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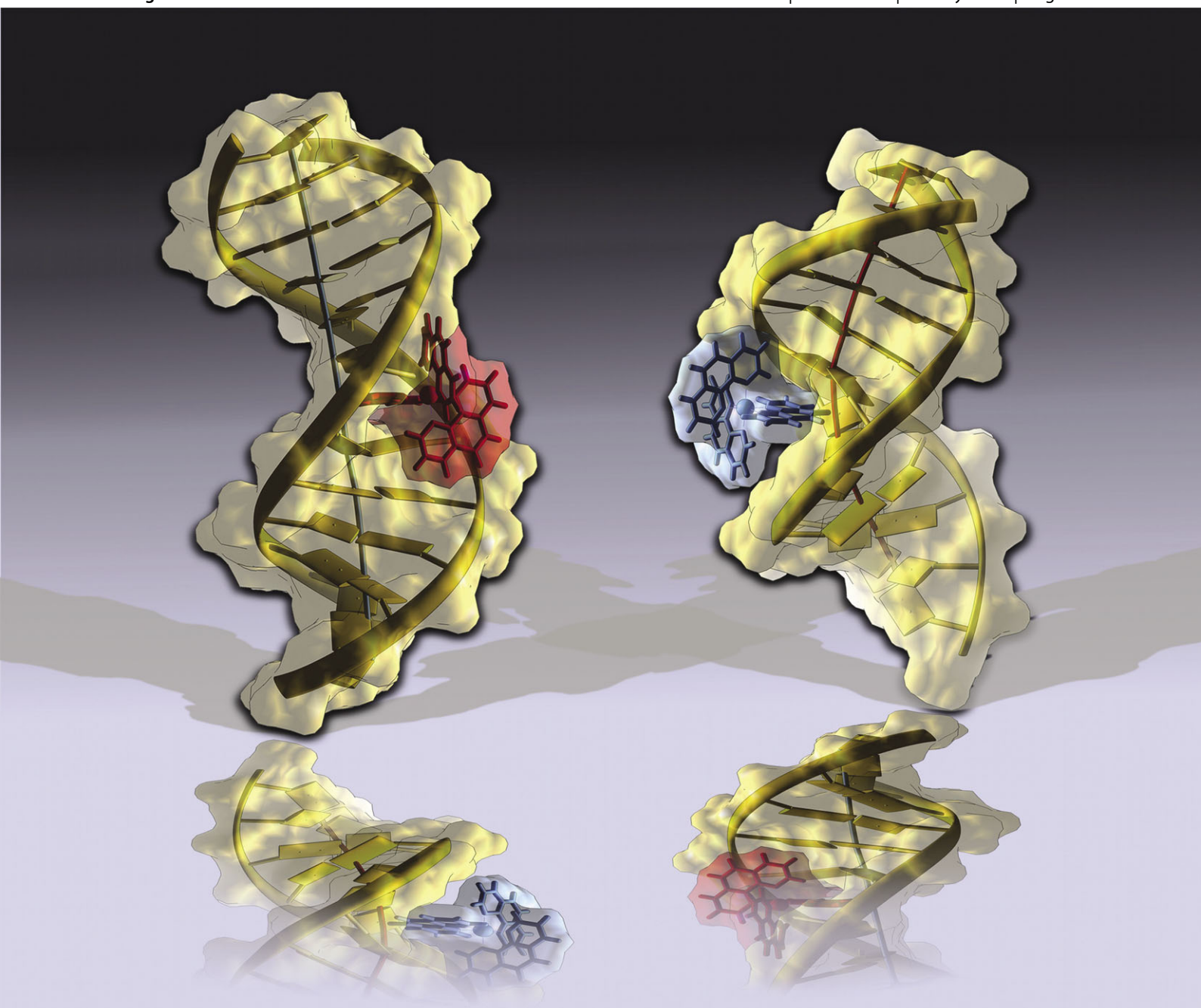

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Enantiospecific kinking of DNA by a partially intercalating metal complex†

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Opposite enantiomers of [Ru(phenanthroline)$_3$]$^{2+}$ affect the persistence length of DNA differently, a long speculated effect of helix kinking. Our molecular dynamics simulations confirm a substantial change of duplex secondary structure produced by wedge-intercalation of one but not the other enantiomer. This effect is exploited by several classes of DNA operative proteins.

The DNA binding of chelate compounds of polycyclic heteroaromatics with transition metals has received great attention over the years.[1–7] The remarkable luminescence properties of such systems allow direct and sensitive monitoring of their interaction dynamics with DNA.[3,5] The hydrophobic nature of the auxiliary ligands of these compounds often leads to binding by intercalation of the polycyclic moieties between base pairs of the DNA double helix. Another parameter is the handedness of the intrinsically chiral compounds. The three-bladed propeller compound [Ru(phenanthroline)$_3$]$^{2+}$ thus exists as two enantiomers, Δ and Λ with, respectively, right-handed and left-handed configurations, which can make diastereomeric combinations with the chiral DNA molecule, providing a basis for binding specificity. If one of the three phenanthrolines is replaced by the larger dipyridophenazine ligand, dppz, the compound is found to bind to DNA by intercalating this elongated aromatic moiety into the double helix. As a result of protection of the dppz aza lone-pairs from water by the stacked surrounding nucleobases, [Ru(phenanthroline)$_2$dppz]$^{2+}$ is no longer quenched as it is in bulk water solution but shows a brilliant luminescence which can thus be used to follow intercalation and dissociation kinetics. However, despite extensive studies of the parental [Ru(phen)$_3$]$^{2+}$ compound and its derivative [Ru(phen)$_2$dppz]$^{2+}$ with respect to their interaction with DNA a considerable degree of elusiveness has remained as to how these compounds really interact with DNA at an atomic level. A recent crystallographic study by Hall *et al.*[8] on the DNA complex with Λ-[Ru(tetraazaphenanthrene)$_2$dppz]$^{2+}$, a compound very similar to [Ru(phenanthroline)$_2$dppz]$^{2+}$, has revealed two intercalative binding modes, also resolving a long-lasting discussion of binding location of inert coordination complexes in duplex DNA. The two binding sites corresponding to a typical intercalation of the elongated dppz ring system and a "semi-intercalation" of the shorter tetraazaphenanthrene ligand are both located in the minor groove, the latter giving rise to a DNA helix kink of 51°. This observation is interesting in view of early experimental evidence for kinks in long DNA upon binding of [Ru(phenanthroline)$_3$]$^{2+}$.

In this communication we can explain the origin of this difference and show why it is biologically relevant. Thus, while Δ-[Ru(phenanthroline)$_3$]$^{2+}$ gives rise to a substantial kink evidenced from a reduced flow orientation factor,[5] decreased relative viscosity of DNA[4] as well as electrophoretic mobility,[6] the Λ-enantiomer, by contrast, shows no significant perturbation in any of these respects. Interestingly, analogous experiments performed with [Ru(phenanthroline)$_2$dppz]$^{2+}$ only show minor differences between opposite enantiomers, in agreement with the early conclusion that the elongated dppz moiety behaves as a typical intercalator and does not bend DNA,[9] also confirmed by Hall *et al.*[8]

We shall here address the remarkable difference between the enantiomers of [Ru(phenanthroline)$_3$]$^{2+}$ utilizing advanced MD simulations and demonstrate how the Δ-form of the complex indeed induces a substantial kink of the DNA helix, of 53°, while the Λ-form behaves more like a typical intercalator producing an inconspicuous bending of only 16°. The resulting structures (Fig. 1) indicate that the kink produced by the Δ-enantiomer is partially due to a wedge effect, separating adjacent base pairs by the edge, but also involves asymmetric hydrophobic stacking with one of the furanose sugars along the DNA minor groove. This asymmetry indirectly enhances the effect of bulkiness of the intercalating phen ligand resulting in widening of the minor groove of the DNA helix. The binding spot only remotely resembles an intercalation pocket, since only insignificant helical rise (5.2 Å) and unwinding (9°) are seen.

By way of contrast, the Λ-enantiomer, by hydrophobic stacking of the two non-intercalated phenanthroline wings with DNA sugars above and below the intercalation spot, gains a symmetric orientation and is inserted deeper into the DNA stack. Despite that the phenanthroline ring system is not big enough to insert itself deep into the DNA base stack in a fashion characteristic for a typical intercalator, the intercalation pocket in the case of Λ is still well-defined with 7.2 Å rise and 22° unwinding of DNA. Importantly, this demonstrates that partial intercalation, as observed with the Λ-compound, is not enough for producing a helix kink by a wedge effect at the base-stack level, as was proposed for "semi-intercalation",[5] but also requires steric interactions of the two non-intercalating ligands. This conclusion is supported by the

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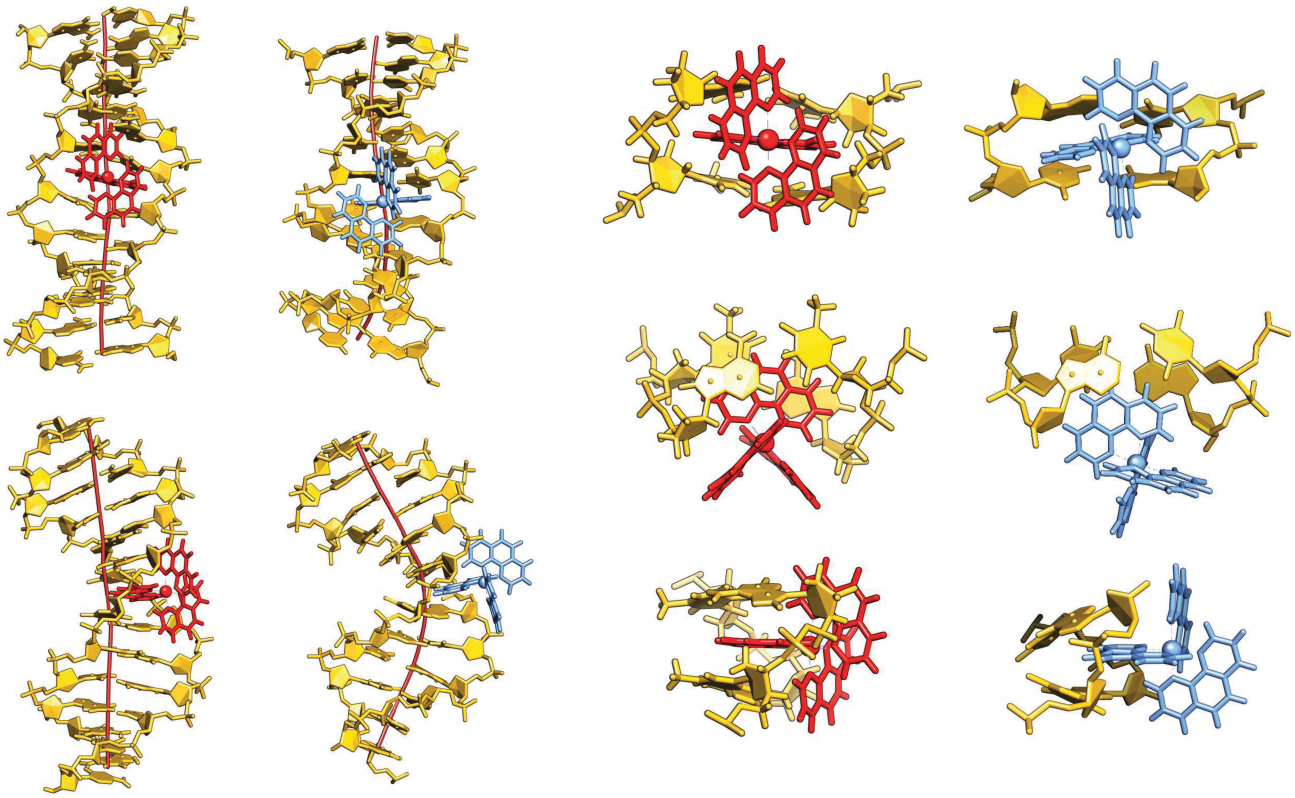

Fig. 1 Binding geometries of the Δ- and Λ-enantiomers of $[Ru(phenanthroline)_3]^{2+}$ with 12mer DNA. Left: the Δ-enantiomer (blue) induces a 53° kink indicated by a helical axis (brown); the Λ-enantiomer (red), in contrast, bends DNA only inconspicuously by 16°. Right: front, top and side zoom-in of DNA intercalation spot illustrating symmetric, resembling typical intercalation, binding of Λ and asymmetric, wedge like, insertion of Δ.

fact that the Δ enantiomer of $[Ru(phenanthroline)_2dppz]^{2+}$ also shows a certain reduction in the orientation factor[5] when the DNA-$[Ru(phen)_2dppz]^{2+}$ system is subjected to shear flow, despite the complete absence of the wedge effect as dppz is fully intercalated. By comparison, with a small-ring complex, $[Ru(bipyridine)_3]^{2+}$, a noticeable drop in the orientation factor is seen for both enantiomers although somewhat bigger for Δ, yet indicating the combinatory nature of DNA kinking as a result of both wedging and hydrophobic plus steric interactions.

Despite the substantial structural differences between the DNA complexes with the enantiomeric forms of $[Ru(phenanthroline)_3]^{2+}$, the experimental binding free energies are almost identical[4] although with small variations depending on the sequence and ionic strength.[3] Association free energies have been evaluated here by the MMPBSA approach[10] from the MD trajectories, into terms that implicitly describe electrostatic interactions ($\Delta G_{PB} + \Delta E_{EL}$), hydrophobic, solvation and dispersive interactions ($\Delta G_{SA} + \Delta E_{VDW}$) and entropic contribution ($T\Delta S$). Taken for what they may be worth, some variations between the calculated values for the enantiomers can be noted (Table 1): Λ is obviously predicted to have a significantly stronger binding energy than Δ, in conflict with experiment. This contrast may indicate that the entropy variation has been underestimated, in agreement with the general finding of extensive entropy–enthalpy compensation in systems involving hydrophobic interaction.[11,12] The smaller predicted electrostatic and hydrophobic binding contributions for Δ correlate with the asymmetric orientation of this enantiomer in its binding pocket with only one of the phenanthroline blades gaining electrostatic interaction with the DNA backbone and the wedging phenanthroline only partially inserted into a hydrophobic environment of the DNA stack. Correspondingly, for the Λ-enantiomer greater values of electrostatic and hydrophobic terms illustrate a substantial stabilizing effect due to both electrostatic and hydrophobic stacking interactions of the two symmetrically placed auxiliary phenanthroline ligands towards sugars at the walls of the minor groove.

It is interesting to note that while more and more elaborate derivatives of $[Ru(phenanthroline)_3]^{2+}$ have emerged in attempts to increase DNA binding specificity, the more strongly binding derivatives such as $[Ru(phenanthroline)_2dppz]^{2+}$ or its dimeric variants generally exhibit poorer enantio- as well

Table 1 Experimental[4] and computed association free energies (kcal mol^{-1}), the latter decoupled into mechanic (ΔE_{IN}), electrostatic ($\Delta G_{PB} + \Delta E_{EL}$), and hydrophobic, solvation and dispersive interactions ($\Delta G_{SA} + \Delta E_{VDW}$), and entropic contribution ($T\Delta S$), for Δ- and Λ-$[Ru(phen)_3]^{2+}$ with 12mer DNA

	ΔE_{IN}	ΔE_{VDW}	ΔE_{EL}	ΔG_{SA}	ΔG_{PB}	$\Delta G_{SA} + \Delta E_{VDW}$	$\Delta G_{PB} + \Delta E_{EL}$	$T\Delta S$	ΔG_{COMP}	ΔG_{EXP}
Δ-$[Ru(phen)_3]^{2+}$	0.5	−33.6	−64.8	−2.7	79.8	−36.3	15.0	−13.4	**−7.9**	**−5.4**
Λ-$[Ru(phen)_3]^{2+}$	0.45	−40.0	−76.0	−3.35	95.0	−43.35	19.0	−13.4	**−10.95**	**−5.5**

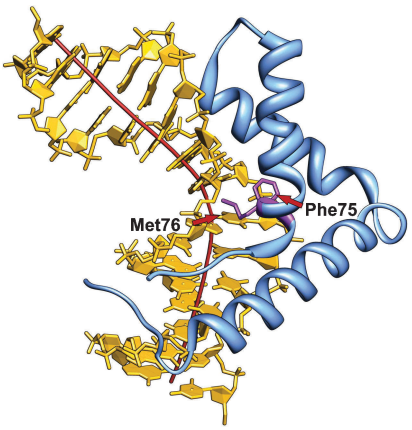

Fig. 2 Binding of thermophile chromosomal protein, Sac7d (PDB ID: 3F27), induces DNA kink of 70°, which is secured by wedging of Met76 and steric hindrance produced by Phe75, residing on the groove.

as sequence-selectivity.[5,13–16] We propose this is a result of a redistribution of relative binding energy: the less selective electrostatic and hydrophobic/dispersive attractive energies dominating over the more selective steric forces. For example, this explains why the strongest enantioselectivity is found for the parent compound $[Ru(phenanthroline)_3]^{2+}$ in the presence of high salt depressing the non-specific electrostatic attraction.[2,3] Even a bis-intercalating Ru-dppz-based compound shows identical binding affinity for Δ–Δ and Λ–Λ except for at very high ionic strength where a slight bias for Λ–Λ is seen.[16]

Understanding the enantioselectivity is not only extremely interesting in the context of chiral recognition and of potential significance in evolutionary selection, but also highly relevant in a direct biological context of nucleic acid–protein interaction. Analogous kinks to the one produced by Δ-$[Ru(phen)_3]^{2+}$ have been observed for several classes of operatory proteins, including the eukaryotic transcription factor TBP (TATA-box binding protein),[17,18] the high mobility group proteins (HMGP),[19,20] as well as for thermophile chromosomal proteins.[21] Remarkably, all these proteins in order to bend DNA use wedging by partial intercalation of a hydrophobic residue, typically methionine or leucine but, in addition, utilize simultaneously stacking interactions of aromatic residues with DNA sugars along the minor groove (Fig. 2). In conclusion, our concept of combined wedging at the base-stack level with steric minor-groove widening as a cause of DNA helix kinking seems to have general impact.

We trust the structural details from our simulations, which involved extensive conformational space sampling using both extended standard and biased conformational sampling simulation techniques, including steered MD and Replica Exchange MD in an explicit solvent environment, at various temperatures, starting from fully separated states. All MD simulations were performed with AMBER11 software package.[22] The DNA molecule was parameterized with AMBER-ff10, the Ru(II)-ion coordination sphere with an earlier described procedure,[23,24] using Gaussian09 software package[25] and AMBER GAFF force field[26] for the parameterization of the remaining atoms of Δ- and Λ-$[Ru(phen)_3]^{2+}$. Using standard protocols, the complex of DNA with either Δ or Λ was solvated and neutralized by sodium ions, energy minimized and equilibrated with decreasing position restraints. MD trajectories were recorded at constant pressure (1 bar) and various temperatures (300–309.5 K). The association free energies were estimated by the MMPBSA[10] approach, testing various sequences of DNA, using a "single-trajectory" setup. Both major and minor groove locations were tested, but only association from the minor groove resulted in stable complexes. The conformational analysis of DNA structures has been performed with Curves+.[27] See ESI† for computational details and simulation protocols.

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