

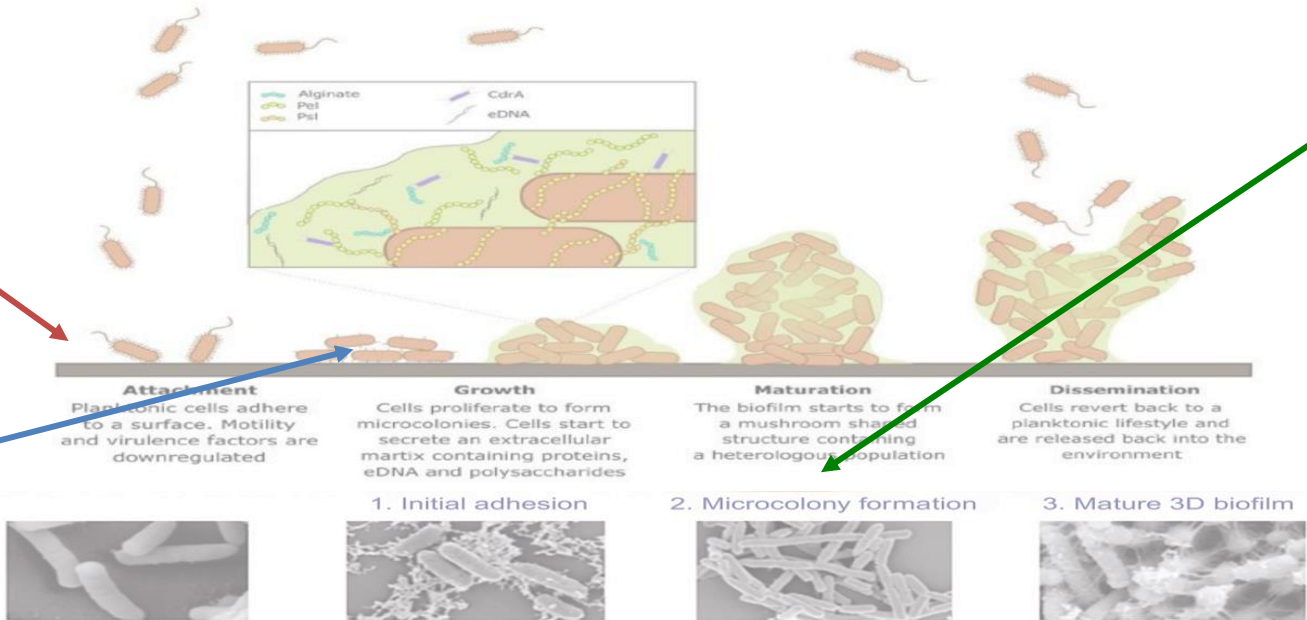
# 2 Dimensional materials for Biofilm, Engineering, Science and Technology – 2D BEST

“unsettled science”

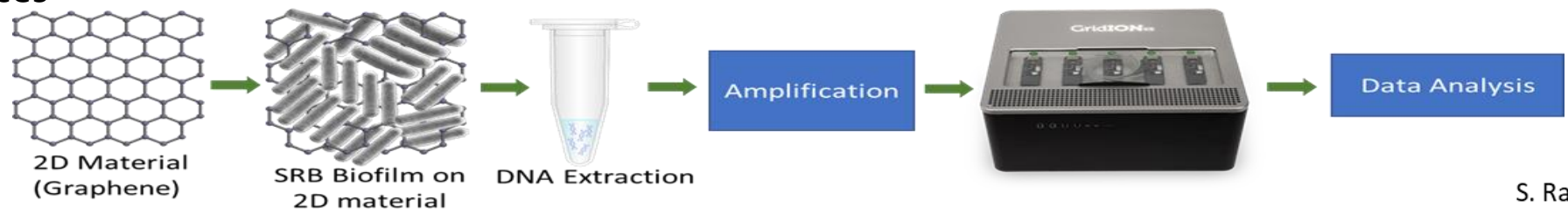
Rationally designed 2D surface properties  
Chemistry  
Engineering  
Physics

Biofilm imaging  
Biology  
Engineering  
Physics  
Life Sciences

Bacterial ‘omics’  
Biology  
Life Sciences



<https://www.sciencedirect.com/topics/engineering/bacterial-biofilm> Maunders and Welch (2017) FEMS Microbiology Letters



S. Rauniyar

# T1 Research Overview

- Hypothesis driven team/convergent science – *atomic scale properties of **2D materials** can be manipulated to influence **genetic** responses of microbes and their biofilm **phenotypes***
- *Two research foci addressing the **Grand Challenges** of food, energy and water*
  - *Area 1 – microbially induced corrosion (water, oil and gas infrastructure integrity)*
  - *Area 2 – microbially enhanced nitrogen fixation (food production)*
  - *Predictive Modeling and Big Data Discovery*

- COVID-19 impact
  - ✓ ~75% of 2D BEST research is experimental
  - ✓ Mar-Jun - Labs shut down except for essential activities, e.g. keeping cells alive
  - ✓ July to date – reduced lab access/activity to accommodate physical distancing
- Outline
  - Preparation for NSF Reverse Site Visit, March 2020
  - Area 1 Overview – **Objectives; Activities; Results, Outcomes & Accomplishments; Progress** and Future Plans
  - Area 2 Overview – **Objectives; Activities; Results, Outcomes & Accomplishments; Progress** and Future Plans
  - Seed Grant Overview
  - Q&A as time permits

# Research Area 1. Stress Resistance

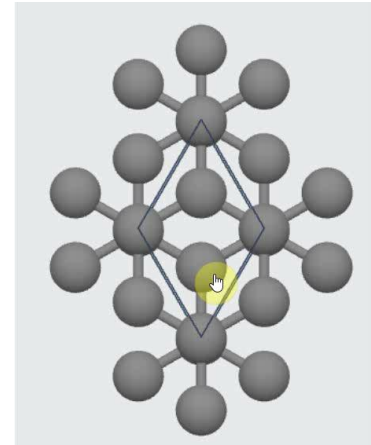
Ramana Gadhamshetty

Dec 10<sup>th</sup>, 2020

All Investigator Zoom Meeting



# Introduction – Area 1



**Problem:** Microbiologically influenced corrosion (MIC) challenges oil and gas industries and water infrastructure. Polymer coatings used to protect metals are prone to microbiologically influenced corrosion (MIC)

**Solution/Goal:** Develop non-invasive protective 2D coatings for protecting metal surfaces exposed to aggressive microbial environments.

**Hypothesis:** Atomic-scale defects in 2D materials used to modify metals will provoke molecular mechanisms that induce stress resistance, biocorrosion, and electronic communication genes in sulfate reducing biofilms.

**Approach:** 2DBEST researchers will: (1) determine the genes and metabolic networks that govern SRB biofilms in response to changes in atomic-scale features (stone-wales defects, point defects, and grain boundaries) and the corresponding barrier and galvanic properties of 2D coatings; (2) match each parameter to SRB molecular markers and biofilm phenotypes (stress resistance; extracellular electron transfer capabilities; corrosion ability; adhesion, morphology and structure) and (3) apply new mechanistic knowledge to design and create a new class of 2D materials to block biocorrosion.

## Research Thrust Area 1:

- Goals
  - Task 1.1 a) Grow SRB biofilms on rationally designed 2D coatings on Cu surfaces and evaluate the SRB stress response as a function of controlled coating properties b) Develop a microfluidic platform that combines single-cell/bioelectrochemistry approaches with fluorescent probes to study how a single SRB cell interacts with corroding metals
  - Task 1.2 Elucidate molecular mechanisms involved in SRB biofilm formation on rationally designed 2D coatings on Cu surfaces
  - Task 1.3 Predict and validate biofilm phenotypes (stress resistance) associated with induced genes using gene knockout/overexpression strategies

## Area-1 Leads



Ramana Gadhamshetty  
(2D materials, SDSMT)

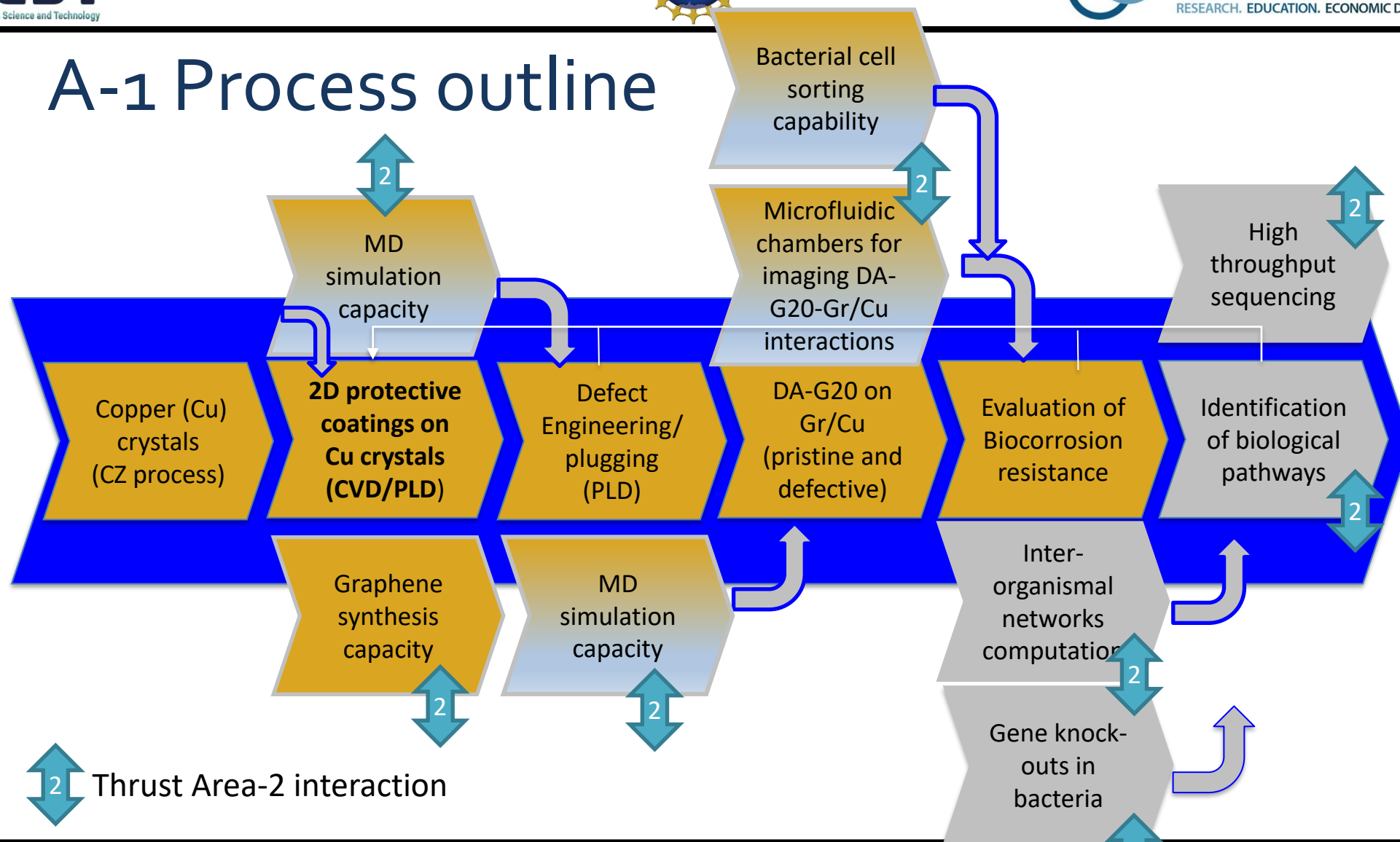


Rajesh Sani  
(Biology, SDSMT)

## Team Members:

Jasthi, Sereda, Dhiman,  
Benjamin, Kalimuthu,  
Lushbough, Gnimpieba, Butzin,  
Gu, Subramanian

# A-1 Process outline



## Task 1.1.1: Develop machine learning tools to identify 2D coatings for biocorrosion



Lushbough



Gnimpieba

- Sub-Tasks/Objectives

- Develop Biofilm-KIDS vo.1
- Develop Data Mining Processes to gather dataset from multiple sources
- Develop GOIP: Gene of Interest prediction tools

- Activities

- 1 SBIR grant submitted on READS system
- 1 Predictive model developed and in production
- 3 papers in preparation

- Results, Outcomes & Accomplishments

- Biofilm-KIDS vo.9 available for beta testing
- 6 Data Mining workflows available
- Broader impact workshop funded



## Task 1.1.1 Machine learning tools Future Activities

### *Progress relative to intellectual merit*

- Research Proposals submitted:
  - 1 NSF Workshop proposal (PI)
  - 1 NSF SBIR (PI)
  - 1 NIH R21, 1 NIH Ro1 (Co-I)
- Awards: 1
- Publications, Presentations
  - 4 publications (1 related to the project)
  - 5 presentations
- Students trained
  - 8 Undergraduates
  - 5 Graduates (MAs, PhD)
  - 2 Post Doc
- Other
  - 2 software packages (R and Python)

### *Plans for future activities*

- Research Proposals submitted
  - NSF PRGP (in development)
  - NIH R21
- Publications, Presentations
  - 2 data mining workflow papers (Material, biofilm)
  - 1 predictive modeling paper (GEPO vo.1)
- Collaborations
  - International Collaboration with NBIC: working group on biofilm to create top priority questions in biofilm discovery
- Other



Lushbough



Gnimpieba

## Task 1.1.2.0: Develop 2D Materials Laboratory (2DML)

- Sub-Tasks/Objectives
  - Develop a new 2DML facility at SDSM&T
  - EasyTube chemical vapor deposition (CVD)
  - Pulsed laser deposition (PLD) with molecular beam epitaxy (MBE)
- Activities
  - Identify and renovate space for 2DML
  - Design, acquire, install and commission CVD for growth of hexagonal boron nitride (hBN)
  - Design, acquire, install and commission PLD
  - Acquire characterization tools



**Gadhamshetty**



**Jasthi**

## Results, Outcomes & Accomplishments

1. Acquired 2DML at Ascent Building, SDSMT. The space is being renovated
2. Received CVD; expecting PLD by 12/30/20
3. CVD training and 2D materials at University of Minnesota



EasyTube CVD (From CVD Corporation).



PLD/MBE 2300 (from PVD Products, Inc.).



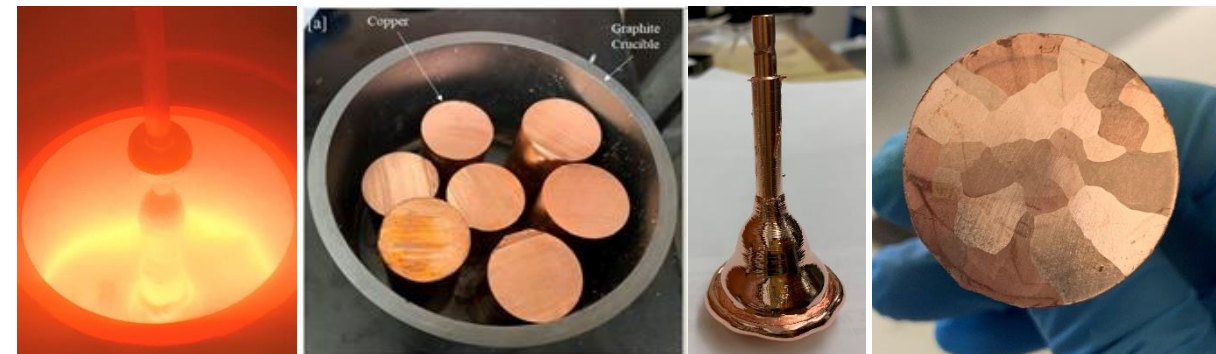
**Jasthi**

## Task 1.1.2a: Czochralski (CZ) process

- Sub-Tasks/Objectives
  - a. Develop and optimize the Czochralski (CZ) Process
  - b. Synthesize and characterize CZ-grown Materials
- Activities
  - Designed a CZ process to grow monocrystalline and polycrystalline (PC) forms of Cu
  - Introduce rationally designed dislocation in Cu crystals (Annealing and cold working)

## Results, Outcomes & Accomplishments

- Obtained CZ-grown poly Cu crystals
- Introduced dislocations and defects into cold worked Cu crystals via cold-working processes







**Gadhamshetty**

## Task 1.1.2b: 2D materials synthesis and characterization

### • Sub-Tasks/Objectives

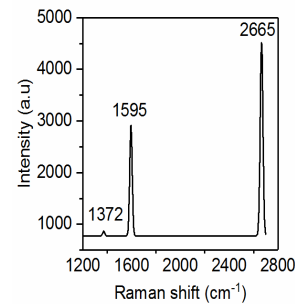
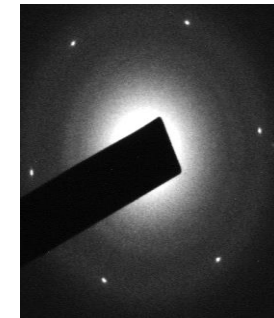
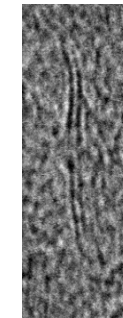
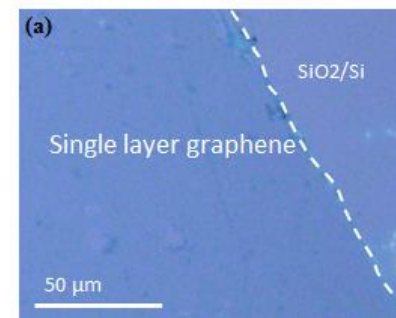
- Synthesize 2D materials on Cu surfaces
- Characterize 2D Materials

### • Activities

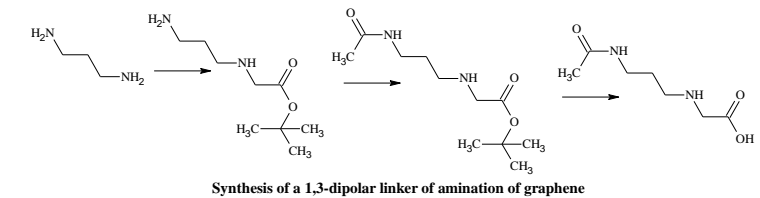
- Trained graduate students in CVD at Midwest Nano Infrastructure Corridor (MINIC) lab
- Acquiring CVD equipment for h-BN (SDSMT) and GR (SDSU)
- Developed methods for characterizing graphene (GR) and hexagonal boron nitride (hBN) materials

### Results, Outcomes & Accomplishments

- Grow SL-GR on PC-Cu crystals
- Ongoing efforts to grow SL-GR on single Cu crystals, CZ-crystals, and annealed and cold-worked Cu
- Methods of assessing signatures of GR on PC-Cu







## Task 1.1.2 2D materials Future Activities:

### *Progress relative to intellectual merit*

- Research Proposals submitted:
  - 1 DOE-NEUP (\$800,000)
  - 1 NAVY SBIR Phase-II (\$200,000)
- Awards: DOE SBIR Phase-IIA, Airforce SBIR Phase-1, SDBOR-i6
- Publications, Presentations
  - ~15 publications (3 related to 2D materials/MIC)
  - >10 presentations
- Students trained
  - 2 Undergraduates
  - 7 Graduates (PhD)
  - 2 Post Doc
- Other
  - CVD and PLD training; CVD software package

### *Plans for future activities*

- Research Proposals
  - NSF NRT (in development)
  - NSF RII T2 (in development)
  - NSF Advance (in development)
  - NSF MRI (XPS) (in development)
- Publications, Presentations
  - >5 pubs (CZ, 2D-material, biofilm)
- Collaborations
  - Collaborations with MINIC, UNL, SENIC
- Others
  - CZ process for single Cu crystals
  - Commission and operate CVD (hBN) and PLD
  - Acquire a 2<sup>nd</sup> CVD for GR
  - Acquire Scanning electrochemical microscopy



## Task 1.1.3: Biocorrosion tests with DA-G20



Gadhamshetty



Kalimuthu

### Sub-Tasks/Objectives

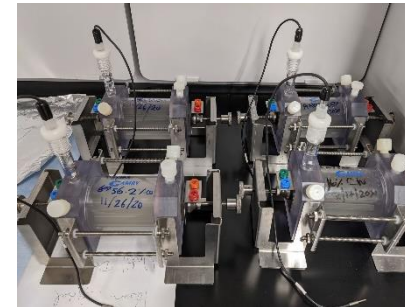
- Assess MIC resistance of PC-Cu and those modified with GR, and hBN- samples
- Test microbial corrosion resistance of defective copper samples, obtained via a cold working (CW) process.
- Carry out electrochemical impedance spectroscopy (EIS) analysis of pristine and defective forms of Cu samples exposed to *D. alaskensis* G20.

### Activities

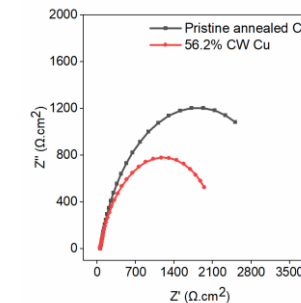
- 16%, 29.5% and 56.2% CW-PC-Cu samples were used for MIC
- Perform EIS, linear polarization resistance (LPR) and Tafel analysis
- Identify corrosion resistance mechanism of defective copper and develop suitable heterostructure coatings
- Study the differential gene expression of DA-G20 exposed to pristine and defective copper samples
- Working in collaboration with Dr. Jasthi on defective copper samples

### Results, Outcomes & Accomplishments

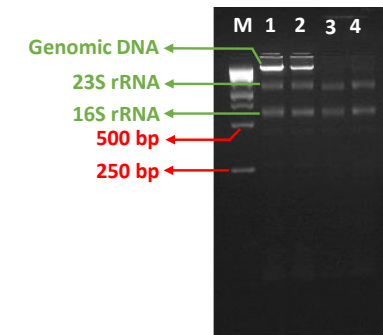
- Established baseline data for MIC resistance of PC-Cu, GR-Cu, and hBN-Cu samples
- Ongoing efforts to assess the effect of dislocations in PC-Cu
  - 56.2% CW copper showed 1.5 folds lesser total resistance than the control copper annealed sample on day 6
  - Phase angle maxima and OCV showed that CW samples showed higher electrochemical activity than the copper annealed samples. Higher MIC of CW samples compared to pristine Cu



Corrosion cells



EIS



RNA from DA-G20



## Task 1.1.3: Biocorrosion tests Future Activities

### *Plans for future activities*

- Establish protocols for biofilm quantification by CV staining and CLSM during biofilm conditioning phase of DA-G20
- Research Proposals: proposals (equipment and research) are planned to be submitted
- One manuscripts is planned in collaboration with Dr. Jasthi
- New collaboration with Montana State University (genetic modifications of DA-G20) will be established
- RNA-seq analysis will be performed to evaluate the genetic mechanism of DA-G20 grown on pristine and defective Cu



Gadhamshetty



Kalimuthu

### *Progress relative to intellectual merit*

- Research Proposals submitted: Please fill in.....
- Awarded: Please fill in.....
- Publications: Two manuscripts are under preparation
- Four graduate students were trained on microbially induced corrosion, Raman spectroscopy, scanning electron microscope, confocal laser scanning microscope and RNA extraction procedure



## Task 1.1.4: Develop a microfluidic platform for single-cell-electrochemistry and live-imaging

Kalimuthu, Butzin, Subramanian, Krishnaraj and Gadhamshetty

### • Sub-Tasks/Objectives

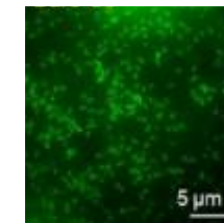
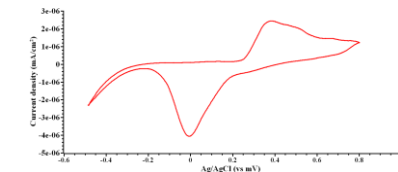
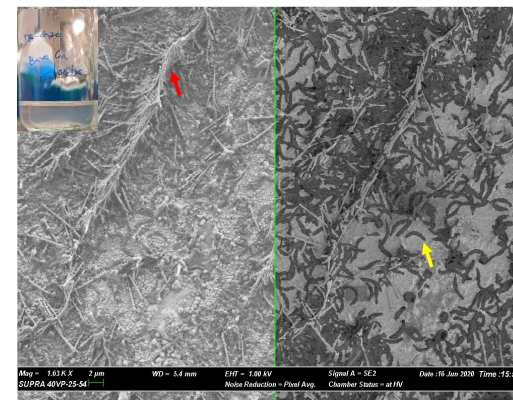
- Grow DA-G2o in glass bottom Petri dishes
- Visualize biofilm using live dead staining/ fluorescent stain
- Tested 3D printed electrodes with DA-G2o

### • Activities

- Analyzed DA-G2o cell structure and biofilm morphology on bare Cu and hBN-Cu
- Analyzed electrochemical response of DA-G2o cells adhered onto 3D printed electrodes
- Working with vendors to acquire screen printed electrodes with Cu as the working electrode

## Results, Outcomes & Accomplishments

- SEM micrographs showed biofilm microstructures (SE detector) and biofilm forming DA-G2o cells (In-lens detector) grown on bare Cu. Red arrows denotes biofilm microstructures and yellow arrow denotes cells.
- Fluorescence microscope image of DA-G2o cells on corroded Cu surface after 48 h exposure and staining with SYTO 9 green-fluorescent nucleic acid



## Task 1.1.4 Microfluidic platform Future Activities

Kalimuthu, Butzin, Subramanian,  
Krishnaraj and Gadhamshetty

### *Progress relative to intellectual merit*

- Research Proposals submitted: NA
- Awarded: NA
- Publications: NA
- Two graduate students are being trained to assess cell adherence; and carry out electrochemistry studies using 3D-printed screen-printed electrodes

### *Plans for future activities*

- Establish protocols for microfluidic device to visualize single cell of DA-G20
- Tag candidate genes involved in biofilm formation using green fluorescent protein
- Research Proposals: Fractal analysis and Rheology Studies
- Fabricate 3D-printed Cu electrodes for analyzing the bioelectrochemical activity of DA-G20 in microfluidic devices

## Task 1.2.1: Analyze epigenetics and omics of SRB biofilms grown on 2D materials



Sani

### • Sub-Task/Objective

- Analyze Epigenetics and Omics of SRB biofilms grown on 2D materials

### • Activities

- Develop infrastructure for epigenetics and transcriptomics studies
- Acquire and install confocal microscopy
- Identify mechanisms involved in DA-G20 Cu homeostasis (Gene expression analysis)
- Study role of epigenetic changes in DA-G20 biofilm formation under Cu stress
- Working in collaboration with Dr. Etienne on comparative genomics and predictive modelling for SRB

### Results, Outcomes & Accomplishments

1. Acquired Nanopore MinION sequencer, Agilent Bioanalyzer, CDC Biofilm reactor, and Anaerobic glove box
2. Installed Spinning Disc Confocal microscope to visualize SRB biofilms
3. Data generation completed for RNA sequencing and Epigenetic analysis for DA-G20 under varying concentrations of Cu ions
4. Gene expression and epigenetics analysis in progress to identify molecular mechanisms in response to Cu stress
5. Pangenome data generated for 110 SRB genomes for the predictive modeling



Nanopore MinION



Agilent Bioanalyzer

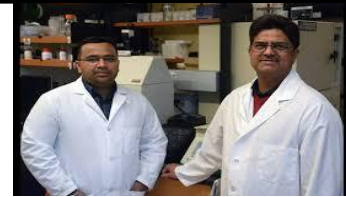


High Precision Computer



Confocal Microscope





Dhiman & Sani

## Task 1.2: Predict and validate biofilm phenotypes

- Sub-Task/Objective
  - Validation of biofilm phenotypes with induced genes
- Activities
  - Targeted genomic modifications to validate results obtained in tasks 1.2.1, 1.3.1, and 1.3.2.
  - Expression of Cas proteins, tracrRNA and crRNA
  - Assembly of CRISPR/Cas system for genome editing

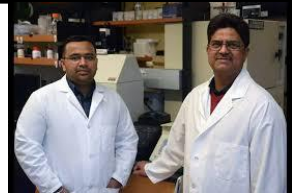
## Results, Outcomes & Accomplishments

This task will start as soon as we get epigenetics and transcriptomic data. We expect to start this Task withing six months.

We will begin with:

- Knockout and overexpression experiments to validate results obtained in omics studies
- Verify the Cu-responsive genes in various metabolic and regulatory pathways





Dhiman & Sani

## Task 1.2 Epigenetics and omics Future Activities

### *Progress relative to intellectual merit*

- Research Proposals submitted: We submitted 8 collaborative proposals (total funding of \$11,333,160) to various agencies
- Awarded: Accelerated carbon mineralization sequestration in cation rich rock formations via microbial augmentation and stimulation, NSF, \$300,000 (Role: Co-PI)
- Publications: Three manuscripts are under preparation
- Four graduate students were trained on Library preparation, Nanopore MinION sequencer and Spinning Disc Confocal Microscope as well as T-Bioinfo training for omics data analysis

### *Plans for future activities*

- Establish protocols for SRB biofilm visualization, quantification of its spatial components (e.g., proteins, lipids, EPSs, carbohydrates), and cell distribution in biofilms
- Research Proposals: Three proposals (equipment and research) are planned to be submitted
- Two manuscripts are planned in collaboration with Dr. Etienne
- New collaborations with Kansas state university and University of Nevada will be established
- Transcriptomics and epigenetics of SRB biofilm grown on 2D material
- Arrange phenomics data analysis training for graduate students

## Task 1.3.1: Evaluate proteins that govern nanowire formation and DA-G20 biofilm attachment on 2D coatings

### • Sub-Tasks/Objectives

- Identify the genes encoding Type IV pilin subunits
- Evaluate the completeness of the identified individual subunit
- Evaluate the molecular interactions of the all identified subunits as nanowire

### • Activities

- Seven genes encoding the nanowire forming subunits were identified
- Completeness of the seven subunits was confirmed and validated
- Structural framework of the four subunits was developed
- Molecular interactions of the four subunits was analyzed
- DFT and MD simulations of amino acids on graphene and copper (with and without water solvent)
- Building towards free energy of binding calculations for proteins in water over pristine and defective graphene and copper 2D surfaces



Dhiman



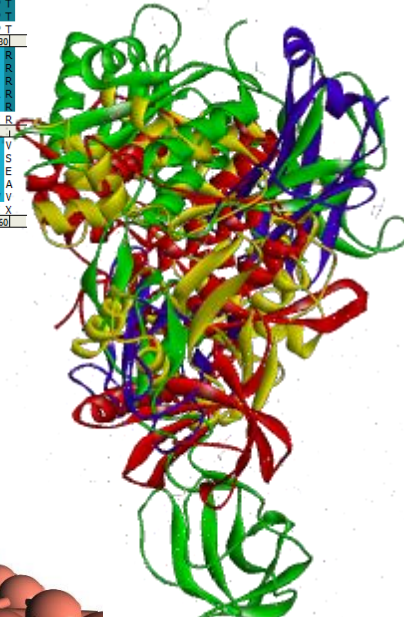
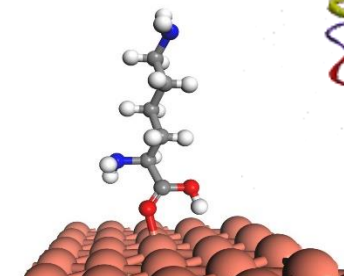
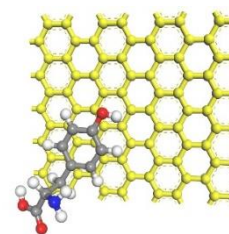
Benjamin

## Results, Outcomes & Accomplishments



### Type IV pilus proteins in G20

- Subunit – 1 # 0203; 134 aa
- Subunit – 2 # 0818; 171 aa
- Subunit – 3 # 0821; 192 aa
- Subunit – 4 # 1029; 230 aa
- Subunit – 5 # 1460; 115 aa
- Subunit – 6 # 1540; 131 aa
- Subunit – 7 # 1849; 113 aa



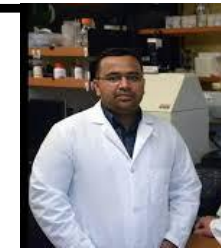
## Task 1.3.2: Validation of selected proteins using QM/MM and rational designing

### • Sub-Tasks/Objectives

- Key residues governing the nanowire formation were selected
- Thermodynamic method was optimized to evaluate the role of the single residue and whole protein

### • Activities

- In-silico mutagenesis was performed to collect the baseline energy value data
- License procurement to perform the calculations
- Building towards implementation of coarse-grained (MARTINI) force field for proteins for MM portion of calculation



Dhiman



Benjamin

## Results, Outcomes & Accomplishments

The thermodynamic calculation methodology was optimized to calculate the mutant's energy for designer proteins.

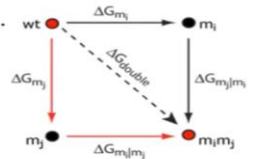


$$\Delta G_{\text{triple}} = \Delta G_{m_i} + \Delta G_{m_j} + \Delta G_{m_k} - \Delta \Delta G_{m_i m_j} - \Delta \Delta G_{m_i m_k} - \Delta \Delta G_{m_j m_k} + \Delta \Delta \Delta G_{m_i m_j m_k}$$

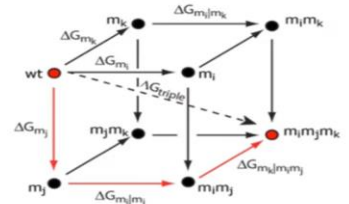
(1) A single mutation...



(2) A double mutation...



(3) A triple mutation...





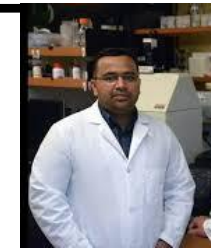
## Task 1.3.3: Validate biofilm phenotypes with induced genes

### • Sub-Tasks/Objectives

- Characterizing the key protein serving as signal molecule or Autoinducer
- Cloning, overexpression and characterization of the QS protein
- External augmentation of overexpressed QS protein to identify the “Significant gene” for biofilm formation via transcriptomics

### • Activities

- QS protein cloned, overexpressed, and purified
- Change of expression vector to reduce formation of inclusion bodies
- Purification of overexpressed protein for biochemical characterization
- Concentration of the purified protein for subsequent crystal structure formation

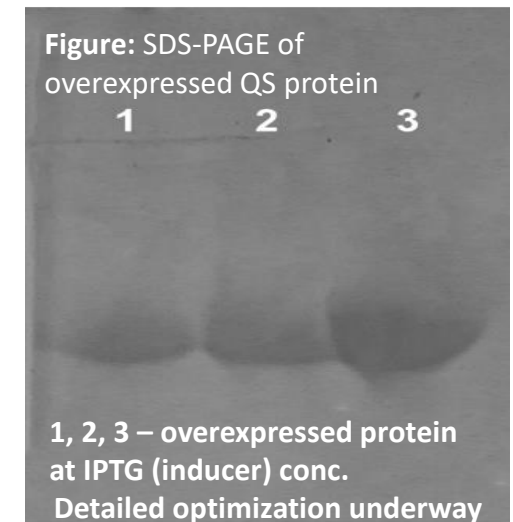
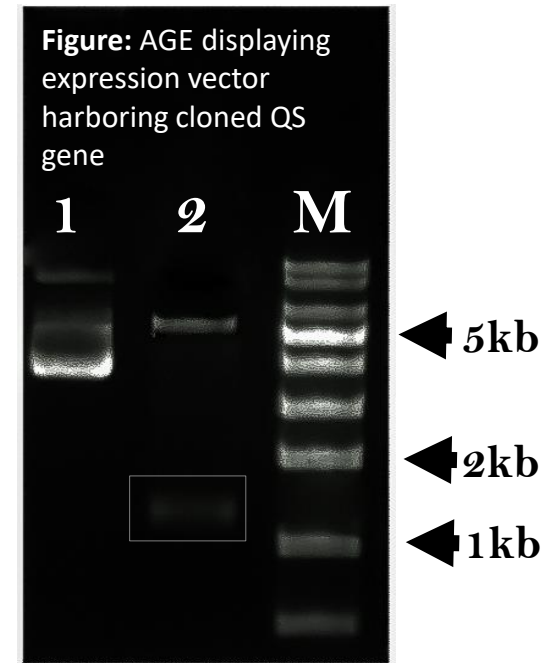


Dhiman



Benjamin

## Results, Outcomes & Accomplishments





## Task 1.1.3 Protein identification and validation Future Activities

### *Progress relative to intellectual merit*

- Research Proposals submitted. N.A
- Awards N.A
- Publications, Presentations
  - Verma, S. and Benjamin, K. M., Density Functional Theory Study of Biomolecule Adsorption to Graphene and Modified Graphene: Molecular Insights into Biofilm Formation and Adhesion. American Institute of Chemical Engineers Annual Conference, November 13, 2019, Orlando, FL, USA.
- Students trained
  - MS thesis/PhD, Sourav Verma
- Collaborations, NA
- Others, NA

### *Plans for future activities*

- Assess structural and functional features of “significant” genes, (pili formation and EPS secretion)
- Design sgRNA for clustered regularly interspaced short palindromic repeat (CRISPR) based genome editing to validate proteins
- AFM analyses to assess surface roughness of defective GR. 2D materials with higher roughness, residues with a higher degree of branched structures will be introduced
- Isothermal titration calorimetry (ITC) will be used to confirm the free energy and binding energy of the active site for the mutant.
- Crystallization of protein controlling the QS mechanisms in DA-G20. The web sgRNA designing tools to create necessary sgRNA for DA-G20.

# Research Area 2. Microbial Resilience

Sen Subramanian

Dec 10<sup>th</sup>, 2020

All Investigator Zoom Meeting

# Introduction – Area 2

**Problem:** Poor competitiveness of high efficiency nitrogen fixing bacteria in occupying root nodules leads to less than optimal nitrogen fixation in soybean

**Solution/Goal:** To increase soybean root nodule occupancy by high efficiency rhizobia

**Hypotheses:**

Designer lectins with higher specificity to desirable bacteria will enhance their root attachment, nodule occupancy and thus increase nitrogen fixation capacity

Increased attachment will elicit the activation of distinct bacterial and plant pathways associated with biofilm resilience against competition

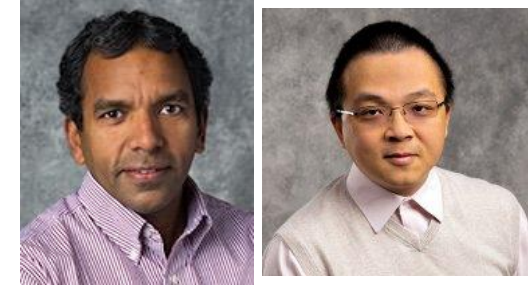
**Approach:** SDBEST researchers will use rational protein engineering to design lectins with better specificity, decorate soybean root surface with these lectins using 2D material technology, evaluate root attachment and internalization using microfluidics enhanced microscopy, determine key plant and bacterial pathways using omics analyses, and use the resulting knowledge to develop strategies to enhance nodule occupancy by desirable rhizobia.

## Research Thrust Area 2:

- **Goals**

- Task 2.1 Determine and design suitable lectin domains that favor attachment by the high efficiency N-fixer *B. diazoefficiens* USDA110
- Task 2.2 Modify soybean root surface with lectins using 2D material technology and evaluate competitive root attachment and biofilm formation
- Task 2.3 Elucidate plant and bacterial genetic pathways associated with rhizobial attachment and biofilm formation under competition.

Sen Subramanian (SDSU, Plant Biology)  
Zhengrong Gu (SDSU, Materials)  
Area Leads

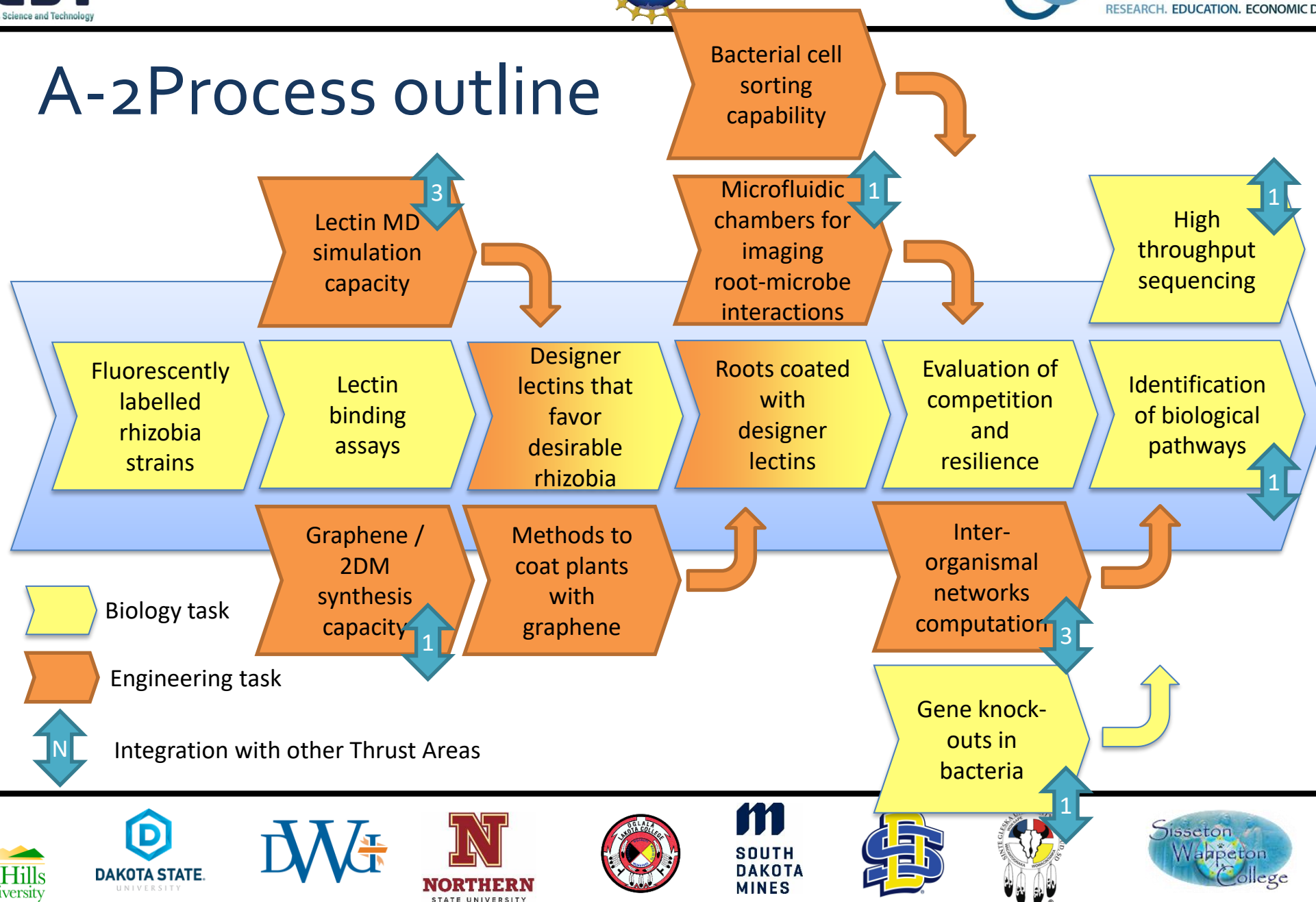


## Task Leads

Benjamin, Brozel, Butzin, Dhiman,  
Erlandson Gadhamshetty, Gnimpieba,  
Gonzalez, Gu, Lushbough,  
Subramanian



# A-2Process outline



## Task 2.1.1: Design a desirable lectin domain using binding assays, molecular dynamics simulations, and predictive modeling

### • Sub-Tasks/Objectives

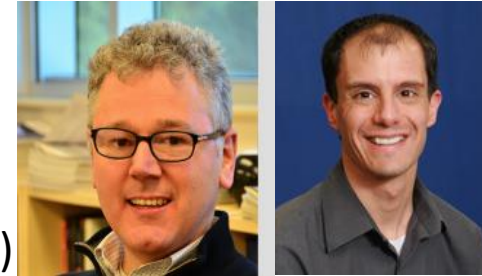
- Screen library of conjugate lectins for binding
- Develop MD simulations of lectin binding
- Perform in-silico mutagenesis and wet-end experimentation to understand rules of lectin binding

### • Activities

- Library of 87 lectins being screened
  - Microscopy-based method optimized
- Surveyed molecular modeling and simulation software options for conducting simulations and made recommendations for future simulation work

Leads:

Volker Brozel (SDSU, Bacteriology);  
Ken Benjamin  
(SD Mines, Simulation)



## Results, Outcomes & Accomplishments

- Native soybean lectin may not distinguish high/low efficiency strains
- Promising other lectins identified
- Differential binding observed in soil medium

		diazo 110	elkani 76	diazo 26	diazo 126	diazo 123
ConA	PSY*	1111	0	11111	11	0
	SEM**	0	0	11111	0	0
DBA	PSY	0	0	1	1	0
	SEM	0	0	0	0	0
PNA	PSY	0	0	1	0	0
	SEM	0	0	0	0	0
RCA 120	PSY	0	0	0	0	0
	SEM	0	0	0	0	0
SBA	PSY	1111	0	11	11111	0
	SEM	0	0	0	11111	0
UEA I	PSY	0	0	1	0	0
	SEM	0	0	111	0	0
WGA	PSY	1111	1	111	0	1
	SEM	1	0	0	1	0

## Task 2.1.2: Protein engineering to obtain and evaluate “designer lectins”

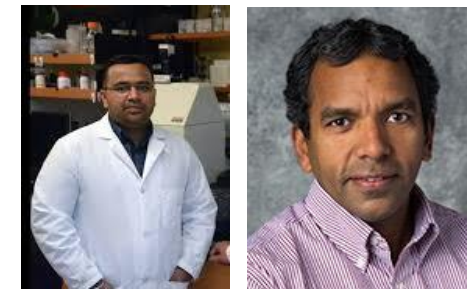
- Sub-Tasks/Objectives

- Introduction of Cys-residue in one of the subunits of lectin protein (Glycine max)
- Validation of designer protein biochemically
- Binding of designer lectin on functionalized 2D material
- Evaluation of binding efficiency of designer protein on functionalized 2D material

- Activities

- Optimization of thermodynamics box for introduction of targeted residues in protein subunit
- In-silico energy validation of Cys-residue introduction
- Functionalization of 2D material for lectin binding

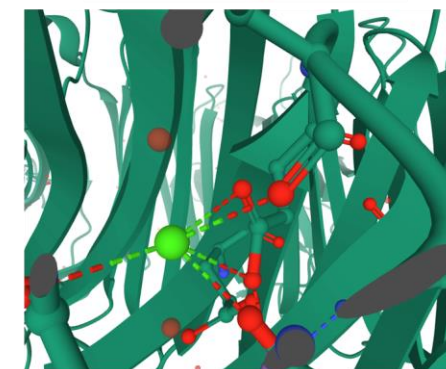
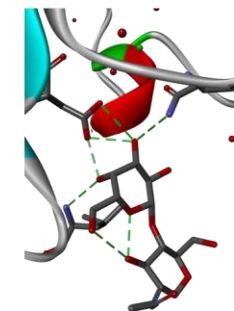
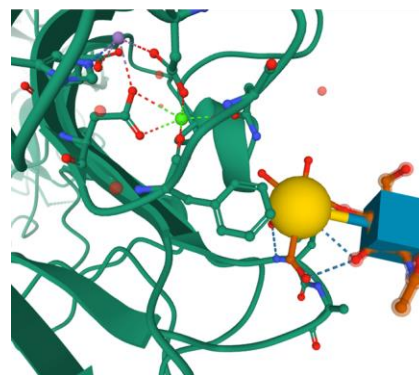
Saurabh Dhiman  
(SD Mines, Simulation)  
Sen Subramanian (SDSU,  
Plant Biology)



## Results, Outcomes & Accomplishments

Challenge - No Cys residue in the lectin protein  
Theory - S-S bond is stronger to hold the biomolecule  
Tested - Functionalization of Gr

Designed protein while maintaining  
Energy (kinetic) of the biomolecule  
No drastic change in the conformation  
Active side residues were avoided (therefore screening of active site is crucial)  
After mutagenesis energy levels were maintained  
Hydration, valency, force fields were optimized





## Task 2.2.1: Develop mimetic root surfaces coated with 2D materials that incorporate lectins of interest

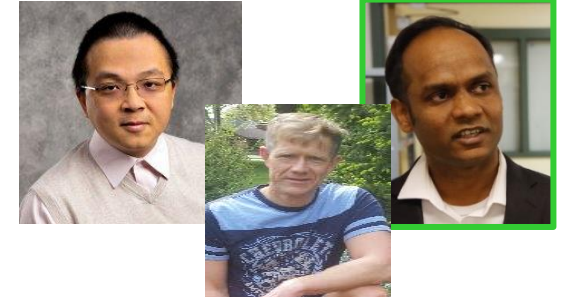
### • Sub-Tasks/Objectives

- Synthesize graphene and 2D materials
- Coating hollow fiber with 2D materials
- Graphene surface functionalization
- Develop methods for root surface modification

### • Activities

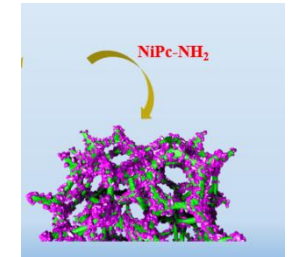
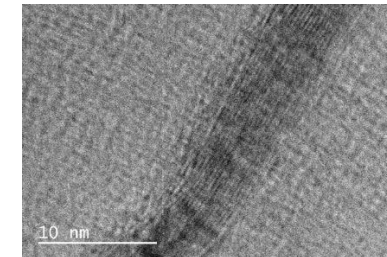
- Develop a high energy plasma process to prepare graphene from lignin
- Installed the plasma enhanced CVD system
- Synthesize 2D MOF (Mg, Ni, Co and Zn)
- Electrochemical synthesis & deposition of 2D MOF on polymer film, metal and cellulose surface
- Electro-deposit graphene on surface of cellulose hollow fiber

Jimmy Gu (SDSU, Materials)  
Ramana Gadhamshetty (SD Mines, Materials)  
Gregory Sereda (USD, Chemistry)



## Results, Outcomes & Accomplishments

- High energy plasma - prepared graphene from lignin at room temperature in < 30 seconds; porous with high surface (~400 m<sup>2</sup>/g) and low microbial toxicity
- The Plasma enhanced CVD system is installed and running now, several graphene samples prepared
- 2D MOF (Mg, Ni, Co and Zn) (non-toxic, electrochemical stability) was synthesized with classic hydro/solvo-thermochemical synthesis
- 2D MOF was synthesized and deposited (on multiple materials) simultaneously with electrochemical process.
- Graphene (commercial) was deposited on hollow fiber through





## Task 2.2.2: Develop methods for root surface modification (Gr coatings incorporated with lectin of choice on the root surface)

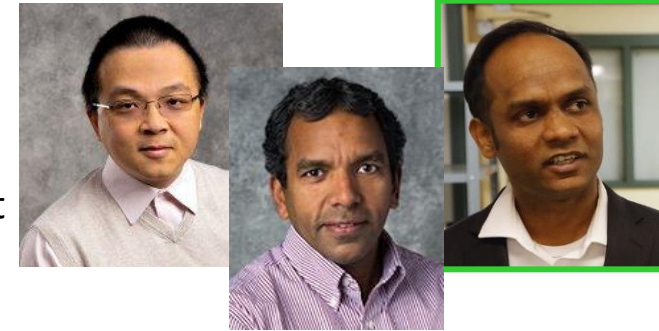
### • Sub-Tasks/Objectives

- Suitability of seed treatment/root dipping for graphene
- Evaluate graphene toxicity on soybean and rhizobia (incl. nitrogen fixation)
- Check distribution and persistence

### • Activities

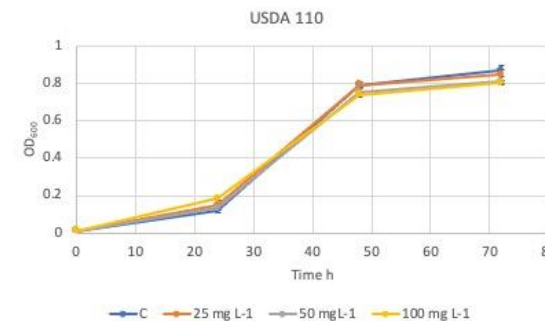
- Evaluated soybean and rhizobia growth in graphene
- Evaluated antioxidant production
- Preliminary analysis of graphene distribution

Jimmy Gu (SDSU, Materials)  
Ramana Gadhamshetty (SD Mines, Materials)  
Sen Subramanian (SDSU, Plant Biology)



## Results, Outcomes & Accomplishments

- Graphene promoted root emergence, but impacted seedling growth at high concentrations
- Graphene differentially inhibited growth of different rhizobia strains
- Impacts are due to reactive oxygen induction



## Task 2.2.3: Design and fabrication of a microfluidic chamber for root-microbe imaging

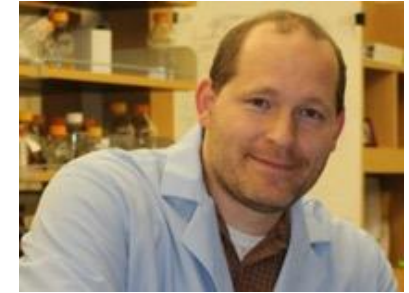
### • Sub-Tasks/Objectives

- Acquire and setup a 3D printer for microfluidics
- Imaging plant/bacteria relationship and microdevice construction

### • Activities

- Identify and renovate space and order 3D Printer and ductless hood
- Test *E. coli* and then *B. japonicum* growth on soybean roots
- Optimize setup

Nicholas Butzin (SDSU,  
Synthetic biology)  
New Faculty (SDSU,  
Microfabrication and  
biosensing)



## Results, Outcomes & Accomplishments

- 3D printer room identified and renovated
- 3D printer ordered (Expected to arrive: Feb 2021)
- Ductless hood ordered (Expected to arrive: Dec 2020)
- Live imaging of bacteria on plant roots



## Task 2.2.4: Evaluate colonization and biofilm formation by *B. japonicum* under different competitive environments using advanced imaging

### • Sub-Tasks/Objectives

- Generate fluorescent strains
- Develop/optimize imaging methods
- Image and track colonization and function

### • Activities

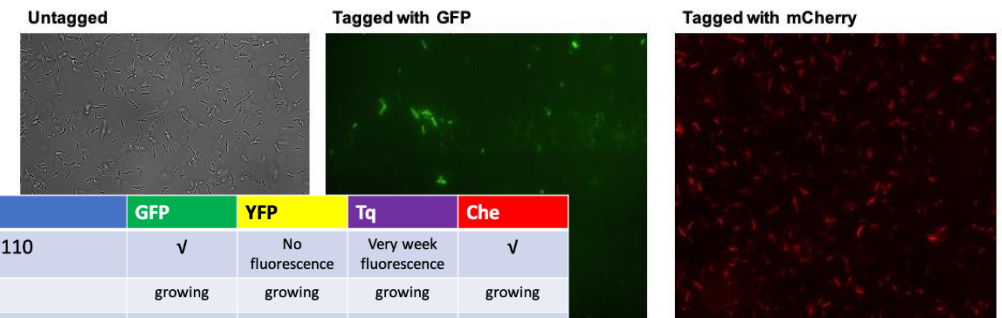
- Acquire relevant genetic materials and transform bacterial strains
- Optimize imaging methods (media, equipment and strains)

Nicholas Butzin (SDSU, Synthetic biology)  
Volker Brozel (SDSU, bacteriology)  
Sen Subramanian (SDSU, Plant biology)  
Rajesh Sani (SD Mines, bacteriology)



## Results, Outcomes & Accomplishments

- Rhizobia conjugation methods optimized
  - Troubleshooting in progress for some strains
- Imaging of fluorescent rhizobia on special plates/devices



Strain	GFP	YFP	Tq	Che
<i>B. diazoefficiens</i> USDA 110	✓	No fluorescence	Very weak fluorescence	✓
<i>B. elkanii</i> USDA 76	growing	growing	growing	growing
<i>B. diazoefficiens</i> USDA 26				
<i>B. diazoefficiens</i> USDA 123				
<i>B. diazoefficiens</i> USDA 126				



## Task 2.3.1: Obtain metatranscriptomes of bacterial cells, and transcriptomics of root segments at selected time points

Jose Gonzalez  
(SDSU, Genomics)  
Sen Subramanian  
(SDSU, Plant  
biology)



### • Sub-Tasks/Objectives

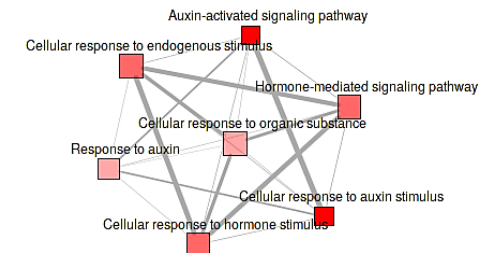
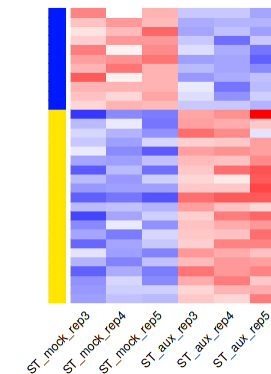
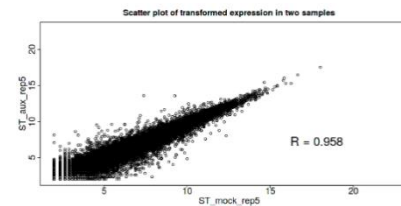
- Optimize bacterial isolation from roots
  - Develop split root systems as alternate
- Construct and analyze gene expression patterns of plants and bacteria

### • Activities

- Analyze existing datasets to train students in gene expression data analysis
- Develop microbiome data analysis capacity

## Results, Outcomes & Accomplishments

- Identified and tested relevant pipelines for DEGs, pathway analysis and identification of key network hubs





Task 2.3.2: Develop an integrated computational pipeline for metatranscriptomic data analysis

Task 2.3.3: Employ a novel workflow to associate microbial and plant pathways

- Sub-Tasks/Objectives

- Develop and utilize integrated analysis pipeline
- Build gene regulatory networks
  - Identify plant and bacterial markers

- Activities

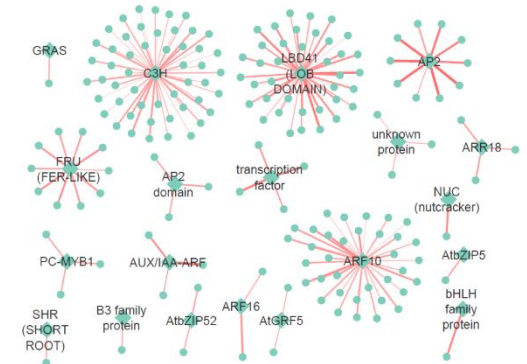
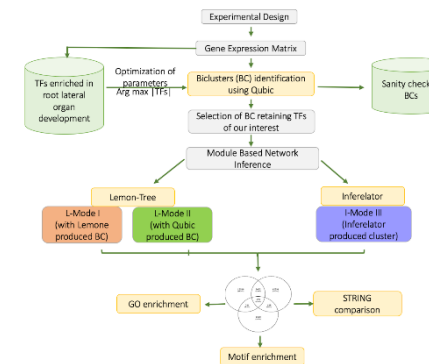
- Build inter-organismal GRNs using existing data (to optimized methods)

Carol Lushbough,  
Etienne Gnimpieba  
(USD, Computer  
Science)  
Sonya Erlandson  
(SDSU, Microbiology)



## Results, Outcomes & Accomplishments

- Evaluated and identified suitable approaches and tools to develop inter-organismal GRNs



## Task 2.3.4: Validate novel microbial pathways involved in attachment and colonization

- Sub-Tasks/Objectives

- Develop gene editing reagents and tools for Bradyrhizobium
- Evaluate mutants for colonization/nodulation capacity

- Activities

- Not started yet

Rajesh Sani (SD Mines, bacteriology)  
Saurabh Dhiman (SD Mines, Modeling)  
Volker Brozel (SDSU, bacteriology)



## Results, Outcomes & Accomplishments

- Nothing to report

## Research Area 2. Microbial Resilience

Benjamin, Brozel, Butzin, Dhiman, Erlandson Gadhamshetty, Gnimpieba, Gonzalez, Gu, Lushbough, Subramanian

### *Progress relative to intellectual merit*

- Research Proposals submitted - 18
- Awards – 3 (excluding seed grants)
  - 1 related to SDBEST (REEU)
- Awards to be leveraged – 2
  - REU and REEU
- Publications, Presentations - 15
- Students trained
  - 10 graduate
  - 6 undergraduate
- Other

### *Plans for future activities*

- Research Proposals submitted
  - 6 planned for next year
- Publications, Presentations
  - 8 in progress
- Collaborations
  - For imaging (2)
  - For simulation/machine learning (2)
  - For expansion to other plants (3)
- Other

# T1 Seed Grant Overview

- Support high risk research leading to submission of research proposals to federal agencies or industrial partners.
  - Provides an opportunity for researchers not currently supported by Track 1 funding to become involved in Track 1 research.
- 
- ✓ 1 year, \$50,000: graduate and undergraduate students, supplies, other activities directly related to research and IDC
  - ✓ YR1 awardees: CY. Jiang, N. Klein, S. Ragi, and S. Roy,
  - ✓ YR2 awardees: P. Diwakar, M. Geza, S. Janaswamy, and A. Maji
  - ✓ **YR3 RFP ~ June 2021**



## Development of Porous Concave Gold Nanoparticles for the SERS Detection of Functional Biofilms

- Goal/Objectives
  - Synthesize concave gold nanomaterials as novel SERS substrates
  - Optimize the adsorption to facilitate the biomaterial SERS detection
- Activities/Aims
  - Synthesize **gold nanoparticles** with concaved surface and porous structures for superior SERS
  - Functionalized the surface with designed ligand for strong **adsorption** of biomaterials.
  - Use SERS to monitor the **biomaterials growth** on surface of dental materials.

### Chaoyang (CY) Jiang

Associate Professor, Chemistry, USD

Post Doc – George Tech 2006-2007

Post Doc – Iowa State Univ. 2003-2006

Post Doc – Mainz Univ. 2000-2003

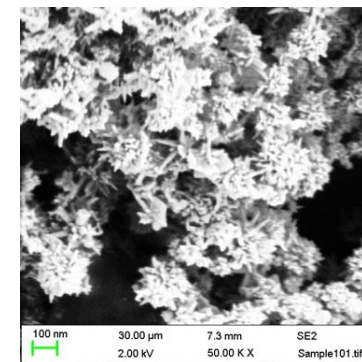
PhD – Chemistry, Nanjing Univ. 2000

BS – Chemistry, Nanjing Univ. 1996



## Results, Outcomes & Accomplishments

1. We have synthesized star-shape gold/silver nanomaterials and examined their SERS activities.
2. We have studied the ligand adsorption on SERS substrates using SERS and zeta size/potential measurements.
3. We have provided gold nanoparticles to Dr. Sereda to working on SERS active microfluidic channel devices.



# Development of Porous Concave Gold Nanoparticles for the SERS Detection of Functional Biofilms, Dr. Chaoyang Jiang



## *Progress relative to intellectual merit*

- A NASA EPSCoR Major Research Grant pre-proposal was submitted for the state-selection (\$750,000, Dr. Jiang is a co-PI).
- The graduate student, Yifeng Huo, received Graduate Research and Creative Scholarship grant from the USD Graduate School. (\$750)
- We are working on one review paper and one journal article. These manuscripts will be submitted soon.
- One PhD student in Materials Chemistry was participated in this project.
- A collaboration with Dr. Sereda was strengthened and we are working on the project of using SERS detection in microfluidic channels for biomaterial monitoring.

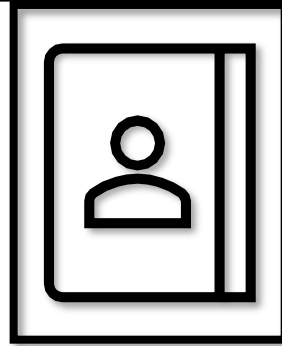
## *Plans for future activities*

- Write a research Proposals on molecular design of stable core-shell plasmonic nanostructures for ultrasensitive SERS study and submit it to NSF
- Submit the two manuscripts for publishing and then work on more manuscripts based the experimental data.
- Present our research results on SERS at ACS national meetings and in an invited talk to local institutes.
- Establish collaborations with others that working on the bio analytical Chemistry and biofilms in the center.
- Secure new research facilities (such as a confocal Raman system) and expand the use the Raman and SERS in bioanalysis
- Training graduate students, especially new users on confocal Raman imaging and spectroscopy

## Characterizing nitrogen fixing biofilms of indigenous food plants *Pedimelum esculentum* (prairie turnip) and *Shepherdia argentea* (buffalo berry)

**Nick Klein**

Faculty, Sinte Gleska University  
PhD – Ocean Science, University of Southern California 2016  
BA – Biology, Augustana University (SD) 2009



- **Goal/Objectives**

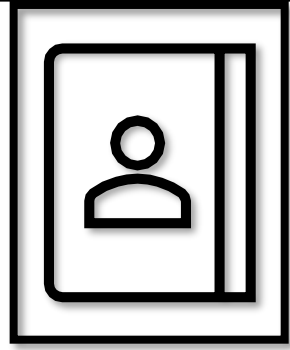
- Characterize microbiomes and transcriptomes of atypical nitrogen-fixing indigenous food plants
- Strengthen research capacity at SGU
- Build collaborative relationship between SGU and SDSU

- **Activities/Aims**

- Sample and sequence plant material
- Recruit students
- PIs and students reciprocal visits to partner campuses

## Results, Outcomes & Accomplishments

1. Stereoscope purchased for tissue imaging (SGU)
2. Masters students (Horvath, Lyotte) for ongoing research and diversity work
3. Microbiome and transcriptome analysis delayed on SGU end but forthcoming



## *Wahúta woksápe olé tǐnpsila maštínčá phuté*

Characterizing nitrogen fixing biofilms of indigenous food plants *Pedimelum esculentum* (prairie turnip) and *Shepherdia argentea* (buffalo berry)

PI: Nick Klein (SGU)

Co-PIs: Jose Gonzalez-Hernandez, Sen Subramanian (SDSU)

### *Progress relative to intellectual merit*

- Recruited-  
Graduate students: K. Horvath, D. Iyotte  
Undergraduates: G. Bordeaux, S. White Hat
- Stereoscope delivered

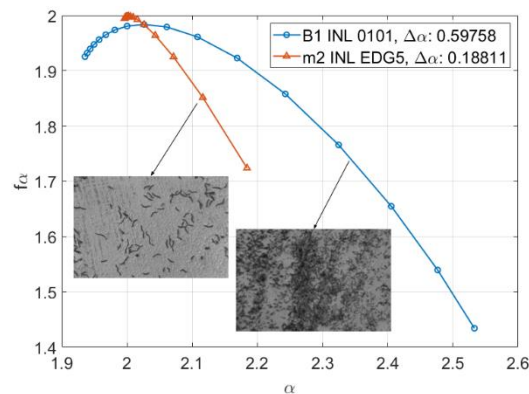
### *Plans for future activities*

- Finish bioinformatics
- Publish results and secure external funding for future research
- Cultivate and sample/sequence other native N-fixing species in 2021
- Sequence plant genomes for *P. esculentum* and *S. argentea*
- Reciprocal site visits



# Exploring Biofilm Rheology via Multifractal and Multiscale Data Analytics

- Goal/Objectives (bullets)
  - Develop a computational approach to estimate the mechanical strength properties (i.e., visco-elastic moduli) of biofilms
  - Calibrate and validate the model's performance against ground truth obtained from the micro-rheology experiments.
- Activities/Aims (bullets)
  - Computational track: develop multi-fractal models to extract fractal properties (multifractal spectra graphs) of biofilms from microscale SEM images
  - Experimental track: conduct SRB biofilm growth experiments and perform micro-rheology experiments to extract the mechanical strength properties of biofilms at various growth stages



Spread of the fractal dimensions increase with the microstructure density

**Shankarachary Ragi**

Assistant Professor, EE, SDSMT

Post Doc – Arizona State Univ, 2016-18

PhD – EE, Colorado State Univ, 2014

BS/MS – EE, IIT Madras, 2009



## Results, Outcomes & Accomplishments

1. Multifractal spectra characteristics (e.g., spread of fractal dimensions) are heavily correlated with the microstructure density present in the biofilm images as confirmed by the results from our box-counting methods we implemented to measure the multifractal dimensions.
2. A frugal setup has been designed and built which can be utilized with an inverted microscope to perform microrheology of *D. alaskensis* G20 biofilm grown on bare copper and multi-layered hexagonal boron nitride coated copper. Two different sizes of fluorescent particles were investigated to increase the Brownian motion in the biofilm. We are growing the biofilm now and will be completing microrheology experiments in the upcoming days.
3. *D. alaskensis* G20 strain exposed to bare copper and multi-layered hexagonal boron nitride coated copper showed differential biofilm microstructures when analyzed through scanning electron microscope

# Exploring Biofilm Rheology via Multifractal and Multiscale Data Analytics, Shankarachary Ragi



*Progress relative to intellectual merit*

- Research Proposals submitted:
  - (PI: Ragi) RII Track-4: AIR-2DML: Artificial Intelligence Research Infrastructure for 2D Material Characterization Laboratory, NSF Track 4 (**declined**)
  - (PI: Ragi) Collaborative Research: Adaptive Waveform Design to Reinforce Radar-Communications Coexistence, NSF ([under review](#))
  - (PI: Ragi) Near real-time wildfire smoke detection and monitoring from satellite imagery using artificial intelligence, SD NASA EPSCoR RIG (**funded**)
  - (PI: Walker) Anaerobic Enhanced Membrane Bioreactor (AEMBR), SDBOR Research & Commercialization Grants (**funded**)
  - (PI: Walker) REU Site: Advancing Voxel-level Engineering (RAVE) at SD Mines, NSF ([under review](#))
  - (PI: Walker) FMRG: GOALI: Nano-Technology In Future Manufacturing for Surfaces/Interfaces with Antimicrobial Functionality through Engineering, NSF (**declined**)
  - (PI: Walker) Microfluidic Device for Growth Observation and Mechanical Characterization of DA-G20 Biofilm, 2DBEST (**declined**)
  - (PI: Walker) Design and optimization of an anaerobic membrane-based water recovery system to mitigate the biofilm interaction and development, NASA ROSES FINESST (**declined**)
- Publications and/or Presentations made
  - H. Rahman, J. Duckworth, S. Ragi, P. Chundi, G. Chilkoor, and V. Gadhamshetty, "Deep Learning Approach to Extract Geometric Features of Bacterial Cells in Biofilms," in The 16th International Conference on Data Science (ICDATA'20), Las Vegas, NV, July 27--30, 2020.
- Students trained
  - Hafizur Rahman, M.S. student, EE, SDSMT
  - Jamison Duckworth, M.S. student, EE, SDSMT
  - Maryam Amouamouha, Ph.D. candidate, CBE, SDSMT

- Awards received
- Publications and/or Presentations made
  - H. Rahman, J. Duckworth, S. Ragi, P. Chundi, G. Chilkoor, and V. Gadhamshetty, "Deep Learning Approach to Extract Geometric Features of Bacterial Cells in Biofilms," in The 16th International Conference on Data Science (ICDATA'20), Las Vegas, NV, July 27--30, 2020.
- Students trained
  - Hafizur Rahman, M.S. student, EE, SDSMT
  - Jamison Duckworth, M.S. student, EE, SDSMT
  - Maryam Amouamouha, Ph.D. candidate, CBE, SDSMT

• Other

*Plans for future activities*

- Research Proposals submitted
  - Using the T1 seed grant results as preliminary evidence, we plan to submit a proposal to NSF's Nanoscale Interaction program by end of Dec 2020.
- Publications, Presentations
  - J. Duckworth, M. Amouamouha, J. Kalimuthu, T. Walker, S. Ragi, "A multifractal approach to estimate mechanical strength properties of biofilms" *In progress*.
  - M. Amouamouha, J.R. Kalimuthu, S. Ragi, T.W. Walker, "Microrheology of Bio-Corrosive Biofilms." *In progress*.
- Collaborations
- Other

# Radio Frequency Transparent Coating Solution for Preventing Microbial Corrosion on Antennas in Harsh Environments

- Goal/Objectives
  - Study the interaction of electromagnetic wave propagation in nanometer thin non-conductive 2D material at GHz ( $10^9$  Hz) frequency spectrum for radiofrequency applications.
  - Evaluate the performance of antimicrobial conformal coating by 2D material(s) on antenna surfaces in harsh environments.
- Activities/Aims
  - Analytical study
  - Measurement verification
  - Corrosion Testing
  - Build in-house RF testing facilities for 2D surfaces

**Sayan Roy**

Assist Prof, EE, SD Mines

Post Doc – University of North Dakota, 2017-18

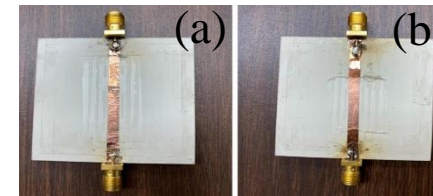
PhD – ECE, North Dakota State University, 2017

MS – ECE, North Dakota State University, 2012

BS – ECE, West Bengal University of Tech, 2010

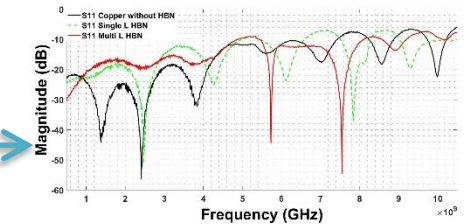


## Results, Outcomes & Accomplishments

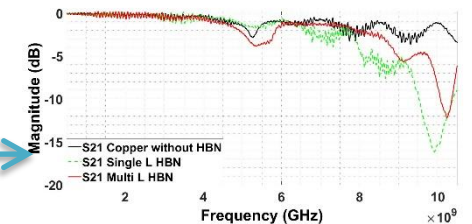


50  $\Omega$  Transmission Line with (a.) 2D hBN coated copper and (b.) blank copper on the 50.8×50.8×1.57 mm<sup>3</sup> Rogers TMM4 ( $\epsilon_r = 4.5$  and  $\tan\delta = 0.002$ ) substrate. Dimension of two identical transmission line: 50×3×0.02 mm<sup>3</sup>. Thickness of 2D hBN on the Transmission Line is 17 nm.

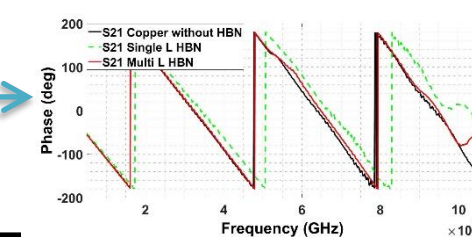
Comparison of the magnitude of return loss in dB from 100 MHz to 10 GHz.



Comparison of the magnitude of forward loss in dB from 100 MHz to 10 GHz.



Comparison of the phase of transmitted signals from 100 MHz to 10 GHz.





# Radio Frequency Transparent Coating Solution for Preventing Microbial Corrosion on Antennas in Harsh Environments, Sayan Roy



## *Progress relative to intellectual merit*

- Research Proposals submitted
  - 4 Proposals (PI: 3, SP: 1)
  - 1 White Paper
  - 1 Accepted NSF EPSCoR Track 2 Pre-proposal
- Awards received
  - South Dakota Board of Regents Competitive Research Grant, \$61.6K
  - South Dakota Bioscience Commercialization Alliance i6, \$11K
- Publications and/or Presentations made
  - 1 Journal paper accepted (Applied Computational Electromagnetics Society Journal)
  - 1 Peer-reviewed Conference (accepted), IEEE URSI NRSM 2021
- Students trained
  - 2 MS Students (Ahsan Aqueeb [EE, Sp 2021], Kazi Kabir [EE, Su 2020])
  - 1 BS Student (Ellie Burczek [Sp 2021])

## *Plans for future activities*

- Research Proposals submitted
  - NSF EPSCoR Track 2: RF Nano Device Center
  - NSF CAREER
- Publications, Presentations
  - 1 Journal (under preparation)
  - Present the accepted paper at IEEE URSI NRSM 2021
- Collaborations
  - Intra-Jurisdiction
  - Inter-Jurisdictions
- Other
  - Search Committee Member, Assistant or Associate Professor-2D Materials



## Plasma Jet Coating for Biofilm Applications

- Goal/Objectives
  - Development of Cold Atmospheric Plasma for 2-D graphene coating
  - Spectroscopic diagnostics of CAP 2-D graphene coating layers
  - Assess the biocorrosion resistance of cold plasma graphene/Cu samples
- Activities/Aims
  - Design of experiments for Parametric optimization of CAP for 2-D graphene coating
  - Recruiting of graduate and undergraduate students
  - PI and student interactions and meetings with 2D-BEST team for resource utilization and development

### Prasoon Diwakar

Assist Prof, ME, SDSMT

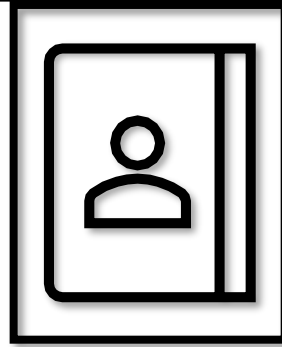
Post Doc – Purdue University, 2012-2018

Post Doc – CDC NIOSH, 2009-2011

PhD – ME, Univ of Florida, 2009

MS – ME, Univ of Florida, 2006

BS – ME, IIT Kanpur, India, 2003



## Results, Outcomes & Accomplishments

1. Meetings with 2D-BEST team accomplished to identify synergistic resource utilizations for success of the project
2. Samples have been procured to initiate the experiments
3. Graduate and undergraduate students have been identified and onboarding is in progress
4. Cold Atmospheric Plasma and Raman spectroscopy experiments on graphene samples have been designed and initiated

# Plasma Jet Coating for Biofilm Applications, Prasoon Diwakar

## *Progress relative to intellectual merit*

- Graduate Student (Kamtung Chen) have been identified and onboarding process has been started
- Undergraduate students have been identified and onboarding process will start soon
- Review of Raman Spectroscopy and LIBS spectroscopy on 2D graphene coating is currently being carried

## *Plans for future activities*

- Parametric optimization of CAP for 2-D graphene coating
- Comparison of Acetylene precursor gas vs aerosolized graphene flakes for 2D graphene layer
- Biocorrosion studies and assessment
- Submission of abstracts to conferences SCIX 2021 in Rhode Island (Oct 2021), Pittcon 2021 in New Orleans (March 2021), APS Plasma meeting in Pittsburgh (Nov 2021), SDSMT student symposium (April 2021), SD EPSCoR undergraduate symposium (Summer 2021).
- Submission of proposals to DOD (Apr 2021) and NSF (May 2021)
- Publish the results from these studies in high impact journals

# Quantifying biofilm growth impacts on performance of wastewater infiltration systems modified with 2D materials

**Stu Geza**

Assistant Professor, CEE, SDSMT



- Goal/Objectives

- Study molecular mechanisms of bioclogging
- Correlate bioclogging, soil hydraulic properties and wastewater infiltration rates using metagenomic analysis, infiltration experiments & modeling.

- Activities/Aims

- Develop a conceptual model describing change in water flux due to bioclogging
- Conduct infiltration experiments
- Metagenomic analysis
- Pore s and transport scale modeling

## Results, Outcomes & Accomplishments

1. Literature review
2. Preparing proposals on:
  - Seed grant topic area
  - Fate and transport of 2D nanomaterials

# Quantifying biofilm growth impacts on performance of wastewater infiltration systems modified with 2D materials, *Stu Geza*



## *Progress relative to intellectual merit*

- Research Proposals submitted: Planning to submit
- Awards received: None
- Publications and/or Presentations made: None
- Students trained: None
- Other

## *Plans for future activities*

- Research Proposals
- 2 planned proposal in 2020/2021
  - Bio clogging in natural and 2D materials modified infiltration systems
  - 2D materials fate and transport in the environment

## Publications, Presentations

- 1 Peer Reviewed publication from seed grant research
- Additional publications if funded
- Collaborations
  - SDSMT CEE, SDSMT NANO, CEE NDSU
- Other



# Synthetic Nodules using Polysaccharide Beads for Sustainable Plant Nitrogen Nutrition

- Goal/Objectives
  - Evaluate polysaccharide beads in the presence of various divalent and trivalent cations to produce beads with desirable properties as synthetic nodules.
  - Evaluate the impact of energy source and biofilm substrate on the rate and amount of nitrogen fixed.

## Srinivas Janaswamy

Assist Prof, Carbohydrate Chemistry, SDSU

Post Doc – Purdue University, 1999

PhD – Structural chemistry, 1997

MS – Physics, 1989

BS – Physics, 1985



## Results, Outcomes & Accomplishments

- Preparing and characterizing beads in the presence of divalent and trivalent cations.

# Synthetic Nodules using Polysaccharide Beads for Sustainable Plant Nitrogen Nutrition, Srinivas Janaswamy



## *Progress relative to intellectual merit*

- One proposal entitled “Synthetic nodules for nitrogen nutrition in cereal crops” was submitted to 2021 South Dakota Nutrient Research and Education Council

## *Plans for future activities*

- Evaluate the amount nitrogen fixed by each bead
- Application of beads to corn fields
- Scouting for funds from NIFA-USDA & NSF

# Role of priority effect on gut microbiota assembly on gut mucosal interface

## Goal/Objectives:

- Determine how mucin adherent bacteria alter microbiome assembly.
- Determine the changes in the transcriptional signaling in mucin in mucin adherent bacteria when assembled as a single species and multi-species biofilm.

## Aims/Activities:

- Screened a Self-developed gut culture library for the mucosal adherence.
- Validated the selected adherent gut microbial species by Confocal and SE Microscopy.
- Studied the adherence dynamics by confocal microscopy and gut like model

**Abhijit Maji**

Post doctoral fellow, SDSU,  
Brookings 2018

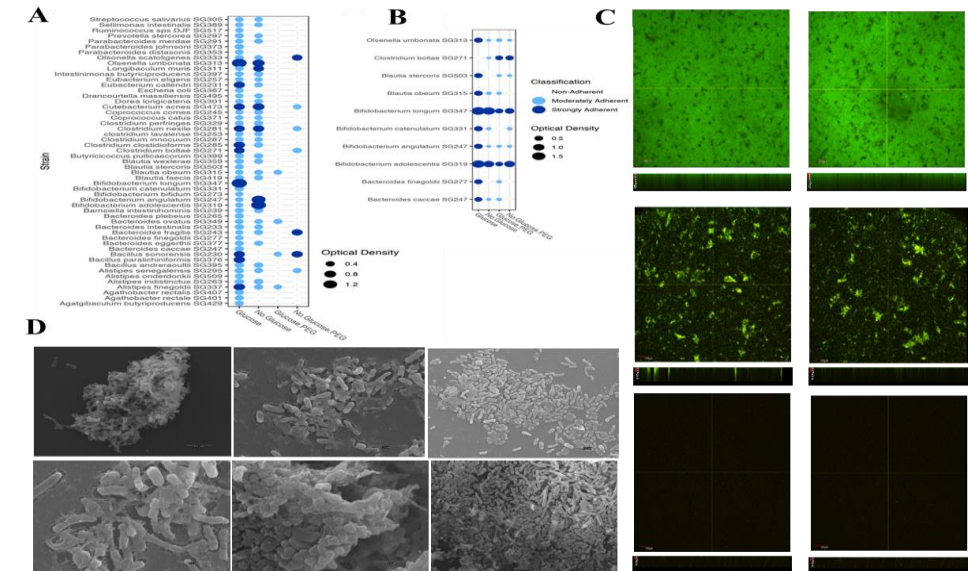
**Ph.D.** in Biotechnology, 2016

**M.Sc** in Microbiology, 2009

**B.Sc** in Microbiology, 2006



Results/Outcomes: Biofilm screening of gut microbial culture library



# Role of priority effect on gut microbiota assembly on gut mucosal interface, Abhijit Maji



## *Progress relative to intellectual merit*

- Research Proposals submitted: Yes
- Awards received: Yes
- Publications : We anticipate to publish at least two peer-reviewed publications based on the proposed experiments in this project.
- Students trained: One
- Other

## *Plans for future activities*

- Research Proposals submitted
- Publications, Presentations
- Collaborations
- Other



# Questions???

## Please join a breakout session this afternoon.