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Enzymatic Ligation Reactions of DNA "Words" on Surfaces for DNA Computing

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Abstract: The enzymatic manipulation of sets of short, 16-base oligonucleotides, or DNA "words", is demonstrated with applications toward DNA computing on surfaces. The enzyme T4 DNA ligase is used to ligate (join) DNA words on a chemically modified gold thin film. The efficiency of this surface ligation reaction is 80%, as determined by removal of the ligated molecules from the surface followed by gel electrophoresis. This surface ligation reaction is used in two new operations for DNA computing on surfaces. In a "Surface Word Append" operation, the complexity and information density of DNA word strands attached to gold surfaces are increased by appending additional words onto these word strands. The ligation reaction is also utilized as part of a "Two-Word Mark and Destroy" operation in which singly marked two-word DNA strands are selectively removed from the surface in the presence of doubly marked two-word strands. These new operations are essential for manipulation of the large combinatorial sets of linked DNA word strands required for DNA computing.

I. Introduction

The field of DNA computing was initiated in 1994 by Adleman,[1] who proposed that the tools of molecular biology could be used to solve instances of difficult mathematical problems known as NP-complete problems.[2] We have adapted these ideas to combinatorial mixtures of DNA molecules attached to surfaces and have proposed to perform logical manipulations of large sets of data by the hybridization and enzymatic manipulation of the attached oligonucleotides.[3−6] In a recent paper,[3] we demonstrated a word design strategy for DNA computing on surfaces which utilizes 16-base oligonucleotides, or DNA "words", attached to chemically modified gold thin films. By linking these words together into word strands, the longer DNA molecules required to make large combinatorial sets of oligonucleotides can be created.

To solve computational problems with single word strands, a set of "operations" was developed previously to manipulate these surface-bound DNA words. These operations are the following: "Mark", in which subsets of the DNA word strands are tagged by the hybridization of complementary words; "Destroy", in which DNA words that are not tagged are removed from the surface; and "Unmark", in which tagged molecules are untagged. To solve more complex computational problems

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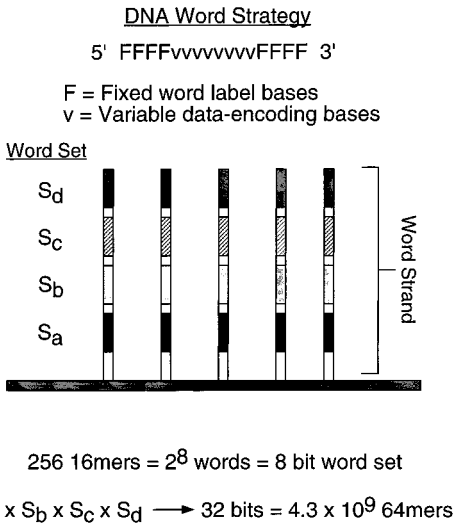

Figure 1. DNA word strategy. DNA words are 16 base oligonucleotides (16mers) that contain 8 fixed word label bases and 8 variable bases which code the data contained in each word. The word label sequence is the same ("Fixed") for every 16mer in a word set; additional word sets are generated by varying the word label sequence. By linking different word sets together to form DNA word strands, large combinatorial mixtures can be created. For example, a set of 256 distinct 16mers contains 2^8 words and is defined as an 8 bit word set. Linking four words together from four different 8 bit word sets would produce a combinatorial set of 4.3×10^9 unique 64mers.

with linked sets of DNA words, additional operations will be necessary. In this paper we describe two new word operations that utilize the enzyme T4 DNA ligase to create and manipulate linked word strands: (i) a "Surface Word Append" operation to selectively append additional DNA words onto surface-bound word strands, and (ii) a "Two-Word Mark and Destroy" operation to selectively join two adjacently hybridized word complements to form a two-word complement. These operations will be incorporated into DNA computing strategies that utilize combinatorial mixtures of linked DNA words.

II. Background

To understand how these new operations can be applied to the manipulation of DNA words on surfaces, the following sections explain some important terminology and concepts in DNA computing. More detailed information can be found in refs 3−6.

Word Design. DNA words have been defined[3] as 16-base oligonucleotides (16mers) that have the following sequence design:

$$5'\text{- FFFFvvvvvvvvFFFFF -3}' \qquad (1)$$

where the 8 bases labeled "F" are denoted as the "word label" and are the same ("Fixed") for every 16mer in a word set, and the 8 bases labeled "v" are the "variable bases" that code the data contained in each of the words. By linking different word sets together into word strands, the longer DNA molecules required to make large combinatorial sets of oligonucleotides can be created (see Figure 1). For example, a set of 256 distinct 16mers contains 2^8 words and is defined as an 8 bit word set. Each member of the word set possesses the same word label sequence, and by varying this word label sequence other 8 bit sets can be created. Linking four words together from four different 8 bit sets would produce a combinatorial set of 4.3×10^9 unique 64mers. The set size of 256 was chosen for illustrative purposes and is smaller than the maximum number of 65 536.

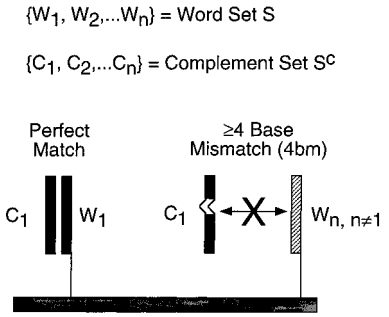

Figure 2. Explanation of 4bm complements. To facilitate discrimination by hybridization, DNA words are designed to differ from each other in at least 4 base locations. Each member W_n of the word set S will form a DNA duplex with the corresponding perfectly complementary word C_n. This pair of molecules is referred to as the "perfect match". All other pairs of words will contain mismatched base pairs. A DNA duplex that contains *n* mismatched base pairs is denoted as an "*n*-base mismatch" (*n*bm), and a set of molecules in which all mismatches are greater than or equal to *n* is denoted as a set of "*n*bm complements." The DNA words used in this paper are part of the set of 108 4bm complements identified previously.[3]

4bm Complement Sets. Though in a given set there is a maximum of 65 536 (4^8) different possible 16mers, the usable set size is much smaller. To be uniquely identified, each word in a set must be distinguishable from all other words on the surface by the hybridization ("marking") of complementary 16mer words. Because our previous results indicated that it was difficult to completely discriminate by hybridization two 16mers that differ by only one base,[5] a more robust strategy in which the 16mers all differ in at least 4 base locations was devised. This design strategy is depicted in Figure 2. Each member W_n of the word set S will form a DNA duplex with the corresponding complementary word C_n in which all of the bases are hydrogen bonded to the correct complementary base. This pair of molecules W_n/C_n is referred to as the "perfect match". All other pairs of words $W_n/C_{m,m\neq n}$ will contain mismatched base pairs. A DNA duplex that contains *n* mismatched base pairs is denoted as an "*n*-base mismatch" (*n*bm), and a set of molecules in which all mismatches are greater than or equal to *n* is denoted as a set of "*n*bm complements". Thus, the word set depicted in Figure 2 is a 4bm complement set because C_m has at least a 4 base mismatch with every $W_{n,n\neq m}$. A set of 108 variable base sequences that are 4bm complements and sets of 4 and 12 word labels that are 8bm and 6bm complements, respectively, have been identified.[3] This 4bm complement strategy was chosen because 4 mismatches are the minimum number needed to achieve satisfactory discrimination by hybridization.[5]

DNA Word Operations. Surface-bound DNA word strands can be manipulated by using the tools of the molecular biologist: hybridization, denaturation, and a host of enzymatic reactions including digestion of single-stranded DNA. These tools have been developed into a set of "operations" for the manipulation of word strands in solving computational problems:

(i) Mark: The "hybridization adsorption" of DNA word complements to surface-bound DNA word strands. In this operation, the surface is exposed to a combinatorial mixture of DNA words; those strands that find a complement on the surface will bind to form a duplex. Thus, "marked" strands will be double-stranded and "unmarked" strands will be single-stranded.

(ii) Destroy: The enzymatic digestion of all single-stranded ("unmarked") word strands in the presence of double-stranded

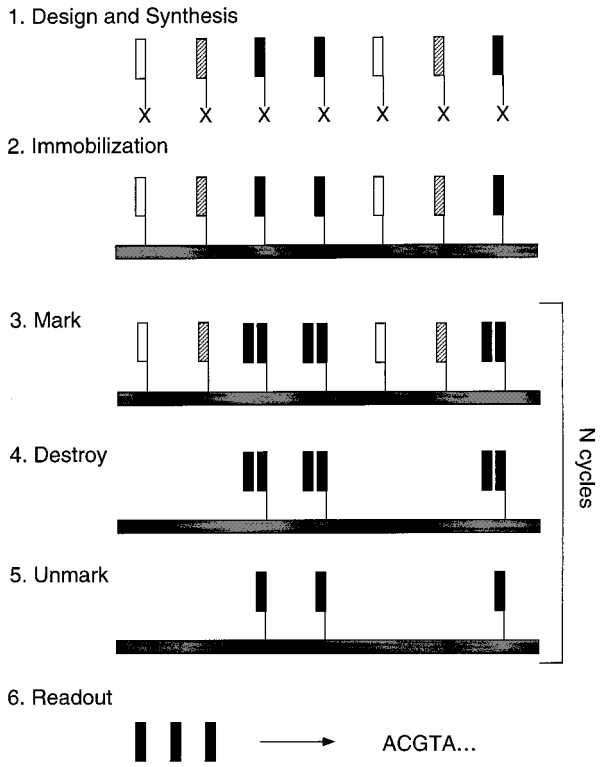

Figure 3. Overview of DNA computing on surfaces. A combinatorial set of DNA molecules representing all possible solutions to a given problem is synthesized and immobilized on a surface via a reactive functional group X. Subsets of the surface-bound combinatorial mixture are tagged by the hybridization of complementary molecules in a "Mark" operation. Thus, "marked" words are double-stranded and "unmarked" words are single-stranded. After the Mark operation, a Destroy operation removes from the surface all unmarked words. The surface is then regenerated by removing all adsorbed molecules in an Unmark operation. Through repeated cycles of the operations Mark, Destroy, and Unmark all strands which do not satisfy the problem are removed from the surface. At the end of N cycles, only those strands which are solutions to the problem remain. The sequence(s) of these surface-bound DNA words is then determined in a Readout operation by either conventional electrophoresis-based DNA sequencing or hybridization to DNA arrays.

("marked") word strands by exposure to a solution containing the enzyme *E. coli* Exonuclease I.

(iii) Unmark: The removal from the surface of all adsorbed word complements by exposure to a solution of 8.3 M urea at 37 °C.

When performed in repeated cycles, these operations can be used to compute, as depicted in Figure 3.

Computing with DNA: A Single Word SAT Calculation. To illustrate how these operations can be used to manipulate DNA words and solve computational problems, consider the famous Satisfiability problem (SAT)[2]. A simple example of a SAT problem is:

$$(x \text{ or } z) \text{ and } (\text{not } y) \qquad (2)$$

This example consists of two clauses separated by the Boolean operator "and" over the three variables *x*, *y*, and *z*. The SAT problem is to determine if there is an assignment of true/false values to the variables that satisfy all the clauses simultaneously. To determine this, every possible truth assignment (e.g., TTT, TFF, etc.) of the variables is represented as a unique DNA word. In this example each of the three variables can be either true or false and hence there are $2^3 = 8$ truth assignment combinations. This combinatorial set of 8 words is synthesized and immobilized on a surface. For each clause in the problem a series of Mark, Destroy, and Unmark operations is performed which removes from the surface all strands which do not satisfy the clause under consideration. Specifically, for the first clause in eq 2, all strands in which *x* is set to true or *z* is set to true are Marked. Thus, the six strands which satisfy the clause are double-stranded, while the two other strands (FTF, FFF) which do not satisfy the clause remain single-stranded. The Destroy operation is then used to remove from the surface those words which are unmarked (single-stranded). Following an Unmark operation that regenerates the surface, the process is repeated for the next clause. At the end of the two cycles, only those sequences which simultaneously satisfy both clauses remain on the surface. The sequence(s) of these surface-bound DNA words is then ascertained in a Readout operation by either conventional electrophoresis-based DNA sequencing or hybridization to DNA arrays. In general, for problems containing *N* clauses, the cycle Mark, Destroy, Unmark is repeated *N* times. The previously established set of single DNA words will be able to encode enough information for a 6-variable SAT problem. To solve more complex computational problems, linked DNA word sets and additional DNA word operations will be necessary. This paper demonstrates how surface enzymatic ligation reactions can be used to create these new operations.

III. Experimental Considerations

A. Materials. The chemicals 11-mercaptoundecanoic acid (MUA) (Aldrich), poly(L-lysine) hydrobromide (PL) (Sigma), sulfosuccinimidyl 4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate (SSMCC) (Pierce), urea (Bio-Rad Laboratories), and triethanolamine hydrochloride (TEA) (Sigma) were all used as received. Gold substrates were prepared by vapor deposition onto microscope slide covers (Fisher No. 2, 18 × 18 mm) that had been silanized with (3-mercaptopropyl)trimethoxysilane (Aldrich) in a manner similar to that reported by Goss et al.[7] Millipore filtered water was used for all aqueous solutions and rinsing. All oligonucleotides were synthesized on an ABI DNA synthesizer at the University of Wisconsin Biotechnology Center. Glen Research 5′-Thiol-Modifier C6, Chemical Phosphorylation Reagent, and ABI 6-FAM were used for 5′-thiol-modified, 5′-phosphorylated, and 5′-fluorescein-modified oligonucleotides, respectively. Prior to purification, thiol-modified oligonucleotides were deprotected as outlined by Glen Research Corp.[8] Before use, each oligonucleotide was purified by reverse-phase binary gradient elution HPLC (Shimadzu SCL-6A). All thiol oligonucleotides were used immediately after purification. Because thiol oligonucleotides slowly oxidize to form disulfide dimers, care must be taken to store free thiol oligonucleotides under an inert atmosphere. DNA concentrations were verified prior to use with an HP8452A UV−vis spectrophotometer. The 5′-thiol DNA solutions used in the surface attachment reactions had a DNA concentration of 1 mM in a pH 7, 100 mM triethanolamine (TEA) buffer. DNA hybridization and rinsing employed a pH 7.4 "2xSSPE/0.2% SDS" buffer that consisted of 300 mM NaCl, 20 mM sodium phosphate, 2 mM EDTA, and 6.9 mM sodium dodecyl sulfate. Removal of hybridized complementary molecules (referred to as "Unmark") was accomplished by immersing the sample in 8.3 M urea at 37 °C for 15 min.

B. DNA Surface Attachment Chemistry. DNA oligonucleotides were immobilized onto gold thin films via a four-step chemical modification described elsewhere.[9] Briefly, a gold thin film was modified with a monolayer of the alkanethiol 11-mercaptoundecanoic

(7) Goss, C. A.; Charych, D. H.; Majda, M. *Anal. Chem.* **1991**, *63*, 85−88.

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acid, followed by the electrostatic adsorption of a poly-L-lysine (PL) monolayer.[10] As shown previously,[11] these steps create an amine-terminated surface that can then be reacted with the heterobifunctional linker sulfosuccinimidyl 4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate (SSMCC), creating a thiol-reactive, maleimide-terminated surface. 5′-Thiol-modified DNA word strands were covalently attached to this maleimide-terminated surface by placing a 0.8 μL drop of a solution containing 1 mM DNA onto the surface and reacting for at least 12 h in a humid environment to prevent evaporation. The drops of DNA spread out on the surface to a diameter of ~3 mm. After exposure to the DNA solution, the surface was rinsed with water and soaked for at least 1 h in 2xSSPE/0.2% SDS at 37 °C. From previous measurements[9] the DNA word strand surface density was estimated to be 5×10^{12} molecules/cm^2.

C. Surface Fluorescence Measurements. Surface fluorescence measurements of hybridization adsorption were performed on a Molecular Dynamics FluorImager 575. Hybridization to the attached DNA word strands was accomplished by exposure of the surface to a 2 μM solution of 5′-fluorescein-labeled oligonucleotides in 2xSSPE/0.2% SDS. A 20 μL drop of this solution was placed onto the gold surface and then spread over the entire surface by placing a clean coverslip on top of the sample. Hybridization adsorption was allowed to proceed for 30 min, after which the sample was immersed in a beaker of 2xSSPE/0.2% SDS buffer for 10 min. The sample was then placed face down on top of a glass scanner tray with a droplet of 2xSSPE/0.2% SDS buffer between the gold surface and tray and then scanned with the FluorImager.

D. Surface Ligation Experiments. Enzymatic ligation reactions were performed by reacting the surface with 100 μL of a solution containing 3.4 U/μL T4 DNA Ligase (New England Biolabs) in 1X T4 DNA Ligase Buffer (New England Biolabs) which consisted of 50 mM Tris−HCl (pH 7.8), 10 mM $MgCl_2$, 10 mM DTT, 1 mM ATP, and 50 μg/mL BSA. The reaction was allowed to proceed for 3 h at room temperature. The enzymatic digestion of single-stranded oligonucleotides in the presence of double-stranded DNA molecules on the gold surface was accomplished by reacting the surface with 100−200 μL of a solution containing 0.2 U/μL of the single-strand-specific enzyme *E. coli* Exonuclease I (Amersham) as outlined previously.[3] Enzymatic digestion was allowed to proceed for 3 h at room temperature after which the surface was rinsed with water.

E. Melting Temperature Measurements. DNA melting curves were obtained by monitoring the absorbance of DNA solutions at 260 nm as a function of temperature with an HP8452A UV−vis spectrophotometer equipped with an HP89090A Peltier temperature control accessory. Melting temperatures were measured in 2xSSPE/0.2% SDS buffer solutions containing 2 μM oligonucleotide. A ramp rate of 1 °C/min with a hold time of 1 min was used over the range 25 to 95 °C to record the DNA melting curve. The Tm was determined as the temperature at which the first derivative of the raw UV absorbance curve was a maximum. Tm data are estimated to be accurate within ±1.5 °C.

F. Surface Ligation Efficiency. Following surface ligation, all adsorbed complements were desorbed from the surface by assembling the sample in an In Situ Reagent Containment System (Perkin-Elmer) containing 100 μL of water and heating at 95 °C for 10 min in a GeneAmp In Situ PCR System 1000 (Perkin-Elmer). The solution was collected, concentrated to a volume of ~5 μL and run on a 20% polyacrylamide gel containing 7 M urea.

IV. Results and Discussion

Before developing these two new word operations, the ability to ligate (i.e., join) two DNA words on a surface was demonstrated. First, a modified gold surface was prepared onto which the two-word strand W_{1ab} was covalently attached. (The DNA molecules used in all the experiments in this paper are listed in Table 1 and are part of the set of 108 4bm complements identified previously.[3]) Next, the word complements C_{1a} and C_{1b} were hybridized to adjacent positions on W_{1ab}, as shown in Figure 4A, using a "Mark {C_{1a}, C_{1b}}" operation. The notation "Mark {*x*,*y*,*z*}" denotes exposure of a surface to a solution containing the molecules *x*,*y*,*z*. These one-word complements (C_{1a}, C_{1b}) were then ligated together on the surface to form the two-word complement C_{1ab} by exposure to a solution containing the enzyme T4 DNA ligase (see Figure 4B). All molecules hybridized to W_{1ab} were then removed and collected as described in Section III-F, and analyzed with polyacrylamide gel electrophoresis. Two bands were present in the gel, which comigrated with known 16- and 32mers and corresponded to unligated and ligated molecules, respectively. Based on the band intensities, a surface ligation efficiency (defined as the ratio of the 32mer band intensity to the sum of the intensities of the 32mer and 16mer bands) of 80% was obtained. The efficiency of ligating these same molecules in solution with T4 DNA ligase was measured to be 92%. This surface ligation efficiency is comparable to the ligation efficiency reported by Zhang and Seeman[12] for oligonucleotides attached to Teflon-based supports. Surface ligation reactions have also been reported on oligonucleotides bound to magnetic beads,[13−15]

Table 1. DNA Words[a] Used in the Ligation Reactions

Word	5'.....3'	5' Modification
W_a	AACGatgcaggaGCAA	Thiol[b]
W_b	GCTTaaccaccaTTCG	Phosphate
C_b	CGAAtggtggttAAGC	Fluorescein
C_{ab}	CGAAtggtggttAAGCTTGCtcctgcatCGTT	Fluorescein
W_{1ab}	AACGatgcaggaGCAAGCTTatcgagcttTTCG	Thiol[b]
W_{2ab}	AACGatcgagctGCAAGCTTatgcaggaTTCG	Thiol[b]
C_{1a}	TTGCtcctgcatCGTT	Phosphate
C_{1b}	CGAAagctcgatAAGC	Fluorescein
C_{2b}	CGAAtcctgcatAAGC	Fluorescein

[a] Uppercase letters are word label bases; lowercase letters comprise the variable base regions. [b] Modified with 5′ HS-$(CH_2)_6$-$(T)_{15}$.

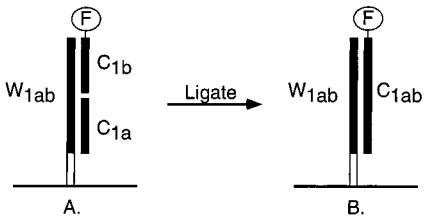

Figure 4. Surface ligation reaction. A modified gold surface was prepared onto which the two-word strand W_{1ab} was covalently attached. The word complements C_{1a} and C_{1b} were hybridized in adjacent positions on W_{1ab} as shown in (A) by exposing the surface to a solution containing C_{1a} and C_{1b}. These one-word complements were ligated together on the surface to form the two-word complement C1ab as shown in (B) by exposing the surface to a solution containing the enzyme T4 DNA ligase. A surface ligation efficiency of 80% was determined by removal of all molecules hybridized to W1ab followed by gel electrophoresis.

(10) Jordan, C. E.; Frey, B. L.; Kornguth, S.; Corn, R. M. *Langmuir* **1994**, *10*, 3642−3648.
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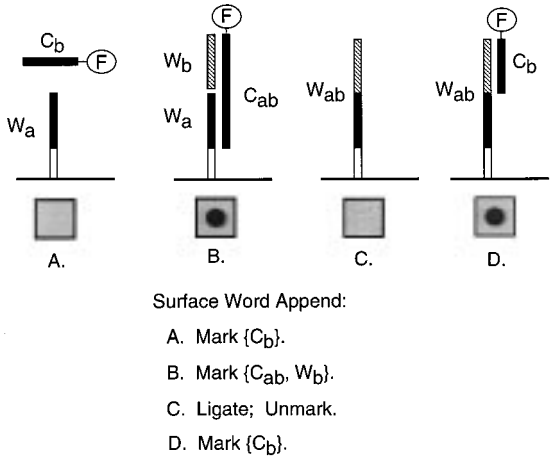

Figure 5. Demonstration of the Surface Word Append operation. The word W_b was appended to the one-word strand W_a in the following manner. W_a was covalently attached to a chemically modified gold surface. A Mark $\{C_{ab}, W_b\}$ operation was performed by exposing the surface to a solution containing the molecules C_{ab} and W_b which resulted in the image shown in (B). Word W_b was ligated to W_a by exposing the surface to a solution of the enzyme T4 DNA ligase, thus creating the two-word strand W_{ab}. C_{ab} was then removed in an Unmark operation by exposing the surface to a solution of 8.3M urea. The sample was then scanned to give the image shown in (C). Successful ligation was confirmed by a Mark $\{C_b\}$ operation performed both before and after ligation. Prior to the ligation of W_b to W_a, no fluorescence was observed after this Mark operation as shown in (A). In contrast, significant fluorescence was observed after ligation as shown in (D).

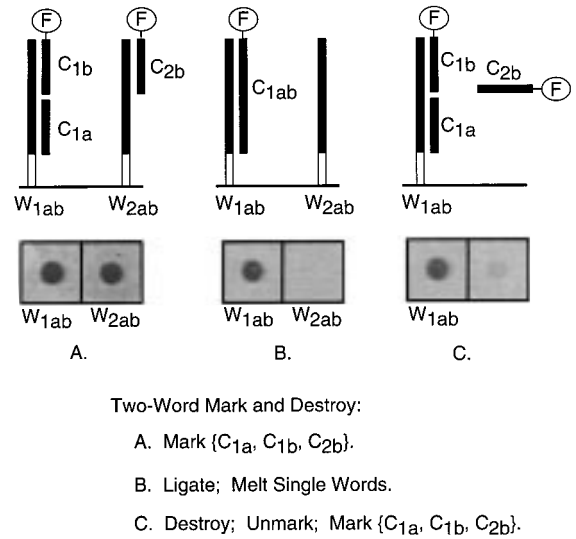

Figure 6. Demonstration of the Two-Word Mark and Destroy operation. A modified gold surface containing the two-word strands W_{1ab} and W_{2ab} attached in distinct regions on the surface was prepared and exposed to a solution containing the one-word complements C_{1a}, C_{1b}, and C_{2b}. Following this Mark $\{C_{1a}, C_{1b}, C_{2b}\}$ operation the surface was scanned to give the image shown in (A). A ligation step was then performed by exposing the surface to a solution of the enzyme T4 DNA ligase, which resulted in the joining of the one-word complements C_{1a} and C_{1b} to form the two-word complement C_{1ab}. This was followed by a "Melt Single Words" step in which all single (i.e. unligated) words were melted off the surface by soaking the sample in a buffer solution for 10 min at 62 °C, giving the image shown in (B). The surface was then exposed to the single-strand-specific enzyme Exonuclease I to remove word strand W_{2ab} from the surface in a Destroy operation. All adsorbed molecules were subsequently removed in an Unmark operation and then a Mark $\{C_{1a}, C_{1b}, C_{2b}\}$ operation using the same solution as in (A) was performed to give the image shown in (C). Analysis of the residual fluorescence due to the hybridization of C_{2b} to any remaining W_{2ab} shows a diminution in intensity of ~94% compared to the original signal in (A).

dextran matrixes,[16] and Sephacryl particles[17] and immobilized in gels.[18]

After demonstrating the ability to perform surface ligation reactions with DNA words, this surface ligation reaction was then used to create a new operation for DNA computing that appends additional DNA words onto a surface. This new operation is denoted as a "Surface Word Append", and was demonstrated by ligating the word W_b to the one-word strand W_a to create the two-word strand W_{ab} as outlined in Figure 5. Specifically, a Mark $\{C_{ab}, W_b\}$ operation with the two-word complement C_{ab} and the single word W_b was used to form the duplex shown in Figure 5B. W_b and W_a were then ligated together to create the two-word strand W_{ab}. C_{ab} was subsequently removed with an Unmark operation and the sample was scanned to give the image shown in Figure 5C. To confirm that the ligation was successful and to demonstrate that the ligated word was accessible to hybridization, a Mark $\{C_b\}$ operation was performed both prior to and after ligation. Before the ligation of W_b to W_a, no fluorescence was observed, as seen in Figure 5A. However, as shown in Figure 5D, significant fluorescence was observed after ligation, indicating that W_b was appended to W_a. Note that more fluorescence was observed in Figure 5B than in Figure 5D. This difference is attributed in part to a difference in the surface fluorescence intensity of an adsorbed 32mer duplex vs an adsorbed 16mer duplex. This point was verified in a separate experiment (data not shown) in which it was observed that the fluorescence intensity of C_b in Figure 5D increased when the unlabeled 16mer C_a was simultaneously hybridized to W_{ab}. One possible explanation for this behavior is that the fluorescence is quenched more efficiently by the gold surface for the shorter 16mer duplex than for the longer 32mer duplex. The increased rigidity of the longer 32mer duplex structure presumably causes the fluorophore present at the end of the duplex to be located farther away from the surface compared to the 16mer duplex. In general, the efficiency of the surface word append operation is limited by (i) the ligation efficiency of W_b and W_a and (ii) the hybridization efficiency of W_b and C_{ab}. In another control experiment (data not shown) performed on a surface containing two different DNA word strands, it was shown that the Surface Word Append operation could be used to selectively append words onto one word strand in the presence of another. This surface word selectivity reflects the high degree of hybridization discrimination afforded by the 4bm complement word design strategy.

In a final set of surface reactions, the surface ligation operation was used to selectively remove from a surface two-word strands that were singly marked in the presence of two-word strands that were doubly marked. This "Two-Word Mark and Destroy" operation is accomplished as outlined in Figure 6 and is comprised of a sequence of surface ligation, differential melting, and exonuclease digestion steps. This new operation will be important for marking and readout strategies of linked DNA word sets. To demonstrate this operation, a surface containing both singly and doubly marked words was prepared by a Mark $\{C_{1a}, C_{1b}, C_{2b}\}$ operation on a surface containing two different two-word strands, W_{1ab} and W_{2ab} (see Figure 6A).

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C_{1a} and C_{1b} were then ligated together by exposure of the surface to a solution of T4 DNA ligase to create the two-word complement C_{1ab}. The one-word complement C_{2b} was then removed from the surface in a "differential melting" or "Melt Single Words" step in which the surface was placed in a 62 °C buffer solution for 10 min. The longer, more stable two-word complement C_{1ab} was retained on the surface. Solution melting temperatures of the duplexes W_{2ab}/C_{2b} and W_{1ab}/C_{1ab} were measured to be 63 and 82 °C, respectively. A schematic representation of the surface after these ligation and differential melting steps is shown in Figure 6B. Notice that W_{2ab} is now single-stranded ("unmarked") while W_{1ab} is double-stranded ("marked"). W_{2ab} was then removed from the surface by a Destroy operation by using the single-strand-specific enzyme *E. coli* Exonuclease I.[3] This was followed by an Unmark operation to remove C_{1ab} from the surface.

To determine the efficiency of the Two-Word Mark and Destroy operation, a Mark $\{C_{1a}, C_{1b}, C_{2b}\}$ operation was performed with the same solution as in (A) to give the fluorescence image shown in Figure 6C. Analysis of the residual fluorescence due to the hybridization of C_{2b} to any remaining W_{2ab} showed a diminution in intensity of ∼94% compared to the original signal in Figure 6A. This number is in agreement with our previously published work on the efficiency of exonuclease digestion on surfaces.[3]

This paper has shown that the enzyme T4 DNA ligase retains its activity on a poly-L-lysine modified gold film and can be used in the manipulation of linked DNA words on surfaces. In a Surface Word Append operation, enzymatic ligation was used to append additional DNA words onto surface-bound word strands. This append operation can be extended to create combinatorial mixtures of DNA molecules attached to surfaces. T4 DNA ligase was also used as part of a Two-Word Mark and Destroy operation in which singly marked two-word strands were selectively removed from a surface in the presence of doubly marked strands. The selectivity demonstrated in these experiments is a direct result of the word design strategy employed, which facilitates discrimination by hybridization through the use of 4bm complement word sets. These surface ligation reactions may also be used in conjunction with PCR amplification for multi-word "Readout" operations as proposed elsewhere.[4] Experiments are in progress to increase the surface ligation efficiency above 80%, and future experiments will demonstrate the use of other enzymes such as DNA polymerases and restriction endonucleases for use in additional DNA computing operations.

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