

## Assembly of hybrid dsDNA-RNA substrate.

1) 90nt synthetic oligos consisting of 21 nt ssDNA (black) and 69 nt RNA (red).

**AGCGGTATCAGCTCACTCATAGAUGCAGACAUAUAUGAUACAAUUUGAUCAGUAUAAAGAUAGUUAU  
GACCGGGCGCGGUGGCGCGGCC**

2) Synthetic 18 nt ssDNA for annealing with 21 nt ssDNA.

**TATGAGTGAGCTGATACC**

3) Annealing of 18 nt ssDNA (2) with 90 nt oligos (1) to create partial 18-dsDNA region (shown as blue thick lines) and 69 nt RNA tail.

4) Ligation of annealed 18-dsDNA containing 69 nt RNA end with 124 bp dsDNA fragment (shown as red thick lines), to get 145 bp dsDNA tag with 69 nt RNA tail.

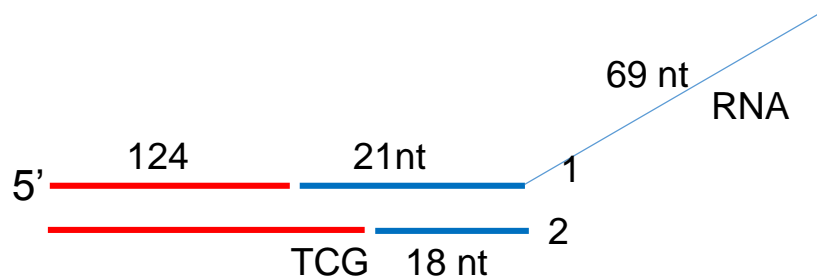


Figure S1. Schematic representation of 4 steps for the assembly of the dsDNA-RNA substrate.

## Gel electrophoresis data

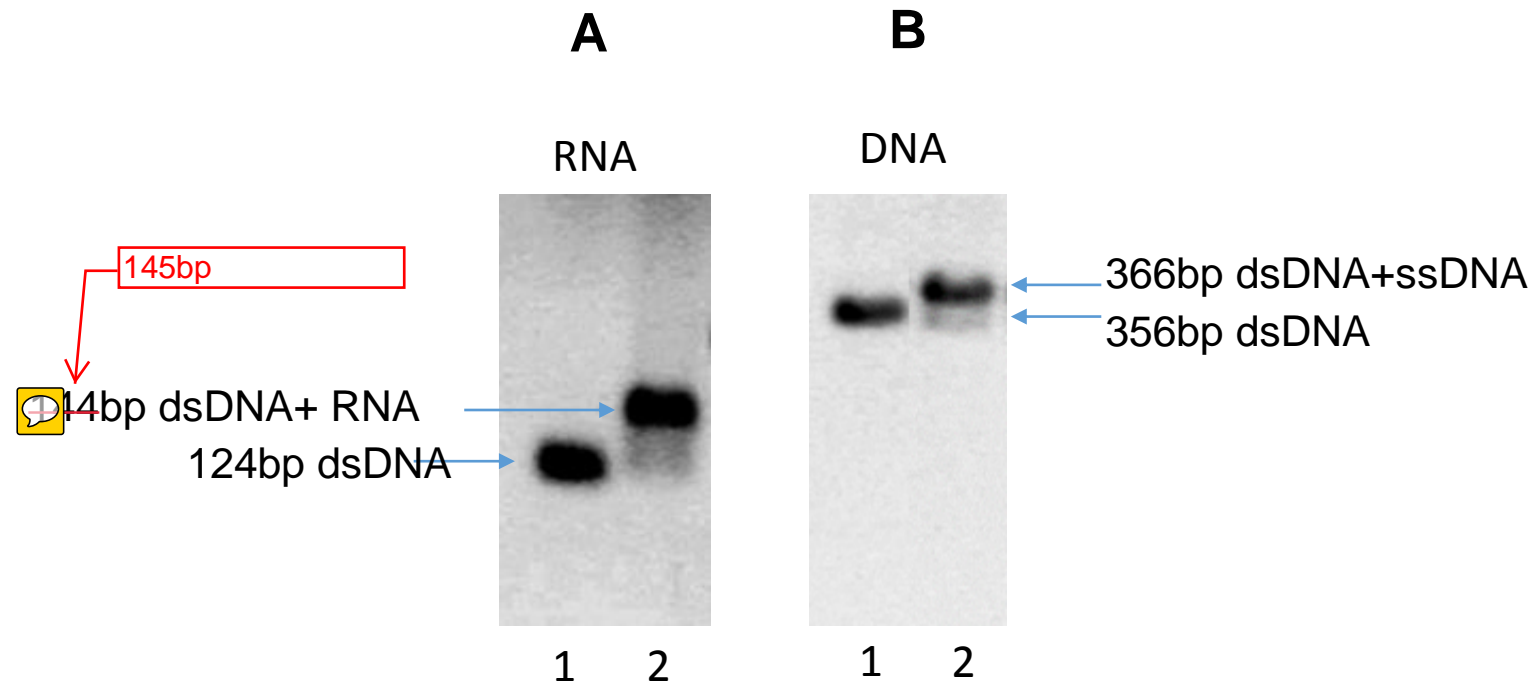


Figure S2. Gel electrophoretic mobility of the ligated products for RNA (A) and ssDNA (B) substrates. A- lane 1- dsDNA 124bp duplex and lane 2- duplex ligated with RNA. The yield of the ligated RNA hybrid is 78%. B- lane 1- dsDNA 356bp duplex and lane 2- duplex ligated with ssDNA. The yield of the ligated product is 90%. The length of the RNA and ssDNA tails in the hybrids is 69nt.

# Assembly of A3G complexes with RNA and ssDNA hybrids

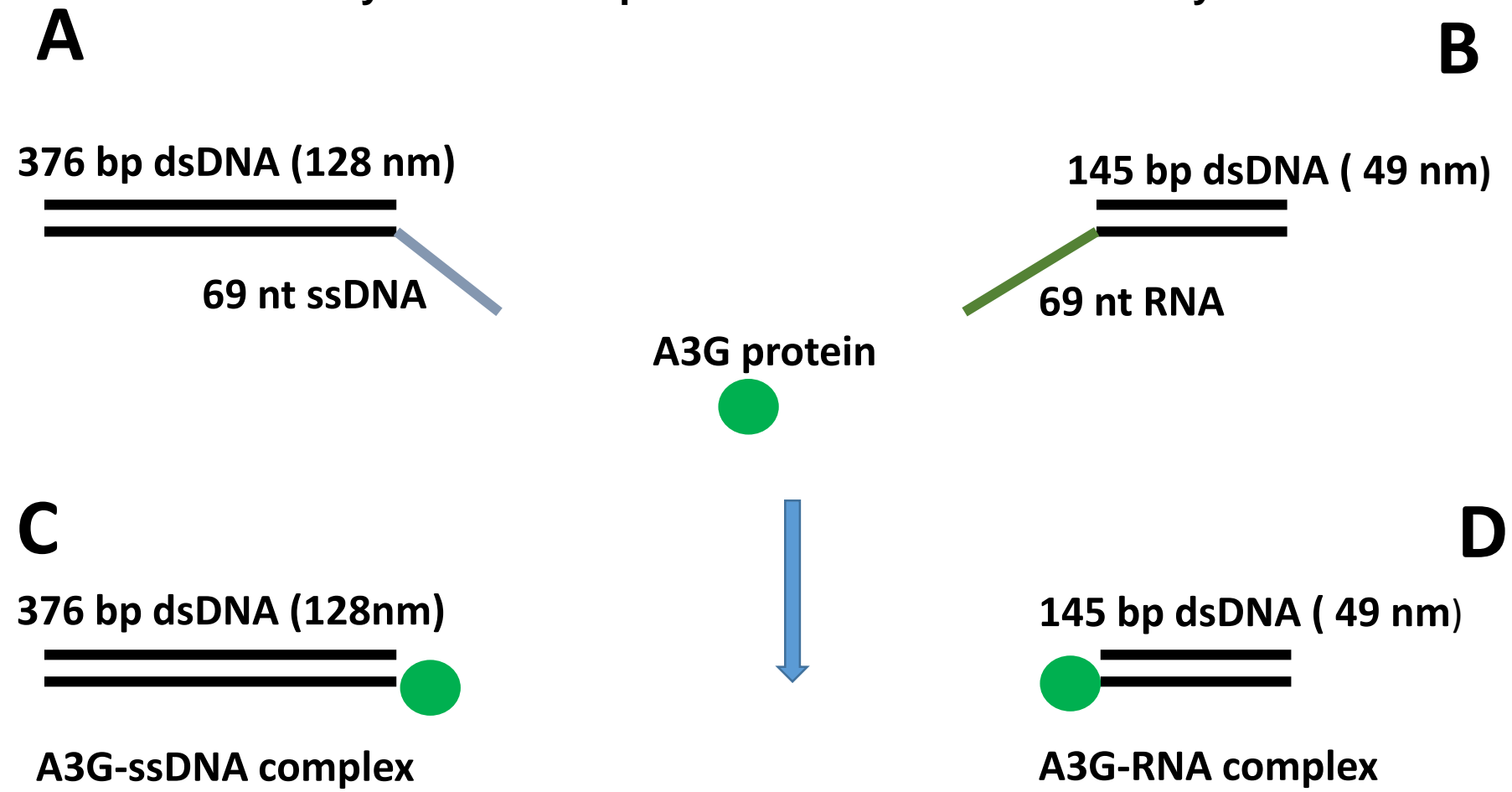


Figure S3. Assembled ssDNA hybrid (A) and RNA hybrid (B) consisting of a long dsDNA tag fused with 69nt ssDNA (A) and a shorter dsDNA tag fused with 69nt ssRNA (B). A3G protein (green ball) forms complexes with ssDNA hybrid (C) and RNA hybrid (D).

## AA sequences of NTD and CTD domains

sNTD-F126: Mw = 21656.47

After precision cut-

GPHMDPDTFSYNFNNRPILSRRNTVWLCYEVKTKGPSRPP  
LDAKIFRGQVYSEDKYHPEMRFLSLVSKWKLHRDQEYEV  
WYISWSPCTKCARDMATFLQENTHVTLTIFVARLYYFWDP  
DYQEALRSLAQAGATIKIMNYDEFQHCWSK FVYSQGAPFQ  
PWDGLDEHSQALSGRLGEILRHS

CTD-191-384-E259A: Mw = 23073.99

After precision cut-

GPHEILRHSMDPPATFTFNFNNEPWVRGRHETYL CYEVER  
MHNDTWVKLNQRRGFLANQAPHKHGFLEGRHAALCFLDVI  
PFWKLDLDQDYRVTCFTSWSPCFSCAQEMAKFISKNKHVS  
LCIKTARIYDDQGRAQEGLRTLAEAGAKISIMTYSEFKHC  
WDTFVDHQGAPFQPWDGLDEHSQDLSGRLRAILQNQEN

Figure S4. The amino acids sequences of CTD-191-384-E259A and sNTD domains.

**AFM images of DNA complexed with CTD-191-384-E259A and sNTD domains.**

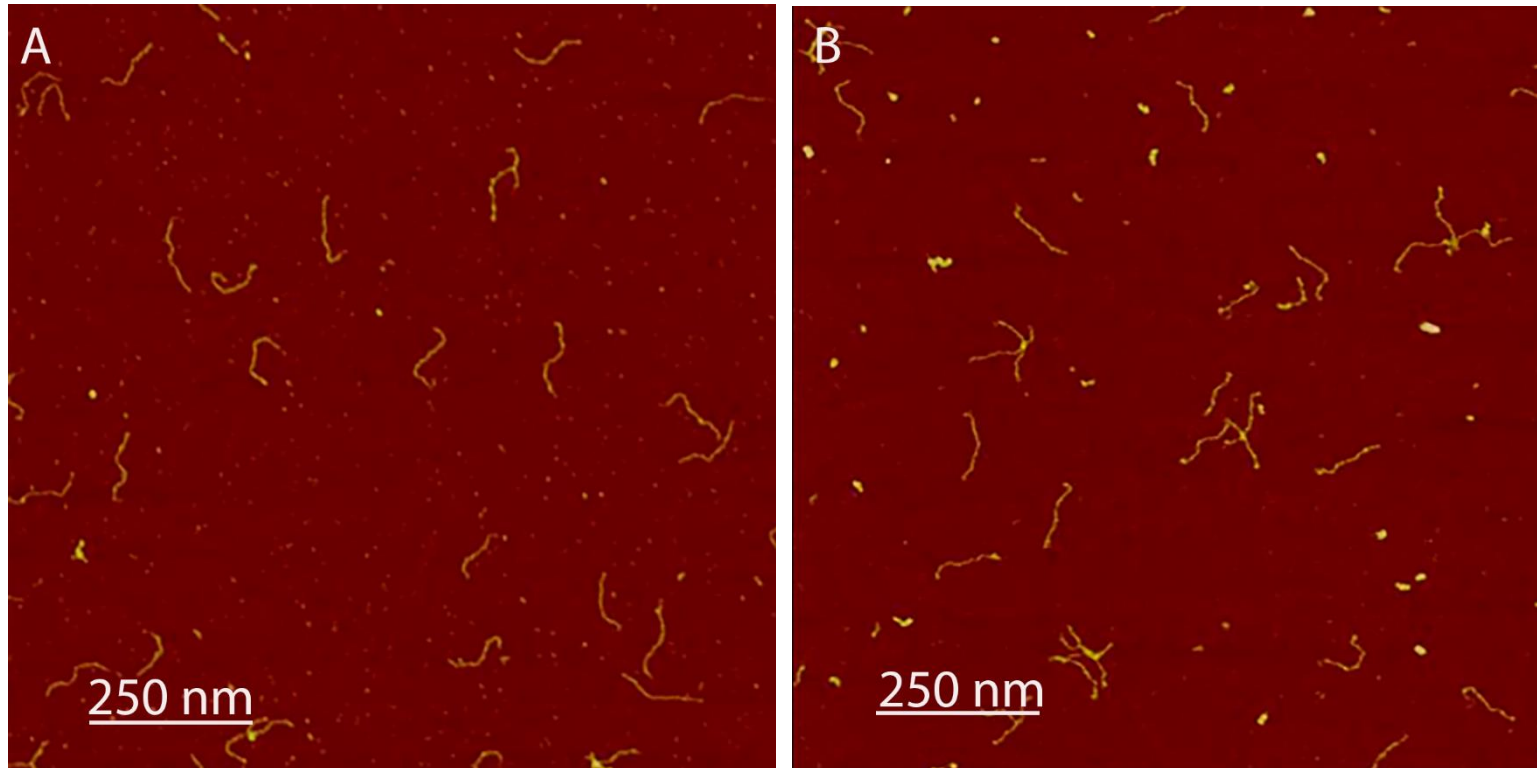


Figure S5. The ssDNA complexes formed with CTD-191-384-E259A (A) and sNTD (B) domains respectively. The protein to ssDNA ratio is 48:1.

## Histogram of protein volume for free A3G dissociated from A3G-RNA and A3G-ssDNA complexes

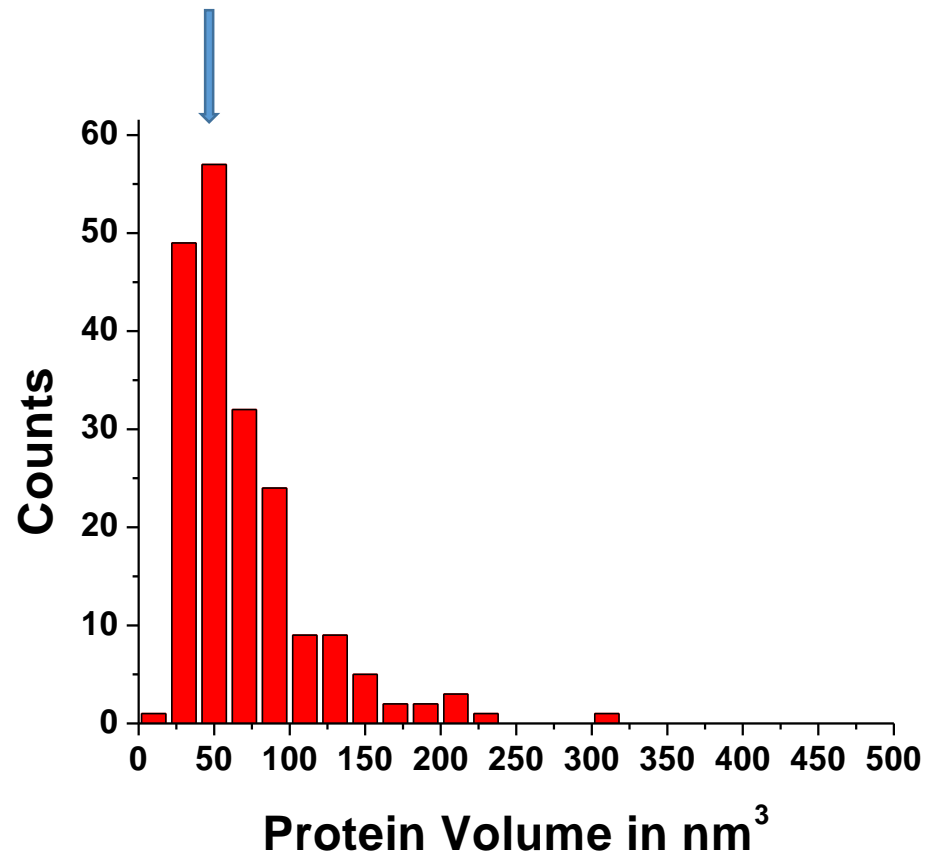


Figure S6. The protein volume of free A3G that dissociated from the complexes. Analysis of the data obtained from dry samples. The arrow points to the monomeric size of the protein.