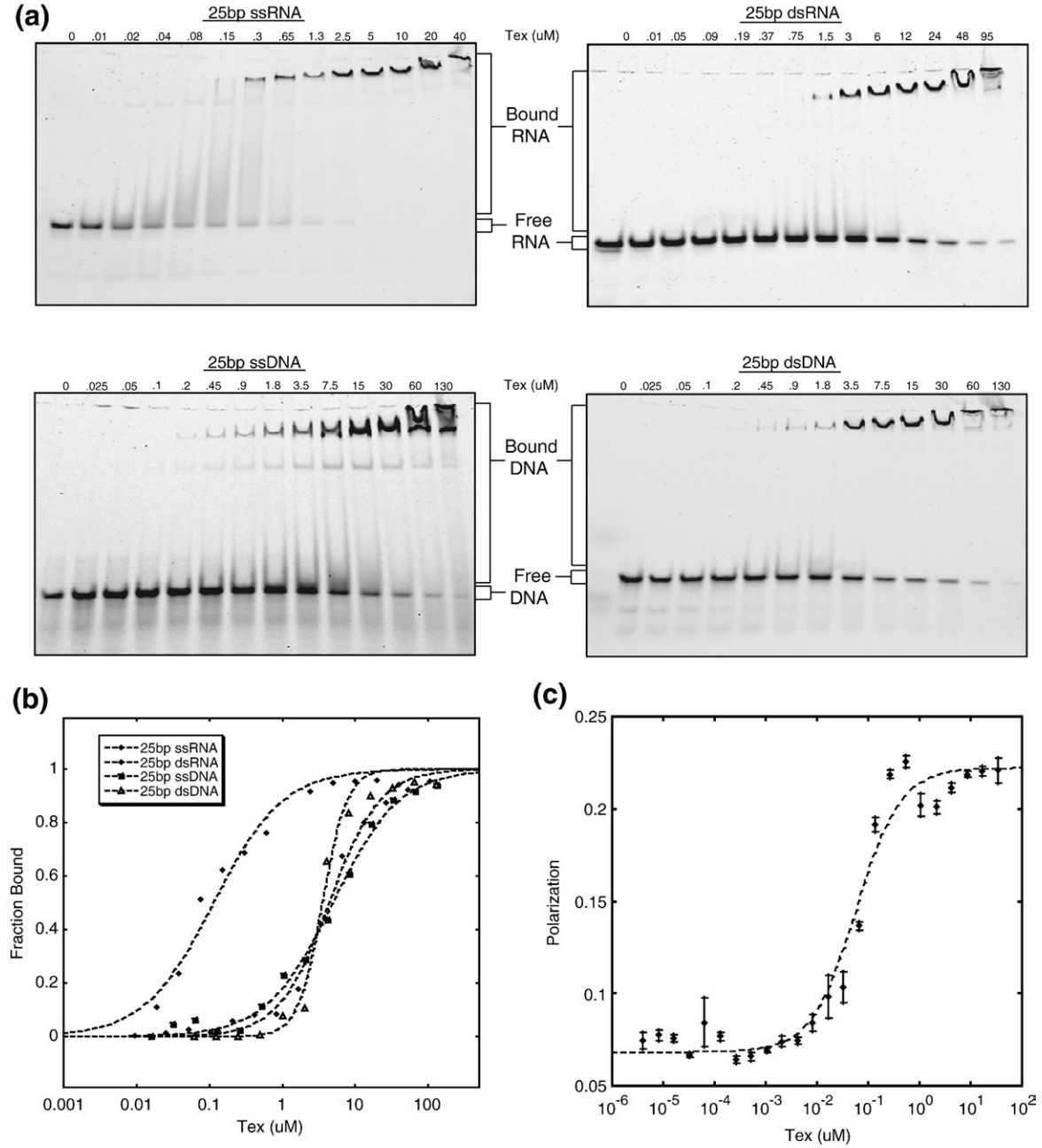

Fig. 7. Electrophoretic mobility shift data for Tex binding different substrates. (a) Full-length Tex protein was added in increasing concentrations to 5′-fluorescein-labeled ssRNA (top left), dsRNA (top right), ssDNA (bottom left), or dsDNA (bottom right). Nucleic-acid-bound Tex complexes were resolved from free substrate by native gel electrophoresis. (b) Representative binding isotherms for the gel shifts represented in (a). Shifts and respective isotherms were repeated at least three times for each substrate. Resulting K_d values with standard error (in micromolar) were 0.21±0.05 for 25 bp ssRNA, 4.2±1.47 for 25 bp dsRNA, 5.1±1.5 for 25 bp ssDNA, and 3.7±0.15 for 25 bp ssDNA. (c) FP binding isotherm for Tex binding to fluorescein-labeled 25 bp ssRNA. Data points with error bars (standard error) representing average with polarization values (P) were measured for increasing concentrations of Tex. K_d (±standard error) for Tex binding ssRNA based on FP experiments is 0.057±0.006 μM.

and is composed of a number of putative nucleic acid binding motifs, binding does not occur in the absence of the S1 domain.

In the numerous S1/OB-fold complex structures determined to date, nucleic acid substrate is coordinated in the binding cleft via surface-exposed aromatic side chains such as phenylalanine and more polar groups such as lysine or arginine.[30,35] The structure of Tex reveals the characteristic S1/OB binding cleft and candidate contact residues: F668, F671, H683, and R718 (Fig. 5). When using DALI[18] to align the Tex S1 domain with other S1 structures, Tex residues F668, F671, H683, and R718 superimpose closely with comparable residues in the S1 domain RNA-binding clefts of PNPase (PDB accession code: 1SRO, 73 C^{α}, RMSD=2.0 Å), aIFα (PDB