High Throughput Screening at EU-OPENSCREEN

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European Research Infrastructure for Chemical Biology and Early Drug Discovery

EU-OPENSCREEN provides access to

- Technologies (screening platforms)
- Resources (compound collections)
- Expertise (assay development, medicinal chemistry)
- Data (compound structures, bioactivity)
- Training

Collaboration partners from academia and industry


eu openscreen

Member Countries and Partner Sites

- EU-OPENSCREEN is a distributed RI with 24 partner sites across Europe
- 3 partner site categories:
  - Screening platforms (17)
  - Chemistry groups (6)
  - Database host (1)
- Partner site accreditation is a 3-step procedure:
  - Nomination of site by ministry
  - Evaluation by external reviewers
  - Approval of individual sites by all ERIC member countries, based on evaluation reports

Screening & Chemistry Partner Sites

Denmark

Finland

Czech Republic

Latvia

Poland

Norway

Spain

Germany

in Braunschweig

Ursula Bilitewski

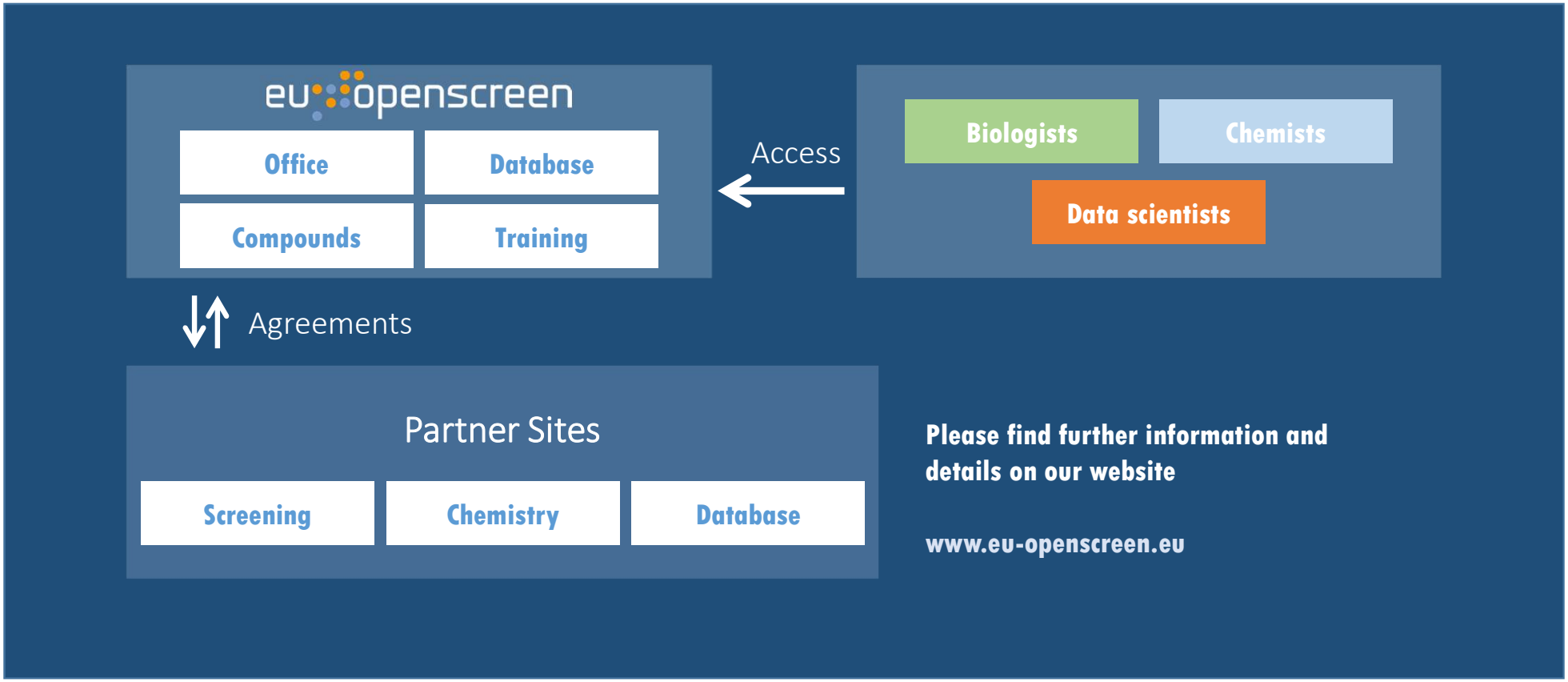
in Hamburg

Phil Gribbon

in Berlin

Jens von Kries
Marc Nazaré

Access model

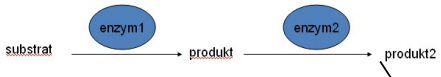


Two Libraries available for Screening at the FMP

Internal FMP Library	EU-OPENSCREEN Library
75.000 cmpds	100.000 cmpds
Acquired 2010 - 2020	Acquired in 2020-2021
Selection based on different concepts (highly diverse, spiro, sp3 rich, macrocycles, natural derived cmpds, bioactives, fragments)	Selection based on 5 different computational groups with 5 methods (highly diverse, 2464 bioactives, fragments)
25 % of structures visible	Structures visible in the ECBD
Structures of primary hits visible	Structures of primary hits visible
Comprehensive knowledge of properties	Property analysis initiated
User control of the data	User control of the data (obligation to publish after 3 years embargo in the ECBD)
8.000 academic cmdps available	Process initiated, target 40.000 cmpds
Costs and conditions individually negogiated	Cost and conditions in the EU-OPENSCREEN network (e.g. replenishment fee)
Library only accessible at the Screening Unit	Library accessible at 10 different Partner Sites
No structural overlap between the libraries	

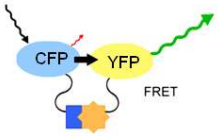
Which Screening Technologies to Apply?

Enzyme activity

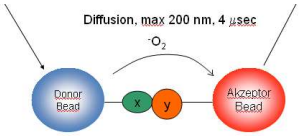


Peptide Posphorylation

Protein-Protein binding

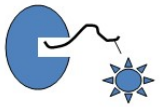


FRET/TR-FRET

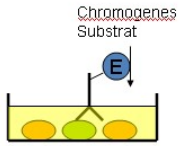


Alphascreen

Protein-Peptide binding

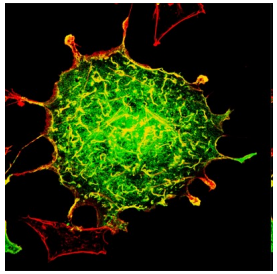


Fluorescence polarisation



ELISA

High Content Screening



luminescene

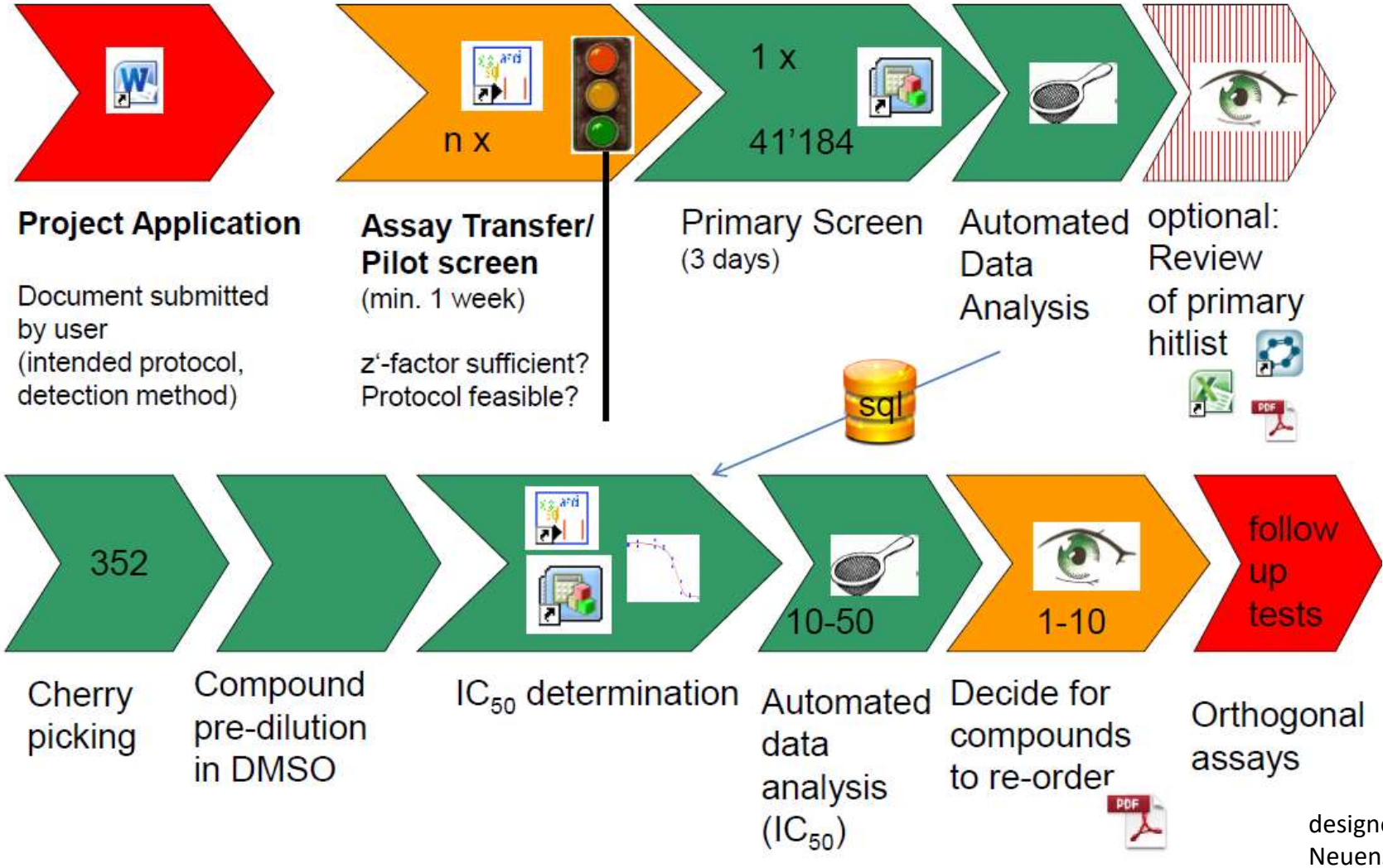
Capillary Electrophoresis

FLIPR

Plate Readers

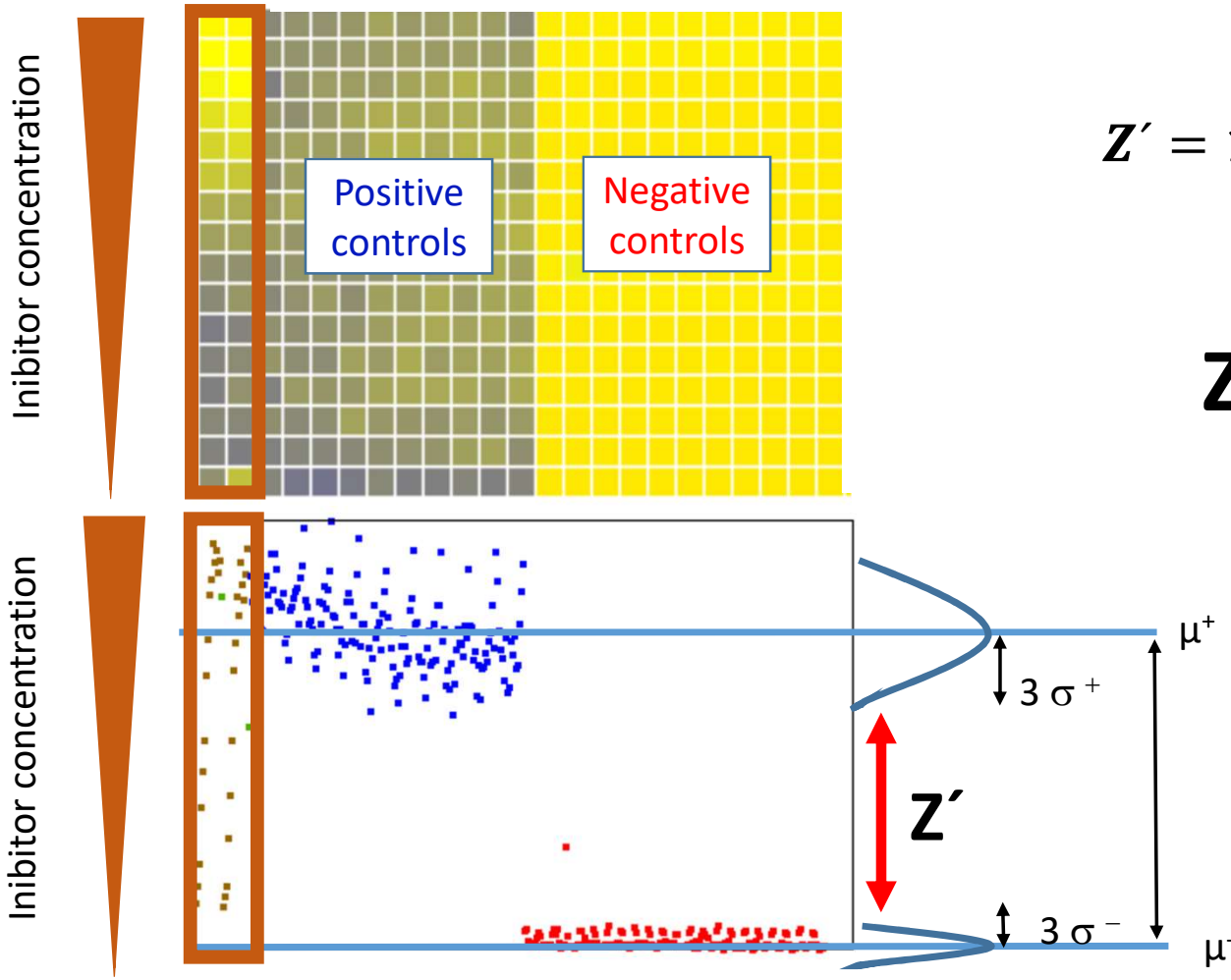
High Content Microscopes

Our Workflow



designed by Martin Neuenschwander
Neuenschwander@fmp-berlin.de

Assay Quality, Z´ Prime Factor



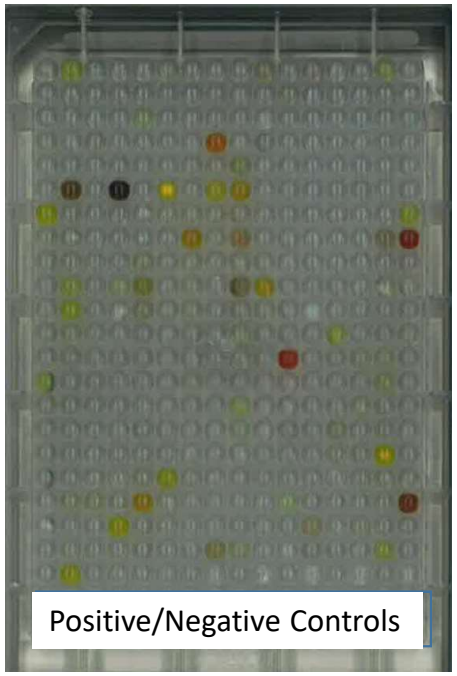
$$Z' = 1 - \frac{3\,(\sigma^- + \sigma^+)}{|\mu^- - \mu^+|}$$

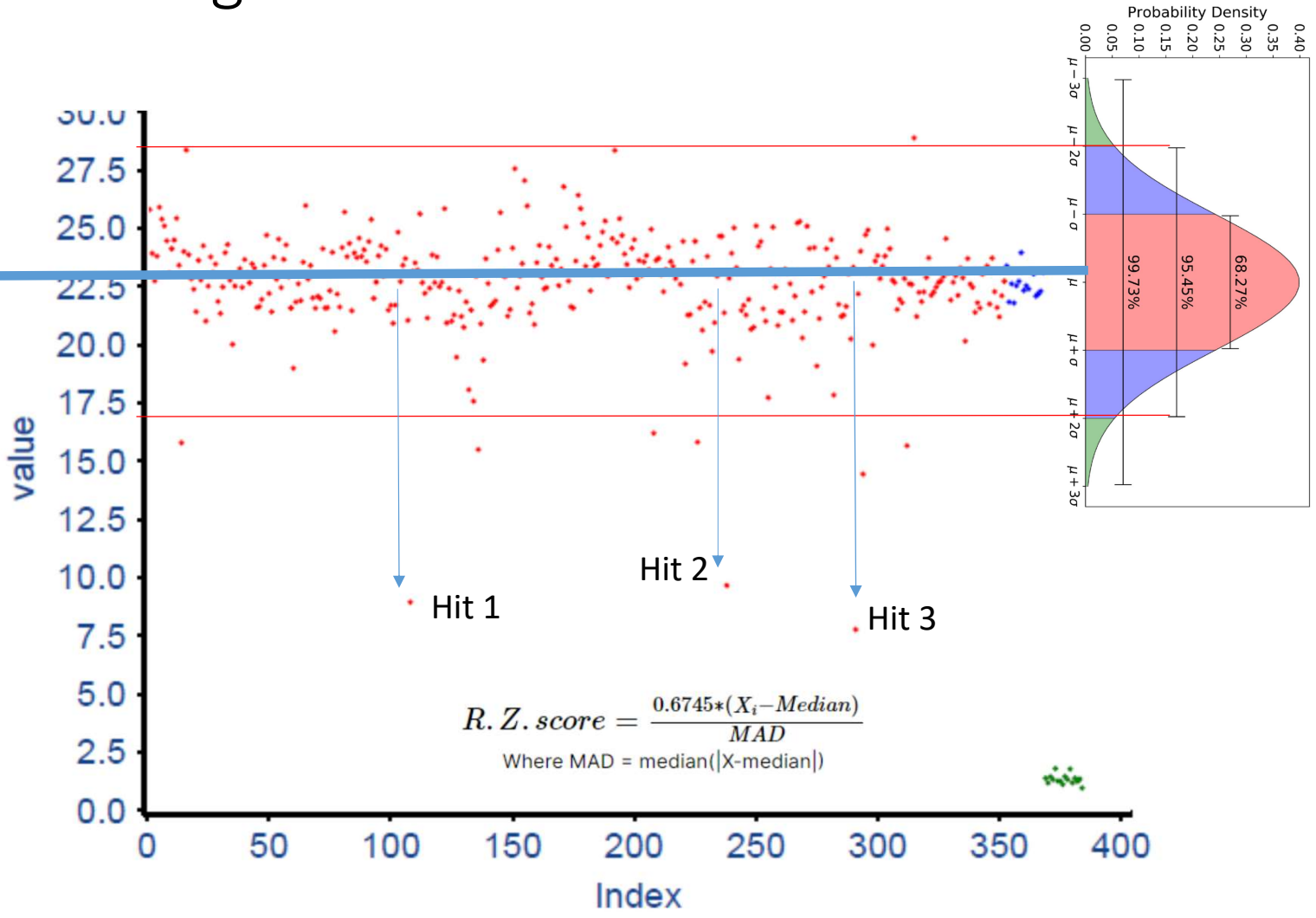
Z´ > 0.5 ☺

Iversen PW, Beck B, Chen YF, et al. HTS *assay validation*. http://www.ncbi.nlm.nih.gov/books/NBK83783/ ; Zhang JH, et al. *A simple statistical parameter for use in evaluation and validation of high throughput screening assays*, J. Biomol. Screening, 1999, Volume 4, Number 2, 67-73.

Robust Statistics against Outliers: the Median

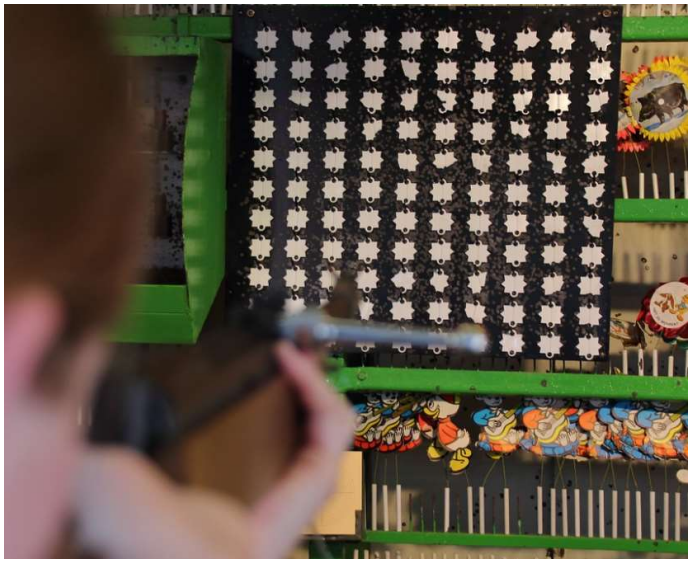
Median instead of Mean



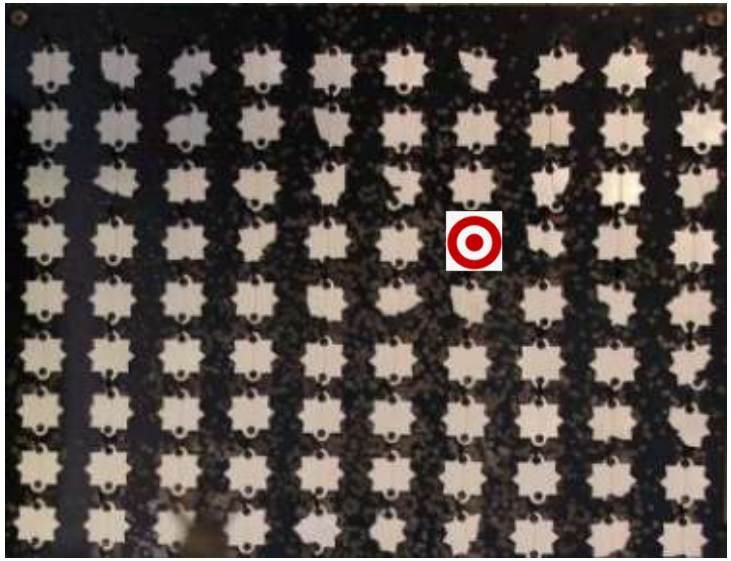


Brideau C., et al. *Statistical methods for hit selection in high-throughput screening*, J Biomol. Screening, 2003, 8, 634-647.

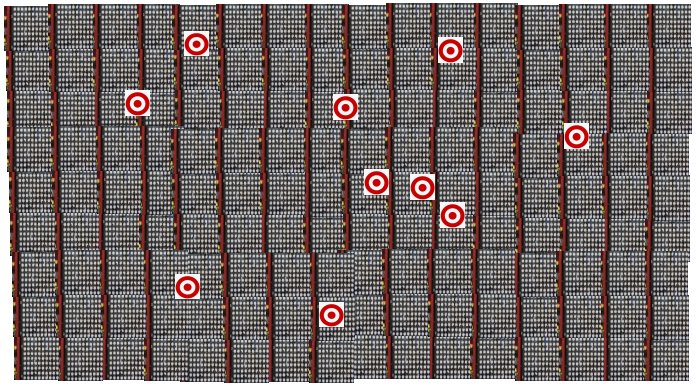
Empirical Hit Rate versus Assay Specificity



Source: Pixabay



Shooting specificity 99 %



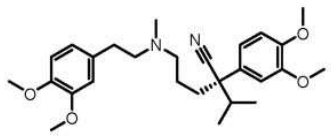
100.000 Cmpds library with an empirical hit rate of true positives
0.01 % => 100.000 x 0.00001 = **10** Hits

Assay/Shooting Specificity of 1%
100.000 x 0.01 = 1.000 Cmpds
=> **990** shots misleading

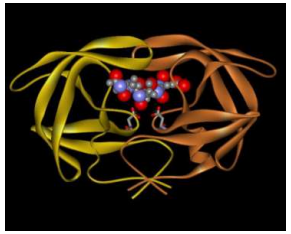
The large majority of hits are stochastic false positives

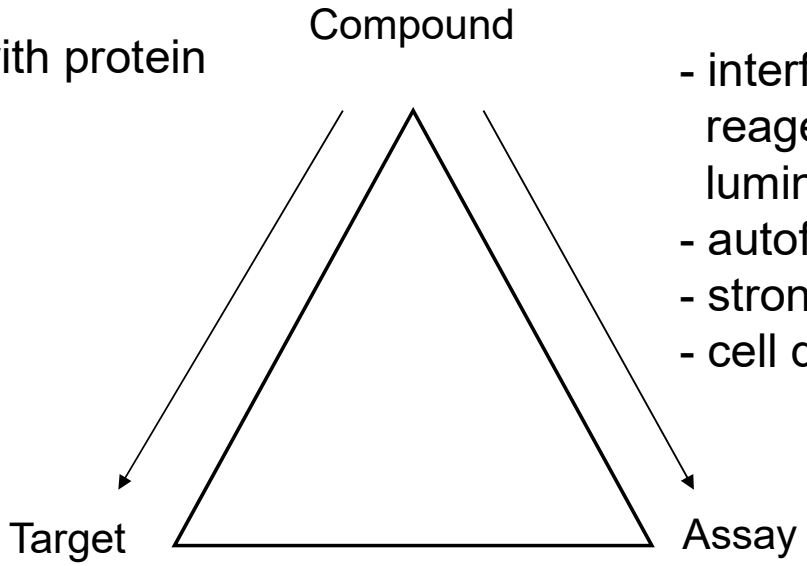
Zhu T., et al. *Hit Identification and Optimization in Virtual Screening: Practical Recommendations Based Upon a Critical Literature Analysis,* J Med. Chem., 2013, 56(17), 6560-6572.

How to find Property based False Positives?

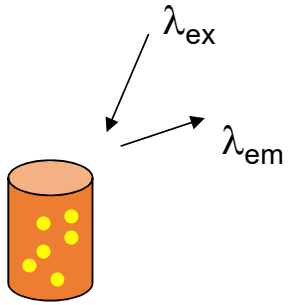


- covalent reaction with protein
- unspecific binding
- aggregation



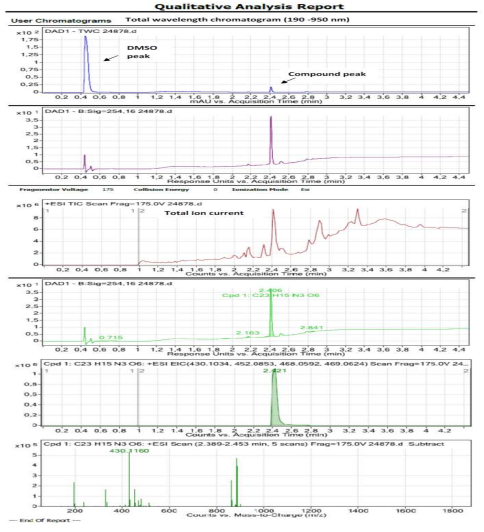


- interference with detection reagents (alphascreen, luminescence)
- autofluorescence (FRET, FP)
- strong color (luminescence)
- cell death

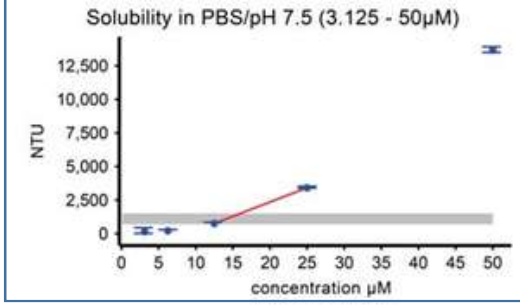


Analyse the Properties of Compounds

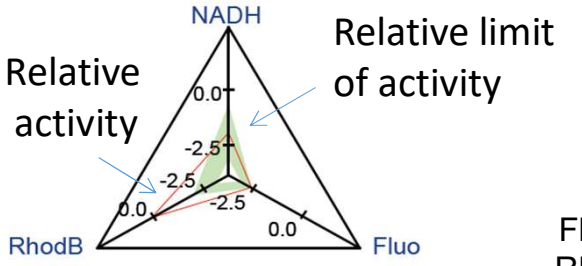
Purity of each cmpd

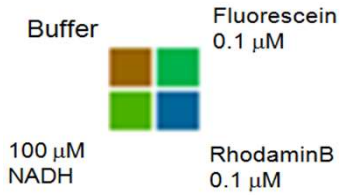


Solubility of each cmpd



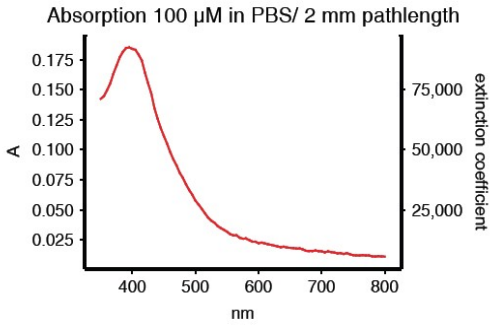
Fluorescence of each cmpd

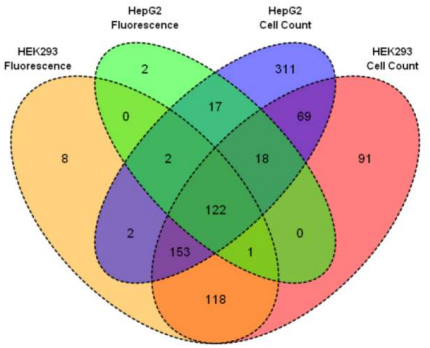




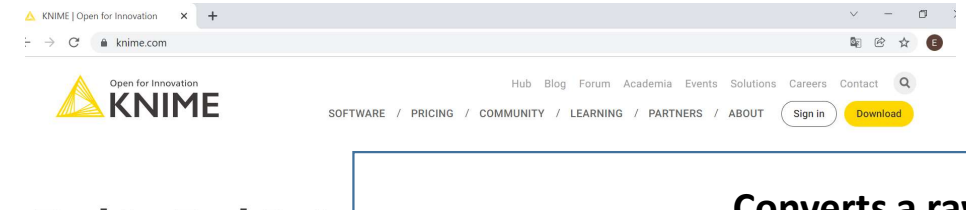
NADH: 340/465 nm
Fluorescein: 485/535 nm
Rhodamine: 560/610 nm

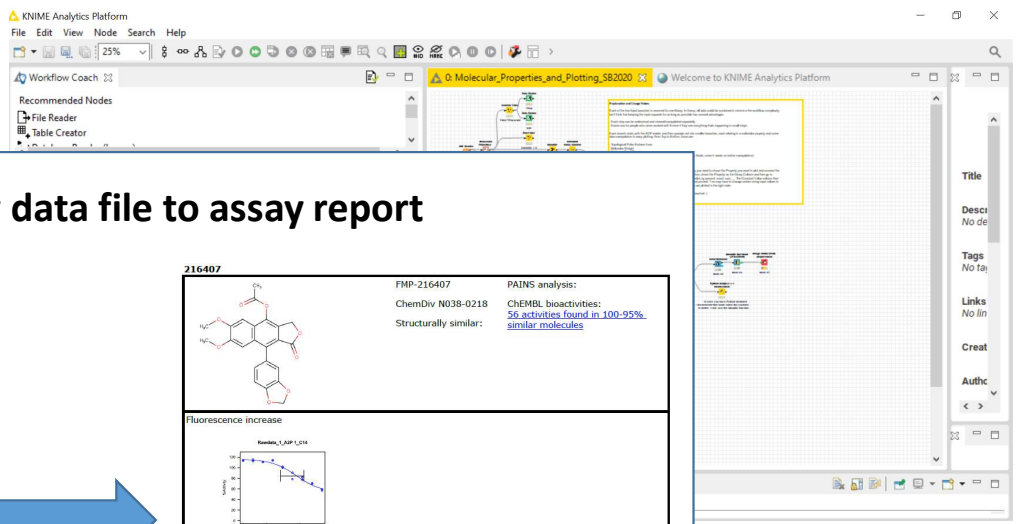
Absorbance and cytotoxicity of each cmpd

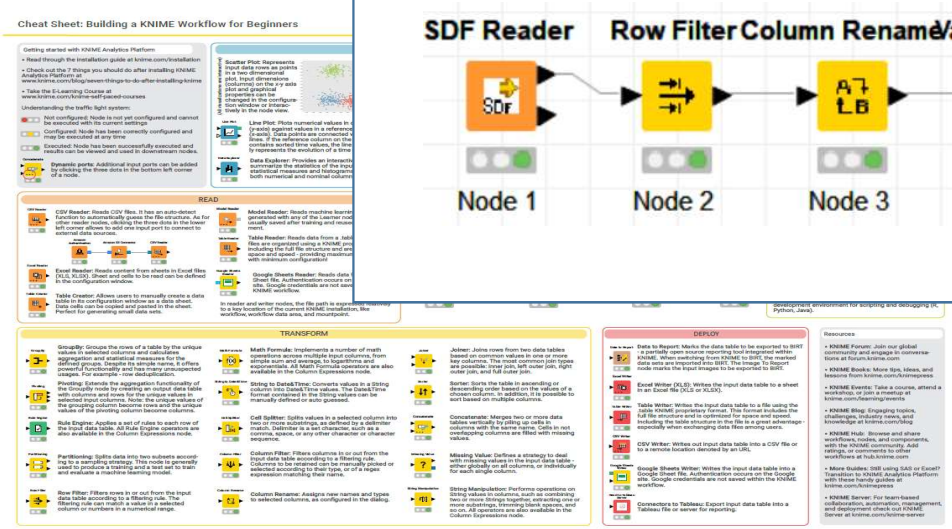


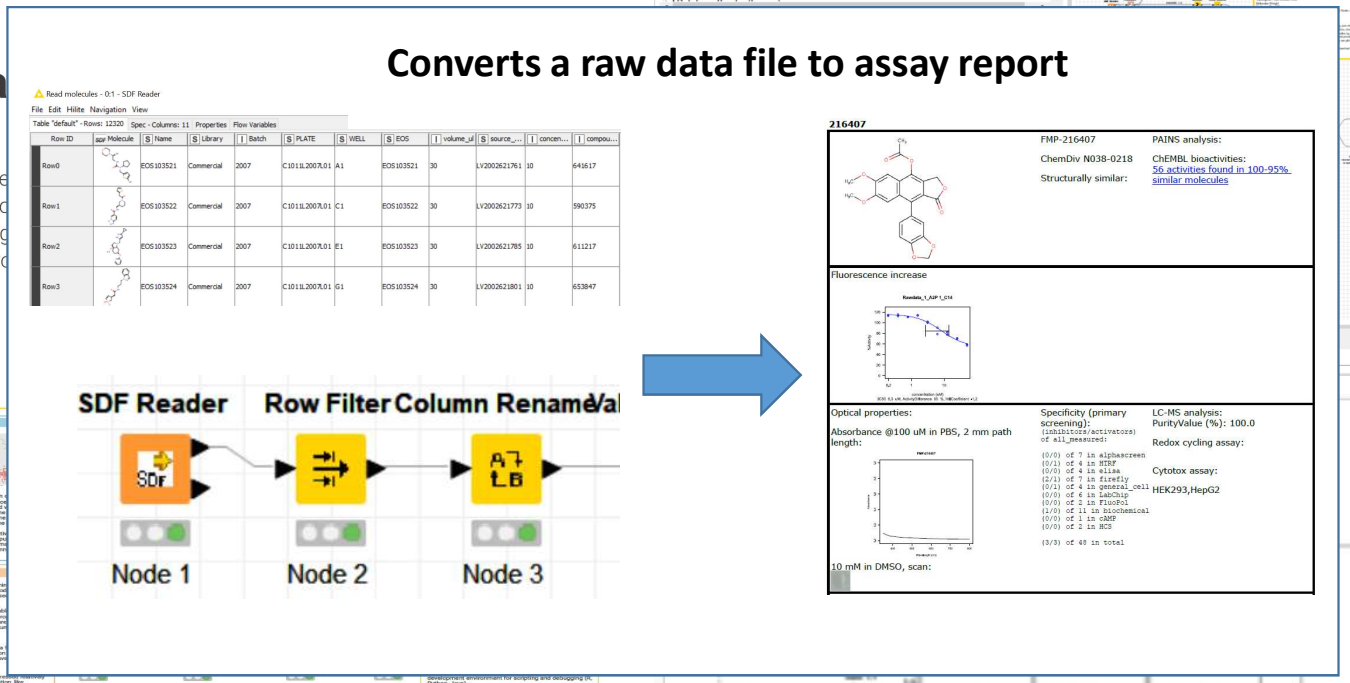


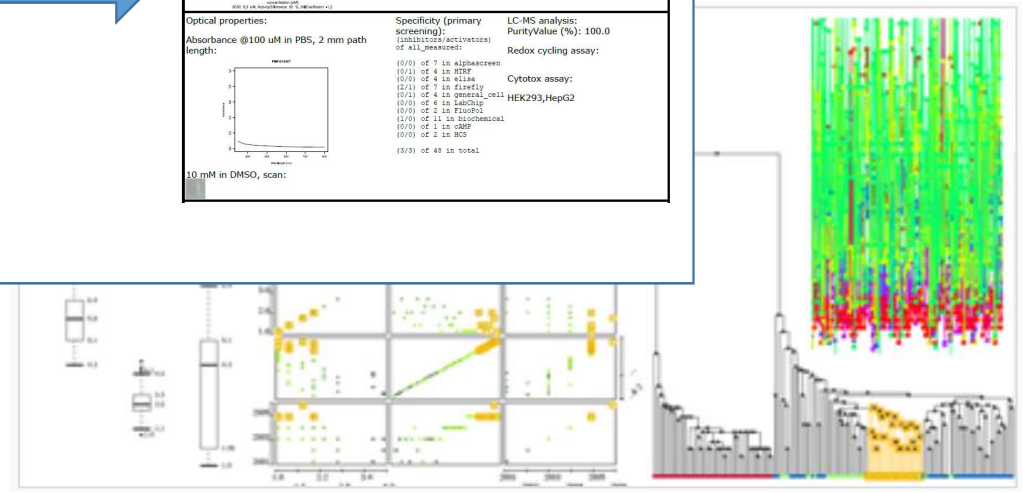
Konstanz Information Miner (www.knime.org)





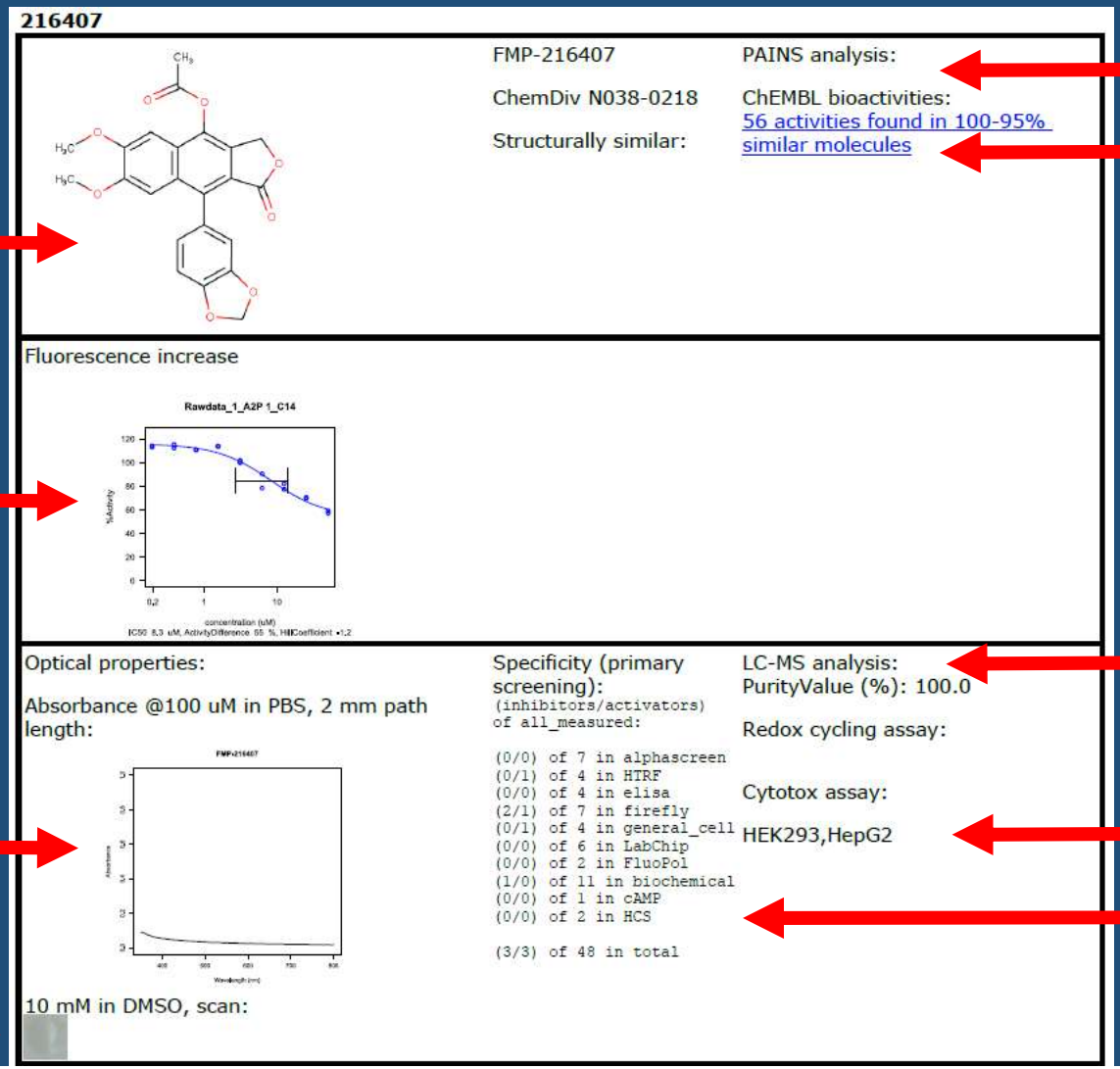






http://informationandvisualization.de/blog/knime-interactive-views#box

Example of Assay Report



Cmpd structure

IC_{50}-Evaluation

Wavelength scan

Well picture

No PAINS structure

56 activities in ChEMBL

LC-MS Purity

Cytotoxic in two cell types

Active in 3 assays

designed by Martin Neuenschwander
Neuenschwander@fmp-berlin.de

EU-OPENSCREEN Libraries

European Chemical Biology Library (ECBL)

Diversity library

- 99.096 structurally highly diverse compounds
- Average MW=350 g/mol
- 0.05 % of PAINS

Horvath D. *et al.*, ChemMedChem 2014, *9*, 2309

CHEMMEDCHEM FULL PAPERS — ChemPubSoc Europe

DOI: 10.1002/cmdc.201402126

Design of a General-Purpose European Compound Screening Library for EU-OPENSCREEN

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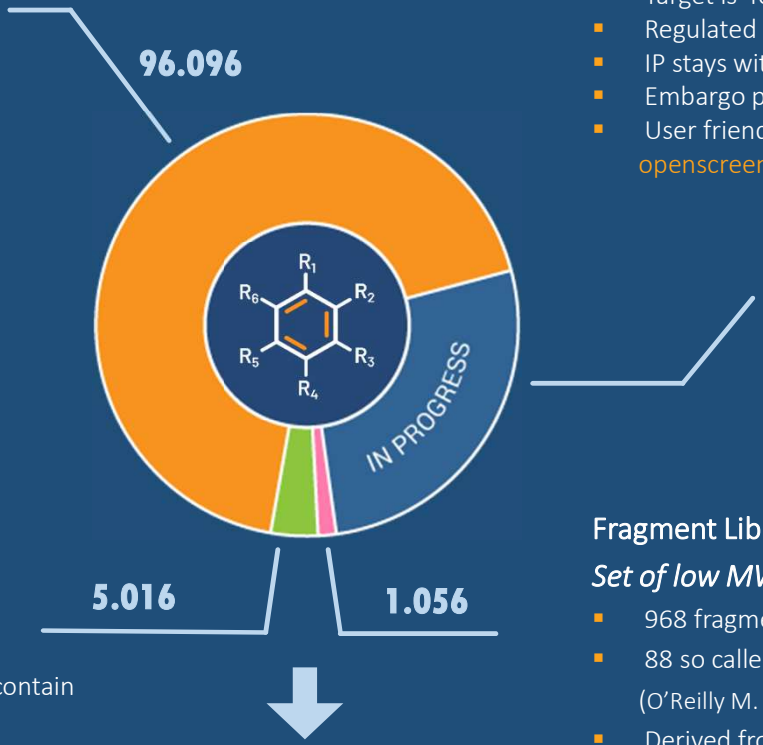


European Chemical Biology Library (ECBL)

Pilot library

- 2.464 bioactives: active against 1039 different targets, contain 654 approved drugs and 368 highly selective probes
- 2.464 representative compounds of the diversity library
- 88 assay interference compounds in 4 dilutions

European Chemical Biology Database

https://ecbd.eu/

The European Academic Compound Library (EACL)

Novel compounds sourced from chemists worldwide

- Target is 40.000 compounds
- Regulated and confidential access (e.g. MTA)
- IP stays with the chemist
- Embargo period up to 3 years
- User friendly online submission: http://www.eu-openscreen-cmpds-donation.eu/login.php

Fragment Library NEW in 2020!

Set of low MW and ultra-low MW fragments

- 968 fragments with HAC > 8 in DMSO-d_6
- 88 so called "minifrags" with HAC < 8 in DMSO-d_6 (O'Reilly M. *et al.*, Drug Discov. Today **2019**, *24*, 1081)
- Derived from the fragment space of the ECBL
- Collaboration with INSTRUCT/ iNEXT-Discovery sites

Library Quality and Questionnaire for Screening

- **Highly diverse** with a plethora of different selection concepts
- **Small clusters** of similar compounds for early SAR
- Lower numbers in property distribution compared to Rule of 5 (Lipsinski Rules) e.g Rule of 3
- How unique are the structures? What is known about similar compounds in the literature?
- Comparison with **Chembl** (publications: https://www.ebi.ac.uk/chembl/)
- Comparison with **SureChembl** (patents: https://www.surechembl.org/)
- Cmpds are **synthesizable, easily to derivatize and scalable?**
- Open access to **negative screening results?**
- **Transparency about the selection** process of the hit list?
- **User involvement in the selection** process of the hit list?
- Access to specific libraries for specific targets and applications
- Cmpds property knowledge to filter for false positives.

Comparison to External Libraries

NIH Library (USA)
882.649 commercial and academic compounds (PubChem)

Chimiothèque Nationale CN (France)
62.824 academic compounds (2018)

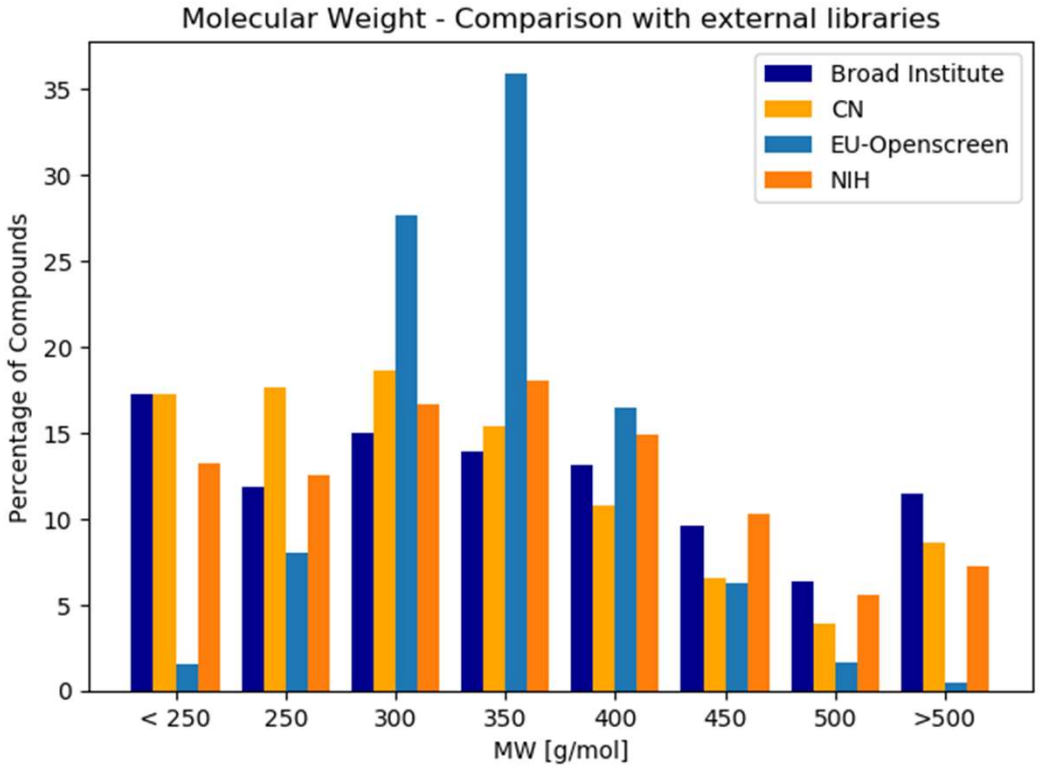
Broad Institute (USA, Boston)
5.681 bio-annotated compounds

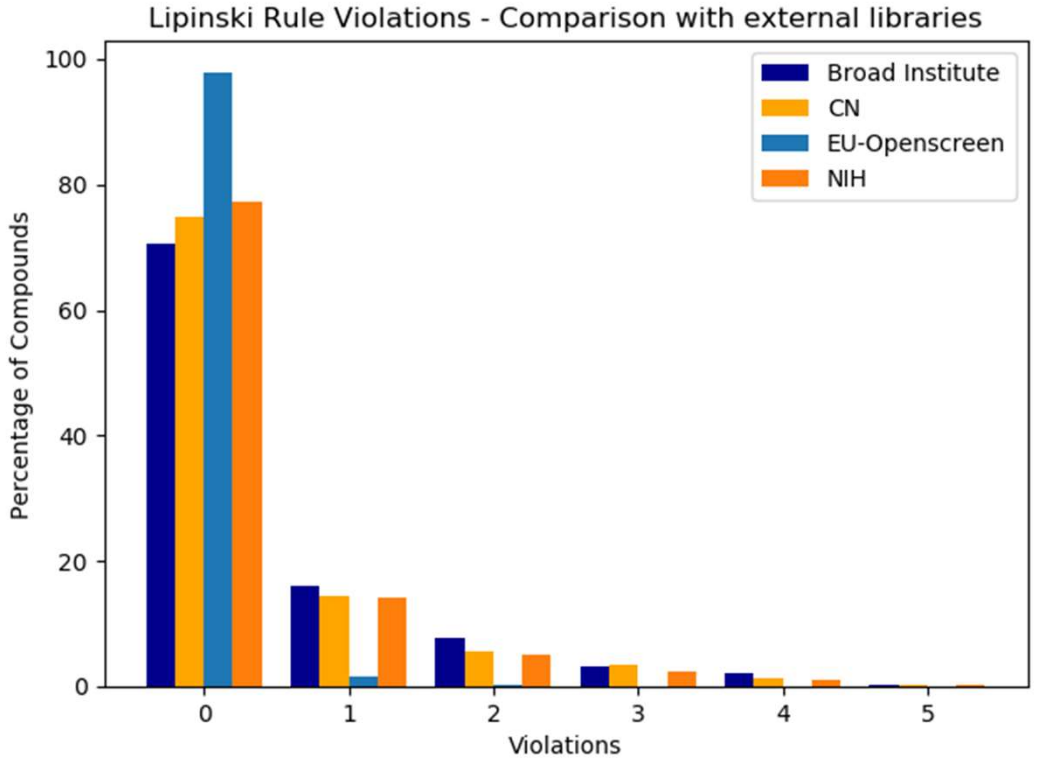
All Libraries suitable for Screening

NIH, Broad and CN with broader portfolio of compound properties

EU-OPENSCREEN with lower number of Lipinski violations: 25 year old concept

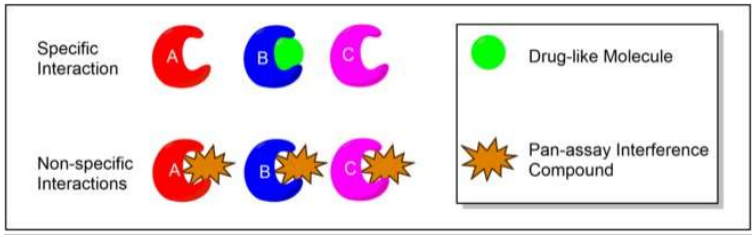
(Ro5: MW < 500 Da, CLogP < 5, HBD < 5, HBA < 10)



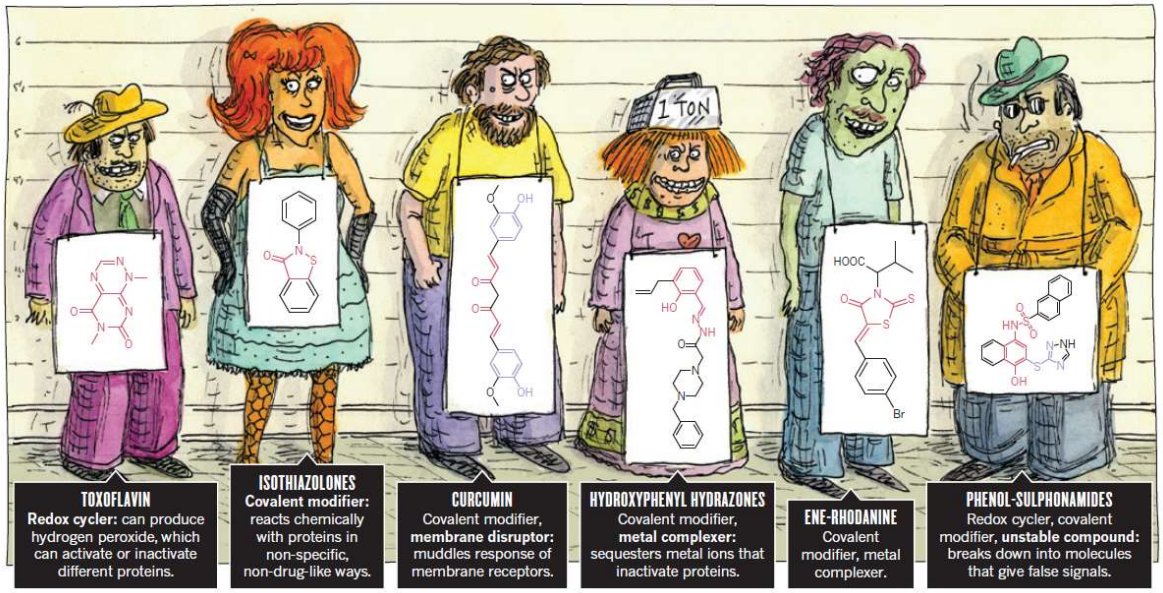


Lipinski CA, et al. *Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings, Adv. Drug. Del. Rev.*, 1997, Vol. 23, 3-25.

PAINS (Pan Assay INterference compoundS)



Baell JB, Holloway GA. *New substructure filters for removal of pan assay interference compounds (PAINS) from screening libraries and for their exclusion in bioassays*, Journal of Medicinal Chemistry, 2010, 53, (7): 2719–40.

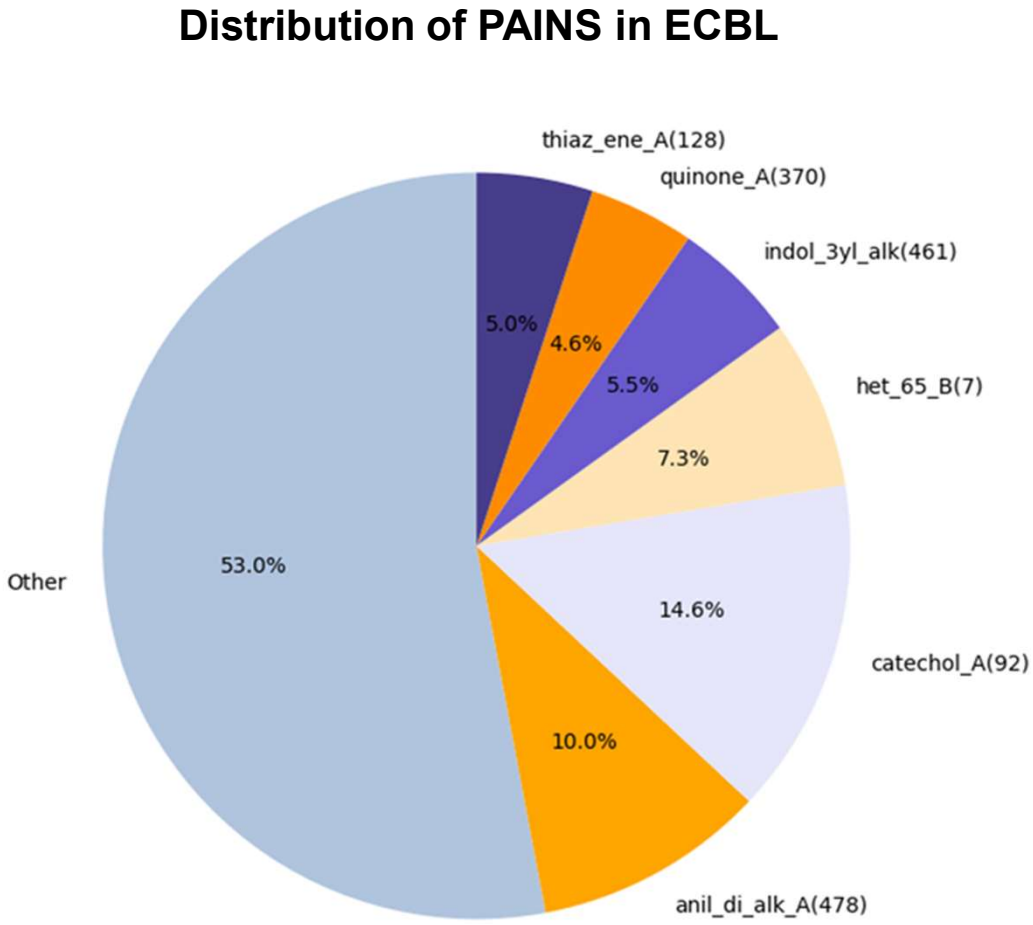


Jonathan Baell and Michael A. Walters. *Chemistry: Chemical con artists foil drug discovery Nature*, 2014, 513 (7519): 481–483.

PAINS Compounds

219 out of 101.276 compounds are classified as PAINS → 0.2 %

without bioactives library classified as PAINS → 0.05 %

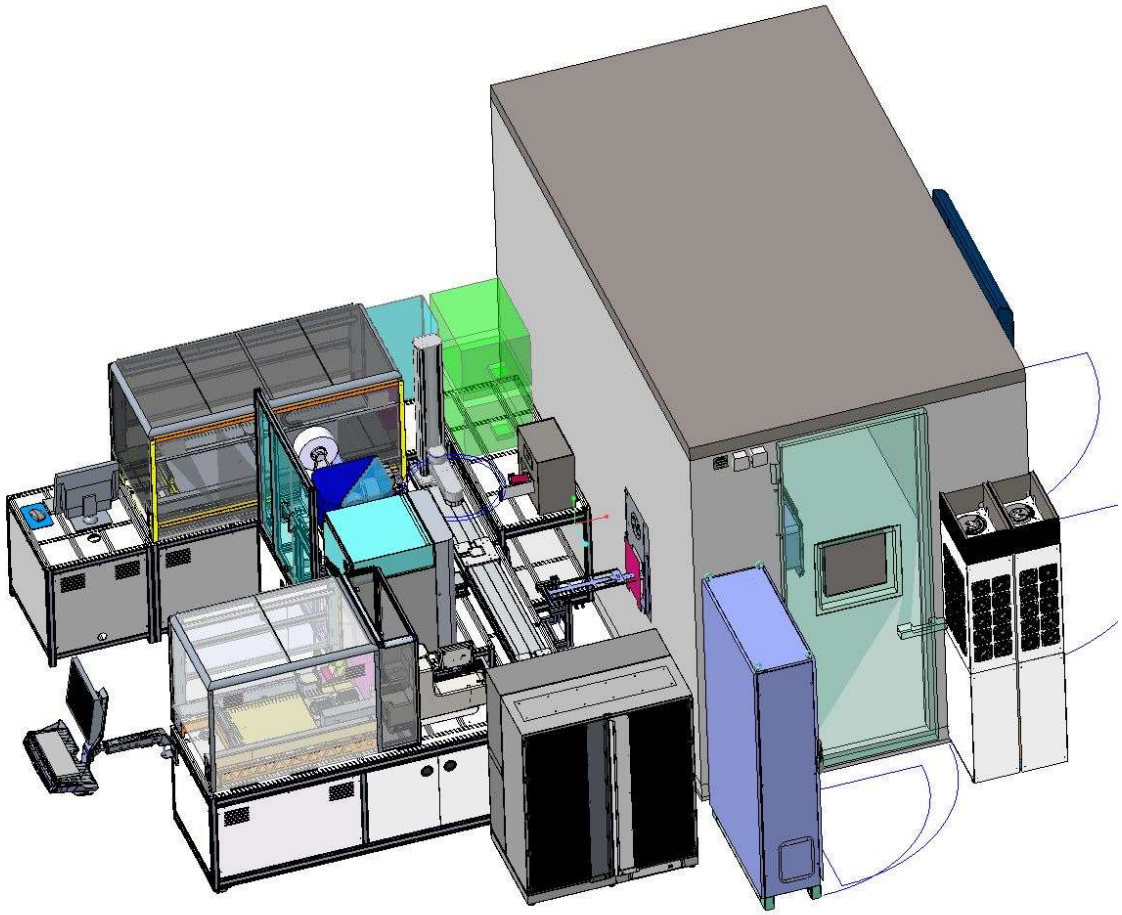


Delivery of Compounds in 384 Well Plates

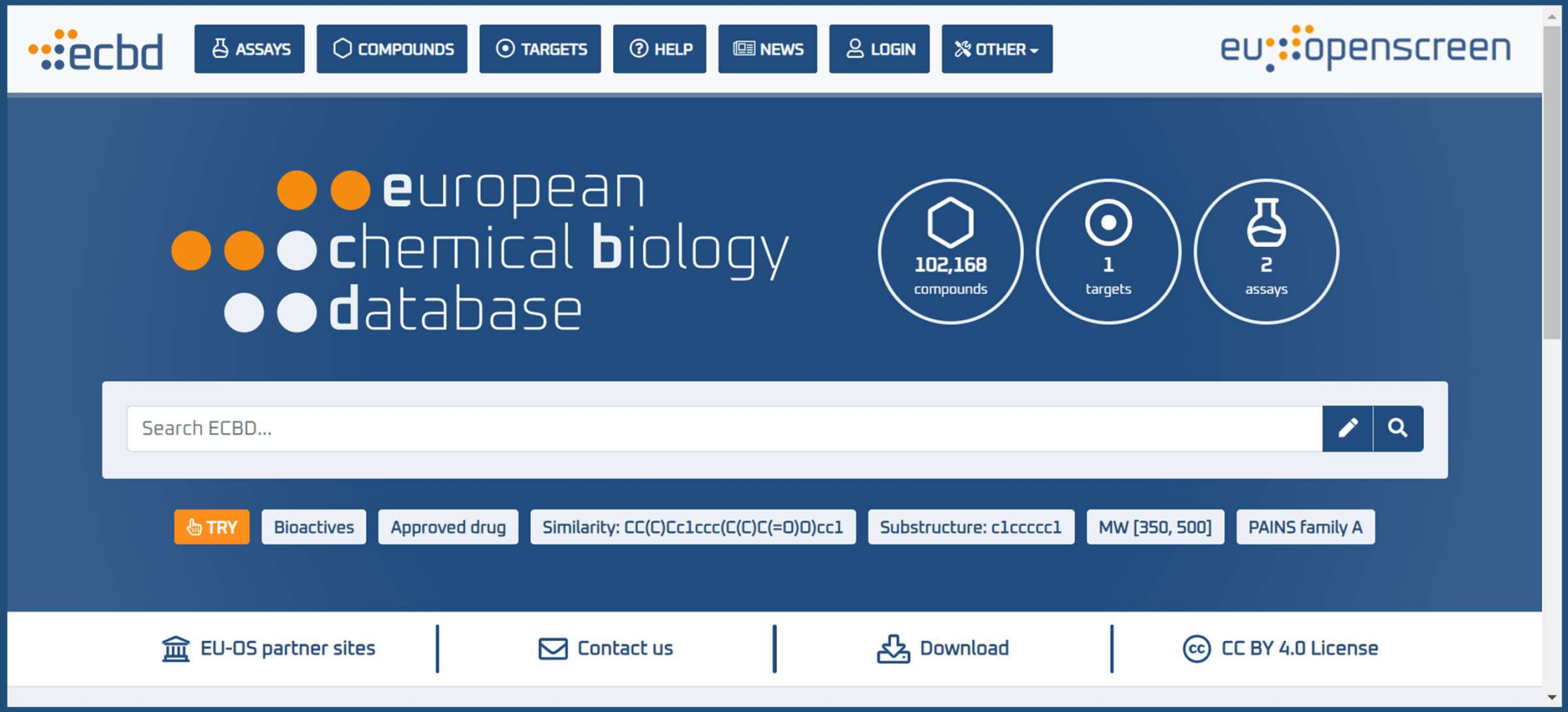
290 plates with 352 compounds to 10 Partner Sites in 1-3 copies
=> 1.400.000 Compounds

Hamilton Verso Store with Hamilton Star Roboter

Capacity of about 300.000 Tubes

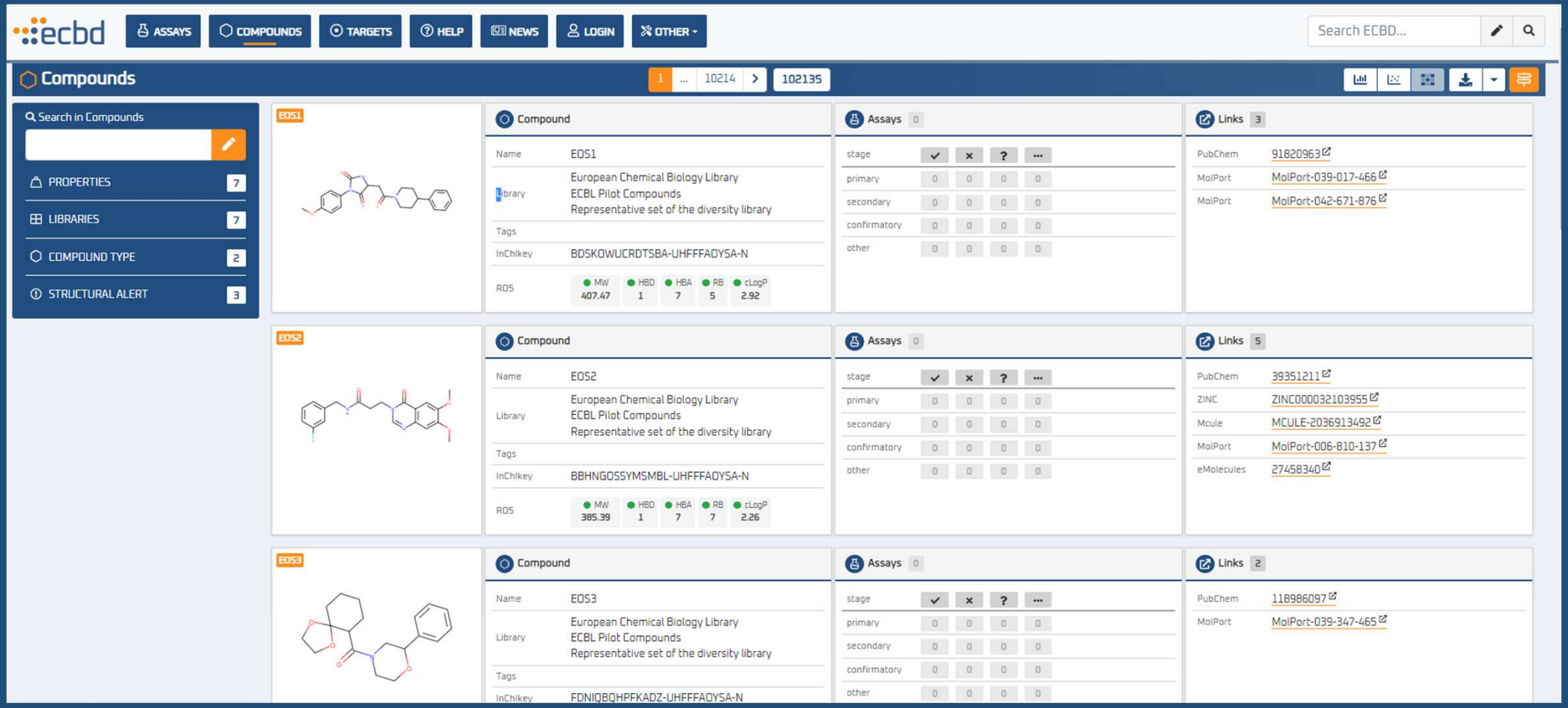


https://ecbd.eu



https://ecbd.eu; Institute of Molecular Genetics of the ASCR (CZ-OPENSCREEN: National Infrastructure of Chemical Biology)

European Chemical Biology Database (ECBD)



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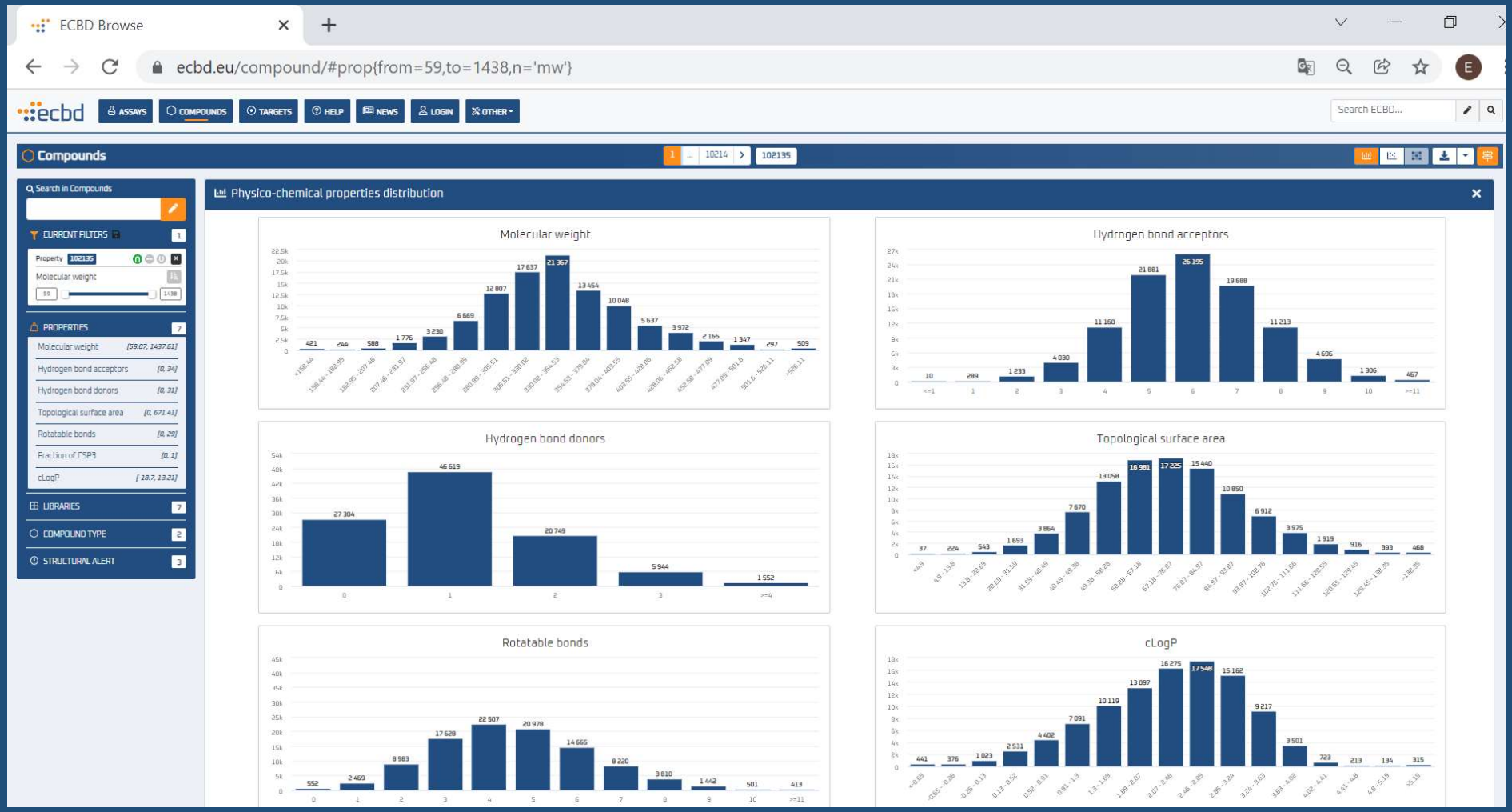
Compound Property Distribution



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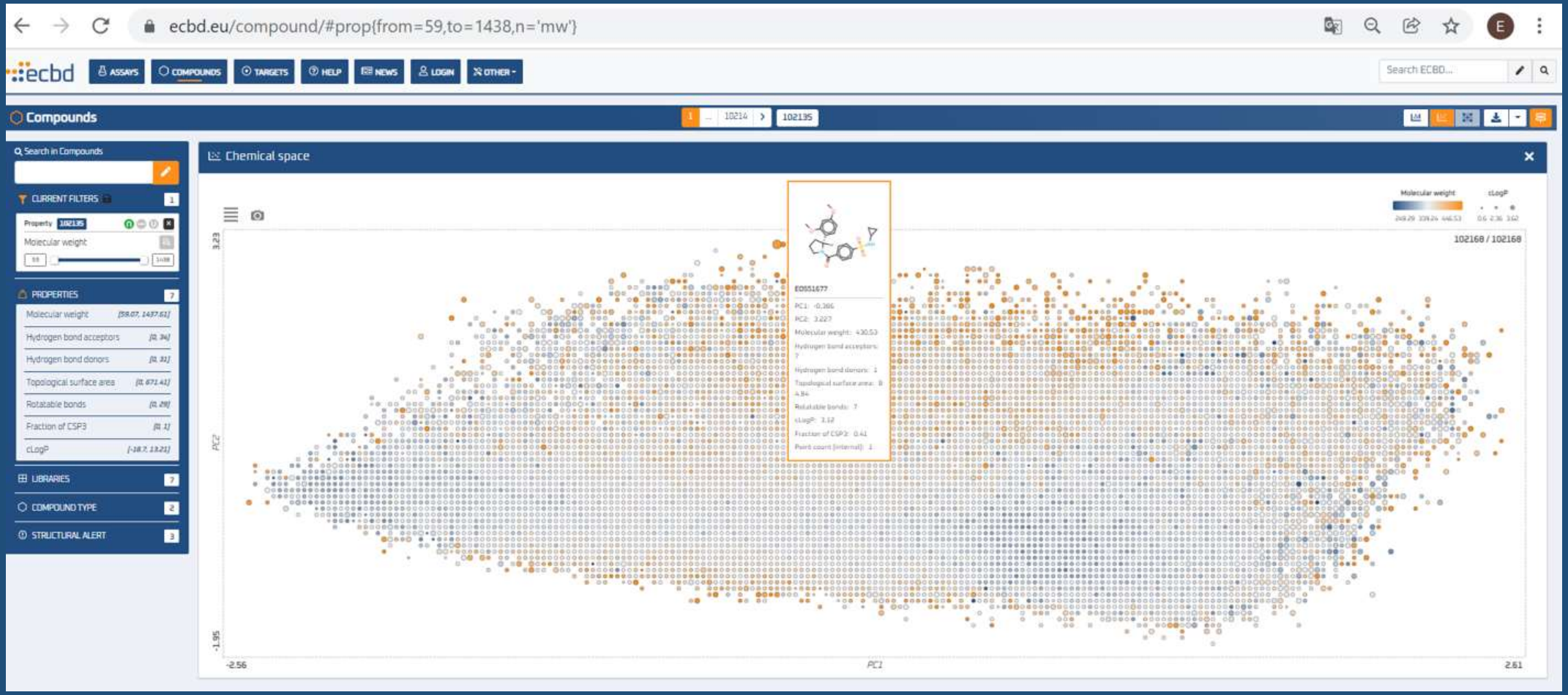
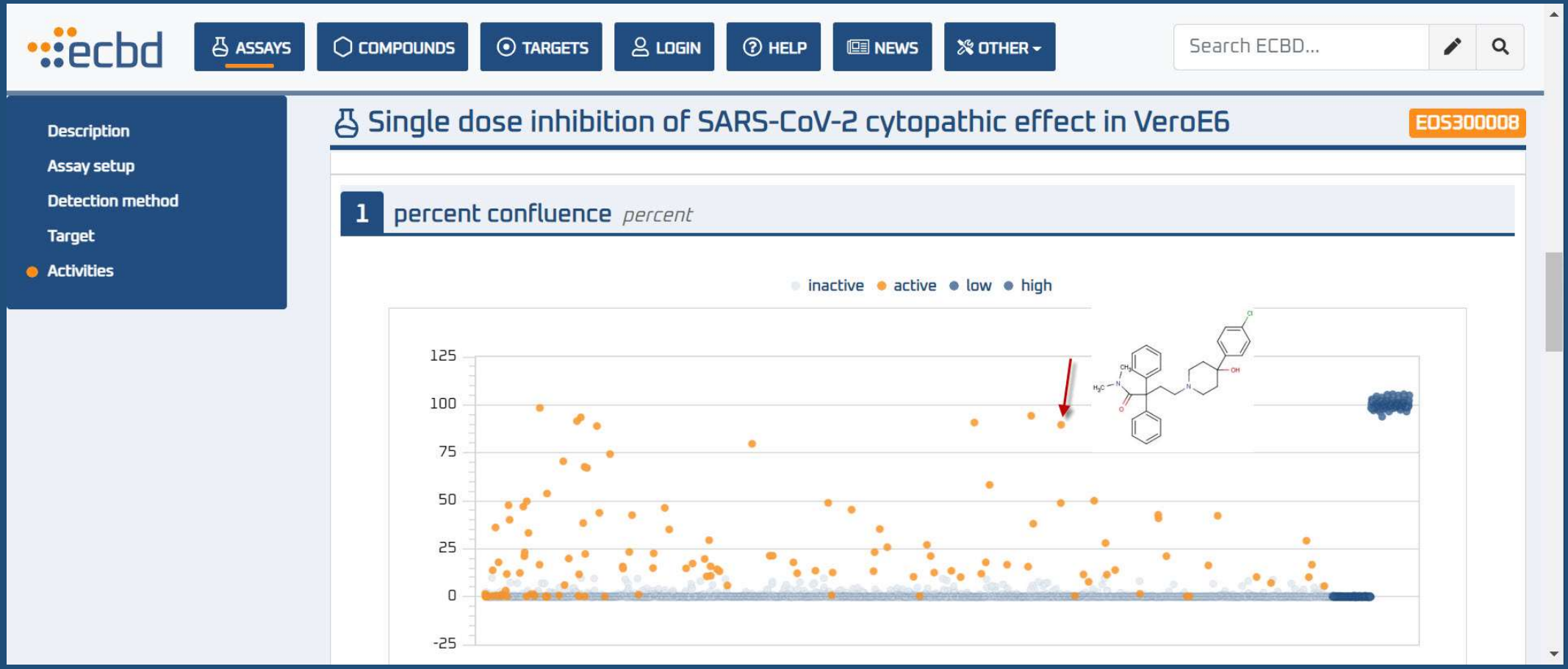
Coverage of Chemical Space



https://ecbd.eu; Institute of Molecular Genetics of the ASCR (CZ-OPENSCREEN: National Infrastructure of Chemical Biology)

First Screening Assay Published on the ECBD Website



https://ecbd.eu; Institute of Molecular Genetics of the ASCR (CZ-OPENSCREEN: National Infrastructure of Chemical Biology)

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Please get an overview about the Partner Sites:

https://www.eu-openscreen.eu/services/screening.html for Screening contacts

https://www.eu-openscreen.eu/services/medicinal-chemistry.html for MedChem contacts

https://www.eu-openscreen.eu/services/database.html for the Database contact

or everyone in the EU-OPENSCREEN Team:

https://www.eu-openscreen.eu/about/contact-team.html

Please also visit the overview at ECBD

https://ecbd.eu/organization/

Any information of the FMP library can be found here:

https://www.leibniz-fmp.de/compound-management or https://www.leibniz-fmp.de/the-screening-unit/downloads