

RESEARCH ARTICLE

Conformational stability of the bacterial adhesin, FimH, with an inactivating mutation

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Funding information

Achievement Rewards for College Scientists Foundation Illinois Chapter; National Institute of General Medical Sciences, Grant/Award Number: T32GM008152; National Science Foundation, Grant/Award Number: 1764421; Northwestern University Quest High Performance Computing Cluster, Grant/Award Number: 30658; Office of Naval Research, Grant/Award Number: N00014163175 N000141512701; Paul and Daisy Soros Fellowships for New Americans; Simons Foundation, Grant/Award Number: 597491-01

Abstract

Allostery governing two conformational states is one of the proposed mechanisms for catch-bond behavior in adhesive proteins. In FimH, a catch-bond protein expressed by pathogenic bacteria, separation of two domains disrupts inhibition by the pilin domain. Thus, tensile force can induce a conformational change in the lectin domain, from an inactive state to an active state with high affinity. To better understand allosteric inhibition in two-domain FimH (H2 inactive), we use molecular dynamics simulations to study the lectin domain alone, which has high affinity (HL active), and also the lectin domain stabilized in the low-affinity conformation by an Arg-60-Pro mutation (HL mutant). Because ligand-binding induces an allostery-like conformational change in HL mutant, this more experimentally tractable version has been proposed as a “minimal model” for FimH. We find that HL mutant has larger backbone fluctuations than both H2 inactive and HL active, at the binding pocket and allosteric interdomain region. We use an internal coordinate system of dihedral angles to identify protein regions with differences in backbone and side chain dynamics beyond the putative allosteric pathway sites. By characterizing HL mutant dynamics for the first time, we provide additional insight into the transmission of allosteric information across the lectin domain and build upon structural and thermodynamic data in the literature to further support the use of HL mutant as a “minimal model.” Understanding how to alter protein dynamics to prevent the allosteric conformational change may guide drug development to prevent infection by blocking FimH adhesion.

KEYWORDS

adhesins, allosteric regulation, *Escherichia coli*, infections, protein conformation, protein domains, molecular dynamics simulation

1 | INTRODUCTION

The bacterial adhesin FimH is one of the most well characterized model proteins for catch-bond behavior, in which tensile force paradoxically increases ligand affinity and lengthens the adhesion time.^{1,2} For many catch-bond proteins,² including FimH, P-selectin,³ α -catenin,⁴ and the $\alpha\beta$ T-cell receptor,⁵ tensile force exposes previously buried regions and induces an allosteric conformational

change.^{6–8} A better understanding of the protein dynamics involved in the activation of FimH⁹ could lead to treatments that target the allosteric site, which could prevent bacterial adhesion to the host during infections.¹⁰

FimH is a 30 kDa two-domain bacterial adhesin found on the fimbrial tips of uropathogenic *Escherichia coli* that binds to mannose ligands on urothelial cells.¹¹ Because it enables bacterial adhesion, which is one of the first steps in urinary tract infections, FimH is a

critical virulence factor.¹¹ During urination, shear forces introduce tension into the FimH-mannose interaction to produce catch-bond behavior, causing FimH to bind more tightly and for longer duration.^{2,9,12,13} This catch-bond behavior distinguishes mannosylated ligands on the urinary tract from decoys and limits bacterial removal.^{11,14} Several glycomimetic drugs (mannosides) have been developed that competitively inhibit FimH and thus prevent bacterial adhesion.^{15,16} However, it is currently not known how to target the allosteric site(s) to block the conformational change.

The mechanism underlying catch-bond behavior involves an allosteric conformational change between an inactive state with low affinity and an active state with high affinity.² In the inactive state, the two domains of FimH are close together, which stabilizes the interdomain region between the ligand-binding lectin domain (HL) and the allosterically inhibitory pilin domain (HP).^{14,17,18} The transition to the active state is thought to occur after the ligand binds to the lectin domain and tensile force pulls the domains apart.¹⁹ Exposing the interdomain region to water, which disrupts inhibition by the pilin domain, is thought to induce a conformational change in the lectin domain.^{18,20} The conformational change has been described by the width of the β -sandwich fold,^{14,17,21} as well as the putative allosteric pathway sites connecting the interdomain region to the binding pocket.^{12,20,22}

While two-domain FimH in the inactive conformation (H2 inactive) can undergo a force-induced, allosteric conformational change to the active state, a truncated protein consisting of the lectin domain alone (HL active) is constitutively active.^{2,18,22} However, while the lectin domain with a single Arg-60-Pro amino acid mutation (HL mutant) is stabilized in the inactive conformation,¹² HL mutant undergoes an allostery-like conformational change upon binding mannoside.²² As a result, Rabbani et al²² have proposed HL mutant as a "minimal model" for FimH allostery that is smaller, consists of a single domain, and is more experimentally tractable than full-length FimH. Although the structure and function of the HL mutant have been well characterized, its dynamics have not yet been investigated experimentally or computationally. Studying the dynamics of HL mutant will make it possible to identify protein regions with dynamical differences compared to H2 inactive, which can provide insight into the allosteric conformational change and help design additional mutations that lock HL mutant in the inactive state.

The impact of the Arg-60-Pro mutation on the structure and function of HL mutant has also been well characterized. After Rodriguez et al¹² selected the Arg-60-Pro mutation to energetically favor the inactive state from a set of trial mutations tested in RosettaDesign, Rabbani et al²² then confirmed that the backbone structure of HL mutant matches the lectin domain of H2 inactive using crystallography and chemical shift mapping from 1H-15N-HSQC NMR spectroscopy. The structural similarity between HL mutant and H2 inactive suggests that the β -bulge has stronger allosteric coupling to the clamp segment than the interdomain regions, which are missing in HL mutant. Thus, different sites along the allosteric pathway may vary in their "coupling" strength to the binding pocket.^{20,22} It has also been demonstrated that HL mutant has more than 10 times higher

ligand association and dissociation rate constants than H2 inactive, despite the structural similarity at the binding pocket.²² Differences in binding affinity may arise from the fact that allosteric conformational changes from the pilin to the mannoside binding site are thought to be propagated in a dynamic manner,^{17,22} affecting the fluctuations of the clamp segment. This hypothesis suggests the need for a deeper investigation into the relative contributions of backbone and side chain dynamics towards allosteric "information transfer" to the clamp segment, such as by comparing the fluctuations of HL mutant and H2 inactive. Rabbani et al²² also found that the addition of a mannoside ligand (n-heptyl α -D-mannopyranoside) induced a conformational change in HL mutant with NMR peaks that matched HL active at the binding pocket and the β -sandwich, but not near the mutation or the interdomain region. The conformational change of HL mutant was hypothesized to be similar to that of full-length FimH due to mannoside binding.¹⁷ However, HL mutant has more than seven times higher affinity for mannoside than H2 inactive.²² The difference in binding affinity, despite the similarity in backbone structure, suggests further differences in the backbone dynamics or the side chains.

The dynamics of HL mutant have not yet been studied using molecular dynamics (MD) simulations. However, Interlandi and Thomas²⁰ isolated the lectin domain from a crystal structure of H2 inactive and used nanosecond-timescale MD simulations in order to identify protein regions with different dynamics than HL active, focusing on the putative allosteric pathway.

We seek here to identify the protein regions with dynamical differences across HL mutant, H2 inactive, and HL active, for both the backbone and side chains. In contrast to the top-down approach that starts with identifying landmarks along the putative allosteric pathway, we employ a bottom-up approach without defining sites a priori by directly comparing the structure and dynamics across the sequence of the lectin domain. Moreover, because using external Cartesian coordinates introduces artifacts from rigid body motion and relative domain motion, we use a system of internal coordinates based on the dihedral angles.²³ To this end, we perform 20 ns-long all-atomistic MD simulations of HL mutant, H2 inactive, and HL active to identify protein regions with differences in dynamics.

We find that structural differences between HL mutant and H2 inactive are not limited to the clamp segment in the binding pocket, the interdomain loops, or the β -bulge region with the mutation. For the backbone dynamics, we find the greatest differences in the interdomain region and the binding pocket. There are also differences in side chain orientation and dynamics, beyond the localized effect of having Arg or Pro at position 60. Our analyses using similarity matrices, a common tool in data science, confirms that the differences in backbone dynamics are more distinct than the differences in backbone structure or in side chain orientation and dynamics.

The implications of our study extend beyond FimH. Our investigation demonstrates the advantages of a system of internal coordinates over Cartesian coordinates for quantifying dynamics. The regions we identify with dynamical differences across HL mutant, H2 inactive, and HL active using dihedral angles are not restricted to the identified landmarks on the putative allosteric pathway and further

highlights the power of a bottom-up approach, which may be applicable to proteins where these landmarks are not yet identified.

2 | MATERIALS AND METHODS

2.1 | FimH structures

We retrieved crystal structures for HL mutant, H2 inactive, and HL active from the Protein Data Bank (RRID:SCR_012820), as detailed in Table 1. We denote the full-length FimH protein in the inactive conformation as H2 inactive and denote the truncated structures that only include the lectin domain as HL mutant and HL active. For H2 inactive, we focus on two structures (4XOD and 5JQI) that were crystallized by separate groups. Due to natural sequence variations, different strains of *E. coli* produce FimH with slightly different amino acid sequences. To directly compare side chain dynamics with matching sequences, we mutated 4XOD at three positions in silico to match HL mutant and HL active (Table 1). In contrast, we made no changes to 5JQI. Due to the sequence mismatch, side chain comparisons involving 5JQI were limited, but backbone comparisons were not affected.

HL mutant has a single Arg-60-Pro mutation. To explore the impact of the residue identity alone, without otherwise changing the structure, we used MODELLER²⁵ (RRID:SCR_008395) to introduce a “perturbation” in silico by changing back the Pro at position 60 to Arg for HL mutant; we made the opposite change for HL active (Supplementary Information). For all structures, we investigated systems with and without the in silico addition of mannose from 1KLF.

2.2 | Equilibrium simulations

We performed all-atomistic MD simulations using NAMD,²⁶ with the CHARMM force field.²⁷ Our NAMD simulation parameters and system details are listed in Table 2. We prepared all simulation systems using visual molecular dynamics.²⁸ We solvated each protein with enough water molecules to prevent interactions with itself through the periodic boundary conditions.

We performed six replicate simulations for each system. Because we found that replicates starting from a single solvated system were too similar to each other, we solvated, minimized, and equilibrated our replicates separately.

2.3 | Backbone and side chain dihedral angles

We focus on dihedral angles to compare protein structures and dynamics instead of Cartesian coordinates because dihedral angles are better suited for identifying the critical regions driving collective motion in Cartesian space, such as hinges that displace distal elements.³⁰ Moreover, using dihedral angles avoids artifacts from structural alignment and better captures angular motions that affect large segments in hinged proteins, such as FimH.^{23,30,31} We describe backbone motion using the dihedral angles ϕ and ψ (see Figure S5). We

TABLE 2 Details of molecular dynamics simulations

Setup and visualization	VMD ²⁸ 1.9.3 (RRID:SCR_001820)
System dimensions	60 Å × 62 Å × 93 Å for HL active 60 Å × 72 Å × 123 Å for H2 inactive
System sizes	> 32 000 atoms for HL active > 60 000 atoms for H2 inactive
Solvation	TIP3P water model with 16 Å padding on each side
Ionization	NaCl ions to neutralize and achieve 50 mM salt conc.
Simulation engine	NAMD ²⁶ 2.13 (RRID:SCR_014894)
Ensemble	NPT
Temperature	300 K
Pressure	1 atm
Non-bonded interactions	Lennard-Jones potential (12 Å cutoff ²⁹)
Electrostatic interactions	Particle-Mesh Ewald sum method ²⁶
Force field	CHARMM c36 July 2018 update
Timestep	1 fs
Coordinates saved every	1 ps
Energy-minimization	Conjugate gradient algorithm in NAMD 10 000 steps with protein fixed 10 000 steps with no atoms fixed
Equilibration	1 ns
Simulation	No ligand: 20 ns Ligand: disassociation or up to 20 ns

Name	HL mutant ²²	HL active ²⁴	H2 inactive v1 ¹⁷	H2 inactive v2 ¹⁴
PDBID	5MCA	4AUU	4XOD	5JQI
Resolution (Å)	1.604	1.5	1.14	1.982
Residue 60	Pro	Arg	Arg	Arg
Residue 27	Val	Val	Ala-27-Val	Ala
Residue 70	Asn	Asn	Ser-70-Asn	Ser
Residue 78	Ser	Ser	Asn-78-Ser	Asn

Note: For H2 inactive, both 4XOD and 5JQI had differences in the primary sequence. For 4XOD, we mutated three positions to match the primary sequence for HL active.

TABLE 1 PDB structures studied

describe side chain motion using both rotamer angles (χ) and custom-defined pseudo-dihedral angles (ν). Each amino acid has one to five rotamer angles, except for Ala and Gly. To compare the wild-type with the Arg-60-Pro mutation, we omit the last three rotamer angles in Arg. We define the pseudo-dihedral angles, ν , to compare side chain dynamics when there are differences in the primary sequence, due to either the Arg-60-Pro mutation or the two versions of H2 inactive. We define one pseudo-dihedral angle per amino acid using four atoms: N, CA, and the last two atoms from the most distal χ angle. For example, since Arg has χ_5 calculated by CD-NE-CZ-Nh1, we calculate ν from N-CA-CZ-Nh1 (Figure S5). For Ala, we use N-CA-CB-H1, and we do not define ν for Gly.

We calculate the average, variance, and standard deviation (SD) using circular statistics, which account for the periodic wrapping from $-\pi$ to π .²³ We use the SD to assess the range of backbone and side chain fluctuations. We compare the SD at each residue to identify protein regions with differences in fluctuation size among the starting structures. Because the backbone dynamics were similar in the presence or absence of mannose, as well as the amino acid at position 60, we pooled replicates for backbone dynamics to increase the statistical power. Specifically, we found no significant dynamical differences in the presence or absence of mannose, and extremely small, yet statistically significant, differences at isolated sites due to the amino acid at position 60. However, having Arg or Pro at position 60 affected the side chain dynamics, so we did not pool replicates. For statistical significance testing, we use Mood's median test from the python package *scipy* (RRID:SCR_008058).³² To correct for multiple comparisons, we use the two-stage Benjamini-Krieger-Yekutieli procedure from the python package *statsmodels* (RRID:SCR_016074),^{33,34} with a false discovery rate of $\alpha = 0.05$. For robustness testing, we also implement the Bonferroni correction for multiple comparisons with $p = .05$.

2.4 | Similarity matrix

After we identified differences between groups of HL mutant and H2 inactive trajectories, we quantify the extent of the difference by constructing similarity matrices that compare each pair of trajectories. As references, we choose one type of comparison where we expect low similarity and two types of comparisons where we expect high similarity. We expect low similarity, or large differences, when we compare HL active with both versions of H2 inactive. In contrast, we expect high similarity when we compare the backbone for both versions of H2 inactive, as well as when we compare replicates. Using this framework with the low- and high-similarity references, we use similarity matrices to compare the structure and dynamics of both the backbone and side chains. Each similarity value is a nonparametric (rank-based) correlation. While we analyze dynamics with Spearman's correlation, we analyze structure using the equivalent correlation for circular variables described by Mardia and modified by Fisher and Lee.³⁵ Using these similarity matrices, we quantify the similarity between HL mutant, H2 inactive, and HL active and perform statistical testing

using Mood's median test with a Bonferroni correction for multiple comparisons.

2.5 | Dihedral principal component analysis

We applied principal component analysis to dihedral angle fluctuations (dPCA) to identify protein regions with coupled dynamics.²³ We chose to use dihedral angle dynamics because they have been shown to capture some motions missed by $C\alpha$ fluctuations due to "artifacts" during the alignment step, particularly during hinged motion and for both large- and small-amplitude motions.^{23,30}

To identify protein regions with coupled motion, we calculate the first through fourth lowest-frequency eigenmodes from the circular covariance matrix for each simulation. We then compare the eigenmodes of HL mutant, H2 inactive, and HL active. We focus on the amount of motion at a particular angle described by these eigenmodes, so we square the eigenvector value at each residue and sum across eigenmodes. Since the eigenvectors are unit vectors, we weight the summation by the eigenvalues, which measure the variance in the angle changes described by the eigenvectors. Moreover, we also calculate the unweighted average, using the eigenvectors that explained 70% of the variation, after finding similar patterns for 50-80% of the variation explained.

3 | RESULTS

3.1 | Crystal structure comparisons

We use root mean squared displacement of the backbone $C\alpha$ atoms ($C\alpha$ -RMSD) to measure crystal structure differences and to broadly identify structural differences across HL mutant, H2 inactive, and HL active (Figure 1 and Figure S3B). As expected, our analyses show that the $C\alpha$ -RMSD between HL mutant (5MCA) and the two versions of H2 inactive are quite small (0.76 Å to 4XOD and 0.93 Å to 5JQI). This similarity is consistent with the similarity between HL mutant and another version of H2 inactive (3JWN) reported by Rabbani et al.²² While HL mutant and H2 inactive have similar crystal structures, they are both quite different from HL active (4AUU). The $C\alpha$ -RMSD between HL active and HL mutant is 3.15 Å and between HL active and the two versions of H2 inactive are 3.30 Å (4XOD) and 3.25 Å (5JQI).

Even though the structural differences between HL mutant and H2 inactive are small, we nonetheless examine the backbone regions with the greatest contributions to the $C\alpha$ -RMSD differences (Figure 1B and Figure S3B). As described by Rabbani et al.,²² we find the largest difference at the clamp segment in the binding pocket and few differences within the " β -sandwich." We also detect some differences in an unnamed region on the opposite side of the binding pocket, as well as in the swing and insertion loops in the interdomain region (see Figure 2 and Figure S1 for named regions).

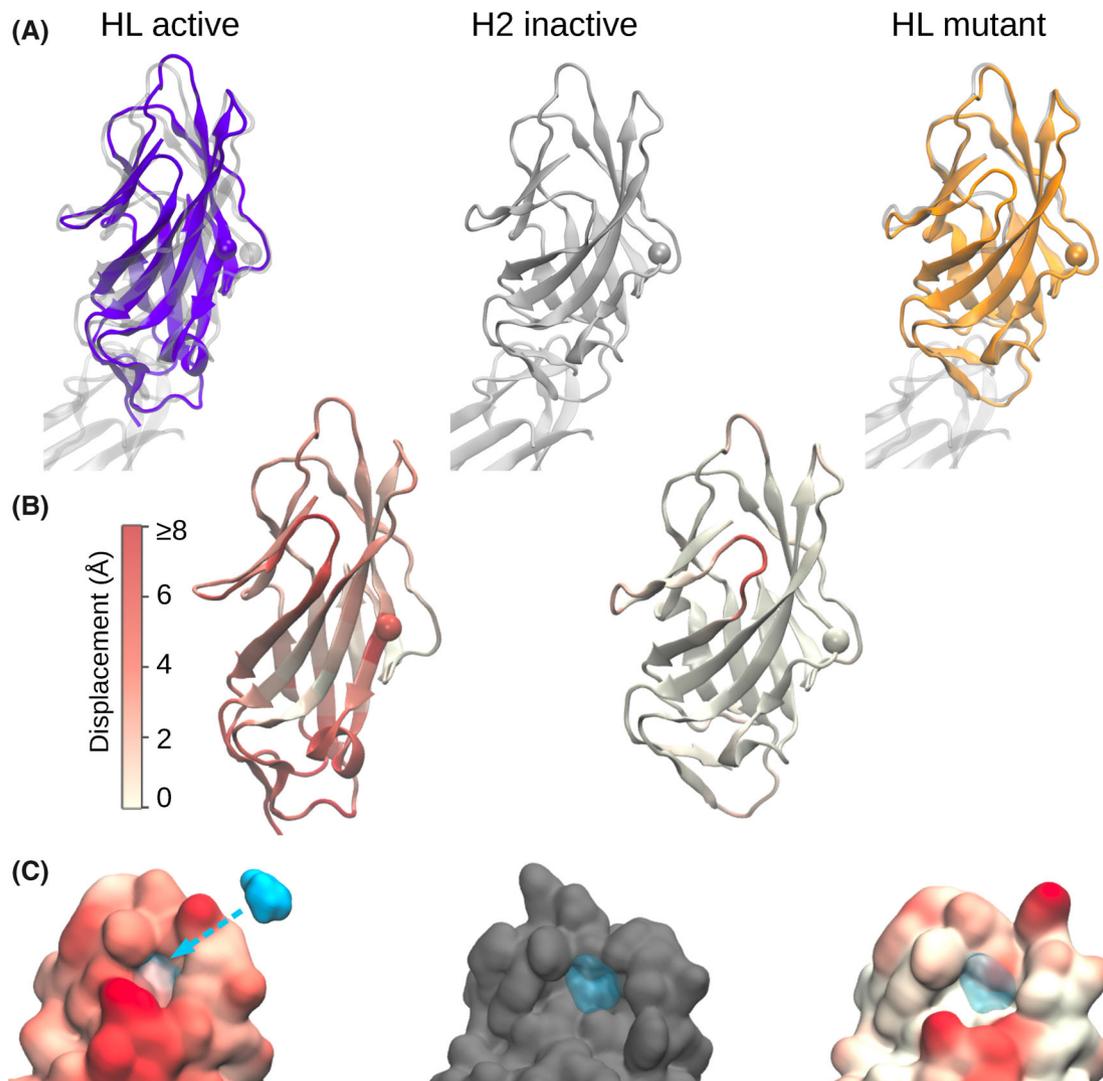


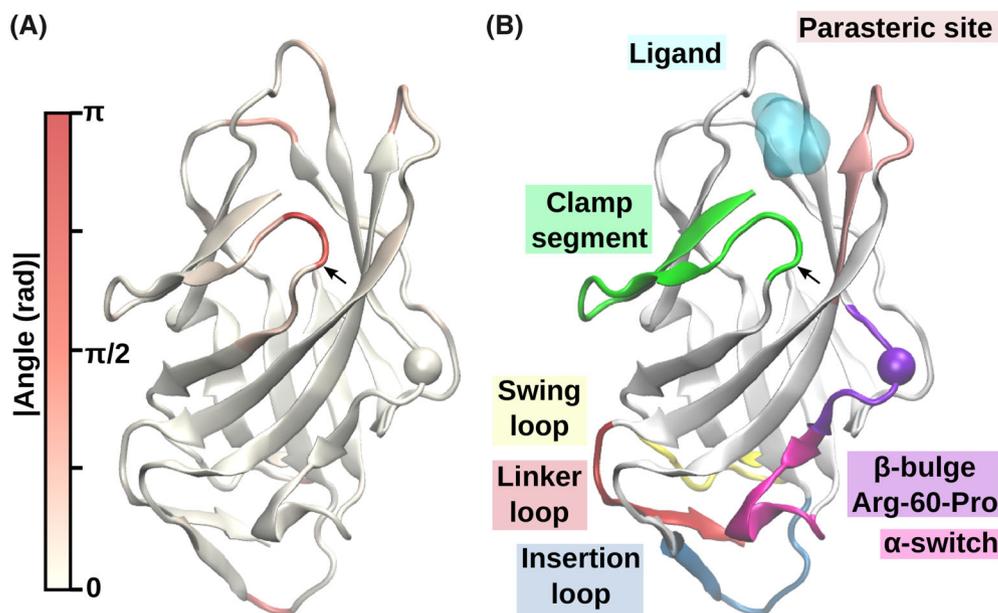
FIGURE 1 The Arg-60-Pro mutant has a backbone structure similar to that of inactive FimH but with a partially closed binding pocket. A, NewCartoon representation of the backbone structures for H2 inactive with the pilin domain cropped (gray, PDBID 4XOD, low-affinity) overlaid by the lectin domain structures: HL active (PDBID 4AUU, orange, high-affinity) and HL mutant with Arg-60-Pro (PDBID 5MCA, purple). We highlight residue 60, the mutation site, with a bead. We use color to illustrate the displacement from H2 inactive for B, the backbone and C, the surface rendering of predicted side chain orientation. The mannose ligand is shown in blue and was positioned after alignment with high-affinity, two-domain FimH (PDBID 1KLF) [Color figure can be viewed at wileyonlinelibrary.com]

The difference between HL mutant and H2 inactive at the clamp segment has been attributed to its flexibility in the absence of ligand.²² In particular, the angular differences found in the backbone dihedrals at the C-terminal end of the clamp segment could act like a “hinge” that displaces distal elements (arrow in Figure 2). This hinge motion may cause the large displacements at the tip of the clamp identified by $C\alpha$ -RMSD (Figure 1B). Compared to the localized angular differences (Figures S3 and S4), the overly broad protein regions identified by $C\alpha$ -RMSD reveals the limitations of calculations involving structural alignment, which have previously been reported in the literature.^{23,30} For this reason, residues with large dihedral angle motion may be more dynamically important and specific than the regions of several residues identified by large $C\alpha$ -RMSD.

3.2 | Backbone dynamics using internal coordinates

We thus consider an internal coordinate system that uses the average backbone dihedral angles²³ to identify differences between average structures obtained from the MD simulations. Using the dihedral angles to compare HL mutant and H2 inactive, we mostly find small differences in backbone angle orientation across the lectin domains (Figure 3B). In contrast, we find large differences at the insertion loop and the hinge at the C-terminal end of the clamp segment (arrow label in Figure 2). Because we also find differences at the hinge in our crystal structure comparisons, we think that the difference in the orientation of the clamp segment is not resolved within the 20-ns simulation

FIGURE 2 Magnitude of angle differences between HL mutant and H2 inactive. A, NewCartoon representation of the HL mutant backbone shows large changes in backbone dihedral angle differences at the C-terminal end of the clamp segment that may act like a hinge-joint (arrow). B, Putative allosteric pathway sites,¹² the parasteric site,¹⁰ and residue 60 (bead) [Color figure can be viewed at wileyonlinelibrary.com]



timescale. In our simulations of longer than 200 ns, we find clamp segment conformations accessed by both HL mutant and H2 inactive, as well as conformations only found in our HL mutant simulations. However, we did not find interdomain region conformations shared by both HL mutant and H2 inactive.

Since we find the largest angle change at the hinge-joint and the largest contribution to $C\alpha$ -RMSD at the center of the clamp segment, we interpret these results to demonstrate that our approach enables us to detect the joints of regions displaying hinge-like motion in an automated fashion. The hinges we identify are those relevant to the 20 ns timescale (Figure 3C), and our smaller number of replicates at the 200 ns timescale suggests the existence of additional hinges (Figure S12B).

We next investigate backbone dynamics for the dihedral angles found in these hinge regions and use the SD to compare the magnitude of the fluctuations (Figures 3C and 4). We find that HL mutant has larger fluctuations than H2 inactive in the insertion and swing loops of the interdomain region and in the clamp segment of the binding pocket (Figure 3C). Thus, the backbone regions with larger dynamical differences are also the regions with larger structural differences.

Since it has been hypothesized that the difference in dynamics is related to the exposed interdomain region of HL mutant,²² we next compare the dynamics of HL mutant and HL active, as both have the interdomain region exposed. We find that HL mutant also has larger fluctuations than HL active in the insertion and swing loops of the interdomain region (Figure 4B). For the clamp segment, we find that HL mutant is more dynamic at the hinge joint, while HL active is only slightly more dynamic at the tip of the clamp.

Surprisingly, we also find that HL active and H2 inactive display small differences in dynamics across the protein (Figure 4C). HL active is only slightly more dynamic in the clamp segment and the C-terminus, with minute differences in dynamics across the protein. We interpret this finding to show that HL active and H2 inactive are more

stable compared to HL mutant. The interdomain regions in H2 inactive and HL active may be stabilized by different mechanisms: contacts with the pilin domain for H2 inactive,^{17,18} and a more rigid fold that reduces the solvent-accessibility of the hydrophobic interdomain region for HL active.²⁰ Our data supports the idea that different allosteric pathway sites have different coupling strengths to the binding pocket.^{12,22}

The presence or absence of the mannose ligand does not result in statistically significant differences for the backbone structure or dynamics. We interpret these results to indicate that the initial protein structure has the largest impact on both structure and dynamics, at least for the up to 20 ns duration of our MD simulations.

3.3 | Comparing side chain orientations and fluctuations

For many proteins, including FimH, ligand binding also depends on side chain flexibility and optimal side chain-ligand interactions.²⁴ To separate side chain motion from displacements due to backbone motion, we again use internal angles. We quantify side chain structure and dynamics using two alternative sets of angles: χ dihedral angles (or rotamer angles) and the custom-defined pseudo-dihedral angles ν . We define one ν angle, using N, $C\alpha$, and two distal atoms, for each amino acid except for Gly (see Methods (section 2.3) and Figure S5). Each amino acid, except for Gly and Ala, also has one to five χ rotamer angles. By construction, the ν angles have broader angle distributions than the χ angles and enable us to compare the two versions of H2 inactive in spite of their minor sequence variations.

While HL mutant and H2 inactive have similar backbone structures, side chain orientations estimated from the crystal structures suggest that the geometries of their binding pockets are quite different (Figure 1C). To separate side chain motion from displacements

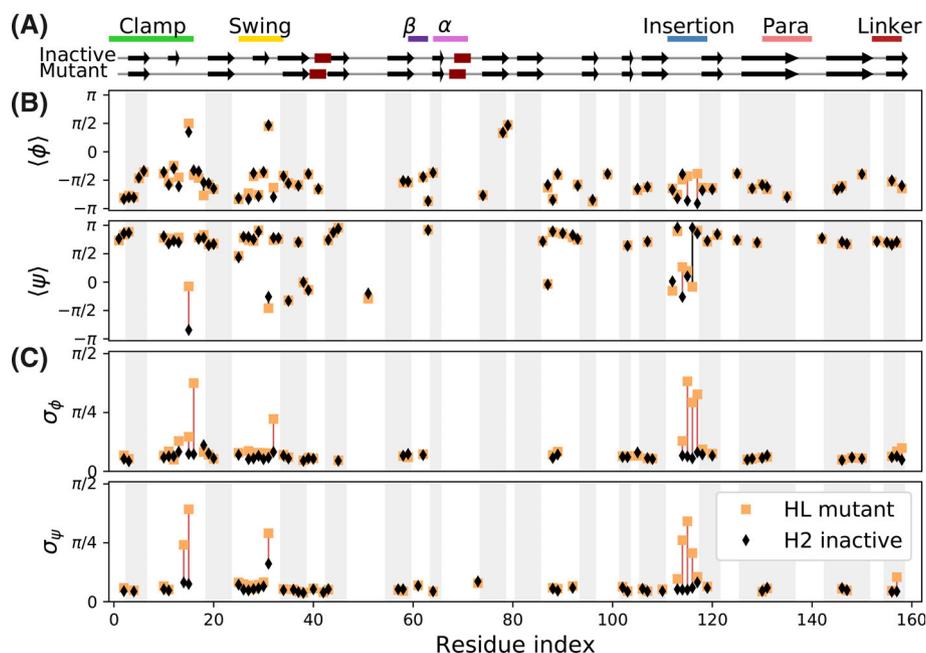


FIGURE 3 Localized differences in the structure between HL mutant and H2 inactive cause differences in dynamics. A, Secondary structure of H2 inactive (top) and HL mutant (bottom) with features. B, Protein structure comparison between HL mutant (orange squares) and H2 inactive (black diamonds) used average backbone dihedral angles ϕ and ψ , which provide an internal frame of reference. Structural differences are localized to the hinge of the clamp loop in the binding pocket and the insertion loop in the interdomain region, which is exposed to water in HL mutant. C, Backbone dynamics comparison used the SDs of ϕ and ψ , quantifying the magnitude of angle fluctuations. HL mutant has larger fluctuations (red lines) at the clamp loop and the insertion loop. For statistically significant differences, the median values are shown [Color figure can be viewed at wileyonlinelibrary.com]

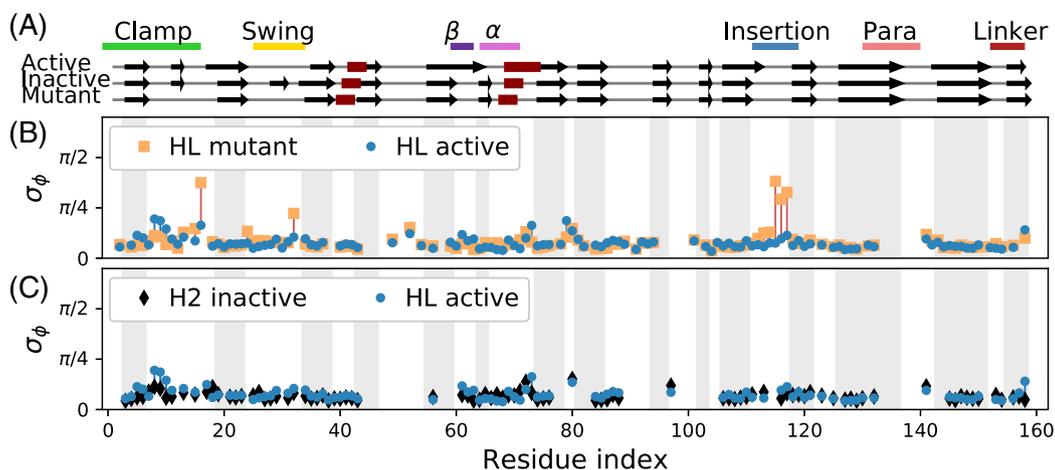


FIGURE 4 Comparisons of backbone dynamics show HL mutant has larger fluctuations than H2 inactive and HL active. Backbone dynamics were compared using the SDs of ϕ (see Figure S11 for ψ). A, Secondary structure of HL active (top), H2 inactive (middle), and HL mutant (bottom) with features. B, HL mutant (orange squares) has larger backbone fluctuations in the insertion and swing loops than HL active (blue circle). C, HL active and H2 inactive (black diamonds) have small differences in backbone dynamics throughout the lectin domain [Color figure can be viewed at wileyonlinelibrary.com]

due to backbone motion, we first compare the average side chain orientations of HL mutant and H2 inactive using ν (Figure 5B) and χ (Figure S14B) angles. Our data show that the differences extend beyond the β -bulge segment with the Arg-60-Pro mutation. Indeed, in addition to the β -bulge segment, we find nine large differences in side

chain orientation across the protein, including the hinge-joint of the clamp segment, the swing loop, the parasteric site, and several unnamed regions. For example, we identify a difference in the side chain orientation of Phe-144 in the β -sheet between the parasteric site and the linker loop. Phe-144 touches the tip of the clamp

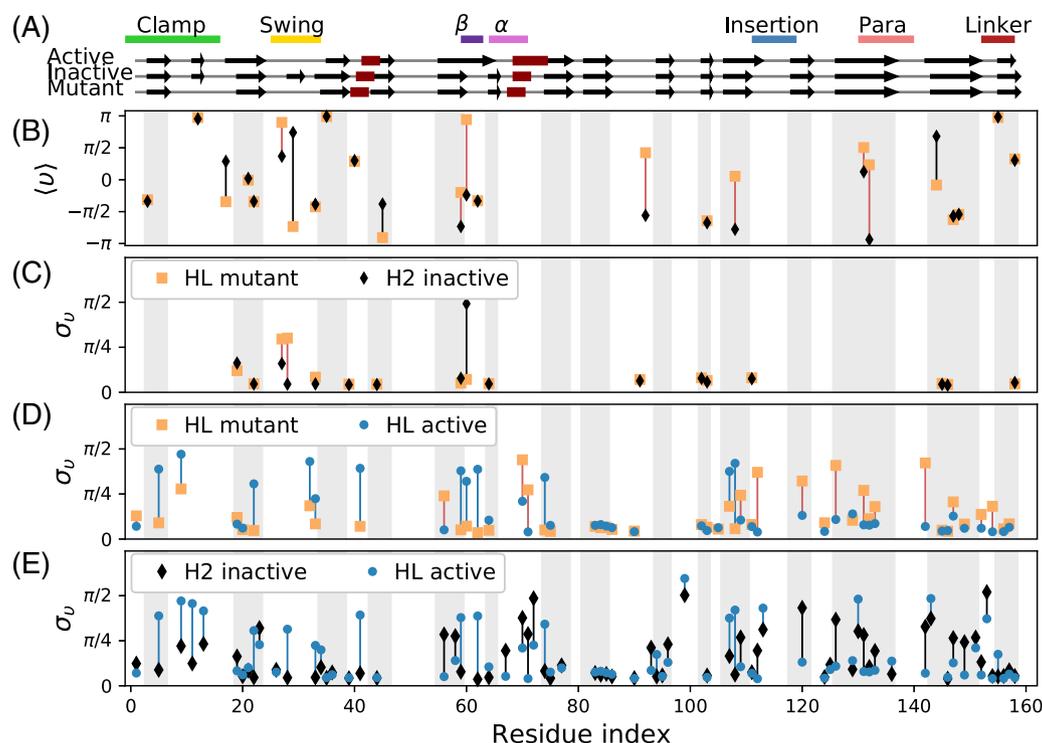


FIGURE 5 Differences in the side chain dynamics between HL mutant and H2 inactive affect the binding pocket, and both are different from HL active. A, Secondary structure of HL active (top), H2 inactive (middle), and HL mutant (bottom) with features. Side chain structure and dynamics were compared using custom dihedral angles ψ . B, At the β -bulge, the hinge of the clamp segment, the parasteric region, the swing loop, and several other sites, the average pseudo-dihedral angle was different between HL mutant (orange squares) and H2 inactive (black diamonds). C, Arg-60 in H2 inactive has larger fluctuations than Pro-60 in HL mutant. HL mutant also has larger fluctuations in the exposed swing loop. D and E, Comparisons of HL active (blue circles) against HL mutant and H2 inactive show differences in side chain dynamics beyond the allosteric pathway sites [Color figure can be viewed at wileyonlinelibrary.com]

segment in HL mutant but not in H2 inactive. We find large differences in the orientation of Phe-43, Tyr-108, and Phe-144 using both ψ and χ , but we only find a statistically significant difference at Arg-92 using ψ , which combines all five χ angles for Arg into one measure between the base and the tip (Figure 5B and Figure S14B).

We also find differences in the side chain orientation and dynamics between HL mutant and H2 inactive. As expected, the longer Arg-60 in H2 inactive has larger fluctuations than Pro-60 in HL mutant. We also find regions with dynamical differences beyond the site of the Arg-60-Pro mutation. Using ψ , the larger fluctuations in the swing loop of HL mutant (Figure 5C) may be attributed to the missing inter-domain contacts that are present in H2 inactive. Moreover, the swing loop of H2 inactive is structurally stabilized as a β -strand with the linker loop (Figure 1 and Figure S1). Using χ instead of ψ , we find HL mutant also has larger fluctuations within the clamp segment (-Figure S14C). This suggests that HL mutant may have a more flexible clamp segment, which is consistent with the larger backbone fluctuations.

As expected, we find that HL active is different from both HL mutant and H2 inactive in both side chain structure and dynamics at many positions across the protein (Figure 5D and Figure S14D and Table S1). In particular, many of these differences fall outside of the landmarks of the putative allosteric pathway,¹² which are frequently

the focus of attention and are primarily studied using backbone dynamics.^{20,22} Overall, the protein regions identified using χ and ψ are very similar.

3.4 | Robustness checking using a similarity matrix approach

In the previous section, we identified protein regions on HL mutant and H2 inactive with statistically significant differences in structure and dynamics by comparing groups of trajectories. Nonetheless, it remains difficult to quantify how different HL mutant is from H2 inactive relative to variations that may occur across replicates. To more fully assess the dissimilarity between the dynamical behavior of HL mutant and H2 inactive, we directly compare pairs of trajectories by calculating rank-based correlations and constructing similarity matrices (Figure 6). We use the Spearman correlation for angle fluctuation size and an equivalent correlation for circular variables for average angle orientation. The similarity matrices allow us to compare each pair of trajectories, complementing our previous comparisons across groups.

We define the reference similarity values as: the low similarity expected for HL active vs H2 inactive, very high similarities for

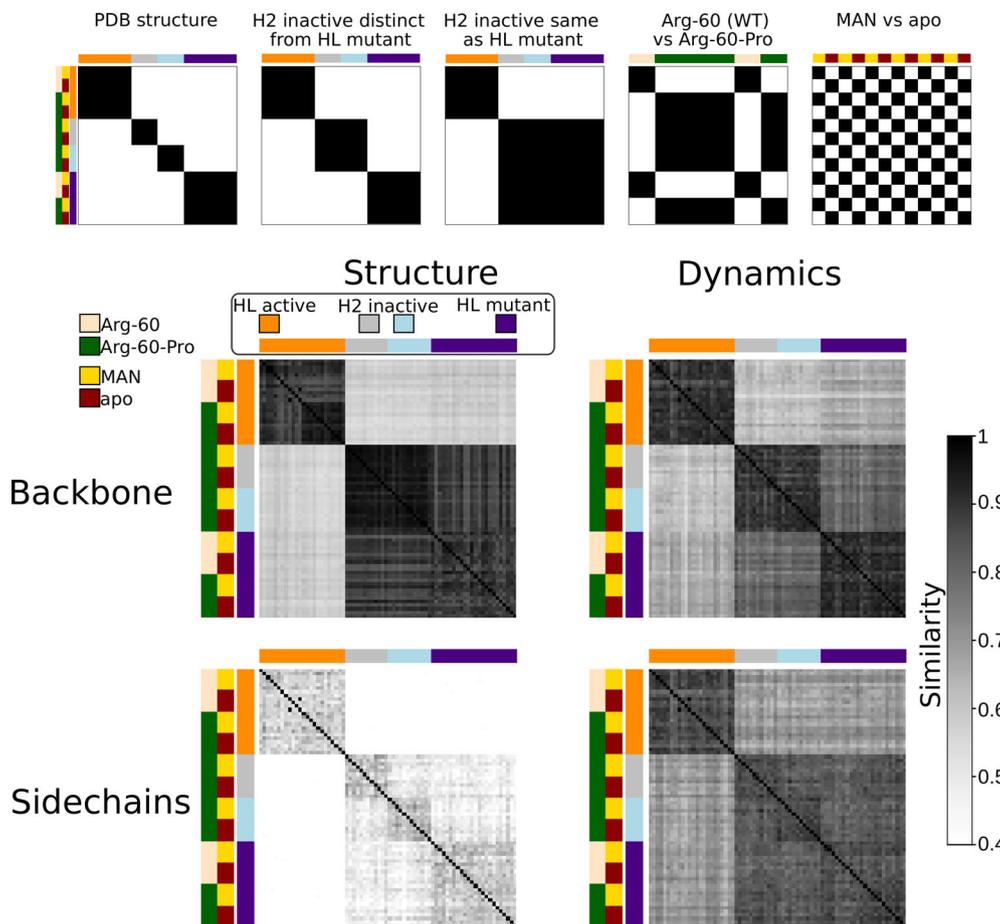


FIGURE 6 Differences between HL mutant and H2 inactive in comparisons of backbone dynamics and side chain orientation (top row, from left to right). Expected patterns based on the hypotheses that similarity depends on: the four PDB structures; the three protein states; grouping HL mutant with H2 inactive; the amino acid at position 60; and the presence of ligand. We constructed similarity matrices by comparing backbone dihedral (upper) and side chain pseudo-dihedral (lower) angles, using the average angle for structure (left) and the SD, for dynamics (right). The greyscale for similarity is the same for all four matrices. The system setup is described using a three-layer color code for the initial protein structure, mutation at position 60, and ligand. The purpose is to compare trajectories from different initial protein structures. HL active is distinct from both H2 inactive and HL mutant for all measures. For backbone dynamics, HL mutant is distinct from H2 inactive. For side chain structure, the two versions of H2 inactive are different, and HL mutant is distinct from H2 inactive [Color figure can be viewed at wileyonlinelibrary.com]

replicates, and high similarity for backbone comparisons of the two versions of H2 inactive. We use these reference values to compare the differences between HL mutant and H2 inactive.

As expected, we find that HL active is very different from both HL mutant and H2 inactive for all similarity matrices, and HL active replicates are relatively similar to each other. We find that the two H2 inactive structures are very similar to each other for backbone comparisons, but different for side chain comparisons. These differences are smaller than the ones between H2 inactive and HL active, but are slightly larger than the ones between replicates of each version of H2 inactive (Figures S15 and S16).

We find the replicates for HL mutant are similar to each other, except for the average backbone structure of HL mutant. One possible explanation is that the larger fluctuations found in HL mutant make the replicates less similar to each other. We find that having separate solvation, minimization, and equilibration preparations

accentuated the difference between technical replicates, compared to having replicate production runs following a single preparation. Thus, for backbone structure, we find the lower similarity values for the comparisons of HL mutant vs H2 inactive is about the same as those for replicates of HL mutant (Figure S15). In comparison, for backbone dynamics, we find clear differences between HL mutant and H2 inactive that are larger than the differences between replicates, yet smaller than the differences for HL active compared to both H2 inactive and HL mutant (Figure S16).

We also find that average side chain orientations had lower similarity overall for both the rotamer (χ) and the custom pseudo-dihedral (ν) angles. Despite overall lower similarities, we are still able to use the pseudo-dihedral angles to distinguish the two versions of H2 inactive, which have differences in the amino acid sequence at three positions in the lectin domain (see Table 1). We also identify differences between HL mutant and H2 inactive (Figure S15). For side chain

dynamics, we find slightly lower similarity values between HL mutant and H2 inactive relative to the replicates, which is comparable to the low similarity between the two versions of H2 inactive.

We find that the reduction in similarity for HL mutant and H2 inactive, relative to the high similarity between replicates, is smaller for side chain dynamics than for backbone dynamics (Figure S16). Across the four types of comparisons, we find that the differences primarily depend on whether the initial protein structure is HL mutant, H2 inactive, or HL active (Figure 6). We find larger differences due to the initial protein structure than those due to the amino acid at position 60 or to the presence of the ligand, which is consistent with our results in the previous section.

3.5 | Coupled backbone motions

We apply dPCA²³ to the backbone dynamics. To evaluate coupled backbone dynamics, or collective motion, we focus on the magnitude at each backbone dihedral by summing the eigenmodes weighted by the eigenvalues. Comparing HL mutant and H2 inactive for the first eigenmode, we find that HL mutant has more motion in the insertion loop and less motion at position 60 in the β -bulge segment. We find that after adding the second eigenmode, the difference in the amount of motion is larger (Figure 7). After adding the third eigenmode, we find that HL mutant has more motion in the linker loop in the interdomain region and the clamp segment of the binding pocket, while

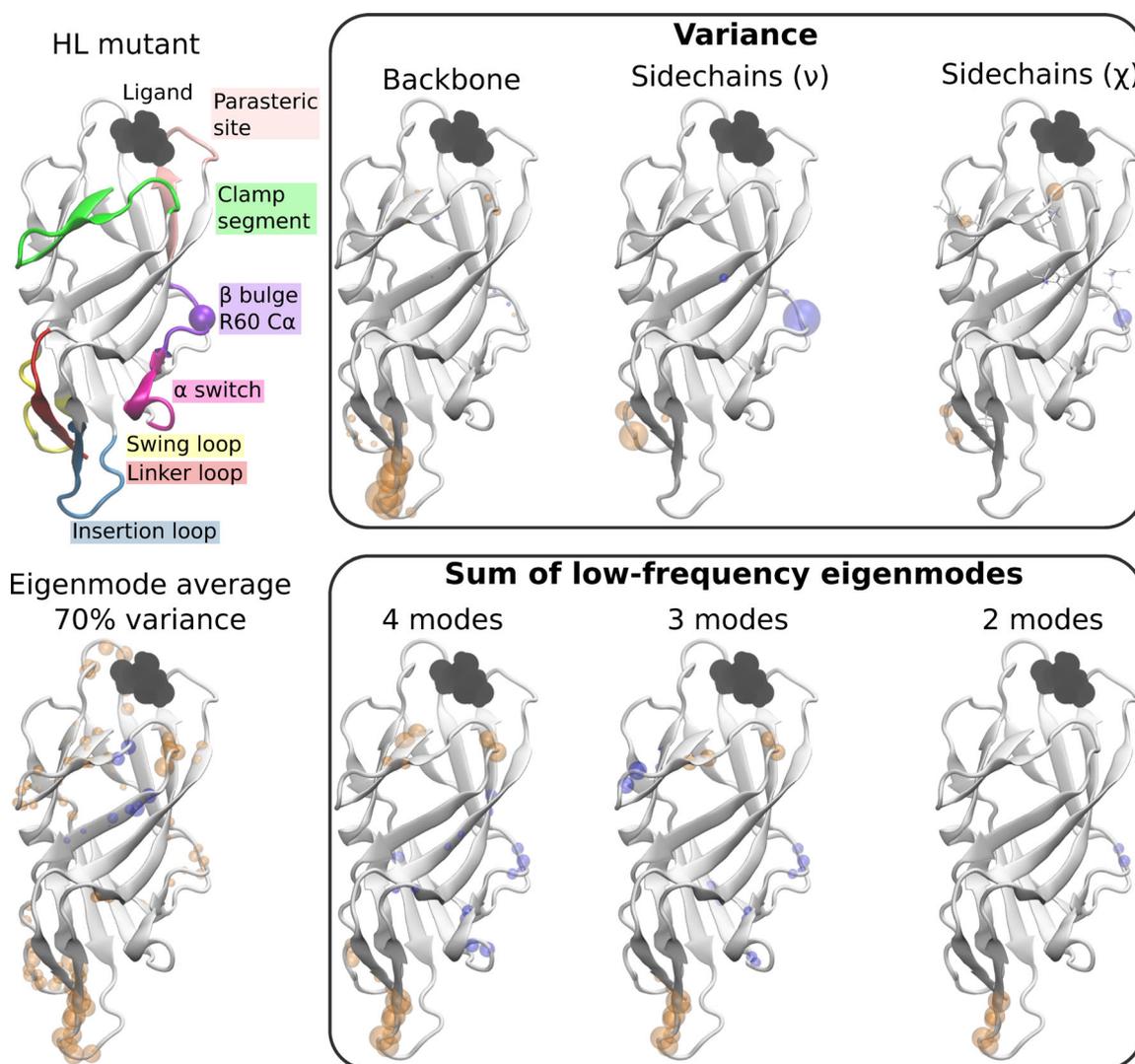


FIGURE 7 HL mutant has larger fluctuations in the interdomain region and clamp segment. (top) Backbone and side chain angles with larger fluctuations for HL mutant (orange) or H2 inactive (blue) are shown as spheres on the protein backbone. The landmarks are shown on HL mutant, and the Arg-60-Pro mutation is indicated with a purple sphere at C α . Fluctuation size, or dihedral variance, is shown as the sphere volume. For backbone dihedrals from Figure 3, ϕ is shown at N, while ψ is shown at the carbonyl C. Side chain pseudo-dihedrals (ψ) from Figure 5 are shown on C α . Side chain dihedrals χ are shown on the second atom defining the dihedral angle, which was usually C α . The side chain was drawn in the licorice representation for distal angles. (bottom) The backbone eigenmodes were compared two ways, using the eigen modes responsible for 70% of the variance and also using the low-frequency eigenmodes

there is less motion in the α -switch segment connected to the β -bulge segment. This is also magnified after adding the fourth eigenmode. We interpret these comparisons between HL mutant and H2 inactive to mean that there may be more coupled motion in HL mutant between the interdomain region and the clamp segment of the binding pocket. In contrast, we find decreased dynamics in HL mutant within the β -bulge/ α -switch segment, which may be due to Pro-60 limiting backbone dynamics compared to wild-type Arg-60 in H2 inactive.

In addition, we also calculate the unweighted average of the eigenvector magnitudes for the eigenmodes that explain 70% of the variance (Figure 7). Similar to the comparison of backbone dynamics, we find that HL mutant has larger fluctuations than H2 inactive, primarily in the insertion, swing, and linker loops of the interdomain region and the clamp loop of the binding pocket. However, we find that HL mutant had smaller fluctuations for the hinge region of the clamp segment. We identified similar regions by averaging eigenmodes that explain 50% to 80% of the variance. We interpret the similar regions identified by both methods to indicate that HL mutant is missing stabilizing interactions in the interdomain region that are present in H2 inactive.¹⁸ The more exposed HL mutant structure may lead to larger fluctuations that may be coupled to the binding pocket.

3.6 | Conclusion

To compare the protein regions with differences in backbone and side chain fluctuations, we show the regions identified with the variance as the volume of the sphere (Figure 7). Different regions are identified for backbone and side chain dynamics. For example, position 60 in the β -bulge and nearby regions showed a larger difference for Pro vs Arg in the side chain than in the backbone. The regions where HL mutant had larger backbone fluctuations than H2 inactive include the interdomain region and the hinge of the clamp segment. Larger fluctuations at the binding and allosteric sites may explain why HL mutant can undergo an allostery-like conformational change without the tensile force required to separate the two domains of H2 inactive.

4 | DISCUSSION

HL mutant (Arg-60-Pro) has been proposed as a "minimal model" of FimH allostery because the single-domain structure is more experimentally tractable than H2 inactive. Moreover, HL mutant undergoes an allostery-like conformational change upon binding mannoside ligand in contrast to the wild-type HL active.²² The HL mutant crystal structure was shown to match H2 inactive, except at the clamp segment of the binding pocket, and this difference was attributed to the flexibility of the clamp segment.²² However, the dynamics of HL mutant have not yet been studied experimentally, and this is the first computational characterization using MD simulations.

While HL mutant and H2 inactive overall share a similar structure, our analysis shows that differences in average backbone structure

exist in the interdomain loops, in addition to the clamp segment of the binding pocket. In these regions, HL mutant also has larger backbone fluctuations than H2 inactive in our 20 ns simulations, which we also observed in the simulations longer than 200 ns. Over longer simulations, many clamp segment conformations are accessed by both HL mutant and H2 inactive, but we only found some conformations in HL mutant. The floppier clamp segment in HL mutant may contribute to the almost 14 times lower binding rate compared to H2 inactive, and possibly the unfavorable loss of conformational entropy upon binding mannoside.²² We further used a technique from data science to show that these differences are robust compared to the variation among replicate simulations.

These structural and dynamical differences likely contribute to the more than seven times higher affinity to mannoside for HL mutant compared to H2 inactive,²² but their role in the mannoside-induced conformational change remains unknown. Rabbani et al²² has proposed studying additional mutations to HL mutant in order to prevent the conformational change. This would avoid the high-affinity state that strengthens bacterial adhesion during urination, which limits clearance and prolongs infections.^{11,15} Studies of single mutations suggest that the allosteric coupling to the binding pocket is stronger at position 60 than at the interdomain loops.^{12,20,22} This is further supported by our finding that HL mutant has larger fluctuations at the binding pocket and the interdomain loops than H2 inactive. Thus, our dynamics characterization further supports the need to determine the structure of the mannoside-bound HL mutant.²² In addition, greater backbone flexibility in the insertion and swing loops of the interdomain region may be useful for predicting additional mutations. Mutations that stabilize the interdomain region may inhibit the conformational change upon mannoside-binding. Comparing the dPCA eigenmodes for HL mutant and H2 inactive suggest these dynamical differences are related to the allosteric coupling between the interdomain region and the clamp segment. Longer simulations may provide additional insight into the mannoside-induced conformational change.

In addition to comparing HL mutant and H2 inactive, we also compared these structures to HL active. Interlandi and Thomas²⁰ found that differences between inactive and active FimH were isolated to the six sites defining the putative allosteric pathway in their study of backbone structure and dynamics using $C\alpha$ -RMSD. In a later study, Rabbani et al²² focused on a subset of these sites when comparing inactive and active FimH using $C\alpha$ -RMSD. However, the alignment step in $C\alpha$ -RMSD calculations results in broader regions of interest since angular fluctuations displace distal elements.^{23,30,31} Because we use an internal coordinate system of dihedral angles, we identify more localized differences. Thus, our backbone comparisons identify large differences at specific angles along the allosteric pathway.

We then extended our analyses of dihedral angles in the backbone to the side chains (using both χ and ν) in order to compare the structure and dynamics. We find that HL active is dynamically different from HL mutant and H2 inactive at multiple regions beyond the putative allosteric pathway identified from the backbone. Further

characterization of these sites may be useful for studying the role of side chain dynamics in the allosteric mechanism in more detail. One goal may be to identify mutations that could be combined with Arg-60-Pro to produce a mutant locked in the inactive state despite the addition of mannoside ligand, a goal proposed by Rabbani et al.²² Thus, our comparisons of HL mutant, H2 inactive, and HL active demonstrate that a bottom-up approach can identify differences in structure and dynamics that are not restricted to the putative allosteric pathway. Beyond our analyses of a “minimal model” for FimH allostery, our bottom-up approach and use of techniques from data science may be applicable to comparing the structure and dynamics of other proteins.

ACKNOWLEDGMENTS

We are extremely grateful to Kerim Dansuk for his helpful discussions and to Martha Dunbar for her help with the MD simulations. Jenny Liu thanks the Paul and Daisy Soros Fellowship, the Achievement Rewards for College Scientists (ARCS) Illinois chapter, the Northwestern Quest High Performance Computing Cluster, and the National Institute of General Medical Sciences T32GM008152. This project was also supported by the Office of Naval Research N00014163175 and N000141512701 (Sinan Keten), as well as by the National Science Foundation 1764421-01 and the Simons Foundation 597491-01 (Luis Amaral).

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How to cite this article: Liu J, Amaral LAN, Keten S. Conformational stability of the bacterial adhesin, FimH, with an inactivating mutation. *Proteins*. 2021;89:276-288. <https://doi.org/10.1002/prot.26013>