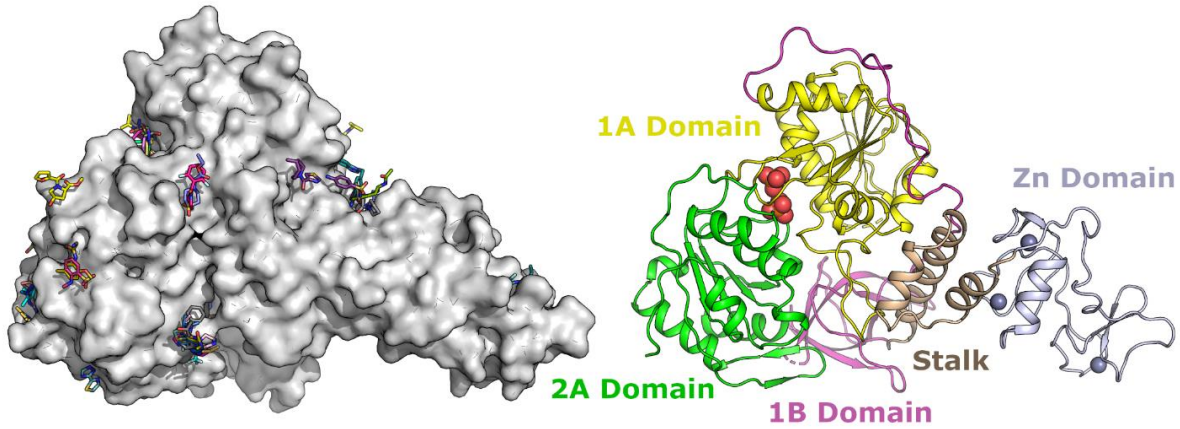
NSP13 Helicase crystal structure and XChem fragment screen

Summary



To contribute towards the development of novel anti-viral therapeutics targeting the current and future emerging coronavirus threats the Gileadi lab at the University of Oxford together with the XChem team at Diamond Light Source have teamed up to perform a crystallographic fragment screen against SARS-CoV-2 NSP13 helicase.

NSP13 is a large 67 kDa protein that utilizes the energy of nucleotide triphosphate hydrolysis to catalyze the unwinding of double stranded DNA or RNA in a 5' to 3' direction. NSP13 is a Upf1-like helicase from Superfamily 1B, that contains 5 domains: a N-terminal Zinc binding domain that coordinates 3 structural Zinc ions; a helical "stalk" domain; a beta-barrel 1B domain; and two "RecA like" helicase subdomains 1A and 2A that contain the residues responsible for nucleotide binding and hydrolysis.

Whilst its precise role in the viral life cycle is not currently well-defined, it is believed to act in concert with the replication-transcription complex (NSP7/NSP8$_2$/NSP12), conceivably involved in either disrupting downstream RNA secondary structures or template switching, and playing an essential role in the life cycle of SARS-CoV-2.

Previously, crystal structures of NSP13 were solved for MERS-CoV and the highly related SARS-CoV, to 3.0 Å and 2.8 Å respectively. These indicated that the protein may be crystallizable, although the resolution of these structures would have been too low to reliably detect fragment binders in a X-ray Fragment screen.

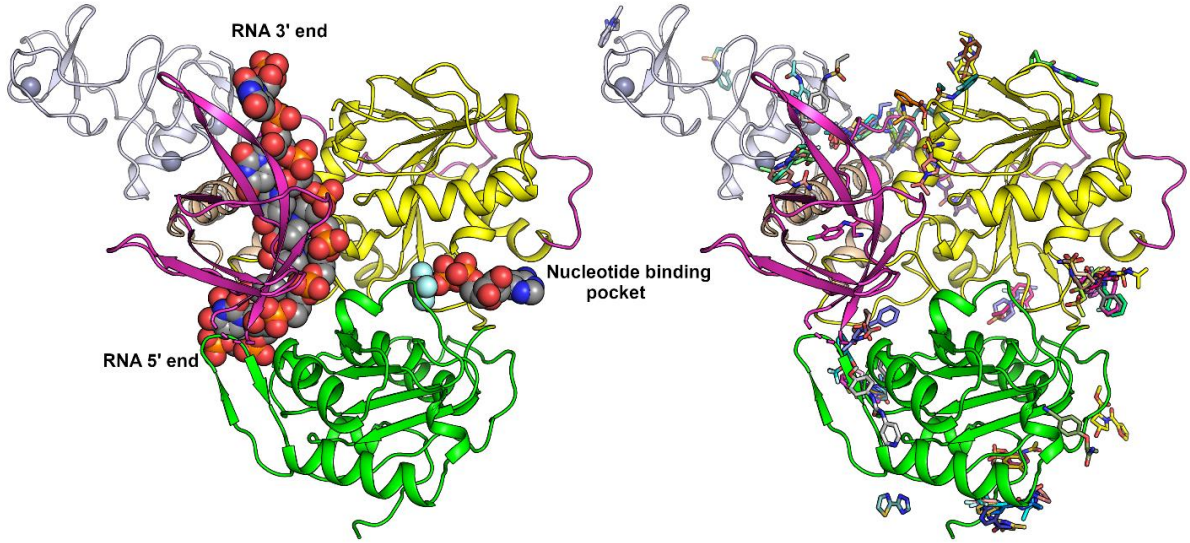
We began lab work on this project in mid-April and by using previous structures to define domain boundaries, were rapidly able to obtain a new crystal form for the full-length protein that is reproducible and diffracts routinely to around 2.0 Å. We deposited the SARS-CoV-2Nnsp13 structure in the pdb (6ZSL).

Potential sites of inhibition of NSP13 include the nucleotide binding pocket, the DNA/RNA binding pocket, and putative allosteric pockets that might block domain movements that are required as part of the NSP13 catalytic cycle. Initial druggability analysis indicates both nucleotide and DNA/RNA binding pockets as being druggable, and amongst the most well conserved pockets in the entire SARS-CoV-2 genome.

XChem Fragment Screen

Details about the purification and crystallization of NSP13 can be found here. The protein was produced and optimized crystallization and seeding conditions were found in the Gileadi lab at the University of Oxford. Further crystallization, crystal mounting, crystal

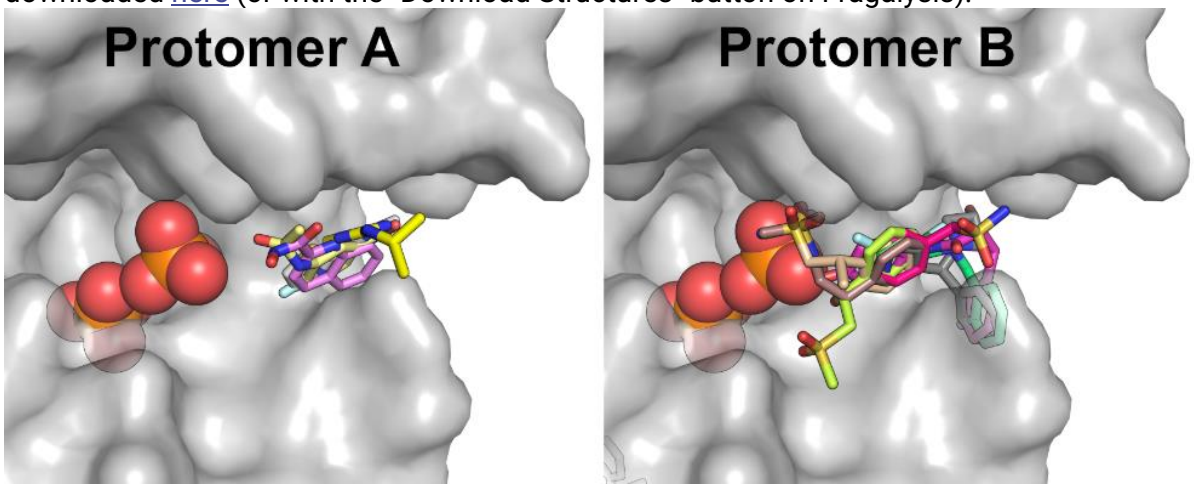
soaking and X-ray data collection was performed by the XChem team at Diamond Light Source. The fragment screen encompassed the full DSI-poised Library, with an additional small selection of fragments from libraries available at XChem.
The NSP13 crystal form contains two protomers and the fragment screen revealed a total of 63 fragment hits across 51 datasets. These datasets have been submitted to the PDB and are also available to view in fragalysis.
There are several potential sites of inhibition of NSP13 helicase activity, namely the nucleotide and RNA binding sites, and putative allosteric sites that may block inter-domain movements or interactions with protein partners. Structures of NSP13 in complex with nucleotides or nucleic acid substrates are not known but we have modelled in the expected positions based on the Upf-1 RNA complex structure (2XZL) which shares sequence homology with NSP13. Here, the nucleotide binds in a cleft between the 1A and 2A domains, whilst the RNA passes through a cavity formed between the IB domain and the 1A and 2A domains. Our X-ray fragment screen yielded fragment hits in all these sites.



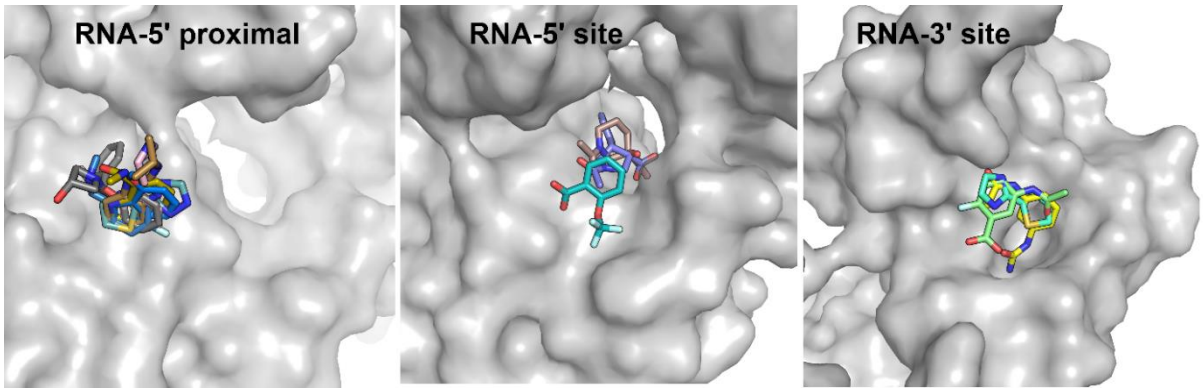
Left: Structural model of NSP13 with the RNA and nucleotide bound in their expected positions based on the crystal structure of Upf-1 RNA complex 2XZL (Chakrabarti et. al. 2011).
Right: NSP13 fragment hits viewed from the same orientation.

Interactive views and downloads

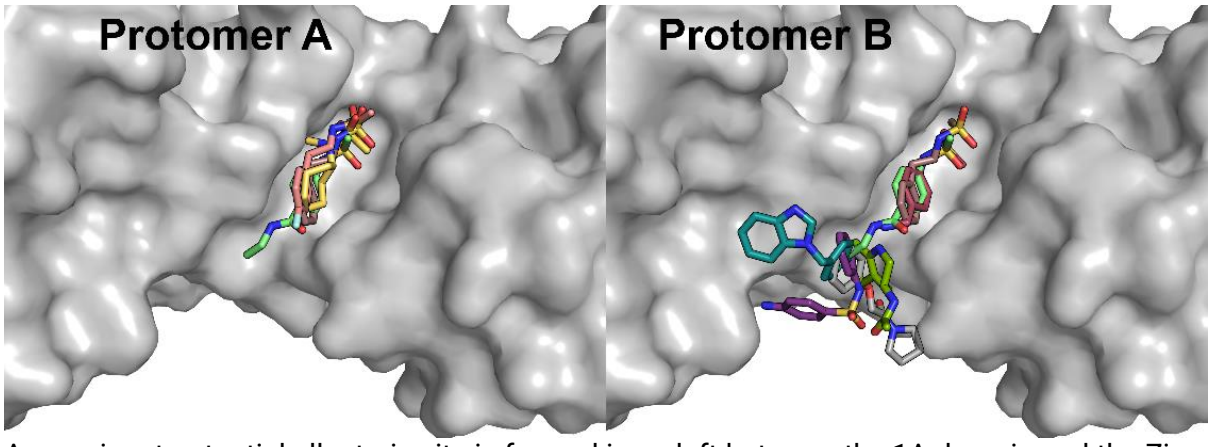
All hit structures of NSP13 can be viewed on Fragalysis. These files can also be downloaded here (or with the "Download Structures" button on Fragalysis).



Nucleotide site for protomer A and protomer B (the phosphates are present as a component of the crystallization condition)



Three fragment clusters were found in the RNA binding channel, one close to the 5’ end, one within the modelled RNA at the 5’ end and one close to the 3’ end.



A prominent potential allosteric site is formed in a cleft between the 1A domain and the Zinc domain, hits were found in a similar pose for protomer A and protomer B.

Credits

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