BIOGRAPHICAL SKETCH

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NAME: Fordyce, Polly

eRA COMMONS USER NAME (credential, e.g., agency login): PFORDYCE

POSITION TITLE: Associate Professor of Genetics and Bioengineering

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of Colorado at Boulder	B.A.	06/2000	Physics, Biology
Stanford University	Ph.D.	01/2007	Physics
University of California, San Francisco	NA	08/2014	Microfluidics/Genomics

Important Mentors:
Ph.D. thesis advisor: Prof. Steve Block, Applied Physics & Biology, Stanford University
Postdoctoral advisor: Prof. Joe DeRisi, Biochemistry & Biophysics, UCSF
Postdoctoral co-advisor: Prof. Steve Quake, Bioengineering, Stanford University

A. Personal Statement

My laboratory focuses on developing novel microfluidic platforms for high-throughput and quantitative biochemistry, biophysics, and single-cell biology. In recent work, we have developed platforms that allow for high-throughput recombinant expression, purification, and biophysical characterization of >1,500 protein constructs in parallel and used this to probe how transcription factors recognize their DNA targets and protein partners (STAMMP, for Simultaneous Transcription Factor Affinity Measurements via Microfluidic Protein arrays; STAMMPPING, for STAMMP-Protein Interaction Network Generator) and how enzymes achieve their exquisite efficiency and specificity (HT-MEK, for High-Throughput Microfluidic Enzyme Kinetics). We can also produce and distinguish spectrally encoded hydrogel beads with >1,000 distinct codes (MRBLEs, for Microspheres with Ratiometric Barcode Lanthanide Encoding) and have used these beads for high-throughput, quantitative measurements of weak protein-peptide interactions (MRBLE-pep) and to profile the force- and sequence-dependence of T cell activation for 1000s of single cells interacting with 10s of different candidate peptide agonists (BATTLES). Finally, we have developed methods for FACS-based sorting of single cells encapsulated within microfluidic water/oil/water double emulsion droplets (sdDE-FACS and Dropception) and a sequencing-based technology (BET-seq for Binding Energy Topography by sequencing) that quantitatively measures relative binding affinities between a single transcription factor and up to 10^6 DNA sequences in parallel. We believe strongly in the power of open science and deposit all manuscripts on preprint servers upon submission, make all data and design files publicly available within OSF repositories, and publish all code for free download on Github.

Four peer-reviewed publications specifically highlight my experience in developing new technologies for high-throughput and quantitative functional characterization of proteins:

1. Feng, Y., Zhao, X., White, A.K., Garcia, K.C., & **Fordyce, P.M.** 2022. A bead-based method for high-throughput mapping of the sequence- and force-dependence of T cell activation. *Nature Methods* (epub ahead of print) (PMID 36064771).
2. Markin, C.J.*, Mokhtari, D.A.*, Sunden, F., Appel, M.J., Akiva, E., Longwell, S.A., Sabatti, C., Herschlag, D.*, & **Fordyce, P.M.***. 2021. Revealing enzyme functional architecture via high-throughput microfluidic enzyme kinetics. *Science* 373:eabf8761 (PMID 34437092) (* denotes shared authorship).
3. Aditham, A.K., Markin, C.J., Mokhtari, D.A., DelRosso, N.V., & **Fordyce, P.M.** 2020. High-throughput

binding affinity measurements for mutations spanning a transcription factor-DNA interface reveal affinity and specificity determinants. *Cell Systems* 14:S2405-4712 (PMID 33340452).

4. Nguyen, H.Q., Roy, J., Harink, B., Damle, N., Baxter, B., Brower, K., Kortemme, T., Thorn, K., Cyert, M., and **Fordyce, P.M.** 2020. Quantitative mapping of protein-peptide affinity landscapes using spectrally encoded beads. *eLife* (2019): e40499 (PMC6728138).

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2023-present	Associate Professor, Stanford University, Departments of Bioengineering & Genetics
2023-present	Co-Founder and Scientific Advisory Board Member, Velocity Bio
2022-2025	Scientific Advisory Board Member, Evozyne
2020-present	Advisory Board Member, Cell Systems
2017-present	Investigator, Chan Zuckerberg Biohub
2015-present	Director, Stanford Microfluidics Foundry
2014-present	Institute Scholar, Stanford ChEM-H
2017-2022	Assistant Professor, Stanford University, Department of Bioengineering
2014-2022	Assistant Professor, Stanford University, Department of Genetics
2014	Associate Research Specialist, University of California, San Francisco
2011-2012	Consultant, GigaGen, Inc.
2007-2014	Postdoctoral Fellow, University of California, San Francisco

Honors and Awards

2024	AAAS (American Academy for the Advancement of Science) Fellow
2024	AIMBE (American Institute of Medical and Biological Engineering) Fellow
2024	Stanford President's Award for Excellence Through Diversity
2023-2028	NIH Pioneer Award (DP1)
2023	Protein Society Young Investigator Award
2023	Eli Lilly Award in Biological Chemistry
2022-2027	Chan Zuckerberg Biohub Investigator
2022-2027	NSF CAREER Award
2021	Stanford Bioengineering Justice, Equity, Diversity, & Inclusion Award
2019-2022	Ono Pharma Foundation Breakthrough Science Initiative Award
2017-2022	Chan Zuckerberg Biohub Investigator
2017-2019	Alfred P. Sloan Foundation Research Fellow
2016-2021	NIH New Innovator Award (DP2)
2016, 2017	Research Corporation/Gordon & Betty Moore Foundation Scialog Fellow (Funded)
2015-2017	Stanford University McCormick and Gabilan Fellowship
2012-2017	NIH Pathway to Independence Award (K99/R00)
2008-2011	Helen Hay Whitney Postdoctoral Fellowship
2007	NIH Kirschstein NRSA Award (declined)
2003-2004	G.J. Lieberman Fellow
2002-2005	NSF Graduate Research Fellow
2002	Centennial Teaching Award
2001	NSF REU Grant Recipient

C. Contributions to Science

1. **High-throughput microfluidic enzyme kinetics.** Enzymes underpin cellular metabolism and regulation, are the targets of many therapeutics, are altered in genetic diseases, and play critical roles in industrial processes. A deeper understanding of how enzymes function and how these functions are altered by mutation could therefore have profound impacts on medicine and biotechnology. To enable this, we developed a novel microfluidic platform (HT-MEK, for High-Throughput Microfluidic Enzyme Kinetics), which allows high-throughput expression and purification of >1500 enzyme variants in hours, followed by iterative measurement of reaction rates in the presence of multiple substrates and inhibitors to yield a wealth of quantitative biochemical and thermodynamic constants in days. To develop and demonstrate HT-MEK, we profiled the effects of glycine and valine substitutions at every position throughout PafA, an Alkaline Phosphatase superfamily member (1,036 mutants; >650,000 reactions

yielding >6,000 kinetic and thermodynamic constants). HT-MEK experiments using a battery of substrates, inhibitors, and reaction conditions allowed us to separate folding from catalytic effects and revealed spatially contiguous "regions" of residues linked to particular aspects of function. In subsequent work, we have applied HT-MEK to understand how enzymes sequence encodes transition state stabilization and developed new arrays to make similar measurements without need for a device.

a. Markin, C.J.[*], Mokhtari, D.A.[*], Sunden, F., Appel, M.J., Akiva, E., Longwell, S.A., Sabatti, C., Herschlag, D.[‡], & **Fordyce, P.M.[‡]** 2021. Revealing enzyme architecture via high-throughput microfluidic enzyme kinetics. *Science* 373(6553) (PMID 34437092).
b. Markin, C.J., Mokhtari, D.A., Du, S., Doukov, T., Sunden, F., **Fordyce, P.M.[‡]**, & Herschlag, D.[‡] 2022. High-throughput enzymology reveals mutations throughout a phosphatase that decouple catalysis and transition state analog affinity. *PNAS* 120:e2219074120 (PMID 37428919).
c. Appel, M.J., Longwell, S.A., Morri, M., Neff, N., Herschlag, D., & **Fordyce, P.M.** 2021. uPIC-M: Efficient and scalable preparation of clonal single mutant libraries for high-throughput protein biochemistry. *ACS Omega* 6(30542-30554) (PMID 34805683).
d. Lee, B., Sunden, F., Miller, M., Pak, B., Krebber, A., Lutz, S., & **Fordyce, P.M.** 2024. Hydrophilic/omniphobic droplet arrays for high-throughput and quantitative enzymology. *bioRXiv* (doi: 10.1101/2024.07.19.604368).

2. **High-throughput measurements of transcription factor binding affinities.** During my postdoctoral research, I sought to understand how transcription factor (TF) binding to DNA targets could dramatically change cellular phenotypes. Working between the DeRisi laboratory at UCSF and the Quake laboratory at Stanford University, I developed a microfluidic platform (MITOMI 2.0) capable of directly measuring binding affinities between a single TF and up to 4,000 sequences in parallel. More recently, my lab has married these devices with next-generation sequencing to quantitatively measure >10^6 TF-DNA binding affinities in parallel (BET-seq), developed platforms for recombinant expression, purification, and biophysical characterization of 1000s of TF variants in parallel (STAMMP, STAMMPPING), and applied them to reveal that short tandem repeats within the genome regulate gene expression by altering regulatory site affinities and kinetics.
    a. Horton, C.A., Alexandari, A.M., Hayes, M.G.B., Marklund, E., Schaepe, J.M., Aditham, A.K., Shah, N., Shrikumar, A., Afek, A., Greenleaf, W.J., Gordan, R., Zeitlinger, J., Kundaje, A., & **Fordyce, P.M**. 2023. Short tandem repeats bind transcription factors to tune eukaryotic gene expression. *Science* 381:eadd1250 (PMID 37733848).
    b. Aditham, A.K., Markin, C.J., Mokhtari, D.A., DelRosso, N.V., & **Fordyce, P.M.** 2020. High-throughput binding affinity measurements for mutations spanning a transcription factor-DNA interface reveal affinity and specificity determinants. *Cell Systems* 14:S2405-4712 (PMID 33340452).
    c. DelRosso, N.*, Suzuki, P.H.*, Griffith, D., Lotthammer, J.M., Novak, B., Kocalar, S., Sheth, M.U., Holehouse, A.S., Bintu, L.[‡], **Fordyce, P.M.[‡].** 2024. High-throughput affinity measurements of direct interactions between activation domains and co-activators. *bioRXiv* (doi:10.1101/2024.08.19.608698).
    d. Le, D.L.*, Shimko, T.C.*, Aditham, A.K., Keys, A.M., Longwell, S.A., Orenstein, Y., & **Fordyce, P.M.** 2018. Comprehensive, high-resolution binding energy landscapes reveal context dependencies of transcription factor binding. *PNAS* 115(16):E3702-3711 (PMC5910820).

3. **Leveraging spectrally encoded beads for high-throughput measurement of macromolecular interactions.** Bead-based assays have the potential to investigate a very large number of analytes simultaneously but require an encoding strategy to identify the analyte coupled to each bead. As a postdoc, I worked with colleagues to develope a new automated spectral encoding method based on ratiometric incorporation of lanthanide nanophosphors within polymer beads. In my own lab, we have demonstrated the ability to produce and distinguish >1100 unique spectral codes with high confidence (MRBLEs, for Microspheres with Ratiometric Barco,de Lanthanide Encoding). We have since used these MRBLEs as the foundation for a new assay that allows quantitative measurement of hundreds of weak protein-peptide interactions in parallel using 1000-fold less material than traditional quantitative assays (MRBLE-pep) and for high-throughput single T cell mechanobiology (BATTLEs). In recent work, we have simplified bead production and functionalization to enhance translation across labs.

a. Feng, Y., Zhao, X., White, A.K., Garcia, K.C., & **Fordyce, P.M.** 2021. Structure-activity mapping of the peptide- and force-dependent landscape of T-cell activation. *Nat. Methods* 19:1295-1305 (PMID: 36064771).
b. Feng, Y., White, A.K., Hein, J.B., Appel, E.A., & **Fordyce, P.M**. 2020. MRBLEs 2.0: High-throughput generation of chemically functionalized spectrally and magnetically-encoded hydrogel beads using a simple single-layer microfluidic device, *Microsystems and Nanoengineering* 6:109 (PMC7704393).
c. Nguyen, H.Q., Baxter, B.C., Brower, K., Diaz-Botia, C.A., DeRisi, J.L, **Fordyce, P.M.**‡, and Thorn, K.S.‡. 2017. Programmable microfluidic synthesis of over one thousand uniquely identifiable spectral codes, *Adv. Opt. Mat.* 5(3) (PMC5604317) (* denotes co-corresponding authors).
d. Nguyen, H.Q., Roy, J., Harink, B., Damle, N., Baxter, B., Brower, K., Kortemme, T., Thorn, K., Cyert, M., and **Fordyce, P.M.** 2020. Quantitative mapping of protein-peptide affinity landscapes using spectrally encoded beads. *eLife* (2019): e40499 (PMC6728138).

4. **High-throughput studies of single cells using microfluidic double emulsion droplets.** Droplet microfluidics has enabled single-cell genomic, epigenomic, and transcriptomic assays at unprecedented scale. The next great challenge is to link observed differences in these cellular *genotypes* with their corresponding effects on cellular *phenotypes*, which can be accomplished by phenotyping (*e.g.* measuring a fluorescence signal associated with each cell) and sorting or isolating cells prior to sequencing. We recently demonstrated that mammalian cells can be encapsulated within *double* emulsion (DE) water-in-oil-in-water droplets and then screened and individually isolated using a commercial FACS machine. This novel technology opens up exciting new opportunities for single-cell analysis, including: (1) sorting based on single-cell phenotypes not currently measurable via FACS (*e.g.* presence of secreted molecules, catalytic activity, presence of transcripts, and genomic variation), and (3) multi-omic measurements on the *same* single cell by performing the first reaction in a droplet and the second in a plate.
    a. Brower, K.K.*, Khariton, M.*, Suzuki, P., Still, C., Kim, G., Calhoun, S., Qi, S., Wang, B.‡, & **Fordyce, P.M.**‡. 2020. Double emulsion picoreactors for high-throughput single-cell encapsulation and phenotyping via FACS, *Anal. Chem.* 92:13262-13270 (PMC7670281).
    b. Brower, K.K., Carswell-Crumpton, C., Klemm, S., Cruz, B., Kim, G., Calhoun, S., Nichols, L., & **Fordyce, P.M.** 2020. Optimized double emulsion flow cytometry with high-throughput single droplet isolation. *Lab on a Chip* 20:2062-2074 (PMC7670282).
    c. Calhoun, S.G.K., Brower, K.K., Suja, V.C., Kim, G., Wang, N., McCully, A.L., Kusumaatmaja, H., Fuller, G.G., & **Fordyce, P.M.** 2022. "Systematic characterization of double emulsion droplets for biological applications", *Lab on a Chip* 22(12):2315-2330 (PMID 35593127).
    d. Lashkaripour A., McIntyre, D.P., Calhoun, S.G.K., Krauth, K., Densmore, D.M., & **Fordyce, P.M.** 2024. Design automation of microfluidic single and double emulsion droplets with machine learning. *Nature Communications* 15:83 (PMID 38167827).

5. **First demonstration of combined optical trapping and single-molecule fluorescence.** These papers describe the design and construction of the world's first microscope capable of simultaneous optical trapping and single-molecule fluorescence. This instrument provided a unique window on the nanoscale world, allowing the simultaneous application of force to a molecule (using an optical trap) and readout of its effects (using fluorescence). Using this microscope, we resolved longstanding controversies surrounding how kinesin motor proteins couple ATP hydrolysis to stepping to drive processive motion.
    a. Lang, M.J.*, **Fordyce, P.M.***, Engh, A.E., Neuman, K.C., and Block, S.M. 2004. Simultaneous, coincident optical trapping and single-molecule fluorescence. *Nat. Methods* 1:133-139 (PMC1483847).
    b. Valentine, M.T.*, **Fordyce, P.M.***, Krzysiak, T.C., Gilbert, S.P., and Block, S.M. 2006. Individual dimers of the mitotic kinesin motor Eg5 step processively and support substantial loads *in vitro*. *Nat. Cell Biol.* 8:470-476 (PMC1523314).
    c. Lang, M.J., **Fordyce, P.M.**, and Block, S.M. 2003. Combined optical trapping and single-molecule fluorescence. 2003. *J. Biol.* 2:6-10 (PMC156597).

d. Rosenfeld, S.S., **Fordyce, P.M.**, Jefferson, P.M., King, G.H., and Block, S.M. 2003. Stepping and stretching – how kinesin uses internal strain to walk processively. *J. Biol. Chem.* 278:18550-18556 (PMC1533991).

Complete List of Published Work in MyBibliography:

http://www.ncbi.nlm.nih.gov/sites/myncbi/polly.fordyce.1/bibliography/44210510/public/?sort=date&direction=ascending