Antibody binding increases the flexibility of the prion protein

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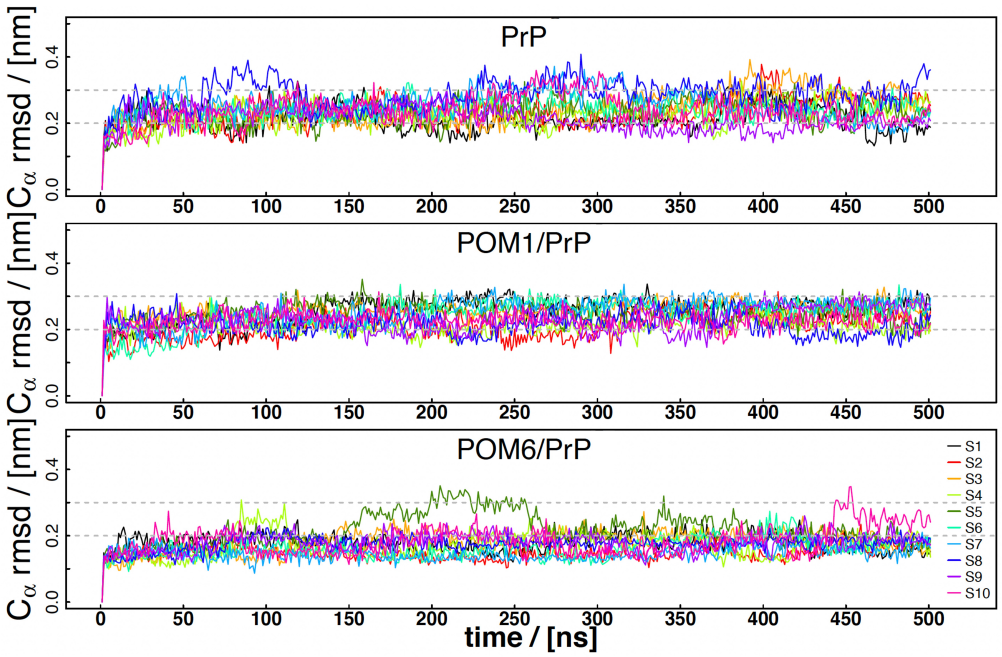

Figure 1: **Structural stability of the PrP globular domain.** The plot shows the temporal evolution of the root-mean-square deviation (rmsd) of the C_α atoms of PrP. The reference structures are the solution NMR structure of the globular domain of mouse PrP[1], the crystal structures of PrP in complex with POM1 [2] and POM6 [3]. The RMSD is calculated for the C_α atoms as $RMSD = \sqrt{\frac{1}{N}\sum_{i=1}^{N}(\mathbf{r_i} - \mathbf{r_i^{ref}})^2}$, where $\mathbf{r_i^{ref}}$ represents the reference position of atom i, and N the C_α atoms belonging to residues in PrP. The alignment of the system is done on the C_α atoms of PrP. The average RMSDs (0.25 ± 0.02 nm for free PrP or complexed with POM1 and 0.18 ± 0.02 nm for PrP in complex with POM6) were calculated over the last 200 ns of each simulation. The standard error of the mean was calculated as the standard deviation of the 10 average values over the last 200 ns of the 10 individual runs.

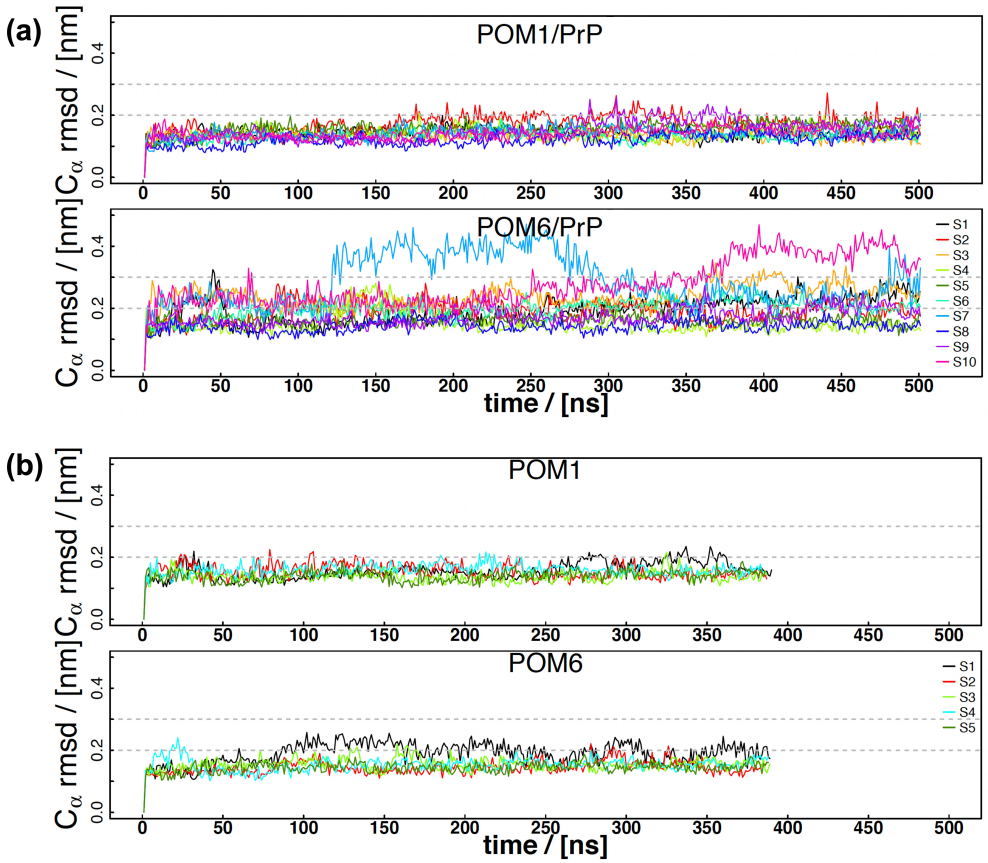

Figure 2: **Structural stability of the antibody variable domain.** The plot shows the temporal evolution of the root-mean-square deviation (rmsd) of the C_α atoms of the variable domains of POM1 and POM6 (a) in complex with PrP and (b) in absence of PrP. The reference structures are the crystal structures of PrP in complex with POM1 [2] and POM6 [3]. The alignment of the system is done on the C_α atoms of the variable domains of the antibodies. The variable domains of the antibodies are structurally stable both in complex with PrP (0.15 $\pm$ 0.02 nm for POM1 and 0.21 $\pm$ 0.06 for POM6) and in absence of PrP (0.15 $\pm$ 0.01 nm for POM1 and 0.16 $\pm$ 0.02 for POM6). The average RMSDs were calculated over the last 200 ns of each simulation. The standard error of the mean was calculated as the standard deviation of the 10 average values over the last 200 ns of the 10 individual runs (5 runs for the simulations with the antibodies alone).

			Comments
1	Lys204 (NZ)	Glu146 (CD)	- Connects α_3 with α_1
2	Glu146 (CD)	Asn143 (ND)	- N-terminus α_1
3	Arg208 (CZ)	Glu146 (CD)	- Connects α_1 to α_3
4	Arg208 (CZ)	Hid140 (O)	- Previously associated with toxicity - Connects α_1 to α_3
5	Arg208 (CZ)	Glu211 (CD)	- Sidechains in α_3 (stabilize α_3)
6	Arg156 (CZ)	Asp202 (CG)	- Connects $\alpha 3$ with β_1- α_1 loop
7	Arg156 (CZ)	Asn153 (OD)	- Connect β_1-α_2 to α_1
8	Arg156 (CZ)	Glu196 (CD)	- Connects C-terminus α_1 to the α_2- α_3 loop
9	Arg156 (CZ)	Tyr149 (OH)	- Connects α_1 to α_1- β_2 loop
10	Glu146 (CD)	Met205 (CE)	- Connects α_1 to α_3
11	Arg164 (CZ)	Asp178 (CG)	- Connects α_2 to β_2
12	Arg148 (CZ)	Glu152 (CD)	- Sidechains in α_1 (stabilize α_1)
13	Hid177 (NE2)	Glu211 (CD)	- Connects α_2 to α_3
14	Hid177 (ND1)	Glu211 (CD)	- Connects α_2 to α_3
15	Arg208 (CZ)	Phe141 (CG)	- Connects α_3 to the β_1- α_1 loop
16	Arg208 (CZ)	Phe141 (O)	- Connects α_3 to the β_1- α_1 loop
17	Tyr169 (OH)	Arg164 (CZ)	- Stabilizes the β_2- α_2 loop
18	Tyr169 (OH)	Arg164 (NE)	- Stabilizes the β_2- α_2 loop
19	Tyr169 (OH)	Asp179 (CG)	- Connects α_2 to β_2- α_2 loop
20	Tyr128 (OH)	Arg164 (CZ)	- Stabilizes the β-sheet
21	Tyr128 (OH)	Asp178 (CG)	- Proximity of β-sheet to α_2
22	Arg164 (CZ)	Asp167 (CG)	- Stabilizes the β_2- α_2 loop

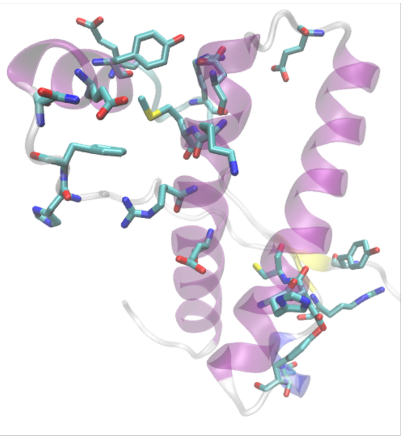

Figure 3: **Interatomic distances used for the SAPPHIRE based clustering.** The distances in the table on the left were used for building the progress index and were selected from literature and by visual inspection. They contribute to the intrinsic plasticity of PrP. The used residues are highlighted in the right snapshot.

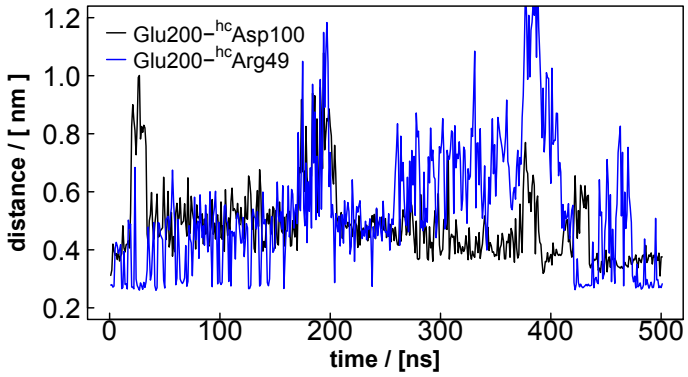

Figure 4: **Distance timelines for simulation S6.** Shown are the timelines of the distances between the side-chains of Glu200 – ^{hc}Asp100 and Glu200 – ^{hc}Arg49. The timelines show that the repulsion between Glu200 of PrP and ^{hc}Asp100 of the POM6 heavy chain disrupts the Glu200–^{hc}Arg49 salt bridge.

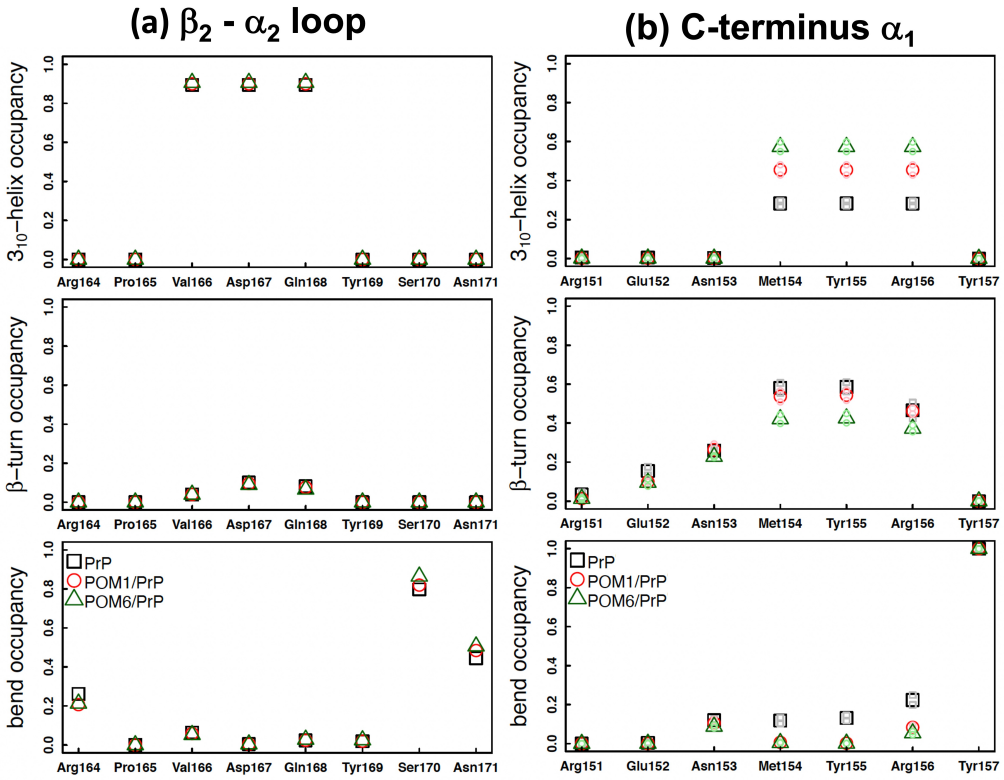

Figure 5: **The antibodies have minor influence on the secondary structure**. The propensity for 3_{10}-helix (top), β-turn (middle), and bend (bottom) was calculated by using CAMPARI version 4 (http://campari.sourceforge.net). To asses the statistical significance, the average values of the propensity were calculated for two separate blocks of the 10 (6 for the POM6/PrP complex) trajectories for each system (light colors).

References

[1] A. D. Gossert, S. Bonjour, D. A. Lysek, F. Fiorito, K. Wüthrich, Prion protein nmr structures of elk and of mouse/elk hybrids, Proc. Nat. Acad. Sci. 102 (3) (2005) 646–650.

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[3] P. K. Baral, M. Swayampakula, A. Aguzzi, M. N. G. James, Structural characterization of pom6 fab and mouse prion protein complex identifies key regions for prions conformational conversion, FEBS J. 285 (9) (2018) 1701–1714.