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Engineering Proteins with Enhanced Mechanical Stability by Force Specific Sequence Motifs

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Abstract

Use of atomic force microscopy (AFM) has recently led to a better understanding of the molecular mechanisms of the unfolding process by mechanical forces; however, the rational design of novel proteins with specific mechanical strength remains challenging. We have approached this problem from a new perspective that generates linear physical-chemical properties (PCP) motifs from a limited AFM data set. Guided by our linear sequence analysis we designed and analyzed four new mutants of the titin I1 domain with the goal of increasing the domain's mechanical strength. All four mutants could be cloned and expressed as soluble proteins. AFM data indicate that at least two of the mutants have increased molecular mechanical strength. This observation suggests that the PCP method is useful to graft sequences specific for high mechanical stability to weak proteins to increase their mechanical stability, and represents an additional tool in the design of novel proteins besides steered molecular dynamics calculations, coarse grained simulations and phi-value analysis of the transition state.

Keywords

titin; atomic force microscopy; CD spectroscopy; protein design; physical-chemical property motifs; molecular dynamics calculations

Introduction

Many cellular activities of proteins, such as such cell adhesion, translocation or ligand binding, require a specific mechanical strength of the protein fold[1-5]. In the case of the muscle protein titin, the main biological function of the protein is to provide structural resistance to unfolding forces during stretching of the muscle sarcomere and restoring the native fold once external forces are no longer applied[6-11]. Even some proteins with no obvious mechanical functions, like the B1 domain of protein G or ubiquitin, show a remarkably high mechanical stability[12-14]. Progress in understanding how proteins react to external unfolding forces, using single molecule force spectroscopy by atomic force

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microscopy (AFM) or laser optical tweezers, combined with steered molecular dynamics calculations has been made in recent years[5,15], yet general rules that govern the mechanical stability of proteins are still elusive.

Steered Molecular Dynamics (SMD) calculations on titin, fibronectin and other β-barrel domains provided evidence that the topology of secondary structures in proteins plays an important role in their mechanical strength[8,16-20]. It was observed that two β-strands, forming hydrogen bonds between the N and the C-terminal end of the polypeptide chain, function as a mechanical clamp that resists the shear forces applied to the termini. The importance of the hydrogen bond network was verified by AFM experiments on mutant proteins with point mutations that disturb these hydrogen bonds [21,22]. More recently it was found that the shear mechanical clamp motif occurs also in other mechanically stable proteins such as the scaffoldings [23]. Thus it was proposed that the shear mechanical clamp was responsible for the main force barrier observed in the AFM experiments. However, the fact that different Ig domains with homologous folds could have various unfolding forces ranging from 100pN to 350pN (at pulling velocity of ~0.5nm/ms) [24] implies that the mechanical clamp is not the only contributor, and that the side chains and the hydrophobic interactions of the core are also involved in the mechanical stability.

For the rational design of proteins with specified molecular strengths one needs a comprehensive theory of the contribution of individual residues to the experimentally observed mechanical strength. Particularly challenging is the design of novel proteins with increased mechanical strength [5,25,26]. Our design strategy uses force-specific linear sequence motifs that we recently determined for the titin domains[27]. The approach took advantage of the differences in the mechanical stabilities of the Ig domains in the I-band of titin, where the Ig domains close to the N-terminus (weak domains) unfold at forces ~100pN lower than the Ig domains close to the C-terminus of the I-band (strong domains) [24]. We have used sequence analysis techniques with the software PCPMer[28] to search for conserved physical-chemical properties of residues among weak domains and across species and compared them to those physical-chemical properties found in strong domains. We identified two sequence motifs that are unique among strong Ig domains and two motifs that are unique for the weak Ig domains of titin. To experimentally test the impact of these motifs on the mechanical stability of the Ig domains we now designed four new mutants of the weak I1 domain of titin guided by these motifs. The analysis of the mechanical strengths of these mutants by AFM experiments confirmed that at least two of the mutants have increased mechanical strength as predicted by our design. This encouraging result represents the first step to further delineate the contribution of individual motifs to the overall mechanical strength and to establish a quantitative predictive method of mechanical stability for domains with identical folds but large sequence variations.

Methods

Expression and purification of polyproteins

The DNA sequences for the four engineered mutants of the I1 domain were synthesized by DNA2.0 Inc. Polyproteins with five sequential arranged domains were constructed for each designed domain to measure the effects on the mechanical stability by AFM experiments. Each polyprotein is composed of four wild type I27 domains flanking one designed target domain in the middle. The wild type I27 domain is used as an internal fingerprint since its mechanical property is well characterized. A 6His-tag was attached to the N-terminal for purification and anchoring to the cover slip. The dipeptide Cys-Cys was added to the N-terminal for the attachment to the AFM probe. The polyproteins were expressed in *E.coli BL21* using home-made plasmid *pAFM* [29] and purified by Ni-affinity chromatography.

CD spectroscopy

CD spectra of all the polyproteins were recorded with *JASCO J-815* at room temperature. A 10mM Phosphate Buffer was used as buffer for the proteins and its signal was later subtracted from the spectra. The path length of the cuvette was 1mm. The concentrations of proteins were determined by Bradford assay for the fitting of the CD spectra. Each spectrum was averaged from three scans from 200nm to 260nm at scanning speed of 50nm/min.

Atomic Force Microscopy

The unfolding forces of the novel proteins were measured with our home-built AFM instrument at room temperature. The polyproteins were diluted with 10mM PBS buffer, and 10mM Dithiothreitol (DTT) was added to the solution to prevent polyproteins from forming dimmers. A drop of ~10μl solution was incubated on the Ni-coated cover slips for 15min and then rinsed with PBS to remove unbound polyproteins. The AFM probe used in the experiments was *MLCT* (*Bruker, CA*) with typical spring constant of 0.03N/m, and the pulling speed was ~0.5nm/ms.

Homology modeling—Three-dimensional structural models for 38 Ig domains with unknown 3D structures in the I-band of cardiac titin and the four designed domains I1s1s2, I1s1s2-w1w3, I1_I27AB and I1_I27AD were generated with standard methods successfully used in our laboratory in the past[28,30]. First, the sequences of the 38 titin domains were submitted to the meta-server, Genesilico [31] to search for the best template structure. All possible templates obtained from the meta-server were analyzed manually and the best template was selected for homology modeling. Homology model structures were generated using our homology modeling software package MPACK, and the software package modeller [32]. The model structures obtained from MPACK were further energy minimized using the FANTOM program. The RMSD value between the template and model structures obtained from MPACK and modeller was found to be less than 0.5 Å. Finally, the model structures and the X-ray crystal structures of I1 and I27 were energy minimized in a water box with TIP3P water molecules for 10000 iterations with the NAMD software tool [33] to test the stability of the model structures. The RMSD between the final model structures and the templates was calculated by Pymol (The PyMOL Molecular Graphics System, Version 1.3, Schrödinger, LLC).

Results

Design of I1 like domains of titin with increased mechanical stability

The sequences of the different cardiac muscle Ig domains of the I-band are quite diverse with an average sequence identity of only 25%[27]. On the other hand the available experimental 3D structures of titin domains, as the NMR solution structures of I27[6] and M5[7], or the X-ray crystal structure of I1[10] and our comprehensive 3D modeling for all the other 38 Ig cardiac titin domains indicate that all titin Ig domains share the same Ig-like fold despite the large variation amino acid sequences. We analyzed the homologous sequences from up to 10 different species of titin domains for conservation of their physical-chemical properties (PCPs) of individual residues. By generating a multiple sequence alignment of all sequences of the weak domains I1, I4 and I5 and across different species and correspondingly from the strong domains I27, I28, I32 and I34 we identified physical-chemical property (PCP) motifs separately for weak and strong Ig domains by our software PCPMer[28,34-37]. PCPMer defines motifs PCP motifs by conservation of 5 quantitative descriptors of amino acids [38]. Some of the motifs are common to both families, reflecting the similar 3D fold of the domains. However, we were able to find also unique motifs for each family[27]. We consider those motifs as force specific that can be used to characterize weak and strong domains by a sequence signature.

These unique motifs, two strong motifs s1 and s2, and two weak motifs, w1 and w3, are mapped on the amino acid sequences of wild type I27 and I1 domains (Fig. 1). The design goal was to increase the mechanical strength of the weak domain I1 by introducing motifs from the strong domains, or to introduce segments encompassing these motifs. The unfolding force of wild type I1 is ~100pN at pulling velocity of ~0.5nm/ms [21], compared to ~200pN of I27 [22]. In the alignment of the sequences of the domains I1 and I27, derived from a structural alignment of their 3D structures, we substituted homologous positions in I1 by residues corresponding the strong motifs s1 and s2 in I27 (construct domain I1s1s2). In another construct, I1s1s2-w1w3, the weak motifs w1 and w3 in I1 were in addition replaced by corresponding residues from I27. To ensure the side chain interactions and hydrophobic interactions in the core are also considered, we constructed two other hybrid proteins, I1_I27AB by introducing the whole segment of A-B strands and I1_I27AD by introducing A-D strands. Homology models for all four engineered proteins were generated using I1 (PDB: 1G1C) as template and the substituted resides were mapped on the models (Fig. 2 ABCD). All four designed domains could be energy refined to similar low energy values as the 3D models of the other Ig domains of cardiac titin and the experimentally known structures of I1 and I27 indicating that the designed domains have a stable fold (Fig. 2E).

Stability and folds of the designed domains

The CD spectra of the four polyproteins confirmed the stabilities of the designed domains. We measured far-UV CD spectra to evaluate the extent of the secondary structures using a polyprotein with the wild type I1 as control. All the engineered proteins showed spectra of similar patterns, characteristic for β-strands proteins (Fig. 3). The results confirmed that the engineered domains fold properly into β-sandwich structures.

The mechanical strengths of the engineered novel proteins

The mechanical strengths of the designed domains were measured by AFM in the context of a polyprotein with five domains (Fig. 4A). The target domain containing the four designed domains or the domain I1 (as a control), is fused to two wild type I27 domains on either side. The wt I27 domain serves as a calibration domain for the force measurement. The molecular weight of the polyprotein is ~50KD, which was verified in SDS-PAGE (Fig. 4B). The AFM measurements were carried out in 10mM PBS buffer and 10mM Dithiothreitol (DTT) was added to the solution to prevent polyproteins forming dimers.

With the wild type I1 as target domain, we observed one low peak for the I1 domain and four high peaks for the I27 domain in the force extension profile as expected (Fig. 4C). The four peaks around 200pN correspond to the mechanical unfolding of the four I27 domains and the lower peak around 100pN corresponds to the unfolding of wild type I1 domain. Statistical analysis of the peaks in 44 recordings confirmed this assignment with a mean force of ~100pN (98 ± 22pN) in 44 low peaks and ~200pN (201± 27 pN) for 176 high peaks (Fig. 4D). The bimodal distribution of these unfolding forces demonstrates the significant difference in the mechanical strengths of the I1 and I27 domains.

For the polyprotein containing the I1s1s2 target domain, we observed 5 peaks with approximately the same unfolding force (Fig. 5A). As one of these peaks must correspond to the unfolding of the target domain, the designed domain I1s1s2 has approximately the same strength of 200 pN as the wild type I27. Thus introducing the two strong motifs in I1 increased the mechanical strength of the designed domain by about 100pN. This finding is further verified by the unimodal distribution of the unfolding forces in all 216 peaks from repeated AFM experiments (Fig. 5B). The average value of 204 pN of the unfolding forces coincides with the expected value for wt I27. The force-extension curves observed for the polyprotein with I1s1s2-w1w3 as target domain similarly show four or five peaks with

maxima values close to that of the wild type I27 (Fig. 5C) and the histogram of the unfolding forces in 156 experiments is unimodal as for the I1s1s2 domain, albeit slightly shifted to lower unfolding forces with an average value of 187 pN (Fig. 5D). This observation indicates that the mechanical strength of the designed domain I1s1s2-w1w3 increased by about 90pN. In the case of the I1_I27AB polyprotein we found that many of the force-extension curves show a low peak of about 100 pN (figure 5E). The histogram of the force barriers found in the recordings of 206 experiments shows a bimodal distribution with many unfolding force peaks near 100pN (figure 5F). Hence the I1_I27AB mutant does not change the I1 mechanical stability. We were not able to obtain clean records for the polyprotein containing the domain I1_I27AD as target, probably due to the low stability of that domain. The AFM results for the designed domains I1s1s2 and I1s1s2-w1w3 clearly demonstrate the success of the design strategy and encourage us to further pursue this strategy with other domain structures and other folds.

Discussion

Experimental data on the mechanical strength for about 100 protein domains has been determined so far and is available in the BSDB database [39] and promising progress has been achieved to computationally predict the unfolding forces from the sequences and 3D structures by steered molecular dynamics calculations [15,40] and Go-like models [41-43]. However, the rational design of proteins with specific mechanical properties remains challenging [5,25].

We propose a new approach, based on force-specific sequence motifs that can guide the engineering of proteins with specific mechanical strengths. We have used titin as an experimental model system to develop our design strategy and to test our computational predictions for the mechanical strength. The use of AFM allows the determination of the mechanical strengths of titin, and has advanced to the point that one can measure the response of individual domains to an applied external force [4,24]. Our approach introduces a new sequence-based perspective that has the potential to be applicable to all protein fold types, and is particularly relevant for all cases where AFM data of structurally related proteins are available.

Based on previous work on linear sequence motifs of weak and strong titin domains [27] we tested the usefulness of the sequence motifs for the design of novel proteins with increased mechanical strength. We provide clear experimental evidence by the AFM recordings of the designed domains (Fig. 4 and Fig. 5) that the mechanical strength of two of our constructs have increased the mechanical strength from about 100 pN to about 200 pN. This dramatic increase of the mechanical strength is remarkable as studies with similar titin domains were only partially successful [25]. For example, steered molecular dynamics simulations of the unfolding pathway of the Ig domain I27 indicated that backbone hydrogen bonds between the A′ and G strands of I27 played a key role in its mechanical stability [44]. However, proline mutagenesis experiments aimed at breaking these key bonds showed that there are also other important interactions [22]. In addition, Sharma et al [45] shuffled large segments between I27 and I32 in an attempt to generate hybrid domains that would share mechanical properties of their parent domains. They found that the A′-G patch is not the only structural region responsible for the mechanical stability of titin Ig domains, a conclusion confirmed by our sequence analysis [27].

Our paper describes the first experimental study to evaluate physical-chemical property (PCP) motifs for the design of mechanically stronger domains. We do not claim that our approach solves the rational design problem, but we present encouraging experimental results that demonstrate the potential of the method. We suggest that the method might be

most useful in combination with steered molecular dynamics calculations[15,46], analysis of side-chain packing in the hydrophobic core [47] and phi-value analysis of the transition state [26,48]. As protein engineering guided by those approaches involves a careful selection of the residues for evaluation, our method can be used as an additional filter in the screening of the residues.

The computational analysis of AFM data has been pioneered by K. Schulten's group using steered molecular dynamics calculations [8,9]. These studies on the mechanical stability of titin Ig domains have provided us with the A′G clamp as an important determinant of the high mechanical stability of titin domains. However, it is still an open question to what extent other interactions, such as side chain packing can influence the mechanical strength. Clarke's group used the analysis of hydrophobic core packing as a guide in the re-engineering of a fibronectin type III domain[49]. The side chain core of the third domain (TNfn3) of fibronectin was compared to that of the homologous tenth domain (FNfn10). The hydrophobic core of FNfn10 was then replaced with that of the homologous, mechanically stronger TNfn3 domain, increasing the mechanical stability of the engineered FNfn10. In a recent study of the macro domain AF1521, a protein from the thermophilic bacteria *Archaeoglobus fulgidus*, showed that water-accessibility of the load bearing hydrogen bonds was correlated with mechanical stability [50]. Another interesting attempt was by Li group to engineer metal chelation sites into various locations of GB1. The binding of metal ions stabilized native state over unfolding transition state[51]. Calculations with Go-like models identified a series of structural motifs that might be correlated with high tensile forces, for example cysteine slipknots were observed in several proteins with high unfolding forces[52]. It remains to be seen to what extent Go-like models or modified SMD strategies, such as PUFF [53], can be combined with the sequence-specific motif approach to provide a general strategy for designing proteins with specific mechanical properties. All these examples make clear that we are far from a comprehensive understanding of the molecular nature of the mechanical strength of a given protein, and there are no established general approaches to re-engineer homologous domains with a specific mechanical strength.

The evaluation of our different constructs by AFM measurements is not completely consistent with our predictions and several open questions remain. In the construct I1s1s2-w1w3 we planned to ‘remove’ the weak motifs in I1 by replacing the residues in I1 in those motif regions with the corresponding residues in I27. The reengineered protein was stronger than I1, but with a similar increase as I1s1s2. Thus ‘removing’ the weak motif did not further strengthen the domain. Hence the importance of weak motifs in the design process is not completely clear. Also we did not analyze the relative contribution of the individual motifs to the mechanical strength of a domain. This will be the topics of future studies.

The motif based approach might also uncover the molecular mechanism of genetic mutations in the muscle protein titin that have been linked to dilated cardiomyopathy (DCM) [54], hypertrophic cardiomyopathy [55] and tibial muscular dystrophy, TMD [56]. DCM and associated heart failure are major causes of human morbidity and mortality. The reported disease-causing missense mutations target Ig domains that localize to the molecule's Z-disk region and the elastic I-band. Mutations may uncouple domains from their binding partners and lead to stress-sensing defects, myocyte dysfunction and cardiac chamber dilation. We can apply our force specific motif approach to those pathogenic mutations and predict which of these mutations are directly related to modification of the mechanical stability of these proteins.

Conclusions

Here we show the results of a first experimental study to evaluate the usefulness of physical-chemical property (PCP) motifs in the design of mechanically stronger domains. The mechanical strengths of two of our re-engineered proteins constructs have significantly increased, demonstrating the potential of our method. Although several open questions regarding the optimal strategy for combining motifs and the usefulness of weak motifs remain, we suggest that the method can be used in combination with other methods as a filter to select relevant residues. Our approach could be similarly applied to other types of protein fold and be used to explain the experimentally observed hierarchy of mechanical strength of all titin Ig domains.

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References

1. Bustamante C, Chemla YR, Forde NR, Izhaky D. Mechanical processes in biochemistry. Annu Rev Biochem. 2004; 73:705–748. [PubMed: 15189157]
2. Forman JR, Clarke J. Mechanical unfolding of proteins: insights into biology, structure and folding. Curr Opin Struct Biol. 2007; 17(1):58–66. [PubMed: 17251000]
3. Puchner EM, Gaub HE. Force and function: probing proteins with AFM-based force spectroscopy. Curr Opin Struct Biol. 2009; 19(5):605–614. [PubMed: 19822417]
4. Oberhauser AF, Carrion-Vazquez M. Mechanical biochemistry of proteins one molecule at a time. J Biol Chem. 2008; 283(11):6617–6621. [PubMed: 18195002]
5. Galera-Prat A, Gomez-Sicilia A, Oberhauser AF, Cieplak M, Carrion-Vazquez M. Understanding biology by stretching proteins: recent progress. Curr Opin Struct Biol. 2010; 20(20138503):63–69. [PubMed: 20138503]
6. Improta S, Politou AS, Pastore A. Immunoglobulin-like modules from titin I-band: extensible components of muscle elasticity. Structure. 1996; 4(8805538):323–337. [PubMed: 8805538]
7. Pfuhl M, Improta S, Politou AS, Pastore A. When a module is also a domain: the role of the N terminus in the stability and the dynamics of immunoglobulin domains from titin. J Mol Biol. 1997; 265(2):242–256. [PubMed: 9020985]
8. Lu H, Isralewitz B, Krammer A, Vogel V, Schulten K. Unfolding of titin immunoglobulin domains by steered molecular dynamics simulation. Biophys J. 1998; 75(9675168):662–671. [PubMed: 9675168]
9. Marszalek PE, Lu H, Li H, Carrion-Vazquez M, Oberhauser AF, Schulten K, Fernandez JM. Mechanical unfolding intermediates in titin modules. Nature. 1999; 402(10573426):100–103. [PubMed: 10573426]
10. Mayans O, Wuerges J, Canela S, Gautel M, Wilmanns M. Structural evidence for a possible role of reversible disulphide bridge formation in the elasticity of the muscle protein titin. Structure. 2001; 9(11525170):331–340. [PubMed: 11525170]
11. Williams PM, Fowler SB, Best RB, Toca-Herrera JL, Scott KA, Steward A, Clarke J. Hidden complexity in the mechanical properties of titin. Nature. 2003; 422(6930):446–449. [PubMed: 12660787]
12. Li HB, Cao Y, Lam C, Wang MJ. Nonmechanical protein can have significant mechanical stability. Angewandte Chemie-International Edition. 2006; 45(4):642–645.
13. Li PC, Makarov DE. Ubiquitin-like protein domains show high resistance to mechanical unfolding similar to that of the 127 domain in titin: Evidence from simulations. Journal of Physical Chemistry B. 2004; 108(2):745–749.

14. Li PC, Huang L, Makarov DE. Mechanical unfolding of segment-swapped protein G dimer: results from replica exchange molecular dynamics simulations. J Phys Chem B. 2006; 110(29):14469–14474. [PubMed: 16854158]
15. Hsin J, Strumpfer J, Lee EH, Schulten K. Molecular origin of the hierarchical elasticity of titin: simulation, experiment, and theory. Annu Rev Biophys. 2011; 40:187–203. [PubMed: 21332356]
16. Gao M, Wilmanns M, Schulten K. Steered molecular dynamics studies of titin I1 domain unfolding. Biophys J. 2002; 83(6):3435–3445. [PubMed: 12496110]
17. Paci E, Karplus M. Forced unfolding of fibronectin type 3 modules: an analysis by biased molecular dynamics simulations. J Mol Biol. 1999; 288(10329153):441–459. [PubMed: 10329153]
18. Gao M, Lu H, Schulten K. Unfolding of titin domains studied by molecular dynamics simulations. J Muscle Res Cell Motil. 2002; 23(5-6):513–521. [PubMed: 12785101]
19. Klimov DK, Thirumalai D. Native topology determines force-induced unfolding pathways in globular proteins. Proc Natl Acad Sci U S A. 2000; 97(13):7254–7259. [PubMed: 10860990]
20. Eom K, Li PC, Makarov DE, Rodin GJ. Relationship between the mechanical properties and topology of cross-linked polymer molecules: Parallel strands maximize the strength of model polymers and protein domains. Journal of Physical Chemistry B. 2003; 107(34):8730–8733.
21. Li H, Fernandez JM. Mechanical design of the first proximal Ig domain of human cardiac titin revealed by single molecule force spectroscopy. J Mol Biol. 2003; 334(14596801):75–86. [PubMed: 14596801]
22. Li H, Carrion-Vazquez M, Oberhauser AF, Marszalek PE, Fernandez JM. Point mutations alter the mechanical stability of immunoglobulin modules. Nat Struct Biol. 2000; 7(11101892):1117–1120. [PubMed: 11101892]
23. Valbuena A, Oroz J, Hervas R, Vera AM, Rodriguez D, Menendez M, Sulkowska JI, Cieplak M, Carrion-Vazquez M. On the remarkable mechanostability of scaffoldins and the mechanical clamp motif. Proc Natl Acad Sci U S A. 2009; 106(33):13791–13796. [PubMed: 19666489]
24. Li H, Linke WA, Oberhauser AF, Carrion-Vazquez M, Kerkvliet JG, Lu H, Marszalek PE, Fernandez JM. Reverse engineering of the giant muscle protein titin. Nature. 2002; 418(12198551):998–991002. [PubMed: 12198551]
25. Crampton N, Brockwell DJ. Unravelling the design principles for single protein mechanical strength. Curr Opin Struct Biol. 2010; 20(20542682):508–517. [PubMed: 20542682]
26. Borgia A, Steward A, Clarke J. An effective strategy for the design of proteins with enhanced mechanical stability. Angew Chem Int Ed Engl. 2008; 47(18666188):6900–6903. [PubMed: 18666188]
27. Garcia TI, Oberhauser AF, Braun W. Mechanical stability and differentially conserved physical-chemical properties of titin Ig-domains. Proteins. 2009; 75(19003986):706–718. [PubMed: 19003986]
28. Ivanciuc O, Oezguen N, Mathura VS, Schein CH, Xu Y, Braun W. Using property based sequence motifs and 3D modeling to determine structure and functional regions of proteins. Curr Med Chem. 2004; 11(5):583–593. [PubMed: 15032606]
29. Steward A, Toca-Herrera JL, Clarke J. Versatile cloning system for construction of multimeric proteins for use in atomic force microscopy. Protein Sci. 2002; 11(9):2179–2183. [PubMed: 12192073]
30. Oezguen N, Zhou B, Negi SS, Ivanciuc O, Schein CH, Labesse G, Braun W. Comprehensive 3D-modeling of allergenic proteins and amino acid composition of potential conformational IgE epitopes. Mol Immunol. 2008; 45(14):3740–3747. [PubMed: 18621419]
31. Bujnicki JM, Elofsson A, Fischer D, Rychlewski L. Structure prediction meta server. Bioinformatics. 2001; 17(8):750–751. [PubMed: 11524381]
32. Eswar N, Webb B, Marti-Renom MA, Madhusudhan MS, Eramian D, Shen MY, Pieper U, Sali A. Comparative protein structure modeling using Modeller. Curr Protoc Bioinformatics. 2006; Chapter 5 Unit 5 6.
33. Phillips JC, Braun R, Wang W, Gumbart J, Tajkhorshid E, Villa E, Chipot C, Skeel RD, Kale L, Schulten K. Scalable molecular dynamics with NAMD. J Comput Chem. 2005; 26(16):1781–1802. [PubMed: 16222654]

34. Oezguen N, Kumar S, Hindupur A, Braun W, Muralidhara BK, Halpert JR. Identification and analysis of conserved sequence motifs in cytochrome P450 family 2. Functional and structural role of a motif 187RFDYKD192 in CYP2B enzymes. J Biol Chem. 2008; 283(31):21808–21816. [PubMed: 18495666]
35. Negi SS, Kolokoltsov AA, Schein CH, Davey RA, Braun W. Determining functionally important amino acid residues of the E1 protein of Venezuelan equine encephalitis virus. J Mol Model. 2006; 12(6):921–929. [PubMed: 16607494]
36. Schein CH, Zhou B, Oezguen N, Mathura VS, Braun W. Molego-based definition of the architecture and specificity of metal-binding sites. Proteins. 2005; 58(1):200–210. [PubMed: 15505785]
37. Mathura VS, Schein CH, Braun W. Identifying property based sequence motifs in protein families and superfamilies: application to DNase-1 related endonucleases. Bioinformatics. 2003; 19(12874050):1381–1390. [PubMed: 12874050]
38. Venkatarajan MS, Braun W. New quantitative descriptors of amino acids based on multidimensional scaling of a large number of physical-chemical properties. Journal of Molecular Modeling. 2001; 7(12):445–453.
39. Sikora M, Sulkowska JI, Witkowski BS, Cieplak M. BSDB: the biomolecule stretching database. Nucleic Acids Res. 2011; 39(Database issue):D443–450. [PubMed: 20929872]
40. Wang Y, Harrison CB, Schulten K, McCammon JA. Implementation of Accelerated Molecular Dynamics in NAMD. Comput Sci Discov. 2011; 4(1)
41. Go N, Abe H. Noninteracting local-structure model of folding and unfolding transition in globular proteins. I. Formulation. Biopolymers. 1981; 20(5):991–1011. [PubMed: 7225531]
42. Sulkowska JI, Cieplak M. Selection of optimal variants of Go-like models of proteins through studies of stretching. Biophys J. 2008; 95(7):3174–3191. [PubMed: 18567634]
43. Faisca PF, Travasso RD, Charters T, Nunes A, Cieplak M. The folding of knotted proteins: insights from lattice simulations. Phys Biol. 2010; 7(1):16009. [PubMed: 20130340]
44. Lu H, Schulten K. The key event in force-induced unfolding of Titin's immunoglobulin domains. Biophys J. 2000; 79(10866937):51–65. [PubMed: 10866937]
45. Sharma D, Cao Y, Li H. Engineering proteins with novel mechanical properties by recombination of protein fragments. Angew Chem Int Ed Engl. 2006; 45(34):5633–5638. [PubMed: 16856192]
46. Lee EH, Hsin J, Sotomayor M, Comellas G, Schulten K. Discovery through the computational microscope. Structure. 2009; 17(19836330):1295–1306. [PubMed: 19836330]
47. Sadler DP, Petrik E, Taniguchi Y, Pullen JR, Kawakami M, Radford SE, Brockwell DJ. Identification of a mechanical rheostat in the hydrophobic core of protein L. J Mol Biol. 2009; 393(19683005):237–248. [PubMed: 19683005]
48. Best RB, Fowler SB, Herrera JL, Steward A, Paci E, Clarke J. Mechanical unfolding of a titin Ig domain: structure of transition state revealed by combining atomic force microscopy, protein engineering and molecular dynamics simulations. J Mol Biol. 2003; 330(4):867–877. [PubMed: 12850153]
49. Ng SP, Billings KS, Ohashi T, Allen MD, Best RB, Randles LG, Erickson HP, Clarke J. Designing an extracellular matrix protein with enhanced mechanical stability. Proc Natl Acad Sci U S A. 2007; 104(23):9633–9637. [PubMed: 17535921]
50. Guzman DL, Randall A, Baldi P, Guan Z. Computational and single-molecule force studies of a macro domain protein reveal a key molecular determinant for mechanical stability. Proc Natl Acad Sci U S A. 2010; 107(20080695):1989–1994. [PubMed: 20080695]
51. Cao Y, Yoo T, Li H. Single molecule force spectroscopy reveals engineered metal chelation is a general approach to enhance mechanical stability of proteins. Proc Natl Acad Sci U S A. 2008; 105(32):11152–11157. [PubMed: 18685107]
52. Sulkowska JI, Sulkowski P, Szymczak P, Cieplak M. Untying knots in proteins. J Am Chem Soc. 2010; 132(40):13954–13956. [PubMed: 20857930]
53. Ho BK, Agard DA. An improved strategy for generating forces in steered molecular dynamics: the mechanical unfolding of titin, e2lip3 and ubiquitin. PLoS One. 2010; 5(20927369)
54. Seidman JG, Seidman C. The genetic basis for cardiomyopathy: from mutation identification to mechanistic paradigms. Cell. 2001; 104(4):557–567. [PubMed: 11239412]

55. Satoh M, Takahashi M, Sakamoto T, Hiroe M, Marumo F, Kimura A. Structural analysis of the titin gene in hypertrophic cardiomyopathy: identification of a novel disease gene. Biochem Biophys Res Commun. 1999; 262(2):411–417. [PubMed: 10462489]

56. Hackman P, Vihola A, Haravuori H, Marchand S, Sarparanta J, De Seze J, Labeit S, Witt C, Peltonen L, Richard I, Udd B. Tibial muscular dystrophy is a titinopathy caused by mutations in TTN, the gene encoding the giant skeletal-muscle protein titin. Am J Hum Genet. 2002; 71(3): 492–500. [PubMed: 12145747]

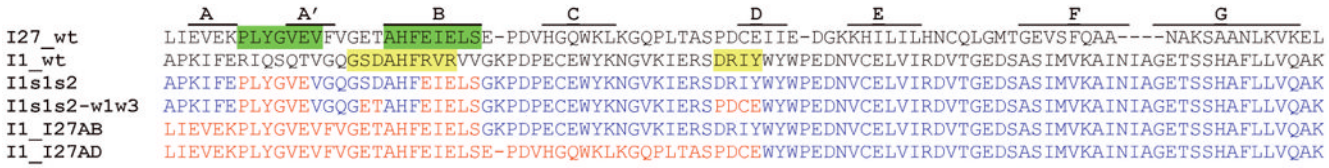

Figure 1.

Sequences of the four engineered proteins. Location of the two unique motifs (s1, s2) for the strong Ig domains of titin are indicated in green on the sequence of wild type I27 (I27_wt), and the two unique motifs of the weak Ig domains are mapped on the sequence of wild type I1 (w1, w3, yellow). In the sequences of the four designed domains, I1s1s2, I1s1s2-w1w3, I1_I27AB and I1_I27AD, residues originating from I1 are shown in blue, and residues from i27 are shown in red.

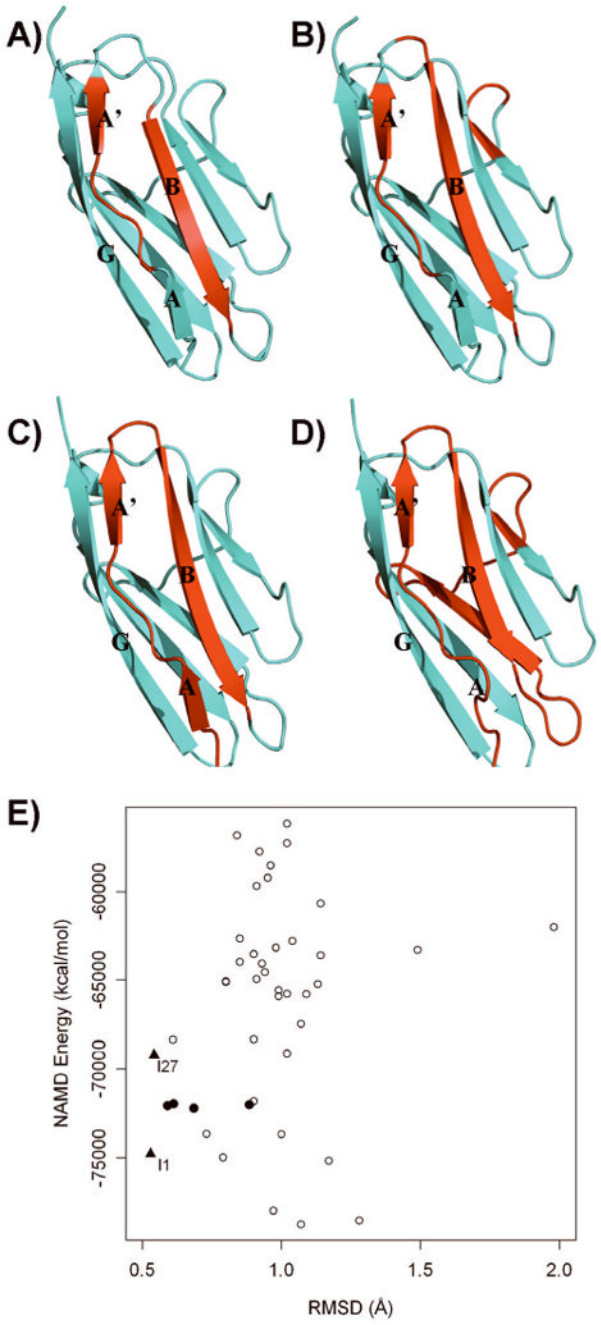

Figure 2.

Homology models of the four engineered proteins. A), B), C), D) are homology models for I1s1s2, I1s1s2-w1w3, I1_I27AB and I1_I27AD. The substituted residues were mapped in red. E), Deviations between energy refined models and templates (RMSD values in Å, filled circles) and NAMD energy values are plotted and compared to the corresponding values of the X-ray crystal structures of I1 and I27 (PDB: 1G1C and 1WAA, triangles), and the 3D models of the remaining 38 Ig like domains in the I-band of titin (empty circles). All the models were stable with low RMSD and minimized energy. The RMSD for the four engineered proteins are 0.61 Å, 0.68 Å, 0.59 Å and 0.88 Å.

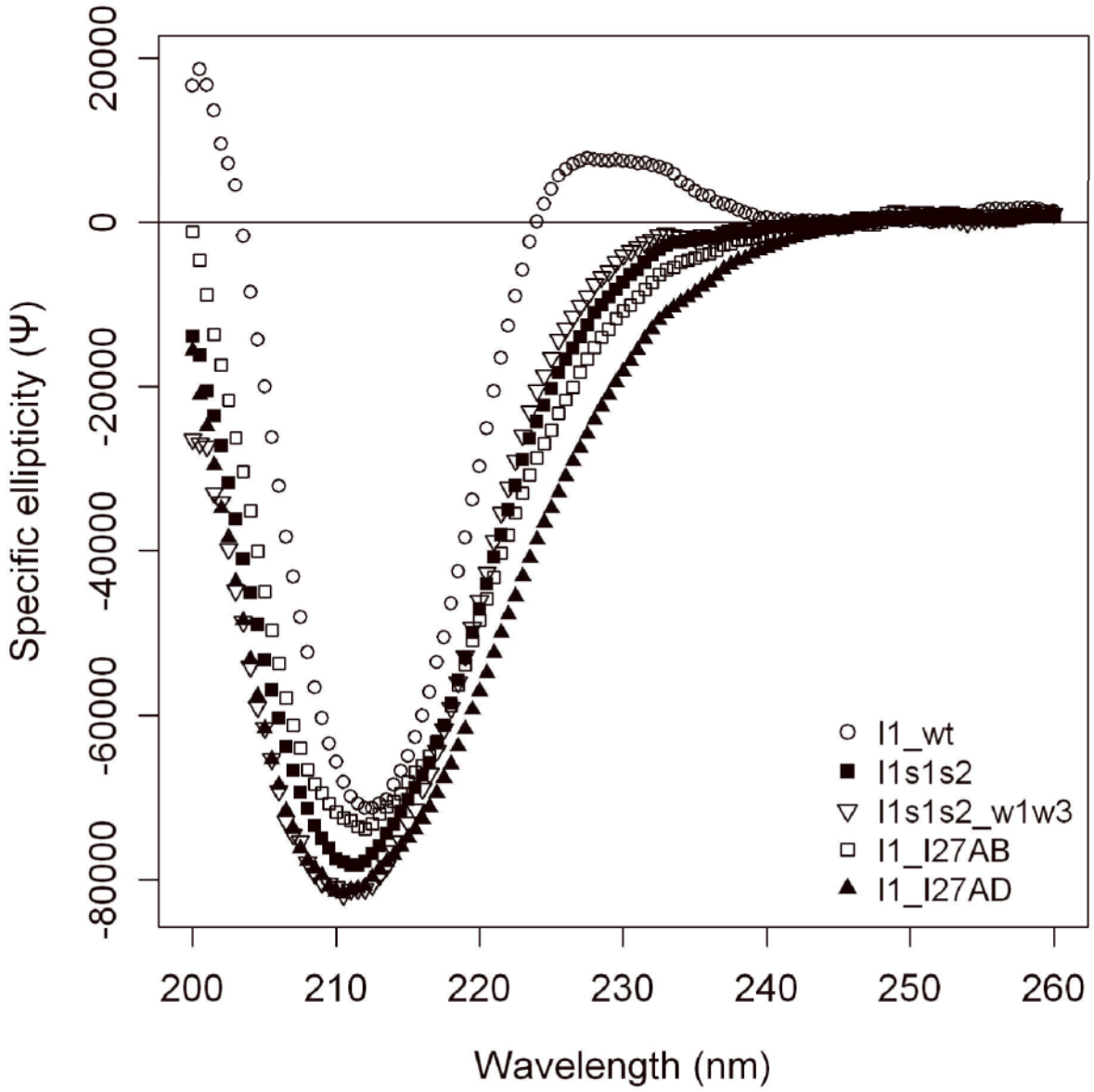

Figure 3.
Far-UV CD spectra for the engineered proteins. The spectra of all engineered proteins showed features of predominantly β-strands secondary structures similar to the spectrum in the wild type I1.

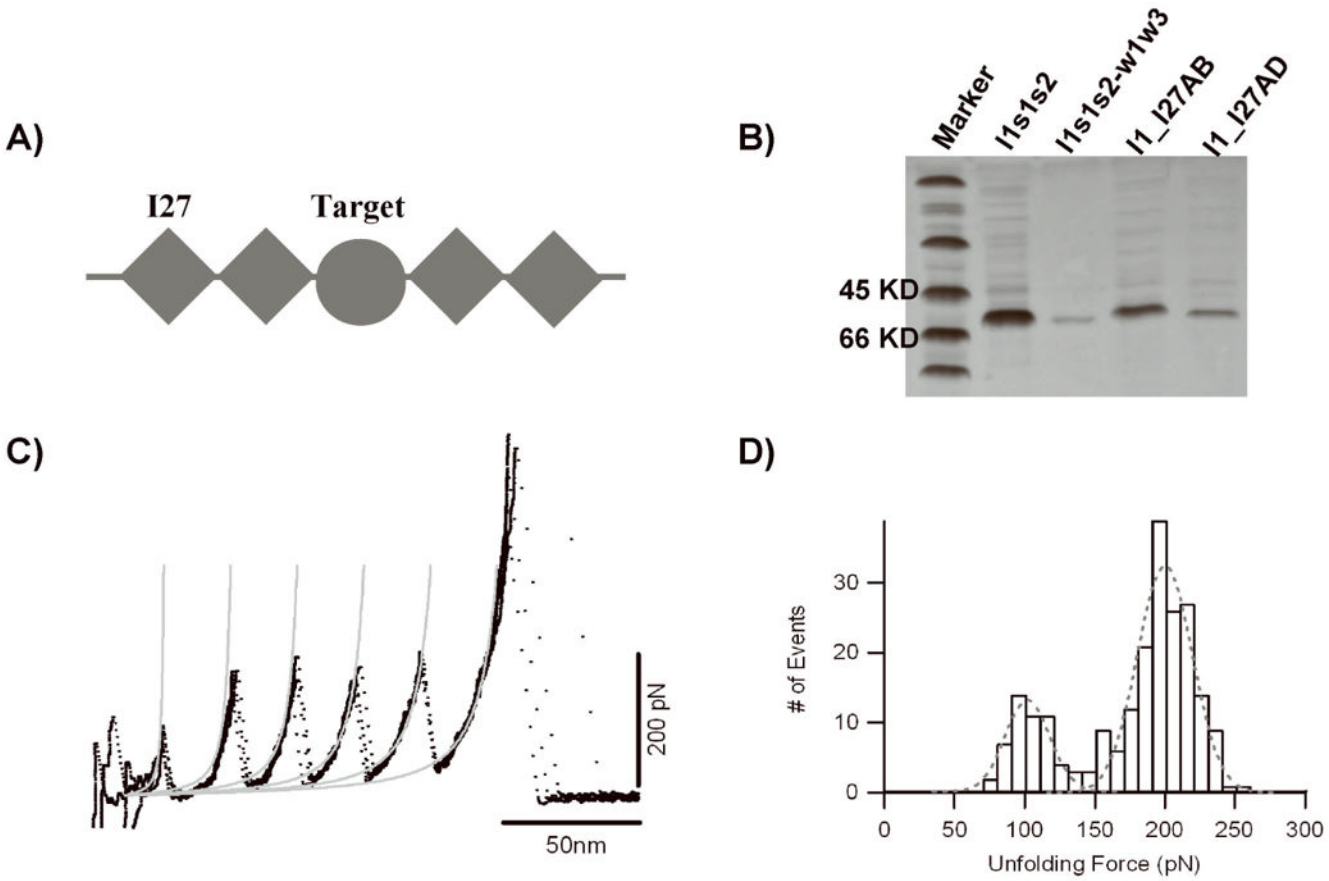

Figure 4.

Poly protein and Force-extension profile of wild type I1. A), the poly protein consists of four I27 domains (diamond) and one target domain (circle). The target domain could be wild type I1 or engineered proteins. B), the SDS-PAGE shows the MW of the poly proteins are ~50KD. C), the poly protein containing wild type I1 domain was used as a control. The force–extension curve shows four peaks for I27 and one low peak for I1. Four curves were overlaid in the figure. Grey curves show the fitting of worm-like chain. D), the histogram of unfolding forces shows two separate peaks. The peak at 98±22 pN, n=44 corresponds to wild type I1, and wild type I27 unfolds at 201±27 pN, n=176. Dashed lines are Gaussian fit to the histogram.

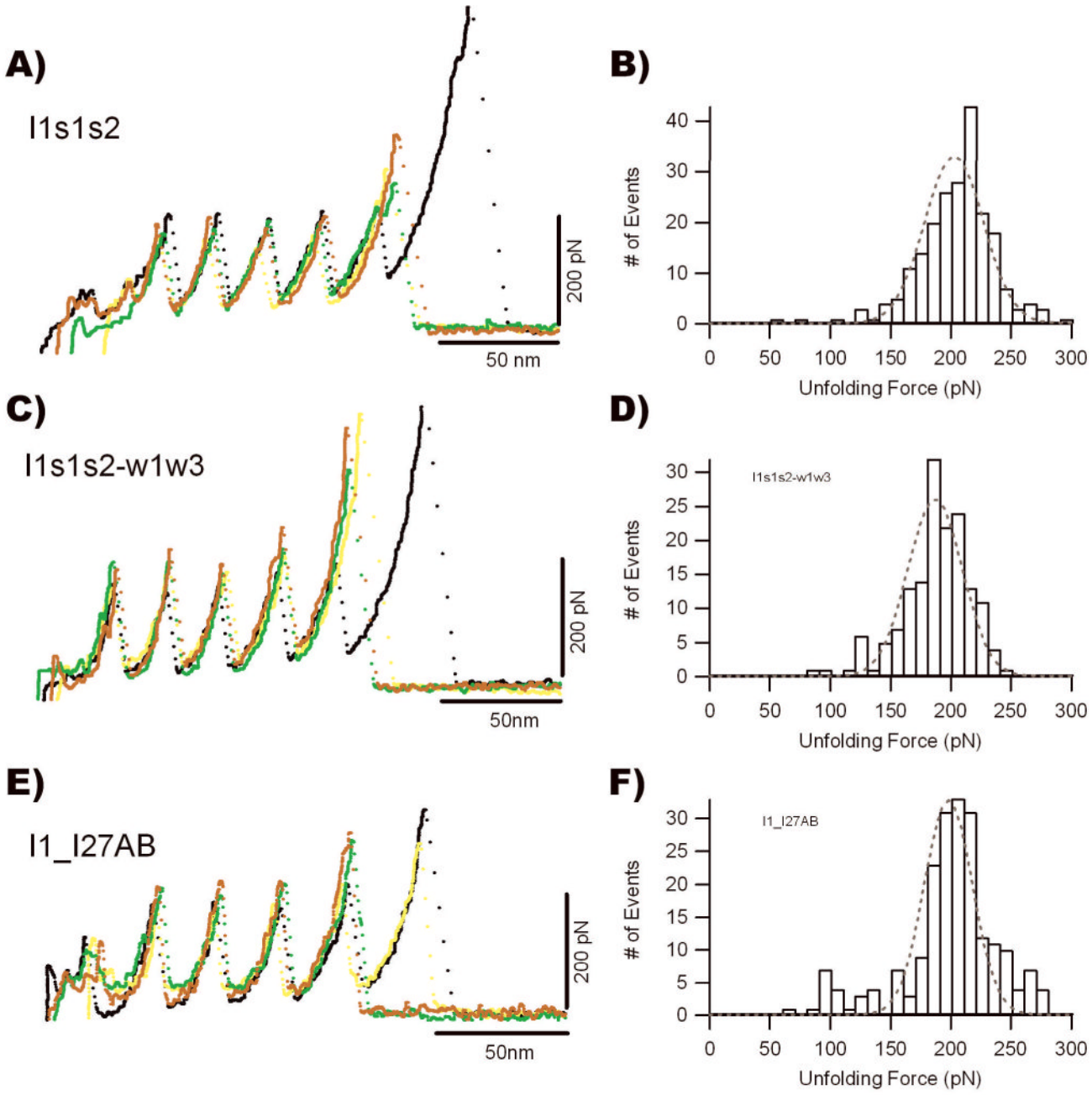

Figure 5.

Force-extension profiles of the engineered proteins with I1s1s2, I1s1s2-w1w3 and I1_I27AB as targets. A) Four saw-tooth recordings, shown in different colors (black, green, red and yellow), and B) histogram of the unfolding forces for all the peaks of the engineered protein with I1s1s2 as target (average 204±32 pN, sample size n=216). Average unfolding forces and standard deviations were calculated according to the Gaussian fits. C), D), extension profile and histogram of the unfolding forces of the construct with I1s1s2-w1w3 as target (187±28 pN, n=156) E), F), data for I1_I27AB. Many of the extension profiles for I1_I27AB show a small peak at the beginning. The histogram also has a shoulder at ~100pN (198±40 pN, n=206).