The effect of molecular shape on oligomerization of hydrophobic drugs : molecular simulations of ciprofloxacin and nutlin

Li, Jianguo; Beuerman, Roger; Verma, Chandra

2018

Li, J., Beuerman, R., & Verma, C. (2018). The effect of molecular shape on oligomerization of hydrophobic drugs : molecular simulations of ciprofloxacin and nutlin. *The Journal of Chemical Physics*, 148(10), 104902-. doi:10.1063/1.5013056

<https://hdl.handle.net/10356/106542>

<https://doi.org/10.1063/1.5013056>

© 2018 The Author(s). All rights reserved. This paper was published by AIP Publishing in *Journal of Chemical Physics* and is made available with permission of The Author(s).

Downloaded on 26 Apr 2023 18:17:35 SGT

The effect of molecular shape on oligomerization of hydrophobic drugs: Molecular simulations of ciprofloxacin and nutlin

Jianguo Li,^{1,2,3} Roger Beuerman,^{1,3,4,5,a)} and Chandra Verma^{1,2,6,7,a)}

¹Singapore Eye Research Institute, 11 Third Hospital Avenue, #06-00, Singapore 168751

²Bioinformatics Institute (A*-STAR), 30 Biopolis Street, #07-01 Matrix, Singapore 138671

³Duke-NUS Medical School, Ophthalmology Academic Clinical Program, Singapore

⁴Department of Ophthalmology, National University of Singapore, 1E Kent Ridge Road, Singapore 119074

⁵School of Chemical and Biomedical Engineering, Nanyang Technological University, 62 Nanyang Drive, Singapore 637459

⁶School of Biological Sciences, Nanyang Technological University, 60 Nanyang Drive, Singapore 637551

⁷Department of Biological Sciences, National University of Singapore, 14 Science Drive 4, Singapore 117543

(Received 10 November 2017; accepted 20 February 2018; published online 13 March 2018)

Molecular aggregation plays a significant role in modulating the solubility, permeability, and bioactivity of drugs. The propensity to aggregate depends on hydrophobicity and on molecular shape. Molecular dynamics simulations coupled with enhanced sampling methods are used to explore the early stages of oligomerization of two drug molecules which have a strong aggregation propensity, but with contrasting molecule shapes: the antibiotic ciprofloxacin and the anticancer drug Nutlin-3A. The planar shape of ciprofloxacin induces the formation of stable oligomers at all cluster sizes. The aggregation of ciprofloxacin is driven by two-body interactions, and transferring one ciprofloxacin molecule to an existing cluster involves the desolvation of two faces and the concomitant hydrophobic interactions between the two faces; thus, the corresponding free energy of oligomerization weakly depends on the oligomer size. By contrast, Nutlin-3A has a star-shape and hence can only form stable oligomers when the cluster size is greater than 8. Free energy simulations further confirmed that the free energy of oligomer formation for Nutlin-3A becomes more favorable as the oligomer becomes larger. The aggregation of star-shaped Nutlin-3A results from many-body interactions and hence the free energy of cluster formation is strongly dependent on the size. The findings of this study provide atomistic insights into how molecular shape modulates the aggregation behavior of molecules and may be factored into the design of drugs or nano-particles. *Published by AIP Publishing.* <https://doi.org/10.1063/1.5013056>

I. INTRODUCTION

Bioavailability of a drug is a basic pharmaceutical property and plays important roles in lead selection during drug discovery.¹ For orally administered drugs, bioavailability is largely determined by the interplay between aqueous solubility and membrane permeability.² Both solubility and permeability often contrastingly depend on the hydrophobicity of the drug molecule. Reduction in the hydrophobicity of drug molecules enhances aqueous solubility but may lead to low membrane permeability. By contrast, increasing the drug hydrophobicity favors membrane permeation, but results in low solubility due to an increased tendency to aggregate. Thus an appropriate balance between membrane permeability and solubility is needed for reasonable bioavailability.

According to the classical nucleation theory (CNT),³ the aggregation of drug molecules is determined by the free energy change ΔG defined as

$$\Delta G = \frac{4}{3}\pi r^3 \Delta g + 4\pi r^2 \sigma, \quad (1)$$

where Δg is the free energy density of the aggregates, r is the radius of the aggregates, and σ is the surface tension. The first term is the bulk contribution of the free energy of the aggregates which is negative and favorable, while the second term is the surface contribution which is always positive and unfavorable. Equation (1) assumes that the aggregates are spherical and Δg is independent of cluster size. However, this may not be the case when the shapes of the particle and the aggregate are non-spherical. The CNT in Eq. (1) describes the state where well-defined nucleus is formed; it may not hold true in the early stage of aggregation where only a low degree of oligomers has occurred. Hence, the free energy of aggregation of non-spherical molecules and its dependence on cluster size during the initial stage of aggregation can be different from that of spherical molecules.

Aggregation of a hydrophobic drug is primarily driven by hydrophobic interactions, which depend on both the hydrophobicity of the molecule and on the desolvation surface area.⁴ Moreover, surface complementarity is known to favor aggregation, suggesting the important role of molecular shape in modulating aggregation.⁵ Although numerous studies have been carried out to understand the effect of hydrophobicity on aggregation, few have focused on the effects of shape anisotropy. Molecules having similar hydrophobicity but with different molecular shapes may have different aggregation

^{a)}Authors to whom correspondence should be addressed: rwbeuerman@gmail.com and chandra@bii.a-star.edu.sg

FIG. 1. (a) Structures of Nutlin-3A and ciprofloxacin and (b) potential of mean force of Nutlin-3A and ciprofloxacin in water.

behavior and aggregate into a variety of oligomers with varying shapes. To understand the role of molecular shape in aggregation, we examined the aggregation behavior of two model compounds with different shapes: Nutlin-3A and ciprofloxacin [Fig. 1(a)]. Nutlin-3A is an anti-cancer compound whose derivatives are currently under clinical trials. It has a star-like shape resulting from branched aromatic moieties. This molecule and its derivatives are being pursued as inhibitors of the mouse double minute 2 (MDM2) homolog protein for cancer treatment.⁶ Nutlin-3A is usually formulated in suspensions in 2% Klucel and 0.5% Tween 80 in oral administration.^{6,7} By contrast, the planar-shaped ciprofloxacin is a representative fluoroquinolone and is a Food and Drug Administration (FDA) approved second line antibiotic which targets the DNA gyrase in bacteria.⁸ The oral formulation of ciprofloxacin is in the form of either tablets or suspensions in diluent.^{9,10} Both compounds are insoluble in water but can be soluble in organic solvents, suggesting strong aggregation propensity in aqueous solutions.^{11–14} There is one study that investigated the mechanism of translocation of ciprofloxacin across membranes and observed that the ciprofloxacin molecules stacked close to the membrane surface but translocate across the membrane as monomers.¹³ From a thermodynamic perspective, the translocation of ciprofloxacin across the membrane requires the creation of cavities in the membrane, which incurs entropic penalties. The monomeric form is favored compared to the oligomeric form because it will engage smaller cavities and hence will incur lower entropic penalty. Given that in solution, the monomers are likely in equilibrium with different sizes of oligomers, the equilibrium between the monomer and oligomers of ciprofloxacin modulates its membrane permeation. In addition, a study using theoretical modeling and NMR experiments also reported stacking in ciprofloxacin.¹⁴ However, there are no reports of the energetics underlying this stacking process. Aggregation is known to modulate the biological properties of drugs.¹⁵ Both drugs bind to hydrophobic cavities (Nutlin-3A to its receptor MDM2 and ciprofloxacin to its receptor DNA gyrase) as monomers. Understanding the population distribution of oligomers with different sizes of oligomers will provide useful insights into their structure-activity relationships (SARs). In particular, it could provide crucial insights into the process that enables a monomer to partition into the binding site on its receptor and this knowledge could be used to enhance binding to the receptor. Although some studies have been carried out to investigate the population distribution of oligomers for planar aromatic molecules, these largely employ analytical equations

to model the aggregation process and assume the formation of one-dimensional aggregates.^{14,16,17} In this study, we employ detailed molecular dynamics (MD) simulations to explore the effect of shape on the aggregation behavior of the two drugs as model compounds: the planar-shaped ciprofloxacin and star-shaped Nutlin-3A. To this purpose, for each drug, we first calculated the potential of mean force (PMF) between two monomers using umbrella sampling. Next we examined the stability of different oligomers via classical MD simulations. Finally, to have a thermodynamic understanding of the dependence of cluster size on aggregation, we calculated the free energy of oligomerization via a combination of umbrella sampling and Hamiltonian replica exchange MD (HREMD) simulations. The results can complement biophysical experiments such as NMR and affinity measurements towards providing a detailed thermodynamic understanding of their mechanisms of aggregation and guiding the design of efficient formulation and delivery strategies.

II. METHODS

We used three sets of simulations to study the effect of molecular shape on the aggregation of Nutlin-3A and ciprofloxacin. In the first set, the potential of mean force (PMF) for both compounds was calculated using either umbrella sampling¹⁸ or HREMD simulations.^{19,20} In umbrella sampling simulations, the distance between two monomers was used as the reaction coordinate and a series of biased simulations in windows with bin size of 0.15 nm were carried out. Each umbrella window was run for 50 ns, with a biased potential of 1000 (kJ/mol/nm²) added to enhance phase space sampling. The full PMF was constructed based on the last 25 ns using the weighted histogram analysis method (WHAM).^{21,22} To account for the entropic contributions, a correction term of $2kT \ln(r)$ was added, where r is the distance along the reaction coordinate.^{23,24} As the PMF for Nutlin-3A showed a surprisingly low free energy minimum, we also performed an HREMD simulation for the Nutlin-3A dimer to validate the convergence of umbrella sampling. In the HREMD simulations, 8 replicas were run in parallel for 15 ns each (totally 120 ns). The first replica corresponds to the case of normal Hamiltonian and in the last replica, the interactions involving the Nutlin-3A molecules were rescaled by a factor of 0.5. Intermediate replicas were added with rescaling between 1 and 0.5 to ensure that the potential energy distributions of adjacent replicas overlap sufficiently. At 1 ps intervals, a Monte Carlo move was tried to exchange adjacent replicas and the

acceptance was based on the Metropolis criterion.²⁵ The PMF was constructed using the following equation (2):

$$\Delta G = -kT \ln(\text{RDF}), \quad (2)$$

where RDF is the radial distribution function between the centers of mass of the two monomers and k and T are the Boltzmann constant and temperature, respectively.²⁶

In the second set, we performed a series of 200 ns classical MD simulations of Nutlin-3A and ciprofloxacin to study the effect of cluster size on the oligomer stability at both 300 K and 310 K. For each compound, 2, 4, 8, and 16 molecules were used and a compound/water ratio was kept at 1/1500, resulting in the same molar concentrations for all sizes of oligomers. In each MD simulation, the required number of compounds was randomly placed in a simulation box, followed by solvation with water molecules. The compound/water ratio corresponds to a molar concentration of 0.037M, which is above the solubility of each compound. The shape, radius of gyration, and the cluster size distribution of the oligomers were used to characterize the stability of the aggregates.

To gain further insights into the thermodynamics of oligomer stability, we calculated the free energy of forming a cluster of N monomers $\Delta G(N)$ by adding single monomers to an existing cluster of $N - 1$ monomers using umbrella sampling. The bin size, simulation time, and force constant were the same as in the PMF calculation. The aggregation numbers used in the free energy simulations, including simulations of the dimer, were 2, 4, 8, and 16 for ciprofloxacin and 2, 4, 8, 12, 16, and 20 for Nutlin-3A. Entropic contributions were accounted for by adding $2kT \ln(r)$, where r is the distance along the reaction coordinate.^{23,24} To prevent the dissociation of clusters in umbrella sampling simulations, wall potentials in the x , y , and z directions were used to restrict the monomers of the cluster to a particular region in the simulation box. Our simulation results revealed that ciprofloxacin forms very stable oligomers at all values of the aggregation number N . Hence at large N (e.g., $N = 8$ and 16), the umbrella sampling simulations likely depend on the initial conformation of the ciprofloxacin aggregates, resulting in insufficient sampling of the phase space of the aggregates. In order to enhance sampling in each umbrella sampling window

so that the aggregates can rearrange the oligomer structure, we also carried out HREMD simulations for each umbrella sampling window for ciprofloxacin at $N = 8$ and 16. For the case of $N = 8$, 8 replicas were used and for $N = 16$, 10 replicas were used to ensure sufficient overlap in the potential energy distributions between the adjacent replica. The error bars of each PMF profile were estimated using the bootstrap method.²²

In all simulations, the AMBER general force field²⁷ was used to model both Nutlin-3A and ciprofloxacin and the TIP3P model was used for the water molecules.²⁸ Short-range electrostatics and Lennard-Jones interactions were calculated with a cut-off distance of 1.0 nm, while long-range electrostatic interactions were calculated using Particle Mesh Ewald (PME).²⁹ All simulations were performed in the NPT ensemble with pressure and temperature maintained at 1 bar and 300 K, respectively. All simulations were performed using the GROMACS package patched with PLUMED.^{30–32}

III. RESULTS

A. Potential of mean force

To understand the aggregation propensities of ciprofloxacin and Nutlin-3A, we begin by calculating the potential of mean force (PMF) using umbrella sampling MD simulations.^{18,21,22} The PMF is the free energy of dimerization of two monomers with the intermolecular distance being the reaction coordinate, and it has been widely used to characterize the aggregation propensity of a molecule.^{33,34} Figure 1 shows that ciprofloxacin has a very favorable free energy of association between two monomers, with a free energy minimum of -27 kJ/mol, suggesting a high propensity for aggregation. The free energy minimum locates at 0.45 nm, corresponding to two ciprofloxacin molecules stacking together (Fig. 2). On the contrary, although Nutlin-3A contains more aromatic rings and has a larger molecular size, the strength of the free energy of association between two Nutlin-3A monomers is much weaker (-7 kJ/mol), indicating that the Nutlin-3A dimer is thermodynamically less stable, which is not correlated with the experimentally determined low solubility of Nutlin-3A. One may wonder if this inconsistency arises from

FIG. 2. Snapshots of aggregates of ciprofloxacin and Nutlin-3A at different cluster sizes during classical MD simulations.

the non-convergence of the umbrella sampling simulations. To examine this, we performed an HREMD simulation of two monomers of Nutlin-3A. Free energies of association as a function of distance between the centers of mass of the two monomers were calculated based on Eq. (2). The free energy from HREMD is identical to that computed using umbrella sampling (Fig. S1 of the [supplementary material](#)) thus ruling out the possibility of non-convergence of the umbrella sampling simulations. In fact, during the HREMD simulations, the two monomers were found to undergo several events of association and dissociation, as can be seen from the variations in the distance between the two monomers during the simulations, thus further suggesting that the Nutlin-3A dimer is not stable. Thus this inconsistency between the instability of the Nutlin-3A dimer and its low solubility suggests that the PMF between two monomers clearly does not account for the known aggregation propensity of Nutlin-3A.

Although both molecules contain aromatic moieties, Nutlin-3A is characterized by a branched topology, while the aromatic rings in ciprofloxacin form a planar shape. When forming a dimer, only part of the Nutlin-3A monomer participates in the interactions with the other monomer. The insufficient number of atomic contacts and low degree of desolvation results in a Nutlin-3A dimer with low stability. This suggests that the two-body interaction (e.g., the interaction between two monomers) is not strong enough to maintain the Nutlin-3A dimer. It suggests that the aggregation of Nutlin-3A may result from many-body interactions and the free energy of association may be a function of the cluster size. Considering the low solubility of Nutlin-3A, the Nutlin-3A oligomers must become stable as the cluster size increases. We next attempt to understand this.

B. Effect of cluster size on oligomer stability

To understand the effect of the cluster size on oligomer stability, we performed classical MD simulations for Nutlin-3A

and ciprofloxacin by varying the number of monomers in the simulation box. As expected, the aggregation behavior of the two molecules differed, as can be seen from the number of clusters during the 200 ns simulations (Fig. 3). When there are 2 and 4 monomers, Nutlin-3A molecules were found to undergo repeated events of association and dissociation, as revealed by the large fluctuations in the number of Nutlin-3A clusters, resulting in polydispersed aggregates. As the aggregation number N further increases to 8 [Fig. 3(c)] and 16 [Fig. 3(d)], all the monomers form one cluster (Fig. 2), suggesting that the stability of the aggregate depends on the cluster size. By contrast, ciprofloxacin forms one stable cluster at all cluster sizes. Snapshots show that in each aggregate, ciprofloxacin molecules stack together, which naturally results from its planarity (Fig. 2). The stacking of ciprofloxacin monomers can be characterized by the relative orientations of monomers (Fig. S2 of the [supplementary material](#)). In the ciprofloxacin dimer, the probability distribution of the angle between the two normal vectors of each planar monomer has a much higher population near zero degrees, suggesting that the monomers assume similar orientations upon aggregation. For Nutlin-3A, the angle is between the two normal vectors of the center ring, which is widely distributed, suggesting a loosely packed dimer, and is consistent with the PMF results we saw earlier. A similar trend was also observed for the radius of gyration of the oligomers (Fig. S3 of the [supplementary material](#)). Nutlin-3A displays high fluctuations when the cluster size is 2 and 4 but converges to a constant value as the cluster size further increases to 8 and 16, while the radius of gyration of ciprofloxacin was found to fluctuate much less, suggesting stable oligomers for each cluster size. Moreover, because of the difference in the aggregation mechanisms, Nutlin-3A and ciprofloxacin form oligomers with different shapes. Ciprofloxacin forms elongated oligomers resulting from the strong aromatic stacking, while Nutlin-3A oligomers have a spherical shape. To understand this, we calculate the shape parameter during the MD simulations, which is defined as the ratio of the longest axis to

FIG. 3. Number of clusters during 200 ns classical MD simulations for Nutlin-3A and ciprofloxacin at 300 K.

the shortest axis of the oligomers (Fig. S4 of the [supplementary material](#)). The shape parameter of the aggregate is about 2 for ciprofloxacin, suggesting an anisotropic shape, while it is around 1 for Nutlin-3A, suggesting a spherical shape. The results confirm our hypothesis that the oligomer stability depends on the shape of the molecule as well as on the size of the oligomer, and differences in the molecular shapes result in different shapes of oligomers.

For hydrophobic molecules, such as Nutlin-3A and ciprofloxacin, aggregation can originate from two driving forces: direct interactions (e.g., direct van der Waals and electrostatic interactions between polar groups) and hydrophobic interactions (e.g., solvent mediated forces). To further understand the driving force behind the oligomerization of Nutlin-3A and ciprofloxacin, we calculated the hydration number and the number of contacts in the various aggregates (Fig. S5 of the [supplementary material](#)). The hydration number is defined as the number of water molecules within 0.5 nm of any non-hydrogen atom of the solute molecule. Two non-hydrogen atoms are considered to be in contact if they belong to different monomers and are separated by a maximum of 0.5 nm. As the aggregation size N increases, the average hydration number of the ciprofloxacin oligomers displays a near linear increase. By contrast, the hydration number for Nutlin-3A increases sharply from monomer to tetramer, followed by a gradual increase beyond the tetramer [Fig. S5(a) of the [supplementary material](#)]. The results confirm that Nutlin-3A cannot form a stable dimer or tetramer due to the high degree of hydration. It appears that only when the oligomer is large enough (e.g., $N \geq 8$), Nutlin-3A can form stable aggregates. The same behavior can be seen from the number of contacts [Fig. S5(b) of the [supplementary material](#)]. Ciprofloxacin displays a linear increase in the number of atomic contacts as N increases, while Nutlin-3A showed a small increase in the number of contacts from dimers to tetramers, suggesting that the dimers and tetramers in the latter are more dynamic. Moreover, ciprofloxacin and Nutlin-3A display different distributions in their 2-D

plots of hydration and atomic contacts [Fig. S5(c) of the [supplementary material](#)]. For ciprofloxacin, the cluster becomes more dynamic as the oligomer size increases, while for Nutlin-3A, the oligomer becomes more stable (less dynamic) as the oligomer size increases. To understand the aggregation of ciprofloxacin and Nutlin-3A at physiological temperatures, we also carried out aggregation simulations at 310 K. The cluster distribution analysis showed very similar results to those at 300 K (Fig. S6 of the [supplementary material](#)). Together, these observations suggest that small oligomers of ciprofloxacin are thermodynamically more stable than large ones, while Nutlin-3A is more stable as larger oligomers.

C. Free energy of oligomerization

To further understand the effect of aggregation number on the thermodynamic stability of the aggregates, we calculated the free energy changes characterizing the following process:

where $\Delta G(N)$ in units of kJ/mol is the free energy change of adding a single monomer to an aggregate of $(N - 1)$ monomers to form an aggregate of N monomers, corresponding to 1 mol of oligomer M_{N-1} and 1 mol of monomer M_1 forming 1 mol of oligomer M_N .

Figures 4(a) and 4(b) show the corresponding free energies of cluster formation for Nutlin-3A. As the aggregation number N increases, the free energy change incurred upon the addition of one Nutlin-3A monomer to an existing aggregate to form a cluster with N monomers $\Delta G(N)$ decreases with aggregation number N . Moreover, as N increases, the reduction in $\Delta G(N)$ decreases, and $\Delta G(N)$ reaches -23 kJ/mol when N reaches 20. Visual inspections of the N -mer aggregate revealed that the aggregates of Nutlin-3A take a globular shape, particularly for aggregates with large N , similar to the aggregates observed in Fig. 2. The difference in $\Delta G(N)$ suggests

FIG. 4. Changes in the free energy on adding one monomer to a cluster vs. final cluster size N . (a) Nutlin-3A, (b) ciprofloxacin, and (c) the free energy depth of different cluster size N .

that Nutlin-3A monomers in small clusters experience a different environment compared to that around large clusters. In small clusters, the Nutlin-3A monomers cannot achieve close packing and complete desolvation due to its star-like shape and small cluster sizes. Note that there are two contributions to the favorable $\Delta G(N)$ when transferring one Nutlin-3A monomer to an existing aggregate: (i) desolvation of the aromatic moieties; (ii) formation of atomic contacts with other monomers in the aggregate. Apparently both contributions depend on the aggregation number N . When N is small, such as is the case of a dimer or a tetramer, all Nutlin-3A molecules locate at the surface of the aggregate and thus are only partially desolvated and the number of atomic contacts between Nutlin-3A monomers is small. As a result, $\Delta G(N)$ is only slightly favorable. When N is relatively large (e.g., 16-mer or 20-mer), transferring one Nutlin-3A monomer to an existing Nutlin-3A cluster results in not only complete desolvation but also in the formation of large number of contacts with other monomers via close packing, forming a stable oligomer and resulting in a much more favorable $\Delta G(N)$. At sufficiently large N , no additional gain is seen in desolvation and number of contacts; thus, $\Delta G(N)$ converges to a constant value.

In the case of ciprofloxacin, $\Delta G(N)$ varies slightly as N increases [Figs. 4(b) and 4(c)]. Given its planar structure, aggregation of ciprofloxacin molecules is driven by stacking. In any size of aggregates, adding one ciprofloxacin monomer to an existing aggregate involves the desolvation of two faces of the aromatic moiety and the formation of an aromatic stack. Therefore $\Delta G(N)$ is relatively weakly dependent on cluster size N .

IV. DISCUSSIONS AND IMPLICATIONS

The above results present new atomistic insights into understanding the effect of molecular shape on drug aggregation. A widely used thermodynamic parameter to characterize molecular aggregation is the potential of mean force (PMF) between two monomers, namely, the free energy of dimerization $\Delta G(2)$, in which, except for the distance, all other factors are considered implicitly. Although $\Delta G(2)$ is widely used to characterize the aggregation propensities of proteins or colloidal particles, the results must be interpreted with caution. In this study, we examined the differences in $\Delta G(2)$ for two therapeutic molecules Nutlin-3A and ciprofloxacin. When Nutlin-3A forms a dimer, the lack of many-body interactions prevent the formation of close contacts between the two monomers, resulting in a thermodynamically not very favorable free energy minimum. This, however, does not correlate with the low solubility of Nutlin-3A. By contrast, the planar-shaped molecule ciprofloxacin has a more favorable $\Delta G(2)$, consistent with its low solubility. Together these observations suggest the important role of shape complementarity in the free energy of dimerization. The effect of molecular shape on $\Delta G(2)$ has also been observed for other molecules. For example, *n*-pentane and neo-pentane, both with the same number of carbon and hydrogen atoms, display different dimerization free energies $\Delta G(2)$.³⁵ More generally, it is found that branching increases solubility and decreases the boiling point of hydrocarbons and the changes correlate well with the

number of branches.^{36,37} Our results indicate that $\Delta G(2)$, or the PMF, fails to account for solubility in cases where the aggregation driving force is dominated by many-body interactions. In such cases, the free energy of aggregation also depends on the cluster size and the free energy of cluster formation needs to be considered.

As the cluster size N increases, Nutlin-3A and ciprofloxacin display different trends in the free energy of cluster formation. For ciprofloxacin, the aggregation process is largely driven by aromatic stacking between two molecules (e.g., two-body interactions). Transferring a monomer of ciprofloxacin to an existing ciprofloxacin cluster results in desolvation of two planes and the formation of a single stacked pair. Thus the corresponding free energy change $\Delta G(N)$ is only weakly dependent on the cluster size N . For Nutlin-3A, $\Delta G(N)$ was shown to be strongly dependent on the cluster size N . As N increases, $\Delta G(N)$ decreases and converges to ~ -23 kJ/mol when N is larger than 16, at which point, transferring a Nutlin-3A monomer to an existing Nutlin-3A cluster results in almost complete desolvation of the monomer. The free energy dependence on the cluster size clearly demonstrates that the aggregation of Nutlin-3A involves many-body interactions and thus is a cooperative process. Interestingly, the size dependence of the free energy change is also observed for aggregation of other hydrophobic molecules, ranging from molecules as small as methane in water to large molecules such as fullerene C60 in water.^{38,39} The observed cooperativity in cluster formation is also supported by recent experiments using real-time monitoring of aggregation in a microfluidic mixer; the study demonstrates that the free energy of aggregation of hexaphenylsilole, a molecule that has a star-shape similar to that of Nutlin-3A, depends on the cluster size.⁴⁰ Furthermore, the free energy dependence on many-body interactions is similar to the cooperative hydrophobic collapse that occurs during the initial events of protein folding,⁴¹ suggesting that cooperative hydrophobic association is a general phenomenon governing biomolecular aggregation.

Comparison of $\Delta G(N)$ for Nutlin-3A and ciprofloxacin has revealed different aggregation mechanisms. Due to the planar shape, the two-body interactions play an important role in the aggregation of ciprofloxacin, but for the star-shaped molecule Nutlin-3A, many-body interactions are required to induce stable oligomers. As a result, ciprofloxacin and Nutlin-3A display different population distributions of oligomers, which have important implications in drug design and delivery. For a drug such as Nutlin-3A whose aggregation is driven by many-body interactions, the low stability of oligomers shifts the equilibrium towards the monomeric state. This is favorable for the drugs as it is easier to permeate a membrane as a monomer, which requires the creation of a small cavity in the membrane, thus incurring low entropic penalty. In addition, this shift in equilibrium is further advantageous because most drugs bind to their receptors as monomers. In a broader context, the shape anisotropy will also play important roles in controlling the self-assembly of lipids, macromolecules (e.g., proteins), and nano-particles as the underlying driving forces of aggregation are conserved. In fact, it has been shown that the structure of protein aggregates strongly depends on the

shape anisotropy of the protein molecule and proteins with complementary shapes have much higher aggregation propensities.^{5,42} Moreover, the shape of a lipid molecule determines the structural features of the aggregates. For example, lipid molecules with big head groups and small lipid tails form micelles, while lipid molecules with smaller head groups form bilayers.⁴³ The effect of shape anisotropy is even more evident for the self-assembly of nano-particles.^{44,45}

V. CONCLUSIONS

In summary, we have studied the aggregation behavior of two hydrophobic molecules with contrasting molecular shapes. Potential of mean force calculations revealed a thermodynamically favorable free energy minimum for ciprofloxacin but a shallow free energy minimum for Nutlin-3A. Classical MD simulations showed that ciprofloxacin forms stable oligomers for all sizes of clusters, but Nutlin-3A can only form stable oligomers when the size of the oligomer is larger than 8. Extensive simulations revealed that the free energy of cluster formation of ciprofloxacin is weakly dependent on the cluster size, while the free energy of cluster formation for Nutlin-3A decreases as the cluster becomes larger and converges to ~ 23 kJ/mol when the cluster size is larger than 20. The difference in the aggregation behavior suggests that molecular shape has significant effects on the aggregation mechanism. The aggregation of planar-shaped molecules such as ciprofloxacin is driven by two-body interactions while that of star-shaped molecules such as Nutlin-3A is dominated by many-body interactions.

SUPPLEMENTARY MATERIAL

See [supplementary material](#) for the potential of mean force between two Nutlin-3A molecules using HREMD; the 2-D plot of hydration vs number of contacts of different sizes of aggregates; the radius of gyration of different oligomers, the shape parameter for different oligomers and the cluster distribution at 310 K.

ACKNOWLEDGMENTS

This work is supported by the grant from Nos. NMRC/TCR/002-SERI/2008/R618 and NMRC/BNIG/2016/2014, Singapore. The authors also thank A*STAR, NSCC for providing the computational facilities.

The authors declare no competing financial interests.

- ¹I. Gomez-Orellana, "Strategies to improve oral drug bioavailability," *Expert Opin. Drug Delivery* **2**, 419–433 (2005).
- ²A. Dahan, A. Beig, D. Lindley, and J. M. Miller, "The solubility-permeability interplay and oral drug formulation design: Two heads are better than one," *Adv. Drug Delivery Rev.* **101**, 99–107 (2016).
- ³P. S. Richard, "Nucleation: Theory and applications to protein solutions and colloidal suspensions," *J. Phys.: Condens. Matter* **19**, 033101 (2007).
- ⁴D. Chandler, "Interfaces and the driving force of hydrophobic assembly," *Nature* **437**, 640–647 (2005).
- ⁵Y. Li, X. Zhang, and D. Cao, "The role of shape complementarity in the protein-protein interactions," *Sci. Rep.* **3**, 3271 (2013).
- ⁶L. T. Vassilev, B. T. Vu, B. Graves, D. Carvajal, F. Podlaski, Z. Filipovic, N. Kong, U. Kammlott, C. Lukacs, C. Klein, N. Fotouhi, and E. A. Liu,

- "*In vivo* activation of the p53 pathway by small-molecule antagonists of MDM2," *Science* **303**, 844–848 (2004).
- ⁷F. Zhang, M. Tegen, S. Throm, J. Mallari, L. Miller, R. K. Guy, M. A. Dyer, R. T. Williams, M. F. Roussel, K. Nemeth, F. Zhu, J. Zhang, M. Lu, J. C. Panetta, N. Boulos, and C. F. Stewart, "Whole-body physiologically based pharmacokinetic model for Nutlin-3A in mice after intravenous and oral administration," *Drug Metab. Dispos.* **39**, 15–21 (2011).
- ⁸L. Becnel Boyd, M. J. Maynard, S. K. Morgan-Linnell, L. B. Horton, R. Sugang, R. J. Hamill, J. R. Jimenez, J. Versalovic, D. Steffen, and L. Zechiedrich, "Relationships among ciprofloxacin, gatifloxacin, levofloxacin, and norfloxacin MICs for fluoroquinolone-resistant *Escherichia coli* clinical isolates," *Antimicrob. Agents Chemother.* **53**, 229–234 (2009).
- ⁹I. H. Gomolin, P. F. Siami, J. Reuning-Scherer, D. C. Haverstock, A. Heyd, and The Oral Suspension Study Group, "Efficacy and safety of ciprofloxacin oral suspension versus trimethoprim-sulfamethoxazole oral suspension for treatment of older women with acute urinary tract infection," *J. Am. Geriatr. Soc.* **49**, 1606–1613 (2001).
- ¹⁰A. Shah, M. C. Liu, D. Vaughan, and A. H. Heller, "Oral bioequivalence of three ciprofloxacin formulations following single-dose administration: 500 mg tablet compared with 500 mg 10 mL or 500 mg 5 mL suspension and the effect of food on the absorption of ciprofloxacin oral suspension," *J. Antimicrob. Chemother.* **43**, 49–54 (1999).
- ¹¹M. Das, F. Dilnawaz, and S. K. Sahoo, "Targeted nutlin-3a loaded nanoparticles inhibiting p53-MDM2 interaction: Novel strategy for breast cancer therapy," *Nanomedicine* **6**, 489–507 (2011).
- ¹²S. Kalepu and V. Nekkanti, "Insoluble drug delivery strategies: Review of recent advances and business prospects," *Acta Pharm. Sin. B* **5**, 442–453 (2015).
- ¹³O. Cramariuc, T. Rog, M. Javanainen, L. Monticelli, A. V. Polishchuk, and I. Vattulainen, "Mechanism for translocation of fluoroquinolones across lipid membranes," *Biochim. Biophys. Acta, Biomembr.* **1818**, 2563–2571 (2012).
- ¹⁴I. Turcu and M. Bogdan, "Size dependence of molecular self-assembling in stacked aggregates. 1. NMR investigation of ciprofloxacin self-association," *J. Phys. Chem. B* **116**, 6488–6498 (2012).
- ¹⁵A. Sosnik, "Drug self-assembly: A phenomenon at the nanometer scale with major impact in the structure-biological properties relationship and the treatment of disease," *Prog. Mater. Sci.* **82**, 39–82 (2016).
- ¹⁶R. B. Martin, "Comparisons of indefinite self-association models," *Chem. Rev.* **96**, 3043–3064 (1996).
- ¹⁷D. A. Beshnova, A. O. Lantushenko, D. B. Davies, and M. P. Evstigneev, "Profiles of equilibrium constants for self-association of aromatic molecules," *J. Chem. Phys.* **130**, 165105 (2009).
- ¹⁸G. M. Torrie and J. P. Valleau, "Nonphysical sampling distributions in Monte Carlo free-energy estimation: Umbrella sampling," *J. Comput. Phys.* **23**, 187–199 (1977).
- ¹⁹P. Liu, B. Kim, R. A. Friesner, and B. J. Berne, "Replica exchange with solute tempering: A method for sampling biological systems in explicit water," *Proc. Natl. Acad. Sci. U. S. A.* **102**, 13749–13754 (2005).
- ²⁰G. Bussi, "Hamiltonian replica exchange in GROMACS: A flexible implementation," *Mol. Phys.* **112**, 379–384 (2014).
- ²¹S. Kumar, J. M. Rosenberg, D. Bouzida, R. H. Swendsen, and P. A. Kollman, "THE weighted histogram analysis method for free-energy calculations on biomolecules. I. The method," *J. Comput. Chem.* **13**, 1011–1021 (1992).
- ²²J. S. Hub, B. L. de Groot, and D. van der Spoel, "g-wham—A free weighted histogram analysis implementation including robust error and autocorrelation estimates," *J. Chem. Theory Comput.* **6**, 3713–3720 (2010).
- ²³R. M. Neumann, "Entropic approach to Brownian movement," *Am. J. Phys.* **48**, 354–357 (1980).
- ²⁴S. Boresch and M. Karplus, "The Jacobian factor in free energy simulations," *J. Chem. Phys.* **105**, 5145–5154 (1996).
- ²⁵N. Metropolis, A. W. Rosenbluth, M. N. Rosenbluth, A. H. Teller, and E. Teller, "Equation of state calculations by fast computing machines," *J. Chem. Phys.* **21**, 1087–1092 (1953).
- ²⁶M. J. Sippl, M. Ortner, M. Jaritz, P. Lackner, and H. Flöckner, "Helmholtz free energies of atom pair interactions in proteins," *Folding Des.* **1**, 289–298 (1996).
- ²⁷J. Wang, R. M. Wolf, J. W. Caldwell, P. A. Kollman, and D. A. Case, "Development and testing of a general amber force field," *J. Comput. Chem.* **25**, 1157–1174 (2004).
- ²⁸W. L. Jorgensen, J. Chandrasekhar, J. D. Madura, R. W. Impey, and M. L. Klein, "Comparison of simple potential functions for simulating liquid water," *J. Chem. Phys.* **79**, 926–935 (1983).

- ²⁹U. Essmann, L. Perera, M. L. Berkowitz, T. Darden, H. Lee, and L. G. Pedersen, "A smooth particle mesh Ewald method," *J. Chem. Phys.* **103**, 8577–8593 (1995).
- ³⁰P. Bjelkmar, P. Larsson, M. A. Cuendet, B. Hess, and E. Lindahl, "Implementation of the CHARMM force field in GROMACS: Analysis of protein stability effects from correction maps, virtual interaction sites, and water models," *J. Chem. Theory Comput.* **6**, 459–466 (2010).
- ³¹M. Bonomi, D. Branduardi, G. Bussi, C. Camilloni, D. Provasi, P. Raiteri, D. Donadio, F. Marinelli, F. Pietrucci, R. A. Broglia, and M. Parrinello, "PLUMED: A portable plugin for free-energy calculations with molecular dynamics," *Comput. Phys. Commun.* **180**, 1961–1972 (2009).
- ³²T. Terakawa, T. Kameda, and S. Takada, "On easy implementation of a variant of the replica exchange with solute tempering in GROMACS," *J. Comput. Chem.* **32**, 1228–1234 (2011).
- ³³M. Makowski, C. Czaplewski, A. Liwo, and H. A. Scheraga, "Potential of mean force of association of large hydrophobic particles: Towards the nanoscale limit," *J. Phys. Chem. B* **114**, 993–1003 (2010).
- ³⁴M. Brunsteiner, M. Flock, and B. Nidetzky, "Structure based descriptors for the estimation of colloidal interactions and protein aggregation propensities," *PLoS One* **8**, e59797 (2013).
- ³⁵A. Bartosik, M. Wiśniewska, and M. Makowski, "Potentials of mean force for hydrophobic interactions between hydrocarbons in water solution: Dependence on temperature, solute shape, and solute size," *J. Phys. Org. Chem.* **28**, 10–16 (2015).
- ³⁶M. Randic, "Characterization of molecular branching," *J. Am. Chem. Soc.* **97**, 6609–6615 (1975).
- ³⁷C. McAuliffe, "Solubility in water of paraffin, cycloparaffin, olefin, acetylene, cycloolefin, and aromatic hydrocarbons," *J. Phys. Chem.* **70**, 1267–1275 (1966).
- ³⁸T. M. Raschke, J. Tsai, and M. Levitt, "Quantification of the hydrophobic interaction by simulations of the aggregation of small hydrophobic solutes in water," *Proc. Natl. Acad. Sci. U. S. A.* **98**, 5965–5969 (2001).
- ³⁹H. Kim, D. Bedrov, and G. D. Smith, "Molecular dynamics simulation study of the influence of cluster geometry on formation of C60 fullerene clusters in aqueous solution," *J. Chem. Theory Comput.* **4**, 335–340 (2008).
- ⁴⁰L. Jiang, S. Cao, P. P.-H. Cheung, X. Zheng, C. W. T. Leung, Q. Peng, Z. Shuai, B. Z. Tang, S. Yao, and X. Huang, "Real-time monitoring of hydrophobic aggregation reveals a critical role of cooperativity in hydrophobic effect," *Nat. Commun.* **8**, 15639 (2017).
- ⁴¹J. Tsai, M. Gerstein, and M. Levitt, "Simulating the minimum core for hydrophobic collapse in globular proteins," *Protein Sci.* **6**, 2606–2616 (1997).
- ⁴²R. Vácha and D. Frenkel, "Relation between molecular shape and the morphology of self-assembling aggregates: A simulation study," *Biophys. J.* **101**, 1432–1439 (2011).
- ⁴³J. N. Israelachvili, D. J. Mitchell, and B. W. Ninham, "Theory of self-assembly of hydrocarbon amphiphiles into micelles and bilayers," *J. Chem. Soc., Faraday Trans. 2* **72**, 1525–1568 (1976).
- ⁴⁴S. C. Glotzer and M. J. Solomon, "Anisotropy of building blocks and their assembly into complex structures," *Nat. Mater.* **6**, 557–562 (2007).
- ⁴⁵P. F. Damasceno, M. Engel, and S. C. Glotzer, "Predictive self-assembly of polyhedra into complex structures," *Science* **337**, 453–457 (2012).