2-Year REPORT FOR THE PERIOD AUG2017-DEC2018

Title of BAG:
ISMB application, Biological Sciences, Birkbeck; Structural and Molecular Biology, School of Pharmacy & NPP, UCL; Biological and Chemical Sciences, Queen Mary College London

Principal Investigator: Prof. Gabriel Waksman

Principal Investigator Affiliation: School of Crystallography Birkbeck College

Proposal Reference Number: MX1983

Period Covering: August 2017 – December 2018

Number of PDB submissions: 5

Number of Publications: 3 (plus 2 in preparation)

Highlights

1. Irving group

Intrahepatic heteropolymerization of M and Z alpha-1-antitrypsin (doi: 10.1172/jci.insight.135459)

The α-1-antitrypsin (or alpha-1-antitrypsin, A1AT) Z variant is the primary cause of severe A1AT deficiency and forms polymeric chains that aggregate in the endoplasmic reticulum of hepatocytes. Around 2%–5% of Europeans are heterozygous for the Z and WT M allele, and there is evidence of increased risk of liver disease when compared with MM A1AT individuals. We have shown that Z and M A1AT can copolymerize in cell models, but there has been no direct observation of heteropolymer formation in vivo. To this end, we developed a monoclonal antibody (mAb2H2) that specifically binds to M in preference to Z A1AT, localized its epitope using crystallography to a region perturbed by the Z (Glu342Lys) substitution, and used Fab fragments to label polymers isolated from an MZ heterozygote liver explant. Glu342 is critical to the affinity of mAb2H2, since it also recognized the mild S-deficiency variant (Glu264Val) present in circulating polymers from SZ heterozygotes. Negative-stain electron microscopy of the Fab2H2-labeled liver polymers revealed that M comprises around 6% of the polymer subunits in the MZ liver sample. These data demonstrate that Z A1AT can form heteropolymers with polymerization-inert variants in vivo with implications for liver disease in heterozygous individuals.

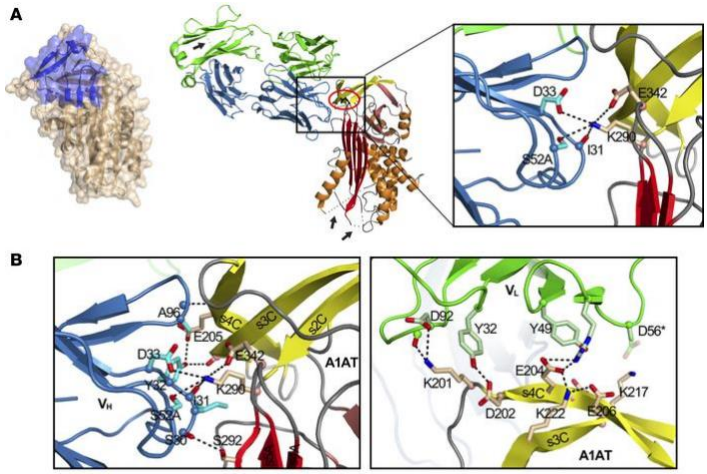
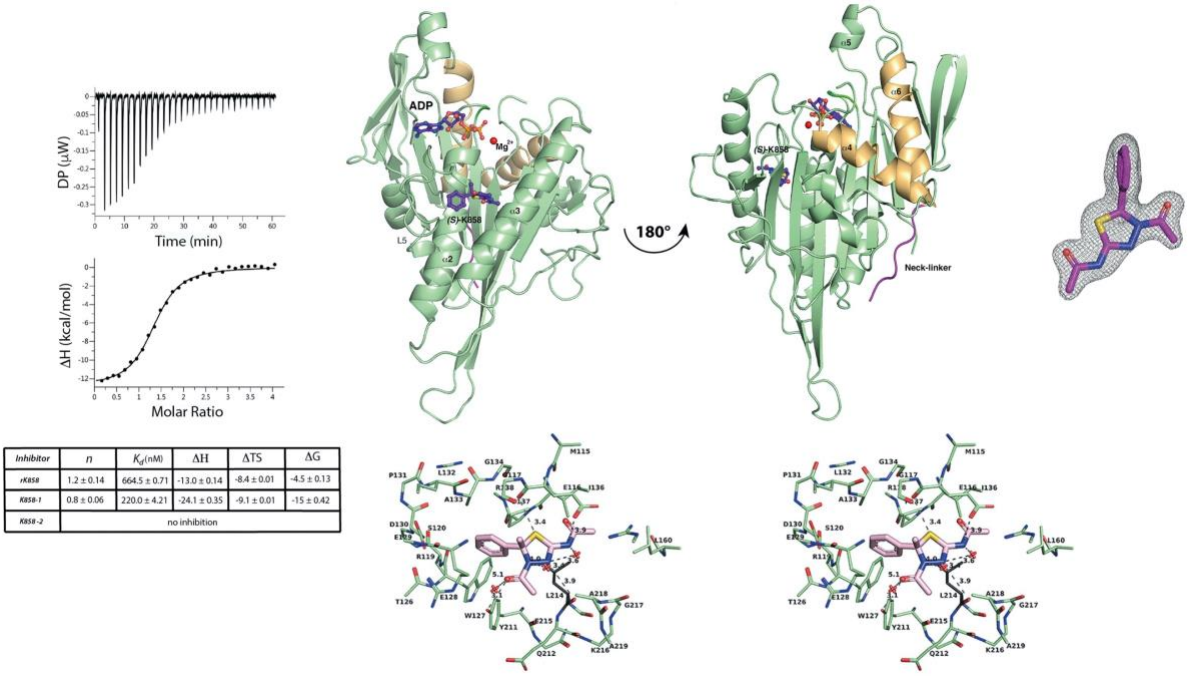


Figure 1: (A) Central panel: the A1AT-Fab2H2 complex (PDB accession 6I3Z) is shown, with the Fab heavy chain colored blue; the light chain colored green; β-sheets A, B, and C colored red, salmon, and yellow, respectively; and the site of the Z mutation indicated by a red ellipse. Arrows denote regions disordered in the crystal structure; none of these occur near the binding site. Left panel: the cleaved A1AT component of the complex is shown as surface-on-cartoon, with the Fab2H2 binding site colored blue. Right panel: detail of interactions at the site of the Z mutation, with Lys290 at the center of a cluster of polar residues. (B) Detail of residues at the interface between A1AT and the Fab2H2 heavy chain (VH, left panel) or light chain (VL, right panel).

2 Kozielski group

Arry-520 is an advanced drug candidate from the Eg5 inhibitor class undergoing clinical evaluation in patients with relapsed or refractory multiple myeloma. Here we show by structural analysis that Arry-520 binds stoichiometrically to the motor domain of Eg5 in the conventional allosteric loop L5 pocket in a complex that suggests the same structural mechanism as other Eg5 inhibitors. We have previously shown that acquired resistance through mutations in the allosteric binding site located at loop L5 in the Eg5 structure appears to be independent of the inhibitors' scaffold, which suggests that Arry-520 will ultimately have the same fate. When Arry-520 was assessed in two cell lines selected for the expression of either Eg5(D130A) or Eg5(L214A) STLC-resistant alleles, mutations previously shown to convey resistance to this class of inhibitors, it was inactive in both. Surprisingly, when the cells were challenged with ispinesib, another Eg5 inhibitor that has been evaluated in clinical trials, the Eg5(D130A) cells were resistant, but those expressing Eg5(L214A) were strikingly sensitive. Molecular dynamics simulations suggest that subtle differences in ligand binding may alter allosteric transmission from the loop L5 site that do not necessarily result in reduced inhibitory activity in mutated Eg5 structures. Whilst we predict that cells challenged with Arry-520 in the clinical setting are likely to acquire resistance through point mutations in the Eg5 binding site, the data for ispinesib suggests that this resistance mechanism is not scaffold independent as previously thought, and new inhibitors can be designed that retain inhibitory activity in these resistant cells.



Inhibitor	n	K_d(nM)	ΔH	ΔTS	ΔG
rK858	1.2 ± 0.14	664.5 ± 0.71	-13.0 ± 0.14	-8.4 ± 0.01	-4.5 ± 0.13
K858-1	0.8 ± 0.06	220.0 ± 4.21	-24.1 ± 0.35	-9.1 ± 0.01	-15 ± 0.42
K858-2	no inhibition				

Figure 1: Left: ITC analysis of the Eg5-K858 interaction. The raw ITC data of the binding reaction between Eg5 and rK858 is shown in the upper panel. The lower panel shows normalised ITC data plotted against the molar ratio of inhibitor to protein. The data fit very well to the single-site binding model. Up middle: The structure of the Eg5 motor domain with bound Mg^{2+}ADP in the nucleotide-binding pocket. Right: (S)-K858 and the f_o-f_c map contoured at 3σ Lower right: Stereoplot of the magnification of the inhibitor-binding pocket.

ALL PUBLICATIONS directly resulting from the use of data recorded on ESRF beamlines since last full 2-year report: (1): ESRF data only, (2): from more than one source

- Talapatra, S.K., Tham, C.L., Guglielmi, P., Cirilli, R., Chandrasekaran, B., Karpoormath, R., Carradori, S., Kozielski, F. **(2019)**. Crystal structure of the Eg5 - K858 complex and implications for structure-based design of thiadiazole-containing inhibitors. *Eur. J. Med. Chem*. **156**, 641-651. (1)

- Riccio, F., Talapatra, S.K., Oxenford, S., Angell, R., Mazzon, M., Kozielski, F. **(2019)**. Development and validation of RdRp Screen, a crystallization screen for viral RNA-dependent RNA polymerases. *Biol Open* Jan 2;8(1). (2)

- Laffranchi, M., Elliston, E.L.K., Miranda, E., Perez, J., Jagger, A.M., Fra, A., Lomas, D.A., Irving, J.A. (**2020**), Intrahepatic heteropolymerization of M and Z alpha-1-antitrypsin. JCI Insight **5(14)** (2)

List of PDB submissions as a direct result of ESRF time
6G6Y: Eg5-inhibitor complex, ID30a-1
6G6Z: Eg5-inhibitor complex, ID30a-1
6HX4 "Fab fragment of a native monomer-selective antibody in complex with alpha-1-antitrypsin" ESRF ID30B
6I3Z "Fab fragment of an antibody selective for wild-type alpha-1-antitrypsin in complex with its antigen" ESRF ID29
6QU9 "Fab fragment of an antibody that inhibits polymerisation of alpha-1-antitrypsin" ESRF ID30B

Summary Report from individual groups
The **Waksman group** was working on a Legionella pneumophila DotB homologue, which was screened on ID23 on the 6/3/2017. Even though no improvement of resolution was observed, the session was essential to improve the crystallisation conditions at a later stage. This work resulted a publication in Protein Science (doi: 10.1002/pro.3439)
The **Wallace group** was examining the transcriptional regulation in Mycobacterium tuberculosis. They screened 12 crystals on ID23 and 4 datasets were collected ranging in resolution from 3.15 Å to 5.44 Å but none of them resulted a publishable structure. On a second visit in April 2018 the aim was to test crystals of the transmembrane pore of a sodium channel. Unfortunately, there were problems with the beamline that made impossible to collect any data.
The **Barrett group** attempted to collect crystals of Tip69 on Id23-2 (11/6/2018). While some of the crystals that were tested didn't diffract sufficiently, there was in addition a sample changer problem that resulted a significant loss of beam time. On a second visit all the Tip49-telomeric DNA crystals diffracted to very low resolution where only test shots could be obtained.
The **Kozielski group** solved two structures of human Eg5 in complex with K858. This structure provided a platform for structure-based drug design and was essential for rational design of inhibitor analogues. In addition, they developed a screen for the crystallisation of viral RNA-dependent RNA polymerases (RdRps) and confirmed its usefulness by solving

two RdRp-inhibitor complexes of dengue virus RdRp. Several human MCAK structures in various nucleotide states have been solved and the structures are being prepared for submission and a manuscript in preparation. They also collected data on a large variety of crystals of *M. tuberculosis* FtsZ and DENV RdRp in complex with fragments. These structures will reveal novel inhibitors binding sites and will allow structure-based drug design (in progress). These complex structures will serve as a basis to develop inhibitors with higher potency and efficacy. For example, data collected from the Massif beamtime for Dengue RdRp in complex with fragments (about 200 data sets) has been processed by standard methods but without any success, but the same data has been processed using the PanDDA software from DLS and a range of fragments binding to DENV RdRp has been identifed.
The **Irving group** have been characterising mutants of the disease-associated plasma protein alpha-1-antitrypsin (AAT) and conformationally-selective antibodies that act as probes of structural change. They have also expended effort attempting to obtain high resolution structures of AAT bound to small molecules, which has necessitated screening of a significant number of crystals. Structures of mutants with substitutions that modify packing in the 'breach' regulatory site of AAT have been solved, as have structures of Fab domains both alone and in complex with AAT. The latter have been used to interpret single-particle reconstructions from negative stain images of oligomers isolated from patients and decorated with Fabs; these data are being written up into two manuscripts which will be submitted in due course. One of the Fab complexes is with an antibody that is being used to screen patient plasma and will represent a third paper.

	Experiment title: ISMB application, Birkbeck; School of Pharmacy & NPP, UCL; Biological and Chemical Sciences, Queen Mary College London. Wallace group	**Experiment number**: MX1983
Beamline: ID30B	**Date of experiment**: from: 21/04/2018 to: 22/04/2018	**Date of report**: 25/02/2019
Shifts: 3	**Local contact(s)**: G Leonard	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Giulia Montini, Wallace group, ISMB, Birkbeck College

Report: The Wallace Group's work is focused on elucidating the nature of the transmembrane fenestrations in voltage-gated sodium channels. These are the sites for the ingress of hydrophobic drug compounds to their binding sites in the centre of the transmembrane pore. These studies were to have included comparison of native and mutant sodium channels, where the mutations were designed to block the fenestrations and drug binding.

We had one partial session for data collection, but unfortunately there were problems with the robot and we were not able collect a single data set. These will be the subject of future beamtime applications.

	Experiment title: ISMB application, Birkbeck; School of Pharmacy & NPP, UCL; Biological and Chemical Sciences, Queen Mary College London. Kozielski group	**Experiment number**: MX1983
Beamline: ID23-2 ID30A-1 ID29 ID30A-3	**Date of experiment**: from: 23/04/2018 to: 24/04/2018 from: 30/06/2018 to: 01/07/2018 from: 12/07/2018 to: 13/07/2018 from: 1/12/2018 to: 02/12/2018	**Date of report**: 25/02/2019
Shifts: 12	**Local contact(s)**: 23/4 N/A 30/06: Bowler M 12/07: De Sanctis D 1/12: Melnikov I	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Sandeep Talapatra, School of Pharmacy, UCL

Frank Kozielsky, School of Pharmacy, UCL

Report:

Frank Kozielski's group solved two structures of human Eg5 in complex with K858 (1). This structure provided a platform for structure-based drug design and was essential for rational design of inhibitor analogues. This work has recently been published.

We have developed a screen for the crystallisation of viral RNA-dependent RNA polymerases (RdRps) and confirmed its usefulness by solving two RdRp-inhibitor complexes of dengue virus RdRp (2). This work has recently been published.

Human MCAK structures in various nucleotide states have been solved and the structures are being prepared for submission. The manuscript is in preparation.

We have collected data on a large variety of crystals of *M. tuberculosis* FtsZ and DENV RdRp in complex with fragments. These structures will reveal novel inhibitors binding sites and will allow structure-based drug design (in progress). These complex structures will serve as a basis to develop

inhibitors with higher potency and efficacy. For example, data collected from the Massif beamtime for Dengue RdRp in complex with fragments (about 200 data sets) has been processed by standard methods but without any success, but the same data has been processed using the PanDDA software from DLS and a range of fragments binding to DENV RdRp has been identifed.

List of PDB submissions as a direct result of ESRF time
6G6Y
6G6Z

List of publications attributable to ESRF time
1. Talapatra, S.K., Tham, C.L., Guglielmi, P., Cirilli, R., Chandrasekaran, B., Karpoormath, R., Carradori, S., Kozielski, F. **(2019)**. Crystal structure of the Eg5 - K858 complex and implications for structure-based design of thiadiazole-containing inhibitors. *Eur. J. Med. Chem*. **156**, 641-651.

2. Riccio, F., Talapatra, S.K., Oxenford, S., Angell, R., Mazzon, M., Kozielski, F. **(2019)**. Development and validation of RdRp Screen, a crystallization screen for viral RNA-dependent RNA polymerases. *Biol Open* Jan 2;8(1).

	Experiment title: ISMB application, Birkbeck; School of Pharmacy & NPP, UCL; Biological and Chemical Sciences, Queen Mary College London. Barrett group	**Experiment number**: MX1983
Beamline: ID23-2 ID29	**Date of experiment**: from: 11/06/2018 to: 12/06/2018 from: 12/07/2018 to: 13/07/2018	**Date of report**: 25/02/2019
Shifts: 6	**Local contact(s)**: Nanao M De Sanctis D	*Received at ESRF:*
Names and affiliations of applicants (* indicates experimentalists): Tracey Barrett, Birkbeck College, Institute of Structural and Molecular Biology		

Report:

ID23-2. 11 June 2018 / 12 June 2018:

Attempts were made to collect data on crystals of Tip49 incubated with human telomeric DNA. Whilst we were able to analyse some crystals that failed to diffract, most couldn't be tested owing to both soft and hardware problems. These included frequent connection losses and freezing of the sample changer robot that in the end resulted in useful beamtime being reduced by several hours.

ID29. 12 July 2018 / 13 July 2018

We were awarded this time based on the problems encountered on the previous ID-23 trip. Whilst all software and hardware functioned smoothly, the Tip49-telomeric DNA crystals diffracted to very low resolution where only test shots could be obtained.

	Experiment title: ISMB application, Birkbeck; School of Pharmacy & NPP, UCL; Biological and Chemical Sciences, Queen Mary College London. Irving group	**Experiment number**: MX1983
Beamline: ID30B ID23-2 ID29 ID30A-3	**Date of experiment**: from: 21/04/2018 to: 22/04/2018 from: 11/06/2018 to: 12/06/2018 from: 12/07/2018 to: 13/07/2018 from: 1/12/2018 to: 02/12/2018	**Date of report**: 25/02/2019
Shifts: 12	**Local contact(s)**: Leonard G Nanao M De Sanctis D Melnikov I	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

James Irving, University of London UCL, Institute of Structural and Molecular Biology

Report: We have been characterising mutants of the disease-associated plasma protein alpha-1-antitrypsin (AAT) and conformationally-selective antibodies that act as probes of structural change. We have also expended effort attempting to obtain high resolution structures of AAT bound to small molecules, which has necessitated screening of a significant number of crystals. Structures of mutants with substitutions that modify packing in the 'breach' regulatory site of AAT have been solved, as have structures of Fab domains both alone and in complex with AAT. The latter have been used to interpret single-particle reconstructions from negative stain images of oligomers isolated from patients and decorated with Fabs; these data are being written up into two manuscripts which will be submitted in due course. One of the Fab complexes is with an antibody that is being used to screen patient plasma and will represent a third paper.

The following structures have been deposited at the PDB based on ESRF data, with three more to be deposited in due course:

6HX4 "Fab fragment of a native monomer-selective antibody in complex with alpha-1-antitrypsin" ESRF ID30B

6I3Z "Fab fragment of an antibody selective for wild-type alpha-1-antitrypsin in complex with its antigen" ESRF ID29
6QU9 "Fab fragment of an antibody that inhibits polymerisation of alpha-1-antitrypsin" ESRF ID30B

	Experiment title: ISMB application, Birkbeck; School of Pharmacy & NPP, UCL; Biological and Chemical Sciences, Queen Mary College London. SAXS-Barrett & Cheung groups	**Experiment number**: MX1983
Beamline: BM29	**Date of experiment**: from: 21/11/2018 to: 22/11/2018	**Date of report**: 25/02/2019
Shifts: 12	**Local contact(s)**: 23/4 N/A 30/06: Bowler M 12/07: De Sanctis D 1/12: Melnikov I	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Nikos Pinotsis, ISMB, Birkbeck College

Tracey Barrett, ISMB, Birkbeck College

Emir Aciyan, Cheung group, ISMB, Birkbeck College

Report:

Cheung group:

The Cheung group has made progress in understanding the structure and architecture of the S. cerevisiae complex: TINTIN, which plays a role in transcription elongation rates via a proposed interaction with RNA Pol II. The complex has yet to be structurally characterised to a high resolution through crystallography or electron microscopy. SAXS data obtained at ESRF, has enabled us to confirm the oligomeric state of the complex, as well as a proposed highly extended conformation. We will use this data in parallel with native mass spectrometry data to better understand the structure of the complex which may lead to functional insights

Barrett group:

During this trip, SAXS data were successfully collected on complexes involving vFLIP (native and several mutants) and IKKγ. This trip was very successful resulting in full datasets being obtained for all samples in both batch and HPLC data collection modes. The data is currently being analysed.