



STRUCTURAL
BIOLOGY

Volume 79 (2023)

Supporting information for article:

**Novel starting points for fragment-based drug design against
mycobacterial thioredoxin reductase identified using
crystallographic fragment screening**

**Friederike T. Füsser, Jan Wollenhaupt, Manfred S. Weiss, Daniel Kümmel and
Oliver Koch**

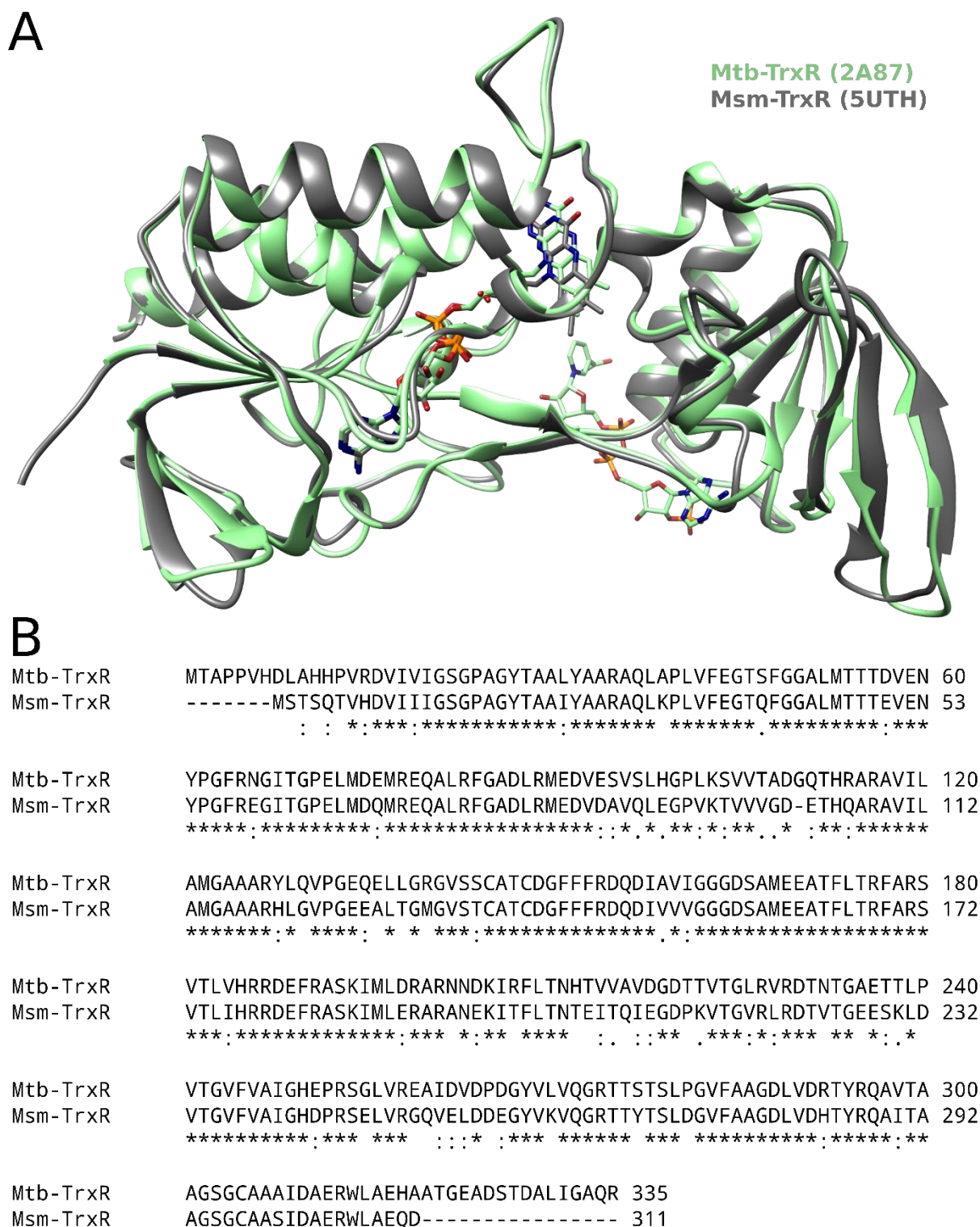


Figure S1 Comparison of mycobacterial TrxR. **(A)** The Structural comparison of Mtb-TrxR (PDB 2A87, green) and Msm-TrxR (5UTH, gray) lead to an RMSD of 0.879 Å using MOE. **(B)** A sequence alignment using clustal omega showed a sequence identity of 77%.

Table S1 Macromolecule production information for PDB 8CCI

| | |
|---|---|
| Source organism | Mycobacterium smegmatis MC2 155 |
| DNA source | |
| Expression vector | BG1816 |
| Plasmid construction method | <i>Provided by SSGCID</i> |
| Expression host | Escherichia coli BL21(DE3) |
| Expression details | Tb-medium, induced by 1 mM IPTG, 16 hours expression at 16°C |
| Complete amino-acid sequence of the protein | MAHHHHHHMSTSQTVHDVIIIIGSGPAGYTAAIYAAR AQLKPLVFEGTQFGGALMTTTEVENYPGFREGITGPE LMDQMREQALRFGADLRMEDVDAVQLEGPVKTVV VGDETHQARAVILAMGAAARHLGVPGEEALTGMGV STCATCDGFFFRDQDIVVGGGDSAMEEATFLTRFA RSVTLIHRRDEFRASKIMLERARANEEKITFLTNTTEITQI EGDPKVTGVRLRDTVTGEESKLDVTGVFVAIGH DPR SELVRGQVELDDEGYVKVQGRTTYTSLDGVFAAGDL VDHTYRQAITAAGSGCAASIDAERWLAEQD- |

Table S2 Crystallization data for PDB 8CCI

| | |
|--|--|
| Method | Vapor diffusion, sitting drop |
| Plate type | 48 well MRC Maxi Plate (Jena Bioscience) |
| Temperature (°C) | 20 |
| Protein concentration | 23 mg mL ⁻¹ |
| Buffer composition of protein solution | 10 mM Tris at pH 8.0, 10 mM DTT |
| Composition of reservoir solution | 2.16 M Sodium malonate dibasic monohydrate, 10% glycerol |
| Volume and ratio of drop | 2 µL 1:1 |
| Volume of reservoir | 100 µL |
| Composition of the cryoprotectant | 2.16 M Sodium malonate dibasic monohydrate, 10% glycerol, 5% DMSO |
| Drop setting | Specify if manual |
| Seeding | no |

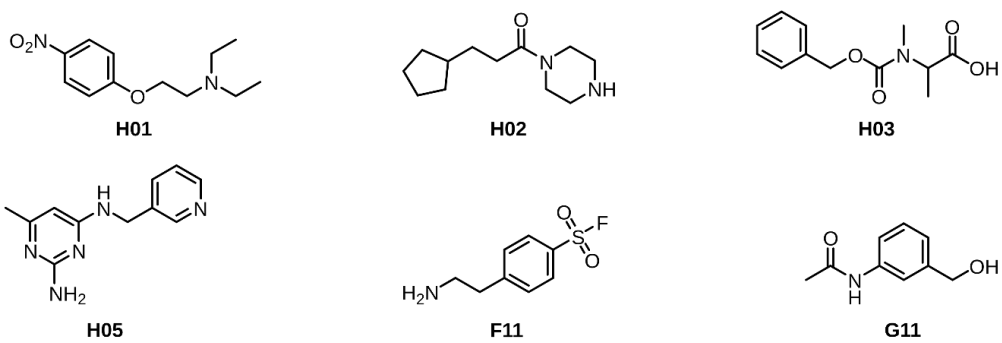


Figure S2 Depicted fragments lead to loss of diffraction after soaking for 3 hours on Msm-TrxR crystals. Fragment F11 contains a warhead that could lead to a covalent binding.

Figure S3 Fragment found to bind after LigandFit analyses in their binding pocket. Corresponding electron density maps (2Fo-DFc) are contoured at $\sigma = 1$ (blue) and (Fo-DFc)-maps are contoured at $\sigma = 3$ (green).

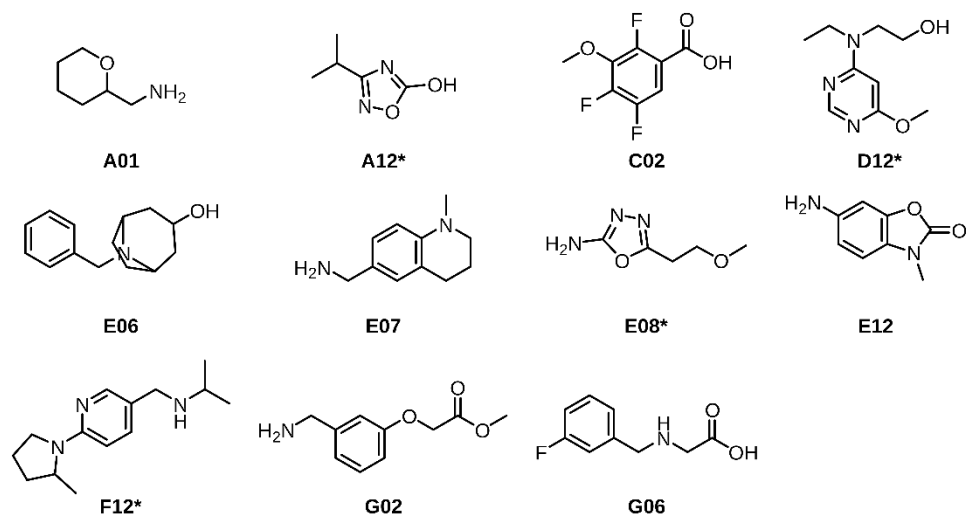


Figure S4 Fragments found binding to the surface of Msm-TrxR. Four fragments, bound between the NADPH- and FAD-domain are marked with (*).

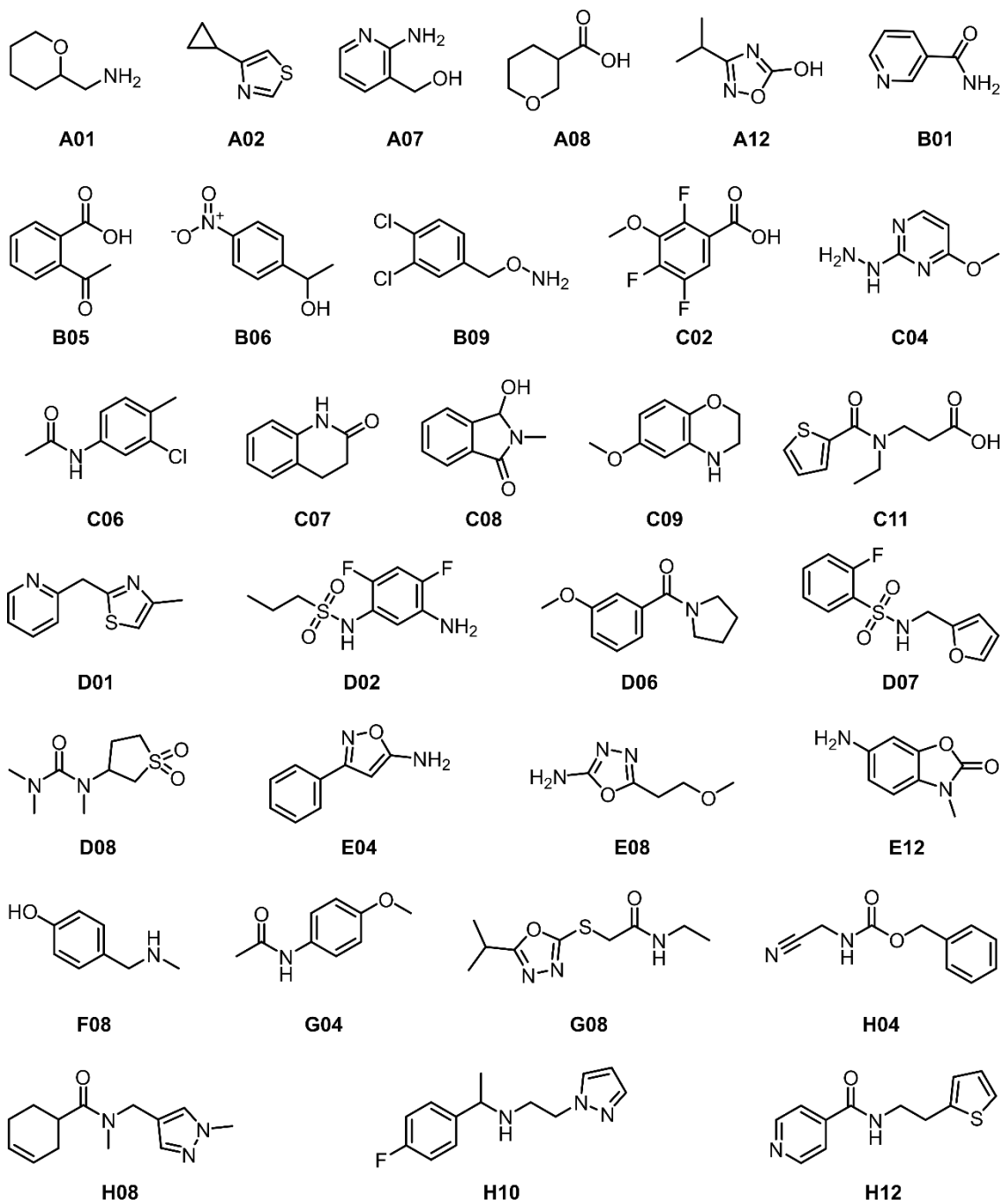


Figure S5 Fragments found binding to the dimer site of Msm-TrxR.

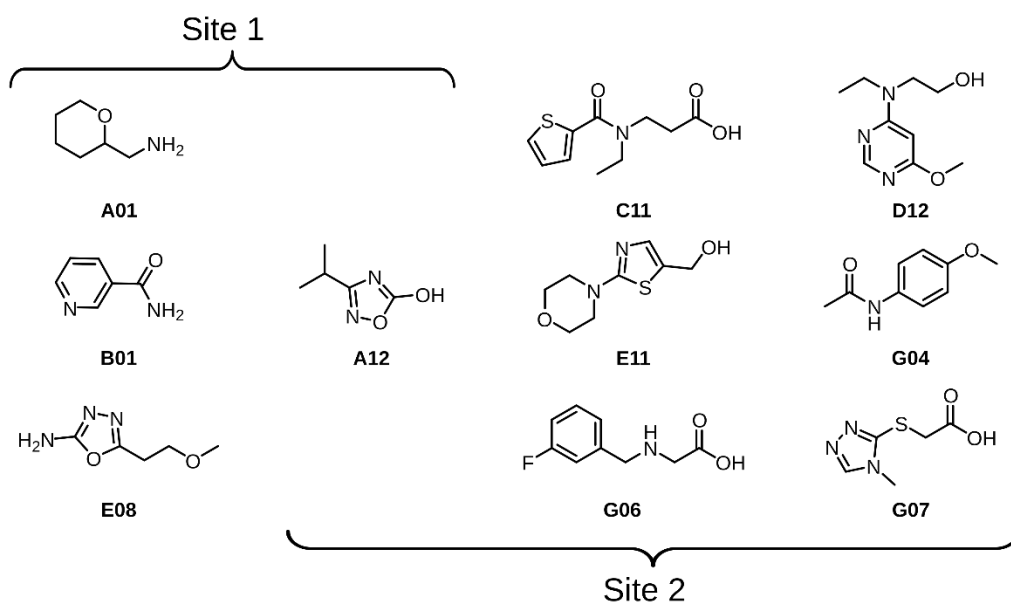


Figure S6 Depicted fragments bound to Trx binding site. Fragments A01, B01, and E08 bind to the first site, A12 can be found in both clusters, while C11, D12, E06, E11, G04, and G07 bind in the second part of the binding pocket.

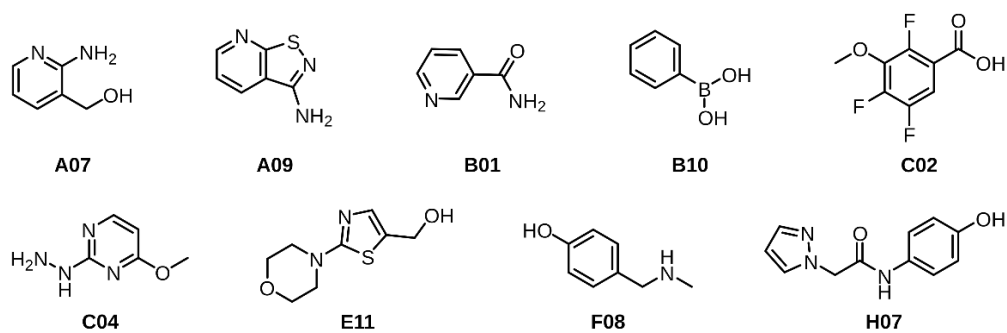


Figure S7 Fragments found binding to the NADPH binding site

Table S3 Available PanDDA results. The datasets can be downloaded from the mentioned DOI.

| Fragment | Crystal | Binding site | Processing program | Refinement pipeline | DOI | Data Path to corresponding PDB file |
|----------|---------|-----------------------------|--------------------|---------------------|----------------------|---|
| A01 | a | Dimer Surface Surface | xdsapp | plex | 10.17879/69998577695 | FF-PanDDA-Daten2/xdsapp_plex/pandda/processed_datasets/FF-F2XEntry-A01a_1/modelled_structures/FF-F2XEntry-A01a_1-pandda-model.pdb |
| A01 | a | Trx1 | xdsapp | dimple | 10.17879/69998577695 | FF-PanDDA-Daten2/xdsapp_dimple/pandda/processed_datasets/FF-F2XEntry-A01a_1/modelled_structures/FF-F2XEntry-A01a_1-pandda-model.pdb |
| A02 | a | Dimer | frag | plex | 10.17879/69998577695 | FF-PanDDA-Daten2/frag_plex/pandda/processed_datasets/FF-F2XEntry-A02a_1/modelled_structures/FF-F2XEntry-A02a_1-pandda-model.pdb |
| A07 | b | Dimer NADPH | frag | plex | 10.17879/69998577695 | FF-PanDDA-Daten2/frag_plex/pandda/processed_datasets/FF-F2XEntry-A07b_1/modelled_structures/FF-F2XEntry-A07b_1-pandda-model.pdb |
| A08 | b | Dimer | xdsapp | dimple | 10.17879/69998577695 | FF-PanDDA-Daten2/xdsapp_dimple/pandda/processed_datasets/FF-F2XEntry-A08b_1/modelled_structures/FF-F2XEntry-A08b_1-pandda-model.pdb |
| A09 | b | NADPH | frag | plex | 10.17879/69998577695 | FF-PanDDA-Daten2/frag_plex/pandda/processed_datasets/FF-F2XEntry-A09b_1/modelled_structures/FF-F2XEntry-A09b_1-pandda-model.pdb |
| A12 | b | Dimer | frag | plex | 10.17879/69998577695 | FF-PanDDA-Daten2/frag_plex/pandda/processed_datasets/FF-F2XEntry-A12b_1/modelled_structures/FF-F2XEntry-A12b_1-pandda-model.pdb |
| A12 | a | Trx1 | frag | plex | 10.17879/69998577695 | FF-PanDDA-Daten2/frag_plex/pandda/processed_datasets/FF-F2XEntry-A12a_1/modelled_structures/FF-F2XEntry-A12a_1-pandda-model.pdb |
| A12 | a | Trx2 | xdsapp | dimple | 10.17879/69998577695 | FF-PanDDA-Daten2/xdsapp_dimple/pandda/processed_datasets/FF-F2XEntry-A12a_1/modelled_structures/FF-F2XEntry-A12a_1-pandda-model.pdb |
| A12 | b | Surface | xdsapp | plex | 10.17879/69998577695 | FF-PanDDA-Daten2/xdsapp_plex/pandda/processed_datasets/FF-F2XEntry-A12b_1/modelled_structures/FF-F2XEntry-A12b_1-pandda-model.pdb |

| | | | | | | |
|-----|---|---------------|--------|--------|----------------------|---|
| A12 | b | Surface | xdsapp | dimple | 10.17879/69998577695 | FF-PanDDA-Daten2/xdsapp_dimple/pandda/processed_datasets/FF-F2XEntry-A12b_1/modelled_structures/FF-F2XEntry-A12b_1-pandda-model.pdb |
| B01 | a | Dimer | xdsapp | plex | 10.17879/69998577695 | FF-PanDDA-Daten2/xdsapp_plex/pandda/processed_datasets/FF-F2XEntry-B01a_1/modelled_structures/FF-F2XEntry-B01a_1-pandda-model.pdb |
| B01 | a | NADPH Trx1 | dials | dimple | 10.17879/40019550156 | FF-PanDDA-Daten1/dials_dimple/pandda/processed_datasets/FF-F2XEntry-B01a_1/modelled_structures/FF-F2XEntry-B01a_1-pandda-model.pdb |
| B05 | b | Dimer | dials | plex | 10.17879/40019550156 | FF-PanDDA-Daten1/dials_plex/pandda/processed_datasets/FF-F2XEntry-B05b_1/modelled_structures/FF-F2XEntry-B05b_1-pandda-model.pdb |
| B06 | b | Dimer | frag | plex | 10.17879/69998577695 | FF-PanDDA-Daten2/frag_plex/pandda/processed_datasets/FF-F2XEntry-B06b_1/modelled_structures/FF-F2XEntry-B06b_1-pandda-model.pdb |
| B09 | b | Dimer | xdsapp | plex | 10.17879/69998577695 | FF-PanDDA-Daten2/xdsapp_plex/pandda/processed_datasets/FF-F2XEntry-B09b_1/modelled_structures/FF-F2XEntry-B09b_1-pandda-model.pdb |
| B10 | a | NADPH | frag | plex | 10.17879/69998577695 | FF-PanDDA-Daten2/frag_plex/pandda/processed_datasets/FF-F2XEntry-B10a_1/modelled_structures/FF-F2XEntry-B10a_1-pandda-model.pdb |
| C02 | a | Dimer | xdsapp | dimple | 10.17879/69998577695 | FF-PanDDA-Daten2/xdsapp_dimple/pandda/processed_datasets/FF-F2XEntry-C02a_1/modelled_structures/FF-F2XEntry-C02a_1-pandda-model.pdb |
| C02 | a | NADPH | dials | dimple | 10.17879/40019550156 | FF-PanDDA-Daten1/dials_dimple/pandda/processed_datasets/FF-F2XEntry-C02a_1/modelled_structures/FF-F2XEntry-C02a_1-pandda-model.pdb |
| C02 | a | Surface | xdsapp | dimple | 10.17879/69998577695 | FF-PanDDA-Daten2/xdsapp_dimple/pandda/processed_datasets/FF-F2XEntry-C02a_1/modelled_structures/FF-F2XEntry-C02a_1-pandda-model.pdb |
| C04 | b | Dimer | xdsapp | dimple | 10.17879/69998577695 | FF-PanDDA-Daten2/xdsapp_dimple/pandda/processed_datasets/FF-F2XEntry-C04b_1/modelled_structures/FF-F2XEntry-C04b_1-pandda-model.pdb |

| | | | | | | |
|-----|---|-----------------|--------|------------|----------------------|--|
| C04 | b | NADPH | frag | plex | 10.17879/69998577695 | FF-PanDDA-Daten2/frag_plex/pandda/processed_datasets/FF-F2XEntry-C04b_1/modelled_structures/FF-F2XEntry-C04b_1-pandda-model.pdb |
| C06 | b | Dimer | dials | dimple | 10.17879/40019550156 | FF-PanDDA-Daten1/dials_dimple/pandda/processed_datasets/FF-F2XEntry-C06b_1/modelled_structures/FF-F2XEntry-C06b_1-pandda-model.pdb |
| C07 | a | Dimer | xdsapp | dimple | 10.17879/69998577695 | FF-PanDDA-Daten2/xdsapp_dimple/pandda/processed_datasets/FF-F2XEntry-C07a_1/modelled_structures/FF-F2XEntry-C07a_1-pandda-model.pdb |
| C08 | b | Dimer | xdsapp | dimple | 10.17879/69998577695 | FF-PanDDA-Daten2/xdsapp_dimple/pandda/processed_datasets/FF-F2XEntry-C08b_1/modelled_structures/FF-F2XEntry-C08b_1-pandda-model.pdb |
| C09 | a | Dimer | frag | plex | 10.17879/69998577695 | FF-PanDDA-Daten2/frag_plex/pandda/processed_datasets/FF-F2XEntry-C09a_1/modelled_structures/FF-F2XEntry-C09a_1-pandda-model.pdb |
| C11 | a | Dimer | dials | fspipeline | 10.17879/40019550156 | FF-PanDDA-Daten1/dials_fspipeline/pandda/processed_datasets/FF-F2XEntry-C11a_1/modelled_structures/FF-F2XEntry-C11a_1-pandda-model.pdb |
| C11 | b | Surface Trx2 | frag | plex | 10.17879/69998577695 | FF-PanDDA-Daten2/frag_plex/pandda/processed_datasets/FF-F2XEntry-C11b_1/modelled_structures/FF-F2XEntry-C11b_1-pandda-model.pdb |
| D01 | b | Dimer | frag | plex | 10.17879/69998577695 | FF-PanDDA-Daten2/frag_plex/pandda/processed_datasets/FF-F2XEntry-D01b_1/modelled_structures/FF-F2XEntry-D01b_1-pandda-model.pdb |
| D02 | b | Dimer | frag | plex | 10.17879/69998577695 | FF-PanDDA-Daten2/frag_plex/pandda/processed_datasets/FF-F2XEntry-D02b_1/modelled_structures/FF-F2XEntry-D02b_1-pandda-model.pdb |
| D06 | a | Dimer | frag | plex | 10.17879/69998577695 | FF-PanDDA-Daten2/frag_plex/pandda/processed_datasets/FF-F2XEntry-D06a_1/modelled_structures/FF-F2XEntry-D06a_1-pandda-model.pdb |
| D07 | a | Dimer | frag | plex | 10.17879/69998577695 | FF-PanDDA-Daten2/frag_plex/pandda/processed_datasets/FF-F2XEntry-D07a_1/modelled_structures/FF-F2XEntry-D07a_1-pandda-model.pdb |

| | | | | | | |
|-----|---|-------------------------------------|--------|--------|----------------------|---|
| D08 | b | Dimer | frag | plex | 10.17879/69998577695 | FF-PanDDA-Daten2/frag_plex/pandda/processed_datasets/FF-F2XEntry-D08b_1/modelled_structures/FF-F2XEntry-D08b_1-pandda-model.pdb |
| D12 | a | Surface Trx2 | xdsapp | dimple | 10.17879/69998577695 | FF-PanDDA-Daten2/xdsapp_dimple/pandda/processed_datasets/FF-F2XEntry-D12a_1/modelled_structures/FF-F2XEntry-D12a_1-pandda-model.pdb |
| D12 | b | Surface | dials | dimple | 10.17879/40019550156 | FF-PanDDA-Daten1/dials_dimple/pandda/processed_datasets/FF-F2XEntry-D12b_1/modelled_structures/FF-F2XEntry-D12b_1-pandda-model.pdb |
| E04 | b | Dimer | xdsapp | dimple | 10.17879/69998577695 | FF-PanDDA-Daten2/xdsapp_dimple/pandda/processed_datasets/FF-F2XEntry-E04b_1/modelled_structures/FF-F2XEntry-E04b_1-pandda-model.pdb |
| E06 | b | Surface | xdsapp | plex | 10.17879/69998577695 | FF-PanDDA-Daten2/xdsapp_plex/pandda/processed_datasets/FF-F2XEntry-E06b_1/modelled_structures/FF-F2XEntry-E06b_1-pandda-model.pdb |
| E06 | b | Surface | xdsapp | plex | 10.17879/69998577695 | FF-PanDDA-Daten2/xdsapp_plex/pandda/processed_datasets/FF-F2XEntry-E06b_1/modelled_structures/FF-F2XEntry-E06b_1-pandda-model.pdb |
| E07 | b | Surface | xdsapp | dimple | 10.17879/69998577695 | FF-PanDDA-Daten2/xdsapp_dimple/pandda/processed_datasets/FF-F2XEntry-E07b_1/modelled_structures/FF-F2XEntry-E07b_1-pandda-model.pdb |
| E08 | b | Dimer Trx1 Surface Surface | xdsapp | dimple | 10.17879/69998577695 | FF-PanDDA-Daten2/xdsapp_dimple/pandda/processed_datasets/FF-F2XEntry-E08b_1/modelled_structures/FF-F2XEntry-E08b_1-pandda-model.pdb |
| E11 | a | NADPH | frag | plex | 10.17879/69998577695 | FF-PanDDA-Daten2/frag_plex/pandda/processed_datasets/FF-F2XEntry-E11a_1/modelled_structures/FF-F2XEntry-E11a_1-pandda-model.pdb |
| E11 | b | Trx2 | xdsapp | dimple | 10.17879/69998577695 | FF-PanDDA-Daten2/xdsapp_dimple/pandda/processed_datasets/FF-F2XEntry-E11b_1/modelled_structures/FF-F2XEntry-E11b_1-pandda-model.pdb |

| | | | | | | |
|-----|---|--------------------|--------|--------|----------------------|---|
| E12 | b | Dimer Surface | xdsapp | dimple | 10.17879/69998577695 | FF-PanDDA-Daten2/xdsapp_dimple/pandda/processed_datasets/FF-F2XEntry-E12b_1/modelled_structures/FF-F2XEntry-E12b_1-pandda-model.pdb |
| F08 | b | Dimer | dials | dimple | 10.17879/40019550156 | FF-PanDDA-Daten1/dials_dimple/pandda/processed_datasets/FF-F2XEntry-F08b_1/modelled_structures/FF-F2XEntry-F08b_1-pandda-model.pdb |
| F08 | b | NADPH | xdsapp | plex | 10.17879/69998577695 | FF-PanDDA-Daten2/xdsapp_plex/pandda/processed_datasets/FF-F2XEntry-F08b_1/modelled_structures/FF-F2XEntry-F08b_1-pandda-model.pdb |
| F12 | a | Surface | xdsapp | plex | 10.17879/69998577695 | FF-PanDDA-Daten2/xdsapp_plex/pandda/processed_datasets/FF-F2XEntry-F12a_1/modelled_structures/FF-F2XEntry-F12a_1-pandda-model.pdb |
| G02 | a | Surface | xdsapp | dimple | 10.17879/69998577695 | FF-PanDDA-Daten2/xdsapp_dimple/pandda/processed_datasets/FF-F2XEntry-G02a_1/modelled_structures/FF-F2XEntry-G02a_1-pandda-model.pdb |
| G04 | b | Dimer | frag | plex | 10.17879/69998577695 | FF-PanDDA-Daten2/frag_plex/pandda/processed_datasets/FF-F2XEntry-G04b_1/modelled_structures/FF-F2XEntry-G04b_1-pandda-model.pdb |
| G04 | b | Trx2 | dials | plex | 10.17879/40019550156 | FF-PanDDA-Daten1/dials_plex/pandda/processed_datasets/FF-F2XEntry-G04b_1/modelled_structures/FF-F2XEntry-G04b_1-pandda-model.pdb |
| G06 | a | Surface Surface | xdsapp | dimple | 10.17879/69998577695 | FF-PanDDA-Daten2/xdsapp_dimple/pandda/processed_datasets/FF-F2XEntry-G06a_1/modelled_structures/FF-F2XEntry-G06a_1-pandda-model.pdb |
| G07 | b | Trx2 | xdsapp | dimple | 10.17879/69998577695 | FF-PanDDA-Daten2/xdsapp_dimple/pandda/processed_datasets/FF-F2XEntry-G07b_1/modelled_structures/FF-F2XEntry-G07b_1-pandda-model.pdb |
| G08 | b | Dimer | xdsapp | dimple | 10.17879/69998577695 | FF-PanDDA-Daten2/xdsapp_dimple/pandda/processed_datasets/FF-F2XEntry-G08b_1/modelled_structures/FF-F2XEntry-G08b_1-pandda-model.pdb |
| H04 | a | Dimer | frag | plex | 10.17879/69998577695 | FF-PanDDA-Daten2/frag_plex/pandda/processed_datasets/FF-F2XEntry-H04a_1/modelled_structures/FF-F2XEntry-H04a_1-pandda-model.pdb |

| | | | | | | |
|-----|---|-------|--------|--------|----------------------|---|
| H07 | a | NADPH | xdsapp | dimple | 10.17879/69998577695 | FF-PanDDA-Daten2/xdsapp_dimple/pandda/processed_datasets/FF-F2XEntry-H07a_1/modelled_structures/FF-F2XEntry-H07a_1-pandda-model.pdb |
| H08 | a | Dimer | xdsapp | dimple | 10.17879/69998577695 | FF-PanDDA-Daten2/xdsapp_dimple/pandda/processed_datasets/FF-F2XEntry-H08a_1/modelled_structures/FF-F2XEntry-H08a_1-pandda-model.pdb |
| H10 | a | Dimer | frag | plex | 10.17879/69998577695 | FF-PanDDA-Daten2/frag_plex/pandda/processed_datasets/FF-F2XEntry-H10a_1/modelled_structures/FF-F2XEntry-H10a_1-pandda-model.pdb |
| H12 | a | Dimer | frag | plex | 10.17879/69998577695 | FF-PanDDA-Daten2/frag_plex/pandda/processed_datasets/FF-F2XEntry-H12a_1/modelled_structures/FF-F2XEntry-H12a_1-pandda-model.pdb |