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Characterization of Folic Acid and Poly(amidoamine) Dendrimer Interactions with Folate Binding Protein: A Force-Pulling Study

Pascale R. Leroueil¹, Stassi DiMaggio³, Abigail N. Leistra⁴, Craig D. Blanchette⁵, Christine Orme⁵, Kumar Sinniah⁴, Bradford G. Orr², and Mark M. Banaszak Holl¹

¹Department of Chemistry, University of Michigan, Ann Arbor, MI 48109.

²Department of Physics, University of Michigan, Ann Arbor, MI 48109.

³Department of Chemistry, Xavier University, New Orleans, LA 70125.

⁴Department of Chemistry & Biochemistry, Calvin College, Grand Rapids, MI 49546.

⁵Physical and Life Sciences Division Lawrence Livermore National Laboratory, Livermore, CA 94550.

Abstract

Atomic force microscopy force-pulling experiments have been used to measure the binding forces between folic acid (FA) conjugated poly(amidoamine) (PAMAM) dendrimers and folate binding protein (FBP). The generation 5 (G5) PAMAM conjugates contained an average of 2.7, 4.7, and 7.2 FA per dendrimer. The most probable rupture force was measured to be 83, 201, and 189 pN for G5-FA_{2.7}, G5-FA_{4.7}, and G5-FA_{7.2}, respectively. Folic acid blocking experiments for G5-FA_{7.2} reduced the frequency of successful binding events and increased the magnitude of the average rupture force to 274 pN. The force data is interpreted as arising from a network of van der Waals and electrostatic interactions that form between FBP and G5 PAMAM dendrimer resulting in a binding strength far greater than that expected for an interaction between FA and FBP alone.

Folic acid-conjugates have been explored extensively for targeted drug and imaging agent delivery¹⁻¹² and for targeted polymer vectors.¹³⁻¹⁶ Folic acid (FA) is necessary for thymidine production as part of de-novo DNA biosynthesis. In order to facilitate rapid division, cancer cells increase the concentration of folate receptors (FRs) on plasma membrane surfaces. To date, seven FA-targeted cancer therapeutics have advanced to clinical trials, but none have progressed to full clinical development. Dose-limiting toxicity due to uptake by healthy cells remains a problem. Additionally, the expression of FRs on the surfaces of tumor cells is highly variable both from individual to individual and within a given cancer type.

Mechanistic studies of the interactions have generally employed folate binding protein (FBP) as a model for the membrane bound FR. FBP is a ~30 kDa glycoprotein containing 222 amino acids that is closely related to membrane-bound FR- α and FR- β , which are

Supporting Information Available: Rupture length distribution of the FBP-G5-FA_n interaction in the presence and absence of “free” FA is provided in Figure S1. This material is available free of charge via the Internet at <http://pubs.acs.org>.

connected to plasma cell membrane via a glycosylphosphatidylinositol (GPI) anchor.^{17,18} A third isoform, FR- γ , is a secreted protein and lacks the signal for modification with a GPI anchor. Soluble FBP likely originates from FR- α that has undergone cleavage of the GPI anchor and from FR- γ , which inherently lacks a GPI modification. X-ray crystal structures of the FA-bound protein and molecular dynamics studies of soluble bovine FBP were recently reported.¹⁹⁻²¹

Multivalent FA-polymer conjugates have been studied in an attempt to develop delivery vectors that would have greater binding strength, and perhaps also greater selectivity, to cancer cells.^{14-16,22} In particular, the Baker laboratory has pursued extensive cell culture and *in vivo* studies of generation 5 poly(amidoamine) (G5 PAMAM) materials for use as targeted delivery agents for both cancer and inflammatory disease applications.^{14,23-26} The promising biological results they report, combined with the relatively narrow dispersity of the PAMAM platform as compared to many other polymer vectors, led to the choice of this system for detailed physical analysis. Recently, the slow-onset, tight-binding mechanism²⁷ by which FA binds to FBP has been found to play a major role in the binding mechanism of PAMAM dendrimer-FA and dendrimer-methotrexate conjugates to the protein (Figure 1).^{28,29} The initial, reversible FA/FBP interaction is followed by a reorganization of the protein structure (denoted as FBP*), leading to the observed nanomolar FA-FBP* binding constant. The change in protein structure, characterized in the literature by a quenching of the FBP tryptophan fluorescence,^{30,31} is hypothesized to lead to a reduction in the number of hydrophobic residues on the protein surface. A network of van der Waals interactions, including a mixture of hydrogen bonding, other dipole-dipole, and dispersion interactions, can then form between FBP* and G5 PAMAM dendrimer resulting in the irreversible binding measured by surface plasmon resonance (SPR).²⁹ In the SPR experiments, increasing the number of FA per dendrimer increased the rate of polymer binding to the surface; however, only a single FA per dendrimer was required to generate the strong, irreversible interaction illustrated in Figure 1.

In order to obtain a more detailed understanding of the binding interaction between G5-FA and FBP protein, we embarked on force-pulling experiments using atomic force microscopy (AFM). Force-distance curves were measured for G5 PAMAM dendrimers containing an average of 2.7, 4.7, and 7.2 FA per dendrimer. The most probable rupture force was measured to be 83, 201, and 189 pN for G5-FA_{2.7}, G5-FA_{4.7}, and G5-FA_{7.2}, respectively. In all three cases a range of rupture forces were observed. The majority of events for G5-FA_{2.7} occurred between 45 and 190 pN, whereas the majority of events for G5-FA_{4.7} and G5-FA_{7.2} occurred between 80 and 400 pN. Blocking with excess folic acid reduced the likelihood of a successful interaction between G5-FA_{7.2} and the FBP surface by roughly an order of magnitude. Surprisingly, the remaining events occurred over a higher range of rupture forces. The magnitude, trend in rupture force as function of number of FA conjugated, reduction in interactions with FA blocking, and the increase in rupture force for remaining events after blocking is consistent with a combination of 1) the general model for binding proposed by Licata and Tkachenko³² and 2) recent SPR binding studies quantifying the binding interactions of G5-FA_n with FBP.²⁹

Experimental Section

Preparation of dendrimers

G5 PAMAM dendrimers were synthesized and purified to remove low molecular weight impurities as well as high molecular weight dimers, according to previous reports.^{22,33} After purification, the dendrimers were partially acetylated using the method of Majoros et al. resulting in G5-Ac₇₀ (70 of the 110 total primary amines).³⁴ The G5-Ac₇₀ was allowed to react in H₂O with FA pre-activated by 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide/HCl (EDC) in DMF/DMSO at different molar ratios (0:1, 3:1, 6:1, 9:1) of FA to G5-Ac₇₀. Following further purification, the dendrimers were characterized as described by Hong et al.²² Based on GPC and UV-Vis results, the following conjugates were formed: G5-Ac₇₀-FA_{2.7}, G5-Ac₇₀-FA_{4.7}, and G5-Ac₇₀-FA_{7.2}.

Preparation of AFM tips for force pulling³⁵

Prior to tip modification, silicon nitride (Si₃N₄) tips (Veeco Probes, NP-S) were placed in a piranha solution (sulfuric acid/H₂O₂, 7:3) for 20 minutes. The tips were rinsed 3 times with Millipore water and then 3 times with 200-proof ethanol (EtOH), dried using a nitrogen stream and then placed in a 110°C oven for 10 minutes to remove any remaining water. The tips were then silanized by suspending them in a nitrogen-purged glass vial for 1 hour containing a 1:15 ratio of aminopropyltriethoxysilane (APTES) to methyltriethoxysilane (MTES) dissolved in chloroform. The silanized tips were subsequently placed in a 110°C oven for 10 minutes. Expected surface coverage based on respective vapor pressures (APTES, 0.02 hPa @20°C; MTES, 14.66 hPa @20°C) is 1:100 APTES:MTES. Tips were pegylated by placing tips in a 10 mg/mL solution of succinimidyl α -methylbutanoate(SMB)-poly(ethyleneglycol)(PEG)-SMB in chloroform (3400 Da nominal molecular weight, Nektar) for two minutes and then rinsed 3 times with fresh chloroform and steam dried with nitrogen. G5-Ac₇₀-FA_n dendrimers were attached by covering tips in a solution containing equal volumes of 1 mg/mL G5-Ac₇₀-FA_n and a pH 8 phosphate buffer. Tips were left undisturbed for 30 minutes and subsequently washed 3 times in a pH 7 phosphate buffer. Modified tips were utilized the same day they were prepared and kept in a nitrogen box when not in use. Based on tip dimensions and APTES:MTES surface coverage, ~2 G5-Ac₇₀-FA_n were expected to be in contact with the FBP substrate during measurements.

Preparation of FBP substrates

Folate binding protein (FBP) substrates were prepared in the same manner as those used in SPR binding studies.²² Substrates were prepared using a BIAcore X (Pharmacia Biosensor AB, Uppsala, Sweden). FBP (Sigma-Aldrich, St. Louis, MO) was immobilized on the sensor chip surface of a carboxylated dextran-coated gold film (CM 5 sensor chip) by amine coupling as described elsewhere.^{24,36-38} Briefly, 70 μ L of a mixed solution of NHS/ECD (1:1, v/v) was first injected into the BIAcore to activate the carboxylated dextran, followed by injection of 70 μ L of 2.5 mg/mL FBP dissolved in 100 mM potassium phosphate buffer, pH 5.0, supplemented with 4 mM mercaptoethanol and 10% (v/v) glycerol. 1 M ethanolamine in water, pH 8.5 was then injected to deactivate residual NHS-esters on the sensor chip. The immobilization process was performed at a flow rate of 10 μ L/min, resulting in the binding of ~6 ng/mm² (~ 5,900 RU) of FBP per channel.²²

Force-distance measurements

Measurements were performed using an Asylum MFP-3D atomic force microscope (AFM) equipped with an Asylum fluid cell. Tips modified with solutions of one of the four platforms (G5-Ac₇₀-FA_n, where $n = 0, 2.7, 4.7$ or 7.2) were brought into contact with a FBP substrate and then retracted. This process was recorded and the force required to rupture the FA-FBP bonds was extracted as described below. Note that forces obtained are *relative* and not absolute.³⁹ 2000 force-distance curves (up down motions) were obtained for G5-Ac₇₀, G5-Ac₇₀-FA_{2.7}, G5-Ac₇₀-FA_{4.7} and G5-Ac₇₀-FA_{7.2}. The position of the tip in relation to the FBP substrate was changed 10 times, or after every 200 force-distance curves. The distance retracted from the surface was 300-500 nm. The rate used for these measurements was 1 Hz with a tip-surface dwell time of 1 sec. A small fraction of the force curves (3, 24, and 83 out of 2000 for G5-Ac₇₀-FA_{2.7}, G5-Ac₇₀-FA_{4.7} and G5-Ac₇₀-FA_{7.2}, respectively) exhibited multiple rupture events containing the characteristic PEG-stretch. Spring constants were calculated for each modified tip using the built-in thermal tune software. Spring constants ranged from 0.046 to 0.065 N/m. A fixed loading rate was used for all force pulling measurements. All measurements were made in phosphate buffered solution (pH 7). All forces include in the analysis have rupture lengths between 5 and 40 nm and forces of less than 1000 pN.

Blocking experiments using free FA were completed to test if the rupture forces measured were related to FA-FBP binding. Blocking experiments were conducted by first incubating the FBP substrate with 10 μ M free FA for 40 minutes before obtaining 2000 force vs. distance curves using a tip modified with G5-Ac₇₀-FA_{7.2}. This experiment was completed with the same tip used for the unblocked G5-Ac₇₀-FA_{7.2}/FBP measurements.

Results

Rupture force densities from force-distance curves

An exemplar approach curve and retraction curve is shown in Figure 2. Non-specific adhesion forces were broken first (region ab). As the tip continued to retract from the surface, the PEG polymer linked to the G5-Ac₇₀-FA_n stretched resulting in the characteristic PEG-stretch (region bc) until finally the (G5-Ac₇₀-FA_n)-FBP' interactions were broken (cd). We use FBP' to indicate the mixture of FBP and FBP* structural forms available for interaction with the PAMAM dendrimer. In these experiments, a total of 2000 curves were obtained for each dendrimer-modified tip (G5-Ac₇₀, G5-Ac₇₀-FA_{2.7}, G5-Ac₇₀-FA_{4.7}, and G5-Ac₇₀-FA_{7.2}). The first force event (region ab) was present for 70% of the curves whereas the remaining 30% smoothly retracted with an additional 2nd force interaction and PEG stretch (region bc) (Figure 2). The second force event (bc) was present for 6 (G5-Ac₇₀), 513 (G5-Ac₇₀-FA_{2.7}), 379 (G5-Ac₇₀-FA_{4.7}), and 593 (G5-Ac₇₀-FA_{7.2}) of the 2000 curves measured. The rupture force distributions of this second rupture force (cd) are displayed in Figure 3 (bar graphs). The most probable rupture force (cd) arising from the force distribution of G5-Ac₇₀-FA_n/FBP' rupture events was obtained by fitting the force distribution data to a Gaussian kernel density (Figure 3 solid lines). All density curves reported were generated using 256 points and the Gaussian kernel function. The most probable rupture force occurs at 83 ± 2 pN (G5-Ac₇₀-FA_{2.7}), 201 ± 9 pN (G5-Ac₇₀-FA_{4.7}),

and 189 ± 5 pN (G5-Ac₇₀-FA_{7.2}). The uncertainties are reported to $\pm 2\sigma$. A small fraction of the force curves exhibited multiple rupture events in the PEG-stretch region. The numbers of these events increased as a function of average number of FA per dendrimer and were present in 0.15%, 1.2% and 4.2% of force curves for G5-Ac₇₀-FA_{2.7}, G5-Ac₇₀-FA_{4.7} and G5-Ac₇₀-FA_{7.2}, respectively. The forces for these separate PEG stretches were similar in magnitude to forces for curves exhibiting a single stretch. Each rupture event from the multiple event curves is included in the force distribution plotted in Figures 3. The tip-sample separation distances (rupture length) at which the FA-FBP' rupture occurs is shown in Figure 4. The rupture length spans a range from 5-80 nm with the peak rupture length $\sim$ 20-25 nm for G5-Ac₇₀-FA_{2.7}, G5-Ac₇₀-FA_{4.7} and G5-Ac₇₀-FA_{7.2}. The wide distribution in rupture lengths is most likely due: a) the polydispersity in the length of the PEG used for linking the dendrimer conjugates to the AFM tip and/or b) the relative location of tip linker and FA conjugation on the dendrimer arms. We also observed a small number of rupture events (0.3% of force curves) for G5-Ac₇₀-FA₀ with a magnitude of 670 ± 69 pN (inset Figure 3). These events were largely outside of the magnitudes assigned as FA/FBP interactions and occurred much more rarely (0.3% as compared to 22-30% for G5-Ac₇₀-FA_{2.7}, G5-Ac₇₀-FA_{4.7} and G5-Ac₇₀-FA_{7.2} force curves).

Rupture force density following preincubation of FBP-substrate with FA

The FBP surface was incubated with free FA prior to force pulling measurements to test whether the observed rupture forces were related to FA/FBP binding. We elected to perform the FA-blocking experiment using the G5-Ac₇₀-FA_{7.2} modified tip since this experiment exhibited a combination of the greatest range of interactions energetically and frequency of interactions. The incubation of 10 μ M free FA with the FBP substrate prior to performing force measurements using a G5-Ac₇₀-FA_{7.2} tip resulted in 70 force events (region bc) from 2000 measured curves. Rupture force densities (cd) are displayed in Figure 5. This is a 91% reduction in counted force events as compared to the measurement performed with no free folic acid present for the 0-400 pN region and an 88% reduction for the 0-800 pN region. The most probable rupture force in the presence of 10 μ M free FA was 274 ± 36 pN in comparison to the 189 ± 5 pN obtained in the absence of free FA. The observed increase in rupture force was not accompanied by a change in rupture lengths (Figure S1).

DISCUSSION

The data summary provided above includes 6 events for the dendrimer containing no FA (G5-Ac₇₀-FA₀; Figure 3 inset; region bc) that we believe are not related to FA/FBP' binding. These 6 events are localized in the 400 to 800 pN force range, which does not overlap with the major portion of the force densities for G5-Ac₇₀-FA_{2.7}, G5-Ac₇₀-FA_{4.7}, or G5-Ac₇₀-FA_{7.2} (Figure 3). The measured rupture forces are summarized below with the data in the 0-400 pN range. The most probable rupture forces for G5-Ac₇₀-FA_{2.7}, G5-Ac₇₀-FA_{4.7}, and G5-Ac₇₀-FA_{7.2} are 83, 201, 189 pN, respectively. Force distribution plots from Figure 3 show a distinctly different distribution for G5-Ac₇₀-FA_{2.7} compared with either G5-Ac₇₀-FA_{4.7}, or G5-Ac₇₀-FA_{7.2}. G5-Ac₇₀-FA_{2.7} is centered at lower average value of rupture force and the width of this distribution is narrower compared to FA_{4.7} and FA_{7.2} conjugated dendrimers. However, G5-Ac₇₀-FA_{2.7} does exhibit a distinct tailing to higher

rupture forces up to 400 pN that includes the range of the majority of the forces measured for G5-Ac₇₀-FA_{4.7}, or G5-Ac₇₀-FA_{7.2}. Recently we measured the rupture force of riboflavin (RF) conjugated to a G5 dendrimer with riboflavin binding protein (RFBP).⁴⁰ The rupture force was measured to be ~67 pN for similar loading rates. By way of contrast, the rupture force from the very strong biotin-avidin interaction was determined to be 75 pN.⁴¹ In light of these comparisons, the average 83 pN force measured for the G5-Ac₇₀-FA_{2.7}/FBP* interaction appears to be quite large for a single FA/FBP* interaction alone. A recent theoretical model by Licata and Tkachenko (L-T)³² suggests that FA binding to FBP keys an interaction resulting in strong van der Waals and electrostatic interactions between the dendrimer and the protein surface. This model was supported by recent surface plasmon resonance (SPR) studies of G5-Ac-FA_n binding to FBP that indicated specific, irreversible binding to FBP that could be triggered by a single FA per dendrimer.²⁹ In this model, the initial FA/FBP interaction is followed by a reorganization of the protein structure (FBP*),^{30,31} and strong van der Waals interaction between the polymer-protein surface leading to the observed nanomolar G5-FA/FBP* binding constant. The data presented here, including both magnitude and width of the force distribution, suggests that G5-FA_n interacts with multiple FBP* and/or FPB surface proteins as illustrated in Figure 6. The G5-FA/FPB* interaction is hypothesized to generate the Gaussian distribution of force values centered at 83 pN and is illustrated schematically in Figure 1 and as structure (i) in Figure 6A. In the previously published SPR experiments, the G5-FA conjugates were fully acetylated; however, synthetic approaches used to conjugate the materials to the PEG linkers left the materials employed for the force-pulling studies partially acetylated. The materials contain primary amines that will protonate in the physiological pH of the buffers employed. Therefore, addition to the network of van der Waals interactions (dipole-dipole forces including H-bonding, dispersion forces), there are static charges in this case that could also result in electrostatic contributions to the interaction network. The force-pulling values measured thus represent an upper bound of the forces expected for the fully neutralized material studied by SPR.²⁹

The high tail of rupture forces to about 400 pN is difficult to explain based upon single G5-FA/FBP* interactions and a multivalent G5-FA/FBP or G5-FA/FBP* interaction hypothesis is at odds with both theory and experiment.^{29,32} A possible mechanism to explain this data is the interaction of the dendrimer conjugate with multiple FBP* or multiple FBP* and FBP surface proteins. In principle, this could occur via the interaction of 2 FA molecules conjugated to a single dendrimer (Figure 6A, structure ii) or by proximate binding of two single G5-FA interacting dendrimers such that two polymers had access to two or more FBP* or FBP* and FPB for polymer/protein surface interactions (Figure 6A, structure iii). Such a multiple dendrimer interaction is possible based on tip dimensions and the APTES:MTES surface coverage used during tip functionalization. About 2 G5-Ac₇₀-FA_n are available to come in contact with the FBP substrate during measurements (see Material and Methods). Either or both of the mechanisms are consistent with the tail of higher rupture force interactions observed.

The number of measured rupture events for all three conjugates were similar with 513 (G5-Ac₇₀-FA_{2.7}), 379 (G5-Ac₇₀-FA_{4.7}), and 593 (G5-Ac₇₀-FA_{7.2}) events each. A comparison of the distribution plots for G5-Ac₇₀-FA_{4.7}, and G5-Ac₇₀-FA_{7.2} show remarkably similar

rupture force distributions. This implies that increasing the average FA valency on the G5 dendrimer did not lead to a greater number of FA's interacting with FBP and is consistent with prior assessment that the binding strength is not altered by FA-FBP multivalency. In the previous SPR studies of binding, one FA interaction was sufficient to trigger irreversible interaction with the FBP surface and therefore no differential in binding strength could be assessed as a function of valency.²⁹ In this study, the two higher average FA valencies (4.7 and 7.2) appear to be able to generate a saturated number of FBP* for each dendrimer polymer to interact with on the surface. The model (ii) illustrated in Figure 6A is appealing from this point of view as the material with an average of 2.7 FA has less likelihood to bind and convert fewer FBP to FBP* under the dendrimer footprint than the materials with an average of 4.7 or 7.2 FA. Using a Poisson distribution estimate of the numbers of FA present per particle for each dendrimer one estimates that roughly 50% of G5-Ac₇₀-FA_{2.7} material has two or less FA available to bind whereas this is true for only 14% and 2% of G5-Ac₇₀-FA_{4.7} and G5-Ac₇₀-FA_{7.2}, respectively. However, this hypothesis is at odds with the conclusions of the model of L-T, which states that multivalent FA/FBP interactions cannot occur over the short time frame of the experiment.³² From this point of view model (iii) in Figure 6A appears preferable, although it is not clear why the 4.7 and 7.2 FA average materials would be more likely to form the proximate pairs required for enhanced binding.

The assignment of the forces measured in the 0-600 pN range as being FA specific was tested by pre-incubating the FBP surface with 10 μ M free FA. Figure 5 illustrates the dramatic decrease in successful binding interactions achieved and an *increase* in average rupture force (from 189 to 274 pN). This observation provides yet another piece of evidence against a multivalent FA/FBP interaction as the mechanism of surface binding. As illustrated in Figure 6B, this is the expected force trend for the binding mechanism highlighted in Figure 1, polymer to protein surface binding, if the polymer is able to form van der Waals/electrostatic interactions with multiple FBP' (i.e. some mixture of FBP* and FBP) proteins on the surface. In the latter case, pre-treatment with FA converts the surface to FBP* proteins that bind the polymer more strongly. For the most part, the presence of the FA also prevents the initial interaction of the G5-FA conjugate with the surface; however, in the instances where an open site is found, there is then a far greater likelihood that nearby FBP protein are in the FBP* form and this enhances the overall G5-FA interaction with the surface.

The relation of these observations to G5-FA binding to cell membrane bound folate receptor α (FR- α), FR- β , or to serum FBP (which lacks the glycosylphosphatidylinositol (GPI) membrane anchor) needs additional scrutiny.⁴² The binding mechanism proposed here is consistent with the membrane aggregation of FR- α that is proposed to occur upon FA binding and/or the presence of FR- α domains prior to binding. The enhanced binding provided by the G5-FA/FBP' interactions can result in the longer residence on the cell surface previously ascribed to multivalency.²²

SUMMARY

Multivalent polymer conjugates of FA have been extensively explored for drug and imaging agent delivery applications. In this study, AFM has been employed to measure rupture force

distributions of the G5-FA_n conjugates (n = 2.7, 4.7, 7.2) interacting with FBP surfaces. Consistent with recent theoretical and experimental studies,^{29,32} the force distributions for these binding experiments are found to be most consistent with a slow-onset, tight-binding mechanism in which the FA binds to FBP and induces structural change in the protein (FBP*), which is then followed by the formation of van der Waals and electrostatic interactions between the dendrimer and protein surfaces. The measured rupture forces arise from breaking this dendrimer/protein van der Waals/electrostatic interaction. This interpretation is further bolstered by FA blocking experiments that reduce the frequency of binding but enhance overall binding strength for the remaining events. Previous SPR experiments were unable to measure binding strength as a function of number of FA conjugated to the dendrimer since even one FA per dendrimer bound irreversibly to the FBP substrate.²⁴ The force pulling experiments indicate that conjugates with an average of 2.7 FA per dendrimer bind to the surface more weakly than conjugates containing an average of 4.7 or 7.2 FA per dendrimer. We hypothesize that this is related to differing number of FBP* available for interaction with dendritic polymer surface.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1. G5 PAMAM dendrimer FA conjugate binding to FBP. In an initial reversible step, FA binds to the protein receptor site, inducing structural change in the protein, and triggering the polymer-protein interactions. In the second irreversible step, the polymer and protein form a network of van der Waals and electrostatic interactions leading to very strong binding.

Figure 2.

A representative force curve for the interaction between G5-Ac₇₀-FA_n and FBP. Rupture forces were extracted from force-distance curves by determining the magnitude of (d-c).

Figure 3. Histogram and kernel density (solid line) of rupture force distribution for G5-AC₇₀-FA_n/FBP' interactions. The most probable (peak) rupture forces are 670 ± 69 pN (G5-AC₇₀-FA₀, inset), 83 ± 2 pN (G5-AC₇₀-FA_{2.7}), 201 ± 9 pN (G5-AC₇₀-FA_{4.7}), and 189 ± 5 pN (G5-AC₇₀-FA_{7.2}).

Figure 4. Histograms of rupture lengths (tip-sample separation distance) for G5-Ac₇₀-FA_n/FBP interactions. a) G5-Ac₇₀-FA_{2.7}, b) G5-Ac₇₀-FA_{4.7}, c) G5-Ac₇₀-FA_{7.2}.

Figure 5.

Histogram of rupture forces for G5-Ac₇₀-FA_n/FBP' interactions following pre- incubation with free FA. Pre- incubation with FA prompts a shift in the most probable (peak) rupture force from 189 ± 5 pN (G5-AC₇₀-FA_{7.2}) to 274 ± 36 pN (G5-AC₇₀-FA_{7.2}+ Free FA). This is accompanied by a 88% reduction in counted force events as compared to the measurement performed with no free folic acid present for the 0-800 pN region and a 91% reduction for the 0-400 pN region.

Figure 6.

Schematic summary of G5-FA_n/FBP binding interactions. A) Mostly likely binding modes for G5-FA_n. (i) indicates an example where a single FBP* has been induced by FA binding. (ii) indicates an example where multiple FBP* have been induced by FA binding and the polymer has the potential to interact with more than one FBP* as well as FBP. (iii) indicates a multiple FBP* binding by interaction with FBP converted to FBP* by an FA conjugated to a separate dendrimer. (iv) indicates an example where FA binding to FBP was sterically prevented from achieving sufficient binding to induce the structural change to FBP*. B) Preincubation gives FA binding and induces structural change to FBP* for most of the FBP on the surface. Subsequent binding by G5-FA_n generates an interaction with the maximum extent of G5-FA_n/FBP* binding and hence the maximum measured binding forces.