

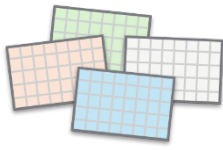
## Combinatorial 4-way and 6-way Strand Displacement for Programmable Reversible Data Manipulation on DNA Origami

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DNA-based data storage systems offer exceptional information density and longevity, yet current approaches face critical barriers: sequence-based methods require costly *de novo* synthesis for each write operation, while read operations depend on complex sequencing infrastructure or specialised super-resolution microscopy, limiting scalability and accessibility. We developed a combinatorial strand displacement architecture for synthesis-free, programmable, and reversible data manipulation on DNA nanostructures using geometric bit encoding readable by atomic force microscopy, eliminating dependencies on costly synthesis and complex readout infrastructure. Our rectangular DNA origami platforms contain 168 addressable positions encoding binary information as presence (1) or absence (0) of molecular "bumps" written and deleted using 4-way and 6-way strand displacement mechanisms. A dual-toehold addressing scheme with separate domains for position and structure recognition enables exponential scaling: with  $M$  position-specific and  $N$  structure-specific recognition domains, the system addresses  $D = M \times N$  unique bit locations using only  $M + N$  pre-synthesised strands forming writing and deletion complexes rather than  $M \times N$  strands. We monitored kinetics via real-time fluorescence (FRET and Cy3 signals), and validated bit placement using atomic force microscopy with dumbbell-modified writing complexes appearing as bright features on origami surfaces. At a single origami position, 4-way strand displacement writing achieved ~80% yield within 1 hour. The novel 6-way deletion mechanism successfully erased written bits at one origami position, confirmed by T-E and A-R waste product formation, and direct FRET/Cy3 measurements. Testing 8 E-R eraser variants (4x2 toehold combinations) individually against pre-written bits at one origami position revealed sequence-dependent deletion kinetics (50% completion: ~2.5-3 hours for optimal pairs, ~6-8 hours for two slower pairs). Orthogonality testing of 81 T-A writing variants (9x9 toehold combinations) in solution showed half-lives of 13-30 minutes with minimal cross-reactivity for mismatched pairs. Leak reaction analysis for 6-way deletion on DNA origami with 2 of 4 toeholds mismatched showed most combinations maintain good selectivity (13-20% cross-reactivity), though some exhibited variable leak behaviour up to 30%, confirming the need for systematic toehold optimisation. This work establishes design principles for programmable data manipulation on DNA nanostructures through combinatorial addressing. By eliminating synthesis requirements while maintaining precise spatial control, our approach removes the primary cost barrier preventing large-scale deployment and enables cost-effective parallel operations for practical synthesis-free DNA storage platforms.

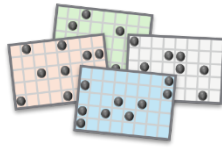
Empty nanostructures



$M$  addressable positions per structure



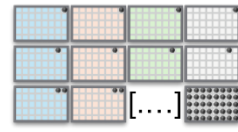
Written nanostructures



□ = 0 (no bump);  
● = 1 (molecular bump)



Scalable array of  $M \times N$  bits



$N$  structures for scale up