



5èmes Rencontres  
scientifiques des Grands  
Causses

La Séparation de Phase  
Liquide-Liquide

# Chromatin structure from high resolution microscopy: scaling laws and micro-phase separation

Millau - 03/10/2023

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Laboratoire Charles Coulomb

Systèmes Complexes et Phénomènes Non-linéaires

Under the supervision of

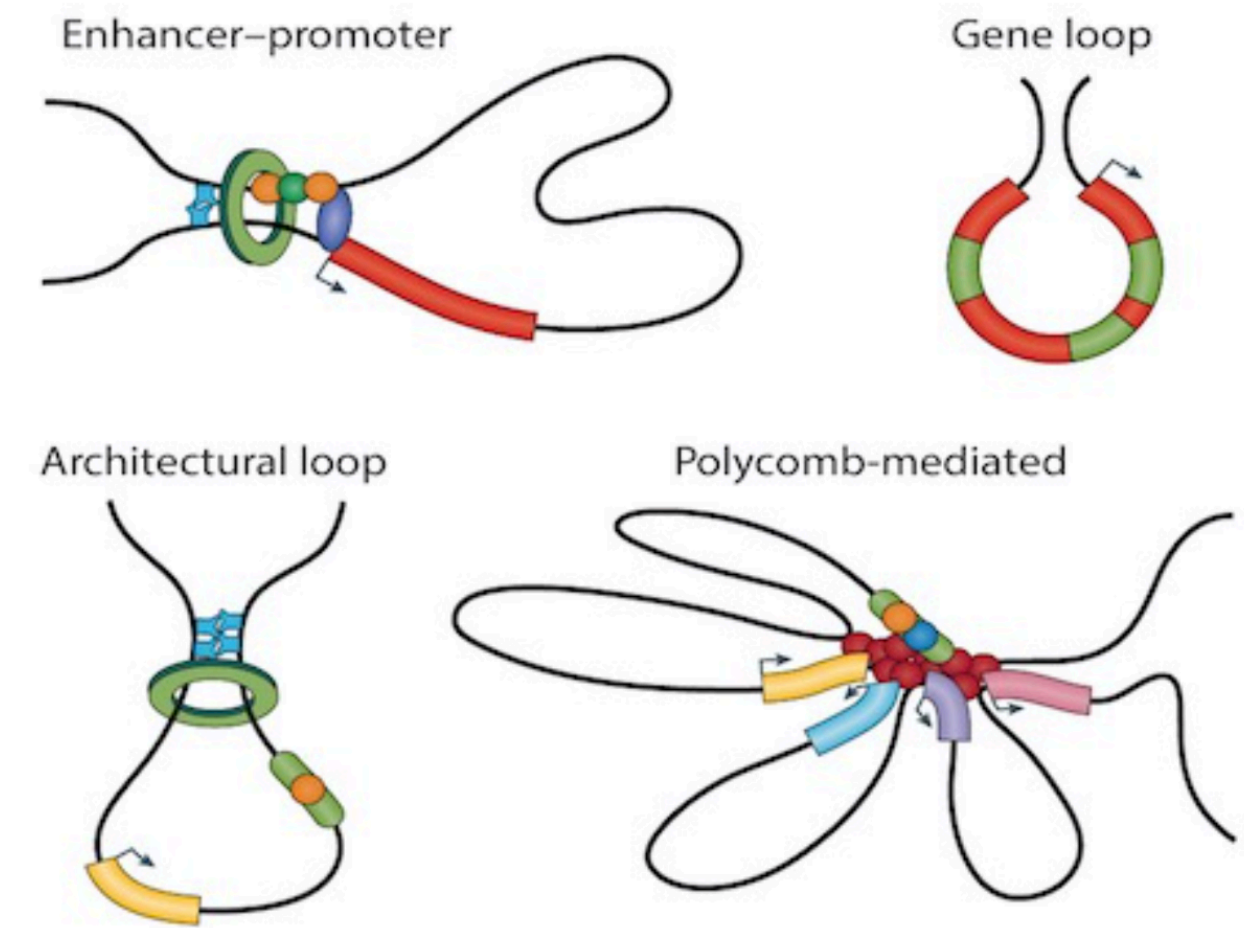
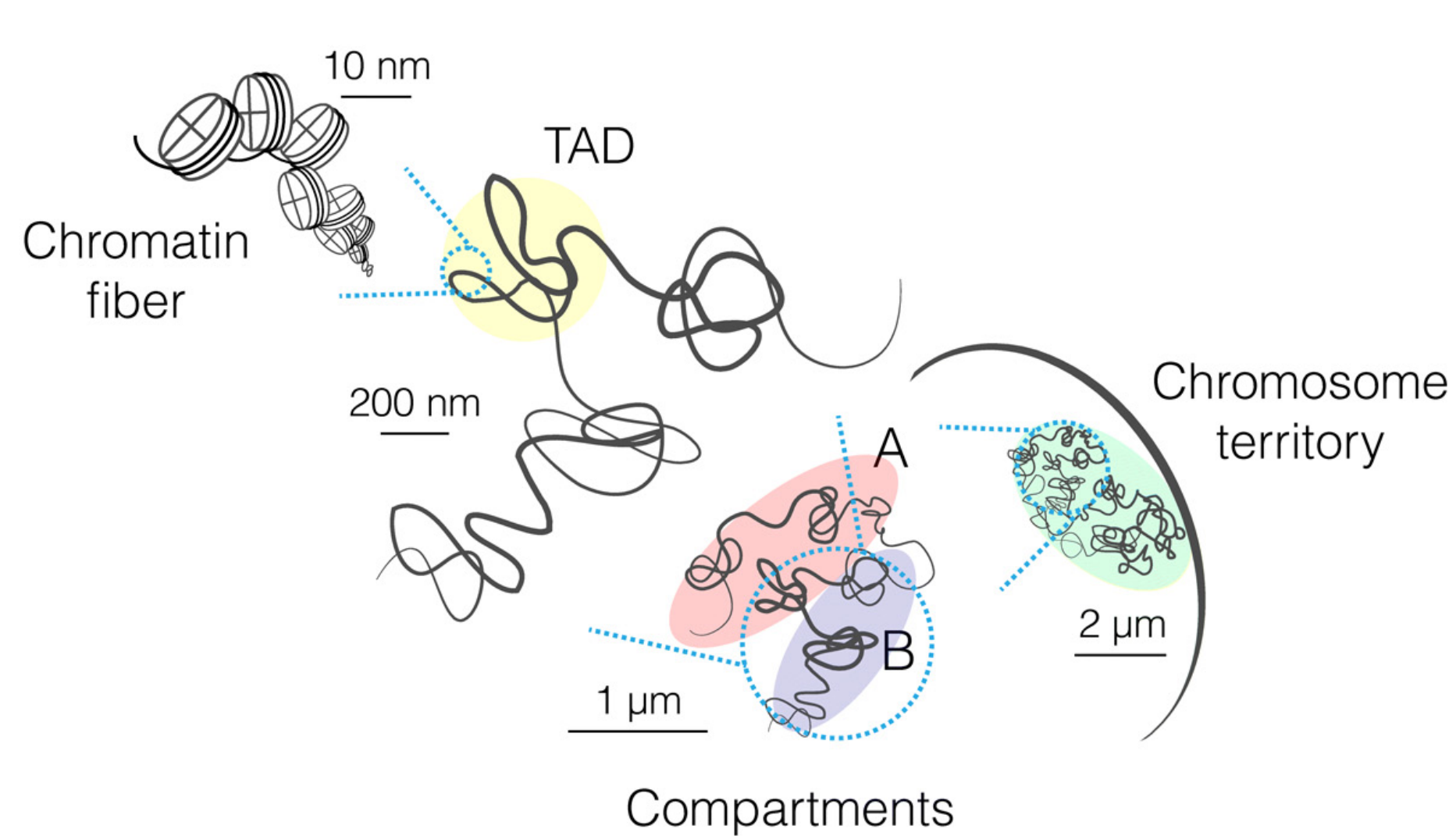
Andrea Parmeggiani



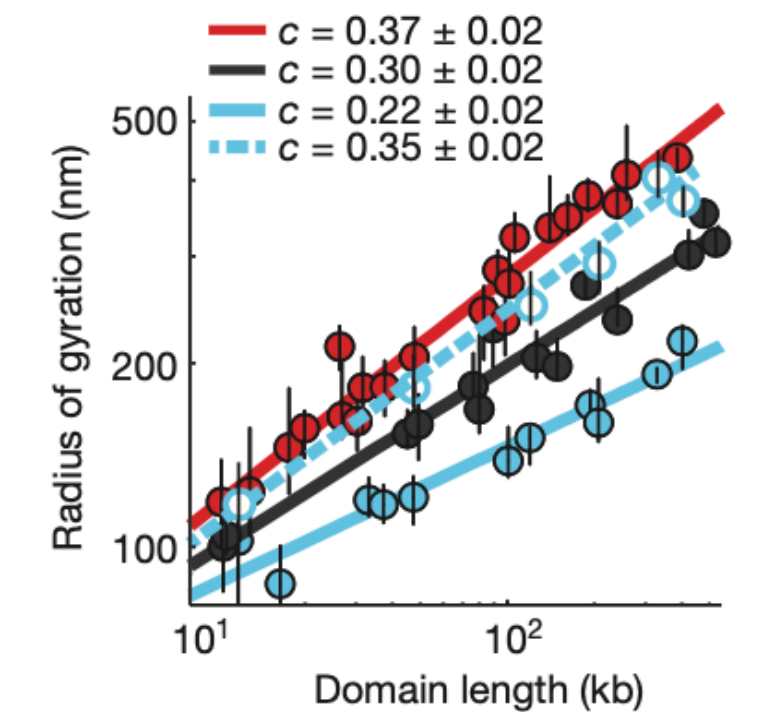
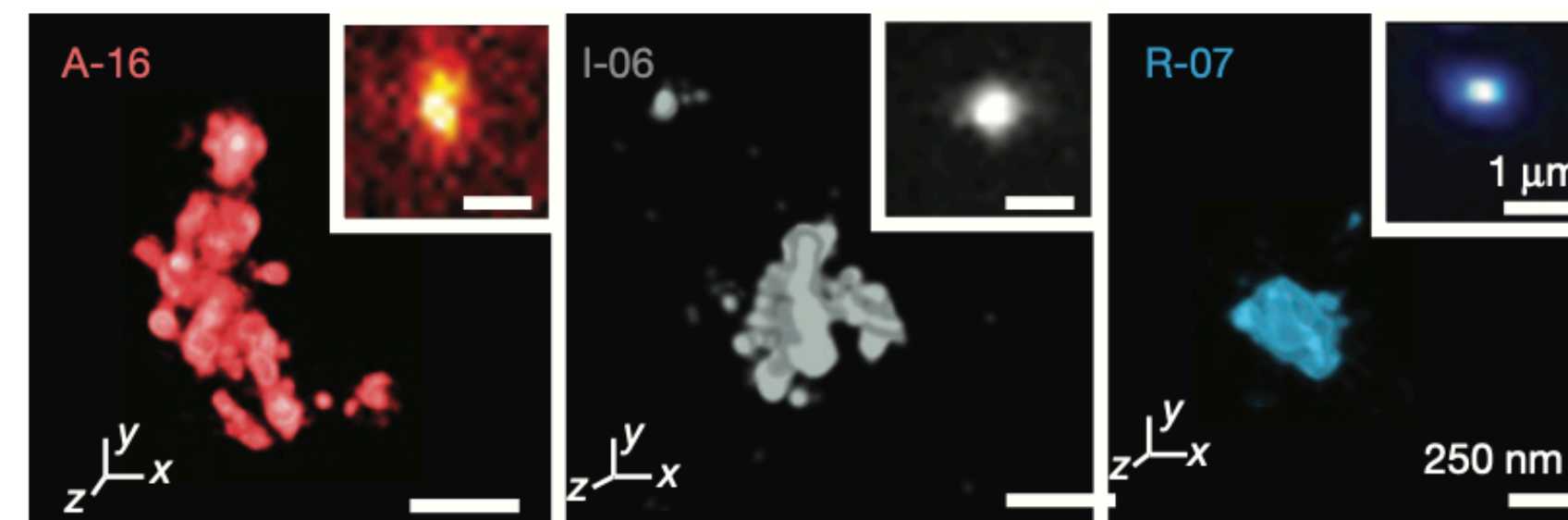
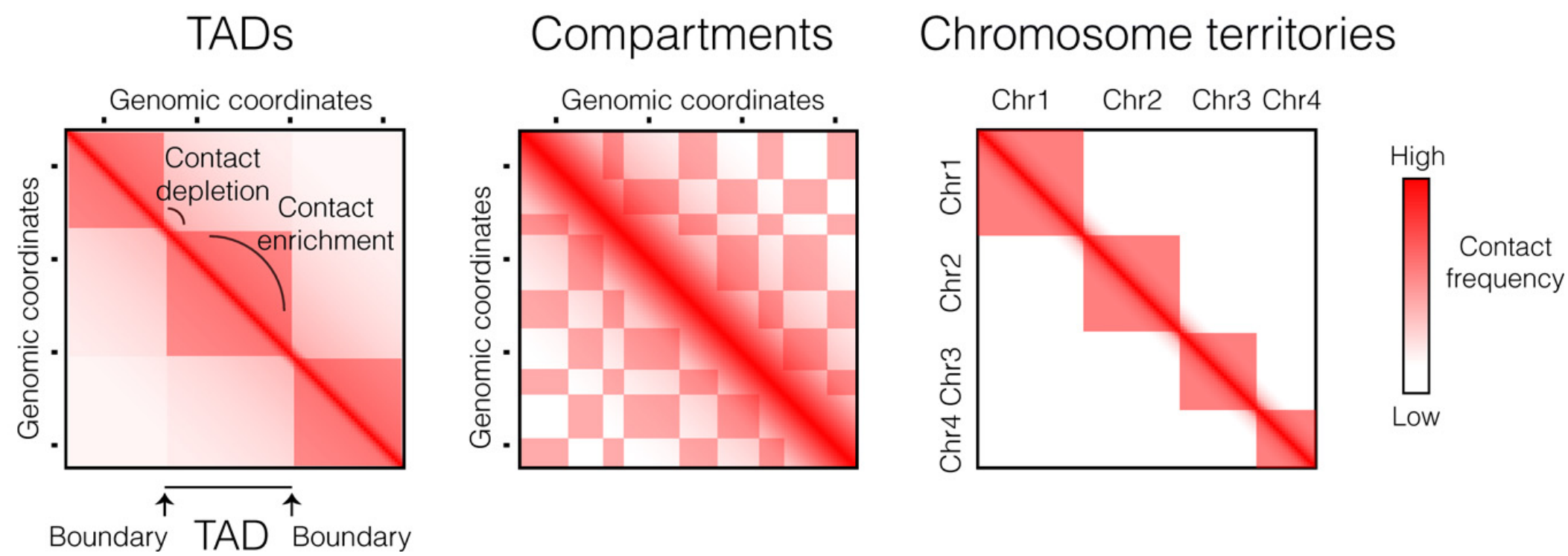
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# Introduction: Higher-order organization in the cell nucleus



[2]



● Active   ● Inactive   ● Repressed   ● Repressed (Ph KD)

[3]

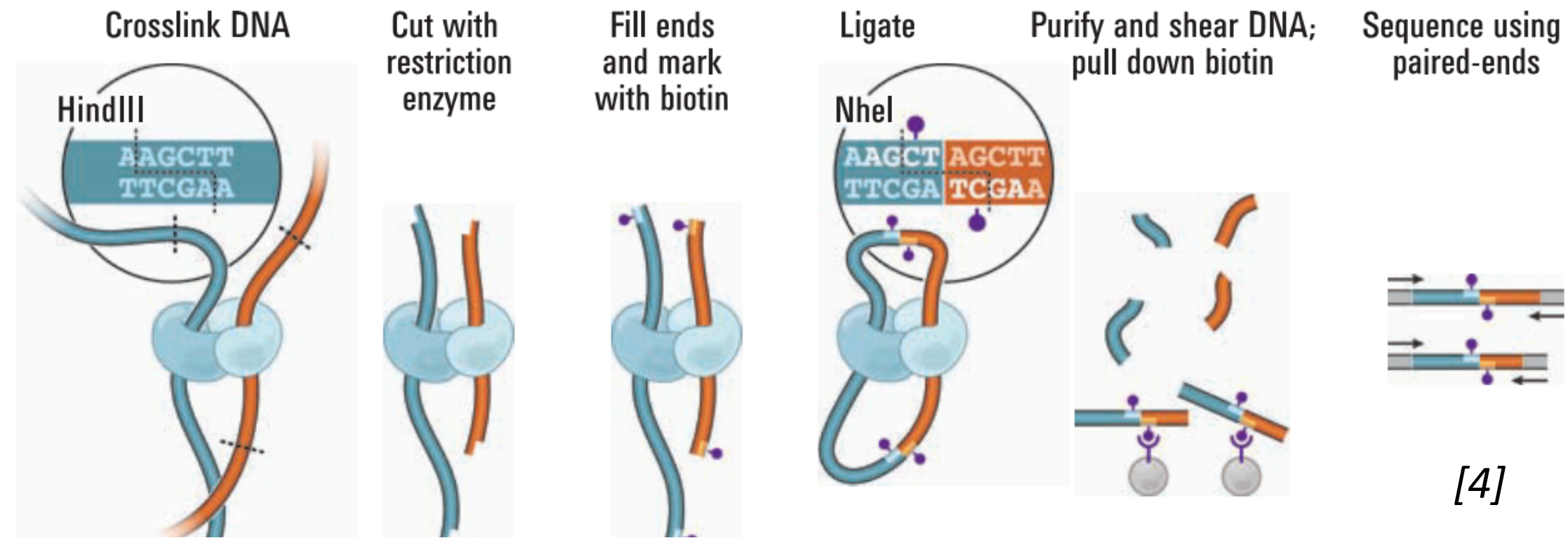
[1]

[1] Szabo et al 2019  
 [2] Adapted from Bonev et al 2019  
 [3] Boettinger et al 2016

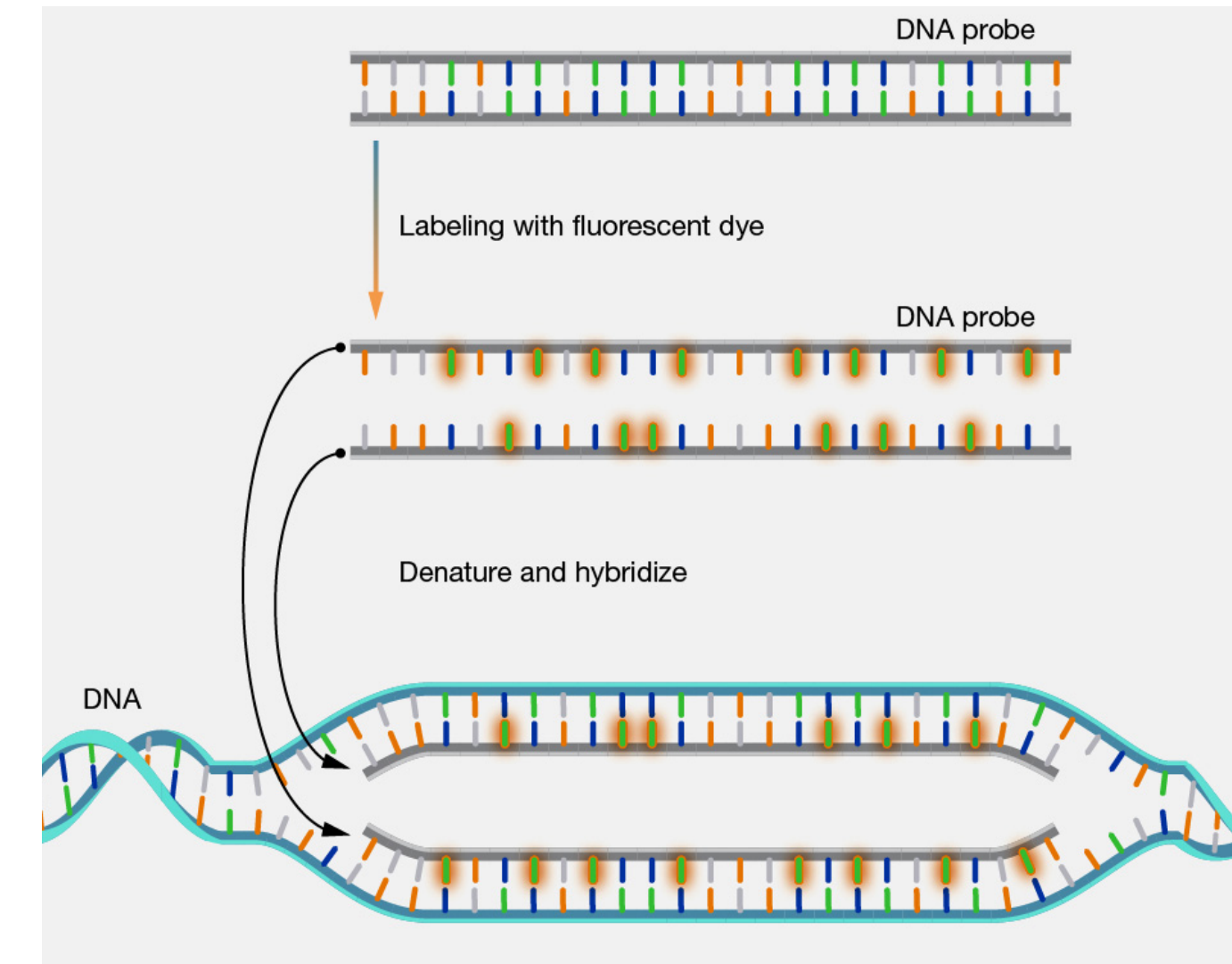


# Experimental tools

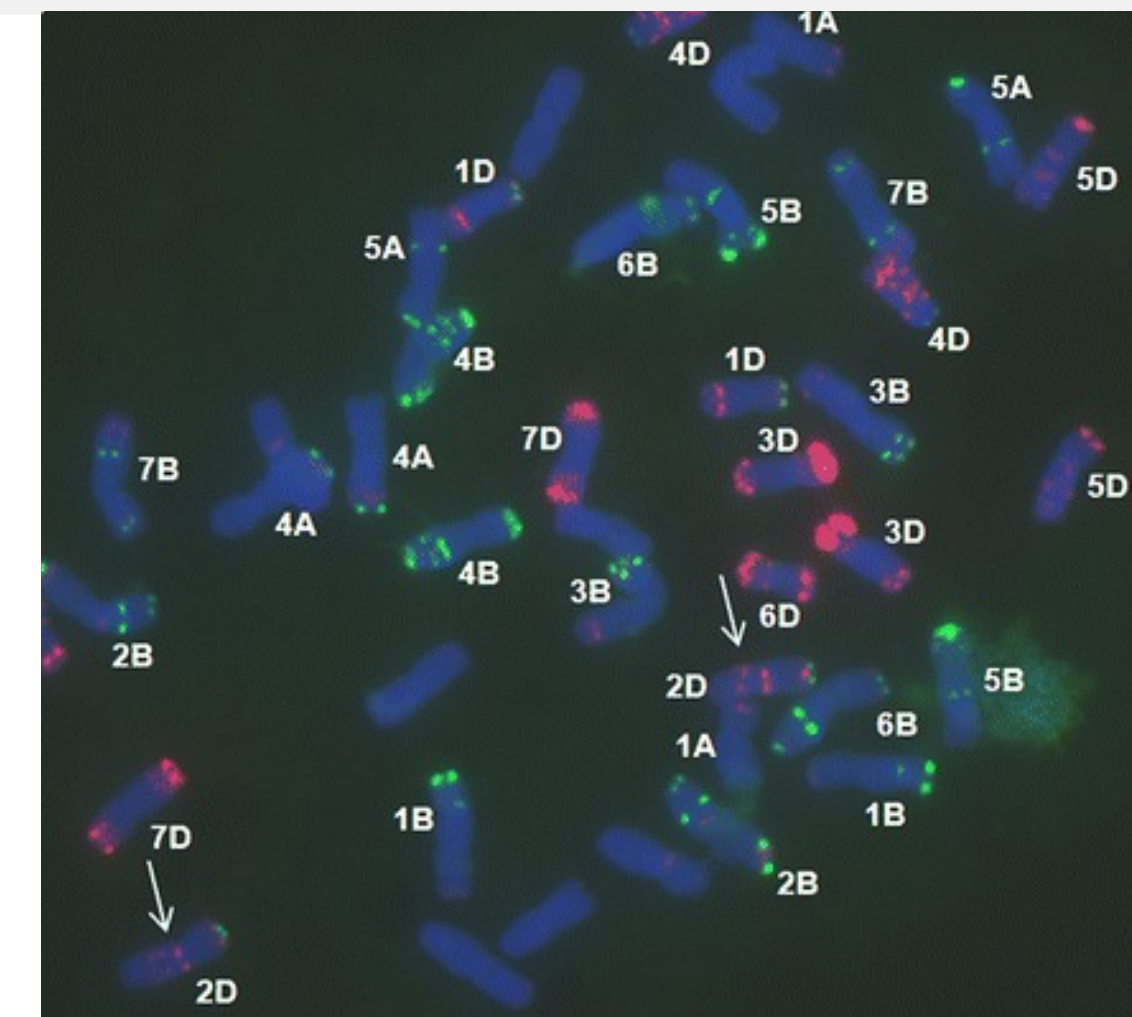
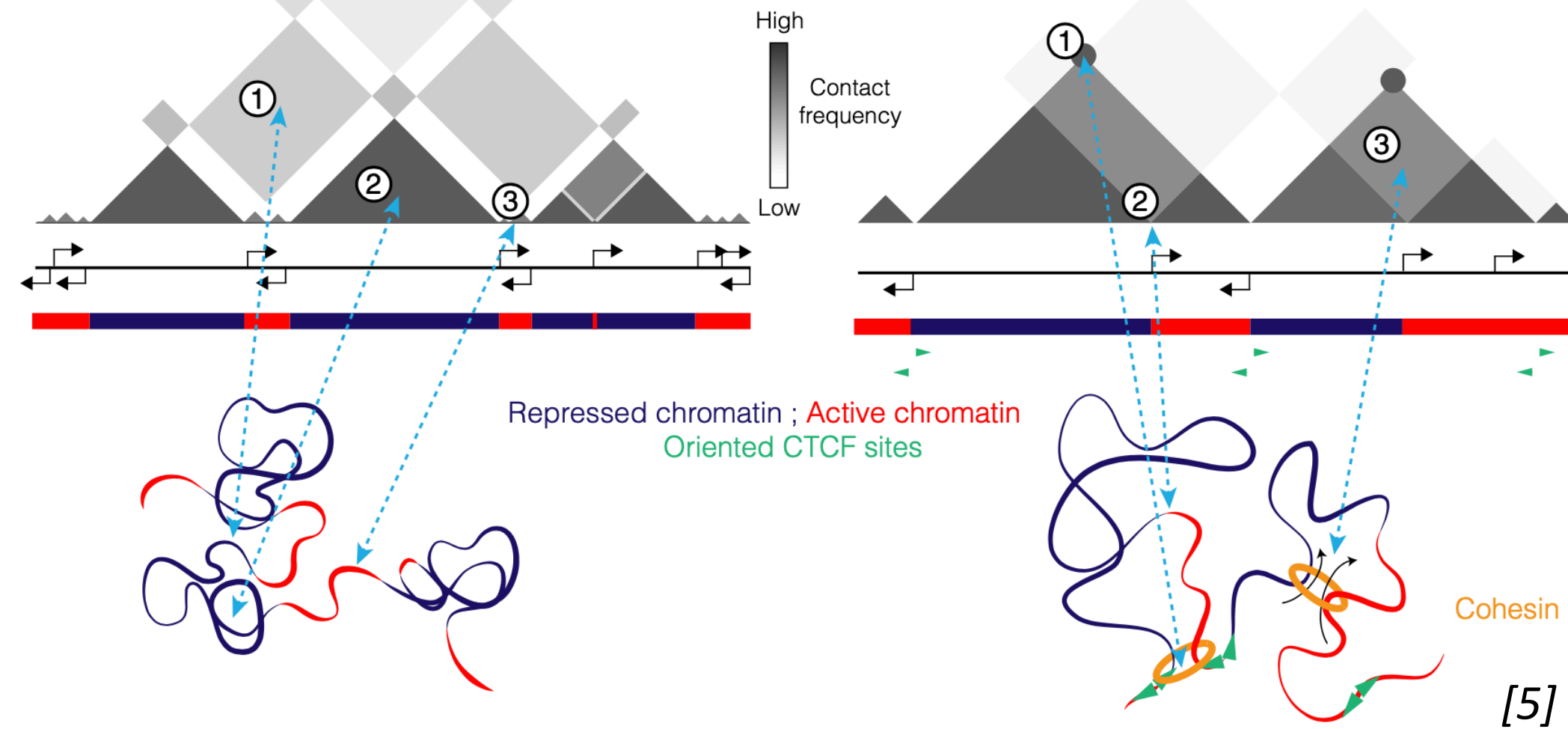
## Hi-C



## Multicolor-FISH



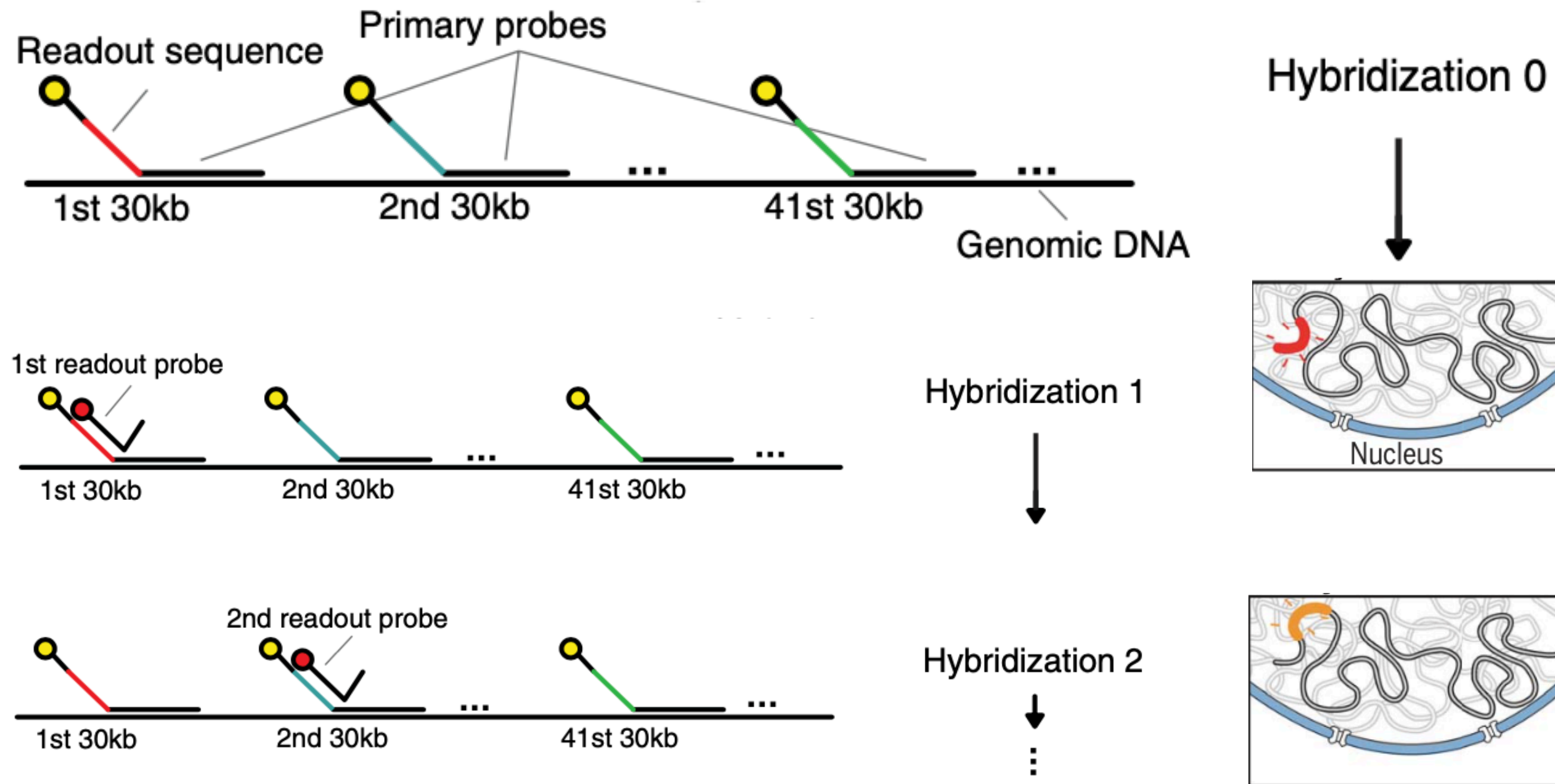
Hi-C  
+  
Chip-Seq



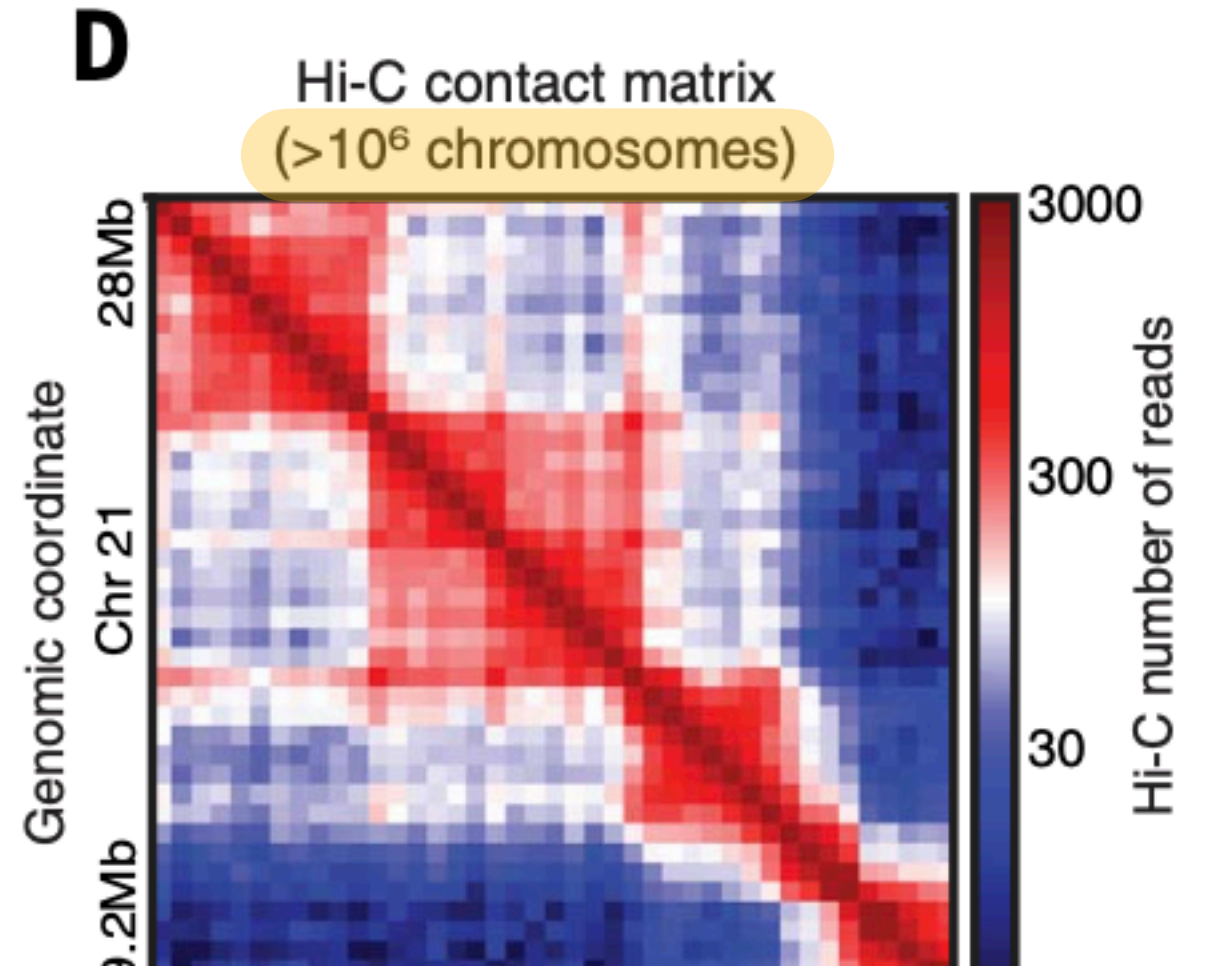
[4] Lieberman-aiden et al, 2009  
[5] Adapted from Szabo et al, 2019

[6] NIH website, updated July 1st 2023  
[7] Chen et al, 2012

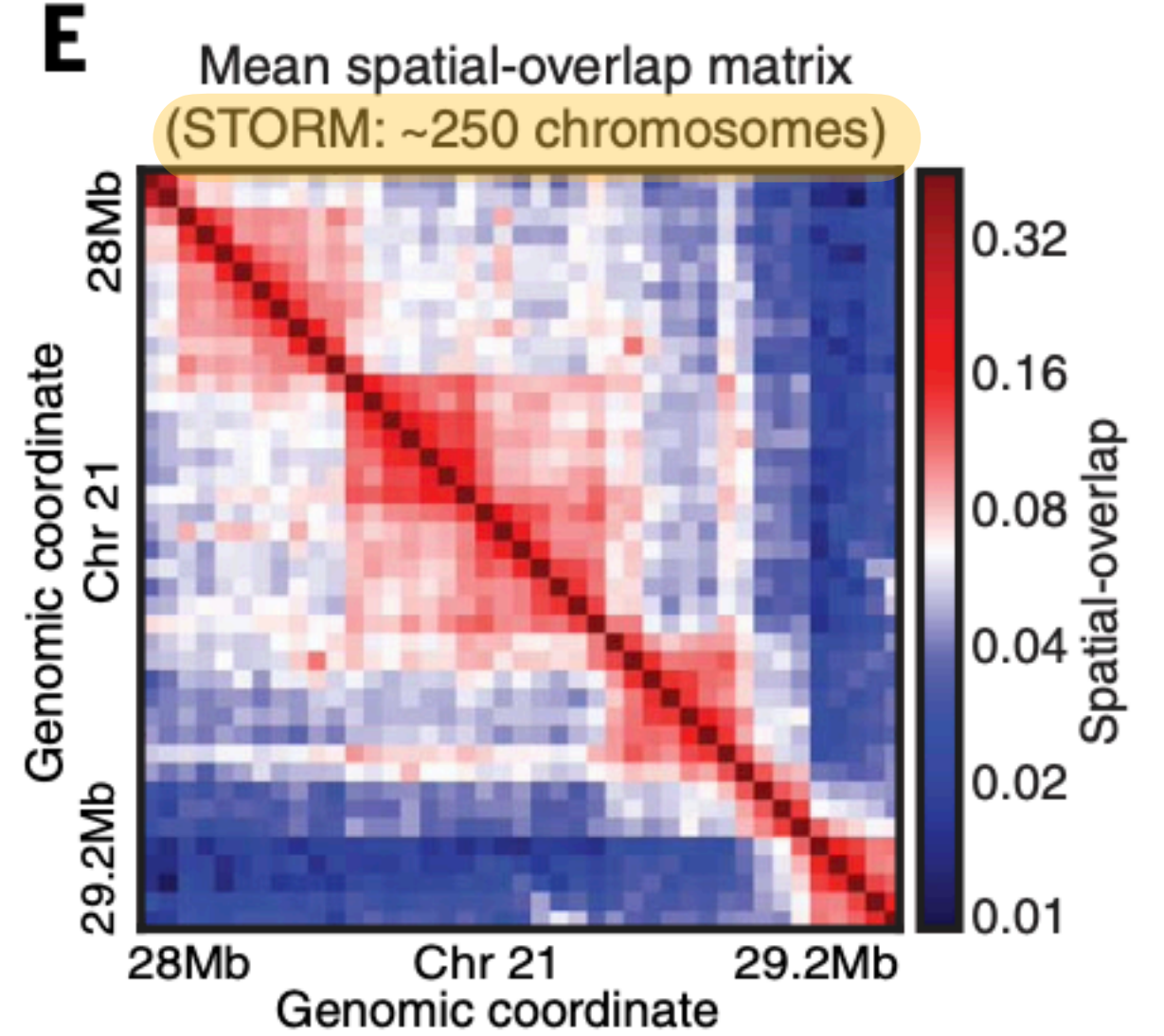
# Experimental tools: Multiplexed-FISH



Hi-C

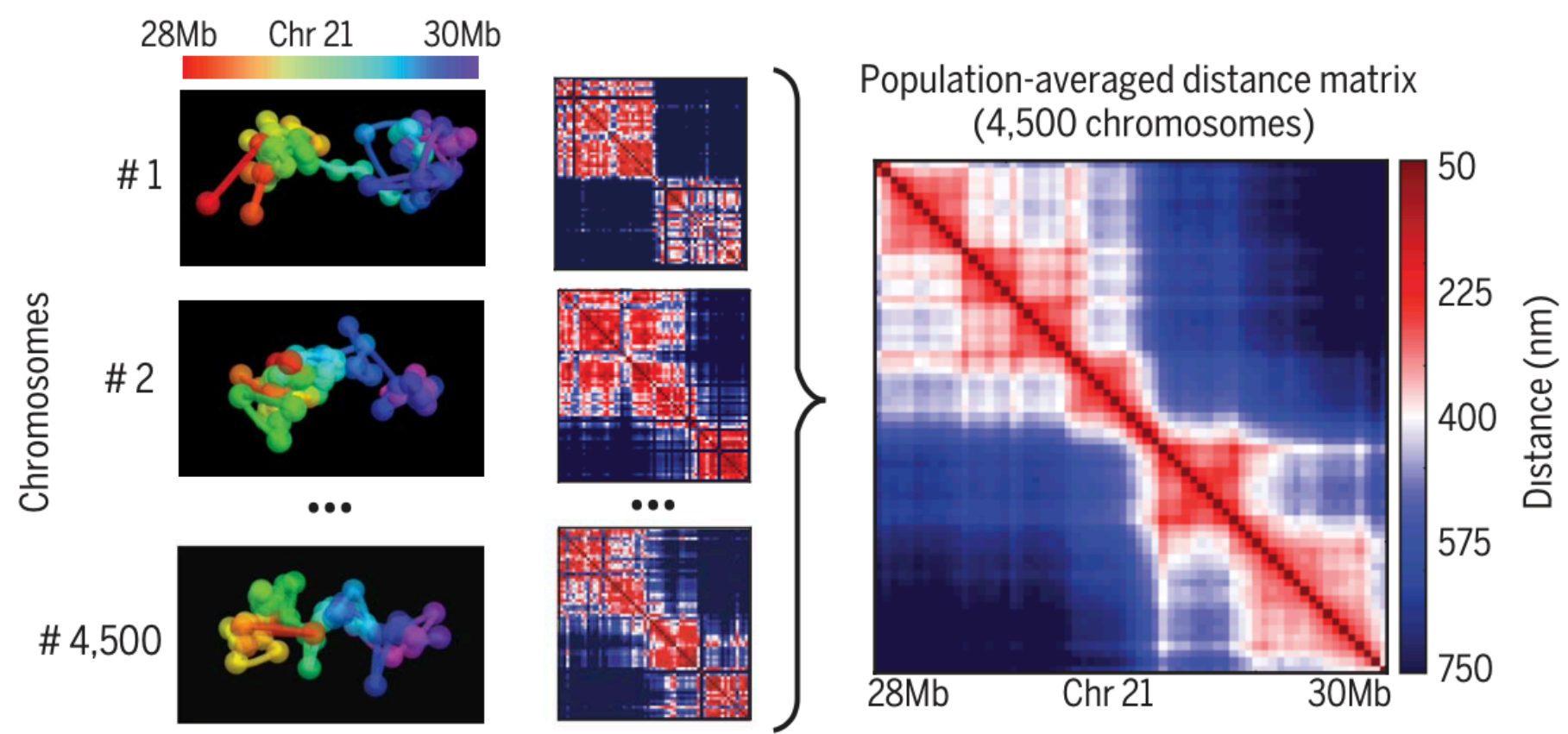
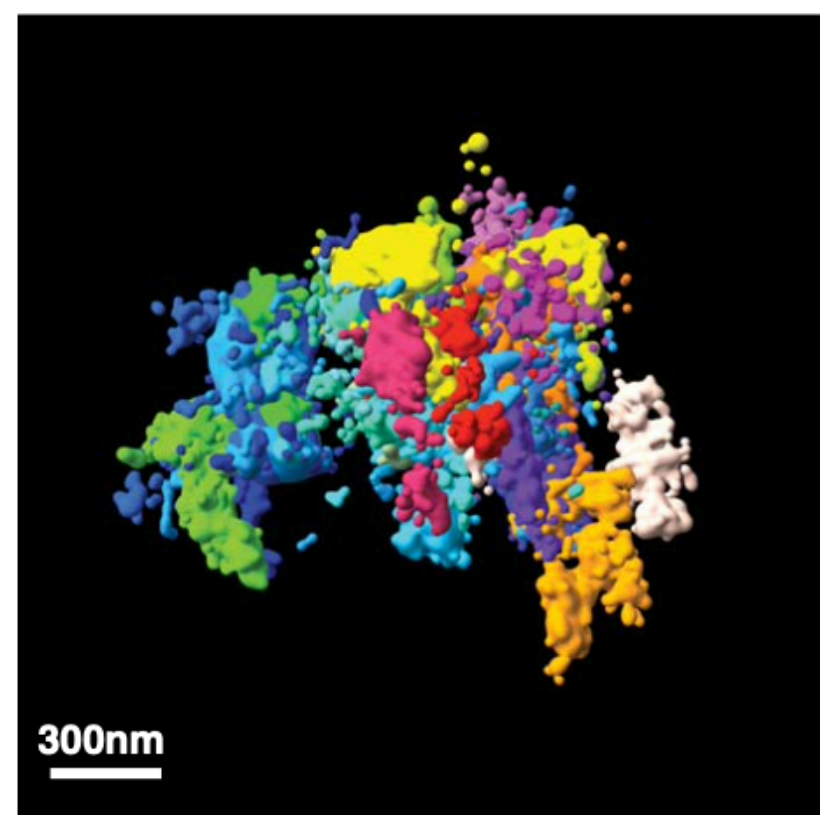


mFISH



Artificial coloring

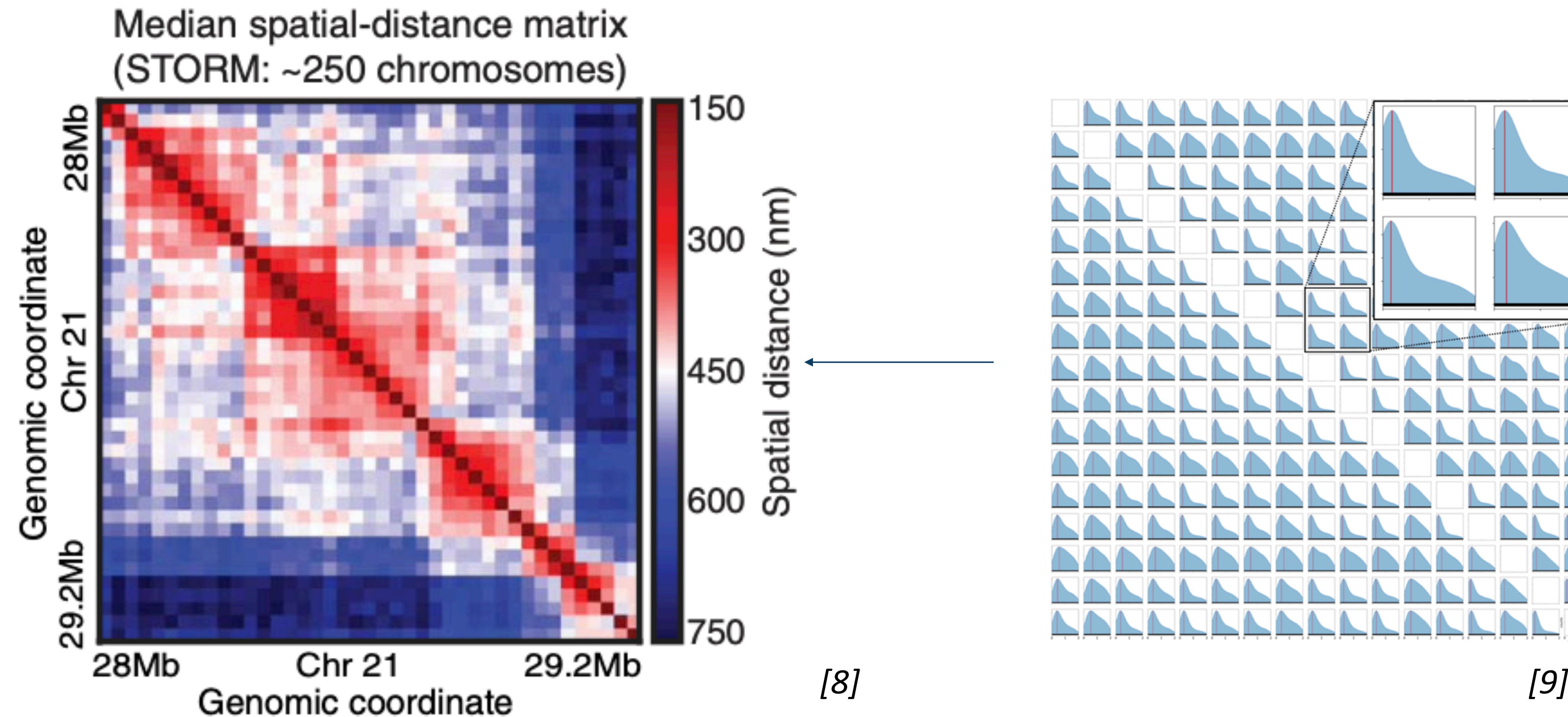
Single cell distance matrices



[8]

[8] Adapted from Bintu et al, 2018

## Raw data encapsulate rich informations

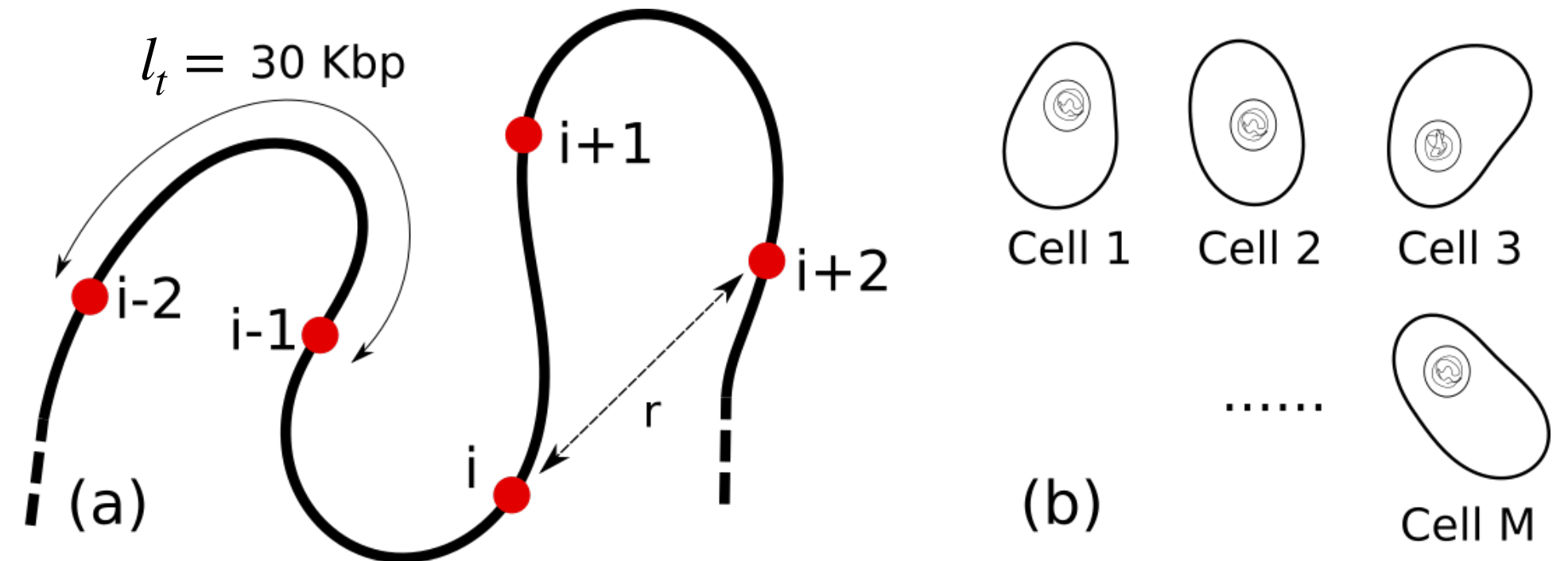


- Each pixel from the contact/distance map hides a pairwise distance distribution.
- The value attributed to a pixel only reflects one information (mean, median or maximum value)

What can we learn from these distributions about the chromatin 3D organization?

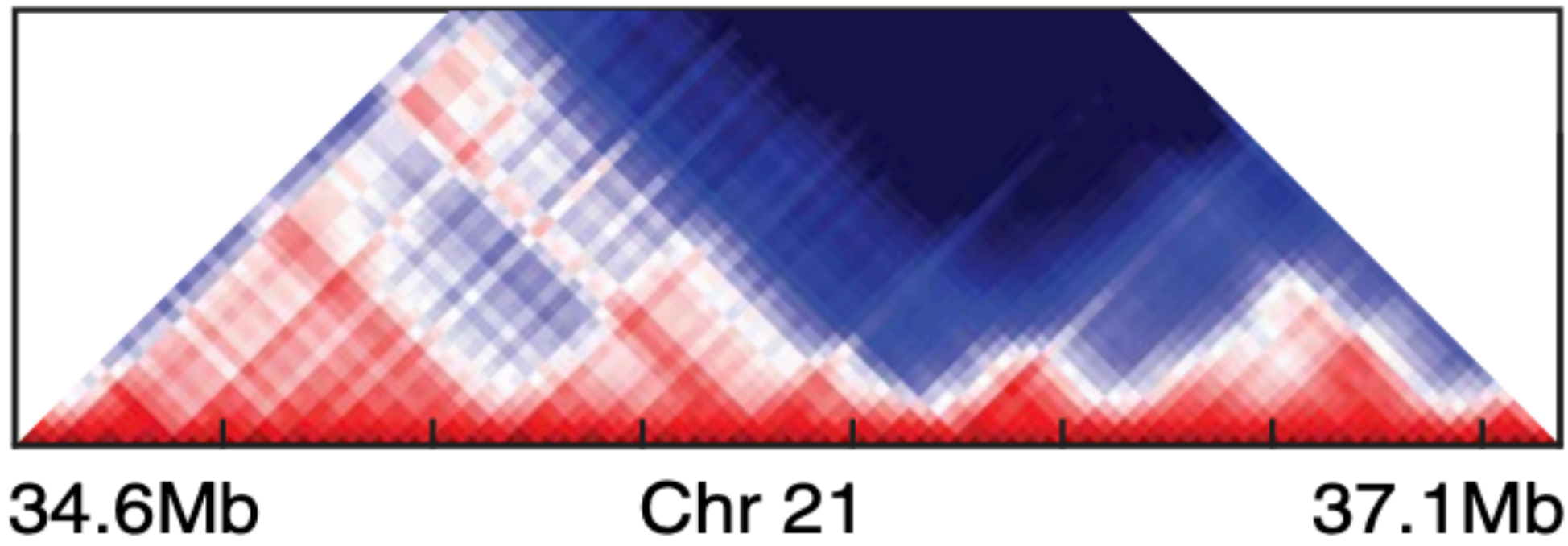
Raw data encapsulate rich informations

Cell line	Chromosomic region	$N$	$M$
HCT116 (untreated)	chr21 [34-37] Mb	83	11631
HCT116 (+Auxin 6h)	chr21 [34-37] Mb	83	9526
IMR90	chr21 [28-30] Mb	65	4871
K562	chr21 [28-30] Mb	65	13997



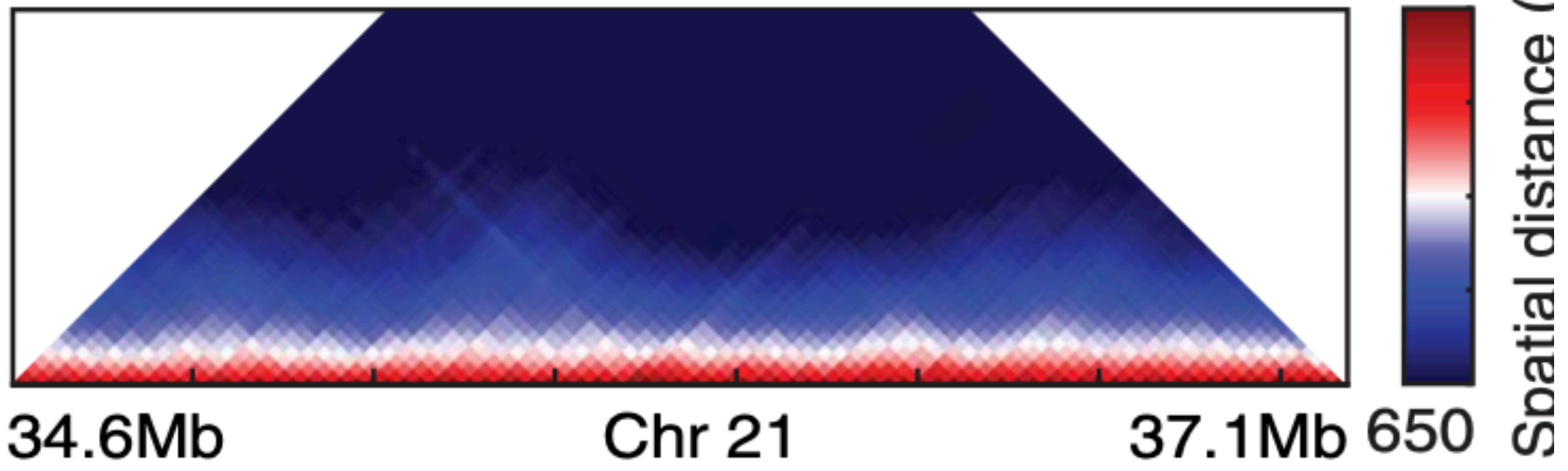
HCT116 ( - AUXIN)

Median spatial-distance matrix (~12,000 chromosomes)



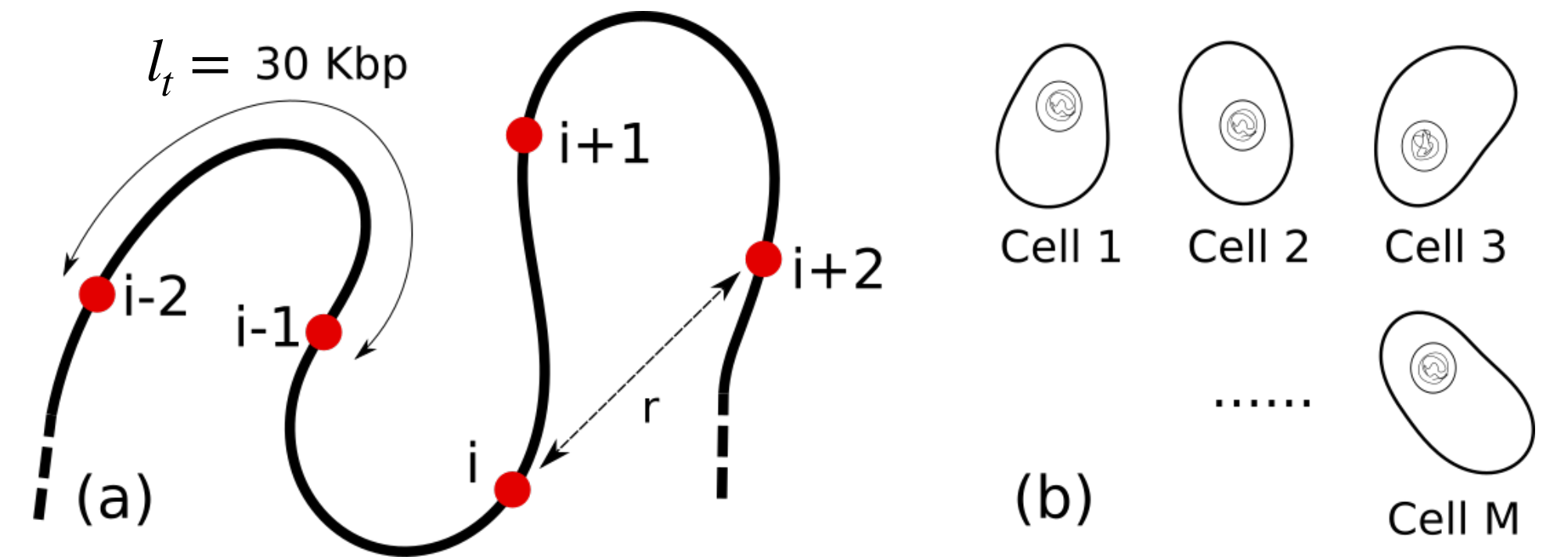
HCT116 ( + AUXIN 6h)

Median spatial-distance matrix (~10,000 chromosomes)



## Raw data encapsulate rich informations

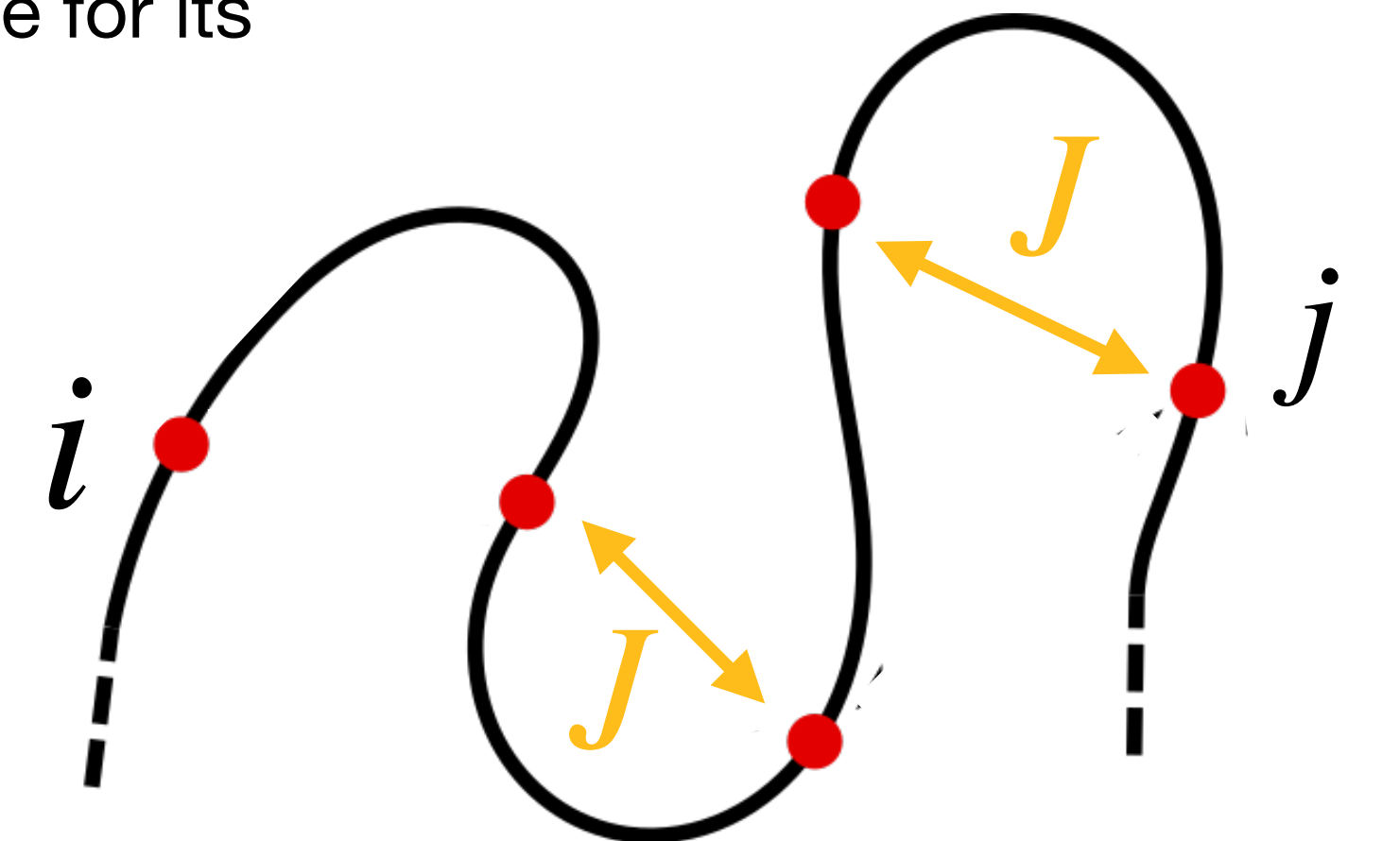
Cell line	Chromosomic region	$N$	$M$
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K562	chr21 [28-30] Mb	65	13997



For an ideal polymer the distance distribution is a gaussian in the vector distance  $\vec{r}$ , while for its length  $r = |\vec{r}|$  takes the form:

$$P_{ij}(r) = 4\pi r^2 \left( \frac{3}{2\pi R^2} \right)^{3/2} e^{-3r^2/(2R^2)}$$

The root of the mean square radius of an ideal polymer scales as:  $R \sim |i - j|^{1/2}$

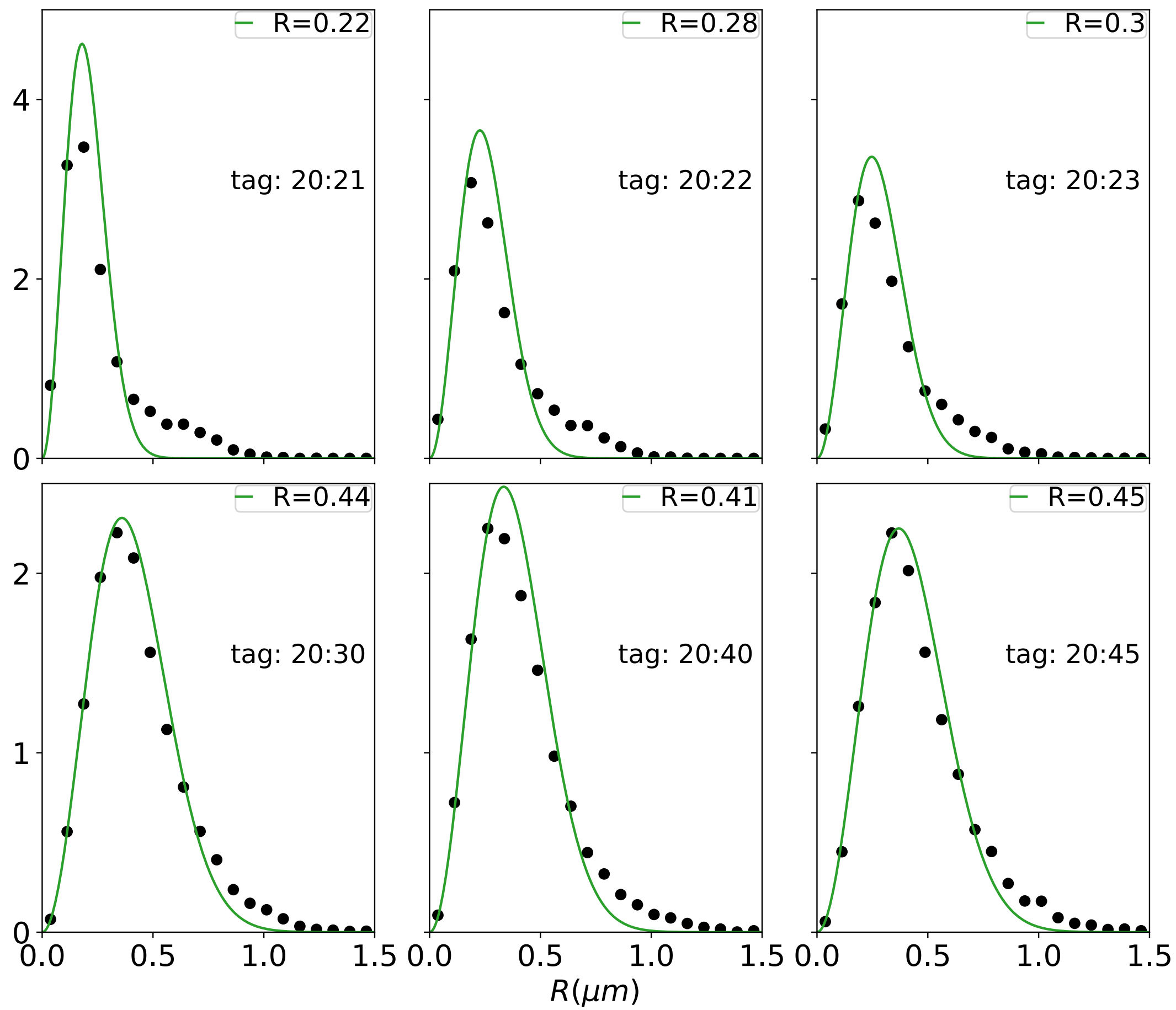


The introduction of suitable monomer-monomer interactions potential  $J$  in the model leads to an equilibrium distance scaling as:  $R \sim |i - j|^\nu$

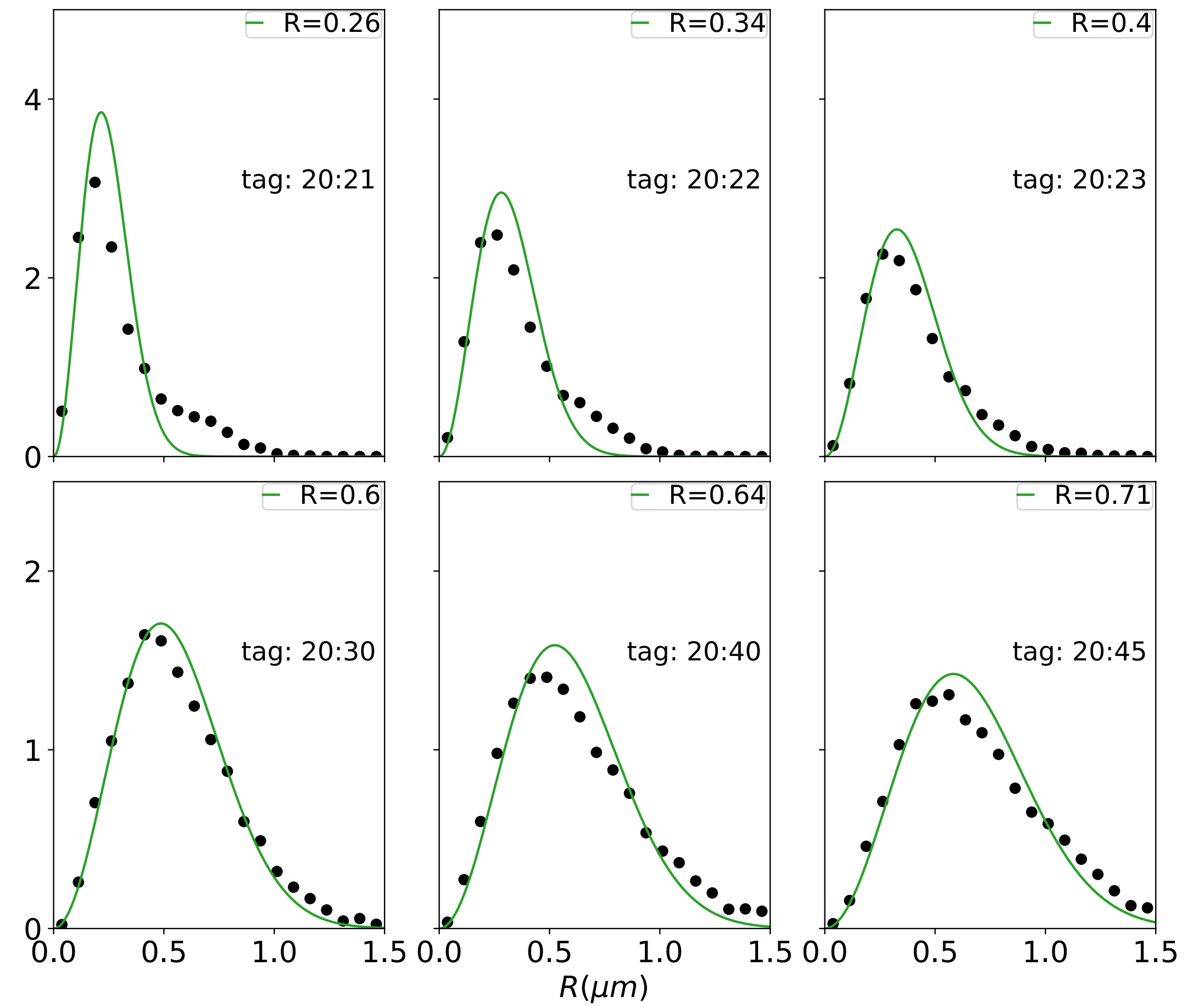
By tuning the interactions one can generate any values of the exponent in the interval  $1/3 \leq \nu \leq 1/2$  englobing a board range of polymer models

# Raw data encapsulate rich informations

## HCT116 (untreated)



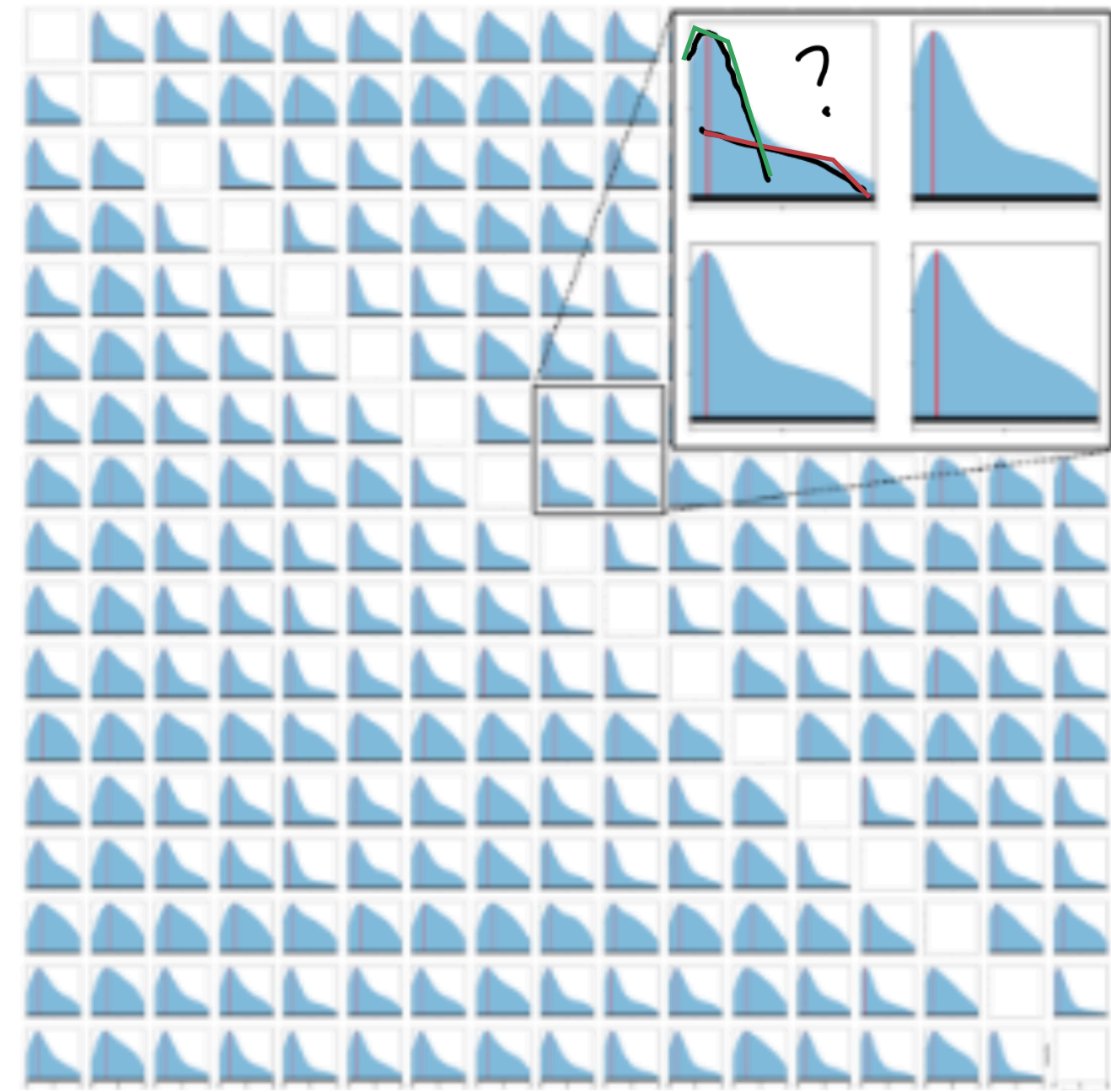
## HCT116 (+Auxin 6h)



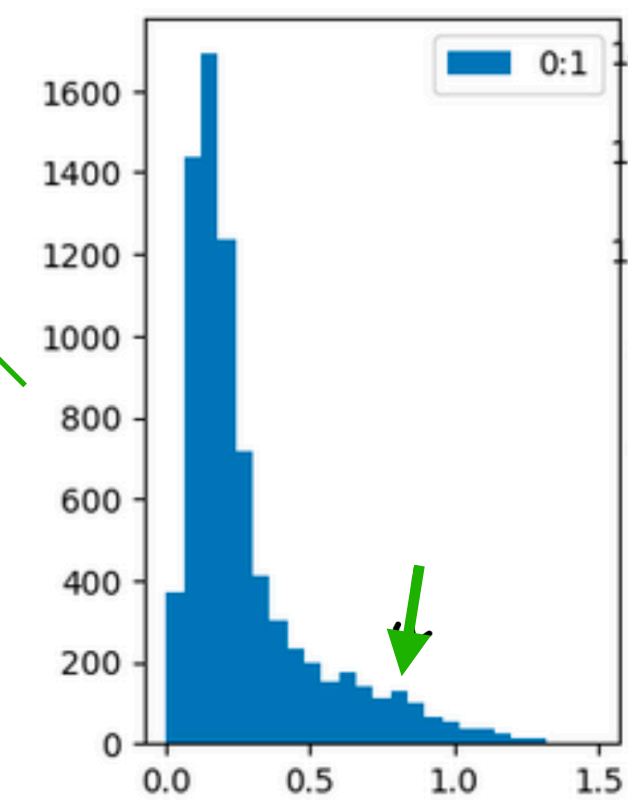
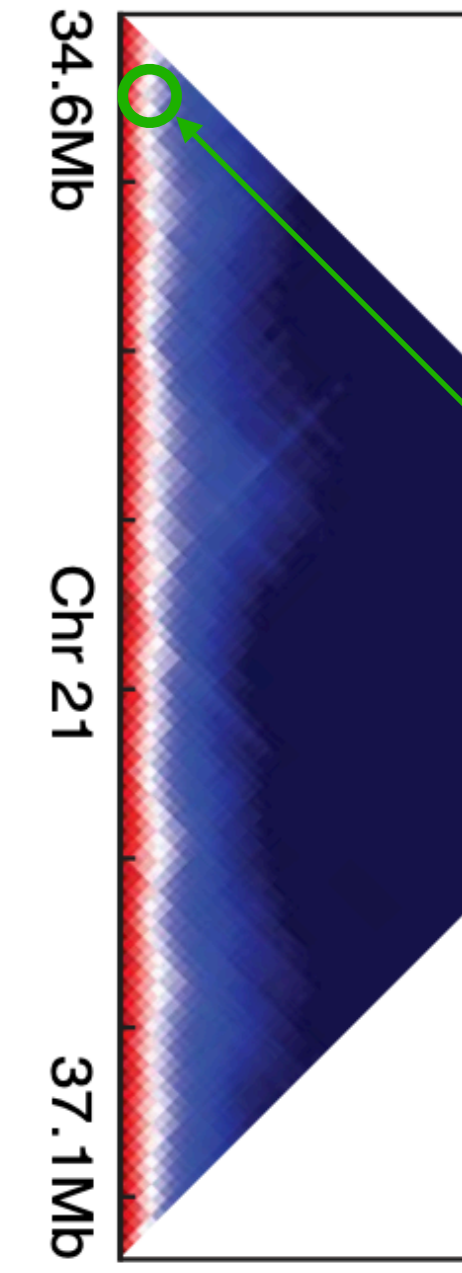
- Experimental histogram
- Gaussian distribution



# Raw data encapsulate rich informations



[9]



[8]

The histograms for small  $|i - j|$  suggest that experiments describe two coexisting populations, therefore we fit the data using:

$$P_{ij}(r) = f_{\alpha} g(r; R_{\alpha}) + (1 - f_{\alpha}) g(r; R_{\beta})$$

With:  $R_{\alpha} < R_{\beta}$

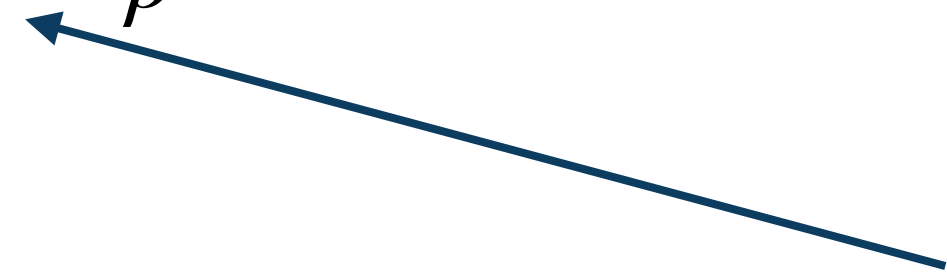
Fraction of samples in the  $\alpha$  phase



The gaussian distribution of the  $\alpha$  phase



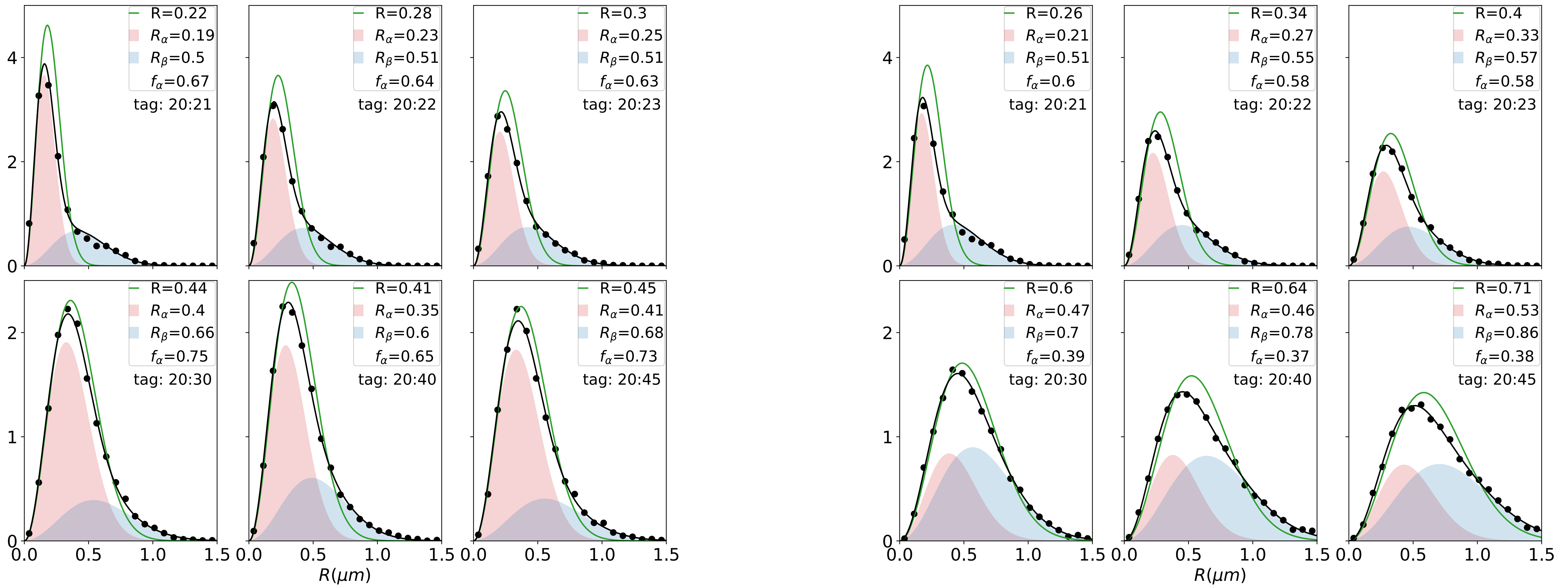
The gaussian distribution of the  $\beta$  phase



# Polymer phase coexistence

**HCT116 (untreated)**

**HCT116 (+Auxin 6h)**



- Experimental histogram
- Gaussian distribution
- Two phases distribution
- $\alpha$  phase contribution
- $\beta$  phase contribution

[10]

[10] Remini et al, 2023 (Pre-print)



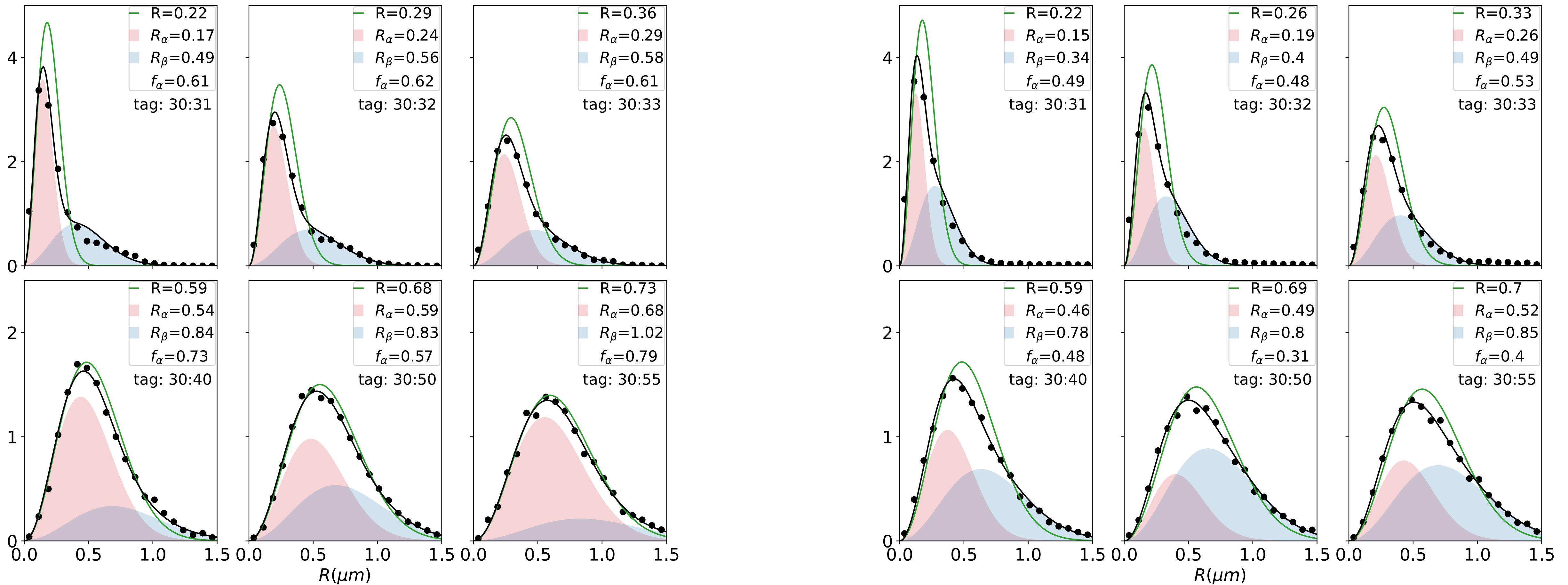
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# Polymer phase coexistence

IMR90

K562



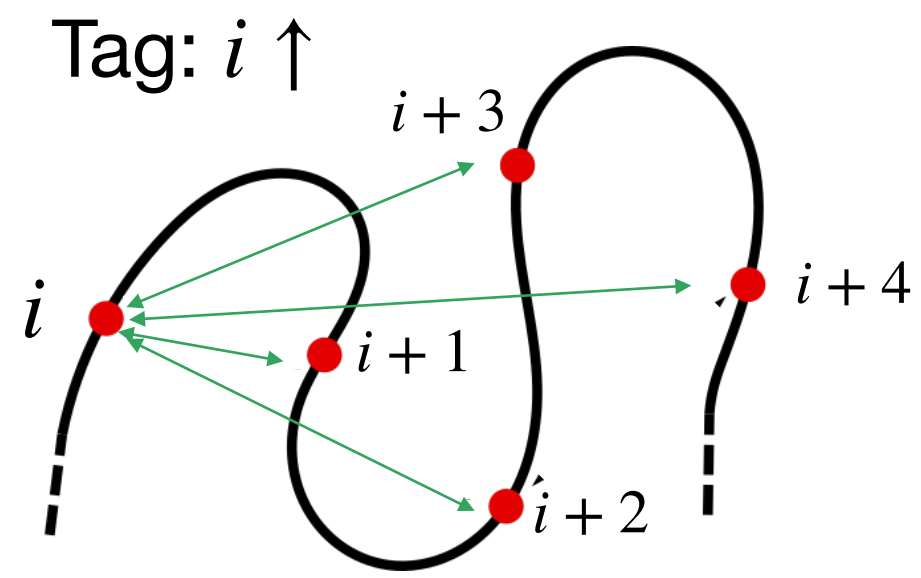
- Experimental histogram
- Gaussian distribution
- Two phases distribution
- $\alpha$  phase contribution
- $\beta$  phase contribution

[10]

[10] Remini et al, 2023 (Pre-print)

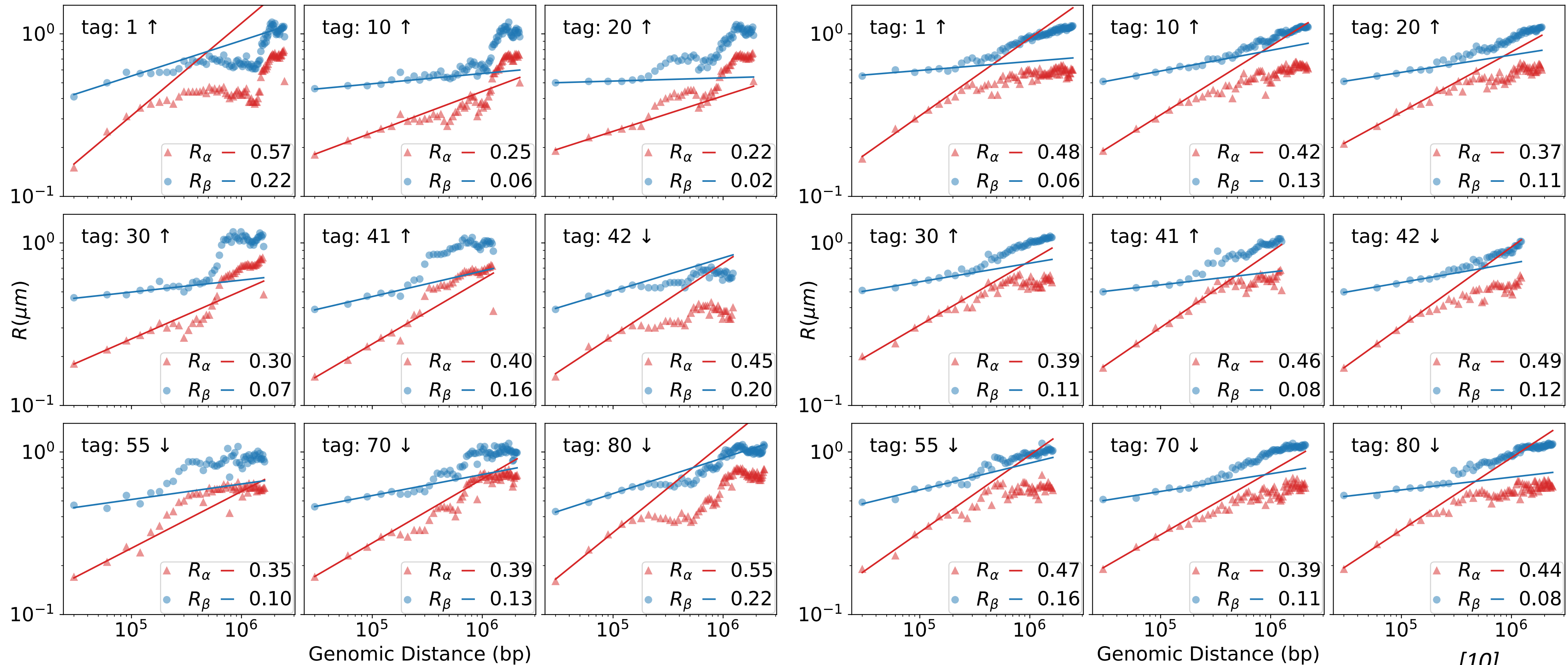
# Polymer phase coexistence

if:  $i < N/2$

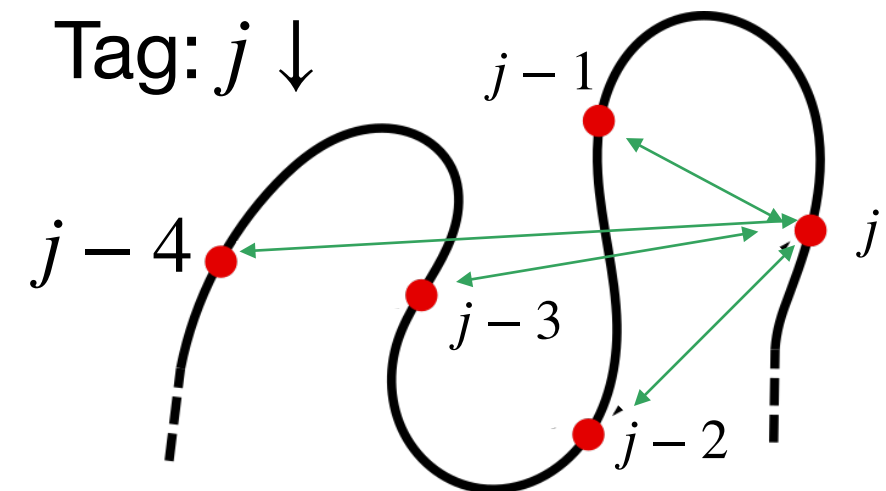


**HCT116 (untreated)**

**HCT116 (+Auxin 6h)**



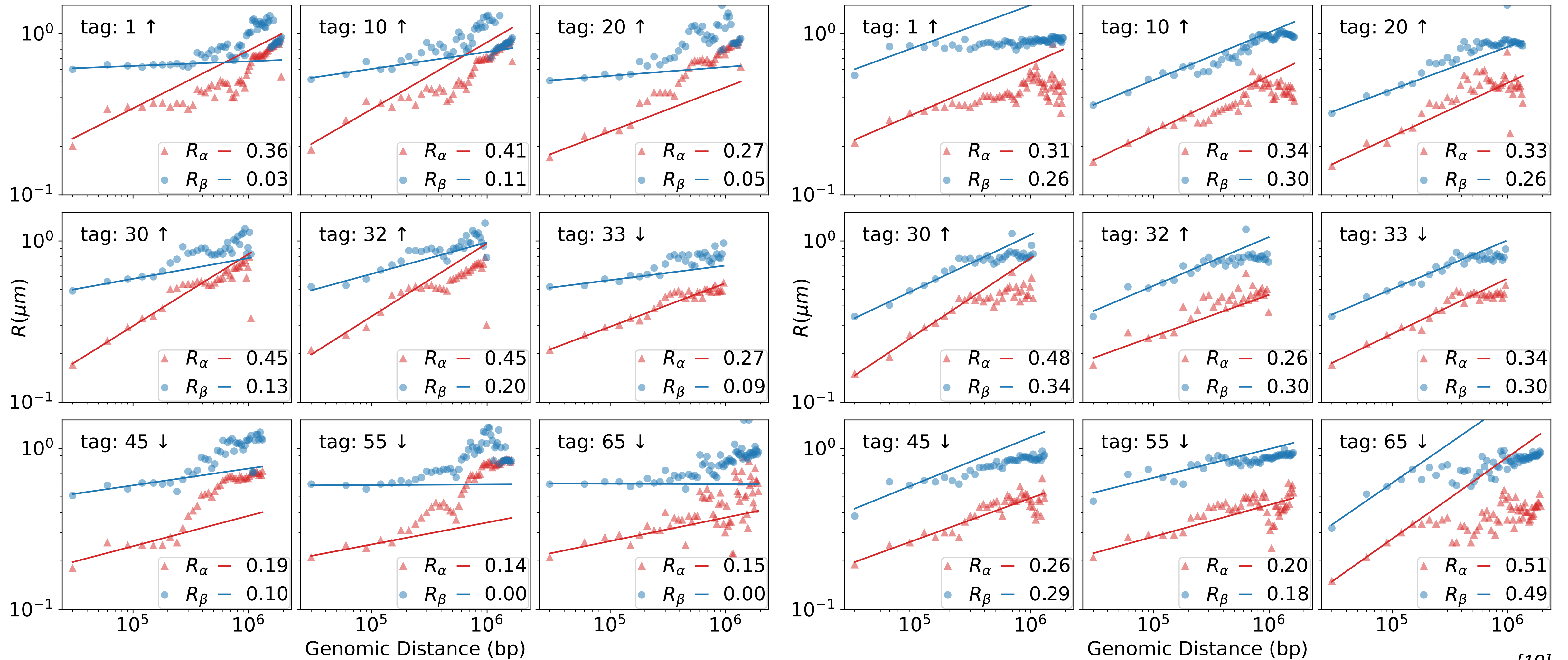
if:  $j > N/2$



# Polymer phase coexistence

**IMR90**

**K562**

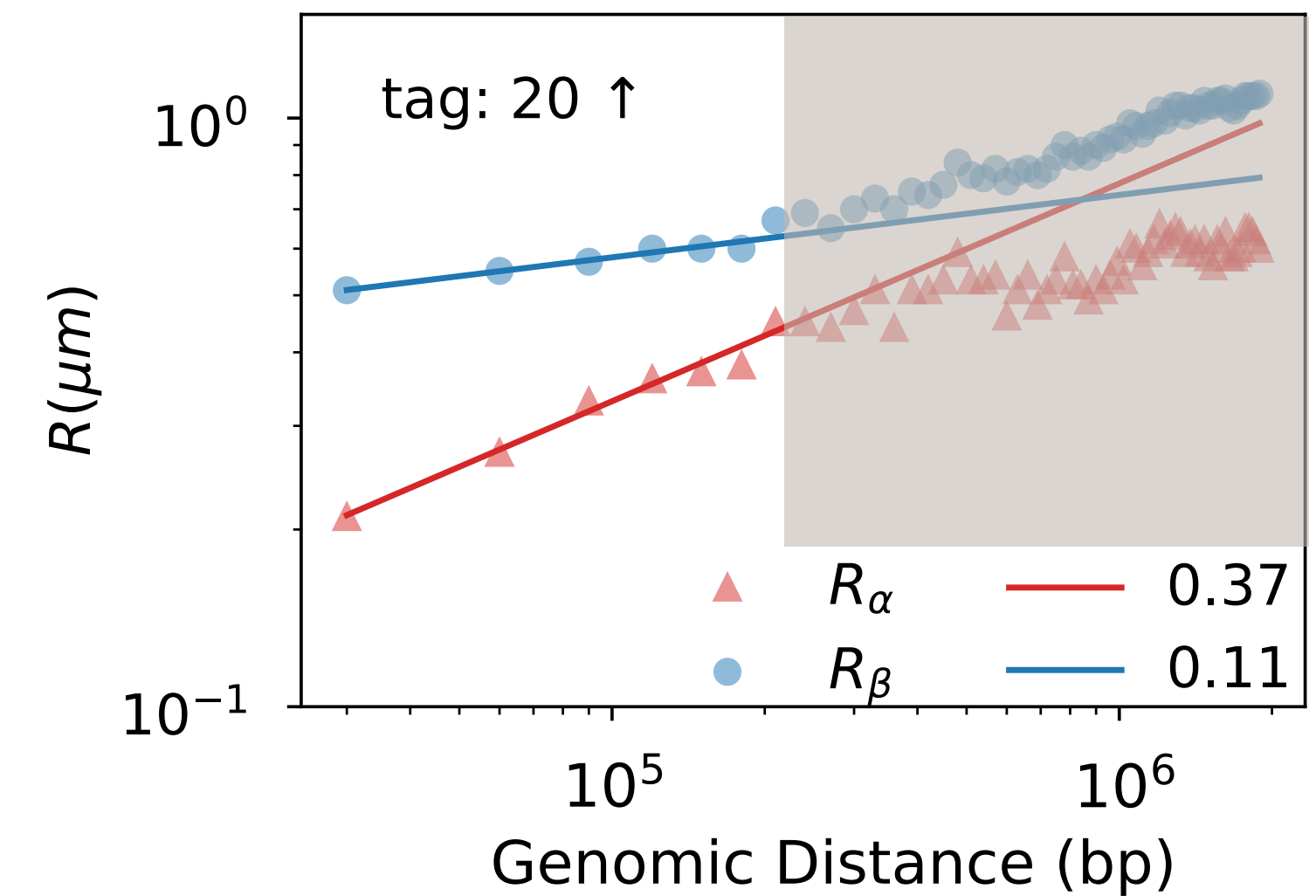
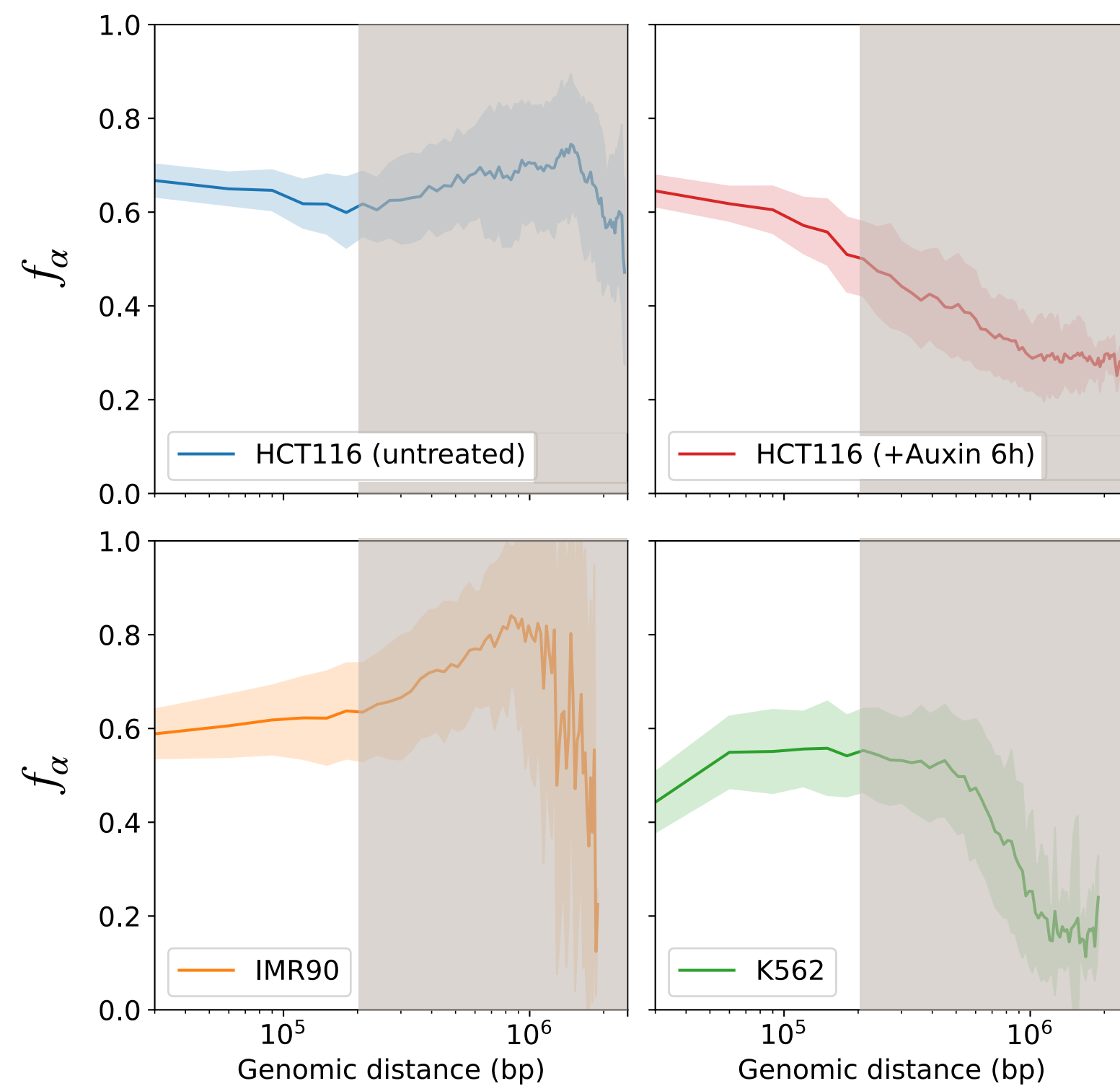


[10]

# Polymer phase coexistence

$$R_x \sim |i - j|^{\nu_x}$$

We characterize the scaling behavior  $\nu_\alpha$  and  $\nu_\beta$  of the radii fitting  $R_\alpha$  and  $R_\beta$  for close tags, i.e.  $|i - j| \leq 6$  ( $\equiv 180$  kbp)



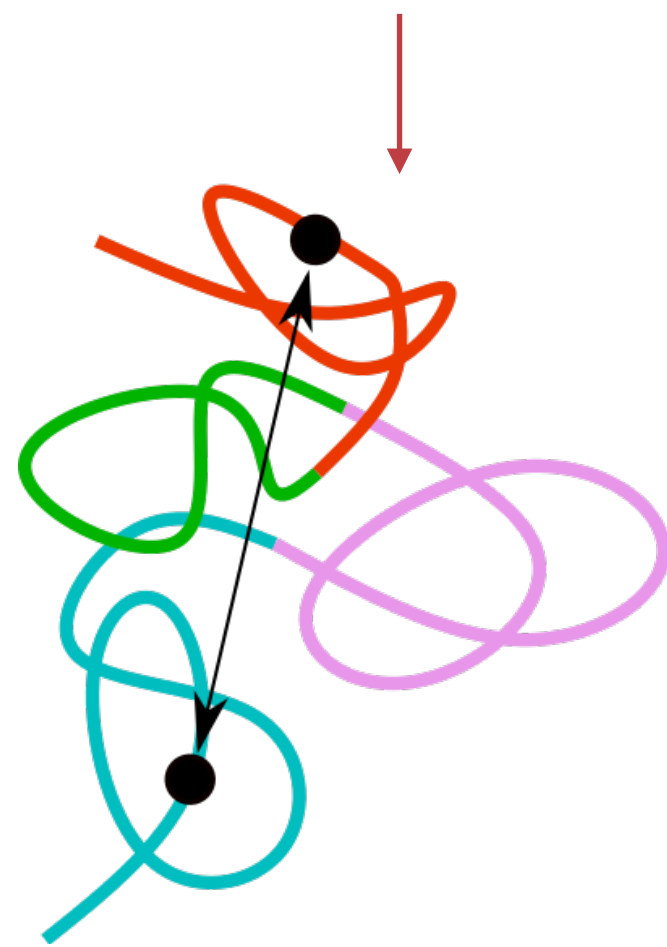
- We observe that the scaling of the radii follows an approximate power-law behavior for a limited distance between tags
- The values of the radii are more accurately determined from fitting histograms for small  $|i - j|$  as well since there is a bigger gap between  $R_\alpha$  and  $R_\beta$
- The value of the fit parameter  $f_\alpha$  remains stable in this range

[10]

# Micro-phase coexistence

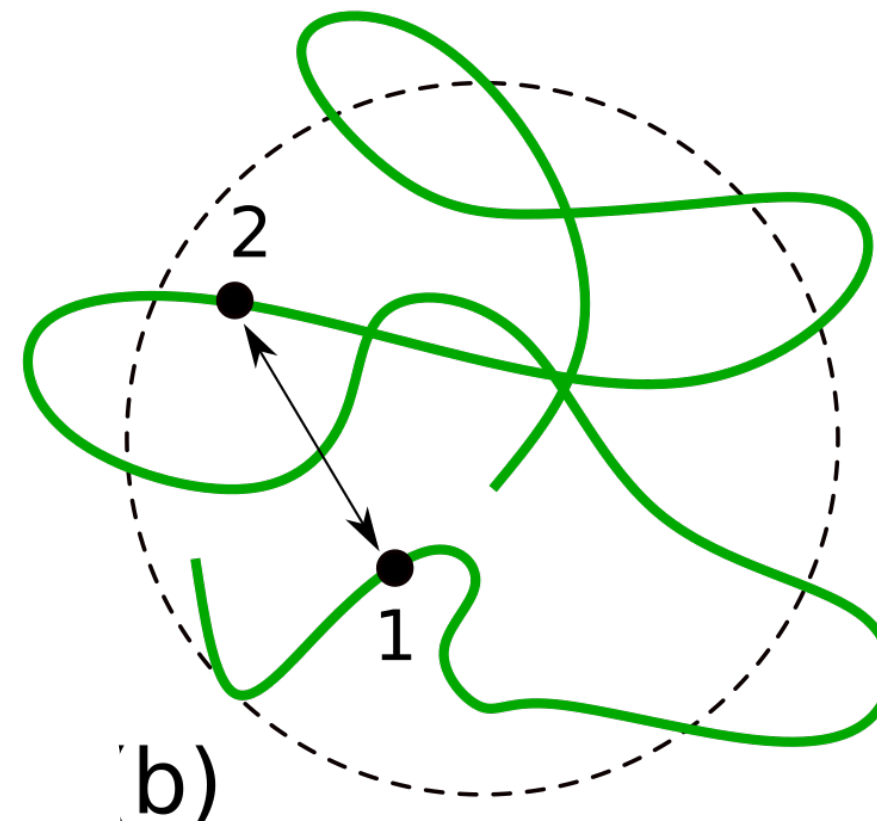
Data set	$\nu_\alpha$	$\nu_\beta$	$f_\alpha$
HCT116 (untreated)	0.35(10)	0.11(7)	0.64(5)
HCT116 (+Auxin 6h)	0.40(6)	0.10(3)	0.60(5)
IMR90	0.31(11)	0.11(6)	0.61(8)
K562	0.38(8)	0.35(7)	0.53(8)

$$\nu_\alpha \approx 1/3$$

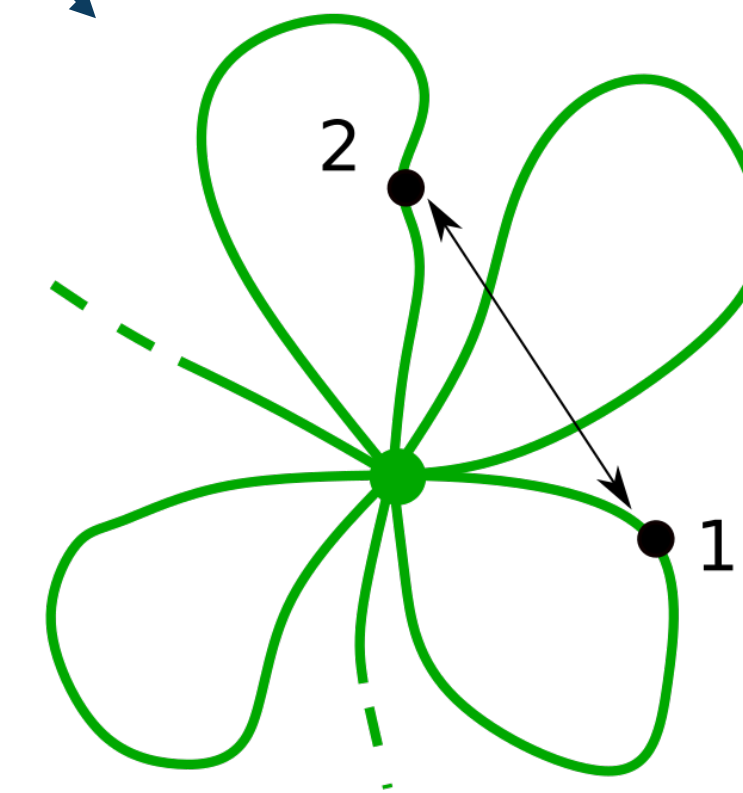


Crumpled globule model

$$\nu_\beta \ll 1/3$$



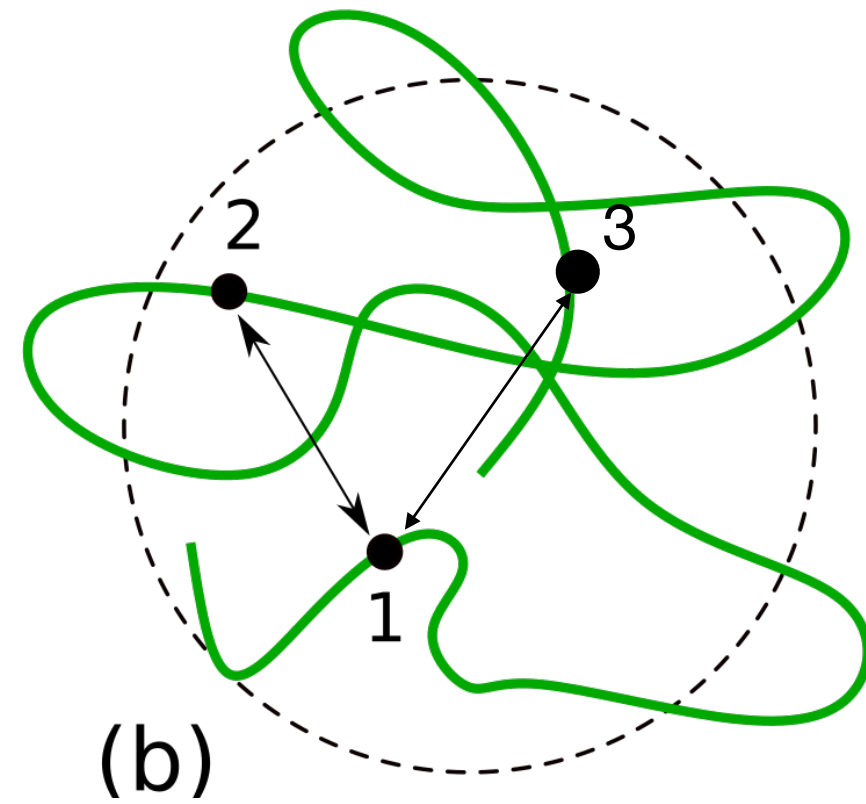
Droplet-like potential



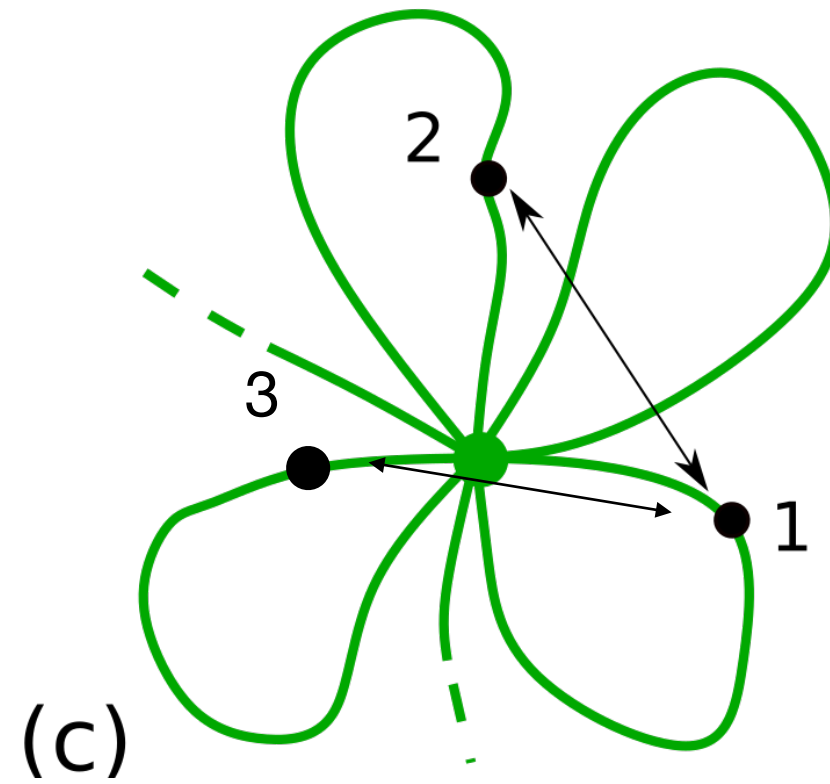
Flower shaped polymer

[10]

# Micro-phase coexistence



(b) Droplet-like potential



(c) Flower shaped polymer

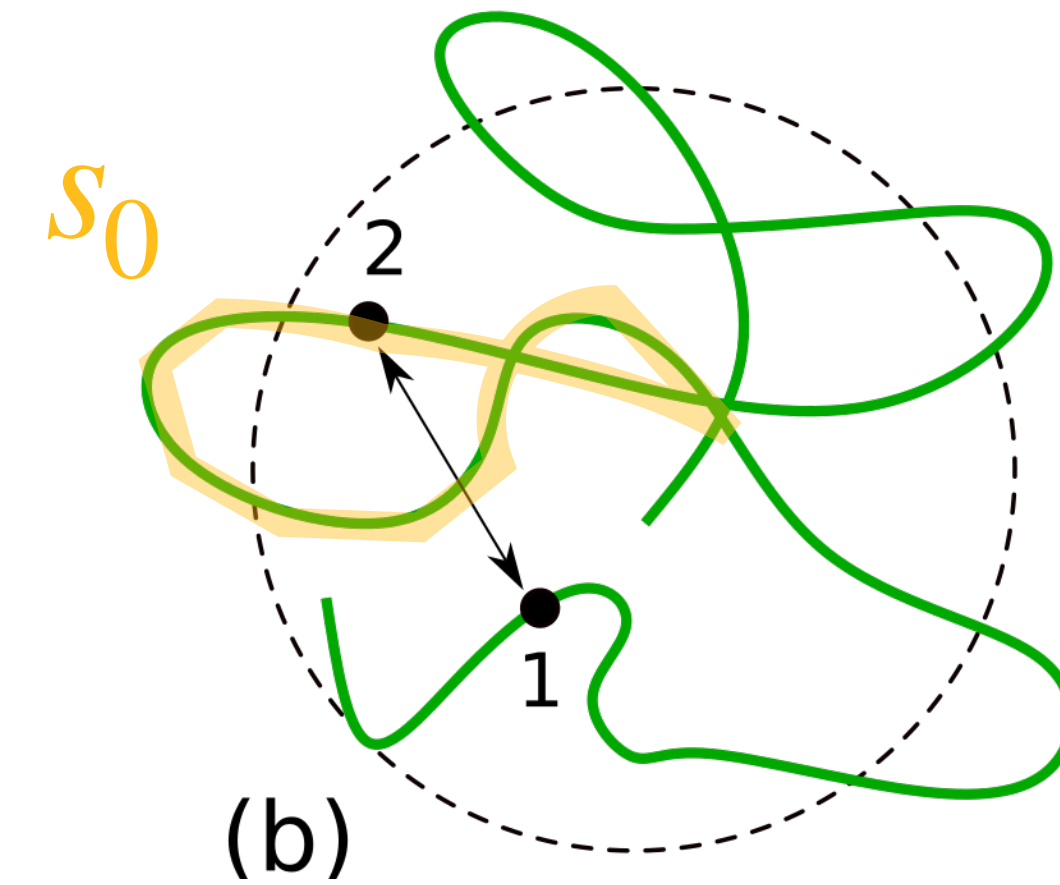
$$R_{\beta}(s) \sim s_0 \quad \text{if } s > s_0$$

where  $s_0$  is dependent on the typical genomic length needed by the polymer to reach the droplet surface and bounce back to the origin in (b) or the typical loop genomic size in (c).

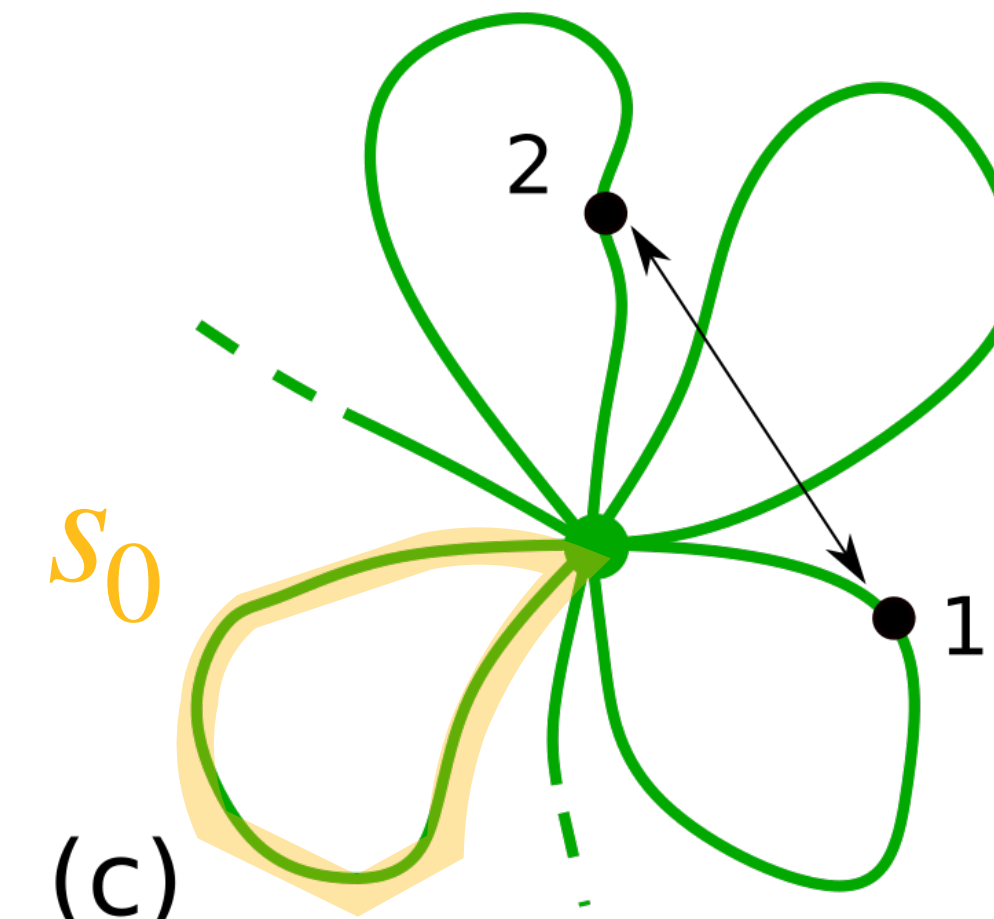
Experimental data show no “ideal” confinement ( $\nu = 0$ ) although very small  $\nu_{\beta} \approx 0.1$ .

The genomic distance between neighboring tags is constant:

$$s_{1,3} = 2 \times s_{1,2}$$



(b)



(c)

[10]



# Cell-to-cell variability

The  $\beta$  phase probability at tag  $i$  for a given cell

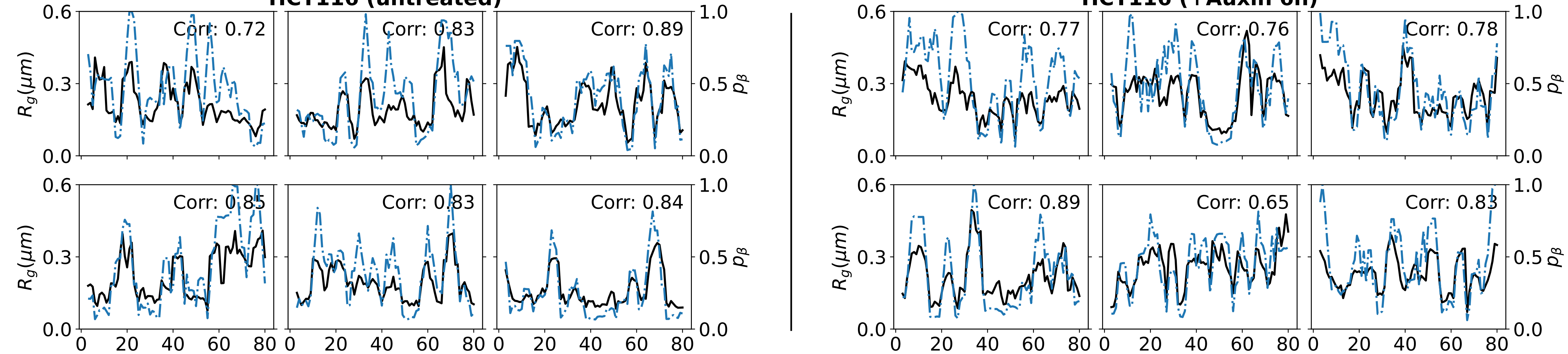
The  $\beta$  phase contribution to a given single cell organization

$$p_{\beta}(i) = \frac{(1 - f_{\alpha})g(r; R_{\beta})}{\langle P_{i,i+1}(r) \rangle_i}$$

Average histogram obtained From all cells of the same cell line for  $(i, i + 1)$

**HCT116 (untreated)**

**HCT116 (+Auxin 6h)**



High probability  $\beta$  regions inferred from the micro-phases coexistence model co-localize with direct measurements of the sliding radius of gyration in single cells with a high correlation score

— Experimental  $R_g(5)$

[10]

.....  $\beta$ -phase probability



[10] Remini et al, 2023 (Pre-print)



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## Conclusion

- Multiplexed-FISH distance maps encapsulate under-exploited informations
- Pairwise distances distribution analysis reveals at least two coexisting micro-phases
  - A **compact  $\alpha$  phase** characterized by a **crumpled globule** scaling behavior
  - A **less-compact  $\beta$  phase** characterized by a strong confinement at long range ( $\nu_\beta \approx 0.1$ )
- We propose two models; a **polymer under a droplet-like confinement** and a **Rosette polymer** to explain the  $\beta$  phase scaling
- We studied cell-to-cell variability and confronted the micro-phase coexistence model's predictions to a measured local quantity ( $R_g$ )

Thanks for your attention!

L2C - SCPN

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Jean-Charles Walter

John Palmeri

Nils-Ole Walliser

Jérôme Dorignac

Frédéric Geniet



Soft matter physics

Enrico Carlon

Midas Segers

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