular Mechanistic Origin of the Toughness of Natural Adhesives, Fibres and Composites. *Nature* **1999**, *399*, 761–763.
28. Evans, E.; Ritchie, K. Dynamic Strength of Molecular Adhesion Bonds. *Biophys. J.* **1997**, *72*, 1541–1555.
29. Baumgartner, W.; Gruber, H. J.; Hinterdorfer, P.; Drenkhahn, D. Affinity of Trans-Interacting VE-Cadherin Determined by Atomic Force Microscopy. *Single Mol.* **2000**, *1*, 119–122.
30. Zhao, H.; Shen, Z. M.; Kahn, P. C.; Lipke, P. N. Interaction of Alpha-Agglutinin and A-Agglutinin *Saccharomyces cerevisiae* Sexual Cell Adhesion Molecules. *J. Bacteriol.* **2001**, *183*, 2874–2880.