BIOGRAPHICAL SKETCH

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NAME: Fordyce, Polly

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

POSITION TITLE: Assistant Professor of Bioengineering and Genetics

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

A. Personal Statement

As a PhD student in Steven Block's laboratory at Stanford University, I constructed the world's first microscope capable of simultaneous optical trapping and single-molecule fluorescence and used it to characterize both conventional kinesins and human mitotic kinesins. Through this research, I gained extensive training in optics, electronics, microscopy, surface chemistry, single-molecule assay development, data acquisition and analysis, and programming. For my postdoctoral training, I joined Joseph DeRisi's laboratory at UCSF and shifted my focus from measuring individual molecules towards measurements at the genome-wide scale. In collaboration with Stephen Quake's laboratory at Stanford University, I developed a microfluidic platform capable of measuring affinities for a single transcription factor interacting with 4,000 different DNA sequences (MITOMI 2.0).

I launched my independent laboratory at Stanford University in September 2014, where we focus on developing novel microfluidic platforms for high-throughput and quantitative biochemistry, biophysics, and single-cell biology. *Since launching my laboratory, I have published or submitted 25 papers (16 of them as corresponding or co-corresponding author).* We recently developed a novel technology (BET-seq for Binding Energy Topography by sequencing) that couples MITOMI microfluidic devices to a deep sequencing readout to quantitatively measure relative binding affinities between a single transcription factor and up to 10^6 DNA sequences in parallel. In addition, we have demonstrated the ability to produce and distinguish spectrally encoded beads with >1,000 distinct codes (MRBLEs, for Microspheres with Ratiometric Barcode Lanthanide Encoding) and have since used these beads for high-throughput, quantitative measurements of weak protein-peptide interactions (MRBLE-pep). In recent work, we have developed platforms that allow for high-throughput recombinant expression, purification, and deep biophysical characterization of > 1,500 different protein constructs in parallel and used this to probe how transcription factor proteins recognize their DNA target sites (STAMMP, for Simultaneous Transcription Factor Affinity Measurements via Microfluidic Protein arrays) and how enzymes achieve their exquisite efficiency and specificity (HT-MEK, for High-Throughput Microfluidic Enzyme Kinetics). Finally, we have also recently developed a novel platform that facilitate FACS-based sorting of single cells encapsulated within microfluidic water/oil/water double emulsion droplets (sdDE-FACS and Dropception). 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.

Along with research, I am dedicated to mentoring the students and postdoctoral scholars in my laboratory. To date, I have mentored or am mentoring: 12 Ph.D. students from the Departments of Bioengineering, Genetics, and Biochemistry (2 of whom recently graduated and founded their own companies); 11 postdoctoral fellows (5 of whom have moved on from the lab and have all successfully continued as scientists in industry, at startups, or at research institutions (Genentech, Illumina, Leiden Measurement Technologies, and the University of

Copenhagen); 3 research technicians (1 of whom is a Biology PhD student at MIT, 1 of whom now works at CZ Biohub, and 1 who is about to start a PhD program); and multiple undergraduates. I provide all new trainees with an evolving document with details describing my expectations, and I give my lab the opportunity to respond to an anonymous feedback form about ways to improve lab culture and my own leadership once a year.

Relevant to this proposal, four peer-reviewed publications specifically highlight my experience in developing new technologies for high-throughput *in vitro* investigations of how proteins find and bind their DNA and protein targets:

1. Aditham, A.K., Markin, C.J., Mokhtari, D.A., DelRosso, N.V., & **Fordyce, P.M.** High-throughput binding affinity measurements for mutations spanning a transcription factor-DNA interface reveal affinity and specificity determinants. 2020. *Cell Systems* 14:S2405-2712 (PMID 33340452).
2. 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). (* denotes shared authorship).
3. **Fordyce, P.M.***, Gerber, D.*, Tran, D., Zheng, J., Li, H., DeRisi, J.L., and Quake, S.R. 2010. *De novo* identification and biophysical characterization of transcription-factor binding sites with microfluidic affinity analysis. *Nat. Biotechnol.* 28(9):970-5 (PMC2937095). (* denotes equal authorship).
4. Nguyen, H.Q., Roy, J., Harink, B., Damle, N., Baxter, B., Brower, K., Kortemme, T., Thorn, K., Cyert, M., and **Fordyce, P.M.** 2019. Quantitative mapping of protein-peptide affinity landscapes using spectrally encoded beads. *eLife*: e40499 (PMC6728138).

B. Positions and Honors

Employment/Experience

2007-2014	Postdoctoral Fellow, University of California, San Francisco
2011-2012	Consultant, GigaGen, Inc.
2014	Associate Research Specialist, University of California, San Francisco
2014-current	Assistant Professor, Stanford University, Department of Genetics
2014-current	Institute Scholar, Stanford University, ChEM-H
2015-current	Assistant Professor, Stanford University, Department of Bioengineering
2015-current	Co-director, Stanford Microfluidics Foundry
2017-current	Investigator, Chan Zuckerberg Biohub

Professional Activities/Honors

2000	B.A. awarded with highest honors
2000	National Science Foundation REU Grant Recipient
2002-2005	National Science Foundation Graduate Research Fellow
2002	Centennial Teaching Award
2003-2004	J.G. Lieberman Fellow
2008	NIH Ruth L. Kirschstein NRSA Postdoctoral Fellow (declined)
2008-2011	Helen Hay Whitney Postdoctoral Fellow
2012-2017	NIH K99 Pathway to Independence Award
2015-2017	McCormick and Gabilan Fellow
2016-2021	NIH New Innovator Award
2017-2019	Sloan Research Fellow
2017-2022	Chan Zuckerberg Investigator
2019-2024	Ono Pharma Foundation Breakthrough Prize Recipient

Ad hoc reviewer: NIH Special Emphasis Panel (2017), DOD PRMRP Grant Panel (2018), NIH Instrumentation and Systems Development study section (2019), NIH Macromolecular Structure and Function B (2021)
2020-current Editorial Board, Cell Systems

C. Contributions to Science

1. **High-throughput measurements of transcription factor binding affinities.** During my postdoctoral research, I sought to understand how simple binding interactions between transcription factors (TFs) and their DNA targets could dramatically change cellular phenotypes. Although there existed multiple methods for making qualitative measurements of transcription factor binding preferences, none of these methods

could provide quantitative information about the strength of binding, which is required for building physically motivated models of transcriptional regulation. Working jointly 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. By using this technique to measure binding to a large combinatorial DNA library, I showed that it was possible to discover transcription factor target sites without prior information about binding preferences and to map the binding energy landscape of these interactions. In my own lab, we have developed a novel assay (BET-seq) that combines microfluidic devices and next-generation sequencing to quantitatively measure >10^6 TF-DNA binding affinities in parallel as well as a platform for recombinant expression, purification, and biophysical characterization of 1000s of TF variants in parallel.

a. **Fordyce, P.M.***, Gerber, D.*, Tran, D., Zheng, J., Li, H., DeRisi, J.L., and Quake, S.R. 2010. *De novo* identification and biophysical characterization of transcription-factor binding sites with microfluidic affinity analysis. *Nat. Biotechnol.* 28(9):970-5 (PMC2937095).
b. Orenstein, Y., Puccinelli, R., Kim, R., **Fordyce, P.,** and Berger, B. 2017. Optimized sequence library design for efficient *in vitro* interaction mapping. *Cell Systems* 5(3):230-6. (PMC5661997).
c. 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) (* denotes shared authorship).
d. 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).

2. **Combining in vitro affinity assays with in vivo measurements of transcription factor binding and gene expression to understand transcriptional networks.** Initial microfluidic affinity assays provided surprising evidence that Hac1, a well-studied *S. cerevisiae* TF involved in the unfolded protein response, can bind two very different target sites. Working in collaboration with Peter Walter's laboratory at UCSF, we developed an *in vivo* genetic screen that both confirmed this binding plasticity and revealed the protein residues involved, establishing that even structurally simple TFs can exhibit complex binding. In collaboration with Sandy Johnson's laboratory at UCSF, we also interrogated binding specificities for multiple TFs from *Candida albicans*, generating data that led to the discovery of a new family of sequence-specific DNA binding proteins and proved crucial for reconstructing the transcriptional network that governs white-opaque switching.

a. **Fordyce, P.M.***, Pincus, D., Kimmig, P., Nelson, C.S., El-Samad, H., Walter, P., and DeRisi, J.L. 2012. The basic leucine zipper transcription factor Hac1 binds DNA in two modes as revealed by microfluidic analyses. *PNAS*. 109(45):E3084-93 (PMC3494901).
b. Lohse, M.B., Hernday, A.D., **Fordyce, P.M.,** Noiman, L., Sorrells, T.R., Hanson-Smith, V., Nobile, C.J., DeRisi, J.L., and Johnson, A.D. 2013. Identification and characterization of a previously undescribed family of sequence-specific DNA-binding domains. *PNAS.* 110:7660-5 (PMC3651432).
c. Hernday, A.D., Lohse, M.B.*, **Fordyce, P.M.***, Nobile, C.J., DeRisi, J.L., and Johnson, A.D. 2013. Structure of the transcriptional network controlling white-opaque switching in *Candida albicans*. *Molecular Microbiology.* 90:22-35 (PMC3888361).
d. Perez, J.C., **Fordyce, P.M.**, Lohse, M.B., Hanson-Smith, V., DeRisi, J.L., and Johnson, A.D. 2014. How duplicated transcription regulators can diversify to govern the expression of non-overlapping sets of genes. *Genes & Development.* 28:1272-1272 (PMC4066398).

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. To date, technical issues with encoding methods have limited these assays to ~100-500 parallel interactions, making them unsuitable for genome-scale studies. To overcome these limitations, I developed 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 Barcode 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). By applying MRBLE-pep to study the binding specificity landscape for calcineurin, a conserved phosphatase essential for the human immune response, we discovered that amino acids flanking the known binding site play large roles for determining specificity.

a. Gerver, R.E.*, Gomez-Sjoberg, R.*, Baxter, B.C.*, Thorn, K.S.*, **Fordyce, P.M.***, Diaz-Botia, C.A., Helms, B.A., and DeRisi, J.L. 2012. Programmable microfluidic synthesis of spectrally encoded microspheres. *Lab on a Chip*. 12(22):4716-23 (PMID 23042484). (* denotes equal authorship, author order was determined by random draw).
b. 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).
c. Nguyen, H.Q., Roy, J., Harink, B., Damle, N., Baxter, B., Brower, K., Kortemme, T., Thorn, K., Cyert, M., and **Fordyce, P.M.** 2019. Quantitative mapping of protein-peptide affinity landscapes using spectrally encoded beads. *eLife*: e40499 (PMC6728138).
d. 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).

4. **High-throughput encapsulation and phenotypic sorting of single cells using microfluidic double emulsion droplets.** Droplet microfluidics has enabled single-cell genomic, epigenomic, and transcriptomic assays at unprecedented scale, revealing critical insights into cellular heterogeneity, tissue organization, and organism development. 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. Unfortunately, *sorting* standard single emulsion (water-in-oil) droplets has remained technically challenging, requiring extensive custom equipment and operating at relatively slow rates for single-droplet isolation (~1 Hz). By encapsulating reagents within *double* emulsion (DE) water-in-oil-in-water droplets, we recently demonstrated the ability to: (1) encapsulate, measure, sort, and isolate *individual* droplets of interest at high-throughput (1 kHz) and with >99% target enrichment using a commercial FACS instrument, and (2) recover >95% of any encapsulated nucleic acids without cross-contamination. 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).

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

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing Research Support

NIH 1DP2 GM123641-01 PI: Fordyce 09/30/2016-06/30/2021
Leveraging spectral encoding for high dimensional biological multiplexing
The goal of this study is to build upon a technology we have recently developed for producing spectrally encoded beads with a high potential code space for: (1) profiling protein-peptide interactions for many peptides, and (2) linking genotype and phenotype information for many single cells in parallel at low cost. There is no overlap with the current proposal.

Chan Zuckerberg Biohub PI: Fordyce 03/01/2017-02/28/2022
Microfluidic Platforms for High-Throughput Characterization of Molecular Phenotypes
The goal of this study is to develop new microfluidic tools for high-throughput, quantitative measurements of transcription factor binding interactions and enzymatic turnover. There is no overlap with the current proposal.

NIH R01 GM064798-09 PI: Herschlag, Fordyce 07/01/2019-06/20/2024
Quantitative, High-Throughput Mechanistic Enzymology
The goal of this study is to apply a new high-throughput technology for quantitative enzymology towards measuring metagenomic bacterial phosphatases to understand sequence-structure-function relationships within the alkaline phosphatase superfamily. There is no overlap with the current proposal.

Ono Pharma Foundation PI: Fordyce, Herschlag 09/01/2019-08/31/2022
High-Throughput Microfluidic Enzyme Kinetics to Identify and Manipulate Allosteric Handles for Enzyme Control
The goal of this study is to leverage a new high-throughput technology for quantitative enzymology towards mapping allosteric networks within human protein tyrosine phosphatases and revealing potential allosteric “handles” that can be used as target interfaces for allosteric control.

Gordon and Betty Moore Foundation PI: Fordyce, Herschlag 02/01/2019-11/30/2022
Unraveling the Second Secret of Life: Discovering and Manipulating Allostery for Enzymatic Control
The goal of this study is to combine molecular dynamics simulations and high-throughput experimental measurements to understand the mechanistic origins of long-range allosteric networks within enzymes.