

FRAGMENT-BASED DRUG DISCOVERY

OUR CAPABILITIES, SKILLS AND EXPERTISE

PROTEIN PRODUCTION

- Proprietary high throughput platform incorporates parallel construct design, molecular biology, protein expression and protein purification
- Expertise in using *E.coli*, yeast, insect and mammalian cells expression systems

FRAGMENT LIBRARY

- 30,000 fragments optimally designed for quality, diversity and novelty to provide tractable starting points for optimisation
- Carefully selected by medicinal chemists as well as strict proprietary computational filters and algorithms

FLUORESCENCE CORRELATION SPECTROSCOPY (FCS) SCREENING

- Proprietary biochemical, highly sensitive and high throughput detection (FCS⁺plus) technology enabling information-rich, single molecule detection coupled with multiple read-out parameters, thus reducing false positives
- Enables the identification of active fragments in a relevant biochemical environment

NMR SCREENING

- Ligand and protein-observed NMR screening
- Worldwide licence to Abbott's powerful and highly sensitive SAR-by-NMRTM technology, enabling characterisation of binding site and determination of binding constraints

SPR SCREENING

- Orthogonal screening technology used for hit confirmation / hit validation, resulting in reduced false positives
- Off-rate determination

PROTEIN CRYSTALLOGRAPHY

- Co-crystallisation and soaking capabilities, significantly reducing false negatives from crystallisation
- In-house X-ray facilities and Diamond Light Source synchrotron (Oxfordshire, UK) are used to collect diffraction data
- Expertise for *de novo* X-ray crystallographic structure elucidation and repeat ligand complexes, facilitating novel structural information for structure-based drug design

Fragment-based drug discovery (FBDD) is a new paradigm in drug discovery that utilises very small molecules, fragments of larger molecules, to generate efficient starting points for drug discovery thereby requiring a highly sensitive screening technology.

EVolutionTM is Evotec's fragment-based drug discovery platform giving access to:

- A high quality library of 30,000 fragments that provide tractable starting points for subsequent optimisation
- A validated set of capabilities integrating a high throughput protein production technology, fragment screening, protein crystallography and fragment optimisation expertise
- Parallel purification technology for isolation of challenging proteins
- A portfolio of orthogonal screening technologies that combine proprietary biochemical FCS⁺plus screening, Nuclear Magnetic Resonance (NMR) and Surface Plasmon Resonance (SPR) for identification of active fragments
- A number of technologies and expertise for the optimisation of fragments into lead molecules

The proven benefits of EVolutionTM are:

- Increased speed of lead generation
- Lower attrition
- Identification of novel chemical IP

MEDICINAL CHEMISTRY AND STRUCTURE-BASED DRUG DESIGN

- Multiple fragment optimisation approaches, maximising the probability of achieving optimisation targets including: SAR-by-Nearest-Neighbour, Fragment Evolution and Fragment Linking
- Structure-based drug design platform integrating structural biology and computational chemistry capabilities
- A fully integrated biology team for *in vitro* and *in vivo* support during fragment-to-lead and lead optimisation

BACE case study: Fragment approach delivers novel starting points for challenging target

BACE1 is a protein implicated in the pathogenesis of Alzheimer's disease. Evotec has identified novel fragment inhibitors of BACE1 from a biochemical fragment-based screen. Co-crystal structures for fragments and optimised inhibitors have been obtained and form the basis for subsequent structure-guided medicinal chemistry program.

EVOTEC'S APPROACH TO IDENTIFYING NOVEL BACE1 INHIBITORS INCLUDES:

- A high throughput fragment screen of Evotec's diverse fragment library, utilising Evotec's sensitive FCS⁺plus functional BACE1 assay
- Counter-screening with a secondary

assay for hit validation
— Confirmation of ligand binding by SPR experiments using an Inhibition in Solution Assay (ISA)
— *In vivo* model for rapid testing of A β lowering drugs for driving a medicinal chemistry program

20,000 FRAGMENTS

- ▼ Primary screening

60 HITS

- ▼ Secondary assay including SPR

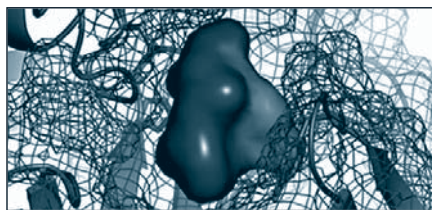
30 HITS

- ▼ Aqueous solubility, Ligand efficiency, Chemical tractability

7 SCAFFOLDS

- ▼ Docking analogues, Binding assay

CRYSTAL TRIALS, MULTIPLE PROTEIN-LIGAND STRUCTURES ELUCIDATED



FRAGMENT	1	1.1	1.2
BACE1 IC ₅₀	800 μ M	16 μ M	7 μ M
A β secretion IC ₅₀	---	---	12 μ M
LE	0.29	0.28	0.29
MW	226	349	363
clogP	1.52	3.75	4.63
TPSA	64	64	73
Lipinski violations	0	0	0
Co-crystal	1.8 \AA	2.1 \AA	2.4 \AA

Structure-based design using the elucidated structure of the Fragment1:BACE1 complex. One cycle of optimisation improved the potency of a fragment in series 1 by a 100-fold, from an 800 μ M fragment to a 7 μ M inhibitor. This compound is functionally active in a cellular A β cleavage assay at 12 μ M IC₅₀. The co-crystal structure of the compound was solved at 2.4 \AA resolution, offering an excellent starting point for further optimisation.

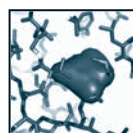
◀ Co-crystal structure of fragment 1 in the active site of BACE1 @ 1.8 \AA resolution

Hsp90 case study: Fragment approach delivers novel starting points for "well- screened" target

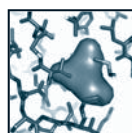
Evotec identified novel small molecules that are potent Hsp90 inhibitors from a high throughput biochemical fragment screen. The fragment hits were rapidly optimised using two complementary strategies. Two fragments binding in distinct pockets were linked resulting in a 1,000-fold increase in potency. A third fragment was optimised using a combination of *in silico* analogue selection, synthesis and structure-based design. Further optimisation is ongoing.

SAMPLE OF Hsp90 FRAGMENT HITS:

Promising fragment hits were submitted to co-crystallisation and soaking experiments with the N-terminal domain of Hsp90.



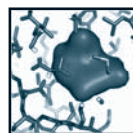
IC₅₀ 15 μ M
Soak 50 mM
1.9 \AA I222



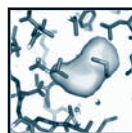
IC₅₀ 55 μ M
Soak 50 mM
1.9 \AA P2,2,2



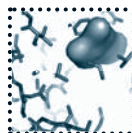
IC₅₀ 155 μ M
Soak 50 mM
1.8 \AA I222



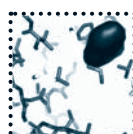
IC₅₀ 555 μ M
Soak 50 mM
1.9 \AA P2,2,2



IC₅₀ 570 μ M
Co-crystal 50 mM
1.8 \AA I222



IC₅₀ 1,030 μ M
Co-crystal 50 mM
1.9 \AA I222



IC₅₀ 1,040 μ M
Co-crystal 50 mM
1.8 \AA I222

LEGEND ▼

ATP POCKET

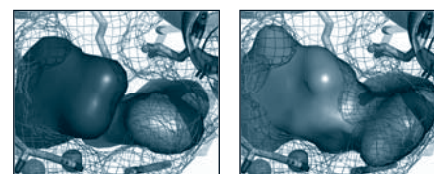
OPEN/HELICAL CLEFT

ATP POCKET AND
OPEN/HELICAL CLEFT

FRAGMENT EVOLUTION: FROM SUB-mM TO SUB- μ M IN 10 COMPOUNDS

— Sub-structure searches performed against 3.8 million available compounds
— Hits docked (GOLDTM), scored and visually inspected for key interactions
— Compounds purchased and tested
— Analogues synthesised and tested
— Virtual library designed and docked. Design focussed on introducing interaction with helical pocket
— Synthesised compounds show further increases in potency

FRAGMENT LINKING: 1,000-FOLD INCREASE IN POTENCY, IN A SINGLE STEP



Two fragments co-crystallised together

Two fragments binding in distinct pockets were linked allowing a 1,000-fold increase in potency