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Perfluorophenyl Azide Immobilization Chemistry for Single Molecule Force Spectroscopy of the Concanavalin A/Mannose Interaction

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The versatility of perfluorophenyl azide (PFPA) derivatives makes them useful for attaching a wide variety of biomolecules and polymers to surfaces. Herein, a single molecule force spectroscopy (SMFS) study of the concanavalin A/mannose interaction was carried out using PFPA immobilization chemistry. SMFS of the concanavalin A/mannose interaction yielded an average unbinding force of 70−80 pN for loading rates between 8000 and 40 000 pN/s for mannose surfaces on aminated glass, and an unbinding force of 57 ± 20 pN at 6960 pN/s for mannose surfaces on gold-coated glass. Dynamic force spectroscopy was used to determine the dissociation rate constant, k_{off}, for this interaction to be 0.16 s^{-1}.

Protein/carbohydrate interactions are of great biological/biochemical interest as they play a crucial role in a variety of molecular recognition events involved in biological processes, ranging from cell−cell recognition in viral/bacterial infections and signal transduction to glycoprotein transport, inflammation, organogenesis, and fertilization.[1] In efforts to further understand their biological role and to develop new strategies to fight diseases, addressing the interactions involved in protein/carbohydrate recognition is essential.[2] Lectins form a family of proteins that recognize and specifically bind different types of carbohydrate sequences. Due to their high binding affinity, lectins have been employed in various types of bioassays for studying carbohydrate recognition processes in mitogenic assays, characterization of malignant cells, and purification of glycoproteins, glycolipids, and proteoglycans.[3] Concanavalin A (ConA) is a lectin that specifically binds α-mannosyl and α-glucosyl groups found in sugars, glycoproteins, and glycolipids. Extracted from the jack bean *Canavalia ensiformis*, ConA is the first commercially available lectin that is widely used in biological as well as biochemical applications to characterize and purify sugar-containing entities.[4]

In recent years, force-based techniques have been used extensively to address, detect, and measure biomolecular interactions at the single molecule level.[5] Single molecule force spectroscopy (SMFS) is a powerful technique given (i) its ability to access a wide range of forces with a high degree of sensitivity as well as specificity, (ii) its ability to measure forces under biologically relevant conditions, and (iii) the variety of surface functionalization chemistries available. SMFS has indeed opened new horizons for measuring the strength of biomolecular interactions at their most fundamental level.[6−9] Force spectroscopy studies investigating the interaction between ConA and mannose have been previously reported.[2,10−17] Herein, we report a new and versatile strategy for force spectroscopy involving the photochemistry of arylazides, in order to immobilize carbohydrates on surfaces. The covalent attachment of carbohydrate moieties is based on the photochemical reactivity of perfluorophenylazides (PFPAs). PFPA-modified carbohydrates can be attached to virtually any organic surface with this simple photochemical protocol.[18] Using this PFPA strategy, a force spectroscopy investigation was carried out to determine unbinding parameters for the interaction of ConA with mannose (i.e., rupture forces, dissociation rate constant, and the distance from transition state on the energy landscape). Recently, a considerable number of functionalization chemistries have been described for attaching both natural as well as synthetic carbohydrate derivatives to hard substrates (e.g., glass, mica, or gold). Examples include (i) noncovalent attachment of biotinylated carbohydrates to streptavidin-functionalized dextran matrices, (ii) covalent attachment of amino-modified carbohydrates to commercial carboxylated surfaces using conventional EDC/NHS

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(1) Varki, A. *Glycobiology* **1993**, *3*, 97–130.
(2) Ratto, T. V.; Rudd, R. E.; Langry, K. C.; Balhorn, R. L.; McElfresh, M. W. *Langmuir* **2006**, *22*, 1749–1757.
(3) Naeem, A.; Saleemuddin, M.; Khan, R. H. *Curr. Protein Pept. Sci.* **2007**, *8*, 261–271.
(4) Lis, H.; Sharon, N. *Chem. Rev.* **1998**, *98*, 637–674.
(5) *Handbook of Molecular Force Spectroscopy*; Noy, A., Ed.; Springer: New York, 2008.
(6) Neuman, K. C.; Nagy, A. *Nat. Methods* **2008**, *5*, 491–505.
(7) Zlatanova, J.; Lindsay, S. M.; Leuba, S. H. *Prog. Biophys. Mol. Biol.* **2000**, *74*, 37–61.
(8) Fuhrmann, A.; Ros, R. *Nanomedicine* (*London, U. K.*) **2010**, *5*, 657–666.
(9) Bizzarri, A. R.; Cannistraro, S. *Chem. Soc. Rev.* **2010**, *39*, 734–749.
(10) Gad, M.; Itoh, A.; Ikai, A. *Cell Biol. Int.* **1997**, *21*, 697–706.
(11) Chen, A.; Moy, V. T. *Biophys. J.* **2000**, *78*, 2814–2820.
(12) Touhami, A.; Hoffmann, B.; Vasella, A.; Denis, F. A.; Dufrêne, Y. F. *Langmuir* **2003**, *19*, 1745–1751.
(13) Ratto, T. V.; Langry, K. C.; Rudd, R. E.; Balhorn, R. L.; Allen, M. J.; McElfresh, M. W. *Biophys. J.* **2004**, *86*, 2430–2437.
(14) Lekka, M.; Laidler, P.; Dulinska, J.; Labedz, M.; Pyka, G. *Eur. Biophys. J.* **2004**, *33*, 644–650.
(15) Lebed, K.; Pyka-Fościak, G.; Raczkowska, J.; Lekka, M.; Styczeń, J. *J. Phys.: Condens. Matter* **2005**, *17*, S1447–S1458.
(16) Lebed, K.; Kulik, A. J.; Forro, L.; Lekka, M. *Acta Phys. Pol., A* **2007**, *111*, 273–286.
(17) Zhang, X.; Yadavalli, V. K. *Anal. Chim. Acta* **2009**, *649*, 1–7.
(18) Pei, Z. C.; Yu, H.; Theurer, M.; Walden, A.; Nilsson, P.; Yan, M. D.; Ramström, O. *ChemBioChem* **2007**, *8*, 166–168.

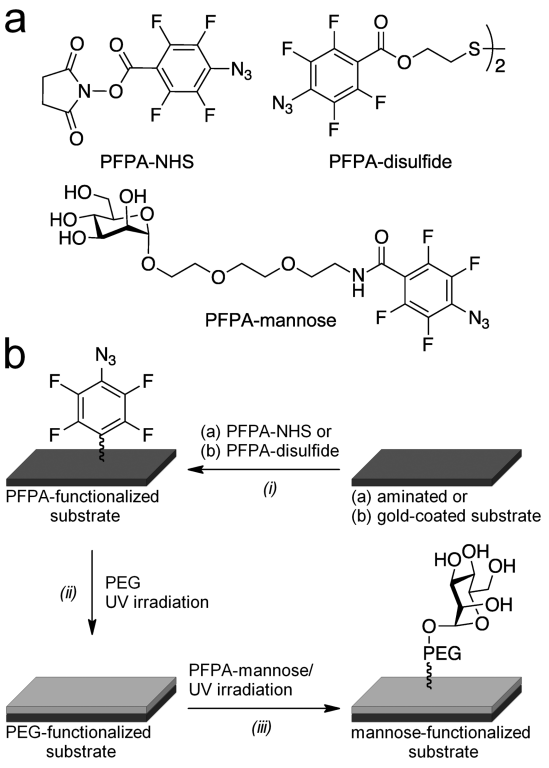

Figure 1. (a) PFPA-disulfide, PFPA-NHS, PFPA-mannoside, and PFPA-galactoside were synthesized as previously reported.[18] (b) Surface functionalization: (i) a layer of PFPA is formed on aminated or gold-coated glass surfaces by reacting with either NHS-PFPA or PFPA-disulfide, (ii) a thin PEG layer (PEG MW 20 000) is covalently attached to the surface using PFPA photochemistry, and (iii) PFPA-modified carbohydrates are attached to this surface via UV irradiation.

chemistry, (iii) covalent attachment of thiolated carbohydrates to gold-coated surfaces using alkanethiol self-assembled monolayers (SAMs), and (iv) covalent attachment of thiolated-carbohydrates to gold-coated surfaces using bifunctionalized poly(ethylene glycol (PEG) spacers.[19] The covalent attachment used in this study is based on the photochemistry of PFPAs, where the azide functionality is converted into a nitrene upon UV irradiation. This highly reactive perfluorophenyl nitrene can then insert preferentially into neighboring CH and NH bonds. This PFPA photochemical method has indeed been popular for surface modification and introducing functional groups into fullerenes, proteins, polymers, as well as carbohydrates.[20] Following this approach, mannose and galactose immobilization on glass surfaces was achieved using PFPA-modified carbohydrates. Due to the high reactivity of PFPAs under UV irradiation, the surface modification chemistry described in this study is an effective alternative to other surface functionalization strategies (Figure 1).

With these carbohydrate-modified surfaces in hand, a force spectroscopy investigation of their interaction with ConA-functionalized atomic force microscopy (AFM) tips was carried out. The force required to break the contact between ConA tips and mannose surfaces reflects the ConA/mannose interaction strength. As a control, the nonspecific interaction with galactose surfaces was also explored. AFM tips functionalized with ConA were prepared using a bifunctional PEG, NHS-PEG-NHS, as previously reported (Figure 2).[13] The PEG spacers allow the

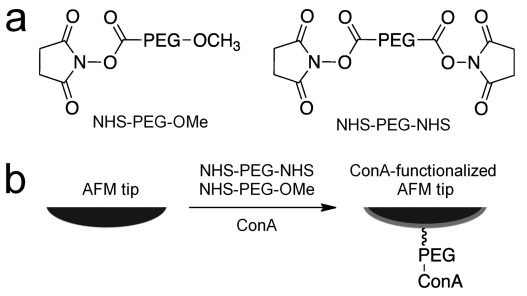

Figure 2. (a) Monofunctional CH_3O-PEG-NHS (PEG MW 750) and bifunctional NHS-PEG-NHS (PEG MW 6000). (b) Tip functionalization: formation of an amide linkage to the terminal amino group or surface-exposed lysine residues of ConA.

differentiation of nonspecific interactions from the desired specific interactions under investigation. The successful use of PEG (or PEO) as a spacer in single molecule force spectroscopy unbinding studies is general and well documented.[21−25] Using an excess of monofunctionalized PEG, NHS-PEG-OMe, limits the number of active proteins attached to the AFM tip, thus increasing the probability of obtaining single molecule events.[26] It is important to note that tip functionalization, as well as force measurements, were carried out at pH values less than 7 in order to avoid measuring multiple ConA/mannose interactions since ConA exists as a dimer rather than a tetramer at this pH.[13]

The interaction force between single ConA/mannose pairs is obtained by force−distance measurements, where the ConA tip is first approached to the mannose surface and subsequently retracted. During the approach−retract cycle, the deflection of the cantilever (x), which can be directly converted into force (f) using Hooke's law ($f = kx$), is continuously measured and plotted as a function of tip−surface separation. As the tip first approaches the surface, the cantilever deflection remains constant until the tip comes into contact with the surface. Further extension at the surface (controlled with a relative trigger at 100 pN), coupled with a 1 s delay time, allows the interactions between ConA and mannose to be established, and subsequent tip retraction allows the measurement of the unbinding force required to detach ConA from mannose. The shape of this nonlinear rupture peak reflects the elastic properties of the tethering PEG polymer, whereas the height of the rupture peak is governed by the strength of the ConA/mannose interaction at a particular tip velocity. No recognition events (i.e., rupture peaks) are observed when the ConA on the tip does not interact with the mannose on the surface. This is often the case, since the probability of observing single molecule events with minimally functionalized AFM tips is very low. In fact, for the retraction of a ConA-functionalized AFM tip from a PFPA-mannose-functionalized surface, curves with no interactions and curves displaying nonspecific interactions represent over 90% of the raw curves (Supporting Information, Figure S1a and b and Table S1). Force curves displaying rupture events are fit with the simplified extended freely jointed chain model (e-FJC$_{PEG}$) in order to determine if the events are due to breaking the interaction between PEG-attached partners

(19) Ebner, A.; Wildling, L.; Zhu, R.; Rankl, C.; Haselgrübler, T.; Hinterdorfer, P.; Gruber, H. J. *Top. Curr. Chem.* **2008**, *285*, 29–76.
(20) Yan, M. *Chem.—Eur. J.* **2007**, *13*, 4138–4144.
(21) Zhang, Y.; Liu, C.; Shi, W.; Wang, Z.; Dai, L.; Zhang, X. *Langmuir* **2007**, *23*, 7911–7915.
(22) Zhang, Y.; Yu, Y.; Jiang, Z.; Xu, H.; Wang, Z.; Zhang, X.; Oda, M.; Ishizuka, T.; Jiang, D.; Chi, L.; Fuchs, H. *Langmuir* **2009**, *25*, 6627–6632.
(23) Liu, C.; Jiang, Z.; Zhang, Y.; Wang, Z.; Zhang, X.; Feng, F.; Wang, S. *Langmuir* **2007**, *23*, 9140–9142.
(24) Strunz, T.; Oroszlan, K.; Schäfer, R.; Güntherodt, H.-J. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 11277–11282.
(25) Kada, G.; Kienberger, F.; Hinterdorfer, P. *Nano Today* **2008**, *3*, 12–19.
(26) Hinterdorfer, P.; Dufrêne, Y. F. *Nat. Methods* **2006**, *3*, 347–355.

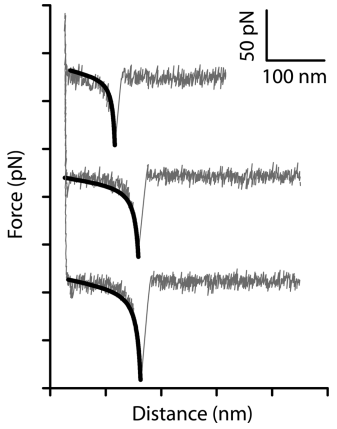

Figure 3. Typical retraction force curves for the unbinding of a ConA with a PFPA-mannoside-functionalized surface. The loading rate was 7120 pN/sec (tip velocity 400 nm/s, cantilever spring constant 17.8 pN/nm) with a relative trigger of 100 pN toward the surface and a dwell time of 1 s. The fits to a simplified extended freely jointed chain model (e-FJC$_{PEG}$) are shown as solid lines. The Kuhn lengths determined from the fits are: 868, 708, and 649 pm from top to bottom.

(Figure 3 and Supporting Information Figure S1c).[27] In order to confirm that the observed rupture peaks indeed represent ConA/mannose unbinding, control experiments must be performed. In this study, these involved eliminating or blocking the ConA/mannose interaction: (i) addition of free mannose to the buffer to saturate the ConA binding sites, (ii) using monofunctionalized PEG that does not link to ConA, and (iii) using galactose-functionalized surfaces that do not specifically bind ConA. The absence of specific interactions in these cases ($<0.3\%$) demonstrates the specificity of ConA/mannose binding (Supporting Information, Table S2). These three control experiments reinforce the reliability and confidence of the reported force results.

At each tip velocity, curves with PEG Kuhn lengths between 600 and 900 pm are chosen and represent about 4% of the overall collected data (Figure 4a and Supporting Information Table S1).[28] An important disadvantage to this functionalization strategy is that the rupture distance cannot be used as a discriminating factor in analyzing the force curves. High molecular weight PEG (20 000 MW: ca. 470 nm) is used in the functionalization where photoactivated PFPA carbohydrates can insert into bonds all along the length of the PEG. This results in varying tether distances throughout the entire surface and a random distribution in the rupture distance histogram (data not shown). The distribution of unbinding forces of accepted curves is analyzed using a Gaussian fit resulting in the most probable unbinding force for the ConA/mannose interaction, which is typically reported with its corresponding loading rate (i.e., the product of the probe velocity and the spring constant) (Figure 4b).[9] SMFS of ConA/mannose yields an average unbinding force of 70−80 pN for loading rates between 8000 and 40 000 pN/s for mannose surfaces on aminated glass (Supporting Information, surface functionalization method A, Figures S2−S6, and Figure 4) and an unbinding force of 57 ± 20 pN at 6960 pN/s (tip velocity 400 nm/s, cantilever spring constant 17.4 pN/nm, $n = 253$) for mannose surfaces on gold-coated glass (Supporting Information, surface functionalization method B).

Our results are in agreement with previously reported unbinding forces in related ConA/mannose studies. For example, in a comprehensive study by Ratto et al., unbinding forces of 46, 68, and 85 pN at a loading rate of 10 000 pN/s were attributed to the monovalent, divalent, and trivalent interactions of ConA with mannose, respectively.[2] Variability in unbinding results is attributed to (i) the polyvalent nature of the ConA/mannose interaction, (ii) different loading rates, and (iii) the possibility of parallel multiple PEG stretching events. The measured unbinding force increases with increasing loading rate, as illustrated in our results (Figure 5). At sufficiently low loading rates, the system is being pulled apart slowly, and therefore, there is enough time for thermal fluctuations to help the system overcome the transition state energy barrier resulting in smaller unbinding forces.[9]

According to the theory introduced by Bell[29] and expanded upon by Evans and Ritchie,[30] measuring the rupture force of

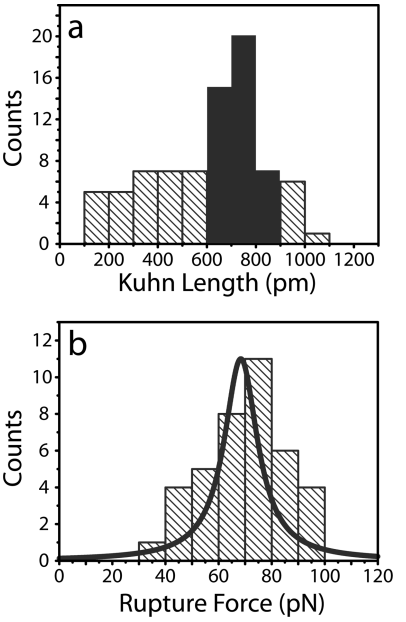

Figure 4. Histograms of single molecule force spectroscopy events for unbinding of ConA from mannose at a loading rate of 8140 pN/sec (tip velocity 400 nm/s, cantilever spring constant 20.35 pN/nm): (a) Distribution frequency of Kuhn lengths ($n = 80$); the highlighted curves between 600 and 900 pm were selected for further analysis. (b) Distribution frequency of unbinding forces ($n = 39$); a Gaussian fit was used to determine the most probable unbinding force to be 69.5 ± 2.7 pN.

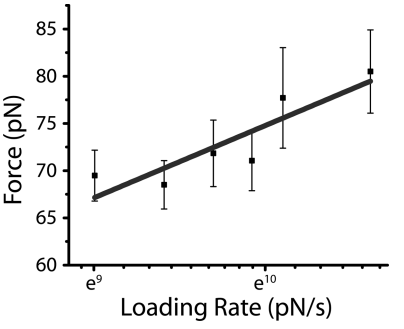

Figure 5. Dynamic force spectroscopy of ConA/mannose unbinding at loading rates between 8140 and 40 700 pN/s. The dissociation rate constant, k_{off}, determined from the intercept of the vertical axis is 0.16 s^{-1}, and the distance between the binding complex and the transition state, x_β, is 5.3 Å.

(27) Guo, S.; Ray, C.; Kirkpatrick, A.; Lad, N.; Akhremitchev, B. B. *Biophys. J.* **2008**, *95*, 3964–3976.
(28) Oesterhelt, F.; Rief, M.; Gaub, H. E. *New J. Phys.* **1999**, *1*, 6.1–6.11.
(29) Bell, G. I. *Science* **1978**, *200*, 618–627.
(30) Evans, E.; Ritchie, K. *Biophys. J.* **1997**, *72*, 1541–1555.

biomolecular interactions at variable probe loading velocities (dynamic force spectroscopy) allows exploring their kinetics by determining the dissociation rate constant (k_{off}) and the distance between the binding complex and the transition state (x_β) (eq 1).

$$f^* = \frac{k_B T}{x_\beta} \ln\left(\frac{x_\beta}{k_{off} k_B T}\right) + \frac{k_B T}{x_\beta} \ln r_l \quad (1)$$

In this equation, r_l is the loading rate which is the product of the probe velocity (nm/s) and the spring constant (pN/nm) and f^* is the most probable rupture force estimated from the histogram of a set of measurements performed at a specific probe velocity. k_B is the Boltzmann constant and T is the absolute temperature. In our experiments, we varied the loading rates from 8140 to 40 700 pN/s. The presence of a single linear region in the ConA/mannose dynamic force spectroscopy plot (Figure 5) indicates the presence of a single energy barrier in the thermally activated regime of the energy landscape.[9] From this plot, k_{off} and x_β were determined to be 0.16 s^{-1} and 5.3 Å, respectively. These results are virtually the same as those previously reported for Con A/mannosyl dynamic force spectroscopy following varying functionalization strategies.[11,16,31]

In this investigation, a combination of optimal tip chemistry (i.e., minimal functionalization and the use of a molecular spacer) as well as an efficient and robust surface chemistry (i.e., photoactivation of PFPA-functionalized reagents) proved to be applicable for SMFS experiments. The versatility of PFPA derivatives makes them useful for attaching a wide variety of biomolecules and polymers to surfaces.

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Supporting Information Available: Experimental details for surface and tip functionalization, single molecule force spectroscopy protocol, and dynamic force spectroscopy histograms. This material is available free of charge via the Internet at http://pubs.acs.org.

(31) Krieg, M.; Helenius, J.; Heisenberg, C. P.; Müller, D. J. *Angew. Chem., Int. Ed.* **2008**, *47*, 9775–9777.