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Molecular energy dissipation in nanoscale networks of Dentin Matrix Protein 1 is strongly dependent on ion valence

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Abstract

The fracture resistance of biomineralized tissues such as bone, dentin, and abalone is greatly enhanced through the nanoscale interactions of stiff inorganic mineral components with soft organic adhesive components. A proper understanding of the interactions that occur within the organic component, and between the organic and inorganic components, is therefore critical for a complete understanding of the mechanics of these tissues. In this paper, we use Atomic Force Microscope (AFM) force spectroscopy and dynamic force spectroscopy to explore the effect of ionic interactions within a nanoscale system consisting of networks of Dentin Matrix Protein 1 (DMP1) (a component of both bone and dentin organic matrix), a mica surface, and an AFM tip. We find that DMP1 is capable of dissipating large amounts of energy through an ion-mediated mechanism, and that the effectiveness increases with increasing ion valence.

1. Introduction

Natural biocomposites, such as abalone and conch shell, marine sponge skeleton, and bone, exhibit very good fracture resistance by combining a mineral component with a small amount of organic components [1–7]. The organic components may serve, in part, as adhesives, and dissipate energy by resisting the separation of mineral segments [1]. Due to the severe health implications of both the mechanical degradation and mechanical failure of bone, it is a biocomposite of great medical importance. If the origins of mechanical strength and the fracture mechanisms that occur in bone could be understood at a molecular level then it might be possible to engineer better artificial bone tissue and to devise therapies to increase the mechanical strength and fracture resistance of bone in patients.

The nanoscale structural components of bone consist of type I collagen fibrils, mineral particles, and a non-fibrillar organic matrix [8]. A more detailed understanding of the nanoscale failure mechanisms of bone has shed light on the importance of the non-fibrillar organic matrix in providing bone with its remarkable mechanical properties, particularly within specific regions such as cement lines that lack substantial amounts of collagen matrix. Nanometer-level deformation studies have shown that bone deformation is distributed between tensile deformation of the stiff mineralized fibre material, and shearing of the more ductile non-fibrillar matrix [7,9]. The compression yield strength of bone was found to be augmented by nanogranular friction from mineral-mineral interactions [10]. A nanoscale energy dissipative mechanism, by which the non-fibrillar organic matrix resists separation of mineralized fibrils, was shown to be highly dependent on the presence of Ca^{2+} ions in the surrounding solution

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[4]. Recent work has shown that two acidic members of the SIBLING (Small Integrin-Binding Ligand N-linked Glycoprotein) family of mineralized tissue-enriched proteins, osteopontin (OPN) and bone sialoprotein (BSP), are capable of dissipating large amounts of energy through a Ca^{2+} mediated mechanism [11], and that homeotypic OPN-OPN bonds may provide physiologically significant strength [12]. Because OPN and related proteins are highly anionic, it is possible that interactions between negatively charged side chains and positively charged ions form the sacrificial bonds in the observed networks.

In this report, we explore the contribution that ionic interactions may play in the energy dissipation of networks of non-collagenous proteins, by measuring the effect of the counter ion valence on the energy dissipation, using a combination of AFM force spectroscopy and dynamic force spectroscopy. We used a model system of DMP1 deposited on mica to mimic the interactions that occur between DMP1 and mineral particles in vivo. We show that DMP1 displays an energy dissipation mechanism that is highly dependent on the ion valence.

2. Materials and Methods

2.1. Purification of Dentin Matrix Protein 1

Recombinant bovine DMP1 was produced in human bone marrow fibroblasts infected with an adenoviral vector carrying the full-length message driven by a strong promoter (CMV) as previously described [13]. Briefly, serum-free media collected from the infected cell culture was purified by HPLC ion exchange chromatography to >95% purity as defined by Coomassie blue and StainsAll-stained SDS polyacrylamide gel electrophoresis. The purified product was ~90% intact DMP1, with the remainder being the 57 kDa DMP1 fragment.

2.2. Sample Preparation

A small quantity of purified DMP1 was dissolved in purified water to a concentration of 0.2 $\mu\text{g}/\mu\text{l}$. Then, 4 μl of this solution was deposited onto a freshly cleaved mica surface and allowed to dry. The sample was subsequently placed in the fluid cell of a MultiMode IV AFM system with PicoForce (Veeco Inc., Santa Barbara, CA) equipped with Au-coated (including tip) SiN cantilevers (Model: OBL-105, Olympus Corp. Tokyo, Japan) with a nominal spring constant of 0.026 N/m. Samples were then rehydrated in a Na buffer solution (150 mM NaCl, 10 mM HEPES, pH 7.4).

2.3. AFM Force Spectroscopy

For testing the effect of divalent ions on DMP1 molecular energy dissipation, we used (in addition to the Na buffer) a Ca buffer solution (110 mM NaCl, 40 mM CaCl_2 , 10 mM HEPES, pH 7.4). A La buffer solution (110mM NaCl, 40 mM LaCl_3 , 10 mM HEPES, pH 7.4) was used for testing the effect of a trivalent ions. A solution of ethylenediaminetetraacetic acid (EDTA) (250 mM EDTA, pH 8.0) was used to chelate the di- and trivalent ions. The concentrations of the solutions were chosen to match our earlier work [4,6,11]. 200 μl buffer solution was flushed through the fluid cell (fluid cell approximate volume 30 μl). A set of 25 force curves were then conducted in a 5 by 5 grid pattern with a spacing of 1 μm . The approach and retract speed for the force curves was set at 951 nm/s. The cantilever was held on the surface for 3s at a deflection force of 500 pN, to allow for non-specific attachment of protein to the tip. This entire sequence, including a new flush with the solution under test, was then repeated 3 more times, for a total of 100 force curves, 4 at each grid position, per solution. The location of the grid was not changed throughout the experiment. To investigate the effects of Na, Ca, and EDTA, we used the following solution test order: Na, Ca, Na, Ca, Na, Ca, Na, Ca, EDTA, Ca, EDTA, Ca (with each solution being injected 4 times before moving to the next solution). For comparing the effects of La^{3+} ions to Ca^{2+} , the solution test order was: Na, Ca, EDTA, La, EDTA, Ca, EDTA,

La, EDTA, Ca, EDTA, La (again, each solution was injected 4 times before moving to the next solution).

2.4. AFM Dynamic Force Spectroscopy

To obtain the dynamic force spectrum of DMP1 networks, retraction speeds in the range from 200 nm/s to 3490 nm/s were investigated. The 3490 nm/s retraction speed was used as a baseline measurement throughout the experiment. A constant approach speed of 1050 nm/s was used. For each speed investigated, a set of 25 force curves were taken in a 5 by 5 grid pattern with a spacing of 1 μ m. The grid position was kept constant throughout the experiment. Three solutions (Na buffer, Ca buffer and La buffer) were tested. For each solution, the order for the experiment was (numbers are retraction speeds in nm/s): 3490, 200, 3490, 300, 3490, 600, 3490, 1000, 3490, 2000. Between solutions, 200 μ l EDTA was flushed through the fluid cell, and a set of 25 force curves were taken at the baseline retraction rate. Then, 200 μ l of buffer solution was flushed through the fluid cell, and another set of 25 force curves were taken at the baseline retraction rate. A further 200 μ l of buffer solution was flushed through the fluid cell before beginning the retraction rate variation sequence.

2.5. Data Analysis

The force curves were analyzed with custom data analysis software written in Labview 7.1 (National Instruments, Austin, TX) to obtain energy dissipated, maximum force and pull length for each curve.

3. Results

Atomic Force Microscopy (AFM) single molecule force spectroscopy experiments can reveal a wealth of information about the mechanical characteristics of individual proteins [14–17]. Typically, those experiments require the existence of well-defined attachment points to a single molecule with a well-defined tertiary structure. However, not all molecular systems of importance in biomechanics involve isolated molecules with well-defined tertiary structure. For experiments not involving single molecules or involving molecules without well-defined tertiary structures, the pulling curves can become much more complex [18,19]. There are nevertheless a number of parameters that can be used to characterize the molecular mechanics of complex or unknown pulling arrangements. Figure 1 shows three parameters which may be measured in the absence of any well-defined pulling construct. Similar to results found for OPN, BSP, gluten and aggrecan-hyaluronic acid complexes [11,18], the pulling signature of DMP1 (with characteristics such as force peaks followed by both higher and lower peaks, and stepwise decreasing peaks) indicates that the protein readily forms extended networks consisting of multiple molecules. The curve in figure 1 illustrates the example signature of force peaks followed by both higher and lower peaks. We observed force curves with a pull length of order 1 μ m and longer, also illustrated in figure 1, a distance much longer than the stretched length of DMP1 (at 510 residues and an average length of 0.35 nm per residue, DMP1 should have a stretched length of approximately 180 nm).

Figure 2 shows the effect of introducing Ca^{2+} ions into the surrounding solution. Upon the introduction of Ca buffer, the energy dissipated increased about three-fold (from an average level of about 0.2×10^{-16} J to about 0.6×10^{-16} J). The maximum force increased a similar amount (from 0.2 nN to 0.7 nN), and the pull length remained roughly equal. The ability of the network to retain Ca^{2+} ions was tested by flushing the fluid cell with Na buffer, which should remove Ca^{2+} ions from the surrounding solution and provide Na^+ ions for binding competition. The first 200 μ l flush of Na buffer after Ca^{2+} ions reduced the energy dissipation and maximum force by about 50%. Additional solution changes were needed before the energy dissipated and maximum force returned back to the initial level with Na^+ ions. We also tested

removal of Ca^{2+} ions by chemical means through chelation with EDTA. In contrast to the results from flushing with Na buffer, for EDTA the change in energy dissipation and maximum force after the first 200 μl EDTA flush was much greater than the change in those parameters after the second EDTA flush.

Two further effects were observed after the use of EDTA. When returning to test with Ca buffer after EDTA, there was a lag of one flush in the rise of the energy dissipation and maximum force levels. The lag was not observable in the change from Na buffer to Ca buffer. This is likely due to the fact that EDTA was present in the fluid cell when new Ca buffer solution was flushed through. Thus, many of the ions in the incoming solution would have been immediately chelated, and the effective concentration of incoming Ca^{2+} ions drastically reduced. Additionally, there appears to be a larger increase in the energy dissipated and maximum force levels upon re-introduction of Ca buffer after EDTA treatment, compared to the energy dissipated and maximum force levels in Ca buffer before EDTA treatment. This effect could be due to the EDTA removing any impurity ions initially present in the system, or to EDTA causing enough unbinding to make previously inaccessible, tangled regions accessible.

The response of DMP1 to pulling in solutions containing another divalent ion was also tested, by a substitution of Sr^{2+} for Ca^{2+} (100 mM NaCl, 40 mM SrCl_2 , 10 mM HEPES, pH 7.4). By cycling between Ca and Sr test solutions, interspersed with EDTA, we were unable to find any reproducible difference between the energy dissipation of DMP1 networks in Ca buffer and in Sr buffer. It appears that at least this divalent ion from the same chemical group produces a similar effect as divalent Ca^{2+} ions.

To investigate the effect of a higher valence ion, La^{3+} was chosen due to its solubility at physiological pH. The average level of energy dissipated in La buffer was significantly higher than the level in Ca buffer (figure 3). Part of this large increase was due to the enhancing effect after EDTA treatment described previously. However, cycling between Ca and La buffers revealed that La^{3+} ions caused consistently higher energy dissipation levels, as shown in figure 3. In all three parameters, energy dissipated, maximum force, and pull length, increased values in La buffer are observed. Table 1 summarizes the results. The energy dissipated and maximum force showed larger increases in La buffer (approximately an order of magnitude above the Na buffer levels) than the pull length (only about 1.5 to 2-fold above the Na buffer levels). The results for Ca^{2+} compared with Na^+ are in good agreement with the data shown earlier in figure 2.

We also tested the response of DMP1 to pulling rate variations from 200 nm/s to 3490 nm/s with three different ions, Na^+ , Ca^{2+} and La^{3+} . As expected, the maximum force increased with increasing retraction rate, for all three ions tested. Due to the complex, random, multiple-molecule interactions taking place during separation of the DMP1 networks, it was not obvious that a linear dependence on the loading rate would be observed, as would be expected for a simple ligand-receptor bond rupture [20]. However, as can be seen in figure 4, the data are well approximated by a linear fit to the logarithm of the pulling rate. The slope of the linear fit to the La and Ca data were measured at 460 ± 80 pN/decade(nm/s) and 500 ± 100 pN/decade(nm/s) respectively, and are equal to each other within statistical uncertainty of the linear fit. There is, however, a notable vertical shift in the La data over the Ca data. The slope for the Na data, at 60 ± 18 pN/decade(nm/s), was found to be significantly lower than the slope of either Ca or La. Degradation over time of the absolute maximum force value was observed during the course of the dynamic force spectroscopy experiments. A similar degradation of the absolute maximum forces with time can be seen in figure 3. We do not know the source of this degradation, but speculate that it may be due to such things as a change in the amount of material adsorbed on one or more of the surfaces, or a change in one or more of the surface properties, or possibly through such events as breaking molecules, breaking bonds that do not readily

reform, or the formation of knots or tangles which cannot be unfolded. To correct for this effect, the data shown in figure 4 have been adjusted for a change in time of the baseline measurement. A 2nd order polynomial was fit to the maximum force vs. time 3490 nm/s baseline data for each solution tested, and the data rescaled by this baseline fit.

4. Discussion

DMP 1 is an acidic protein and member of the SIBLING family [21]. It contains many negatively charged residues at physiological pH, and many post-translational modifications such as phosphate and acid carbohydrate (e.g. sialic acid) groups, adding further negatively charged groups. Although initially postulated to be dentin-specific [22], it has been located in bone matrix [23] and many non-mineralizing tissues such as specific active ductal epithelial tissues [24]. DMP1 is believed to bind strongly to calcium and display a high affinity for mineral platelets [25]; thus, when highly expressed in mineralized tissues it has a suspected functional role in the mineralization process itself [26–30]. Tartaix et al. found that, in its native form, DMP1 inhibits mineralization, but, when cleaved into highly phosphorylated 57 kDa peptides or dephosphorylated in recombinant form, it may initialize mineralization [26]. He et al. found that nucleation of mineral platelets via DMP1 began by DMP1 binding Ca. A self-assembly process, whereby DMP1 was able to oligomerize in the presence of Ca²⁺ ions, was found essential for mineral nucleation [25]. Our results provide evidence that DMP1 strongly binds not only Ca²⁺ ions, but other multivalent ions as well. We have additionally shown that the energy dissipation of networks of DMP1 resisting tip-mica separation is highly dependent on the valence of the ion in the surrounding solution. We observed large increases in both the measured energy dissipation and maximum force of the pulling curves that followed increases in the valence of the ions in the buffer solution. In contrast, the measured pull length of the force curves increased by only a small amount in Ca buffer, and by only a slightly larger amount in La buffer. This suggests that the DMP1 protein-protein and protein-surface interactions are present with monovalent ions in solution, but are significantly enhanced with addition of multivalent ions.

The levels of energy dissipated and maximum force observed are quite large on a molecular scale. For example, the energy dissipated in stretching a single worm-like-chain molecule with persistence length 0.3 nm (typical for a flexible polypeptide [31]) and contour length 180 nm (the approximate contour length of DMP1) to a maximum force 2 nN is on the order of 0.1×10^{-16} J, about 1% to 10% of the energy dissipated observed in our experiments. The combination of multiple DMP1 molecules interacting through the sacrificial-bond and hidden-length mechanism would match well with the observed force curves, and could explain the large values of energy dissipation [1,18]. In this mechanism, the majority of the energy is dissipated through work against entropic elasticity. Initially, the network is highly tangled and held together by “sacrificial” bonds which are weaker than the molecular backbone. Only the accessible length of the molecules is subject to the applied force. As the pulling force increases and the molecules are stretched, sacrificial bonds are ruptured. Additional “hidden” length is revealed and subjected to the applied force, and further work against entropic elasticity must be done to stretch the molecule. The random nature of the sacrificial bonds, along with the continual release of hidden length leads to complex, jagged force curves, as seen in figure 1. We believe that sacrificial bonds in this system are likely formed by positively charged ions bound from the buffer solution acting as salt bridges between the many negatively charged groups in DMP1, such as phosphorylated or carboxylated residues. Thus, higher valence positive ions would make better bridges between the negative groups. We note that, although the majority of the energy is dissipated as work against entropic elasticity, the entropic elasticity does not change with the introduction of higher valence ions. Rather, it is the increased sacrificial bond strength, allowing the pulling forces to rise higher before rupture, which results in increased energy dissipation.

Several different interactions are possible in the observed force curves: protein-protein, protein-mica and protein-tip. Since DMP1 contains one cysteine residue, it is possible for the protein to bind strongly to the Au-coated AFM tip. We observed only minimal adhesion when testing a used tip on a clean mica surface, suggesting that while some material does bind to the tip, there is not a large aggregation on the tip. DMP1 could also bind strongly to the mica surface (through multivalent ions linking the negative side group charges on the protein and the negative surface charges on the mica). Based on these reasons, we speculate that primary rupture occurs in the DMP1 network. Furthermore, because the pull length may extend much greater distances than an individual DMP1 molecule and is not consistent from one force curve to the next, there must be some rupture event along the DMP1 bridge between mica and tip. However, we have also on occasion observed plateaus in the force curves characteristic of polyelectrolyte desorption from a surface [32], indicating DMP1 desorption from the mica surface. Additionally, the degradation in absolute maximum forces may be indicative of an effect due predominantly to a change in the protein-tip interaction, for example by a reduction in the amount of material binding the tip or a change in the adsorption of DMP1 to the tip. Our experimental method precludes us from accurately distinguishing these different interactions, leaving open the question of which is most influential.

Other proteins in the SIBLING family (OPN and BSP) have been shown to be unstructured in solution [33]. In that study, insufficient quantities of DMP1 were available to assess its structure, however, based on its hydrophilic sequence, it was predicted to be mostly if not entirely flexible. This flexibility may be important for the recoverability of the energy dissipative mechanism following EDTA treatment. In a recent study by Sun et al., divalent Ca^{2+} and Mg^{2+} ions were found to play a significant role in maintaining the stickiness of a glue secreted by the marine worm *Phragmatopoma californica* [34]. There, the glue performance was greatly reduced by treatment with EDTA, as in this study on DMP1. In contrast to this study on DMP1, the initial performance levels of that marine worm glue could not be recovered upon reintroduction of Ca^{2+} and Mg^{2+} ions. A structural collapse of glue was observable following EDTA treatment, leading to the conclusion that the adhesive function depends in part on ions maintaining a specific conformation of the proteins involved. Because we observe recoverable adhesive properties by reintroducing multivalent ions following EDTA treatment, the energy dissipative mechanism in DMP1 networks does not appear to exhibit this type of conformation dependence, a result consistent with the prediction that DMP1 is predominantly unstructured.

The mica surface used in our experiment presents similar chemical groups to mineral platelets that DMP1 would experience in-vivo, specifically, negatively charged surface hydroxyl groups, and as such provides an adequate model system for studying interactions of DMP1 with mineral platelets. Our model nanoscale system provides insights into the mechanics of tissues involving DMP1 and other similar, negatively charged flexible molecules. The large energy dissipation of DMP1 and the robust, self-healing capability, as evidenced by repeated hysteretic force curves and recovery following EDTA treatment, are good characteristics for a providing self-healing and fracture resistance to a biocomposite. The results of our model system indicate that, for biomaterials such as bone and teeth, one possible additional role of DMP1, and other similar molecules, may be to dissipate energy by resisting the separation of mineral crystals (particularly in regions such as cement lines that are known to be rich in SIBLING proteins but are relatively collagen deficient) through protein networks governed by an ion mediated mechanism.

The absolute values of the measured parameters strongly depended on the amount of material being investigated, making a direct numerical comparison of samples difficult. However, a measurement of the relative effects of the different ions tested remained comparable between samples. This indicates that knowing the amount of protein we investigate in a single experiment is not critical, as long as it is roughly consistent over the region investigated in a

single experiment. Assuming densely packed DMP1 has a density of 1.35 kg/l and the adsorbed protein on the mica spreads uniformly to a diameter of 1 μm , we calculate the thickness of the protein layer to be approximately 7.5 nm. Because DMP1 is mainly negatively charged, there is likely some degree of electrostatic repulsion in the adsorbed layer, making this thickness estimate a lower bound. AFM imaging of the mica surface following the initial protein deposition and following completion of the experiment revealed a dense, uniform surface coverage over length-scales relevant to our test procedure, in rough agreement with expectation. The surface dwell time before tip retraction also has an influence on the absolute values of the measured data. The general trend is for more adhesion with greater resting time [11]. For this reason, we used a constant dwell time of 3 seconds.

Obtaining a detailed understanding of the energy landscape from our dynamic force spectrum is made extremely complex due to the random arrangement of molecules in our experimental technique. However, we do have indications from the force curves that many of the bonds are loaded in parallel, and thus can make an initial comparison of our results with models for the detachment of multiple parallel bonds. The appearance of a linear dependence on the logarithm of the loading rate matches more closely with modelling and experimental observations for low numbers of multiple streptavidin-biotin bonds [35] than to alternate relationships predicted by simulations [36] or analytical analysis [37] and observed experimentally [38] for a larger number of bonds. This suggests that our results may be better described by a stochastic as opposed to deterministic model. It also appears, from the change in height of the force spectrum between the Ca^{2+} and La^{3+} buffer solutions, that the strength of the bonds in the DMP1 system are significantly increased with increasing counterion valence. It is interesting to note that an effect most likely due to a change in the ionic interactions between protein side chains manifests itself in a measurable change in the dynamic force spectrum of a random network of molecules. Thus, the dynamic force spectrum may present useful information for studying ion-dependent effects in multiple-bond biomolecular interactions.

5. Conclusion

There is great importance, as well as considerable experimental difficulty, in studying the networks of molecules that are important for the mechanics of tissues, including hard tissues such as dentin and bone. Here we show that it is possible to make a start on this important, but difficult, problem by using the techniques of force spectroscopy in a simple model system: DMP1 adsorbed on a mica surface, probed with an Au-coated AFM tip. We found that molecular energy dissipation was substantially increased with the addition of Ca^{2+} ions into the surrounding solution, and further increased with the addition of La^{3+} ions. The large number of negatively charged residues and the strong dependence of energy dissipation on ion valence suggest that DMP1 dissipates energy through sacrificial bond interactions of negatively charged side groups linked by positive multivalent ions. This simple system serves as a crude model for the in vivo interactions of DMP1 networks with mineral platelets, thus providing indication that DMP1 has an adhesive role between mineral platelets in mineralized tissue such as dentin and bone, in which it is known to be present. This is not to say that DMP1 is the dominant or even the most important, adhesive molecule in these systems. Further research will be needed to quantify the relative contributions of different molecules in these systems.

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References

1. Smith BL, et al. Molecular mechanistic origin of the toughness of natural adhesives, fibres and composites. *Nature* 1999;399:761–3.
2. Kamat S, Su X, Ballarini R, Heuer AH. Structural basis for the fracture toughness of the shell of the conch *Strombus gigas*. *Nature* 2000;405:1036–40. [PubMed: 10890440]
3. Aizenberg J, Weaver JC, Thanawala MS, Sundar VC, Morse DE, Fratzl P. Skeleton of *Euplectella* sp.: Structural hierarchy from the nanoscale to the macroscale. *Science* 2005;309:275–8. [PubMed: 16002612]
4. Fantner GE, et al. Sacrificial Bonds and Hidden Length Dissipate Energy as Mineralized Fibrils Separate During Bone Fracture. *Nat Mater* 2005;4:612–6. [PubMed: 16025123]
5. Fantner GE, et al. Influence of the degradation of the organic matrix on the microscopic fracture behavior of trabecular bone. *Bone* 2004;35:1013–22. [PubMed: 15542025]
6. Thompson JB, Kindt JH, Drake B, Hansma HG, Morse DE, Hansma PK. Bone indentation recovery time correlates with bond reforming time. *Nature* 2001;414:773–6. [PubMed: 11742405]
7. Gupta HS, Wagermaier W, Zickler GA, Aroush DRB, Funari SS, Roschger P, Wagner HD, Fratzl P. Nanoscale deformation mechanisms in bone. *Nano Lett* 2005;5:2108–11. [PubMed: 16218747]
8. Fantner GE, et al. Hierarchical interconnections in the nano-composite material bone: Fibrillar cross-links resist fracture on several length scales. *Compos Sci Technol* 2006;66:1205–11.
9. Gupta HS, Seto J, Wagermaier W, Zaslansky P, Boesecke P, Fratzl P. Cooperative deformation of mineral and collagen in bone at the nanoscale. *Proc Natl Acad Sci USA* 2006;103:17741–6. [PubMed: 17095608]
10. Tai K, Ulm FJ, Ortiz C. Nanogranular origins of the strength of bone. *Nano Lett* 2006;6:2520–5. [PubMed: 17090084]
11. Fantner GE, Adams J, Turner P, Thurner PJ, Fisher LW, Hansma PK. Nanoscale ion mediated networks in bone: Osteopontin can repeatedly dissipate large amounts of energy. *Nano Lett* 2007;7:2491–8. [PubMed: 17645366]
12. Goldsmith HL, Labrosse JM, McIntosh FA, Maenpaa PH, Kaartinen MT, McKee MD. Homotypic interactions of soluble and immobilized osteopontin. *Ann Biomed Eng* 2002;30:840–50. [PubMed: 12220083]
13. Jain A, Karadag A, Fohr B, Fisher LW, Fedarko NS. Three SIBLINGs (small integrin-binding ligand, N-linked glycoproteins) enhance factor H's cofactor activity enabling MCP-like cellular evasion of complement-mediated attack. *J Biol Chem* 2002;277:13700–8. [PubMed: 11825898]
14. Marszalek PE, Lu H, Li HB, Carrion-Vazquez M, Oberhauser AF, Schulten K, Fernandez JM. Mechanical unfolding intermediates in titin modules. *Nature* 1999;402:100–3. [PubMed: 10573426]
15. Rief M, Gautel M, Oesterhelt F, Fernandez JM, Gaub HE. Reversible unfolding of individual titin immunoglobulin domains by AFM. *Science* 1997;276:1109–12. [PubMed: 9148804]
16. Rief M, Oesterhelt F, Heymann B, Gaub HE. Single molecule force spectroscopy on polysaccharides by atomic force microscopy. *Science* 1997;275:1295–7. [PubMed: 9036852]
17. Janovjak H, Struckmeier J, Hubain M, Kedrov A, Kessler M, Muller DJ. Probing the energy landscape of the membrane protein bacteriorhodopsin. *Structure* 2004;12:871–9. [PubMed: 15130479]
18. Fantner GE, et al. Sacrificial bonds and hidden length: Unraveling molecular mesostructures in tough materials. *Biophys J* 2006;90:1411–8. [PubMed: 16326907]
19. Qi HJ, Ortiz C, Boyce MC. Mechanics of biomacromolecular networks containing folded domains. *J Eng Mater Tech-T ASME* 2006;128:509–18.
20. Merkel R, Nassoy P, Leung A, Ritchie K, Evans E. Energy landscapes of receptor-ligand bonds explored with dynamic force spectroscopy. *Nature* 1999;397:50–3. [PubMed: 9892352]
21. George A, Sabsay B, Simonian PAL, Veis A. Characterization of a Novel Dentin Matrix Acidic Phosphoprotein - Implications for Induction of Biomineralization. *J Biol Chem* 1993;268:12624–30. [PubMed: 8509401]

22. George A, Silberstein R, Veis A. In situ hybridization shows Dmp1 (AG1) to be a developmentally regulated dentin-specific protein produced by mature odontoblasts. *Connect Tissue Res* 1995;33:67–72. [PubMed: 7554964]
23. MacDougall M, Gu TT, Luan XG, Simmons D, Chen JK. Identification of a novel isoform of mouse dentin matrix protein 1: Spatial expression in mineralized tissues. *J Bone Miner Res* 1998;13:422–31. [PubMed: 9525343]
24. Ogbureke KUE, Fisher LW. SIBLING expression patterns in duct epithelia reflect the degree of metabolic activity. *J Histochem Cytochem* 2007;55:403–9. [PubMed: 17210923]
25. He G, Dahl T, Veis A, George A. Nucleation of apatite crystals in vitro by self-assembled dentin matrix protein, 1. *Nat Mater* 2003;2:552–8. [PubMed: 12872163]
26. Tartaix PH, et al. In vitro effects of dentin matrix protein-1 on hydroxyapatite formation provide insights into in vivo functions. *J Biol Chem* 2004;279:18115–20. [PubMed: 14769788]
27. Ling YF, Rios HF, Myers ER, Lu YB, Feng JQ, Boskey AL. DMP1 depletion decreases bone mineralization in vivo: An FTIR imaging analysis. *J Bone Miner Res* 2005;20:2169–77. [PubMed: 16294270]
28. Qin C, Baba O, Butler WT. Post-translational modifications of sibling proteins and their roles in osteogenesis and dentinogenesis. *Crit Rev Oral Biol M* 2004;15:126–36. [PubMed: 15187031]
29. Narayanan K, Ramachandran A, Hao JJ, He G, Park KW, Cho M, George A. Dual functional roles of dentin matrix protein 1 - Implications in biomineralization and gene transcription by activation of intracellular Ca²⁺ store. *J Biol Chem* 2003;278:17500–8. [PubMed: 12615915]
30. Toyosawa S, Kanatani N, Shintani S, Kobata M, Yuki M, Kishino M, Ijuhin N, Komori T. Expression of dentin matrix protein 1 (DMP1) during fracture healing. *Bone* 2004;35:553–61. [PubMed: 15268908]
31. Zhou HX. Polymer models of protein stability, folding, and interactions. *Biochemistry* 2004;43:2141–54. [PubMed: 14979710]
32. Hugel T, Seitz M. The study of molecular interactions by AFM force spectroscopy. *Macromol Rapid Comm* 2001;22:989–1016.
33. Fisher LW, Torchia DA, Fohr B, Young MF, Fedarko NS. Flexible structures of SIBLING proteins, bone sialoprotein, and osteopontin. *Biochem Bioph Res Co* 2001;280:460–5.
34. Sun C, Fantner GE, Adams J, Hansma PK, Waite JH. The role of calcium and magnesium in the concrete tubes of the sandcastle worm. *J Exp Biol* 2007;210:1481–8. [PubMed: 17401131]
35. Erdmann T, Pierrat S, Nassoy P, Schwarz US. Dynamic force spectroscopy on multiple bonds: Experiments and model. *Europhys Lett* 2008;81:48001.
36. Li F, Leckband D. Dynamic strength of molecularly bonded surfaces. *J Chem Phys* 2006;125:194702. [PubMed: 17129145]
37. Seifert U. Rupture of multiple parallel molecular bonds under dynamic loading. *Phys Rev Lett* 2000;84:2750–3. [PubMed: 11017316]
38. Prechtel K, Bausch AR, Marchi-Artzner V, Kantelehner M, Kessler H, Merkel R. Dynamic force Spectroscopy to probe adhesion strength of living cells. *Phys Rev Lett* 2002;89:028101. [PubMed: 12097018]

Figure 1.

Parameters that may be used to characterize molecular energy dissipation in the absence of well-defined pulling constructs. The energy dissipated (grey area) is the hysteresis between the approach curve (bottom) and retraction curve (top). Maximum force is defined as the point of greatest difference between the retraction curve and the zero-deflection force level. The pull length is the length from zero separation to the maximum distance at which there is a non-zero positive deflection. The example force curve shown is DMP1 in Ca buffer. A pull length of almost 1 μm , compared with a stretched length of 180 nm for a single DMP1 molecule, and a network force curve signature of force peaks followed by both higher and lower subsequent peaks, indicate the interaction of multiple molecules in a network.

Figure 2.

Ca²⁺ ions increase the molecular energy dissipation of DMP1. Each bar shows the average and standard deviation for 25 individual pulling curves following a 200 μ l flush of the indicated solution. The energy dissipated, maximum force and pull length are shown. Large increases are observable in energy dissipation and maximum force upon the introduction of Ca²⁺ ions. The changes in pull length are much smaller. Thus the increase in energy dissipation is primarily due to the increase in maximum force. The increase is reduced gradually by flushing out the Ca²⁺ ions with Na buffer, and reduced in a much quicker fashion by chelating the Ca²⁺ ions with EDTA. Following either of these treatments, a return to elevated energy dissipation levels is achieved with a reintroduction of Ca²⁺ ions

Figure 3.

La³⁺ ions further increase the molecular energy dissipation of DMP1 over Ca²⁺ ions. Each bar shows the average and standard deviation for 25 individual pulling curves following a 200 μl flush of the indicated solution. Large increases in energy dissipation and maximum force are observed in La buffer compared with both Na and Ca buffer. The ionic-enhancement effect is reversible with EDTA treatment and may be cycled repeatedly many times.

Figure 4.

The dynamic force spectrum of DMP1 in Na buffer, Ca buffer and La buffer. The data for each solution tested appear to be well described by a linear dependence on the logarithm of the separation rate. The slope in La buffer and Ca buffer, at 460 ± 80 pN/decade(nm/s) and 500 ± 100 pN/decade(nm/s) respectively, are equal to each other within experimental error. The slope in Na buffer, at 60 ± 18 pN/decade(nm/s), is significantly different from either.

Absolute and relative comparison of energy dissipated, maximum force, and pulling length in Na buffer, Ca buffer and La buffer, corresponding to the data presented in figure 3.

Table 1

Dataset	Energy dissipated (10^{-16} J)	Energy dissipated (normalized)	Maximum force (pN)	Maximum force (normalized)	Pulling length (nm)	Pulling length (normalized)
Na ⁺ average	0.08	1	130	1	150	1
Ca ²⁺ range	0.2 – 0.6	2.5 – 7.5	325 – 670	2.5 – 5	150 – 220	1 – 1.5
La ³⁺ range	0.8 – 1.7	10 – 21	750 – 1100	6 – 8.5	200 – 330	1.5 – 2.2