Engineering Protein Scaffolds for Interactions with Nanoparticles

Laralynne Przybyla, Biochemistry and Molecular Biology, Purdue University
NNIN REU Site: NanoTech User Facility, University of Washington

NNIN REU Principal Investigator: Dr. Beth Traxler, Microbiology, University of Washington
NNIN REU Mentor: Eliora Gachelet, Microbiology, University of Washington
Contact: lprzybyl@purdue.edu, btraxler@u.washington.edu

Abstract:

The focus of this project is to exploit the self-assembly properties of proteins to arrange inorganic compounds in predictable patterns. Part of this effort has involved the genetic manipulation of the *E. coli* TraI protein, a DNA helicase involved in F plasmid conjugation. TraI has a high affinity for DNA, and we are using TraI-derivatives with specific metal binding capacities to make DNA-protein-nanoparticle assemblies. Polypeptide sequences that mediate binding to gold were incorporated into previously identified TraI-permissive sites, without altering the *in vivo* function of the TraI protein.

Two of these TraI proteins, TraI-GBP7Q and TraI-GBP5Q, have been purified and subsequently imaged using an atomic force microscope (AFM). The proteins have been observed binding to both single-stranded DNA and gold nanoparticles, thus producing a linear ordered array of nanoAu. These conjugates of DNA-TraI-nanoAu, which demonstrate a binding, and ordering of gold nanoparticles along a DNA strand may be used in nanowire applications, or as a proof-of-concept for making devices.

Introduction:

TraI, also known as *E. Coli* DNA helicase I, has the function of nicking, then unwinding, DNA during F plasmid conjugation—the transfer of DNA between bacteria, as seen in Figure 1 [4].

Derivatives of the TraI protein have been created which are capable of binding metal ions. We have used TraI derivatives with gold-binding polypeptide sequences inserted into the previously identified permissive sites Q369 and L1753 (Traxler et al, in preparation). These gold-binding sequences (GBP) were of either 5 or 7 repeats of a gold-binding domain identified by Brown [1].

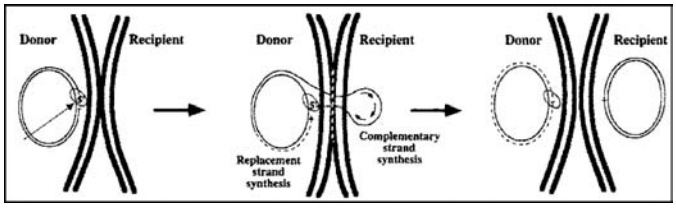

Figure 1: F plasmid conjugation (Firth et al 1996). Left arrow indicates the TraI protein.

AFM imaging was then performed on the TraI derivatives TraI-GBP7Q and TraI-GBP5Q. The proteins were examined bound to both single-stranded DNA and gold nanoparticles in order to analyze the structure of the proteins in this configuration and to see if the AFM is a useful tool to directly image these proteins. The idea was to create a linear ordered array of nanoparticles using a DNA substrate, which could have many applications, including creating nanowires or semiconductors.

Procedures:

TraI-GBP derivatives were purified from the soluble fraction of cell extracts. TraI-GBP was separated from other soluble proteins first on a DEAE column using the following buffer at increasing NaCl concentrations: 20 mM TrisCl pH 7.6, 0.1 mM EDTA, and 10% glycerol. The 0.15 M NaCl fractions were pooled and dialyzed. The TraI then was further purified using a heparin column with the above buffer, and the 0.40 M NaCl fractions were collected and stored at -80°C.

The *in vivo* function of the TraI-GBP derivatives was measured in a conjugation assay where donors (XK1502 F'lac ΔTraI with various plasmids) and recipients (BT8) were each diluted 1:10 into LB and incubated at 37°C for 45 min. Dilutions were plated onto lactose minimal with streptomycin plates, and colonies were counted after incubation at 37°C.

To prepare the samples for use in the AFM, ssDNA, protein, and/or gold colloid were incubated together at 4°C for 1 hour, in 20 mM TRIS, pH-7.5, 5 mM KCl, 5 mM gCl_2, 1 mM DTT (Sattin). 20 μl of this solution was then deposited on 1 cm x 1 cm freshly cleaved mica and allowed to bind to the mica substrate at room temperature for 20 minutes. Next, the mica chip was rinsed drop-wise with dH_2O and dried under an Ar stream.

The AFM was operated on tapping mode, at which the probe oscillates at its resonant frequency. The AFM

used was the Nanoscope IIIa/MMAFM system (Veeco, Inc). ssDNA was prepared by boiling λ dsDNA (NEB) cut with BstEII for 10 minutes, then immediately cooling in liquid nitrogen. 15 nm Au particles were purchased in colloid form from Ted Pella, Inc.

Results and Conclusions:

TraI-GBP proteins were purified, and assays were done on the TraI derivative proteins to ensure their functionality. The quantitative mating assay checked the efficiency of F plasmid conjugation with the TraI-GBP and demonstrated that they functioned properly in vivo. The results of this assay are shown in Figure 2.

Donor Strain	Avg. Mating Efficiency	% of Wild Type
pTrc99A (vector control)	0	0
pTrc99traI+ (positive control)	3.588 * 10-5	100
p99I::i31Q (5Q/7Q parent)	4.806 * 10-3	13393
p99I::GBP5Q	2.668 * 10-3	7437
p99I::GBP7Q	2.180 * 10-3	6076
p99I::GBP5L	3.925 * 10-5	109
p99I::GBP7L	1.201 * 10-3	3348

Figure 2: Quantitative mating assay.

We next demonstrated that the TraI-GBP derivatives could be manipulated *in vitro*. We imaged these proteins bound to DNA and simultaneously to DNA and gold nanoparticles, as shown in the AFM images (Figures 3 and 4). Figure 4 shows a quantitation, in which the percentage of gold particles bound to DNA and thus presumably to the TraI protein was found to be 68±18%.

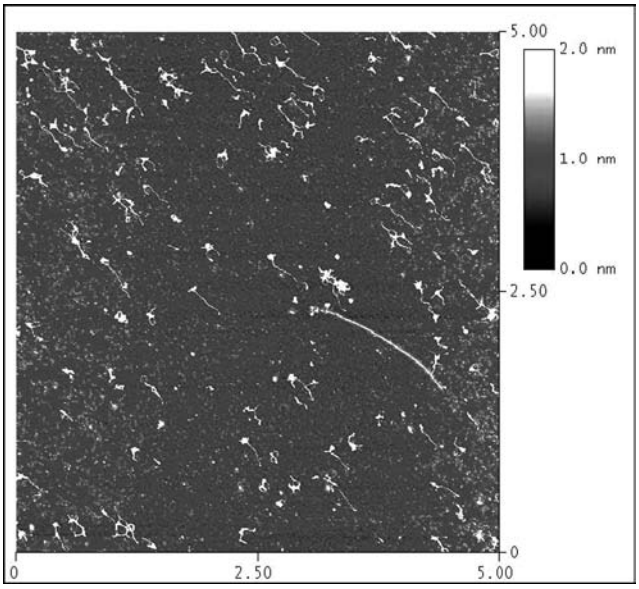

Figure 3: ssDNA and TraI: 30 µg/ml DNA, 3 µg/ml TraI-GBP7Q.

Future Work:

In the future, TraI could be imaged with different metal binding sites, including platinum, chromium, and cuprous oxide. We could also use TraI with more than one type of metal binding site, for example, TraI with a gold binding site at Q369 and a cuprous oxide binding site at L1753, to create composite nanowires.

Using conjugates of DNA, TraI, and nanoAu, we could create a hydrophilic/hydrophobic pattern by contact printing, then deposit the conjugate onto the pattern. We expect that the conjugates will have affinity for hydrophilic surface and therefore will only deposit on the hydrophilic part of the pattern. This process could be used as a proof-of-concept for making devices.

Acknowledgments:

The NNIN REU program, lab members Rembrandt Haft and Jessica Smith, Dr. Sarikaya's lab, and Hanson Fong for assistance with the AFM.

References:

[1] Nat. Biotech. 1997 15:269-272 Metal-recognition by repeating polypeptides Brown S.

[2] Ann. Rev. Mat. Sci. 1997 27: 175-222 Characterization of Polymer Surfaces with Atomic Force Microscopy Magonov SN, Reneker DH.

[3] Biophysical J. 2004 87(5):3430-6 Direct observation of the assembly of RecA/DNA complexes by atomic force microscopy Sattin BD, Goh MC.

[4] "Escherichia coli and Salmonella: cellular and molecular biology" 1996, pp. 2377-2401. Firth NK, Ippen-Ihler K, Skurray R. Structure and function of the F factor and mechanism of conjugation (ASM Press).

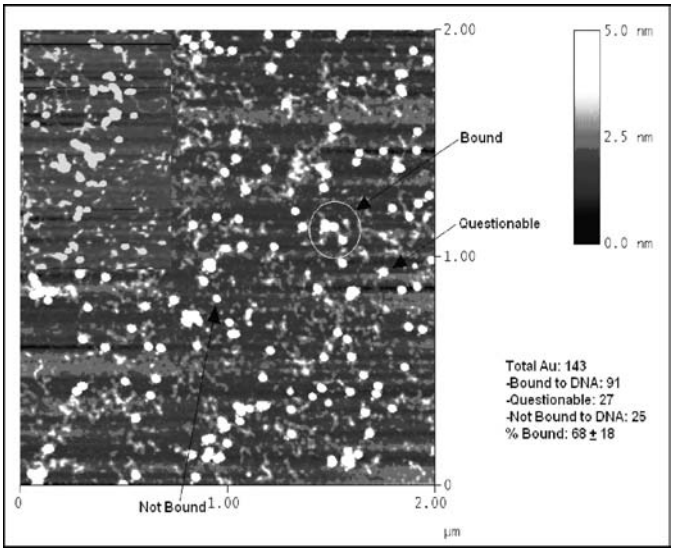

Figure 4: DNA, TraI, nanoAu: 25 µg/ml DNA, 10 µg/ml TraI-GBP7Q.