Supplementary Table 1. Apparent DNA binding affinities of LigIII proteins used in this study. DNA binding activity was measured in TR-FRET buffer containing 80 mM NaCl by fluorescence polarization. A FITC labelled DNA was used to form a 1:1 complex of LigIII bound to a DNA duplex.

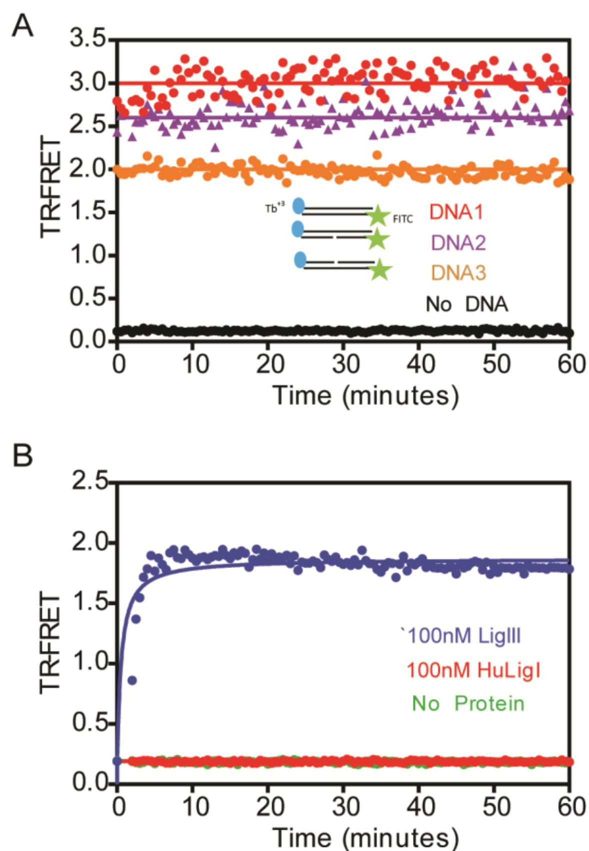
	K_{app} (nM)
LigIII	155 ± 4
ΔZnF LigIII	166 ±20
ΔOBD LigIII	308 ±13

Supplementary Table 2. Structural parameters for LigIII and DNA complexes from SAXS data

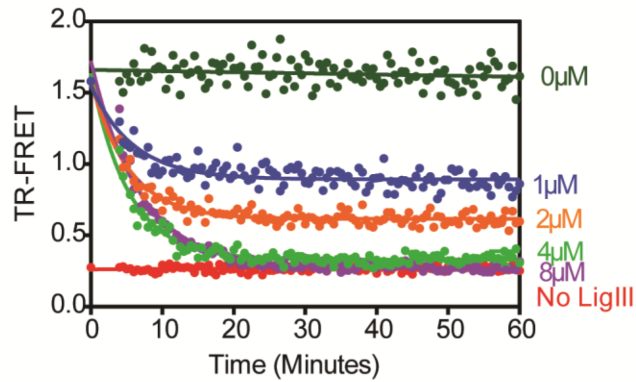
SAXS sample	D_{max} (Å)	R_g[#] (Å)
LigIII	~198	59
LigIII with DNA (3 nucleotide overhang)	~200	59
LigIII with a nicked DNA duplex	~200	59
ΔZnF LigIII	~130	38
ΔZnF LigIII with DNA (3 nucleotide overhang)	~117	34
ΔZnF LigIII with a nicked DNA duplex	~115	33
ΔOBD LigIII	~141	46
ΔOBD LigIII with DNA (3 nucleotide overhang)	~130	43
ΔOBD LigIII with a nicked DNA duplex	~125	40

[#] R_g is the radius of gyration calculated from the Guinier approximation. D_{max} is the maximum dimension of the protein estimated from the pair distribution (*cf.* Figure 6).

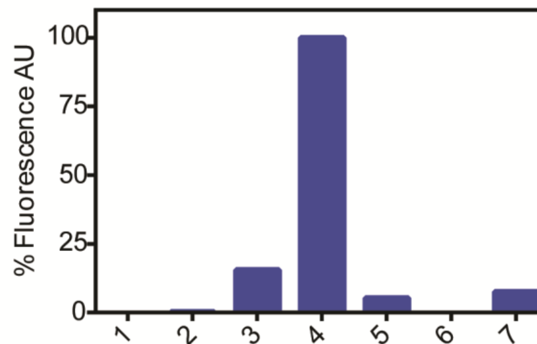
Supplementary Figure 1: Positive and Negative controls for the TR-FRET DNA bridging assay. **(A)** An intact DNA duplex (DNA1) labeled with Tb^{3+} and FITC on both 5' ends served as a standard to determine the maximum TR-FRET signal generated by our assay conditions. Dually labeled DNA1 (10 nM) was mixed with unlabeled DNA (500 nM) to mimic standard assay conditions. This sample produced a constant TR-FRET signal of 3.0, approximately 1.5- to 2-fold higher than the TR-FRET signal generated in a DNA bridging assay, which uses a limiting concentration of LigIII (see Figure 2 and Methods). Dual labeled, nicked DNAs (DNA2, DNA3) showed intermediate values of TR-FRET (2.0 to 2.5) that may reflect differences in the labeling efficiencies for these DNAs. **(B)** Human DNA ligase 1 (100nM) failed to generate a TR-FRET signal and lacks the zinc finger domain that is required for DNA bridging by LigIII.



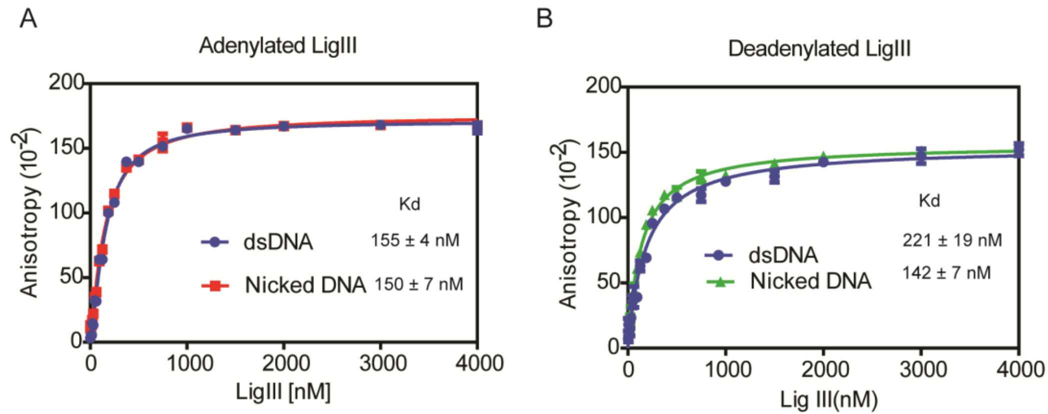
Supplementary Figure 2: Dissociation of labeled DNAs from the bridging complex. Addition of an unlabeled competitor DNA resulted in the loss of the TR-FRET signal with an apparent rate of 0.1 min^{-1} in the presence of a saturating amount of competitor DNA.



Supplementary Figure 3: Pull-down of blunt-ended DNAs in a bridging complex. The pull-down assay was performed as described in the Methods, using a pair of blunt-ended DNAs. LigIII forms a bridging complex with blunt-ended DNAs in a manner similar to the complex with three-nucleotide overhangs shown in Figure 8.



Supplementary Figure 4: DNA binding of adenylated and deadenylated LigIII. FITC labelled dsDNA (25 nM) or a nicked DNA duplex (25 nM) was titrated with increasing concentrations of (**A**) adenylated LigIII or (**B**) deadenylated LigIII. The formation of a protein-DNA complex is indicated by an increase in fluorescence anisotropy. Adenylated and deadenylated LigIII show comparable DNA binding activity.



Supplementary Figure 5: Intermolecular DNA ligation by LigIII. The time course of DNA ligation was monitored for substrates with complementary 3-nucleotide overhangs (**A**) or with blunt ends (**B**). The rate of blunt end ligation catalyzed by LigIII is significantly slower than the ligation of DNAs with complementary overhangs.