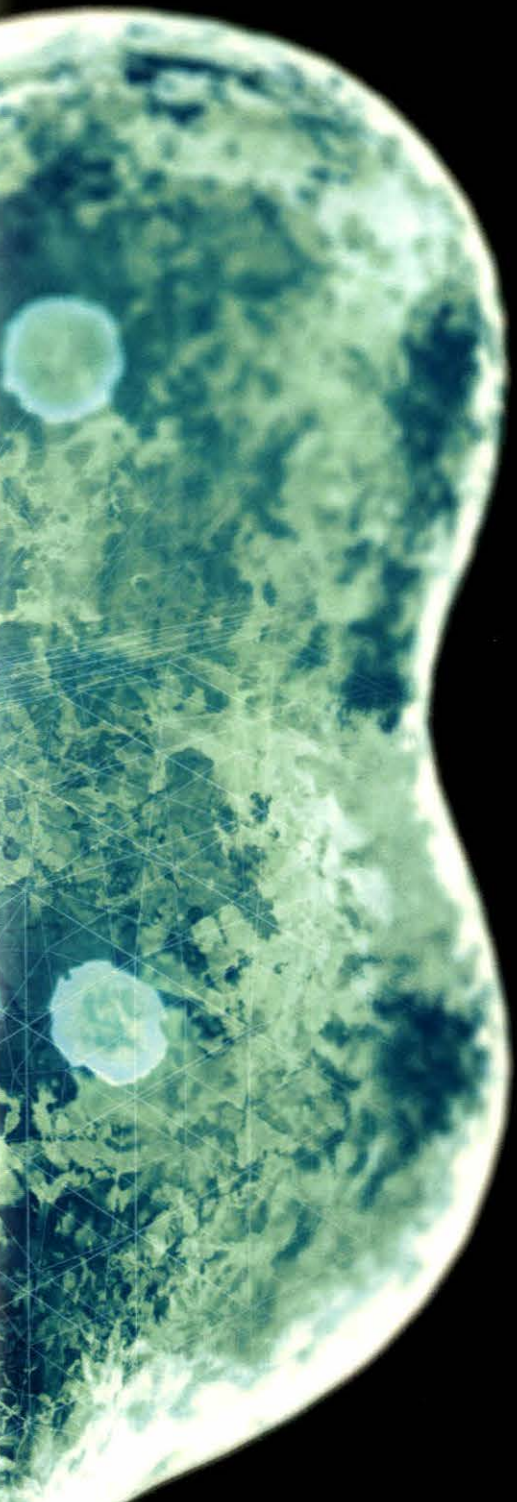
CURJ



WINTER 2015
VOL. 16 NO. 1

CALTECH UNDERGRADUATE
RESEARCH JOURNAL

We're hiring. You do the math.

D E Shaw & Co

www.deshaw.com

CURJ

Interview

Research

Me MECHANICAL ENGINEERING

Be BIO ENGINEERING

Bc BIO CHEMISTRY

Art Center College of Design

One of the world's preeminent art and design colleges, Art Center College of Design is renowned for its culture of innovation, rigorous transdisciplinary curriculum, superb professional faculty and extensive relationships with industry. For 80 years, Art Center has prepared an influential group of artists and designers to become leaders both in their chosen fields and the world at large.

UNDERGRADUATE PROGRAMS

Advertising
Entertainment Design
Environmental Design
Film
Fine Art
Graphic Design
Illustration
Photography and Imaging
Product Design
Transportation Design

GRADUATE PROGRAMS

Art
Broadcast Cinema
Industrial Design
Media Design

CURJ IS A COLLABORATIVE EFFORT BY STUDENTS AT ART CENTER COLLEGE OF DESIGN AND THE CALIFORNIA INSTITUTE OF TECHNOLOGY.

● Art Center College of Design

1700 Lida Street, Pasadena, CA 91103
626.396.2373 artcenter.edu

From the editors

CALTECH UNDERGRADUATE RESEARCH JOURNAL vol. 16 no. 1

This fall marks the end of yet another summer of intense undergraduate research at Caltech and JPL. Every year, hundreds of students from around the country and the globe are drawn to Pasadena for ten weeks of research under the leadership of talented faculty mentors. Through the guidance of these dedicated mentors, as well as the tireless efforts of Caltech's Student Faculty Programs office, these students have been able to take their first steps of what will become a long journey of scientific curiosity and discovery.

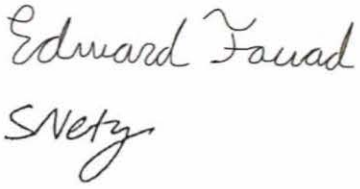
This issue of the Caltech Undergraduate Research Journal highlights the exceptional research of three young scholars: Alejandra Rios, Doreen Chan, and Simon Kalouche. With topics ranging from organic antifreeze proteins to stem cell differentiation to gecko-based climbing robots, these three articles exemplify the great diversity of work currently being performed by undergraduate students.

In this issue, CURJ is pleased to feature an interview with Professor Mikhail Shapiro, a new member of Caltech's chemical engineering faculty. His work uses biophysical methods such as magnetic resonance, ultrasound, and electrophysiology to develop novel noninvasive techniques to image and control biological systems. Professor Shapiro's accomplishments as well as his enthusiasm and dedication to pushing the limits of engineering serve as an inspiration to all young scientists at Caltech.

We encourage you to visit our website at curj.caltech.edu, where you can find past CURJ issues and more information about the journal. We welcome your comments and feedback. Thank you for picking up this latest issue!

Best regards,

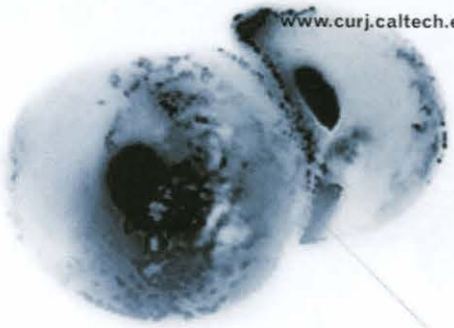
Edward Fouad and Suchita Nety



Interview with Professor Mikhail Shapiro

What does your lab work on?

We develop technologies that allow us to study biological systems in new ways. The idea is to study molecular and cellular processes as they happen within the context of the living, breathing organism. We want to see things like gene expression, cell signaling and cell migration, as it happens in the middle of the brain. We want to be able to interact with biological systems and turn things on and off, and observe what happens. Most of the work to be able to do this requires us to engineer new molecules that allow us to interact with them using forms of energy, like magnetic fields and sound waves, that can penetrate into the organism.



What excites you most about your work?
It's really intellectually fascinating to work with exotic proteins and other biological structures that have these physical properties that allow them to interact with magnetic fields and sound waves. Looking around in the world of biology, at all the organisms that have evolved over a billion years, there's an amazing variety in the kind of structures they have made to adapt to their biological niche, and now we're going to use them for a completely different purpose.

I'm also very excited about what we'll be able to do once we develop these technologies. There are many questions we want to answer – for example, how the brain works – that you can't study in a dish. By developing technologies, we'll be able to obtain information from the intact brain that will help us start addressing some of these questions.

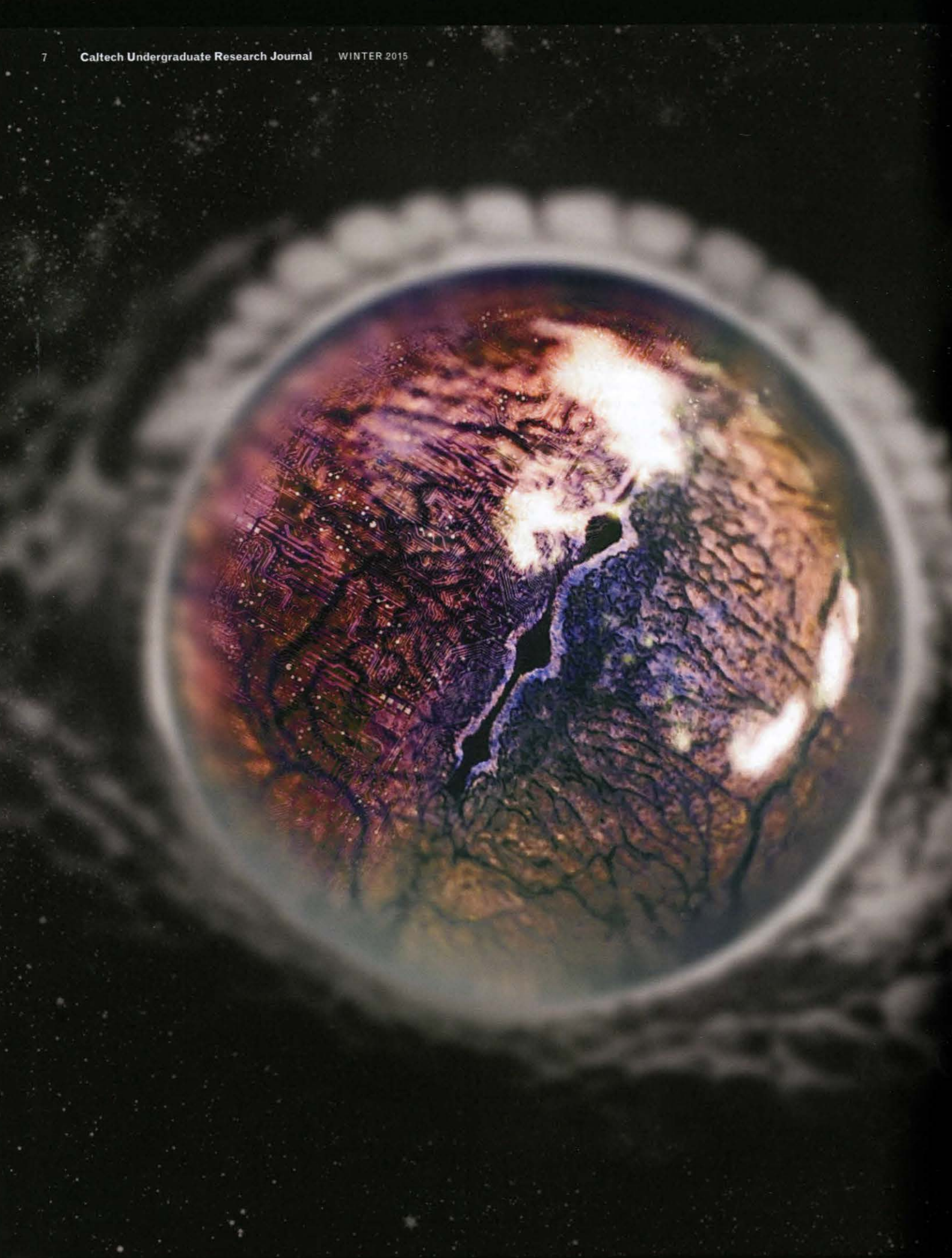
What was your path to academia?
My research career started in 2001 when I was an undergrad at Brown. I started out as a political science and philosophy major and then I got really interested in neuroscience. I was fascinated by the mystery of how the brain works and how there's so much unknown about it. My first research project was working on brain-computer interfaces, which are implants that go into the brain. That experience made me realize that the biggest leaps in neuroscience are made by developing new technologies that let you look at biological systems in new ways. That motivated me to become an engineer, and I went to MIT for a Ph.D. in bioengineering. Progressively since then, I've been delving more into the biophysics and chemistry of the different molecules that I've been working with. Initially, I was driven by "I want molecules that do X," then I discovered that they don't exist and I had to go make the molecules that do X. Now, I've realized that I love working with these types of molecules. So the main driver is still to have these molecules and use them, but the intellectual challenge of working with these proteins and structures is just as exciting.

What drew you to Caltech?
Caltech is awesome. When I was a grad student I started a collaboration with Frances Arnold and I used to visit once or twice a year and I always loved the intellectual environment here. Caltech is surprisingly small and intimate for the impact that it makes in the world and that's something you don't appreciate until you set foot on campus and see it for yourself. For a new professor, I don't think there's a better place in the whole world. Caltech is like a playground with unlimited sand and it's up to you to imagine new sandcastles.

What are your future hopes for your research?
The exciting and challenging part of this work is that we need to make some breakthroughs. We've got some hypotheses and are working on projects to get where we want to go, but the mission of being able to do things at a precise and detailed level deep inside the organism is probably going to take another 30 years. Of course, it would be exciting if we just solved it right away, but I would also be very disappointed if it were that easy.

What hobbies or interests do you have?
I like to play sports like tennis and basketball. However, last time I played basketball in the Caltech gym it was a pretty rough game and I got a rib injury and had to get 3 stitches. My wife and I also like to look at art and go to concerts. We've been to the Walt Disney Concert Hall a few times.

Do you have any advice for Caltech students?
Find research that you're really passionate about, that thing that can really sustain your interest in science for several decades. Think about different areas and find the thing that's going to make you wake up in the morning and go to bed at night thinking about how to solve that problem.



DESIGN OF A ZERO-GRAVITY CLIMBING ROBOT USING ON/OFF GECKO ADHESIVES.

SIMON R. KALOUCHE
Mentor: Aaron Parness
NASA Jet Propulsion Laboratory

We present ACROBOT II, a gecko adhesive enabled robot capable of climbing surfaces of any gravitational orientation or in full zero gravity (W 1). ACROBOT II is being developed as a prototype for inspection of the International Space Station (ISS). There are current voids in the inspection coverage of the ISS due to the inaccessibility of certain areas to astronauts. Equipment in these areas that remains uninspected poses a risk of component failure and threatens the long-term success of the ISS. A specific area of interest for service and inspection is a narrow gap, approximately 2 inches wide, between the outer shell of the ISS and the external equipment racks. A small robotic platform, used in both autonomous and remote-controlled operation, would be advantageous for its ability to precisely navigate in tight spatial conditions. Due to the operating environment of a vacuum with no significant gravitational forces, a method of adhesion is required to ensure constant contact between the robot and the surface of the ISS. Gecko adhesives can be actuated ON and OFF by means of an applied shear force. They leave no residues, are highly reusable, and can create adhesion in a vacuum, making them a viable and promising option for space applications.

Biography
Name: Simon Kalouche
Hometown: Doylestown, PA
College: The Ohio State University
Major: Mechanical Engineering
Graduation Year: 2014
Hobbies: Basketball, Mechatronics / Robotics Projects

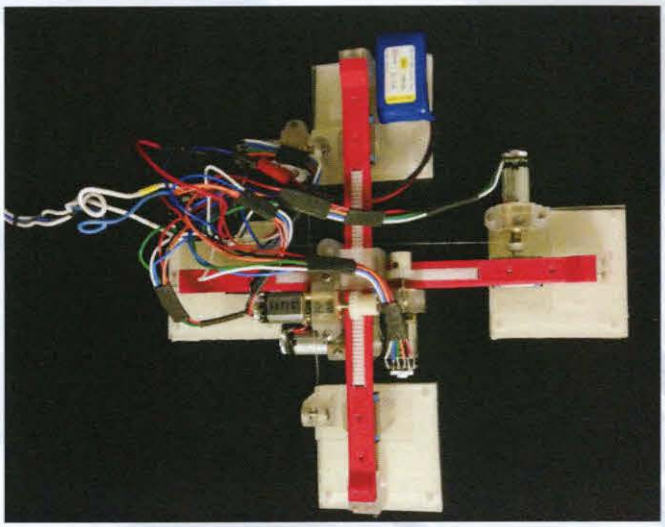


Figure 1: Complete functional, climbing, prototype of ACROBOT II.

I. MIMICKING THE SKIN OF GECKOS

Studying the biological structure of gecko's feet and toes led to the fabrication of a synthetic, gecko-like adhesive material. Gecko toes are composed of a hierarchy of several layers. Each toe has tens of millimeter-sized flaps called lamellae, on which grow arrays of millions of micron-sized 'hairs' called setae. Setae branch further at the tip into thousands of nano-sized hairs called spatulae. The setae add a layer of suspension to help conform to surface roughness of order 20-100 microns on the surface the gecko is climbing. The spatulae are then able to create very intimate contact with the surface being climbed, increasing the real area of contact (RAC) between the gecko's foot and the wall's surface.

As the RAC between the two surfaces increases, the net van der Waals intermolecular interactions subsequently increase, resulting in a significant adhesive force. To mimic this method of adhesion for climbing robots, synthetic gecko adhesives were fabricated. Using a complex multi exposure stereo lithography process, quartz molds were made with an array of 60-micron tall wedged-shaped structures. Silicone mixes were then poured into the mold, put into a vacuum, spun at a high rpm, and then set aside to cure. Post treatment processes were performed once the original molded silicone had cured to enhance the shape of the microfibrillar wedge's tips.

The synthetic gecko pads are a dual tiered structure. There exists a silicone foam suspension layer and an array of micro fibrillar wedges (the actual adhesive layer). The suspension layer allows the gecko-adhesive layer to conform to slight surface roughness and misalignments while evenly distributing loads across it. The micro fibrillar wedge shaped structure is composed of a 2-dimensional array of 60-micron tall directionally-biased wedges designed to increase RAC when under an applied directional shear force. The RAC between the synthetic gecko pad and the surface is small while no shear load is applied, as the only points of contact between the pad and the surface are the tips of the

Gecko adhesives leave no residues, are highly reusable, have low sensitivity to temperature and pressure, are self-cleaning, and can operate within a vacuum–all ideal characteristics of an adhesion source for applications in space.

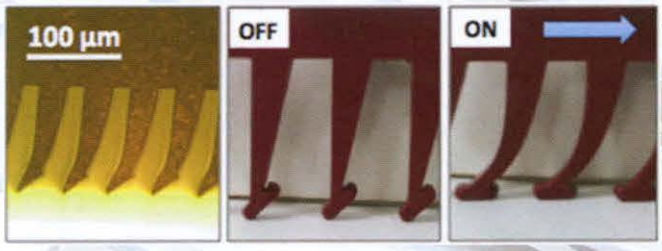


Figure 2: Gecko adhesive microstructure (left), 1000x mockup of adhesive in OFF state (center), and 1000x mockup of adhesive in ON state (right).

micron-sized wedges. However, when a shear force is applied to the gecko layer, the compliant wedges bend so that a much larger portion of the micro-fibrillar wedges are in intimate contact with the surface being adhered to, thus increasing the RAC by orders of magnitude and turning the adhesive ON. The microstructured adhesive can be seen in Figure 2 in both the ON and OFF states.

This synthetic gecko adhesive design provides a direct method of turning the adhesive ON and OFF. By simply applying a shear force in the direction of the wedge bending bias, the adhesive is turned ON. By applying a shear force in the opposite direction, against the bias of the micro wedges, the adhesive is turned OFF. Therefore, gecko adhesives are advantageous in comparison to traditional pressure sensitive adhesives (tape, glue, etc.) because large normal forces are not required to generate the adhesive forces, and a peeling motion is not required to remove the adhesive forces. Large normal forces are undesirable for climbing applications, because a large pull off force could potentially rip the whole robot off the wall; additionally, a large normal preload force would be very difficult to generate in space without the robot simply pushing itself off the surface. Gecko adhesives leave no residues, are highly reusable, have low sensitivity to temperature and pressure, are self-cleaning, and can operate within a vacuum—all ideal characteristics of an adhesion source for applications in space.

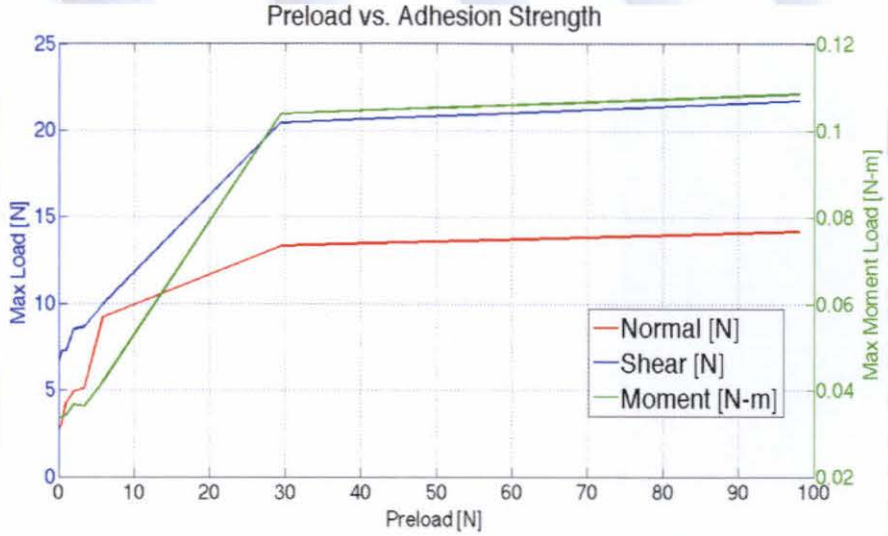


Figure 3: Characterization shows of the gecko adhesives show that adhesion is dependent on applied normal preload force when turning pads ON until about 30N, where the gecko layer is presumably coplanar with the climbing surface.

II. DESIGN

Robot Objectives and Design Approach

The main objective was to design a robot capable of climbing surfaces of any gravitational orientation and in full zero gravity. Because gecko adhesives require coplanar alignment to the climbing surface within 0.5 degrees, the mechanism responsible for placing the gecko pads onto the climbing surface must keep the pads parallel to the surface at all times or have a method for correcting angular misalignment. Gecko adhesive pads have a theoretical and experimental maximum carrying capacity under normal, shear, and moment loads. Load testing of the specific gecko pads being used has yielded the maximum load the pads can support before the adhesive fails for a given preload. As seen in Figure 3, the gecko adhesive's max load increases with increased initial preload. Theoretically, if the gecko pads are perfectly parallel and coplanar to the climbing surface, they do not require any normal preload force to turn the adhesive ON. However, slight manufacturing and assembly errors typically prevent the gecko pads from being coplanar with the surface; thus, applying a normal (into the climbing surface) preload force onto the gecko pad essentially forces out any slight misalignments by the foam suspension layer's passive distribution of the load. Therefore, a critical goal is to design a mechanism that can create high normal preload forces while maintaining coplanar pad-climbing surface alignment.

Another design goal is to minimize the mass of the robot so as to not exceed the adhesive's load carrying capacity, and to optimize the location of the robot's center of mass to minimize moment loads that could peel the gecko adhesive off the climbing surface. The maximum height of the robot is to be kept within a 2" limit to allow the robot to access areas inaccessible to astronauts aboard the ISS—the robot's main operating environment. Limiting the height of the robot will also reduce moment loads acting on the gecko pads in Earth gravity. To enable ACROBOT II to climb, a controllable mechanism must be designed that is capable of consistently toggling its adhesives between their ON and OFF state when desired. A power efficient mechanism is desired so as to use minimal actuation and allow for on-board power supply.

It is desired for ACROBOT II to have complete 2-dimensional mobility across a single plane. It is also desirable that the robot be designed to have future capabilities enabling the transition between orthogonal planes (i.e. wall to ceiling, floor to wall, etc.).

Adhesive Actuation Mechanism

The mechanism responsible for actuating the gecko adhesives between their ON and OFF states was designed to use a guide rail and carriage where a pair of gecko pads were oriented in directionally biased opposition. Using a single guide rail with a carriage or linear bearing mounted to each gecko pad ensures the two pads are always coplanar and parallel with respect to the other. The linear rail also constrains the motion of the pads to 1 translational degree of freedom. Extension springs are fixed to each of the gecko pads on one end and onto the end of the rail at the other end. A winch system was designed to wind up a tendon that couples both the gecko pads as seen in Figure 4. By coupling the two gecko pads (aligned in directional opposition), the pair of pads can be toggled between their ON and OFF states via one actuator rather than two. The use of extension springs creatively allows for a shear force to be applied to the gecko pads indefinitely without the use of any power. This allows the robot to remain adhered to a surface indefinitely without the need to consume energy or actuate any motors.

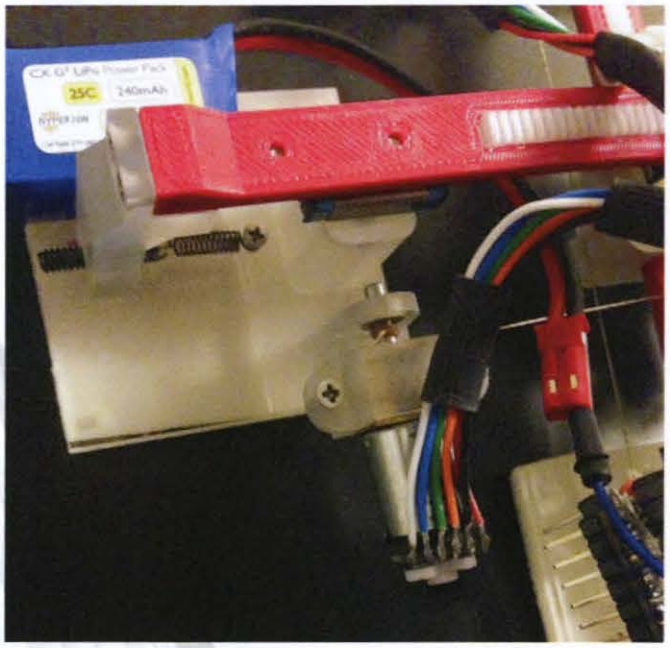


Figure 4: The winch mechanism responsible for turning the adhesives ON and OFF. The brushed DC micro-gearmotor has a tendon wrapped around its shaft and can wind it up to put the extension springs into tension.

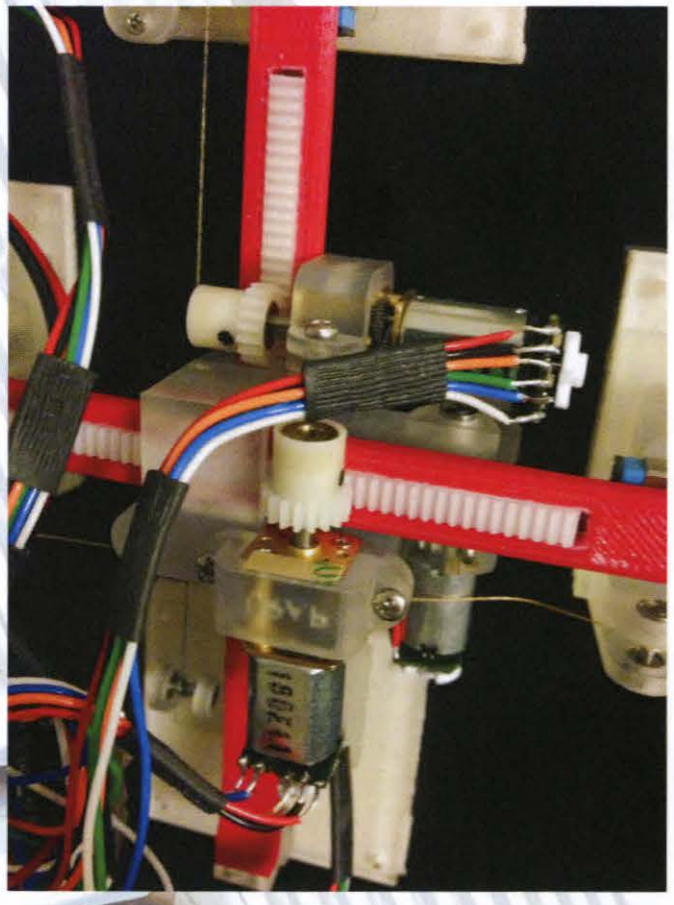


Figure 5: Prototype of ACROBOT II showing the 2 orthogonal rack and pinion inch-worm mechanisms actuated by identical brushed DC 298:1 micro-gearmotors.

Mobility Mechanisms

ACROBOT II can achieve translational inch-worm style locomotion in two perpendicular directions using two micro rack and pinion systems aligned orthogonally to one another as seen in Figure 5. A central unit composed of two components (upper and lower) was designed to house three motors which actuate both upper and lower rack and pinions and the cam motor. By actuating only one rack and pinion, ACROBOT II will travel linearly in one direction (e.g. vertical). If the other rack and pinion is actuated, ACROBOT II will travel in a direction perpendicular to the other rack and pinion (e.g. horizontal). To travel along a diagonal line, the two rack and pinions can be actuated in an alternating pattern to achieve complete 2-dimensional mobility.

A cam is used to raise and lower each pad-pair onto and off of the climbing surface. The orientation of the cam determines which pair of pads is in contact with the surface and which pair of pads is lifted off the surface so that it can translate forward without sliding or rubbing on the climbing surface. The cam has 4 dwells designed for 4 static positions: (1) upper rack and pinion pad-pair in contact with surface, (2) both rack and pinion pad-pairs in contact with the surface, (3) lower rack and pinion pad pair in contact with the surface, (4) both rack and pinion pad-pairs in contact with the surface. This cam allows for the cam motor to be only actuated in one direction while the sequence of dwells corresponds to the appropriate sequence needed for taking a step.

This design addresses and fulfills the major design criteria assessed in the preliminary design stages. The two components in the central unit are constrained to 1 translational degree of freedom relative to one another. The cam mechanism controls this single degree of freedom by translating rotary motion from the motor into linear motion between the two central unit components. By constraining the two rack and pinions to one relative, translational, degree of freedom, the design ensures that the pads will always be parallel to each other and to the climbing surface. Furthermore, this design ensures that the pads will be placed onto the climbing surface with an ideal normal preload force. To maximize effective preload force, the cam and winch mechanisms can be actuated in a manner that allows the set of un-adhered pads to be preloaded with a force less than or equal to the maximum normal load of the adhesive pad pair that would be currently turned ON. Therefore, rather than simply turning OFF the pad-pair by removing the shear load from extension spring with the winch, the cam can rotate to the position in which the pair of pads that is currently ON would be lifted off the surface and the pair of that is currently OFF would be pressed onto the surface. This would effectively use the maximum normal load capacity of the gecko pads to preload the non-adhered pair while pulling off the adhered pair.

Modular Design and Future Mobility

ACROBOT II has a modular design in that it uses the same motor to actuate all of its mechanisms. The motor used is a Pololu Micro Metal gearmotor, which outputs nearly 1.5 times the torque offered by servo of comparable size. The use of an optical sensor for shaft encoding allows the motor to be smaller than available hobby servos, able to produce more torque, and have equivalent resolution for angular positioning tasks.

ACROBOT II's design is also modular in its ability to operate in parallel with one or more identical robots, making each individual robot a module in a multi-robot system. When large structures are assembled on Earth, the construction is done in a parallel manner by small units that move as the structure grows, for instance, a colony of termites building a 10 m mound or construction workers on moveable scaffolds building a skyscraper. The proposed is a similar approach where swarms of hundreds or thousands of microrobots autonomously maneuver the various components of the structure into position. These segments would then be mated with a passive connector (ball and socket, magnetic, TBD) or, less optimally, delivered to a traditional dexterous robot arm and manipulator for final integration into the assembly. Using this approach, massive apertures, solar arrays, or mirror assemblies could be constructed in days instead of years – with the components literally walking themselves into place and plugging in.

Two or more ACROBOT II robots could also be coupled by a high degree of freedom (DOF) serial arm allowing for this configuration to achieve mobility across orthogonal planes such as inner and outer edges. This modular configuration could also allow for manipulative tasks where the robots would essentially act as grippers in the overall system rather than individual climbers.

The symmetrical architecture and design of ACROBOT II is also conducive to the implementation of four rotors above each gecko pad, essentially making this robot a quadrotor flying and climbing, mulit-modal mobility platform. This architecture could allow for short periods of flying and long periods of perching, effectively elongating the mission time of currently existing quadrotors for terrestrial applications.

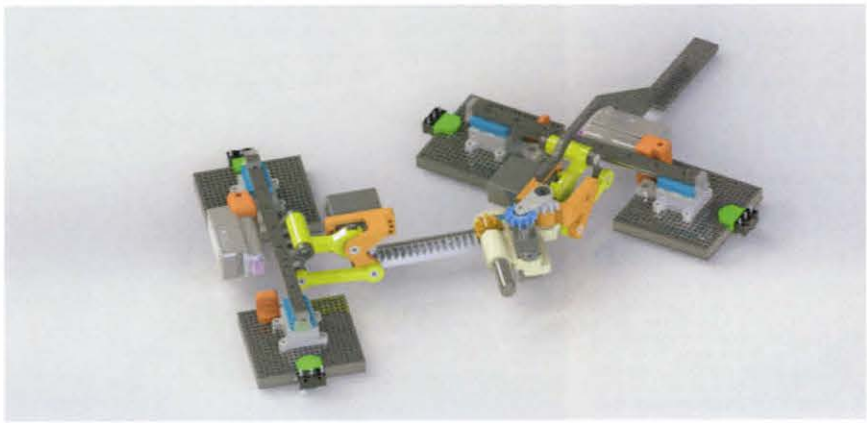


Figure 6: ACROBOT I used kinematically synthesized compliant 4-bar linkages to lift pads onto and off of the climbing surface.

ACROBOT II has successfully demonstrated climbing on sloped, vertical, and 30 degree inverted planes in Earth's gravity during tele-operation.

III. CONTROL & SENSING

Controller

ACROBOT II uses a Pololu Baby Orangutan robot controller which houses an Atmel ATmega328P microcontroller, offers two internal channel bidirectional motor drivers, 16 general purpose digital I/O pins (8 of which can be used as analog input channels). Six of the digital I/O pins are used to send Pulse Width Modulation (PWM) signals to external dual h-bridge motor drivers to power three additional motors. With this configuration, up to 6 actuators can be powered; however only 5 are needed in ACROBOT's current architecture.

Sensors

ACROBOT II is equipped with several sensors to provide feedback on internal motion and position to close the loops on motor actuation. Each of ACROBOT II's 5 identical brushed DC micro gear motors is configured with an optical sensor used for shaft encoding. The motors have an extended shaft, which spins at the frequency of the motor output (before the gearbox). Using a three tooth encoder wheel, the optical sensor has a resolution of 6 ticks or increments per motor output revolution. Thus, the output gearbox shaft has a resolution of 6 times 298, which is 1,788 increments per 360 degrees or 1 revolution of the gearbox shaft. This resolution is more than sufficient for this application.

Hall effect sensors are also used in parallel with the motor encoders for the two winch mechanisms to determine the displacement in the extension springs which would allow for ACROBOT II to generate the optimized shear load onto the gecko layer which differs when climbing on different materials or using different pad sizes. The Hall effect sensors are also being implemented to sense whether or not adhesion was created when attempting to turn the gecko pads ON.

Future work may include the implementation of novel, soft multi-axis force sensors, using a multi-layered array of channels containing conductive liquid metal. Changes in resistance due to deformations in the cross section of the fluid channels can provide force data at the gecko layer. This can be used to determine an optimized cam actuation pattern so as to not exceed the load carrying capacity of the adhered pair of pads while preloading the non-adhered pair.

Software

ACROBOT's control software is written in C via the Arduino environment. Code has been written to print sensor output data to a serial window, to allow for serial control and tele-operation of each of the motors individually, and to allow ACROBOT II to climb autonomously and execute multiple step sequences. ACROBOT II can currently climb vertical and slightly inverted walls autonomously using open-loop control; however, climbing is not yet robust at this point. Implementation of the aforementioned sensors would close the loop on applied force data at the gecko layer. This would enable optimal adhesion to be generated and maintained during climbing.

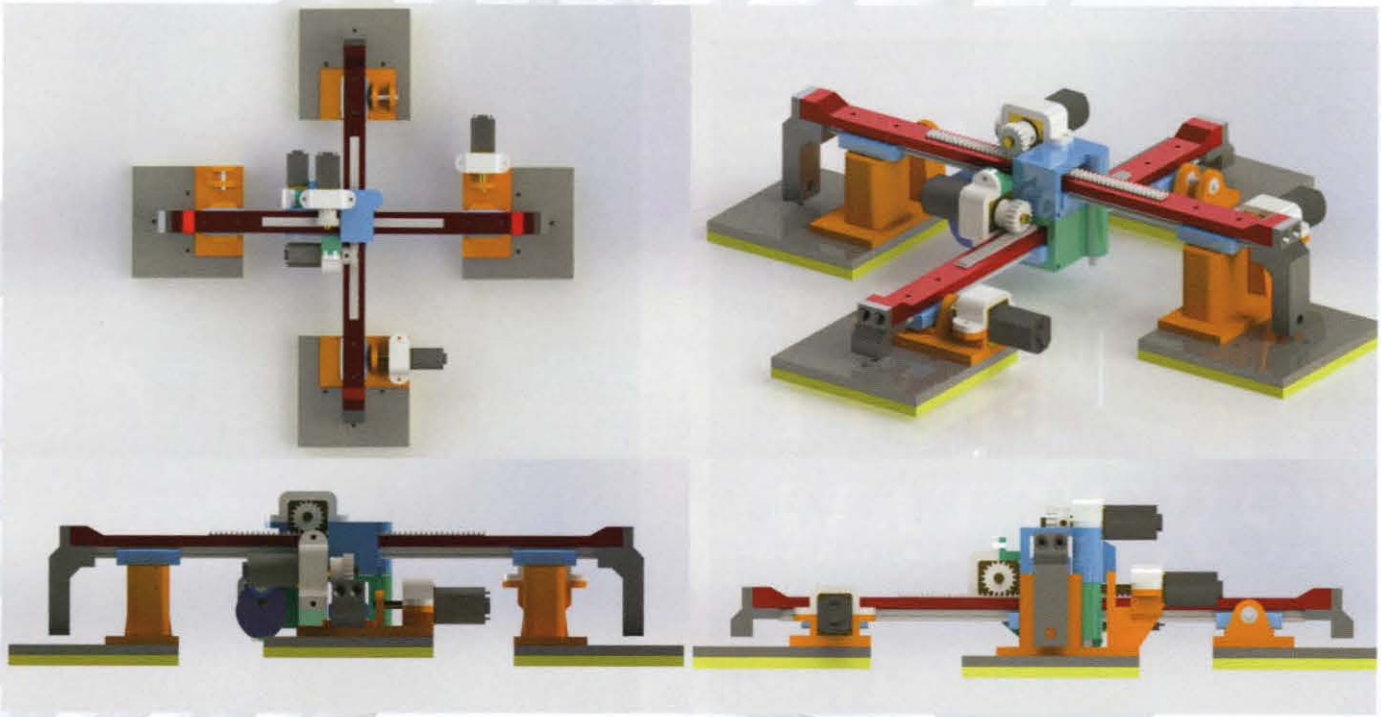


Figure 7: Top, front, right side, and isometric views of ACROBOT II. The top view shows all five of the actuators and their locations within the robot.

IV. TESTING & RESULTS

Gecko Pad Suspension Testing

In controlled laboratory tests, the directional gecko adhesive requires almost no preload to engage. However, on the robot, tolerances and manufacturing imperfections lead to slight pad-surface and pad-pad misalignments that are significant at the micro-scale of the adhesive. Using a hierarchical suspension layer and a slight preload orthogonal to the climbing surface can overcome these misalignments; however, maximum adhesive performance is sacrificed. Testing of pad pairs shows adhesion strength increases with preload until reaching a critical value, presumably due to good alignment. Therefore, ACROBOT II was designed using mechanisms synthesized to maintain parallel pad-surface interface while producing sufficient normal preload forces. Figure 3 shows the characterization of a pair of 4 in^2 gecko pads under applied normal, shear, and moment loads as a function of applied normal preload force.

ACROBOT II Climbing Tests

ACROBOT II has successfully demonstrated climbing on sloped, vertical, and 30 degree inverted planes in Earth's gravity during tele-operation. While tele-operation produces more robust climbing, due to operator feedback and control adjustment, the climbing is significantly slower as each actuator needs to be actuated independently. ACROBOT II is also programmed to climb autonomously in all orthogonal directions in any sequence. Climbing performance in autonomous mode, however, is not robust due to the current lack of feedback at the gecko layer. The robot operates open-loop, meaning it has no sensing capability between itself and its environment at this point in development. ACROBOT II's only sensing capabilities are the encoders and Hall-effect sensors, which are used to sense internal kinematic orientations of its actuation mechanisms. Future sensor implementations will close the loop on force sensing at the gecko layer which can significantly improve autonomous climbing performance to match that of tele-operated performance.

Improvements Over Previous Design

The previous generation ACROBOT I (see Figure 6), was designed, iterated, and tested thoroughly before the design of ACROBOT II (see Figure 7). ACROBOT I used similar actuation mechanisms to toggle between the adhesives ON and OFF states using springs to apply indefinite actuating shear forces on the gecko layer. This conserved significant amounts of energy and elongated mission time, thus this mechanism design was incorporated in a similar way into ACROBOT II. ACROBOT II uses a winch and tendon rather than a linear actuator to put a spring in tensions. ACROBOT I used two compliant, kinematically synthesized, 4-bar linkages to place and lift the adhesive pads onto and off of the climbing surface. The 4-bar linkage was synthesized to rotate the gecko pads through 3 task positions. The first two kept the gecko pads nearly parallel to the climbing surface for single plane locomotion while the 3rd task position rotated the gecko pads to be orthogonal to the original climbing surface for plane-to-plane transitions. ACROBOT II uses a cam to alternate between which orthogonal pair of pads is in contact with the surface. ACROBOT II solves angular misalignment issues by constraining the pads to 1 translational degree of freedom with the pads parallel to the climbing surface at all times. ACROBOT I turns while ACROBOT II has orthogonal bi-directional mobil-

ity. Both versions of the robot use a stable inchworm style gait. ACROBOT II's main advantages are that it keeps its gecko pads parallel to the climbing surface and that its center of gravity is always between the pair of pads that is adhered at all positions during climbing sequences. This minimizes the adhesives main failure mode caused by applied moment loads.

V. FUTURE WORK

The next step in progressing to a further flight readiness level for this robotic platform would be to continue testing and slightly modifying the design to increase performance on surfaces of all gravitational orientations (i.e. vertical, inverted, etc.). Once ACROBOT is climbing consistently in Earth gravity, testing will be done on climbing in micro gravity via NASA's reduced gravity simulating parabolic flights. ACROBOT is expected to perform better under zero gravity due to the absence of gravity induced loads and moments on the gecko pads. Cameras, LEDs, and other inspection tools will eventually be mounted onto the robot for testing readiness of inspecting the ISS.

VI. ACKNOWLEDGMENTS

Simon R. Kalouche acknowledges and thanks Aaron Parness and the other members in JPL 347C for their assistance, advice, mentorship, and continued support throughout his research experience at the Jet Propulsion Laboratory. In addition, he would like to thank the California Institute of Technology, JPLSIP, and the JPL Education Office for making this opportunity possible.

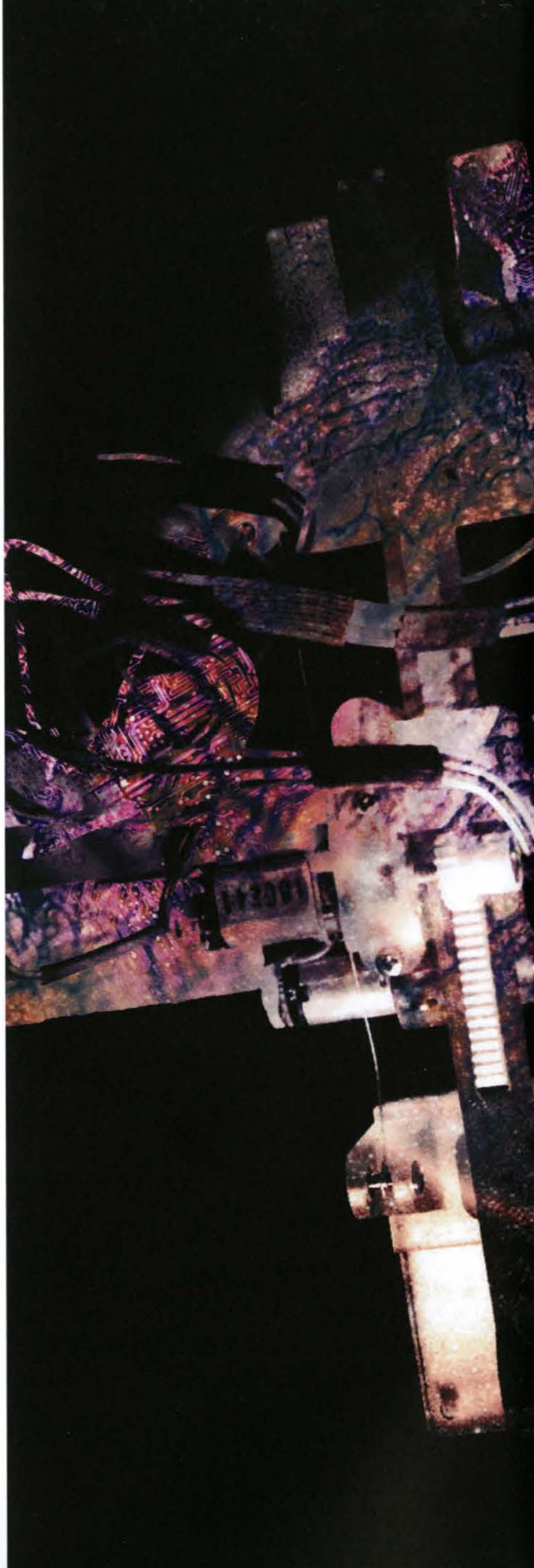
VII. FURTHER READING

[1] S. Kalouche, N. Wiltsie, H. Su, A. Parness. "Inchworm Style Gecko Adhesive Climbing Robot", Interna- tional Conference on Intelligent Robots and Systems, Chicago, USA 2014.

[2] S. Kim, M. Spenko, S. Trujillo, B. Heyneman, D. Santos, and M. Cutkosky, "Smooth vertical surface climbing with directional adhesion," Robotics, IEEE Transactions on, vol. 24, no. 1, pp. 65 – 74, 2008.

[3] A. Parness, T. Hilgendorf, P. Daniel, M. Frost, V. White, and B. Kennedy, "Controllable on-off adhesives for earth orbit grappling applications," IEEE Aerospace Conference, 2013.

[4] E. Hawkes, D. Christensen, E. Eason, M. Estrada, M. Heverly, E. Hilgemann, M. Pope, A. Parness, and M. Cutkosky, "Dynamic surface grasping with directional adhesion," IEEE/RSJ IROS, 2013.



Probing the Binding Site of Antifreeze Proteins to Trehalose Crystals

Alejandra Rios
Mentors: William A. Goddard III, Soo-Kyung Kim, and Fan Liu
California Institute of Technology

Biography
Name: Alejandra Rios
Hometown: Eagle Rock, CA
College: California State University, Los Angeles
Major: Biochemistry and Physics
Expected Graduation Year: 2014
Hobbies: Running, jujutsu and capoeira, drawing, reading science fiction.

In most animals, intra and extracellular freezing causes tissue damage resulting in death. However, some cold-adapted organisms use antifreeze proteins to resist extreme climates. It is thought that these unique proteins bind to ice crystals to inhibit their further growth, but the detailed mechanism of Anti-Freeze Protein(AFP) and ice crystal binding is unclear. Insect AFPs usually have high antifreeze activity, which can be enhanced by certain small molecules such as trehalose, citrate and glycerol. The antifreeze activity enhancement effect of DAFP-1, an AFP from the beetle ***Dendroides candensis***, by small molecules has been extensively studied in Wen lab at California State University, Los Angeles (CSULA) and the model of the three dimensional structure of DAFP-1 was first obtained in Goddard lab at California Institute of Technology (Caltech).

Direction	Face	Length (Angstroms)
[100]	a	17.8065
[010]	b	12.1662
[001]	c	7.5395

Table 1.
Trehalose dihydrate orthorhombic crystal characteristics.

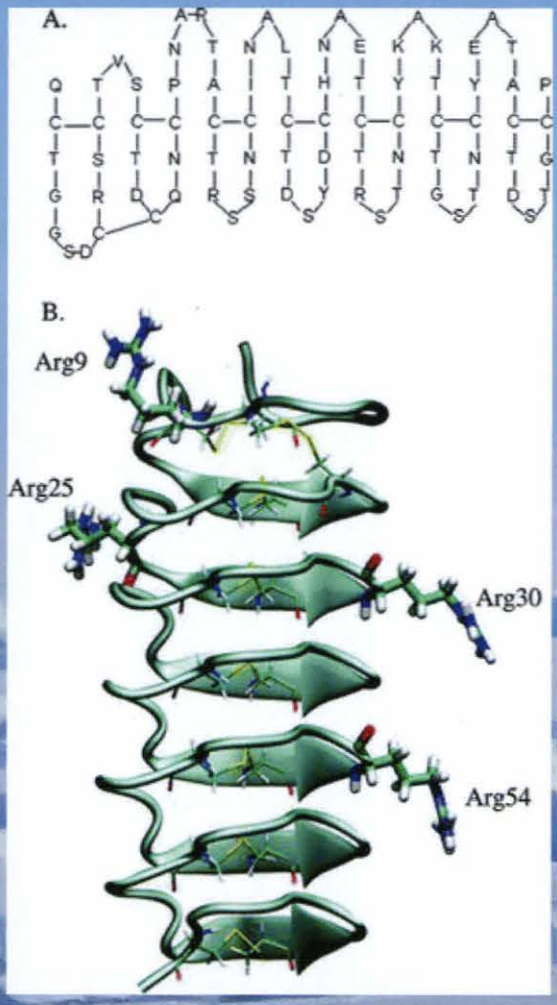


Figure 1.
Antifreeze protein DAFP1 illustration
a) sequence of DAFP-1
b) 3-D model of DAFP1.

Properties of DAFP1

DAFP1 is composed of 84 residues and has a molecular weight of 8969.857 Da. It contains 8 disulfide bonds, which are significant contributors to the stability of this protein, as well as, 7 negatively charged residues and 6 positively charged residues. This results in a net charge of -1. The structure of DAFP-1 gives vital clues about its mechanism of binding to small molecules such as trehalose.

Properties of trehalose dihydrate

Trehalose, a type of sugar disaccharide, is one cryoprotectant utilized as energy by Dendroides candensis. It has been experimentally observed that a trehalose dihydrate crystal has a more favorable crystal growth rate on the (-110) plane and a least favorable rate on the (0-11) plane. In addition, it has been noticed that trehalose dihydrate crystals do not precipitate at temperatures lower than its freezing point in the presence of DAFP-1, from 5 degrees Celsius to -15 degrees Celsius.

Through software programs and molecular dynamics simulation, an approximation can be made from this structural data to propose a binding model for trehalose and DAFP-1.

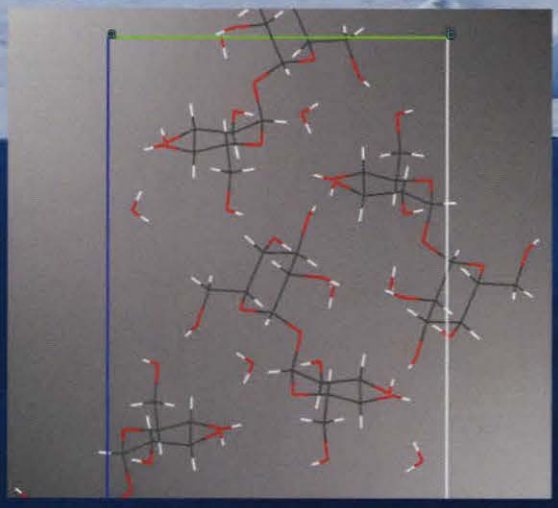
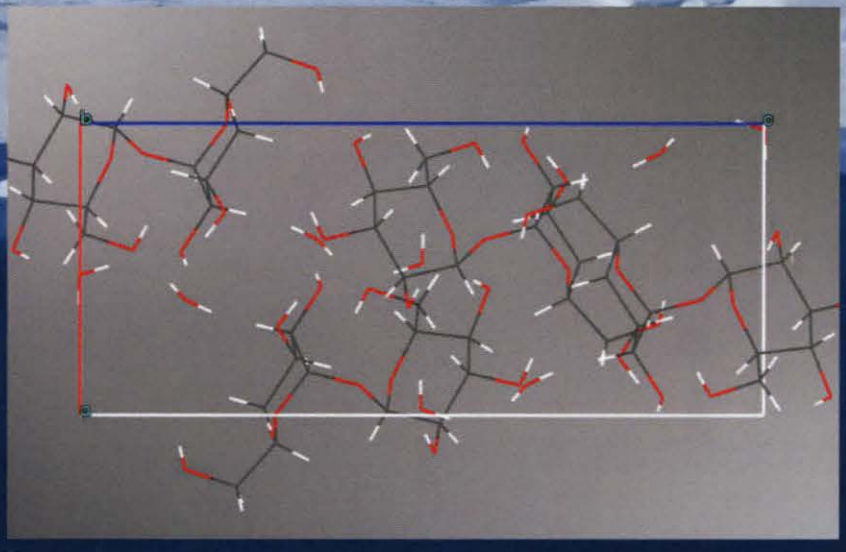


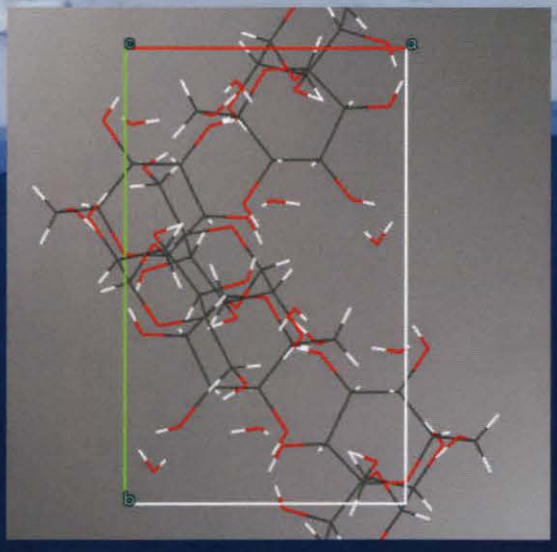
Figure 2.a)
Unit cell of trehalose dihydrate orthorhombic crystal. Figures created with Cerius software.
a) projection along the [100] direction

Amino acid	Symbol	# Of amino acids in protein	% Composition in protein
Ala	A	9	10.5%
Arg	R	4	4.7%
Asn	N	7	8.1%
Asp	D	5	5.8%
Cys	C	16	18.6%
Gln	Q	2	2.3%
Gly	G	4	4.7%
His	H	1	1.2%
Ile	I	1	1.2%
Leu	L	1	1.2%
Lys	K	2	2.3%
Met	M	1	1.2%
Phe	F	0	0.0%
Pro	P	2	2.3%
Ser	S	9	10.5%
Thr	T	16	18.6%
Trp	W	0	0.0%
Tyr	Y	3	3.5%
Val	V	1	1.2%
Pyl	O	0	0.0%
Sec	U	0	0.0%

Table 2.
Amino acid composition of protein DAFP1.
Total number of negatively charged residues (Asp + Glu): 7
Total number of positively charged residues (Arg + Lys): 6



2.b) along [010]



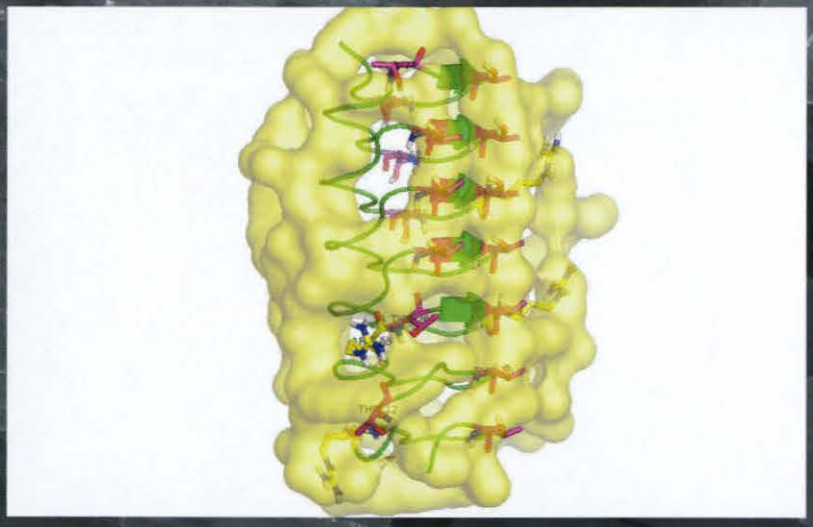
2.c) along [001].

Determination of DAFP1 binding site

Using programs such as Pymol, Maestro, Lingraf Linux and VMD, we modeled the proteins, performed trajectory analyses, and visualized possible protein interaction models. the binding site of DAFP1 was determined by using Darwin Docking, an analysis method.

Darwin Docking was used to narrow down the possibilities of the binding sites of a single unit of trehalose with the protein DAFP-1 from 35000 possible binding sites to ~25 possible binding sites (Fig. 2) In order to further isolate potential binding sites, mutants of DAFP1 were created. Point mutations were introduced into possible binding sites. If antifreeze activity was perturbed, then the mutated site may be involved in DAFP1 binding. A particular mutant of interest was the Q1T_S14T_A27T_A75T. Where the residue Q (Gln) in position 1 of the sequence was replace with a T (Thr), residue S (Ser) in position 14 was replaced with a T (Thr) and so forth, as shown in Fig. 3. The antifreeze activity of this mutant had the most significant increase compared to the wild type, as shown in Fig. 4. Combining these results, a better approximation of the binding site of the protein was proposed.

The antifreeze activity of this mutant had the most significant increase compared to the wild type.... Combining these results, a better approximation of the binding site of the protein was proposed."



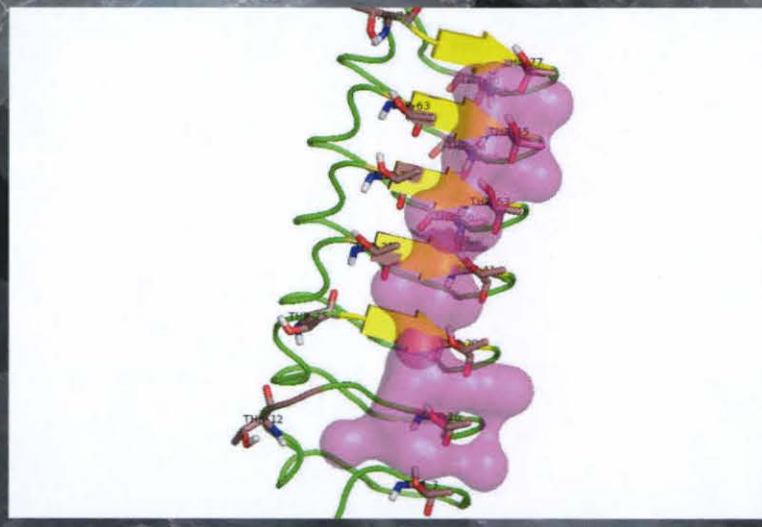


Figure 3.
(a) The yellow spheres show the possible binding sites on DAFP1 (3500 possibilities).

(b) The most favorable binding sites on DAFP1 (pink spheres found on Threonine, Thr, region), determined by Darwin Docking analysis. Figures created using Python.

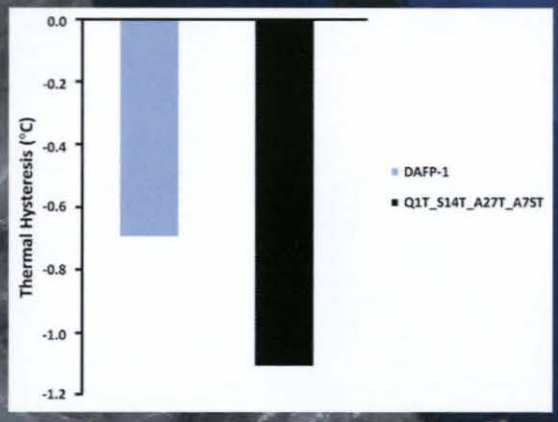


Figure 4.
Comparison of antifreeze activity of mutant Q1T_S14T_A27T_A75T with wild DAFP1 protein.

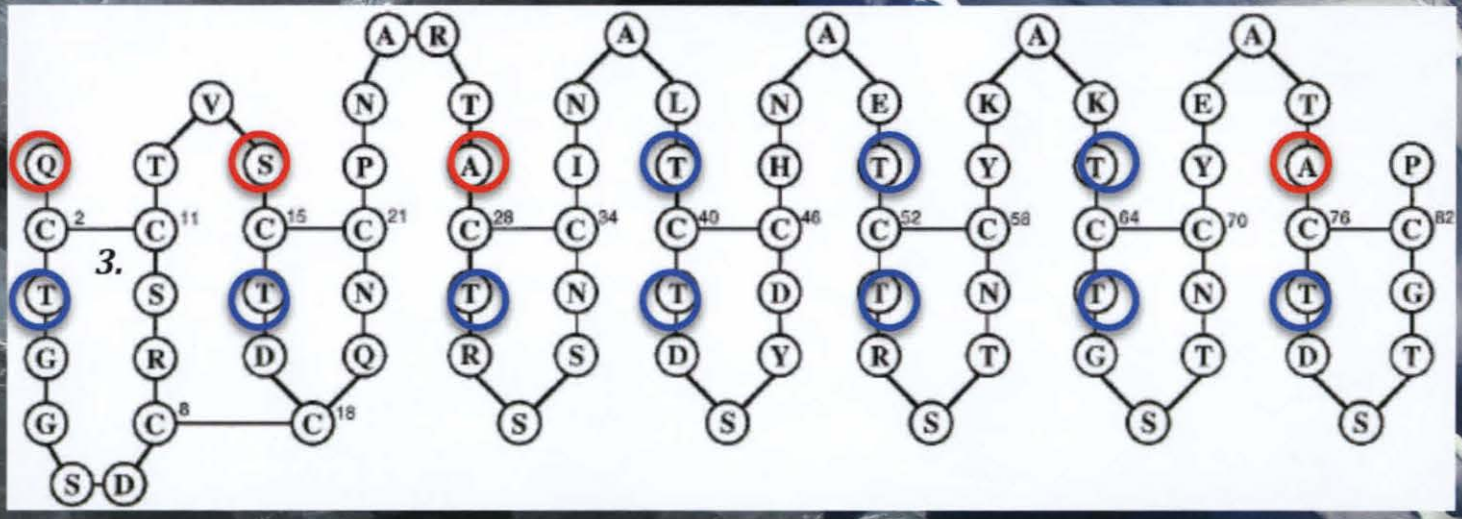


Figure 5.
DAFP1 sequence. The red circles indicate the amino acids changed to Threonine to form the mutant Q1T_S14T_A27T_A75T. Blue circles indicate the unchanged Threonine amino acids.

Plane	# Of trehalose molecules	# Of water molecules	Area of mesh (A2)	# Of atoms
(-110)	32	84	1019.45	1692
(0-11)	24	56	1248	1248
(001)	12	56	366.908	624
(100)	32	64	866.55	1632
(101)	36	72	941.026	1836
(111)	44	84	1083.47	2236
[010]	45	90	1208.27	2295

Table 3.
Properties of different trehalose dihydrate (2x2x2 super cell) planes.

Trehalose planes

Additionally, we modeled the structure of trehalose dehydrate crystals. Experimentally, it was observed that a trehalose dihydrate crystal has a more favorable crystal growth rate on the (-110) plane and a least favorable rate on the (0-11) plane. To have an understanding of how the protein interacts with both planes, a super cell 2x2x2 was made from the original crystal structure of trehalose dihydrate, figure 2, to fit the dimensions of the protein DAFP1. The planes were cut from the super cell.

Super cell 2x2x2 contains 1632 atoms, density of 1.53854 g/mol, volume 13066.7 A3, number of Carbons 384, Hydrogen 832 and Oxygen 416. ⍰ = 90°, ⍰ = 90°, ⍰ = 90°, c = 15.079 Å, b = 24.3324 Å, and a = 35.6130 Å.

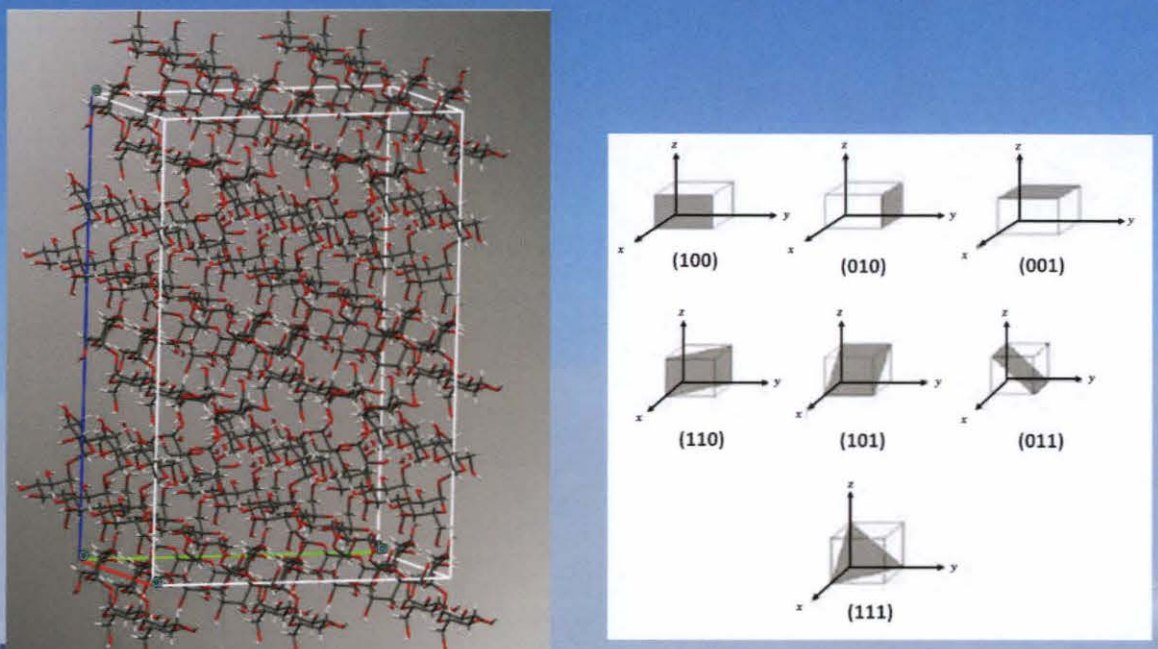
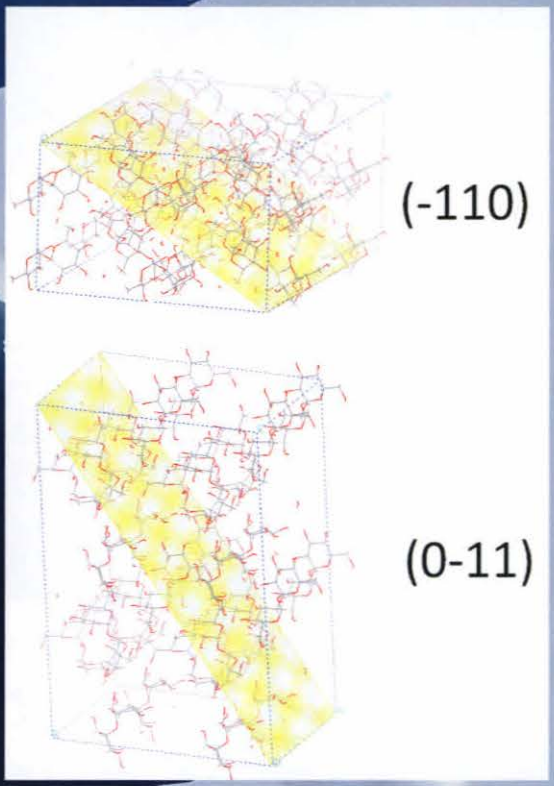


Figure 6.
Super cell 2x2x2 and selected planes of study.



DAFP1 and trehalose dihydrate.

Finally, we visualized potential interactions of DAFP1 with trehalose dihydrate crystal faces. The structure of DAFP1 was compared to the planes (-110))and (0-11) of trehalose dihydrate, $C_{12}H_{22}O_{11}$ * $2H_2O$, to have a better understanding on how the protein may interact with two of the many planes of the crystal. Geometrical comparisons between both planes and DAFP1 were made to understand how the protein could interact with these planes. A lattice was formed on the Threonine of the protein and was compared with similar lattice on both trehalose dihydrate planes. The results showed a matching lattice on the (-110) plane with the Threonine residues of DAFP1 and a no matching lattice on the (0-11) plane.

As shown in Fig. 7d, the lattice of DAFP1 and trehalose (-110) are almost symmetric. This complex was run in molecular dynamics to explore the interaction this system. The complex was run in a tip4 water box at 277 K, NAMD (Nano scale molecular dynamics), force field AMBER, and ensemble NVT (constant number of molecules, constant volume, and constant temperature). After 6 nanoseconds, some hydrogen bonding interactions were formedWW between DAFP1 and trehalose dihydrate (-110) plane. However, more calculations need to be done in order to obtain the free energy of this system. In addition, similar procedures must be performed on all the planes of trehalose dihydrate to have congruent results of the interaction of the protein and trehalose dihydrate and compare them with the experimental results.

Figure 7.
Comparison of DAFP-1 and trehalose dihydrate planes, (a) projection of protein lattice compared to the (0-11) plane. Due to the concave shape of (0-11), finding a matching lattice with the protein was difficult. (b) Projection of DAFP1 lattice and trehalose dihydrate (-110) plane. A matching lattice was found which shows a better interaction of the protein with (-110). (c) and (d) show both lattices and their symmetrical comparison on different views. Images were made with VMD.

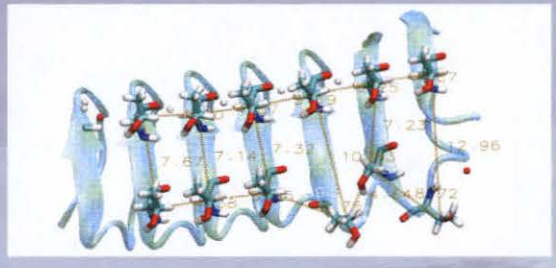
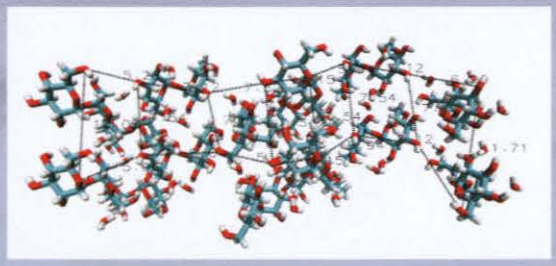


Figure 7. a



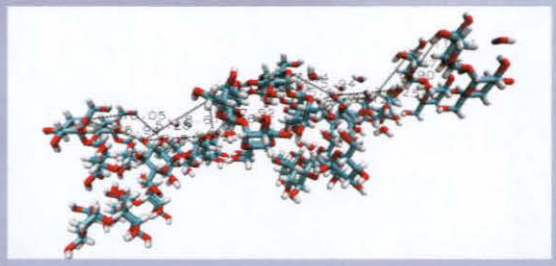
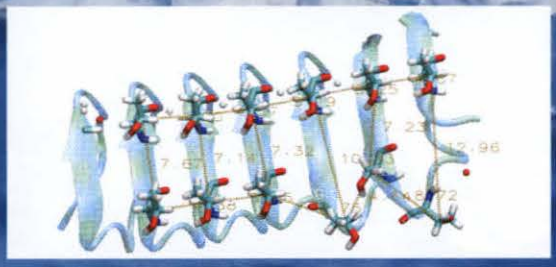


Figure 7. b



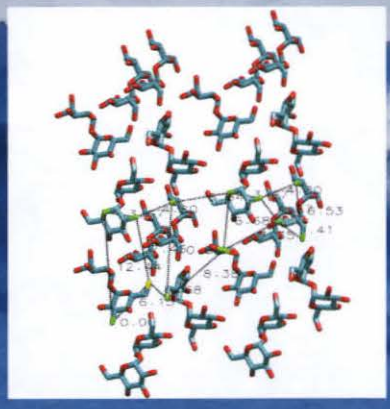


Figure 7. c

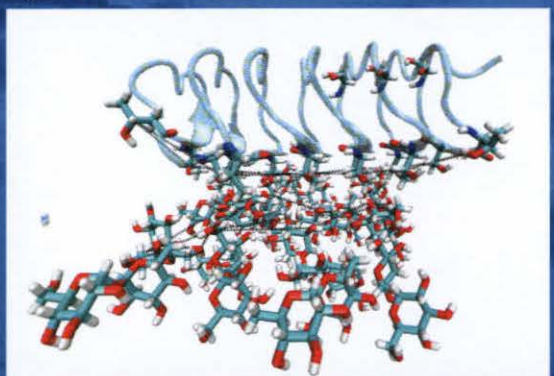
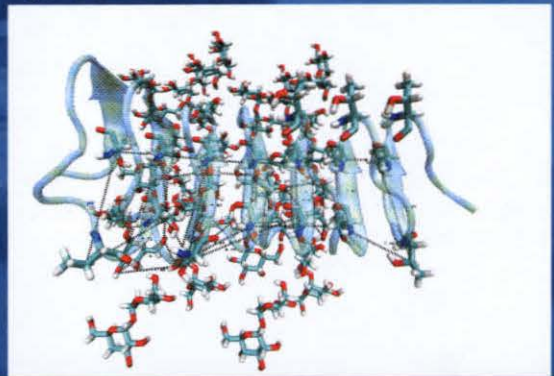


Figure 7. d



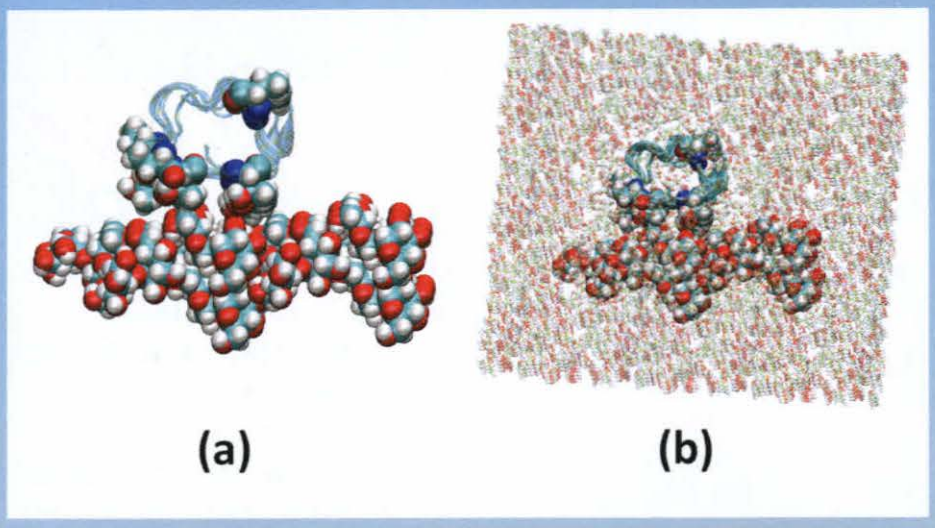


Figure 8.
System setup for Molecular Dynamics.DAFP1 and trehalose dihydrate plane (-110) at 277 K. a) DAFP-1 and trehalose dihydrate (-110) plane complex, matching lattice. b) DAFP-1/Trehalose dihydrate complex surrounded by water molecules Tip4 at 277 K.

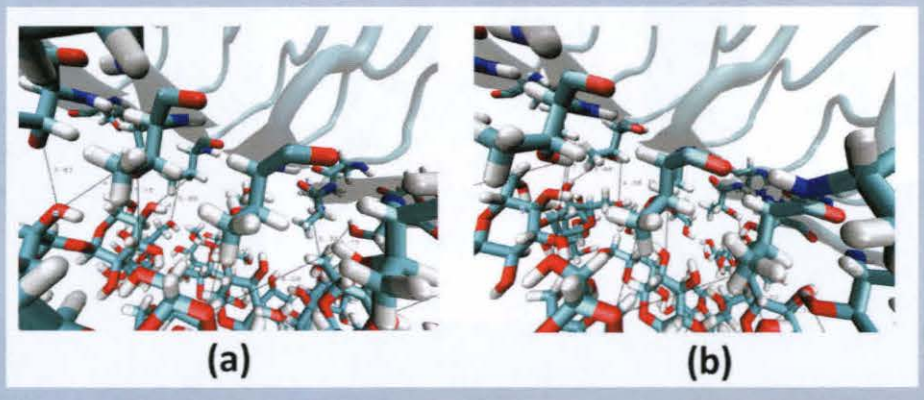


Figure 9.
DAFP-1 and trehalose dihydrate plane (-110) hydrogen bond interaction after 6 nanoseconds of MD at 277 K. a) Initial bond length between DAFP1 and hydroxyl groups in trehalose (0 nanoseconds). b) Bond length after 6 nanoseconds shows possible hydrogen bonding interaction.

AFP binding conformations may yield a better understanding of the function of these remarkable proteins.

Conclusion

In this study, we have proposed one possible binding model between DAFP-1 and trehalose dyhydrate. Future studies will continue these analyses as well as attempt to experimentally confirm these predictions. It is of great interest to understand the details of how AFPs recognize their ligands and control the crystal growth of these substances. In addition, AFP binding conformations may yield a better understanding of the function of these remarkable proteins. Not only are AFP's vital adaption mechanisms for many organisms, crystal growth control is essential in many scientific fields including chemistry, materials science, and pharmaceutical development. Our findings have greatly expanded the molecular recognition repertoire of AFPs from ice-like crystals to non-ice like crystals.

Acknowledgements
This research was funded by Howard Hughes Medical Institute MURF Fellow. The author would like to thank the MURF Undergraduate Research Fellowships, the Student Faculty Programs (SFP) at Caltech, Dr. William A. Goddard III and Dr. Soo-Kyung Kim, graduate students in the Material and Simulation Center, and Dr. Xin Wen's Laboraty Group.

Further Reading

(1) Jia, Z. C.; Davies, P. L. Trends in Biochemical Sciences 2002, 27, 101-106.
(2) Wang, S.; Amornwittawat, N.; Juwita, V.; Kao, Y.; Duman, J. G.; Pascal, T. A.; Goddard, W. A.; Wen, X. Biochemistry 2009, 48, 9696-9703.
(3) Wang, S.; Wen, X.; Nikolovski, P.; Juwita, V.; Fnu Arifin, J. Chem. Commun. 2012, 11555-11557.

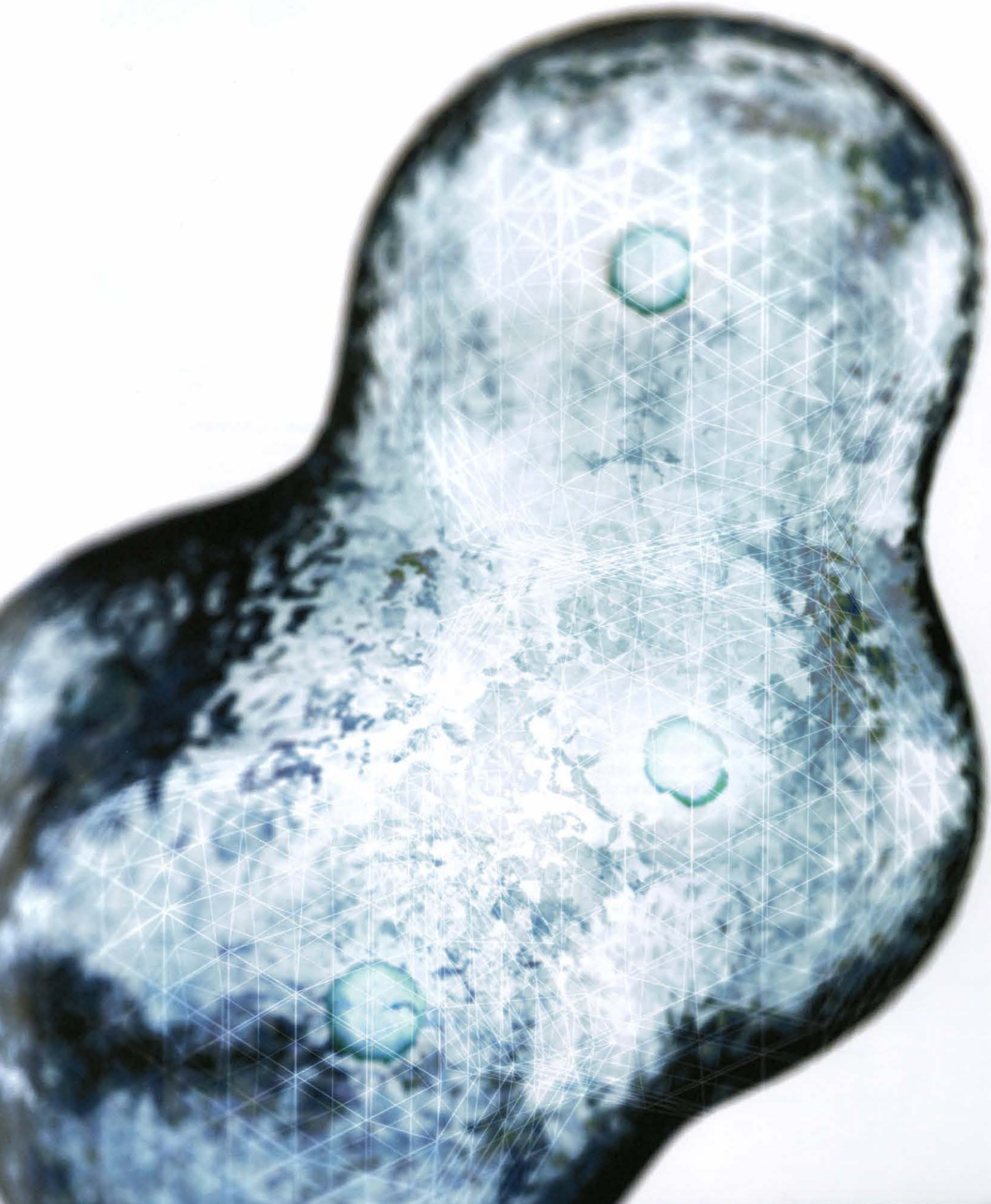
MIMICKING THE EXTRACELLULAR MATRIX: INDUCING STEM CELL DIFFERENTIATION WITH NANOSTRUCTURED SURFACES

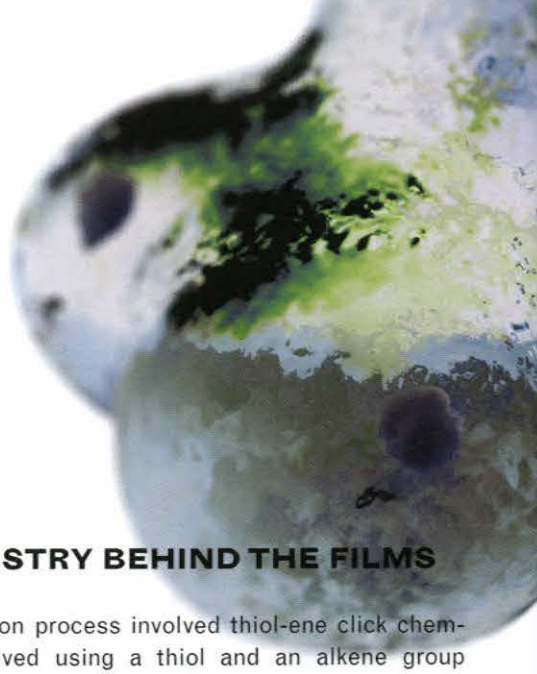
Doreen Chan
Mentors: Professor Luis M. Campos and Helen Tran
Columbia University, New York, NY

INTRODUCTION

Stem cells offer a distinct approach to regenerative therapies; they regrow tissues and organs that the body cannot regrow alone. Mesenchymal stem cells are multipotent; they possess the ability to differentiate into a few cell types, such as adipogenic, chondrogenic, and osteogenic cells (fat, cartilage, and bone, respectively). Similarly, pluripotent stem cells have the potential to differentiate into many types of cells, but their differentiation mechanism is not well understood. This process of differentiation holds incredible potential for the engineering of safe and reliable tissue growth for applications in regenerative therapies, but before such progress can take place, the driving force and mechanism behind differentiation must be better understood.[1]

Biography
Name: Doreen Chan
Hometown: Huntingtown, MD
College: California Institute of Technology
Major: Chemistry
Expected Graduation Year: 2015
Hobbies: cooking, traveling, playing the flute





MIMICKING THE HUMAN BODY

The extracellular matrix (ECM), a structural tissue that provides support for the cells, plays a critical role in healthy cell growth. It provides a scaffold for cells to adhere to; the ECM is a vital factor in stem cell renewal and differentiation. The links between the cell and the ECM are cell integrins, surface receptors that mediate signals and recognize motifs on the ECM, exchanging signals with the cell that are necessary for survival. These cell integrins are receptor proteins on the nanometer scale, spanning the cellular membrane and interacting through peptide binding sequences, which, when presented on biomimetic cell scaffolds, induce cell adhesion and influence different cell processes. Cells respond to many factors on ECM, including matrix stiffness, topography, and the pattern of these biomolecules.

Our objective was to mimic the ECM by synthesizing a material on which to grow cells. We hoped to pattern nanoscale biomolecules on this material; the biomolecules would be the same size scale as cell integrins, which would enable us to investigate various factors that affect the mechanism of stem cell differentiation.

Another group has previously shown that stem cells, when plated on films, respond to nanoscale patterns as well as microscale patterns. Within microscale patterns, nanoscale patterns can be designed, creating hierarchical patterns.
We will examine four main objectives to design a material that will mimic the ECM in a cost-efficient, quick, and effective manner: 1) synthesize films, 2) modify the film properties, 3) image the films to verify desired properties were achieved, and 4) conduct cell studies on fabricated films. This material utilizes "click" chemistry to create a film with easily tunable properties, one that is inexpensive and robust. It holds great potential for biomaterials because it has easy processability.

THE CHEMISTRY BEHIND THE FILMS

Our film fabrication process involved thiol-ene click chemistry, which involved using a thiol and an alkene group to make the polymer film. The thiol that we used was poly[(mercaptopropyl)methylsiloxane] (PMMS). This material is a derivative of polydimethylsiloxane (PDMS), a substrate used typically for cell studies. Our alkene was a mixture of two polymers: triallyl cyanurate (TAC) and diacrylate of ethoxylated bisphenol A (BPADMA). This mixture of two alkenes allowed for modifiable mechanical properties and varying stiffness of the film. To initiate a chemical reaction, we needed a photoinitiator that would cross-link these three starting materials, so that the mixture would solidify into a solid polymer film. We used 2,2-dimethoxy-2-phenylacetophenone (DMPA). To achieve micro-scale patterns on the surface of our film, we pressed glass slides to a sleeve of Teflon to create a small envelope to which we added our polymer.
When these starting materials were irradiated under UV light at 365 nm for 2 minutes, they formed a crosslinked polymer (Figure 1).

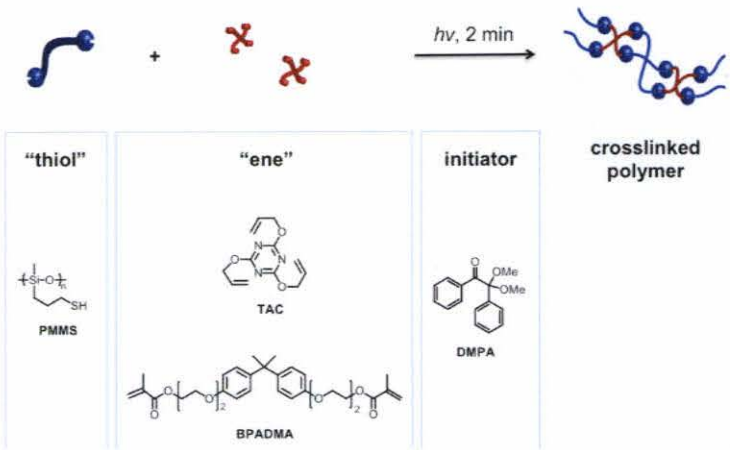


Figure 1. Crosslinked with the alkenes, TAC and BPADMA (alkenes), formed a crosslinked polymer after a two-minute irradiation with DMPA (initiator).

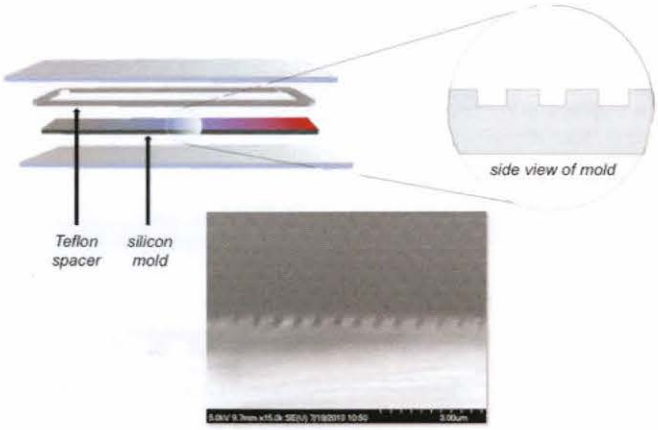


Figure 2. Mold set up for film fabrication, with SEM image of a side view of the mold.

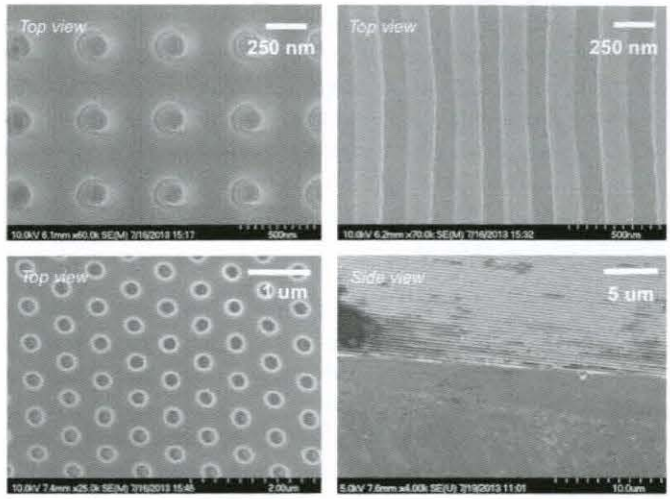


Figure 3. SEM images of polymer film surfaces, showing different nanoscale patterns are achievable using different silicon molds.

A micropatterned silicon chip was sandwiched in between two glass slides (Figure 2). This enabled our polymer to form the inverse pattern on the chip's surface; the chip was easily peeled off the glass slide surface. Different patterns, including a square array of circles, stripes, and a hexagonal array of circles, were achieved (Figure 3).

APPROACH A: PHYSISORPTION

As the extracellular matrix has integrins that present biomolecules on its surface, we wanted our films to have patterned biomolecules as well. The method we used was physisorption, which involves weak van der Waals attraction between two substances. During physisoprtion, a loose bond forms between the two substances but does not modify either of them. The peptide we wished to adhere to the surface was RGD (arginine-gluatamic acid-aspartic acid) (Figure 4). To accomplish this, the silicon mold was initially dipped into a solution of RGD peptide to physisorb on the surface of the silicon. When the polymer was pipetted into the mold, the loose bond between the peptide and the silicon wafer was broken and the peptide cross-linked to the surface of the polymer film (Figure 5).

APPROACH B: TMPDE-BIOTIN STAMPS

Another approach to creating nanopatterned biomolecules that we considered was utilizing a stamping process, much like that of an inked stamp pressed on paper. This method utilized the biotin-strept avidin motif, two molecules that have an extremely strong and specific binding affinity for each another. By incorporating biotin in our starting materials in the film, we would then be able to "stamp" any molecule with strept avidin (Figure 6).

The first challenge was incorporating biotin into our films. Because biotin does not cross-link, another alkene, trimethylolpropane diallyl ether (TMPDE), was reacted with biotin to form the molecule in Figure 10. The four starting materials found in Figure 10 were exposed as before to UV light at 365 nm for two minutes, thus incorporating biotin to the film, some of which would be randomly distributed on the surface of the film. Next, PDMS stamps were fabricated to press onto the biotinylated films. The PDMS stamps were micropatterned, thus allowing for hierarchicalt patterns when pressed on nanopatterned films. To test the concept, PDMS stamps were then dipped in a solution of strept-avidin Alexa-Fluor to physisorb onto the stamp. Then, it was pressed onto a nanopatterened biotin film. Fluorescent images were taken to verify the concept's success.

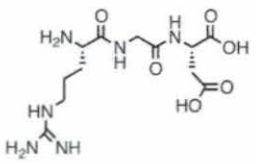


Figure 4. Structure of RGD peptide sequence, a biomolecule found on the integrin surface of the ECM.

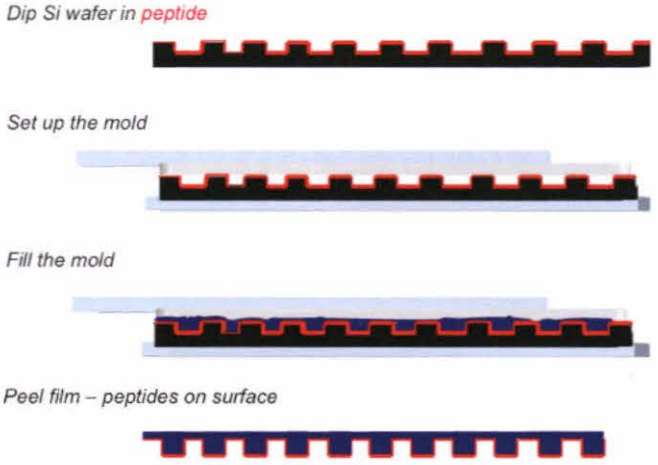


Figure 5. Schematic of physisorption of peptide onto silicon mold, which transfers onto the film surface and cross-links with the thiol, PMMS.

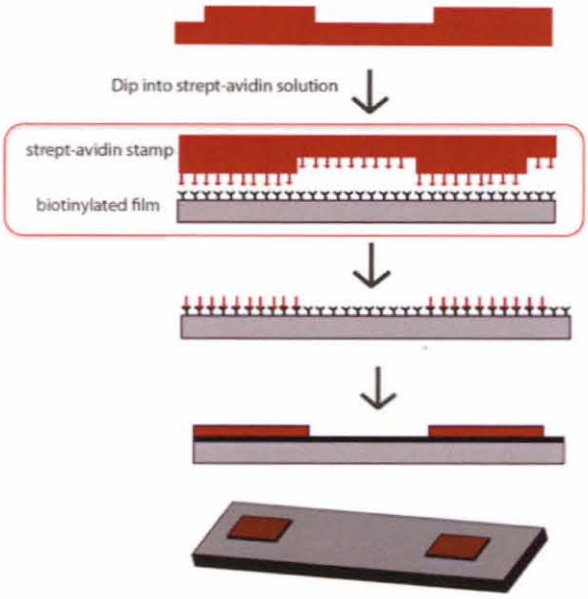


Figure 6. Structure of RGD peptide sequence, a biomolecules found on the integrin surface of the ECM.

WHERE'S THE PEPTIDE?

Verification of the physisorption of peptide through Approach A was difficult. Infrared spectroscopy (IR) was used to obtain spectra in which amide peaks were anticipated from the peptide sequence. However, both films with and without triallyl cyanurate, which are speculated to mask the peptide sequence, showed no peaks at the amide region. IR was unable to deduce the peptide's presence; this, however, could be accounted for by the extremely low and possibly undetectable presence of the peptide on the film surface.

Cell studies with collaborators in the Sheetz lab were more promising. When myocytes (muscle cells) were plated for an hour on films that had the peptide on the surface, the cells had noticeably better adhesion and spreading than cells plated on films without the peptide (Figure 8). Focal adhesions and stress fiber formations were more visible with the former set of cells, indicating that the RGD peptide was playing a role in cell growth. However, this method of physisorption of peptide could not be completely verified, so approach B was also considered. First, we imaged the PDMS stamps to ensure they had micropatterns on its surface. (Figure 9). After running the Biotin-TMPDE reaction (Figure 10), it was verified by nuclear magnetic resonance (NMR) (Figure 11). This additional starting material, which contains an alkene, was added to the mixture of the other starting materials and synthesized as before, but this time some of the biotin was incor-

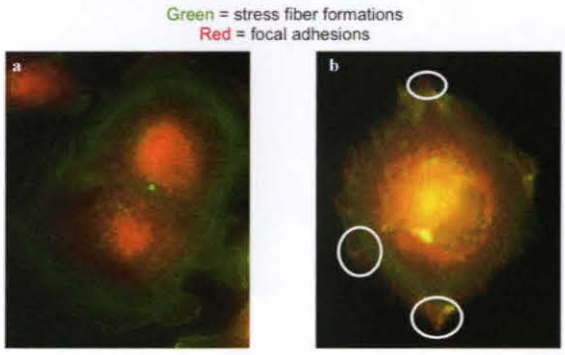


Figure 8. Cell studies showed more adhesion and fiber formations when plated on peptide-surfaced films. a) Myocyte cell plated on film fabricated with nanopatterned silicon. b) Myocyte cell plated on film fabricated with silicon that had physisorbed peptide.

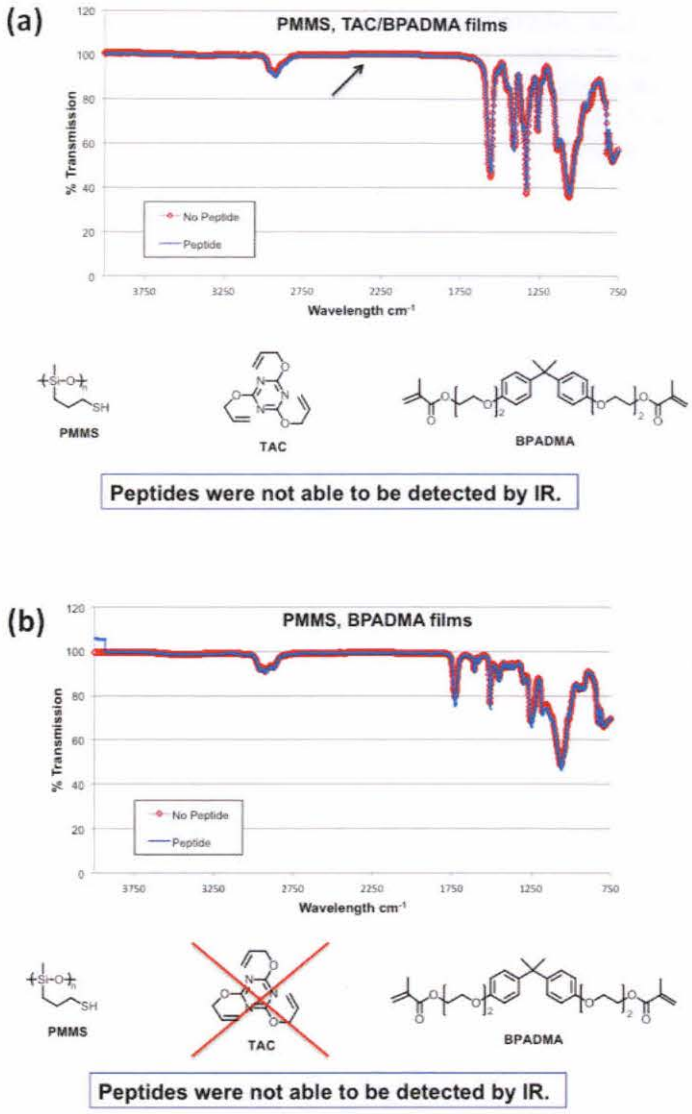


Figure 7. Peptides were not detected by IR. a) Structure of RGD peptide sequence, b) biomolecules found on the integrin surface of the ECM.

porated onto the film surface. The PDMS stamps were then soaked in strept-fluorophores and pressed uniformly onto the biotinylated films. As the binding between strept avidin and biotin is very strong and specific, this interaction would allow for the incorporation of other biomolecules with strept-avidin onto the surface of our films. Verification through fluorescent microscopy showed us that this stamping method was successful and had patterns of molecules on the surface of our films (Figure 12).

THE FUTURE

Embryonic stem cells are no longer the only option for regenerative therapies, which enables researchers to avoid the controversy associated with using this type of cell. While the methods presented here require much optimization, the fabrication of patterned films holds great promise for understanding how different factors influence cell fate. Cell integrins present more factors than just the patterned biomolecules as we have tried to mimic here. By examining each characteristic of the cell integrin and incorporating it into our film, we can hope to understand how all of these factors influence the fate of a cell. We hope that one day, chemistry can be used to create an environment similar to the extracellular matrix and thus replicate a cell's niche; we hope that when that day arrives, we will have the power to control the differentiation of stem cells.

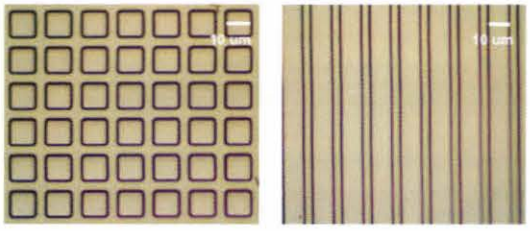


Figure 9. Confocal microscopy of PDMS stamps with microstructured patterns.

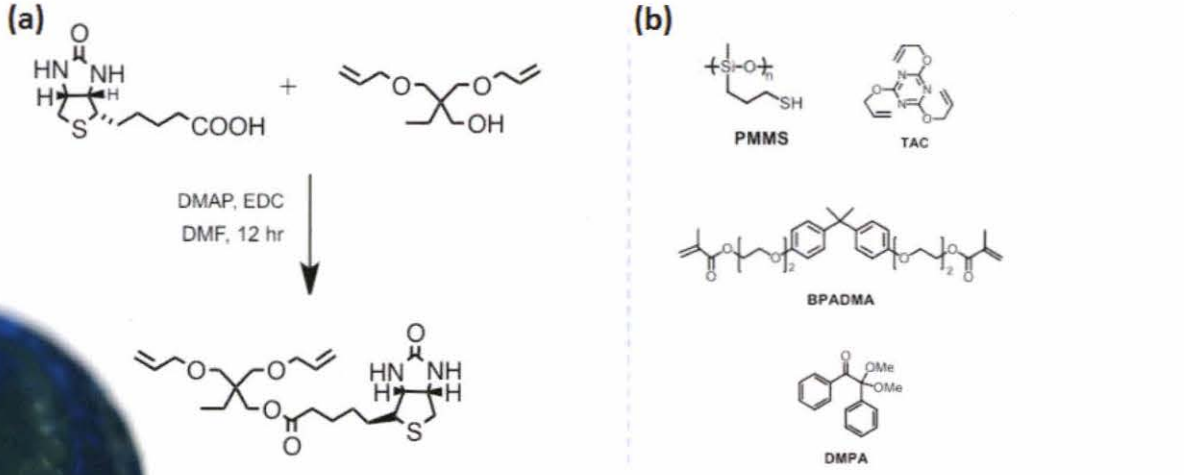


Figure 10. a) Synthesis of biotin-TMPDE. b) Four starting materials used to synthesize biotinylated film.

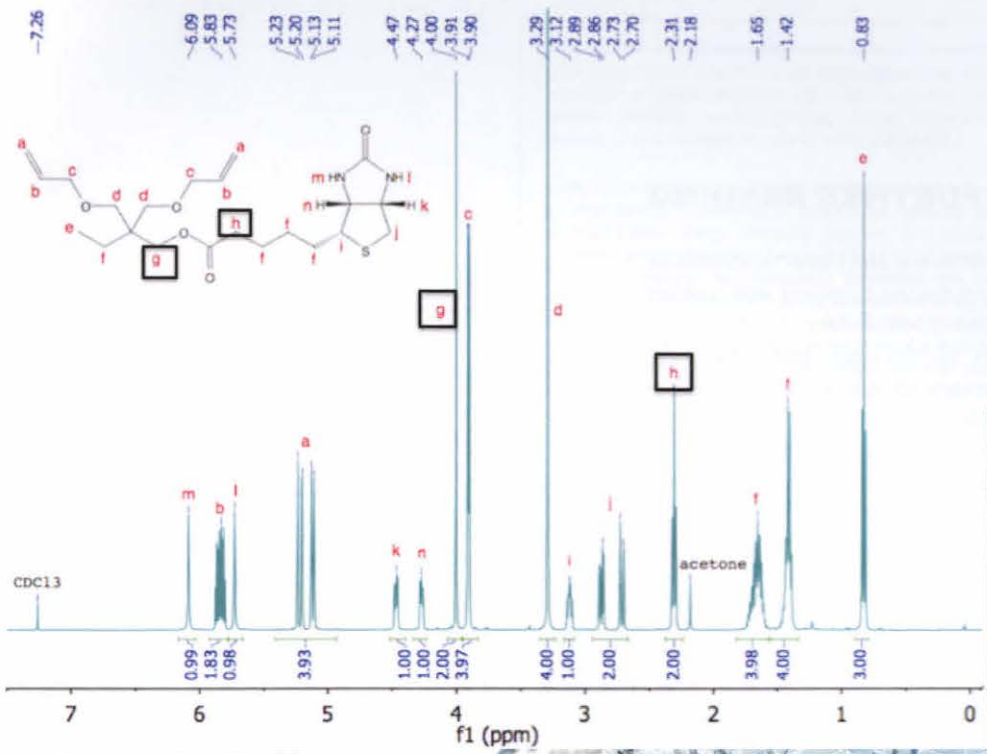


Figure 11. Synthesis of biotin-TMPDE was verified by 1H NMR. Protons g and h in particular verified reaction described in Figure 10 to yield biotin-TMPDE. Deuterated chloroform was used as the solvent.

Figure 12. Fluorescent microscopy of biotinylated films stamped with strept-avidin AlexaFluor. While hierarchicalt patterns are not visible here, they are present due to the preexisting nanopatterns on the films. Pattern preservation of the stamp was preserved, indicating that this method could be used with any particle with strept-avidin.

REFERENCES AND FURTHER READING

1 Discher, Dennis E., David J. Mooney, and Peter W. Zandstra. "Growth factors, matrices, and forces combine and control stem cells." Science 324 (2009): 1673-77. Print.
2 Frantz, Christian, Kathleen M. Stewart, and Valerie M. Weaver. "The extracellular matrix at a glance." Journal of Cell Science 123 (2010): 4195-200. Print.
3 Killops, Kato L., et al. "Nanopatterning Biomolecules by Block Copolymers Self-Assembly." ACS Macro Letters 1 (2012): 758-63. Print.
4 J. Polym. Sci. Part A: Polym. Chem.: Vol. 48 (2010)

ACKNOWLEDGMENTS

Many thanks to Professor Luis M. Campos, Helen Tran, Nevette Bailey, the Campos group, and our Sheetz lab collaborator, Thomas Iskratsch, for invaluable guidance, discussion, and mentoring. Funding was graciously provided by Robert and Delpha Noland, Caltech, and Columbia University.