SUPPLEMENTARY MATERIALS

Figure S-1. ***Primer extension assay of DNAP in presence of Ca^{2+} and Mg^{2+} divalent metal cofactors.*** **A** and **B** show the extended products of 0mm-T construct (sequence shown at the top) with Klenow and Klentaq respectively. **A.** Primer extension in the presence of all dNTPs in Mg^{2+} (lane 1); primer extension in the presence of all dNTPs in Ca^{2+} (lane 2); free construct (lane 3). **B.** The left and the right panels represent products obtained in the presence of Mg^{2+} and Ca^{2+} respectively. Primer extension in the presence of all dNTPs (lane 1); primer extension in presence of dATP (lane 2); free construct (lane 3).

Figure S-2. ***CD spectra for Klenow-DNA complexes in Ca^{2+} and Mg^{2+} buffers.*** **A-D** represent the CD spectra for Klenow-ssP16-1 (**A-B**) and Klenow-P16-2 (**C-D**) complexes in the presence of Ca^{2+} (**A** and **C**) and Mg^{2+} (**B** and **D**) buffers. Color coding is the same for all the panels: dark green, ssP16-1; red, P16-2 construct; pink, P16-2 construct plus Klenow.

Figure S-3. ***Klenow and Klentaq DNA polymerases bound to matched P/T DNA at low [Mg^{2+}] conditions.*** **A-C** show the low energy CD of the complexes formed with 0mm-A construct and Klenow (**A-B**) and Klentaq (**C**) in different buffer conditions (see Experimental Procedures for buffer compositions). Color coding same for all the panels; dark green, ssP16-1; red, 0mm-A construct; pink, 0mm-A construct + DNAP in no Mg^{2+} buffer; purple, 0mm-A construct + DNAP in Mg^{2+} + EDTA buffer; blue, 0mm-A construct + DNAP in EDTA buffer; light green, 0mm-A construct + Klenow + dTTP in Mg^{2+} + EDTA buffer.

Figure S-4. ***Klenow binding to the P/T constructs exhibit a 1:1 stoichiometry.*** Panels **A-D** show calculated *c(s)* plots for Klenow polymerase in the presence and absence of various DNA constructs in different buffers. (**A**) Klenow alone in EDTA buffer. **(B)** Klenow plus the 3mm construct in Ca^{2+} buffer. (**C**) Klenow plus the 0mm-A construct in EDTA buffer. (**D**) Klenow plus 0mm-A construct in Ca^{2+} buffer. (**E**) The $s_{20,w}$ values and the estimated and calculated molecular weights for each of the above complexes. The molecular weights estimated from the s_{20w} values confirm that stoichiometric 1:1 complexes of Klenow were formed with the DNA constructs under all the buffer conditions used. The partial specific volume used for Klenow alone was 0.73 ml/g and that for the Klenow-DNA complexes was 0.695 ml/g. Sedfit analysis of Klenow alone and Klenow-DNA complexes yielded frictional ratios of ~1.1 and ~1.3 respectively, suggesting that both are roughly globular in shape. The rmsd values for all the fits were ~0.009.

Figure S-1.

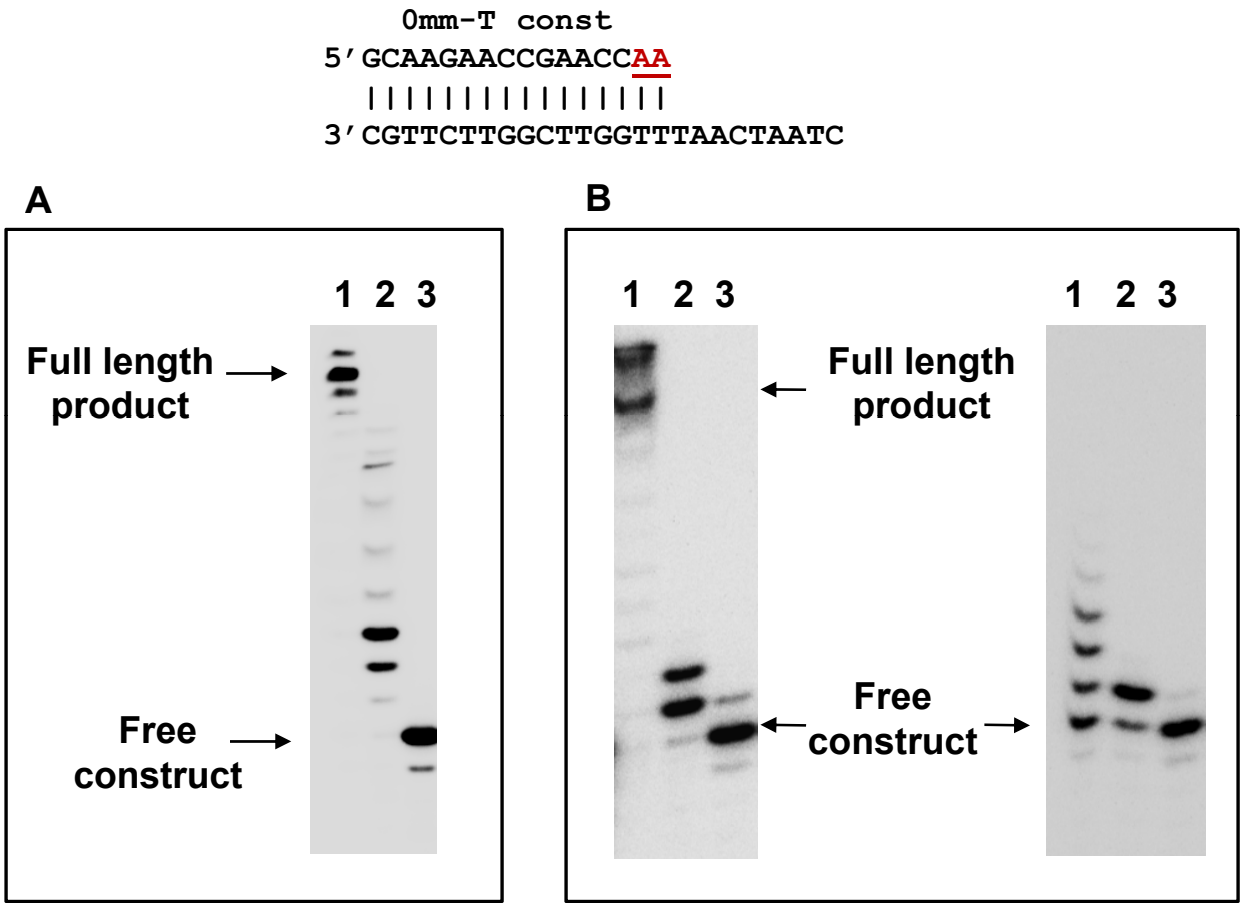

0mm-T const
5′GCAAGAACCGAACCAA
3′CGTTCTTGGCTTGGTTTAACTAATC
A
1 2 3
Full length product
Free construct
B
1 2 3
Full length product
Free construct
1 2 3

Figure S-2.

ssP16-1
5′GCAAGAACCGAACCAA

P16-2 construct
5′GCAAGAACCGAACCAA
|||||||||||||||||
3′CGTTCTTGGCTTGGTTAAACTAATC

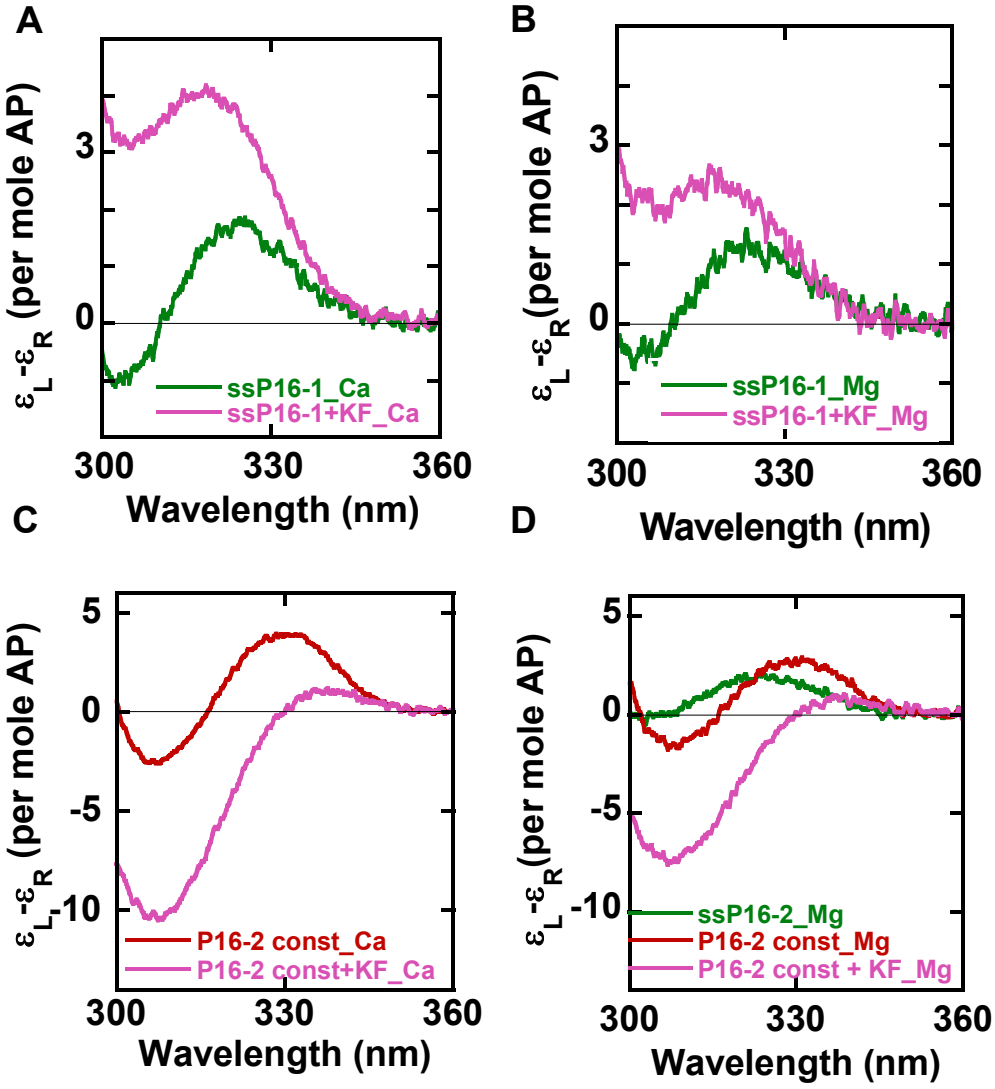


Figure S-3.

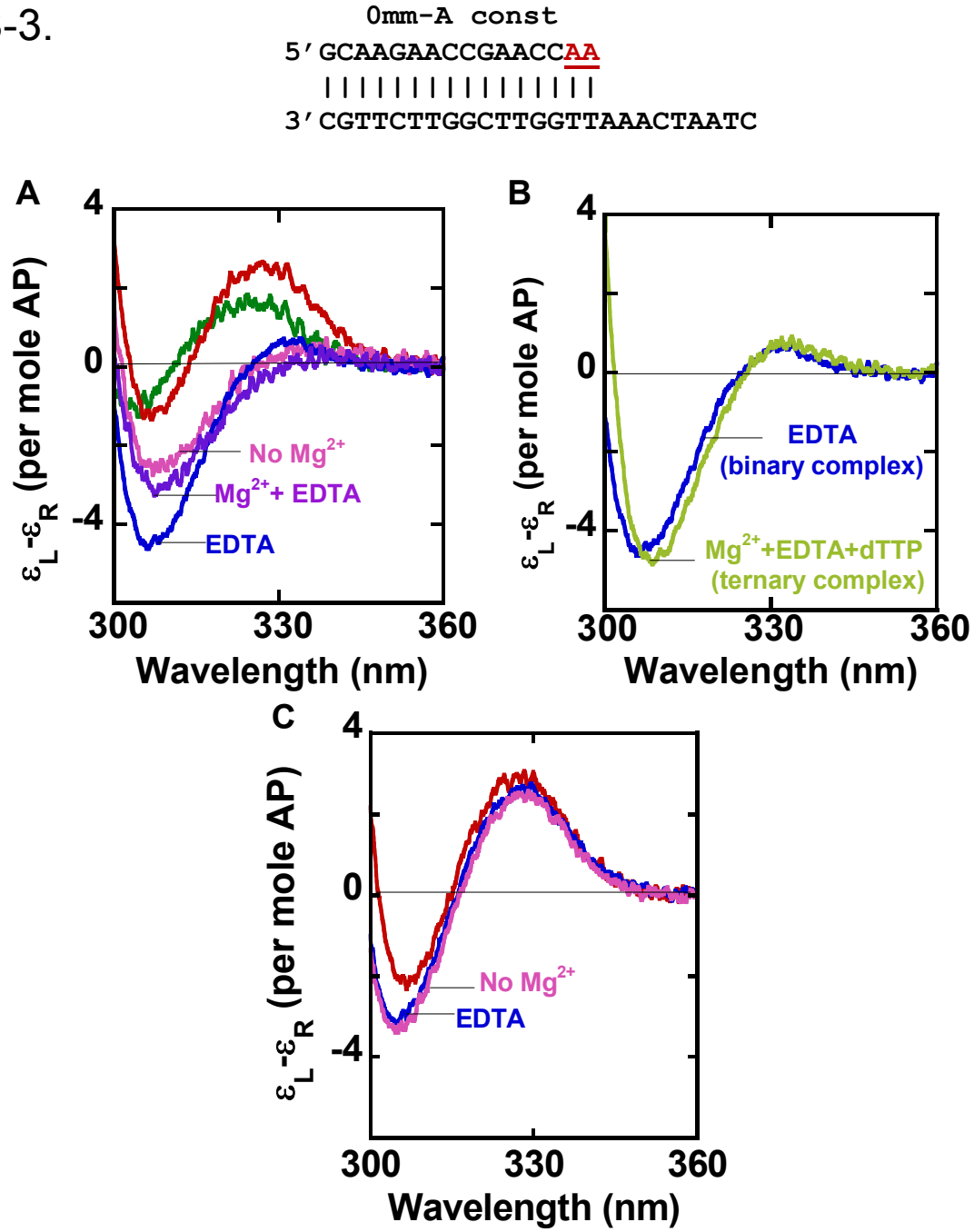
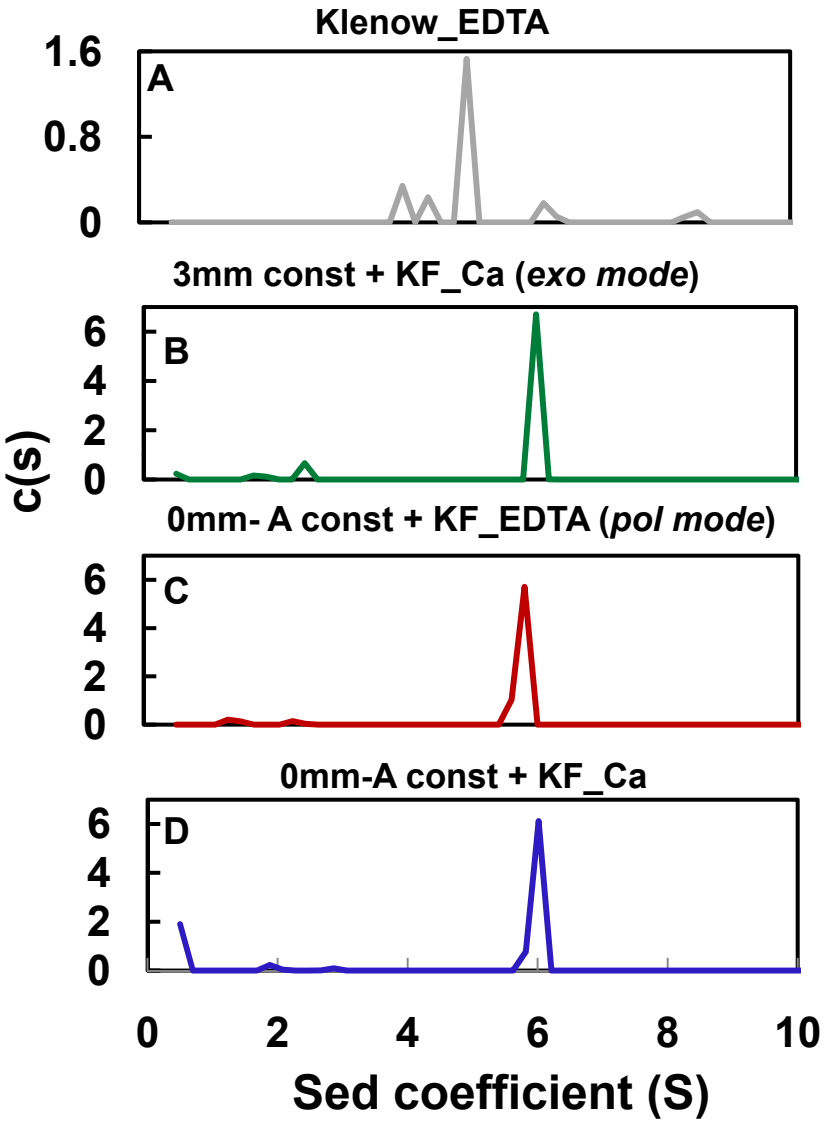

0mm-A const
5′GCAAGAACCGAACCAA
3′CGTTCTTGGCTTGGTTAAACTAATC
A
B
C
ε_L-ε_R (per mole AP)
4
0
-4
300
330
360
Wavelength (nm)
No Mg2+
Mg2++ EDTA
EDTA
EDTA
(binary complex)
Mg2++EDTA+dTTP
(ternary complex)

Figure S-4.



E

Sample	S_{20w} (S)	Estimated MW (Da)	Calculated MW (Da)
Klenow_EDTA	5.05 (±0.08)	68294	68000
3mm const+KF(Ca^{2+})	6.04 (±0.08)	80556	80469
0mm const+KF(EDTA)	5.82 (±0.11)	80901	80516
0mm const+KF(Ca^{2+})	6.02 (±0.10)	80200	80516