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Matrix metalloproteinase interactions with collagen and elastin

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Abstract

Most abundant in the extracellular matrix are collagens, joined by elastin that confers elastic recoil to the lung, aorta, and skin. These fibrils are highly resistant to proteolysis but can succumb to a minority of the matrix metalloproteinases (MMPs). Considerable inroads to understanding how such MMPs move to the susceptible sites in collagen and then unwind the triple helix of collagen monomers have been gained. The essential role in unwinding of the hemopexin-like domain of interstitial collagenases or the collagen binding domain of gelatinases is highlighted. Elastolysis is also facilitated by the collagen binding domain in the cases of MMP-2 and MMP-9, and remote exosites of the catalytic domain in the case of MMP-12.

Keywords

Collagen triple helix; Elastin; Exosite; Matrix metalloproteinases

Introduction

Collagens and elastin comprise highly abundant fibrils that are each repetitive in sequence, enriched in polyproline II conformation, cross-linked, insoluble when assembled, and resistant to most proteolytic enzymes. The “monomer” unit of type I collagen comprises two extended $\alpha 1$ chains and one $\alpha 2$ chain twisted together into a triple helix. The detailed structural features of collagen, the many types of collagen, and the supramolecular assembly of the fibrils have been reviewed [1]. Elastin provides the extraordinary, enduring elasticity of the aorta and lung and is integrated with other proteins from the extracellular matrix in elastic fibrils [2–5]. The tropoelastin monomer is boot-shaped and contains the elasticity in the elongated N-terminal coil region [5,6]. The foot-like C-terminal end can bind cells and was proposed to grasp the next monomer in a head-to-tail manner in the extended polymer [5]. Proteolytic fragments of elastin are highly chemotactic and stimulating of inflammation, proliferation, and angiogenesis [7].

Collagenolysis and elastolysis by matrix metalloproteinases (MMPs) occur in development, wound healing, and major inflammatory diseases [7,8]. The MMPs proposed to be elastolytic have been MMP-2, MMP-7, MMP-9, MMP-12, and MT1-MMP, but with

MMP-3 and MMP-10 in doubt [4]. Experiments using highly elastolytic human monocyte-derived macrophages (MDMs) asserted MMP-7 to be the principal elastolytic MMP under the very elastolytic conditions when activated by a urokinase-type plasminogen activator pathway [9]. Parallel experiments using the MDMs suggested the unlikelihood of direct elastolysis by MMP-9, and rather that MMP-12 deposited on elastin fibrils is the MMP required for digesting elastin in the absence of plasminogen. The authors proposed that MMP-12 might influence elastolysis indirectly by digesting chemokines and other extracellular proteins [9]. (Chemokines and numerous non-matrix proteins have been identified as physiological substrates of MMP-12 [10–12]). Degradation of interstitial collagen fibrils, e.g., types I and III, to generate the classic 3/4 and 1/4 fragments is catalyzed by MMP-1, MMP-8, MMP-13, MT1-MMP, MT3-MMP, and presumably MT2-MMP [8]. MMP-2 digests *solubilized* monomers of collagens I, II, and III [13–15]. MMP-9 digests solubilized collagen I and III monomers [16]. Mechanistic insights into MMP binding and hydrolysis of fibrillar collagens and elastin are surveyed below. The specific questions considered regard how do MMPs (i) move across collagens to sites for attack, (ii) interact with and distort the sites of cleavage in collagen, and (iii) employ multiple sites of interaction to engage elastin?

MMP movements to susceptible sites in collagen

The question of how do MMPs move to their sites of cleavage in collagen has been answered with great insight by biophysical approaches, though some methods do not recognize arrival at the site of cleavage. MMP-2 and MMP-9 randomly diffuse laterally on intact collagen fibrils [17]. Atomic force microscopy visualized MMP-9 diffusion along the MMP-8-generated 3/4 and 1/4 fragments of collagen II, especially the preferred 3/4 fragments, resulting in MMP-9 accumulation at their termini [18]. The MMP-9 first unwound the termini into an expanded, gelatin-like state prior to proteolysis. Bound to collagen this way, MMP-9 itself became more compact with its domains drawing close. The helicase activity of MMP-9 at the termini distinguishes it from MMP-1, 8 and 13 that unwind and cleave collagen internally at the 3/4–1/4 site [18]. Collagen fibrils first cleaved by MMP-1, 8, or 13 may subsequently experience MMP-2 and MMP-9 diffusing across them and then unwinding and digesting the fibrils further [17–19].

The diffusion of MMP-1 and MT1-MMP along collagen is biased into a single direction, requiring the proteolytic activity which “burns bridges” to prevent regress [17,20]. The probability of fibril-bound MMP-1 digesting the collagen (~5% when MMP-1 is active) is largely sufficient to model the diffusion. Inhibition of the collagenolytic activity of MMP-1 or MT1-MMP converts the diffusion on the collagen fibril to mostly bidirectional and random [17,20].

In intact collagen fibrils, MMP-1 cannot reach the vulnerable sites in the collagen monomer, apparently because they are covered by the C-terminal telopeptide [21]. This could account for around 90% of MMP-1 on collagen fibrils being hindered. The paused states are either (i) shorter in duration and non-periodic or (ii) longer-lasting (near 1.1 s) and periodically at 1.3 and 1.5 μm intervals along the fibril [22]. After long pauses, wild-type MMP-1 moved faster and farther than did inactivated MMP-1. The active MMP-1 was propelled in the C-

terminal direction along the fibril by the burnt bridges effect [22]. Only 5% of the longer pauses of MMP-1 on the periodic hotspots appear to be productively associated with an average of 13 to 15 irreversible steps of escape, attributed to a rapid succession of proteolysis spaced 67 nm apart [22]. Removal of the collagen C-terminus was proposed to be necessary to expose the scissile bond en route to digestion of the outer layer of monomers in the collagen fibril [21]. The large size of the thermal activation energy for collagenolysis [23] probably includes disruption of the steric obstacle of the collagen C-terminus impeding collagenolysis [22]. Removal of the structural barriers to collagen digestion may be integral to the kinetically hindered, intermittent, and directional behaviors [22].

Association of collagen fibrils with cell surfaces and MMPs was hypothesized to allow cells to move on collagen [20], e.g. keratinocyte migration on collagen [24]. Also fulfilling this hypothesis are the collagenolytic activities of (i) MMP-8 supporting neutrophil migration [25] and (ii) MT1-MMP in developing the full force of cells migrating through 3D collagen-based tissue models [17]. Since all of the components of the MMP-2/TIMP-2/MT1-MMP complexes of cell surfaces diffuse readily on collagen fibrils, their complexes were proposed to support cell movement on collagen, together with integrins and the cytoskeleton [17]. The extended shape and mobility between domains of these MMPs was likened to DNA-binding proteins and restriction enzymes diffusing on DNA [17]. The extreme flexibility between the MMP-9 catalytic and HPX domains was proposed to aid interactions between substrates and cells on the move [26], possibly through inchworm-like extension and retraction between domains [27].

Vulnerability of cleavage sites in fibrillar collagens

Reasons have been sought for collagenolytic MMPs cleaving specifically the 3/4–1/4 locus in interstitial collagen monomers, despite the many non-specific sites of MMP binding imaged [18,28]. The sequences recognized by MMP-1, MMP-8, MMP-13, and MT1-MMP span about 30 residues from P₁₃ through P₁₇' positions in the sequences tested [29–33]. The N-terminal side of each cleavage site has tightly wound triple helix rich in the imino acids Pro and Hyp. The C-terminal side of the scissile bond has more loosely wound triple helix that is relatively poor in the imino acids [29]. While pepsin-treated collagen fibrils thermally melt at 42 °C [34], collagen monomers are less stable and melt around body temperature [35]. Less thermally stable segments are found at many positions across the long triple helix [34,36–38] and are attributable to imino-poor sequences [29,37]. Among these destabilized loci, MMP cleavage of the 3/4–1/4 locus could be attributed to burial within the fibril and the proteolytic removal of the C-terminus to expose the 3/4–1/4 site [21], plus MMP preferences that restrict the choices in the collagen sequence [31,39].

The triple helix of monomers of collagens I and III was simulated to undergo localized separation of one chain from the other two chains near the scissile bond [40,41]. Such “vulnerable states” were proposed to be inherent to the sites of cleavage in collagen and to be recognizable by a collagenolytic MMP [41,42]. The dynamics of the isoleucine at the scissile peptide linkage in a collagen III mimic [39] is in accord with the vulnerable states hypothesis but suggests a more localized separation. The infrequency of productive collagen

degradation resulting from MMP-1 pausing at hotspots suggested vulnerable states there to be necessary but insufficient for initiating collagenolysis [22].

Mechanism of collagen triple helix engagement and distortion

Once the collagenolytic MMP has reached its site of cleavage in the collagen, how does the triple-helical collagen “monomer” get cleaved by the collagenolytic MMP? The diameter of the collagen triple helix of ~ 13 Å exceeds the ~ 5 Å passage through the S_1' to S_3' subsites of the active site cleft, necessitating separation of a chain from the triple helix in order to fit this narrow channel for proteolysis [43]. How collagenolytic MMPs might unwind and digest the triple helix has continued to be intriguing. Many possible means of mechanical manipulation that could separate the chains twisted together were imagined [44]. One MMP-1 enzyme molecule, even inactivated, is capable of presenting a collagen triple helix sufficiently unwound to another MMP or non-collagenolytic protease for successful digestion, implying that unwinding by MMP-1 precedes its proteolysis of the susceptible site [45]. Instructive is that the greater thermal barrier to unwinding of homotrimeric collagen impedes its digestion by MMP-1 [46]. The unwinding of homotrimeric collagen I preceding proteolysis appears to result not so much from the hypothesized MMP-1 capture of a vulnerable, melted bubble [40], but rather from MMP-1 shifting the equilibrium to establish a small population of locally unfolded triple helix [46]. Supporting this latter interpretation is the weakening of contacts between chains of the triple helix only after binding of MMP-1 [47].

The collagen triple helix binds the β -propeller fold of the HPX domain of MMP-1 around Ile290 and Arg291 of blade I, contributing to the S_{10}' pocket, as well as around Val318 and Asp338 in blade II [32,48,49]. Structural models of MMP-1 based on NMR data in solution or on crystallography place inactivated MMP-1 catalytic and HPX domains side-by-side, with the collagen triple helix bridging between them (cyan in Fig. 1). In the crystal structure, the triple helix from collagen II is anchored by inserting leucine at P_{10}' into the S_{10}' subsite in the HPX domain, but is not in a productive position with respect to catalytic domain [49]. The authors proposed a 108° rotation of the triple helix about its longitudinal axis and a shift of one residue to move the triple helix into a productive mode of binding. These authors further suggested that a small bend of the triple helix from the rotation of the domains of MMP-1 combined with the local thermal instability of the collagen monomer could release a chain from the triple helix to insert into the active site cleft [49]. The NMR-based model instead placed the collagen triple-helical peptide (THP) in a position already nearly productive for cleaving scissile bond [47]. The high mobility between the domains of MMP-1 in solution [50,51] has restriction imposed upon it by the binding of the THP, which is thought to draw the catalytic domain closer to the HPX domain [8,47]. These authors proposed a subsequent rotation of the catalytic and HPX domains to their closed orientation in the crystal structure [52]. This is consistent with the report of a phenylalanine from the HPX domain inserting into a pocket in the catalytic domain [48]. This hugging of catalytic and HPX domains would result in the bending of the triple helix, which could release a chain from the triple helix to enter the catalytic cleft [8,47]. Finally, the mode of binding of the hydrolyzed, exposed chain may be represented by crystallographic snapshots of a

cleaved peptide substrate with a collagen-based sequence within the active site of MMP-12 [47,53].

Simulations of the catalytic domain of MMP-2 bound to a collagen V-derived THP substrate predicted the chains of the triple helix to separate around the scissile Gly–Leu linkage, coinciding with the mobile and deformable segment of the unbound THP [54]. Though simulations of two alternative THP chains inserted in the active site differed in the bend of the triple helix and occupation of the subsites, the patterns of hydrogen bonding between THP and MMP-2 were shared with each other [54] and with many crystal structures of MMP complexes with peptide inhibitors [55]. A chain drawn out of the triple helix and into the active site cleft is likely to traverse residues found important in digesting triple-helical collagen substrates: Tyr210/189 in MMP-1 [30,56]; Asn188 to Tyr189 in MMP-8 [57]; and Phe202, Thr210, Thr239, and Lys241 in MMP-12 [58].

The HPX domain was originally postulated to fold over to sandwich the collagen triple helix between it and the catalytic domain [59], contrasting the side-by-side orientation observed in the MMP-1 complexes with THPs. A very recent NMR solution structural model of a THP substrate bound to the HPX domain of MT1-MMP renews the possibility of the triple helix becoming sandwiched between its catalytic and HPX domains [60] (PDB ID: 2mq5). While the THP (same sequence used by [47]) crosses the HPX domains of MT1-MMP and MMP-1 at the same angle, the scissile bond is shifted around 2.5 to 3 nm closer to the HPX domain of MT1-MMP [60], out of the apparent reach of the catalytic domain when the domains sit side-by-side (Fig. 1). For the scissile bond to reach the active site of MT1-MMP, either of two conformational changes could achieve this: (i) the triple helix could slide ~2.5 nm if the domains lie side-by-side or perhaps more likely (ii) the catalytic domain could fold over the scissile bond region bound near the HPX to sandwich the triple helix between these domains of MT1-MMP (Fig. 1) [60], as Bode [59] postulated.

MMP-2 and MMP-9 instead rely mainly on their CBD, especially its second and third fibronectin II-like modules, to support their activities towards *soluble* collagen monomers, including those from fibrillar collagens V and XI [61–65]. The CBD is inserted in a loop at the “primed” end of the catalytic channel [66]. The collagen binding sites lie at bowls in the fibronectin-like modules lined by hydrophobic residues [67–70]. CBD binding of collagens may enable bending modes of motion of the triple helix, recently simulated, which may potentially facilitate unwinding of the triple helix [71].

Elastin engagement by MMPs relies on exosites

Structure-function studies of elastolysis have focused on MMP-12, MMP-2, and MMP-9 and have mapped extensive binding sites for elastin. MMP-2 and MMP-9 require their fibronectin-like modules to bind and digest elastin [72]. Deposition of MMP-12 on elastin fibrils it digests was suggested both by this protease found bound to the damaged elastin fibrils of sections of aorta surgically removed from aneurysms [73] and by experiments *in vitro* using MDMs and elastin fibrils [9]. MMP-12 digests elastin at about 40 sites. The 36 sites identified suggest the most frequent sequence around the scissile bond to be: Ala/Gly at P₂, Gly at P₁, Leu at P₁′, and Gly at P₂′ [74]. Structural insights into elastin interactions with

the MMP-12 catalytic domain, also known as macrophage elastase or metalloelastase, are the focus below.

While solubilized elastin interacts with both catalytic and HPX domains of MMP-12 [75], the catalytic domain is sufficient for elastolysis by MMP-12 [58]. Ten residues on the periphery of the active site cleft were found to contribute to the specific activity of MMP-12 towards an enhanced elastin substrate and to distinguish it from other MMPs [58] (Fig. 2, green). Eight of these were located around the “primed” side of the active site that engages residues on the C-terminal side of the scissile bond, while two map to the unprimed side of the active site. The two residues nearest the catalytic center *boost the rate* of catalytic turnover of the elastin substrate, whereas the eight residues more distant instead enhance the K_m or apparent affinity [58]. Multiple elastin-derived peptides were proposed to bind the full length of the active site cleft of MMP-12 in an extended conformation [75]. Such modes of binding place peptides between the nine residues bordering the cleft implicated in elastolysis (Fig. 2, green) [58,76]. However, ~10-fold longer elastin peptides also protected much more extensive and remote surfaces of the catalytic domain from a paramagnetic NMR probe, including a swath across the β -sheet and broad patch most distant from the catalytic cleft [58]. Such far more extensive interactions could help explain the embedding of MMP-12 among the degraded fibrils from aneurysms. Two patches of residues on either end of the elastin-binding patch on the β -sheet were found to enhance elastin degradation: exosite 1 containing four confirmed residues and exosite 2 containing two confirmed residues (Fig. 2, red and orange) [76]. The importance of both exosites was underscored by one mutation in each exosite plus one in the active site combining to drop elastin-degrading activity to the level of MMP-3 considered poorly elastolytic [76]. These remote sites suggest allostery in elastolysis by MMP-12. Moreover, these exosites for elastin reside within the *catalytic* domain of MMP-12.

Major achievements have thus been realized regarding the diffusion of MMPs on collagen fibrils, the binding of collagen and elastin to exosites of MMPs, and the means of MMP-catalyzed opening of collagen for digestion.

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Abbreviations used

CBD	collagen binding domain
HPX	hemopexin-like
NMR	nuclear magnetic resonance spectroscopy
THP	triple-helical peptide

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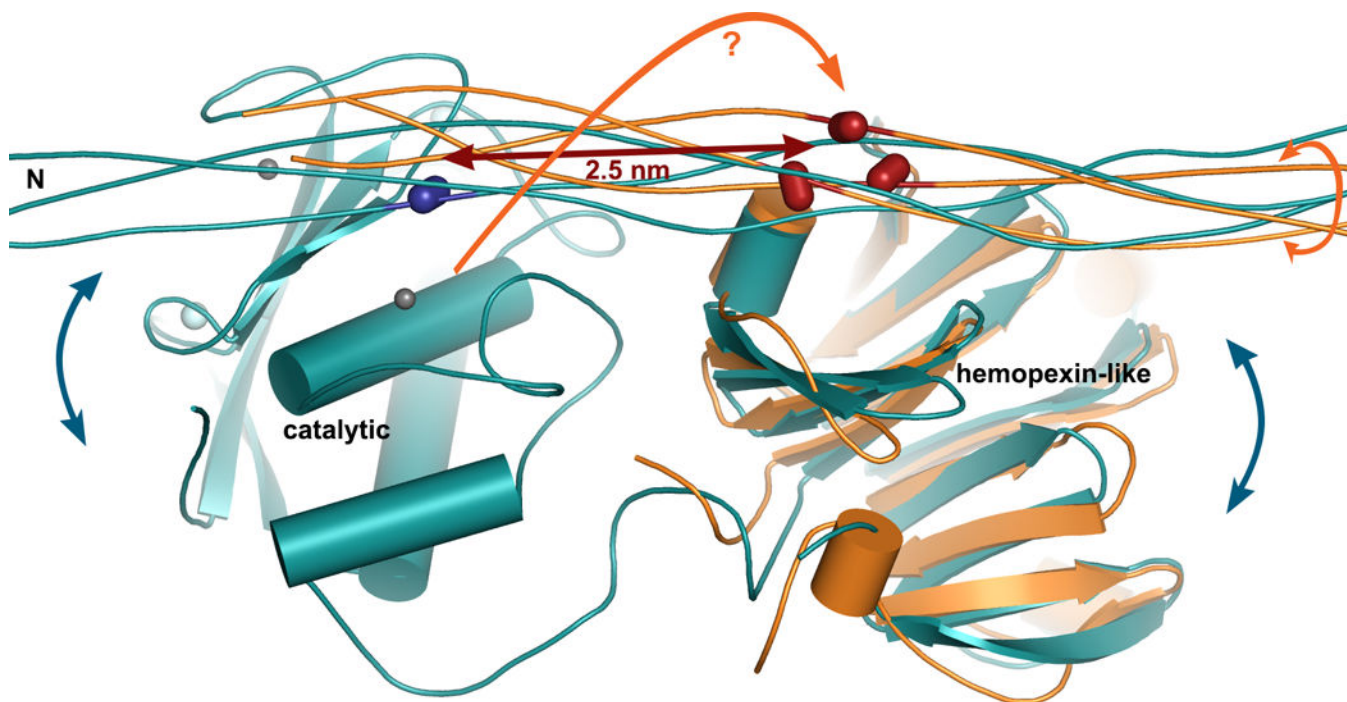


Fig. 1. The collagen triple helix bridges between domains of MMP-1 (cyan) [47,49] and crosses the HPX domain of MT1-MMP (orange) with the same angle but a shift in sequence [60]. The cyan arrows symbolize the interdomain motions proposed to open the triple helix in order to release a single chain into the active site [47,49]. The blue sticks represent the scissile Gly-Ile peptide bond over the MMP-1 active site and the red sticks mark the equivalent scissile Gly-Ile peptide bonds proximal to the HPX domain of MT1-MMP. The red arrow points out the ~2.5 nm shift of these scissile bonds and THP between the two complexes. The large orange arrow symbolizes the question of how does the catalytic cleft of MT1-MMP meet a scissile bond? The small orange arrow refers to the rotational averaging observed in the complex of a THP with the HPX domain of MT1-MMP [60].

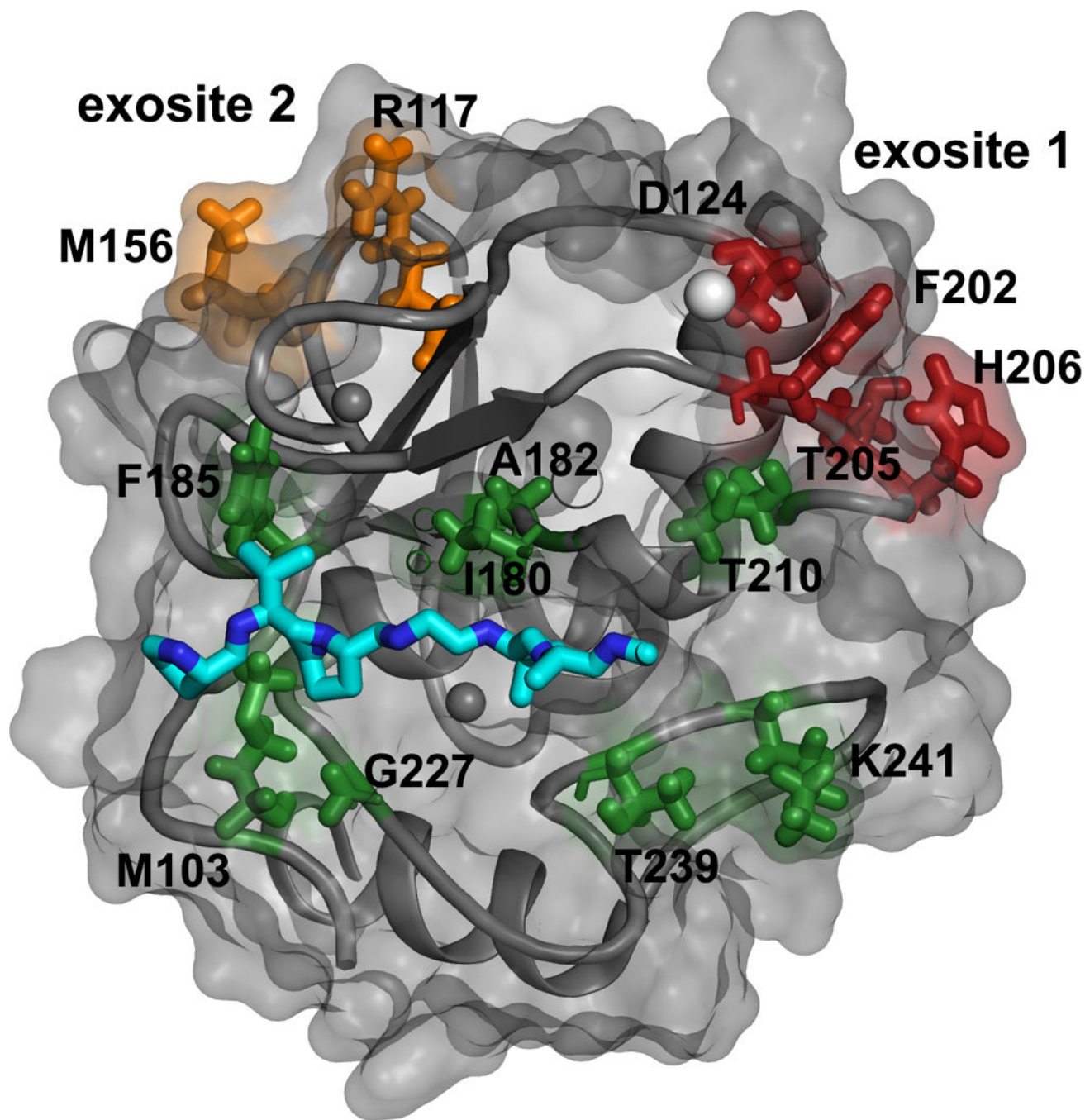


Fig. 2. The functional sites supporting elastolysis extend beyond the active site to exosites 1 and 2 of the catalytic domain of MMP-12. Sites of mutations impairing proteolysis of an elastin substrate [58,76] are plotted and labeled on the NMR structure of MMP-12 [77]. Gray spheres mark zinc ions and the white sphere a calcium ion. The peptide PVPGG~LAG (human elastin residues 65–72, with ~ marking the scissile bond) matches the preferred sequence for cleavage [74] and is modeled into the active site (cyan and blue) using as template the coordinates of a long peptide complex with MMP-13 [78] (PDB code: 4FU4,

chains A and C). This binding trajectory satisfies active site contacts but fails to pass by the remote exosites (red and orange) accessed by larger fragments of elastin as substrates [58,76].

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