Contributions to Science:

Contribution 1: Discovery of a new type of carbon pump that drives CO_2 concentration in bacteria:

1.1: Historical background Rubisco is the enzyme responsible for fixing the vast majority of all the CO_2 that enters the biosphere each year and is essential for all plants, algae, and most autotrophic bacteria. However, Rubisco evolved before the great oxygenation event and is competitively inhibited by the presence of O_2. This is a problem that all autotrophs that use rubisco must overcome to survive in the the modern atmosphere, with its 20% O_2 and only 0.04% CO_2. Many autotrophic bacteria including a wide range of chemolithoautotrophs and most cyanobacteria overcome this issue using an α-carboxysome based CO_2 concentrating mechanism (CCM). These CCMs work by creating a compartment inside the bacteria where the concentration of CO_2 is raised high enough to saturate rubisco with CO_2 and out-compete O_2. While our theoretical understanding and previous experimental work suggested that an inorganic carbon (C_i) transporter was absolutely required for the functioning of the CCM, no such transporter was known in the model chemolithoautotroph *H. neapolitanus*.

1.2: Central finding I performed a genome-wide genetic screen for CCM components in *H. neapolitanus*. I identified the essential components of the CCM in *H. neapolitanus*. I cloned putative C_i transporters, called DABs, then confirmed their activity with reporter strain and C_i uptake assays in *E. coli*. I showed that data was consistent with these transporters acting not as direct transporters but as a new family of energy coupled carbonic anhydrase (CA) enzymes. These CAs concentrate C_i by converting membrane permeable CO_2 into membrane impermeable HCO_3^- causing a net flow of C_i into the cell. I further showed that similar operons in the human pathogens *V. Cholera* and *B. anthracis* had the same function.

1.3: Influence/Application This work was instrumental in ongoing study of the CCM including a successful effort to reconstitute a functional α-carboxysome CCM in *E. coli*, and work on CCM evolution that I will discuss in contribution 2. Since this work was published, it was shown that a similar operon in *S. aureus* has the same function and is essential for growth in atmospheric CO_2 concentrations. Considering the activity of energy coupled CA transporters has been shown to be necessary to understand the carbon isotope fractionation data in very old rock strata. There has been interest in investigating the potential applications of DABs in engineering crop plants and autotrophic bio-fuel production hosts for increased yield.

1.4: My role I was the primary author on this work. I conceived and designed the experiments, performed the genetic screen, analyzed the sequencing data, did cloning, performed the biochemistry experiments, and performed the reporter strain experiments.

John J Desmarais, ... et al. (Dec. 2019). "DABs are inorganic carbon pumps found throughout prokaryotic phyla". en. In: *Nat Microbiol* 4.12, pp. 2204–2215. ISSN: 2058-5276. DOI: 10.1038/s41564-019-0520-8. URL: http://dx.doi.org/10.1038/s41564-019-0520-8.

Contribution 2: Characterization of potential evolutionary paths for developing carbon dioxide concentrating mechanisms:

2.1: Historical background The α-carboxysome based CO_2 concentrating mechanism (CCM) required several major evolutionary steps to evolve. These were acquiring CA activity, gaining C_i transport, and encapsulating CA and rubisco in an α-carboxysome. However, all of these components are need for the effect of the CCM and removing even one of these components is lethal in modern autotrophs. Since there is no apparent fitness benefit for a partial system, it is not clear how the system could have evolved. Geochemical evidence suggests that the atmosphere was very different when the CCM first evolved, with much higher levels of CO_2 and much lower levels of O_2 and a mixture of biological and geochemical processes has slowly changed the atmospheric composition to the current mixture. This information lead us to the hypothesis that evolutionary intermediates of the CCM may have provided fitness benefits at intermediate atmospheric compositions.

2.2: Central finding

2.3: Influence/Application

2.4: My role

Avi I Flamholz, ..., John J Desmarais, ... et al. (2022). "Trajectories for the evolution of bacterial CO_2-concentrating mechanisms". In: *Proceedings of the National Academy of Sciences* 119.49,

e2210539119. DOI: 10.1073/pnas.2210539119. eprint: https://www.pnas.org/doi/pdf/10.1073/pnas.2210539119. URL: https://www.pnas.org/doi/abs/10.1073/pnas.2210539119.

Contribution 3: Development of nuclease chain reaction signal amplification for viral diagnostics:
3.1: Historical background
3.2: Central finding
3.3: Influence/Application
3.4: My role

Tina Y Liu, ..., John J Desmarais, ... et al. (Aug. 2021). "Accelerated RNA detection using tandem CRISPR nucleases". en. In: *Nat. Chem. Biol.*, pp. 1–7. ISSN: 1552-4450. DOI: 10.1101/2021.03.19.21253328. URL: https://www.nature.com/articles/s41589-021-00842-2.

Contribution 4: Application of general epistasis techniques to protein design:
4.1: Historical background
4.2: Central finding
4.3: Influence/Application
4.4: My role
Contribution 5: Discovery of CasX:
5.1: Historical background
5.2: Central finding
5.3: Influence/Application
5.4: My role

Jun-Jie Liu, ..., John Desmarais, ... et al. (Feb. 2019). "CasX enzymes comprise a distinct family of RNA-guided genome editors". en. In: *Nature*, p. 1. ISSN: 0028-0836. DOI: 10.1038/s41586-019-0908-x. URL: https://www.nature.com/articles/s41586-019-0908-x.

Contribution 6: Improved production of chemicals in E. coli through nitrogen limitation:
6.1: Historical background
6.2: Central finding
6.3: Influence/Application
6.4: My role

Victor Chubukov, John James Desmarais, ... et al. (Jan. 2017). "Engineering glucose metabolism of Escherichia coli under nitrogen starvation". In: *NPJ Syst Biol Appl* 3, p. 16035. ISSN: 2056-7189. DOI: 10.1038/npjsba.2016.35. URL: http://www.ncbi.nlm.nih.gov/pmc/articles/PMC5516864/.