Development of High-Throughput Screening Assay to Identify Inhibitors of the SARS-CoV-2 Guanine-N7-Methyltransferase Using RapidFire-MS

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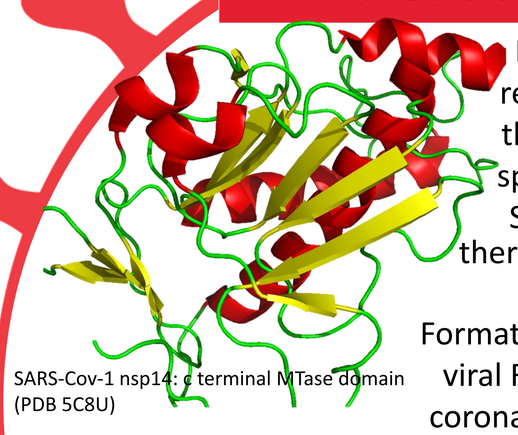
Drug Discovery Unit

The Drug Discovery Unit is rare in a British university, offering integrated, industry-standard capabilities to translate basic biomedical research into novel drug targets with in vivo proof-of-concept and to develop candidate medicines.

Introduction



SARS-Cov-1 nsp14: c terminal MTase domain (PDB 5C8U)

Despite similarity to related coronaviruses, there are currently no specific treatments for SARS-CoV-2. There is therefore an urgent need to develop therapies.

Formation of the cap at the 5′ end of viral RNA has been shown to help coronaviruses evade host defences.

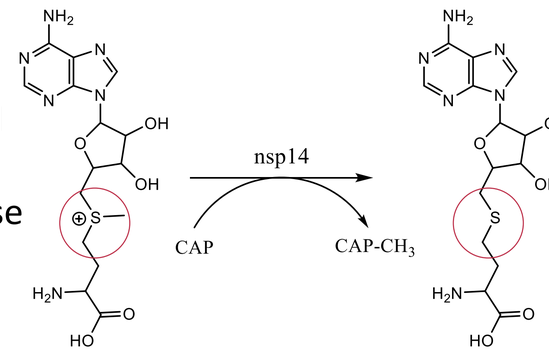
Non-structural protein 14 (nsp14) is responsible for N7-methylation of the cap guanosine in coronaviruses, & is highly conserved.

Using RapidFire technology we characterised & developed a high-throughput assay for nsp14 of SARS-CoV-2.

We used this to screen a library of 1,771 FDA approved drugs in order to identify potential starting points for drug development with a potentially faster path to the clinic.

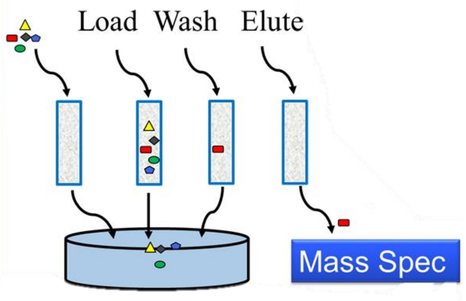
Rapid Fire method for detecting MTAse activity

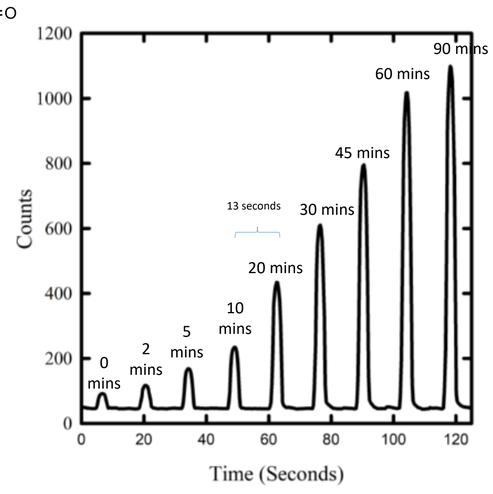
Nsp14 is a SAM dependent methyltransferase



SAM donates a methyl group to the cap guanosine and is turned over to SAH.

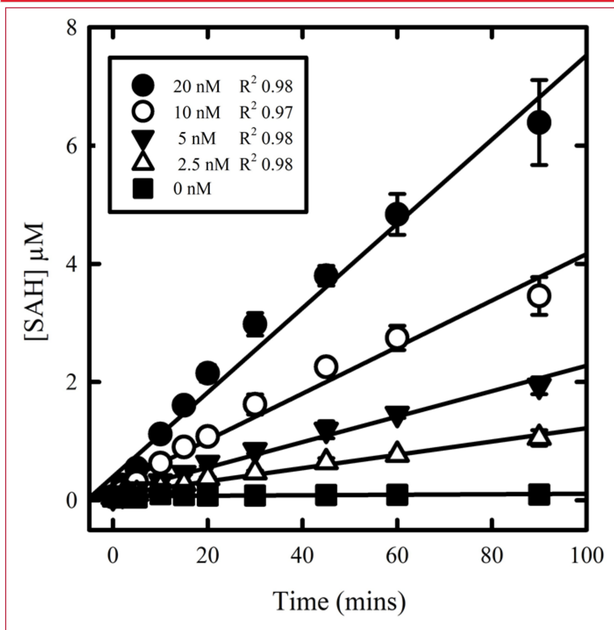
The RapidFire detects SAH, with reference to an internal standard, d4SAH.





Representative chromatograph of time course of SAH production by nsp14

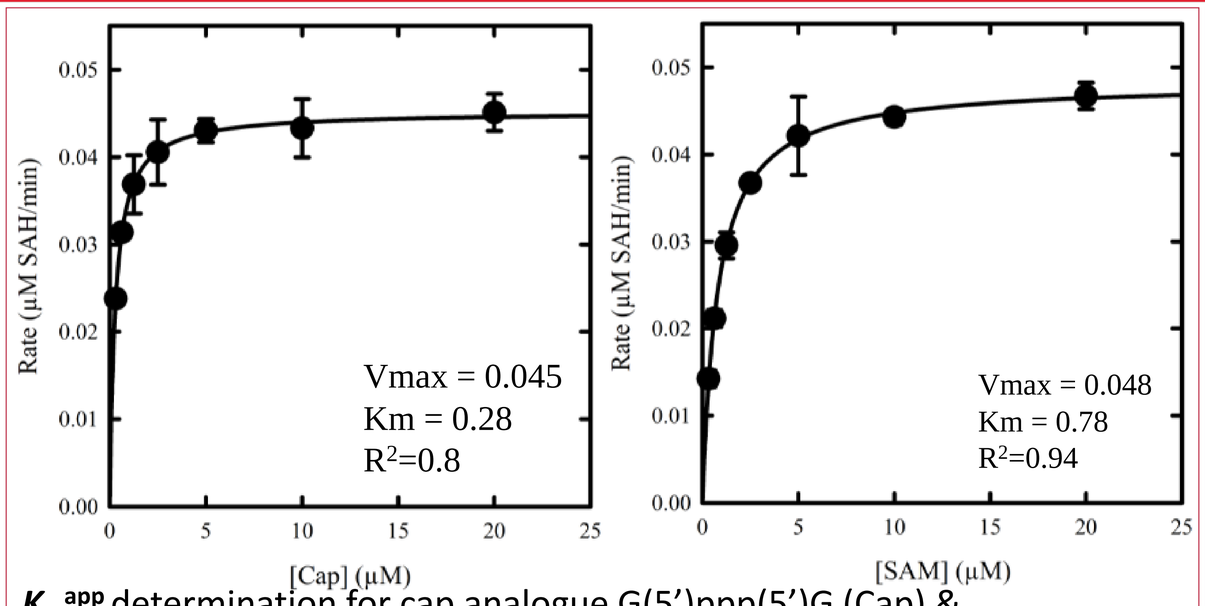
Characterisation of the protein



Titration with a time course:

Protein activity confirmed

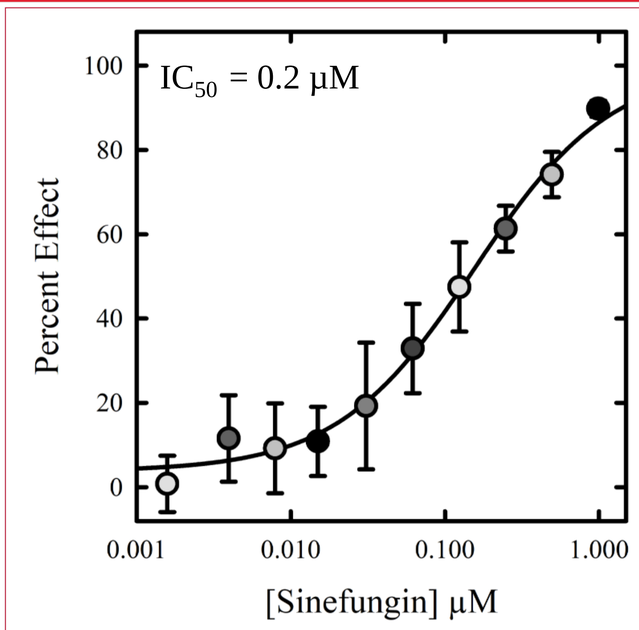
Appropriate concentration selected



K_m^{app} determination for cap analogue G(5′)ppp(5′)G (Cap) & S-Adenosylmethionine (SAM)

Substrate screening concentrations selected at or near K_m^{app}

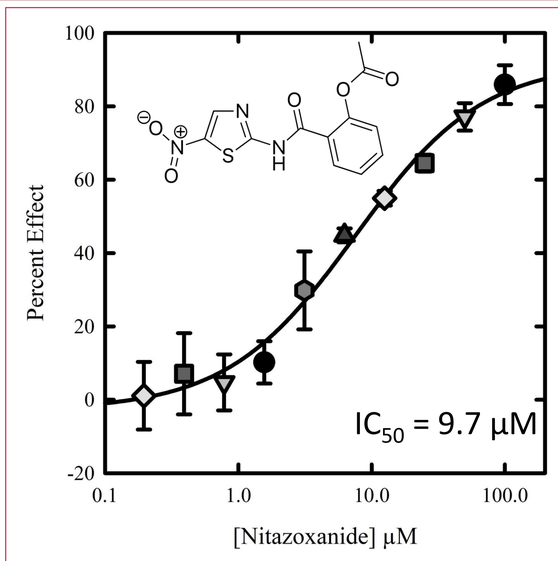
'Balanced' conditions give the best chance of detecting diverse inhibitor types



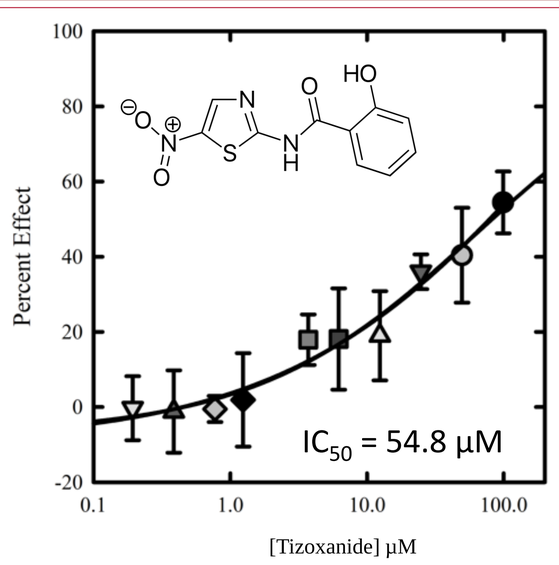
Assay validated with **Sinefungin:**

SAM competitive inhibitor with confirmed activity against SARS-Cov-1 nsp14

Identifying Inhibitors



SP screen identified **Nitazoxanide:** commercial anti-infective, active against range of coronaviruses, incl. Middle East respiratory syndrome coronavirus (MERS-CoV). Shown to block SARS-CoV-2 *in vitro* infections at low µmolar concentrations.



Nitazoxanide metabolite **tizoxanide** also showed activity against nsp14.

Conclusion:

Following characterisation of **nsp14** we developed a high-throughput assay which we used to screen against 1,771 FDA approved drugs.

The results identified a validated inhibitor of this important viral target, which, along with its metabolite inhibits the target at concentrations achievable by doses known to be safe in people.

For more detail, see our paper in July SLAS Discovery

Acknowledgments:
This work would not have been possible without the hard work of the compound management team, and the advice and support of Tony Hope.
Thanks go to Fraser Cunningham for his help with structures.
This work is funded by the Medical Research Council, Wellcome Trust and European Research Council (ERC) Horizon 2020 programme.

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