

Allosteric Communications between Domains Modulate the Activity of Matrix Metalloprotease-1

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ABSTRACT An understanding of the structure-dynamics relationship is essential for understanding how a protein works. Prior research has shown that the activity of a protein correlates with intradomain dynamics occurring at picosecond to millisecond timescales. However, the correlation between interdomain dynamics and the function of a protein is poorly understood. Here, we show that communications between the catalytic and hemopexin domains of matrix metalloprotease-1 (MMP1) on type 1 collagen fibrils correlate with its activity. Using single-molecule Förster resonance energy transfer, we identified functionally relevant open conformations in which the two MMP1 domains are well separated, which were significantly absent for catalytically inactive point mutant (E219Q) of MMP1 and could be modulated by an inhibitor or an enhancer of activity. The observed relevance of open conformations resolves the debate about the roles of open and closed MMP1 structures in function. We fitted the histograms of single-molecule Förster resonance energy transfer values to a sum of two Gaussians and the autocorrelations to an exponential and power law. We used a two-state Poisson process to describe the dynamics and calculate the kinetic rates from the fit parameters. All-atom and coarse-grained simulations reproduced some of the experimental features and revealed substrate-dependent MMP1 dynamics. Our results suggest that an interdomain separation facilitates opening up the catalytic pocket so that the collagen chains come closer to the MMP1 active site. Coordination of functional conformations at different parts of MMP1 occurs via allosteric communications that can take place via interactions mediated by collagen even if the linker between the domains is absent. Modeling dynamics as a Poisson process enables connecting the picosecond timescales of molecular dynamics simulations with the millisecond timescales of single-molecule measurements. Water-soluble MMP1 interacting with water-insoluble collagen fibrils poses challenges for biochemical studies that the single-molecule tracking can overcome for other insoluble substrates. Interdomain communications are likely important for multidomain proteins.

SIGNIFICANCE It is often challenging to distinguish functionally important dynamics because proteins are inherently flexible. MMP1 is a model enzyme because both the catalytic and hemopexin domains are necessary to degrade triple-helical type 1 collagen, the highly proteolysis-resistant structural component of the extracellular matrix. We report, for the first time, to our knowledge, measurements of MMP1 interdomain dynamics on type 1 collagen fibrils. We have identified functionally relevant MMP1 conformations in which the two domains are far apart. Mutations and ligands can allosterically modulate the dynamics that correlate with activity. The dynamics follow a two-state Poisson process that connects the picosecond timescales of MD simulations with the millisecond timescales of experiments. The two domains can functionally communicate via collagen even when the physical linker is absent.

INTRODUCTION

Researchers have argued both in favor of (1) and against (2) the roles of protein conformational dynamics in the activity.

A direct link between the timescale hierarchies of the intradomain dynamics, from pico- to milliseconds, and function has been argued based on experimental and computational studies (3). However, researchers do not entirely know how the interdomain dynamics of a protein at different timescales influence its activity. Collagen degradation by MMP1 provides a unique opportunity to define the relationship between the interdomain dynamics and function because both

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the catalytic and hemopexin domains of MMP1 are necessary to degrade structurally intact collagen (4). Collagen is the primary component of the extracellular matrix that provides a scaffold for cells to maintain tissue integrity. Degradation of fibrils by matrix metalloproteases (MMPs) is an integral part of tissue remodeling (5). MMP1, a collagenase in the 23-member MMP family, can degrade type 1 collagen. MMP1 consists of a catalytic domain that degrades collagen, a hemopexin domain that helps MMPs bind to collagen, and a linker that mediates communications between the two (6). Even though MMPs have differences in activity and substrate specificity, the catalytic domain sequence across the MMP family is very similar to MMP1 (7). The functional difference, despite the catalytic domain similarity, suggests that the two domains communicate allosterically, which means a signal originating at one site transfers to a distant site where the protein's function, such as catalysis, occurs (8).

Most studies on MMP1-collagen interactions have used water-soluble collagen monomers instead of water-insoluble collagen fibrils. Each 300-nm-long collagen monomer with a diameter of 1.5 nm consists of three left-handed chains forming a right-handed triple-helical structure that hides the cleavage sites and makes collagen resistant to degradation (9). Biochemical studies on collagen monomers revealed that MMP1 actively unwinds the three strands (4). Follow-up studies reinterpreted the same results (4) to introduce the concept of collagen breathing, which leads to a partial unwinding of collagen because of thermal fluctuations, enabling MMP1 to bind and cleave collagen (10). Additional experimental studies supported such passive unwinding of collagen because of temperature (11). Although the catalytic domain alone can degrade denatured collagen, it cannot degrade triple-helical collagen without the hemopexin domain (4). The essential role of the hemopexin domain in function suggests communications between distant parts of MMPs. Further research on MMP1 (12,13) and MMP3 (14) provided additional support for allosteric interactions between domains. One set of studies suggests that a larger separation (open conformation) between the catalytic and hemopexin domains is necessary for activity based on NMR and small-angle x-ray scattering (SAXS) experiments and all-atom molecular dynamics (MD) simulations (13,15–17). Another set of studies suggests that the two domains are closer (closed conformation) based on x-ray crystallography (12). We need to resolve the debate about the open and closed conformations.

However, the process of degrading physiologically relevant collagen fibrils is different from that of degrading monomers for several reasons. First, collagen fibrils are water insoluble and macroscopic, greatly complicating ensemble biochemical and kinetics studies. Fibrils are larger than MMPs in size, and MMPs bind to fibrils nonspecifically as well as at the partially unwound vulner-

able sites created during fibril assembly (18,19). As such, binding to the cleavage sites is not a simple diffusion-limited process determined by the diffusion constant in solution. Also, the combined MMP1-fibril system is not statistically time independent because the fibril itself changes upon degradation by MMP1. Second, because monomers self-assemble into fibrils, the cleavage sites become less accessible compared with monomers because of covering by the C-terminal telopeptides (20). Saffarian et al. (21) showed that collagen degradation biases the motion of MMP1 on type 1 collagen fibrils using fluorescence correlation spectroscopy. Using single-molecule tracking of labeled MMP1 on type 1 collagen fibrils, we showed that MMP1 diffusion is both biased and hindered because of cleavage (19). MMP1 spends more than 90% of its time on the collagen fibril by diffusing, binding, and pausing without initiating degradation. Such extensive binding and pausing reduce the apparent catalytic rate of MMP1. The subsequent publication showed that fibrils also play a role in MMP1 activity because of the vulnerable sites on fibrils are caused by the relaxation of the strain during fibril assembly (18). In summary, the overall catalytic rate of MMP1 on fibrils depends on random motion (19) and substrate properties (18) as well as on active site catalysis (12) and conformational dynamics (13) applicable to both fibrils and monomers. However, there is no report of MMP1 conformational dynamics on fibrils and their roles in the active site catalysis.

Here, we focus on collagen fibrils as opposed to collagen monomers and show that functionally relevant MMP1 conformations have the catalytic and hemopexin domains distant. These conformations are present in active MMP1 but are significantly absent in inactive MMP1. Tetracycline, an antibiotic that is known to inhibit MMP1 activity (22,23), inhibits these conformations. In contrast, MMP9, another member of the MMP family that cannot degrade triple-helical collagen (24), enhances the low Förster resonance energy transfer (FRET) conformations. MD simulations of active and inactive MMP1 bound to a model triple-helical collagen reproduced some of the experimental observations. MD simulations further revealed that MMP1 opens its catalytic domain more compared with inactive MMP1. Also, the root-mean-square (rms) distance between the MMP1 catalytic site and the cleavage sites on the collagen chains has a lower average value for active MMP1 compared with inactive MMP1.

MATERIALS AND METHODS

Selection of labeling sites on MMP1

For a fluorescence-based study of interdomain motions, fluorescent dyes need to be attached to MMP1. For this purpose, we analyzed the crystal structure of MMP1 bound to a model of triple-helical collagen monomer (Protein Data Bank, PDB: 4AUO) for suitable sites for labeling on the catalytic and hemopexin domains. Because there are numerous

surface-exposed lysine residues, labeling using dyes with *N*-hydroxysuccinimide ester is not appropriate. Because disulfide bonds between cysteine residues are generally crucial for structural stability (25), two cysteine residues (CYS278 and CYS466) joined by a disulfide bond in MMP1 are not good candidates for mutations. However, CYS278 and CYS466 are relatively hidden, making them less labile. Consequently, we chose to mutate one serine residue from each domain to cysteine residues. We considered several factors before selecting the two sites for mutations: 1) the distance between the locations should be around the Förster radius of the dye pair chosen for the single-molecule FRET (smFRET), 2) the amino acid at the site should be solvent exposed to facilitate labeling, 3) the amino acid selected should be similar to cysteine to minimize the effect of a mutation on activity, 4) the amino acid should not be in a conserved region, 5) the amino acid should not be near the collagen-binding site, and 6) the amino acid should preferably be on the relatively stable helices. Based on these criteria, we mutated SER142 on the catalytic domain and SER366 on the hemopexin domain to cysteines (Fig. 1 A).

Purification, labeling, and ensemble activity measurements

We expressed full-length recombinant MMPs with pro domains in *Escherichia coli*. We cleaved off pro domains using 0.1 mg/mL trypsin to activate both active and catalytically inactive point mutant (E219Q) MMP1 as well as active MMP9 (26). We purified activated MMPs using a protease-based purification method (27). We labeled purified MMP1 with Alexa Fluor 555 (catalog no. A20346, donor dye; Thermo Fisher Scientific, Waltham, MA) and Alexa Fluor 647 (catalog no. A20347, acceptor dye; Thermo Fisher Scientific) using maleimide chemistry. 1 mL of MMP1 at a concentration of 1 mg/mL was incubated with 20 μ L of 1 mg/mL Alexa Fluor 555 and Alexa Fluor 647 (Thermo Fisher Scientific) for 60 min in a 5-mL glass vial with continuous nitrogen flow to avoid oxidation of the dyes. After incubation, we filtered the sample three times using a 30-kDa cutoff Amicon filter (Sigma-Aldrich, St. Louis, MO) to remove free dyes from the solution. We analyzed the integrity of labeled MMP1 using 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis (Fig. 1 B, left panel). We compared the activities of labeled MMP1 and unlabeled MMP1 (Fig. 1 B, right panel) on the synthetic substrate, MCA-Lys-Pro-Leu-Gly-Leu-DPA-Ala-Arg-NH₂ (catalog no. ES010; R&D Systems, Minneapolis, MN) (27). MMP9 was not labeled.

Single-molecule measurements

We mixed 90 μ L of 4 mg/mL solution of type 1 collagen monomers (RatColl Type 1 Collagen for three-dimensional (3D) gels, catalog no. 5153; Advanced BioMatrix, Carlsbad, CA) with 10 μ L of neutralizing solution (Neutralization Solution, catalog no. 5155; Advanced BioMatrix) to create a reaction volume of 100 μ L. We created a thin layer of reconstituted type 1 collagen fibrils on a quartz slide using the 100- μ L reaction. After incubation for 1 h at 37°C, we made a flow cell using a piece of double-sided tape sandwiched between the quartz slide and a glass coverslip. We mixed 50 μ L of 0.1 mg/mL labeled MMP1 with 50 μ L of 1) protein buffer (50 mM Tris and 100 mM NaCl (pH 8.0)), 2) 1 mg/mL MMP9, and 3) 100 μ g/mL tetracycline. We incubated the mixtures for 30 min at 22°C. We serially diluted the labeled MMP1 to prepare a concentration of \sim 100 pM. Before flowing in labeled MMPs into the flow cell, we photobleached the slide using the highest available power of the laser in our total internal reflection fluorescence (TIRF) microscope (25-mW laser power at 532 nm focused using a 10-cm plano-convex lens). As a result, bright spots because of impurities photobleached, and image frames became spot free. Then, we flowed labeled MMPs and acquired videos. Alexa Fluor 555 dyes (Thermo Fisher Scientific) attached to MMP1 were excited at 532 nm using the evanescent wave created at the interface of the quartz slide and sample solution in a TIRF microscope as described before (18,19). We separated Alexa Fluor 647 and Alexa Fluor 555 (Thermo Fisher Scientific) emissions into different channels using the Quad View Simultaneous Imaging System (QV2; Teledyne Photometrics, Tucson, AR) and imaged using an EMCCD camera (Andor iXon; Andor Technology, Belfast, UK) at 22°C with a 100-ms time resolution. We superimposed the two emission channels using a pairwise stitching plugin of ImageJ and extracted the emission intensities from the two dyes for further analysis using MATLAB (The MathWorks, Natick, MA). For each experimental condition (no ligand, with MMP9, and with tetracycline), we acquired at least 10 videos with a 100-ms time resolution with 3000 frames for both active and inactive MMP1. The number of anticorrelated trajectories was \sim 20 \pm 14% (mean \pm SD) of the total number of spots in a video.

Analysis of potential labeling issues

Upon activation by trypsin, MMP1 is cleaved at the F100-V101 bond (27), i.e., MMP1 that we used for our experiments has amino acids between V101 and N469. The analysis of the amino acid sequence showed that, between

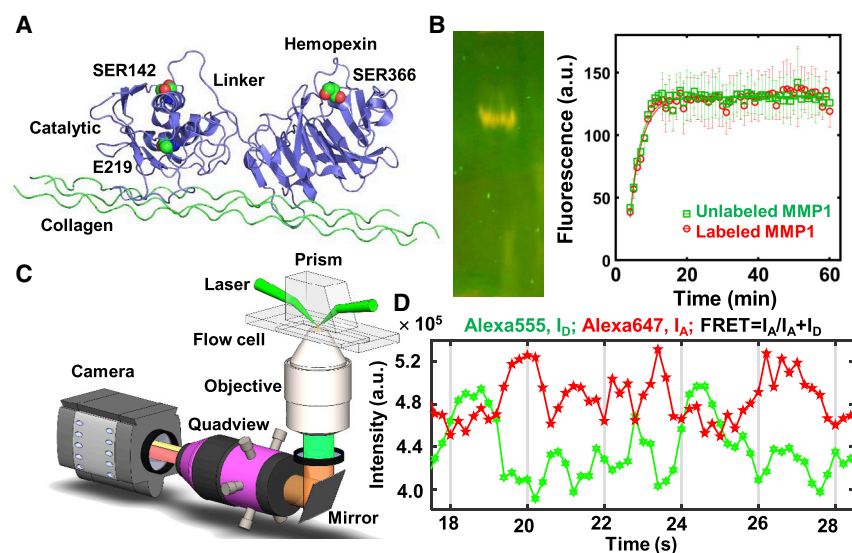


FIGURE 1 Single-molecule measurement of MMP1 dynamics on type 1 collagen fibril. (A) Relative positions of the MMP1 domains and residues (green and red spheres) created using 4AUO. (B) Left panel: 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis of labeled MMP1; right panel: fluorescence from degraded peptide substrate as a function of time for unlabeled (green squares) and labeled (red circles) MMP1 at 37°C. Solid lines are respective best fits to $y = a - b \times \exp(-kt)$. After calibration, the specific activity is \sim 1000 pmol/min/ μ g. The error bars are the SDs of three technical repeats. (C) Schematics of the TIRF microscope. (D) Emission intensities of the two dyes attached to active MMP1. Low FRET conformations lead to high Alexa Fluor 555 (Thermo Fisher Scientific) emission, whereas high FRET conformations lead to low Alexa Fluor 555 (Thermo Fisher Scientific) emission. Anticorrelated Alexa Fluor 647 and Alexa Fluor 555 (Thermo Fisher Scientific) emissions, I_A and I_D, respectively, indicate the conformational dynamics of MMP1. To see this figure in color, go online.

V101 and N469, there are total four CYS residues at C142 (mutated from S142), C278 (native), C366 (mutated from S366), and C466 (native). Note that both active and inactive MMP1 have the same sequence except the E219Q point mutation for inactive MMP1. The analysis of MMP1 crystal structure (4AUO) showed that C278 and C466 are relatively hidden and, therefore, likely to be less labile. Also, we measured the distances between possible combinations of four CYS residues in the open (S142–S366 = 5.2 nm) and closed (S142–S366 = 4.5 nm) conformations of MMP1. Only the distance between C142 and C366 changes significantly (~1 nm) as MMP1 undergoes interdomain dynamics compared with the changes (~0.1 nm) in other CYS-pair distances. In other words, C278 and C466 would not interfere with smFRET results even if they were labeled. However, there are four possibilities of labeling C142 and C366: 1) no fluorophore attached, 2) only one CYS is labeled, 3) both are labeled with the same type of fluorophore, and 4) both are labeled with different fluorophores. We would detect smFRET only for the fourth possibility, with both C142 and C366 labeled with different dyes. The photophysical properties of fluorophores may also cause problems because fluorescence depends on solution conditions (pH, temperature, chemical composition, etc.) (28). Furthermore, blinking because of the transition into metastable states (29,30) and photobleaching because of the reaction with oxygen in the excited state (31) can complicate the tracking of fluorophores. Additionally, the flexible linker between a protein and Alexa Fluor dyes (Thermo Fisher Scientific) (32) and the enzyme microenvironment around the fluorophores can change smFRET (33). Previous studies have elucidated the combined complexity of smFRET, solution conditions, and simulations (34). Because active and inactive MMP1 would be affected equally by these complications, we could distinguish the effects due to enzymatic activity. For further confirmation, we also used inactive MMP1 as the control for all-atom MD simulations (see the next paragraph).

All-atom MD simulations

We performed all-atom MD simulations using GROMACS version 2018.2 (35). We applied a leapfrog integration time step of 2 fs and constrained all bonded interactions using the LINCS constraint algorithm (36). The short-range neighbor interaction list cutoff was updated every 10 fs and fixed to 0.8 nm. We modeled the short-range interactions using a 12-6 Lennard-Jones potential truncated at 0.8 nm and truncated electrostatic interactions at 0.8 nm. There is no standard choice for truncation. We used 0.8 nm to be consistent with previous publications (37–39). We calculated the long-range electrostatic interactions using the particle mesh Ewald scheme (40). We maintained the temperature at 310 K using the Nosé-Hoover thermostat (41). We set the pressure at 1 atm using an anisotropic Parrinello-Rahman coupling (42). We defined the simulation cell using periodic boundary conditions in three dimensions and saved the coordinates, velocities, and energies every 5 ps. We used the crystal structure (PDB: 4AUO) of inactive MMP1 (E219A) bound to a model of the triple-helical collagen monomer. Note that E219 (27) is the same residue as E200 (12). The number differs because the first residues are different for our full-length MMP1 before activation and the crystal structure 4AUO. The sequence of MMP1 is in Fig. S1.

We modified residues in 4AUO to match the amino acid sequences of active and inactive MMP1 used in smFRET experiments using Schrödinger Maestro (Schrödinger Release 2015-4). We incorporated the two serine-to-cysteine (SER142CYS and SER366CYS) substitutions used for labeling MMP1. Additionally, we included an alanine-to-glutamine (A219Q) substitution to match the sequence of inactive MMP1. We repeated procedures in earlier work (37) for 1) initialization of collagen-bound MMP1 complex crystal structure (PDB: 4AUO), 2) parameterization and implementation steps for the bond distances and angles, and 3) respective force constants and partial charges of the zinc-binding sites.

We placed each protein complex at the center of a cubic unit cell sufficiently large enough to avoid periodic boundary artifacts. We solvated

unit cells using TIP4P water molecules and added counterions to neutralize the system. We subjected solvated protein complexes to a steepest descent energy minimization with a maximal force of 1000 kJ/mol stopping criteria for correcting steric clashes between atoms. The minimized structures underwent a 10-ps MD simulation using the NVT (fixed particles, volume, and temperature) ensemble with position restraints of 1000 kJ/mol to all heavy atoms. We maintained the position restraints and extended the simulation by 200 ps with constant pressure using the Parrinello-Rahman pressure coupling scheme. We simulated a series of 200-ps intervals, systematically reducing the position restraints to a final 10 kJ/mol. We removed all position restraints with the exception of those on the collagen α -carbon atoms, which we increased to 150 kJ/mol to prevent the fraying of the short collagen during the structure relaxation. With restrained collagen, we performed 225-ns NPT (fixed particles, pressure, and temperature) simulation. We removed the position restraints and performed 700-ns NPT simulation without constraints.

Normal mode analysis using Anisotropic Network Model

We computed preferred conformations or “normal modes” of MMP1 interacting with collagen using the anisotropic network model (ANM) (43). The ANM shows each amino acid as a bead and creates a virtual bond network connecting these beads (43,44). By modeling these virtual bonds as harmonic oscillators, ANM calculates normal modes and enables analysis of conformations that are accessible to a 3D protein structure. For the MMP1-collagen system, we used the crystal structure of MMP1 (12) in complex with a collagen peptide (PDB: 4UAO) using the ANM web server 2.1 with default parameters (45).

The output of ANM simulations is an $N \times N$ matrix, where N is the number of beads (amino acids) in the system, which provides $3N - 6$ nonzero eigenvalues and $3N - 6$ eigenvectors that represent the respective frequencies and shapes of the individual normal modes (46). The smaller the frequency, the slower the motion that a mode represents. For each mode, the collective motion of beads and springs is a series of frames. The ANM server allows for the generation of bead coordinates from frames of each mode. We obtained the 3D coordinates of beads from 20 frames of motion for the 20 slowest modes. For each frame, we calculated the desired distances using PyMOL (Version 1.8; The PyMOL Molecular Graphics System). We used 1) the distance between S142 and S366 to measure the interdomain separation, 2) the distance between N171 and T230 to measure the catalytic pocket opening, and 3) the distances between A219 and the cleavage site G-L (12) to quantify the proximity of MMP1 to the collagen chains.

We obtained the bound configurations of MMP1 domains (for example, see Fig. 6) by removing parts from the crystal structure of collagen-bound MMP1 (PDB: 4AUO). We used chains A (for MMP1) and C, D, and E (for collagen) as inputs to ANM. We obtained the different lengths of the enzyme (two domains connected by the linker, two domains separated, or the catalytic domain only) by removing the appropriate 3D coordinates from the original PDB file (4AUO): 1) removing no residue, 2) removing the linker region, and 3) removing the hemopexin domain and the linker region. Because 4AUO has amino acids between F100 and C466, we aligned PDB: 1SU3 (MMP1 structure having amino acids between A32 and C466 with the pro domain) with 4AUO and generated the structure for collagen-bound pro MMP1. ANM is significantly faster than the all-atom MD simulations and provides significant insights. However, there is an intrinsic limitation of the ANM method. Although the all-atom MD simulations can distinguish the effect of single point mutations, ANM cannot because each amino acid, regardless of its chemical nature, is modeled as a single bead. As such, a mutation GLU to GLN or GLU to ALA would not have any impact on ANM results if the input structure is the same.

RESULTS AND DISCUSSION

Single-molecule measurement of MMP1 interdomain dynamics on reconstituted type 1 collagen fibril

For our studies, we differentiated the role of activity by comparing the results for active (E219) with catalytically inactive (E219Q) MMP1. We mutated SER142 in the catalytic domain and SER366 in the hemopexin domain to cysteines (Fig. 1 A). Note that the residue E219 (27) is the same as E200 (12), which differs because of the first residue used for counting. The distance between the central carbon atoms of the two selected amino acids is ~ 4.5 nm, which is similar to the Förster radius of 5.1 nm (47) for the dye pair Alexa Fluor 555 and Alexa Fluor 647 (Thermo Fisher Scientific). We labeled MMP1 with Alexa Fluor 555 and Alexa Fluor 647 (Thermo Fisher Scientific) using maleimide chemistry. The left panel of Fig. 1 B shows the gel electrophoresis of labeled MMP1. The right panel of Fig. 1 B presents the specific activities of labeled and unlabeled MMP1 on the synthetic peptide substrate, MCA-Lys-Pro-Leu-Gly-Leu-DPA-Ala-Arg-NH₂ (48). Labeling did not affect the function of MMP1. We used a TIRF microscope because many MMPs can be imaged simultaneously, in contrast to a confocal microscope with point detection. We used a TIRF microscope in a prism-type configuration (Fig. 1 C) because it has lower background noise because of the separation of excitation and emission paths in contrast to the objective-type TIRF microscope for which the excitation and emission paths share optics. As labeled MMPs moved and underwent interdomain dynamics on the surface of a fibril, fluorescence emissions from the donor and acceptor

dyes were detected using two quadrants (channels) of an electron multiplying charge-coupled device camera. We superimposed the two channels and tracked the intensity and location of spots to measure the interdomain dynamics. When the two domains are within the Förster radius, the energy from Alexa Fluor 555 (Thermo Fisher Scientific) is nonradiatively transferred to Alexa Fluor 647 (Thermo Fisher Scientific) because of FRET. As a result, the fluorescence from Alexa Fluor 555 (Thermo Fisher Scientific) decreases, leading to a simultaneous increase in the emission from Alexa Fluor 647 (Thermo Fisher Scientific). Fig. 1 D shows an example of anticorrelated fluorescence from the two dyes because of MMP1 interdomain dynamics. See Materials and Methods for the detailed procedures.

Well-separated catalytic and hemopexin domains correlate with the activity of MMP1

The role of dynamics in function is not clear: Warshel proposed that protein dynamics and enzyme catalysis are not coupled (2,49), but Karplus proposed that enzyme motions can lower the activation energy (1,3). We measured the interdomain dynamics of active and inactive MMP1 on reconstituted type 1 collagen fibrils using smFRET (Fig. 2). The histograms of FRET values revealed the equilibrium distributions of interdomain distance, whereas the autocorrelations showed how conformations correlate with each other at different time points. We fitted a sum of two Gaussians to the histograms (Fig. 2 A). The best-fit parameters for the centers of Gaussians are the following: 0.44 ± 0.01 and 0.55 ± 0.01 for active MMP1 and 0.56 ± 0.01 and 0.68 ± 0.01 for inactive MMP1 (Table S1). These results

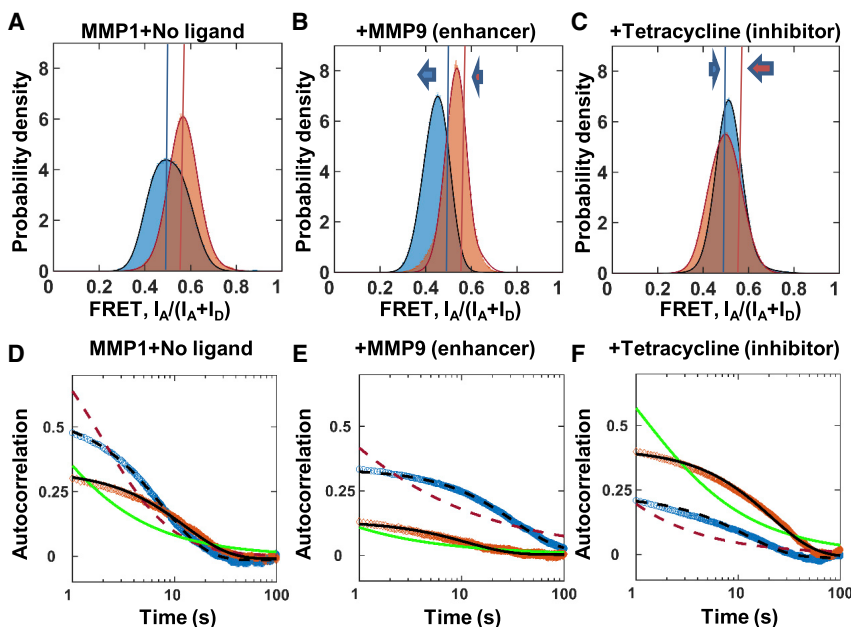


FIGURE 2 Activity-dependent interdomain dynamics of MMP1 on reconstituted type 1 collagen fibrils at 22°C with a 100-ms time resolution. Area-normalized histograms of MMP1 interdomain distance (more than 300,000 FRET values for each condition; bin size = 0.005) (A) without ligand, (B) in the presence of MMP9 (an enhancer), and (C) in the presence of tetracycline (an inhibitor) for active (blue) and inactive (orange) MMP1. All histograms are fitted to a sum of two Gaussians (active: solid blue line; inactive: solid red line). Blue and orange lines indicate the peak positions for active and inactive MMP1 without ligands, whereas blue and orange arrows indicate the directions of shifts of the FRET peaks in the presence of ligands. Autocorrelations of MMP1 interdomain distance (D) without ligand, (E) in the presence of MMP9, and (F) in the presence of tetracycline for active (blue) and inactive (orange) MMP1. All autocorrelations are fitted to exponentials and power laws (exponential fit to active: dashed black line; power law fit to active: dashed red line; exponential fit to inactive: solid black line; power law fit to inactive: solid green line). The error bars in histograms and autocorrelations represent the square roots of the bin counts and the standard errors of the means (SE) and are too small to be seen. The Supporting Materials and Methods contain the fit equations and the best-fit parameters for histograms (Table S1) and autocorrelations (Table S2). To see this figure in color, go online.

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suggest that the low FRET conformation at 0.44 ± 0.01 , at which the two MMP1 domains are distant (the open conformation), are relevant for the catalytic activity of MMP1. The relevance of the open structure in function is in agreement with previous results for collagen monomers based on NMR and SAXS experiments and all-atom MD simulations (13,15–17) that revealed the importance of a larger separation between the catalytic and hemopexin domains for activity. As such, the open conformation with more interdomain distance is functionally relevant for MMP1 interactions with both collagen monomers and fibrils.

To further delineate the relation between interdomain dynamics and activity, we used two ligands that modulate MMP1 activity: MMP9 (an enhancer) and tetracycline (an inhibitor). MMP9, another member of the MMP family, is generally thought to be unable to degrade structurally intact triple-helical type 1 collagen (50). Both pro (51,52) and activated forms (53–56) of MMP9 exist in numerous physiological conditions. MMP9 and MMP1 form a stable complex (24), and computational studies have predicted enhancement of MMP1 activity (57). Tetracycline plays dual roles as an antibiotic and an MMP inhibitor (23). Our measurements confirmed that MMP9 is an enhancer, whereas tetracycline is an inhibitor, of MMP1 activity (Fig. S2 I). The surface morphology of fibrils treated with MMP1 changed in the presence of MMP9 and tetracycline (Fig. S2, A–H).

In the presence of MMP9, the peaks shift toward the left to 0.43 ± 0.01 and 0.48 ± 0.01 for active MMP1 (Fig. 2 B). In contrast, tetracycline inhibits low FRET conformations and appears to move the peaks toward the right to 0.51 ± 0.01 and 0.55 ± 0.01 for active MMP1 (Fig. 2 C). In other words, MMP1 stays more or less in the open conformation (low FRET) as its activity increases or decreases in the presence of MMP9 and tetracycline, respectively. In contrast, inactive MMP1 primarily stays in a closed conformation corresponding to FRET values at ~ 0.50 or higher (Table S1). In the presence of tetracycline, both active and inactive MMP1 primarily adopt conformations with FRET values at ~ 0.50 (Table S1). We performed molecular docking to investigate why tetracycline affected active and inactive MMP1 similarly. We observed that tetracycline forms two hydrogen bonds with each domain (Fig. S4) and appears to hold MMP1 in place, preventing interdomain dynamics. As such, the molecular docking provides an explanation for similar FRET values for active and inactive MMP1 in the presence of tetracycline. These results on activity-dependent interdomain dynamics collectively confirm that open MMP1 conformation and catalytic activity of MMP1 are functionally related.

Next, we studied correlation in the dynamics because correlated motions indicate a decrease in conformational entropy. A reduction in entropy may affect kinetics and thermodynamics of biological processes, including catalysis (58). Without any ligand, active MMP1 had more time-

correlated conformational dynamics at short times than inactive MMP1 (Fig. 2 D). In the presence of MMP9, the time correlations were longer for both active and inactive MMP1 (Fig. 2 E). MMP9 not only led to more low FRET conformations of MMP1, but it also stabilized low FRET conformations, as indicated by narrow widths of histograms (Fig. 2 B; Table S1) and longer correlation times (Fig. 2 E; Table S2). Interestingly, tetracycline reversed the trend, and inactive MMP1 had longer time correlation than active MMP1 (Fig. 2 F; Table S2). In other words, ligands can alter conformational entropy of MMP1, leading to a change in function.

The observed modulation of interdomain separation and correlation could be a “consequence” or the “cause” of catalytic activity. We argue that low FRET conformations are likely the cause for several reasons. First, a mutation E219Q at the catalytic site not only renders MMP1 inactive, but it also induces changes in the distance allosterically to alter the interdomain dynamics (Fig. S5). Second, changes in the linker region also have been shown to affect activity allosterically (59). Third, the catalytic domains of MMPs are mostly similar (7), suggesting a crucial role of the hemopexin domains in substrate specificity (60). Fourth, the catalytic domain alone can degrade denatured collagen, where the cleavage sites are easily accessible. However, the catalytic domain requires the hemopexin domain to degrade the basic building block of collagen fibrils, i.e., triple-helical collagen, where the cleavage sites are hidden (4,61,62). Overall, it appears that the hemopexin domain communicates with the catalytic domain and induces necessary changes to enable collagen degradation. Further studies are required to establish causality.

MMP1 interdomain dynamics is a two-state Poisson process

MMP1 interdomain dynamics are random (stochastic) time series that can be described by a Poisson process or a set of Poisson processes. A Poisson process has a constant probability of occurring at each temporal or spatial point. Previously, we applied the Poisson process approach to diverse phenomena (63), including MMP1-collagen interactions (18,19). To investigate whether the Poisson process approach could be applied to describe MMP1 interdomain dynamics, we performed stochastic time series simulations of the distance between SER142CYS and SER366CYS. We analyzed simulations and experimental FRET measurements similarly.

The centers of the Gaussian fits (Table S1, the *parameters* $b1$ and $b2$) were defined to be the two states, S1 (lower FRET) and S2 (higher FRET). We considered that MMP1 interconverts between S1 and S2 with kinetic rates $k1$ ($S1 \rightarrow S2$) and $k2$ ($S2 \rightarrow S1$). We calculated the ratio $k1/k2$ from the ratio $\text{Area}(S2)/\text{Area}(S1)$ determined by the Gaussian fits to FRET histograms (Fig. 2, A–C). We derived

the sum $k_1 + k_2$ from the decay rate e (Table S2) determined by the exponential fits (Fig. 2, D and E) because the autocorrelation for a two-state system decays as the sum of two rates (64). The ratio k_1/k_2 and sum $k_1 + k_2$ determined k_1 and k_2 (Table S3) for different experimental conditions. We considered that if MMP1 is in state S1, there is a constant probability, given by $P(t)dt = k_1 \times \exp(-k_1 \times dt) \times dt$, of going to state S2 at each time step dt . Similarly, if MMP1 is in state S2, there is a constant probability, given by $P(t)dt = k_2 \times \exp(-k_2 \times dt) \times dt$, of going to state S1 at each time step dt . To implement this kinetic mechanism, we used the “expnrnd” function in MATLAB to generate the exponentially distributed dwell times in each state with and without noise.

As an example, we considered the interdomain dynamics of MMP1 without ligands and simulated 350 FRET trajectories with (Fig. 3) and without (Fig. S3) noise with 100-ms time resolution, with each being 1000 s long. We added Gaussian-distributed noise to the simulated FRET trajectories determined by the Gaussian fit parameters (Table S1) for the experimental histograms (Fig. 2, A–C). Fig. S3 shows examples of FRET trajectories for active and inactive MMP1 without noise (Fig. S3 A), normalized histograms (Fig. S3 B), and auto-

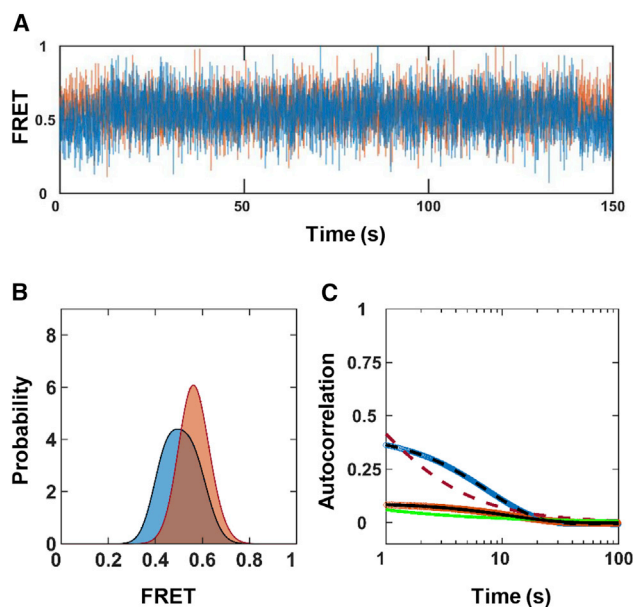


FIGURE 3 Simulated MMP1 interdomain dynamics as a Poisson process. (A) Examples of simulated two-state FRET trajectories with noise for active (blue) and inactive (orange) MMP1. (B) Histograms of simulated FRET values. (C) Autocorrelations of simulated trajectories recover the sum, $k_1 + k_2$, from exponential fits (active: dashed black line; inactive: solid black line). As expected, the power law does not fit autocorrelations (active: dashed red line; inactive: solid green line). Exponential fits recover $k_1 + k_2$ with and without noise. The addition of noise changes the width of the FRET histograms and y-intercepts. The error bars are the SEs for histograms and autocorrelations and are too small to be seen. The Supporting Materials and Methods contain the best-fit parameters for histograms (Table S4) and autocorrelations (Table S5). To see this figure in color, go online.

correlations (Fig. S3 C). The histograms of simulated FRET values reproduced the locations of states (Table S4) and an exponential fit of the autocorrelations, then power laws, as expected. The analysis of simulated trajectories recovered the experimentally determined input rates for both active and inactive MMP1 without noise (Table S5). We also simulated FRET trajectories using the same parameters but with noise. Fig. 3 shows examples of FRET trajectories with noise for active and inactive MMP1 (Fig. S3 A), normalized histograms (Fig. 3 B), and autocorrelations (Fig. 3 C). Even with noise, Gaussian fits recovered the simulated FRET values (Table S4) and the ratio of rates, whereas exponential fits recovered the sum of decay rates (Table S5). Note that the y-intercept of autocorrelation depends on S1, S2, k_1 , k_2 , and noise. Without noise, a closed-form expression for the y-intercept can be derived (64). In summary, MMP1’s interdomain dynamics can be described by a Poisson process at the experimental timescales.

A two-state Poisson process description of MMP1 interdomain dynamics enables a more in-depth interpretation of autocorrelations (Fig. 2, D and E) and exponential fit parameters (Table S3). For example, if low FRET values at $\sim 0.44 \pm 0.01$ for active MMP1 with and without MMP9 represent catalytically relevant open conformations, we would expect MMP1 to stay in this state longer as the activity increases. In other words, MMP9 would facilitate MMP1 by staying in the low FRET state longer, leading to a smaller k_1 , which is indeed the case, as indicated by the decrease of k_1 from 0.08 to 0.01 s^{-1} in the presence of MMP9 (Table S3). In other words, the kinetic rates of interconversion between open and closed MMP1 conformations correlate with the activity.

A larger interdomain distance often accompanies a larger catalytic pocket opening of MMP1 and closer proximity to the collagen chains

The MMP1 catalytic cleft (~ 0.5 nm wide) is too narrow to accommodate the collagen monomer (1.5 nm in diameter) (13). Therefore, a larger opening of the MMP1 catalytic pocket is needed to bring collagen closer to the active site and must accompany more interdomain separation if low FRET conformations are indeed relevant. To this end, we investigated the activity-dependent interdomain dynamics of active and inactive MMP1 using all-atom simulations (see Materials and Methods). As shown in Fig. 4 A, the all-atom simulations reproduced that active MMP1 has more low FRET conformations (i.e., interdomain distances represented by the blue shoulder around 5 nm) with the collagen backbone restrained. In contrast, MMP1 has fewer low FRET states with the collagen backbone unrestrained (Fig. 4 C). Table S6 contains the best-fit parameters for Gaussian fits to the histograms. The autocorrelations (Fig. 4, B and D) of simulated interdomain distances show

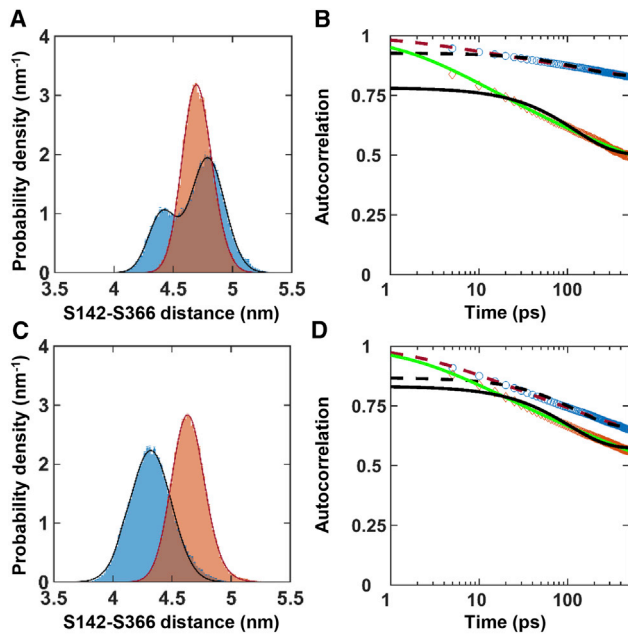


FIGURE 4 All-atom MD simulations with collagen backbone restrained (*A* and *B*) and unrestrained (*C* and *D*). Simulated dynamics using GRO-MACS simulation package at 37°C with a 2-fs time step, data saved every 5 ps, and 225- and 700-ns simulations for restrained and unrestrained, respectively. (*A*) Area-normalized histograms of simulated dynamics with bin size = 0.02 nm and (*B*) autocorrelations for active (blue) and inactive (orange) with the collagen backbone restrained by an energy penalty of 1000 kJ/mol. (*C*) Area-normalized histograms of simulated dynamics and (*D*) autocorrelations for active (blue) and inactive (orange) with the collagen backbone unrestrained. All histograms are fitted to a sum of two Gaussians (active: solid blue line; inactive: solid red line). All autocorrelations are fitted to exponentials and power laws (exponential fit to active: dashed black line; power law fit to active: dashed red line; exponential fit to inactive: solid black line; power law fit to inactive: solid green line). The error bars in the histograms represent the square roots of the bin counts and are too small to be seen, whereas the autocorrelations do not have error bars. The [Supporting Materials and Methods](#) contain the best-fit parameters for histograms ([Table S6](#)) and autocorrelations ([Table S7](#)). To see this figure in color, go online.

that active MMP1 has more time-correlated dynamics at short times than inactive MMP1, in agreement with the experiments. Note that an exponential fit autocorrelates better than power law for smFRET measurements ([Fig. 2, D–F](#)). In contrast, power law fits autocorrelations better than an exponential distribution for simulations ([Fig. 4, B and D](#)). The best-fit parameters for the power law $(a \times t + 1)^{-b}$ are in [Table S7](#). The error bars are the SEs. There are reports on power law distribution of conformational dynamics ([65,66](#)). However, we need further studies to understand the transition from power law to an exponential distribution at the two extreme timescales.

For unrestrained collagen backbone, the histograms of simulated conformations ([Fig. 4 C](#)) showed that active MMP1 adopts more high FRET conformations (smaller interdomain distances), in contrast to the experimental observations that active MMP1 adopts more low FRET con-

formations ([Fig. 2 A](#)). In other words, the all-atom simulations with a restrained collagen backbone agree more with the experiments, which may be explained by the fact that the organization of fibrils restrains collagen monomers in comparison with collagen monomers in solution. Also, the difference in autocorrelations for active and inactive MMP1 on unrestrained collagen ([Fig. 4 D](#)) is significantly lower than those on restrained collagen ([Fig. 4 B](#)). Furthermore, simulations also suggest that MMP1 dynamics vary depending on the substrate properties, i.e., enzyme-substrate interactions are mutually affected by each other. Thus, experiments and simulations provide a synergistic combination to gain insights into MMP1 activity. Informed by single-molecule measurements of MMP1 interdomain dynamics, we used MD simulations to identify catalytically relevant conformations more precisely. We assumed that the catalytic pocket should open more before catalysis so that the catalytic residues can approach the cleavage sites on the three collagen chains. We measured the catalytic pocket opening by the distance between N171 and T230. We quantified the proximity to the collagen chains by the rms distance between the MMP1 residue (E/Q219) and the collagen cleavage sites (G-L).

Three-dimensional scatter plots ([Fig. 5](#)) show the patterns in the MMP1 conformational landscape. [Fig. 5 A](#) suggests that the encircled clusters are likely the functionally relevant conformations of MMP1 because these conformations are significantly absent in inactive MMP1 ([Fig. 5 B](#)). When the collagen backbone is free, the detailed structures of the conformational space disappear ([Fig. 5, C and D](#)). Interestingly, the number of conformational peaks depends on the projection plane (reaction coordinates). Overall, we compared simulations with experiments to gain further insights into the MMP1-collagen system and learned that an increase in interdomain separation correlates with a larger catalytic pocket opening and closer proximity to the collagen chains.

The all-atom MD simulations also explained why the E219Q mutation in the catalytic domain alters interdomain dynamics in the hemopexin domain. We calculated the rms side-chain fluctuation (RMSF) for residues in active and inactive MMP1 ([Fig. S5](#)). The E219Q mutation caused significant changes in fluctuations allosterically in the hemopexin domain. Surprisingly, variations in the catalytic domain are similar for active and inactive MMP1. The mean \pm SDs of RMSF ([Fig. S5](#)) across all the amino acids are 3.6 ± 0.7 nm for active and 4.6 ± 0.4 nm for catalytically inactive MMP1. The higher SD of RMSF for active MMP1 might explain the broader widths of histograms for active MMP1. To further delineate the difference between active and inactive MMP1, we performed principal component analysis of the fluctuations ([67](#)) (see [Supporting Materials and Methods](#)). We observed a significant difference between active and inactive MMP1 ([Figs. S6–S8](#)).

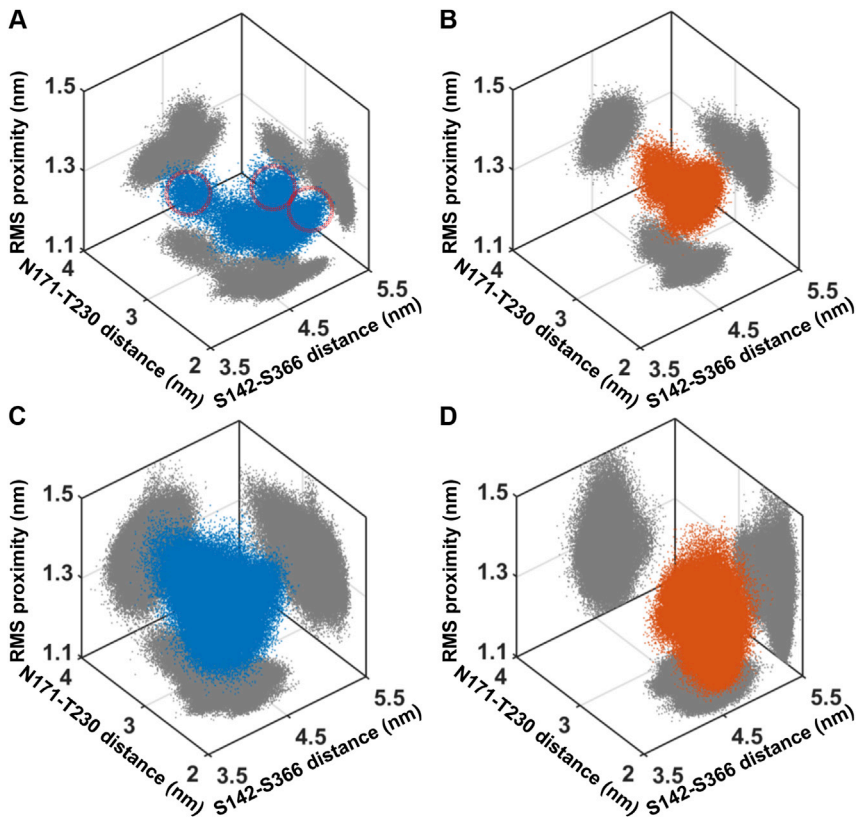


FIGURE 5 Insights from all-atom MD simulations with collagen backbone restrained (A and B) and unrestrained (C and D). Three-dimensional scatter plots of S142–S366 distance (represents interdomain dynamics), N171–T230 distance (represents the opening of the MMP1 catalytic pocket), and rms distance between the MMP1 catalytic site and the cleavage sites on three collagen chains for active (blue) and inactive (orange) MMP1. Two-dimensional projections of the scatter plots are in gray. The clusters encircled in red in (A) represent the plausible catalytically relevant conformations. To see this figure in color, go online.

MMP1 undergoes substrate-dependent adaptive conformational dynamics

Both the experiments (Fig. 2) on collagen fibrils at a 100-ms timescale and the simulations (Fig. 4) with restrained collagen monomer at a 5-ps timescale showed that active MMP1 adopts more conformations with larger interdomain distances. Such changes in protein conformations can occur because of the interactions with a ligand or substrate and can be understood based on the general principles of classical or quantum mechanics. If the interactions are weak, we can still use the conformations proteins without interactions and treat any conformational changes as perturbations. For weak interactions, one can envision applying “lock-and-key” (68), “induced fit” (69), and “conformational selection” (70) theories of enzyme specificity and function (71). If the interactions are strong, however, conformations, normal modes, and eigenfunctions are not represented by those of individual systems. For the MMP1-collagen system, the interactions are so strong that there is negligible unbinding once MMP1 binds collagen (19,21). As a result, collagen binding can lead to a new set of allowed MMP1 conformations. Indeed, MMP1 undergoes conformational dynamics in a substrate-dependent manner because restraining the collagen backbone changes the structures and correlations (Fig. 4). Besides, the mutation E219Q at the catalytic site of MMP1 also changes the set of allowed conformations

that appear at the two extreme timescales of the all-atom simulations (Figs. 3 and 4) and experiments (Fig. 2). Therefore, we can propose an alternative model of “adaptive conformational dynamics” in which substrate recognition leads to a new set of protein conformations that can be dynamically influenced by mutations and ligands (Fig. 2). It is also plausible to consider adaptive conformational dynamics as a time-dependent disorder or randomness, which is known to alter chemical rates (72).

Poisson process connects experimental and all-atom simulation timescales

The agreements between experiments and simulations suggest similar initial conformations of collagen-bound MMP1 at picoseconds as well as milliseconds. Note that crystal structures represent thermodynamically (lowest energy) or kinetically (lower activation barriers) states (73). Because we used crystal structures for simulations and we detected collagen-bound MMP1 for smFRET measurements, similar initial conformations are plausible. As such, we postulated that functionally relevant MMP1 interdomain conformations have a finite probability of occurring even at picoseconds, and dynamics might be connected using a Poisson process approach.

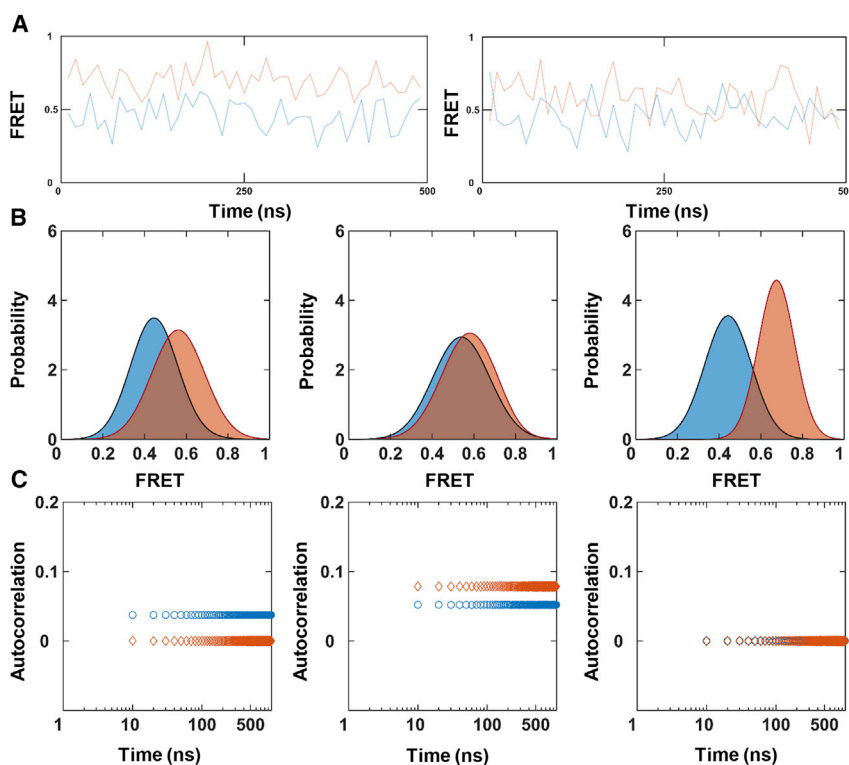


FIGURE 6 Two-state simulations at faster nanosecond timescales. Different trials with the same underlying parameters produced different (A) trajectories (blue: active MMP1; orange: inactive MMP1), (B) histograms with bin size = 0.005 (active: solid blue line; inactive: solid orange line), and (C) autocorrelations (active: blue circle; inactive: orange diamond). Different trials of simulations lead to different results even though the underlying process is the same two-state Poisson process. It is challenging to see hidden transitions in the example trajectories because the difference between S1 and S2 (~ 0.1) is similar to the noise levels (~ 0.1). For simulated trajectories with the same parameters but without noise, see Fig. S3 A. To see this figure in color, go online.

We modeled MMP1 interdomain dynamics as a two-state Poisson process, and Poisson-process-based stochastic simulations reproduced the kinetics of interconversion. In principle, a Poisson process at second timescales of experiments should also be a Poisson process at picosecond timescales of simulations. However, the probability of transition between states will be significantly lower at the picosecond according to the equation for probability for the exponential distribution $P(t)dt = k \times \exp(-k \times dt) \times dt$. For example, k is around 0.1 s^{-1} for MMP1 (Table S3), and chances for a transition at each time step are 0.0099 and 5×10^{-13} for 100-ms (smFRET experiments) and 5-ps (all-atom MD simulations) time steps, respectively. As a result, stochastic simulations at second timescales are more likely to have transitions and would reproduce the experimental features. However, simulations at picosecond to nanosecond timescales are less probable to have interconversion during the length of simulated trajectories and would give different results at different trials (Fig. 6) and may not reproduce the correlations even if the underlying process is a Poisson process.

To determine if we could apply the Poisson process approach to the faster timescales of MD simulations, we simulated 1000-ns-long trajectories with time resolution, $dt = 10 \text{ ns}$, using the same experimentally determined parameters, S1, S2, k_1 , and k_2 , for MMP1 without ligands (Tables S1 and S3). As shown in Fig. 6, both histograms and autocorrelations show different results at different trials because of the statistical nature of transitions. Without transitions, a trajectory is simply a constant value (S1 or S2)

plus noise, leading to zero autocorrelations (Fig. 6). If there are transitions, autocorrelations appear (Fig. 6).

The power law behavior of correlations (74) at picosecond timescales is intriguing because it usually occurs when there is a long-range order (for example, phase transitions (74,75)) or a correlated random walk (for example, stock market fluctuations (76) and DNA sequences (77,78)). We have several possible explanations. First, an exponential can become linear for short timescales if the condition $kt \ll 1$ is valid. Second, two-state Poisson process simulations showed that exponential correlations (Fig. 3 C) in the presence of noise changed into power law correlations in the absence of noise (Fig. S3 C) for the same underlying parameters. Indeed, fluctuations can transform a Lorentzian line shape into a Gaussian line shape (79). Variations in measurements can originate from samples, instruments, and tracking methods, leading to changes in the observed pattern (80). Third, Fig. 6 also provides one explanation for the power law behavior at short timescales because the presence of correlations stochastically depends on the number of transitions during the simulated length of trajectories. Without any transition, the correlation is zero. With transitions, the correlation is not zero but does not decay in picosecond to nanosecond timescales because the rates of conversion between the two states ($\sim 0.1 \text{ s}^{-1}$) are slow. As a result, the simulated correlation fits better with a power law. Further studies are needed to explain the power law behavior of autocorrelations at picosecond timescales.

Both the linker and the collagen substrate facilitate interdomain communications

We computed preferred conformations or “normal modes” of collagen-bound MMP1 using the ANM simulation (Fig. 7; (43)). The ANM is a prediction method that models each amino acid as a bead and creates a virtual bond network that connects these beads based on a user-defined cutoff distance (43,44). By modeling these virtual bonds as harmonic oscillators, we obtained an integrated view of the restrictions in conformational space experienced by each bead and, in turn, predicted what motions are accessible to MMP1. ANM cannot distinguish between active and inactive MMP1. Because active MMP1 with the pro domain attached (proMMP1) is also catalytically inactive, we calculated normal modes for proMMP1 as a proxy for inactive MMP1. Note that we produced both active (E219) and inactive (E219Q mutant) MMP1 as pro enzymes with the pro domain attached (27). MMPs are catalytically inactive with the pro domains attached. The pro domains need to be cleaved to activate MMPs. We activated proMMP1 using trypsin, as described in our previous publication (27). For ANM simulations, we used activated (pro domain cleaved) and pro (pro domain attached) MMP1. With the pro domain attached, we expected that the catalytic pocket opening and proximity to the collagen chains for proMMP1 would be restricted.

We calculated normal modes for collagen-bound (Fig. 7, A and B) and free (Fig. 7, C and D) MMP1 for activated and

proMMP1. As shown in Fig. 7, free and collagen-bound MMP1 have some similarities in allowed normal modes. Some modes of free MMP1 disappear, and some new modes appear for collagen-bound MMP1. When MMP1 is bound to collagen, the interdomain distance between S142 and S366 has larger values for activated MMP1 than pro MMP1. The larger interdomain distances accompanied larger catalytic pocket openings between N171 and T230 and a smaller rms proximity to the collagen cleavage sites for activated MMP1 (Fig. 7 A) in comparison with pro MMP1 (Fig. 7 B). When MMP1 was not bound to collagen, the ANM calculations showed that the catalytic pocket openings have a broader range for activated MMP1 (Fig. 7 C) in comparison with pro MMP1 (Fig. 7 D), but the interdomain distances are similar. These results further confirm that the collagen substrate can significantly change the dynamics of MMP1.

We investigated whether both the linker and collagen can allosterically communicate between the two domains. Fig. 8 shows that the catalytic domain opening has the largest SD (0.52 nm) when both the linker and collagen are present (Fig. 8 A). The catalytic pocket opening has the smallest SD (0.19 nm) when only the catalytic domain interacts with collagen (Fig. 8 C). The changes in opening suggest the essential role of the hemopexin domain in MMP1 activity. We computed conformations without the linker and observed that the hemopexin domain can still influence the catalytic domain plausibly via collagen and increase the catalytic pocket opening (Fig. 8 B). In this case,

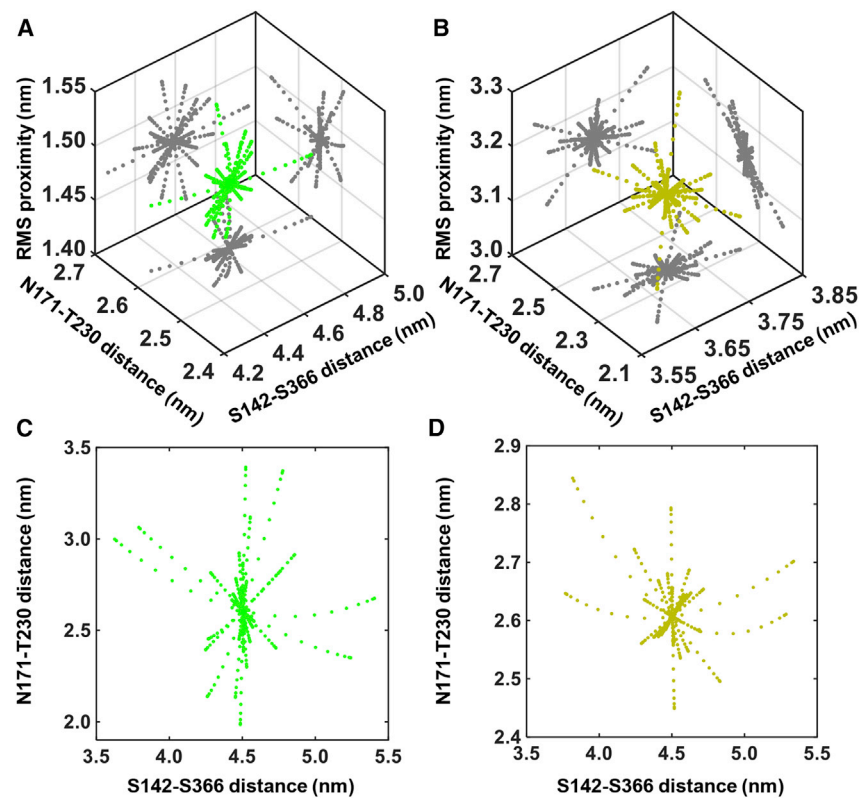


FIGURE 7 Normal mode analysis of MMP1 using the ANM. Three-dimensional scatter plots of S142–S366 distance (represents interdomain dynamics), N171–T230 distance (represents the opening of the MMP1 catalytic pocket), and rms distance between the MMP1 catalytic site and the cleavage sites on three collagen chains for (A) collagen-bound activated (green) and (B) pro (greenish-yellow) MMP1. Two-dimensional projections in (A) and (B) of the scatter plots are in gray. Two-dimensional scatter plots of S142–S366 distance and N171–T230 distance for free (C) activated (green) and (D) pro (greenish-yellow) MMP1. To see this figure in color, go online.

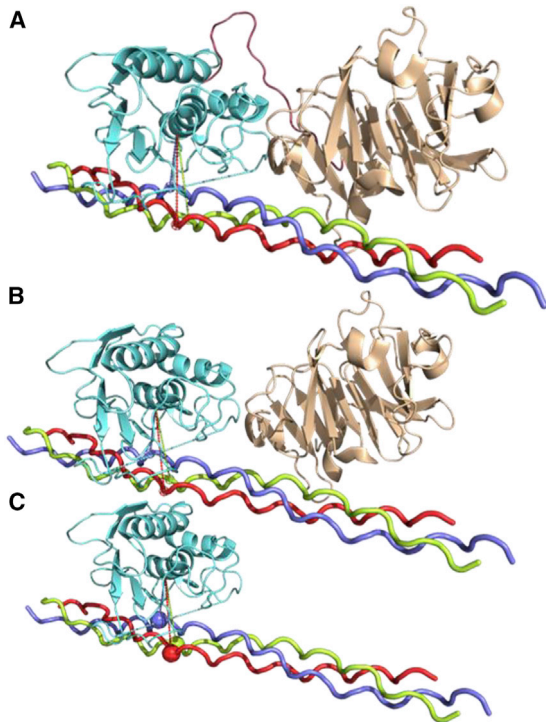


FIGURE 8 Interdomain communications via collagen even without the linker. The catalytic pocket openings as measured by the distance between N171 and T230 (cyan line) are (A) 2.56 ± 0.52 , (B) 2.56 ± 0.26 , and (C) 2.55 ± 0.19 nm. The error bars represent the SDs of 60 measurements of the catalytic pocket opening obtained from 20 frames each for the three slowest normal modes. The red, blue, and green spheres on collagen represent the cleavage sites. The catalytic domain (cyan), the linker (brown), and the hemopexin (wheat) domains represent the residue ranges F100–Y260, G261–C278, and D279–C466, respectively. To see this figure in color, go online.

the catalytic pocket opening has an SD of 0.26 nm, which is between 0.52 and 0.19 nm (Fig. 8). In other words, the two MMP1 domains can communicate via collagen even when the linker does not physically connect the two domains. Previous studies reported that a mixture of the two MMP1 domains purified separately could degrade triple-helical collagen (4), but the reason was not apparent. A larger catalytic opening because of allosteric communications between the two MMP1 domains via collagen explains why even a mixture of the two MMP1 domains can degrade triple-helical collagen. Such allosteric communications may lead to the observed correlations in interdomain motions (Fig. 2, D–F). Note that both autocorrelation and entropy are measures of randomness and order (81). A higher correlation suggests a lower amount of randomness and a higher amount of order. The randomness and order also change entropy that connects to kinetics and thermodynamics. In other words, any correlated motion or stabilization of conformations because of allosteric communications indicates a decrease in randomness (entropy). Enabling access to the cleavage sites on triple-helical collagen via interdomain dynamics of MMP1 is

an entropy-driven process, which can lead to an increased rate of cleavage.

In summary, we measured the interdomain dynamics of MMP1 on type 1 collagen fibrils at the single-molecule level. To distinguish the functionally relevant MMP1 conformations, we used inactive MMP1, an enhancer of MMP1 activity (MMP9), and an inhibitor of MMP1 activity (tetracycline). We found that an open conformation, which has the two domains well separated, is essential for function. Tetracycline forms two hydrogen bonds with both domains and inhibits dynamics, resulting in similar histograms for active and inactive MMP1. Two-state stochastic simulations, coarse-grained ANM simulations, and all-atom MD simulations reproduced some features observed in single-molecule measurements. We modeled MMP1 dynamics as a two-state stochastic system, which enabled using histograms and autocorrelations to calculate the kinetic rates. MD and ANM simulations also provided new insights into MMP1 dynamics to show that a larger interdomain motion of MMP1 correlates with a larger catalytic pocket opening of the catalytic domain and a smaller rms distance between the MMP1 catalytic site and the cleavage sites on collagen.

Our results resolve the debate on whether the two MMP1 domains are well separated (open low FRET state) or not (closed high FRET state). Based on NMR and SAXS experiments and the all-atom MD simulation studies (13,15–17), previous studies argued that a larger separation between the catalytic and hemopexin domains is necessary for activity. In contrast, x-ray crystallography suggested that the two domains need to be closer (12). Also, it is well-established that MMP1 exists in an equilibrium between open and closed conformations (15,16,82). Our single-molecule measurements, all-atom simulations, and two-state stochastic simulations also support that MMP1 exists in equilibrium between the two conformations. However, the ratio between the two states differs between active and inactive MMP1. Our results suggest that active MMP1 prefers the open conformation, whereas inactive MMP1 prefers the closed state. Because the crystal structure of MMP1 bound to a model of triple-helical collagen (PDB: 4AUO) used inactive MMP1, it is not surprising that x-ray crystallography suggests a preference for the closed conformation (12).

In contrast to previous studies on water-soluble triple-helical collagen monomers, we measured MMP1 dynamics on water-insoluble type 1 collagen fibrils using smFRET. MMP1 dynamics on fibrils show similarities with dynamics on monomers and support previous mechanistic insights obtained from monomer-based studies. However, we know that MMP1 activity on monomers and fibrils are different. To this end, we assumed that monomers in solution will be more flexible than monomers in fibrils and performed MD simulations with restrained and unrestrained collagen backbones. Interestingly, inactive MMP1 did not show a significant change in preferred conformations between restrained and unrestrained collagen, but active MMP1

showed more preference for open conformations with the collagen backbone restrained. We found that two MMP1 domains can allosterically communicate to induce a larger catalytic pocket opening via the linker as well as the collagen. The E219Q point mutant in the catalytic domain significantly changed the fluctuations of residues in the hemopexin domain. Our approach is readily applicable to other multi-domain proteins, including surface receptors, enzymes, and intracellular signaling proteins.

SUPPORTING MATERIAL

Supporting Material can be found online at <https://doi.org/10.1016/j.bpj.2020.06.010>.

AUTHOR CONTRIBUTIONS

S.K.S. conceived and designed the overall project. L.K. and S.K.S. designed the experiments. L.K. performed the experiments. A.N. performed the all-atom simulations. C.H. wrote the codes for analyzing the smFRET data and drew the TIRF schematics. J.P.-I. performed the ANM simulations and molecular docking. L.K., A.N., J.P.-I., D.W., J.K.-S., and S.K.S. analyzed the data. S.K.S. wrote the manuscript. All authors read and edited the manuscript.

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REFERENCES

- Karplus, M. 2010. Role of conformation transitions in adenylate kinase. *Proc. Natl. Acad. Sci. USA*. 107:E71–E72.
- Pisliakov, A. V., J. Cao, ..., A. Warshel. 2009. Enzyme millisecond conformational dynamics do not catalyze the chemical step. *Proc. Natl. Acad. Sci. USA*. 106:17359–17364.
- Henzler-Wildman, K. A., M. Lei, ..., D. Kern. 2007. A hierarchy of timescales in protein dynamics is linked to enzyme catalysis. *Nature*. 450:913–916.
- Chung, L., D. Dinakarpanian, ..., H. Nagase. 2004. Collagenase unwinds triple-helical collagen prior to peptide bond hydrolysis. *EMBO J*. 23:3020–3030.
- Page-McCaw, A., A. J. Ewald, and Z. Werb. 2007. Matrix metalloproteinases and the regulation of tissue remodeling. *Nat. Rev. Mol. Cell Biol*. 8:221–233.
- Jackson, B. C., D. W. Nebert, and V. Vasiliou. 2010. Update of human and mouse matrix metalloproteinase families. *Hum. Genomics*. 4:194–201.
- Ratnikov, B. I., P. Cieplak, ..., J. W. Smith. 2014. Basis for substrate recognition and distinction by matrix metalloproteinases. *Proc. Natl. Acad. Sci. USA*. 111:E4148–E4155.
- Gunasekaran, K., B. Ma, and R. Nussinov. 2004. Is allostery an intrinsic property of all dynamic proteins? *Proteins*. 57:433–443.
- Kadler, K. E. 2017. Fell Muir Lecture: collagen fibril formation in vitro and in vivo. *Int. J. Exp. Pathol*. 98:4–16.
- Nerenberg, P. S., R. Salsas-Escat, and C. M. Stultz. 2008. Do collagenases unwind triple-helical collagen before peptide bond hydrolysis? Reinterpreting experimental observations with mathematical models. *Proteins*. 70:1154–1161.
- Leikina, E., M. V. Merts, ..., S. Leikin. 2002. Type I collagen is thermally unstable at body temperature. *Proc. Natl. Acad. Sci. USA*. 99:1314–1318.
- Manka, S. W., F. Carafoli, ..., H. Nagase. 2012. Structural insights into triple-helical collagen cleavage by matrix metalloproteinase 1. *Proc. Natl. Acad. Sci. USA*. 109:12461–12466.
- Karabancheva-Christova, T. G., C. Z. Christov, and G. B. Fields. 2018. Conformational dynamics of matrix metalloproteinase-1 triple-helical peptide complexes. *J. Phys. Chem. B*. 122:5316–5326.
- Manka, S. W., D. Bihan, and R. W. Farndale. 2019. Structural studies of the MMP-3 interaction with triple-helical collagen introduce new roles for the enzyme in tissue remodeling. *Sci. Rep*. 9:18785.
- Bertini, I., M. Fragai, ..., G. B. Fields. 2012. Structural basis for matrix metalloproteinase 1-catalyzed collagenolysis. *J. Am. Chem. Soc*. 134:2100–2110.
- Cerofolini, L., G. B. Fields, ..., J. M. Teixeira. 2013. Examination of matrix metalloproteinase-1 in solution: a preference for the pre-collagenolysis state. *J. Biol. Chem*. 288:30659–30671.
- Arnold, L. H., L. E. Butt, ..., A. R. Pickford. 2011. The interface between catalytic and hemopexin domains in matrix metalloproteinase-1 conceals a collagen binding exosite. *J. Biol. Chem*. 286:45073–45082.
- Dittmore, A., J. Silver, ..., K. C. Neuman. 2016. Internal strain drives spontaneous periodic buckling in collagen and regulates remodeling. *Proc. Natl. Acad. Sci. USA*. 113:8436–8441.
- Sarkar, S. K., B. Marmer, ..., K. C. Neuman. 2012. Single-molecule tracking of collagenase on native type I collagen fibrils reveals degradation mechanism. *Curr. Biol*. 22:1047–1056.
- Perumal, S., O. Antipova, and J. P. R. O. Orgel. 2008. Collagen fibril architecture, domain organization, and triple-helical conformation govern its proteolysis. *Proc. Natl. Acad. Sci. USA*. 105:2824–2829.
- Saffarian, S., I. E. Collier, ..., G. Goldberg. 2004. Interstitial collagenase is a Brownian ratchet driven by proteolysis of collagen. *Science*. 306:108–111.
- Greenwald, R. A., L. M. Golub, ..., T. Sorsa. 1998. In vitro sensitivity of the three mammalian collagenases to tetracycline inhibition: relationship to bone and cartilage degradation. *Bone*. 22:33–38.
- Golub, L. M., N. Ramamurthy, ..., H. Perry. 1984. Tetracyclines inhibit tissue collagenase activity. A new mechanism in the treatment of periodontal disease. *J. Periodontal Res*. 19:651–655.
- Goldberg, G. I., A. Strongin, ..., B. L. Marmer. 1992. Interaction of 92-kDa type IV collagenase with the tissue inhibitor of metalloproteinases prevents dimerization, complex formation with interstitial collagenase, and activation of the proenzyme with stromelysin. *J. Biol. Chem*. 267:4583–4591.
- Messens, J., and J.-F. Collet. 2006. Pathways of disulfide bond formation in *Escherichia coli*. *Int. J. Biochem. Cell Biol*. 38:1050–1062.
- Rowell, S., P. Hawtin, ..., R. A. Pauptit. 2002. Crystal structure of human MMP9 in complex with a reverse hydroxamate inhibitor. *J. Mol. Biol*. 319:173–181.
- Kumar, L., W. Colomb, ..., S. K. Sarkar. 2018. Efficient protease based purification of recombinant matrix metalloprotease-1 in *E. coli*. *Protein Expr. Purif*. 148:59–67.
- Stennett, E. M., M. A. Ciuba, and M. Levitus. 2014. Photophysical processes in single molecule organic fluorescent probes. *Chem. Soc. Rev*. 43:1057–1075.
- Cordes, T., J. Vogelsang, and P. Tinnefeld. 2009. On the mechanism of Trolox as antiblinking and antibleaching reagent. *J. Am. Chem. Soc*. 131:5018–5019.

30. Rasnik, I., S. A. McKinney, and T. Ha. 2006. Nonblinking and long-lasting single-molecule fluorescence imaging. *Nat. Methods*. 3:891–893.
31. Kochevar, I. E., and R. W. Redmond. 2000. Photosensitized production of singlet oxygen. *Methods Enzymol.* 319:20–28.
32. Best, R. B., K. A. Merchant, ..., W. A. Eaton. 2007. Effect of flexibility and cis residues in single-molecule FRET studies of polyproline. *Proc. Natl. Acad. Sci. USA*. 104:18964–18969.
33. Levitus, M., and S. Ranjit. 2011. Cyanine dyes in biophysical research: the photophysics of polymethine fluorescent dyes in biomolecular environments. *Q. Rev. Biophys.* 44:123–151.
34. Merchant, K. A., R. B. Best, ..., W. A. Eaton. 2007. Characterizing the unfolded states of proteins using single-molecule FRET spectroscopy and molecular simulations. *Proc. Natl. Acad. Sci. USA*. 104:1528–1533.
35. Abraham, M. J., T. Murtola, ..., E. Lindahl. 2015. GROMACS: high performance molecular simulations through multi-level parallelism from laptops to supercomputers. *SoftwareX*. 1:19–25.
36. Hess, B., H. Bekker, ..., J. G. Fraaije. 1997. LINCS: a linear constraint solver for molecular simulations. *J. Comput. Chem.* 18:1463–1472.
37. Nash, A., H. L. Birch, and N. H. de Leeuw. 2017. Mapping intermolecular interactions and active site conformations: from human MMP-1 crystal structure to molecular dynamics free energy calculations. *J. Biomol. Struct. Dyn.* 35:564–573.
38. Collier, T. A., A. Nash, ..., N. H. de Leeuw. 2016. Intra-molecular lysine-arginine derived advanced glycation end-product cross-linking in Type I collagen: a molecular dynamics simulation study. *Biophys. Chem.* 218:42–46.
39. Collier, T. A., A. Nash, ..., N. H. de Leeuw. 2015. Preferential sites for intramolecular glucosylated cross-link formation in type I collagen: a thermodynamic study. *Matrix Biol.* 48:78–88.
40. Darden, T., D. York, and L. Pedersen. 1993. Particle mesh Ewald: an $N \cdot \log(N)$ method for Ewald sums in large systems. *J. Chem. Phys.* 98:10089–10092.
41. Hoover, W. G. 1985. Canonical dynamics: equilibrium phase-space distributions. *Phys. Rev. A Gen. Phys.* 31:1695–1697.
42. Parrinello, M., and A. Rahman. 1981. Polymorphic transitions in single crystals: a new molecular dynamics method. *J. Appl. Phys.* 52:7182–7190.
43. Eyal, E., L.-W. Yang, and I. Bahar. 2006. Anisotropic network model: systematic evaluation and a new web interface. *Bioinformatics*. 22:2619–2627.
44. Isin, B., K. C. Tirupula, ..., I. Bahar. 2012. Identification of motions in membrane proteins by elastic network models and their experimental validation. *Methods Mol. Biol.* 914:285–317.
45. Eyal, E., G. Lum, and I. Bahar. 2015. The anisotropic network model web server at 2015 (ANM 2.0). *Bioinformatics*. 31:1487–1489.
46. Atilgan, A. R., S. R. Durell, ..., I. Bahar. 2001. Anisotropy of fluctuation dynamics of proteins with an elastic network model. *Biophys. J.* 80:505–515.
47. Haugland, R. P. 2002. Handbook of Fluorescent Probes and Research Products. Molecular Probes Inc, Eugene, OR.
48. Neumann, U., H. Kubota, ..., D. Leppert. 2004. Characterization of Mca-Lys-Pro-Leu-Gly-Leu-Dpa-Ala-Arg-NH₂, a fluorogenic substrate with increased specificity constants for collagenases and tumor necrosis factor converting enzyme. *Anal. Biochem.* 328:166–173.
49. Kamerlin, S. C., and A. Warshel. 2010. Reply to Karplus: conformational dynamics have no role in the chemical step. *Proc. Natl. Acad. Sci. USA*. 107:E72.
50. Patterson, M. L., S. J. Atkinson, ..., G. Murphy. 2001. Specific collagenolysis by gelatinase A, MMP-2, is determined by the hemopexin domain and not the fibronectin-like domain. *FEBS Lett.* 503:158–162.
51. Saito, S., M. J. Trovato, ..., P. J. Pappas. 2001. Role of matrix metalloproteinases 1, 2, and 9 and tissue inhibitor of matrix metalloproteinase-1 in chronic venous insufficiency. *J. Vasc. Surg.* 34:930–938.
52. Asahi, M., K. Asahi, ..., E. H. Lo. 2000. Role for matrix metalloproteinase 9 after focal cerebral ischemia: effects of gene knockout and enzyme inhibition with BB-94. *J. Cereb. Blood Flow Metab.* 20:1681–1689.
53. Fujimura, M., Y. Gasche, ..., P. H. Chan. 1999. Early appearance of activated matrix metalloproteinase-9 and blood-brain barrier disruption in mice after focal cerebral ischemia and reperfusion. *Brain Res.* 842:92–100.
54. Hanemaaijer, R., H. Visser, ..., J. H. Verheijen. 1998. A novel and simple immunocapture assay for determination of gelatinase-B (MMP-9) activities in biological fluids: saliva from patients with Sjögren's syndrome contain increased latent and active gelatinase-B levels. *Matrix Biol.* 17:657–665.
55. Koshiha, T., R. Hosotani, ..., M. Imamura. 1997. Detection of matrix metalloproteinase activity in human pancreatic cancer. *Surg. Today*. 27:302–304.
56. Sakalihasan, N., P. Delvenne, ..., C. M. Lapière. 1996. Activated forms of MMP2 and MMP9 in abdominal aortic aneurysms. *J. Vasc. Surg.* 24:127–133.
57. Powell, B., D. C. Malaspina, ..., Y. Dhaer. 2019. Effect of collagenase-gelatinase ratio on the mechanical properties of a collagen fibril: a combined Monte Carlo-molecular dynamics study. *Biomech. Model. Mechanobiol.* 18:1809–1819.
58. Jarymowycz, V. A., and M. J. Stone. 2006. Fast time scale dynamics of protein backbones: NMR relaxation methods, applications, and functional consequences. *Chem. Rev.* 106:1624–1671.
59. Singh, W., G. B. Fields, ..., T. G. Karabancheva-Christova. 2016. Importance of the linker region in matrix metalloproteinase-1 domain interactions. *RSC Advances*. 6:23223–23232.
60. Piccard, H., P. E. Van den Steen, and G. Opendakker. 2007. Hemopexin domains as multifunctional liganding modules in matrix metalloproteinases and other proteins. *J. Leukoc. Biol.* 81:870–892.
61. Overall, C. M. 2002. Molecular determinants of metalloproteinase substrate specificity: matrix metalloproteinase substrate binding domains, modules, and exosites. *Mol. Biotechnol.* 22:51–86.
62. Clark, I. M., and T. E. Cawston. 1989. Fragments of human fibroblast collagenase. Purification and characterization. *Biochem. J.* 263:201–206.
63. Sarkar, S. K. 2016. Single Molecule Biophysics and Poisson Process Approach to Statistical Mechanics. Morgan & Claypool Publishers, San Rafael, CA.
64. Zumbusch, A., L. Fleury, ..., M. Orrit. 1993. Probing individual two-level systems in a polymer by correlation of single molecule fluorescence. *Phys. Rev. Lett.* 70:3584–3587.
65. Wang, J., L. Xu, ..., E. Wang. 2008. Exploring the origin of power law distribution in single-molecule conformation dynamics: energy landscape perspectives. *Chem. Phys. Lett.* 463:405–409.
66. Tang, J., and R. A. Marcus. 2006. Chain dynamics and power-law distance fluctuations of single-molecule systems. *Phys. Rev. E Stat. Nonlin. Soft Matter Phys.* 73:022102.
67. Maisuradze, G. G., A. Liwo, and H. A. Scheraga. 2009. Principal component analysis for protein folding dynamics. *J. Mol. Biol.* 385:312–329.
68. Koshland, D. E., Jr. 1995. The key-lock theory and the induced fit theory. *Angew. Chem. Int. Engl.* 33:2375–2378.
69. Koshland, D. E. 1958. Application of a theory of enzyme specificity to protein synthesis. *Proc. Natl. Acad. Sci. USA*. 44:98–104.
70. Monod, J., J. Wyman, and J. P. Changeux. 1965. On the nature of allosteric transitions: a plausible model. *J. Mol. Biol.* 12:88–118.
71. Boehr, D. D., R. Nussinov, and P. E. Wright. 2009. The role of dynamic conformational ensembles in biomolecular recognition. *Nat. Chem. Biol.* 5:789–796.
72. Zwanzig, R. 1990. Rate processes with dynamical disorder. *Acc. Chem. Res.* 23:148–152.
73. Desiraju, G. R. 2002. Cryptic crystallography. *Nat. Mater.* 1:77–79.

74. Stanley, H., S. Buldyrev, ..., M. Simons. 1993. Long-range power-law correlations in condensed matter physics and biophysics. *Physica A*. 200:4–24.
75. Buldyrev, S. V. 2006. Power law correlations in DNA sequences. *In Power Laws, Scale-Free Networks and Genome Biology*. E. V. Koonin, Y. Wolf, and G. Karev, eds. Springer, pp. 123–164.
76. Grau-Carles, P. 2001. Long-range power-law correlations in stock returns. *Physica A*. 299:521–527.
77. Stanley, H., S. Buldyrev, ..., M. Simons. 1993. Scaling concepts and complex fluids: long-range power-law correlations in DNA. *Le Journal de Physique IV*. 3:C1-15–C1-26.
78. Luscombe, N. M., J. Qian, ..., M. Gerstein. 2002. The dominance of the population by a selected few: power-law behaviour applies to a wide variety of genomic properties. *Genome Biol.* 3:RESEARCH0040.
79. Wertheim, G., M. Butler, ..., D. Buchanan. 1974. Determination of the Gaussian and Lorentzian content of experimental line shapes. *Rev. Sci. Instrum.* 45:1369–1371.
80. Colomb, W., J. Czerski, ..., S. K. Sarkar. 2017. Estimation of microscope drift using fluorescent nanodiamonds as fiducial markers. *J. Microsc.* 266:298–306.
81. Chapeau-Blondeau, F. 2007. Autocorrelation versus entropy-based autoinformation for measuring dependence in random signal. *Physica A*. 380:1–18.
82. Bertini, I., M. Fragai, ..., D. I. Svergun. 2009. Interdomain flexibility in full-length matrix metalloproteinase-1 (MMP-1). *J. Biol. Chem.* 284:12821–12828.