DI
GA
TN
BI

MOLECULAR MECHANISMS OF INFECTION BY CHLAMYDIA

Allocation: GLCPC/1,107 Knh
PI: Juan Perilla[1]

[1]University of Delaware

EXECUTIVE SUMMARY

The most common sexually transmitted bacterium, *Chlamydia trachomatis*, is an intracellular pathogen responsible for a swath of debilitating conditions including blinding trachoma, which afflicts roughly 84 million persons worldwide and has led to total blindness in eight million people. Despite what is well understood about *C. trachomatis*, important aspects remain mysterious: particularly, how this organism accomplishes membrane fusion. Postinfection, cellular inclusions undergo this critical step whereby separate inclusions are fused together inside the host cell before maturation and expulsion. In 2016, it was shown that the virulence of *C. trachomatis* is wholly dependent on this stage of its lifecycle, and completion thereof was traced to one protein known as IncA. With the structure of IncA provided by experimental collaborators and with access to Blue Waters, the PI performed studies that could be accomplished with no other scientific instrument: simulating the dynamics of IncA over microseconds to uncover key structural and conformational factors of this important cell machinery.

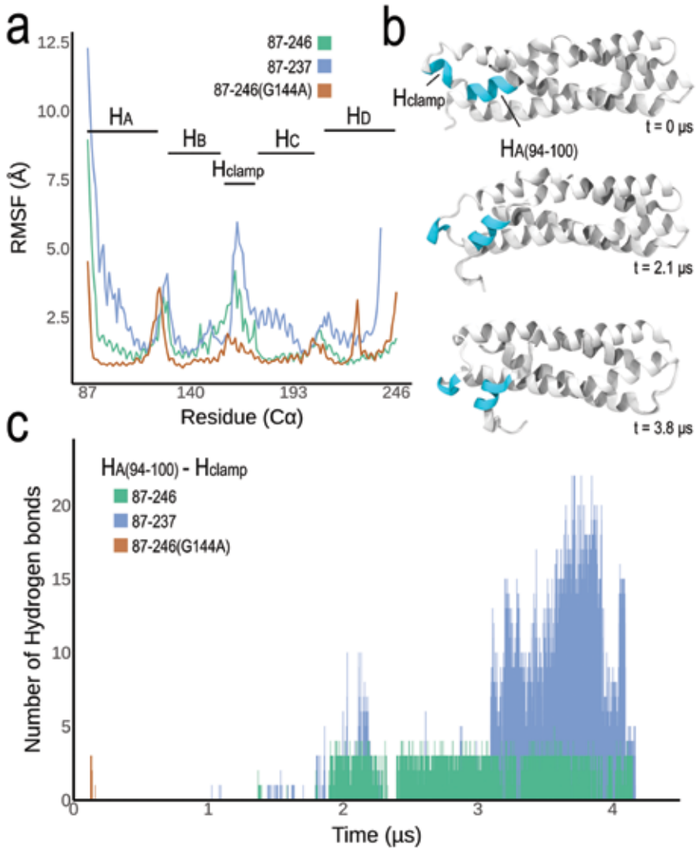

Figure 1: Dynamic characterization of Chlamydia's inclusion body protein (IncA). Results of molecular dynamics study of wild-type IncA ($IncA_{87–246}$) and two variants, a truncated mutant ($IncA_{87–237}$) and a point-mutant ($IncA_{87–246(G144A)}$). a) Root-mean-square fluctuation (RMSF) of each residue in the three IncA variants tested. b) Snapshots of $IncA_{87–237}$ trajectory, showing large-scale conformational change and interactions between the H_{clamp} region and a section of helix A ($H_{A(94–100)}$). c) Trace of the hydrogen bonds between H_{clamp} and $H_{A(94–100)}$ for each of the three constructs over time.

RESEARCH CHALLENGE

Using a high-resolution crystal structure of wild-type IncA provided by experimental collaborators—$IncA_{87–246}$—the PI aimed to assess if the coordinates represented a thermodynamic minimum or a metastable intermediate capable of reorganizing into a fusion-competent state. For comparison, two other constructs identified experimentally were tested: a truncated mutant $IncA_{87–237}$ and a point-mutant $IncA_{87–246(G144A)}$. Without understanding the dynamics of *C. trachomatis*' key fusion protein, and what conformation might be assumed to initiate fusion, no conclusions leading to drug targets may be formed. In a world where classic antibiotic treatments are leading to super-resistant pathogens, the need for new drug targets cannot be overstated.

METHODS & CODES

The simulations used the NAMD molecular dynamics engine, which is optimized to take full advantage of high-performance distributed-memory architectures. Using NAMD, the PI sampled all three systems for an aggregate of twelve microseconds. These trajectories provided invaluable insights into the dynamics of IncA variants and will be used in further in-depth analyses. Blue Waters not only made collecting these data practical but enabled the researcher to efficiently gather evidence identifying key structural features of IncA that help regulate its overall structure and flexibility. Similarly, parallel I/O capabilities of Blue Waters' Lustre file system made postprocessing of the obtained trajectories feasible.

RESULTS & IMPACT

The simulations showed that the crystal structure of $IncA_{87–246}$ represents a thermodynamic minimum that is not likely to unfold to initiate fusion. Analysis of obtained trajectories showed that the C-terminal domain of the fusion peptide is essential to regulating global structural flexibility, particularly in the region known as the H_{clamp}, which was shown in simulations to have a much higher mobility than the rest of the structure (Fig. 1a). Importantly, because IncA is thought to recognize a conspecific or separate regulatory protein before fusion, the high mobility of H_{clamp} hints at its significance in the fusion process. Truncated mutant $IncA_{87–237}$—which does not have C-terminal domain—showed a wildly fluctuating H_{clamp} region as well as a poorly maintained global structure (Fig. 1a, 1b), explaining the experimentally observed inability of this truncated construct to even initiate fusion. Additionally, when the distance between the C-terminal domain and H_{clamp} is narrowed down, as was tested with the point-mutant $IncA_{87–246(G144A)}$, overall structural stability increases. Increased stability, observed as decreased root-mean-square fluctuation (RMSF), is most apparent in the H_{clamp} region of $IncA_{87–246(G144A)}$ (Fig. 1a). Consequently, this prevented the H_{clamp} from forming any lasting hydrogen bonds with helix A, which were observed in the other constructs (Fig. 1c). This finding supports the idea that the H_{clamp} region and its interactions with the C-terminal domain are critical determinants of structure and dynamics. Moreover, it provides insights into why the G144A construct could not form a homodimer in solution in experiments.

WHY BLUE WATERS

NAMD, the molecular dynamics engine used in this research, is well suited for distributed-memory parallel architectures and is particularly well-optimized on Blue Waters. These qualities make NAMD an ideal choice for the type of calculations the PI does in his research. Together, NAMD and Blue Waters enabled the researcher to obtain new results quickly and efficiently, whereas Blue Waters' Lustre file system enabled the high-throughput postprocessing of the obtained results.

PUBLICATIONS & DATA SETS

G. Cingolani *et al.*, "Structural basis for the homotypic fusion of chlamydial inclusions by the SNARE-like protein IncA," *Nat. Commun.*, no. 10, p. 2747, Jun. 2019.

Protein data bank entries: 6E7E, 6E6A. [Online]. Available: https://www.rcsb.org/structure/6E7E and https://www.rcsb.org/structure/6E6A

A. Bryer, J. A. Hadden–Perilla, J. E. Stone, and J. R. Perilla, "High-performance analysis of biomolecular containers to measure small-molecule transport, trans bilayer lipid diffusion, and protein cavities," *J. Chem. Inf. Model.*, Sep. 2019, doi: 10.1021/acs.jcim.9b00324.