evotec

#RESEARCHNEVERSTOPS

Hit and Lead ID Capabilities

Track record, expertise and cutting-edge technologies

Agenda

1. Evotec overview
2. Evotec’s Hit ID platform
3. Assay Development and Screening Strategies
4. Summary and flexible scientific & commercial solutions

Bringing the industry closer together

Our contribution to the industry

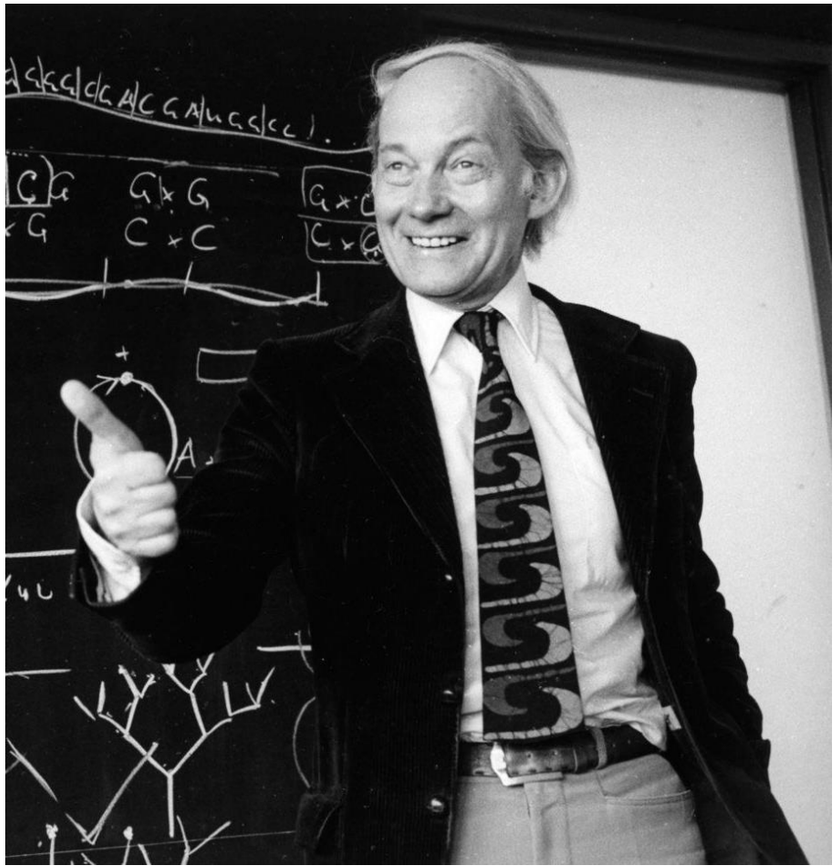
„The goal of Evolution is not one single human, it is mankind.“

We design medicines for difficult to treat diseases in **efficient collaborations**

We focus on data driven precision medicine and early disease relevance to **improve Probabilities of Success**

We built the “shared economy” in R&D, designed to result in **a large royalty pool**

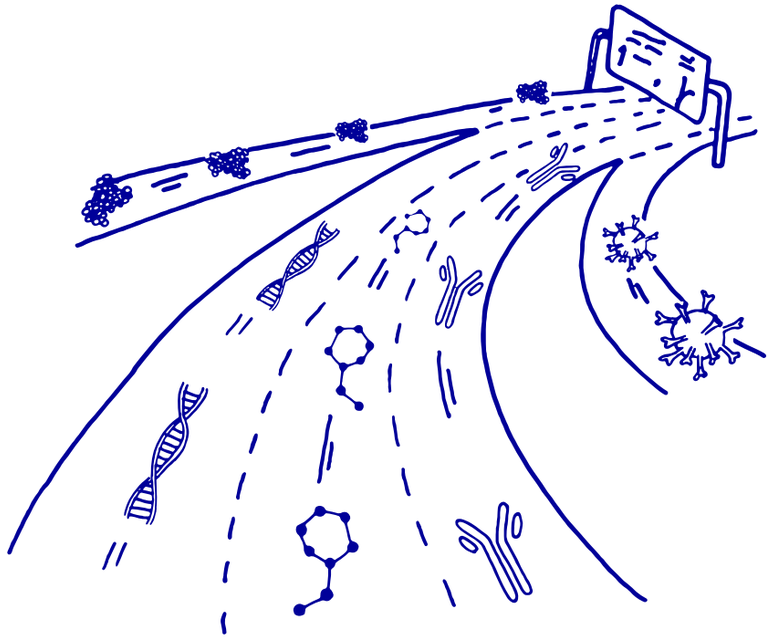
evotec



Manfred Eigen (1927–2019)
Co-founder of Evotec, Nobel Prize 1967

The multi-modality pipeline

Linking the best target to the best modality, the best biomarker and the best patient



ML & AI will accelerate Small Molecule and Antibody/ADC discovery

- Unbiased application of right tools and modalities to novel biology will make drug discovery much more data driven and cost effective
- Determining the clinical path will be a core consideration from the start

An expanded biologics' panel increases opportunities for drugs

- A multi-faceted approach to targeting RNA: small molecule, RNAi & ASO
- Protein and peptide-based therapeutic optimization and production
- Exploration of exosome therapeutic applications

Re-defining cell therapy & gene therapy lowers the hurdle for patients

- Investing in innovative, 'off-the-shelf' platforms to make these therapies even more accessible to patients
- Discovery through to development platforms

Platforms & technologies are high-tech driven & fully integrated

The drug discovery & development innovation hub – Capabilities & expertise overview

Industry needs

Capabilities & expertise (illustrative)

R&D efficiency platforms[1]

Target ID & validation | Hit identification | Lead optimisation | Sample management | Chemistry | DMPK & ADME-Tox | Research informatics | Bio Reagents | *In vitro* biology | *In vivo* Pharmacology | Biomarker discovery | Antibody discovery | INDiGO | Integrated CMC | Integrated pre-clinical development | Clinical development solution

Precision medicine platforms

EVOpanOmics

EVOpanHunter

EVOgnostic

E.iPSC DRUG DISCOVERY

ScreenSeq HIGH-THROUGHPUT TRANSCRIPTOMICS

ScreenPep HIGH-THROUGHPUT PROTEOMICS

E.MPD TRANSLATIONAL MOLECULAR PATIENT DATABASE

E.SAFETY TOX AND SAFETY PREDICTION

Just – Evotec Biologics[1]

J.DISCOVERY™ MOLECULE DISCOVERY

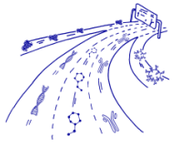
J.HAL℠ AI DESIGNED mAB LIBRARY

J.MD™ MOLECULE DESIGN

JP3® PROCESS & PRODUCT DESIGN

J.POD® MANUFACTURING DESIGN

Right modality drug design



EVOcells

EVOgenes

Antibodies & Bifunctionals

Small molecules

Protein degradation

Antisense

Exosomes

E.RNA TARGETING RNA SPACE

E.INVENT-AI MOLECULAR DESIGN EXCELLENCE

1 Also partly accessible as stand alone “Fee for Service” or FTE-rate based offerings

Faster and more learning curves illustrate … “just the beginning”

“Evotec inside” (selected KPI’s 2023)

High throughput screening at Evotec in a nutshell

10 Screening systems	**>12 m** Investments in past 5 years	**>850 k** Compounds available for screening	**>25** Different readouts supported

>1,500 Assays developed	**>750** HTS campaigns in total	**>300** HTS campaigns in past 5 years	**>80** Screening campaigns for year (~400 cpds per screen)

>20 Years of operation	**>175** Scientists in HIT Identification	**30%** PhDs	**>1,000** Cumulated years of expertise in HTS business

>5,000 Proteins produced in past 5 years	**~1,600** Cell lines generated in past 5 years	**>1,750** Protein ligand complexes delivered in past 5 years	**>250** Secondary targets assessed

Comprehensive integrated research and development

Illustrative functional capabilities along Evotec value chain (Pipeline Co-Creation)[1]

Sourcing of novel ideas	Target ID/ validation	Hit identify-cation	Lead optimi-sation	Pre-clinical de-velopment	Phase I	Phase II	Phase III	Approval	Market
• Exploratory biology • Hit-finding technologies • Chemistry • DMPK • Sample management	• Disease area expertise • Biology, translational biology • Design/chemistry • DMPK • PK:PD	• Translational biology • Design/chemistry • DMPK/physical chem • Formulation, PK/PD and ADME • Safety • Biomarkers • Clinical planning and project management	• Translational biology • API process development & manufacturing • Formulation & drug product for clinical testing • Safety / safety prediction	• Commercial API and drug product manufacturing					

Pipeline Co-Creatinon

- Focused, inter-disciplinary teams
- Comprehensive “under ONE” roof offering of technologies, experience, and expertise
- Operational excellence and AI/ML-driven predictive science driving rapid progress and successful outcomes

1 Illustrative for small molecules, but in full build-up also for biologics as well as cell therapy processes

One platform – more efficiency, better precision, higher speed

Evotec footprint – 17 Sites & ~5,000 employees

Hamburg (GER – HQ)
Manfred Eigen Campus – A major hub for integrated drug discovery including variety of HTS screening activities; home of neuroscience experts & the basis for leading end-to-end iPSC platform

Göttingen (GER)
Manfred Eigen Campus – home of multi-omics data analysis PanHunter, E.MPD & iPSC-derived cells

Cologne (GER)
Induced pluripotent stem cell (iPSC) technology

Halle (GER)
Centre of excellence for rare disease drug substance manufacturing

Munich (GER)
Dedicated to unrivalled proteomics and bioinformatics; unique mass spectrometry-based "omics" platform

Seattle (US)
Dedicated to biologics

J.POD® Redmond (US)
Biologics development & cGMP commercial manufacturing

Branford site (US)
Dedicated Sample Management Facility

Princeton (US)
Gertrude B. Elion Campus, dedicated to cell & protein production

Framingham (US)
US site of the ADME-Tox capabilities

Alderley Park (UK)
Focused on antimicrobial and infectious disease; Cyprotex – global leader in DMPK/ADME-tox

Abingdon (UK)
Dorothee Hodgins Campus, integrated drug discovery & development

Toulouse (FR)
Campus Curie – Oncology & immuno-oncology centre of excellence; integrated drug discovery; 2nd J.POD®

Lyon (FR)
Anti-infective drug discovery; BSL 3 laboratory set up

Vienna (AU)
Dedicated to gene therapy

Modena (IT)
Cell therapy manufacturing

Verona (IT)
Campus Levi-Montalcini Integrated drug discovery & development

Serving all key parts of the industry

Central infrastructure for partners with different missions

Partners	Collaboration priorities	Examples
> 40 Pharma	Flexible access to technologies and assets	novo nordisk, Bristol Myers Squibb, janssen, Boehringer Ingelheim, MERCK
> 400 Biotech	Integrated drug discovery & development processes	Sernova Corp, CHINOOK THERAPEUTICS, ALPINEImmuneSciences, Exscientia, RAPPTA THERAPEUTICS, almirall
> 30 Academia	Funding & operations for industrial translation	University of Oxford, Harvard, Yale, EMBL, Stanford University
> 10 Foundations	Data pooling & advanced analytics of patient data	CHDI, Bill & Melinda Gates foundation, The Michael J. Fox Foundation for Parkinson's Research

Trusted partner through >25 years of delivery

Track record and current capacity of >3,000 scientists

Strong track record

- **>700** assay developments and high-throughput screens
- **>150** Hit-to-lead campaigns
- **>300** patents with Evotec scientists as named inventors
- **>100** pre-clinical candidates delivered
- **>50** compounds approved for clinical trials
- Decades of new technology development (FCS+plus, iPSCs, FMO, advanced proteomics, novel disruption of cell signalling)

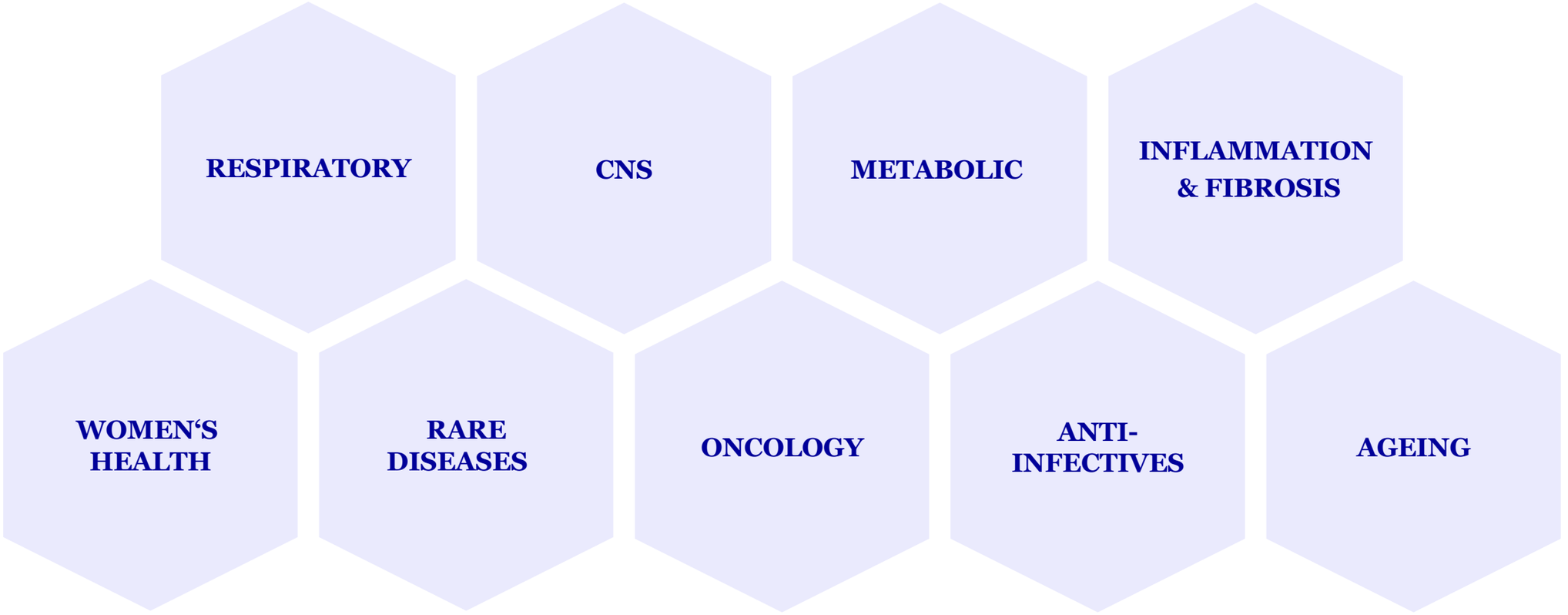
Globally leading current state & accelerating

- **~80** high-throughput screens per year, including BSL2^{+} and BSL3, phenotypic, biophysical screens
- **~20** Hit-to-lead campaigns per year
- **>40** concurrent integrated drug discovery projects in 2018
- **>16** pre-clinical INDiGO packages ongoing
- **30** peer-reviewed publications in 2018
- Numerous continuations, new strategic partnerships, milestones, and clinical progressions announced in 2018

FMO – Fragment Molecular Orbital theory: quantum mechanical calculations for advanced structure based drug design

Evotec focuses on diseases with high unmet medical need

Broad range of therapeutic area expertise at Evotec



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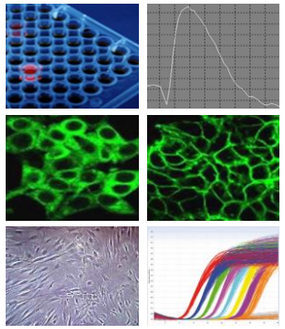
Hit ID platforms and philosophy

Addressing disease-relevant biological events

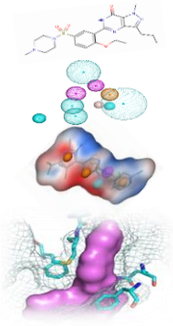
Target directed Hit ID

- Biochemical and cellular HTS
- Lead-like libraries (Evotec and partner libraries)
- Biophysical, label free HTS
- Covalent library screening
- Rapid hit follow up



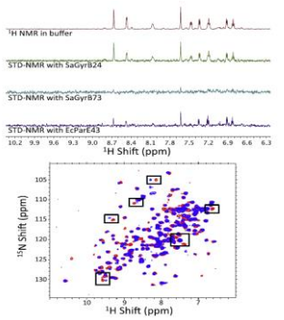
In silico approaches

- Ligand & structure-based approaches
- *De novo* designed virtual libraries
- Structure based (docking and pharmacophore) searches
- High-throughput docking



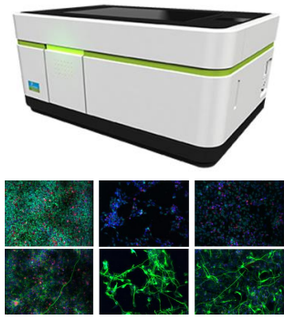
Structure based Drug Discovery

- Fragment screening (biophysical and high concentration biochemical screening)
- Fragment libraries
- Virtual and Biophysical FBS
- HT X-ray and fragment expansion



Phenotypic Hit ID

- Disease related functional & pathway assay systems
- HT phenotypic screening
- Secondary assay systems
- Poly-pharmacological models
- Target decon (e.g. proteomics)



- Unbiased technological approach giving rapid access to tractable hit series at lowest attrition rates and disease-relevant pharmacological profile
- Ability to hit previously un-druggable targets by multiple / novel technologies

High Throughput & Content Screening

Overview

- Deep institutional knowledge and expertise due to technological leadership
- Global operation of 10 integrated (u)HTS platforms including BSL2⁺/3 capabilities
- Multiple plate formats up to fully miniaturized HTS (1536-well)
- Global capacity for > 80 HTS projects per year
- High throughput platforms for genomic screening by RT-qPCR, biophysical (SPR, NMR) and mass spectrometry-based screening (RapidFire, AEMS[1], ASMS[2] and intact protein MS for covalent cpds)
- Numerous automated microscopes from Perkin Elmer for imaging-based assay system
- High throughput Ion Channel & Transporter Discovery platform
- World-class centralized Compound Management platform
- Centralized data statistics and discovery informatics support
- Relevance, quality, efficiency

PerkinElmer · paa peak analysis & automation · HighRes biosolutions · ttplabtech · LABCYTE · MOSAIC · Genedata solutions in silico

1 Acoustic Ejection Mass Spectrometry (ECHO-MS)
2 Affinity Selection Mass Spectrometry

Global Screening Centers

Evotec's screening sites and profiling capabilities

	Hamburg (83)	Toulouse (73)	Princeton[1] (20)
Biochemical and cellular	Fluorescence, FP, FRET, HTRF, Luminescence, Absorbance, AlphaScreen	Fluorescence, FP, FRET, HTRF, Luminescence, Absorbance, AlphaScreen	Fluorescence, FP, FRET, HTRF, Luminescence, Absorbance, AlphaScreen
Radiometric	-	–	Various isotopes
Mass Spectrometry	RapidFire MS, AEMS	RapidFire MS, online SEC-MS (ASMS)	RapidFire MS, online SEC-MS (ASMS), intact protein MS for covalent cpds
Anti-infective BSL2/2$^+$, BSL3	–	Anti-Infective, micro-organism (BSL2 / BSL2$^+$, BSL3)	–
Cellular	FLIPR 384 and 1536 (Ca-Flux, FMP, Thallium), Cellular Second messenger, reporter gene, MSD	1,536 and 384-well RT-qPCR, Cellular Second messenger, reporter gene	FLIPR (Ca/Na-Flux), Cellular Second messenger, reporter gene, MSD
Imaging / HCS	OPERA / Phenix	Operetta CLS	ViewLux, Licor Odyssey
Ephys	Manual PatchClamp, APC, IonWorks, SyncroPatch	–	–
Biophysics	SPR, NMR, DSF (384), MST, HDX, switchSense	SPR, DSF (384, 1,536), MST	–
Infrastructure	• 3 PE & 2 HighRes platforms, 1,536 & 348 well • Tecan, PMP, CyBi vario, TTP tube stores & Mosquito, ECHO Access stations	• 3 PAA + 2 HighRes platforms 1,536 & 384, 3 Beckmann stations • RTS stores, Tecan, Agilent, Beckman, TTP, Echo Access	• PE platforms, 384-well & Varispan; ECHO, Apricot, Multidrop Combo • FLIPR, ViewLux, Envision

1 PRI focus on high throughput profiling, Hit to Lead, LO support, MS screening

Evotec Screening Approaches

Developing strategies to identify small molecule hit compounds

Possible approaches for identifying hit compounds:

- HTS campaign with various assay systems → possibility to differentiate from competitor approaches
- For structurally enabled targets consider fragment screening by biophysical methods or X-ray crystallography
- Virtual Screening as primary knowledge-based approach or to augment hit population with VS derived hits
- DEL screening optional with external partner

Options for HTS assays (examples)

Type	Reagents	Assay format
Cell-based with reporter gene	Target expressing reporter cells	Luciferase reporter assay
Functional cellular assay	Target expressing cell line	Functional FLIPR readout
Cellular protein level	Target expressing cell line / type	Bioluminescent HiBiT detection
Phenotypic high content screen	Relevant cell line / cell type	Imaging
Biochemical activity assay	Recombinant protein	Enzyme activity, fluorescence or mass spec
Biochemical binding assay	Recombinant protein, fluorescent probe for displacement assay	Displacement assay or label free affinity selection MS
Protein::protein interaction	Purified proteins with appropriate tags	TR-FRET biochemical assay
Fragment screening by SPR/NMR X-ray screening	Recombinant protein with appropriate tag Isotope labelled protein Soakable X-ray system	SPR binding assay Protein observed NMR X-ray crystallography

1 External partner used for DEL screening, if required

Best quality start-points for chemistry

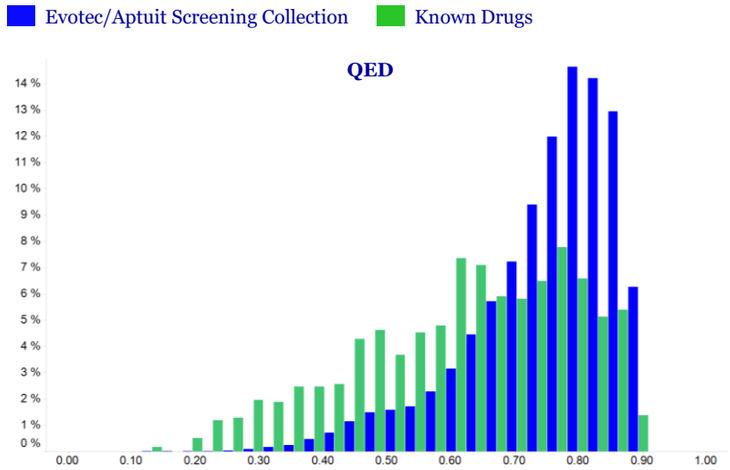
Our knowledge and experience, for our client's benefit

Tailored screen design • Know-how and experience • Flexible modules

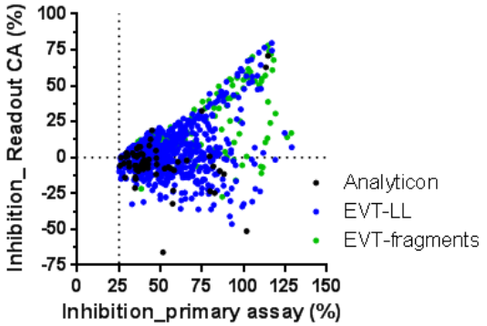
Attractive chemical library | **Assay development** | **Marker library** | **Primary Screen & Confirmation** | **Analysis** | **Profiling / counter screens**

- **Highest quality chemical start points**
- **Best value**

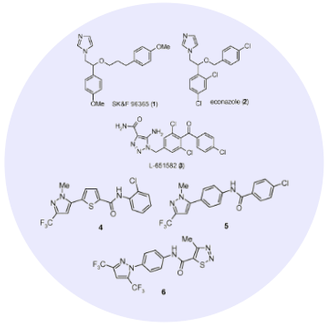
Ongoing investment in screening collection



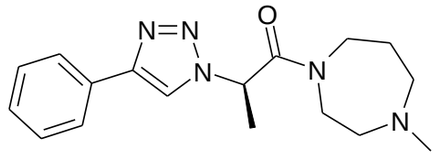
Integrated Biology and Medchem Process to identify most suitable hits for progression into hit expansion



2,500 confirmed hits



50-100 hits for follow up



Desirable feature	Molecular term	Value
QED	Composite property	0.91
3 D shape	–	Chiral
LipE	clogD	1.1
LE	MW	314

HTS Data Review Process – MedChem Assessment

A data driven process

Initiation of Screen:
Formation of review team (scientists from Hit ID, *in vitro* pharmacology and medicinal chemistry)

Genedata solutions in silico

Primary screening

Hit confirmation

dotmatics knowledge solutions

Medicinal chemistry review Stage 1:
Review of confirmed hits and recommendation for compound selection for hit profiling

Genedata solutions in silico

Hit Profiling
(Dose Response and QC)

dotmatics knowledge solutions

Medicinal chemistry review Stage 2:
Review of dose response data, recommendation for selection of most suitable hits for progression

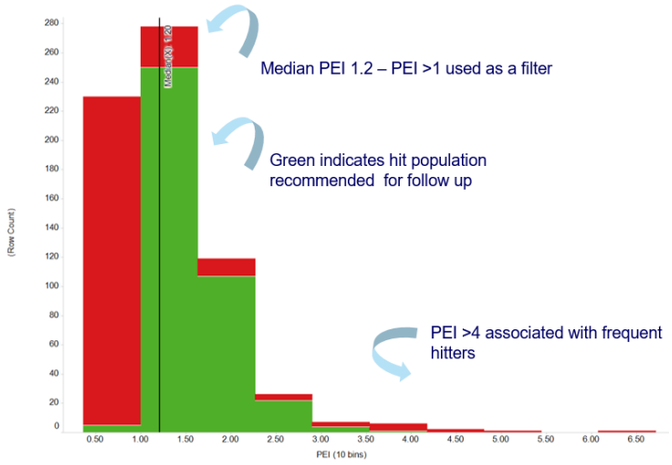
Report Generation:
Summary of Medicinal Chemistry assessment & follow-up recommendations

Data driven selection at each stage to avoid subjectivity and cognitive bias, process informed by literature

Graphical data representation using key metrics to aid selection of most lead like hits.
Metrics applied include: PEI, LE, LipE, QED[1], PFI, CNS MPO score

Consideration of potential promiscuity of hits; Structural motifs e.g. PAINs[2,3] Evotec structural flags; Frequent hitter information

Consideration of structural diversity within hit population; Clustering of hits, SAR analysis

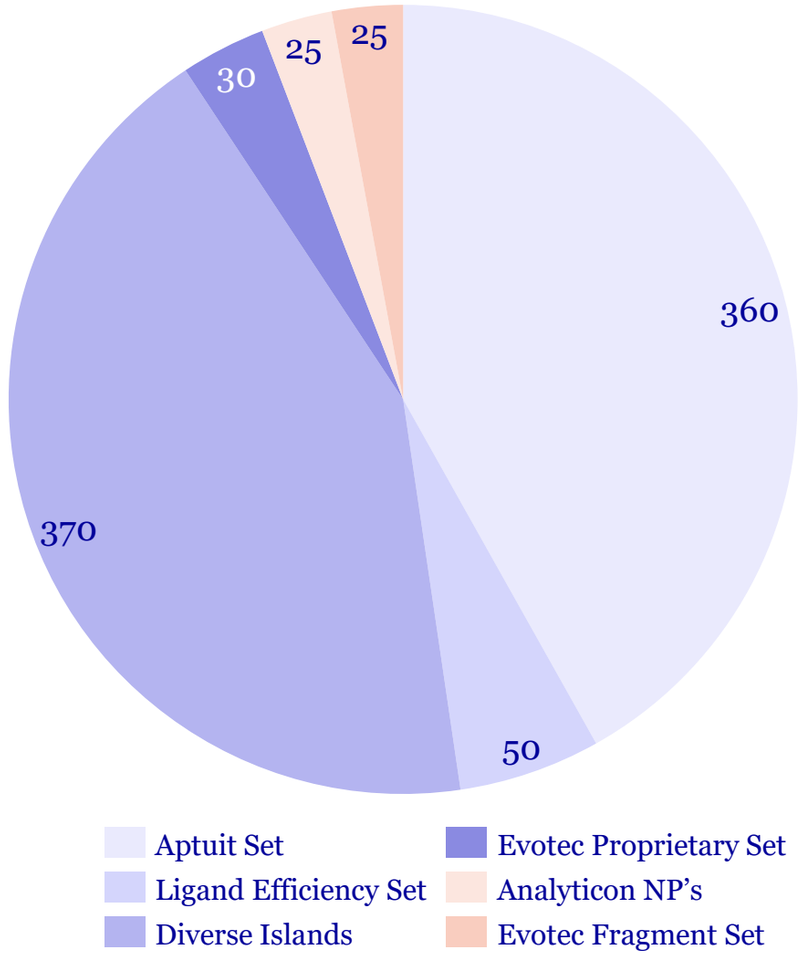


1 Nature Chemistry, 2012, 4, 90-98
2 Future Medchem, 2014, 6, 1265-1290
3 Nature, 2014, 513, 482

Evotec/Aptuit Lead Discovery Library

What is the general make up of our collection?

- Lead-like screening sets available to screen novel targets, typical sizes of screened libraries are 250k or 400k cpds
- Both screening sets provide access to highly attractive physico-chemical property space
- Optimized compound storage for long term stability
- Transition of library to acoustic tubes 2023/2024
- Ongoing library enhancement process (substructure filters, purity, attractiveness), replacement of significant part of the library in 2023/24
- Diverse "islands" of similar compounds facilitate hit series selection and generate preliminary SAR from screening data
- Proprietary set: access to novel lead-like chemical space
- Libraries available as pooled sets for mass spec affinity screening
- Ligand Efficiency Set & Fragment sets for FBDD approaches
- 5k Covalent Library in preparation, available Q2 onwards
- 30,000 natural products for PPIs and other targets
  a) 4,500 pure Natural Products (MEGx)
  b) 1,500 macrocycles
  c) 24,000 semi-synthetic compounds (NATx)
- 4,000 fractionated natural products from unique source[1]

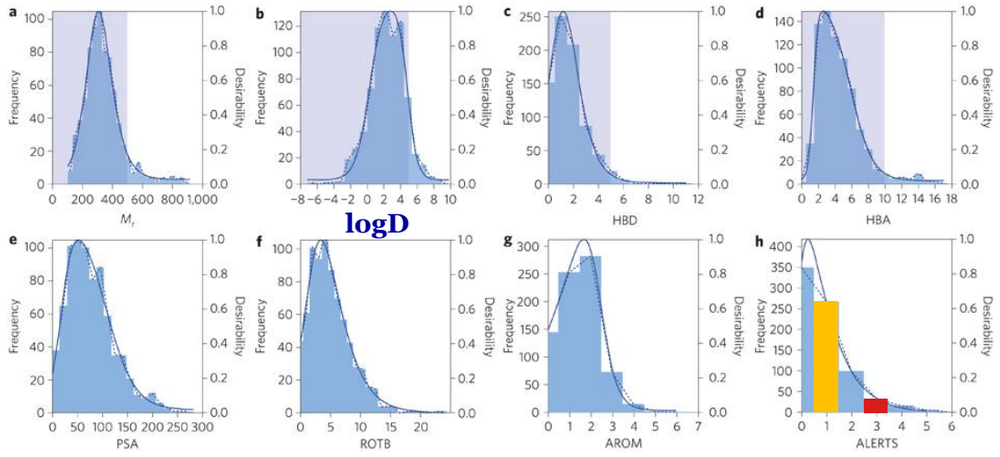


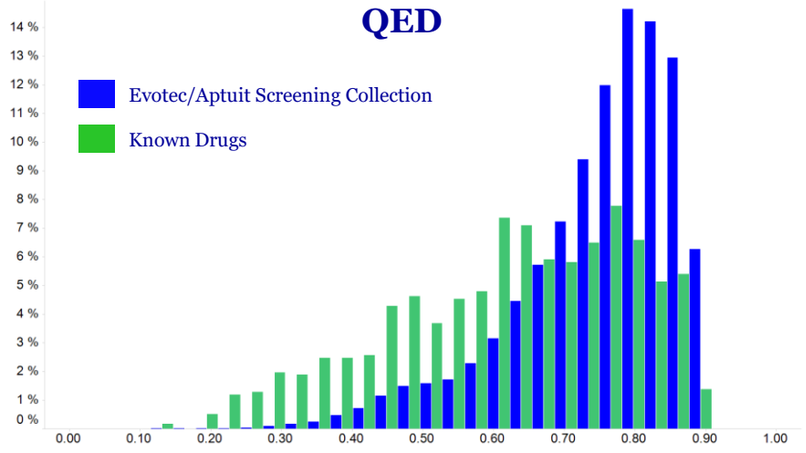
1 Fractionated NP's available via collaboration with Adapsyn

Modified QED score of chemical desirability

QED: Multi-parameter drug-likeness assessment

- Multi-parameter “chemical beauty” score calculated based on 8 molecular descriptors http://www.nature.com/nchem/journal/v4/n2/full/nchem.1243.html
- Adapted for Evotec's purposes, substituting clogP for clogD, and introducing a 2-tier alerts system, validated by Evotec medicinal chemists
- Evotec Screening collection with QED distribution comparable to known drugs





The Evotec & Aptuit screening libraries provide access to highly attractive chemical space

1 Fractionated NP’s available via collaboration with Adapsyn

Compounds selection for phenotypic screening

Maximise chemical and biological diversity despite limited throughput

1 Phenotarget set of bioannotated cpds

~5,500 biologically diverse annotated compounds

DRUGS | **TOOLS** | **PROBES**

Approved drugs
- *Repositioning*

Non-selective bioactives
- *Early indication of potential targets for validation*

Selective pharmacological probes
- *Aimed at single target/cellular pathway*
- *New disease biology insight*

2 Diverse phenotypic library

- *Maximal diversity with IP and hit to lead series potential*

~400,000 Evotec/Chembridge diverse lead-like collections

~20,000 Diverse Phenotypic Library

High diversity subset for limited assays
- Dissimilar from the ~5k annotated cpd set
- Chemical clustering with representatives to maximise predicted biological diversity (domain based fingerprint)
- Exclusion of pan-target predicted inactives (legacy data) & compounds with potential liabilities (solubility, frequent hitter, etc.)
- *High discovery potential: Novel target ID*

3 Natural products library (AnalytiCon)[1]

AnalytiCon discovery | A BRAIN Group company

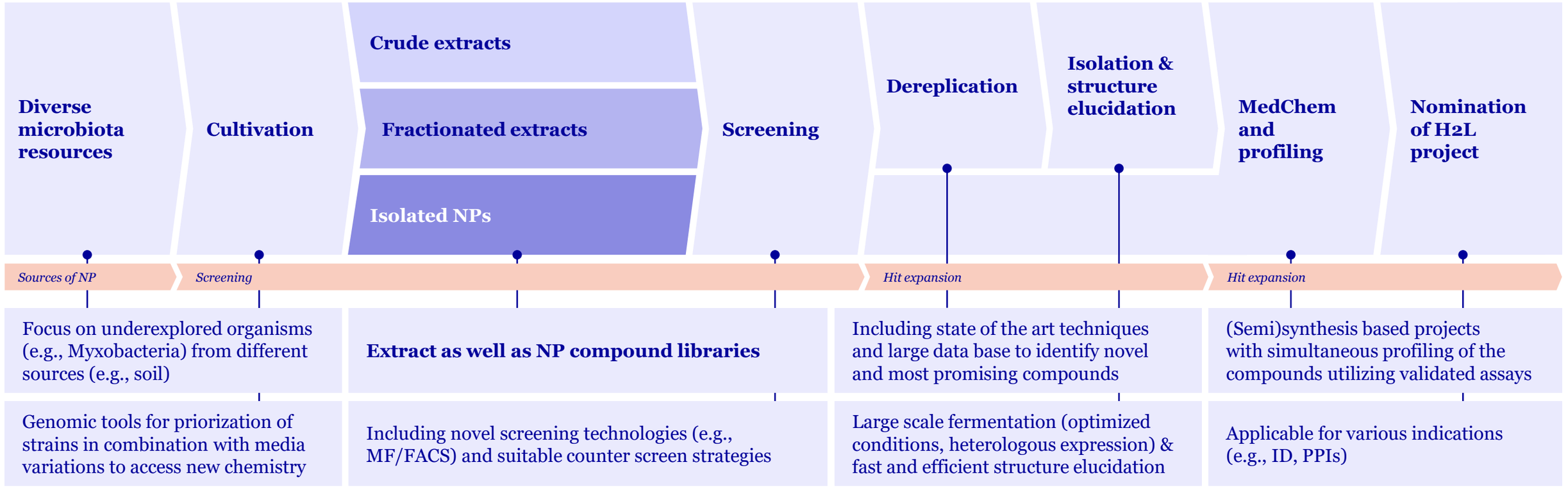
~4,500 natural compounds
~24,000 NP derivatives
1,500 macrocycles

Semi-synthetic & natural products
- NATx: Semi-synthetic library based on natural product scaffolds
- MEGx: Purified natural products from plants & microorganisms
- *Accesses novel physico-chemical property space*

1 Library does not belong to Evotec & requires payment of a compound access fee

GoTo Natural Products platform at Evotec

Accessing NP diversity for different indications from various resources



From extracts/NPs to H2L project within 9–14 months ⟶

1. *Projects together with external partner(s)*
2. *Evotec full ownership on project progression*

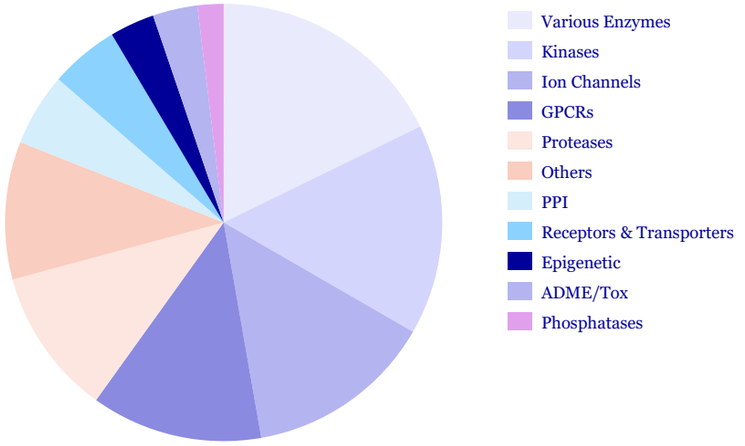
NP: natural product; MedChem: medicinal chemistry; MF: microfluidics; FACS: Fluorescence-activated cell sorting; ID: infectious diseases; PPI: protein-protein interaction; H2L: hit to lead.

Track record in assay development and screening

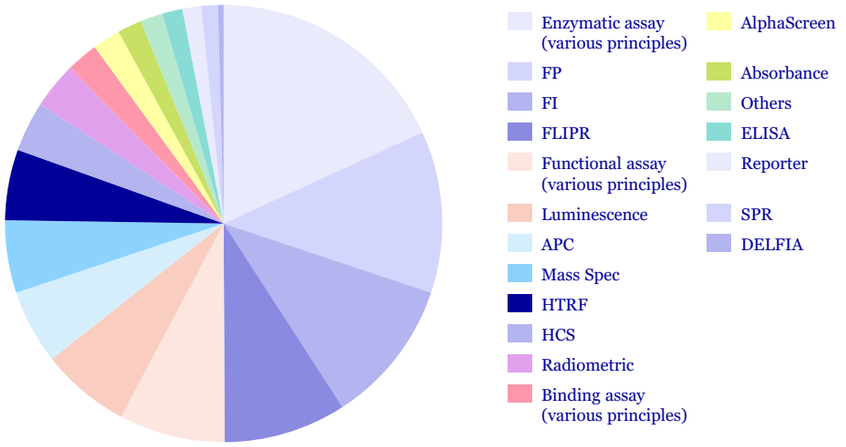
A know-how developed by >20 years of serving our customers

- Vast amount of expertise in assay development, validation and automation
  - More than 1,500 assays developed
  - 60% biochemical, 40% cell based screening campaigns
  - More than 750 HTS projects completed
- Approximately 50% of our hit identification projects continue with hit expansion, hit-to-lead and lead optimisation projects at Evotec
- Track record in addressing challenging targets
  - PAM/NAM for GPCRs and Ion Channels
  - Allosteric modulators of enzymes, PPI's
  - Complex assay systems (stem cells, mitochondria, co-cultures, microorganisms, primary cells, blood cells, isolated proteins)
- Deep integration of alternative hit identification routes
  - Multiple assay modalities per target
  - High Throughput early liability assessment (eADMET panel)
  - Virtual screening

Target classes



Assay principles



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High Throughput Screening

Typical HTS project workflow at Evotec

1 Agreement on screening strategy
Assay development / transfer including confirmation of robustness, sensitivity and pharmacology

2 Assay adaptation to HTS platform
Pilot screen at two compound concentrations (n = 3)
HTS with in-process controls (robustness, sensitivity, n = 1)
Hit confirmation of primary hits (n = 3), MedChem assessment (stage 1)

3 Hit profiling (11-point IC50, n = 2) including an appropriate counter-screen for artifact detection,
Confirmation of compound identity and purity
MedChem assessment (stage 2), tractability of hit compounds

4 *Optional*: Hit validation with orthogonal assays (LC/MS, SPR, NMR) from fresh powder samples / hit evolution / expansion, secondary assays, DMPK, structural biology …

- Project Team including reagent production, sample management, research informatics, *in vitro* biology, medicinal chemistry and others
- Regular teleconferences
- Progress reports
- If required, adjustment of screening strategy
- Final report and data package

Flexibility to be responsive to client needs

Assay Development or Assay Transfer

Assay Development at Evotec

Setup of novel assays

- Assay development from scratch
- No literature precedence and/or special assay requirements
- May require a feasibility study

Existing assay technologies

In-depth assay development
(e.g. literature precedence with special requirements on robustness and sensitivity)

Basic assay development or adaption to Evotec equipment
(e.g. existing assay protocol and/or literature precedence)

Assay Transfer

Assay Development by client

- Transfer of assay developed by client for Screening at Evotec
- If desired: transfer of assay developed by Evotec for use at client site

Screening

Assay Transfer

Biochemical assay technologies

Combination of traditional and label free read-outs

Traditional readouts for HTS

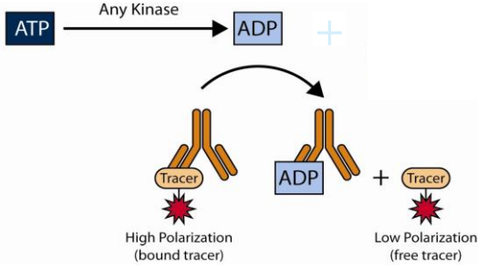
- Fluorescence Intensity
- Fluorescence Polarisation
- TR-FRET
- AlphaLISA® / AlphaScreen® (PE)
- Luminescence
- Radiometric [^{3}H], [^{14}C], [^{35}S], [^{32}P], [^{125}I], [^{33}P] & [^{59}Fe]

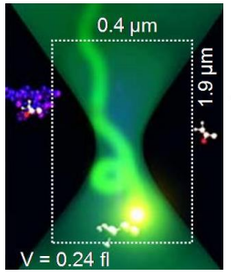
Orthogonal read-out technologies

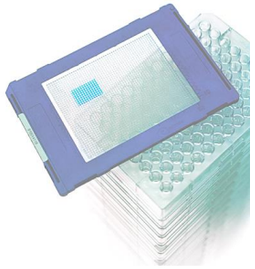
- Surface Plasmon Resonance / SPR
- Mass Spectrometry / LC-MS/MS
- Nuclear Magnetic Resonance / NMR
- Hydrogen-Deuterium Exchange / HDX
- Thermal-shift
- Mesoscale
- Nephelometry
- Microscale Thermophoresis
- Flow assays

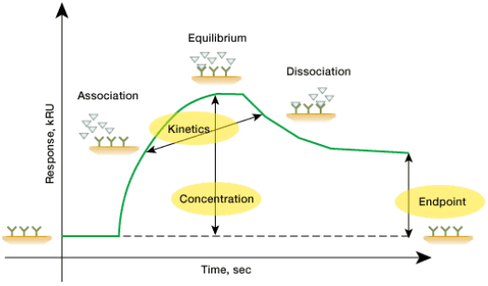
Hardware

- PE EnVision including AlphaScreen
- Tecan Safire II, Tecan Ultra, Tecan Infinite 500
- BMG PHERAstar
- BioRad Thermo Cyclers for PCR
- PE MicroBeta²
- Waters AQUITY TQD UPLC-MS/MS
- Agilent 1290 UPLC QTRAP4000
- 7 Agilent RapidFire™ systems
- 3 ASMS systems, LCT Premier XE, XEVO
- HDX-MS Leap HDX-3, LTQ Orbitrap Velos
- 5 Biacore T200
- Biacore 4000, 5 Biacore 8K/8K+
- 600 MHz NMR, Avance III, 1.7mm CryoProbe head
- FluoDia T70, VIIA7, LightCycler, Thermo QS7
- BMG NEPHELOstar
- Monolith NT.LabelFree, NT.115
- Dynamic Biosensors SwitchSense









Cellular assay technologies

Large technology panel for target-directed and phenotypic assays

Assay principles

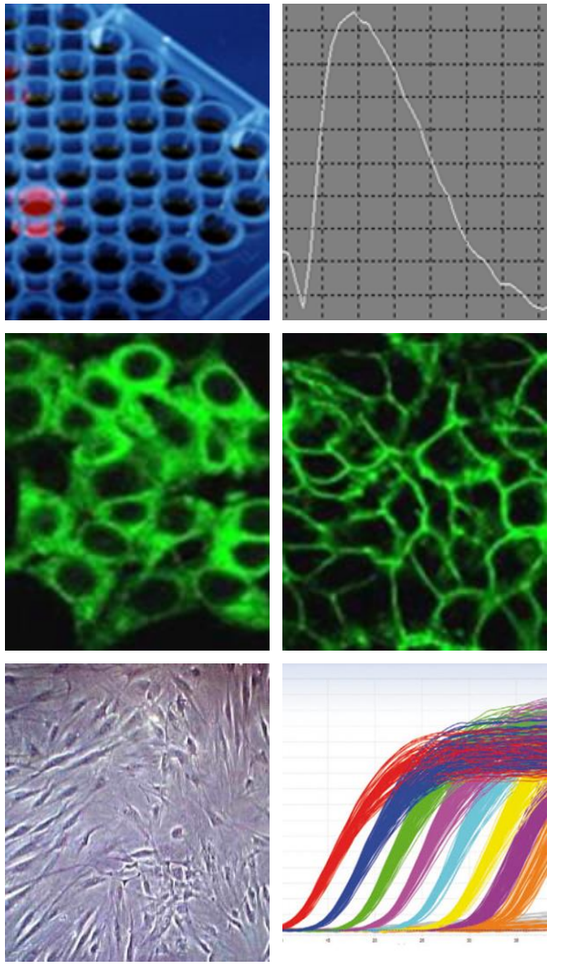
- Ca^{2+} flux (FLIPR)
- Second messenger (cAMP, IP_3)
- Membrane potential (ion channels)
- Reporter gene assays
  (Luciferase, SEAP, β-lactamase, β-galactosidase)
- Whole cell fluorescence
- ELISA, Mesoscale, HTRF
- Patch-clamp
- Primary cells & stem cells
- Migration assays
- Cytometry (cell cycle regulation, receptor expression studies)
- Metabolic assays (radiometric, O_2 consumption and pH change, metabolite production via LC/MS)
- RTqPCR on endogenous genes
- HiBiT protein detection

Hardware

- Opera Phenix™
- Operetta CLS™
- Various multimode readers
- FLIPR^{Tetra}
- Mesoscale (MSD)
- Seahorse
- LC/MS
- LightCycler 1,536
- Flow cytometers

Main target classes

- GPCRs
- Ion channels
- Nuclear hormone receptors
- Transporters
- Kinases (membrane and cytosolic)
- Transcription factors
- HDACs, HMTs
- Phenotypic

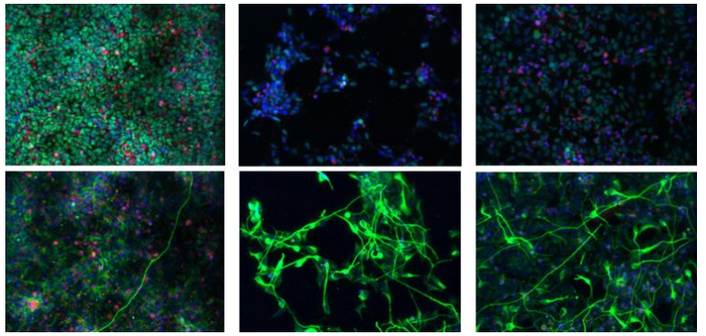


The robust application of Evotec´s cellular assays platform enables monitoring of events in larger panels of cell lines

High Content Screening for Target ID and profiling

Strengths and benefits of HCS at Evotec

1. Use relevant phenotypic assays to gain access to **new target and chemical space**
2. Take advantage of **primary cells** and **stem cells** to achieve tissue-specific cellular models
3. High-throughput & high-quality histology: HCS read-outs on tissue slices for **target validation & efficacy studies**
4. **>10** years of expertise in the development of the OPERA flows into Evotec´s HCS drug discovery platform
5. **Multifactorial data analysis** integrated into SAR analysis delivering more informative data at earlier stages

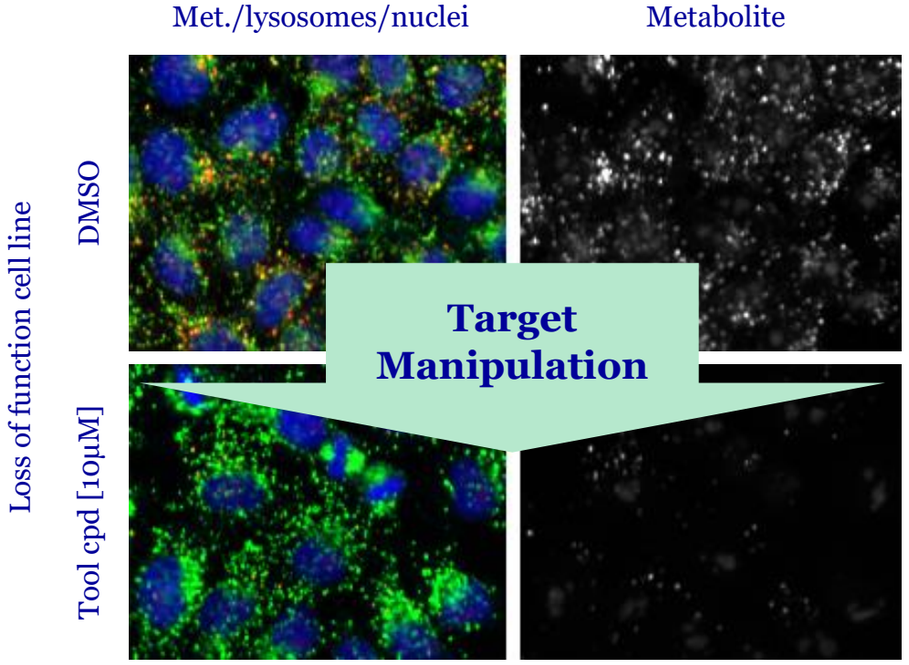


Unlocking Target-based drug discovery

Arrayed CRISPR complements Phenotypic Screening

Compound Library	**Assay Set Up, Phenotypic Screen**	**Hit validation, Hit-to-Lead**	**Target & Pathway Deconvolution**	**Lead Optimisation**
CRISPR Library		**HTS**	**Hit validation, Hit-to-Lead**	

Disease relevant assay



Phenotypic Screening Identification of Compounds

Similarity of Phenotypes

CRISPR Screening Identification of Targets

- **Enhanced utilisation** of disease relevant assay
- **Reduced risk** by using complementary approaches
- **Increased entry points** for follow-up development
- **Value maximisation for Drug Discovery campaign**

iPSC Automation

iPSC Cell Culture and Screening Automation

- The system is used to maintain iPSC and iPSC-derived cell cultures in 384-well plate format
- Smart, real time QC of iPSC cultures
- Support of iPSC screening
- Supplier: Perkin Elmer
  - cell::explorer robotic workstation
  - plate::works scheduling Software
  - 2x plate::handler II Flex SCARA robots
  - Enclosure cabinet with lighting
  - Three fan filter units with HEPA (U16) filter
  - Imaging read outs (PE EnSight)
- Throughput: Plate storage and handling capacity of approx. 230 384-well plates, support of several iPSC work flows in parallel

Integrated Instrumentation

- 3x Thermo Cytomat 2C (40 plates) and 1x Cytomat 5C (110 plates) benchtop incubators, BioTek ELx406 washer/dispenser, Multi-Drop 384, LPX 220 plate hotel
- PE EnSight multi mode reader
- PE JANUS Mini liquid handler MDT
- Millipore MilliQ®-Water supply system

BSL2/BSL2$^+$ & BSL3 Screening Capabilities

HTS & MTS on micro-organisms

- >15 years of screening expertise in anti-infective space
  - Antibacterials (e.g. ESKAPE, Gram positive, *Mycobacterium*,…)
  - Antivirals (e.g. HBV, hPIV)
- Support for back screening and hit expansion and secondary assays for hit characterization
- BSL2/BSL2$^+$ screening capabilities for HTS/MTS
  - 2 BSL2/BSL2$^+$ HTS platforms (ET-1/2) and 1 BSL2 MTS platform (Agilent workstation)
- BSL3 screening capabilities for MTS
  - BSL3 lab (~200 m^2) including controlled access, autoclave and H_2O_2 SAS for waste handling
  - BSL3 trained people
  - Basic equipment [safety cabinets (7), incubators, refrigerators and freezers (-20°C and -80°C)]
  - Automation equipment under safety cabinet
    - Dispenser/washer (ELX406, Biotek) for cell/reagent dispensing/washing
    - Pipettor (Cybiwell, Cybio) for compound addition
    - Multimode plate reader (PE EnVision) with stackers (30 plates)
  - Screening in semi-automatic process (384-well format)

ET-1

ET-2

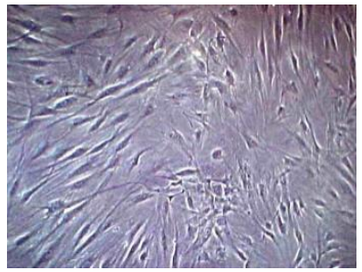
BSL3

BSL3

HTS – RT-qPCR Screening

384-well plate-based RT-qPCR screening

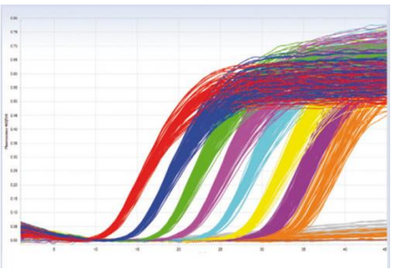
Biology



Automation

Real time PCR reaction

Data management



Cell seeding 384 wells → **Compound addition** → **Cell lysis** → **Lysat transfer in PCR plates** → **Addition mix PCR** → **RTqPCR duplex** → **Data analysis**

- High throughput approach to gene expression profiling with RT-qPCR as a primary screening tool
- Fully automated and integrated work flow for 384 well assays, multiplexing possible for up to 4 genes of interest
- Secondary screening to qualify hits initially identified with other screening approaches

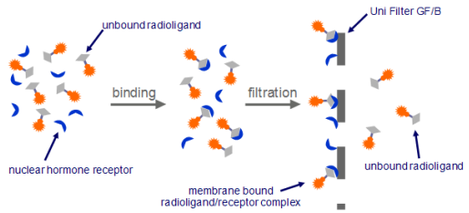
Integrated platform provides access to unique high throughput RT-qPCR screening

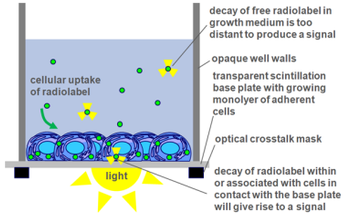
Radiometric Assay Technologies

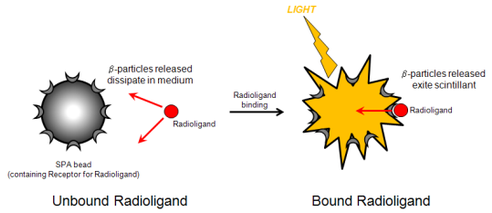
Hit identification and validation using a variety of assay technologies

Portfolio of radiometric assay technologies

- Radioligand binding assays (RLBA) – Filtration assays and SPA
- Uptake assays – SPA using PerkinElmer Cytostar-T™ Scintillating Microplates (applied for HTS)
- Enzymatic assays – FlashPlate® Assays and SPA (applied for HTS)
- GTPγS assay – binding of GTP-γ-[^{35}S] to cell membranes
- Isotopes in use: [^{3}H], [^{14}C], [^{35}S], [^{32}P], [^{125}I] (handling permit also for the use of [^{33}P] & [^{59}Fe])
- Biochemical and cellular assays







1 Ability to screen targets using multiple radio-metric screening assays

2 Capabilities enable screening of smaller sized compound decks

3 Orthogonal and counter screening for hit profiling

Capabilities and experience to screen various target classes in radiometric formats

Radiometric Assay Technologies

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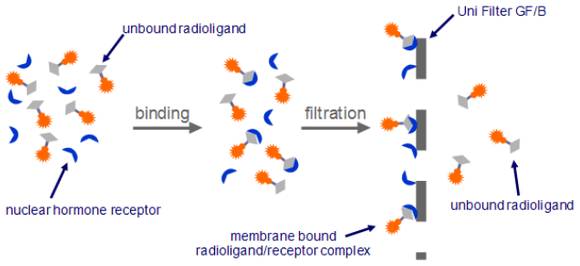

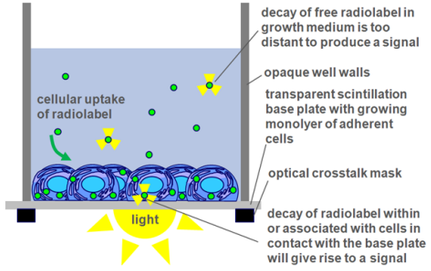

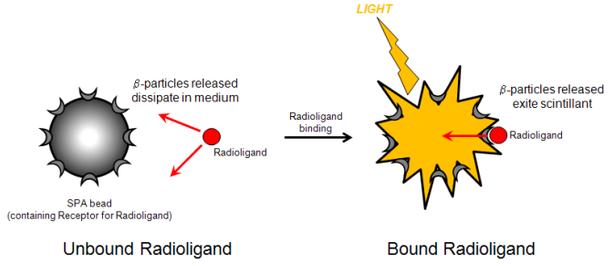

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Ion Channel & Transporter Discovery Platform

Electrophysiology at every stage of the discovery process

QUBE 384

Qpatch HTX®

Patch Liner®

Dynaflow® Pro II

Manual Patch-Clamp

SyncroPatch 384PE

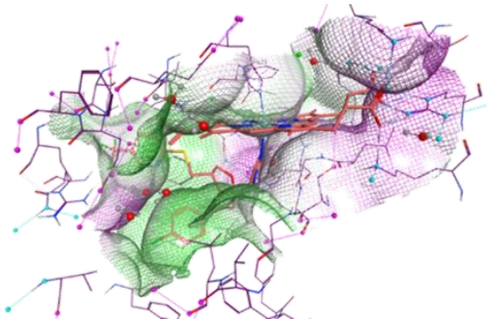
	N	Format	**Throughput** (Compounds per week)	**Utility**
SyncroPatch384PE	2	384-well	4,000-10,000	High throughput screening, hit-to-lead
QUBE 384	1	384-well	>20,000	High throughput screening, hit-to-lead
Q-patch HTX®	1	48-well	150-450	Hit-to-lead, lead optimisation & early safety assessment
PatchLiner©	3	16-well	75-150	Hit-to-lead, lead optimisation & early safety assessment
Port-A-Patch®	2	1-well	25-50	Hit-to-Lead, Lead optimization
Manual Electrophysiology (Dynaflow™ optional)	5	"1-well"	1-25	Lead optimisation, safety pharmacology
SURFE2R N96	1	96-well	10-20	Membrane transporters

Ability to screen ion channels and transporters in multiple formats including HT applications

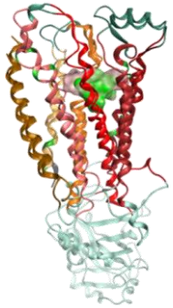
Virtual Screening Positioning

Some success stories

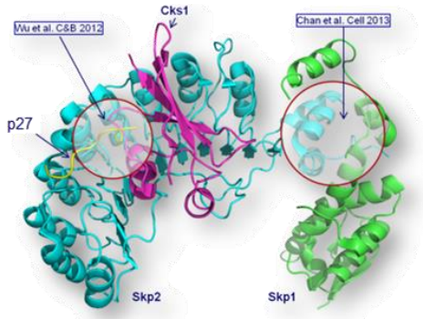
Heme containing active site



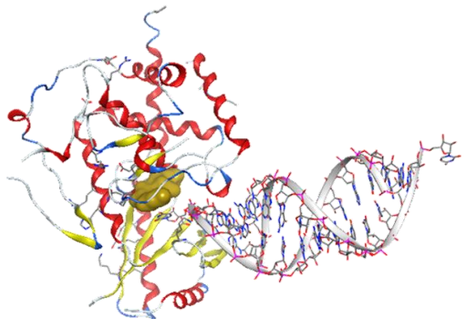
GPCR homology model directed screen



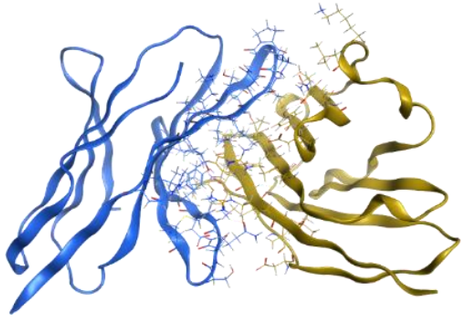
Protein//Protein interaction SBDD/LBDD screens[1]



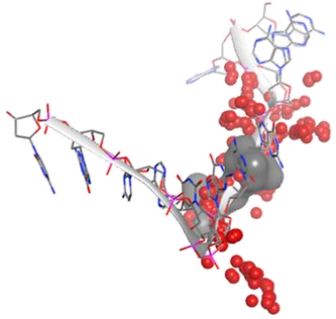
Protein//RNA transferase SBDD/LBDD screens



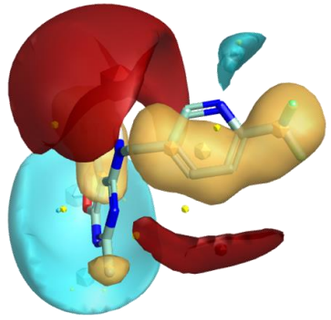
Protein//Protein interaction SBDD/LBDD screens



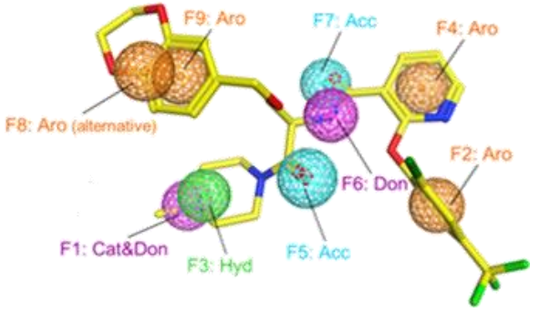
Protein//DNA endonuclease SBDD/LBDD screens



Field Pharmacophore guided LBDD



Pharmacophore guided LBDD excluded volumes



1 Assoc. Amer. Canc. Res., 2017

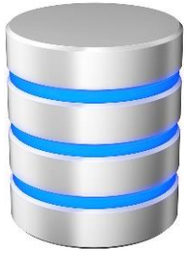
Hit ID by Virtual Screening

Knowledge based identification of novel chemotypes

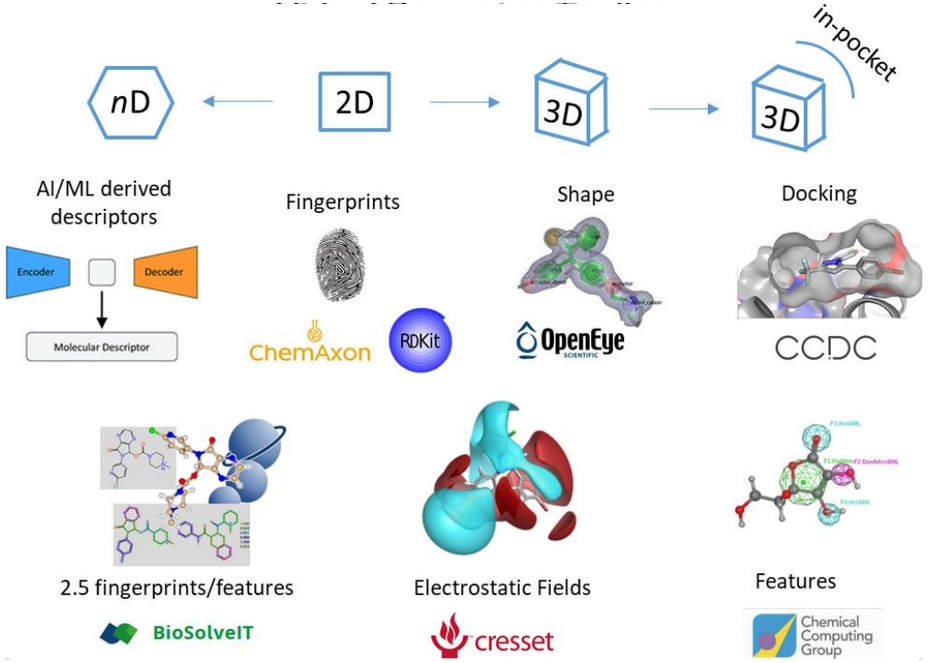
EvoSource

MolPort

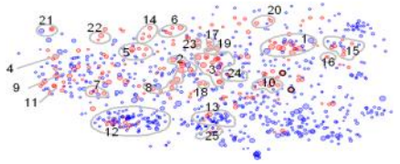
Enamine Real (Diversity)



Virtual screening toolbox



Data analytics, cheminformatics and clustering



Structural analysis and assimilation of data → **Establish virtual screening protocol** → **Run prospective virtual screen** → **Cluster virtual hits, select representatives**

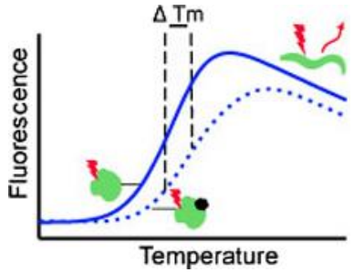
- Large collections of real and virtual compound libraries including a manually curated commercial supplier deck
- Custom virtual screening workflows are designed and developed by a highly skilled and experienced member of the computational chemistry team
- Proven track record of hit delivery for a diverse range of targets

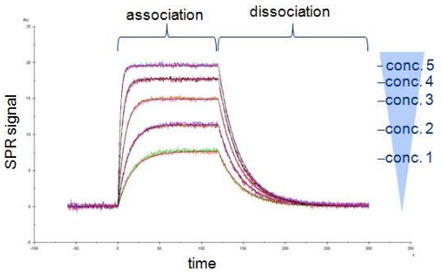
Label-free and Biophysical Screening

Orthogonal Approaches to Conventional Screening Assays

Molecular Interactions

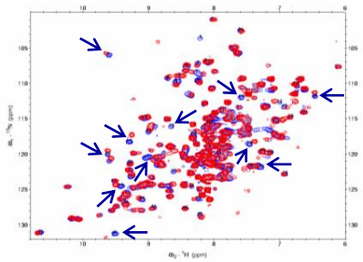
- Surface Plasmon Resonance (SPR, Biacore T200, 4000, 8K, 8K+)
  - Binding affinity, kinetics of interaction
- Differential Scanning Fluorimetry (DSF, FluoDia T70, ViiA7)
- Microscale Thermophoresis (MST, NT.LabelFree, NT.115)
- Isothermal Titration Calorimetry (ITC, iTC200)
- SwitchSense technology (Dynamic Biosensors DRX)

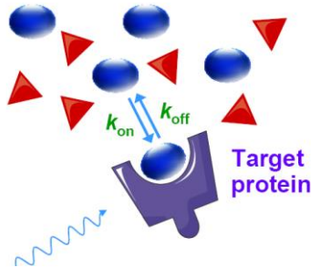




NMR

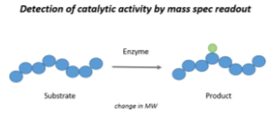
- Bruker 600MHz, 1.7 mm TXI CryoProbe, Avance III HD
  - Protein-observed NMR (chemical shift perturbations)
  - Ligand-detected NMR – STD and WaterLOGSY

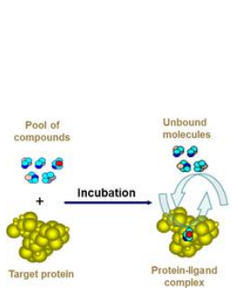


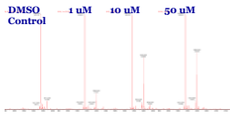


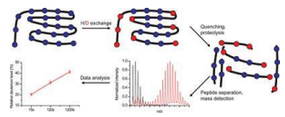
Mass Spectrometry

- HTMS: RapidFire™ systems (catalytic activity with native substrates)
- Online SEC-MS screening (affinity selection of binders by ms, ASMS)
- Intact Mass for covalent modifiers
- Hydrogen-deuterium exchange (HD-X)









Biophysical Screening Technologies

Properties and requirements in particular for fragment approaches

	Typical library size at Evotec	Protein requirements	Limitations	Information provided
NMR – ligand obs. (STD, WaterLOGSY)	3,000 as mixtures	low µM, increasing sensitivity with size	Fragment binding with very high affinity (false negatives, K_d <<µM)	Binding, binding site in displacement mode
NMR – protein obs. (HSQC, TROSY)	3,000 as mixtures	µM, decreasing sensitivity with size	Isotope label on protein req. 1H, 15N-HSQC (≤35kDa), 1H, 15N-TROSY (≤60kDa)	Binding including binding site
SPR binding assay	3,000-20,000	µM to mM, decreasing sensitivity with size	Functional immobilization required	Binding, k_{on}, k_{off}, K_d
Thermal Shift (TS)	20,000 +	µM, decreasing sensitivity with size	Thermal stability of the protein	Binding, stabilization of complex, K_d
Fluorescence based bioassay	20,000 +	nM to µM	Fluorescence labelling required	Binding or functional activity
MS activity and binding readout	20,000 +	nM to µM	Catalytic activity, sufficient affinity of binders	Functional activity or confirmed binding
Intact Mass screening	1,000 +	up to mM	Off rates of reversible covalent binders	Covalent binding, stoichiometry
X-ray crystallography	500 singletons	µM to mM	Small libraries, risk to miss hits by soaking	Complex structure
HDX-MS	<10	nM to µM	Very low throughput	Binding, structural information, dynamics

MOA Studies and Thermodynamic Signatures

Impacting MedChem optimization

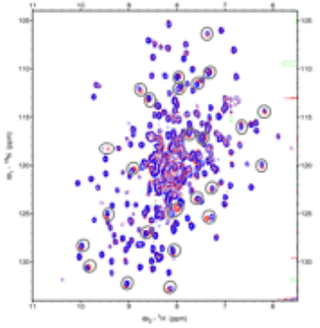
Orthosteric vs. allosteric modulation

- Allosteric modulation targeted to achieve improved selectivity
  - Positive and negative allosteric modulation of e.g. GPCR's
  - Allosteric enzyme inhibitors / activators (e.g. phosphatases)
- Co-operativity of allosteric binders with orthosteric ligands

Apo protein

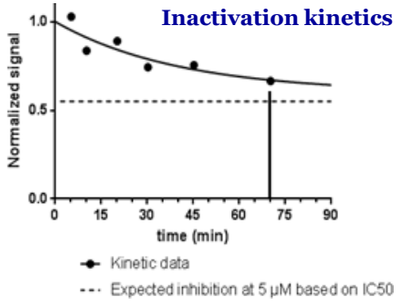
Protein + allosteric tool compound

Perturbation patterns define regions of interest (ROI)



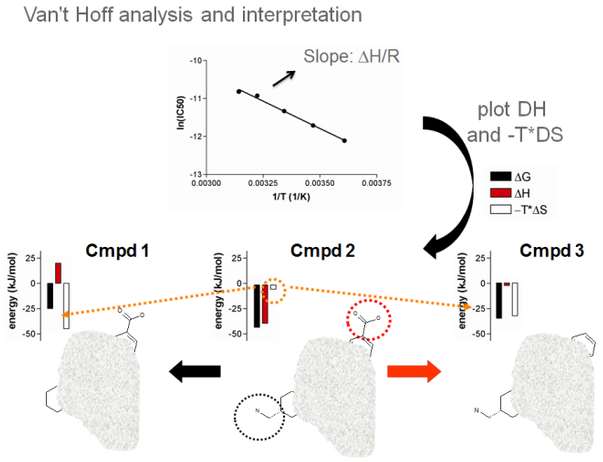
Rapid reversible vs. covalent / irreversible inhibition

- Type of reversible interaction (competitive, uncompetitive, or non-competitive inhibitors)
- Slow off-rate binders with improved residence time
- Inactivation kinetics of irreversible inhibitors (k_{inact}/K_i)



Thermodynamic signatures of small molecules

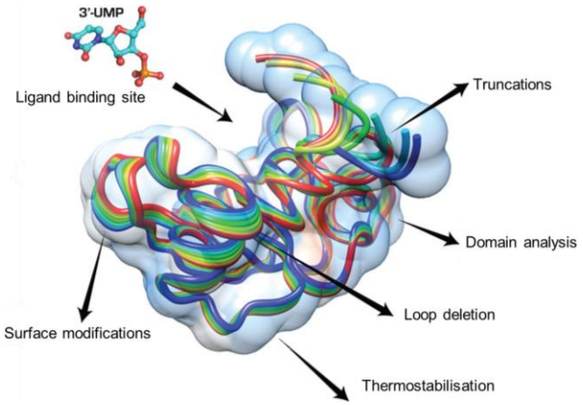
- Free energy changes upon formation of small molecule / protein complexes
- Enthalpic versus entropic contribution to binding

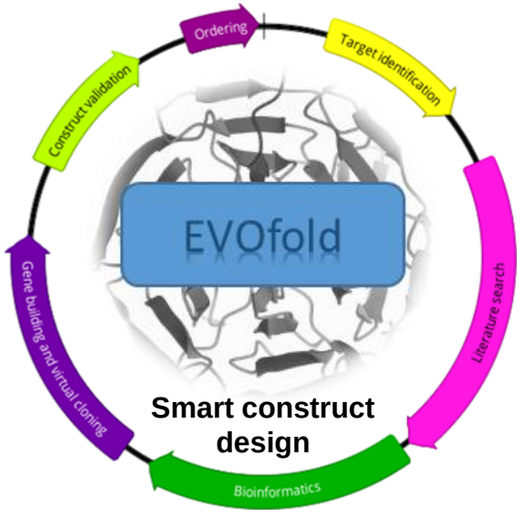


Detailed mechanistic and energetic analysis enables more efficient medicinal chemistry optimisation

Protein Science > 165 FTEs across four sites

From gene to protein – flexible entry points and smart design





E. coli

Insect

Mammalian

Yeast

Fit for purpose

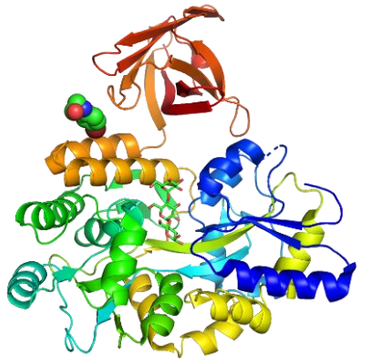
- Structural biology
  - **Xray**
  - **Cryo-EM**
- Tagging
  - **SPR**
- Isotope labelling
  - **NMR**
- Endotoxin-free
  - ***In vivo***
- Complexes
  - **Bio Chem**
- Antibody antigen
  - **Antigen**

EVOfold = integrates AlphaFold2; RosettaFold; OpenFold

Structural Biology

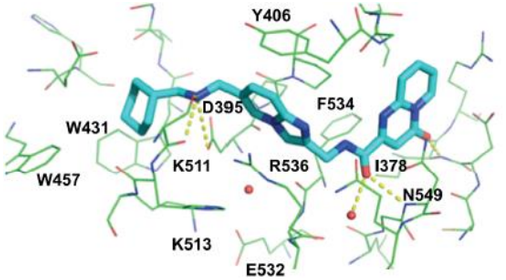
Team of 35 PhD structural biologists across two sites

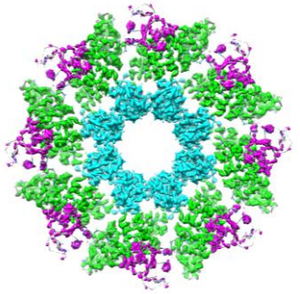
- Team established 2003, currently >35 PhD structural biologists in two sites
  - Crystallography
  - Cryo-EM
  - BIOSAXS
- Weekly synchrotron visits for crystallography data collection, automated data pipelines and cutting-edge software
- Multiple entry points for microscope access
- In house grid freezing capabilities
- Ability to solve structural problems across protein classes
  - Soluble proteins to macromolecular complexes (DNA and RNA) and membrane proteins
  - X-ray fragment screening
- Co-located with computational and medicinal chemistry teams



TREM-2 IG domain
Fragment Screen with MSD[1]

METTL3 Clinical
Candidate with Storm[2]





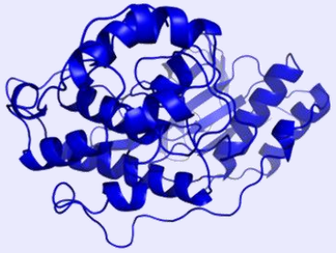
SARM1 *apo* CryoEM
structure Disarm[3]

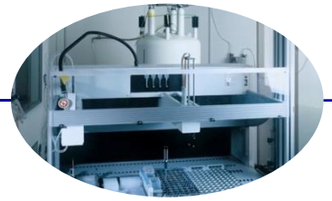
1 Byrne *et al.* Prot Expr & Purif 179 (2021), Development of a robust crystallization platform for immune receptor TREM2 using a crystallization chaperone strategy
2 Yankova, E. *et al.* Nature (2021), Small-molecule inhibition of METTL3 as a strategy against myeloid leukaemia
3 Figley *et al.* Neuron 2021, SARM1 is a metabolic sensor activated by an increased NMN / NAD + ratio to trigger axon degeneration

NMR Assays in Drug Discovery Projects

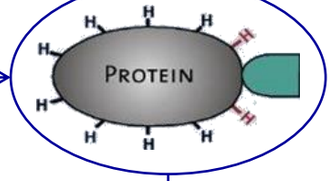
More than one option to detect ligand binding by NMR

Drug Target

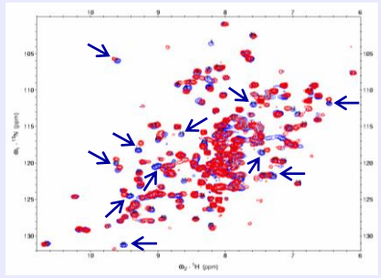




Fragment Library



Protein observed fragment screening (SAR-by-NMR)

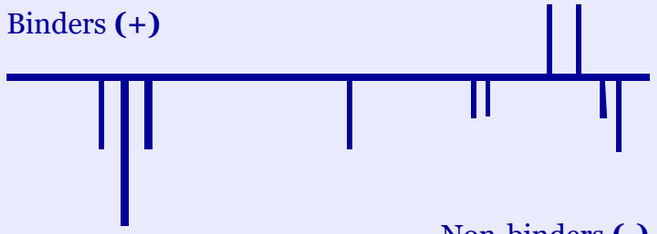


Protein alone

Protein + Fragment Hit

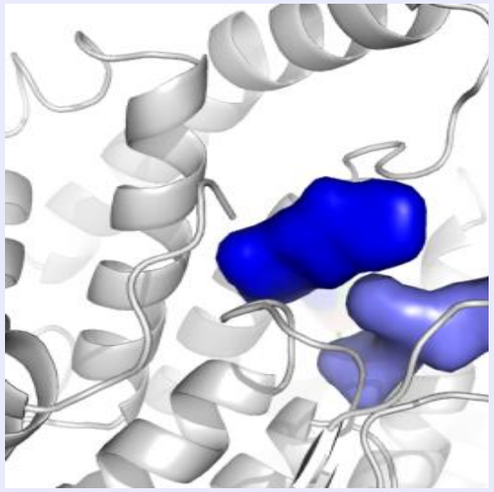
Ligand observed fragment screening
(e.g. STD-NMR, WaterLOGSY)

Binders **(+)**



Non-binders **(-)**

Fragment Hits



Ligand observed screening approaches also applicable to RNA targets.

Screening for Allosteric Site Binders

Protein observed NMR assay allows selection of relevant screening hits

5,000 fragments

NMR-based screening

33 hits

ROI analysis

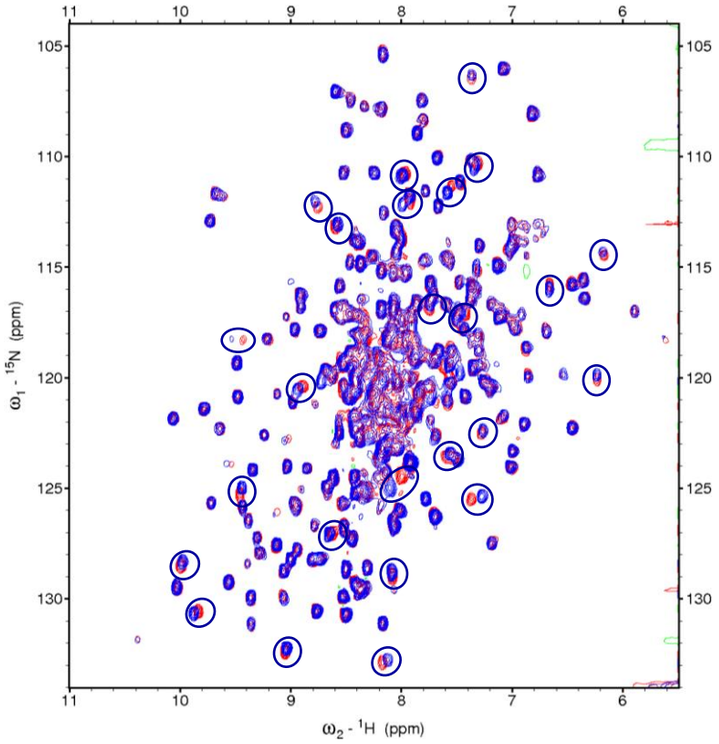
26 site-specific hits

K_D titrations

KD = 50–450 µM

Scaffolds prioritized for crystallography

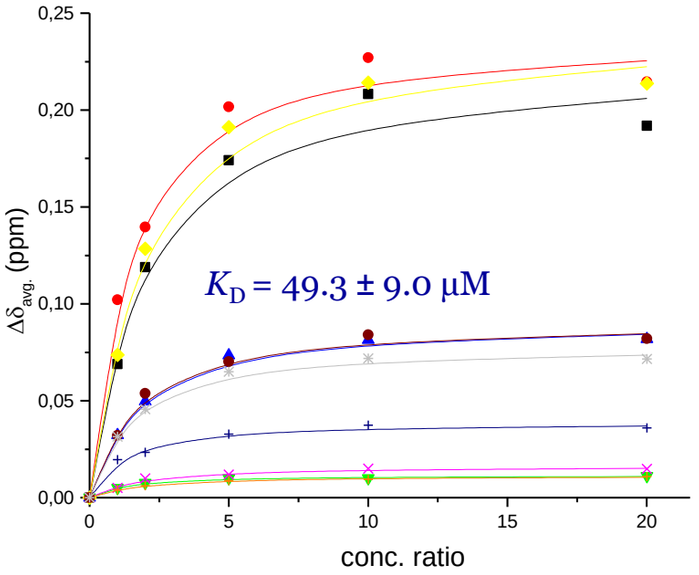
$^{1}H,^{15}N$-TROSY (40 kDa kinase)



Apo protein / Protein + Tool Compound

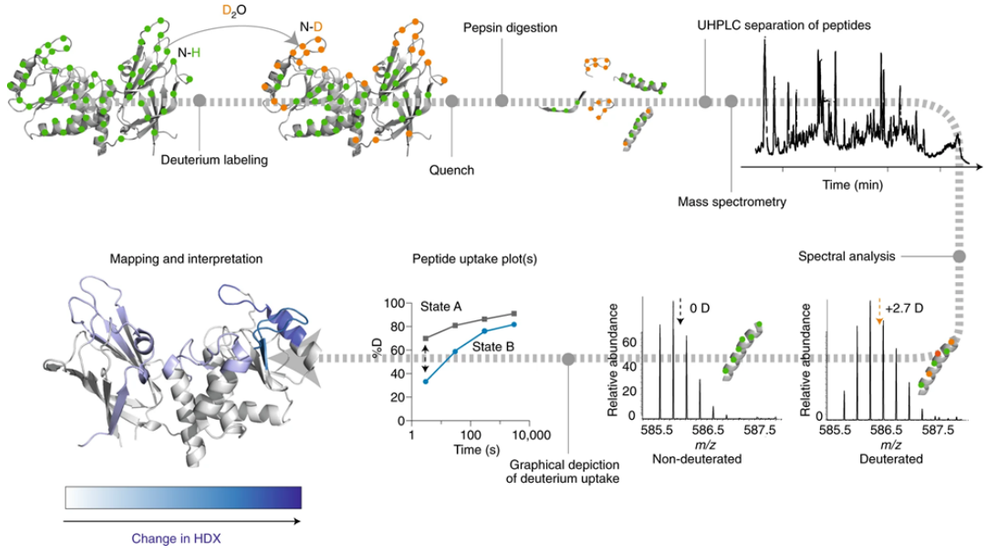
◯ = resonance perturbed by tool compound

- Desired Focus: Allosteric Binding Site
- Resonance Assignments available
- Region of interest (ROI) defined by a tool compound
- Ranking of hit potency by Kd



Hydrogen-Deuterium Exchange Mass Spectrometry (HDX-MS)

Protein dynamics, folding and interactions



- Amine hydrogens are in constant exchange with solvent hydrogens
- Using D_2O based solvents, these hydrogens can be exchanged by deuterium
- Exchange speed depends on solvent access (reduced by ligands) and can be quenched by low pH and low temperature
- Deuterium incorporation can be monitored by mass spectrometry (after pepsin digestion)

Applications

- Confirmation and localization of protein-ligand interactions (e.g. small molecules)
- Epitope mapping of antigen-antibody interactions
- Protein stability / folding studies
- Protein structure changes and protein dynamics

Full automation is enabled by

- Leap HDX-3 extended System
- UltiMate NCS-3500RS pumps
- LTQ Orbitrap Velos

Advantages

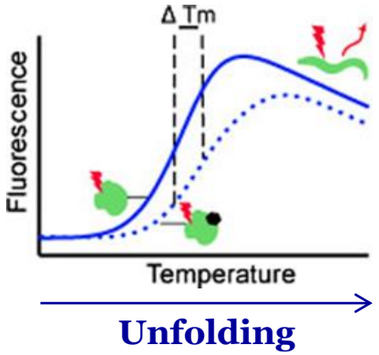
- High experimental robustness by maximized automation and fully controlled environmental conditions (chilled syringes, fluidics, column chambers)
- No intrinsic limitations regarding protein size
- Studies can be performed with native proteins in solution
- Comparatively low protein consumption

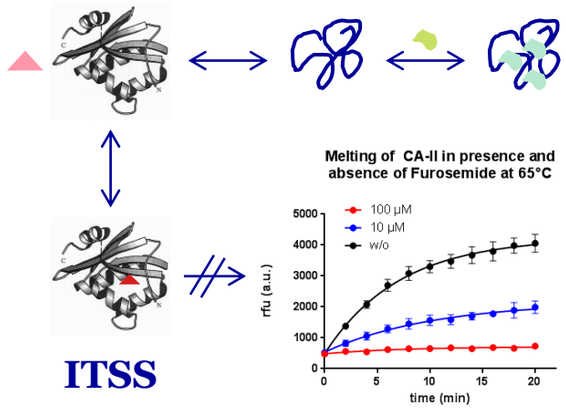
Figure from https://doi.org/10.1038/s41592-019-0459-y

Hit ID with alternative biophysical techniques

Differential Scanning Fluorimetry (DSF)

- Differential Scanning Fluorimetry for screening
  - High Throughput Screening for stabilizing ligands can have a valuable approach to directly access the underlying mode of action of a disease (e.g. pharmacological chaperones)
  - Thermodynamic effect relating changes of protein stability and potency/stoichiometry of ligand binding
  - Ligand binding can induce protein stabilization
- Both classical DSF but also Isothermal Stabilization Screening (ITSS) established to screen large compound collections
  - HT DSF in 384 well plates, fully integrated process
  - Further improved throughput by screening at constant, increased temperature = ITSS, compatible with 384 well screening
  - Data analysis by A+ or Genedata TSA / Protein Thermal Shift software
- Validity of approach demonstrated with projects delivering complex structures of identified hits from DSF screens

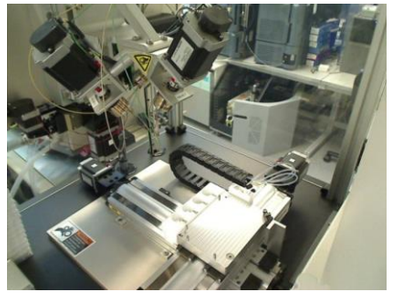




DSF as alternative approach to identify novel chemical starting points with good drug like properties

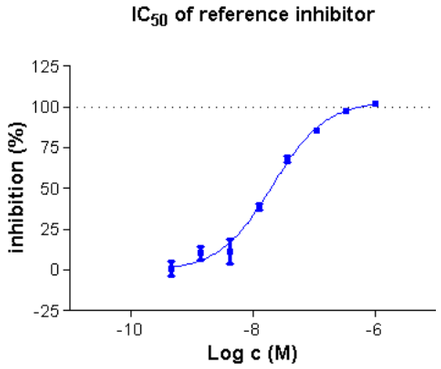
Mass Spectrometry Screening

RapidFire Screening Technology

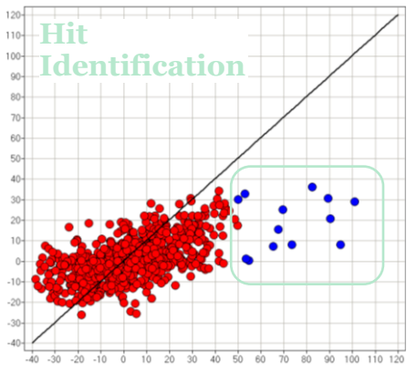


- Increasing demand for label-free screening using mass spec
- Hit identification by High Throughput MS screening
  - Mass spectrometry readout (MS/MS) on RapidFire-MS/MS
  - HTMS systems: 8 RapidFire systems available[1] enable screening of larger compound collections incl. fragments
  - Throughput: on average 7k/day/RFMS (depending of extraction time)
  - Systems fully integrated into Evotec's Lead Discovery screening platform
- Fast progression of qualified hits into early drug discovery stages enables more efficient drug discovery processes

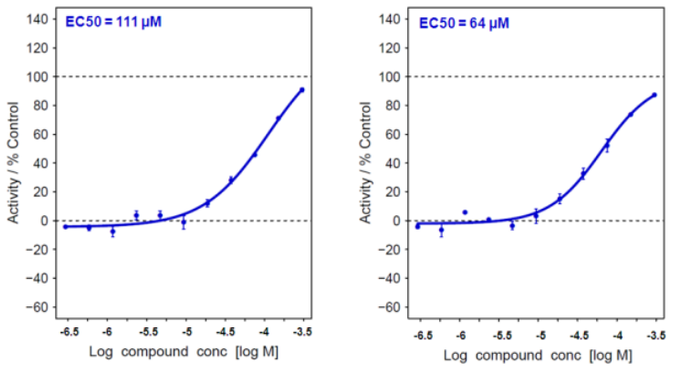
Assay development
robust, reproducible and sensitive assays



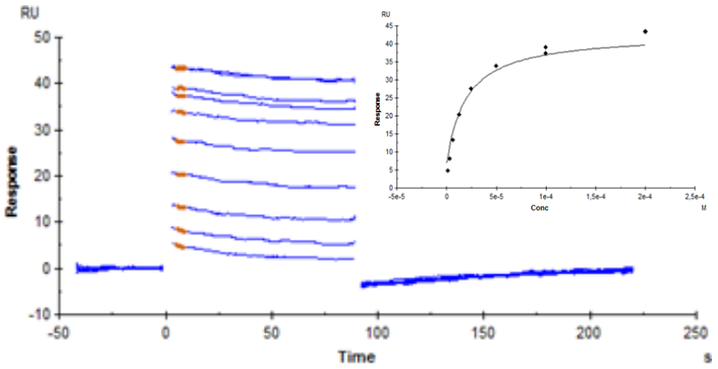
Fragment Screen on RapidFire HTMS
20K fragments at 2 conc



Hit Profiling
10pt dilution series in duplicates



Hit Validation in orthogonal assays
SPR, NMR, ITC



1 Strategic partnership with Agilent in place

HT Mass Spectrometry Screening (HTMS)

RapidFire screening

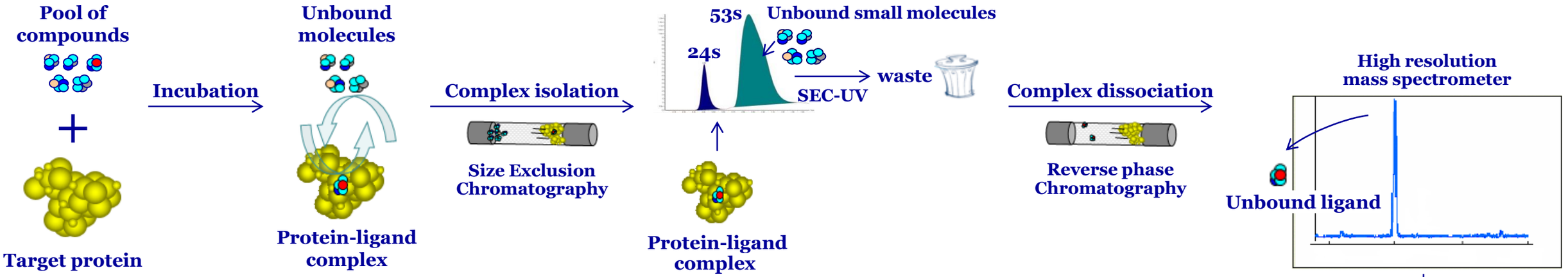
Target classes suitable for mass spec screening with demonstrated track record

- Very broad range of various indications
- Native analyte detection enabling also otherwise challenging targets

Proteases	**Kinases / Phosphatases**	**Protein Hydroxylases**
Methylases / Demethylases	**Acetylases / Deacetylases**	**Small Molecule Hydroxylases**
Transferases	**Dehydroxylases**	**Fatty Acid Amide Hydrolases**
Desaturases	**Reductases**	**Small Molecule Transporter**
Fatty Acid Elongases	**RNA Modifying Enzymes**	***… and more …***

Affinity Screening by ASMS

Principle Affinity Selection Mass Spectrometry



Target classes with demonstrated track record

Phosphatases	Nuclear receptor	Protein Hydroxylases
Secretases	Coagulation factors	Convertases
Transferases	Infectious agent proteins	TLRs
Kinases	RNAs	*... and more ...*

Agenda

1. Evotec overview
2. Evotec’s Hit ID platform
3. Assay Development and Screening Strategies
4. Summary and flexible scientific & commercial solutions

Summary of Evotec's Hit ID platform

Track record, expertise and cutting-edge technologies

1 State-of-the-art biology capabilities, capacities and scientific excellence maximize probability of success in converting biochemical and functional targets into lead generation projects and beyond

2 Our target-dependent, tailored HitID process design and workup provides partners with the highest quality chemical start points in the most cost-efficient manner

3 Expertise in molecular interaction analysis from a portfolio of biophysical and *in silico* technologies provide a fully integrated screening platform including FBDD

4 Industry-leading proteomics capabilities driven by high-end quantitative mass spectrometry incl. maximizing the chance to discover the most relevant biomarker candidates

Flexible solutions for success

Examples for drug discovery program structures

Partner	Programme	Evotec's contribution	Collaboration structure	Outcome
ucb	Hit ID, H2L and LO	HTS, *in vitro*, *in vivo* biology, chemistry, DMPK, comp. chem, compound management	Integrated collaboration *Shared risks*	H2L, late LO/pre-clinical candidates
CHDI FOUNDATION, Inc.	Hit ID, H2L and LO	HTS, *in vitro*, *in vivo* biology, chemistry, DMPK, comp. chem, compound management	Integrated collaboration *FTE-based*	H2L, Late LO/ Pre-clinical candidates
BAYER	Hit ID, H2L and LO	HTS, structural biology, *in vitro* and *in vivo* biology, medicinal and comp. chemistry, DMPK	Integrated collaboration *Shared risks*	H2L, Late LO/Pre-clinical, Phase I clinical development
Padlock THERAPEUTICS	Hit ID, H2L	HTS, structural biology, *in vitro* and *in vivo* biology, medicinal and comp. chemistry, DMPK	Integrated collaboration *FTE based, shared risks*	Development candidate, acquisition by BMS
Genentech A Member of the Roche Group	Hit ID, H2L and LO	HTS, HCS, structural biology, *in vitro* biology, electro-physiology, DMPK, proteomics	Integrated collaboration *FTE based*	2 PDC's, several joint publications
STORM THERAPEUTICS	Hit ID, H2L, LO, development	HTS, structural biology, *in vitro* biology, chemistry, DMPK, computational chemistry	Integrated collaboration *FTE-based*	Multiple H2L, LO, Phase 1 clinical study[1]

1 STORMS lead candidate STC-15 is advancing its ongoing Phase 1 study. The details of the study can be found on clinicaltrials.gov under the identifier NCT05584111.

Our commercial offering

Flexible deal structures

Individual services from our platform to help your drug discovery programmes

Agreed-upon timelines to reach goals

- Fee-for-service or FTE-based deal

STAND-ALONE SERVICES & INTEGRATED COLLABORATIONS

Integrated chemistry & biology projects from target to pre-clinic

Flexibility to adjust the make-up of the team within the scientific functions to respond to project needs & deliver upon timelines

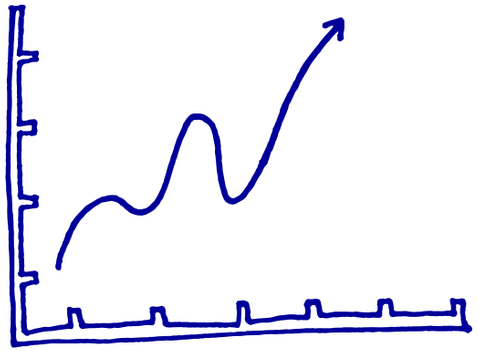
- **Traditional**
  - Fully funded FTEs / defined number of FTEs
  - Client manages resource allocation
- **Shared Risk**
  - Reduced FTE rate
  - Success-based milestones and/or royalties
- **Deliverable**
  - Fixed cost to deliver agreed-upon milestones (e.g. lead compound and pre-clinical candidate)
  - Evotec manages resource allocation

Why us?

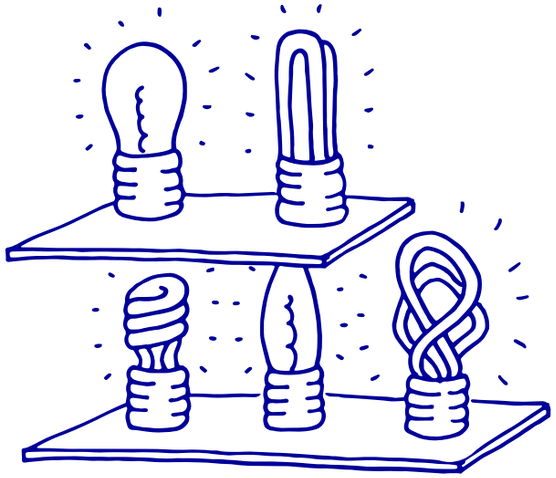
Evotec – The right choice

A track record of success means that we consistently deliver on our clients' needs

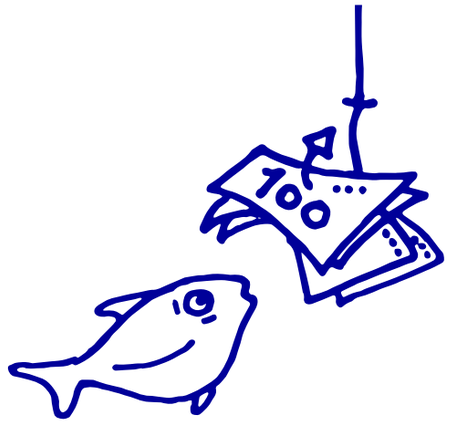
State-of-the-art capabilities and scientific excellence will maximise your chances of success



Fully integrated drug discovery platform and project management expertise will accelerate your drug discovery programme



Evotec is a low-risk outsourcing partner who is continually investing in its platform to the benefit of the customer



evotec

#RESEARCHNEVERSTOPS

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