# Summary

Hi-C studies let us find regions of DNA that are folded near each other in TADs (**T**opologically **A**ssociated **D**omains).

This tells us proximity of regions, but most of the spatial layout cannot be determined from this.

In this paper, researchers applied a modified FISH (**F**luorescence **I**n **S**itu **H**ybridization) to directly observe the locations of each TAD in the nucleus.

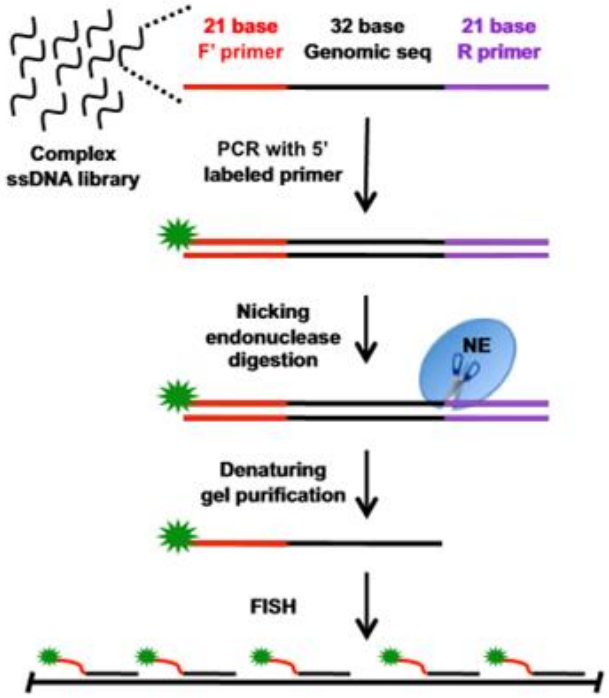
Used multiplexed FISH probes targeting sequential TADs to map 3D structure of human chromosomes.

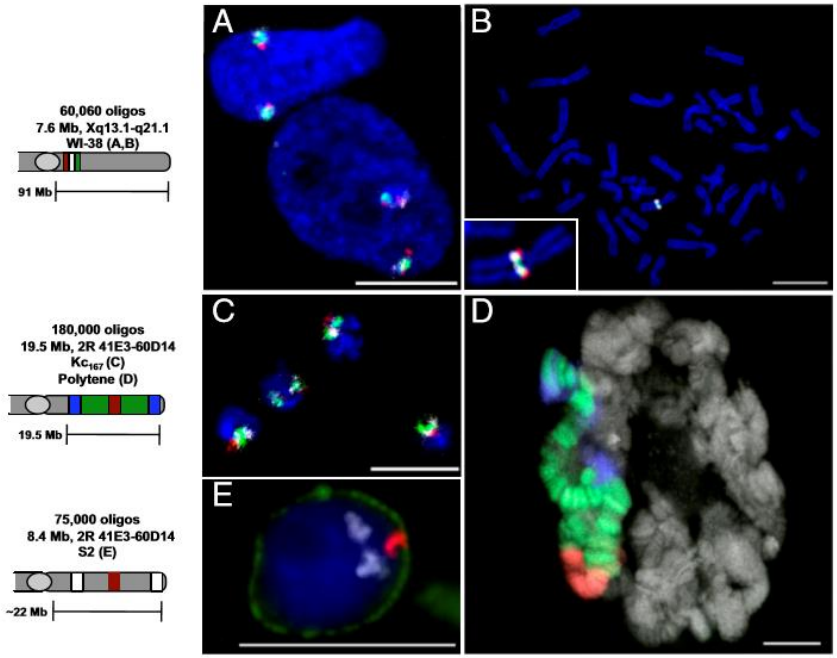
Cross-validated their observations with Hi-C results

# Background

# Background - Oligopaint (Beliveau et. al)

short (32 b), strand-specific probe

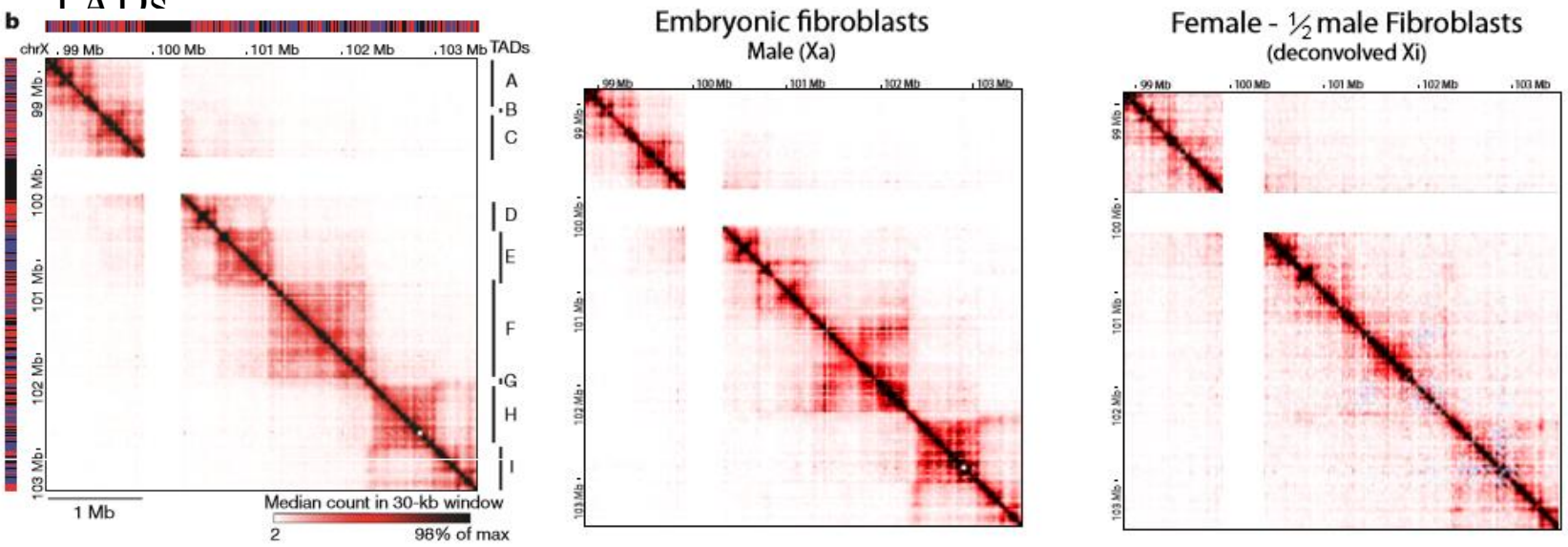


# Background - TADs (Nora et. al)

Long-range contacts within discrete genomic blocks

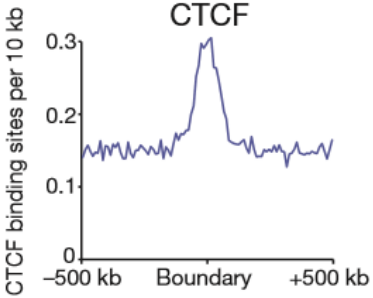
X-inactivation leads to the reduction of chromosomal contact frequencies within TADs

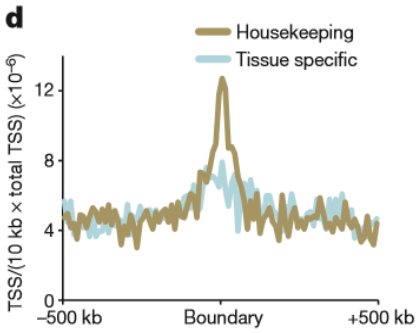


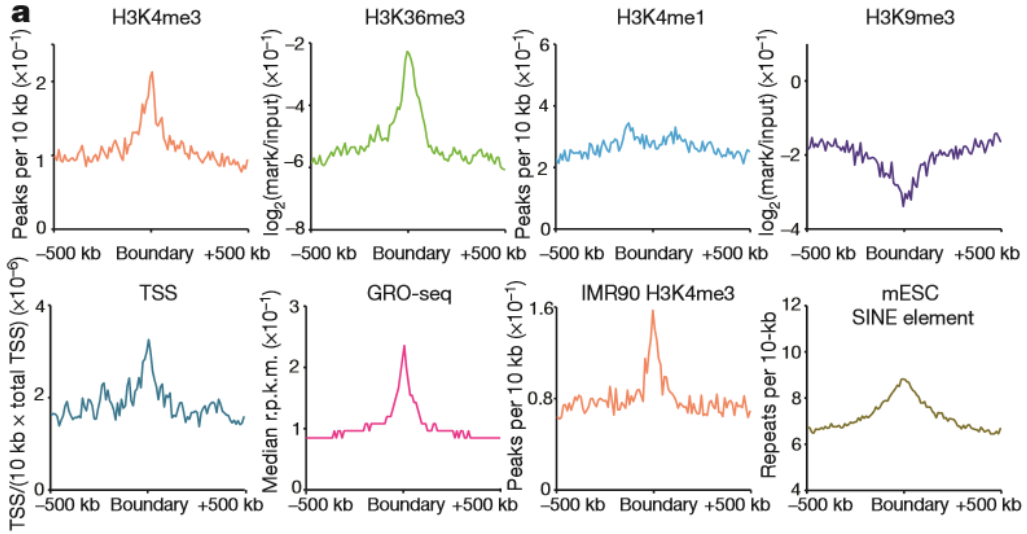
# Background - TADs (Dixon et. al)

Discovered highly self-interacting regions, termed topological domains

Identified factors associated with boundary formation



# Methods

# Methods - Multiplexed FISH

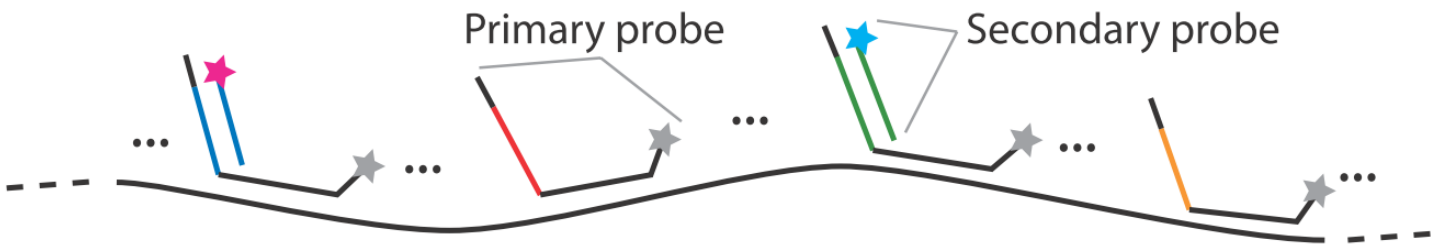
Targeted central 100-kb regions of each TAD with 1000 multi-probes.

Each Primary Probe has two parts:

Targeting Sequence - complementary to a target location in the genome

Mainstreet - A non-genomic sequence shared by all probes for a certain TAD, which is complementary to the Secondary Probes.

Each



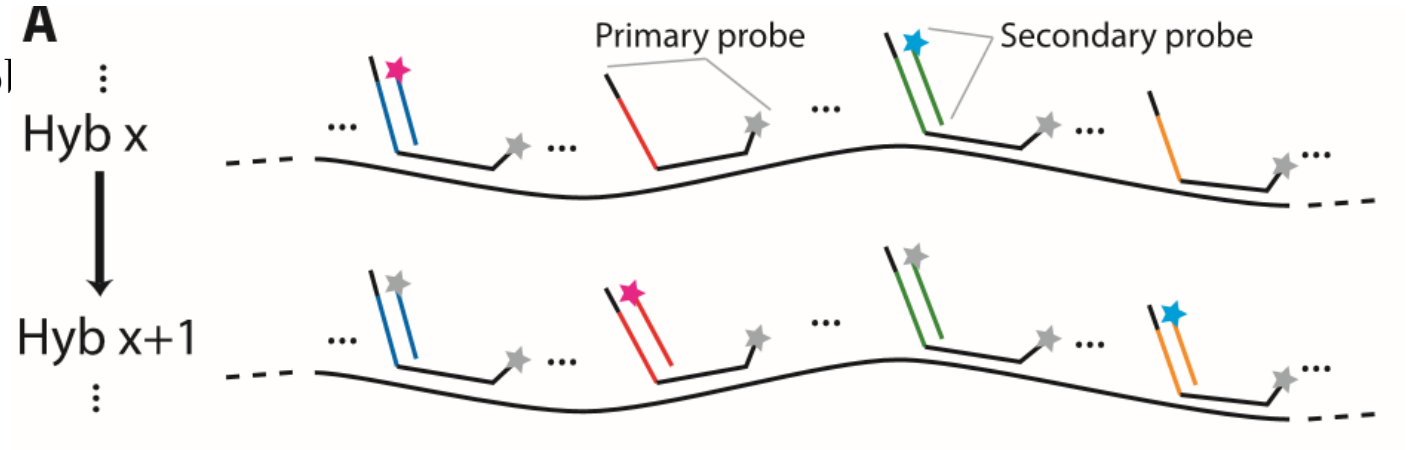
# Methods - Sequential Rounds

First, all primary probes are hybridized to the target chromosome

In rounds, they add secondary probes to image the TADs in sequential order

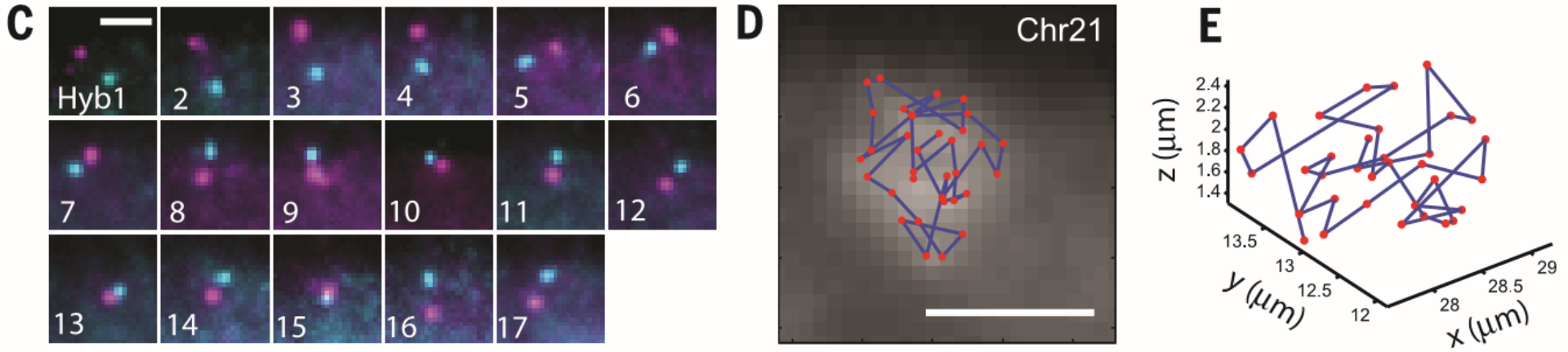
Two colors of probes were used, allowing them to find 2 TADs per round

Photol



# Methods - Fluorescence Imaging

After secondary hybridization

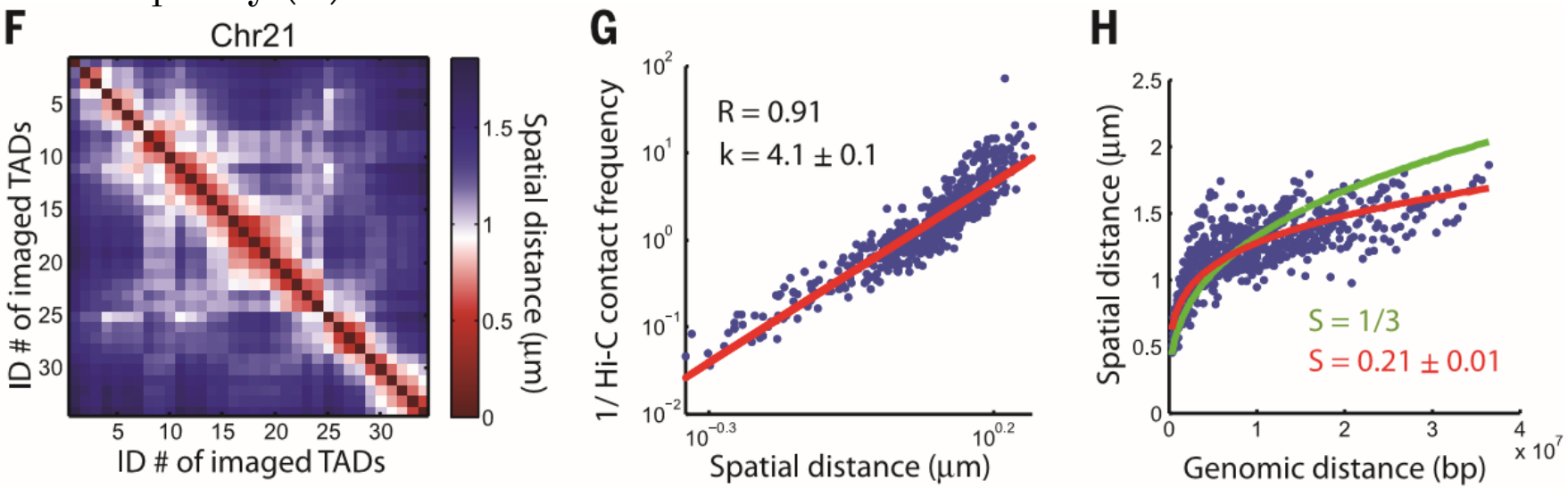


# Findings

# Findings - Correlation with Hi-C

Matrix **F** shows mean distance between TADs

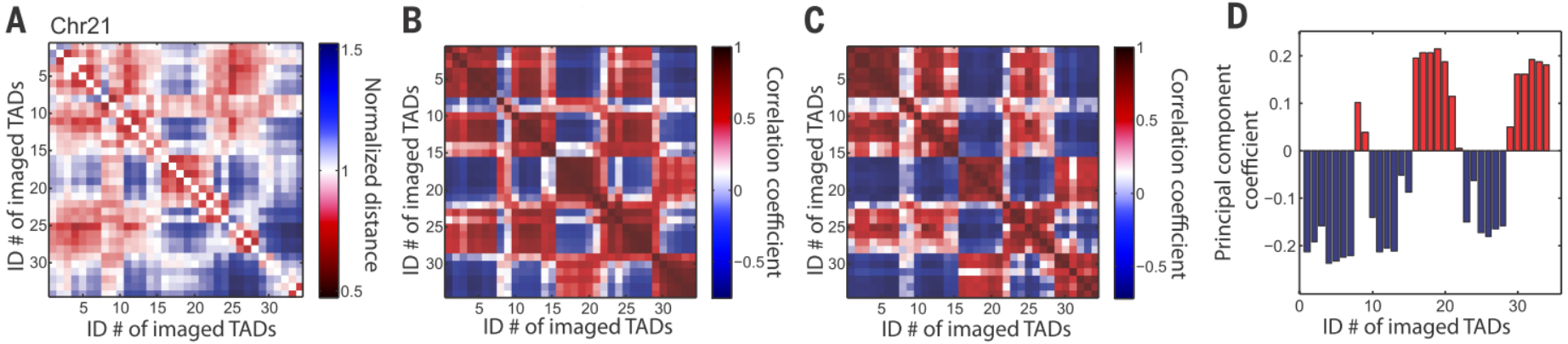
Strong correlation between mean spatial distance and inverse Hi-C contact frequency (**G**)



# Findings - Compartments

Normalized the mean distance matrix (**A**) using expected distance (based on genomic distance) (**B**)

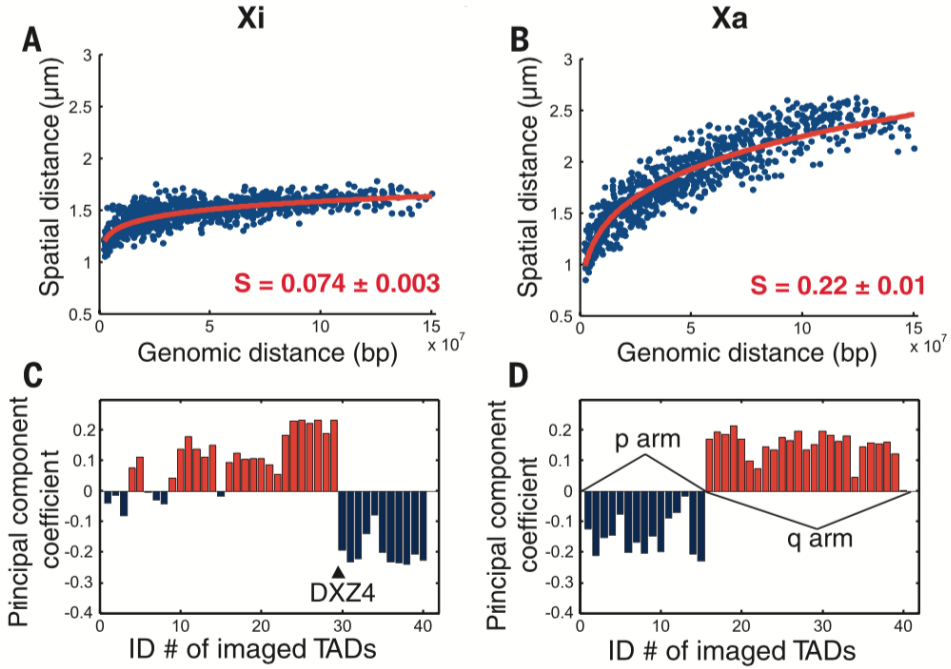
This closely matches the compartments found through Hi-C (**C**)



# Findings - Compartments in Inactive and Active X

They applied this imaging technique to compare chromosome folding in active and inactive X chromosomes

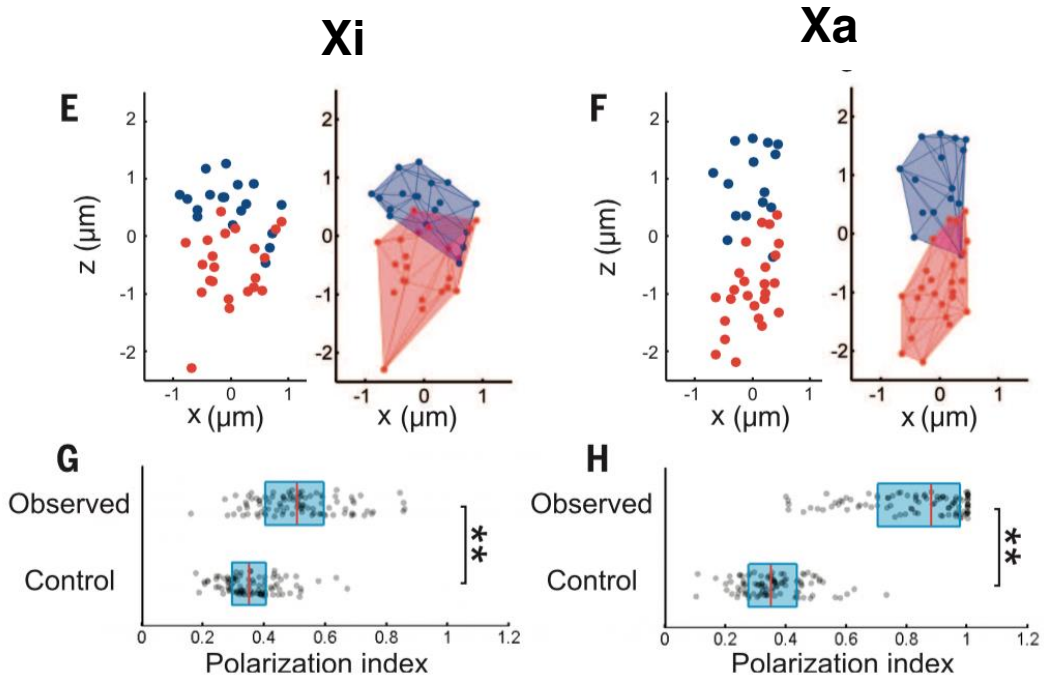
Found significantly different compartments (**C** and **D**) and different power law scaling between genomic distance and spatial distance (**A** and **B**)



# Findings - Compartments in Inactive and Active X

They also measured the polarization of the A and B compartments (using their 3D convex hulls)

Inactive X was significantly less polarized

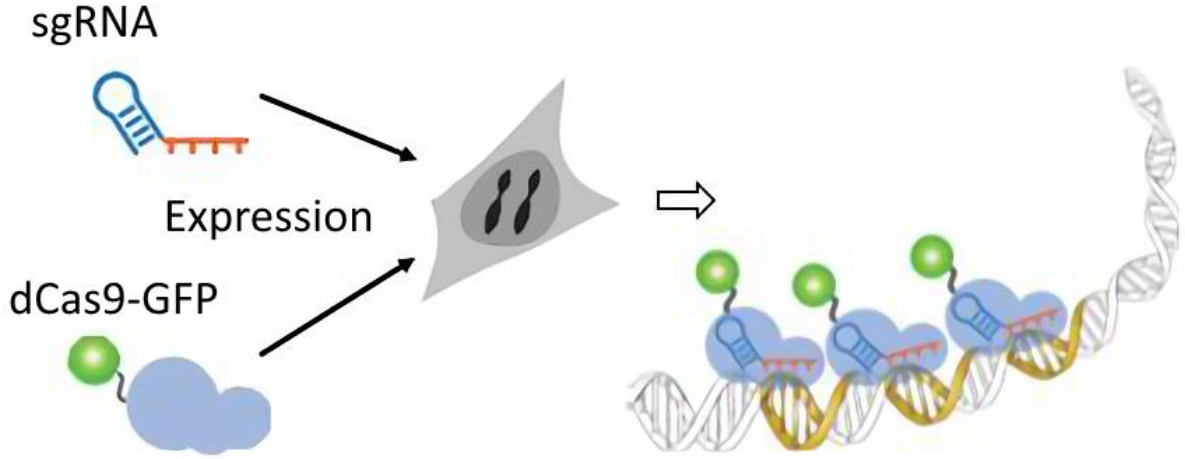


# Further Research

# Crispy FISH

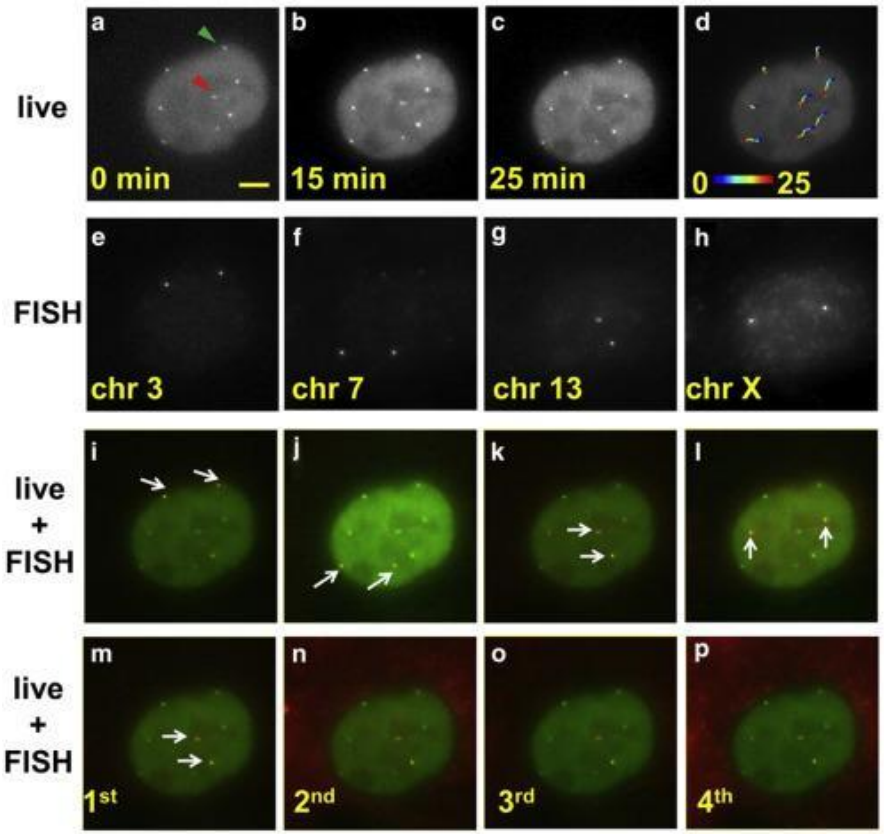
Tracking Multiple Genomic Elements Using Correlative CRISPR Imaging and Sequential DNA FISH. Guan et al. 2017

Used live CRISPR imaging to track position of genes over time, and then sequential FISH to identify the genes



# Crispy FISH

Figure shows the CRISPR imaging and drift of points over time, then the mapping to specific FISH tags



# Other Reasons for Citation

Conformation of compartmentalized structure of DNA

Specific values found from data for use in models

- ⅕ power law value for relation between bp and spacial distance

Citing the existence of FISH

# Questions?