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The Pharmaceutical Biochemistry Group: Where Pharmaceutical Chemistry Meets Biology and Drug Delivery

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Abstract: Successful drug discovery and development of new therapeutics is a long, expensive multidisciplinary process needing innovation and the integration of smart cutting edge science and technology to overcome the challenges in taking a drug from the bench to the bedside. The research activities of the Pharmaceutical Biochemistry group span the drug discovery and development process, providing an interface that brings together pharmaceutical chemistry, biochemistry, structural biology, computational chemistry and biopharmaceutics. Formulation and drug delivery are brought into play at an earlier stage when facing the perennial challenge of transforming a potent molecule *in vitro* into a therapeutic agent *in vivo*. Concomitantly, drug delivery results can be understood at a molecular level. This broad range of interdisciplinary research activities and competences enables us to address key challenges in modern drug discovery and development, provides a powerful collaborative platform for other universities and the pharmaceutical industry and an excellent training platform for pharmacists and pharmaceutical scientists who will later be involved in drug discovery and development.

Keywords: Cancer · Drug delivery · Drug discovery and development · Neglected diseases · Pharmaceutical biochemistry and chemistry · Transdermal

Research Activities of the Pharmaceutical Biochemistry Group

The research of the Pharmaceutical Biochemistry Group aims at addressing some of the challenges in taking a drug from the bench to the patient[1,2] by integrating cutting-edge science and technology[3] using an interdisciplinary approach. Solving the twin problems of lack of efficacy and high toxicity goes through the selection of the right target, the right molecule and the appropriate delivery technology and formulation for the appropriate clinical trial.[4] The research of the group tackles the issues related to choosing the right targets, molecules and formulations by combining two complementary research fields. Research in the Pharmaceutical Biochemistry/Chemistry field led by Prof. Scapozza and Dr. Perozzo focuses on molecular recognition for a better understanding of ligand–macromolecule interactions to develop therapeutic strategies involving new chemical entities and targets, using an approach based on the combination of biochemistry/biophysics, organic chemistry and computational chemistry/molecular modelling techniques, and covers three main topics:

• Cancer: we have two main objectives, namely the development of inhibitors of the tyrosine kinase domain of oncogenic fusion proteins involved in signalling pathways and the development of a thymidine kinase-based safety and monitoring tool for stem cell therapy.

• Antibiotics: in the context of a Sinergia (SNF) project we aim to find antibiotics with novel mechanisms of action that inhibit bacterial virulence.

• Neglected diseases: we aim to elucidate and to validate new drug targets against the major parasitic diseases of the Third World (malaria, leishmaniasis, African trypanosomiasis) and to find active lead compounds.

In addition, a new activity has recently been initiated to develop a molecular recognition-based approach for improving antibody formulation.

Research on Cutaneous Drug Delivery led by Dr. Kalia focuses on topical and transdermal delivery of therapeutic agents by investigating the effect of molecular properties on both passive and active transport processes and exploring how innovative technologies can be used to increase the range of therapeutic agents that can be delivered across the skin and increase options for therapy. The main areas of research are:

• Synthesis and characterization of prodrugs optimized for topical and transdermal administration.

• Development of new formulations to increase topical and transdermal drug delivery.

• Development of new techniques for the non- and minimally invasive delivery of biotechnology-derived therapeutics across the skin; evaluation of alternatives to parenteral administration.

Here, we present a brief overview of some of our research activities.

Cancer Research: Tyrosine Kinase Plasticity and the Development of Protein Kinase Inhibitors

Protein tyrosine kinases (PTyrKs) are critical components of the cellular signalling pathways[5] that are involved in the control of cell growth, metabolism and apoptosis.[6] Deregulated protein kinases have been linked to numerous diseases including cancer, diabetes and inflammation.[7] Targeted therapy using small molecule tyrosine kinase (TyrK) inhibitors is now well established, *e.g.* imatinib is an Abl TyrK inhibitor used as first line treatment in chronic myeloid leukemia (CML) and several solid tumours. The central feature of the TyrK enzymatic regulation is the protein plasticity which allows reversible transition between active and inactive confor-

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mations. Intensive research has been done to design novel protein kinase inhibitors targeting oncogenic fusion proteins and to identify the key amino acids involved in the regulation of the conformational changes and, hence, the relationship between mutations and resistance observed in patients undergoing targeted therapies.[8,9]

Development of Small Molecules Inhibiting Oncogenic Npm-ALK

In anaplastic large-cell lymphomas, chromosomal translocations involving the kinase domain of anaplastic lymphoma kinase (ALK) and nucleophosmin produce highly oncogenic ALK fusion proteins that deregulate the cell cycle, differentiation, and apoptosis. Different fusion oncoproteins involving ALK kinase have been found in patients with non-small cell lung, breast, neuroblastoma and colorectal cancers.[10] Within the framework of the ProKinase European project, research has focused on developing inhibitors for targeted therapy of these ALK-positive tumours. Combining a virtual screening approach with the knowledge of two known inhibitors targeting inactive kinases, doramapimod and sorafenib, we designed new urea derivatives as ALK inhibitors with IC_{50} values in the high nanomolar range and selective antiproliferative activity on ALK-positive cells (Fig. 1).[11–13] Within the same framework, two further series of Npm-ALK inhibitors (thiazole derivatives and α-Carboline) have been developed. Both series show nanomolar inhibition of Npm-ALK and its gatekeeper mutant L1196M Npm-ALK and clear structure–activity relationships as well as selective antiproliferative activity on ALK and ALK-mutant-positive cells with IC_{50} values in the high nanomolar range.[12,13] *In vivo* tests using a Karpas299 cells xenograft model demonstrated that 8 h treatment with these inhibitors produced a noticeable reduction in ALK-phosphorylation and activity (Prof. Gambacorti, personal communication). More extended preclinical studies are now ongoing with newly designed and optimized compounds.

Binding Kinetics of Protein Kinase Inhibitors make the Difference

Resistance to imatinib is an important clinical issue in the treatment of Philadelphia chromosome positive leukemias[8,14] which is being tackled by the development of new, more potent drugs, such as the dual Src/Abl inhibitors dasatinib and bosutinib.[15,16] To increase the potency and eventually overcome some of the resistance related to mutations distant from the ATP binding site,[8] we designed and synthesized cmp-584, a compound with enhanced shape complementarity with the kinase domain of Abl (Fig. 2) and with ~20–300-fold greater cellular activity *in vitro* and *in vivo* than imatinib, which was attributed to a slower off-rate.[17] To our knowledge, this represented the first evidence of higher anti-Abl activity potentially caused by a slower off-rate emphasising the importance of understanding binding kinetics in drug discovery.[18] Studies of binding kinetics are now implemented in the process of the hit to lead optimization and to develop quantitative structure kinetics relationships (QSKR).

The Difference in Flexibility Regulates the Accessibility of Druggable Conformations

Despite intensive research, it is not completely known which amino acids are involved in the regulation of the changes between active and inactive conformations of tyrosine kinases[19–23] and the question of the conformational propensity of tyrosine kinases and thus their targetability by small molecule inhibitors remains partially unanswered. A comparative structural analysis of all tyrosine kinases from the human genome using molecular modelling identified a pool of residues at the hydrophobic interface that appears to be important for protein conformation and motion.[24] In collaboration with Dr. Gervasio of the CNIO (Madrid), we analyzed the DFG conformational transition of the two model kinases, c-Src and Abl, that show a very different imatinib sensitivity despite high homology.[19] The results of massive molecular dynamics simulations, free energy calculations and isothermal titration calorimetry led to the hypothesis that the different flexibility of the two kinases results in a different stability of the DFG-out conformation and this might be the main determinant of imatinib selectivity.[25,26] We have also recently identified a single

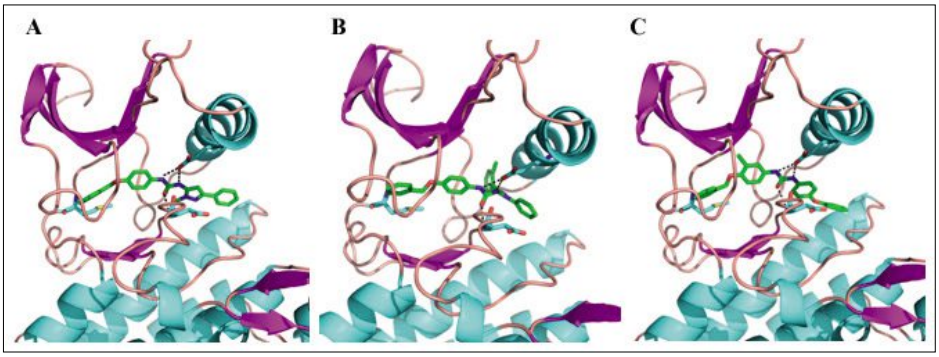

Fig. 1. Binding mode of representative urea derivatives as depicted by docking into the inactive ALK model. M259 of the hinge region, E227 of the C-helix and D330 of the DFG motif are shown as sticks with carbon atoms in cyan. H-bonds are shown as black dotted lines. For the sake of clarity, the gatekeeper L256 is not displayed. A: Compound 1 (IC_{50} on purified ALK = 2.3 μM, IC_{50} on Npm-ALK transduced BaF3 cells = 49 μM, IC_{50} on parental BaF3 cells = 74 μM) representing the 1-methylpyrazolyl urea derivatives. B: Compound 17 (IC_{50} on purified ALK = 0.6 μM, IC_{50} on Npm-ALK transduced BaF3 cells = 0.7 μM, IC_{50} on parental BaF3 cells = 0.7 μM) representing the 1-phenylpyrazolyl urea derivatives. C: Compound 19 (IC_{50} on purified ALK = 5 μM, IC_{50} on Npm-ALK transduced BaF3 cells = 1.5 μM, IC_{50} on parental BaF3 cells = 10 μM) of the phenyl urea derivatives (Reprinted with permission from ref. [11]. Copyright Wiley 2011)

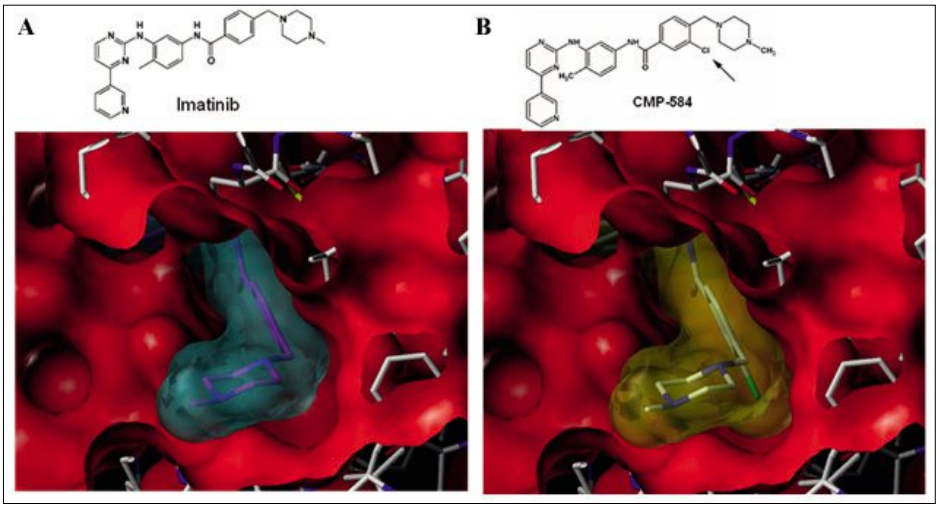

Fig. 2. cmp-584 shows higher shape complementarity with the Abl kinase domain. Structural formula and binding mode of imatinib (A) and cmp-584 (B) in Abl tyrosine kinase (PDB code: 1IEP) The Connolly surface of the protein is in red while the one of imatinib and cmp-584 is in blue and yellow respectively. The inhibitors as well as some amino acids forming the binding pockets are shown as colour coded capped stick model.

amino acid that is crucial for the conformational transition and DFG flip of c-Src by combining mutagenesis, X-ray crystallography, activity measurements, isothermal titration calorimetry and full atomistic very long molecular dynamics including free energy calculations performed in collaboration with Dr. Gervasio. The mutation of this single amino acid at the H1-H2 interface produces a 6000-fold increase in the binding affinity for the TyrK inhibitor imatinib.[27] Further questions such as the role of such mutations in the cellular context are addressed.

Development of a Thymidine Kinase-based Safety and Monitoring Tool for Stem Cells Therapy

Herpes simplex type I thymidine kinase (HSV1 TK) displays a broad substrate acceptance and is able to phosphorylate both pyrimidine and purine analogues featuring modified sugar mimicry such as the antiviral drugs aciclovir and ganciclovir. Due to this broader substrate diversity in comparison to their cellular counterpart,[28] they represent a selectivity filter for antiviral therapy using aciclovir and its analogues. More recently it emerged as a suitable suicide gene to be used as safety tools for stem cell transplantation (SCT) within cancer therapy,[29] gene replacement therapy[30] and as a powerful reporter gene for *in situ* monitoring of protein expression and localization of stem cells.[31]

Our research focuses on characterizing the molecular mechanism governing the broad substrate specificity of HSV1 TK compared to human cytosolic TK[32–38] in order to address the problem of resistance towards antiviral compounds and the clinical limitations such as immunogenicity and treatment incompatibility discovered during the clinical application of the suicide gene approach based on HSV1 TK/ganciclovir[39] using the interdisciplinary approach of the group. An effort has been made in order to design and develop new PET tracers for monitoring cancer and stem cells *in vivo*[40–43] in collaboration with Prof. Ametamey of the ETHZ.

Antibiotic Research: Identification and Characterization of Novel Antibacterial Compounds

Bacterial infections are a major public health problem worldwide. According to estimates of the World Health Organization, 16 million people died from infectious diseases in 2002. (*http://www.who.int/healthinfo/bodgbd2002revised/en/index.html*). Approximately 50% of the fatalities were due to bacterial pathogens, which if not lethal might also cause debilitating and maiming diseases. The threat is aggravated by the evolution of drug resistance, a paucity of new antibiotics, and the emergence of previously unrecognized infectious agents.[44] For these reasons, the identification of novel antibacterial drugs is of major importance.

Identification and Characterization of Novel Antibacterial Compounds using Protozoan Hosts

To tackle this issue a consortium of five groups with the necessary expertise in experimental microbiology and pharmaceutical chemistry was formed in 2010 and a SNF-sponsored Sinergia project (CRSI33-130016) initiated. This consortium is coordinated by the Pharmaceutical Biochemistry group which is responsible for virtual screening and elucidation of structure–activity relationships. The other members of the consortium are Profs. Pierre Cosson and Thierry Soldati of the University of Geneva and Prof. Hubert Hilbi of Ludwig-Maximilians-University in Munich who have long-standing experience in studying virulence of different bacterial pathogens using non-mammalian host models and Prof. John McKinney of the EPFL who is an expert in mammalian phagocytes to study bacterial virulence and in microfluidic system development for single cell analysis. The aim of this project is to discover and to characterize novel antibacterial compounds that can prevent or treat bacterial infections. We are focusing primarily on antivirulence compounds that selectively target bacterial virulence factors, including adhesins, toxins and secretion systems, specific metabolic pathways, the bacterial capacity to spread from cell to cell, or biofilm formation.[45] Since they exert no direct selective pressure on bacteria, the hope is that resistance against these compounds will develop less efficiently and more slowly, if at all. The first screening using a highly diverse low size chemical library, which targets pathways essential for bacterial virulence and host resistance, and which covers the chemical and biological space of the compounds available for screening, led to the identification of a few validated hits with activities in the low micromolar range. Detailed studies to establish SAR and mechanism of actions have begun.

Neglected Diseases Research: An Interdisciplinary Approach

Among the neglected diseases, tropical diseases describe a group of disabling conditions that include the most common chronic infections in people living in areas of extreme poverty. When taken together, the disease burden of neglected diseases accounts for 90% of the global disease burden. Remarkably, given their incidence, of the new drugs developed over 30 years (1975–2004) only 1.3% are dedicated to neglected diseases.[46,47] Despite the recent progress in understanding parasite biology and host–parasite interactions, this has not led to new products, resulting in a severe lack not only of safe, affordable, effective and field-adapted drugs, but also vaccines and diagnostics.

Identifying and Developing New Therapeutic Concepts for the Treatment of Neglected Diseases

It is our aim to study and to understand the fundamentals of important metabolic pathways suitable for medical intervention in parasites and thus to propose new therapeutic concepts against the diseases and to supply structural information as well as molecular probes for validation purposes and lead structures that could be further optimized.

Using a genome/target-based approach we combine molecular biology with structural biology and computational methods to characterize the targets and develop molecular probes that are needed to chemically validate the targets and further understand the biology of the parasite. This approach has been applied successfully for the validation of the enzymes involved in the fatty acid biosynthesis pathway of *P. falciparum* as a novel drug target. We have cloned and expressed all four enzymes involved in chain elongation and crystallized three of them either in their apo form or as complex with substrate and/or inhibitors. All necessary assays are established, and chemical library screening and natural product screening afforded a series of highly active hits/leads.[48–55] More recently, we started activities in the area of methionine salvage in *Trypanosoma brucei*. We have cloned genes for both methionine synthase and homocysteine S-methyltransferase (recently identified drug targets[56]) and are now producing recombinant protein for target characterization and hit/lead discovery.

For compounds that arise within the probe/lead finding process and that exhibit high activity against parasites, but for which the target is unknown or seems different from that hypothesized, we follow two approaches to isolate and to identify the corresponding target. The chemical proteomics approach, based on affinity chromatography techniques, has been applied successfully for the identification of adenosine kinase (AK) as the target of 4-[5-(4-phenoxyphenyl)-2*H*-pyrazol-3-yl]-morpholine in *Trypanosoma brucei rhodesiense*.[57,58] Chemical validation using recombinant protein indeed showed specific and tight interaction. Interestingly,

the compound toxicity is conferred by enzyme activation. We have constructed an overexpressing *ak* strain, an *ak* knockdown strain, and work is in progress to produce the corresponding full *ak* knockout strain, aiming at the genetic validation and elucidation of the mechanism of action of 4-[5-(4-phenoxyphenyl)-2*H*-pyrazol-3-yl]-morpholine at the cellular level. We have also solved the crystal structure of AK in complex with a bisubstrate inhibitor and with 4-[5-(4-phenoxyphenyl)-2*H*-pyrazol-3-yl]-morpholine.[59] Similar projects are currently ongoing and aim to isolate and identify targets in *Plasmodium falciparum* K1 and *Leishmania spp*. The *in silico* inverse screening[60,61] aimed at target prediction/discovery is a complementary tool to the more labour-intensive chemical proteomics. We have successfully applied such tools for the isolation and identification of targets of Triclosan in *Plasmodium falciparum* K1, which provided two enzymes with the potential to serve as drug targets. The cloning, expression, characterization and validation of the putative targets are subject of ongoing research in our laboratory.

Molecular Recognition-based Formulation of Antibodies

Therapeutic antibodies are susceptible to deamidation, isomerisation, racemisation, oxidation, denaturation, aggregation and other covalent modifications.[62] In addition to regulatory and quality control burdens, antibody aggregation can result in decreased efficacy and increased immunogenicity during clinical trials. Antibody 'stabilizing' agents include polysorbate-based surfactants, amino acids, glucose, sorbitol, and dextran sulphate but neither these nor mutation of single amino acids in Igs that target hydrophobic patches implicated in aggregation has had much success.[63] Our work, in collaboration with Prof. Robert Gurny of the University of Geneva, has aimed at finding novel excipients able to reduce antibody aggregation of the monoclonal IgG1 antibody bevacizumab (Avastin®). It has involved the *in silico* discovery and *in vitro* validation of small molecule aggregation breakers exploiting specific molecular recognition patterns formed during the aggregation process. Early results showed that dexamethasone phosphate stabilized bevacizumab by reducing dimer formation or by converting aggregates into an essentially monomeric state.[64] Monomer and a dimer aggregation model of bevacizumab, built using homology modelling, revealed a unique contact region between the Fc of one monomer and one Fab of the adjacent monomer, which is conserved in marketed IgG1 antibodies like trastuzumab (Herceptin®) and rituximab (MabThera®). A ligand-based search for similar aggregation breaking molecules based on a pharmacophore derived from the tested steroids yielded a small molecule[61,65,66] that is generally recognized as safe by the FDA. This was confirmed by both docking and experimental validation to be an aggregation breaker. Given the conserved primary aggregation region in IgG1 antibodies, this molecule could be broadly applicable to current antibody aggregation problems.[61,65,66] This hypothesis is now being tested and small molecules with aggregation breaking properties further developed.

New Formulation Strategies and Technologies for Topical and Transdermal Delivery

The skin is the largest organ in the body and is an easily accessible portal for the administration of medicinal agents for local or systemic therapy. However, one of its principal physiologic functions is to prevent the entry of substances into the body. This protective barrier function resides principally in the stratum corneum (SC) – the outermost layer of the epidermis – which is particularly effective in limiting the permeation of hydrosoluble polar substances. So how can we overcome the challenge posed by Mother Nature if we wish to exploit this delivery route? This is the question addressed by the research of the Cutaneous Drug Delivery Group. Some specific areas of interest are described below.

Dermatopharmacokinetics and the Development of New Formulations to Increase Cutaneous Bioavailability

A pharmaceutical company wishing to introduce a generic topical dermatology product must demonstrate bioequivalence to the existing product. Although it is possible to measure drug concentrations in the blood or urine, they do not necessarily represent drug levels at the target site – the skin. In order to simplify and to accelerate the assessment of topical bioavailability and bioequivalence, the US FDA explored the possibility of introducing a dermatopharmacokinetic approach. The drug formulation is in contact with the skin for a fixed application period, after which its concentration in the skin is determined as a function of time. In this context, we investigated the topical delivery of terbinafine and ibuprofen in human volunteers and determined drug concentrations as a function of position in the SC.[67,68] Mathematical models were used to separate the contributions of thermodynamic and kinetic parameters to drug transport, which can be of use in formulation optimisation; in addition, they enable short-exposure data to be used for the prediction of drug bioavailability at longer time-points.

In 2010, we began to explore the potential of using polymeric micellar systems based on amphiphilic MPEG-hexPLA block copolymers to improve and to target delivery of dermatology therapeutics.[69] A recent study comparing the topical delivery of econazole nitrate from an aqueous micellar formulation to that from a marketed product (Pevaryl®) into porcine and human skin *in vitro* demonstrated a significant increase in econazole nitrate delivery over the marketed formulation.[69] Studies are currently underway to explore other potential applications of these micellar systems in dermatology.

Iontophoresis: Using Electrically-assisted Transport to Deliver Charged Molecules across Intact Skin

Iontophoresis involves the application of a small electric potential to drive ions across the skin; transport is due to the concentration and potential gradients across the skin and given that passive diffusion is poor for these species, it is dominated by electromigration. Transport rates are controlled by the intensity, duration and profile of current application and these can be modulated to obtain precise/individualized drug delivery kinetics.[70]

We have investigated the iontophoretic transport of small molecules for topical action (*e.g.* herpes labialis[71] and the local delivery of anti-oxdiants[72]) and for systemic effect (*e.g.* migraine[73,74] and chemotherapy-induced emesis[75–77]), therapeutic peptides (triptorelin and vapreotide),[78,79] and conducted mechanistic studies that resulted in the first published reports on the non-invasive transdermal delivery of functional proteins across the skin.[80–83]

Experiments with valaciclovir, a cleavable valine ester prodrug of aciclovir with superior oral bioavailability, showed that its transdermal iontophoresis resulted in several hundred-fold increases in aciclovir delivery due to the charged amino group.[71] Sumatriptan and zolmitriptan are $5HT_{1B/1D}$ agonists used in the treatment of acute migraine episodes; their iontophoretic administration was investigated *in vitro* and *in vivo* and the results illustrated how the current profile controlled iontophoretic delivery kinetics and, as a result, drug concentrations in the plasma.[73,74] The iontophoretic delivery of therapeutic amounts of anti-emetics used in the treatment of chemotherapy induced nausea and vomiting has also been successfully demonstrated *in vitro* and *in vivo*.[75–77] Ongoing projects continue to investigate the controlled iontopho-

retic delivery of therapeutics to treat CNS disorders and the iontophoretic transport of glycomimetics.[84,85] In 2007, we published the first report on the transdermal iontophoresis of an intact protein (cytochrome c; 12.4 kDa) across intact skin.[80] Prior to this report it was generally thought that iontophoresis could only be used with molecules of lower molecular weight (of up to a few kDa).[86] Recently completed studies into the transdermal iontophoresis of ribonuclease A[81] (13.6 kDa), human basic fibroblast growth factor[83] (17.4 kDa) and negatively charged ribonuclease T1[82] (11.1 kDa) demonstrate that intact, biologically active proteins can be delivered non-invasively across the skin (Fig. 3).

Laser-assisted Microporation: A New Approach to Minimally Invasive Transdermal Delivery

In 2007, we began a collaboration to investigate a new Er.YAG laser-based microporation technology (P.L.E.A.S.E.®) for minimally invasive drug delivery into and across the skin.[69,87–92] The P.L.E.A.S.E.® device applies short duration energy pulses (a few μs) at 2.94 μm that excite water molecules in the epidermis and result in their evaporation. This leads to the formation of transport channels whose depth can be controlled by varying the applied energy. Several hundred pores with reproducible size can be created in a few seconds. Experimental studies have been conducted into the delivery of small molecules (*e.g.* lidocaine,[89] prednisone[91] and diclofenac[93]), peptides (*e.g.* exenatide[88]) and proteins (*e.g.* cytochrome c[87] and follicle stimulating hormone). Laser microporation increases both the rate and extent of drug delivery; *e.g.* for lidocaine, a local anaesthetic, it was shown that significant levels were present in the skin after a 5 min application following microporation.[89] It has recently been shown that this technology can also be used for the local 'needle-less' delivery of functional therapeutic antibodies (anti-thymocyte globulin (ATG) and basiliximab (~150 kDa)) into the skin.[92] Biologics are playing an increasing role in dermatology and their targeted local delivery may expand potential therapeutic applications since high local concentrations may be achievable without the risk of systemic side-effects. These will be investigated in collaboration with clinical partners.

In summary, these results clearly show the extensive experience of Cutaneous Drug Delivery Group in drug formulation and the use of new technologies to expand the range of drug molecules including cosmeceuticals[94] that can be administered into and across the skin. The published studies with biopharmaceuticals challenge the perceived limitations of topical and transdermal delivery and suggest that there are less invasive, more patient-friendly alternatives to the parenteral administration of these therapeutics. It is no longer necessary to consider the transdermal route as being exclusively reserved for small, moderately lipophilic compounds.

Conclusions

The broad range of interdisciplinary research activities and competences within the Pharmaceutical Biochemistry group enable it to address some of the key challenges in modern drug discovery and development and to offer a powerful collaborative platform for other universities, the pharmaceutical industry and for the training of future pharmaceutical chemists and technologists.

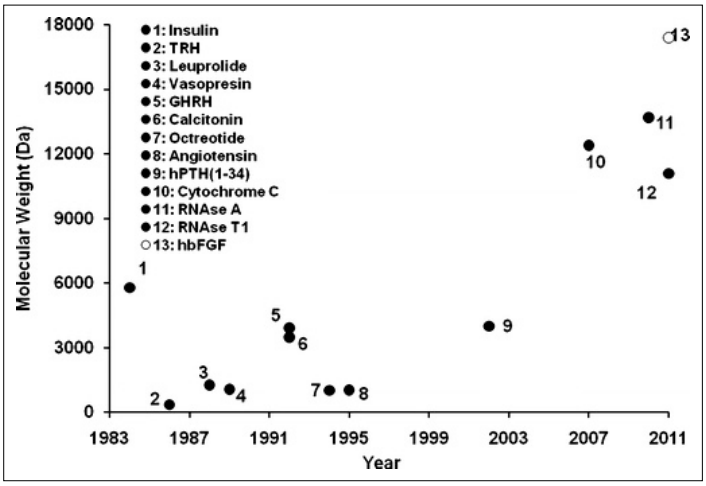

Fig. 3. A chronology of the studies into the transdermal iontophoretic delivery of different peptides and proteins showing the evolution in the size of the molecules under investigation. (Non-exhaustive compilation). To-date, hbFGF (17.4 kDa; open circle) is the largest (functional) protein delivered by iontophoresis across intact skin. (Reprinted with permission from ref. [83]. Copyright American Chemical Society 2011)

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[1] M. E. Bunnage, *Nat. Chem. Biol.* **2011**, *7*, 335.
[2] I. Kola, J. Landis, *Nat. Rev. Drug Discov.* **2004**, *3*, 711.
[3] I. Collins, P. Workman, *Nat. Chem. Biol.* **2006**, *2*, 689.
[4] T. A. Yap, C. P. Carden, S. B. Kaye, *Nat. Rev. Cancer* **2009**, *9*, 167.
[5] S. R. Hubbard, J. H. Till, *Annu. Rev. Biochem.* **2000**, *69*, 373.
[6] G. Manning, D. B. Whyte, R. Martinez, T. Hunter, S. Sudarsanam, *Science* **2002**, *298*, 1912.
[7] D. J. Matthews, M. E. Gerritsen, 'Targeting Protein Kinases for Cancer Therapy', John Wiley & Sons, Hoboken, NJ, ISBN-13: 978-0-470-22965-1, pp 702. Wiley, **2011**.
[8] C. B. Gambacorti-Passerini, R. H. Gunby, R. Piazza, A. Galietta, R. Rostagno, L. Scapozza, *Lancet Oncol.* **2003**, *4,* 75.
[9] S. Salemi, S. Yousefi, D. Simon, I. Schmid, L. Moretti, L. Scapozza, H. U. Simon, *Allergy* **2009**, *64*, 913.
[10] C. J. Tartari, L. Scapozza, C. Gambacorti-Passerini, *Curr. Top Med. Chem.* **2011**, *11*, 1406.
[11] G. B. af Gennas, L. Mologni, S. Ahmed, M. Rajaratnam, O. Marin, N. Lindholm, M. Viltadi, C. Gambacorti-Passerini, L. Scapozza, J. Yli-Kauhaluoma, *ChemMedChem* **2011**, *6*, 1680.
[12] L. Scapozza, P. Goekjian, A. Zambon, C. Gambacorti, Patent EP2107054, WO2009121535 **2008**.
[13] L. Scapozza, P. Goekjian, C. Gambacorti, Patent EP2161271, WO2010025872 **2008**.
[14] M. E. Gorre, M. Mohammed, K. Ellwood, N. Hsu, R. Paquette, P. N. Rao, C. L. Sawyers, *Science* **2001**, *293*, 876.
[15] C. Gambacorti-Passerini, M. Gasser, S. Ahmed, S. Assouline, L. Scapozza, *Leukemia* **2005**, *19*, 1267.
[16] M. Puttini, A. M. Coluccia, F. Boschelli, L. Cleris, E. Marchesi, A. Donella-Deana, S. Ahmed, S. Redaelli, R. Piazza, V. Magistroni, F. Andreoni, L. Scapozza, F. Formelli, C. Gambacorti-Passerini, *Cancer Res*. **2006**, *66*, 11314.
[17] M. Puttini, S. Redaelli, L. Moretti, S. Brussolo, R. H. Gunby, L. Mologni, E. Marchesi, L. Cleris, A. Donella-Deana, P. Drueckes, E. Sala, V. Lucchini, M. Kubbutat, F. Formelli, A. Zambon, L. Scapozza, C. Gambacorti-Passerini, *Haematologica* **2008**, *93*, 653.
[18] R. A. Copeland, D. L. Pompliano, T. D. Meek, *Nat. Rev. Drug. Discov.* **2006**, *5*, 730.
[19] M. A. Seeliger, B. Nagar, F. Frank, X. Cao, M. N. Henderson, J. Kuriyan, *Structure* **2007**, *15*, 299.
[20] M. Azam, M. A. Seeliger, N. S. Gray, J. Kuriyan, G. Q. Daley, *Nat. Struct. Mol. Biol.* **2008**, *15*, 1109.
[21] A. P. Kornev, S. S. Taylor, L. F. Ten Eyck, *Proc. Natl. Acad. Sci. USA* **2008**, *105*, 14377.
[22] H. Chen, J. Ma, W. Li, A. V. Eliseenkova, C. Xu, T. A. Neubert, W. T. Miller, M. Mohammadi, *Mol. Cell* **2007**, *27*, 717.
[23] A. Cavalli, C. Dezi, G. Folkers, L. Scapozza, M. Recanatini, *Proteins* **2001**, *45*, 478.
[24] L. Moretti, Thesis, University Geneva **2007**, no. 3904.
[25] R. Campos-Olivas, M. Marenchino, L. Scapozza, F.L. Gervasio, *Biomol. NMR Assign.* **2011**, *5*, 221.
[26] S. Lovera, L. Sutto, R. Boubeva, L. Scapozza, N. Dolker, F. L. Gervasio, *J. Am. Chem. Soc.* **2012**, *134*, 2496.
[27] R. Boubeva, Thesis, University Geneva **2011**, no. 4300.
[28] G. B. Elion, P. A. Furman, J. A. Fyfe, P. de Miranda, L. Beauchamp, H. J. Schaeffer, *Proc. Natl. Acad. Sci. USA* **1977**, *74*, 5716.
[29] C. Bonini, G. Ferrari, S. Verzeletti, P. Servida, E. Zappone, L. Ruggieri, M. Ponzoni, S. Rossini, F. Mavilio, C. Traversari, C. Bordignon, *Science* **1997**, *276*, 1719.
[30] D. B. Kohn, M. Sadelain, C. Dunbar, D. Bodine, H. P. Kiem, F. Candotti, J. Tisdale, I. Riviere, C. A. Blau, R. E. Richard, B. Sorrentino, J. Nolta, H. Malech, M. Brenner, K. Cornetta, J. Cavagnaro, K. High, J. Glorioso, *Mol. Ther.* **2003**, *8*, 180.
[31] G. Koehne, M. Doubrovin, E. Doubrovina, P. Zanzonico, H. F. Gallardo, A. Ivanova, J. Balatoni, J. Teruya-Feldstein, G. Heller, C. May, V. Ponomarev, S. Ruan, R. Finn, R.G. Blasberg, W. Bornmann, I. Riviere, M. Sadelain, R. J. O'Reilly, S. M. Larson, J. G. Tjuvajev, *Nat. Biotechnol.* **2003**, *21*, 405.
[32] M. S. Birringer, M. T. Claus, G. Folkers, D. P. Kloer, G. E. Schulz, L. Scapozza, *FEBS Lett.* **2005**, *579*, 1376.
[33] F. C. Christians, L. Scapozza, A. Crameri, G. Folkers, W. P. Stemmer, *Nat. Biotechnol.* **1999**, *17*, 259.
[34] R. Perozzo, I. Jelesarov, H. R. Bosshard, G. Folkers, L. Scapozza, *J. Biol. Chem.* **2000**, *275*, 16139.
[35] P. Russ, P. Schelling, L. Scapozza, G. Folkers, E. D. Clercq, V. E. Marquez, *J. Med. Chem.* **2003**, *46*, 5045.
[36] P. Schelling, M. T. Claus, R. Johner, V. E. Marquez, G. E. Schulz, L. Scapozza, *J. Biol. Chem*. **2004**, *279*, 32832.
[37] P. Schelling, G. Folkers, L. Scapozza, *Anal. Biochem*. **2001**, *295*, 82.
[38] M. Sulpizi, P. Schelling, G. Folkers, P. Carloni, L. Scapozza, *J. Biol. Chem*. **2001**, *276*, 21692.
[39] S. Verzeletti, C. Bonini, S. Marktel, N. Nobili, F. Ciceri, C. Traversari, C. Bordignon, *Hum. Gene Ther.* **1998**, *9*, 2243.
[40] S. Kristafor, I. Novakovic, T. Gazivoda Kraljevic, S. Kraljevic Pavelic, P. Lucin, Y. Westermaier, L. Pernot, L. Scapozza, S. M. Ametamey, S. Raic-Malic, *Bioorg. Med. Chem. Lett*. **2011**, *21*, 6161.
[41] M. Martic, L. Pernot, Y. Westermaier, R. Perozzo, T. G. Kraljevic, S. Kristafor, S. Raic-Malic, L. Scapozza, S. Ametamey, *Nucleos. Nucleot. Nucl.* **2011**, *30*, 293.
[42] S. Prekupec, D. Svedruzic, T. Gazivoda, D. Mrvos-Sermek, A. Nagl, M. Grdisa, K. Pavelic, J. Balzarini, E. De Clercq, G. Folkers, L. Scapozza, M. Mintas, S. Raic-Malic, *J. Med. Chem.* **2003**, *46*, 5763.
[43] S. Raic-Malic, A. Johayem, S. M. Ametamey, S. Batinac, E. De Clercq, G. Folkers, L. Scapozza, *Nucleos. Nucleot. Nucl.* **2004**, *23*, 1707.
[44] D. J. Payne, M. N. Gwynn, D. J. Holmes, D. L. Pompliano, *Nat. Rev. Drug Discov.* **2007**, *6*, 29.
[45] L. Cegelski, G. R. Marshall, G. R. Eldridge, S. J. Hultgren, *Nat. Rev. Microbiol.* **2008**, *6*, 17.
[46] P. Chirac, E. Torreele, *Lancet* **2006**, *367*, 1560.
[47] P. Trouiller, P. Olliaro, E. Torreele, J. Orbinski, R. Laing, N. Ford, *Lancet* **2002**, *359*, 2188.
[48] H. Kirmizibekmez, I. Calis, R. Perozzo, R. Brun, A. A. Donmez, A. Linden, P. Ruedi, D. Tasdemir, *Planta Med.* **2004**, *70*, 711.
[49] D. Kostrewa, F. K. Winkler, G. Folkers, L. Scapozza, R. Perozzo, *Protein Sci.* **2005**, *14*, 1570.
[50] M. R. Kuo, H. R. Morbidoni, D. Alland, S. F. Sneddon, B. B. Gourlie, M. M. Staveski, M. Leonard, J. S. Gregory, A. D. Janjigian, C. Yee, J. M. Musser, B. Kreiswirth, H. Iwamoto, R. Perozzo, W. R. Jacobs, Jr., J. C. Sacchettini, D. A. Fidock, *J. Biol. Chem.* **2003**, *278*, 20851.
[51] G. Lack, E. Homberger-Zizzari, G. Folkers, L. Scapozza, R. Perozzo, *J. Biol. Chem.* **2006**, *281*, 9538.
[52] R. Perozzo, M. Kuo, A. S. Sidhu, J. T. Valiyaveettil, R. Bittman, W. R. Jacobs, Jr., D. A. Fidock, J. C. Sacchettini, *J. Biol. Chem.* **2002**, *277*, 13106.
[53] D. Tasdemir, G. Lack, R. Brun, P. Ruedi, L. Scapozza, R. Perozzo, *J. Med. Chem.* **2006**, *49*, 3345.
[54] D. Tasdemir, D. Sanabria, I. L. Lauinger, A. Tarun, R. Herman, R. Perozzo, M. Zloh, S. H. Kappe, R. Brun, N. M. Carballeira, *Bioorg. Med. Chem*. **2010**, *18*, 7475.
[55] F. Colizzi, R. Perozzo, L. Scapozza, M. Recanatini, A. Cavalli, *J. Am. Chem. Soc*. **2010**, *132*, 7361.
[56] B. Nerima, D. Nilsson, P. Maser, *BMC Genomics* **2010**, *11*, 217.
[57] S. Kuettel, M. Mosimann, P. Maser, M. Kaiser, R. Brun, L. Scapozza, R. Perozzo, *PLoS Negl. Trop. Dis*. **2009**, *3*, e506.
[58] S. Kuettel, A. Zambon, M. Kaiser, R. Brun, L. Scapozza, R. Perozzo, *J. Med. Chem.* **2007**, *50*, 5833.
[59] S. Kuettel, J. Greenwald, D. Kostrewa, S. Ahmed, L. Scapozza, R. Perozzo, *PLoS Negl. Trop. Dis.* **2011**, *5*, e1164.
[60] P. Muller, G. Lena, E. Boilard, S. Bezzine, G. Lambeau, G. Guichard, D. Rognan, *J. Med. Chem.* **2006**, *49*, 6768.
[61] Y. Westermaier, Thesis, University Geneva **2011**, no. 4295.
[62] M. C. Manning, D. K. Chou, B. M. Murphy, R. W. Payne, D. S. Katayama, *Pharm. Res*. **2010**, *27*, 544.
[63] N. Chennamsetty, V. Voynov, V. Kayser, B. Helk, B. L. Trout, *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 11937.
[64] M. Veurink, C. Stella, C. Tabatabay, C. J. Pournaras, R. Gurny, *Eur. J. Pharm. Biopharm*. **2011**, *78*, 271.
[65] L. Scapozza, Y. Westermaier, M. Veurink, R. Gurny, Patent appl. WO2011121560 **2010**.
[66] L. Scapozza, Y. Westermaier, M. Veurink, R. Gurny, Patent appl. WO2011121559 **2010**.
[67] I. Alberti, Y. N. Kalia, A. Naik, R. H. Guy, *Pharm. Res.* **2001**, *18*, 1472.
[68] C. Herkenne, A. Naik, Y. N. Kalia, J. Hadgraft, R. H. Guy, *J. Invest. Dermatol.* **2007**, *127*, 887.
[69] Y. G. Bachhav, K. Mondon, Y. N. Kalia, R. Gurny, M. Moller, *J. Control. Release* **2011**, *153*, 126.
[70] Y. N. Kalia, A. Naik, J. Garrison, R. H. Guy, *Adv. Drug Deliv. Rev*. **2004**, *56*, 619.
[71] N. Abla, A. Naik, R. H. Guy, Y. N. Kalia, *Pharm. Res.* **2006**, *23*, 1842.
[72] F. Marra, J. L. Levy, P. Santi, Y. N. Kalia, *J. Cosmet. Dermatol.* **2008**, *7*, 105.
[73] S. R. Patel, H. Zhong, A. Sharma, Y. N. Kalia, *Eur. J. Pharm. Biopharm*. **2007**, *66*, 296.
[74] S. R. Patel, H. Zhong, A. Sharma, Y. N. Kalia, *Eur. J. Pharm. Biopharm.* **2009**, *72*, 304.
[75] J. Cazares-Delgadillo, I. B. Aziza, C. Balaguer-Fernandez, A. Calatayud-Pascual, A. Ganem-Rondero, D. Quintanar-Guerrero, A. C. Castellano, V. Merino, Y. N. Kalia, *Eur. J. Pharm. Sci.* **2010**, *41*, 353.
[76] J. Cazares-Delgadillo, C. Balaguer-Fernandez, A. Calatayud-Pascual, A. Ganem-Rondero, D. Quintanar-Guerrero, A. C. Lopez-Castellano, V. Merino, Y. N. Kalia, *Eur. J. Pharm. Biopharm.* **2010**, *75*, 173.
[77] J. Cazares-Delgadillo, A. Ganem-Rondero, D. Quintanar-Guerrero, A. C. Lopez-Castellano, V. Merino, Y. N. Kalia, *Eur. J. Pharm. Sci.* **2010**, *39*, 387.
[78] Y. B. Schuetz, A. Naik, R. H. Guy, E. Vuaridel, Y. N. Kalia, *J. Pharm. Sci.* **2005**, *94*, 2175.
[79] Y. B. Schuetz, A. Naik, R. H. Guy, E. Vuaridel, Y. N. Kalia, *Pharm. Res.* **2005**, *22*, 1305.
[80] J. Cazares-Delgadillo, A. Naik, A. Ganem-Rondero, D. Quintanar-Guerrero, Y. N. Kalia, *Pharm. Res*. **2007**, *24*, 1360.
[81] S. Dubey, Y. N. Kalia, *J. Control. Release* **2010**, *145*, 203.
[82] S. Dubey, Y. N. Kalia, *J. Control. Release* **2011**, *152*, 356.
[83] S. Dubey, R. Perozzo, L. Scapozza, Y. N. Kalia, *Mol. Pharm.* **2011**, *8*, 1322.

[84] T. Gratieri, B. Wagner, D. Kalaria, B. Ernst, Y. N. Kalia, *Biomed. Chromatogr.* **2012**, *26*, 507.
[85] T. Gratieri, B. Wagner, D. Kalaria, B. Ernst, Y. N. Kalia, *Exp. Dermatol*. **2012**, *21*, 226.
[86] T. Gratieri, D. Kalaria, Y. N. Kalia, *Expert Opin. Drug Deliv.* **2011**, *8*, 645.
[87] Y. G. Bachhav, Y. N. Kalia, *Biomed. Chromatogr*. **2010**, *24*, 732.
[88] Y. G. Bachhav, Y. N. Kalia, *Biomed. Chromatogr.* **2011**, *25*, 838.
[89] Y. G. Bachhav, S. Summer, A. Heinrich, T. Bragagna, C. Bohler, Y. N. Kalia, *J. Control. Release* **2010**, *146*, 31.
[90] Y. N. Kalia, Y. G. Bachhav, T. Bragagna, C. Bohler, *Drug Del. Technol*. **2008**, *8*, 26.
[91] J. Yu, Y. G. Bachhav, S. Summer, A. Heinrich, T. Bragagna, C. Bohler, Y. N. Kalia, *J. Control. Release* **2010**, *148*, e71.
[92] J. Yu, D. R. Kalaria, Y. N. Kalia, *J. Control. Release* **2011**, *156*, 53.
[93] Y. G. Bachhav, A. Heinrich, Y. N. Kalia, *Eur. J. Pharm. Biopharm*. **2011**, *78*, 408.
[94] T. Gratieri, Y. N. Kalia, *Kosmetische Medezin* **2011**, *11*, 165.