LILACs: Looking Inside Living Algal Cell Walls

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Why study Ulva species?

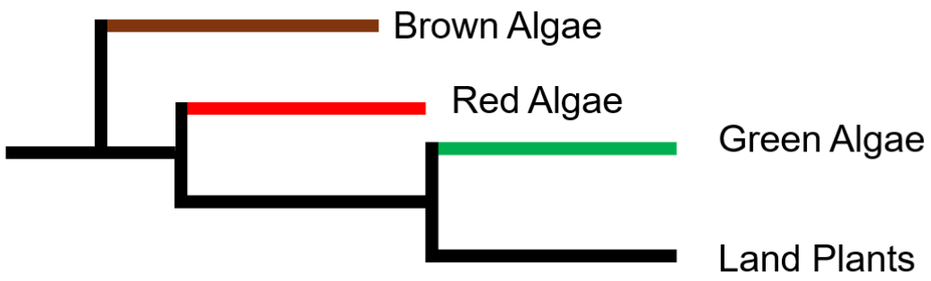
- *Ulva compressa* has already had had its genome sequenced so is an ideal model macroalgal system (1)

- Green tides, the rapid growth of unattached green macroalgae, commonly consist of ulva species
  - They lead to substantial environmental issues
  - An increasing issue in eutrophic marine environments
  - Full causes not understood (2)

Figure 1: Green tide in the lagoon Ria Formosa (Portugal) (1)

- Cell walls are particularly of interest as they have had to evolve to withstand the extreme environmental changes seaweed undergoes

- **They are evolutionarily distinct from land plants**



Current model of *Ulva sp.* cell wall structure

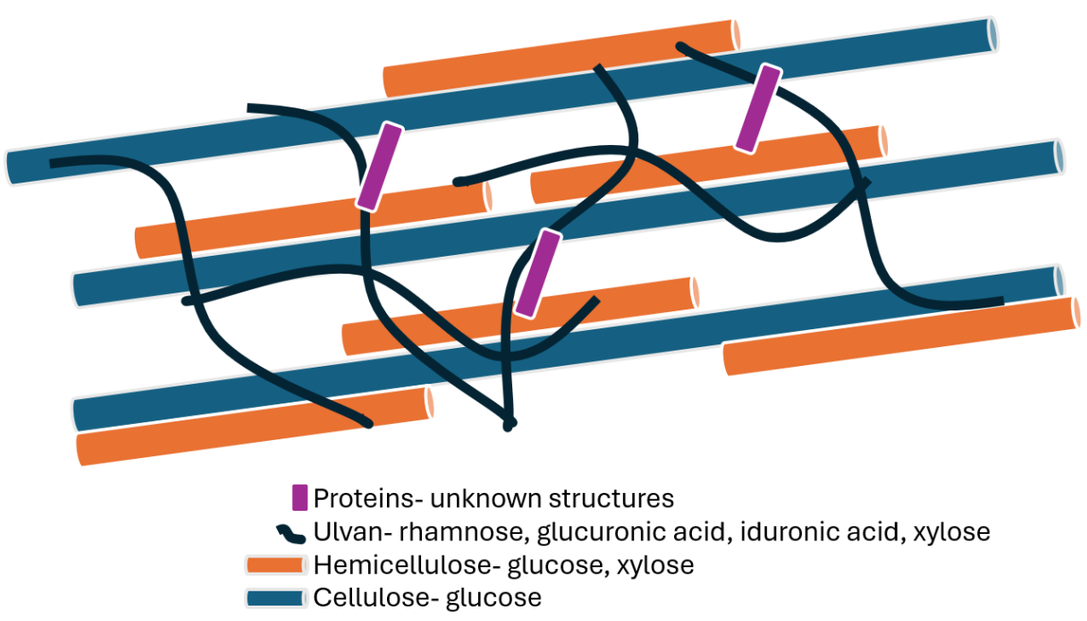


Figure 2: Simplified diagram of cell wall structure

- **We aim to study the properties of the cell wall components in live cells as well as through extraction**

Extraction of cell wall proteins

- 9 to 26% (dry weight) of the green algal cell wall is protein
- We are interested in how they influence cell wall structural properties and developing specific labelling methods
- Extraction protocol adapted from (3)

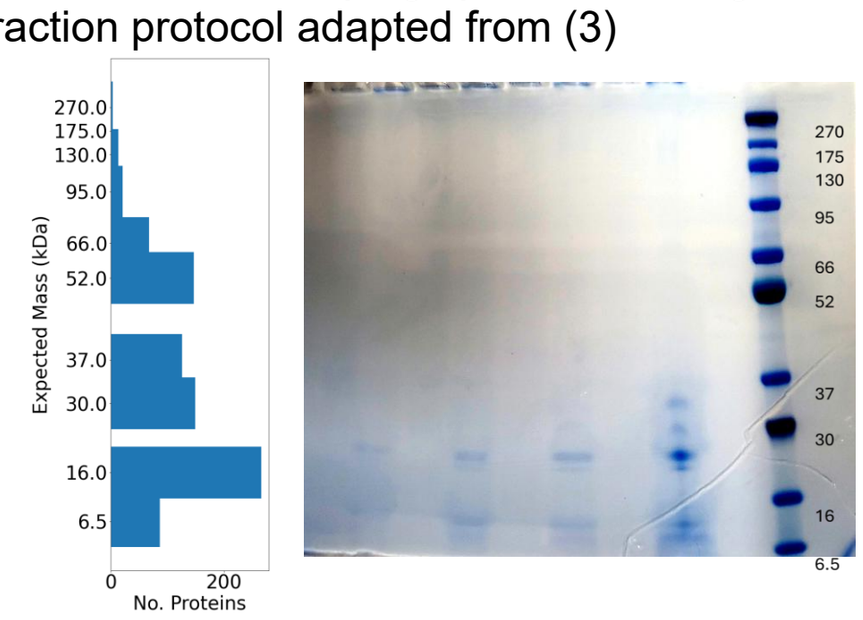


Figure 3: SDS-PAGE Gel showing proteins extracted from $CaCl_2$ wash and predicted masses of extracellular proteins from genome (1)

Challenges:
- Separating the cell wall proteins from other proteins
- Keeping the proteins in solution
- Removing carbohydrate contamination

Fluorescence microscopy of cell walls

1) Nonspecific binding of AF488-DCBO

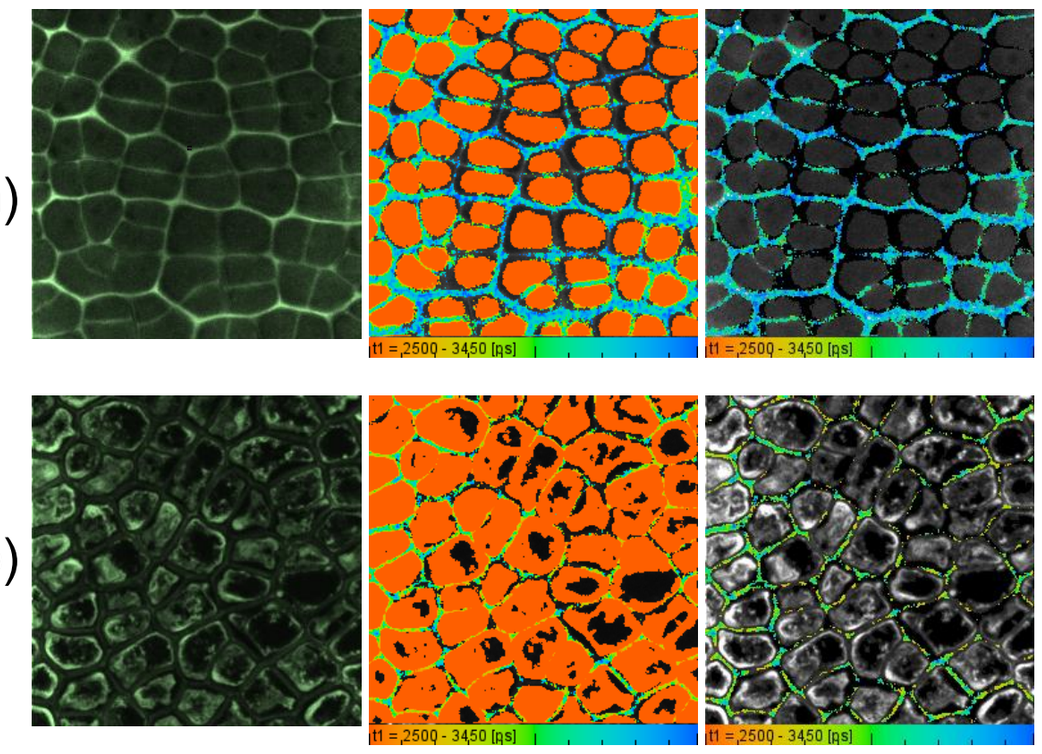


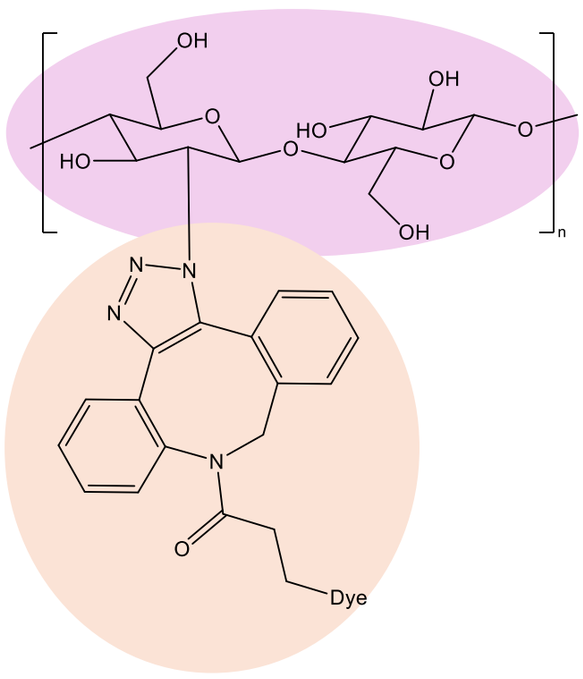
Figure 4: Fluorescence intensity (left) and lifetime (middle and right) representation of lead like seaweed stained with AF488-DCBO a) before and b) after heating to 46°C for 7 minutes

- DCBO allows the dye to access the cell wall
- See that after heating dye begins to penetrate the cell showing cell wall becoming more permeable

Pros:
- Minimal damage to cells so able to study effect of environmental changes

Challenges:
- Understanding the binding mechanism

2) Modified glucose labelling, adapted from (4)



Incubate sample with modified sugar for a few day

↓

Wash away free sugar

↓

Incubate with the DCBO-Dye

↓

Wash with methanol

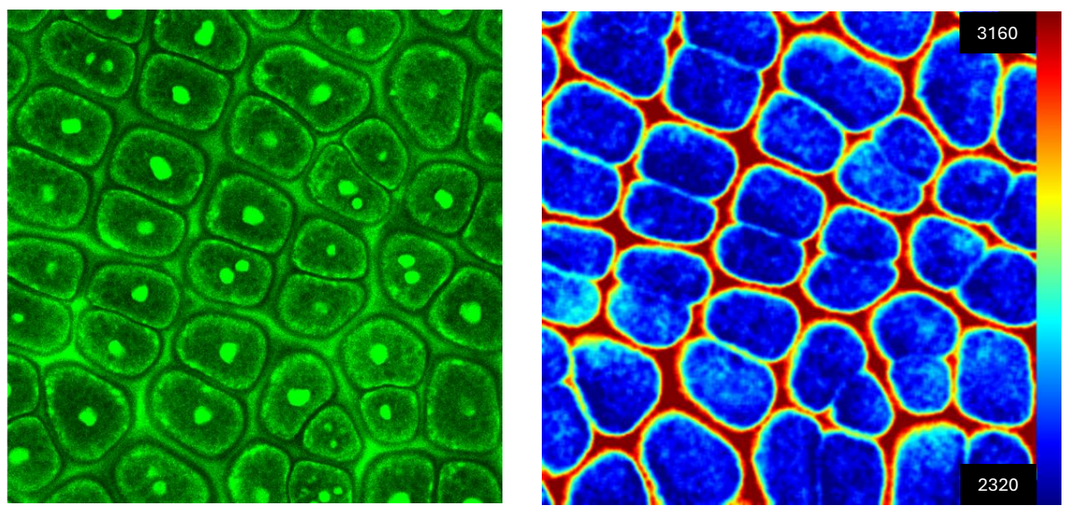


Figure 5: Fluorescence intensity (left) and lifetime (right) representation of lead like seaweed stained with AF488-DCBO via a modified glucose molecule

Pros:
- Specific labelling of defined components of the cell wall (in the case of glucose, cellulose)

Challenges:
- Removing the dye without damaging the cell

References and Acknowledgements

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