Mutation effects on ankyrin repeat mechanics

Mutation of conserved histidines alters the tertiary structure and nanomechanics of consensus ankyrin repeats

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Background: The H-R mutation in a native ankyrin-R protein is responsible for converting RBCs to spherocytes which causes hereditary spherocytosis (HS).

Results: The mutant unfolds and refolds at lower forces as compared to the wild-type.

Conclusion: The H-R mutation weakens the mechanical stability of ankyrin repeats.

Significance: The H-R mutation in ankyrin-R may cause HS by decreasing the mechanical stability and affecting its structure recovery ability.

ABSTRACT

The conserved TPLH tetrapeptide motif of ankyrin repeats (ARs) plays an important role in stabilizing AR proteins, and histidine (TPLH) to arginine (TPLR) mutations in this motif have been associated with a hereditary human anemia, spherocytosis (HS). Here, we used a combination of Atomic Force Microscopy (AFM)-based single-molecule force spectroscopy and molecular dynamics (MD) simulations to examine the mechanical effects of H->R substitutions in TPLH motifs in a model ankyrin repeat protein, NI6C. Our MD results show that the mutated protein is less mechanically stable than the wild-type (WT). Our AFM results indicate that the mechanical energy input necessary to fully unfold the mutated protein is only half of that necessary to unfold the WT protein (53 kcal/mol vs. 106 kcal/mol). In addition, the ability of the mutant to generate refolding forces is also reduced. Moreover, the mutant protein subjected to cyclic stretch-relax measurements displays mechanical fatigue, which is absent in the WT. Taken together, these results indicate that the H->R substitutions in TPLH motifs compromise mechanical properties of ARs and suggest that the origin of HS may be related to mechanical failure of ankyrin repeats.

Ankyrin repeats (ARs) are one of the most common motifs of repeat proteins with a high degree of amino acid sequence homology. ARs fold into nearly identical helix1-helix2-loop structures, and stack to form elongated, superhelical domains that frequently mediate protein-protein interactions (1-3). Previously, single molecule force spectroscopy experiments of a few AR proteins revealed that mechanically unfolded ARs refold rapidly and generate very robust refolding forces (4-9). Very recently we examined the mechanical properties of a model synthetic AR protein Ni6C (7) in detail. Ni6C is composed of six identical internal ARs based on a consensus sequence and two capping repeats (10). Our atomic force microscopy manipulations on Ni6C presented very interesting mechanical properties. We observed that Ni6C unfolds stepwise, repeat by repeat, and individual unfolding events produce regular, well-resolved force peaks of ~22 pN. In addition, relaxation traces of Ni6C captured robust
refolding force peaks and revealed no elastic hysteresis or fatigue, suggesting this protein possesses superior elastic properties (7).

It has been suggested that the conserved TPLH tetrapeptide motif of ARs plays an important role in stabilizing AR proteins (2,11-13). Some mutations in the TPLH motif, including T/A, H/A and/or H/Q substitutions were found to significantly affect the thermal stability of gankyrin (11). However, the role of the TPLH motif in the mechanical stability of ARs has not been studied.

Hereditary spherocytosis (HS) is a life threatening human anemia in which erythrocytes (RBC) lose their characteristic shape and become spherical and very fragile (14,15). HS is caused by defects in proteins that mediate membrane-cytoskeleton connections in erythrocytes such as ankyrin-R, spectrins and band 3 (1,14,16). There are two missense mutations identified so far in the ankyrin repeats segment of ankyrin-R that are related to HS: H277R and V463I (14,16). Importantly, the H-R substitution involves a highly conserved histidine of the TPLH motif.

Since RBCs undergo repeated cycles of stress and deformation while flowing through narrow capillaries and slits of the spleen (16,17), the elastic response of the membrane and associated cytoskeletal proteins to the applied forces is essential for maintenance of the RBCs integrity and to enable recovery. Force-induced unfolding of spectrin was already captured in live RBCs under physiological stress (18). It is therefore possible that ankyrin-R, depending on (presently unknown) direction and magnitude of applied forces also undergoes stretch-relax cycles in vivo, which may involve all or some of its ankyrin repeats.

The goal of this work is to test the hypothesis that H→R substitutions in the TPLH motif disrupt stabilizing interactions and compromise mechanical properties of ARs. Because the membrane binding domain of Ankyrin-R is very large and its crystal structure is not yet available, we decided to carry out our initial studies of the mechanical effect of H→R substitution in the TPLH motif in NI6C, which proved to be an excellent model AR system amenable to single molecule force spectroscopy by AFM (7). To amplify the possible effect of the H-R mutation, we substituted TPLH histidines in four internal ARs within NI6C (Fig. 1B). We used a combination of AFM-based single molecule force spectroscopy (19-27) and molecular dynamics simulations (9,28-34) to examine how mutations in this motif affect the mechanical and structural properties of NI6C when the proteins are pulled by their N and C termini. We found that for the particular pulling geometry used in this study (stretching by the N and C termini), the mechanical unfolding forces of the mutant were significantly reduced, and as a result the total consumed mechanical energy during the unfolding process decreased by half (53 kcal/mol vs. 106 kcal/mol) as compared to the WT NI6C, suggesting that H-R substitutions indeed alter the mechanical properties of ARs. Furthermore, we registered that the mechanical refolding forces of the mutant were significantly reduced and observed the mechanical fatigue of the mutant over cyclic stretch-relax experiments. Taken together, these observations suggest that the H277R mutation possibly reduces the mechanical robustness of ankyrin-R, and may ultimately cause its mechanical failure, leading to HS.

**MATERIALS AND METHODS**

**Cloning, expression and purification of repeat proteins**

The gene of the mutant protein NI(I_{H7R})_{4}IC was synthesized by Genescript (Piscataway, NJ). In the mutant protein, four internal consensus ARs (repeat # 2, 3, 4, 5) were modified by substituting the histidines at position 7 with arginines (Fig. 1B). Please note that in Fig. 1B, the TPLH histidines are in position 9. However, this position corresponds to position 7 within AR sequence according to the residue-numbering scheme of ARs introduced by Michaely (2). The NI(I_{H7R})_{4}IC gene was inserted into the Poly-I27 pRSETa vector [a kind gift from Jane Clark (35)] using KpnI and NheI restriction sites, and the 8th I27 module was replaced by strep-tag and a STOP codon. The engineered plasmids were transformed into E.coli C41 (DE3) and expressed using IPTG induction. The expressed proteins were purified.
using a nickel affinity column (GE Healthcare #17-5248-01) followed by a streptag column (IBA #2-1202-0250). Proteins were determined to be greater than 95% pure by SDS-PAGE analysis. Then the purified proteins were dialyzed in 150 mM NaCl, 1mM EDTA, 2mM TCEP (Thermo Scientific, product # 77720) and 10 mM Tris buffer (ph 7.4). The final concentration of the purified protein was ~0.3 mg/ml.

AFM-based single molecule force spectroscopy
All AFM measurements were carried out on custom-built AFM instruments (20,36,37) equipped with an AFM detector head from Veeco Metrology Group, and high-resolution piezoelectric stages from Physik Instrumente, equipped with capacitive or strain-gauge position sensors (vertical resolution of 0.1 nm). The spring constant \( k_c \) of each cantilever was calibrated in solution using the energy equipartition theorem as described in Ref. (38). All force-extension measurements were performed in solution using biolever AFM cantilevers (Veeco, \( k_c \approx 6 \) pN/nm) at pulling speeds of 0.03 ~ 0.1 nm/ms at room temperature. Stock protein solutions were diluted to 1~10 g/ml using 150 mM NaCl, 1mM EDTA, 2mM TCEP and 10 mM Tris buffer (ph 7.4). 50 l of the diluted solution was deposited on clean glass substrate, gently washed after 30 min incubation, and used for AFM pulling experiments.

Single-hit approach
For AFM force spectroscopy measurements, proteins were picked up by an AFM tip by gently contacting the sample substrate at forces lower than 1 nN. Typically, the nonspecific adsorption of proteins to the AFM tip requires multiple attempts to be carried out on the same location before a successful event occurs. However, this approach may result in the mechanical denaturing of proteins. To avoid possible protein damage, we modified this protocol and performed single measurements at a given location, which we call ‘single-hit approach’. If the first attempt was not successful, the AFM tip was moved to a different location for another attempt to pick up a molecule until a successful event occurred. The six I27 domains of titin flanking NI6C and NI(1iR61)IC proteins (three on each N- and C-terminus sides of ARs) served as pulling handles and as a force spectroscopy reference for identifying single molecule recordings. To establish a mechanical fingerprint of ARs, we fully stretched constructs (extensions of ~400 nm) and selected unfolding force-extension curves with more than four characteristic I27 force peaks (~200 pN, ~28 nm) for single molecule identification. We used these recordings as the reference force spectrogram to identify unfolding events of ARs in other measurements.

Cyclic measurements
In some experiments, after a molecule was picked up by using single hit approach, we performed cyclic stretch-relax measurements on it. In those measurements, the extension was limited so I27 domains were not unfolded while the AFM tip was slightly lifted above the sample substrate (5 ~ 10 nm) to prevent adsorption of other molecules.

Mechanical unfolding energy analysis.
The WT NI6C contains eight ARs: six identical, consensus repeats flanked by slightly different N and C capping repeats (Fig. 2, (7)). WT ARs unfold sequentially, generating 5~6 almost uniform unfolding force peaks of ~23 pN, spaced by ~11 nm. Supported by molecular dynamics simulations, these sharp and regular 5~6 unfolding force peaks are interpreted to report the unfolding of six internal consensus repeats. The unfolding of the capping repeats is typically unresolved. Since ankyrin repeats unfold sequentially and we mutated only the internal four repeats, we expect that the middle peaks in force-extension curves of the mutant (see Fig. 2C) are generated when the mutated internal repeats are unraveled. Often the unfolding force peaks of the mutant were not sharp but smeared and merged producing a force curve with a shape of a force plateau. For these reasons it was rather difficult to determine the individual unfolding force peaks and their amplitudes. To quantify mechanically the effect of the mutation, the consumed energy during the unfolding process was calculated for each curve (WT and mutant). As an estimate of this energy input, the area enclosed by the unfolding force-extension curve of ARs (corresponding to the extension between 18 nm and 81 nm) and the WLC curve drawn through the first I27 unfolding
peak was calculated (yellow area in Fig. S2 C-D). The average “mechanical unfolding energy” defined this way was 53.2± 10.9 kcal/mol (n = 50) for the mutant, and 106.5± 12.7 kcal/mol (n = 12) for the WT protein. Thus, the four internal AR \( H \rightarrow R \) mutations cause the mechanical unfolding work input to decrease by about 50% as compared to that of the wile-type.

**MD simulations**

The initial geometry of NI6C (253 amino acids, (7)) was built based on the PDB structure 2QYJ corresponding to NI3C consensus AR protein (10). In NI6C, the two capping and all internal repeat amino acid sequences are the same as in NI3C, only the number of internal repeats is increased in NI6C from 3 to 6. Structure building details are described in (7). The histidines of 4 internal repeats of NI6C (Fig. 1B) were mutated to arginines by using the program VMD (39). Equilibrium MD simulations of both NI6C and the mutant NI(\( I_{HTR} \))IC structures in periodic water boxes (110x100x100 Å\(^3\)) with 150 mM NaCl, using the CHARMM22 force-field (40), were performed with NAMD (42). The force-response of each structure was subsequently probed by steered MD (SMD) simulations (41), where the C-terminal C of the protein is pulled with a constant velocity (by an attached spring force) \( v \), while the N-terminal C is held fixed. Each structure was extended by 50 nm, using a pulling velocity of \( v = 5 \) Å/ns and a spring constant of 3 k\(_B\)T Å\(^2\). Simulations were carried out at 310 K and at 1 atm. Table S1 presents a summary of all simulations performed.

**RESULTS AND DISCUSSION**

Fig. 1A shows the ribbon diagram of the TPLH region in the WT NI6C (7,42). The TPLH motifs are located between the hydrophobic helical bundles and the solvent-exposed loop regions (2). The histidines in the TPLH motifs are located at the beginning of helix-1. As marked with dotted lines in Fig. 1A, histidines at position 7 and tyrosines at position 4 in TPLH motifs form a hydrogen-bond (H-bond) network. Each TPLH histidine forms three H-bonds with TPLH tyrosine within the same repeat, and one H-bond with the TPLH tyrosine of the next AR. The intra-repeat H-bonds are T4H\(^N\)-H7N\(^{\delta1}\), T4O\(^{\gamma1}\)-H7H\(^N\) and T4H\(^{\gamma1}\)-H7N\(^{\delta1}\). The inter-repeat H-bond is between H7H\(^2\) and the T4O of the next repeat. Because of these extensive interactions, the conserved TPLH motif is vital to the protein structure and its stability(11). For these reasons, we anticipate that \( H \rightarrow R \) substitutions in the TPLH motif will likely affect both the structure and stability of AR polypeptides.

**Equilibrium structures of NI6C and NI(\( I_{HTR} \))IC**

First we employed molecular dynamics (MD) simulations to test whether the structure of NI6C changes from the H–R mutations. Four internal consensus ARs (repeats # 2, 3, 4 and 5; Fig1B) of the WT NI6C (7) were modified in the mutant NI(\( I_{HTR} \))IC by mutating histidines at position 7 to arginines. Equilibrium all-atom MD simulations of both NI6C and NI(\( I_{HTR} \))IC were performed, totaling 200 ns (see Table S1 in supplementary information for details). The WT NI6C showed little deviation from the initial structures in the equilibrium simulations, with an average backbone root-mean squared deviations (C \_RMSDs) of 1.14 Å, 1.14 Å and 1.33 Å in each NI6C simulation (Fig. 1C). The mutant NI(\( I_{HTR} \))IC showed significantly more deviation from the initial structure, with average C \_RMSDs of 1.8 Å, 2.1 Å and 2.2 Å in each simulation. Much of the deviation occurred in the loop regions between the repeats, with an extension of the overall structure from an average length of 73.5 Å for NI6C to 76.2 Å for NI(\( I_{HTR} \))IC. Significant rearrangement of hydrogen bonding in the TPLH motifs occurs due to the H–R mutation: the bulkier arginine displaces the inter-AR loop and forms a single hydrogen bond with the aspartic acid in position 32 (in the loop region between ARs), thus altering the average loop orientation with respect to the AR -helices (Fig 1D). The reduction from three hydrogen bonds, made by the histidine in NI6C, to only one by the arginine in NI(\( I_{HTR} \))IC results in a destabilization of the mutant structure, especially the loop regions, as shown by the root mean squared fluctuations calculated from the equilibrium MD trajectories (Fig. 1E).

**In silico force-unfolding of NI6C and NI(\( I_{HTR} \))IC**

Following equilibrium simulation we performed two SMD simulations for each NI6C and NI(\( I_{HTR} \))IC, pulling on the C-terminal C using a
pulling velocity of \( v = 5 \text{ Å/ns} \). The resulting force-extension curves from the simulations are shown in Fig. S1A. The force-extension curves show as much difference between the WT and the mutant structures as between repeated SMD simulations of the same structure. All simulations showed major force-rupture peaks spaced every 10 nm, which corresponded to the rupture of each AR. Unfolding of each AR occurred for both WT and mutant from the C-terminus to the N-terminus, as previously reported (7). We note however that the unfolding pathways as determined through SMD simulations may differ from the pathways explored by the proteins in AFM unfolding measurements and these issues have to be further examined in future studies. Visual analysis of the SMD simulations shows that the major force-rupture peaks are due to the dissociation of the intra-repeat helices (Fig S1B). Given the amplitude of fluctuations seen in equilibrium simulations (Fig. 1E), slower pulling velocities than those achievable with current computational resources are likely required to capture the mechanistic differences between the force-unfolding of NI6C and NI(I_{H7R})_{4}IC.

**Mechanical unfolding of NI(I_{H7R})_{4}IC.**

To examine the mechanical unfolding behavior of an intact NI(I_{H7R})_{4}IC mutant, we performed ‘single-hit’ stretching measurements on NI(I_{H7R})_{4}IC-I27 constructs, which were designed to limit possible mechanical damage to the protein resulting from multiple attempts to pick it up by an AFM tip (Methods). To ease single molecule force spectroscopy measurements and their interpretations, the mutant protein NI(I_{H7R})_{4}IC, similar to NI6C protein (7), was flanked on each side by three I27 domains of titin, serving as pulling handles and as a force spectroscopy reference for identifying single-molecule recordings (8,24,35,44,45). Fig. 2A inset shows the schematic ribbon diagrams of the chimeric proteins (I27)_{3}-NI6C-(I27)_{3} and (I27)_{3}-NI(I_{H7R})_{4}IC-(I27)_{3}, which were examined by AFM. Since we studied the mechanical property of the WT NI6C previously (7), we compared the AFM results of NI(I_{H7R})_{4}IC to the force spectra of NI6C. In Fig. 2A, we show the representative unfolding force-extension traces that were recorded in single molecule AFM measurements of NI6C-I27 and NI(I_{H7R})_{4}IC-I27 constructs. Both curves were obtained at the same pulling speed of 0.1 nm/ms. The five I27 unfolding force peaks at extensions above 100 nm provide direct evidence that the whole measurement was obtained on a single molecule containing all eight ARs. Small unfolding force peaks at protein extensions below 100 nm in red and green curves strongly suggest that they correspond to the sequential unfolding of NI6C and NI(I_{H7R})_{4}IC, respectively. We used these record as the reference force spectra to identify unfolding events of ARs in other measurements on the WT and the mutant.

In Fig. S2 A and B, we superimposed AFM recordings of different molecules of the mutated protein and WT, respectively. In Fig. 2C, we compare the AFM recordings of mutant with a typical unfolding force-extension curve of the WT NI6C (7). It is clear that the force-extension curves of the mutant overlap reasonably well with the force-extension curve of WT NI6C at the beginning and at the end. However, at the intermediate extensions (marked as a green bar in Fig. 2C), unfolding forces of the mutant are significantly lower as compared with the unfolding forces of the WT. These observations are consistent with the design of the mutant NI6C, in which only the four internal repeats were mutated. We determined the mechanical energy consumed during the AR unfolding process by integrating the area between each force-extension curves and the WLC curves (yellow areas in Fig. S2 C-D). The average energy input necessary to mechanically unfold the mutant was 53.2±10.9 kcal/mol (n = 50), and 106.5±12.7 kcal/mol (n = 12) for the WT. Thus, four internal AR H→R mutations resulted in the ~53 kcal/mol decrease of the work necessary to unravel the mutated ARs as compared to the wild-type ARs. In summary, the mechanical unfolding forces of the mutated ARs are significantly lower than those of the WT ARs. These observations confirm our expectations that the H-R mutations in TPLH motifs may reduce the mechanical stability of ARs.

**Mechanical refolding of NI(I_{H7R})_{4}IC in cyclic measurements**

To examine the mechanical refolding behavior of NI(I_{H7R})_{4}IC, we performed cyclic stretch-relax...
measurements on the NI(II_R)IC-I27 construct. During these measurements, the stretching distance was limited to unfold most of the ARs of the NI(II_R)IC insert without unfolding of any of the I27 domains. Since the I27 domains remained folded, they did not interfere with the folding of ARs during the relaxation step. In Fig. 3A, a typical stretching force extension curve of the mutant NI(II_R)IC protein obtained under such partial unfolding conditions is superimposed with its refolding force extension trace. In contrast to the large and sharp refolding force peaks of the WT NI6C protein (black trace in Fig. 3B, ref. (7)), the refolding force peaks of the mutant are smaller and somewhat smeared. The refolding traces of the WT and the mutant are superimposed in Fig. 3C. During repetitive stretch-relax measurements on the mutant, the refolding force peaks diminished further indicating some refolding “fatigue” (Fig. 4 and Fig. S3A-C) that was not observed for the WT (Fig. S3 D-F; Supporting information in ref. (7)). In summary, H→R substitution in the TPLH motif seems to significantly affect the mechanical unfolding and refolding behavior of consensus ARs.

Our MD and AFM results revealed that H→R substitutions in TPLH motifs: 1) eliminate inter-repeat hydrogen bonds, greatly increasing the flexibility in the loop regions, which lessens the mechanical stability of the mutated ARs; 2) decreased the observed unfolding and refolding forces as compared to those of the WT when both proteins are stretched by their termini (mechanical unfolding energy of 53 kcal/mol vs. 106 kcal/mol); 3) caused mechanical fatigue, which is absent in the WT protein. These results indicate that the mutated ARs in NI(II_R)IC unfold easily and refold in a less springy, less robust manner as compared to the WT protein. We hypothesize that the H→R mutation in ankyrin-R may cause HS by altering its tertiary structure and decreasing its mechanical robustness. Repetitive deformations of ankyrin-R during circulation of RBCs may induce the HS mutant to progressively lose its structure and elastic properties leading to the mechanical failure of ankyrin-R. This in turn could perturb the protein network mediated by ankyrin-R and disrupt important membrane-cytoskeleton connections.

REFERENCES


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**FIGURE LEGENDS**

**FIGURE 1.** Structures of the wild-type NI6C and the mutant NI(I_{H7R})IC.

A, Putative hydrogen bonds (H-bonds) of a histidine in the TPLH motif in NI6C. These structures are determined based on Ref. (7,42). The program MolProbity was used to add hydrogen atoms to the structure with the Asn/Gln/His flip-optimization option on (46-48). Intra- and inter-repeat H-bonds between histidine and tyrosine are depicted with dotted lines.

B, The amino acid sequence of NI(I_{H7R})IC. Histidines of 4 internal repeats were mutated to arginines using VMD(39).

C, RMSD from initial structure in the simulations, with wild-type show in shades of pink and mutant in shades of green.

D, Ribbon diagram showing every 200 ps of the equilibrium MD trajectories for internal repeats 3 and 4 (totaling 100ns for each wild-type and mutant).

E, Root-mean-squared fluctuations averaged over all trajectories for the wild-type (pink) and mutant (green) equilibrium MD simulations. Vertical grey bars indicate the position of the H7R mutation.

**FIGURE 2.** Unfolding force-extension traces of the wild-type NI6C and mutant NI(I_{H7R})IC.

A, The schematic diagrams of NI6C-I27 and NI(I_{H7R})IC-I27 chimeric proteins (inset) and their representative unfolding traces at the pulling speed of 0.1 nm/ms. Force peaks of NI6C-I27 are fitted to two families of WLC curves, with contour length increments ΔLc = 10.5 nm (blue dashed lines, persistence length, p, ~ 0.7 nm) and 28 nm (gray dashed lines, p ~ 0.36 nm) corresponding to the unfolding of individual ARs (33 aa * 0.365 nm/aa ~ 0.8 nm (folded length)) and of I27 domains (89 aa * 0.365 nm/aa ~ 4 nm (folded length)), respectively.

B, The magnified portion of the unfolding force-extension curve of NI(I_{H7R})IC-I27 in A corresponding to the extension between 25 nm and 100 nm. The gray trace and a black dashed line show the force baseline.

C, The mutant force-extension trace (green, the same trace in A and) is compared with the unfolding force-extension trace of the wild-type (red, the same trace in A). The unfolding force peaks are divided into two groups: middle peaks (green bar), and last peaks (blue bar).
FIGURE 3. Unfolding and refolding force-extension traces of the wild-type Ni6C and mutant Ni(IHTR)4IC. 
A, A set of unfolding (green) and refolding (blue) force-extension traces of Ni(IHTR)4IC. The gray trace and a black dashed line show the force baseline. 
B, A set of unfolding (red) and refolding (black) force-extension traces of Ni6C. The black dashed line shows the force baseline. 
C, A comparison of the refolding traces of Ni(IHTR)4IC (blue trace in A) and Ni6C (black trace in B).

FIGURE 4. Cyclic stretch-relax measurements of Ni(IHTR)4IC captured the mechanical fatigue. A Ni(IHTR)4IC molecule is partially stretched and relaxed at the extension rate of 0.03 nm/ms. After one stretch-relax measurements, a molecule was re-stretched after waiting 15~30 sec.
Figure 1

Figure 1 (A) shows a close-up of the ankyrin repeat domain, highlighting key residues such as Thr and His. The corresponding amino acid sequences are shown below the figure:

- DLGKKLLL EAARAGQDDEVRLMANGADVAK N
- DKDGYTPLHLAAREGHEIVEVLLKAGADVAK
- DKDGYTPLHLAAREGHEIVEVLLKAGADVAK 2
- DKDGYTPLHLAAREGHEIVEVLLKAGADVAK
- DKDGYTPLHLAAREGHEIVEVLLKAGADVAK
- DKDGYTPLHLAAREGHEIVEVLLKAGADVAK 3
- DKDGYTPLHLAAREGHEIVEVLLKAGADVAK
- DKDGYTPLHLAAREGHEIVEVLLKAGADVAK 4
- DKDGYTPLHLAAREGHEIVEVLLKAGADVAK
- DKDGYTPLHLAAREGHEIVEVLLKAGADVQ 6
- DKFGKTAFDISIDNGNEDSIMAILQ

Figure 1 (B) presents a bar chart showing the RMSD values over time for different conditions labeled as Ni6C - 1, Ni6C - 2, Ni6C - 3, and Ni((Glu))6C - 1, Ni((Glu))6C - 2, Ni((Glu))6C - 3. The x-axis represents time in nanoseconds (t/ ns) ranging from 0 to 40, while the y-axis shows RMSD in Ångstrom (Å) ranging from 0.5 to 4.

Figure 1 (C) displays a similar bar chart, but with a focus on the Ca RMSD values. The graph shows a detailed representation of how Ca RMSD changes with respect to time and conditions.

Figure 1 (D) shows a comparison of the structures of different conditions: Ni6C and Ni((Glu))6C. The figures highlight the structural differences and similarities between these conditions.

Figure 1 (E) illustrates the RMSF (RMS fluctuation) values for different residues labeled as N, I1, I2, I3, I4, I5, I6, and C. The RMSF values are shown for both Ni6C and Ni((Glu))6C conditions, with the x-axis representing residue numbers from 20 to 260, and the y-axis indicating RMSF values from 0.5 to 4 Å.
Figure 2

A

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Extension / nm

28 nm

10.5 nm

B

C

pN

20 nm

20 nm

middle

last
Figure 3
Figure 4
Mutation of conserved histidines alters tertiary structure and nanomechanics of consensus ankyrin repeats.

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Under “Results and Discussion,” line 5 should read as follows. “As marked by dotted lines in Fig. 1A, histidines at position 7 and threonine at position 4 in TPLH motifs form an H-bond network. Each TPLH histidine forms three H-bonds with the TPLH threonine within the same repeat and one H-bond with the TPLH threonine of the next AR.”

In the legend to Fig. 1, lines 2 and 3 should read as follows. “Intra- and inter-repeat H-bonds between histidine and threonine are depicted by dotted lines.”

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