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Structure and mechanism of DHHC protein acyltransferases

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Abstract

S-acylation, whereby a fatty acid chain is covalently linked to a cysteine residue by a thioester linkage, is the most prevalent kind of lipid modification of proteins. Thousands of proteins are targets of this post-translational modification, which is catalyzed by a family of eukaryotic integral membrane enzymes known as DHHC protein acyltransferases (DHHC-PATs). Our knowledge of the repertoire of S-acylated proteins has been rapidly expanding owing to development of the chemoproteomic techniques. There has also been an increasing number of reports in the literature documenting the importance of S-acylation in human physiology and disease. Recently, the first atomic structures of two different DHHC-PATs were determined using x-ray crystallography. This review will focus on the insights gained into the molecular mechanism of DHHC-PATs from these structures and highlight representative data from the biochemical literature that they help explain.

Introduction

Protein lipidation brings together two crucial classes of macromolecules, proteins and lipids, by means of a covalent bond. Because proteins are inherently larger than lipids, the result is a relatively small perturbation to the local properties of the protein. However, lipidation can result in dramatic changes in localization and other physiological fates of the target protein. By numbers, lipidation is one of the most prevalent classes of post-translational modifications (PTM) that expand the stereochemical diversity of proteins [1]. This review will focus on S-acylation, a specific form of protein lipidation whereby a long chain fatty acid, derived from acyl-CoA, becomes attached to a cysteine by a thioester bond. What is unique about S-acylation among other forms of lipidation is that it is reversible, through the action of cellular thioesterases that can catalyze its cleavage (Figure 1A). The fundamental physicochemical effect of S-acylation on the target protein is an increase in its hydrophobicity as a consequence of the attached fatty acid chain. This change in hydrophobicity can translate into a wide variety of physiological consequences, ranging

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from altered protein assembly[2], stability [3–5], aggregation [6, 7] trafficking [8–11], and activity [12, 13]. S-acylation is also commonly known as palmitoylation, because 16-carbon palmitate is the most predominant fatty acid that gets attached to target proteins through this modification [14, 15]. However, there are two important points worth noting here: first, fatty acids other than C16 (palmitoyl) can and do get attached through S-acylation; second, and more importantly, the inherent heterogeneity of the fatty acyl modification is obscured by the current *in cellulo* methods of investigating S-acylation. In one form or another, ~ 3500 human proteins are currently known to be S-acylated, according to the SwissPalm database [16]. The targets of protein S-acylation touch almost all aspects of cellular physiology, including receptors, ion-channels, transporters, cell-adhesion proteins, enzymes, scaffold proteins and small GTPases among others [17, 18]. Not surprisingly, S-acylation plays important roles in biological processes including but not limited to, cellular signaling [8, 19], meiosis [20], massive endocytosis[21], and metabolism [22]. S-acylation has also been found to be an important component of host-pathogen interactions. Pathogenic bacteria have been shown to recruit host-cell DHHC enzymes, which bacteria use to modify effector proteins that are then injected back into the host cell for survival and proliferation [23–25]. Parasites such as *Plasmodium falciparum*, which causes malaria, and *Toxoplasma gondii*, which causes toxoplasmosis, encode 12 and 18 DHHC enzymes in their genomes respectively, some of which are essential [26–28]. S-acylation has also shown to be critically important for viruses, including Influenza, SARS and Hepatitis C viruses[29–33]. Of these Influenza hemagglutinin (HA) has been the most well-studied case where it has been shown that S-acylation is essential for viral replication and impacts viral assembly and fusion pore formation. S-acylation has also been shown to be important for plant growth and development [34, 35].

Although S-acylation/palmitoylation was first discovered in the 1970s [36], for a long time it remained controversial whether this process was spontaneous or enzyme-catalyzed. Only in the early 2000s were the enzymes that catalyze S-acylation discovered, owing to landmark efforts in the Linder, Deschenes and Davis labs[37, 38]. Due to the highly-conserved Asp-His-His-Cys tetrapeptide motif necessary for catalysis, these enzymes are known as DHHC protein acyltransferases (DHHC-PATs). Prokaryotes lack DHHC-PATs although bacterial proteins can be S-acylated in an eukaryotic host [23–25]. Yeast encode five to seven DHHC members and this number increases to 23 in humans [39, 40]. DHHC-PATs are polytopic integral membrane proteins that localize to organellar membranes such as the Golgi apparatus and endoplasmic reticulum, as well as the plasma membrane [41]. Expression of DHHC-PATs are also tissue specific with some members being expressed ubiquitously [41, 42].

In recent years, remarkable developments in chemical biology tools, especially metabolic biorthogonal labeling in combination with global profiling based on mass spectrometry, have led to a rapid expansion in the repertoire of proteins known to be modified by S-acylation, the so-called cellular “palmitoylome” [12, 27, 43–48]. These proteome wide datasets have been complemented by more targeted investigations of S-acylation of specific proteins. With the discovery of newer targets and newer pathways connected to S-acylation, the importance of this PTM in cellular physiology [17] and diseases [49–51] is becoming ever more evident. Intriguingly, protein palmitoylation has been linked to neuropsychiatric disorders and

various cancers [52]. Two DHHC-PATs have recently been proposed as therapeutic targets for development of cancer therapy [13, 53]. However, until recently our understanding of the mechanism of DHHC-PATs was severely limited by the lack of high-resolution structures of any member of this family. DHHC-PATs, being eukaryotic multipass transmembrane proteins, contributed to this challenge both in terms of biochemical purification and structure determination. Thus, knowledge of essential features such as the three-dimensional organization of DHHC-PATs, the site and mode of binding of acyl CoA and the specific mechanistic roles of highly conserved sequence motifs were completely lacking. Our lab has recently achieved a breakthrough in this direction by obtaining the first high-resolution structures of two DHHC-PATs, namely DHHC15 and DHHC20 [54]. Immediately prior to that, we had also solved the structure of the complex between the substrate-binding domain of human DHHC17 and a peptide fragment of a substrate [55]. These structures have led to novel insights into the structure and mechanism of DHHC-PATs, which will be the focus of this review. For a more expansive overview of the field of S-acylation, readers are directed to several excellent reviews [17, 18, 56–60].

Structural overview of the DHHC-PATs

DHHC-PATs derive their name from a highly conserved Asp-His-His-Cys(D-H-H-C) tetrapeptide motif [61], which is necessary for catalysis. Surrounding the DHHC motif, a stretch of ~ 50 residues rich in cysteine and histidine residues is also well conserved (Figure 1B). Together, this is known as the DHHC-Cysteine rich domain (CRD) with the CRD thought to be involved in binding zinc-ions [39, 61]. Apart from the DHHC-CRD, there is considerable sequence variation in the N- and C-terminal domains of the enzyme. This variability is reflected in vastly different protein sizes across the DHHC family, with DHHC22 at one end, with ~ 263 residues, and DHHC8 at the other, exceeding ~750 residues. Besides the DHHC-CRD, other conserved motifs have been identified, i.e., Asp-Pro-Gly (DPG), Thr-Thr-x-Glu (TTxE), and PaCCT (palmitoyltransferase conserved C-terminus) [39, 62]. The canonical topology of DHHCs consists of four transmembrane (TM) helices with the DHHC-CRD facing the cytosol represented by DHHC15 and DHHC20[54]. DHHC13 and 17 are predicted to have six TM helices from the experimentally-determined topology of Akr1[63], which is orthologous to DHHC17. However, the transmembrane topologies of other DHHC members have not been established experimentally and this remains an open and interesting question [59]. Topology prediction algorithms indicate that there may be other DHHC members that depart from the canonical four-TM architecture.

Our group recently solved the atomic structures of two different DHHC-PATs, namely human DHHC20 (hDHHC20) and a catalytically inactive mutant of zebrafish DHHC15 (zfDHHS15). We also solved the structure of an irreversibly inhibited form of hDHHC20 bound to the suicide inhibitor 2-bromopalmitate, which allowed us to define the lipid binding cavity [54]. The structures show that the transmembrane helices of DHHC-PATs form a tepee like arrangement, with the DHHC-CRD and the C-terminus projecting into the cytosol (Figures 2A, 2B). The structures also revealed that the C-terminal domain interacts extensively with both the DHHC-CRD and the transmembrane domains. Although the C-terminal domain is the most sequence divergent part of the protein within the family, the structures showed that conserved residues such as the TTxE and the PaCCT motifs make

some of the crucial contacts, thus finally elucidating their role. Interestingly, the *second* threonine of the TTxE motif directly interacts with the aspartic acid of the DHHC motif. The exact chemical role of this contact in catalysis is presently unclear. However, it is worth noting that two members with well characterized enzymatic activity, DHHC3 and DHHC7 have TGIE and TEIE respectively in place of a TTxE motif, yet retains enzymatic activity [64, 65]. The glutamate of TTxE forms a salt bridge with a conserved arginine (Arg138 in hDHHC20) in the DHHC-CRD. The highly conserved Asn (Asn266 in hDHHC20) of the PaCCT motif utilizes all of its H-bonding capabilities, thus making a unique demand for this sidechain to be an amide and is likely important for the structural integrity of the enzyme. The structures also show two Zn^{+2} ions bound to the DHHC-CRD in CCHC zinc-finger domains [54, 66]. However, unlike in some Zn^{+2} metalloenzymes where the Zn^{+2} activates a catalytic cysteine by direct coordination [67, 68], in DHHC-PATs the ions are coordinated by conserved cysteines and histidines. Thus, their role appears to be structural, namely to help position the active-site cysteine nucleophile, which is critical in catalytic chemistry. This architecture constrains the active site at the membrane-cytoplasm interface which explains the only known unifying feature of cysteines that get palmitoylated – their proximity to the membrane [16, 69]. Above the active site, the four TM helices form a cavity where the fatty acyl chain binds. It is worth noting that the three structures obtained thus far are essentially identical (Figure 2C), even though they represent two different DHHC members, two different states of human DHHC20 itself and quite different crystallization conditions (lipidic cubic phase vs. hanging drop vapor diffusion), suggesting that these structural features will be shared by others members of the DHHC family of enzymes.

Mechanism of catalysis

The DHHC enzyme active site contains the well-known catalytic triad Asp-His-Cys [70]. This triad is however arranged atypically, in that the three active site residues (Asp153, Hisl54, and Cys156) are positioned sequentially *and* linearly, in contrast to the three-dimensional arrangements observed in the serine proteases such as alpha-chymotrypsin [71] and rhinovirus 3C cysteine protease [72][61]. Nonetheless, the geometry of the triad is not substantially different, and the distances between the Cys/Ser-His and His-Asp pair are ~ 4Å and 3Å, respectively (Figure 2D). In the catalytic triad mechanism, His154 is polarized by Asp153 and acts as a base in extracting a proton from Cys156, thereby transforming this residue into a thiolate nucleophile (Figure 3A). Cys156 thiolate then attacks the carbonyl carbon in the fatty acyl-CoA thioester, resulting in an autoacylated DHHC which is an intermediate in the catalytic cycle. Subsequently, the fatty acyl chain is transferred to a protein substrate thus regenerating the DHHC enzyme for another round of catalysis. Interestingly, the Deschenes group found that yeast ERF2-ERF4 with a DAHC mutation could autoacylate but not acylate the protein substrate[73]. Based on the DHHC20 structure, we proposed that in the substrate palmitoylation step, the protonated first histidine activates the carbonyl of the thioesterified DHHC, thus triggering the cysteine in the substrate to attack the carbonyl carbon. Thus in this proposal, the first histidine plays an important role in both the autoacylation step as well as the substrate palmitoylation steps. The mutational results would suggest that in ERF2-ERF4, the first histidine plays a more important role in the substrate palmitoylation step than the autoacylation step. Another possible explanation

for this discrepancy is a distinct mechanism operating due to the association of the ERF4 subunit.

Studies of yeast DHHCs Swf1 and Pfa4 by the Taubas group, in which the active site cysteine was mutated to arginine to give DHHR, reported the enzymes remained active [74]. One plausible reason why replacement of the catalytic cysteine by an arginine could result in a catalytically active enzyme is that the mutation leads to a reaction mechanism that proceeds through a ternary complex (i.e. DHHC, fatty acyl CoA and protein substrate), rather than a ping-pong mechanism [64]. This finding might also imply that S-acylation might not necessarily require an autoacylated DHHC intermediate. Ohno et al could not detect autoacylated DHHC13, 19, and 22 using tritiated palmitate [75]. The Chamberlain group did not detect autoacylated DHHC13 and 17 either, [76] and more recently we were not able to detect autoacylated DHHC17 using alkyne fatty acid analog [55]. Therefore, the absolute requirement for an autoacylated intermediate in DHHC-mediated S-acylation remains an open question. Intriguingly, a unique DQHC motif is found in DHHC13. Mutating the glutamine to histidine reconstituted the canonical DHHC motif but did not impart any extra catalytic ability to DHHC13. Conversely, replacing DHHC with DQHC in the closely related DHHC17 enzyme abolished its activity [76]. These observations show that the DHHC and DQHC motifs are not interchangeable and that there are specific structural and chemical requirements for catalysis by a DQHC motif. Contrary to these results, in yeast Swf1, a DHHC to DQHC mutant still retained some activity – although this activity seems dependent on the reactivity of the protein substrates' cysteines [74]. However, it is important to keep in mind that activities of mutants are hard to gauge from cell-based experiments and have to be confirmed with *in vitro* enzymatic measurements using purified mutants of the abovementioned proteins before definite hypotheses could be formulated and/or tested. Since DHHC13 and DHHC17 have specific substrate-binding domains that are separate from the DHHC-CRD domain, these mutants retain the capability of binding to the substrate. It is conceivable that these mutants, which can presumably still bind fatty acyl CoA, lead to a localized increase of fatty acyl CoA and the substrate, thus resulting in substrate S-acylation.

The chemical mechanism by which the fatty acyl chain is transferred to the protein substrate from an autoacylated DHHC also remains to be fully understood. Presumably this transfer occurs by a nucleophilic attack of the cysteine in the substrate on the carbonyl group of the acylated DHHC enzyme. In the structure of the 2-BP modified DHHC20, the protonated histidine is in the right position to activate the carbonyl of the autoacylated enzyme. This observation led us to propose that the simplest mechanism for the transpalmitoylation step is one whereby the fatty acyl thioester is activated so that the various substrate cysteine nucleophiles can attack [54]. Given the fact that each DHHC enzyme works on multiple substrates, this model bypasses the requirement for a single DHHC enzyme to activate a multitude of target cysteines which likely reside in different stereochemical microenvironments. It has been shown in certain cases where cysteines that are S-acylated can have low background reactivity [77–79]. Therefore, bringing them proximal to an activated thioester may be sufficient for substrate palmitoylation. Interestingly, there are only a few cases of self-acylation known and it is instructive to compare them in this context. In the cases of TEAD, a transcription factor [80] and Bet3, a component of the transport

protein particle (TRAPP) complex involved in vesicular trafficking [81, 82], where structures of the S-acylated proteins are available, the acyl chain binds in a hydrophobic groove with the cysteine positioned in an optimal fashion so as to facilitate the self-acylation reaction (Figure 3C). Thus self S-acylation, when it occurs in a few cases, is structurally analogous to the autoacylation of DHHC enzymes where the structure of the protein generates a hydrophobic groove/cavity with a cysteine positioned at the mouth. Presumably, the pKa of the cysteine is perturbed such that upon acyl CoA binding to the hydrophobic groove/cavity, S-acylation proceeds in the forward direction.

Membrane deformation fosters catalysis

The proposed mechanism of catalysis requires the catalytic cysteine to become a thiolate nucleophile, as previously mentioned. In the crystal structure of hDHHC20, however, this cysteine appears to be within the hydrophobic TM region of the protein. In such environment, the formation of the thiolate nucleophile intermediate would be energetically costly, hindering catalysis. To try to reconcile the structure with the catalytic function of this site, we recently used atomically-detailed Molecular Dynamics (MD) simulations [83]. We found that hDHHC20 induces a local deformation of the membrane, particularly on the cytoplasmic site where the catalytic cysteine resides (Figure 4A). This deformation causes this cysteine to be continuously accessible to water; as a result of this hydrophilic environment, the thiolate nucleophile intermediate becomes viable. Interestingly, the deformation also exposes the catalytic cysteine to the inner ester layer of the lipid membrane. In complementary MD simulations of several fatty acyl CoAs known to react with hDHHC20, we found that the position of the thioester group in the membrane coincides with the ester layer, irrespective of the acyl-chain length. (Figure 4B). Thus, it appears that the membrane deformation caused by hDHHC20 positions the catalytic Cys optimally for the autoacylation reaction with a fatty acyl CoA. That the position of the thioester group is largely identical for fatty acyl CoAs of different chain lengths (Figure 4C) also suggests that the observed chain-length selectivity of hDHHC20, and probably other enzymes in this family, does not stem from this initial recognition step.

To date, hDHHC20 is the only DHHC-PATs that has been examined using MD simulations, and hence it remains unclear whether the membrane deformation caused by this enzyme and the resulting position of the catalytic site are conserved features across the family. Nonetheless, similar membrane perturbations near the catalytic site of the rhomboid protease GlpG have been reported, based on MD simulations [84, 85] and EPR spectroscopy [86]. MD simulations have also shown that oligomycin recognition by the ATP synthase also requires a local membrane depression [87]; this kind of deformation also appears likely near the active site of the isoprenylcysteine carboxyl methyltransferase [88], and near the ubiquinone binding site of respiratory complex I [89]. The common denominator of these systems is that their ligands are amphipathic, which are naturally adsorbed on the membrane surface and must therefore be recognized laterally, rather than from bulk solvent. Indeed, the ligands of many membrane-integral enzymes and transporters are also amphipathic, or hydrophobic, including transferases [88, 90–93], some translocases [94], synthases [95], polymerases [96] and proteases [97]. While little is known about how many of these proteins interact with the membrane, the emerging model from existing analyses is that that many of

these proteins have evolved highly specific ways to perturb the membrane to facilitate the recognition of substrates and ligands.

Substrate recognition and binding

DHHC-PATs have two substrates – fatty acyl CoA and the protein substrate. In our structural studies of hDHHC20 [54], we detected electron density for a putative small molecule ligand that apparently co-purified and crystallized with the enzyme. Based on the features of this density and likely moieties given the enzymatic chemistry and crystallization conditions, we refined as free coenzyme A. Two conserved residues from the DHHC-CRD, namely Lys135 and His140, interact with the phosphates on the ligand. The distance from the active site is consistent with these interactions serving to anchor the CoA headgroup of an intact fatty acyl CoA upon binding, albeit in a different orientation than what is seen in the current structure. An interesting possibility remains whether binding of a ligand at this site could regulate the function of DHHC20 [98]. Further structural and biochemical investigations will help elucidate the role of this ligand binding site in the mechanism of DHHC enzymes. Another intriguing question is the mechanism of entrance and exit of the fatty acyl chain into and from the cavity. The subcellular distribution of long chain fatty acyl CoAs are not known with certainty, but most likely a fraction is embedded in the membrane. Thus, a plausible hypothesis is that membrane embedded acyl CoA enters laterally by “breathing motions” of the transmembrane domain; a competing possibility is that the lipid is delivered from the cytosol by the acyl CoA binding protein (ACBP). There may be aspects about this mechanism that are shared by other integral membrane proteins that bind long chain fatty acyl CoA. Shedding light on the detailed mechanism of acyl chain entry and exit from the cavity will likely require computational studies together with supporting biochemical experiments.

Fatty acyl chain selectivity

Although the term “palmitoylation” is commonly used to refer to S-acylation, fatty acids other than 16-carbon palmitate can get incorporated onto substrate proteins [14, 15]. For example, a pioneering *in vitro* study by the Linder lab reported that DHHC2 and 3 enzymes had significantly different fatty acyl CoA preference [64]. While DHHC3 was strikingly stringent against fatty acid chains longer than 16-carbon, DHHC2 was much more promiscuous. This study further showed that fatty acyl CoA selectivity for the substrate was determined at the autoacylation stage by the DHHC enzyme, implying that DHHC enzymes transferred to the substrate whichever fatty acid chain they were autoacylated with [64]. The Chamberlain group followed up on these results using a cell-based S-acylation assay with azide-alkyne fatty acids and click chemistry [65], and showed that DHHC3 and DHHC7 were strikingly different in their acyl selectivity, despite being in the same phylogenetic group. In particular, DHHC3 was much more selective compared to DHHC7. Interestingly, this difference in fatty acyl selectivity between DHHC3 and DHHC7 appears to result from a single residue on TM3. DHHC3 has an isoleucine at this position and prefers shorter chain fatty acids while DHHC7 features a serine and is more promiscuous. The structural-chemical rationale for these effects was finally revealed by the structure of hDHHC20 with a fatty acid chain covalently attached to its active site cysteine [54]. The fatty acid chain is inserted in a cavity formed by the four TM helices, with TM3 contributing the most residues

in direct contact with the fatty acid chain. In hDHHC20 the homologous residue to the Ile in TM3 of DHHC3 is Tyr181 which forms an H-bond with Ser29 on TM1 (Figure 5B). Together, the Tyr-Ser H-bond pair closes off one side of the top end of the fatty acid chain binding cavity. Mutation of Ser29 that introduces a bulky side-chain and presumably reduces the size of the cavity leads to hDHHC20 preferring shorter fatty acid chains, whereas mutation of Tyr181 to a smaller residue shifts the preference to longer fatty acyl chains (Figure 5C). Therefore, as a first approximation, the cavity can be thought of as a molecular ruler that contributes to determining the fatty acid chain length selectivity of the enzyme (Figure 5A). It is worth noting, however, that comparison of the primary sequences of different members of the DHHC family shows that the chemical features of the cavity-lining residues vary in a complex manner. Thus, understanding the fatty acyl selectivity of different DHHC-PATs will require high resolution structures of representative members of the family as well as careful functional and computational analyses to understand the detailed interactions of the fatty acyl chain with the cavity across the DHHC family.

Intriguingly, in influenza HA it was found that among three S-acylation sites, two were modified by a 16-carbon palmitoyl chain while the third one was exclusively an 18-carbon stearoyl chain [99, 100]. The key determinant seems to be that the cysteine residue modified by the stearoyl chain is in the transmembrane region while the other two are in the cytoplasmic tail of HA [100]. Although the set of DHHC-PATs that target HA has been recently narrowed down to DHHC2, DHHC8, DHHC15 and DHHC20 [101], it has not yet been established which member acylates each site. It is quite likely that this differential fatty acylation stems from the fatty acyl chain length selectivity of the corresponding DHHC enzyme. In a separate study, an analysis of DHHC protein substrate specificity by expressing the individual members in yeast had determined DHHC2 and 20 among others to be more active towards integral membrane proteins [75]. DHHC2 and 20 are also capable of using longer fatty acyl chains [54, 64]. These observations point to the possibility of a global bias towards cysteines embedded in the membrane to be modified with longer fatty acyl chains. S-acylation of proteins is not only limited to palmitate and stearate but has also been shown to involve longer, as well as unsaturated acyl chains[102–104]. However, to our knowledge, specific DHHC members have not been assigned for catalyzing these reactions. In an *in vitro* assay, it has been shown that DHHC20 can use 16:1, i.e. palmitoleic acid in autoacylation reactions[105]. In order to explain how an unsaturated acyl chain be accommodated in the cavity of DHHC20, it was proposed that rotations about the carbon-carbon single bonds can achieve an approximately linear conformation of the unsaturated acyl chain, albeit with a local kink. Although our knowledge about cellular proteins that are modified by S-acylation has rapidly expanded in recent years, information about specific acyl chains at each of these cysteines is still unavailable. One reason is that common methods of examining S-acylation, such as acyl biotin exchange (ABE), do not identify the heterogeneity in fatty acyl modification at individual sites [106]. Alkyne labeling by palmitic acid analogs are also not definitive because unnatural fatty acid analogs can be metabolized by cells into longer and unsaturated chains [65].

An added layer of complexity could possibly arise from the differential distribution of fatty acyl CoAs of different lengths in the plasma membrane and various organellar membranes, and from the specific localization of different DHHC-PATs to a subset of these membranes.

It is quite likely that a detailed knowledge about the subcellular distribution of various forms of fatty acyl CoA, exact chemical identification of fatty acyl modification at individual cysteines of substrate proteins and intrinsic localization of each substrate could lead to insights about cellular regulation of fatty acylation and DHHC-PATs. In addition, it will be essential to get structures of the individual DHHC-PATs, ideally in different states of the enzymatic reaction, to elucidate these important mechanistic details at the atomic level.

Interaction with protein substrates

One of the most intriguing aspects about protein S-acylation by DHHC-PATs has been the mechanisms that control their interactions with substrate proteins. Since the number of potential substrate proteins is orders of magnitude greater than that of DHHC-PATs, each of these enzymes is highly likely to work on multiple substrates. However several studies have now shown that individual substrates can be S-acylated by multiple DHHC-PATs leading to views that S-acylation is non-specific and proximity based [107]. This is contrary to the observations that some DHHC-PATs feature protein-protein interaction domains, which argues for specific enzyme-substrate interactions. Specifically, among the human orthologs DHHC3, 7, 5, 8, 14, 16, 17, 20, and 21 are predicted to have PDZ-binding motifs [40], and DHHC6 is predicted to have an SH3 domain [108, 109]. It has also been shown that some DHHC-PATs interact with their substrates quite specifically [110–112]. The clearest examples are DHHC13 and DHHC17, each of which contains an ankyrin-repeat (AR) domain at the N-terminus. A recent important discovery by the Chamberlain group was the identification of a substrate recognition motif, [VIAP][VIT]XXQP, in substrates of DHHC13 and DHHC17, which interacts with the AR domains [113]. However, the structural underpinnings of this interaction came to fore when our lab solved the crystal structure of the complex of the AR domain of DHHC17 (ANK17) with a peptide fragment of SNAP25, a substrate of DHHC17 [55]. This structure revealed, for the first time, the atomic details of the interactions between a DHHC enzyme and its substrate. It showed that the ANK17 domain forms a concave shape. In the substrate, a conserved proline residue in the aforementioned recognition sequence causes a kink in its structure, which then binds to the ANK17 domain in a shape-complementary way (Figure 6). A highly conserved tryptophan residue in the ANK17 domain makes the most crucial contact with this proline in the substrate, which forms the anchor point of this interaction. Mutation of this proline caused a marked decrease of SNAP25 palmitoylation by human DHHC17 in an *in cellulo* assay. While the identity of the consensus motif has now been expanded using peptide arrays [114], the proline has remained invariant, highlighting the significance of the ANK17-peptide complex structure in understanding the mechanistic basis of the peptide-AR domain interaction.

The structures of hDHHC20 and zfDHHS15 do not lend any obvious insights into where substrate proteins can bind, however. Given that the four TM helices enclose the fatty acyl chain in the autoacylated enzyme and that the acyl chain has to be eventually transferred to the substrate, leaves one obvious direction from which to approach the active site for a successful S-acylation event [54]. Nevertheless, the question remains as to what interactions between the DHHC-PAT and the substrate dictate that process of recognition. The last ~ 30 residues, predicted to be unstructured, are missing from the hDHHC20 structure. One

possibility is that the C-terminus engages with the substrate and presents it to the active site. Since the C-terminal domain contains, unexpectedly, an amphipathic helix that wraps around the protein, it is unclear if the missing residues are adequate to bind and present the protein substrate close to the active site for S-acylation or whether this would entail considerable rearrangement of interactions between the C-terminal domain and the TM helices, as well as a different placement of the amphipathic helix.

Paradoxically, while DHHC13 and 17 strongly bind SNAP25 and CSP (mediated by the AR domain-peptide interaction) and DHHC3 and 7 do not, DHHC3 and 7 S-acylate SNAP25 and CSP more efficiently than DHHC13 and 17 [76]. How is this possible? It is important to note that DHHC13 and DHHC17 do not seem to have a stable autoacylated state. Perhaps because DHHC13 and 17 S-acylate without an autoacylated intermediate, they are less efficient. In our own *in vitro* studies, while hDHHC20 efficiently S-acylates GobX, the binding is not stable enough for pulldown. Although DHHC3 may not bind strongly to SNAP25 or CSP, it seems to do so with Herpes Simplex Virus 1 protein UL20 [115]. In another example, DHHC5 binds GRIP1b using its PDZ-binding motif [116] while it uses an unstructured, ~ 120-residue C-terminal domain to bind the cardiac phosphoprotein phospholemman [117]. These studies seem to indicate that transient/weak DHHC enzyme-substrate interactions can still be catalytically productive. It is worthwhile to recall that in the cellular context, these interactions occur in close proximity to the membrane which is likely a critical third component in engaging the enzyme-substrate interactions. In accordance with this, the GobX protein is thought to interact with membranes using an amphipathic helix [24]. However, the details of how, if at all, specific membrane components play a role in DHHC enzyme-substrate interactions is far from clear at this stage and will need careful reconstitution-based studies. It is important to point out here that very few substrate S-acylations have been studied *in vitro* using purified components. Together with high-resolution structures, it is direly needed to dissect this aspect of DHHC-PATs with a range of different substrates using reconstitution-based approaches such as nanodiscs. Such a bottom-up approach will help bridge the structural data with the cell-biological data and bring out salient aspects of substrate-DHHC interactions that are still very poorly understood.

Concluding remarks

The recently determined structures of hDHHC20 and zfDHHS15, and of the ANK17 domain of human DHHC17 in complex with the consensus motif peptide, have finally given a structural framework to begin to understand and further study DHHC-PATs. Important questions remain, among them protein substrate specificity of DHHC-PATs other than DHHC13 and 17. Engineering DHHC-PATs that can selectively use unnatural fatty acids is a promising avenue to address this question, in analogy to the bump-hole approach for protein kinases [54, 118]. The DHHC enzyme family is large and diverse. Although the catalytic core and the fatty acid binding cavity is expected to be well conserved, the structures of DHHC-PATs containing more than four helices are of outstanding interest. Some DHHC-PATs, such as the yeast ERF2 require obligate partners, namely ERF4, to function [119]. Clearly, structures of topologically distinct DHHC-PATs, enzyme-substrate complexes, and DHHC-PATs at different stages of the enzymatic cycle are essential to obtain further atomic

resolution understanding into the structure and mechanism of this very important enzyme family. Aided by computational studies such as molecular dynamics simulations, such structures will lead to more fine-grained mechanistic hypotheses that can be tested and validated using both *in vitro* and *in vivo* experimental systems.

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References

[1]. Walsh C Posttranslational Modification of Proteins: Expanding Nature's Inventory: W. H. Freeman; 2006.

[2]. Drisdel RC, Manzana E, Green WN. The role of palmitoylation in functional expression of nicotinic alpha7 receptors. J Neurosci. 2004;24:10502–10. [PubMed: 15548665]

[3]. Valdez-Taubas J, Pelham H. Swf1-dependent palmitoylation of the SNARE Tlg1 prevents its ubiquitination and degradation. EMBO J. 2005;24:2524–32. [PubMed: 15973437]

[4]. Murphy J, Kolandaivelu S. Palmitoylation of Progressive Rod-Cone Degeneration (PRCD) Regulates Protein Stability and Localization. J Biol Chem. 2016;291:23036–46. [PubMed: 27613864]

[5]. Rossin A, Durivault J, Chakhtoura-Feghali T, Lounnas N, Gagnoux-Palacios L, Hueber AO. Fas palmitoylation by the palmitoyl acyltransferase DHHC7 regulates Fas stability. Cell Death Differ. 2015;22:643–53. [PubMed: 25301068]

[6]. Yanai A, Huang K, Kang R, Singaraja RR, Arstikaitis P, Gan L, et al. Palmitoylation of huntingtin by HIP14 is essential for its trafficking and function. Nat Neurosci. 2006;9:824–31. [PubMed: 16699508]

[7]. Greaves J, Lemonidis K, Gorleku OA, Cruchaga C, Grefen C, Chamberlain LH. Palmitoylation-induced aggregation of cysteine-string protein mutants that cause neuronal ceroid lipofuscinosis. The Journal of biological chemistry. 2012;287:37330–9. [PubMed: 22902780]

[8]. Rocks O, Peyker A, Kahms M, Verveer PJ, Koerner C, Lumbierres M, et al. An acylation cycle regulates localization and activity of palmitoylated Ras isoforms. Science. 2005;307:1746–52. [PubMed: 15705808]

[9]. Craven SE, El-Husseini AE, Bredt DS. Synaptic targeting of the postsynaptic density protein PSD-95 mediated by lipid and protein motifs. Neuron. 1999;22:497–509. [PubMed: 10197530]

[10]. Michaelson D, Ahearn I, Bergo M, Young S, Philips M. Membrane trafficking of heterotrimeric G proteins via the endoplasmic reticulum and Golgi. Mol Biol Cell. 2002;13:3294–302. [PubMed: 12221133]

[11]. Hayashi T, Thomas GM, Huganir RL. Dual palmitoylation of NR2 subunits regulates NMDA receptor trafficking. Neuron. 2009;64:213–26. [PubMed: 19874789]

[12]. Yount JS, Moltedo B, Yang YY, Charron G, Moran TM, Lopez CB, et al. Palmitoylome profiling reveals S-palmitoylation-dependent antiviral activity of IFITM3. Nature chemical biology. 2010;6:610–4. [PubMed: 20601941]

[13]. Runkle KB, Kharbanda A, Stypulkowski E, Cao XJ, Wang W, Garcia BA, et al. Inhibition of DHHC20-Mediated EGFR Palmitoylation Creates a Dependence on EGFR Signaling. Molecular cell. 2016;62:385–96. [PubMed: 27153536]

[14]. Bizzozero OA, McGarry JF, Lees MB. Acylation of rat brain myelin proteolipid protein with different fatty acids. J Neurochem. 1986;47:772–8. [PubMed: 2426408]

[15]. Muszbek L, Haramura G, Cluette-Brown JE, Van Cott EM, Laposata M. The pool of fatty acids covalently bound to platelet proteins by thioester linkages can be altered by exogenously supplied fatty acids. Lipids. 1999;34 Suppl:S331–7. [PubMed: 10419194]

[16]. Blanc M, David F, Abrami L, Migliozzi D, Armand F, Burgi J, et al. SwissPalm: Protein Palmitoylation database. F1000Res. 2015;4:261. [PubMed: 26339475]

[17]. Chamberlain LH, Shipston MJ. The physiology of protein S-acylation. Physiol Rev. 2015;95:341–76. [PubMed: 25834228]

[18]. Jiang H, Zhang X, Chen X, Aramsangtienchai P, Tong Z, Lin H. Protein Lipidation: Occurrence, Mechanisms, Biological Functions, and Enabling Technologies. Chemical reviews. 2018;118:919–88. [PubMed: 29292991]

[19]. Holland SM, Collura KM, Ketschek A, Noma K, Ferguson TA, Jin Y, et al. Palmitoylation controls DLK localization, interactions and activity to ensure effective axonal injury signaling. Proc Natl Acad Sci U S A. 2016;113:763–8. [PubMed: 26719418]

[20]. Zhang MM, Wu PY, Kelly FD, Nurse P, Hang HC. Quantitative control of protein S-palmitoylation regulates meiotic entry in fission yeast. PLoS Biol. 2013;11:e1001597. [PubMed: 23843742]

[21]. Hilgemann DW, Fine M, Linder ME, Jennings BC, Lin MJ. Massive endocytosis triggered by surface membrane palmitoylation under mitochondrial control in BHK fibroblasts. Elife. 2013;2:e01293. [PubMed: 24282236]

[22]. Lin DTS, Davis NG, Conibear E. Targeting the Ras palmitoylation/depalmitoylation cycle in cancer. Biochem Soc Trans. 2017;45:913–21. [PubMed: 28630138]

[23]. Hicks SW, Charron G, Hang HC, Galan JE. Subcellular targeting of Salmonella virulence proteins by host-mediated S-palmitoylation. Cell Host Microbe. 2011;10:9–20. [PubMed: 21767808]

[24]. Lin YH, Doms AG, Cheng E, Kim B, Evans TR, Machner MP. Host Cell-catalyzed S-Palmitoylation Mediates Golgi Targeting of the Legionella Ubiquitin Ligase GobX. The Journal of biological chemistry. 2015;290:25766–81. [PubMed: 26316537]

[25]. Schroeder GN, Aurass P, Oates CV, Tate EW, Hartland EL, Flieger A, et al. Legionella pneumophila Effector LpdA Is a Palmitoylated Phospholipase D Virulence Factor. Infect Immun. 2015;83:3989–4002. [PubMed: 26216420]

[26]. Russo I, Oksman A, Goldberg DE. Fatty acid acylation regulates trafficking of the unusual Plasmodium falciparum calpain to the nucleolus. Mol Microbiol. 2009;72:229–45. [PubMed: 19239622]

[27]. Jones ML, Collins MO, Goulding D, Choudhary JS, Rayner JC. Analysis of protein palmitoylation reveals a pervasive role in Plasmodium development and pathogenesis. Cell Host Microbe. 2012;12:246–58. [PubMed: 22901544]

[28]. Frenal K, Tay CL, Mueller C, Bushell ES, Jia Y, Graindorge A, et al. Global analysis of apicomplexan protein S-acyl transferases reveals an enzyme essential for invasion. Traffic. 2013;14:895–911. [PubMed: 23638681]

[29]. Shulla A, Gallagher T. Role of spike protein endodomains in regulating coronavirus entry. J Biol Chem. 2009;284:32725–34. [PubMed: 19801669]

[30]. Majeau N, Fromentin R, Savard C, Duval M, Tremblay MJ, Leclerc D. Palmitoylation of hepatitis C virus core protein is important for virion production. J Biol Chem. 2009;284:33915–25. [PubMed: 19783655]

[31]. Wagner R, Herwig A, Azzouz N, Klenk HD. Acylation-mediated membrane anchoring of avian influenza virus hemagglutinin is essential for fusion pore formation and virus infectivity. J Virol. 2005;79:6449–58. [PubMed: 15858028]

[32]. Chen BJ, Takeda M, Lamb RA. Influenza virus hemagglutinin (H3 subtype) requires palmitoylation of its cytoplasmic tail for assembly: M1 proteins of two subtypes differ in their ability to support assembly. J Virol. 2005;79:13673–84. [PubMed: 16227287]

[33]. Petit CM, Chouljenko VN, Iyer A, Colgrove R, Farzan M, Knipe DM, et al. Palmitoylation of the cysteine-rich endodomain of the SARS-coronavirus spike glycoprotein is important for spike-mediated cell fusion. Virology. 2007;360:264–74. [PubMed: 17134730]

[34]. Hemsley PA, Kemp AC, Grierson CS. The TIP GROWTH DEFECTIVE1 S-acyl transferase regulates plant cell growth in Arabidopsis. Plant Cell. 2005;17:2554–63. [PubMed: 16100337]

[35]. Zhou LZ, Li S, Feng QN, Zhang YL, Zhao X, Zeng YL, et al. Protein S-ACYL Transferase10 is critical for development and salt tolerance in Arabidopsis. Plant Cell. 2013;25:1093–107. [PubMed: 23482856]

[36]. Schmidt MF, Schlesinger MJ. Fatty acid binding to vesicular stomatitis virus glycoprotein: a new type of post-translational modification of the viral glycoprotein. Cell. 1979;17:813–9. [PubMed: 226266]

[37]. Lobo S, Greentree WK, Linder ME, Deschenes RJ. Identification of a Ras palmitoyltransferase in Saccharomyces cerevisiae. J Biol Chem. 2002;277:41268–73. [PubMed: 12193598]

[38]. Roth AF, Feng Y, Chen L, Davis NG. The yeast DHHC cysteine-rich domain protein Akr1p is a palmitoyl transferase. The Journal of cell biology. 2002;159:23–8. [PubMed: 12370247]

[39]. Mitchell DA, Vasudevan A, Linder ME, Deschenes RJ. Protein palmitoylation by a family of DHHC protein S-acyltransferases. Journal of lipid research. 2006;47:1118–27. [PubMed: 16582420]

[40]. Thomas GM, Hayashi T. Smarter neuronal signaling complexes from existing components: how regulatory modifications were acquired during animal evolution: evolution of palmitoylation-dependent regulation of AMPA-type ionotropic glutamate receptors. Bioessays. 2013;35:929–39. [PubMed: 23943381]

[41]. Ohno Y, Kihara A, Sano T, Igarashi Y. Intracellular localization and tissue-specific distribution of human and yeast DHHC cysteine-rich domain-containing proteins. Biochimica et biophysica acta. 2006;1761:474–83. [PubMed: 16647879]

[42]. Fukata M, Fukata Y, Adesnik H, Nicoll RA, Bredt DS. Identification of PSD-95 palmitoylating enzymes. Neuron. 2004;44:987–96. [PubMed: 15603741]

[43]. Roth AF, Wan J, Bailey AO, Sun B, Kuchar JA, Green WN, et al. Global analysis of protein palmitoylation in yeast. Cell. 2006;125:1003–13. [PubMed: 16751107]

[44]. Martin BR, Cravatt BF. Large-scale profiling of protein palmitoylation in mammalian cells. Nat Methods. 2009;6:135–8. [PubMed: 19137006]

[45]. Kang R, Wan J, Arstikaitis P, Takahashi H, Huang K, Bailey AO, et al. Neural palmitoyl-proteomics reveals dynamic synaptic palmitoylation. Nature. 2008;456:904–9. [PubMed: 19092927]

[46]. Ren W, Jhala US, Du K. Proteomic analysis of protein palmitoylation in adipocytes. Adipocyte. 2013;2:17–28. [PubMed: 23599907]

[47]. Hemsley PA, Weimar T, Lilley K, Dupree P, Grierson C. Palmitoylation in plants: new insights through proteomics. Plant signaling & behavior. 2013;8.

[48]. Martin BR, Wang C, Adibekian A, Tully SE, Cravatt BF. Global profiling of dynamic protein palmitoylation. Nat Methods. 2011;9:84–9. [PubMed: 22056678]

[49]. Yeste-Velasco M, Linder ME, Lu YJ. Protein S-palmitoylation and cancer. Biochim Biophys Acta. 2015;1856:107–20. [PubMed: 26112306]

[50]. Cho E, Park M. Palmitoylation in Alzheimer's disease and other neurodegenerative diseases. Pharmacol Res. 2016;111:133–51. [PubMed: 27293050]

[51]. Resh MD. Palmitoylation of proteins in cancer. Biochemical Society transactions. 2017;45:409–16. [PubMed: 28408481]

[52]. Fraser NJ, Howie J, Wypijewski KJ, Fuller W. Therapeutic targeting of protein S-acylation for the treatment of disease. Biochem Soc Trans. 2019.

[53]. Sharma C, Wang HX, Li Q, Knoblich K, Reisenbichler ES, Richardson AL, et al. Protein Acyltransferase DHHC3 Regulates Breast Tumor Growth, Oxidative Stress, and Senescence. Cancer Res. 2017;77:6880–90. [PubMed: 29055014]

[54]. Rana MS, Kumar P, Lee CJ, Verardi R, Rajashankar KR, Banerjee A. Fatty acyl recognition and transfer by an integral membrane S-acyltransferase. Science. 2018;359. [PubMed: 29700239]

[55]. Verardi R, Kim JS, Ghirlando R, Banerjee A. Structural Basis for Substrate Recognition by the Ankyrin Repeat Domain of Human DHHC17 Palmitoyltransferase. Structure. 2017;25:1337–47 e6. [PubMed: 28757145]

[56]. Greaves J, Chamberlain LH. DHHC palmitoyl transferases: substrate interactions and (patho)physiology. Trends in biochemical sciences. 2011;36:245–53. [PubMed: 21388813]

[57]. Korycka J, Lach A, Heger E, Boguslawska DM, Wolny M, Toporkiewicz M, et al. Human DHHC proteins: a spotlight on the hidden player of palmitoylation. Eur J Cell Biol. 2012;91:107–17. [PubMed: 22178113]

[58]. Chen B, Sun Y, Niu J, Jarugumilli GK, Wu X. Protein Lipidation in Cell Signaling and Diseases: Function, Regulation, and Therapeutic Opportunities. Cell Chem Biol. 2018;25:817–31. [PubMed: 29861273]

[59]. Zaballa ME, van der Goot FG. The molecular era of protein S-acylation: spotlight on structure, mechanisms, and dynamics. Crit Rev Biochem Mol Biol. 2018;53:420–51. [PubMed: 29999430]

[60]. De I, Sadhukhan S. Emerging Roles of DHHC-mediated Protein S-palmitoylation in Physiological and Pathophysiological Context. Eur J Cell Biol. 2018;97:319–38. [PubMed: 29602512]

[61]. Putilina T, Wong P, Gentleman S. The DHHC domain: a new highly conserved cysteine-rich motif. Mol Cell Biochem. 1999;195:219–26. [PubMed: 10395086]

[62]. Gonzalez A, Quiroga R, Maccioni HJ, Valdez Taubas J. A novel motif at the C-terminus of palmitoyltransferases is essential for Swf1 and Pfa3 function in vivo. Biochem J. 2009;419:301–8. [PubMed: 19138168]

[63]. Politis EG, Roth AF, Davis NG. Transmembrane topology of the protein palmitoyl transferase Akr1. J Biol Chem. 2005;280:10156–63. [PubMed: 15632165]

[64]. Jennings BC, Linder ME. DHHC protein S-acyltransferases use similar ping-pong kinetic mechanisms but display different acyl-CoA specificities. J Biol Chem. 2012;287:7236–45. [PubMed: 22247542]

[65]. Greaves J, Munro KR, Davidson SC, Riviere M, Wojno J, Smith TK, et al. Molecular basis of fatty acid selectivity in the zDHHC family of S-acyltransferases revealed by click chemistry. Proc Natl Acad Sci U S A. 2017;114:E1365–E74. [PubMed: 28167757]

[66]. Gonzalez Montoro A, Quiroga R, Valdez Taubas J. Zinc co-ordination by the DHHC cysteine-rich domain of the palmitoyltransferase Swf1. Biochem J. 2013;454:427–35. [PubMed: 23790227]

[67]. Long SB, Casey PJ, Beese LS. Reaction path of protein farnesyltransferase at atomic resolution. Nature. 2002;419:645–50. [PubMed: 12374986]

[68]. He C, Hus JC, Sun LJ, Zhou P, Norman DP, Dotsch V, et al. A methylation-dependent electrostatic switch controls DNA repair and transcriptional activation by E. coli ada. Mol Cell. 2005;20:117–29. [PubMed: 16209950]

[69]. Reddy KD, Malipeddi J, DeForte S, Pejaver V, Radivojac P, Uversky VN, et al. Physicochemical sequence characteristics that influence S-palmitoylation propensity. J Biomol Struct Dyn. 2017;35:2337–50. [PubMed: 27498722]

[70]. Dodson G, Wlodawer A. Catalytic triads and their relatives. Trends Biochem Sci. 1998;23:347–52. [PubMed: 9787641]

[71]. Birktoft JJ, Blow DM. Structure of crystalline -chymotrypsin. V. The atomic structure of tosyl- -chymotrypsin at 2 A resolution. J Mol Biol. 1972;68:187–240. [PubMed: 5069789]

[72]. Baxter A, Chambers M, Edfeldt F, Edman K, Freeman A, Johansson C, et al. Non-covalent inhibitors of rhinovirus 3C protease. Bioorg Med Chem Lett. 2011;21:777–80. [PubMed: 21183345]

[73]. Mitchell DA, Mitchell G, Ling Y, Budde C, Deschenes RJ. Mutational analysis of Saccharomyces cerevisiae Erf2 reveals a two-step reaction mechanism for protein palmitoylation by DHHC enzymes. The Journal of biological chemistry. 2010;285:38104–14. [PubMed: 20851885]

[74]. Gonzalez Montoro A, Chumpen Ramirez S, Valdez Taubas J. The canonical DHHC motif is not absolutely required for the activity of the yeast S-acyltransferases Swf1 and Pfa4. J Biol Chem. 2015;290:22448–59. [PubMed: 26224664]

[75]. Ohno Y, Kashio A, Ogata R, Ishitomi A, Yamazaki Y, Kihara A. Analysis of substrate specificity of human DHHC protein acyltransferases using a yeast expression system. Molecular biology of the cell. 2012;23:4543–51. [PubMed: 23034182]

[76]. Lemonidis K, Gorleku OA, Sanchez-Perez MC, Grefen C, Chamberlain LH. The Golgi S-acylation machinery comprises zDHHC enzymes with major differences in substrate affinity and S-acylation activity. Molecular biology of the cell. 2014;25:3870–83. [PubMed: 25253725]

[77]. Bharadwaj M, Bizzozero OA. Myelin P0 glycoprotein and a synthetic peptide containing the palmitoylation site are both autoacylated. J Neurochem. 1995;65:1805–15. [PubMed: 7561879]

[78]. Duncan JA, Gilman AG. Autoacylation of G protein alpha subunits. The Journal of biological chemistry. 1996;271:23594–600. [PubMed: 8798571]

[79]. Veit M, Sachs K, Heckelmann M, Maretzki D, Hofmann KP, Schmidt MF. Palmitoylation of rhodopsin with S-protein acyltransferase: enzyme catalyzed reaction versus autocatalytic acylation. Biochim Biophys Acta. 1998;1394:90–8. [PubMed: 9767130]

[80]. Chan P, Han X, Zheng B, DeRan M, Yu J, Jarugumilli GK, et al. Autopalmitoylation of TEAD proteins regulates transcriptional output of the Hippo pathway. Nat Chem Biol. 2016;12:282–9. [PubMed: 26900866]

[81]. Turnbull AP, Kummel D, Prinz B, Holz C, Schultchen J, Lang C, et al. Structure of palmitoylated BET3: insights into TRAPP complex assembly and membrane localization. EMBO J. 2005;24:875–84. [PubMed: 15692564]

[82]. Kim YG, Sohn EJ, Seo J, Lee KJ, Lee HS, Hwang I, et al. Crystal structure of bet3 reveals a novel mechanism for Golgi localization of tethering factor TRAPP. Nat Struct Mol Biol. 2005;12:38–45. [PubMed: 15608655]

[83]. Stix R, Song J, Banerjee A, Faraldo-Gomez JD. DHHC20 Palmitoyl-Transferase Reshapes the Membrane to Foster Catalysis. Biophys J. 2019.

[84]. Bondar AN, del Val C, White SH. Rhomboid protease dynamics and lipid interactions. Structure. 2009;17:395–405. [PubMed: 19278654]

[85]. Bondar AN. Mechanisms by Which Lipids Influence Conformational Dynamics of the GlpG Intramembrane Protease. J Phys Chem B. 2019;123:4159–72. [PubMed: 31059259]

[86]. Kreutzberger AJB, Ji M, Aaron J, Mihaljevic L, Urban S. Rhomboid distorts lipids to break the viscosity-imposed speed limit of membrane diffusion. Science. 2019;363.

[87]. Zhou W, Faraldo-Gomez JD. Membrane plasticity facilitates recognition of the inhibitor oligomycin by the mitochondrial ATP synthase rotor. Biochim Biophys Acta Bioenerg. 2018;1859:789–96. [PubMed: 29630891]

[88]. Diver MM, Pedi L, Koide A, Koide S, Long SB. Atomic structure of the eukaryotic intramembrane RAS methyltransferase ICMT. Nature. 2018;553:526–9. [PubMed: 29342140]

[89]. Parey K, Haapanen O, Sharma V, Kofeler H, Zullig T, Prinz S, et al. High-resolution cryo-EM structures of respiratory complex I: Mechanism, assembly, and disease. Sci Adv. 2019;5:eaax9484. [PubMed: 31844670]

[90]. Mao G, Zhao Y, Kang X, Li Z, Zhang Y, Wang X, et al. Crystal structure of E. coli lipoprotein diacylglyceryl transferase. Nat Commun. 2016;7:10198. [PubMed: 26729647]

[91]. Lu G, Xu Y, Zhang K, Xiong Y, Li H, Cui L, et al. Crystal structure of E. coli apolipoprotein N-acyl transferase. Nat Commun. 2017;8:15948. [PubMed: 28885614]

[92]. Wiktor M, Weichert D, Howe N, Huang CY, Olieric V, Boland C, et al. Structural insights into the mechanism of the membrane integral N-acyltransferase step in bacterial lipoprotein synthesis. Nat Commun. 2017;8:15952. [PubMed: 28675161]

[93]. Anandan A, Evans GL, Condic-Jurkic K, O'Mara ML, John CM, Phillips NJ, et al. Structure of a lipid A phosphoethanolamine transferase suggests how conformational changes govern substrate binding. Proc Natl Acad Sci U S A. 2017;114:2218–23. [PubMed: 28193899]

[94]. Chung BC, Zhao J, Gillespie RA, Kwon DY, Guan Z, Hong J, et al. Crystal structure of MraY, an essential membrane enzyme for bacterial cell wall synthesis. Science. 2013;341:1012–6. [PubMed: 23990562]

[95]. Liu X, Yin Y, Wu J, Liu Z. Structure and mechanism of an intramembrane liponucleotide synthetase central for phospholipid biosynthesis. Nat Commun. 2014;5:4244. [PubMed: 24968740]

[96]. Sjodt M, Brock K, Dobihal G, Rohs PDA, Green AG, Hopf TA, et al. Structure of the peptidoglycan polymerase RodA resolved by evolutionary coupling analysis. Nature. 2018;556:118–21. [PubMed: 29590088]

[97]. Wang Y, Zhang Y, Ha Y. Crystal structure of a rhomboid family intramembrane protease. Nature. 2006;444:179–80. [PubMed: 17051161]

[98]. Dietrich LE, Ungermann C. On the mechanism of protein palmitoylation. EMBO Rep. 2004;5:1053–7. [PubMed: 15520806]

[99]. Kordyukova LV, Serebryakova MV, Baratova LA, Veit M. S acylation of the hemagglutinin of influenza viruses: mass spectrometry reveals site-specific attachment of stearic acid to a transmembrane cysteine. J Virol. 2008;82:9288–92. [PubMed: 18596092]

[100]. Brett K, Kordyukova LV, Serebryakova MV, Mintaev RR, Alexeevski AV, Veit M. Site-specific S-acylation of influenza virus hemagglutinin: the location of the acylation site relative to the membrane border is the decisive factor for attachment of stearate. J Biol Chem. 2014;289:34978–89. [PubMed: 25349209]

[101]. Gadalla MR, Abrami L, van der Goot FG, Veit M. Hemagglutinin of Influenza A, but not of Influenza B and C viruses is acylated by ZDHHC2, 8, 15 and 20. Biochem J. 2020;477:285–303. [PubMed: 31872235]

[102]. Muszbek L, Laposata M. Covalent modification of proteins by arachidonate and eicosapentaenoate in platelets. J Biol Chem. 1993;268:18243–8. [PubMed: 8349700]

[103]. Hallak H, Muszbek L, Laposata M, Belmonte E, Brass LF, Manning DR. Covalent binding of arachidonate to G protein alpha subunits of human platelets. J Biol Chem. 1994;269:4713–6. [PubMed: 8106438]

[104]. Liang X, Nazarian A, Erdjument-Bromage H, Bornmann W, Tempst P, Resh MD. Heterogeneous fatty acylation of Src family kinases with polyunsaturated fatty acids regulates raft localization and signal transduction. J Biol Chem. 2001;276:30987–94. [PubMed: 11423543]

[105]. Lee CJ, Rana MS, Bae C, Li Y, Banerjee A. In vitro reconstitution of Wnt acylation reveals structural determinants of substrate recognition by the acyltransferase human Porcupine. J Biol Chem. 2019;294:231–45. [PubMed: 30420431]

[106]. Drisdel RC, Alexander JK, Sayeed A, Green WN. Assays of protein palmitoylation. Methods. 2006;40:127–34. [PubMed: 17012024]

[107]. Rocks O, Gerauer M, Vartak N, Koch S, Huang ZP, Pechlivanis M, et al. The palmitoylation machinery is a spatially organizing system for peripheral membrane proteins. Cell. 2010;141:458–71. [PubMed: 20416930]

[108]. Fredericks GJ, Hoffmann FW, Rose AH, Osterheld HJ, Hess FM, Mercier F, et al. Stable expression and function of the inositol 1,4,5-triphosphate receptor requires palmitoylation by a DHHC6/selenoprotein K complex. Proc Natl Acad Sci U S A. 2014;111:16478–83. [PubMed: 25368151]

[109]. Ebsen H, Lettau M, Kabelitz D, Janssen O. Identification of SH3 domain proteins interacting with the cytoplasmic tail of the a disintegrin and metalloprotease 10 (ADAM10). PLoS One. 2014;9:e102899. [PubMed: 25036101]

[110]. Gonzalez Montoro A, Chumpen Ramirez S, Quiroga R, Valdez Taubas J. Specificity of transmembrane protein palmitoylation in yeast. PLoS One. 2011;6:e16969. [PubMed: 21383992]

[111]. Smotrys JE, Schoenfish MJ, Stutz MA, Linder ME. The vacuolar DHHC-CRD protein Pfa3p is a protein acyltransferase for Vac8p. The Journal of cell biology. 2005;170:1091–9. [PubMed: 16186255]

[112]. Nadolski MJ, Linder ME. Molecular recognition of the palmitoylation substrate Vac8 by its palmitoyltransferase Pfa3. The Journal of biological chemistry. 2009;284:17720–30. [PubMed: 19416974]

[113]. Lemonidis K, Sanchez-Perez MC, Chamberlain LH. Identification of a Novel Sequence Motif Recognized by the Ankyrin Repeat Domain of zDHHC17/13 S-Acyltransferases. The Journal of biological chemistry. 2015;290:21939–50. [PubMed: 26198635]

[114]. Lemonidis K, MacLeod R, Baillie GS, Chamberlain LH. Peptide array-based screening reveals a large number of proteins interacting with the ankyrin-repeat domain of the zDHHC17 S-acyltransferase. The Journal of biological chemistry. 2017;292:17190–202. [PubMed: 28882895]

[115]. Wang S, Mott KR, Wawrowsky K, Kousoulas KG, Luscher B, Ghiasi H. Binding of Herpes Simplex Virus 1 UL20 to GODZ (DHHC3) Affects Its Palmitoylation and Is Essential for Infectivity and Proper Targeting and Localization of UL20 and Glycoprotein K. J Virol. 2017;91.

[116]. Thomas GM, Hayashi T, Chiu SL, Chen CM, Huganir RL. Palmitoylation by DHHC5/8 targets GRIP1 to dendritic endosomes to regulate AMPA-R trafficking. Neuron. 2012;73:482–96. [PubMed: 22325201]

[117]. Howie J, Reilly L, Fraser NJ, Vlachaki Walker JM, Wypijewski KJ, Ashford ML, et al. Substrate recognition by the cell surface palmitoyl transferase DHHC5. Proc Natl Acad Sci U S A. 2014;111:17534–9. [PubMed: 25422474]

[118]. Shah K, Liu Y, Deirmengian C, Shokat KM. Engineering unnatural nucleotide specificity for Rous sarcoma virus tyrosine kinase to uniquely label its direct substrates. Proceedings of the National Academy of Sciences of the United States of America. 1997;94:3565–70. [PubMed: 9108016]

[119]. Mitchell DA, Hamel LD, Ishizuka K, Mitchell G, Schaefer LM, Deschenes RJ. The Erf4 subunit of the yeast Ras palmitoyl acyltransferase is required for stability of the Acyl-Erf2 intermediate and palmitoyl transfer to a Ras2 substrate. The Journal of biological chemistry. 2012;287:34337–48. [PubMed: 22904317]

Research Highlights

- Protein S-acylation is one of the most abundant forms of protein lipidation.
- Members of the DHHC family of integral membrane enzymes catalyze protein S-acylation in eukaryotes.
- High-resolution structures of two members of the family revealed a tremendous amount of information about organization and mechanism of DHHC enzymes.
- Atomistic molecular dynamics simulation revealed DHHC enzymes deform the membrane to facilitate catalysis.

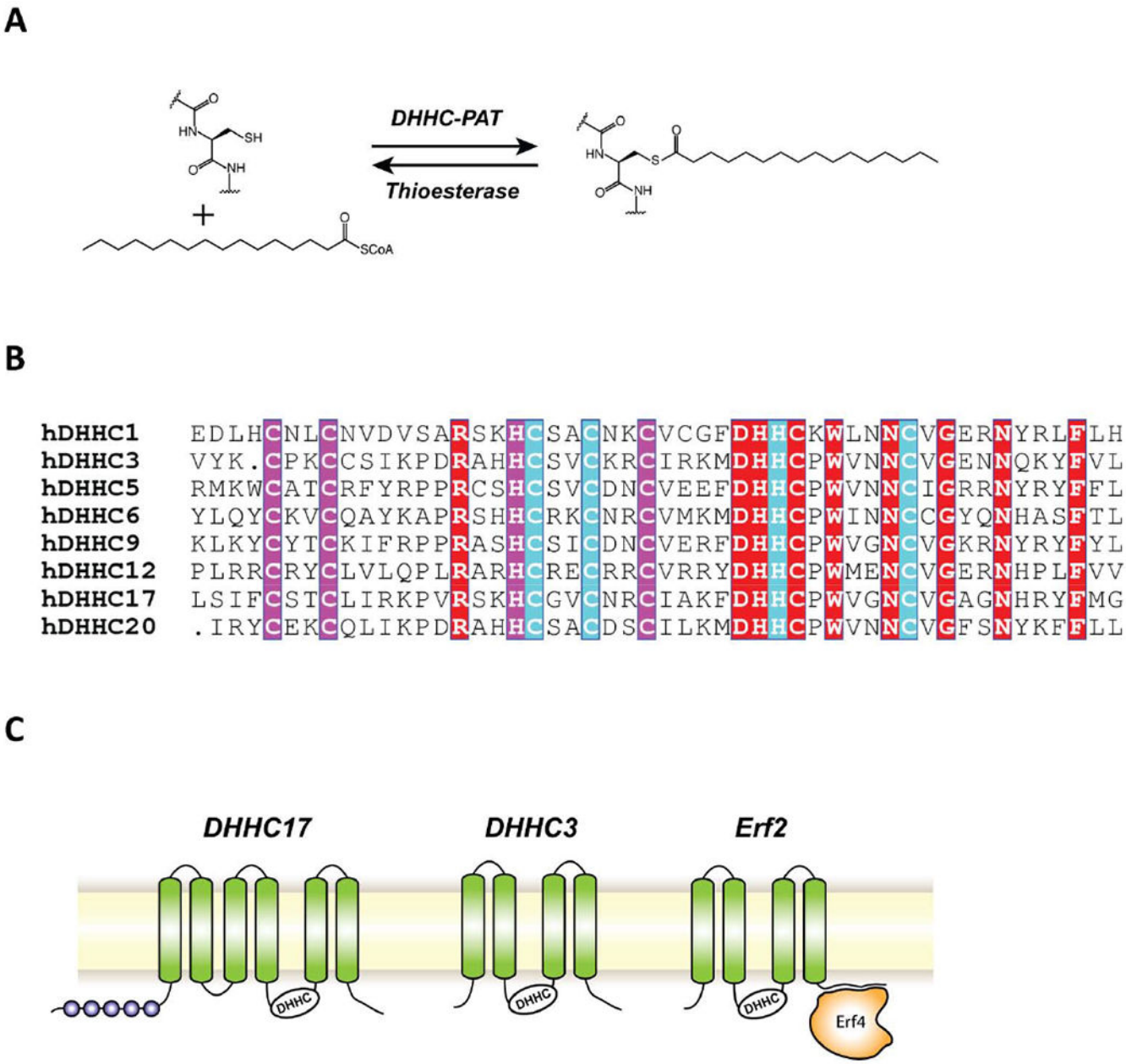

Figure 1. S-acylation and DHHC-PATs

(**A**) Scheme of protein S-acylation by DHHC-PATs and deacylation by thioesterases. Fatty acyl CoA is the fatty acid chain donor. (**B**) Multiple sequence alignment of the DHHC-CRD of select human DHHC-PATs. The two CCHC zinc-finger motifs are color coded as magenta and cyan. (**C**) Diverse membrane topologies of the DHHC-PATs. DHHC17 has six transmembrane helices with an ankyrin-repeat domain at the N-terminus. DHHC3 has four transmembrane helices like most DHHC-PATs. The yeast ERF2 has four transmembrane helices but requires ERF4 and forms a heteromeric complex. DHHC24 is predicted to have five transmembrane helices.

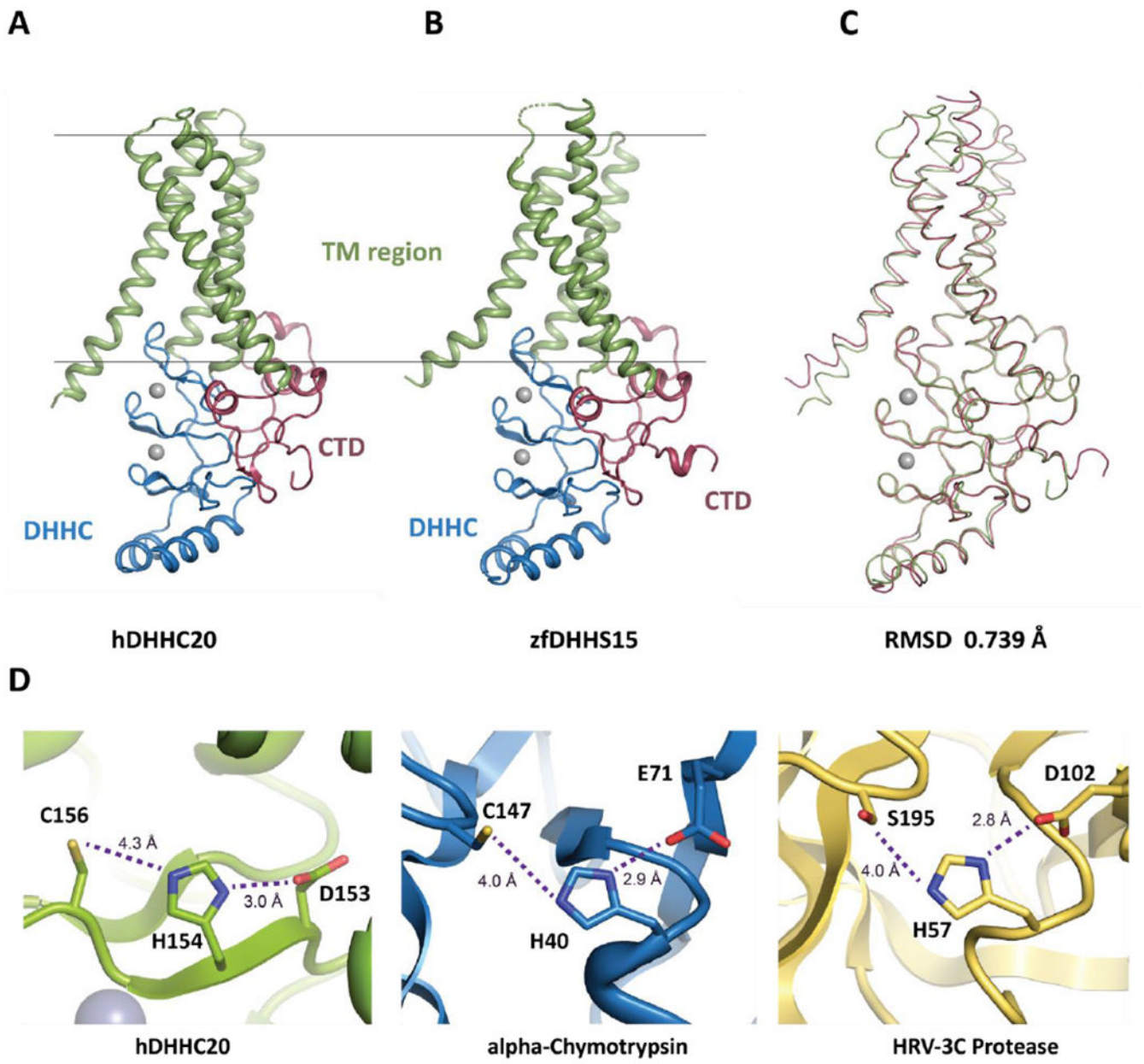

Figure 2. Structure of DHHC-PATs

Overall structure of (A) human DHHC20 and (B) zebrafish DHHS15 shown in cartoon. The transmembrane domains are colored in green, the DHHC-CRD domains in blue, and the C-terminal domains in orange. The two zinc-ions are represented as grey spheres. (C) Superimposition of the backbones of hDHHC20 (green) and zfDHHS15 (purple), Cα rmsd is 0.7Å.(**D**) Close-up views of the active-site of hDHHC20 (PDB ID: 6BMN), alpha-chymotrypsin (PDB ID: 2CHA) and human rhinovirus 3C cysteine protease (PDB ID: 2XYA), showing the similarity in the arrangement of the catalytic triad.

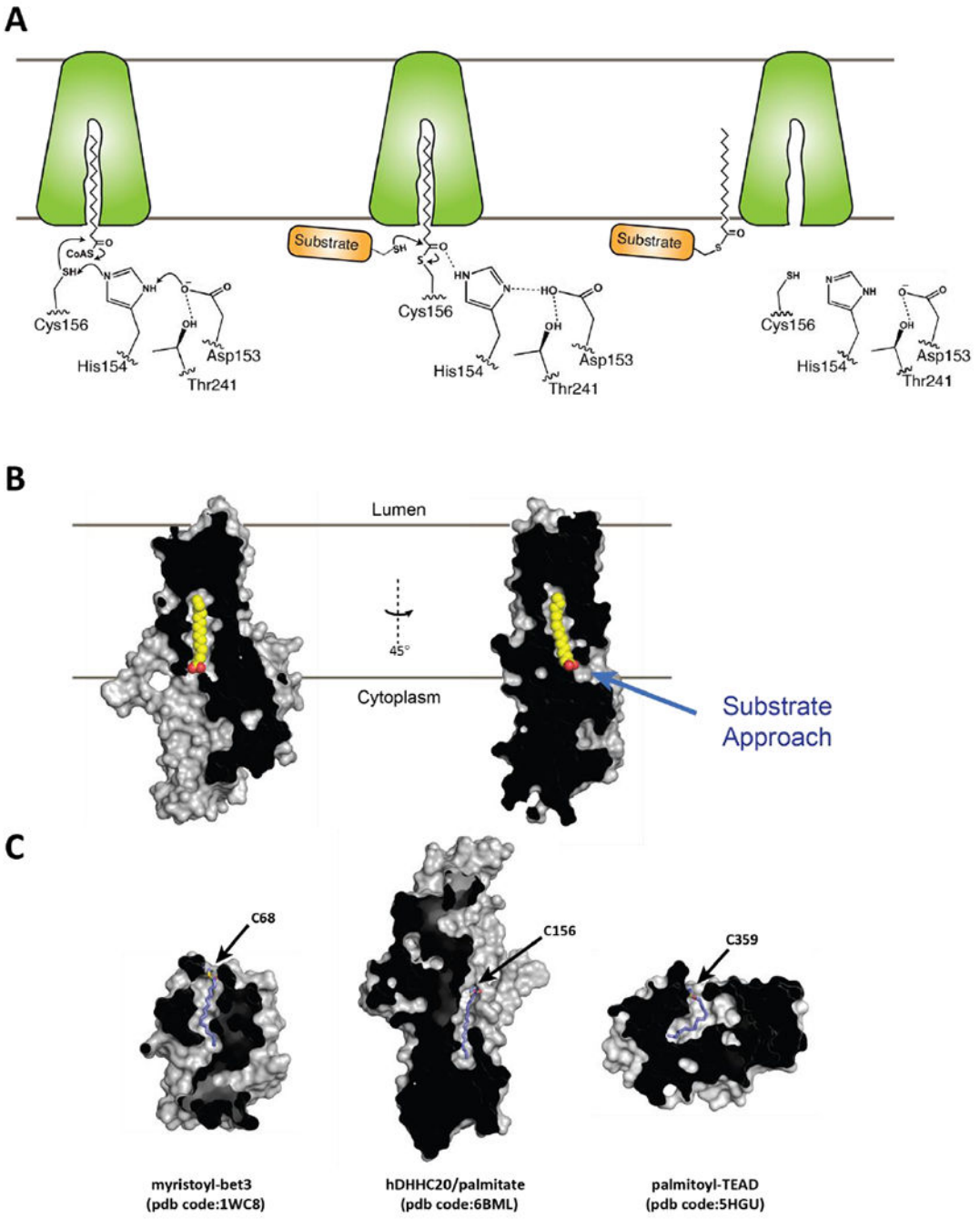

Figure 3. Proposed mechanism of catalysis.

(A) DHHC shown schematically in green. In the first step, the His154 (in DHHC20) deprotonates Cys156 to activate for autoacylation. In the second step, His154 activates the carbonyl of the thioester for substrate acylation. **(B)** Proposed direction of approach by substrate. (C) Cutaway views of the hydrophobic groove of Bet3, hDHHC20, and TEAD, showing the acyl chains linked to the conserved cysteine residues.

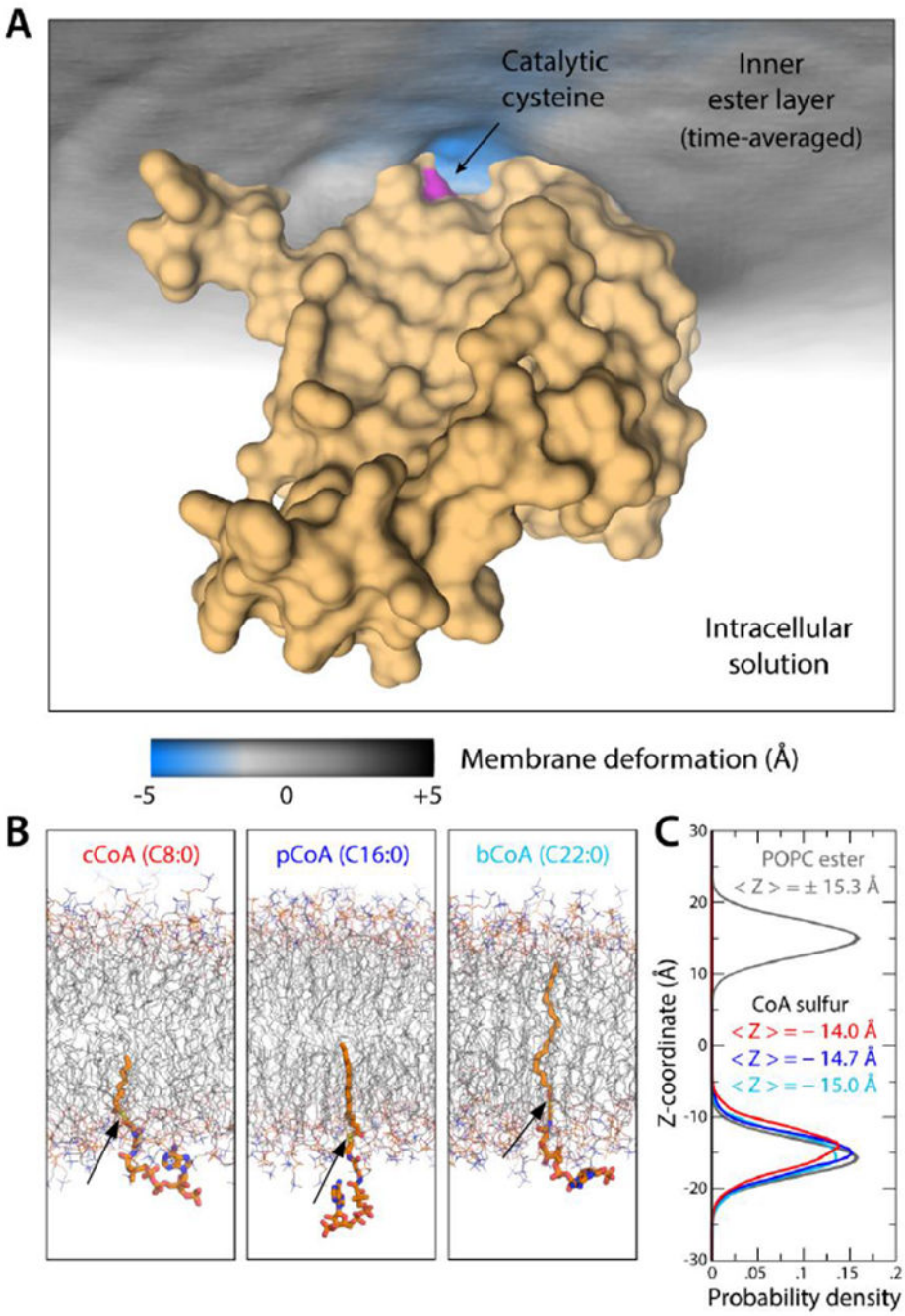

Figure 4. Molecular dynamics simulations of hDHHC20 and acyl-CoA in a POPC bilayer.

(**A**) hDHHC20 deforms the surrounding membrane to expose the catalytic Cys 156 (magenta) to solvent. Shown are the surface of the protein (tan) and of the ester layer of POPC, on the cytoplasmic side, when averaged over the simulated time. The color bar indicates the degree of perturbation of the membrane, relative to a point far away from the protein; e.g. blue indicates a depression towards the bilayer center. These data are based on two MD trajectories of 1 μs each. (**B**) Snapshots of MD simulations of caprylyl-CoA (cCoA), palmitoyl-CoA (pCoA) and behenyl-CoA (bCoA) in POPC. Water, ions and

hydrogens are omitted for clarity. The positions of sulfur atoms have been indicated with an arrow. (**C**) For each form of acyl-CoA, the plots quantify the most probable location of the sulfur atom where acyl-CoA is cleaved off, relative to the membrane center and to the two ester layers in POPC. The distributions derive from three 1-μs MD trajectories (one for each form of acyl-CoA).

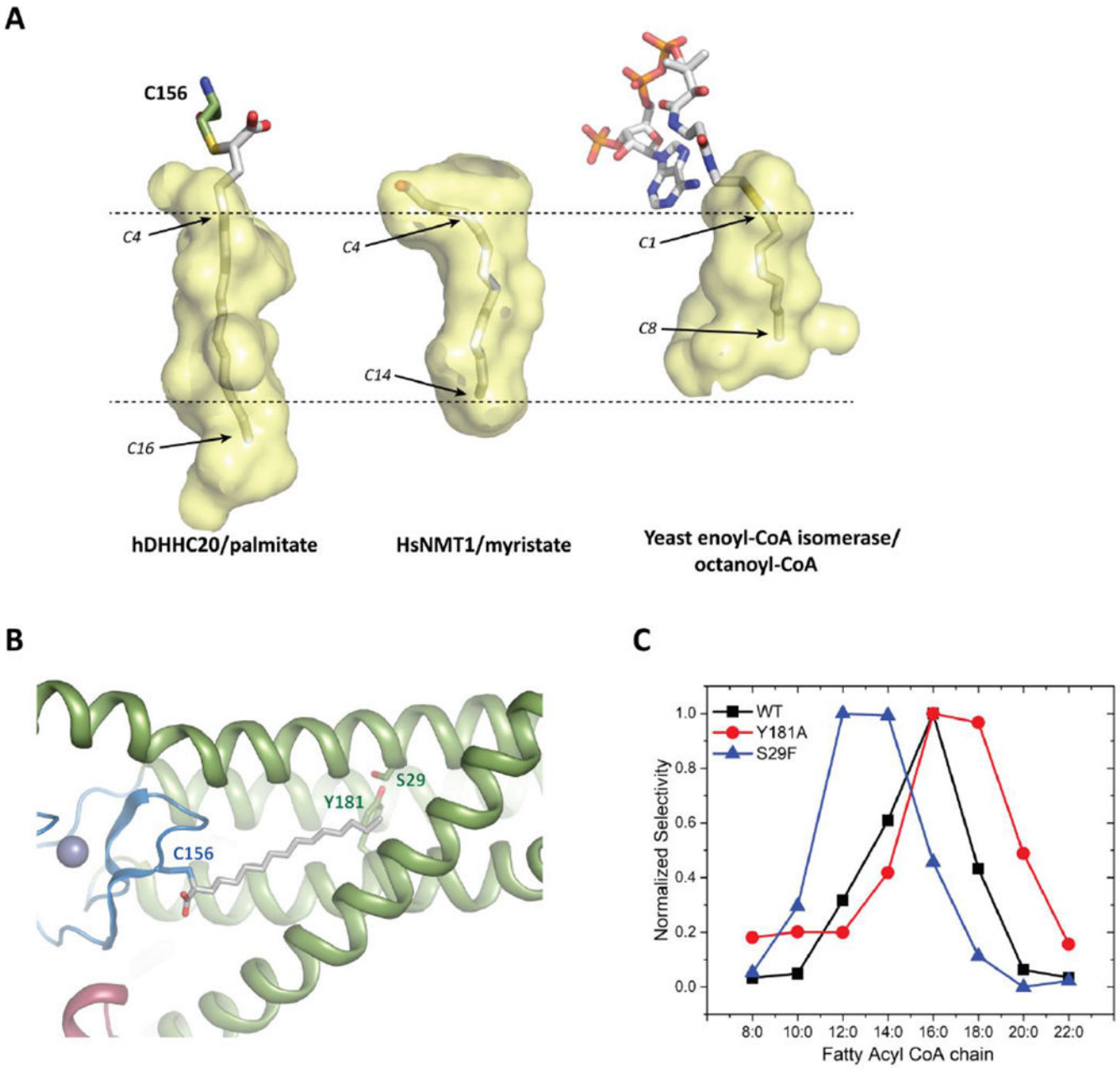

Figure 5. Structural basis of fatty acyl CoA selectivity by DHHC-PATs

(**A**) Comparison of the fatty acid binding cavity of human DHHC20 with a covalently linked fatty acid (PDB ID: 2BML), human N-myristoyltransferase in complex with CoA and a myristoylated peptide (PDB ID: 5O9S), and yeast enoyl-CoA isomerase complexed with octanoyl-CoA (PDB ID: 4ZDC). (**B**) Fatty acid chain binding cavity of hDHHC20 formed by the transmembrane domain. The covalently linked fatty acid chain is shown in grey. The critical Tyr181-Ser29 H-bond interaction that limits the cavity is shown as dotted lines. (**C**) The shift in fatty acyl chain length selectivity upon mutagenesis to increase or decrease the hDHHC20 cavity.

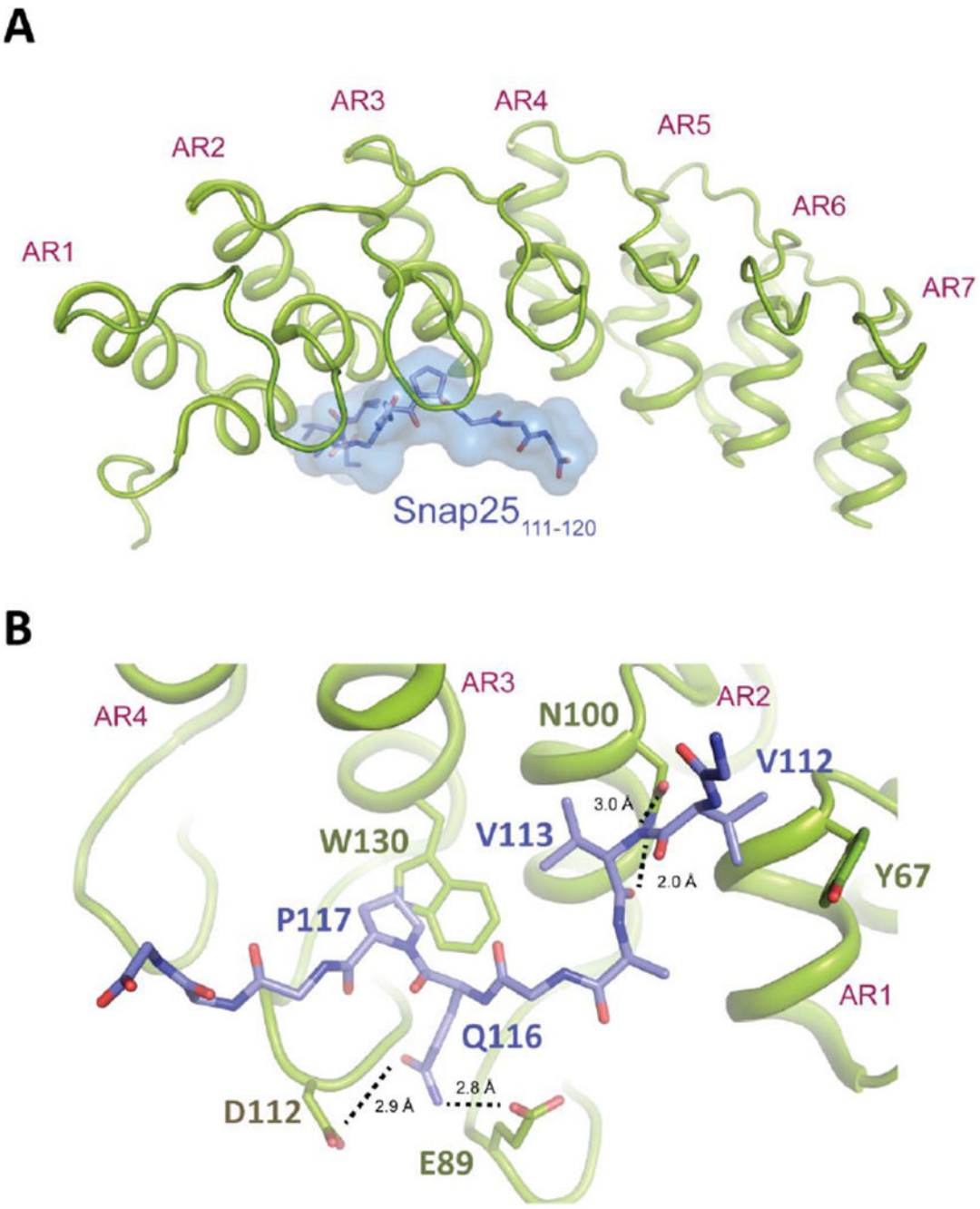

Figure 6. Substrate binding by the ankyrin-repeat (AR) domain of human DHHC17.
(A) Molecular structure of the complex between the N-terminal AR domain of DHHC17 (light yellow) and the consensus motif peptide of the protein SNAP25b (purple) showing the shape-selective recognition. **(B)** Pro117, which interacts with Trp130 of the AR domain, introduces a kink in the peptide giving rise to shape complementarity to the concave AR domain structure. The proline residue is essentially invariant in the consensus motif. PDB ID for the structure is 5W7I.