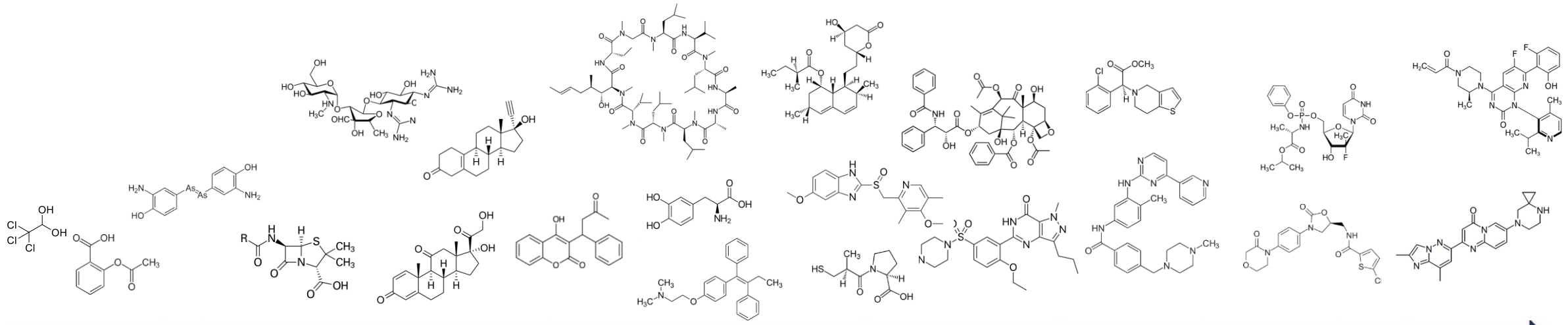
An Introduction to Chemical Biology – The art of identifying & profiling targets and molecules – an industrial perspective

Dr. Sebastian Essig

Chemical Biology, Omics, Imaging
Drug Discovery Sciences

Bayer AG

Medicinal Chemistry has a very long and successfuly history



1832 1900 1920 1940 1960 1980 2000 2020

1832 Chloral Hydrate Sleeping Drug (first synthetic drug)

1899 Aspirin (acetylsalicylic acid) NSAID, antiplatelet

1910 Salvarsan (arsphenamine) antibiotic

1941 Penicillin (penicillin) antibiotic

1946 Streptomycin (streptomycin) antibiotic

1949 Mustargen (mechlorethamine) cancer

1952 Thorazine (chlorpromazine) psychosis

1954 Coumadin (warfarin) anticoagulant

1955 Meticorten (prednisone) anti-inflam.

1958 Diuril (chlorothiazide) diuretic

1960 Enovid (NTD + MES) first "pill"

1963 Valium (diazepam) anxiolytic

1964 Lasix (furosemide) diuretic

1965 Inderal (propanolol) hypertension

1969 Ventolin (salbutamol) asthma

1973 Madopar (levodopa) Parkinson´s

1975 Novaldex (tamoxifen) cancer

1981 Capoten (captopril) hypertens.

1983 Sandimmune (ciclopsporin) immunosuppr.

1987 Mecavor (lovastatin) lipid-lowering

1987 Retrovir (AZT) anti-HIV

1988 Losec (omeprazole) gastric ulcers

1988 Prozac (fluoxetine) depression

1992 Taxol (paclitaxel) cancer

1998 Plavix (clopidogrel) antiplatelet

1998 Viagra (sildenafil) erectile dysfunction

2000 Zyvoxid (linezolid) antibiotic

2001 Gleevec (imatinib) cancer

2008 Xarelto (rivaroxaban) anticoagulant

2010 Gilenya (fingolimod) MS

2012 Farxiga (dapagliflozin) diabetes/CKD

2013 Sovaldi (sofosbuvir) anti-HCV

2015 Entresto (SAC + VAL) heart failure

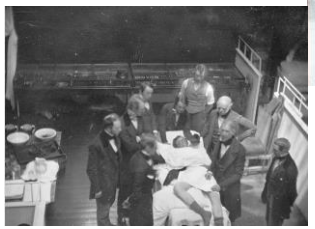
2018 Vitrakvi (larotrectinib) cancer

2020 Evrysdi (risdiplam) SMA

2021 Lumakras (sotorasib) cancer

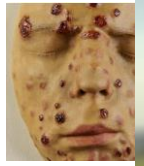
H. Beck, M. Härter, B. Haß, C. Schmeck, L. Baerfacker, *Drug Discov. Today* **2022**, *27*, 1560-1574.

Medicinal Chemistry has a huge influence on society



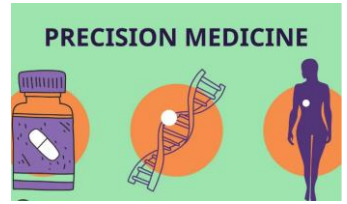
Discovery of Anesthesia

Synthetic killer



Treatment syphilli

Treatment of Orphan Diseases



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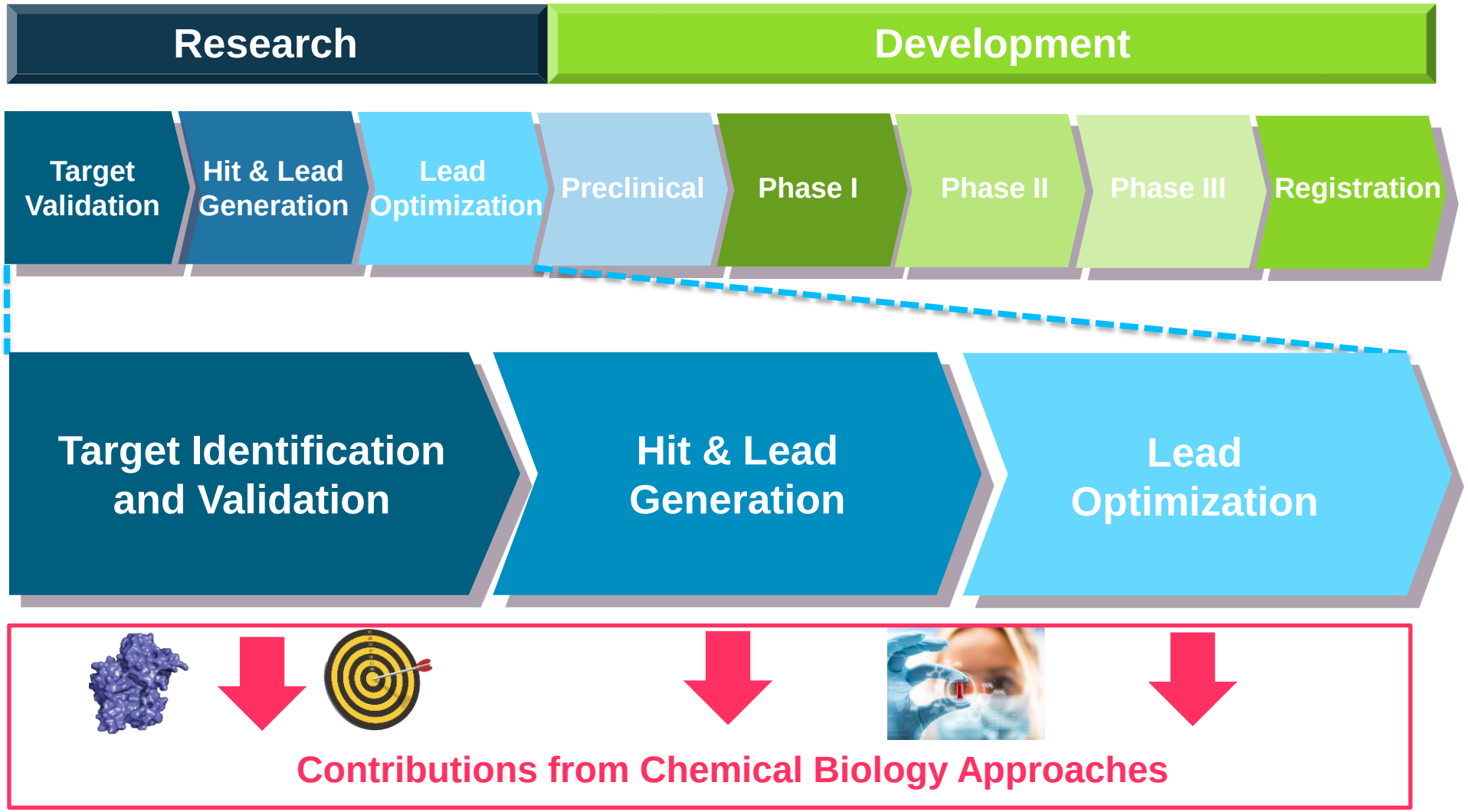
H. Beck, M. Härter, B. Haß, C. Schmeck, L. Baerfacker, *Drug Discov. Today* **2022**, *27*, 1560-1574.

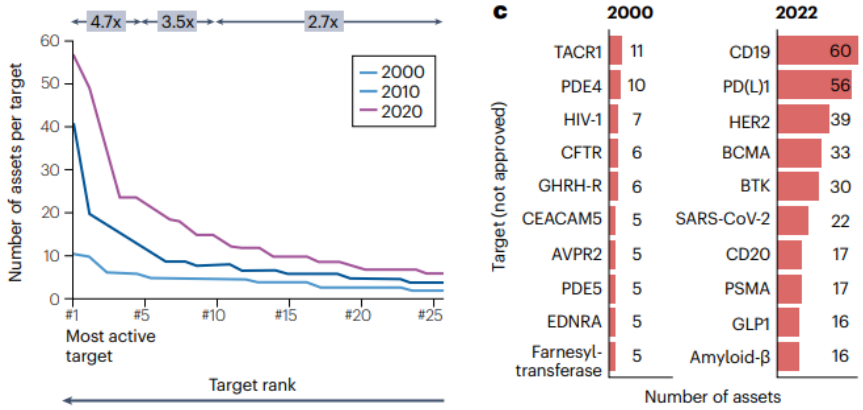
The different Stages of a Drug Discovery & Development Program



Current Challenges in Drug Discovery

Herding in the drug development pipeline

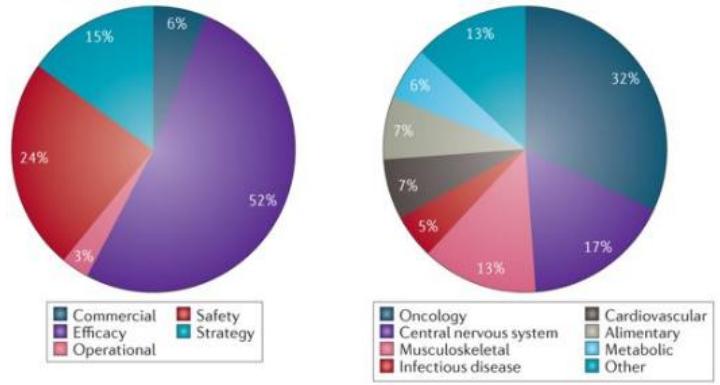
Nat. Rev. Drug Disc 2023



The number of targets has grown just 3.4% per year.

Translational failure in clinical phases

R. K. Harrison, *Nat. Rev. Drug Discov.* 2016, *15*, 817.

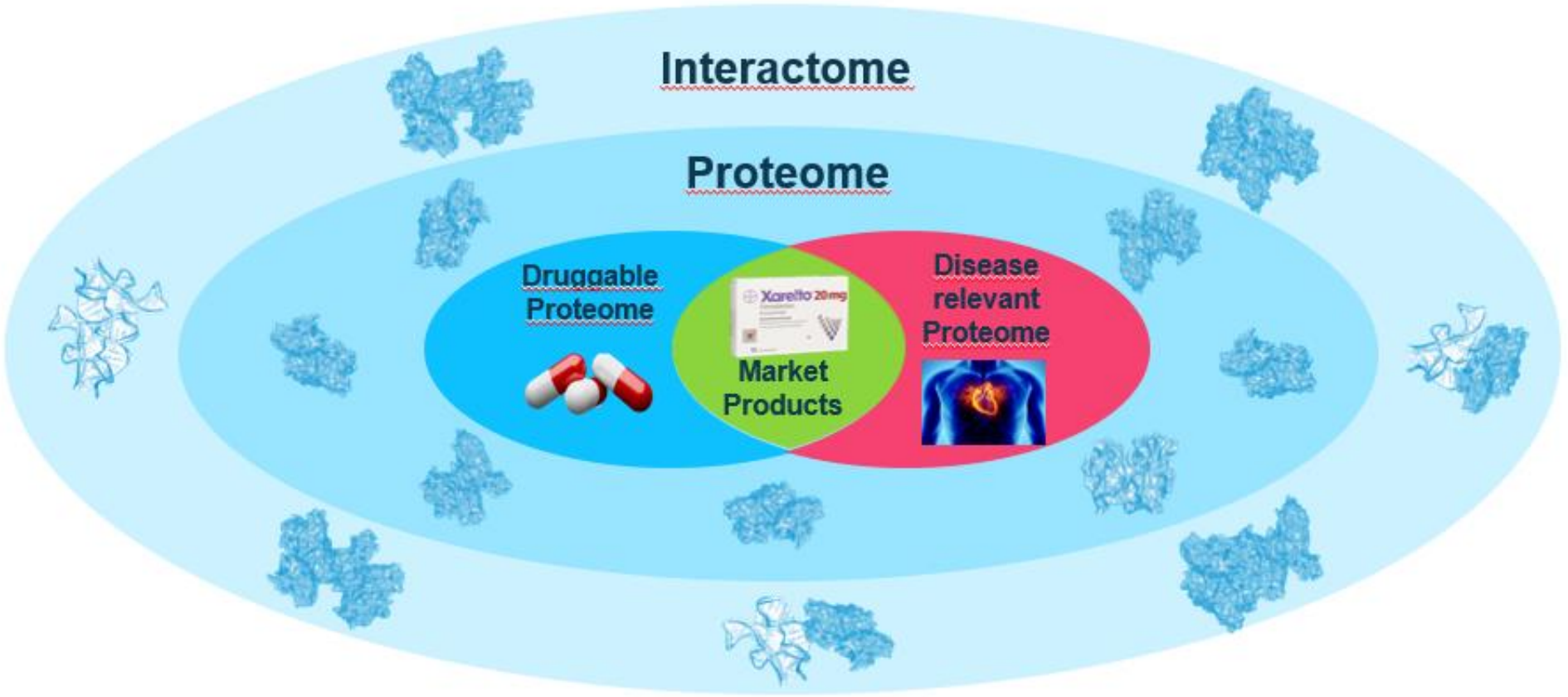


76% of clinical programs fail due to efficacy or safety.

Current Bottlenecks to increase R&D productivity: Access to robust and cost effective tools towards…

- // novel targets
- // clear understanding of target to disease link
- // choosing right targeting modalities
- // better understanding of on/off-target activity and mode of action

There is a high need to expand the druggable space



A. L. Hopkins, C. R. Groom, *Nat. Rev. Drug Discov.* **2002**, *1*, 727.

Intro Quiz Questions

How many Genes are present in the human genome?

a) 140.000

b) 20.435

c) 49.131

d) still unclear

2.) How many proteins are expressed in human cells?

a) 42.320

b) 192.917

c) 1.000000

d) 20.359

3.) How many proteins are currently druggable?

a) 4479

b) >667

c) 2282

d) not enough

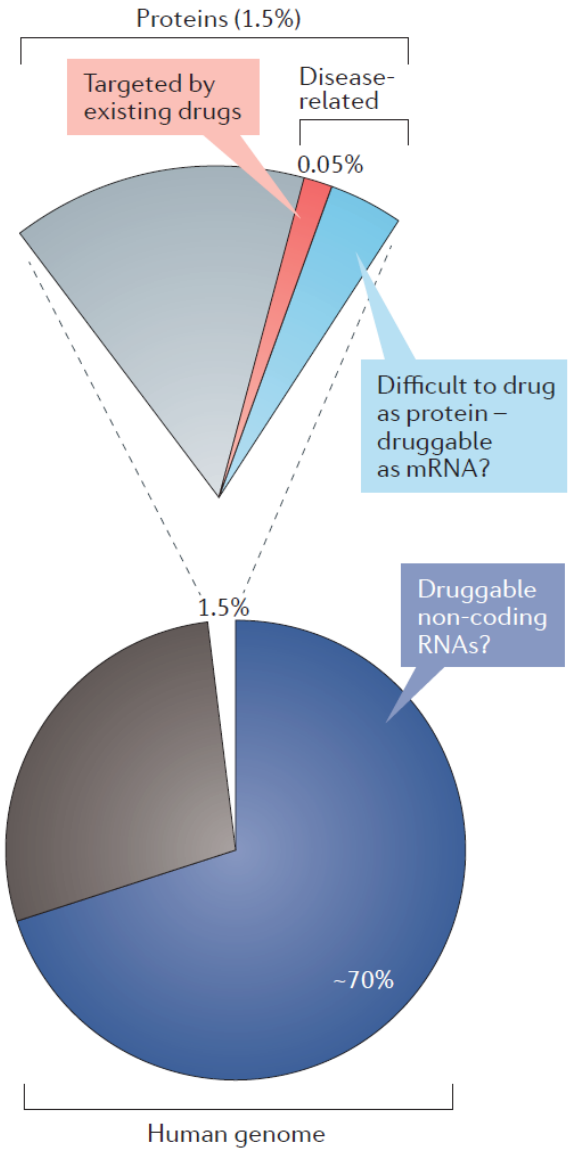
TTD: Therapeutic Target Database (idrblab.net)

Aebersold, R., Agar, J., Amster, I. *et al.*, *Nat Chem Biol* **14**, 206–214 (2018)

The druggable space is currently limited

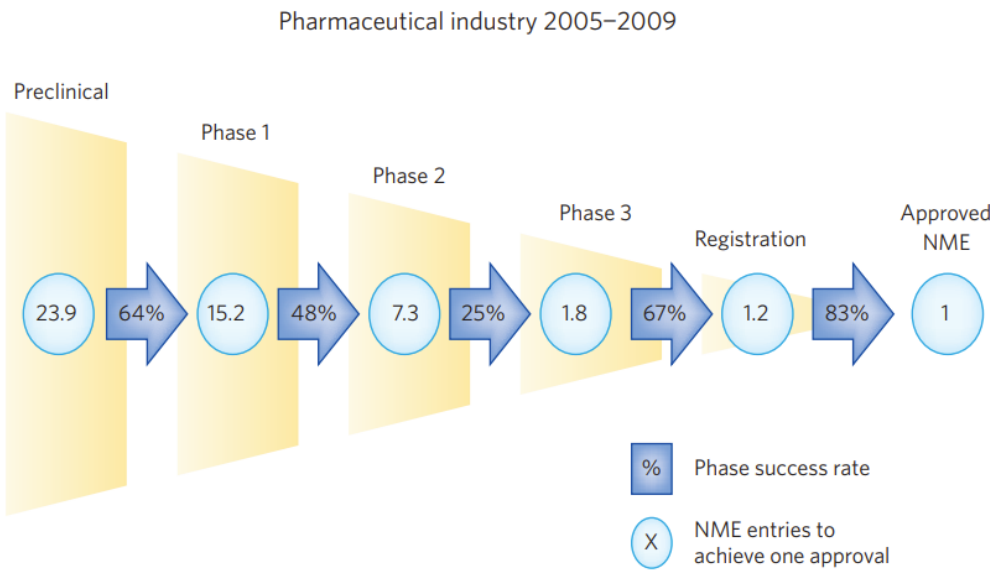
- Number of drug targets of approved drugs: **>667**
- Number of disease relevant proteins: **2000 - 3000**
- Number of druggable proteins: **4479**
- Size of the humane transcriptome: **> 200.000 transcribed RNAs**, covering about 70% of the human genome

Drug target class	Targets			Drugs		
	Total targets	Small-molecule drug targets	Biologic drug targets	Total drugs	Small molecules	Biologics
Human protein	667	549	146	1,194	999	195
Pathogen protein	189	184	7	220	215	5
Other human biomolecules	28	9	22	98	63	35
Other pathogen biomolecules	9	7	4	79	71	8



Hopkins & Groom, Nature Reviews Drug Discovery 1 (2002) 727–730; Overington et al., Nature Reviews Drug Discovery 1 (2006) 993–996; Dixon & Stockwell, Curr Opin Chem Biol 13 (2009) 549–555
Santos et al., Nature Reviews Drug Discovery 16 (2017) 19-34 ; Warner et al., Nature Reviews Drug Discovery 17 (2018) 547-558

The Big Pharma R&D Dilemma:

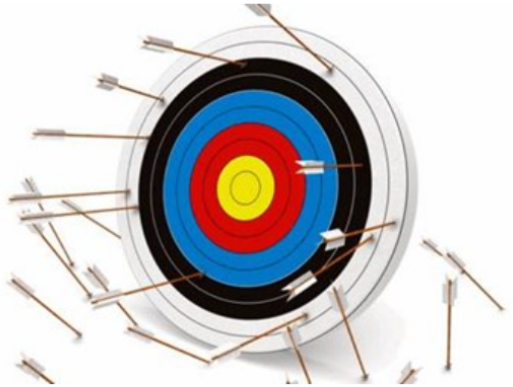


The 'big pharma' dilemma: develop new drugs or promote existing ones?

Guest Column | October 3, 2016

The High Price Of Failed Clinical Trials: Time To Rethink The Model

By Ralf Huss, MD, Chief Medical Officer, Definiens



Back in 2014, a study in Nature Biotech showed that only 32% of drugs have a probability of making it to Phase 3 trials, and only one in 10 drugs overall actually makes it to market. Things haven't improved since then.

BIO recently put out a study reporting that the average overall likelihood of approval (LOA) by FDA from Phase I was 9.6 percent – a 1 in 10 chance. The rate is even lower for major disease areas like oncology. Phase II clinical programs continue to experience low success rates as well, with only 30.7 percent of candidates advancing to Phase III, a slightly worse rate than it was a few years ago.

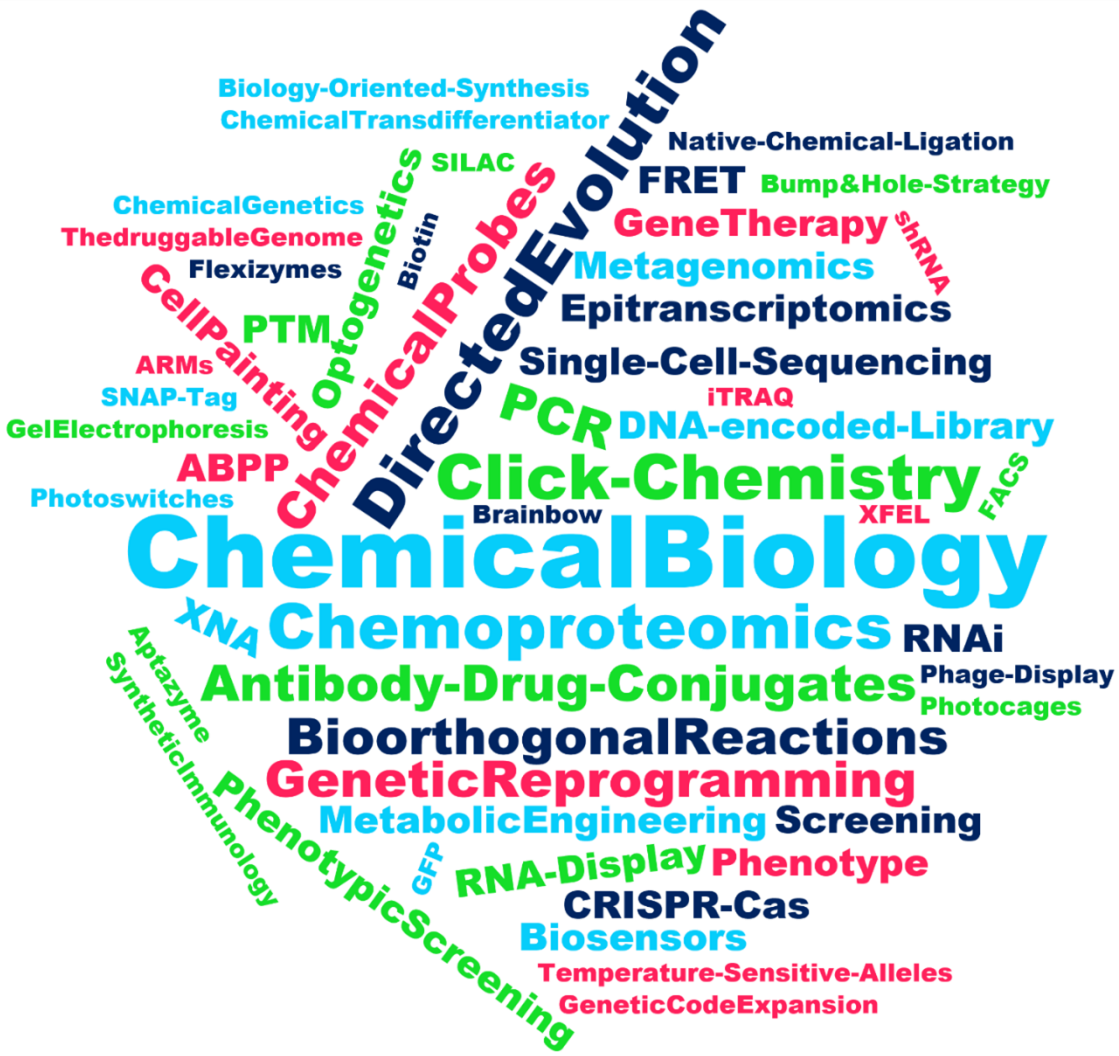
The cost of failed clinical trials is high, and the industry needs to focus on ways to reduce the

Weiss, D., Naik, P. & Weiss, R. *Nat Rev Drug Discov* **8**, 533–534 (2009)

Bunnage *et al. Nature Chemical Biology* **2011**, *7*, 335

Is there a new way to increase pharma R&D productivity?

Chemical Biology provides a modern and diverse toolbox



M. Bucci, C. Goodman, T. L. Sheppard, *Nat. Chem. Biol.* **2010**, *6*, 847-854.

Chemical Biology in Pharma Research – A new way to increase success rates

Know your target, know your molecule

Mark E Bunnage, Adam M Gilbert, Lyn H Jones & Erik C Hett

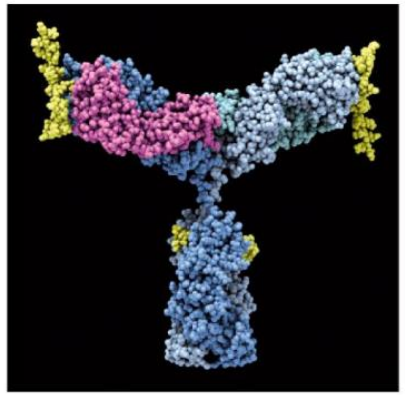
The pharmaceutical industry continues to experience significant attrition of drug candidates during phase 2 proof-of-concept clinical studies. We describe some questions about the characteristics of protein targets and small-molecule drugs that may be important to consider in drug-discovery projects and could improve prospects for future clinical success.

RSC Drug Discovery

Edited by Mark E Bunnage

New Frontiers in Chemical Biology

Enabling Drug Discovery



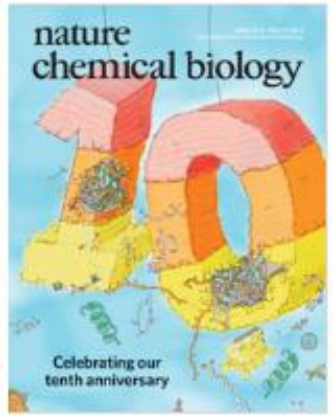
RSC Publishing

Frontiers in drug discovery

Integrating chemistry and biology in the search for the next generation of therapeutics.

A decade of chemical biology

With insights from a panel of experts, the Nature Chemical Biology editors examine the evolution and current era of chemical biology.



IN THE PIPELINE

Derek Lowe's commentary on drug discovery and the pharma industry. An editorially independent blog from the publishers of *Science Translational Medicine*. All content is Derek's own, and he does not in any way speak for his employer.

By Derek Lowe

ANALYTICAL CHEMISTRY

Chemical Biology – The Future?

By Derek Lowe | 23 September, 2010

nature chemical biology

EDITORIAL

An emerging role for chemical biology

Chemical Biology is currently an important topic in the Pharmaceutical Industry

NOVARTIS

CB Culture: High-profile CB-Scientist as Boss

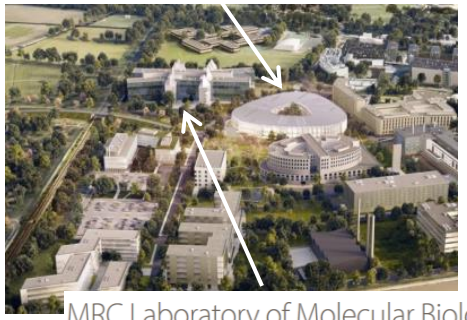
Former President of the Novartis Institutes for BioMedical Research (NIBR), Head of research @ Amgen

Chemical Biology at NIBR

Our Approach to Early Research and Discovery

We organize our early discovery efforts around a scientific discipline called chemical biology, which combines biology, chemistry and computer science.

AstraZeneca



MRC Laboratory of Molecular Biology

Right target

- Strong link between target and disease
- Differentiated efficacy
- Available and predictive biomarkers

target as epicenter @ 5 R Strategy

MERCK

INVENTING FOR LIFE

Merck & Co. Opens Cambridge Exploratory Science Center, Plans to Hire 30 More Employees

Education Minimum Requirement:

- PhD in Chemistry and a minimum of 7 years of experience in chemical biology /medicinal/synthetic chemistry

Required Experience and Skills:

- Prior experience in chemical biology
- Chemical Biology and drug discovery knowledge and experience

Thursday - August 5, 2021

BAYER

Bayer strengthens drug discovery platform through acquisition of Vividion Therapeutics

VIVIDION Therapeutics

P. Morgan et al., *Nat. Rev. Drug Discov.* **2018**, *17*, 167-181.

How can Chemical Biology be defined?

The Historical Roots of Chemical Biology

THE BIRTH OF CHEMICAL BIOLOGY.

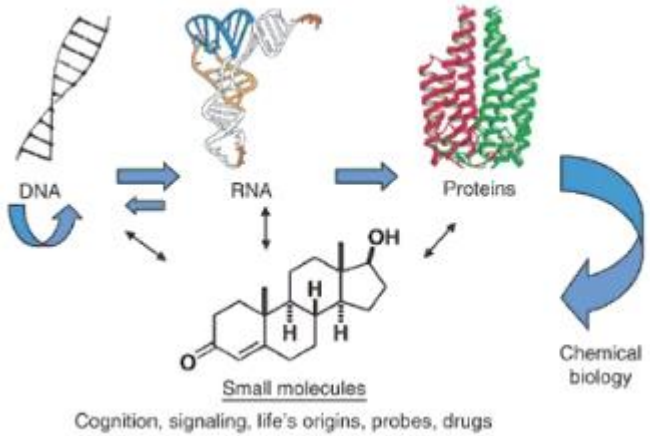
The Harveian Oration delivered before the Royal College of Physicians of London on Oct. 18th, 1930,

BY J. B. LEATHES, M.B. OXF., F.R.S.,

FELLOW OF THE COLLEGE; PROFESSOR OF PHYSIOLOGY IN THE UNIVERSITY OF SHEFFIELD.

at the birth of biological chemistry, a science to whicl chemistry owes as much as biology, and without which chemistry would have but three legs to stand on. If the ideas in his work did not all originate

J. B. Leathes, *The Lancet* **1930**, *216*, 889-895.



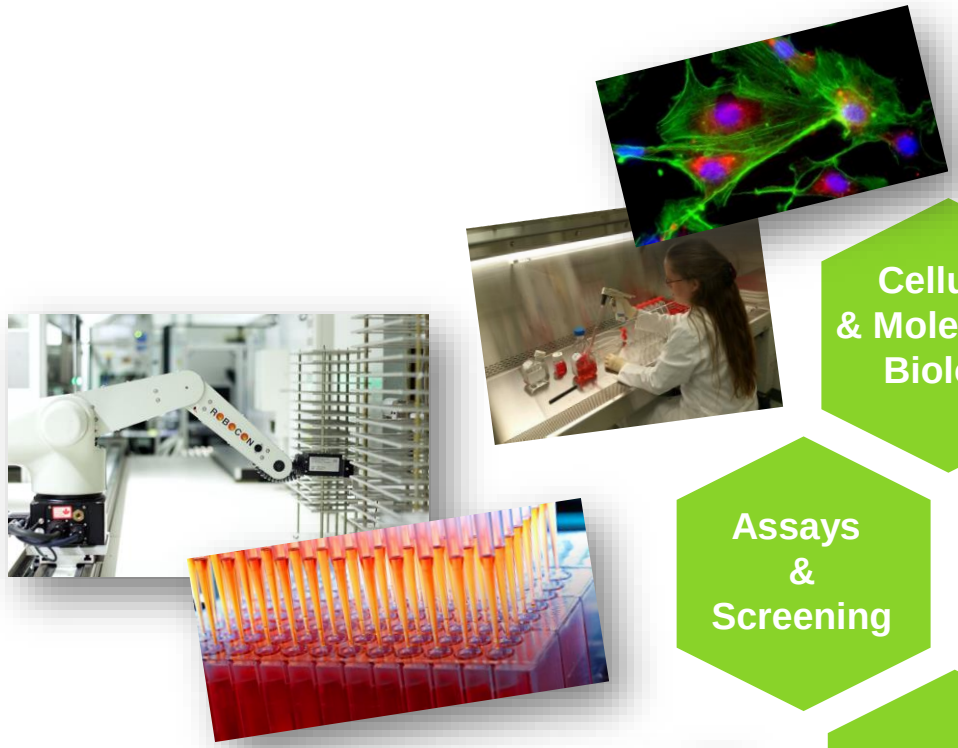
Small molecules: the missing link in the central dogma

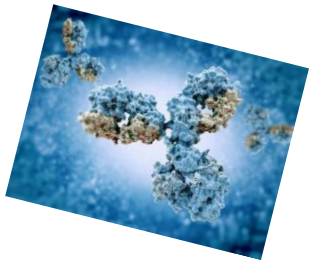
Schreiber, S. *Nat Chem Biol* **1**, 64–66 (2005)

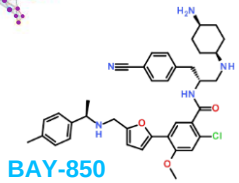
A Personal Definition:

Design of Synthetic Probes &
Technological Tools to Identify & Study
Novel Druggable Mode of Actions

Chemical Biology: An Interdisciplinary Research Approach







Cellular & Molecular Biology

Bioinformatics & Datascience

Assays & Screening

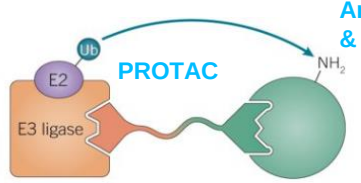
Chemical Biology

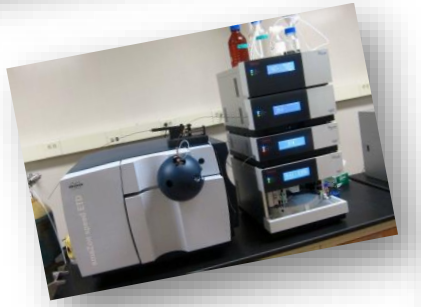
Medicinal Chemistry & Modalities

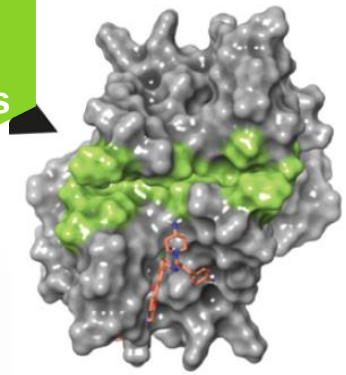
Proteomics & Genomics

Structural Biology & Biophysics

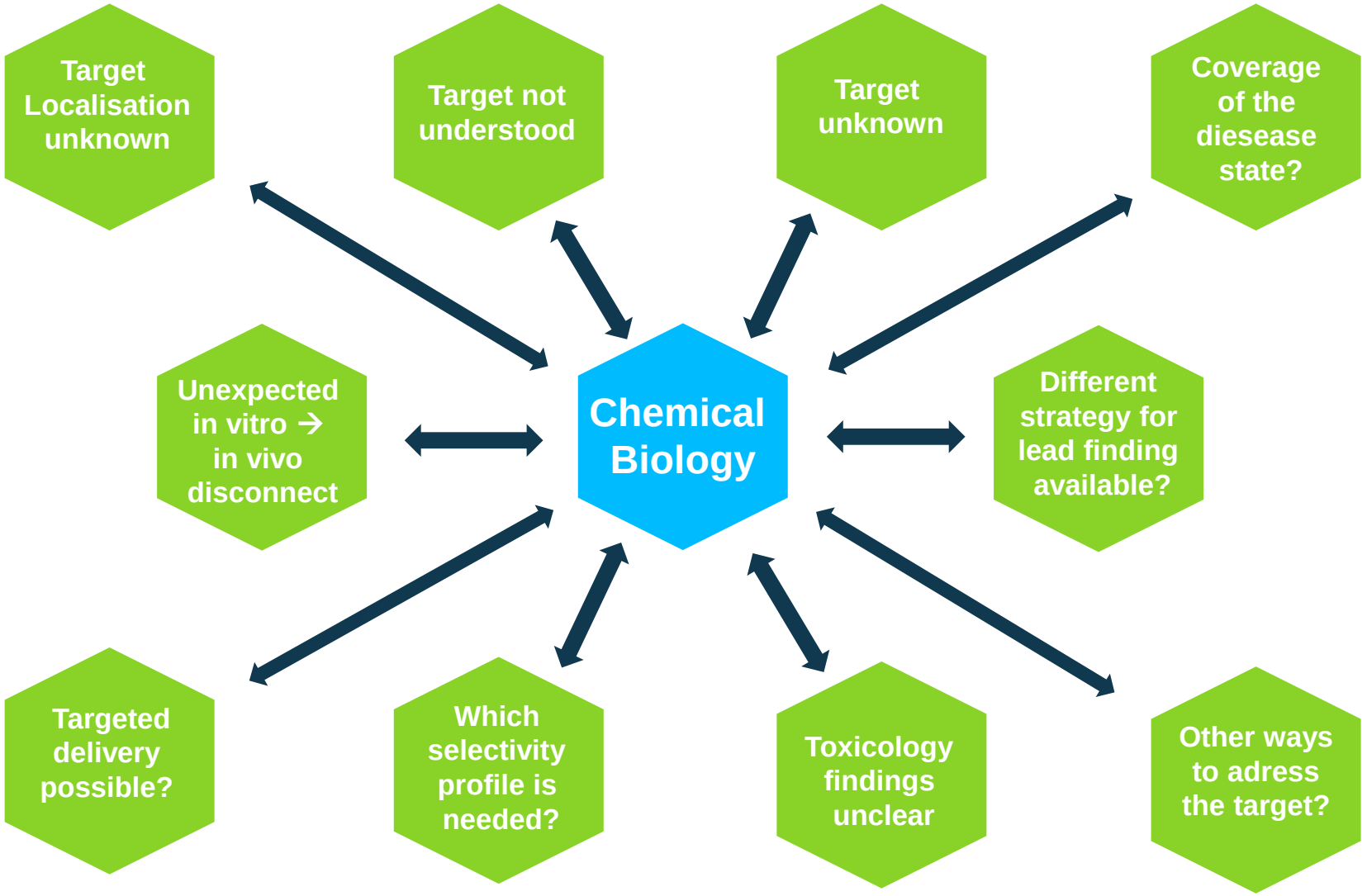
Antibodies, Proteins & Peptides







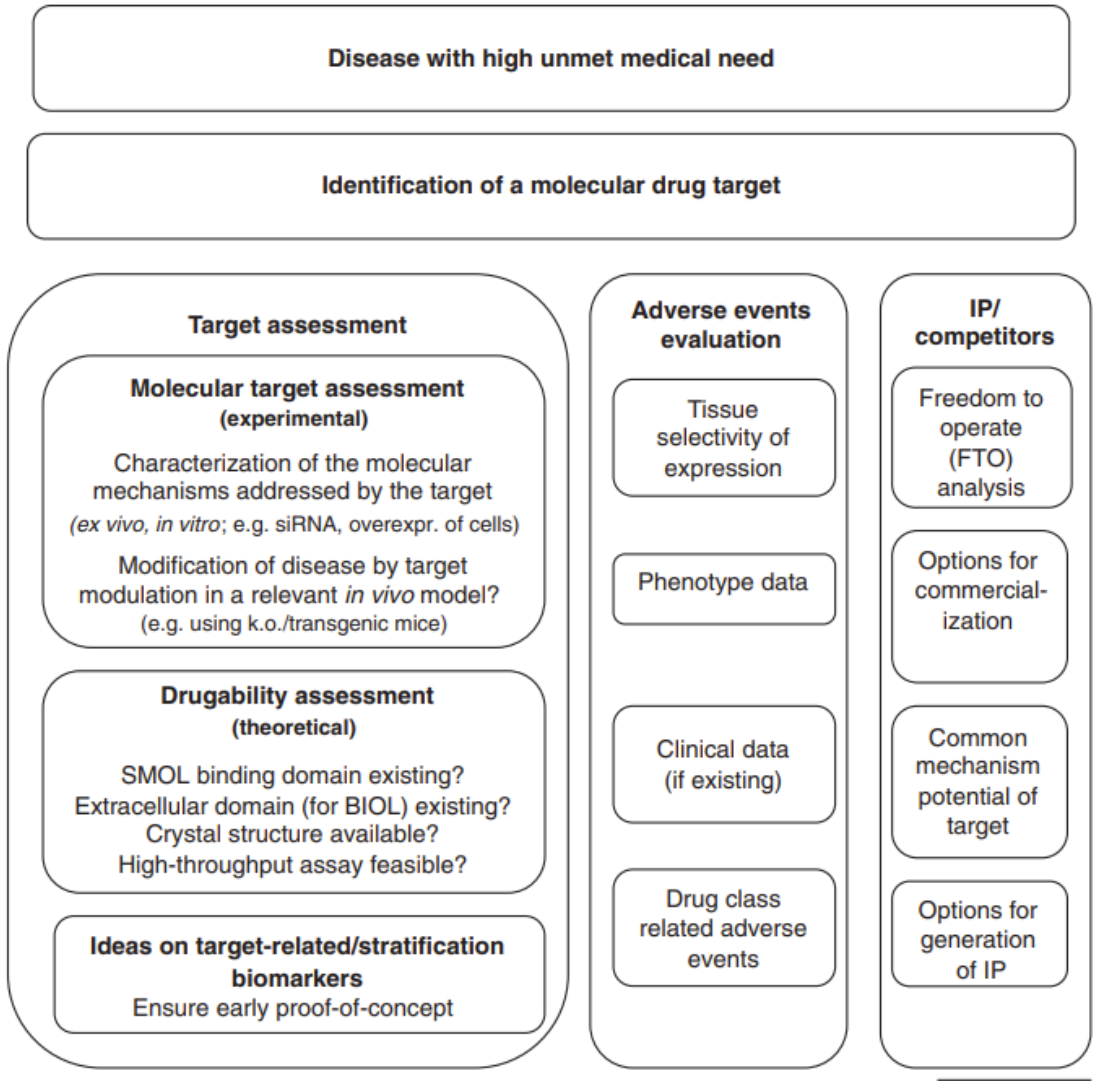
Chemical Biology Questions – What can you do with the toolbox???



One application for Chemical Biology tools: Target Identification

What makes a good drug target?

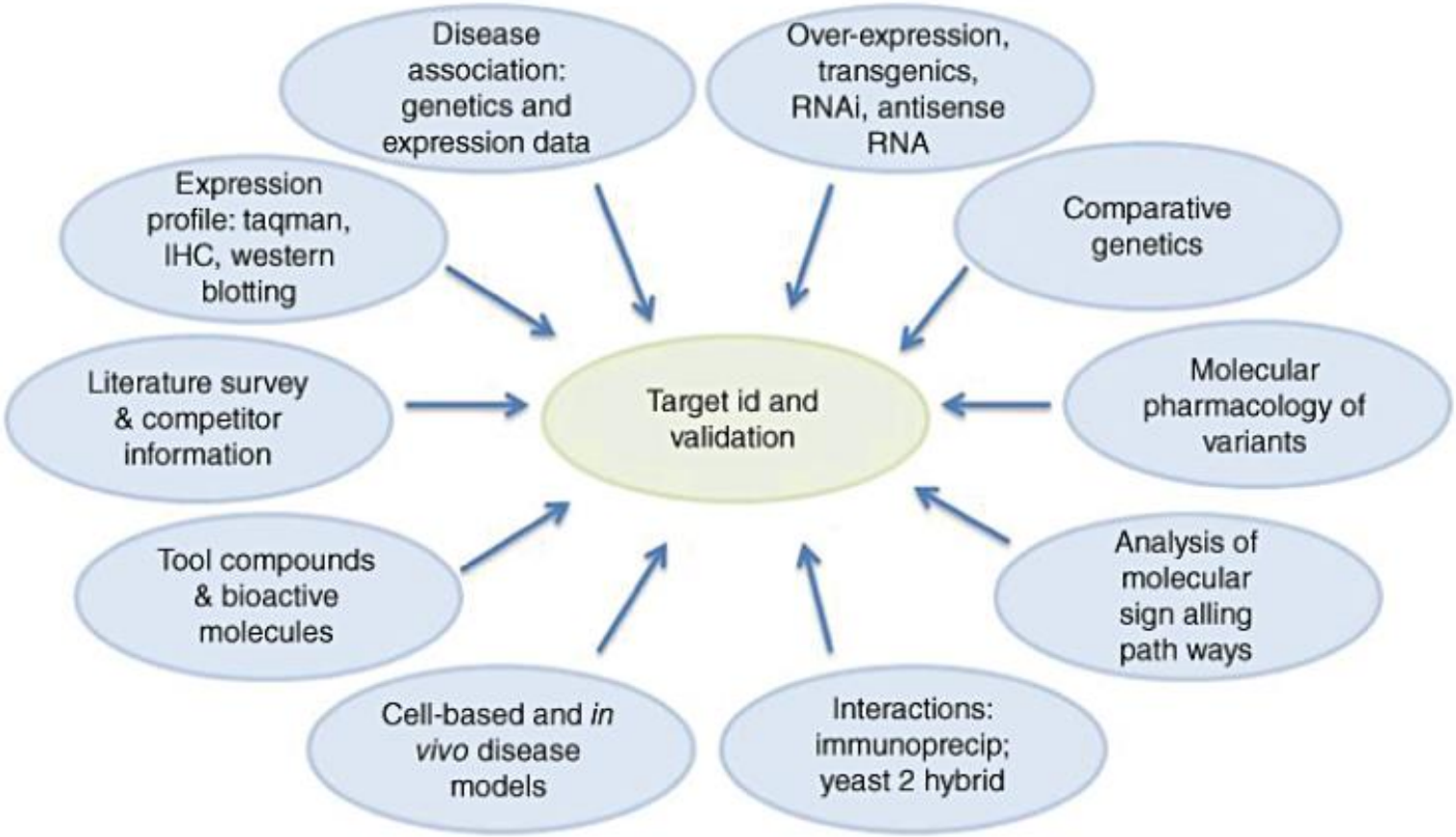
Criteria for a good drug target are complex and connected



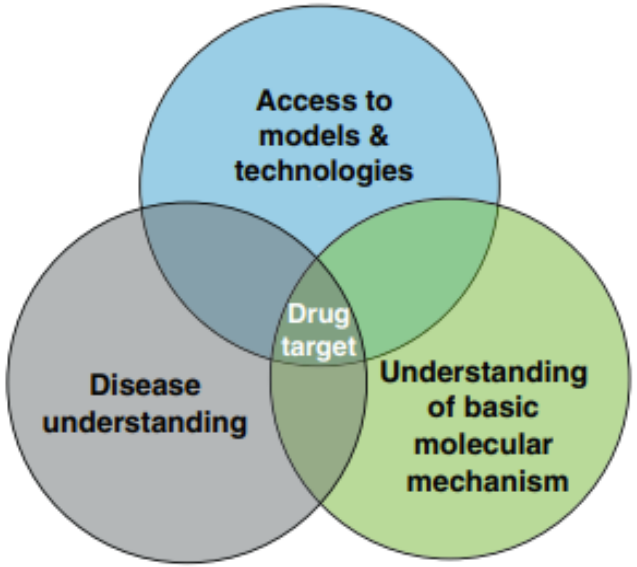
I. Gashaw, P. Ellinghaus, A. Sommer, K. Asadullah, *Drug Discovery Today*,*16*, 23–24, **2011**,1037-1043.

The option space for target identification is increasing:

There are multiple sources to identify a drug target:

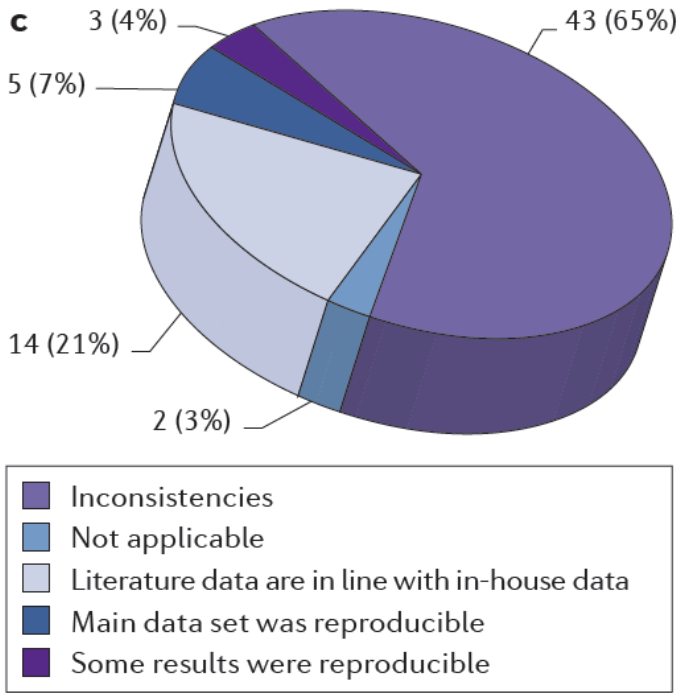


The Drug target space expansion depends on:



Hughes, J., Rees, S., Kalindjian, S. and Philpott, K. (2011), British Journal of Pharmacology, 1239-1249.
I. Gashaw, P. Ellinghaus, A. Sommer, K. Asadullah, *Drug Discovery Today*,*16*, 23–24, **2011**,1037-1043.

How good is the academic literature as a source for drug targets?



Bayer study:

67 projects investigated (70% from oncology)
In **2/3 of the investigated projects inconsistencies between published and in-house data**

Amgen study:

Data from 53 landmark papers reproduced internally
Scientific findings were confirmed in 11% of cases

Many industrial drug discovery projects **start from literature reports on new targets**

A lack of reproducibility of published data causes severe problems

F. Prinz *et al. Nat. Rev. Drug Disc.* **2011**, *10*, 712. C. G. Begley *et al. Nature* **2012**, *483*, 531.
M. Baker & D. Penny *Nature* **2016**, *454*, 452. William G. Kaelin *Nat. Rev. Canc.* **2017**, *17*, 425.

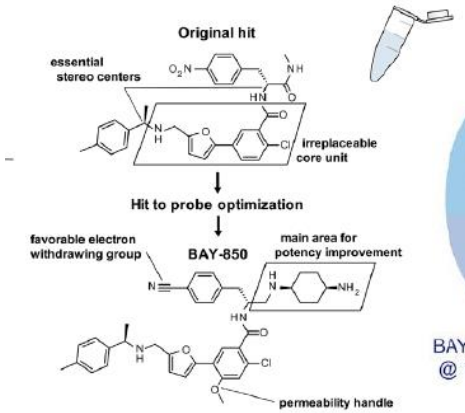
Many academic authors are also ignoring target validation work from industry

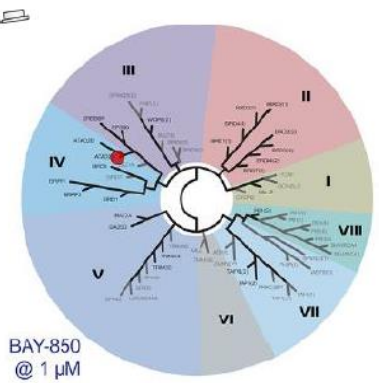
Integrated Genomic Analysis of the 8q24 Amplification in Endometrial Cancers Identifies *ATAD2* as Essential to *MYC*-Dependent Cancers

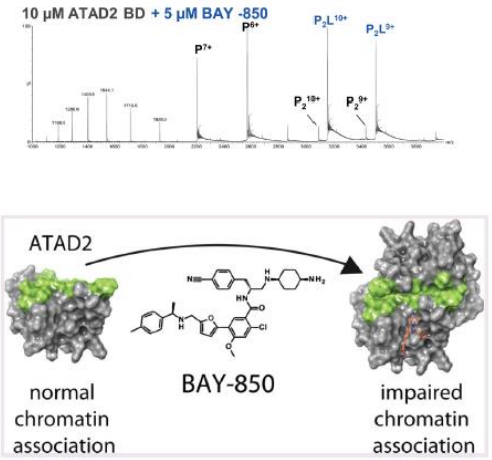
Maria B. Raeder, Even Birkeland, Jone Trovik, Camilla Krakstad, Shyemaa Shehata, Steven Schumacher, Travis I. Zack, Antje Krohn, Henrica MJ. Werner, Susan E. Moody, Elisabeth Wik, Ingunn M. Stefansson, Frederik Holst, [...], Helga B. Salvesen

[view all]

Published: February 5, 2013 • https://doi.org/10.1371/journal.pone.0054873







ATAD2 is a driver and a therapeutic target in ovarian cancer that functions by upregulating CENPE

Praveen Guruvaiah, Suresh Chava, Chiao-Wang Sun, Nirupama Singh, Courtney A. Penn & Romi Gupta

Cell Death & Disease **14**, Article number: 456 (2023) | Cite this article

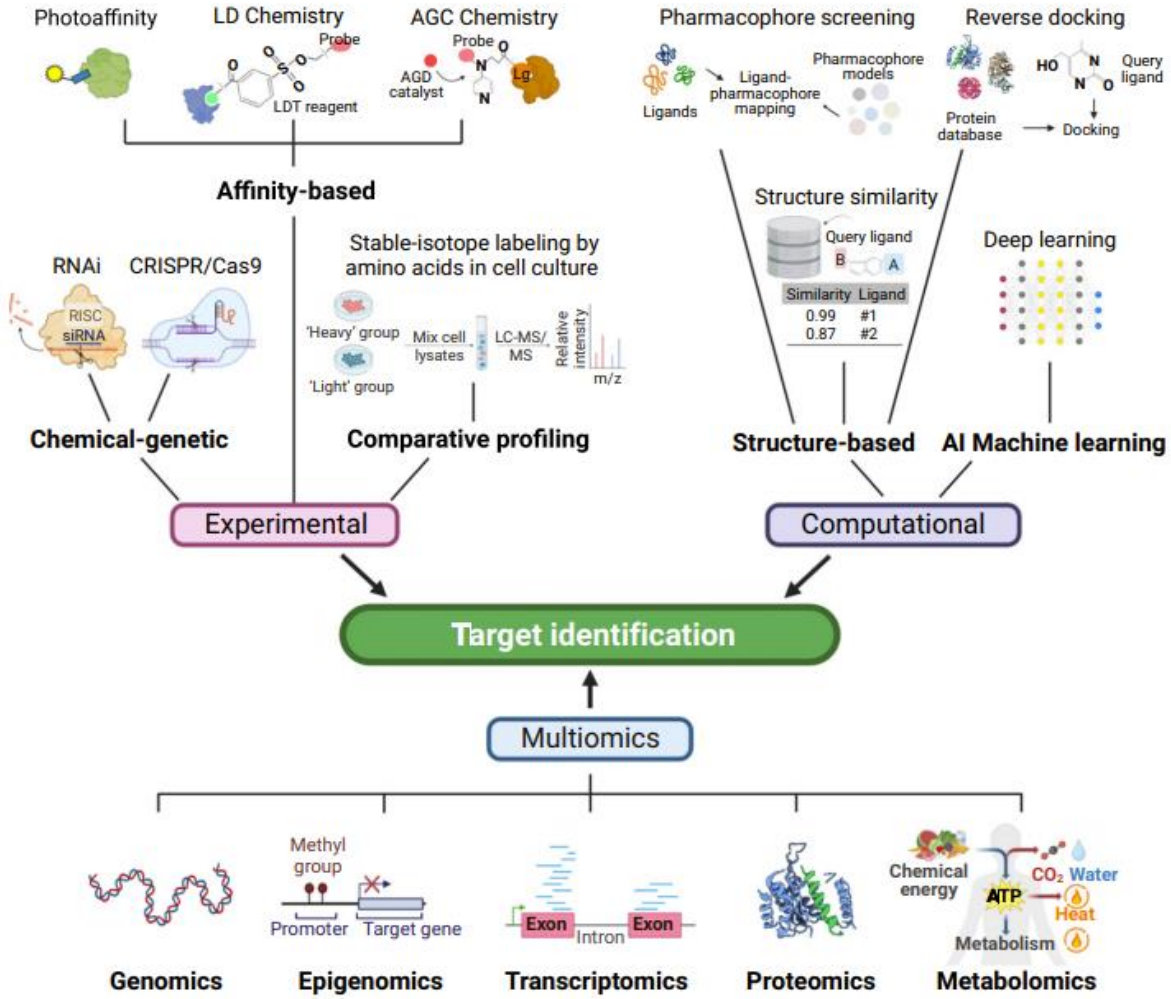
1118 Accesses | **1** Citations | **6** Altmetric | Metrics

active on the nontransformed and cancer cells (Figure4E). Thus, we concluded that the cytotoxic effects displayed by BAY-850 cannot be unmistakably linked to ATAD2 BD inhibition. Further evidence for a disconnect between the observed growth inhibition and the inhibition of ATAD2 BD was provided by gene expression studies, in which BAY-850 treatment did not affect the expression of some of the previously identified ATAD2 target genes[11,12] (Supporting Information Figure 7). Consistently, other recently published ATAD2 BD inhibitors also failed to demonstrate significant effects on target gene expression and cancer cell survival below 20 µM despite engaging ATAD2 BD in living cells.[9] Potential

ACS Chem. Biol. 2017, 12, 11, 2730–2736.

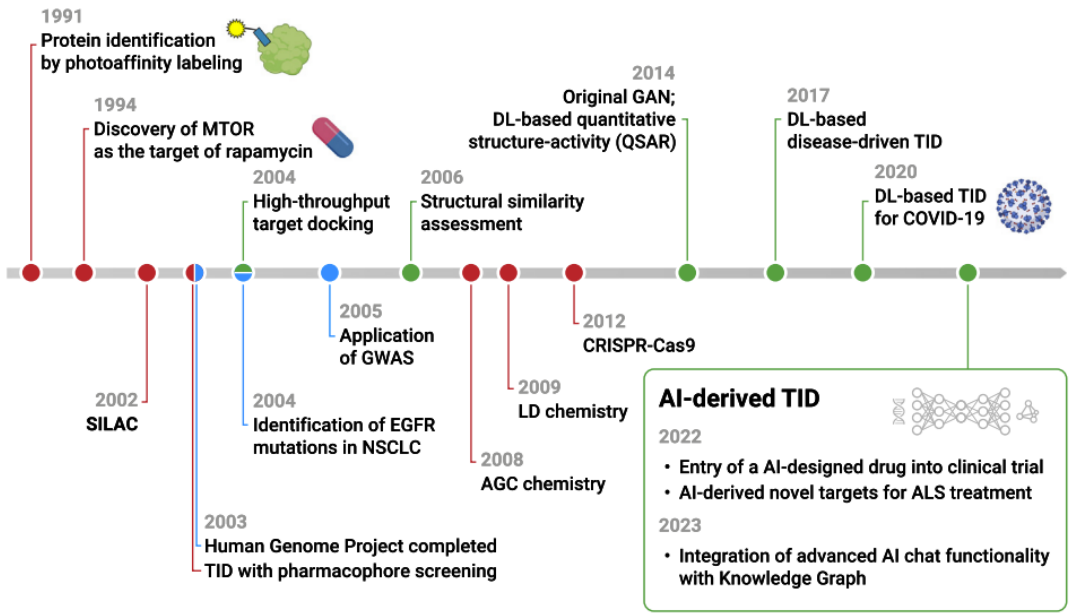
Are there modern ways to find drug targets?

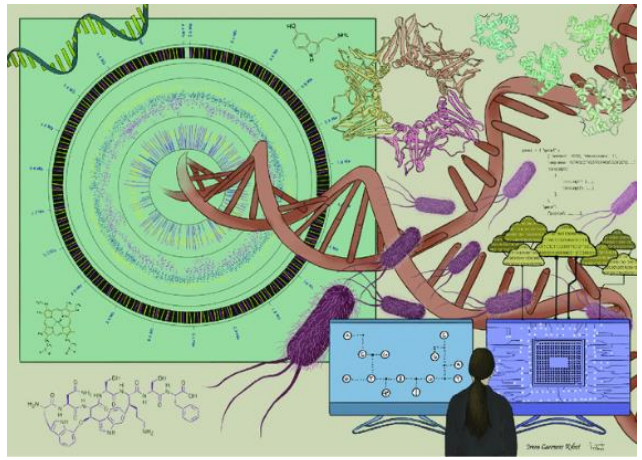
Modern Chemical Biology tools offer a flexible for Target Discovery

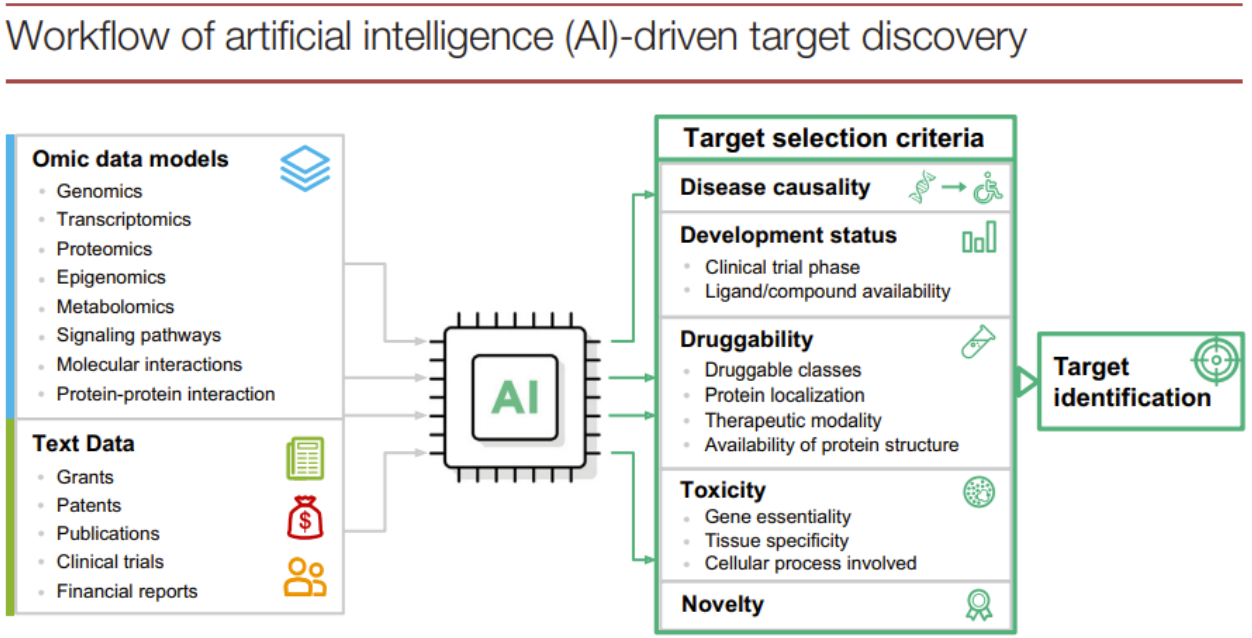


Pun, Frank W. et al. Trends in Pharmacological Sciences, 44, 561 – 572.

AI might play an important role to deal with the complexity of PhenOMICs data



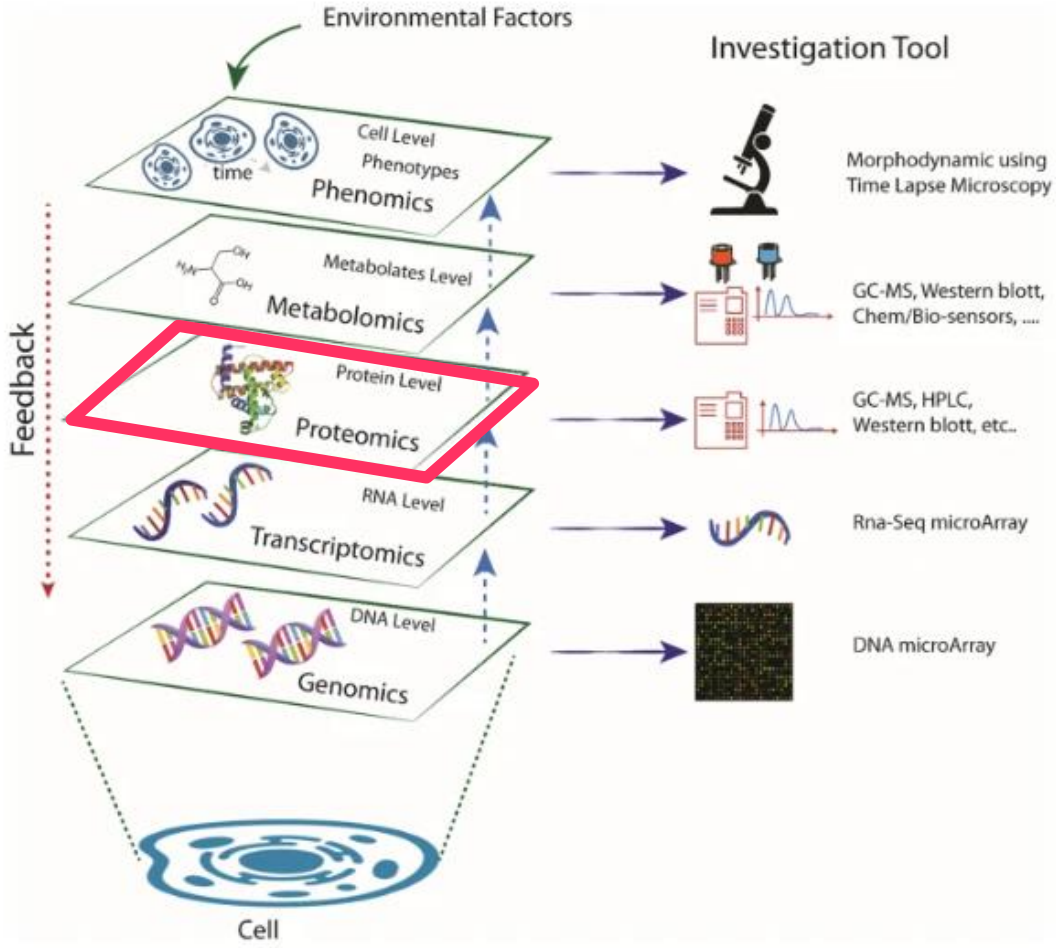




Pun, Frank W. et al. Trends in Pharmacological Sciences, 44, 561 – 572.

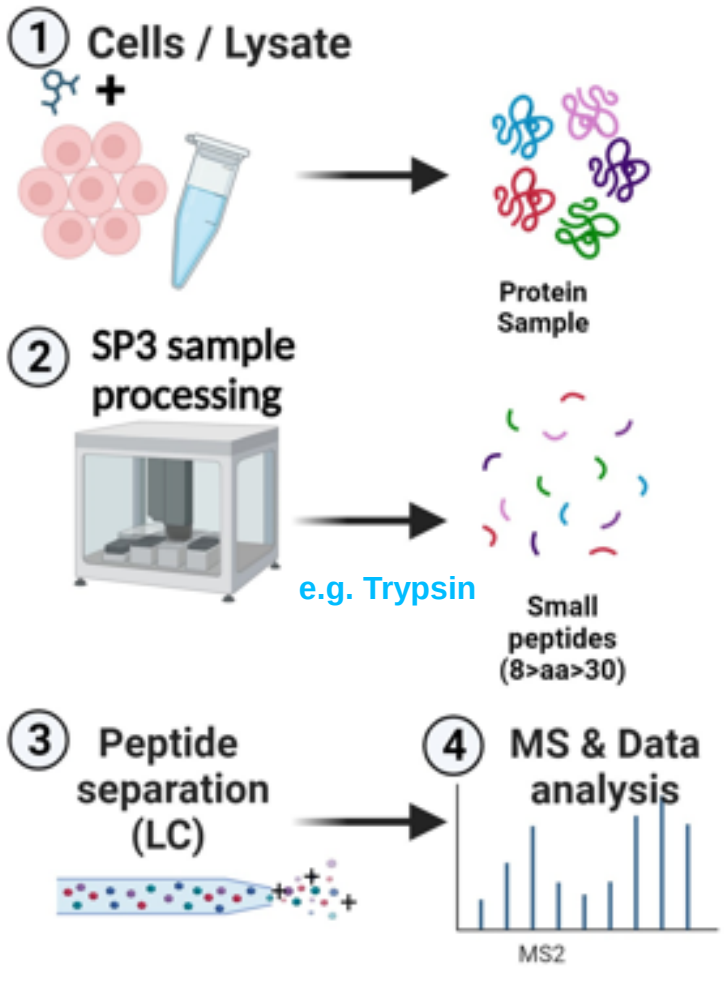
Overview PhenOMICs tools - Use case example Proteomics

„Analysis of genetic, disease associated and/or modality mediated effects on relevant biological systems with image-based, functional genomics, sequencing and mass spectrometric methods”

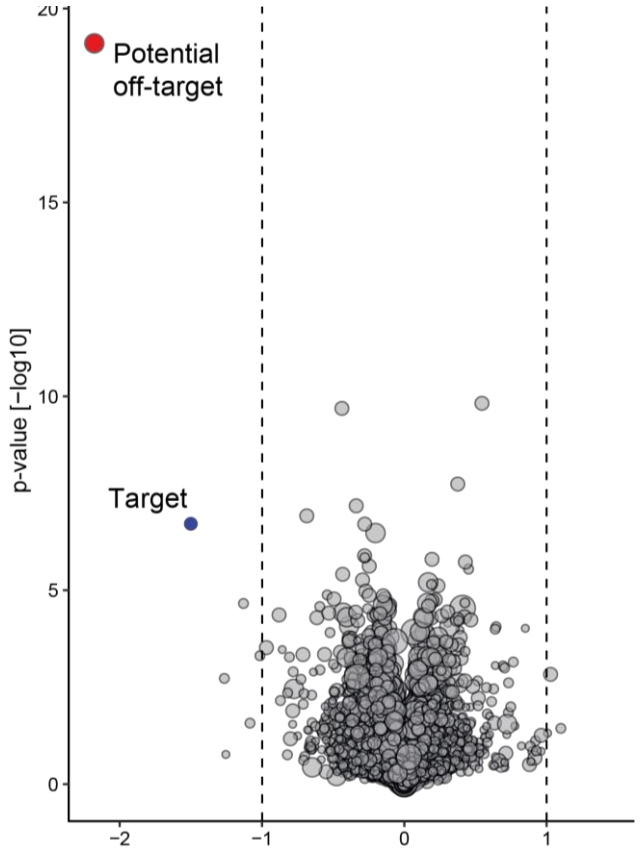


D’Orazio, M., Murdocca, M., Mencattini, A. *et al. Sci Rep* **12**, 8545 (2022).

PhenOMICs Tools – Overview Proteomics



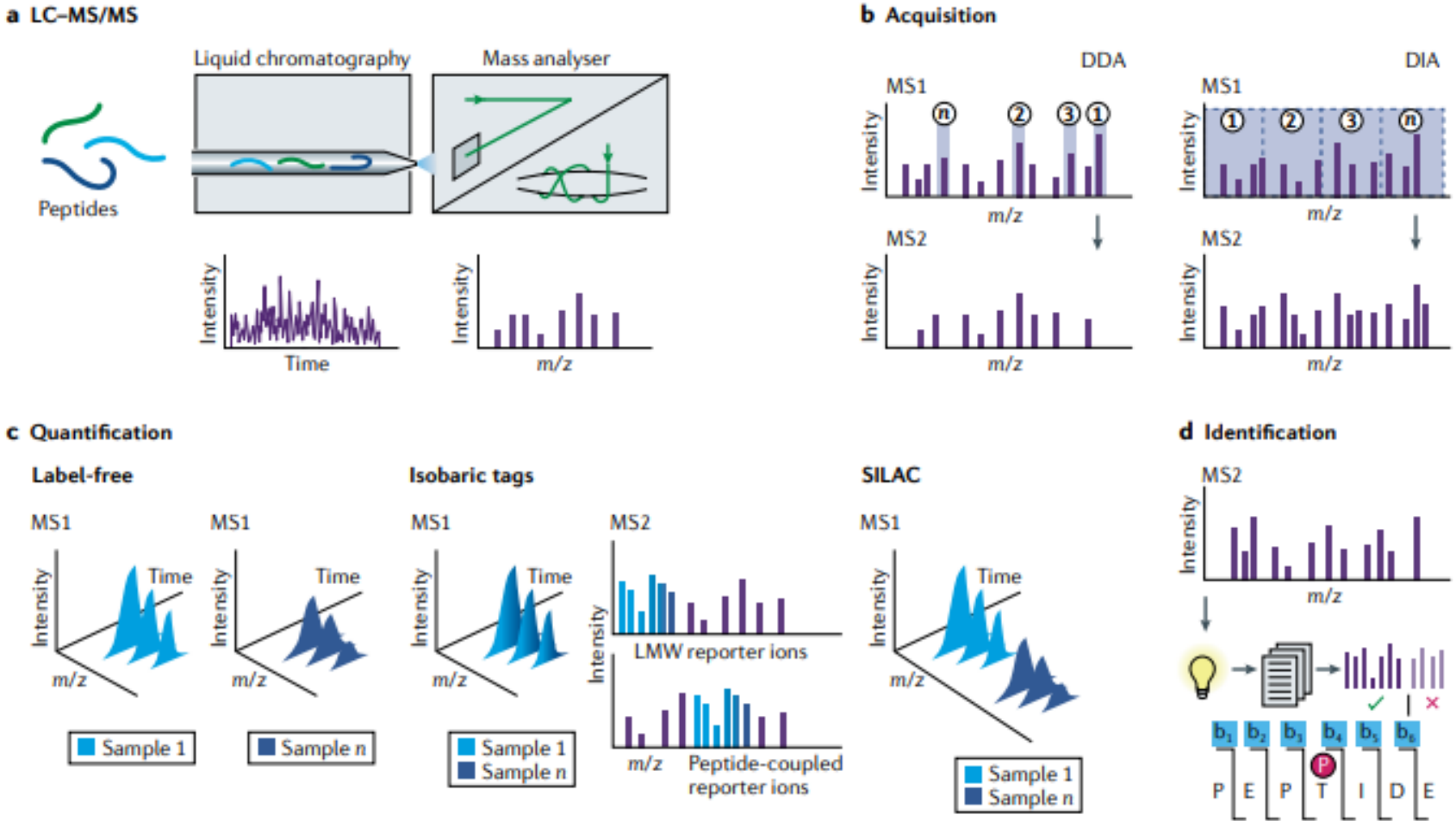
Data representation: Vulcano-Plot



Downregulation or degradation

Upregulation or stabilization

Proteomics workflows and their technological improvements



Vocabulary:

DDA: Data Dependend Acquisition

→ Fragmentation of the most intense

→ peptide mode in MS1

DIA: Data independent acquisition

→ Fragmentation of all peptide

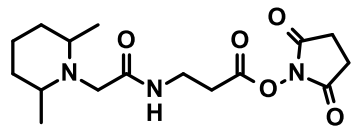
→ precursors in a narrow window in MS1

TMT: tandem mass tag

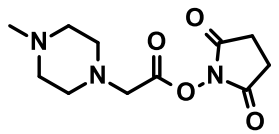
→ e.g. TMT18 (18 Isotopes)

ITRAQ: Isobaric tags for relative and absolutequantification

SILAC: stable isotope labeling by/with amino acids in cell culture



TMT Tag



iTRAQ

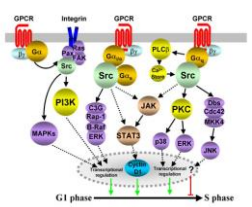
Meissner, F., *Nat Rev Drug Discov* **21**, 637–654 (2022).

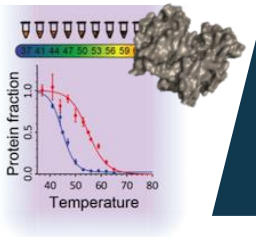
Chemoproteomics – A short overview

// (Chemo-)**Proteomics** allows the **qualitative and quantitative** analysis of **protein expression patterns** in large scale by mass spectrometry (before and after chemical modulation)
// **Significant technological improvements** in the last decade → analysis and quantification of >8000 proteins possible with reduced sample amounts and measurements times
// Fast **evolving field** → chemoproteomics, tissue proteomics, single cell proteomics, phosphoproteomics

For State of the Art Applications

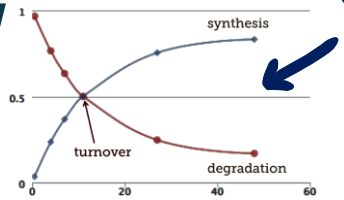
… as broad applicable **target validation tool** (complementary to genomics data)





… to support phenotypic screens by **identifying** novel **targets** & **protein-protein interaction networks**

… to prove target engagement, e.g. by measuring **protein degradation** levels of PROTACs

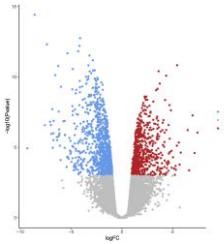


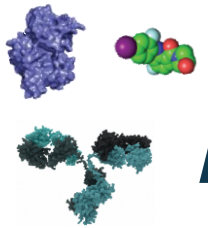
(Chemo-) Proteomics can be used …

For Visionary Applications

… to analyse **healthy vs. disease state,** e.g. quality check for disease hypothesis/models

… for a **better characterization** of assets, e.g. off-targets, biomarkers …

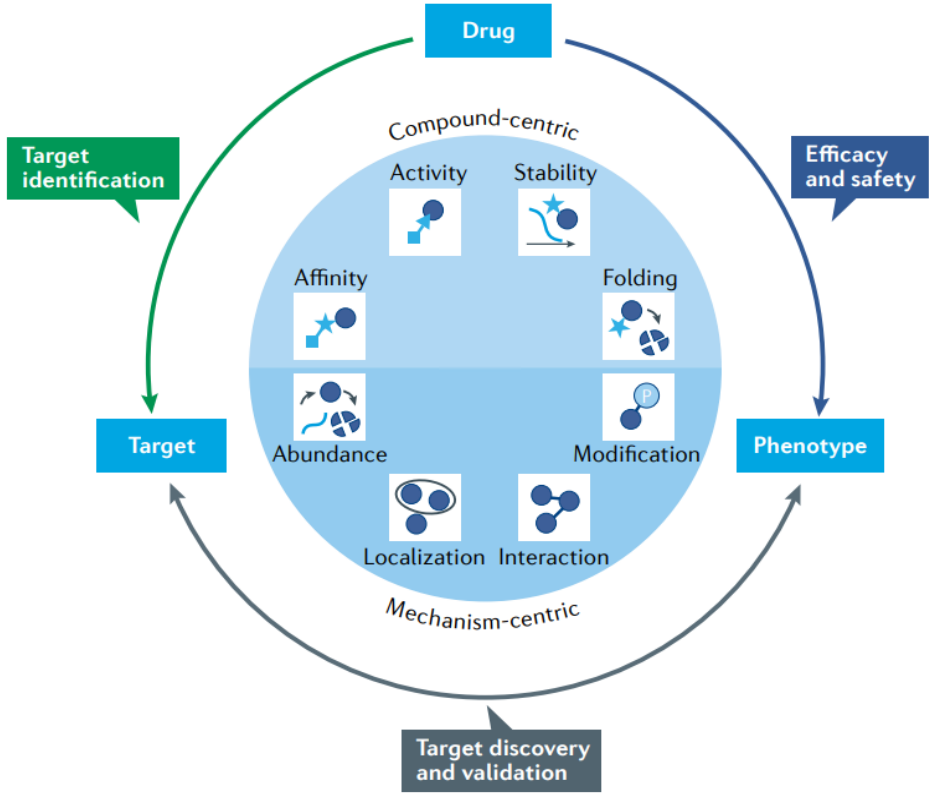




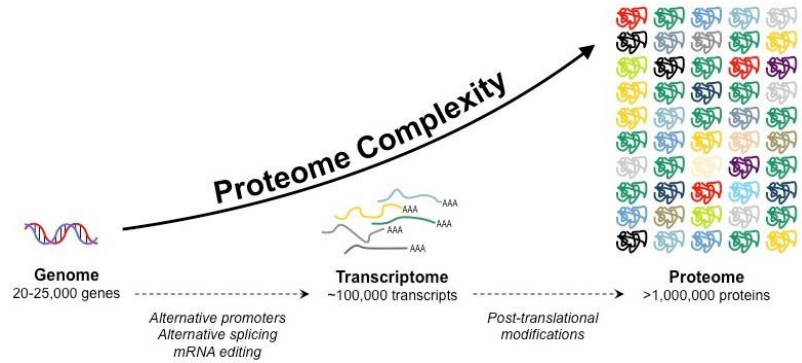
… to screen for **novel lead structures** for currently undruggable targets

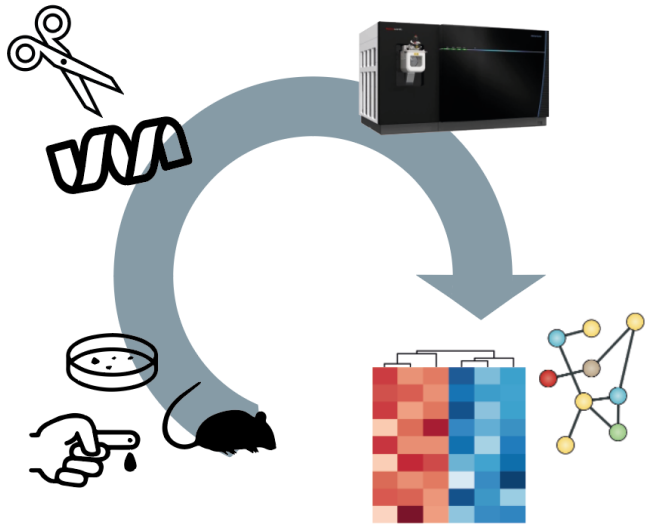
Proteomics tools are the currently most impactful tool in the Chemical Biology field

Proteomics applications in the preclinical drug discovery process



Proteomics enables characterization of the complex human proteome





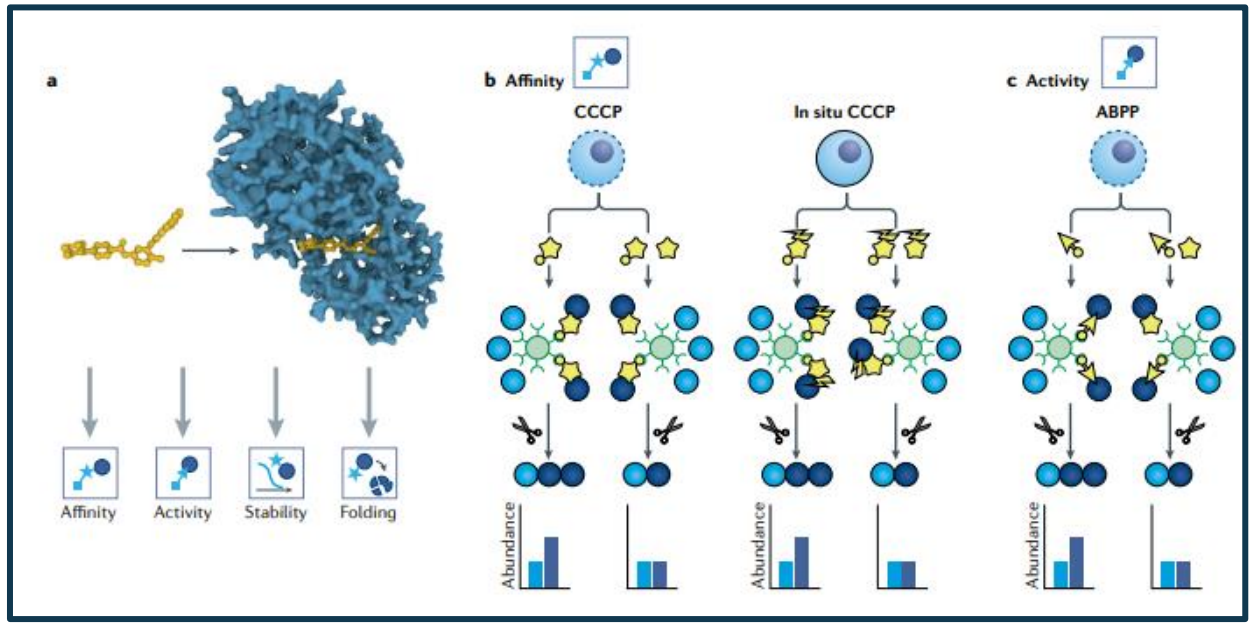
Current Limitations: Throughput due to complexity

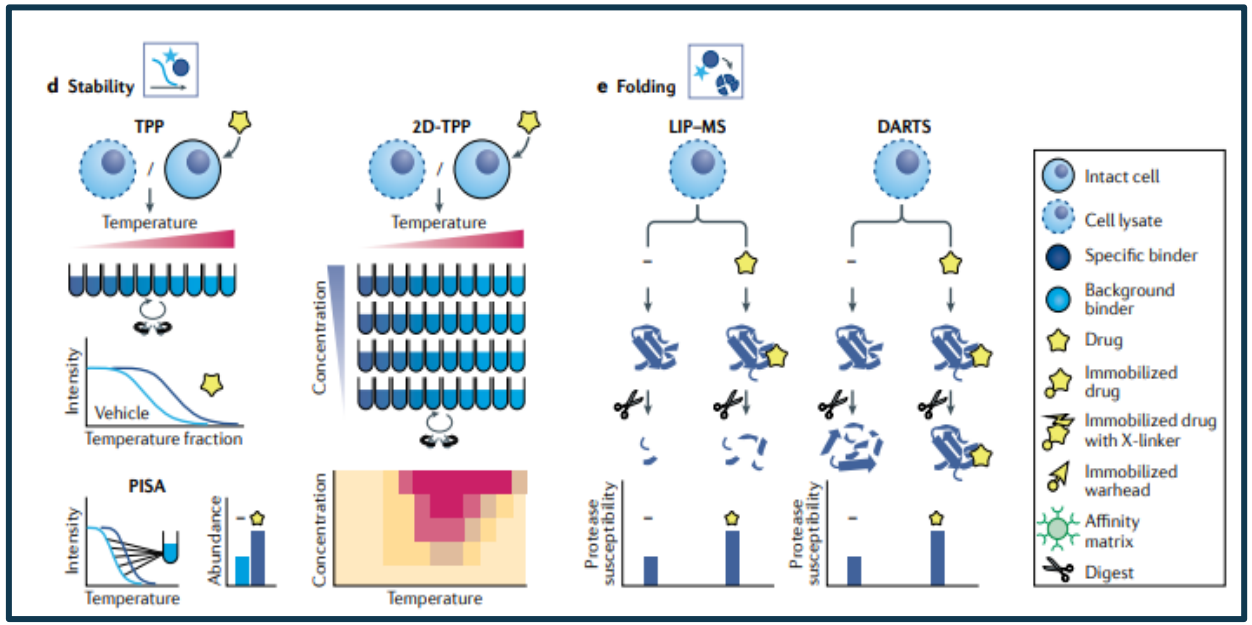
Meissner, F., *Nat Rev Drug Discov* **21**, 637–654 (2022).

Overview: Chemoproteomics Use Cases in Drug Discovery

Affinity and Activity Methods

Stability and Folding Methods





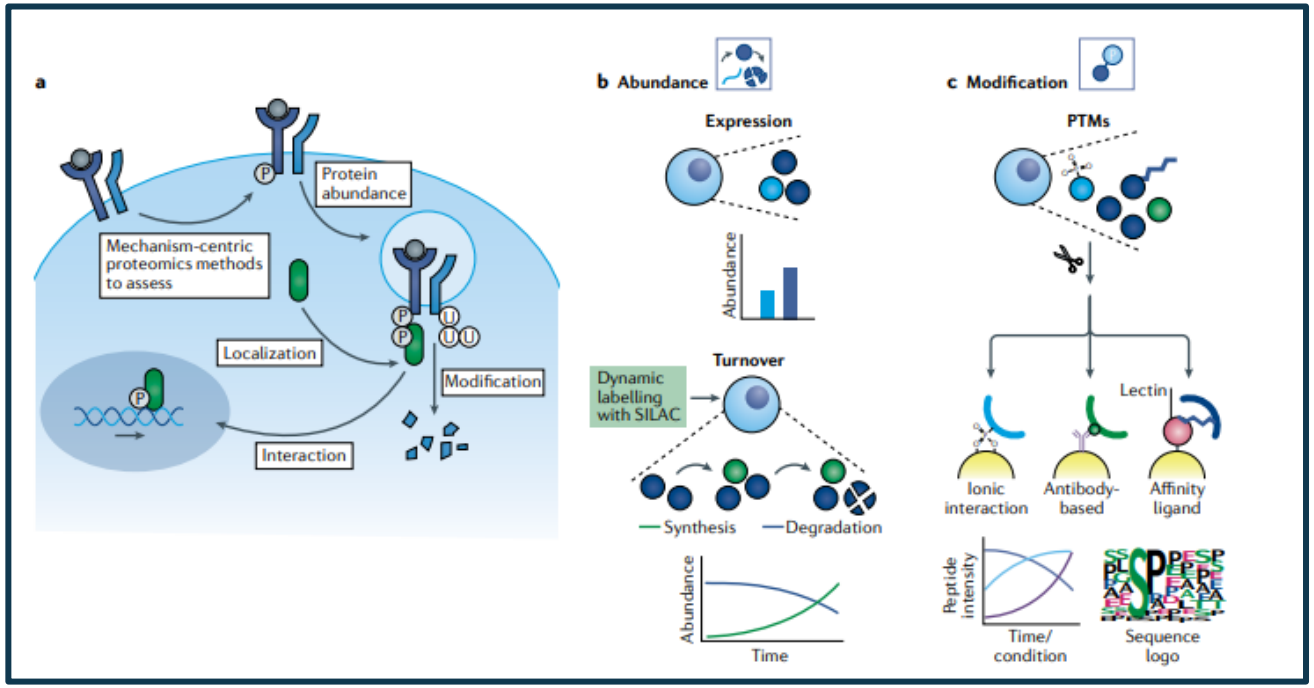
CCCP = Compound-Centric Chemical Proteomics
ABPP = Affinitiy/Activity based Protein Profiling

TPP = Thermal Proteome Profiling
LIP-MS = Limited Proteolysis coupled to Mass spectrometry
DARTS = Drug affinity responsive targets stability

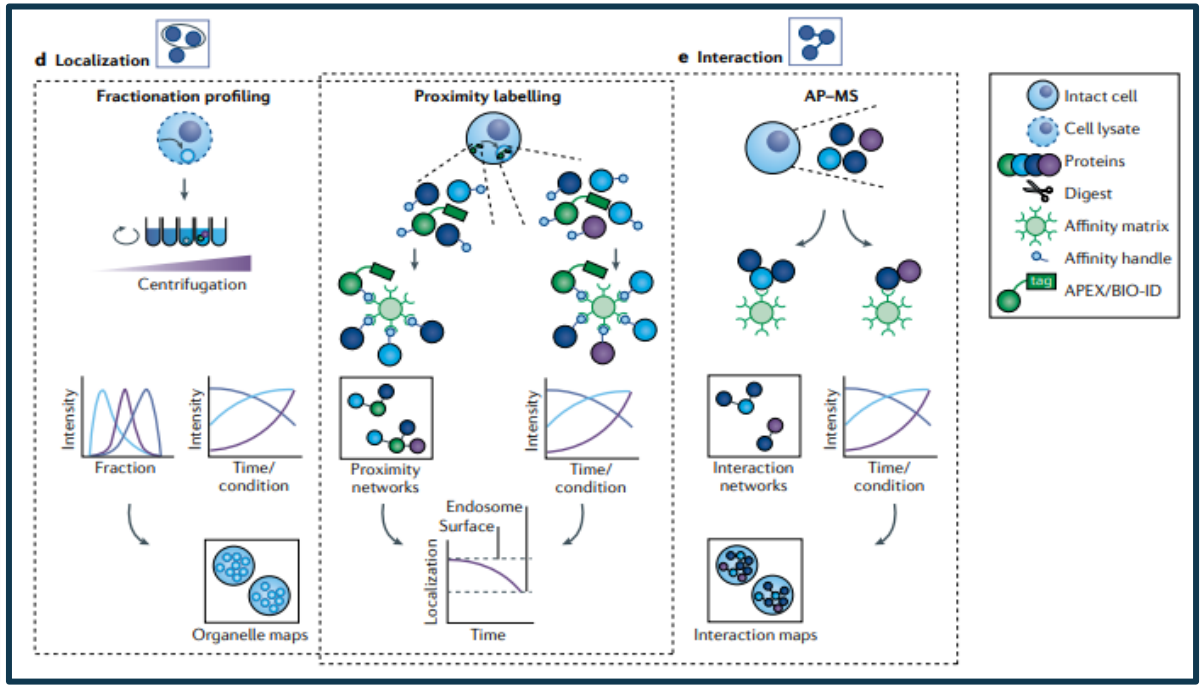
Meissner, F., *Nat Rev Drug Discov* **21**, 637–654 (2022).

Overview: Chemoproteomics Use Cases in Drug Discovery

Expression and Modification Methods



Localization and Interaction Methods

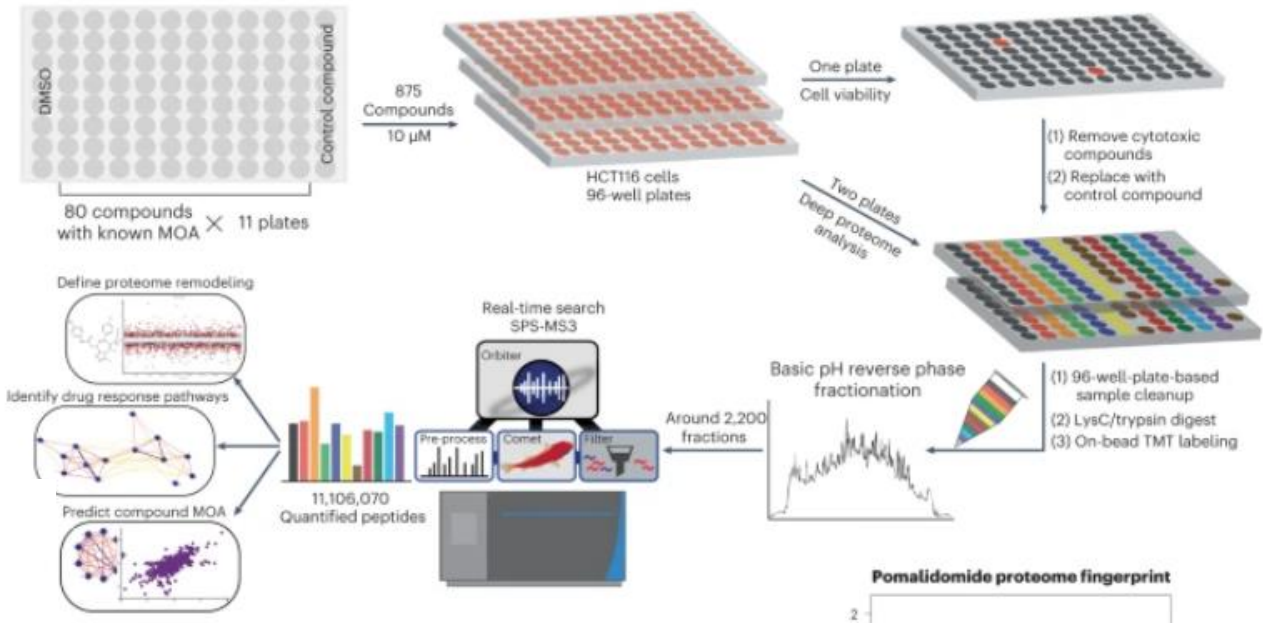


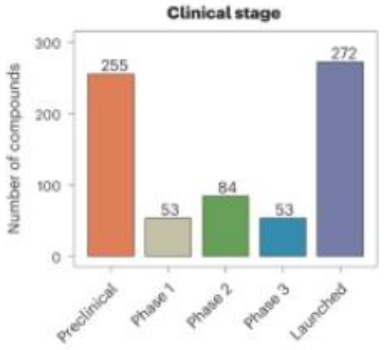
PTM = Post translational modification

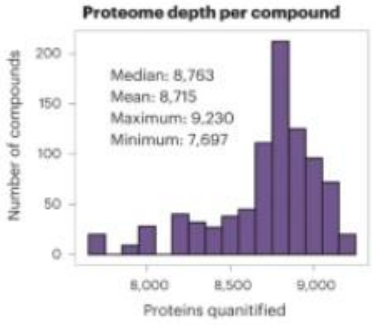
AP-MS = Affinity Purification Mass Spectroscopy

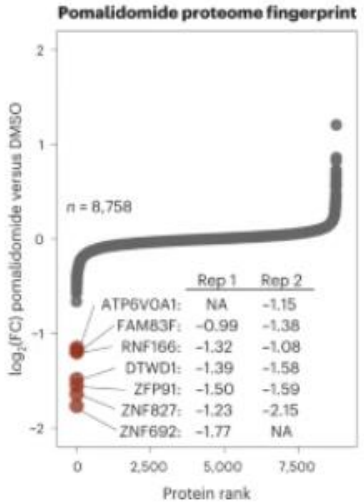
Meissner, F., *Nat Rev Drug Discov* **21**, 637–654 (2022).

Currently largest study: A proteome-wide fingerprinting atlas of drug mechanism of action

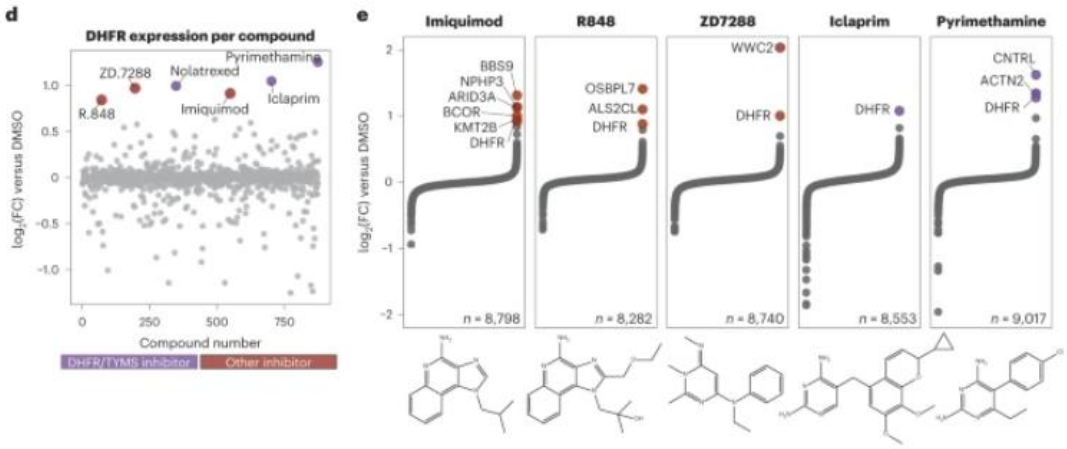




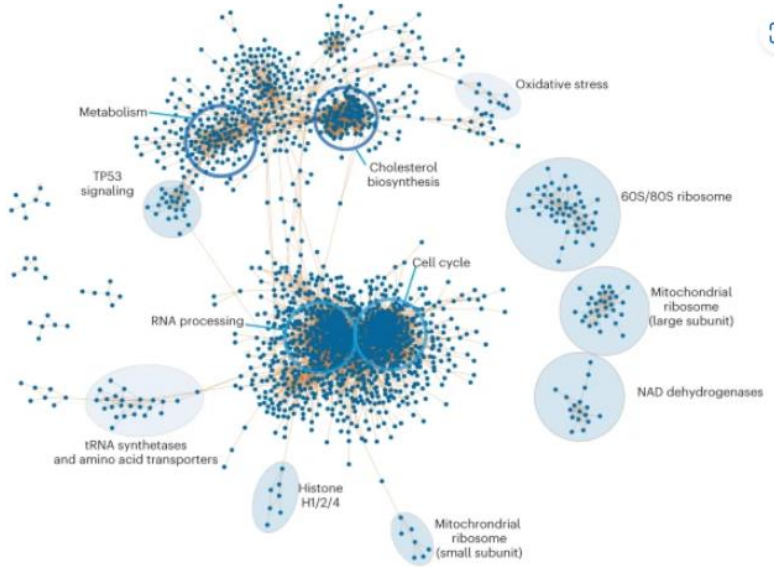




Example: DHFR expression modulators

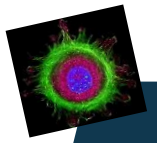


An atlas allows proteome wide compound clustering



Mitchell DC, *Nat. Biotechnol.* **2023**, 41, 845-857.

Examples for advanced Chemoproteomics tools: Target engagement and deconvolution by Cellular Thermal Shift Assays (CETSA)



Wide range of sample types (cells, tissue, lysates, organelles)

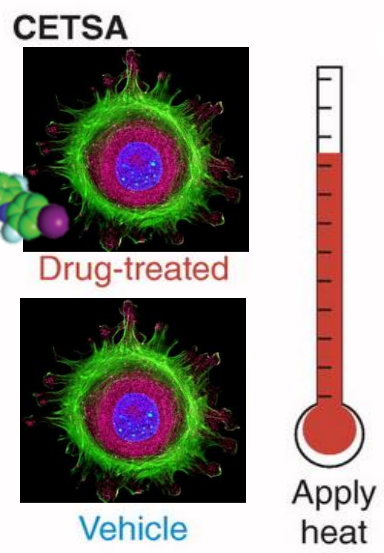
Target validation

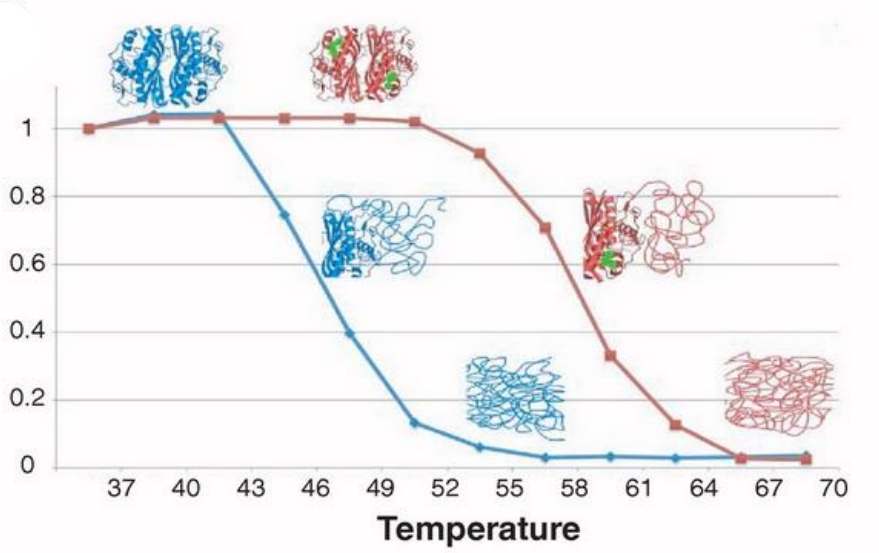
Label free

Target/ Off target identification

Target engagement in cells

Target deconvolution





Basic Principle : Heating of cells with subsequent **quantification of the presence of target protein** in the soluble fraction by a broad **variety of detection methods** (e.g. Western Blot or Mass spectrometry)

Daniel Martinez Molina, *Science* **341**, 84-87 (2013).
A.Jensen, Future Medicinal Chemistry 2015, 7, 975-978.

Sample

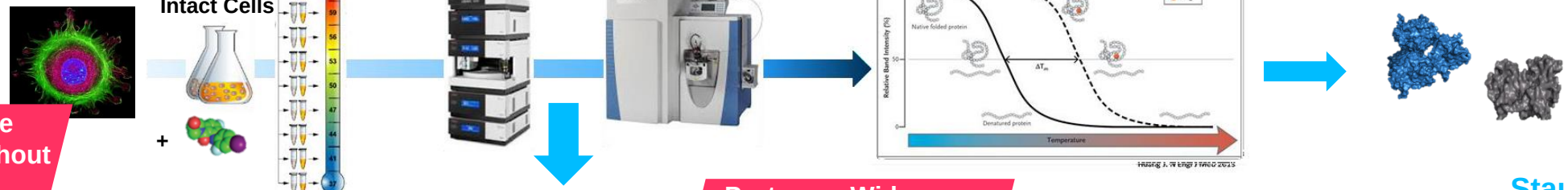
CETSA

Intact Cells

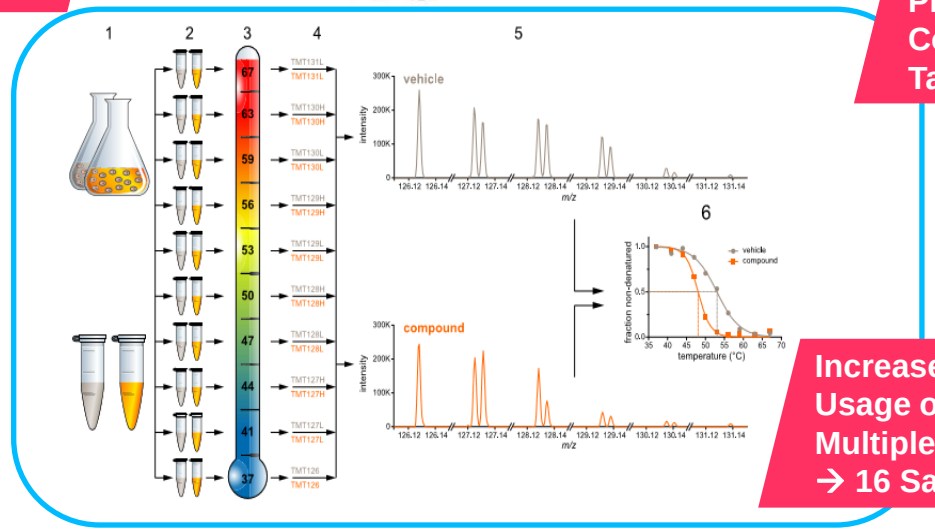
LC-MS/MS Analysis

Data Analysis

Target or off-Target Candidates

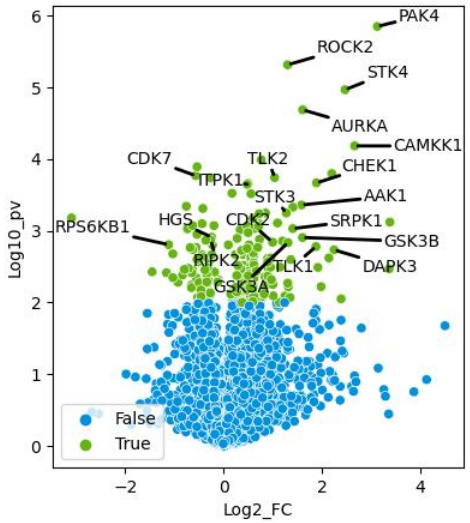


Usage of label free Compound (= without modifications)

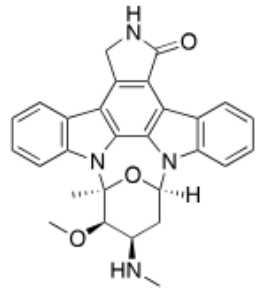


Proteome Wide Coverage of Potential Target interactions

Increase of Throughput by Usage of 16 TMT Multiplexing and MS-setup → 16 Samples in 1 MS-Run



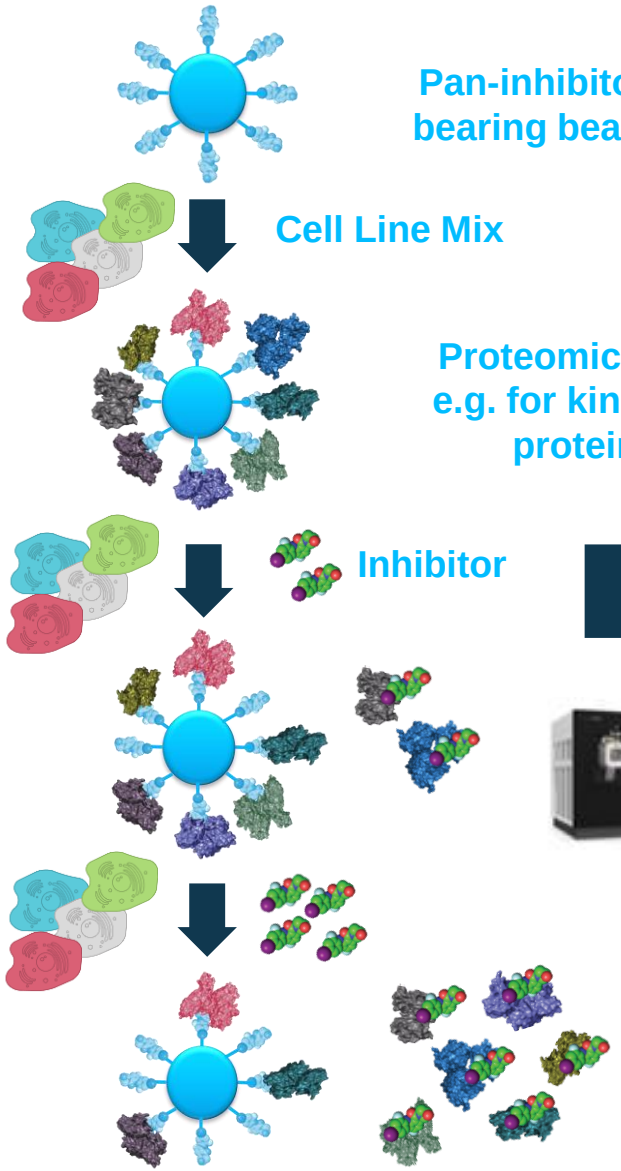
Staurosporine



// **The difference in thermal stability** can be used to monitor **protein-drug interactions across the whole proteome** in living cells or lysates enabling target engagement in an **unbiased way**

// CETSA MS allows the **identification of markers for drug efficacy** and **toxicity** as well as

Chemoproteomics based screens – Basic Principle



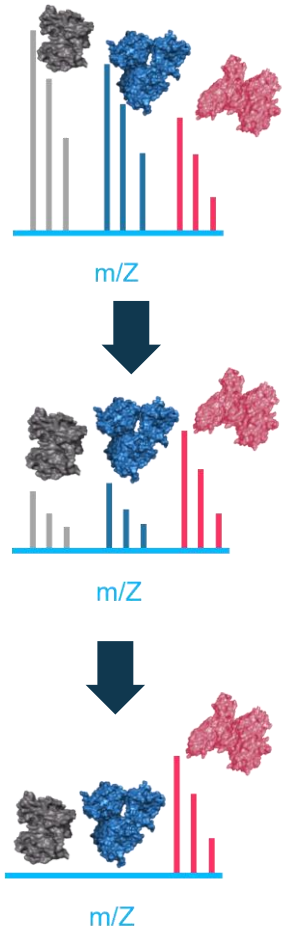
Pan-inhibitor bearing beads

Cell Line Mix

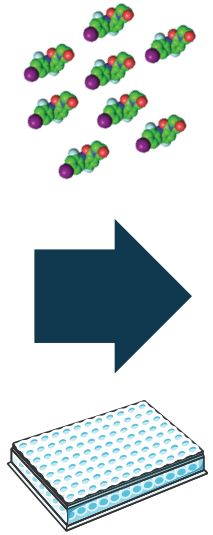
Proteomics readout for e.g. for kinases or other protein classes

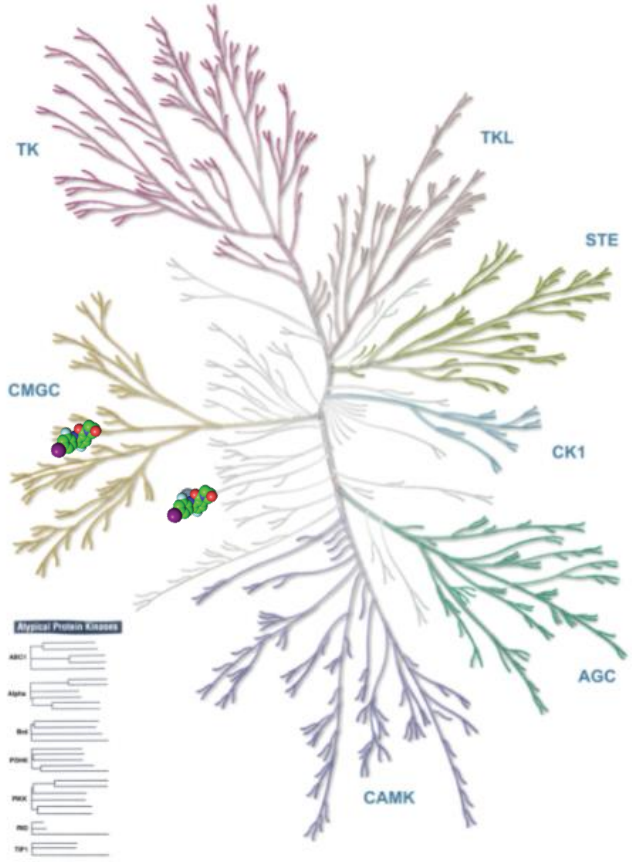
Inhibitor

Determination of IC50 curves



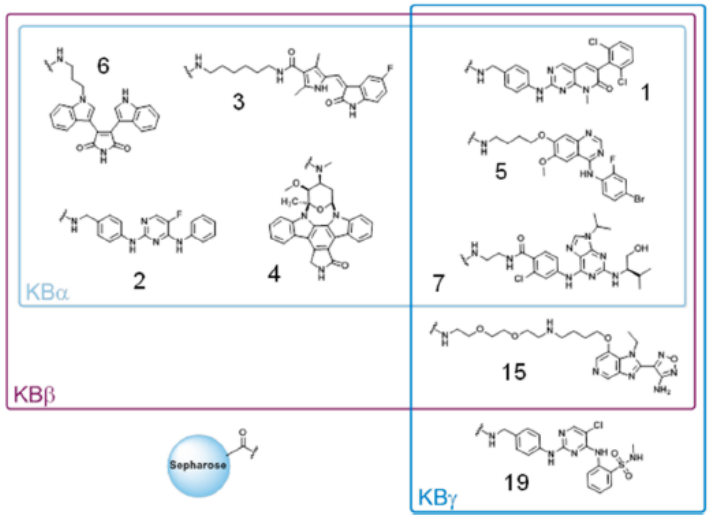
Selection of suitable library compounds



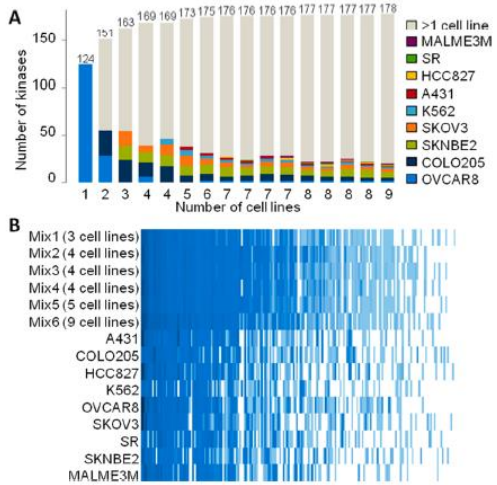


Chemoproteomics Example – The KinoBead Technology

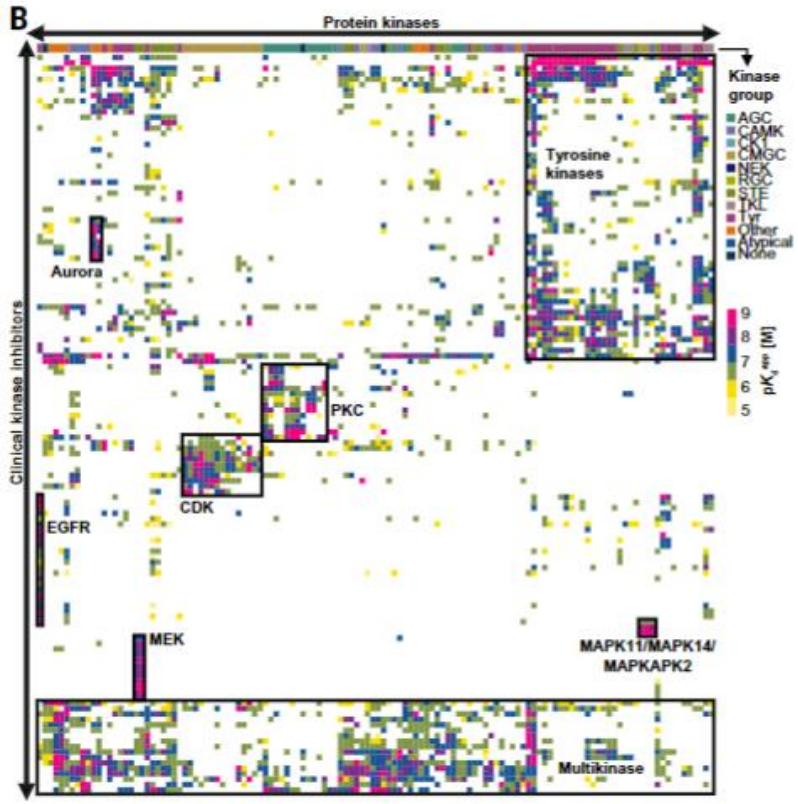
Examples for the Pan-Kinase Inhibitors



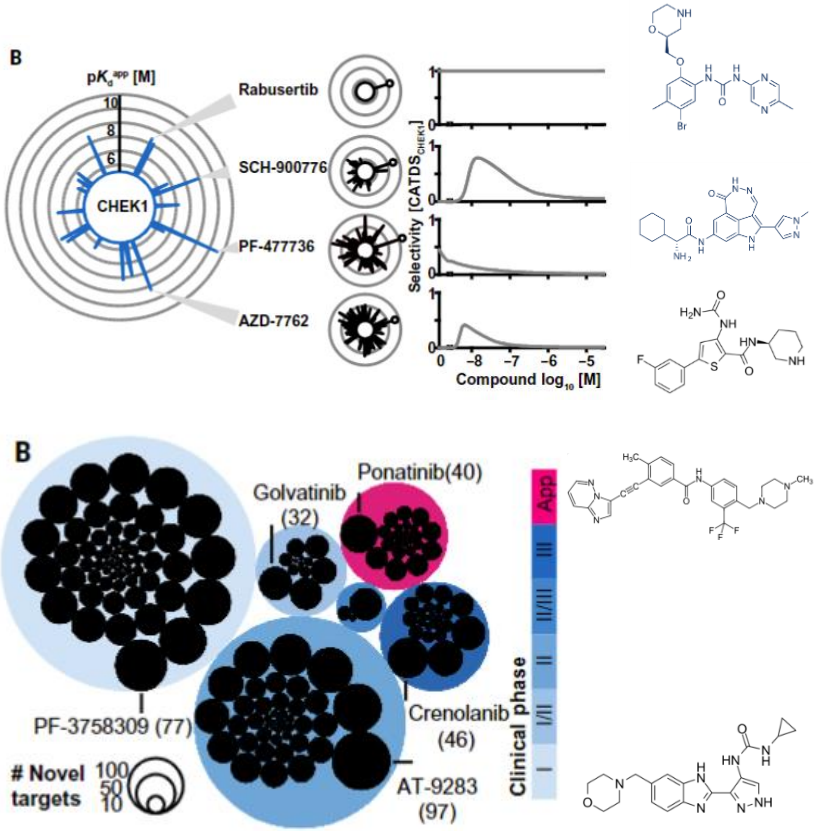
Optimization of the cell line mixtures



Profiling of clinical Kinase Inhibitors on Scale



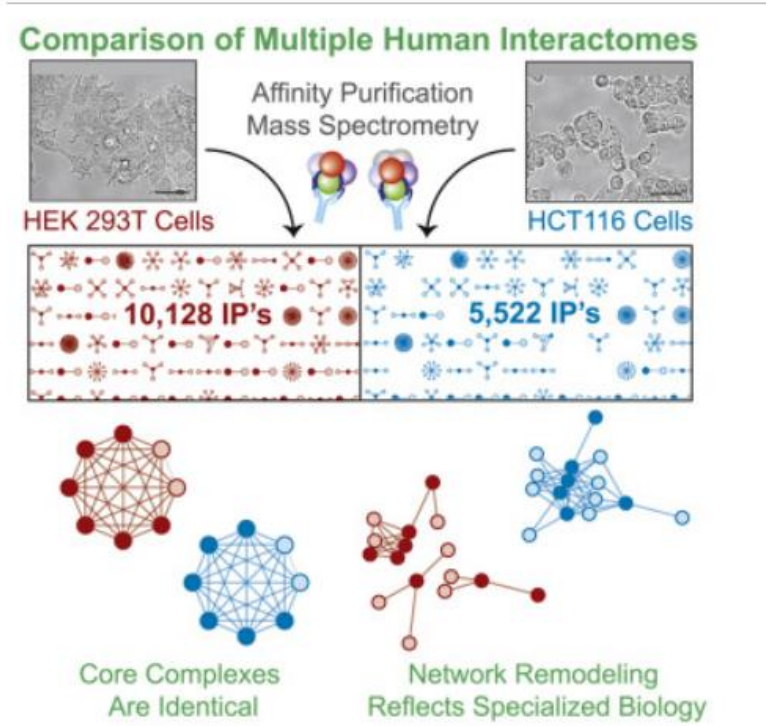
Outcome: Several off-targets were not known before and might reflect clincial success rate



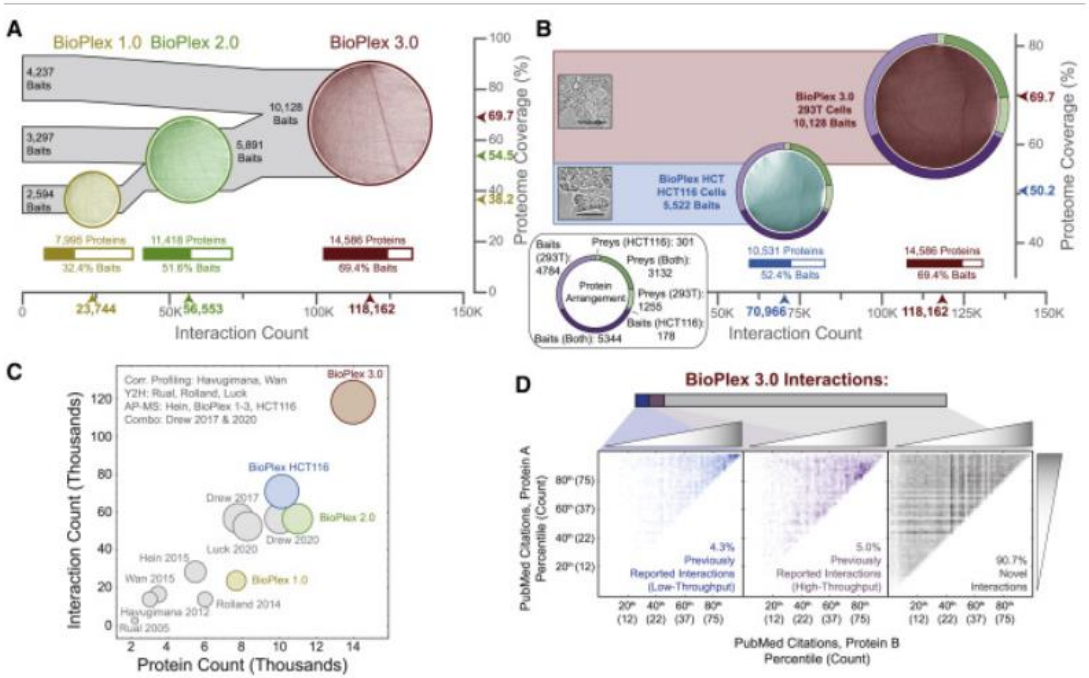
M. Bantscheff et al., *Nat. Biotechnol.* **2007**, *25*, 1035-1044.
G. Médard et al., *J. Proteome Res.* **2015**, *14*, 1574-1586.
S. Klaeger et al., *Science* **2017**, *358*, 4368.

Proteomics based interactome analysis: Bioplex database

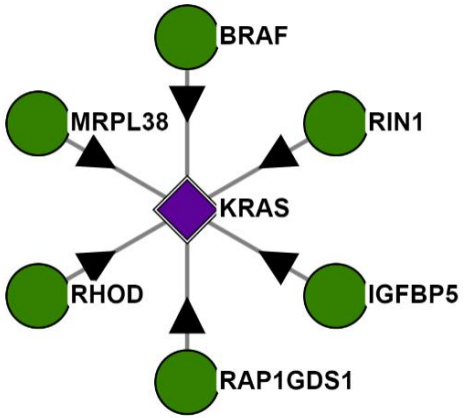
Immunoprecipitation setup:



Bioplex database construction:



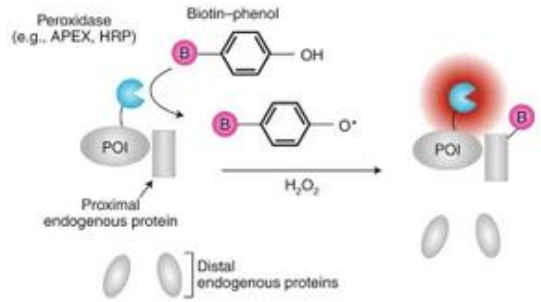
Example: KRAS



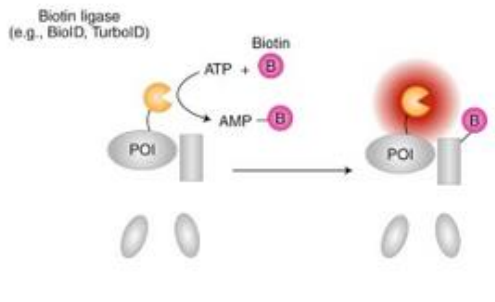
S. P. Gygi et al. *Cell* **2021**, *184*, 3022-3040

Unraveling Interactomes by Proximity Labeling

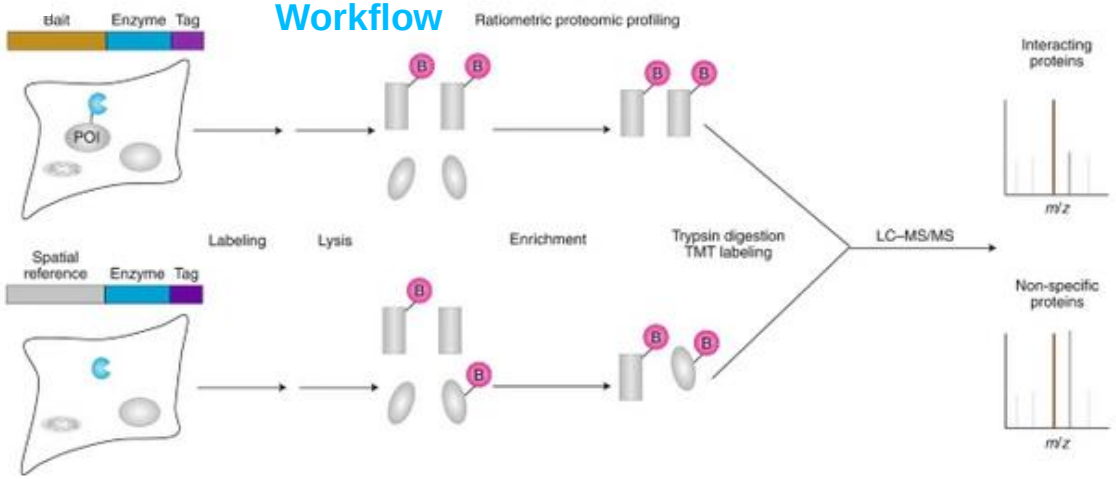
APEX



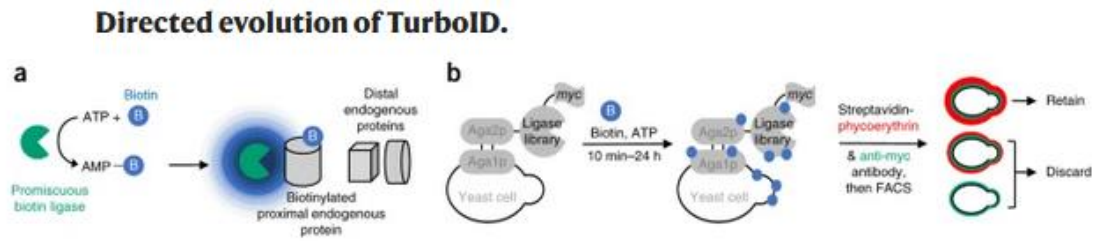
BioID

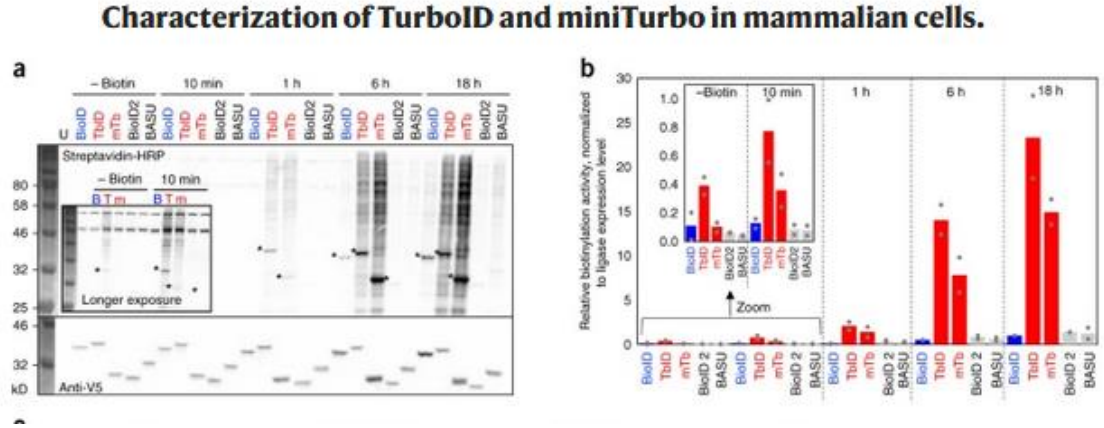


Workflow



TurboID: Tool improvements via protein engineering





Proximity Labeling needs a fusion of an APEX or Biotin ligase tag to the POI (Protein of interest) and allows a Chemoproteomics analysis of the labeled proteins

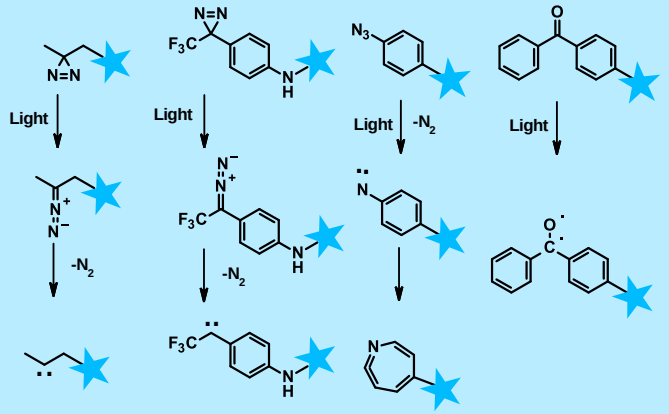
Qin, W., Cho, K.F., Cavanagh, P.E. *et al. Nat Methods* **18**, 133–143 (2021).

Branon, T., Bosch, J., Sanchez, A. *et al. Nat Biotechnol* **36**, 880–887 (2018).

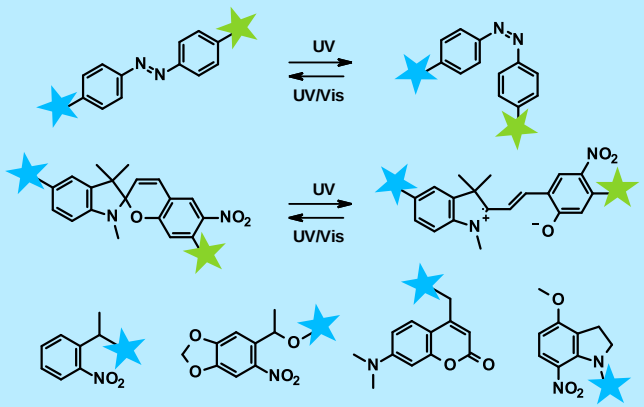
The Central Element for Chemical Biology: development of the right Chemical Probing concepts

Overview: Chemical Biology Probe Chemistry

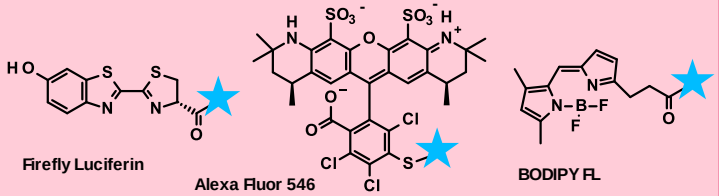
Photoreactive Probes



Photoswitches & Photocages

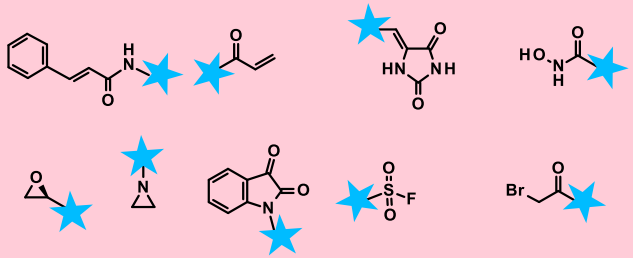


Fluorescence Groups

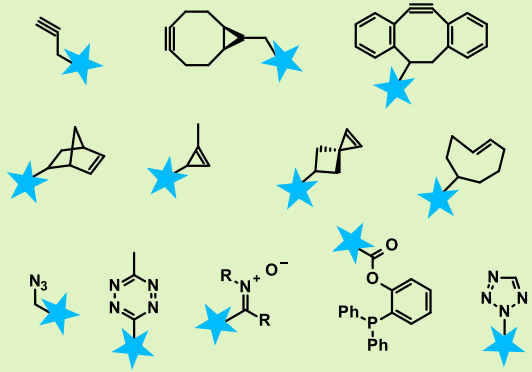


Chemical Biology Probe Molecules

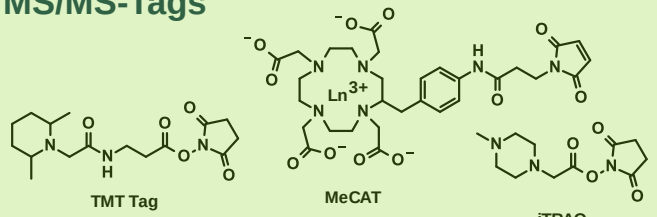
Warheads



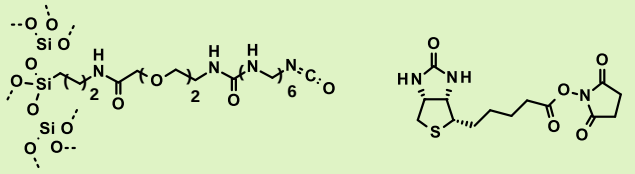
Bioorthogonal Click-Groups



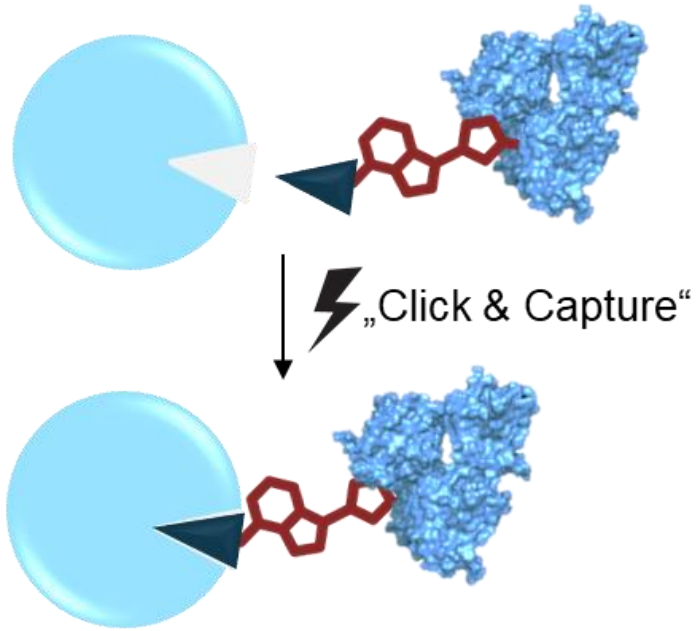
MS/MS-Tags

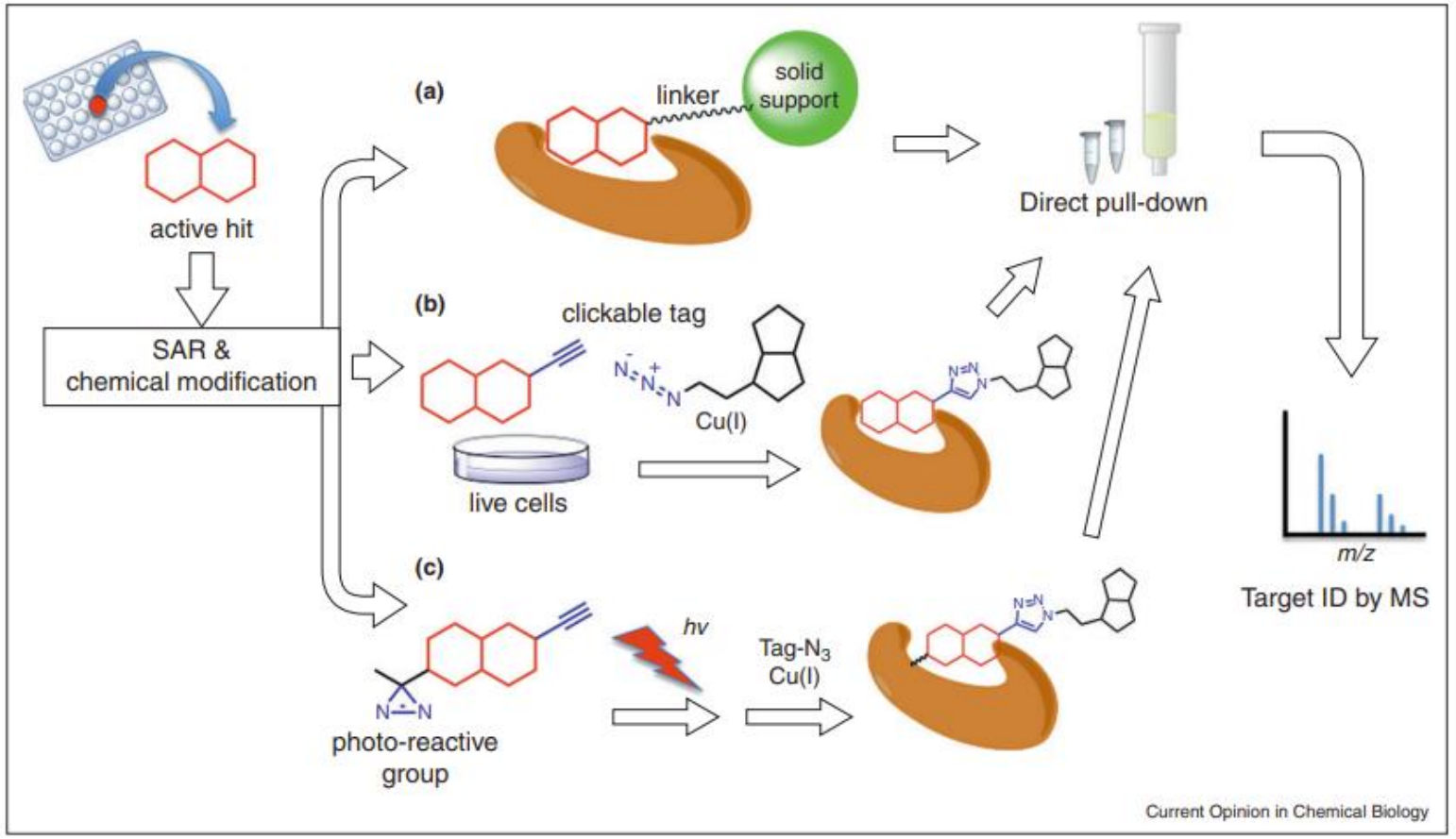


Immobilization



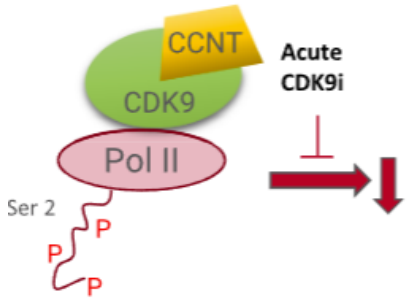
Pulldown/Affinity Proteomics – Basic Principle





J. Lee, M. Bogyo, *Curr. Opin. Chem. Biol.* **2013**, *17*, 118-126.

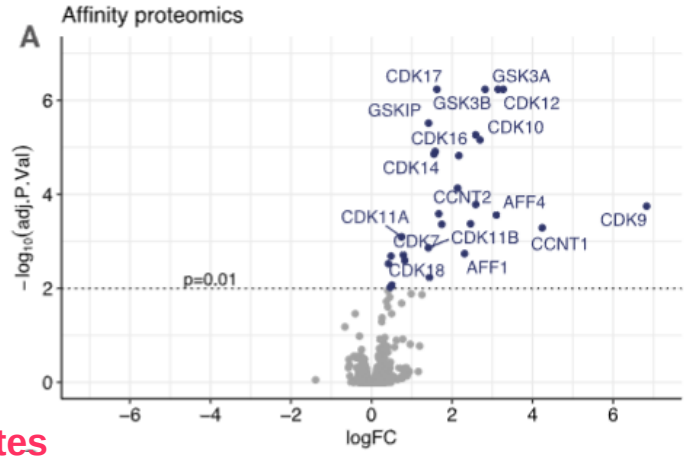
Example for classical Affinity Proteomics – Astra Zeneca CDK 9 program

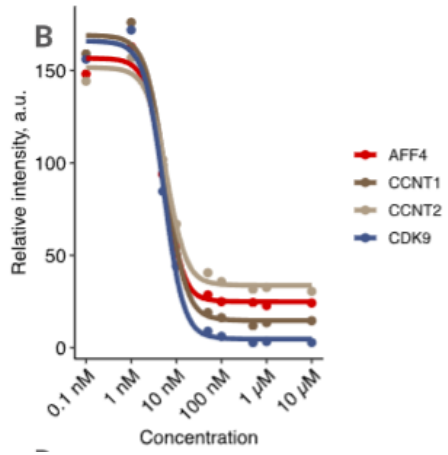


Acute CDK9i

Short half-life transcripts/ labile proteins, *e.g.*
Mcl-1 – Survival
MYC – Survival/growth

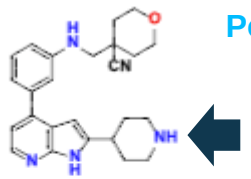
The Affinity pulldown results is way broader than a Kinase profiling

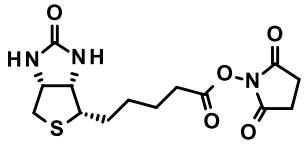




Biotin Attachment Point for Pulldown

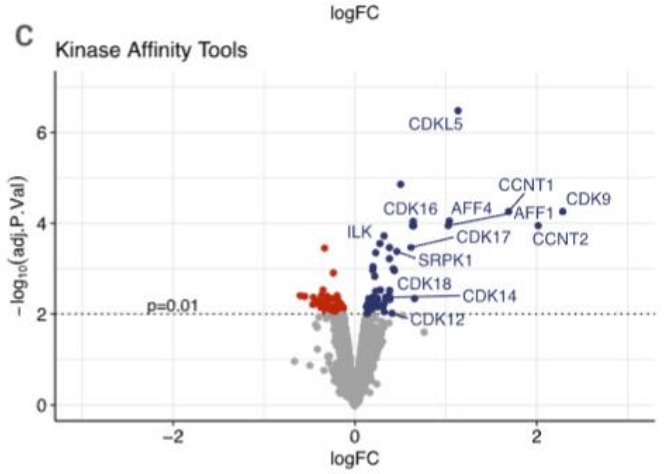
!!! Limitation: only applicable for lysates

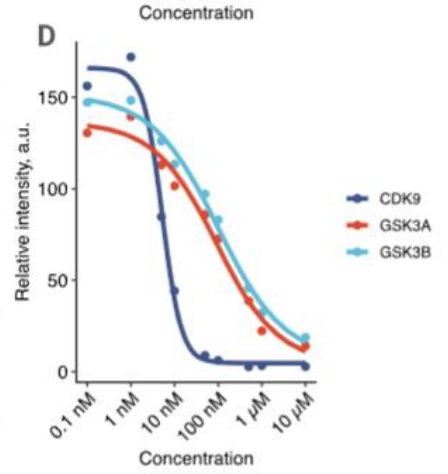




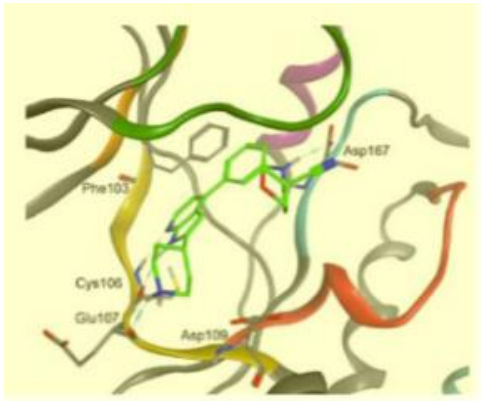
Compound 1

Biochemical Assay	Potency
Km ATP	< 3 nM
5 mM ATP	< 3 nM



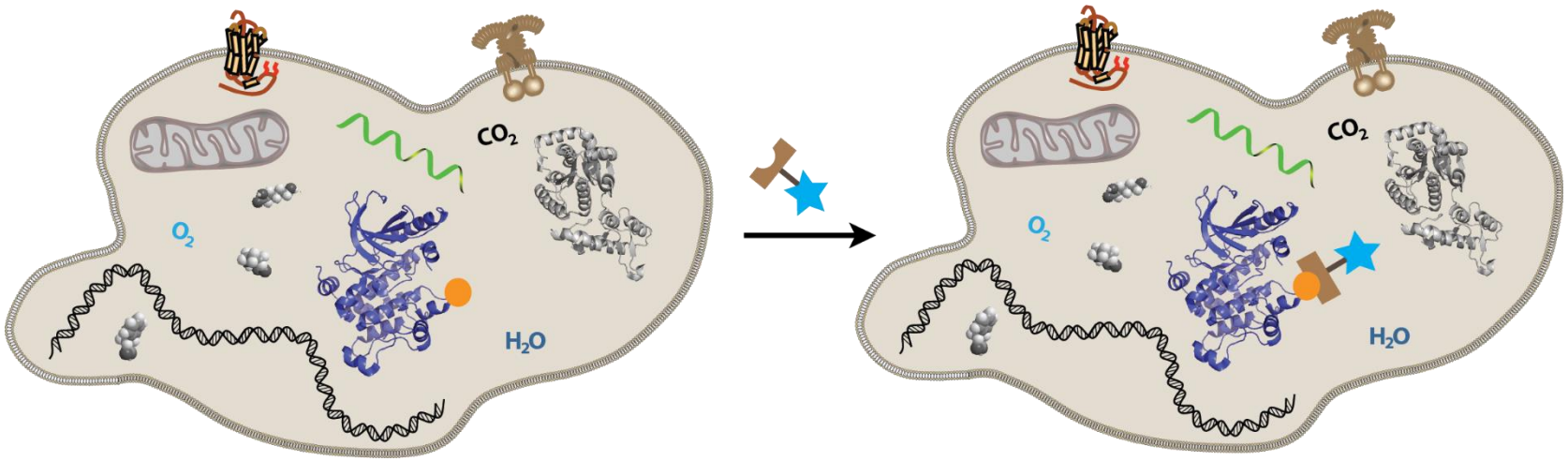


!!! SAR understanding for Probe design is crucial



J. A. Hendricks et al., *ACS Chem. Biol.* **2022**, *17*, 54-67.

Bioorthogonal and Click Chemistry – Basic principle



● and) react without interfering with native biochemical processes

★ additional molecular probe

Nobel Prize 2022

Carolyn R. Bertozzi

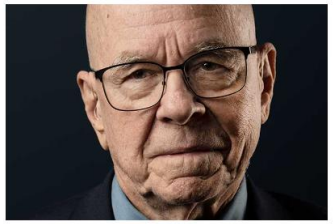
"for the development of click chemistry and bioorthogonal chemistry"

Morten Meldal

"for the development of click chemistry and bioorthogonal chemistry"

K. Barry Sharpless

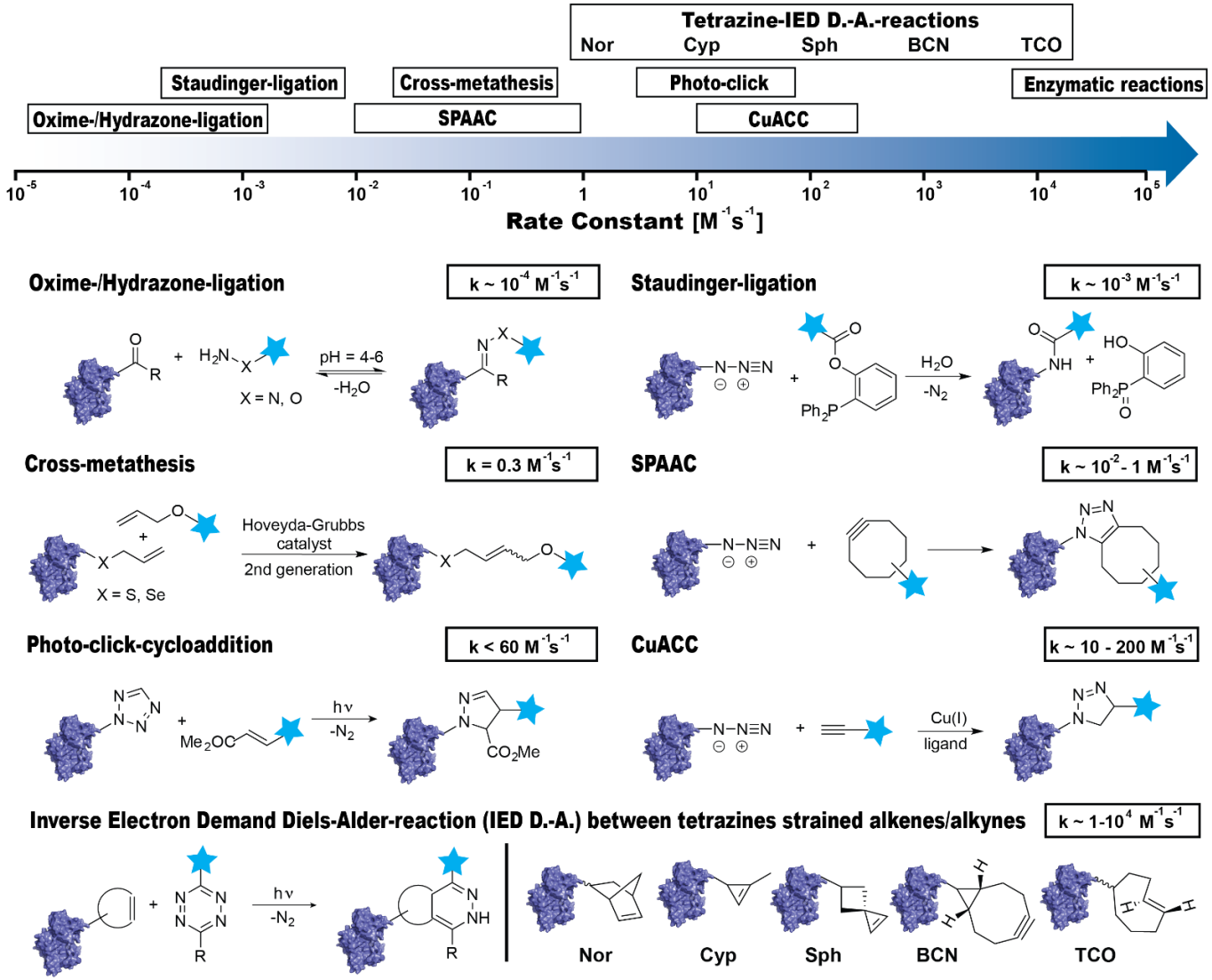
"for the development of click chemistry and bioorthogonal chemistry"



E. M. Sletten, C. R. Bertozzi, *Angew. Chem. Int. Ed.* **2009**, *48*, 6974-6998.

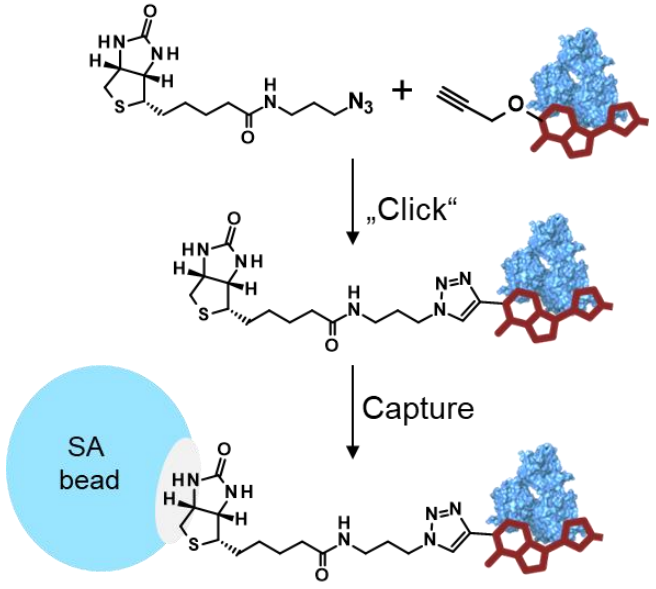
R. E. Bird, S. A. Lemmel, X. Yu, Q. A. Zhou, *Bioconjugate Chemistry* **2021**, *32*, 2457-2479.

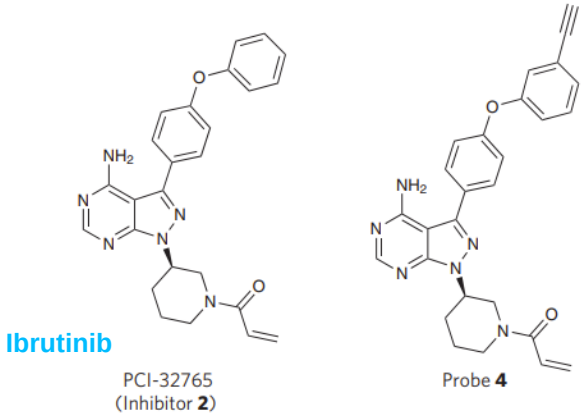
Bioorthogonal Chemistry – Overview about modern tools



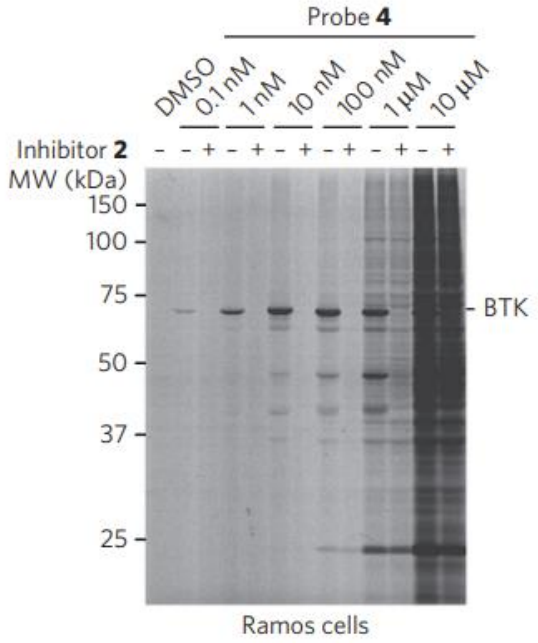
K. Lang, J. W. Chin, *ACS Chem. Biol.* **2014**, *9*, 16-20.
K. Lang, J. W. Chin, *Chem. Rev.* **2014**, *114*, 4764-4806.

Application Example for Click Chemistry: Target Deconvolution by Activity based Protein Profiling (ABPP)

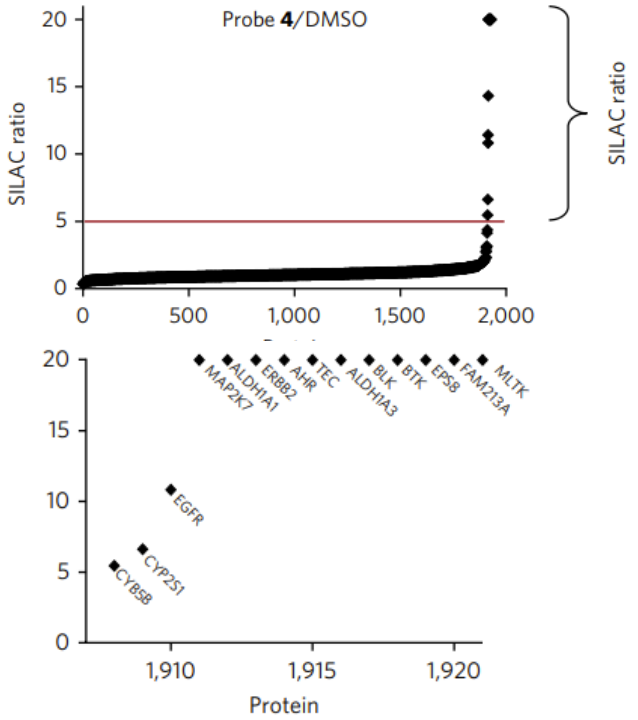




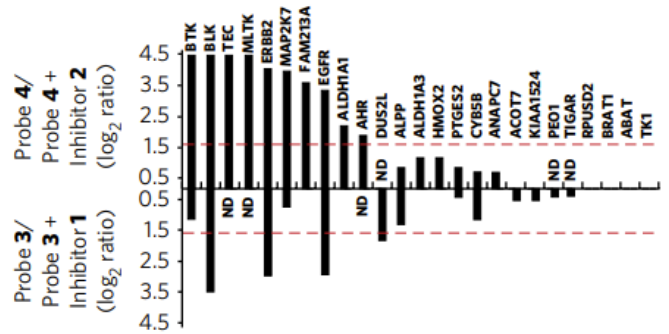
Western Blot analysis



SILAC Proteomics

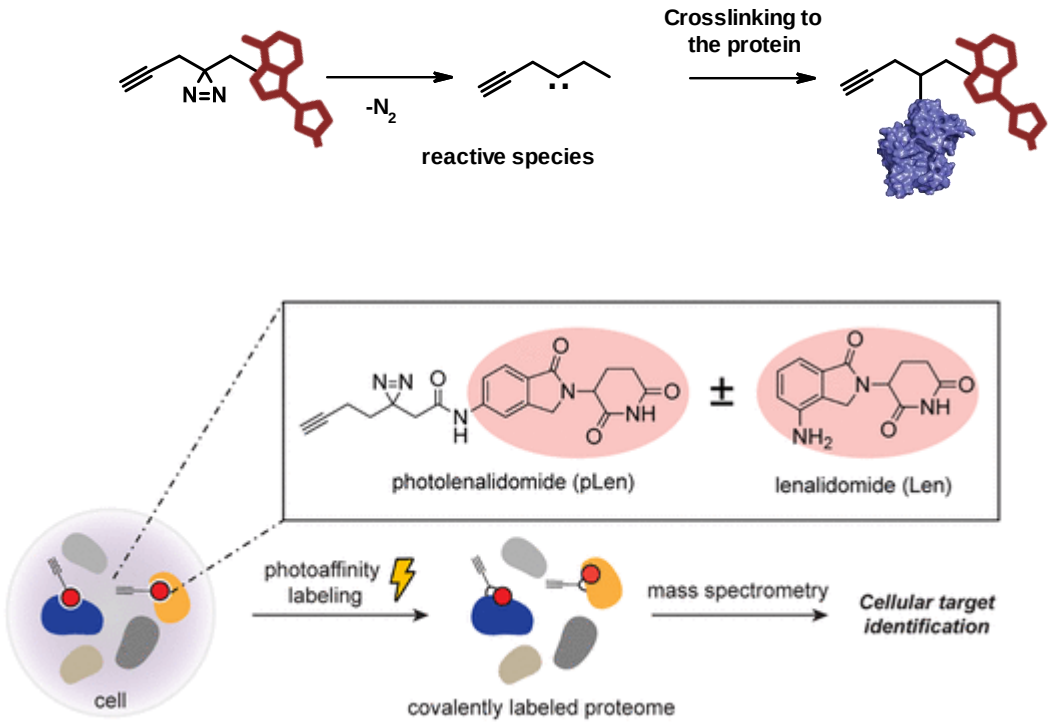


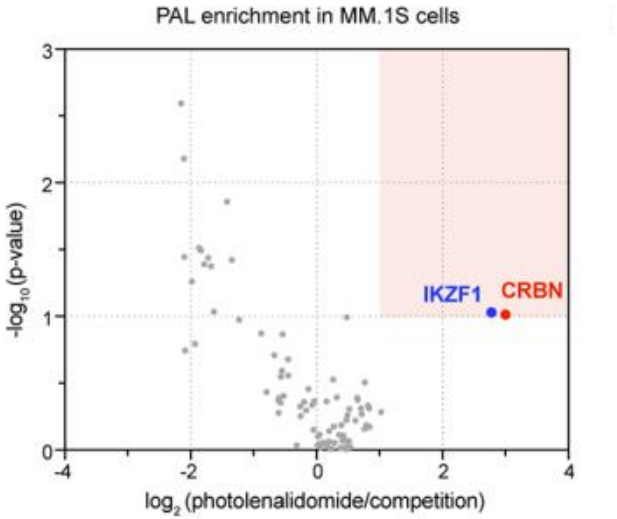
Competition Ratio Proteomics

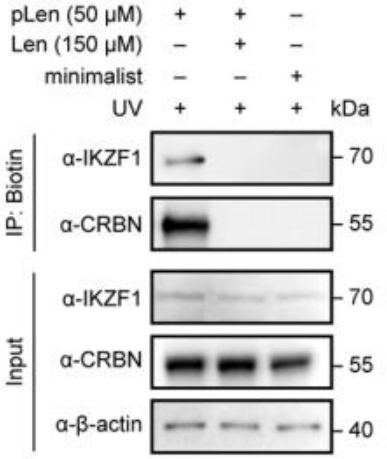


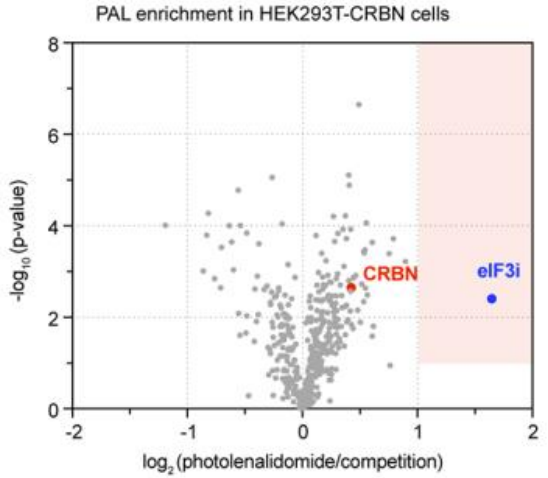
B. F. Cravatt et al., *Nat. Chem. Biol.* **2014**, *10*, 760-767.

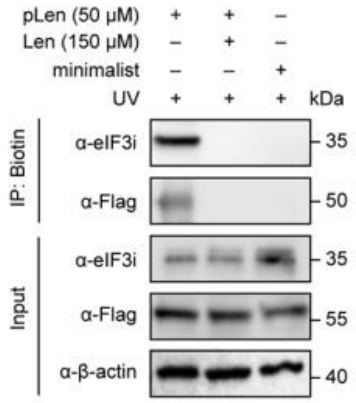
The labeling efficiency of Activity based Protein Profiling (ABPP) experiments can be improved by Photoaffinity tags (PAL)





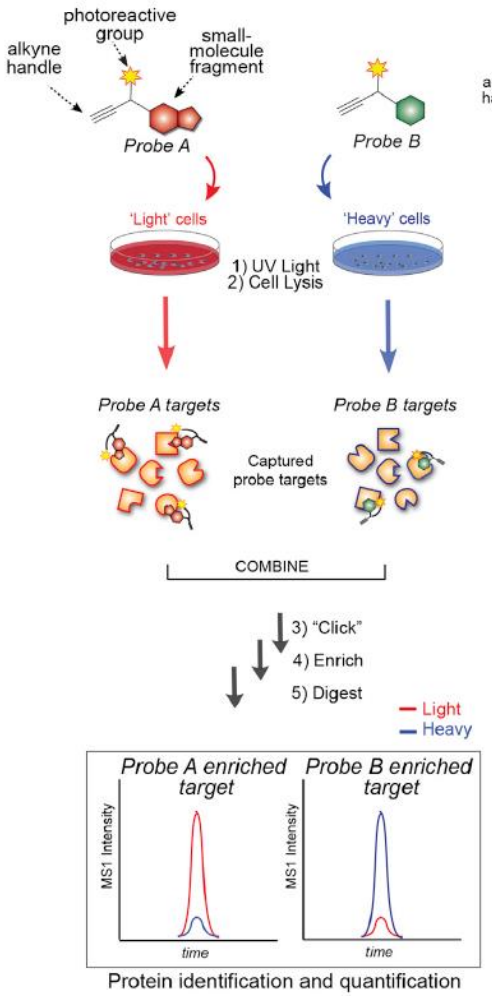


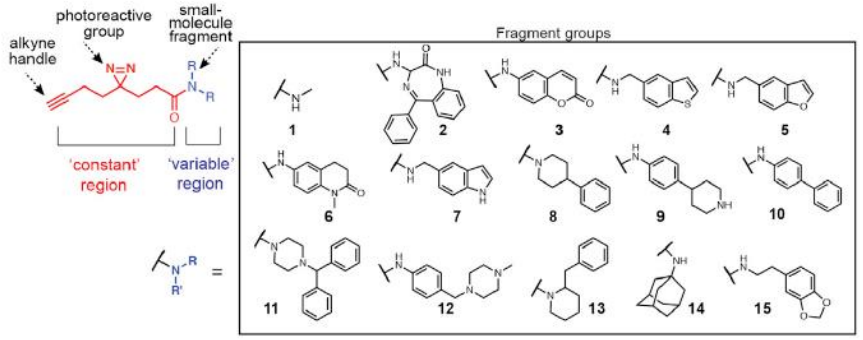


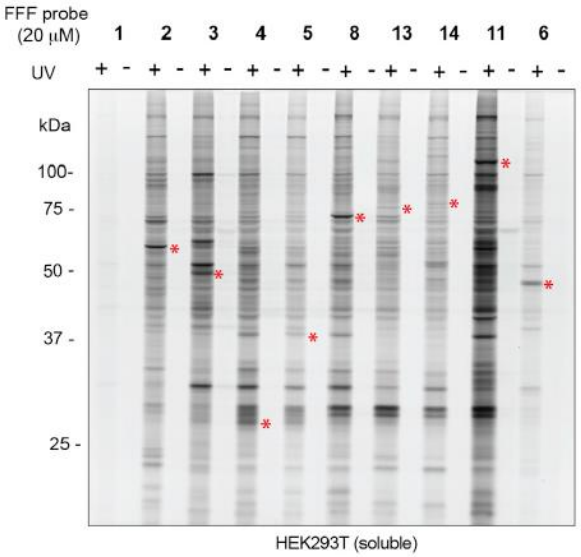


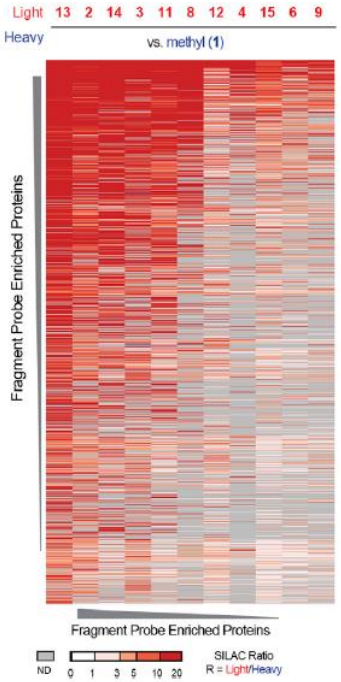
J. Am. Chem. Soc. 2022, 144, 1, 606–614

Cellular Fragment-Based Screening by Photoaffinity based Protein Profiling (ABPP)

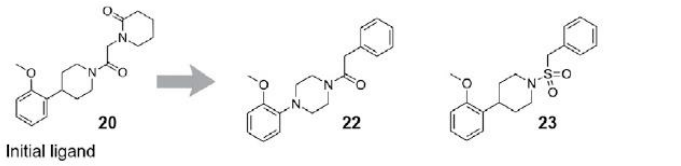


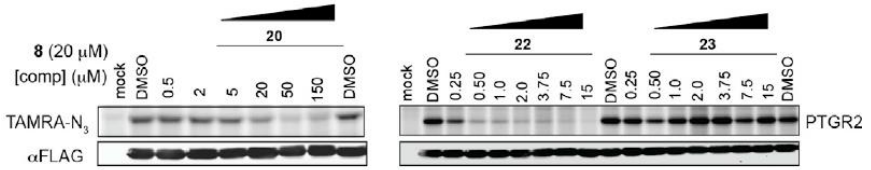






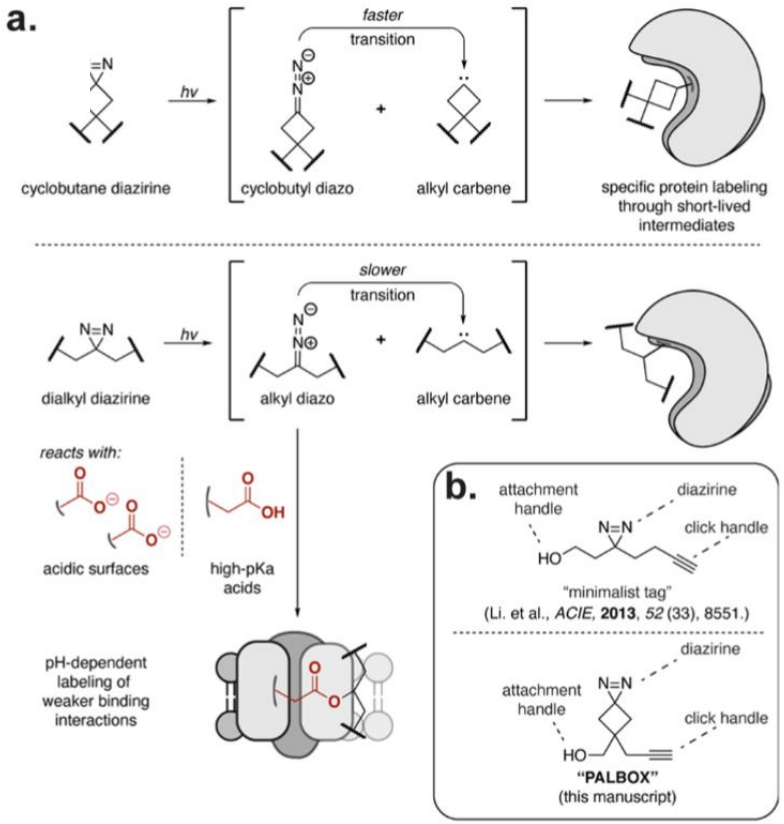
Fragment Enrichment Clustering, Target Selection & Fragment Optimization

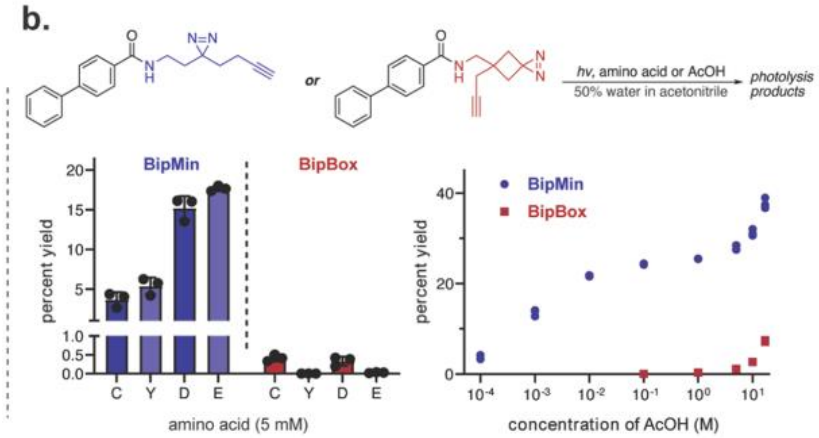


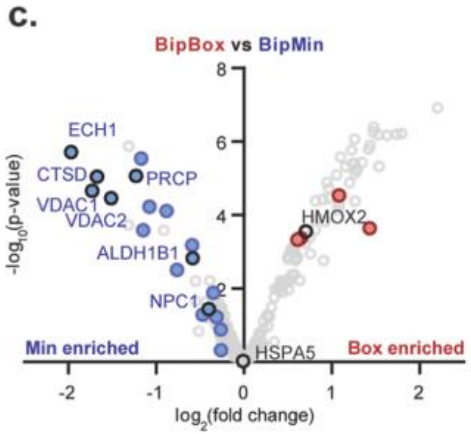


C. G. Parker, A. Galmozzi, Y. Wang, B. E. Correia, K. Sasaki, C. M. Joslyn, A. S. Kim, C. L. Cavallaro, R. M. Lawrence, S. R. Johnson, I. Narvaiza, E. Saez, B. F. Cravatt, *Cell* **2017**, *168*, 527-541.

The Chemical Nature of the Probe has significant influence on the labeling efficiency and biological outcome of a Photoaffinity labeling experiment

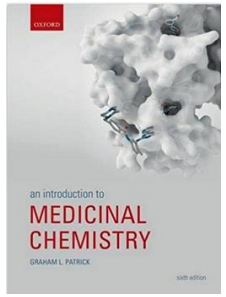


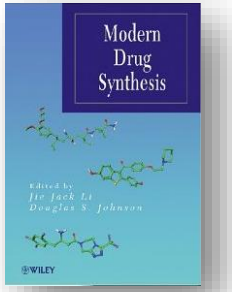




A. V. West, Y. Amako, C. M. Woo, *JACS* **2022**, *144*, 21174-21183.

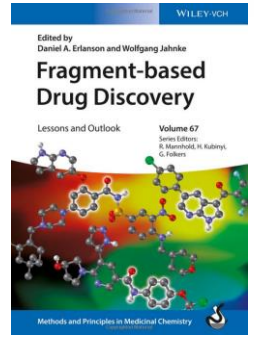
Recommended Literature

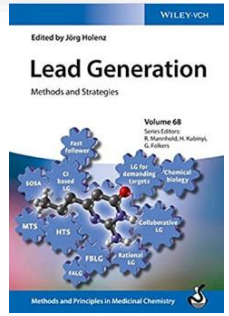




Medicinal Chemistry:

P. Graham: An Introduction to Medicinal Chemistry, 2017
G. Klebe: Drug Design: Methodology, Concepts, and Mode-of-Action, 2013
D. Erlanson, Wolfgang Jahnke: Fragment-based Drug Discovery, 2016
J. J. Lie, D. S. Johnson, Modern Drug Synthesis (Case Studies), Wiley, 2013
J. Holenz, Lead Generation: Methods, Strategies, and Case Studies, Wiley, 2016
Methods and Principles in Medicinal Chemistry: Several Volumes with different topics
D.A. Smith: Metabolism, Pharmacokinetics and Toxicity of Functional Groups, 2010
B. Rupp: Biomolecular Crystallography, 2009
Swiss course on Medicinal chemistry: https://scg.ch/component/eventbooking/scmc22



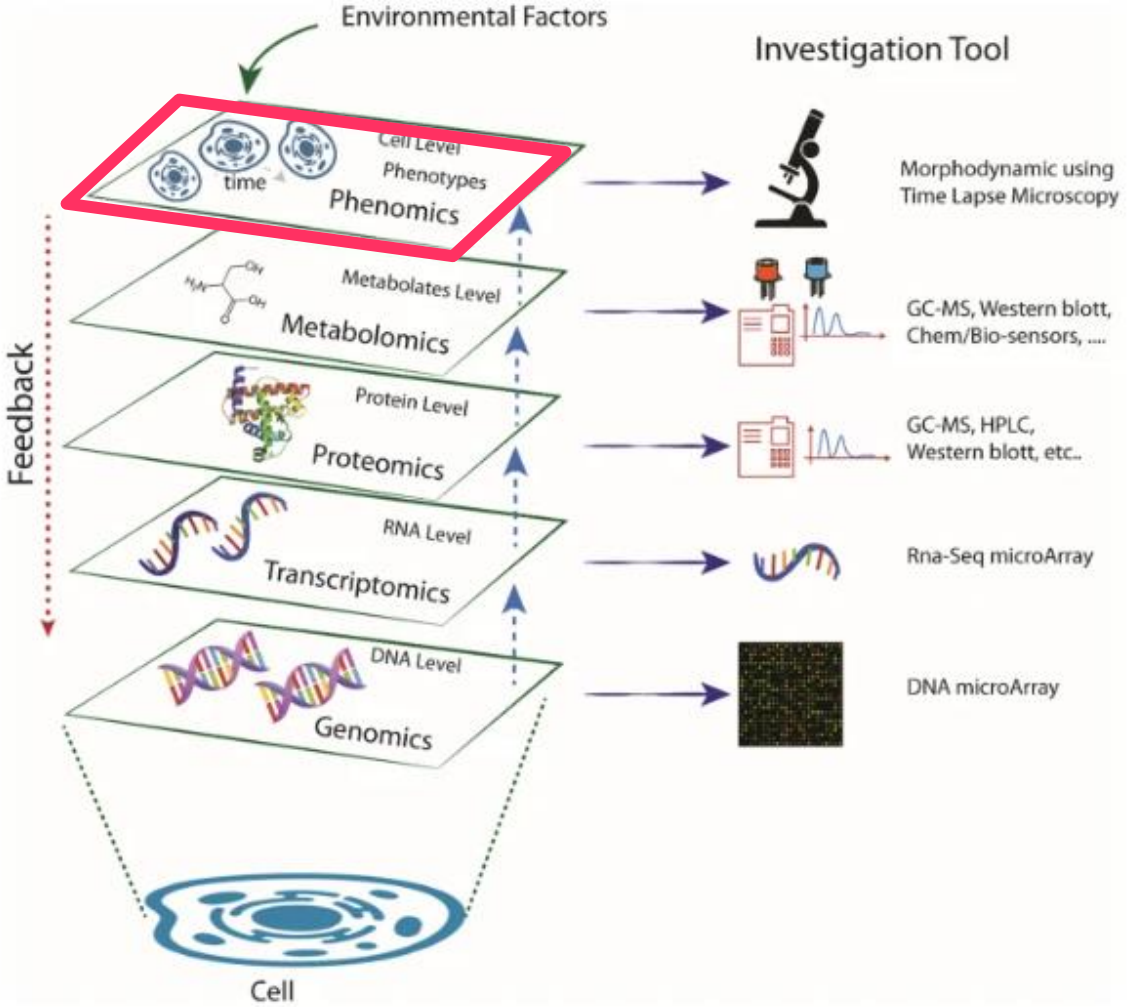


Chemical Biology:

A. Plowright, R. Mannhold, H. Buschmann, J. Holenz: Target Discovery and Validation: Methods and Strategies for Drug Discovery , 2019
H. Waldmann, P. Janning: Concepts and Case Studies in Chemical Biology, 2014
A. D. Miller, J. A. Tanner: Essentials of Chemical Biology
S. Schreiber, T. Kapoor, G. Wess: Chemical Biology, 2007
P. Cromm: Inducing Targeted Protein Degradation: From Chemical Biology to Drug Discovery and Clinical Applications, 2022
P. Wyatt: Proteomics. Principles, Techniques and Analysis, 2018
T. Engel, J. Gasteiger: Chemoinformatics: Basic Concepts and Methods: Basic Concepts and Methods, 2018
F. Lottspeich, J. Engels: Bioanalytics: Analytical Methods and Concepts in Biochemistry and Molecular Biology, 2018

Thank You for your attention!!! Happy to take questions???

Overview PhenOMICs tools

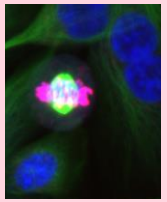


D'Orazio, M., Murdocca, M., Mencattini, A. *et al. Sci Rep* **12**, 8545 (2022).

PhenOMICs Tools IV – Overview Image based approaches

Phenotypic Approach

What is the underlying target or mechanism?



Discovery of phenotypic differences

Example: Mitotic phenotype under Sagopilone treatment

Definition:

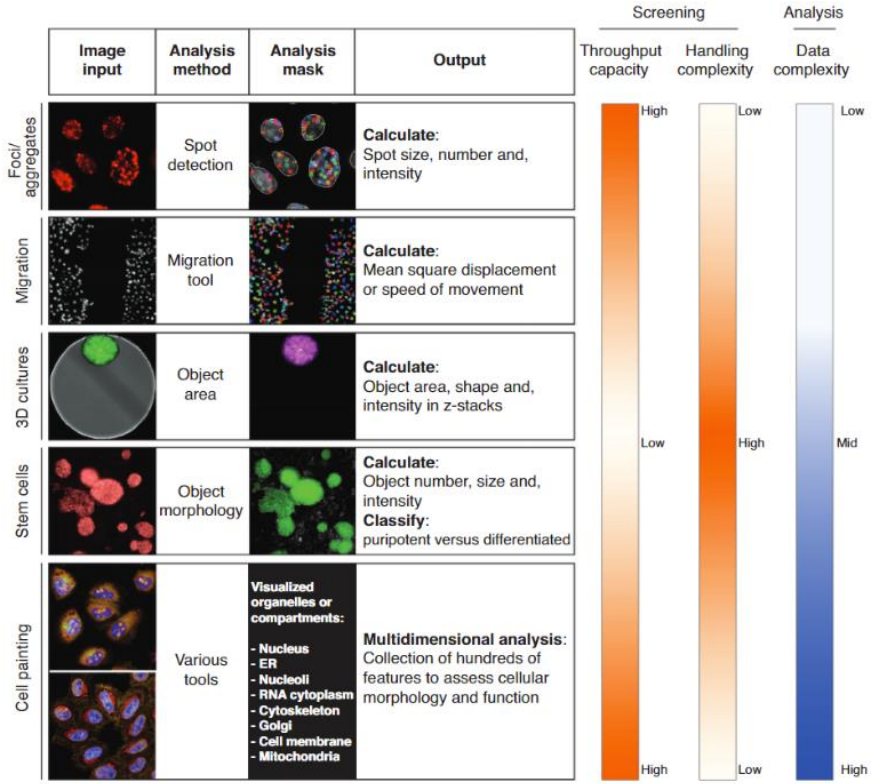
// Characterization of compound induced effects on cellular phenotypes

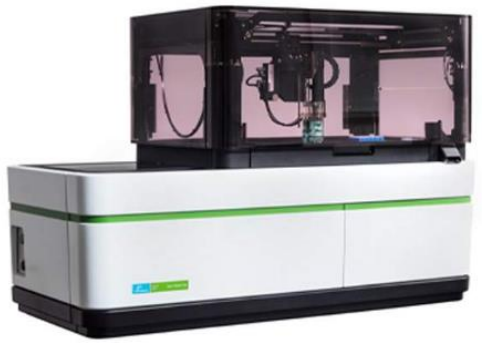
// Multiple readouts possible (Imaging, Omics, ELISA, Histology), however in most cases by **fluorescence microscopy**

// Quantification of effects via **image analysis** covering typically several parameters (High-Content-Screening (HCS))

// Single timepoint analysis and/or kinetic monitoring

// High Importance of target deconvolution strategies





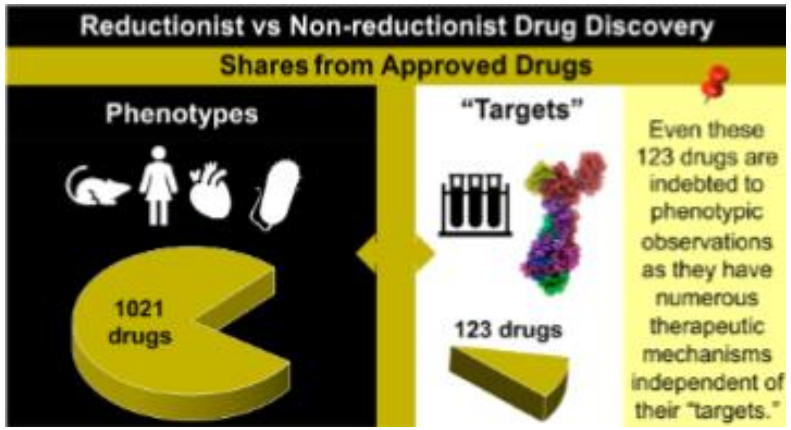
Example of an Automated High-Content Screening Systems

S. Lin, K. Schorpp, I. Rothenaigner, K. Hadian, *Drug Discov. Today* **2020**, *25*, 1348-1361.

The fundamental question: Is target-based drug discovery efficient? A critical view on R&D productivity

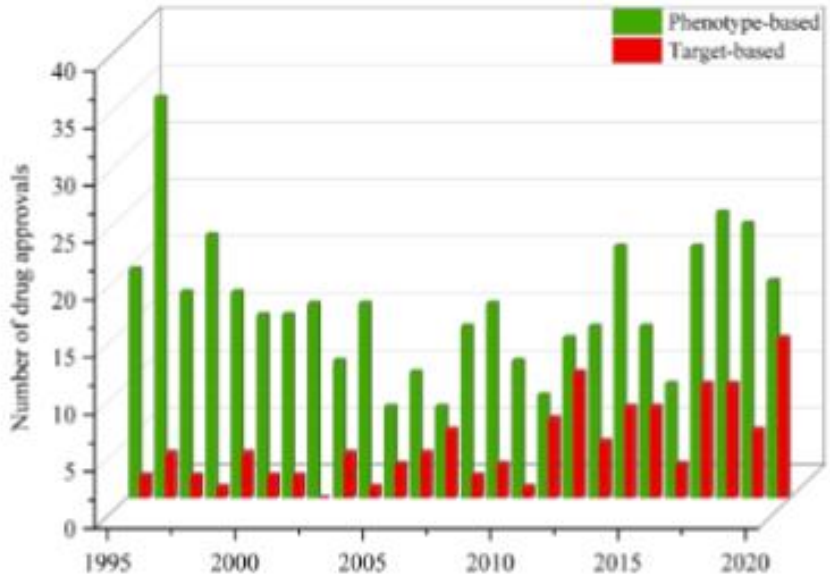
Is Target-Based Drug Discovery Efficient? Discovery and "Off-Target" Mechanisms of All Drugs

Arash Sadri*



- This is the first systematic and comprehensive assessment of the real-world efficiency of target-based drug discovery.
- Merely 9.4% of approved small-molecule drugs have been discovered by this approach.
- Even these supposedly target-based drugs depend on numerous off-target mechanisms for their therapeutic effects.
- Reductionist target-based drug discovery has thus far been inefficient and maybe a cause of the productivity crisis.
- Approaches that prioritize higher-level observations are potentially more efficient based on both observational and theoretical evidence.

Source of FDA approved drugs per year:



J. Med. Chem. 2023, 66, 18, 12651–12677

Many approved and successful drugs are hitting multiple targets although they were optimized for one main target

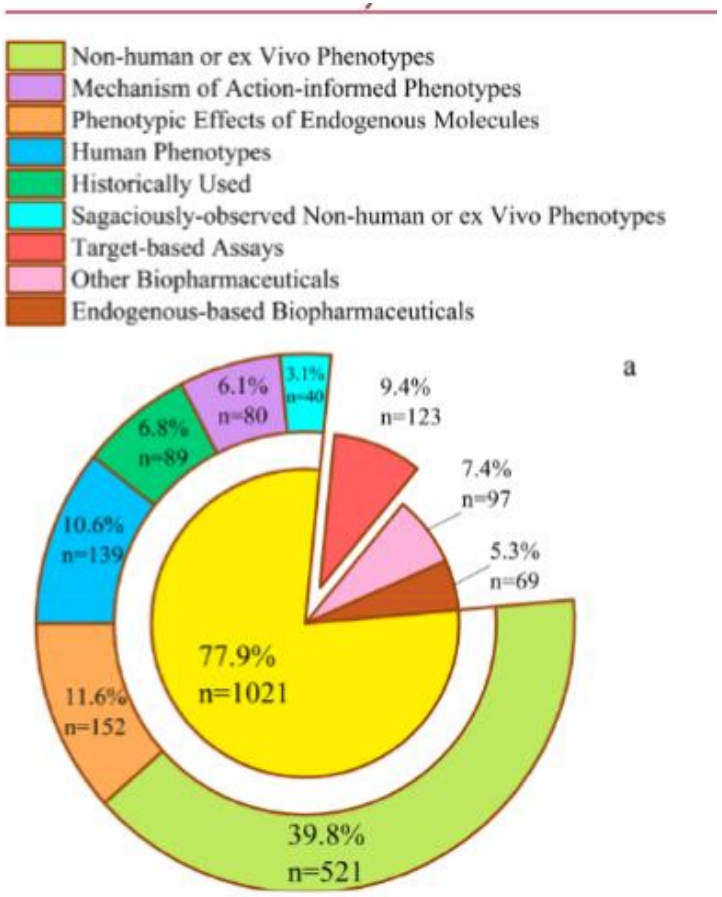
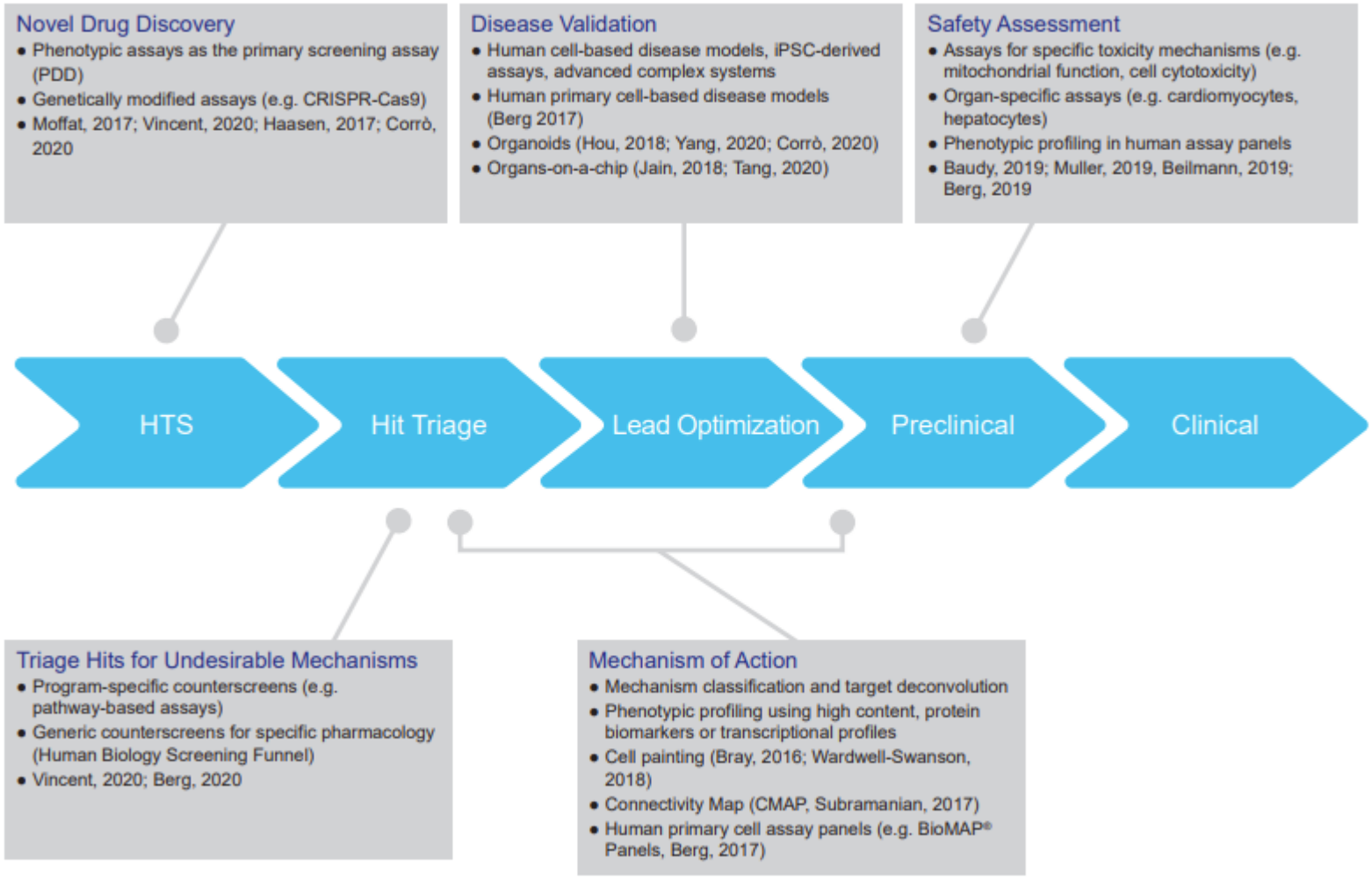


Table 2. Counts of "Off-Target" Therapeutic Mechanisms of "Target-Based" Drugs[a]

DONEPEZIL	40	BORTEZOMIB	69	ORLISTAT	10
ACARBOSE	14	CARFILZOMIB	2	RIMEGEPANT	1
ALISKIREN	12	EFAVIRENZ	2	ROFLUMILAST	9
RIVAROXABAN	7	NINTEDANIB	229	SACUBITRIL	2
EDOXABAN	1	GEFITINIB	111	TIRBANIBULIN	2
ZANAMIVIR	1	ERLOTINIB	109	VENETOCLAX	2
OSELTAMIVIR	1	LAPATINIB	16	SAQUINAVIR	11
FOMEPIZOLE	2	VANDETANIB	114	RITONAVIR	8
SITAGLIPTIN	13	AFATINIB	41	INDINAVIR	7
SAXAGLIPTIN	20	OSIMERTINIB	8	NELFINAVIR	8
LINAGLIPTIN	1	NERATINIB	4	LOPINAVIR	2
ALOGLIPTIN	1	SORAFENIB	140	ATAZANAVIR	1
ELTROMBOPAG	1	PAZOPANIB	107	MARAVIROC	3
ARGATROBAN	1	AXITINIB	102	ELTROMBOPAG	1
DABIGATRAN	2	REGORAFENIB	20	PALBOCICLIB	38
MIRABEGRON	2	LENVATINIB	10	RIBOCICLIB	14
DASATINIB	158	IMATINIB	78	ABEMACICLIB	17
NILOTINIB	63	CERITINIB	9	TALAZOPARIB	3
PONATINIB	13	OLAPARIB	4	CABOZANTINIB	5
BOSUTINIB	74	RUCAPARIB	17	ACALABRUTINIB	3
CRIZOTINIB	148	NIRAPARIB	2	VEMURAFENIB	18
TOFACITINIB	34	IBRUTINIB	40	SUNITINIB	270

J. Med. Chem. **2023**, *66*, 18, 12651–12677

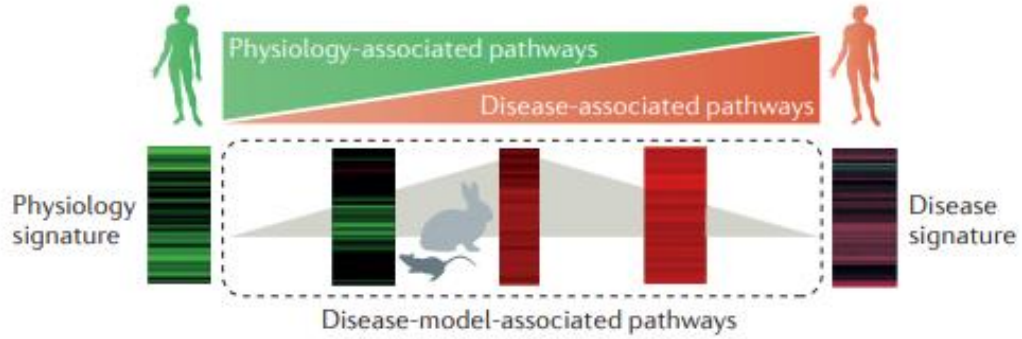
Phenotypic drug discovery opens a different perspective to assess the polypharmacological behaviour of small molecules



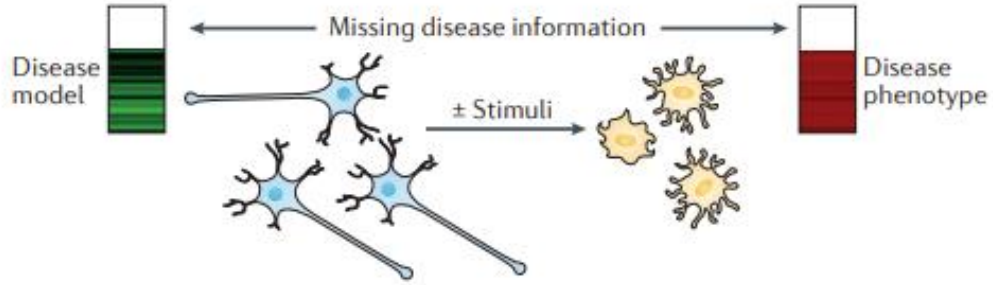
E. L. Berg, *Cell Chem Bio* **2021**, *28*, 424-430

Phenotypic Drug Discovery – step by step

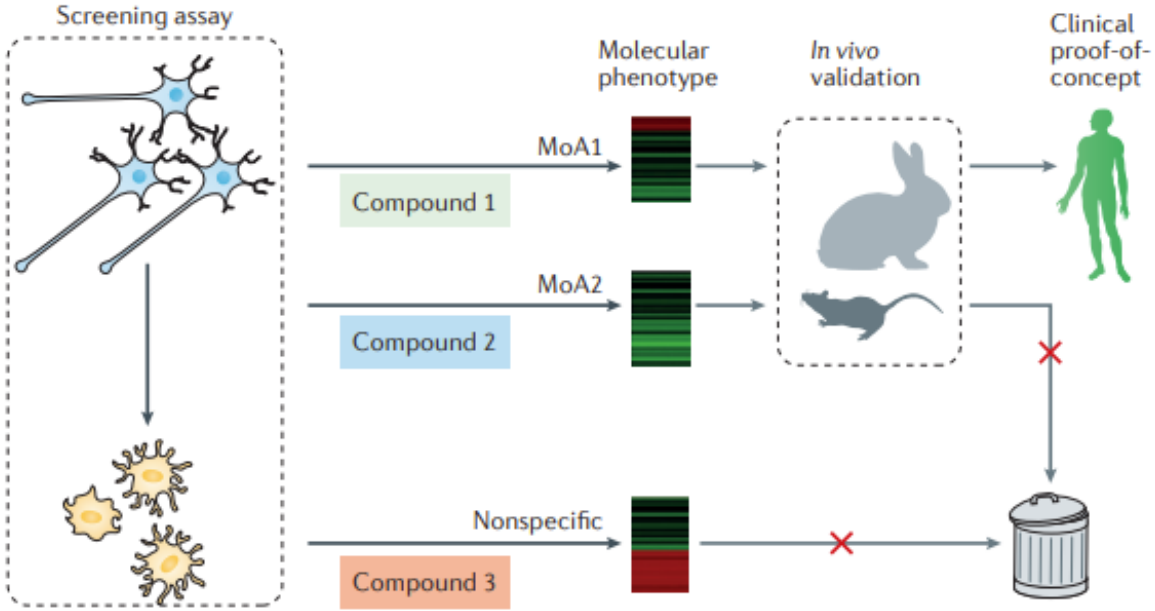
Step 1: Definition of the phenotypic signature



Step 2: Implementation of the phenotypic model



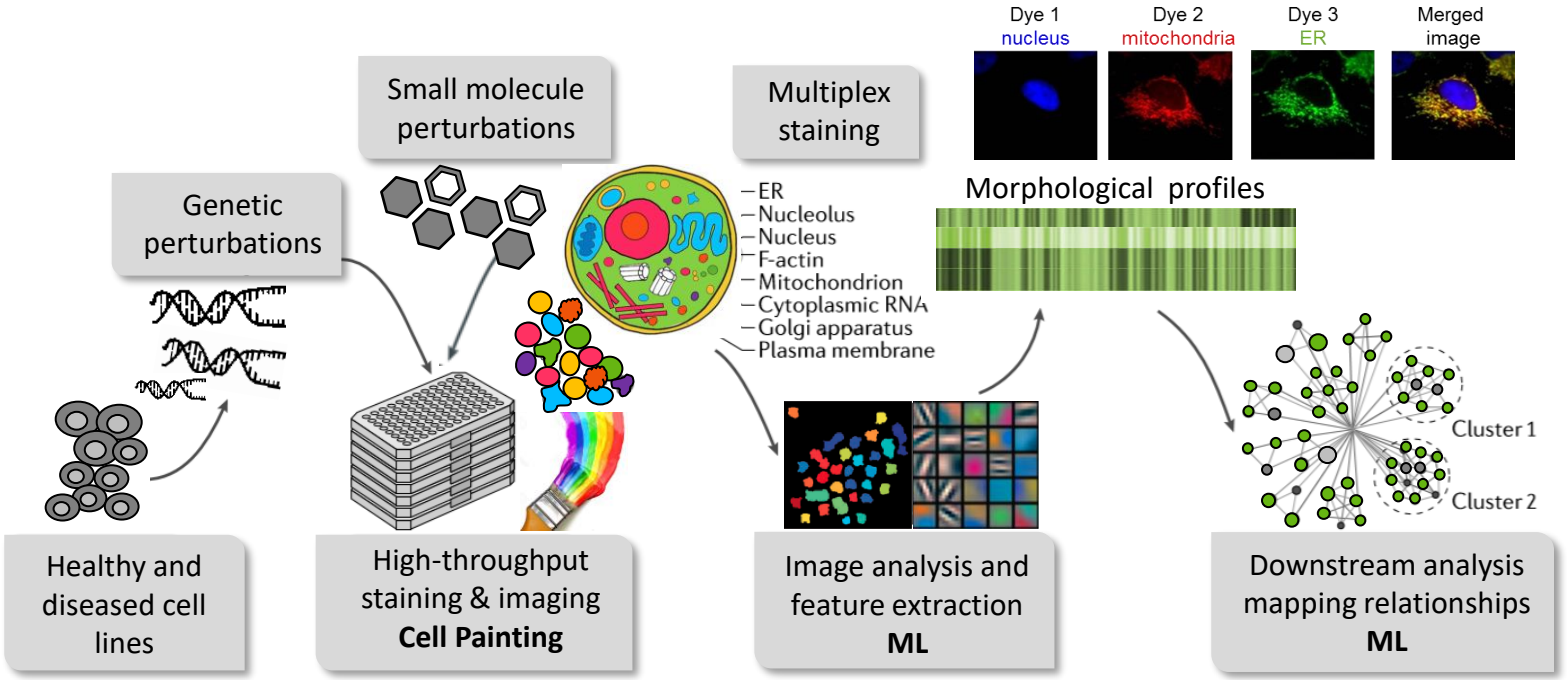
Step 3: Screening and MoA Deconvolution

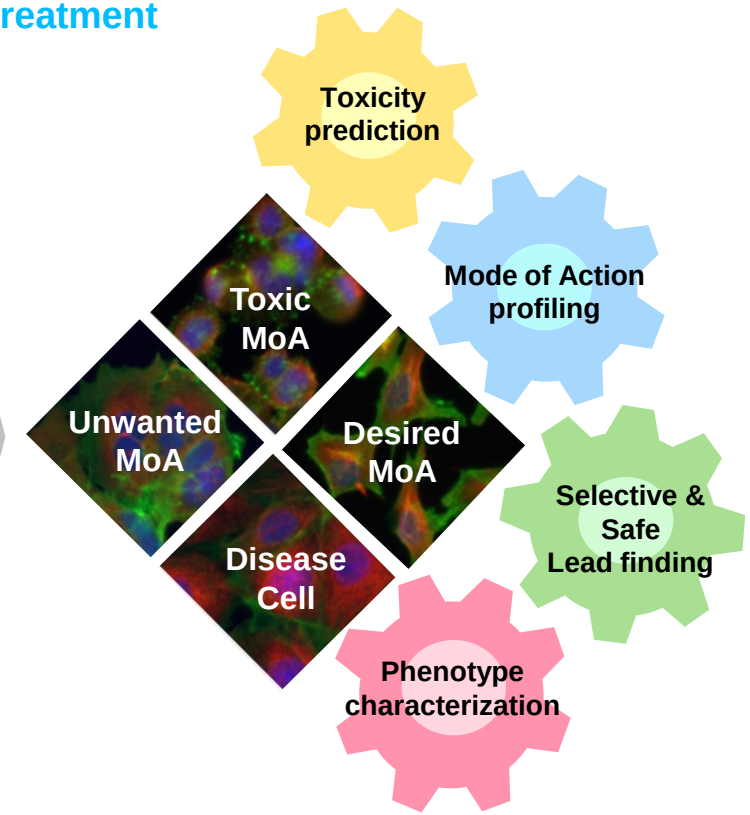


J. G. Moffat, F. Vincent, J. A. Lee, J. Eder, M. Prunotto, *Nat. Rev. Drug Discov.* **2017**, *16*, 531-543.

Cell Painting: A novel Imaging approach for cellular phenotyping

Cell Painting allows the fingerprinting of a huge variety of cellular changes after compound treatment

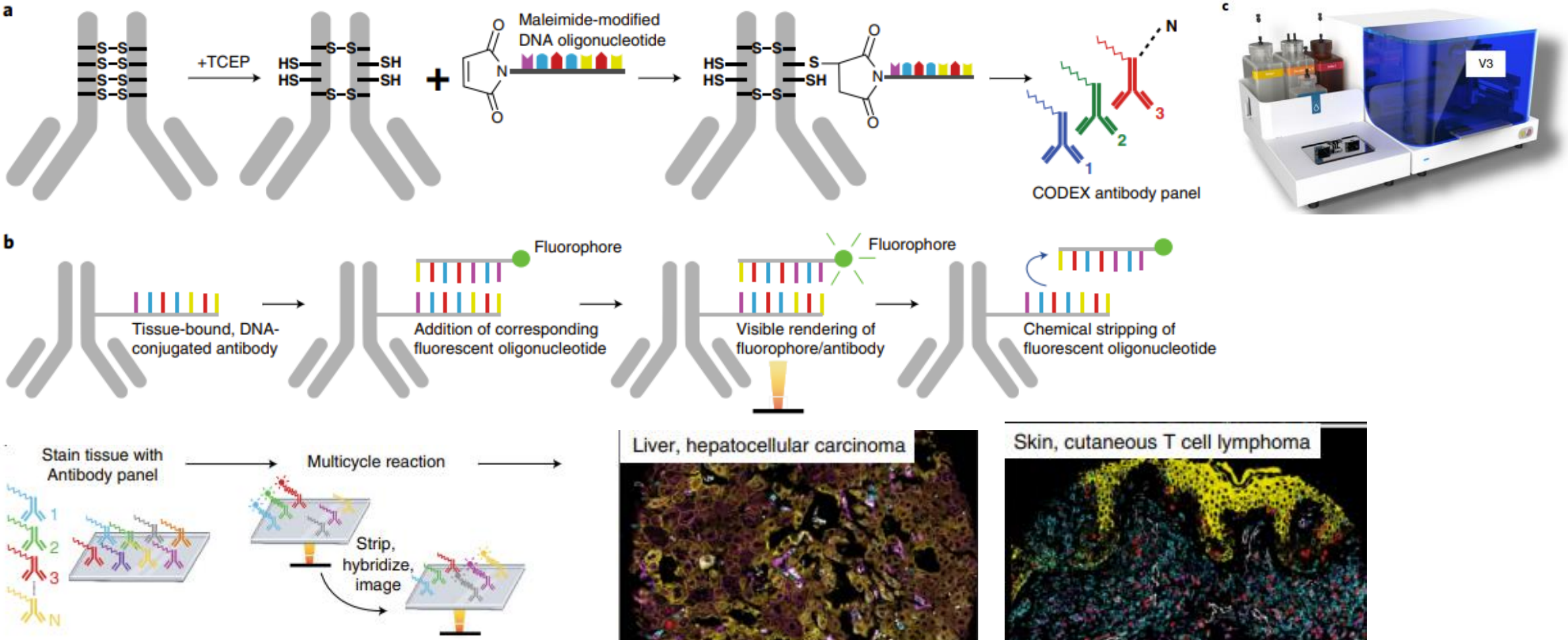




Combination with Machine Learning and AI approaches gives access to a novel phenotypic target and lead structure space

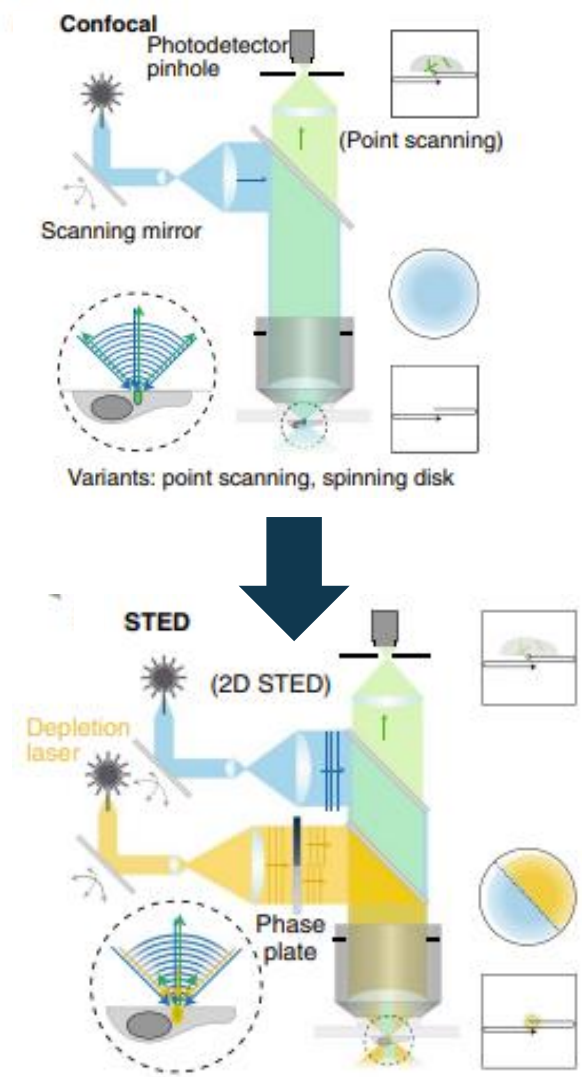
A. E. Carpenter et al., *Nat. Protoc.* **2016**, *11*, 1757-1774.

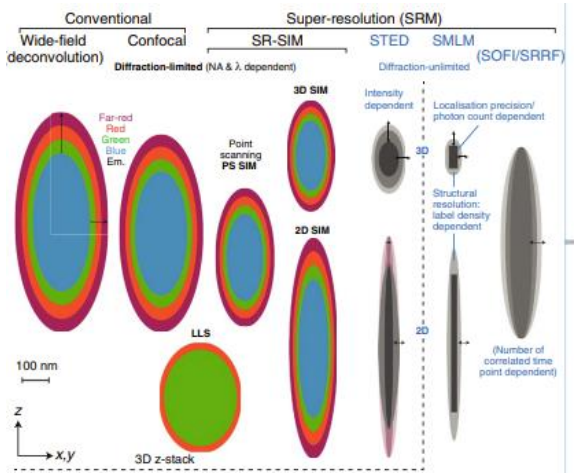
Advances in labeling techniques have huge influence on phenotypic drug discovery: Example CODEX technology



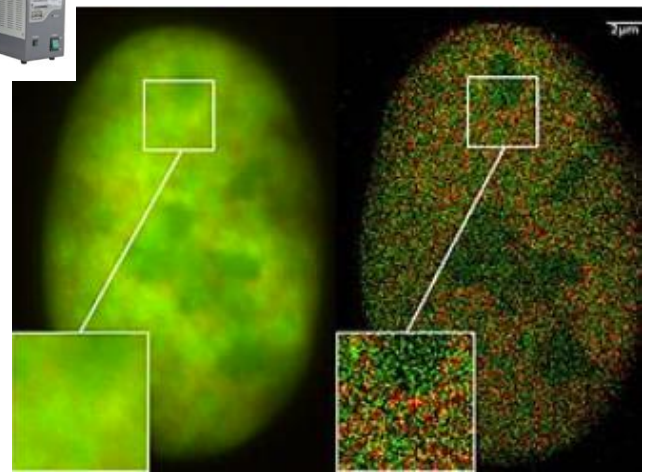
Black, S., Phillips, D., Hickey, J.W. *et al*. *Nat Protoc* **16**, 3802–3835 (2021).

Outlook: The resolution becomes constantly better: Super-Resolution Microscopy

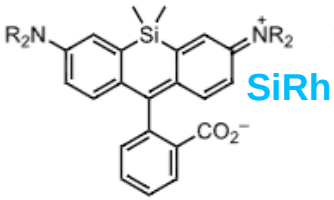




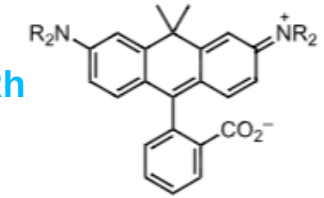
conventional vs super-resolution

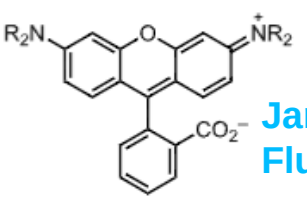


Examples for super resolution dyes:

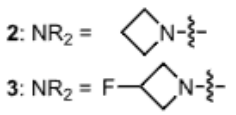


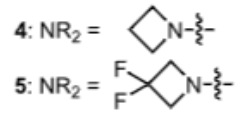
SiRh





Janelia Fluor



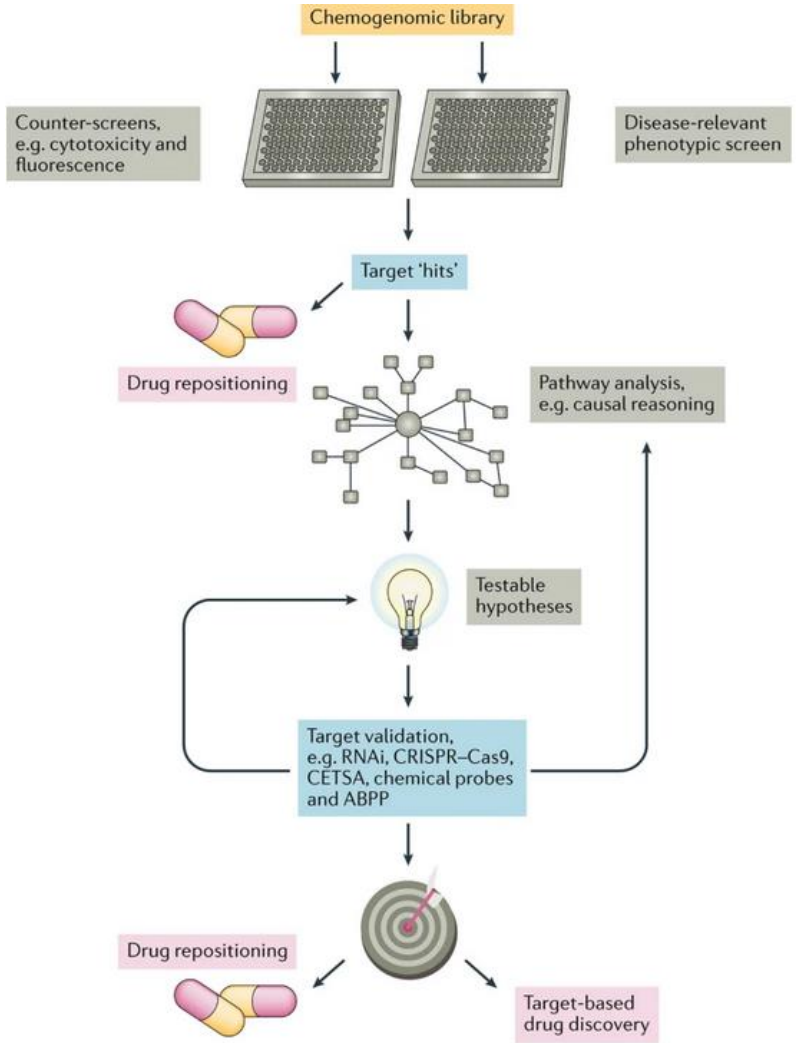


Schermelleh, L., Ferrand, A., Huser, T. *et al.* Super-resolution microscopy demystified. *Nat Cell Biol* **21**, 72–84 (2019).

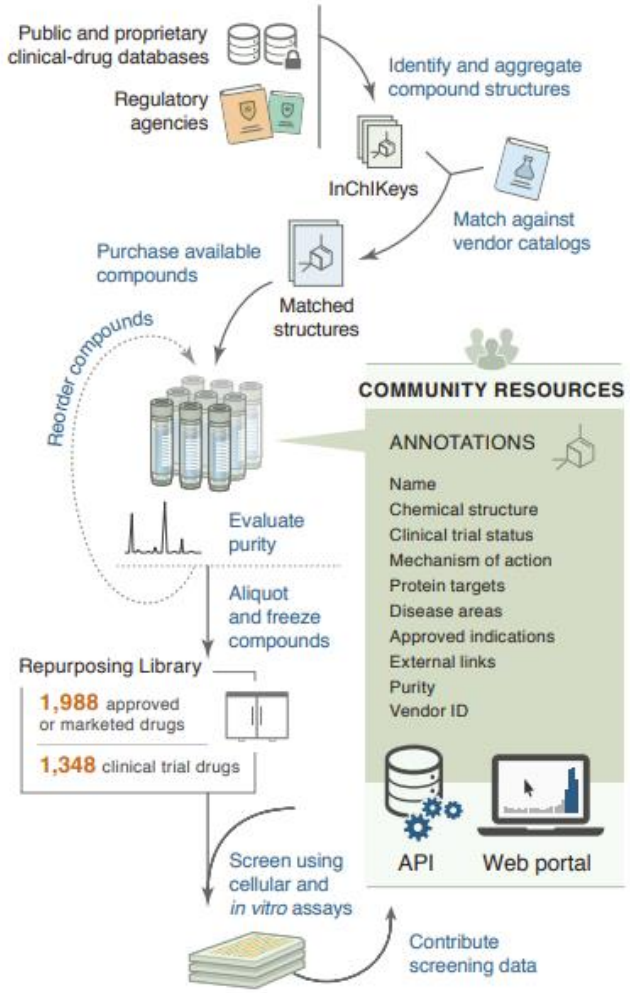
Q. Zheng, A. X. Ayala, I. Chung, A. V. Weigel, A. Ranjan, N. Falco, J. B. Grimm, A. N. Tkachuk, C. Wu, J. Lippincott-Schwartz, R. H. Singer, L. D. Lavis, *ACS Cent. Sci.* **2019**, *5*, 1602-1613.

The basis of PhenOmics based drug discovery: A well annotated Chemogenomics Library

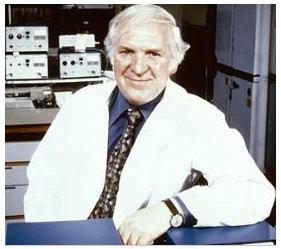
Use cases:



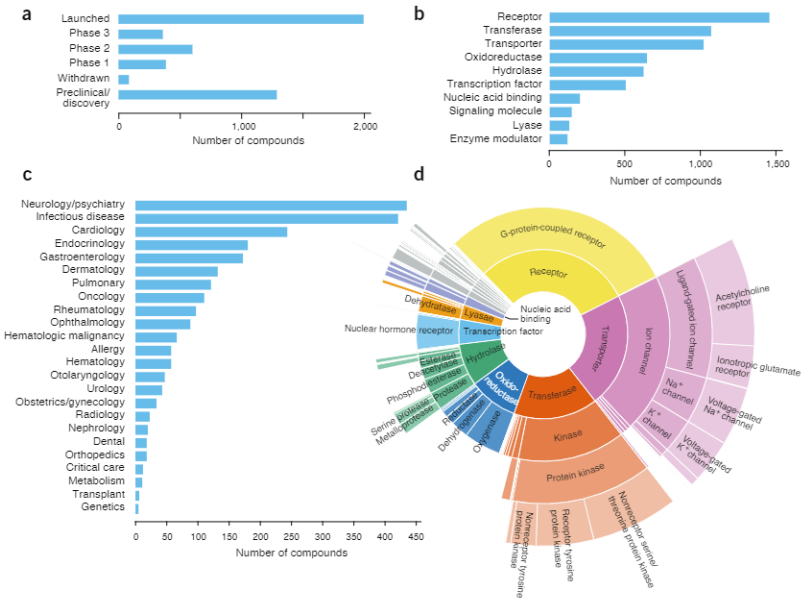
Typical construction workflow:



„The best way to discover a new drug is to start with an old one“
- James Black



Composition example:



L. H. Jones, M. E. Bunnage, *Nat. Rev. Drug Disc.* **2017**, *16*, 285-296.

Corsello, S., Bittker, J., Liu, Z. *et al.* *Nat Med* **23**, 405–408 (2017).