Leveraging Super High Optical Resolution Microscopy to Probe the Interaction Zone Between *Clostridium thermocellum* and Biomass

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CBI

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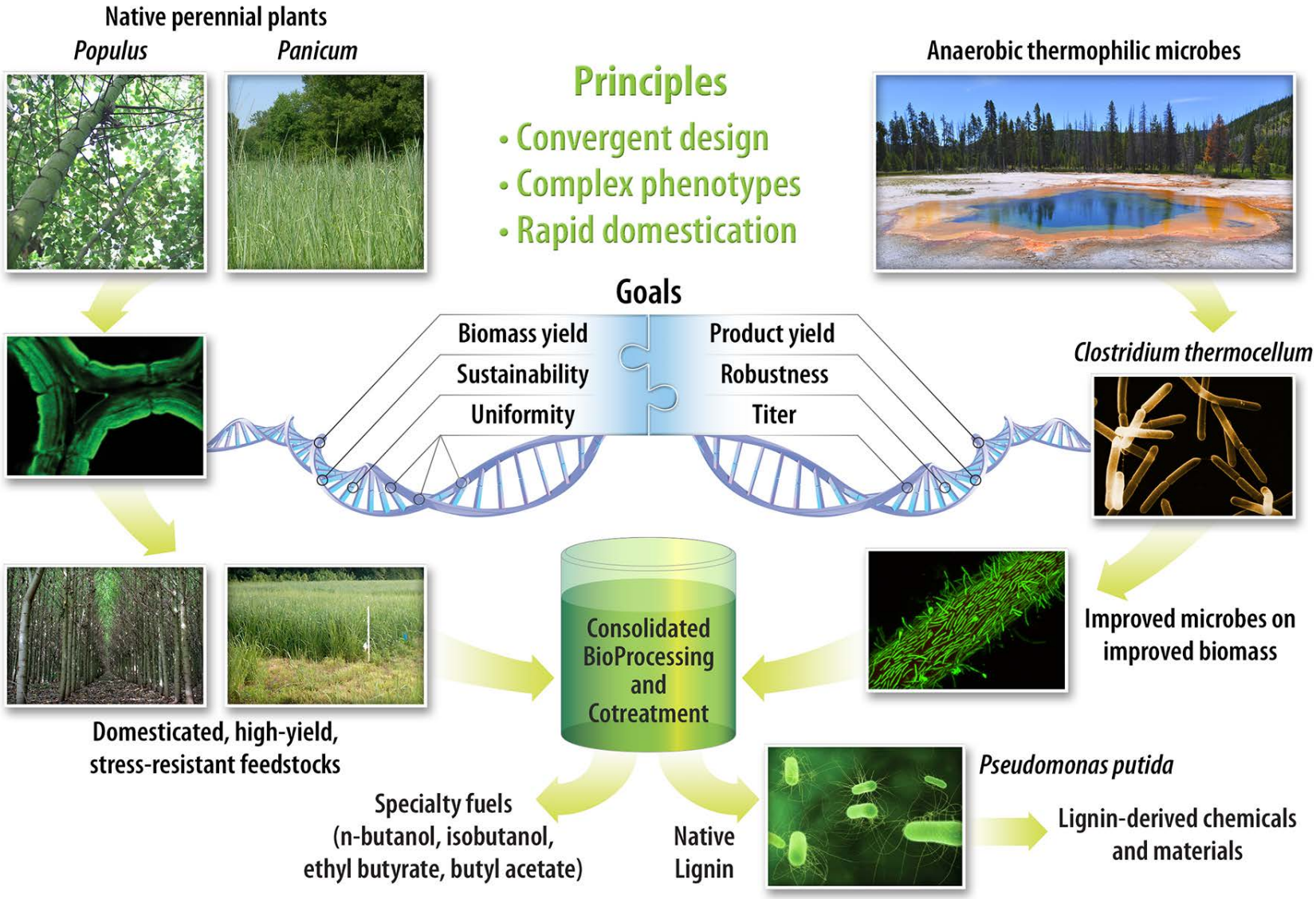
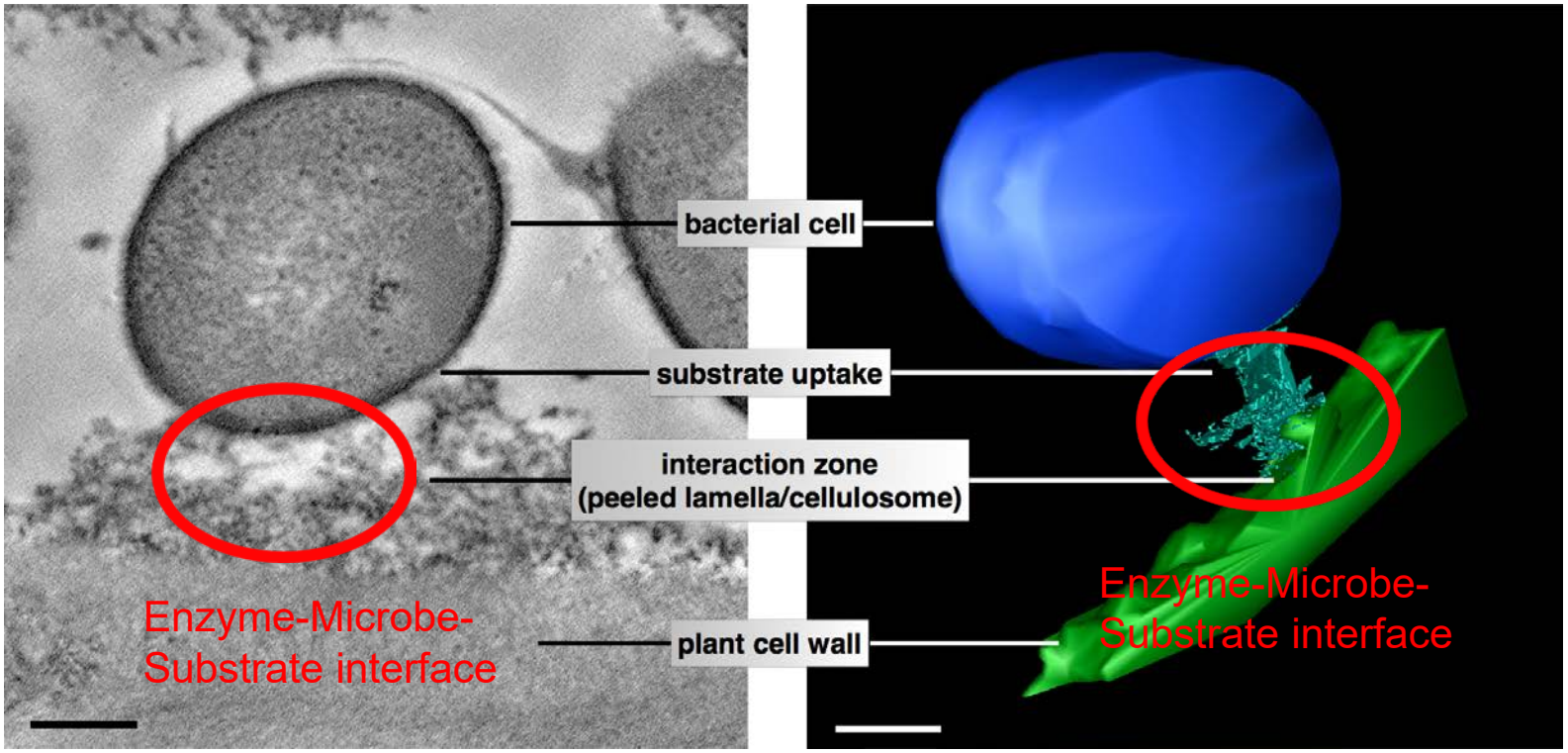
Convergent improvement of plants and microbes for feedstocks, fuels and products



C. thermocellum is, so far, the most efficient cellulolytic bacterium that has been isolated.

C. thermocellum can solubilize more than 60% of C5 and C6 sugars in 4-5 days from non-pretreated mature biomass such as switchgrass, far exceeding the performance of fungal preparations.



Is the key in the Enzyme-Microbe-Substrate interface?

Optimal techniques used to study the microbial/surface interactions

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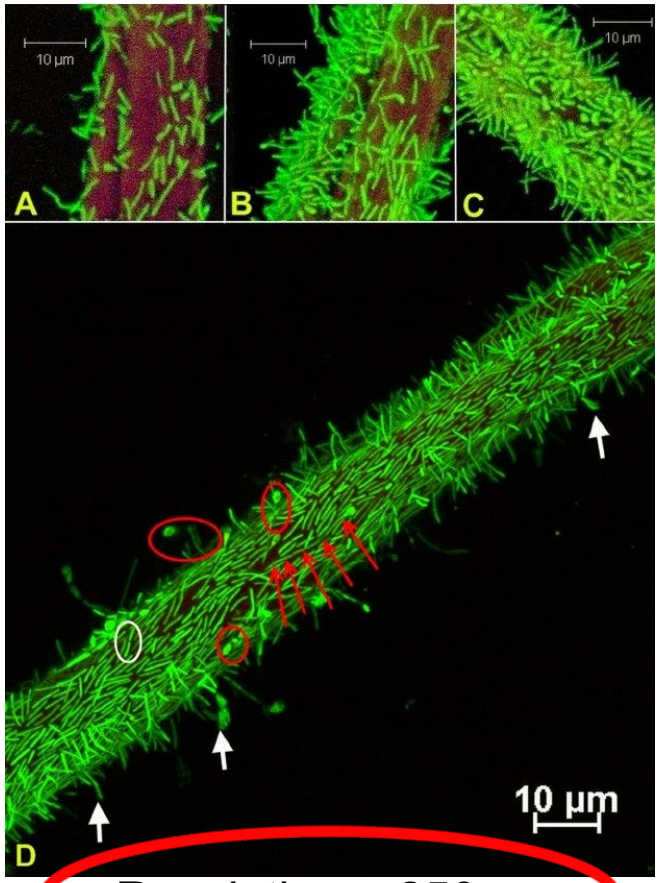
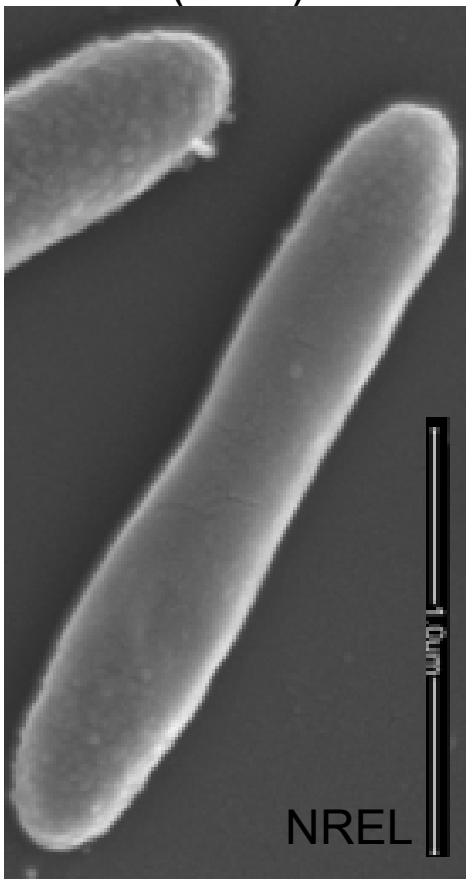
Optical Imaging



Resolution > 250nm

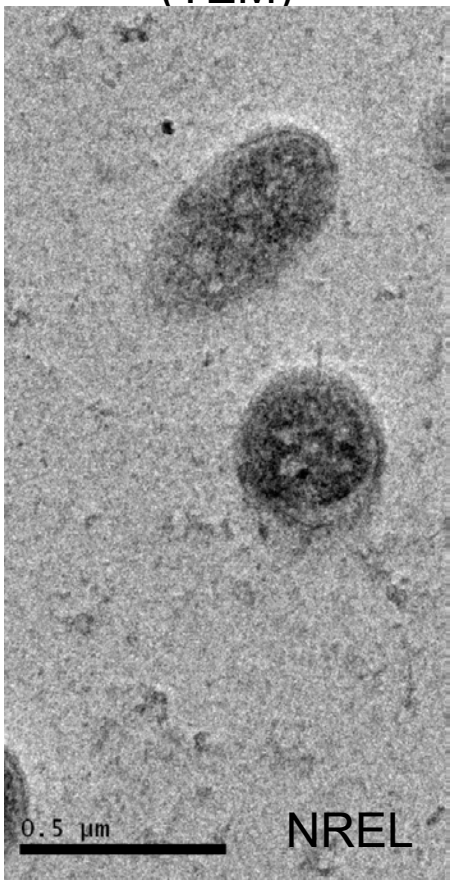
Source: Dumitrache, Alexander. et al. 2013. "Form and Function of *Clostridium thermocellum* Biofilms". AEM, January 2013 https://aem.asm.org/content/79/1/231

Scanning Electron Microscopy Imaging (SEM)



Resolution > 10nm

Transmission Electron Microscopy Imaging (TEM)



Resolution > 0.2nm

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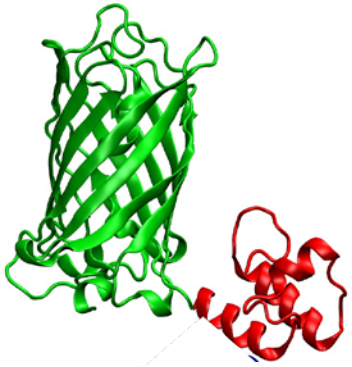
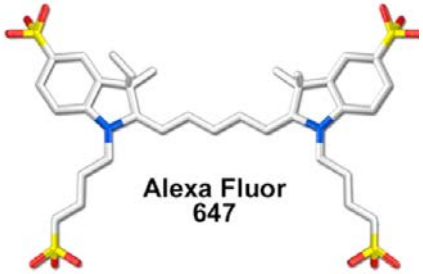
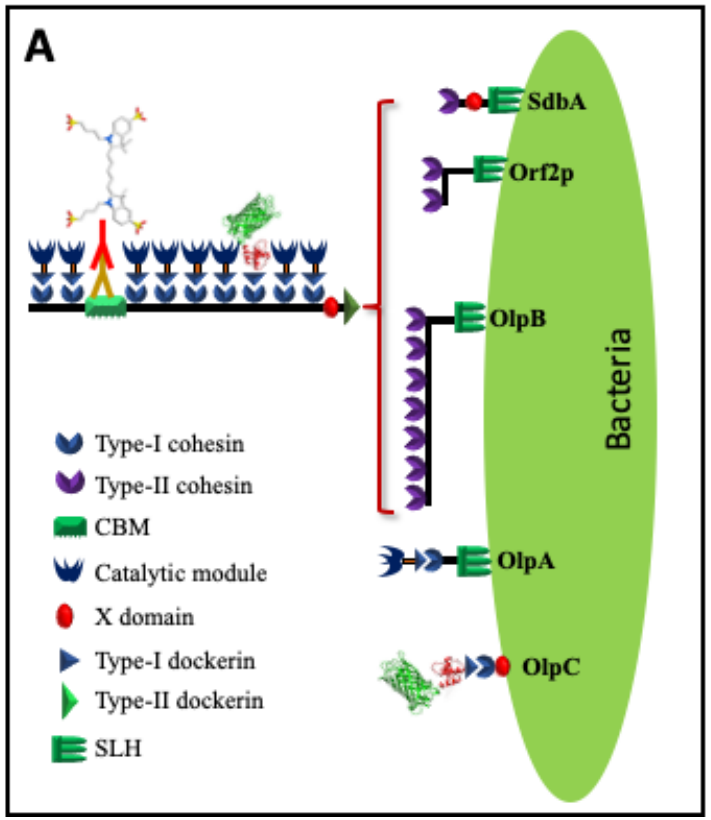
Labeling *C. thermocellum* for Super High resolution imaging



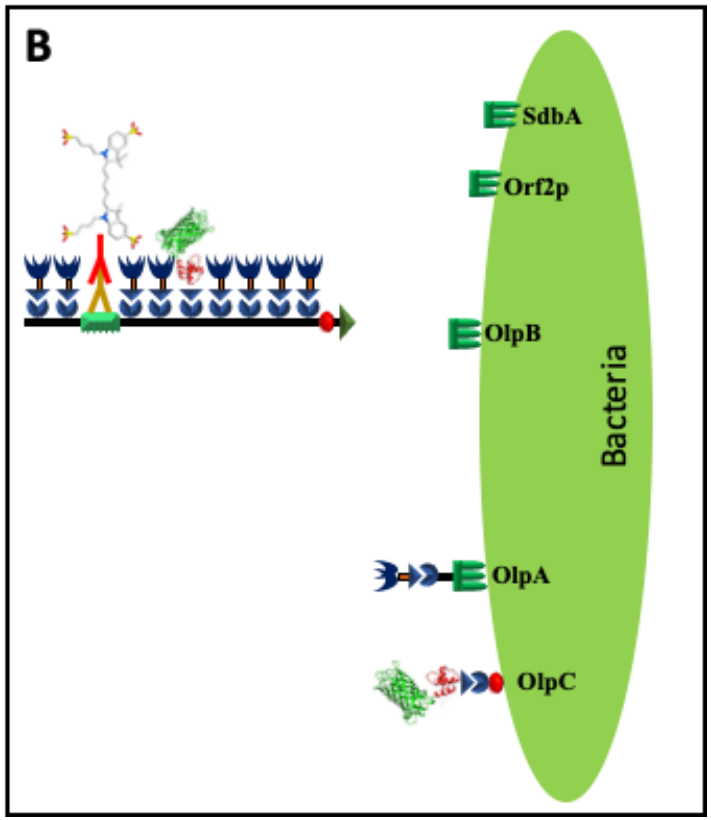
PA-GFP with a dockerin



Wild Type



CTN5 mutant



C. therm grown on cellobiose: TEM vs Super high resolution imaging

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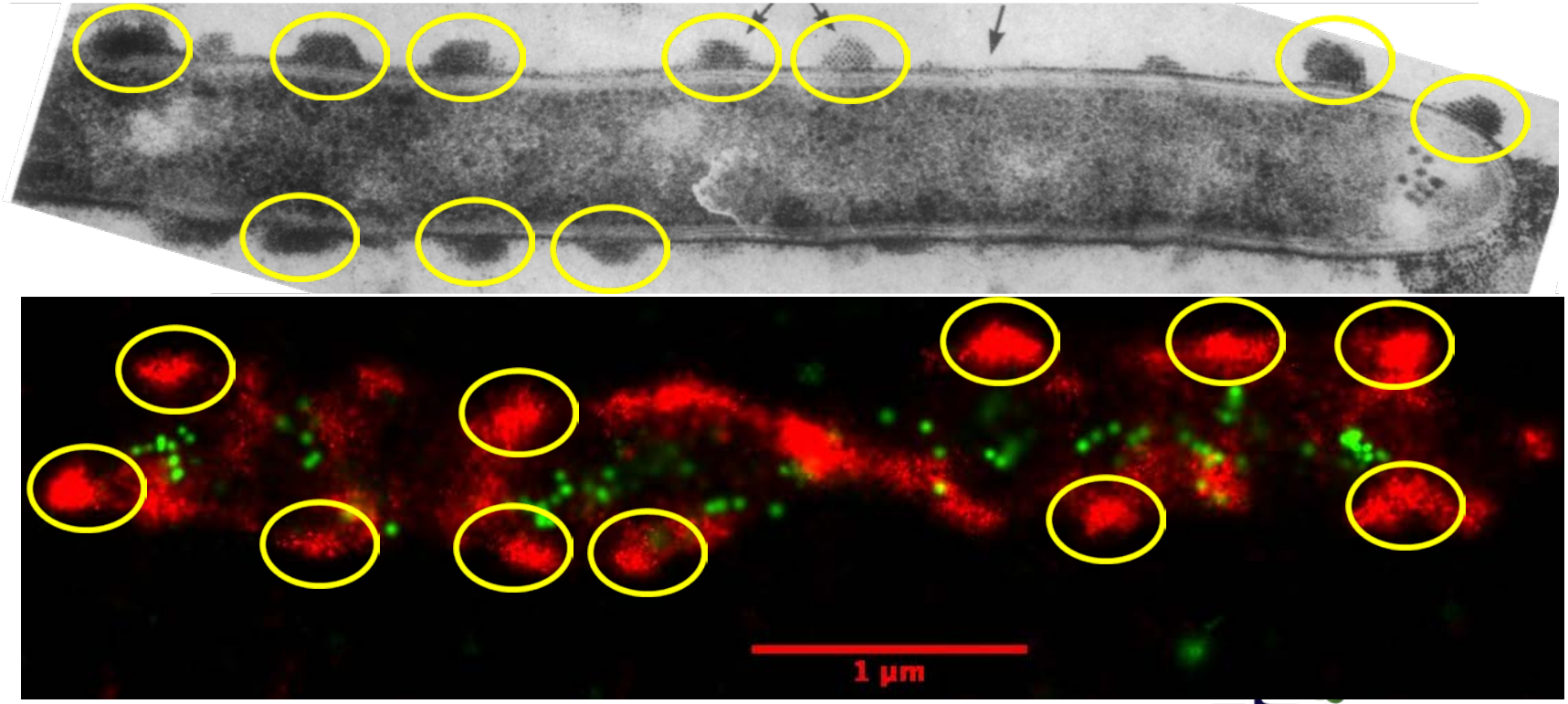
Polycellulosomal protuberance

- Identified back in 1986 by Ed Bayer et al and highlighted with yellow circles
  - These protuberances relationship to cellulosomes which varied in both size and longitudinally arrangement reveled through the use of antibodies were specific to cellulosomes
- bacterial cell within the optical images is decorated with fluorescence protuberances (yellow circles)
  - very similar in structure as the protuberance shown by Bayer et al



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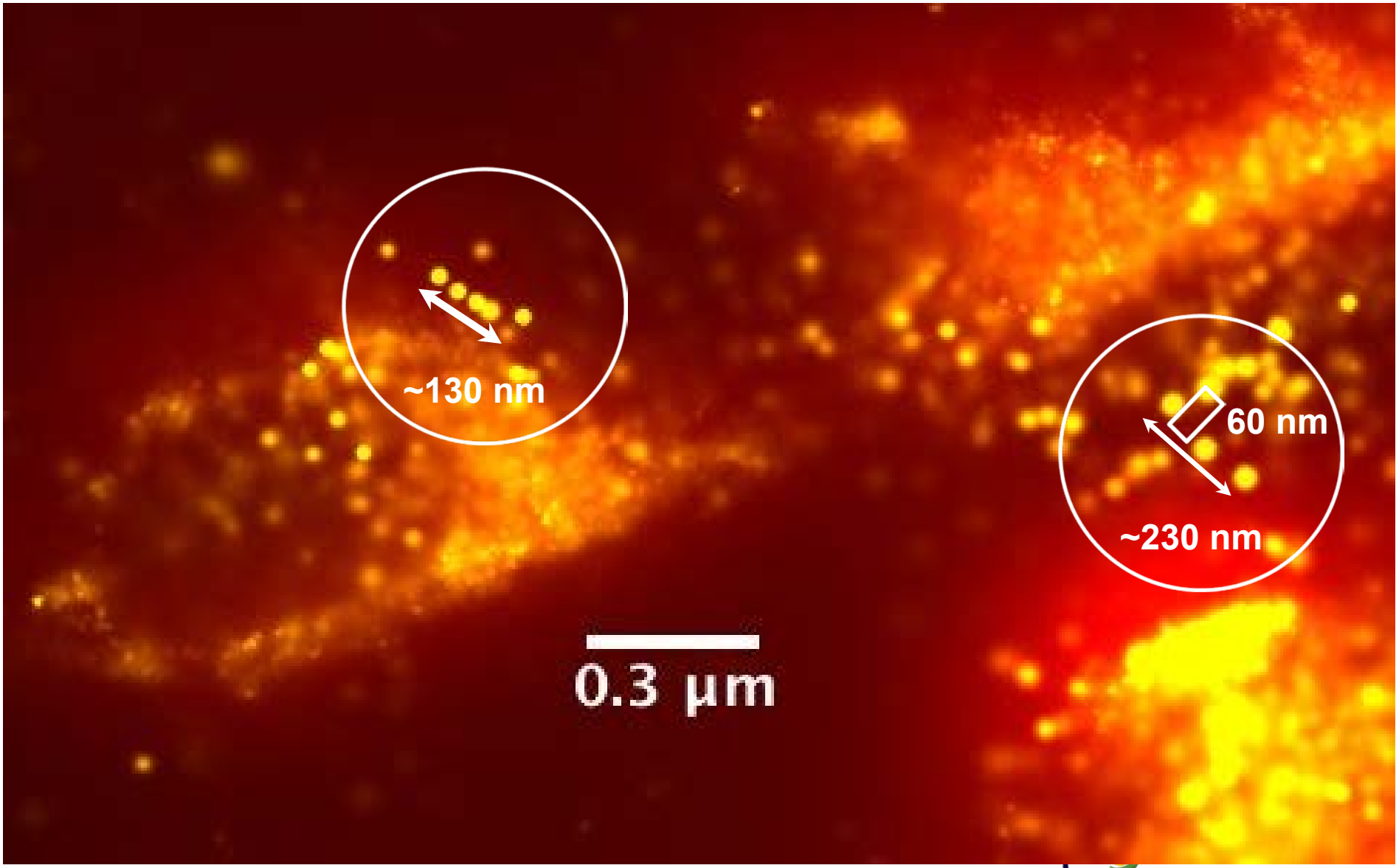
6 Ultrastructure of the Cell Surface Cellulosome of Clostridium thermocellum and Its Interaction with Cellulose,, Edward Bareyer and Raphaell Amed, Journal of Bacteriology, Sept. 1986, p. 828-836

Super high resolution imaging with *C. therm* grown on Avicel



Far-field and STORM fluorescence combined

Super high resolution imaging with *C. therm* grown on Cellobiose – Need for Quantification

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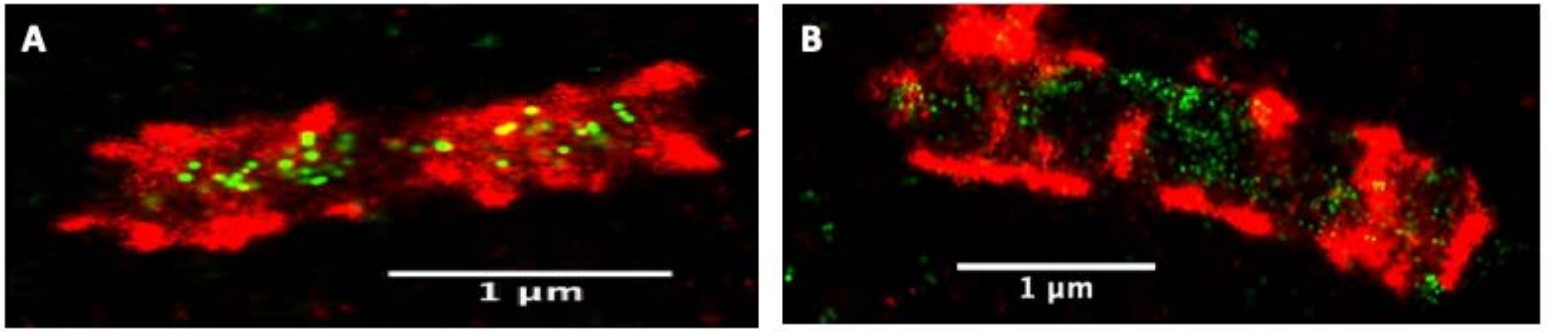
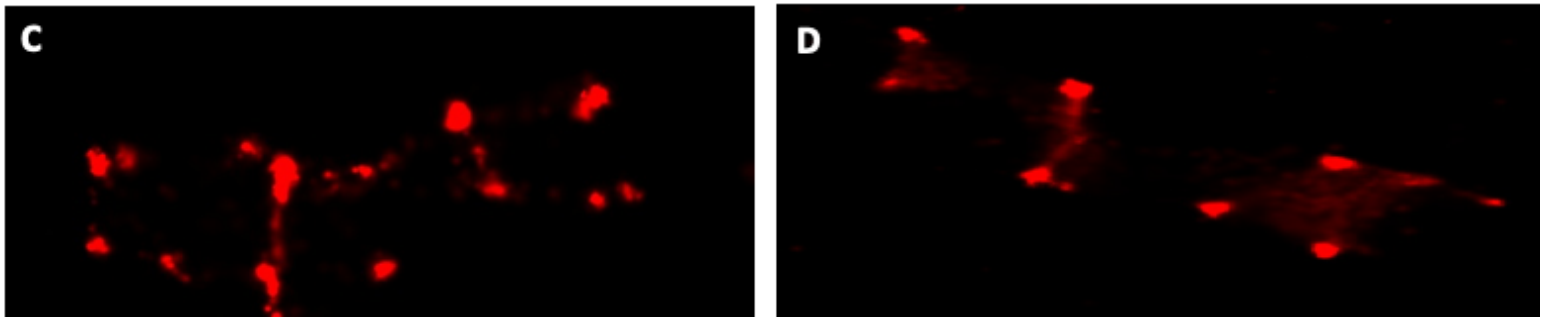
C. Thermocellum grown on cellobiose and captured in log phase



C. Thermocellum grown on cellobiose and captured in stationary phase



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Super high resolution imaging with *C. therm* grown on Avicel with multiple probes

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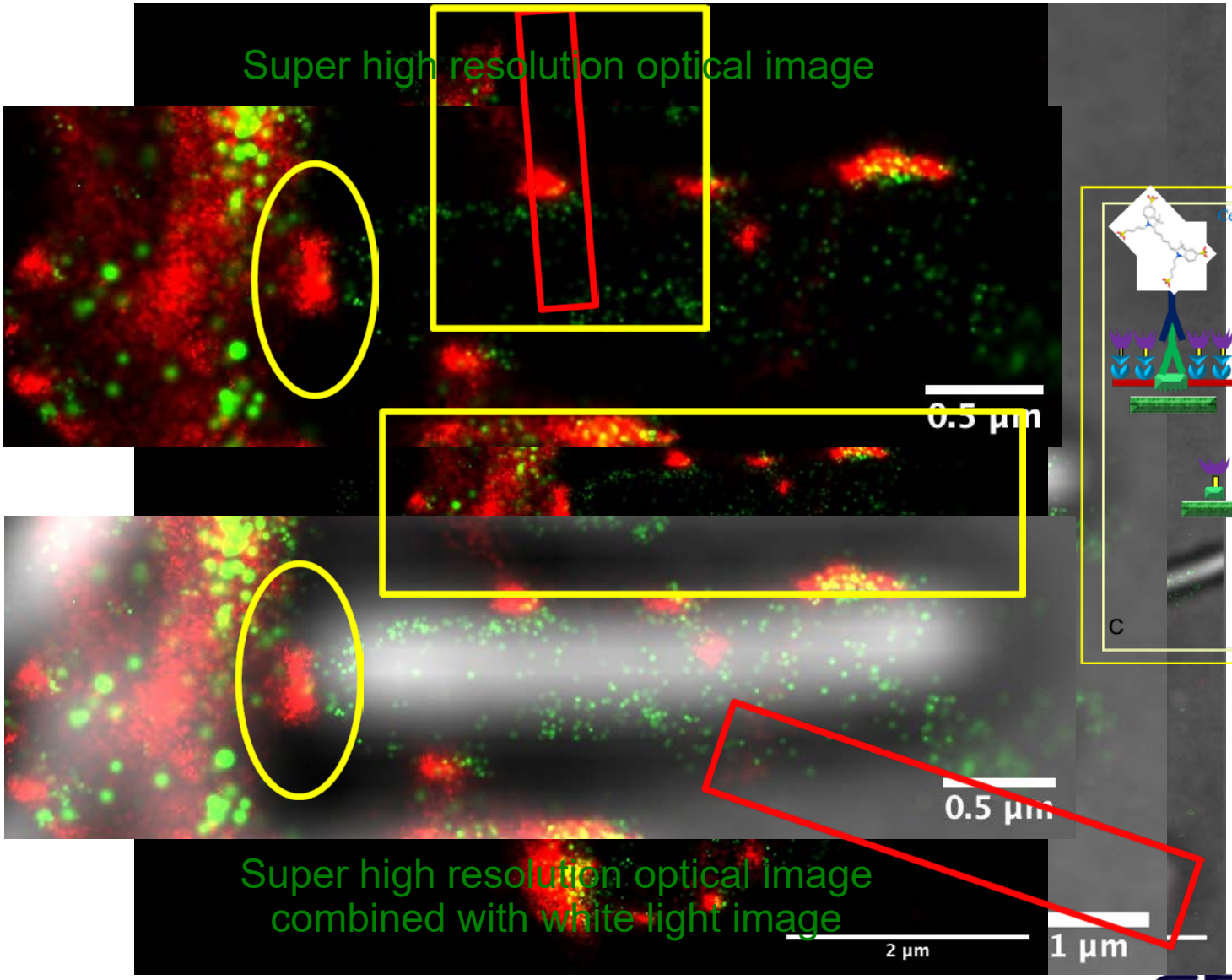

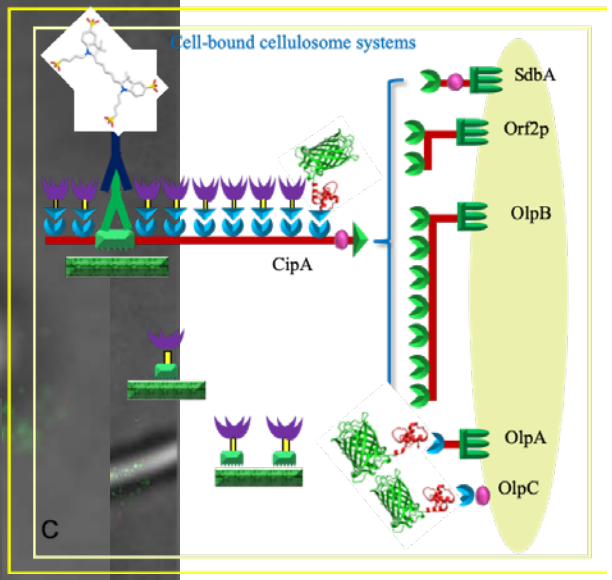

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Quantitative analysis of the distribution of cellulosomes: Density-based spatial clustering of applications with noise (DBSCAN)

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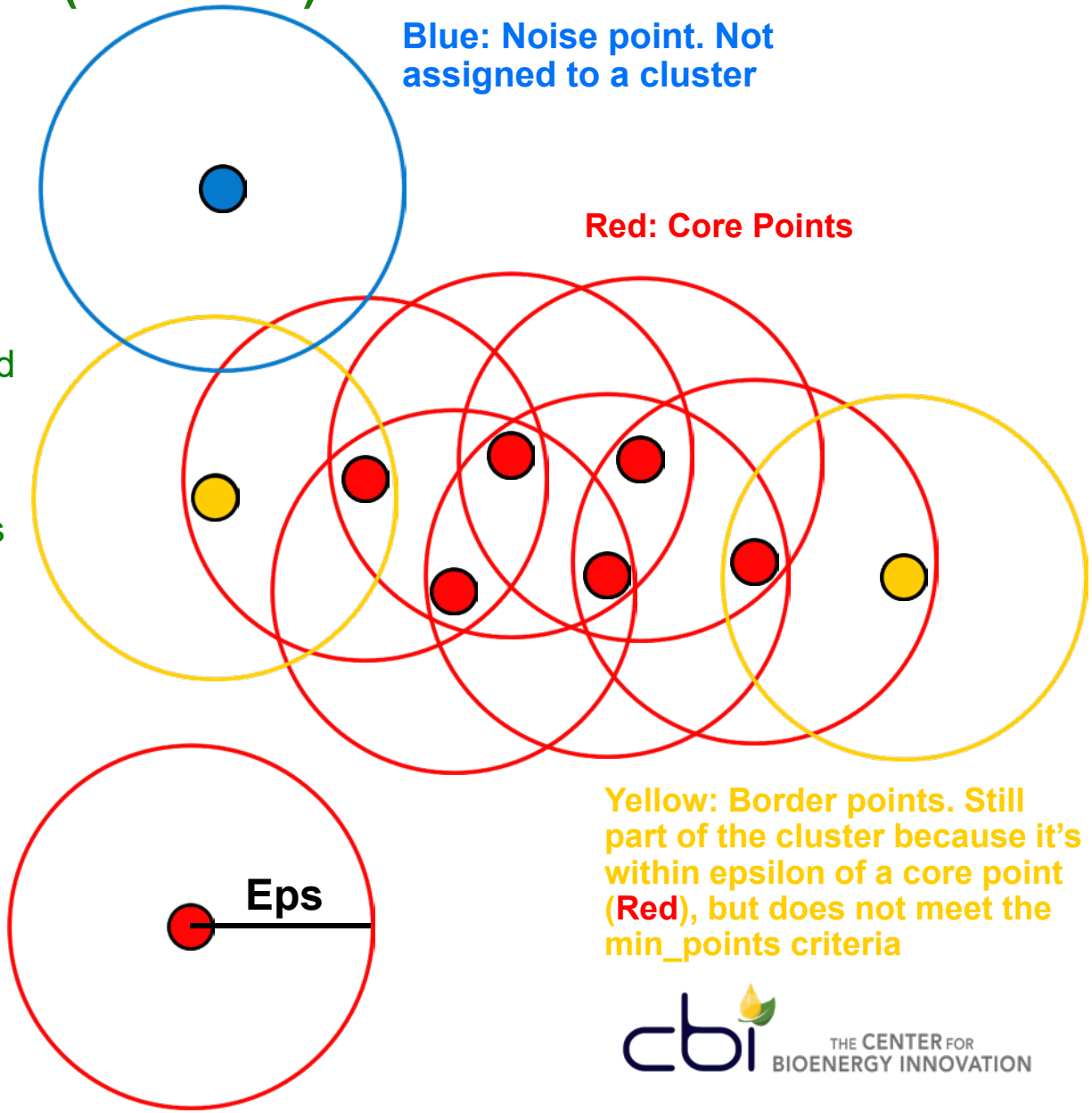
DBSCAN:

- unsupervised machine learning referred to as cluster analysis
- propagative cluster detection method
- linking points that are closely packed together
- detects outliers using user defined epsilon radius and minimum number of points
- detect arbitrary shaped clusters and is quick and robust to outliers
- User defined
  - Epsilon radius (Eps)
  - Minimum number of points, or in this case, the number of fluorescent molecules
- For this work
  - Eps set at 75nm (diameter is 150nm, approximate size of cellulosome
  - Minimum number of points varied from 10 to 1200



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DBSCAN analysis on C. therm grown on Avicel

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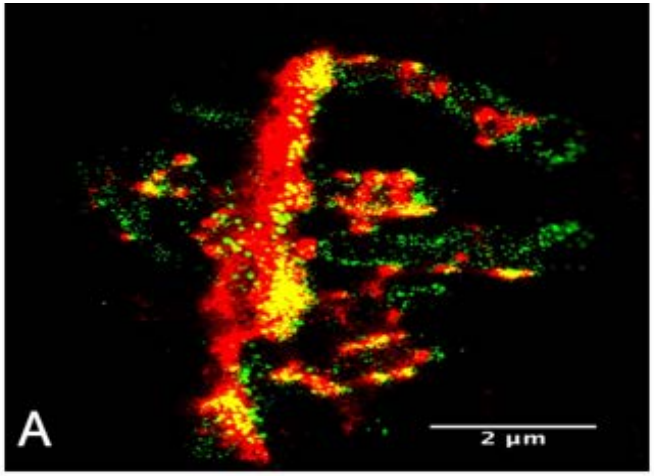
DBSCAN

- Raw data set contains x-y coordinates of each fluorescent event
  - Have the ability to separate channels (cbm3 and GFP)
  - This data can be used with the DBSCAN algorithms



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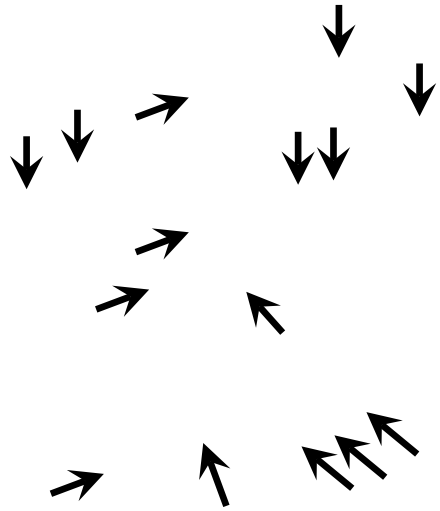
DBSCAN analysis on C. therm grown on Avicel

DBSCAN initial results on individual Avicel particle

- 49 clusters with identified with having at least 500 molecuels/epsilon (75nm radius)
- 27 clusters of 500 on bacteria cell wall for cellulosome
- 22 clusters are located on the Avicel



Observations

- Increased number of molecules and larger cluster size at the interaction zone (**CBM3**)
- Larger Clusters size at the interaction zone with the with **GFP**

DBSCAN analysis on *C. therm* grown on Cellobise

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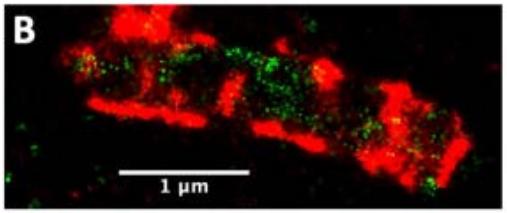
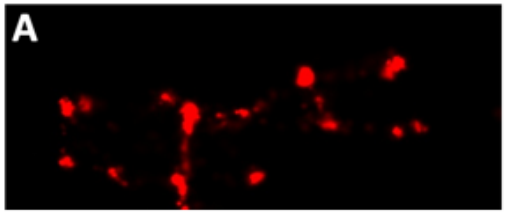
C. thermocellum in Log phase on cellbiose



C. thermocellum in stationary phase on cellbiose



DBSCAN analysis on *C. therm* grown on Cellobise: Averaging through the individual cells

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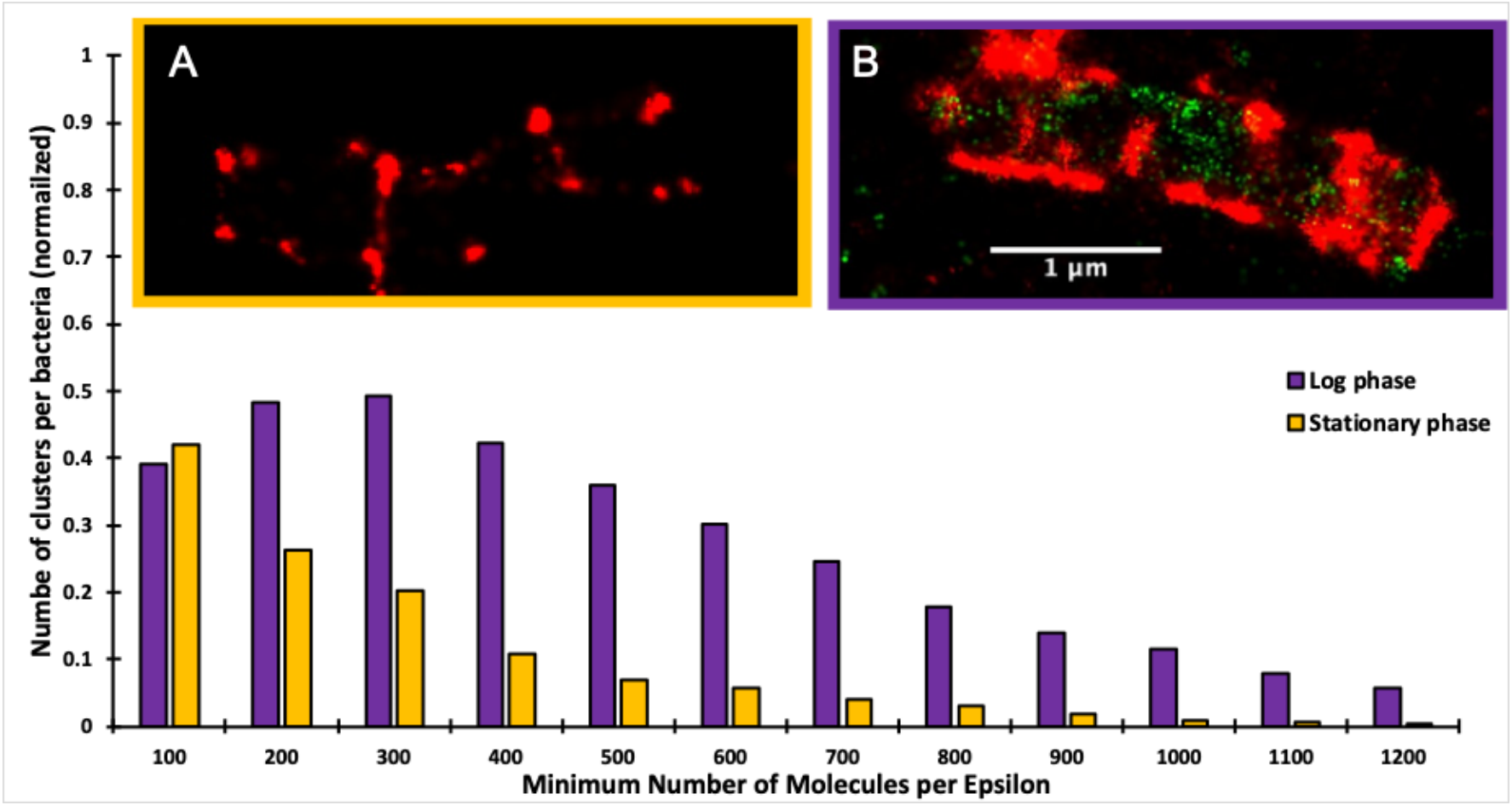
DBSCAN initial results on individual Cellobiose

- 65 individual bacteria cells analyzed in log phase grown on cellobiose
- 70 individual bacteria cells analyzed in stationary phase grown on cellobiose
- All DBSCAN results for each bacteria cell are normalized and the average of the normalized number of clusters are plotted



DBSCAN analysis on *C. therm* grown on Avicel: Averaging through the individual cells and Avicel particles

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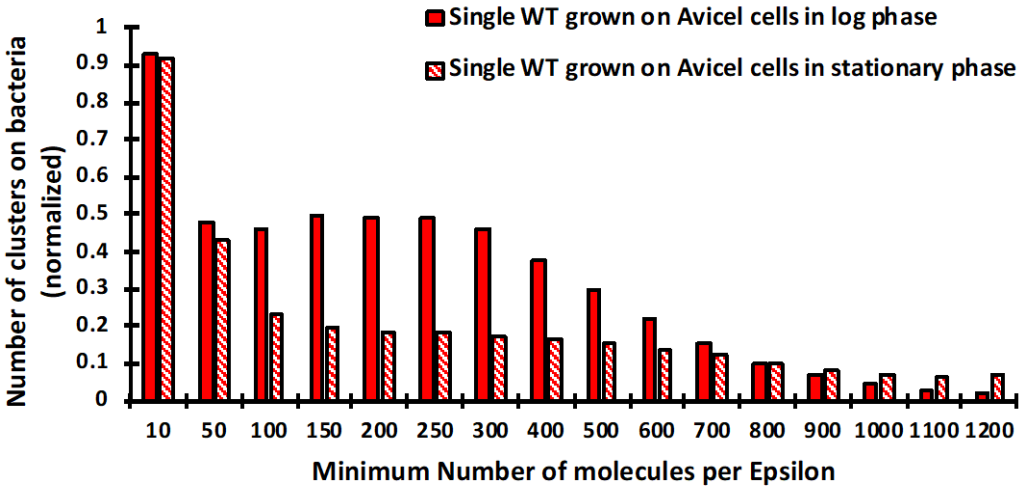
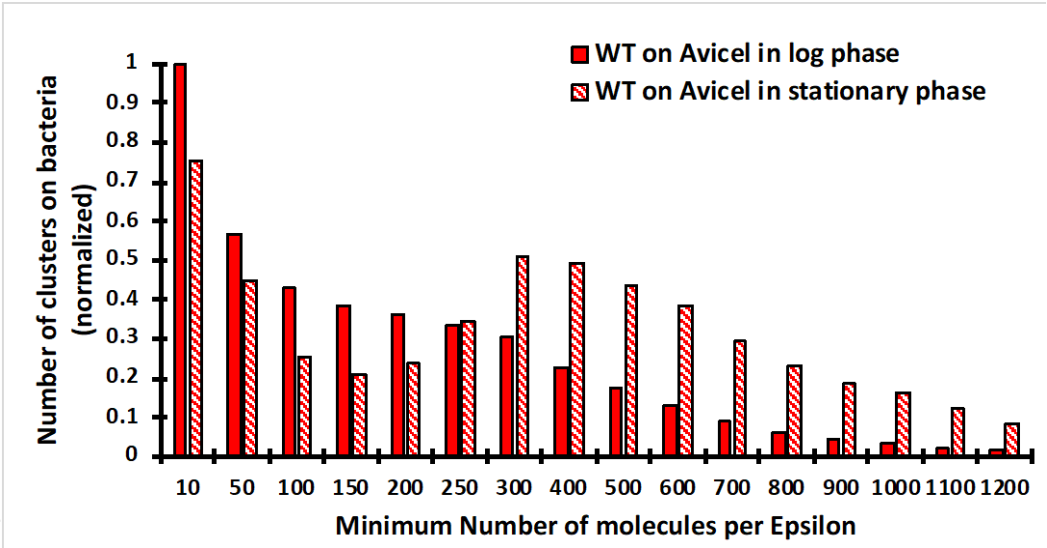
DBSCAN results on individual bacteria cells grown in the presence of Avicel

- 73 individual bacteria cells analyzed in log phase grown on Avicel
- 62 individual bacteria cells analyzed in stationary phase grown on Avicel
- All DBSCAN results for each bacteria cell are normalized and the average of the normalized number of clusters are plotted





DBSCAN results Avicel particles with bacteria attached

- 6 individual Avicel particles analyzed in log phase grown
- 8 individual Avicel particles analyzed in stationary phase grown
- All DBSCAN results for each Avicel particle are normalized and the average of the normalized number of clusters are plotted

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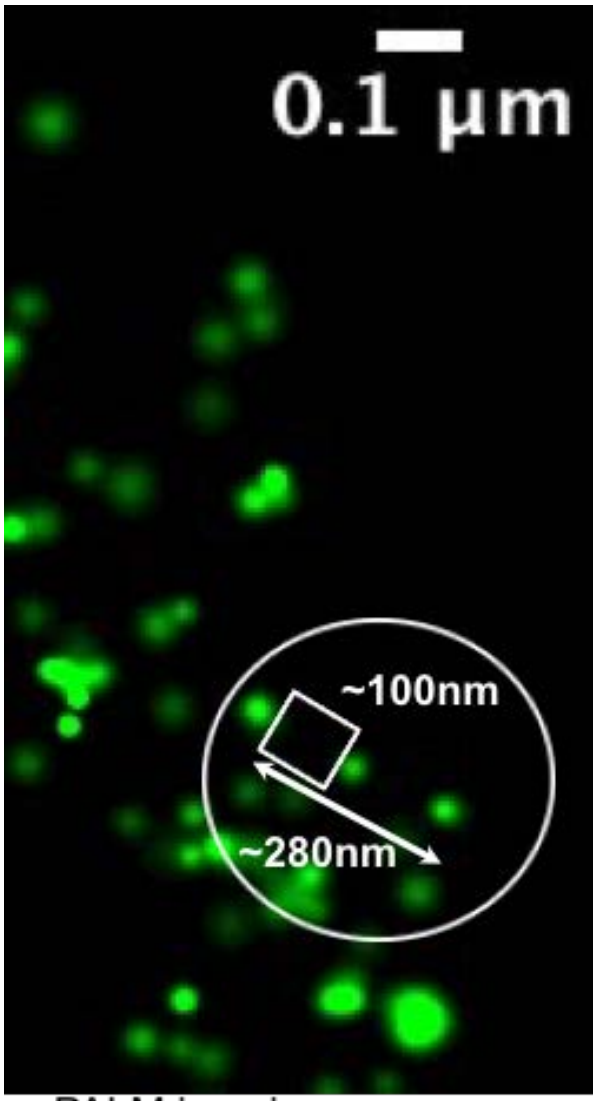
Conclusions for C. thermocellum and Avicel



PALM imaging

- Utilizing PALM and STORM super high resolution imaging enables us to get optical resolution down to <40nm

- Can be used to study biomass deconstruction by *C. thermocellum* and finally understand the enzyme-microbe-substrate interaction region, cellulosome formation, and the evolution of cellulosomes during growth.

- Polycellulosomal protuberance distribution CBM3
  - Wild Type in Log Phase
    - Clusters with larger minimum number of molecules per epsilon
  - While Type in Stationary Phase
    - Clusters with smaller minimum number of molecules per epsilon
  - Clear difference in how the bacteria cells look during different growth phases
  - Higher clustering of cellulosomes on Avicel as the cells progress from log phase to stationary phase
    - Suggest the bacteria is shuttling the cellulosomes onto the surface of Avicel
    - Significant increase in the number of cellulosomes at the interaction zone – increases the anchoring of the bacteria to the surface of Avicel

Thank you

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Questions