I. INTRODUCTION AND MOTIVATION

The idealization of dilute conditions in conventional in vitro biophysical experiments has long been recognized to ignore the important aspect of crowding. In typical cellular environments, the experimental, computational, and theoretical results have shown crowding agents to affect the stability of proteins and the rates of protein folding. The measured and predicted effects of crowding, however, are varied and seem to be dependent on both the protein and the crowders themselves. For a summary of the recent developments in the field since 2004; see the excellent review by Zhou, Rivas, and Minton.\(^1\) Crowding effects are still actively being explored, with most efforts focusing on the entropic effects to elucidate the response common to all crowding agents. While energetic interactions may exist between the protein and the crowding agents, a simplified yet effective treatment of a crowder is that of a steric, inert particle, a concept utilized by the experiments done by Gai and co-workers\(^8\) to study the statistical mechanical behaviors of folding of \(\beta\)-sheet peptides. Using a simple bead-lattice model, we are able to consider, separately, the conformational entropy involving the bond angles along the backbone and the orientational entropy associated with the dihedral angles. We use a Ising-like model to partially account for the dihedral angle entropy and, implicitly, the hydrogen-bond formations. We also compare our results to recent experiments and find good quantitative agreement on the predicted folded fraction. On the basis of the predictions from the scaled particle theory, we investigate changes in the melting temperature of the protein, suggesting crowding enhanced stability for a variant of trpzip hairpin and a slight instability for the larger \(\beta\)-sheet designed proteins.

ABSTRACT: In this paper we introduce the idea of the implicit crowding method to study the statistical mechanical behaviors of folding of \(\beta\)-sheet peptides. Using a simple bead-lattice model, we are able to consider, separately, the conformational entropy involving the bond angles along the backbone and the orientational entropy associated with the dihedral angles. We use a Ising-like model to partially account for the dihedral angle entropy and, implicitly, the hydrogen-bond formations. We also compare our results to recent experiments and find good quantitative agreement on the predicted folded fraction. On the basis of the predictions from the scaled particle theory, we investigate changes in the melting temperature of the protein, suggesting crowding enhanced stability for a variant of trpzip hairpin and a slight instability for the larger \(\beta\)-sheet designed proteins.
the conformations of the density of states and the ability to compute thermodynamic quantities to high accuracy.

The organization of the article is as follows. In section II we introduce the Hamiltonian and the effective free energies associated with crowding and the dihedral angles. In this section, we explicitly define the cost, both enthalpically and entropically for each conformation. We demonstrate how the density of states can be factored into two terms, greatly speeding up the calculation using the Wang–Landau algorithm. We then describe the experimental results in section III and fit our model to the experimental observations. We use the SPT to determine the effect of crowders on the thermodynamic quantities and discuss the implications. Finally, we use the results to make predictions for future experiments.

II. METHODS

Our protein is coarse grained to a chain of beads and projected as a self-avoiding walk onto a fcc lattice, with a “bead” representing an amino acid residue. The fcc lattice was chosen over the traditional cubic lattice to provide more degrees of freedom. Previous works have found the fcc lattice to be a more natural fit to the secondary structures of α-helices and β-sheets. The choice of lattice is not arbitrary, as higher coordination numbers and different symmetries may better represent the underlying structure. For a summary on the effect of lattice choice see Pieri et al.

Two beads are considered nearest-neighbors if they are on adjacent sites on the lattice. With the underlying lattice being defined by a set of primitive vectors \( e \), we define two lattice points \( \mathbf{x}_i, \mathbf{x}_j \) to be nearest neighbors if there exists a vector \( \mathbf{v} \in e \) such that \( \mathbf{x}_j = \mathbf{x}_i + \mathbf{v} \). For convenience, we define the twelve lattice steps in Cartesian coordinate space \((x, y, z)\) that form the base set of a face-centered cubic lattice

\[
e = \begin{bmatrix}
1 & 1 & 1 & 0 & 0 & 0 & 0 & -1 & -1 & -1 & 1 & 0 \\
1 & -1 & 0 & 0 & 1 & 1 & -1 & 1 & 0 & 0 & 1 & -1 \\
0 & 0 & 1 & -1 & 1 & -1 & 1 & 1 & 1 & -1 & 0 & 0
\end{bmatrix}^T
\]

These twelve vectors define the nearest-neighbors for a given lattice point. Here \( l = 3.8 \, \text{Å} \) is the length scale of the lattice, which is the average spacing between two \( \text{C}_\alpha \) atoms. Let the set of all backbone conformations be denoted by \( C \) with the vector \( \mathbf{c} \in C \) representing an individual conformation on the lattice.

Our model Hamiltonian is a modification of the Go model, where the only energetic contributions are either from the attraction of the residues that are predefined native contacts or from the repulsion of the nonnative ones. The Go model, primarily a model of minimal frustration, typically ignores the potential from non-native contacts. Models with the repulsive terms added create a frustrated energy landscape since more structural information is encoded in the Hamiltonian. In addition, the high coordination number of the fcc lattice does not always admit a unique native state for some structures without the nonnative term. We let \( G \) be a symmetric matrix of native contacts, \( G_{ij} = 1 \) if positions \( i \) and \( j \) are native contacts of the protein, otherwise \( G_{ij} = 0 \).

In addition to the Go-like native contacts we further require that the beads have the correct orientations. To achieve this, each bead has a binary internal state, representing the correct (or incorrect) range of values of its dihedral angles. This is similar to the ideas presented in the Munoz–Eaton (ME) model where each amino acid is allowed two internal states, folded or unfolded. While the ME model has been solved exactly in a restricted form and incorporated into more extensive models, we exploit the fact that the ME model generates a density of internal states that is easily decoupled with the positional microstates, thus leading to more precise estimate of the thermodynamic variables. The permutations of these internal states generate an ensemble of microstates; let the set of all such state sequences be denoted \( J’, \) with the vector \( s \in J’ \) representing a particular sequence of the internal states. It will be useful to refer to the total number of folded beads for a state \( \sigma = \sum s_{ij} \) with the unfolded/folded states defined, indexed by \( s_i = 0/1 \), and amino acid residue count \( L \).

Our Hamiltonian depends on the number of native and non-native contacts of all the beads on the lattice and on their internal state

\[
\mathcal{H}(c, s) = -\sum_{i=1}^{L} \sum_{j=1}^{L} \alpha_{ij} J_{ij} - \sum_{k=0}^{L} \beta_{k} G_{kk}
\]

where \( \alpha_{ij} = 1 \) if residues \( i \) and \( j \) are nearest-neighbors on the lattice and \( J_{ij} \) represent the strength of the Go model’s native and non-native contacts, respectively. A more intuitive form can be written by counting the number of contributions from the native \( k_+ \) and non-native \( k_- \) contacts:

\[
\mathcal{H}(c, s) = -\sum_{i=1}^{L} \sum_{j=1}^{L} \alpha_{ij} J_{ij} \quad k_+ \quad k_-
\]

where \( \alpha_{ij} = 1 \) if residues \( i \) and \( j \) are nearest-neighbors on the lattice and \( J_{ij} \) represent the strength of the Go model’s native and non-native contacts, respectively. A more intuitive form can be written by counting the number of contributions from the native \( k_+ \) and non-native \( k_- \) contacts:

\[
\mathcal{F}(c, s) = \mathcal{H}(c, s) - \beta \Delta \psi(\sigma) - \beta \Delta \mu(c)
\]

Here \( \beta = 1/k_B T, \) \( \Delta \psi(\sigma) \) is the free energy term associated with the entropy of the dihedral angle orientation, and \( \beta \Delta \mu(c) \) is an entropic cost of inserting the protein into a solution of crowders (both terms to be defined in later sections). When the crowders are implicitly modeled as hard-particles, there is only entropic cost for insertion; the term is truly a free energy contribution. If, however, the crowder specifically interacts with the protein, this contribution must be included in the Hamiltonian. The model admits three fitting parameters \( \{J_{ij}, J_{ij}, \beta \} \), with \( h \) setting the energy scale of the dihedral angle term \( \beta \Delta \psi(\sigma) \). Additionally, the crowding term, \( \beta \Delta \mu(c) \), depends on the concentration and the geometry of the crowders.

The positional conformation \( c \) determines the number of non-native contacts \( k_- \); thus we take \( \Omega(c, \sigma, k_-) \) as the density of states. The partition function can be factored by summing up to the maximum number of native contacts \( k_+ \):

\[
Z = \sum_{c} \sum_{\sigma} e^{-\beta \mathcal{F}(\sigma)} = \sum_{c} \sum_{\sigma} \left[ \prod_{i=1}^{N} \left( C(c_i, \sigma_i, k_+) \right) e^{\beta \Delta \psi(\sigma)} \right] \sum_{s_{ij} \in \mathcal{J}, \sum_{k=0}^{L} s_{kk} = 0} e^{-\beta \mathcal{F}(s_{ij})}
\]

A. Conformational Entropy of Dihedral Angles. Associating an entropic cost with the correct dihedral angles is an idea that
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ARTICLE

The finite-graph system is calculated by considering, over all possible finite-graph for a spin-state system. The density of states for this hence the conformations. Each automorphism group defines a

graphs in the same automorphism group are isomorphic to

permutation group, whose members are related by mapping

c

automorphism group to which

belongs. Each Aut((c)) is a
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defined for

models. Usually, when a Potts/Ising-type system is solved,

the underlying graph has a high degree of symmetry (cubic lattices or Cayley trees are common examples). For a typical graph produced by our model, this symmetry is broken, forcing us to numerically compute \( \Omega(c, \sigma, k_{+}) \times X(c) \). However, the number of edges in the graph determine the maximum number of favorable connections. Since this number is small, the convergence of the Wang–Landau algorithm on this portion of the DOS is rapid.

In general, grouping a particular \( c \) to its automorphism group requires solving the graph isomorphism problem multiple times. While specialized algorithms exist,\(^{31}\) the computational solution is unique in its complexity class and lacks a simple invariant that definitively determines isomorphism.\(^{32}\) The problem is greatly simplified if the energetic matrix \( G \) permits only a few, highly degenerate sets of graphs. The Hamiltonian defined for \( \beta \)-sheets happens to be one of these favorable partitions. Since the \( \beta \)-sheet structure is essentially planar, moving perpendicular to the strand direction identifies a column of connections. When abstracted to a graph, the only structure possible is that of a linear chain (unary tree), whose length is limited by the number of strands. The set of conformations \( \Omega \) for \( \beta \)-sheets create graph structures that have a high degeneracy and consequently low cardinality in the set of unique automorphism groups. Additionally, checking for graph isomorphism of linear chains is trivial since these graphs can be

\( \frac{\beta \Delta \psi(\sigma)}{h} \Sigma_{i=1}^{l} s_{i} = h \sigma \)  

\( X_{\sigma}(c) = G_{\sigma} \omega_{\sigma}(c) \)

goest back to the original Zimm–Bragg (ZB)\(^{26}\) and Lifson–Roig (LR) models.\(^{27}\) These models were first used for helix–coil transitions and later extended to include sheets.\(^{28,29}\) Letting each bead have an internal state, native or non-native, allows us to capture some of the detail present in more complex models yet still retain the simplicity inherent in lattice models. It is the lack of spatial degrees of freedom that separate the ZR, LR type models from the one presented here. In our model each conformation defines a new Ising-like subproblem, where we consider the entropy associated with the ensemble of “spins” of only the nearest neighbor contacts. Our model is actually the generalized variant of the spin systems, commonly referred to as the Potts model. There are two major distinctions between the Ising and Potts models; the spin directions are not necessarily restricted to two states and the strength of spins in contact are determined by an interaction matrix. We still retain the two-state model, folded/ unfolded, but our interaction matrix has only a single nonzero connection is favorable (\( G_{ij} = 1 \) if \( |i - j| > 1 \)) in the particular conformation shown in Figure 1a. All of the native connections, which in this case are simply all nearest neighbors, are shown in Figure 1b. Abstracting the representation to a graph in Figure 1c shows the finite system that solves the density of states over the Potts model. Usually, when a Potts/Ising-type system is solved, the underlying graph has a high degree of symmetry (cubic lattices or Cayley trees are common examples). For a typical graph produced by our model, this symmetry is broken, forcing us to numerically compute \( \Omega(c, \sigma, k_{+}) \times X(c) \). However, the number of edges in the graph determine the maximum number of favorable connections. Since this number is small, the convergence of the Wang–Landau algorithm on this portion of the DOS is rapid.

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uniquely determined by a single number, the chain length. These two facts combined significantly speed the computation of the decoupled density of states $\Omega_i(c) \Omega_j(c_k \rightarrow X(c))$.

C. Implicit Crowding Effects. We study the effects of crowders to our protein system using the scaled particle theory. In one of the original formulations of SPT, one calculates the work done to expand a spherical cavity of radius $R$ in a hard sphere fluid of radius $r_c$. The centers of the fluid particles are excluded from the cavity region, which implies that meaningful values of $R$ must be greater than $r_c$. This work $W(R)$ is the configurational part of the chemical potential for a single solute particle $\Delta \mu(c(R))$. The relationship between the probability of any particular configuration and the work required to create it is

$$W(-r_c \leq R \leq 0) = -kT \ln(1 - (4/3) \pi \eta (R + r_c)^3)$$

where $\eta$ is the number density of the solvent. One expands $W(R)$ around $R = 0$ to the second order (noting that the leading term for large $R$ must be the pressure-volume term $4/3 \pi \rho R^3$ with $p$ as the pressure of the fluid) and obtains

$$\beta \Delta \mu(c(R)) = \beta \Delta \mu(c(0)) + \frac{1}{2} \Delta \mu''(c(0)) R^2 + \frac{4}{3} \beta \pi p R^3$$

These terms can be computed by the continuity of $W(R)$ and its first two derivatives at $R = 0$. The pressure can be found by substituting in the exact solution of the Percus-Yevick equation, yielding a density approximation as a function of the packing fraction $\phi$. This density route is not unique among thermodynamic pathways. Expressions have been worked out for both compressibility and viral routes. Each of these pathways amounts to a smoothing in the structural information of the fluid as one "turns-on" the density field. The compressibility and viral routes tend to yield better approximations to the solvation free energy, giving

$$\langle \beta \Delta \mu \rangle_{\text{spherical}} = \frac{\phi(2 - 2\phi - 11\phi^2)}{2(1 - \phi)^3} - \ln(1 - \phi) + \frac{18\phi^3 - 18\phi^2(1 + \phi)}{2r_c} \left( \frac{R}{2r_c} \right)^2 + \frac{8\phi(1 + \phi + \phi^2)}{(1 - \phi)^3} \left( \frac{R}{2r_c} \right)^3$$

The above treatment by SPT assumes, however, that the cavity created is spherical, a condition that is not rigorously satisfied for the crowders nor the proteins examined in this study. The native states of the proteins studied in this work can be well approximated by a right circular cylinder, as the $\beta$-sheet structures are disk-like. Additionally our crowder, Ficoll 70, is known to have an elongated shape and recent predictions model them as spherocylinders with diameters of 28 Å and an end-to-end length of 184 Å.

The extension of SPT to work with aspherical mixtures can be expressed through the activity coefficient $\gamma_i$. The activity coefficient $\gamma_i$ is the measure of the deviation of the $i$th species at the actual composition of the solution from the chemical potential of an ideal solution as given by the equation

$$RT \ln \gamma_i = \mu_i - \mu_i^0$$

where $\mu_i^0$ is the chemical potential of a reference state. For hard-convex particles the nonideality of a particular species of interest can be found by computing an expression as a function of the volume $V_i$, surface area $S$, and the Kihara support function $H_i$ of that species, given by

$$\ln \gamma_i = -\ln(1 - \langle V \rangle) + \frac{H_i(S) + S \langle H \rangle + V_i(1)}{1 - \langle V \rangle} + \frac{H_i^2(S)^2}{2(1 - \langle V \rangle)^2} + \frac{V_i(H^2)(S)^2}{3(1 - \langle V \rangle)^3}$$

where $\langle X \rangle = \Sigma \rho_i X_i$ and $\langle 1 \rangle = \Sigma \rho_i$. If we scale the radius and length so all beads fit inside. Using eq 11 and 12, we can determine the free energy due to crowders in our system.

D. The Wang–Landau Density of States Method. Wang–Landau (WL) sampling is a generic algorithm to calculate the relative density of states (DOS) for a given system. The algorithm starts off with the initial a priori ansatz that all conformational states are equally likely $\Omega(\zeta) = 1$, where $\zeta$ is a conformation of the system. Traditionally, the calculation of the density of states was computed as a function of the energy of the system. Like others, we use the Wang–Landau method to determine the density of states for a conformation of the system rather than the energy explicitly. Each conformation is still directly related to a numerical energy. By calculating the DOS for conformations, we can delay the calculation of the energy. This has the advantage that multiple simulations are not required for each set of the system parameters ($J_i$, $J_{ii}$, $h_i$, $\phi_i$, $r_c$).

In the WL method, the density of states is iteratively refined, crudely at first to ensure a large sampling, and then with greater precision as $\Omega$ converges. Similar to a typical Monte Carlo simulation, the algorithm has an acceptance rate. However, unlike traditional Metropolis–Hastings simulations, the process can not be modeled as a Markov chain since the transition matrix itself is iteratively refined. The WL acceptance rate is

$$P(\zeta_A \rightarrow \zeta_B) = \min \left( \frac{\Omega(\zeta_A)}{\Omega(\zeta_B)} \frac{n_B - A}{n_A} \right)$$

where $n_A$ is the number of outgoing moves from states A and $n_{A-B}$ is the number of moves from A to B (similarly defined for $n_B$ and $n_{B-A}$). If the moves are reversible, then $n_{B-A}/n_{A-B} = 1$. These factors are necessary for detailed balance if the move set
chosen has a variable number of moves from each state. Once any particular state had been selected, the density of states is modified by Ω(ζ) → Ω(ζ), where j is a constant that is slowly reduced to unity during the simulation. All of the computations carried out in this paper took f0 = ε̃' = 2.71828, final = ε̃' − 9, and f_j+1 = (f_j)^(1/2).

Each time a conformation was selected, the DOS is updated along with a histogram of visits for that conformation H(ζ). The factor j was reduced when H(ζ) was no less than 90% of the average number of visits for all conformations (H(ζ)). Once the factor j had been reduced, we reset the histogram of visits for all conformations, H(ζ) = 0, and began the process again.

Our move set consists of pull moves, which were first defined over a cubic lattice and later for triangular lattice models. Pull moves are an ergodic, reversible move set that modify the positional conformations by moving the chain along a path defined by a pair of beads adjacent in chain sequence (i, i + 1). Since the number of moves are finite and easily computable, we can quickly determine the factors necessary for detailed balance (i.e., n_m, n_m−1, etc.).

When converged, the WL method gives a flat histogram. That is, the averaged fraction of time spent at each macrostate approaches the same constant. Here we define a macrostate as the set of all conformations with the same energy level. Not every microstate is visited during the simulation, nor would it be possible due to the exponential growth in the DOS as a function of chain length. We assume that the visits to each state are ergodic; subsequent visits to a macrostate will visit each conformation an equal number of times over long averages. This idea is reasonable when we consider detailed balance is obeyed for the Monte Carlo simulation and is employed by Wust and Landau.

We exploit this observation to determine a probability distribution for a second observable as a function of the first. For instance, we can step through the conformations to compute the probability distribution of the activity coefficient for the protein in a particular conformation c. Doing so prevents the need for a multiplicative increase in the density of states (thus speeding up the convergence of the WL algorithm), yet it still provides us with a reasonable estimate of an extended DOS Ω(c,r_c).

III. RESULTS

A. Model Calibration. Unlike α-helices, the β-sheet motif has been difficult to study experimentally due to its propensity to aggregate. Recently there has been a spate of designed peptides that exhibit the β-sheet motif, albeit with extremely broad thermal transitions. We consider and describe below, three experimentally designed β-sheets peptides used in this study. These designed proteins were specifically chosen to test the positional conformations by moving the chain along a path defined by a pair of beads adjacent in chain sequence (i, i + 1). Since the number of moves are finite and easily computable, we can quickly determine the factors necessary for detailed balance (i.e., n_m, n_m−1, etc.).

The first peptide (sequence: RFSEV[P][PG]KKFITS[P][PG]-KTYTEV[P][PG]KKILQ, nicknamed [PG]P(P)P) is a 28-residue chain with a natural four-strand β-sheet structure. This designed peptide was studied experimentally by Xu et al. as an extension of the peptide [P]P-P-II first proposed by Gellman and co-workers. A schematic model of the native state for the peptide is shown in Figure 2a. The second peptide (sequence: RFIEVP[P][PG]KKFITS[P][PG]KTYTE, nicknamed [P]P(P)P) is a 20-residue chain with a natural three-strand β-sheet secondary structure.

The final peptide (sequence: GEWTD[AT][K]TWTTWE, nicknamed trpzip4-m1) is a 16-residue variant of the tryptophan zippers studied by Cochran et al. and later by Du et al. Compared to the designed peptides, the tryptophan zippers have significantly higher stabilities (with a difference of approximately ΔG_change ≈ 1.0 kcal mol⁻¹ at 298 K).

An artifact of the fcc lattice forces the β-hairpin to be made over an odd number of lattice sites; thus we replace the Pro-Gly residues in the first two designed proteins and the Ala-Thr residues in the third peptide trpzip4-m1, with a combined residue (denoted with a square bracket, e.g., [PG]). The experimental measurements on these peptides have been carried out using temperature jump experiments, for details see refs 8 and 10.

We use the WL method to calculate the conformations of the positional Ω_1(c) and orientational Ω_2(σ,ζ,ξ(c)) density of states. The model is calibrated by fitting J_+, J_−, and h to the data of three experiments. We calculate the fraction of β-sheet contacts, observable from the experiments, by taking the expectation of (ζ(θ(α))). Here θ is the standard Boltzmann average, θ is the Heaviside step function, and ζ measures how close the protein is to its native state. Since the experimental data measure the fraction of β-sheet contacts (inferred from a circular dichroism measurement), we calibrated our model with physically similar observable,

$$\alpha(c) = \langle k_+ - k_- \rangle / k_+$$  (14)

This fraction of β-sheet contacts was used to calibrate the three free parameters. We note that without the non-native term J_− in the Hamiltonian in eq 2 the fraction of sheet contacts would be, as usual, k_+/k_+. In Figure 3 we show the fits of the two proteins trpzip4-m1 and [P]P(P)P with the fitting parameters given in Table 1. The fits are quite good, encouraging us to make predictions about the system behavior as a function of crowding packing fraction. It is worth noting that the three- and four-stranded designed β-sheets had the best fits with J_− = 0, implying that additional stabilization provided by the term was needed only to model trpzip4-m1. This may not be surprising when the larger melting points and the broad thermal transitions of the designed proteins are considered versus those of the smaller β-hairpin peptide (listed in Table 2).

B. Effects of Crowders. In this section we use the model defined above to study the effects of crowders on peptide/ protein structures and stability. For one of the peptides we have the experimental results on crowding effects to compare with. In
Figure 3. Experimental data of fraction folded versus temperature for trpzip4-m1 (blue circles) and the three stranded β-sheet ΔFΔPΔP (red diamonds). Model fits are shown with dashed lines of the same color. The thin (black) vertical lines are shown to mark the critical temperatures at 36.1 and 48.5 °C for three-stranded and trpzip4-m1 peptides, respectively. The fit for the four-stranded β-sheet ΔFΔPΔP is similar to the three strand and is not shown for clarity.

The paper by Mukherjee et al.9 a significant change (approximately 12 °C) in the melting point of trpzip4-m1 was observed under crowded conditions. The crowder chosen for this experiment was Ficoll 70 (F70) at a concentration of 200 mg/mL. F70 is a compact, highly cross-linked branched copolymer of sucrose and epichlorohydrin with an average molecular weight of 70 000.46 At 200 and 300 mg/mL the packing fractions are approximately \( \phi = 0.13 \) and \( \phi = 0.20 \), respectively.47,48 We study the effects of crowders by considering the specific heat, \( C_v(T) = \beta^2 \left( \langle E^2 \rangle - \langle E \rangle^2 \right) \) and note that in all cases, we observe only a single maxima. We identify this maxima as the melting temperature \( T_c \) (alternatively, \( (\partial C_v / \partial T)|_{\gamma} = 0 \)).

Heat capacity as a function of temperature for trpzip4-m1 is shown in Figure 4 while the melting points for all peptides are listed in Table 2. As expected, trpzip4-m1 displays crowding enhanced stability with the change of critical temperature \( \Delta T_c = [1.03, 1.65] °C \) at packing fractions \( \phi = [0.13, 0.20] \), respectively. However, the three- and four-stranded β-sheets exhibit a slight decrease in their critical temperatures with \( \phi \), indicating an entropically based instability caused by the crowders.

The native state for the three- and four-stranded β-sheets are highly aspherical. When the entropic effects of crowders are considered, the system prefers compact conformations that minimize the excluded volume effect. As a consequence of this, the native state ceases to be the minimum free energy conformation at large enough \( \phi \). Crowding-induced conformational change of the native state has been observed experimentally in a recent work by Dhar et al.48 who studied phosphoglycerate kinase (PGK) with the same crowders as our simulations (Ficoll 70). In this study, the conformational states were changed dramatically with crowders; an optimal nonzero packing fraction of crowders was found to increase the protein’s activity. In our simulation, there was no shift to a new distinct native state at higher crowding concentrations. Rather, we observed a gradual shift toward more compact conformations at the expense of breaking energetically favored bonds, a general collapse of the β-sheet. As an example of the native conformation, which is enthalpically favored, versus an entropically favored one (Figure 5). Previous studies that showed crowding enhanced stability often dealt with globular wild-type proteins, whose natural environment required them to operate in crowded conditions. In contrast to the PGK study, the two larger peptides in our study were not wild-type, rather they were designed and studied because of the fact that they folded into β-like conformations at realistic temperatures without aggregation. This suggests further experimentation on the designed peptides to determine if the destabilization of the native state against those of the unfolded and intermediate states under crowded conditions can be observed experimentally.

To assess the effect on the conformational states, we examine the Boltzmann averaged excess chemical potential from the native state as a function of temperature and \( \phi \)

\[
\beta \langle \Delta \mu_{\text{ex}}(T) \rangle = \langle \ln \gamma_i - \ln \gamma_N \rangle
\]  

Figure 6 of this free energy term for trpzip4-m1 illuminates several interesting structural features from an ensemble perspective. At large temperatures we see that this excess chemical potential approaches a constant, proportional to the change in the unfolded states due to the crowders. Conversely, at very low temperatures crowders have no effect on the only viable con-

Table 2. List of the Experimental and Model Melting Points (°C) for Each Peptide

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Experiment</th>
<th>( \phi = 0 )</th>
<th>( \phi = 0.13 )</th>
<th>( \phi = 0.20 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>trpzip4-m1</td>
<td>32.1 ± 0.9</td>
<td>36.12</td>
<td>37.15</td>
<td>37.76</td>
</tr>
<tr>
<td>three-stranded</td>
<td>52.6 ± 0.4</td>
<td>48.49</td>
<td>48.23</td>
<td>48.10</td>
</tr>
<tr>
<td>four-stranded</td>
<td>50.5 ± 0.8</td>
<td>49.18</td>
<td>49.01</td>
<td>49.04</td>
</tr>
</tbody>
</table>

* The experimental melting points are taken from refs 8 – 10 in a dilute solution without crowders. The calculated melting points from the model are given at the listed values of the packing fraction. Not shown is the experimental value of trpzip4-m1 in the Ficoll 70 solution of 200 mg/mL with \( T_c = 44.0 ± 0.2 °C \).
The predictions of SPT, we found crowding-induced stability, in qualitative agreement with experiment for the smaller peptide, trpzip4-m1. The effect predicted by this model showed a modest change of about $\approx 1\, ^\circ C$, in contrast to the large change observed in the experiments of Mukherjee.\(^9\) We note, however, that these coarse-grained models are approximations and selectively ignore various interactions. The study presented here is an entropic one. If the crowders have enthalpic interactions with the peptide, then these predicted effects will be incomplete. We attribute this underestimation to effects that cannot be explained by excluded volume effects alone.

We found that that the model predicted instability for the designed three- and four-stranded $\beta$-sheet peptides. This is consistent with the observation that their native state does not minimize excluded volume effects (it is disk-like rather then globular). This observation alone, however, is not sufficient to predict of crowding based instability. Even if the native state is nonideal, one has to consider the entire ensemble of states as a whole. This was possible using the Wang–Landau method, which allowed us to accurately determine the density of states under the constraints of our model.

The extension of the Go-like contact map to a finite graph presented here is not limited to the $\beta$-sheet motif. This entropic model of conformational states can be extended to $\alpha$-helices or a mixture of secondary structures, as long as the contact graph structure can be decomposed into simple degenerate forms.

## AUTHOR INFORMATION

**Corresponding Author**

*E-mail: hoppe@drexel.edu.*

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