Molecular modelling and simulation studies studies to validate the binding of SELEX-derived high-affinity aptamers to the Ebolavirus nucleoprotein

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Ebola is a fatal, highly infectious and one of the deadliest viruses in the world, with a mortality rate of 90%. The 2014 outbreak, in West Africa, was the largest and most complex Ebola outbreak since the virus was first discovered in 1976. There were more cases and deaths in this outbreak than the ones that occurred in 1976, 2013, 2016, 2018, 2020 and 2021, respectively. Reverse transcriptase-polymerase chain reaction (RT-PCR) has been the standard molecular diagnostic modality for Ebola virus disease. However, it is time-consuming as it takes 2-6 hours, requires trained personnel, is expensive (USD 100) and requires large volumes of whole human blood. Immunoassays and lateral flow assays (LFAs) can be alternative diagnostic tools, though they make use of antibodies that are timely and expensive to produce and have batch-to-batch variations. WHO has called upon the scientific community to come up with improved, sensitive and specific rapid diagnostic devices that could be used at the point- of-care (POC). Recently, DNA aptamers have emerged as superior alternatives to antibodies, based on their high binding affinities, improved sensitivity, no batch-to-batch variations, their stability in extreme temperatures and because they are cheap to produce. Using systemic evolution of ligands by exponential enrichment (SELEX), three aptamers (Apt1, Apt2 and Apt3) were isolated experimentally that bind with higher specificities and affinities to the Ebola viral nucleoprotein (EBOVNP). Microscale thermophoresis (MST) reported dissociation constants (KD) of 39, 56 and 140 nM for Apt1, Apt2 and Apt3, respectively. These experimental findings were validated by *in silico* methods using molecular docking and molecular dynamics (MD) simulations. Briefly, the EBOVNP and the aptamers were prepared by Phyre2, RNAfold and RNAComposer web-based softwares. Their qualities were assessed and validated using SAVES web server and PYMOL software, before being subjected to HADDOCK web server for *in silico* docking calculations. Phyre2 web-based software generated one complete model of EBOVNP with a confidence coverage of >90%, while RNAfold and RNAComposer yielded high-quality RNA 3D models. SAVES web server assessed and validated the modelled EBOVNP, while PYMOL aligned the nucleic acid residues of the aptamers and HADDOCK yielded bound clusters. Based on the Z-score (-2.4, -1.3, -1.6, -1.8 and -1.3), number of hydrogen bonds (11, 13, 14, 2 and 5) and salt-bridges (8, 1, 6, 1 and 7), Apt1 bound with a stronger affinity to the EBOVNP, followed by Apt3 and finally Apt2. Interestingly, the molecular dynamic simulation analysis was in agreement with the docking results, with the EBOVNP-Apt1 complex being more stable and displaying less flexible regions compared to Apt2 and Apt3 EBOVNP complexes. Similarly,

higher non-bonded interaction energy values were found for EBOVNP-Apt1 complex having - 809.7kcal/mol, compared to EBOVNP-Apt2 and EBOVNP-Apt3 each having -700.7kcal/mol and
-599.4kcal/mol), respectively. Our methodology can therefore be used as a guide for aptamer selection before wet lab binding validation studies. In addition, using *in silico* approach, aptamers would be selected within few days and cheaply, and this technique would lead to the development of an aptamer-based LF diagnostic device, which would promote early identification of infected patients, and subsequently interrupt the chain of viral transmission to allow quick provision of treatment programs.

Keywords: SELEX, free minimum energy, dissociation constant (KD), molecular docking calculations, molecular dynamics (MD) simulations, hydrogen bond, salt-bridges, RMSD, Z- score and HADDOCK score.