

Direct Measurement of Intermolecular Mechanical Force for Nonspecific Interactions between Small Molecules

Shankar Pandey, Yuan Xiang, Dirk Friedrich, Yongsheng Leng, and Hanbin Mao*



Cite This: <https://doi.org/10.1021/acs.jpclett.1c03142>



Read Online

ACCESS |



Metrics & More

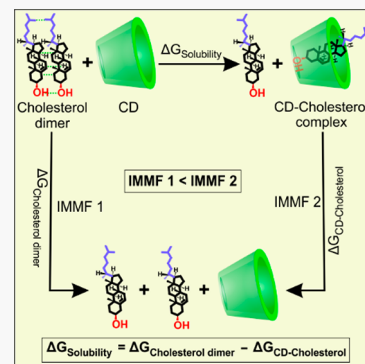


Article Recommendations



Supporting Information

ABSTRACT: Mechanical force can evaluate intramolecular interactions in macromolecules. Because of the rapid motion of small molecules, it is extremely challenging to measure mechanical forces of nonspecific intermolecular interactions. Here, we used optical tweezers to directly examine the intermolecular mechanical force (IMMF) of nonspecific interactions between two cholesterols. We found that IMMFs of dimeric cholesterol complexes were dependent on the orientation of the interaction. The surprisingly high IMMF in cholesterol dimers (~ 30 pN) is comparable to the mechanical stability of DNA secondary structures. Using Hess-like cycles, we quantified that changes in free energy of solubilizing cholesterol ($\Delta G_{\text{solubility}}$) by β -cyclodextrin (β CD) and methylated β CD (Me- β CD) were as low as -16 and -27 kcal/mol, respectively. Compared to the $\Delta G_{\text{solubility}}$ of cholesterols in water (5.1 kcal/mol), these values indicated that cyclodextrins can easily solubilize cholesterols. Our results demonstrated that the IMMF can serve as a generic and multipurpose variable to dissect nonspecific intermolecular interactions among small molecules into orientational components.



When two molecules interact with each other, intermolecular force (IMF)² is used to evaluate the strength of the interaction. IMF can be quantified by chemical energy, which offers a convenient way to directly compare different types of IMF. However, as a thermodynamic variable, chemical energy is pathway independent and cannot probe the directionality of molecular interactions. Both pathway and directionality, however, are essential factors to render a full picture of kinetic intermolecular interactions. For example, folding or unfolding energy trajectories of proteins are dependent on the direction of a process in which intermediates with different local energy minima are located.

Mechanical force is a kinetic variable that is dependent on both pathway and direction of a process.^{3,4} Recent technical advances on the application and measurement of piconewton forces⁵ are instrumental to the development of interdisciplinary fields, such as mechanobiology^{6–8} and mechanochemistry,^{9,10} in which mechanical forces participate in biology and chemistry processes, respectively. Many mechanical properties of polymers and biomacromolecules, such as proteins and nucleic acids, have been portrayed by single molecule force spectroscopy (SMFS)^{11–13} in optical tweezers, magnetic tweezers, or AFM instrument.¹¹ However, almost all measurements are focused on the intramolecular interactions inside a particular molecule. It is rare to follow intermolecular interactions from a mechanical perspective.¹⁴ It is even more challenging to investigate the mechanochemistry between two small molecules which have large freedom of motion. One reason for this difficulty is the low throughput of the SMFS. It is difficult to repeatedly probe the interaction between two small molecules. Upon dissociation of the two interacting

molecules, it is time-consuming to locate and track the next pair of molecules for measurement. In addition, once two molecules are forced apart, it is almost impossible to evaluate the same molecule pair again, which increases the measurement noise due to the stochastic variation in consecutive sampling practice.

Our recent success in the investigation of individual host–guest pairs has provided a solution to increase the throughput of SMFS and to address the issue of the variation in stochastic sampling. This has been achieved by introducing a linker between two interacting molecules so that the two components do not escape to the surroundings after mechanical dissociation. As a result, the same molecular pair can repeatedly form an interacting complex for the next round of measurement on a single-molecule platform.

In this work, this strategy has been catapulted to probe the intermolecular mechanical force (IMMF)¹⁵ during the interaction of two cholesterol molecules. As a small molecule, cholesterol is an essential constituent of cell membrane and a precursor for many important biomolecules such as corticosteroids hormone, sex hormones, and vitamin D.^{16,17} Cholesterol contains a large hydrophobic motif (central planar fused rings and a flexible hydrocarbon tail, see Figure 1) and a

Received: September 24, 2021

Accepted: November 11, 2021



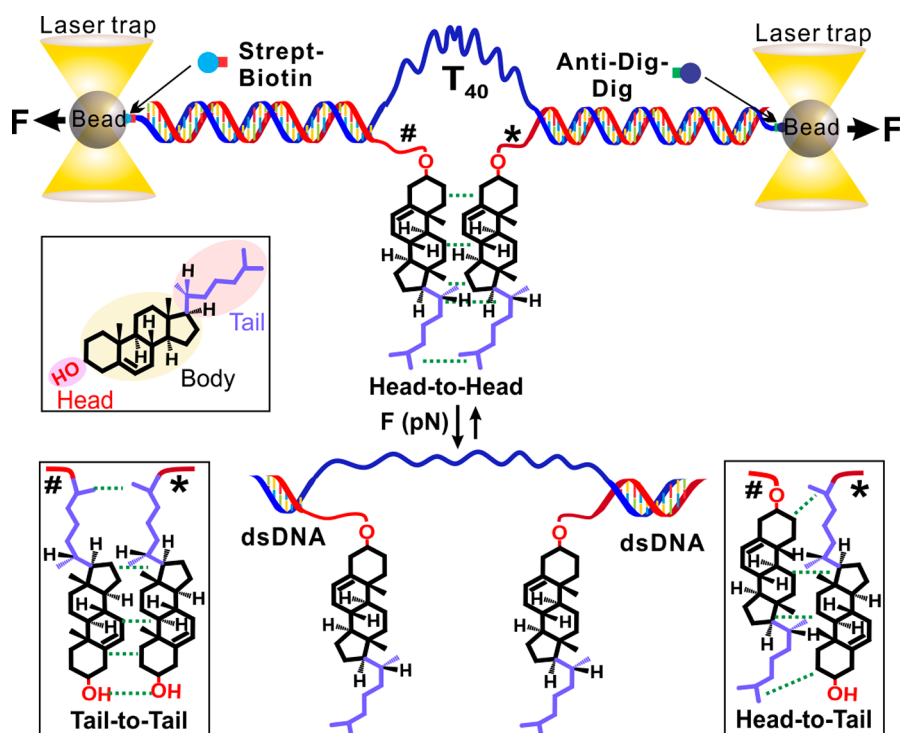


Figure 1. Schematic diagram for single molecular mechanical unfolding of cholesterol dimers with different orientations (bottom insets). The self-assembled construct is sandwiched between two dsDNA handles, which are tethered between two optically trapped polystyrene beads by streptavidin (strep)/biotin and digoxigenin (dig)/antidigoxigenin (anti-dig) interactions. Cholesterol structure is shown in the top inset.

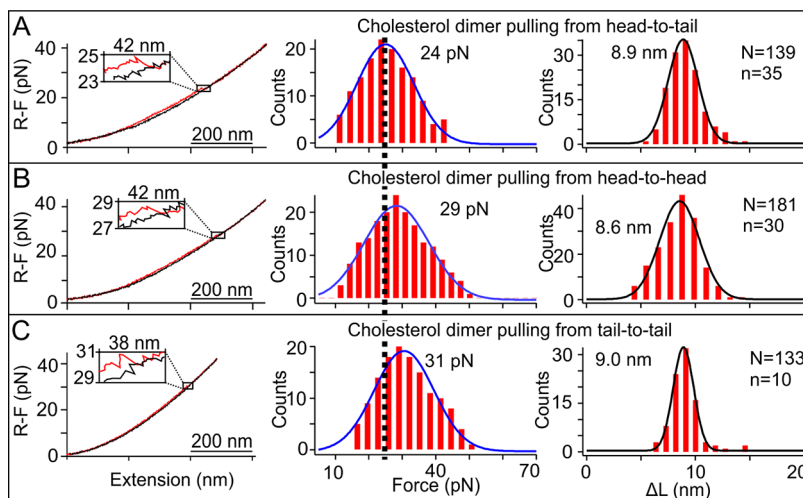


Figure 2. Mechanical dissociation of cholesterol dimers with different orientations. A typical stretching (red) and relaxing (black) force–extension curve, rupture force (IMMF) histogram, and change-in-contour-length (ΔL) histogram for a cholesterol dimer pulled from (A) head-to-tail direction, (B) head-to-head direction, and (C) tail-to-tail direction. The dotted line in the middle panel depicts 24 pN. N and n represent the total numbers of data points and molecules, respectively, for each experiment. The histograms are fitted by Gaussian curves. See SI Figure S15 for fitting based on the equation proposed by Dudko.¹

small hydrophilic motif (the hydroxyl headgroup). The central hydrophobic rings have two faces: a flat and smooth face that has no substituents (the α face) and a rough face with methyl groups (the β face). Owing to its structural complexity, cholesterol may form a dimer in which two α faces interact with each other.¹⁸ Cholesterol dimers play a fundamental role in many functions such as those that occur in the cell membrane.^{19–24} When cholesterol molecules aggregate, the dimer is the simplest unit based on which the cholesterol

crystal may form in artery plaques, which can cause atherosclerosis conditions.²⁵

Given the importance of cholesterol molecules in various physiological and pathological processes, here we directly measured the IMMF between two interacting cholesterol molecules (dimer) in aqueous buffer by a single-molecule mechanochemical assay in an optical tweezers instrument (Figure 1). Unlike the IMF which is a thermodynamic variable, IMMF provides a mechanical information between cholesterol molecules. This produces an unprecedented perspective on the kinetic aspect

of small molecule interactions. Surprisingly, we found that the IMMFs between two nonspecifically interacting cholesterol molecules are comparable to the unfolding force of DNA tetraplexes,^{26–28} while higher than the unzipping force of DNA duplexes.²⁹ By uniquely manipulating orientations of individual cholesterol molecules, we further evaluated the mechanical anisotropy in the interaction of the cholesterol dimers. Finally, the changes in free energy of dissociation of cholesterol dimers were compared with those of cholesterol–cyclodextrin complexes. This allowed us to determine, for the first time, the change in free energy of solubility of cholesterol in β -cyclodextrin (β CD) or methylated β CD (Me- β CD), two compounds widely used to extract cholesterol from cell membranes or artery plaques. This IMMF measurement therefore provides convenient and direct quantification of the fundamental nonspecific intermolecular processes among small molecules.

Based on different cholesterol alignments, a cholesterol dimer can have three possible orientations: head-to-head, head-to-tail, and tail-to-tail (Figure 1). To evaluate the IMMF of cholesterol dimers with these three orientations, we modified cholesterol either at the alkyl tail with an alkyne group or the hydroxyl head extended with an azide group. The modified cholesterol was conjugated to a single-stranded DNA by copper(I) catalyzed cycloaddition reaction (Figures S1–S11).^{15,30} The conjugate was finally incorporated in our single-molecule platform by self-assembly of DNA strands (Figure 1). The platform contained a polythymidine (T_{40}) ssDNA linker which helps keep two cholesterol in close proximity even after the dimer is dissociated. This linker facilitated repeated intercholesterol association and dissociation events. The entire molecular setup was anchored to two optically trapped beads by using affinity linkages of streptavidin/biotin and digoxigenin/antibody, respectively (Figure 1).

Using this setup, we first evaluated the mechanical stability of the cholesterol dimer with a misaligned orientation. This was achieved by pulling one cholesterol from the hydroxy head and the other cholesterol from the alkyl tail end (Figure 1, inset). Mechanical dissociation and association of cholesterol dimers were carried out by moving one trapped bead away from the other. This process increased tension in the DNA tether, leading to the dissociation of the cholesterol dimer, which was manifested by a sudden rupture event in a force–extension (F – X) curve (Figure 2A, left). After mechanical breaking of the cholesterol dimer, one bead was moved toward another, relaxing the tension in the DNA tether. Since two dissociated cholesterol molecules were still in proximity due to the presence of the T_{40} linker, the cholesterol pair would interact again for another cycle of dissociation–association experiment. From these repetitive force ramping experiments, we constructed a rupture force histogram (Figure 2A, middle), which revealed an average IMMF of 24 pN for this type of cholesterol dimer orientation. After the cholesterol dimer is dissociated at 24 pN, the T_{40} linker will be fully stretched, which leads to an extended molecular construct. By calculating the change in the contour length (ΔL) due to this extension (Figure 2A, right), we found the ΔL value was close to that expected from the release of the T_{40} linker after breakage of the cholesterol dimer (see SI and Figure S12 for detailed calculation). This suggested that observed rupture events were due to the dissociation of cholesterol dimers at a specific IMMF.

To confirm that the observed IMMF was indeed due to the dissociation of two cholesterol, we performed two control experiments. In the first control, we incorporated only one tail-modified cholesterol in the single-molecule platform. In the second control, we integrated a head-modified cholesterol in our DNA platform. In each control, we did not observe any rupture event, suggesting there should be no interaction between cholesterol and the DNA template used in the IMMF measurement platform. Thereby, the rupture events observed in the F – X curves in Figure 2 were indeed dissociations of cholesterol dimers.

Next, we changed the association/dissociation of the cholesterol dimer from the head–tail to the head–head orientation. This was achieved by attaching both cholesterol to the IMMF measurement platform via their hydroxy heads (head-to-head) (Figure 1). The rupture force histogram showed an IMMF of 29 pN (Figure 2B), a value higher than the head-to-tail pulling (24 pN). This can be attributed to a better match in molecular shape between two cholesterol for this head-to-head orientation, which increased the interfacial area of the cholesterol dimer associated via hydrophobic interactions. To test this hypothesis, we prepared another construct in which cholesterol dimers are associated and dissociated from the alkyl tails (tail-to-tail) (Figure 1). We found that the average IMMF for this orientation was 31 pN (Figure 2C). This IMMF was again higher than that of the head-to-tail orientation, suggesting that tail-to-tail orientation also had more contact area between two interacting cholesterol molecules than that in the head-to-tail orientation. Such an orientational effect is of high clinical importance since interaction strength among cholesterol molecules can decide how well cholesterol crystals in the artery plaques are dissolved by chemical agents.

To understand the molecular mechanisms of the cholesterol–cholesterol interactions, we performed molecular dynamic (MD) simulations (see SI for details) on the mechanical dissociation of the three different dimer conformations studied in single-molecule force measurements. All simulations of dimer dissociation were set to be along either in-plane or out-of-plane of the cholesterol α faces. However, the final rupture conformation shows the strong tendency of dimer dissociation along the in-plane trajectory, except for the misaligned case, signaling this is most likely the lowest energy pathway of the dimer rupture. MD simulations gave two different values of dissociation force, ~ 97 pN and ~ 82 pN for the head-to-head/tail-to-tail and the head-to-tail orientations, respectively (Figure S16 for the in-plane and Figure S17 for the out-of-plane dissociations). In Figure S16, we showed that the head-to-head/tail-to-tail dissociation forces were larger than that of head-to-tail misaligned contact conformation with statistical significance. These results were consistent with the trend of force histograms shown in Figure 2. However, because of the long flexible DNA handles connected to cholesterol dimers, we could not completely exclude the possible misaligned orientations. But it was clear that the majority of dimer conformations upon association followed the aligned orientation. It is noteworthy that MD simulation gave higher force values due to the requirement of a much higher pulling rate (0.002 Å/ps here) used in the field.^{15,31–33}

To quantify the contact area in the cholesterol dimer, we counted the number of C and O atoms within 5 Å between the two cholesterol molecules (blue and orange atoms in Figure S17). For the head-to-head/tail-to-tail contact, we counted 40

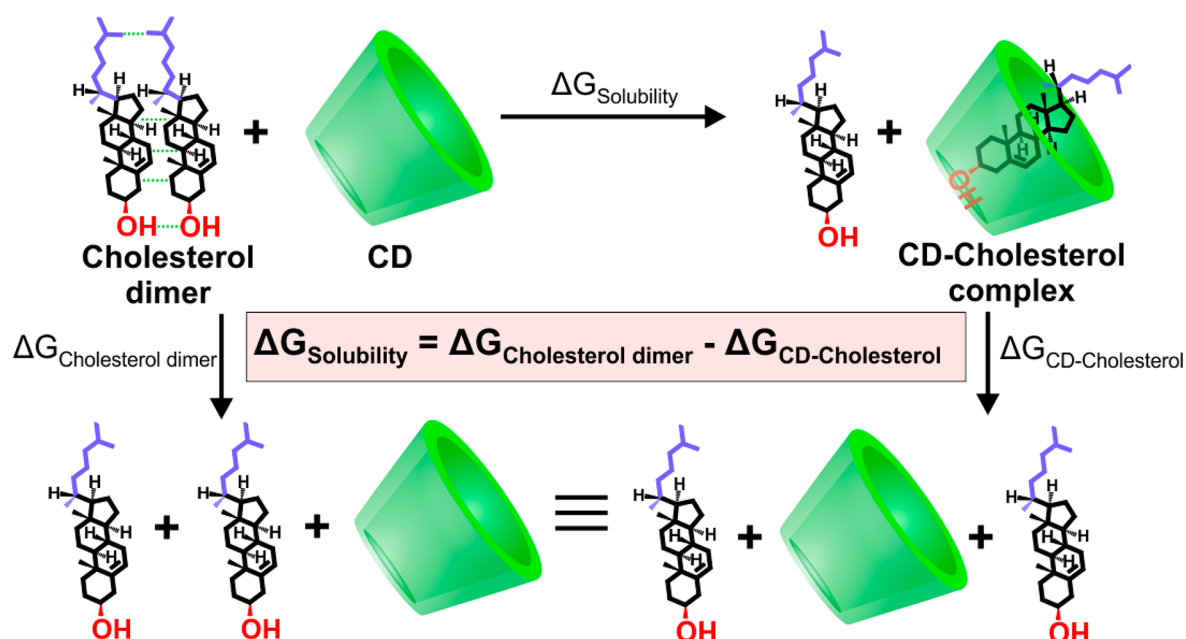


Figure 3. A schematic diagram to determine the change in free energy of solubilization ($\Delta G_{\text{solubility}}$) of cholesterol in the Me- β CD or β CD molecule (green barrel) by using a process analogous to the Hess cycle.

215 ± 2 atoms, while in the head-to-tail, we only found 33 ± 4
 216 atoms in their contact plane. This result signifies that the
 217 contact area in the head-to-tail dimer is smaller than that in the
 218 head-to-head or tail-to-tail dimer, while the latter two clearly
 219 have more stable α - α face-to-face contact conformation
 220 (Figure S16A). Such a finding explains why the head-to-tail
 221 contact often cannot maintain the face-to-face separation (flip/
 222 rolling separation) during the pulling, leading to a lower
 223 dissociation force which is in accordance with the experimental
 224 findings.

225 After measurements of IMMFS in all three cholesterol
 226 dimers with different orientations, we further evaluated the
 227 stability of cholesterol dimers from free energy perspectives.
 228 To this end, we calculated the dissociation works of these three
 229 cholesterol dimers, from which changes in free energy of
 230 dissociation ($\Delta G_{\text{dissociation}}$) were retrieved by using Jarzynski
 231 nonequilibrium equation (see Supporting Information).^{27,34,35}
 232 We found $\Delta G_{\text{dissociation}}$ equal to 9.2 ± 1.6 , 11.6 ± 0.7 , and 13.4
 233 ± 0.9 kcal/mol, respectively, for the head-to-tail, head-to-head,
 234 and tail-to-tail orientations (see Table S2 and Figure S13 for
 235 the dissociation work histograms). It is noteworthy that each
 236 $\Delta G_{\text{dissociation}}$ represents the combination of the ΔG to break a
 237 cholesterol dimer and that to stretch the T40 loop (Figure 1),
 238 the latter of which remains the same for all intermolecular
 239 systems using the T40 loop. It is clear that the thermodynamic
 240 stability of the tail-to-tail cholesterol dimer is significantly
 241 different from that of the head-to-tail or the head-to-head
 242 dimers at the confidence level of 99.9% (Student's t test),
 243 which is consistent with different intercholesterol contact areas
 244 revealed by MD simulations above.

245 With this set of $\Delta G_{\text{dissociation}}$ for different cholesterol dimers,
 246 we proceeded to calculate the change in free energy of
 247 solubilization ($\Delta G_{\text{solubility}}$) of cholesterol in β -cyclodextrins (β -
 248 CD) or methylated β -CD, two well-known biomolecules for
 249 cholesterol extractions.^{36–39} By using the Hess-like cycle
 250 (Figure 3), $\Delta G_{\text{solubility}}$ can be calculated in eq 1,

$$\Delta G_{\text{solubility}} = \Delta G_{\text{cholesterol dimer}} - \Delta G_{\text{host-cholesterol}} \quad (1)$$

where $\Delta G_{\text{cholesterol dimer}}$ is the change in free energy of
 dissociating cholesterol dimers (either the head–tail, head–
 head, or tail–tail orientation) and $\Delta G_{\text{host-cholesterol}}$ is the free
 energy change to dissociate host–cholesterol complex (host:
 Me- β CD or β CD). By obtaining the $\Delta G_{\text{host-cholesterol}}$ from our
 recent work (Figure 4A and Figure S14, here $\Delta G_{\text{host-cholesterol}}$
 contains the ΔG to break a host-cholesterol complex and to
 stretch the T40 loop, the latter of which remains the same for
 all the systems using the T40 loops),¹⁵ we found that all
 $\Delta G_{\text{solubility}}$ values of cholesterol inside the β CDs are negative.
 In contrast, the $\Delta G_{\text{solubility}}$ of cholesterol in water was found to
 be positive in the literature (5.1 kcal/mol).⁴⁰ These data
 indicate that solubilization of cholesterols in β CD or Me- β CD
 is a spontaneous process. In addition, the $\Delta G_{\text{solubility}}$ of
 cholesterol in Me- β CD (-16.05 kcal/mol) is more negative
 than β CD (-8.9 kcal/mol), which is in full agreement with the
 finding that Me- β CD is more effective with respect to β CD to
 solubilize cholesterol due to its increased size of the
 hydrophobic cavity.¹⁵ Similarly, we discovered that $\Delta G_{\text{solubility}}$
 of the cholesterol in misaligned cholesterol dimers is more
 negative than that in aligned cholesterol dimers (Figure 4B).
 This result indicated that fully aligned cholesterol molecules
 are more difficult to dissolve, which is in agreement with
 increased IMMFS for these dimers. Finally, when cholesterols
 enter cyclodextrins with a head(hydroxyl)-on fashion, the
 cholesterol solubility becomes larger (Figure 4B). This result
 has been rationalized by simulations in which stronger
 interactions persist between the hydroxy head of a cholesterol
 and the wide opening of the cyclodextrin.¹⁵

Bulk methods such as isothermal titration calorimetry (ITC)
 and surface plasmon resonance (SPR) have been used to
 investigate affinity complexes of biological molecules with
 defined interaction orientations. However, in small molecules
 such as cholesterols discussed here, the interactions are often
 nonspecific without a predominant binding mode. For these
 cases, the ensemble average approaches can only give an
 average description of overall nonspecific interactions. Our
 IMMFS allows to precisely dissect specific interactions among

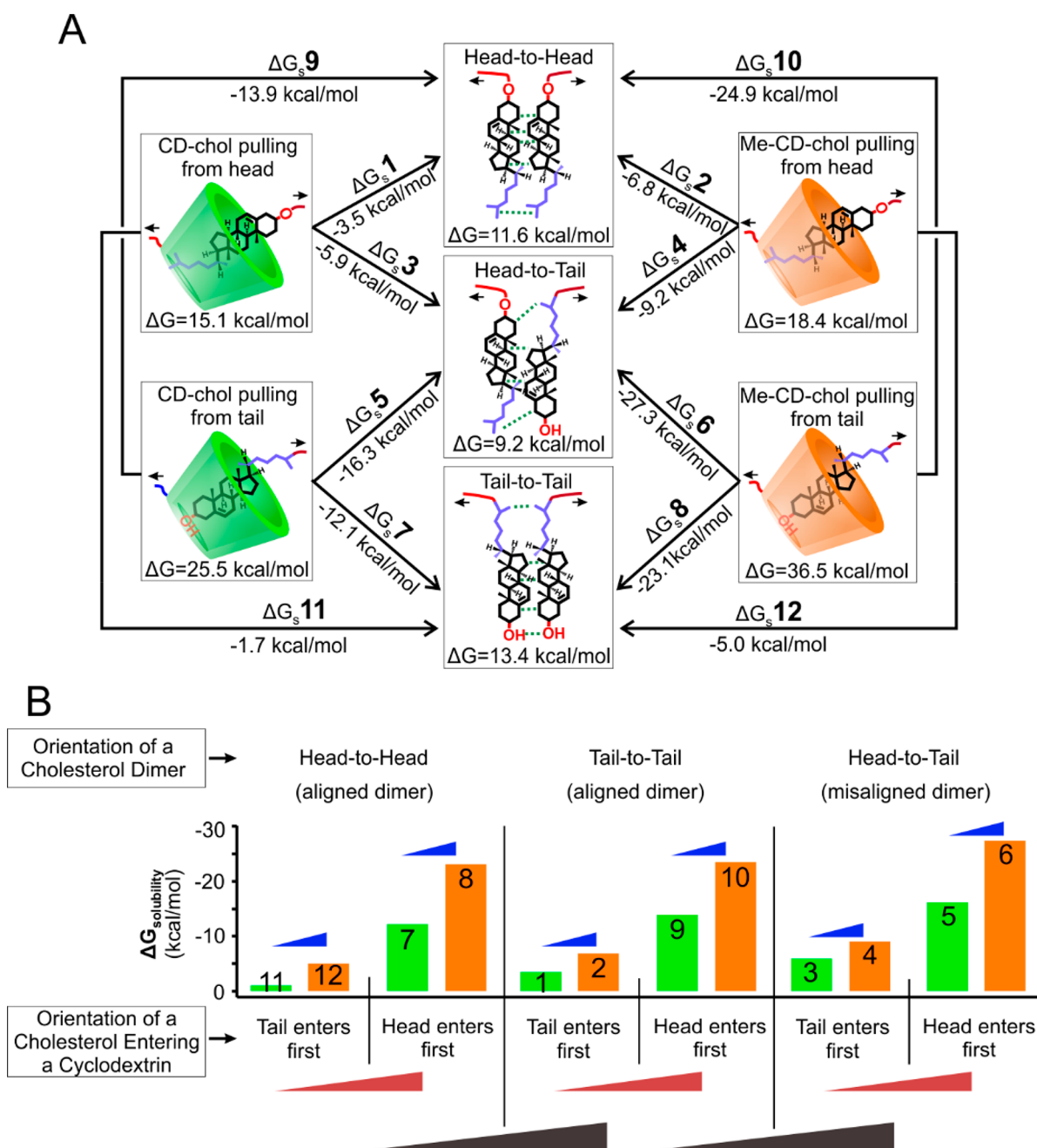


Figure 4. Summary diagram for changes in the free energy of solubilization of cholesterols in β CD or Me- β CD with different interaction directions. (A) Left: Change in free energy (ΔG) associated with the dissociation of β CD-cholesterol complex pulled from the head (top) and tail (bottom) of the cholesterol (chol). Middle: Change in free energy (ΔG) associated with the dissociation of the cholesterol dimer pulled from the head-to-head (top), head-to-tail (middle), and tail-to-tail (bottom) orientations. Right: Change in free energy (ΔG) associated with the dissociation of Me- β CD-cholesterol complex pulled from the head (top) and the tail (bottom) of the cholesterol. (B) Changes in free energy of solubilization ($\Delta G_{\text{solubility}}$) of cholesterols are dependent on the disruption orientations of cholesterol-cholesterol dimers (top) as well as on the orientation of a cholesterol (bottom notation) entering either β -CD (green) or Me- β -CD (brown). Slanted arrowheads depict increasing magnitudes of $\Delta G_{\text{solubility}}$.

many nonspecific binding modes, such as the head-to-head/tail-to-tail and the head-to-tail interacted cholesterol dimers. This offers an unprecedented opportunity to understand molecular interactions, such as solubilities, from a perspective of defined orientations (Figure 4).

In conclusion, we demonstrated that the intermolecular mechanical force (IMMF) can be directly measured for interactions between small molecules. Such IMMF indicated that fully aligned cholesterol dimers have a stronger interaction than misaligned dimers. The solubility of cholesterol in β CD

has been rationalized by the magnitude of IMMF, which represents a new and generic variable to describe solubility of chemicals beyond the cholesterols studied here. Given the importance of the cholesterol in various cellular and biological processes, the mechanisms of and the insights on the cholesterol dimer interaction revealed here are expected to understand a range of physiological and pathological processes involving cholesterols.

■ ASSOCIATED CONTENT

■ Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.jpclett.1c03142>.

Methods and experiments; synthesis of cholesterol functionalized DNA; Method to determine the change-in-contour-length; additional figures and tables as described in the text (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Hanbin Mao – Department of Chemistry and Biochemistry, Kent State University, Kent, Ohio 44242, United States; orcid.org/0000-0002-6720-9429; Email: hmao@kent.edu

Authors

Shankar Pandey – Department of Chemistry and Biochemistry, Kent State University, Kent, Ohio 44242, United States; orcid.org/0000-0001-5576-6714

Yuan Xiang – Department of Mechanical and Aerospace Engineering, The George Washington University, Washington, D.C. 20052, United States; orcid.org/0000-0002-4665-3360

Dirk Friedrich – Department of Chemistry and Biochemistry, Kent State University, Kent, Ohio 44242, United States

Yongsheng Leng – Department of Mechanical and Aerospace Engineering, The George Washington University, Washington, D.C. 20052, United States; orcid.org/0000-0002-3558-5016

Complete contact information is available at:

<https://pubs.acs.org/doi/10.1021/acs.jpclett.1c03142>

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

H.M. thanks prof. Janarthanan Jayawickramarajah and his graduate student, Dilanka V. D. Walpita Kankanamalage from Tulane University for providing 3' azido-propyl DNA during the synthesis of 3' cholesterol-propyl DNA. H.M. thanks NIH (R01 CA236350, for construct preparation) and NSF (CBET-1904921 for mechanical unfolding) for financial support. Y.L. is grateful for the NSF grant (CBET-1817394) for the computational studies of membrane fouling and separations.

■ REFERENCES

- (1) Dudko, O. K.; Hummer, G.; Szabo, A. Theory, analysis, and interpretation of single-molecule force spectroscopy experiments. *Proc. Natl. Acad. Sci. U. S. A.* **2008**, *105* (41), 15755–15760.
- (2) Silberberg, M. S. *Principles of general chemistry*; McGraw-Hill: New York, 2013.
- (3) Tinoco, I.; T. X. Li, P.; Bustamante, C. Determination of thermodynamics and kinetics of RNA reactions by force. *Q. Rev. Biophys.* **2006**, *39* (4), 325–360.
- (4) Evans, E. Probing the relation between force-lifetime-and chemistry in single molecular bonds. *Annu. Rev. Biophys. Biomol. Struct.* **2001**, *30*, 105–28.
- (5) Moffitt, J. R.; Chemla, Y. R.; Smith, S. B.; Bustamante, C. Recent advances in optical tweezers. *Annu. Rev. Biochem.* **2008**, *77*, 205–228.
- (6) Mehta, A. D.; Rief, M.; Spudich, J. A.; Smith, D. A.; Simmons, R. M. Single-Molecule Biomechanics with Optical Methods. *Science* **1999**, *283* (5408), 1689–1695.

- (7) Freikamp, A.; Cost, A.-L.; Grashoff, C. The piconewton force awakens: quantifying mechanics in cells. *Trends Cell Biol.* **2016**, *26* (11), 838–847.
- (8) Shao, J.-Y. Measuring piconewton forces and its application in cellular and molecular biomechanics. *Advances in Biomechanics* **2001**, 47–51.
- (9) Keller, D.; Bustamante, C. The Mechanochemistry of Molecular Motors. *Biophys. J.* **2000**, *78*, 541–556.
- (10) Caruso, M. M.; Davis, D. A.; Shen, Q.; Odom, S. A.; Sottos, N. R.; White, S. R.; Moore, J. S. Mechanically-induced Chemical Changes in Polymeric Materials. *Chem. Rev.* **2009**, *109*, 5755–5798.
- (11) Neuman, K. C.; Nagy, A. Single-molecule force spectroscopy: optical tweezers, magnetic tweezers and atomic force microscopy. *Nat. Methods* **2008**, *5* (6), 491–505.
- (12) Wang, L.; Qian, Y.; Sun, Y.; Liu, B.; Wei, G. Single-molecule force spectroscopy: A facile technique for studying the interactions between biomolecules and materials interfaces. *Rev. Anal. Chem.* **2020**, *39* (1), 116–129.
- (13) Carrion-Vazquez, M.; Oberhauser, A. F.; Fisher, T. E.; Marszalek, P.; Li, H.; Fernandez, J. M. Mechanical design of proteins studied by single-molecule force spectroscopy and protein engineering. *Prog. Biophys. Mol. Biol.* **2000**, *74*, 63–91.
- (14) Pandey, S.; Kankanamalage, D. V. W.; Zhou, X.; Hu, C.; Isaacs, L.; Jayawickramarajah, J.; Mao, H. Chaperone-Assisted Host–Guest Interactions Revealed by Single-Molecule Force Spectroscopy. *J. Am. Chem. Soc.* **2019**, *141* (46), 18385–18389.
- (15) Pandey, S.; Xiang, Y.; Walpita Kankanamalage, D. V. D.; Jayawickramarajah, J.; Leng, Y.; Mao, H. Measurement of Single-Molecule Forces in Cholesterol and Cyclodextrin Host–Guest Complexes. *J. Phys. Chem. B* **2021**, *125* (40), 11112–11121.
- (16) Ramamoorthy, S.; Cidlowski, J. A. Corticosteroids: Mechanisms of Action in Health and Disease. *Rheum Dis Clin North Am.* **2016**, *42* (1), 15–vii.
- (17) Berg, J.; Tymoczko, J.; Stryer, L. Important derivatives of cholesterol include bile salts and steroid hormones. *Biochemistry*, 5th ed.; WH Freeman: New York, 2002; section 26.4.
- (18) Elkins, M. R.; Bandara, A.; Pantelopulos, G. A.; Straub, J. E.; Hong, M. Direct Observation of Cholesterol Dimers and Tetramers in Lipid Bilayers. *J. Phys. Chem. B* **2021**, *125* (7), 1825–1837.
- (19) Hung, W.-C.; Lee, M.-T.; Chen, F.-Y.; Huang, H. W. The Condensing Effect of Cholesterol in Lipid Bilayers. *Biophys. J.* **2007**, *92* (11), 3960–3967.
- (20) Miao, L.; Nielsen, M.; Thewalt, J.; Ipsen, J. H.; Bloom, M.; Zuckermann, M. J.; Mouritsen, O. G. From Lanosterol to Cholesterol: Structural Evolution and Differential Effects on Lipid Bilayers. *Biophys. J.* **2002**, *82* (3), 1429–1444.
- (21) Simons, K.; Toomre, D. Lipid rafts and signal transduction. *Nat. Rev. Mol. Cell Biol.* **2000**, *1* (1), 31–39.
- (22) Tewary, P.; Veena, K.; Pucadyil, T. J.; Chattopadhyay, A.; Madhubala, R. The sterol-binding antibiotic nystatin inhibits entry of non-opsonized Leishmania donovani into macrophages. *Biochem. Biophys. Res. Commun.* **2006**, *339* (2), 661–666.
- (23) Kumar, G. A.; Jafurulla, M.; Chattopadhyay, A. The membrane as the gatekeeper of infection: Cholesterol in host–pathogen interaction. *Chem. Phys. Lipids* **2016**, *199*, 179–185.
- (24) Silvius, J. R. Role of cholesterol in lipid raft formation: lessons from lipid model systems. *Biochim. Biophys. Acta, Biomembr.* **2003**, *1610* (2), 174–183.
- (25) Varsano, N.; Beghi, F.; Elad, N.; Pereiro, E.; Dadosh, T.; Pinkas, I.; Perez-Berna, A. J.; Jin, X.; Kruth, H. S.; Leiserowitz, L.; Addadi, L. Two polymorphic cholesterol monohydrate crystal structures form in macrophage culture models of atherosclerosis. *Proc. Natl. Acad. Sci. U. S. A.* **2018**, *115* (30), 7662–7669.
- (26) Dhakal, S.; Yu, Z.; Konik, R.; Cui, Y.; Koirala, D.; Mao, H. G-Quadruplex and i-Motif Are Mutually Exclusive in ILPR Double-Stranded DNA. *Biophys. J.* **2012**, *102* (June), 2575–2584.
- (27) Yu, Z.; Schonhoft, J. D.; Dhakal, S.; Bajracharya, R.; Hegde, R.; Basu, S.; Mao, H. ILPR G-Quadruplexes Formed in Seconds

- 434 Demonstrate High Mechanical Stabilities. *J. Am. Chem. Soc.* **2009**, *131*
435 (5), 1876–1882.
- 436 (28) Koirala, D.; Dhakal, S.; Ashbridge, B.; Sannohe, Y.; Rodriguez,
437 R.; Sugiyama, H.; Balasubramanian, S.; Mao, H. A Single-Molecule
438 Platform for Investigation of Interactions between G-quadruplexes
439 and Small-Molecule Ligands. *Nat. Chem.* **2011**, *3*, 782–787.
- 440 (29) Woodside, M. T.; Behnke-Parks, W. M.; Larizadeh, K.; Travers,
441 K.; Herschlag, D.; Block, S. M. Nanomechanical measurements of the
442 sequence-dependent folding landscapes of single nucleic acid hairpins.
443 *Proc. Natl. Acad. Sci. U. S. A.* **2006**, *103* (16), 6190–6195.
- 444 (30) Kolb, H. C.; Finn, M. G.; Sharpless, K. B. Click Chemistry:
445 Diverse Chemical Function from a Few Good Reactions. *Angew.*
446 *Chem., Int. Ed.* **2001**, *40*, 2004–2021.
- 447 (31) Park, S.; Khalili-Araghi, F.; Tajkhorshid, E.; Schulten, K. Free
448 energy calculation from steered molecular dynamics simulations using
449 Jarzynski's equality. *J. Chem. Phys.* **2003**, *119* (6), 3559–3566.
- 450 (32) Park, S.; Schulten, K. Calculating potentials of mean force from
451 steered molecular dynamics simulations. *J. Chem. Phys.* **2004**, *120*
452 (13), 5946–5961.
- 453 (33) Makarov, D. E. Communication: Does force spectroscopy of
454 biomolecules probe their intrinsic dynamic properties? *J. Chem. Phys.*
455 **2014**, *141* (24), 241103.
- 456 (34) Jarzynski, C. Nonequilibrium Equality for Free Energy
457 Differences. *Phys. Rev. Lett.* **1997**, *78*, 2690–2693.
- 458 (35) Liphardt, J.; Dumont, S.; Smith, S. B.; Tinoco, I., Jr.;
459 Bustamante, C. Equilibrium information from nonequilibrium
460 measurements in an experimental test of Jarzynski's equality. *Science*
461 **2002**, *296* (5574), 1832–1835.
- 462 (36) Irie, T.; Otagiri, M.; Sunada, M.; Uekama, K.; Ohtani, Y.;
463 Yamada, Y.; Sugiyama, Y. Cyclodextrin-induced hemolysis and shape
464 changes of human erythrocytes in vitro. *J. Pharmacobio-Dyn.* **1982**, *5*
465 (9), 741–744.
- 466 (37) Zimmer, S.; Grebe, A.; Bakke, S. S.; Bode, N.; Halvorsen, B.;
467 Ulas, T.; Skjelland, M.; De Nardo, D.; Labzin, L. I.; Kerksiek, A.;
468 Hempel, C.; Heneka, M. T.; Hawxhurst, V.; Fitzgerald, M. L.;
469 Trebicka, J.; Björkhem, I.; Gustafsson, J.-Å.; Westerterp, M.; Tall, A.
470 R.; Wright, S. D.; Espevik, T.; Schultze, J. L.; Nickenig, G.; Lütjohann,
471 D.; Latz, E. Cyclodextrin promotes atherosclerosis regression via
472 macrophage reprogramming. *Sci. Transl. Med.* **2016**, *8* (333), 333ra50.
- 473 (38) Kim, H.; Han, J.; Park, J.-H. Cyclodextrin polymer improves
474 atherosclerosis therapy and reduces ototoxicity. *J. Controlled Release*
475 **2020**, *319*, 77–86.
- 476 (39) Beseničar, M. P.; Bavdek, A.; Kladnik, A.; Maček, P.; Anderluh,
477 G. Kinetics of cholesterol extraction from lipid membranes by methyl-
478 β -cyclodextrin—A surface plasmon resonance approach. *Biochim.*
479 *Biophys. Acta, Biomembr.* **2008**, *1778* (1), 175–184.
- 480 (40) Bux, K.; Moin, S. T. Solvation of cholesterol in different
481 solvents: a molecular dynamics simulation study. *Phys. Chem. Chem.*
482 *Phys.* **2020**, *22* (3), 1154–1167.