DNA Structures

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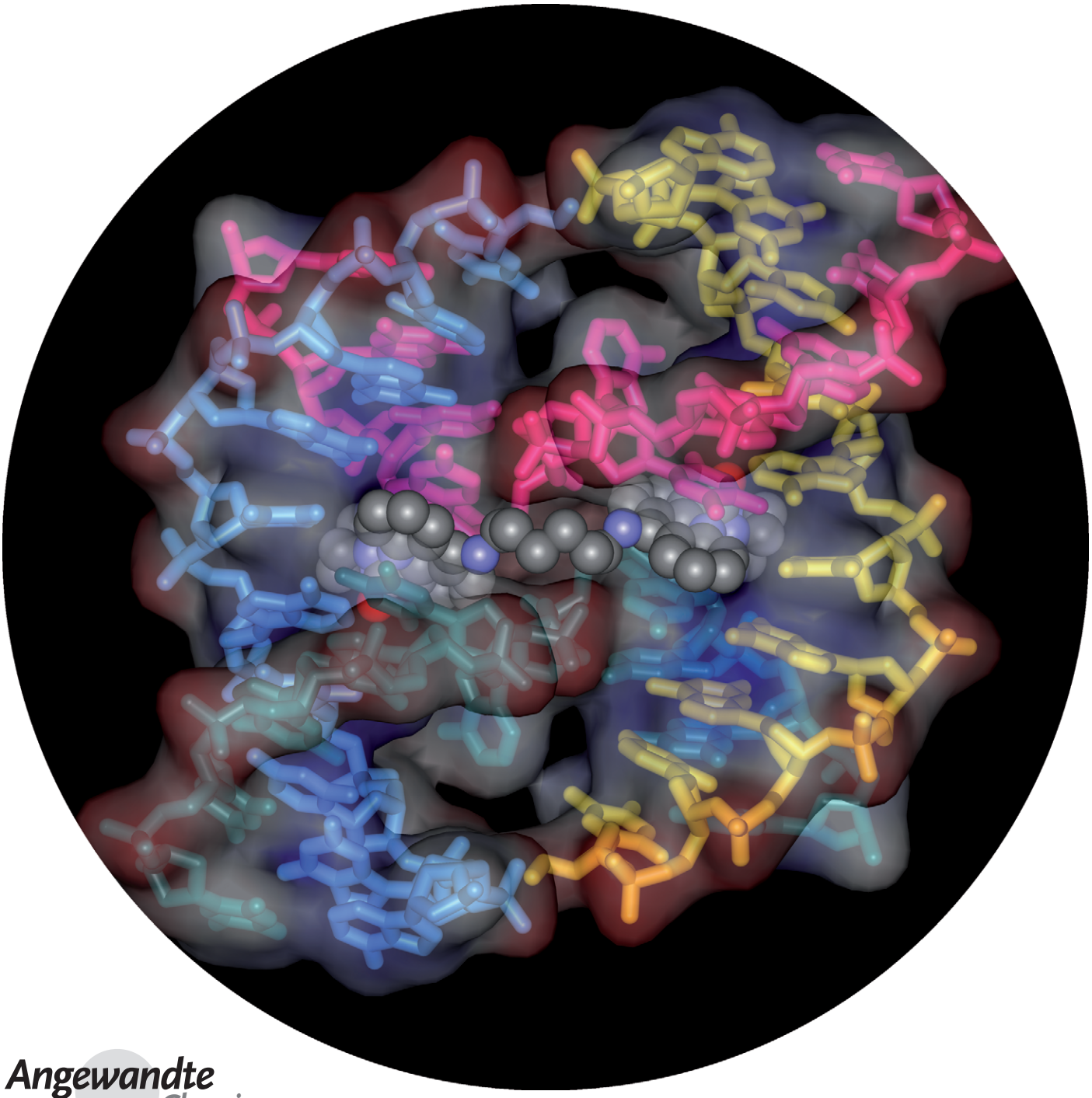
Ligand Bridging of the DNA Holliday Junction: Molecular Recognition of a Stacked-X Four-Way Junction by a Small Molecule**

*Anna L. Brogden, Nicholas H. Hopcroft, Mark Searcey, and Christine J. Cardin**



Homologous recombination (HR) in the repair of a collapsed replication fork is a process that involves strand invasion, branch migration, and resolution of the resulting Holliday junction (HJ). The HJ is a four-way DNA junction,[1] and the crystal structure of the stacked-X conformation was first reported in 1999.[2] Since then, the sequence dependence of spontaneous junction formation has been carefully studied[3–5] by crystallography and, most recently, the formation of a tetrameric species in solution by the d(CCGGTACCGG) decamer sequence has been confirmed.[6] That work provides a convincing demonstration that the solution data agree well with the crystal structure[7,8] and suggest that each of the two ACC core trinucleotides at the HJ crossover region contribute between -3.9 and -4.1 kcal mol^{-1} to the stabilization of the HJ in solution.

Recent studies have shown that cells that are deficient in HR are sensitive to inhibitors of poly(ADP-ribose) polymerase (PARP1; ADP = adenosine diphosphate).[9,10] The HJ can thus be seen as a target for the design of selective inhibitors of HJ resolution, which may lead to general PARP1 sensitivity in replicating tumor cells, without the requirement for defective genes such as BRCA2. A second area of possible therapeutic relevance is the ALT (alternative lengthening of telomeres) pathway,[11,12] which has been described as an alternative mechanism for the extension of telomeres in immortalized cells (such as tumor cells) that lack active telomerase. This pathway involves HR, and the role of the HJ in this process could be probed with an appropriate specific molecule.

Small molecules have been described that target higher-order DNA structures such as G quadruplexes[13] and, more recently, a three-way junction.[14] To probe HJ binding, we designed a series of prototype compounds that could potentially reach across the junction and bind in an intercalative fashion. Similar compounds have previously been shown to bind to duplex DNA through either mono- or bis-intercalation.[15,16] This family of compounds, based upon alkyl-chain-linked dimers of 9-aminoacridine-4-carboxamide, was particularly appealing as well-established chemistry allowed the variation of interchromophore distance to probe the interhelical space of the four-way junction. The eight-carbon-linked bis-acridine derivative has previously been shown to bind to a quadruplex-like structure,[17] which suggested that HJ binding was feasible. Herein, we describe the crystal structure of an unexpected and entirely new mode of binding in which a bis-acridine molecule, $\mathbf{C_6}$, binds across the center of the junction and the two adenines either side of the junction crossover are replaced by the acridine chromophores.

Five compounds ($\mathbf{C_4}$, $\mathbf{C_6}$, $\mathbf{C_8}$, $\mathbf{C_{10}}$, and $\mathbf{C_{12}}$, labeled according to their linker length; see Figure 1a and Figure S1 in the Supporting Information) were synthesized through previously described chemistry[18] and isolated as their dihydrochloride salts. Cocrystallization experiments were carried out with these compounds and the oligonucleotide d(TCGGTACCGA). Crystals were found in those conditions containing $\mathbf{C_6}$. This DNA sequence has previously been shown to crystallize as a four-way junction in the presence of metal cations.[3] Unexpectedly, the complex of $\mathbf{C_6}$ with sequence d(TCGGTACCGA) crystallized isomorphously to the native structures solved.[3] The structure of the complex was initially solved by a rigid body refinement of the native model against X-ray data to 2.7 Å resolution. This revealed additional electron density at the junction crossover region, clearly indicating the presence of the six-carbon linker spanning across the central junction cavity. During the early stages of refinement, the extra electron density corresponding to the bridging linker was the most obvious difference when compared to data for native HJ structures (Figure 1c and d). This suggested that the model would have to incorporate an unprecedented displacement of the adenine bases (A6(A) and A6(A′) at the crossover region to allow the ligand chromophores to fit in. Replacement of the central adenines A6(A) and A6(A′ with the acridine chromophores greatly improved the electron density map, and the refined structure of the HJ complex revealed a $\mathbf{C_6}$ molecule that is bound noncovalently at the junction crossover region (Figure 2a). The initial model was a good fit for the rest of the HJ structure, requiring only local realignment of the backbone to accommodate the presumed position of the adenosyl (A6) position. We suggest that the presence of a positively charged $\mathbf{C_6}$ molecule at the junction center helps to neutralize the high charge density in this region that is necessary for the formation of the stacked-X HJ. This is provided by Mg^{2+} in vivo and by the presence of metal cations (as noted in previous reports[3]) for synthetic examples. The model was refined to 1.7 Å resolution and the refined structure showed well-ordered packing, with 129 water molecules located within the asymmetric unit, surrounding the junction and lying within the minor grooves of the junction arms. The number of water molecules present in this structure is less than in previous unliganded structures, which may be due to the hydrophobic side chains of $\mathbf{C_6}$ in the minor grooves, restricting the level of hydration. Two spermine molecules were also located in the junction crossover region, lying along the phosphate backbones. This tetracationic species, in

[*] Dr. A. L. Brogden, Dr. N. H. Hopcroft,[+] Prof. C. J. Cardin
School of Chemistry
University of Reading
Whiteknights, Reading, Berkshire, RG6 6AD (UK)
Fax: (+44) 118-931-6331
E-mail: c.j.cardin@rdg.ac.uk
M. Searcey[++]
School of Pharmacy
University of London
29-39 Brunswick Sq, London, WC1N 1AX (UK)
[+] Current Address: School of Chemistry, University of Nottingham
University Park, Nottingham, NG7 2RD (UK)
[++] Current Address: School of Chemical Sciences and Pharmacy
University of East Anglia, Norwich NR4 7TJ (UK)
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Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

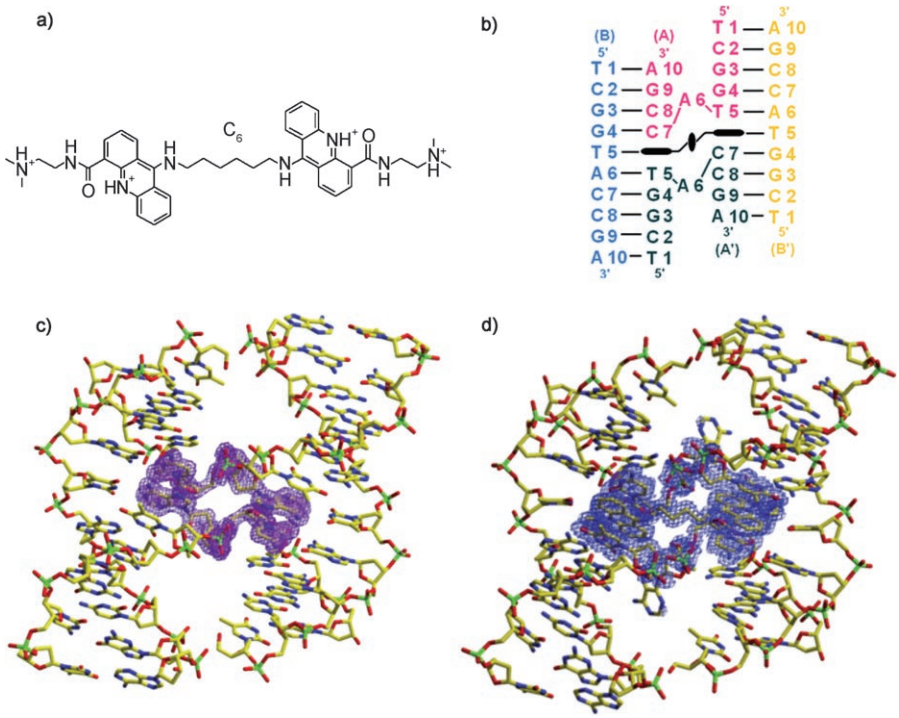

Figure 1. Overview of the HJ structure complexed with $\mathbf{C_6}$. a) Line drawing of the bis-acridine $\mathbf{C_6}$. Note that the subscripted 6 refers to the length of the linker, that is, $\mathbf{C_{10}}$ has a 10 carbon linker. b) Schematic representation of the nucleotide numbering used with each strand colored differently for clarity. Note that all further figures employ the same color and numbering scheme. The position of $\mathbf{C_6}$ can be seen in black at the crossover region. There is a twofold axis, as marked, so that the asymmetric unit of the structure consists of two DNA strands and half a bis-acridine molecule. The numbering scheme chosen reflects the symmetry. c) A view of the electron density at the crossover region of a native HJ structure (PDB code: 1NQS) formed from the same sequence $d(TCGGTACCGA)_4$. The complete model is shown viewed from the major-groove side. d) A view of the electron density at the crossover region for the HJ with $\mathbf{C_6}$ bound. The partial map is again superimposed on the complete model and viewed from the major-groove side. Clear electron density can be seen for the bis-acridine linker in this case. All maps are σA weighted ($2F_o-F_c$) maps that were determined by using 10.0–1.7 Å data and with contouring at 1σ.

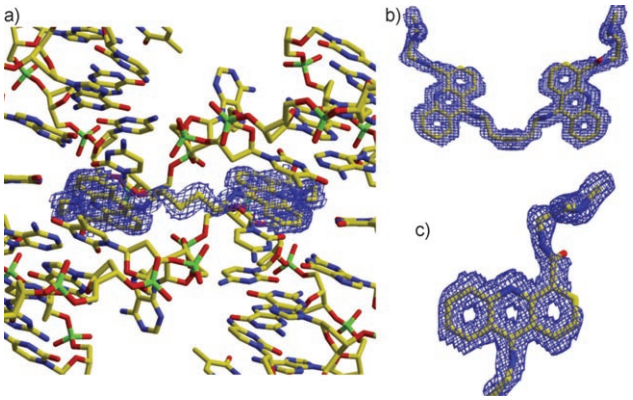

Figure 2. a) A view of the electron density of the bound bis-acridine molecule $\mathbf{C_6}$ at the center of the junction. The model is viewed from the major-groove side. b) A view of the electron density for the complete bound molecule. The rest of the structure has been omitted for clarity. This view shows the twofold symmetry and the wraparound effect of the conformation adopted. c) A view looking directly onto one of the chromophores of $\mathbf{C_6}$. All maps are σA weighted ($2F_o-F_c$) maps determined by using 10.0–1.7 Å data and with contouring at 1σ.

addition to the ligand, aids in stabilizing the structure through DNA negative-charge neutralization.

The map obtained for the $\mathbf{C_6}$ molecule in position at the junction crossover region shows that the chromophores are related by a twofold axis of symmetry through the center of the linker. The asymmetric unit is, thus, half of the junction. The quality of the electron density around the ligand after the final refinement was very good (Figure 2). The novel mode of binding observed for $\mathbf{C_6}$ is possible because the adenines A6(A) and A6(A′) at the crossover region can flip out of the base-pair stack towards the major groove, allowing the acridine chromophores to replace them. The chromophores have extensive π–π stacking interactions with the bases of C7 and T5 in the crossover DNA strands, with T5 stacking on the unsubstituted acridine ring and C7 stacking on the central heterocyclic ring (Figure 3). We have observed in previous structures that the AT hydrogen bonds are exceptionally long at this site, T5(B)–A6(A) (3.30 Å O4–N6; 3.14 Å N3–N1), when compared with the canonical B-DNA. The displaced adenine bases could be modeled in several orientations between the junction arms, none of which are preferred over any other conformation by virtue of stacking or hydrogen bonds, explaining the lack of electron density for this nucleotide. The thymines T5(B) and T5(B′) opposite the acridine chromophores have also shifted position (Figure 4a). They no longer stack with the adjacent bases above and below as was seen in the native structure (Figure 4b), but are instead twisted within the stack due to close contact with the $CH_2CH_2NMe_2H^+$ group of the ligand side chains.

The chromophore side chains lie in the minor grooves of the HJ structure with remarkably clear electron density for this type of functional group. Previous reports[15,16] in which similar mono-intercalators have bound to duplex DNA show poor electron density around the essentially flexible side chain. In the current structure (Figure 5a), there are a number of interesting features that could account for the stability and immobility of these side chains. Perhaps the most striking interaction is the hydrogen bond that is observed between the hydrogen of the amide bond and the O2 of cytosine C7(A), directly above the chromophore. The amide hydrogen is fixed in position in the plane of the amide bond, effectively immobile. Upon formation of the hydrogen bond with O2, the cytosine base is pulled down and out of alignment with its guanine base pair (Figure 5a). This is most obvious when compared with the native structure where instead a water molecule interacts through a hydrogen bond with the same O2 on cytosine. The water is not fixed in position so the cytosine is not pulled out of alignment with the guanine (Figure 5b). Owing to the hydrogen-bond interaction

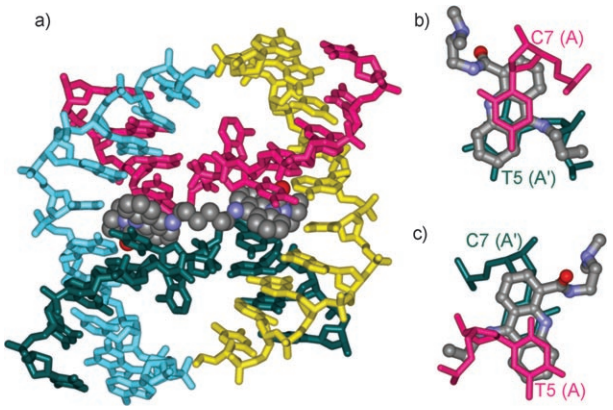

Figure 3. The HJ and its stacking interactions. a) A view of the overall structure showing the bound bis-acridine molecule at the junction crossover region, again from the major groove, and looking down the twofold axis. b) A view of the pyrimidine–acridine overlaps within the junction structure, viewed from the C7 side. Clear π–π stacking interactions can be seen with both the lower aromatic ring on the chromophore and the thymine (T5(A')) base below and the central aromatic ring and the cytosine (C7(A)) above the ring. c) The same pyrimidine–acridine stacking viewed from the thymine (T5(A')) direction. The symmetry-related chromophores are used for clarity.

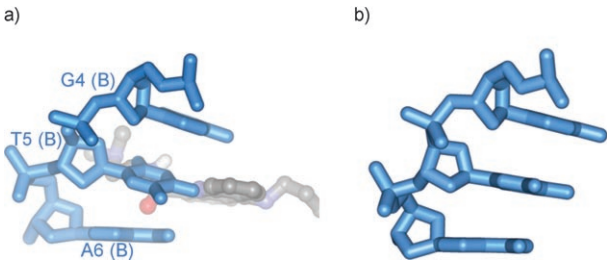

Figure 4. a) Steric clashing of the side-chain alkyl group with the thymine base T5(B). The thymine can be seen twisted out of alignment with the bases above and below owing to this effect. b) The same thymine base in the native structure (PDB code: 1NQS).

between the cytosine C7(A) and the hydrogen in the amide, the hydrogen bonds between this cytosine and its base-paired guanine G4(B) become longer. These can be compared to the equivalent base-pair lengths in the native structure (Figure 5d). As one side of the cytosine C7(A) is pulled away towards the amide, the opposite side is pushed away from the guanine G4(B), resulting in an increase in the length of the two bonds furthest from the O2 hydrogen-bond interaction with the ligand side chain (Figure 5c). In this conformation, the side chain is in a stable arrangement, preventing any rotation about the carbon–chromophore bond.

Design of further HJ-binding molecules will be based upon an analysis of such close contacts in this high-resolution structure to generate HJ-specific binders while also moving away from compounds known to bind to duplex DNA. Small peptides that interact with the HJ have previously been described, although their interactions have not been structurally characterized.[19] The HJ has also been shown to covalently bind the light-activated compound psoralen,

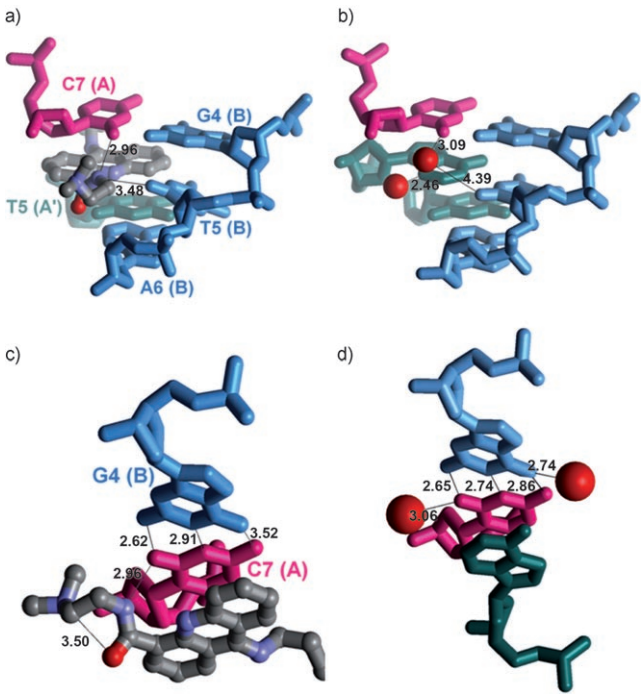

Figure 5. a) Hydrogen-bonding interactions between the bis-acridine side chain and the surrounding bases. The amide can be seen forming a hydrogen bond with the oxygen of (C7(A)). This causes the cytosine base to be pulled out of alignment with G4(B). b) Similar interactions are also seen in the native structure. A water molecule appears to form the same hydrogen bonding interaction as described above, however, its position is not so close and therefore the interaction is weaker. c) Hydrogen bonding between the cytosine C7(A) and the guanine base pair G4(B) above the bis-acridine chromophore. The bonds furthest from the side-chain interaction are longer due to the pull of the hydrogen bond between the oxygen O2 of the cytosine and the amide hydrogen. d) The same bonds in the native structure, with the exception that the bonds are more equal across the bonding pair.

which cross-links the thymine bases across the complementary strands.[20] That work provides a unique example of the stabilization of a HJ structure by covalent drug binding in a DNA molecule that does not otherwise form a junction, and is consistent with the known effect of psoralen in promoting recombination. However, the same mode of psoralen binding is also well established for duplex DNA[21] and is associated with helix destabilization and unwinding. The binding mode described herein, in contrast, is HJ specific and does not cause unwinding. In conclusion, we have described a new mode of binding to the stacked-X, four-way Holliday junction in which a $\mathbf{C}_6$ molecule binds across the center of the junction and two adenines are replaced by the acridine chromophores either side of the crossover. The biological consequences of this binding and the design of HJ-selective molecules are currently under investigation.

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