KYLE G. DANIELS, Ph.D.
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RESEARCH INTERESTS

My laboratory is interested in understanding how information is encoded by the biophysical and structural characteristics of receptors, signaling adapters, and gene regulatory elements of immune cell signaling systems. The goals of our work are to decode natural signaling systems to understand their functions, and to encode specific instructions in synthetic signaling systems to engineer therapeutic cells. We use synthetic biology, high-throughput library screening, and machine learning to explore how various signaling modules control cell function (Daniels KG, Wang S, et al. *Science*. 2022). We are particularly interested in understanding how modular domains can be recombined in new combinations and arrangements to give rise to diverse cellular behaviors. To explore cell signaling we curate a modular toolkit of domains involved in cell signaling, combine them in novel arrangements to make combinatorial libraries of hundreds to thousands of synthetic signaling molecules, and test these libraries for their effects on human immune cell phenotypes. Using neural networks, we build predictive models to quantitatively and systematically understand cell signaling networks, decode structure-function relationships, and design new immune cell therapeutics. We are also using innovative library-on-library screening approaches to optimize multiple proteins or cell-types at once to develop next-generation therapies. This potentially transformative vision for cell therapy is discussed in our recent review article (Capponi S & Daniels KG, *Immunol. Rev. 2023*). Through our interdisciplinary research in biophysics, immune engineering, and machine learning, we hope to train a new generation of scientists capable of combining emerging technologies to solve problems in human health.

EDUCATION AND TRAINING

Postdoctoral Fellow, UCSF, Advisor: Wendell A. Lim……September 2015-June 2023

Ph.D., Duke University, Biochemistry & Biophysics, Advisor: Terrence G. Oas.……
……..…….August 2010-August 2015

B.S., University of Maryland-College Park, Biochemistry……...September 2006-May 2010

PUBLICATIONS (9)

Peer Reviewed Original Research Articles (8):

- **Daniels KG**, Wang S, Simic MS, Bhargava HK, Capponi S, Tonai Y, Yu W, Bianco S, Lim WA. (2022) "Decoding CAR T Cell Phenotypes Using Combinatorial Signaling Motif Libraries and Machine Learning". Science. 378(6625), 1194-1200. Available from: 10.1126/science.abq0225
- O'Donoghue G, Bugaj L, Anderson W, **Daniels KG**, Rawlings D, Lim WA. (2021) "T Cells Selectively Filter Oscillatory Signals on the Minutes Timescale". PNAS. 118(9), e2019285118. Available from: 10.1073/pnas.2019285118
- **Daniels KG**, Suo Y, Oas TG. (2015) "Conformational Kinetics Reveals Affinities of Protein Conformational States". PNAS. 112(30), 9352-9357. Available from: 10.1073/pnas.1502084112
- Deis LN, Wu Q**,** Wang Y, Qi Y, **Daniels KG**, Zhou P, Oas TG. (2015) "Suppression of Conformational Heterogeneity at a Protein-Protein Interface". PNAS. 112(29), 9028-9033. Available from: 10.1073/pnas.1424724112
- Mosley PA, **Daniels KG** , Oas TG. (2015) "Electrostatic Energetics of Bacillus subtilis Ribonuclease P Protein Determined by NMR-based Histidine pKa Measurements". Biochemistry. 54(35), 5379-5388. Available from: 10.1021/acs.biochem.5b00138
- **Daniels KG**, Tonthat NK, McClure DR, Chang Y, Liu X, Schumacher MA, Fierke CA, Schmidler SC, Oas TG. (2014) "Ligand Concentration Regulates the Pathways of Coupled Protein Folding and Binding". J. Am. Chem. Soc. 136(3), 822–825. Available from: 10.1021/ja4086726
- DeArmond PD, Xu Y, Strickland EC, **Daniels KG**, Fitzgerald MC. (2011) "Thermodynamic Analysis of Protein-Ligand Interactions in Complex Biological Mixtures Using a Shotgun Proteomics Approach". J. Proteome Res. 10(11), 4948-4958. Available from: 10.1021/pr200403c

- **Daniels KG** and Beckett D. (2010) "Biochemical Properties and Biological Function of a Monofunctional Microbial Biotin Protein Ligase". Biochemistry. 49(25), 5358–5365. Available from: 10.1021/bi1003958

Review Articles (1):

- Capponi S & **Daniels KG**. (2023) "Harnessing the power of artificial intelligence to advance cell therapy". Immunological Reviews. 320(1), 147-165. Available from: 10.1111/imr.13236

GRANTS AWARDED

Active:

- Stanford Cancer Institute Innovation Award……..…...October 2023-September 2024
- Hypothesis Fund Pilot Grant.……May 2023-October 2024

Completed:

- IBM Exploratory Sciences Grant……..September 2021-July 2023
- NIH CURE Diversity Supplement for U54……June 2020-December 2021
- Damon Runyon Cancer Research Foundation Postdoctoral Fellowship……...July 2016-June 2020
- Burroughs Wellcome Fund Postdoctoral Enrichment Program Award……...September 2016- August 2019
- National Science Foundation Graduate Research Fellowship…….August 2012-July 2015

HONORS AND AWARDS

- Duke University Dean's Fellowship 2010-2013
- Duke University Diversity Enhancement Fellowship 2010
- Merck Index Award, University of Maryland Dept. of Chemistry and Biochemistry 2010
- Howard Hughes Medical Institute Undergraduate Research Fellowship 2007-2010
- Banneker-Key Scholarship: full cost of tuition, housing, food, and fees 2006-2010
- First Place in Biochemical Sciences, UMBC Undergraduate Research Symposium in Chemical and Biological Sciences 2009

PATENTS

Lim, Wendell & **Daniels, Kyle**. 2021. Immune Receptors with Synthetic Co-stimulatory Domains. 63/279,578, filed November 15, 2021. Patent Pending.

RESEARCH EXPERIENCE

Postdoctoral Research: T cells are quickly becoming a vehicle for cell-based immunotherapy in cancer and autoimmune diseases. To effectively use T cells to treat disease will require the ability to modulate the many variable properties of T cells—proliferation, persistence, memory formation, killing, metabolism, and cytokine release—all of which are controlled by cell signaling events. I focused my postdoctoral research on understanding how immune signaling events control T cell function. To do this, I developed a platform to rapidly construct libraries of thousands of synthetic receptors (CARs) that differ in their signaling domains, with each receptor containing a different combination and arrangement of conserved signaling motifs. I screened these libraries in primary human CAR T cells to find receptors (and signaling motif combinations) that promote or inhibit activation, proliferation, cytotoxicity, and persistence. In a collaboration with scientists at IBM, we used neural networks to learn and predict how combinations of signaling motifs dictate T cell phenotype and function such as stemness and anti-tumor cytotoxicity. I developed an analysis to quantify the contribution of signaling motifs to each of these biological functions. This work is the first implementation of combined high-throughput screening and machine learning/artificial intelligence to create an improved cell therapy prototype with enhanced in vitro and in mouse efficacy. It both improves our understanding of how signaling shapes T cell function and

provides a roadmap for accelerating design of receptors that give immune cells enhanced anti-cancer phenotypes.

Graduate Research: My graduate research focused on understanding the kinetic mechanisms of coupled binding and conformational changes in proteins and RNAs. In this work I performed kinetic, thermodynamic, and structural experiments and used machine learning to globally fit a complex model to the data. The results of my research provided the first experimental demonstration that molecular recognition could occur through both induced fit (binding before conformational change) and conformational selection (conformational change before binding) in the same molecule. This challenged the current view of the field, which traditionally described molecular recognition as happening either exclusively through induced fit or conformational selection, but not both. My results also demonstrated that molecular recognition for complex systems can be well described in terms of flux, and that the flux through various molecular recognition pathways depends on the concentration of ligand and protein or RNA. This work to understand coupled binding and conformational change improves our understanding of biological regulation and conformational changes relevant to drug design.

Undergraduate Research: My early career contributions as an undergraduate were in determining the biochemical characteristics that make biotin protein ligases either able to transfer biotin (monofunctional) or able to both transfer biotin and regulate biotin biosynthesis (bifunctional). I measured the ability of the monofunctional biotin protein ligase from *Pyrococcus horikoshii* to bind biotin and ATP and its ability to dimerize. By comparing these biochemical properties to the properties of the bifunctional biotin protein ligase from *Escherichia coli*, I showed that the biotin-dependent dimerization is a critical feature of bifunctional (but not monofunctional) biotin protein ligases. The quantitative approach used here to understand protein structure-function relationships still informs some of my work.

INVITED TALKS

- **Daniels, KD.** "Decoding the Language of Signaling Domains to Control Cell Function." Hong Kong University Stem Cell Seminar Series, January 15, 2024. Virtual Seminar.
- **Daniels, KD.** "Decoding the Language of Signaling Domains to Control Cell Function." 10th Annual Mammalian Synthetic Biology Workshop, June 23, 2023. San Jose, CA.
- **Daniels, KD.** "High-throughput screening and artificial intelligence as tools for cell therapy development." The SITC Immuno-Engineering Workshop, May 19, 2023. Boston, MA.
- **Daniels, KD.** "Forward Engineering of CARs: Using Libraries and AI to Learn Design Rules." Immuno Oncology Translation Network Steering Committee Meeting, November 3, 2022. Virtual Symposium.
- **Daniels, KD.** "Towards forward engineering receptors for immune cell control." Harvard University Systems Biology Seminar, September 19, 2022. Boston, Massachusetts.
- **Daniels, KD.** "Towards Forward Engineering of CARs: Combining Synthetic Biology and AI to Learn Design Rules." Duke University Biomedical Engineering, April 18, 2022. Durham, NC.
- **Daniels, KD.** "Towards Forward Engineering of CARs: Combining Synthetic Biology and AI to Learn Design Rules." University of Chicago Pritzker Molecular Engineering, March 9, 2022. Chicago, IL.
- **Daniels, KD.** "Towards Forward Engineering of CARs: Combining Synthetic Biology and AI to Learn Design Rules." Princeton University Bioengineering Initiative, March 4, 2022. Princeton, NJ.
- **Daniels, KD.** "Towards Forward Engineering of CARs: Combining Synthetic Biology and AI to Learn Design Rules." Immuno Oncology Translational Network, January 26, 2022. Virtual Seminar.

- **Daniels, KD.** "Towards Forward Engineering of CARs: Combining Synthetic Biology and AI to Learn Design Rules." Stanford University Mackall Lab Journal Club, January 14, 2022. Virtual Seminar.
- **Daniels, KD.** "A Combinatorial Signaling Motif Language Systematically Encodes CAR T Cell Phenotype." Duke University Pharmacology Cancer Biology, November 17, 2021. Durham, NC.
- **Daniels, KD.** "High-throughput Screening of Synthetic Costimulatory Domains to Modulate CAR T Cell Function." Stanford University Bioengineering and Genetics, January 12, 2021. Virtual Seminar.
- **Daniels, KD.** "High-throughput Screening of Synthetic Costimulatory Domains to Modulate CAR T Cell Phenotype." Duke University Biochemistry Seminar Series, November 20, 2020. Virtual Seminar.
- **Daniels, KD.** "High-throughput Screening of Synthetic Costimulatory Domains to Modulate CAR T Cell Function." Memorial Sloan-Kettering Cancer Biology & Genetics Seminar Series, November 16, 2020. Virtual Seminar.
- **Daniels, KD.** "High-throughput Screening of Synthetic Costimulatory Domains to Modulate CAR T Cell Function." University of Pennsylvania Bioengineering Seminar Series, October 22, 2020. Virtual Seminar.
- **Daniels, KD.** "High-throughput Screening of Synthetic Costimulatory Domains to Modulate CAR T Cell Function." Stanford-UCSF-Berkeley Next Generation Faculty Symposium, October 20, 2020. Virtual Seminar.

MENTORING

Alexandra Beckett, Postdoctoral Researcher, Stanford University	2023-Present
Wansang Cho, Postdoctoral Researcher, Stanford University	2023-Present
Kamal Obbad, MD/Ph.D. Student, Stanford University	2023-Present
Jodie Lunger, Ph.D. Candidate, Stanford University	2023-Present
Nakoa Po, Technician, Stanford University	2023-Present
Jenny Liu, Technician, Stanford University	2023-Present
Ben Ma, Masters Student, UCSF	2022-Present
William Baumbacher, Student/B.S. Candidate, St. Ignatius College Preparatory/UCLA	2016-2018
Jonathan Grego, B.S. Candidate, Duke University	2014-2015
Thu Nguyen, B.S. Candidate, Duke University	2013-2015
Bryan Lockwood, B.S. Candidate, Duke University	2012-2013
Chanelle Simmons, B.S. Candidate, Swarthmore College	2012-2013