

Open access HTS platform providing WDI derived screening collections

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The mission of ChemBioNet is dedicated to provide a „real“ link between biologists, chemists and other scientific disciplines highlighted as novel discipline termed Chemical Biology. ChemBioNet has built up a professional infrastructure with modern screening technologies and a compound library management which ensures an efficient system of data storage. Hitherto, about 40000 compounds have been deposited in the ChemBioNet screening library from national and international groups. The ChemBioNet supports the scientific community with the open access HTS platform which is used by either academic or industrial research groups.

1. Compound selection

The objective of the ChemBioNet is the rational assembly of a screening collection enriched with bioactive and chemical diverse drug-like compounds. For this purpose the following strategy has been developed.

1. Removal of reactive and unstable compounds from vendor libraries of 12 million compounds (Fig.1) with a self-compiled reactivity filter¹.
2. Search for bioactive compounds derived from the WDI using a maximum common substructure concept^{1,2}.
3. Extraction of the most diverse compounds from each set of substructures from the WDI^{1,2}.
4. Scrutiny of compound selection in accordance with the criteria of the „Lipinski rules“³.

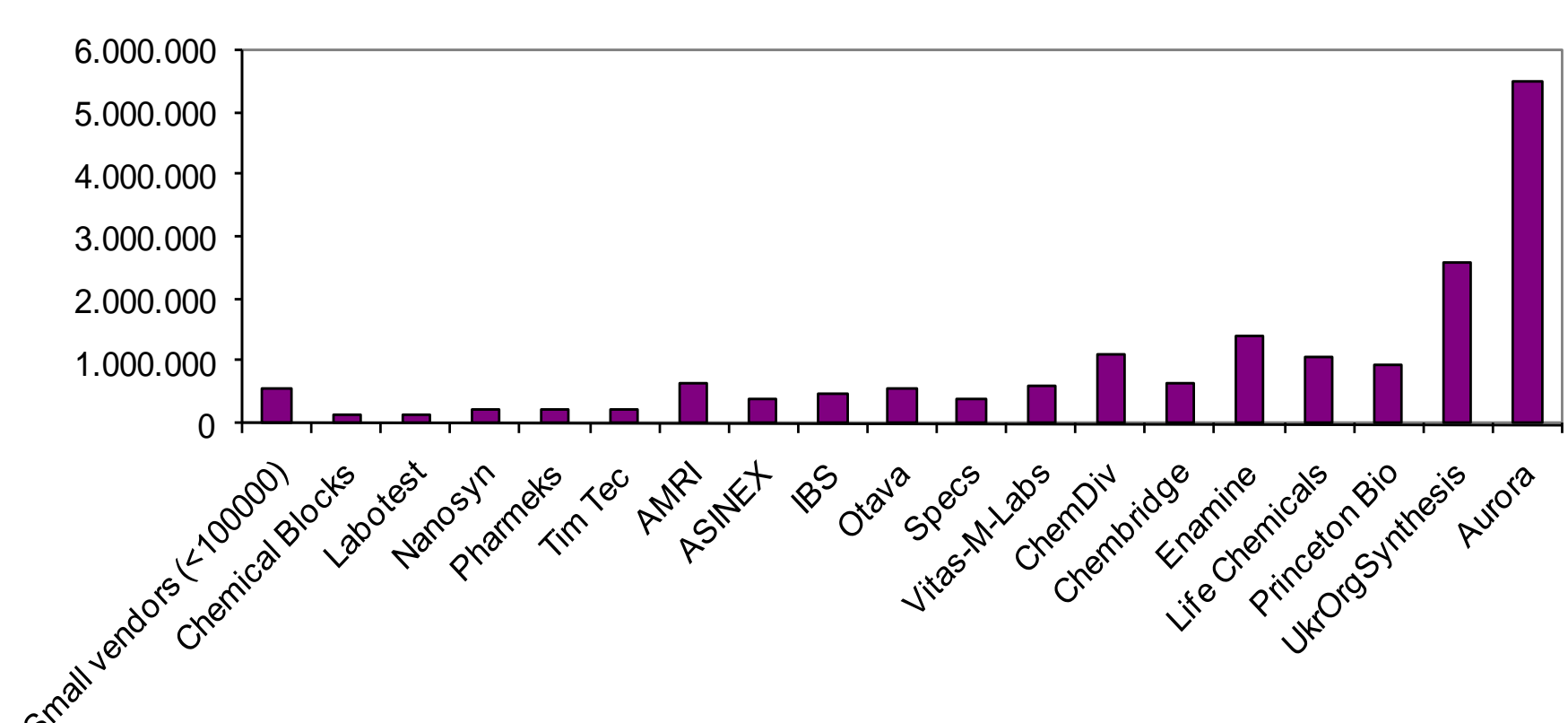


Fig.1: Graphical representation of commercially available compounds in the libraries of various vendors.

If a common substructure (e.g. piperazine) occurs more than 5 times in the WDI (35345 compounds) it will be extracted and added to a list where 561 substructures were finally stored. Exemplarily we refer to the kinase inhibitor imatinib (Fig.2).

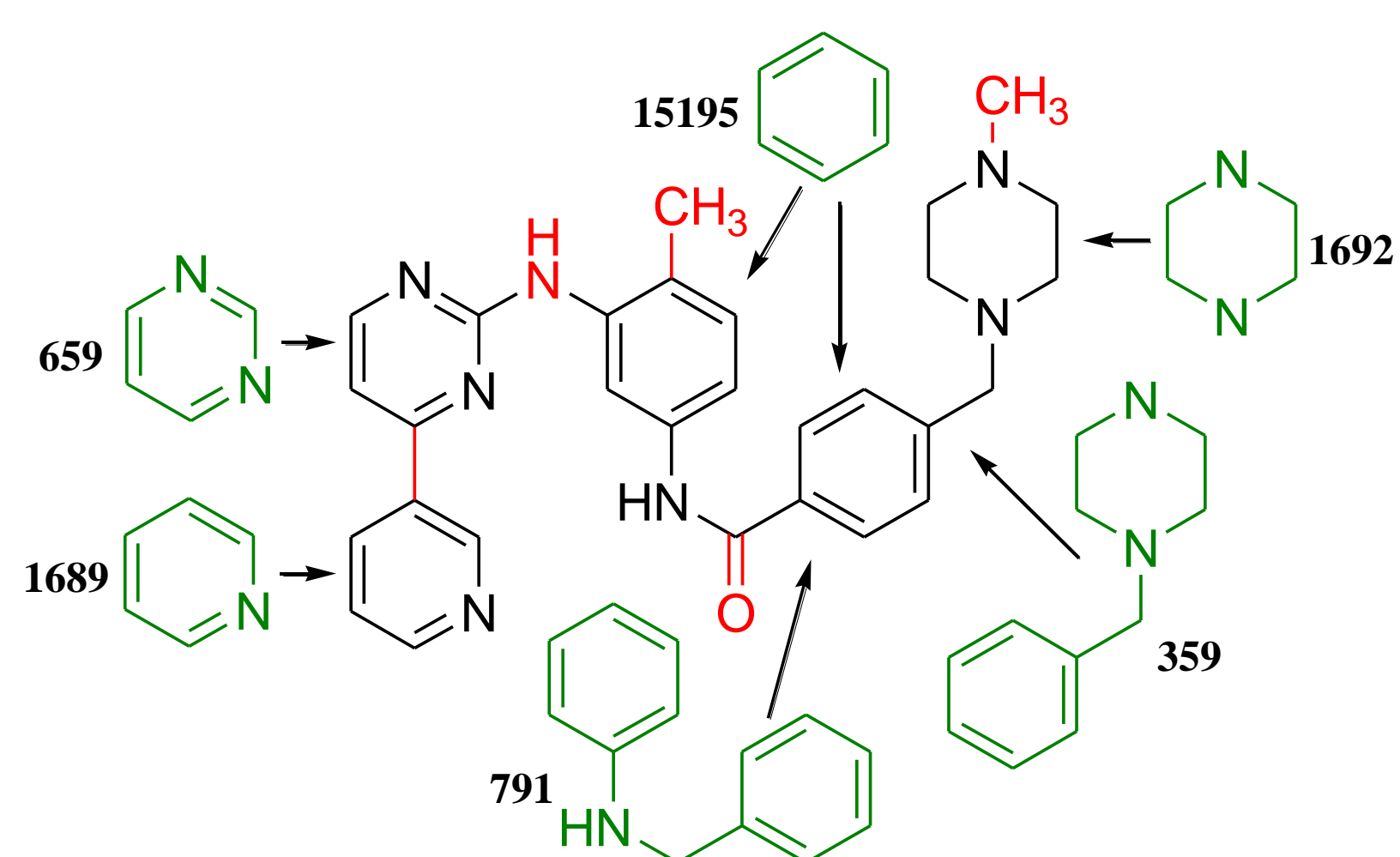


Fig.2: Extraction of substructures (green) from the kinase inhibitor imatinib (black) and total count (bold numbers) of substructures within the library. (e.g. piperazine is found once within imatinib whereas the total count as substructure sums up to 1692).

A collection of 100 substructures with the highest frequency in the WDI is displayed in Figure 3.

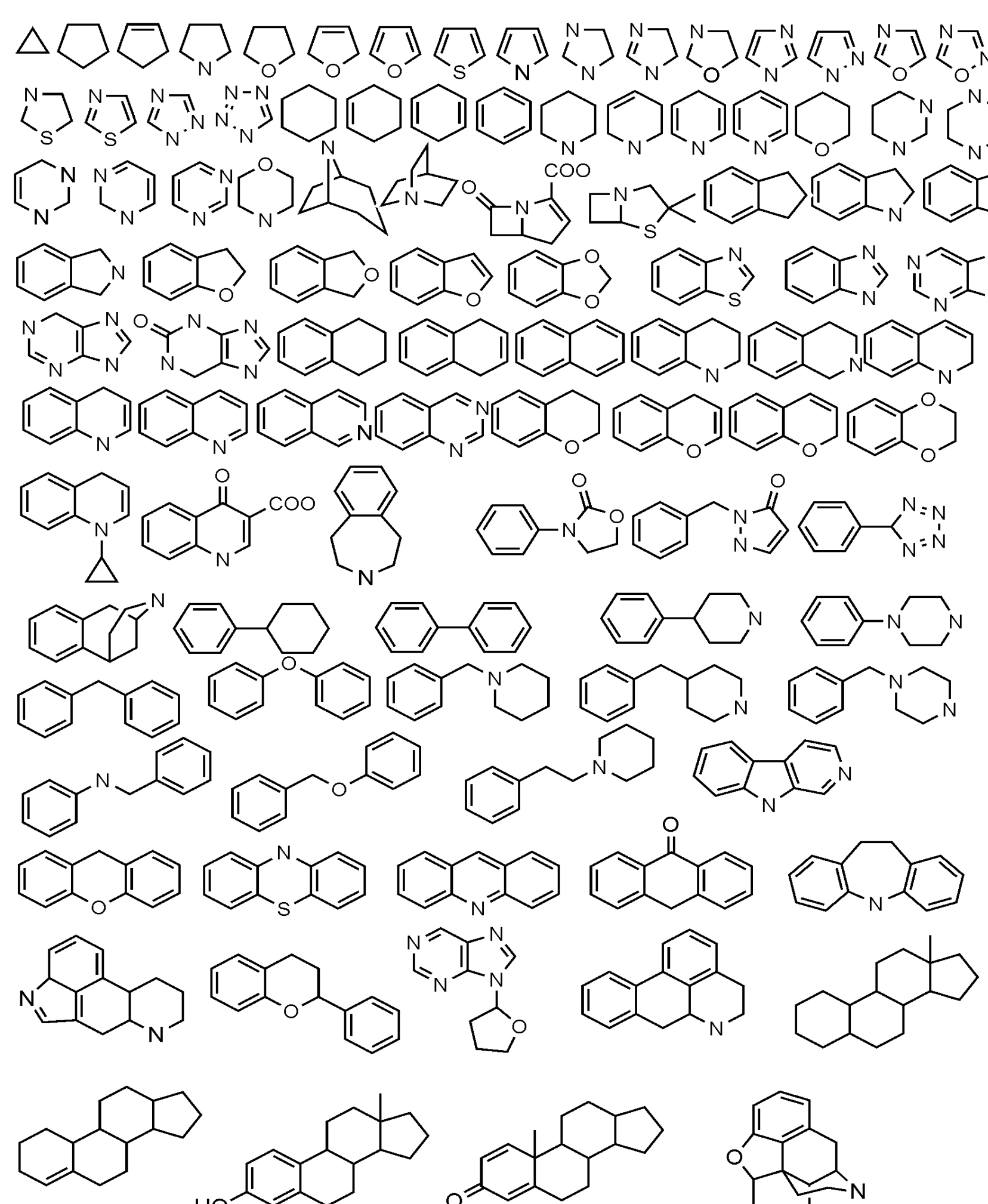


Fig.3: Overview of 100 substructures of out 561 with the highest frequency in the WDI. The classification was derived by ClassPharmer⁴ or Pipeline Pilot⁵, standardized with chemaxon standardizer⁶ and substructures are ordered by increasing complexity.

2. Remp storage system

If a vendor supplies the compounds in a 384 well plate format the compounds will be solubilized in DMSO and transferred to 4 x 96 Remp well plates (Fig. 4). By means of a Tecan robot work station the Remp plates are stored in a RempSSS freezer at -20°C which stores and registers the plates via barcode detection in a database (Fig.5).

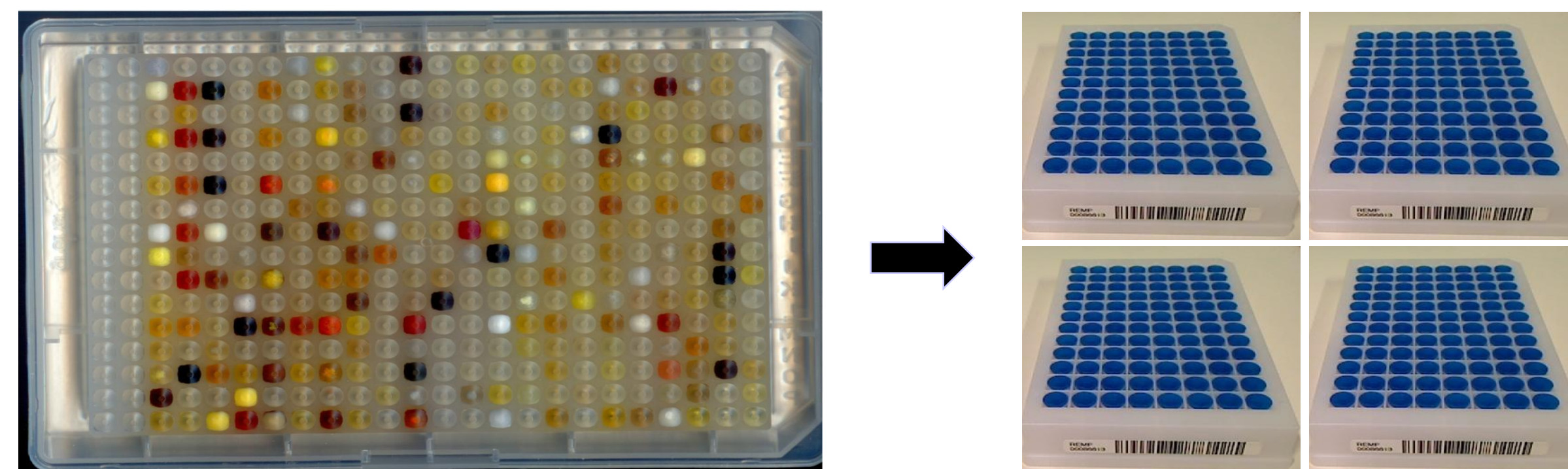


Fig.4: Picture of a 384 well plate with 352 compounds solubilized in DMSO. Transfer of 4 x 88 compounds to a 4 x 96 well plates (Z-format). Last columns are assigned for positive and negative control.

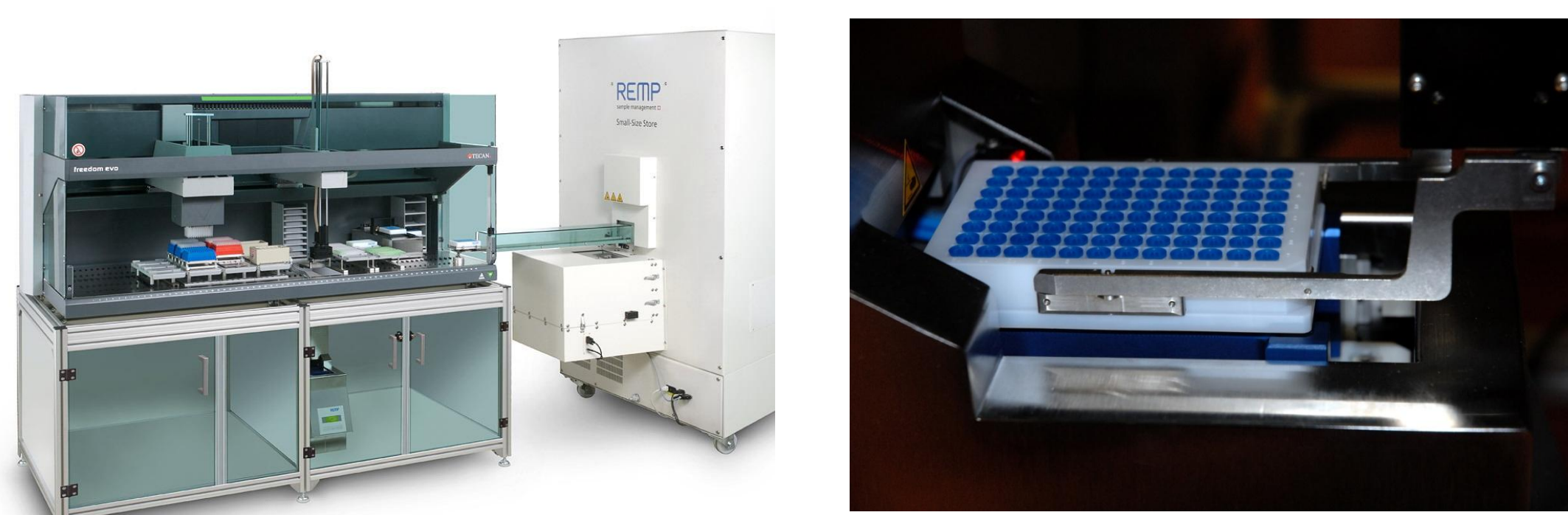


Fig.5: Tecan robot workstation connected to the REMP SSS freezer. A robot arm transfers a Remp plate into the freezer.

3. Equipment and assay technologies

If a screening is conducted with our ChemBioNet platform we will invite the user to join us at the FMP in Berlin in order to adapt the assay to the 384 well plate format. In order to screen 40000 compounds in 113 x 384 well plates in 2-3 days we use Tecan robot workstations which run the optimized assay set up automatically (Fig. 6). The ChemBioNet platform offers a variety of new assay technologies. Beside standard application like ELISA, fluorescence polarization, FRET based systems and UV absorption we have established luminescence/alphascreen technology (Perkin Elmer, Envision Fig. 7), on-chip capillary electrophoresis (Caliper, LabChip 3000 Fig. 8)⁸, impedance measurements (Roche, xCELLigence system Fig. 9)^{9,10}, High content screen (Cellomics, array scan Fig. 10)¹⁰, genome-wide-RNA-interference (in process) and SPR measurements (Biacore, T-100 planned).



Fig.6: By means of a Tecan robot workstation aliquots of 40000 compounds are automatically added to the assay samples provided as aq. buffers in 113 x 384 well plates.

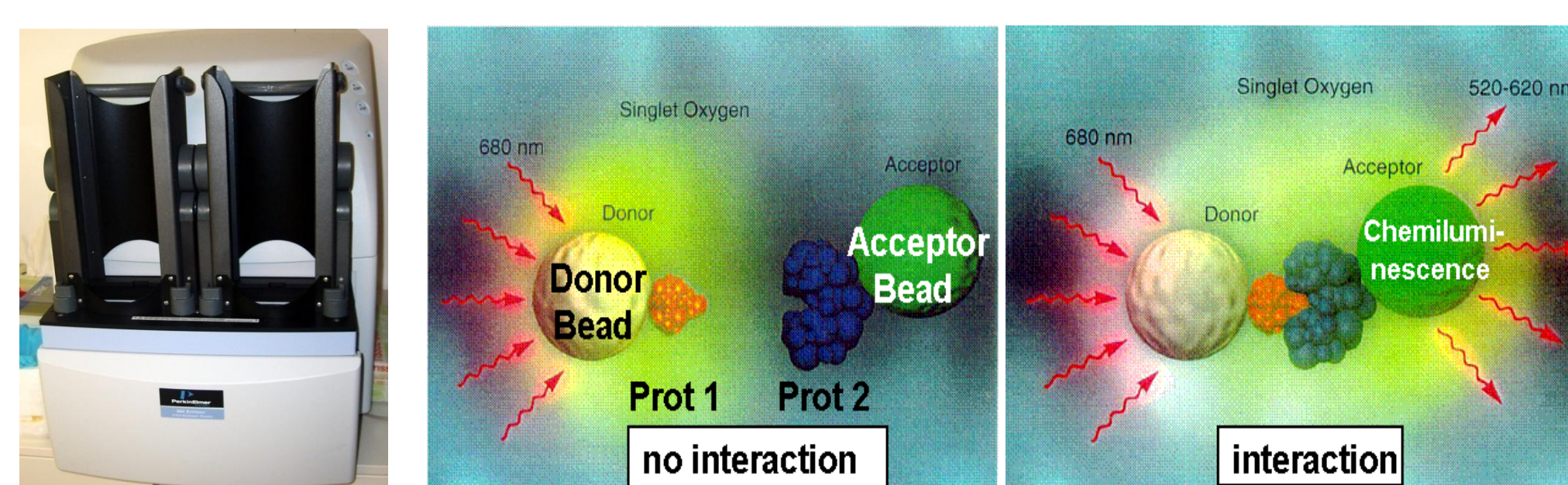


Fig.7: Highly sensitive luminescence method for the detection of molecular binding events using hydrogel coated donor and acceptor beads („Alpha-Screen“). Both beads are bioconjugated with potential binding partners (e. g. receptor-ligand-interaction). If the beads come to close proximity a laser excitation (680 nm) of the donor beads will transfer a signal via a singlet oxygen to the acceptor beads which emit luminescence at 520-620 nm.

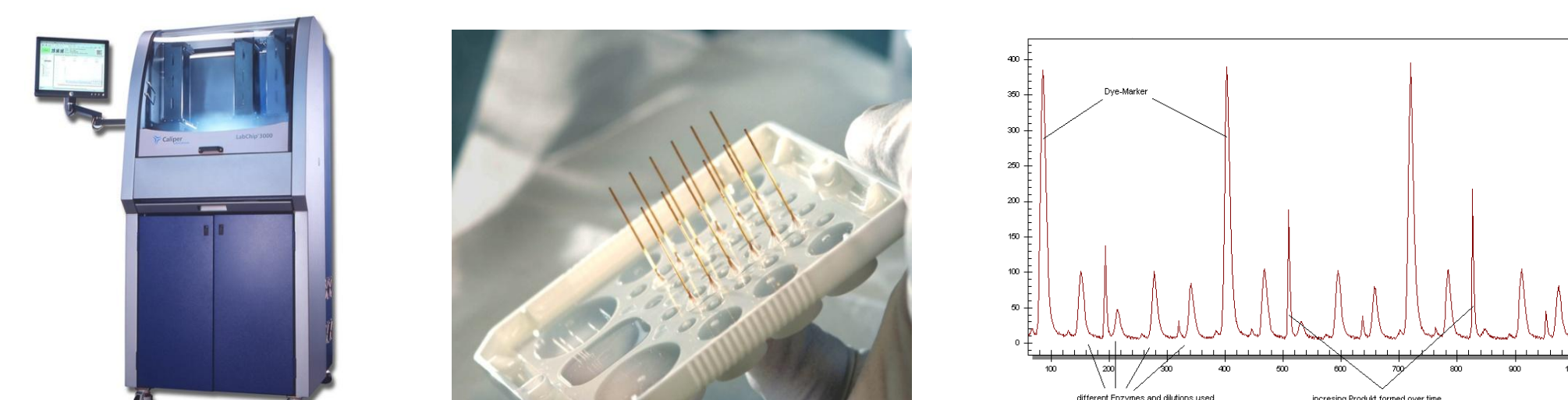


Fig.8: On-chip capillary electrophoresis for kinase, phosphatase and protease assays. Increased effective separation of substrates and products in comparison to well-based assays.

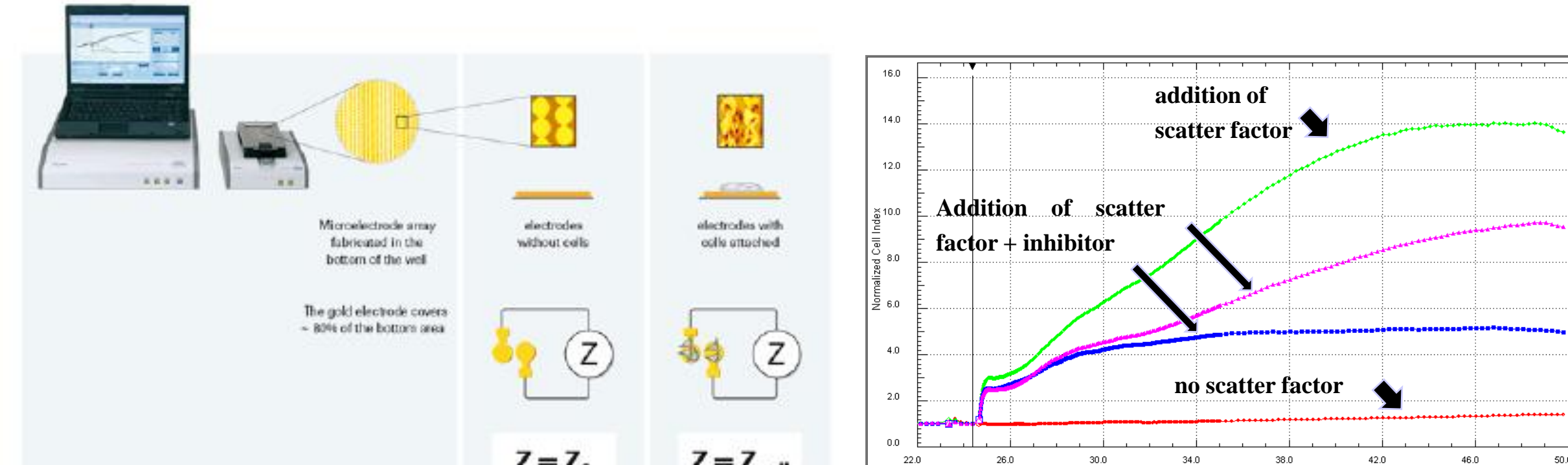


Fig.9: xCelligence system is used for the real time measurement of cell growth and/or proliferation. The cells on top of the electrodes will affect the local ionic environment at the electrode/solution interface. The more cells are attached to the electrode, the larger the increase in the electrode impedance. The graph shows the cell index plotted against the time in presence or absence of a scatter factor^{9,10}.

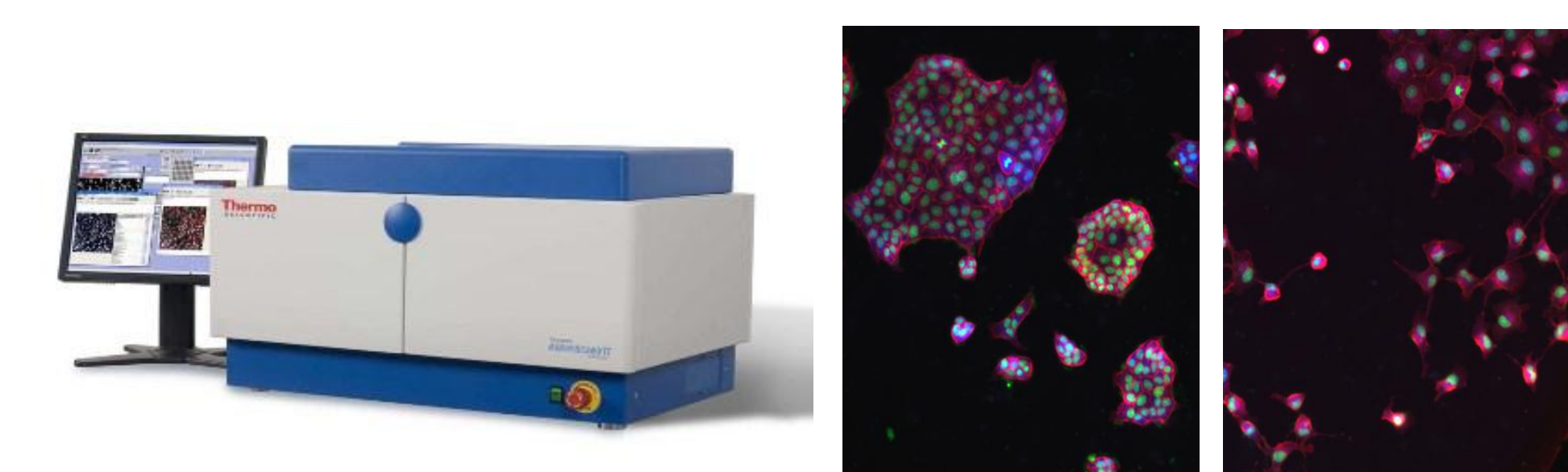


Fig.10: High content microscopy: cells are stained with phalloidin B (red) for actin and with Hoechst 3342 (green) for nuclei. Images are documented and analysed with the Array Scan VTI. Pictures show behavior of MDCK cells before and after addition of a scatter factor⁸.

4. Statistical analysis and evaluation

When a primary screening has been finished we provide a comprehensive statistical analysis for the primary hit evaluation process. Raw data will be available in terms of a distribution pattern (Fig. 11), a heat map (Fig. 12), a structure data file of the „best“ 352 hit compounds (Fig. 13) and a time related plot with positive and negative controls for each plate (Fig. 14)⁵.

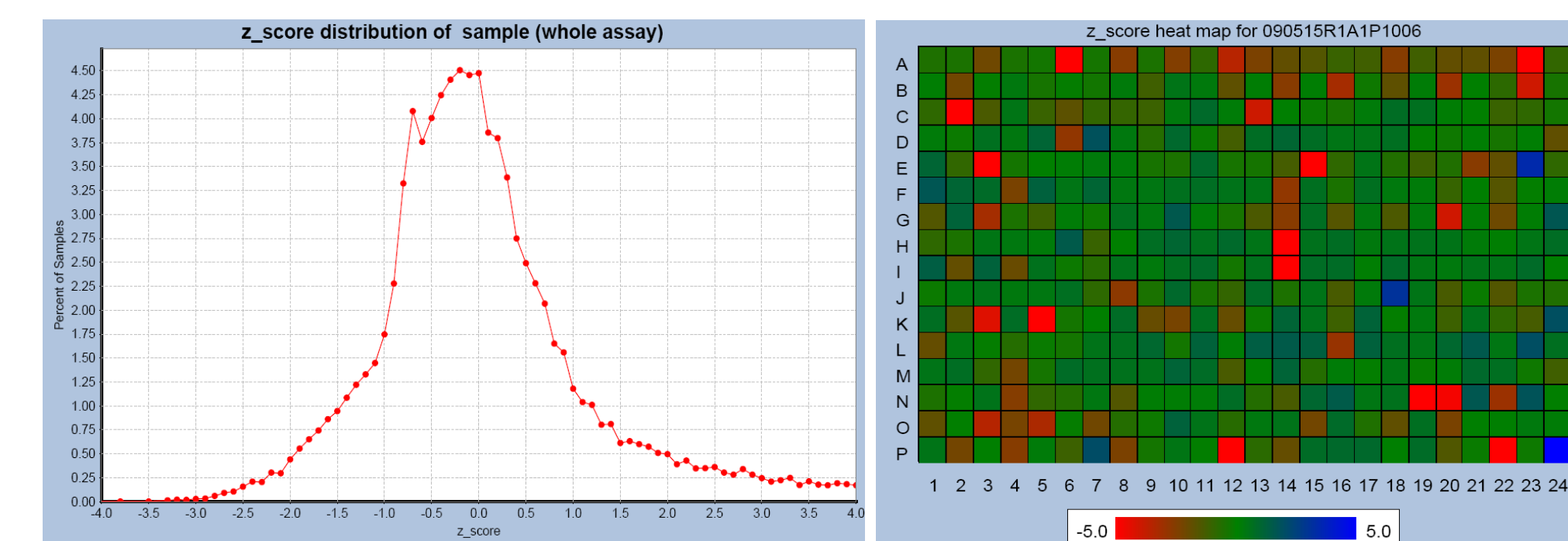


Fig.11: Distribution pattern of each sample type over the whole assay is visualized.

Fig.12: Heat-map plots of normalized data allow detection of edge effects and instrument artifacts.

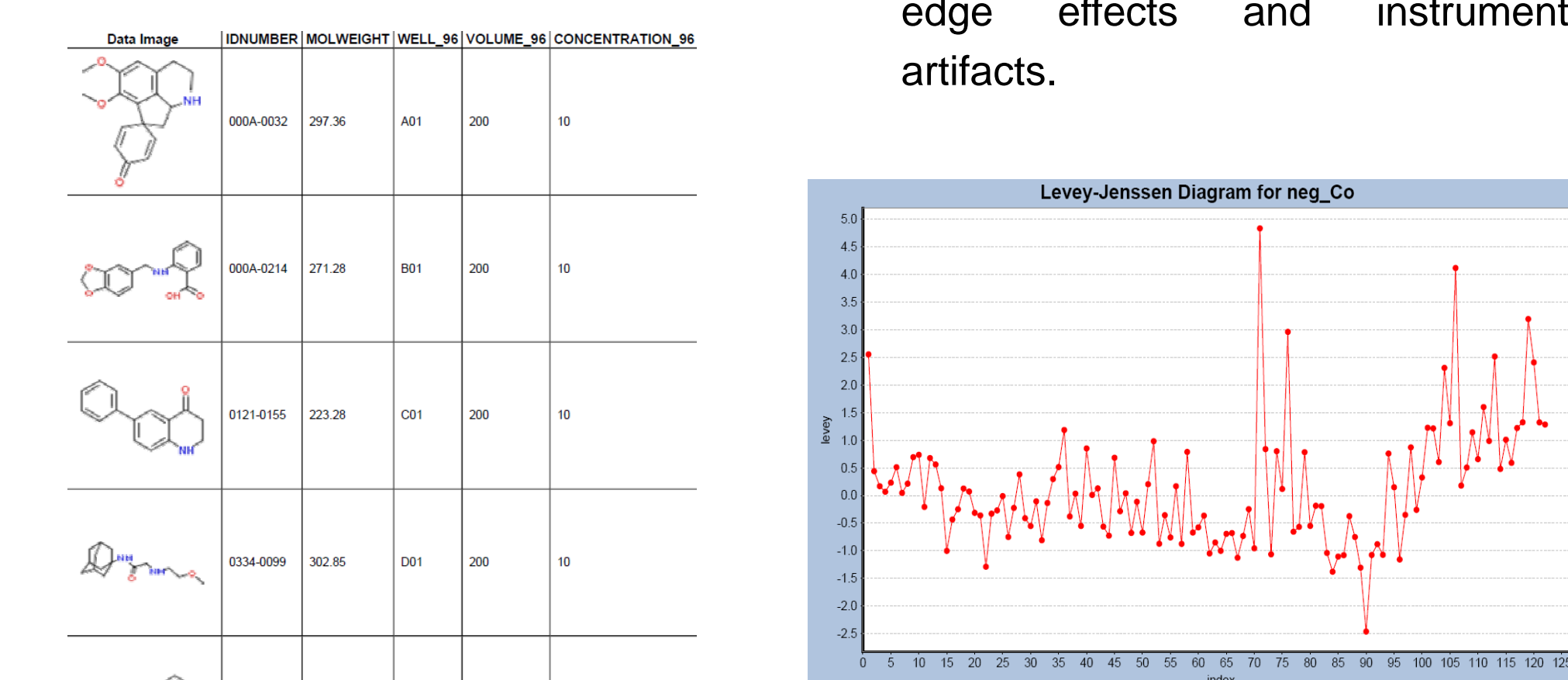


Fig.13: Assay data are stored in structure-data-file format and visualized via pipeline pilot software. Fig.14: For each sample type and plate time-indexed plots permit detection of signal changes over assay execution time.

While the primary screening is conducted with a defined compound concentration (5 or 10 µM) the secondary screen involves measurements based on concentration dependence of hit compounds. Final selection of hits is evaluated with cluster analysis, synthetic and commercial availability and LC/MS data provided for 352 hit compounds (Fig. 15).

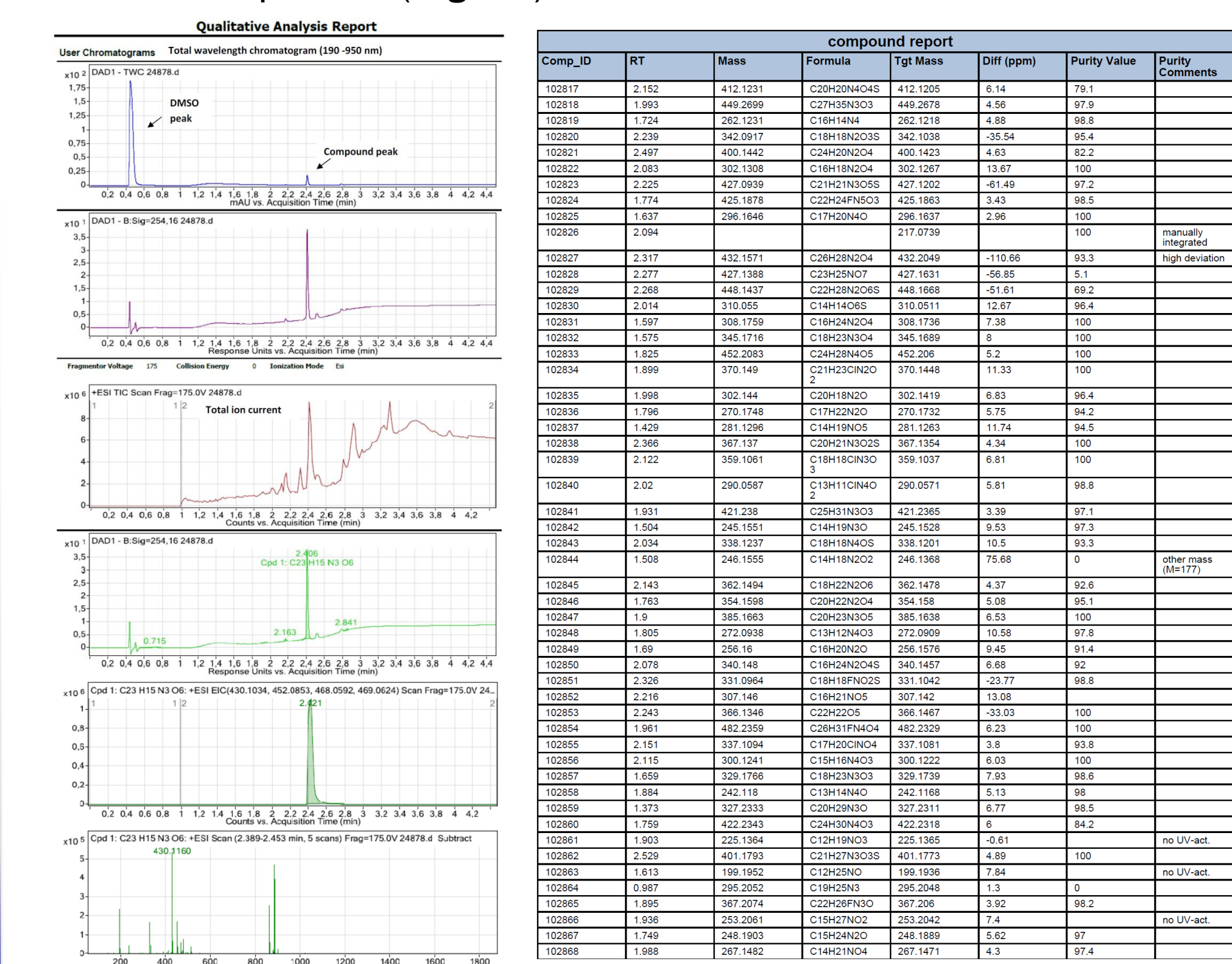


Fig.15: The quality analysis report consists of a total wavelength scan, a scan at 254 nm, a total ion current measurements and the automatic determination of the correct mass according to the molecular formula. The compound report displays a list of hit compounds with their Comp_ID, retention time, measured mass, molecular formula, target mass, difference between measured and target mass and the purity according to the integration of peaks at 254 nm.

References:

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