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Supporting information for article:

***FragMAXapp*: crystallographic fragment screening data analysis
and project management system**

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Costantino, Tatjana Barthel, Jan Wollenhaupt, Manfred S. Weiss and Uwe
Mueller**

Project Settings

Set experiment definitions

Protein Protein acronym

Library Name Library name

Library Fragments Choose file No file chosen

Proposal Number 20180489

Shifts 20190622,20190629

Data Processing Encrypted Mode

CREATE CANCEL

Figure S1 Project Setting page. The minimum required information is validated before project creation.

Data Collection Overview

UPDATE PROJECT DATABASE

Search for Sample names...

Legend: Successful (Green), Not started (Yellow), Failed (Red)

Info/Process	Protein	Sample name	Run	Proc Status	Ref Status	Lig Status	Resolution [Å]	Frames	Crystal Picture	Ligand
INFO	PrtK	A10a	1	Pipedream XIAZ/DIALS XIAZ/XDS XDSAPP autoPROC EDNA_proc fastdp	Pipedream DIMPLE BUSTER LigandFit	Pipedream rhoFit	1.24	3600		<chem>Nc1ccc(O)c(O)c1</chem>
INFO	PrtK	A11a	1	Pipedream XIAZ/DIALS XIAZ/XDS XDSAPP autoPROC EDNA_proc fastdp	Pipedream DIMPLE BUSTER LigandFit	Pipedream rhoFit	1.24	3600		<chem>Nc1ccc(O)c(O)c1</chem>

Figure S2 Fragment screening project Home Page, displaying information about the data collection parameters, the crystal, fragment and data processing progress.

The screenshot shows the 'FragMAX - Library view' web interface. On the left is a dark sidebar with navigation options: PrtK - JBS, Project, PDBe, Library view, Project info, Home, Data Analysis, PanDDa, Results, Download, PDB export tool, Check HPC, and Logout. The main content area has a 'New Library CSV' section with a 'Choose file' button and an 'UPDATE' button. Below this is a message: 'Fragment missing from your project based on dataset names'. The 'JBS Library' section displays a grid of eight chemical structures labeled A10a, A11a, A12a, A1a, A2a, A3a, A4a, and A5a. Each structure is accompanied by its SMILES string: A10a (NCC(=O)c1ccc(Br)cc1), A11a (Fc1ccc(cc1F)C1=NNC2=NCCN2C1), A12a (O[C@@H](C(=O)O)c1ccccc1)c1), A1a (Cc1ccccc1C(=O)NN), A2a, A3a, A4a, and A5a.

Figure S3 Full reproduction of sample management page interface.

The screenshot shows the 'FragMAX - Data Analysis' web interface. The sidebar on the left includes: hCAII - FMLv03, Project, Home, Data Analysis, PanDDa, Results, Download, PDB export tool, Check HPC, and Logout. The main area is titled 'hCAII Data Analysis' and features a notification: 'New actions will process/reprocess your datasets using at MAX IV HPC and replace your current result'. There are three checkboxes: 'Process all datasets' (checked), 'Process new datasets', and 'Select ALL datasets'. A 'Select dataset' dropdown is present. The interface is divided into three columns: 'Data processing', 'Structure Refinement', and 'Ligand fitting'. Each column has a 'Software' section with checkboxes for Pipedream, XIA2/DIALS, XIA2/XDS, XDSAPP, and autoPROC. The 'Data processing' column also has a 'Space group' field (P43212) and 'Cell parameters' (a, b, c, α , β , γ). The 'Structure Refinement' column has a 'PDB model' dropdown and a 'Space group' field (P43212). The 'Ligand fitting' column has a 'Software' section with checkboxes for Pipedream, RhoFit, LigandFit, and PanDDA, and radio buttons for GRADE, AceDRG, and eLBOW. At the bottom of each column is a 'RUN' button: 'RUN DATA PROCESSING', 'RUN REFINEMENT', and 'RUN LIGAND FITTING'. A footer bar contains tabs for each software package: XIA2/DIALS, XIA2/XDS_XSCALE, XDSAPP, autoPROC, DIMPLE, BUSTER, fspipeline, RhoFit, LigandFit, PanDDA, and Pipedream.

Figure S4 Full reproduction Data Analysis interface. In this view, the submission buttons are enabled due to the selection of pipelines.

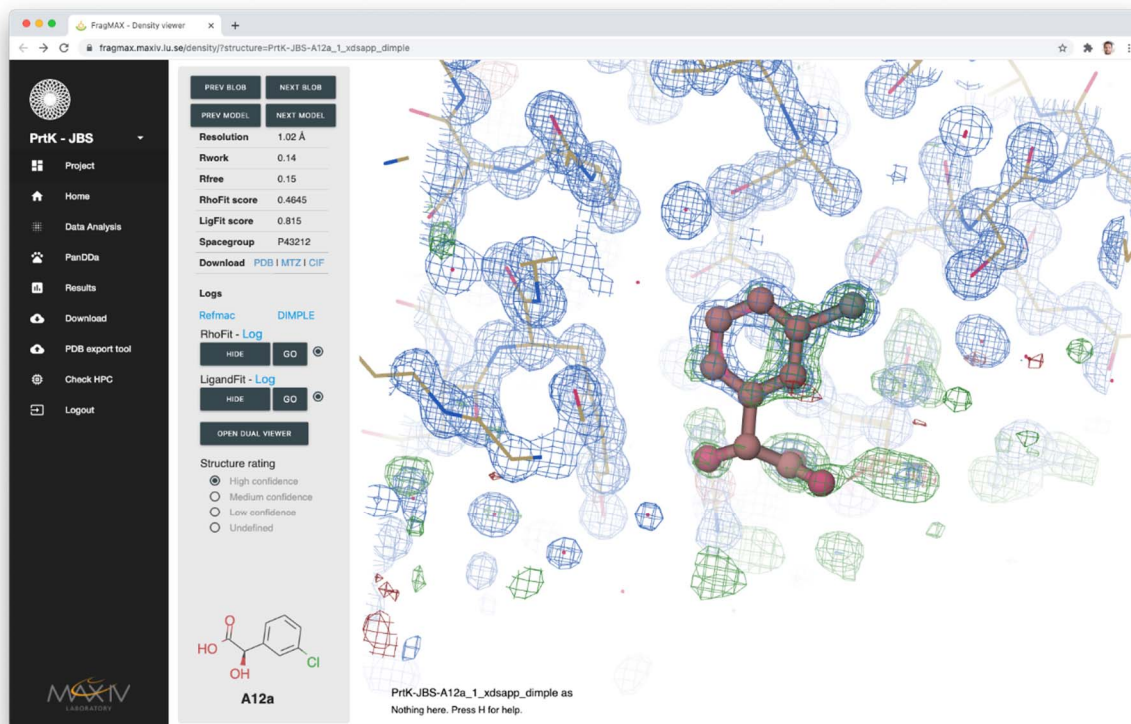


Figure S5 Full reproduction of Results view.

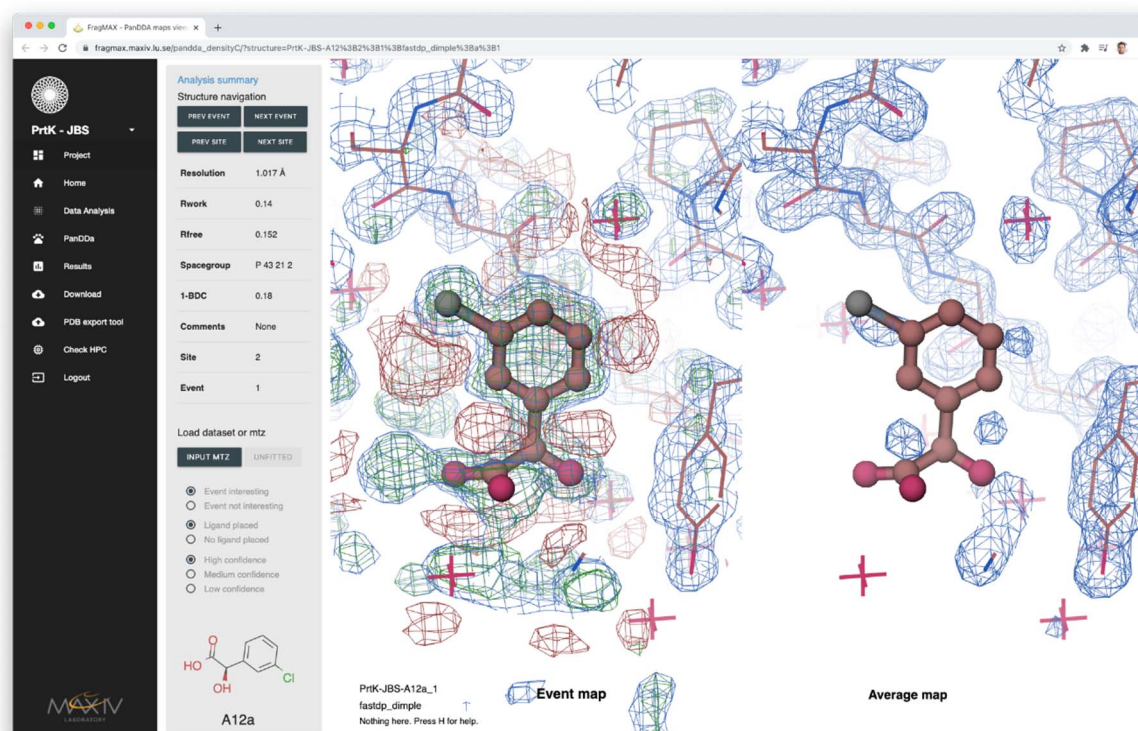


Figure S6 PanDDA events viewer. The side-by-side comparison between the event and average maps from PanDDA help identify false-positive events during the analysis. From the webapp, it is

possible to see information about the event and the user-annotated confidence level. The navigation function moves the view to different site centromeres.

S1. Proteinase K fragment screening

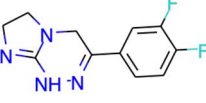
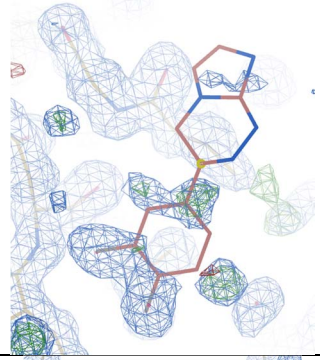
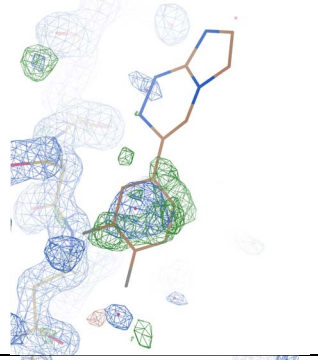
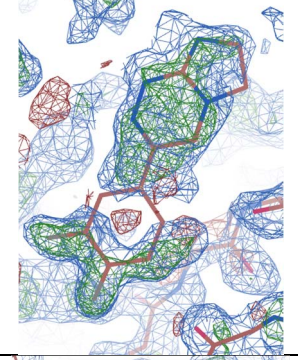
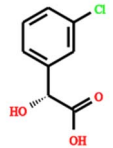
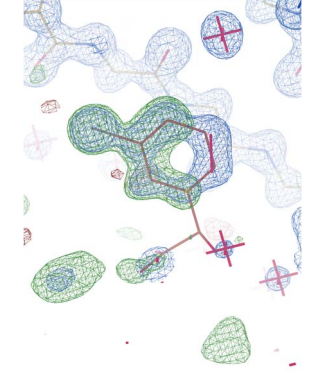
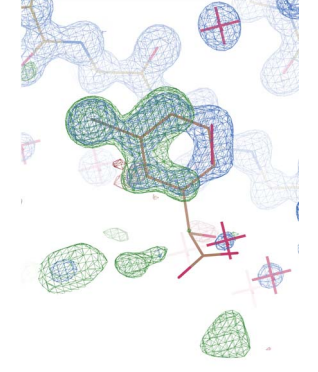
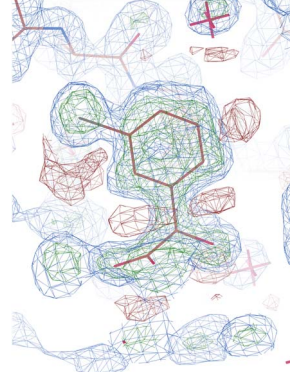
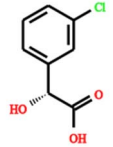
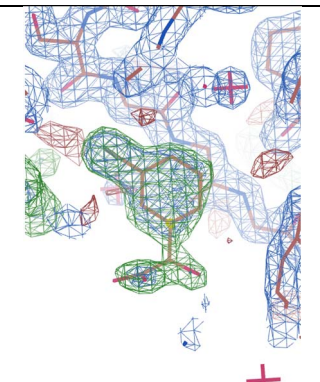
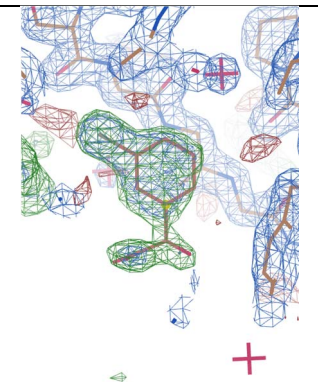
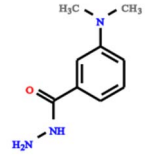
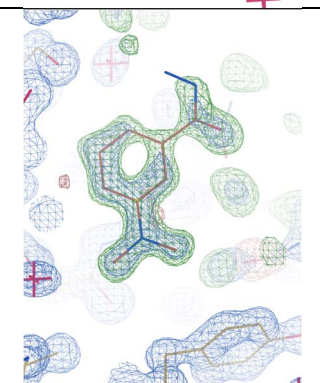
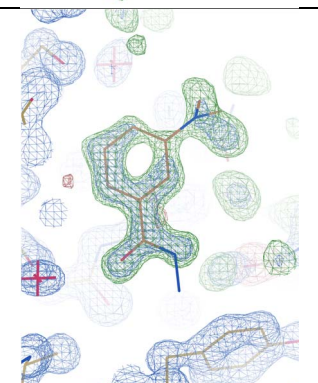
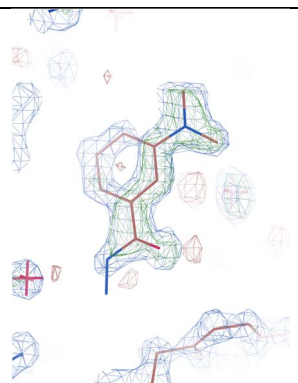
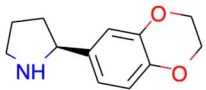
S1.1. Material and Methods

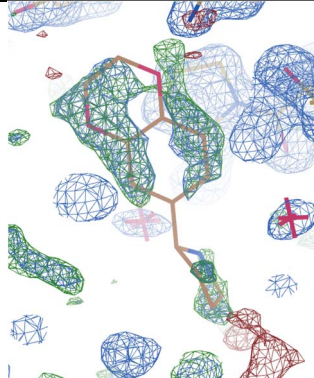
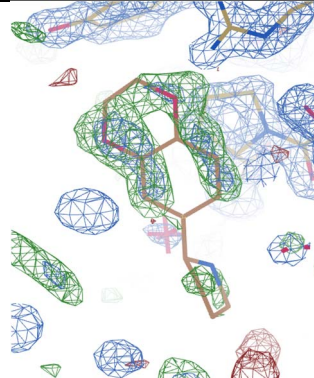
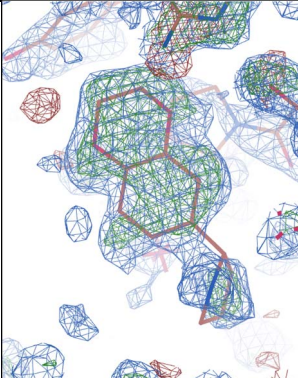
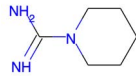
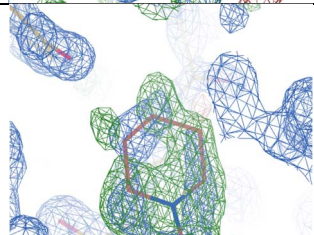
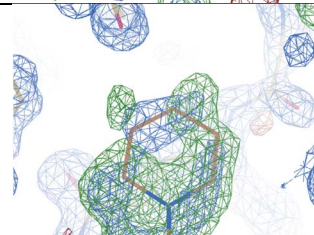
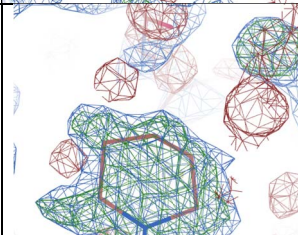
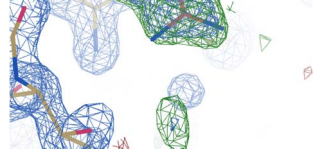

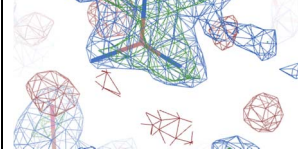
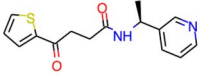
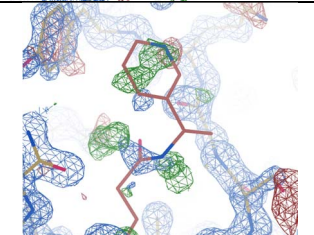
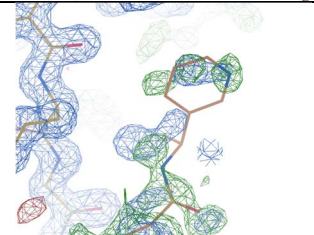
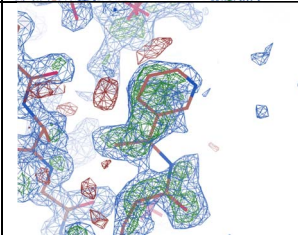
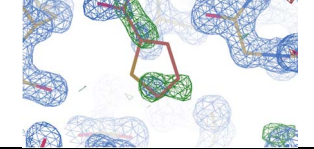

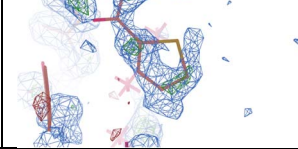
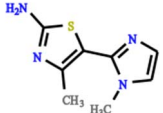
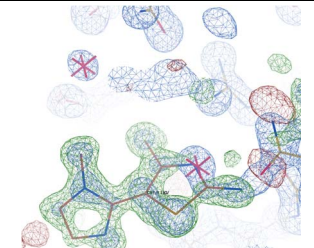
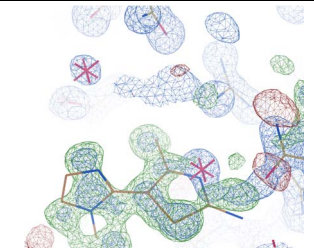
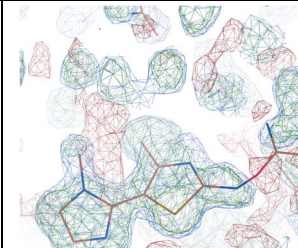


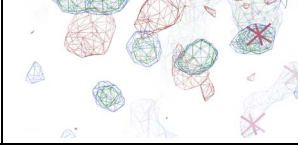
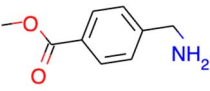
The protein was purchased (Jena Bioscience, Germany) and crystallised using sitting drop method as described in previous work (Larson *et al.*, 2009) on MRC3 plates (SWISSCI, Switzerland) using a Mosquito liquid handling system (TTP Labtech, UK). The fragment library Frag Xtal Screen was purchased (Jena BioScience, Germany) and handled according to the manual instructions. For this experiment, we renamed the fragments from its original convention (1 to 96) to an alphanumeric convention equivalent to its position in the 96-well storage plate (A1 to H12). The crystallisation drops were 300 nL containing a mixture of 150 nL of precipitating solution (1.2 M Ammonium Sulfate, 0.1 M Tris-HCl, pH 8.0) and 150 nL of 10 mg.mL⁻¹ Proteinase K solution. Suitable single crystals were obtained after 48 h, with sizes ranging from 50 to 100 µm. The crystals were soaked for 2 hours using 300 nL at 50 nM of each fragment dissolved in a precipitant solution containing 20% DMSO, for a final DMSO concentration of 10% after addition to the crystallisation drop. To transfer the fragment solutions on top of the crystallisation drop, the Crystal Shifter (Oxford Labtec, UK) was used with the crystal plate in one of its plate holders and the solubilised fragment plate in the second one. After soaking, crystals were harvested with Crystal Shifter assistance and cryo-cooled in LN2. Diffraction data of the identified hits from the cryo-experiment were also collected at room temperature (RT). The samples for RT experiment were prepared using the same protocol described in this section, mounting the crystal loops inside MicroRT capillaries (MiTeGen, USA) instead of cryo-cooling in LN2. Data collection was performed at BioMAX beamline, with experimental parameters specified in Table 1.

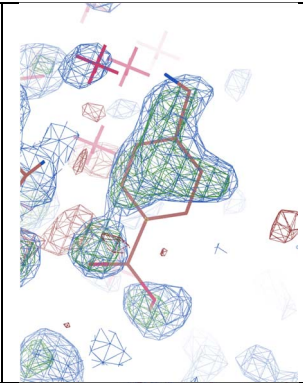
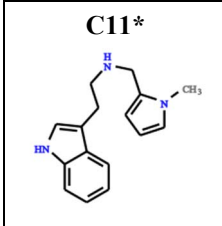
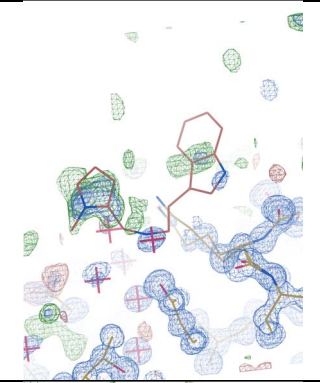
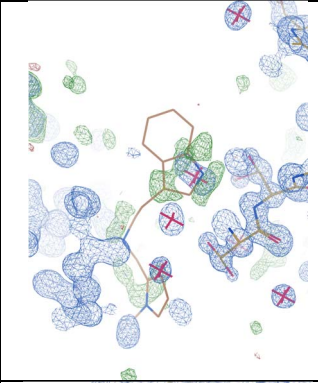
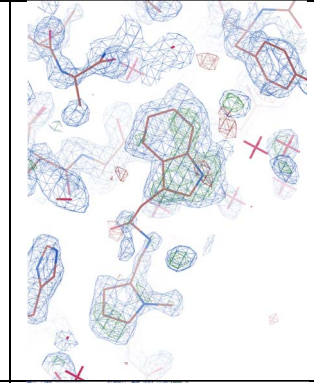
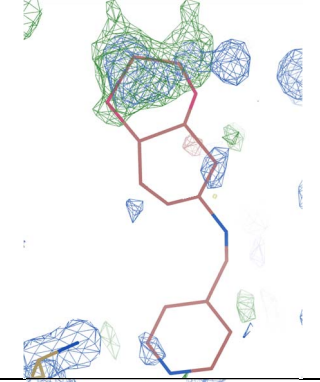
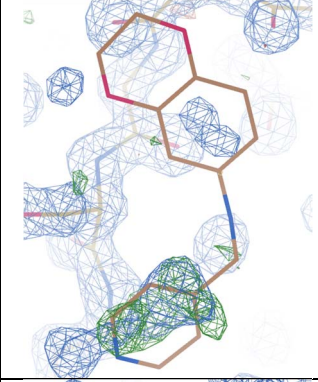
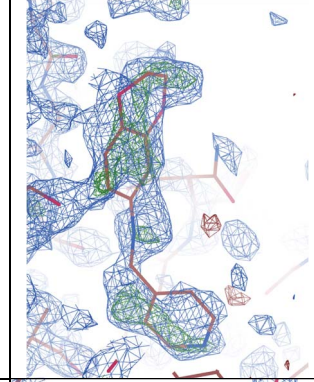
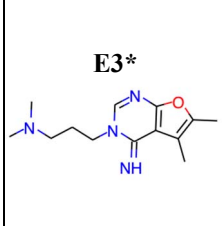
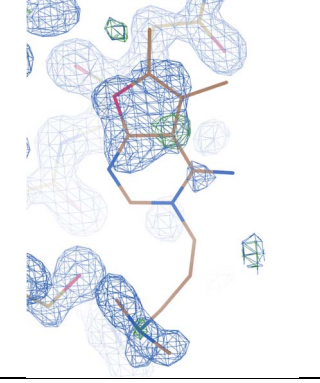
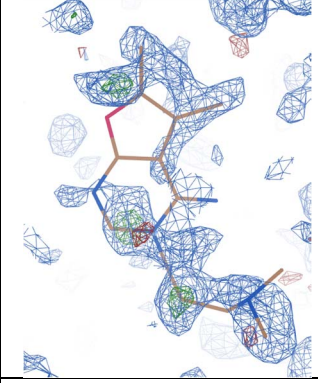
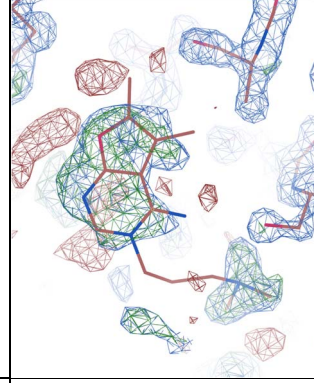
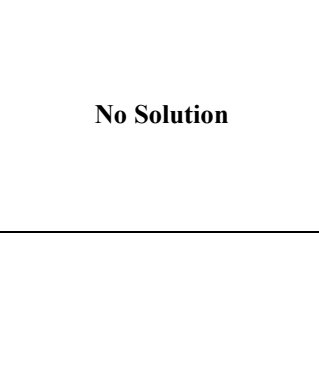
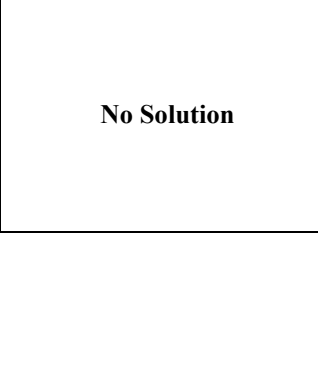
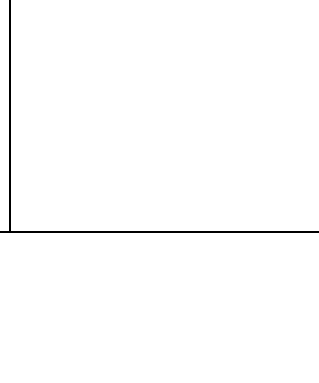




S1.2. Results

Using FragPLEX selection and PanDDA to analyse the data, 18 fragments out of 96 were found, with an 18,75% hit rate (Table S1).

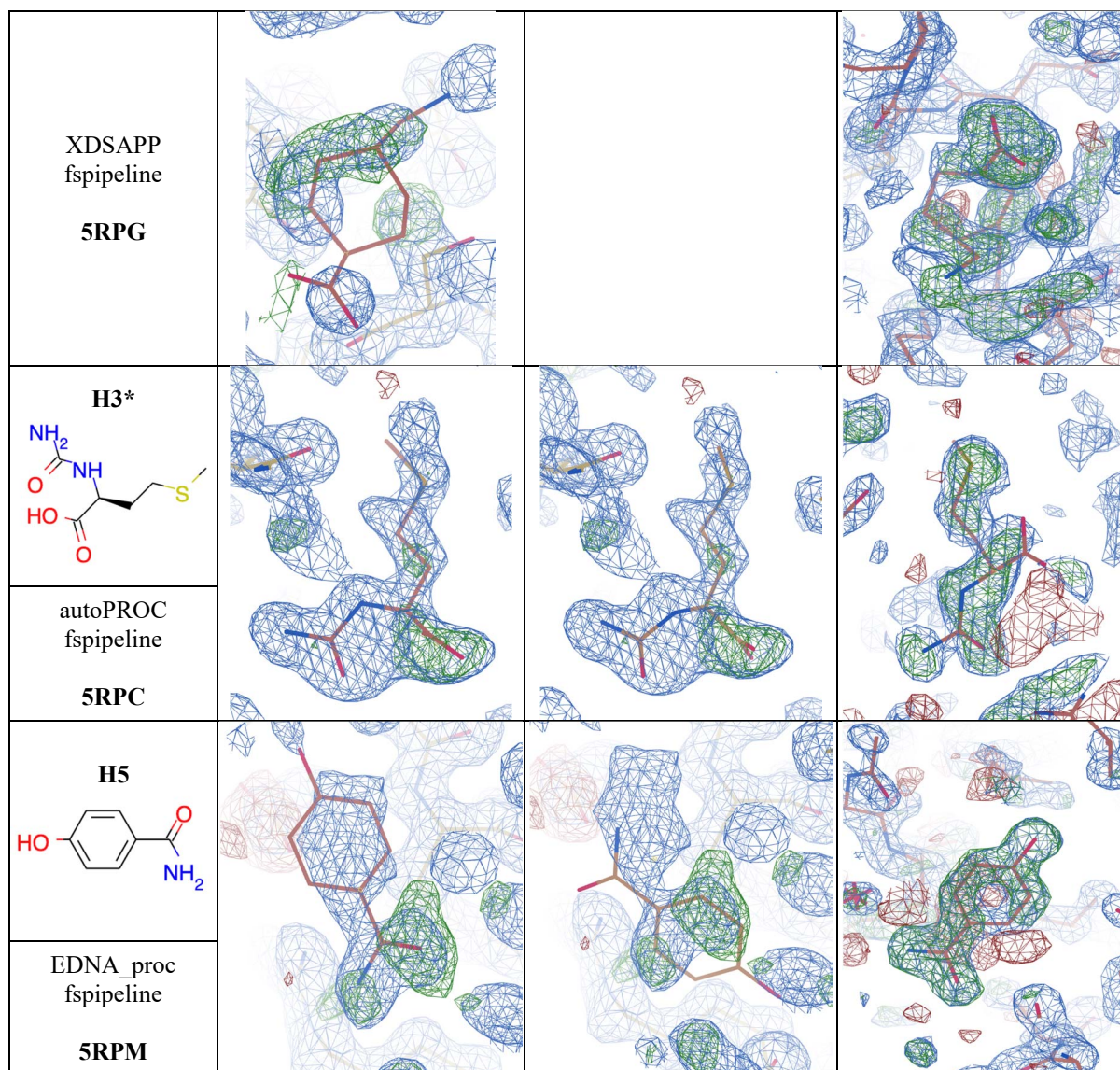
Table S1 Complete automated ligand searching comparison. Every hit found using PanDDA is compared to other automated methods. The dataset information column displays the fragment used during soaking, the methods used to process and refine the dataset, and the PDB ID of the final structure.

Dataset Information	LigandFit	RhoFit	PanDDA
<p>A11*</p> 			
<p>fastdp fspipeline</p> <p>5RPH</p>			
<p>A12</p> 			
<p>autoPROC fspipeline</p> <p>5ROQ</p>			
<p>A12 RT</p> 			<p>No Solution</p>
<p>xia2/DIALS fspipeline</p> <p>5ROP</p>			
<p>B5</p> 			
<p>EDNA_proc fspipeline</p> <p>5ROL</p>			
<p>B7</p> 			

<p>fastdp fspipeline</p> <p>5RP9</p>			
<p>B9</p> 			
<p>xia2/XDS DIMPLE</p> <p>5RPK</p>			
<p>B12</p> 			
<p>fastdp fspipeline</p> <p>5RPJ</p>			
<p>C2</p> 			
<p>fastdp fspipeline</p> <p>5RP6</p>			
<p>C8</p> 	<p>No solution</p>	<p>No solution</p>	

<p>autoPROC fspipeline</p> <p>5RPL</p>			
<p>C11*</p> 			
<p>autoPROC fspipeline</p> <p>5ROF</p>			
<p>D12</p> 			
<p>xia2/DIALS fspipeline</p> <p>5RP7</p>			
<p>E3*</p> 			
<p>autoPROC fspipeline</p> <p>5RPA</p>			
<p>E4</p>	No Solution	No Solution	

<p>xia2/DIALS fspipeline</p> <p>5RON</p>			
<p>F1</p>	No solution	No solution	
<p>XDSAPP fspipeline</p> <p>5ROR</p>			
<p>F6</p>			
<p>xia2/DIALS fspipeline</p> <p>5ROW</p>			
<p>F12*</p>			
<p>xia2/DIALS BUSTER</p> <p>5RPD</p>			
<p>H2</p>		No solution	



* The binding site identified by RhoFit, LigandFit and PanDDA is not the same.