# Chip'N'Fish™: Novel chemical proteomics platform for rapid identification of small molecule drug candidates against disease-specific targets

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## Introduction

Identification and selection of disease-specific targets is one of the most critical steps in post-genomic drug-discovery. Overexpression of genes and their protein products is often the best starting point for dissecting the pathways that are responsible for the development of specific diseases. Such proteins often serve as molecular targets particularly in cancer, where small molecules are designed to inhibit directly oncogenic proteins that are mutated or overexpressed.

## Our approach

The Chip'N'Fish approach allows identifying overexpressed proteins directly from cell extracts of disease origin using healthy counterparts as controls. Figure 1 shows the scheme of our approach. The advantage of this approach lies on the fact that the 'positive' small molecules can serve as drug candidates as well as tools for target isolation and identification through affinity-based methods. Such drug candidate molecules *(without having the linker)* can also be validated in cytotoxicity assays.

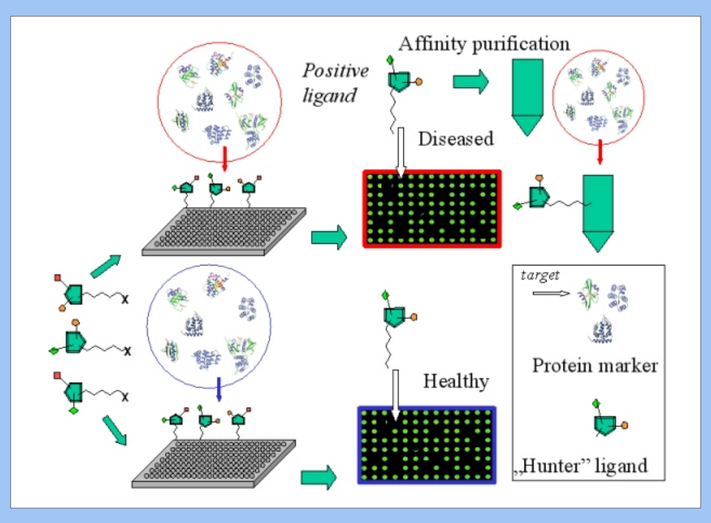


Fig.1.

## Realization

A ligand microarray was generated containing duplicates of 600 compounds with a diverse structure immobilized on reactive glass-slides [1]. Representative compounds are shown in Fig. 2. The array was used for medium-throughput screening of protein-ligand interaction. Entire protein extract from two melanoma cell lines, A375 and RVH, and from primary melanocyte cells were fluorescently labeled with Cy5-dye and incubated on separate microarrays. After scanning the slides intensity data of each spot representing one compound on the array was determined.

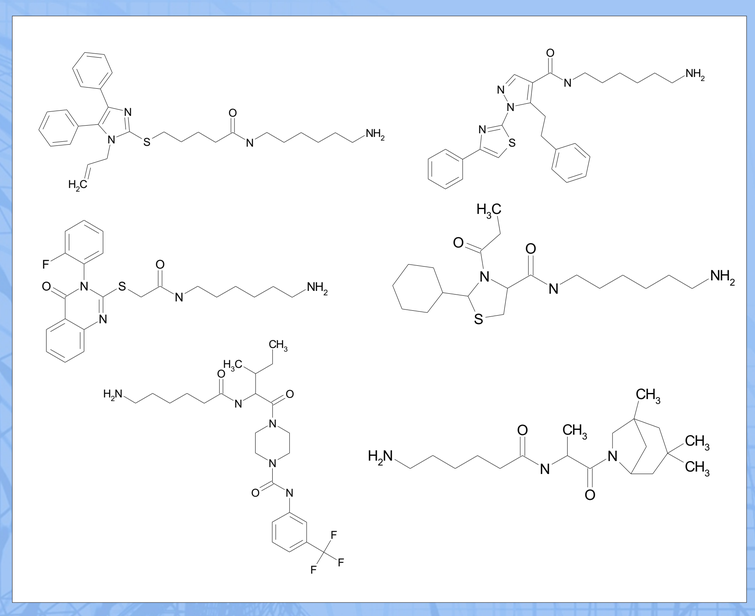

Fig.2.

Those spots which gave different signal intensities on the arrays when compared the melanoma cell extracts with the primary cell extracts, were determined. Fig. 3.

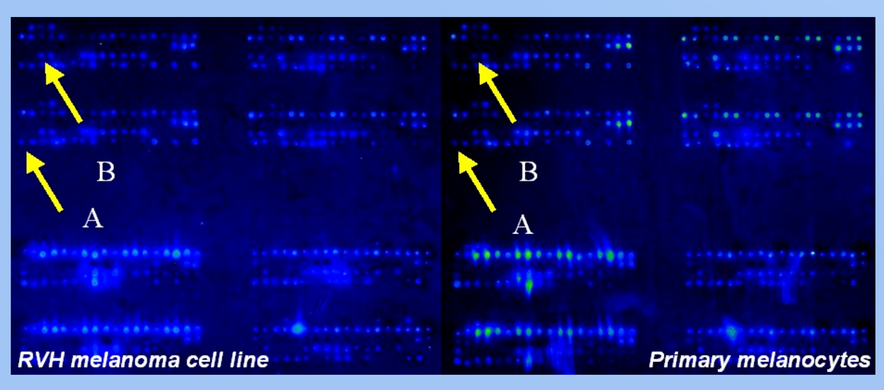


Fig. 3.

## Results

### *1. Microarray analysis*

Out of 600 compounds 354 gave significantly higher intensity value compared to the background. 2 compounds exhibited decreased and 3 compounds had higher intensities than in the control array. 3 compounds (M2123, M3520 and M1931) gave higher signals in case of melanoma cell line RVH than in the A375 cells when compared to the primary control cells. In response to M3520 A375 gave slightly higher intensity value than the control cells (1.75-fold). The RVH signal was significantly higher (2.59-fold) than the melanocyte one. In case of M2123 and M1931 an average of 2-fold difference could be detected.

### *2. In vitro analysis of selected, interacting molecules*

The selected compounds (M2123, M3520 and M1931), without bearing the linker side-arm, were further tested in cell cultures for analyzing their phenotypic effects *in vitro*. 500 cells were incubated with compounds at 10 and 30 μM concentrations for 72 hours. Number of viable cells were determined (Fig. 4.). At 10 μM concentration M3520 had the most pronounced effects on melanoma cells. At higher concentrations RVH cells were more sensitive to all of the compounds tested. The number of viable cells decreased dramatically. Treatment with M3520 completely inhibited cell division, resulted in 1/10 cells compared to untreated cells.

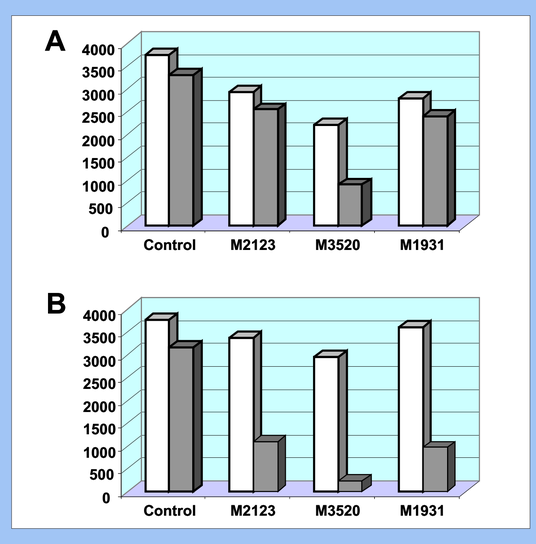


Fig. 4.

## Summary

600 compounds immobilized on glass-chips were used in differential protein binding assay using cell extracts of healthy and disease origin. We concentrated on those spots where intensity values were higher using one of the melanoma cells than obtained from the primary cells, as they might represent ligands that interact with protein targets having over-expressed in the melanoma cells.
We hypothesized that the expression of the interacting protein(s) is induced in the melanoma cell lines and could be responsible for certain cell functions which relate to malignancy or increased cell division potency.
We found that one of the 3 selected compounds expressed significant cytotoxicity.
These results provide a preliminary proof-of-concept of our Chip'N'Fish approach. The extension of the validation is currently under progress, including identification of the exact protein target with affinity based methods.

## References

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[3] G Dormán and F Darvas: Utilizing small molecules in chemical genomics: toward HT approaches, In: *Chemical Genomics*, Eds. F Darvas, A Guttman, G Dormán, Marcel Dekker, New York, Basel, 2004, pp. 137-197.