Nano Meets Micro: Synthesis of Proteins for the Organization of Nanostructures

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Abstract:

Manufacturing reliable nanoelectronics can be challenging, simply because of the scale on which these devices are built. In the future, it may be effective to find a way to direct their self-assembly. Certain proteins can be genetically engineered to assist in this process. Permissive sites, which allow for the insertion of short amino acid sequences without a loss of function, have been identified in two different proteins from the bacterium *Escherichia coli*, LacI and TraI. A short polypeptide sequence with a known affinity for silver particles was inserted into different permissive sites in each protein, and the resulting modified proteins were characterized. LacI derivatives successfully repressed expression of β-galactosidase, an enzyme whose transcription is controlled by LacI. TraI derivatives participated successfully in conjugation. An assay was attempted to measure the proteins' affinity for silver, but the results were inconclusive; a different assay will be needed in the future. The expected ability of these modified proteins to bind strongly to both deoxyribonucleic acid (DNA) and inorganic particles suggests they could play a key role in the organization of nanostructures by arranging silver particles in defined patterns along a DNA template.

Introduction:

The broad goal of this project is to create a library of proteins that can bind to both DNA and inorganic particles. These proteins can be used to determine where in a cell a certain protein is most concentrated, or to organize nanoparticles along a DNA scaffold, perhaps leading to self-assembling structures with applications in nano- electronics and -photonics. Researchers have had success modifying proteins to bind to several inorganics; this work builds on their work by modifying two proteins to bind to silver.

Many proteins made by the bacterium *E. coli* naturally have the ability to bind to DNA. Researchers have already identified permissive sites in several of these, including LacI and TraI. LacI is a transcriptional repressor: when there is no lactose present in a cell, LacI binds to dsDNA at the operator sequence and prevents the transcription of the genes that follow. These genes encode the enzyme β-galactosidase which breaks down lactose. If lactose is present in the cell, LacI releases, and the genes are transcribed.

TraI is involved in F-plasmid conjugation, the process by which bacteria transfer DNA to one another. TraI binds to a double-stranded DNA plasmid in the donor bacterium and nicks it, and then helps it unwind so that one strand can be transferred to the recipient. Both bacteria then synthesize the complementary strand of DNA. While LacI binds only at its 30 base dsDNA operator sequence, TraI has the ability to bind non-specifically on both ds and ssDNA. Different applications in the future may require sequence-specific or sequence-independent binding, so both proteins were used.

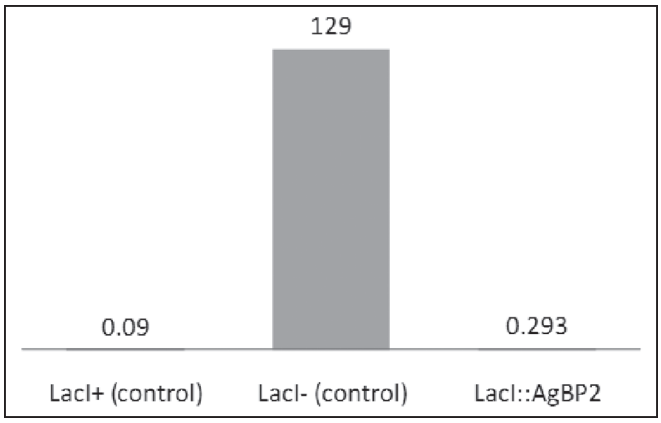

Figure 1: Level of β-galactosidase activity for cells carrying three different versions of LacI

Using a phage display system, researchers have identified a cysteine-constrained 12 amino acid peptide (EQLGVRKELRGV), known as AgBP2, with a high affinity for silver. This sequence was inserted into the permissive sites of LacI and TraI after the 317th and 369th amino acids, respectively, to produce LacI::AgBP2 and TraI::AgBP2. These derivatives were identified using polymerase chain reaction (PCR) screening, DNA sequencing, and expression samples, and then characterized for their affinities to DNA and silver.

Characterizing DNA-Binding:

Since LacI represses synthesis of β-galactosidase, a high level of DNA binding by LacI::AgBP2 would be associated with a low level of • β-galactosidase activity. Figure 1 shows the level of β-galactosidase activity for cells carrying three different versions of LacI; the units shown in the chart are proportional to the level of β-galactosidase activity per cell per unit time. The LacI::AgBP2 showed wild type-level β-galactosidase repression.

Because the ability of TraI to bind to DNA is necessary for conjugation, a mating assay was performed to characterize TraI::AgBP2 DNA-binding activity. TraI::AgBP2 had wild type-level mating activity.

Characterizing Silver-Binding:

To determine the affinity of LacI::AgBP2 and TraI::AgBP2 for silver, clarified cell extracts were mixed with silver powder. After suspension and centrifugation, the supernatant was saved, and the powder was washed twice and then resuspended. Ideally, proteins exhibiting no binding would be found in the initial supernatant, those showing nonspecific binding would come off in one of the washes, and those with a strong affinity for silver would remain with the powder until the end. However, as shown in Figure 2, the affinity of modified proteins for silver was not demonstrated to be any stronger than that of the unmodified proteins. This particular method of measuring binding ability had sometimes been ineffective in the past, and the silver nanoparticles agglomerated into larger particles during the pre-test preparation phase, indicating that this test for Ag binding is not accurate. Future characterization will assess binding on Ag surfaces with purified proteins.

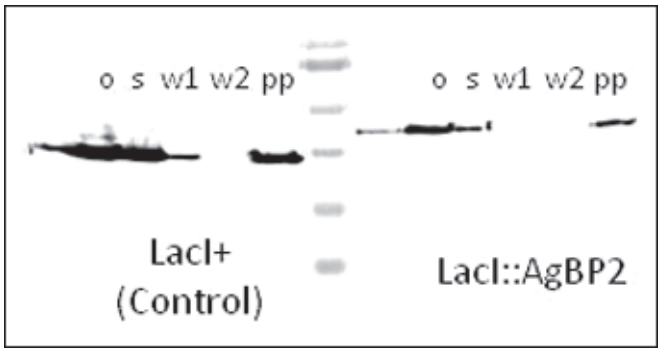

Figure 2: Results of silver binding assay. o = original amount of protein; s = supernatant; w1 = wash 1; w2 = wash 2; pp = powder pellet.

Conclusions and Future Work:

Derivatives of LacI and TraI containing a silver-binding peptide have been synthesized successfully, and their sequences have been confirmed. LacI::AgBP2 shows wild type-level β-galactosidase repression, verifying its *in vivo* binding to DNA at the *lac* operator. TraI::AgBP2 exhibits wild type-level mating activity, indicating wild type-level DNA binding. The abilities of both derivatives to bind to silver have not been demonstrated. To further characterize these proteins, a more accurate and quantitative assay is needed. Surface plasmon resonance or quartz crystal microbalance will be used to characterize silver binding. Silver and DNA binding will be visualized via atomic force or electron microscopy. Such images could confirm ability of the proteins to bind to silver and DNA simultaneously, and perhaps demonstrate the expected sequence-specific binding of LacI derivatives and sequence-independent binding of TraI derivatives.

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