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Molecular biophysics of FAAH: structural dynamics, mechanistic insights, and therapeutic translation for neurological disorders

Khanh Huyen Thi Pham
Dongguk University
Dung N.A Hoang
Université de Sherbrooke
Thuy Van Dinh
Phenikaa University
Thuy Duong T Khuat
Phenikaa University
Minh Duc Nguyen
Phenikaa University
Tan Khanh Nguyen
khanhnt@donga.edu.vn
Dong A University

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Abstract

Fatty acid amide hydrolase (FAAH) is a key regulator of endocannabinoid and N-acylethanolamine signaling, with important implications for pain, stress responses, neuronal excitability, and neuroinflammation. Beyond its catalytic function, FAAH exhibits distinctive biophysical properties, including membrane-associated substrate access, conformationally flexible binding channels, and inter-subunit communication, which collectively influence substrate recognition, enzymatic activity, and inhibitor interactions. This review examines the molecular and structural basis of FAAH function and its relevance to neurological and neuropsychiatric disorders, with particular emphasis on the relationship between protein dynamics, lipid signaling, and therapeutic modulation. We discuss the structural organization and catalytic mechanisms of FAAH1, the emerging biological significance of FAAH2, and the implications of their differences for inhibitor selectivity and preclinical-to-clinical translation. We further synthesize experimental and clinical evidence supporting FAAH modulation in pain, anxiety, post-traumatic stress disorder, epilepsy, and neurodegenerative conditions. Despite promising preclinical findings, clinical investigations have revealed substantial challenges, including inconsistent therapeutic efficacy despite robust target engagement and safety concerns associated with insufficient inhibitor selectivity. Advances in covalent and non-covalent inhibitor development are therefore discussed alongside emerging computational strategies, including molecular simulations, explainable artificial intelligence, and generative molecular design. By integrating structural biophysics, neurological mechanisms, and pharmacological evidence, this review highlights the importance of understanding FAAH as a dynamic, membrane-associated regulator of lipid signaling rather than a static enzymatic target. Such an integrated perspective may guide the development of more selective inhibitors and support improved translation of FAAH-targeted therapeutic strategies.

Introduction

Fatty acid amide hydrolase (FAAH) lies at the interface between membrane biophysics, lipid signaling, and neurobiology. By regulating the turnover of anandamide (AEA) and related N-acylethanolamines, FAAH influences lipid signals involved in pain, stress, fear, neuronal excitability, and neuroinflammation (Bracey et al. 2002; Palermo, Rothlisberger, et al. 2015). Its function, however, depends on more than catalytic chemistry: membrane association, substrate access, conformational dynamics, active-site organization, and oligomeric coupling together shape how efficiently lipid substrates are processed. Although FAAH has been extensively investigated by structural biology, molecular simulation, pharmacology, and clinical studies, these areas are often considered separately. This review provides an updated overview of structural studies of FAAH, with particular emphasis on how its membrane-associated organization and conformational properties influence substrate access, catalytic activity, and inhibitor binding. These structural insights are further discussed in the context of drug design, highlighting how the architecture and dynamics of FAAH can inform the development and optimization of inhibitors. Particular attention is also given to FAAH2, a related FAAH found in humans and other primates but absent in rodents, an important species difference that may complicate the translation of preclinical findings to humans (Palermo, Rothlisberger, et al. 2015). In parallel, the review updates current evidence linking FAAH activity and endocannabinoid metabolism to neurological and neuropsychiatric disorders. Finally, it summarizes the development of FAAH inhibitors, with a focus on compounds that have progressed into clinical evaluation, their outcomes, and the challenges that have

limited the therapeutic translation of FAAH inhibition. Taken together, these advances are discussed to identify current gaps in FAAH biology and to consider how structural biology, computational biophysics, and emerging AI-based approaches may support future FAAH-targeted drug discovery.

Structural architecture and functional dynamics of FAAH1

FAAH belongs to the amidase-signature (AS) family, a diverse group of amide-hydrolyzing enzymes found in both prokaryotes and eukaryotes. Members of this family share a conserved ~ 130-residue AS region within an α/β/α structural core, enriched in serine and glycine residues and organized around an unusual Ser–Ser–Lys catalytic machinery, rather than the classical Ser–His–Asp triad of many serine hydrolases.

FAAH represents a specialized AS-family enzyme adapted for hydrolysis of hydrophobic fatty acid amides, including AEA and oleamide (Palermo, Bauer, et al. 2015). While its catalytic core retains the conserved chemistry of the AS family, surrounding structural elements are specialized for membrane association and lipid-substrate access. FAAH can therefore be viewed as a conserved amidase catalytic scaffold modified to process membrane-derived signaling lipids.

Fatty acid amide hydrolase 1 (FAAH1) is the prototypical mammalian member of the AS family, a diverse group of amide-hydrolyzing enzymes found across bacteria, archaea, and eukaryotes. AS enzymes share a conserved, serine- and glycine-rich catalytic region embedded within an alpha/beta/alpha core and employ an unconventional Ser-Ser-Lys catalytic system rather than the classical Ser-His-Asp triad of many serine hydrolases (Giang et al. 1997). FAAH retains this conserved chemical machinery but is distinguished by structural adaptations that couple catalysis to the membrane environment. These adaptations enable the enzyme to recruit and hydrolyze hydrophobic signaling lipids, including AEA, oleamide, and related fatty acid amides, at the membrane-water interface (Cravatt et al. 1996).

The available structural, biochemical, and simulation data therefore support a model in which FAAH function emerges from the integration of five features: parallel dimerization, monotopic membrane association, a branched and dynamic substrate-trafficking cavity, an unusual proton-transfer network, and longer-range regulation by interprotomer and protein-lipid interactions (McKinney et al. 2005). Importantly, these features are not supported equally by the evidence. The global fold, catalytic residues, and principal cavities are defined directly by crystallography and mutagenesis, whereas substrate-trafficking pathways, allosteric coupling, and sterol-dependent regulation remain more dependent on molecular dynamics, docking, and indirect biochemical measurements.

Quaternary structure and conserved amidase fold

Crystallographic studies of rat FAAH and humanized rat variants have established a parallel homodimeric architecture in which the membrane-binding surfaces and active-site entrances of both protomers face the same side of the complex (Bracey et al. 2002; Mileni et al. 2010). This organization differs from the antiparallel arrangement found in some bacterial AS enzymes and is geometrically compatible with simultaneous engagement of both protomers with a single membrane surface (Fig. 1A). Each approximately 63-kDa protomer contains 579 residues and adopts the conserved AS fold, comprising a central twisted

beta-sheet of 11 mixed strands surrounded by an extensive alpha-helical scaffold of approximately 24 helices (Bracey et al. 2002; Mileni et al. 2008).

Each protomer contains a complete catalytic triad and substrate-access architecture, indicating that the neighboring subunit is not required to construct the active site (Fig. 1A). Dimerization may nevertheless stabilize the membrane-facing orientation, constrain conformational fluctuations, and permit communication between the two catalytic centers (Patricelli et al. 1998). The latter possibility is discussed below, but it should not be inferred solely from the dimeric crystal structure. Although the parallel dimer provides a persuasive structural framework for membrane-associated catalysis, whether FAAH behaves as a strictly obligate dimer in every physiological environment and whether its active sites display classical kinetic cooperativity remain incompletely resolved (Dainese et al. 2014).

Structural basis of membrane association

Unlike most AS-family hydrolases, FAAH is adapted to function at intracellular membranes, particularly the endoplasmic reticulum and, in some cellular contexts, the outer mitochondrial membrane (Giang and Cravatt 1997; Kaczocha et al. 2009). This localization is mechanistically relevant because AEA and related N-acylethanolamines partition strongly into lipid bilayers. FAAH must therefore achieve more than stable membrane attachment, it must orient its buried active site so that membrane-resident substrates can reach the catalytic center without first undergoing energetically unfavorable desorption into bulk water (Dainese et al. 2014; McKinney and Cravatt 2005).

Membrane association involves at least two structural elements. The N-terminal hydrophobic segment, encompassing approximately residues 7–29, contributes to membrane targeting and may influence higher-order organization (Palermo, Bauer, et al. 2015). However, N-terminally truncated FAAH remains catalytically active and strongly membrane associated, demonstrating that this segment is neither the sole membrane anchor nor an essential component of the chemical reaction. A second, more distinctive determinant is the hydrophobic plateau formed primarily by the alpha18-alpha19 helix-turn-helix region around residues 410–438 (Fig. 1A, B). This insertion interrupts the otherwise conserved AS fold and creates a relatively flat membrane-facing surface adjacent to the substrate-entry pathway (Patricelli et al. 1998). Hydrophobic side chains can penetrate one leaflet of the bilayer, whereas neighboring basic residues can interact with phospholipid headgroups, supporting a monotopic rather than a conventional multipass topology (McKinney and Cravatt 2005).

Active-site architecture and the branched substrate-trafficking network

The FAAH catalytic center is buried within the protein and communicates with the membrane and cytosol through a branched cavity conventionally divided into the membrane-access channel (MAC), the acyl-chain-binding pocket (ABP, also termed the ACB pocket), and the cytosolic port (CP) (Fig. 1C). These regions are functionally distinct but not physically isolated. The MAC and ABP merge near the catalytic core and their apparent boundaries depend on ligand occupancy and side-chain conformation (Fig. 1C). Therefore, the frequently used description of a “three-channel system” is useful schematically but should not imply three equivalent, permanently open tunnels.

The amphipathic MAC originates near the hydrophobic plateau and provides the most plausible route for substrate entry from the bilayer (Fig. 1D). Asp403 and Arg486 contribute a polar component that may assist movement of the fatty-acid-amide headgroup, whereas the surrounding hydrophobic surface remains compatible with the lipid acyl chain. Trp531– contributes to the inner boundary of this pathway. Direct visualization of a complete substrate-entry event is not yet available, however, and the trafficking model is derived from the orientation of ligand-bound structures, mutagenesis, and membrane-embedded molecular dynamics simulations rather than from experimentally resolved transport intermediates.

Adjacent to the MAC, the ABP forms a predominantly hydrophobic branch that accommodates and repositions the fatty-acyl chain. Residues commonly associated with the pocket include Leu192, Phe194, Phe244, Leu380, Phe381, Leu404, Phe432, Met436, and Ile491 (Fig. 1E). The pocket is therefore better considered a conformationally adaptable binding region than a rigid container. Its local geometry helps align the scissile amide bond with Ser241 while allowing structurally diverse substrates to sample alternative acyl-chain conformations.

Species-dependent substitutions further emphasize the pharmacological importance of this region. Six positions commonly highlighted in comparisons of rat and human FAAH are Leu192/Phe192, Phe194/Tyr194, Ala377/Thr377, Ser435/Asn435, Ile491/Val491, and Val495/Met495, respectively (Fig. 2A). These substitutions preserve the global fold but alter local sterics, polarity, aromaticity, hydration, and ligand-induced rearrangements. Differences in inhibitor potency between rodent and human FAAH should therefore not be reduced to cavity volume or to a single residue, particularly when extrapolating preclinical pharmacology to the human enzyme (Mileni et al. 2008).

The branched cavity is conformationally heterogeneous. Briefly, Phe432 and Trp531 form a dynamic boundary between the MAC and ABP (Fig. 1F). Combined molecular dynamics, mutagenesis, and kinetic analyses suggest that these residues act as a “dynamic paddle,” redistributing cavity volume and guiding flexible acyl chains between the MAC, a transition region, and the ABP (Palermo, Bauer, et al. 2015). AEA samples curved, pre-reactive conformations more readily than less efficiently hydrolyzed substrates such as oleamide or palmitoylethanolamide (PEA), supporting the view that selectivity depends not only on equilibrium affinity but also on the probability of reaching catalytically competent geometries (Palermo et al. 2013). Even so, the paddle model remains a dynamic interpretation rather than a directly visualized molecular trajectory, and its proposed motions should be described as MD-supported and experimentally constrained rather than definitive.

Catalytic machinery: the unconventional Ser-Ser-Lys triad

At the center of the cavity, FAAH employs the conserved Ser241-Ser217-Lys142 triad characteristic of the AS family. Ser241 is the catalytic nucleophile: its hydroxyl attacks the substrate carbonyl to generate a tetrahedral intermediate, which collapses to form a covalent fatty-acyl-enzyme species (Fig. 2C). Mutation of Ser241 abolishes hydrolytic activity and prevents covalent labeling by serine-reactive fluorophosphonate probes, establishing its essential catalytic role (McKinney et al. 2003).

Lys142 serves as the principal general acid-base catalyst. During acylation, a neutral Lys142 is proposed to facilitate activation of Ser241, after nucleophilic attack, protonated Lys142 contributes to protonation of the leaving group (Palermo, Bauer, et al. 2015). During deacylation, the same network activates an incoming water molecule and subsequently regenerates the free serine (Mileni et al. 2010). Because the intrinsic solution pKa of lysine would not favor this behavior, the catalytic function of Lys142 depends on the electrostatic and hydration environment of the active site rather than on the isolated properties of the side chain (Fig. 2B)

Ser217 bridges the spatial separation between Ser241 and Lys142 and is commonly described as a proton-transfer relay. Its replacement by alanine reduces hydrolytic activity by approximately 2,000-fold, supporting a central role in catalysis (McKinney and Cravatt 2003). Nevertheless, a simple linear “proton shuttle” model is probably incomplete. Proton transfer is more likely to involve coordinated rearrangement of the triad, substrate or leaving group, and active-site water network, with the dominant pathway potentially changing between acylation and diacylation (Palermo et al. 2013).

Tetrahedral intermediates are stabilized by an oxyanion hole formed by the backbone amide groups of Ile238, Gly239, Gly240, and Ser241 (Fig. 2C). FAAH catalysis therefore couples substrate positioning by the MAC-ABP network to nucleophilic attack by Ser241, proton transfer through the Ser217-Lys142 network, and electrostatic stabilization by the oxyanion hole (Bracey et al. 2002; Palermo, Bauer, et al. 2015). This overall mechanism is well supported, but the temporal ordering of individual proton-transfer events and the identity and exchange pathway of catalytically relevant waters remain areas of continued mechanistic refinement.

Interprotomer allostery and membrane sterol interactions

FAAH may be regulated at two levels beyond the local catalytic cavity: communication across the dimer interface and modulation by the surrounding lipid environment. Biochemical studies have reported that partial occupation of the dimer by inhibitors can suppress activity more strongly than expected for two fully independent sites. Trp445 has been implicated in this behavior, and mutation at this position can alter the apparent cooperative response while retaining substantial catalytic activity (Palermo, Bauer, et al. 2015). Structurally, Trp445 lies near the CP and participates in interprotomer contacts, providing a plausible route by which ligand-dependent changes in one active site could influence hydration, channel geometry, or conformational populations in the neighboring protomer (Fig. 2D) (Bracey et al. 2002).

This proposed interprotomer coupling should be distinguished from Phe432-Trp531 gating within a single active site (Sasso et al. 2015). The latter concerns local substrate positioning between the MAC and ABP, whereas Trp445-mediated communication is proposed to operate across the dimer interface (Fig. 1F, 2D). Evidence for the two processes is not equivalent. Indeed, local gating is supported by simulation, mutagenesis, and substrate-dependent kinetics, while classical allostery across the dimer still requires more rigorous demonstration through binding stoichiometry, quantitative kinetic models, and structural characterization of asymmetric states in membrane-like environments (Palermo, Bauer, et al. 2015). Apparent asymmetry in crystal structures alone could also reflect crystal packing, incomplete occupancy, or ligand-specific conformational selection.

The hydrophobic plateau has additionally been proposed to accommodate cholesterol and steroid-related molecules, including pregnenolone, progesterone, and testosterone (Dainese et al. 2014; Sabatucci et al. 2019). Such interactions would occur outside the Ser241-centered catalytic machinery and could modulate membrane affinity, monotopic insertion, or MAC dynamics rather than directly compete with substrates at the catalytic site. However, the location of a discrete steroid-binding site has been inferred mainly from docking and membrane-binding measurements rather than directly resolved crystallographically (Sabatucci et al. 2019).

Taken together, FAAH is not adequately described as a rigid serine hydrolase with a passive lipid-binding tunnel. Its activity depends on the coupling of membrane orientation, conformationally adaptable substrate-access cavities, catalytic proton and water networks, and potentially longer-range dimer and lipid-mediated regulation. The strongest evidence supports the global fold, membrane-facing architecture, branched active-site cavity, and Ser241-Ser217-Lys142 catalytic machinery. By contrast, the detailed trajectories of substrate entry, the extent of interprotomer cooperativity, and the existence of discrete regulatory sterol sites remain working models that require further validation by time-resolved structural methods, quantitative biophysics, and experiments in native-like membranes.

Key target for covalent inhibitor design

FAAH is particularly attractive for covalent inhibitor design because its catalytic mechanism depends on the nucleophilic Ser241. Electrophilic inhibitor groups can be positioned within the active site so that Ser241 attacks the inhibitor, generating a stable or long-lived covalent adduct that blocks catalytic turnover. This strategy has been successfully exploited by several FAAH inhibitor classes, including carbamates such as URB597 and urea-based inhibitors such as PF-750 and PF-622.

Importantly, effective covalent inhibition depends not simply on electrophilic reactivity but also on selective recognition and correct positioning of the warhead relative to Ser241. FAAH therefore provides a well-characterized catalytic nucleophile within a defined binding environment, making it a useful target for structure-guided covalent inhibitor development.

FAAH2 as off-target or another promising therapeutic target

Humans also express FAAH2, a relevant secondary target of FAAH inhibitors but its potential as an independent therapeutic target remains unestablished. Discovered in 2006, FAAH2 shares approximately 20% amino acid sequence identity with the canonical enzyme FAAH1 (Fig. 3A). However, structural superposition of human FAAH2 and FAAH1 revealed a similar overall protein fold, with all three catalytic residues conserved between the two proteins (Fig. 3B and 3C). Both enzymes hydrolyze oleamide, whereas FAAH1 exhibits greater activity towards N-acelethanolamines, including AEA. Importantly, FAAH2 is present in humans but absent from mice and rats, creating a potential limitation when translating FAAH biology and pharmacology from conventional rodent models to humans (Wei et al. 2006).

The biological rationale for targeting FAAH2 derives partly from its distinct intracellular localization. In transfected cells, FAAH2 associates with lipid droplets, whereas FAAH1 localizes to the endoplasmic

reticulum. Despite relatively low activity in homogenates, FAAH2-mediated hydrolyssi of AEA and PEA in intact cells reached approximately 30–40% of that observed with FAAH1. These findings support a contribution to compartmentalized lipid-amide metabolism, although they do not establish the contribution of endogenous FAAH2 in individual human tissues. Selective inhibition could therefore modulate lipid pools differently from FAAH1 inhibition, but this remains a hypothesis requiring direct testing (Kaczocha et al. 2010).

Whether FAAH2 should be considered an off-target depends on the intended pharmacological profile. For a compound developed to inhibit FAAH1 selectively, FAAH2 engagement represents additional activity that requires characterization. ASP8477, for example, inhibited human FAAH1 and FAAH2 in vitro with IC50 values of 3.99 and 57.3 nM, respectively (Watabiki et al. 2017). Nevertheless, inhibition of both enzymes is not necessary to increase circulating fatty acid ethanolamides: JNJ-40355003, whichh showed no detectable FAAH2 inhibition in the reported assay, elevated plasma AEA, oleoylethanolamide (OEA) and PEA in cynomolgus monkeys.Thus, broader substrate preservation provides a possible rationale for dual inhibition, but and additional therapeutic benefit cannot be assumed (Keith et al. 2012)

FAAH2 engagement also should not be equated with toxicity. Activity-based protein profiling identified FAAH2 as an additional target of PF-04457845 at higher tested concentrations. BIA 10-2472 inhibited FAAH2 together with other serine hydrolases, including ABHD6, CES2 and PNPLA6, and produced alterations in lipid networks in human cortical neurons. These findings support investigating the consequences of broad hydrolase inhibition, but do not identify FAAH2 as the cause of the neurological toxicity observed in the BIA 10-2474 trial. Selectivity should therefore be evaluated in human systems across relevant exposure ranges, with FAAH2 assessed separately from other intended targets (van Esbroeck et al. 2017).

The strongest experimental rationale for therapeutic investigation concerns stress-associated behavior. Genetic disruption of faah2a in larval zebrafish attenuated locomotor responses to hyperosmotic stress without impairing the response to nicotine. This supports a role for FAAH2-related signaling in stress responses, but is not evidence that a selective human FAAH2 inhibitor would provide anxiolytic efficacy (Krug et al. 2018). Human genetics also argues for caution when inferring the desired direction of modulation. The p.Ala458Ser variant was associated with partial enzyme inactivation and altered lipid metabolism in a patient with neurological and psychiatric symptoms (Sirrs et al. 2015). A subsequent report described p.Gln330Lys in a patient with neurodevelopmental and metabolic abnormalities, with structural modelling suggesting an effect on the AS domain. These individual observations do not establish causality, and lifelong genetic impartiment cannot be considered equivalent to pharmacological inhibition initiated in adulthood (Vinci et al. 2025).

FAAH2 should therefore be included in the selectivity assessment of FAAH1 inhibitors while remaining an exploratory therapeutic target. Its validation requires selective chemical probes, genetic confirmation of target-dependent effects, and lipidomic analyses in human models expressing endogenous FAAH2. Comparison of FAAH1-selective, FAAH2-selecitve and dual inhibition should establish whether FAAH2 engagement adds efficacy and whether that benefit is compatible with preserved lipid homeostasis and predictable drug disposition. The current evidence supports investigation of FAAH2, but does not yet justify describing its inhibition as a validated therapeutic strategy.

Neurological and neuropsychiatric relevance: a mechanism-first synthesis

The neurological relevance of FAAH stems from its structural control over AE) and related N-acylethanolamine turnover. Membrane association, substrate-channel accessibility, catalytic efficiency, and conformational dynamics together determine how long these lipid signals persist and therefore how strongly they influence neuronal signaling **(**Fig. 4**).**

Pain and nociceptive processing

Nociceptive processing (transduction, transmission, modulation, perception) is shaped by FAAH, which acts as a critical “gatekeeper” controlling the availability and duration of endogenous AEA signaling (Elgohary et al. 2026; Karcz et al. 2024). On-demand AEA acts retrogradely on presynaptic CB1 receptors (CB1R), whose Gi/o signaling inhibits voltage-gated Ca^{2+} channels, activates K^+ channels, and suppresses the release of glutamate, substance P, and CGRP, a mechanism recently reviewed at the spinal nociceptive synapse (Elgohary and Baraka 2026; Spicarova et al. 2024). FAAH-sensitive lipid mediators, including PEA and OEA, further contribute to antinociception through PPARα-related and neuroimmune pathways (Grim et al. 2014), while AEA also acts on TRPV1, producing pronociceptive or desensitizing effects depending on context (Spicarova and Palecek 2024; Carey et al. 2016).

Pharmacological or genetic FAAH inhibition raises endogenous AEA and related lipids, producing analgesic effects across inflammatory, visceral, neuropathic (URB597 (Larauche et al. 2024), PF-3845 (Grim et al. 2014)), and trigeminal (URB937 (Sasso et al. 2012), JZP327A (Greco et al. 2023)) pain models. Within the dorsal periaqueductal gray, nerve injury increases FAAH and depletes AEA, contributing to hyperalgesia and impaired sympathetic regulation that local FAAH inhibition reverses via CB1R (Woyach et al. 2022). Combined FAAH + COX inhibition or dual FAAH/MAGL inhibition (JZL195, AKU-005) further enhances antinociception (Greco et al. 2024). However, effects remain context-dependent: FAAH deficiency can produce TRPV1-mediated hyperalgesia under challenges such as capsaicin (Carey et al. 2016).

Human genetics reinforces FAAH’s role in pain sensitivity: a microdeletion at the *FAAH-OUT* locus markedly reduces FAAH expression and is associated with a rare pain-insensitivity phenotype, reduced anxiety, and faster wound healing, with subsequent studies implicating BDNF-, WNT-, and ACKR3-related pathways (Mikaeili et al. 2023). Clinical translation, however, remains limited—the selective inhibitor PF-04457845 achieved > 96% FAAH inhibition yet failed to produce significant analgesia in knee osteoarthritis (Huggins et al. 2012). FAAH is thus best viewed as a context-dependent regulator of nociceptive signaling, shaped by interactions among CB1R, CB2R, TRPV1, and PPARα, rather than a simple analgesic switch (Carey et al. 2016; Grim et al. 2014; Mikaeili et al. 2023).

Anxiety, stress, fear extinction, and post-traumatic stress disorder

FAAH regulates stress and anxiety by controlling AEA/CB1R signaling in corticolimbic circuits. Acute stress increases CRH/CRHR1-driven FAAH activity in the basolateral amygdala (BLA) and mPFC, reducing AEA

availability, weakening inhibitory control of glutamatergic signaling, and promoting HPA-axis activation and anxiety-like behavior; repeated stress sustains FAAH elevation and is associated with BLA structural remodeling (Gunduz-Cinar et al. 2013). During fear extinction, BLA AEA release promotes CB1R-dependent inhibitory long-term depression, supporting extinction-memory consolidation and recall (Gunduz-Cinar et al. 2013; Mayo et al. 2020). Consistently, the *FAAH* C385A (rs324420) variant reduces enzyme stability and increases AEA availability; A-allele carriers show blunted amygdala threat reactivity, stronger prefrontal–amygdala connectivity, and generally enhanced extinction learning or recall, although not all behavioral effects are consistently replicated (Dincheva et al. 2015; Mayo et al. 2020; Spagnolo et al. 2016).

Pharmacological FAAH inhibition (URB597, PF-04457845, URB694, SSR411298) prevents stress-induced AEA depletion and reduces anxiety- and stress-related behaviors, including in preclinical post-traumatic stress disorder (PTSD) models (Carnevali et al. 2025; Gunduz-Cinar et al. 2013). In healthy humans, PF-04457845 increased circulating AEA approximately tenfold, enhanced 24-h extinction recall, and attenuated autonomic and subjective stress responses without altering fear acquisition (Mayo et al. 2020), while JNJ-42165279 reduced amygdala, ACC, and insular responses during emotional-face processing (Paulus et al. 2021). Positive signals in cannabis withdrawal and social anxiety further suggest that the clinical effects of FAAH inhibition may be disorder-specific (D'Souza et al. 2019).

These findings are particularly relevant to PTSD, which is characterized by impaired fear extinction and recall, threat overgeneralization, hyperarousal, and dysregulated stress responses (Carnevali et al. 2025). Chronic PTSD has been associated with reduced circulating AEA and impaired endocannabinoid mobilization during stress, while higher AEA availability or the *FAAH* C385A A allele has been linked to lower PTSD symptom severity, particularly hyperarousal, following trauma exposure (Dincheva et al. 2015; Mayo et al. 2020). Translation to PTSD, however, remains limited: a 2025 RCT of JNJ-42165279 in 100 patients increased AEA but showed no improvement in CAPS-5, PCL-5, or neuroimaging outcomes compared with placebo plus iCBT, and JZP150 (formerly PF-04457845) likewise failed to meet its primary PTSD endpoint (Mayo et al. 2025; Flynn 2023). Proposed explanations include persistent and generalized traumatic memories, altered CB1R function, and the possibility that increasing AEA alone is insufficient to restore dysfunctional fear-regulation circuits; differences in CB1R efficacy between AEA and 2-AG have also motivated interest in MAGL inhibition (Mayo et al. 2025). FAAH thus remains a biologically relevant regulator of stress resilience and fear extinction, with its therapeutic efficacy in PTSD likely depending on disease state, CB1R function, and the broader endocannabinoid and neuroimmune context.

Epilepsy and neuronal excitability

Beyond its roles in synaptic and stress signaling, FAAH-dependent AEA/CB1R signaling contributes to the control of pathological neuronal excitability (Grim et al. 2014; Morais et al. 2026; Sugaya et al. 2021). URB597 reduces kainic-acid-induced hippocampal pyramidal-cell firing and burst activity, partly through CB1R, while sparing basal firing (Coomber et al. 2008). In the basolateral amygdala and dentate gyrus, FAAH inhibition also restores tonic CB1-mediated control of glutamate release and reduces seizure-related afterdischarges, suggesting preferential modulation of excessive neuronal activity rather than global suppression (Coomber, O'Donoghue, and Mason 2008; Colangeli et al. 2017).

These effects on neuronal excitability are consistent with the anticonvulsant activity of FAAH inhibitors across kainate, bicuculline, PTZ, electrical-stimulation, and absence-epilepsy models. Notably, PF-04457845 markedly suppresses spike-wave discharges in GAERS rats by enhancing endogenous AEA signaling in thalamocortical circuits, and direct infusion into the ventrobasal thalamus reproduces this effect (Morais et al. 2026). Dual FAAH/MAGL inhibition with AM6701 provides greater neuroprotection than selective FAAH inhibition in kainate models, preserving spectrin, GluA1, synapsin IIb, and pGluA1-Ser845 (Naidoo et al. 2012). However, complete FAAH deficiency causes excessive AEA accumulation and worsens severe seizures and hippocampal injury, potentially through excessive CB1-mediated suppression of GABA release and/or TRPV1 activation. Thus, FAAH modulation may have a therapeutic window in which moderate AEA elevation is protective, whereas excessive accumulation becomes detrimental (Clement et al. 2003; Sugaya and Kano 2021).

Importantly, this therapeutic potential may extend beyond seizure suppression because FAAH inhibition can preserve synaptic plasticity and influence epilepsy-associated behavioral abnormalities. Unlike WIN55,212-2, which suppresses LTP and short-term potentiation, URB597 preserves normal plasticity and can improve seizure-associated deficits, while restoration of AEA signaling may reduce kindling-related anxiety-like and fear-memory abnormalities (Colangeli et al. 2017). However, human genetic evidence remains limited: in 105 Chinese children with epilepsy, FAAH C385A (rs324420) was not significantly associated with antiseizure-medication response or drug-induced liver injury, suggesting that this variant may have less direct relevance to seizure control than to the affective phenotypes discussed above (Wang et al. 2025).

Neurodegeneration and neuroinflammation

FAAH regulates neuroinflammatory tone by controlling the availability of endogenous N-acylethanolamines with homeostatic and neuroprotective functions. Hydrolysis of AEA, PEA, and OEA limits their anti-inflammatory signaling, while AEA hydrolysis also generates arachidonic acid, which can contribute to COX-2-dependent prostaglandin production (Arnanz et al. 2025; Spicarova and Palecek 2024; Tchantchou et al. 2014; Jain et al. 2022). In neurodegenerative and brain-injury settings, altered FAAH activity has been associated with changes in lipid signaling and glial inflammatory responses (de Ceballos 2025; Jain et al. 2022). Conversely, pharmacological inhibition of FAAH, including with URB597 or PF-3845, can increase endogenous N-acylethanolamines and reduce inflammatory mediators such as TNF-α, IL-1β, IL-6, CCL2, iNOS, and COX-2 (Arnanz et al. 2025; Cammarota et al. 2023; de Ceballos 2025; Rivera et al. 2018; Armeli et al. 2024; Grieco et al. 2021). In traumatic brain injury, PF-3845 further reduced cortical lesion volume and neuronal degeneration and preserved proteins associated with neuronal and synaptic integrity, supporting a role for FAAH-regulated lipid signaling in limiting secondary neural damage (Tchantchou et al. 2014).

Beyond the regulation of inflammatory signaling, these effects may influence several pathological processes underlying neurodegeneration. In Alzheimer's disease (AD) models, FAAH inhibition or genetic deletion has been associated with reduced BACE1 expression and Aβ production, with recent evidence supporting a CB1-dependent mechanism that may involve epigenetic regulation of the *Bace1* promoter. FAAH inhibition can also favor non-amyloidogenic APP processing and enhance microglial uptake and clearance of Aβ (de Ceballos 2025). In addition, URB597 has been reported to improve autophagic function and preserve dendritic spines, synaptic plasticity, and cognitive performance in experimental models. These protective

effects are not restricted to AD (Armeli et al. 2024), as URB597 reduced neuronal loss and gliosis in a TDP-43 model of frontotemporal dementia (Santos-Garcia et al. 2023). Together, these findings suggest that FAAH may influence neurodegenerative progression through interconnected effects on lipid homeostasis, glial activity, protein clearance, and neuronal integrity.

However, the relationship between FAAH and neuroinflammation is strongly context-dependent, particularly because FAAH is expressed mainly in neurons and astrocytes rather than microglia (Arnanz et al. 2025; Cammarota et al. 2023; Jain et al. 2022). Changes in neuronal or astrocytic FAAH can therefore modify microglial behavior through heterocellular lipid signaling. Notably, complete FAAH deletion in 5xFAD mice increased selected inflammatory signals while promoting a disease-associated, phagocytic microglial phenotype, enhancing Aβ uptake and reducing plaque growth; this illustrates that increased inflammatory markers do not necessarily indicate a detrimental microglial response (Arnanz et al. 2025; Ruiz-Perez et al. 2021; Vazquez et al. 2015). Moreover, AEA can undergo COX-2-dependent conversion to prostamides, providing another pathway through which increased AEA availability may produce context-dependent effects (Grim et al. 2014; Santos-Garcia et al. 2023). From a therapeutic perspective, FAAH inhibition may provide a more physiologically constrained approach to enhancing endocannabinoid signaling than direct CB1 agonism; however, the severe and fatal neurotoxicity associated with BIA 10-2474 in a 2016 clinical trial highlights the importance of inhibitor selectivity and off-target safety (Mikaeili et al. 2023). Thus, the therapeutic value of FAAH inhibition may depend not simply on increasing AEA levels, but on achieving appropriate target selectivity, cellular modulation, and exposure.

Development of FAAH inhibitors

Overview

A large number of FAAH inhibitors have been reported with diverse chemical scaffolds and pharmacological profiles. These compounds can be broadly classified into irreversible covalent, reversible-covalent, and reversible non-covalent inhibitors, according to their interactions with the catalytic site, particularly Ser241 (Keith et al. 2015; Tuo et al. 2017; Nicoara et al. 2025; Landolfi et al. 2026; Santana et al. 2026; Tripathi 2020). Early development focused on covalent inhibitors such as URB597, PF-3845, and PF-04457845, which provided high potency and prolonged target engagement. Reversible-covalent inhibitors, exemplified by OL-135 and related α-ketoheterocycles, were developed to combine covalent recognition with controlled reversibility (Jain et al. 2025; Keith et al. 2015; Santana et al. 2026).

Reversible non-covalent inhibitors have also been developed from diverse scaffolds, including aryl isoxazoles, aryloxyacetamides, pyrimidines, and ketobenzimidazoles (Tripathi 2020). In parallel, multitarget-directed ligands (MTDLs) have combined FAAH inhibition with modulation of MAGL, COX, or soluble epoxide hydrolase (sEH). Importantly, multitarget activity represents a separate design strategy from the covalent/non-covalent classification (Tripathi 2020; Landolfi et al. 2026; Deplano et al. 2017). Given the extensive number of FAAH inhibitors reported in the literature, this review does not aim to provide an exhaustive catalogue, but instead highlights representative compounds that capture major mechanistic

classes, medicinal chemistry advances, multitarget strategies, and key lessons from clinical development (Table 1).

Irreversible covalent FAAH inhibitors

Carbamates and aryl ureas established irreversible covalent inhibition as an effective strategy for FAAH. URB597 became a widely used pharmacological tool (Sasso et al. 2012; Tuo et al. 2016), whereas URB937 demonstrated the potential of peripheral FAAH inhibition through restricted tissue distribution (Landolfi et al. 2026; Couttas et al. 2026; Moreno-Sanz et al. 2013). Subsequent aryl ureas, including PF-3845 and PF-750, improved potency and selectivity (Ahn et al. 2009; Mileni et al. 2008).

PF-04457845 represented a major advance, showing nanomolar potency and high selectivity for FAAH (Ahn et al. 2011). Its clinical development, however, highlighted the distinction between target engagement and therapeutic efficacy: despite producing > 96% FAAH inhibition in patients with knee osteoarthritis, it did not significantly improve pain outcomes (Couttas et al. 2026; Huggins et al. 2012).

JNJ-42165279 is another potent covalent FAAH inhibitor with prolonged central and peripheral target engagement (Keith et al. 2015; van Egmond et al. 2021). Preclinical studies demonstrated covalent FAAH inactivation, while PET studies confirmed substantial brain FAAH occupancy in humans. Because hydrolytic reversal of the enzyme–inhibitor adduct has also been described, JNJ-42165279 is better characterized as a covalent inhibitor with prolonged target engagement rather than being assigned rigidly to either irreversible or reversible-covalent categories (Keith et al. 2015; Postnov et al. 2018).

Reversible-covalent FAAH inhibitors

OL-135 is a representative α-ketoheterocycle that forms a transient covalent hemiketal with Ser241, producing reversible FAAH inhibition and increasing brain AEA levels (Alexander et al. 2005; Keith et al. 2015). Further optimization introduced additional electrophilic interactions with Cys269, resulting in prolonged enzyme inhibition and sustained elevation of endogenous FAAH substrates (Otrubova et al. 2013). These studies demonstrated that covalent inhibitor design can be tuned by controlling both chemical reactivity and the residues involved.

Clinically investigated reversible FAAH inhibitors such as V158866 provided an alternative strategy based on sustained pharmacological modulation without relying on permanent enzyme modification (Rocha et al. 2022). Together, these approaches illustrate efforts to balance potency, duration of action, and control over enzyme recovery.

Reversible non-covalent inhibitors and multitarget strategies

Non-covalent FAAH inhibitors were developed to avoid permanent modification of the enzyme. Representative chemotypes include 3-carboxamido-5-arylisoxazoles (Andrzejak et al. 2011; Tourteau et al. 2014), N-aryl-2-aryloxyacetamides (Sunduru et al. 2017), pyrimidines (Keith et al. 2014; Qiu et al. 2016), and ketobenzimidazoles (Min et al. 2011). AZ513 provides a mechanistically informative example: it is a non-covalent, reversible, noncompetitive FAAH inhibitor that does not form a covalent adduct and appears to bind differently from the covalent inhibitor OL-135 (Scott et al. 2011).

MTDL strategies have expanded FAAH inhibitor development beyond selective FAAH blockade. JZL195 and related compounds simultaneously inhibit FAAH and MAGL, thereby increasing both AEA and 2-AG (Landolfi et al. 2026; Long et al. 2009). FAAH/COX hybrids provide another approach; ARN2508, for example, combines covalent FAAH inhibition through Ser241 with non-covalent COX inhibition (Migliore et al. 2016; Palermo et al. 2016). Thus, multitarget compounds can integrate distinct mechanisms within a single molecular framework. FAAH/sEH dual inhibition has also been explored, although many such compounds remain at the preclinical stage (Landolfi et al. 2026; Tsang et al. 2026).

Clinical translation and safety

Clinical studies have demonstrated that strong FAAH target engagement does not necessarily translate into clinical efficacy. PF-04457845 achieved near-complete FAAH inhibition without improving osteoarthritis pain (Couttas et al. 2026; Huggins et al. 2012). More recently, a 2026 phase IIa trial of JNJ-42165279 in major depressive disorder with anxious distress showed marked increases in circulating fatty acid amides, confirming pharmacodynamic target engagement, but no significant difference from placebo in either the primary or secondary clinical outcomes (Schmidt et al. 2026). These findings highlight that biochemical or biomarker-level target engagement alone may be insufficient to predict therapeutic benefit.

A major challenge in FAAH inhibitor development is off-target activity, particularly for covalent inhibitors containing electrophilic warheads (Dider et al. 2016). Insufficiently selective compounds may react with other serine hydrolases, especially at higher systemic exposures. Early activity-based protein profiling (ABPP) studies showed that several FAAH inhibitors, including URB597, can inhibit carboxylesterases and other serine hydrolases (Zhang et al. 2007). The clinical development of BIA 10-2474 further highlighted this concern. Although BIA 10-2474 covalently inhibits FAAH, it also inhibited additional lipid-processing enzymes, including ABHD6, ABHD11, PNPLA6, and carboxylesterases, particularly at higher exposures. It also produced broader lipidomic alterations in human cortical neurons, whereas PF-04457845 showed substantially greater selectivity and mainly increased endogenous N-acylethanolamines (van Esbroeck et al. 2017; Bonifacio et al. 2020). Thus, high FAAH potency does not necessarily predict proteome-wide selectivity or preservation of neuronal lipid homeostasis.

Off-target activity may also arise from drug metabolites. Demethylated metabolites of BIA 10-2474 were shown to covalently modify catalytic cysteines in aldehyde dehydrogenases, including ALDH2. Although their contribution to the neurological toxicity observed clinically remains unresolved, these findings emphasize the need to evaluate both parent compounds and metabolites for proteome-wide reactivity. Importantly, the BIA 10-2474 case should not be interpreted as evidence that FAAH inhibition itself is intrinsically neurotoxic (Huang et al. 2019).

Off-target liability is compound-dependent rather than an inherent property of all FAAH inhibitors (Zhang et al. 2007). PF-3845 and PF-04457845 showed substantially improved selectivity, whereas LY2183240 inhibited FAAH together with multiple brain serine hydrolases (Ahn et al. 2009; Ahn et al. 2011; Alexander et al. 2006; Johnson et al. 2011). Therefore, biological effects observed with FAAH inhibitors should be interpreted in the context of the selectivity profile of each compound. Future development should integrate ABPP, human-cell and tissue-based assays, metabolite characterization, and lipidomics early in the

discovery process (van Esbroeck et al. 2017; Huang et al. 2019). In parallel, AI- and in silico-based approaches, including proteome-wide target prediction, molecular modeling, covalent-reactivity prediction, and machine-learning-guided off-target screening, could help identify potential liabilities before experimental testing and guide the design of more selective inhibitors (Luong et al. 2026).

More recent programs, including BMS-986368, are continuing to investigate dual FAAH/MAGL inhibition in phase II clinical studies (Francois Bethoux 2026; Home ; Home). Overall, FAAH inhibitor development has progressed from maximizing potency toward optimizing target engagement, proteome-wide selectivity, reversibility, tissue distribution, off-target reactivity, metabolite safety, and disease-specific pharmacology.

Table 1
Representative FAAH inhibitors: pharmacological and clinical profiles

Compound	Chemical class / mechanism	FAAH potency	Selectivity / major off-targets	Development status	Source
URB597	O-Aryl carbamate; irreversible covalent	hFAAH IC_{50} ~3 nM; rFAAH ~ 5 nM	Carboxylesterases; FAAH2	Preclinical; early clinical investigation	(Fegley et al. 2005; Mileni et al. 2008)
URB937	O-Aryl carbamate; peripherally restricted	hFAAH IC_{50} ~27 nM	Restricted CNS penetration	Preclinical	(Vozella et al. 2019)
PF-3845	Piperidine urea; irreversible covalent	Ki = 0.23 μM	High selectivity; minimal carboxylesterase/FAAH2 activity	Preclinical	(Ahn et al. 2009)
PF-04457845	Piperidine pyridazine urea; irreversible covalent	hFAAH IC_{50} 7.2 nM	Highly selective; limited FAAH2 activity	Phase II: positive in cannabis use disorder; negative in knee OA pain	(Johnson et al. 2011; Huggins et al. 2012)
JNJ-42165279	Heteroaryl piperazinyl urea; slowly reversible covalent	hFAAH IC_{50} ~70 nM	High selectivity; transient liver enzyme elevations at high doses	Phase II/IIa: SAD, ASD, MDD with anxious distress (negative primary endpoints, 2026)	(Keith et al. 2015; Schmidt et al. 2026)
BIA 10-2474	Imidazole urea; irreversible covalent (genuine on-target)	~ 50–70 nM in situ	ABHD6, ABHD11, PNPLA6, CES1–3, LIPE, FAAH2; metabolites affect ALDH2	Phase I: terminated after fatal neurotoxicity (2016)	(Huang et al. 2019; Bonifacio et al. 2020; Kiss et al. 2018)
OL-135	α-Ketoheterocycle; reversible-covalent	~ 4–15 nM	Relatively selective; reversible Ser241 adduct	Preclinical	(Mileni et al. 2009)
AZ513	Non-covalent, noncompetitive	hFAAH IC_{50} = 551 nM, rat brain FAAH = 27 nM	No covalent Ser241 modification; distinct binding mode	Preclinical	(Scott et al. 2011)
V158866	Azetidine carboxamide; reversible	~ 38 nM	No major off-target liability identified	Phase II: spinal cord injury pain	(Pawsey et al. 2016)

Compound	Chemical class / mechanism	FAAH potency	Selectivity / major off-targets	Development status	Source
				(insufficient efficacy)	
SSR411298	Dioxane carbamate; reversible	mFAAH ~ 62.5 nM	Minimal off-target activity in broad screening	Phase II: MDD and cancer pain (both discontinued)	(Griebel et al. 2018)
JZL195	Dual FAAH/MAGL; covalent	FAAH ~ 2 nM; MAGL ~ 4 nM	Elevates AEA and 2-AG	Preclinical	(Long et al. 2009)
ARN2508	FAAH/COX hybrid; covalent FAAH + non-covalent COX inhibition	rFAAH ~ 31 nM	Dual FAAH/COX	Preclinical	(Palermo et al. 2016)
BMS-986368	Dual FAAH/MAGL; irreversible covalent	Nanomolar range	Selectivity/safety under evaluation	Phase II: MS spasticity and Alzheimer's agitation (recruiting 2026)	(Francois Bethoux 2026; Home ; Home)

Computational biophysics and Artifact Intelligence (AI): future tools for FAAH inhibitor design

We retrieved FAAH inhibitor data from ChEMBL (target ID: CHEMBL2243). Following data cleaning, standardization, and removal of unsuitable entries, the curated dataset contained approximately 1,100 compounds with pIC_{50} values ranging from approximately 6 to 10. Key physicochemical properties were calculated using RDKit, and the majority of compounds were found to be consistent with Lipinski's rule of five, suggesting generally favorable drug-like characteristics. In addition, most compounds exhibited a topological polar surface area (TPSA) below 120 Å², indicating physicochemical properties compatible with cellular permeability. This consideration is particularly relevant for FAAH inhibitor development, as centrally acting FAAH inhibitors may need to penetrate the blood–brain barrier to effectively modulate FAAH activity within the central nervous system. However, BBB permeability depends on multiple molecular properties, and TPSA alone is not sufficient to predict CNS penetration (Fig. 5A). This relatively large and chemically diverse dataset provides a valuable foundation not only for developing predictive machine-learning models with potentially high accuracy, but also for constructing explainable artificial intelligence (XAI) frameworks capable of identifying structural features, scaffolds, and molecular descriptors associated with FAAH inhibitory activity. The diversity of physicochemical properties represented in the dataset may further facilitate exploration of broader chemical space and support the design of structurally diverse FAAH inhibitors.

Applications of modern AI to FAAH inhibitor discovery, however, remain relatively recent and limited compared with conventional computational approaches. In 2026, the FRAIL framework provided an example of generative AI applied specifically to FAAH inhibitor discovery by integrating deep generative modeling, fragment-based molecular design, reinforcement learning, predicted pIC_{50} values, and molecular modeling to generate novel FAAH-focused chemical structures (Fig. 5B**)** (Luong et al. 2026). Nevertheless, the reported compounds were primarily computationally prioritized and lacked extensive experimental validation, highlighting an important gap between AI-driven molecular generation and biological confirmation. Beyond ligand-based prediction, advances in structural and computational biophysics provide an additional layer of information for rational FAAH inhibitor design. Detailed knowledge of FAAH structure, catalytic mechanism, active-site architecture, and ligand–protein interactions can be integrated with AI models to guide the design and prioritization of compounds with mechanistically relevant binding modes. This is particularly important for the development of covalent FAAH inhibitors, in which both molecular recognition and the positioning and reactivity of the electrophilic warhead toward key catalytic-site residues must be carefully optimized. Combining structural modeling, molecular dynamics simulations, covalent docking, XAI, and generative AI may therefore enable future design strategies that move beyond activity prediction toward mechanism-informed generation and optimization of novel FAAH inhibitors.

Conclusions

Several conclusions emerge from the molecular and biophysical features of FAAH discussed here. First, FAAH function is intrinsically coupled to membrane organization: membrane association, the MAC–ABP architecture, and conformational flexibility together determine how lipid substrates access the catalytic Ser–Ser–Lys machinery. Second, FAAH should not be viewed as a rigid monomeric active site, because local channel dynamics and inter-subunit communication within the homodimer introduce additional levels of regulation that can influence substrate processing and inhibitor response. Third, inhibitor efficacy depends on more than affinity alone; covalent reactivity, residence time, conformational state, FAAH1/FAAH2 selectivity, and proteome-wide off-target effects all contribute to pharmacological outcome. Finally, the clinical translation of FAAH inhibition remains constrained by species differences, human-specific FAAH2 biology, spatially heterogeneous lipid signaling, and the disconnect between strong target engagement and variable therapeutic benefit. Taken together, these observations support a multiscale view of FAAH as a dynamic membrane-coupled regulator of lipid signaling rather than a static hydrolase target. Future progress will require closer integration of structural biology, membrane biophysics, molecular simulation, proteome-wide selectivity profiling, and physics-informed AI to resolve conformational ensembles, improve inhibitor design, and better predict how molecular perturbations propagate from FAAH inhibition to neural and clinical outcomes.

Declarations

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Figures

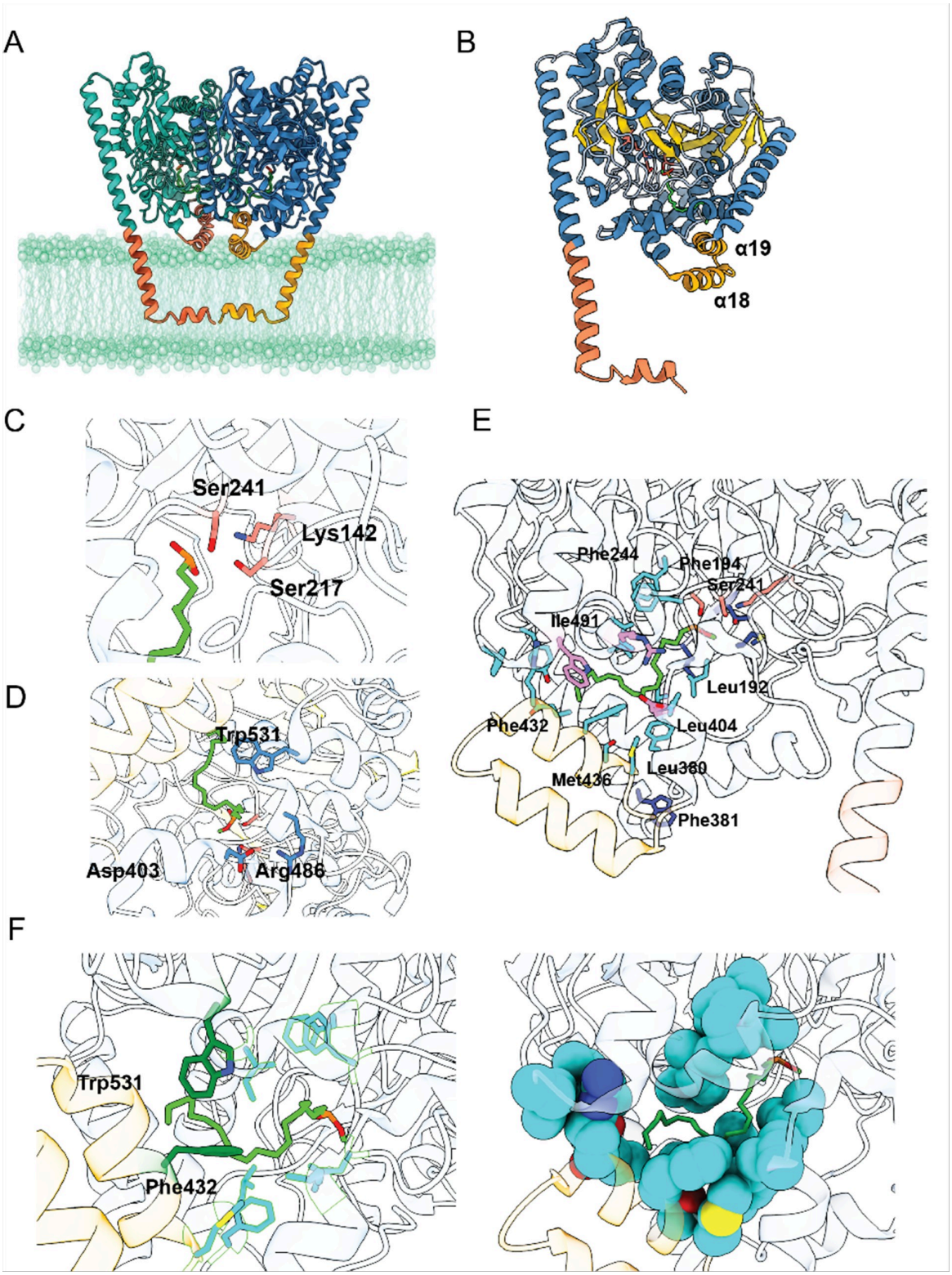

Figure 1

Structural architecture, membrane association, and substrate-access pathways of fatty acid amide hydrolase (FAAH). **A** Membrane-associated FAAH homodimer. The two protomers are shown in teal and blue, the modeled lipid bilayer in pale green, and the N-terminal membrane anchors in orange and red. The hydrophobic plateaus are highlighted in orange. **B** FAAH monomer showing the α-helical scaffold in blue, central β-sheet in yellow, N-terminal membrane anchor in orange-red, and membrane-interacting helices α18

and α19 in orange. **C** Close-up of the unconventional catalytic triad Ser241–Ser217–Lys142, with hydrogen-bonding interactions indicated by dashed lines. **D** Membrane-access region showing the polar residues Asp403 and Arg486 and the gating residues Phe432 and Trp531. **E** Acyl-chain-binding pocket containing the covalently bound methyl arachidonyl fluorophosphonate (MAFP), shown in magenta/cyan, with selected pocket-lining residues labeled. **F** Enlarged views of the Phe432–Trp531 gate at the junction of the membrane-access channel and acyl-chain-binding pocket; pocket-lining residues are shown as sticks on the left and cyan molecular surfaces on the right. Surrounding residues are represented as thin gray lines for structural context. Structures were generated from the rat FAAH–MAFP complex (PDB: 1MT5); the N-terminal membrane anchor, absent from the crystal structure, was modeled from the human FAAH AlphaFold prediction AF-O00519-F1

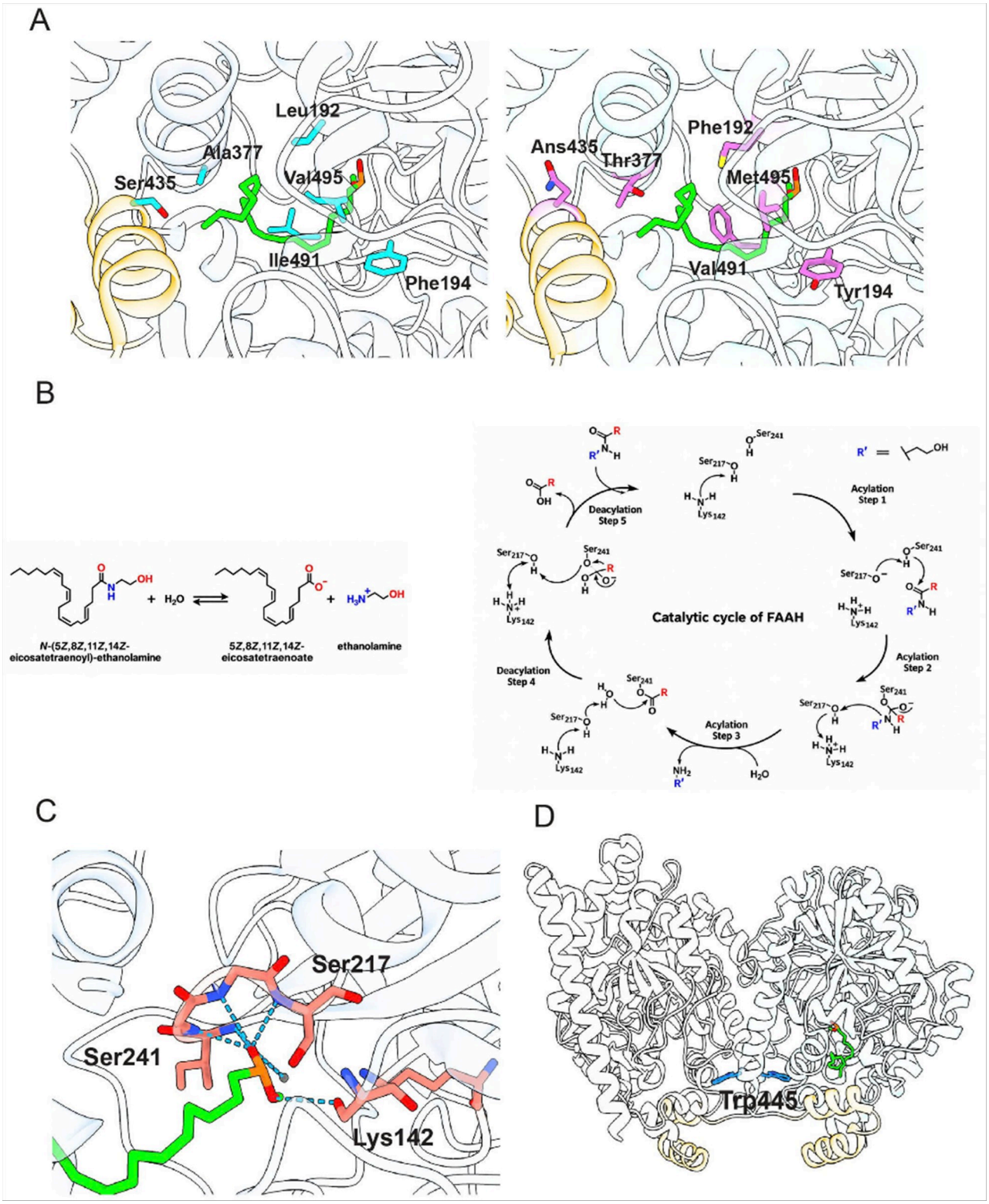

Figure 2

Species-dependent ligand recognition, catalytic mechanism, and interprotomer organization of FAAH. **A** Comparison of the acyl-chain-binding pockets of rat FAAH (left, 1TM5) and humanized FAAH (right, 2VYA). The six species-variable positions are highlighted in cyan/green for rat FAAH (Leu192, Phe194, Ala377, Ser435, Ile491, and Val495) and magenta for their human counterparts (Phe192, Tyr194, Thr377, Asn435, Val491, and Met495). The bound ligand is shown in green, and helices α18–α19 are colored pale yellow. **B**

Proposed two-stage catalytic mechanism showing substrate acylation of Ser241, formation of the acyl-enzyme intermediate, water-mediated deacylation, and regeneration of the free enzyme (adapted from Palermo, Campomanes, et al. 2015). **C** Close-up of the Ser241–Ser217–Lys142 catalytic network. Catalytic residues are shown as red sticks, the covalently bound ligand in green, and ordered water molecules as blue spheres; dashed lines indicate hydrogen-bonding interactions involved in proton transfer and water organization. **D** FAAH homodimer showing the two protomers in light and dark gray, bound ligand of one promotor in green, membrane-interacting α18–α19 regions in pale yellow, and Trp445 in magenta at the interprotomer interface.

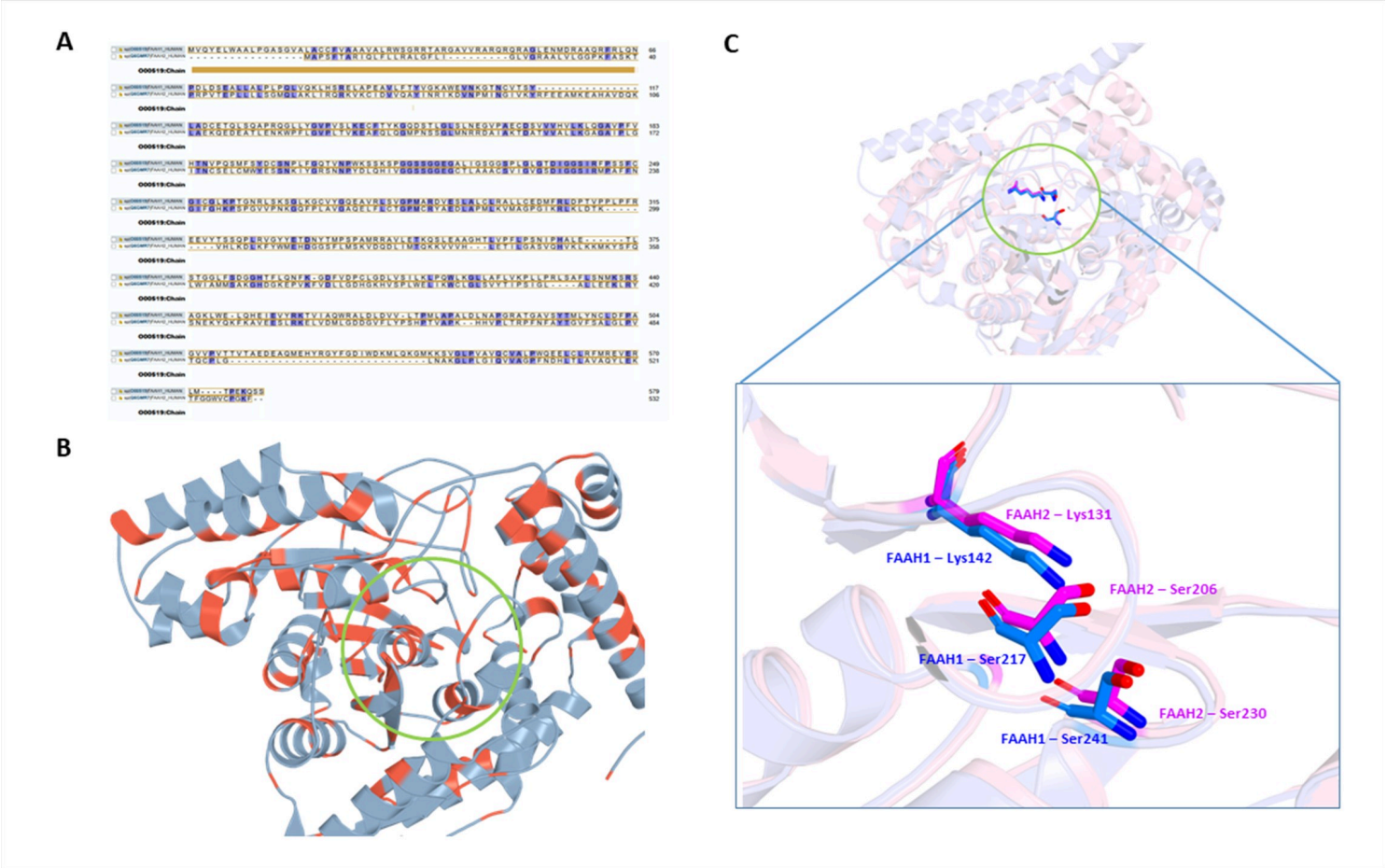

Figure 3

Sequence and structural comparison of human FAAH1 and FAAH2, highlighting conservation of the catalytic triad. **A** Sequence alignment of human FAAH1 and FAAH2 using sequences retrieved from UniProt and visualized with Jalview, showing 25.4% sequence identity. **B** Structural superposition of the top-ranked models of human FAAH1 (AF-O00519-F1) and FAAH2 (AF-Q6GMR7-F1), predicted from their amino acid sequences using AlphaFold Server. **C** Comparison of the three conserved catalytic residues in the active sites of FAAH1 and FAAH2, highlighting the spatial conservation of the catalytic triad.

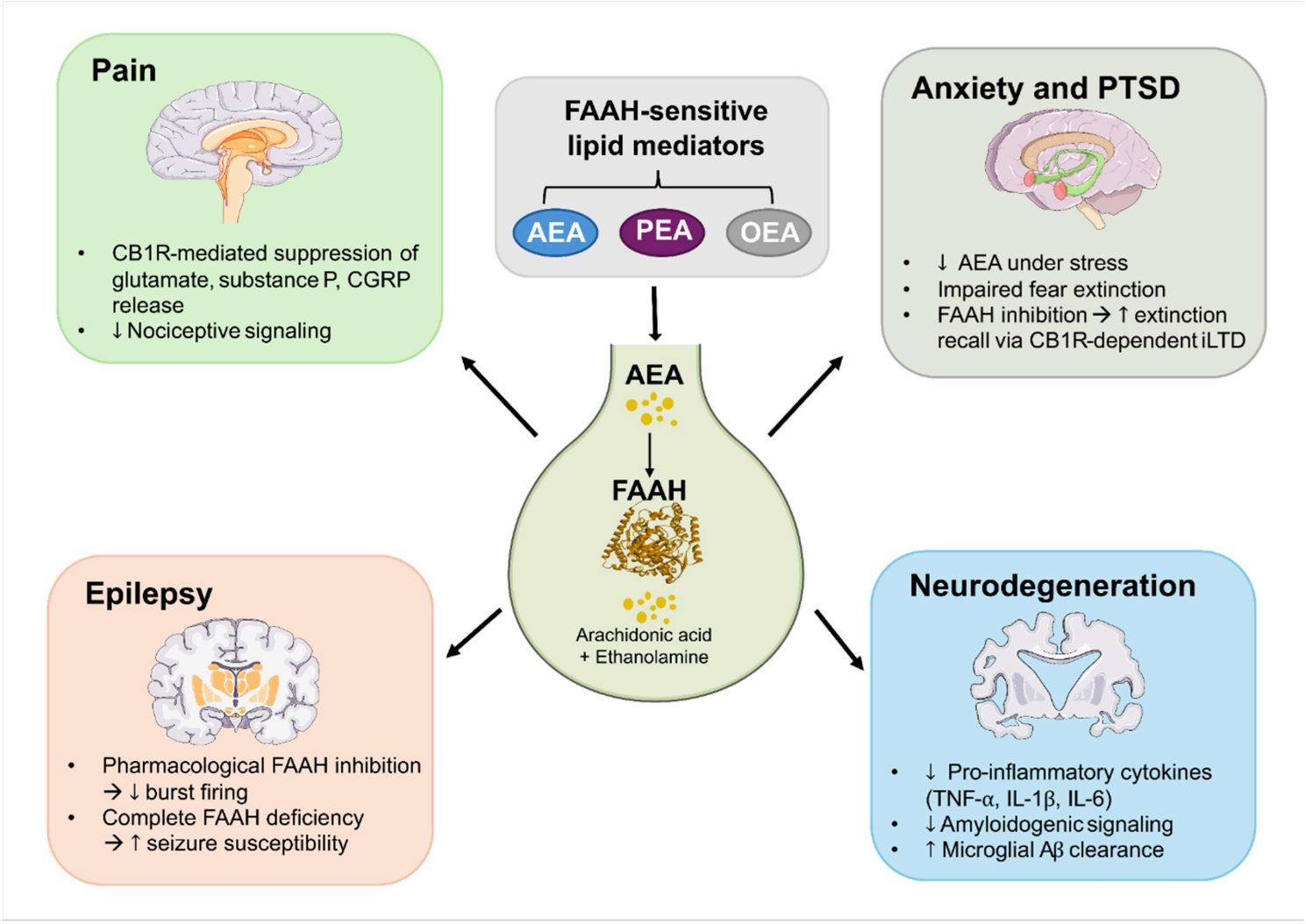

Figure 4

The multifaceted roles of FAAH in neurological and psychiatric conditions. FAAH regulates the levels of bioactive lipid mediators, including AEA, PEA, and OEA. The modulation of this system impacts various central nervous system disorders, highlighting its therapeutic potential in pain management, epilepsy, anxiety/PTSD, and neurodegenerative diseases.

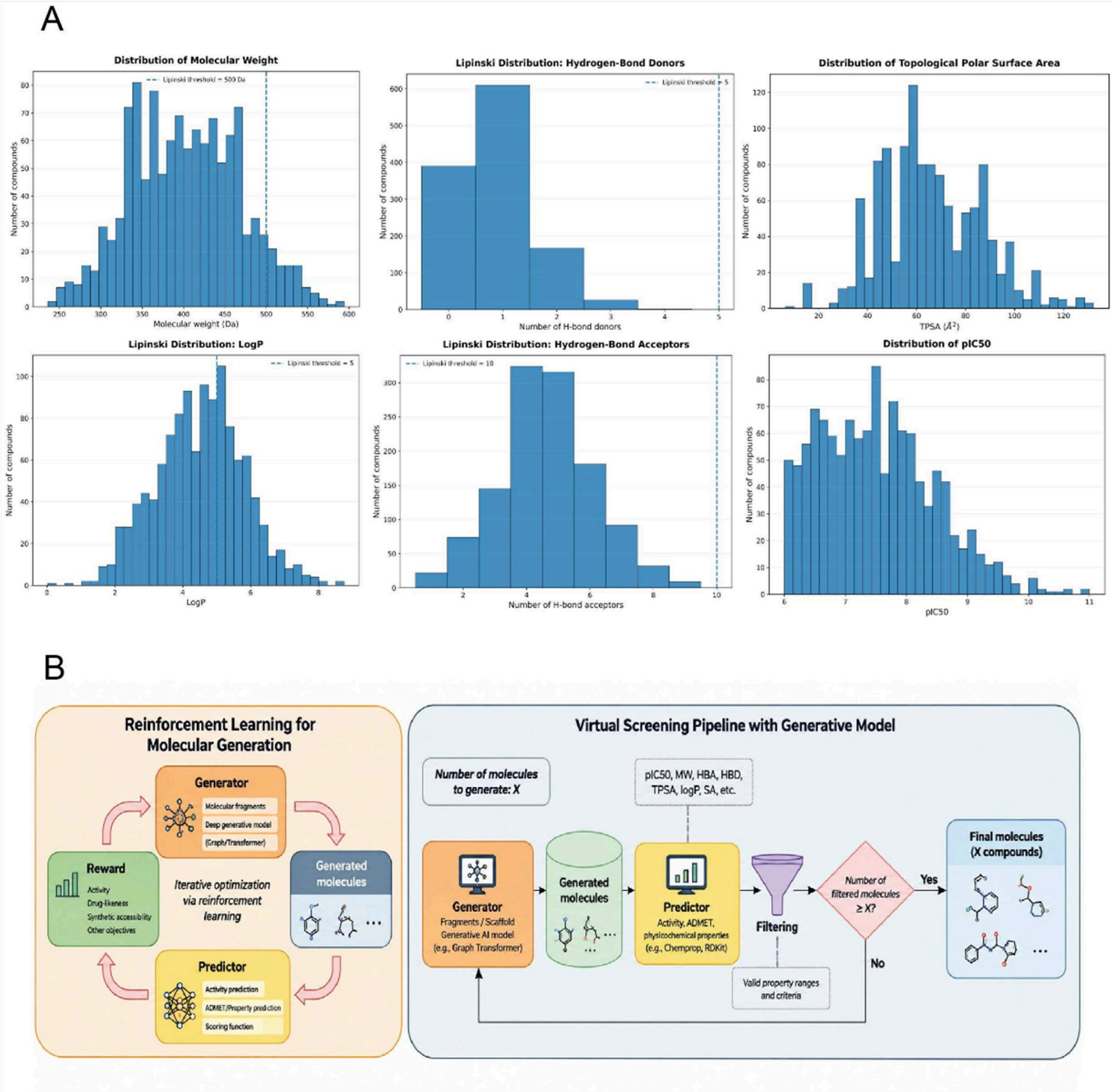

Figure 5

Bioactivity Landscape and Generative AI-Driven Design of FAAH Inhibitors. **A** Distribution of bioactivity and physicochemical properties of FAAH-targeting compounds. **B** Generative AI-based approach for designing novel FAAH inhibitors.