Poster

Crystallographic structure of whole nsp14 from SARS-CoV-2 stabilised by a nanobody

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The Coronavirus Replication/Transcription Complex (RTC) is composed of 7 proteins, in addition to the viral polymerase. One of these proteins is the non-structural protein 14 (nsp14), a bi-functional multi-domain enzyme.

The N-terminal domain of nsp14 is a viral 3'-to-5' exonuclease (ExoN), which enables the correction of errors generated by the polymerase. Its activity is regulated by the cofactor nsp10. The C-terminal N7-Methyltransferase (MTase) domain is involved in the formation of the 5' cap structure (cap 0) of viral RNA (vRNA), ensuring RNA stability. These two enzymatic activities make nsp14 an interesting therapeutic target in the fight against Coronaviruses [1].

Obtaining crystallogenesis conditions would enable co-crystallisation screening campaigns to be carried out in search of inhibitors. However, No conditions for the crystallogenesis of whole nsp14, or of the nsp14:nsp10 complex of SARS-CoV-2 have yet been published, probably due to the flexibility between the ExoN and N7-MTase domains of nsp14, as well as a poorly structured N-terminal domain in the absence of nsp10.

To reduce this flexibility, camelid 'nanobodies' (or VHHs) targeting nsp14 were produced. We used biophysical analysis to characterise their affinities for nsp14 and the nsp14:nsp10 complex, as well as their effects on the enzymatic activities of the two nsp14 domains. SEC-MALS-SAXS structural data showed that the nsp14:nsp10:VHH complex was transiently possible in solution, but that during crystallogenesis, nsp10 is excluded in favour of a complex consisting solely of nsp14-VHH.

Here we present the structure of the nsp14:VHH complex at 2.4 Å resolution. This has enabled us to define the conditions for structural screening of potential inhibitors of the ExoN [2] and MTase [3] activities of nsp14. The search for potential applications for these anti-nsp14 VHHs is underway.

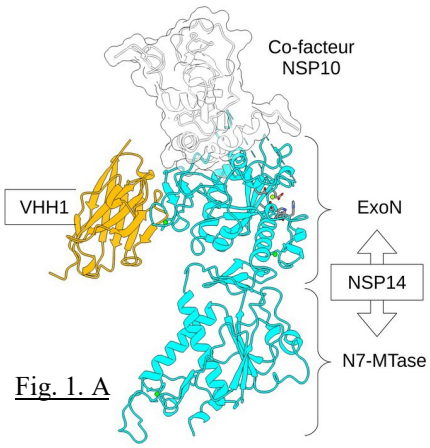

Fig. 1. A

Fig. 1. B

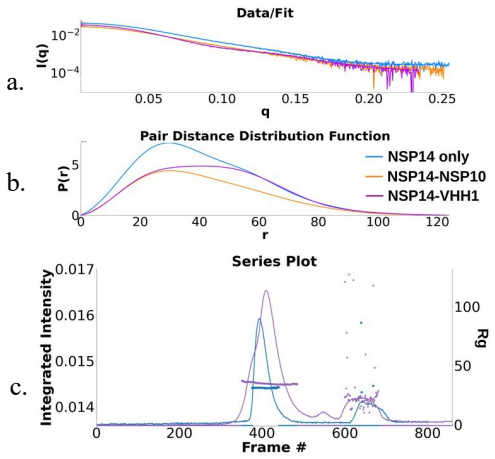

Figure 1. **A.** Crystallographic structure of the nsp14 (cyan)-VHH complex (yellow). The theoretical position of nsp10 in the nsp14:10 complex is shown on the surface (white) (7DIY). **B.** SEC-SAXS characterisation **a.** Guinier plot **b.** P(r) function **c.** SEC-SAXS profiles and analysis of Radius of gyration (Å) versus frame number. nsp14 (blue), nsp14-VHH (violet) and nsp14-nsp10 (orange).

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