however, the processes underlying this important feature of meiosis are not yet understood. In this work, we show that even in the absence of proteins or additional polymers, individual dsDNA molecules of 5 kb or longer in solutions containing only monovalent ions, can self-assemble into sequence dependent pairs, where this homologous dependent pairing can occur at lower dsDNA concentrations than those typically found in vivo. We have used magnetic tweezers to probe the stability of these molecular pairs and demonstrate that at room temperature they remain bound for shear forces up to 10 pN. To elucidate the mechanisms that underlie the observed homologous association of pairs, we studied the effect of sequence, dsDNA concentration, non specific competitors, temperature, and salt.

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Conformational Equilibria Of Bulged Sites In Duplex Dna Studied By EPR Spectroscopy And Differential Scanning Calorimetry

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Conformational flexibility in nucleic acids provides a basis for complex structures, binding, and signaling. One-base bulges directly neighboring single-base mismatches in nucleic acids can be present in a minimum of two distinct conformations, complicating the examination of the thermodynamics by calorimetry or UV-monitored melting techniques. To provide additional information about such structures, we demonstrate how electron paramagnetic resonance (EPR) active spin-labeled base analogues, base-specifically incorporated into the DNA, are monitors of the superposition of different bulge-mismatch conformations. EPR spectra provide information in terms of ''dynamic signatures'' that have an underlying basis in structural variations. By examining the changes in the equilibrium of the different states across a range of temperatures, the enthalpy and entropy of the interconversion among possible conformations can be determined. The DNA constructs with a single bulge neighboring a single-base mismatch (''bulge-mismatches'') may be approximately modeled as an equilibrium between two possible conformations. Experiments on the bulge-mismatches show that basepairing across the helix can be understood in terms of purine and pyrimidine interactions, rather than specific bases. Measurements of the enthalpy and entropy of formation for the bulge-mismatches by differential scanning calorimetry and UV-monitored melting confirm that the formation of bulge-mismatches is in fact more complicated than a simple two-state process, consistent with the base-specific spectral data that bulge-mismatches exist in multiple conformations in the pre-melting temperature region. We find that the predictions of the nearest-neighbor (NN) model, based on data from DNA denaturation experiments, do not correlate well with the structures inferred from the base-specific EPR dynamics probe. We report that the base-specific spin probes are able to identify a bi-stable, temperature dependent, switching between conformations for a particular complex bulged construct.

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Closing The Lid On Dna End-to-end Stacking Interactions

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Recent experiments suggest that short DNA strands associate by end-to-end stacking. The stacking of hydrophobic ends of double-strand DNA molecules may provide the favorable free energy required for association. Here, we report interactions between DNAs with modified ends. DNA duplexes, 20 bp long, were capped with short T_4 loops at 2, 1 or 0 ends, and were placed in solutions dialyzed against buffer containing 20mM Mg^{2+}. Association was not observed in constructs with both ends capped. DNA-DNA interactions were characterized by measuring variations in small angle X-ray scattering (SAXS) curves at the lowest scattering angles. Second virial coefficients were computed from the SAXS data to further confirm that end-to-end stacking plays an important role in short strand DNA-DNA interactions.

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A Designed 3D Self-Assembled Crystalline DNA Array

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The precise control of the structure of matter is a central concern of the natural sciences. To this end, numerous investigators have developed self-assembling systems to produce targets of interest. Taking its cue from biological systems, structural DNA nanotechnology has used branched DNA motifs combined with cohesive ends to produce objects, nanomechanical devices and designed two-dimensional lattices. The details of these 2D lattices have been studied primarily by atomic force microscopy, whose resolution is 3-10 nm in typical analyses. The criteria for three dimensional self-assemblies are more strict, because the primary technique for their analysis is x-ray crystallography, whose resolution is limited only by the wavelength of the source (about 1 A). Previous efforts to produce self-assembled three-dimensional lattices have produced lattices that conformed to the design, but whose resolution was no better than 10 A, not really capable of revealing molecular structure. Here, we report the crystal structure at 5 A resolution of a self-assembled designed three-dimensional lattice based on the tensegrity triangle. Each edge of the tensegrity triangle contains two turns of DNA. The structure and sticky ends have been designed to be 3-fold symmetric, and at this resolution it appears to be so. The data are of sufficient quality to demonstrate clearly that it is possible to design a 3D lattice using the techniques of self-assembly based on molecular recognition.

This research supported by NIGMS, NSF, ARO and the W.M. Keck Foundation.

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Helix-Coil Transitions of Unusual DNA Structures by Measuring the Fluorescence Changes of 2-Aminopurine When Incorporated into DNA

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One focus of our research is to investigate the helix-coil transition of unusual DNA structures by comparing optical and calorimetric melting curves with fluorescence melts obtained by observing the fluorescence changes of 2-aminopurine (*AP*) when incorporated into DNA. In this work, we used a combination of temperature-dependent UV, circular dichroism (CD) and fluorescence spectroscopies, and differential scanning calorimetric (DSC) techniques to investigate the unfolding of: a) an intramolecular pyrimidine triplex, $A_3APA_3C_5T_7C_5T_7$; b) a G-quadruplex, $G_2T_2G_2TAPTG_2T_2G_2$; and c) a dodecamer duplex, CGCGA*AP*TTCGCG. Our experimental observations are as follows.

a) All melting curves of the triplex show similar monophasic transitions with T_Ms of 31 °C, ΔH_{vH}s of 38 kcal/mol and ΔH_{DSC} of 70 kcal/mol. Thus, this triplex unfolds in a non-two state fashion similar to the unfolding of the unmodified triplex but with a lower endothermic heat (by 12 kcal/mol). This indicates the presence of *AP* contributes with lower stacking interactions.

b) The G-quadruplex also unfolds in similar monophasic transitions: T_Ms of 52°C, ΔH_{vH}s of 44 kcal/mol and ΔH_{DSC} of 36 kcal/mol. The unstacking of *AP* follows the overall cooperative unfolding of the whole molecule. This quadruplex unfolds in two-state fashion similar to the unfolding of the unmodified quadruplex but with a higher endothermic heat (by 14 kcal/mol). This indicates that the presence of *AP* induces higher stacking contributions.

c) All melting curves of the dodecamer duplex show biphasic transitions. The T_M of the first transition depends on strand concentration while the T_M of the second one is independent, consistent with the presence of a duplex ← → hairpin ← → random coil equilibria. The central placement of *AP* destabilizes the duplex state, while does not affect the hairpin state.

Supported by Grant MCB-0616005 from NSF.

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Understanding the Chemistry of Cytosine Methylation Effects

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Proteins bind to DNA to initiate several important biological processes including DNA replication and transcription of genes, these processes are also highly regulated. A common method of bioregulation is cytosine methylation. Recent research suggests that cytosine methylation quenches DNA dynamics, preventing the DNA deformation often necessary for efficient protein-DNA complex formation. Using ^{31}P NMR, we have shown that methylation induced rigidity may stem from conformational changes in the backbone from BI to BII conformation. To explore the mechanism of these conformational changes, we have turned to the study of cytosine fluorination.