

Kinetics of base stacking-aided DNA hybridization

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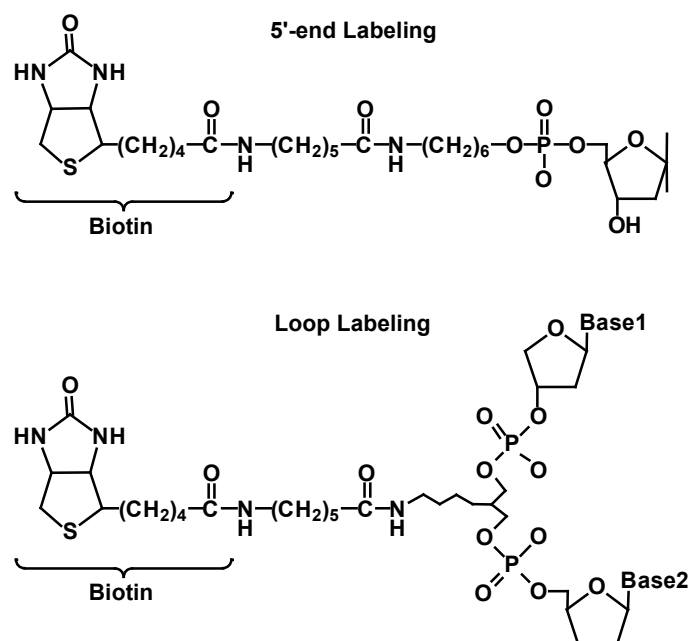
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Oligonucleotide sequences

Base pairing	Sequences and Stacking Scheme
Biotin-L6 + L6	
Biotin-H6 + L6	
Biotin-H6 + H6	
Biotin-L11 + L11	
Biotin-H11 + L11	
Biotin-H11 + H11	
Biotin-L22 + L22	
Biotin-H22 + L22	
Biotin-H22 + H22	

Oligonucleotides were synthesized by TaKaRa Biotech (Dalian, China). The hairpin forming sequences were designed to fold to form via self-complementation a hairpin structure with a poly-T, a 16 bp stem and a single-stranded 6, 11 or 22 nt single-stranded overhang. The capture oligonucleotides were either labeled at the 5' end or at the middle of the loop with a biotin as illustrated below.

Oligonucleotide labeling and immobilization



Biotinylated capture oligonucleotide was immobilized on CM5 sensor chip (BIAcore, Sweden) via biotin-streptavidin interaction using a BIAcore X optical biosensor (BIAcore, Sweden) (24). 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 $\mu\text{l}/\text{min}$ into one flow cell resulting in a capture of about 100 to 200 RU of oligonucleotide.

Measurement

Measurements were carried out at 25 $^{\circ}\text{C}$ on a BIAcore X optical biosensor (BIAcore, Switzerland) and extraction of kinetic parameters was performed as previously described (24). For each sensorgram recording, sensor chip was regenerated with an injection of 5 μl of 20 mM NaOH at 30 $\mu\text{l}/\text{min}$ followed by equilibration with the HBS buffer. Hybridization was initiated by injecting 45 μl of target oligonucleotide at 20 $\mu\text{l}/\text{min}$ 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 target 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.