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Conformational Modulation of DNA by Polyamide Binding: Structural Effects of f-Im-Py-Im Based Derivatives on 5'-ACGCGT-3'

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Abstract

The DNA sequence 5'-ACGCGT-3' is in the core site of the Mlu1 cell-cycle box, a transcriptional element in the promoter region of human Dbf4 gene which is highly correlated with a large number of aggressive solid cancers. The polyamide formamido-Imidazole-Pyrrole-Imidazole-Amine⁺ (f-Im-Py-Im-Am⁺) can target the minor groove of 5'-ACGCGT-3' as an antiparallel stacked dimer and has shown good activity in inhibiting transcription factor (TF) binding. Recently, f-Im-Py-Im-Am⁺ derivatives that involve different orthogonally positioned substituents were synthesized to target the same binding site, and some of them have displayed improved binding and pharmacological properties. In this study, the gel electrophoresis-ligation ladders assay was used to evaluate the conformational effects of f-Im-Py-Im-Am⁺ and derivatives on the target DNA, an essential factor for establishing the molecular basis of polyamide-DNA complexes and their TF inhibition. The results show that the ACGCGT site in DNA has a relatively wide minor groove and a B-form like overall structure. After binding with f-Im-Py-Im-Am⁺ derivatives, the DNA conformation is changed as indicated by the different mobilities in the gel. These conformational effects on DNA will at least help to point to the mechanism for the observed Mlu 1 inhibition activity of these polyamides. Therefore, modulating DNA transcription by locking the DNA shape or altering the minor groove geometry to affect the binding affinity of certain TFs is an attractive possible therapeutic mechanism for polyamides. Some of the substituents are charged with electrostatic interactions with DNA phosphate groups and their charge effects on DNA gel mobility have been observed.

Keywords

polyamides; functional side chain; charge effects; conformational changes; DNA rigidity; transcription modulation; Mlu 1

Introduction

From naturally occurring polyamides (PAs), such as distamycin and netropsin, to synthetic pyrrole (Py) and imidazole (Im) containing PAs, remarkable effort has been put into developing PAs for new applications as DNA complexes (Bando and Sugiyama 2006; Chavda *et al.*, 2011; Dervan 2001; Lacy *et al.*, 2003; Satz and Bruice 2002). Starting with the original studies on the distamycin stacked dimer complex (Pelton and Wemmer 1988; Pelton and Wemmer 1989; Pelton and Wemmer 1990), minor groove binding PAs have

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been designed to recognize a variety of DNA sequences with high affinity (Bando *et al.*, 2002; Buchmueller *et al.*, 2005a; Wemmer 2000). PAs have potential use for development as therapeutics, for example, by inhibiting binding of transcription factors to their DNA recognition sequences (Chenoweth and Dervan 2009; Oyoshi *et al.*, 2003; Raskatov *et al.*, 2012; Wang *et al.*, 2010). This inhibition effect of PAs is highly related to binding affinity and also to the ability to alter the local geometry of DNA upon binding (Chenoweth and Dervan 2010; Wang *et al.*, 2011). Therefore, besides improvement of binding affinity, sequence selectivity, solubility and cellular uptake, the conformational effect of PAs on DNA is an important aspect for the design of therapeutic candidates. A better understanding of PA effects on DNA structure is also essential for establishing the molecular recognition basis of PA-DNA complexes.

In our efforts to develop PAs as potential drugs we have focused on three-ring monomer derivatives that can bind tightly to specific DNA sequences. These PAs also have good pharmacological potential. We have placed specific focus on the formamido-Imidazole-Pyrrole-Imidazole-Amine⁺ (f-Im-Py-Im-Am⁺) derivative which has unusually tight binding to the DNA sequence 5'-ACGCGT-3' (Buchmueller *et al.*, 2005a; Buchmueller *et al.*, 2005b; Buchmueller *et al.*, 2006; Lacy *et al.*, 2002). This sequence is of interest since it is found in the core sequence of the Mlu1 cell-cycle box (MCB), a transcriptional element in the promoter region of the human Dbf4 gene. The level of Dbf4 is critical for the activation of Cdc 7 kinase and has been implicated with a large number of aggressive solid cancers (Hess *et al.*, 1998; Verma *et al.*, 1991; Wu and Lee 2002).

To improve the DNA binding and pharmacological properties of f-Im-Py-Im-Am⁺ based derivatives, a variety of modified analogs have been synthesized to target the same ACGCGT binding site (Satam *et al.*, 2012a; Satam *et al.*, 2012b) (Figure 1). Some of the modifications have led to both improved DNA binding and solution properties of f-Im-Py-Im-Am⁺. The structural influences of PAs on DNA, however, are unclear. To evaluate the conformational effects of the f-Im-Py-Im-Am⁺ derivatives, we have used gel electrophoresis with ligation ladders. We wish to determine: i) how the binding of f-Im-Py-Im-Am⁺ stacked dimers affects the DNA structure at the CGCG site, and ii) how the additional functional groups influence the local geometry of minor groove. The results provide a novel mechanism for allosteric modulation of DNA structure, which is complementary to other demonstrated mechanisms for changing DNA groove geometry (Chenoweth and Dervan 2009).

Materials and Methods

DNA-Binding PAs

All the PAs in this study were synthesized as described previously (Ramos *et al.*, 2013; Satam *et al.*, 2012a; Satam *et al.*, 2012b), and their structures are shown in Figure 1.

DNA Oligonucleotides and Ligation Ladders

A 10 bp self-complementary duplex with a CGCG site flanking with AT base pairs (Figure 1) was purchased from Integrated DNA Technologies, Inc. (IDT, Coralville, IA) with HPLC purification. Double distilled water was added to the lyophilized DNA oligomers to bring the concentration to approximately 1.0 mM, based on the reported amount of DNA from IDT. The actual concentration of the single strand DNA was then determined using a Cary 300 UV-visible spectrophotometer (Agilent, Santa Clara, CA) at A₂₆₀ with extinction coefficient calculated by the nearest-neighbor method (Fasman 1975). The self-complementary single strands were annealed in ligation reaction buffer (New England Biolabs, Ipswich, MA) containing 50 mM Tris-HCl, 10 mM MgCl₂, 10 mM dithiothreitol

and 1 mM ATP. A 20 base-pair ladder (20 bp, Bayou Biolabs, Metairie, LA), which is a commercially available standard sequence with a double intensity band at 100 bp, was used for gel mobility reference.

The ligation procedure is as described previously (Tevis *et al.*, 2009). In brief, annealed duplexes were 5' phosphorylated using T4 polynucleotide kinase (New England Biolabs) and then ligated with T4 DNA ligase (New England Biolabs) in the buffer provided. Kinasing reactions were incubated for 30 min at 37 °C followed by enzyme deactivation for 20 min at 65 °C. Ligation reactions in 200 μ L contained 2 μ M DNA with 2400 U of ligase and were done at room temperature overnight. The ligated DNA solutions were stored at 4 °C.

Gel Electrophoresis and Calculation of R_L

Ligation ladders were separated by 8% native polyacrylamide gels (1.5 mm thick, 20 cm long) prepared from a 40% acrylamide solution (29:1, acrylamide: bisacrylamide, Acros Organic, NJ). Electrophoresis was carried out at 200 V (10 V/cm) for 170 min at 25 °C in a BioRad Protean II xi gel apparatus with TBE buffer (0.089 M Tris, 0.089 M boric acid, 2.0 mM EDTA, pH 8.3) and a BioRad PowerPac Basic 300. Each sample had a final volume of 20 μ L containing 12 μ L of 2 μ M DNA, 4 μ L of 6 \times load dye (Promega, Madison, WI), and the amount of compounds needed for the desired ratios of compound to binding site. After electrophoresis, gels were stained with SYBR Gold Nucleic Acid Gel Stain (Invitrogen, Carlsbad, CA). The stained gels were imaged using an UltraLum Omega 10 gD Molecular Imaging System (UltraLum, Claremont, CA).

Molecular weight assignments were obtained by adding 10 bp to each sequential gel band starting from the bottom most bands and using the 20 bp ligation ladders as the reference (Figure 2A). The value was in bp and termed as the actual length (L_{actual}) for each gel band. Using Image Quant TL (GE Healthcare, Piscataway, NJ), the apparent length (L_{apparent}) of each band was determined based on the distance it migrated in the gel. Relative mobility R_L , where $R_L = L_{\text{apparent}}/L_{\text{actual}}$ (Ross *et al.*, 1999; Tevis *et al.*, 2009), was calculated for each ligation ladder and plotted as a function of L_{actual} for each sample. The R_L value for each unbound CGCG duplex was normalized to 1.00 in order to compare the mobility changes induced by ligands binding more easily. The relative mobilities were reproducible within ± 0.01 experimental error.

Molecular Docking

Molecular docking of PA into the CGCG site was performed by employing the SYBYL-X 1.1 software package (Tripos Inc. St Louis 2010) on a Fedora Core 5 Linux work station. PDB 1BOS, which has an f-Im-Im-Im-Am⁺ dimer in complex with 5'-GAACGGTTC-3' (Yang *et al.*, 1999), was used as a template. The target DNA duplex 5'-GAACGCGTTC-3' was constructed by editing the 1BOS sequence with the Biopolymer Modules in SYBYL. The target sequence was then minimized by a maximum of 100 iterations using the Conjugate Gradient algorithm and Tripos force field with a termination conjugate gradient of 0.1 kcal/ mol \AA (Collar *et al.*, 2010). f-Im-Py(C₃NH₃)⁺-Im-Am⁺ (Figure 1) dimer was assigned Gasteiger-Hückel charges and then minimized for 1000 maximum iterations using the Tripos force field until a terminating conjugate gradient of 0.01 kcal/ mol \AA was reached. The f-Im-Im-Im-Am⁺ dimer in 1BOS was employed as a reference for aligning f-Im-Py(C₃NH₃)⁺-Im-Am⁺ dimer to the DNA sequence and then was removed. f-Im-Py(C₃NH₃)⁺-Im-Am⁺ dimer was moved to a separate molecular area in order to move independently of the DNA in the FlexiDock genetic algorithm (Liu *et al.*, 2012). The FlexiDock module of the SYBYL software package was employed implementing five different random numbers. The docking consisted of 87000 generations, which was

calculated by (the number of rotatable bonds + 6) \times 500, to ensure that it received the lowest energy conformations. Both DNA and f-Im-Py(C₃NH₃)⁺-Im-Am⁺ dimer were permitted torsional, rotational and translational flexibility in the docking process.

Results

Effects on DNA gel mobility of f-Im-Py-Im-Am⁺ and analogs with orthogonally positioned charged alkyl amine side chain

Ligation ladders of the target sequence CGCG, with f-Im-Py-Im-Am⁺ and analogs that contain an additional positively charged alkyl amine side chain that either attaches on the pyrrole moiety or on the formamido-terminus imidazole, f-Im-Py(C₃NH₃)⁺-Im-Am⁺ and f-Im(C₃NH₃)⁺-Py-Im-Am⁺, were compared using gel electrophoresis (Figure 2B). PAs were incubated with ligated sequences at five concentrations to achieve 2:1, 4:1, 6:1, 8:1 and 10:1 ligand to binding site ratios. Higher molar ratios than binding stoichiometries were used to overcome partial dissociation of the positively charged PA dimers during the electrophoresis experiment. As indicated by the colored arrows (Figure 2B), the 200 bp ligated multimers were used to visually compare the migration behaviors of the target sequence CGCG with and without ligands. This length of multimers was selected for two reasons: (1) they migrated far enough in the gel to be fully separated from other ligated multimers and (2) at this length the multimers can display structural features more prominently than shorter ligated oligomers. The gel results show that the free CGCG duplex migrated in the gel at approximately the same rate as the ligated 20 bp random sequence, indicating that this DNA site does not have significant bends. After binding with the monocation f-Im-Py-Im-Am⁺ dimer, CGCG migrated faster than the free DNA. The mobility changed with an increasing amount of compound until a molar ratio of 6:1. Bands were somewhat smeared at the 4:1 ratio suggesting some exchange between free and bound DNA in the gel experiment. The extent of migration change of CGCG bound with dications f-Im-Py(C₃NH₃)⁺-Im-Am⁺ and f-Im(C₃NH₃)⁺-Py-Im-Am⁺ dimers was less significant and it could only be detected at high molar ratios of ligand to DNA binding sites i.e. at ratios of 8:1 and 10:1.

To compare the DNA mobility changes induced by the binding of PAs, the relative mobility, R_L , was determined and is plotted as a function of L_{actual} (Figure 2C a–c). Since the R_L values of free DNA have been normalized to 1.00, a bound sequence with an R_L value below 1 indicates that it has a higher mobility than the free DNA, while a value greater than 1 means lower mobility. As shown in Figure 2C a, the dimer formation of monocation f-Im-Py-Im-Am⁺ in the minor groove increased the DNA mobility. Similar mobility changes have been detected for the two dication complexes but only in longer DNA multimers and are less significant than those induced by the monocation dimer (Figure 2C b–c). The dicationic PAs also had quite divergent behavior depending on the oligomer length: oligomers less than around 80–100 bp had mobilities less than free DNA while larger oligomers had higher mobility. 50 bp ladders were used as the starting bands for the mobility analysis and all the R_L values of each sample were subtracted by the difference between the R_L value of its 50 bp ligation ladder and 1.00 as shown in Figure 2C d–f. In this way the R_L values of each PA-DNA complex are adjusted to start at the same value for direct comparison. This aspect of the analysis is described in more detail in the Discussion Section.

Effects on DNA gel mobility of modification of the side chain structure and charge

To further test the effects of the addition of a functional side chain on DNA mobility, four more f-Im-Py-Im-Am⁺ analogs, f-Im-Py(C₃NHAc)-Im-Am⁺, f-Im-Py(C₃NHGly)⁺-Im-Am⁺, f-Im-Py(C₃Cl)-Im-Am⁺ and f-Im-Py(C₃N₃)-Im-Am⁺, were studied using PAGE (Figure 3A). All complexes had small mobility changes. Dimer formation of monocations f-Im-Py(C₃NHAc)-Im-Am⁺, f-Im-Py(C₃Cl)-Im-Am⁺ and f-Im-Py(C₃N₃)-Im-Am⁺ slightly

increased the DNA mobility but by smaller extents than parent PA f-Im-Py-Im-Am⁺. In contrast, a slight mobility retardation was detected in the samples containing f-Im-Py(C₃NHGly)⁺-Im-Am⁺. The amount of retardation decreased with increasing lengths of ligation ladders. R_L values were plotted as a function of the length of DNA segment with unbound sequences serving as the reference in Figure 3B a–d. To compare the mobility changes more effectively, the R_L values were corrected using the 50 bp ladders as the starting bands as described above (Figure 3B e–h).

Effects on DNA gel mobility of incorporation of an amino terminal benzamide

Two more f-Im-Py-Im-Am⁺ analogs with a benzamide in place of the terminal formamido group, phenyl-Im-Py-Im-Am⁺ and phenyl-Im-Py(C₃NH₃)⁺-Im-Am⁺ (Figure 1), were incubated with the target CGCG ligated duplex and evaluated by PAGE (Figure 4A). Phenyl-Im-Py-Im-Am⁺ slightly accelerated the DNA mobility but the dication analog phenyl-Im-Py(C₃NH₃)⁺-Im-Am⁺ significantly retarded the DNA migration. This decreased mobility became more remarkable with longer sequences, and its effects were more pronounced than the retardation induced by f-Im-Py(C₃NHGly)⁺-Im-Am⁺. Figure 4B shows the R_L values as a function of L_{actual} and obviously displays two different effects on DNA mobility, acceleration and retardation by the phenyl attached monocation and dication, respectively. The corrected R_L values using the same method above are shown as well.

Docking of f-Im-Py(C₃NH₃)⁺-Im-Am⁺ to cognate DNA

To provide a better understanding of the binding mode of PAs in this work, the f-Im-Py(C₃NH₃)⁺-Im-Am⁺ dimer was docked into the 5'-GAACGCGTTC-3' target sequence (Figure 5). The left view in Figure 5 is directly into the minor groove while the right view is rotated 90 degrees. Two f-Im-Py(C₃NH₃)⁺-Im-Am⁺ molecules form an antiparallel dimer in the minor groove within the central CGCG region and the Im-Py-Im heterocycles form H-bonds with the CGCG sequence at the floor of the groove. The positively charged alkyl amine (C₃NH₃)⁺ side chains extend out in opposite directions and interact with the phosphate groups of G₇ and G₁₇. This interaction helps explain the extra stabilization of the dimer complex that is provided by the alkyl amine substituents. It was also found that the distance between the two alkyl amine side chains and the neighboring phosphate groups of G₇ and G₁₇ are too large to form H-bonds. The f-Im-Py(C₃NH₃)⁺-Im-Am⁺ dimer also has intermolecular H-bonds between the charged tail amide NH and formamido O groups. Since the DNA sequence is symmetric, there are similar interactions between the two molecules and the bases on complementary strands.

Discussion

1. Factors that determine DNA gel mobility

a. DNA bending and minor groove geometry—The ligation ladder-PAGE assay has been validated as a sensitive and qualitative tool to analyze global DNA curvature and the local variation in DNA minor groove geometry through comparison of DNA mobilities (Crothers and Drak 1992; Diekmann *et al.*, 1992; Ross *et al.*, 1999). For example, DNA sequences with a narrow minor groove, such as phased A tracts (Digabriele *et al.*, 1989), have a locally bent structure that yields a curved helix axis and anomalously migration in gels (Koo and Crothers 1988; Lu and Stellwagen 2008; Maki *et al.*, 2004). Sequences possessing a wider, more B-form like, minor groove, like alternating AT sequences (Yoon *et al.*, 1988), are less curved and display higher gel mobility (Crothers and Drak 1992; Tevis *et al.*, 2009; Wang *et al.*, 2011). This ligation ladder-PAGE method has been extended to detect DNA conformational changes induced by the binding of small molecules in the minor groove (Hunt *et al.*, 2011; Wilson *et al.*, 2008). DNA mobility changes observed upon ligand binding demonstrate that compounds, such as netropsin (Tevis *et al.*, 2009) and distamycin

(Wang *et al.*, 2011), which target AT rich minor groove sites can constrict or widen the minor groove and thus cause bending or straightening of DNA structures.

In this study, a DNA sequence, which contains in phase CGCG sites, has been investigated with minor groove binding PAs by the ligation ladder PAGE method for the first time. Unlike the relatively slow migration for sequences containing A tracts, the target DNA CGCG displayed a mobility in the gel that is similar to the reference sequence, a random 20 bp ladder, lacking A/T or G/C binding sites for the PAs in Figure 1. This observation indicates that the target CGCG DNA has a relatively straight B-form structure with a wider minor groove binding site than with A tracts. This result is in good agreement with crystallographic analysis that the introduction of G/C base pairs widens the minor groove (Goodsell *et al.*, 1993; Heineman and Alings 1989) relative to that for AT rich sites.

b. Ligation ladder length and the ligand charge effects—Experimental and modeling results (Allison *et al.*, 2002; Diekmann *et al.*, 1992) illustrate that, in general, DNA ligation ladders that are shorter than 50 bp are too short to display gel mobility differences in 8% polyacrylamide gel (Tevis *et al.*, 2009; Wilson *et al.*, 2008). Therefore, the relative mobility R_L values for any 50 bp or shorter DNA sequence are similar to the migration standard, around 1.0 after normalization under the gel conditions used in the experiments reported here. With increasing length of DNA, the helix axis and global curvature become more pronounced, and their effects on mobility can be detected. Another factor that can affect the DNA mobility is the positive charges of the bound ligands. Cationic ligands result in less negatively charged complexes that can migrate slower than the free DNAs under the same conditions. The local influence of single or double positive charges from a ligand on DNA mobility has not had a significant effect during the gel electrophoresis in compounds evaluated to date, however, perhaps due to counterion release from DNA on binding of the cationic ligands (Chaires 1997; Record *et al.*, 1978).

The nine PAs in this study have the same basic Im-Py-Im heterocyclic structure but with various substituents. This set allows evaluation of any steric or the structural effects on DNA of compound modifications but also provides the possibility to detect differential charge effects on mobility. All of the PAs caused at least some changes of the DNA mobility, either acceleration or retardation, but both the magnitude and direction of the effects were dependent on the PAs substituents. The relative mobilities R_L values for the 50 bp complexes with monocation derivatives are quite similar to that for the unbound DNA due to the lack of PAGE sensitivity for curvature of this length of DNA, as discussed above. However, an unusual phenomenon was observed with the R_L values of the dimer complexes involving dication derivatives, f-Im-Py(C₃NH₃)⁺-Im-Am⁺, f-Im(C₃NH₃)⁺-Py-Im-Am⁺ and f-Im-Py(C₃NHGly)⁺-Im-Am⁺. The complexes formed with short ligation ladder migrated slower than the unbound DNA, while the ones with longer sequence moved faster relative to the unbound DNA. This inconsistent DNA length dependent mobility change can be explained by functional group charge effects. The PAs bind in the minor groove as stacked dimers and when each PA is a dication, this gives a tetracationic minor groove complex that could exceed the local ability of counterion release. The decreased DNA motilities resulting from charge effects are observed at short DNA segments, up to around 100 bp in length (Figures 2C b–c). As the target CGCG sequence gets longer, the structural influence by PA binding accumulates and becomes larger than charge effects.

Among the dications, f-Im-Py(C₃NHGly)⁺-Im-Am⁺ showed the most remarkable charge effects (Figures 2C b–c, 3B b). This may be due to its longer positively charged side chain relative to the alkyl amide of the other two derivatives (Figure S1). This longer and more extended side chain, C₃NHGly⁺, could interact with two phosphate groups on the DNA

backbone rather than one, as the shorter $C_3NH_3^+$ does (Figure 5) and this may account for its more significant charge effects on the DNA gel mobility.

2. A novel mechanism for allosteric modulation of DNA transcription

For the better comparison of the structural effect of all derivatives, 50 bp ladders were chosen as the starting multimers since they rarely display conformational effects. In this case charge effects can be eliminated by subtracting the difference between the values of 50 bp bound DNA and the normalized free CGCG from each of the R_L . In this way, the R_L values of the nine 200 bp complexes were corrected to 1.0 at 50 bp (Figure 6). The R_L values with charge effect corrections show us that the mobility of the target sequence CGCG has been enhanced to some degree by dimer formation of all the PAs tested in this study except phenyl-f-Im-Py($C_3NH_3^+$)-Im-Am⁺ (Figure 6). The increased migration indicates the DNA is less curved or the flexibility is reduced by the PA binding. When the rigidity is enhanced, the DNA migrates through the gel pores faster with less resistance than with similar but more flexible duplexes (Anderson 1986; Ohyama *et al.*, 1998; Stellwagen 2009). Some compounds targeting the narrow and curved minor groove of A-tract sites have shown the ability to increase the DNA mobility by straightening the DNA (Tevis *et al.*, 2009). For the target sequence CGCG in this work, the overall structure of free DNA is essentially straight, relative to the control sequence, and the minor groove is relatively wide as discussed above. It seems likely that increased rigidity of the duplex structure allows the DNA to move more easily through the gel. The wide minor groove is an ideal location for the dimer formation, and this excellent space fitting by the dimer complex increases the energetic cost for the DNA to be flexible and thus can rigidify the DNA structure. The reduced flexibility effectively straightens the average structure of the DNA helix axis.

Besides accelerating DNA mobility, the rigidifying effect might provide new insight on how PAs could act as allosteric modulators of DNA transcription. By locking the DNA structure with regard to the groove width and overall curvature, PAs can inhibit the binding of certain transcription factors that modify DNA structure on binding. This is a novel possible allosteric modulation mechanism that could be involved in inhibition of the binding of Mlu I to the MCB site (5'-ACGCGT-3') of the human Dbf4 gene by f-Im-Py-Im-Am⁺. This inhibition mechanism is based on an increased energy barrier of DNA conformational changes and it is complementary to changing DNA groove geometry by PAs as found, for example, by Chenoweth and Dervan (Chenoweth and Dervan 2009; Chenoweth and Dervan 2010). Bruice and coworkers (reviewed in Satz and Bruice 2002) synthesized and studied positively charged tripyrrole PAs with several different cationic groups on the central pyrrole. Their compounds are all similar to f-Im-Py($C_3NH_3^+$)-Im-Am⁺ but have only pyrroles. They found that their compounds bind very strongly to AT-rich DNA sequences. Some of their PAs can bend AT-rich DNAs and change the DNA conformation based on the NMR and AFM results.

3. Effects of the modified side chains on DNA structure

Figure 6 shows that PAs affect the target DNA in the following order: f-Im-Py-Im-Am⁺ > f-Im($C_3NH_3^+$)-Py-Im-Am⁺ > f-Im-Py(C_3NHAc)-Im-Am⁺ > f-Im-Py(C_3N_3)-Im-Am⁺ > f-Im-Py(C_3NHGly^+)-Im-Am⁺ > f-Im-Py($C_3NH_3^+$)-Im-Am⁺ > f-Im-Py(C_3Cl)-Im-Am⁺ > phenyl-Im-Py-Im-Am⁺ > phenyl-Im-Py($C_3NH_3^+$)-Im-Am⁺. Based on the classification of the various modifications on these compounds, the order above is: compound without side chain > compounds with side chain on Im ring > compounds with side chain on Py ring > compound with an additional phenyl ring > compound with both a phenyl ring and a side chain on Py. This observation suggests that the side chains on the Py ring, no matter what their charge states, have weaker effects on DNA structure relative to modification on the formamido-terminal Im ring. The Py rings of two ligands forming the antiparallel dimer are

closer in structure over the two formamido-terminal Im rings (Collar *et al.*, 2010) and thus the side chains attaching on Py have stronger steric and electrostatic repulsion if the side chain is positively charged. For the derivatives with an uncharged side chain, there is no electrostatic interaction between the neutral group and the DNA backbone, which results in more unfavorable steric clash between the side chains on the dimer complex. These repulsion forces or steric effects between side chains will reduce the stacking intensity between the Im and Py rings, and the flexibility of the PA-DNA complexes will be increased as a consequence.

Phenyl rings—The migration properties of phenyl-containing PAs on CGCG multimers were significantly influenced by the cationic side chain. The binding of monocation phenyl-Im-Py-Im-Am⁺ increased the DNA mobility in a smaller extent than all the non-phenyl analogs. The dication, phenyl-Im-Py(C₃NH₃)⁺-Im-Am⁺, possesses both a side chain on Py and a terminal benzamide, and it significantly decreased the DNA migration (Figure 4). This PA, in contrast to the monocation, causes DNA bending. It could be due to the fact that the minor groove with a wide CGCG site has been narrowed by the dimer formation of phenyl-Im-Py(C₃NH₃)⁺-Im-Am⁺. To obtain a better understanding of how the phenyl ring may play a role in structural influence, both the binding modes of f-Im-Py-Im-Am⁺ and those with terminal benzamide are illustrated in Figure 7. It is clear that there are two pairs of stacked rings, Im/Py and Py/Im, in the dimer involving ligands without the phenyl (Figure 7A). With the addition of terminal phenyl rings, the imidazole ring in the charge terminus in one ligand can be stacked together with the phenyl ring in the other ligand through the π - π interaction. This means that the introduction of phenyl rings brings two more pairs of stacked rings into the dimer unit (Figure 7B). Once the positively charged alkyl amide side chains are attached on the central Py ring, they will attract the DNA backbones to fold towards the minor groove for a special compensation through the electrostatic interaction. Therefore, the CGCG minor groove would become narrower and DNA structure would become curved with decreased gel mobilities.

Conclusions

In conclusion, the dimer formation of PA in minor groove has the ability to stiffen the overall DNA structure without grossly affecting DNA conformation. The PAs can also bend DNA through inducing a narrower minor groove with ligand modifications. These various conformational effects might allow these PAs to be allosteric modulators of DNA transcription by locking the DNA shape or altering the minor groove width to reduce or enhance the binding affinity of DNA transcription factors that bind in the major groove of the same sequence.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations

PAGE polyacrylamide gel electrophoresis

bp	base pair
f	formamido
Im	imidazole
Py	pyrrole
Am	amine

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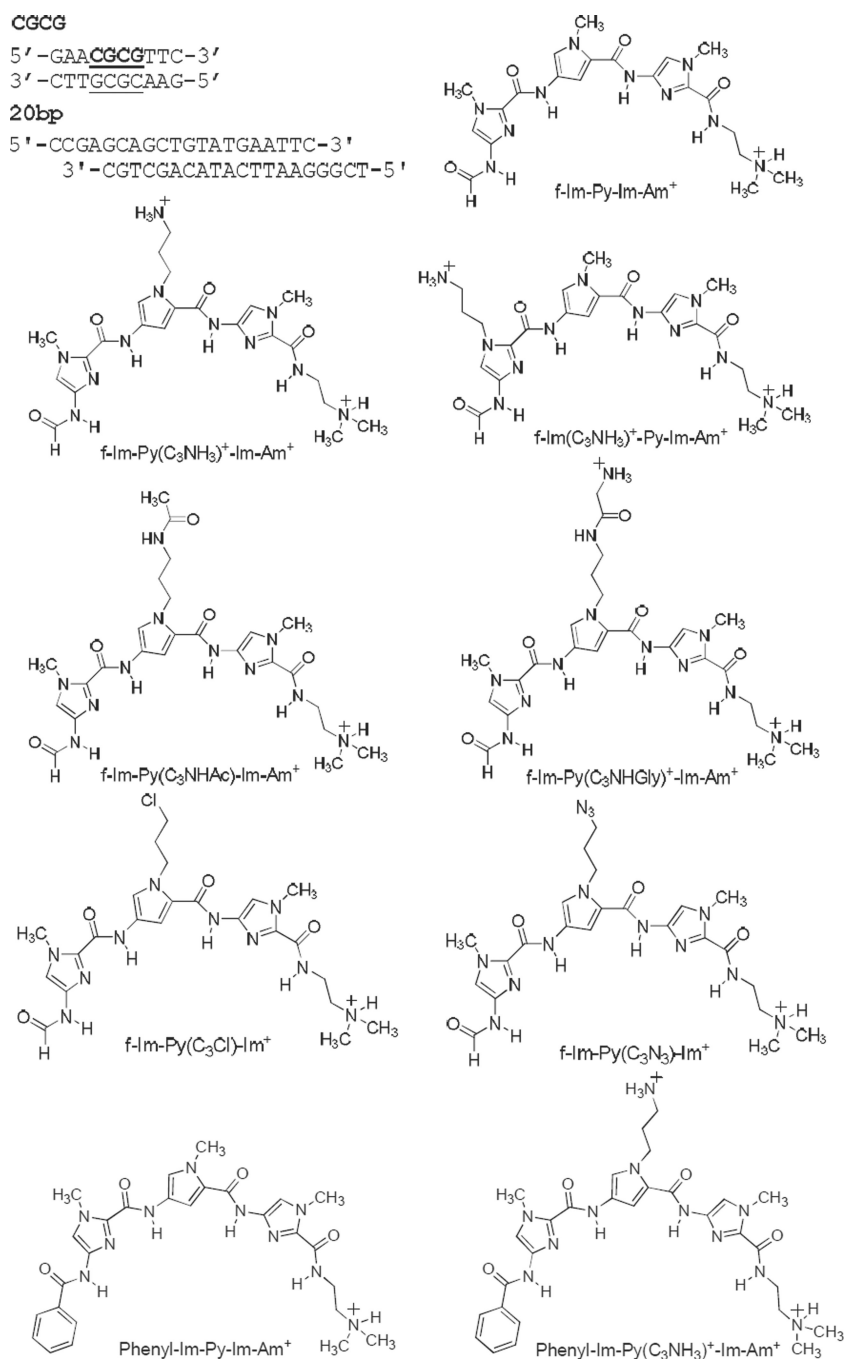
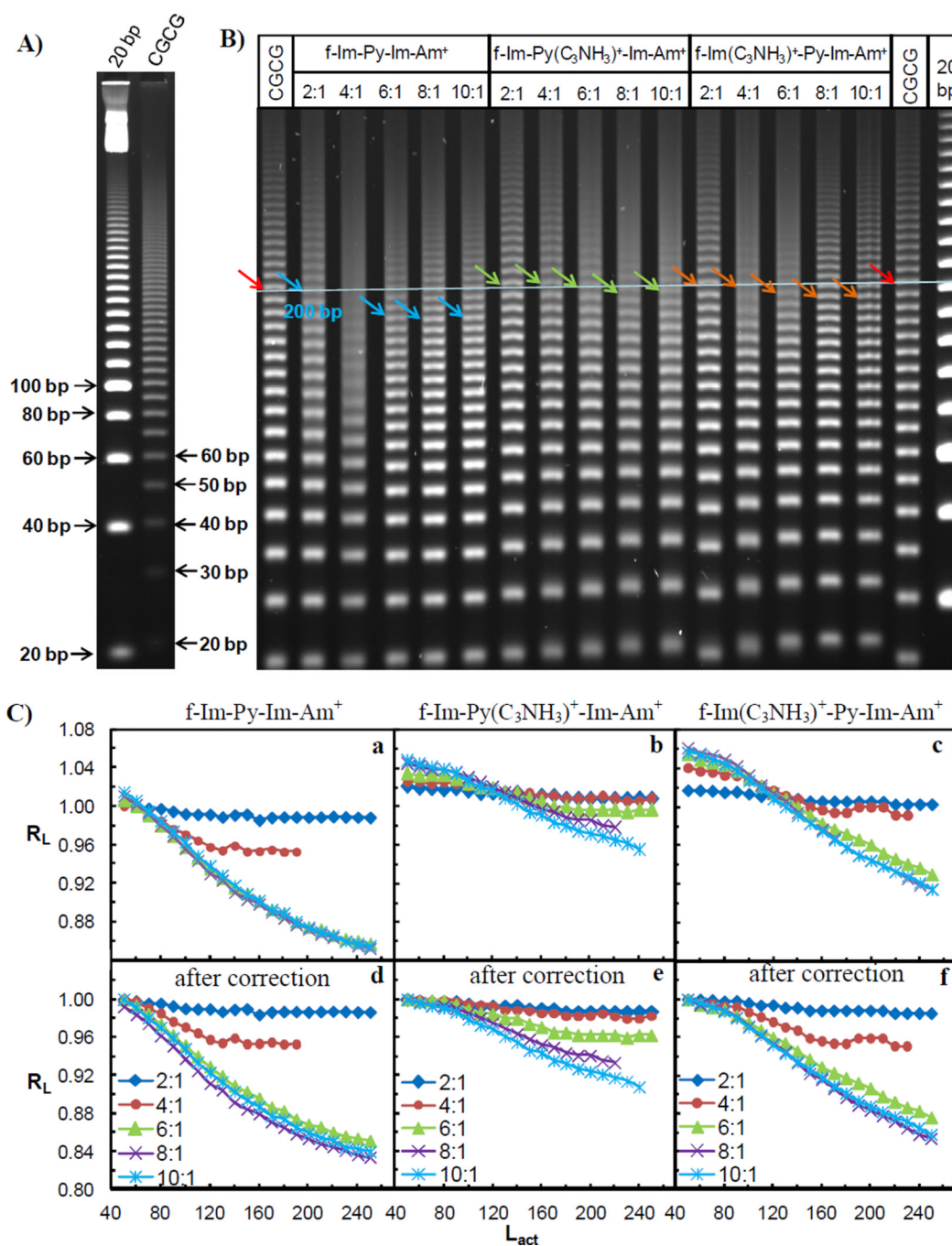
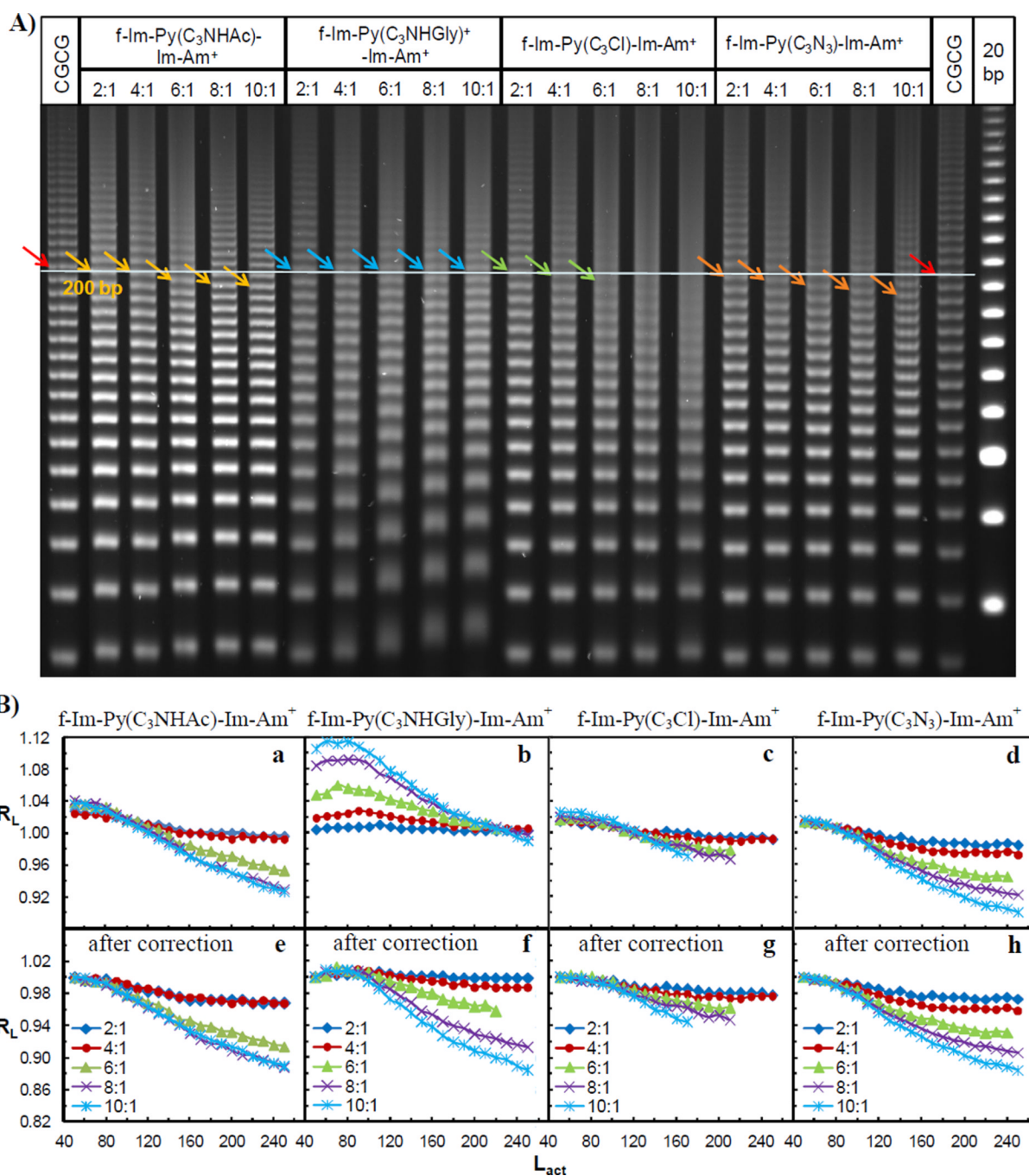


Figure 1.
 DNA sequence and compounds used in this study.

**Figure 2.**

A) 8% (29:1) native PAGE gel of unbound target DNA CGCG and the 20 bp reference sequence. The arrows indicate the molecular weights of the ligation ladders. **B)** 8% (29:1) native PAGE gel of monocation f-Im-Py-Im-Am⁺, dications f-Im-Py(C₃NH₃)⁺-Im-Am⁺ and f-Im(C₃NH₃)⁺-Py-Im-Am⁺ with CGCG. Ratios on top refer to compound per binding site of DNA. Free DNA and a 20 bp marker are shown as the migration standards. Colored arrows indicate 200 bp ligated linear multimers. **C)** Plot of the relative mobility, R_L , as a function of L_{actual} for the CGCG bound with three PAs before (a–c) and after (d–f) the correction of charge effect. Ratios are as in Figure 2B.

**Figure 3.**

A) 8% (29:1) native PAGE gel of f-Im-Py(C₃NHAc)-Im-Am⁺, f-Im-Py(C₃NHGly)⁺-Im-Am⁺, f-Im-Py(C₃Cl)-Im-Am⁺ and f-Im-Py(C₃N₃)-Im-Am⁺ with target DNA CGCG. Ratios on top are as in Figure 2. Colored arrows indicate 200 bp ligated linear multimers. **B)** Plot of the relative mobility, R_L , as a function of L_{actual} for the CGCG bound with four PAs before (a–d) and after (e–h) the correction of charge effect. Ratios are as in Figure 2.

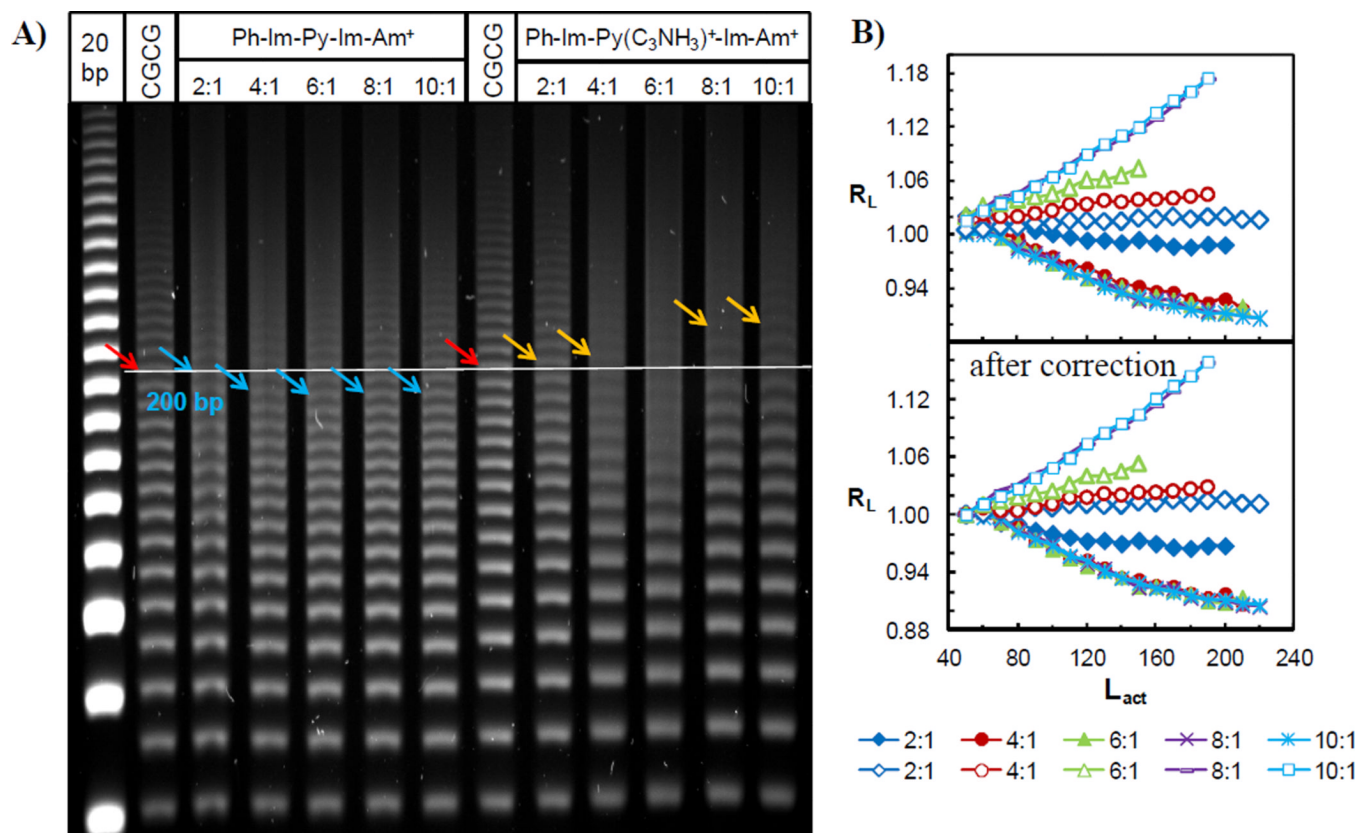


Figure 4.

A) 8% (29:1) native PAGE gel of phenyl-Im-Py-Im-Am⁺ and phenyl-Im-Py(C₃NH₃)⁺-Im-Am⁺ with target DNA CGCG. Ratios on top are as in Figure 2. Colored arrows represent the 200 bp sequences. **B)** Plot of R_L as a function of L_{actual} for phenyl-Im-Py-Im-Am⁺ (filled symbols) and phenyl-Im-Py(C₃NH₃)⁺-Im-Am⁺ (empty symbols) before and after the correction of charge effect.

5'- G₁ A₂ A₃ C₄ G₅ C₆ G₇ T₈ T₉ C₁₀ -3'
 3'- C₂₀ T₁₉ T₁₈ G₁₇ C₁₆ G₁₅ C₁₄ A₁₃ A₁₂ G₁₁ -5'

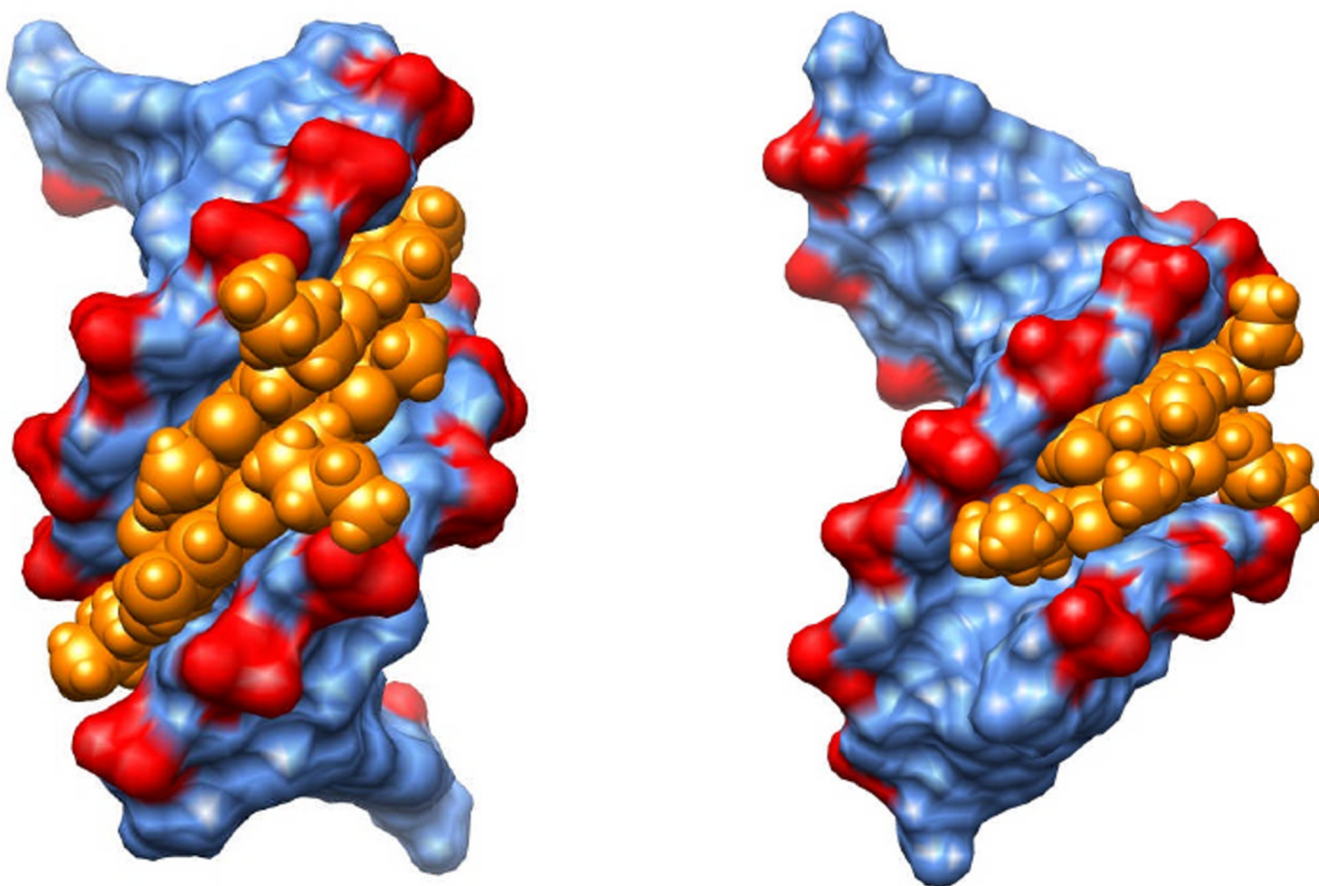


Figure 5. Docking of f-Im-Py(C₃NH₃)⁺-Im-Am⁺ to its cognate DNA sequence 5 - GAACGCGTTC-3 . Front (left) and side (right) views of the docked model in the minor groove of CGCG site were built with Chimera. The PA is shown in golden and DNA sequence is colored as blue with the highlighted phosphate groups in red. The full sequence with bases numbered is shown at the top of the figure.

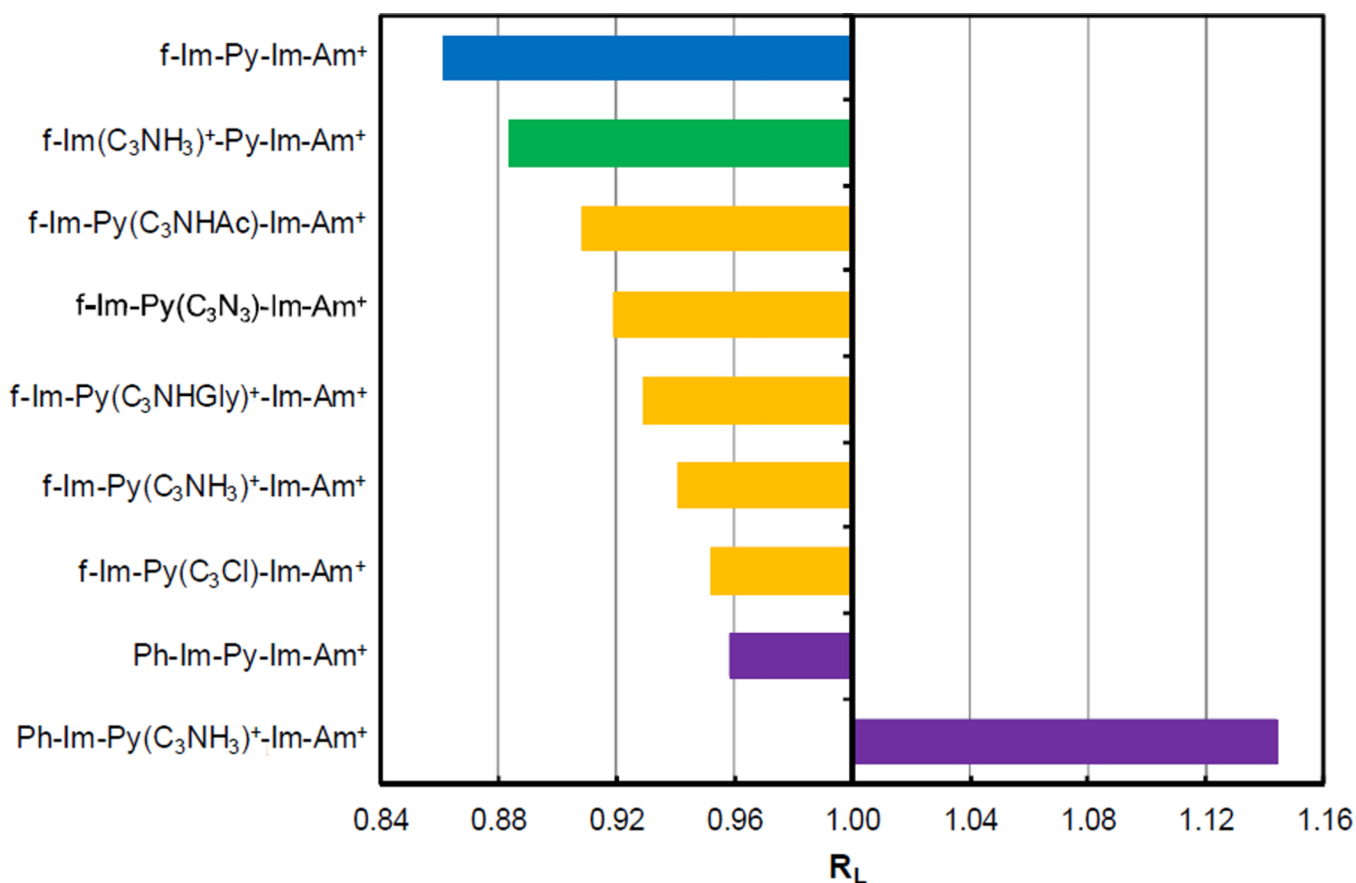


Figure 6.

Plot of R_L values of 200 bp ladders as a function of L_{actual} after charge effect correction. The target sequence CGCG is shown with f-Im-Py-Im-Am⁺ and other eight derivatives in an 8:1 compound: DNA molar ratio. Compounds are classified by colors and the structure is indicated at the left.

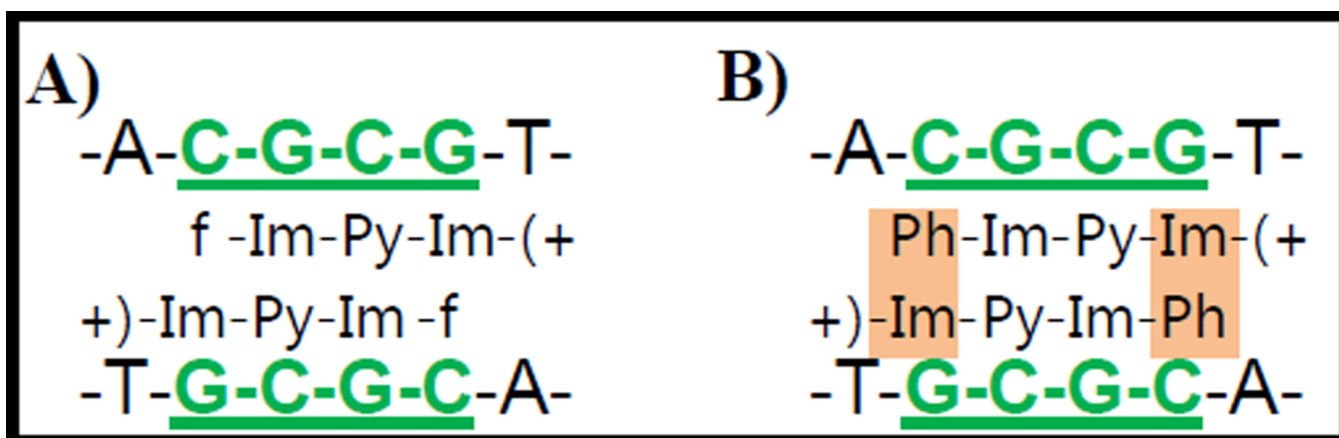


Figure 7.
Binding modes of f-Im-Py-Im-Am⁺ and N-benzamide analogs.