- 10 enumerated diamine core libraries, 93'026 molecules per library = ~ 1 million compounds.
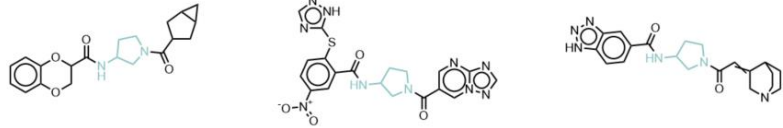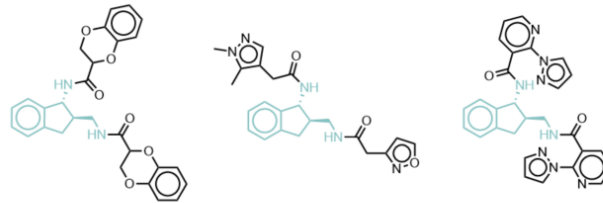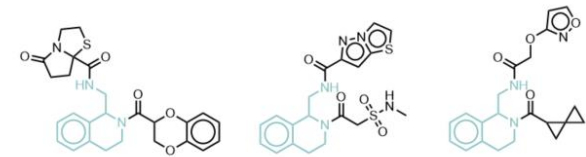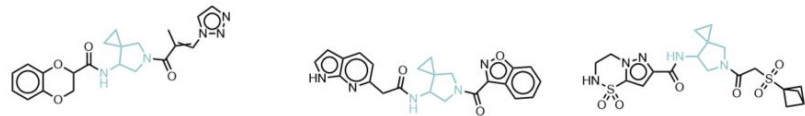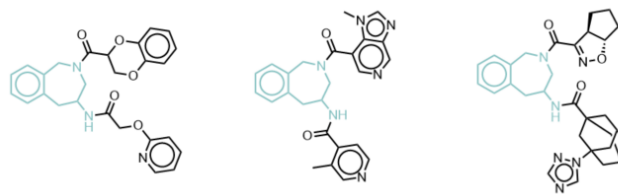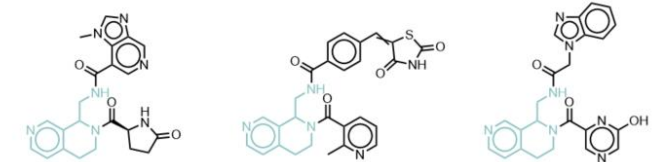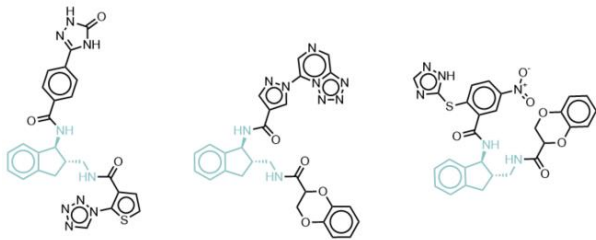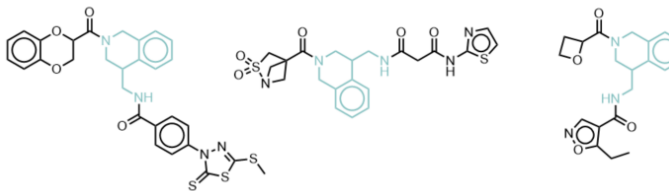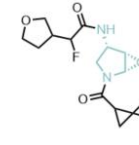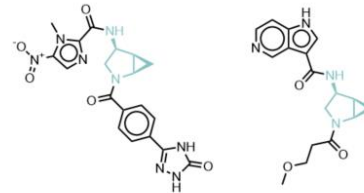
Benzoazepine diamide core series "molecular staples"
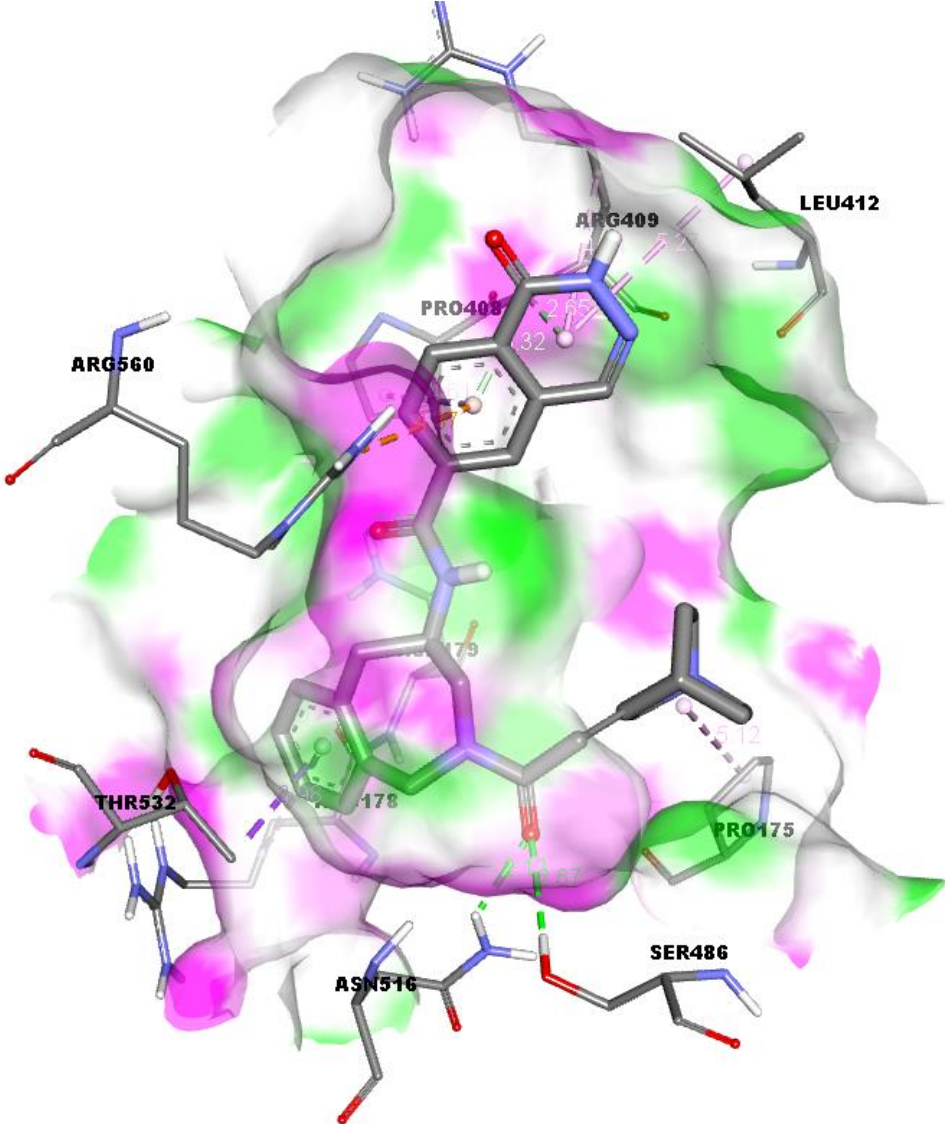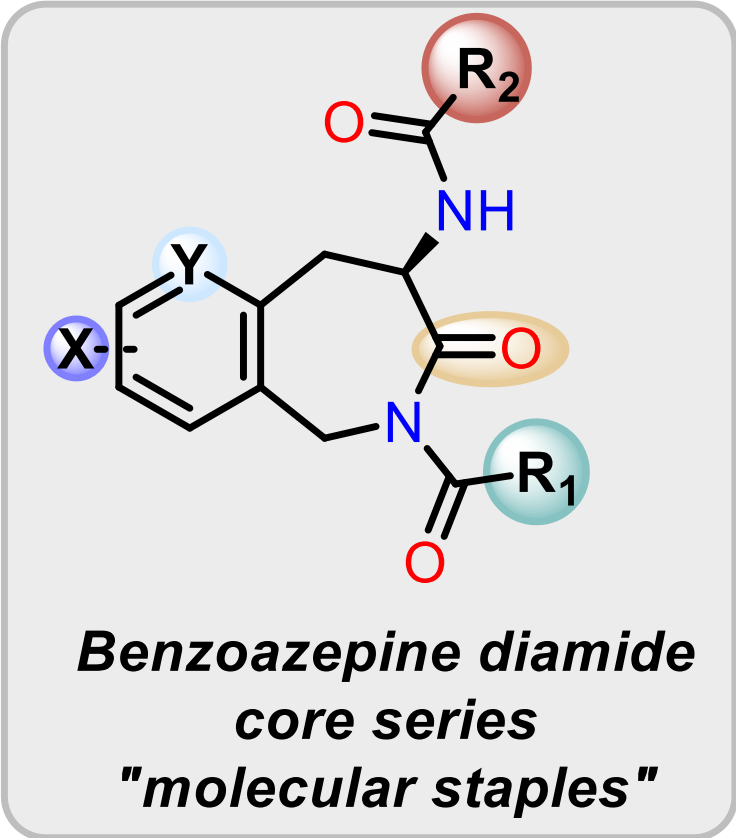
Top 100 compounds: *de novo A.I.* generated compounds
Benzoazepines
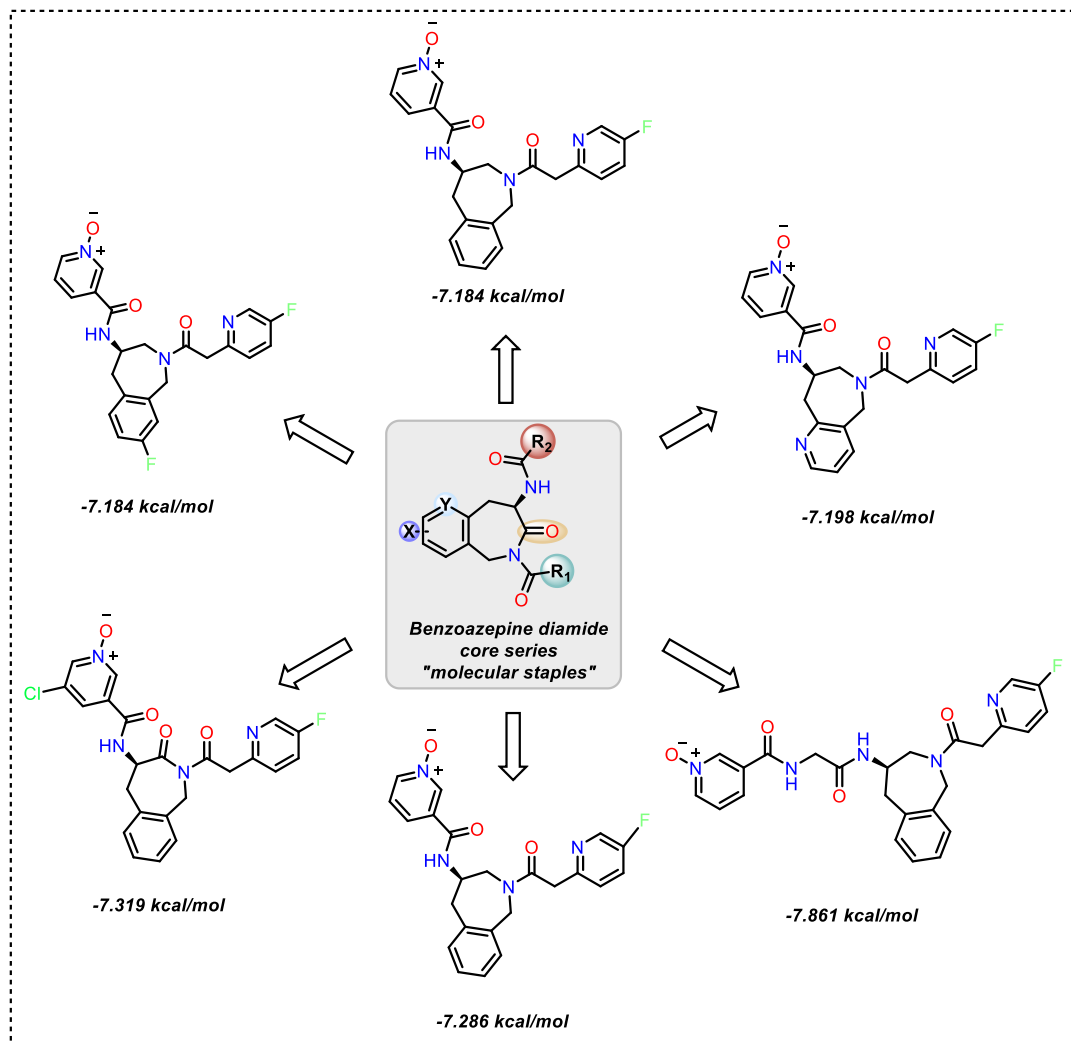
- ATPase/SPR screening of the Enamine N-oxide set (51 compounds) afforded no results – therefore, we have decided to maintain these cores and diversify the R groups.

- One core of interest includes the benzoazepines, found in 6/100 of the original de-novo top-scoring Glide hits for Rec2A allosteric site ($\Delta G_{Binding}$ ~ -7.2 kcal/mol, see left).

- This core is non-commercial, rigid with tunable flexibility (amide vs diamine), and allows diverse difunctionalisation (N-capping).
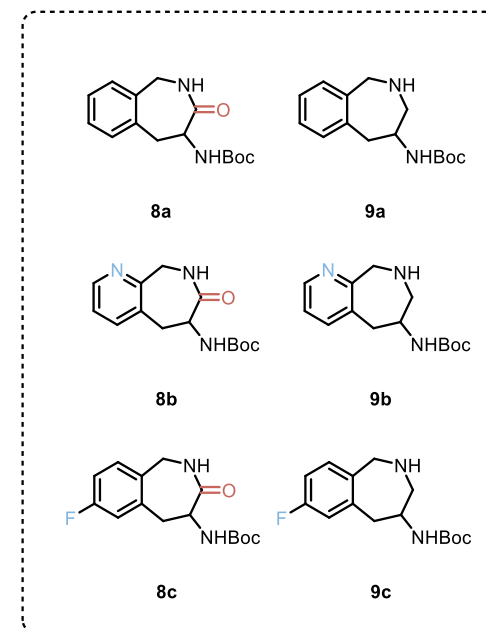
- Chemistry has focused on easy access to this core for diversification at the R1 and R2 positions, as well as scaffold hopping on the aromatic ring (SAR) and amide vs amine (rigidity).
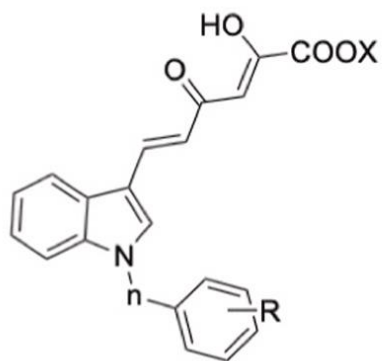
https://doi.org/10.1016/j.antiviral.2023.105697
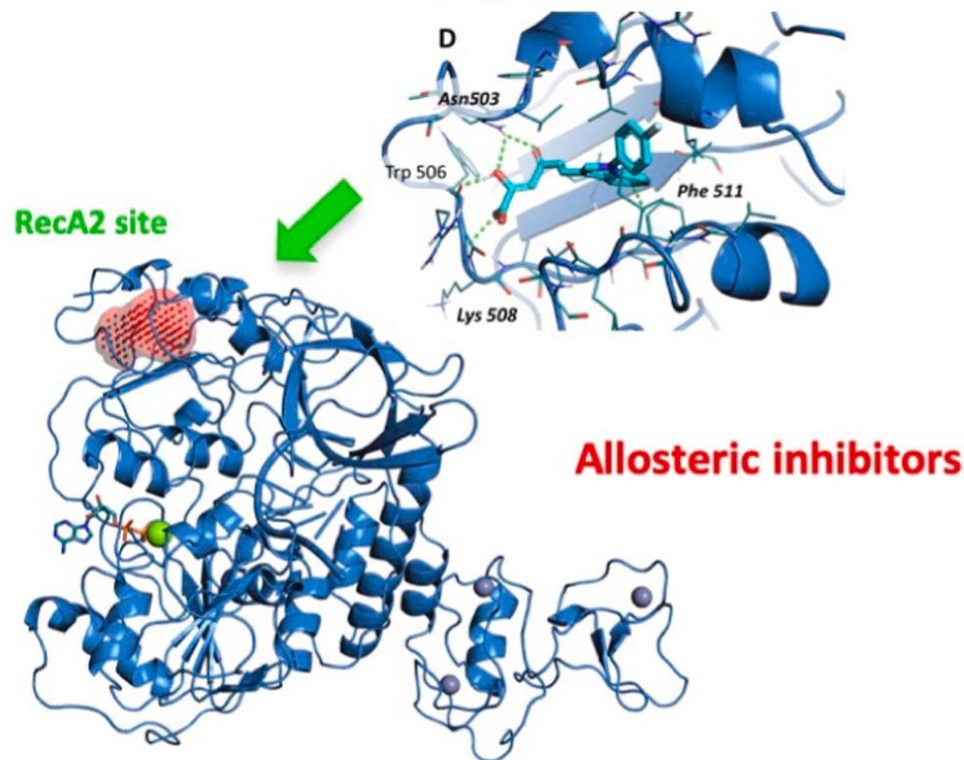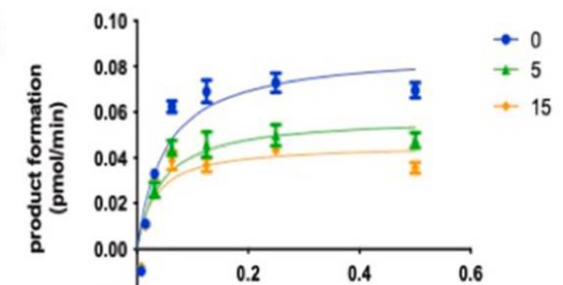

Chemical synthesis

n = 0, CH_2
R = H, F

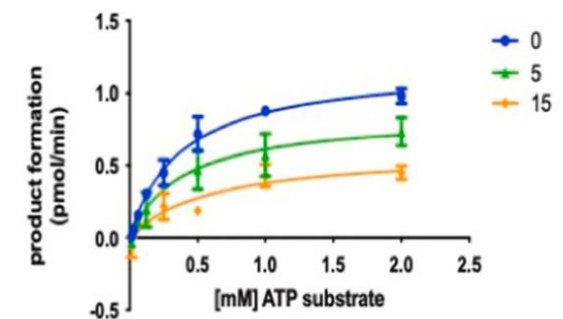**New class of nsp13 inhibitors**

Dual acting inhibitors
Effective vs SARS-CoV-2
infected cells

Docking studies

RecA2 site

Allosteric inhibitors

Kinetics of inhibition

De-Novo Generated Top 100 Scoring Compounds:
Benzoazepines: Moving away from Pyridine N-oxides

1) Diversification of the amine cores with commercially available and diverse Enamine carboxylic acids afforded 93'026 compounds, which were sent for virtual screening (Maestro Glide).

2) Filtering (DW):
Total molweight < 500 Da
cLogP < 5
cLogS < 1
H Acceptors < 10
H Donors < 5
Rotatable bonds < 3
Polar Surface Area < 140 Angstroms
Mutagenic **X**
Tumorigenic **X**
Reproductive effective **X**
Irritant **X**
Nasty functions **X**

3) Virtual Screening (PyRx/Vina)

- Reduced to 20'226 molecules.

- DataWarrior's 'diversity selection rank' in order of structural diversity.

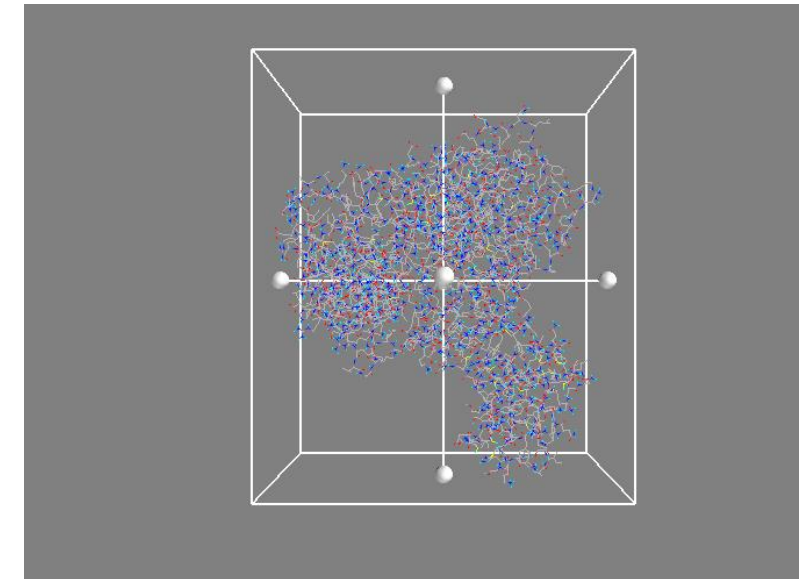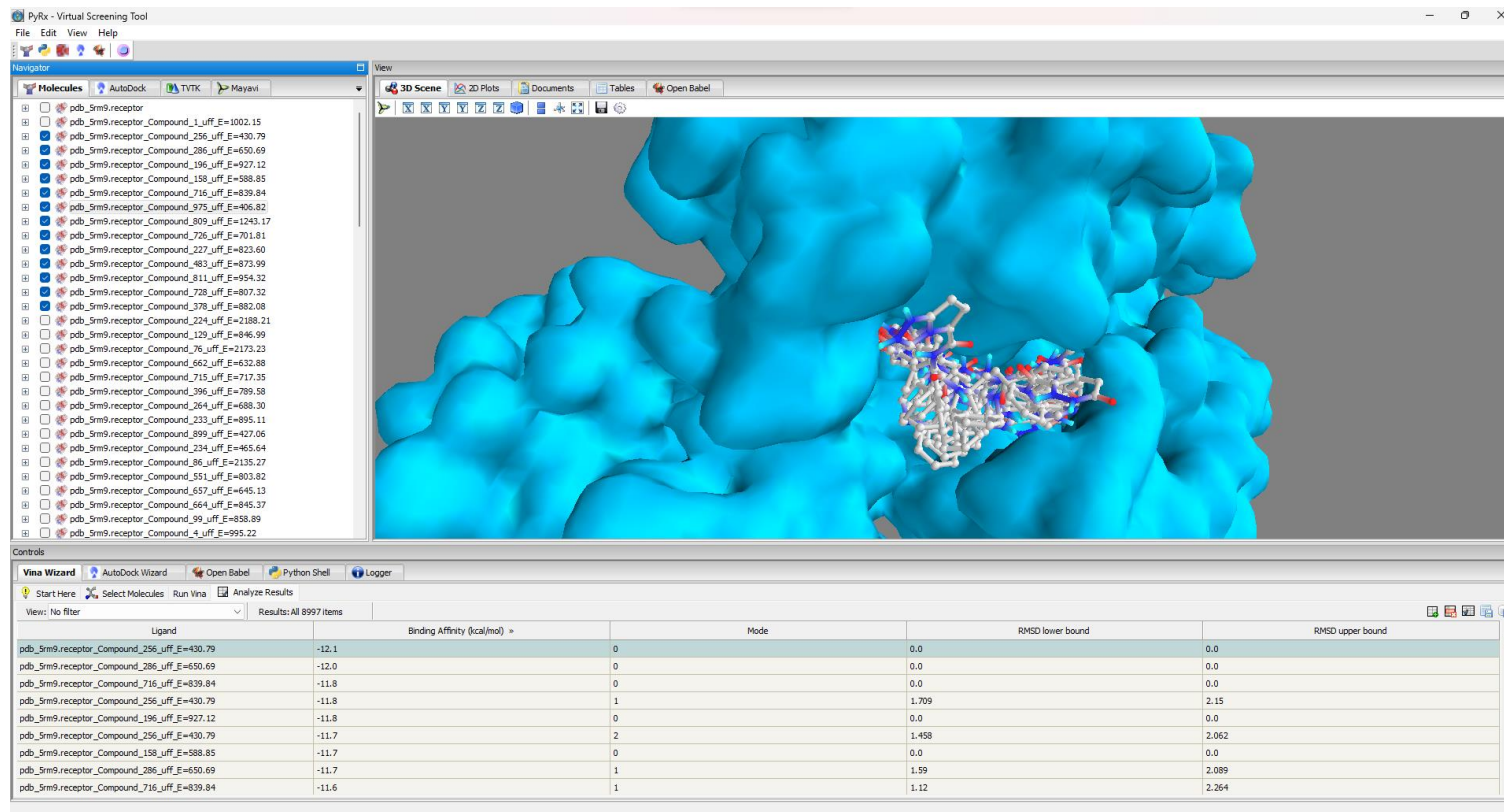- Selected the top 1000 most diverse compounds for virtual screening using PyRx 0.8/AutoDock Vina. PyRx - Virtual Screening Tool download | SourceForge.net
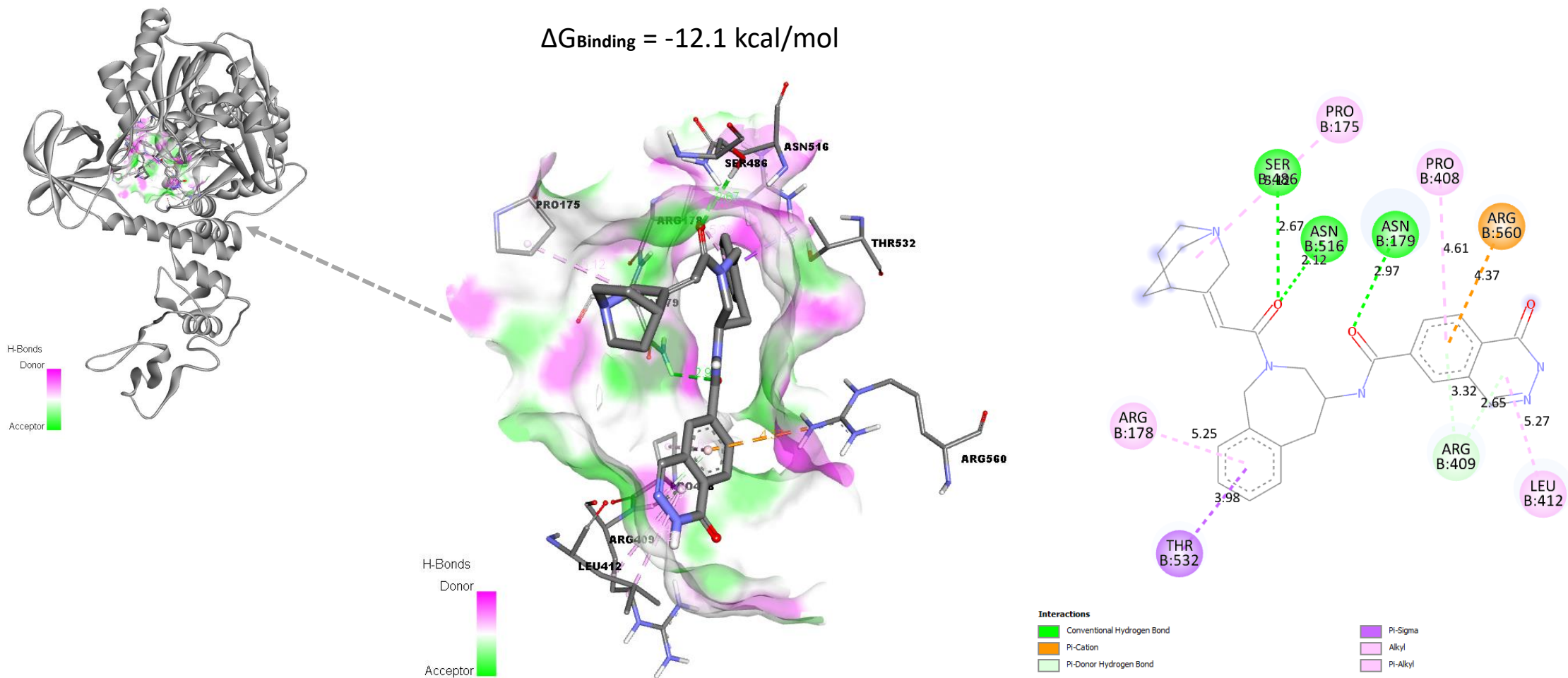
De-Novo Generated Top 100 Scoring Compounds:
Virtual Screen with PyRx 0.8 / AutoDock VINA

- Unbiased virtual screen (whole protein grid) using energy-minimised ligands and exhaustiveness = 8 conformers.

- Scores as low as $\Delta G_{Binding}$ = -12.1 kcal/mol, but virtually all poses were at the **RNA binding site**, with a few at the ATP binding site, but none at the Rec2A allosteric pocket.

- Binding free energies are lower than those of the de-novo generative set (N-oxides) at the RNA binding site – not surprising given these were designed from a site 3 pharmacophore.

$\Delta G_{Binding}$ = -12.1 kcal/mol

- PDB of lowest-energy pose with Geoff for MD analysis (AMBER20).

$\Delta G_{Binding} = -9.7$ kcal/mol

These bifunctional molecules interact with both protein and RNA components to lock them together.

RNA helicases offer multiple opportunities for drug development.

Small molecules that block protein:helicase interaction, interact with the ATP binding pocket, nucleic acid interaction interface, or inhibit ATPase hydrolysis have been described.

A flexible linker connects the RecA-like domains of helicase proteins. Their opening and closing are essential to helicase function with each pose having the potential to accommodate different small molecules.

Compounds that "staple" a DEAD-box protein onto RNA have been described. Currently, there are only a small number of RNA helicases against which small molecule inhibitors have been identified.

Targeting DEAD-box RNA helicases: The emergence of molecular staples https://doi.org/10.1002/wrna.1738
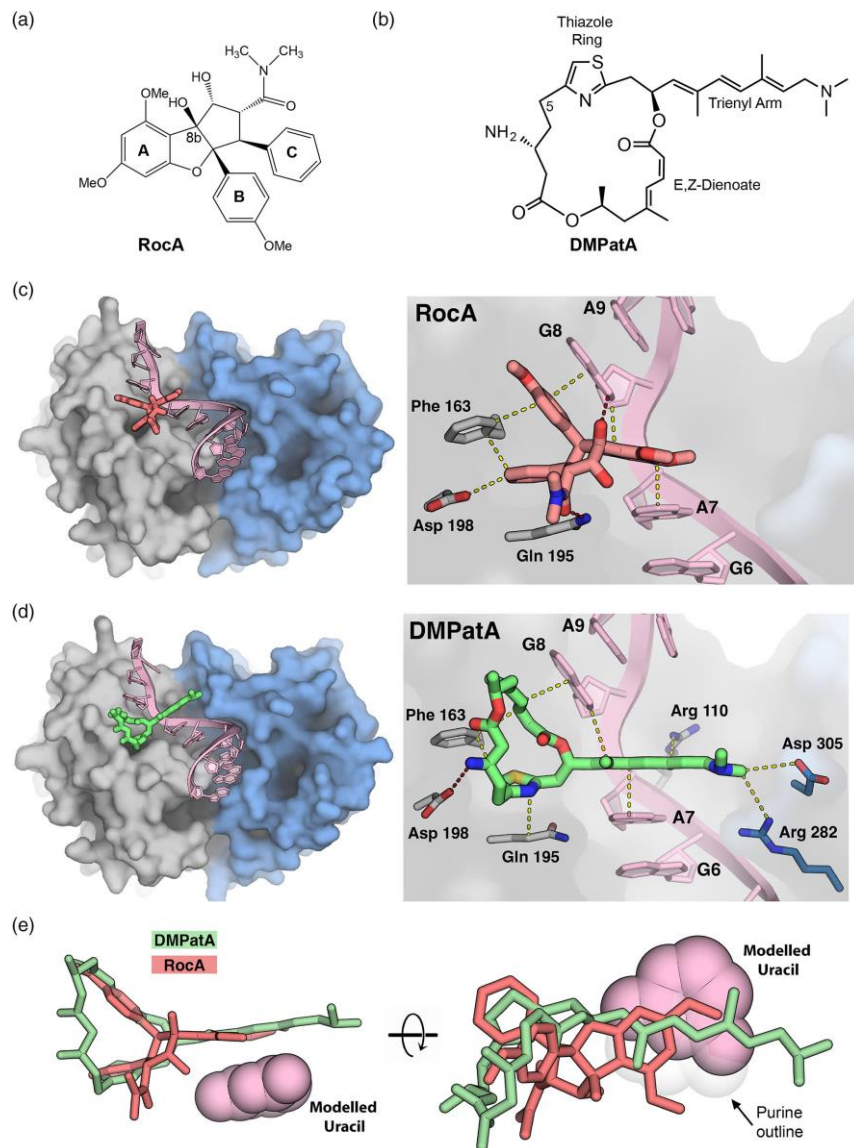
Compounds that "staple" a DEAD-box protein onto RNA have been described. Currently, there are only a small number of RNA helicases against which small molecule inhibitors have been identified.

De-Novo Generated Top 100 Scoring Compounds:
Benzoazepines optimised route

DMAP cyclisation RPC pure
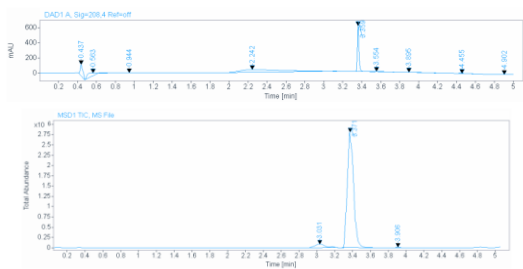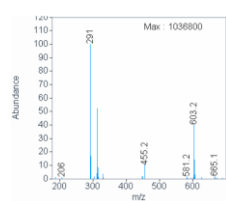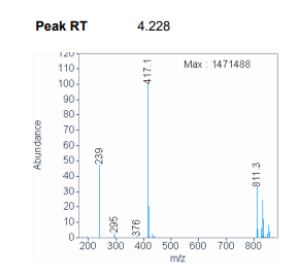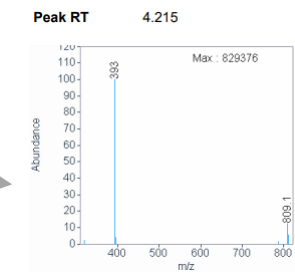
Decarboxy Rt=1 h, 100degC 1M HCl

BENZO AMINE rpc a1

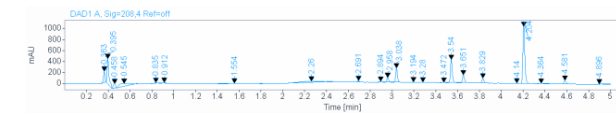# ^{19}F fragment screening @ SoP
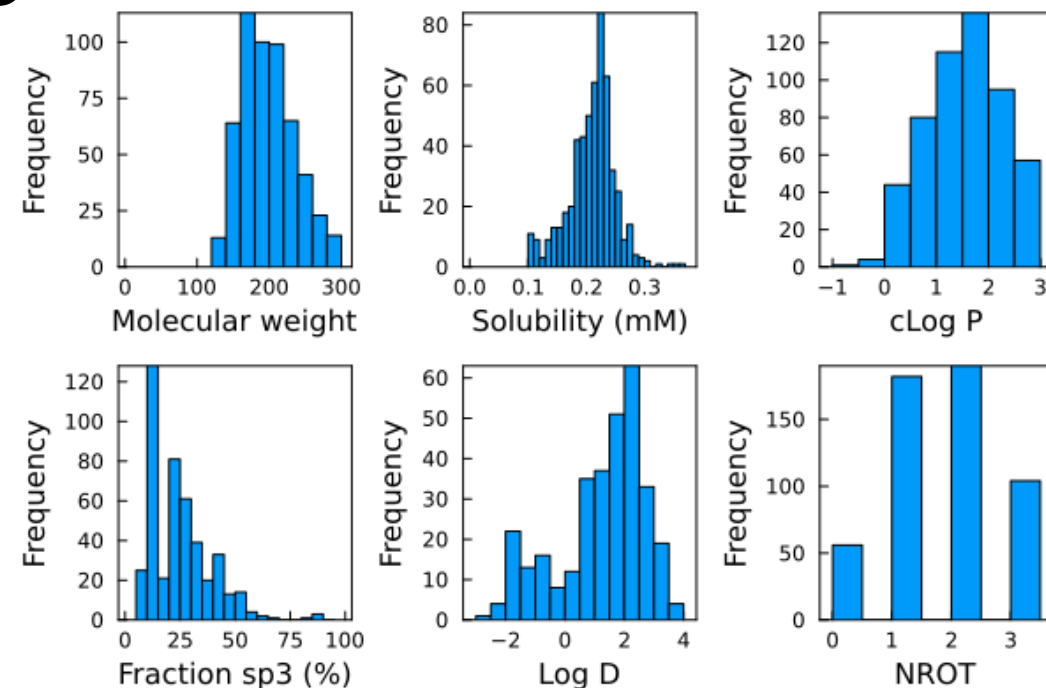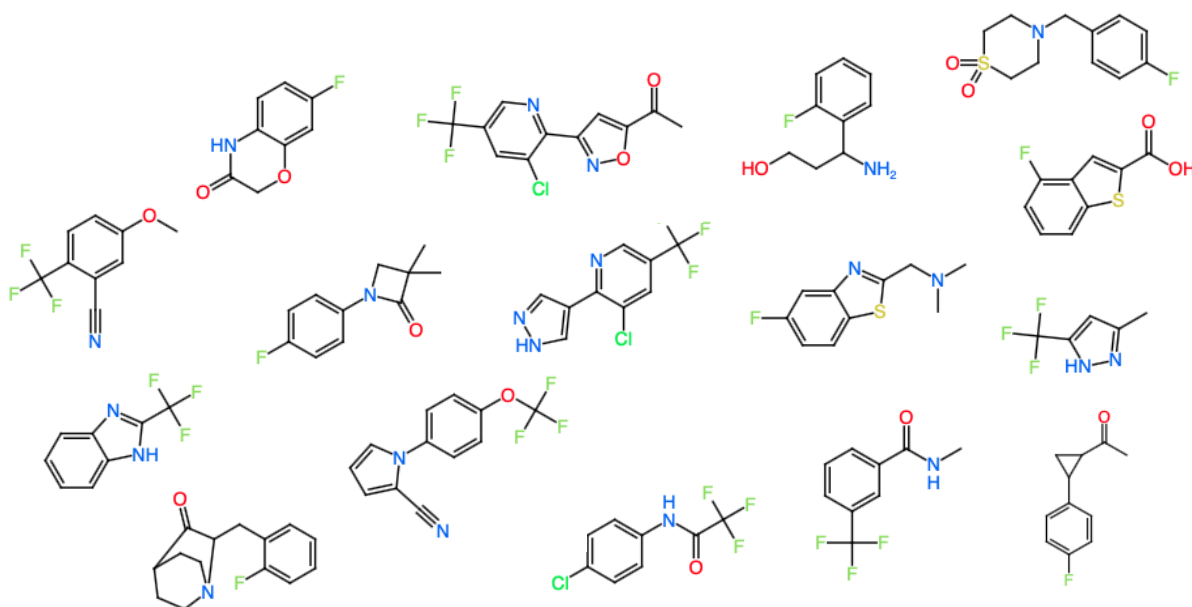
SoP 600 MHz QCI-F spectrometer
Full automation + liquid handling robotics
20 min measurement time / cocktail of 10 fragments
[R_2 weighted ^{19}F{^{1}H} and ^{1}H, ^{1}H waterLOGSY]
Two samples required (+/- fragment)
Approx. 36 hr total acquisition time
ca. £1600 (UKRI rate) + £250 NMR tubes

BioNET ^{19}F library
Ro3 compliant, solubility tested, PAINS free
No usage/license restrictions
500 fragments x 10 mg
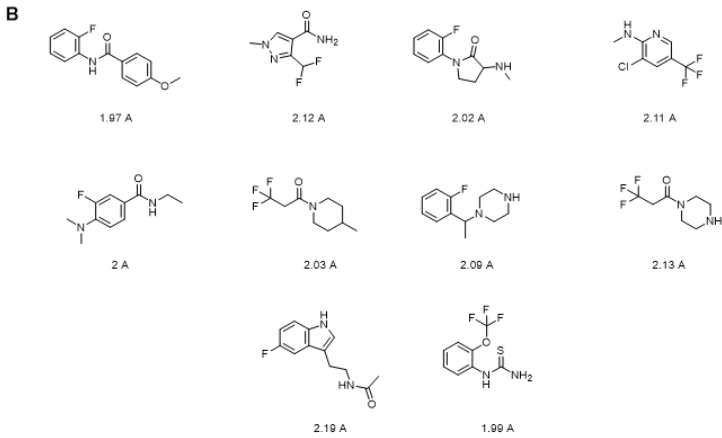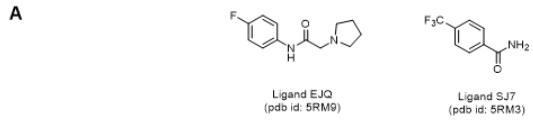ca. £11k – enough material for 700 screens
(2 x 170 µL x 200 µM required per screen)
[to determine – library storage, maintenance]

Total cost ca. £2.1k / screen, assuming we run 50 screens

https://pubs.acs.org/doi/10.1021/acs.jmedchem.3c00656

Nsp13 FAXS NMR Assay Design


Competition binding FAXS (T₂ filtered)

A) Spy molecules EJQ (pdb id: 5rm9) and SJ7 (pdb id: 5rm3) against SARS-CoV-2 nsp13 site 3. B) Negative control compounds for competitive FAXS screen with good solubility in crystallisation buffer. Newman *et al.*, 2021. Diffraction resolutions for each compound are given in Angstroms. C) 2-(trifluoromethyl)benzoic acid (TFMBA) for ^{19}F chemical shift referencing.
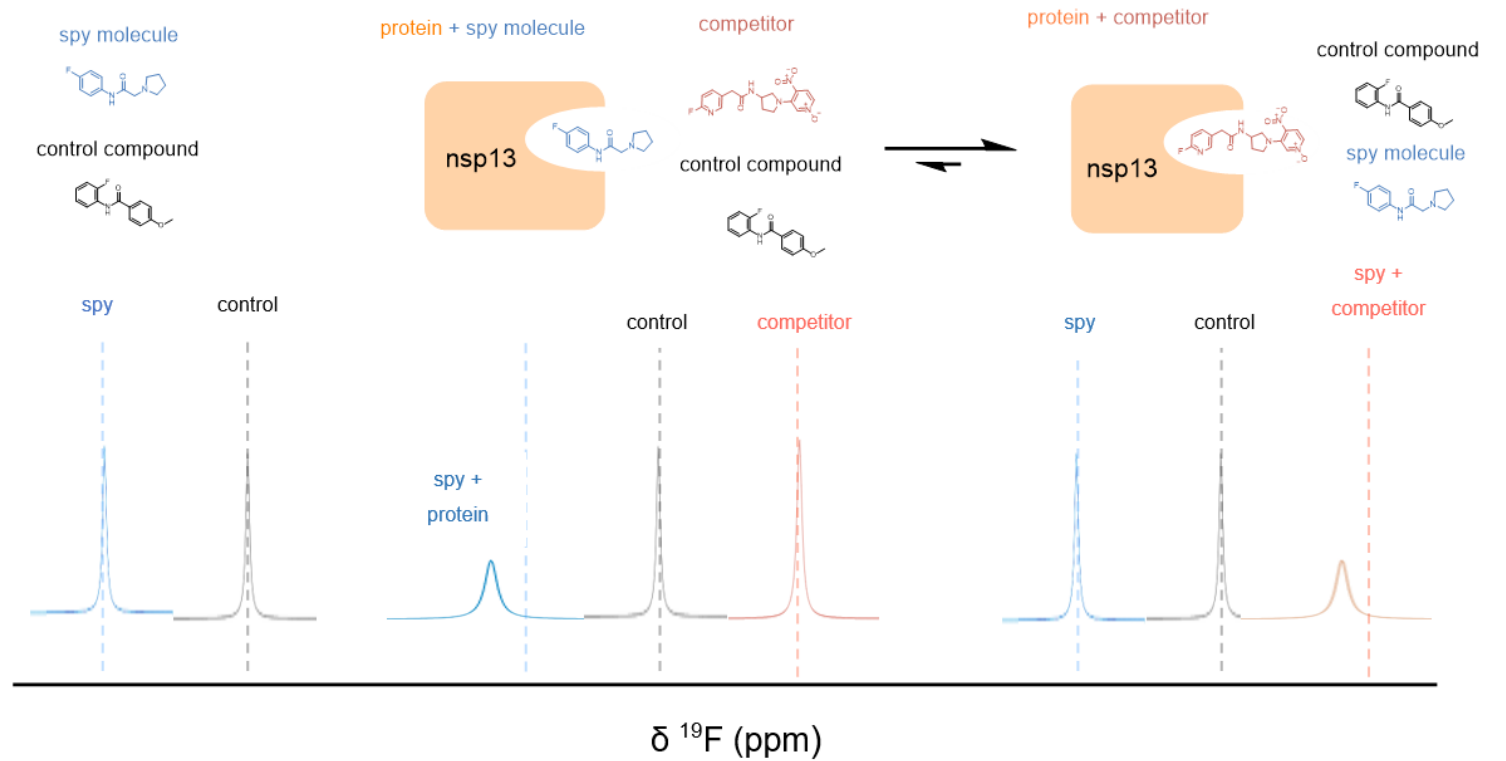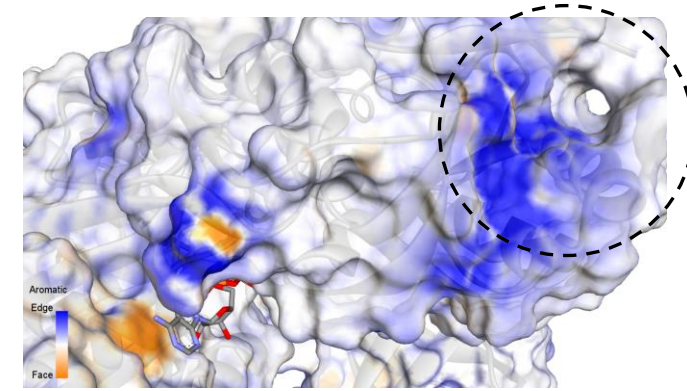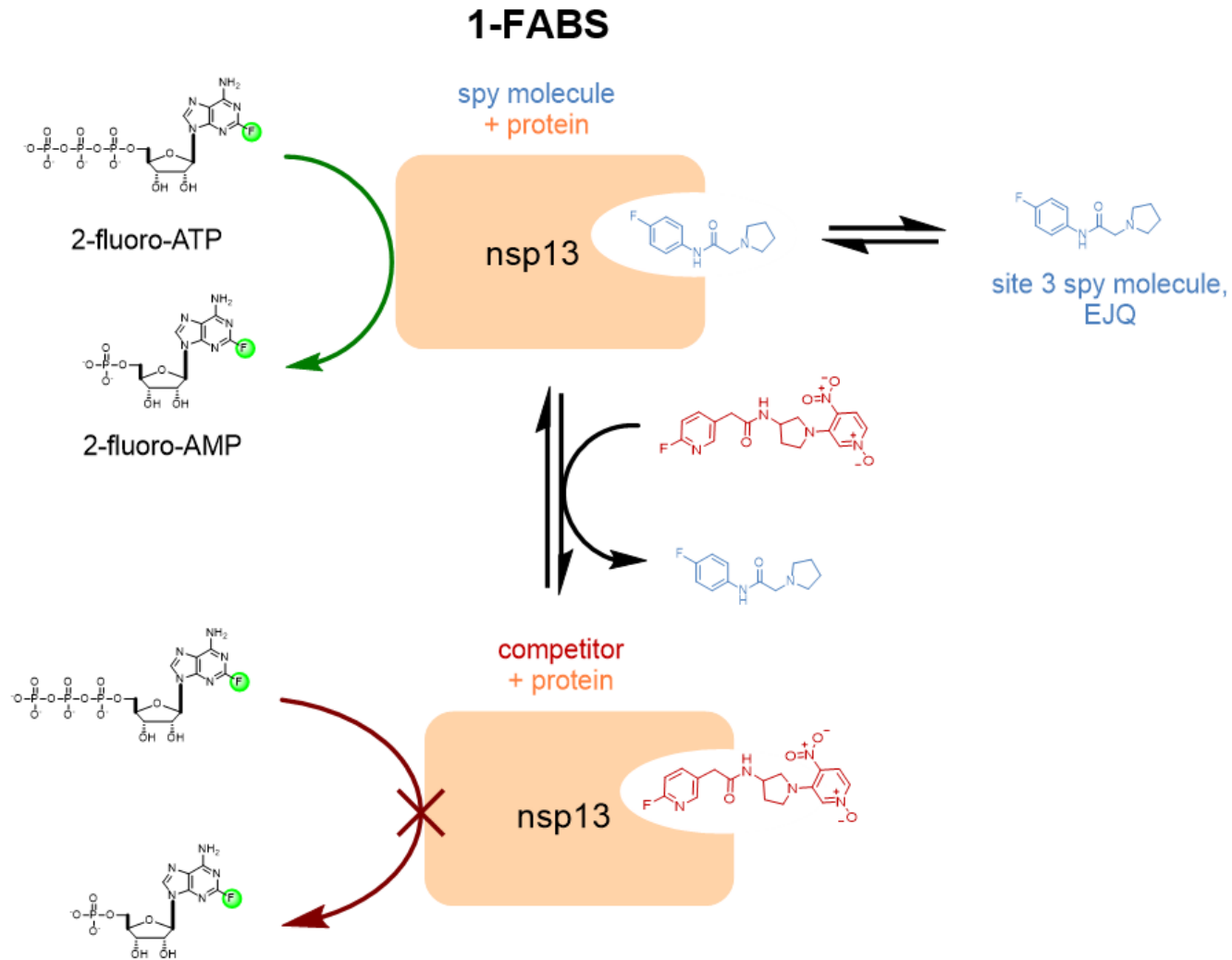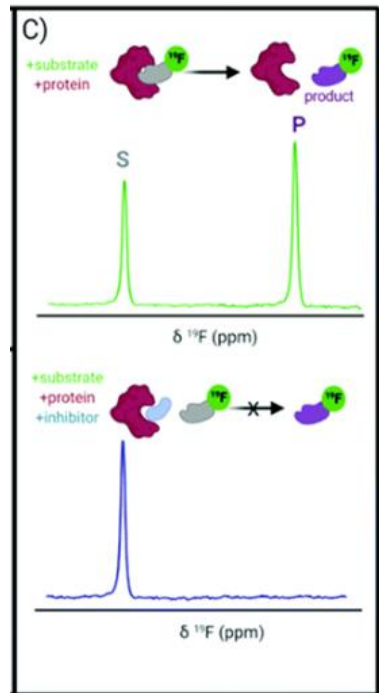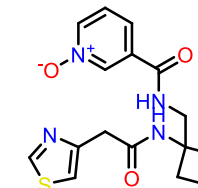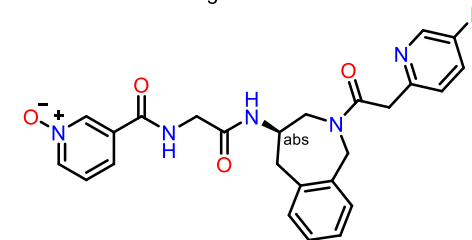
AMP-PNP/NSP13 complex, pdb 7NN0.
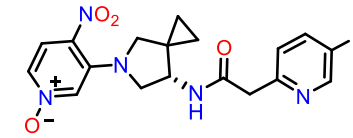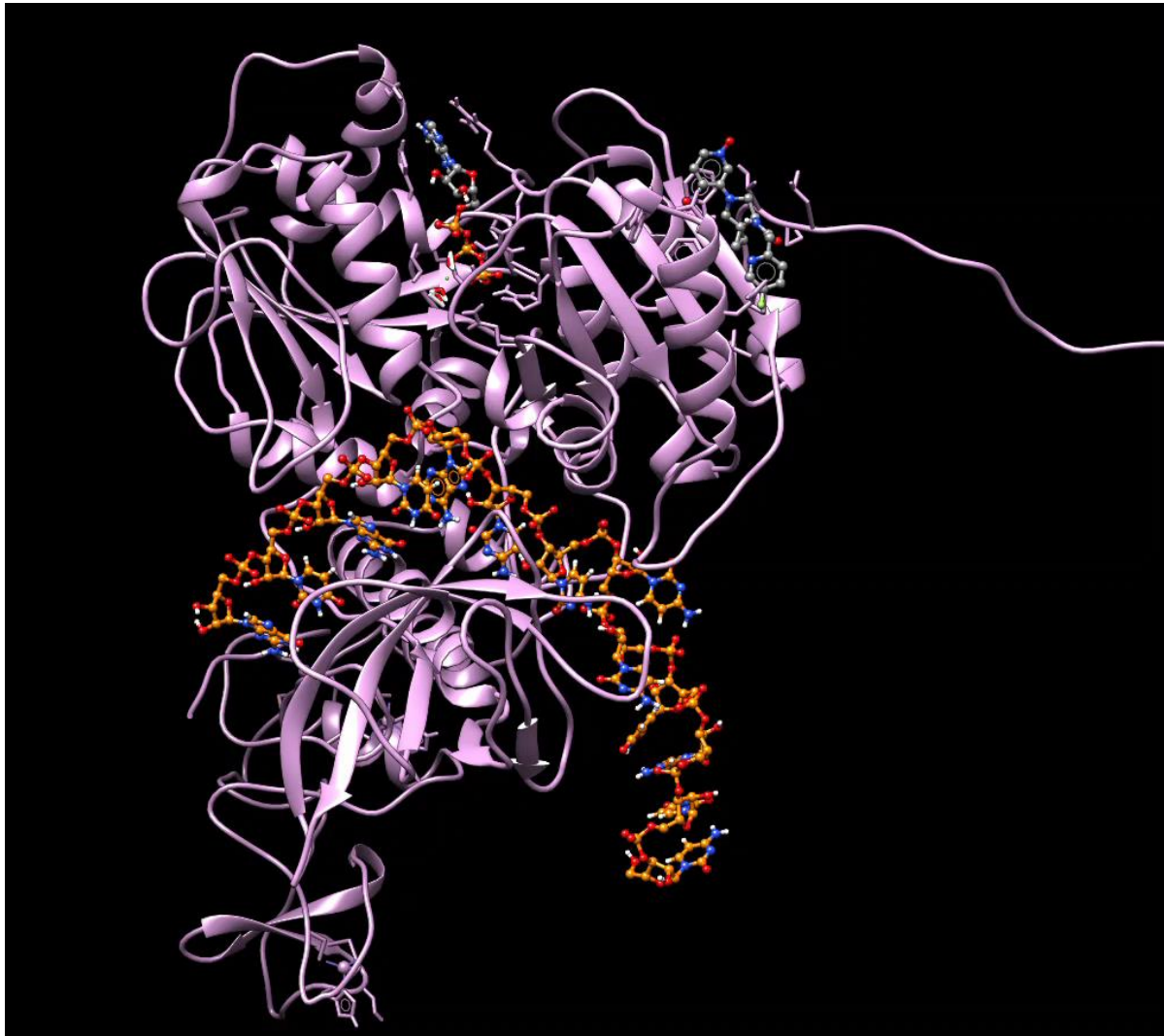
2c
VINA: -5.8 kcal/mol

glide -7.869
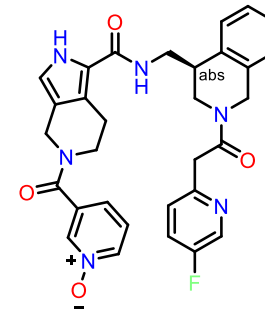
RA-0001264-01

ATPase IC50 = 48 µM

glide -7.861

5RM3

Molecular Docking (AutoDock VINA) and Dynamics (AMBER20):
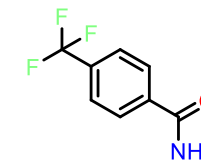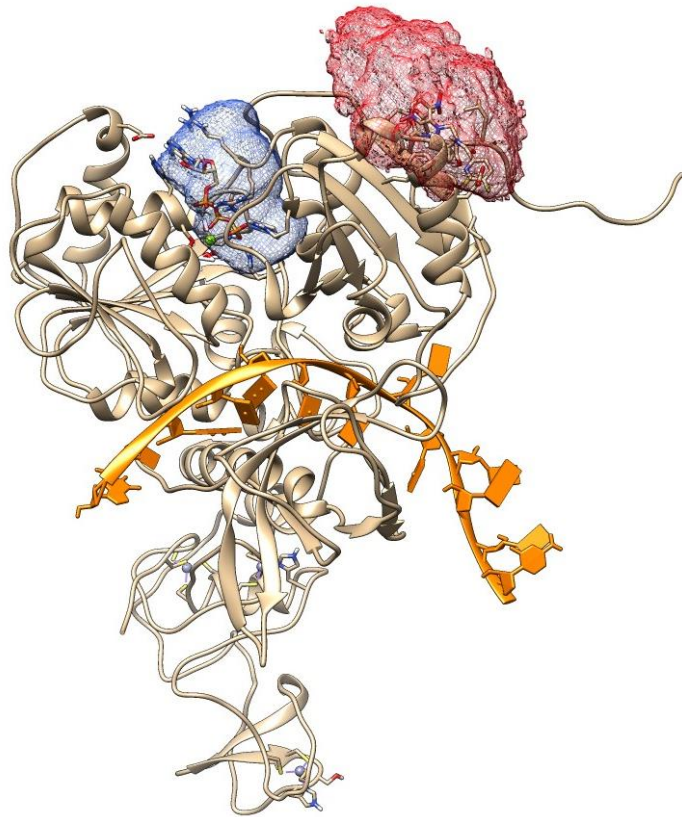
Ligand 2c atomic occupancy volume

RMSD map
Ligand 2c (red) vs ATP (blue) vs frame 1
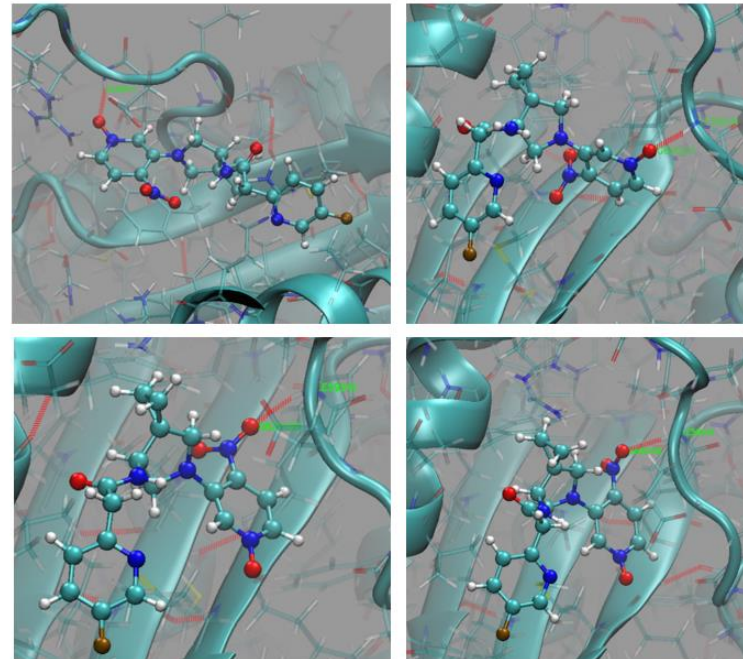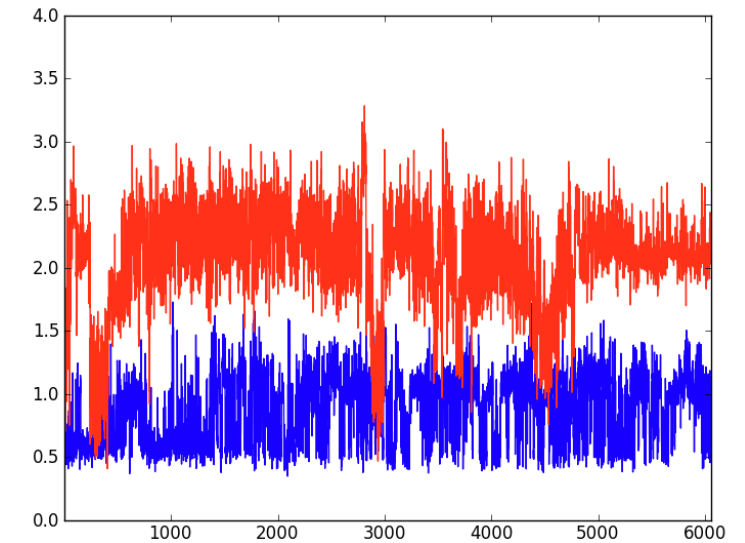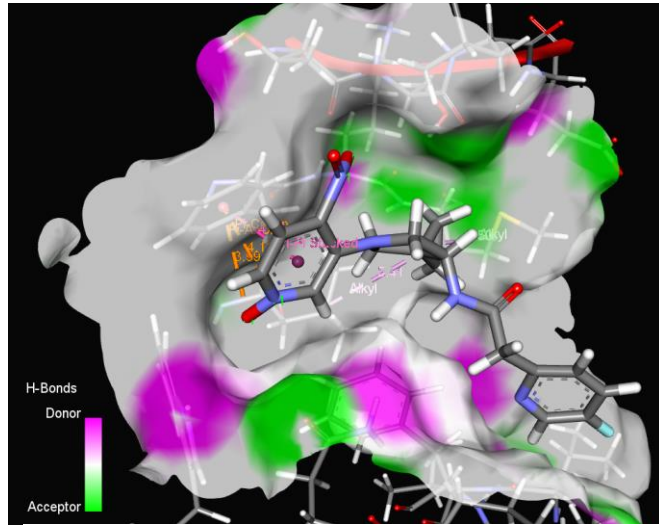

Molecular dynamics (MD) simulation of Nsp13-ATP-ssRNA-Ligand B7 complex showing hydrogen bonding (< 3 Angstroms, red) interactions at 373.0ns and 535.2ns (top, N-oxide N-O···HN ILE592), 384.3ns and 437.9ns (middle, nitro N-O···HN ILE592), and 356.8ns (bottom, amide NH···O GLU591).

Molecular Docking (AutoDock VINA) and Dynamics (AMBER20): Fragment-bound vs ATP/ssRNA bound states (Geoff Wells + Tom)
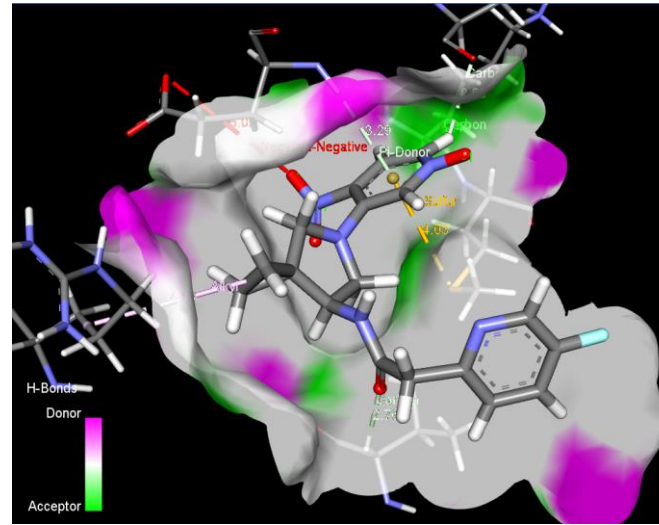
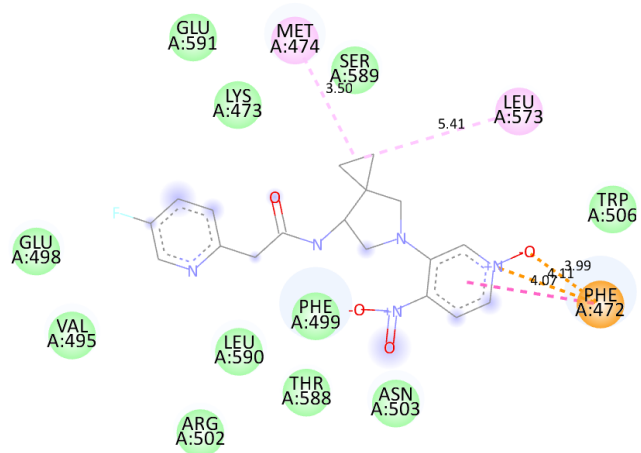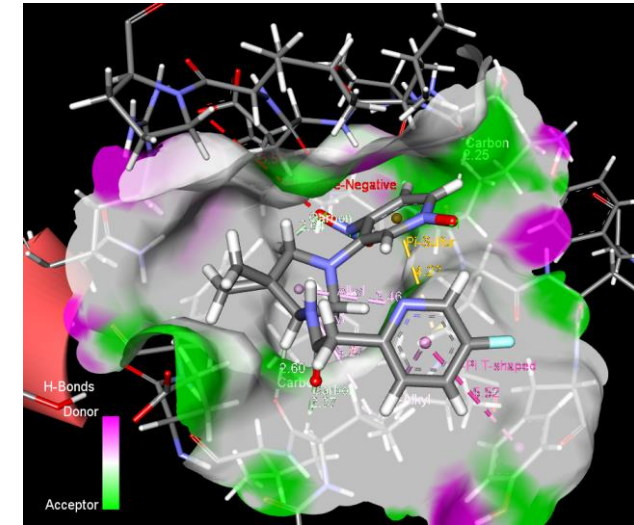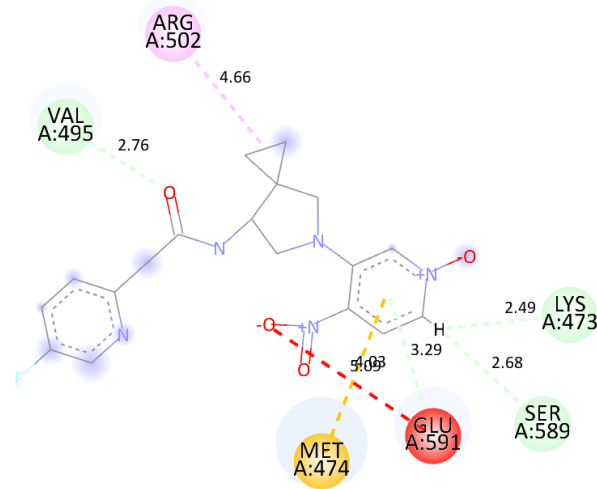# Molecular Docking (AutoDock VINA) and Dynamics (AMBER20): Fragment-bound vs ATP/ssRNA bound states (Geoff Wells + Tom)