

Understanding chitosan-induced flocculation mechanism in *C. vulgaris* using atomic force microscopy (AFM)

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Large-scale harvesting of microalgae is a challenge



Microalgae grow at low concentration
(0.3-3 g/L)

Harvesting techniques

- ultracentrifugation
 - filtration
 - flocculation

Require high energy input + cell damaged!
Important costs (1/3 of total production cost)

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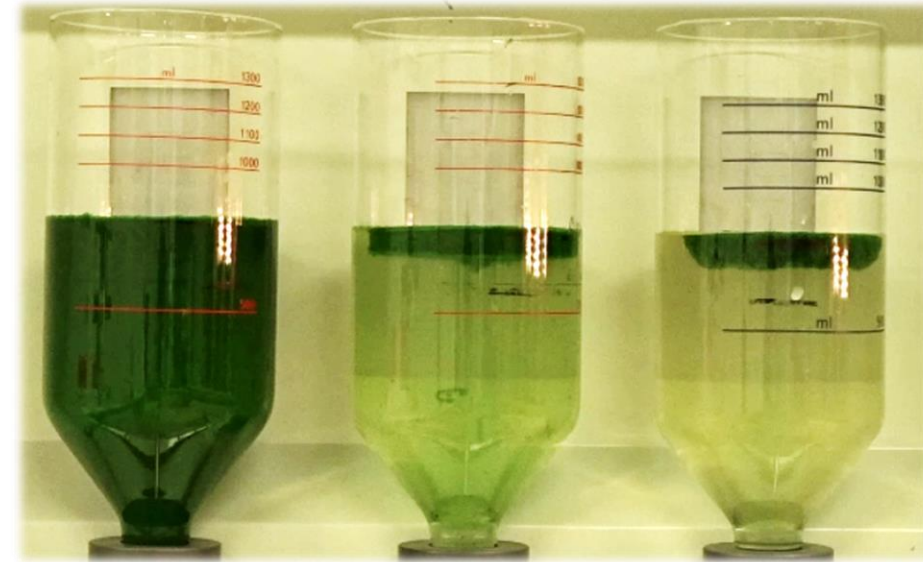
Harvesting techniques

- **ultracentrifugation**
 - **filtration**
 - **flocculation**

Require high energy input + cell damaged!
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FLOTATION

Cell capture by rising air-bubble in a microalgal suspension



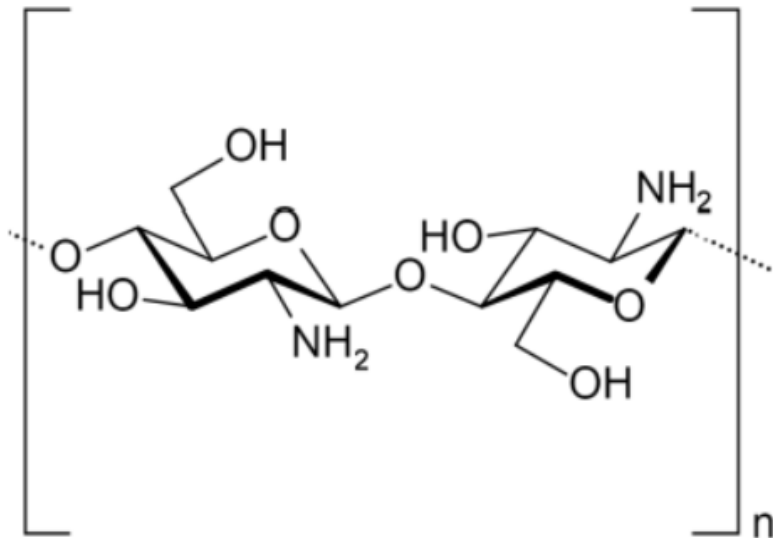
- ▶ Cheap and non-damaging technique
- ▶ Challenging with microalgae, often need further application or pretreatment

FLOCCULATION

**Use of chemical or bio-flocculants to form large flocs easily
captured by the bubble**

Identification of a natural flocculant for *C. vulgaris* → chitosan

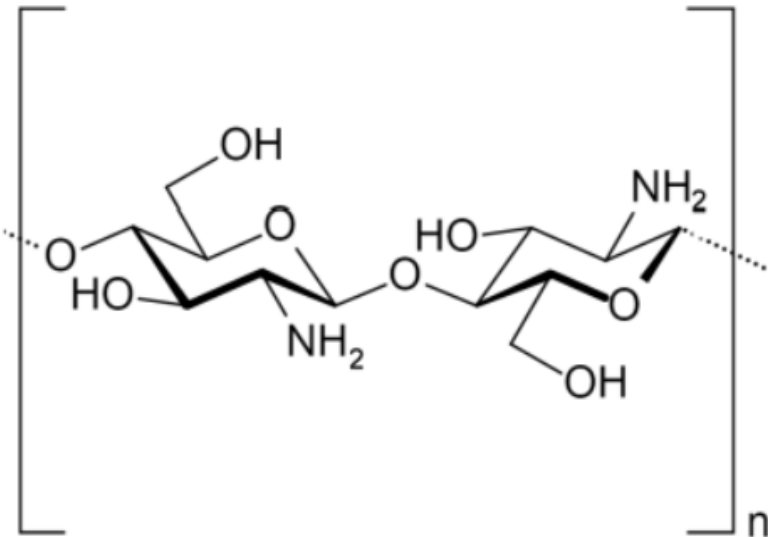
Chitosan



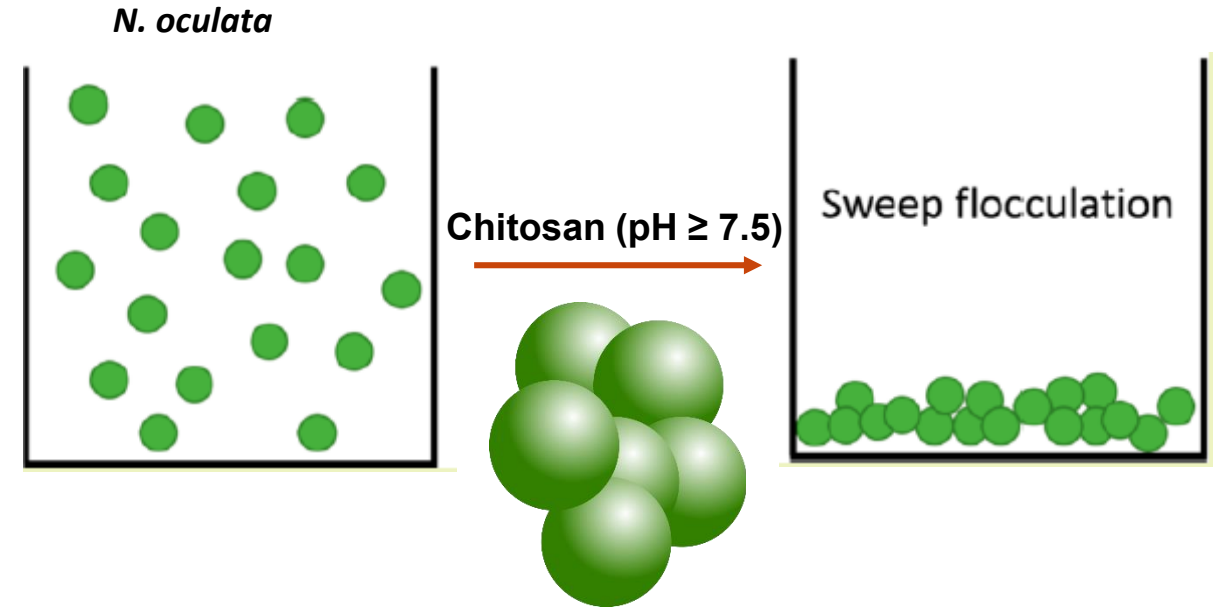
- ▶ Chitosan, a natural molecule, is able to flocculate the cells at low pH
- ▶ Positively charged at pH lower its pKa (6.5).

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Chitosan

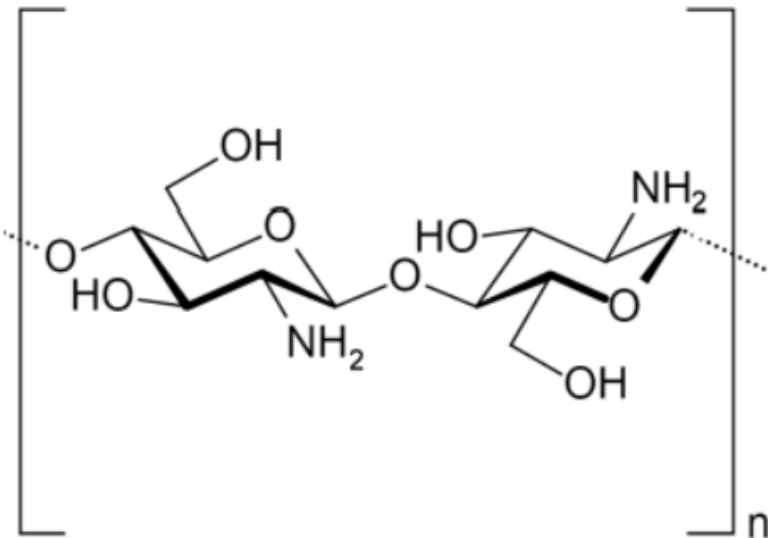


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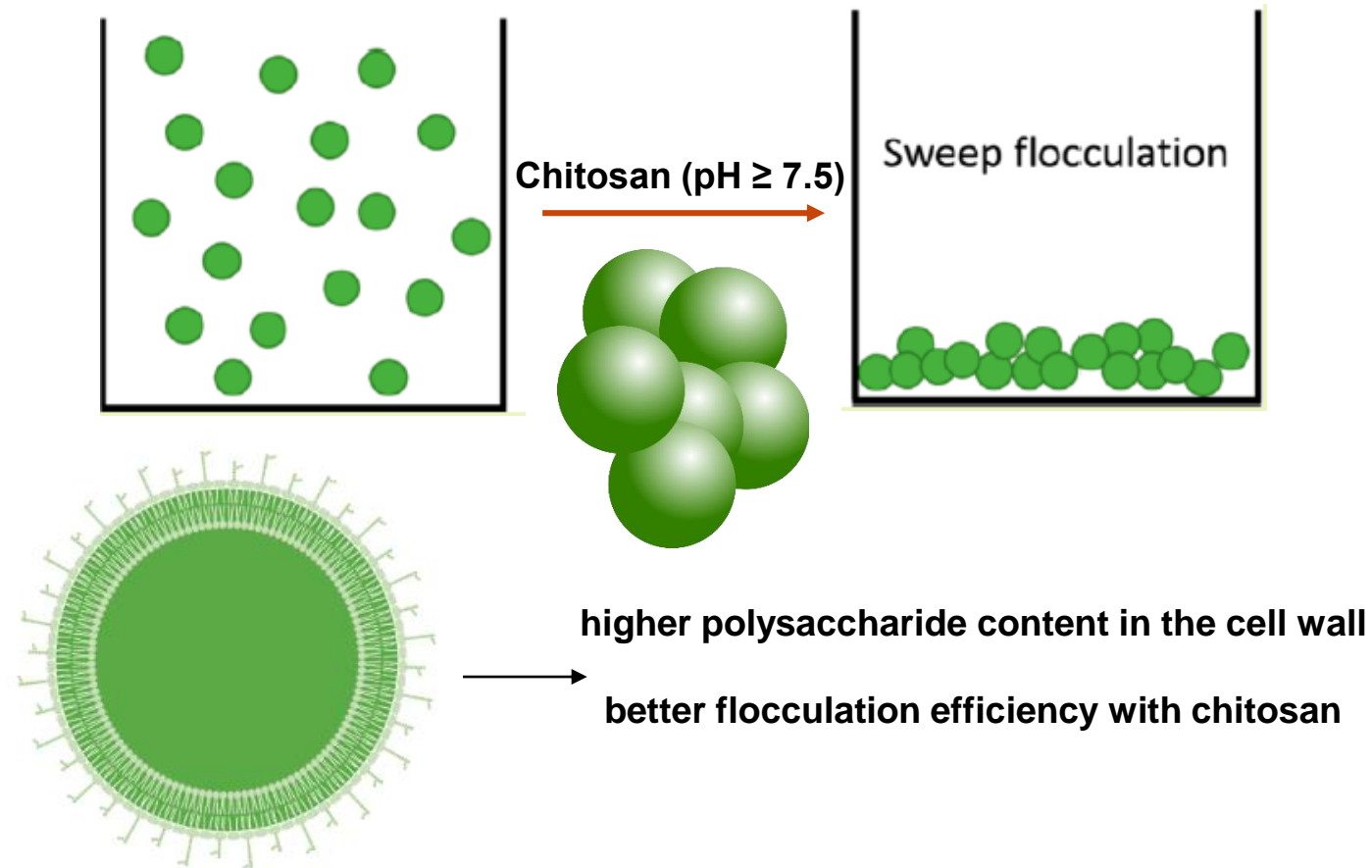
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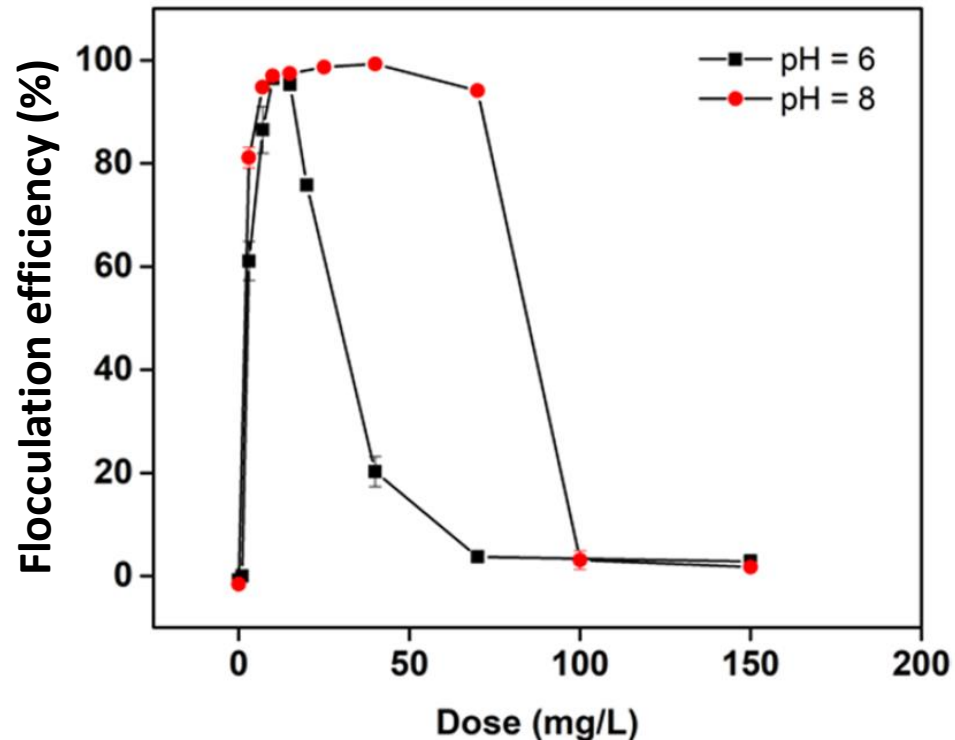
N. oculata



Suggesting that the flocculation mechanism may rely on different interactions between chitosan and cells than only electrostatic interactions

Bio-Flocculation of *C. vulgaris* with Chitosan and CNCs

Flocculation experiments



Chitosan flocculate the cells at both pH 6 and 8

Macroscopic observations

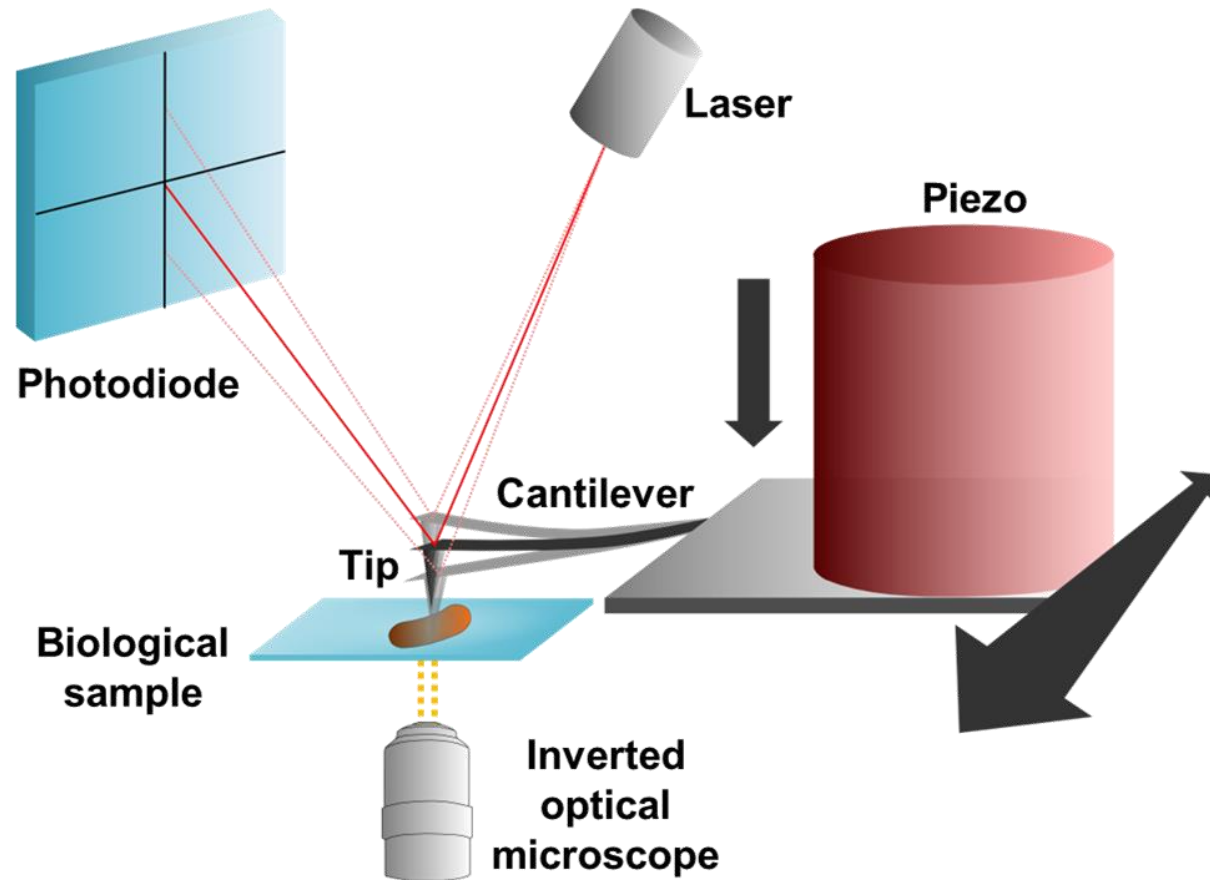
Identification of a natural flocculant for *C. vulgaris* → chitosan



Macroscopic Observations Show Chitosan Does Not flocculate the cells at pH 8

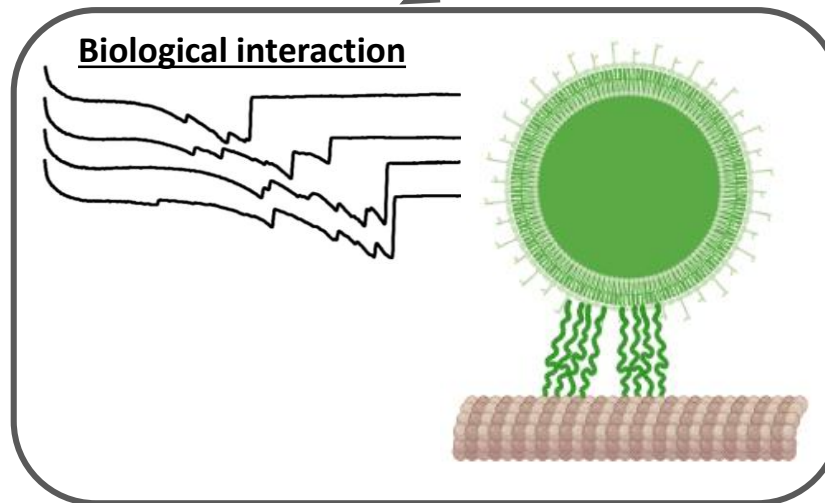
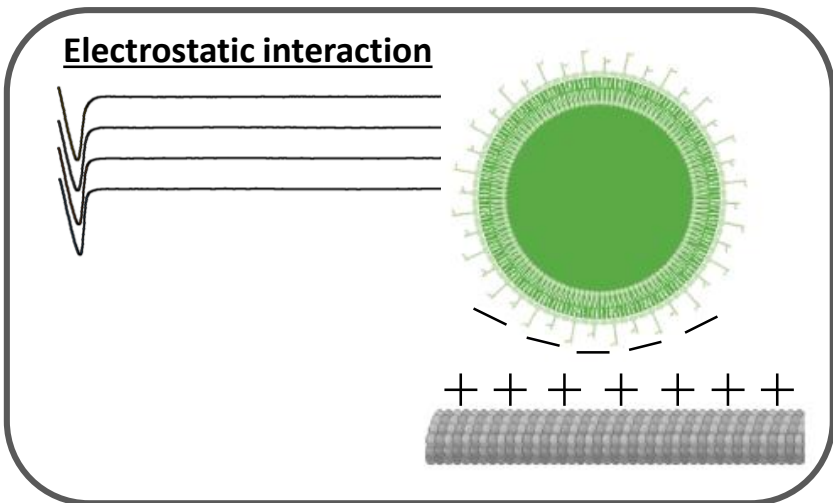
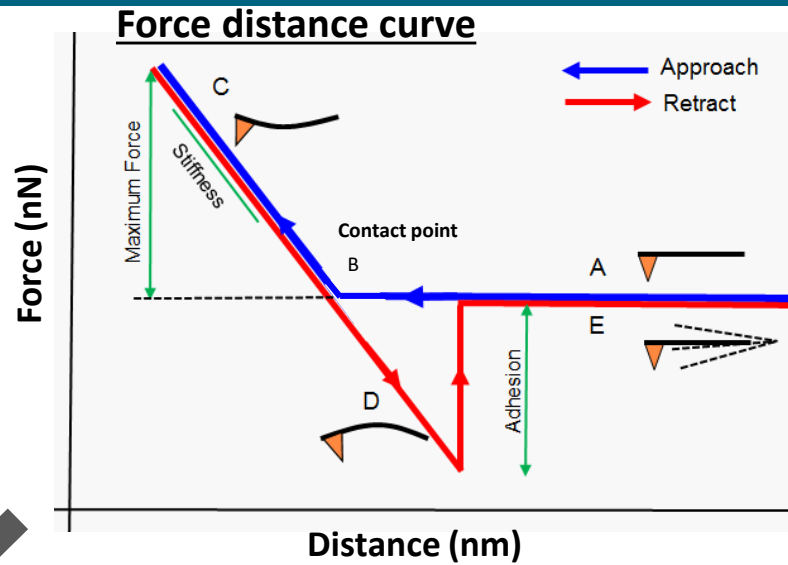
Then, what is the mechanism involved?

Atomic Force Microscopy (AFM) to identify the flocculation mechanism at play

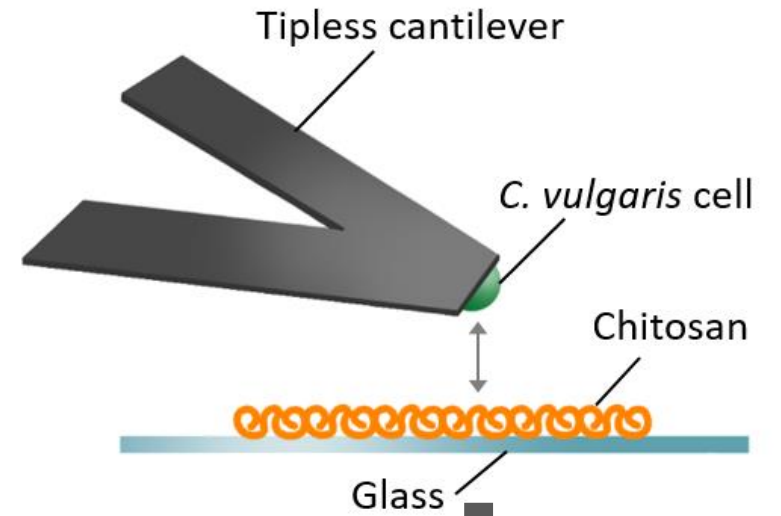
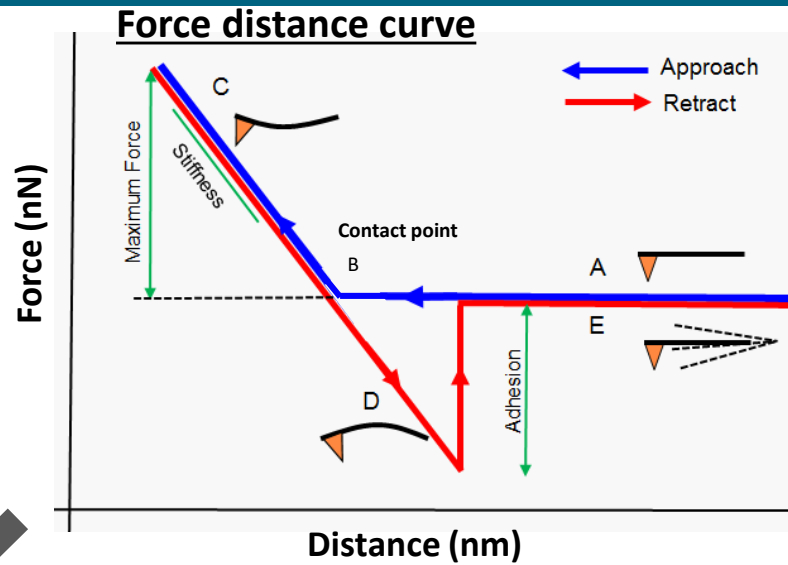


- Force measurements to access adhesive properties
 - Operates in liquid
 - High-resolution imaging
- Roughness and Nano-mechanical analysis
 - AFM tip functionalization with chemicals, biological molecules, inert particles, living cells

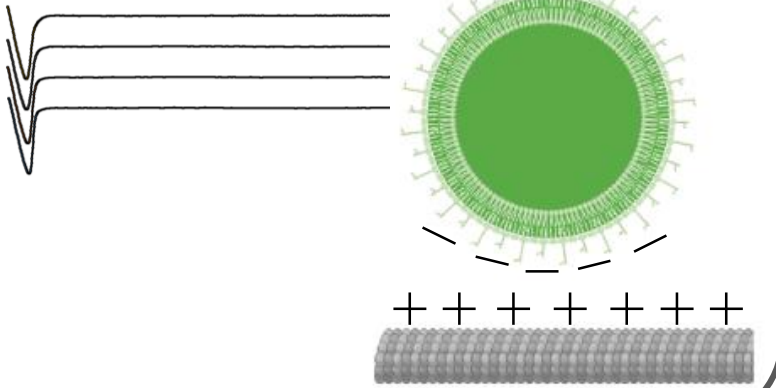
AFM to probe cell surface adhesion



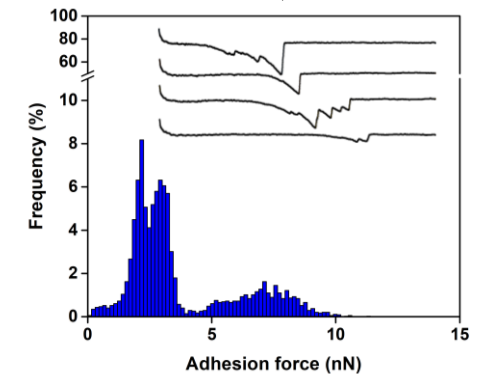
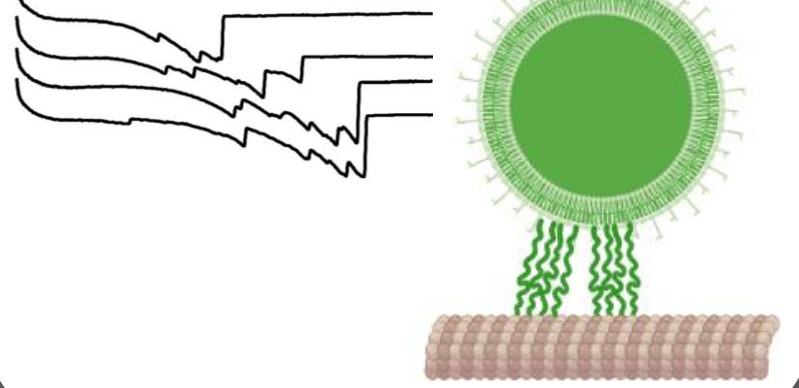
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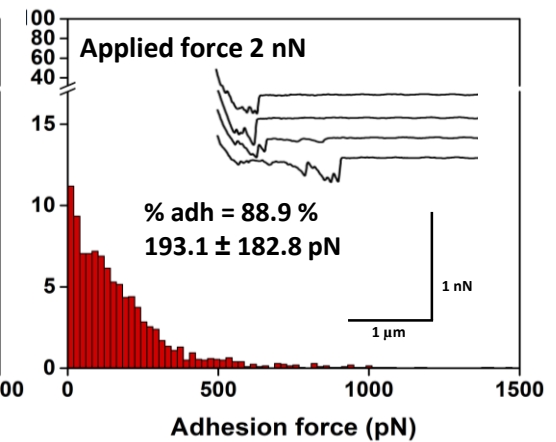
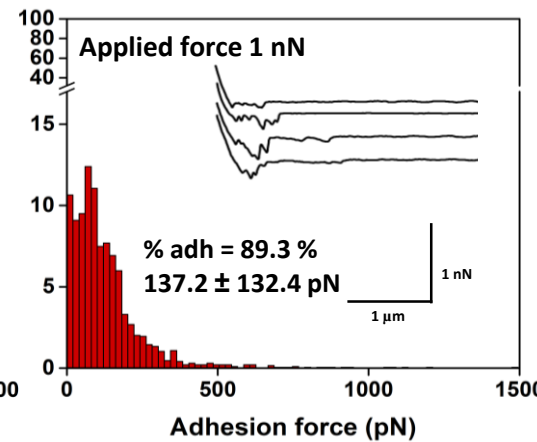
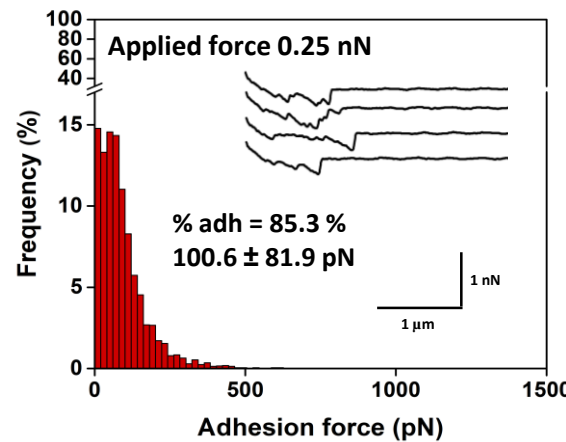
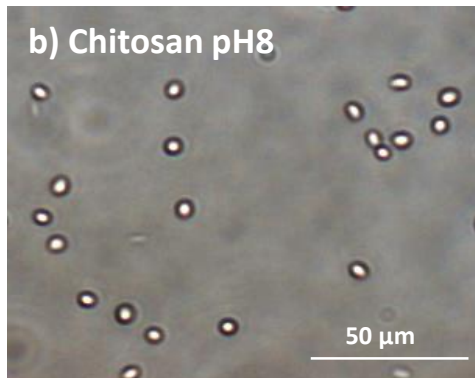
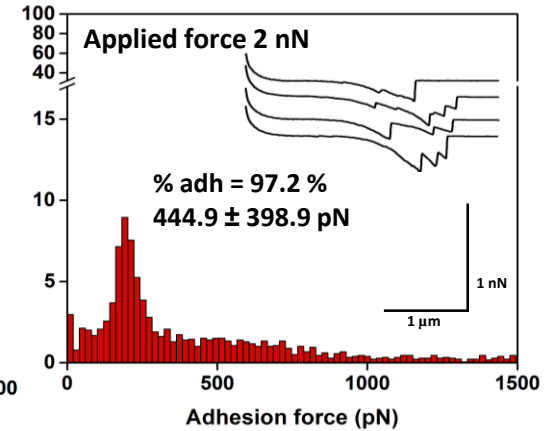
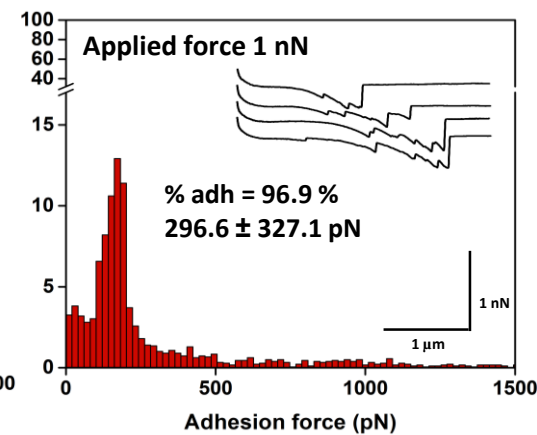
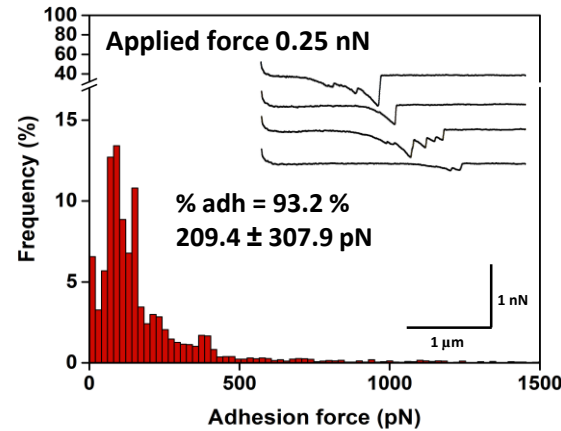
Electrostatic interaction



Biological interaction

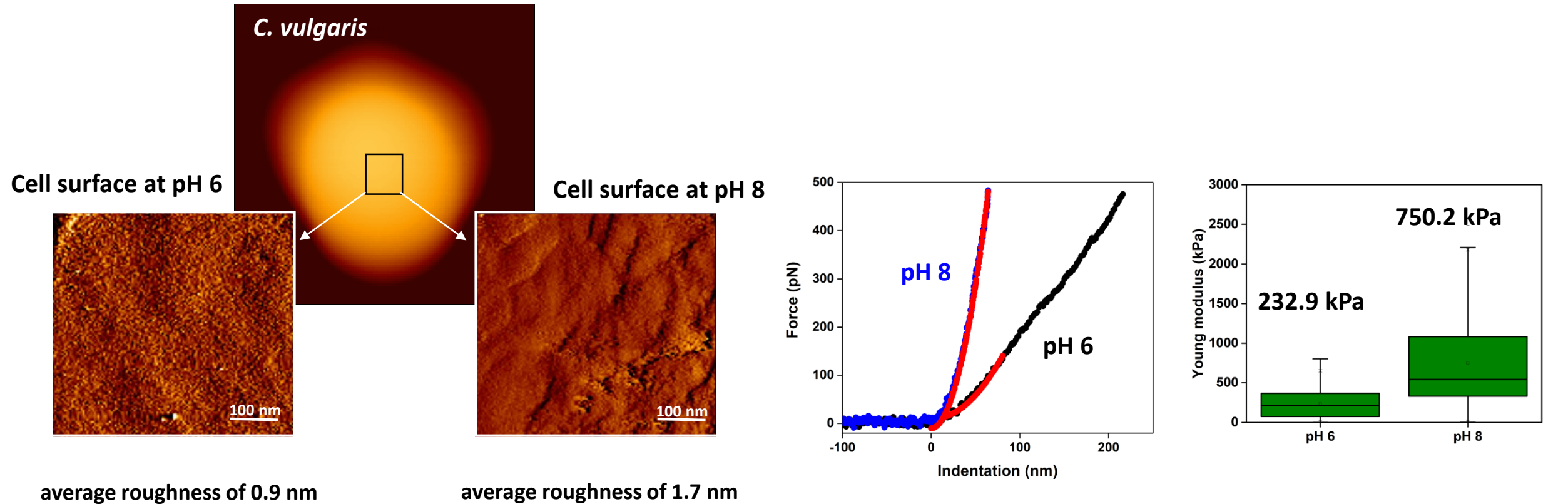


Chitosan is interact specifically with polymers present at the surface of the cells



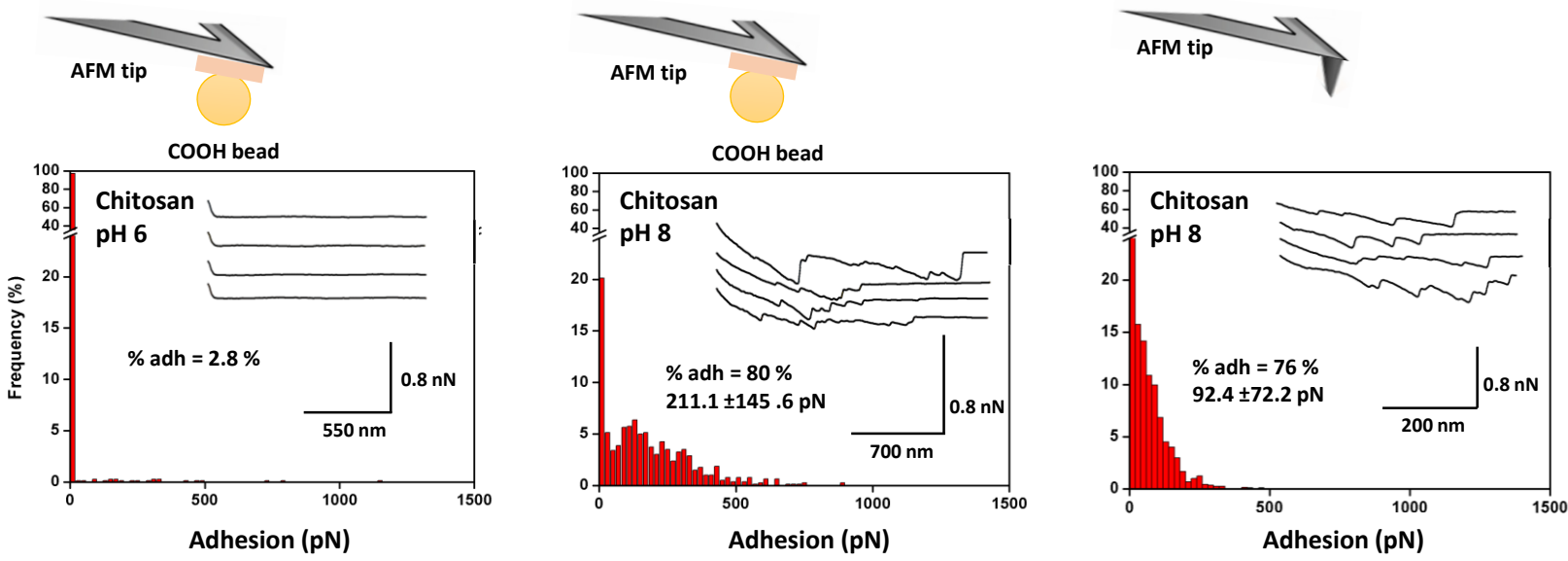
AFM Nanoscale Experiments Reveal the Role of Biological Interactions
between *C. vulgaris* and Chitosan during Flocculation

Cell wall properties change based on pH

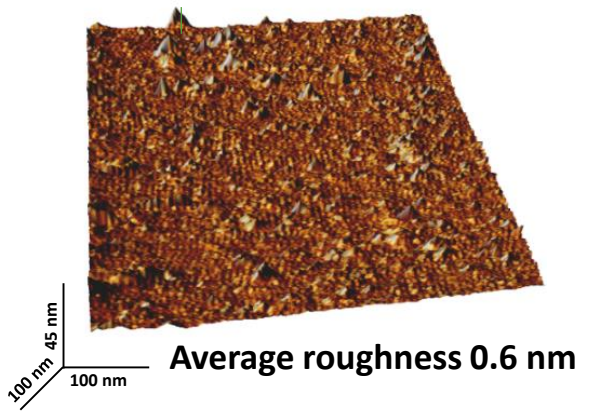


Biophysical analysis of the cell wall of *C. vulgaris* indicates that a pH increase from 6 to 8 clearly affects its rigidity and its roughness

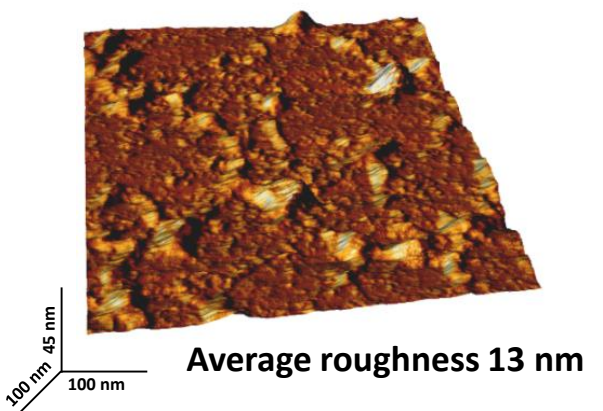
Chitosan Induced-Flocculation of *C. vulgaris* at Higher pH Does Not Occur through the Same Mechanism



a) Chitosan surface at pH 6

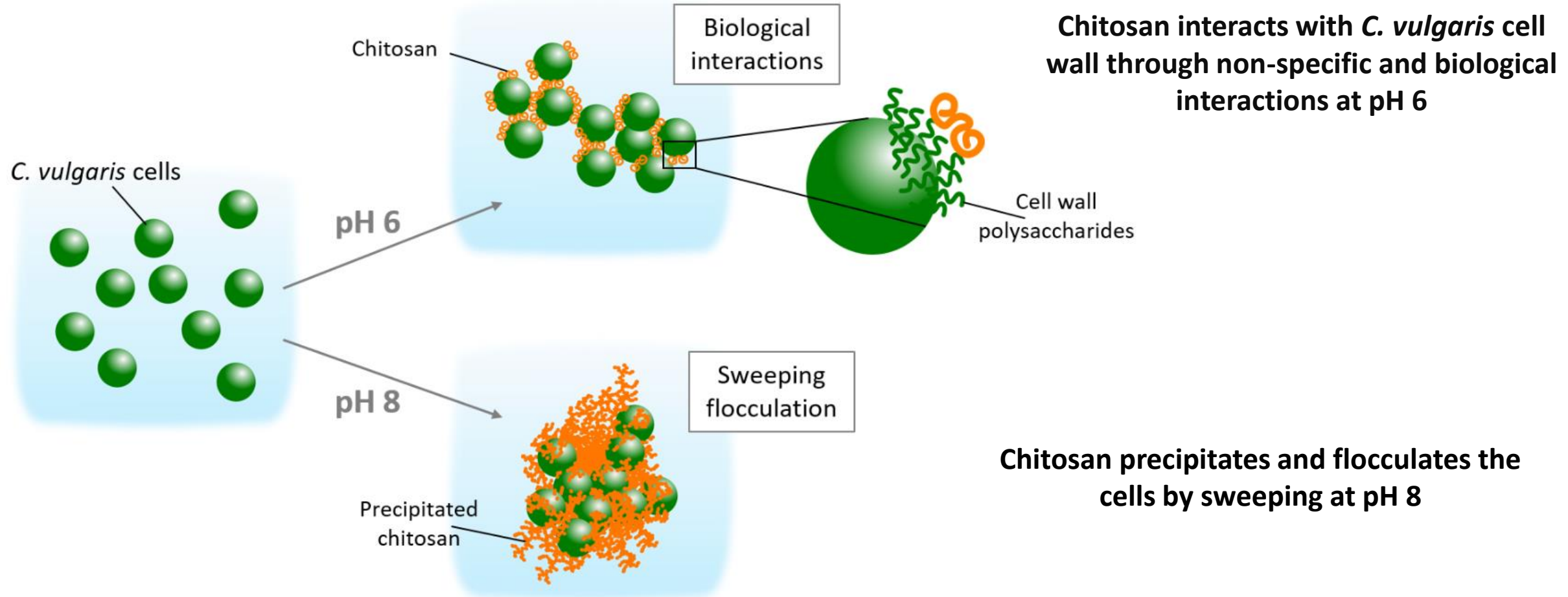


b) Chitosan surface at pH 8



pH has an important effect on the structure of chitosan, which precipitates and gets detached from the surface at pH 8

Our results enlighten the chitosan induced flocculation mechanism



Understand flocculation mechanism contributing general usage of chitosan to harvest microalgae

Funding



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