Bio Circuit Nano Ninjas

Enhancing BioCircuits

Group 6:
Jacob Bautista, Kendra Krueger,
Alyanna Pilapil, Jesus Quiroz,
Zaden Yet, Marius Held

Abstract

- Address unsustainable power sources using microbial fuel cells
- Utilize Geopilin domain 1 Protein - pilA-N
- Improve conductance of electrons

Fig. 1: PilA-N from Geopilin domain

Our proposal aims to provide a more efficient, accessible, and scalable solution to address increasing global energy demand, resource scarcity, and toxic waste

Background & Significance

- **Issue: Rising global energy demand & depletion of rare earth minerals**
  - Solar panels and hydroelectric power
  - Increase of 0.2% in carbon emissions

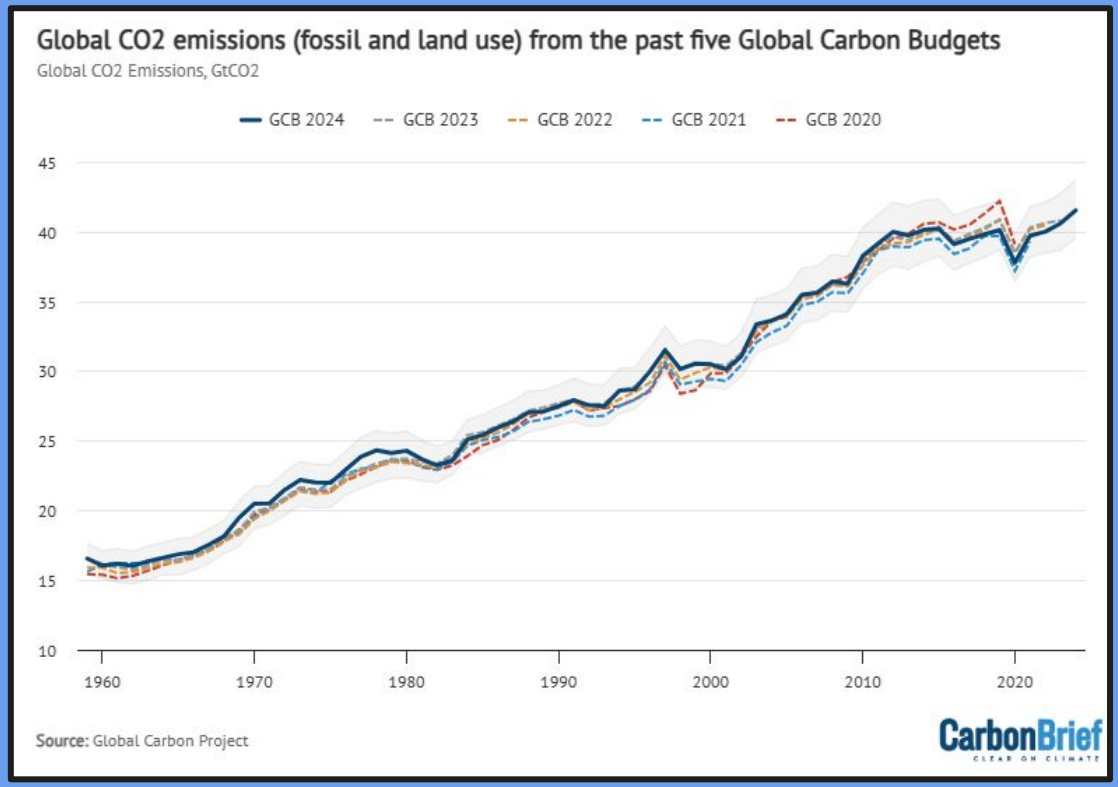


Fig. 2: Annual total global CO2 emissions

Background & Significance

- **Proposed solution: microbial bioelectrochemical systems (BES)**
  - Utilizing bacteria to generate electricity through extracellular e- transport (EET)

- **However...**
  - Limited conductivity of bacterial nanowires → suboptimal aromatic π-stacking

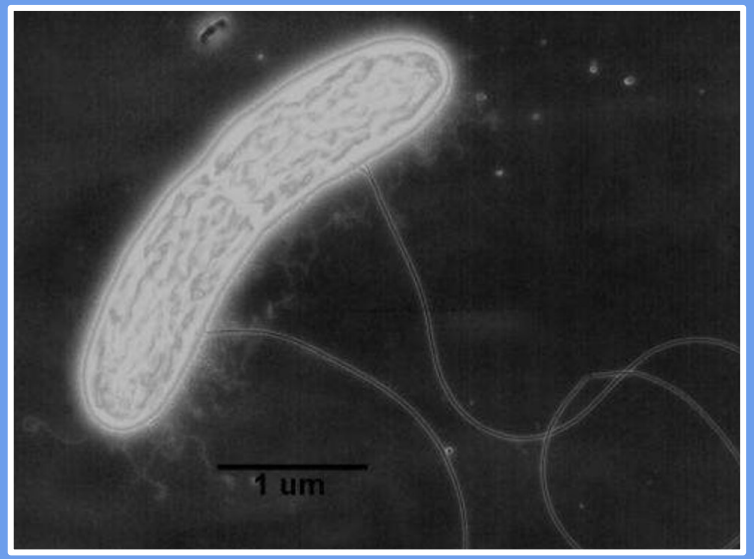


Fig. 3: Nanowires of Geobacter sulfurreducens

Background & Significance

- **Goal: engineer the Geopilin domain 1 protein (pilA-N) → key structural component of bacterial pili**
  - Enhance its conductivity

- **Introducing <u>aromatic residues</u>:**
  - Improve π-stacking networks
  - Reduce spatial constraints
  - Optimize hydrogen bonding interactions

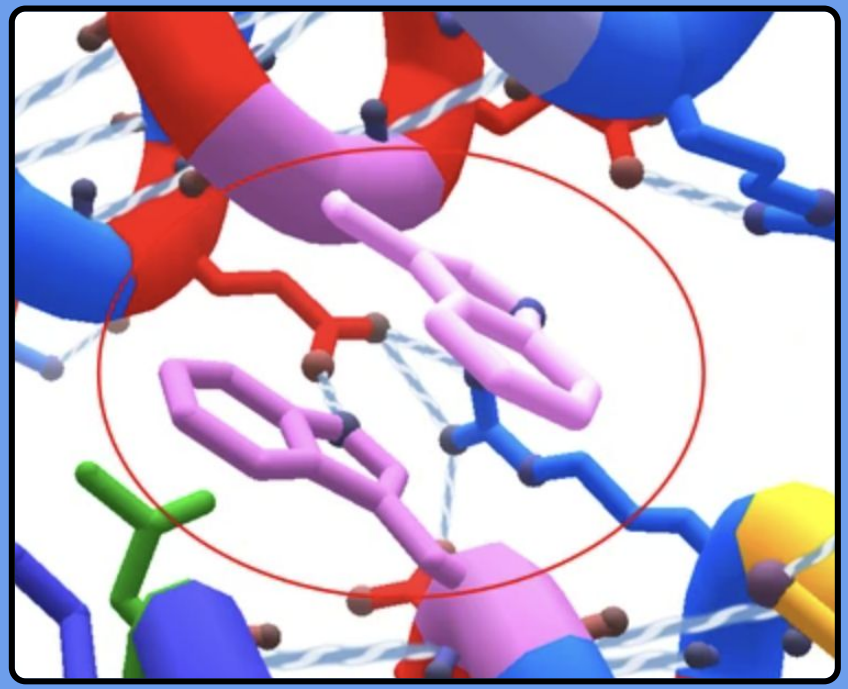


Fig. 4: π stacking between two tryptophan residues

Background & Significance

- **Critical roles of OmcS and OmcZ cytochromes c genes in e- transfer**
  - Heme cofactors for redox reactions of Fe(III) oxide → direct e- transfer to electrodes
  - Improving conductivity
- **Complementary role to PilA-N**
  - Synergize w/ native conductive components → enhanced efficiency
- **Note: deletion of cytochrome c genes vs. PilA-N**

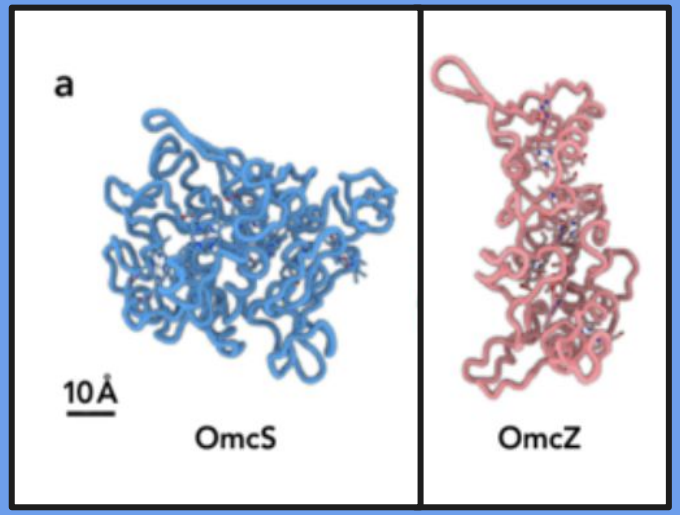


Fig. 5: Protein fold of OmcS and OmcZ

Main Goal

Objective: To engineer the Geopilin domain 1 protein, pilA-N, to enhance its electron conductance

- Introduce aromatic amino acids to key sites through combination of comparative modeling and rational design
- Employ directed evolution to further fine-tune the protein's conductive properties
  - Explore a wide mutational landscape and help identify beneficial mutations

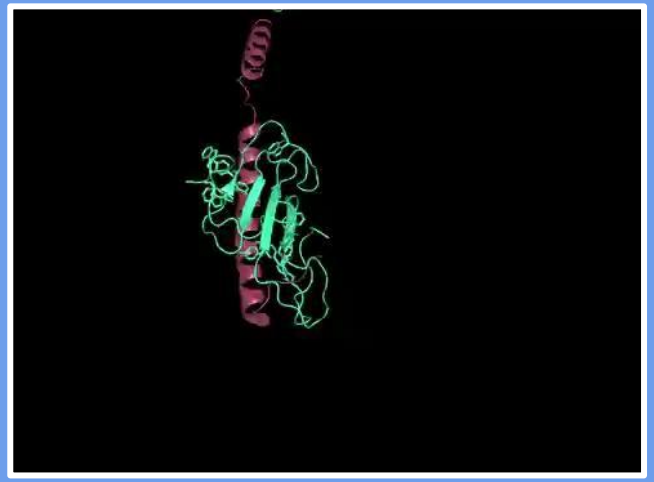


Fig. 6: One pilA-N domain showcasing native aromatic side chains, Pymol

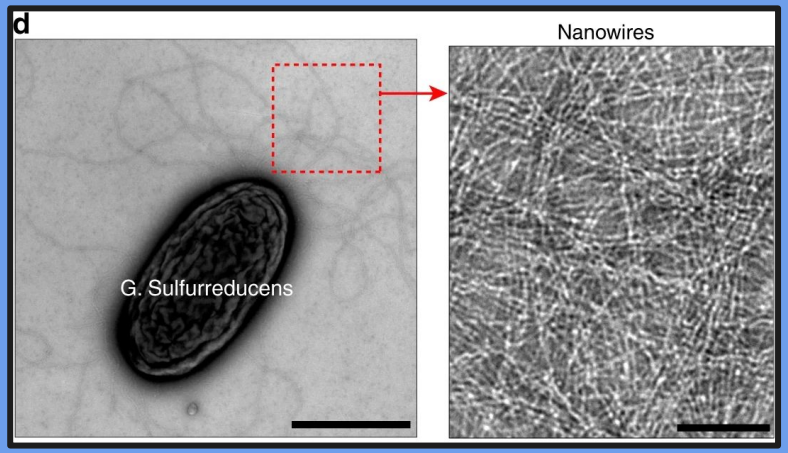


Fig. 7: Geobacter sulfurreducens and its bacterial nanowires. Tianda Fu, Xiaomeng Liu, Hongyan Gao,

Why pila-N?

pilA-N is a subunit protein of the pilus structure in Geobacter sulfurreducens

- part of the Geopilin domain 1 family
- refers to the N-terminal domain of the PilA protein.

Properties of pilA-N

- Superior native conductance, structural stability, and well-characterized filament structure
- Forms a stable heterodimer with pilA-C into filaments
- More extensive research through Cryo-EM

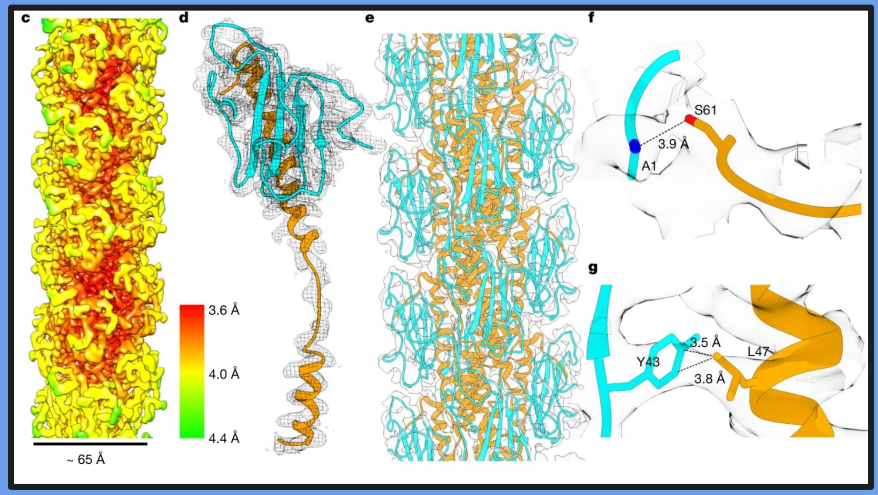


Fig. 8: a–c, Cryo-EM micrograph (a), two-dimensional average(b). d, e, PilA-N (orange) and PilA-C (cyan) form a heterodimer (e). f, Charge interactions between S61 of PilA-N and A1 of PilA-C. g, Hydrophobic interactions between PilA-N and PilA-C.

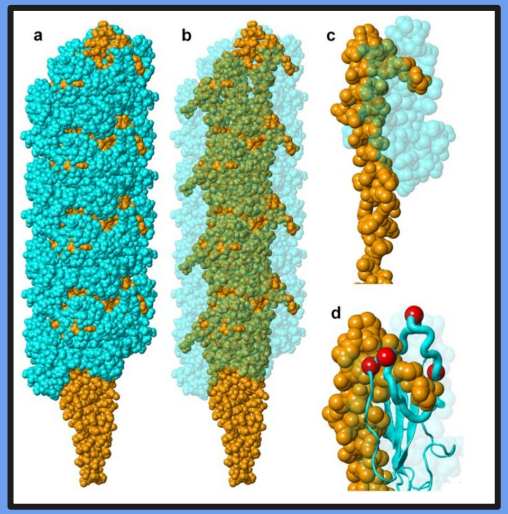


Fig. 9: a Pilus model showcasing contacts between PilA-N (orange) and PilA-C (cyan)

Experimental Approach

Outline:

- **Comparative Design**
- **Rational Design**
- **Direct Evolution**
  - Knock In of Variant PilA-N into wild type G. sulfurreducens PCA
- **Screening**
  - Ferrozine Assay
- **Iterate through Direct Evolution**
- **Protein Purification**

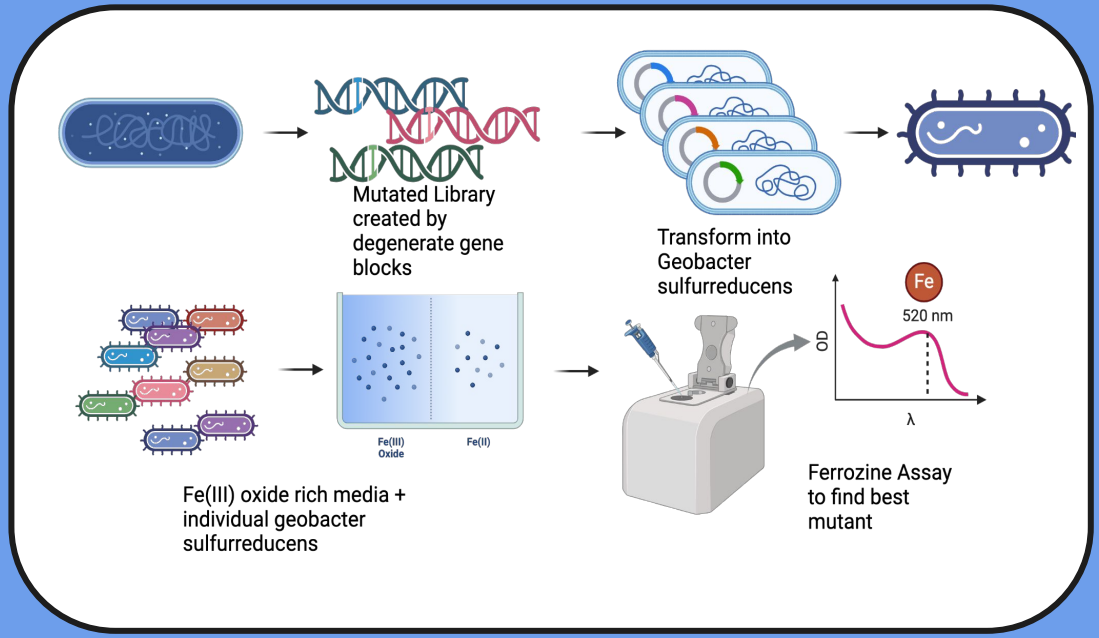


Fig. 10: Experimental Design made through BioRender
Create mutant strain - create library - introduce variant pilA-N into mutant - test with Fe(III) oxide rich medium - measure Fe(II) using Ferrozine Assay

Experimental Approach

Comparative Design (Fig. 1)

- UniProt MSA Accession ID: Q74D23
- Geopilin domain 1 protein with gene name PilA-N

11 targeted residues

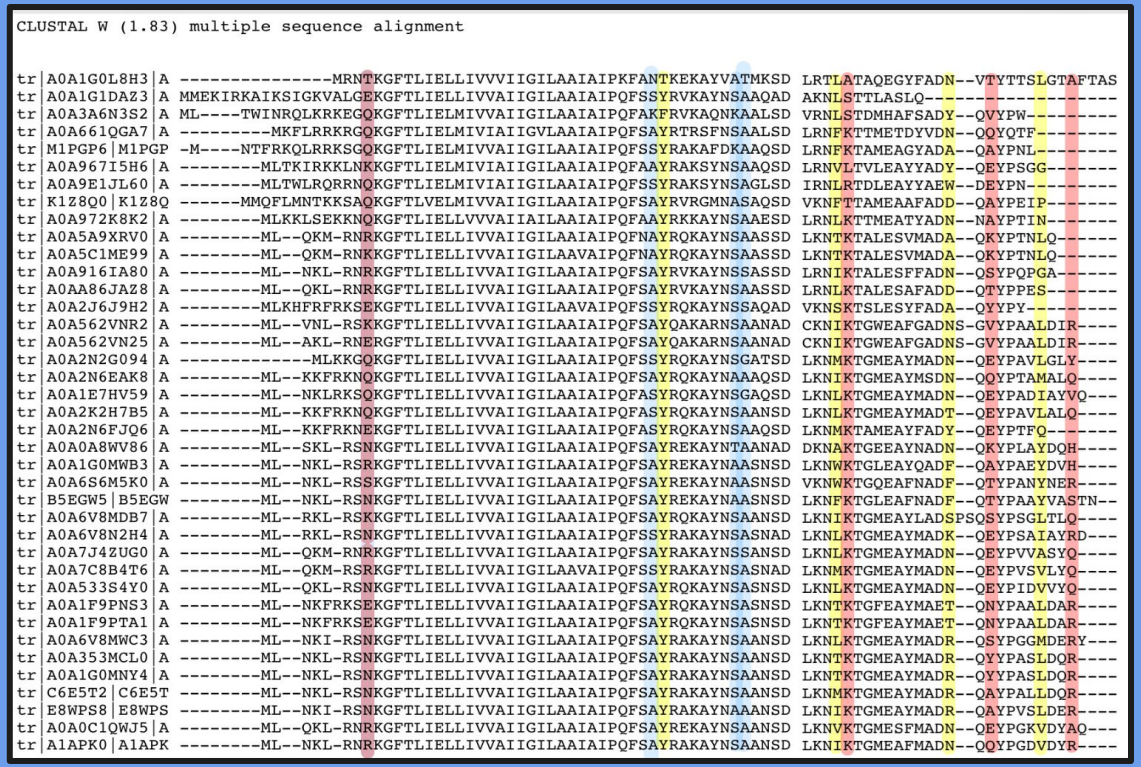


Fig. 11: Residues for further investigation under altered state. Made by UniProt MSA
Aromatic residues (Y): Position 33 (T to Y), 48 (L to F), 60(N to Y), 67 (L to Y). Are in the transmembrane domain.
Charged residues (R): Position 4 (T to), 50 (A to), 61 (T to) 69 (A to)
Hydrophobic residues (B): Position 4, 14. Are in the transmembrane domain. Position 32, 41 not in transmembrane domain A) 1st half of compared Pilus assembly protein sequence B) 2nd half of compared Pilus assembly protein sequence

Experimental Approach

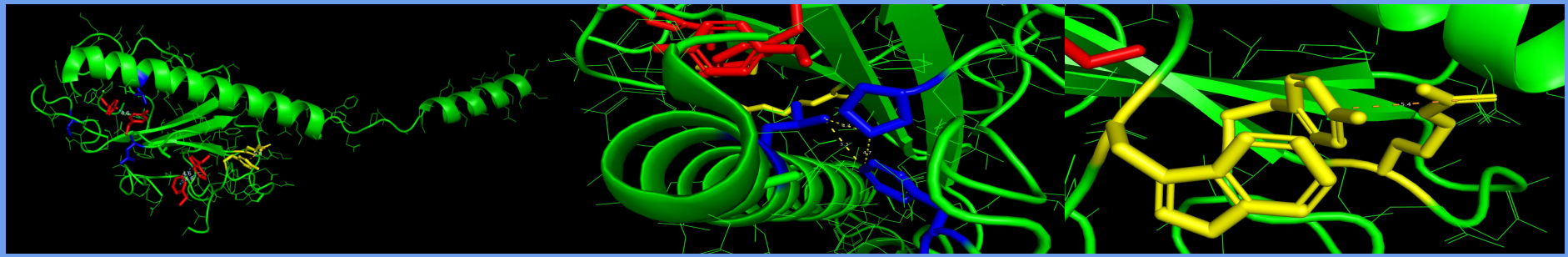
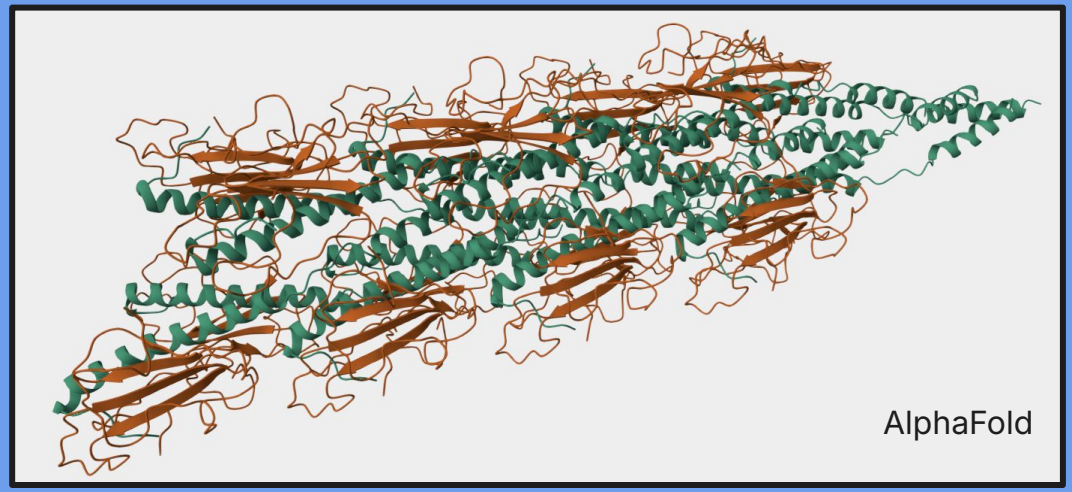


Fig. 12: PilA-N from Geopilin domain|monomer|PDB: 7TGG made with PyMol. Red = pi-pi interactions, Yellow = pi-cation interaction which can be altered for pi-pi interactions Blue = target residues for aromatic alteration

Rational Design (Fig. 2)

- PDB: 7TGG
- Want more Pi-Pi stacking
- Consider: whole pilA-N protein in an oligomer



Experimental Approach

Directed Evolution

- **Library Construction - NNK gene blocks / Oligonucleotide mutagenesis**
  - Synthetic gene blocks/primers containing degeneracy at targeted site
  - 11 targeted residues = 20×11 = 220 library size
  - Gibson Cloning Fragment into suicide vector pK18mobsacB

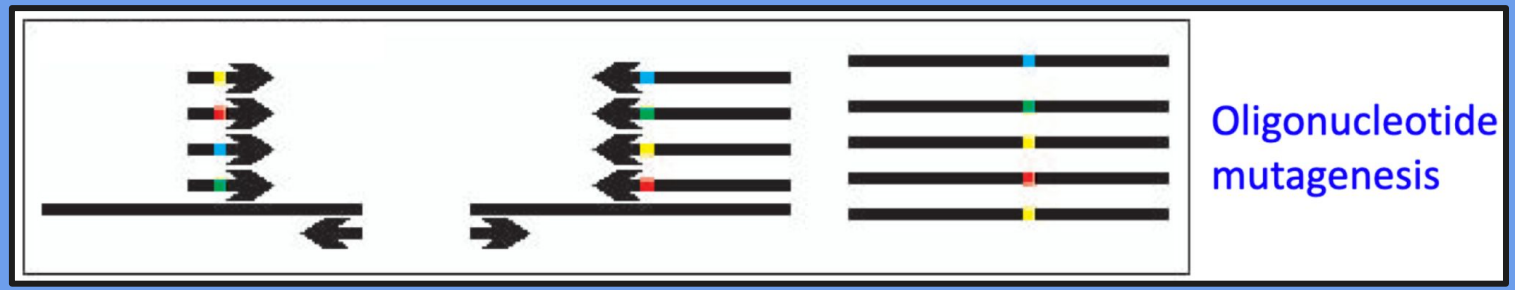


Fig. 13: Example of oligonucleotide mutagenesis taken from slide 47 from Andy Yeh presentation 4

G. Sulfurreducens as a Protein Expression System

Use Geobacter to express PilA-N given

- **6 to 22 hours doubling time**
- **Conserve native expression & translocation machinery**
- **Well established synthetic biology methods for EET studies**
  - Suicide vectors for homologous recombination
  - Ferrozine assay
  - Protein Purification

Experimental Approach- Genomic Knockout WT & Knock-in Mutants

Why knock in alterations into G. sulfurreducens genome?

- **Allows for conductance screening assay without relying on other expression systems**
  - Eliminate native PilA-N
  - Express Altered form

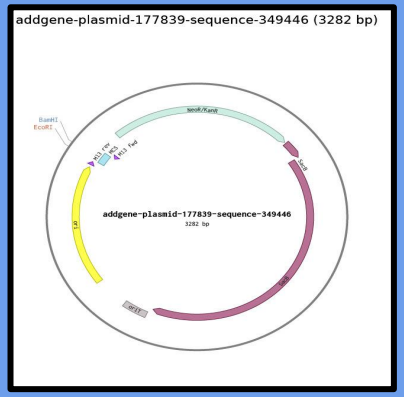


Fig. 15: pK18mobsacB Plasmid Backbone generated from benchling and sourced from addgene

Details:

- **Using suicide vector(pK18mobsacB)**
- **Ordered sequence contains**
  - Homology arms, locus tag(GSU1496), mutant sequence, regulatory regions, restriction sites
- **Use electroporation for transformation**

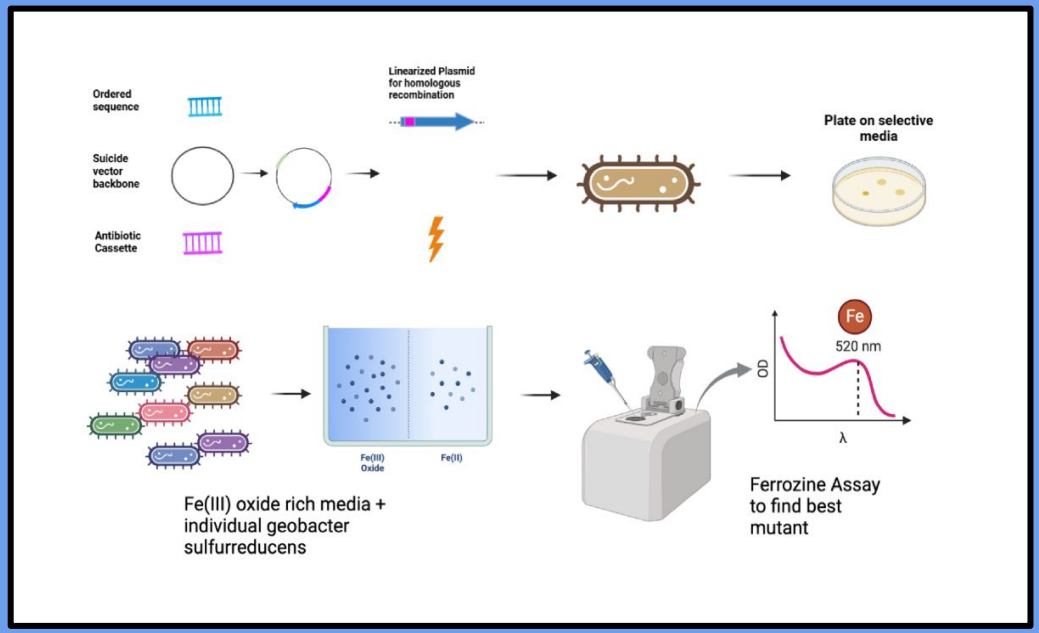
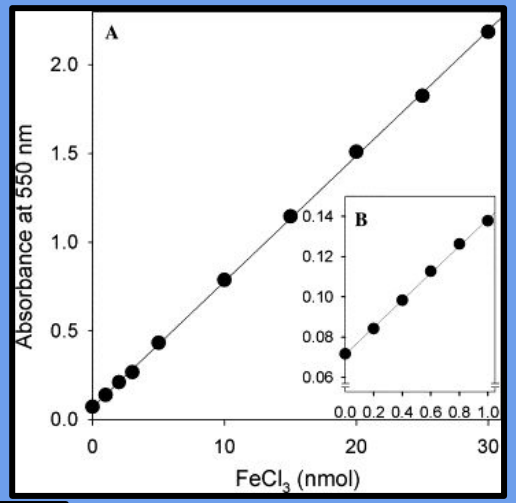


Fig. 14: Experimental Design & workflow made with BioRender
Create mutant strain -> create library -> introduce variant pilA-N into mutant -> test with Fe(III) oxide rich medium -> measure Fe(II) using Ferrozine Assay

Experimental Approach

Screening Library using Ferrozine Assay

- G. Sulfurceddens reductase ability and extracellular electron transport
- Measuring Fe(II) production
- Growing lactase, hydrogen, and Fe(III) oxide-rich media
- Dye complex formed with Fe(II) measured at 550 nm



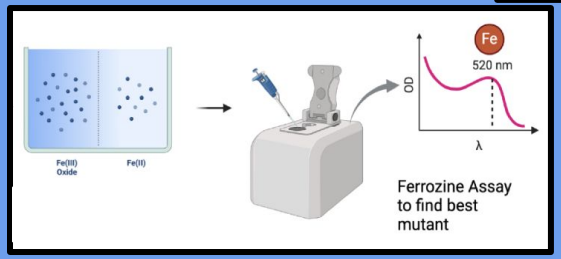


Fig. 16. Absorbance of the Fe2+–ferrozine complex formed with increasing concentration of the standard FeCl3. The amount of iron indicated on the abscissa was contained in a volume of 280 µL in wells of a microtiter plate. The data represent means ± SD of triplicates of a representative experiment. The SDs are smaller than the symbols representing the mean values. The increase in absorbance was linear between 1 and 30 nmol (A) and between 0.2 and 1 nmol (B) of FeCl3. For both concentration ranges the correlation coefficients were 0.999.

Experimental Approach - Protein Purification

- **Mutants plated on graphite electrodes**
  - Electron acceptors
  - Mimics environment where bacteria naturally EET
- **Shear cells**
- **Several wash steps**
  - Overnight 10% ammonium sulfate
- **Verify using Western Blot and SDS Page**
  - ~6.5 kDa

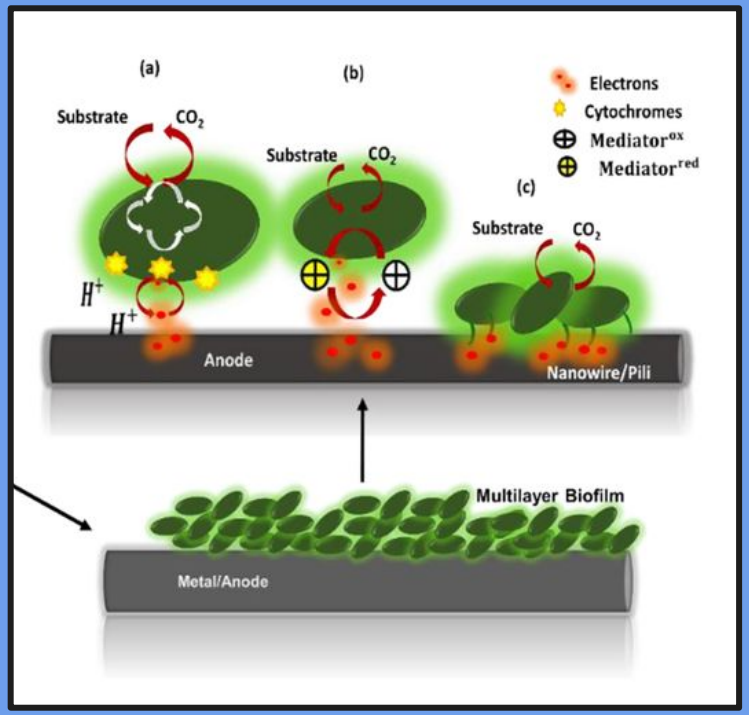


Fig. 17- External Electron Transfer using graphite electrode. Manisha Verma, Vishal Singh, Vishal Mishra

Anticipated hurdles & alternatives

Because of Oligomer state, proposed protein purification alternative:

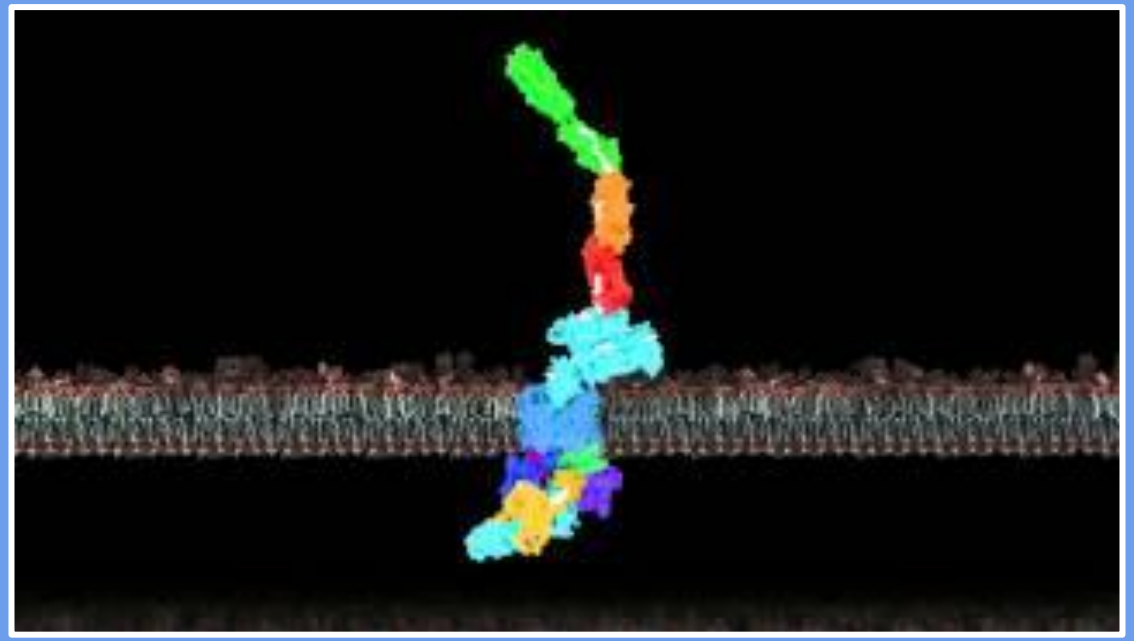


Fig. 18 Gabriel Waksman, Pilus biogenesis at the outer membrane of Gram-negative bacteria, https://youtu.be/WQ_g1z8rJY0?t=35

Anticipated hurdles

Hurdles for Protein Purification:

- **Mechanical shearing or physical disruption of bacterial cells**
- **The use of ammonium sulfate precipitation might not fully separate pilA-N**
    - Less pure sample
        - Acquire an unclear SDS-PAGE and Western blot

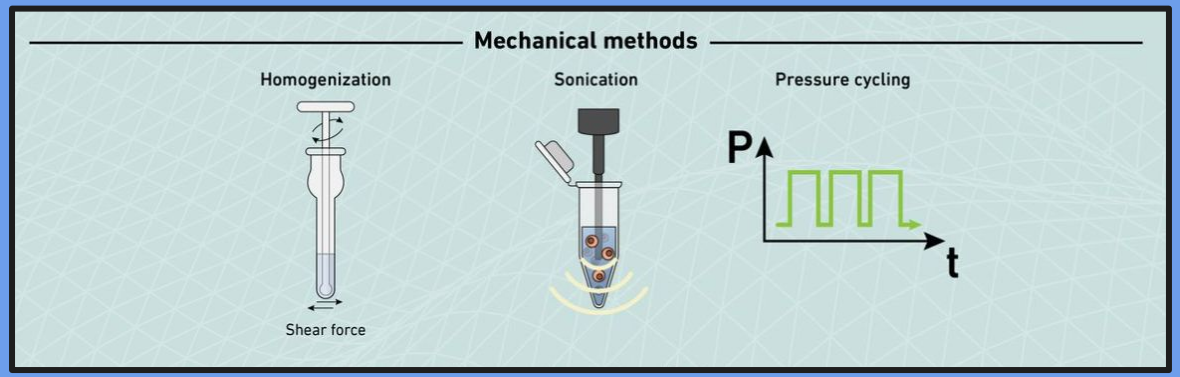


Fig. 19: Examples of mechanical methods to lyse the bacterial cells.
Héctor Zamora Carreras, PhD

Alternatives/ possible solutions

- Incorporate a Glycine-Serine (GS) linker sequence between the pilA-N protein and 6x His tags
- Further isolate the pilA-N protein using ion exchange chromatography by choosing an ion-exchange resin based on the isoelectric point (pI) of pilA-N
  - Use ExPASy Compute pI/Mw tool

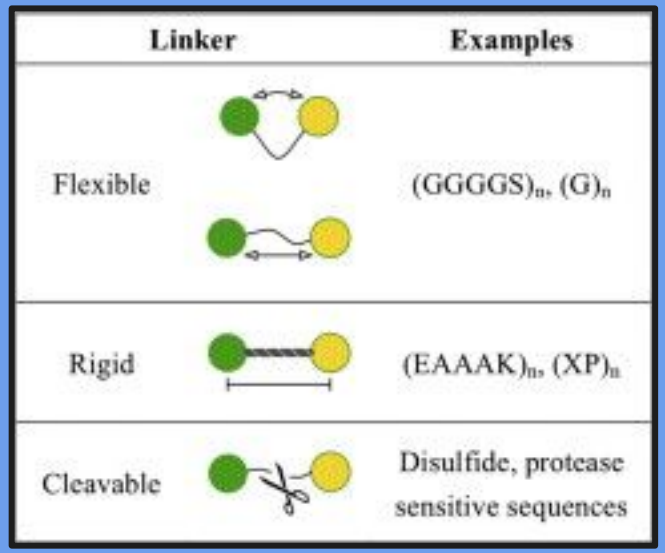


Fig. 20: Different fusion protein linkers. Xiaoying Chen. atl

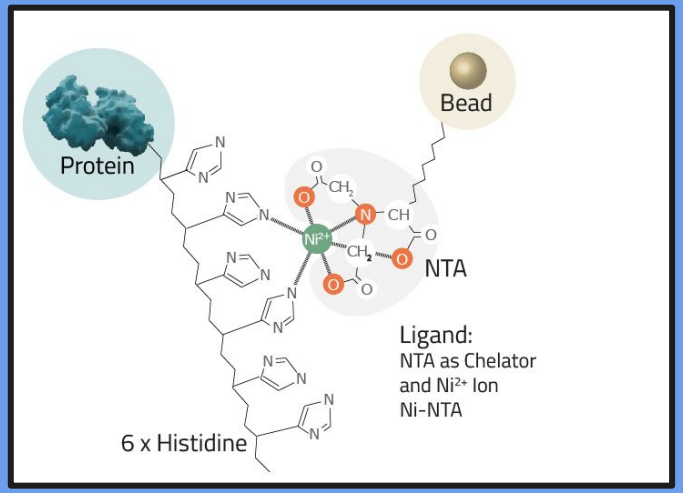


Fig. 21: 6x His tags. Cube Biotech site.

Citations

- [1] Biological wires carry electricity thanks to special amino acids. (n.d.-a). https://phys.org/news/2013-03-biological-wires-electricity-special-amino.html
- [2] Wiki, C. to F. (n.d.). Pi stacking. Foldit Wiki. https://foldit.fandom.com/wiki/Pi_Stacking
- [3] OMCZ differs from OmcS/OmcE in protein fold, heme ... (n.d.-b). https://www.researchgate.net/figure/OmcZ-differs-from-OmcS-OmcE-in-protein-fold-heme-arrangements-and-coordination-a-The_fig1_363315423
- [4] Tianda Fu, Xiaomeng Liu, Hongyan Gao, Joy E. Ward, Xiaorong Liu, Bing Yin, Zhongrui Wang, Ye Zhuo, David J. F. Walker, J. Joshua Yang, Jianhan Chen, Derek R. Lovley & Jun Yao - https://www.nature.com/articles/s41467-020-15759-y
- [5]Gu Y, Srikanth V, Salazar-Morales AI, Jain R, O'Brien JP, Yi SM, Soni RK, Samatey FA, Yalcin SE, Malvankar NS. Structure of Geobacter pili reveals secretory rather than nanowire behaviour. Nature. 2021 Sep;597(7876):430-434. doi: 10.1038/s41586-021-03857-w. Epub 2021 Sep 1. PMID: 34471289; PMCID: PMC9127704.
- [6]Carreras, Héctor Zamora, “An Introduction to Protein Purification: Methods, Technologies and Applications” https://www.technologynetworks.com/analysis/articles/an-introduction-to-protein-purification-methods-technologies-and-applications-388443
- [7] Xiaoying Chen, Jennica L. Zaro, Wei-Chiang Shen,Fusion protein linkers: Property, design and functionality,Advanced Drug Delivery Reviews,Volume 65, Issue 10, 2013, Pages 1357-1369,ISSN 0169-409X, https://doi.org/10.1016/j.addr.2012.09.039.
- [8] (1)Cube Biotech. His-Tag | Definition & Data. Cube Biotech. https://cube-biotech.com/our-science/protein-purification/his-tag/.


Thank
You