Biophysical Journal, Volume *118*

Supplemental Information

The Anti-Aggregation Holdase Hsp33 Promotes the Formation of Folded Protein Structures

Fatemeh Moayed, Sergey Bezrukavnikov, Mohsin M. Naqvi, Bastian Groitl, Claudia M. Cremers, Guenter Kramer, Kingshuk Ghosh, Ursula Jakob, and Sander J. Tans

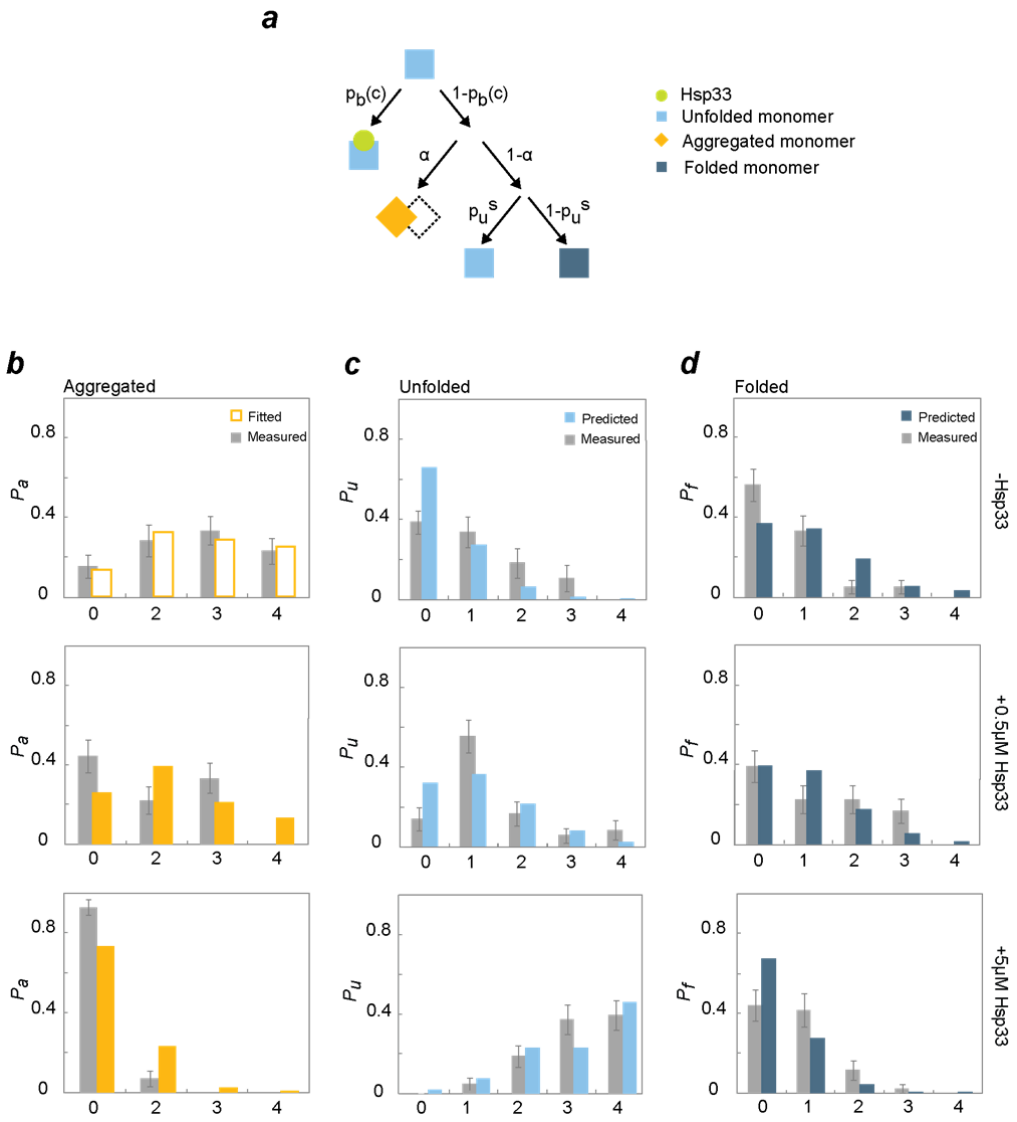

Fig S1: Prediction of 4MBP data set (a) Substrate transitions for model 2 (see Methods). Each monomer in 4MBP can adopt three different states; aggregated, folded, and unfolded (bound or unbound by Hsp33). In the presence of Hsp33, monomers can bind to the chaperone molecules with the probability of p_b(c). Monomers bound by Hsp33 are assumed to remain unfolded and do not aggregate. Unbound monomers can aggregate with the probability of α. The non-aggregated unbound monomers are assumed to have the same probability as sMBP to fold (p_f^s) or remain unfolded (p_u^s). (b) Using the experimentally measured value of P_a^i in the absence of chaperone (i = 0, 2, 3, 4), we determined α (see open bars for fit). The α value together with the experimental data of sMBP refolding with and without Hsp33 was used to predict the probability of forming i aggregated (b), unfolded (c) and folded (d) monomers in the presence of Hsp33. Error bars show 95% confidence intervals estimated by bootstrapping.

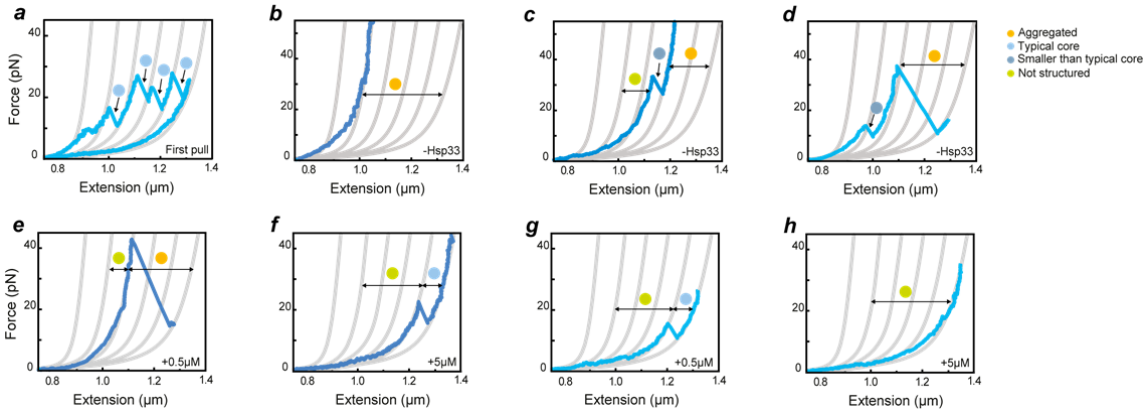

Fig S2: 4MBP pulling-stretching experiments. (a) For a native 4MBP construct, the first pull initially shows a gradual transition indicating the detachment of the external alpha-helices for all 4 MBP repeats (unmarked, in between first two grey WLC curves), followed by 4 consecutive unfolding steps, each corresponds to one typical core (80 nm < L_u < 104 nm and F_u < 35 pN) (light blue circles). (b-h) examples of stretching experiments after the first pull and relaxing to 0 pN. The unfolded peptide segments corresponding to the 4 core structures can now adopt the following fates, as detected in subsequent stretching (shown blue curves): a non-native aggregated structure (L_u > 104 nm and/or F_u > 35 pN) (yellow circles), a folded structure smaller than one core (L_u < 80 nm, 5<F_u <35 pN) (dark blue circles), a typical native-like folded core (light blue circles), and an unstructured polypeptide (F_u<5 pN hollow circles). Gray lines are theoretical worm like chain (WLC) models of the DNA-protein construct in different states.

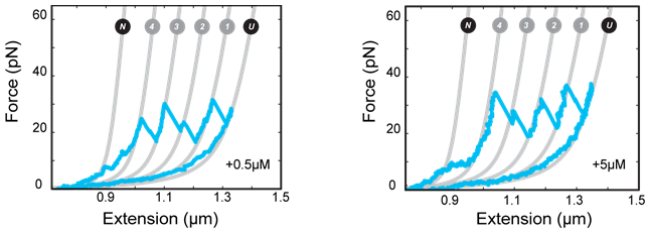

Fig S3: First stretching and relaxation curve of 4MBP construct in the presence of 0.5μM (left) and 5μM (right) Hsp33. Gray lines are theoretical worm like chain (WLC) model of the DNA-protein construct in different states. After C-terminal unfolding (N→4), four core unfolding events (4→3→2→1→U) are observed.

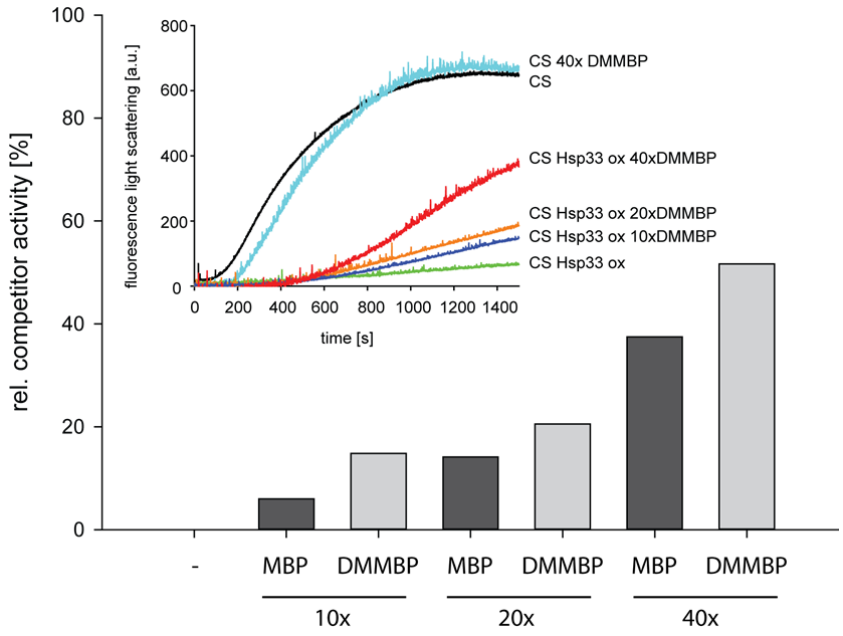

Fig S4: MBP binding to Hsp33. The competitor activity of MBP and DMMBP was monitored by determining their ability to compete with 0.15 µM of thermally unfolded Citrate Synthase for binding to 0.6 µM $Hsp33_{ox}$ at 43°C. Complexes between active $Hsp33_{ox}$ and competitors were allowed to form for 4 min at 43°C before CS was added. Light-scattering measurements of CS at 43 °C were conducted as a read-out for competitor activity. Light-scattering of CS measured in the presence of fully active chaperones was set to 0% competitor activity, and the light-scattering signal of CS in the absence of functional chaperone was set to 100%. MBP and DMMBP did not significantly influence the aggregation propensity of CS in the absence of chaperones and did not aggregate when incubated alone at 43°C. Shown are the relative competitor activities for a ten-fold, twenty-fold, and forty-fold excess of MBP (dark grey) or DMMBP (light grey) to CS. The data show that both MBP and DMMBP can compete with CS for Hsp33 binding. DMMBP is slightly more efficient, consistent with its reduced stability and suggestive of a higher affinity for Hsp33 binding. Insert shows a subset of light scattering data that were used for generating the bar graph. A loss in the prevention of aggregation of CS by Hsp33 upon the addition of DMMBP indicates binding of DMMBP to Hsp33. DMMBP had no effect on the aggregation behavior of CS.

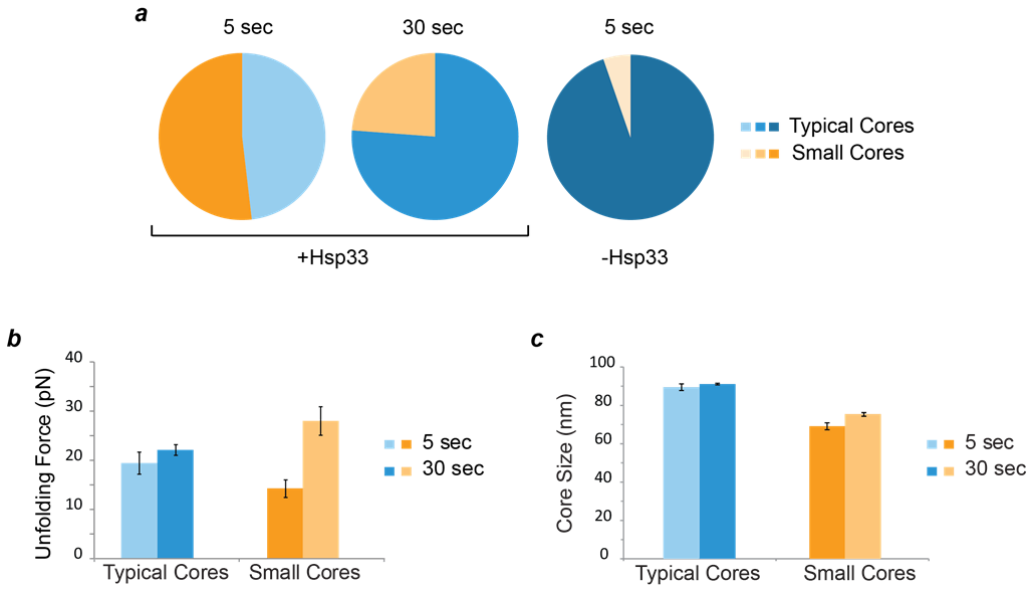

Fig S5: Dependence on waiting time at 0 pN. A single MBP is exposed to cycles of stretching, waiting at 0 pN for 5 s (N=27) or 30 s (N=42), and stretching, in the presence of 0.5 μM chaperone. Stretching results in full unfolding, the waiting time at 0 pN provides a time window to form tertiary structure, and subsequent stretching informs of the latter. Stretching indicates unfolding events with changes in length that correspond to small (50-80 nm) or typical core structures (80-104 nm). (a) Pie charts indicating the fraction of cycles that showed the formation of small (orange) and typical (blue) core structures. No chaperone data were collected with 5 sec waiting time (N=41) (b) Corresponding observed unfolding forces. Error bars are ±1 se. (c) Corresponding observed core sizes. Error bars are ±1 se. Overall, these data show that waiting longer at 0 pN increased the fraction of typical cores and decreased the fraction of small cores ($p < 0.05$). The mean size and unfolding force of the small-core category also increased ($p < 0.05$) with increased waiting time, whereas the size and unfolding force of typical cores are not significantly increased. These findings are consistent with a maturation of the core structures over time.