Kinetics of base stacking-aided DNA hybridization†

Bi-feng Yuan,‡[ab] Xing-ying Zhuang,[b] Yu-hua Hao[a] and Zheng Tan*[ab]

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The association and dissociation rate constants (k_a and k_d) of DNA hybridizations involving dual, single or no stacking with different base-pairing sizes were measured, which reveals the advantage of stacking hybridization in both the kinetic and steady state.

DNA hybridization is an indispensable component of many biological processes and modern molecular biology techniques used in a variety of analytical and diagnostic applications. In such applications, stable duplex formation is a prerequisite for success. While duplex stability is affected by several factors, such as sequence composition, secondary structure and strand length, ionic strength and temperature,[1] two factors contributed the major forces for the stability of DNA duplex: base pairing between complementary strands and stacking between adjacent bases.[2] Base stacking is the dipole induced dipole–dipole interaction between the planar aromatic bases in two contiguous nucleotides.[2] When two contiguous tandem sequences are annealed on a longer strand, base stacking at the nick brings additional stability[3–8] and efficiency[9–11] to hybridization. This property has found applications in many hybridization-based methods.[9–13]

Many studies on the enhancement in stacking hybridization mostly focused on the thermodynamics,[3–8] but only a couple of indirect or qualitative analysis on the kinetics has been reported.[10,14] To gain more detailed information, we studied the real-time kinetics of hybridizations involving dual, single and no stacking using an optical biosensor based on surface plasmon resonance (SPR) technique, a convenient platform for measuring the association and dissociation rate constant of macromolecular interactions[15] such as DNA hybridization.[16–18]

This study used hairpin and linear oligonucleotides for both target and probe. The hairpin oligonucleotides carried a single-stranded 6, 11 or 22 nt overhang beyond the duplex stem for stacking interaction (Table 1). The target oligonucleotides were either labeled at the 5′ end or at the middle of the loop with a biotin (Fig. 1) and then immobilized on sensing chip. Hybridizations of different target–probe combinations were initiated by injecting probe across the sensing surface and monitored in real-time on an BIAcore X§ that records changes in mass of the analyte at sensing surface by changes in refractive index.[15] The hybridization during probe injection was reflected by an increase in response units (RU) in proportion to the quantity of captured probe and the dissociation during washing was reflected by a decrease in RU in each sensorgram (Fig. 2).

Fig. 2 shows the sensorgrams of hybridization in different stacking schemes. The association (k_a) and dissociation (k_d) rate constants derived from the sensorgrams and the dissociation equilibrium constants (K_D) calculated are given in Table 2. The values of our k_a and k_d for the 22 and 11 bp hybridizations without stacking are similar to those reported of 21, 20 and 11 bp hybridizations using similar techniques.[18–20] Our data revealed several features of stacking hybridization. In general, base stacking improved hybridization as a result of increased association and decreased dissociation. The introduction of one stacking event decreased the K_D by roughly one order of magnitude or better. Stacking decreased the k_d by about 4 times or more. However, the influence on association was somewhat dependent of the size of hybridization region. Comparing with the single stacking, the dual stacking of the 6 bp hybridization increased the k_a by more than 7 times. However, for the 22 bp hybridization, enhancement was only observed for the single stacking over non-stacking format, but not for the dual over the single stacking. As a result, the improvement in k_a was more significant for shorter sequence. It can be seen from the sensorgrams that the complementation of 6 nt without stacking showed little hybridization. The introduction of a single stacking led to obvious hybridization. An additional stacking further increased the k_a by more than 7 times and decreased the k_d by more than 4 times (Table 2) resulting in an decrease in the K_D by more than 30 times. The increase of k_a by base stacking, even with the more bulky hairpin probe, suggests

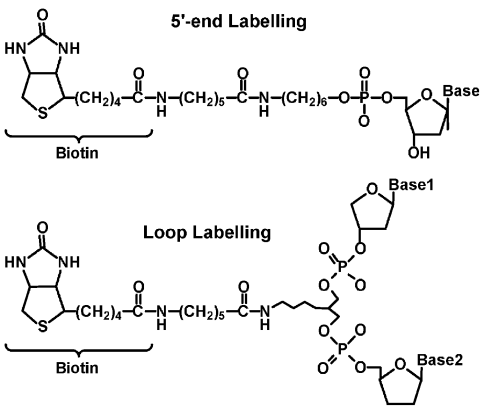

Fig. 1 Oligonucleotide labelling with biotin.

[a] *State Key Laboratory of Biomembrane and Membrane Biotechnology, Institute of Zoology, Chinese Academy of Sciences, Beijing, 100101, China. E-mail: z.tan@ioz.ac.cn. E-mail: tanclswu@public.wh.hb.cn*
[b] *Laboratory of Biochemistry and Biophysics, College of Life Sciences, Wuhan University, Wuhan, 430072, China*

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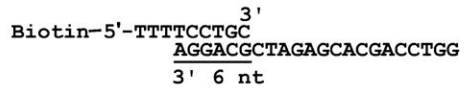
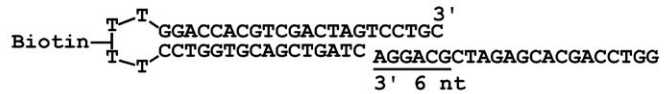
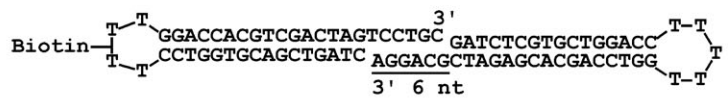
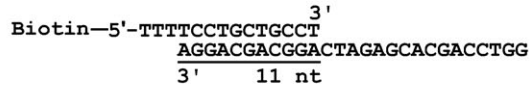
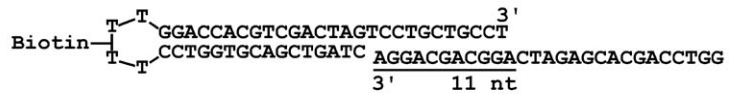
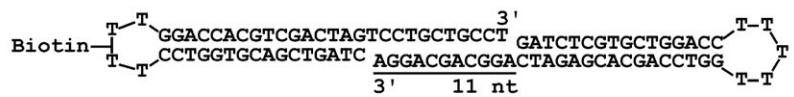
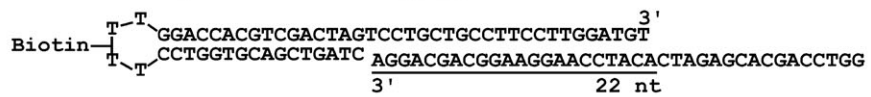
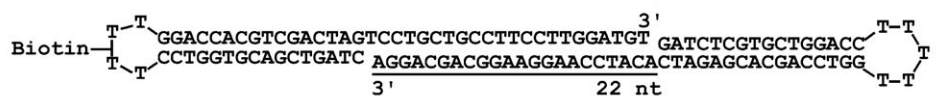
‡ Present address: Department of Chemistry, University of California, Riverside, CA 92521, USA.

Table 1 Oligonucleotide sequences and stacking schemes

Base pairing	Sequences and Stacking Schemes
Target-L6 + Probe-L6	
Target-H6 + Probe-L6	
Target-H6 + Probe-H6	
Target-L11 + Probe-L11	
Target-H11 + Probe-L11	
Target-H11 + Probe-H11	
Target-L22 + Probe-L22	
Target-H22 + Probe-L22	
Target-H22 + Probe-H22	

that the target/probe association is not a primary process but consistent with a nucleation-zipper model.[21,22] Stacking increases the stability of transient intermediates in the hybridization interaction.

In the two previous studies on the kinetics of stacking hybridization,[10,14] one reported association equilibrium constant (K_A) at 25 °C for single stacking and non-stacking hybridizations.[10] This work used the same interaction schemes to analyze the single stacking and non-stacking hybridizations as we did. In that work, biotinylated target was immobilized onto microtiter plate wells to hybridize with probe that was labeled with a fluorescent dye FITC at its 5′ end. The captured probe was then quantified with anti-FITC antibody. This quantitation was indirect and was preceded with six washings to remove non-specific binding, which might result in significant underestimation of the hybridization and thus hampered the quantification. For instance, the K_A for their non-stacking hybridization was several orders of magnitude lower than the many published values.[17–20,23,24]

Our results show that base stacking interaction leads to faster association, slower dissociation of probe DNA and significantly enhanced capture sensitivity, which can be used to improve probe detection in hybridization-based applications. The parameter K_D indicates the probe concentration

Table 2 Kinetic parameters of DNA hybridizations in different stacking schemes

Base pairing	Stacking	$k_a/M^{-1}\ s^{-1}$	k_d/s^{-1}	K_D/M^{-1}
Target-L6 + Probe-L6	No	ND	ND	ND
Target-H6 + Probe-L6	Single	3.34×10^4 (1.0×10^4)	8.84×10^{-2} (8.3×10^{-3})	2.65×10^{-6}
Target-H6 + Probe-H6	Dual	2.42×10^5 (3.7×10^3)	2.08×10^{-2} (1.3×10^{-4})	8.58×10^{-8}
Target-L11 + Probe-L11	No	1.40×10^5 (5.5×10^3)	5.93×10^{-4} (1.7×10^{-5})	4.24×10^{-9}
Target-H11 + Probe-L11	Single	2.44×10^5 (5.7×10^3)	1.30×10^{-4} (7.2×10^{-6})	5.33×10^{-10}
Target-H11 + Probe-H11	Dual	6.55×10^5 (2.3×10^3)	2.72×10^{-5} (4.9×10^{-6})	4.15×10^{-11}
Target-L22 + Probe-L22	No	2.90×10^5 (4.5×10^3)	1.24×10^{-4} (5.6×10^{-6})	4.28×10^{-10}
Target-H22 + Probe-L22	Single	5.87×10^5 (8.2×10^3)	3.37×10^{-5} (7.9×10^{-6})	5.74×10^{-11}
Target-H22 + Probe-H22	Dual	5.71×10^5 (4.7×10^3)	3.16×10^{-7} (3.4×10^{-6})	5.54×10^{-13}

Numbers in parenthesis are standard errors. K_D was calculated as k_d/k_a. ND: not determined.

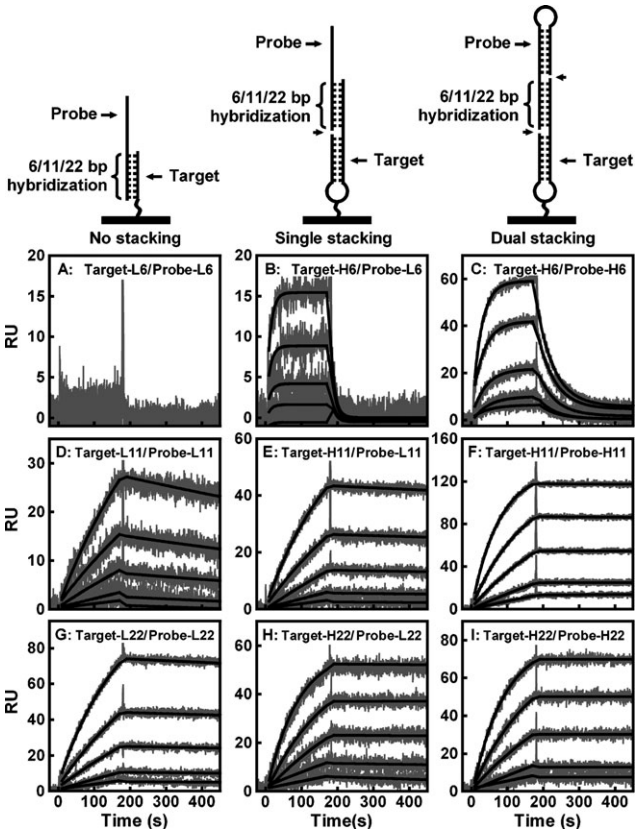


Fig. 2 Hybridization sensorgrams obtained at 25 °C, pH 7.4 in 150 mM NaCl solution. Hybridization scheme was indicated above the panels. Probe oligonucleotide was injected at 5, 10, 20, 40, 80 nM for the 6 bp (panels A–C) and 1, 2, 5, 10, 20 nM for the 11 (panels E–F) and 22 (panels G–I) bp hybridizations. Sensorgrams were globally fitted (dark line) to the Langmuir binding model to extract kinetic parameters.

required to bind half of the immobilized target, thus can be used to evaluate detecting sensitivity. For instance, the single stacking format with 6 nt complementation will require probe concentration above μM level. Dual stacking will lower the detecting level by over 30-fold. For the hybridization of 11 bp, the require probe concentration will be around nM level that can be lowered by one or two orders of magnitude by adopting single or dual stacking. In the case of 22 bp hybridization, this requirement can be further lowered to subpicomolar level.

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Notes and references

§ Biotinylated target oligonucleotide was immobilized on CM5 sensor chip (BIAcore, Sweden) *via* biotin-streptavidin interaction using a BIAcore X optical biosensor (BIAcore, Sweden).[25] Streptavidin was coupled to the carboxy-methylated dextran coating using the Amine Coupling Kit (BIAcore, Sweden) according to manufacturer's instruction. 60 μl of 50 nM biotinylated oligonucleotide in HEPES buffered saline (HBS, 10 mM HEPES, pH 7.4, 0.15 M NaCl, 3.4 mM EDTA, and 0.005% (v/v) surfactant P20) was injected at 20 μl min^{-1} into one flow cell resulting in a capture of about 100 to 200 RU of oligonucleotide. Hybridization measurements were carried out at 25 °C on the BIAcore X optical biosensor as previously described.[25] For each sensorgram recording, sensor chip was regenerated with an injection of 5 μl of 20 mM NaOH at 30 μl min^{-1} followed by equilibration with the HBS buffer. Hybridization was initiated by injecting 45 μl of probe oligonucleotide at 20 μl min^{-1} followed by flow of HBS. Both the association and dissociation phase was recorded and simultaneous signal from a blank cell was subtracted as background. For each measurement, sensorgrams of five injections of different concentrations of probe oligonucleotide were recorded. The dissociation rate constant k_d was first extracted by globally fitting the dissociation phase and the association rate constant k_a was then extracted by globally fitting the association phase, using the BIAevaluation 3.0 software supplied by the manufacturer of BIAcore and the built-in Langmuir binding model.

1 J. G. Wetmur, *Annu. Rev. Biophys. Bioeng.*, 1976, **5**, 337–361.
2 E. T. Kool, *Annu. Rev. Biophys. Biomol. Struct.*, 2001, **30**, 1–22.
3 P. Yakovchuk, E. Protozanova and M. D. Frank-Kamenetskii, *Nucleic Acids Res.*, 2006, **34**, 564–574.
4 M. Kohandel and B. Y. Ha, *Phys. Rev. E Stat. Nonlinear Soft Matter Phys.*, 2006, **73**, 011905.
5 D. Murphy, H. Doyle, R. Eritja and G. Redmond, *J. Biomol. Struct. Dyn.*, 2004, **22**, 195–204.
6 D. V. Pyshnyi, I. Pyshnaya, A. Levina, E. Goldberg, V. Zarytova, D. Knorre and E. Ivanova, *J. Biomol. Struct. Dyn.*, 2001, **19**, 555–570.
7 V. A. Vasiliskov, D. V. Prokopenko and A. D. Mirzabekov, *Nucleic Acids Res.*, 2001, **29**, 2303–2313.
8 S. Bommarito, N. Peyret and J. SantaLucia, Jr, *Nucleic Acids Res.*, 2000, **28**, 1929–1934.
9 N. E. Broude, K. Woodward, R. Cavallo, C. R. Cantor and D. Englert, *Nucleic Acids Res.*, 2001, **29**, E92.
10 P. V. Riccelli, F. Merante, K. T. Leung, S. Bortolin, R. L. Zastawny, R. Janeczko and A. S. Benight, *Nucleic Acids Res.*, 2001, **29**, 996–1004.
11 D. Wang, L. Zhu, D. Jiang, X. Ma, Y. Zhou and J. Cheng, *J. Biochem. Biophys. Methods*, 2004, **59**, 109–120.
12 A. A. Stomakhin, V. A. Vasiliskov, E. Timofeev, D. Schulga, R. J. Cotter and A. D. Mirzabekov, *Nucleic Acids Res.*, 2000, **28**, 1193–1198.
13 X. Zhao, S. Nampalli, A. J. Serino and S. Kumar, *Nucleic Acids Res.*, 2001, **29**, 955–959.
14 D. O'Meara, P. Nilsson, P. A. Nygren, M. Uhlen and J. Lundeberg, *Anal. Biochem.*, 1998, **255**, 195–203.
15 M. Fivash, E. M. Towler and R. J. Fisher, *Curr. Opin. Biotechnol.*, 1998, **9**, 97–101.
16 V. V. Koval, O. V. Gnedenko, Y. D. Ivanov, O. S. Fedorova, A. I. Archakov and D. G. Knorre, *IUBMB Life*, 1999, **48**, 317–320.
17 K. K. Jensen, H. Orum, P. E. Nielsen and B. Norden, *Biochemistry*, 1997, **36**, 5072–5077.
18 M. Gotoh, Y. Hasegawa, Y. Shinohara, M. Shimizu and M. Tosu, *DNA Res.*, 1995, **2**, 285–293.
19 C. M. Niemeyer, W. Burger and R. M. Hoedemakers, *Bioconjugate Chem.*, 1998, **9**, 168–175.
20 K. Tawa, D. Yao and W. Knoll, *Biosens. Bioelectron.*, 2005, **21**, 322–329.
21 M. Spodheim and E. Neumann, *Biopolymers*, 1977, **16**, 289–298.
22 D. Porschke and M. Eigen, *J. Mol. Biol.*, 1971, **62**, 361–381.
23 K. Tawa and W. Knoll, *Nucleic Acids Res.*, 2004, **32**, 2372–2377.
24 F. Yu, D. Yao and W. Knoll, *Nucleic Acids Res.*, 2004, **32**, e75.
25 Y. Zhao, Z. Y. Kan, Z. X. Zeng, Y. H. Hao, H. Chen and Z. Tan, *J. Am. Chem. Soc.*, 2004, **126**, 13255–13264.