Fig 6 – Supplement 1: CMG displacement of streptavidin from ssDNA

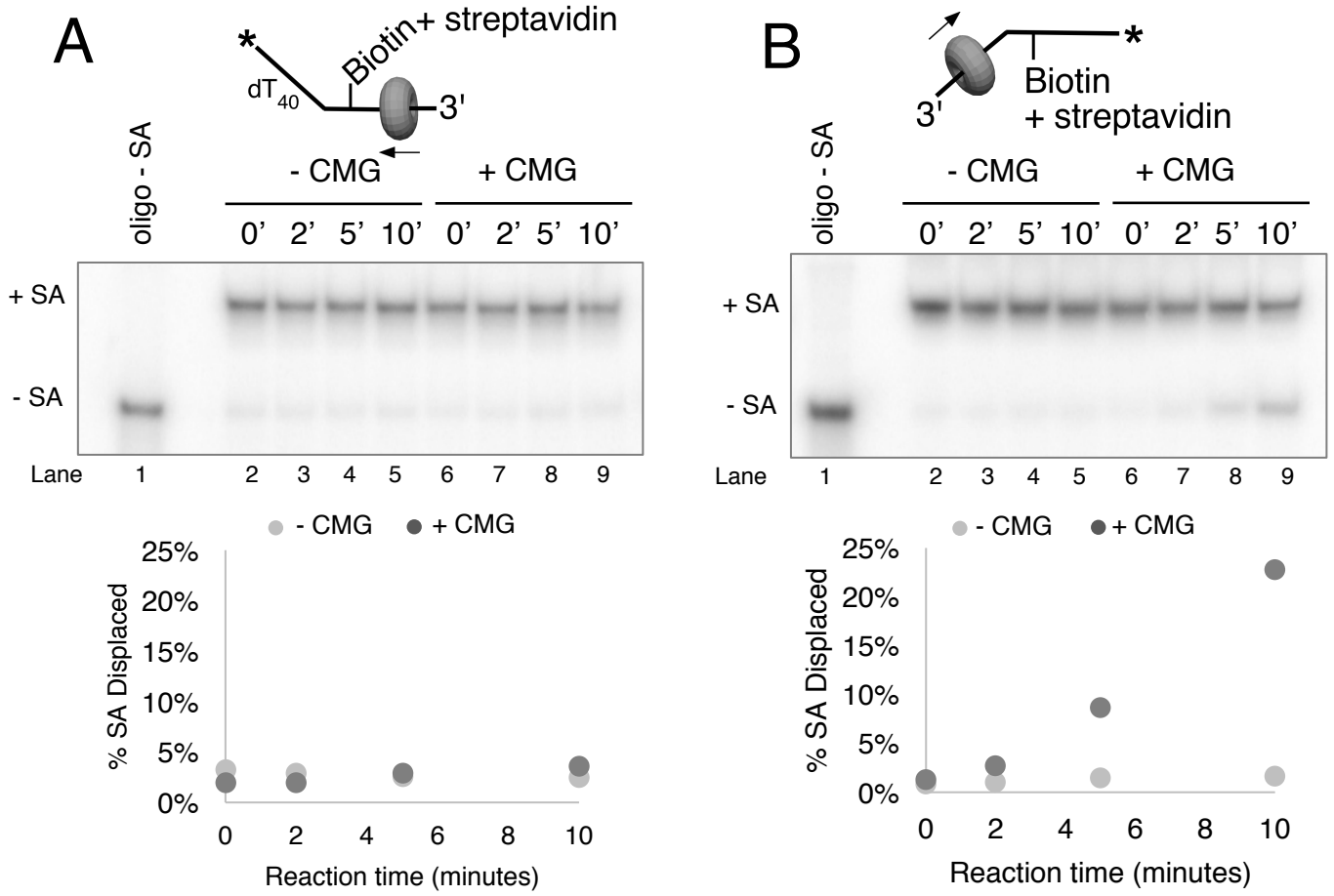

Figure 6 – Supplement 1: **CMG displacement of streptavidin from ssDNA.**
The ability of CMG to remove streptavidin from the lagging strand ssDNA (A) and leading strand ssDNA (B) was examined. In order to remove streptavidin from an ssDNA oligo, CMG must load onto the 3' end and translocate along ssDNA in order to displace streptavidin from biotin, presumably by the force of translocation. As such, this assay examines the efficiency with which CMG loads onto the 3' end of a given ssDNA. The radiolabeled ssDNA substrates (0.5 nM) were pre-incubated at 30°C for 5' in the presence of 2 μg/ml streptavidin and then placed on ice for 10' before addition of 25 nM CMG and 750 nM free biotin (final concentrations) to a total reaction volume of 60 μl. Reactions were started by incubating at 30° C and 12 μl aliquots were removed, stopped with EDTA/SDS, and flash frozen in liquid nitrogen at the time points indicated in the Figures.

(A) The ability of CMG to remove streptavidin from lagging strand template ssDNA was examined. The substrate is 50duplex LAG single biotin (see Table I) radiolabeled at the 5' end. Lane 1 shows the migration of the substrate in the absence of streptavidin and lanes 2-5 show a time course of spontaneous dissociation of streptavidin in the presence of the trap (no CMG). Lanes 6-10 show removal of streptavidin by CMG translocating along the ssDNA substrate in the presence of the trap. As shown in the graph below the gel, both spontaneous dissociation (- CMG) and active removal of streptavidin (+ CMG) are negligible over the 10' time course.

(B) The ability of CMG to remove streptavidin from leading strand ssDNA was examined. The reactions in (A) were repeated with 50duplex LEAD single biotin (Table I) radiolabeled at the 5' end as substrate. As shown in the graph below the gel, CMG removes streptavidin much more efficiently from leading strand ssDNA than from lagging strand ssDNA (A). Based on the results of Figure 2 – Supplement 1, we presume that the presence of dT_{40} at the 3' end of the leading strand oligo confers better loading than the dN_{50} at the 3' end of the lagging strand oligo in (A).