DNA Force Analysis

DOI: 10.1002/anie.200703007

Recognition of "Mirror-Image" DNA by Small Molecules**

Christian Dose, Dominik Ho, Hermann E. Gaub, Peter B. Dervan, and Christian H. Albrecht**

Pyrrole-imidazole polyamides are synthetic oligomers with affinities and specificities for DNA comparable to naturally occurring DNA-binding proteins.[1] The molecular recognition of the minor groove of DNA by polyamides arises from interactions of pairs of the aromatic amino acids *N*-methylpyrrole, *N*-methylimidazole, and *N*-methylhydroxypyrrole with the edges of Watson–Crick DNA base pairs.[2] Introduction of an amino group in the *R* configuration to the γ-turn unit of hairpin polyamide oligomers confers a chiral substituent and increases the DNA-binding affinity, whereas *S*-configured molecules provide lower affinities relative to unsubstituted hairpins.[3] L-DNA is the "mirror-image" of the natural occurring D conformation[4] and has been applied in nucleic acid chemistry for developing anti-HIV agents,[5] in the study of aptamers,[6] transcription factors,[7] mechanisms of antitumor drugs,[8] and as microarray platforms.[9] Herein we report that mirror-image hairpin polyamides can distinguish L-DNA in presence of natural DNA. To detect this specificity we introduce a symmetric molecular force balance to simultaneously measure rupture forces of diastereomeric DNA–ligand complexes. We show that the chirality of polyamides is suitable to enhance the sensitivity of the measurement to determine effects of subtle structural changes in a single experiment.

Single-molecule force spectroscopy that operates in the range of piconewton forces has contributed detailed insights into the understanding of host–ligand interactions.[10] A differential approach to study binding forces was recently introduced, wherein rupture forces of a target complex are directly probed against a known reference complex.[11] This differential measurement format has been applied to distinguish single base-pair mismatches within 30mer DNA duplexes,[12] for studying differences of antibody/antigen interactions,[13] and to eliminate cross-reactions on protein microarrays.[14] Although a large number of molecules are measured simultaneously, the actual measurement is performed at a single-molecule level. One duplex is compared to one reference duplex.

Our design of the molecular force balance comprises D-DNA duplex **1**·**2**, which is covalently linked to a glass slide (bottom), and L-DNA duplex **3**·**4**, which is attached by a biotin–streptavidin bond to a silicone stamp (top, Figure 1a). These DNA duplexes contain identical DNA sequences (20 base pairs) and are linked by an oligothymine spacer (12 bases) that is fluorescently labeled. Upon separation of the two surfaces, force is applied to the molecular balance until one of the duplexes ruptures. If no ligand is bound to the balance, there is a 50% probability for each of the duplexes to survive and the fluorophore will distribute equally between stamp and slide. When ligands are bound to one of the duplexes, the symmetry of the assay is broken and, as a result, there is a higher probability for the fluorophore to end up on the side of the stronger DNA duplex. Parent hairpin polyamide **5**, and mirror-image polyamides (*R*)-**6** and (*S*)-**6** were programmed to bind the six-base-pair DNA sequence 5′-TGGTCA-3′ embedded in D-DNA and L-DNA duplexes **1**·**2** and **3**·**4**, respectively (Figure 1b). To investigate the difference in rupture forces, we first examined chiral hairpin polyamide (*R*)-**6** in the molecular force balance. The survival probability of D-DNA duplex **1**·**2**, which corresponds to the remaining Cy3-fluorescence intensity on the glass slide, was monitored in presence of increasing polyamide concentrations (0–100 nM). Background fluorescence intensities, caused by incomplete biotin–streptavidin bond formations, were subtracted to ensure that the determined values were only the result of ruptured DNA complexes.[12] Figure 2a shows that the survival probability was significantly changed by increasing the polyamide concentration; for example, the probability was 0.69 at 25 nM. Interestingly, at 100 nM (*R*)-**6**, a reduced survival probability of 0.56 was observed. We assume that both DNA duplexes were occupied at higher concentrations of polyamide (*R*)-**6**, but stabilization of D-DNA duplex relative to L-DNA duplex was still favored. Mirror-image polyamide (*S*)-**6** should bind to the L-DNA duplex rather than the D-DNA duplex. Indeed, addition of (*S*)-**6** to the molecular force balance provided an inverted analysis course containing a survival probability of 0.21 at 25 nM. Removal of the chiral information in the polyamides (as in **5**) abolished the mirror-image specificity, as represented by an almost consistent survival probability course across the entire concentration gradient.

Next, we examined the difference in rupture forces between D-DNA duplex and L-DNA duplex by introducing

[*] Dr. C. Dose,[+] Prof. P. B. Dervan
Division of Chemistry and Chemical Engineering
California Institute of Technology
Pasadena, California 91125 (USA)
Fax: (+1) 626-683-8753
E-mail: dervan@caltech.edu
Homepage: http://dervan.caltech.edu/
D. Ho,[+] Prof. H. E. Gaub, Dr. C. H. Albrecht
Lehrstuhl für Angewandte Physik and Center for Nanoscience,
Ludwig-Maximilians-Universität
Amalienstrasse 54, 80799 Munich (Germany)
Fax: (+49) 89-2180-2050
E-mail: christian.albrecht@physik.uni-muenchen.de
[+] These authors contributed equally to this work.

[**] Support was provided by the National Institutes of Health, the Deutsche Forschungsgemeinschaft, and the Fonds der Chemischen Industrie. C.D. is grateful to the Alexander von Humboldt foundation for a postdoctoral fellowship. D.H. is grateful to the Elite Network of Bavaria (IDK-NBT) for a doctoral fellowship.

Supporting information for this article is available on the WWW under http://www.angewandte.org or from the author.

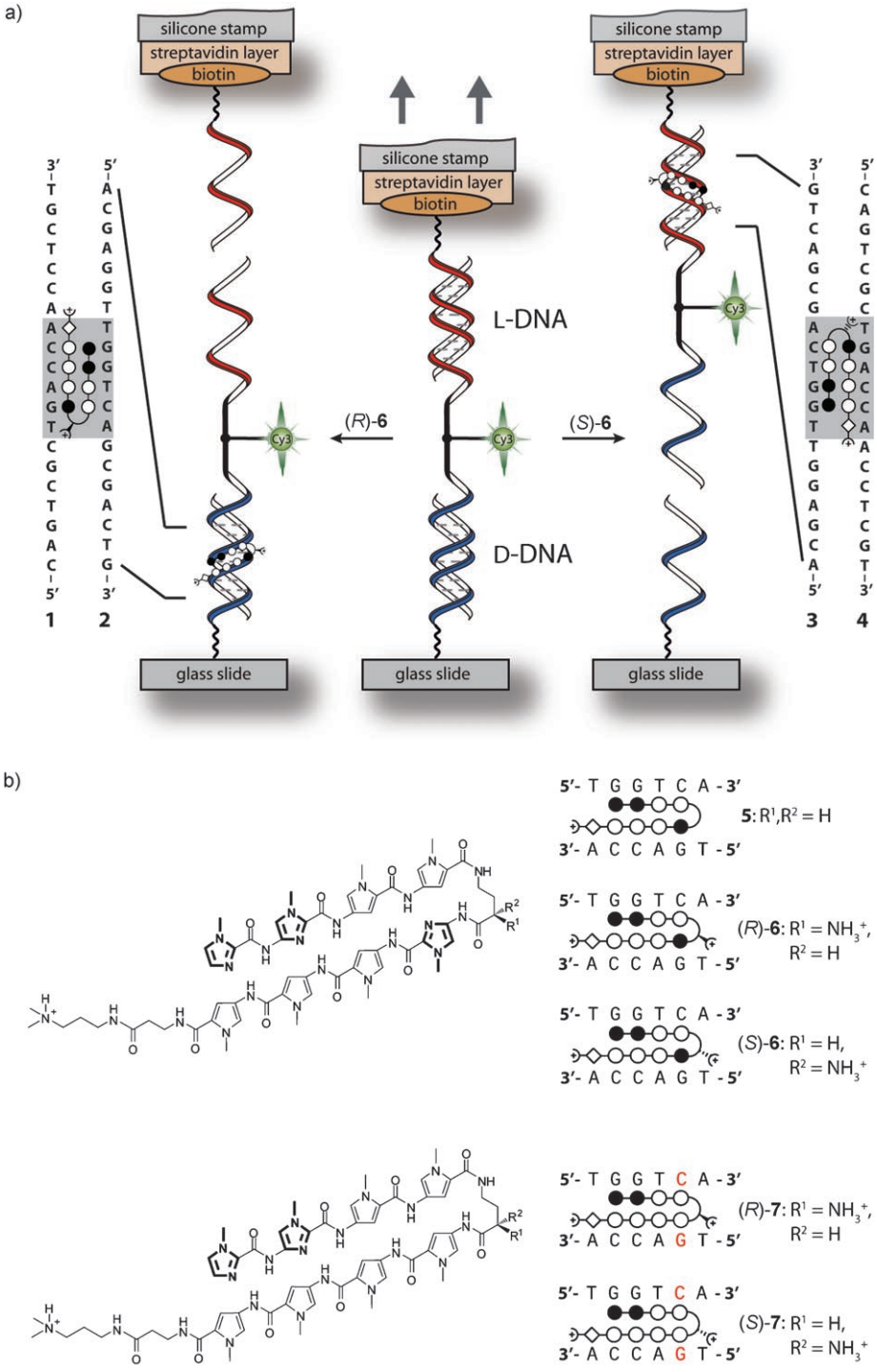

Figure 1. a) Schematic representation of the molecular force balance containing D-DNA and L-DNA duplexes in the presence of chiral hairpin polyamides (*R*)-**6** or (*S*)-**6**; the bases indicated by filled circles are those highlighted in the corresponding structures shown in (b). b) Chemical structures and binding motifs of matched hairpin polyamides **5**, (*R*)-**6**, (*S*)-**6**, and single-base-pair-mismatched compounds (*R*)-**7**, (*S*)-**7**. Mismatched positions are represented by red-colored base pairs.

single-base-pair-mismatched polyamides (*R*)-**7** and (*S*)-**7** to the molecular balance. Both chiral polyamides led to changes in survival probability: 0.59 and 0.27, respectively, at 25 nM concentration (Figure 3). Comparison to the matched polyamides revealed that the change in signal was decreased approximately 0.6-fold. We assumed that the differences in survival probability were caused by less stabilized reference complexes as a result of the discriminative power arising from chirality and mismatched DNA base-pairing of polyamides (*R*)-**7** and (*S*)-**7**. This combination reduces the sensitivity of the molecular force balance for measuring differences between matched and single-base-pair-mismatched DNA–polyamide complexes. To increase the sensitivity, we applied a mixture of matched *R*-configured polyamide (*R*)-**6** and single-base-pair-mismatched *S*-configured polyamide (*S*)-**7** to the molecular force balance (Figure 3). As a result, a single-base-pair-mismatched DNA–polyamide complex is directly compared with a perfectly matched DNA–polyamide complex. Indeed, using a 1:1 mixture of (*R*)-**6**:(*S*)-**7** significantly increased the survival probability relative to the parent single-base-pair-mismatched analysis (0.66 at 25 nM concentration). An almost identical change (survival probability = 0.21) was observed by using the complementary polyamide mixture (*S*)-**6**:(*R*)-**7**. As a control, differential force analysis in the presence of enantiomeric mismatched polyamides (*R*)-**7**:(*S*)-**7** gave a balanced ratio. It can be concluded that the resolution of the measurement for matched and single-base-pair-mismatched DNA–polyamide complexes is enhanced by applying mixtures of chiral hairpin polyamides. By this means, polyamides provide a versatile tool to modify reference duplexes for improving the sensitivity of the molecular force balance.

To ensure that the differences in rupture forces were a result of the DNA duplex stabilization by hairpin polyamides, we compared the melting temperatures (T_m) of the DNA–polyamide complexes.[15] Melting analysis containing nonchiral polyamide **5** revealed an identical increase of 7 °C for complexes **1**·**2**·**5** (T_m = 77 °C) and **3**·**4**·**5** (T_m = 77 °C) relative to the D-DNA and L-DNA duplexes **1**·**2** and **3**·**4** (T_m = 70 °C), respectively (Table 1). Addition of chiral polyamide (*R*)-**6** to D-DNA duplex **1**·**2**

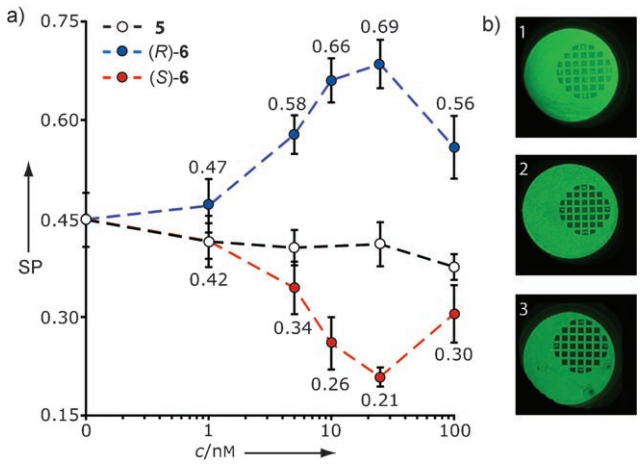

Figure 2. a) Molecular force balance analysis in the presence of increasing concentrations of polyamides **5** (white circles), (*R*)-**6** (blue circles), and (*S*)-**6** (red circles). The survival probability (SP) represents the Cy3-fluorescence intensity on the glass slide after separation of the silicone stamp. b) Cy3-fluorescence images of a spot on the glass slide showing the imprint of the silicone stamp after separation. Images 1, 2, and 3 represent the fluorescence intensities in presence of 25 nM polyamide (*R*)-**6**, **5**, and (*S*)-**6**, respectively.

Table 1: Melting temperatures for DNA–polyamide complexes.[a]

Polyamide	D-DNA duplex [°C] **1·2**	ΔT_m [°C]	L-DNA duplex [°C] **3·4**
–	70	–	70
5	77	–	77
(*R*)-**6**	83	8	75
(*S*)-**6**	75	8	83
(*R*)-**7**	76	5	71
(*S*)-**7**	71	5	76

[a] Values reported are the mean values of at least three melting temperature measurements.

provided a higher stabilization leading to a $T_m = 83\,°C$ for complex **1·2**·(*R*)-**6**. In contrast, melting temperature of L-DNA–polyamide complex **3·4**·(*R*)-**6** was significantly lower ($T_m = 75\,°C$) and revealed a ΔT_m value of 8 °C, which confirms a binding preference of polyamide (*R*)-**6** for D-DNA duplex **1·2**. An identical value of $\Delta T_m = 8\,°C$ was observed for DNA–polyamide complexes **1·2**·(*S*)-**6** and **3·4**·(*S*)-**6**. As expected, melting analysis of DNA–polyamide complexes **1·2**·(*R*)-**7** ($T_m = 76\,°C$) and **3·4**·(*R*)-**7** ($T_m = 71\,°C$), containing single-base-pair-mismatched polyamide (*R*)-**7**, revealed a decrease in stabilization leading to a reduced ΔT_m value of 5 °C relative to the matched complexes. This result was confirmed by an identical difference in T_m values for complexes **1·2**·(*S*)-**7** ($T_m = 71\,°C$) and **3·4**·(*S*)-**7** ($T_m = 76\,°C$).

In summary, we have introduced a symmetric molecular force balance by using the mirror-image forms of DNA as differentiation modules. A single chiral amine substituent of enantiomeric hairpin polyamides affords diastereoselective complexes for D-DNA and L-DNA duplexes. This feature expands the scope of polyamides and provides an easy tool to modify reference complexes to increase the rupture force resolution of the molecular force balance. The investigations

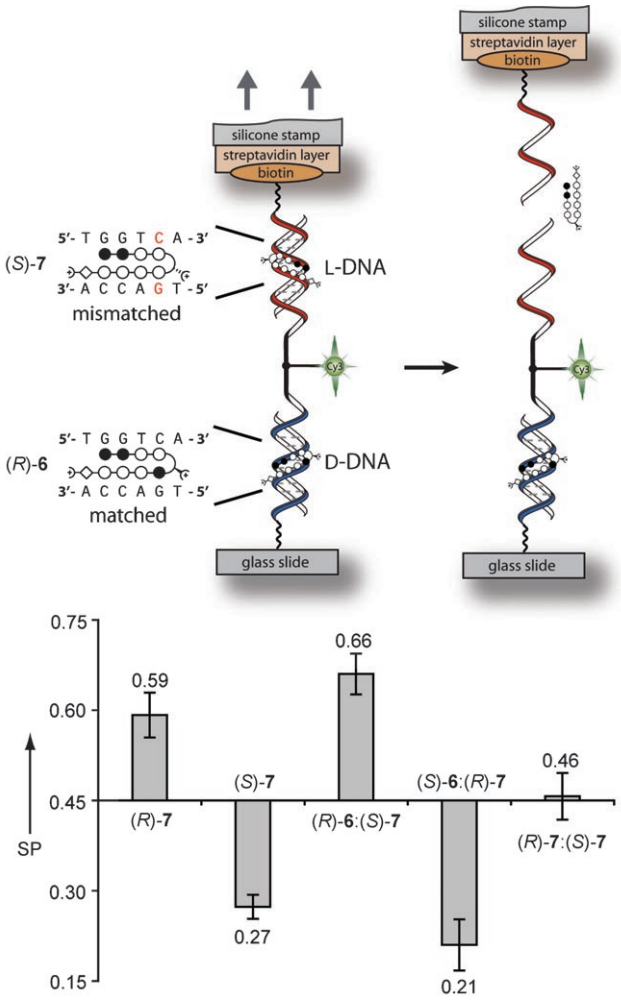

Figure 3. Schematic representation of the molecular force balance for measuring mixtures of polyamides and analysis data of single-base-pair-mismatched polyamides (*R*)-**7** and (*S*)-**7** as well as polyamide mixtures (1:1) of (*R*)-**6**, (*S*)-**6**, (*R*)-**7**, and (*S*)-**7**, respectively, at 25 nM concentration (SP = survival probability).

demonstrate the potential to determine effects of structural changes within a single experiment. Because of its sensitivity and amenability to high-throughput screenings, one could imagine exploring further chiral DNA-binding molecules by the molecular force balance, for example, intercalators, transcription factors, and restriction enzymes.

Received: July 6, 2007
Revised: August 8, 2007
Published online: September 26, 2007

Keywords: chiral resolution · DNA recognition · nanotechnology · polyamides · single-molecule studies

[1] J. W. Trauger, E. E. Baird, P. B. Dervan, *Nature* **1996**, *382*, 559–561.
[2] P. B. Dervan, B. S. Edelson, *Curr. Opin. Struct. Biol.* **2003**, *13*, 284–299.

[3] D. M. Herman, E. E. Baird, P. B. Dervan, *J. Am. Chem. Soc.* **1998**, *120*, 1382 – 1391.
[4] H. Urata, K. Shinohara, E. Ogura, Y. Ueda, M. Akagi, *J. Am. Chem. Soc.* **1991**, *113*, 8174 – 8175.
[5] H. Urata, T. Kumashiro, T. Kawahata, T. Otake, M. Akagi, *Biochem. Biophys. Res. Commun.* **2004**, *313*, 55 – 61.
[6] K. P. Williams, X. H. Liu, T. N. M. Schumacher, H. Y. Lin, D. A. Ausiello, P. S. Kim, D. P. Bartel, *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 11285 – 11290.
[7] S. Negi, M. Dhanasekaran, T. Hirata, H. Urata, Y. Sugiura, *Chirality* **2006**, *18*, 254 – 258.
[8] H. Urata, Y. Ueda, Y. Usami, M. Akagi, *J. Am. Chem. Soc.* **1993**, *115*, 7135 – 7138.
[9] a) N. C. Hauser, R. Martinez, A. Jacob, S. Rupp, J. D. Hoheisel, S. Matysiak, *Nucleic Acids Res.* **2006**, *34*, 5101 – 5111; b) G. Hayashi, M. Hagihara, A. Kobori, K. Nakatani, *ChemBioChem* **2007**, *8*, 169 – 171.
[10] a) V. T. Moy, E. L. Florin, H. E. Gaub, *Science* **1994**, *266*, 257 – 259; b) R. Merkel, P. Nassoy, A. Leung, K. Ritchie, E. Evans, *Nature* **1999**, *397*, 50 – 53; c) H. Clausen-Schaumann, M. Seitz, R. Krautbauer, H. E. Gaub, *Curr. Opin. Chem. Biol.* **2000**, *4*, 524 – 530; d) C. Bustamante, Z. Bryant, S. B. Smith, *Nature* **2003**, *421*, 423 – 427; e) P. B. Tarsa, R. R. Brau, M. Barch, J. M. Ferrer, Y. Freyzon, P. Matsudaira, M. J. Lang, *Angew. Chem.* **2007**, *119*, 2045 – 2047; *Angew. Chem. Int. Ed.* **2007**, *46*, 1999 – 2001; f) Y. J. Jung, B. J. Hong, W. Zhang, S. J. B. Tendler, P. M. Williams, S. Allen, J. W. Park, *J. Am. Chem. Soc.* **2007**, *129*, 9349 – 9355.
[11] C. H. Albrecht, K. Blank, M. Lalic-Multhaler, S. Hirler, T. Mai, I. Gilbert, S. Schiffmann, T. Bayer, H. Clausen-Schaumann, H. E. Gaub, *Science* **2003**, *301*, 367 – 370.
[12] C. H. Albrecht, H. Clausen-Schaumann, H. E. Gaub, *J. Phys. Condens. Matter* **2006**, *18*, S581 – S599.
[13] G. Neuert, C. H. Albrecht, H. E. Gaub, *Biophys. J.* **2007**, *93*, 1215 – 1223.
[14] K. Blank, T. Mai, I. Gilbert, S. Schiffmann, J. Rankl, R. Zivin, C. Tackney, T. Nicolaus, K. Spinnler, F. Oesterhelt, M. Benoit, H. Clausen-Schaumann, H. E. Gaub, *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 11356 – 11360.
[15] D. S. Pilch, N. Poklar, C. A. Gelfand, S. M. Law, K. J. Breslauer, E. E. Baird, P. B. Dervan, *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 8306 – 8311.

