

Characterization of dsDNA Binding Protein SlyA for Nanostructure Assembly

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Abstract and Introduction:

This project explored the suitability of the *Salmonella typhimurium* deoxyribonucleic acid (DNA)-binding protein SlyA (17kDa) for use in the self-assembly of nanoscale inorganics on a DNA scaffold. Self-assembly techniques are attractive because they manufacture structures from the bottom-up. DNA is a potential scaffold for nanostructures because it is stable at room temperature and self-assembles via base pairing.

Previous experimentation with engineered derivatives of the DNA-binding protein TraI (192kDa) demonstrated its ability to spontaneously assemble gold nanoparticles (AuNPs) on DNA [1]. Utility of this TraI derivative is limited by its large size, non-specific affinity for various metals, and multiple DNA-binding domains.

SlyA is a smaller protein, so it may exhibit less nonspecific metal binding than TraI, and its single DNA binding domain allows for increased control during assembly. Additionally, its protein family (MarR) is well characterized and its crystal structure is known (Figure 1) [2].

SlyA was purified to high concentrations via a 10x Histidine-tag. Subsequent efforts focused on cleaving the His-tag from

purified His-SlyA via Factor Xa protease, since the His-tag would likely exhibit background binding to a multitude of materials.

Localized surface plasmon resonance (LSPR) spectrometry characterized His-SlyA binding to 15 nm AuNPs. His-SlyA exhibited high binding overall and caused aggregates at 0.25 μM and higher. Cloning gold binding peptide (GBP1-7x or AuBP2) sequences into the SlyA gene efforts are ongoing [3,4].

Methods:

His-SlyA Purification. *Escherichia coli* (*E. coli*) cultures were induced to over express His-SlyA and then lysed. The resulting supernatant was mixed with NiNTA resin, which bound to the His-tag, and then loaded into a column. After 100 mM imidazole washes eliminated untagged proteins, 500 mM imidazole washes eluted His-SlyA from the resin.

Factor Xa Cleavage. 0.2 U/ μl Factor Xa protease was incubated with His-SlyA for 4.5 hours at room temperature to cleave the His-tag. Factor Xa resin removed the protease; NiNTA resin removed cleaved-His-tags and His-SlyA.

Gold-Binding Characterization. His-SlyA was diluted in solutions of 0.01 μM to 1 μM and mixed with 15 nm AuNPs. A LSPR spectrometer was used to obtain absorbance vs. wavelength. The peak absorbance wavelength of plain AuNPs was also obtained and subtracted from the peak absorbance wavelength of each dilution to obtain peak wavelength shift vs. His-SlyA concentration.

Insertion of GBP1-7x and AuBP2. The GBP1-7x sequence (MHGKTQATSGTIQS), AuBP2 sequence (WALRRSIRRQSY), and the pet16B plasmid containing the SlyA gene were digested with NdeI [5,6]. Each digested peptide sequence was ligated with varying concentrations of digested pet16B. The region of the plasmid containing the NdeI site was observed through gel electrophoresis to determine if the insert annealed with the plasmid.

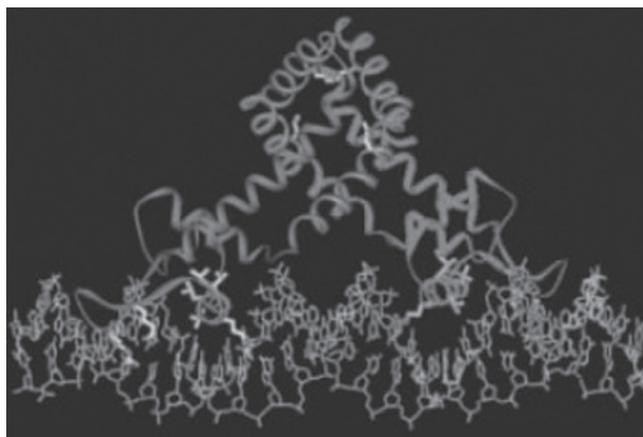


Figure 1: SlyA in complex with DNA [2].

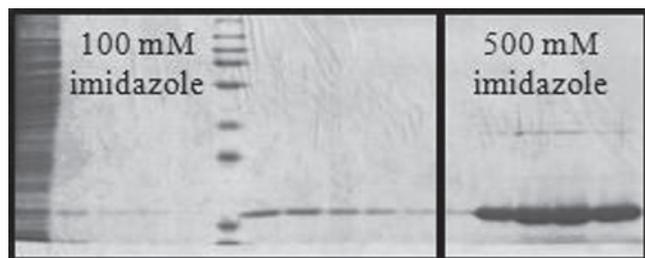


Figure 2: Purification gel of His-SlyA.

Results and Discussion:

His-SlyA purification was effective, repeatable, and high-yield. The imidazole washes successfully eliminated untagged proteins and separated the His-tag from the resin, resulting in 0.66 mg/ μ l His-SlyA (Figure 2). Thus, purification via His-tag is a good and reliable method that should work for SlyA variants.

Both the His-SlyA cleavage and subsequent removal of Factor Xa protease were effective. 0.2 U/ μ l Factor Xa protease incubation for 4.5 hours cleaved most of the His-SlyA, and Factor Xa resin completely removed the protease (Figure 3). However, SlyA bound to the NiNTA resin during post-cleavage purification, even when imidazole was added to decrease that non-specific binding (Figure 3).

His-SlyA binds gold with high affinity, which is not surprising as histidine is a known gold-binder [7]. A visible color change due to gold aggregation was observed with 0.25 μ M to 1 μ M protein, indicating that the 0.01 μ M to 0.25 μ M range should be further investigated when examining SlyA.

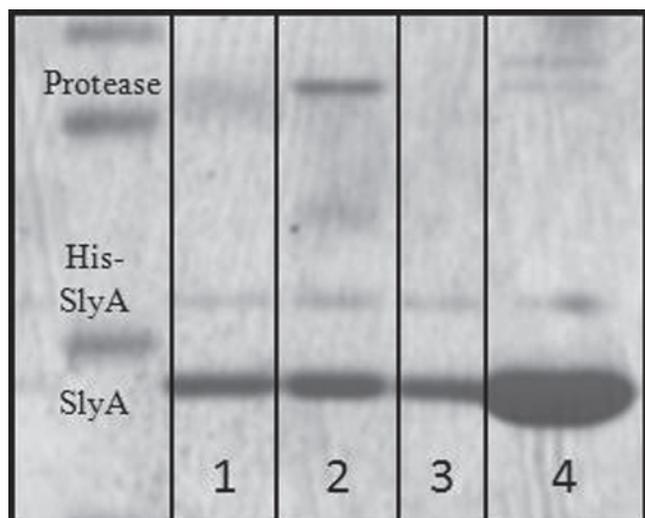


Figure 3: Factor Xa cleavage gel of His-SlyA.

Left to right: post-cleavage, protease resin, post-protease resin.

Conclusions:

Although SlyA has potential for assembling AuNPs on a DNA scaffold, it should have low background binding to gold to validate its use. Significant progress was made in the initial steps to discover SlyA's gold binding. It was determined that a 10x His-tag is a good way to purify SlyA and that the His-tag strongly affects background binding to gold, so it is necessary to cleave the tag and isolate SlyA post-cleavage.

Future work includes troubleshooting the post-cleavage purification process to isolate SlyA, and using LSPR spectrometry to determine SlyA's innate affinity for gold. Additionally, SlyA gold-binding variants SlyA::GBP1-7x and SlyA::AuBP2 will be engineered, their gold affinities examined to determine which is most effective, and their DNA affinities measured to ensure addition of a gold binding motif does not eliminate SlyA's original DNA-binding function.

Acknowledgements:

I would like to thank my mentor, Ruth Hall Sedlak, and Principal Investigator, Beth Traxler, for their guidance, suggestions, and support. I would also like to thank the rest of Traxler Lab. Funding was provided by the National Nanotechnology Infrastructure Network Research Experience for Undergraduates (NNIN REU) Program and the National Science Foundation.

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