

The NIH Common Fund 4DNucleome Program

**Council of Councils Meeting
January 19, 2022**

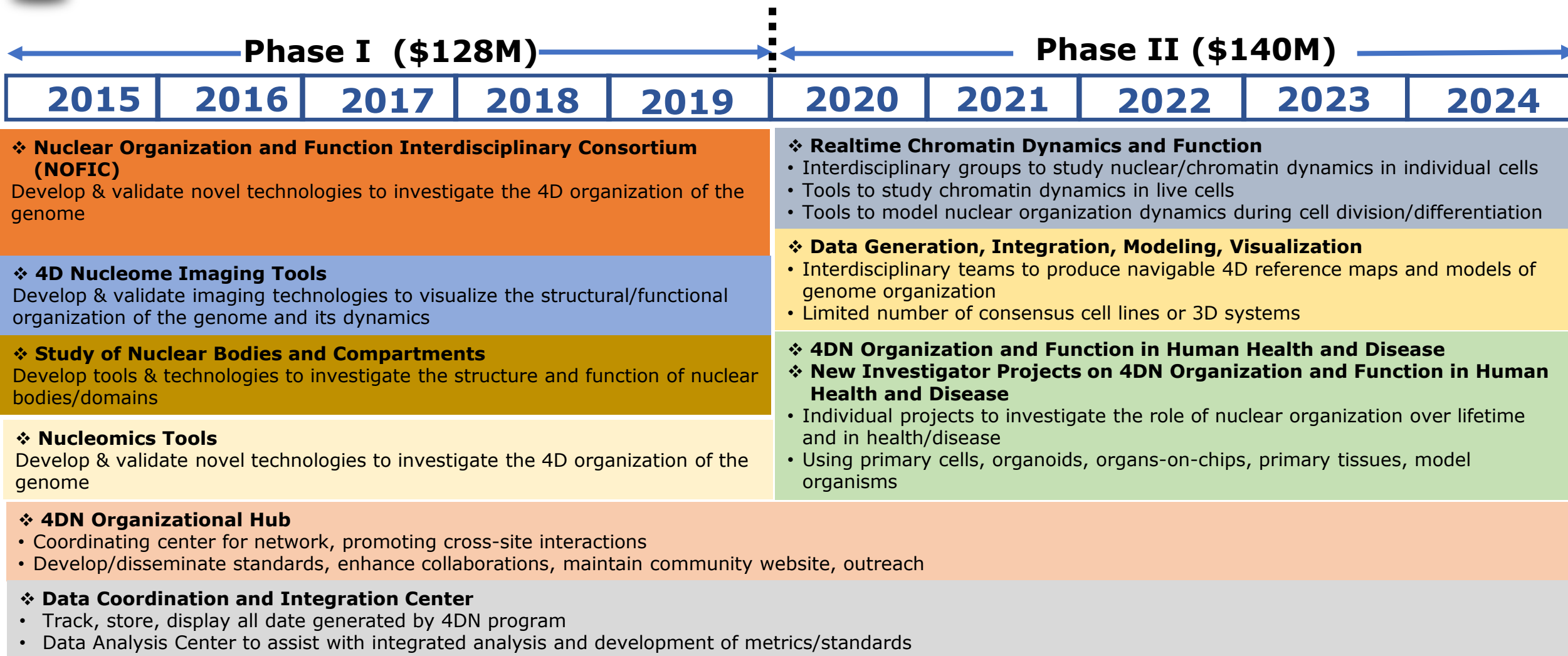
Ananda L Roy, PhD

**Assistant Director, Transformational Science and Discovery
Office of Strategic Coordination/Common Fund**



National Institutes of Health
Office of Strategic Coordination–The Common Fund

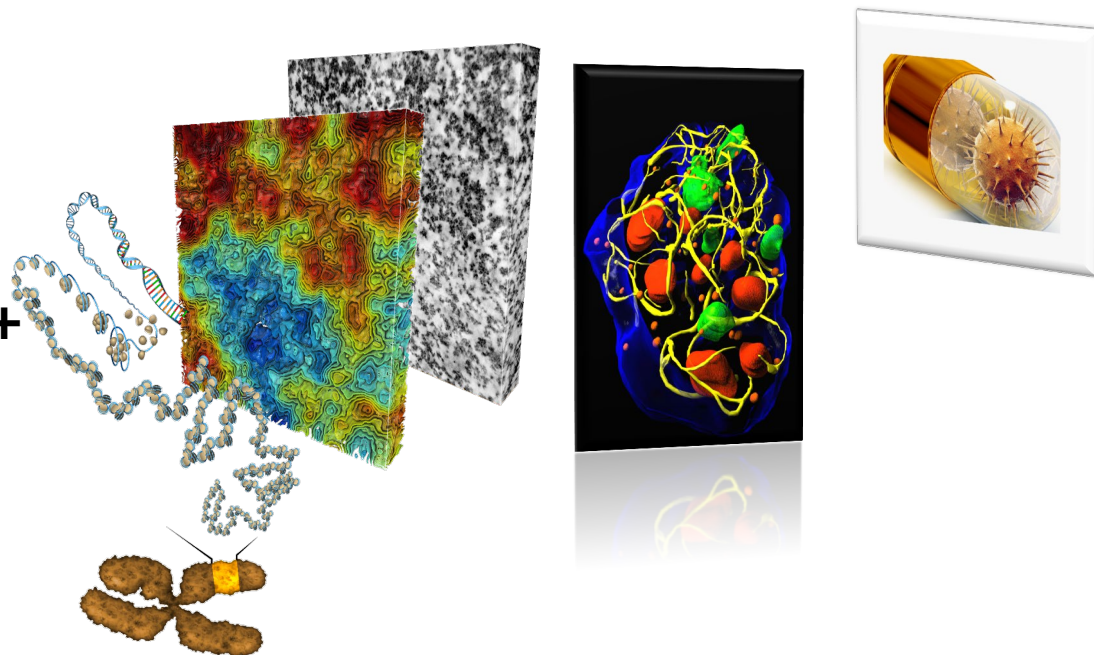
4DN Timeline & Budget





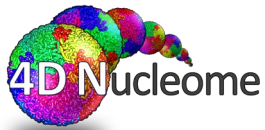
4DN Phase 1 Accomplishments

- **~30 omics and ~25 imaging technologies developed**
- **50+ analysis and software packages**
- **~1100 publicly available datasets supported by 9+ analysis pipelines, 10+ protocols; 17 cell lines; Support for community download and analysis (JupyterHub)**
- **6 Collections, inc.: Joint Analysis Portal for 4DN and ENCODE data; 4DN & Allen Institute; Inflammation / Stress**
- **Standardization of imaging – chromatin tracing, SPT, FISH, segmentation, MicroMeta interface, microscope calibration**
- **First major biology consortium to adopt pre-print/data sharing of publications through bioRxiv**



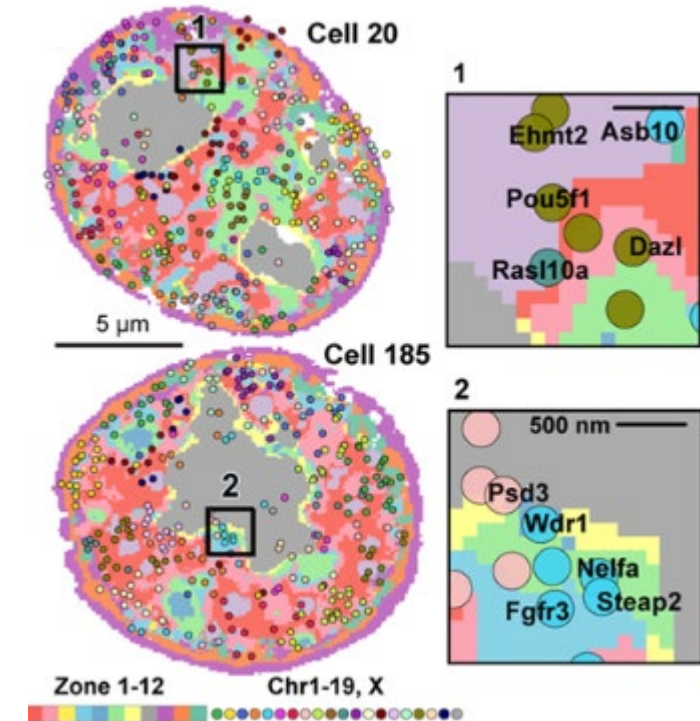
ChromeEMT: Visualizing 3D chromatin structure & compaction in interphase & mitotic cells
Clodagh C. O'Shea, 2017

<https://data.4dnucleome.org>

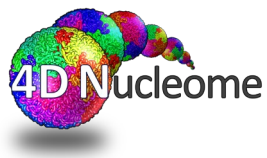


4DN Phase 2 Progress

- **~30 projects; ~\$28M/year; 194 publications; phase 1 publication package in progress**
- **Now 1750+ datasets available on Data Portal, growing number (180+) from tissue and primary cells**
- **sc4ALL Collaborative Project; Common Coordinate Framework**
- **Collaboration with SCGE on progeria; regular meetings between 4DN-CFDE**
- **4DN tutorials; increasing use of 4DN data & publications (4+) by external groups**



Reconstructions for nuclear zones and DNA loci at a single z plane using DNA seqFISH+
Nature. 2021 Feb; 590(7845): 344–350.
Long Cai (U01DK127420)



4DN NIH Working Group

4DN Co-Chairs

Dinah Singer (NCI)
Carolyn Hutter (NHGRI)

4DN Co-Coordination

Judy Mietz (NCI)
Olivier Blondel (NIDDK)
Mike Pazin (NHGRI)

4DN Common Fund Leader

Richard Conroy (OD)

4DN Program Director

Ian Fingerman (NCI)

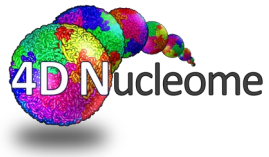
4DN WG

David Balasundaram (CSR)
Anthony Carter (NIGMS)
Jason Shockey (OD)
Max Guo (NIA)
Sean Hanlon (NCI)

Lisa Postow (NHLBI)
Robert Riddle (NINDS)
John Satterlee (NIDA)
Geetha Senthil (NIMH)
Veronica Taylor (OD)
Fred Tyson (NIEHS)

4DN Program Analyst

Michael Mensah (NIDDK)

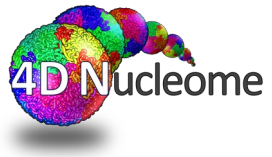


4D Nucleome Consortium Update

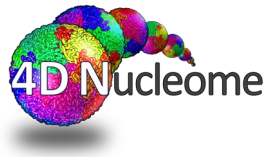
Ana Pombo – Max Delbrück Center for Molecular Medicine, Berlin

Jian Ma – Carnegie Mellon University, Pittsburgh

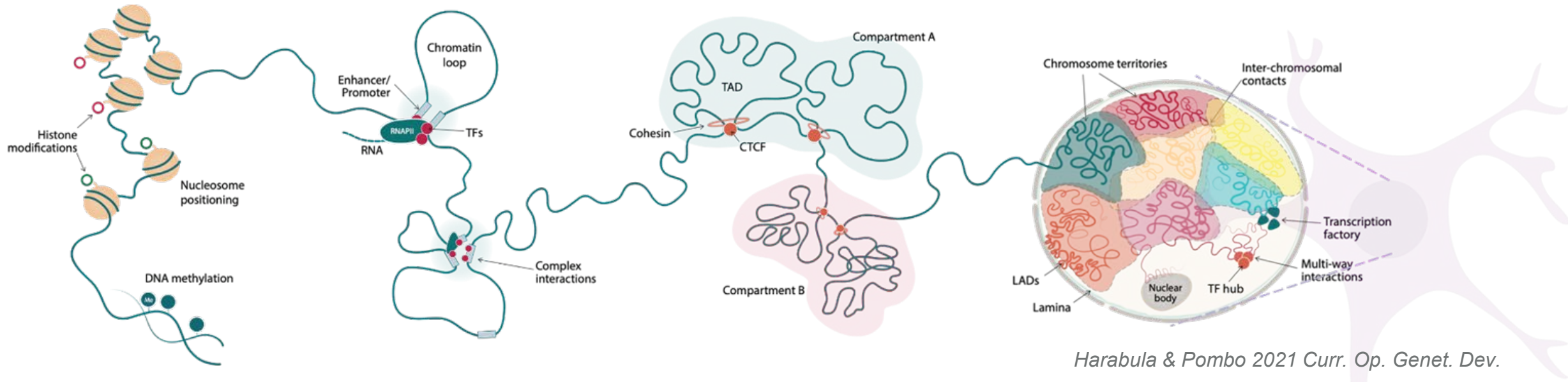
Geeta Narlikar – University of California, San Francisco



Importance of accelerating the mapping of the dynamic nuclear architecture



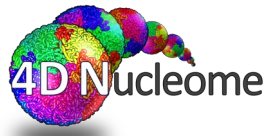
4D-Nucleome: diverse hierarchical topologies of genome organization with different underlying mechanisms



3D genome structure

layer of intrinsic information to enable/restrict cell identity, cell fate transitions, homeostasis

**heterogeneity of mechanisms
across scales and cellular
conditions (e.g., tissue types)**



Mapping and decoding 3D genome structure

Complex and rare diseases

Often polygenic and not always familial
(e.g. cancer, neurodevelopmental, and degenerative diseases)

Environmental factors

(e.g. drug exposure, neurodegeneration)

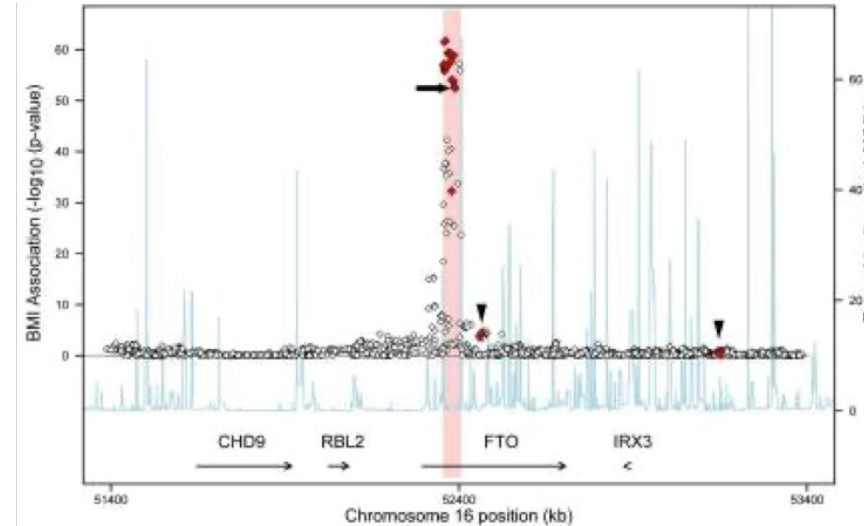
Few patients

(challenge/cost of genetic associations, molecular phenotyping of patient samples)

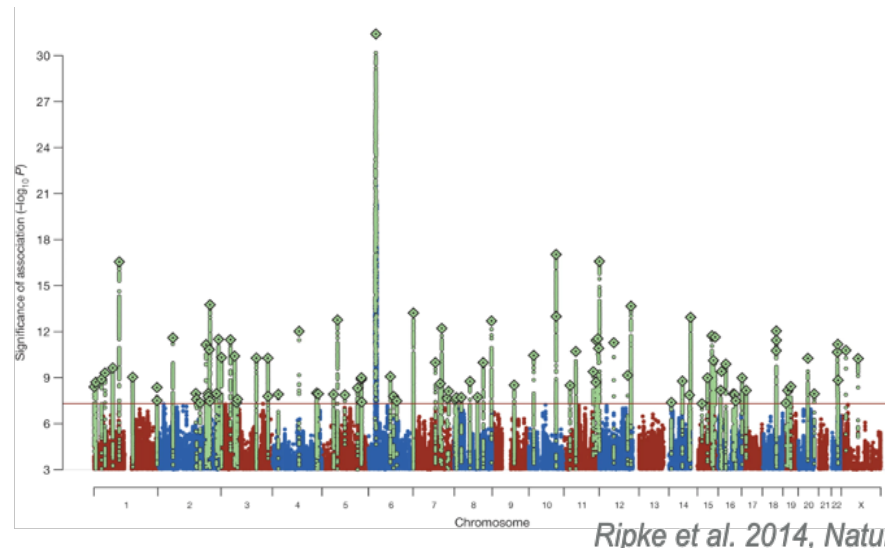
Genetic variation/mutations

in non-coding regions

(e.g. obesity, cancer, and schizophrenia)

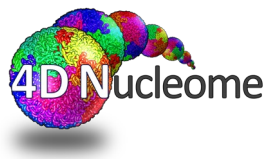


Obesity-associated non-coding genetic variation within the FTO gene alters the expression of IRX3



Most schizophrenia associated genetic variants are non-coding: challenge to discover target genes

Ripke et al. 2014, Nature

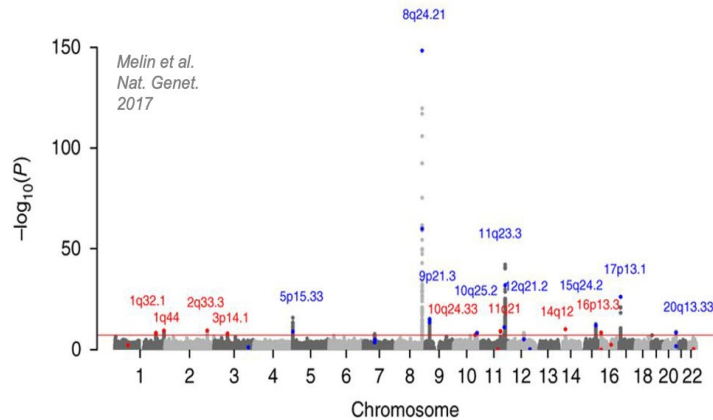


Cell-to-cell variability: using 4DN data to dissect disease mechanisms in adult low-grade glioma

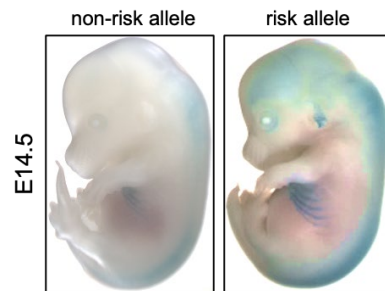
Low grade glioma

Mean survival of ~5 years
No treatment outside surgery/radiation

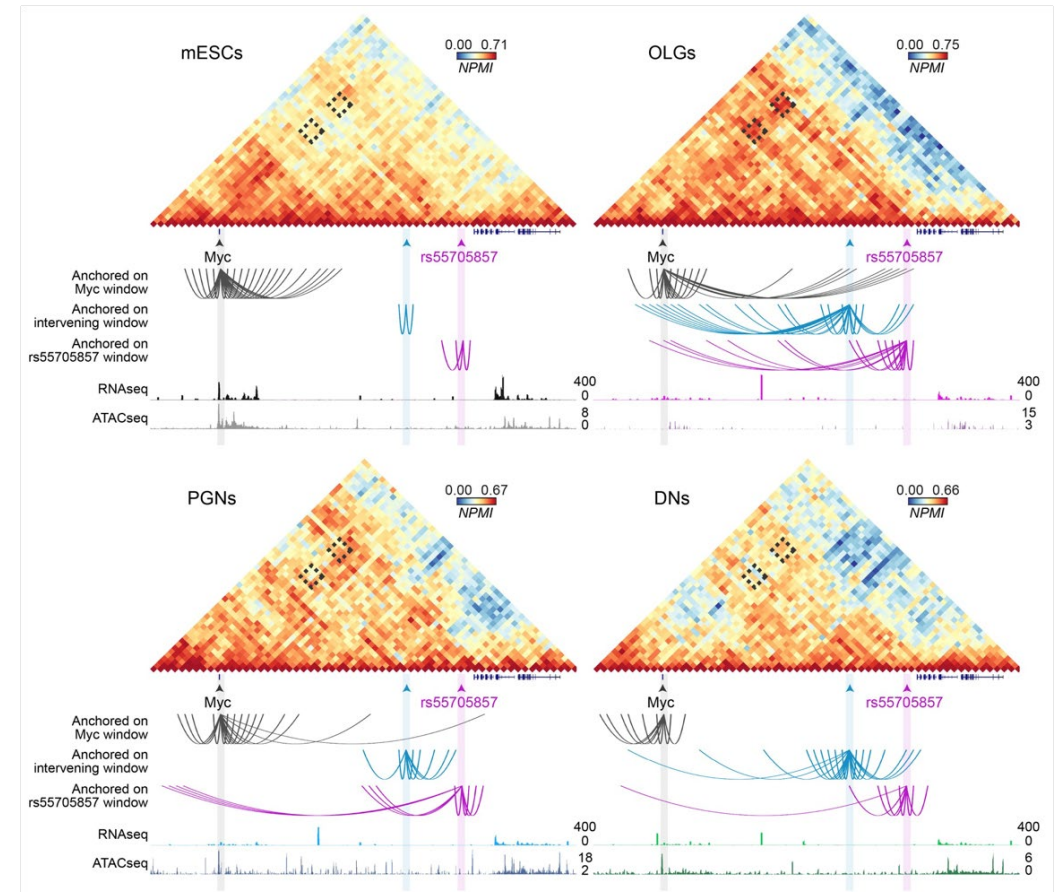
Causal glioma risk variant at 8q24.21: 6-fold risk



rs55705857 (A-to-G) SNP has enhancer activity in neuronal tissues



rs557 interacts with *Myc* specifically in oligodendroglia (1.7Mb)



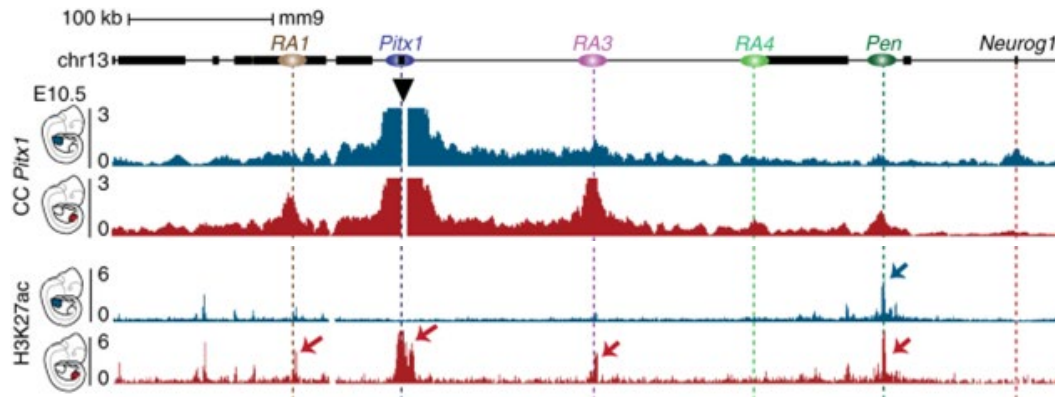
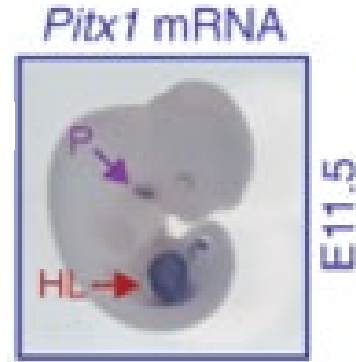
Yanchus et al. 2022 Science

4DN GAM data: Winick-Ng et al. 2021 Nature (4DN PI: Pombo)

Cell-to-cell variability: chromatin conformation is sufficient to restrict the activity of enhancers

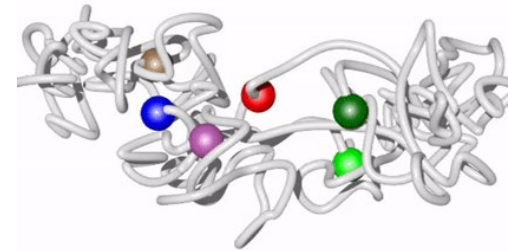
Hindlimb-specific expression of *Pitx1*

is controlled by an enhancer which is active in **both** forelimbs and hindlimbs

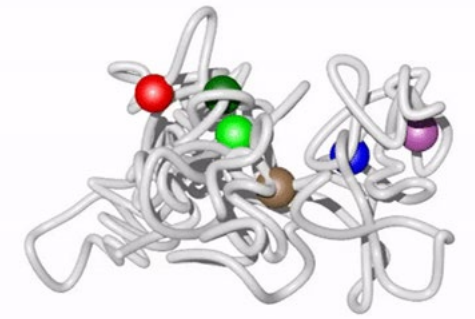


Chromatin configuration restricts **enhancer** activity to hindlimbs; **enhancer** and *Pitx1* are separated in forelimbs

Forelimb



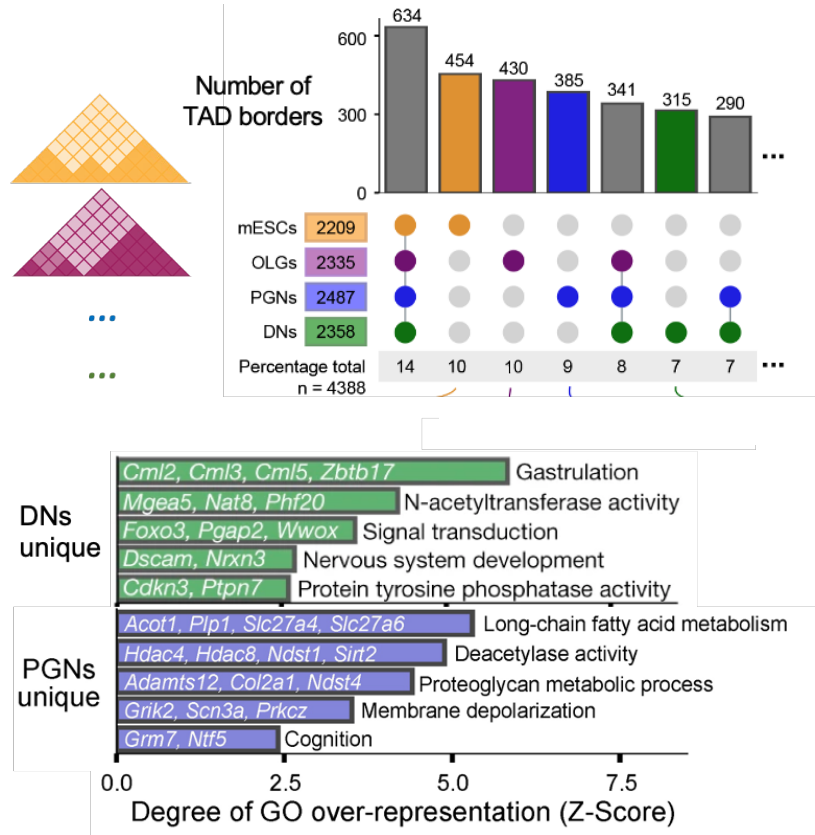
Hindlimb





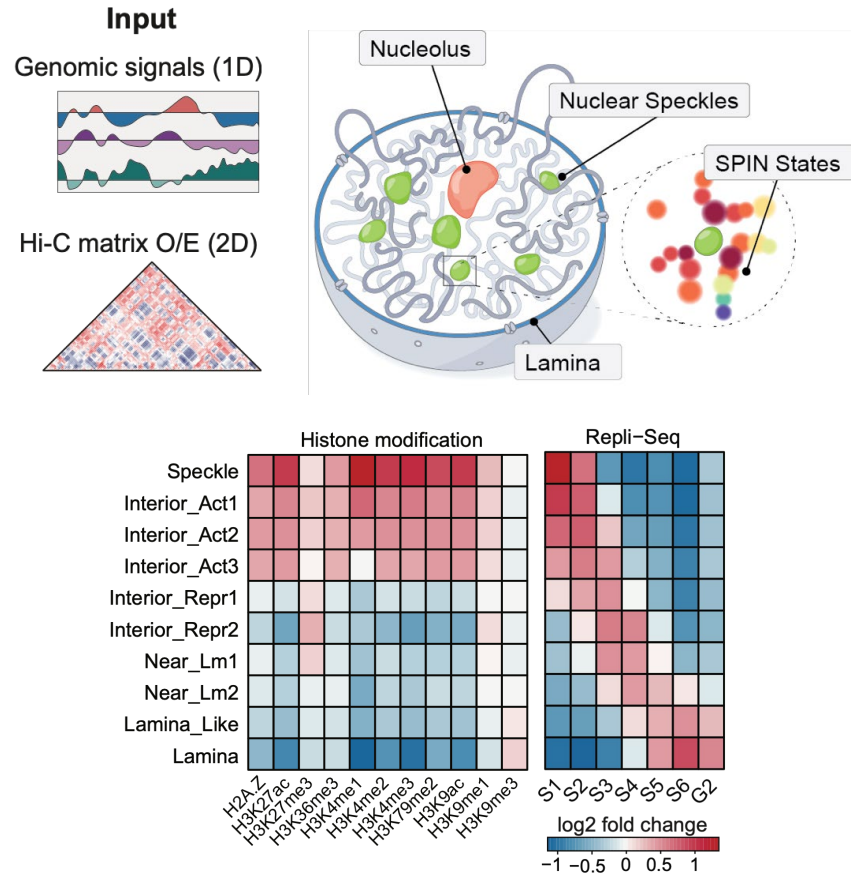
4D Nucleome: cell-type diversity at different scales

Reorganisation of topological domains



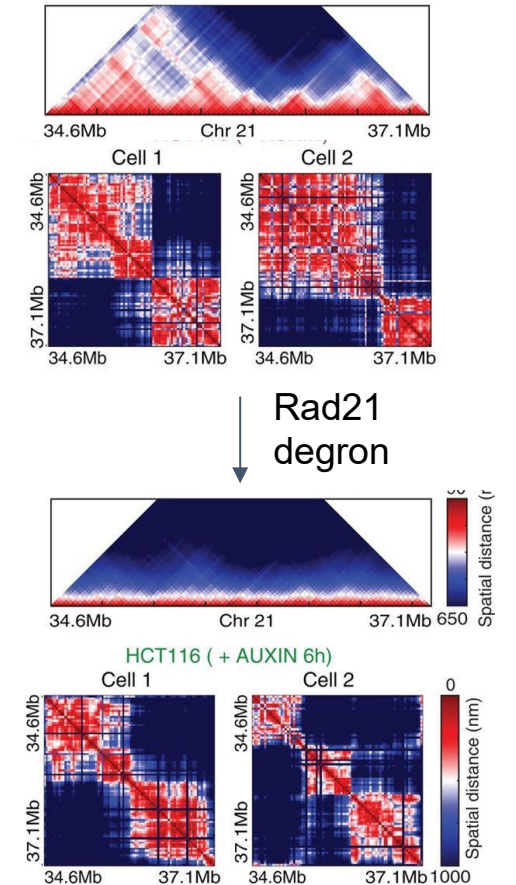
MacArthur & Capra, 2021 AJHG
Winick-Ng et al. 2021 Nature
(4DN PI: Pombo)

Chromatin compartmentalization relative to nuclear bodies



Wang et al. 2021 Genome Biol
(4DN PIs: Ma, Belmont, van Steensel, Gilbert)

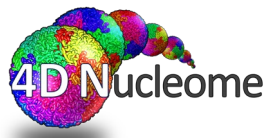
Chromatin domains within cell populations



Bintu et al. 2018 Science
(4DN Pls: Boettinger, Zhuang)

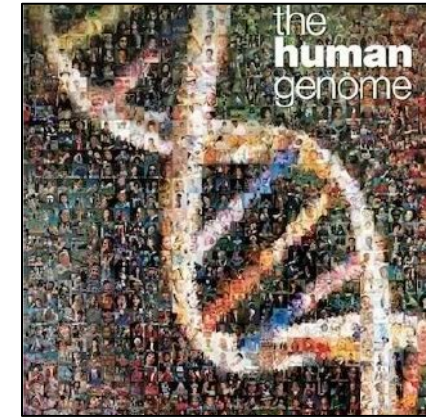


The value of a consortium



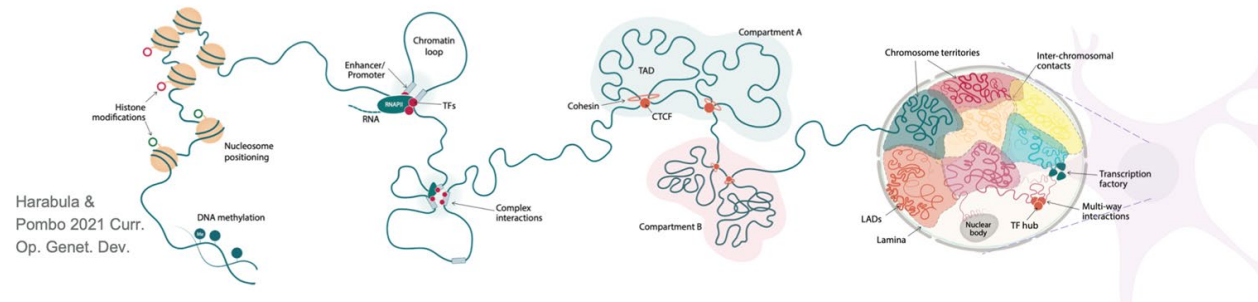
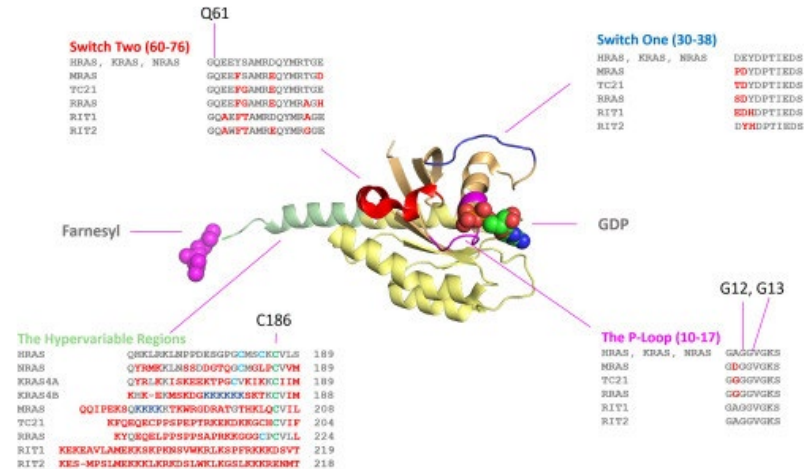
A Next-Gen Consortium

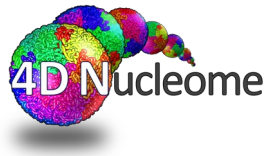
1990 – 2003, Human Genome Project:
Singular goal of sequencing the human genome



2013 – current, Ras initiative:
Accommodates heterogeneity of Ras Cancers

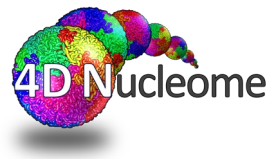
2016 – current, 4D Nucleome:
Accommodates heterogeneity of mechanisms across scales and tissues



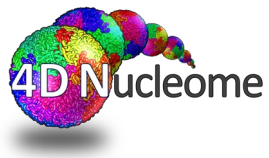


4DN specific value

- **Tackles biological diversity** by concurrent intersection of projects with different conceptual and technical expertise
- **Encourages creative conflict** between internal and external members (inclusion of Allen Institute for Cell Science and others)
- **Fosters open sharing** of data, new ideas, papers before peer-review
- **Enables Standardization** of methods, controls and analyses
- **Makes affordable** mapping of the nucleus, **democratizes technologies** that enable individual R01s, and **reaches stakeholders** broadly
- **Promotes next generation** by inclusion, mentorship and visibility of junior scientists and early career researchers at the forefront of 4DN efforts



Early successes enabled by 4DN collaboration



Comparison and joint analysis of multimodal datasets

Joint effort in data collection and cohesive integration of genomic and imaging datasets for genome annotations

Two human cell lines:

H1 hESC

HFF fibroblasts

Genomic methods:

Hi-C, micro-C

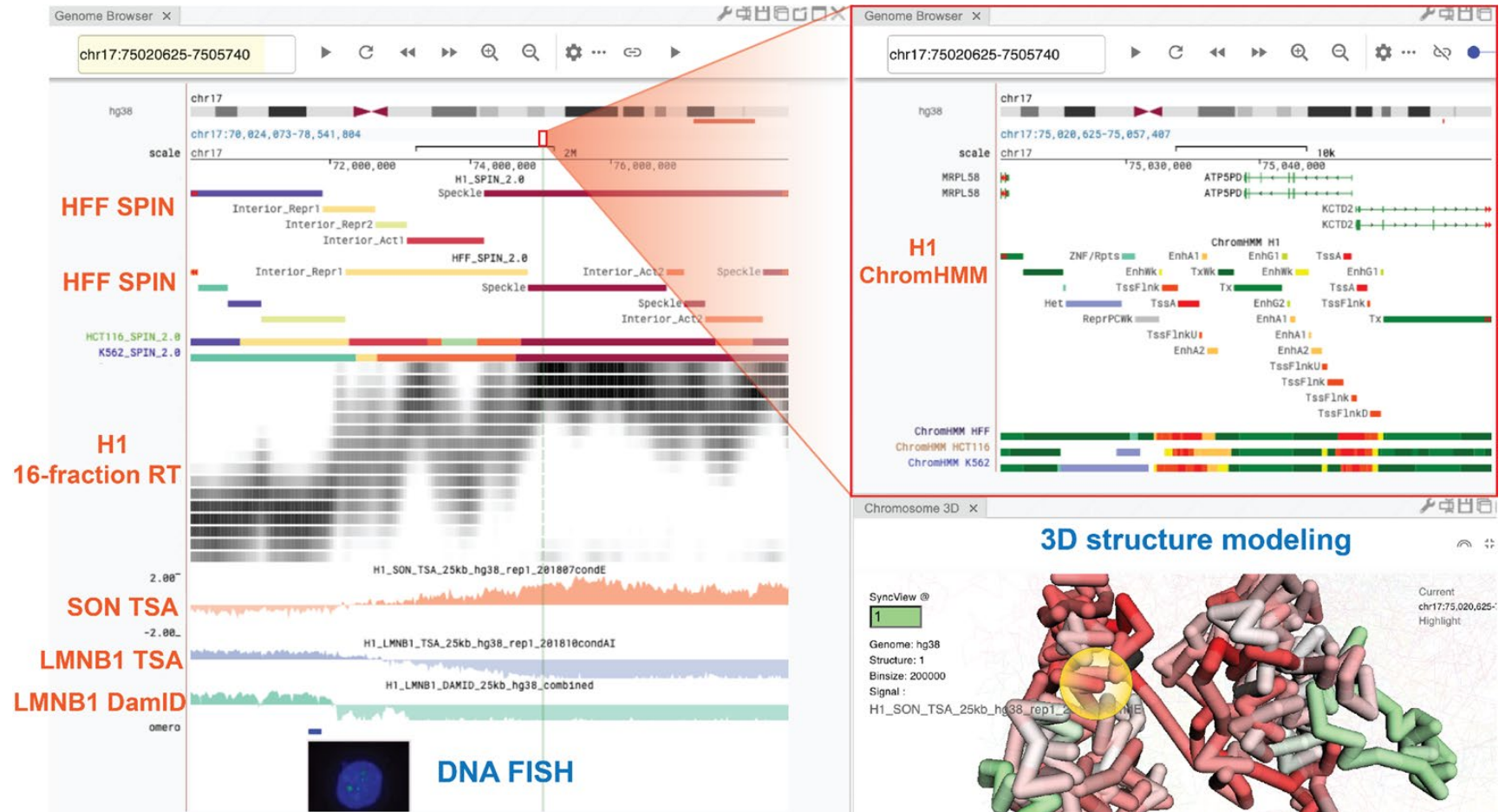
TSA-seq

DamID

SPRITE

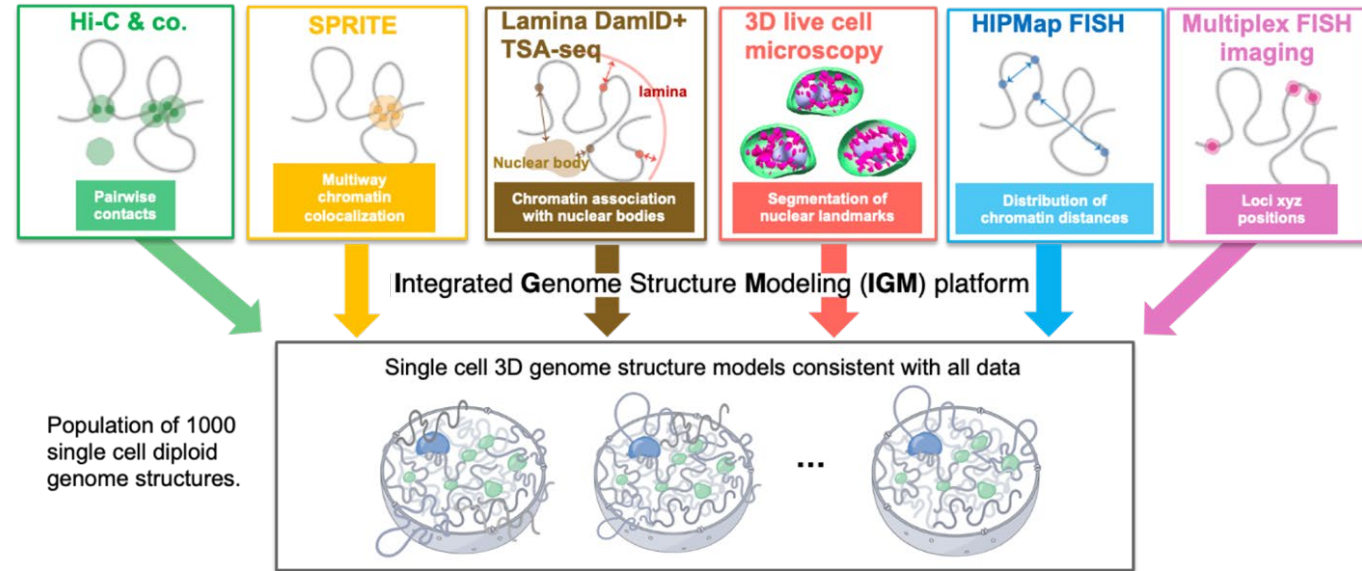
GAM

Diverse set of analysis tools

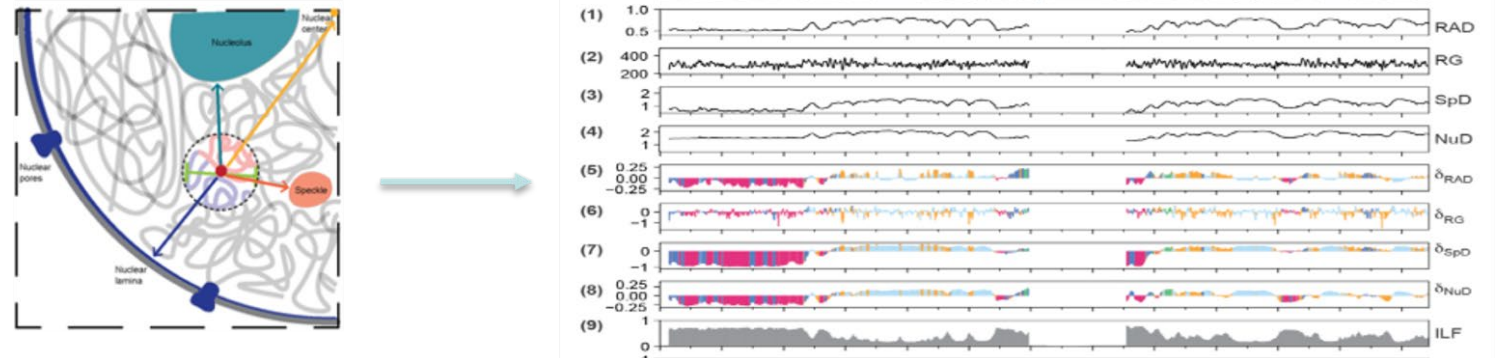


Realistic 3D structures for structure-function analysis

- Integrative analysis, prediction, and data-driven realistic structure modeling
- Produce single-cell structures that are statistically consistent with multimodal 4DN datasets
- Unique tool to study cell-to-cell variability and structure-function connections



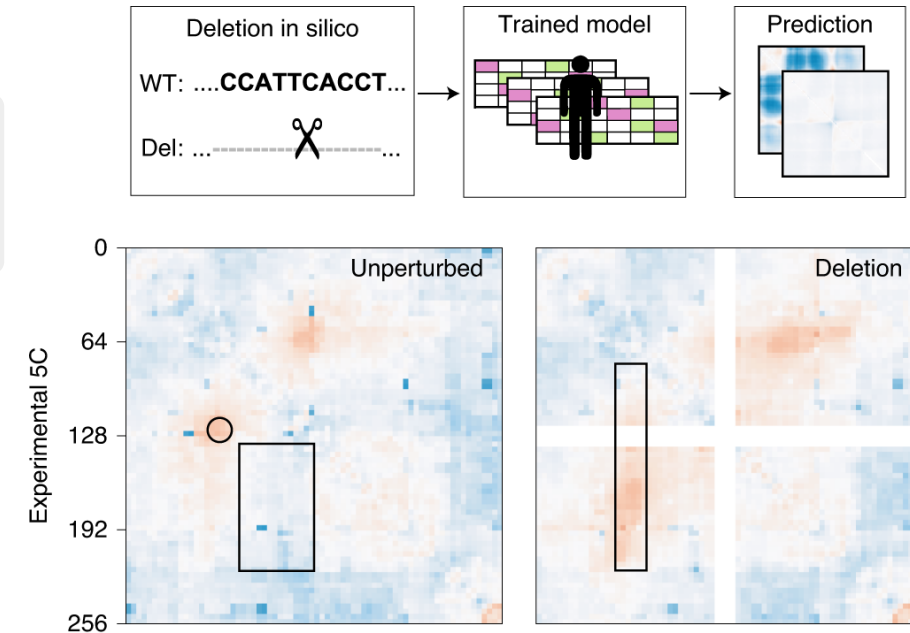
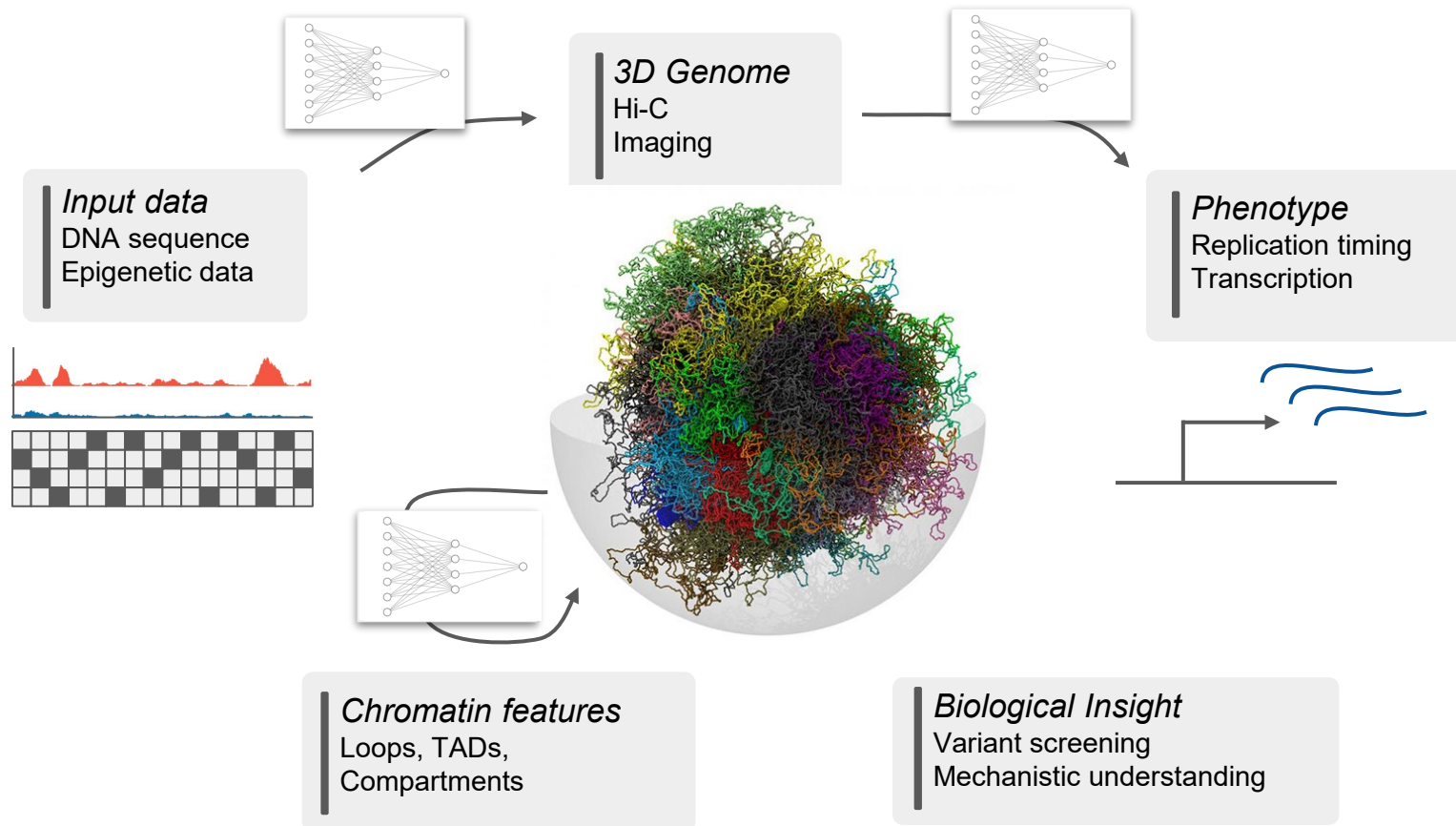
Extract single cell genome structure properties to answer biological questions



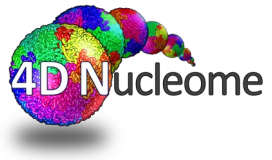
Boninsegna et al. Nat Methods 2022; Yildirim et al. bioRxiv; Hua et al. Nat Prot 2018
(4DN PIs: Alber, Ma, Dekker, Ren, Guttman, Rafelski, Belmont, Gilbert, van Steensel, Zhuang, Misteli)

Machine learning to guide the next experiments

Modern AI/ML predictive methods to identify important genetic variants through the lens of 3D genome folding



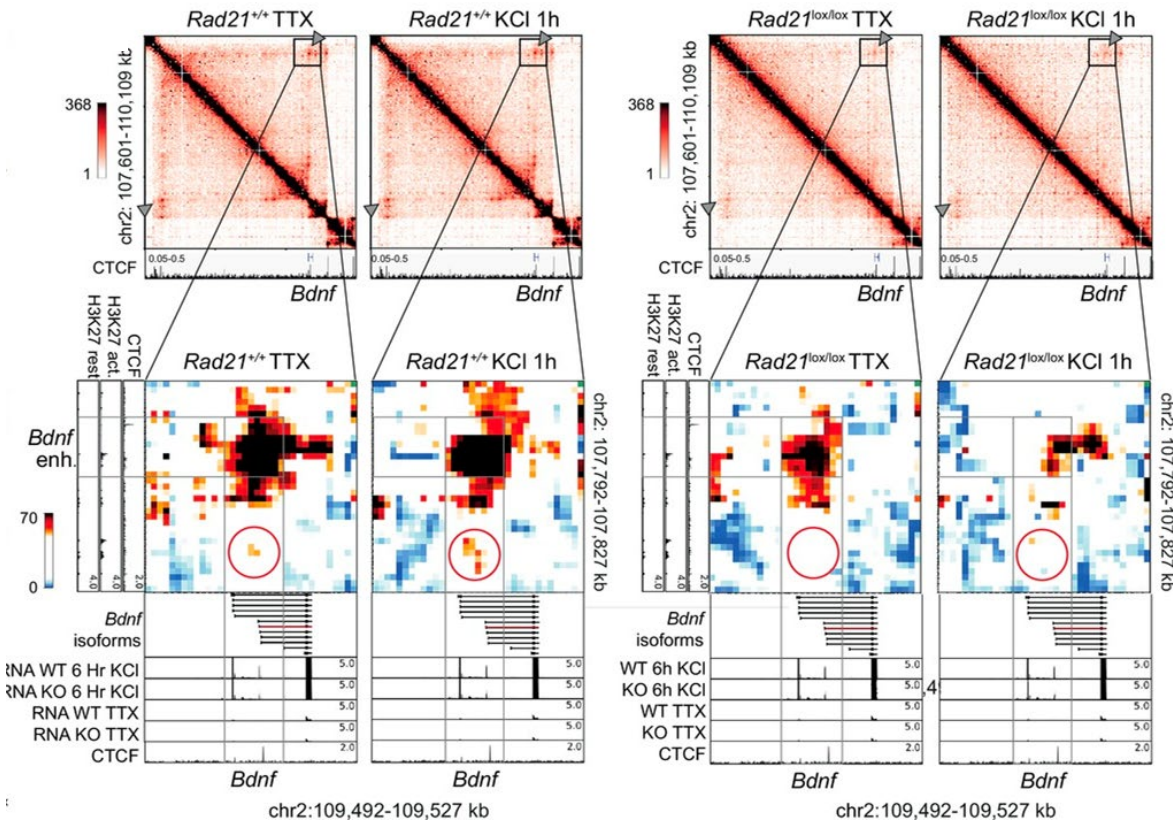
*Fudenberg, Kelley & Pollard, Nat Methods 2020
(4DN PIs: Pollard, Leslie)*



Case studies of ongoing work and unique opportunities of 4DN phase 2

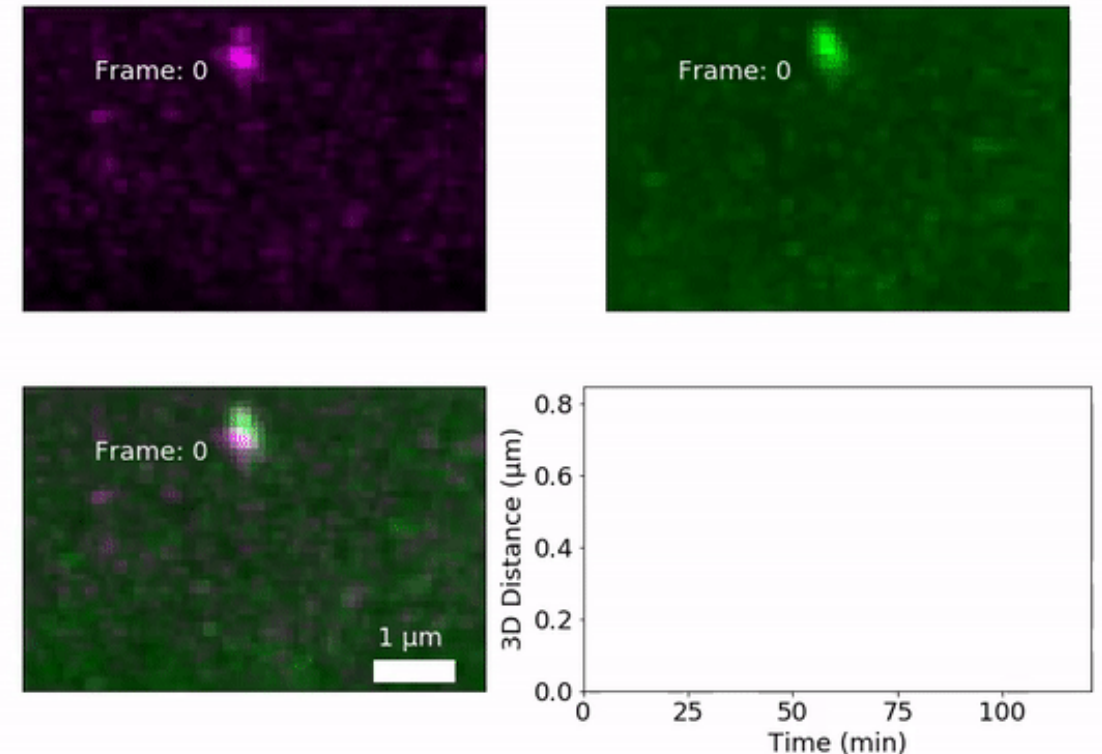
Moving beyond cell lines and beyond static images

Essential roles of cohesin in cortical excitatory neurons

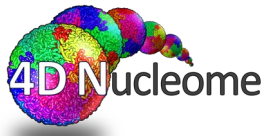


Calderon et al. 2022 eLife
(4DN PI: Phillips-Cremins)

Capturing dynamics of genome folding and unfolding

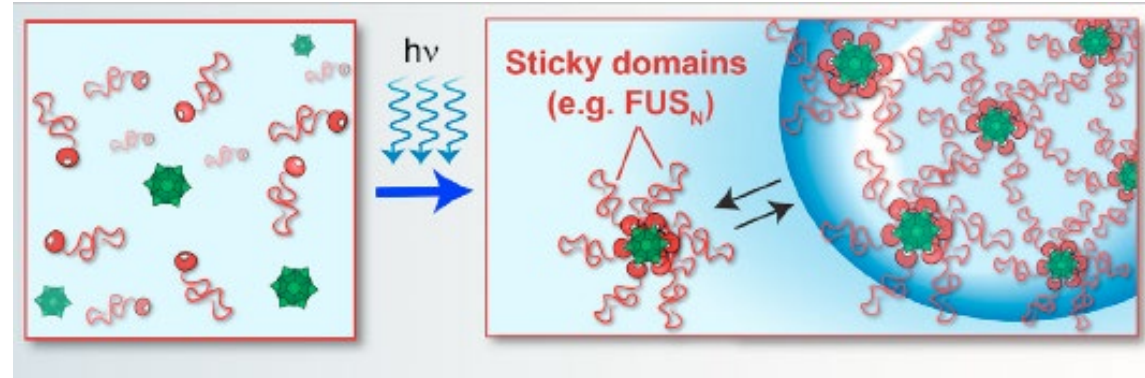


Gabriele et al. 2022 Science
(4DN PI: Anders Hansen)



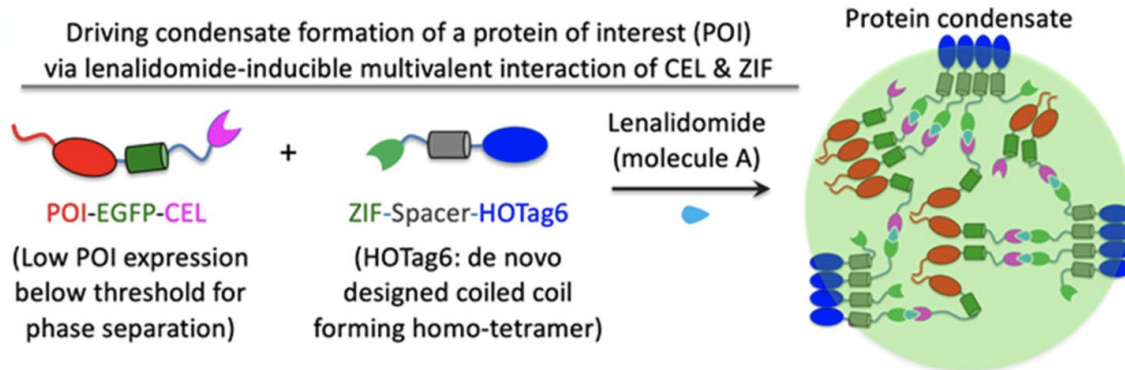
Biophysical mechanisms: investigating roles of phase-separation by perturbation technologies

4DN phase 1: *Light-controlled method to reversibly induce defined phases in the nucleus*



*Bracha et al.
2019 Cell
(4DN PI:
Brangwynne)*

4DN phase 2: *Chemical genetic method to control phase-separation at endogenous protein expression levels (4DN PIs: Shu, Narlikar, Huang, Ramani)*

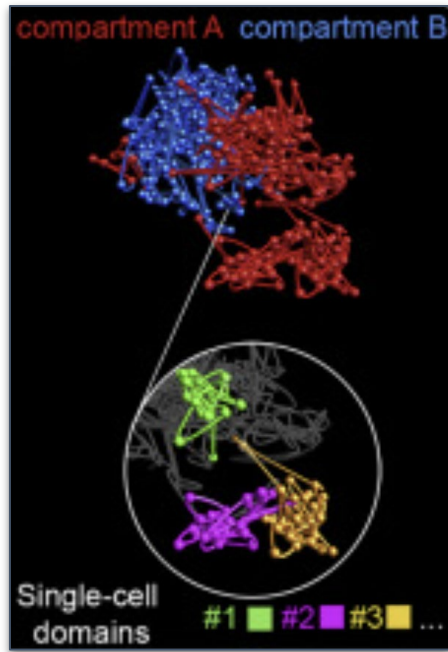


Dissolving condensate via rapamycin-inducible recruitment of highly soluble protein, e.g. SUMO

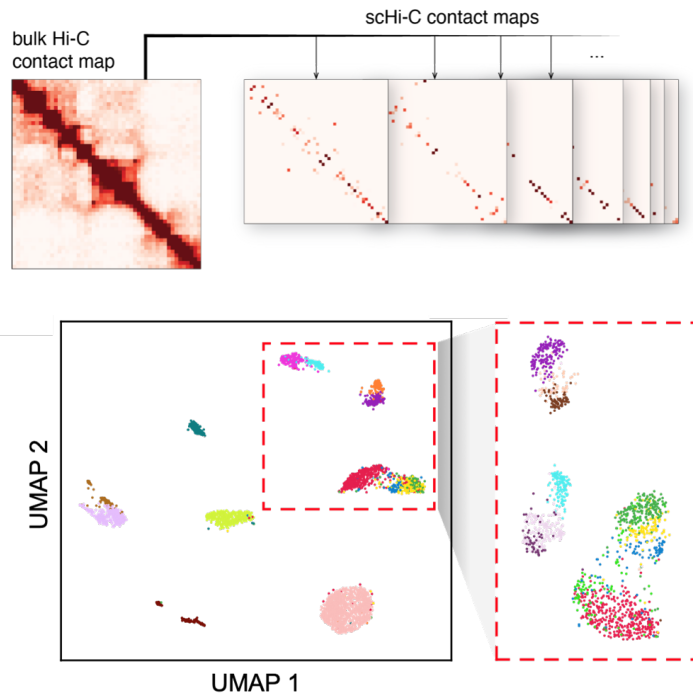


Structure function in single cells

4DNsc4ALL – Phase 2 consortium-wide collaboration

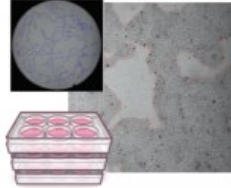


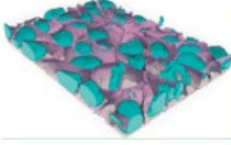

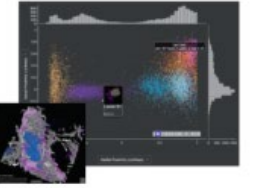
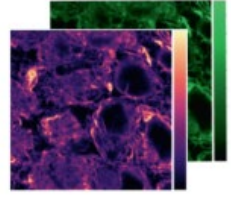
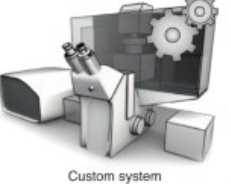
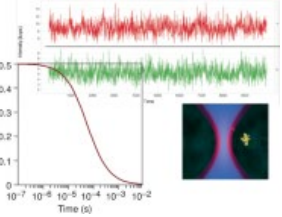


Chromatin tracing
(4DN PIs: Zhuang, Wu, Cai, Boettinger)

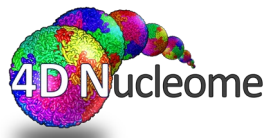


Single-cell Hi-C
(4DN PIs: Ren, Ecker, Hu, Ma)

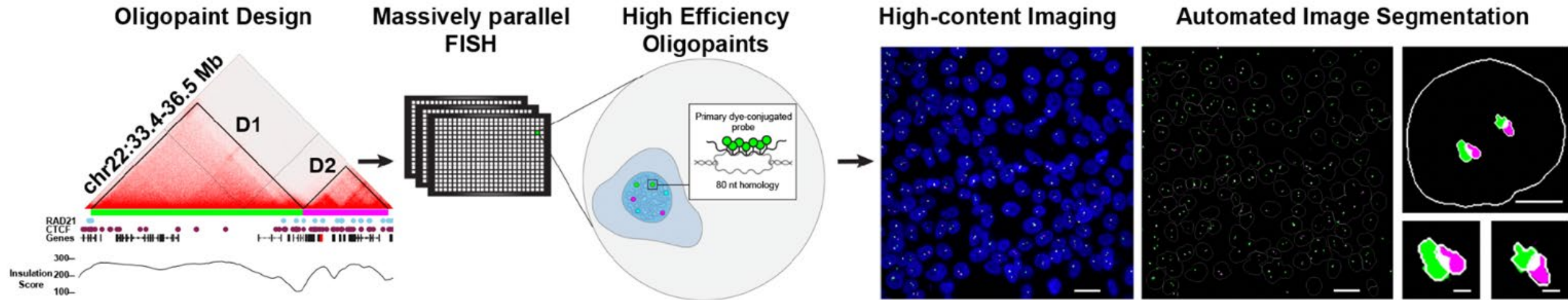
Standards for imaging data sharing

	Experiment and images	Microscope and modality	Results and analysis
Tier 1 Minimum information/ qualitative or basic quantification/ materials and methods			
Tier 2 Advanced quantification			
Tier 3 Manufacturing/ technical development/ full documentation		 <p>Custom system</p>	

Hammer et al. 2021 Nature Methods
(4DN PIs: Grunwald, Strambio-De-Castilla)



Identifying druggable genome regulators



High-throughput Oligopaint screen identifies druggable regulators of genome folding

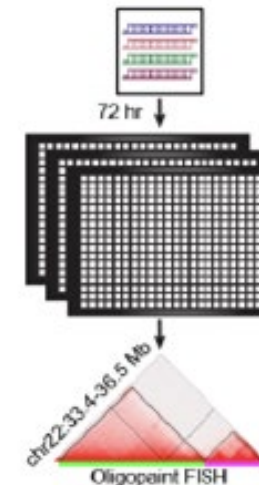
Daniel S. Park, Son C. Nguyen, Randi Isenhardt, Parisha P. Shah, Wonho Kim, R. Jordan Barnett, Aditi Chandra, Jennifer M. Luppino, Jailyann Harke, May Wai, Rachel Yang, Yemin Lan, Sora Yoon, Rebecca Yunker, Golnaz Vahedi, Jennifer E. Phillips-Cremins, Rajan Jain, Eric F. Joyce

doi: <https://doi.org/10.1101/2022.04.08.487672>

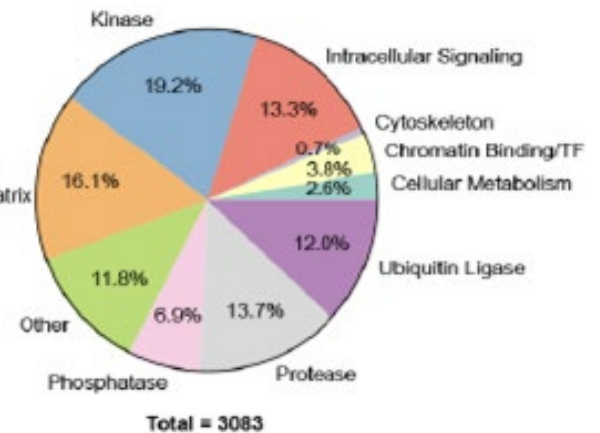


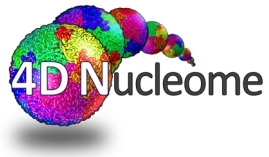
bioRxiv
THE PREPRINT SERVER FOR BIOLOGY

Pooled siRNA Library



Druggable Genome





Outlook

- 1. Accelerate debate and discussion on key concepts of genome organization and function**
- 2. Generate integrative approaches that are readily portable for studying multiple cell types**
- 3. Accelerate conversion of curiosity based science to improvements in human health**