REPORT DOCUMENTATION PAGE

Form Approved OMB NO. 0704-0188

1. REPORT DATE (DD-MM-YYYY)	2. REPORT TYPE	3. DATES COVERED (From - To)
27-05-2020	Final Report	1-Jun-2016 - 30-Nov-2019

4. TITLE AND SUBTITLE
Final Report: 7.4 Reactive Chemical Systems: Effects of Nanoscale Chemical Heterogeneity on Hydrophobic Interactions and Molecular Assembly at Surfaces

5a. CONTRACT NUMBER
W911NF-16-1-0154

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER
611102

6. AUTHORS

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS (ES)
U.S. Army Research Office
P.O. Box 12211
Research Triangle Park, NC 27709-2211

10. SPONSOR/MONITOR'S ACRONYM(S)
ARO

11. SPONSOR/MONITOR'S REPORT NUMBER(S)
68753-CH.4

12. DISTRIBUTION AVAILIBILITY STATEMENT
Approved for public release; distribution is unlimited.

13. SUPPLEMENTARY NOTES
The views, opinions and/or findings contained in this report are those of the author(s) and should not contrued as an official Department of the Army position, policy or decision, unless so designated by other documentation.

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	15. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Samuel Gellman
UU	UU	UU	UU		19b. TELEPHONE NUMBER 608-262-3303

RPPR Final Report
as of 02-Jun-2020

Agency Code:

Proposal Number: 68753CH **Agreement Number: W911NF-16-1-0154**
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DUNS Number: 161202122 EIN: 396006492
Report Date: 29-Feb-2020 Date Received: 27-May-2020
Final Report for Period Beginning 01-Jun-2016 and Ending 30-Nov-2019
Title: 7.4 Reactive Chemical Systems: Effects of Nanoscale Chemical Heterogeneity on Hydrophobic Interactions and Molecular Assembly at Surfaces
Begin Performance Period: 01-Jun-2016 **End Performance Period:** 30-Nov-2019
Report Term: 0-Other
Submitted By: Samuel Gellman Email: gellman@chem.wisc.edu
Phone: (608) 262-3303
Distribution Statement: 1-Approved for public release; distribution is unlimited.

STEM Degrees: 0 **STEM Participants:**

Major Goals: Overview: This ARO grant enabled our collaborative team to undertake fundamental studies of hydrophobically driven association processes. Because hydrophobic interactions underlie many phenomena of biological and technological importance, the surprising insights generated by this research suggest a need for broad reconsideration of the role of hydrophobic forces in folding, recognition and assembly phenomena that occur in water. ARO support enabled us to apply a powerful and nontraditional combination of molecular design, chemical synthesis and physical analysis to elucidate the modulation of hydrophobic forces by proximal polar functionality. This multidisciplinary research provided an outstanding training environment for the participating students.

Accomplishments: See attached pdf for details

Training Opportunities: Nothing to Report

Results Dissemination: Nothing to Report

Honors and Awards: Nothing to Report

Protocol Activity Status:

Technology Transfer: Nothing to Report

PARTICIPANTS:

Participant Type: PD/PI
Participant: Samuel Gellman
Person Months Worked: 1.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: Y
Other Collaborators:

RPPR Final Report
as of 02-Jun-2020

Participant Type: PD/PI
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Person Months Worked: 1.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: Y
Other Collaborators:

Participant Type: Graduate Student (research assistant)
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Person Months Worked: 15.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Graduate Student (research assistant)
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Person Months Worked: 10.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

Participant Type: Postdoctoral (scholar, fellow or other postdoctoral position)
Participant: Chenxuan Wang
Person Months Worked: 14.00 **Funding Support:**
Project Contribution:
International Collaboration:
International Travel:
National Academy Member: N
Other Collaborators:

ARTICLES:

RPPR Final Report
as of 02-Jun-2020

Publication Type: Journal Article Peer Reviewed: Y **Publication Status:** 1-Published
Journal: Langmuir
Publication Identifier Type: Publication Identifier: DOI
Volume: 33 Issue: 19 First Page #: 4628
Date Submitted: 8/15/17 12:00AM Date Published: 4/18/17 6:27PM
Publication Location:
Article Title: Influence of order within non-polar monolayers on hydrophobic interactions
Authors: Hongseung Yeon, Chenxuan Wang, Reid C. Van Lehn, and Nicholas L. Abbott
Keywords: non-polar monolayers
Abstract: We report an experimental investigation of the influence of molecular-level order (crystallinity) within nonpolar monolayers on hydrophobic interactions. The measurements were performed using gold film-supported monolayers formed from alkanethiols (CH3(CH2)nSH, with n ranging from 3 to 17), which we confirmed by using polarization–modulation infrared reflection–adsorption spectroscopy to exhibit chain-length-dependent order (methylene peak moves from 2926 to 2919 cm–1, corresponding to a transition from liquid- to quasi-crystalline-like order) in the absence of substantial changes in chain density (constant methyl peak intensity). By using monolayer-covered surfaces immersed in either aqueous triethanolamine (TEA, 10 mM, pH 7.0) or pure methanol, we quantified hydrophobic and van der Waals contributions to adhesive interactions between identical pairs of surfaces (measured using an atomic force microscope) as a function of the length and order of the aliphatic chains within the monolay
Distribution Statement: 3-Distribution authorized to U.S. Government Agencies and their contractors
Acknowledged Federal Support: **Y**

Publication Type: Journal Article Peer Reviewed: Y **Publication Status:** 1-Published
Journal: Journal of American Chemical Society
Publication Identifier Type: Other Publication Identifier:
Volume: 17 Issue: 139 First Page #: 18536
Date Submitted: 8/29/18 12:00AM Date Published: 8/15/17 8:00PM
Publication Location:
Article Title: Non-Additive Interactions Mediated by Water at Chemically Heterogeneous Surfaces: Non-ionic Polar Groups and Hydrophobic Interactions
Authors: Chenxuan Wang†, Chi-Kuen Derek Ma, Hongseung Yeon, Xiaoguang Wang, Samuel H. Gellman, and M
Keywords: non-ionic polar groups
Abstract: We explore how two non-ionic polar groups (primary amine and primary amide) influence hydrophobic interactions of neighboring non-polar domains. Our experimental design is based on a stable ?-peptide secondary structure, the 14-helix, that has approximately three residues per turn. We designed sequences that generated globally amphiphilic (GA) helices, each with a discrete non-polar domain formed by six cyclohexyl side chains arranged along one side of the 14-helix. The other side of the helix was dominated by three polar side chains, from ?3-homolysine (K) and/or ?3-homoglutamine (Q) residues. Variations in this polar side chain array included exclusively ?3-hLys (GA-KKK) and ?3- hLys/?3-hGln mixtures (e.g., GA-QKK and GA-QQK). Comparative chemical force measurements in aqueous solution vs. methanol allowed quantification of the effects of these polar side chain variations on hydrophobic interactions of the ?- peptide with the non-polar tip of an atomic force microscope (AFM). At pH 1
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Acknowledged Federal Support: **Y**

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as of 02-Jun-2020

Final Report - Scientific Progress and Accomplishments

Below we summarize key scientific progress and accomplishments for the duration of our grant from the ARO.

Overview: This ARO grant enabled our collaborative team to undertake fundamental studies of hydrophobically driven association processes. Because hydrophobic interactions underlie many phenomena of biological and technological importance, the surprising insights generated by this research suggest a need for broad reconsideration of the role of hydrophobic forces in folding, recognition and assembly phenomena that occur in water. ARO support enabled us to apply a powerful and non-traditional combination of molecular design, chemical synthesis and physical analysis to elucidate the modulation of hydrophobic forces by proximal polar functionality. This multidisciplinary research provided an outstanding training environment for the participating students.

1. Non-additive interactions mediated by water at chemically heterogeneous surfaces: effects of proximal primary amine vs. primary amide groups on hydrophobic interactions.

Our experimental approach for exploring the effects of proximal amine vs amide groups on hydrophobic interactions leveraged our prior experiments and understanding of the effects of β^3-hLys residues on hydrophobic interactions. Specifically, we explored the influence of replacing β^3-hLys residues in GA-KKK with β^3-homoglutamine (β^3-hGln or Q) residues on hydrophobic interactions of globally amphiphilic β-peptides by performing AFM measurements with GA-QKK and GA-QQK (Figures 1-2). The side chain of Q presents a primary amide group, which is polar but uncharged under all conditions explored in our study (pH between 7 and 10.5). At high pH, where the amino group of K is also not charged, our experimental design permitted direct comparison of the effects of two non-ionic polar groups, primary amine versus primary amide, on hydrophobic interactions.

Our measurements revealed that the introduction of Q into the GA peptides led to changes in the hydrophobic interaction of the non-polar ACHC domains of the GA peptides with the non-polar AFM tip. This result is most evident in our comparison of hydrophobic interactions of GA-KKK and GA-QQK with the AFM tip at pH 10.5 (Figure 2). Whereas we measured a hydrophobic interaction with GA-KKK, no measurable hydrophobic interaction was detected with GA-QQK. A comparable conclusion was also drawn from complementary measurements that we performed with mixed monolayers presenting alkyl and either amide or amine groups at pH 10.5.

Overall, these results, when correlated with the hydrogen bonding characteristics and relative hydration free energies of the amine and primary amide functional groups, generate the hypothesis that the divergent effects of amide and amine groups on hydrophobic interactions arise from the relative propensities of these two groups to perturb the structure of water in contact with adjacent non-polar domains. Our observations that primary amide and primary amine groups differ greatly in their influence on hydrophobic interactions involving neighboring non-polar groups provide a fresh perspective for understanding the water-mediated interactions that control associations with chemically heterogeneous surfaces and self-assembly in chemical and biological systems. These findings also complement our earlier demonstration that the structure of a cationic group influences the hydrophobic interactions of neighboring non-polar domains.

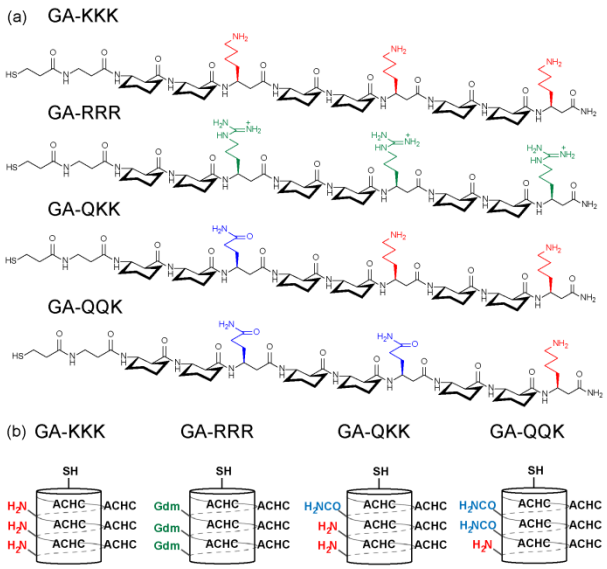

Figure 1: Globally amphiphilic (GA) β-peptides. The chemical structures (a) and helical representations (b) of globally amphiphilic β-peptides, GA-KKK, GA-RRR, GA-QKK, and GA-QQK.

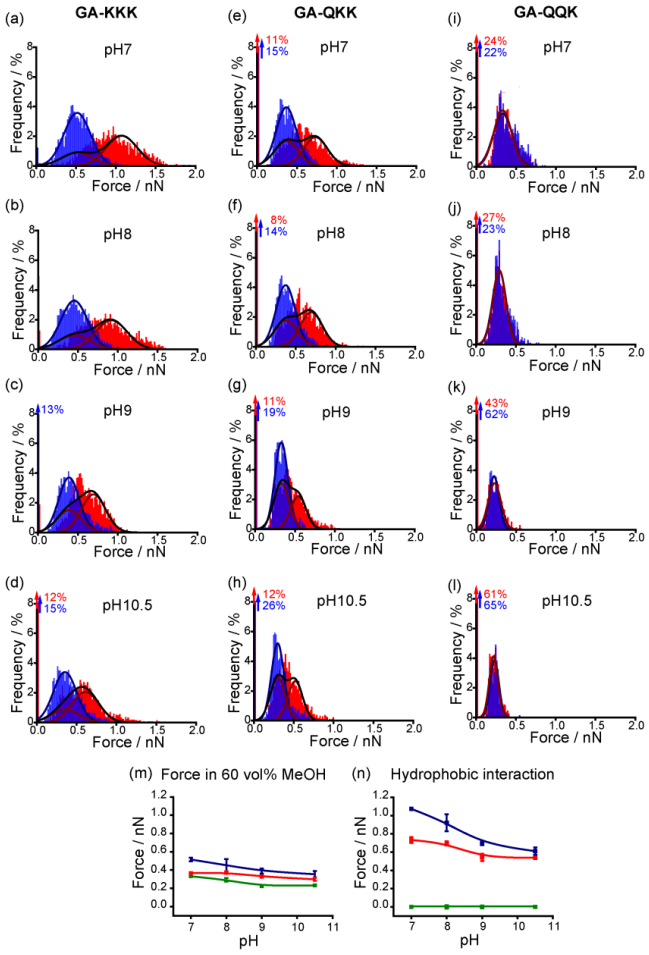

Figure 2: Influence of β[3]-homoglutamine side chain to mediate hydrophobic interactions of globally amphiphilic (GA) β-peptides. Histograms of pull-off forces measured using immobilized GA-KKK (a-d), GA-QKK (e-h), and GA-QQK (i-l) in either 10 mM TEA buffer (red) or 60 vol% MeOH (blue) as a function of pH. The dependence on pH of pull-off forces measured in 60 vol% MeOH (m) or hydrophobic interactions measured in aqueous 10 mM TEA (n) using GA-KKK (blue), GA-QKK (red), and GA-QQK (green).

2. Cationic Side Chain Identity Directs Hydrophobically-Driven Self-Assembly of Amphiphilic β-Peptides in Aqueous Solution.

Our previously reported single molecule force measurements with globally amphiphilic (GA) β-peptides (GA-Lys and GA-Arg) revealed that replacement of β[3]-homolysine (βLys) with β[3]-homoarginine (βArg) eliminates hydrophobic interactions between a non-polar surface displayed by the β-peptide and the non-polar surface of an atomic force microscope (AFM) tip. During the grant period, we asked whether the profound difference between proximal ammonium and guanidinium groups detected for surface-confined β-peptides is pertinent to hydrophobically-driven β-peptide assembly in bulk aqueous solution. Our new studies were conducted in 10 mM triethanolamine (TEA) buffer at pH 7, where both βLys (ammonium) and βArg (guanidinium) side chains are substantially protonated. As in the AFM studies, addition of 60 vol % methanol was used to eliminate the majority of hydrophobic interactions. Comparisons of light scattering measurements and cryo-electron micrographs before and after addition of MeOH indicated very different outcomes of hydrophobically-driven assembly of AcY-GA-Lys vs. AcY-GA-Arg (AcY denotes an N-acetylated-β[3]-homotyrosine (βTyr) at each N-terminus; Figure 3). Nuclear magnetic resonance and analytical ultracentrifugation confirmed that AcY-GA-Lys assembles into large aggregates in aqueous buffer, whereas AcY-GA-Arg at comparable concentrations forms only small oligomers. Titration of AcY-GA-Arg into aqueous solutions of AcY-GA-Lys revealed that the driving force for AcY-GA-Lys association is far stronger than that for AcY-GA-Arg association. Overall, these results established that the identity of proximal cationic groups, ammonium vs. guanidinium, profoundly modulates the hydrophobically driven self-assembly of β-peptides in bulk solution.

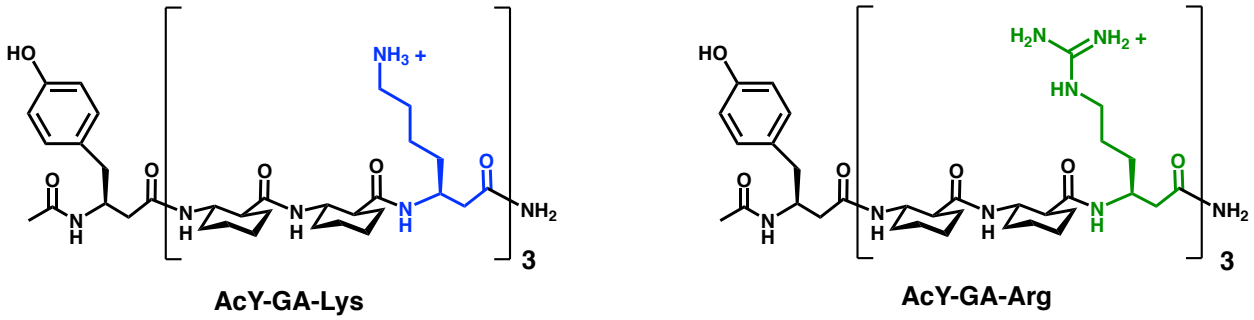

Figure 3: Molecular structures of β-peptides using in our studies of self-assembly in bulk aqueous solution.

The findings are very significant because there is no precedent for the concept that the degree to which a nonpolar surface manifests hydrophobicity is influenced by the identity of cationic groups within the same molecule. It was not obvious before these studies were undertaken that the surprising trends we previously discovered via single-molecule AFM measurements would apply as well to self-assembly in bulk aqueous solution. Our discoveries suggest a new perspective on interactions involving macromolecules, both biological and synthetic that display complex and functionally diverse surfaces.

3. Dependence of Hydrophobic Interactions on Nonpolar Domain Area

Molecular simulations of model non-polar solutes and complementary experiments have established that hydrophobic interactions scale in strength in proportion to nonpolar domain area (at constant interfacial curvature). It is not understood, however, if this scaling changes in chemically heterogeneous systems. To address this gap in knowledge, we performed experiments to determine how ammonium groups, when immobilized adjacent to nonpolar domains, change the dependence of hydrophobic interactions on nonpolar domain area. Specifically, by using atomic force microscopy (AFM), we quantified the force required to detach surface-immobilized globally-amphiphilic (GA) β-peptides from adhesive contact in water with a nonpolar monolayer formed on an AFM tip. We tested a series of GA β-peptides comprising repeats (n) of the triad ACHC-ACHC-K, where ACHC is trans-2-aminocyclohexanecarboxylic acid and K is β^3-homolysine, with n = 2, 3 or 4. At pH 10.5, where the side chains of β^3-homolysine residues are largely deprotonated (amine), we measured a linear scaling of hydrophobic adhesive force with nonpolar domain area (i.e., there was no statistically significant difference among the hydrophobic forces per repeat of the triad for n = 2-4; Figure 4, right). In contrast, at pH 7.0, where the amine groups will be protonated, we measured the strength of hydrophobic adhesion to scale non-linearly with nonpolar domain area (i.e., the hydrophobic force per repeat of the triad increased with n from n = 2 to n = 4 at a significance level of 99%). These results revealed that charged groups, when immobilized adjacent to nonpolar domains, have a profound influence on the way in which hydrophobic interactions scale with nonpolar domain area. This result hints at new design rules for hydrophobically-driven self-assembly based on the engineering of immobilized charge groups.

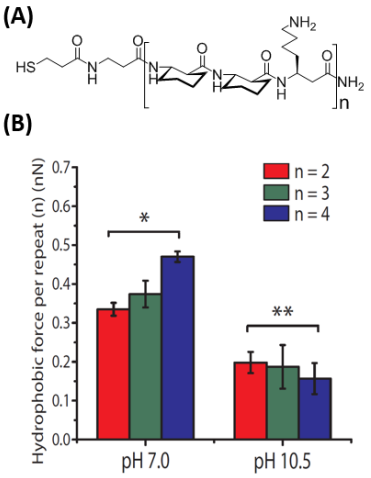

Figure 4. (A) Chemical structure of GA β-peptides where 'n' indicates the number of repeating triad with n = 2-4. (B) Hydrophobic force per repeat of the triad of GA-K-n (n = 2, 3 and 4) measured at pH 7.0 and 10.5, respectively. P values are < 0.01 (*) and 0.07 (**), respectively.

4. Influence of Immobilized Cations on the Enthalpy-Entropy Compensation underlying Hydrophobic Interactions

Previously, we had reported experiments demonstrating that protonation of amine groups immobilized at nonpolar surfaces enhances the strength of hydrophobic adhesion, whereas guanidinium cations diminish the strength of hydrophobic adhesion relative to ammonium cations. To understand the thermodynamic origin of the strikingly different effects of ammonium and guanidinium cations on hydrophobic interactions, we measured the temperature-dependence of these interactions. We analyzed the measurements to identify the influence of cation identity on the enthalpy-entropy compensation underlying the hydrophobic interactions. Specifically, we used AFM to measure adhesive forces between a mixed-component self-assembled monolayer (comprising 40% 11-aminoundecanethiol or 40% 11-guanidinoundecanethiol and 60% 1-decanethiol) and a nonpolar AFM tip, at temperatures between 298 K to 328 K and pH values ranging from 3.5 to 10.5. We interpreted the measurements using the Johnson-Kendall-Roberts (JKR) model in terms of a free energy change associated with transfer of a mixed monolayer surface from aqueous TEA containing 60 vol % methanol to aqueous TEA (i.e., transfer free energy, ΔG_{tr}, representing a key part of the free energy change underlying the hydrophobic interaction).

The temperature-dependence of this transfer free energy was used to identify the enthalpic and entropic contributions to the hydrophobic interaction (Figure 5).

At pH 3.5, where both amine and guanidine groups are largely protonated, we measured ΔG_{tr} to be less favorable for mixed monolayer surfaces containing ammonium (40% Am^+) than for mixed monolayer surfaces containing guanidinium (40% Gdm^+). This result is consistent with our past observation that ammonium cations mediate a stronger hydrophobic interaction than guanidinium cation when immobilized at non-polar surfaces. We also established that an unfavorable (endothermic) enthalpy contribution to ΔG_{tr} underlies the stronger hydrophobic adhesion measured with ammonium mixed monolayer surfaces as compared to the guanidinium mixed monolayer surfaces.

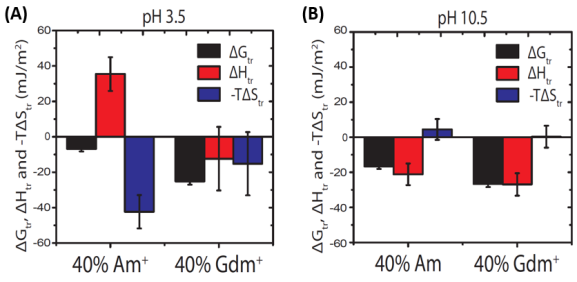

Figure 5. Transfer free energy (ΔG_{tr}, black), transfer enthalpy (ΔH_{tr}, red), and transfer entropy (ΔS_{tr}, blue) of mixed monolayer surfaces containing amine (40% Am^+ or 40% Am) or guanidine groups (40% Gdm^+) at pH 3.5 (A) and 10.5 (B) at 298K.

Additionally, at pH 10.5 where amine groups are largely deprotonated, we measured ΔG_{tr} for mixed monolayer surfaces containing amine groups (40% Am) to be more favorable (i.e., less hydrophobic) than the ammonium-containing mixed monolayers surfaces (pH 3.5; 40% Am^+). The change in ΔG_{tr} with pH was found to be dominated by a pH-dependent enthalpy of transfer (changes from endothermic to exothermic with increase in pH). Overall, these results provide insight into the enthalpy-entropy compensation that underlies the effects of immobilized cations on hydrophobic effects.

5. Characterization of Interfacial Water Structure Relevant to Hydrophobic Hydration

The temperature-dependent AFM measurements described above provided insight to the thermodynamic origins of the divergent effects of ammonium and guanidinium cations on hydrophobic interactions. These observations and others made over the past three years led us to explore how the identity of immobilized cations impacts the organization of water (solvation/hydration) near non-polar domains. To this end, we used Raman spectroscopy to characterize the influence of the cationic groups of hexylammonium chloride (HA HCl) or hexylguanidinium chloride (HG HCl) on the hydration of the aliphatic tails of these surfactants. We used the Self-Modeling Curve Resolution (SMCR) method to separate solute-correlated (SC) spectra, which contain features of the both solute and solute-induced ordering of interfacial water, from the spectra of bulk water. We performed these measurements using methylammonium chloride (MA HCl) and methylguanidinium chloride (MG HCl) as reference samples.

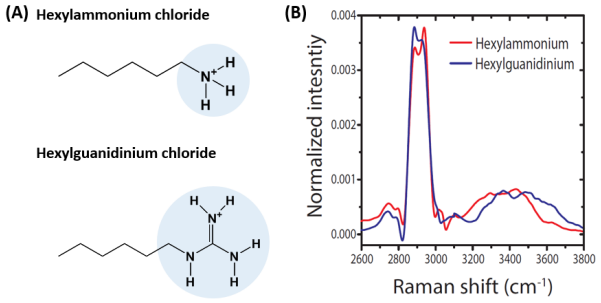

Figure 6. (A) Chemical structure of hexylammonium chloride (top) and hexylguanidinium chloride (bottom) showing hydrogen bonded water. (B) Solute-correlated spectra of hexylammonium chloride (red) and hexylguanidinium chloride (blue) measured relative to the Raman spectra of methylated cations.

We found the Raman spectra of water near the non-polar tails of HA HCl (red data in Figure 6) to be similar to bulk water (i.e., two major OH stretching peaks at 3,200 cm^{-1} and 3,400 cm^{-1}). In contrast, the Raman spectra of water near the aliphatic tails of HG HCl are blue shifted (i.e., a broad OH stretching peak from 3,000 cm^{-1} to 3,800 cm^{-1}; blue data in Figure 6), indicating that water that is weakly hydrogen bonded relative to bulk water. These Raman spectra generate a number of interesting future directions of inquiry. For example, the measurements predict that the entropy of micellization of HG HCl will be smaller (less negative) than for HA HCl, a prediction that we can test by experiment. More broadly, these results provide insight into the changes in organization of water that underlie our previous observation that cation identity changes hydrophobic interactions.

6. Hydrophobic Interactions Encoded by Alpha-Helical Coiled-Coil Peptides

In our studies described above, we used β-peptides to examine the interplay between charged and nonpolar moieties within functionally complex molecules enabled by the conformational rigidity that can be encoded into these oligomers. In more recent studies, we performed experiments to explore if our AFM-based methods for characterization of intermolecular forces, including hydrophobic interactions, developed initially with β-peptides, can be extended to conventional peptides (i.e., peptides comprised of α-amino acid residues). This question is important because proteins contain exclusively α-amino acid residues, and the α-peptide backbone has high intrinsic flexibility. To begin to address this question, we used oligopeptides (Amph-Lysine and Amph-Arginine peptides shown in Figure 7) that form α-helical coiled-coil dimers, via an assembly process that is driven by burial of hydrophobic side chains at the inter-helical interface. The coiled-coil is the best-understood quaternary structures in the protein realm.

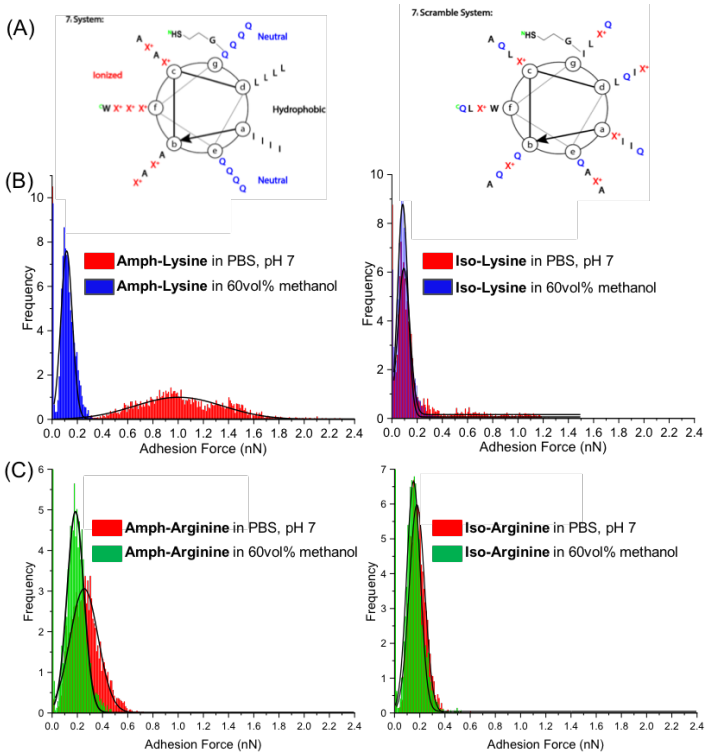

Figure 7. (A) Helical wheel diagram of Amphiphilic (left) and Isomeric (right) α-peptides. (B) Force histograms measured between Amph-Lysine (left) or Iso-Lysine (right) and a nonpolar AFM tip in PBS buffer (red) or 60 vol % methanol added to PBS buffer (blue), respectively. (C) Force histograms measured between Amph-Arginine (left) or Iso-Arginine (right) and a nonpolar AFM tip in PBS buffer (red) or 60 vol % methanol added to PBS buffer (green), respectively.

We performed single-molecule AFM measurements using either Amph-Lysine or Amph-Arginine (or an isomeric oligopeptides called "Iso-Lysine" and "Iso-Arginine") and a nonpolar AFM tip to quantify the strength of hydrophobic interaction encoded by these peptides. When folded into helices, Amph-Lysine and Amph-Arginine peptides display a global segregation of side chains bearing nonpolar and charged/polar functional groups. In contrast, the Iso-Lysine and Iso-Arginine peptides project both

nonpolar and charged/polar functional groups around the entire periphery (the oligopeptides have identical composition of amino acids but differ in their sequence) (Figure 7A). We found that Amph-Lysine and Iso-Lysine peptides generate qualitatively different adhesion force histograms when characterized in aqueous PBS or aqueous PBS to which 60% methanol was added (Figure 7B). This result suggests that the two peptides present distinct patterns of non-polar and polar functional groups to the AFM tip. Additionally, we found evidence of hydrophobic adhesion between Amph-Lysine and the nonpolar AFM tip (0.88 nN) whereas the Iso-Lysine peptides generated adhesive forces that were not significantly different in the two aqueous solvents used in our experiments. We interpret these results to indicate that the helical secondary structure of Amph-Lysine is sufficiently well-defined during these experiments that it presents a clustering of non-polar side-chains that mediate a hydrophobic interaction; the absence of measurable hydrophobic interaction with Iso-Lysine provides further support for the conclusion that these oligopeptides possess sufficiently well-defined secondary structures that they generate sequence-dependent interactions with non-polar surfaces. In contrast to Amph-Lysine, the replacement of lysine by arginine (i.e., Amph-Arginine) greatly diminished hydrophobic interactions, as evidenced by the small difference in forces measured between Amph-Arginine and the non-polar AFM tip in PBS versus PBS to which 60 vol. % MeOH was added (Figure 7C). Similar to Iso-Lysine, the force distribution of Iso-Arginine in PBS and 60 vol % MeOH overlapped, suggesting the absence of any measurable hydrophobic interaction. Overall, these results suggest that our AFM methodology developed previously for characterization of β-peptides is also a promising approach for characterization of context-dependent hydrophobic interactions using α-oligopeptides and ultimately proteins.

7. Hydrophobically-Driven Coiled-Coil Dimerization of Peptides in Bulk Aqueous Solution

We completed a series of experiments to evaluate how the identity of proximal cationic groups, guanidinium vs. ammonium, influences hydrophobically driven assembly of conventional peptides (i.e., α-peptides) in aqueous solution. This study focused on homodimerization via formation of a parallel coiled coil, which is a very well-studied quaternary structure motif in proteins. In the α-helical conformation, peptides competent for coiled-coil dimerization display a stripe of hydrophobic residues along one side, and dimerization is driven by burial of hydrophobic surfaces against one another. We re-engineered a previously reported coiled-coil-forming sequence to enable placement of cationic residues, either Arg (guanidinium) or Lys (ammonium) on the helix side that is diametrically opposite to the hydrophobic stripe. In contrast to the helical β-peptides we have studied,

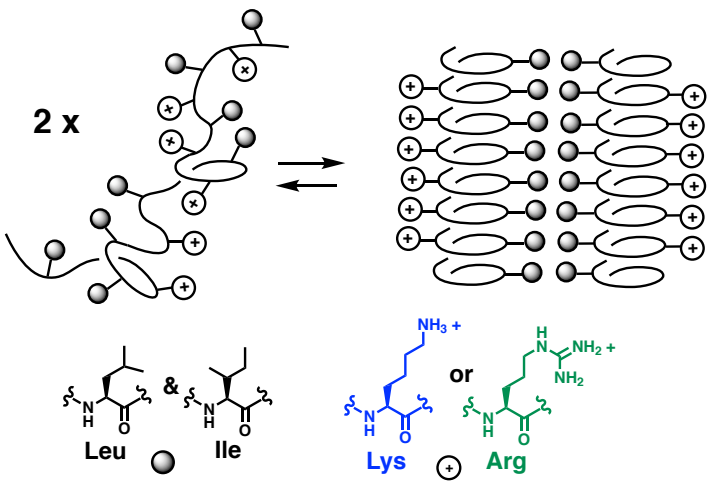

Figure 8. Cartoon illustration of coiled-coil dimerization. This depiction emphasizes the fact that helicity develops concomitantly with dimer formation; peptides in the monomeric state are unfolded.

these α-peptides are unfolded in the monomeric state. Thus, coiled-coil formation involves development of helical secondary structure concomitantly with dimerization (Figure 8).

We determined a crystal structure of the Arg version of our new peptide design. This structure demonstrated that despite the substantial modification of side chain positions relative to a conventional coiled-coil-forming peptide, our sequence supports formation of a canonical coiled-coil dimer (Figure 9). We were therefore surprised to find that the Arg peptide dimer is slightly more stable than the dimer formed by the Lys peptide. Extrapolation from our β-peptide results would have predicted the opposite trend, because the β-peptide containing βLys self-associates more avidly than does the analogous β-peptide containing βArg. The coiled coil trend was established by two methods, variable-temperature circular dichroism (CD) and differential scanning calorimetry (DSC).

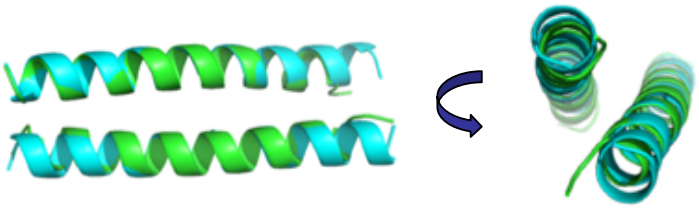

Figure 9. Backbone-level comparison of our designed Arg-rich coiled coil dimer (green; PDB 6OWD) with a previously reported coiled coil that has a canonical sequence pattern (blue; PDB 4DZM).

We attribute the change in trend between the α-peptide and β-peptide systems to the intrinsic flexibility of the α-peptide backbone. In the coiled-coil system (α-peptide), slight differences in helix propensity between Arg and Lys can exert a substantial impact on dimer stability, because helical folding is coordinated with dimerization (Figure 8). In contrast, the high helix stability enforced by cyclohexane-based residues in the β-peptides eliminates any analogous factor arising from propensity differences between βArg and βLys. We conclude that the small difference in helical propensity favoring Arg relative to Lys has obscured the effects we sought to measure with our coiled-coil system.

The second phase of our α-peptide studies involves modified coiled-coil-forming sequences that contain side chain-to-side chain crosslinks that stabilize an α-helical conformation. Our intent is to enforce α-helicity sufficiently that residue-based variations in helix propensity become irrelevant. Preliminary studies reveal that the crosslinks eliminate cooperativity in thermal transitions, which is motivating us to pursue new strategies for characterizing monomer-dimer equilibria in solution. In addition, we are working to identify associations between fully folded proteins that might allow us to compare the effects of proximal Lys vs. Arg residues.