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14. ABSTRACT					
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RPPR Final Report
as of 06-Jan-2023

Agency Code: 21XD

Proposal Number: 66543ESSR

Agreement Number: W911NF-15-2-0119

INVESTIGATOR(S):

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Report Date: 31-Dec-2020

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Final Report for Period Beginning 01-Oct-2015 and Ending 30-Sep-2020

Title: How Do Coupled Noncovalent Forces Affect the Properties of Complex Structures?

Begin Performance Period: 01-Oct-2015

End Performance Period: 30-Sep-2020

Report Term: 0-Other

Submitted By: Senior Research Scientist, ARO Stephen L Email: stephen.j.lee28.civ@army.mil

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STEM Degrees: 2

STEM Participants: 2

Major Goals: This proposal outlined an experimental plan for characterizing how perturbations in a noncovalent network of coupled interactions were accommodated by the structure's network. Such questions are fundamental to the properties of complex materials such as proteins, polymers, and many other entities. Preliminary evidence suggested that such distortions were minimized by a combination of enthalpic and entropic response mechanisms. The research plan focused on delineating how both the structure and dynamics responded to changes in the noncovalent network of a complex [2]-catenane model. This model provided enough complexity to create a functioning noncovalent network, but was sufficiently manipulable and characterizable to extract high-resolution structure-property relationships for mutated variants. The outcome of these efforts were molecular level descriptions of how non-covalent networks can facilitate structural changes and stabilize insults to their structure.

Accomplishments: see attached document

Training Opportunities: 1 Ph D Student, 1 undergraduate, 3 postdoctoral fellows trained, each of whom has continued in the research space and are contributing to new science

Results Dissemination: "Tetrameric Psuedo-Peptide Receptors with Allosteric Properties" M. K. Chung, S. J. Lee, M. L. Waters, M. R. Gagné, Chem. Commun. 2016, 52, 8103.

"Investigation of a Catenane with a Responsive Noncovalent Network: Mimicking a Long Range Responses in Proteins" M. K. Chung, P.S. White, S. J. Lee, M. R. Gagné, M. L. Waters, J. Am. Chem. Soc. 2016, 138, 13344-13352.

"Gold(I) catalyzed dearomative Claisen rearrangement of allyl, allenyl methyl, and propargyl aryl ethers" M. T. Peruzzi, S. J. Lee, M. R. Gagné, Org. Lett. 2017, 19, 6256-6259.

"Chemoselective amide reductions by heteroleptic fluoroaryl boron Lewis acids" M. T. Peruzzi, a Q. Q. Mei, a S. J. Lee, b M. R Gagné, Chem Commun. 2018, 54, 5855-5858.

"Harnessing the Reactivity of Poly(methylhydrosiloxane) for the Reduction and Cyclization of Biomass to High-Value Products" N. M. Hein, Y. Seo, S. J. Lee, M. R. Gagné, Green Chem. 2019, 21, 2662-2669.

"Site selective amide reduction of cyclosporine A opens new structural space for an important cyclic peptide" M. T. Peruzzi, F. Gallou, S. J. Lee, M. R. Gagné Org. Lett. 2019, 21, 3451-3455.

RPPR Final Report
as of 06-Jan-2023

Honors and Awards: Michel R. Gagné, 2016, Fellow of the Royal Society of Chemistry
Michel R. Gagné, 2016, Royal Society "Catalysis in Organic Synthesis" Award

Protocol Activity Status:

Technology Transfer: Nothing to Report

PARTICIPANTS:

Participant Type: Faculty

Participant: Michel R Gagné

Person Months Worked: 1.00

Project Contribution:

National Academy Member: N

Funding Support:

Participant Type: Postdoctoral (scholar, fellow or other postdoctoral position)

Participant: Nicholas Hein

Person Months Worked: 12.00

Project Contribution:

National Academy Member: N

Funding Support:

Participant Type: Postdoctoral (scholar, fellow or other postdoctoral position)

Participant: Mee-Kyung Chung

Person Months Worked: 6.00

Project Contribution:

National Academy Member: N

Funding Support:

Participant Type: Postdoctoral (scholar, fellow or other postdoctoral position)

Participant: Youngran Seo

Person Months Worked: 1.00

Project Contribution:

National Academy Member: N

Funding Support:

Participant Type: Graduate Student (research assistant)

Participant: Michael Perruzi

Person Months Worked: 12.00

Project Contribution:

National Academy Member: N

Funding Support:

Participant Type: Undergraduate Student

Participant: Qiong Qiong Mei

Person Months Worked: 1.00

Project Contribution:

National Academy Member: N

Funding Support:

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Journal: Chem. Commun.

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Publication Identifier: 10.1039/C6CC03296F

Volume: 52

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Date Submitted: 11/29/16 12:00AM

Date Published:

Publication Location:

Article Title: Tetrameric psuedo-peptide receptors with allosteric properties

Authors: Mee-Kyung Chung, Stephen J. Lee, Marcey L. Waters, Michel R. Gagné

Keywords: receptors

Abstract: This paper reports the binding properties of tetrameric pseudo-peptide receptors for protonated cytidines. The receptors, which were isolated from a dynamic combinatorial chemistry (DCC) experiment, bind the analytes with affinities that depend on the presence or absence of excess acid, and with a stoichiometry that is both concentration and temperature dependent.

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 the American Chemical Society

Publication Identifier Type: DOI

Publication Identifier: 10.1021/jacs.6b07833

Volume: 138

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Date Submitted: 11/29/16 12:00AM

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Publication Location:

Article Title: Investigation of a Catenane with a Responsive Noncovalent Network: Mimicking Long-Range Responses in Proteins

Authors: Mee-Kyung Chung, Peter S. White, Stephen J. Lee, Michel R. Gagné, Marcey L. Waters

Keywords: non covalent networks proteins

Abstract: We report a functional synthetic model for studying the noncovalent networks (NCNs) required for complex protein functions. The model [2]-catenane is self-assembled from dipeptide building blocks and contains an extensive network of hydrogen bonds and aromatic interactions. Perturbations to the catenane cause compensating changes in the NCNs structure and dynamics, resulting in long-distance changes reminiscent of a protein. Key findings include the notion that NCNs require regions of negative cooperativity, or "frustrated" noncovalent interactions, as a source of potential energy for driving the response. We refer to this potential energy as latent free energy and describe a mechanistic and energetic model for responsive systems.

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Publication Identifier Type: DOI

Publication Identifier: 10.1021/acs.orglett.7b03306

Volume: 19

Issue: 22

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Date Published: 11/1/17 4:00AM

Publication Location:

Article Title: Gold(I) Catalyzed Dearomative Claisen Rearrangement of Allyl, Allenyl Methyl, and Propargyl Aryl Ethers

Authors: Michael T. Peruzzi, Stephen J. Lee, Michel R. Gagné

Keywords: catalysis

Abstract: Claisen rearrangements of allyl aryl ethers to generate enones bearing all carbon quaternary centers are accelerated by Ph₃PAuNTf₂ under mild conditions in good yields. Multiple C–C bond containing variants of the allyl fragment are viable, including alkylidenecyclopropanes, allenes, and alkynes, which generate all-carbon stereogenic centers substituted with vinyl cyclopropanes, 1,3-butadienyl, and allenyl substituents, respectively, for subsequent synthetic manipulation. With allyl phenyl ethers, the product of the [3,3] rearrangements can be trapped by a tandem [4 + 2] cycloaddition to generate complex molecular scaffolds from readily available, achiral starting materials.

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Publication Type: Journal Article Peer Reviewed: Y **Publication Status:** 1-Published

Journal: Chemical Communications

Publication Identifier Type: DOI

Publication Identifier: 10.1039/C8CC01863D

Volume: 54

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Date Submitted: 10/5/18 12:00AM

Date Published:

Publication Location:

Article Title: Chemoselective amide reductions by heteroleptic fluoroaryl boron Lewis acids

Authors: Michael T. Peruzzi, Qiong Qiong Mei, Stephen J. Lee, Michel R. Gagné

Keywords: catalysis

Abstract: Claisen rearrangements of allyl aryl ethers to generate enones bearing all carbon quaternary centers are accelerated by Ph₃PAuNTf₂ under mild conditions in good yields. Multiple C–C bond containing variants of the allyl fragment are viable, including alkylidenecyclopropanes, allenes, and alkynes, which generate all-carbon stereogenic centers substituted with vinyl cyclopropanes, 1,3-butadienyl, and allenyl substituents, respectively, for subsequent synthetic manipulation. With allyl phenyl ethers, the product of the [3,3] rearrangements can be trapped by a tandem [4 + 2] cycloaddition to generate complex molecular scaffolds from readily available, achiral starting materials.

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Peer Reviewed: Y

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Journal: Organic Letters

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Publication Identifier: 10.1021/acs.orglett.9b01245

Volume: 21

Issue: 9

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Date Submitted: 7/27/20 12:00AM

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Publication Location:

Article Title: Site Selective Amide Reduction of Cyclosporine A Enables Diverse Derivation of an Important Cyclic Peptide

Authors: Michael T. Peruzzi, Fabrice Gallou, Stephen J. Lee, Michel R. Gagné;

Keywords: catalysis, peptides

Abstract: Site selective amide reductions of the cyclic undecapeptide, cyclosporine A, have been developed using the combination of a heteroleptic borane catalyst and a silane reductant. Tertiary silane Me₂EtSiH provides two unique cyclosporine A derivatives, one of which can be readily diversified in subsequent reactions. The secondary silane Et₂SiH₂ enables divergent reactivity that uses a free hydroxyl group to direct the reduction. The transient O-silyl hemiaminal intermediate of this reduction can additionally be trapped by reducing to the amine or by reductive cyanation.

Distribution Statement: 2-Distribution Limited to U.S. Government agencies only; report contains proprietary info
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Publication Type: Journal Article

Peer Reviewed: Y

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Journal: Green Chemistry

Publication Identifier Type: DOI

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Volume: 21

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Publication Location:

Article Title: Harnessing the reactivity of poly(methylhydrosiloxane) for the reduction and cyclization of biomass to high-value products

Authors: Nicholas M. Hein, Youngran Seo, Stephen J. Lee, Michel R. Gagné;

Keywords: catalysis, green chemistry

Abstract: Site selective amide reductions of the cyclic undecapeptide, cyclosporine A, have been developed using the combination of a heteroleptic borane catalyst and a silane reductant. Tertiary silane Me₂EtSiH provides two unique cyclosporine A derivatives, one of which can be readily diversified in subsequent reactions. The secondary silane Et₂SiH₂ enables divergent reactivity that uses a free hydroxyl group to direct the reduction. The transient O-silyl hemiaminal intermediate of this reduction can additionally be trapped by reducing to the amine or by reductive cyanation.

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Journal: Organometallics

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Date Submitted: 11/2/20 12:00AM

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Publication Location:

Article Title: Spectroscopic and Computational Assessment of Silicon's Electrophilicity in Phosphinosilylium Cations

Authors: Kathleen M. Riley, Andreas L. Wierschen, Stephen J. Lee, and Michel R. Gagne?

Keywords: silylium, NMR

Abstract: An experimental method of determining the electrophilicity of silicon in phosphinosilylium cations is reported and compared to Djukic's DFT method of computing relative intrinsic silylicity, χ . We also establish linear correlations between silicon electrophilicity and ^{29}Si NMR chemical shifts, DFT-computed silylicities, and the Tolman electronic parameter of the phosphine. These correlations were not universal, as deviations were observed for the most sterically hindered phosphines. Intermolecular silylium transfer experiments between phosphinosilyliums and added phosphines provided a thermodynamic assessment of the electrophilic character at silicon. This confirmed that relative intrinsic silylicity (χ) and ^{29}Si NMR chemical shifts are useful parameters for semiquantitatively determining the electrophilicity of the silyl group in a Lewis pair.

Distribution Statement: 2-Distribution Limited to U.S. Government agencies only; report contains proprietary info
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Publication Type: Journal Article Peer Reviewed: Y **Publication Status:** 1-Published

Journal: Journal of the American Chemical Society

Publication Identifier Type: DOI

Publication Identifier: 10.1021/jacs.9b08178

Volume: 141

Issue:

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Date Submitted: 11/2/20 12:00AM

Date Published: 9/18/19 4:00AM

Publication Location:

Article Title: Silylpalladium Cations Enable the Oxidative Addition of $\text{C}(\text{sp}^3)\text{O}$ Bonds

Authors: Andreas L. Wierschen,[†] Neyen Romano,^{†,§} Stephen J. Lee,[‡] and Michel R. Gagne?

Keywords: palladium silicon

Abstract: An experimental method of determining the electrophilicity of silicon in phosphinosilylium cations is reported and compared to Djukic's DFT method of computing relative intrinsic silylicity, χ . We also establish linear correlations between silicon electrophilicity and ^{29}Si NMR chemical shifts, DFT-computed silylicities, and the Tolman electronic parameter of the phosphine. These correlations were not universal, as deviations were observed for the most sterically hindered phosphines. Intermolecular silylium transfer experiments between phosphinosilyliums and added phosphines provided a thermodynamic assessment of the electrophilic character at silicon. This confirmed that relative intrinsic silylicity (χ) and ^{29}Si NMR chemical shifts are useful parameters for semiquantitatively determining the electrophilicity of the silyl group in a Lewis pair.

Distribution Statement: 2-Distribution Limited to U.S. Government agencies only; report contains proprietary info
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RPPR Final Report as of 06-Jan-2023

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Peer Reviewed: Y

Publication Status: 1-Published

Journal: Organometallics

Publication Identifier Type: DOI

Publication Identifier: 10.1021/acs.organomet.0c00034

Volume: 39

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Publication Location:

Article Title: Silylpalladium Cations Enable the Cleavage of Nitrile C≡N Bonds

Authors: Andreas L. Wierschen, Jared Lowe,§ Neyen Romano,? Stephen J. Lee, and Michel R. Gagne?

Keywords: palladium, silicon

Abstract: The stoichiometric addition of aryl and alkyl nitriles to the silylpalladium cation [(PCy₃)₂Pd⁺SiMe₂Et][(C₆F₅)₄B⁻] (1) resulted in the cleavage of the nitrile C≡N bond and the formation of palladium⁺aryl and palladium⁺alkyl cations. Low-temperature experiments support a mechanism where silylpalladium cation 1 transfers silylium to nitrile, generating a silylnitrilium cation and the reduced, zerovalent complex Pd(PCy₃)₂. These two intermediates readily recombine at low temperatures to generate palladium⁺(N-silyl)iminoacyl complexes, which upon warming can undergo aryl/alkyl group migration from the iminoacyl ligand to the metal. Additional experiments show that palladium⁺(N-silyl)iminoacyl cations are potent sources of silylium, capable of cleaving the methyl C–O bond of an ether, while DFT calculations confirm the electrophilicity of the silyl moiety.

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Acknowledged Federal Support: Y

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Publication Type: Thesis or Dissertation

Institution: University of North Carolina at Chapel Hill

Date Received:

Completion Date: 4/30/19 6:16PM

Title: New Catalysts for Site-Selective Amide Reductions

Authors: Michael Peruzzi

Acknowledged Federal Support: Y

Partners

I certify that the information in the report is complete and accurate:

Signature: michel gagne

Signature Date: 9/6/22 4:15PM

“How Do Coupled Noncovalent Forces Affect the Properties of Complex Structures?”

Final Research Report Stephen J. Lee

W911NF-15-2-0119

Abstract. This proposal outlined an experimental plan for characterizing how perturbations in a noncovalent network of coupled interactions were accommodated by the structure’s network. Such questions are fundamental to the properties of complex materials such as proteins, polymers, and many other entities. Preliminary evidence suggested that such distortions were minimized by a combination of enthalpic and entropic response mechanisms. The research plan focused on delineating how both the structure and dynamics responded to changes in the noncovalent network of a complex [2]-catenane model. This model provided enough complexity to create a functioning noncovalent network, but was sufficiently manipulable and characterizable to extract high-resolution structure-property relationships for mutated variants. The outcome of these efforts were molecular level descriptions of how non-covalent networks can facilitate structural changes and stabilize insults to their structure.

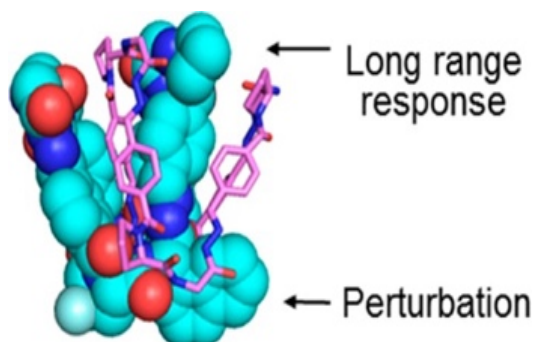
Introduction. Proteins are complex 3-D assemblies that utilize the fold propensities of a primary amino acid sequence in combination with a large number of interresidue noncovalent interactions like H-bonds, hydrophobic forces, electrostatic interactions, and various aromatic interactions (pi-pi, CH-pi, etc) to arrive at a low energy structure. It is the noncovalent interactions in their aggregate that drive the folding behavior of proteins. An important, but under-appreciated microscopic property of complex structures is that their individual noncovalent interactions couple with one another to create a web of individually weak, but collectively strong forces that engender flexibility and adaptability to the protein.

By describing a non-covalent network as a collection of forces that are attractive or repulsive, one can begin asking important questions about the fundamental properties of noncovalent networks, and how these affect the function of complex structures. One can therefore describe the lowest energy structure as one that minimizes the energy of a collection of interactions, but by its nature these interactions are inherently tensioned, as the lowest energy of the system does not necessarily correspond to the lowest energy of each individual noncovalent interaction. *Complexity forces individual components to adopt compromise structures.* Consequently, intramolecular attractive and repulsive forces either act to reinforce or oppose one another and one can describe a minimum energy structure as one where the structure’s attractive forces are optimized and repulsive forces minimized, and additionally that structural perturbations creates more repulsive forces than attractive. We sought to determine whether this was an appropriate way to describe a noncovalent network in a complex structure?

Our research followed two main paths, described in this report. In Aim 1, we investigated a series of self-assembled catenated receptors to explore compensatory non-covalent interactions and long-range allostery in a complex, but high-resolution structure. These involved time-resolved experiments, static solid-state structural perturbations, and thermo-chemical investigations to arrive at a conclusion that supported the above hypothesis. A second Aim, was also experimental and sought to develop catalysts able to functionalize highly structured cyclic peptides with well defined tertiary structures. Cyclosporin A was chosen for these studies as it adopts two different well-defined conformations, one for apolar environments where the polar amides point inward and a second where the the amides point outwards to stabilize the molecule in polar environments. These amazing properties provide Cyclosporin with an ability to cross cell membranes. Our principal aim was to understand selectivity in the context of a non-covalent network of H-bonds and hydrophobic side chain packing forces.

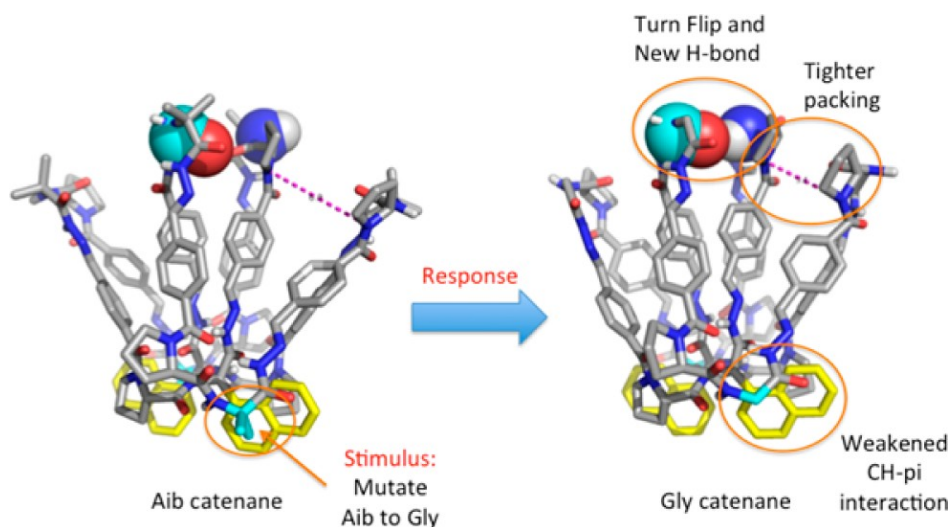
Aim 1: Investigation of a Catenane with a Responsive Noncovalent Network:Mimicking Long-Range Responses in Proteins

ABSTRACT: We report a functional synthetic model for studying the noncovalent networks (NCNs) required for complex protein functions. The model [2]-catenane is self-assembled from dipeptide building blocks and contains an extensive network of hydrogen bonds and aromatic interactions. Perturbations to the catenane cause compensating changes in the NCNs structure and dynamics, resulting in long-distance changes reminiscent of a protein. Key findings include the notion that NCNs require regions of negative cooperativity, or “frustrated” noncovalent interactions, as a source of potential energy for driving the response. We refer to this potential energy as *latent free energy* and describe a mechanistic and energetic model for responsive systems.



INTRODUCTION

Many protein functions depend on long-distance changes in response to a stimulus. Examples include allostery,¹ signal transduction,² and even binding through induced fit or conformational selection mechanisms³ and catalysis.⁴ Moreover, recent work has shown that this response can be structural (e.g., a change in the folded structure) or dynamic (e.g., a change in protein motion).⁵ This type of communication through a protein structure is mediated by a noncovalent network (NCN) of interactions inherent in a folded protein.⁶ Despite the importance of NCNs in protein function, molecular models for their mode(s) of action are lacking, in part due to the ambiguating complexity of proteins that utilize them for function. Furthermore, while communication through a network is a common mechanism in proteins, there are very few examples in synthetic systems, as small molecule models are generally insufficient to create functional NCNs.⁷ While many molecular machines demonstrate switching capabilities between two states in response to a stimulus, these systems generally rely on the on/off switching of one or two dominant interactions rather than a network of weak noncovalent interactions as is typical of a protein. Thus, the ability to mimic, and hence utilize, actuation through a noncovalent network is in its infancy.



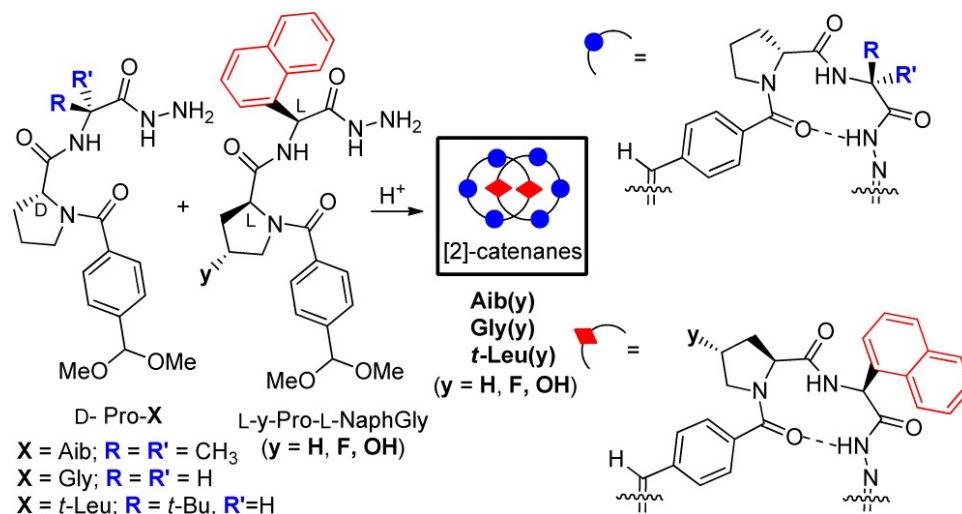
Scheme 1. Schematic Representation of the Long-Range Response in the NCN Arising from the Aib to Gly Mutation in the Catenane Model System

Herein, we utilize a synthetic NCN composed of biomimetic noncovalent interactions (H-bonds, π - π , and CH- π interactions) of intermediate complexity (~ 3000 Da),⁸ which combine the molecular features of high manipulability and high-resolution characterizability to systematically study the response of a NCN to mutation (Scheme 1). This study articulates molecular and energetic mechanisms by which synthetic NCNs can respond to a stress and the key notion that “frustrated” noncovalent interactions displaying negative cooperativity are required to provide the driving force for the response. We refer to this driving force as *latent free energy* and describe a mechanistic and energetic model for responsive systems. These insights provide a new framework for considering the role of NCNs in complex function while also establishing a basis for designing new artificial stimuli-responsive systems.

RESULTS

System Design. Our model is a self-assembled C_2 -symmetric [2]-catenane which we identified by dynamic combinatorial chemistry.⁹ The two interlocked macrocyclic rings are each created via hydrazone linkages from four dipeptide monomers with exchangeable (with 50 equiv of CF_3COOH only) hydrazide and aldehyde termini (Scheme 2).⁸ Once isolated, the catenanes are stable in the absence of TFA and amenable to full characterization.

Scheme 2. Synthetic Scheme for the Self-Assembly of the [2]-Catenane Models with 50 Equiv of TFA



Each macrocycle contains three D-Pro-X ($\text{X} = \text{Aib}$, Gly, or t-Leu) and one L-Pro-L-NaphGly subunit to form $(\text{L-Pro-L-NaphGly})_2(\text{D-Pro-X})_6$ catenanes, with the arylGly monomer being required for catenane formation.¹⁰ To facilitate X-ray analysis several L-Pro ($\text{y} = \text{H}$, OH, F) variants were also synthesized; our analysis shows the influence of y to be minimal (see SI Tables 7–9 and SI Figures 3a and 4a). The catenanes herein will be referred to by their X residue with the L-Pro substituent y noted in parentheses; the parent is thus Aib(H) (Scheme 2).

The X-ray structure of Aib(H) was previously reported (see ref 8c). Its NCN is assessed in more detail in this report as it provides a baseline for comparison to the mutants, Gly(H) and t-Leu(H) . Structural analysis of Aib(H), which is formed in 63% yield,⁸ revealed a highly organized compact structure with little free volume, consisting of 8 β -turns and multiple inter- and intramacrocyclic noncovalent interactions including Hbonds, a CH- π -CH sandwich made up of L-Pro, naphthylGly, and a CH_3 group from Aib,^{11,12} and an aryl barrel consisting of multiple edge-face and offset stacked π - π interactions (Figure 1a and 1b).

The necessity of the arylGly monomer in the self-assembly suggested that a conserved pair of CH- π -CH sandwiches was critical to catenane stability (Figure 1a and 1c), a notion that was tested by varying the components of the sandwich. Surprisingly, mutations designed to destabilize the CH component in Aib(H) were much less detrimental than expected. For example, deleting the methyl group (Aib to Gly mutation)

and thereby removing two Me- π interactions worth approximately 1–1.9 kcal/mol each based on literature values (for a sum of 3–3.8 kcal/mol)^{12,13} or increasing the alkyl size (Aib for *t*-Leu) and introducing a steric clash still yielded significant quantities of catenane (54% and 17% yield, respectively, cf. 66%).¹⁴ While we expected these mutations to abolish catenane formation, they were accommodated in some way by the densely packed structure. To validate that yield corresponds to stability, we analyzed the speciation of these libraries and performed a mixed monomer experiment, described below. Our analysis of the equilibrium speciation of the self-assembly indicates that like the parent Aib(H) catenane, no unusually stable cyclic dimers, trimers, or tetramers are affecting the catenane equilibrium concentrations in the Gly(H) library. Moreover, a mixed-monomer experiment with both D-Pro-Aib and D-Pro-Gly monomers in combination with L-Pro-NaphGly shows that in addition to predominantly generating catenanes that are a mix of D-Pro-X units (by LC-MS), these mixed experiments generate both pure D-Pro-Gly- and D-Pro-Aib-derived catenanes in comparable quantities (within a factor of 2; see SI Figure 1).

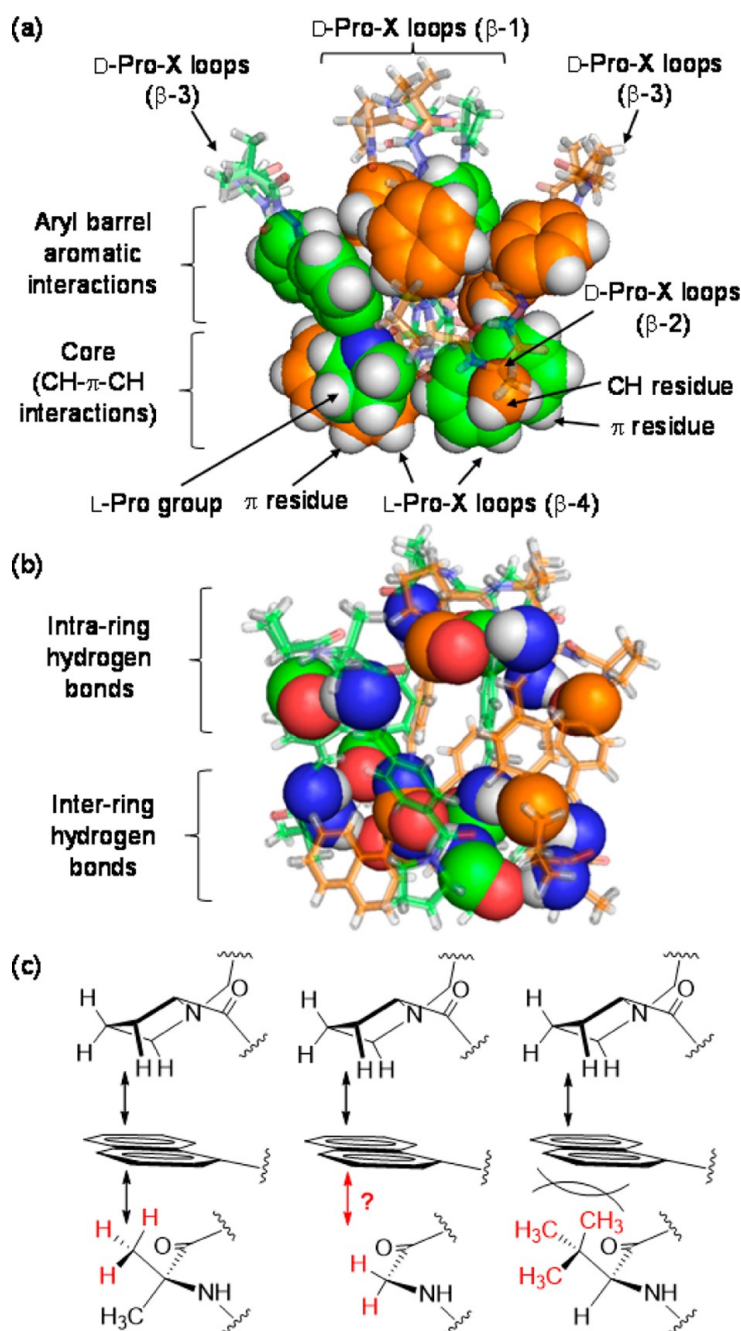


Figure 1. (a) Aryl barrel and the CH- π -CH networks (in space filling) in Aib(H). (b) H-bond network (in space filling) in Aib(H). (c) Schematic showing the Aib to Gly or *t*-Leu mutations. Green and orange represent the carbons in the two interlocking rings.

This experimental approach to validating the hypothesis that the D-Pro-Gly and the D-Pro-Aib catenanes are similar in energy complements numerical simulation approaches that we and others have investigated.¹⁵ These studies indicate that it is inherent stability in the D-Pro-Gly catenane that is responsible for its significant concentration at equilibrium.

Taking yield as a measure of stability, in the Gly(H) case, the mutant is only ~ 0.3 kcal/mol^{8b} less stable than the parent Aib(H).¹⁶ On the basis of a theoretical maximum for the CH– π interaction (1.9 kcal/mol), the loss of two CH– π interactions in the absence of other changes to the structure is expected to destabilize the catenane up to 3.8 kcal/mol. Thus, the NCN provides up to 3.5 kcal/mol of compensating stabilization through its reoptimization. Even with a more modest value for a CH– π interaction of 1 kcal/mol, as measured in a torsion balance in CDCl₃,¹³ the NCN still provides ~ 1.7 kcal/mol in stabilization.

Leu groups should destabilize the catenane by 6 kcal/mol in the absence of reoptimization of the NCN. Instead, the observed loss in stability, based on yields, is only about 1.2 kcal/mol. Thus, rebalancing the NCN provides approximately 4.8 kcal/mol in stabilization. Herein, we investigate the catenane structures and dynamics to gain molecular level insight into how the NCN provided this compensatory stabilization. As discussed below, we propose that this compensating energy can be thought of as latent free energy that is harnessed upon perturbation of the parent structure.

X-ray Structural Analysis. Differences in β -Turns. X-ray structures of Gly(F) and *t*-Leu(OH) were obtained to provide insight into how the NCN responds to the mutations relative to Aib(H) (Figure 2).¹⁷ The most dramatic change observed is that, in contrast to Aib(H) and *t*-Leu(OH), Gly(F) has its C_2 -symmetry broken by the flipping of one β -1 turn from type II' in Aib(H) to type I'. This turn flip creates an additional H bond between the β -1 and the β -1' turns (Figure 2c).¹⁸ Furthermore, the 1/1', 2/2', and 3/3' turns in Gly(F) all exhibit deviations of more than 10° in one or more dihedral angle, indicating that methyl group loss creates changes across the entire structure. Changes in *t*-Leu(OH) are less pronounced with each turn remaining type II' (Figure 2d) but with some deviations of $>10^\circ$ in turns 1/1', 2/2', and 3/3'. In all three catenanes, however, β -turn 4, which contains the L-Pro-arylGly unit in the core, is invariant, despite making up part of the altered CH– π -CH sandwich (see Table 1 and SI Table 2).

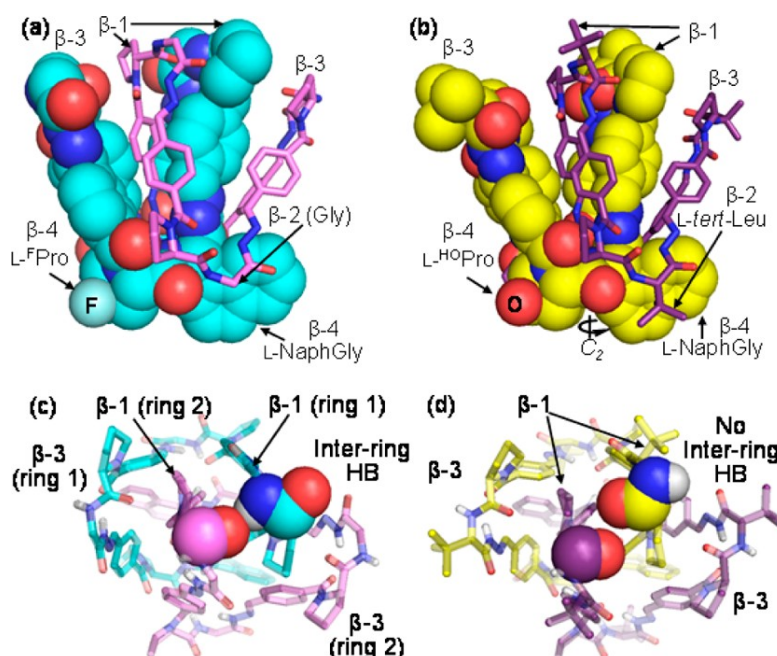


Figure 2. X-ray structures of (a) Gly(F) and (b) *t*-Leu(OH); one ring in space filling. (c) New H bond formed upon flipping of the β -1 turns from II' to I' in Gly(F). (d) Equivalent region of *t*-Leu(OH) (no turn flip, no H bond).

Analysis of CH– π -CH Interactions. The X-ray structures show intact CH– π -CH sandwiches, though they are maintained to different degrees in response to the variation in one CH component (Figure 3). The non- C_2 -symmetric Gly(F) exhibits two slightly different weak Gly–naphthyl interactions. As shown in Figure 3a and 3b for one of these interactions, the Gly residue is displaced from both the center of the naphthyl ring and its plane, with Gly C α distances to the naphthyl centroid lengthening to 4.85 and 4.93 Å (cf. 3.48 Å for the Aib methyl to centroid distance, see Figure 3c), at the extreme limit of what might be described as a CH– π interaction. The (expected) diminishment/loss of the Gly CH– π interaction is, however, counterbalanced by a tighter prolyl– π interaction (3.72 and 3.74 Å for the F-Pro groups versus 4.08 Å for Aib(H)).¹⁹

The *t*-Leu(OH) catenane also displays significant plasticity, as it accommodates the bulky *tert*-butyl group by shifting the entire *t*-Leu-containing β -turn away from the naphthyl ring to place a CH₃ in the same position as the Aib CH₃ (CH₃– π = 3.28 Å; Figure 3d). This shift pushes the proline away from the center of the naphthyl ring (angle between the proline centroid and the naphthyl ring = 109.2° versus 93.3° for Aib(H)) but 0.3 Å closer than for Aib(H) (Figure 3d and SI Table 3). Thus, in both Gly(F) and *t*-Leu(OH) the NCN has flexed to optimize the critical CH– π -CH sandwich.

Table 1. Direct Comparison of Metrical β -Turn Parameters of Aib(H), Gly(F), and *t*-Leu(OH) (dihedral angle differences > 10° in red bold italics)

		Dihedral Angle (°) of Aib(H) , (turn type)	Δ Gly(F) – Aib(H) (°) ^{a,b} , (turn type)		Δ t-Leu(OH) – Aib(H) (°) ^{a,b} , (turn type)
			Ring 1	Ring 2	
β -1 (loop)	Φ_{i+1}	60.1	0.9	-2.0	-2.1
	Ψ_{i+1}	-145.9	176.9	11.7	5.0
	Φ_{i+2}	-52.3	162.3	-23.7	-13.0
	Ψ_{i+2}	-35.9	9.9	26.9	11.7
β -2 (core)	Φ_{i+1}	58.7	7.4	6.4	12.2
	Ψ_{i+1}	-128.9	1.6	6.4	1.3
	Φ_{i+2}	-62.7	-13.3	-20.3	-9.1
	Ψ_{i+2}	-13.0	15.0	16.0	-1.2
β -3 (loop)	Φ_{i+1}	58.5	10.4	3.5	10.2
	Ψ_{i+1}	-126.6	-5.5	13.6	4.3
	Φ_{i+2}	-62.5	-20.5	-13.5	-8.8
	Ψ_{i+2}	-21.9	27.9	14.9	4.9
β -4 (core)	Φ_{i+1}	-66.1	-5.4	-6.5	-2.5
	Ψ_{i+1}	-30.7	-2.5	-1.6	3.2
	Φ_{i+2}	-159.0	-1.0	6.1	4.6
	Ψ_{i+2}	83.0	-8.2	1.0	-4.1

^aThe corresponding dihedral angle differences in Aib(H) vs Gly(F) and *t*-Leu(OH). Two values are reported for Gly(F) as this compound is non- C_2 symmetric in the solid state. The ring containing a flipped β -1 turn (type I') is assigned ring 1 (see Figure 2c). ^bThe RMSD metrics of structural similarity to the parent structure (Aib(H)) obtained by PyMOL pair fitting using 48 arbitrarily chosen backbone atom pairs for Gly(F) vs Aib(H) and *t*-Leu(OH) vs Aib(H) are 0.811 and 0.642 Å, respectively.

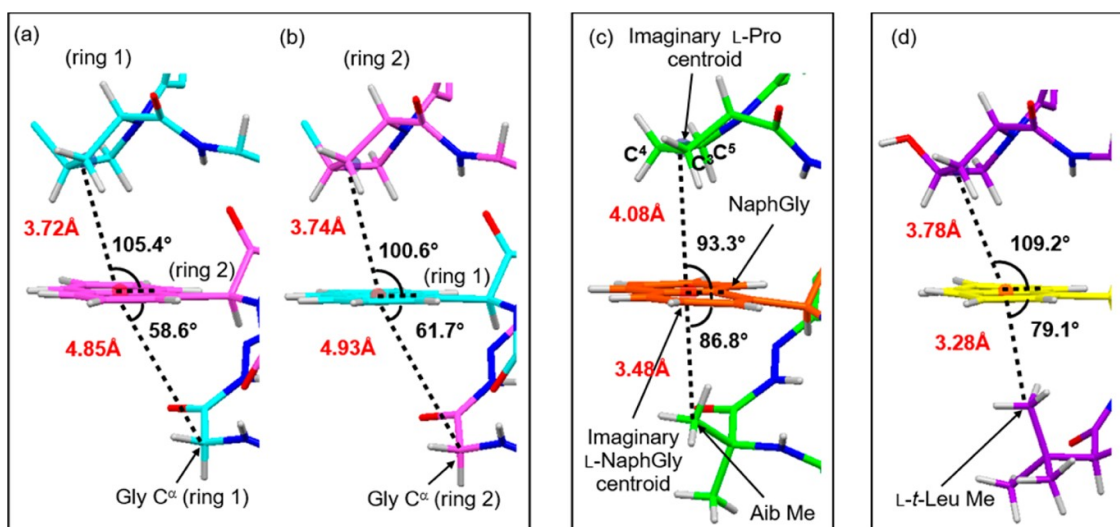


Figure 3. Direct comparison of the distances (red bold, Angstroms) and angles (bold, degrees) characterizing CH- π interactions (computed from the imaginary proline and L-NaphGly centroids and from the imaginary L-NaphGly centroid and Gly CH₂, Aib Me, and L-*t*-Leu Me, respectively) in (a and b) Gly(F) (The ring containing a flipped β -1 turn (type I') is assigned ring 1), (c) Aib(H), and (d) *t*-Leu(OH); distance cutoff is X...M $\leq$ 5.0 Å (see Shanthi, V.; Ramamathan, K.; Sethumadhavan, R. *Appl. Biochem. Biotechnol.* 2010, 160, 1473–1483). The imaginary proline centroid was generated from three carbons of an L-Pro (C³, C⁴, C⁵) and an imaginary L-NaphGly centroid computed from 10 carbons of the L-Naph ring.

Table 2. Direct Comparison of the Distances (Angstroms) between O and the N Atoms of the Main Chain C=O and NHgroups forming Intraturn and Inter-Ring Hydrogen Bonds in Aib(H), Gly(F), and *t*-Leu(OH)

		N-O distance (Å) in Aib(H)	Δ Gly(F) – Aib(H) ^a		Δ <i>t</i> -Leu(OH) – Aib(H) ^a
			ring 1 ^b	ring 2 ^b	
Intra-turn hydrogen bonds of Gly(F)					
	β -1	3.14	-0.27	-0.29	-0.20
	β -2	2.96	-0.01	0.01	0.07
	β -3	2.91	-0.02	0.20	0.10
Inter-ring hydrogen bonds of Gly(F)					
	N (amide) in β -2 to O in β -4	2.99	-0.10	-0.15	-0.11
	N (hydrazone) in β -4 to O in β -2	2.95	-0.00	0.01	-0.02
	N (amide) in β -4 to O in β -4	2.86	0.03	-0.02	0.02
	N (amide) in β -1 to O in β -1'	none	2.99	none	none
	N (amide) in β -1 to O in β -1'	none	2.99	none	none

^aThe differences in the corresponding H-bond distances forming intraturn and inter-ring hydrogen bonds in Aib(H) vs Gly(F) and Aib(H) vs *t*-Leu(OH). The negative values represent the shorter distance compared to those in Aib(H). ^bThe ring containing the N atom of an NH group forming inter-ring hydrogen bonds. The ring contained a flipped β -1 turn (type I') is assigned as ring 1 (see Figure 2c).

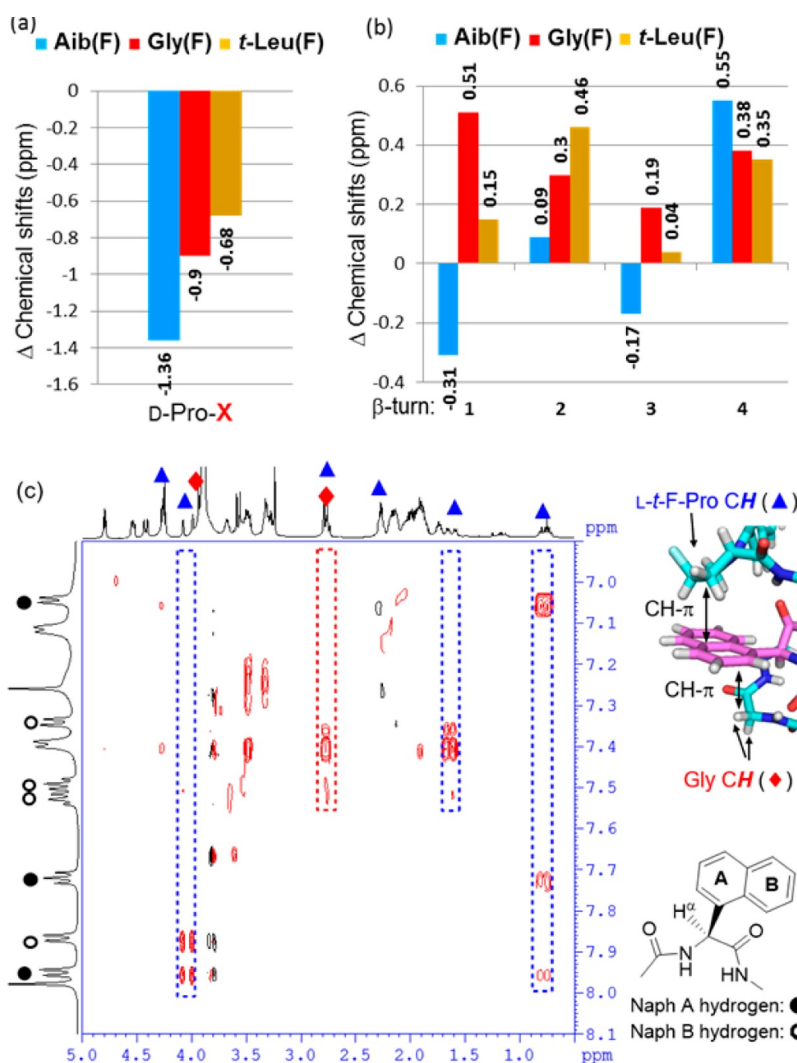


Figure 4. Direct chemical shift comparisons of (a) catenane D-Pro-X residue X signal (β -2 turn) and (b) catenane amide NHs. Series of catenanes are Aib(F), Gly(F), and *t*-Leu(F). (c) Portion of the 2D 600.1 MHz ROESY spectrum of Gly(F) (F1 δ = 6.9–8.1 ppm; F2 δ = 0.0–5.0 ppm; MeOH-*d*₄/5CDCl₃, 25 °C) showing CH- π interactions between L-*trans*-F-Pro or a Gly CH₂ group and the NaphGly residue, respectively.

Analysis of the H-Bond Network. Compensatory changes also appear as strengthened H-bond contributors to the NCN. Aib(H) contains six pairs of symmetry-related H bonds with N–O distances of 2.8–3.1 Å. As mentioned above, the turn flip at β -1 in Gly(F) creates a new H bond with a N–O distance of 3 Å (Figure 2c and Table 2). Also shortened are the β -1 intraring H bonds (by 0.3 Å) and the inter-ring H bonds in the core between β -2 and β -4 (by 0.1 Å).²⁰ Except for the new β -1/ β -1' H bond, these same H-bonds are also strengthened in *t*-Leu(OH) (see Table 2), implying that the NCN can reoptimize specific local and distant noncovalent interactions to compensate for the mutations.

Changes in the Aryl Barrel. Changes to the CH- π -CH sandwich and H bonds are also accompanied by constriction in the aryl barrel in both the Gly and the *t*-Leu catenanes relative to the Aib catenane (see SI Tables 5 and 6 for metrical parameters), suggesting plasticity in the aryl barrel that accommodates changes elsewhere in the structure. Together this structural analysis indicates that both mutated catenanes accommodate the perturbation of the CH- π interaction via reoptimization of the entire NCN rather than just local changes.

NMR Structural Analysis of Gly- and *t*-Leu Catenanes. NMR analysis of the *trans*-F-Pro version of all three catenanes was also undertaken to provide additional structural and dynamic information.²¹ At RT, the catenanes all display *C*₂-symmetric ¹H NMR spectra, despite the asymmetric X-ray structure of Gly(F) (but see VT NMR of Gly(F) vide infra).

Analysis of CH– π -CH Interactions. Inspection of several key chemical shifts relative to their monomers gives molecular level insights into the response of the NCN to individual perturbations of the CH– π interaction. The chemical shifts of the CH– π participating methyl and CH resonances in Aib(F) and Gly(F), respectively, show a weaker (less populated) CH– π interaction for Gly(F) than for Aib(F) (normalizing for the number of hydrogens, 0.9 ppm and $1.36 \times 3 = 4.08$ ppm upfield shifted for Gly(F) and Aib(F) relative to monomer, respectively, see Figure 4a). Despite the smaller shift for Gly(F), the upfield shifting still suggests that the CH is near the face of the aromatic group, in agreement with the X-ray structure (see Figure 3a and 3b). The CH₃ groups of the *t*-Bu residue in *t*-Leu(F) are upfield shifted by 0.68 ppm. This is an average shift for the three equilibrating methyl groups, thus providing a net upfield shifting of $0.68 \times 9 = 6.12$ ppm when normalized for the number of hydrogens to compare to Gly(F) above. While this may be a slight overestimate, this large shift is in agreement with the close contact observed in the X-ray structure (see Figure 3c). Thus, the apparent order of CH– π interactions between the mutated residue and the naphthyl group is *t*-Leu(F) > Aib(F) > Gly(F). The variation in the proline chemical shifts that participates in the Pro– π interaction is much smaller (see SI Figure 3), suggesting that the interaction is of similar magnitude across all three catenanes.

Analysis of the H-Bond Network. Comparison of the amide NH chemical shifts also revealed changes in the H-bonding network (see Figure 4b). The β -1 amide, which is in the central “stem” of the catenane (Table 2), is 0.8 ppm farther downfield in Gly(F) relative to Aib(F), consistent with the former’s new inter-ring H bond (X-ray, Figure 2c). The β -2 amide NH in the core also becomes increasingly downfield shifted on going from Aib to Gly to *t*-Leu, suggesting that this inter-ring H bond is stronger (more populated) in the mutants. Conversely, the β -4 amide NH (which is buried in the core of the catenane and forms an inter-ring H-bond) is 0.2 ppm less downfield shifted in Gly(F) and *t*-Leu(F), suggesting a slightly weaker (less populated) H bond in the core relative to Aib(F). *Taken together these H-bond shifts suggest that in addition to the already noted changes in the CH– π interactions, Gly(F) and t-Leu(F) also compensate for their mutations by reoptimizing their entire H-bond networks.*

Changes in the Aryl Barrel. Analysis of the aryl rings in the aryl barrel indicate that only minor changes occur on mutation of Aib to Gly or *t*-Leu (see SI Table 8). In the *t*-Leu case, there is no significant change in the chemical shifts of the aryl protons (<0.1 ppm in all positions). In the Gly(F) catenane, most protons exhibit upfield shifting of ~0.5 ppm relative to Aib(H), and one proton is shifted upfield by ~0.2 ppm, suggesting a modestly more favorable edge–face interaction. *In sum, this demonstrates that while the aryl barrel is able to transmit a signal (mutation) from one end of the catenane to the other, it is quite malleable and does not appear to directly contribute significant stability to the mutants through new or optimized noncovalent interactions.*

Dynamics by H/D Exchange. H/D exchange was used to extract dynamics information from the various catenanes, as has been done in proteins such as streptavidin.²² While noncyclized monomers undergo complete H/D exchange in <5 min, amide exchange times in these closed structures is much slower and structurally variable. For example, the exchange half-lives for Aib(F) vary from minutes in the periphery to days in the core β -4 amide positions (Figure 5). By contrast, the amide exchange behavior of Gly(F) is characterized by slow exchange at β -4 and fast exchange at all other positions (<20 min). Interestingly, the exchange rates for *t*-Leu(F) exhibit a different pattern in which exchange is faster than Aib(F) and Gly(F) at the most buried β -4 amide (factor of 20) but similar to Aib(F) at the slightly more exposed β -2 amide positions (factor of 3) (Figure 5c and SI Table 10).

VT NMR of Gly(F). VT NMR indicates that Gly(F) displays more significant dynamics than the other catenanes (see Figure 6 and SI Figures 17 and 18). While its RT ¹H NMR displays net *C*₂ symmetry, cooling desymmetrizes the spectra into two sets of resonances (<–26 °C) that are consistent with the asymmetry observed in the X-ray structure. This process does not occur in Aib(F) or *t*-Leu(F) even as low as –80 °C. These millisecond dynamics combined with enhanced rates of H/D exchange show Gly(F) to be far more dynamic than Aib(F), while *t*-Leu(F) is intermediate. *As discussed below, one intriguing implication is that part of the unexpected*

stability in Gly(F) is due to enhanced dynamics.

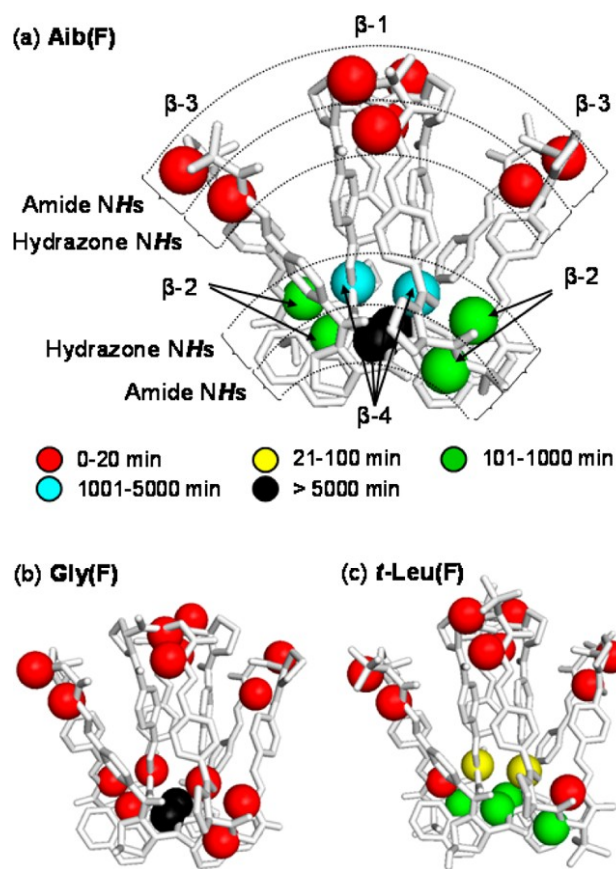


Figure 5. Half-life for position-dependent H/D exchange in (a) Aib(F), (b) Gly(F), and (c) *t*-Leu(F) determined by NMR spectroscopy. Reaction conditions: MeOH-*d*₄/5CDCl₃, 20 °C.

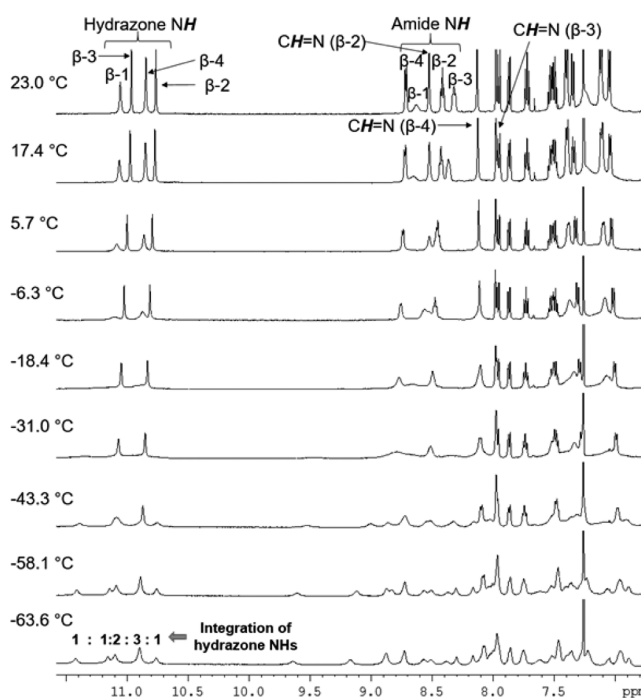


Figure 6. Portion of the ¹H variable-temperature (VT) NMR spectra of Gly(F) (δ = 7.4–11.6 ppm, 500.1 MHz, MeOH-*d*₄/5CDCl₃) showing the decoalescence of hydrazone and amide NHs.

DISCUSSION

These studies describe the molecular level response of a NCN to a mutation-induced stress. In the mutation of Aib to Gly, the stress is a loss of a key $\text{CH}_3-\pi$ interaction, whereas in the Aib to *t*-Leu mutation, a steric clash is introduced. In the absence of any changes in the catenane structure, these mutations would be expected to abolish the thermodynamically controlled self-assembly. However, due to long-range restructuring of the NCN in combination with changes in dynamics, these mutants still provide stable, well-folded catenanes. Our detailed structural (X-ray and NMR) and dynamics studies provide sufficient resolution to describe this phenomenon in terms of specific molecular features.

NMR and X-ray data indicate that the $\text{CH}-\pi$ interaction is in fact weakened or lost in the Gly(X) catenane, while the $\text{Pro}-\pi$ interaction is maintained. These structural studies show a number of short- and long-range changes to the baseline interactions that together enthalpically compensate for this loss. Most prominent is the long-distance turn flip that introduces a new H bond between the β -1 and the β -1' rings. Other H-bonds in turns 2 and 3 are more populated as well, based on NH chemical shifts. Structural changes are also observed in the aryl barrel, although with minimal changes to chemical shifts. Interestingly, this system appears to employ a seesaw type of mechanism, where a large perturbation at one end of the NCN ($\text{CH}-\pi$ interaction) induces a large change at a distal site (turn flip and new H bond), suggesting that the plasticity of the aryl barrel may facilitate communication from one end of the catenane to the other. *Thus, while the responses to the mutations can be assessed on an individual residue level, they are best viewed as a network of perturbations that propagate through the NCN of hydrogen bonds and aromatic noncovalent interactions.*

Entropy also contributes to the stabilization of Gly(X), including a millisecond time scale conformational switching term ($R \ln 2 = 0.4 \text{ kcal/mol}$)²³ as well as longer time scale (min +) structure-dependent (Gly(F) vs Aib(F) vs *t*-Leu(F)) and position-dependent H/D exchange processes. Importantly, entropy and enthalpy are not always opposed in these systems, as some amides became more dynamic while simultaneously exhibiting stronger H-bonds based on chemical shifts.

The *t*-Leu catenane also displays both enthalpic and entropic stabilization throughout the NCN. The $\text{CH}-\pi-\text{CH}$ sandwich shifts to accommodate the *t*-Leu, resulting in a $\text{CH}-\pi$ interaction that is stronger relative to Aib, based on $\text{CH}_3-\pi$ distances and CH_3 chemical shifts. As with Gly(F), *t*-Leu(F) also exhibits several more populated H bonds based on amide NH chemical shifts, while its dynamics, based on H/D exchange, are intermediate to those of Gly(F) and Aib(F). In this case, however, no turn flip was induced, and so no additional H-bond was gained. *Thus, each mutant utilizes a unique rebalancing of the NCN to maintain stability.*

These two mutants exhibit a number of protein-like qualities. The Aib-to-*t*-Leu mutant is reminiscent of a nonlethal protein mutation which is accommodated by many small changes throughout the folded structure. The Aib-to-Gly mutant, which induces the aforementioned long-distance turn flip and the creation of a new H bond, is a model for actuation through a network. Signal transduction through a membrane relies on the propagation of a signal (structural perturbation) at one end of the protein or protein complex into a commensurate structural change over the long distances characteristic of membrane spanning proteins. Furthermore, the opposite mutation from Gly to Aib (i.e., introducing a methyl group) exhibits characteristics of binding by conformational selection.²⁴ The Gly catenane exhibits significant conformational flexibility, but introduction of the methyl group in the Aib catenane causes one conformation to be stabilized over the others. In this case, the conformational selection is the result of mutation rather than binding.

While the stimulus-response mechanisms observed in these catenanes are comparable to those observed in proteins, it is significantly different than typical designed stimuli-responsive systems, such as molecular shuttles or designed allosteric systems.²⁵ Most designed stimulus-responsive systems utilize simple “on–off” interactions rather than networked interactions as observed here (and utilized by proteins).

Analysis of the changes in the NCN in these systems leads to a novel energetic model regarding how NCNs are able to respond to a stimulus as described below. First, consider a case in which every individual noncovalent interaction in a NCN is fully optimized. Introduction of a stimulus (in this case a mutation that results in loss of a favorable interaction) would necessarily destabilize the network by the same amount. In this scenario, the

network would be unable to respond. However, if the NCN is in its lowest energy accessible state but *not every individual* noncovalent interaction can access its optimal geometry, perturbation at one position in the network *could be compensated for by improvements in different positions*, i.e. loss of one interaction allows for formation/optimization of others. This reoptimization of the network can result in long-distance structural and dynamic changes, as are required for complex protein functions such as allostery and signal transduction. Stated another way, *some interactions in the initial NCN must exhibit negative cooperativity for the NCN to be responsive to a stimulus*. In a broad sense, such a *responsive NCN* represents an example of systems chemistry and is conceptually equivalent to dynamic combinatorial chemistry, in which a system finds a new lowest energy state in response to a stimulus.²⁶

In this description, a *responsive NCN* is therefore one that stores potential energy the potential energy of the non-optimal noncovalent interactions. We refer to this stored energy as latent free energy (LFE). In the Aib-to-Gly mutation, for example, latent free energy is released in the form of new and strengthened H-bonds and increased entropy, the combination of which compensate for the loss of the CH- π interactions. While this concept of latent free energy is related to cooperativity, the concepts describe different phenomena. Cooperativity defines the energetic relationship between individual noncovalent interactions within a network relative to an unnetworked reference state, whereas latent free energy is associated with the ability of a cooperative network of interactions to re-equilibrate in response to a stimulus. The concept of a responsive NCN being one that stores latent free energy may be related to the observation that functional sites in proteins (both binding and catalytic sites and allosteric sites) contain a higher degree of “frustration”, as described by Wolynes.²⁷ Frustration at such sites may correlate with the storage/buildup of latent free energy and hence responsiveness of the network. We suggest that this conceptual framework connecting molecular level changes to an energetic model of latent free energy will be valuable for developing new synthetic systems with more complex function.

CONCLUSIONS

Our model demonstrates that a stimulus-triggered rebalancing of attractive and repulsive forces in a NCN can drive complex responses and thereby link molecular observables to an energetic framework. The outcome of our studies on a responsive NCN provides a strategy for mimicking similar, albeit more complex, protein functions involving actuation. In general, a corollary of our LFE model of NCN properties is that de novo designed proteins may be overdesigned for stability rather than for the latent free energy required to drive function.²⁸ Moreover, this work provides a conceptual basis for incorporating complex NCN-coupled functions in novel abiotic systems.

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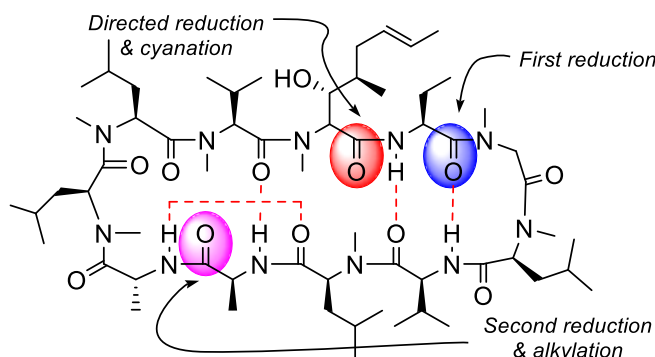
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- (17) By NMR these catenane variants are symmetric in solution (see [SI Tables 7–9](#) and [SI Figures 3 and 4](#)).
- (18) A similar turn flip was observed in a catenane with D-Pro- cyclobutylglycine in which the core CH– π -CH interaction was weakened (see refs [8a](#) and [b](#)). To the best of our knowledge, a type I' turn has not previously been observed in a D-Pro-Gly containing natural peptide, suggesting that it is the context of the catenane not the inherent turn preference that induced the turn flip. (a) Gopi, H. N.; Roy, R. S.; Raghothama, S. R.; Karle, I. L.; Balaram, P. *Helv. Chim. Acta* 2002, *85*, 3313–3330. (b) Wilmot, C. M.; Thornton, J. M. *J. Mol. Biol.* 1988, *203*, 221–232.
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- (20) These two H bonds are similarly shortened in *t*-Leu(OH) (see [Table 2](#) and [SI Table 4](#)).
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Aim 2: Site selective amide reduction of cyclosporine A opens new structural space for an important cyclic peptide

ABSTRACT

Site selective amide reductions of the cyclic undecapeptide, cyclosporine A, have been developed using the combination of a heteroleptic borane catalyst and a silane reductant. Tertiary silane Me_2EtSiH provides two unique cyclosporine A derivatives, one of which can be readily diversified in subsequent reactions. The secondary silane Et_2SiH_2 enables divergent reactivity that uses a free hydroxyl group to direct the reduction. The transient O-silyl hemiaminal intermediate of this reduction can additionally be trapped by reducing to the amine or by reductive cyanation.



Achieving specificity over which chemically distinct but functionally identical groups are modified in a complex molecule (site selectivity), represents a significant challenge in homogenous catalysis.^{1,2} Nowhere is this challenge more acute than in the realm of late-stage functionalization, a strategy in medicinal chemistry where complex natural products or drug candidates are site-selectively modified to optimize their physical, biological or pharmaceutical properties.³⁻⁷ The targeted diversification of an already bioactive structure removes the bottleneck imposed by de novo synthesis. While orthogonal reactivity can be exploited to achieve chemoselectivity, the challenge is magnified when a substrate bears multiple copies of the same reactive functional group. In these cases strategic use of catalysts and reagents can provide uniquely enabling methods.

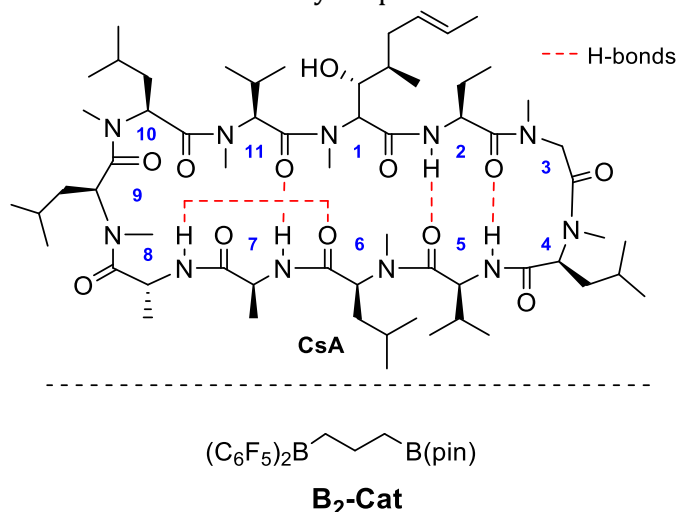
Approaches for site-selective amide reduction in polyamides is not well studied.^{8,9} Although many ways to reduce amides exist^{10,11}, including stoichiometric metal hydrides (i.e. LiAlH_4), these reactive reagents are not prone to tunable selectivity. Metal catalyzed hydrogenation of amides¹²⁻¹⁴ are in principle possible, but usually require extreme conditions (temperature and pressure), which are inhospitable to selectivity. Arguably, the mildest subclass of amide reductions are those employing silane reductants. Various metal catalysts¹⁵ (Ir ^{16,17}, Rh ^{8,18}, Pt ¹⁹, Zn ²⁰, Ni ²¹) are effective and display varying degrees of chemoselectivity. Metal free variations exist and use boranes^{9,22-26} and boronic acids²⁷ as catalysts. We have recently shown that heteroleptic perfluoroaryl/alkyl borane catalysts, such as **B₂-Cat** are highly selective for amide reductions relative to their homoleptic counterparts (i.e. $\text{B}(\text{C}_6\text{F}_5)_3$). Given the successful application of heteroleptic borane catalysts on substrates with the complexity of di- and tri-peptides²⁸, we questioned whether they may also enable site-selective reductions in complex polyamide substrates.

The cyclic undecapeptide natural product, cyclosporine A (**CsA**, **Scheme 1**), was used to investigate this question as it provides an excellent test bed for developing site selective amide chemistries.^{8,9} **CsA** is a potent immunosuppressant used in the clinic to treat graft rejection, rheumatoid arthritis, and psoriasis. Its immunosuppressant activity stems from its ability to inhibit calcineurin (T cell activator) through formation of the ternary complex cyclophilin·CsA·calcineurin.²⁹ Since the protein cyclophilin is also a target for treating viral infections (hepatitis and HIV) and neural protection, considerable effort has gone into discovering non-immunosuppressant analogs of **CsA**³⁰⁻³², i.e. those that on binding cyclophilin don't inhibit calcineurin.

The conversion of an amide to an amine alters a peptide's metabolism, membrane permeability, and bioavailability, all of which can be useful in optimizing a drug's properties.^{33,34} Site selective reductions of **CsA**

therefore enable interesting but largely inaccessible structural spaces to be populated. Positionally selective reduction of **CsA**'s secondary amides is particularly intriguing as the resulting secondary amines provide new handles for further diversification. Catalysts able to reduce secondary amides, however, are rare.^{16,18,21,26}

Scheme 1: Structure of cyclosporine A and borane catalyst

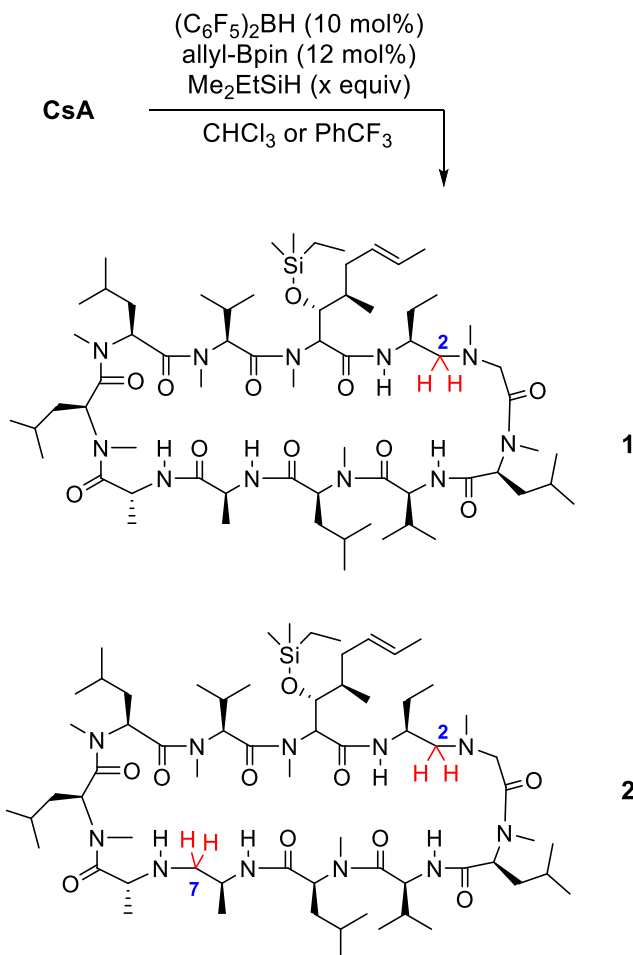


We began our studies using the heteroleptic catalyst, **B₂-Cat**, which is conveniently prepared in situ by reacting Piers borane^{35,36} $((\text{C}_6\text{F}_5)_2\text{BH})$ with allyl-B(pin). **B₂-Cat** in combination with a tertiary silane such as Me_2EtSiH in toluene can reduce tertiary amides in the presence of secondary amides, alcohols, heterocycles, etc. Dehydrogenative silylation of the free hydroxyl group of **CsA** was fast as assessed by the rapid effervescence of the solution. Since the subsequent amide reduction was relatively slow, the actual substrate for the reaction is the OH-silylated, **CsA-OSiR₃**. A brief solvent screen indicated that CHCl_3 and trifluorotoluene gave higher conversions over 24 hours and were thus used for further studies (**Scheme 2**).

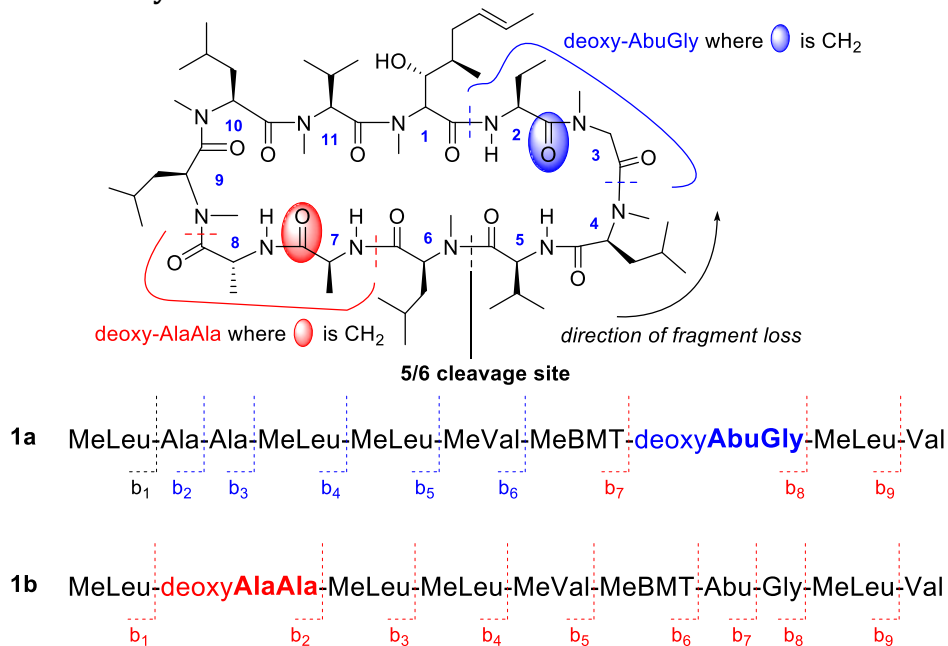
Analysis of the reaction mixture by LC-MS revealed two major products, a single and a double amide reduction. The same products were observed across the solvents tested. The mono- (**1**, 37%) and double reduction (**2**, 16%) products were isolable by flash column chromatography. The structure of the double reduction (**2**) product was ascertained by a suite of ^1H and ^{13}C NMR experiments. Briefly, the amide region of the ^{13}C NMR spectrum confirmed the loss of two amides; the amide region of the ^1H NMR spectrum revealed that one secondary amide had been reduced; this was surprising as small molecule testing typically show secondary peptide linkages to be inert.²⁸ Selectively irradiating the remaining amide NH resonances via 1D-TOCSY experiments localized the reduction to either Ala7 or Ala8. A combination of 2D (HSQC, HMBC, and COSY) spectra identified the site to be Ala7. The site of tertiary amide reduction could then be traced to Abu2 by analysis of the 2D spectra (HSQC, HMBC, COSY).

In contrast to **2**, the NMR spectra of **1** were significantly broadened. Since resubjecting **1** to reaction conditions smoothly converted it to **2**, the site of reduction was logically either Abu2 or Ala7. This was disambiguated by adapting a peptide sequencing mass spectrometry experiment. The characterization of peptides via MS/MS is a powerful proteomics technique, but applying these methods to cyclic n-mer peptides is problematic as the first (ring-opening) fragmentation can occur at any of the (O)C–N bonds to give “n” distinct primary ions, all of which have the same molecular weight. We sought to distinguish between Abu2 or Ala7 being the first site reduced by analyzing the fragmentation of each of the 10 possible ions created by ring opening of a monoreduced **CsA**. To illustrate, consider the putative linear peptides that result from a ring-opening cleavage at the 5/6 junction in the mass spectrometer of the two isomeric monoreduced possibilities (**1a** and **1b**, **Scheme 3**). Although the two parent ions (**1a** and **1b**) have the same m/z, sequential fragment loss from these ions predicts a set of b-series ions that are unique to the two possible reduction sites.³⁷ Using a sensitivity threshold of 6σ, 247 fragments were found for **1** in the MS/MS experiment. These ions were then matched to those predicted for **1a** and **1b**. As indicated by the blue (observed) and red (not observed) coding in **Scheme 3**, the fidelity of the predicted/observed ions of **deoxy Ala7** is a significantly poorer match than that predicted for **deoxy Abu2**. The analysis of the other nine ring opening sites were similarly consistent with **1** being deoxy-Abu2.

Scheme 2: Reduction of **1** with Me₂EtSiH



Scheme 3: Sample MS² analysis of **1**



Having successfully identified **1** and **2** we sought to optimize the reaction conditions. As shown in Table 1, the best conditions (entry 7) utilized 15 equivalents of silane and was robust to scale up (x8, ~500 mg **CsA**). The reaction was quite sensitive to the sterics of the silane as the bulkier Et₃SiH (5 or 15 equivalents) stops after dehydrocoupling of the free OH.

Table 1. Optimization of the reaction in Scheme 2 with Me₂EtSiH

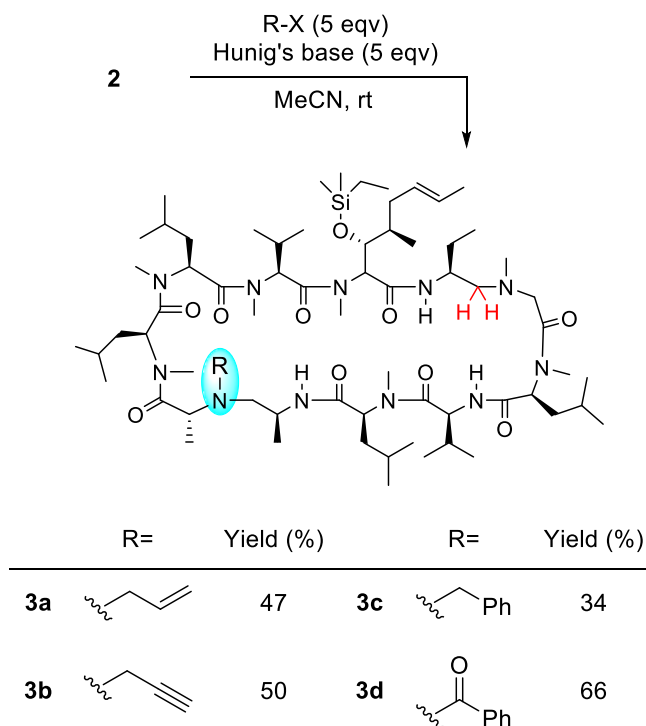
entry	Solvent	X Eqv. Me ₂ EtSiH	Yield (%) 1	Yield (%) 2
1	PhCF ₃	5	24	13
2	PhCF ₃	9	38	16
3	CHCl ₃	5	37	16
4 ^a	CHCl ₃	5	31	17
5	CHCl ₃	9	39	26
6 ^b	CHCl ₃	9	40	26
7	CHCl ₃	15	35	44

All reactions were performed with 10 mol % **B₂-Cat.** on 0.05 mmol scale. Isolated yields are given. ^aReaction performed at 50 °C. ^bReaction performed on 0.4 mmol scale

B₂-Cat's selectivity for first reducing a tertiary (Abu2) over a secondary site in **1** agrees with results on simple substrates. We surmise that **B₂-Cat** selects Abu2 over the tertiary valine and isoleucine sites on steric grounds, however, its preference over the normally more reactive Gly3 is unprecedented. For example, Gly3 is the site reduced by Beller in his Rh catalyzed hydrosilylation protocol⁸ and it is also the site activated by [Me₃O][BF₄]³⁸ en route to acyclic **CsA** derivatives (after MeOH treatment). The preferred reduction sites of **B₂-Cat** are therefore *not* those that are most sterically accessible and they additionally differ from previous derivatization efforts.

The secondary amine created in **2** resides at the edge of the calcineurin binding domain but could also modulate its binding to cyclophilin A.^{30–32} Despite being a difficult nitrogen to alkylate in **CsA**³⁰, the amine form in **2** is readily allylated, propargylated, benzylated, and benzoylated in moderate yields (**Scheme 4**). The allyl and propargyl group provide excellent handles for further derivations.^{39–41} Traces of over alkylation was observable by LC-MS. In situ protection of the alcohol under the reduction conditions conveniently prevents O-alkylation in the follow up derivatization steps.

Scheme 4: Derivations of **3**



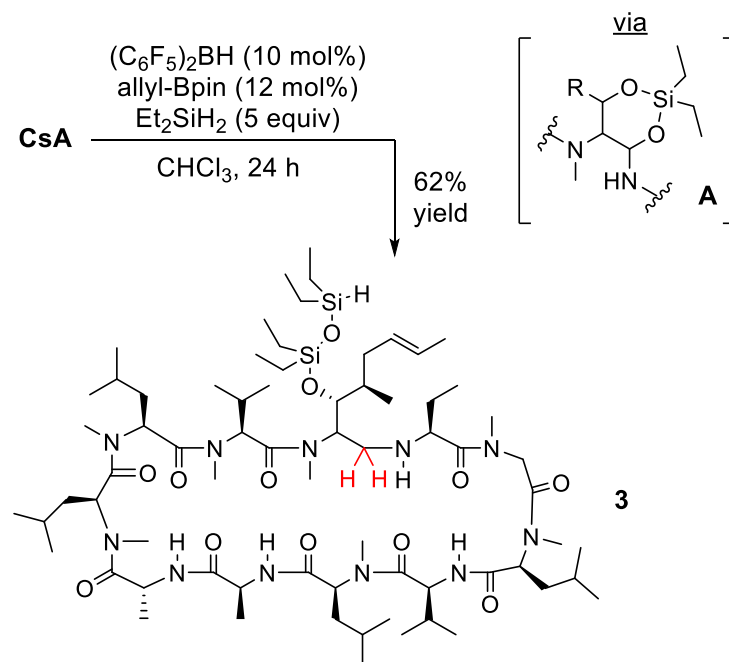
We next envisioned that the free hydroxyl group in **CsA** could be used to direct reactivity to proximal amide

sites as we previously demonstrated with natamycin.² While preparing this manuscript, the Kanai group applied this strategy using $(\text{C}_6\text{F}_5)_3\text{B}$ and PhMeSiH_2 .⁹ Our own conditions (**Scheme 5**) similarly provided the directed reduction product **3** (at MeBmT1) in 62% yield. HRMS analysis of the product indicated that the residual silyl group is the bis silyl ether that would result from ring opening the intermediate O-silyl hemiaminal. As shown in **Scheme 5**, the targeted proximal amide generates the 6-membered ring intermediate **A**. Reaching back to Val11 was also considered feasible but this would require a 7-membered ring intermediate. Since NMR spectra of **3** were sharp, the site of reduction could be identified through selective irradiation of the amide proton resonances in 1D-TOCSY experiments. The reaction also performed well on a half gram scale giving 50% isolated yield of chromatographically purified **3**.

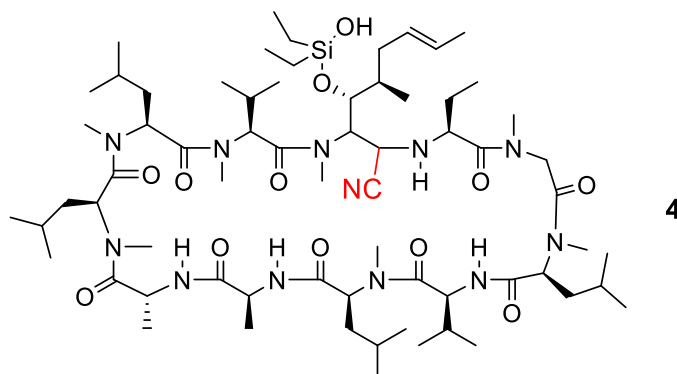
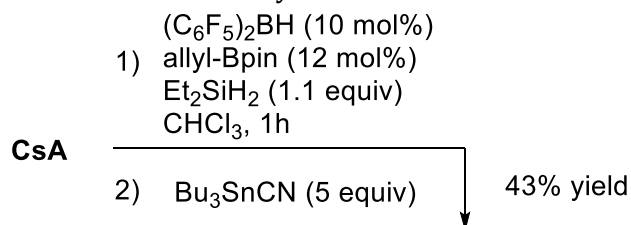
O-silyl hemiaminals such as **A** are additionally prone to selective chemistry.^{42–45} Although we have not directly observed this intermediate by in situ ^{13}C or ^1H NMR spectroscopy we hoped that it could be intercepted productively. To this end, **CsA** was reacted first with stoichiometric Et_2SiH_2 to putatively generate **A**. After one hour, 5 equivalents of $\text{Bu}_3\text{Sn-CN}$ was added and the residual **B₂-Cat** was able to catalyze the opening/cyanation to yield the alpha amino nitrile **4** in 43% yield as a single diastereomer. Alpha-amino nitriles can also be diversely transformed to other potentially interesting structures.^{42,46}

The development of amide reduction catalysts able to function under mild conditions has enabled the development of site selective reactions on a complex polyamide structure. Despite being the subject of extensive synthetic studies for its diverse medicinal effects, the observed selectivities with tertiary silanes are novel. Even though the origins of the innate site-selectivity is not yet clear, the **B₂-Cat** system reduces amide sites not targeted in other systems, and doubles the number of known amide reduced **CsA** derivatives. Reduction of secondary amide Ala7 additionally reveals a secondary amine that is prone to diversity generating reactivity. Furthermore, the **B₂-Cat** catalyzed amide reductions can be directed using secondary silanes, in this case using the MeBmT1 hydroxyl to select for MeBmT1 reduction over other sites. We also report that the O-silyl hemiaminal intermediate (**A**) can be intercepted to form new C–C bonds, and products that can be further diversified. These studies provide a framework for using non-metal catalysts to site-selectively diversify complex poly-amides with important biological properties.

Scheme 5: Directed reduction of **1**



Scheme 6: Reductive cyanation of CsA



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