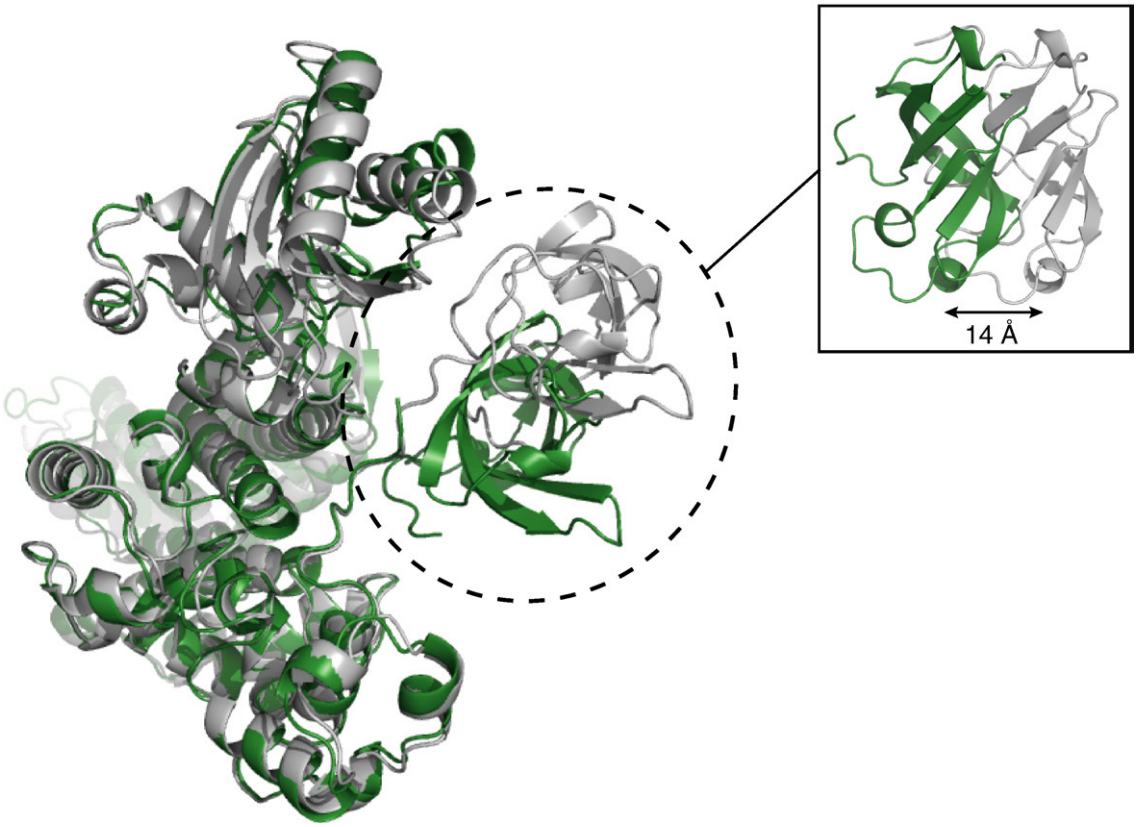

Fig. 6. S1 domain mobility. Significant rearrangement of the S1 domain is observed in different crystal forms of the Tex structure. The 2.5-Å (crystal form I, gray) and 2.3-Å (crystal form II, green) crystal structures are superimposed. A side view of the superimposed crystal structures (inset) highlights a 14-Å shift between the two S1 domains.

tic mobility shift likely results from differences in equilibrium considerations in solution studies *versus* gel-based electrophoretic methods.[33]

The length dependence of ssRNA binding was examined using 10-mer, 13-mer, 16-mer, 20-mer, and 25-mer sequences. Negligible differences in binding were observed between the 20-mer and 25-mer sequences, and a modest reduction in binding was observed for the 16-mer sequence. In contrast, the 13-nt ssRNA bound with markedly reduced affinity, and binding was not detected with the 10-mer sequence.

Our electrophoretic mobility shift assays reveal two distinct, shifted bands for ssRNA and ssDNA (Fig. 7). Although this may be an artifact of the native gel electrophoresis, it may also suggest non-stoichiometric binding or protein multimerization during the binding event. Studies of other proteins, such as RNase E and PNPase, have shown that S1-domain-mediated multimerization may be critical for substrate binding and enzymatic activity.[31,34] We therefore investigated Tex RNA-binding stoichiometry using FP and gel filtration. Apo Tex elutes from a gel filtration column as a single peak with a retention time expected for a monomer, even at a very high concentration. Similarly, a Tex:ssRNA complex, prepared by premixing Tex and an excess of 20 nt ssRNA, elutes from the sizing column as a single peak with the retention time expected for a 1:1 stoichiometry. FP was also used to estimate stoichiometry by titrating Tex protein into a solution containing a saturating concentration (>20-fold above K_d) of 25 nt ssRNA. Polarization values (P) were read at each Tex concentration, and the values were plotted as P *versus* the molar ratio of Tex to ssRNA. The inflection in this plot represents the point at which Tex has saturated all the binding sites on the RNA substrate. The inflection point for this experiment occurred at a molar ratio of 1:1, indicating that a single molecule of Tex binds one 25-nt ssRNA molecule (data not shown).

Oligonucleic acids bind the Tex S1 domain

Tex's preference for binding ssRNA made the S1 domain an obvious candidate for mediating binding of nucleic acid. In support of this possibility, we found that protein lacking the S1 domain (TexΔS1) was unable to bind ssRNA, dsRNA, ssDNA, or dsDNA in our electrophoretic mobility shift assays (Table 2). It is unlikely that the loss of nucleic acid binding is due to misfolding because the TexΔS1 protein behaves similarly to the full-length protein throughout the purification process, including gel filtration. Furthermore, significant repositioning of the S1 domain (with respect to the rest of the protein) in different Tex crystal forms has little effect on the rest of the Tex structure. These data indicate that although Tex binds a variety of nucleic acids