evotec

'RESEARCH NEVER STOPS'

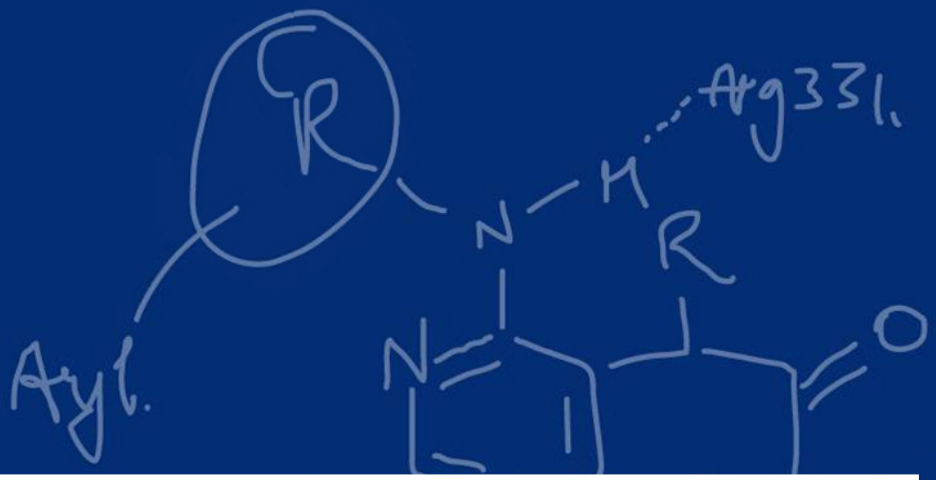
Structural Data Analysis workflow in the context of industrial uHTS

Creating integrated drug discovery innovation alliances

KNIME

3rd KNIME Users Meeting and Workshop

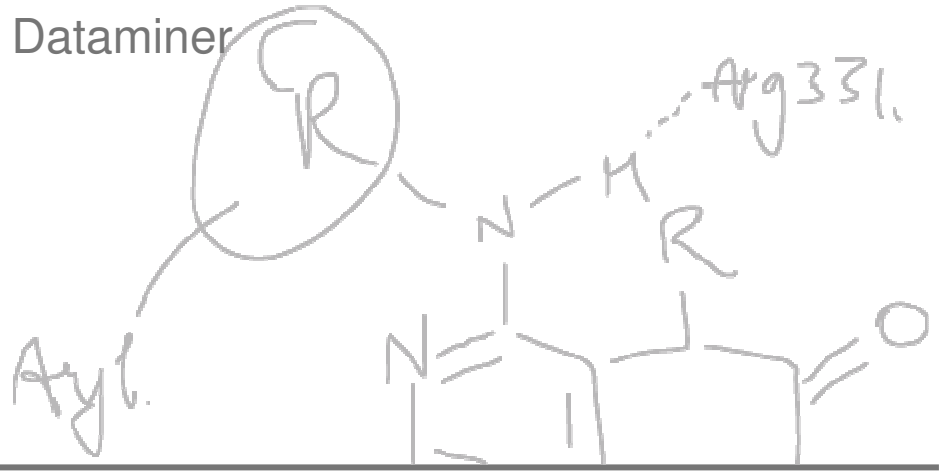
February 22-27, 2010, Zurich, Switzerland



Evotec AG, Knime, Evotec spectral clustering, Tripos HTS Benchware explorer, 22th -27th February 2010

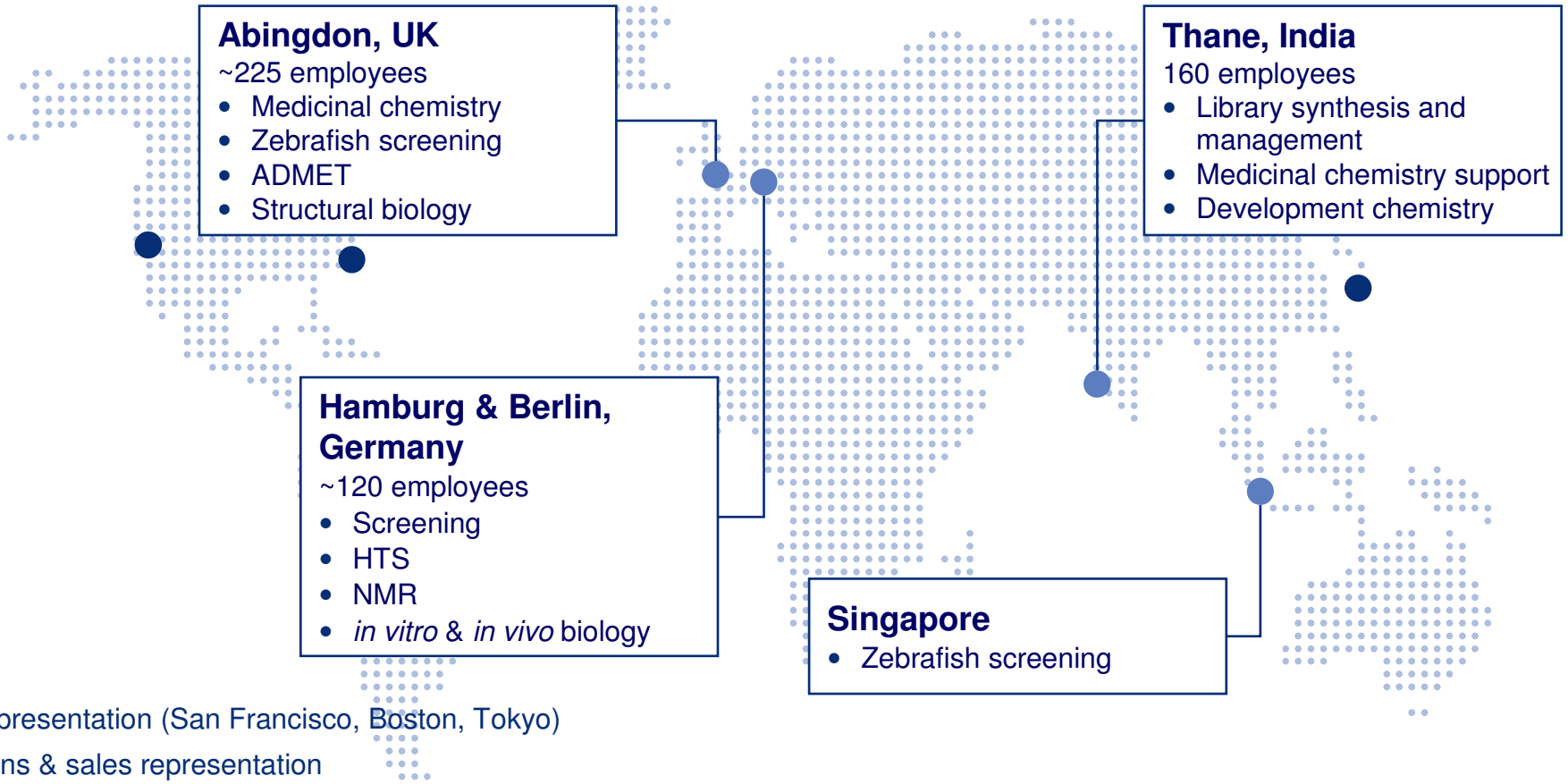
Agenda

- Introduction
- Data analysis in the context of uHTS
  - Knime workflow
  - Spectral Clustering
  - HTS Benchware Dataminer
- Examples
- Summary



Global reach for global projects

Ca. 500 Evotec employees worldwide



We create innovation alliances to discover drugs

Evotec business model

Discovery & technology platform

- Strong track record after >15 years of drug discovery history
  - >100 lead series
  - 20 preclinical candidates
  - 16 clinical compounds
- Leading technology platforms
  1. Assay development & screening
  2. Fragment-based drug discovery
  3. Comp. chem./structural based drug discovery
  4. Medicinal chemistry
  5. Early ADMET& zebrafish

Discovery alliances with the top pharma & biotech companies

Boehringer Ingelheim
NOVARTIS
Roche
ONO
CHDI FOUNDATION, Inc.
biogen idec
Pfizer

Product franchises

- Core expertise with clinical candidates in
  - Treatment-Resistant Depression
  - Others
- Wealth of preclinical assets for partnering or own development

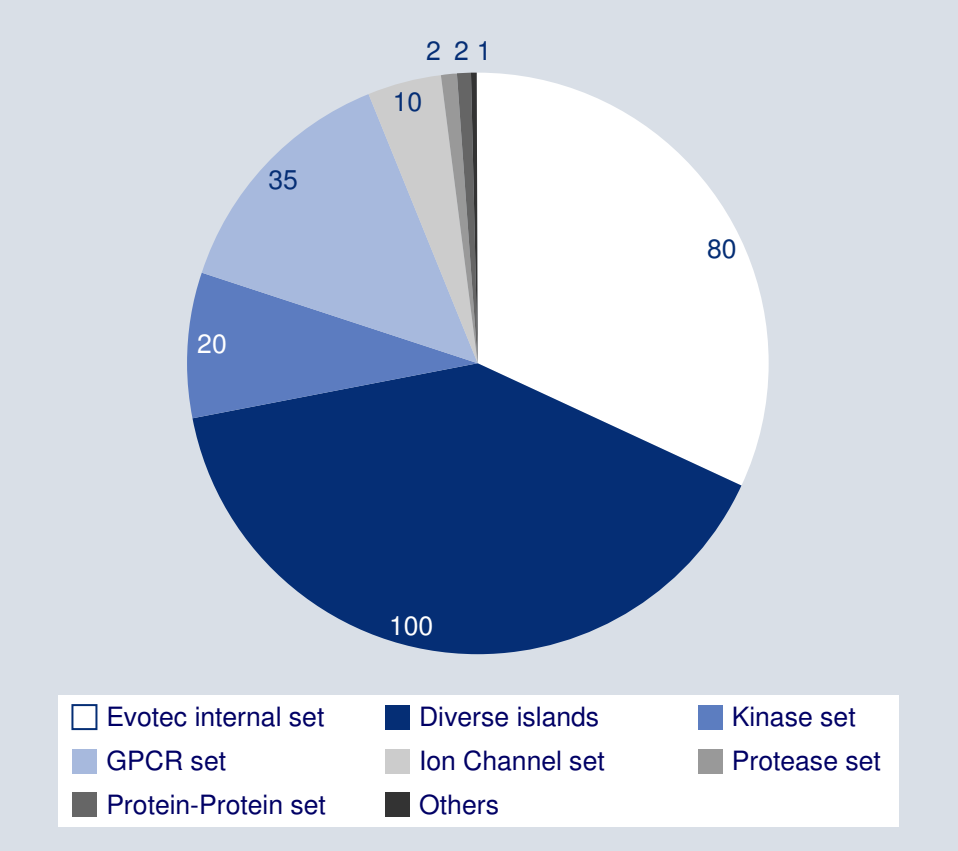
Our value proposition

- Expand highly profitable and cash generating discovery alliance business into globally leading Contract Research Organization
- Finance innovative R&D through
  - DAB profits
  - Development partnerships

Evotec‘s Screening Library – 250K HTS screening collection

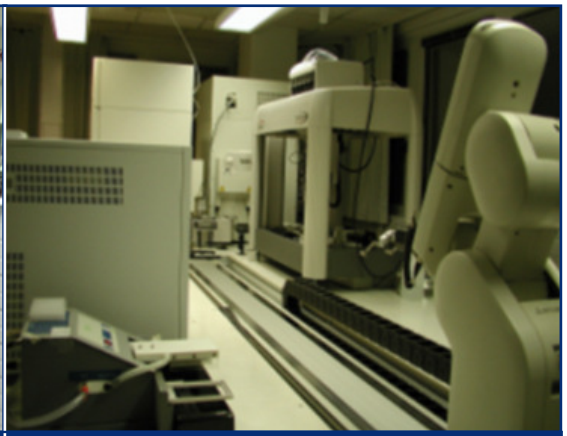
HTS collections

- 250,000 diverse lead-like compound for uHTS
- Optimised compound storage for long term stability
- LC-MS based continuous quality management
- Proven enhanced hit-rate from focused sets
- Diverse ‘islands’ of similar compounds facilitate hit series selection and generate SAR from screening data
- 30,000 fragments for fragment screening



A powerful and multipurpose technology platform

Screening hardware - proprietary and commercial



	EVOscreen Mark II	EVOscreen Mark III	384-HTS System
No. of Systems	2	1	1
Volume	1µl/well	1-50µl/well	10-50µl/well
Plates	2080-well	384, 1536, 2080-well	384-well
Formats	Biochemical	Cellular + Biochemical	Cellular + ELISA
Readers	FCS	EnVision, Opera, FCS etc	Acquest, FLIPR3, Safire
Detection	FCS+*plus*	FCS, fluo, lumi, abs	fluo, lumi, abs
Throughput	100,000 per day	100,000 per day	30,000 per day

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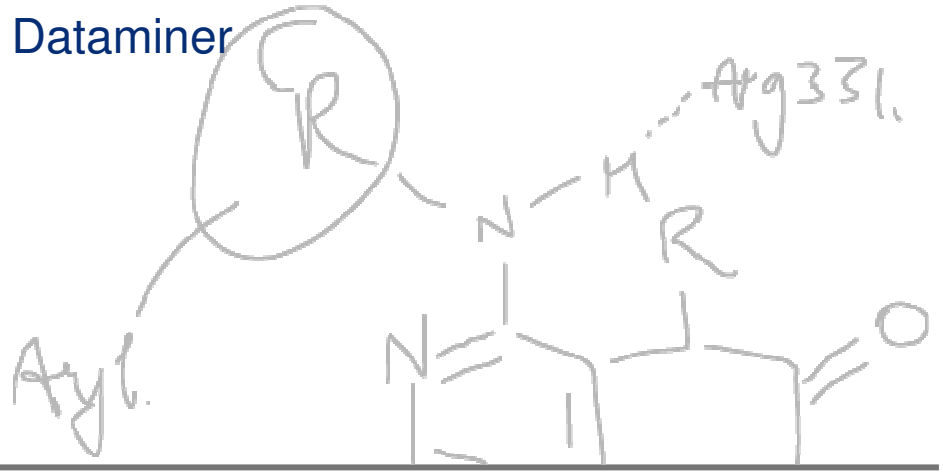
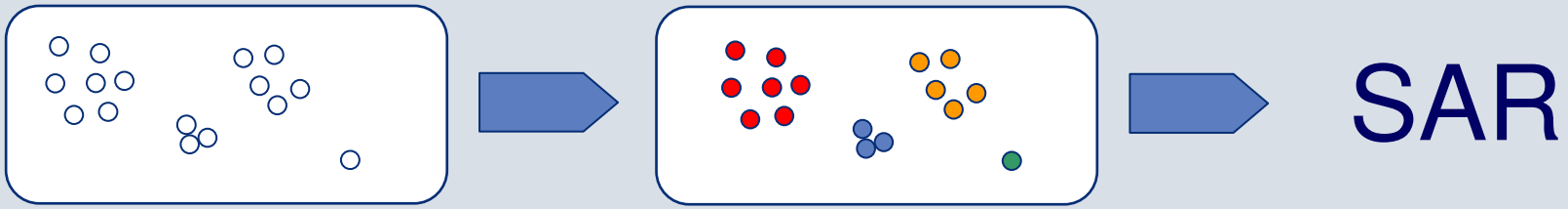


Analyze large hit populations and understand SAR

Industry standard - clustering

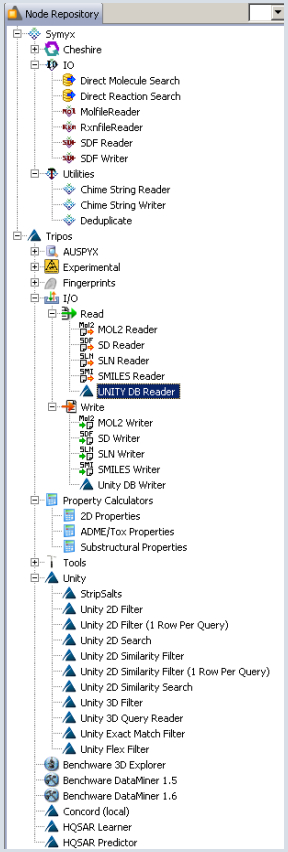
- Our interest: develop an automated procedure to classify 2D molecular structures
  - assess the number and representation of different scaffolds



- Assignment of descriptors
  - assess the number and representation of different scaffolds
  - one dimensional properties (e.g. molecular weight)
  - more complex properties (e.g. molecular electrostatic potential)
- Quantify molecular similarity
  - highly dependent on the molecular descriptors
- Clustering algorithm
  - compute intermolecular similarities and arrange the molecules into clusters

Excellent case study for Knime workflow

All required nodes implemented/available



- Obtain activity data from Evotec analysis platform “A+”
  - tabulated text file
- Split IDs and retrieve structures from Symyx ISIS host database via ISIS direct
  - alternative: read SDfile
- Convert structures to Sybyl line notation
- Calculate Tripos UNITY fingerprints
- Feed fingerprints to cluster algorithm (Evotec spectral clustering)
- Feed merged dataset to HTS Benchware explorer 1.6

- Manual analysis and review of data

- Feed final product to Knime reporting functionality
- Write pdf / Word document

Knime Workflow

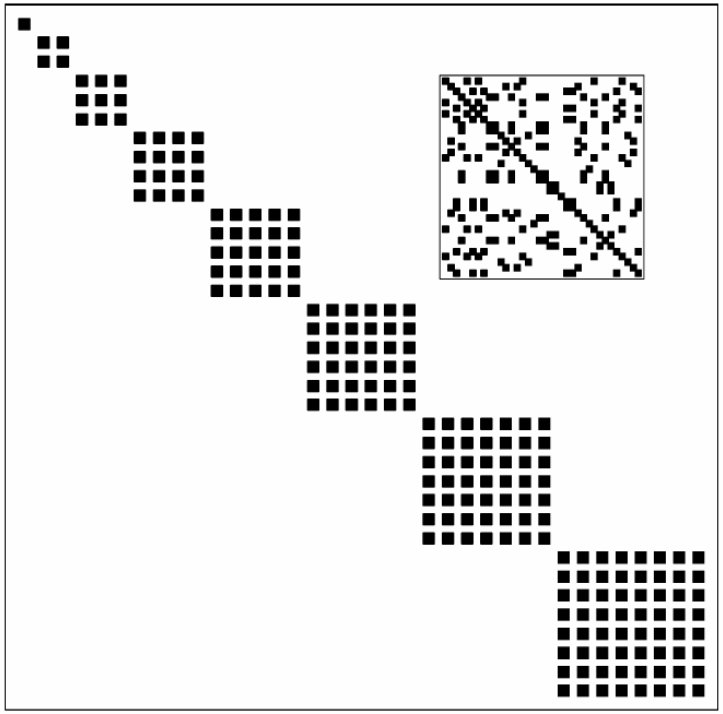
advantages / caveats

- Automated process, easy modification of workflow
- Full documentation via graphical frontend
- Integration of all Evotec available SW vendors (Tripos, Symyx, R-Project, Moe, …)
- Automated reporting available
- No server deployment yet
- Licensing of target applications might prevent company wide role out
- Evotec final platform decision (Linux, Windows) pending

Spectral clustering*

Eigenvalue and eigenvector decomposition of similarity matrix

- *N* molecules produce a similarity matrix *S* (*N x N*)
  - ideally: *k* distinct clusters
  - molecules with similarity $S_{xy} = 1$ belong to one cluster
  - not similar $S_{xy} = 0$ belong to different clusters

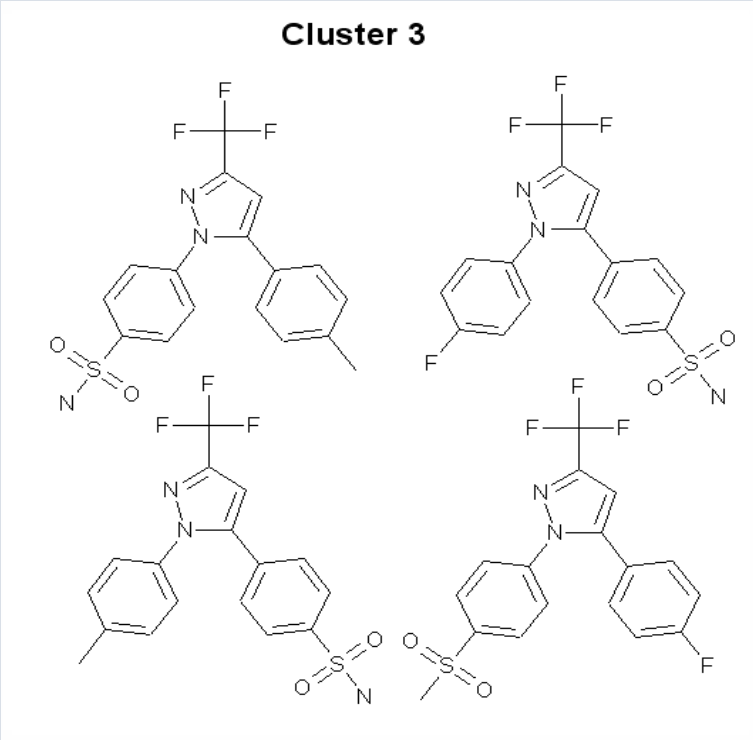


* Brewer, ML J Chem Inf Model. 2007 Sep-Oct;47(5):1727-33

Spectral clustering*

Eigenvalue and eigenvector decomposition of similarity matrix

- Reorder similarity matrix in block diagonal form
  - by definition *S* will have *k* non zero eigenvalues; values are equal to the cluster populations p_i
  - each nonzero eigenvalue has an associated eigenvector that has p_i nonzero elements and the indices of these elements correspond to the molecules belonging to that cluster
- Conclusion: with the eigenvectors at hand it will be possible to reorder the matrix to a block form and reveal the distinct clusters
  - validated on a published test dataset (COX-2)
  - method widely used in image analysis



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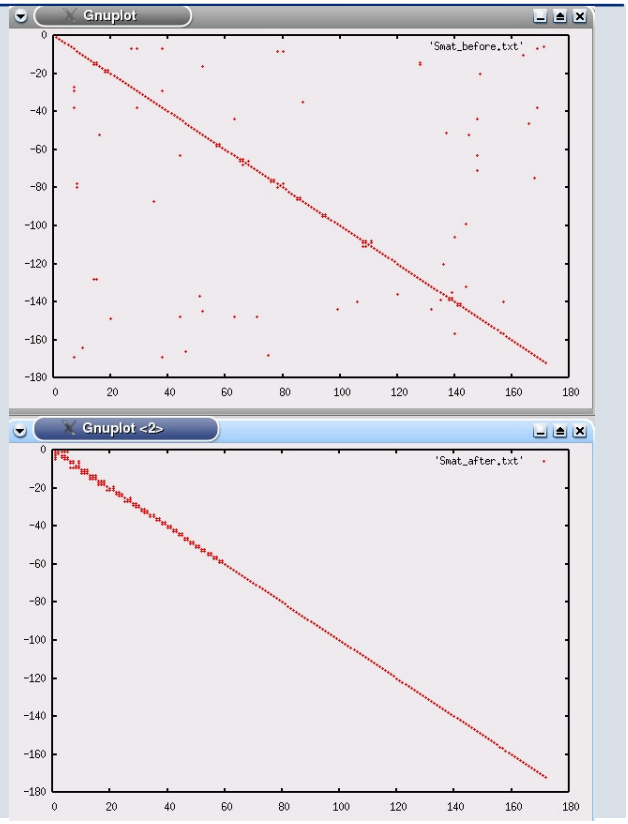
Workflow integration and extensive testing

Pre-calculate clusters at different similarity thresholds

- Automated procedure (initially perl script now Knime workflow)
  - retrieve structures from database (by list of compound IDs)
  - convert to UNITY database and calculate 2D fingerprints
  - calculate similarity matrix
  - reorder matrix and identify clusters (spectral clustering)
  - merge to compound IDs, potency data and compound properties (Lipinski and ADMET)
- Manual review of cluster
  - ranking of clusters by relevance / chemical interest
- Extensive testing on real datasets



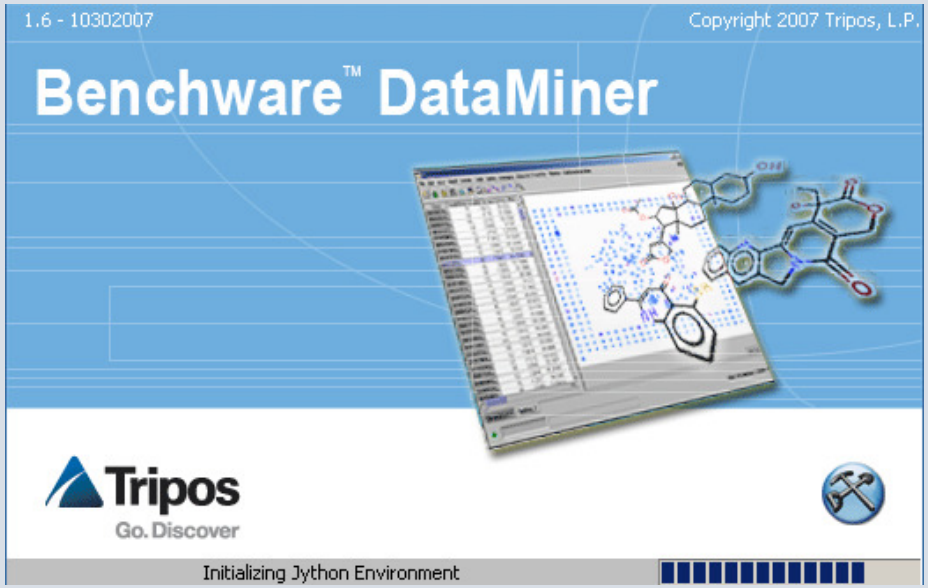
Spectral clustering

advantages / caveats

- Fast and reliable calculation
- Free to integrate into Evotec software tools
- Fully automated cluster assignment
- Applicable to a variety of similarity matrices (different fingerprints)
- No visualization of chemistry space
- No substructures integration
- Cluster ranking independent of target potency information

Tripos HTS Benchware Dataminer

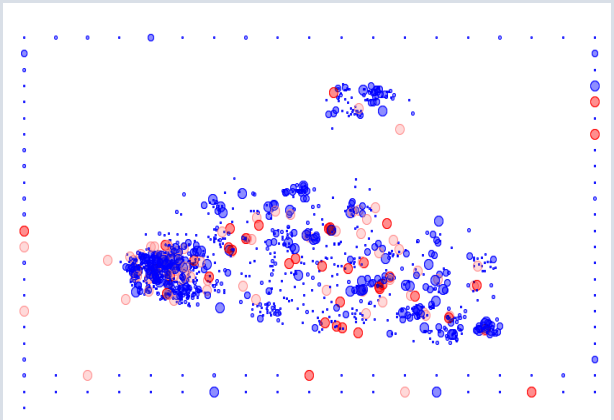
- Workflow integration
- Examples



HTS Benchware Dataminer

Practical Workflow

- Compile dataset (prepare SDFile via Knime Workflow)
  - target potency data (log IC50 values / % inhibition)
  - selectivity information
  - readout artefact behaviour
  - compound purity information
  - predicted Lipinski or ADMET properties

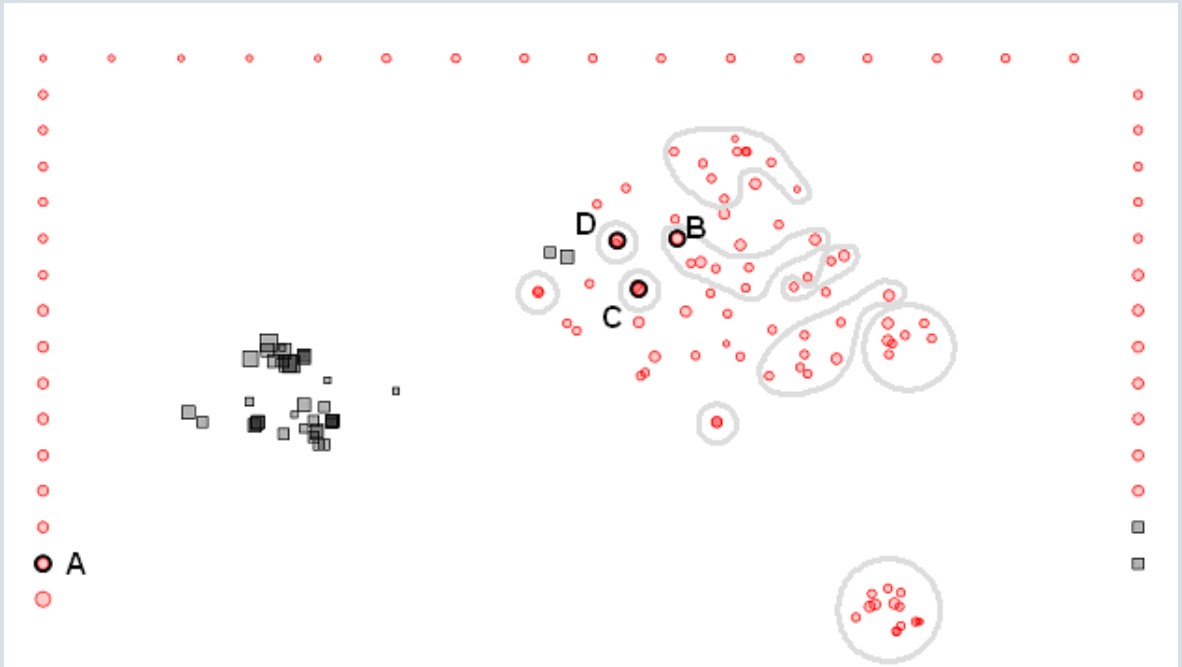


- 2D projection of chemistry space (PCA & NLM)
- Manually identify and rank clusters
- Calculate mcs and add substructure search results to cluster
- Characterize identified clusters

Identification of novel Kv1.3 blockers using a fluorescent cell-based ion channel assay

Comparison to literature known actives

- HTS hits (red circles)
- Literature known actives (black squares)
- Size = potency
- A-D confirmed by patch clamp method
- Singletons at the border of projection



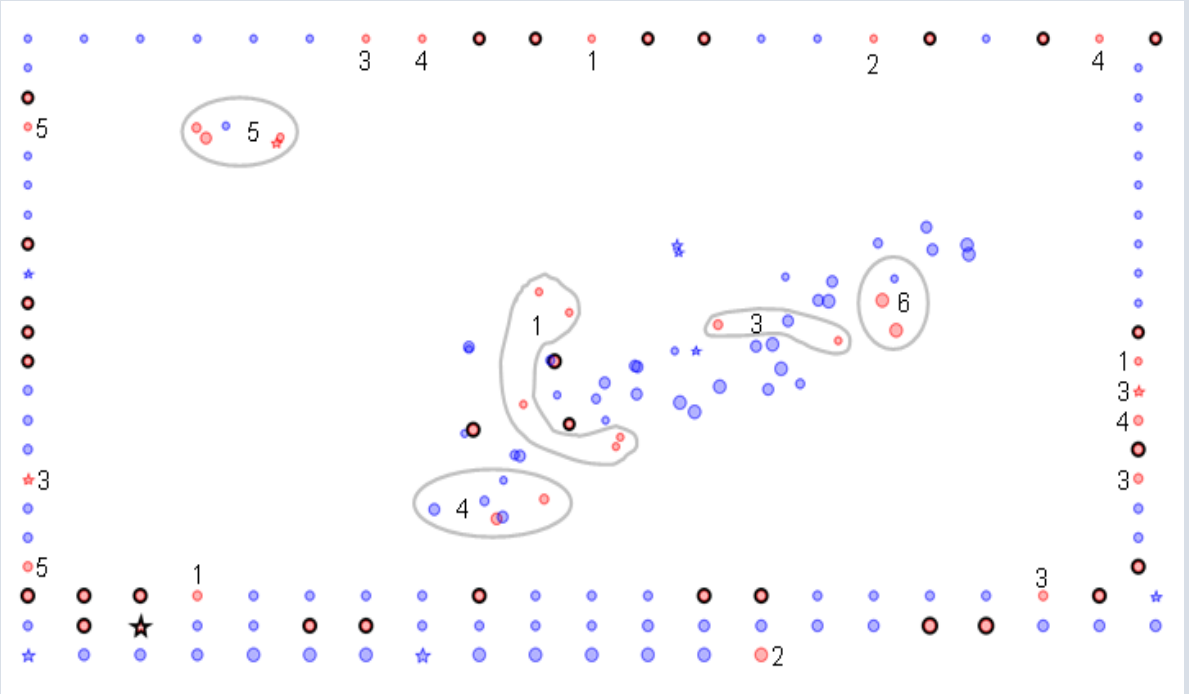
* Slack M et al J Biomol Screen. 2006 Feb;11(1):57-64. Epub 2005 Nov 28

Identification of selective kinase modulators

Inhibitors not interacting with ATP binding pocket

- Difficult target

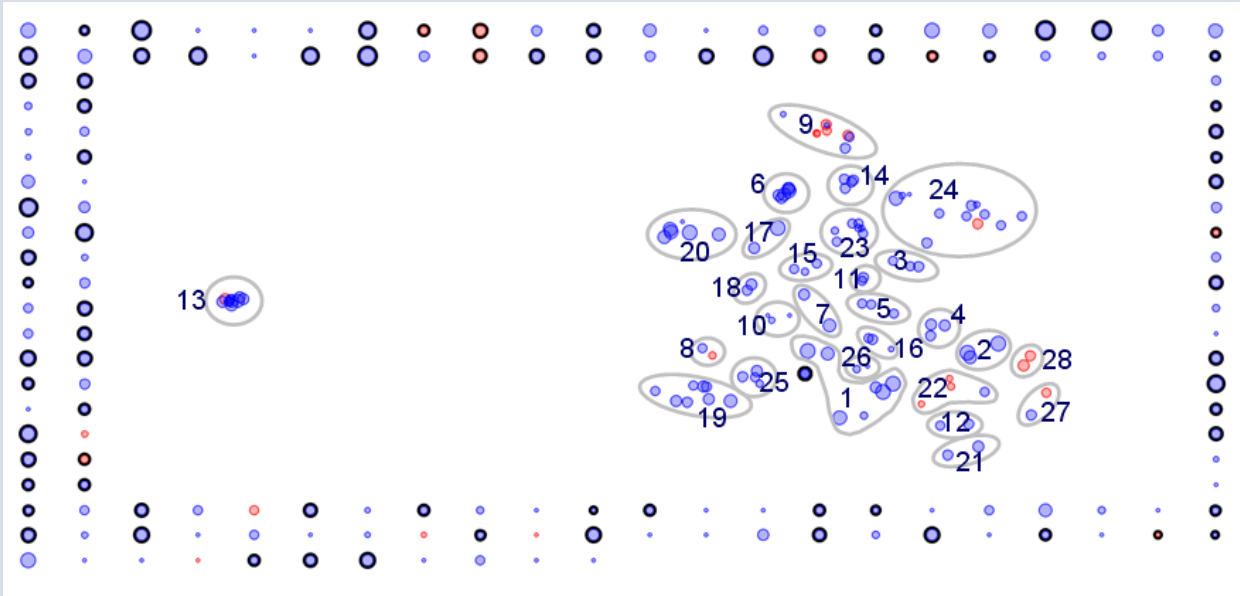
- Size = kinase potency
- red = selective
- blue = ATP active
- star = QC failures



Identification of enzyme inhibitors

Target in field of epigenetics / HTRF assay

- Potency (size)
- Counter screen active (red)
- 30 singletons (black circles)



HTS Benchware dataminer

advantages / caveats

- Easy and intuitive to use application; endpoint for Knime workflow
- Full integration of required workflows
  - Structure import, clustering, visualization, determine mcs, substructure search
- Excellent 2D projection of chemical space
- Manual review and ranking required
- Reporting functionality could be improved (export plots), no Knime input node
- Improvement of spreadsheet functionality
- Only applicable to UNITY fingerprints
- Small user base

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and colleagues

Knime.com

Organizing committee

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