

# 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<sup>+</sup>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-NMR<sup>TM</sup> 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.

### EVOLUTION<sup>SM</sup> RESEARCH SERVICES OFFERED

- EVolution<sup>SM</sup> 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<sup>+</sup>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 EVolution<sup>SM</sup> 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<sup>+</sup>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 $\beta$  lowering drugs for driving a medicinal chemistry program

### 20,000 FRAGMENTS

- ▼ Primary screening

### 60 HITS

- ▼ Secondary assay including SPR

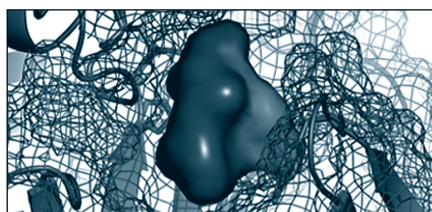
### 30 HITS

- ▼ 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 <sub>50</sub>	800 $\mu$ M	16 $\mu$ M	7 $\mu$ M
A $\beta$ secretion IC <sub>50</sub>	---	---	12 $\mu$ 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 $\text{\AA}$	2.1 $\text{\AA}$	2.4 $\text{\AA}$

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  $\mu$ M fragment to a 7  $\mu$ M inhibitor. This compound is functionally active in a cellular A $\beta$  cleavage assay at 12  $\mu$ M IC<sub>50</sub>. The co-crystal structure of the compound was solved at 2.4  $\text{\AA}$  resolution, offering an excellent starting point for further optimisation.

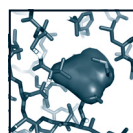
◀ Co-crystal structure of fragment 1 in the active site of BACE1 @ 1.8  $\text{\AA}$  resolution

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

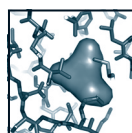
Evotec identified novel small molecules that are potent Hsp90 inhibitors from a high throughput biochemical fragment screen. The fragment hits were rapidly optimised using two complementary strategies. Two fragments binding in distinct pockets were linked resulting in a 1,000-fold increase in potency. 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:

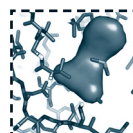
Promising fragment hits were submitted to co-crystallisation and soaking experiments with the N-terminal domain of Hsp90.



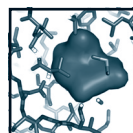
IC<sub>50</sub> 15  $\mu$ M  
Soak 50 mM  
1.9  $\text{\AA}$  I222



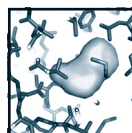
IC<sub>50</sub> 55  $\mu$ M  
Soak 50 mM  
1.9  $\text{\AA}$  P2,2,2



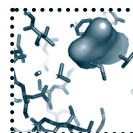
IC<sub>50</sub> 155  $\mu$ M  
Soak 50 mM  
1.8  $\text{\AA}$  I222



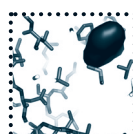
IC<sub>50</sub> 555  $\mu$ M  
Soak 50 mM  
1.9  $\text{\AA}$  P2,2,2



IC<sub>50</sub> 570  $\mu$ M  
CO-crystal 50 mM  
1.8  $\text{\AA}$  I222



IC<sub>50</sub> 1,030  $\mu$ M  
Co-crystal 50 mM  
1.9  $\text{\AA}$  I222



IC<sub>50</sub> 1,040  $\mu$ M  
Co-crystal 50 mM  
1.8  $\text{\AA}$  I222

### LEGEND ▼

ATP POCKET

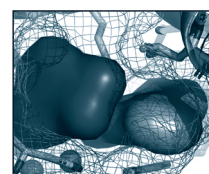
OPEN/HELICAL CLEFT

ATP POCKET AND  
OPEN/HELICAL CLEFT

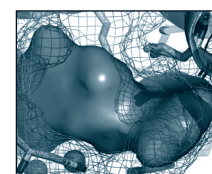
### FRAGMENT EVOLUTION: FROM SUB-mM TO SUB- $\mu$ M IN 10 COMPOUNDS

- Sub-structure searches performed against 3.8 million available compounds
- Hits docked (GOLD<sup>TM</sup>), 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