

# Image Analysis on Biopolymer Networks

Characterization using graphs

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## Motivation: Role of network architecture

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## Sources of Strain-Stiffening

- [Storm et al., 2005]: strain stiffening arises from non linearity of single chains. Under the assumption that the network is isotropic and homogeneous. What does happens under other architectures?
- [Onck et al., 2005], at the same time an alternative explanation of strain stiffening arised exclusively from network connectivity and relative orientation of the fibers.

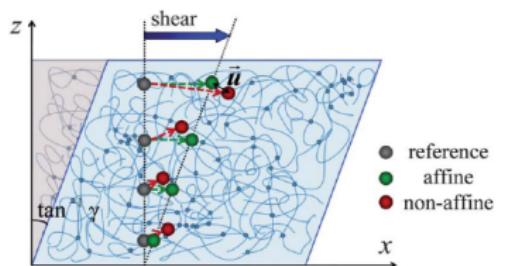
# Strain-Stiffening: Validity of Affine Deformation approximation in semi-flexible polymers

## Affine transformations valid when:

In Homogeneous and Isotropic Network

- Stiff Chains
- Dense Networks

[Wilhelm and Frey, 2003]



[Basu et al., 2011; Wen et al., 2012]

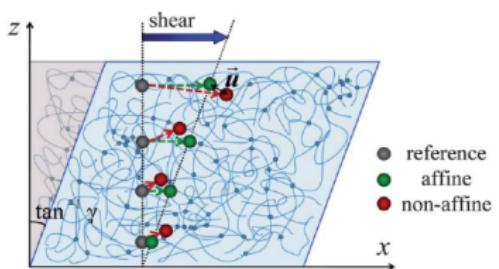
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Non-Affine range when:

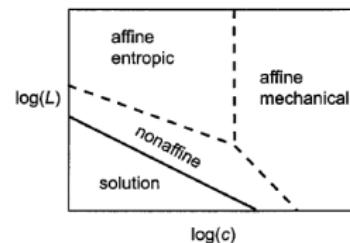
In Homogeneous and Isotropic Network

- Compliant Chains
- Coarse Networks

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$L$ : Molecular Weight,  $c$ : Concentration  
[Head et al., 2005]

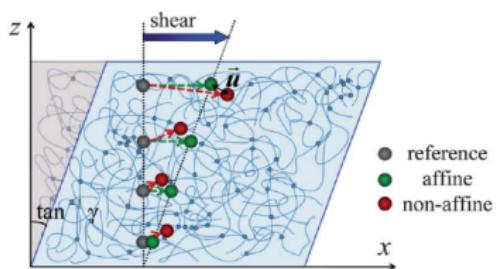
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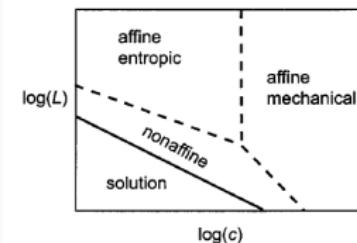
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## Pre-stress and architecture role:

Affine approx. is valid at constrained networks

- Initial pre-stress constrains networks.  
[Cioroianu et al., 2015]

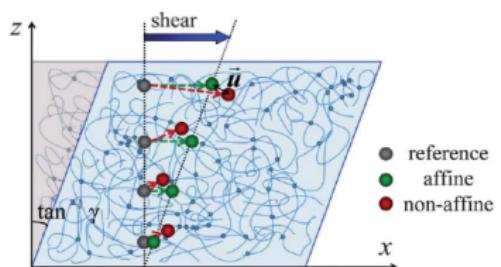
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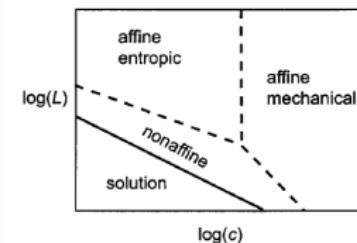
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Pre-stress and architecture role:

Affine approx. is valid at constrained networks

- Initial pre-stress constrains networks.  
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- Role of **Anisotropy or Architecture?**

## Goals and research questions

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- Going beyond the homogeneous and isotropic model of biopolymer networks: **Characterize the architecture** of connected filaments using images from different microscopy techniques depending on the size of the biopolymer.

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- Going beyond the homogeneous and isotropic model of biopolymer networks: **Characterize the architecture** of connected filaments using images from different microscopy techniques depending on the size of the biopolymer.
- How different architectures do influent the **mechanical properties?** Are completely different biopolymers sharing similar geometries?

## Goals and research questions

- Going beyond the homogeneous and isotropic model of biopolymer networks: **Characterize the architecture** of connected filaments using images from different microscopy techniques depending on the size of the biopolymer.
- How different architectures do influent the **mechanical properties?** Are completely different biopolymers sharing similar geometries?
- Develop an open-source, free, user friendly, tested, and well documented software for others to use with microscopy images.

## Steps to characterize the network

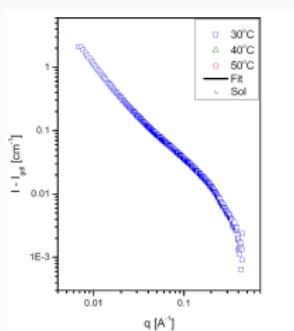
- Gathering the images. Microscopy.
- Image analysis. Skeletonization.
- Characterize the image with a graph representation.

## Gathering structure data:

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# Scattering: SAXS and SANS

- Study of peaks and fractality at different  $q$  values provides an **averaged** structural information (mesh size, width of single-chains)
- It does not require special sample preparation.
- Good statistics, fast, allow study of dynamics.
- **But**, it does not provide explicit 3D structure of exact connectivity.



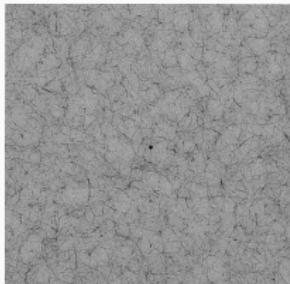
$I$  vs  $q$  Pectin from SAXS

# Microscopy: TEM and Confocal

To get better architecture we need **3D microscopy**.

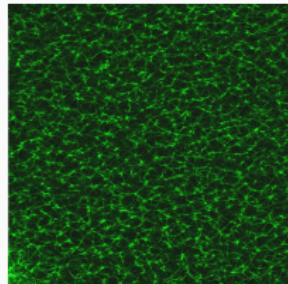
## TEM tomography

- Necessary to reach polysaccharides scale.
- Sample preparation is hard.  
Artifacts?
- **Working on validation method** comparing it with scattering.



## Confocal

- Suitable for most protein biopolymer networks.
- Sample preparation is more reliable.



# Image Analysis

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# Skeletonization I: Ridges of Distance Map

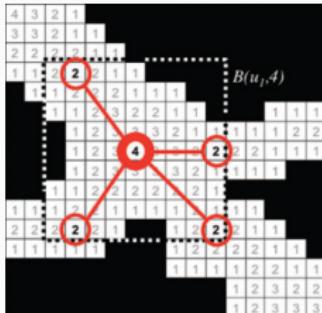
## Goal:

Process raw image data to get a spatial graph representation of the network.

**Anisotropic denoise:** Denoise conserving edges.

**Binarization:** Choose threshold that best conserves architecture.

**Distance Map:** Distance to the background for each pixel.



# Skeletonization I: Ridges of Distance Map

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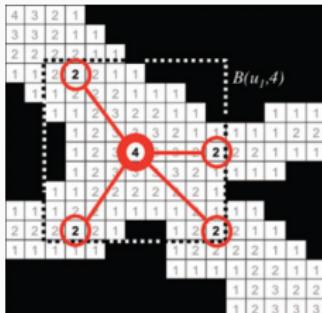
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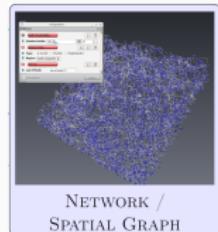
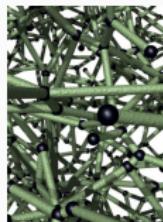
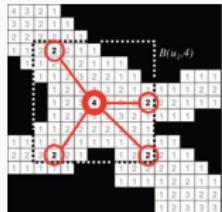
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**Ridge Detection:** Follow the highest values of the distance map.

**Branch Merging and Pruning:** Fill gaps and remove short branches.



# Skeletonization: Different methods and pre-processing

## Pre-processing techniques

1. Hessian Matrix: Eigenvalues to detect vesselness.
2. Anisotropic Diffusion: Denoise, keeping edges information.
3. Multiscale characterization with gaussian operator.

## Other Skeleton Methods

1. Open Contours: Snakes
2. Template Matching
3. Morphology Operators

## **Graph Characterization**

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# Graphs: A Link to Complex Systems

We can now link our spatial graph to standard complex network libraries.

**IGraph** C, R, Python (GPL licence)

**Boost: Graph and GraphParallel** C++ (MIT licence)

**NetworkX** Python (MIT licence)



# Generating Software Tools

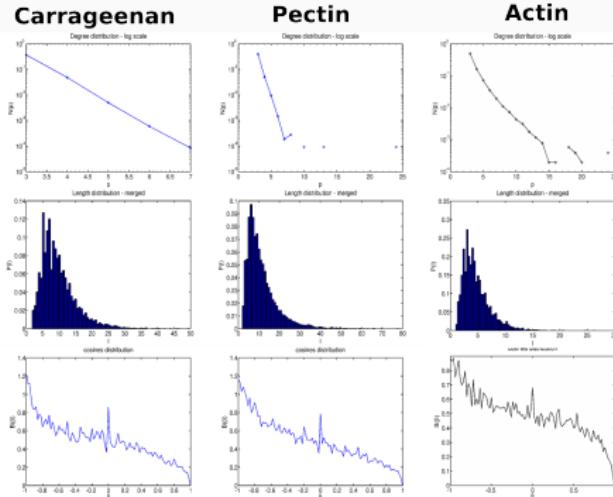
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# Results: Proof of concept

Three Networks analyzed:

- Polysaccharides using TEM: Pectin, Carrageenan

- Proteins using Confocal: Actin (Collagen from literature [Lindstrm et al., 2013] ).



Degree: Geometrical distribution

$$N(p) = q(1-q)^{p-3}$$

where  $q = 1/(Z-2)$ , and  $Z$  is the average node degree.

Node-to-node dist.: Log-normal

$$P(\ell) = \frac{1}{\ell s \sqrt{2\pi}} \exp\left(-\frac{(\mu - \ln \ell)^2}{2s^2}\right)$$

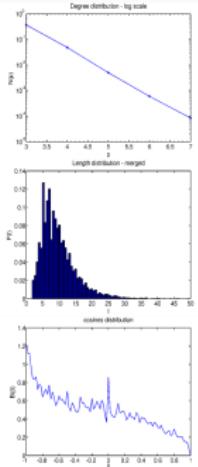
Edge relative orientation: Power-Series

$$B(\beta) = \sum_{k=1}^m b_k (1-\beta)^{2k-1}$$

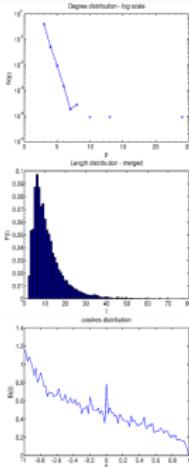
# Results: Proof of concept

Statistical distributions of local graph properties: **Showing same functional forms**

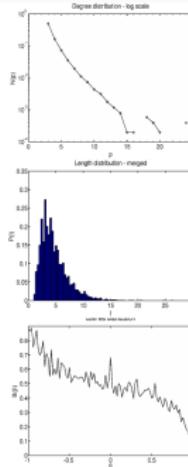
**Carageenan**



**Pectin**



**Actin**



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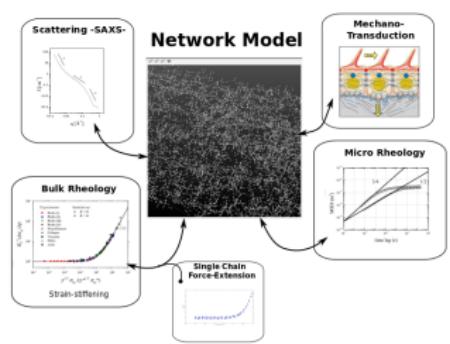
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# Summary

## Digging up the network geometry

- When does architecture matter? What is its role at lower scales.
- Gather best image analysis techniques for biopolymers.
- Link to complex network libraries for graph analysis. **Reconstruct in-silico networks from it**
- The dream would be to input force-extension curve of single chains + network architecture to get bulk rheology. But initial configuration setup and pre-stress play a role in simulations.



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- Leif Lundin. SP Food and Bioscience. Sweden.

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**Thanks for your attention!**

# Follow development live in github

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Keep up to date with further development at my github page:

[github.com/phcerdan](https://github.com/phcerdan)

Get this beamer theme from:

[github.com/matze/mtheme](https://github.com/matze/mtheme)



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