

STRUCTURAL BIOLOGY

Medicinal Chemistry and Early Development

OUR CAPABILITIES, SKILLS AND EXPERTISE

PROTEIN EXPRESSION

- Effective construct design of targets by an experienced team of scientists
- Mutants designed to increase protein solubility and stability
- Proven expertise in the parallelisation of bacterial expression studies in a 24 well footprint
- Extensive protein expression know-how in insect, yeast and mammalian cells for hard-to-handle targets

PROTEIN PURIFICATION

- Demonstrated skills in protein refolding, delivering crystal grade material
- Semi-automated high throughput purification process, providing superior quality proteins efficiently

PROTEIN CHARACTERISATION

- Implementation of stringent quality controls guaranteeing only the most suitable proteins enter crystal trials
- Automated and routinely applied SDS-PAGE analysis, Mass spectrometry, dynamic light scattering, analytical size exclusion and 1-D NMR techniques
- Access to 320+ off-the-shelf assays to confirm activity of recombinant protein samples

CRYSTALLISATION

- Workflow driven process for efficient X-ray crystallography
- Expertise in design of crystallisation experiments for optimal ligand binding
- Proven application of automated systems for rapid, high throughput crystallisation on nanolitre scale
- Expertise in exploiting automated image capture of random crystallisation screens to optimise crystal growth

X-RAY DIFFRACTION DATA COLLECTION

- In-house access to comprehensive X-ray diffraction facilities
- Secure and routine access to the high throughput facilities for crystal screening and highest resolution diffraction data acquisition (Diamond Light Source, UK)

STRUCTURE ELUCIDATION

- Suite of state-of-the-art software programmes used for the rapid solution of X-Ray crystal structures
- Heavy atoms introduced as required (e.g. Selenium), to aid structure solution
- Expertise in the delivery of novel ligand:protein complex structures (See table of available off-the-shelf targets) ▶

With its high throughput protein production facilities and expertise in the rapid elucidation of protein:ligand structures, Evotec provides superior quality support of structure-guided drug discovery programmes.

The benefits of using Evotec's structural biology expertise include:

- Rapid parallel protein production of multiple target constructs
- Early entry into crystallisation trials
- Expertise with inhibitor profiling
- Demonstrated success across multiple targets of protein:ligand complexes

Combining its structural biology and proprietary screening expertise, Evotec has also developed a superior technology for high throughput fragment screening with the distinct advantage of reliable delivery of protein:fragment complexes, thus efficiency and effectively guiding fragment optimisation programmes.

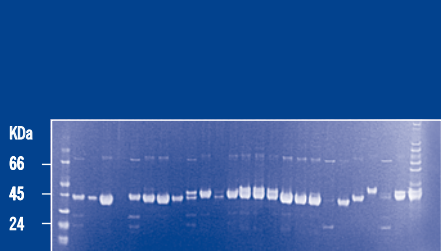
TARGET CLASS	TARGET	EVOTEC PDB CODE(S)
Kinases	Casein Kinase 2	-
	BRAF	-
	MK2	3GOK
	MK3	3FHR, 3FXW
	P38α	3KQ7
Metallo-proteinases	Thimet	-
	Oligopeptidase	-
Miscellaneous metallo-enzymes	Glyoxalase	-
	JMJD2A	-
Other enzymes	PDF (<i>E. coli</i>)	3K6L
	MIF	3HOF
ATPases	Serine racemase	3HMK, 3FKP, 3FDA
	Hsp90	3FT8, 3FT5
Phosphodiesterases	Kif11 (Eg5)	3K5E
	PDE4a	3I8V
	PDE4b	3KKT
	PDE4d	3IAK, 3K4S
Viral enzymes	PDE10a	2WEY
	HCV Helicase	-
	HCV polymerase 1a	-
	HCV polymerase 1b	3H98
Protein:protein interaction	Bcl-xL	-

MAPKAP KINASE

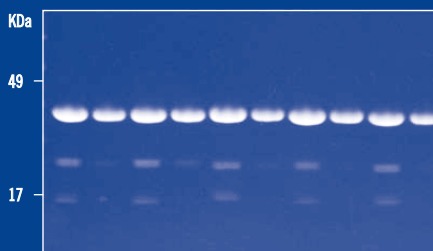
- Gene to novel structure, suitable for ligand studies, in three months
- 24 constructs designed and expressed
- Five constructs selected for large scaled purification and characterisation
- One construct of the 24 delivered diffraction grade crystals
- Structure of inhibitor complex elucidated to 1.9 Å

Case study – MAPKAP Kinase: Gene to novel structure in 3 months

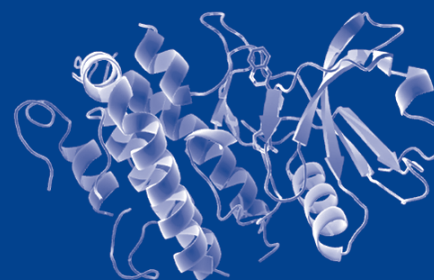
- Crystal system demonstrated for ligand and soaking to provide the structure of complexes of potent inhibitors
- Rapid and efficient identification of a novel construct suitable for structure-guided drug discovery



Expression levels of soluble MK3



Purification of five constructs

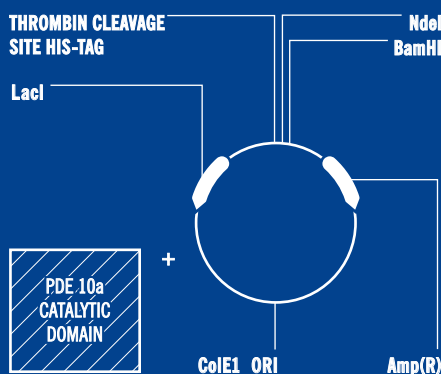


1.9 Å structure of MK3

PHOSPHODIESTERASE 10a

- Flow chart of the protein production procedure

Case Study – Phosphodiesterase 10a: Multiple novel ligand:protein complexes delivered



- Multiple novel crystal structures of complexes of hits delivered from a fragment and virtual screen
- Diverse structures delivered of inhibitor complexes ranging from micromolar to nanomolar potency
- High resolution structures of scaffolds delivered early in the programme allowing informed prioritisation of hits
- Foundation provided for an effective structure-guided medicinal chemistry programme

EXAMPLE OF FRAGMENT COMPLEXES OF PDE10a

