IMI2 Project ID 101005077

CARE – Corona Accelerated R&D in Europe

WP 2 Target-based drug discovery and design

D2.3 - Crystal or Cryo-EM structures of the nsp14 and Spike protein

A crystal or cryo-EM structure of i) the nsp14 with or without the nsp10 co-factor at a resolution lower than 2.5 Å, allowing structure-guided inhibitor screening and design (M60); 2) the Spike protein either in complex with a peptidic/non-peptidic inhibitor or with different conformation along the fusion process(M60)

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Document History

Version	Date	Description
1.00	20 Feb 2025	Final report on D2.3 v1
2.00	27 Mar 2025	Final report

The CARE project has received funding from the Innovative Medicines Initiative 2 Joint Undertaking (JU) under grant agreement No 101005077. The JU receives support from the European Union's Horizon 2020 research and innovation programme and EFPIA and BILL & MELINDA GATES FOUNDATION, GLOBAL HEALTH DRUG DISCOVERY INSTITUTE, UNIVERSITY OF DUNDEE.

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Abstract

A crystal or cryo-EM structure of the nsp14 with or without the nsp10 co-factor at a resolution lower than 2.5 Å, allowing structure-guided inhibitor screening and design

The Replication/Transcription Complex (RTC) of the Coronavirus viral genome has an error correction system mediated by a 3'-to-5' exonuclease (ExoN). This function is carried out by the non-structural protein 14 (nsp14), regulated by the co-factor nsp10. Nsp14 is a bi-functional multi-domain protein. N-terminal is the ExoN domain and C-terminal is an N7-Methyltransferase (MTase) domain involved in the process of forming the viral RNA (vRNA) cap structure: 5'-viral RNA cap 0. These two enzymatic activities make nsp14 an interesting therapeutic target in the fight against Coronaviruses.

Obtaining a high-resolution structure by crystallography would enable docking and co-crystallization assays for inhibitor research. In contrast to SARS-CoV-1, no crystallogenesis conditions for the whole nsp14, or the nsp14:nsp10 complex of SARS-CoV-2, have yet been published, due to the high flexibility of the ExoN and N7-MTase domains of nsp14, as well as a poorly structured amino-terminal domain in the absence of nsp10.

To reduce this flexibility, three camelid 'nanobodies' (or VHHs) targeting nsp14 were produced, and their affinities were characterized by Bio-layer interferometry (BLI) against nsp14 and the nsp14:nsp10 complex. The influence of these nanobodies on the enzymatic activities of the nsp14:nsp10 complex was also studied. The various possible complexes were analysed by SEC-MALS and SEC-SAXS. Structural data showed that the nsp14:nsp10:vhh1 complex is possible in solution, but that during crystallogenesis, nsp10 is excluded in favour of a complex consisting solely of nsp14-VHH1.

This work enabled us to obtain the structure of the entire nsp14 of SARS-CoV-2 stabilized by VHH1 at 2.4 Å resolution, and we demonstrated that the VHH does not alter the activity of the enzyme. Structural screening of potential inhibitors against of ExoN and MTase activity of nsp14 have been attempted by soaking and/or co-crystallisation without affecting diffraction. The structure and SAXS data were deposited at PDB under code 9H01 and SASDB under codes *SASDVU8/SASDVV8/SASDVW8/SASDVX8* respectively. A paper is being written.

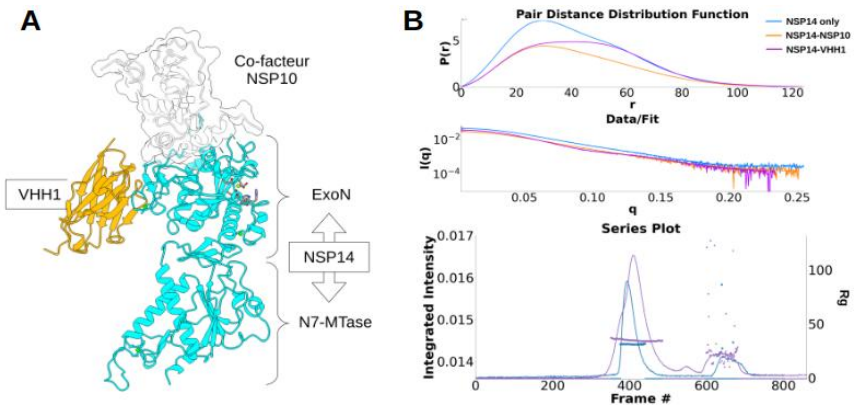

Figure 1. A) Crystallographic structure of the NSP14-VHH1 complex (2.4 Å) superimposed on the NSP10 structure (7DIY). B) SEC-SAXS characterization Top. Graph names for NSP14 (blue), NSP14-NSP10 (orange) and NSP14-VHH1 (purple) Bottom. SEX-SAXS profiles of NSP14 (blue) and NSP14-VHH1 (violet), and analysis of Radius of gyration (Å) versus frame number.

The Spike protein either in complex with a peptidic/non-peptidic inhibitor or with different conformation along the fusion process

UU have developed cyclic peptide inhibitors of the SARS-CoV-2 spike protein and used cryo-EM to determine their structure and mechanism of action. The first of these was published in 2023 (PMID: 37339194) – see Figure 2A. Following this, UU embarked developed 96 derivatives of this peptide and selected three molecules with improved potency and solubility, H04, H04-P4S and I8V+V15T. Synthesized monovalent peptides were tested by JU for i) activity against authentic SARS-CoV-2, both Wuhan and BA.2.86 variant. ii) Toxicity in A549 and Vero cells. Subsequently, the efficacy of these monovalent peptides was tested in HAE cells. At a fixed concentration of 10 uM, all peptides inhibited replication of the Wuhan and BA.2.86 isolate. To understand the structural basis for their improved potency, cryo-EM structures were determined for the three monovalent peptides. The results of these analysis indicate that peptide H04 binds to an altered quaternary binding site in which the NTD has rotated inwards and the RBD has rotated upwards (Figure 2B).

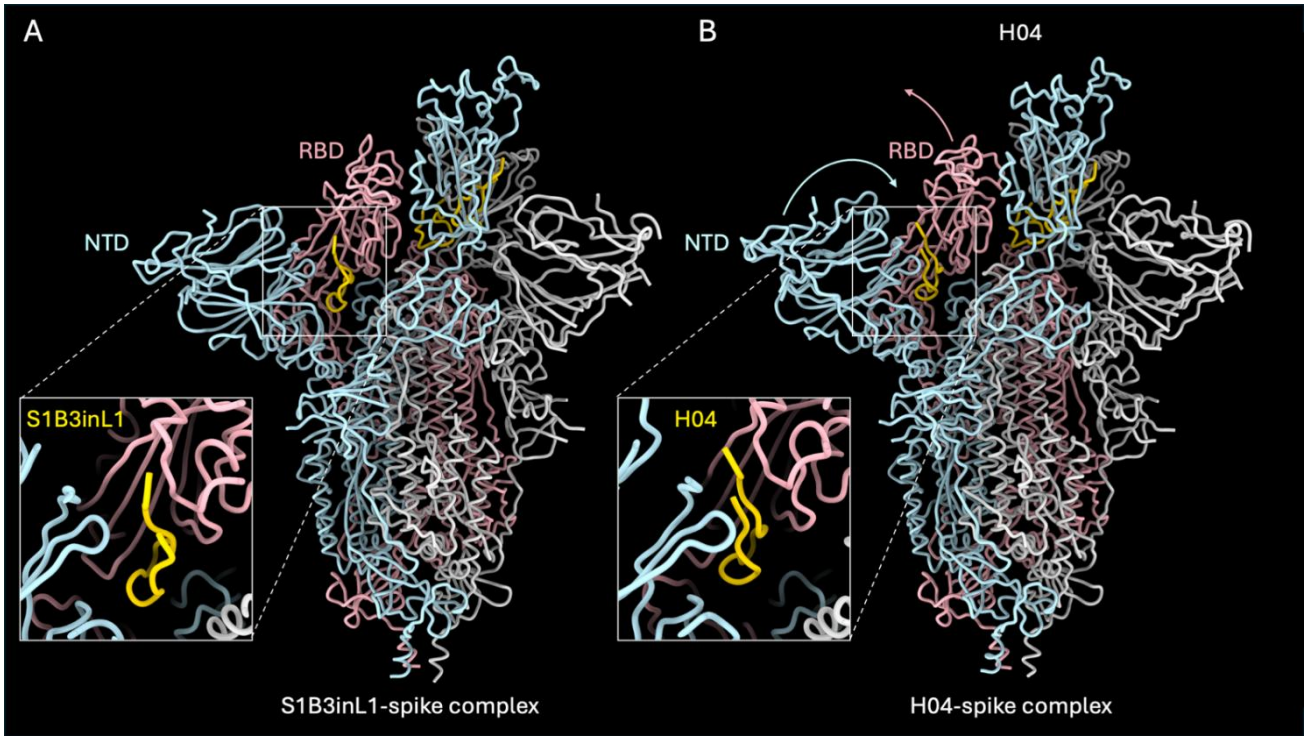

Figure 2) (A) Atomic model of the SARS-CoV-2 spike in complex with the parental macrocyclic peptide, S1B3inL1 (PMID: 37339194). (B) New structure of SARS-CoV-2 spike in complex with the more potent H04 peptide. Differences in the orientation of the spike RBD and NTD are observed between the two complexes.