

Fig 5 – Supplement 1: Free Biotin Trap prevents binding of streptavidin to biotinylated oligo when added before streptavidin but not when added after streptavidin

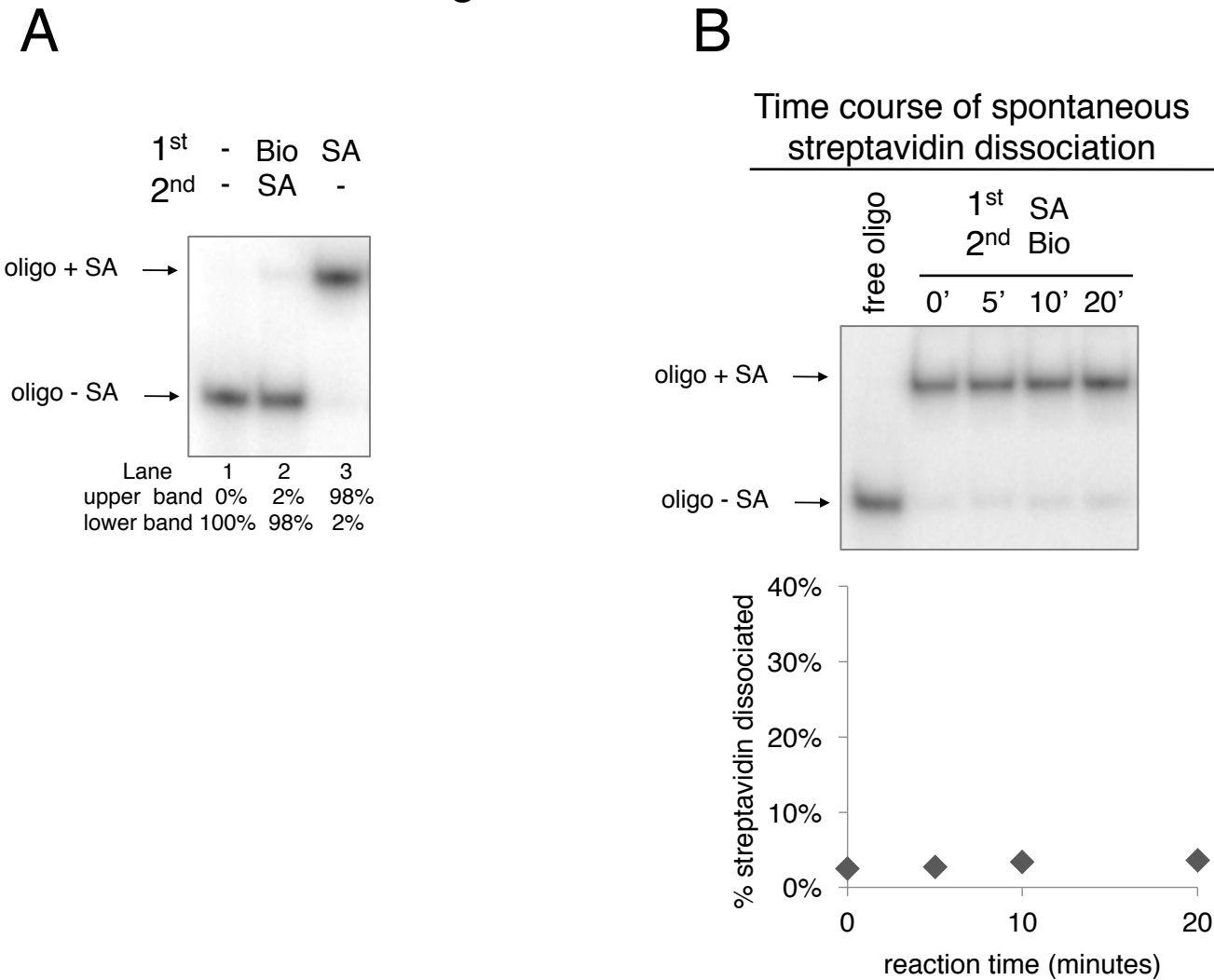
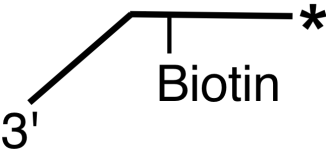


Figure 5 – Supplement 1: **Free biotin trap prevents binding of streptavidin to biotinylated oligo when added before streptavidin but not when added after streptavidin.**

(A) To validate the use of free biotin as a trap for displaced streptavidin, we examined the migration of the biotinylated leading strand ssDNA (50duplex LEAD single biotin, see Table I) in the absence of streptavidin (lane 1), when biotin is added before streptavidin (lane 2), and when streptavidin is added without biotin (lane 3). The radiolabeled substrate was present at 0.5 nM and streptavidin and free biotin were at 2 μg/ml and 750 nM respectively (final concentrations). The substrate was pre-incubated at 30°C for 5' with biotin (lane 2), streptavidin (lane 3) or neither (lane 1) and then placed on ice for 10'. Then streptavidin was added to the reaction in lane 2 and all three reactions were incubated at 30°C for 10' before loading on the gel.