

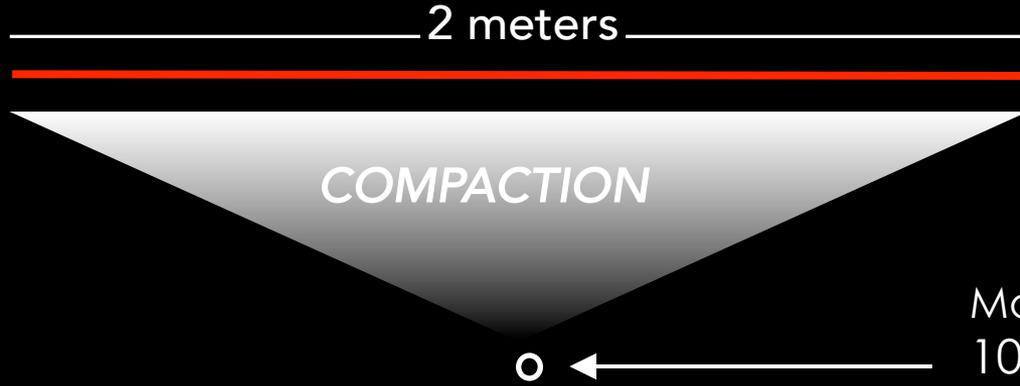
*Imaging Nanoscale Chromatin compaction
in vivo using FRET approach*

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*Robert Feil Group
Genomic Imprinting and Development*

*Institute of Molecular Genetics of Montpellier,
France*

Genome Organization



Nucleosomal

0-10 Kb

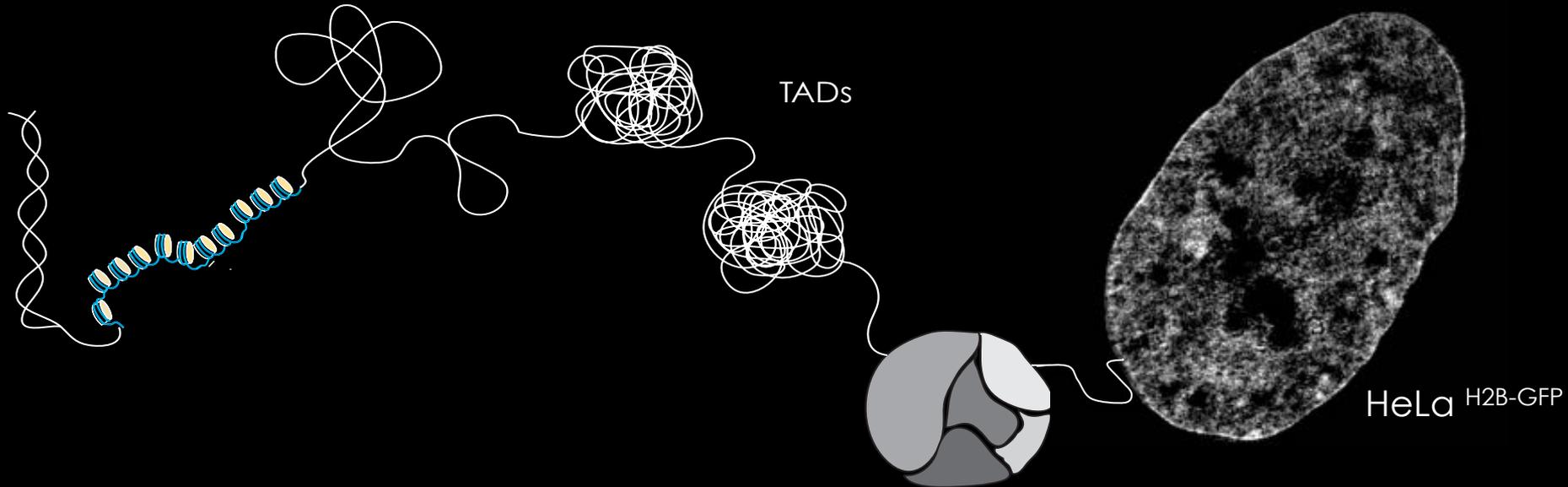
Supra-nucleosomal

10-500 Kb

500 kb -1Mb

Nuclear

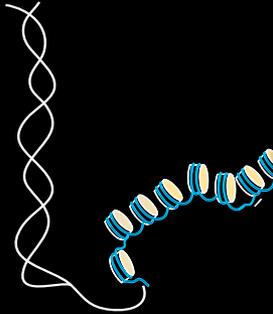
>Mb



Genome Organization

Nucleosomal

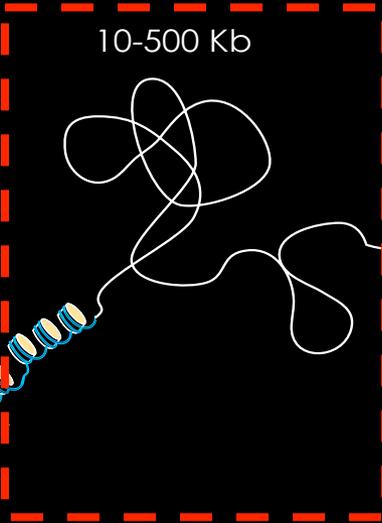
0-10 Kb



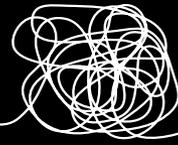
- CHIP
- Microscopy electronic
- In vitro reconstitution
- Super-resolution microscopy

Supra-nucleosomal

10-500 Kb



500 kb - 1 Mb

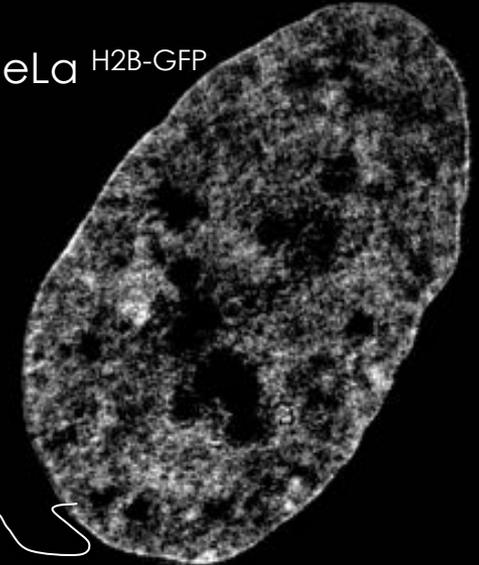


TADs

- Chromosome Conformation Capture
- FISH

Nuclear

HeLa H2B-GFP

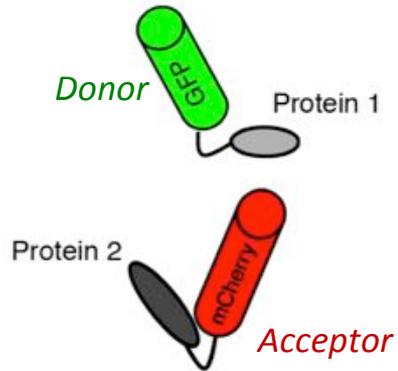


- Cell imaging
- FISH approaches

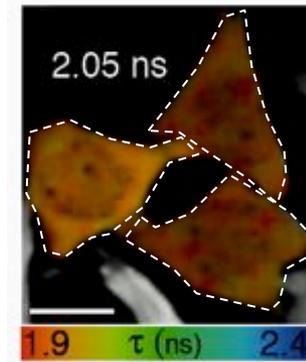
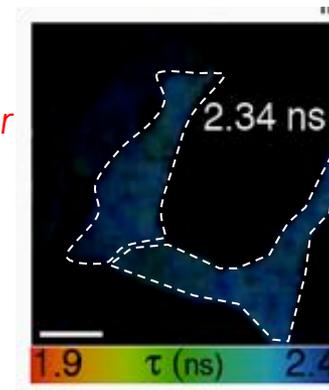
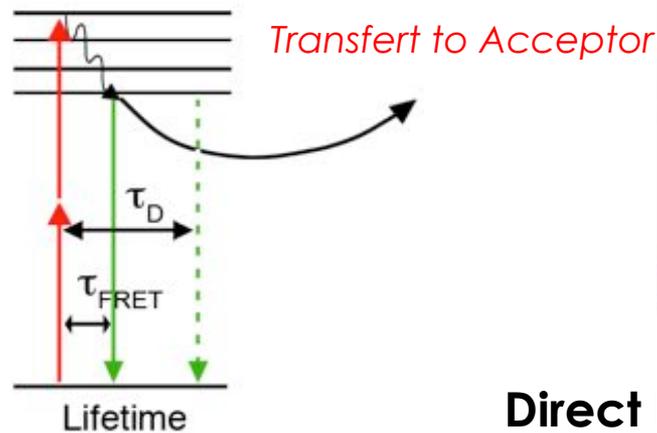
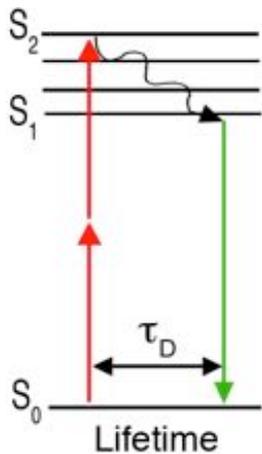
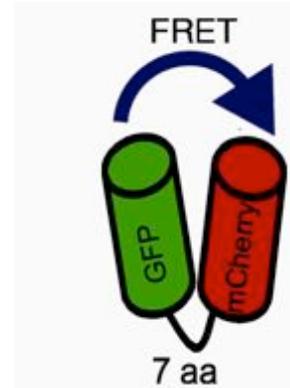
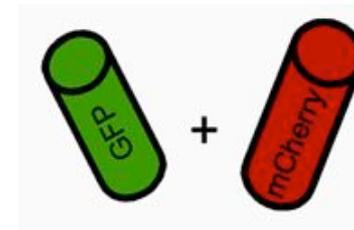
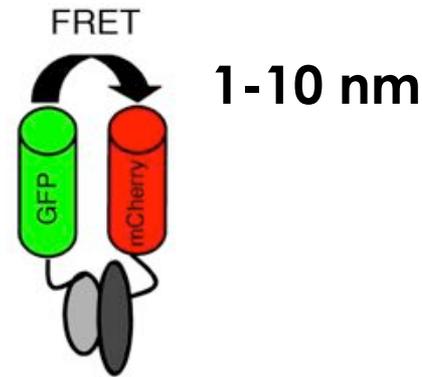
In vivo single-cell approach

FRET resolution in the range of the nucleosome array

No interaction



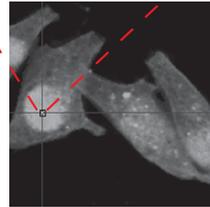
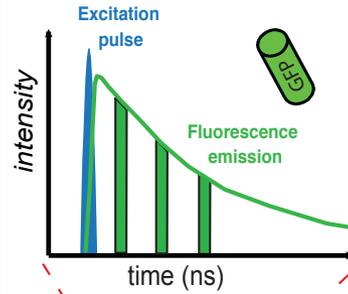
Interaction



Direct Interaction : Reduced Lifetime

FLIM-FRET principle

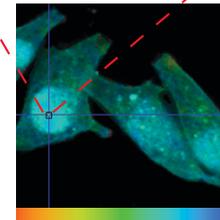
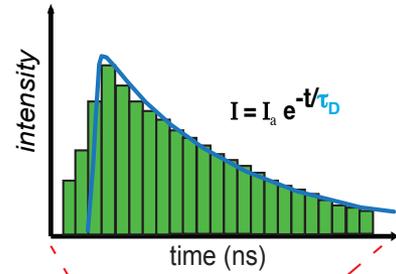
Single photon



EGFP intensity

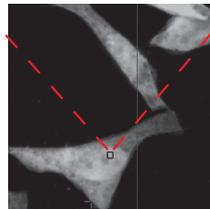
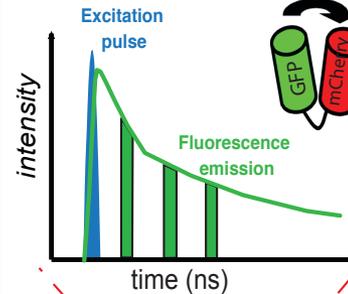
Fluorescence decay component

collection of n photons



1.25 ns 2.4 ns
EGFP lifetime

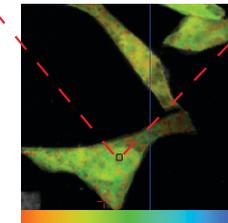
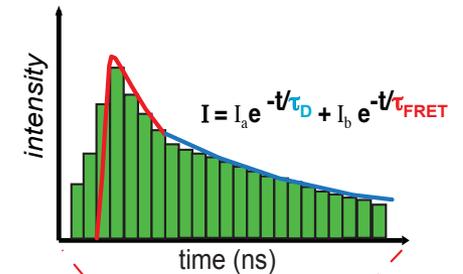
Single photon



EGFP intensity

Fluorescence decay components in FRET

collection of n photons

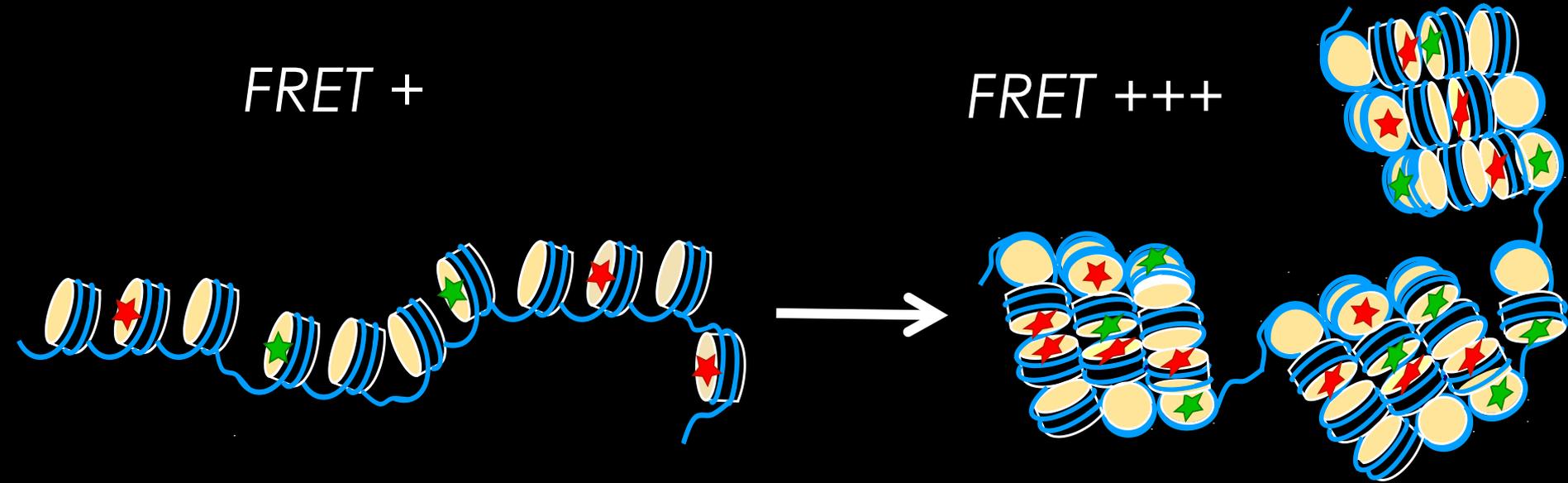


1.25 ns 2.4 ns
EGFP lifetime

FRET resolution in the range of the nucleosomal compaction

FRET +

FRET +++



Nucleosome



mCherry-H2B



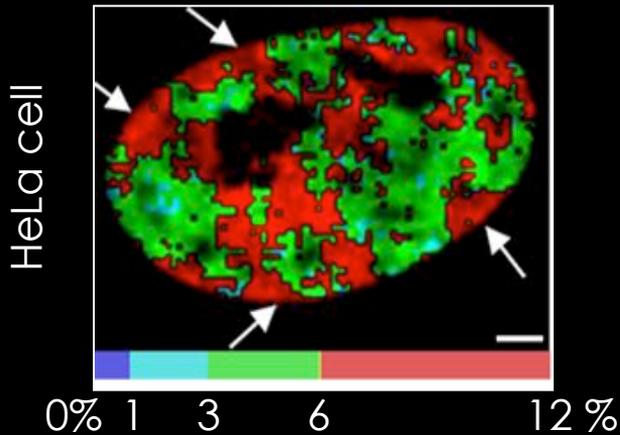
H2B-GFP



DNA

Nucleosome-nucleosome interactions contribute to the FRET readout

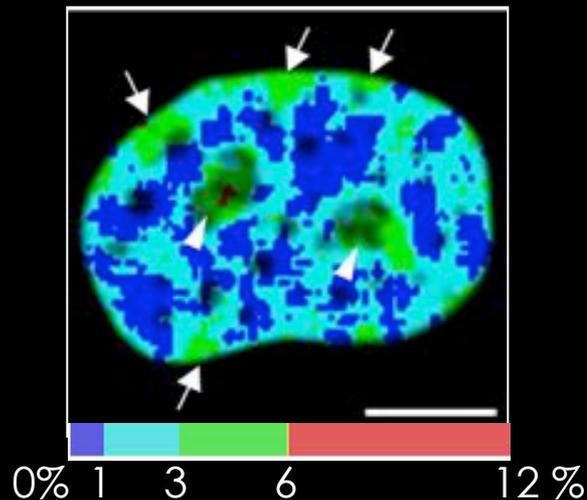
FRET (%) map



- Localisation & Quantification of chromatin domains compaction in living cells



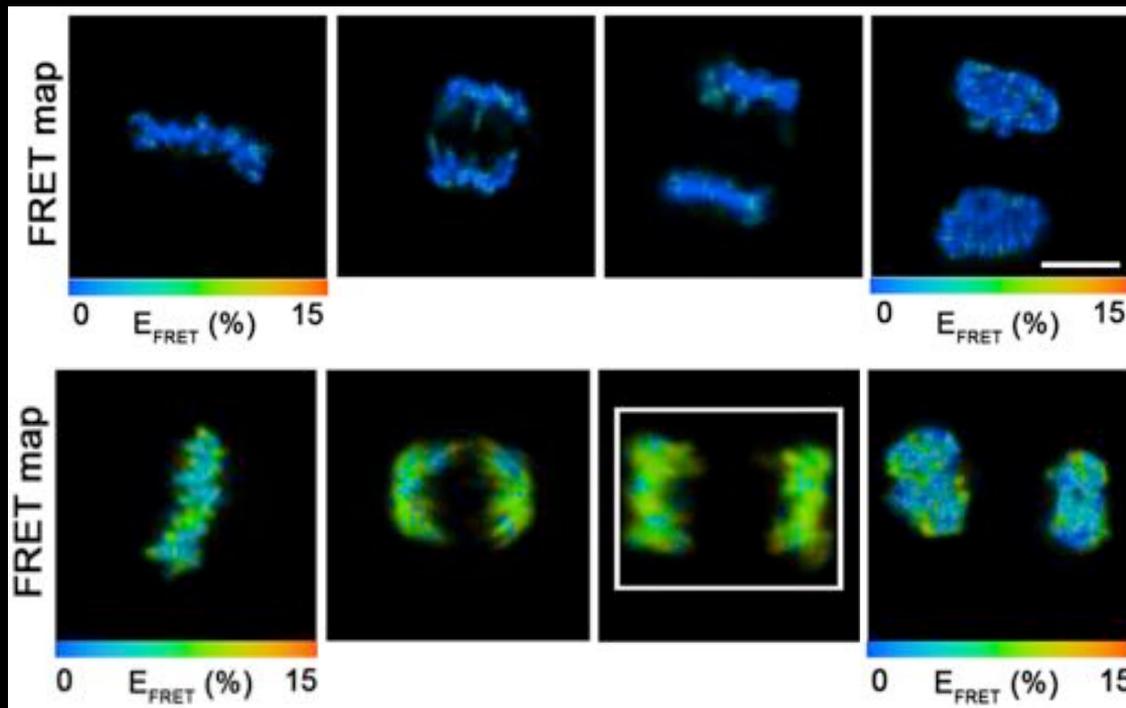
HDAC inhibitor (TSA)



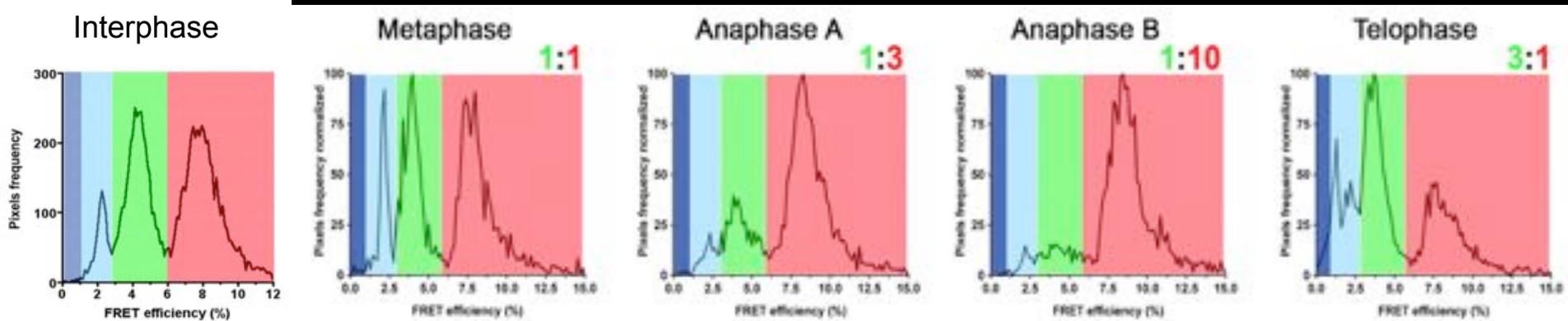
- Chromatin domain compaction is controlled by histone modifications

Maintenance of chromatin domains along mitosis

HeLa H2B-GFP

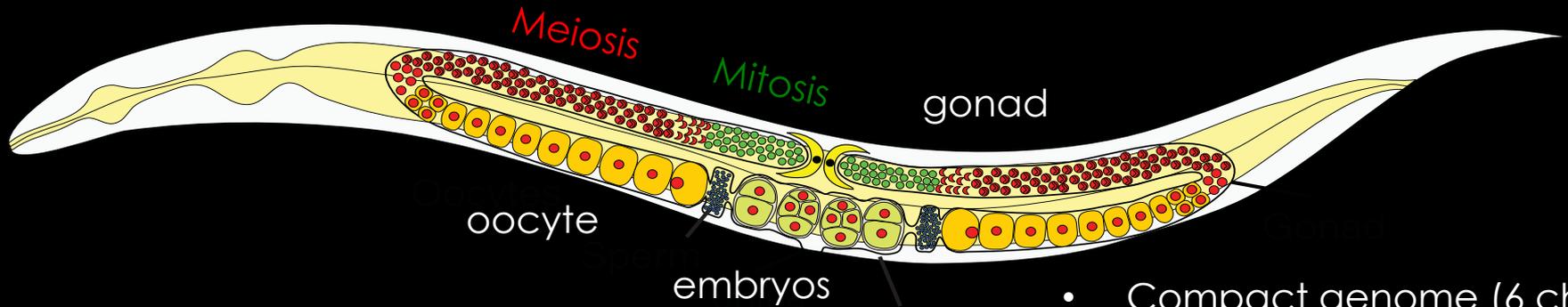


HeLa H2B-GFP/mCherry-H2B



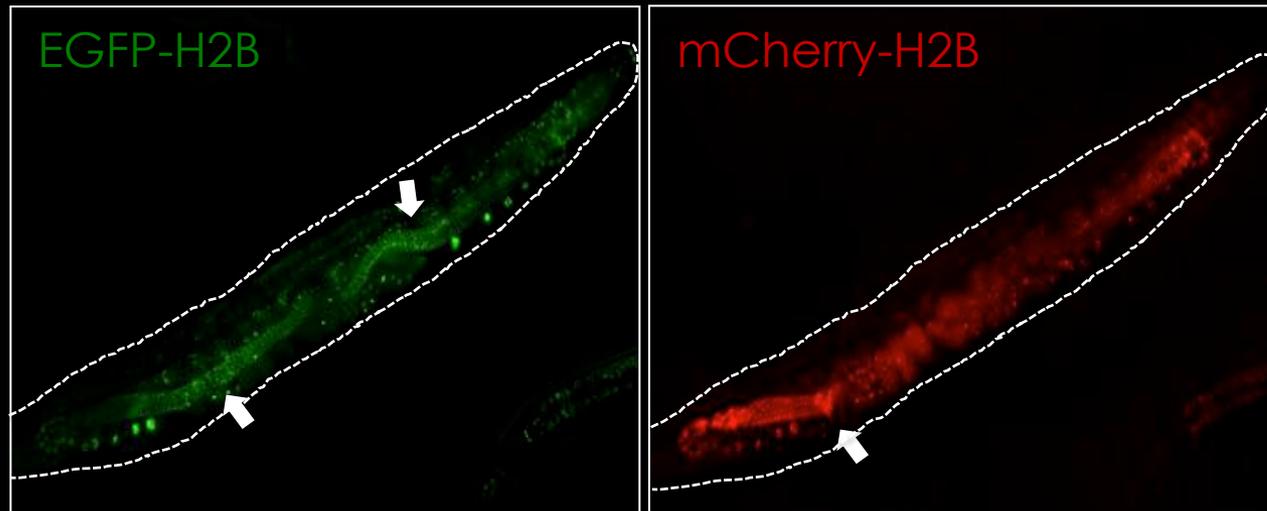
Measuring chromatin compaction in an organism

Caenorhabditis elegans as an organism model

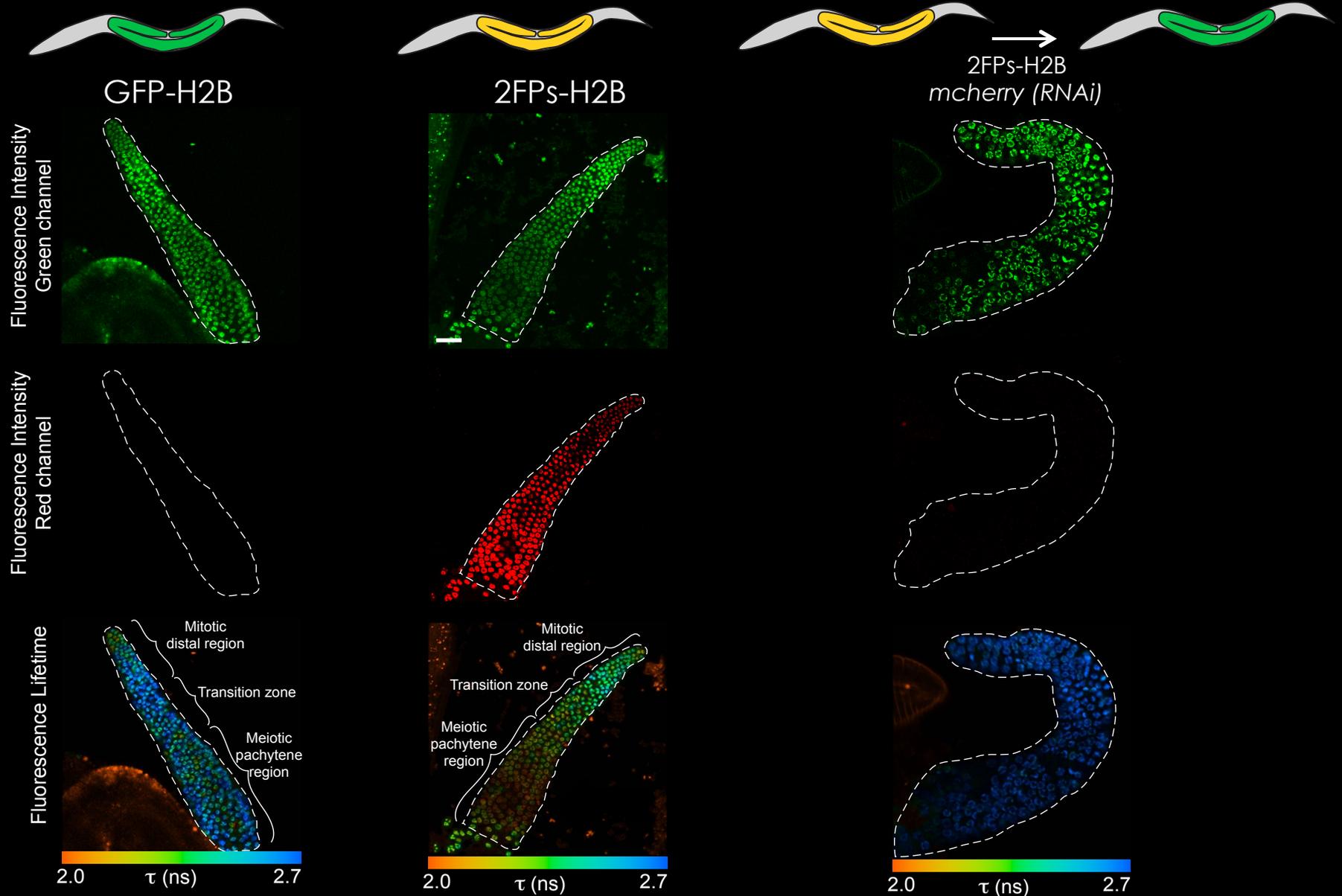


- Compact genome (6 chr)
- Transparent
- High degree of conservation

Hermaphrodites *c. elegans* 2FPs-H2B



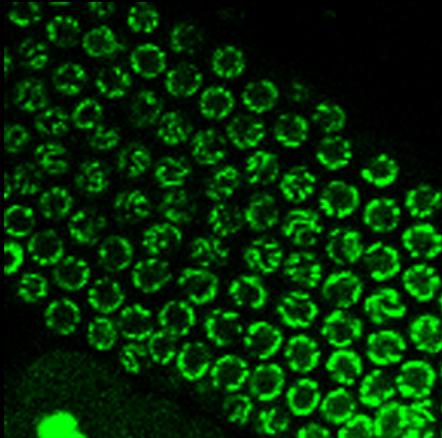
FLIM-FRET measurements in *C.elegans* gonads



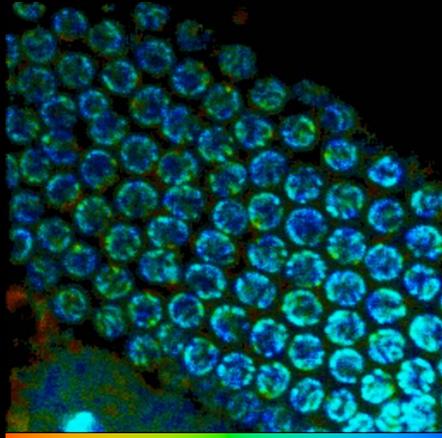
Highest chromatin compaction in meiosis

C.elegans GFP-H2B

Fluorescence Intensity
GFP::H2B



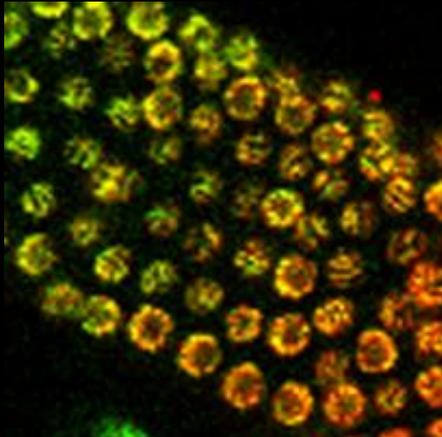
Fluorescence Lifetime



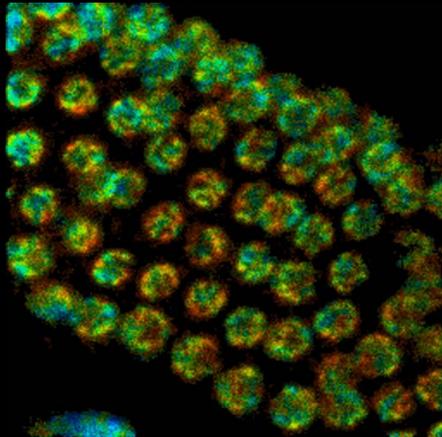
2.0 τ (ns) 2.8

C.elegans 2FPs-H2B

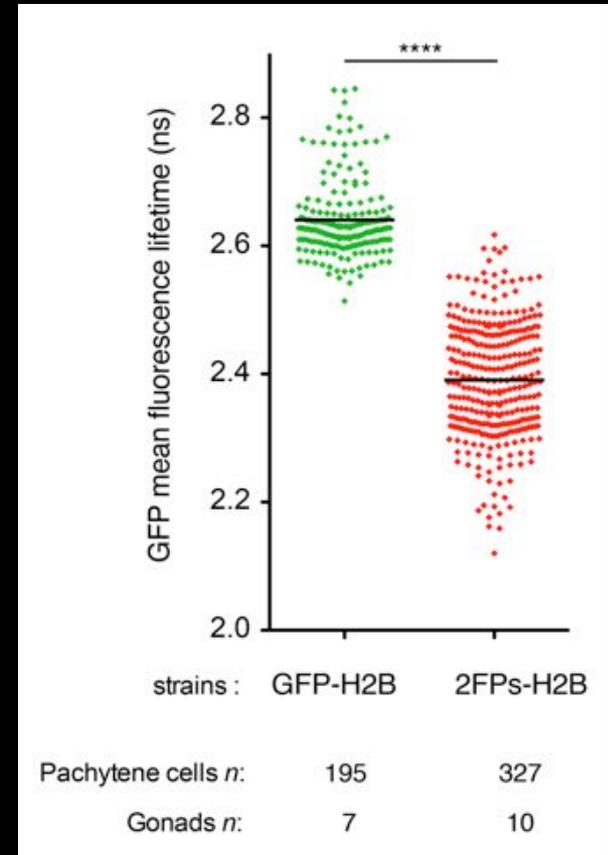
Fluorescence Intensity
GFP::H2B mCherry::H2B



Fluorescence Lifetime

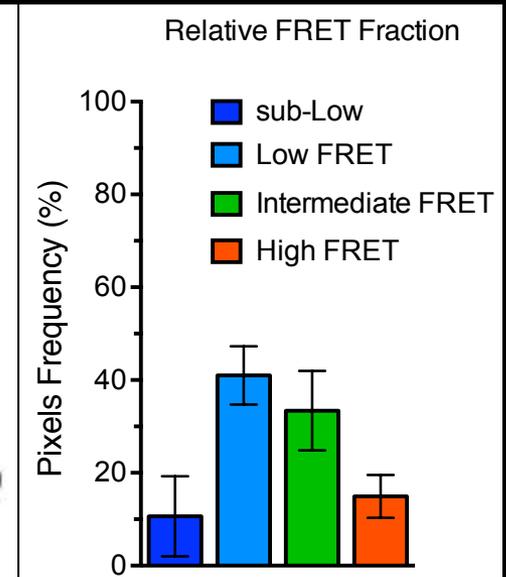
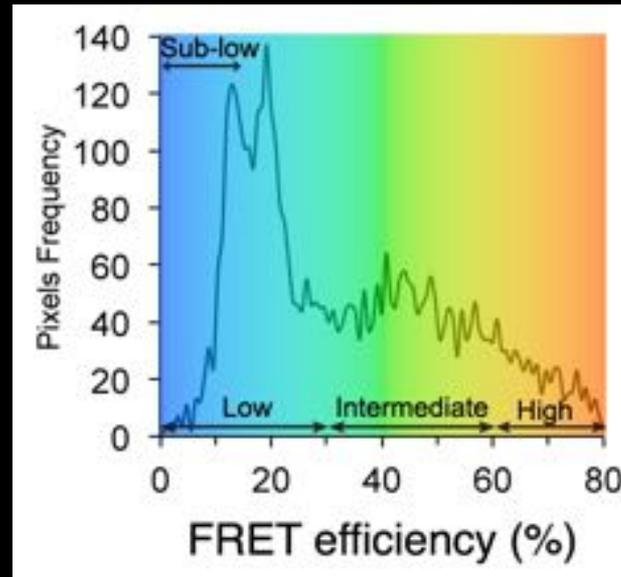
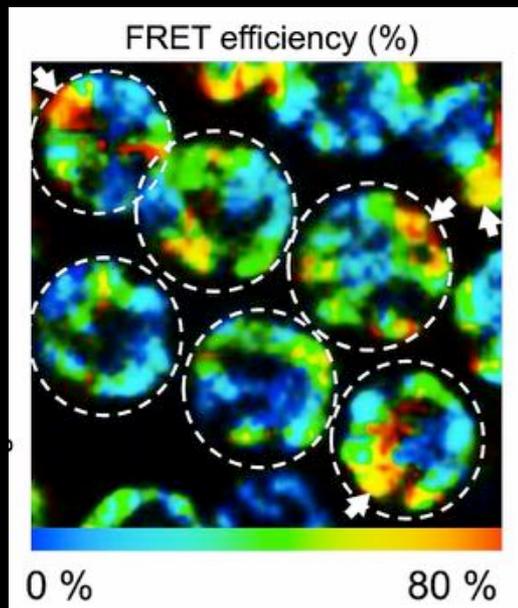
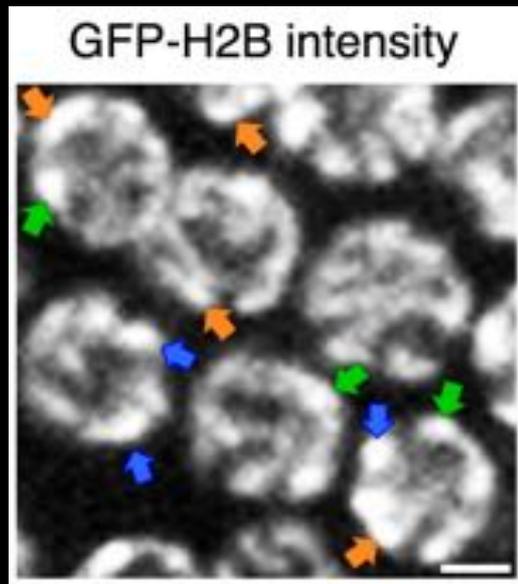


2.0 τ (ns) 2.8

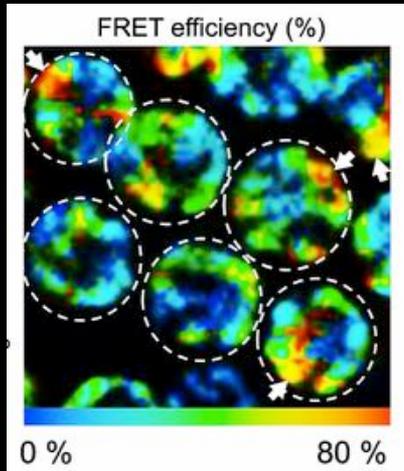


Llères D, Bailly A et al. Cell reports. 2017

Distinct chromatin domain structures in pachytene-stage chromosomes

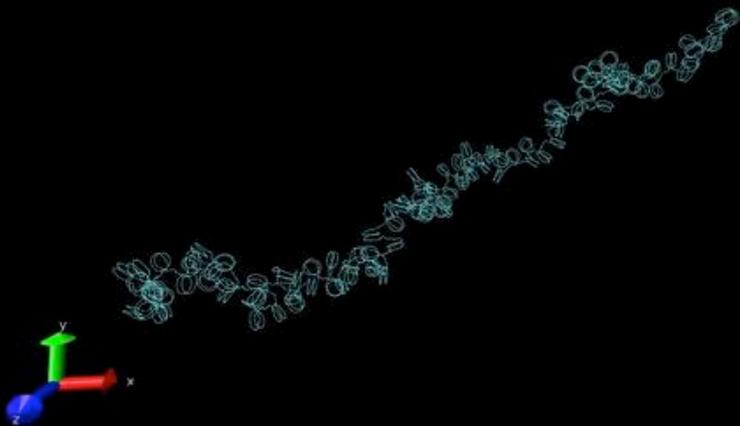


Ongoing work: Modeling Chromatin domain structures in pachytene-stage chromosomes

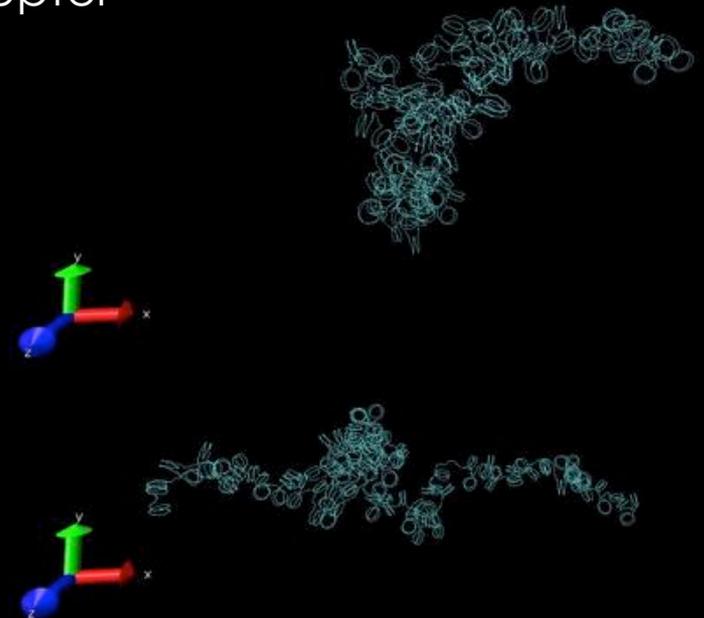


- Low FRET interactions
- High FRET interactions
- Fraction of donor interacting with acceptor

Coarse grained modeling



Open loops

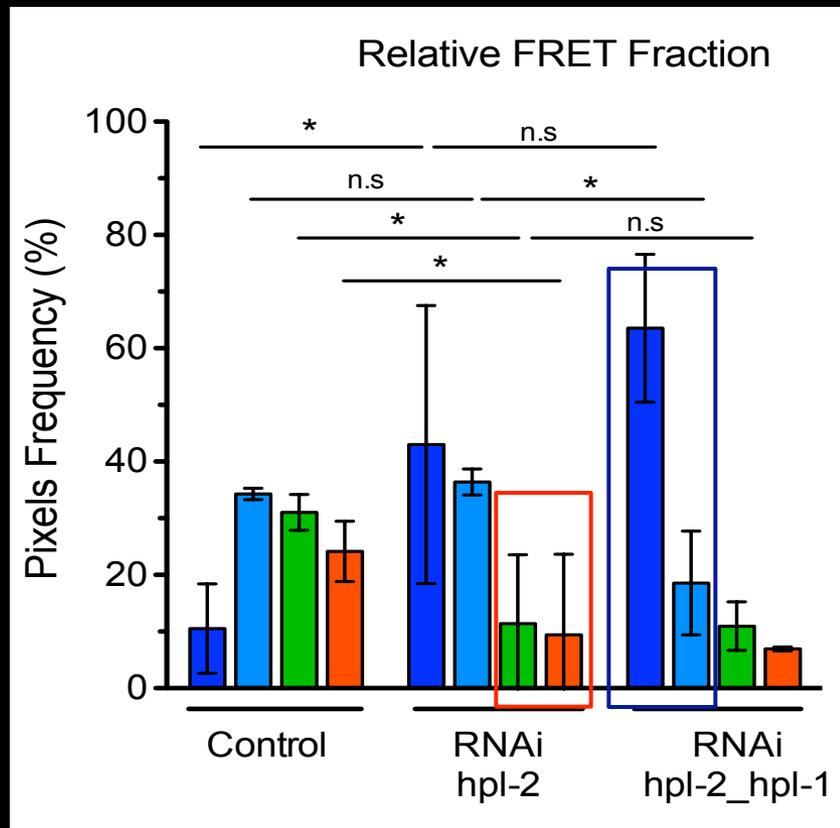
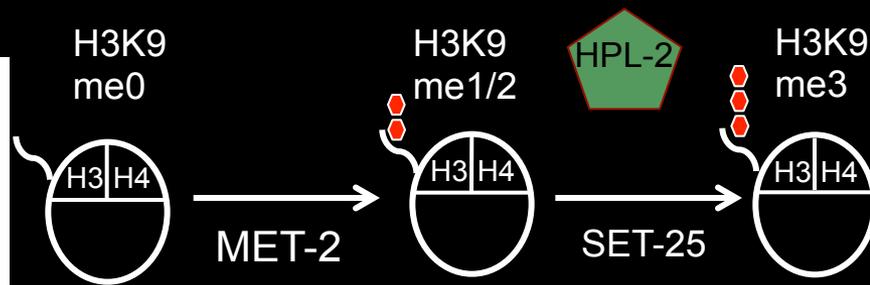
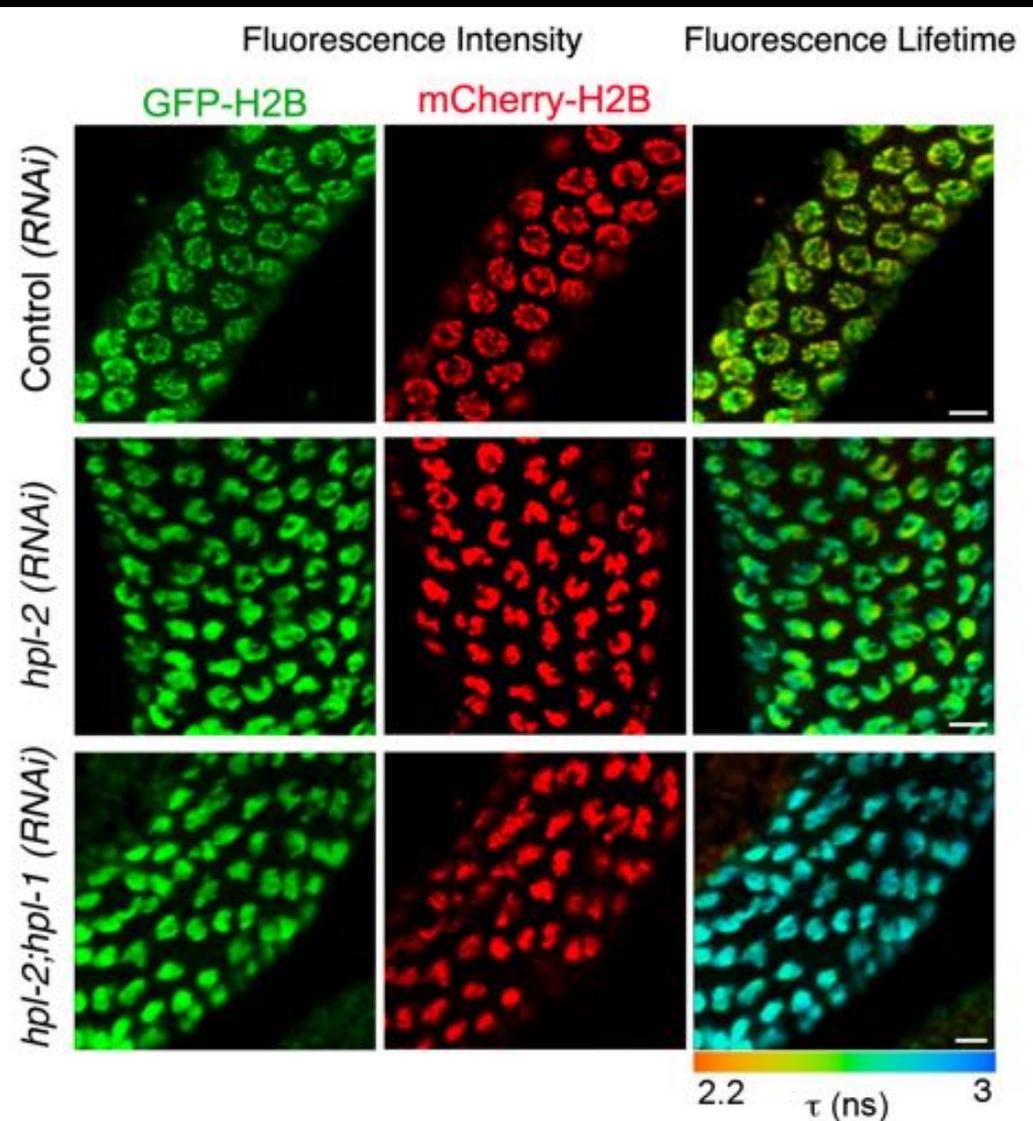


Dense loops
Intermingling interactions

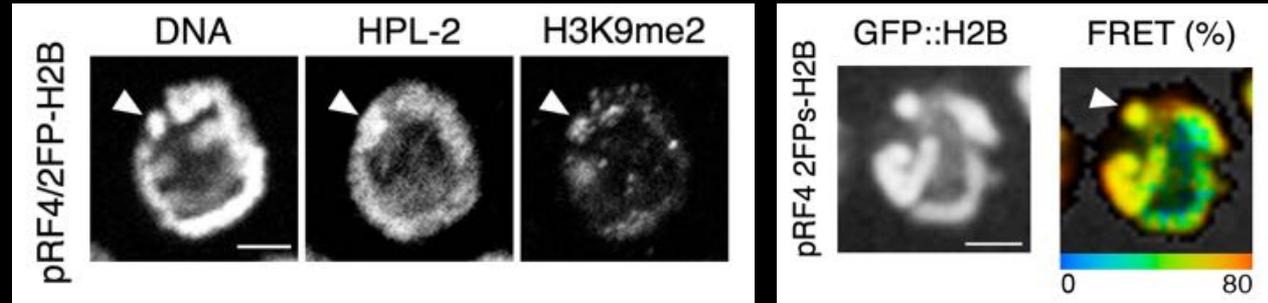
What do these compaction states correspond to ?

How are they controlled ?

HP1 homolog is essential for heterochromatin compaction



Silenced repeated sequences acquired structurally compacted chromatin controlled by HP1



concatemerization

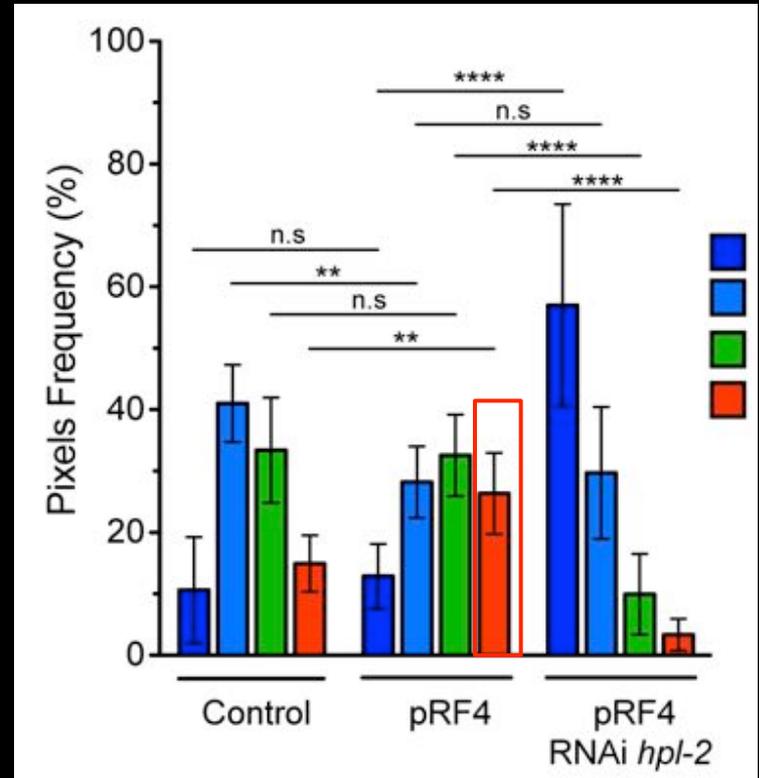


pRF4 extra-chromosomal array

- Silenced in germ cells

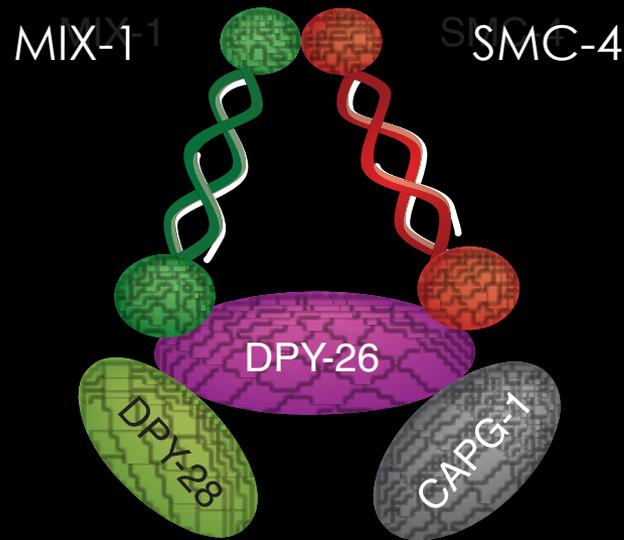
- Expressed in somatic cells

ROLLER phenotype



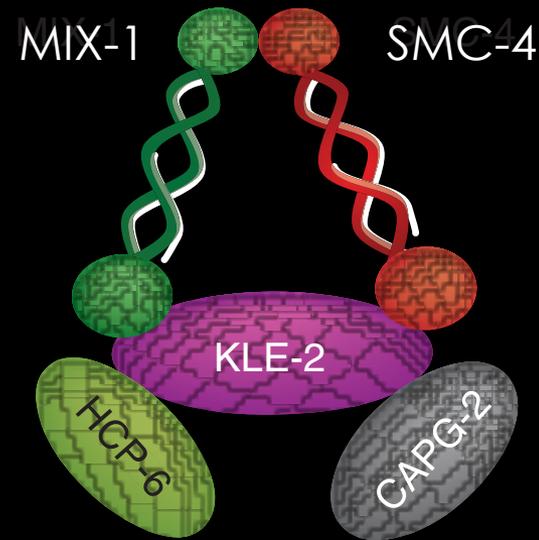
Structural regulators of chromosomes: Condensin complexes

Condensin I



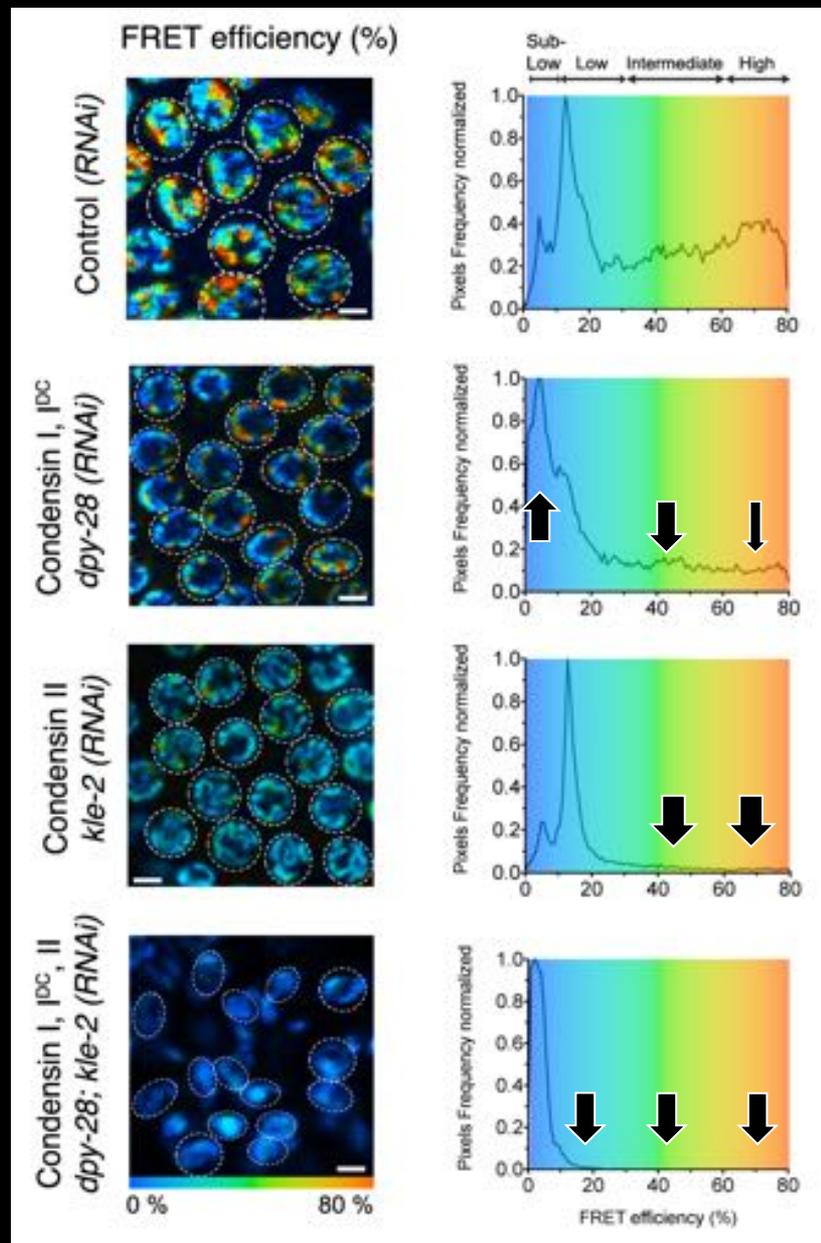
Controls chromosomes
in early meiosis

Condensin II

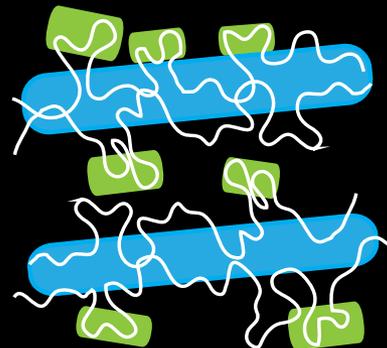
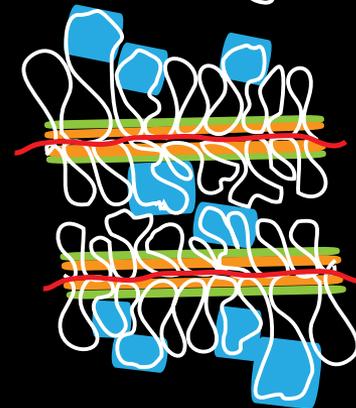
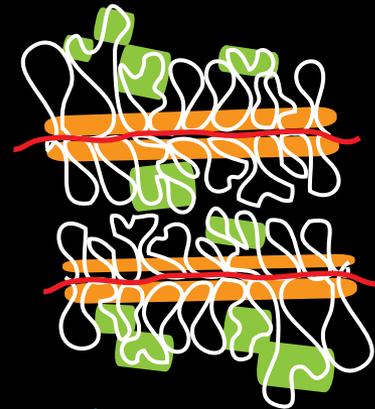
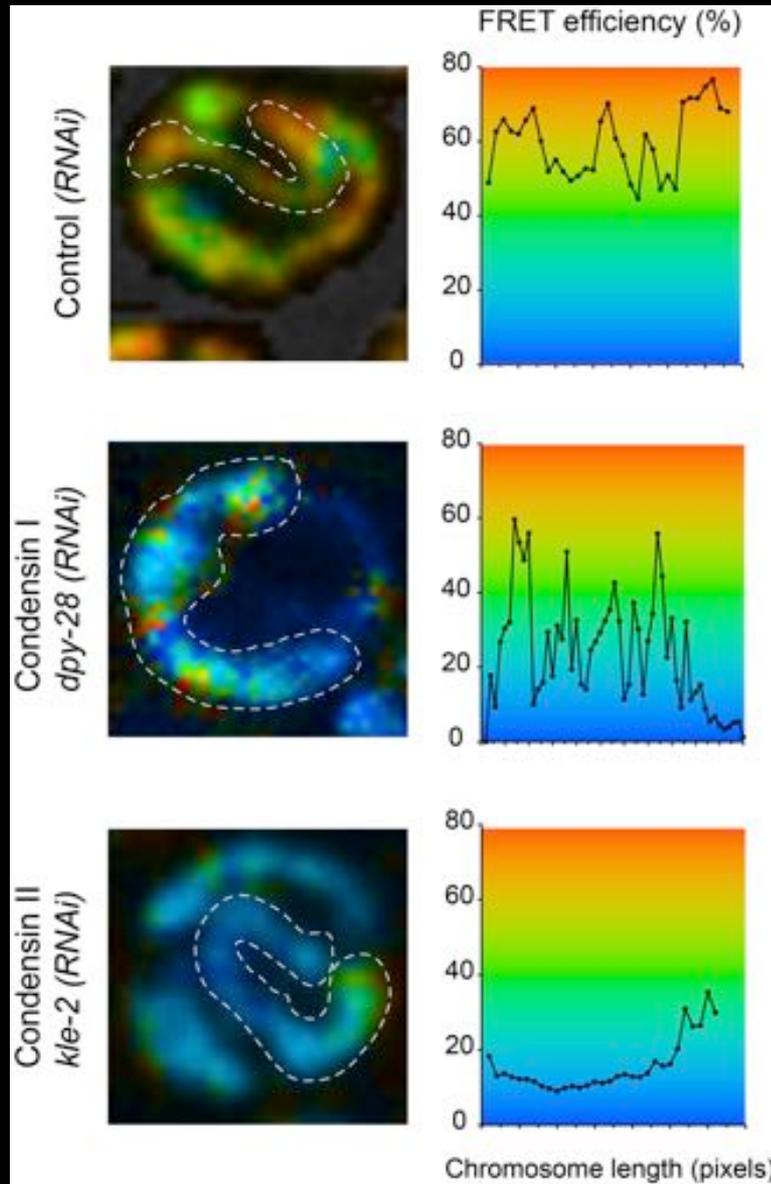


Restructure meiotic
chromosomes after
pachytene exit

Condensin complexes differentially regulate compaction

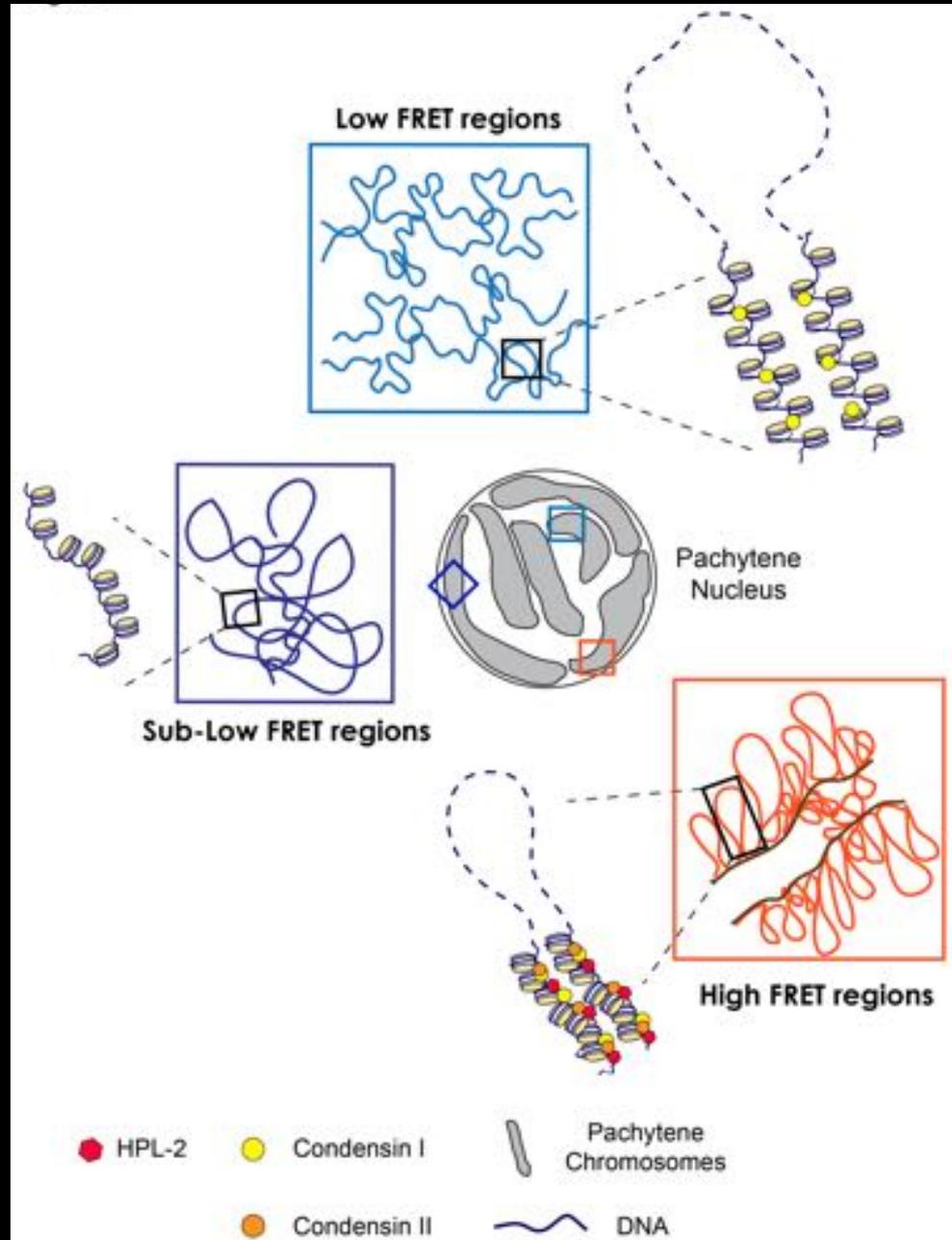


Relationship between Condensin complexes and lampbrush meiotic architecture



- Low FRET
- Inter FRET
- High FRET

Summary



Conclusions

- Meiotic chromosomes in living *C. elegans* display heterogeneous chromatin domain structures
- Heterochromatin architecture revealed a highly compacted nanoscale organisation *in vivo* controlled by HP1
- Tandem repeat-enriched ectopic chromosomes acquire heterochromatic structure
- Condensin I and II show differential effects on chromosome structure

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Lamond lab (GRE, Dundee)

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Aymeric Bailly
Aurélien Perrin

Modesto Orozco lab (IRB, Barcelona)
Jurgen Walther
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Montpellier Ressources Imagerie



MRI
Montpellier Ressources
Imagerie

