Structural Characterization of σ^{54} Core-Binding Domain Truncation

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Introduction

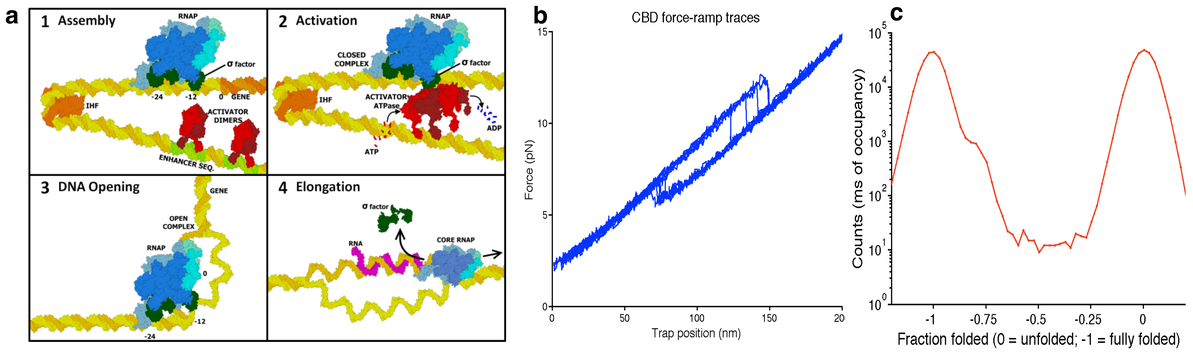
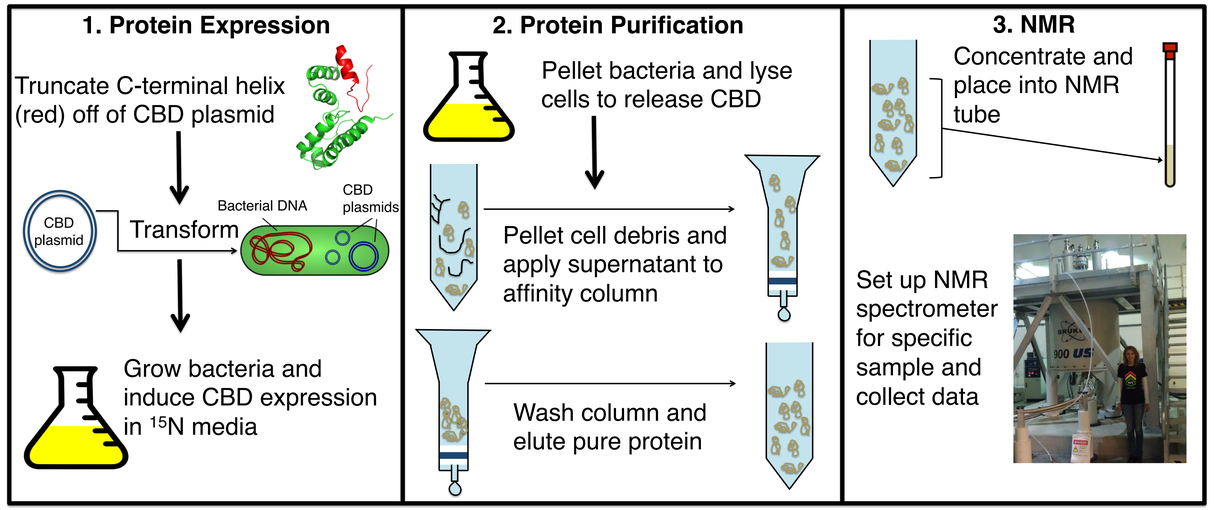


Fig. 1 a A cartoon depiction of transcriptional activation by σ^{54}. σ^{54} (green) bound to RNA polymerase (blue) cannot initiate transcription until ATP hydrolysis by the activator ATPase (red). σ^{54} might require pulling through the action of the ATPase domains of the activator to initiate transcription. **b** Force-extension cycles generated by stretching and unfolding Core-Binding Domain (CBD) of σ^{54} with molecular tweezers. **c** Amount of time CBD spends in various folded and unfolded states during molecular tweezers experiment.

Methods



Results

a

Sequence Comparison of Truncated σ^{54} Core-Binding Domain (red) with Original (blue)

Helix 1 Helix 2 Helix 3

MRGSHHHHHHGSLVPRGSENLYFQGYTPSELEELQQNIKLELEGKEQELALELLNYLNEKGFLSKSVEEISDVLRCS
MRGSHHHHHHGSLVPRGSENLYFQGCTPSELEELQQNIKLELEGKEQELALELLNYLNEKGFLSKSVEEISDVLRAS

Helix 4 Helix 5 Helix 6 Helix 7

VEELEKVRQKVLRLEPLGVCSKDVWEFLELQIEEIYPEEEEILKKALRDLKRGKKLKPEIKGKLSRLRLFPLSSSAEK
VEELEKVRQKVLRLEPLGVASKDVWEFLELQIEEIYPEEEEILKKALRDLKRGKKLC

	Contacting Residues	Helix 1 + linker	Helix 2 + linker	Helix 3 + linker	Helix 4 + linker	Helix 5 + linker
Average Distance (ppm) away from original peak	0.12	0.21	0.20	0.52	0.23	0.52
Truncated CBD peaks/ total residues	3/10	5/19	9/22	5/11	9/23	4/16

b

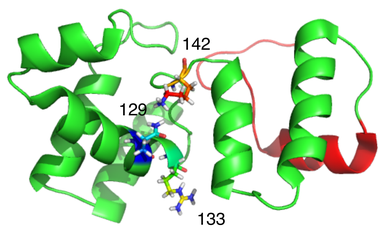


Table 1 Average distance (ppm) between peaks in the truncated spectrum and peaks in the original spectrum. Only well-shifted peaks were averaged and sorted according to the secondary structure they make up.

c

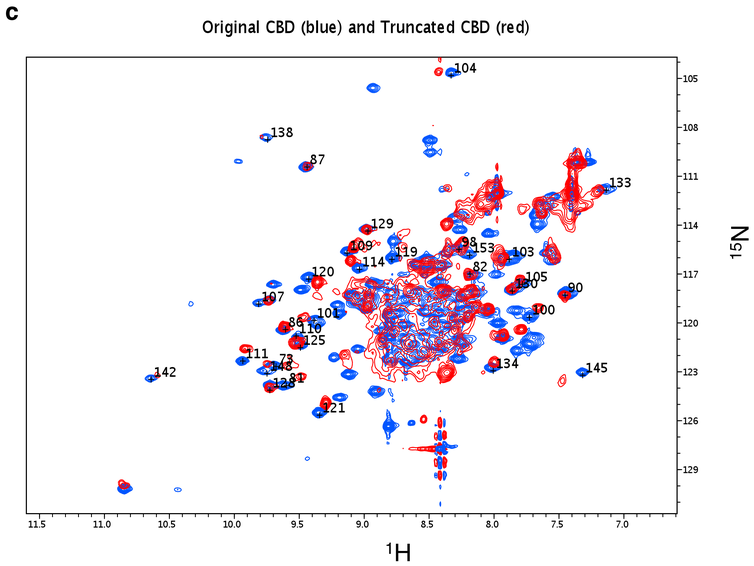


Fig. 2 a Amino acid sequence comparison of the original CBD construct (blue) and the truncated CBD construct (red). QuikChange mutagenesis was used to insert a cysteine and a stop codon after amino acid 176. **b** A cartoon depiction of CBD with the side chains that make contacts between the two helix bundles exposed. The truncated helix is colored red. **c** HSQC spectrum overlay of the original CBD spectrum (blue) [1] and the truncated CBD construct (red) with selected peaks.

Conclusions

a

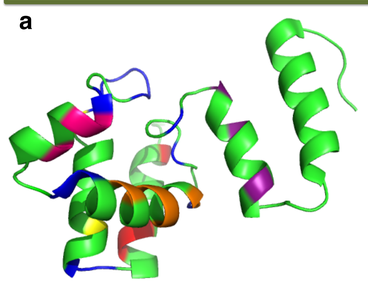


Fig 3 a A cartoon depiction of σ^{54} CBD with the last helix truncated. Residues in the HSQC spectrum of the truncated CBD that can be identified and matched to the original spectrum are highlighted in different colors. Because these residues are throughout the domain, it is likely that the truncated CBD construct is folded similarly to the original construct. Molecular tweezers experiments may now be performed to study the unfolding behavior of the domain under applied force.

Acknowledgements

Special thanks to my mentor, Professor David Wemmer, as well as Alex Siegel, Professor Wenshu Wang, Dr. Kwang Seo Kim and Dr. Eunmi Hong. Thanks to the UC Berkeley Amgen Scholars Summer Research Program and to the Genentech Scholars Program.

References

1. 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.