CBC | CHICAGO BIOMEDICAL CONSORTIUM

CBC Catalyst Award Proposal Title Page

Title of the proposal *(no more than 100 characters including white space)*

Determining the role of CUX1 in genome architecture

Name, degree, title, institution, and contact information including the email address of each PI

Megan McNerney, MD/PhD, Associate Professor, Departments of Pathology and Pediatrics
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Do you have any current or pending grant applications that potentially overlap with this application?
Check ONE:

YES ☐ **NO** ☒

If YES, please identify them.

N/A

Explain the overlap in the Biosketch section.

Does the proposed research involve animal subjects?	**Check ONE:**	**YES** ☐	**NO** ☒
Does the proposed research involve human subjects?	**Check ONE:**	**YES** ☐	**NO** ☒
Does the proposed research involve embryonic stem cells?	**Check ONE:**	**YES** ☐	**NO** ☒

(Note: if the data entered exceeds one page, it is acceptable to submit it as two pages.)

LAY SUMMARY. Each human cell contains approximately five feet of DNA that is systematically folded and organized within the cell. Organization of the DNA is important for normal tissue development and function and loss of this structure can lead to diseases including cancer. How DNA is arranged in a three-dimensional manner remains poorly understood. We have identified a master-regulatory gene, CUX1, that is essential for the development of multiple tissue types and in the prevention of cancer. Our preliminary data implicate CUX1 in DNA folding. In the current proposal we will rigorously establish the role for CUX1 in DNA three-dimensional architecture using the expertise of genome biologist, Dr. Yue, and physician-scientist, Dr. McNerney. Accomplishing this work will identify CUX1 as a novel protein necessary for DNA organization and reveal the mechanism by which CUX1 regulates normal cells and prevents disease.

IMPACT. With rapid advances in genomic technologies, the critical role of genome organization in development and disease has become evident. Structural proteins such as CTCF and cohesin function as architectural elements, however, DNA looping is a dynamic process during tissue-specific development. It is thought that sequence-specific transcription factors are necessary to direct these structural proteins to regulate 3D genome organization, yet these putative factors remain elusive. We have compelling preliminary data that the widely expressed HOX-family transcription factor, CUX1, is involved in DNA looping. In the current proposal, we test this model with cutting-edge tools, functional genomics, and data analysis, leveraging the complimentary expertise of the Feng and McNerney labs. These studies will break new ground in the fields of genome biology, developmental biology, and cancer pathogenesis. Accomplishing the proposed work will provide essential reagents and preliminary data necessary for the successful of a multiple PI R01 to complete this work.

BACKGROUND AND SIGNIFICANCE. **CUX1 is essential in tissue development.** CUX1 is a non-clustered HOX family transcription factor (1). Across species, CUX1 is essential for development and controls several cellular processes including proliferation, apoptosis, and cell identity in multiple tissue types (2). In *Drosophila*, the ortholog of CUX1 has dosage-dependent roles in differentiation (3,4). Similarly, we reported that CUX1 haploinsufficiency increases myelopoiesis at the expense of erythropoiesis in mice (5).

***CUX1* mutations are recurrent in myeloid neoplasia.** -7/del(7q) is recurrent in myeloid malignancies, including myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) (1). In almost all cases, -7/del(7q) carries a poor prognosis; there is an urgent need for new therapies for these patients. We reported that *CUX1* is a haploinsufficient tumor-suppressor gene encoded on chromosome 7 and loss of *CUX1* is sufficient to cause MDS in mice (5). *CUX1* loss-of-function mutations are recurrent in myeloid malignancies, are an independent prognostic indicator of poor outcome, and can be initiating events in disease (1).

The role of CUX1 in DNA looping. Changes in 3D DNA architecture are critical to the execution of cell fate decisions (6). DNA looping mediates the recruitment of enhancers to regulate promoter activity or the formation of insulated neighbors to prevent precocious gene expression. The role of transcription factors in this process remains poorly understood (6). Multiple lines of evidence implicate CUX1 in the formation and/or maintenance of DNA looping: i) Across hundreds of functional genomic datasets, features, and multiple cell lines, CUX1 genomic occupancy is one of the strongest predictors of enhancer-promoter looping (7); ii) We reported that CUX1 is enriched at sites of DNA looping, as measured by cohesin complex occupancy and Hi-C contact points, and this looping was predictive of CUX1 regulation of target genes (8); and iii) CUX1 family member, SATB1, is also critical in genome organization (9). The current proposal is designed to directly test the role of CUX1 in establishing and/or maintaining genome architecture.

INNOVATION. 1) Conceptually, we propose that CUX1 is a novel regulator of DNA looping. Further, we posit that the dosage of CUX1 a determinant of DNA looping and lineage specification. With few exceptions, the majority of research on transcriptional regulators are “present” or “absent” studies. With our innovative tools we are poised to make more nuanced and discretized analyses. 2) Degron technology is a powerful system for rapid target degradation and is titratable, modeling distinct levels of knockdown (10). The system is reversible – a powerful capability enabling rescue experiments. 3) We continue to innovate tools for 3D data analysis including NeoLoopFinder, Peakachu, HiCPlus, HiCRep, and the 3D Genome Browser (11-14).

PRELIMINARY STUDIES. We performed ChIP-seq for CUX1 in the K562 human AML cell line and found that of 5,520 CUX1 binding sites are located at DNA loop anchors as determined by Hi-C analysis. 35.8% of these sites are located at enhancer-promoter loops, while the majority of the remaining loops connect enhancer-enhancer or promoter-promoter interactions. An example is shown in Fig. 1. These results support the premise that CUX1 is involved in linking *cis*-regulatory elements to target genes and support the feasibility of the proposed experiments.

APPROACH. In addition to functioning as a tumor suppressor, CUX1 is broadly expressed and implicated in the homeostasis of multiple tissue types. A **major barrier** in understanding CUX1 biology is that the molecular mechanism(s) by which CUX1 exerts transcriptional regulation remains unclear. The **overall objective** of this proposal is to determine the role for CUX1 in genome architecture and gene regulation. In preliminary data,

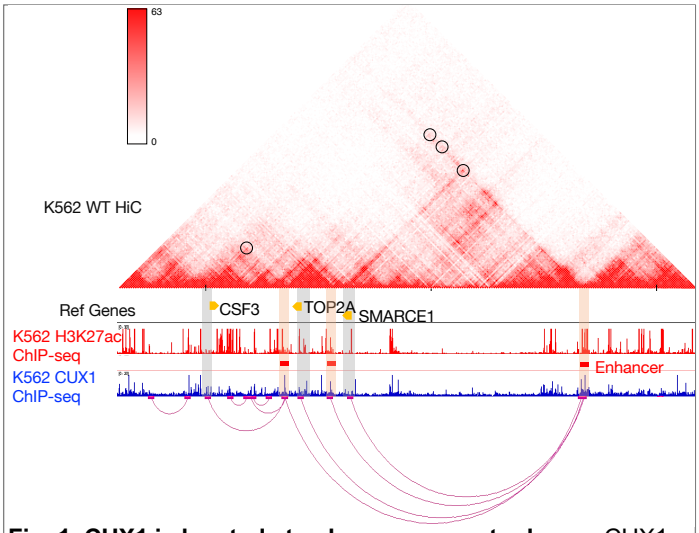

Fig. 1. CUX1 is located at enhancer-promoter loops. CUX1 ChIP-seq and Hi-C data support the premise that CUX1 is located at Hi-C contact points in the K562 human AML cell line.

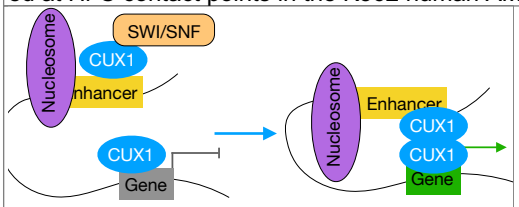

Fig. 2. Models for Aims 1 & 2. In unpublished data, we identified a role for CUX1 in "pioneer" activity wherein CUX1 binds enhancers and recruits the SWI/SNF chromatin remodeling complex to increase enhancer accessibility. We predict that CUX1 monomers or homodimers promote enhancer-promoter looping to regulate gene expression.

we demonstrate that CUX1 is enriched at sites of Hi-C contact points (Fig. 1). Based on these data, our **central hypothesis** is that CUX1 mediates the recruitment of enhancers to lineage-specific promoters to drive differentiation (Fig. 2). The **rationale** for this work is to provide fundamental understanding of the role of CUX1 in cell fate specification and ultimately strategic therapeutic strategies for patients with CUX1 haploinsufficiency. This work will help achieve our **long-term goal,** to improve the length and quality of life for patients with high-risk malignancies.

Aim 1: Hypothesis – CUX1 regulates hematopoietic differentiation genes. **Establish cell lines with degradable endogenous CUX1.** Inducible protein degraders have enabled new insights into genome folding due to nearly complete elimination of the target protein within hours (15). We will establish three human AML cell lines with the endogenous *CUX1* locus tagged with a FKBP12^{V36} degron (16) at the C-terminal end: K562 is a tier-1 ENCODE cell line with substantial existing functional genomics datasets to leverage; KG-1 is a complex karyotype leukemia with mutant *TP53*, modeling high-risk AML; and MONO-7 has a single *CUX1* allele for simplified genomic modification. We chose the 3' end for tagging as a similarly placed GFP tag does not disrupt CUX1 nuclear localization or DNA binding in K562 (ENCODE, ENCSR178NTX). All cell lines are readily transfectable by Neon (Thermo Fisher). To tag endogenous *CUX1* we will use a gRNA targeting the *CUX1* 3'UTR and a homology mediated repair strategy (Synbio) as we published (17). We will validate correct integration by PCR as previously (17). We will generate two single cell clones per cell line. We will test the half-life of tagged CUX1 by cycloheximide and immunoblotting. We will perform a time course and concentration gradient for dTAGV-1 (Tocris, 6914) ligand-induced CUX1 protein knockdown with vehicle controls.

Determine the cellular phenotype of CUX1 knockdown. To test the impact of acute CUX1 elimination, we will treat CUX1-tagged cell lines and negative controls with dTAGV-1 concentrations that lead to 50% and 100% CUX1 degradation (or vehicle control) and measure proliferation, cell cycle, and apoptosis for 0-72 hours, using ATP-lite and flow cytometry as we performed previously (5,8). We anticipate that 50% and near 100% removal of CUX1 will increase proliferation, increase progression through the cell cycle, and not impact apoptosis as we observed in shRNA-based knockdown experiments (5,8).

Identify direct CUX1 target genes. We will perform fastGRO nascent RNA-seq (18) at 0, 4, and 24 hours post-CUX1 50% and 100% degradation (n=3 biological replicates per AML cell line). Data will be analyzed as previously (8,19) with spike-in normalization. We will perform CUT&RUN to identify CUX1 genomic binding sites in these same cells as previously (8). We anticipate identifying CUX1 differentially expressed target genes that are enriched for regulators of proliferation and differentiation.

Aim 2: Hypothesis – CUX1 is required for enhancer-promoter looping in hematopoietic cells. **Determine the role of CUX1 in DNA looping.** We will perform *in situ* Hi-C experiments in the three human AML cell lines described above, K562, Kg-1, and Mono-7, with CUX1-degron tagged. Cells will be assayed at 0, 4, and 24 hours post-CUX1 degradation to assess the time course of the impact of CUX1 on DNA folding. We will perform one biological replicate per sample, with two independent clones per sample, for a total of n=2 biological replicate per condition. We will repeat these experiments with ~50% CUX1 knockdown to test the impact of CUX1 haploinsufficient expression levels on looping. We will employ methods and data analyses as we have previously described (11,20-22). We will integrate these results with CUX1 CUT&RUN data from the same cell lines. We anticipate that complete CUX1 knockdown by 24 hours will not have large effects on topological associated domain (TAD) boundaries, but instead will decrease sub-TAD enhancer-promoter looping, particularly at CUX1 binding sites. We predict that many of these changes will already be present at the four hour time point. In the haploinsufficient state, we anticipate that either: i) a subset of potentially lower affinity CUX1 binding sites will have reduced DNA looping; or ii) alternatively we may find that all CUX1 binding sites have reduced contacts. These data will shed light on the impact of CUX1 haploinsufficiency on genome folding. We will integrate fastGRO data with these genomic assays to measure the impact of changes in DNA

looping with target gene expression. We expect that those genes with reduced enhancer-promoter looping will have decreased expression in a CUX1 dosage-dependent manner.

4C validation of CUX1 regulated DNA contacts. We will prioritize three sites of CUX regulated DNA looping events for validation based on: i) change in predicted target gene expression, and ii) known involvement of the target gene in hematopoietic differentiation. Based on our prior transcriptome analysis of CUX1-dependent differentially expressed genes in mouse hematopoietic stem cells, we expect CUX1 target genes to include master regulators of differentiation such as *HLF*, *MEIS1*, *CEBPA*, and *GATA2* (19). We will perform 4C experiments using techniques we reported (11). We will validate changes in gene expression by qRT-PCR.

Identify the role of CUX1 in DNA looping in primary human hematopoietic stem and progenitor cells. To increase the translational relevance of our findings, we will extend our studies to include human CD34+ hematopoietic stem and progenitor cells (Fred Hutch). We will culture the cells as we described (23). Given the lower cell numbers available, we will perform HiChip, requiring ~ 1 million cells for transcription factor targets (24). For these experiments we will use our in-house generated, ChIP-grade anti-CUX1 antibody (PUC) (19). We will perform two biological replicates in cells CRISPR edited for *CUX1* or *HPRT* control locus, using methods we previously reported which routinely provide > 60% *CUX1* editing in bulk cultures (23). The resulting data will be integrated with our previously generated RNA-seq data in human CD34+ cells post-CUX1 knockdown (5) as well as our unpublished CUX1 CUT&RUN data from the same cell types. We anticipate that CUX1 knockdown will decrease enhancer promoter looping at CUX1 binding sites, and these changes will correlate with CUX1-regulated differentially expressed genes.

Expected outcomes. Overall, we anticipate establishing critical reagents for rapid and titratable control of CUX1 levels. We expect that complete CUX1 removal will disrupt the genomic architecture of hematopoietic leukemia and normal counterparts. We predict that these changes will be enriched at sub-TAD looping of enhancers bound by CUX1 with target gene promoters that have decreased expression after CUX1 knockdown. We may find that 50% reduction of CUX1 leads to partial reduction of contacts at all CUX1-regulated looping events, or we may find that a subset of CUX1 genomic targets is affected by reduced CUX1 levels. Both results are informative in interpreting the *cis*-regulatory logic of a the transcriptional misregulation caused by haploinsufficiency. We expect that CUX1 drives the expression of genes involved in lineage specification.

Potential problems and alternative strategies. If we have low efficiency of homology mediated repair in Aim 1 and/or challenges acquiring cells with biallelic degron tagging, there are multiple compounds at our disposal to enhance homologous repair (25). If these efforts also encounter challenges, we will use AML cell lines that only harbor one allele of *CUX1*, of which we have several. In the unlikely event that we are unable to establish degron-labeled CUX1 cell lines, we have two alternative approaches. First, we will perform degron tagging of the N-terminal end of CUX1. Second, we will perform the proposed experiments in the setting of CRISPR-mediated *CUX1* inactivation. If we encounter difficulties with the fastGRO protocol, there are multiple alternative methods for nascent RNA-seq (26). Similarly, there are multiple alternative approaches for chromosome conformation capture for limited cell numbers, as proposed in Aim 2. Micro-C is one such alternative (27). If the anti-CUX1 antibody does not perform well in HiChIP experiments, we will execute the studies with validated antibodies against cohesin complex members, which will remain informative of DNA looping (24).

MEASURING SUCCESS. We will measure our success by meeting the milestones indicated in our timeline.

TIMELINE Year:	1	2
Aim 1 Complete degron tagged lines	X X	
Aim 1 Phenotype, transcriptome, CUT&RUN analysis		X X
Aim 2 Generate Hi-C data	X X	
Aim 2 Data analysis, integration, & validation		X X
Submit multi-PI R01		X

INTER-INSTITUTIONAL COLLABORATION. **Dr. McNerney, MD/PhD,** is a physician-scientist investigating the pathogenesis of high-risk myeloid malignancies. Her seminal work includes the identification of *CUX1* as a chr. 7 tumor suppressor gene. Her lab is a leader in CUX1 biology with unique expertise, tools, and mouse models to study this complex gene. **Dr. Yue, PhD,** is the founding director of the Center for Cancer Genomics at NU and is investigating the role of epigenomics and 3D organization in hematopoietic and solid tumors. In addition to his fundamental discoveries in the pathogenesis of cancer genome biology, his lab has developed computational software widely used in the field. Through these complementary expertise, we are uniquely poised to uncover the role of CUX1 in genome architecture.

FUTURE DIRECTIONS. Accomplishing the proposed studies will set the foundation for a success NHLBI R01 application. We anticipate the following Specific Aims building on the data generated in the current proposal: 1) Determine the role of CUX1 dosage in genome architecture during differentiation of primary hematopoietic stem and progenitors and how haploinsufficiency disrupts this process in primary AML patient samples with CUX1 deficiency. 2) Identify the CUX1 protein domains, dimerization, and interaction partners necessary for the formation of DNA looping. Overall, this work will identify a novel mechanism of action for a critical regulator of tissue homeostasis and provide a missing link connecting CTCF and cohesin with tissue-specific transcriptional regulation.

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Program Director/Principal Investigator (Last, First, Middle): McNerney, Megan

DETAILED BUDGET FOR INITIAL BUDGET PERIOD DIRECT COSTS ONLY

FROM 6/1/2023 THROUGH 5/31/2025

List PERSONNEL *(Applicant organization only)*
Use Cal, Acad, or Summer to Enter Months Devoted to Project
Enter Dollar Amounts Requested *(omit cents)* for Salary Requested and Fringe Benefits

NAME	ROLE ON PROJECT	Cal. Mnths	Acad. Mnths	Summer Mnths	INST.BASE SALARY	SALARY REQUESTED	FRINGE BENEFITS	TOTAL
Megan McNerney	PD/PI	0.24				0		
Saira Khan	Senior Technician	6			56,700	28,350	7,150	35,500
SUBTOTALS →								35,500

CONSULTANT COSTS	
EQUIPMENT *(Itemize)*	
SUPPLIES *(Itemize by category)*	21,000
TRAVEL	
INPATIENT CARE COSTS	
OUTPATIENT CARE COSTS	
ALTERATIONS AND RENOVATIONS *(Itemize by category)*	
OTHER EXPENSES *(Itemize by category)*	6,000

CONSORTIUM/CONTRACTUAL COSTS	DIRECT COSTS	
SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD *(Item 7a, Face Page)*		$ 62,500
CONSORTIUM/CONTRACTUAL COSTS	FACILITIES AND ADMINISTRATIVE COSTS	
TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD		$ 62,500

Program Director/Principal Investigator (Last, First, Middle): McNerney, Megan

BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD
DIRECT COSTS ONLY

BUDGET CATEGORY TOTALS	INITIAL BUDGET PERIOD *(from Form Page 4)*	2nd ADDITIONAL YEAR OF SUPPORT REQUESTED	3rd ADDITIONAL YEAR OF SUPPORT REQUESTED	4th ADDITIONAL YEAR OF SUPPORT REQUESTED	5th ADDITIONAL YEAR OF SUPPORT REQUESTED
PERSONNEL: *Salary and fringe benefits. Applicant organization only.*	35,000	35,000			
CONSULTANT COSTS					
EQUIPMENT					
SUPPLIES	21,500	21,500			
TRAVEL					
INPATIENT CARE COSTS					
OUTPATIENT CARE COSTS					
ALTERATIONS AND RENOVATIONS					
OTHER EXPENSES	6,000	6,000			
DIRECT CONSORTIUM/ CONTRACTUAL COSTS					
SUBTOTAL DIRECT COSTS *(Sum = Item 8a, Face Page)*					
F&A CONSORTIUM/ CONTRACTUAL COSTS					
TOTAL DIRECT COSTS	62,500	62,500			

TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD	**$** 125,000

JUSTIFICATION. Follow the budget justification instructions exactly. Use continuation pages as needed.

BUDGET JUSTIFICATION – MCNERNEY LAB

The inclusion of faculty salary in this budget reflects the policy of the Division of the Biological Sciences (BSD) and the Pritzker School of Medicine. It is expected that investigators will recover reasonable and appropriate salary support from grants and contracts proportionate to the fraction of their time and effort devoted to the project. BSD faculty appointments are made on a twelve-month basis and the requested salary is based upon the investigator's total University compensation. If an award is made in a reduced amount, the investigator will be expected to retain an appropriate amount of salary support in the budget.

PERSONNEL:

Megan McNerney, M.D. Ph.D. (Principal Investigator, 0.24 calendar months): Dr. McNerney is an Associate Professor in the Departments of Pediatrics Hematology/Oncology and Pathology, an Attending in the Genomic and Molecular Pathology clinical lab, and Assistant Program Leader of the Molecular Mechanisms of Cancer Program, The University of Chicago NCI-Designated Comprehensive Cancer Center. Dr. McNerney will provide overall leadership to the project and will oversee all personnel in her lab responsible for the day-to-day operations and experiments. Dr. McNerney is responsible for all aspects of the project, ensuring that research goals are met in a timely manner with scientific integrity, that the work is done within budgeted amounts, and is in compliance with the University and funding agency regulations. She will help design experiments, analyze genomics data and interpret results, and plan subsequent experiments. She will also be responsible for assisting in the writing and editing manuscripts resulting from this work. Dr. McNerney's salary is recovered from her Leukemia and Lymphoma Society Scholar award. Thus, she is providing effort but without requesting salary recovery.

Saira Khan (Senior Research Technician, 6 calendar months): Ms. Khan is an experienced Senior Research Technologist in the McNerney lab. She received two Master's degrees in Biological Sciences and Zoology. Ms. Khan has extensive experience in protein biochemistry, cell biology, cell culture, molecular biology, and functional genomics assays. She will be performing the experimental work within Aim 1 of the proposed project, including reagent development, tissue culture experiments, and the proposed biochemical experiments. She will coordinate with the Feng lab to support the execution of the proposed experiments in Aim 2.

Fringe benefit rate is calculated at 25.3%.

SUPPLIES:

Tissue culture ($8,000/year): We are requesting funds for purchasing human CD34+ cells, culturing cells, proliferation assays, and antibodies for detecting differentiation markers. For these activities we need media, growth factors, serum, antibiotics, plates, gloves, pipettes, tips, and other miscellaneous supplies.

Molecular reagents ($8,000/year): We are requesting funds for general molecular biology supplies for this project. Required reagents include Taq DNA polymerase, primers, DNA and RNA isolation reagents, dNTPs, agarose, DNA ladders, RT-PCR reagents, transfection reagents, SYBR Green, BrdU, gRNAs, Cas9, repair templates, $dTAG^V$-1 and vectors.

General lab supplies ($5,000/year): Funds are needed to cover the costs of consumable supplies not indicated above. Necessary reagents include sterile filters, pipettes, tips, centrifuge tubes, Eppendorf tubes, buffers, flasks, dishes, antibodies for western blots including anti-GAPDH, western blot gels, protein ladders, film, HRP detection, protein quantification kits, bacterial growth media, competent cell costs, glassware, dry ice, etc.

OTHER EXPENSES:

Next generation sequencing: ($2,000/year): Funds are requested for fastGro experiments including library preparation kits, performing Bioanalyzer sample quality control, and next-generation sequencing for transcriptome profiling.

Sanger sequencing ($2,000/year): Sanger sequencing is necessary for plasmid quality control, testing gRNA efficiency, and identifying correctly modified cells.

Flow cytometry ($2,000/year): Cell sorting on the Aria is $90 per hour. Benchtop analyzers are billed at $30/hour. We also request funds for fluorescently tagged antibodies and viability dyes.

Program Director/Principal Investigator (Last, First, Middle): Yue, Feng

DETAILED BUDGET FOR INITIAL BUDGET PERIOD DIRECT COSTS ONLY

FROM 6/1/2023 THROUGH 5/30/2025

List PERSONNEL *(Applicant organization only)*
Use Cal, Acad, or Summer to Enter Months Devoted to Project
Enter Dollar Amounts Requested *(omit cents)* for Salary Requested and Fringe Benefits

NAME	ROLE ON PROJECT	Cal. Mnths	Acad. Mnths	Summer Mnths	INST.BASE SALARY	SALARY REQUESTED	FRINGE BENEFITS	TOTAL
Feng Yue	PD/PI	0.12				4,074	1,031	5,105
TBD	Post-doc fellow	6			52,704	26,352	6,667	33,019
SUBTOTALS →								38,124

CONSULTANT COSTS	
EQUIPMENT *(Itemize)*	
SUPPLIES *(Itemize by category)*	4,000
TRAVEL	
INPATIENT CARE COSTS	
OUTPATIENT CARE COSTS	
ALTERATIONS AND RENOVATIONS *(Itemize by category)*	
OTHER EXPENSES *(Itemize by category)*	20,376

CONSORTIUM/CONTRACTUAL COSTS	DIRECT COSTS	
SUBTOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD *(Item 7a, Face Page)*		$ 62,500
CONSORTIUM/CONTRACTUAL COSTS	FACILITIES AND ADMINISTRATIVE COSTS	
TOTAL DIRECT COSTS FOR INITIAL BUDGET PERIOD		$ 62,500

Program Director/Principal Investigator (Last, First, Middle): Yue, Feng

BUDGET FOR ENTIRE PROPOSED PROJECT PERIOD
DIRECT COSTS ONLY

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PERSONNEL: *Salary and fringe benefits. Applicant organization only.*	38,124	38,124			
CONSULTANT COSTS					
EQUIPMENT					
SUPPLIES	4,000	4,000			
TRAVEL					
INPATIENT CARE COSTS					
OUTPATIENT CARE COSTS					
ALTERATIONS AND RENOVATIONS					
OTHER EXPENSES	20,376	20,376			
DIRECT CONSORTIUM/ CONTRACTUAL COSTS					
SUBTOTAL DIRECT COSTS *(Sum = Item 8a, Face Page)*					
F&A CONSORTIUM/ CONTRACTUAL COSTS					
TOTAL DIRECT COSTS	62,500	62,500			

TOTAL DIRECT COSTS FOR ENTIRE PROPOSED PROJECT PERIOD	**$** 125,000

JUSTIFICATION. Follow the budget justification instructions exactly. Use continuation pages as needed.

BUDGET JUSTIFICATION – YUE LAB

Key/senior Personnel

Feng Yue, Ph.D., Principal Investigator (0.12 CM)

Dr. Yue is the Duane and Susan Burnham Professor of Molecular Medicine, founding director of the Center for Cancer Genomics, and director of the Center for Advanced Molecular Analysis at Northwestern University Feinberg School of Medicine. He has extensive experience in coordinating large collaborative research. He led the overall integrative analysis effort for the mouse ENCODE consortium and currently serves as co-chair for the Joint Analysis Workgroup in the 4D Nucleome Project, and also co-chairs the Steering Committee for the NIH IGVF Consortium. Dr. Yue co-chaired the ENCODE consortium outreach workgroup from 2015 - 2017 and organized many outreach sessions in prestigious conferences like ASHG and Keystone Symposia. He co-chaired the ENCODE Users Meetings with more than 130 registrants in 2015 and 2016. Due to his scientific leadership, innovative research, and deep commitment to scientific outreach, he was nominated for the Presidential Early Career Award for Scientists and Engineers (PECASE) by NHGRI in 2019. He will lead the Hi-C effort at Northwestern site.

Postdoc fellows, TBD, (6 CM)

This position needs to have extensive experience working with different omics technologies such as Hi-C and ChIP-Seq. This person will also perform integrative data analysis.

Supplies

Molecular reagents ($4,000 per year)

Reagents needed for Hi-C and 4-C library preparation include restriction enzymes, primers, biotinylated adapters, RNase A, DNase I, Klenow fragment, polymerase, library preparation kits, AMPure beads, end repair kit, Dynabeads, nucleotides, media, and buffers.

Other expenses

Next-generation sequencing ($20,376 per year)

A major cost of this arm of the project is Illumina sequencing of Hi-C and 4C libraries. We propose to perform *in situ* Hi-C on three human AML cell lines at 3 different time points with 3 different conditions (wildtype, 50% CUX1 knockdown, and near complete CUX1 knockdown) with n=2 biological replicates per condition, totaling 54 independent experiments. We will need ~ 600 million paired-end reads per sample. 4C experiments will be performed on 3 independent sites (n=2 replicates per site).

BIOGRAPHICAL SKETCH

NAME: **McNerney, Megan E.**

eRA COMMONS USER NAME: MMCNERNEY

POSITION TITLE: Associate Professor, Dept. of Pediatrics and Dept. of Pathology, The University of Chicago

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE	Completion Date	FIELD OF STUDY
Northwestern University, Evanston, IL	B.A.	06/1999	Molecular & Cell Biology
The University of Chicago, Chicago, IL	Ph.D.	06/2005	Immunology
The University of Chicago, Chicago, IL	M.D.	06/2007	Medicine
The University of Chicago, Chicago, IL	Residency	07/2010	Clinical Pathology
The University of Chicago, Chicago, IL	Postdoctoral	09/2014	Genomics & Systems Biology

A. PERSONAL STATEMENT

I am a cancer genomicist and physician-scientist investigating how genetic changes alter normal hematopoiesis and drive malignancy. I also serve as an Attending in the Genomic and Molecular Pathology clinical laboratory where I sign-out next-generation sequencing panels for oncology patients. This work provides a clinical perspective that informs my research program. Over 50,000 people are diagnosed with a myeloid neoplasm every year in the U.S. alone. A high-risk subset of patients is unresponsive to treatment and their survival is less than a year. The long-term goal of my lab is to improve the outcome for these patients. To this end, our research focuses on understanding the underlying genomic abnormalities in high-risk myeloid neoplasms. Half of adverse-risk myeloid neoplasms have lost all or part of chromosome 7 [-7/del(7q)]. To understand the pathogenesis of -7/del(7q), we have developed and employed an arsenal of innovative methods and tools, including genetically accurate pre-clinical mouse models, functional genomics, single-cell approaches, CRISPR screening, proteomics, and machine learning. We have identified pathogenic tumor suppressor genes on chromosome 7, including the CUX1 transcription factor, and shown that loss of CUX1 is sufficient to cause myeloid disease. We demonstrated that pre-existing CUX1-deficiency, in the context of clonal hematopoiesis, disrupts DNA repair and sensitizes mice to therapy-related myeloid neoplasms (t-MN) after chemotherapy, supporting a paradigm shift in understanding the etiology of t-MN. We repudiated the existence of the p75 isoform of CUX1 and undermined the literature that CUX1 is oncogenic. Ongoing work includes determining the mechanistic role of CUX1 in development and gene expression and how 7q tumor suppressor genes cooperate in leukemogenesis. Feng Yue and I first interacted as participants in the Chicago Blood Stem Cell Group, which held joint Quarterly meetings with The University of Chicago, Northwestern, Loyola, and University of Illinois at Chicago for several years. Dr. Yue's expertise in genome biology and bioinformatics will dovetail perfectly with my lab's expertise in leukemogenesis, hematopoiesis, and CUX1 biology to successfully execute the proposed studies.

Ongoing projects that I would like to highlight include:

R01 CA231880-01 NIH/NCI (McNerney) 9/01/2018-8/31/2023
The impact of chromosome 7q deletions in myeloid malignancies of childhood.

R01 HL142782-01 NIH/NHLBI (McNerney) 7/15/2018-6/30/2023
Regulation of hematopoiesis by CUX1

Leukemia and Lymphoma Society Scholar Award (McNerney) 7/1/2022-6/30/2027
Genomic interrogation of high-risk myeloid neoplasms to identify new therapies

Janet D. Rowley Discovery Fund Award (M. McNerney) 7/1/2022-6/30/2023
The role of CUX1 in intestinal epithelium homeostasis

American Cancer Society Research Scholar Award (McNerney) 1/1/2019-12/31/2022
The genetic and environmental etiology of therapy-related myeloid neoplasms

DoD Peer Reviewed Cancer, Impact Award (Kron) 7/1/2020-6/30/2023
Preventing blood cancers and other malignancies in military personnel at risk due to occupational radiation exposure

NIH/NCI P30 CA014599 (Ondunsi) 05/22/2018-03/31/2023
Cancer Center Support Grant

Overlap: none

B. POSITIONS, SCIENTIFIC APPOINTMENTS, AND HONORS

Positions and Employment

2020-present Associate Professor, Dept. of Pathology and Dept. of Pediatrics, Section of Hematology/Oncology, The University of Chicago
2020-present Assistant Program Leader of the Molecular Mechanisms of Cancer Program, The University of Chicago NCI-Designated Comprehensive Cancer Center
2014-present Attending Pathologist, Division of Genomic and Molecular Pathology
2014-present Fellow, Institute for Genomics and Systems Biology
2014-present Committee on Genetics, Genomics, and Systems Biology
2014-present Committee on Cancer Biology
2014-2019 Assistant Professor, Dept. of Pathology and Dept. of Pediatrics, Section of Hematology/Oncology, The University of Chicago
2013-present Member, The University of Chicago NCI-Designated Comprehensive Cancer Center
2012-2014 Instructor, Department of Pathology, The University of Chicago
2010-2012 Postdoctoral Fellow and Advanced Research Fellow, Departments of Pathology and Human Genetics, The University of Chicago
2007-2010 Resident, Clinical Pathology, Department of Pathology, The University of Chicago

Other Experience and Professional Memberships

2010 American Board of Pathology Certification, Clinical Pathology
2010- Member, American Society of Hematology
2007 State of Illinois Medical License (License Number 036.124163)

Honors

2022 Leukemia and Lymphoma Society Scholar Award
2020 Faculty Award for Excellence in Graduate Teaching and Mentoring
2019 American Cancer Society Research Scholar Award
2017 FASEB Hematologic Malignancies Conference Mini-Talk Award
2017 American Society of Hematology Junior Faculty Scholar Award
2014 “V” Foundation in Cancer Research Scholar Award
2013 Leukemia and Lymphoma Society, Illinois Chapter Researcher of the Year
2013 Evans Travel Award, FASEB Hematologic Malignancies Science Research Conference
2010 Robert W. Wissler Fellowship Award, Department of Pathology, University of Chicago
2010 Leukemia and Lymphoma Society Fellow Award
2009 Resident Research Grant, College of American Pathologists
2007 Leon Jacobson Prize for Senior Scientific Presentation, Pritzker School of Medicine
2006 Committee on Immunology Best PhD Thesis Award, University of Chicago
2006 Honorable Mention Best PhD Dissertation Award, Biological Sciences, University of Chicago
2005 Robert E. Priest Pathology Merit Award, Department of Pathology, University of Chicago
2004 Doolittle-Harrison Travel Fellowship, University of Chicago
2004 Best Oral Presentation, International NK Cell Workshop, Noordwijkerhout, The Netherlands
2003 Best Graduate Student Poster, Committee on Immunology Retreat, University of Chicago
2003-2007 Elaine Frank Family Fellowship for Medical Research, MSTP, University of Chicago
2000-2007 Medical Scientist Training Program, NIH
1999 Graduation Cum Laude, Northwestern University
1999 Graduation with Departmental Honors, Biological Sciences, Northwestern University
1997 Edgar Macey Scholarship for Summer Research, Northwestern University

Professional Activities and Service

2022-2026 NIH R01 Study Section, Gene Regulation in Cancer (GRIC), permanent member
2022 NIH R01 Study Section, Special Emphasis Panel/Scientific Review Group ZRG1 VH-C (02)

2022 NIH NCI P01 Study Section, Special Emphasis Panel ZCA1 RPRB-L M1 S
2022 V Scholar Pediatric Cancer Research Grant review committee
2021-present American Society of Hematology Scientific Committee on Epigenetics and Genomics
2021-present American Cancer Society Illinois Area Board Research Delegate
2021 NIH R01 Study Section, Cancer Molecular Pathology (CAMP)
2021 NIH R01 Study Section, Hemostasis, Thrombosis, Blood Cells, Transfusion Medicine (HTBT)
2021 ASH Scholar Award Basic/Translational grant review
2020 NIH R01 Study Section, Molecular and Cellular Hematology (MCH)
2020 NIH R01 Study Section, Special Emphasis Panel/Scientific Review Group ZRG1 VH-C (02)
2019 NIH R01 Study Section, Special Emphasis Panel/Scientific Review Group ZRG1 OBT-H (02) M
2018 NIH R01 Study Section, Early Career Reviewer, Cancer Molecular Pathology (CAMP)
2018 V Foundation Robin Roberts Cancer Survivorship grant review

C. CONTRIBUTIONS TO SCIENCE (38 manuscripts) in MyBibliography:
https://www.ncbi.nlm.nih.gov/myncbi/megan.mcnerney.1/bibliography/public/

1. Identifying the pathogenesis of -7/del(7q) in myeloid neoplasms.

My seminal discovery in cancer research was the identification of *CUX1* as a tumor suppressor gene on chromosome 7. Over 40 years ago, it was found that 20% of myeloid cancers have lost all or a portion of chromosome 7 [-7/del(7q)], portending a dismal prognosis of less than one-year survival. -7/del(7q) also occurs in 50% of therapy-related myeloid neoplasms which are a devastating side-effect of chemotherapy/radiation that occurs patients that survived a prior cancer. Many investigators have sought to identify the putative tumor suppressor gene(s) on chromosome 7. I used high-resolution genomic technologies to map *CUX1*, and discovered it is a highly conserved tumor suppressor in humans and even *Drosophila*.[a] This was a major breakthrough for the field. I identified the spectrum of mutations that co-occur with -7/del(7q) in forty patients with high-risk acute myeloid leukemia (AML).[b] I discovered that -7/del(7q) leukemias have a distinct mutational profile characterized by RAS pathway activating mutations. Using an array-based CRISPR screen and orthogonal machine learning approach, we identified additional chromosome 7 tumor suppressor genes and proposed the novel concept that chromosome 7 is a contiguous gene syndrome.[c] Finally, in collaboration with my clinical colleagues, I provided next-generation sequencing analyses to characterize the genetics of high-risk patients that respond to new treatment protocols.[d]

a. **McNerney ME***, Brown, CD, Wang, X, Bartom, ET, Karmakar, S, Bandlamudi, C, Yu, S, Ko, J, Sandall, BP, Stricker, T, Anastasi, J, Grossman, RL, Cunningham, JM, Le Beau, MM, and White, KP*. (2013) *CUX1* is a haploinsufficient tumor suppressor gene on chromosome 7 frequently inactivated in acute myeloid leukemia. **Blood**. 121:975-983. PMC3567344. *Co-corresponding author.
b. **McNerney ME**, Brown, CD, Peterson, AL, Banerjee, M, Larson, RA, Le Beau, MM, and White, KP. (2014) Frequent RAS activating mutations in -7/del(7q) acute myeloid leukemia. **British Journal of Haematology**. 166:550-556. PMC5479678
c. Baeten, JT, Liu, W, Preddy, I, Zhou, N, and **McNerney, ME**. (2022) CRISPR screening in human hematopoietic stem and progenitor cells reveals an enrichment for tumor suppressor genes within chromosome 7 commonly deleted regions. **Leukemia.** DOI: 10.1038/s41375-021-01491-z
d. Cahill, KE,*, Karimi, YH,* Karrison, TG, Jain, N, Green, M, Weiner, H, Fulton, N, Kadri, S Godley, LA, Artz, AS, Liu, H, Thirman, MJ, Le Beau, MM, **McNerney, ME**, Segal, J, Larson, RA, Stock, W, and Odenike, O (2020) A phase I study of azacitidine with high-dose cytarabine and mitoxantrone in high-risk acute myeloid leukemia**. Blood Advances**. 4(4):599-606. * These authors contributed equally to this work

2. Determining the molecular mechanisms of CUX1 tumor suppressor activity.

To determine the role for CUX1 in hematopoiesis, we generated two CUX1-knockdown mouse models which spontaneously develop MDS and MDS/MPN.[a] In diseased mice, restoration of CUX1 expression was sufficient to reverse the disease. This is significant as it demonstrates that sustained reduction of this single 7q gene is necessary and sufficient to cause disease. Mechanistically, we showed that CUX1 is regulating expression of *Pik3ip1*, an inhibitor of PI3K signaling, to control normal cell growth. To determine how CUX1 acts as a transcriptional regulator, we determined CUX1 transcriptional genomic targets in three human cell types, including AML, and characterized the mechanism by which haploinsufficiency is encoded at the *cis*-regulatory level.[b] By understanding the networks and logic of transcriptional misregulation in *CUX1*-deleted leukemia, I will reveal new therapeutic vulnerabilities in this disease.[c] Lastly, we charactered the CUX1 isoforms present in hematopoietic cells and report that they express the full-length p200 CUX1.[c] Through the course of these

studies, we demonstrated that the short, p75 isoform does not exist and is likely an artefact. Based on these results, prior studies of p75 require reevaluation, including the interpretation of oncogenic roles previously attributed to short CUX1 isoforms. Overall, my lab has established itself as a leader on CUX1 biology.[d]

a. An, N, Khan, S, Imgruet, M, Gurbuxani, SK, Konecki, SN, Burgess, M, and **McNerney, ME** (2018) Gene dosage effect of CUX1 in a murine model disrupts HSC homeostasis and controls the severity and mortality of MDS. **Blood**. 131:2682-2697. PMC6032890.
b. Arthur*, R, An*, N, Khan, S, and **McNerney, ME** (2017) The haploinsufficient tumor suppressor, CUX1, is an analog transcriptional regulator that controls target genes through distal enhancers that loop to target promoters. **Nucleic Acids Research**. 45:6350-6361. PMC5499738. *Co-first authors
c. Krishnan, M, Wolfgeher, DJ, Senagolage, MD, Baeten, JT, Kron, SJ, and **McNerney, ME**. (2022) Genomic studies controvert the existence of the CUX1 p75 isoform. **Scientific Reports.** 12:151.
d. Jotte, M and **McNerney, ME**. (2022) The significance of CUX1 and chromosome 7 in myeloid malignancies. **Current Opinion in Hematology***. 29*:92-102*.*

3. Uncovering the etiology of therapy-related myeloid neoplasms

Therapy-related myeloid neoplasms (t-MNs) are high-risk late effects with poorly understood pathogenesis in cancer survivors. It has been postulated that, in some cases, hematopoietic stem and progenitor cells (HSPCs) harboring mutations are selected for by cytotoxic exposures and transform. We evaluated this model in the context of deficiency of CUX1, which is deleted in half of t-MN cases. We report that CUX1 has a critical early role in the DNA repair process in HSPCs.[a] Mechanistically, CUX1 recruits the histone methyltransferase EHMT2 to DNA breaks to promote downstream H3K9 and H3K27 methylation, phosphorylated ATM retention, and subsequent γH2AX focus formation and propagation. As a consequence, preexisting CUX1 deficiency predisposes mice to highly penetrant -MN. These findings establish the importance of epigenetic regulation of HSPC DNA repair and position CUX1 as a gatekeeper in myeloid transformation. I have collaborated with multiple investigators, including Michelle Le Beau, in unraveling the complexity of the genomics and pathogenesis of t-MN. In these studies, I have contributed to the design, bioinformatic analysis, and interpretation of functional genomic datasets.[b,c] Finally, I have demonstrated expertise on the topic of t-MN by a first author review in Nature Reviews Cancer.[d] Overall, this work has helped determine how somatic changes drive t-MN.

a. Imgruet, MK, Lutze, J, An, N, Hu, B, Khan, S, Kurkewich, Martinez, TC, Wolfgeher, DJ, , Gurbuxani, S, Kron, SJ, and **McNerney, ME**. (2021) Loss of a 7q gene, *CUX1,* disrupts epigenetic-driven DNA repair and causes therapy-related myeloid neoplasm. **Blood**. 138:790–805. PMC8414261
b. Stoddart, A, Wang, J, Fernald, AA, Davis, EM, Johnson, CR, Hu, C, Cheng, JX, **McNerney, ME**, and Le Beau, MM. (2020) Cytotoxic therapy-induced effects on both hematopoietic and marrow stromal cells promotes therapy-related myeloid neoplasms. **Blood Cancer Discovery**. DOI: 10.1158/2643-3230.BCD-19-0028
c. Stoddart, A, Fernald, AA, Davis, EM, **McNerney, ME**, and Le Beau, MM. (2022) EGR1 haploinsufficiency confers a fitness advantage to hematopoietic stem cells following chemotherapy. **Experimental Hematology**. *In press.*
d. **McNerney, ME**, Godley, LA, and Le Beau, MM. (2017) Therapy-related myeloid neoplasms: when genetics and environment collide. **Nature Reviews Cancer***.* Invited review. *17*:513-27.

4. The role of CD244 (2B4) regulation of natural killer cells

Natural killer (NK) cells are critical in the immune response to tumor cells, virally infected cells, and bone marrow allografts. In my graduate work with Vinay Kumar, I focused on how NK cells are regulated to kill tumor cells while remaining tolerant of normal cells. 2B4 (CD244) is a receptor expressed on all NK cells and the ligand for 2B4, CD48, is expressed on hematopoietic cells. I reported that 2B4-CD48 is a previously unrecognized receptor-ligand system of NK cell inhibition.[a] I discussed the implications of this novel system of self-tolerance in a review in Nature Reviews Immunology.[d] I published two additional first or co-first author papers exploring this system further, as it relates to anti-tumor immunity and tolerance of allogeneic bone marrow transplants.[b,c] This work is of immediate relevance for the treatment of oncology patients, as efforts are currently being made to modulate NK cell activity to improve the host vs. leukemia response while preventing host rejection of therapeutic bone marrow transplants.

a. Lee, KM*, **McNerney, ME***, Stepp, SE, Mathew, PA, Schatzle, JD, Bennett, M, and Kumar, V. (2004) 2B4 acts as a non-MHC binding inhibitory receptor on mouse NK cells. **Journal of Experimental Medicine**. 199:1245-1254. *Authors contributed equally. PMC2211902

b. **McNerney, ME**, Guzior, D, and Kumar, V. (2005) 2B4 (CD244) – CD48 interactions provide a novel MHC class I-independent system for NK cell self-tolerance in mice. **Blood**. 106:1337-1340. PMC1895194
c. Lee, KM*, Forman, J*, **McNerney, ME***, Stepp, S, Kuppireddi, S, Guzior, D, Latchman, YE, Sayegh, MH, Yagita, H, Park, CK, Oh, SB, Wulfing, C, Schatzle, J, Mathew, PA, Sharpe, AH, and Kumar, V. (2006) Requirement of homotypic NK cell interactions through 2B4(CD244)/CD48 in the generation of NK effector functions. **Blood**. 107:3181-3188. *Authors contributed equally. PMC1895752
d. Kumar, V and **McNerney, ME**. (2005) A new self: MHC class I independent NK cell self-tolerance. **Nature Reviews Immunology**. 5:363-374.

5. Innate immune regulation in transplant and tumor immunology

To achieve donor-specific immune tolerance to allogeneic organ transplants, it is imperative to understand the cell types involved in acute allograft rejection. As a graduate student, I helped demonstrate that when T-cell responses are suboptimal in mice, simulating transplanted patients taking immunosuppressive drugs, the participation of NK cells becomes apparent.[a,b] We found that subsets of NK were required for cardiac allograft rejection, thus, therapies aimed at specific subsets of NK cells may facilitate transplantation tolerance. In addition, I co-authored a paper where I contributed to the observation that 2B4 modulates the NK cell response to tumor cells.[c] As a resident, I continued my scholarly activity of the response of the immune system to allogeneic transplantation. I wrote a first-author manuscript of a case report and review of the literature on the rapid development of pathogenic antibodies in a patient in response to a single red blood cell transfusion.[d] Overall, I have built a body of literature on the regulation of the innate immune system to alloantigens.

a. **McNerney, ME***, Lee, KM*, Zhou, P*, Molinero, L, Mashayekhi, M, Guzior, D, Sattar, H, Kuppireddi, S, Wang, CR, Kumar, V, and Alegre, ML. (2006) Role of natural killer cell subsets in cardiac allograft rejection. **American Journal of Transplantation**. 6:505-513. *Authors contributed equally.
b. Alegre, ML and **McNerney, ME**. (2007) NK cell subsets in allograft rejection and tolerance. **Current Opinion in Organ Transplantation**. 12:10-16.
c. Vaidya SV, Stepp SE, **McNerney ME**, Lee JK, Bennett M, Lee KM, Stewart CL, Kumar V, Mathew PA. (2005) Targeted disruption of the 2B4 gene in mice reveals an *in vivo* role of 2B4 (CD244) in the rejection of B16 melanoma cells. **Journal of Immunology***. 174:*800-807.
d. **McNerney, ME**, Baron, BW, Volchenboum, SL, Papari, M, Keith, M, Williams, K, and Richa, E. (2010) Development of warm auto- and alloantibodies in a three year-old boy with sickle cell hemoglobinopathy following his first transfusion of a single unit of red blood cells. **Blood Transfusion***. 8*:126-128. PMC2851217

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: Yue, Feng

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

POSITION TITLE: Duane and Susan Burnham Professor of Molecular Medicine

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
Peking University, Beijing, China	B.A.	07/2000	English
University of South Carolina, Columbia, SC	M.S.	08/2003	Computer Science
University of South Carolina, Columbia, SC	Ph.D.	05/2008	Computer Science
University of California, San Diego, California	Post-doc	09/2013	Functional Genomics, Epigenomics

A. Personal Statement

My long-term goal is to study gene regulation and the impact of genetic variations in human diseases. I was initially trained as a computer scientist specializing on algorithm development, and then received rigorous training in functional genomics in Dr. Bing Ren's group at UC San Diego. During the period, I was an essential team member that discovered the topologically associating domains (TADs) and also co-led an effort to generate the most comprehensive cis-regulation element annotation in the mouse genome at the time.

I started my own lab in the Pennsylvania State University in 2013 and was recruited to Northwestern University as the founding director of the Center for Cancer Genomics at Lurie Comprehensive Cancer Center in 2019. I have been a long-time member of several large NIH-funded consortia (such as the ENCODE and the Roadmap Epigenomics Project). I led the overall organizing and analysis effort for the mouse ENCODE consortium (Yue et al. Nature 2014). Currently, I serve as the co-chair for the Joint Analysis Working group in the 4D Nucleome Project (4DN), leading the consortium effort to integrate multiple data types to profile the 3D genome organization and its relationship with gene regulation and diseases. I co-chair the Steering Committee of the NIH Impact of Genomic Variation on Function Consortium (IGVF).

My lab is well-versed in both computational biology and functional genomics. Recently, our group produced the most comprehensive map of enhancers, silencers, whole genome methylome, and 3D genome organization in up to 30 acute myeloid leukemia (AML) patients (Xu et al. Nature 2022), by performing RNA-Seq, ATAC-Seq, CUT&Tag for multiple histone modifications, and Hi-C experiments. We also constructed disease-specific gene regulatory network in this work and identified complex relationship between key TFs and their downstream target genes. My group also has demonstrated how epigenome and 3D genome structure are altered and led to gene dysregulation in different types of solid tumor as well, such as bladder cancer and brain tumor (Ding et al. Leukemia 2019, Iyyanki et al. Genome Biol. 2021, Wang et al. Science Adv. 2021).

I am dedicated to education and outreach. My lab has graduated seven Ph.D. students and each of them has at least one first-author publication upon their graduation in journals such as *Genome Research*, *Nature Communications*, or *Nature Genetics*. Two of them continued their training in Md/PhD program; the rest of them either joined pharmaceutical companies or pursue postdocs. I have been passionate about community outreach. I co-chaired the ENCODE consortium Outreach workgroup for several years and actively organized the 4D Nucleome Project outreach activities as well, including chairing education sessions in conferences like ASHG annual meetings and Keystone Symposia.

Ongoing and recently completed projects that I would like to highlight include:

R35GM124820
Yue (PI)
09/01/2017-07/31/2027
High throughput interrogation of non-coding variants and 3D genome organization

U24HG012070
Yue (MPI), Ting Wang (contact PI)
08/24/2021-05/31/2026
WashU-Northwestern Genomic Variation and Function Data and Administrative Coordinating Center

UM1HG012649
Yue (MPI), Mazhar Adli (Contact PI)
09/22/2022-06/30/2027
Molecular and cellular characterization of essential human genes.

R01HG011207
Yue (PI)
06/07/2021-03/31/2025
Computational methods to identify neo-TADs and enhancer-hijacking in rearranged genomes

1R01HG009906
Yue (PI)
01/08/2018-12/31/2022
Visualization, modeling and validation of chromatin interaction data

Citations:

1. Xu J, Fan S, Lyu H, Kobayashi M, Zhang B., Zhao Z, Hou Y, Wang X, Luan Y, Jia B, Stasiak L, Wong JH, Wang Q, Jin Q, Jin Q, Fu Y, Yang H, Hardison RC, Dovat S, Platanias LC, Diao Y, Yang Y, Yamada T, Viny AD, Levine RL, Claxon D, Broach JR, Zheng H, and **Yue F**. (2022) Subtype-specific 3D genome alteration in acute myeloid leukaemia. **Nature**; 611(7935):387-398. doi: 10.1038/s41586-022-05365-x. PMID: 36289338.
2. Wang X, Xu J, Zhang B, Hou Y, Song F, Lyu H, **Yue F**. (2021) Genome-wide detection of enhancer-hijacking events from chromatin interaction data in rearranged genomes. **Nature Methods** 18, 661–668 (2021). PMID: 34092790; PMCID: PMC8191102.
3. Yang H, Luan Y, Liu T, Lee HJ, Fang L, Wang Y, Wang X, Zhang B, Jin Q, Ang KC, Xing X, Wang J, Xu J, Song F, Sriranga I, Khunsriraksakul C, Salameh T, Li D, Choudhary MNK, Topczewski J, Wang K, Gerhard GS, Hardison RC, Wang T, Cheng KC, **Yue F**. (2020) A map of cis-regulatory elements and 3D genome structures in zebrafish. **Nature**;588(7837):337-343. doi: 10.1038/s41586-020-2962-9. PMID: 33239788; PMCID: PMC8183574.
4. Dixon JR, Xu J, Dileep V, Zhan Y, Song F, *et al.*, Noble WS, Dekker J, Gilbert DM, **Yue F**. (2018) Integrative Detection and Analysis of Structural Variation in Cancer Genomes. **Nat Genet**; 50(10):1388-1398. PubMed PMID: 30202056. PubMed Central PMCID: PMC6301019.

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2022.09 –	Professor, Department of Biochemistry and Molecular Genetics Professor, Department of Pathology
2020.03 –	Founding Director, Center for Advanced Molecular Analysis Northwestern Institute for Augmented Intelligence in Medicine

2019.07 –	Founding Director, Center for Cancer Genomics, Robert H. Lurie Comprehensive Cancer Center of Northwestern University
2019.07 –	Duane and Susan Burnham Professor of Molecular Medicine
2019.07–2022.08	Associate Professor with tenure, Dept. of Biochemistry and Molecular Genetics Northwestern University Feinberg School of Medicine
2015.01–2019.06	Director, Bioinformatics Division, Institute for Personalized Medicine, Pennsylvania State University
2013.10–2019.06	Assistant Professor, Pennsylvania State University, College of Medicine, Hershey, PA

Other Experience and Professional Memberships

2021.07 – 2024.06	Chartered member, Genomics, Computational Biology and Technology (GCAT) study section.
2021.04	NIDDK Catalyst Award (DP1, RFA-DK-20-024), Stage 1 reviewer.
2021.03	NIGMS Maximizing Investigators' Research Award (MIRA) (R35) for Early Stage Investigators, co-chair.
2020.11	NIGMS Maximizing Investigators' Research Award (MIRA) (R35) for Established Investigators, co-chair.
2020.07	NIDDK Special Emphasis Panel GRB-J O1 - Type 1 Diabetes Disease-associated Variants
2020.06	NIGMS Maximizing Investigators' Research Award (MIRA) (R35) for Established Investigators
2020.03	NIGMS Maximizing Investigators' Research Award (MIRA) (R35) for Early Stage Investigators
2019.11	NCI Special Emphasis Panel, Beau Biden Cancer Moonshot Initiatives, Develop New Cancer Technologies
2019.10	Genomics, Computational Biology and Technology (GCAT) study section, *ad hoc* member.
2019.07	NIDDK Special Emphasis Panel, Ancillary Studies to the NIDDK Inflammatory Bowel Disease Genetics Consortium (RFA-DK-18-017)
2019.04	NIDDK Special Emphasis Panel, Contribution of T1D Disease- associated Variants
2018.07	NIDDK Special Emphasis Panel, Ancillary Studies to the NIDDK Inflammatory Bowel Disease Genetics Consortium (RFA-DK-17-017)

Editorial Boards/Journal Reviewer:

Editorial Board: Science Advances, Genome Research, Leukemia & Lymphoma.

Reviewer: Nature, Nature Biotechnology, Nature Genetics, Nature Methods, Nature Cancer, Molecular Cell, Genome Biology, Genome Research, etc.

Honors

2019	Northwestern University Endowed Chair, Duane and Susan Burnham Professor of Molecular Medicine
2019	Presidential Early Career Award for Scientists and Engineers (PECASE), nominated by NHGRI.
2016	Leukemia Research Foundation Young Investigator Award
2015	Recipient of phRMA Research Starter Grant in Informatics
2015	23rd International conference on Intelligent Systems for Molecular Biology (ISMB 2015) Travel Fellowship
2008	Outstanding Graduate Student, College of Engineering and Computing, University of South Carolina

C. Contribution to Science

1. **Discovery and characterization of *cis*-regulatory elements and 3D genome organization in vertebrate genomes**: My research has been focused on understanding mechanisms that control gene expression in human and vertebrate model systems, through computational and experimental methods. As an active member of ENCODE consortium and a *lead analyst* for the mouse ENCODE consortium, I have extensive experience in large scale integrative analysis of gene expression and distal regulatory elements in the mammalian genome. I played an essential role in coordinating the organization and performing many key analysis of more than 1,000 data sets of gene expression (RNA-Seq), DNase I hypersensitivity (DNase-Seq), transcription factor binding and histone modification (ChIP-Seq) in a collection of 123 mouse tissue and cell types. In another recently work (Yang et al. 2020), we profiled the most comprehensive annotation in the zebrafish genome and studied the how the gene regulatory network was conserved between zebrafish, mice and human.
   a. Shen Y*, **Yue F***, McCleary DF, Ye Z, Edsall L, Kuan S, Wagner U, Dixon J, Lee L, Lobanenkov VV, Ren B. (2012) A map of the cis-regulatory sequences in the mouse genome. Nature;488(7409):116-20. PMID: 22763441; PMCID: PMC4041622. (*co-first author)
   b. **Yue F***, Cheng Y*, Breschi A*, Vierstra J*, Wu W*, Ryba T*, Sandstrom R*, Ma Z*, Davis C*, Pope BD*, Shen Y*, Pervouchine DD, … and the Mouse ENCODE Consortium. (2014) A comparative encyclopedia of DNA elements in the mouse genome. Nature; 515(7527):355-64. PMID: 25409824; PMCID: PMC4266106. (* equal contribution)
   c. Yang H, Luan Y, Liu T, Lee HJ, Fang L, Wang Y, Wang X, Zhang B, Jin Q, Ang KC, Xing X, Wang J, Xu J, Song F, Sriranga I, Khunsriraksakul C, Salameh T, Li D, Choudhary MNK, Topczewski J, Wang K, Gerhard GS, Hardison RC, Wang T, Cheng KC, **Yue F**. (2020) A map of cis-regulatory elements and 3D genome structures in zebrafish. Nature.;588(7837):337-343. doi: 10.1038/s41586-020-2962-9. Epub 2020 Nov 25. PMID: 33239788; PMCID: PMC8183574.
2. **Altered gene regulation in different types of cancer**: My lab has been working on identifying distal regulatory elements in difference types of cancer. By working with a group of PIs from the ENCODE consortium, we show that Hi-C can be used as tool for systematic discovery of SVs in the genome. We performed and analyzed Hi-C data in over 20 cancer cell lines and observed widespread neo-TADs and enhancer hijacking events, which potentially contribute to gene misregulation in cancer cells.
   a. Dixon JR[#], Xu J, Dileep V, Zhan Y, Song F, et al., Noble WS[#], Dekker J[#], Gilbert DM[#], **Yue F**[#]. (2018) Integrative Detection and Analysis of Structural Variation in Cancer Genomes. Nat Genet. 2018; 50(10):1388-1398. PubMed PMID: 30202056. PMCID: PMC6301019.
   b. Jing Zhang, Donghoon Lee, Vineet Dhiman, Peng Jiang, Jie Xu, Patrick McGillivray, Hongbo Yang, ..., **Feng Yue[#],** X. Shirley Liu[#], Kevin White[#], Mark Gerstein[#]. (2020) An integrative ENCODE resource for cancer genomics. Nat Commun; (11)3696. PMID: 32728046. PMCID: PMC7391744. ([#] Co-corresponding author. Our lab performed all the functional experiments in this work).
   c. Iyyanki T, Zhang B, Wang Q, Hou Y, Jin Q, Xu J, Yang H, Liu T, Wang X, Song F, Luan Y, Yamashita H, Chien R, Lyu H, Zhang L, Wang L, Warrick J, Raman JD, Meeks JJ, DeGraff DJ[#], **Yue F**[#]. (2021) Subtype-associated epigenomic landscape and 3D genome structure in bladder cancer. Genome Biol; 22(1):105. doi: 10.1186/s13059-021-02325-y. PubMed PMID: 33858483; PubMed Central PMCID: PMC8048365.
   d. Xu J, Fan S, Lyu H, Kobayashi M, Zhang B., Zhao Z, Hou Y, Wang X, Luan Y, Jia B, Stasiak L, Wong JH, Wang Q, Jin Q, Jin Q, Fu Y, Yang H, Hardison RC, Dovat S, Platanias LC, Diao Y, Yang Y, Yamada T, Viny AD, Levine RL, Claxon D, Broach JR, Zheng H, and **Yue F**. (2022) Subtype-specific 3D genome alteration in acute myeloid leukaemia. **Nature**; 611(7935):387-398. doi: 10.1038/s41586-022-05365-x. PMID: 36289338.
3. **Three-dimensional (3D) genome organization**: I have extensive experience studying the 3D spatial organization of the mammalian genome. I was a key member of the research team at Dr. Bing Ren's lab that discovered the mammalian genomes are organized into mega-base size self-interacting domains, commonly referred as topologically associated domains (TADs). After establishing my group in 2013, my lab has published a series of algorithms related with the analysis of 3D genome structure data and also performed Hi-C experiments to study the function of TAD and chromatin loops in the context of human diseases.

a. Dixon JR, Selvaraj S, **Yue F**, Kim A, Li Y, Shen Y, Hu M, Liu JS, Ren B. (2012) Topological domains in mammalian genomes identified by analysis of chromatin interactions. Nature; 485(7398):376-80. PMID: 22495300; PMCID: PMC3356448.

b. Zhang Y, An L, Xu J, Zhang B, Zheng WJ, Hu M, Tang J, **Yue F**. (2018) Enhancing Hi-C data resolution with deep convolutional neural network HiCPlus. Nat Commun; 9(1):750. PMID: 29467363; PMCID: PMC5821732.

c. Wang Y, Song F, Zhang B, Zhang L, Xu J, Kuang D, Li D, Choudhary MNK, Li Y, Hu M, Hardison R, Wang T, **Yue F**. (2018) The 3D Genome Browser: a web-based browser for visualizing 3D genome organization and long-range chromatin interactions. Genome Biol;19(1):151, PMID: 30286773. PMCID: PMC6172833.

d. Wang X, Xu J, Zhang B, Hou Y, Song F, Lyu H, **Yue F**. (2021) Genome-wide detection of enhancer-hijacking events from chromatin interaction data in rearranged genomes. Nat Methods;18(6):661-668. doi: 10.1038/s41592-021-01164-w. Epub 2021 Jun 3. PMID: 34092790; PMCID: PMC8191102.

4. **Algorithm development and data visualization:** In the past a few years, we have developed a series of algorithms on 3D genome organization, such as evaluating Hi-C data reproducibility (HiCRep), enhance Hi-C data resolution with deep learning (HiCPlus), and use supervised learning to predict chromatin loops on a genome-wide chromatin interaction matrix (Peakachu). My lab built one of the most popular tools for visualizing chromatin interaction data (The 3D genome browser), which has been visited >1,000,000 times by users from over 100 countries.

   a. Yang T, Zhang F, Yardımcı GG, Song F, Hardison RC, Noble WS, **Yue F**[#], Li Q[#]. (2017) HiCRep: assessing the reproducibility of Hi-C data using a stratum-adjusted correlation coefficient. Genome Res; 27(11):1939-1949. PMID: 28855260; PMCID: PMC5668950 ([#] Co-corresponding author).

   b. Yardımcı GG, Ozadam H, Sauria MEG, Ursu O, Yan KK, Yang T, Chakraborty A, Kaul A, Lajoie BR, Song F, Zhan Y, Ay F, Gerstein M, Kundaje A, Li Q, Taylor J, **Yue F**, Dekker J, Noble WS. (2019) Measuring the reproducibility and quality of Hi-C data. Genome Biol; 20(1):57. doi: 10.1186/s13059-019-1658-7. PMID: 30890172; PMCID: PMC6423771.

   c. Zhang Y, An L, Xu J, Zhang B, Zheng WJ, Hu M, Tang J, **Yue F.** (2018) Enhancing Hi-C data resolution with deep convolutional neural network HiCPlus. Nat Commun; 9(1):750. PubMed PMID: 29467363; PubMed Central PMCID : PMC5821732.

   d. Salameh T, Wang X, Song F, Zhang B, Wright SM, Khunsriraksakul C, **Yue F**. (2020) A supervised learning framework for chromatin loop detection in genome-wide contact maps. Nat Commun. 11: 3428. PMID: 32647330 PMCID: PMC7347923

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