Engineering Hcp1 to Bind DNA

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Introduction:

Proteins are appealing building blocks for nanoscale systems because they are inexpensive, biocompatible, and have self-assembling properties. Specifically, protein nanotubes, such as viral capsids, microtubules, and tobacco mosaic viruses have been engineered for nanotechnological applications including nanowire synthesis, drug delivery, and gene delivery [1-3].

Current deoxyribonucleic acid (DNA) processing methods used for DNA sequencing and microarray analysis rely on restriction enzymes that produce oligonucleotides of heterogeneous lengths. Homogenous length DNA would be an advantage to chip based DNA analysis technologies as a way to reduce false positives (incorrect matches of complementary DNA). Nanotubes of discrete lengths could serve as a template for length specific DNA processing.

Our lab has previously demonstrated that Heme Carrier Protein 1 (Hcp1), a homohexameric ring protein secreted by *Pseudomonas aeruginosa*, forms stable nanotubes [4]. Currently, our lab is focused on optimizing Hcp1 to form tubes of discrete length. The potential to control nanotube length makes Hcp1 an ideal candidate for DNA scaffolding applications.

In this study we report progress toward DNA encapsulation with Hcp1. The work presented here, shows that the introduction of positively charged lysines to the interior of Hcp1 can mediate non-specific interactions between Hcp1 and the negatively charged backbone of DNA.

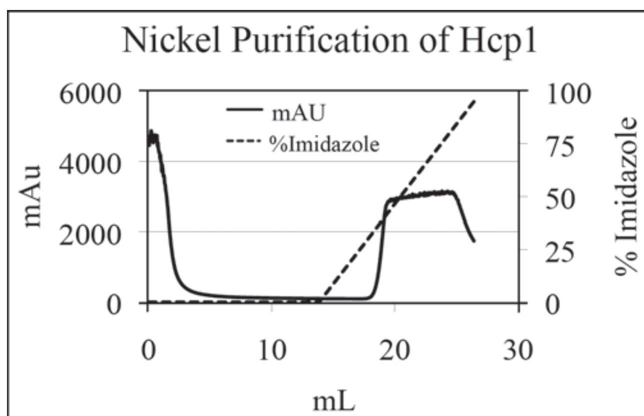

Figure 1: Representative elution profile of Hcp1 lysine mutants.

Materials and Methods:

Lysine Mutant Production and Purification. We produced two lysine modified Hcp1 mutants—Asp55Lys and Ser31Lys Asp55Lys—via site-directed mutagenesis. Hcp1 mutants were expressed in *E. coli* and purified over a His-Trap column with an increasing concentration gradient of imidazole buffer and were further purified on a size exclusion column.

Hcp1-DNA Electrophoretic Mobility Shift Assay. 1 μM of 40 base pair (bp) DNA and various protein concentrations of Hcp1 wild type Hcp1 (WT), Hcp1 (Asp55Lys) and Hcp1 (Ser31Lys Asp55Lys) mutants were mixed and incubated for one hour on ice. Next, the reactions were run on a 2% TBE agarose gel at 50 V for two hours, stained with ethidium bromide, rinsed and photographed using UV imaging. The second electrophoretic mobility shift assay (EMSA) incubated 100 nM plasmid DNA with various concentrations of Hcp1 WT, Hcp1 Asp55Lys and Hcp1 Ser31Lys Asp55Lys for 1 hour on ice. The mixtures were run on a 1.5% gel for 4 hours at 50 V, stained with ethidium bromide, rinsed and visualized by UV transillumination using a gel documentation system.

TEM Analysis of Hcp1 Asp55Lys with Cysteine Modifications. Hcp1 lysine mutants with engineered cysteine residues were produced and proteins purified as previously described. Protein samples were stained with uranyl formate and imaged by transmission electron microscopy (TEM).

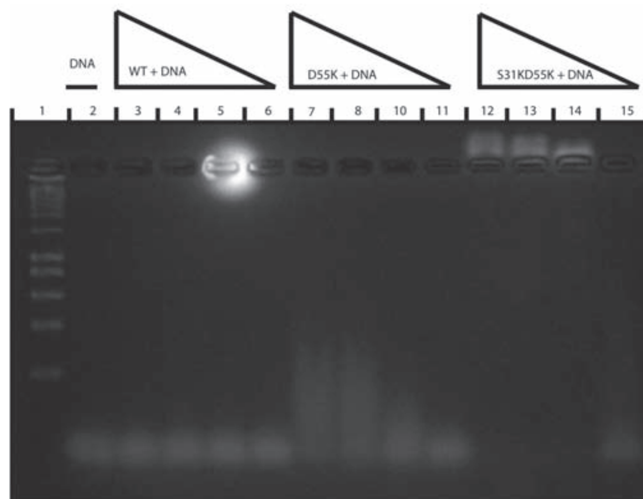

Figure 2: EMSA of DNA in the presence of Hcp1.

Results:

Hcp1-DNA EMSA. We performed electrophoretic mobility shift assays (EMSA) to determine if Hcp1 mutants would bind DNA with greater affinity than the WT. Figure 2 shows varied concentrations of Asp55Lys and Ser31Lys Asp55Lys in the presence of a 1 μM 40 base pair (bp) DNA strand. (Lane 1, DNA Ladder; lane 2, DNA control, lanes 3-6, lanes 7-11 and lanes 12-15 contained WT, Asp55Lys and Ser31Lys Asp55Lys respectively) (10 μM, 7 μM, 4 μM and 1 μM). At high concentrations of Asp55Lys and low concentrations of Ser31Lys Asp55Lys, a smear of DNA is observed, which is not seen with the WT. At high protein concentrations, Ser31Lys Asp55Lys shifts in the slightly positive direction. Additionally, low concentrations of Ser31Lys Asp55Lys weaken the DNA band compared to the DNA control.

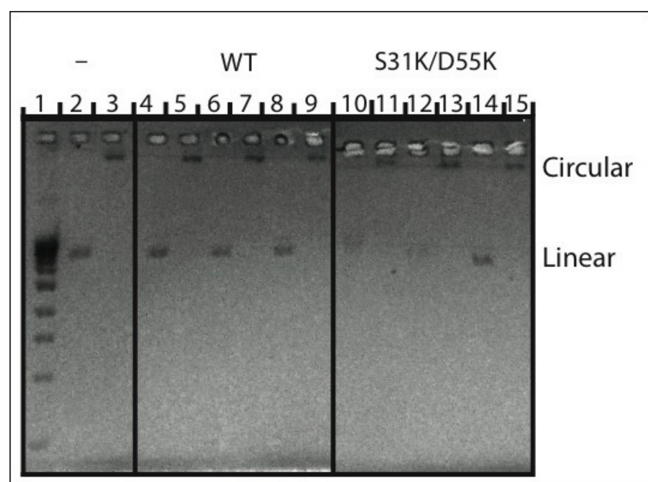

Figure 3: EMSA of circular vs. linear plasmid DNA.

Linear vs. Plasmid DNA EMSA. We utilized an EMSA with circular and linear plasmids to determine if DNA was binding to the interior of the protein. Figure 3 shows Ser31Lys Asp55Lys altered DNA migration patterns of the linear plasmid but not the circular plasmid. Protein concentration decreased left to right; 8 μM, 4 μM, 1 μM in lanes 1 and 2, 3 and 4, and lanes 5 and 6 respectively. The linear DNA shifted at high concentrations of Ser31Lys Asp55Lys.

TEM Hcp1 Asp55Lys with Cysteine Modifications. Transmission electron microscopy (TEM) visually confirmed Hcp1 Asp55Lys tube formation as previously described by Ballister et. al. [4] (Figure 4).

Discussion:

This work demonstrates the preliminary steps towards producing DNA encapsulating tubes. We show the introduction of positively charged lysine to the interior of Hcp1 promotes a non-specific DNA-protein interaction. At high concentrations of Ser31Lys Asp55Lys, an upward shift indicates a positively charged and possibly large DNA-protein complex. However, these results may suggest an aggregated complex of DNA and misfolded protein. Similarly, DNA smears can stem from a variety of possibilities including DNA being released during electrophoresis, numerous complexes forming and the presence of various charged species. Therefore, this experiment is inconclusive.

Preliminary evidence suggests DNA is binding to the interior of Hcp1. First, the protein eluted at the volume expected for the hexamer (data not shown). The linear plasmid migration pattern was altered in the presence of Ser31Lys Asp55Lys whereas the circular plasmid did not appear to show shift (Figure 3). This suggests DNA is binding to the interior of the protein because the linear DNA has exposed ends to allow for binding to the interior of Hcp1. This evidence depends on stable ring formation, which has not been conclusively determined yet. Although clear evidence of stable ring formation is not available for the Ser31Lys Asp55Lys mutant, we did observe intact ring conformation and tube formation for Asp55Lys (Figure 4).

Future steps include imaging the interaction between Hcp1 mutants and DNA encapsulation for tubes. The introduction of a di-copper complex at the top and bottom of an Hcp1 tube could cut DNA based on tube length. Di-metal complexes have shown to non-specifically cut the backbone of DNA5. This system would serve as an excellent scaffold to synthesize homogenous length DNA strands.

Acknowledgements:

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References:

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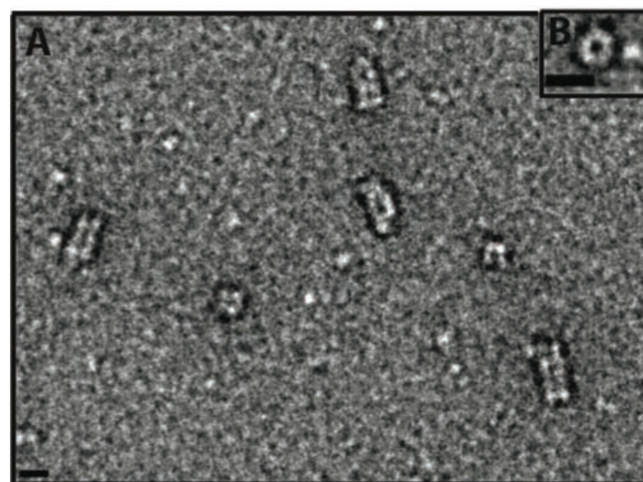

Figure 4: TEM of Hcp1 D55K with cystiene modifications.