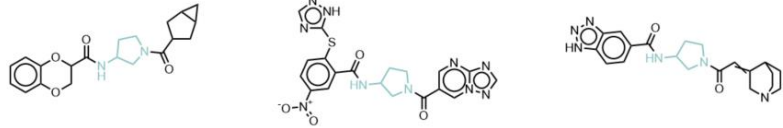


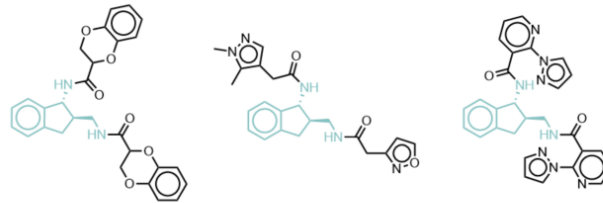
# De-Novo Generated Top 100 Scoring Compounds: *Moving away from Pyridine N-oxides*

- 10 enumerated diamine core libraries, 93'026 molecules per library = ~ 1 million compounds.

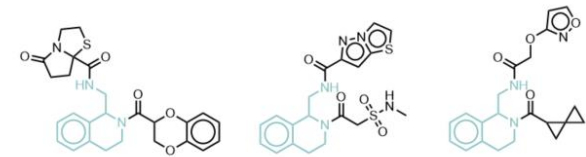
Core 1



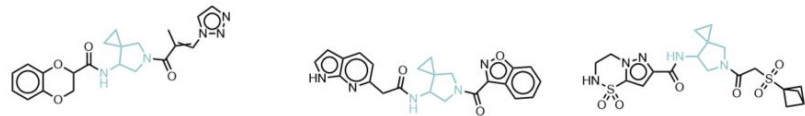
Core 3b



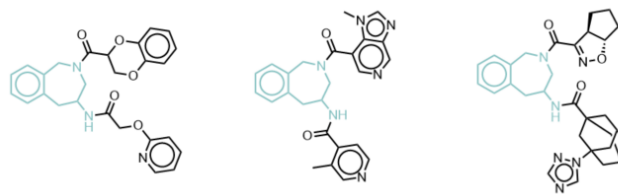
Core 6



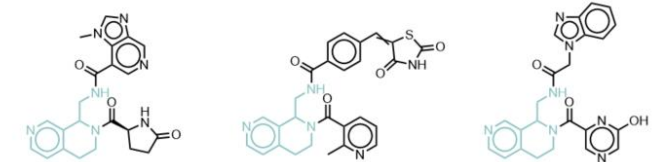
Core 2



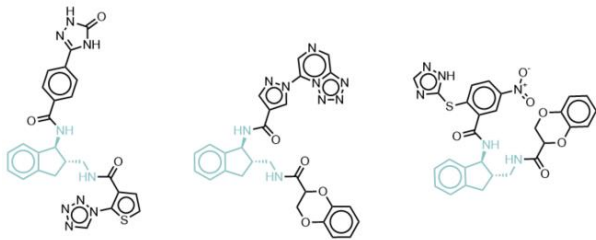
Core 4



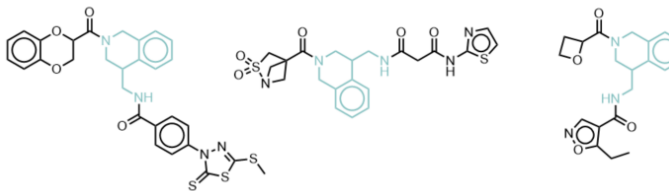
Core 7



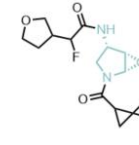
Core 3a



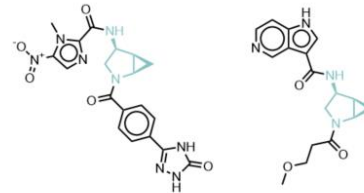
Core 5



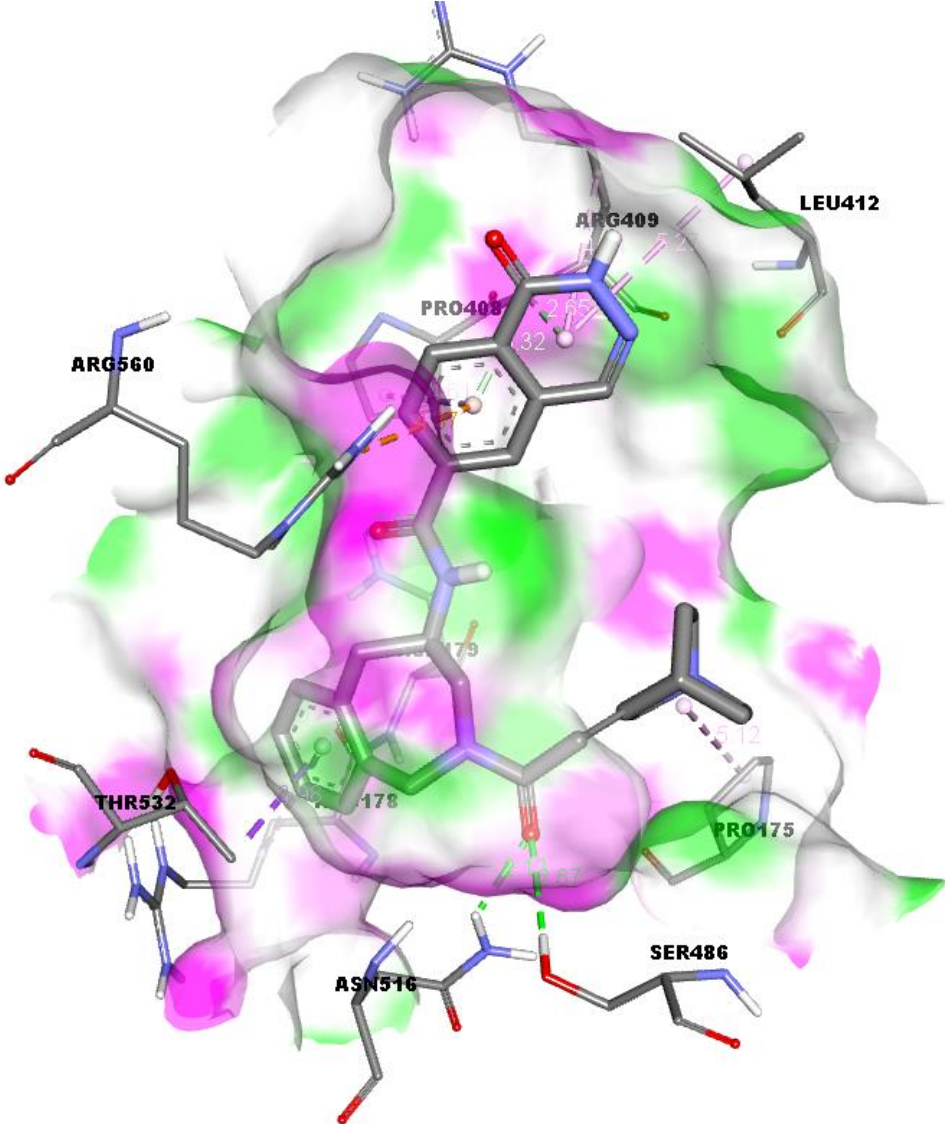
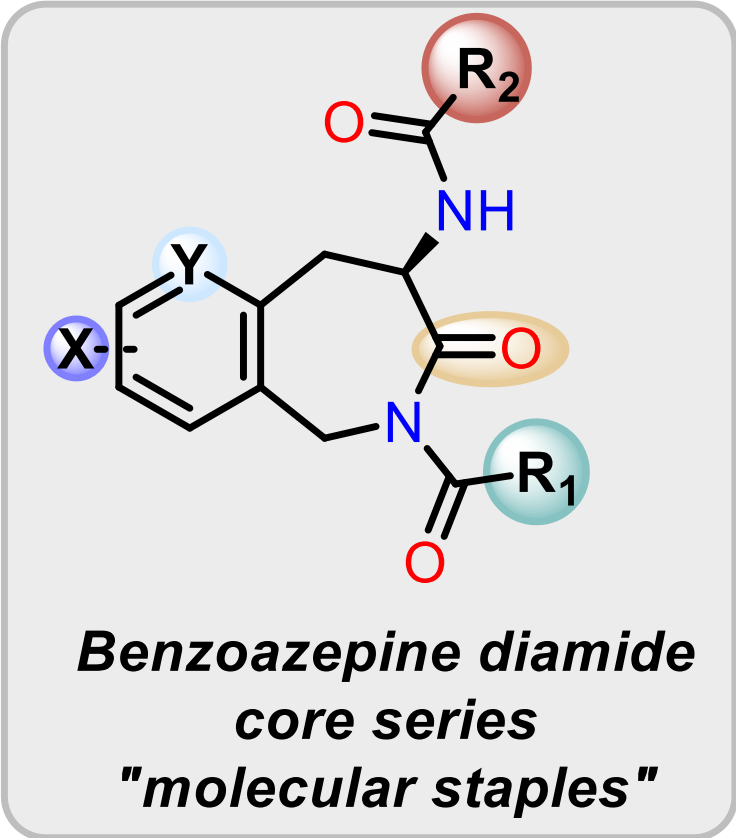
Core 8a



Core 8b

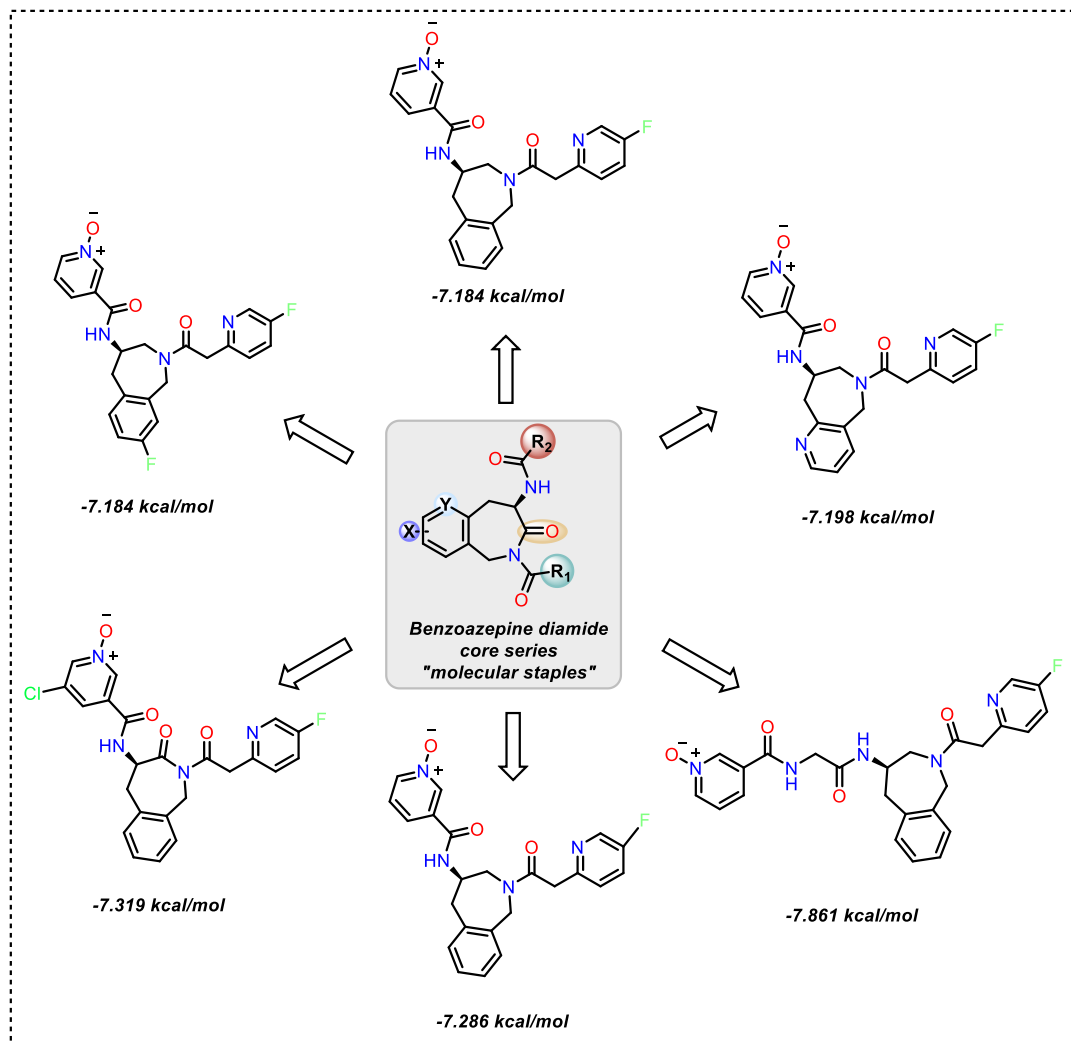


# Benzoazepine diamides as “molecular staple” cores targeting RecA-like viral helicases

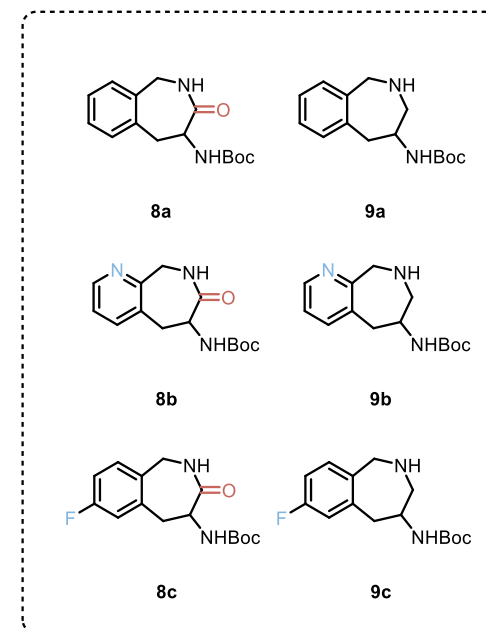


# Top 100 compounds: *de novo* A.I. generated compounds

## 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 ( $\Delta G_{\text{Binding}} \sim -7.2$  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).

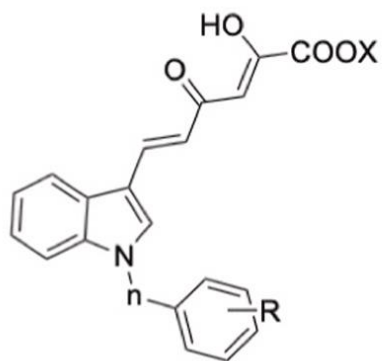




# De-Novo Generated Top 100 Scoring Compounds: *Peter Commercial carboxylic acid caps (93'025)*

<https://doi.org/10.1016/j.antiviral.2023.105697>

## Chemical synthesis

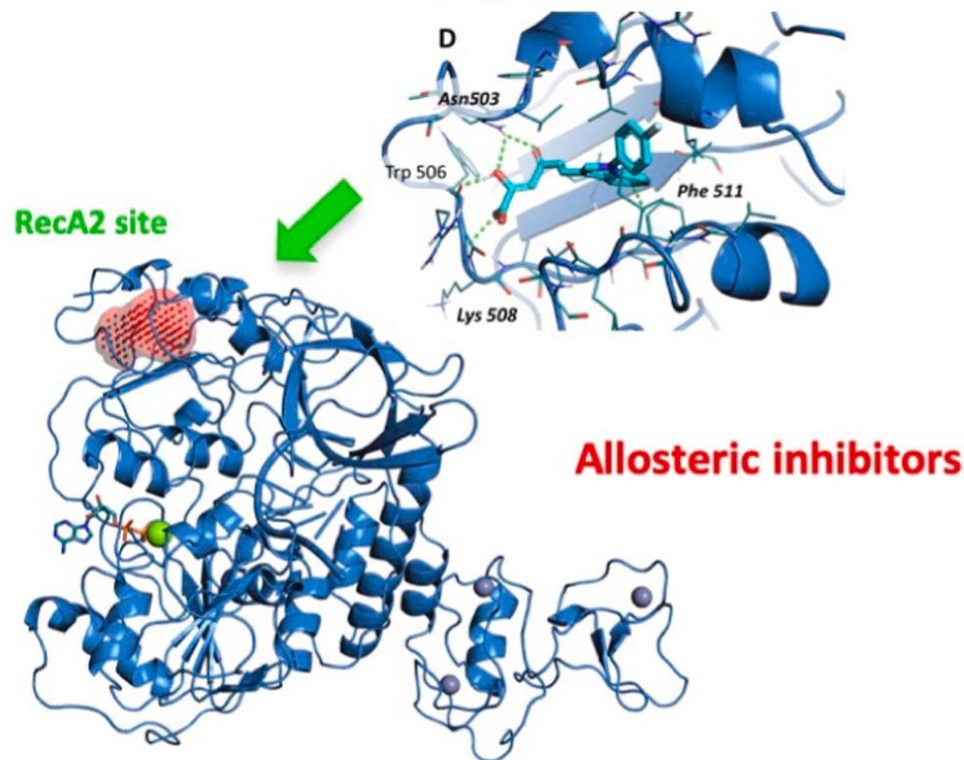


n = 0, CH<sub>2</sub>  
R = H, F

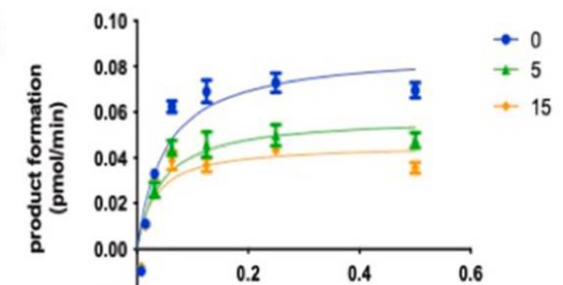
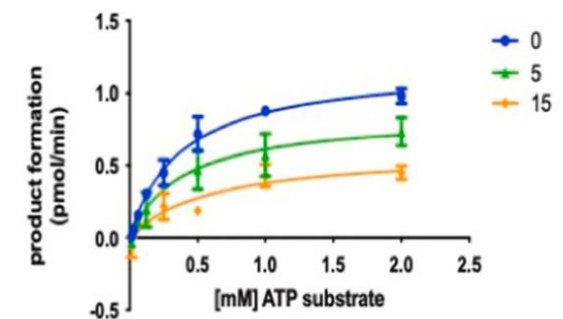
## New class of nsp13 inhibitors

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

## Docking studies



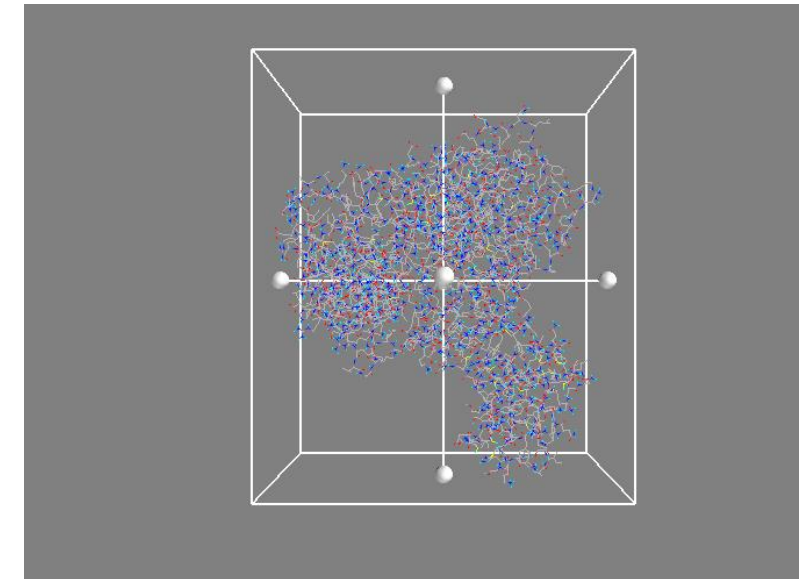
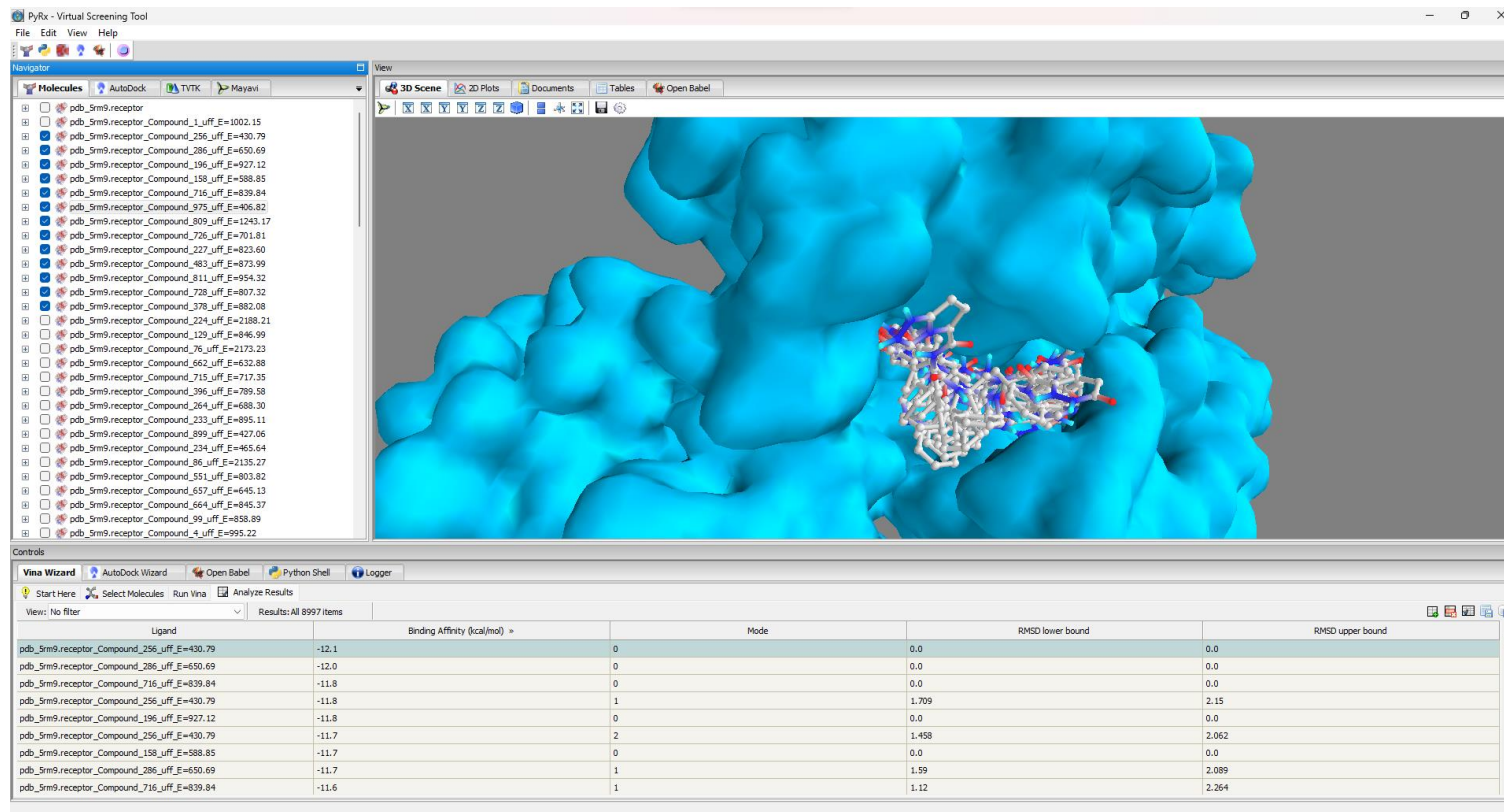
## Kinetics of inhibition





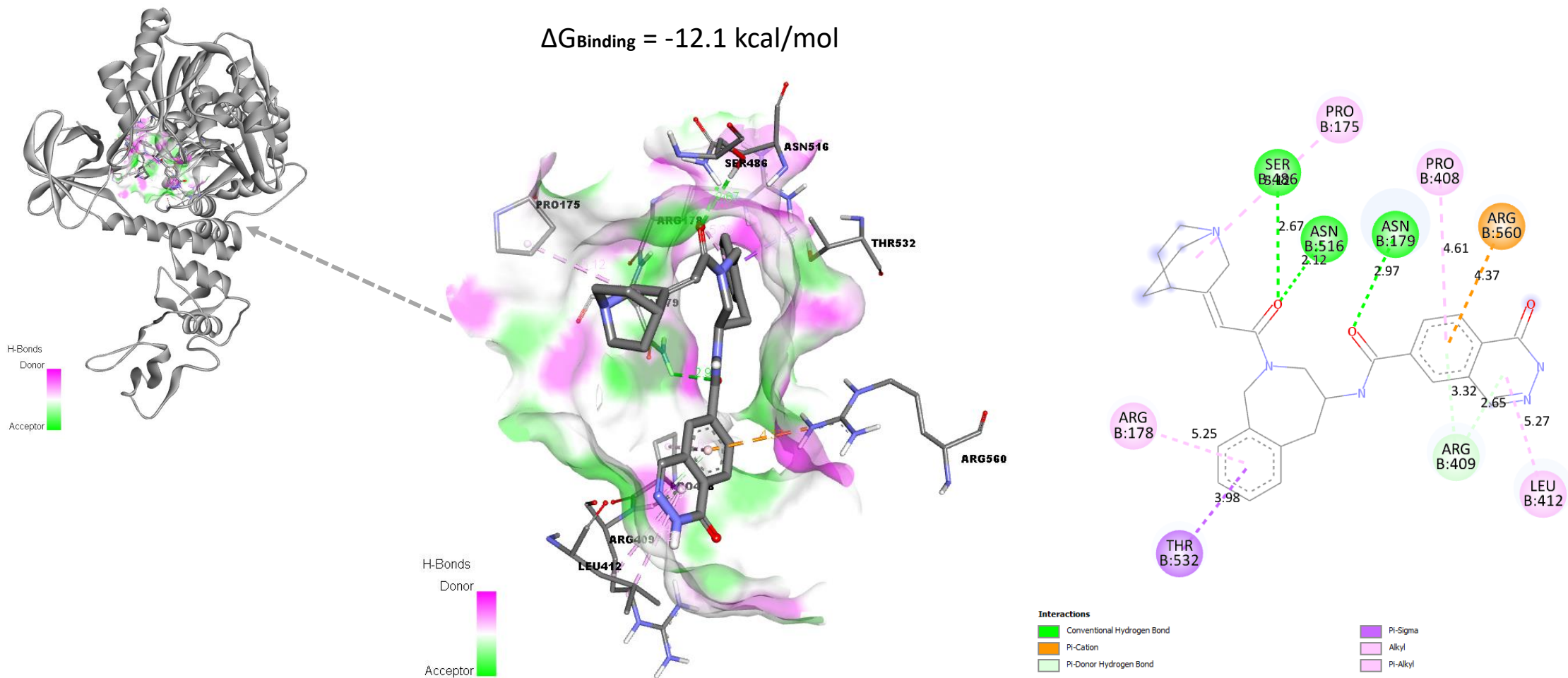
# De-Novo Generated Top 100 Scoring Compounds: Virtual Screen with PyRx 0.8 / AutoDock VINA

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



- Scores as low as  $\Delta G_{\text{Binding}} = -12.1$  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.

# De-Novo Generated Top 100 Scoring Compounds: *AutoDock VINA Pose (best scoring hit)*

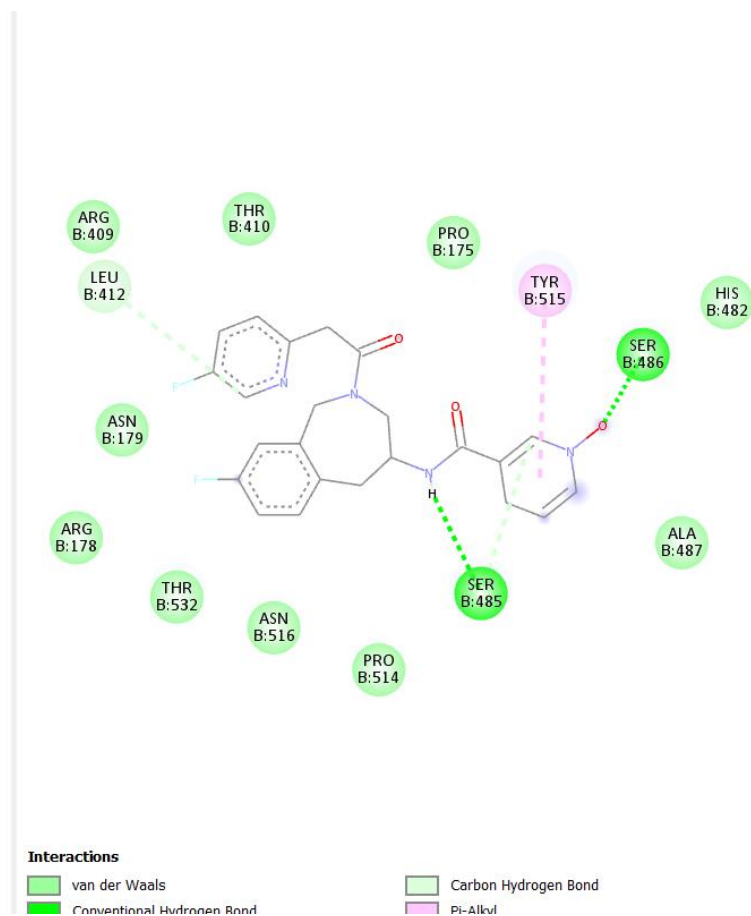
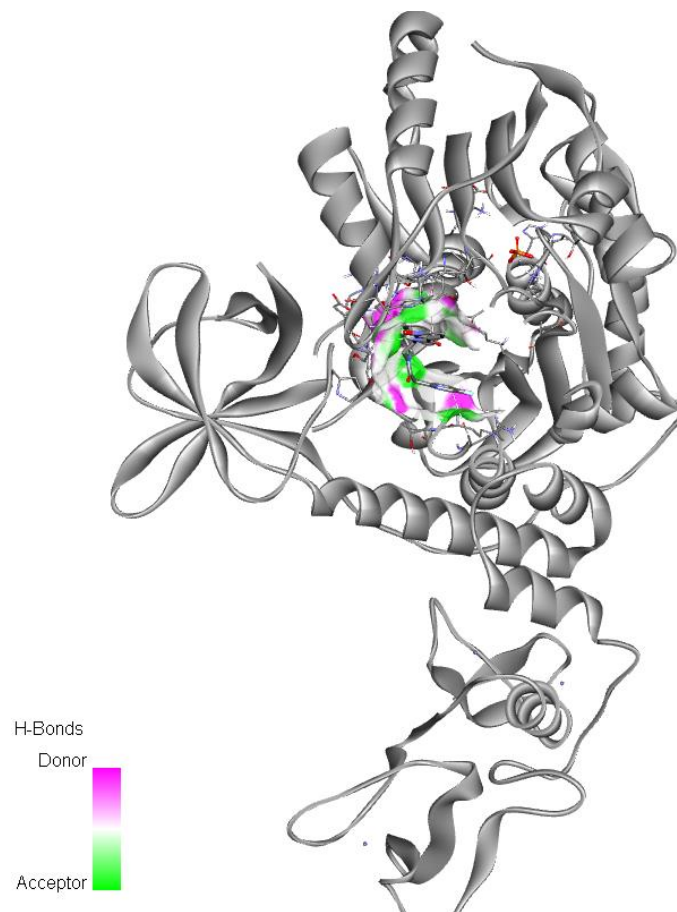


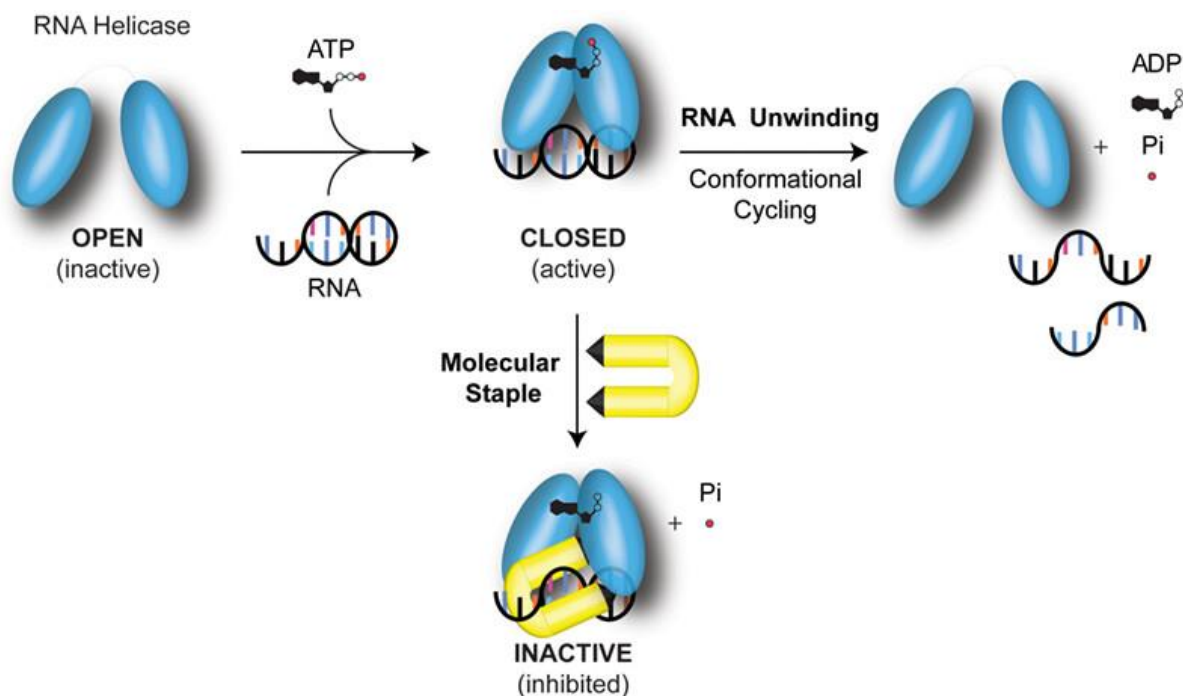
- PDB of lowest-energy pose with Geoff for MD analysis (AMBER20).



# De-Novo Generated Top 100 Scoring Compounds: Virtual Screen with PyRx 0.8 / AutoDock VINA

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





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

RNA helicases offer multiple opportunities for drug development.

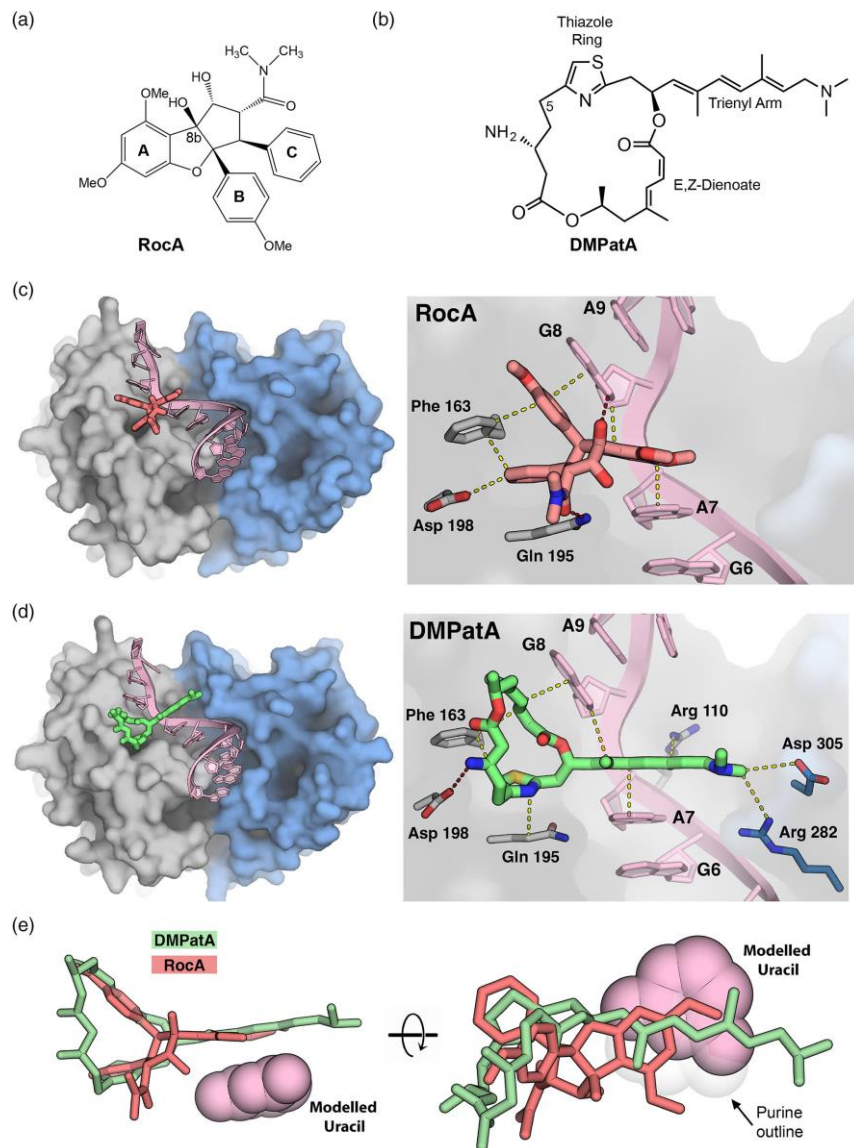
Small molecules that block protein:helicase interaction, interact with the ATP binding pocket, nucleic acid interaction interface, or inhibit ATPase hydrolysis have been described.

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.

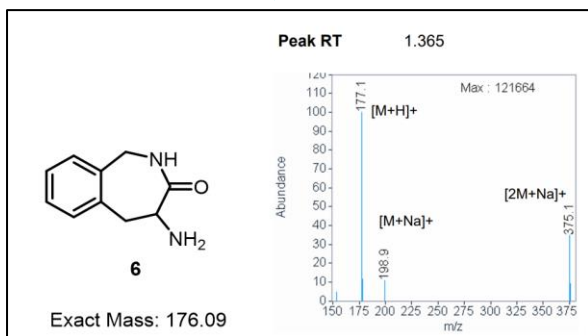
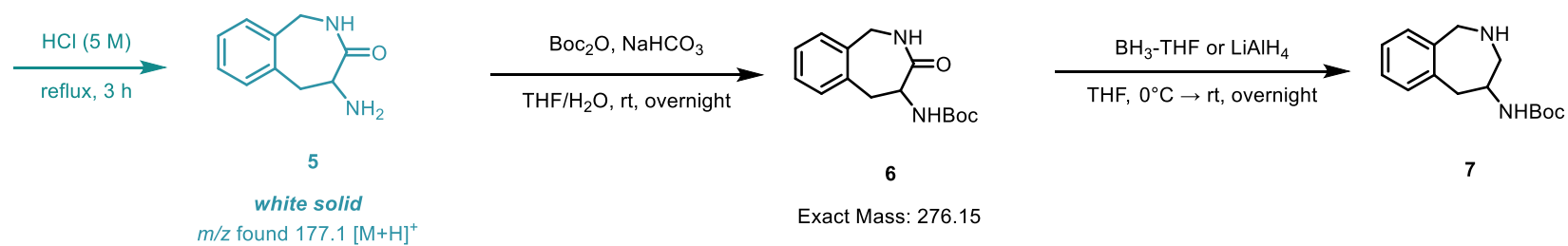
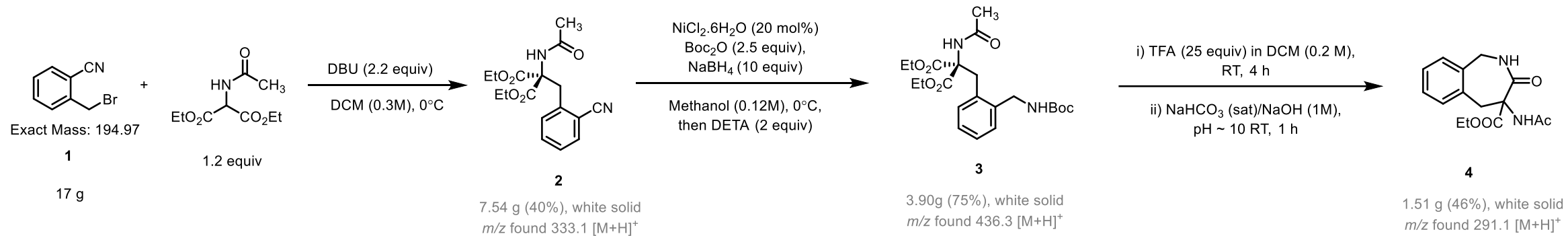
# Top 100 compounds: *de novo* A.I. generated compounds

## Molecular Staples



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.

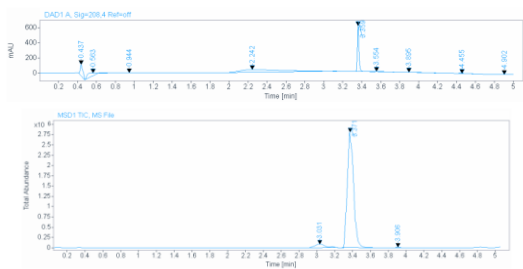
# De-Novo Generated Top 100 Scoring Compounds: Benzoazepines optimised route



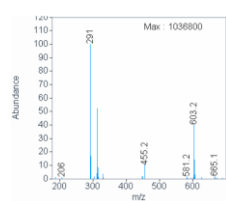
# De-Novo Generated Top 100 Scoring Compounds: Benzoazepines optimised route



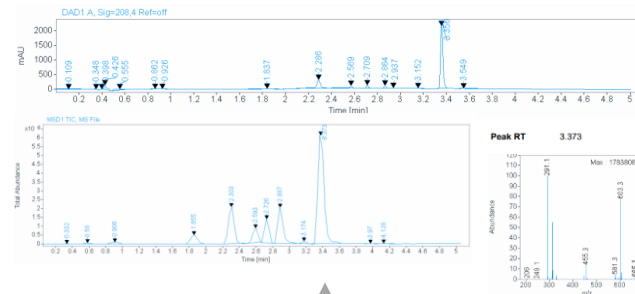
## DMAP cyclisation RPC pure



Peak RT 3.371



## Decarboxy Rt=1 h, 100degC 1M HCl



## BENZO AMINE rpc a1

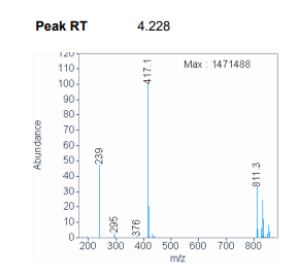
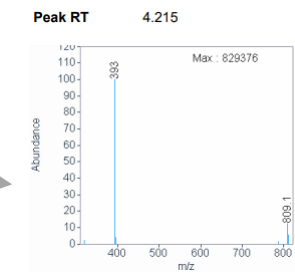
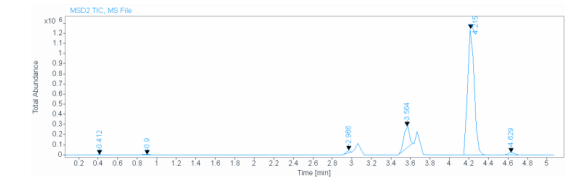
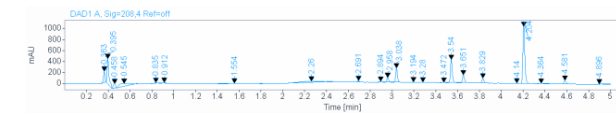
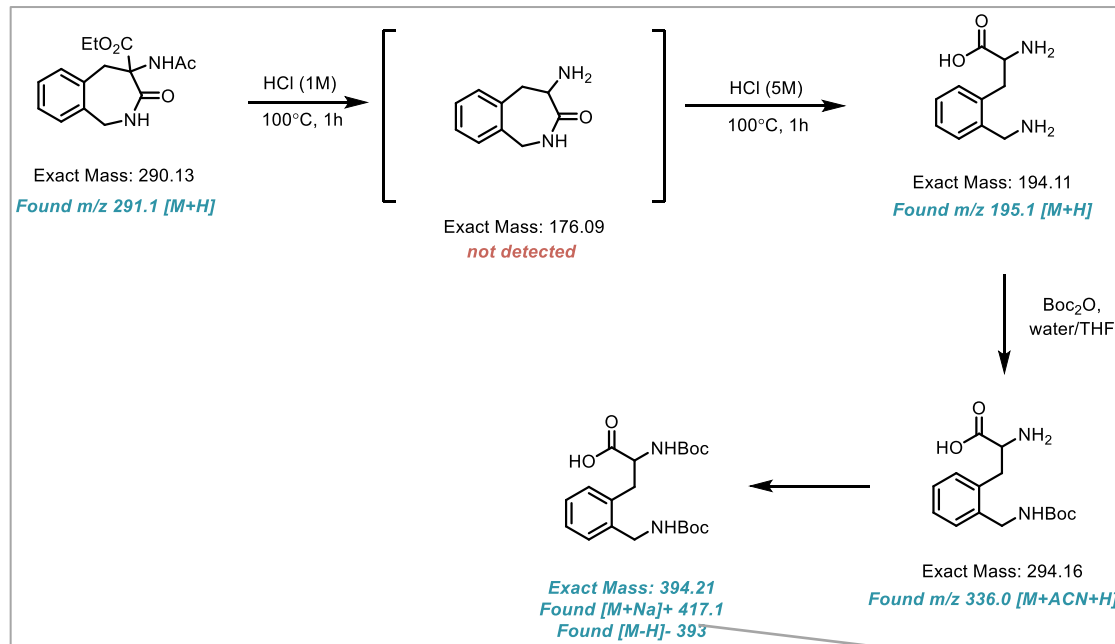
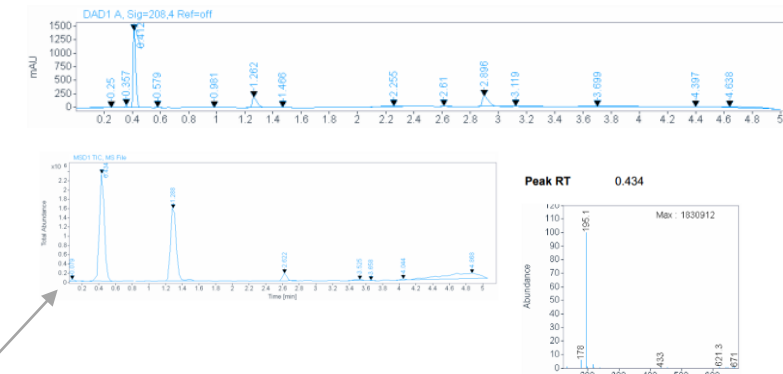
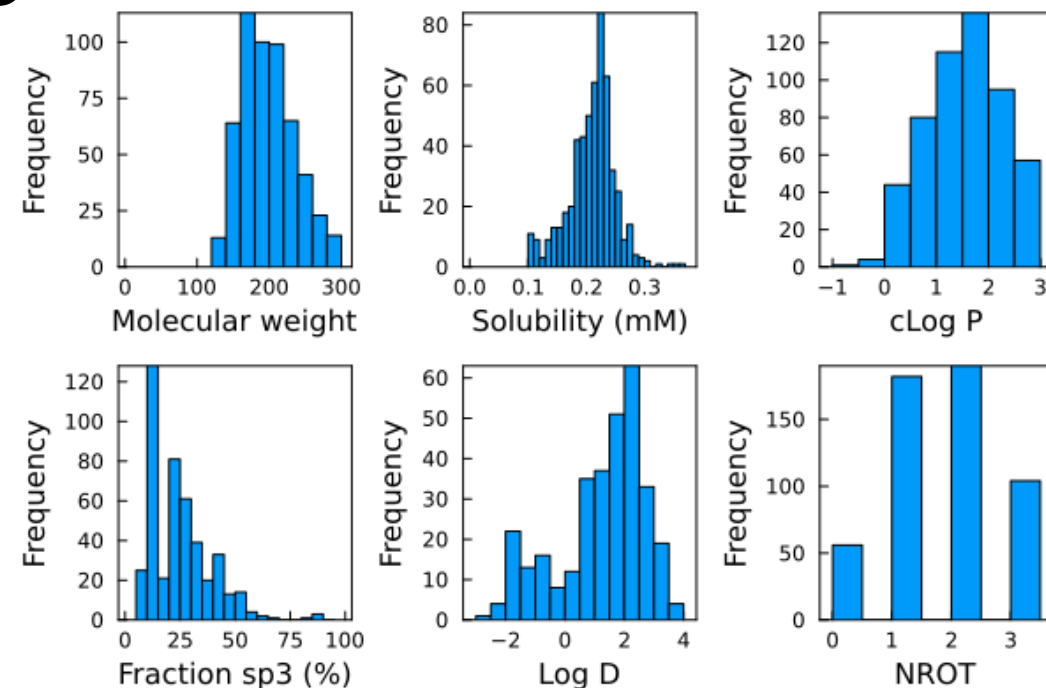
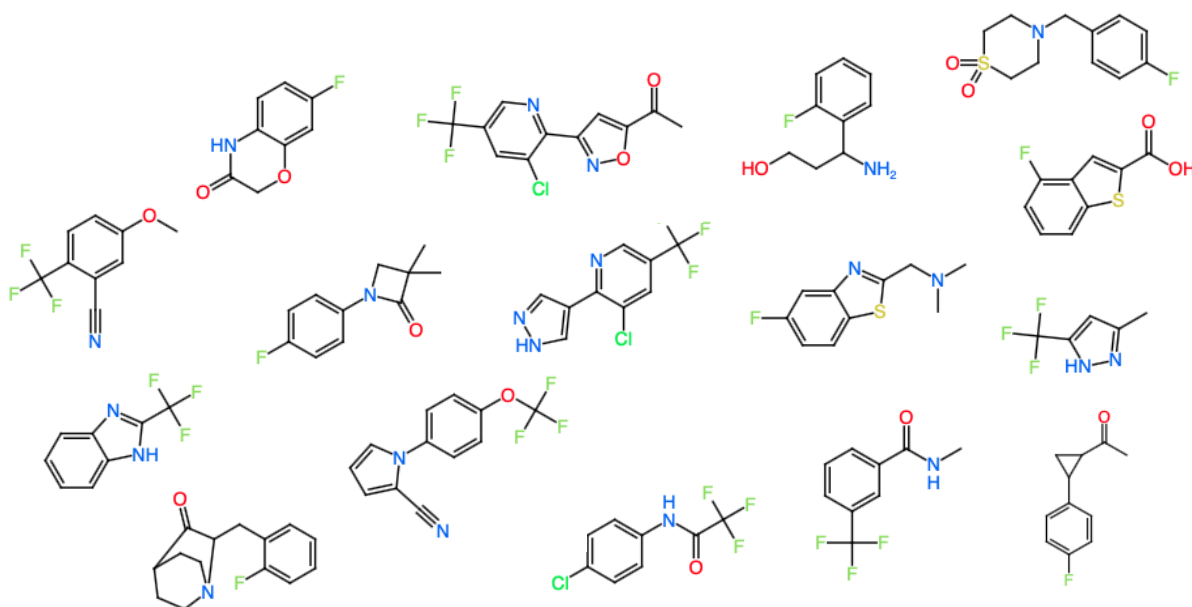


Table 1. Monoisotopic exact masses of molecular ion adducts often observed in ESI mass spectra

Ion name	Ion mass	Charge Mult	Mass	Your RT here 476.09000	Result:	Your M+X or M-X 336	Reverse:
1. Positive ion mode							
[M+3H] <sup>3+</sup>	M/3 + 1.007276	3+	0.33	1.007276	59.703943	110.659391	
[M+2H+Na] <sup>3+</sup>	M/3 + 8.334590	3+	0.33	8.334590	67.031257	103.332077	
[M+2H+K] <sup>3+</sup>	M/3 + 15.7661904	3+	0.33	15.662453	74.359120	96.004213	
[M+3Na] <sup>3+</sup>	M/3 + 22.989218	3+	0.33	22.989218	81.685885	88.677449	
[M+2H] <sup>2+</sup>	M/2 + 1.007276	2+	0.5	1.007276	89.052276	166.492724	
[M+H+NH <sub>4</sub> ] <sup>2+</sup>	M/2 + 9.520550	2+	0.5	9.520550	97.555550	157.979450	
[M+H+K] <sup>2+</sup>	M/2 + 11.988247	2+	0.5	11.988247	100.043247	155.501753	
[M+H+K] <sup>2+</sup>	M/2 + 19.985217	2+	0.5	19.985217	108.030217	147.514783	
[M+ACN+2H] <sup>2+</sup>	M/2 + 21.520550	2+	0.5	21.520550	109.565550	145.979450	
[M+2Na] <sup>2+</sup>	M/2 + 22.989218	2+	0.5	22.989218	111.034218	144.510782	
[M+2ACN+2H] <sup>2+</sup>	M/2 + 42.033823	2+	0.5	42.033823	130.078823	125.466177	
[M+3ACN+2H] <sup>2+</sup>	M/2 + 62.547097	2+	0.5	62.547097	150.592097	104.952903	
[M+H] <sup>+</sup>	M + 1.007276	1+	1	1.007276	177.097276	333.992724	
[M+NH <sub>4</sub> ] <sup>+</sup>	M + 18.033823	1+	1	18.033823	194.123823	316.966177	
[M+Na] <sup>+</sup>	M + 22.989218	1+	1	22.989218	190.079218	312.010782	
[M+CH <sub>3</sub> OH+H] <sup>+</sup>	M + 33.033489	1+	1	33.033489	209.123489	301.966511	
[M+K] <sup>+</sup>	M + 38.963158	1+	1	38.963158	215.053158	296.036842	
[M+ACN+H] <sup>+</sup>	M + 42.033823	1+	1	42.033823	218.123823	292.966177	
[M+2H+H] <sup>+</sup>	M + 44.971160	1+	1	44.971160	221.061160	290.028940	
[M+isoProp+H] <sup>+</sup>	M + 61.065340	1+	1	61.065340	237.155340	273.934560	
[M+ACN+Na] <sup>+</sup>	M + 64.015765	1+	1	64.015765	240.105765	270.984235	
[M+2K+H] <sup>+</sup>	M + 76.919040	1+	1	76.919040	253.009040	258.080960	
[M+EMSO+H] <sup>+</sup>	M + 79.02122	1+	1	79.021220	255.111220	255.978780	
[M+2ACN+H] <sup>+</sup>	M + 83.060370	1+	1	83.060370	258.150370	251.938630	
[M+isoProp+Na+H] <sup>+</sup>	M + 84.05511	1+	1	84.055110	260.145110	250.944890	
[2M+H] <sup>+</sup>	2M + 1.007276	1+	2	1.007276	353.187276	668.992724	
[2M+NH <sub>4</sub> ] <sup>+</sup>	2M + 18.033823	1+	2	18.033823	370.213823	651.966177	
[2M+Na] <sup>+</sup>	2M + 22.989218	1+	2	22.989218	375.169218	647.010782	
[2M+K] <sup>+</sup>	2M + 38.963158	1+	2	38.963158	391.143158	631.036842	
[2M+ACN+H] <sup>+</sup>	2M + 42.033823	1+	2	42.033823	394.213823	627.966177	
[2M+ACN+Na] <sup>+</sup>	2M + 64.015765	1+	2	64.015765	416.195765	605.984235	

# $^{19}\text{F}$ 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  $^{19}\text{F}\{^1\text{H}\}$  and  $^1\text{H}$ ,  $^1\text{H}$  waterLOGSY]

Two samples required (+/- fragment)

Approx. 36 hr total acquisition time

ca. £1600 (UKRI rate) + £250 NMR tubes

## BioNET $^{19}\text{F}$ library

Ro3 compliant, solubility tested, PAINS free

No usage/license restrictions

500 fragments x 10 mg

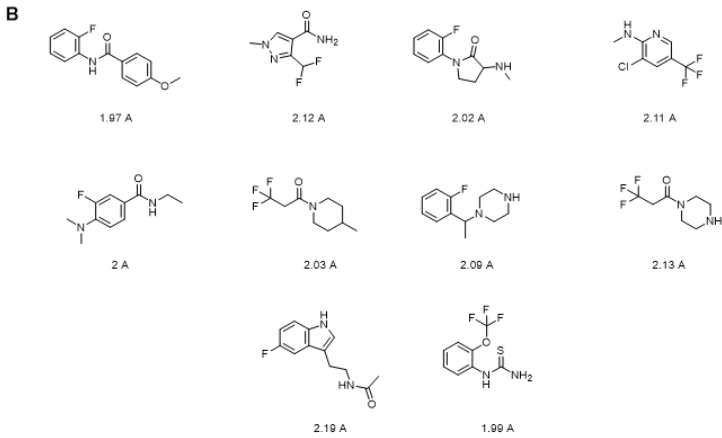
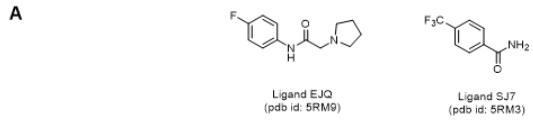
ca. £11k – enough material for 700 screens

(2 x 170  $\mu\text{L}$  x 200  $\mu\text{M}$  required per screen)

*[to determine – library storage, maintenance]*

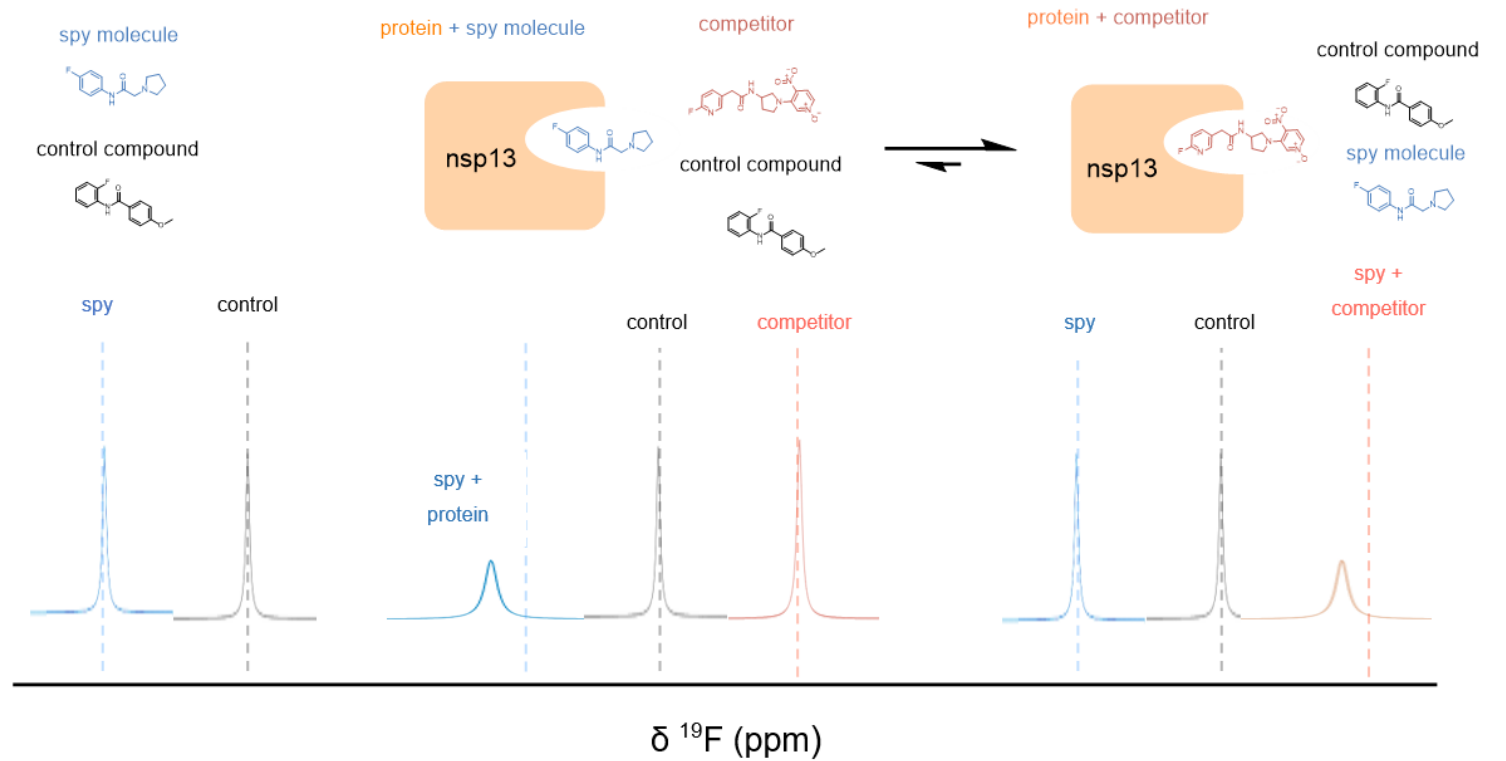
Total cost ca. £2.1k / screen, assuming we run 50 screens

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

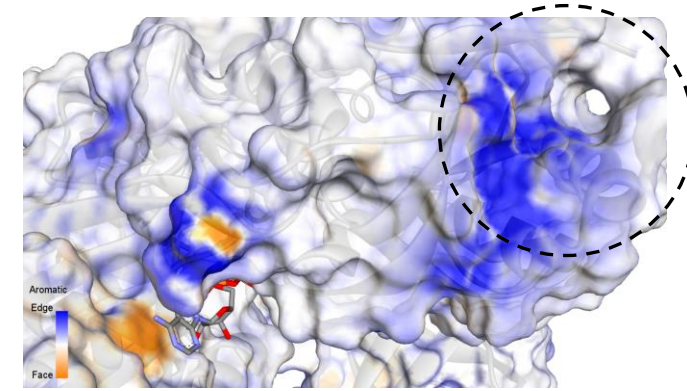
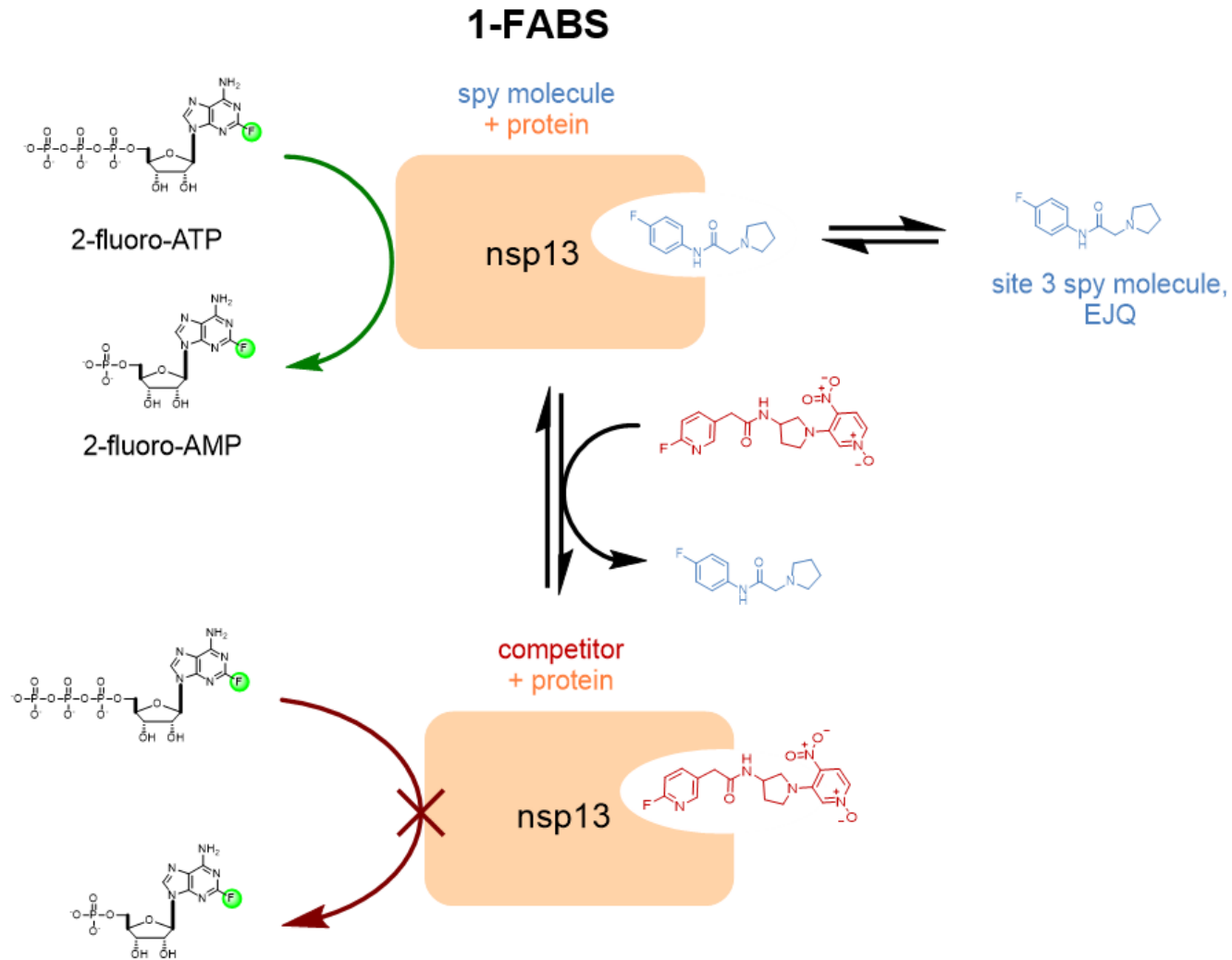
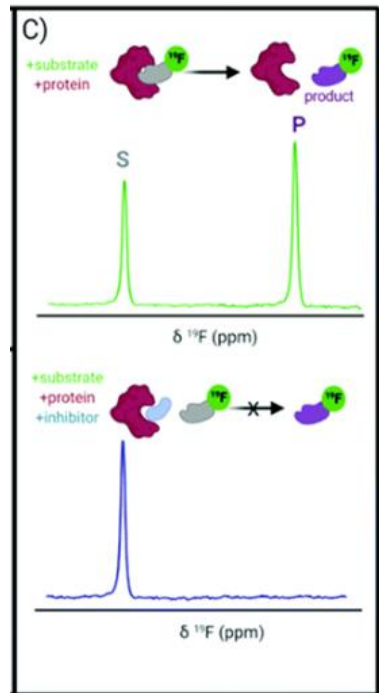


A) Spy molecules EJQ (pdb id: 5rm9) and SJ7 (pdb id: 5rm3) against SARS-CoV-2 nsp13 site 3. B) Negative control compounds for competitive FAXS screen with good solubility in crystallisation buffer. Newman *et al.*, 2021. Diffraction resolutions for each compound are given in Angstroms. C) 2-(trifluoromethyl)benzoic acid (TFMBA) for <sup>19</sup>F chemical shift referencing.

## Competition binding FAXS (T<sub>2</sub> filtered)



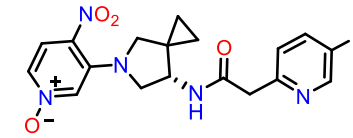
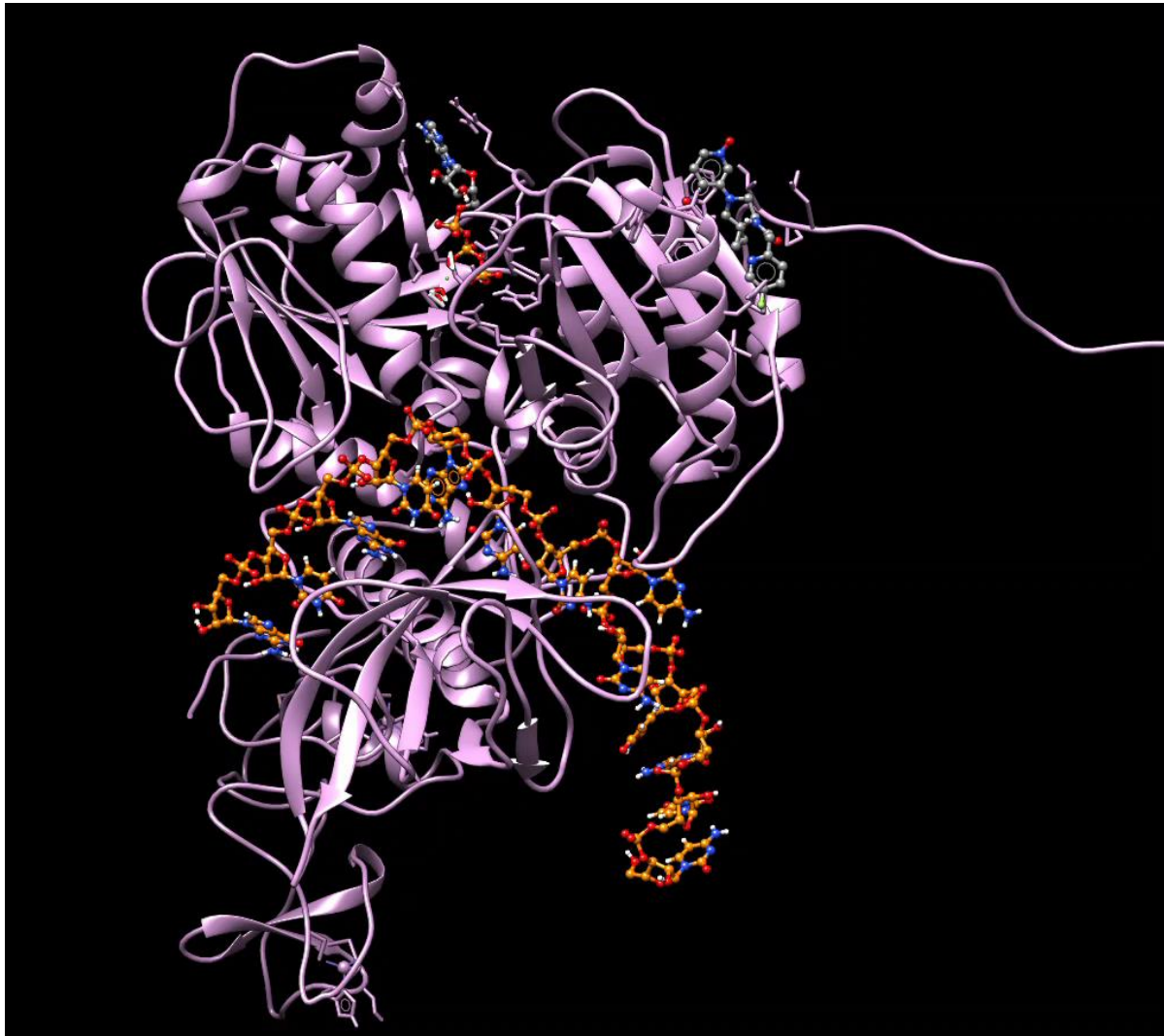
# Nsp13 N-FABS NMR Assay Design



AMP-PNP/nsp13 complex,  
pdb 7NN0.

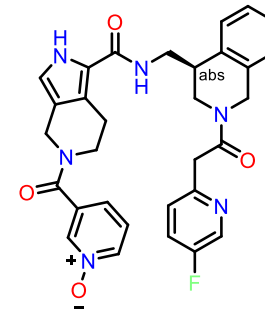


# Molecular Dynamics (AMBER) simulation and docking: Nsp13-ATP-ssRNA + N-oxide ligand (Geoff Wells + Tom)



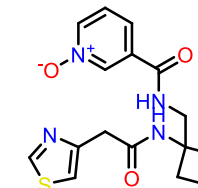
**2c**  
VINA: -5.8 kcal/mol

glide -7.869

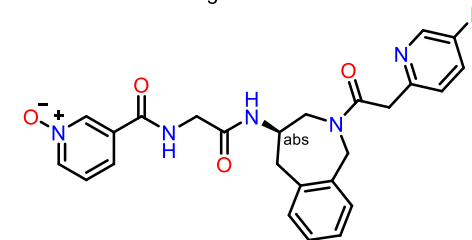


**RA-0001264-01**

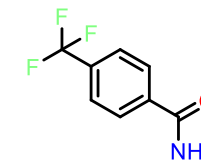
ATPase IC50 = 48  $\mu$ M



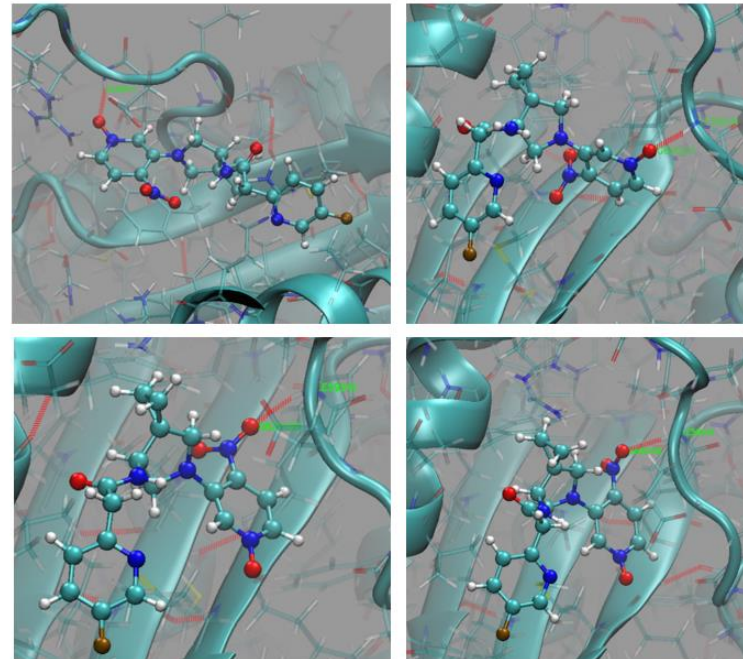
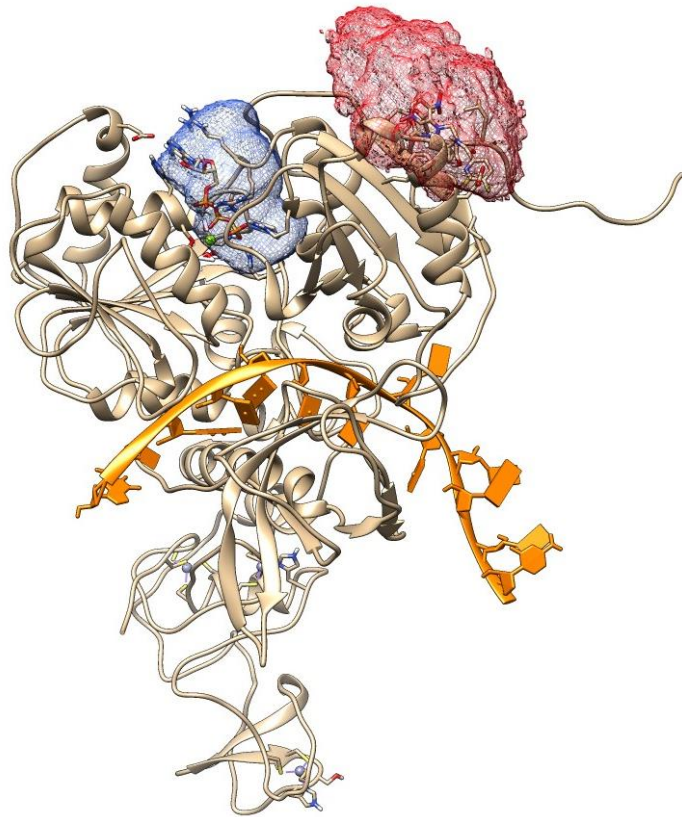
glide -7.861



**5RM3**

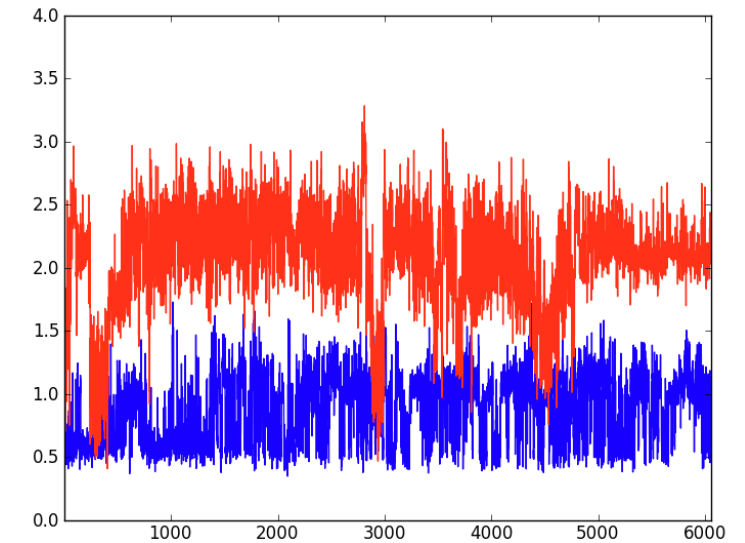


Ligand 2c atomic occupancy volume



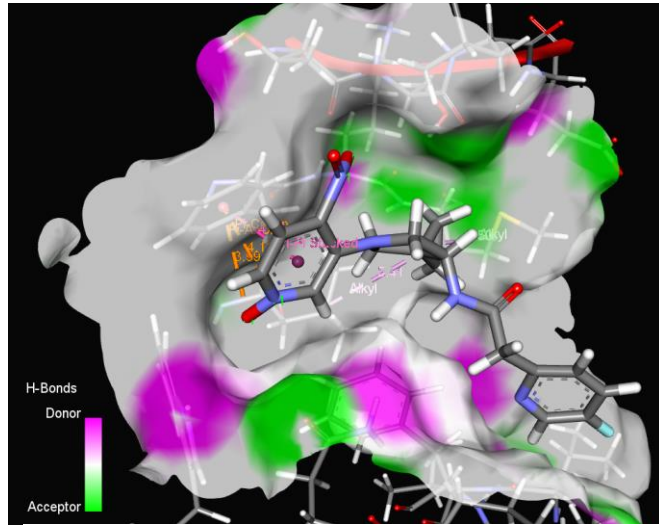
Molecular dynamics (MD) simulation of Nsp13-ATP-ssRNA-Ligand B7 complex showing hydrogen bonding (< 3 Angstroms, red) interactions at 373.0ns and 535.2ns (top, N-oxide N-O...HN ILE592), 384.3ns and 437.9ns (middle, nitro N-O...HN ILE592), and 356.8ns (bottom, amide NH...O GLU591).

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

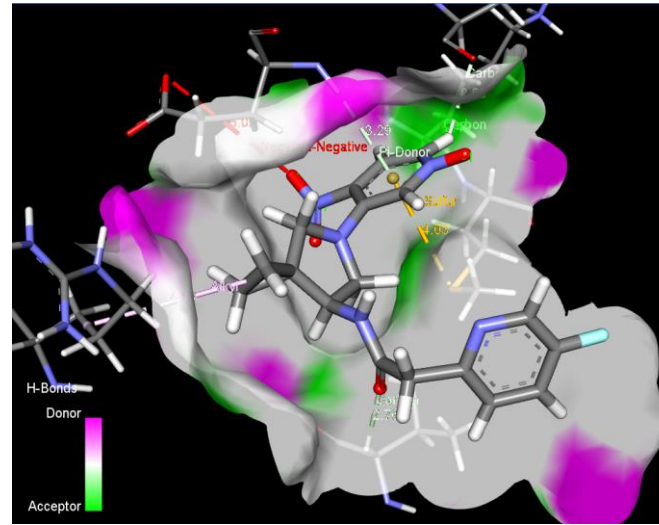


# Molecular Docking (AutoDock Vina) and Dynamics (AMBER20): Fragment-bound vs ATP/ssRNA bound states (Geoff Wells + Tom)

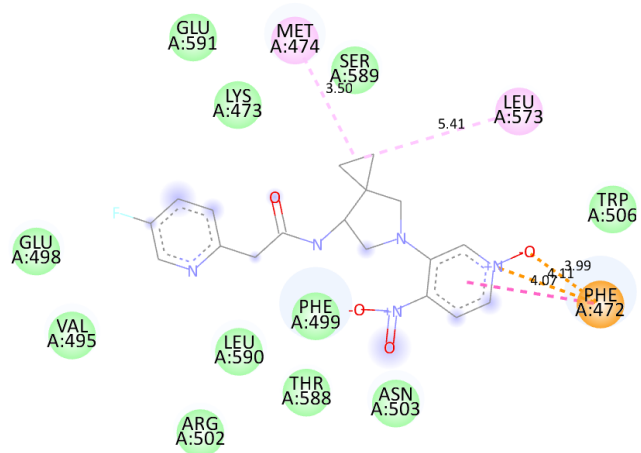
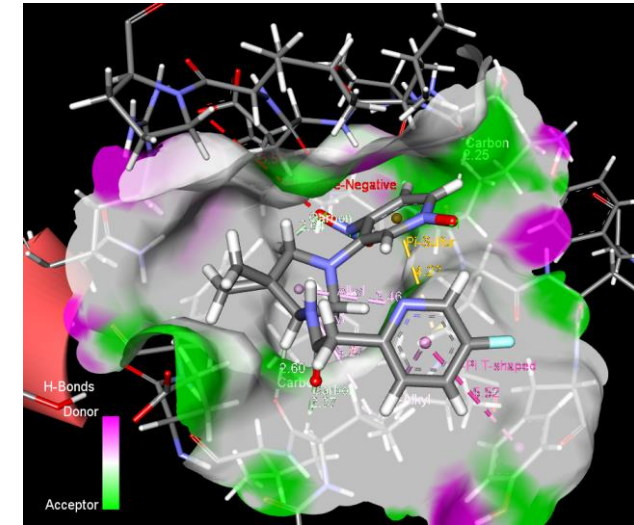
0 ns



100 ns

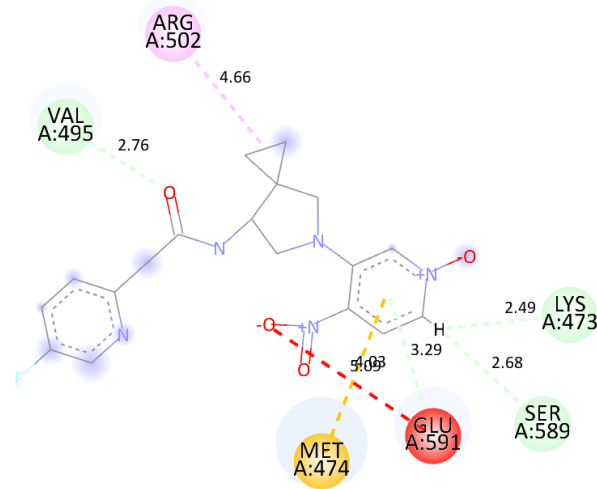


200 ns



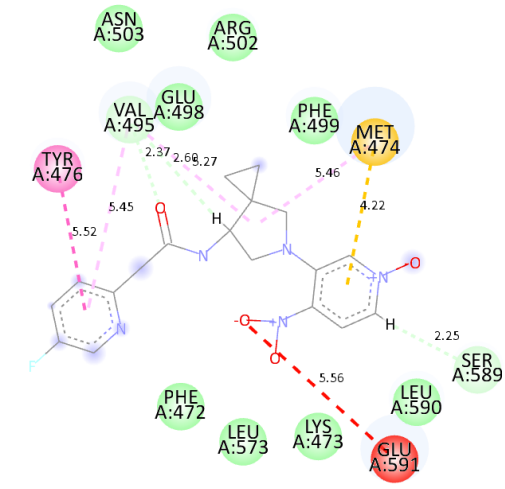
**Interactions**

- van der Waals
- Pi-Cation
- Pi-Anion
- Pi-Pi Stacked
- Alkyl



**Interactions**

- Carbon Hydrogen Bond
- Unfavorable Negative-Negative
- Pi-Donor Hydrogen Bond
- Pi-Sulfur
- Alkyl



**Interactions**

- van der Waals
- Carbon Hydrogen Bond
- Unfavorable Negative-Negative
- Pi-Sulfur
- Pi-Pi T-shaped
- Alkyl
- Pi-Alkyl

# Molecular Docking (AutoDock Vina) and Dynamics (AMBER20):

## Fragment-bound vs ATP/ssRNA bound states (Geoff Wells + Tom)



300 ns

400 ns

500 ns

600 ns

