C-Trap data analysis

An introduction to data analysis using Lakeview and Pylake

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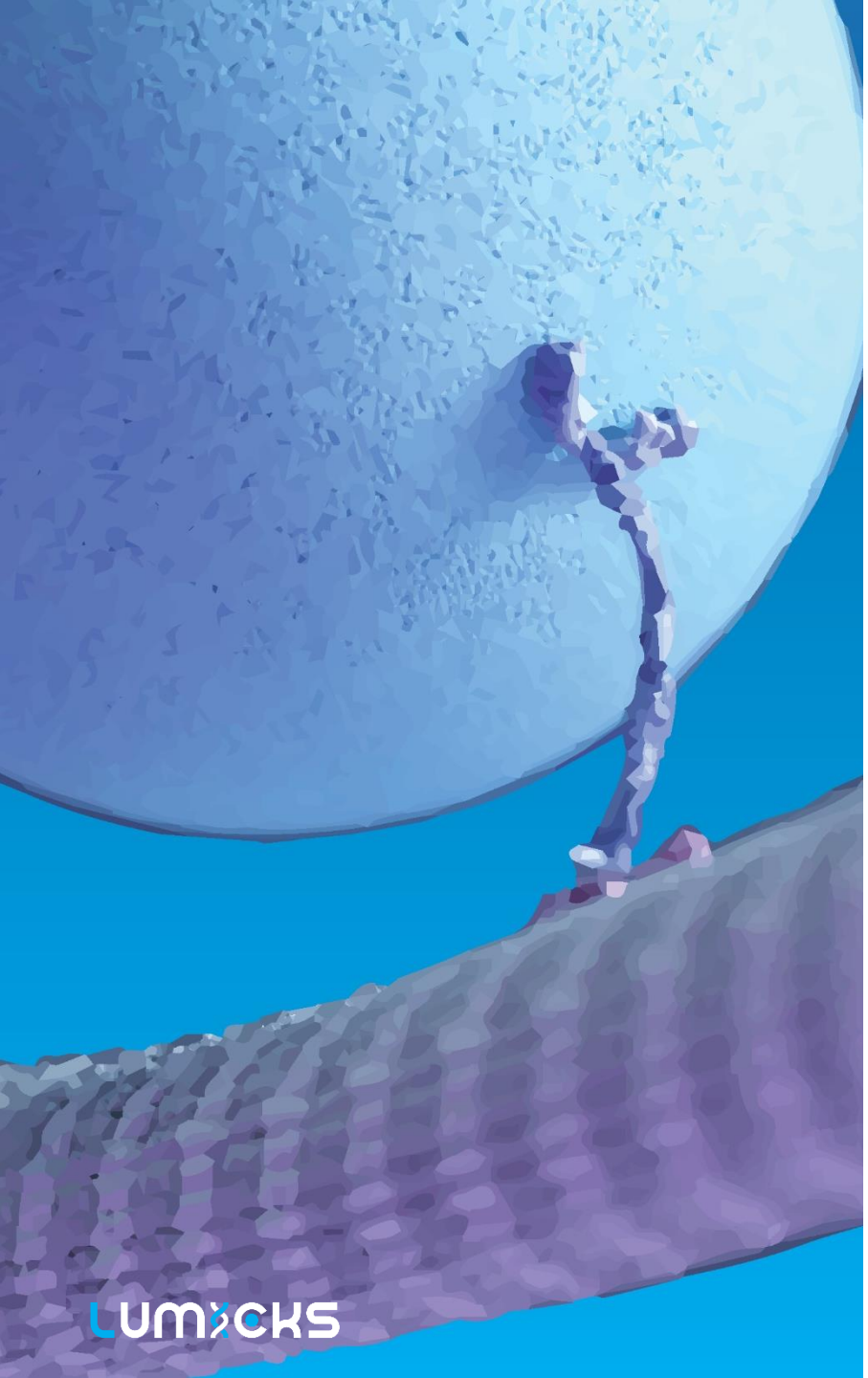

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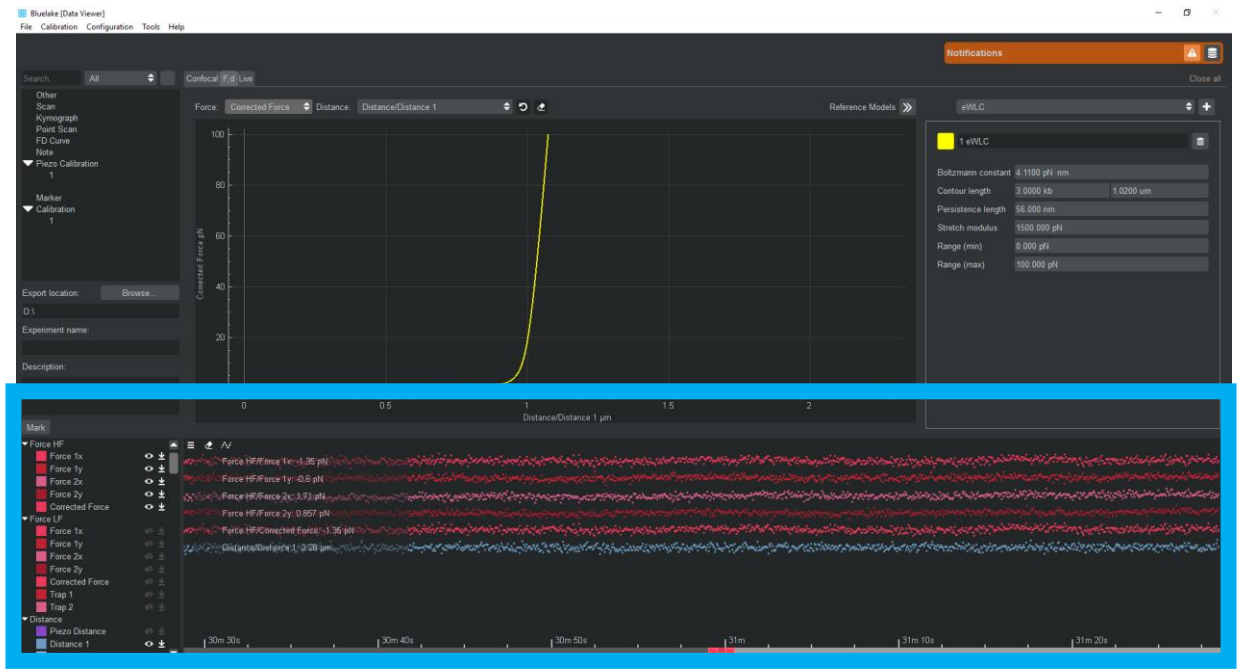
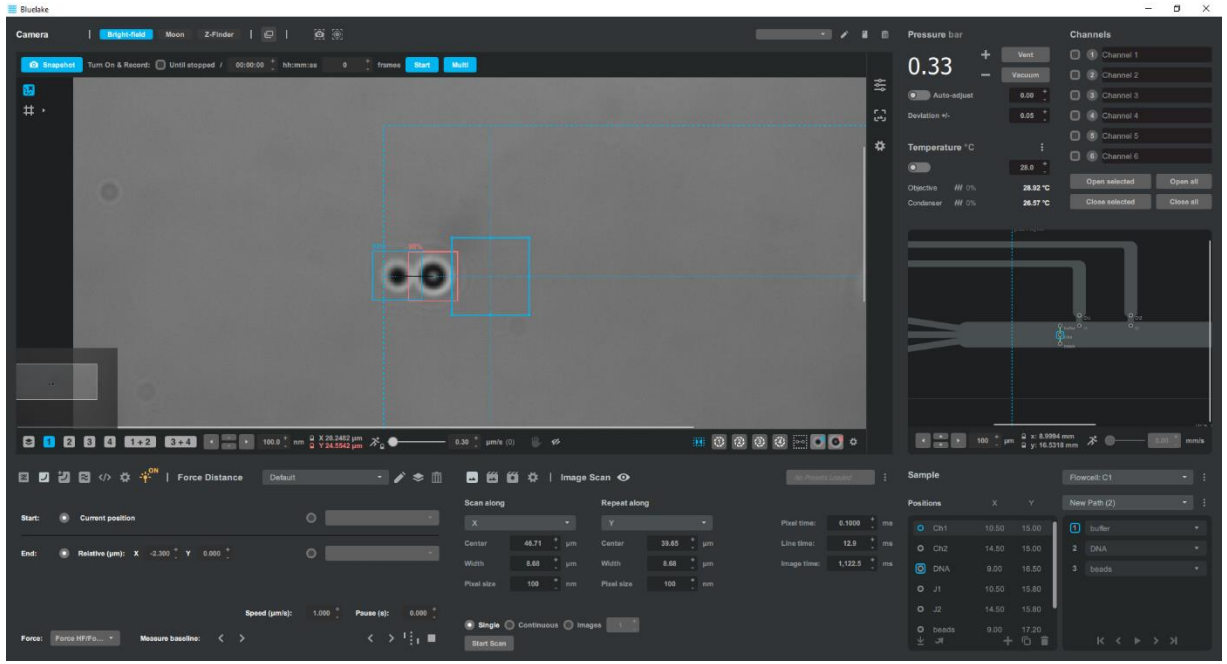
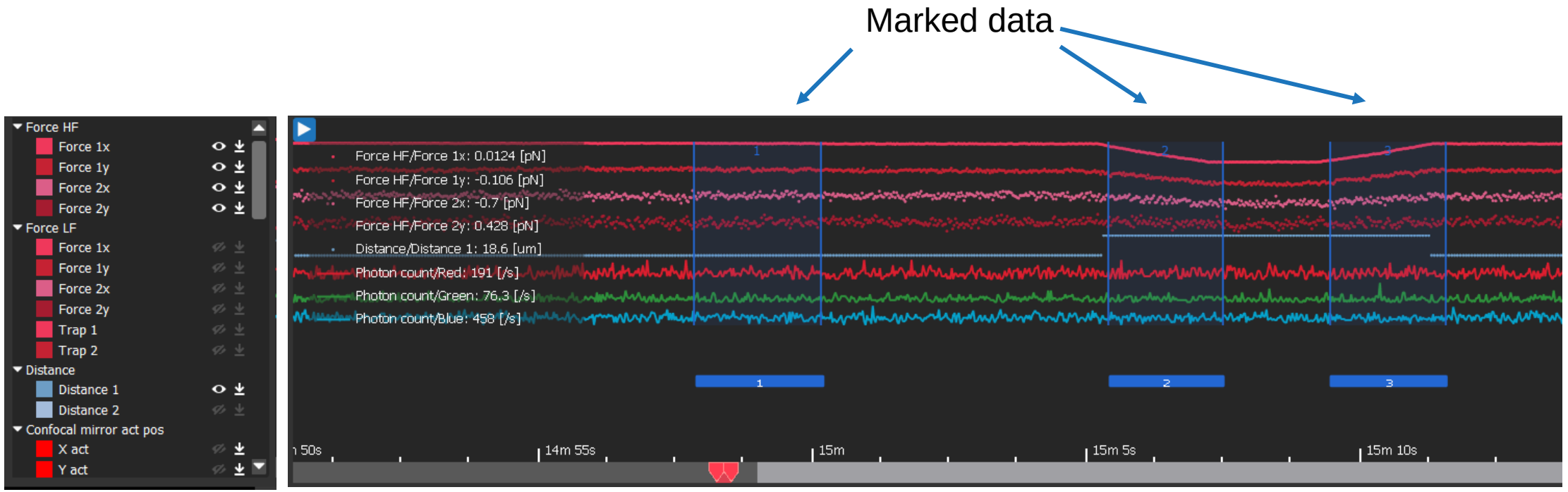


Instrument Control



- Each time Bluelake is opened, a new **session** is created that contains **all data streams**.
- Note: due to the size of session files, they cannot be kept permanently.
- Sessions can only be opened in **Session Viewer**, which can be found on the desktop of the Controller PC.

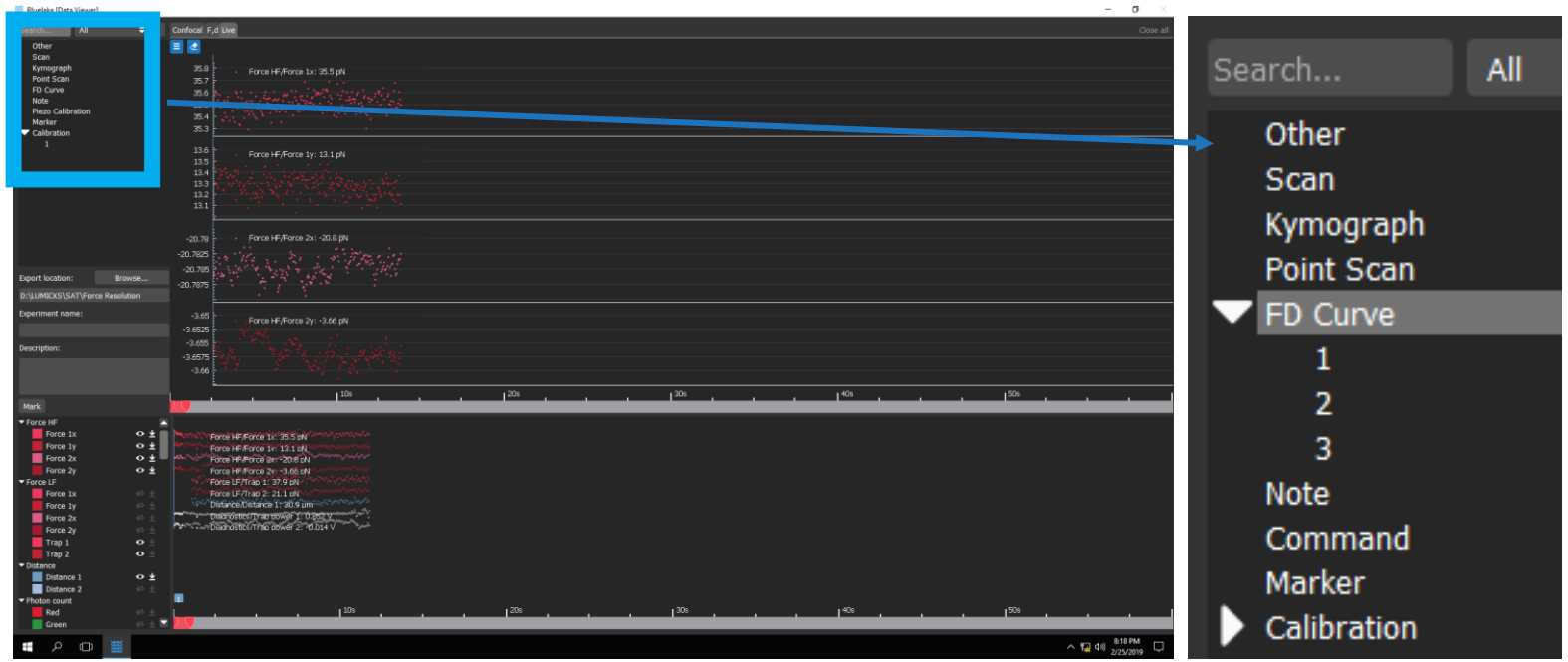
Selected channels in marker can be exported as .h5 file



- Visualize/show

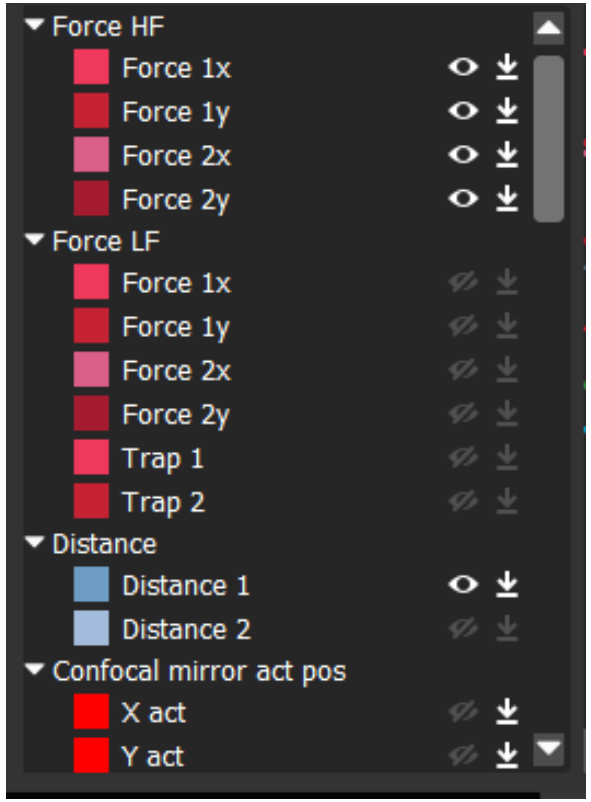
- Download/include channel when exporting file

List of data that can be exported



- List of Scans, Kymographs, Point scans, FD curves, etc that were collected during the experiment
- The data can be exported as .h5 file.
- Scans and kymographs can also be saved as an image

hdf5 files



- Data exported from Bluelake is stored in the hdf5 (.h5) format .
- The HDF5 files contain groups (Force HF, Distance,…) that can hold datasets (Force 1x, Distance 1, …)
- More information about HDF5 files can be found at https://support.hdfgroup.org/HDF5/doc/H5.intro.html
- HDF5 files can be opened with various data analysis software, such as Matlab, Python, origin, prism, …
- LUMICKS has developed
  - Lakeview for direct visualization of hdf5 files
  - Pylake for data analysis

C-Trap data analysis

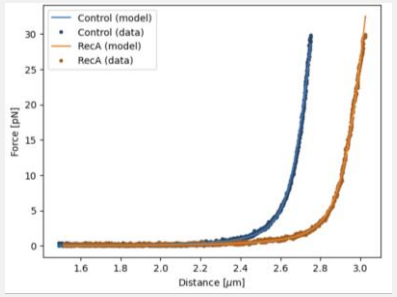
Lakeview

- Quickly visualize C-Trap h5 data
- Export data to a different format
- Perform Kymograph analysis

- Download Lakeview here

Pylake

- Python package for visualizing and analysing C-Trap h5 data, for example:
  - Analyzing fd curves
  - Computing the Piezo distance
  - Example analysis workflows



- Pylake documentation

harbor

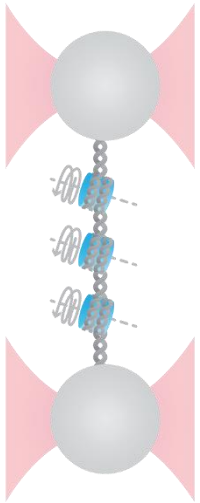
- Script-sharing platform for C-Trap users
- Download data analysis and automation scripts

- harbor.lumicks.com

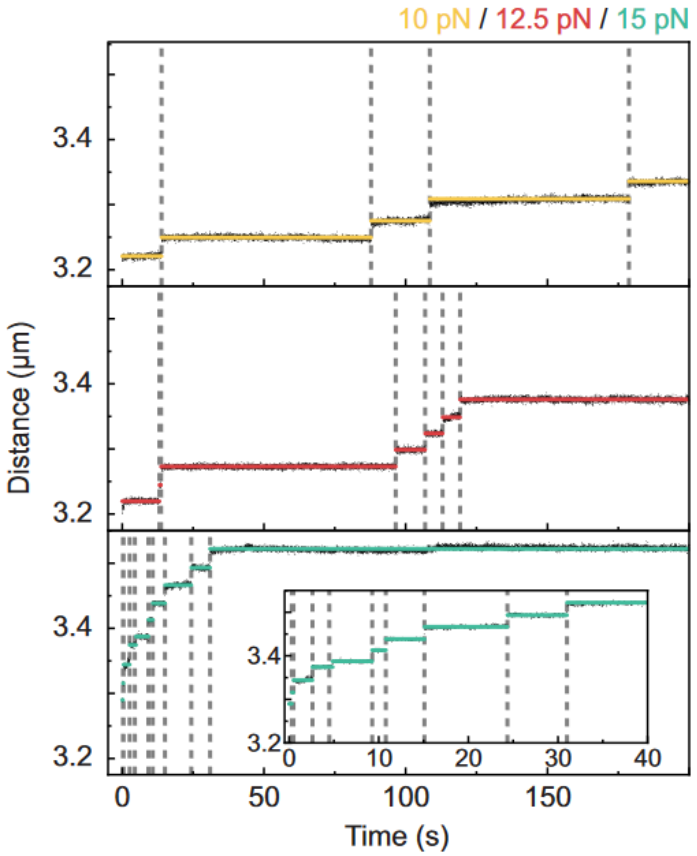
Example data output from Pylake

Data channel over time

Example: sudden distance changes over time reflects the unwrapping of nucleosomes

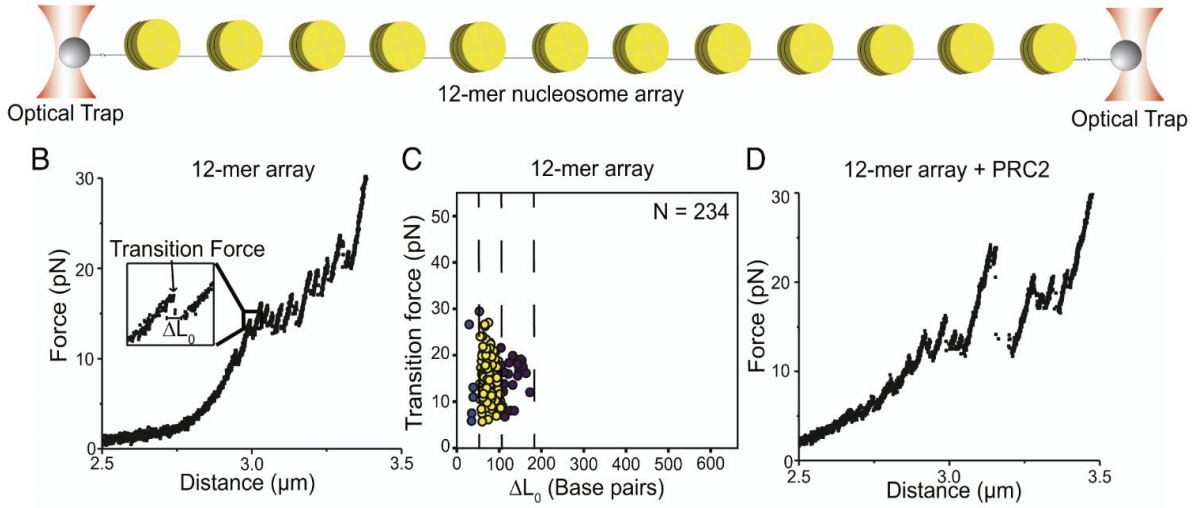


3 out of 12 nucleosomes shown for simplicity



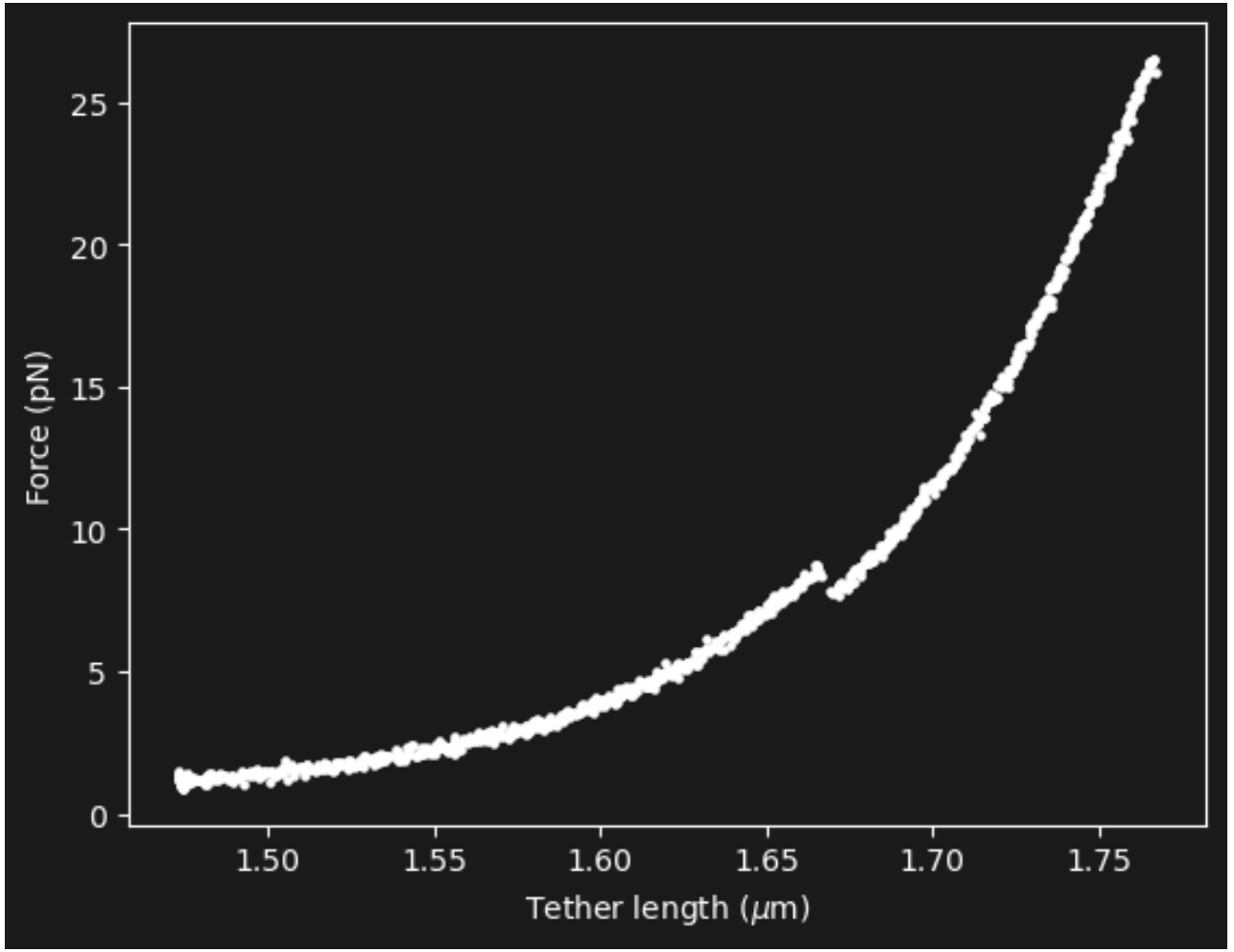
Data channel vs. another data channel

Example: force-distance relationship of dsDNA, analyze DNA compaction

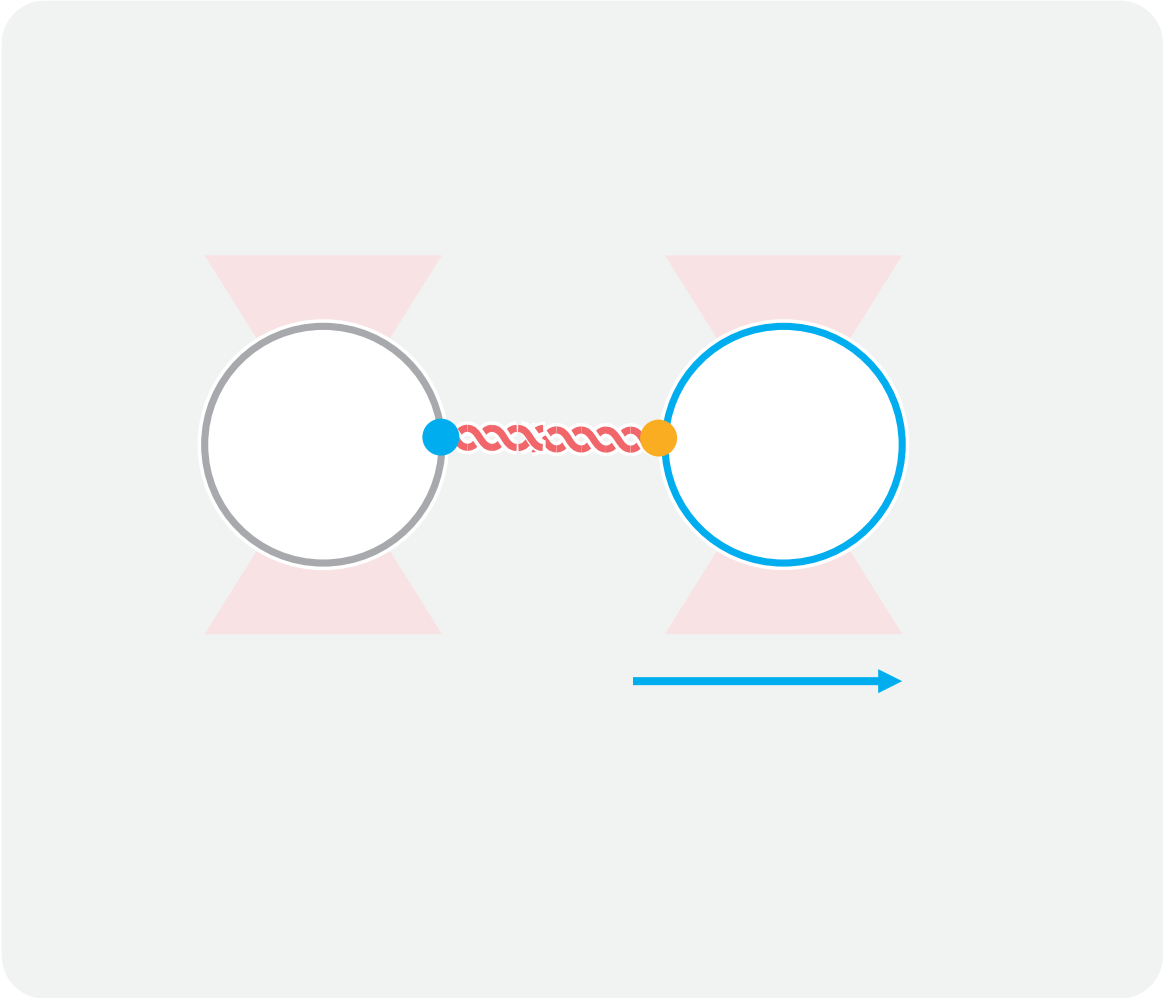


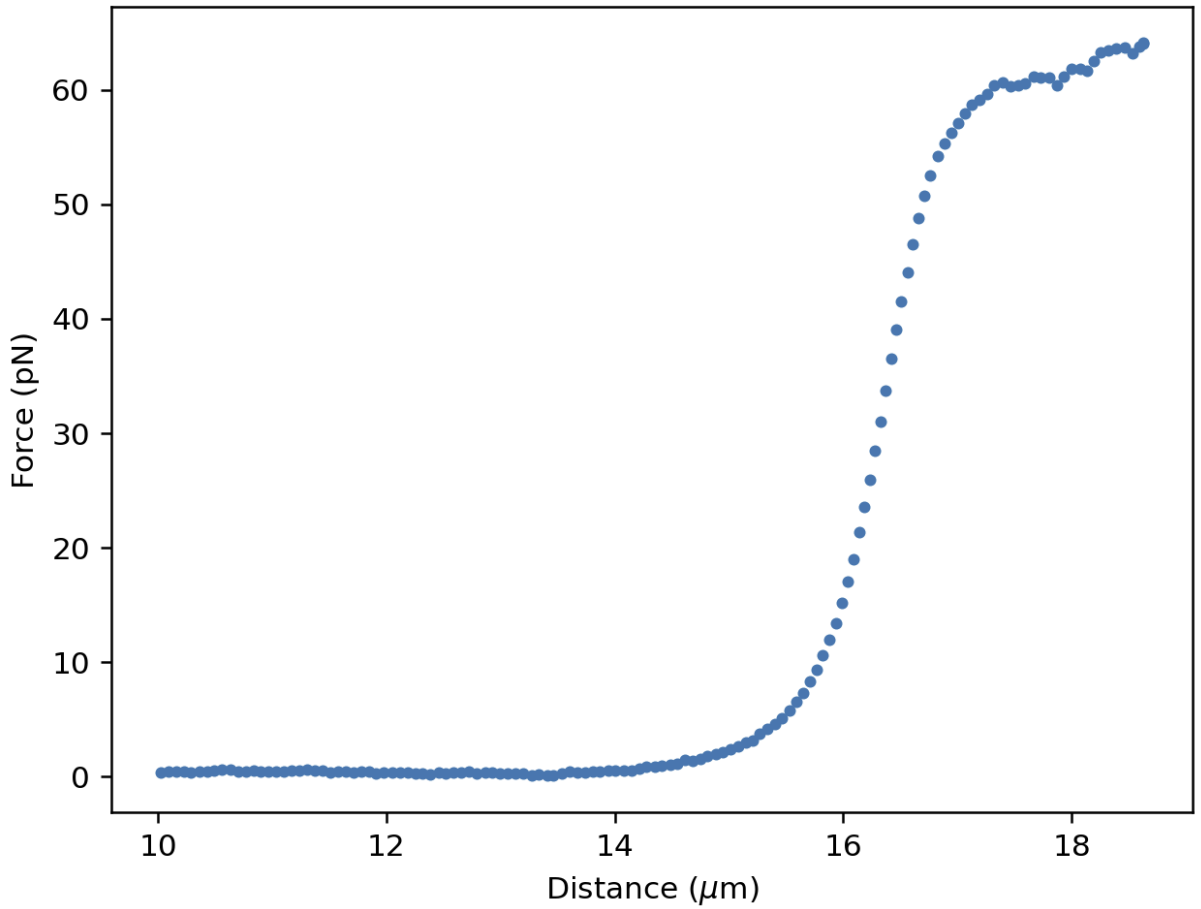
Spakman et al. PICH acts as a force-dependent nucleosome remodeler. Nature Communications 2022

Leicher et al. Single-molecule and in silico dissection of the interaction between Polycomb repressive complex 2 and chromatin, PNAS 2020

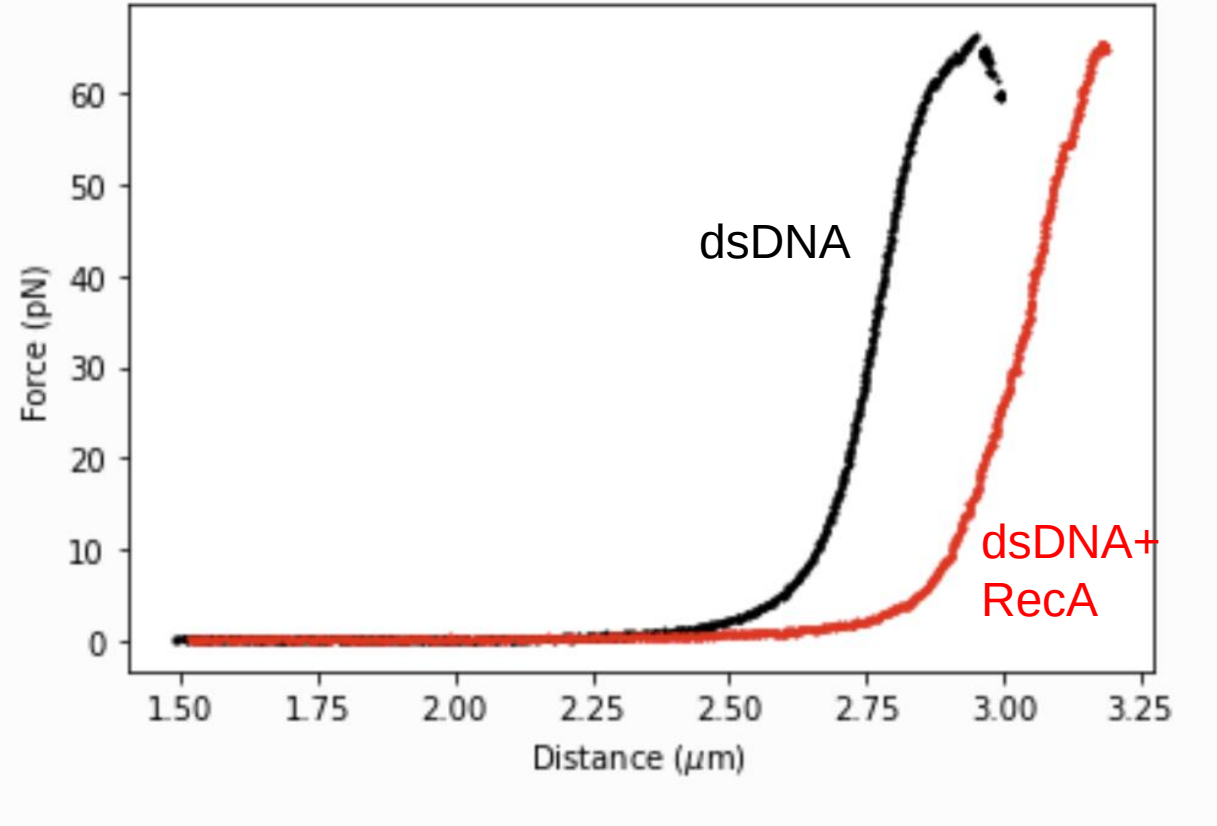

25
20
15
10
5
0
Force (pN)
1.50
1.55
1.60
1.65
1.70
1.75
Tether length (μm)

Force-extension curves

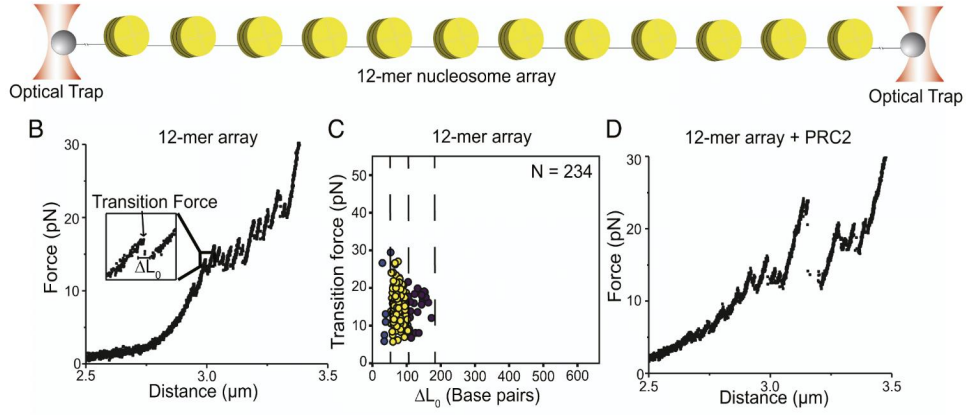




Stretching DNA



- When performing force-extension measurements, you often want to know
  - What is the length of my double stranded DNA?
  - What are the mechanical properties of my DNA?
  - Level of compaction
  - …
- This information can be obtained by fitting a model to the force extension curve

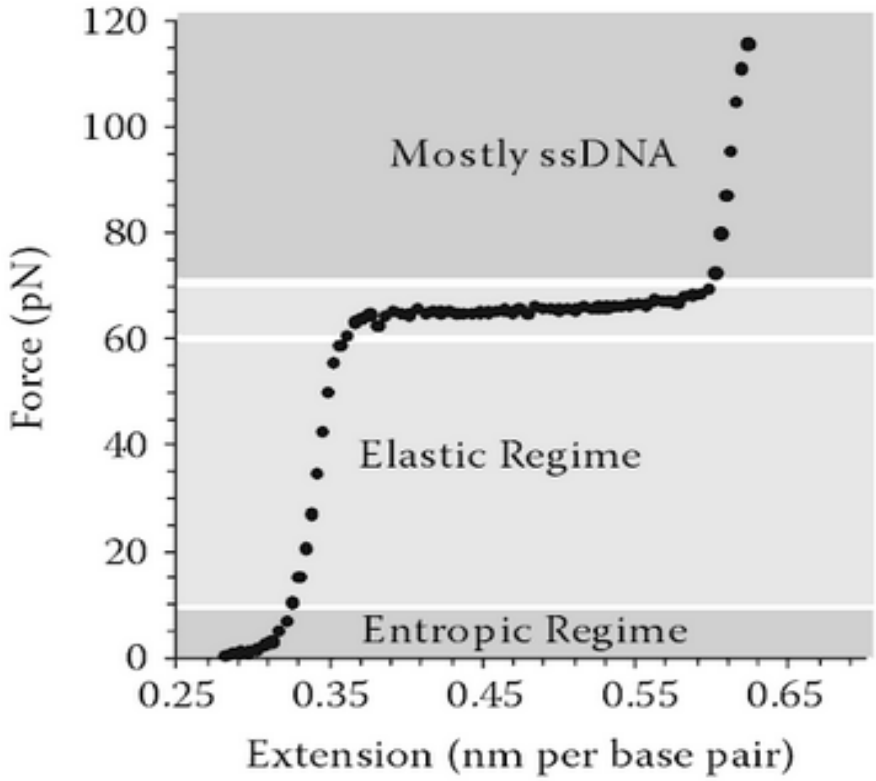


The worm-like chain model

- The Worm-like chain (WLC) model is a good model for fitting DNA force-extension curves

- Variants of the WLC model:
  - WLC (up to 5-10 pN)
  - Extensible WLC (up to 30 pN) -> **most common**
  - Twistable WLC (up to 50 pN)
  - …

- Choose model with as few parameters as possible



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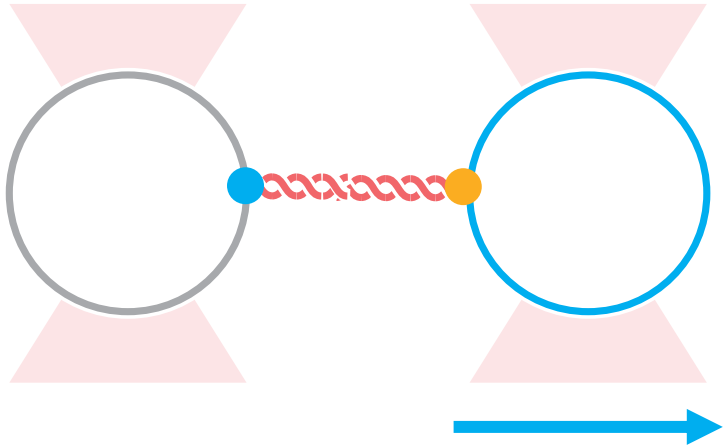
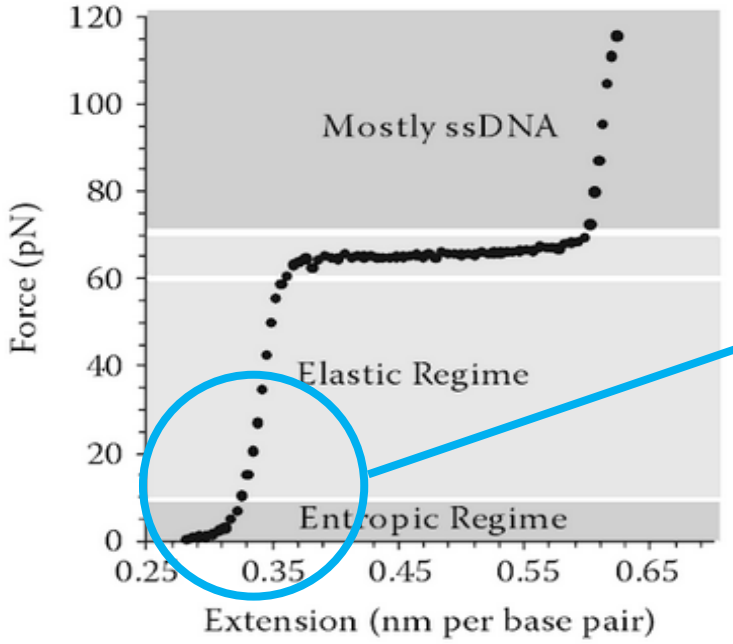
Extensible WLC





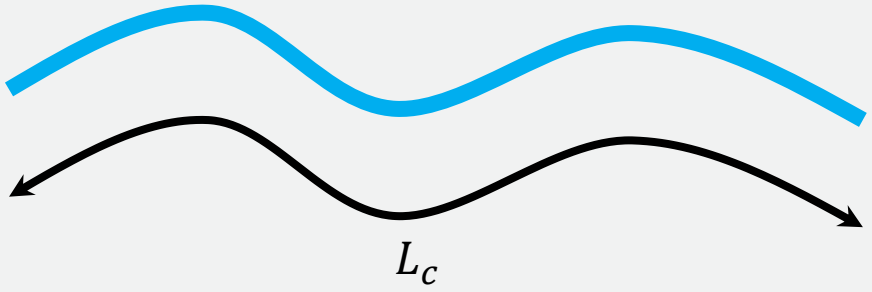
Extensible worm-like chain (up to ~ 30 pN)
Odijk 1995

In Pylake:
`ewlc_odijk_force`
`ewlc_odijk_distance`

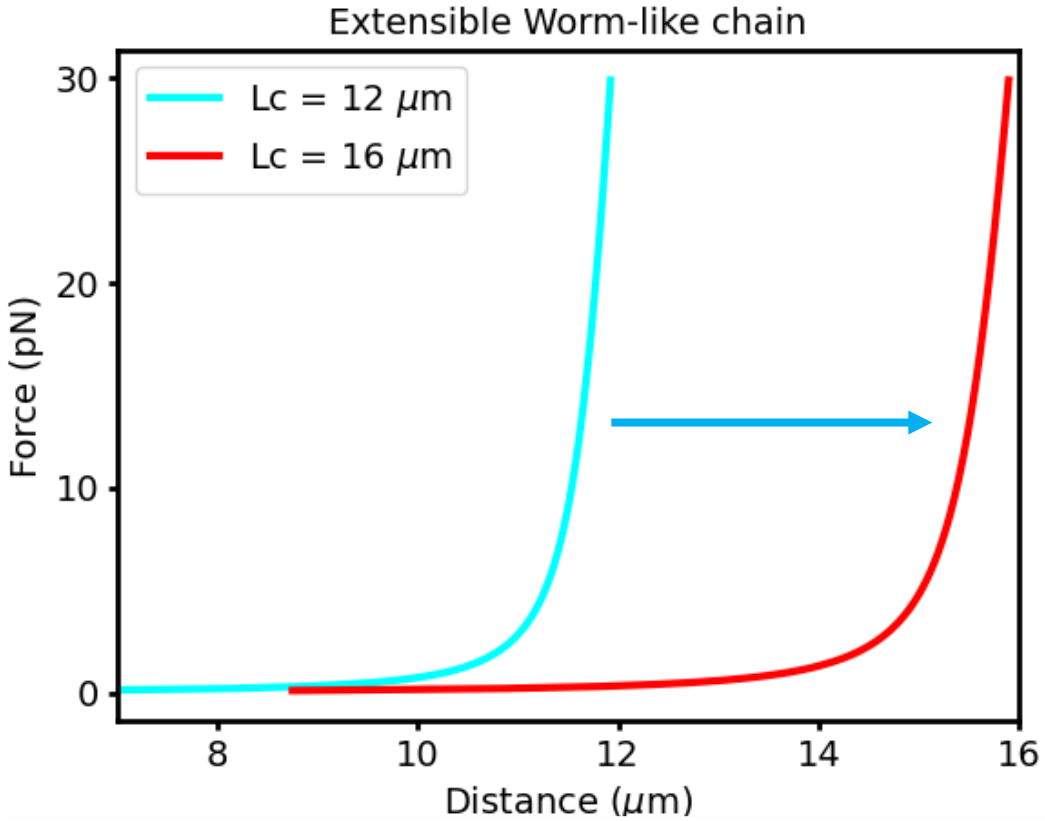
$$d(f) = Lc_{DNA}\left(1 - \frac{1}{2}\sqrt{\frac{kT}{fLp_{DNA}}} + \frac{f}{St_{DNA}}\right)$$

Parameters of the extensible WLC: Contour length

- The contour length, Lc, is the length of the DNA

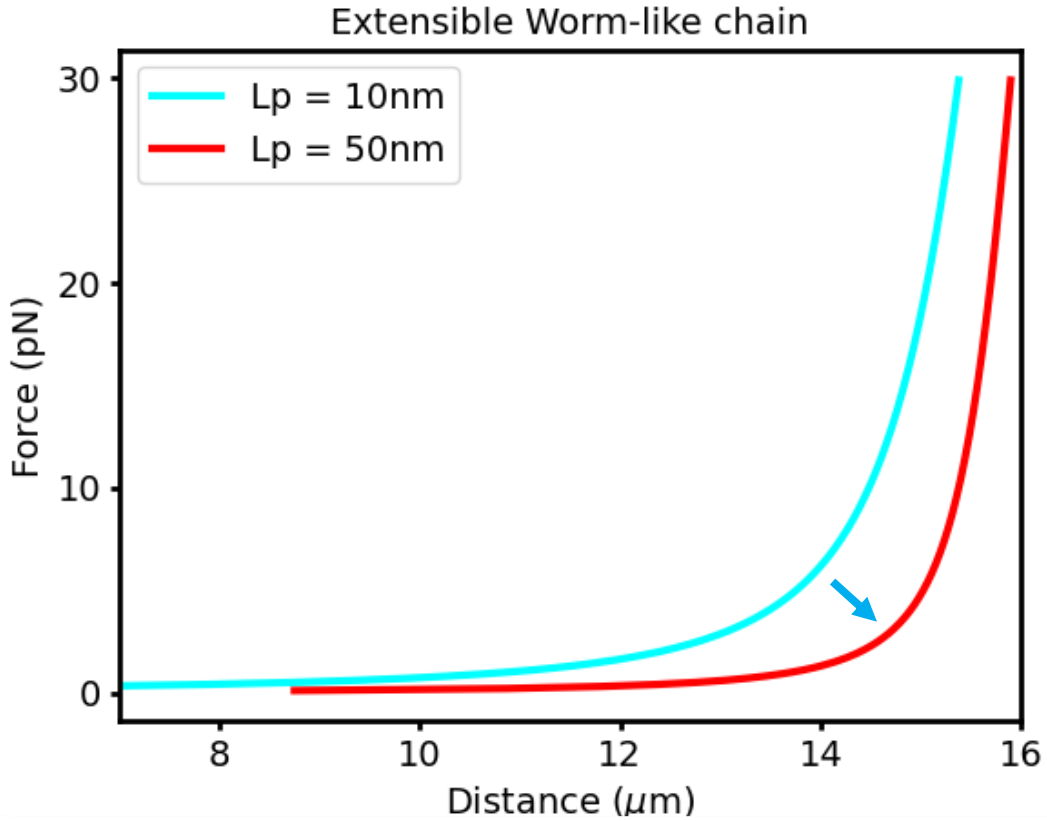


- The contour length can be estimated from the FD curve



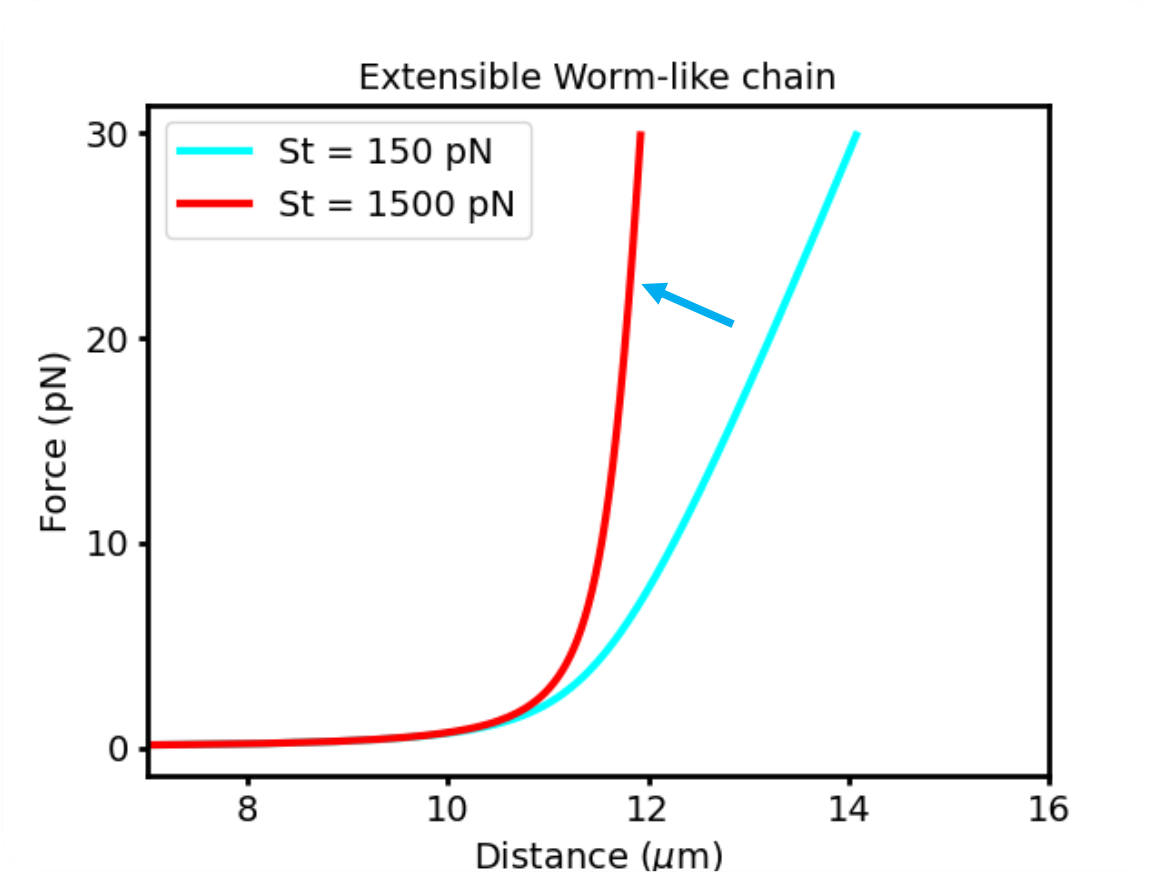
Parameters of the extensible WLC: Persistence length, Lp

- Measure of stiffness: ‘the persistence length is the typical length along which the chain forgets its previous orientation.’
- Typically 50 nm for double-stranded DNA
- Value depends on salt concentration (Baumann 1997)



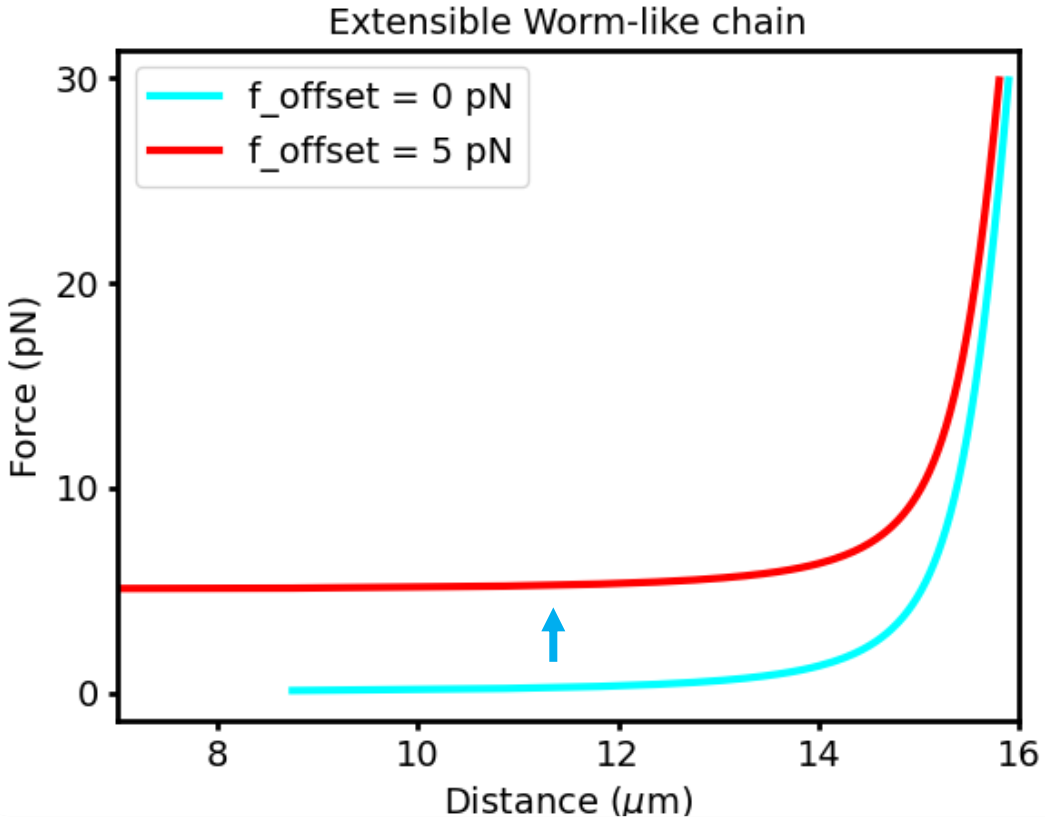
Parameters of the extensible WLC: Stretch modulus

- Higher St means more resistance against stretching
- St important for higher forces
- Typical value for dsDNA: 1500 pN

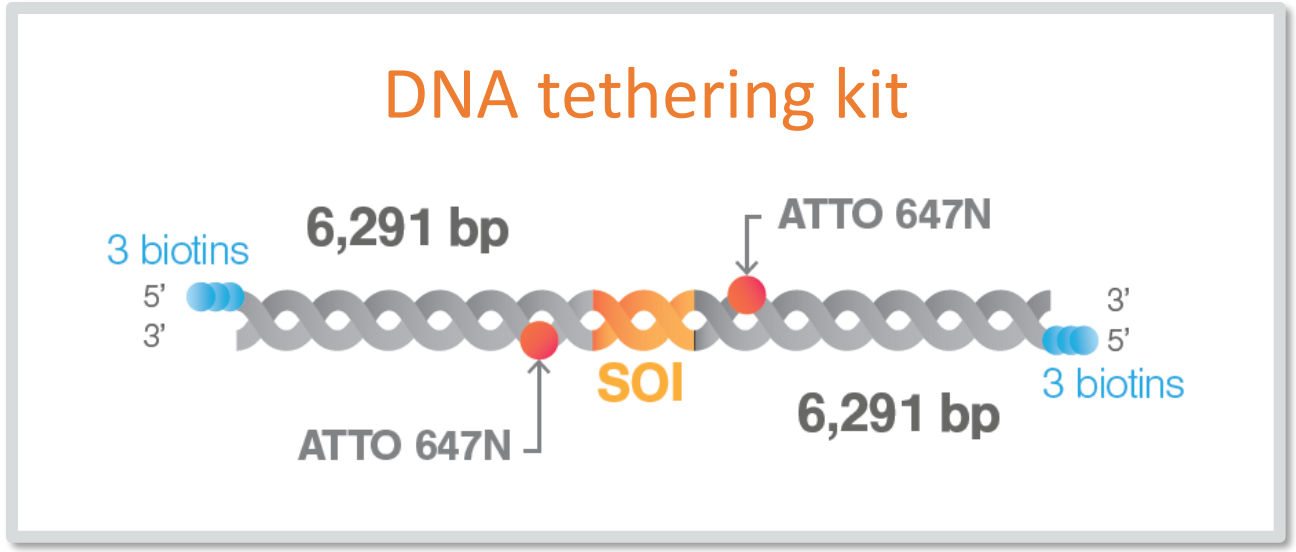


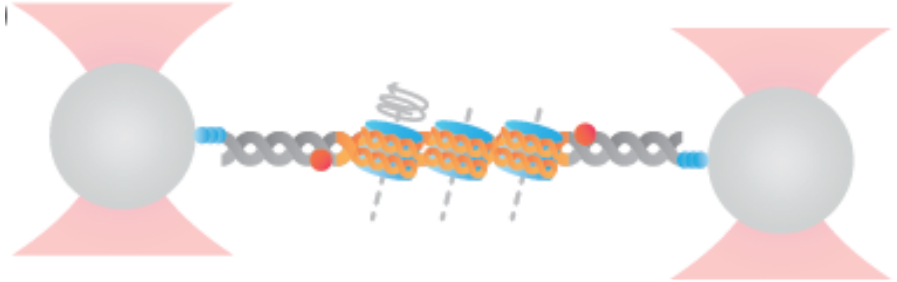
Extra parameter: Force offset

- The force offset can be non-zero when the force was not reset before recording the FD curve
- The force offset vertically shifts the fd curve
- Note: The force offset can be negative, this can give weird effects when using the absolute force. Therefore, it is better to use Force 2x

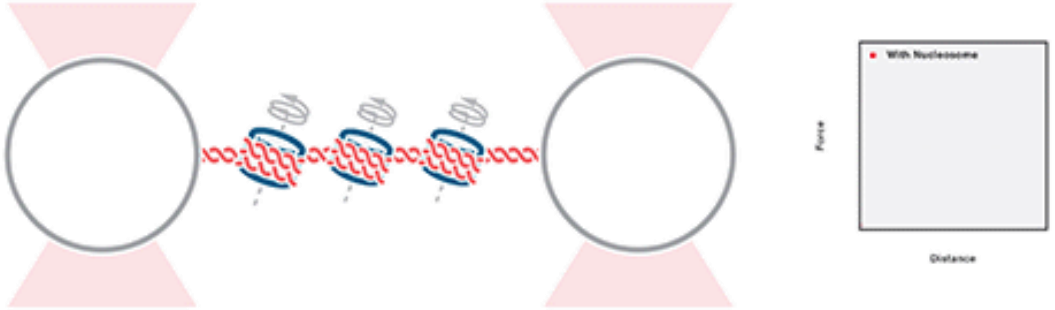


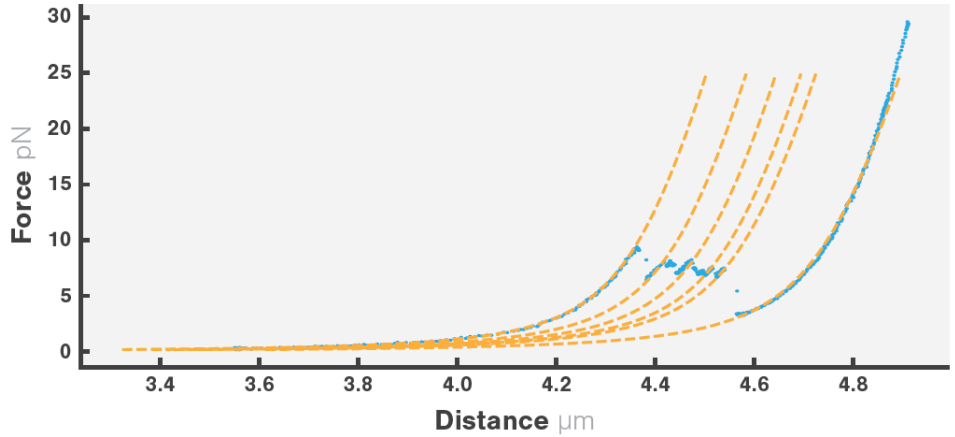
Nucleosome unwrapping





Nucleosome array





Concluding remarks

- Pylake can be cited using the following DOI: 10.5281/zenodo.4280789
- Both questions and feedback can go to the support website or support@lumicks.com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