



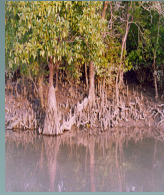
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### Background

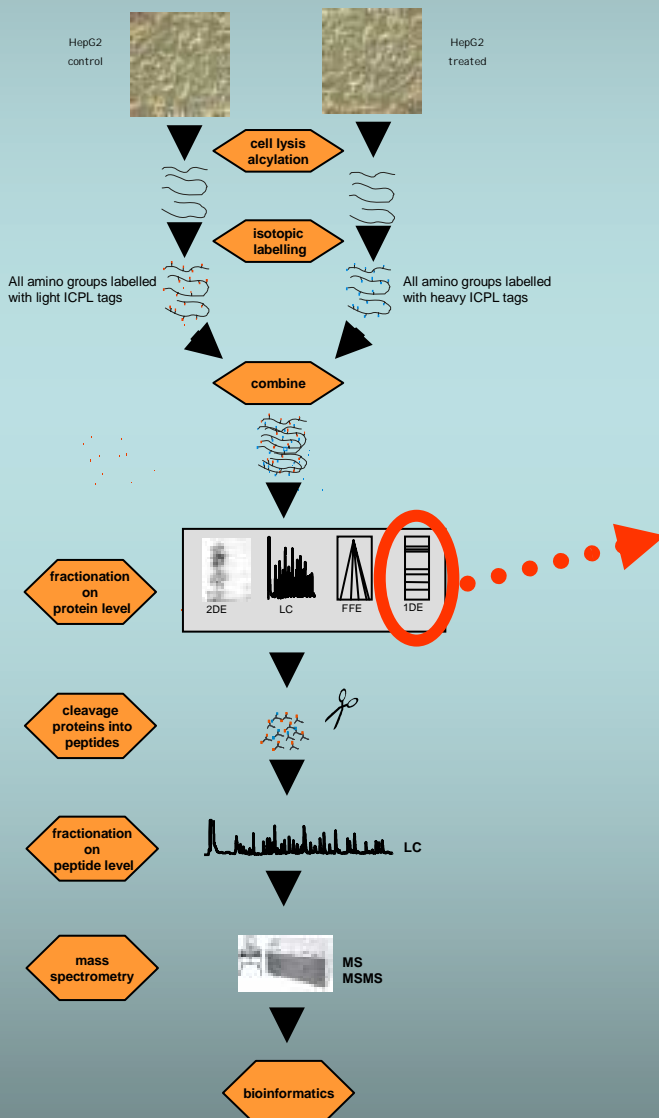
The overall objective of the MONACO project is the identification of new lead compounds for drug discovery from natural sources related to traditional Chinese medicine. In particular, mangrove plants and their associated microorganisms represent a unique ecological system which can be found on tropical and sub-tropical coast lines and turn out to be a rich source for natural products<sup>1)</sup>.



### Proteome Analysis

For functional characterisation of bioactive compounds HepG2 liver tumor cells<sup>2)</sup> are treated under standardised conditions with cytotoxic substances in well defined concentrations. For protein quantification we use a unique isotopic labelling technique termed ICPL<sup>3)</sup>. It is based on differential isotopic labelling of proteins derived from two cell states with either light or heavy tags. After labelling both samples are combined and separated using different proteomic methods. Finally, the labeled peptides are identified by MS/MS analysis (Proteomics analyzer 4700 MALDI-TOF/MS) and protein database search (GPS-Explorer/Mascot). With the help of bioinformatics, functional conclusions can be drawn in order to disclose in which regulatory and metabolic networks the referring compound is involved.

### Workflow



### Cell Cultivation and Compound Treatment

Defined numbers of HepG2 cells, harvested in the logarithmic phase of their growth, were cultivated. After 24h (cell confluence about 50-60%) cytotoxic substances were added to the cultures in defined concentrations. After an incubation period the cells were analysed by both cytotoxicity (Fig.1)<sup>4)</sup> and apoptosis assay (Fig.2)<sup>5)</sup>. First, five standard substances - Adriamycin, Paclitaxel, Cyclosporin A, Trichostatin A and Camptothecin were analysed to evaluate the whole system. Their cytotoxic effect and their target at different distinct metabolic pathways in cells is well known.

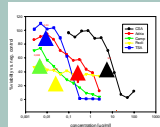


Fig. 1: Cytotoxicity plots of 5 standard compounds - Cyclosporin A, Adriamycin, Camptothecin, Paclitaxel and Trichostatin A on HepG2 cells after incubation over 24h.

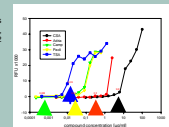
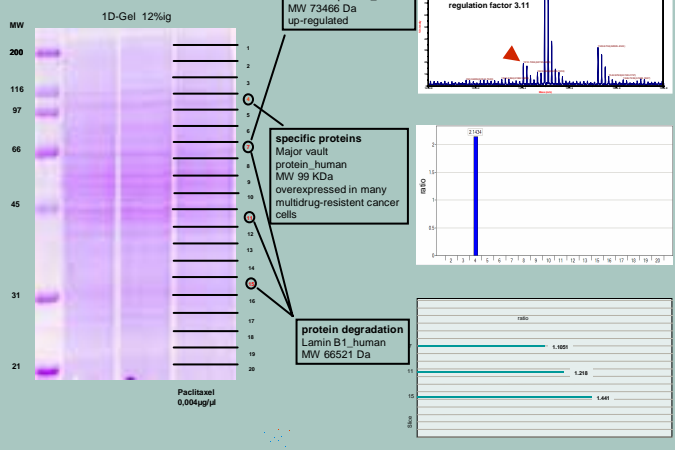


Fig. 2: Photos of apoptosis assay. The arrows show the concentrations, applied in cultivations for the determination of proteomic profiling.

### HepG2 Cells treated with Paclitaxel Identification and Quantification of Proteins

Paclitaxel is the disturbance of the function of microtubules which then leads to cell cycle arrest at G2/M-phase



### Results of Paclitaxel Treatment

> MS	8000
> MSMS	47560
> total identified protein species	2443
> unique proteins	1351
> regulated proteins (>30% regulation)	288
up-regulated	57
down-regulated	231

### References

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### Summary

The ICPL method provides a precise quantitative determination of differential expressed proteins in highly complex protein mixtures. For an accurate quantification and identification of proteins within complex systems the combination of protein labelling, gel-electrophoreses and LC-MS/MS is a powerful approach.