

## Supporting Information

### **Enhanced affinity of racemic phosphorothioate DNA with transcription factor SATB1 arising from diastereomer-specific hydrogen bonds and hydrophobic contacts**

Kazuhiko Yamasaki, Yukie Akutsu, Tomoko Yamasaki, Makoto Miyagishi,  
and Tomomi Kubota

*Biomedical Research Institute, National Institute of Advanced Industrial Science and  
Technology (AIST), 1-1-1 Higashi, Tsukuba, Ibaraki 305-8566, Japan  
E-mail: k-yamasaki@aist.go.jp*

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**Table S1. Crystallographic data for dataset 2 used in the molecular replacement**

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Space group	P 31
Unit cell	
a/b/c (Å)	45.51/45.51/98.27
$\alpha/\beta/\gamma$ (°)	90.0/90.0/120.0
Wavelength (Å)	1.000
Resolution range (outer shell) (Å)	39.41–2.16 (2.27– 2.16)
Total reflections	51,028
Unique reflections	12,236
Completeness (outer shell) (%)	99.5 (99.2)
Redundancy (outer shell)	4.2 (4.2)
$R_{\text{merge}}$ (outer shell) (%)	14.3 (59.7)
Average $I/\sigma(I)$ (outer shell)	6.2 (2.9)

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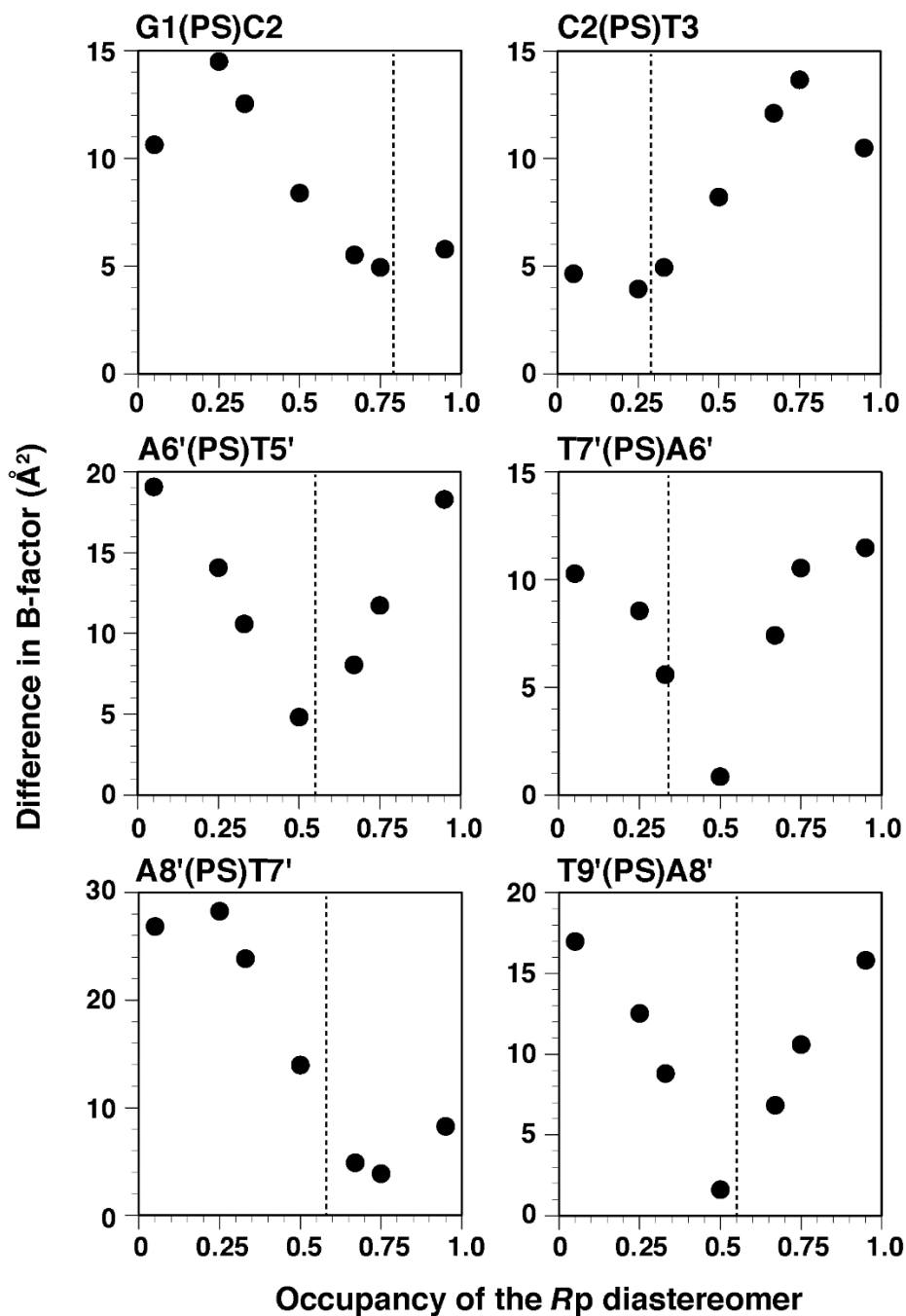


Fig. S1: Validation of occupancies of the diastereomers assuming constancy in the B-factors.  $((B_S(Rp) - B_S(Sp))^2 + (B_O(Rp) - B_O(Sp))^2)^{1/2}$ , as functions of preset occupancy of the *Rp* diastereomer, where  $B(Rp)$  and  $B(Sp)$  stand for the B-factors in the two diastereomers, and the suffixes S and O indicate those of the sulfur and (non-bridging) oxygen atoms, respectively. The vertical broken lines indicate the final occupancy values optimized in the refinement process by Refmac5.

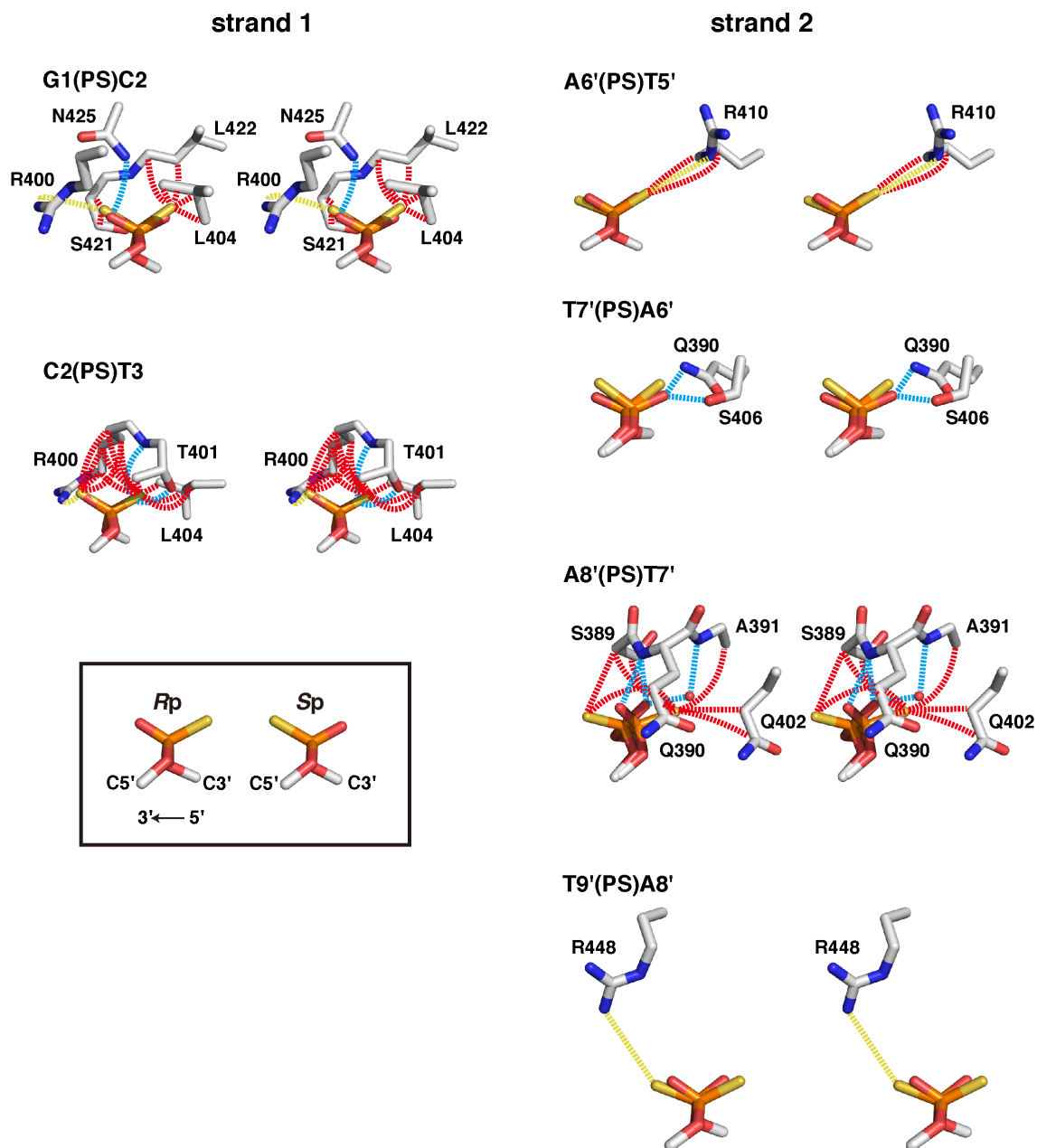


Fig. S2: Intermolecular interactions at the six phosphorothioate-modified sites. Interactions with the two diastereomers are shown together in a stereo view. Dashed lines indicate hydrogen bonds (cyan), hydrophobic contacts (red), and salt bridges (yellow), as defined in Fig. 2 in the main text (see also Fig. 3). Shown in the framed panel are configurations of the two diastereomers, the orientations of which are kept in the other panels. Note that the 5' end of the DNA strand is located in the direction of C3' of phosphorothioate, and vice versa.

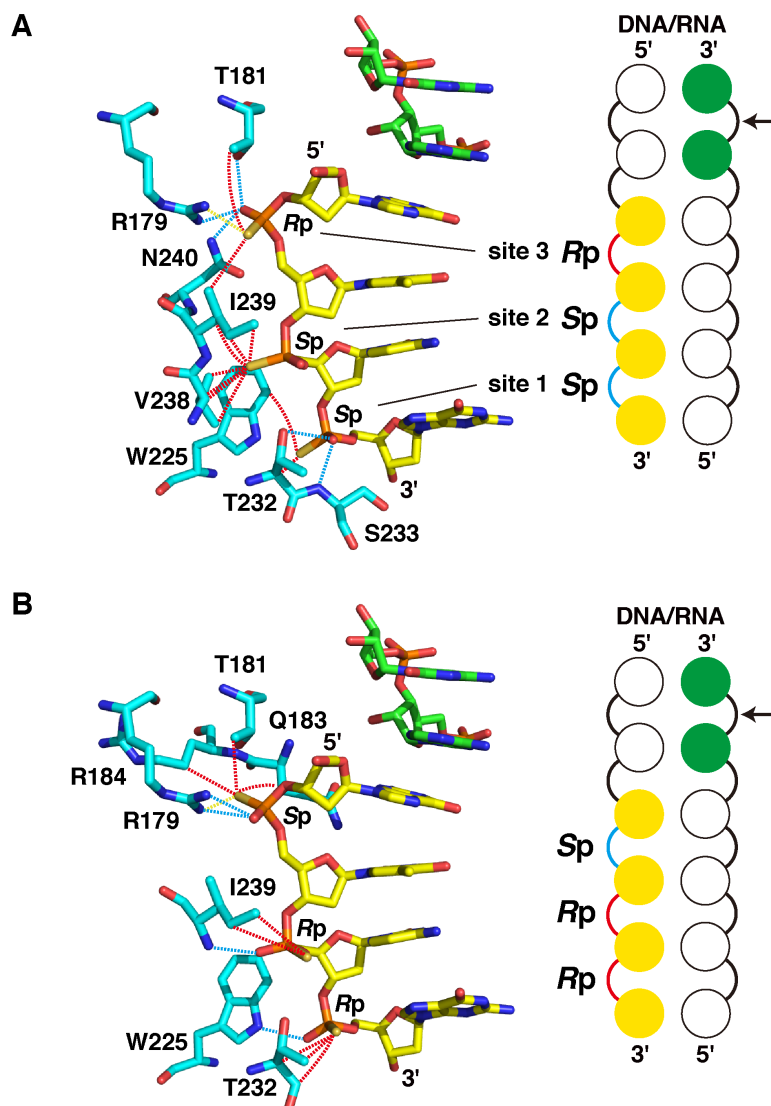


Fig. S3: Structural models of interaction between stereochemically unique antisense oligonucleotides (ASOs) and RNase H. (A) A model of the ASO containing the 3'-SpSpRp-5' motif with enhanced activity (ref. 23 in the main text) in complex with RNase H. This was produced by oxygen–sulfur substitutions in the crystal structure of the complex of RNase H and DNA/RNA duplex (PDB ID: 2QK9; ref. 52 in the main text). DNA, RNA, and protein residues are shown in yellow, green, and cyan-based colors, respectively. Dashed lines indicate hydrogen bonds (cyan), hydrophobic contacts (red), and salt bridges (yellow), as defined in Fig. 2 in the main text. The three sites are numbered from 3' end as in ref. 23. The DNA/RNA hybrid is shown schematically in right, where an arrow indicates the cleavage site and filled circles indicate the nucleotides presented in left. (B) A model of the ASO containing the alternative 3'-RpRpSp-5' motif in complex with RNase H, for comparison. The presentation scheme is the same as in (A).