Antiviral proteins targeting Influenza A hemagglutinin: design, production and characterization

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In recent years we have felt the devastating impact of viral pandemics, highlighting the need for preparation for future pandemics. Antiviral biologics, including small proteins that bind to and block viral targets, are promising therapeutic options that should be explored to increase pandemic preparedness.
One of the viruses with high pandemic potential is influenza, the causative agent of flu. Despite being characterized by annual seasonal epidemics, global pandemics caused by this virus have occurred sporadically and unpredictably1. In the Influenza virus, the fusion of the viral and host membrane (a crucial step in infection) is elicited by hemagglutinin A (HA), a homotrimeric glycoprotein, which engages the virus with sialic acid receptors at the host cell surface and is a privileged target for antivirals2. The focus of this work is the design of Virus-Targeting Antibody-like scaffolds (ViTAIs), which can bind to HA, thereby blocking Influenza A entry into host cells. The design is based on innovative strategies that combine knowledge-based and physics-based computational methods to generate tens of thousands of ViTAIs, which are ranked according to relevant parameters, such as binding free energy and shape complementarity. The folding stability and conformational dynamics of selected designs are studied through molecular dynamics simulations to obtain a deeper knowledge of their properties and discard candidates that are predicted to be unstable. The candidates that pass all the computational filters are then produced in bacteria and tested using a platform based on biolayer interferometry to assess their binding affinity for the target and on differential scanning fluorimetry to evaluate their thermal stability. Those that have a high binding affinity and high thermal stability are produced in higher amounts and characterized using biophysical techniques and will subsequently be validated using in vitro neutralization assays. This work contributes to the development and validation of an innovative strategy that can be applied to tackle a broad range of viruses, including influenza A.