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**Structural properties and role of the endocannabinoid lipases ABHD6 and ABHD12 in lipid signalling and disease**

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## Abstract

The endocannabinoid (eCB) system is an important part of both the human central nervous system (CNS) and peripheral tissues. It is involved in the regulation of various physiological and neuronal processes and has been associated with various diseases. The eCB system is a complex network composed of receptor molecules, their cannabinoid ligands and enzymes regulating the synthesis, release, uptake and degradation of the signalling molecules. Although the eCB system and the molecular processes of eCB signalling have been studied extensively over the last decades, the involved molecules and underlying signalling mechanisms have not been described in full detail. An example pose the two poorly characterized eCB-degrading enzymes  $\alpha/\beta$ -hydrolase domain protein 6 (ABHD6) and ABHD12, which have been shown to hydrolyze 2-arachidonoyl glycerol - the main eCB in the CNS. We review the current knowledge about the eCB system and the role of ABHD6 and ABHD12 within this important signalling system and associated diseases. Homology modelling and multiple sequence alignments highlight the structural features of the studied enzymes and their similarities, as well as the structural basis of disease-related ABHD12 mutations. However, homologies within the ABHD family are very low, and even the closest homologues have widely varying substrate preferences. Detailed experimental analyses at the molecular level will be necessary in order to understand these important enzymes in full detail.

## Keywords

Endocannabinoid ~~signalings~~signalling,  $\alpha/\beta$ -hydrolase, 2-Arachidonoyl glycerol, lipid metabolism, PHARC

## 1. The human endocannabinoid system

Widely distributed in both the human central nervous system (CNS) and peripheral tissues, the endocannabinoid (eCB) system plays a crucial role in regulating different processes, *e.g.* appetite, stress response, memory, immune system, synaptic plasticity, learning, sleep regulation, reproduction, and pain perception (Di Marzo et al. 2004).— Thus, it poses an attractive pharmacological target for the treatment of a variety of diseases.

The discovery of this important ~~signalingsignalling~~ system was mainly due to the common use of marijuana, since researchers were interested in the mechanism and action of the drug. The medical and ritual use of marijuana, consisting of dried leaves and flowers of the plant *Cannabis sativa*, originates thousands of years ago in the ancient Asian culture (Zuardi 2006). Cannabis extract has been applied in the treatment of pain, depression, asthma, spasms, sleep disorders and loss of appetite (Grotenhermen and Muller-Vahl 2012). Due to its psychoactive effect and despite negative effects, including impaired functional connectivity in certain brain regions, resulting in decreased awareness, learning, and memory, marijuana is the world's most commonly used illicit drug (Volkow et al. 2014).

Research on the molecular effects of marijuana started in the 1960s, when the two main components of cannabis were extracted from *Cannabis sativa* L. (Gaoni and Mechoulam 1971). The isolation and synthesis of the main psychoactive compound  $\Delta^9$ -tetrahydrocannabinol (THC) in marijuana finally led to the discovery of the molecular components of the eCB system (Mechoulam and Gaoni 1965; Wilson and Nicoll 2002). The eCB system has become an important field of neurological research, since drugs modulating the components of the system could possibly treat diseases like inflammation, pain, multiple sclerosis, epilepsy, emesis, as well as ~~ases~~ Parkinson's, Huntington's, and Alzheimer's disease (Kaur et al. 2016).

### 1.1. Components of the endocannabinoid system

The eCB system is a complex network constituted of membrane-integrated receptor molecules, endogenous cannabinoid ligands, and several enzymes regulating the synthesis, release, uptake, and degradation of these ~~signalingsignalling~~ molecules- (Fig. 2). These components are introduced in the following chapters.

#### Cannabinoid receptors

Two types of cannabinoid receptors, CB1 and CB2, are currently known, both belonging to the superfamily of G-protein-coupled receptors (GPCRs) and exhibiting a sequence identity of 48% (Mechoulam and Parker 2013). Both CB1 and CB2 contain seven transmembrane (7TM)  $\alpha$ -helices (I to VII) connected by three extracellular (ECL1–3) and three intracellular loops (ICL1–3). Both receptor types possess a glycosylated N-terminal extracellular domain and a C-terminal intracellular domain coupled to a G-protein complex (Malfitano et al. 2014; Hua et al. 2016; Svizenska et al. 2008).

CB1 is one of the most abundant receptors in the brain, which emphasizes its importance in neuronal processes, such as memory, perception, and the control of movement (Ameri 1999). Autoradiographic studies revealed that CB1 is expressed heterogeneously within the brain (Herkenham et al. 1990). Although CB1 is most prevalent in brain tissue, like the basal

ganglia, cerebellum, hippocampus, substantia nigra, and globus pallidus, it has also been found in peripheral tissues (Crocì et al. 1998; Wagner et al. 2001; Svizenska et al. 2008). The fact that it is expressed, preferentially pre-synaptically, both in excitatory (glutamatergic) and inhibitory (GABAergic) neurons indicates that it may be involved in the regulation of signalling via glutamate and  $\gamma$ -aminobutyric acid (GABA) and thereby regulate synaptic plasticity (Tsou et al. 1999; Gerdeman and Lovinger 2001; Hill et al. 2007; Katona et al. 1999; Katona et al. 2006).

CB2 is mainly expressed in the peripheral nervous system (PNS) and the immune system, with the highest expression levels observed in thymus, tonsils, B/T lymphocytes, macrophages, monocytes, and natural killer cells. This distribution pattern could explain the cannabinoid immunomodulatory effects of cannabis preparations, whereby CB2 activation by phytocannabinoids results in the inhibition of cytokine production, decrease in antigen presentation, and modulation of cell migration (Lynn and Herkenham 1994; Berdyshev 2000; Ehrhart et al. 2005). CB2 receptors have also been detected in the CNS, *e.g.* in neurons and microglial cells (Cabral and Griffin-Thomas 2009).

Knockout studies of— CB1 or CB2, applying genetic modification or inhibitor-based inactivation, have shown that certain effects of eCBs are still present and thus seem to be mediated by other receptors (Begg et al. 2005). Since these eCB-induced CB1/CB2-independent *in vitro* effects are sensitive to pertussin toxin (PTX), members of the GPCR protein family have been suggested as candidates (De Petrocellis and Di Marzo 2010).

### Receptor ligands

Ligands of the eCB system can be endogenous (eCBs), plant-derived (phytocannabinoids), or synthetic molecules acting as agonists or antagonists. All of them can bind to eCB receptors (CB1/CB2) and thereby affect signalling.

eCBs are unsaturated fatty acid glycerols, glycerol ethers, or ethanolamines (Reggio 2010). The most active and best characterized eCBs are 2-arachidonoyl glycerol (2-AG) and N-arachidonoyl ethanolamine (anandamine / AEA), which are both lipid eCBs and exhibit similar synthesis, internalization, and degradation (Murataeva et al. 2014; Fonseca et al. 2013). Both are arachidonic acid (AA) derivatives, sharing a 19-carbon backbone structure, whereby 2-AG is formed from an glycerol/AA ester, and AEA belongs to amides (Fig. 1) (Fonseca et al. 2013).

2-AG and AEA can diffuse across the cell membrane and are synthesized and released 'on demand'. Unlike the typical neurotransmitters acetylcholine, serotonin, and dopamine, which are stored in vesicles, 2-AG and AEA are synthesized from lipid precursors present in the cell membrane, where they mostly remain until release (Placzek et al. 2008). Upon release from the postsynaptic cell, the lipophilic molecules travel through the pericellular space and activate eCB receptors at the presynaptic terminal, classifying them as retrograde messengers.

AEA exhibits a higher affinity for CB1 than for CB2, but also acts as an agonist on transient receptor potential vanilloid 1 (TRPV1) channel and peroxisome proliferator-activated receptors (PPARs) (Pertwee et al. 2010; Zygmunt et al. 1999; O'Sullivan 2007). With the exception of TRPV1-receptors, 2-AG shows similar receptor binding ability. When comparing AEA and 2-AG, the latter has higher affinity and efficacy for CB1 and CB2 and is present in

the CNS in higher amounts, suggesting that 2-AG plays a crucial role in eCB ~~signaling~~signalling (Fonseca et al. 2013).

Additionally to AEA and 2-AG, several potential eCBs have been identified, although mostly present at lower concentrations. Examples are the CB1/CB2 agonist 2-arachidonoyl glycerol ether (noladin ether), the CB1 and TRPV1 ligand *N*-arachidonoyl-dopamine (NADA), and the CB1 antagonist *O*-arachidonoyl ethanolamine (virodhamine) (Hanus et al. 2001; Shoemaker et al. 2005; Redmond et al. 2016; Porter et al. 2002; Huang et al. 2002).

The effects of eCBs can be mimicked by phytocannabinoids, *e.g.* THC, cannabidiol (CBD), and cannabitol (CBN), which are the main psychoactive compounds of *Cannabis sativa* extracts (Mechoulam et al. 2014). Similarly to eCBs, these plant-derived ligands are lipids and lead – *via* binding to CB1 and CB2 – to hypokinesia, hypothermia, antinociception, and catalepsy (Little et al. 1988).

Artificial ligands acting as agonists or antagonists for eCB receptors have been synthesized. The discovery of the antagonists SR141716A and AM630/SR144528 for CB1 and CB2, respectively, helped to identify additional signalling pathways involving the cannabinoid receptors, and to understand the receptor specificity of cannabinoid-induced effects (Mechoulam et al. 2014; Rinaldi-Carmona et al. 1995; Rinaldi-Carmona et al. 1994; Pertwee et al. 1995; Rinaldi-Carmona et al. 1998). Much effort has been put into the development of additional, selective antagonists and inverse agonists, which could be used to treat diseases associated with CB1/CB2. SR141716A, an inverse agonist for CB1, has been approved for the treatment of obesity-related syndromes, such as dyslipidaemia and diabetes (Nissen et al. 2008; Van Gaal et al. 2005).

#### Enzymes involved in ligand synthesis, internalization, and degradation

Both of the main eCBs, 2-AG and AEA, are believed to be synthesized 'on demand' prior to release and have distinct biosynthesis pathways, which are mostly Ca<sup>2+</sup>-dependent. Hence, a depolarization-induced increase of Ca<sup>2+</sup> concentration by voltage gated calcium channels (VGCCs) or release from intracellular Ca<sup>2+</sup> stores induces eCB synthesis (Wilson and Nicoll 2002). In another, Ca<sup>2+</sup>-independent, ~~signaling~~signalling pathway, activation of group I metabotropic glutamate receptors (mGluRs) leads to the mobilization of eCBs (Varma et al. 2001; Maejima et al. 2001).

Since 2-AG is not only involved in eCB ~~signaling~~signalling, but is an important intermediate in lipid metabolism, it is not surprising that different biosynthesis pathways have been identified. The main route for 2-AG biosynthesis has been suggested to include the hydrolysis of membrane phospholipids (PLs) by phospholipase C (PLC), resulting in the 2-AG precursor 1,2-diacylglycerol (DAG), which – as a substrate for diacylglycerol lipase (DAGL) – in turn gets converted into 2-AG (Fonseca et al. 2013). Alternative synthesis pathways include ~~the~~ phospholipase A1 (PLA1) and ~~the~~ lyso-phospholipase C (lysoPLC), by which a 2-arachidonoyl-lysophospholipid is generated and subsequently hydrolyzed to 2-AG (Higgs and Glomset 1994). Lipid phosphatases can act on 2-arachidonoyl-lysophosphatidic acid and thereby form 2-AG (Nakane et al. 2002). 2-AG synthesis by PLC/DAGL is accepted as the main pathway, since 2-AG accumulation is prevented by specific inhibition of PLC and DAGL in neurons (Stella et al. 1997).

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The main pathway for the production of AEA involves the transfer of arachidonic acid from phosphatidylcholine to phosphatidylethanolamine by *N*-acetyltransferase (NAT). *N*-arachidonoyl-phosphatidylethanolamine (NAPE) is a precursor for AEA and gets cleaved by phospholipase D (NAPE-PLD), which releases AEA from NAPE and is located in the inner leaflet of the cell membrane in brain, kidney, heart, lung, liver, and spleen (Fonseca et al. 2013). Knock-out experiments revealed that NAPE-PLD is solely responsible for the cleavage of saturated and monounsaturated NAPEs, but not for polyunsaturated NAPEs, like AEA (Leung et al. 2006). Hence, two additional mechanisms for the synthesis of AEA, involving a PLC-like enzyme or a NAPE-specific hydrolase, have been proposed, in which NAPE is converted into AEA in a 2-step reaction. NAPE hydrolysis by an uncharacterized PLC-like enzyme leads to the release of phosphoanandamide, which subsequently gets dephosphorylated to AEA by a protein tyrosine phosphatase (Liu et al. 2006). Another possibility of AEA synthesis includes the (lyso-)NAPE-specific hydrolase ABHD4, which acts on the AEA precursor *N*-arachidonoyl lyso-NAPE and generates glycerophosphoarachidonoyl ethanolamine (GpAEA), which gets converted by a metal-dependent phosphodiesterase into AEA and glycerol-3-phosphate (Simon and Cravatt 2006; Lord et al. 2013).

Synthesized eCBs are released into the extracellular space, enabling the binding to eCB receptors at the presynaptic terminal. The mechanism of eCB release is unknown. However, the formation of extracellular vesicles (EVs), as observed in microglia, or facilitated transport by an eCB membrane transporter (EMT) have been suggested as possible pathways (Gabrielli et al. 2015; Fowler 2013).

In addition to the synthesis and release rate, the ligand amount at the presynaptic neuron is regulated by eCB uptake. Due to their lipophilic character, the ligands can diffuse freely across the cell membrane – a process driven by a concentration gradient between the extra- and intracellular spaces. The intracellular concentration of eCBs is regulated by degradative enzymes, which are localized in intracellular structures (Fonseca et al. 2013). Since eCBs are water-insoluble, their intracellular trafficking may be dependent on carrier proteins, including heat shock proteins and fatty acid-binding proteins, as shown for AEA (Kaczocha et al. 2009; Fowler 2013; Oddi et al. 2009). Besides passive diffusion across the cell membrane, eCBs may enter the cell *via* a clathrin-independent, caveola/lipid raft-related endocytosis or a carrier-mediated transport *via* a yet-to-be identified EMT, which alleviates diffusion and promotes bidirectional transport across the plasma membrane (McFarland et al. 2004; Fowler 2013).

Once the internalized ligands reach intracellular compartments, eCBs can be degraded by metabolic enzymes, and the breakdown products may be recycled for the biosynthesis of eCBs and other endogenous molecules. Alternatively, they can be converted to other metabolites involved in further signalling processes.

AEA gets hydrolyzed to arachidonic acid and ethanolamine mainly by fatty acid amide hydrolase (FAAH), which belongs to the serine hydrolase family and is a membrane-integrated postsynaptic protein in  $\text{Ca}^{2+}$ -storing organelles, such as mitochondria and the smooth endoplasmic reticulum (Blankman et al. 2007; Cravatt et al. 1996; McKinney and Cravatt 2005; Gulyas et al. 2004). FAAH inhibition with distinct inhibitors or knock-down of the FAAH-encoding gene in rodents results in the blockade of AEA hydrolysis in [the](#) brain (Cravatt et al. 2001; Kathuria et al. 2003).

In contrast to AEA, 2-AG degradation into arachidonic acid and glycerol is catalyzed by several enzymes. Hydrolytic enzymes acting on 2-AG include FAAH, neuropathy target esterase (NTE), hormone-sensitive lipase (HSL), monoacylglycerol lipase (MAGL), and the poorly characterized enzymes  $\alpha/\beta$ -hydrolase domain protein 6 (ABHD6) and  $\alpha/\beta$ -hydrolase domain protein 12 (ABHD12) (Goparaju et al. 1998; van Tienhoven et al. 2002; Dinh et al. 2002; Belfrage et al. 1977; Blankman et al. 2007). The presynaptic enzyme MAGL is a key player by being responsible for 85% of 2-AG degradation in rat cerebellar membranes and 50% of soluble rat brain fractions (Dinh et al. 2004; Saario et al. 2005; Gulyas et al. 2004). Quantitative profiling of mouse brain 2-AG hydrolases revealed that MAGL, ABHD6, and ABHD12 collectively account for approximately 98% of the total 2-AG hydrolase activity. The fact that these three hydrolases are distributed heterogeneously within the cell suggests that they might regulate different 2-AG pools within the CNS (Blankman et al. 2007).

## 1.2. Endocannabinoid ~~signalingsignalling~~

### Retrograde ~~signalingsignalling~~

The eCB system possesses a peculiar synaptic transmission process. Not only is the lipophilic character of endocannabinoids rare amongst other signalling systems, but also the mechanism of action of the ligands is unique. Following the synthesis of the ligands in response to depolarization-induced VGCC-gated  $\text{Ca}^{2+}$  influx or mGluR activation, eCBs are released from the postsynaptic neuron and travel backwards through the synaptic cleft, ~~where they binding~~ to their receptors ~~in~~ the presynaptic membrane. This retrograde signalling, in addition to other downstream signals, leads to the inhibition of neurotransmitter release, forming a negative feedback loop, by which short- and long-term synaptic plasticity are mediated (Wilson and Nicoll 2002; Castillo et al. 2012). This phenomenon was first observed in cerebellar Purkinje cells and hippocampal pyramidal cells, where a depolarization of the cell led to the suppression of inhibitory GABAergic response – a process known as “depolarization-induced suppression of inhibition” (DSI) (Llano et al. 1991; Pittler and Alger 1992). An analogous phenomenon, accordingly referred to as “depolarization-induced suppression of excitation” (DSE), was observed in glutamatergic (excitatory) synapses in the cerebellum (Kreitzer and Regehr 2001). ~~eCB~~-induced DSI and DSE are involved in short-term plasticity in the brain (Wilson and Nicoll 2002). The retrograde ~~signalingsignalling~~ of eCBs contradicts the common pattern of a one-way signal transmission at the synapse.

eCB stimulation of neuroblastoma cells and CB1/CB2-expressing ~~C~~hinese hamster ovary (CHO) cells leads to a decrease in cyclic adenosine monophosphate (cAMP) levels, suggesting G-protein-mediated negative coupling to adenylyl cyclase (Vogel et al. 1993; Felder et al. 1993; Howlett and Mukhopadhyay 2000). Indeed, eCB signalling mechanisms of short- and long-term plasticity involve G-protein activation (Castillo et al. 2012). Short-term plasticity effects by DSI and DSE can be evoked by presynaptic ligand binding to the receptor and subsequent G-protein-dependent inactivation of VGCCs, leading to a decrease in  $\text{Ca}^{2+}$  influx and the associated inhibition of neurotransmitter release from the presynaptic cell. The process of 2-AG-induced DSE at glutamatergic synapses is shown schematically in Fig. 23.

eCB-induced long-term depression (eCB-LTD) has been shown in both excitatory and inhibitory synapses and involves mainly the G-protein-coupled inhibition of adenylyl cyclase, resulting in downregulation of the cAMP/PKA pathway (Castillo et al. 2012; Gerdeman et al.

2002; Chevalleyre and Castillo 2003). By modulating the cAMP production, and thereby the phosphorylation of target proteins by protein kinase A (PKA), eCBs can alter cellular activity by binding to their receptors (Howlett and Mukhopadhyay 2000). One example of eCB-regulated neuronal response is the signalling via voltage-dependent K<sup>+</sup>-channels in the hippocampus, whereby their phosphorylation/dephosphorylation mediates neuronal activity (Childers and Deadwyler 1996). Other ion channels affected by cannabinoid binding to CB1 are N-type voltage-gated Ca<sup>2+</sup> channels and Q-type Ca<sup>2+</sup> channels (Mackie et al. 1993; Mackie et al. 1995). In *nucleus accumbens* synapses, the blockade of P/Q-type Ca<sup>2+</sup> channels and PKA activity eliminates eCB-LTD, supporting the hypothesis that these ion channels, and PKA, are important for long-term plasticity (Mato et al. 2008).

CB1 and CB2 are also involved in the regulation of mitogen-activated protein kinase (MAPK) pathways (Bouaboula et al. 1995; Bouaboula et al. 1996). Examples of anandamide-induced activation include the MAP kinase ERK2, involved in prostaglandin E2 production in WI-38 cells, and the pp125-focal adhesion kinase (FAK), which promotes cytoskeletal changes accompanied with signal transduction events (Wartmann et al. 1995; Derkinderen et al. 1996; Howlett and Mukhopadhyay 2000). Hence, MAPK activation may account for eCB-induced synaptic plasticity changes.

Not only neurons are involved in eCB-mediated retrograde signalling, but also glial cells, such as astrocytes, participate in eCB signalling. In Schaffer collateral excitatory synapses of hippocampal pyramidal neurons, the excitation-induced release of eCBs causes the activation of both presynaptic and astrocytic CB1. In contrast to presynaptic CB1 activation, which leads to DSI or DSE, the activation of astrocytic CB1 is associated with an increase in glutamate release and enhanced synaptic transmission (Navarrete and Araque 2008).

### Non-retrograde ~~signalingsignalling~~

In addition to the retrograde ~~signalingsignalling~~ via CB1/CB2, eCBs can act directly as intracellular messengers by binding to and activating postsynaptic receptors, (Fig. 3), including TRPV1 (van der Stelt et al. 2005). eCB-induced activation of TRPV1 channels has been reported both in peripheral tissues, where ~~its activationit~~ regulates pain perception, and in different brain tissues, where it is associated with postsynaptic plasticity of excitatory synapses (Castillo et al. 2012).

Another process, in which eCBs act in a non-retrograde manner, was studied in neocortical GABAergic interneurons and neocortical pyramidal neurons; repetitive activation leads to a postsynaptic hyperpolarization, decreasing excitability (Bacci et al. 2004; Marinelli et al. 2009). Excitation of the postsynaptic cell resulted in an increase of intracellular Ca<sup>2+</sup> and subsequent mobilization of 2-AG. eCB binding to postsynaptic CB1 activated K<sup>+</sup> channels G-protein-dependently, leading to self-inhibition of postsynaptic cell excitability. Similar observations have been made for CB2 in prefrontal cortical pyramidal neurons. Activation of CB2, localized in intracellular compartments, resulted in postsynaptic self-inhibition via coupling to Ca<sup>2+</sup>-activated Cl<sup>-</sup> channels (den Boon et al. 2012).

### Interaction with other ~~signalingsignalling~~ pathways

In addition to the regulation of synaptic transmission via glutamate and GABA, eCBs can modify the release of different neurotransmitters, including serotonin, ~~opiodsopioids~~.



dopamine, and acetylcholine. Many of these neurotransmitters, in turn, are ligands for GPCRs, which couple to the eCB synthesis machinery (Castillo et al. 2012).

Presynaptic connection to other signalling systems occurs through the co-localization of different receptors with the cannabinoid receptors. Examples are the dopamine D<sub>2</sub>-like receptors, which promote CB1-mediated inhibition of neurotransmitter release, the adenosine A1 receptor, which acts oppositely when ~~tonically~~ *tonically* activated, and the GluK1-containing kainate receptor, by which CB1 action is facilitated (Chiu et al. 2010; Hoffman et al. 2010; Lourenco et al. 2010). Another way of modulating signal transmission is the oligomerization of CB1 with colocalizing receptors, including the opioid, adenosine A2A, and dopamine D<sub>2</sub>-like receptors, which are involved in different signalling pathways- (Hudson et al. 2010; Rios et al. 2006; Carriba et al. 2007; Mackie 2005).

### Endocannabinoid signalling in peripheral tissues

In addition to the ~~previously described~~ pathways *described above*, ~~endocannabinoid-eCB~~ signalling regulates various processes in peripheral tissues. This area of ~~endocannabinoid eCB~~ research has just recently found more attention, and it is very likely that more peripheral ~~endocannabinoid-eCB~~ functions will be discovered. A few examples of peripheral ~~endocannabinoid-eCB~~ actions are given below.

The ~~endocannabinoid-eCB~~ system has been shown to be a key regulator of energy homeostasis and food intake, whereby CB1 is the main receptor involved in eCB signalling (Heyman et al. 2012; Freedland et al. 2000; Simiand et al. 1998). CB1 inhibition leads to a decreased food intake, lowered obesity-mediated insulin resistance, and a ~~loss~~ of body weight (Van Gaal et al. 2005; Rosenstock et al. 2008; Tam et al. 2018). The fact that CB1 expression levels are increased in adipose tissue and liver (Jourdan et al. 2010), kidney (Udi et al. 2017), and possibly in skeletal muscle (Pagotto et al. 2006; Heyman et al. 2012) during diabetes and obesity, suggests an eCB-dependent regulation of the disease state. ~~Mice~~ *Studies on mice* serving as a model for the metabolic syndrome revealed that CB1 is involved in the dysregulation of lipid metabolism observed in adipose tissue as well as ~~the~~ lipogenesis in adipocytes and liver (Osei-Hyiaman et al. 2005; Jourdan et al. 2010). Several other studies give strong evidence that peripheral eCB signalling has a physiological role in energy homeostasis and could be a pharmacological target to treat associated diseases, such as obesity, obesity-/diabetes- induced insulin resistance, and the metabolic syndrome (Tam et al. 2018). In addition, it was observed that eCB levels in the kidney are elevated during obesity, and that CB1 is highly expressed in kidney renal proximal tubular cells (RPTCs) and ~~podocytes~~ *podocytes* in murine models for type 1 and 2 diabetes, which suggests – along with other studies – that peripheral eCB signalling contributes to the development of obesity- and diabetes-induced chronic kidney disease (Udi et al. 2017; Tam 2016). This topic has been extensively reviewed by Tam and colleagues, and the reader is referred to this work for further detailed information (Tam et al. 2018).

Another peripheral involvement of the endocannabinoid system was shown in the process of bone remodeling, which is important for maintaining skeletal integrity and ensures the adaptation of bone strength to mechanical demands. The main components of the eCB system, the eCBs 2-AG and AEA, eCB-synthesizing/degrading enzymes, and ~~CBRs~~ *cannabinoid receptors* are expressed/synthesized by osteoblasts and osteoclasts (Tam et al. 2006; Bab et al. 2008; Rossi et al. 2009). While ~~CBR-cannabinoid receptors~~ may be directly

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involved in ~~the~~ bone turnover and regeneration, which ~~are~~<sup>is</sup> disturbed in CB2-deficient mice (Sophrone et al. 2011), CB1 is mainly expressed on sympathetic nerve endings and has been shown to modulate ~~the~~ CNS activity in bone remodelling by targeting skeletal sympathetic effects (Tam et al. 2008).

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eCB signalling, mainly involving CB1, has also been associated with the regulation of skeletal muscle differentiation. *In vitro* studies with murine C2C12 cells and *in vivo* experiments showed that 2-AG levels are decreased during the formation of myotubes and mouse muscle growth, respectively. CB1-deficient-KO mouse embryos possessed more muscle fibres than controls, and an increased muscle fibre diameter was observed in postnatal CB1 KO-knock-out mice. These studies suggest 2-AG to act as a CB1-mediated endogenous repressor of myoblast differentiation (Iannotti et al. 2014). In the context of skeletal muscle homeostasis, peripheral ~~endocannabinoid-eCB~~ signalling *via* CB1 ~~was shown to~~ negatively modulate insulin sensitivity and substrate oxidation ~~reviewed by Heyman and colleagues~~ (Heyman et al. 2012). This effect could be due to an overactivation of CB1 during obesity, caused by an elevation of ~~endocannabinoid-eCB~~ levels. The endocannabinoid AEA, in contrast, was found to be beneficial in muscle glucose uptake, insulin signalling, and mitochondrial biogenesis, when applied in a sufficient dose. These positive metabolic effects might be triggered by the AEA-mediated activation of TRPV1 and PPAR $\gamma$  receptors (Heyman et al. 2012).

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~~One A~~ recent ~~work studying study on~~ human spermatozoa revealed ~~endocannabinoid-eCB~~ signalling as the underlying mechanism of sperm activation *via* the sex hormone progesterone. Progesterone is involved in various signalling processes and can act *via* a genome-receptor pathway, in which transcriptional regulation is achieved, or by a non-genomic pathway, which modifies intracellular factors and thereby results in a fast signal. Spermatozoa are regulated *via* the latter pathway. Patch-clamp measurements showed a progesterone(P4)-dependent activation of sperm CatSper channels, which leads to an increase of cytoplasmic Ca<sup>2+</sup> and thereby increases sperm motility and initiates acrosomal exocytosis. The authors also demonstrated that CatSper channels are inhibited by certain ~~endocannabinoid-eCBs~~, 1-AG and 2-AG. Further studies, involving a P4 analogue, light-induced crosslinking and pull-down experiments followed by (LC-)MS, indicated that P4 binds to the serine hydrolase ABHD2. *In vitro* activity assays showed that P4 binding increases the ability of ABHD2 to hydrolyse AGs, which suggests ABHD2 as the progesterone receptor in human spermatozoa (Miller et al. 2016).

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## 2. ABHD6 and ABHD12 – two ~~as of~~ yet uncharacterized endocannabinoid hydrolases

As outlined above, the eCB system is an important part of the CNS and PNS, where it accounts for the regulation of signal transmission in processes including synaptic plasticity, learning, memory, stress response, appetite, immune system, sleep regulation, reproduction, and pain perception. Although the eCB system and the associated signalling pathways have been extensively studied, many molecular and mechanistic details are lacking. Further research on ligands, receptors, and regulating enzymes connected with *in vitro* and *in vivo* studies would improve the understanding of this complex signalling system and facilitate drug development.

An example of a poorly characterized part of the eCB system is the degradation ~~the of~~ eCBs, which is essential for the above pathways. For 2-AG, the most prevalent eCB in the CNS, the

degradation into arachidonic acid and glycerol is mainly catalyzed by three enzymes: MAGL, ABHD6, and ABHD12, which belong to the  $\alpha/\beta$  hydrolase superfamily (Blankman et al. 2007; Lord et al. 2013). While MAGL has been structurally and functionally analyzed, ABHD6 and ABHD12 remain uncharacterized at the molecular level (Labar et al. 2010; Bertrand et al. 2010; Blankman et al. 2007).

Although no structural information for ABHD6 and ABHD12 is available so far, bioinformatics and mouse knock-out studies have provided information on possible structure, subcellular distribution, and function in lipid degradation- (Lord et al. 2013; Blankman et al. 2007; Navia-Paldanius et al. 2012; Marrs et al. 2010). The current knowledge about the structure and function of these important enzymes is summarized below. Homology analysis, modelling, and structural alignments give information on the structure and function of ABHD6 and ABHD12.

#### ABHD6 and ABHD12 are related enzymes of the same family

Both ABHD6 and ABHD12 are predicted to belong to the  $\alpha/\beta$  hydrolase domain (ABHD) family (Lord et al. 2013). ABHD family members are involved in lipid synthesis and degradation, and mutations ~~in the~~ in the respective genes are associated with inherited alterations ~~in of~~ in lipid metabolism. The ABHD family is part of the  $\alpha/\beta$  hydrolase superfamily, which includes lipases, proteases, esterases, peroxidases, dehalogenases, and epoxide hydrolases, and the members exhibit a common fold (Nardini and Dijkstra 1999). The canonical  $\alpha/\beta$ -hydrolase fold is formed of an 8-stranded  $\beta$ -sheet, with the second strand being antiparallel, with  $\alpha$ -helices on both sides of the sheet. The position and number of the  $\alpha$ -helices can vary, and various insertions into this principal fold have been observed within the family, adapting for different substrates and intermolecular interactions. Despite the variation between the members, the active site is comprised of a highly conserved catalytic triad, which involves a nucleophilic residue, an acidic residue, and a histidine (Fig. ~~3a4a~~ 3a4a). The nucleophile, which can be ~~a~~-serine, cysteine, or aspartate, is usually located in a sharp turn after strand  $\beta 5$ , being easily accessible for the substrate. The nucleophilic elbow can be identified by the conserved amino acid sequence Sm-X-Nu-X-Sm (Sm, small residue; X, any residue; Nu, nucleophilic residue). Besides positioning the nucleophile, the nucleophilic elbow plays a role in the formation of an oxyanion hole, which stabilizes the anionic reaction intermediate. The acidic residue, ~~glutamate or aspartate~~, of the catalytic triad ~~can be either a glutamate or an aspartate, being~~ is usually present after strand  $\beta 7$ . The His residue is completely conserved and located at the end of the last  $\beta$ -strand (Nardini and Dijkstra 1999). In addition to the nucleophile motif, many members of the ABHD family possess a conserved His-XXXX-Asp motif, which has been associated with acyltransferase activity (Ghosh et al. 2008; Montero-Moran et al. 2010). The active site of  $\alpha/\beta$ -hydrolases can be covered by lids and domains, which shape the substrate-binding site. Especially for lipases, a dynamic lid has been described, whereby enzymatic activity increases, when in contact with a lipid-water interface (Nardini and Dijkstra 1999).

The 2-AG-degrading enzyme MAGL is a member of the  $\alpha/\beta$  hydrolase family, and its structure has been solved by X-ray crystallography (Labar et al. 2010). It possesses the canonical  $\alpha/\beta$  hydrolase fold with the catalytic triad composed of Ser123, Asp239, and His269 (Fig. ~~3b4b~~ 3b4b). The  $\alpha_D$  helix has lipophilic character and is located in the cap domain, presumably enabling ~~the a~~ contact with a membrane, where the lipophilic substrates are

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located. The oxyanion hole is formed by the backbone nitrogen atoms of Ala51 and Met123 (Labar et al. 2010).

### Biochemical features and associated biological processes

Both ABHD6 and ABHD12 are predicted to be membrane-integrated by an N-terminal transmembrane helix (Lord et al. 2013). Glycosidase digestion conducted in ABHD6/ABHD12-transfected COS-7 cells suggests that ABHD6 is a membrane protein facing the cytoplasm, whereas ABHD12 seems to be a transmembrane glycoprotein with its active site facing the luminal/extracellular space (Blankman et al. 2007). With MAGL being a cytoplasmic or peripheral enzyme, these data suggest that the three major 2-AG-degrading hydrolases regulate different eCB pools of the endocannabinoid. By the application of a sensitive fluorescent glycerol assay in eCB hydrolase-overexpressing HEK293 cell lysates, the substrate preferences of the enzymes could be identified. While ABHD6, similarly to MAGL, prefers saturated monoacylglycerols (MAGs) with medium-length chains, ABHD12 catalyzes most efficiently the hydrolysis of 1(3)- and 2-isomers of arachidonoyl glycerol (Navia-Paldanius et al. 2012).

ABHD6 is a 38-kDa, 337-residue hydrolase, and the catalytic triad has been proposed to be constituted of Ser148, Asp278, and His306 (Kaczor et al. 2015; Marrs et al. 2010). This assumption was confirmed by an activity assay and activity-based protein profiling (ABPP), whereby site-directed mutagenesis of these residues led to inactivation (Navia-Paldanius et al. 2012). ABHD6 is ubiquitously expressed, and high mRNA levels have been found in murine small intestine, brown adipose tissue, brain, liver, testis, muscle, kidney, and heart (Poursharifi et al. 2017). With a postsynaptic expression pattern observed for ABHD6, this hydrolase might regulate 2-AG levels at the site of its synthesis (Marrs et al. 2010). Chemical inhibition of ABHD6 in primary neuronal cell cultures decreased 2-AG degradation by 50% and led to 2-AG accumulation. In BV-2 cells, this decrease in 2-AG hydrolysis leads to a stimulation of cell migration, suggesting a regulatory function in CB2-mediated migration (Marrs et al. 2010). In addition to the previously described eCB signalling, ABHD6 is also involved in other physiological processes in different peripheral tissues, which involve signalling processes mediated by its major substrate MAG (Poursharifi et al. 2017). Since the hydrolysis of 2-AG results in the release of arachidonic acid, a precursor of prostaglandins, ABHD6 may be associated with inflammatory processes and autoimmune diseases. Indeed, the blockage of ABHD6 in macrophages leads to anti-inflammatory effects, such as increased synthesis of the anti-inflammatory agent prostaglandin-D2-glycerol ester PGD2-GE (Alhouayek et al. 2013). Studies in ABHD6-K-deficient mice, where inflammatory conditions were induced by high-fated diet, revealed an increase of anti-inflammatory properties compared to wild type mice, shown by increase of FGF and FGF21 circulation and reduced levels of inflammatory indicators, such as ICAM-1, resistin, and RAGE (Zhao et al. 2016). ABHD6 is also believed to be involved in the regulation of glucose-stimulated insulin secretion (GSIS), which is essential for glucose homeostasis in different tissues. 1-MAG is able to bind directly to Munc13-1, which interacts with SNARE proteins and thereby facilitates the priming of secretory vesicles, leading to the exocytosis in different secretory tissues. One example is the Munc13-1-mediated priming of insulin granule vesicles, e.g., in pancreatic  $\beta$ -cells. Thus, 1-MAG, which is synthesized during glucose metabolism, acts as a coupling factor between glucose metabolism and insulin secretion. This process can be negatively controlled by ABHD6-catalyzed hydrolysis of 1-MAG, and a evidenced has been given by mouse studies applying  $\beta$ -cell specific ABHD6 knockdown (Zhao et al. 2014). In

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addition, 1-MAG has been shown to activate ~~peroxisomal proliferator activated receptors (PPARs)~~  $\alpha$  and  $\gamma$ , which are implicated in adipocyte metabolism and adipose browning. ABHD6-deficient mice show elevated 1-MAG levels, leading to enhanced PPAR  $\alpha/\gamma$  activity, which suggests that ABHD6 is a key player in the regulation of energy homeostasis, brown adipose tissue function, and white adipose tissue browning, and could be a potential target for associated malfunctions (Zhao et al. 2016). For further more detailed information about MAG-1 signalling and related ABHD6 functions in peripheral tissues, the reader is referred to ~~thea recently published extensive review by Poursharifi and colleagues (Poursharifi et al. 2017)~~. Different inhibitors have been developed for ABHD6; especially triazole ureas have been proven to be potent in both *in vitro* and *in vivo* (Hsu et al. 2010; Hsu et al. 2013; Hui Deng 2017). A systemic, peripherally active, and bioavailable compound, belonging to the (2-substituted)-piperidyl-1,2,3-triazole urea inhibitors, was reported (Hsu et al. 2013). No mutations in ABHD6 have been linked to human diseases (Lord et al. 2013). ABHD6 expression is possibly linked to the pathogenesis of Epstein-Barr virus (EBV)-related diseases, including Hodgkin's lymphoma, post-transplant lymphoma, and endemic Burkitt's lymphoma (Li et al. 2009; Maier et al. 2006; Lord et al. 2013). High expression levels have been found in European patients with the autoimmune disease systemic lupus erythematosus (SLE) (Oparina et al. 2015). In addition, elevated ABHD6 expression was observed in different tumor cell types, including PC-3 (prostate), U2OS (bone), and Jurkat (leukocyte) cell lines (Li et al. 2009). Exceptionally high ABHD6 expression levels were also found in Ewing family tumors (EFT); however, tumor cell growth of EFT cells was not inhibited by ABHD6 knock down (Max et al. 2009). High expression of ABHD6 was observed in both human and murine pancreatic ductal adenocarcinoma (PDAC) tumors and cell lines, and ABHD6 inhibition reduced tumor metastasis *(in vivo)* and tumor cell proliferation *(in vitro)* (Gruner et al. 2016).

ABHD12 is a 45-kDa, 398-residue transmembrane protein expressed at high levels in murine brain, including microglial cells, although it has also been found in other cell types outside the CNS (Savinainen et al. 2012). By the application of a fluorescent assay ~~ien~~ transfected COS-7 cells, hydrolase activity towards 2-AG was observed, whereby ABHD12 accounts for ~ 9% of the total hydrolase activity (Blankman et al. 2007). The catalytic triad, predicted as Ser246, Asp333, His372, was confirmed by site-directed mutagenesis and a biochemical assay coupled with ABPP (Navia-Paldanius et al. 2012). Mutations in ABHD12 are associated with the rare autosomal-recessive "polyneuropathy, hearing loss, ataxia, retinitis pigmentosa and cataract" disease (PHARC) (Fiskerstrand et al. 2010). The symptoms, typically recognized first in the late teens, include cerebellar atrophy, clouding of the eye lens (cataract), epithelial pigment deposits, pallor of the optic disc, sensorineural hearing loss, and peripheral neuropathy (Fiskerstrand et al. 2010). PHARC is a slow, progressive neurodegenerative disease, which is poorly understood. With the principal ability of hydrolyzing 2-AG, which regulates synaptic plasticity and neuroinflammation, mutated ABHD12 might cause PHARC due to impaired 2-AG hydrolytic activity ~~sis~~ (Fiskerstrand et al. 2010). Clearly, the connection of ABHD12 mutations to alterations in the eCB system during PHARC needs to be further studied. On the other hand, ABHD12 has been identified as lysophospholipase acting on lysophosphatidylserine (LPS), which is accumulated in ABHD12-deficient mice, showing auditory- and motor-defects similar to the PHARC ~~typical~~ phenotype (Blankman et al. 2013). Another condition, Usher syndrome 3, which exhibits similar characteristics to PHARC, is ~~associated-linked~~ to a nonsense mutation in ABHD12 (Eisenberger et al. 2012). Besides mutation-induced diseases, the ABHD12 gene expression profile has been associated to colorectal cancer development, and ABHD12 has been suggested as a biomarker for liver

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enzymes in plasma (Chambers et al. 2011; Yoshida et al. 2010; Lord et al. 2013). Reversible, selective triterpene-based inhibitors for ABHD12 have been discovered. However, structural details of ~~the~~ enzyme-inhibitor interactions are missing (Parkkari et al. 2014), as high-resolution structural data on ABHD12 are not available to date.

#### ABHD6 and ABHD12 as regulators of (lyso-)phospholipid metabolism

ABHD6 and ABHD12 not only play a role in eCB metabolism and signalling, but also have been implicated in (lyso-)phospholipid metabolism (Blankman et al. 2013; Thomas et al. 2013). Lysophospholipids (LPs) are small bioactive molecules, mainly acting as extracellular mediators ~~throughby~~ GPCR activation, while some have additional intracellular functions. LPs are structurally characterized by a single hydrocarbon chain and a polar headgroup, which leads, compared to the ~~if~~ corresponding phospholipids, to enhanced polarity. LPs can be divided into two classes: lysosphingolipids, with a sphingoid base backbone, and lysoglycerophospholipids, possessing a glycerol backbone (Meyer zu Heringdorf and Jakobs 2007). Examples ~~includeeinclude~~ sphingosine-1-phosphate (S1P) and lysophosphatidic acid (LPA), as well as the less characterized lipids lysophosphatidylcholine (LPC), LPS, and sphingosylphosphorylcholine (SPC) (Fig. 45).

LPs regulate various cellular functions, including cell growth and survival, chemotaxis, migration, cytoskeletal architecture, cell adhesion, and  $\text{Ca}^{2+}$ -homeostasis, and ~~they have~~ been associated with processes like angiogenesis, cancer growth and metastasis, lymphocyte trafficking, inflammation, and neurodevelopment (Meyer zu Heringdorf and Jakobs 2007). Signalling through LPs occurs *via* binding to and activation of GPCRs, of which the receptors of the endothelial differentiation gene (EDG) family are characterized best. GPCR activation leads to the inhibition of adenylyl cyclase, stimulation of PLC, and mobilization of intracellular  $\text{Ca}^{2+}$  (Takuwa et al. 2002; Meyer zu Heringdorf and Jakobs 2007). Additional effects of GPCR activation by LPs are the stimulation or inhibition of MAPKs and regulation of cell proliferation, migration, differentiation, and apoptosis (Belcheva and Coscia 2002; Meyer zu Heringdorf and Jakobs 2007). LP binding to a GPCR controls Akt activity, and the GPCR-mediated activation of Rho/Rac leads to alterations in the cytoskeleton, which plays a crucial role in chemotaxis (New et al. 2007; Meyer zu Heringdorf and Jakobs 2007; Spiegel et al. 2002). LPs can also act on intracellular target sites involved in gene regulation, *e.g.* the nuclear transcription factor PPAR $\gamma$ , which binds LPA (McIntyre et al. 2003).

ABHD6 and ABHD12, as well as other members of the ABHD family, play roles in (lyso-)phospholipid metabolism, lipid signal transduction, and related metabolic diseases. *In vivo* mouse experiments revealed an upregulation of ABHD6 expression in the liver and small intestine, induced by high-fat diet (HFD) feeding (Thomas et al. 2013). Transcriptional changes in metabolic tissue suggested an association of ABHD6 with HFD-induced metabolic disease. Knockdown experiments using antisense oligonucleotide (ASO) targeting showed a protective ~~veon~~ effect ~~againstfrom~~ HFD-induced body weight gain, hepatic steatosis, and associated insulin resistance. Since these symptoms are indicative for the metabolic syndrome, the authors concluded that ABHD6 might be a mediator in the etiology of this widespread metabolic disorder. Additional symptoms of the metabolic syndrome are high blood pressure, high blood sugar, elevated serum triacylglycerol (TAG), and decreased high-density lipoprotein (HDL), which were partially weakened in ASO-treated HFD mice. ABHD6 knockdown in HFD mice mitigated the accumulation of hepatic TAG, protected mice against

hyperglycemia, hyperinsulinemia, and, hypercholesterolemia, and improved the tolerance for glucose and insulin (Thomas et al. 2013). Improved glucose tolerance has also been shown in diabetic mice, where-in which glucose-stimulated insulin secretion could be restored upon ABHD6 inhibition (Zhao et al. 2014). This phenomenon could be explained by reduced hydrolysis of MAG, which was found to activate the vesicle priming protein Munc13-1 and induce insulin secretion (Zhao et al. 2014). Another effect of ABHD6 knockdown in HFD mice is the downregulation of gene expression related to *de novo* fatty acid synthesis and lipogenesis (Thomas et al. 2013). Finally, ABHD6 has been shown to hydrolyze LPs, suggesting a function in LP metabolism. A combination of *in vivo* knockdown studies and *in vitro* activity assays with recombinant murine ABHD6 allowed the identification of the main substrates of the enzyme: LPA, lysophosphatidylglycerol (LPG) and lysophosphatidylethanolamine (LPE), whereby the highest activity was observed towards LPG. By knocking down ABHD6, the hydrolysis of bulk LPG is reduced and reesterification leads to enhanced amounts of phosphatidylglycerol (PG), a precursor of complex phospholipids, which might affect mitochondrial and lysosomal metabolic processes (Thomas et al. 2013). ABHD6 also specifically hydrolyzes bis(monoacylglycerol)phosphate (BMP), and *in vivo* experiments revealed that ABHD6 knockdown elevated hepatic BMP levels (Pribasniig et al. 2015). These findings, and the colocalization of ABHD6 with late endosomes/lysosomes, suggest that ABHD6 plays a role in BMP catabolism and may be part of the late endosomal/lysosomal lipid sorting machinery (Pribasniig et al. 2015).

ABHD12 has been implicated in (L)PS pathways, and deregulation might cause PHARC (Blankman et al. 2013). This slowly progressive, autosomal recessive disorder, caused by a missense- or null-mutation in the ABHD12 gene, is associated with demyelination of sensorimotor neurons, cerebellar atrophy, retinal dystrophy, and polymodal sensory and motor defects (Fiskerstrand et al. 2010; Nishiguchi et al. 2014). Brain fractions of ABHD12-deficient mice displayed an altered LPS and PS lipid profile, with a strong increase of very long chain LPS (~~VLS~~ LPS), known to activate the Toll-like receptor 2, involved in immune response (Oliveira-Nascimento et al. 2012; Blankman et al. 2013). Enhanced levels of pro-inflammatory LPS lipids were detected in the early life span of ABHD12<sup>-/-</sup> mice, followed by an age-dependent activation of cerebellar microglia, which play a regulatory role in CNS immunity (Blankman et al. 2013; Yang et al. 2010). *In vitro* activity assays identified ABHD12 as a major contributor to lipase activity of murine brain LPS lipids. An additional effect of ABHD12 deletion in mice was the age-dependent development of a PHARC-related phenotype, including auditory and motor defects. Other PHARC symptoms, such as cerebellar atrophy and changes in ocular structure and function, did not occur in ABHD12<sup>-/-</sup> mice. These findings suggest that ABHD12 plays a crucial role in LPS metabolism, in which deregulation due to ABHD12 knockdown leads to the age-dependent development of PHARC-like abnormalities. The observed activation of microglia suggests that neuroinflammatory changes may provide the basis for the development of PHARC-related phenotypes (Blankman et al. 2013). Since ABHD12 is a major brain LPS lipase and regulator of LPS metabolism, it might also contribute to other (neuro-)immunological disorders. A dynamic axis built by ABHD12 and ABHD16A regulates brain LPS brain-levels and lipopolysaccharide-induced cytokine production, which suggests the enzymes as a potential targets for the treatment of (neuro-)immunological diseases (Kamat et al. 2015).

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### 3. Bioinformatics analyses of ABHD6 and ABHD12

#### Bioinformatics studies on ABHD6

Bioinformatics give an insight into structural features, key residues, and possible substrates of ABHD6 (UniProt ID: Q9BV23). Similarity studies were conducted in order to obtain information about common folding and catalytic regions within the enzyme. A homology search by BLASTp (Altschul et al. 1990) against the Protein data bank (PDB) found exclusively proteins of the  $\alpha/\beta$ -hydrolase family (Table 1). A multiple sequence alignment of ABHD6 and the homologues provides information about conserved regions and putative catalytic residues in ABHD6 (Fig. S1).

Table 1 BLASTp search results for ABHD6 ordered by total score.

Name	Organism	UniProt ID	PDB ID	Identity	Substrate	RMSD (Å)	Reference
Lip1	<i>Acinetobacter baumannii</i> (AYE)	B0V9K7	4OPM	28%		1.7	
Pcryo	<i>Psychrobacter cryohalolentis</i> (K5)	Q1QEU6	4NS4	24%		2.15	
BphD	<i>Paraburkholderia xenovorans</i> (LB400)	P47229	2OG1	26%	HOPDA	1.6	(Horsman et al. 2006)
HsaD	<i>Mycobacterium tuberculosis</i> (CDC 1551/Oshkosh)	P9WNH4	5JZ9	26%	4,9-DSHA	2.68	(Ryan et al. 2017)
CumD	<i>Pseudomonas fluorescens</i>	P96965	1IUN	24%	6-isopropyl- -6-methyl- -6-ethyl- HODA	2.8	(Fushinobu et al. 2002)

The characterized homologues BphD, HsaD, and CumD belong to the class of *meta*-cleavage product (MCP) hydrolases acting on metabolites of different degradative pathways involved in bacterial exploitation of aromatic compounds (Horsman et al. 2006; Ryan et al. 2017; Fushinobu et al. 2002). This process is essential for the global microbial carbon cycle and usually involves oxygenation of the aromatic ring, resulting in a catechol, which is subsequently transformed into an MCP by ring opening. The MCP is typically hydrolysed to 2-hydroxypenta-2,4-dienoic acid (HPD) and a second product, depending on the degradation pathway.

BphD is a *Paraburkholderia xenovorans* (LB400) MCP hydrolase involved in the Biphenyl (Bph) pathway, acting on 2-hydroxy-6-oxo-6-phenylhexa-2,4-dienoic acid (HOPDA), and generating HPD and benzoic acid (Horsman et al. 2006). The MCP hydrolase HsaD from *Mycobacterium tuberculosis* (CDC 1551/Oshkosh) plays an important role within cholesterol metabolism by catalyzing C-C-bond cleavage of the MCP 4,5-9,10-diseco-3-hydroxy-5,9,17-trioxoandrost-1(10),2-diene-4-oic acid (4,9-DHSA) (Van der Geize et al. 2007). The MCP hydrolase CumD, crucial for the cumene (isopropylbenzene) degradation pathway in

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*Pseudomonas fluorescens*, acts on different 2-hydroxy-6-oxohexa-2,4-dienoate (HODA) derivatives: 6-isopropyl-, 6-methyl- and 6-ethyl-HODA (Fushinobu et al. 2002).

All of the above-mentioned enzymes possess the common  $\alpha/\beta$ -hydrolase fold, including the core domain, constituted of an 8-stranded  $\beta$ -sheet with surrounding  $\alpha$ -helices, and a lid, which is formed by  $\alpha$ -helical structures. The substrate binding sites of BphD, HsaD, and CumD are located in the cleft between the core- and lid-domains of the enzymes.

The acyltransferase motif HXXXXD is present on the  $\beta$ 5-strand in ABHD6 (99-HLVCVD) (Lord et al. 2013), but not in all homologues (Fig. S1). The nucleophile motif GXSXG with the catalytic Ser (ABHD6: Ser148) is fully conserved among the aligned enzymes (ABHD6: 146-GTSMG), consistent with their proposed function as serine hydrolases. The Asp (ABHD6: Asp278) of the catalytic triad is located in the turn following the  $\beta$ 7-strand. The third residue constituting the catalytic triad is a conserved His (ABHD6: His306) followed by a conserved Gly positioned behind the last short  $\beta$ 8 strand. Other conserved residues are mainly present in the core domain and might be important for proper folding and stability. The sequence alignment shows that the lid domains vary between the enzymes and could be characteristic for substrate binding and interactions with other proteins or membranes.

A membrane domain prediction was previously conducted for ABHD6, identifying the first N-terminal residues as being incorporated into the membrane (Blankman et al. 2007). A topology prediction by the TMHMM server (Fig. 56-a) suggests that the ABHD6 transmembrane domain is located at the N-terminal end, while the following segment might be oriented to the cytosol (40% probability) or the extracellular space (60% probability). Around residue 150, the protein shows weak characteristics of a transmembrane region, which might highlight a region that peripherally interacts with the membrane.

Homology modelling was conducted in order to get an overview of the folding of ABHD6 (Fig. 56-b-d). The model shows the canonical  $\alpha/\beta$ -hydrolase fold with the core and lid domains (Fig. 56-b). The lid domain is constituted of five  $\alpha$ -helices and arranged in a way that the active site is partially covered (Fig. 56-c). The lid might be important for substrate interaction and is possibly flexible, able to undergo a conformational change upon substrate binding, or when in contact with a lipid-water interface. A detailed view of the catalytic triad shows the positioning of Ser148, Asp278, and His306 (Fig. 56-d).

## Bioinformatics studies on ABHD12

A homology search for ABHD12 (UniProt ID: Q8N2K0) within the PDB by BLASTp revealed similar enzymes, ~~which~~ providing information about putative structure and catalytic characteristics of ABHD12 (Table 2). A multiple sequence alignment for ABHD12 and its five closest homologues in the PDB revealed conserved regions and residues, which could be important for the folding and function of ABHD12 (Fig. S2).

Table 2 Blastp search results for ABHD12 ordered by total score

Name	Organism	UniProt ID	PDB ID	Identity	Substrate	RMSD (Å)	Reference
Yju3	<i>Saccharomyces cerevisiae</i>	P28321	4ZXF	29%	glycerol monoesters of long-chain fatty acids	2.5	(Aschauer et al. 2016)
Avi	<i>Agrobacterium vitis</i>	B9JYM4	3LLC	25%		1.8	
Glac	<i>Aureobacterium anophageum</i>	Q8GJP7	1HKH	24%	$\gamma$ -lactam	1.73	(Line et al. 2004)
DhaA	<i>Bradyrhizobium diazoefficiens</i>	P59337	3A2M	28%	1-haloalkane	1.84	(Prokop et al. 2010)
EstPp	<i>Pseudomonas putida</i> (Arthrobacter siderocapsulatus)	Q3HWU8	1ZOI	23%	DL-MATI	1.6	(Elmi et al. 2005)

Analogously to ABHD6, the homologues found for ABHD12 exclusively belong to the  $\alpha/\beta$ -hydrolase superfamily, whereby the individual proteins belong to different subclasses. Yju3 from *Saccharomyces cerevisiae* is a monoglyceride lipase, which hydrolyzes MAGs (Aschauer et al. 2016). It is thereby functionally related to human MAGL (Fig. 3b4b), and has the same substrate preference as suggested for ABHD6 and ABHD12 (Labar et al. 2010; Rengachari et al. 2012; Blankman et al. 2007). Glac is a (-)- $\gamma$ -lactamase found in an *aureobacterium* species and shows an enantiospecific activity for bicyclic  $\gamma$ -lactam. The enzyme cleaves the amide bond of the heterocyclic ring of (-)- $\gamma$ -lactam, resulting in a free fatty acid, and is therefore required for the exploitation of the N-acyl compound for carbon and energy metabolism (Line et al. 2004). DhaA (DbaA) from *Bradyrhizobium diazoefficiens* is part of the dehalogenase subclass and acts enantioselectively on haloalkanes, whereby halogenated aliphatic molecules are converted into their corresponding alcohols (Prokop et al. 2010). The stereoselective enzyme EstPp from *Pseudomonas putida*, catalyzing the hydrolysis of the methyl ester group within methyl DL- $\beta$ -acetylthioisobutyrate (DL-MATI), is part of the esterase subclass (Elmi et al. 2005). The BLASTp search for ABHD12 shows how diverse the family of  $\alpha/\beta$ -hydrolases is; ~~and that~~ a suggestion for a function is not trivial, as actual sequence identities are very low and concentrate on residues important for catalysis and folding.

The N-terminal part of ABHD12, putatively located in the cytosolic part of the cell, contains both a poly-serine and a poly-alanine stretch. Poly-serines are commonly found to function as flexible linker regions, which are mostly found in modular proteins and can enhance

substrate accessibility (Howard et al. 2004). For ABHD12, this characteristic may not be of relevance, since the C-terminal  $\alpha/\beta$ -hydrolase domain is predicted to be the active, substrate-binding domain. ABHD12 and DhaA possess the acyltransferase motif HXXXXD (ABHD12: 199-HVVTFD), whereby only the Asp is fully conserved amongst the six aligned enzymes (Fig. S2). The acyltransferase motif is followed by two fully conserved glycine residues located in a  $\beta$ - $\alpha$ -loop, which might be important for optimal folding of the  $\alpha/\beta$ -hydrolase core. The nucleophile motif GX SXG (ABHD12: 244-GHSLG) with the catalytic serine (ABHD12: Ser246) is present in all compared enzymes except for DhaA, where only the last glycine is conserved. DhaA acts *via* an  $S_N2$  mechanism, where an Asp acts as the nucleophile in the active site (Prokop et al. 2010). On the other hand, the Asp of the catalytic triad is not present in DhaA (ABHD12: Asp333). The His of the active site (ABHD12: His372) is fully conserved in all compared sequences, suggesting a crucial role in catalysis. Like observed for ABHD6 and [its homologues](#), the lid domains are variable in chain length and residue type.

TMHMM analysis resulted in a definite topology prediction for ABHD12. The N-terminal portion of the protein is most probably located in the cytosol, whereas the longer C-terminal part is predicted to reside on the luminal/extracellular side of the cell (Fig. [67-a](#)). The transmembrane domain is predicted to contain around 50 residues. A homology model was generated for both the full-length protein (Fig. [67-b,-c,-d](#)) and the N-terminal intracellular domain (Fig. [67-e](#)), as identified by TMHMM analysis (Fig. [67-a](#)).

The model for full-length ABHD12 shows that the extracellular domain adopts the conserved  $\alpha/\beta$ -hydrolase fold with the core and lid domains (Fig. [67-b,-c](#)). The nucleophile motif with the catalytic Ser is located in the sharp turn between the  $\beta 5$ -strand and [its following the subsequent](#)  $\alpha$ -helix ( $\alpha 3$ ). The Ser is in close proximity to the other two catalytic residues, Asp333 and His372 (Fig. [67-d](#)). The acyltransferase motif is present on the  $\beta 5$ -strand. The lid domain consists of two  $\alpha$ -helices with connecting loops and might be important for binding to the membrane and interaction with the lipophilic substrate, which is in the membrane. The long segment between the transmembrane domain and the first  $\beta$ -strand might also be involved in peripheral membrane binding.

#### Comparison between ABHD6, ABHD12, and MAGL

Since ABHD6, ABHD12, and MAGL have been proposed to hydrolyze the eCB 2-AG, reasonably high similarities between the protein sequences and structures were anticipated. However, a sequence alignment shows low identity between ABHD12 and MAGL (28%), and between ABHD6 and MAGL (23%). A multiple sequence alignment of the three proteins, excluding the membrane-integrated parts, gives insight into similar regions and domain varieties, which could result from their different cellular localization and orientation (Fig. S3).

The  $\alpha/\beta$ -hydrolase core domains of the three proteins contain several conserved residues, including the catalytic triad. The lid/cap regions, located between the  $\beta 6$ - and  $\beta 7$ -strand, show low similarities, which could indicate that the proteins interact differently with their substrates or [show different interaction with the](#) membranous structures. While ABHD6 and ABHD12 are both predicted membrane proteins and therefore in close contact to the lipid phase, the cytosolic/peripheral protein MAGL might only bind to the membrane when scavenging the membrane-bound substrate 2-AG. A structural alignment of ABHD6, ABHD12, and MAGL reveals similarities within the overall fold but identifies differences within the lid domains (Fig. [78](#)), as expected from the multiple sequence alignment (Fig. S3).

The structure alignment of the  $\alpha/\beta$ -hydrolase core domains (Fig. 78-a) shows that the 8-stranded  $\beta$ -sheet and surrounding  $\alpha$ -helices are arranged in a similar fashion, which is consistent with the residue conservation observed in the multiple sequence alignment. In contrast, the cap domains of the three enzymes have very low similarities (Fig. 78-b). Protein surface representations of MAGL (Fig. 78-c), ABHD6 (Fig. 78-d), and ABHD12 (Fig. 78-e) reveal possible differences in the 2-AG binding pocket, which mainly result from the different architecture of the lid domains, although one cannot deem the homology models reliable with respect structural details of the lid.

### Structural analysis of ABHD12 mutations associated with PHARC

Mutations in the ABHD12 gene are associated with the rare neurodegenerative disease PHARC, but the connection between genetic mutations and the observed phenotype is still not understood/unknown (Tingaud-Sequeira et al. 2017). Most of the mutations identified so far are linked to a premature stop codons, resulting in a truncated version of the protein (Tingaud-Sequeira et al. 2017). Additionally, 14-kb and 59-kb deletions in the first exon of the ABHD12 gene and missense mutations, such as p.T253R and p.H372Q, were have been identified (Fig. 89-a) (Chen et al. 2013; Fiskerstrand et al. 2010; Tingaud-Sequeira et al. 2017; Nishiguchi et al. 2014).

In order to get insight into possible the alterations at the protein level caused by the mutations in the ABHD12 gene, the homology model of ABHD12 was analyzed regarding the known nonsense and missense mutations (p.R65\*, p.D113Ffs\*15, p.H285fs\*1, p.R352\*, p.K377\*, p.H372Q, p.T253R). The different versions of the protein, resulting from nonsense or missense mutations are shown in Fig. 89-b, -c.

The missense mutation p.H372Q affects the catalytic residue His372 and thereby leads to a disruption of the active site (Fig. 89-b). The catalytic His in  $\alpha/\beta$ -hydrolases is involved in the formation of hydrogen bonds to other catalytic residues or the substrate and is important for stabilizing the catalytic serine and shaping the substrate-binding site (Fushinobu et al. 2005; Horsman et al. 2006; Dawson et al. 2011; Line et al. 2004). The p.T253R mutation leads to a variation in the  $\alpha$ 3-helix, whereby the small Thr is substituted with a large Arg (Fig. 89-b). This alteration might destabilize the  $\alpha/\beta$ -hydrolase fold and thus lead to a decreased enzyme activity. The nonsense mutations all result in a truncated variant of ABHD12. The longest of the analyzed truncated variants, induced by the p.K377\* mutation, misses the  $\alpha$ -helix following the last  $\beta$ -strand of the core  $\beta$ -sheet. The loop between these two secondary structure elements contains the catalytic histidine (His372) and is very important for the catalytic triad. This loop might become flexible if the following  $\alpha$ -helix is missing. The mutations causing even shorter variants are expected to disrupt ABHD12 folding completely.

### Concluding remarks

This review has summarized the current knowledge about the eCB system, molecular key players, and signalling events. We focused on the two 2-AG-degrading enzymes ABHD6 and ABHD12, whose role in eCB signalling is poorly understood. Besides the eCB degrading pathway, both ABHD6 and ABHD12 are important for (L)PS hydrolysis, possibly linking the enzymes to lipid metabolism diseases. Bioinformatics analyses give insight into similarities between ABHD6 and ABHD12, as well as the homologous  $\alpha/\beta$ -hydrolase MAGL. Considering their similar substrate preferences, it is intriguing, how low the sequence similarity between the three enzymes is. This low conservation must also lead to significant differences in 3D

structure. Experimental studies at the molecular level are necessary in order to understand the structure and function of ABHD6 and ABHD12 and their role in signalling pathways related to the eCB system, lipid metabolism, and neurodegenerative diseases.

## Figure legends

**Fig. 1** Structure of the eCBs 2-arachidonoyl glycerol (a) and arachidonoyl ethanolamide (b).

**Fig. 2** Schematic overview of retrograde signaling at the synapse, shown for 2-AG-induced DSE2 Components of the eCB system.

**Overview of 2-AG retrograde signaling at the synapse.** Overview of the eCB system components and their spatial arrangement. The membrane-integrated eCBs 2-AG and AEA are synthesized and degraded by various enzymes, such as including PLC, DAGL, NAT, and NAPE-PLD, and as well as MAGL, ABHD6, ABHD12, and FAAH, respectively. They can bind to and activate their respective cannabinoid receptors, CB1 and CB2, which are differently expressed in different tissues (CNS, PNS, immune system (IS)). Signalling in the CNS and PNS normally occurs via postsynaptically synthesized eCBs and presynaptic receptors.

**Fig. 3** Schematic overview of retrograde signalling at the synapse, shown for 2-AG-induced DSE, and non-retrograde signalling pathways.

**Overview of 2-AG retrograde signalling at the synapse.** Postsynaptic 2-AG synthesis by PLC and DAGL is promoted by depolarization-activated VGCCs and G-protein coupled mGluRs. 2-AG is released into the pericellular space by an unknown mechanism and travels backwards to the presynaptic cell. 2-AG binds to its respective GPCR (CB1 in this case), which inhibits VGCC-mediated  $\text{Ca}^{2+}$  influx. The absence of increasing  $\text{Ca}^{2+}$  concentration inhibits neurotransmitter release (glutamate in this case) and leads to postsynaptic short-term depression (DSE in this case). Pericellular 2-AG is presynaptically taken up and gets degraded into AA and glycerol by hydrolases, e.g. MAGL: the hydrolase MAGL. Alternatively, newly synthesized 2-AG can be degraded by the post-synaptic hydrolase ABHD6, which has been shown to physically associate with  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPA), or the pre-synaptic homologue ABHD12, once 2-AG has passed the pericellular space. 2-AG can also act in a non-retrograde manner by activating postsynaptic TRPV1 channels and CB1/2 receptors. Figure adapted from (Benarroch 2014; Ohno-Shosaku et al. 2012).

**Fig. 3-4** ABHD structure.

a) The canonical  $\alpha/\beta$ -hydrolase fold consisting of an eight-stranded  $\beta$ -sheet with surrounding  $\alpha$ -helices and the catalytic triad constituted comprised of a nucleophilic residue, an acidic residue, and a histidine. Figure adapted from (Lord et al. 2013). b) The crystal structure of MAGL as an example of an  $\alpha/\beta$ -hydrolase with the catalytic triad (Ser122, Asp239, His269), lid domain (purple, residues 151-225), and the hydrophobic helix  $\alpha_0$  responsible for membrane interaction, based on PDB entry 3HJU (Labar et al. 2010).

**Fig. 45** Structural formulae of S1P (a) and LPA (b).

**Fig. 56** Membrane topology prediction and homology model for ABHD6.

a) Probability scores for each residual position of ABHD6 to be part of a transmembrane domain or to be located in the cytosolic or extracellular/luminal space. The topology prediction was conducted using TMHMM (Sonnhammer et al. 1998). b-d) Homology models predicted using the Phyre2 server (Kelley et al. 2015) and analyzed by using PyMOL. Colouring based on the multiple sequence alignment (Fig. 9), trans-membrane domain analysis by TMHMM (Fig. 5-a), and domain information for BphD (Horsman et al. 2006). Shown are the N-terminal transmembrane domain (lemon), core domain (grey), lid domain (blue), nucleophile motif 146-GTSMG (yellow), the catalytic triad Ser148, Asp278, His306 (red), and the acyltransferase motif 99-HLVCVD (cyan). b) – full view, c) – top view, d) – catalytic triad. The homology model is based on 6 templates (PDB IDs: 4QLO, 1CR6, 4D9J, 3I28, 3OOS, 5D6O), whereby the first 45 N-terminal residues were modelled *ab initio* and thereby need to be considered with care. This region corresponds to the protein part which has been predicted to be membrane-integrated and is not present in the template structures.

**Fig. 67** Membrane topology prediction and homology model for ABHD12.

a) Probability scores for each residual position of ABHD12 to be in belong to a transmembrane domain or to be located in the cytosolic or extracellular/luminal space. The topology prediction was conducted with TMHMM. b-e) Homology models built using Phyre2 and analyzed by using PyMOL. Colouring based on the multiple sequence alignment (Fig. 10), transmembrane domain analysis by TMHMM (Fig. 6-a), and structural alignment with Yju3. Shown are the N-terminal intracellular domain (blue), transmembrane domain (orange), core domain (grey), lid domain (palecyan), nucleophile motif 244-GHSLG (yellow), the catalytic triad Ser246, Asp333, His372 (red), and the acyltransferase motif 199-HVVTDF (green). b – full view full-length ABHD12, c – top view full-length ABHD12, d – catalytic triad, e – intracellular domain, separately modelled. The Phyre2 model for ABHD12 is based on the alignment with six templates (PDB IDs: 2HU7, 1HLG, 5G59, 3AZQ, 1K8Q, 4Z8Z). Both the N-terminal intracellular domain and the membrane domain were modelled mainly by *ab initio* modelling, which is highly unreliable. Thus, a separate homology model was generated for the intracellular domain (residues 1-68), where one template (PDB ID: 3A1I) was chosen (Fig. 76-e). However, only 17% of the sequence was asere covered and the residual residues were modelled by *ab initio*.

**Fig. 78** Structure comparison of MAGL and the homology models for ABHD6 and ABHD12.

Comparison between the crystal structure of MAGL (grey, PDB ID: 3JW8) and the predicted  $\alpha/\beta$ -hydrolase fold domains of ABHD6 (pink, residues 49-337) and ABHD12 (green, residues 99-398). a) - aligned  $\alpha/\beta$ -hydrolase

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core domains, b) – aligned  $\alpha/\beta$ -hydrolase cap/lid domains, c-e) – surface representation of MAGL, ABHD6, and ABHD12, respectively, with marked-residues of the catalytic triad marked: Ser (red), Asp (orange), His (yellow). A co-crystallized 2-methyl-pentane-2,4-diol molecule (green) in MAGL indicates the tunnel where the 2-AG substrate could be accommodated. For clarity, the N-terminal parts of ABHD6 and ABHD12, predicted to be incorporated into the membrane or residing on the other side of the membrane, were excluded from the homology shown models.

**Fig. 89 Mutations observed in the ABHD12 gene.**

a) Mutations in the 13 ABHD12 exons, including deletions, missense mutations, and nonsense mutations. Underlined are mutations associated with PHARC, the residual mutations are associated with retinitis pigmentosa. Figure adopted from (Tingaud-Sequeira et al. 2017). b,-c) Missense and nonsense mutations in ABHD12 associated with PHARC. The homology model for the catalytic domain was analyzed regarding relevant PHARC mutations. b) – ABHD12 model (grey) with catalytic residues Ser246 and Asp333 (blue) and mutated residues His372 in p.H372Q (pink) and Thr253 in p.T253R (green). c) – Missense mutations leading to truncated versions of ABHD12. Shown are the overlapped structures of the Phyre2 model for p.K377\* (grey), p.R352\* (cyan, grey) and p.H285fs\*1 (purple, cyan, grey) with marked catalytic residues (red).

**Supplementary Information**

**Fig. S1 Multiple sequence alignment of ABHD6 and the five closest homologues in the PDB.**

ABHD6 and homologous  $\alpha/\beta$ -hydrolases were aligned by using Clustal Omega (Sievers et al. 2011). The multiple sequence alignment was visualized using ESPrnt (www.esprnt.ibcp.fr). Blue background highlights identical residues, blue colored-font indicates similar residues. The predicted acyltransferase motif is marked in green and the predicted nucleophile motif in yellow (Lord et al. 2013). The catalytic residues Ser148, Asp278, and His306 are highlighted in pink. Secondary structure elements depicted above the alignment were generated from the crystal structure of Lip1.

**Fig. S2 Multiple sequence alignment for ABHD12 and the five closest PDB homologues.**

ABHD12 and homologous  $\alpha/\beta$ -hydrolases were aligned and colored as is Fig. S1. The poly-serine and poly-alanine regions in the N-terminal part of ABHD12 are marked in orange and faint green, respectively. Secondary structure elements depicted above the alignment were generated from the crystal structure of Yju3.

**Fig. S3 Multiple sequence alignment of ABHD, ABHD12 and MAGL.**

The predicted intracellular domain of ABHD6 (residues 49-337) and extracellular domain of ABHD12 (residues 99-398) were aligned with the sequence of MAGL (UniProt ID: Q99685) using T-coffee (Notredame et al. 2000). The multiple sequence alignment was visualized with ESPrnt. Blue background highlights identical residues, and blue colored-font indicates similar residues. The predicted acyltransferase motif is marked in green, the nucleophile motif is depicted in yellow. Secondary structure elements above the alignment are from the crystal structure of MAGL (PDB ID: 3JW8).

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7 **Acknowledgements**

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9 This study was supported by a training grant from the ERASMUS+ programme.

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11 **Compliance with Ethical Standards**

12 Conflict of Interest: The authors declare that they have no conflict of interest.

13  
14 Ethical approval: This article does not contain any studies with human participants or animals  
15 performed by any of the authors.

16  
17 **Data availability**

18 The data generated and analyzed during the current study are available from the  
19 corresponding author upon reasonable request.  
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## References

- Alhouayek M, Masquelier J, Cani PD, Lambert DM, Muccioli GG (2013) Implication of the anti-inflammatory bioactive lipid prostaglandin D2-glycerol ester in the control of macrophage activation and inflammation by ABHD6. *Proc Natl Acad Sci U S A* 110 (43):17558-17563. doi:10.1073/pnas.1314017110
- Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. *J Mol Biol* 215 (3):403-410. doi:10.1016/S0022-2836(05)80360-2
- Ameri A (1999) The effects of cannabinoids on the brain. *Prog Neurobiol* 58 (4):315-348
- Aschauer P, Rengachari S, Lichtenegger J, Schittmayer M, Das KM, Mayer N, Breinbauer R, Birner-Gruenberger R, Gruber CC, Zimmermann R, Gruber K, Oberer M (2016) Crystal structure of the *Saccharomyces cerevisiae* monoglyceride lipase Yju3p. *Biochim Biophys Acta* 1861 (5):462-470. doi:10.1016/j.bbalip.2016.02.005
- Bab I, Ofek O, Tam J, Rehne J, Zimmer A (2008) Endocannabinoids and the regulation of bone metabolism. *Journal of neuroendocrinology* 20 Suppl 1:69-74. doi:10.1111/j.1365-2826.2008.01675.x
- Bacci A, Huguenard JR, Prince DA (2004) Long-lasting self-inhibition of neocortical interneurons mediated by endocannabinoids. *Nature* 431 (7006):312-316. doi:10.1038/nature02913
- Begg M, Pacher P, Batkai S, Osei-Hyiaman D, Offertaler L, Mo FM, Liu J, Kunos G (2005) Evidence for novel cannabinoid receptors. *Pharmacol Ther* 106 (2):133-145. doi:10.1016/j.pharmthera.2004.11.005
- Belcheva MM, Coscia CJ (2002) Diversity of G protein-coupled receptor signaling pathways to ERK/MAP kinase. *Neurosignals* 11 (1):34-44. doi:10.1159/000057320
- Belfrage P, Jergil B, Stralfors P, Tornqvist H (1977) Hormone-sensitive lipase of rat adipose tissue: identification and some properties of the enzyme protein. *FEBS Lett* 75 (1):259-264
- Benarroch EE (2014) Synaptic effects of cannabinoids: complexity, behavioral effects, and potential clinical implications. *Neurology* 83 (21):1958-1967. doi:10.1212/WNL.0000000000001013
- Berdyshev EV (2000) Cannabinoid receptors and the regulation of immune response. *Chem Phys Lipids* 108 (1-2):169-190
- Bertrand T, Auge F, Houtmann J, Rak A, Vallee F, Mikol V, Berne PF, Michot N, Cheuret D, Hoornaert C, Mathieu M (2010) Structural basis for human monoglyceride lipase inhibition. *J Mol Biol* 396 (3):663-673. doi:10.1016/j.jmb.2009.11.060
- Blankman JL, Long JZ, Trauger SA, Siuzdak G, Cravatt BF (2013) ABHD12 controls brain lysophosphatidylserine pathways that are deregulated in a murine model of the neurodegenerative disease PHARC. *Proc Natl Acad Sci U S A* 110 (4):1500-1505. doi:10.1073/pnas.1217121110
- Blankman JL, Simon GM, Cravatt BF (2007) A comprehensive profile of brain enzymes that hydrolyze the endocannabinoid 2-arachidonoylglycerol. *Chem Biol* 14 (12):1347-1356. doi:10.1016/j.chembiol.2007.11.006
- Bouaboula M, Poinot-Chazel C, Bourrie B, Canat X, Calandra B, Rinaldi-Carmona M, Le Fur G, Casellas P (1995) Activation of mitogen-activated protein kinases by stimulation of the central cannabinoid receptor CB1. *Biochem J* 312 ( Pt 2):637-641
- Bouaboula M, Poinot-Chazel C, Marchand J, Canat X, Bourrie B, Rinaldi-Carmona M, Calandra B, Le Fur G, Casellas P (1996) Signaling pathway associated with stimulation of CB2 peripheral cannabinoid receptor. Involvement of both mitogen-activated protein kinase and induction of Krox-24 expression. *Eur J Biochem* 237 (3):704-711
- Cabral GA, Griffin-Thomas L (2009) Emerging role of the cannabinoid receptor CB2 in immune regulation: therapeutic prospects for neuroinflammation. *Expert Rev Mol Med* 11:e3. doi:10.1017/S1462399409000957
- Carriba P, Ortiz O, Patkar K, Justinova Z, Stroik J, Themann A, Muller C, Woods AS, Hope BT, Ciruela F, Casado V, Canela EI, Lluís C, Goldberg SR, Moratalla R, Franco R, Ferre S (2007) Striatal adenosine A2A and cannabinoid CB1 receptors form functional heteromeric complexes that

- [mediate the motor effects of cannabinoids. Neuropsychopharmacology 32 \(11\):2249-2259. doi:10.1038/sj.npp.1301375](#)
- Castillo PE, Younts TJ, Chavez AE, Hashimoto-dani Y (2012) Endocannabinoid signaling and synaptic function. *Neuron* 76 (1):70-81. doi:10.1016/j.neuron.2012.09.020
- Chambers JC, Zhang W, Sehmi J, Li X, Wass MN, Van der Harst P, Holm H, Sanna S, Kavousi M, Baumeister SE, Coin LJ, Deng G, Gieger C, Heard-Costa NL, Hottenga JJ, Kuhnel B, Kumar V, Lagou V, Liang L, Luan J, Vidal PM, Mateo Leach I, O'Reilly PF, Peden JF, Rahmioglu N, Soininen P, Speliotes EK, Yuan X, Thorleifsson G, Alizadeh BZ, Atwood LD, Borecki IB, Brown MJ, Charoen P, Cucca F, Das D, de Geus EJ, Dixon AL, Doring A, Ehret G, Eyjolfsson GI, Farrall M, Forouhi NG, Friedrich N, Goessling W, Gudbjartsson DF, Harris TB, Hartikainen AL, Heath S, Hirschfeld GM, Hofman A, Homuth G, Hypponen E, Janssen HL, Johnson T, Kangas AJ, Kema IP, Kuhn JP, Lai S, Lathrop M, Lerch MM, Li Y, Liang TJ, Lin JP, Loos RJ, Martin NG, Moffatt MF, Montgomery GW, Munroe PB, Musunuru K, Nakamura Y, O'Donnell CJ, Olafsson I, Penninx BW, Pouta A, Prins BP, Prokopenko I, Puls R, Ruokonen A, Savolainen MJ, Schlessinger D, Schouten JN, Seedorf U, Sen-Chowdhry S, Siminovitch KA, Smit JH, Spector TD, Tan W, Teslovich TM, Tukiainen T, Uitterlinden AG, Van der Klauw MM, Vasani RS, Wallace C, Wallaschowski H, Wichmann HE, Willemsen G, Wurtz P, Xu C, Yerges-Armstrong LM, Alcohol Genome-wide Association C, Diabetes Genetics R, Meta-analyses S, Genetic Investigation of Anthropometric Traits C, Global Lipids Genetics C, Genetics of Liver Disease C, International Consortium for Blood P, Meta-analyses of G, Insulin-Related Traits C, Abecasis GR, Ahmadi KR, Boomsma DI, Caulfield M, Cookson WO, van Duijn CM, Froguel P, Matsuda K, McCarthy MI, Meisinger C, Mooser V, Pietilainen KH, Schumann G, Snieder H, Sternberg MJ, Stolk RP, Thomas HC, Thorsteinsdottir U, Uda M, Waeber G, Wareham NJ, Waterworth DM, Watkins H, Whitfield JB, Witteman JC, Wolffenbuttel BH, Fox CS, Ala-Korpela M, Stefansson K, Vollenweider P, Volzke H, Schadt EE, Scott J, Jarvelin MR, Elliott P, Kooner JS (2011) Genome-wide association study identifies loci influencing concentrations of liver enzymes in plasma. *Nat Genet* 43 (11):1131-1138. doi:10.1038/ng.970
- Chen DH, Naydenov A, Blankman JL, Mefford HC, Davis M, Sul Y, Barloon AS, Bonkowski E, Wolff J, Matsushita M, Smith C, Cravatt BF, Mackie K, Raskind WH, Stella N, Bird TD (2013) Two novel mutations in ABHD12: expansion of the mutation spectrum in PHARC and assessment of their functional effects. *Human mutation* 34 (12):1672-1678. doi:10.1002/humu.22437
- Chevalere V, Castillo PE (2003) Heterosynaptic LTD of hippocampal GABAergic synapses: a novel role of endocannabinoids in regulating excitability. *Neuron* 38 (3):461-472
- Childers SR, Deadwyler SA (1996) Role of cyclic AMP in the actions of cannabinoid receptors. *Biochem Pharmacol* 52 (6):819-827
- Chiu CQ, Puente N, Grandes P, Castillo PE (2010) Dopaminergic modulation of endocannabinoid-mediated plasticity at GABAergic synapses in the prefrontal cortex. *J Neurosci* 30 (21):7236-7248. doi:10.1523/JNEUROSCI.0736-10.2010
- Cravatt BF, Demarest K, Patricelli MP, Bracey MH, Giang DK, Martin BR, Lichtman AH (2001) Supersensitivity to anandamide and enhanced endogenous cannabinoid signaling in mice lacking fatty acid amide hydrolase. *Proc Natl Acad Sci U S A* 98 (16):9371-9376. doi:10.1073/pnas.161191698
- Cravatt BF, Giang DK, Mayfield SP, Boger DL, Lerner RA, Gilula NB (1996) Molecular characterization of an enzyme that degrades neuromodulatory fatty-acid amides. *Nature* 384 (6604):83-87. doi:10.1038/384083a0
- Croci T, Manara L, Aureggi G, Guagnini F, Rinaldi-Carmona M, Maffrand JP, Le Fur G, Mukenge S, Ferla G (1998) In vitro functional evidence of neuronal cannabinoid CB1 receptors in human ileum. *Br J Pharmacol* 125 (7):1393-1395. doi:10.1038/sj.bjp.0702190
- Dawson A, Fyfe PK, Gillet F, Hunter WN (2011) Exploiting the high-resolution crystal structure of *Staphylococcus aureus* MenH to gain insight into enzyme activity. *BMC structural biology* 11:19. doi:10.1186/1472-6807-11-19

- De Petrocellis L, Di Marzo V (2010) Non-CB1, non-CB2 receptors for endocannabinoids, plant cannabinoids, and synthetic cannabimimetics: focus on G-protein-coupled receptors and transient receptor potential channels. *J Neuroimmune Pharmacol* 5 (1):103-121. doi:10.1007/s11481-009-9177-z
- den Boon FS, Chameau P, Schaafsma-Zhao Q, van Aken W, Bari M, Oddi S, Kruse CG, Maccarrone M, Wadman WJ, Werkman TR (2012) Excitability of prefrontal cortical pyramidal neurons is modulated by activation of intracellular type-2 cannabinoid receptors. *Proc Natl Acad Sci U S A* 109 (9):3534-3539. doi:10.1073/pnas.1118167109
- Derkinderen P, Toutant M, Burgaya F, Le Bert M, Siciliano JC, de Franciscis V, Gelman M, Girault JA (1996) Regulation of a neuronal form of focal adhesion kinase by anandamide. *Science* 273 (5282):1719-1722
- Di Marzo V, Bifulco M, De Petrocellis L (2004) The endocannabinoid system and its therapeutic exploitation. *Nat Rev Drug Discov* 3 (9):771-784. doi:10.1038/nrd1495
- Dinh TP, Freund TF, Piomelli D (2002) A role for monoglyceride lipase in 2-arachidonoylglycerol inactivation. *Chem Phys Lipids* 121 (1-2):149-158
- Dinh TP, Kathuria S, Piomelli D (2004) RNA interference suggests a primary role for monoacylglycerol lipase in the degradation of the endocannabinoid 2-arachidonoylglycerol. *Mol Pharmacol* 66 (5):1260-1264. doi:10.1124/mol.104.002071
- Ehrhart J, Obregon D, Mori T, Hou H, Sun N, Bai Y, Klein T, Fernandez F, Tan J, Shytle RD (2005) Stimulation of cannabinoid receptor 2 (CB2) suppresses microglial activation. *J Neuroinflammation* 2:29. doi:10.1186/1742-2094-2-29
- Eisenberger T, Slim R, Mansour A, Nauck M, Nurnberg G, Nurnberg P, Decker C, Dafinger C, Ebermann I, Bergmann C, Bolz HJ (2012) Targeted next-generation sequencing identifies a homozygous nonsense mutation in ABHD12, the gene underlying PHARC, in a family clinically diagnosed with Usher syndrome type 3. *Orphanet J Rare Dis* 7:59. doi:10.1186/1750-1172-7-59
- Elmi F, Lee HT, Huang JY, Hsieh YC, Wang YL, Chen YJ, Shaw SY, Chen CJ (2005) Stereoselective esterase from *Pseudomonas putida* IFO12996 reveals alpha/beta hydrolase folds for D-beta-acetylthioisobutyric acid synthesis. *Journal of bacteriology* 187 (24):8470-8476. doi:10.1128/JB.187.24.8470-8476.2005
- Felder CC, Briley EM, Axelrod J, Simpson JT, Mackie K, Devane WA (1993) Anandamide, an endogenous cannabimimetic eicosanoid, binds to the cloned human cannabinoid receptor and stimulates receptor-mediated signal transduction. *Proc Natl Acad Sci U S A* 90 (16):7656-7660
- Fiskerstrand T, H'Mida-Ben Brahim D, Johansson S, M'Zahem A, Haukanes BI, Drouot N, Zimmermann J, Cole AJ, Vedeler C, Bredrup C, Assoum M, Tazir M, Klockgether T, Hamri A, Steen VM, Boman H, Bindoff LA, Koenig M, Knappskog PM (2010) Mutations in ABHD12 cause the neurodegenerative disease PHARC: An inborn error of endocannabinoid metabolism. *Am J Hum Genet* 87 (3):410-417. doi:10.1016/j.ajhg.2010.08.002
- Fonseca BM, Costa MA, Almada M, Correia-da-Silva G, Teixeira NA (2013) Endogenous cannabinoids revisited: a biochemistry perspective. *Prostaglandins Other Lipid Mediat* 102:103-130. doi:10.1016/j.prostaglandins.2013.02.002
- Fowler CJ (2013) Transport of endocannabinoids across the plasma membrane and within the cell. *FEBS J* 280 (9):1895-1904. doi:10.1111/febs.12212
- Freedland CS, Poston JS, Porrino LJ (2000) Effects of SR141716A, a central cannabinoid receptor antagonist, on food-maintained responding. *Pharmacol Biochem Behav* 67 (2):265-270
- Fushinobu S, Jun SY, Hidaka M, Nojiri H, Yamane H, Shoun H, Omori T, Wakagi T (2005) A series of crystal structures of a meta-cleavage product hydrolase from *Pseudomonas fluorescens* IP01 (CumD) complexed with various cleavage products. *Biosci Biotechnol Biochem* 69 (3):491-498. doi:10.1271/bbb.69.491
- Fushinobu S, Saku T, Hidaka M, Jun SY, Nojiri H, Yamane H, Shoun H, Omori T, Wakagi T (2002) Crystal structures of a meta-cleavage product hydrolase from *Pseudomonas fluorescens* IP01

- (CumD) complexed with cleavage products. *Protein Sci* 11 (9):2184-2195. doi:10.1110/ps.0209602
- Gabrielli M, Battista N, Riganti L, Prada I, Antonucci F, Cantone L, Matteoli M, Maccarrone M, Verderio C (2015) Active endocannabinoids are secreted on extracellular membrane vesicles. *EMBO Rep* 16 (2):213-220. doi:10.15252/embr.201439668
- Gaoni Y, Mechoulam R (1971) The isolation and structure of delta-1-tetrahydrocannabinol and other neutral cannabinoids from hashish. *J Am Chem Soc* 93 (1):217-224
- Gerdeman G, Lovinger DM (2001) CB1 cannabinoid receptor inhibits synaptic release of glutamate in rat dorsolateral striatum. *J Neurophysiol* 85 (1):468-471
- Gerdeman GL, Ronesi J, Lovinger DM (2002) Postsynaptic endocannabinoid release is critical to long-term depression in the striatum. *Nat Neurosci* 5 (5):446-451. doi:10.1038/nn832
- Ghosh AK, Ramakrishnan G, Chandramohan C, Rajasekharan R (2008) CGI-58, the causative gene for Chanarin-Dorfman syndrome, mediates acylation of lysophosphatidic acid. *J Biol Chem* 283 (36):24525-24533. doi:10.1074/jbc.M801783200
- Goparaju SK, Ueda N, Yamaguchi H, Yamamoto S (1998) Anandamide amidohydrolase reacting with 2-arachidonoylglycerol, another cannabinoid receptor ligand. *FEBS Lett* 422 (1):69-73
- Grotenhermen F, Muller-Vahl K (2012) The therapeutic potential of cannabis and cannabinoids. *Dtsch Arztebl Int* 109 (29-30):495-501. doi:10.3238/arztebl.2012.0495
- [Gruner BM, Schulze CJ, Yang D, Ogasawara D, Dix MM, Rogers ZN, Chuang CH, McFarland CD, Chiou SH, Brown JM, Cravatt BF, Bogoy M, Winslow MM \(2016\) An in vivo multiplexed small-molecule screening platform. \*Nature methods\* 13 \(10\):883-889. doi:10.1038/nmeth.3992](#)
- Gulyas AI, Cravatt BF, Bracey MH, Dinh TP, Piomelli D, Boscia F, Freund TF (2004) Segregation of two endocannabinoid-hydrolyzing enzymes into pre- and postsynaptic compartments in the rat hippocampus, cerebellum and amygdala. *Eur J Neurosci* 20 (2):441-458. doi:10.1111/j.1460-9568.2004.03428.x
- Hanus L, Abu-Lafi S, Fride E, Breuer A, Vogel Z, Shalev DE, Kustanovich I, Mechoulam R (2001) 2-arachidonoyl glyceryl ether, an endogenous agonist of the cannabinoid CB1 receptor. *Proc Natl Acad Sci U S A* 98 (7):3662-3665. doi:10.1073/pnas.061029898
- Herkenham M, Lynn AB, Little MD, Johnson MR, Melvin LS, de Costa BR, Rice KC (1990) Cannabinoid receptor localization in brain. *Proc Natl Acad Sci U S A* 87 (5):1932-1936
- [Heyman E, Gamelin FX, Aucouturier J, Di Marzo V \(2012\) The role of the endocannabinoid system in skeletal muscle and metabolic adaptations to exercise: potential implications for the treatment of obesity. \*Obesity reviews : an official journal of the International Association for the Study of Obesity\* 13 \(12\):1110-1124. doi:10.1111/j.1467-789X.2012.01026.x](#)
- Higgs HN, Glomset JA (1994) Identification of a phosphatidic acid-preferring phospholipase A1 from bovine brain and testis. *Proc Natl Acad Sci U S A* 91 (20):9574-9578
- Hill EL, Gallopin T, Ferezou I, Cauli B, Rossier J, Schweitzer P, Lambolez B (2007) Functional CB1 receptors are broadly expressed in neocortical GABAergic and glutamatergic neurons. *J Neurophysiol* 97 (4):2580-2589. doi:10.1152/jn.00603.2006
- Hoffman AF, Laaris N, Kawamura M, Masino SA, Lupica CR (2010) Control of cannabinoid CB1 receptor function on glutamate axon terminals by endogenous adenosine acting at A1 receptors. *J Neurosci* 30 (2):545-555. doi:10.1523/JNEUROSCI.4920-09.2010
- Horsman GP, Ke J, Dai S, Seah SY, Bolin JT, Eltis LD (2006) Kinetic and structural insight into the mechanism of BphD, a C-C bond hydrolase from the biphenyl degradation pathway. *Biochemistry* 45 (37):11071-11086. doi:10.1021/bi0611098
- Howard MB, Ekborg NA, Taylor LE, Hutcheson SW, Weiner RM (2004) Identification and analysis of polyserine linker domains in prokaryotic proteins with emphasis on the marine bacterium *Microbulbifer degradans*. *Protein Sci* 13 (5):1422-1425. doi:10.1110/ps.03511604
- Howlett AC, Mukhopadhyay S (2000) Cellular signal transduction by anandamide and 2-arachidonoylglycerol. *Chem Phys Lipids* 108 (1-2):53-70
- Hsu KL, Tsuboi K, Chang JW, Whitby LR, Speers AE, Pugh H, Cravatt BF (2013) Discovery and optimization of piperidyl-1,2,3-triazole ureas as potent, selective, and in vivo-active inhibitors

- of alpha/beta-hydrolase domain containing 6 (ABHD6). *J Med Chem* 56 (21):8270-8279. doi:10.1021/jm400899c
- Hsu KL, Tsuboi K, Speers AE, Brown SJ, Spicer T, Fernandez-Vega V, Ferguson J, Cravatt BF, Hodder P, Rosen H (2010) Optimization and characterization of triazole urea inhibitors for abhydrolase domain containing protein 6 (ABHD6). In: *Probe Reports from the NIH Molecular Libraries Program*. Bethesda (MD),
- Hua T, Vemuri K, Pu M, Qu L, Han GW, Wu Y, Zhao S, Shui W, Li S, Korde A, Laprairie RB, Stahl EL, Ho JH, Zvonok N, Zhou H, Kufareva I, Wu B, Zhao Q, Hanson MA, Bohn LM, Makriyannis A, Stevens RC, Liu ZJ (2016) Crystal Structure of the Human Cannabinoid Receptor CB1. *Cell* 167 (3):750-762 e714. doi:10.1016/j.cell.2016.10.004
- Huang SM, Bisogno T, Trevisani M, Al-Hayani A, De Petrocellis L, Fezza F, Tognetto M, Petros TJ, Krey JF, Chu CJ, Miller JD, Davies SN, Geppetti P, Walker JM, Di Marzo V (2002) An endogenous capsaicin-like substance with high potency at recombinant and native vanilloid VR1 receptors. *Proc Natl Acad Sci U S A* 99 (12):8400-8405. doi:10.1073/pnas.122196999
- Hudson BD, Hebert TE, Kelly ME (2010) Physical and functional interaction between CB1 cannabinoid receptors and beta2-adrenoceptors. *Br J Pharmacol* 160 (3):627-642. doi:10.1111/j.1476-5381.2010.00681.x
- Hui Deng TvdW, Richard J. B. H. N. van den Berg, Adrianus M. C. H. van den Nieuwendijk, Freek J. Janssen, Marc P. Baggelaar, Hermen S. Overkleeftb and Mario van der Stelt (2017) Chiral disubstituted piperidiny ureas: a class of dual diacylglycerol lipase- $\alpha$  and ABHD6 inhibitors. *MedChemComm* 8
- Iannotti FA, Silvestri C, Mazzarella E, Martella A, Calvigioni D, Piscitelli F, Ambrosino P, Petrosino S, Czirfa G, Biro T, Harkany T, Tagliatela M, Di Marzo V (2014) The endocannabinoid 2-AG controls skeletal muscle cell differentiation via CB1 receptor-dependent inhibition of Kv7 channels. *Proc Natl Acad Sci U S A* 111 (24):E2472-2481. doi:10.1073/pnas.1406728111
- Jourdan T, Diaoui L, Demizieux L, Gresti J, Verges B, Degrace P (2010) CB1 antagonism exerts specific molecular effects on visceral and subcutaneous fat and reverses liver steatosis in diet-induced obese mice. *Diabetes* 59 (4):926-934. doi:10.2337/db09-1482
- Kaczocha M, Glaser ST, Deutsch DG (2009) Identification of intracellular carriers for the endocannabinoid anandamide. *Proc Natl Acad Sci U S A* 106 (15):6375-6380. doi:10.1073/pnas.0901515106
- Kaczor AA, Targowska-Duda KM, Patel JZ, Laitinen T, Parkkari T, Adams Y, Nevalainen TJ, Poso A (2015) Comparative molecular field analysis and molecular dynamics studies of alpha/beta hydrolase domain containing 6 (ABHD6) inhibitors. *J Mol Model* 21 (10):250. doi:10.1007/s00894-015-2789-8
- Kamat SS, Camara K, Parsons WH, Chen DH, Dix MM, Bird TD, Howell AR, Cravatt BF (2015) Immunomodulatory lysophosphatidylserines are regulated by ABHD16A and ABHD12 interplay. *Nat Chem Biol* 11 (2):164-171. doi:10.1038/nchembio.1721
- Kathuria S, Gaetani S, Fegley D, Valino F, Duranti A, Tontini A, Mor M, Tarzia G, La Rana G, Calignano A, Giustino A, Tattoli M, Palmery M, Cuomo V, Piomelli D (2003) Modulation of anxiety through blockade of anandamide hydrolysis. *Nat Med* 9 (1):76-81. doi:10.1038/nm803
- Katona I, Sperlagh B, Sik A, Kafalvi A, Vizi ES, Mackie K, Freund TF (1999) Presynaptically located CB1 cannabinoid receptors regulate GABA release from axon terminals of specific hippocampal interneurons. *J Neurosci* 19 (11):4544-4558
- Katona I, Urban GM, Wallace M, Ledent C, Jung KM, Piomelli D, Mackie K, Freund TF (2006) Molecular composition of the endocannabinoid system at glutamatergic synapses. *J Neurosci* 26 (21):5628-5637. doi:10.1523/JNEUROSCI.0309-06.2006
- Kaur R, Ambwani SR, Singh S (2016) Endocannabinoid System: A Multi-Facet Therapeutic Target. *Curr Clin Pharmacol* 11 (2):110-117
- Kelley LA, Mezulis S, Yates CM, Wass MN, Sternberg MJ (2015) The Phyre2 web portal for protein modeling, prediction and analysis. *Nature protocols* 10 (6):845-858. doi:10.1038/nprot.2015.053

- Kreitzer AC, Regehr WG (2001) Retrograde inhibition of presynaptic calcium influx by endogenous cannabinoids at excitatory synapses onto Purkinje cells. *Neuron* 29 (3):717-727
- Labar G, Bauvois C, Borel F, Ferrer JL, Wouters J, Lambert DM (2010) Crystal structure of the human monoacylglycerol lipase, a key actor in endocannabinoid signaling. *Chembiochem* 11 (2):218-227. doi:10.1002/cbic.200900621
- Leung D, Saghatelian A, Simon GM, Cravatt BF (2006) Inactivation of N-acyl phosphatidylethanolamine phospholipase D reveals multiple mechanisms for the biosynthesis of endocannabinoids. *Biochemistry* 45 (15):4720-4726. doi:10.1021/bi060163l
- Li F, Fei X, Xu J, Ji C (2009) An unannotated alpha/beta hydrolase superfamily member, ABHD6 differentially expressed among cancer cell lines. *Mol Biol Rep* 36 (4):691-696. doi:10.1007/s11033-008-9230-7
- Line K, Isupov MN, Littlechild JA (2004) The crystal structure of a (-) gamma-lactamase from an *Aureobacterium* species reveals a tetrahedral intermediate in the active site. *J Mol Biol* 338 (3):519-532. doi:10.1016/j.jmb.2004.03.001
- Little PJ, Compton DR, Johnson MR, Melvin LS, Martin BR (1988) Pharmacology and stereoselectivity of structurally novel cannabinoids in mice. *J Pharmacol Exp Ther* 247 (3):1046-1051
- Liu J, Wang L, Harvey-White J, Osei-Hyiaman D, Razdan R, Gong Q, Chan AC, Zhou Z, Huang BX, Kim HY, Kunos G (2006) A biosynthetic pathway for anandamide. *Proc Natl Acad Sci U S A* 103 (36):13345-13350. doi:10.1073/pnas.0601832103
- Llano I, Leresche N, Marty A (1991) Calcium entry increases the sensitivity of cerebellar Purkinje cells to applied GABA and decreases inhibitory synaptic currents. *Neuron* 6 (4):565-574
- Lord CC, Thomas G, Brown JM (2013) Mammalian alpha beta hydrolase domain (ABHD) proteins: Lipid metabolizing enzymes at the interface of cell signaling and energy metabolism. *Biochim Biophys Acta* 1831 (4):792-802. doi:10.1016/j.bbalip.2013.01.002
- Lourenco J, Cannich A, Carta M, Coussen F, Mulle C, Marsicano G (2010) Synaptic activation of kainate receptors gates presynaptic CB(1) signaling at GABAergic synapses. *Nat Neurosci* 13 (2):197-204. doi:10.1038/nn.2481
- Lynn AB, Herkenham M (1994) Localization of cannabinoid receptors and nonsaturable high-density cannabinoid binding sites in peripheral tissues of the rat: implications for receptor-mediated immune modulation by cannabinoids. *J Pharmacol Exp Ther* 268 (3):1612-1623
- Mackie K (2005) Cannabinoid receptor homo- and heterodimerization. *Life Sci* 77 (14):1667-1673. doi:10.1016/j.lfs.2005.05.011
- Mackie K, Devane WA, Hille B (1993) Anandamide, an endogenous cannabinoid, inhibits calcium currents as a partial agonist in N18 neuroblastoma cells. *Mol Pharmacol* 44 (3):498-503
- Mackie K, Lai Y, Westenbroek R, Mitchell R (1995) Cannabinoids activate an inwardly rectifying potassium conductance and inhibit Q-type calcium currents in AtT20 cells transfected with rat brain cannabinoid receptor. *J Neurosci* 15 (10):6552-6561
- Maejima T, Hashimoto K, Yoshida T, Aiba A, Kano M (2001) Presynaptic inhibition caused by retrograde signal from metabotropic glutamate to cannabinoid receptors. *Neuron* 31 (3):463-475
- Maier S, Staffler G, Hartmann A, Hock J, Henning K, Grabusic K, Mailhammer R, Hoffmann R, Wilmanns M, Lang R, Mages J, Kempkes B (2006) Cellular target genes of Epstein-Barr virus nuclear antigen 2. *J Virol* 80 (19):9761-9771. doi:10.1128/JVI.00665-06
- Malfitano AM, Basu S, Maresz K, Bifulco M, Dittel BN (2014) What we know and do not know about the cannabinoid receptor 2 (CB2). *Semin Immunol* 26 (5):369-379. doi:10.1016/j.smim.2014.04.002
- Marinelli S, Pacioni S, Cannich A, Marsicano G, Bacci A (2009) Self-modulation of neocortical pyramidal neurons by endocannabinoids. *Nat Neurosci* 12 (12):1488-1490. doi:10.1038/nn.2430
- Marrs WR, Blankman JL, Horne EA, Thomazeau A, Lin YH, Coy J, Bodor AL, Muccioli GG, Hu SS, Woodruff G, Fung S, Lafourcade M, Alexander JP, Long JZ, Li W, Xu C, Moller T, Mackie K, Manzoni OJ, Cravatt BF, Stella N (2010) The serine hydrolase ABHD6 controls the



- accumulation and efficacy of 2-AG at cannabinoid receptors. *Nat Neurosci* 13 (8):951-957. doi:10.1038/nn.2601
- Mato S, Lafourcade M, Robbe D, Bakiri Y, Manzoni OJ (2008) Role of the cyclic-AMP/PKA cascade and of P/Q-type Ca<sup>++</sup> channels in endocannabinoid-mediated long-term depression in the nucleus accumbens. *Neuropharmacology* 54 (1):87-94. doi:10.1016/j.neuropharm.2007.04.014
- Max D, Hesse M, Volkmer I, Staeger MS (2009) High expression of the evolutionarily conserved alpha/beta hydrolase domain containing 6 (ABHD6) in Ewing tumors. *Cancer science* 100 (12):2383-2389. doi:10.1111/j.1349-7006.2009.01347.x
- McFarland MJ, Porter AC, Rakhshan FR, Rawat DS, Gibbs RA, Barker EL (2004) A role for caveolae/lipid rafts in the uptake and recycling of the endogenous cannabinoid anandamide. *J Biol Chem* 279 (40):41991-41997. doi:10.1074/jbc.M407250200
- McIntyre TM, Pontsler AV, Silva AR, St Hilaire A, Xu Y, Hinshaw JC, Zimmerman GA, Hama K, Aoki J, Arai H, Prestwich GD (2003) Identification of an intracellular receptor for lysophosphatidic acid (LPA): LPA is a transcellular PPARgamma agonist. *Proc Natl Acad Sci U S A* 100 (1):131-136. doi:10.1073/pnas.0135855100
- McKinney MK, Cravatt BF (2005) Structure and function of fatty acid amide hydrolase. *Annu Rev Biochem* 74:411-432. doi:10.1146/annurev.biochem.74.082803.133450
- Mechoulam R, Gaoni Y (1965) A Total Synthesis of Di-Delta-1-Tetrahydrocannabinol, the Active Constituent of Hashish. *J Am Chem Soc* 87:3273-3275
- Mechoulam R, Hanus LO, Pertwee R, Howlett AC (2014) Early phytocannabinoid chemistry to endocannabinoids and beyond. *Nat Rev Neurosci* 15 (11):757-764. doi:10.1038/nrn3811
- Mechoulam R, Parker LA (2013) The endocannabinoid system and the brain. *Annu Rev Psychol* 64:21-47. doi:10.1146/annurev-psych-113011-143739
- Meyer zu Heringdorf D, Jakobs KH (2007) Lysophospholipid receptors: signalling signaling, pharmacology and regulation by lysophospholipid metabolism. *Biochim Biophys Acta* 1768 (4):923-940. doi:10.1016/j.bbmem.2006.09.026
- Miller MR, Mannowetz N, Iavarone AT, Safavi R, Gracheva EO, Smith JF, Hill RZ, Bautista DM, Kirichok Y, Lishko PV (2016) Unconventional endocannabinoid signaling governs sperm activation via the sex hormone progesterone. *Science* 352 (6285):555-559. doi:10.1126/science.aad6887
- Montero-Moran G, Caviglia JM, McMahon D, Rothenberg A, Subramanian V, Xu Z, Lara-Gonzalez S, Storch J, Carman GM, Brasaemle DL (2010) CGI-58/ABHD5 is a coenzyme A-dependent lysophosphatidic acid acyltransferase. *J Lipid Res* 51 (4):709-719. doi:10.1194/jlr.M001917
- Murataeva N, Straiker A, Mackie K (2014) Parsing the players: 2-arachidonoylglycerol synthesis and degradation in the CNS. *Br J Pharmacol* 171 (6):1379-1391. doi:10.1111/bph.12411
- Nakane S, Oka S, Arai S, Waku K, Ishima Y, Tokumura A, Sugiura T (2002) 2-Arachidonoyl-sn-glycero-3-phosphate, an arachidonic acid-containing lysophosphatidic acid: occurrence and rapid enzymatic conversion to 2-arachidonoyl-sn-glycerol, a cannabinoid receptor ligand, in rat brain. *Arch Biochem Biophys* 402 (1):51-58. doi:10.1016/S0003-9861(02)00038-3
- Nardini M, Dijkstra BW (1999) Alpha/beta hydrolase fold enzymes: the family keeps growing. *Curr Opin Struct Biol* 9 (6):732-737
- Navarrete M, Araque A (2008) Endocannabinoids mediate neuron-astrocyte communication. *Neuron* 57 (6):883-893. doi:10.1016/j.neuron.2008.01.029
- Navia-Paldanius D, Savinainen JR, Laitinen JT (2012) Biochemical and pharmacological characterization of human alpha/beta-hydrolase domain containing 6 (ABHD6) and 12 (ABHD12). *J Lipid Res* 53 (11):2413-2424. doi:10.1194/jlr.M030411
- New DC, Wu K, Kwok AW, Wong YH (2007) G protein-coupled receptor-induced Akt activity in cellular proliferation and apoptosis. *FEBS J* 274 (23):6025-6036. doi:10.1111/j.1742-4658.2007.06116.x
- Nishiguchi KM, Avila-Fernandez A, van Huet RA, Corton M, Perez-Carro R, Martin-Garrido E, Lopez-Molina MI, Blanco-Kelly F, Hoefsloot LH, van Zelst-Stams WA, Garcia-Ruiz PJ, Del Val J, Di Gioia SA, Klevering BJ, van de Warrenburg BP, Vazquez C, Cremers FP, Garcia-Sandoval B,

- Hoyng CB, Collin RW, Rivolta C, Ayuso C (2014) Exome sequencing extends the phenotypic spectrum for ABHD12 mutations: from syndromic to nonsyndromic retinal degeneration. *Ophthalmology* 121 (8):1620-1627. doi:10.1016/j.ophtha.2014.02.008
- Nissen SE, Nicholls SJ, Wolski K, Rodes-Cabau J, Cannon CP, Deanfield JE, Despres JP, Kastelein JJ, Steinhubl SR, Kapadia S, Yasin M, Ruzyllo W, Gaudin C, Job B, Hu B, Bhatt DL, Lincoff AM, Tuzcu EM, Investigators S (2008) Effect of rimonabant on progression of atherosclerosis in patients with abdominal obesity and coronary artery disease: the STRADIVARIUS randomized controlled trial. *JAMA* 299 (13):1547-1560. doi:10.1001/jama.299.13.1547
- Notredame C, Higgins DG, Heringa J (2000) T-Coffee: A novel method for fast and accurate multiple sequence alignment. *J Mol Biol* 302 (1):205-217. doi:10.1006/jmbi.2000.4042
- O'Sullivan SE (2007) Cannabinoids go nuclear: evidence for activation of peroxisome proliferator-activated receptors. *Br J Pharmacol* 152 (5):576-582. doi:10.1038/sj.bjp.0707423
- Oddi S, Fezza F, Pasquariello N, D'Agostino A, Catanzaro G, De Simone C, Rapino C, Finazzi-Agro A, Maccarrone M (2009) Molecular identification of albumin and Hsp70 as cytosolic anandamide-binding proteins. *Chem Biol* 16 (6):624-632. doi:10.1016/j.chembiol.2009.05.004
- Ohno-Shosaku T, Tanimura A, Hashimoto-dani Y, Kano M (2012) Endocannabinoids and retrograde modulation of synaptic transmission. *Neuroscientist* 18 (2):119-132. doi:10.1177/1073858410397377
- Oliveira-Nascimento L, Massari P, Wetzler LM (2012) The Role of TLR2 in Infection and Immunity. *Front Immunol* 3:79. doi:10.3389/fimmu.2012.00079
- Oparina NY, Delgado-Vega AM, Martinez-Bueno M, Magro-Checa C, Fernandez C, Castro RO, Pons-Estel BA, D'Alfonso S, Sebastiani GD, Witte T, Lauwerys BR, Endreffy E, Kovacs L, Escudero A, Lopez-Pedraza C, Vasconcelos C, da Silva BM, Frostegard J, Truedsson L, Martin J, Raya E, Ortego-Centeno N, de Los Angeles Aguirre M, de Ramon Garrido E, Palma MJ, Alarcon-Riquelme ME, Kozyrev SV (2015) PDK locus in systemic lupus erythematosus: fine mapping and functional analysis reveals novel susceptibility gene ABHD6. *Annals of the rheumatic diseases* 74 (3):e14. doi:10.1136/annrheumdis-2013-204909
- Osei-Hyiaman D, DePetrillo M, Pacher P, Liu J, Radaeva S, Batkai S, Harvey-White J, Mackie K, Offertaler L, Wang L, Kunos G (2005) Endocannabinoid activation at hepatic CB1 receptors stimulates fatty acid synthesis and contributes to diet-induced obesity. *The Journal of clinical investigation* 115 (5):1298-1305. doi:10.1172/JCI23057
- Pagotto U, Marsicano G, Cota D, Lutz B, Pasquali R (2006) The emerging role of the endocannabinoid system in endocrine regulation and energy balance. *Endocrine reviews* 27 (1):73-100. doi:10.1210/er.2005-0009
- Parkkari T, Haavikko R, Laitinen T, Navia-Paldanius D, Ryttilahti R, Vaara M, Lehtonen M, Alakurtti S, Yli-Kauhaluoma J, Nevalainen T, Savinainen JR, Laitinen JT (2014) Discovery of triterpenoids as reversible inhibitors of alpha/beta-hydrolase domain containing 12 (ABHD12). *PLoS One* 9 (5):e98286. doi:10.1371/journal.pone.0098286
- Pertwee R, Griffin G, Fernando S, Li X, Hill A, Makriyannis A (1995) AM630, a competitive cannabinoid receptor antagonist. *Life Sci* 56 (23-24):1949-1955
- Pertwee RG, Howlett AC, Abood ME, Alexander SP, Di Marzo V, Elphick MR, Greasley PJ, Hansen HS, Kunos G, Mackie K, Mechoulam R, Ross RA (2010) International Union of Basic and Clinical Pharmacology. LXXIX. Cannabinoid receptors and their ligands: beyond CB(1) and CB(2). *Pharmacol Rev* 62 (4):588-631. doi:10.1124/pr.110.003004
- Pitler TA, Alger BE (1992) Postsynaptic spike firing reduces synaptic GABA responses in hippocampal pyramidal cells. *J Neurosci* 12 (10):4122-4132
- Placzek EA, Okamoto Y, Ueda N, Barker EL (2008) Mechanisms for recycling and biosynthesis of endogenous cannabinoids anandamide and 2-arachidonylglycerol. *J Neurochem* 107 (4):987-1000. doi:10.1111/j.1471-4159.2008.05659.x



- Porter AC, Sauer JM, Knierman MD, Becker GW, Berna MJ, Bao J, Nomikos GG, Carter P, Bymaster FP, Leese AB, Felder CC (2002) Characterization of a novel endocannabinoid, virodhamine, with antagonist activity at the CB1 receptor. *J Pharmacol Exp Ther* 301 (3):1020-1024
- [Poursharifi P, Madiraju SRM, Prentki M \(2017\) Monoacylglycerol signaling and ABHD6 in health and disease. \*Diabetes, obesity & metabolism\* 19 Suppl 1:76-89. doi:10.1111/dom.13008](#)
- Pribasni MA, Mrak I, Grabner GF, Taschler U, Knittelfelder O, Scherz B, Eichmann TO, Heier C, Grumet L, Kowaliuk J, Romauch M, Holler S, Anderl F, Wolinski H, Lass A, Breinbauer R, Marsche G, Brown JM, Zimmermann R (2015) alpha/beta Hydrolase Domain-containing 6 (ABHD6) Degrades the Late Endosomal/Lysosomal Lipid Bis(monoacylglycerol)phosphate. *J Biol Chem* 290 (50):29869-29881. doi:10.1074/jbc.M115.669168
- Prokop Z, Sato Y, Brezovsky J, Mozga T, Chaloupkova R, Koudelakova T, Jerabek P, Stepankova V, Natsume R, van Leeuwen JG, Janssen DB, Florian J, Nagata Y, Senda T, Damborsky J (2010) Enantioselectivity of haloalkane dehalogenases and its modulation by surface loop engineering. *Angewandte Chemie* 49 (35):6111-6115. doi:10.1002/anie.201001753
- Redmond WJ, Cawston EE, Grimsey NL, Stuart J, Edington AR, Glass M, Connor M (2016) Identification of N-arachidonoyl dopamine as a highly biased ligand at cannabinoid CB1 receptors. *Br J Pharmacol* 173 (1):115-127. doi:10.1111/bph.13341
- Reggio PH (2010) Endocannabinoid binding to the cannabinoid receptors: what is known and what remains unknown. *Curr Med Chem* 17 (14):1468-1486
- Rengachari S, Bezerra GA, Riegler-Berket L, Gruber CC, Sturm C, Taschler U, Boeszoermyeni A, Drevenyi I, Zimmermann R, Gruber K, Oberer M (2012) The structure of monoacylglycerol lipase from *Bacillus* sp. H257 reveals unexpected conservation of the cap architecture between bacterial and human enzymes. *Biochim Biophys Acta* 1821 (7):1012-1021. doi:10.1016/j.bbalip.2012.04.006
- Rinaldi-Carmona M, Barth F, Heaulme M, Alonso R, Shire D, Congy C, Soubrie P, Breliere JC, Le Fur G (1995) Biochemical and pharmacological characterisation of SR141716A, the first potent and selective brain cannabinoid receptor antagonist. *Life Sci* 56 (23-24):1941-1947
- Rinaldi-Carmona M, Barth F, Heaulme M, Shire D, Calandra B, Congy C, Martinez S, Maruani J, Neliat G, Caput D, et al. (1994) SR141716A, a potent and selective antagonist of the brain cannabinoid receptor. *FEBS Lett* 350 (2-3):240-244
- Rinaldi-Carmona M, Barth F, Millan J, Derocq JM, Casellas P, Congy C, Oustric D, Sarran M, Bouaboula M, Calandra B, Portier M, Shire D, Breliere JC, Le Fur GL (1998) SR 144528, the first potent and selective antagonist of the CB2 cannabinoid receptor. *J Pharmacol Exp Ther* 284 (2):644-650
- [Rios C, Gomes I, Devi LA \(2006\) mu opioid and CB1 cannabinoid receptor interactions: reciprocal inhibition of receptor signaling and neuritogenesis. \*Br J Pharmacol\* 148 \(4\):387-395. doi:10.1038/sj.bjp.0706757](#)
- [Rosenstock J, Hollander P, Chevalier S, Iranmanesh A, SERENADE Study Group \(2008\) SERENADE: the Study Evaluating Rimonabant Efficacy in Drug-naïve Diabetic Patients: effects of monotherapy with rimonabant, the first selective CB1 receptor antagonist, on glycemic control, body weight, and lipid profile in drug-naïve type 2 diabetes. \*Diabetes care\* 31 \(11\):2169-2176. doi:10.2337/dc08-0386](#)
- [Rossi F, Siniscalco D, Luongo L, De Petrocellis L, Bellini G, Petrosino S, Torella M, Santoro C, Nobili B, Perrotta S, Di Marzo V, Maione S \(2009\) The endovanilloid/endocannabinoid system in human osteoclasts: possible involvement in bone formation and resorption. \*Bone\* 44 \(3\):476-484. doi:10.1016/j.bone.2008.10.056](#)
- Ryan A, Polycarpou E, Lack NA, Evangelopoulos D, Sieg C, Halman A, Bhakta S, Eleftheriadou O, McHugh TD, Keany S, Lowe ED, Ballet R, Abuhammad A, Jacobs WR, Jr., Ciulli A, Sim E (2017) Investigation of the mycobacterial enzyme HsaD as a potential novel target for anti-tubercular agents using a fragment-based drug design approach. *Br J Pharmacol* 174 (14):2209-2224. doi:10.1111/bph.13810

- Saario SM, Salo OM, Nevalainen T, Poso A, Laitinen JT, Jarvinen T, Niemi R (2005) Characterization of the sulfhydryl-sensitive site in the enzyme responsible for hydrolysis of 2-arachidonoyl-glycerol in rat cerebellar membranes. *Chem Biol* 12 (6):649-656. doi:10.1016/j.chembiol.2005.04.013
- Savinainen JR, Saario SM, Laitinen JT (2012) The serine hydrolases MAGL, ABHD6 and ABHD12 as guardians of 2-arachidonoylglycerol ~~signalling~~ [signaling](#) through cannabinoid receptors. *Acta Physiol (Oxf)* 204 (2):267-276. doi:10.1111/j.1748-1716.2011.02280.x
- Shoemaker JL, Joseph BK, Ruckle MB, Mayeux PR, Prather PL (2005) The endocannabinoid noladin ether acts as a full agonist at human CB2 cannabinoid receptors. *J Pharmacol Exp Ther* 314 (2):868-875. doi:10.1124/jpet.105.085282
- Sievers F, Wilm A, Dineen D, Gibson TJ, Karplus K, Li W, Lopez R, McWilliam H, Remmert M, Soding J, Thompson JD, Higgins DG (2011) Fast, scalable generation of high-quality protein multiple sequence alignments using Clustal Omega. *Mol Syst Biol* 7:539. doi:10.1038/msb.2011.75
- [Simiand J, Keane M, Keane PE, Soubrie P \(1998\) SR 141716, a CB1 cannabinoid receptor antagonist, selectively reduces sweet food intake in marmoset. \*Behavioural pharmacology\* 9 \(2\):179-181](#)
- Simon GM, Cravatt BF (2006) Endocannabinoid biosynthesis proceeding through glycerophospho-N-acyl ethanolamine and a role for alpha/beta-hydrolase 4 in this pathway. *J Biol Chem* 281 (36):26465-26472. doi:10.1074/jbc.M604660200
- Sonnhammer EL, von Heijne G, Krogh A (1998) A hidden Markov model for predicting transmembrane helices in protein sequences. *Proceedings International Conference on Intelligent Systems for Molecular Biology* 6:175-182
- [Sophocleous A, Lando-Bassonga E, Van't Hof RJ, Idris AI, Ralston SH \(2011\) The type 2 cannabinoid receptor regulates bone mass and ovariectomy-induced bone loss by affecting osteoblast differentiation and bone formation. \*Endocrinology\* 152 \(6\):2141-2149. doi:10.1210/en.2010-0930](#)
- Spiegel S, English D, Milstien S (2002) Sphingosine 1-phosphate signaling: providing cells with a sense of direction. *Trends Cell Biol* 12 (5):236-242
- Stella N, Schweitzer P, Piomelli D (1997) A second endogenous cannabinoid that modulates long-term potentiation. *Nature* 388 (6644):773-778. doi:10.1038/42015
- Svizenska I, Dubovy P, Sulcova A (2008) Cannabinoid receptors 1 and 2 (CB1 and CB2), their distribution, ligands and functional involvement in nervous system structures--a short review. *Pharmacol Biochem Behav* 90 (4):501-511. doi:10.1016/j.pbb.2008.05.010
- Takuwa Y, Takuwa N, Sugimoto N (2002) The Edg family G protein-coupled receptors for lysophospholipids: their signaling properties and biological activities. *J Biochem* 131 (6):767-771
- [Tam J \(2016\) The emerging role of the endocannabinoid system in the pathogenesis and treatment of kidney diseases. \*Journal of basic and clinical physiology and pharmacology\* 27 \(3\):267-276. doi:10.1515/jbcpp-2015-0055](#)
- [Tam J, Hinden L, Drori A, Udi S, Azar S, Baraghithy S \(2018\) The therapeutic potential of targeting the peripheral endocannabinoid/CB1 receptor system. \*European journal of internal medicine\* 49:23-29. doi:10.1016/j.ejim.2018.01.009](#)
- [Tam J, Ofek O, Fridé E, Ledent C, Gabet Y, Muller R, Zimmer A, Mackie K, Mechoulam R, Shohami E, Bab I \(2006\) Involvement of neuronal cannabinoid receptor CB1 in regulation of bone mass and bone remodeling. \*Mol Pharmacol\* 70 \(3\):786-792. doi:10.1124/mol.106.026435](#)
- [Tam J, Trembovler V, Di Marzo V, Petrosino S, Leo G, Alexandrovich A, Regev E, Casap N, Shteyer A, Ledent C, Karsak M, Zimmer A, Mechoulam R, Yirmiya R, Shohami E, Bab I \(2008\) The cannabinoid CB1 receptor regulates bone formation by modulating adrenergic signaling. \*FASEB J\* 22 \(1\):285-294. doi:10.1096/fj.06-7957com](#)
- Thomas G, Betterers JL, Lord CC, Brown AL, Marshall S, Ferguson D, Sawyer J, Davis MA, Melchior JT, Blume LC, Howlett AC, Ivanova PT, Milne SB, Myers DS, Mrak I, Leber V, Heier C, Taschler U, Blankman JL, Cravatt BF, Lee RG, Crooke RM, Graham MJ, Zimmermann R, Brown HA, Brown

- JM (2013) The serine hydrolase ABHD6 Is a critical regulator of the metabolic syndrome. *Cell Rep* 5 (2):508-520. doi:10.1016/j.celrep.2013.08.047
- Tingaud-Sequeira A, Raldua D, Lavie J, Mathieu G, Bordier M, Knoll-Gellida A, Rambeau P, Couprie I, Andre M, Malm E, Moller C, Andreasson S, Rendtorff ND, Tranebjaerg L, Koenig M, Lacombe D, Goizet C, Babin PJ (2017) Functional validation of ABHD12 mutations in the neurodegenerative disease PHARC. *Neurobiology of disease* 98:36-51. doi:10.1016/j.nbd.2016.11.008
- Tsou K, Mackie K, Sanudo-Pena MC, Walker JM (1999) Cannabinoid CB1 receptors are localized primarily on cholecystokinin-containing GABAergic interneurons in the rat hippocampal formation. *Neuroscience* 93 (3):969-975
- Udi S, Hinden L, Earley B, Drori A, Reuveni N, Hadar R, Cinar R, Nemirovski A, Tam J (2017) Proximal Tubular Cannabinoid-1 Receptor Regulates Obesity-Induced CKD. *Journal of the American Society of Nephrology* : JASN 28 (12):3518-3532. doi:10.1681/ASN.2016101085
- Van der Geize R, Yam K, Heuser T, Wilbrink MH, Hara H, Anderton MC, Sim E, Dijkhuizen L, Davies JE, Mohn WW, Eltis LD (2007) A gene cluster encoding cholesterol catabolism in a soil actinomycete provides insight into Mycobacterium tuberculosis survival in macrophages. *Proc Natl Acad Sci U S A* 104 (6):1947-1952. doi:10.1073/pnas.0605728104
- van der Stelt M, Trevisani M, Vellani V, De Petrocellis L, Schiano Moriello A, Campi B, McNaughton P, Geppetti P, Di Marzo V (2005) Anandamide acts as an intracellular messenger amplifying Ca<sup>2+</sup> influx via TRPV1 channels. *EMBO J* 24 (17):3026-3037. doi:10.1038/sj.emboj.7600784
- Van Gaal LF, Rissanen AM, Scheen AJ, Ziegler O, Rossner S, RIO-Europe Study Group-R-ES (2005) Effects of the cannabinoid-1 receptor blocker rimonabant on weight reduction and cardiovascular risk factors in overweight patients: 1-year experience from the RIO-Europe study. *Lancet* 365 (9468):1389-1397. doi:10.1016/S0140-6736(05)66374-X
- van Tienhoven M, Atkins J, Li Y, Glynn P (2002) Human neuropathy target esterase catalyzes hydrolysis of membrane lipids. *J Biol Chem* 277 (23):20942-20948. doi:10.1074/jbc.M200330200
- Varma N, Carlson GC, Ledent C, Alger BE (2001) Metabotropic glutamate receptors drive the endocannabinoid system in hippocampus. *J Neurosci* 21 (24):RC188
- Vogel Z, Barg J, Levy R, Saya D, Heldman E, Mechoulam R (1993) Anandamide, a brain endogenous compound, interacts specifically with cannabinoid receptors and inhibits adenylate cyclase. *J Neurochem* 61 (1):352-355
- Volkow ND, Baler RD, Compton WM, Weiss SR (2014) Adverse health effects of marijuana use. *N Engl J Med* 370 (23):2219-2227. doi:10.1056/NEJMr1402309
- Wagner JA, Jarai Z, Batkai S, Kunos G (2001) Hemodynamic effects of cannabinoids: coronary and cerebral vasodilation mediated by cannabinoid CB(1) receptors. *Eur J Pharmacol* 423 (2-3):203-210
- Wartmann M, Campbell D, Subramanian A, Burstein SH, Davis RJ (1995) The MAP kinase signal transduction pathway is activated by the endogenous cannabinoid anandamide. *FEBS Lett* 359 (2-3):133-136
- Wilson RI, Nicoll RA (2002) Endocannabinoid signaling in the brain. *Science* 296 (5568):678-682. doi:10.1126/science.1063545
- Yang I, Han SJ, Kaur G, Crane C, Parsa AT (2010) The role of microglia in central nervous system immunity and glioma immunology. *J Clin Neurosci* 17 (1):6-10. doi:10.1016/j.jocn.2009.05.006
- Yoshida T, Kobayashi T, Itoda M, Muto T, Miyaguchi K, Mogushi K, Shoji S, Shimokawa K, Iida S, Uetake H, Ishikawa T, Sugihara K, Mizushima H, Tanaka H (2010) Clinical omics analysis of colorectal cancer incorporating copy number aberrations and gene expression data. *Cancer Inform* 9:147-161
- Zhao S, Mugabo Y, Ballentine G, Attane C, Iglesias J, Poursharifi P, Zhang D, Nguyen TA, Erb H, Prentki R, Peyot ML, Joly E, Tobin S, Fulton S, Brown JM, Madiraju SR, Prentki M (2016) alpha/beta-

[Hydrolase Domain 6 Deletion Induces Adipose Browning and Prevents Obesity and Type 2 Diabetes. Cell Rep 14 \(12\):2872-2888. doi:10.1016/j.celrep.2016.02.076](#)

Zhao S, Mugabo Y, Iglesias J, Xie L, Delghingaro-Augusto V, Lussier R, Peyot ML, Joly E, Taib B, Davis MA, Brown JM, Abousalham A, Gaisano H, Madiraju SR, Prentki M (2014) alpha/beta-Hydrolase domain-6-accessible monoacylglycerol controls glucose-stimulated insulin secretion. Cell Metab 19 (6):993-1007. doi:10.1016/j.cmet.2014.04.003

Zuardi AW (2006) History of cannabis as a medicine: a review. Rev Bras Psiquiatr 28 (2):153-157. doi:/S1516-44462006000200015

Zygmunt PM, Petersson J, Andersson DA, Chuang H, Sorgard M, Di Marzo V, Julius D, Hogestatt ED (1999) Vanilloid receptors on sensory nerves mediate the vasodilator action of anandamide. Nature 400 (6743):452-457. doi:10.1038/22761

Figure 1

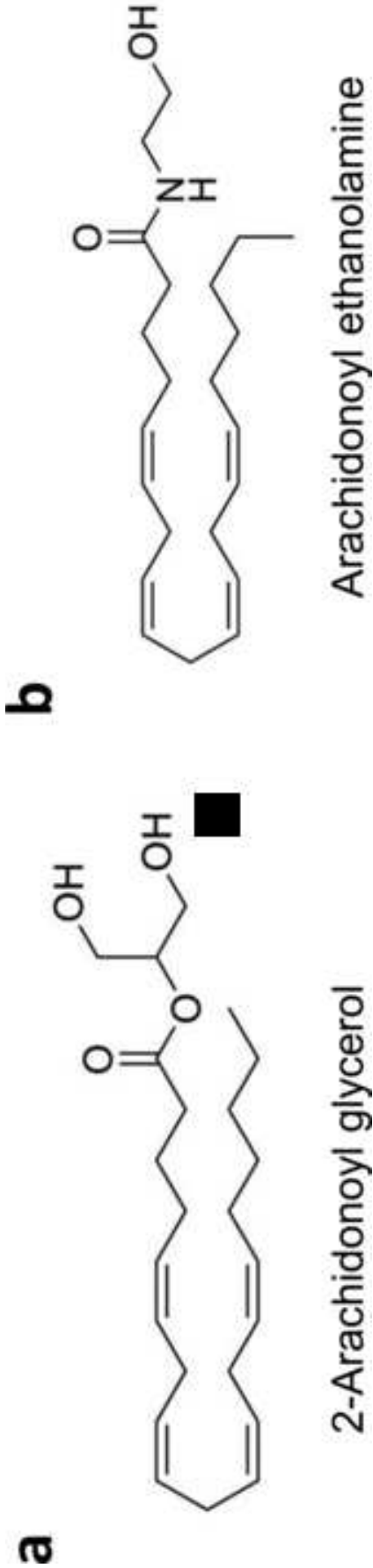


Figure 2

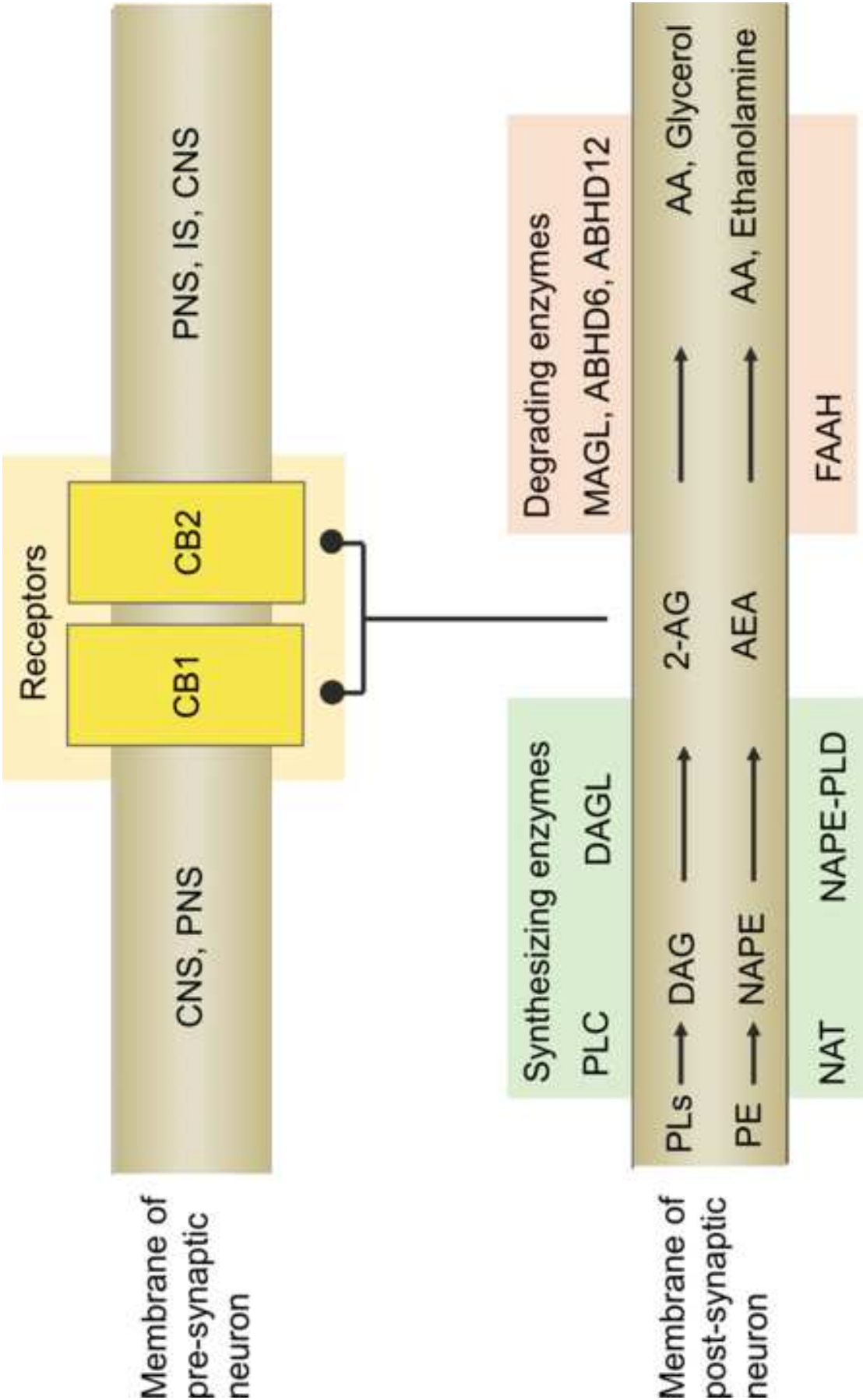


Figure 3

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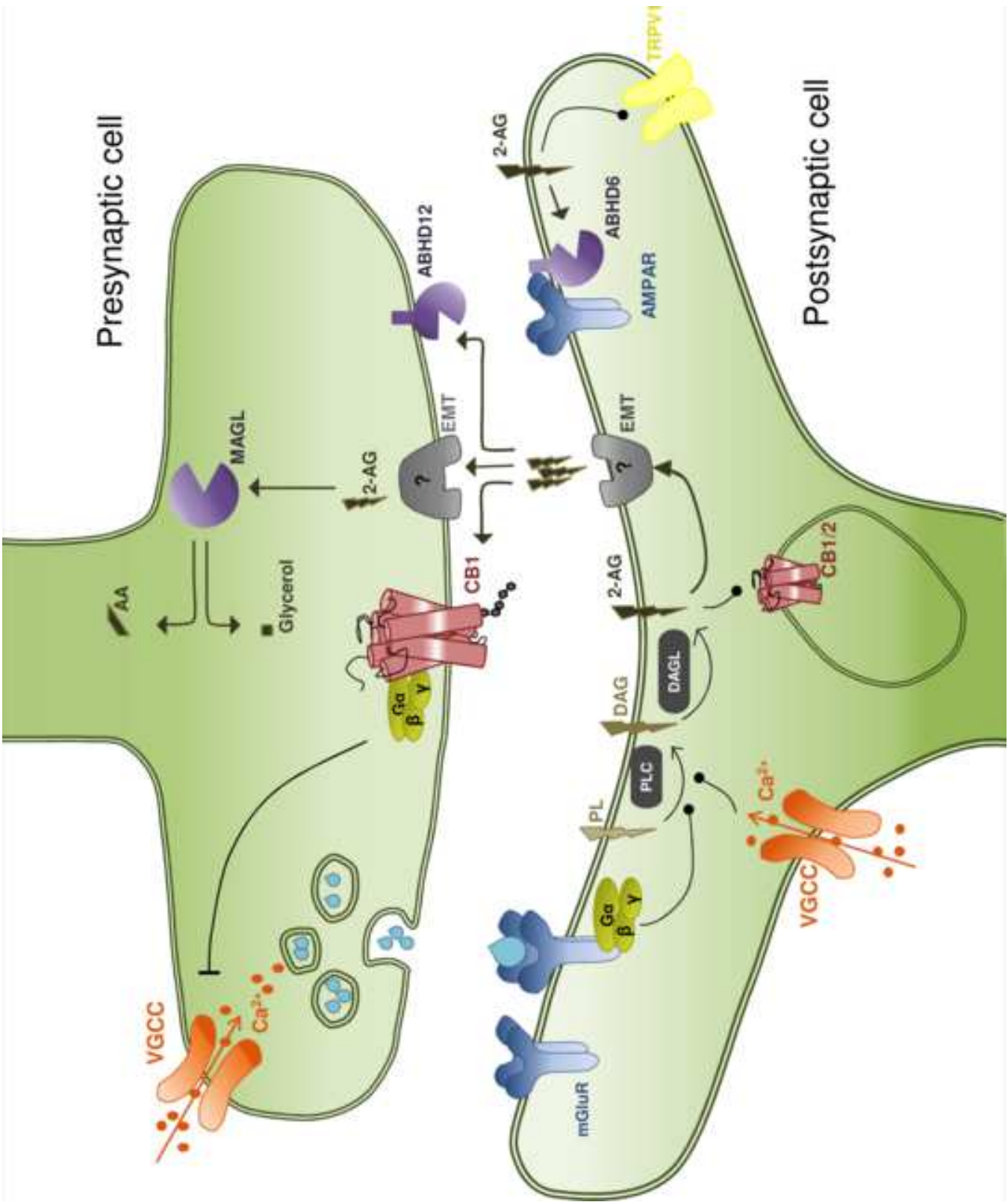








Figure 5

**a**



Sphingosine-1-phosphate

**b**



Lysophosphatidic acid

Figure 6

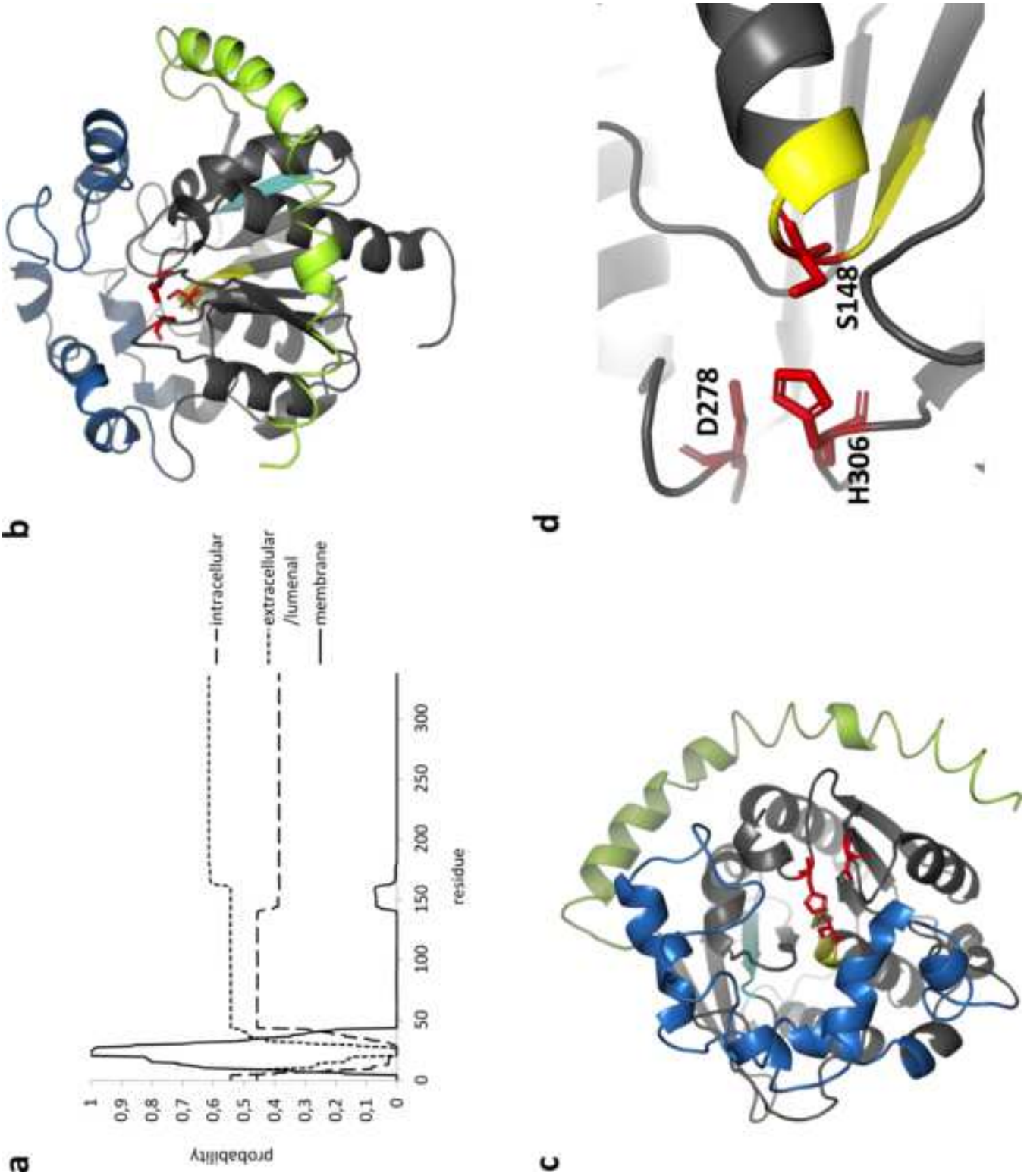


Figure 7

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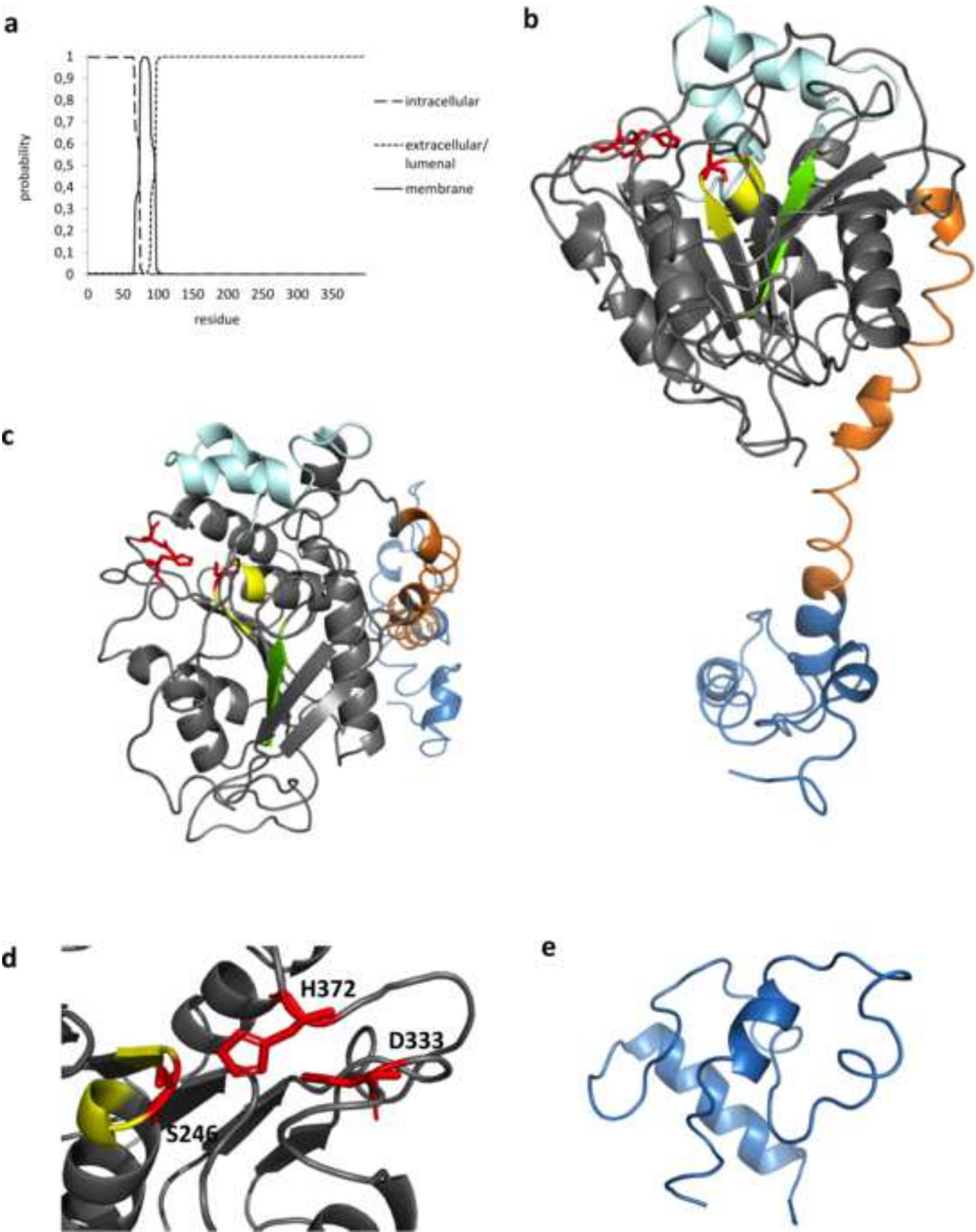


Figure 8

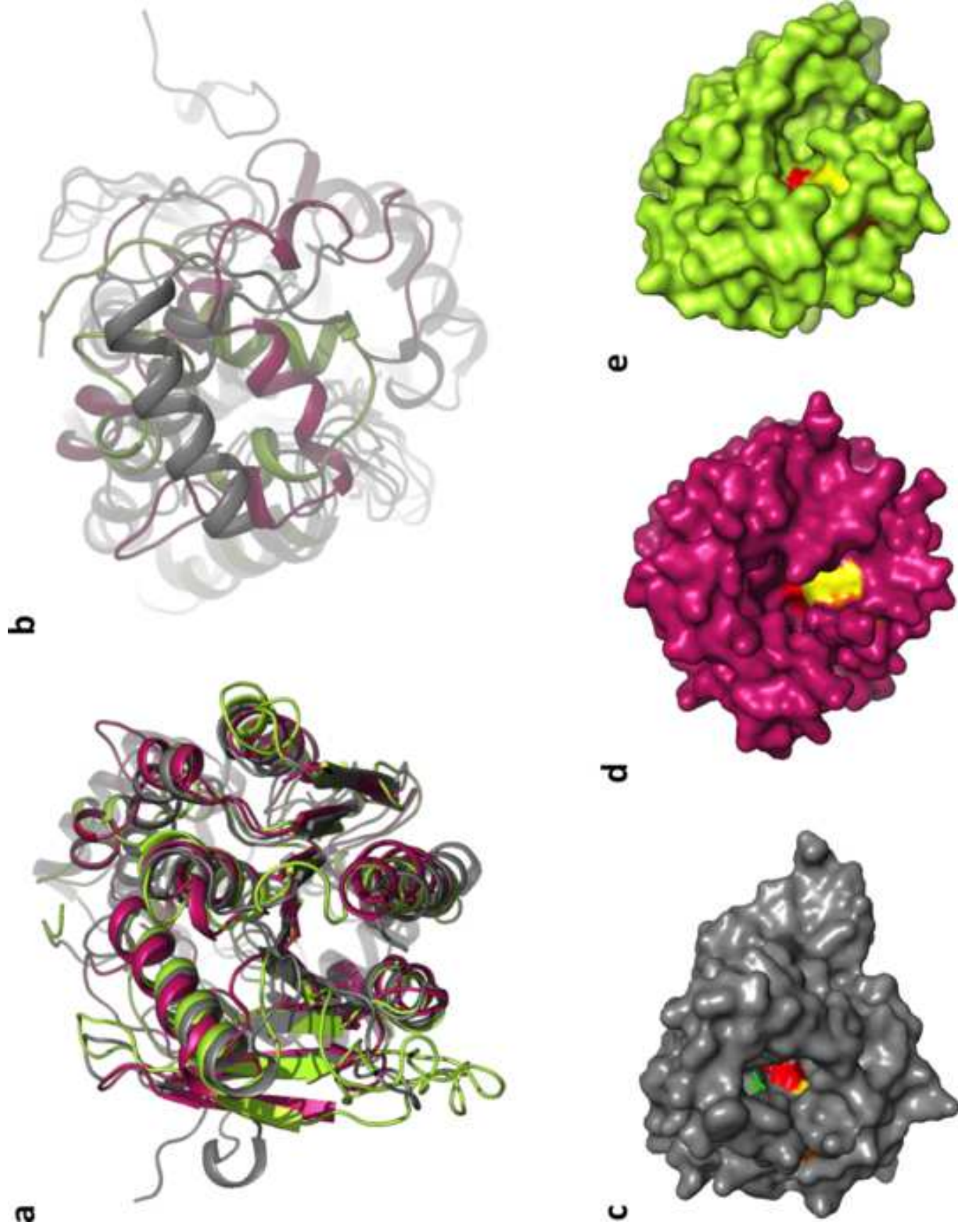




Figure 9

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