Use of Molecular Tweezers to Investigate the Effect of Applied Force on σ^{54} Core-Binding Domain

Katie Amberg-Johnson[1] Alex Siegel[2] Dave Wemmer[3]
[1]College of Natural Resources, [2]Biophysics Graduate Group, [3]Department of Chemistry UC Berkeley

Introduction

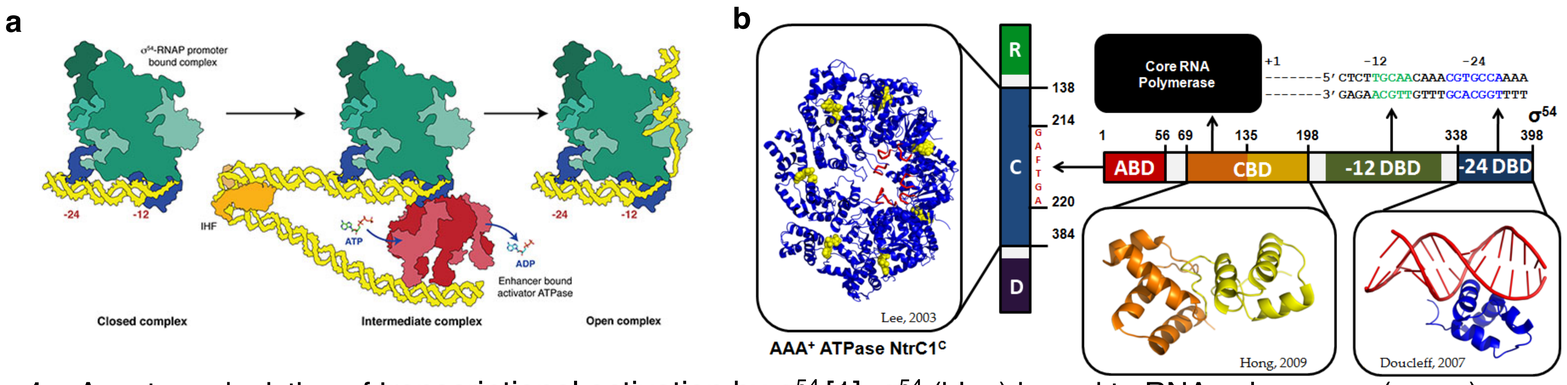
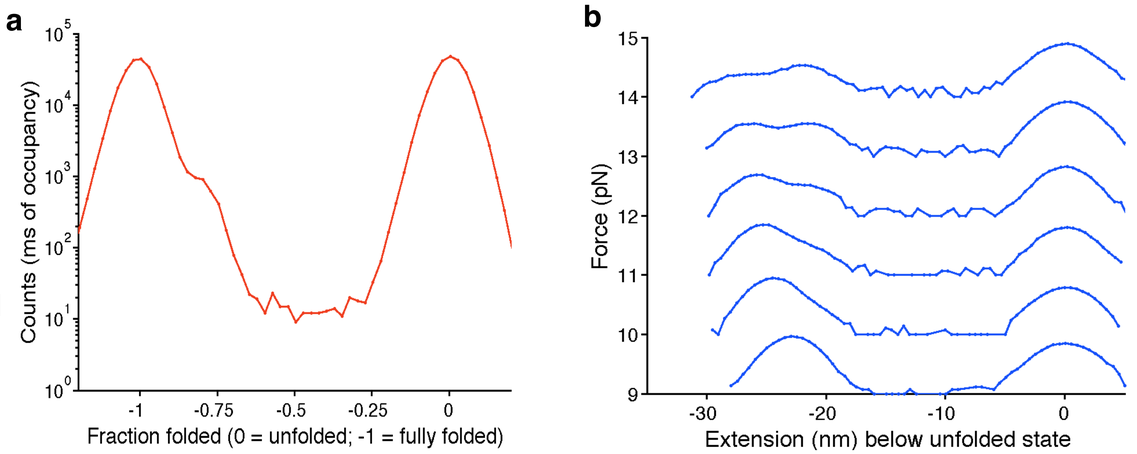


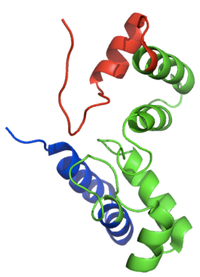
Fig. 1 a A cartoon depiction of transcriptional activation by σ^{54} [1]. σ^{54} (blue) bound to RNA polymerase (green) cannot initiate transcription until multiple rounds of ATP hydrolysis by the activator ATPase. **b** A schematic of the domains of *Aquifex aeolicus* σ^{54}, select solved structures, and molecules that interact with the individual domains.

Results

Fig 3 a Amount of time CBD spends in various folded and unfolded states during force-extension cycles obtained by stretching and relaxing CBD molecules with the molecular tweezers. **b** Force-extension cycles observed by stretching and relaxing CBD at different forces.



Discussion



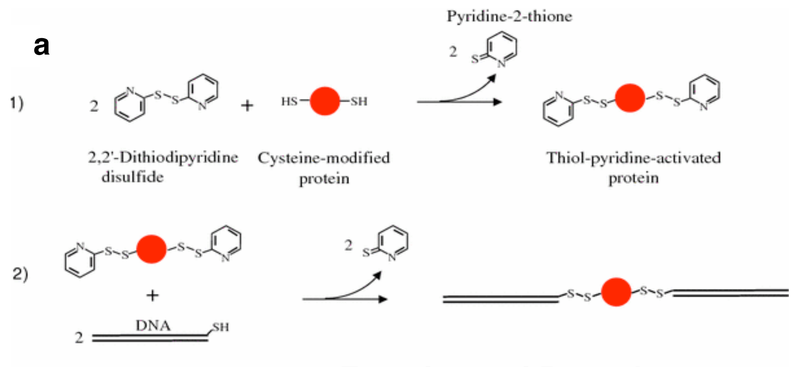
- 20% of the molecule unfolds first, followed by the remaining 80%
- The C-terminal helix is about 20% of the protein and is the likely cause of the first unfolding transition (supported by NMR data as well)

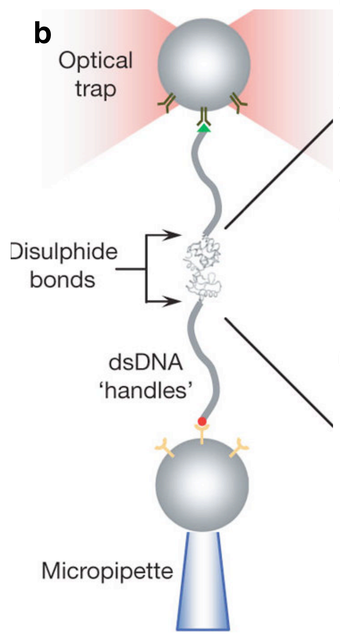
Fig. 4 Cartoon structure of CBD [2].

Future Work

- Truncate the C-terminal helix and perform another molecular tweezers experiment to confirm whether the C-terminal helix unfolds separately
- Bind full-length σ^{54} to RNA Polymerase and to promoter DNA. Perform another molecular tweezers experiment to try to activate σ^{54} in vitro.

Materials and Methods





Experimental Procedure:

1. Generate 500bp DNA-handles
2. Express and purify CBD
3. Denature CBD and react with DTDP
4. React DNA-handles with CBD
5. Bind the DNA-CBD construct to polystyrene beads coated in streptavidin or anti-digoxigenin antibodies
6. Use molecular tweezers to trap and pull on individual molecules

Fig 2 a Schematic of the reactions used to (1) modify the protein with DTDP and (2) attach the modified protein to the DNA-handles [3]. **b** Cartoon depiction of the molecular tweezers set-up [4].

Acknowledgments

Special thanks to my mentor Professor David Wemmer as well as Alex Siegel, Professor Wenshu Wang, Dr. Jesse Dill, Dr. Barat Jagannathan, Dr. Christian Wilson, and Matthew Draelos for their assistance and contributions.

References

1. Wigneshweraraj, S. R., P. C. Burrows, et al. (2005). The second paradigm for activation of transcription. Progress in Nucleic Acid Research and Molecular Biology, Academic Press. Volume 79: 339-369.
2. Hong, E., Doucleff, M. & Wemmer, D.E. Structure of the RNA polymerase core-binding domain of sigma(54) reveals a likely conformational fracture point. Journal of molecular biology 390, 70-82 (2009).[6] Barrios, H., Valderrama, B., Morett, E. (1999) Compilation and analysis of sigma(54)-dependent promoter sequences. Nuc. Ac. res. 27, 4305-13.
3. Cecconi, C., Shank, E., Marquesee, S., Bustamante, C. (2008). Protein-DNA chimeras for single molecule mechanical folding studies with the optical tweezers. *Eur Biophys J.* **37,** 729-738.
4. Shank, E., Cecconi, C. Dill, J., Marqusee, S., Bustamante, C. (2010). The folding cooperativity of a protein is controlled by its chain topology. *Nature*. **465**, 637-640.