Nano Meets Micro

Use of Engineered Proteins for the Organization of Nanostructures

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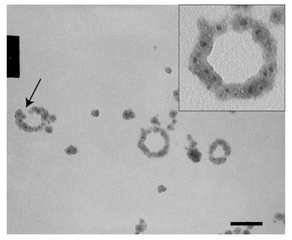
Background

The interdisciplinary Genetically Engineered Materials Science and Engineering Center at the UW, GEMSEC, seeks to create a library of proteins that can bind to both DNA and inorganic particles. These proteins can be used for protein localization within a cell, or to organize nanoparticles in a certain order along a DNA scaffold for applications in electronics and photonics. Researchers have already had success modifying proteins to bind to several inorganics. This project continues this work by creating two DNA binding proteins that also bind to silver.



*Cu_2O-binding derivatives of TraI on DNA; Dai, H., et al. J. Am. Chem. Soc. **2005**, 127, 15637-15643*

Using a phage display system, researchers have identified an 12 amino acid peptide (EQLGVRKELRGV), known as AgBP2, with a high affinity for silver. This sequence was inserted into two *E. coli* proteins: the *lac* operon repressor LacI and the conjugation protein TraI. The resulting derivatives were then characterized for their DNA- and silver-binding abilities.

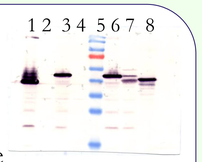
Identifying Candidates

Genes coding for the modified proteins suspected of carrying the AgBP2 insertion were subjected to PCR screening using a forward primer corresponding to the AgBP2 sequence and a reverse primer corresponding to the native protein after the insert. The presence of any PCR product implied the presence of the AgBP2 insert. 50 LacI and 50 TraI transformants were screened, and four LacI and two TraI candidates were identified.

Lanes 1, 2, and 7 show PCR-positive candidates

Checking for Expression

PCR-positive candidates were grown up, and the size of the modified proteins was compared to that of the wild type. Two of the LacI candidates and one of the TraI candidates were shown to be larger than wild type, consistent with the expectation that they could contain the AgBP2 insertion.

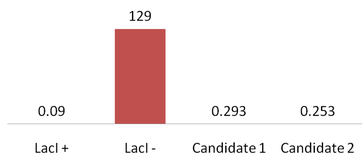


Western Blot: LacI candidates (lanes 3 and 6) are larger than wild type (lane 1) and LacI with a 31-a.a. insertion (lane 8)

DNA Binding

PCR-positive candidates were then tested for their ability to bind DNA. Wild type LacI represses production of β-galactosidase, an enzyme used to break down lactose, so the levels of lactose break-down products were measured. Units shown in the chart below are proportional to the amount of lactose broken down per cell per unit time. Both remaining LacI candidates showed wild-type level β-galactosidase repression.

LacI candidates demonstrated β-galactosidase repression similar to that of the positive control



TraI helps with DNA processing during conjugation, the transfer of DNA between bacteria, so the DNA-binding properties of TraI candidates were measured by measuring the conjugation efficiency of a host strain when compared with a control. The remaining TraI candidate showed a conjugation efficiency comparable to that of the control, suggesting that the TraI candidate is fully functional for DNA binding.

Sequencing

Remaining candidates were sequenced to further verify their composition. Sequencing confirmed that all candidates contained exactly one copy of the expected AgBP2 insert.

Conclusions

Both LacI and TraI have been successfully modified to include a known silver binding peptide insertion. These modified proteins are approximately the expected size, and preserve wild-type DNA-binding abilities.

Future Directions

In the short term, several experiments should be completed to further characterize these candidates. All candidates should be subjected to a silver binding assay to determine their affinity for powdered silver. Only after that experiment has been completed will it be possible to conclude that the candidates do possess all the desired DNA- and silver-binding traits.

In the longer term, applications of these candidates can be explored. TraI derivatives have already demonstrated the ability to organize metal particles around a loop of DNA, an experiment that could be repeated with this candidate. The ability of the LacI candidates to position metal particles at the *lac* operator sequence could be investigated. And finally, more modified proteins with the ability to bind to different metals and DNA sequences could be created, leading to a library of proteins for use in future applications.

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