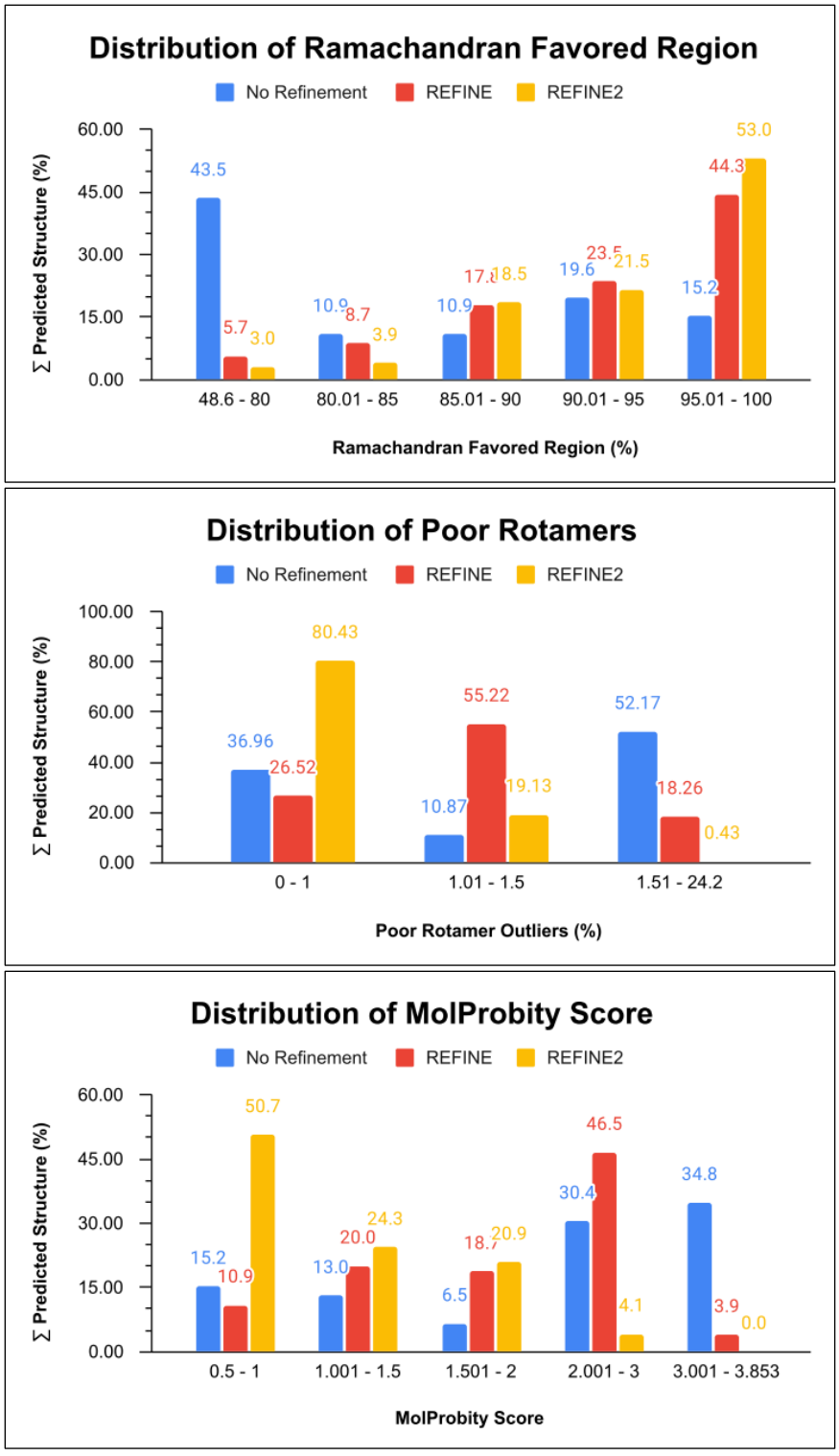

Figure S1. Distribution of 46 predicted and 690 refined structures by GalaxyWEB in three parameters: Ramachandran favored region percentage (top), poor rotamers (middle), and MolProbity score (bottom). In each bar chart, Galaxy's REFINE2 algorithm improve the sturcture quality significantly. Better quality are indicated by high percentage of Ramachandran favored region, low value of poor rotamers, and low MolProbity score.

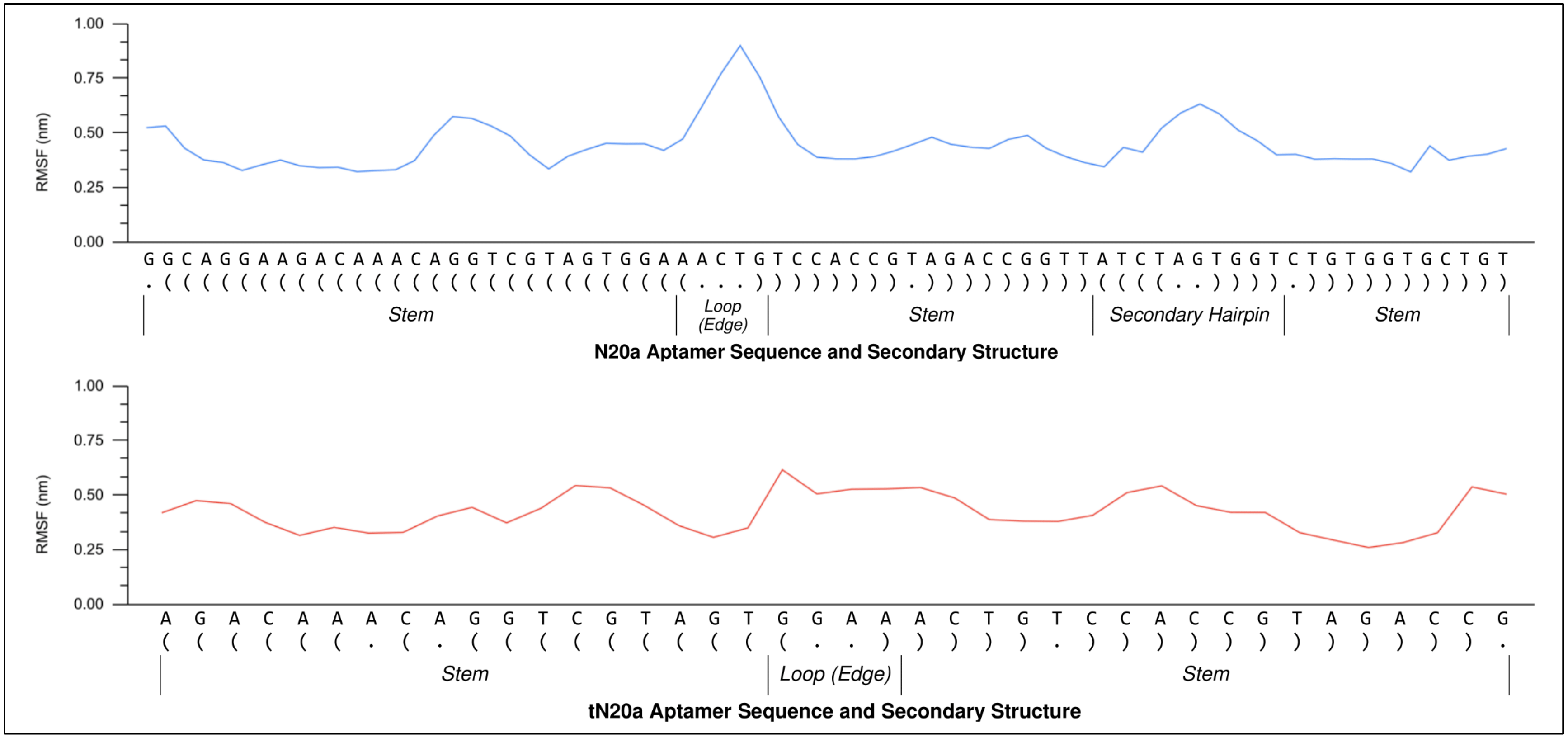

Figure S2. Root Mean Square Fluctuation (RMSF) of aptamers. The less stable regions of aptamer, which are demonstrated by higher RMSF than other regions, are predominantly contributed by unpaired bases in the loop structures. Aptamer truncation (secondary hairpin removal from N20a aptamer) results in tN20a, but its RMSF still shows high yet fluctuating plot indicating its unstability. Random mutations produced tN20a-fm32 and tN20a-sm14 aptamers which have relatively lower RMSF value means the stability are increased.

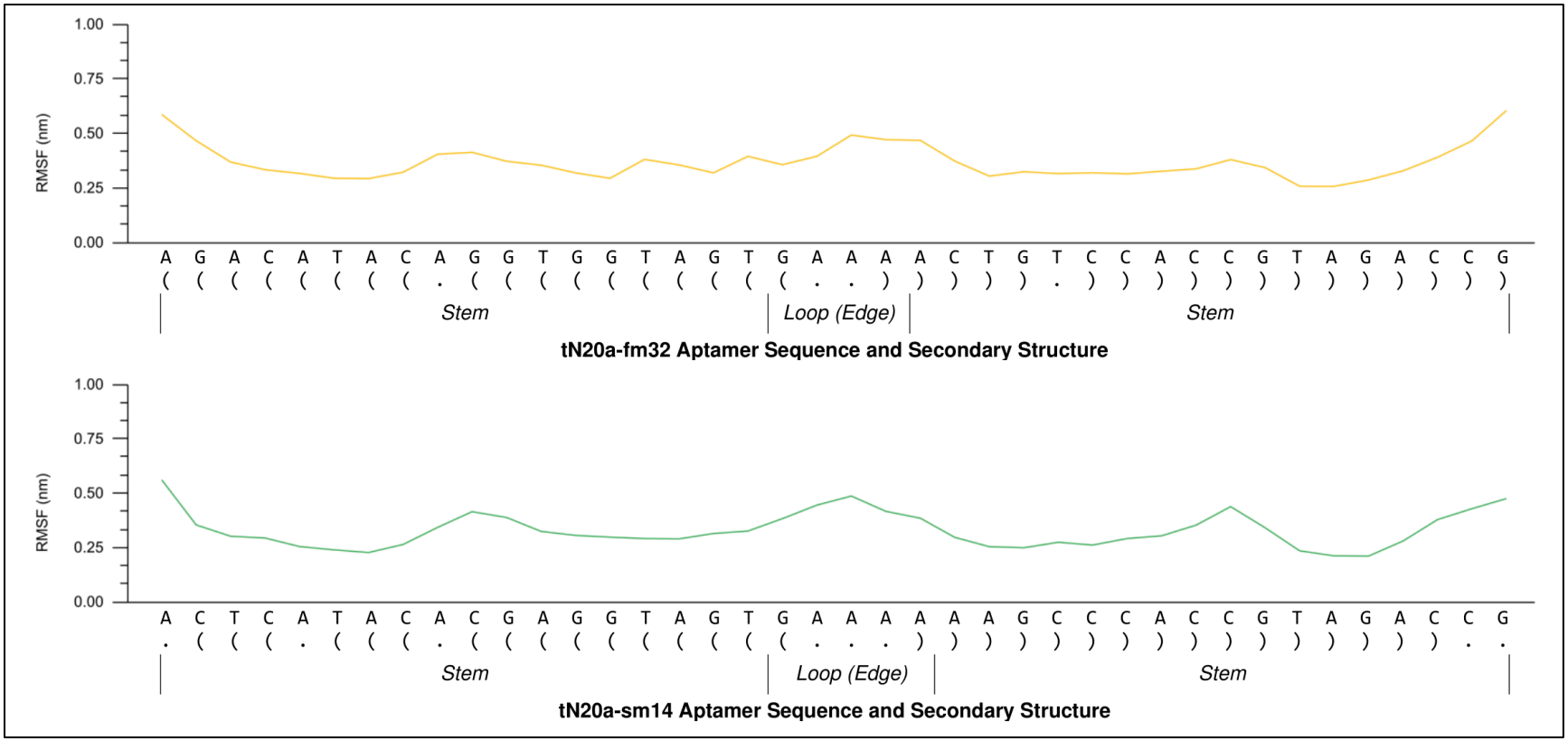

Figure S2. ***(cont.)*** Root Mean Square Fluctuation (RMSF) of aptamers. The less stable regions of aptamer, which are demonstrated by higher RMSF than other regions, are predominantly contributed by unpaired bases in the loop structures. Aptamer truncation (secondary hairpin removal from N20a aptamer) results in tN20a, but its RMSF still shows high yet fluctuating plot indicating its unstability. Random mutations produced tN20a-fm32 and tN20a-sm14 aptamers which have relatively lower RMSF value means the stability are increased.

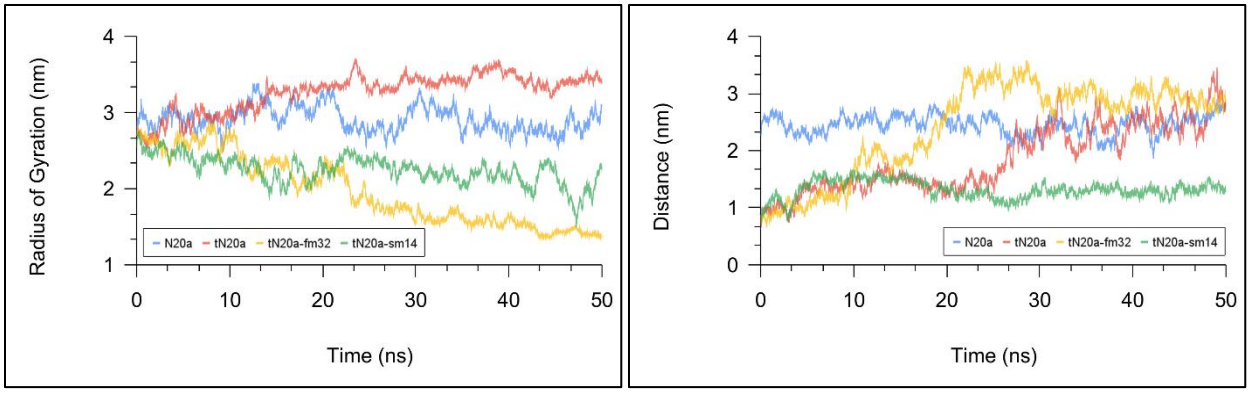

Figure S3. Additional analysis of structural stability between aptamer and NT-proBNP protein complex, including radius of gyration plot of protein (left) and aptamer-protein's center of mass plot (right). Radius of gyration indicates structure compactness. The tN20a-fm32 aptamer fail to maintain its complex stability as NT-proBNP compactness continued to decreasing whereas tN20a-sm14's complex have slightly similar trend of protein compactness with the native N20a aptamer, indicating the similar stability. Center of mass between aptamer and protein is also showing same trends as N20a and tN20a-sm14 plots have the most non-fluctuating graph during 50 ns MD simulation.

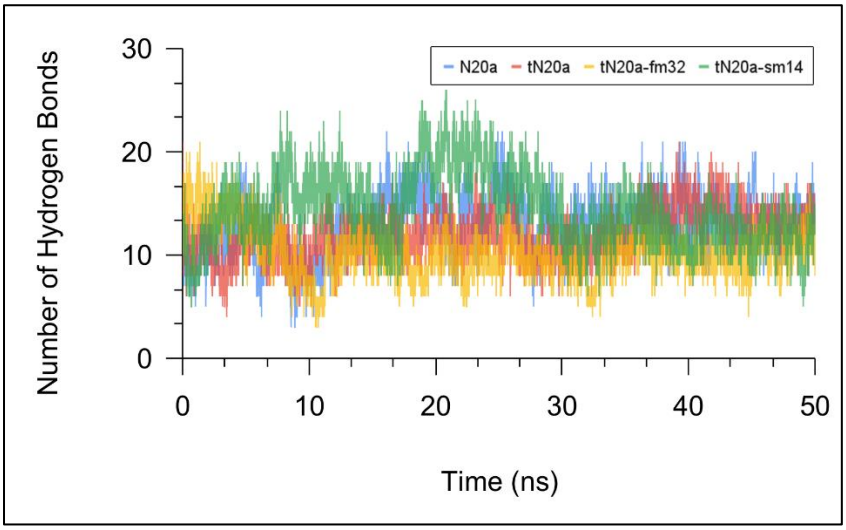

Figure S4. The number of contact hydrogen bonds plot between aptamer bases and NT-proBNP residues. The tN20a-sm14 system showed relatively higher plot with averages at 14.3 ± 3.5, the highest value among N20a, tN20a, and tN20a-fm32 with averages at 12.9 ± 2.8, 11.9 ± 2.4, 10.2 ± 2.5, respectively. As hydrogen bonds contribute to complex interaction, high number showed greater interactions strength.