Mutations Alter RNA-Mediated Conversion of Human Prions
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Supporting Information

ABSTRACT: Prion diseases are connected with self-replication and self-propagation of misfolded proteins. The rate-limiting factor is the formation of the initial seed. We have recently studied the early stages in the conversion between functional PrP^{C} and the infectious scrapie PrP^{SC} form, triggered by the binding of RNA. Here, we study how this process is modulated by the prion sequence. We focus on residues 129 and 178, which are connected to the hereditary neurodegenerative disease fatal familial insomnia.

INTRODUCTION

Prions play a critical role in the maintenance and growth of neuronal synapses in mammalian and avian species, and improperly folded prions are associated with various neurodegenerative diseases. Examples are scrapie in sheep, bovine spongiform encephalopathy in cows, chronic wasting disease in elks and deer; and Creutzfeldt-Jacob, Kuru, fatal familial insomnia, and potentially even the pregnancy-specific disorder pre-eclampsia, in humans. The shared characteristic in all of these illnesses are cytotoxic aggregates formed from misfolded prions.

However, the underlying disease mechanism is difficult to probe as, unlike the functional form PrP^{C}, no structural models of misfolded and toxic PrP^{SC} have been resolved and published in the protein data bank (PDB). Circular dichroism measurements show for this so-called scrapie form PrP^{SC} a reduction in helicity from 43% in PrP^{C} to 30% in PrP^{SC}, coupled with a rise in β-strand frequency of 3% in PrP^{C} to 43% in PrP^{SC}. The mouse prion, it is known that it is primarily the N-terminal region of residues 129 and 178, which are connected to the hereditary neurodegenerative disease fatal familial insomnia.

Figure 1. Structure of the poly-A-RNA fragment. Note that all bonds are flexible during docking, allowing the molecule to bind to the protein surface.

either a polybasic domain of residues 21–31, or with a segment made form residues 144–155, leads to the formation of a characteristic pincer motif between the polybasic domains of residues 21–31 and on helix A. This pincer encapsulates the RNA fragment, and the formation of the resulting complex leads to unfolding of helix A as interactions between the RNA fragment and the side chains of residues 144–148 disrupt locally the backbone hydrogen bonds of helix A.

In the present project, we study whether and how this mechanism changes when going from wild-type prions to mutants associated with severe illnesses. Fatal familial insomnia is a neurodegenerative disorder caused by the loss of neurons in the thalamus; this leads to a progressively worsening psychosis which rapidly degenerates into dementia. There is currently no effective treatment, and patients die within 12 months of the first appearance of symptoms. The disease is associated with a heritable mutation D178N, replacing an acidic aspartic acid (D) by a neutral asparagine (N), which is known to increase the polymorphism of the scrapie form. The disease also requires methionine (M) at position 129 and is not...
accompanied by decay of the N-terminal helix A, the molecular dynamic simulations the formation of a pincer motif. Only when the RNA binds to site 1 and site 3 did we see in site 2 was observed 4 times, and site 3 was observed 3 times. For the 129M-178N mutant, binding site 3 is observed in all of site 1 is found 3 times, and site 2 is seen once. Site 3 is the only one that shows an increased affinity, although less discernible than previous mutations, with −6.8 (1.1) kcal/mol in 129M-178D wild type leading to the unfolding of helix A. The same is observed here for mutant (our system 129M-178N) is only observed at binding this binding site is found corresponds again to a lower average binding energy of −8.4 (0.7) kcal mol (compared to −6.5 (0.8) kcal/mol for the 129M-178D wild type) calculated by Autodock. The residue replacements D178N increases the stability and extension of the pincer. Poly-A-RNA binding to this site that allows for the pincer conversion mechanism proposed by us in earlier work,18 whereas the mutation D178N increases the residue replacements M129V and/or D178N shift the binding site 3 is the only one that shows an increased affinity, although less discernible than previous mutations, with −6.8 (1.1) kcal/mol in 129M-178D wild type. For the 129V-178D sequential variation of the wild type, 8 of the top 10 complexes bind at site 2, with the remaining two complexes binding to site 1. The higher frequency with which this binding site is found corresponds again to a lower average binding energy of −8.4 (0.7) kcal mol (compared to −6.5 (0.8) kcal/mol for the 129M-178D wild type) calculated by Autodock. Little changes are seen for binding site 1: −6.6 (0.7) kcal/mol in 129M-178D wild type, 8 of the top 10 complexes bind at site 2, with the remaining two complexes binding to site 1. The higher frequency with which this binding site is found corresponds again to a lower average binding energy of −8.4 (0.7) kcal mol (compared to −6.5 (0.8) kcal/mol for the 129M-178D wild type) calculated by Autodock. Little changes are seen for binding site 1: −6.6 (0.7) kcal/mol in 129M-178D wild type, 8 of the top 10 complexes bind at site 2, with the remaining two complexes binding to site 1. The higher frequency with which this binding site is found corresponds again to a lower average binding energy of −8.4 (0.7) kcal mol (compared to −6.5 (0.8) kcal/mol for the 129M-178D wild type) calculated by Autodock. Little changes are seen for binding site 1: −6.6 (0.7) kcal/mol in 129V-178N. For the last prion, the mutant 129V-178N, we again observe all three of our previously identified binding sites: site 3 is found 6 times, site 1 is found 3 times, and site 2 is seen once. Site 3 is the only one that shows an increased affinity, although less discernible than previous mutations, with −6.8 (1.1) kcal/mol in 129M-178D rising to −7.9 (0.7) kcal/mol in 129V-178N. As in our previous study of the 129M-178D wild type, we selected for all of the three new systems (129V-178D, 129M-178N, and 129V-178N) the most probable conformation (see methods section for further explanation) of a complex with a certain binding site and followed its time evolution over 300 ns in three independent trajectories. A matrix of the various systems, binding sites, and trajectory numbers is given in Table 1. Visual Inspection and Trajectory Analysis. Fatal familial insomnia is connected with a methionine at residue 129 (129M) and the mutation D178N. Poly-A-RNA binding to this mutant (our system 129M-178N) is only observed at binding site 3, residues 144–155. In the wild type (129M-178D) studied in our previous work, binding of RNA to site 3 always lead to the unfolding of helix A. The same is observed here for the mutant; however, we find two differences. First, in about 60% of all configurations sampled in our molecular dynamics trajectories, residue 178N forms a hydrogen bond with 18D that restrains the movement of helix C and keeps it in close proximity to both helix A and the polybasic domain of residues 21–31, see Figure 3A. This, second, allows for the RNA to interact with both helix A (140–158) and helix C (200–220), observed with a valine (V) at position 129, another often found variant of the wild type.19 In our previous investigation,18 we considered only the predominant 129M variant of the wild type. Here, we extend these investigations and research on how the residue replacements D178N and M129V alter the mechanism that leads to unfolding of helix A. Four systems will be studied: the wild type with 129M and 178D (129M-178D), its variant with 129V (129V-178D), and the two mutants 129M-178N and 129V-178N. The sequence of these four systems is listed in Figure 2. By analyzing long-time molecular dynamics simulations of these four systems, we argue that residue 129 controls the probability of RNA binding to a site that allows for the pincer conversion mechanism proposed by us in earlier work,18 whereas the mutation D178N increases the stability and extension of the pincer.

### RESULTS AND DISCUSSION

In ref 17, we have identified three sites where the poly-A-RNA can bind to the PrPC form of the wild-type 129M-178D. At binding site 1, the RNA interacts with residues 21–31, at binding site 2 with residues 111–121; and binding site 3 consists of residues 144–155. In the 11 stable complexes predicted by Autodock, binding site 1 was observed 5 times, site 2 was observed 4 times, and site 3 was observed 3 times. Only when the RNA binds to site 1 and site 3 did we see in molecular dynamic simulations the formation of a pincer motif followed by decay of the N-terminal helix A, the first step in the conversion to the scrapie form PrPC. On the other hand, binding to site 2 did not alter the stability of helix A or any other helix.

The residue replacements M129V and/or D178N shift the probabilities of the binding sites but we do not find new binding sites in the three prions 129V-178D, 129M-178N, and 129V-178N (sequences shown in Figure 2) now considered. For the 129M-178N mutant, binding site 3 is observed in all of the top 10 complexes predicted by Autodock. Furthermore, the estimated binding affinity is with −9.3 (0.5) kcal/mol, substantially lower than the corresponding binding energies of −6.8 (1.1) kcal/mol for site 3 in the 129M-178D wild type. For the 129V-178D sequential variation of the wild type, 8 of the top 10 complexes bind at site 2, with the remaining two complexes binding to site 1. The higher frequency with which this binding site is found corresponds again to a lower average binding energy of −8.4 (0.7) kcal mol (compared to −6.5 (0.8) kcal/mol for the 129M-178D wild type) calculated by Autodock.

### Table 1. Frequency of Binding Sites Found in the Top 10 Autodock Predictions

| system      | freq site 1 | affinity site 1 | freq site 2 | affinity site 2 | freq site 3 | affinity site 3 | trajectories |
|-------------|-------------|-----------------|-------------|-----------------|-------------|-----------------|--------------|
| 129M-178D   | 4           | −6.6(0.7)       | 3           | −6.5(0.8)       | 3           | −6.8(1.1)       | 3 × 3        |
| 129V-178D   | 2           | −6.3(1.0)       | 8           | −8.4(0.7)       | 0           | N/A             | 3 × 2        |
| 129M-178N   | 0           | N/A             | 0           | N/A             | 10          | −9.3(0.8)       | 3 × 1        |
| 129V-178N   | 1           | −6.0(0.9)       | 3           | −6.3 (1.0)      | 6           | −7.9 (0.7)      | 3 × 3        |

*Listed are also the average binding affinities (in kcal/mol) of these sites as calculated by Autodock. For a given system and binding site the most stable complex is followed in three trajectories over 300 ns.
see Table 2. As a consequence, not only helix A but also most of helix C has dissolved in the final conformation of the 300 ns trajectory. Note that helix C is now also participating in the three-pronged helix-polybasic pincer motif, adding to the polybasic domain of residues 21–31 and the segment of residues 140–161 on helix A additional contacts between the RNA and residues 219–223 on helix C (see Table 2). However, while all trajectories led to unfolding of helix A, this enlarged pincer and the decay of helix C is only seen in two of the three trajectories.

The above picture is quantified in Figure 4B, where we show the time evolution of the RMSD with respect to the start configuration. We compare the complex of poly-A-RNA binding to the prion with that of the isolated prion having the same structure. For comparison, we show in Figure 4A the same quantity as calculated from our previous studies of the wild-type 129M-178D in ref 17. In all systems, we distinguish between complexes where the RNA docks to binding site 1 or 3 (shown in red), and where it docks to binding site 2 (shown in gold). The blue circle indicates the region of the trajectory after which less than 50% of the helical contacts of helix A remain. These are also the regions where we observe large changes in RMSD. This transition region appears much earlier in the trajectory for the mutant system. Note also the second jump in RMSD marked by the black circle where 50% of helical contacts for helix C have decayed. The decrease in helicity is also seen in Table 3. Although the helicity does not change in the control, the average helicity decreases from about 43 to 32% for the wild type when RNA binds to either site 1 or 3. In the 129M-178N mutant, the overall helicity decreases from 45 to 30%, most of it coming from helix A (95 to 20%), with the reduction for helix C from 96 to 38%.

Fatal familial insomnia is not observed in humans carrying the D178N mutation if residue 129 is a valine instead of a methionine (more commonly observed at this position).

Figure 3. Initial configurations of complexes of RNA bound to our three prion models. The 129M-178N mutant binds only to site 3 shown in (A), whereas the 129V-178N binds to site 1 (B), site 2 (C), or site 3 (D). The 129V-178D wild-type variant binds to either site 2 (E) or site 1 (F). Colors denote the following: brown: RNA molecule, blue helix: helix A, yellow helix: helix B, purple helix: helix C, red strand: binding site 1, orange strand: binding site 2, blue ball: N-terminus, and red ball: C-terminus.

Table 2. Contacts Between the Prion Protein and the RNA Fragment that are Formed in More Than 50% of the Time Steps

|                | 129M-178D | 129M-178N | 129V-178N | 129V-178D |
|----------------|-----------|-----------|-----------|-----------|
| **Contacts with Helix A** |           |           |           |           |
| 144D           | 144D      | 144D      | 144D      | 144D      |
| 145W           | 145W      | 145W      | 145W      | 145W      |
| 147D           | 147D      | 147D      | 147D      | 147D      |
| 148R           | 148R      | 146E      | 146E      | 146E      |
| 139H           | 148R      | 148R      | 148R      | 148R      |
| 140F           | 149Y      | 149Y      | 149Y      | 149Y      |
| 146E           | 150Y      | 150Y      | 150Y      | 150Y      |
| 149Y           |           |           |           |           |
| 150Y           |           |           |           |           |
| **Contacts with Helix C** |           |           |           |           |
| N/A            | N/A       | 223Q      | 223Q      | 223Q      |
| 225Y           |           |           |           |           |
| **Contacts Around Residues 21–31** |           |           |           |           |
| 25R            | 25R       | 24K       | 24K       | 24K       |
| 27K            | 27K       | 25R       | 25R       | 25R       |
| 34G            | 34G       | 27K       | 27K       | 27K       |
| 35G            | 35G       | 31N       | 31N       | 31N       |
| 41Q            | 41Q       | 23K       | 31N       | 31N       |

aData are for complexes where the RNA fragment binds to either site 1 or site 3, leading to unfolding of helices.
position 129, the 129V-178N mutant leads to poly-A-RNA binding to all three binding sites that are also observed in our previous simulations\(^{19}\) of the wild-type 129M-178D. Complexes with these three binding sites are shown in Figure 3B–D. Similar to the behavior of the 129M-178D wild type, unraveling of helices for the mutant 129V-178N is observed only for binding at sites 1 or 3, not when the poly-A-RNA fragment binds to site 2. However, Table 2 also shows that if only for binding at sites 1 or 3, not when the poly-A-RNA

The above observations are again quantified by the RMSD plots in Figure 4C. Initially, the docked system resembles the 129M-178D wild type (Figure 4A), with similar regions for the decays of helix A (indicated by blue circles). At 240 ns, we also see a jump in RMSD corresponding to the unraveling of helix C indicated by a black circle, however, the signal is less pronounced than for the 129M-178N mutant. Correspondingly, the decline in helicity in Table 3 is less than that for the 129M-178N mutant, decreasing from 97 to 25% in helix A and 97 to 48% in helix C. Hence, comparing the two mutants 129M-178N and 129V-178N, it appears that the mutation D178N extends the unraveling of helices from helix A to helix C. On the other hand, a valine instead of a methionine as residue 129 seems to increase the frequency of binding to site 2 which does not lead to unraveling of helices in the prion protein.

In the wild type, we observe transient \(\beta\)-strands in the region of helix A that hint at the start of the conversion to the PrP\(^{\text{Sc}}\) state, as shown by the increase in relative \(\beta\)-strand content and occupancy in Table 3. Here, occupancy is defined as the average amount of time a \(\beta\)-strand is observed. Although the total \(\beta\)-strand propensity increases only by about 5%, the average life time of the transient \(\beta\)-strands grows by about 25%, which may indicate that the conversion to the \(\beta\)-sheet-rich PrP\(^{\text{Sc}}\) structure is to begin. Such transient \(\beta\)-strands are also observed in both D178N mutants, with similar values for \(\beta\)-strand content and occupancy. However, even with the more rapid and extensive helical unfolding seen in the D178N mutants, 300 ns is clearly too short for the formation of stable \(\beta\)-sheets expected in the PrP\(^{\text{Sc}}\).

From our comparison of the two mutants 129M-178N and 129V-178N, we would expect to see for the wild type with valine at position 129 (129V-178D) that the frequency of complexes with poly-A-RNA binding to site 2 is larger than that seen for the 129M-178D wild type. We find indeed that for 129V-178D in 80% of the complexes, poly-A-RNA binds to site 2 (Figure 3E), whereas the corresponding number in our previous simulations of 129M-178D is only 40%\(^{19}\). As observed in all our simulations, binding to site 2 does not lead to unfolding of helices. Only in 20% of cases did we find binding to site 1 (Figure 3F), and in no case binding to site 3. On the other hand, binding sites 1 and 3 are observed with a frequency of 80% in the 129M-178D wild type. While complexes with binding site 1 are observed with a lower frequency for the 129V-178D wild type than for the 129M-178D wild type, they lead again to the decay of helix A. However, the interaction is weaker with only a partial unraveling of helix A that starts late after \(\sim 150\) ns and is not preceded by the formation of the

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Table 3. Secondary Structure Averaged Over Specific Periods of the Respective Trajectories\(^{a}\)

| Secondary Structure | 129M-178D | 129V-178D |
|---------------------|-----------|-----------|
|                     | control 0–300 ns | complex 0–100 ns | complex 200–300 ns | control 0–300 ns | complex 0–100 ns | complex 200–300 ns |
| total helicity      | 43% (2%)  | 39% (3%)  | 32% (2%)  | 44% (2%)  | 43% (1%)  | 44% (1%)  |
| helix A             | 92% (2%)  | 56% (6%)  | 22% (4%)  | 97% (1%)  | 97% (1%)  | 97% (2%)  |
| helix C             | 96% (2%)  | 93% (3%)  | 92% (3%)  | 98% (2%)  | 94% (1%)  | 95% (2%)  |
| \(\beta\)-strands   | 4% (1%)   | 7% (2%)   | 9% (2%)   | 5% (1%)   | 4% (1%)   | 4% (1%)   |
| \(\beta\)-strand occupancy | 28% (1%) | 38% (5%) | 58% (4%) | 28% (2%) | 25% (1%) | 27% (1%) |

| Secondary Structure | 129M-178N | 129V-178N |
|---------------------|-----------|-----------|
|                     | control 0–300 ns | complex 0–100 ns | complex 200–300 ns | control 0–300 ns | complex 0–100 ns | complex 200–300 ns |
| total helicity      | 45% (2%)  | 39% (3%)  | 30% (3%)  | 48% (3%)  | 40% (3%)  | 36% (5%)  |
| helix A             | 95% (3%)  | 62% (7%)  | 20% (5%)  | 98% (2%)  | 60% (7%)  | 25% (5%)  |
| helix C             | 96% (4%)  | 73% (3%)  | 38% (6%)  | 97% (3%)  | 81% (4%)  | 48% (5%)  |
| \(\beta\)-strands   | 4% (1%)   | 7% (3%)   | 9% (3%)   | 4% (2%)   | 6% (2%)   | 8% (3%)   |
| \(\beta\)-strand occupancy | 27% (2%) | 40% (3%) | 58% (6%) | 27% (2%) | 40% (4%) | 52% (5%) |

\(^{a}\)Data are for complexes where the RNA fragment binds to either site 1 or site 3.
pincer motif. This can be seen from the RMSD plot in Figure 4D where we do not see a discernible loss in helical contacts until the last 50 ns of the trajectory. In addition, there is a little difference between the RMSD values for binding site 1 (shown in red), binding site 2 (shown in gold), and the undocked system (shown in blue). Hence, it seems that valine instead of methionine at position 129 decreases not only the probability to bind to site 1 or site 3, but also in the rare cases where binding to these sites is seen and hinders or delays the formation of the pincer by increasing the flexibility in critical regions of the N-terminal domain.

Hydrogen Bond Pattern and Structural Flexibility. To understand how the variation in sequence at position 129 and 178 changes the effect of poly-A-RNA binding to prions, we focus on trajectories where the RNA fragment binds to either site 1 or 3. This is because binding to site 2 never lead to unraveling of helices in the prion protein which we assume to be the initial state in the conversion from PrPC to the scrapie form PrPSc. We start by looking in Figure 5 into the root-mean-square fluctuation (RMSF) of each residue in various systems. The presented data are for complexes where the RNA fragment binds to the prion at either site 1 or 3 and are divided by the corresponding values for the undocked prions. A ratio of 1 (marked by a green line) means that a given residue is equally flexible in the bound system and in the isolated prion. Values above the green line indicate elevated flexibility upon interaction with RNA.

As expected, a similar but less pronounced behavior is also observed in the 129V-178N mutant (Figure 5C), with the unraveling of helix C delayed (Table S2). Note especially that the binding of poly-A-RNA to the two mutants does not increase the flexibility of residues 45–90 as it does for the wild-type 129M-178D. The increased flexibility of these residues in the later system results from a loss of contacts between helix A and polar residues of the N-terminal domain upon interaction with the RNA fragment. In the wild type, the frequency of these contacts drops from 32% (7%) for the isolated prion to 12% (3%) when in complex with RNA. However, in the two mutants, there is the possibility for a contact between residue 178N and 18D that stabilizes the segment. As a consequence, the frequency of contacts upon binding of RNA does not drop in the 129M-178N mutant, 36% (5%) versus 39% (4%), Hence, on effect of the D178N, mutation seems to be the stabilization of the N-terminal segment of residues 45–90, which makes it easier to form the pincer that is associated with unraveling of the helices in the prion. This effect is weaker in the 129V-178N mutant, where the frequency of stabilizing contacts still drops from 39% (6%) to 22% (4%).

On the other hand, the drop in frequency of these contacts in the 129V-178D variant of the wild type is similar to that in the 129M-178N form: 31% (4%) down to 11% (6%), however, the relative fluctuations in the N-terminal region are larger than that in the 129M-178D wild type, see Figure 5D. Hence, a valine instead of a methionine at position 129 may not only shift the binding pattern to site 2 but also increase the flexibility of the N-terminus. Because for 129V-178D, the RNA fragment binds only to site 1 (residues 21–31) or 2 (residues 111–121), the higher flexibility of this region may explain the difficulties seen in forming a stable pincer motif and the late unraveling of helix A seen for this variant of the wild type.

Table 4 shows that despite the loss of the backbone hydrogen bonds in helix, the newly formed contacts of residues with the poly-A-RNA result in an overall higher number of hydrogen bonds. For the fatal familial insomnia causing sequence 129M-178N, 14 hydrogen bonds are gained despite the dissolution of helix A and C, possibly indicating the start of forming another ordered structure. For the wild type and the

| Table 4. Frequency of Main-Chain Hydrogen Bonds in Complexes and the Controls, and Their Difference Δ° |
|---------------------------------------------------------------|
| Name | 0−100 ns control | 0−100 ns complex | Δ | 200−300 ns control | 200−300 ns complex | Δ |
| 129M−178D | 75 (3) | 82 (2) | 7 (4) | 82 (4) | 93 (3) | 11 (4) |
| 129V−178D | 73 (4) | 75 (3) | 2 (5) | 80 (4) | 75 (4) | −5 (5) |
| 129M−178N | 80 (3) | 88 (3) | 8 (4) | 87 (3) | 101 (3) | 14 (3) |
| 129V−178N | 77 (3) | 81 (2) | 4 (4) | 75 (3) | 88 (3) | 12 (4) |

°Data are for complexes where the RNA fragment binds to either site 1 or site 3.
nondisease-causing mutant 129V-178N, the gain is still 12 hydrogen bonds. Hence, for the two mutants and the 129M wild type, despite the loss of helix-stabilizing hydrogen bonds, the binding of RNA appears energetically favorable. This is different for the 129V variant of the wild type where because of the increased flexibility of the N-terminus, about five hydrogen bonds are lost.

**CONCLUSIONS**

We have extended a previous investigation of the effect of poly-A-RNA on the conversion of functional PrP\textsuperscript{C} into the toxic and self-propagating scrapie form PrP\textsubscript{SC}, by exploring how this process depends on the prion sequence. Our guiding assumption is that mutants which are associated with fast disease progression and sever symptoms ease the process of seed generation. The example we chose is the D178N mutant which, when going together with a methionine as residue 129, 129M-178N, leads to fatal familial insomnia, but not if residue 129 is a valine (129V-178N). As controls, we also looked into the case of the wild-type variant 129V-178D (instead of the 129M-178D version studied by us in previous work) which has a valine as residue 129 and is known to be associated with a lower frequency of neurodegenerative disorders. In a meta-analysis of Creutzfeldt–Jakob patients, only 10% were found to be homozygous for valine at the 129 position, with ~50% being homozygous for methionine at the 129 position and the remaining being heterozygous.

In all cases, we observe that unraveling of helix A is connected with the appearance of a pincerlike motif between helix A and the polybasic domain that encapsulates the RNA. Formation of the pincer requires binding of the RNA to either the polybasic segment of residues 21–31 (binding site 1) or the segment 141–155 (binding site 3) and traps the RNA. In both D178N mutants, this pincer becomes three-pronged and leads also to the unraveling of helix C. As the molecular dynamic trajectories proceed, formation of the pincer is followed by replacement of helical contacts in helix A (and helix C in the mutants) by short and transient \(\beta\)-strands that eventually may lead to the high \(\beta\)-sheet propensity of the disease-causing misfolded PrP\textsubscript{SC} structure. Hence, RNA binding to the prion can trigger the conversion of the cellular prion protein structure PrP\textsuperscript{C} to its infectious scrapie PrP\textsubscript{SC} form, a process whose early steps (formation of the pincer motif starting unfolding of helix A and in the mutants helix C) could be observed in our 300 ns long molecular dynamics trajectories.

The sequence variation at positions 129 and 178 of the prion protein modulates this process in two ways. First, they alter the probability that RNA binds to the prion protein at a site that allows the formation of the pincer motif, and second, they change the stability and extension of this motif. The gatekeeper for the first effect appears to be residue 129, with a valine at this position skewing the binding of RNA to site 2, which does not lead to unraveling of helices, and further increasing the flexibility of the N-terminus. On the other hand, a methionine at this position increases the chances of binding to site 1 or site 3, resulting in the formation of the pincer and subsequent unfolding of helix A. The second effect is controlled by residue 178. This mutation decreases the frequency of the side chain contact between 178D and 101K, which in turn increases the flexibility in residues 90–105 around binding site 2. Hence, the probability to bind to site 2 while at the same time binding to site 3 will be enhanced. An even more important effect of the mutation D178N is the formation of a stable contact between residues 178N and 18D that restricts the motion of the polybasic domain of residues 21–31, enhancing the appearance of the pincer motif. Furthermore, the 178N-18D contact brings the RNA into close proximity with the C-terminal region of helix C, allowing for the formation of a three-pronged pincer between the polybasic domain and the two helices. This leads to the eventual decay of helical contacts in both helix A and C. We caution that this relation between rigidity of the N-terminal region (residues 21–31) and enhanced prion fibril formation is not general. Protein-docking studies by Schillinger et al. suggest that the increased flexibility enhances the rate of assembly for the human interleukin-6 receptor complex. Conversely, Kouza et al. saw a decline in the rate of aggregation of amyloidogenic monomers with increased flexibility of the peptide. It is likely specific to our system that the increased rigidity of this key region is connected with enhanced unfolding and (presumed) subsequent aggregation. The net effect of the residue combination 129M and 178N is that it both increases the frequency of binding of RNA to sites that allow for the formation of the pincer motif and the strength and extension of this motif. The net effect is a faster conversion of the functional PrP\textsuperscript{C} prion structure to an infectious scrapie PrP\textsubscript{SC} form that may seed the formation of toxic amyloids which then cause the symptoms of familial fatal insomnia.

**MATERIALS AND METHODS**

**Model Generation.** In human systems, prion proteins attach to the cell membrane of neurons in the extracellular space via a glycosylphosphatidylinositol (GPI) anchor which is added to the C-terminus following a prior cleavage of 24 residues from the C-terminal residues. As our previous study showed that an unanchored system is sufficient to model the initial stages of conversion, we save on the computational costs by modeling all our systems in this study with unanchored prions. To exclude erroneous charge interactions in the C-terminal region, we simulate the full-sized, noncleaved prions with all 253 residues. As only the C-terminal region of the native protein structure has been fully resolved, we have used in our previous work the two programs MODELLER and ITASS for prediction of the un-resolved N-terminal segment of residues 1–121. Both methods led to similar models with marginal differences in binding sites and strength. As the ITASS structure preserved slightly the C-terminal helices, we used this protein structure as the starting point for our study. Using ITASS to refine our model to our previously determined template, we took this wild-type PrP\textsuperscript{C} structure and mutated residues 178D and/or 129M into 178N and/or 129V. This led to three structures, corresponding to 129M and 178N, 129V and 178D, 129V and 178N, which were relaxed in short (5 ns) molecular dynamics simulations at 310 K and 1 bar. Configurations were taken from these trajectories at 500 ps intervals (0.5 ns) and assessed using three separate methods for quantifying the model quality: Rampage, ERRAT, and ProQ. For this purpose, we calculate the average score for the corresponding trajectory. If this score is below the cutoff value for any of the three methods, the initial structure is refined using a combination of MODELLER and ITASS, as outlined in ref 36 until the resulting trajectory passes the quality threshold. The so-generated three structures were selected for the next stage of model generation.

**Docking Confirmation.** As in our previous work, we selected Autodock 25 to generate our protein-RNA complexes, a program that has a successful history of modeling similar
A 5-nucleotide snippet of poly-A-RNA was selected as this is the minimal size that let in experiments to consistent prion conversion whereas photodegradation below the five nucleotide threshold drastically lowered the rate of conversion. Our docking protocol allows for free rotation around all single bonds in the RNA molecule. The 10 highest scoring docked systems for all three target structures were collected for the next stage of model generation. These complexes were examined for common regions of protein-RNA interaction and compared to the ones seen in our previous study. The D178N mutation 129M-178N is the most probable binding site for all 10 complexes, the one that we called binding site 3 (residues 135–145) in our previous study. The M129V sequence change (129V-178D) results in eight complexes with binding site 2 (residues 111–121) and two complexes with binding site 1 (residues 21–31). The third system, characterized by residue changes M129V and D178N (129V-178N), has again only binding sites also seen in our previous study: six complexes with binding site 1, one complex with binding site 2, and three complexes with binding site 3. We then evaluated the stability of all 30 complexes in short (10 ns) long molecular dynamics simulations with a temperature of 310 K and pressure of 1 bar, looking for RNA detachment from the protein. No such detachment was seen for the D178N mutant 129M-178N. For the 129V-178D system, the RNA did detach for two of the eight complexes with binding site 2. For the system with both residue alterations M129V and D178N (129V-178N), the RNA detached from the Prion was not only in the sole complex with binding site 2 but also in one of the six complexes with binding site 1. Note that in our previous study of prion RNA interaction, we encountered similar events of RNA detachment, all involving binding site 2. In the present study for long molecular dynamics runs, we considered only complexes where we did not observe RNA detachment in the short runs. Out of the remaining cluster, we selected a start configuration for the long runs for each system and each observed binding site, the one that had the lowest RMSD in the helix A region at the end of the above-described short runs. In this way, we tried to minimize any possible bias in our complexes toward helix instability. Table 1 presents a matrix of systems and binding sites with the number of structures and long molecular dynamics runs for each case. As a control, we also evaluate the stability of the isolated prions, by following molecular dynamics trajectories of the same length and generated with the same simulation protocol, with the prion having the same start configuration as in the corresponding complex.

Simulation Protocol. All our molecular dynamics simulations rely on the GROMACS software package version 4.6.5, using the CHARMM36 force field with associated nucleic acid parameters and TIP3P water model to model interactions between protein, RNA, and water. The prion was put at the center cubic box with at least 12 Å distance between the boundary of the box and the protein-RNA complex, and the box was filled with water molecules. Periodic boundary conditions are used, and the electrostatic interactions are calculated via the PME algorithm. Because of the size of the system and the potential for steric clashes during solvation, the solvated model was relaxed first by steepest descent energy minimization following a 2 ns molecular dynamic simulation using NVT protocol and a subsequent 2 ns simulation using NPT protocol. For integration of the equations of motion, a 2 fs time step is used, with hydrogen atoms constrained by the LINCS algorithm and water constrained with the Settle algorithm. The temperature of the system is kept at 310 K by the Parrinello–Donadio–Bussi thermostat (τ = 0.1 fs) and the pressure at 1 bar by Parrinello–Rahman algorithm (τ = 1 fs). A group cutoff scheme was selected, with a neighbor search using a grid-cutoff scheme with a cutoff distance of 1.5 nm. Electrostatic interactions outside these dimensions are handled by particle mesh Ewald with cubic interpolation and grid dimensions set to 0.15 nm.

For each of the systems listed in Table 1, we run three molecular dynamic trajectories differing in the initial velocity distributions to get a simple estimate of the statistical fluctuations between trajectories. Data are saved every 4 ps for further analysis. Using the internal tools of GROMACS, we measured RMSDs of the Cα atoms, secondary structure contents, contact distances, and hydrogen bonding. Configurations are visualized using PyMOL and VMD.
(2) Westerlund, I.; Christensen, H. M.; Harris, D. A. The cellular prion protein (PrP(C)) its physiological function and role in disease. *Biochim. Biophys. Acta, Mol. Basis Dis.* 2007, 1772, 629—644.

(3) Bremer, J.; Baumann, F.; Tiberi, C.; Wessig, C.; Fischer, H.; Schwarz, P.; Steele, A. D.; Toyka, K. V.; Nave, K.-A.; Weis, J.; Aguzzi, A. Axonal prion protein is required for peripheral myelin maintenance. *Nat. Neurosci.* 2010, 13, 310—318.

(4) Caiati, M. D.; Saffulina, V. F.; Fattorini, G.; Sivakumaran, S.; Legname, G.; Cherubini, E. PrP(C) controls via protein kinase A the direction of synaptic plasticity in the immature hippocampus. *J. Neurosci.* 2013, 33, 2973—2983.

(5) Buhimschi, I. A.; Nayeri, U. A.; Zhao, G.; Shook, L. L.; Pensalfini, A.; Funai, E. F.; Bernstein, I. M.; Glabe, C. G.; Buhimschi, C. S. Protein misfolding, congophilia, oligomerization, and defective amyloid processing in preeclampsia. *Sci. Transl. Med.* 2014, 6, 245ra92.

(6) Kouza, M.; Baneri, J.; Kolinski, A.; Buhimschi, I. A.; Kloczkowski, A. Oligomerization of FVFLM peptides and their ability to inhibit beta amyloid peptides aggregation: consideration as a possible model. *Phys. Chem. Chem. Phys.* 2017, 19, 2990—2999.

(7) Pan, K. M.; Baldwin, M.; Nguyen, J.; Gasset, M.; Serban, A.; Prusiner, S. B. Conversion of alpha-helices into beta-sheets features in protein oligomerization at atomic resolution. *ACS Omega* 2013, 8113—8122.

(8) Norstrom, E. M.; Barcikowska, M.; Liberski, P. P. The prion protein M129V polymorphism. *Neurology* 2009, 73, 8113—8122.

(9) Kouza, M.; Mo, N. T.; Nguyen, P. H.; Kolinski, A.; Li, M. S. Preformed template fluctuations promote fibril formation: insights from lattice and all-atom models. *J. Chem. Phys.* 2015, 142, 145104.

(10) Webb, B.; Sali, A. Comparative Protein Structure Modeling Using MODELLER. *Curr. Protoc. Bioinformatics* 2014, 47, S.6.1.

(11) Caiati, M. D.; Safiulina, V. F.; Fattorini, G.; Sivakumaran, S.; Legname, G.; Cherubini, E. PrP(C) controls via protein kinase A the direction of synaptic plasticity in the immature hippocampus. *J. Neurosci.* 2013, 33, 2973—2983.

(12) Schlepcow, K.; Schwalbe, H. Molecular mechanism of prion protein oligomerization at atomic resolution. *Angew. Chem., Int. Ed. Engl.* 2013, 52, 10002—10005.

(13) Tycko, R.; Savtchenko, R.; Ostapchenko, V. G.; Makarava, N.; Baskakov, I. V. The α-helical C-terminal domain of full-length recombinant PrP converts to an in-register parallel beta-sheet structure in PrP fibrils: evidence from solid state nuclear magnetic resonance. *Biochemistry* 2010, 49, 9488—9497.

(14) Salamat, K.; Moudou, M.; Chauvin, J.; Zuber, A.; Armant, E.; Béringue, V.; Rezea, H.; Pastore, A.; Laude, H.; Dron, M. Integrity of helix 2-helix 3 domain of the PrP protein is not mandatory for prion replication. *J. Biol. Chem.* 2012, 287, 18953—18964.

(15) Prusiner, S. B. Prion diseases and the BSE crisis. *Science* 1997, 278, 245—251.

(16) Singh, J.; Udgaonkar, J. B. Dissection of conformational conversion events during prion amyloid fibril formation using hydrogen exchange and mass spectrometry. *J. Mol. Biol.* 2013, 425, 3510—3521.

(17) Deleault, N. R.; Lucassen, R. W.; Supattapone, S. Prion nucleation site unmasked. *PLoS Pathog.* 2011, 7, No. e1002128.

(18) Deleault, N. R.; Lucassen, R. W.; Supattapone, S. RNA molecules stimulate prion protein conversion. *Nature* 2003, 425, 717—720.

(19) Alfred, E. J.; Nguyen, M.; Martin, M.; Hansmann, U. H. E. Molecular dynamics simulations of early steps in RNA-mediated conversion of prions. *Protein Sci.* 2017, 26, 1524—1534.

(20) Zurausha, A. Z.; Walsh, D. J.; Fortier, S. M.; Chidawanyika, T.; Sengupta, S.; Zilm, K.; Supattapone, S.; Prion nucleation site unmasked by transient interaction with phospholipid cofactor. *Biochemistry* 2014, 53, 68—76.

(21) Malempède, M. A.; Fernández-Vega, I.; Ansoleaga, B.; Blanco, T.; Carmona, M. A.; Antonio Del Rio, J.; Zerr, I.; Llorens, F.; Zarzur, J. R.; Ferrer, I. Fatal familial insomnia: mitochondrial and protein synthesis machinery decline in the mediodorsal thalamus. *Brain Pathol.* 2017, 27, 95—106.
C.; Michnick, S.; Ngo, T.; Nguyen, D. T.; Prodhom, B.; Reiher, W. E.; Roux, B.; Schlenkrich, M.; Smith, J. C.; Stote, R.; Straub, J.; Watanabe, M.; Wiorkiewicz-Kuczera, J.; Yin, D.; Karplus, M. All-atom empirical potential for molecular modeling and dynamics studies of proteins. J. Phys. Chem. B 1998, 102, 3586−3616.

(43) Mackerell, A. D., Jr.; Feig, M.; Brooks, C. L. 3rd Extending the treatment of backbone energetics in protein force fields: limitations of gas-phase quantum mechanics in reproducing protein conformational distributions in molecular dynamics simulations. J. Comput. Chem. 2004, 25, 1400−1415.

(44) Denning, E. J.; Priyakumar, U. D.; Nilsson, L.; Mackerell, A. D., Jr. Impact of 2′-hydroxyl sampling on the conformational properties of RNA: update of the CHARMM all-atom additive force field for RNA. J. Comput. Chem. 2011, 32, 1929−1943.

(45) Best, R. B.; Zhu, X.; Shum, J.; Lopes, P. E.; Mittal, J.; Feig, M.; Mackerell, A. D., Jr. Optimization of the additive CHARMM all-atom protein force field targeting improved sampling of the backbone $\phi$, $\psi$ and side-chain $\chi(1)$ and $\chi(2)$ dihedral angles. J. Chem. Theory Comput. 2012, 8, 3257−3273.

(46) Mahoney, M. W.; Jorgensen, W. L. A five-site model for liquid water and the reproduction of the density anomaly by rigid, nonpolarizable potential functions. J. Chem. Phys. 2000, 112, 8910−8922.

(47) Jorgensen, W. L.; Chandrasekhar, J.; Madura, J. D.; Impey, R. W.; Klein, M. L. Comparison of simple potential functions for simulating liquid water. J. Chem. Phys. 1983, 79, 926−935.

(48) Darden, T.; York, D.; Pedersen, L. Particle mesh Ewald: An N log(N) method for Ewald sums in large systems. J. Chem. Phys. 1993, 98, 10089−10092.

(49) Essmann, U.; Perera, L.; Berkowitz, M. L.; Darden, T.; Lee, H.; Pedersen, L. G. A smooth particle mesh Ewald method. J. Chem. Phys. 1995, 103, 8577−8593.

(50) Hess, B. P-LINCS: A parallel linear constraint solver for molecular simulation. J. Chem. Theory Comput. 2008, 4, 116−122.

(51) Miyamoto, S.; Kollman, P. A. Settle - an analytical version of the shake and rattle algorithm for rigid water models. J. Comput. Chem. 1992, 13, 952−962.

(52) Bussi, G.; Donadio, D.; Parrinello, M. Canonical sampling through velocity rescaling. J. Chem. Phys. 2007, 126, 014101.

(53) Parrinello, M.; Rahman, A. Polymorphic transitions in single crystals: A new molecular dynamics method. J. Appl. Phys. 1981, 52, 7182−7190.

(54) The PyMOL Molecular Graphics System, Version 1.8; Schrödinger, LLC: 2000.