

# Substrate recognition and specificity of double-stranded RNA binding proteins

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Electronic Supporting Information (ESI)



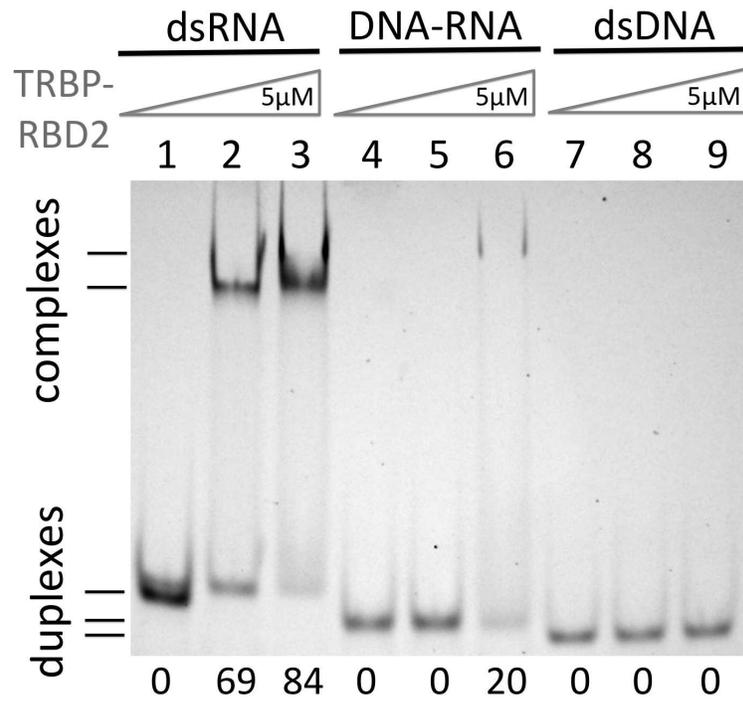


Figure S2: EMSA of 25-bp duplexes incubated with 0, 1, and 5  $\mu$ M of TRBP-RBD2. TRBP-RBD2 binds to dsRNA and DNA-RNA (only 5  $\mu$ M TRBP-RBD2), and does not bind to dsDNA. The quantified binding fraction of TRBP-RBD2 to each duplex is displayed below the gel image.

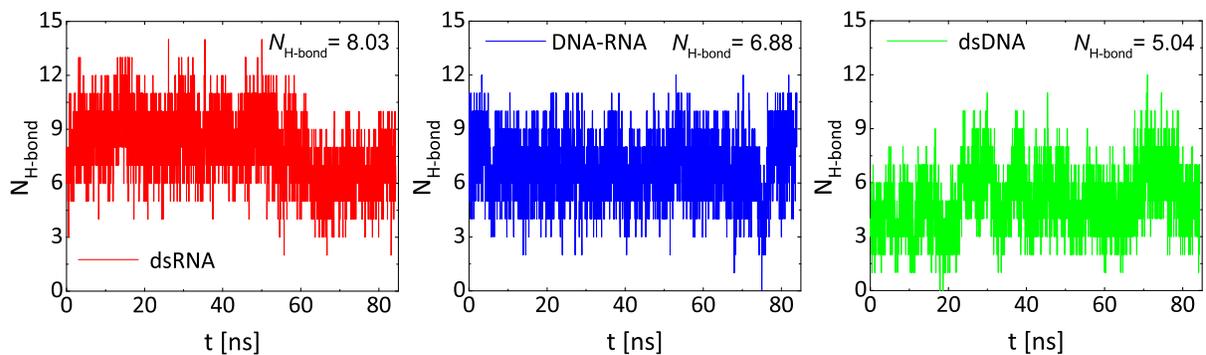


Figure S3: Time dependence of the number of hydrogen bonds at the interface of TRBP-RBD2 with dsRNA (left), DNA-RNA (middle) and dsDNA (right) duplexes. The average number of hydrogen bonds is shown in the top right corner of each plot.

Table S1: Averages of selected helical parameters (twist, slide, and roll) for dsRNA, DNA-RNA, and dsDNA duplexes in complex with TRBP-RBD2. Dihedral angles  $\delta$  and  $\chi$ , and phase angles of pseudorotation,  $P$ , of duplex strands I and II. For DNA-RNA duplex, strands I and II correspond to RNA and DNA strands, respectively. The reported values are evaluated from averaged structures of complexes, which were obtained by averaging the coordinates of each system over the last 40 ns of simulation. Standard deviations are given in brackets. All the values in the table were calculated with 3-DNA software [2].

Duplex	Twist [°]	Slide [Å]	Roll [°]	$\delta_I$ [°]	$\delta_{II}$ [°]	$\chi_I$ [°]	$\chi_{II}$ [°]	$P_I$ [°]	$P_{II}$ [°]
dsRNA	29.5 (1.6)	-1.4 (0.3)	11 (4)	79 (3)	79 (2)	-156 (6)	-155 (6)	17 (5)	18 (4)
DNA-RNA	30.2 (1.6)	-1.1 (0.4)	7 (4)	82 (6)	111 (9)	-153 (8)	-129 (15)	19 (18)	109 (28)
dsDNA	32.2 (2.8)	-0.6 (0.3)	4 (4)	119 (8)	118 (10)	-120 (9)	-123 (13)	126 (17)	125 (17)

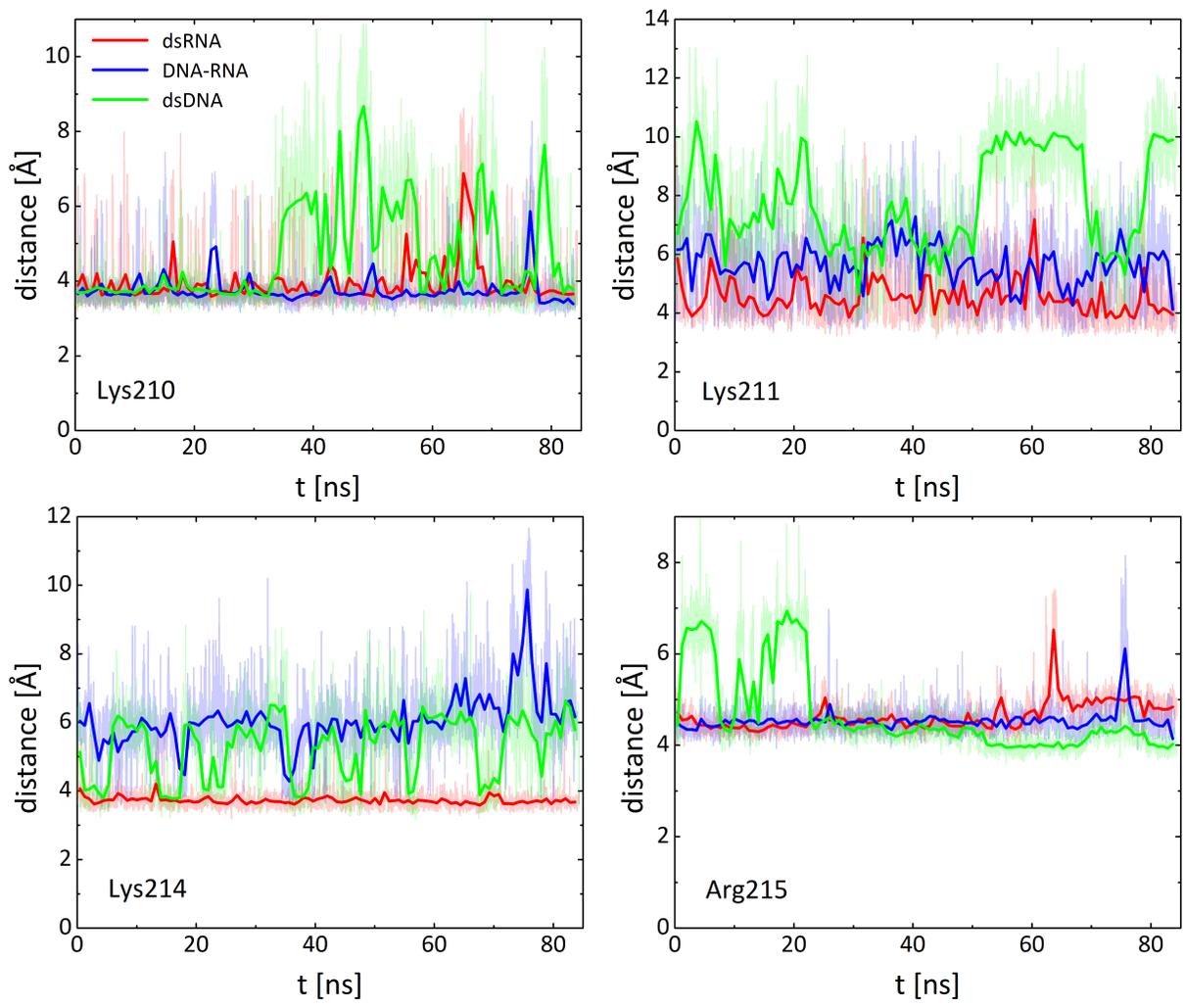


Figure S4: Interactions of positively charged TRBP-RBD2 residues with a major groove of dsRNA, DNA-RNA and dsDNA. Distances of Lys210, Lys211, Lys214 and Arg215 residues from the nearest phosphate groups (P-atoms) are shown for the last 85 ns of simulations. Thin lines correspond to data sampled from trajectories; thick lines show gliding time averages over 800 ps.

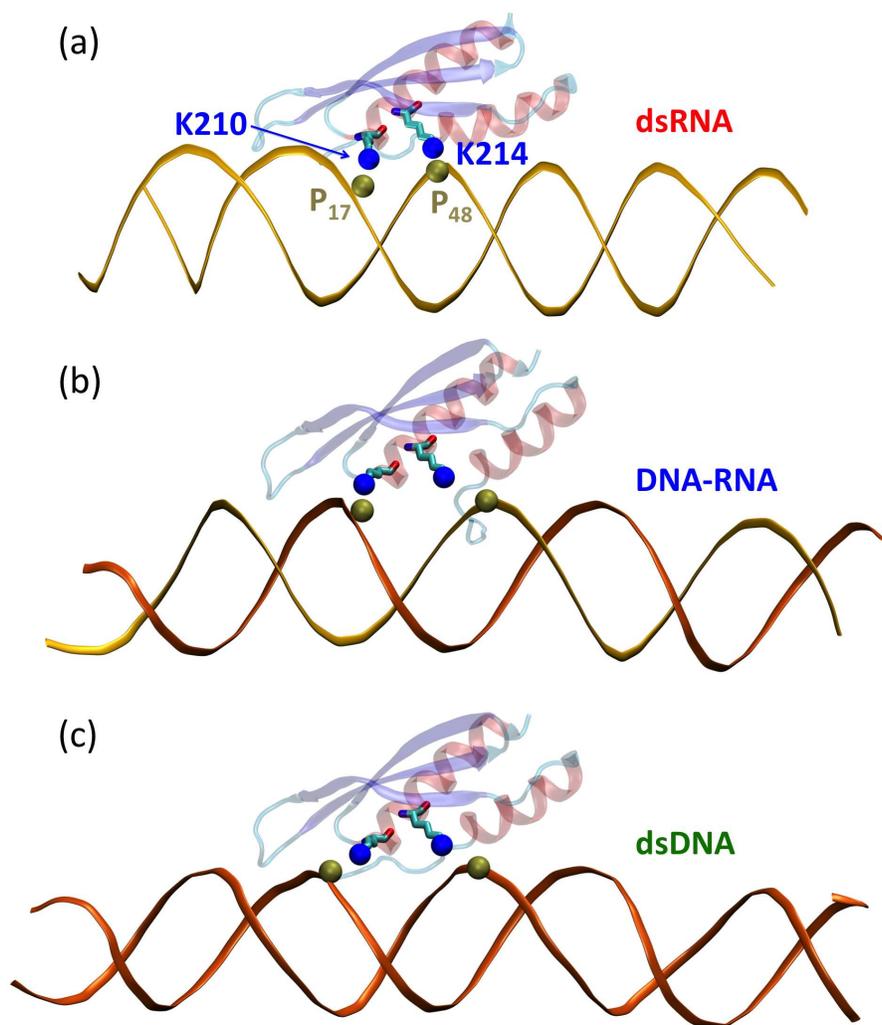


Figure S5: Register fit of Lys210-Lys214 pair in major grooves of dsRNA (a), DNA-RNA (b), and dsDNA (c). Nitrogen atoms in the amino groups of Lys210 and Lys214 are highlighted as blue spheres, and phosphorus atoms, which coordinate the shown amino groups, are highlighted as tan spheres. TRBP-RBD2 is shown as transparent cartoon, and duplexes are shown by backbone contours. The shown complexes are obtained by averaging the coordinates of each system over the last 40 ns of simulation.

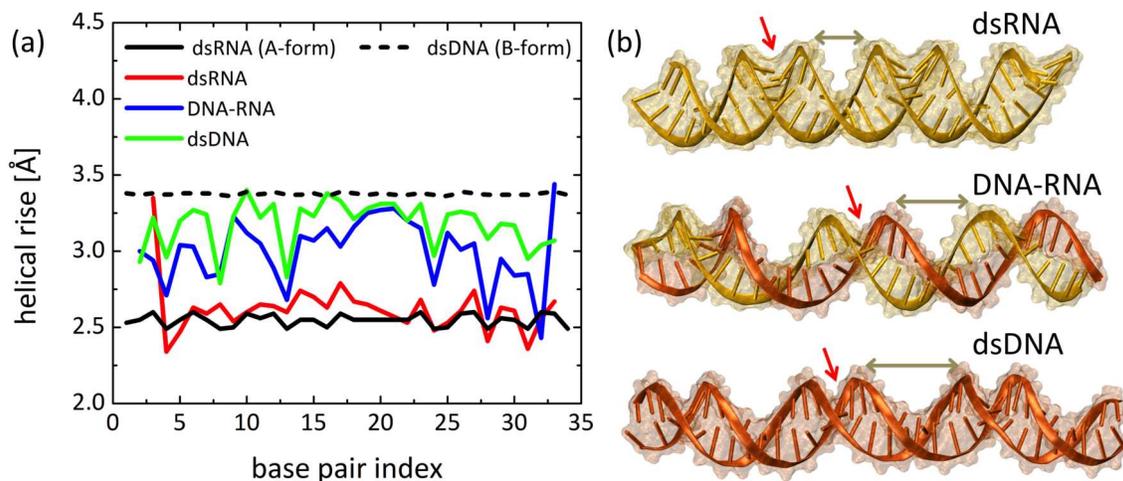


Figure S6: Duplex forms of dsRNA, DNA-RNA and dsDNA simulated in 0.05 M NaCl. The analyzed duplexes are obtained by averaging the coordinates of each system over the last 40 ns of simulation. (a) Helical rise for duplex base pairs of A-form dsRNA, B-form dsDNA, and averaged structures of simulated dsRNA, DNA-RNA, and dsDNA. Helical rise values were calculated with 3-DNA software [2]. (b) Averaged structures of three studied duplexes. RNA strands are shown in gold, and DNA strands are shown in orange. Tan arrows indicate major groove widths and red arrows point to minor grooves.

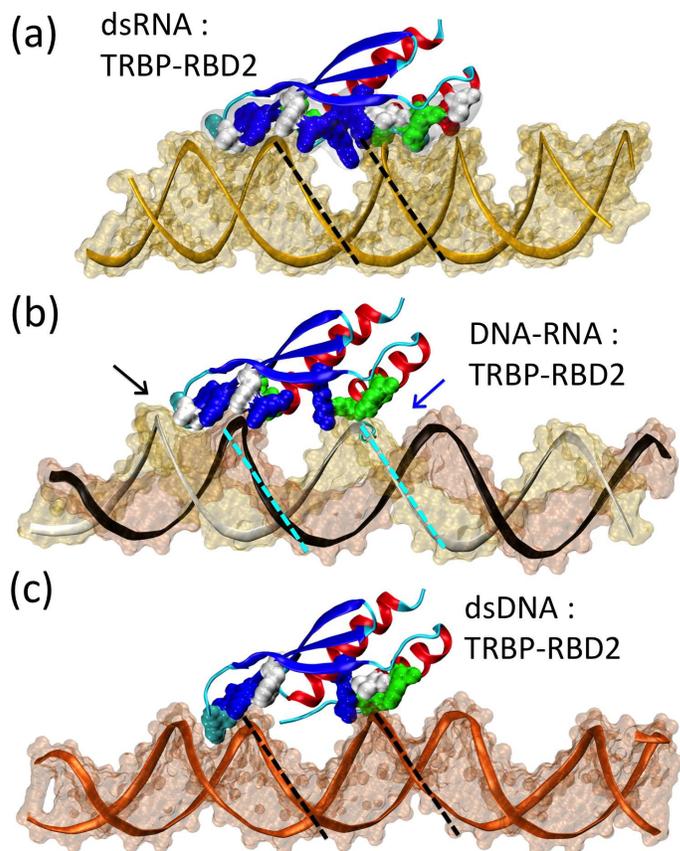


Figure S7: Duplex forms at TRBP-RBD2 binding sites. Averaged structures of TRBP-RBD2 in complex with (a) dsRNA, (b) DNA-RNA and (c) dsDNA. The complexes shown are averaged over the last 40 ns of simulation. The protein domain is colored according to secondary structure elements (red-helix, blue-beta sheet, cyan-loops), and residues within 3.0 Å of duplexes are highlighted according to residue type (blue residues are positively charged, red are negatively charged, green and cyan are polar, and white residues are nonpolar). Central major grooves are highlighted by dashed black (cyan) lines. In (b), the black arrow marks an A-form-like minor groove and the blue arrow marks a B-form-like minor groove in the DNA-RNA duplex bound to TRBP-RBD2.

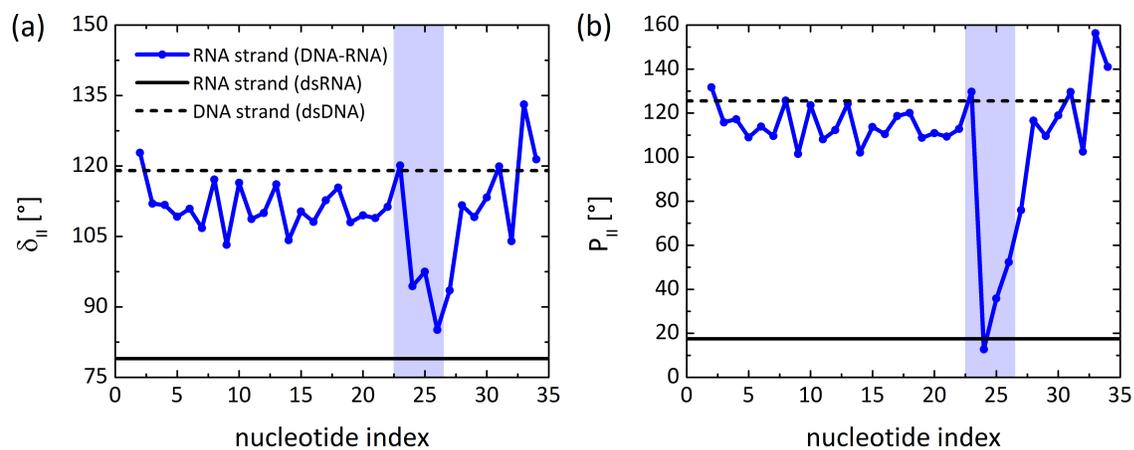


Figure S8: Average values of dihedral  $\delta_{II}$  (a) and phase angle of pseudorotation  $P$  (b) for DNA nucleotides of the DNA-RNA duplex. The values were determined from the averaged structure of the TRBP-RBD2 : DNA-RNA complex, which was obtained by averaging the coordinates of the system over the last 40 ns of simulation. DNA nucleotides with indices 23-26 (index range highlighted in light blue), which form the minor groove II, are in contact with TRBP-RBD2. The plotted values were calculated with 3-DNA software [2].

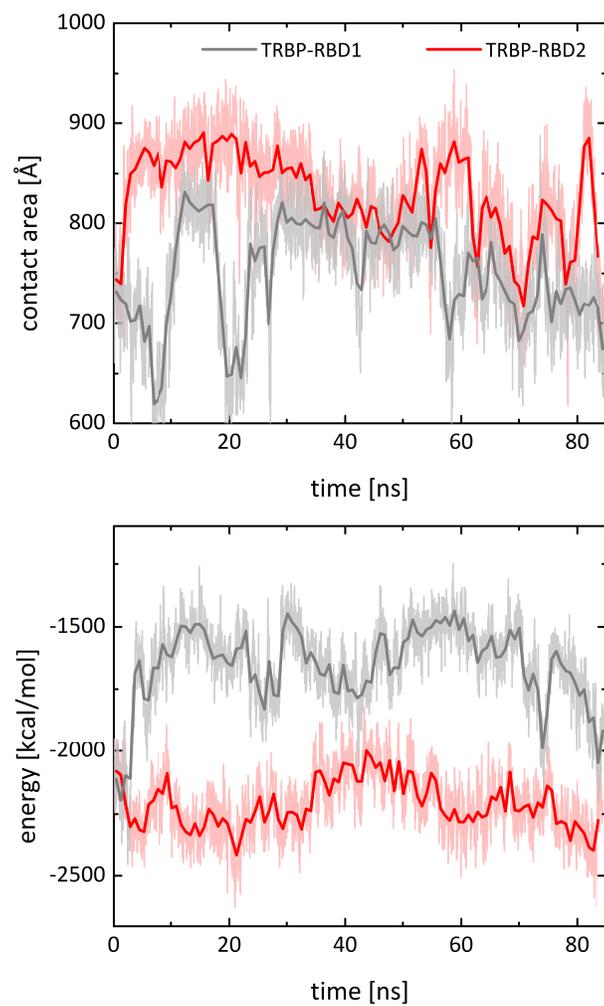


Figure S9: Interaction of TRBP-RBD1 and TRBP-RBD2 with the dsRNA duplex. (Top) Contact areas between TRBP-RBD1 or TRBP-RBD2 and dsRNA. (Bottom) Interaction (nonbonding) energy between TRBP-RBD1 or TRBP-RBD2 and the dsRNA duplex. The plots are shown for the last 85 ns of simulations. Thin lines correspond to data sampled from trajectories; thick lines show gliding time averages over 800 ps.

## References

- [1] Crooks, G. E., Hon, G., Chandonia, J. M. and Brenner, S. E. (2004) WebLogo: A sequence logo generator. *Genome Res.* *14*, 1188-1190.
- [2] Lu, X.-J. and Olson, W. K. (2003) 3DNA: A software package for the analysis, rebuilding and visualization of three-dimensional nucleic acid structures. *Nucleic Acids Res.* *31*, 5108-5121.