EBERHARD KARLS
UNIVERSITÄT
TÜBINGEN

Drug Discovery at the Eberhard Karls Universität Tübingen

Faculty of Science
Department of Pharmacy and Biochemistry – Institute of Pharmacy

Drug Discovery at the Eberhard Karls Universität Tübingen

Medicinal Chemistry is a quite heterogeneous area dealing with all chemical aspects of pharmaceutical drug discovery and development. 1877 can be considered as date of birth of this discipline when Phenacetin, the first fully synthetic drug driven by organic chemistry, was launched to the market.

Today, more than 4.000 molecules are registered as APIs (active pharmaceutical ingredients) and sold under more than 70.000 brand names. However, these 4000 molecules only act on 450 out of 3000 potential disease-relevant targets (druggable genome) underlining both underutilized chances and possibilities for novel drugs. The relatively young discipline Medicinal Chemistry had a substantial impact on both improvement in life quality and improvement in life expectation in this period. Cancer and degenerative diseases, particularly neurodegeneration, are urgent fields of therapeutic innovation for a strongly aging population.

The Medicinal Chemistry at the University of Tübingen is located in the Department of Pharmacy and Biochemistry with a chair for Pharmaceutical/ Medicinal Chemistry. Four professors contribute to this discipline.

Research Profile:

- Major targets are protein kinases and eicosanoids involved in inflammation, autoimmune diseases, neurodegenerative diseases and cancer / cancer chemoprevention.
- Therapeutic intervention in the p53 signalling network provides novel chances for cancer treatment and chemoprevention
- Integration of structural biology and molecular design leads to hypothesis-driven synthesis and rational exploration of the chemical space.
- Computational chemistry and molecular biophysics help to address fundamental questions such as the characterization of new drug-host interactions (e.g. halogen bonding) and are used to complement synthesis.
- Efficient and flexible synthetic methods to vary the lead structure extensively are essential for the derivation of structure-activity relationships, as well as for rapidly obtaining the optimised compound.
- Metabolism and kinetics are included as early as possible in the development process as these properties are major reasons for failure of drug candidates.

- Bioanalytics is fundamental for both metabolism and biological testing. Development of bioassays, high performance liquid chromatography methods coupled with mass spectrometry (HPLC-MS), as well as new dedicated separation materials for metabolomics, biomarker analysis, and biopharmaceutical separation are major research directions.
- This leads to a cyclic, multi-disciplinary iterative approach integrating *in silico* design, synthesis, and biological testing to refine and adapt the design hypothesis (Figure 1).
- The major goal is the development of high quality tools and methods, as well as molecular probes for both *in vitro* and *in vivo* studies. Finally, even preclinical development candidates can be achieved.

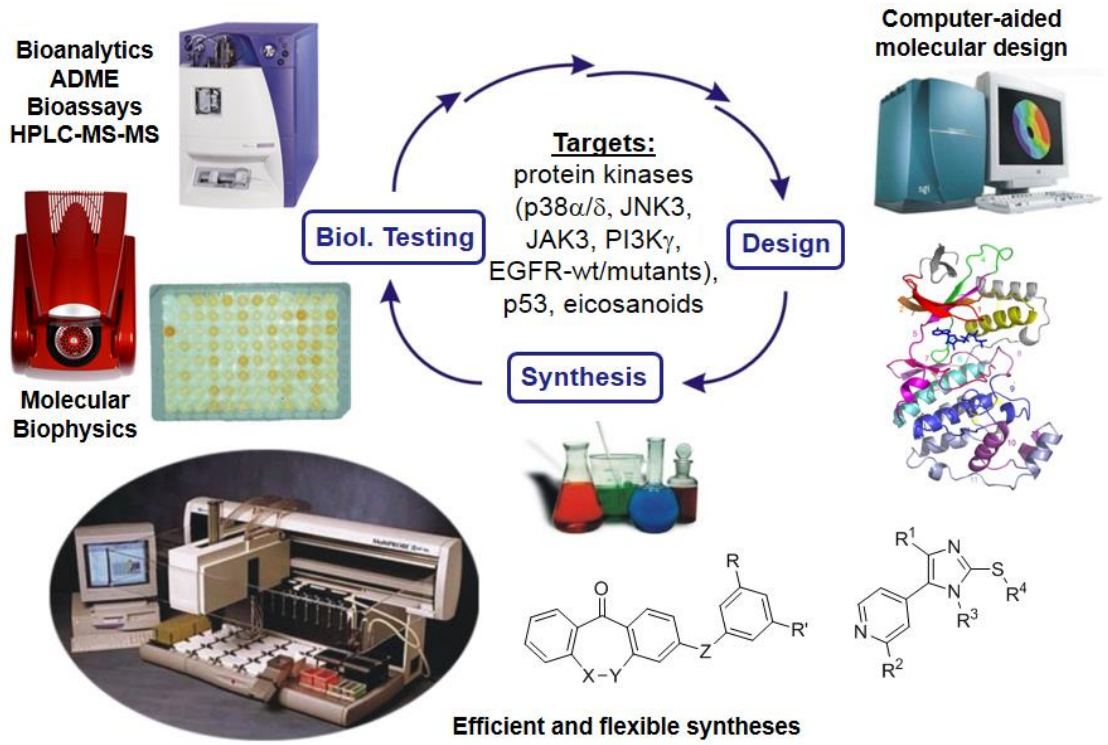

Figure 1: Cyclic iterative approach integrating *in silico* design, synthesis, and biological testing to refine and adapt the design hypothesis.

Protein kinases

Protein kinases are a large and diverse multi-gene family of enzymes (about 1.7% of the human genome), which regulate many different cell proliferation, differentiation, and signaling processes by adding phosphate groups to target protein substrates. Disease arises when signal transduction in a cell breaks down, thereby removing the tight control that typically exists over cellular functions. Devastating diseases such as

cancer, autoimmune diseases, inflammation, psoriasis, allergic reactions, neurological disorders and hormone-related diseases can result from abnormal signal transduction.

At present 518 kinases are identified, in which all of them bind the cofactor ATP in a very similar way (Figure 2, left). The conservation of structural features within the ATP binding cleft initially indicated that the design of selective ATP-competitive small molecule inhibitors would be very challenging. Currently, there are more than 15 drugs on the market that address protein kinase targets.

At the pharmaceutical institute of the Eberhard Karls Universität Tübingen, teardrop binder-type protein kinase inhibitors were designed for their ability to inhibit protein kinases, which can be used for the treatment of inflammation, neurodegenerative diseases, autoimmune diseases and cancer (Figure 2). For the development of novel potential anti-cancer drugs, we are not only focusing on the inhibition of the target protein kinase but also on the inhibition of the mutated protein kinase. The rationale is clear: tumor variability and propensity to resistance demands variation of selection pressure. A drug with one or few targets conferring a tumor suppressive effect will quickly select for resistance. Too many targets will lead to limited tolerance, which will limit dose and, thereby, effect.

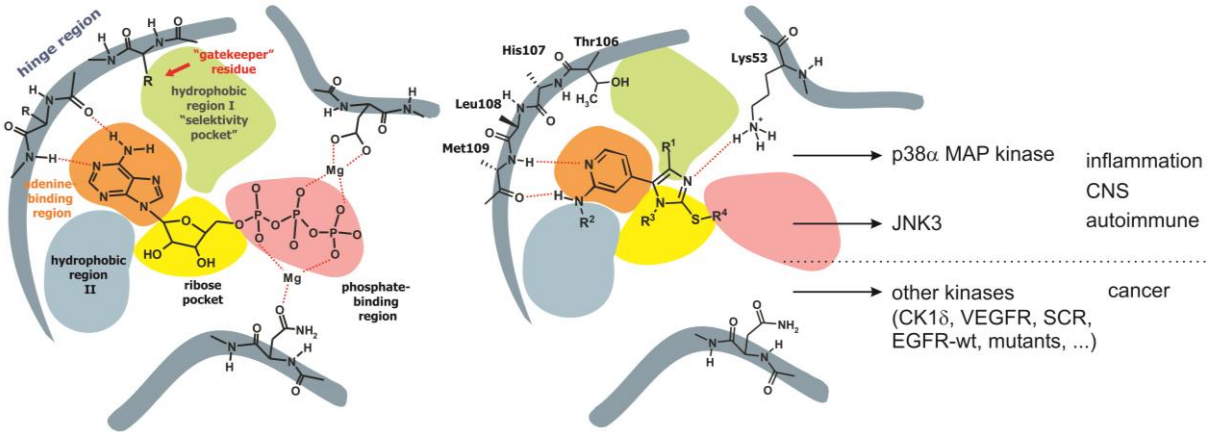

Figure 2: Comparison of the binding mode of ATP (left) and protoypical teardrop binder (right) in the ATP binding site.

We also followed a different approach with compounds, which only interact with the hinge region and the hydrophobic regions (linear binder). We intended to make use of a peptide flip at the hinge region, which is induced by a carbonyl-interaction of the

inhibitor with two backbone NH-groups to address the hydrophobic region I and II with aryl-residues (Figure 3, left). Another structural requirement was reducing conformational flexibility of the inhibitors. A rigid structure should allow only less induced fit to other than the target (off-target) kinases. Starting from initial benzophenone leads, we developed dibenzosuberones and optimized them down to single digit nanomolar IC_{50}-values against p38α MAP kinase and excellent selectivity profiles against other protein kinases. To this end, Skipenone-L, the first ATP-competitive p38α MAP kinase inhibitor with excellent *in vi*vo efficacy and selectivity, was designed. This compound achieved "high quality kinase probe" status at Nature Chemical Biology. The binding mode was confirmed by X-ray crystallographic studies as well as by protein-NMR experiments in solution. Skepinone-L serves as biological probe and *in vivo* tool for pharmacological experiments.

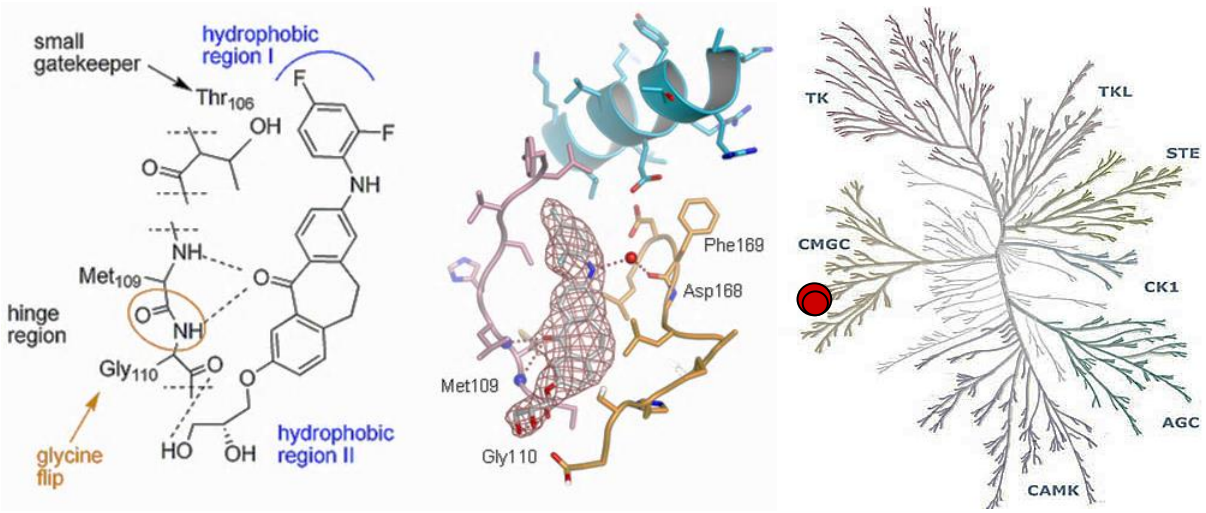

Figure 3: Binding mode and kinome dendrogram of Skepinone-L. Kinase data were generated with the KINOMEscan screening platform.

The compound library at the Medicinal Chemistry Division of the University of Tübingen contains more than 4000 profiled compounds.

Molecular Interactions: Halogen Bonding

Molecular interactions are the key to understanding affinity and selectivity. Until recently they appeared to be fully known and understood. However, more attention is currently drawn to modern, more sophisticated types of interactions, such as halogen bonds. Based on an anisotropic electron distribution of the electron density, a "crown

of positive charge” (σ-hole) opposite of the covalent bond of the halogen allows the formation of attractive interactions with Lewis bases that “donate electron density”. Different “bonding models” (electrostatic, dispersion, polarization and charge transfer to the σ* LUMO orbital of the R-X bond) may contribute to a varying extent in different systems, rendering the description of this type of interaction difficult. We have used quantum chemical methods for investigating small model systems of biological relevance, to understand the dependence of halogen bonding geometry and energy, enabling us to derive predictions for favourable applications of halogen bonds in drug design. We have furthermore pioneered the efficient identification of halogen bonding patterns as essential key interactions in protein binding sites by halogen-enriched fragment libraries (HEFLibs). The high ligand efficiencies that can be found for fragments, frequently lead to optimized interaction networks based on halogen bonds when using HEFLibs. We furthermore have established design algorithms for identifying ligand-protein complexes benefitting from the introduction of halogen bonds and we work toward recognition of halogen bonds in virtual screening campaigns by parameterization of scoring functions.

p53

The tumor suppressor p53 plays a pivotal role in cancer prevention and defense. As a transcription factor, it transactivates a variety of genes, leading to the activation of DNA repair, induction of growth arrest, initiation of apoptosis, or inhibition of angiogenesis. In collaboration with the MRC Laboratory of Molecular Biology (Prof. Sir Alan R. Fersht) we have discovered and developed multiple series of stabilizers of the p53 mutant Y220C (PhiKans), which act as pharmacological chaperones. We have demonstrated that p53 mutant rescue is feasible and can provide new therapeutic opportunities for fighting cancer.

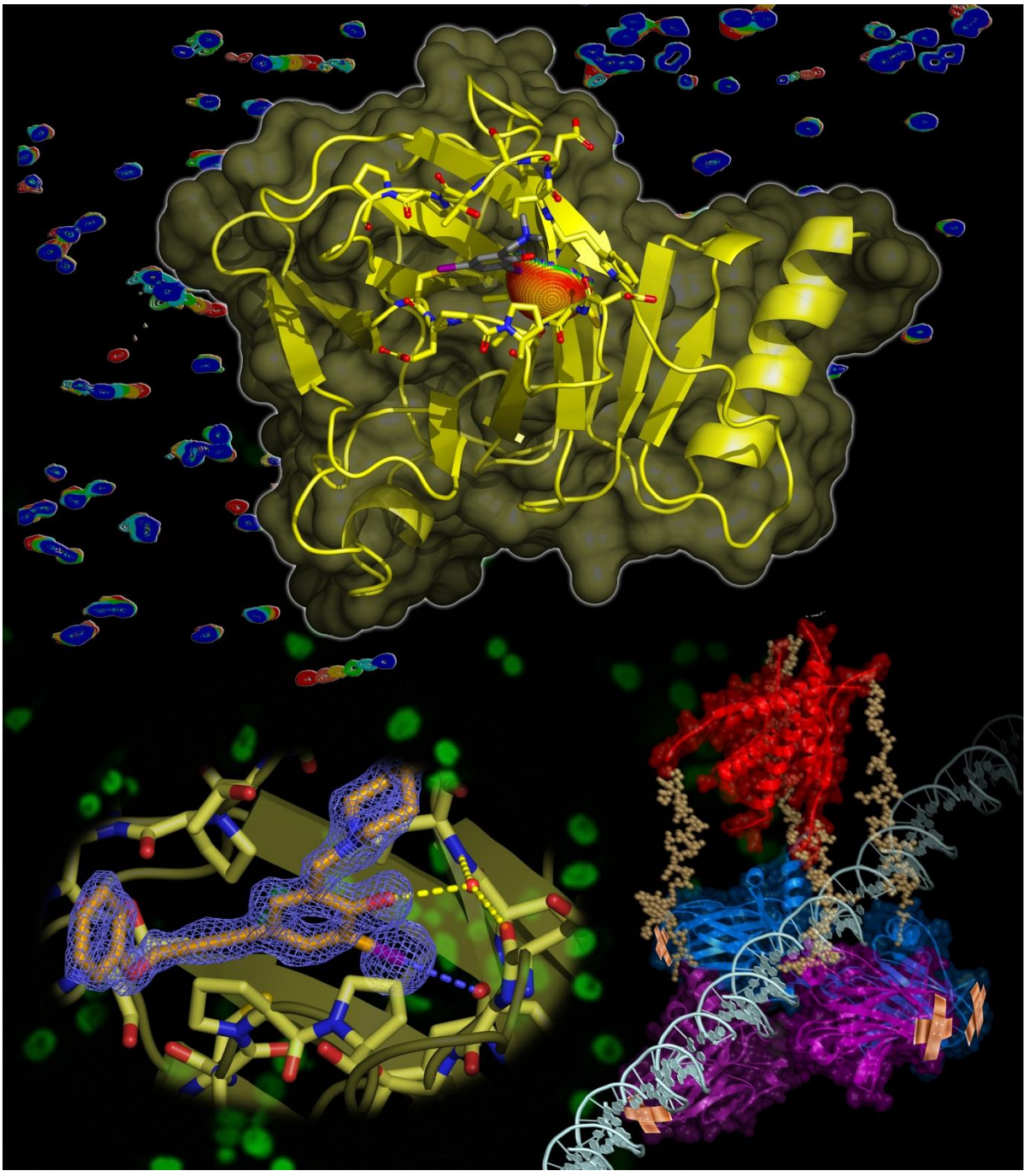

Figure 4: Halogen bonding was exploited for finding mutant p53 activators by screening halogen-enriched fragment libraries (HEFLibs) by biophysical methods (top). The small molecule PhiKan784 binds to the mutation-induced cavity in the p53 mutant Y220C through a halogen-carbonyl interaction (left). Its analogs act as pharmacological chaperones, stabilizing the folded state of the mutant and facilitating its transcriptional activity (right).

Eicosanoids

We have focused for a long time on eicosanoid modulators. The dual LOX/COX inhibitor LICOFELONE (ML3000) has made it up to phase III clinical trials in Osteoarthrits. Other indications like cancer chemoprevention are still under active

research. However, after the failure of the Coxibs, there is still a continuous need for better "3rd generation non-steroidal anti-inflammatory drugs (NSAIDs)", both for classical inflammatory indications as well as cancer.

Prostaglandin (PG) E_2 is one of the most important and powerful prostanoids with diverse biological activity. As a key cyclic lipid mediator derived from arachidonic acid (AA), it is involved in the development and perpetuation of inflammation seen in diseases like rheumatoid arthritis. AA has been implicated in the development of peripheral and central sensitization during nociceptive processing (e.g. hyperalgesia, allodynia) as well as in tumorigenesis. In the eicosanoid pathway, induction of PGE_2 biosynthesis during inflammation requires the enzymatic actions of two cytokine-inducible enzymes: cyclooxygenase (COX) and prostaglandin E synthase (PGES). Inhibition of COX results in a reduced synthesis of prostaglandins (e.g. PGE_2) and thromboxanes (TXA_2) and is the basis for the anti-inflammatory efficacy and probably also for the analgesic activity of NSAIDs. Owing to undesirable adverse effects of COX inhibitors (NSAIDs) and COX-2-selective inhibitors (COXIBs), in the gastrointestinal, renal, and cardiovascular systems, the selective inhibition of PGE_2-forming enzymes downstream of COX, such as the inducible glutathione-dependent microsomal PGE_2 synthase-1 (mPGES-1), has recently been proposed as a more promising approach for development of drugs for anti-inflammatory and pain therapy. mPGES-1 is a member of the MAPEG family, including FLAP, and is the key terminal enzyme in pathology related production of PGE_2 from COX-2 derived PGH_2 (Figure 5).

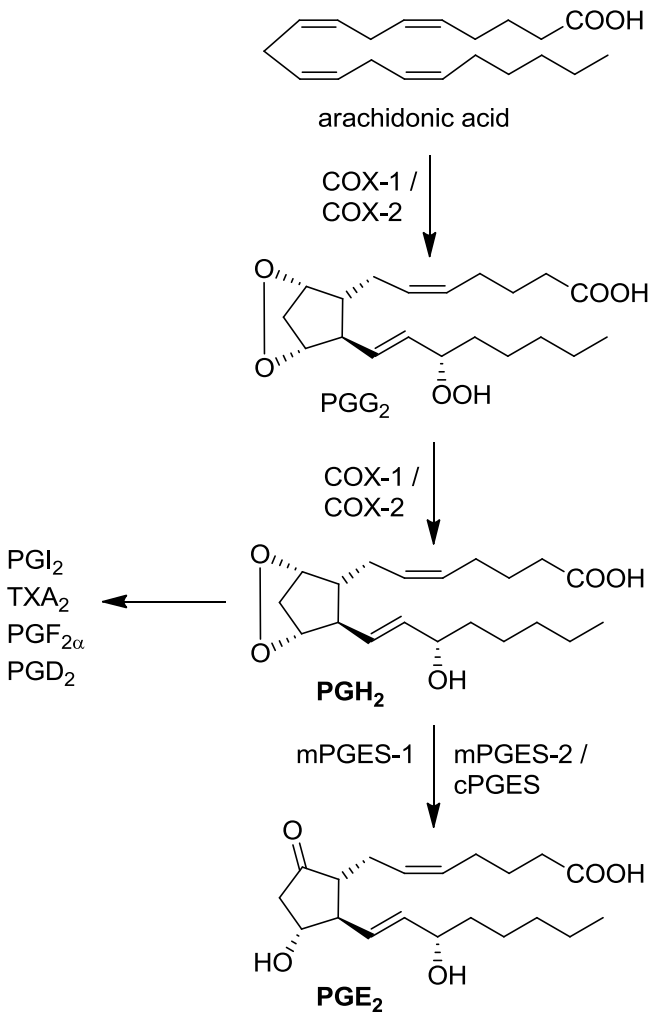

Figure 5: Biosynthetic pathway of PGE_2.

Based on conventional NSAIDs, we have successfully modified the carboxylic acid to sulfonamides in order to minimize COX-1/2 activity and to optimize both mPGES-1 and 5-LOX inhibition (Figure 6).

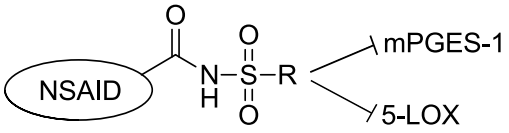

Figure 6: Modification of conventional NSAIDs.

Building knowledge based on a strong modern instrumental (bio)analytics

Integral part of the research in the division of Medicinal Chemistry at the University of Tübingen is a strong commitment to separation science and modern instrumental analysis technologies as well as their integration in fundamental drug discovery research and clinical analysis. Besides fluorescence, NMR and FTIR spectroscopy,

further modern instrumentation comprises (U)HPLC, nano-HPLC, capillary electrophoresis, GC-MS, HPLC-MS^n, and MALDI-TOF besides various other instrumental techniques.

For the biological testing of developed new drug candidates various bioassays for protein kinases, eicosanoids, cytokines as well as *in vitro* metabolism have been established. Moreover, full characterization of drug disposition involves pharmacokinetics analysis by HPLC-MS/MS and elucidation of metabolic pathways of xenobiotics *in vitro* as well as *in vivo*. Currently, workflows are developed for comprehensive metabolomics profiling with the goal to support drug discovery and development as well as early toxicological screenings. Furthermore, developed HPLC-MS/MS platforms have aided bioprocess optimization in biotechnological drug synthesis and biopharmaceuticals production. Future research directions are aiming at using metabolomics and lipidomics approaches to identify biomarkers of disease.

In contrast to these untargeted, comprehensive analysis strategies, new methodologies for the targeted biomarker screening in clinical analysis with high sample throughput capability as well as selective and sensitive MS detection are elaborated. In one research focus, oxidized phospholipids are analyzed in human plasma samples as biomarkers of oxidative stress utilizing functionalized nanoparticles for selective enrichment and MALDI-TOF or HPLC-MS/MS detection.

Another research focus is dedicated to the development of new functionalized separation materials comprising chiral stationary phases, mixed-mode chromatography phases, macroporous monoliths, functionalized nanoparticles, and biochromatography media with chemoaffinity type molecular recognition principles. For example, new stationary phases are devised for the analysis and purification of distinct topological forms of plasmids (pDNA) which are currently under development and clinical evaluation as potentially useful biopharmaceuticals in nonviral gene therapy as well as genetic vaccination and some of which are regarded as impurities. Ligands which can selectively interact with different pDNA isoforms as well as can recognize distinct topoisomers have been elaborated and, after their immobilization onto chromatographic supports, gave rise to highly selective separations of the therapeutic covalently closed circular isoform from impurities such as nicked, linear and oligomeric topologically distinct forms as well as topoisomers. Similar strategies are utilized for the design of new media for analysis and purification of oligonucleotide and protein therapeutics such as monoclonal antibodies.

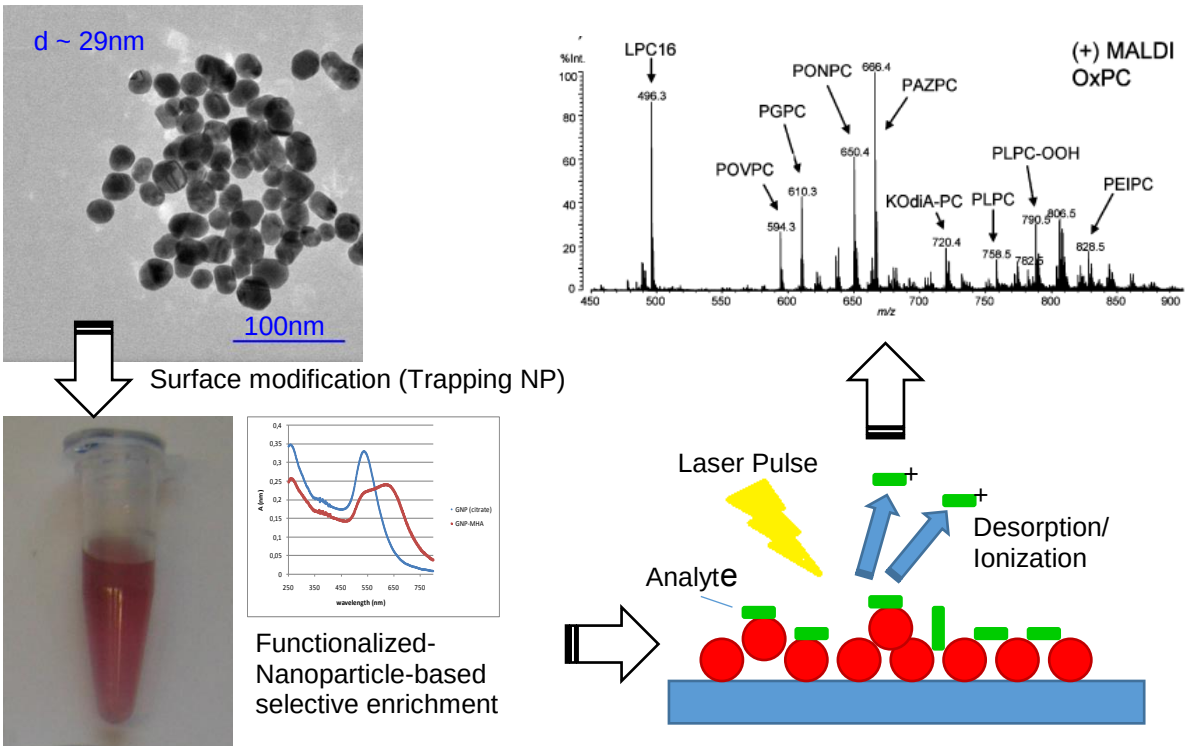

Figure 7: Gold nanoparticle based enrichment and MALDI-TOF detection of oxidized phospholipids as biomarkers of oxidized stress.

Interfaculty Centre of Pharmacogenomics and Drug Research

Medicinal Chemistry needs even more of integration and translational research to develop the full value chain. Therefore, the Interfaculty Centre of Pharmacogenomics and Drug Research (ICEPHA) was created in Tübingen together with the Robert Bosch Foundation and Robert Bosch Hospital in Stuttgart as an interconnection between chemical and biological sciences and human medicine, thus forming a dynamic network in focused research areas across academic expertise and pharmaceutical industry users. ICEPHA is a research network as well as a service and development center for innovative drugs and therapies. Such a construction allows to carry out research projects that cannot be handled efficiently through one single institution.

ICEPHA offers expertise in the full development chain ranging from target identification to proof of concept in human (Phase I/IIa clinical trials) except regulatory safety and toxicology studies.

One major interest of ICEPHA is to provide a basis for tailor-made individualized therapy with patient-directed drugs and dosage (personalized medicine). Therefore, ICEPHA is concentrated on genes which (A) affect the susceptibility of patients to

drugs or (B) are associated with the manifestation of disease. Program (A) provides knowledge for defining and investigating targets for custom-tailored drugs. Program (B) is the basis for a predictable individualized therapy with patient-directed drugs and dosage. This is of prime importance for the maximum benefit for the patient's health after therapeutic intervention, but of equal importance for the economy of the public health system.

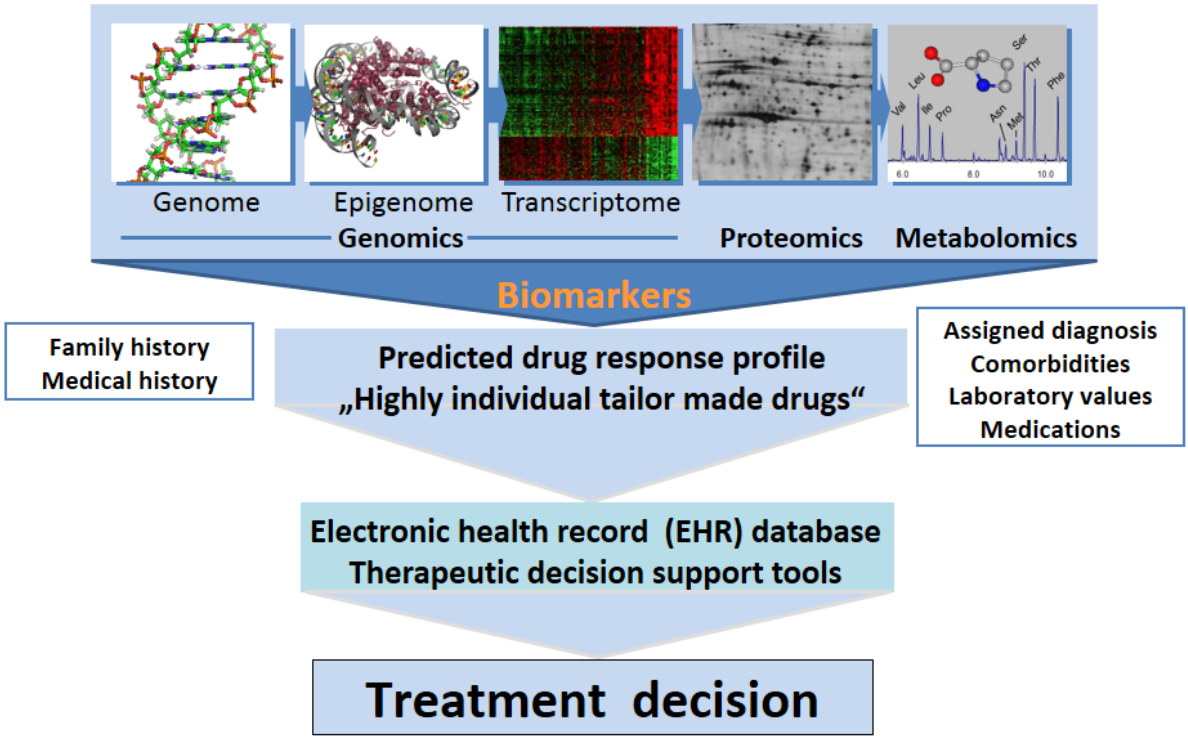

Figure 8: Future progress in personalized therapy by OMICS technologies (modified from: Meyer, Zanger, Schwab *Annu. Rev. Pharmacol. Toxicol.* **2013**).

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ICEPHA profile and competences:

- Target identification
- Target validation (Knock-out mice)
- Assay development
- Bioanalytics
- Medicinal Chemistry (hit to lead to candidate, pharmacological tools and probes)
- Computational chemistry
- Pharmacology
- Mouse clinic

- ADME, preclinical studies ranging from *in vivo*, *in vitro* to genetic modified animal models
- Formulation development
- Clinical trial unit
- GMP facilities
- Patient tissues bank
- Clinical cohorts
- Genotyping

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