Systems pharmacology of the endocannabinoid system

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Chapter 1

General Introduction

Systems thinking in modern drug discovery

Over the last century, the nature of health and disease are increasingly being studied by applying system-based approaches [1]. System-based approaches allow us to overcome the difficulties in predicting the functional state of an intact organism from the functional state of its parts in isolation. This paradigm shift towards more systems-thinking could transform drug discovery and development in a cost-effective manner to produce the right treatment to right patient [2,3]. It has been realized that the current drug discovery approach based on the assumption that a single compound modulates a single target has limitations for the multifactorial abnormalities such as cardiovascular disease, type 2 diabetes, neurological disorders. Furthermore, compounds designed to modulate only the target in a target-based approaches, can have unanticipated effects on "off-target" biochemical mechanisms, and the safety implications of those unwanted effects might not be revealed in early drug development or until a drug candidate is evaluated in large clinical trials. Therefore, it is essential to generate comprehensive molecular descriptions of a disease or the effect of drugs (i.e. drug response) that take into account the breath of biochemical perturbations of an organism [2,4]. Systems pharmacology is a concept that attempts to characterize the molecular network changes due to a disease state and its changes due to a drug: this includes studying drug-perturbed states relative to unperturbed state. The molecular network changes due to diseases are often also referred to as systems pathophysiology or systems biology of diseases.

The concept of having system fingerprints to describe states of complex systems integrating different biochemical components such as genes, transcripts, proteins and endogenous metabolites allows to create system-wide response profiles and study system-state differences [2]. The topological connections between these molecular components define the organization of a given biological system and its function reflects how the system evolves with regards to metabolic fluxes or environmental stimuli [5]. To capture the initial loss of homeostasis within the body, system response profiles can be generated either using blood plasma levels which reflect interactions between organs or by studying cells, complex cellular in-vitro models or tissues in case of multi-factorial diseases. The system response profiles integrate the molecular phenome (different omics), drug response phenotype (Pharmacokinetics-Pharmacodynamics, PK-PD),exposure to environmental factors ranging from toxic substances, drugs to diet (exposome), and the clinical phenome (clinical features and clinical metrics) (**Fig. 1**). System fingerprints, based on biomolecular profiles, to stratify patient populations and to differentiate between responders and non-responders to drugs and

to fine-tune drug interventions are emerging concepts to improve and personalize healthcare approach [6]. In addition, a key tool to achieve inter-species translation of findings in drug development is to have biological networks (gens, proteins and metabolites) that can be comparable between species [7]. To this end metabolomics can serve as an powerful phenotyping platform complementary to other "omics" as it can yield uniform information about disease biochemistry and drug action in both pre-clinical and clinical studies, and helps in improving inter-species translational research [7–9].

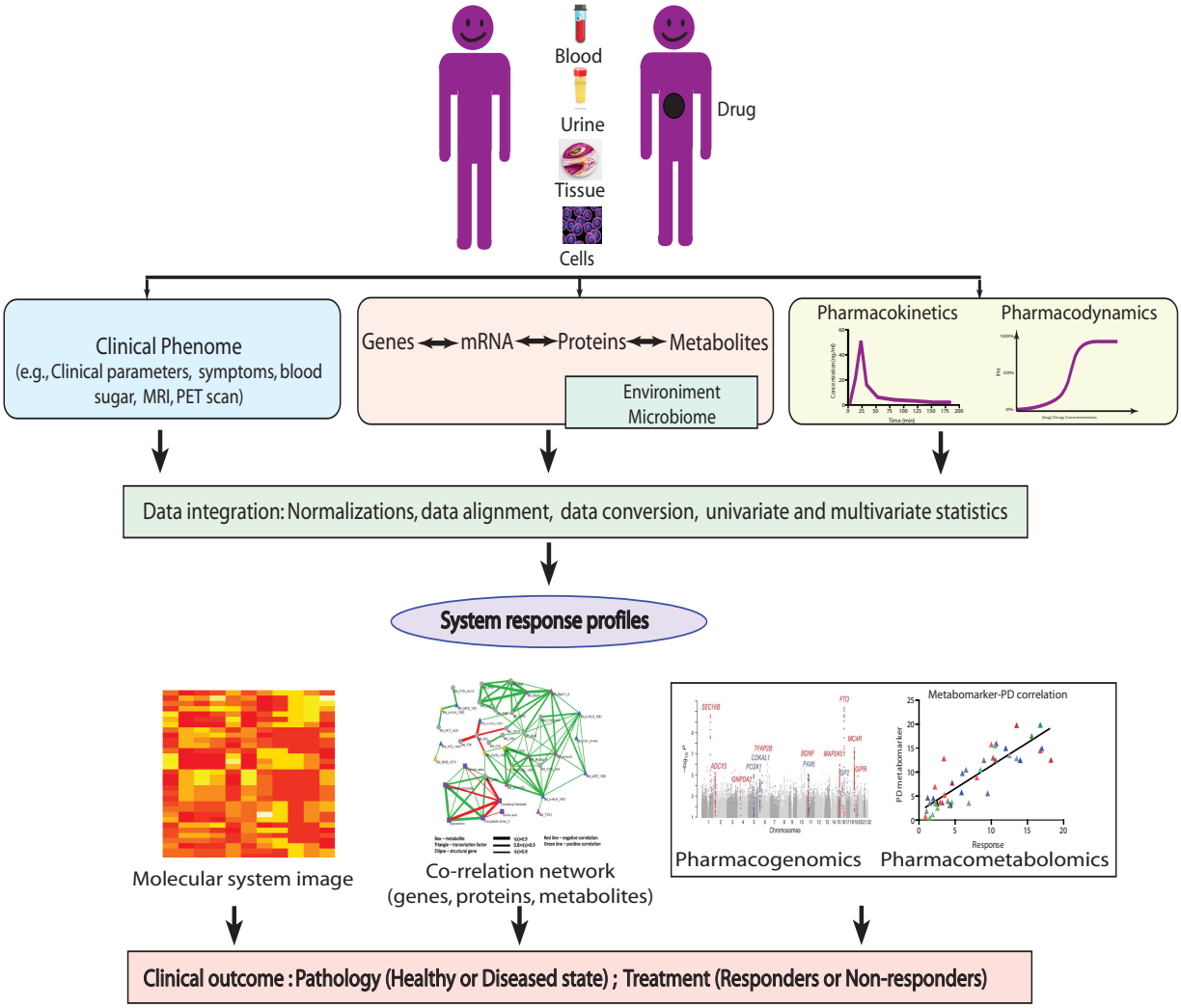

Figure 1: Flowchart for systems pharmacology. An overview of the materials, information and methods that constitute the workflows and outputs of systems pharmacology**.** These different biological, pharmacological and clinical measurements should be studied in a multi-dimensional fashion by taking the inherent spatial and temporal scales of both measurements technology platforms and disease and drug dynamics from the molecular to the population level into account.

Metabolomics and pharmacometabolomics

Metabolomics is a molecular phenotyping tool which provides the comprehensive quantitative and qualitative analysis of metabolites under a given (patho)physiological condition of an individual [10]. Significant progress in genomics and proteomics have increased our understanding of altered genes and protein under a variety of perturbations, including disease conditions and drug effect (**Fig. 2**). Metabolomics serves as an alternative molecular approach to help understand altered metabolic pathways in various perturbations. Metabolomics is especially attractive: subtle changes in genes, transcripts or protein levels can result in substantial changes in the levels and dynamics of metabolite and closely resembles the organism phenotype. In addition, metabolomics is attractive in translational studies, as metabolites have to a major extent identical structures and comparable functions in different species, and localized perturbations in organs due to disease or drug can be measured in blood as metabolites are excreted from the cells and organs, and often reach ultimately the blood stream [7]. The endogenous metabolite profile of an individual is a snapshot of the phenotypic status of an individual resulting from, for instance, age, environmental interactions, and genotype, and inform on an individual's health or disease status [11,12]. Metabolomics or endogenous metabolite profiling may be therefore used as a phenotypic tool to provide new predictive biomarkers and mechanistic markers for individualizing drug treatment [13].

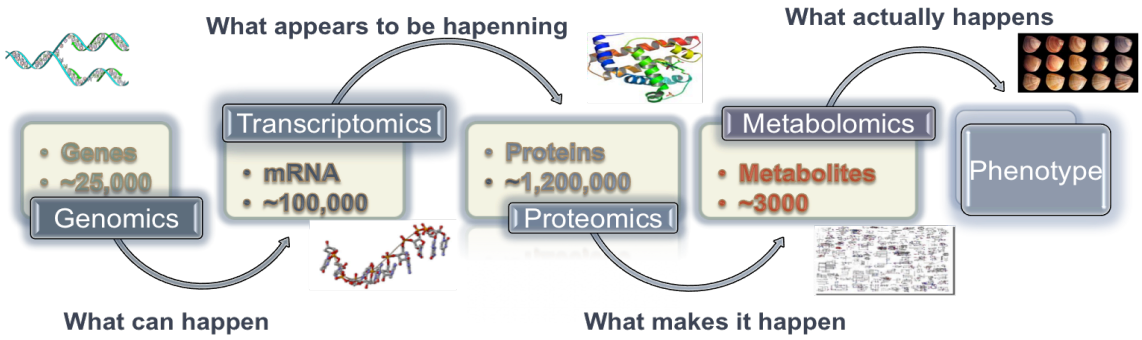

Figure 2: Schematic of 'omic' hierarchy: Genomics, Transcriptomics, Proteomics and Metabolomics. The general flow of information is from genes to transcripts to proteins to metabolites to phenotype (or function). Furthermore, as a feedback mechanisms, metabolites can also regulate enzyme activities and gene expression. The integration of all these 'omic' approaches is the main aim in systems biology.

Pharmacometabolomics is emerging as a sub-discipline of metabolomics that studies the interplay between drug pharmacology and the patients' (patho)physiology, by measuring endogenous metabolites that inform on drug effects and variation in drug response [14–17]. Pharmacokinetics (PK) is the study of the time-course of the drugs in bio fluids and tissues (i.e., what the body does to the drug). Pharmacodynamics (PD) is the study of the biological effects of drugs and their mechanisms of action (i.e., what a drug does to the body). PK-PD plays a key role in drug research, informing about *in vivo* drug and system properties under physiological and pathological conditions [18]. Metabolomics and pharmacometabolomics have the potential to prospectively inform about PK and PD processes, and by that, can take us one step closer to personalized medicine [19]. The systems pharmacology approach we applied in this thesis integrates concepts of metabolomics with PK-PD, in order to gain more insights in disease biochemistry and drug pharmacology.

Evolution of interest in the endocannabinoid system

Cannabis sativa has been used for medicinal and recreational purposes throughout the past millennium; actually, marijuana is still one of the most wide-spread illicit drugs of abuse in the world. It was during the second half of the twentieth century that research finally cast light onto some of its mechanisms of action [20], first with the identification of its major pharmacologically active components, the cannabinoids, and later their molecular targets. (–)-Δ9-Tetrahydrocannabinol (THC) is the most well-known active compound from cannabis and is generally held responsible for the well-known effects such as 'the munchies', a term used for hunger pangs after cannabis use, and central effects on consciousness, such as feeling high and altered time perception [21]. Cannabis also contains many other cannabinoids such as cannabidiol, but for most of these compounds, the pharmacological activity is currently still being investigated (for review on early phytocannabinoid chemistry, refer [22]).

The first guanine-nucleotide-binding protein (G protein)-coupled receptor (GPCR) activated by THC, cannabinoid receptor 1 (CB1; encoded by gene CNR1), was discovered in the brain in 1988 [23] and cloned in 1990 [24], and the crystal structure of human CB1 determined in 2016 [25]and the second GPCR, CB2, was identified in immune cells in 1993 [26]. The tissue distribution of CB1 and CB2 receptors accounts for the well-known psychotropic and peripheral effects of THC. CB1, most abundant GPCR, is distributed in central nervous system (CNS) and also present in the peripheral nervous system [27] and in peripheral metabolic organs (white adipose tissue (WAT), brown adipose tissue (BAT), liver, pancreas, muscle and Gut).

By contrast, CB2 is sparsely present in brain but more abundant in immune tissues and cells [28]. In addition to cannabinoid receptors, the endocannabinoid system (ECS) comprises endogenous ligands, the endocannabinoids and the enzymes responsible for the synthesis and hydrolysis of endocannabinoids.

Pharmacology of the endocannabinoid system

Endocannabinoids are highly lipophilic compounds that are not stored in vesicles after production. They are substantially synthesized "on demand" from membrane phospholipids, and released immediately. The most-studied endocannabinoids are anandamide (N-arachidonoyl ethanolamine, AEA) and 2-arachidonoylglycerol (2-AG). Anandamide is a high-affinity, low-efficacy CB1 agonist, with even lower efficacy at CB2 receptors, whereas 2-AG has lower affinity, but is a fully effective agonist at both CB1 and CB2 [29]. Both AEA and 2-AG are produced at post-synaptic neurons. Although they both derive from cell membrane arachidonic acid derivatives, the levels of these endocannabinoids are differentially regulated (**Fig. 3**). AEA levels depend on synthesis via hydrolysis of N-acyl-phosphatidylethanolamines (NAPE) by a NAPE-specific phospholipase D (NAPE-PLD) [30] and degradation is primarily regulated by fatty acid amide hydrolase (FAAH) [31]. 2-AG levels are regulated by the synthesis enzymes diacylglycerol lipase α and β (DAGL-α and β) [32] and degradation enzyme mono-acylglycerol lipase (MAGL) [33] although ABHD6 (an abbreviation of α,β hydrolase 6) [34] and ABHD12, as well as FAAH [35], are also capable of hydrolysing 2-AG. Both anandamide and 2-AG are also oxidized by the enzyme cyclooxygenase-2 (COX-2; also known as prostaglandin G/H synthase 2), cytochrome p450 and lipooxygenases (LOX) [36]. AEA belongs to a family of bioactive N-acylethanolamines (NAEs), which include N-linoleoylethanolamine (LEA), N-palmitoylethanolamine (PEA), N-oleoylethanolamine (OEA) and N-stearoylethanolamine (SEA), N-docosatetraenylethanolamide (DHEA). These NAEs arise from the same biosynthetic pathway as AEA and are capable to indirectly modulate cannabinoid receptor activity by interfering with endocannabinoid metabolism, known as an "entourage effect" [37]. PEA which acts as a body-own anti-inflammatory agent [38] and, specifically, OEA interacts with nuclear receptor peroxisome proliferator-activated receptor α (PPAR-α), stimulates lipolysis in the liver and adipocytes and acts as a satiety factor [39,40].

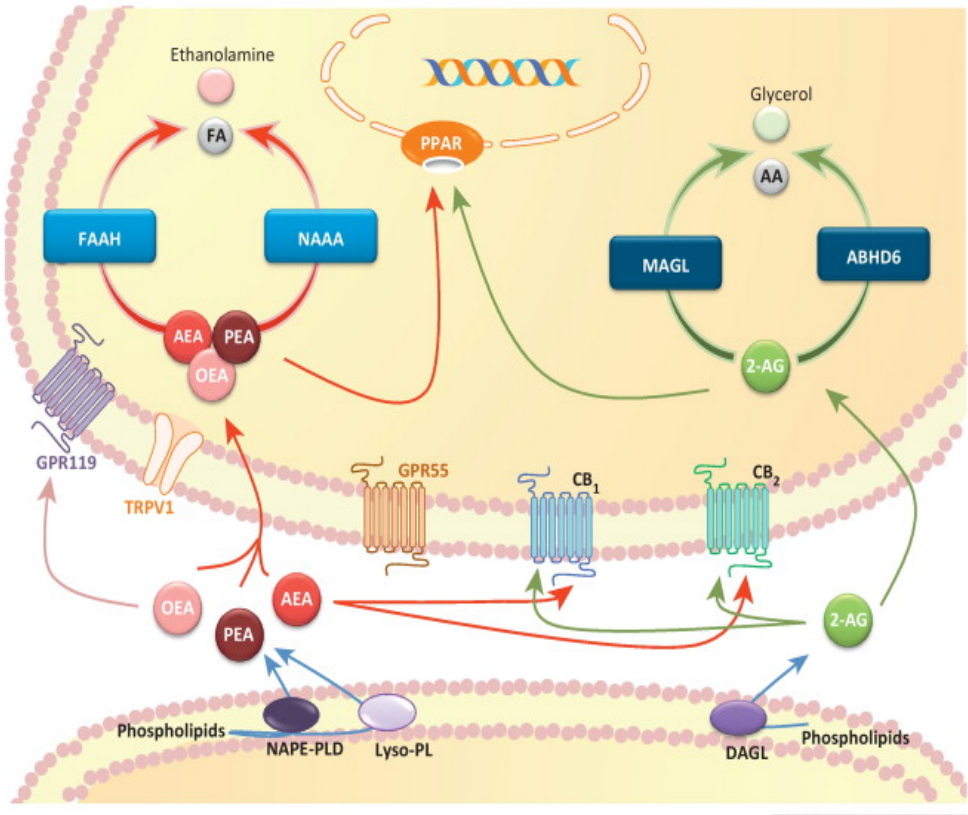

Figure 3: The endocannabinoid system in a nutshell. The figure illustrates the interactions between components of the endocannabinoid system including, receptors (cannabinoid CB1&CB2 and other GPCR relates), biosynthetic &hydrolytic enzymes and ligands (endocannabinoids and N-acylethanolamines). *Adapted from M Alhouayek Trends Mol Med 2012* [41].

Complexity of endocannabinoid system

Endocannabinoids are involved in complex physiological processes that play an important role in a huge number of diseases in almost all areas of medicine. In principle, this makes them appealing targets for drug development, but at the same time challenging. However, because of this complexity and the relatively recent discovery of the endocannabinoid system, its research is still in a premature stage. The endocannabinoid research and drug development is complicated further by a huge number of factors. Firstly, when compared with other pharmacological systems, the endocannabinoid system is one of the most widely distributed systems through the body. This complicates systemic or organ-specific targeting as when aiming to target a specific location, the ubiquitous presence of the system leads to undesirable "side" effects elsewhere in the body, limiting the development of therapeutically tissue-specific drugs. Secondly, the lipophilic nature of the endogenous and exogenous cannabinoids (e.g. Log P values for anandamide and 2-AG are 6.31 and 8.01) respectively leads to difficulties in

optimizing the pharmacokinetic properties and in distributing between blood and organs. Thirdly, the complex physiological network with other pharmacological signaling systems with many layers of regulation and feedback mechanisms makes it difficult to target a specific signaling pathway. This could be one of the reasons why there are so many discrepancies in the results obtained and why it is difficult to draw conclusions on the precise role of endocannabinoid system in various pathophysiological conditions. Fourthly, in case of multicascadic physiological mechanisms, the integration of endocannabinoid system at subcellular levels and the wide range of effects makes it challenging for measurement of changes in their activity, which is essential in drug development.

Endocannabinoid system as a therapeutic drug target

One of the functions of CB1 is to control whole body energy hemostasis [28]. Induction of CB1 receptor stimulates feeding by increasing appetite, whereas blocking CB1 signaling induces hypophagia by an appetite-suppressant effect [42,43]. Since the beginning of the 1990s, the endocannabinoid system has been considered an attractive therapeutic target in numerous diseases. Modulating ECS activity holds therapeutic promise for a broad range of diseases, including neurodegenerative, cardiovascular and inflammatory disorders, obesity/metabolic syndrome, cachexia, chemotherapy-induced nausea and pain, among others [44,45]. An early strategy to treat obesity was to target the cannabinoid receptors directly with exogenous antagonists, like rimonabant or dronabinol. Rimonabant, a CB1 receptor antagonist, was launched as an anti-obesity drug. It showed promise in decreasing appetite and body weight and improved cardio-metabolic risk factors, but was withdrawn from the market due to unwanted side effects on the central nervous system, namely psychiatric effects [46,47] . The most promising alternative strategy to overcome the side effects caused by directly blocking the receptor is to indirectly tune the endogenous ligand concentrations (endocannabinoids) by modulating their synthetic and hydrolysis enzymes [48]. Since the endocannabinoids are produced on demand (in a stimulus-dependent manner), modulating endocannabinoid signaling offers advantages in regulating their biochemistry, thus CB receptor activation, in a tissue-specific manner. Furthermore, the biochemical pathways associated with synthesis and degradation of the endocannabinoids in different tissues have been well elucidated [49], which makes it relatively attractive to chemically synthesize small molecule inhibitors and use them to study the enzyme activities. There are several examples showing the use of covalent enzyme inhibitors, which can be potential drug candidates, for tuning the endogenous endocannabinoid

levels for a beneficial effect. For instance, PF-04457845 is a clinically tested FAAH inhibitor used to increase endocannabinoid tone for beneficial effects such as pain and inflammation [50]. Similarly, LEI105, DH376 has been recently developed to regulate 2-AG levels by inhibiting DAGL enzyme [51,52].

To screen for target engagement, downstream effects, including also to screen for possible off-target effects of drugs, profiling platforms are required. In this regard, Activity based protein profiling (ABPP) can serve as a versatile chemoproteomic method to assess the functional state of entire enzyme classes directly in native biological systems [51,53]. ABPP is unique in its ability to rapidly identify inhibitor interaction and selectivity within large enzyme families in complex proteome samples either by analysis on SDS–polyacrylamide gel electrophoresis (SDS-PAGE) using fluorescent probes or by mass spectrometry (MS)–based analysis for target identification using biotinylated probes. Lipidomics or Metabolomics can be used as another powerful phenotypic platform to identify the target engagement of enzyme substrates or products or to identify the off-target effects of drugs on boarder molecular network [54].

Aim and Scope of Thesis

The overall aim of the research described in this thesis is to develop and apply systems pharmacology approaches to understand and modulate the endocannabinoid system across different models such as in cells, zebrafish, mice and humans (**Fig. 4**). First, the goal is to achieve deeper insights in the functional role of endocannabinoid system in healthy and diseased states. In addition, this research seeks to modulate the endocannabinoid system with newly synthesized drug molecules to understand the drug pharmacology and whether such new compounds may modify the disease biochemistry; this includes the study of target engagement, downstream effects and the off-target effects of new compounds. Furthermore, this research aims to integrate PK and PD concepts and metabolomics to determine the effective and safe drug dosing and drug-response phenotypes in cell and animal models. Finally, this work attempts to translate the findings from humans to animal models, and translate back from animal models to humans in order to improve our understanding of disease biochemistry and to support the discovery and development of novel therapeutic drugs.

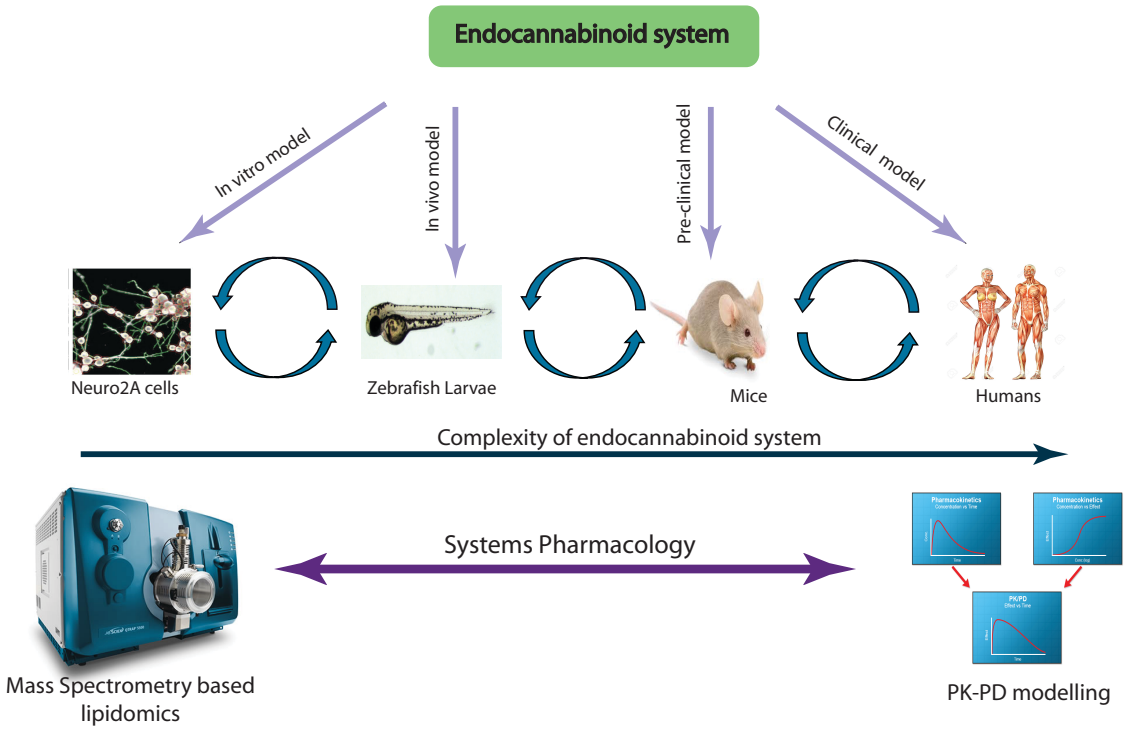

Figure 4: System pharmacology of the endocannabinoid system. This figure illustrates the schematic of overall studies performed in my PhD thesis

In **chapter 2**, the concept of system pharmacology is introduced: the combination of metabolomics with pharmacology is described in order to support the development of personalized medicine. It was highlighted how (pharmaco)metabolomics can predict pharmacokinetic (PK) and pharmacodynamics (PD) processes. In this regard, endogenous biomarkers are therefore indispensable to predict PK-PD.

To study endocannabinoid signaling system, endocannabinoids and their related lipids that are present at very low concentrations (nanomole to picomole range) have to be measured in various biological matrices. Since sensitivity is the key factor, in **Chapter 3** the development of a highly-sensitive nano LC-MS/MS analytical platform is described to quantify endocannabinoids and their congeners in human cerebrospinal fluid (CSF) and later extended to measure in other biological matrices like, cells, zebrafish larvae, plasma (mice and human).

To understand the physiological role of endocannabinoids, especially of 2-AG and AEA, selective inhibitors for their synthetic and hydrolytic enzymes are required. **Chapter 4** describes the use of DH376, a potent *in vivo* active DAGL inhibitor, to investigate which isoform of diacylglycerol lipase (DAGL-α and -β) takes lead in 2-AG biosynthesis in a cellular model. Lipidomics and ABPP were employed to vehicle or DH376 treated wild type and knock-out Neuro2A populations (DAGL-α KO, DAGL-β KO, DAGL-α, -β double KO). These data revelled presence of additional enzymes responsible for 2-AG synthesis. In **chapter 5**, the reason behind the failure of the phase1 clinical trial of the fatty acid amide hydrolase (FAAH) inhibitor BIA 10-2474 was investigated. ABPP and lipidomics profiling platforms were used to measure on-target engagement and off-target effects of this compound. This analysis revealed that the compound interacts with several other lipases and produced substantial alterations in lipid network in human cortical neurons.

In order to gain better understanding of the *in vivo* pharmacology of the endocannabinoid system and to evaluate the systemic effect of drugs, proper *in vivo* model systems are required and need to be properly validated. In this regard, zebrafish larvae were tested as an attractive pre-clinical model system in **chapter 6** which characterizes the pharmacokinetics of paracetamol as a paradigm compound. The zebrafish drug clearance scales reasonably well with the clearance rates in higher vertebrates, including humans, and expanded the current allometric scale by 5 orders of magnitude. To further expand the applicability of zebrafish model in drug research, the pharmacodynamics of an endocannabinoid modulator PF04457845 was evaluated in **chapter 7**. In this chapter, ABPP and lipidomics tools were used to investigate the on-target engagement of PF04457845 on the protein landscape and its

downstream effects on the lipid networks. The results show the *in vivo* exposure of PF04457845 completely inhibited FAAH2 and increased the levels of NAEs, substrates of FAAH, while no cross-reactivity was observed with other serine hydrolases or alterations in lipid networks.

To expand our findings from models to clinical studies, **chapter 8** investigates a possible reason that underlies the disadvantageous metabolic phenotype of South Asians later in life. Quantification endocannabinoids in plasma showed that the South Asian men who have not yet developed a disadvantageous metabolic phenotype have a higher endocannabinoid tone compared to white Caucasian men.

Chapter 9 summarizes the work described in this thesis and its impact on the future researchers studying endocannabinoid system.

References

1. Kitano, H. Computational systems biology. *Nature* **420,** 206–210 (2002).
2. van der Greef, J. & McBurney, R. N. Rescuing drug discovery: in vivo systems pathology and systems pharmacology. *Nat. Rev. Drug Discov.* **4,** 961–967 (2005).
3. Vicini, P. & van der Graaf, P. H. Systems pharmacology for drug discovery and development: paradigm shift or flash in the pan? *Clin. Pharmacol. Ther.* **93,** 379–81 (2013).
4. van der Greef, J. Systems biology, connectivity and the future of medicine. *Syst. Biol. (Stevenage).* **152,** 174–8 (2005).
5. Kitano, H. Systems biology: a brief overview. *Science (80-.).* **295,** 1662–1664 (2002).
6. van der Greef, J., Hankemeier, T. & McBurney, R. N. Metabolomics-based systems biology and personalized medicine: moving towards n = 1 clinical trials? *Pharmacogenomics* **7,** 1087–94 (2006).
7. van der Greef, J., Adourian, A., Muntendam, P. & McBurney, R. N. Lost in translation? Role of metabolomics in solving translational problems in drug discovery and development. *Drug Discov. Today Technol.* **3,** 205–211 (2006).
8. Watkins, S. M. & German, J. B. Metabolomics and biochemical profiling in drug discovery and development. *Curr. Opin. Mol. Ther.* **4,** 224–8 (2002).
9. Kaddurah-Daouk, R., Kristal, B. S. & Weinshilboum, R. M. Metabolomics: a global biochemical approach to drug response and disease. *Annu. Rev. Pharmacol. Toxicol.* **48,** 653–83 (2008).
10. Rabinowitz, J. D., Purdy, J. G., Vastag, L., Shenk, T. & Koyuncu, E. Metabolomics in drug target discovery. *Cold Spring Harb Symp Quant Biol* **76,** 235–246 (2011).
11. Fernie, A. R., Trethewey, R. N., Krotzky, A. J. & Willmitzer, L. Metabolite profiling: from diagnostics to systems biology. *Nat. Rev. Mol. Cell Biol.* **5,** 763–9 (2004).
12. Suhre, K. *et al.* Human metabolic individuality in biomedical and pharmaceutical research. *Nature* **477,** 54–60 (2011).
13. van der Greef, J., Hankemeier, T. & McBurney, R. N. Metabolomics-based systems biology and personalized medicine: moving towards n = 1 clinical trials? *Pharmacogenomics* **7,** 1087–1094 (2006).
14. Clayton, T. A. *et al.* Pharmaco-metabonomic phenotyping and personalized drug treatment. *Nature* **440,** 1073–7 (2006).
15. Kaddurah-Daouk, R. & Weinshilboum, R. Metabolomic Signatures for Drug Response Phenotypes: Pharmacometabolomics Enables Precision Medicine. *Clin. Pharmacol. Ther.* **98,** 71–5 (2015).
16. Nicholson, J. K., Wilson, I. D. & Lindon, J. C. Pharmacometabonomics as an effector for personalized medicine. *Pharmacogenomics* **12,** 103–11 (2011).
17. Ellero-Simatos, S. *et al.* Oxylipid Profile of Low-Dose Aspirin Exposure: A Pharmacometabolomics Study. *J. Am. Heart Assoc.* **4,** e002203 (2015).
18. Gabrielsson, J., Fjellström, O., Ulander, J., Rowley, M. & Van Der Graaf, P. H. Pharmacodynamic-pharmacokinetic integration as a guide to medicinal chemistry. *Curr. Top. Med. Chem.* **11,** 404–418 (2011).
19. Kantae, V. *et al.* Integration of pharmacometabolomics with pharmacokinetics and pharmacodynamics: towards personalized drug therapy. *Metabolomics* **13,** 9 (2017).
20. Mechoulam, R., McCallum, N. K. & Burstein, S. Recent advances in the chemistry and biochemistry of cannabis. *Chem. Rev.* **76,** 75–112 (1976).
21. Foltin, R. W., Fischman, M. W. & Byrne, M. F. Effects of smoked marijuana on food intake and body weight of humans living in a residential laboratory. *Appetite* **11,** 1–14 (1988).

22. Mechoulam, R., Hanuš, L. O., Pertwee, R. & Howlett, A. C. Early phytocannabinoid chemistry to endocannabinoids and beyond. *Nat. Rev. Neurosci.* **15,** 757–764 (2014).
23. Devane, W. A., Dysarz, F. A., Johnson, M. R., Melvin, L. S. & Howlett, A. C. Determination and characterization of a cannabinoid receptor in rat brain. *Mol. Pharmacol.* **34,** 605–13 (1988).
24. Matsuda, L. A., Lolait, S. J., Brownstein, M. J., Young, A. C. & Bonner, T. I. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. *Nature* **346,** 561–4 (1990).
25. Hua, T. *et al.* Crystal Structure of the Human Cannabinoid Receptor CB 1. *Cell* **167,** 750–762.e14 (2016).
26. Munro, S., Thomas, K. L. & Abu-Shaar, M. Molecular characterization of a peripheral receptor for cannabinoids. *Nature* **365,** 61–65 (1993).
27. Mackie, K. Distribution of cannabinoid receptors in the central and peripheral nervous system. *Handb. Exp. Pharmacol.* 299–325 (2005).
28. Silvestri, C. & Di Marzo, V. The Endocannabinoid System in Energy Homeostasis and the Etiopathology of Metabolic Disorders. *Cell Metab.* **17,** 475–490 (2013).
29. Gonsiorek, W. *et al.* Endocannabinoid 2-arachidonyl glycerol is a full agonist through human type 2 cannabinoid receptor: antagonism by anandamide. *Mol. Pharmacol.* **57,** 1045–50 (2000).
30. Okamoto, Y., Morishita, J., Tsuboi, K., Tonai, T. & Ueda, N. Molecular characterization of a phospholipase D generating anandamide and its congeners. *J. Biol. Chem.* **279,** 5298–305 (2004).
31. Cravatt, B. F. *et al.* Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. *Nature* **384,** 83–7 (1996).
32. Bisogno, T. *et al.* Cloning of the first sn1-DAG lipases points to the spatial and temporal regulation of endocannabinoid signaling in the brain. *J. Cell Biol.* **163,** 463–8 (2003).
33. Dinh, T. P. *et al.* Brain monoglyceride lipase participating in endocannabinoid inactivation. *Proc. Natl. Acad. Sci.* **99,** 10819–10824 (2002).
34. Marrs, W. R. *et al.* The serine hydrolase ABHD6 controls the accumulation and efficacy of 2-AG at cannabinoid receptors. *Nat. Neurosci.* **13,** 951–7 (2010).
35. Blankman, J. L., Simon, G. M. & Cravatt, B. F. A comprehensive profile of brain enzymes that hydrolyze the endocannabinoid 2-arachidonoylglycerol. *Chem. Biol.* **14,** 1347–56 (2007).
36. Urquhart, P., Nicolaou, A. & Woodward, D. F. Endocannabinoids and their oxygenation by cyclo-oxygenases, lipoxygenases and other oxygenases. *Biochimica et Biophysica Acta - Molecular and Cell Biology of Lipids* **1851,** 366–376 (2015).
37. Ho, W.-S. V, Barrett, D. A. & Randall, M. D. "Entourage" effects of N-palmitoylethanolamide and N-oleoylethanolamide on vasorelaxation to anandamide occur through TRPV1 receptors. *Br. J. Pharmacol.* **155,** 837–46 (2008).
38. Keppel Hesselink, J. M., de Boer, T. & Witkamp, R. F. Palmitoylethanolamide: A Natural Body-Own Anti-Inflammatory Agent, Effective and Safe against Influenza and Common Cold. *Int. J. Inflam.* **2013,** 151028 (2013).
39. Guzmán, M. *et al.* Oleoylethanolamide stimulates lipolysis by activating the nuclear receptor peroxisome proliferator-activated receptor alpha (PPAR-alpha). *J. Biol. Chem.* **279,** 27849–27854 (2004).
40. Schwartz, G. J. *et al.* The Lipid Messenger OEA Links Dietary Fat Intake to Satiety. *Cell Metab.* **8,** 281–288 (2008).
41. Alhouayek, M. & Muccioli, G. G. The endocannabinoid system in inflammatory bowel diseases: from pathophysiology to therapeutic opportunity. *Trends Mol. Med.* **18,** 615–25 (2012).

42. Koch, M. Cannabinoid Receptor Signaling in Central Regulation of Feeding Behavior: A Mini-Review. *Front. Neurosci.* **11,** 293 (2017).
43. Jager, G. & Witkamp, R. F. The endocannabinoid system and appetite: relevance for food reward. *Nutr. Res. Rev.* **27,** 172–185 (2014).
44. Pacher, P. & Kunos, G. Modulating the endocannabinoid system in human health and disease--successes and failures. *FEBS J.* **280,** 1918–43 (2013).
45. Pacher, P. The Endocannabinoid System as an Emerging Target of Pharmacotherapy. *Pharmacol. Rev.* **58,** 389–462 (2006).
46. Després, J.-P., Golay, A. & Sjöström, L. Effects of Rimonabant on Metabolic Risk Factors in Overweight Patients with Dyslipidemia. *N. Engl. J. Med.* **353,** 2121–2134 (2005).
47. Sam, A. H., Salem, V. & Ghatei, M. A. Rimonabant: From RIO to Ban. *J. Obes.* **2011,** 432607 (2011).
48. Marzo, V. Di. Targeting the endocannabinoid system: to enhance or reduce? *Nat. Rev. Drug Discov.* **7,** 438–455 (2008).
49. Basavarajappa, B. S. Critical enzymes involved in endocannabinoid metabolism. *Protein Pept. Lett.* **14,** 237–46 (2007).
50. Li, G. L. *et al.* Assessment of the pharmacology and tolerability of PF-04457845, an irreversible inhibitor of fatty acid amide hydrolase-1, in healthy subjects. *Br. J. Clin. Pharmacol.* **73,** 706–716 (2012).
51. Baggelaar, M. P. *et al.* Highly Selective, Reversible Inhibitor Identified by Comparative Chemoproteomics Modulates Diacylglycerol Lipase Activity in Neurons. *J. Am. Chem. Soc.* **137,** (2015).
52. Ogasawara, D. *et al.* Rapid and profound rewiring of brain lipid signaling networks by acute diacylglycerol lipase inhibition. *Proc. Natl. Acad. Sci.* **113,** 26–33 (2016).
53. Niphakis, M. J. & Cravatt, B. F. Enzyme Inhibitor Discovery by Activity-Based Protein Profiling. *Annu. Rev. Biochem.* **83,** 341–377 (2014).
54. van Esbroeck, A. C. M. *et al.* Activity-based protein profiling reveals off-target proteins of the FAAH inhibitor BIA 10-2474. *Science (80-.).* **356,** 1084–1087 (2017).