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COMMUNICATION

Neutravidin coated surfaces for single DNA molecule analysis†

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We present a novel approach for single DNA molecule analysis using neutravidin coated surfaces. DNA molecules are elongated and reversibly immobilized on neutravidin coated surfaces with pH and salt controls. We demonstrate restriction enzyme reactions for optical mapping and ligation for tethered DNA molecules.

Effective analysis of individual DNA molecules lays the fundamental basis for construction of large and informative data sets. Since Morikawa and Yanagida first visualized fluorochrome stained DNA molecules in 1981,[1] many significant developments have followed leveraging many advantages of single molecules for studies concerning presentation, biochemistry,[2,3] and biophysics.[4,5] Visualization of single DNA molecules not only provides direct measurements of DNA size, but also offers information regarding specific sequence locations mapped in a DNA backbone under a fluorescent microscope. Single DNA molecules have also been used as platforms for the study of DNA and protein interactions.[6,7]

Large DNA molecules exist as random coils, not appropriate for high resolution DNA analysis, requiring unraveling and stretching to present DNA for optical analysis. Many DNA stretching methods have been developed to take advantage of surface-immobilization, because this allows enough time for analysis of individual fluorochrome-stained DNA molecules. For example, an approach using positively charged surfaces allows massively parallel elongation of DNA molecules.[8] This approach has spurred the development of a powerful whole genome analysis platform, Optical Mapping, which has been used to analyze a broad range of genomes ranging from microorganisms[9] to human.[10]

Although surface immobilization is successful in many cases, surface-induced perturbations may affect molecular properties.[5] To circumvent these surface interactions, supported lipid bilayers are employed to immobilize large DNA molecules.[11,12] Biotinylation and nanopatterned anchoring are added to fix molecular positions on the lipid floating surface.[13] To completely avoid surface interactions, nano-/microfluidic immobilization methods such as nanochannel confinement[14–16] and microfluidic DNA elongation[17,18] have been developed. However, fluidic stretched DNA does not allow additional biochemical reactions in the nano-/microfluidic devices because analytes also flow out when exchanging chemical surroundings for another reaction.

Here we present a new approach that effectively elongates and reversibly immobilizes DNA molecules in a biocompatible environment with the capacity to manipulate the biochemical surroundings. This approach elongates and immobilizes DNA molecules on a protein coated surface. The protein coat is made of neutravidin on top of biotin-labeled bovine serum albumin adsorbed on a glass surface (see ESI†, Methods). Our experimental procedure consists of three steps as shown in Fig. 1. First, loaded λ DNA (48.5 kb) adheres as a random coil at pH 7.5 (Fig. 1a). Then, a flow of pH 8.5 buffer allows DNA molecules to partially detach and elongate (Fig. 1b). Although some are washed away by flow, numerous DNA molecules stretch at pH 8.5 with partial adsorption. Then elongated DNA molecules are immobilized on the surface simply by reducing the pH to 7.5 (Fig. 1c). Here, simple pH control is a key to elongating and immobilizing DNA molecules on the neutravidin surface. Additionally, we found that DNA molecules on the neutravidin surface can be immobilized by "salty" solutions such as 1 mM $MgCl_2$, 1 mM NaCl, and 1 mM $CaCl_2$ in water. As an example, Fig. 1d shows DNA molecules immobilized with 1 mM $MgCl_2$ after the partial desorption at pH 8.5. Since DNA adsorption is reversible, we were able to remove all DNA molecules in Fig. 1c and d with 1 mM NaOH solution. We repeated this experiment with T4 DNA (166 kb). Large T4 DNA molecules are adsorbed as randomly coiled conformations on the surface (Fig. 1e) and fully elongated DNA molecules are immobilized on the surface (Fig. 1f).

To understand why DNA molecules are adsorbed on the neutravidin coated surface, we have examined 3D structures of avidin and related proteins in the Protein Data Bank (www.pdb.org). Distinctively, the avidin structure has a protruded tryptophan with lysine and aspartate residues (DIGDD*W*K).[19] Tryptophan is known to have DNA intercalation capability, a topic which has been intensively studied by Hélène and co-workers.[20,21] They reported strong DNA binding of oligopeptides containing Trp-Lys and also characteristic affinity decrease from pH 8 to pH 9,[20] which is consistent with our observation of DNA partial desorption at pH 8.5 (Fig. 1b). They explained the affinity decrease by the neutralization of $-NH_3^+$ in lysine with pH increase. Also, the effect of salty solution can be explained by a recent crystallographic analysis which presented the role of metal bound aspartates.[22] Similar to our findings, they observed that Na^+ and Ca^{2+} can take the place of a biological cofactor of Mg^{2+} for

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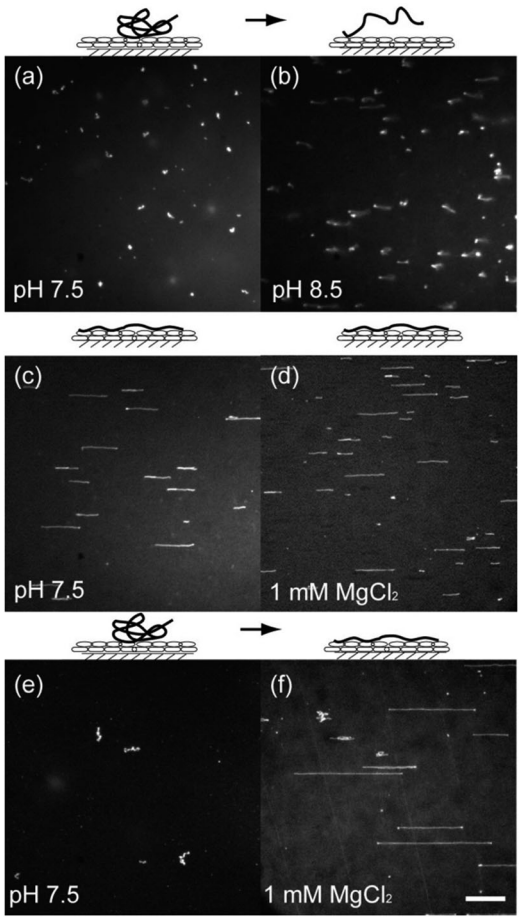

Fig. 1 Reversible immobilization of elongated DNA on a neutravidin coated surface with schematics presenting DNA conformations. (a) λ DNA (48.5 kb) at pH 7.5. (b) Partial desorption with a flow of pH 8.5. (c) Immobilization with pH 7.5. (d) Immobilization with 1 mM $MgCl_2$. (e) T4 DNA (166 kb) at pH 7.5. (f) Immobilization with 1 mM $MgCl_2$ (scale bar: 20 μm).

the DNA–protein intercalation complex. Accordingly, we reason that the mechanism of DNA binding on neutravidin can be attributed to the intercalation of a protruded indole moiety, and pH control and salty solution regulate lysine and metal bound aspartate to facilitate tryptophan intercalation.

A biocompatible environment is the most important characteristic of our approach presented here since elongated DNA molecules are deposited on the protein surface with intercalation. For biochemical reactions, controlled microfluidic flows are able to gently remove old reagents and supply new chemicals. As an example, we demonstrated biocompatibility with restriction enzyme reactions on the neutravidin coated surface. We used the restriction enzyme BbvC1 on T4 DNA to make restriction optical maps.[10] For this reaction, we used a pH 7.5 reaction buffer in order to maintain the adsorption of DNA fragments during the enzyme reaction. Fig. 2a shows typical restriction maps of T4 DNA on the neutravidin surface, which confirms that the neutravidin surface binds DNA molecules tightly enough to retain small restricted fragments. In addition, the restriction map of T4 DNA confirms the circularly permuted genome[23] when aligned against the *in silico* map (Fig. 2b).

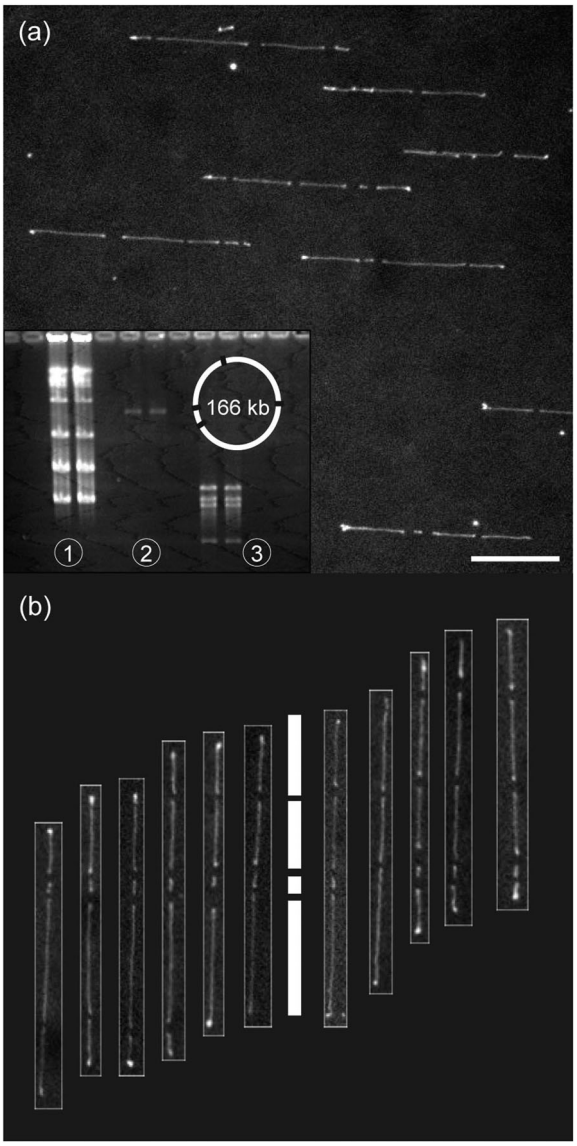

Fig. 2 Optical maps of T4 DNA on a neutravidin coated surface. (a) T4 DNA digested by the BbvCI restriction enzyme (scale bar: 20 μm). The inset shows pulsed field gel electrophoresis results. (1) λ concatemer ladders; (2) T4 DNA, 166 kb; (3) T4 DNA digested by BbvCI, 68.2, 47.7, 39.8, 10.0 kb that corresponds to its circularly permuted map. (b) T4 DNA maps aligned with an *in silico* map (47.7, 39.8, 10.0, 68.2 kb). A series of images illustrate the circularly permutated T4 bacteriophage genome.

In addition to biocompatibility, neutravidin surfaces have a well-known capability of tethering to biotin-labeled molecules. Neutravidin can selectively grab the biotin-labeled end of DNA molecules to make a mushroom conformation, and the DNA molecules are then fully stretched from one end to the other as shown in Fig. 3. In this experiment, we added biotin-labeled DNA oligomers on the neutravidin surface to hybridize the cohesive ends of λ DNA molecules, and then added λ DNA with T4 DNA ligase to covalently connect λ DNA on the DNA oligomers. As a result, λ DNA molecules are linked to the surface as well as ligating additional λ DNA molecules. Fig. 3a demonstrates that a pressure-driven flow stretches λ concatemers up to their full contour lengths, which

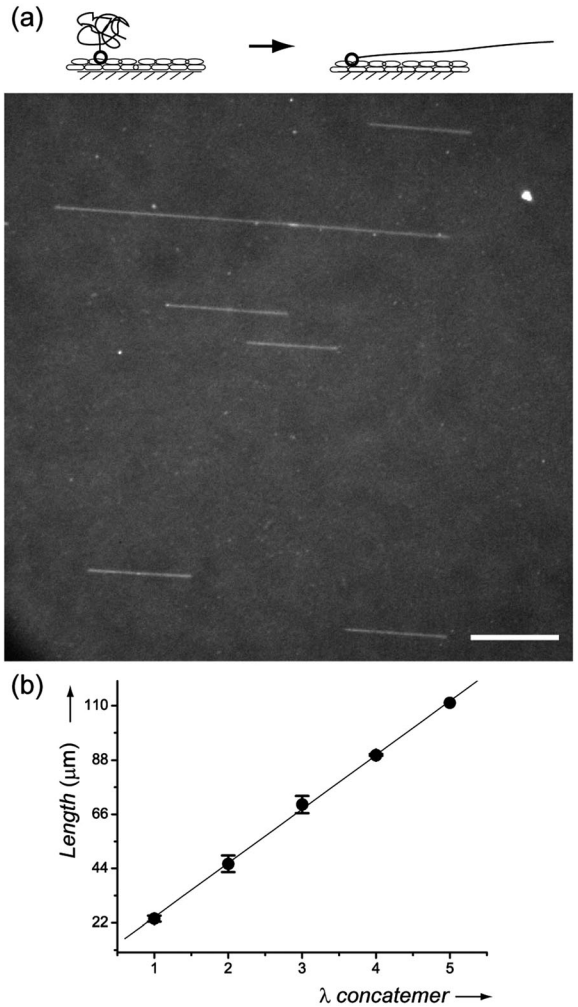

Fig. 3 (a) Dynamic elongation of biotin-tethered λ DNA monomers and a concatemer (scale bar: 20 μm). (b) Measured lengths of λ concatemer. The number of molecules is 471 yet two tetramers and one pentamer are used for the graph. Linear regression gives 22 μm per monomer.

are longer than the immobilized DNA shown in previous figures. Dynamically elongated λ concatemers show an average stretch of 22 μm per monomer, which corresponds to the full contour length of λ DNA (Fig. 3b). In addition, we were able to immobilize these DNA molecules on the neutravidin surface with 1 mM $MgCl_2$ water solution, which is beneficial to further biochemical reactions and analysis based on molecular images. We also observed that these molecules were easily returned to mushroom conformations.

Here we present a novel approach of elongation and reversible immobilization of DNA molecules on neutravidin surfaces by simple control of chemical environments. We demonstrate biocompatibility on the neutravidin surface with restriction enzyme reactions for optical mapping and ligase reactions for tethered DNA. Compared to previous approaches, the use of neutravidin surfaces has unique advantages. First, protein coated surfaces provide biocompatible environments favorable for enzyme reactions. Second, DNA is reversibly immobilized on neutravidin surfaces, which can be beneficial for the development of more complex and integrated genome analysis systems. Finally, neutravidin surfaces are able to tether DNA using biotin labels, which provides a variety of possibilities for the development of advanced genome analysis platforms.

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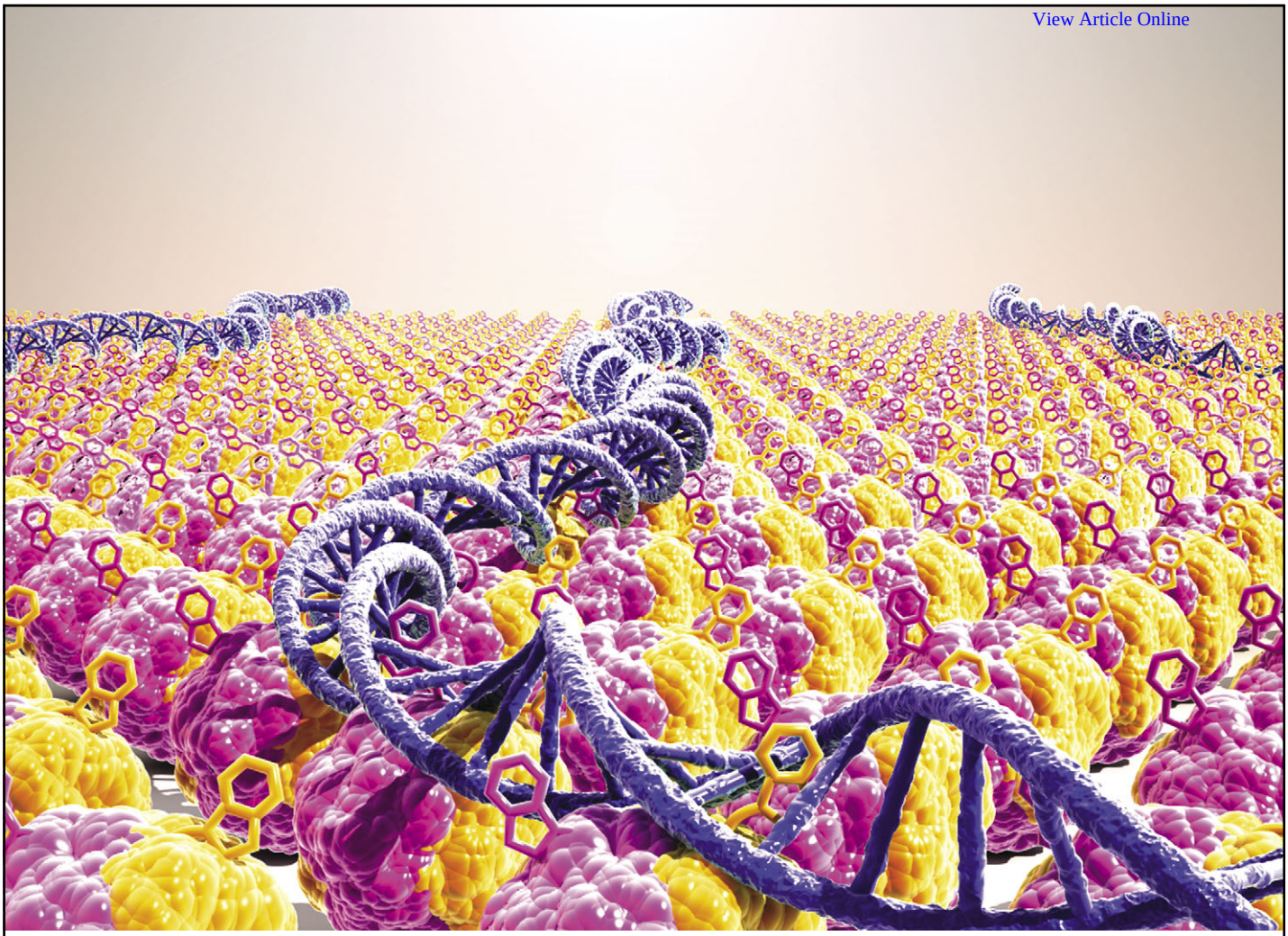
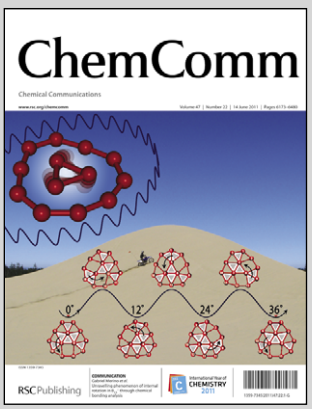


Showcasing work from Dr Kyubong Jo's labs at Sogang University, Korea

Neutravidin Coated Surface for Single DNA Molecule Analysis

Single DNA molecules are immobilized by intercalation of protruded imidazole rigns on the neutravidin coated surface. Reversible DNA binding is controlled by changing pH and salt environments. Neutravidin bound DNA molecules are utilized for biochemical analysis.

As featured in:



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