



OPEN ACCESS

EDITED BY
Cecilia Pozzi,
University of Siena, Italy

REVIEWED BY
Efstratios Stratikos,
National and Kapodistrian University of Athens,
Greece
Yue Zhai,
Air Force Medical University, China

*CORRESPONDENCE
Saleem Bhat,
✉ saleemyousuf26@yahoo.in

RECEIVED 22 November 2025
REVISED 05 January 2026
ACCEPTED 16 January 2026
PUBLISHED 10 February 2026

CITATION
Bhat S (2026) Zinc-dependent
aminopeptidases: new perspectives on
structure, function, and
biomedical applications.
Front. Chem. Biol. 5:1752191.
doi: 10.3389/fchbi.2026.1752191

COPYRIGHT
© 2026 Bhat. This is an open-access article
distributed under the terms of the [Creative
Commons Attribution License \(CC BY\)](#). The use,
distribution or reproduction in other forums is
permitted, provided the original author(s) and
the copyright owner(s) are credited and that the
original publication in this journal is cited, in
accordance with accepted academic practice.
No use, distribution or reproduction is permitted
which does not comply with these terms.

Zinc-dependent aminopeptidases: new perspectives on structure, function, and biomedical applications

Saleem Bhat*

University of Pennsylvania, Philadelphia, PA, United States

Zinc-dependent aminopeptidases are a subclass of exopeptidases implicated in the hydrolysis of N-terminal residues from peptides through a Zn(II) co-factor dependent hydrolytic mechanism. In humans, the prominent members of this class of enzymes (ERAP1/2, IRAP, APN/CD13, APA) catalyze peptide trimming in antigen presentation, peptide hormone regulation, and peptide homeostasis. Since the catalytic activity of these enzymes relies on a tightly coordinated Zn(II) ion in their conserved H-E-X-X-H...E motif, they constitute attractive yet challenging therapeutic targets. In this review, I provide an updated survey of their structural and mechanistic principles, evaluate their physiological and pathological roles, and outline emerging strategies for selective modulation and biotechnological usage. I also discuss current obstacles and future directions in deploying zinc-targeted aminopeptidase chemistry in translational settings.

KEYWORDS

aminopeptidase, APA, APN/CD13, biotechnological tools, ERAP1, immunopeptidome, inhibitor design, IRAP

1 Introduction: zinc as a catalytic and structural cofactor in proteolysis

Zinc is a ubiquitous metal in biology, with roles spanning structural stabilization (e.g., zinc fingers), regulation (e.g., metalloregulatory proteins), and catalysis (metalloenzymes) (Pace and Weerapana, 2014; Li et al., 2022; Vallee and Auld, 1993). Among catalytic roles, Zn(II) is especially suited to metalloenzymes that perform hydrolysis, due to its ability to act as a Lewis acid, stabilize negative charge accumulation, and polarize bound water without undergoing redox cycling (Clemens, S., 2022). The catalytic versatility of Zn has made it the most common metal cofactor in human metalloproteases (Vallee and Auld, 1990; Karlin and Zhu, 1997).

In proteolysis, Zn-dependent enzymes include a wide range of metalloproteases (e.g., MMPs, ADAMs, gluzincins) as well as exopeptidases (aminopeptidases, carboxypeptidases) (Thompson, 2022; Bhat et al., 2018; Bhat and Qureshi, 2020; Bhat and Qureshi, 2021; Bhat and Qureshi, 2024; Vendrell and Avilés, 1999). Within the exopeptidase class, zinc-dependent aminopeptidases operate by binding a Zn(II) in their active site that aids in peptide bond cleavage from the N terminus. The metal typically coordinates to two histidines and a glutamate (or other residue), along with the water nucleophile and sometimes a backbone carbonyl or side chain ligand (Pace and Weerapana, 2014).

TABLE 1 Summary of key mammalian aminopeptidases, their subcellular localization, principal substrates, and established physiological or pathological roles, highlighting their diverse functions in antigen processing, peptide metabolism, and signaling regulation.

Enzyme	Abbreviation/ Gene name	Localization or topology	Major substrates/clients known	Roles based on literature
Endoplasmic reticulum aminopeptidase 1	ERAP1	ER lumen	Trimming N-extended precursor peptides to 8–10 aa	Key role in MHC I antigen processing, immunopeptidome shaping (Tiburcă et al., 2024)
Endoplasmic reticulum aminopeptidase 2	ERAP2	ER lumen	Complementary trimming of certain peptides	Acts in tandem or in heterodimer fashion with ERAP1 (Papakyriakou et al., 2022)
Insulin regulated aminopeptidase	IRAP/LNPEP	Endosomal membrane	Degradation of peptide hormones (e.g., oxytocin, vasopressin, Ang IV)	Implicated in cognitive/metabolic diseases (Barlow and Thompson, 2020)
Aminopeptidase N	APN/CD13	Cell surface ectoenzyme	Broad-spectrum cleavage of oligopeptides	Involvement in angiogenesis, tumor biology, peptide processing (Farsa and Uher, 2025)
Aminopeptidase A	APA/ENPEP	Extracellular/plasma membrane	Cleavage of N-terminal Asp from angiotensins	Key role in renin–angiotensin system regulation, hypertension (Holmes et al., 2017)

Because the Zn(II) site is mechanistically essential, zinc-binding warheads (hydroxamic acids, phosphinic acids, thiols) are widely used in inhibitor design (Albrecht et al., 2011). However, the ubiquity of Zn in many catalytic enzymes presents a key challenge: achieving isoform specificity without off-target chelation or broad metalloprotease inhibition is nontrivial (Zastrow and Pecoraro, 2013; Zastrow and Pecoraro, 2014; Hryczanek et al., 2024).

In this review, I confine most of the discussion to aminopeptidases for which Zn(II) is established as the physiological catalytic metal—primarily members of the M1 (gluzincin) family in mammals (Evnouchidou et al., 2023). I have first review their classification and structural motifs, then dissect their catalytic mechanism, survey their physiological and pathological roles, and finally evaluate strategies for therapeutic and biotechnological exploitation (Table 1). Moreover, this review emphasizes M1 aminopeptidases mostly involved in regulated peptide trimming and signaling; other M1 members such as LTA4H and PSA, which perform functionally distinct roles, are only discussed briefly.

2 Classification and structural motifs of zinc-dependent aminopeptidases

2.1 M1 (gluzincin) aminopeptidase family: domain architecture and sequence signatures

The majority of well-characterized zinc aminopeptidases in humans belong to the M1 (gluzincin) family of metalloproteases. These enzymes share a conserved fold and several characteristic motifs:

- The H-E-X-X-H...E motif (commonly HExxH...E) near the active site is essential for Zn²⁺ binding and general base catalysis. Two histidines coordinate Zn(II), while a glutamate acts as a general base to activate the water nucleophile (Hooper, 1994; Fukasawa et al., 2011).

- The GAMEN (or variant) motif is involved in orienting the substrate N-terminal residue and contributes to substrate binding specificity (Hooper, 1994).
- Many M1 aminopeptidases adopt a multi-domain architecture (typically four structural domains) forming a concave “bowl” that encloses the active site, sometimes with a substrate chamber or internal cavity that imposes a “molecular ruler” behavior i.e., limiting trimming beyond a certain length (Maben et al., 2021).

Structural studies (e.g., ERAP1, APN) show that these domains can undergo conformational changes (open ↔ closed) to allow substrate entry and product release (Chen et al., 2012; Australo-Anglo-American Spondyloarthritis Consortium (TASC) et al., 2011; Nguyen et al., 2011). The closed conformation helps stabilize substrate binding and positioning, while opening allows exchange of substrate or release of trimmed peptide. This dynamic interconversion is central to many regulatory and selectivity properties of these proteins (Australo-Anglo-American Spondyloarthritis Consortium (TASC) et al., 2011; Nguyen et al., 2011; Drinkwater et al., 2017).

2.2 Human Zn-dependent aminopeptidases: key members

ERAP1 and ERAP2 are luminal endoplasmic reticulum aminopeptidases that cooperatively trim N-terminally extended peptides for optimal MHC class I presentation, with ERAP2 acting in a complementary or heterodimeric manner (Table 1). IRAP (LNPEP) is an endosomal aminopeptidase involved in the degradation of peptide hormones and is being explored as a therapeutic target in cognitive, metabolic, and immune disorders. In contrast, APN (CD13) and APA (ENPEP) are cell-surface aminopeptidases with broad extracellular roles, including peptide processing, angiogenesis, tumor biology, and regulation of the renin–angiotensin system (Table 1).

Table 1 below depicts a nonexhaustive list of well-studied human zinc aminopeptidases (all in M1 family) with catalytic Zn(II) dependence.

3 Mechanistic principles of zinc-assisted peptide hydrolysis

3.1 Coordination geometry and catalytic water activation

In the prototypical M1 aminopeptidase active site, the Zn(II) is coordinated in a roughly tetrahedral (or sometimes penta-coordinate) arrangement: two histidine side chains (from HExxH motif), the carboxylate of a catalytic glutamate, and a water molecule (or hydroxide). Often a backbone carbonyl or side-chain group may contribute a weak fifth ligand. The catalytic water (or hydroxide) is polarized and activated by the Zn(II), lowering its pKa and increasing nucleophilicity (Bennett and Holz, 1997; Mucha et al., 2010; Bhat, 2024).

The general catalytic scheme is:

1. Substrate binding: the N-terminal amino group of the peptide interacts with a substrate-binding pocket (which may include residues from the GAMEN motif) and orients the carbonyl toward Zn(II).
2. Activation of water: the bound water, polarized by Zn(II) and aided by the general base glutamate, becomes hydroxide-like and attacks the carbonyl carbon of the scissile bond.
3. Tetrahedral intermediate formation: the Zn(II) ion stabilizes the developing negative charge on the carbonyl oxygen (oxyanion stabilization).
4. Collapse/proton transfer: breakdown of the tetrahedral intermediate yields the cleaved peptide bond, releasing the N-terminal amino acid and shorter peptide product; a proton (from the general base) is transferred to the amine leaving group.
5. Product release and metal–water reconstitution: the active site resets with a water ligand (Chaikuad et al., 2012; Bhat, 2024).

Because Zn(II) is redox-inert in physiological conditions, it does not change oxidation state during catalysis, which provides stability to the enzyme mechanism (Oteiza, 2012).

3.2 Substrate length sensing and trimming regulation

A defining feature of several zinc-dependent aminopeptidases involved in antigen processing, particularly ERAP1, is their ability to trim N-terminally extended peptide precursors to an optimal length for MHC class I presentation while avoiding destructive over-trimming. ERAP1 preferentially generates peptides of 8–10 residues, a property commonly described as a “molecular ruler” mechanism (York et al., 2002; Chang et al., 2005; Saveanu et al., 2005; Australo-Anglo-American Spondyloarthritis Consortium (TASC) et al., 2011; Nguyen et al., 2011).

This length selectivity arises from the presence of a large internal substrate-binding cavity that accommodates peptide substrates in an extended conformation. Structural studies reveal that ERAP1 alternates between open and closed conformations, in which coordinated movements of domains II and IV relative to domains I/III modulate the volume and geometry of the catalytic

chamber. Long peptides can engage both the active site and distal binding regions, promoting domain closure and efficient trimming, whereas shorter peptides fail to stabilize the closed, catalytically competent conformation, thereby reducing further cleavage (Australo-Anglo-American Spondyloarthritis Consortium (TASC) et al., 2011; Evnouchidou and van Endert, 2019).

In addition to this intrinsic length-sensing mechanism, ERAP1 contains a regulatory/allosteric site spatially distinct from the catalytic zinc center. Binding of peptides, peptide fragments, or small-molecule inhibitors at this site can influence enzyme activity by stabilizing specific conformational states (Giastas et al., 2019). Such allosteric modulation affects substrate residence time, trimming rates, and product release, providing an additional layer of control over antigenic peptide generation (Chang et al., 2005; Maben et al., 2019; Maben et al., 2021). Importantly, this regulatory site functionally couples distal substrate interactions to active-site chemistry.

Mutational analyses strongly support this model. Substitutions in residues lining the internal cavity or allosteric regions alter length preference and trimming efficiency without directly disrupting catalytic residues, demonstrating that peptide length regulation is separable from catalysis itself (Australo-Anglo-American Spondyloarthritis Consortium (TASC) et al., 2011; Giastas et al., 2019). Together, these findings establish ERAP1 as a conformationally dynamic enzyme in which internal binding sites and allosteric regulation cooperate to enforce molecular ruler behavior essential for effective antigen presentation.

4 Physiological roles of key zinc-dependent aminopeptidases

4.1 ERAP1 and ERAP2: shaping the immunopeptidome

ERAP1 and ERAP2 reside in the lumen of the endoplasmic reticulum and process proteasome-generated precursor peptides for loading onto MHC class I molecules. The sequential trimming of N-extended peptides is necessary to produce optimal 8–10 mer epitopes; but over-trimming or inefficient trimming can alter the antigenic peptide pool, thereby shaping T cell recognition and immune surveillance (Chang et al., 2005; Hammer et al., 2006; Martín-Esteban et al., 2022; López de Castro, J.A., 2018).

Numerous studies show that polymorphic variants (allotypes) of ERAP1/2 influence trimming specificity, altering which peptide epitopes are presented. Such variability in immunopeptidome composition has been linked to susceptibility to autoimmune diseases (e.g., ankylosing spondylitis, psoriasis) and cancer immune evasion (Australo-Anglo-American Spondyloarthritis Consortium (TASC) et al., 2011; Reeves et al., 2013; Reeves et al., 2014; Sanz-Bravo et al., 2018; López de Castro, J.A., 2018).

Recent computational modeling suggests that ERAP1 and ERAP2 may form heterodimeric complexes, potentially coordinating trimming functions and expanding peptide processing versatility (Papakyriakou et al., 2022). Beyond antigen trimming, ERAP1 has been implicated in roles outside the ER: for instance, secretion by macrophages and modulation of innate immunity has been reported (Goto et al., 2011). Thus, the Zn-

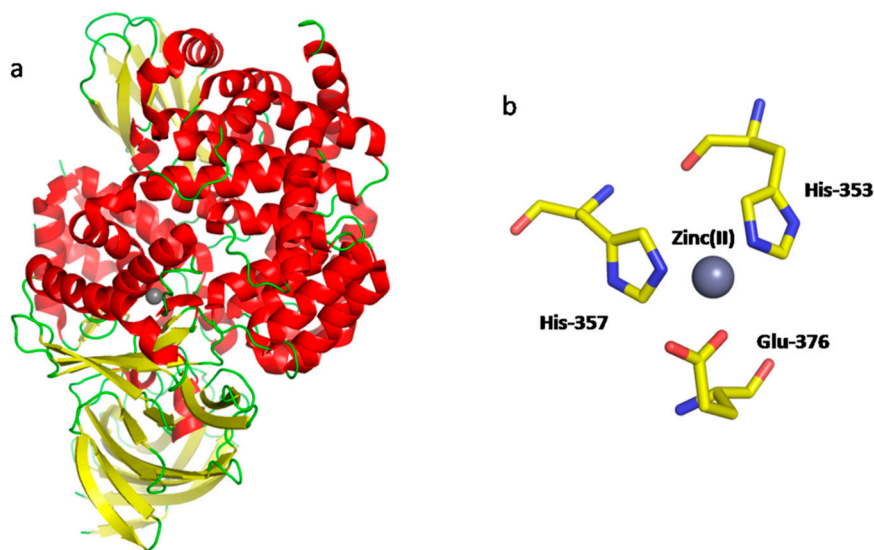


FIGURE 1

(a) Cartoon representation of the structure of human ERAP1 (PDB code: 2YD0). (b) Catalytic residues (in sticks) surrounding a zinc ion which serves as a co-factor during catalysis (sphere).

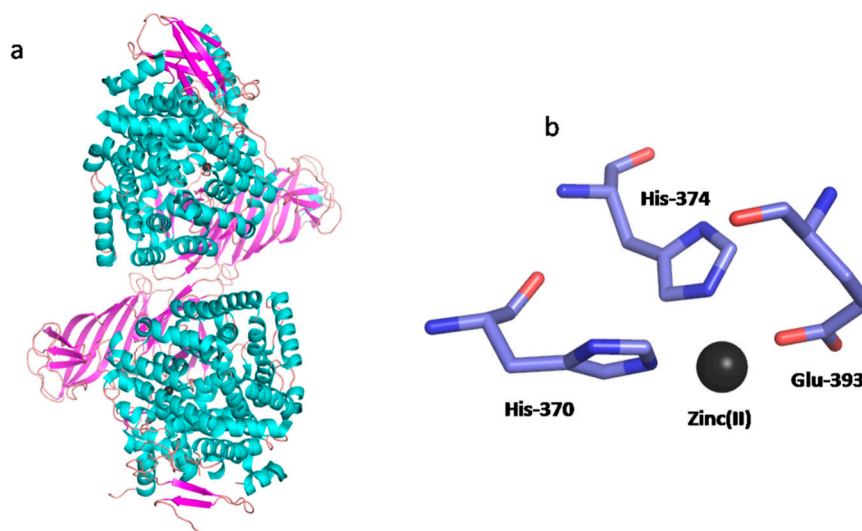


FIGURE 2

(a) Cartoon representation of the structure of dimeric human ERAP2 (PDB code: 7SH0). (b) Conserved catalytic residues shown as sticks surrounding a zinc metal co-factor ion (sphere).

binding catalytic core is not only central to peptide trimming but is embedded in a broader regulatory network influencing immunity.

4.2 Comparative structural basis of ERAP1 and ERAP2 function

ERAP1 and ERAP2 both are categorized into the M1 family zinc aminopeptidases as both share a four-domain architecture typical of M1 zinc metallopeptidases, consisting of an N-terminal cap domain, a catalytic domain harboring the zinc-binding HEXXH motif, a

regulatory hinge domain, and a C-terminal β -sandwich domain (Figures 1, 2). Despite this overall similarity, they exhibit key structural differences that dictate their distinct substrate specificities and biological functions. ERAP1 has a deeper and more hydrophobic substrate-binding cavity that can undergo large conformational changes between open and closed states, enabling it to accommodate and trim longer peptide precursors of 8–16 amino acids (Chang et al., 2005; Australo-Anglo-American Spondyloarthritis Consortium (TASC) et al., 2011). In contrast, ERAP2 has a more compact and positively charged catalytic pocket that favors basic residues such as arginine and lysine and is better

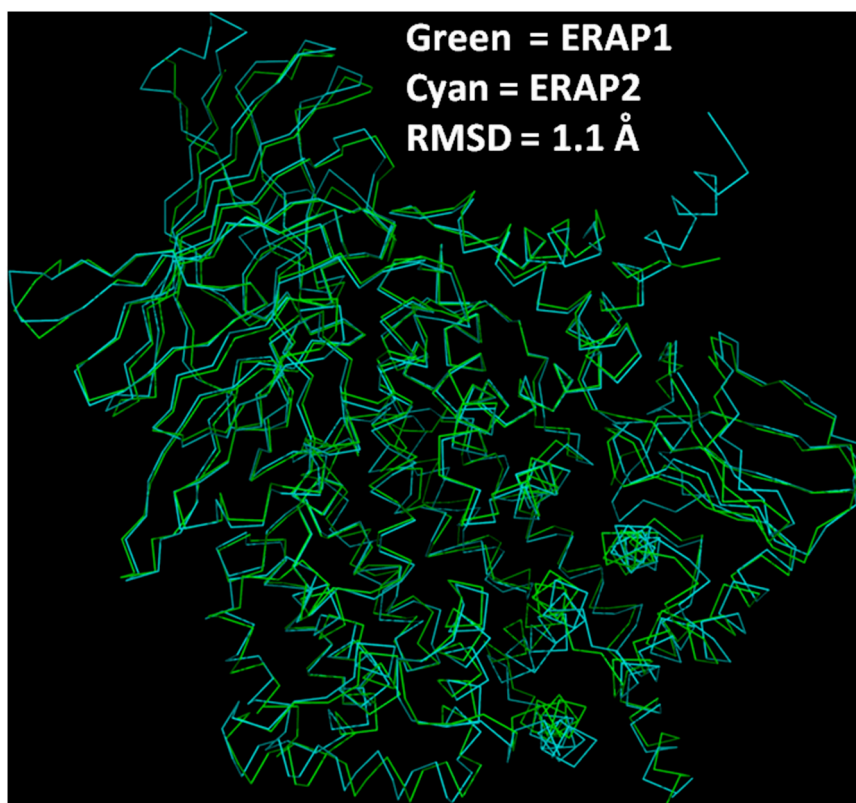


FIGURE 3

(a) Ribbon diagram reflecting the superimposition of human ERAP1 (PDB code: 2YD0 and in green color) with ERAP2 (PDB code: 7SH0 and in cyan color), revealing a backbone RMSD of approximately 1.1 Å. Such small differences in RMSD backbone makes them structurally similar.

suited for processing shorter peptides of 5–8 amino acids (Mpakali et al., 2015). The domain interfaces in ERAP1 are more flexible, allowing a “clamshell-like” movement crucial for substrate binding and product release, whereas ERAP2 displays a more rigid structure with limited domain mobility (Figure 3). Additionally, ERAP1 harbors polymorphic residues arising from coding SNPs, such as Lys528 and Arg725, which influence enzymatic activity, substrate specificity, and pH dependence. These residues appear unique to ERAP1, and contribute to its distinct biochemical properties and trimming behaviors (Reeves et al., 2013; Reeves et al., 2014; Sanz-Bravo et al., 2018). These structural distinctions allow ERAP1 to act as the primary enzyme for trimming long antigenic precursors to optimal lengths for MHC class I presentation, while ERAP2 fine-tunes the final peptide repertoire, often functioning synergistically with ERAP1. Together these peptidases form a complementary system—ERAP1 does coarse trimming; ERAP2 refines the final product and share structural attributes with each other (Figure 3) revealing a RMSD backbone difference of only 1.1 Å (Australo-Anglo-American Spondyloarthritis Consortium (TASC) et al., 2011; Birtley et al., 2012). An important similarity between ERAP 1 and 2 is a conserved active-site tyrosine residue that stabilizes the negatively charged oxyanion of the tetrahedral intermediate through hydrogen bonding during peptide bond hydrolysis (Nguyen et al., 2011). Mutation of this residue abolishes catalytic activity (Stratikos and Stern, 2013). The equivalent conserved tyrosine in ERAP2 fulfills the same

essential mechanistic role symbolizing identical catalytic mechanisms.

4.3 Drug targeting of ERAP1 and ERAP2

Therapeutic strategies aimed at regulating ERAP1 primarily focus on the development of small-molecule inhibitors. The two main classes of ERAP1 inhibitors target either the catalytic site or the allosteric site. Catalytic site inhibitors include phosphinic acid derivatives (Kokkala et al., 2016), which bind potently to the catalytic pocket as demonstrated by structure–activity relationship studies (Giastas et al., 2019). Other catalytic inhibitors, such as DABA analogues and urea derivatives, have been identified but generally exhibit lower potency (Papakyriakou et al., 2013; 2015). Allosteric site inhibitors of ERAP1 encompass several chemical classes, including cyclohexyl acids, clerodane acid (identified through high-throughput library screening), sulfonamides, and benzofurans, which represent some of the most potent inhibitors reported (Maben et al., 2019; Hryczanek et al., 2024; Liddle et al., 2020; Temponeras et al., 2023; Deddouche-Grass et al., 2021). As of 2023, Grey Wolf Therapeutics has advanced an ERAP1 inhibitor, GRWD5769, into Phase I/II clinical trials. This compound is being evaluated for safety, tolerability, efficacy, and pharmacokinetics in patients with virus-associated solid tumors—such as head and neck squamous cell carcinoma,

cervical cancer, and hepatocellular carcinoma—that are particularly sensitive to ERAP1 inhibition. The trials assess GRWD5769 both as a monotherapy and in combination with the PD-1 immune checkpoint inhibitor Libtayo® (cemiplimab) (In news as <https://www.prnewswire.com/news/grey-wolf-therapeutics/>).

Genetic variants and haplotypes (allotypes) of ERAP1 have been linked to numerous inflammatory, infectious, and neoplastic diseases. ERAP1 is a key risk gene identified in genome-wide association studies (GWAS) of MHC-I-associated inflammatory conditions, also known as “MHC-I-opathies,” including ankylosing spondylitis, Behçet’s disease, birdshot uveitis, and psoriasis (Australo-Anglo-American Spondyloarthritis Consortium (TASC) et al., 2011; Kirino et al., 2013). In many of these disorders, ERAP1 exhibits epistatic interactions with the primary risk MHC-I allele. Additional disease associations include insulin-dependent diabetes mellitus, multiple sclerosis, and hypertension—the latter being the first condition historically linked to ERAP1 polymorphisms. Emerging evidence also connects ERAP1 single nucleotide variants (SNVs) to cancer susceptibility and infectious disease outcomes, such as altered resistance to influenza virus infection (Cortes et al., 2015).

Similar to ERAP1, ERAP2 has been targeted predominantly with catalytic-site inhibitors, while bona fide allosteric inhibitors have been reported more recently. Catalytic ERAP2 inhibitors include phosphinic transition-state analogues and phosphorus-containing amino acid or dipeptide derivatives, which exploit subtle differences in active-site architecture between ERAP1 and ERAP2 to achieve selectivity (Kokkala et al., 2016; Mpakali et al., 2017; Giastas et al., 2019; Węglarz-Tomczak et al., 2016). In contrast, ERAP2 allosteric inhibition has been demonstrated primarily by sulfonamide-based scaffolds identified through kinetic target-guided synthesis and structure-based optimization (Camberlein et al., 2022; Arya et al., 2022).

4.4 ERAP1 polymorphism

ERAP1 is highly polymorphic in human populations, and common coding single nucleotide polymorphisms (SNPs) form discrete allotypes that differ in enzymatic activity and substrate specificity (Hutchinson et al., 2021). Structural and biochemical studies of the ten most prevalent allotypes reveal up to ~60-fold variation in trimming efficiency for specific peptide substrates, with some allotypes, such as allotype 10, exhibiting markedly reduced activity (Reeves et al., 2013). These functional differences directly influence the composition of the MHC class I immunopeptidome, affecting peptide length and sequence distribution (Nikopaschou et al., 2025). Importantly, specific allotype combinations are genetically associated with HLA-linked inflammatory diseases, including ankylosing spondylitis (Reeves et al., 2014) and Behçet’s disease (Takeuchi et al., 2016).

4.5 IRAP (insulin-regulated aminopeptidase)

IRAP (also known as LNPEP) is a membrane-associated aminopeptidase predominantly localized in endosomal compartments, where it is often co-trafficked with GLUT4-

containing vesicles in insulin-responsive cells. This localization links IRAP to both peptide processing and vesicular trafficking, providing a unique interface between metabolic regulation and enzymatic function. IRAP is known to cleave a range of bioactive peptide hormones, including oxytocin, vasopressin, and Ang IV (angiotensin IV), highlighting its role in neuroendocrine and cardiovascular signaling pathways (Barlow and Thompson, 2020). Its enzymatic activity is zinc-dependent via the conserved M1 metalloprotease motif, and IRAP shares significant structural homology with ERAP1 and ERAP2, including a similar peptide-binding cavity. This structural similarity has enabled inhibitor development strategies for IRAP to draw on lessons learned from ERAP-targeted drug design.

Several peptide-mimetic inhibitors, including cyclic compounds, have been co-crystallized with IRAP, providing high-resolution insights into subsite specificity, binding conformations, and the molecular determinants of selectivity (Mpakali et al., 2015; Vourloumis et al., 2022). These structural studies have informed both the design of more potent inhibitors and the understanding of how subtle changes in amino acid composition of the catalytic pocket influence substrate recognition. IRAP has also garnered attention in cognitive and metabolic disease research. For example, the interaction of Ang IV with IRAP has been implicated in memory consolidation and cognitive enhancement, while emerging evidence links IRAP activity to immunometabolic and inflammatory pathways, suggesting broader physiological relevance beyond classical peptide cleavage (Albiston et al., 2003). From a therapeutic perspective, small molecules such as benzyloxyhydroxamic acid derivatives have demonstrated significant inhibition of IRAP activity, offering potential avenues for pharmacological intervention in disorders ranging from metabolic dysfunction to neurodegeneration (Beveridge et al., 2024). Together, these functional, structural, and pharmacological insights position IRAP as a promising target at the intersection of metabolism, cognition, and immunomodulation.

4.6 APN/CD13: the multifunctional ectopeptidase

Aminopeptidase N (APN, CD13) is an abundant membrane-bound ectoaminopeptidase expressed in many tissues, particularly the kidneys, small intestine, lung, and in vascular and tumor endothelium (Table 1). APN broadly hydrolyzes oligopeptides and is involved in peptide catabolism, extracellular matrix turnover, and regulation of local peptide concentrations (Farsa and Uher, 2025).

In cancer, APN is often overexpressed and contributes to angiogenesis, cell migration, and tumor invasion. APN is also exploited as a receptor for certain viruses (e.g., human coronavirus 229E) and acts as a “moonlighting” protein with non-catalytic regulatory roles (Kolb et al., 1998; Croix et al., 2000; Farsa and Uher, 2025).

Because APN is an extracellular, membrane-bound enzyme with broad expression across a variety of tumor types, it has become an attractive target for both therapeutic and diagnostic applications (Croix et al., 2000; Farsa and Uher, 2025). Its localization on the cell surface enables direct accessibility to circulating therapeutic agents,

reducing the need for intracellular delivery mechanisms. This feature has been extensively exploited in the design of targeted therapies, including peptide–drug conjugates, prodrugs, and antibody–drug conjugates that are selectively activated by APN enzymatic activity within the tumor microenvironment. For instance, APN-cleavable peptide linkers have been used to release cytotoxic agents specifically in APN-expressing tumors, thereby enhancing local drug concentration while minimizing systemic toxicity (Pasqualini et al., 2000; Li et al., 2016).

In addition to its role in drug activation, APN serves as a tumor-homing receptor for several peptide ligands. Notably, the NGR (Asn–Gly–Arg) motif selectively binds to APN, and NGR-conjugated drug carriers or imaging probes have been employed to achieve targeted delivery of chemotherapeutic agents, nanoparticles, and radiotracers to tumor vasculature. Such strategies have been evaluated in preclinical and clinical settings for cancers such as glioma, melanoma, breast, ovarian, and lung carcinoma, demonstrating improved tumor localization and therapeutic efficacy (Pasqualini et al., 2000; Zou et al., 2012; Li et al., 2016).

APN has also been utilized in molecular imaging and diagnostic approaches. Radiolabeled APN inhibitors and NGR-based probes have enabled non-invasive imaging of APN expression using PET and SPECT modalities, providing valuable insights into tumor progression, angiogenesis, and response to therapy. Furthermore, APN expression correlates with tumor invasiveness and metastatic potential, making it a useful biomarker for disease prognosis and treatment monitoring (Schreiber and Smith, 2018; Murakami et al., 2005).

Overall, APN's dual function as an enzyme involved in tumor angiogenesis and as a surface-accessible biomarker makes it an ideal candidate for enzyme-activated prodrug design, ligand-directed drug delivery, and image-guided therapy. These strategies continue to evolve, with current research focusing on optimizing substrate specificity, improving drug stability, and integrating APN-targeted agents into combination immunotherapy and nanoparticle-based delivery systems to enhance therapeutic outcomes.

4.7 APA (aminopeptidase A/ENPEP)

APA (glutamyl aminopeptidase) acts on angiotensin peptides, specifically converting Ang II to Ang III via removal of an N-terminal Asp residue. Through this, APA regulates the balance of angiotensin peptides and modulates blood pressure and salt balance (Table 1). Its Zn catalytic function is central to its role in the renin–angiotensin system (RAS) (Holmes et al., 2017).

Pharmacologically, Aminopeptidase A (APA/ENPEP) has emerged as a promising target for the treatment of hypertension and cardiovascular diseases, owing to its pivotal role in the renin–angiotensin system (RAS). APA catalyzes the conversion of angiotensin II (Ang II) to angiotensin III (Ang III), a peptide that acts primarily in the brain to regulate blood pressure through stimulation of AT₁ receptors. Inhibition of APA therefore reduces the generation of Ang III, leading to decreased central sympathetic outflow and lower blood pressure. This mechanism represents an alternative to classical RAS-targeting drugs, such as ACE inhibitors and angiotensin receptor blockers, by acting upstream within the brain RAS (Reaux et al., 2000; Schinzari et al., 2025).

Several APA inhibitors, including the well-characterized compound EC33 and its prodrug RB150 (firibastat), have shown significant antihypertensive effects in both animal models and human trials. Firibastat, a brain-penetrant prodrug that releases EC33 after crossing the blood–brain barrier, has demonstrated efficacy in lowering blood pressure in patients with treatment-resistant or salt-sensitive hypertension (Khosla et al., 2022; Ferdinand et al., 2019.) Despite these advances, translation to broader clinical use remains challenging due to the complexity and redundancy of RAS signaling, inter-individual variability in enzyme expression, and compensatory mechanisms that can limit therapeutic benefit.

Ongoing research aims to refine APA inhibitor design, enhance blood–brain barrier permeability, and explore combination therapies with other RAS modulators. These efforts seek to harness APA inhibition as a novel strategy for long-term cardiovascular control while minimizing systemic side effects.

5 Zinc aminopeptidases in disease and therapeutic potential

5.1 Autoimmunity and cancer immunotherapy

Given the central role of ERAP1 and ERAP2 in shaping the MHC class I immunopeptidome, pharmacological modulation of their activity has significant immunotherapeutic implications (Leib et al., 2025). By trimming proteasome-derived precursor peptides within the endoplasmic reticulum, ERAPs determine both the length and sequence composition of peptides available for MHC class I loading. Excessive or overactive trimming can destroy otherwise immunogenic epitopes before they are loaded onto MHC molecules, whereas insufficient or inefficient trimming can result in the accumulation of N-terminally extended peptides that bind poorly to MHC class I and fail to elicit effective CD8⁺ T-cell responses (Koumantou et al., 2019). Consistent with this central role, allelic variation in ERAP1, arising from common coding polymorphisms, has been genetically linked to susceptibility to autoimmune diseases such as ankylosing spondylitis, highlighting how subtle changes in trimming activity can have profound immunological consequences (Cortes et al., 2015 Australo-American Spondyloarthritis Consortium (TASC) et al., 2011).

In the context of cancer immunotherapy, there is growing interest in targeting ER aminopeptidases to reshape tumor antigen presentation. Pharmacological inhibition or modulation of ERAP activity has been shown to alter the tumor immunopeptidome, promoting the surface presentation of novel or subdominant antigenic peptides and increasing tumor immunogenicity (Koumantou et al., 2019; Temponeras et al., 2022; Temponeras et al., 2023). Such approaches have been proposed as a means to enhance tumor visibility to the immune system and potentially improve responses to immune checkpoint blockade. However, therapeutic manipulation of ERAPs carries inherent risks, as excessive or complete inhibition may disrupt normal self-peptide presentation and compromise immune tolerance (Evnouchidou and van Endert, 2019; Mattorre et al., 2022). Consequently, current strategies emphasize selective or partial inhibition of ERAP activity, rather than full enzymatic blockade, to achieve a balance between

enhanced antitumor immunity and the avoidance of autoimmunity (Hutchinson et al., 2021; Leib et al., 2025).

5.2 Infectious disease and viral exploitation

Certain pathogens exploit aminopeptidases, such as Aminopeptidase N (APN/CD13), as receptors for cellular entry, making these enzymes critical determinants of viral infectivity. Pharmacological inhibition or altered expression of APN can reduce viral entry and replication, highlighting their potential as antiviral targets (Kolb et al., 1998). Similarly, modulation of endoplasmic reticulum aminopeptidases (ERAP1/2) or insulin-regulated aminopeptidase (IRAP) can influence antigen processing and presentation, shaping immune responses in infection and vaccination (Saulle et al., 2021; Mattorre et al., 2022). Together, zinc-dependent aminopeptidases occupy a central role at the crossroads of immunity, metabolism, cancer, and host–pathogen interactions, making them attractive targets for therapeutic intervention across multiple disease contexts.

6 Therapeutic and biotechnological exploitation

6.1 Inhibitor chemotypes and selectivity strategies

Designing selective inhibitors for zinc-dependent aminopeptidases remains challenging due to the highly conserved catalytic Zn-binding site. Strategies to achieve potency and specificity typically combine zinc-binding warheads—such as hydroxamates, phosphinates, or thiols—with side chains targeting unique S1, S1', and distal subsites. For example, selective ERAP1 inhibitors have been developed using this approach (Maben et al., 2021). Allosteric modulators, which bind outside the catalytic site, can bias enzymes toward open or closed conformations and are particularly useful for regulating ERAP's molecular-ruler function. Substrate-mimetic peptides or peptidomimetics exploit the GAMEN motif to improve specificity, while prodrug or targeted-activation strategies leverage extracellular aminopeptidases like APN, enabling tumor-selective peptide–drug conjugate activation. Cross-family insights from IRAP inhibitor development further inform medicinal chemistry efforts (Albiston et al., 2011). A notable success is firibastat, a prodrug of a thiol-based APA inhibitor in Phase III trials for resistant hypertension; the prodrug design reduces systemic off-target exposure while maintaining efficacy.

6.2 Diagnostic, imaging and biosensor applications

Several aminopeptidases, particularly extracellular or secreted enzymes such as APN/CD13, can be exploited in diagnostic and imaging applications. Fluorogenic peptide substrates, in which N-terminal quenching is relieved upon cleavage, enable enzyme activity assays and high-throughput inhibitor screening. Activatable

imaging probes for MRI, PET, or optical modalities use peptide-masked reporters that are selectively unmasked by APN in tumor microenvironments, providing localized signal enhancement. Additionally, urinary or plasma APN activity serves as a biomarker for disease states. These strategies leverage both the enzymatic specificity and extracellular accessibility of zinc-dependent aminopeptidases (Schreiber et al., 2018; Trencsényi et al., 2023).

7 Mechanistic and therapeutic challenges in aminopeptidase biology

7.1 Challenges and pitfalls in mechanistic inference

Because many aminopeptidases accept multiple metal ions *in vitro*, it is essential to confirm physiological Zn dependence (e.g., via metal-reconstitution, metal exchange, mutagenesis). Some past inhibitor studies inadvertently exploited non-physiological metalation states, leading to confusing or non-physiological potency claims (Graham et al., 2005). Moreover, because the Zn-binding site is highly conserved (Figures 1a,b, 2a,b), small-molecule inhibitors can inadvertently inhibit off-target metalloproteases unless they extend well beyond the metal-binding moiety into unique subsite interactions. A direct proof of this assertion is depicted in the inhibition of both the ERAP1 and ERAP2 aminopeptidases by the same class of inhibitors (Węglarz-Tomczak et al., 2016).

7.2 Targeting aminopeptidases is challenging

Many aminopeptidases are considered validated drug candidates to treat diseases like malaria and several neglected tropical diseases (Trenholme et al., 2010; Zheng et al., 2016; Bhat, 2024). However, despite their functional diversity, M1 family aminopeptidases share a highly conserved zinc-dependent catalytic architecture (Figures 1a,b, 2a,b), which complicates the development of isoform-selective inhibitors as demonstrated by comparative structural and inhibitor profiling studies (Chang et al., 2005; Australo-Anglo-American Spondyloarthritis Consortium (TASC) et al., 2011; Mpakali et al., 2015). In particular, ERAP1 and ERAP2 display extensive structural and catalytic similarity, differing mainly in residues lining the substrate-binding cavity rather than in the core catalytic machinery (Chang et al., 2005; Australo-Anglo-American Spondyloarthritis Consortium (TASC) et al., 2011). Although these differences allow selectivity in principle, primary biochemical studies have shown that most active-site inhibitors exhibit only ~5–10-fold selectivity between ERAP isoforms (Maben et al., 2019; Mpakali et al., 2015; Zervoudi et al., 2013). In cellular settings, isoform selectivity is further diminished by overlapping substrate preferences and a shared trimming mechanism, as evidenced by immunopeptidomics and cellular inhibition studies (Zervoudi et al., 2013; Reeves et al., 2020). Consequently, many peptide-mimetic compounds, including

classical inhibitors such as amastatin and actinonin, act as broad-spectrum inhibitors of multiple M1 aminopeptidases rather than as ERAP-selective agents (Chang et al., 2005; Maben et al., 2019).

In contrast to classical active-site inhibitors, significant effort has been made to develop allosteric modulators of ERAP1, exploiting its pronounced conformational dynamics during peptide trimming. ERAP1 displays dynamic interconversion between open and closed conformations, and allosteric ligands binding outside the catalytic pocket modulate enzymatic activity in a substrate- and peptide length-dependent manner, resulting in functional regulation rather than complete catalytic blockade (Zervoudi et al., 2013). Such allosteric inhibition has been shown to selectively reshape the cellular immunopeptidome and alter antigen presentation, supporting the concept of functional rather than absolute inhibition (Reeves et al., 2020; Temponeras et al., 2023). Recent structure- and property-guided medicinal chemistry efforts have further demonstrated that targeting non-catalytic regions of ERAP1 can yield inhibitors with improved potency and selectivity profiles compared with peptide-mimetic active-site inhibitors (Hryczanek et al., 2024).

7.3 Metal ion promiscuity and cofactor selectivity in many aminopeptidases

For many aminopeptidases from other metallopeptidase families, such as M20 and M17, Zn(II) is often assumed to be the physiological cofactor; however, *in vitro* assays suggest that this is not always the case (Arfin et al., 1995; Bhat et al., 2020; Bhat et al., 2021). It is often the case that many aminopeptidase enzymes show markedly better activity with metal cofactors such as, Co(II) or Ca(II) or for that matter Mg(II) (Bhat et al., 2020). Our observation is that Zinc tends to be a precipitant of these proteins *in vitro*. In fact, many titrations of this metal beginning in low nanomolar concentrations have most definitely suggested that Zinc (II) is often inhibitory and that both low or high concentrations do not help in driving the aminopeptidase activity significantly (Bhat et al., 2020). These co-factor patterns are well documented for many aminopeptidases such as, M20 Peptidase T that functions as a broad spectrum aminopeptidase with high preference for the hydrolysis of smaller hydrophobic residues like alanine in P1 and basic residues in P2 positions, respectively (Bhat et al., 2020). However, inhibitory or precipitating effects of exogenous Zn(II) reflect non-physiological concentrations or altered coordination environments, not a challenge to Zn(II)'s physiological role as a catalytic cofactor.

8 Selected M1 aminopeptidases with distinct biological roles

8.1 Leukotriene A4 hydrolase (LTA4H)

Leukotriene A4 hydrolase (LTA4H) is a member of the M1 zinc aminopeptidase family, containing the conserved HExxH...E motif and a catalytically essential Zn(II) ion (Haeggstrom, J.Z., 2004). Unlike canonical M1 aminopeptidases that primarily function as peptide-trimming enzymes, LTA4H is functionally bifunctional. In addition to aminopeptidase activity, it catalyzes a dominant epoxide hydrolase reaction that converts leukotriene A4 into leukotriene B4,

a potent lipid mediator of inflammation. Structural and mutational analyses have shown that the aminopeptidase and epoxide hydrolase activities share an overlapping active site, with subtle differences in substrate positioning dictating reaction outcome (Haeggstrom, J.Z., 2004; Haeggström et al., 2007; Thunnissen et al., 2001). Importantly, genetic, biochemical, and pharmacological studies indicate that the physiological relevance of LTA4H is largely driven by its role in inflammatory signaling rather than peptide metabolism (Rudberg et al., 2002; Pal et al., 2019). Consequently, LTA4H is considered a structurally conserved but functionally specialized M1 family member, illustrating how the canonical zinc-dependent aminopeptidase scaffold can evolve to support distinct biochemical and biological functions.

8.2 Puromycin-sensitive aminopeptidase (PSA/NPEPPS)

Puromycin-sensitive aminopeptidase (PSA, also known as NPEPPS) is a cytosolic zinc-dependent member of the M1 aminopeptidase family that contains the conserved HExxH motif and adopts the canonical M1 metalloprotease fold (Bhutani et al., 2007; Madabushi et al., 2023). PSA functions primarily as a broad-specificity exopeptidase involved in intracellular peptide degradation, protein quality control, and the turnover of short peptides generated by proteasomal processing (Johnson and Hersh, 1990; Constam et al., 1995; Thompson and Hersh, 2003; Bhutani et al., 2007). In contrast to ERAP1 and ERAP2, PSA does not exhibit molecular-ruler behavior, regulated length selection, or a role in antigen presentation (Bhutani et al., 2007). Instead, PSA acts as a housekeeping enzyme that contributes to cellular proteostasis (Bhutani et al., 2007). Genetic and biochemical studies have implicated PSA in neuronal homeostasis, with loss or dysregulation of activity linked to neurodegenerative disease pathways, including impaired degradation of polyglutamine-expanded proteins and tau-derived peptides (Kudo et al., 2011). Together, these findings position PSA as a canonical M1 aminopeptidase whose primary biological role lies in intracellular peptide turnover rather than regulated peptide trimming.

9 Challenges and future directions

Achieving selective inhibition of zinc-dependent aminopeptidases remains challenging due to conserved Zinc-binding motifs and potential off-target effects on MMPs, ADAMs, or other metalloproteases. Isoform specificity requires engagement of unique subsites or allosteric pockets, while physiological validation ensures Zn(II) is the relevant catalytic metal. Context-dependent effects *in vivo*—on antigen repertoires, peptide hormones, or other substrates—necessitate systems-level profiling. Intracellular targets like ERAPs and IRAP demand membrane-permeable inhibitors with precise subcellular localization. Integration with patient-specific factors, including HLA genotype and tumor context, guides clinical translation. Future advances in cryo-EM, fragment-based screening, computational design, and natural product discovery promise selective, effective modulation (Maben et al., 2021; Brown et al., 2016; Drag and Salvesen, 2010; López de Castro., 2018).

10 Conclusion

Zinc-dependent aminopeptidases form a mechanistically coherent yet biologically diverse subclass of metalloproteases. Their catalytic dependence on a Zn(II) cofactor, embedded in a dynamic structural framework, makes them attractive yet challenging targets in immunology, cardiovascular disease, cancer, and biotechnology. Advances in structural biology, chemical biology, immunopeptidomics, and targeted delivery now converge to make selective modulation of Zn aminopeptidases a promising frontier. The next wave of progress will likely come from allosteric or conformational modulators, engineered enzyme variants, and biomarker-driven translational strategies.

Author contributions

SB: Investigation, Validation, Writing – review and editing, Software, Formal Analysis, Conceptualization, Visualization, Writing – original draft, Supervision.

Funding

The author(s) declared that financial support was not received for this work and/or its publication.

References

- Albiston, A. L., Mustafa, T., McDowall, S. G., Mendelsohn, F. A., Lee, J., and Chai, S. Y. (2003). AT4 receptor is insulin-regulated membrane aminopeptidase: potential mechanisms of memory enhancement. *Trends Endocrinol. and Metabolism* 14 (2), 72–77. doi:10.1016/s1043-2760(02)00037-1
- Albiston, A. L., Diwakarla, S., Fernando, R. N., Mountford, S. J., Yeatman, H. R., Morgan, B., et al. (2011). Identification and development of specific inhibitors for insulin-regulated aminopeptidase as a new class of cognitive enhancers. *Br. Journal Pharmacology* 164 (1), 37–47. doi:10.1111/j.1476-5381.2011.01402.x
- Albrecht, S., Al-Lakkis-Wehbe, M., Orsini, A., Defoin, A., Pale, P., Salomon, E., et al. (2011). Amino-benzosuberone: a novel warhead for selective inhibition of human aminopeptidase-N/CD13. *Bioorg. and Medicinal Chemistry* 19 (4), 1434–1449. doi:10.1016/j.bmc.2011.01.008
- Arfin, S. M., Kendall, R. L., Hall, L., Weaver, L. H., Stewart, A. E., Matthews, B. W., et al. (1995). Eukaryotic methionyl aminopeptidases: two classes of cobalt-dependent enzymes. *Proc. Natl. Acad. Sci.* 92 (17), 7714–7718. doi:10.1073/pnas.92.17.7714
- Arya, R., Maben, Z., Rane, D., Ali, A., and Stern, L. J. (2022). Phenylsulfamoyl benzoic acid inhibitor of ERAP2 with a novel mode of inhibition. *ACS Chem. Biol.* 17 (7), 1756–1768. doi:10.1021/acscchembio.2c00093
- Australo-Anglo-American Spondyloarthritis Consortium (TASC), Wellcome Trust Case Control Consortium 2 (WTC2), Evans, D. M., Spencer, C. C., Pointon, J. J., Su, Z., Harvey, D., Kochan, G., et al. (2011). Interaction between ERAP1 and HLA-B27 in ankylosing spondylitis implicates peptide handling in the mechanism for HLA-B27 in disease susceptibility. *Nat. Genetics* 43 (8), 761–767. doi:10.1038/ng.873
- Author Anonymous (2023). Grey wolf therapeutics announces dosing of first patient in phase 1/2 clinical study of GRWD5769 in patients with advanced solid tumours.
- Barlow, N., and Thompson, P. E. (2020). IRAP inhibitors: M1-Aminopeptidase family inspiration. *Front. Pharmacol.* 11, 585930. doi:10.3389/fphar.2020.585930
- Bennett, B., and Holz, R. C. (1997). EPR studies on the mono- and dicobalt (II)-substituted forms of the aminopeptidase from *Aeromonas proteolytica*. Insight into the catalytic mechanism of dinuclear hydrolases. *J. Am. Chem. Soc.* 119 (8), 1923–1933. doi:10.1021/ja963021v
- Beveridge, J., Söderström, M., Prieto-Díaz, R., Gutierrez-de-Teran, H., Odell, L. R., Hallberg, M., et al. (2024). Benzylhydroxamic acids as inhibitors of insulin regulated aminopeptidase (IRAP). *Eur. J. Med. Chem. Rep.* 12, 100215. doi:10.1016/j.ejmc.2024.100215
- Bhat, S. Y. (2024). Drug targeting of aminopeptidases: importance of deploying a right metal cofactor. *Biophys. Rev.* 16 (2), 249–256. doi:10.1007/s12551-024-01192-8
- Bhat, S. Y., and Qureshi, I. A. (2020). Mutations of key substrate binding residues of leishmanial peptidase T alter its functional and structural dynamics. *Biochimica Biophysica Acta (BBA)-General Subj.* 1864 (1), 129465. doi:10.1016/j.bbagen.2019.129465
- Bhat, S. Y., and Qureshi, I. A. (2021). Structural and functional basis of potent inhibition of leishmanial leucine aminopeptidase by peptidomimetics. *ACS Omega* 6 (29), 19076–19085. doi:10.1021/acso.6a02386
- Bhat, S. Y., Dey, A., and Qureshi, I. A. (2018). Structural and functional highlights of methionine aminopeptidase 2 from *Leishmania donovani*. *Int. Journal Biological Macromolecules* 115, 940–954. doi:10.1016/j.ijbiomac.2018.04.090
- Bhat, S. Y., Jagruthi, P., Srinivas, A., Arifuddin, M., and Qureshi, I. A. (2020). Synthesis and characterization of quinoline-carbaldehyde derivatives as novel inhibitors for leishmanial methionine aminopeptidase 1. *Eur. J. Med. Chem.* 186, 111860. doi:10.1016/j.ejmech.2019.111860
- Bhat, S. Y., Bhandari, S., Thacker, P. S., Arifuddin, M., and Qureshi, I. A. (2021). Development of quinoline-based hybrid as inhibitor of methionine aminopeptidase 1 from *Leishmania donovani*. *Chem. Biol. and Drug Des.* 97 (2), 315–324. doi:10.1111/cbdd.13783
- Bhutani, N., Venkatraman, P., and Goldberg, A. L. (2007). Puromycin-sensitive aminopeptidase is the major peptidase responsible for digesting polyglutamine sequences released by proteasomes during protein degradation. *EMBO Journal* 26 (5), 1385–1396. doi:10.1038/sj.emboj.7601592
- Birtley, J. R., Saridakis, E., Stratikos, E., and Mavridis, I. M. (2012). The crystal structure of human endoplasmic reticulum aminopeptidase 2 reveals the atomic basis for distinct roles in antigen processing. *Biochemistry* 51 (1), 286–295. doi:10.1021/bi201230p
- Brown, M. A., Kenna, T., and Wordsworth, B. P. (2016). Genetics of ankylosing spondylitis—Insights into pathogenesis. *Nat. Rev. Rheumatol.* 12 (2), 81–91. doi:10.1038/nrrheum.2015.133
- Camberlein, V., Fléau, C., Sierocki, P., Li, L., Gealageas, R., Bosc, D., et al. (2022). Discovery of the first selective nanomolar inhibitors of ERAP2 by kinetic target-guided synthesis. *Angew. Chem. Int. Ed.* 61 (39), e202203560. doi:10.1002/anie.202203560
- Chaikuad, A., Pilka, E. S., De Riso, A., Von Delft, F., Kavanagh, K. L., Vénien-Bryan, C., et al. (2012). Structure of human aspartyl aminopeptidase complexed with substrate analogue: insight into catalytic mechanism, substrate specificity and M18 peptidase family. *BMC Structural Biology* 12 (1), 14. doi:10.1186/1472-6807-12-14
- Chang, S. C., Momburg, F., Bhutani, N., and Goldberg, A. L. (2005). The ER aminopeptidase, ERAP1, trims precursors to lengths of MHC class I peptides by a

Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Generative AI statement

The author(s) declared that generative AI was not used in the creation of this manuscript.

Any alternative text (alt text) provided alongside figures in this article has been generated by Frontiers with the support of artificial intelligence and reasonable efforts have been made to ensure accuracy, including review by the authors wherever possible. If you identify any issues, please contact us.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

- “molecular ruler” mechanism. *Proc. Natl. Acad. Sci.* 102 (47), 17107–17112. doi:10.1073/pnas.0500721102
- Chen, L., Lin, Y. L., Peng, G., and Li, F. (2012). Structural basis for multifunctional roles of Mammalian aminopeptidase N. *Proc. Natl. Acad. Sci.* 109 (44), 17966–17971. doi:10.1073/pnas.1210123109
- Clemens, S. (2022). The cell biology of zinc. *J. Exp. Bot.* 73 (6), 1688–1698. doi:10.1093/jxb/erab481
- Constam, D. B., Tobler, A. R., Rensing-Ehl, A., Kemler, I., Hersh, L. B., and Fontana, A. (1995). Puromycin-sensitive aminopeptidase: sequence analysis, expression, and functional characterization. *J. Biol. Chem.* 270 (45), 26931–26939. doi:10.1074/jbc.270.45.26931
- Cortes, A., Pulit, S. L., Leo, P. J., Pointon, J. J., Robinson, P. C., Weisman, M. H., et al. (2015). Major histocompatibility complex associations of ankylosing spondylitis are complex and involve further epistasis with ERAP1. *Nat. Communications* 6 (1), 7146. doi:10.1038/ncomms8146
- Croix, B. S., Rago, C., Velculescu, V., Traverso, G., Romans, K. E., Montgomery, E., et al. (2000). Genes expressed in human tumor endothelium. *Science* 289 (5482), 1197–1202. doi:10.1126/science.289.5482.1197
- Deddouche-Grass, S., Andouche, C., Barenz, F., Halter, C., Hohwald, A., Lebrun, L., et al. (2021). Discovery and optimization of a series of benzofuran selective ERAP1 inhibitors: biochemical and *in silico* studies. *ACS Med. Chem. Lett.* 12 (7), 1137–1142. doi:10.1021/acsmchemlett.1c00235
- Drag, M., and Salvesen, G. S. (2010). Emerging principles in protease-based drug discovery. *Nat. Reviews Drug Discovery* 9 (9), 690–701. doi:10.1038/nrd3053
- Drinkwater, N., Lee, J., Yang, W., Malcolm, T. R., and McGowan, S. (2017). M1 aminopeptidases as drug targets: broad applications or therapeutic niche? *FEBS Journal* 284 (10), 1473–1488. doi:10.1111/febs.14009
- Evnouchidou, I., and van Endert, P. (2019). Peptide trimming by endoplasmic reticulum aminopeptidases: role of MHC class I binding and ERAP dimerization. *Hum. Immunol.* 80 (5), 290–295. doi:10.1016/j.humimm.2019.01.003
- Evnouchidou, I., Koumantou, D., Nugue, M., and Saveanu, L. (2023). M1-aminopeptidase family—Beyond antigen-trimming activities. *Curr. Opin. Immunol.* 83, 102337. doi:10.1016/j.coi.2023.102337
- Farsa, O., and Uher, T. (2025). Aminopeptidase N: a multifunctional and promising target in medicinal chemistry. *RSC Advances* 15 (32), 26455–26472. doi:10.1039/d5ra03038b
- Ferdinand, K. C., Balavoine, F., Besse, B., Black, H. R., Desbrandes, S., Ditttrich, H. C., et al. (2019). Efficacy and safety of firibastat, a first-in-class brain aminopeptidase A inhibitor, in hypertensive overweight patients of multiple ethnic origins: a phase 2, open-label, multicenter, dose-titrating study. *Circulation* 140 (2), 138–146. doi:10.1161/CIRCULATIONAHA.119.040070
- Fukasawa, K. M., Hata, T., Ono, Y., and Hirose, J. (2011). Metal preferences of zinc-binding motif on metalloproteases. *J. Amino Acids* 2011 (1), 574816. doi:10.4061/2011/574816
- Giastas, P., Mpakali, A., Papakyriakou, A., Lelis, A., Kokkala, P., Neu, M., et al. (2019). Mechanism for antigenic peptide selection by endoplasmic reticulum aminopeptidase 1. *Proc. Natl. Acad. Sci.* 116 (52), 26709–26716. doi:10.1073/pnas.1912070116
- Giastas, P., Neu, M., Rowland, P., and Stratikos, E. (2019). High-resolution crystal structure of endoplasmic reticulum aminopeptidase 1 with bound phosphonic transition-state analogue inhibitor. *ACS Medicinal Chemistry Letters* 10 (5), 708–713. doi:10.1021/acsmchemlett.9b00002
- Goto, Y., Ogawa, K., Hattori, A., and Tsujimoto, M. (2011). Secretion of endoplasmic reticulum aminopeptidase 1 is involved in the activation of macrophages induced by lipopolysaccharide and interferon- γ . *J. Biol. Chem.* 286 (24), 21906–21914. doi:10.1074/jbc.M111.239111
- Graham, S. C., Bond, C. S., Freeman, H. C., and Guss, J. M. (2005). Structural and functional implications of metal ion selection in aminopeptidase P, a metalloprotease with a dinuclear metal center. *Biochemistry* 44 (42), 13820–13836. doi:10.1021/bi0512849
- Haeggstrom, J. Z. (2004). Leukotriene A4 hydrolase/aminopeptidase, the gatekeeper of chemotactic leukotriene B4 biosynthesis. *J. Biol. Chem.* 279 (49), 50639–50642. doi:10.1074/jbc.R400027200
- Haeggström, J. Z., Tholander, F., and Wetterholm, A. (2007). Structure and catalytic mechanisms of leukotriene A4 hydrolase. *Prostagl and Other Lipid Mediators* 83 (3), 198–202. doi:10.1016/j.prostaglandins.2007.01.006
- Hammer, G. E., Gonzalez, F., Champsaur, M., Cado, D., and Shastri, N. (2006). The aminopeptidase ERAAP shapes the peptide repertoire displayed by major histocompatibility complex class I molecules. *Nat. Immunol.* 7 (1), 103–112. doi:10.1038/ni1286
- Holmes, R. S., Spradling-Reeves, K. D., and Cox, L. A. (2017). Mammalian glutamyl aminopeptidase genes (ENPEP) and proteins: comparative studies of a major contributor to arterial hypertension. *J. Data Mining Genomics and Proteomics* 8 (2), 2. doi:10.4172/2153-0602.1000211
- Hooper, N. M. (1994). Families of zinc metalloproteases. *FEBS Letters* 354 (1), 1–6. doi:10.1016/0014-5793(94)01079-x
- Hryczanek, R. P., Hackett, A. S., Rowland, P., Chung, C. W., Convery, M. A., Holmes, D. S., et al. (2024). Optimization of potent and selective cyclohexyl acid ERAP1 inhibitors using structure-and property-based drug design. *ACS Medicinal Chemistry Letters* 15 (12), 2107–2114. doi:10.1021/acsmchemlett.4c00401
- Hutchinson, J. P., Temponeras, I., Kuiper, J., Cortes, A., Korczynska, J., Kitchen, S., et al. (2021). Common allotypes of ER aminopeptidase 1 have substrate-dependent and highly variable enzymatic properties. *J. Biol. Chem.* 296, 100443. doi:10.1016/j.jbc.2021.100443
- Johnson, G. D., and Hersh, L. B. (1990). Studies on the subsite specificity of the rat brain puromycin-sensitive aminopeptidase. *Archives Biochemistry Biophysics* 276 (2), 305–309. doi:10.1016/0003-9861(90)90724-d
- Karlin, S., and Zhu, Z. Y. (1997). Classification of mononuclear zinc metal sites in protein structures. *Proc. Natl. Acad. Sci.* 94 (26), 14231–14236. doi:10.1073/pnas.94.26.14231
- Khosla, J., Aronow, W. S., and Frishman, W. H. (2022). Firibastat: an oral first-in-class brain aminopeptidase A inhibitor for systemic hypertension. *Cardiol. Review* 30 (1), 50–55. doi:10.1097/CRD.0000000000000360
- Kirino, Y., Bertias, G., Ishigatsubo, Y., Mizuki, N., Tugal-Tutkun, I., Seyahi, E., et al. (2013). Genome-wide association analysis identifies new susceptibility loci for behcet’s disease and epistasis between HLA-B* 51 and ERAP1. *Nat. Genetics* 45 (2), 202–207. doi:10.1038/ng.2520
- Kokkala, P., Mpakali, A., Mauvais, F. X., Papakyriakou, A., Daskalaki, I., Petropoulou, I., et al. (2016). Optimization and structure–activity relationships of phosphonic pseudotriptide inhibitors of aminopeptidases that generate antigenic peptides. *J. Med. Chem.* 59 (19), 9107–9123. doi:10.1021/acs.jmedchem.6b01031
- Kolb, A. F., Hegyi, A., Maile, J., Heister, A., Hagemann, M., and Siddell, S. G. (1998). “Molecular analysis of the coronavirus-receptor function of aminopeptidase N,” in *Coronaviruses and arteriviruses* (Boston, MA: Springer US), 61–67.
- Koumantou, D., Barnea, E., Martin-Esteban, A., Maben, Z., Papakyriakou, A., Mpakali, A., et al. (2019). Editing the immunopeptidome of melanoma cells using a potent inhibitor of endoplasmic reticulum aminopeptidase 1 (ERAP1). *Cancer Immunol. Immunother.* 68 (8), 1245–1261. doi:10.1007/s00262-019-02358-0
- Kudo, L. C., Parfenova, L., Ren, G., Vi, N., Hui, M., Ma, Z., et al. (2011). Puromycin-sensitive aminopeptidase (PSA/NPEPPS) impedes development of neuropathology in hPSA/TAUP301L double-transgenic mice. *Hum. Mol. Genet.* 20 (9), 1820–1833. doi:10.1093/hmg/ddr065
- Leib, J., Waeckel-Énée, E., Fabrega, S., Keelan, N., Senni, A., Mauvais, F. X., et al. (2025). ERAP1-dependent extreme antigen processing efficacy can govern MHC class I expression hierarchy. *J. Immunol.* 214 (6), 1147–1159. doi:10.1093/jimmunol/vkaf013
- Li, J. J., Chang, S. F., Liao, I. I., Chan, P. C., Liu, R. S., Yen, S. H., et al. (2016). Targeted antitumor prodrug therapy using CNGRC- γ CD fusion protein in combination with 5-fluorocytosine. *J. Biomedical Science* 23 (1), 15. doi:10.1186/s12929-016-0227-6
- Li, X., Han, M., Zhang, H., Liu, F., Pan, Y., Zhu, J., et al. (2022). Structures and biological functions of zinc finger proteins and their roles in hepatocellular carcinoma. *Biomark. Research* 10 (1), 2. doi:10.1186/s40364-021-00345-1
- Little, J., Hutchinson, J. P., Kitchen, S., Rowland, P., Neu, M., Cecconie, T., et al. (2020). Targeting the regulatory site of ER aminopeptidase 1 leads to the discovery of a natural product modulator of antigen presentation. *J. Med. Chem.* 63 (6), 3348–3358. doi:10.1021/acs.jmedchem.9b02123
- López de Castro, J. A. (2018). How ERAP1 and ERAP2 shape the peptidomes of disease-associated MHC-I proteins. *Front. Immunology* 9, 2463. doi:10.3389/fimmu.2018.02463
- Maben, Z., Arya, R., Rane, D., An, W. F., Metkar, S., Hickey, M., et al. (2019). Discovery of selective inhibitors of endoplasmic reticulum aminopeptidase 1. *J. Medicinal Chemistry* 63 (1), 103–121. doi:10.1021/acs.jmedchem.9b00293
- Maben, Z., Arya, R., Georgiadis, D., Stratikos, E., and Stern, L. J. (2021). Conformational dynamics linked to domain closure and substrate binding explain the ERAP1 allosteric regulation mechanism. *Nat. Commun.* 12 (1), 5302. doi:10.1038/s41467-021-25564-w
- Madabushi, S., Chow, K. M., Song, E. S., Goswami, A., Hersh, L. B., and Rodgers, D. W. (2023). Structure of puromycin-sensitive aminopeptidase and polyglutamine binding. *Plos One* 18 (7), e0287086. doi:10.1371/journal.pone.0287086
- Martin-Esteban, A., Rodriguez, J. C., Peske, D., Lopez de Castro, J. A., Shastri, N., and Sadegh-Nasseri, S. (2022). The ER aminopeptidases, ERAP1 and ERAP2, synergize to self-modulate their respective activities. *Front. Immunology* 13, 1066483. doi:10.3389/fimmu.2022.1066483
- Mattorre, B., Tedeschi, V., Paldino, G., Fiorillo, M. T., Paladini, F., and Sorrentino, R. (2022). The emerging multifunctional roles of ERAP1, ERAP2 and IRAP between antigen processing and renin-angiotensin system modulation. *Front. Immunol.* 13, 1002375. doi:10.3389/fimmu.2022.1002375
- Mpakali, A., Saridakis, E., Harlos, K., Zhao, Y., Papakyriakou, A., Kokkala, P., et al. (2015). Crystal structure of insulin-regulated aminopeptidase with bound substrate analogue provides insight on antigenic epitope precursor recognition and processing. *J. Immunol.* 195 (6), 2842–2851. doi:10.4049/jimmunol.1501103
- Mpakali, A., Giastas, P., Deprez-Poulain, R., Papakyriakou, A., Koumantou, D., Gealageas, R., et al. (2017). Crystal structures of ERAP2 complexed with inhibitors reveal pharmacophore requirements for optimizing inhibitor potency. *ACS Med. Chem. Lett.* 8 (3), 333–337. doi:10.1021/acsmchemlett.6b00505

- Mucha, A., Drag, M., Dalton, J. P., and Kafarski, P. (2010). Metallo-aminopeptidase inhibitors. *Biochimie* 92 (11), 1509–1529. doi:10.1016/j.biochi.2010.04.026
- Murakami, H., Yokoyama, A., Kondo, K., Nakanishi, S., Kohno, N., and Miyake, M. (2005). Circulating aminopeptidase N/CD13 is an independent prognostic factor in patients with non-small cell lung cancer. *Clin. Cancer Research* 11 (24), 8674–8679. doi:10.1158/1078-0432.CCR-05-1005
- Nguyen, T. T., Chang, S. C., Evnouchidou, I., York, I. A., Zikos, C., Rock, K. L., et al. (2011). Structural basis for antigenic peptide precursor processing by the endoplasmic reticulum aminopeptidase ERAP1. *Nat. Structural and Molecular Biology* 18 (5), 604–613. doi:10.1038/nsmb.2021
- Nikopaschou, M., Samiotaki, M., Kannavou, A., Angelis, N. V., Tsitsilonis, O., Panayotou, G., et al. (2025). ERAP1 allotypes 2 and 10 differentially regulate the immunopeptidome of melanocytes. *Immunology* 177 (2), 398–412. doi:10.1111/imm.70056
- Oteiza, P. I. (2012). Zinc and the modulation of redox homeostasis. *Free Radic. Biol. Med.* 53 (9), 1748–1759. doi:10.1016/j.freeradbiomed.2012.08.568
- Pace, N. J., and Weerapana, E. (2014). Zinc-binding cysteines: diverse functions and structural motifs. *Biomolecules* 4 (2), 419–434. doi:10.3390/biom4020419
- Pal, K., Feng, X., Steinke, J. W., Burdick, M. D., Shim, Y. M., Sung, S. S., et al. (2019). Leukotriene A4 hydrolase activation and leukotriene B4 production by eosinophils in severe asthma. *Am. J. Respir. Cell Mol. Biol.* 60 (4), 413–419. doi:10.1165/rcmb.2018-0175OC
- Papakyriakou, A., Zervoudi, E., Theodorakis, E. A., Saveanu, L., Stratikos, E., and Vourloumis, D. (2013). Novel selective inhibitors of aminopeptidases that generate antigenic peptides. *Bioorg. and Medicinal Chemistry Letters* 23 (17), 4832–4836. doi:10.1016/j.bmcl.2013.07.024
- Papakyriakou, A., Zervoudi, E., Tsoukalidou, S., Mauvais, F. X., Sfyroera, G., Mastellos, D. C., et al. (2015). 3, 4-diaminobenzoic acid derivatives as inhibitors of the oxytocinase subfamily of M1 aminopeptidases with immune-regulating properties. *J. Medicinal Chemistry* 58 (3), 1524–1543. doi:10.1021/jm501867s
- Papakyriakou, A., Mpakali, A., and Stratikos, E. (2022). Can ERAP1 and ERAP2 form functional heterodimers? A structural dynamics investigation. *Front. Immunol.* 13, 863529. doi:10.3389/fimmu.2022.863529
- Pasqualini, R., Koivunen, E., Kain, R., Lahdenranta, J., Sakamoto, M., Stryhn, A., et al. (2000). Aminopeptidase N is a receptor for tumor-homing peptides and a target for inhibiting angiogenesis. *Cancer Research* 60 (3), 722–727.
- Reaux, A., Iturriz, X., Vazeux, G., Fournie-Zaluski, M. C., David, C., Roques, B. P., et al. (2000). Aminopeptidase A, which generates one of the main effector peptides of the brain renin-angiotensin system, angiotensin III, has a key role in central control of arterial blood pressure. *Biochem. Soc. Trans.* 28 (4), 435–440. doi:10.1042/bst0280435
- Reeves, E., Edwards, C. J., Elliott, T., and James, E. (2013). Naturally occurring ERAP1 haplotypes encode functionally distinct alleles with fine substrate specificity. *J. Immunol.* 191 (1), 35–43. doi:10.4049/jimmunol.1300598
- Reeves, E., Colebatch-Bourn, A., Elliott, T., Edwards, C. J., and James, E. (2014). Functionally distinct ERAP1 allotype combinations distinguish individuals with ankylosing spondylitis. *Proc. Natl. Acad. Sci.* 111 (49), 17594–17599. doi:10.1073/pnas.1408882111
- Reeves, E., Islam, Y., and James, E. (2020). ERAP1: a potential therapeutic target for a myriad of diseases. *Expert Opinion Therapeutic Targets* 24 (6), 535–544. doi:10.1080/14728222.2020.1751821
- Rudberg, P. C., Tholander, F., Thunnissen, M. M., Samuelsson, B., and Haeggström, J. Z. (2002). Leukotriene A4 hydrolase: selective abrogation of leukotriene B4 formation by mutation of aspartic acid 375. *Proc. Natl. Acad. Sci.* 99 (7), 4215–4220. doi:10.1073/pnas.072090099
- Sanz-Bravo, A., Alvarez-Navarro, C., Martín-Esteban, A., Barnea, E., Admon, A., and de Castro, J. A. L. (2018). Ranking the contribution of ankylosing spondylitis-associated endoplasmic reticulum aminopeptidase 1 (ERAP1) polymorphisms to shaping the HLA-B*27 peptidome. *Mol. and Cell. Proteomics* 17 (7), 1308–1323. doi:10.1074/mcp.ra117.000565
- Saulle, I., Vicentini, C., Clerici, M., and Biasin, M. (2021). Antigen presentation in SARS-CoV-2 infection: the role of class I HLA and ERAP polymorphisms. *Hum. Immunol.* 82 (8), 551–560. doi:10.1016/j.humimm.2021.05.003
- Saveanu, L., Carroll, O., Lindo, V., Del Val, M., Lopez, D., Lepelletier, Y., et al. (2005). Concerted peptide trimming by human ERAP1 and ERAP2 aminopeptidase complexes in the endoplasmic reticulum. *Nat. Immunology* 6 (7), 689–697. doi:10.1038/nii208
- Schinzari, F., Montenero, R., Cardillo, C., and Tesaro, M. (2025). Emerging pharmacological approaches for the treatment of arterial hypertension. *Biomedicine* 13 (4), 790. doi:10.3390/biomedicine13040790
- Schreiber, C. L., and Smith, B. D. (2018). Molecular imaging of aminopeptidase N in cancer and angiogenesis. *Contrast Media and Molecular Imaging* 2018 (1), 5315172. doi:10.1155/2018/5315172
- Stratikos, E., and Stern, L. J. (2013). Antigenic peptide trimming by ER aminopeptidases—Insights from structural studies. *Mol. Immunology* 55 (3–4), 212–219. doi:10.1016/j.molimm.2013.03.002
- Takeuchi, M., Ombrello, M. J., Kirino, Y., Erer, B., Tugal-Tutkun, I., Seyahi, E., et al. (2016). A single endoplasmic reticulum aminopeptidase-1 protein allotype is a strong risk factor for behçet’s disease in HLA-B*51 carriers. *Ann. Rheumatic Diseases* 75 (12), 2208–2211. doi:10.1136/annrheumdis-2015-209059
- Temponeras, I., Stamatakis, G., Samiotaki, M., Georgiadis, D., Pratsinis, H., Panayotou, G., et al. (2022). ERAP2 inhibition induces cell-surface presentation by MOLT-4 leukemia cancer cells of many novel and potentially antigenic peptides. *Int. J. Mol. Sci.* 23 (3), 1913. doi:10.3390/ijms23031913
- Temponeras, I., Samiotaki, M., Koumantou, D., Nikopaschou, M., Kuiper, J. J., Panayotou, G., et al. (2023). Distinct modulation of cellular immunopeptidome by the allosteric regulatory site of ER aminopeptidase 1. *Eur. J. Immunol.* 53 (8), 2350449. doi:10.1002/eji.202350449
- Thompson, M. W. (2022). Regulation of zinc-dependent enzymes by metal carrier proteins. *Biomaterials* 35 (2), 187–213. doi:10.1007/s10534-022-00373-w
- Thompson, M. W., and Hersh, L. B. (2003). Analysis of conserved residues of the human puromycin-sensitive aminopeptidase. *Peptides* 24 (9), 1359–1365. doi:10.1016/j.peptides.2003.07.012
- Thunnissen, M. M., Nordlund, P., and Haeggström, J. Z. (2001). Crystal structure of human leukotriene A4 hydrolase, a bifunctional enzyme in inflammation. *Nat. Structural Biology* 8 (2), 131–135. doi:10.1038/84117
- Țiburcă, L., Zaha, D. C., Jurca, M. C., Severin, E., Jurca, A., and Jurca, A. D. (2024). The role of aminopeptidase ERAP1 in human Pathology—A review. *Curr. Issues Mol. Biol.* 46 (3), 1651–1667. doi:10.3390/cimb46030107
- Trencsényi, G., Halmos, G., and Képes, Z. (2023). Radiolabeled NGR-Based heterodimers for angiogenesis imaging: a review of preclinical studies. *Cancers* 15 (18), 4459. doi:10.3390/cancers15184459
- Trenholme, K., I Brown, C., Skinner-Adams, T., Stack, C., Lowther, J., To, J., et al. (2010). Aminopeptidases of malaria parasites: new targets for chemotherapy. *Infect. Disorders-Drug Targets Formerly Curr. Drug Targets-Infectious Disord.* 10 (3), 217–225. doi:10.2174/187152610791163363
- Vallee, B. L., and Auld, D. S. (1990). Zinc coordination, function, and structure of zinc enzymes and other proteins. *Biochemistry* 29 (24), 5647–5659. doi:10.1021/bi00476a001
- Vallee, B. L., and Auld, D. S. (1993). Zinc: biological functions and coordination motifs. *Accounts Chemical Research* 26 (10), 543–551. doi:10.1021/ar00034a005
- Vendrell, J., and Avilés, F. X. (1999). *Carboxypeptidases*. Proteases new perspectives, 13–34. doi:10.1007/978-3-0348-8737-3_2
- Vourloumis, D., Mavridis, I., Athanasoulis, A., Temponeras, I., Koumantou, D., Giastas, P., et al. (2022). Discovery of selective nanomolar inhibitors for insulin-regulated aminopeptidase based on α -hydroxy- β -amino acid derivatives of bestatin. *J. Medicinal Chemistry* 65 (14), 10098–10117. doi:10.1021/acs.jmedchem.2c00904
- Węglarz-Tomczak, E., Vassiliou, S., and Mucha, A. (2016). Discovery of potent and selective inhibitors of human aminopeptidases ERAP1 and ERAP2 by screening libraries of phosphorus-containing amino acid and dipeptide analogues. *Bioorg. and Med. Chem. Lett.* 26 (16), 4122–4126. doi:10.1016/j.bmcl.2016.06.062
- York, I. A., Chang, S. C., Saric, T., Keys, J. A., Favreau, J. M., Goldberg, A. L., et al. (2002). The ER aminopeptidase ERAP1 enhances or limits antigen presentation by trimming epitopes to 8–9 residues. *Nat. Immunology* 3 (12), 1177–1184. doi:10.1038/nr860
- Zastrow, M. L., and Pecoraro, V. L. (2013). Influence of active site location on catalytic activity in de novo-designed zinc metalloenzymes. *J. Am. Chem. Soc.* 135 (15), 5895–5903. doi:10.1021/ja401537t
- Zastrow, M. L., and Pecoraro, V. L. (2014). Designing hydrolytic zinc metalloenzymes. *Biochemistry* 53 (6), 957–978. doi:10.1021/bi4016617
- Zervoudi, E., Saridakis, E., Birtley, J. R., Seregin, S. S., Reeves, E., Kokkala, P., et al. (2013). Rationally designed inhibitor targeting antigen-trimming aminopeptidases enhances antigen presentation and cytotoxic T-cell responses. *Proc. Natl. Acad. Sci.* 110 (49), 19890–19895. doi:10.1073/pnas.1309781110
- Zheng, J., Cheng, Z., Jia, H., and Zheng, Y. (2016). Characterization of aspartyl aminopeptidase from *Toxoplasma gondii*. *Sci. Rep.* 6 (1), 34448. doi:10.1038/srep34448
- Zou, M., Zhang, L., Xie, Y., and Xu, W. (2012). NGR-Based strategies for targeting delivery of chemotherapeutics to tumor vasculature. *Anti-Cancer Agents Med. Chem. Former. Curr. Med. Chemistry-Anti-Cancer Agents* 12 (3), 239–246. doi:10.2174/187152012800228751