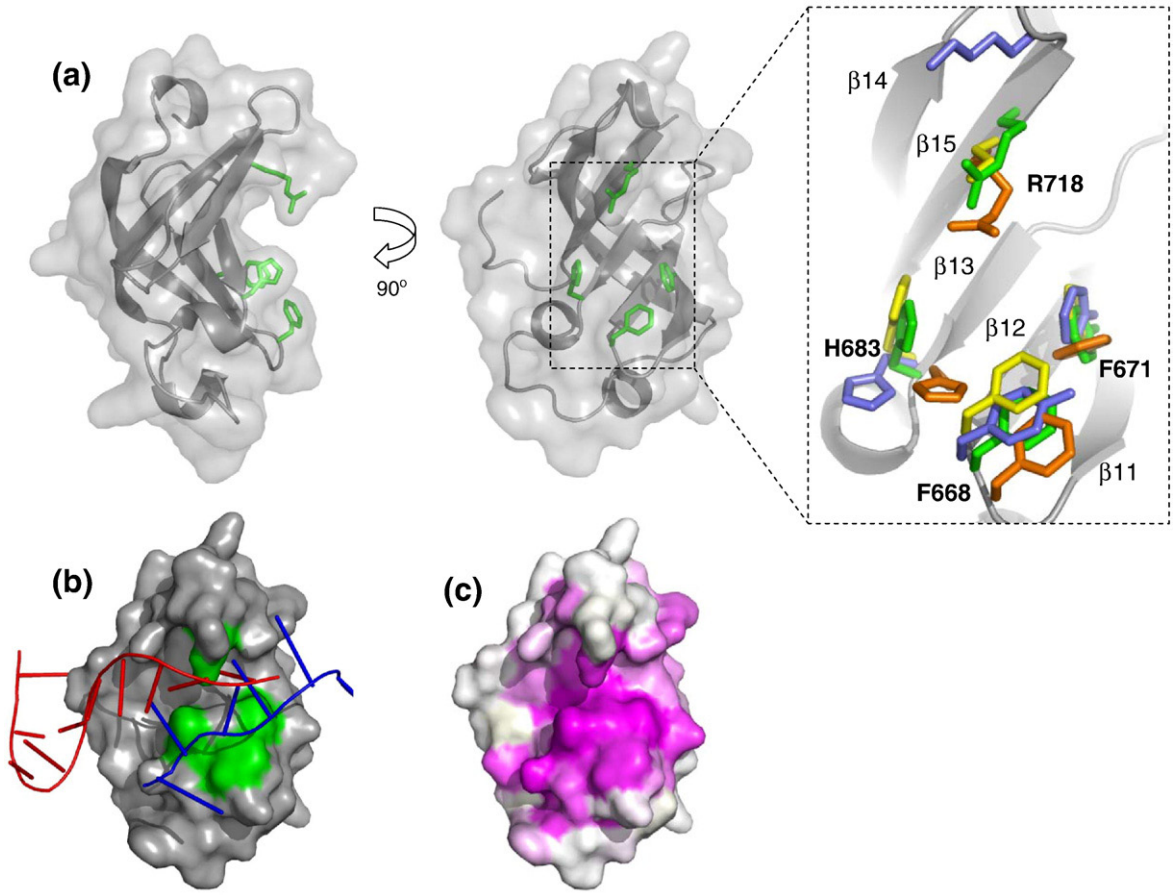

Fig. 5. The Tex S1 domain. (a) Two orthogonal cartoon representations with conserved residues R718, H683, F668, and F671 shown in green. Right, a close-up view of an alignment of conserved residues from structurally related S1 domains: green, Tex; orange, PNPaseS1 (PDB accession code: 1SRO); yellow, archealRPB4/7 (PDB accession code: 1GO3); blue, archealIFα (PDB accession code: 1ZY6). (b) A hypothetical model for RNA binding to the S1 domain binding cleft. The crystal structures of S1 domains with bound RNA from RNase E (PDB accession code: 2COB, red) and RNase II (PDB accession code: 2IX1, blue) were aligned with Tex S1 using DALI.[18] As illustrated, ssRNA binds the same face of the different S1 domains but considerable differences in detail are apparent. (c) Surface representation showing primary sequence conservation as assigned by the ConSurf server.[19] Conservation is indicated as a gradient from magenta (high) to white (low). In contrast to the view shown here, minimal conservation is observed on the opposite face of the S1 domain.

indicating a high degree of flexibility. In addition, very few contacts are observed in either structure between the S1 domain and the rest of the Tex protein. These observations indicate that the S1 domain is relatively unrestrained and able to adopt a range of orientations in solution.

Tex binds oligonucleic acids

Although the precise function of Tex is not known, the structural motifs observed in the Tex structure suggest binding to nucleic acids. It has recently been demonstrated by Southwestern and Northwestern analysis that recombinant *S. pneumoniae* Tex can interact with RNA and DNA,[3] although Tex–nucleic acid interactions have not been explored in detail. We therefore quantified the ability of Tex to bind various nucleic acid substrates using electrophoretic mobility shift assays. Random 25-mer ssDNA, dsDNA, ssRNA, and double-stranded RNA (dsRNA) sequences were tested for binding (Fig. 7). Tex bound all four types of nucleic acids, with a strong preference for ssRNA ($K_d = 210 \pm 50$ nM, $n = 9$), and binding affinities were not altered by the presence of Mg^{2+}. Binding to dsDNA ($K_d = 3720 \pm 150$ nM, $n = 3$), dsRNA ($K_d = 4200 \pm 147$ nM, $n = 4$), and ssDNA ($K_d = 5100 \pm 150$ nM, $n = 4$) was more than 10-fold weaker (Table 2). Binding to an RNA/DNA hybrid was also confirmed, though not quantified. Because Tex binds RNA and DNA chosen at random, including a poly-U ssRNA sequence (data not shown), binding appears to be sequence independent, although the possibility of some strong sequence preferences cannot be excluded at this time.

To supplement our electrophoretic mobility shift data and provide a solution-based estimate of binding, we used fluorescence polarization (FP). The K_d for 25-nt ssRNA binding to Tex was 56.6 ± 6.2 nM ($n = 5$). The approximately fourfold decrease in K_d compared to the value determined by electrophore-