Program Proposal for CF Support – Continuing Program
4D Nucleome

I. TABLE 1 PROGRAM OVERVIEW

Program Title	4D Nucleome
Program Goal:	To enable research that explores the relationship between nuclear organization and function
Working Group Co-Chairs	Dinah Singer (NCI) and Phil Smith (NIDDK)
Program Coordinator(s): IC staff who provide daily oversight and leadership for the program	Olivier Blondel (NIDDK) and Judy Mietz (NCI)
Total # of Proposed Initiatives and their names:	5 initiatives: 1) Centers for Chromatin Dynamics and Function (U54) 2) Centers for data Generation, Integration, Modeling and Visualization (U54) 3) 4DN translation to Primary Cells, Organoids, Tissues and model organisms (U01) 4) Organizational Hub (U01) 5) 4DN Data Coordination & Integration Center (U01)
Total Common Fund $$ Requested for ALL Years of Continuation Phase (millions)	$141.5 M over 5 years.

II. INTRODUCTION AND DESCRIPTION OF PROGRAM EVOLUTION

The 4D Nucleome (4DN) Network of investigators was established to address outstanding technological and conceptual challenges to studying nuclear organization and dynamics in mammalian cells. The 4DN NIH Common Fund Program was launched in 2015 with the goal of developing the tools and resources that would enable the characterization of the three-dimensional structure and dynamics of human and mouse genomes and provide deeper mechanistic insights into how the nucleus is functionally organized. The key deliverables from Stage I include: 1) next-generation genome analysis, imaging and computational tools to explore nuclear organization and its relationship with the regulation of gene expression programs, including in single cells; 2) pilot reference maps of the 3D architecture of the interphase nucleus for a select set of eukaryote cells; 3) validated predictive models of genome conformation/function relationships; 4) next-generation tools to explore nuclear dynamics through controlled disruption of nuclear architecture and imaging in live cells and tissue, and 5) community data and metadata standards.

Towards achieving these goals, Network investigators are actively developing and benchmarking experimental and computational approaches for measuring genome conformation and nuclear organization, and investigating how these contribute to gene regulation and other genome functions. Validated experimental technologies are being combined with biophysical approaches to generate reference maps of nuclear architecture and quantitative models of spatial genome organization in different biological states, both in cell populations and in single cells.

The Program is on target to produce key deliverables by the end of Stage 1, based on progress from both individual projects and from large trans-network collaborations such as the “4DN Joint Analysis Project” and the “4DN/Allen Institute for Cell Science collaboration” that generate large amounts of data from a variety of

complementary technologies on a limited number of consensus cell lines, and integrate multimodal datasets to produce reference models of spatial genome organization for the community.

Basic architectural features of the genome/nucleus are being revealed but higher-definition models are still needed. Application of newly developed tools to primary cells and tissues needs development—although application of such tools in biologically relevant systems and in human diseases have begun. Novel approaches and tools to study nuclear dynamics at various time scales are being developed and need to be validated. Finally, studies applying new tools and knowledge to broadly explore functional roles of nuclear architecture in human health and disease are also needed.

After 4 years of technology-development and basic science discoveries, a number of outstanding questions and technological challenges remain. These include: 1) better means of studying chromatin dynamics in live cells and in complex tissues, which will likely imply a heavier reliance on high-content imaging-based approaches and methods to distill massive amounts of imaging data; 2) better tools for the controlled disruption of nuclear features to better understand structure/function relationships in dynamic experimental systems; 3) tools to explore components of the functional nuclear architecture that are still undefined, including protein complexes, non-coding RNAs, nuclear bodies, compartments and microenvironments; and 4) development of next-generation analytical, visualization and modeling tools that are accessible and usable by the broader scientific community and can lead to reliable 4D models of genome organization.

These outstanding scientific and technology challenges can be met through the creation of a research environment that promotes multidisciplinary approaches, team science, and data integration, resulting in the production of important deliverables such as reference datasets, data standards, benchmarked protocols, community-tested software, and computational models, that can be accessed by the broader community and used to address the role of nuclear organization in health and disease. The proposed Stage 2 of the 4DN program (below) is designed to achieve these goals.

III. INITIATIVES AND BUDGETS

Initiative 1: Chromatin Dynamics and Function

Goal: Develop and apply a comprehensive set of technologies and analytical tools to describe high resolution nuclear/chromatin dynamics in individual cells and how components of 4D genome architecture impact biological processes.

- $1.6M/y TC, 6-8 awards ($10M)
- Interdisciplinary centers that include omics, imaging, biophysics, computational biology, cell biology;
- Develop and combine omics + imaging technologies to study chromatin dynamics in live (either ensemble or single) cells;
- Develop computational analysis tools to model nuclear organization dynamics during fundamental cell transitions (cell division, stem cell differentiation, etc.);
- Develop strategies to perturb aspects of nuclear genome organization, including chromatin looping events, formation and constitution of nuclear compartments and biomolecular condensates; perturbations can be at the DNA level, or architectural components and/or regulators of nuclear structure (remodeling enzymes, transcription factors, nuclear lamins, cohesins, ncRNAs etc); include rapid and controlled perturbations (chemical, optogenetics, etc.), or natural changes such as cell cycle, development, differentiation, aging, circadian or evolutionary time scales;

- Develop approaches to manipulate local (single loci) or genome-wide chromatin structures to study the impact on a variety of nuclear and cellular functions (replication, DNA repair, recombination, gene expression, cell fate and cell state, etc.); focus on both naturally-occurring or abnormal architectural features, such as those characteristic of disease states or defining individual tissue states;
- Develop synthetic biology approaches to define the minimum structural and organizational requirements for specific or optimal genome function;
- Award budgets should include 20% set-aside for collaborative projects;
- $200K/year budget incentive to include a specific aim led by an Early Stage Investigator (ESI) or an investigator with < 10 years of research funding.

Initiative 2: Data Generation, Integration, Modeling and Visualization

Goal: Produce navigable 4D reference maps and models of genome organization through application of robust and complementary technologies to consensus cell types and/or 3D systems.

- $2M/y TC, 4-5 awards ($8M)
- Interdisciplinary centers that include omics, imaging, biophysics, computational biology, cell biology;
- Build on the progress made in Stage 1 to produce a significant number of omics-based and imaging-based reference datasets by applying a wide array of complementary technologies to a limited number of consensus cell lines and/or primary cells in-vitro;
- Develop and apply technologies that describe the contribution of non-DNA components to nuclear organization (protein complexes, ncRNAs, etc.);
- Develop next-generation analytical, visualization and modeling tools for interrogating these datasets;
- Build 4D reference models of genome organization in a limited number of human cell lines and/or mammalian primary cells by combining analytical and visualization tools ("navigable landscapes") to study various aspects of genome organization and chromatin dynamics both spatially (at different scales) and temporally;
- Produce a series of single-cell or ensemble navigable 4DN landscapes to better document the relationships between 4DN structure and cell state or function: assessment of "normal" cell-to-cell variance (differences between single-cell and ensemble maps for the same cell type), maps of enhancer-promoter connectivity, maps of known disease or disease-risk states, maps of developmental stages or tissue specificity, etc. ;
- Award budgets should include 20% set-aside for collaborative projects;
- $200K/year budget incentive to include a specific aim led by an Early Stage Investigator (ESI) or an investigator with < 10 years of research funding.

Initiative 3: Nuclear Architecture over the Lifespan and in Human Health And Disease

Goal: Develop and apply tools to investigate the role of nuclear organization during development and lifespan and in human health and disease.

- $650K/y TC, 8-10 research projects; ($6M)
- Modify and improve on existing 4DN technologies, or develop new tools and approaches, to elucidate genome organization in primary cells, organoids, stem cell-derived organs-on-chip, primary tissues or model organisms; compare organization over developmental time or in health vs disease models

- Develop computational tools to compare and integrate single-cell versus ensemble data, and to facilitate data analysis in 3D tissue models;
- Optimize and share protocols and tools to facilitate adoption by the larger scientific community that can apply them to the study of a variety of cell systems, model organisms or disease models. This would include the development of faster, cheaper and more robust versions of existing assays so that they can be brought to most labs and to the clinic;
- Apply novel tools to resolve chromatin architecture and dynamics in development aging and lifespan; and use of 4DN-related measurements to help predict or understand disease state;
- Encourage applications that apply new tools in a variety of areas, including: role of 4DN in regulation of development and cell fate; role of phase separation in 3D genome organization and function; role of ncRNAs and architectural proteins in 4DN organization; biophysical modelling and/or computational tools to resolve chromatin architecture and dynamics in development aging and lifespan; and use of 4DN-related measurements to help predict or understand disease state;
- Award budgets should include 20% set-aside for collaborative projects within the Network.

Initiative 4: 4DN Organizational Hub

Goal: Provide an organizational support for all 4DN activities.

- $1.5 M/y TC, 1 award
- The Hub will be responsible for: promoting cross-site interaction; developing and disseminating standards for the field; enhancing collaborations among investigators throughout the 4D Nucleome Program through annual meetings, working groups, community website; managing the 4DN Program Steering Committee and relations with External Program Consultants; providing reports on progress and budget to NIH staff; and serving as the focal point for outreach to other relevant research networks and consortia outside of 4DN. This initiative will be open to extramural investigators only.

Initiative: Data Coordination & Integration Center (4DN-DCIC)

Goal: Track, store, and display all data generated by the 4D Nucleome Program

- $2.5 M/y TC, 1 award
- In addition to main goal (above), will provide a Data Analysis Center to assist with integrated analyses and with the development of metrics and standards to be adopted by the community at large. All data generated by 4D Nucleome participants will be rapidly released into public databases.
- This initiative will be open to extramural investigators only.

IV. TABLE 2 BUDGET SUMMARY

Stage 2 4DN	Lead IC	FY20	FY21	FY22	FY23	FY24	total
Initiative 1: Chromatin Dynamics and Function							
	TBD	10	10	10	10	10	50
Initiative 2: Data Generation, Integration, Modeling and Visualization							
	TBD	8	8	8	8	8	40
Initiative 3: Nuclear Architecture over the Lifespan and in Human Health and Disease							
	TBD	6	6	6	6	6	30
Initiative 4: 4DN Organizational Hub (4DN-OH)							
	TBD	1.5	1.5	1.5	1.5	1.5	7.5
Initiative 5: Data Coordination & Integration Center (4DN-DCIC)							
	TBD	2.5	2.5	2.5	2.5	2.5	12.5
RMS – for NIH staff salary and travel; NIH-organized workshops							
	TBD	0.3	0.3	0.3	0.3	0.3	1.5
TOTAL							141.5