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# Dissecting the copper bioinorganic chemistry of the functional and pathological roles of the prion protein: Relevance in Alzheimer's disease and cancer

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**Abstract**

The cellular prion protein ($PrP^C$) is a metal-binding biomolecule that can interact with different protein partners involved in pivotal physiological processes, such as neurogenesis and neuronal plasticity. Recent studies profile copper and $PrP^C$ as important players in the pathological mechanisms of Alzheimer's disease and cancer. Although the copper-$PrP^C$ interaction has been characterized extensively, the role of the metal ion in the physiological and pathological roles of $PrP^C$ has been barely explored. In this article, we discuss how copper binding and proteolytic processing may impact the ability of $PrP^C$ to recruit protein partners for its functional roles. The importance to dissect the role of copper-$PrP^C$ interactions in health and disease is also underscored.

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**Keywords**

Copper, Prion protein, Neurogenesis, Neuroplasticity, Alzheimer's disease, Cancer.

## The cellular prion protein as a multifaced protein partner

The cellular prion protein ($PrP^C$) is a membrane-anchored glycoprotein mainly located within lipid rafts, which are microdomains that favor the formation of signaling complexes [1]. $PrP^C$ is ubiquitously expressed in the body and is particularly abundant in the central nervous system [2]. Although this protein is well-known for its role in neurodegeneration, it has also been associated with pivotal cellular processes, such as neurogenesis and neuronal plasticity. Along with its flexible N-terminal domain, $PrP^C$ has functional regions engaged in copper binding and electrostatic/hydrophobic interactions with different protein partners (Figure 1a–b) [3,4]. $PrP^C$ interacts with proteins involved in neurodegeneration, such as amyloid-beta peptide (Aβ), but it also binds key players in axonal growth and neuronal plasticity, such as the neural cell adhesion molecule (NCAM), the extracellular matrix proteins — laminin (Ln), and vitronectin (Vn) — , the stress-inducible protein 1 (STI1), and glutamate receptors (NMDAR and mGluR1/5) [5]. In these interactions, $PrP^C$ can act as a scaffold, recruiting proteins to form functional complexes at the cellular membrane, and it can also act as a receptor, binding extracellular signals and transmembrane proteins to activate signaling pathways [6,7]. $PrP^C$ undergoes four irreversible proteolytic modifications that can alter its ability to interact with other proteins, shedding releases a full-length soluble protein, while the α-, β-, and γ-cleavages yield C- and N-terminal fragments (Figure 1c) [8]. Although the C-terminal fragments remain attached to the cellular membrane, they lose important regions to interact with other proteins, which might impact the role of $PrP^C$ as a scaffold and as a receptor (Figure 1b–c). The soluble N-terminal fragments and shed $PrP^C$ lack a membrane anchoring but they can bind its protein partners at the extracellular space or perform as signaling agents by binding to cell-surface receptors [9–12]. Understanding the molecular details that drive the interactions of $PrP^C$ with its multiple protein partners is crucial to dissect the role of this protein in health and disease [5,8]. Interestingly, some of these interactions occur in regions engaged in copper binding, and this metal has been implicated in functional and pathological roles of $PrP^C$. This article discusses the impact of the copper-binding properties of $PrP^C$ in its interaction with proteins involved in neuritogenesis, neuronal plasticity, and neurodegeneration.

## Abbreviations

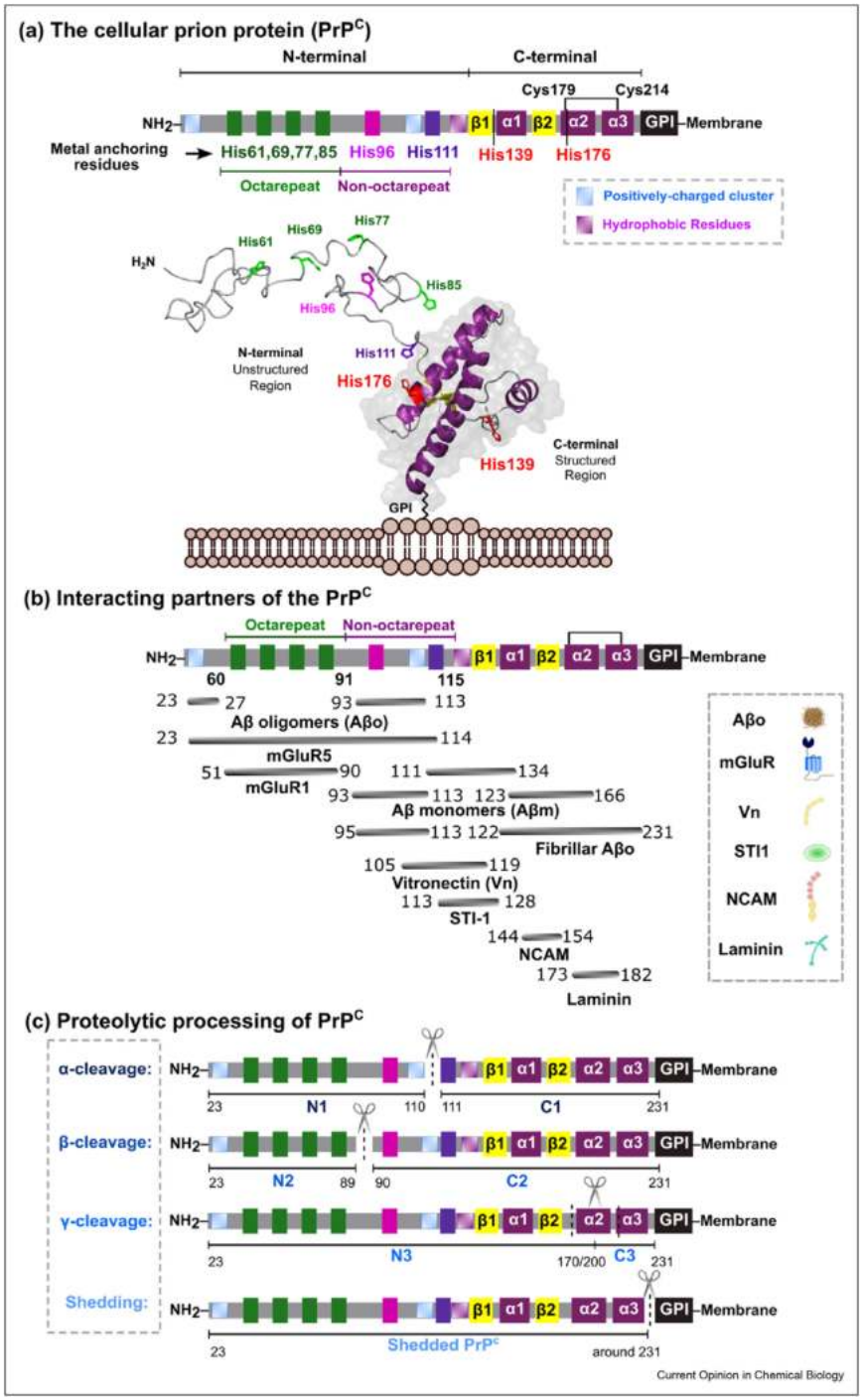
| | |
|---|---|
| $PrP^C$ | cellular prion protein |
| Aβ | amyloid-beta peptide |
| NCAM | neural cell adhesion molecule |
| Ln | laminin |
| Vn | vitronectin |
| STI1 | stress-inducible protein 1 |
| NMDAR | N-methyl-D-Aspartate receptor |
| mGluR1 | metabotropic glutamate receptor 1 |
| mGluR5 | metabotropic glutamate receptor 5 |
| rPrP | recombinant PrP |
| PrP | prion protein |
| Aβo | amyloid-beta oligomers |
| Aβm | amyloid-beta monomers |
| ADAM8 | a disintegrin and metalloproteinase domain-containing protein 8 |
| NHE | normal hydrogen electrode |
| NPC | neuronal progenitor cells |
| α7AChR | α7 nicotinic acetylcholine receptor |
| ECM | extracellular matrix |
| PI3K | the phosphoinositide 3-kinase |
| mTOR | a mammalian target of rapamycin |
| MAPK | the mitogen-activated protein kinase |
| ERK1/2 | the extracellular signal-regulated kinase 1/2 |
| PKA | protein kinase A |
| PKC | protein kinase C |
| RhoA | Rho GTPase A |
| ROCK | Rho kinase |
| LIMK | LIM kinase |
| ATP7A | ATPase copper transporting alpha |
| AD | Alzheimer's disease |
| MEMO | mediator of ERBB2-driven cell motility |
| LOX | lysyl oxidase protein |
| Atox-1 | antioxidant 1 copper chaperone |

## Can copper binding modulate the interaction of $PrP^C$ with its protein partners?

Recombinant PrP (rPrP) can anchor up to six $Cu^{2+}$ ions at its N-terminal, displaying different coordination modes with different affinities (Figure 2c). The coordination properties of PrP are highly dependent on $Cu^{2+}$ concentration, $Cu^{2+}$/protein ratio, and pH (as reviewed in the study by Sanchez-Lopez et al. [3], Evans and Millhauser [4], and Salzano et al. [13]). A recent study demonstrated that $Cu^{2+}$ binding to rPrP favors a contact between the N- and C-terminal domains (*cis*-interdomain) forming a complex that involves three His residues from the octarepeat region and C-terminal His139/176 residues (Figure 2b) [14]. Because multi-His coordination is only favored at low $Cu^{2+}$/PrP ratios, $Cu^{2+}$-binding to PrP could act as a conformational switch; at low $Cu^{2+}$ levels, a multi-His coordination mode stabilizes the *cis*-interdomain interaction (Figure 2bi), while $Cu^{2+}$ coordination modes favored at high $Cu^{2+}$ levels prevent interdomain interactions (Figure 2b–iii) [14]. This conformational switch could drive the lateral movement of $PrP^C$ outside the lipid rafts, a mechanism that depends on the octarepeat region and $Cu^{2+}$ concentration (Figure 2b) [15]. On the other hand, some proteins — such as Ln, Vn, and Aβ oligomers (Aβo) — bind $PrP^C$ involving residues from the non-octarepeat region, such that $Cu^{2+}$ coordination to His96 and His111 could either compete for protein-binding sites and/or form ternary complexes. Hence, $Cu^{2+}$ binding to $PrP^C$ could impact its interactions with other proteins through several mechanisms by controlling its localization at lipid rafts, inducing conformational changes (as *cis*-interdomain interaction) that could be recognized by other proteins, forming ternary protein-$Cu^{2+}$-$PrP^C$ complexes, or competing for protein-binding sites. Moreover, some copper-$PrP^C$ complexes can undergo redox cycling, producing activated forms of oxygen (such as $H_2O_2$) and nitric oxide that are important for cell signaling [16–18]. Overall, the dynamic copper binding to $PrP^C$, involving a wide variety of coordination modes with different metal-binding affinities and redox activities, opens a myriad of ways by which copper could modulate the functional roles of $PrP^C$.

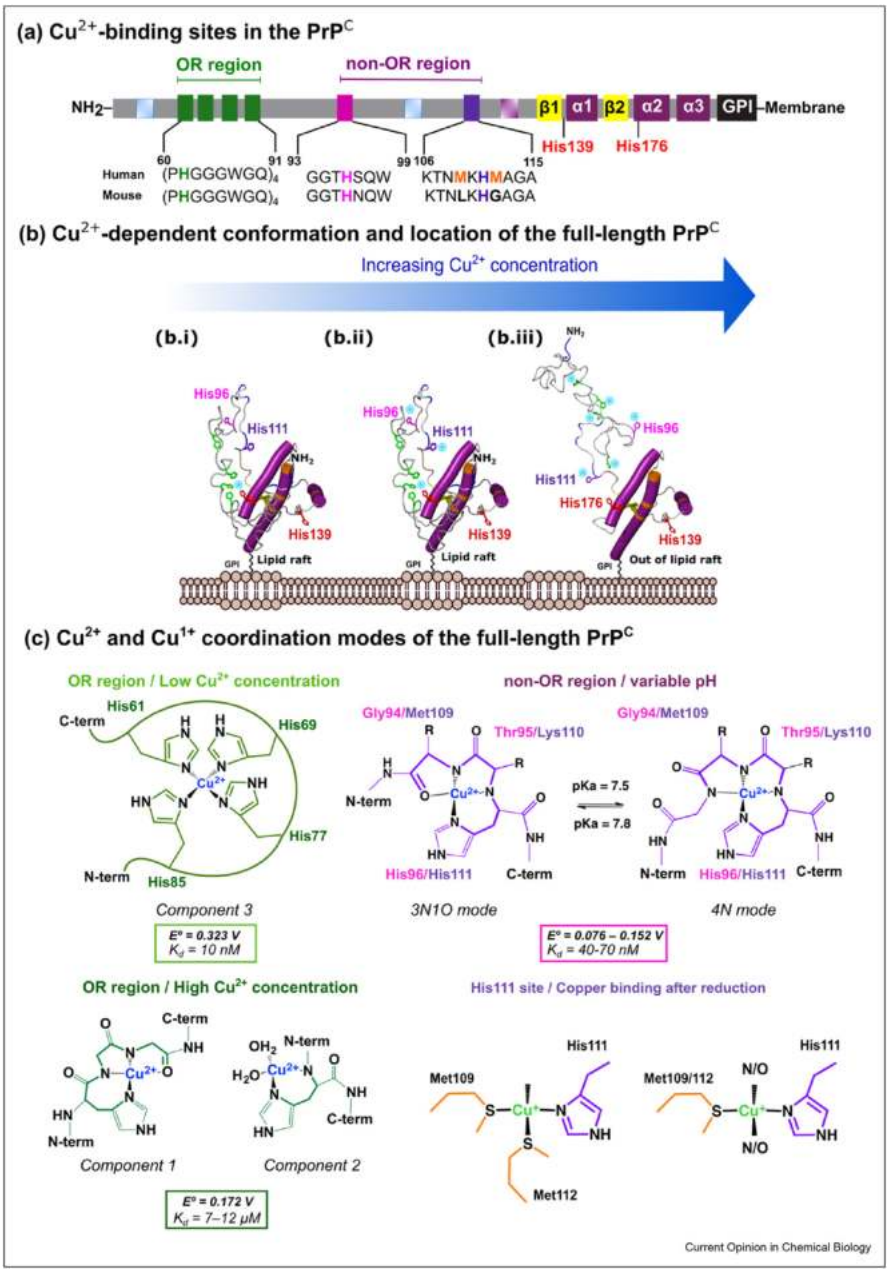
In addition, proteolytic processing of $PrP^C$ impacts its metal-binding properties, contributing to diverse $Cu^{2+}$-$PrP^C$ speciation. Conversely, both α- and β-cleavage product distributions are strongly affected by the presence of copper ions [19]. For instance, $Cu^{2+}$ binding to His111 prevents α-cleavage at the Lys110↓His111 site. Shedding and γ-cleavage of $PrP^C$ generate soluble forms that preserve all its metal-binding sites intact [3,8]. α- and β-cleavages produce soluble fragments (N1 and N2, respectively) with some conserved metal-binding sites and membrane-attached fragments (C1 and C2, respectively), with a free N-terminal group (Figures 1c and 3a) [3,8,20]. A free-$NH_2$ group has an electron pair that can act as a strong anchoring site for $Cu^{2+}$, significantly impacting the metal-binding properties of the protein. Recently, $Cu^{2+}$ binding to a peptide model for the α-cleaved C1 fragment has been studied, finding that it displays two coordination modes, termed Mode I and II, depending on the relative $Cu^{2+}$/PrP concentrations (Figure 3b–c) [20]. Interestingly, Mode I involves two PrP molecules and one $Cu^{2+}$ ion, suggesting that $Cu^{2+}$ might induce dimerization of cleaved-$PrP^C$ [20]. Investigating the possibility of a $Cu^{2+}$-induced dimerization of α-cleaved-$PrP^C$ is particularly important because dimerization of membrane proteins is a common mechanism in cell adhesion or activation of signaling pathways, although the interplay between this effect and the impact of copper ions in the α-cleavage

**Figure 1**



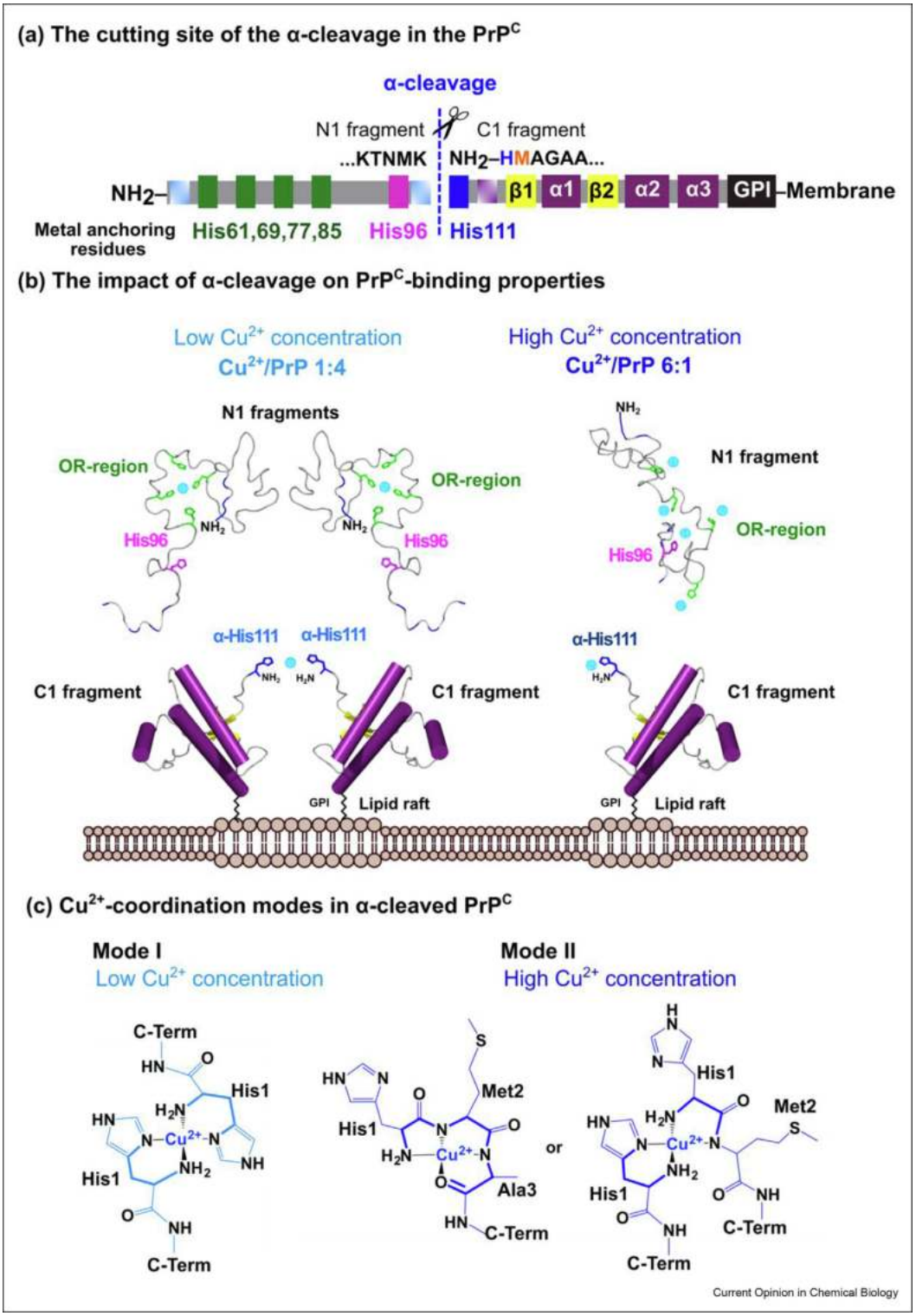

The cellular prion protein: structure, interacting partners and proteolytic processing. **(a)** Structural and functional features of $PrP^C$. The N-terminal domain exhibits a cluster of positively charged residues (shimmer blue); two metal-binding regions, octarepeat (green) containing four His residues, and the non-octarepeat with His96 (pink) and His111 (purple), and one hydrophobic cluster (shimmer purple). The His residues act as anchoring ligands for $Cu^{2+}$ ions. The structured C-terminal domain is folded by three α-helices and two anti-parallel β-sheets, containing His139 and His176 residues that can also participate in $Cu^{2+}$ binding. **(b)** Regions of $PrP^C$ identified as sites of interaction with protein partners. **(c)** Schematic representation of the four cleavage events that $PrP^C$ undergoes (α-, β- and γ-cleavage, and the shedding process). α-cleavage can occur at Lys110↓His111 or the region spanning from Ala117 to Val121; here, only α-cleavage between residues 110 and 111, as performed by ADAM8, is drawn, yielding N1 (23–110) and C1 (111–230) fragments. β-cleavage occurs between residues 89 and 90, to yield C2 and N2 fragments, the latter possibly suffering further fragmentation. γ-cleavage occurs between residues 170 and 200, to yield N3 and C3 fragments. Finally, the shedding process releases a soluble version of the protein (shed $PrP^C$).

**Figure 2**



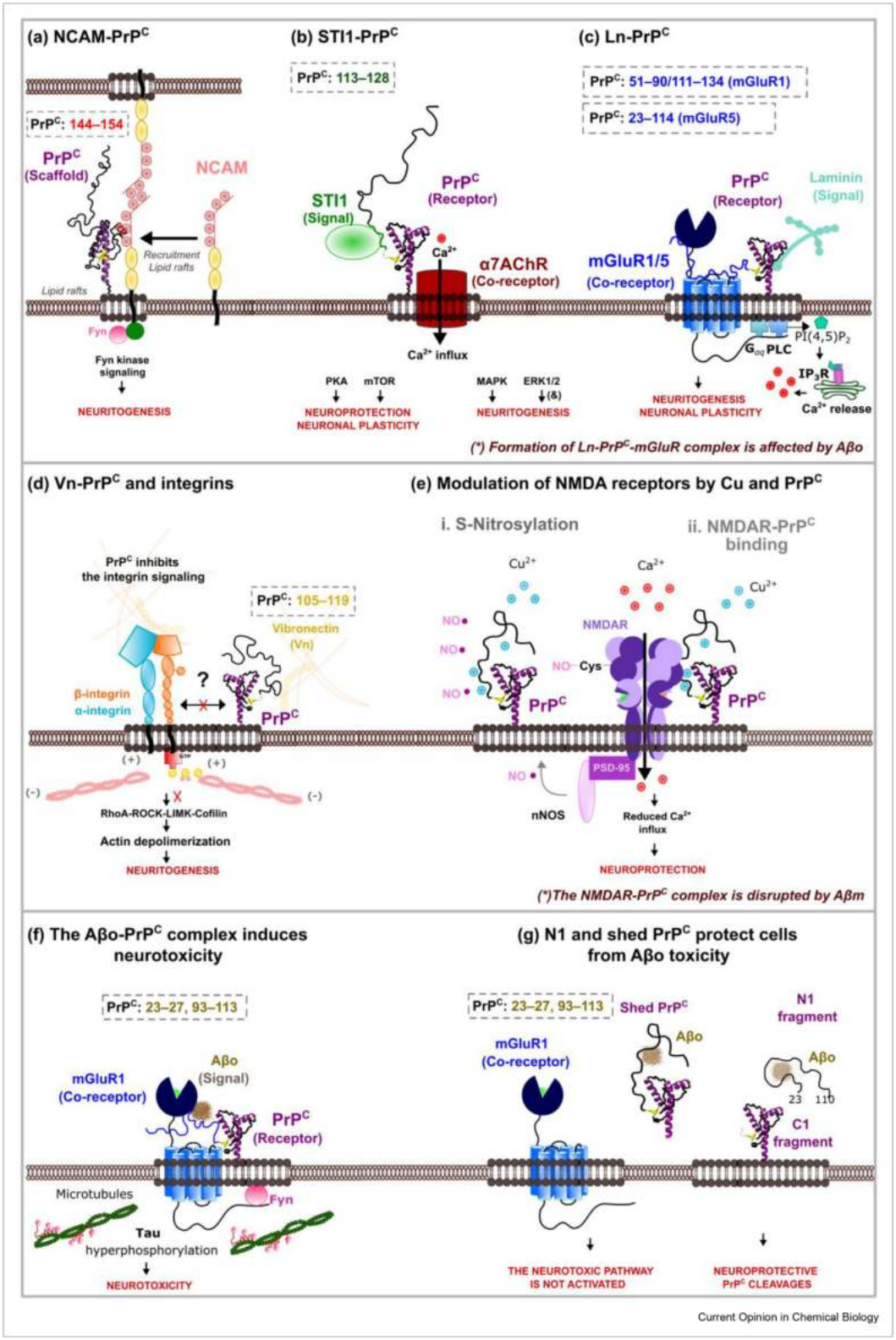

Copper binding to the prion protein. **(a)** The $Cu^{2+}$-anchoring residues include the octarepeat site (green), the His96 and His111 sites in the non-octarepeat region (pink and purple, respectively), and the site including His139 and His176 in the C-terminal domain (red). Amino acid sequences of the metal-anchoring sites from human and mice sequences are compared. The human $PrP^C$ sequence harbors two Met residues (109 and 111) in proximity to His111, which are absent in the mouse sequence. **(b)** Tridimensional representations of the cellular prion protein, illustrating three scenarios of $Cu^{2+}$ bound to the protein. **(b.i)** At a low $Cu^{2+}$/PrP ratio, $Cu^{2+}$ promotes a conformation in $PrP^C$ that involves the interaction between C- and N-terminal domains (*cis*-interdomain interaction), although this bent interaction also occurs in the absence of metal, $Cu^{2+}$ binding to the octarepeat region enhances its stability by forming a multi-His coordination mode. When $PrP^C$ acquires this conformation, it is mainly located in the lipid rafts (see membrane localization of $PrP^C$ in the schematic representation). **(b.ii)** At intermediate $Cu^{2+}$ concentrations (~40–70 nM), the His96 and His111 sites can also coordinate $Cu^{2+}$ ions. **(b.iii)** At high $Cu^{2+}$ concentrations (~100 μM), $PrP^C$ binds up to six $Cu^{2+}$ ions through its N-terminal domain, four at the octarepeat region (green), two at the non-octarepeat region (shown in pink and purple), and one at the free-$NH_2$ group. High $Cu^{2+}$ concentration induces the lateral movement of $PrP^C$ outside the lipid rafts by a mechanism that depends on the octarepeat region, possibly involving the disruption of the *cis*-interdomain interaction. **(c)** Proposed structures for the $Cu^{2+}$-$PrP^C$ complexes that form at the octarepeat region at low and high $Cu^{2+}$ concentrations (dark and light-green, respectively); the His96 and His111 sites in the non-octarepeat region (pink and purple, respectively), which yield similar 3N1O and 4N coordination modes. Metal-binding affinities and redox potentials for each $Cu^{2+}$ coordination mode are given. Although component 3 is reduced easily ($E^o$ = 0.323 V vs NHE), its reoxidation is very difficult, because its estimated binding affinity for $Cu^{1+}$ is three magnitude orders higher than its binding affinity for $Cu^{2+}$. Components 1 and 2 ($E^o$ = 0.172 vs NHE), as well as the 3N1O (0.076 V vs NHE) and 4N ($E^o$ = 0.152 V vs NHE) modes at the His111 site can be reduced by ascorbate and re-oxidized by $O_2$. In the His111 site, $Cu^{1+}$ is stabilized by Met109 and Met112 residues (orange).

**Figure 3**




Copper binding to the α-cleaved $PrP^C$. **(a)** Schematic representation of the α-cleavage of $PrP^C$ at Lys110↓His111, as performed by ADAM8. **(b)** Structural representation of the resulting $Cu^{2+}$-bound N1 and C1 fragments at different $Cu^{2+}$ concentrations: at low $Cu^{2+}$ concentrations (left), N1 fragments could form Component 3, while the anchored C1 fragment could dimerize to generate a bis-His binding site with one $Cu^{2+}$ ion; at high $Cu^{2+}$ concentrations (right), each His site of the N1 fragment can bind one metal ion to yield Components 1 and 2, while the C1 fragment could bind $Cu^{2+}$ in a 1:1 ratio and it would not dimerize. **(c)** $Cu^{2+}$ coordination modes of the α-cleaved $PrP^C$. At low $Cu^{2+}$ concentrations, the free $NH_2$ group and His residues of two C1 fragments coordinate $Cu^{2+}$ to form a 4N coordination mode, termed mode I (light blue); while at high $Cu^{2+}$ concentrations, a 1:1 complex with proposed 4N or 3N1O coordination modes is formed, termed mode II.

**Figure 4**




Physiological and pathological roles of the interaction of $PrP^C$ with its protein partners. **(a)** $PrP^C$ binds NCAMs at the first α-helix (144–154) at the C-terminal domain, recruiting this protein to lipid rafts. The NCAMs-$PrP^C$ complex promotes neuritogenesis through the activation of the Fyn signaling pathway. In this mechanism, $PrP^C$ acts as a scaffold protein (**b)** $PrP^C$ interacts with STI1 close to the non-octarepeat region (113–128). The STI1-$PrP^C$ complex promotes neuritogenesis by activating the PI3K-mTOR, MAPK and ERK1/2 signaling pathways, while this complex also participates in

needs to be taken into account [19]. Finally, the $Cu^{2+}$ binding ability of soluble N1 and N2 fragments must be considered to elucidate the functional and pathological roles of cleaved-$PrP^C$ species.

## How can copper impact the role of $PrP^C$ in pivotal cellular processes?

Copper has been implicated in the same cellular processes as $PrP^C$, such as neurogenesis and neuronal plasticity. The former generates new neurons from neuronal progenitor cells (NPCs) [21], involving cell proliferation, migration, and differentiation [21]. In the adult brain, copper is enriched in the subventricular zone, a niche of NPCs [22]. During neuronal differentiation, intracellular copper demand is increased to synthesize metalloenzymes [23]. Moreover, $PrP^C$ is also upregulated in NPCs, and it is essential for proliferation and differentiation [24]. The role of $PrP^C$ in neuritogenesis is associated with its interaction with NCAMs, STI1, Ln, and Vn (Figure 4) [24–26]. $PrP^C$ promotes neuritogenesis by recruiting NCAMs to lipid rafts (Figure 4a) and activating signal pathways upon binding STI1 and Ln, using α7AChR and mGluR1/5 as co-receptors (Figure 4b–c) [25,27–30]. Although the mechanism by which Vn promotes neuritogenesis is not well understood, $PrP^C$ helps cytoskeleton remodeling by inhibiting the signaling triggered by integrins, which are receptors for Ln and Vn (Figure 4d) [31,32]. Interestingly, Ln, Vn and mGluR1/5 bind $PrP^C$ involving residues that participate in metal binding (Figure 2b) [31,33,34]. Thus, $Cu^{2+}$ may compete with these protein-binding sites and/or promote the formation of ternary complexes. On the other hand, NCAMs bind $PrP^C$ outside its metal-binding regions (Figure 2b); however, its role in neuritogenesis depends on the localization of $PrP^C$ at lipid rafts (Figure 4a), which is modulated by $Cu^{2+}$ [15]. For the mGluR1-$PrP^C$ and STI1-$PrP^C$ complexes, it was concluded that their formation is not affected by high $Cu^{2+}$ concentrations [35,36]; however, such experiments cannot rule out the impact of the high-affinity $Cu^{2+}$-binding sites (Figure 2bi). Although the effect of $Cu^{2+}$ is commonly tested using high $Cu^{2+}$ concentrations or copper chelators, it is important to consider a wide range of $Cu^{2+}$/PrP ratios, given the complexity of $Cu^{2+}$-$PrP^C$ speciation. Indeed, a recent report suggests that $Cu^{2+}$-mediated interactions of $PrP^C$ with Aβ are highly dependent on the relative $Cu^{2+}$/PrP concentrations [37].

In addition to neurogenesis, copper and $PrP^C$ are involved in neuronal plasticity, a process associated with memory and learning that entails functional and morphological changes in neuronal cells in response to experience or injury [38]. PrP knockout mice display alterations in memory and show higher sensitivity to neuronal damage under stress conditions [1]. Interestingly, the STI1-$PrP^C$ and Ln-$PrP^C$-mGluR5 complexes that participate in neuritogenesis are also important for memory formation (Figure 4b–c) [39]. Moreover, $PrP^C$ binds NMDAR, a calcium-permeable channel that drives activity-dependent changes associated with neuronal plasticity (Figure 4e) [40]. Formation of the NMDAR-$PrP^C$ complex requires $Cu^{2+}$ and prevents neuronal damage by reducing $Ca^{2+}$ influx [35]. Recently, it has been demonstrated that this neuroprotective mechanism requires metal-anchoring residues from octarepeat and non-octarepeat regions [41]. In addition, $PrP^C$ and $Cu^{2+}$ protect neurons by promoting S-nitrosylation of Cys residues at NMDAR (Figure 4e), possibly requiring redox cycling of copper-$PrP^C$ complexes [42]. Although these mechanisms require $Cu^{2+}$ ions, the molecular details of these interactions remain unclear, as well as their impact on memory formation and neurogenesis.

Strikingly, there is a high similarity between the $PrP^C$-dependent mechanisms associated with neuritogenesis and neuronal plasticity. In both cases, $PrP^C$ is involved in cell-to-cell and cell-to-extracellular matrix interactions, calcium signaling, and cytoskeleton remodeling [43,44]. These mechanisms participate in cell motility, a process where $PrP^C$ has been recently implicated and dissecting the role of copper-$PrP^C$ interactions would be of great interest.

## Copper in the pathological roles of $PrP^C$: from Alzheimer's disease to cancer

Consistently with its functional role in cell proliferation, differentiation, and motility, $PrP^C$ has been implicated

neuroprotection and neuronal plasticity by activating the PKA and mTOR cascades. (&) Activation of the ERK1/2 cascade requires endocytosis of $PrP^C$. In these mechanisms, $PrP^C$ performs as a receptor using a transmembrane protein as a co-receptor to activate cell signaling. The cholinergic receptor (α7AChR) has been identified as a co-receptor of $PrP^C$ in cell signaling activated by STI1. **(c)** $PrP^C$ binds to laminin (Ln) at the third α-helix (173–182), where is localized the His176 and can participate in the $Cu^{2+}$-mediated *cis*-domain interaction. The Ln-$PrP^C$ complex recruits glutamate receptors (mGluR1/5) and induces $Ca^{2+}$ release from intracellular sources, promoting neuritogenesis by activation of the PKC and ERK1/2 signaling pathways. Here, $PrP^C$ acts as a receptor and mGluR5 as a co-receptor. **(d)** $PrP^C$ interacts with Vn, involving residues around the His111 site (105–119). The Vn-$PrP^C$ complex promotes neuritogenesis, but the co-receptor of $PrP^C$ and the signaling pathways involved in this mechanism have not been identified. $PrP^C$ promotes cytoskeleton remodeling and neuritogenesis by inhibiting integrin signaling (RhoA-ROCK-LIMK-cofilin). **(e)** $PrP^C$ modulates the activity of NMDAR in a $Cu^{2+}$-dependent manner by two mechanisms: **(i)** S-nitrosylation at Cys residues of NMDAR, and **(ii)** direct binding of $PrP^C$ to NMDAR; **(*)** The interaction NMDAR-$PrP^C$ is disrupted by Aβm and Aβo. **(f)** Soluble Aβo bind $PrP^C$ involving residues from the positively charged cluster (23–27) and the non-octarepeat region (93–113). The Aβo-$PrP^C$ complex recruits mGluR5 as a co-receptor and activates Fyn signaling, which is involved in tau hyperphosphorylation and neuronal damage observed in Alzheimer's disease mice models. **(g)** Both, the α-cleavage derived N1 fragment and shed $PrP^C$, bind Aβo extracellularly, preventing the activation of Fyn signaling and its associated neurotoxicity.

in Alzheimer's disease (AD) and cancer. The former is a neurological disorder with progressive loss of brain cells, while the latter is a disease where cells acquire resistance to death, uncontrolled proliferation, and the ability to migrate. In AD, $PrP^C$ acts as a receptor for Aβ, a neurotoxic copper-binding peptide that accumulates in AD brains [45]. $PrP^C$ binds Aβ monomers (Aβm) and Aβo involving the non-octarepeat region (Figure 2b) [46]. Aβo compete for $PrP^C$ binding to Ln, impairing signaling of the Ln-$PrP^C$-mGluR1 complex that is associated with memory formation (Figure 4c) [47]. Moreover, the Aβo-$PrP^C$ complex contributes to the cognitive impairment observed in AD mice models activating a toxic signaling pathway (Figure 4f) [48]. Interestingly, the proteolytic products, N1 and shed $PrP^C$ interact with Aβo, protecting cells from toxic cellular signaling activated by these species (Figure 4g) [11,12]. Although micromolar $Cu^{2+}$ concentrations do not affect Aβo-$PrP^C$ interactions [45], neither the effects of copper chelators and lower $Cu^{2+}$ concentrations have been tested, nor the impact of using Aβo formed in the presence of $Cu^{2+}$. On the other hand, Aβm disrupt the $Cu^{2+}$-dependent formation of the NMDAR-$PrP^C$ complex (Figure 4e) possibly by competing for $Cu^{2+}$ at the non-octarepeat binding sites [35,37,41]. Overall, further studies are needed to dissect the role of copper ions in the Aβ-$PrP^C$ interactions involved in these neuroprotective and neurotoxic mechanisms.

In cancer, $PrP^C$ is overexpressed in several types of tumors, while copper concentration is increased. Both features are associated with invasiveness (metastasis) and multidrug resistance [49,50]. Copper-dependent enzymes, MEMO, and lysyl oxidase protein are critical for metastasis by inducing the cytoskeleton and extracellular matrix remodeling [51,52]. Recent studies show that the $Cu^+$ chaperone Atox-1 mediates cell migration in breast cancer cells by facilitating the $Cu^+$ transport from ATP7A to lysyl oxidase protein [53]. Interestingly, the $PrP^C$ protein partners — STI1, NCAMs, and Ln — implicated in neurogenesis and neuronal plasticity are also involved in the proliferation and invasiveness of cancer cells [54–56]. Undoubtedly, exploring the role of copper-$PrP^C$ interactions in cancer will provide further insights into these mechanisms. Altogether, recent studies highlight copper and $PrP^C$ as therapeutical targets in AD and cancer [49,50,57,58]. Indeed, in both cases, copper chelators have been tested in clinical trials phase II, showing promising results [59,60]. However, understanding the functional and pathological roles of copper-$PrP^C$ interactions is crucial to recognize the limitations of chelating therapies and design specific target-directed drugs.

## Concluding remarks

$PrP^C$ is a copper-binding protein that can interact with different protein partners and is involved in a diverse range of cellular processes. Metal-binding and proteolytic cleavage of $PrP^C$ may impact its conformation and ability to recruit proteins at the cellular membrane contributing to its intricate functions. Considering that the sites for protein interactions and metal-binding overlap, and that copper-binding to $PrP^C$ yields a wide variety of coordination modes with different metal-binding affinities and redox activities, it is important to study how the metal ion could modulate the functional roles of $PrP^C$, including neuritogenesis and neuronal plasticity. On the other hand, $PrP^C$ has been involved in pathologies, such as AD and cancer, where copper has also been implicated. Dissecting the role of copper-$PrP^C$ interactions in these diseases will be of great interest in the field.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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