NMR Mapping of Disordered Segments from a Viral Scaffolding Protein Enclosed in a 23 MDa Procapsid

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Abstract

Scaffolding proteins are required for the capsid shell assembly of many tailed dsDNA bacteriophages, some archaeal viruses, herpesviruses, and adenoviruses. Despite their importance, only one high-resolution structure is available for scaffolding proteins within procapsids. Here we use the inherent size limit of NMR to identify mobile segments of the 303-residue phage P22 scaffolding protein free in solution and when incorporated into a ~23 MDa procapsid complex. Free scaffolding protein gives NMR signals from its acidic N-terminus (residues 1-40) and basic C-terminus (residues 264-303), while NMR signals from the middle segment (residues 41-263) are missing because of intermediate conformational exchange on the NMR chemical shift timescale. When scaffolding protein is incorporated into P22 procapsids, NMR signals from the C-terminal helix-turn-helix domain disappear due to binding to the procapsid interior. Signals from the N-terminal domain persist, indicating that this segment retains flexibility when bound to procapsids. The unstructured character of the N-terminus, coupled with its high content of negative charges, is likely important for dissociation and release of scaffolding protein during the dsDNA genome packaging step accompanying phage maturation.

Introduction

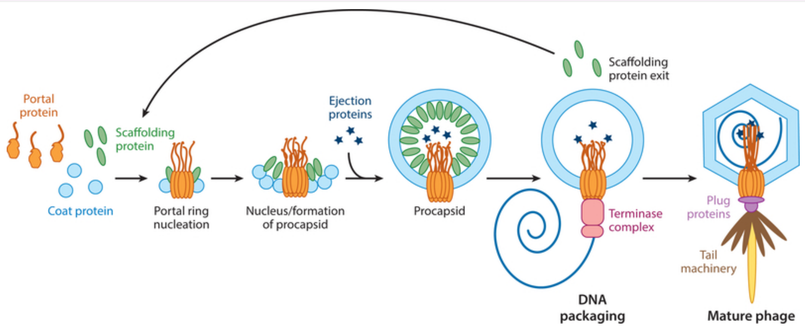


Figure 1: Assembly Pathway of P22[1]

In the bacteriophage P22, scaffolding protein (SP) nucleates the assembly of a dodecameric portal ring complex which functions as a nucleation point for capsid assembly. 60-300 copies of scaffolding protein co-assemble with 415 copies of coat protein and ejection proteins to form an immature capsid, called a procapsid.[1] Scaffolding protein exits through holes in the procapsid and is recycled for further rounds of assembly.[2]

Scaffolding protein is an intrinsically disordered protein despite the large alpha helical character of its CD spectra. It exists as a loosely organized molten globule, without a coordinated hydrophobic core.

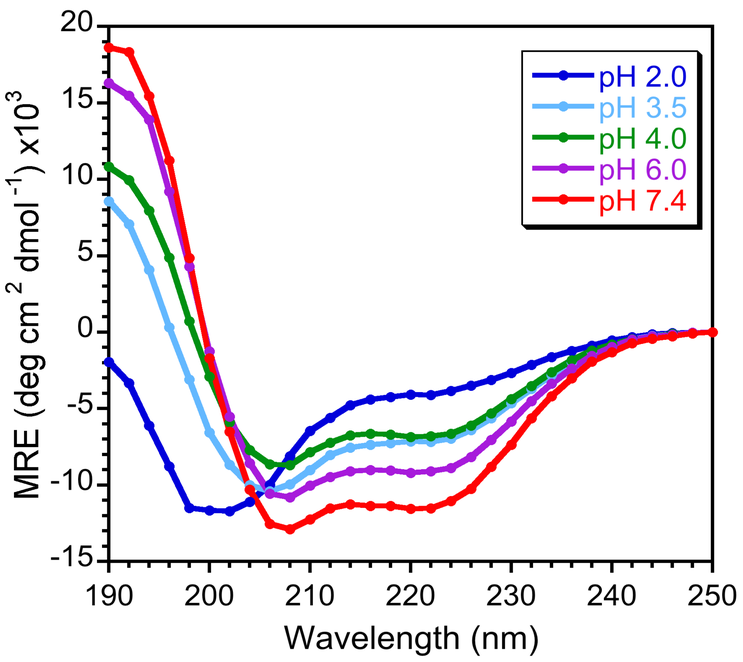


Figure 2: Far UV CD Spec of P22 SP as a function of pH

Results and Discussion

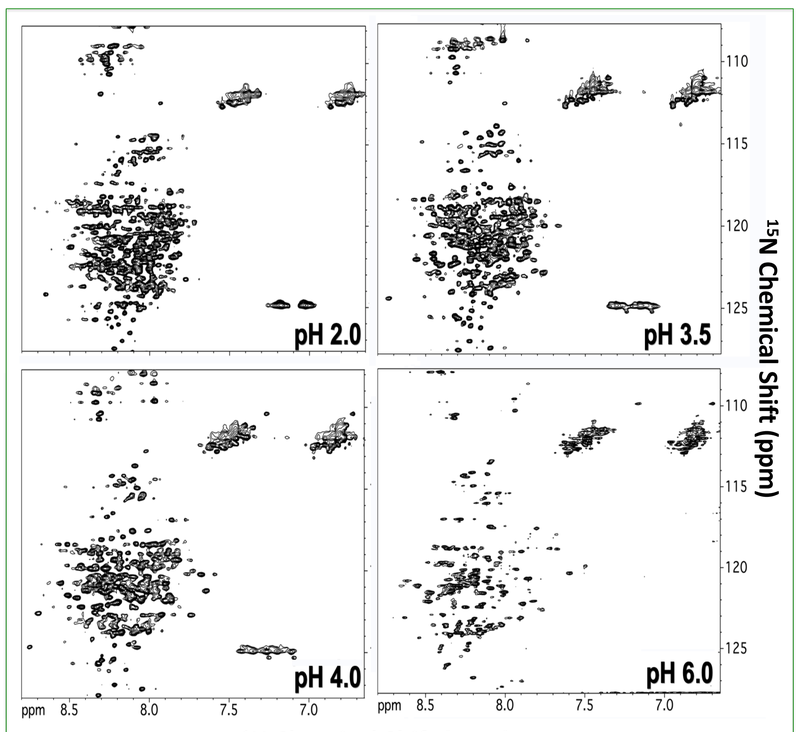


Figure 3: Representative ^{1}H-^{15}N HSQC spectra of P22 SP from a pH titration. At lower pH, more correlations are seen because of unfolding of residues 41—263 in the central part of SP.

- The pH 6 HSQC shows ~100 correlations of the 303 amino acid scaffolding protein
- To make sure this wasn't due to the large protein size (33.6kDa) or the oligomerization state (monomer-dimer-tetramer) we preformed a dilution experiment below the K_D of oligomerization.

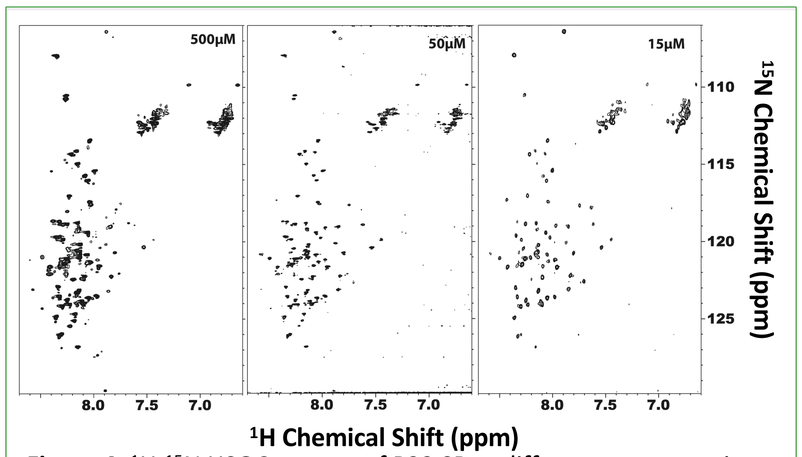
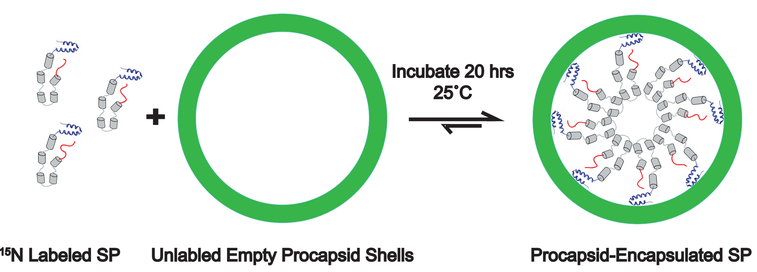


Figure 4: ^{1}H-^{15}N HSQC spectra of P22 SP at different concentrations.

- In the 500uM sample, the protein is predominately dimer/tetramer (>95%), while in 50/15uM samples the protein is mostly monomeric, ~70% and ~90% respectively[3]
- Signal loss is likely from exchange on the NMR timescale

Question: Will NMR signals from the P22 scaffolding protein remain when encapsulated in P22 procapsid like particles?



- ^{15}N Labeled SP was encapsulated into unlabeled P22 Procapsid shells at a ratio of 60:1 to ensure tight binding of the SP[4]
- We first need NMR assignments of the HSQC to get residue specific information about the protein

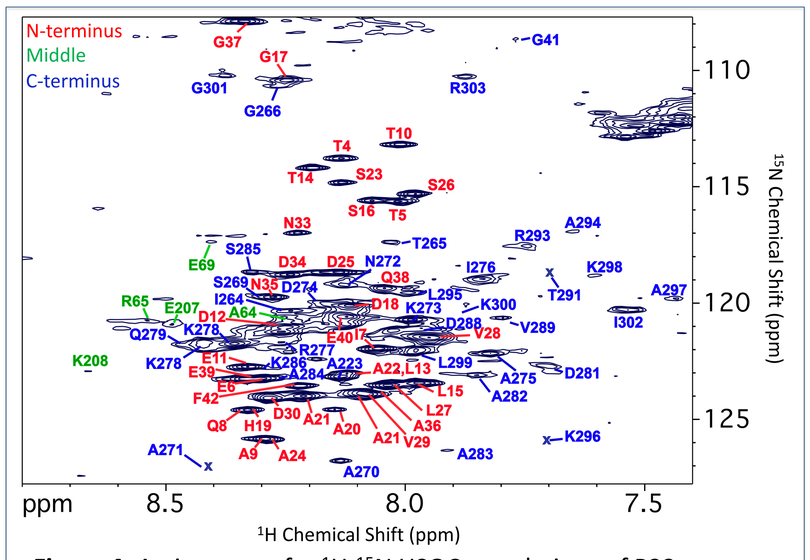


Figure 4: Assignments for ^{1}H-^{15}N HSQC correlations of P22 scaffolding protein at pH6

- The ~100 peaks in our HSQC are from the N and C-terminus of the protein.
- The peaks from the N-terminus of the protein persist even while the protein is encapsulated inside the procapsid

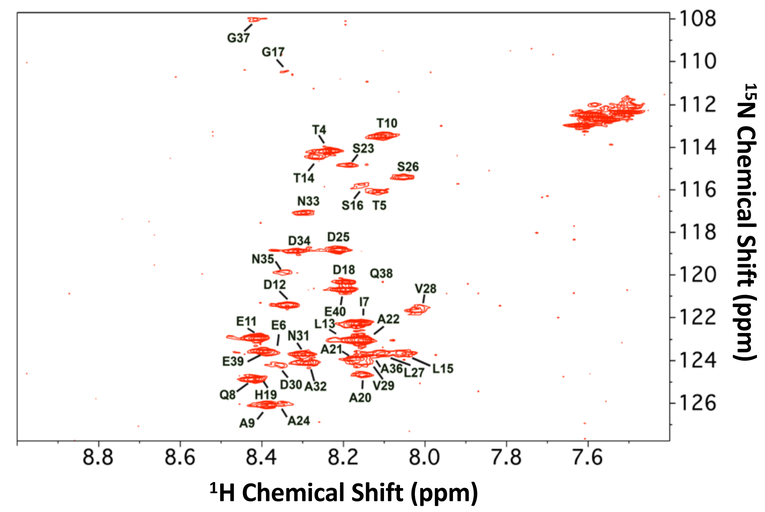


Figure 5: Assignments of ^{1}H-^{15}N HSQC spectra of P22 SP when encapsulated into procapsids. All remaining signals are from N-terminal amino acids 1-40

Conclusions

- SP shows an incomplete set of peaks in the ^{1}H-^{15}N HSQC spectra due to μs-ms motion on the NMR timescale.
- The peaks we can see correspond to the first ~40 N-terminal residues and the last ~40 C-terminal residues.
- When scaffolding protein is encapsulated into procapsids signals from the C-terminus disappear because this area contains the helix turn helix coat binding domain.
- Signals from the N-terminus remain in the encapsulated spectra suggesting this region is flexible both in solution and while bound to the procapsid.

References

1. Dedeo CL, Cingolani G, Teschke CM. Portal Protein: The Orchestrator of Capsid Assembly for the dsDNA Tailed Bacteriophages and Herpesviruses. Annu Rev Virol. 2019 Sep 29;6(1):141-160.
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3. Parker, M. H., W. F. Stafford, III, and P. E. Prevelige, Jr. 1997. Bacteriophage P22 scaffolding protein forms oligomers in solution. J. Mol. Biol. 268:655–665.
4. Parker, M. H., C. G. Brouillette, and P. E. Prevelige, Jr. 2001. Kinetic and calorimetric evidence for two distinct scaffolding protein binding populations within the bacteriophage P22 procapsid. Biochemistry. 40:8962–8970

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