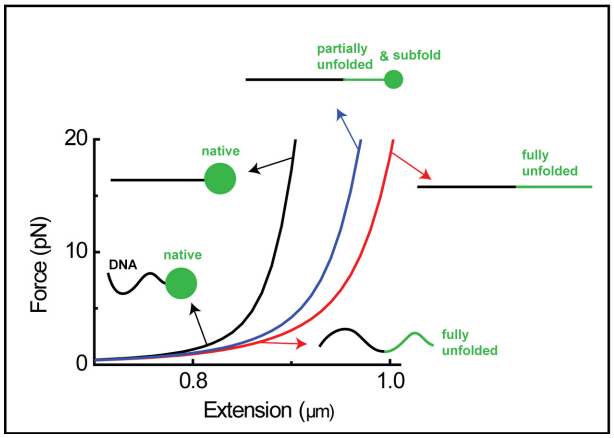
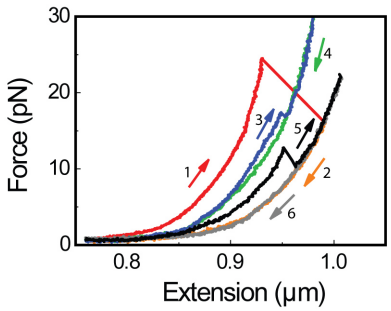
doi:10.1038/nature12293

Figure S1



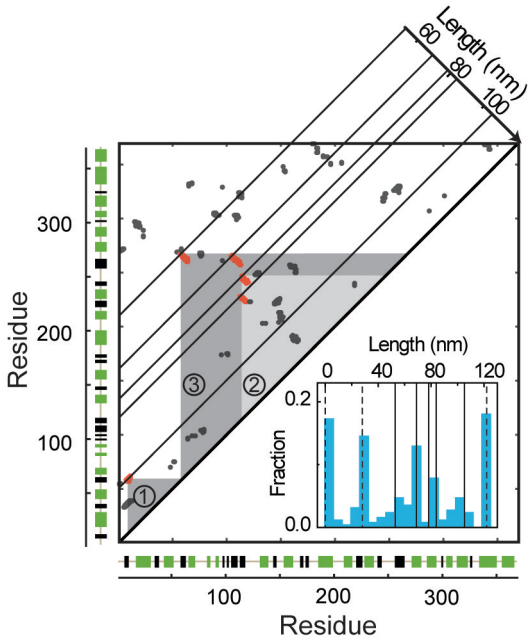
Schematic diagram detailing force-extension behaviour of different folded states. Black line: stable native state. As force and tension increases along the line, only the DNA extends. Blue line: one segment of the protein is unfolded, the other segment is a stable partial fold. Red line: protein is fully unfolded.

Figure S2



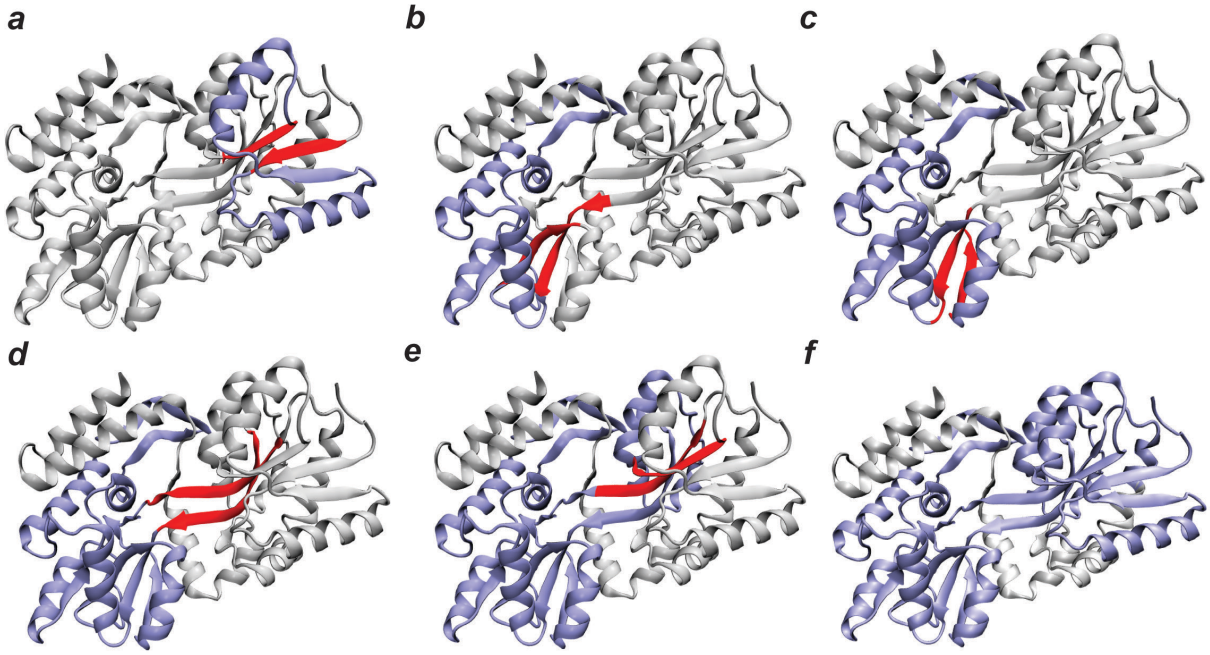
Trigger Factor mediated folding transitions. The small unfolding transition at about 17 pN (experiment 3) indicates the occurrence of small variations in the structures of partial folds, which give rise to broadening of peaks in the protein length distributions (as in Fig. 1D). The transition from experiment 2, where the fully unfolded protein is relaxed, to experiment 3, where a partial fold is stretched, indicates nucleation of a partial within an unfolded polypeptide at zero force. The transition from experiment 4 to 5 shows that a partial fold can unfold partially at zero force, and hence indicates reversibility of partial fold transitions.

Figure S3



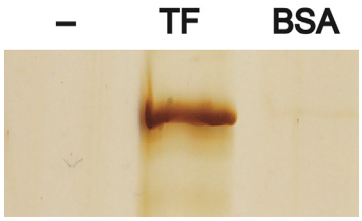
Determination of MBP truncates that can fold only partially. Points are residue contact map of MBP (residue pairs within 7 Å of the crystal structure, see Methods). Residue contacts within 20 positions in sequence (along the diagonal) are omitted for clarity. Red points: Five most stable contact regions, as determined by adding energies of neighbouring contacts (Methods). For each contact region, one can consider the polypeptide segment in between the residues that form the contact region. The black lines indicate the lengths of these polypeptide segments. Each of these five polypeptide segments forms a sub-structure within the larger MBP crystal structure (Figure S4). Three of these five polypeptide segments (truncates) were purified (indicated by the three shaded regions (Methods). Residue positions are for mature MBP lacking the signal sequence. Beta-sheet (black) and alpha-helices (green) are shown along axis. Inset: measured length distribution of folds (Fig. 1D). Protein lengths of the five polypeptide segments as determined by the contact map analysis are indicated by the drawn black lines. Dashed lines: protein lengths for native, I_0, and fully unfolded states.

Figure S4



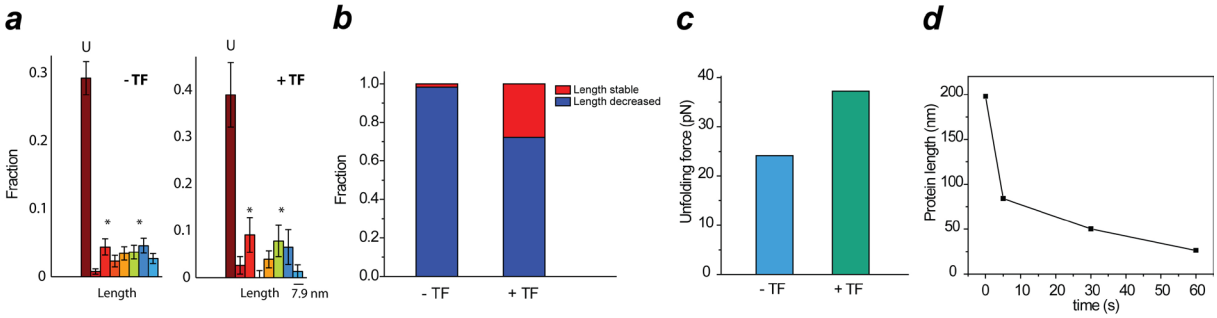
MBP sub-structures. In red are indicated the five MBP contact regions as determined by the contact map analysis (Fig. S3 and Methods). Purple indicates the polypeptide segment in between the residues forming the contact regions. Each panel shows one such sub-structure: ***a***) residues 7-62 and calculated length, L ~ 105 nm ***b***) residues 112-228, L ~ 84 nm ***c***) residues 114-247, L ~ 78 nm ***d***) residues 104-266, L ~ 69 nm ***e***) residues 57-267, L ~ 53 nm. ***f***) residues 1-279, L ~ 92 nm. Residue numbers are for mature MBP lacking the signal sequence.

Figure S5



Results of Amylose resin binding assay as displayed in Fig. 4C. Image shows a SDS-PAGE gel stained with silver, taken after urea denaturation, refolding in the absence or presence of Trigger Factor or BSA (2 μM), binding to amylose resin, and elution with excess of maltose (Methods). Lane 1 (without Trigger Factor) and lane 3 (with BSA) indicate the low amount of protein specifically bound to resin while lane 2 (where refolding efficiency was increased due to the presence of Trigger Factor) show an increased quantity of bound protein and hence recovery of native function.

Figure S6



Trigger Factor effect on the intermediate folds of Luciferase ***a***) Length analysis of the smallest Luciferase folds in absence and presence of TF. The symbol U indicates fully unfolded Luciferase. The histogram indicates the fraction of times a protein length is observed in stretching and relaxation experiments; similar as in Fig. 1. Without TF, in addition to U, two additional characteristic lengths can be discerned (stars). With TF, these characteristic lengths are again observed, and are more pronounced. ***b***) Partial folds at zero force. Lengths during relaxation are compared with lengths during subsequent stretching (after waiting at zero force for 5 seconds). Without TF, the protein length almost exclusively decreased (blue), showing a short lifetime of the partial folds, and tendency to increase the fold. With TF, the length was now often unchanged (red), indicating a longer lifetime. ***c***) Mechanical stability of the partial folds against applied force. With TF the unfolding force is about a third higher. Overall, TF thus binds and stabilizes partial folds in Luciferase as well. ***d***) The average protein length, as observed by stretching, after relaxing fully unfolded luciferase and waiting at zero force. The data shows that when the polypeptide has longer to fold, more of it becomes folded, consistent with the notion that TF allows folding to continue.