



Supplementary Figure 1. Chase experiment with short sense and antisense oligonucleotides does not dissociate pre-formed Lasso-TNF685 target complex. Internally ³²P-labeled Lasso ALR229-5 was incubated in 10 mM MgCl₂ / 50 mM Tris-Cl (pH 8) for a total of 120 minutes at 37°C alone (lane 1), with non-radioactive 0.4 μM TNF-20 (lane 2), and with 0.4 μM TNF685 (lanes 3-5). Lane 4 is the same as lane 3 but chased with a 14-fold excess of competitor antisense RNA, anti-TNF-20 (20-nt) over TNF685. Lane 5 is the same as lane 3 but chased with 7-fold excess of competitor sense TNF-20 (20-nt) over TNF685. Samples were analyzed by 6% denaturing PAGE. Anti-TNF-20 sequence is identical to the antisense sequence incorporated into the Lasso. TNF-20 sequence corresponds to the sequence of the TNF_α mRNA targeted by these Lassos. Abbreviations: **Complex 2** is the Lasso complex with the long target (TNF685); **Complex 1** is the Lasso complex with the short target (TNF-20); **C** is the circular form of fully-processed Lassos; **UPL** is unprocessed pre-Lasso transcripts; **HPL** consists of semi-processed pre-Lassos; **LL** is 5' and 3'-processed, linear Lasso.