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Type	Oral communication
Session	High Resolution RNA Computational Biology
Title	ASO design for the disruption of the NSP1 - 5’UTR interaction of SARS-CoV 2
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Motivation

The 5’ UTR of coronavirus genome contains five structurally conserved loops, namely SL1 to SL5. Among these, SL1 to SL3 are the most conserved and are involved in the interaction with the SARS CoV-2 nonstructural protein 1 (NSP1). The binding of NSP1 to RNAs harbouring the SARS-CoV-2 5’UTR favour the translation of viral subgenomic RNA compared to the host messenger RNAs [1].
The 5’UTR of SARS-CoV-2 has been found modified in two crucial sites, uridine 54 into pseudouridine (PSI) [2] and adenine 74 with m6A. Both these RNA modification change the structure of the RNA sequence and influence its RNA interaction propensity, influencing processes required for the virus lifecycle such as translation of viral subgenomic RNAs and viral replication [3]. In particular, the pseudouridylation of SARS-CoV-2 5’UTR enhances NSP1 binding to subgenomic viral RNAs [3].
Antisense oligonucleotides (ASOs) are synthetic nucleic acid molecules designed to complementarily bind to specific DNA/RNA regions, thus interfering with their biological function. They can be used as therapeutic instruments to modulate gene expression.
The aim of this analysis is to design ASOs able to bind to SL1 to SL3 sequence (SL1_3) of the SARS-CoV-2 5’UTR to affect the recognition and binding of NSP1 as strategy to impair viral replication.

Methods

SL1_3 sequence was obtained from NCBI (accession number NC_045512, nts 1 to 75) and 2D structure was built according to the literature [4].
The ASO sequences were designed using Soligo web server [5] (length 25) starting from SL1_3 sequence, and then all obtained ASOs were hybridized to SL1_3 using the RNAHybrid webserver [6]. For selected complexes the 2D structure was obtained via VFoldCPX webserver [7], and their 3D structure via IsRNA webserver [8].
On SL1_3 structure complexed with the ASOs, U54 was modified in PSI and m6A was applied on A74 of the complexes, to obtain SL1_3psi, SL1_3m6A, respectively, and both nucleotides, to obtain SL1_3psu_m6A. Wild type, psu, m6A, and psu&m6A SL1_3-ASOs complexes were simulated to obtain 100ns of molecular dynamics (MD) in explicit saline solution with Amber22, according to modrna08 and RNA.YIL forcefields. The simulation protocol, includes minimization, heating and equilibration, to sample the conformational space and evaluate the binding stability on the complexes. On MD trajectories, MM/GBSA analysis was applied to evaluate the binding affinity between RNA molecules, and DBSCAN was emplyed to cluster the trajectoies in order to obtain the most representative conformer. NSP1 viral protein 3D structure was obtained from RCSB (PDB_ID: 8AOU), and employed for SL1_3-ASO-NSP1 blind and binding site-based (using NSP1 interaction residues identified in Vankadari et al.[9]) docking via Hdock [8]. RNA-ASO-Protein complexes will undergo to minimization, equilibration and 100ns of production MD under standard protocol with Amber22 and amber14SB forcefield along with modrna08 and RNA.YIL.

Results

After obtaining the ASOs sequences starting from the SL1_3 one, the hybridization energies were obtained for all of them. Selecting complexes with hybridization energy:
$x \leq |mean| \pm SD$
eight ASOs were chosen (namely: 12-36,13-37,14-38,15-39,16-40,17-41,18-42, 43-67). These have an hybridization energy lower or equal to the threshold (-49 kcal/mol), -49.6, -52.8, -52.6, -51.3, -50.7, -49.4, -49.5, and -49 kcal/mol, respectively. The 2D structure was selected based on the minimum free energy among the predictions which respect the 5’UTR stem loops conformation. The first 3D prediction of IsRNA was selected for the wt complex, and psi and m6A modifications were applied on these conformers. All of them underwent to MD simulation as depicted in Methods section. On MD trajectories, MM/GBSA analysis was applied to evaluate the binding affinity between RNA molecules, and DBSCAN was emplyed to cluster the trajectoies in order to obtain the most representative conformer. These conformers will be subject to blind and binding site-based (using NSP1 interaction residues identified in Vankadari et al.[9]) docking with NSP1 via Hdock. A selection of the SL1_3-ASO-NSP1 output docked complexes, based on geometric criteria, to select ASOs that interfere in SL1_3 – NSP1 binding, will undergo to MD as specified in the Methods section.

Info

[1] Cell 2020, 183, 1325–1339

[2] Molecular Therapy: Nucleic Acids, 2023, 34
[3] bioRxiv 2022. 10.1101/2022.10.17.512569
[4] RNA Biol. 2021; 18(4): 447–456
[5] Nucleic Acids Research, 2004, Vol. 32; Web Server issue: W135–W141
[6] Nucleic Acids Research, 2006, Vol. 34(Issue suppl_2): W451–W454
[7] PLoS One. 2016; 11(9): e0163454
[8] J Chem Theory Comput. 2021 Mar 9; 17(3): 1842–185
[9] Nucleic Acids Res. 2017 Jul 3; 45(Web Server issue): W365–W373
[10] J Phys Chem Lett. 2020 Nov 19;11(22):9659-9668

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Figure

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Availability	-

Dissemination Material

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Summary

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