Polypeptide Interactions at Ice and Biomineral Interfaces Are Defined by Secondary Structure-Dependent Chain Orientations†

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The stereospecific interaction of polypeptides with biominerals and ice represents a unique phenomenon in nature. Here, using the theoretical geometric lattice matching algorithm, CHASM (abbreviation for *ch*ain *a*lignment on the *s*urface of *m*aterials), we explore the geometric complementarity of specific C_α polypeptide secondary structures (α-helix, β-sheet, γ-turn) with the [001] planes of the biominerals, aragonite and calcite, and the [001], [100], and [201] faces of hexagonal ice. We find that certain secondary structure−surface pairings exhibit a defined set of orientational minima that agree closely with published results. Moreover, no two secondary structures feature the exact same set of global *and* local orientational minima for a given surface. *This suggests that the molecular complementarity of each secondary structure with a given surface is highly specific and cannot be mimicked by another secondary structure.* These results suggest that the site and orientation of polypeptide adsorption onto biominerals or ice may be influenced by secondary structure type.

Introduction

The stereospecific interaction of polypeptides with biominerals[1] and ice[2,3] represents a unique phenomenon in nature. What underlies these interfacial interactions is the concept of "molecular complementarity";[4,5] i.e., the molecular surface of the polypeptide complements the atom array at the crystalline surface. This type of interfacial interaction results in catalytically or epitaxially induced crystal growth or inhibition.[1−3] A number of mimetic approaches have evolved that emulate polypeptide−crystal interactions; these include surfactant assemblies,[6] Langmuir−Blodgett films,[7] polymer thin films,[8] and microemulsions,[9] all of which can synthesize specific inorganic materials. Biomineral− and ice−polypeptide interactions can also serve as ideal models for understanding the general phenomenon of biopolymer−material interactions. Thus, there is a critical need to explore the energy landscape of the polypeptide−crystal surface.

Recent studies of polypeptide interactions with ice and biominerals indicate that the interfacial stability between the polypeptide chain and a given Miller plane is defined *in part* by specific polypeptide chain *orientations* with respect to the surface lattice vectors.[2,10−14] Recently, we devised a geometric lattice-matching algorithm, CHASM-P (abbreviation for *ch*ain *a*lignment on the *s*urface of *m*aterials, dislocation energy *p*arameter method), which is capable of predicting the backbone orientation of a given polymer chain adsorbed onto a specific material surface, using the lattice unit cell spacings for both species.[15] In this Letter, we outline a novel approach that expands the application of CHASM-P to both well-characterized and hypothetical polypeptide−surface interactions. Instead of requiring the actual lattice coordinates for the polypeptide species, we input a "reduced" C_α lattice[16] representation (i.e., two-dimensional square lattice) for different polypeptide secondary structures. Using this "generic" approach, we examine the angle of polypeptide chain adsorption, θ, versus dislocation energy parameter, P (Figure 1),[15,17] for polypeptide secondary structures with biomineral and ice surfaces (Table 1), with comparison against available published findings or theories.[1−3,10,11,13,18−21] Our findings indicate that secondary structure influences the optimal alignment of the adsorbed polypeptide chain at a given surface, in agreement with other studies.[1−3,10,11,13,18−21] Moreover, we find that the CHASM-P-"reduced" C_α lattice approach can serve as a novel predictive tool for the selection of "initial guess" polypeptide−surface starting geometries, which can serve as starting points for more accurate modeling methods.

Methods and Results

A more detailed description of the methods involved in this study can be found elsewhere in this Letter.[15,17] We first utilize CHASM-P to investigate the nacreous layer of the mollusk *Nautilus repertus*, a composite of aragonite ($CaCO_3$), β-sheet Gly, Ala-rich "silk-fibroin-like" scaffolding polypeptides, and other "acidic" proteins.[1,21,22] The mineral and Ala, Gly-rich "silk-fibroin-like" β-sheet polypeptide display an epitaxial relationship: the $a-b$-plane of aragonite is matched with the planar β-sheet structure of the proteins, as determined by X-ray diffraction.[21] Hence, this system serves as a reasonable experimental benchmark for testing the usefulness of CHASM-P. Using polypeptide lattice parameters derived from X-ray diffraction studies,[21] CHASM-P calculates a global minimum orientation of 0°, in agreement with the experimentally observed alignment for the Ala, Gly-rich "silk-fibroin-like" β-sheet polypeptide with the aragonite [001] plane.[21] As observed for all other data sets in this report, geometrically permissable *local* minima were also identified (Figure 2A, Table 1).[15] For comparison, a "generic" β-sheet structure (i.e., 2.69 Å lattice spacing) representing an aragonite-specific "acidic" biomineralization polypeptide[22] was also matched to the aragonite [001] surface (Figure 2A, Table 1), yielding global minima of 0° and 90°. If we consider the global alignment for a generic α-helix (3.80 Å spacing) with the aragonite [001] surface, we find that the α-helix also exhibits an orientation of 0°. However, *note that the local minima for 3.80 and 2.69 Å lattices are different*.

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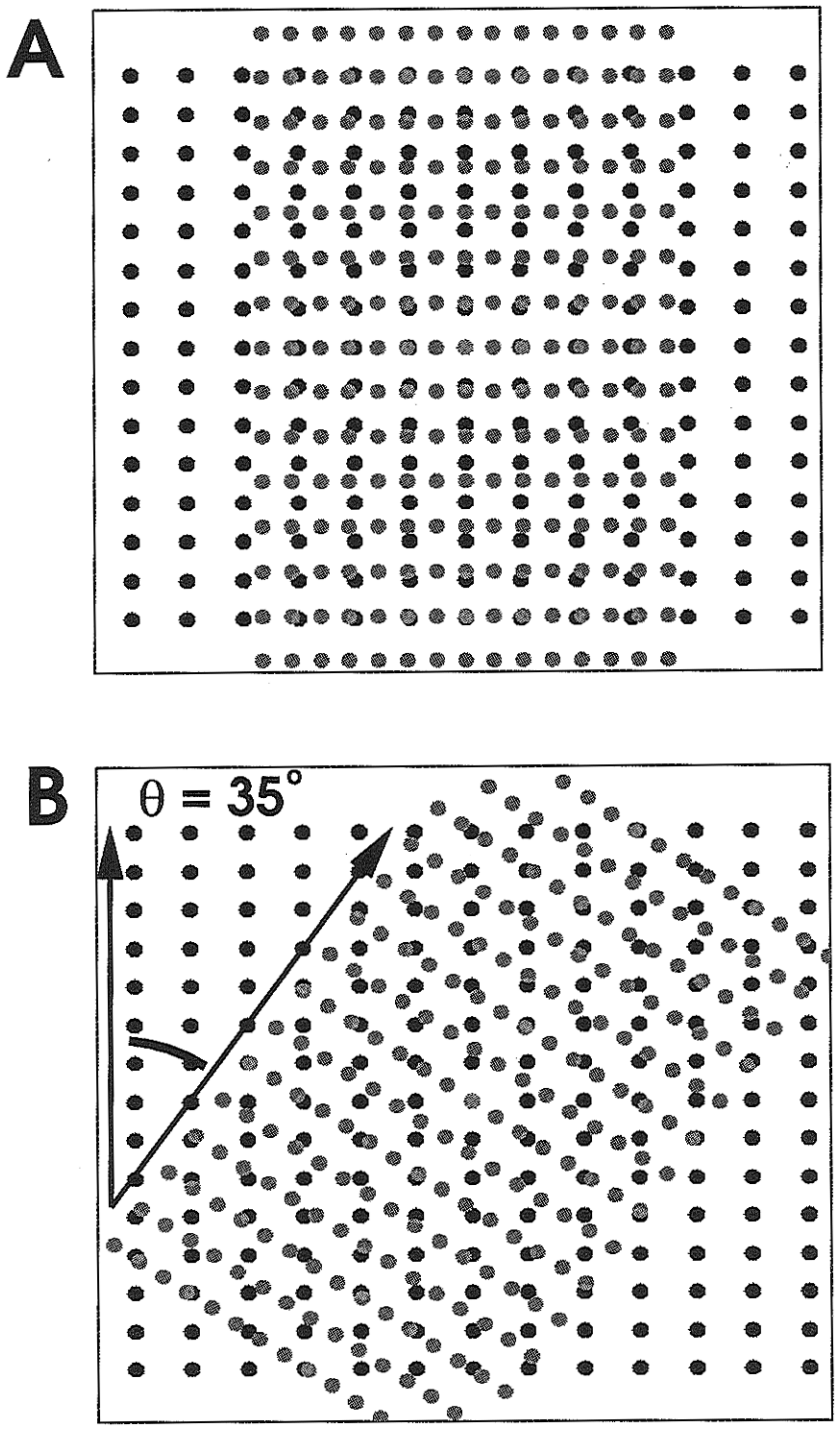

Figure 1. Example of a CHASM-generated θ rotation and resulting moiré pattern for the Ala, Gly-rich "silk-fibroin-like" β-sheet polypeptide (lattice **1**, dark gray points) on the aragonite [001] surface (lattice **2**, medium gray points). (A) $\theta = 0°$; (B) $\theta = 35°$. The angle of lattice **2** rotation is shown in (B). As seen in (A) and (B), a lattice point overlap or coincidence pattern is generated (light gray points), giving rise to a θ-dependent periodic moiré pattern.

To explore this further, we also examined the matching of both types of secondary structures with the calcite [001] plane. The interaction of the predominantly β-sheet acidic proteins[1,21,22] with the [001] calcite surface has been observed *in vitro*.[1,5,22] CHASM-P matching of the calcite [001] plane with each C_α lattice revealed that the global alignment minimum for the β-sheet is 0° (Figure 2B, Table 1) with geometrically equivalent minima at 30, 60, and 90°), in full agreement with previous modeling studies of anionic β-sheet polypeptides with calcite.[13] However, the α-helix possesses an optimal alignment angle of 15° with respect to the [001] surface (Figure 2B, Table 1) with geometrically equivalent minima repeated at 30° intervals). In other words, *the C_α spacing of the α-helix does not permit the polypeptide axis to align with the calcite [001] lattice vectors with the same orientations as the β-sheet*. Hence, each secondary structure exhibits a unique interaction with the basal planes of aragonite and calcite.

Next, we compare secondary structure matching to ice surfaces. Polypeptide interactions with ice take on several forms; for example, many strains of bacteria demonstrate ice-nucleating ability that is conferred by surface-bound membrane proteins comprised of organized antiparallel β-sheet domains.[2] These β-sheet domains have an epitaxial relationship with the basal plane [001] of the hexagonal form of ice (I_h) and are

TABLE 1[a]

lattice **1**[b] **[a** (Å)/**b**(Å)/α (deg)]	lattice **2**[b] [**a** (Å)/**b**(Å)/α (deg)]	CHASM minima ($0 \le \theta \le 90$) (deg)	deduced orientations (deg)[ref]
"silk fibroin-like" [6.9/9.5/90]	aragonite [001] [7.97/4.96/90]	***0***, 35, 50, 60, 90	**0**[21]
α-helix [3.8/3.8/90]	aragonite [001]	*0*, 37, 56	N/A[c]
β-sheet [2.69/2.69/90]	aragonite [001]	***0***, 25, 42, 70, *90*	**0**[21]
α-helix	calcite [001] [4.98/4.98/120]	*15*, *45*, *75*	N/A
β-sheet	calcite [001]	***0***, *30*, *60*, *90*	**0**[13]
α-helix	ice [001] [4.516/4.516/120]	*15*, *45*, *75*	N/A
β-sheet	ice [001]	***0***, *30*, *60*, *90*	**0**[2]
γ_m-turn [3.78/3.78/90]	ice [100] [4.516/7.354/90]	***0***, 56, 90	**0**[18]
β-sheet	ice [100]	0, *42*, 70, 77	N/A
α-helix	ice [201] [15.386/4.516/81.561]	***8***, **62**	**9**, **63**[19,20]
β-sheet	ice [201]	8, *46*	N/A

[a] All θ given in degrees. **Boldface** values indicate correlated minima between CHASM and experiment; *italicized* values represent the smallest *P* value obtained for a given θ, i.e., a value considered to be a global orientation minimum. Note that there are CHASM minima that are not experimentally observed. "Deduced orientations" refer to alignments that were calculated by us from the findings of previous published polypeptide−surface interaction studies. [b] Lattice **1**, **2** refer to CHASM lattices utilized in determining orientational mimina; **a**, **b**, = lattice vectors, α = intervector angle.[15] [c] N/A = data not available.

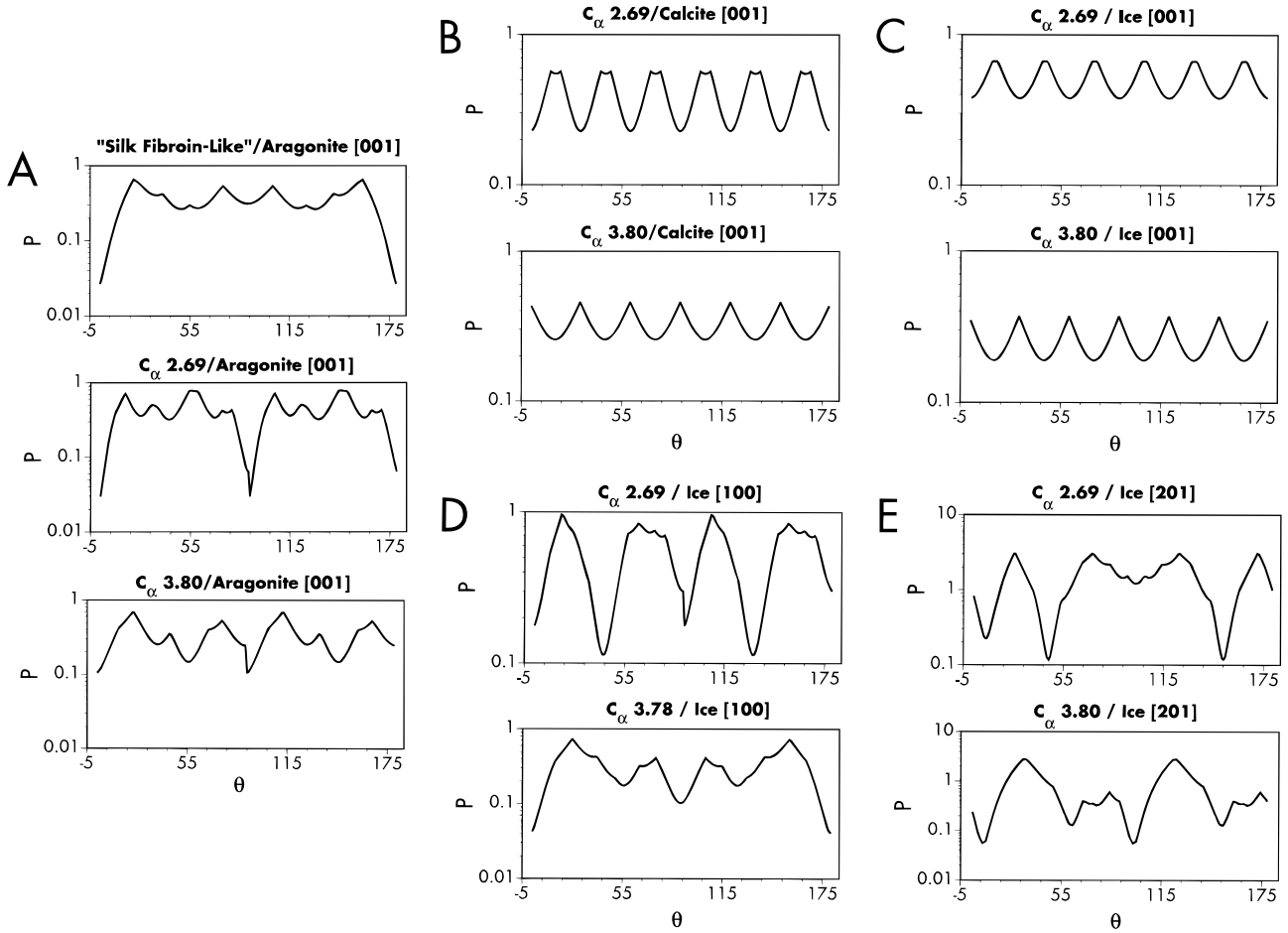

Figure 2. CHASM-P $\theta-P$ semiLog orientation plots for polypeptide−biomineral and polypeptide−ice surfaces: (A) aragonite [001] surface with silk fibroin, *β*-sheet, α-helix; (B) calcite [001] surface with *β*-sheet, α-helix; (C) ice [001] surface with *β*-sheet, α-helix; (D) ice [100] surface with *β*-sheet, γ_m-turn; (E) ice [201] surface with *β*-sheet, α-helix. *P* has dimensionless units.

aligned so that the placement of Ser, Thr, Tyr side chains is superimposable with that of the ice surface.[2] CHASM-P predicts the global orientation of the *β*-sheet to be 0°, and at intervals of 30° from this angle (Figure 2C, Table 1). This predicted global orientation is consistent with previously published theoretical findings.[2] However, the global orientation for the α-helix was found to be at 15°, and at 30° intervals from this angle (Table 1). In contrast to the ice nucleation system, many cold-water fishes utilize polypeptide (AFP)[10−12,19,20,23] and glycopeptide (AFGP)[3,18] antifreeze agents

to inhibit ice formation in their blood sera. A subset of the AFP family, the AFP type I polypeptides, exist primarily as single amphipathic α-helices,[10−12] which bind to the [201] bipyramidal planes of I_h.[11,19,20] The type III AFP polypeptide, which consists of orthogonally arranged β-sheet structures, is presumed to bind to the prismatic {100} face of I_h.[23] The AFGP glycopeptide adopts a mirror-related γ-turn (γ_m),[24,25] and binds to the first-order prismatic plane [100].[3,18] For the I_h [100] plane, CHASM-P predicts that the AFGP γ_m lattice (3.78 Å) preferentially aligns with the *a*-axis of the ice surface (global minimum = 0°) in accord with the deduced orientation (Figure 2D,Table 1).[18] However, the β-sheet, which would presumably represent the AFP Type III polypeptide, exhibits a different *global* orientational minima (Figure 2D, Table 1) but has one orientational *local* minima (0°) that coincides with the global minima for AFGP. At this time, there is no information available for the adsorption orientation for AFP type III for comparison.[23] For polypeptide interactions with the [201] bipyramidal plane, CHASM-P calculations demonstrate that an α-helix exhibits global (8°) and local (62°) alignment angles within 1° of the alignments calculated elsewhere for the ice-binding motifs of the α-helical AFP type I (Figure 2E, Table 1).[19,20] Conversely, the β-sheet complements the [201] plane at a global minima at 46°, with a local minimum at 8°. Hence, *compared to the* β-sheet, a "helical" polypeptide structure (γ_m-turn, α-helix) exhibits a different interaction with the I_h [100], [201], and [001] planes, in agreement with previously published findings for polypeptides and biominerals.

Discussion

As shown herein, CHASM-P predicts backbone global alignment(s) for different pairings of secondary structures and periodic surfaces and demonstrates reasonable correlation with previously published data (Figure 2, Table 1). Moreover, using CHASM-P, we can predict the alignment for polypeptide−interfacial interactions for which no experimental data yet exists. Examples include α-helix and β-sheet adsorbed onto the [001] face of calcite and the [100] plane of ice, respectively (Table 1); these systems could be experimentally examined to verify or refute the theoretical prediction. However, the reader should note that there are potential limitations to the method. The population of a given adsorption minimum can be influenced by factors such as chain−chain interactions, chain-packing densities, side chain−surface interactions, and surface irregularities, such as pits, fissures, lattice defects and dislocations.[15] CHASM-P determines *only* the geometric conditions for lattice matching and will locate *all* possible orientations where there is matching between the superimposed lattices (e.g., the "local" minima given in Table 1).[15] *Obviously, the use of simple 2-D models fails to account for the importance of side chain−surface interactions on minima selection, the three-dimensionality of the polypeptide chain, or the contributions arising from topological features such as surface irregularities, pits, dislocations, and so on.* Hence, CHASM-P should be viewed as a preliminary simulation step to obtain an "initial guess" polypeptide−surface orientation. These initial orientations can then serve as the basis for more rigorous exploration using energy methods such as minimization and molecular dynamics.

Perhaps the single most important finding is that the 3.80, 3.78, and 2.69 Å C_α lattices differ in their geometric matching to the lattice structure of a given biomineral or ice surface. Although similar *global* alignment minima are found for some secondary structures adsorbed onto certain surfaces, *no two secondary structures feature the exact same set of global and local minima for a given surface* (Table 1). This suggests that the interaction of each secondary structure with a given surface may be highly specific and cannot be entirely mimicked by another secondary structure. This conclusion gives rise to an interesting principle: *by utilizing predominately one type of secondary structure or a combination of secondary structures within a polypeptide sequence, the site and orientation of polypeptide adsorption onto a surface could be controlled*. More importantly for the chemist, the manipulation of these principles could lead to vast improvements in nanocomposite material synthesis.

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References and Notes

(1) Lowenstam, H. A.; Weiner, S. *On Biomineralization*; Oxford University Press: New York, 1989; pp 1−100.
(2) Kajava, A. V.; Lindow, S. E. *J. Mol. Biol.* **1993**, *232*, 709.
(3) Wen, D.; Laursen, R. A. *Biophys. J.* **1992**, *63*, 1659.
(4) Mann, S.; Archibald, D. D.; Didymus, J. M.; Douglas, T.; Heywood, B. R.; Meldrum, F. C.; Reeves, N. J. *Science* **1993**, *261*, 1286.
(5) Mann, S. *Nature* **1993**, *365*, 499.
(6) Askay, I. A.; Trau, M.; Manne, S.; Honma, I.; Yao, N.; Zhou, L.; Fenter, P.; Eisenberger, P. M.; Gruner, S. M. *Science* **1996**, *273*, 892.
(7) Hughes, N. P.; Heard, D.; Perry, C. C.; Williams, R. J. P. *J. Phys. D: Appl Phys.* **1991**, *24*, 146.
(8) Bianconi, P. A.; Lin, J.; Strzelecki, A. R. *Nature* **1991**, *349*, 315.
(9) Yang, H.; Coombs, N.; Sokolov, I; Ozin, G. A. *Nature* **1996**, *381*, 589.
(10) Sicherl, F.; Wang, D. S. C. *Nature* **1995**, *375*, 427.
(11) (a) Wen, D.; Laursen, R. A. *Biophys. J.* **1992**, *63*, 1659. (b) Laursen, R. A.; Wen, D.; Knight, C. A. *J. Am. Chem. Soc.* **1994**, *116*, 12057.
(12) Yang, D. S. C.; Sax, M.; Chakrabartty, A.; Hew, C. L. *Nature* **1988**, *333*, 232.
(13) Wierzbicki, A.; Sikes, C. S.; Madura, J. D.; Drake, B. *Calcif. Tissue Int.* **1994**, *54*, 133.
(14) Berman, A.; Hanson, J.; Leiserowitz, L.; Koetzle, T. F.; Weiner, S.; Addadi, L. *J. Phys. Chem.* **1993**, *97*, 5162.
(15) Reeves, N. J.; Evans, J. S. *J. Phys. Chem.* **1996**, *100*, 17297. A two-dimensional lattice is constructed for both the surface and for the polypeptide; typically, lattice **1** represents the largest dimension lattice (see Table 1). We then generate lattice **2** using transformation (**S**) and rotation (**R**) matrix operators; this leads to superimpositioning of the two lattices as a function of the rotation angle, θ. This gives rise to a "coincidence-site" or "o"-lattice, which is a common lattice containing the lattice points of **1** and **2**. For noncommensurate protein−surface lattices, we then evaluate this o-lattice for optimum lattice matching by calculating the dislocation energy parameter, *P* (CHASM-P)

$$P = \frac{|\mathbf{b}_1|^2|\mathbf{p}_2^o|^2 + |\mathbf{b}_2|^2|\mathbf{p}_1^o|^2}{|\mathbf{p}_1^o \times \mathbf{p}_2^o|} \quad (1)$$

where $\mathbf{b}_1$, $\mathbf{b}_2$ are the lattice vectors of lattice **1** and $\mathbf{p}_1^o$, $\mathbf{p}_2^o$ are the o-points of lattices **1** and **2**, respectively.
(16) Godzik, A.; Kolinski, A.; Skolnick, J. *J. Comp. Chem.* **1993**, *14*, 1194.
(17) We create a two-dimensional lattice (i.e., square lattice, with intervector angle α = 90° typical of a three-dimensional protein cubic lattice); we then input the $C_\alpha-C_\alpha$ spacing that approximates a particular secondary structure[16] or is derived directly from experiment (e.g, the Gly, Ala-rich "silk-fibroin-like" scaffolding polypeptides, Table 1). Note that we do not "compress" a three-dimensional polypeptide structure onto a two-dimensional lattice. The "reduced" polypeptide 2-D lattice is a two-dimensional periodic array whose vertices represent only the C_α atoms of the polypeptide. This is then input into the CHASM-P program along with the known lattice parameters (lattice vectors **a**, **b** and intervector angle α) for each interfacial surface (Table 1). By mapping the complementarity of lattice points of the protein lattice with the surface lattice as a function of lattice orientation (θ), we obtain a number of mimina and maxima (Figure 1).[15] A global minimum orientation is defined to occur at the smallest value of *P*, or the dislocation energy parameter, for a given value of θ; all other minima are considered "local".[15] *P* is calculated over a range of lattice orientations (0.1° increments) from 0 to 180° for the purpose of ascertaining continuity in θ vs *P* transitions (which, in fact, are observed; see Figure 2A−E).

(18) Knight, C. A.; Driggers, E.; DeVries, A. L. *Biophys J.* **1993**, *64*, 252.

(19) Knight, C. A.; Cheng, C. C.; DeVries, A. L. *Biophys. J.* **1991**, *59*, 409.

(20) (a) Madura, J. D.; Wierzbicki, A.; Harrington, J. P.; Maughon, R. H.; Raymond, J. A.; Sikes, C. S. *J. Am. Chem. Soc.* **1994**, *116*, 417. (b) Wierzbicki, A.; Talyor, M. S.; Knight C. A.; Madura, J. D.; Harrington, J. P.; Sikes, C. S. *Biophys J.* **1996**, *71*, 8.

(21) (a) Weiner, S.; Traub, W. *FEBS Lett.* **1980**, *111*, 311. (b) Weiner, S.; Talmon, Y.; Traub, W. *Int. J. Biol. Macromol.* **1983**, *5*, 325.

(22) Falini, G.; Albeck, S.; Weiner, S.; Addadi, L. *Science* **1996**, *271*, 67.

(23) (a) Sonnichsen, F. D.; Sykes, B. D.; Chao, H.; Davies, P. L. *Science* **1993**, *259*, 1154. (b) DeLuca, C. I.; Chao, H.; Sonnichsen, F. D.; Sykes, B. D.; Davis, P. L. *Biophys. J.* **1996**, *71*, 2346.

(24) Drewes, J. A.; Rowlen, K. L. *Biophys. J.* **1993**, *65*, 985.

(25) Krimm, S.; Bandekar, J. *Adv Prot. Chem* **1986**, *38*, 181. Using the ϕ, ψ, and ω values for the three-residue γ_m turn, we calculate $C_\alpha - C_\alpha$ to be 3.78 Å, which we utilized along with $\alpha = 90°$ to construct the AFGP polypeptide C_α lattice.