



Dualistic approach – characterizing fucoidan modifying enzymes while solving the structure of fucoidans

MAREN OFTEBRO
PHD AT NORWEGIAN SEAWEED BIOREFINERY PLATFORM (TAREPLATTFORMEN)
DEPARTMENT OF BIOTECHNOLOGY AND FOOD SCIENCE, BIOPOLYMERS AND BIOMATERIALS GROUP

SUPERVISORS:

FINN L. AACHMANN
ANNE TØNDERVIK



Outline



INTRODUCTION TO
FUCOIDAN

STRUCTURAL
CHALLENGES



INTRODUCTION TO
ENZYMES

NEW TOOLS FOR
CHARACTERIZATION



Introduction to fucoidan

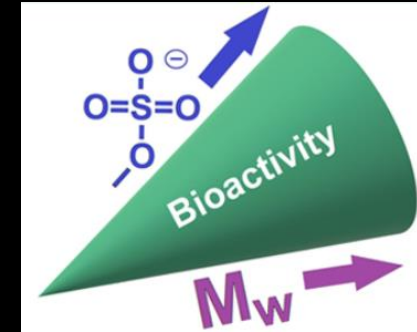
- A diverse family of sulfated polysaccharides occurring predominantly in brown algae
- Fucose-rich backbone with different degrees of sulfation
- Rich in biological activities
- Medical applications require well-defined structures



Laminaria hyperborea (stortare)

Photo: Jan Rueness

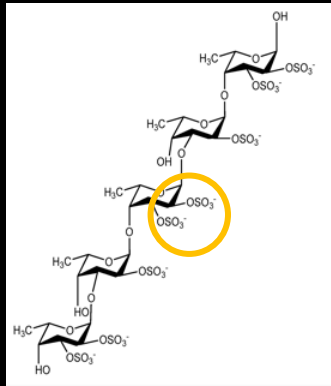
anticoagulant
anti-tumor
antiviral
anti-inflammatory
antithrombotic
immunomodulatory
antioxidant



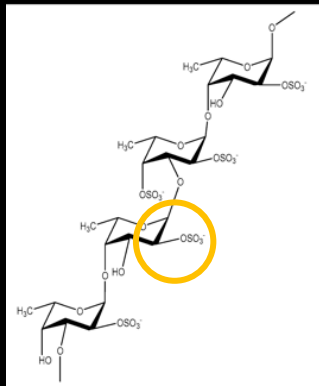


Structural challenges

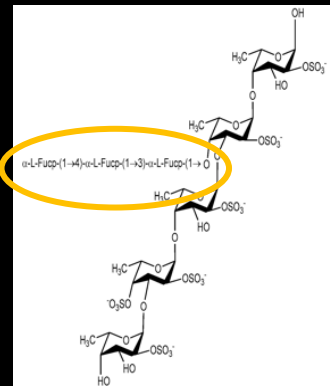
- Heterogeneous composition with variations in sulfation, other sugars, side chains and linkages – α -1,3- or 1,4-linked fucose backbone



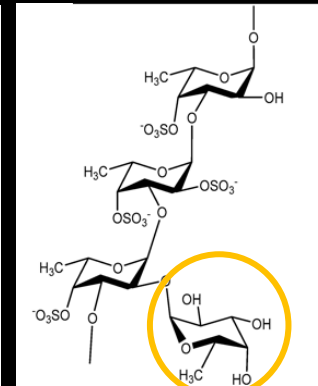
Ascophyllum nodosum
Fucus vesiculosus



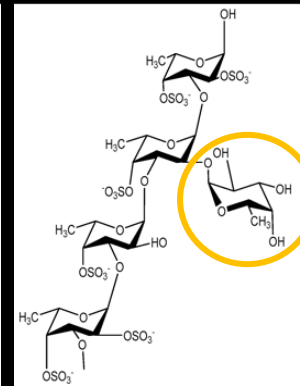
Fucus evanescens C. Ag.



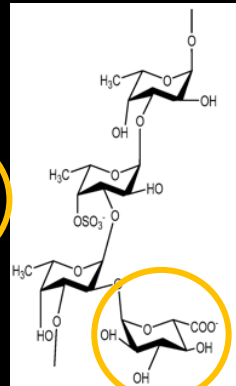
Fucus serratus L.



Laminaria saccharina



Chorda filum

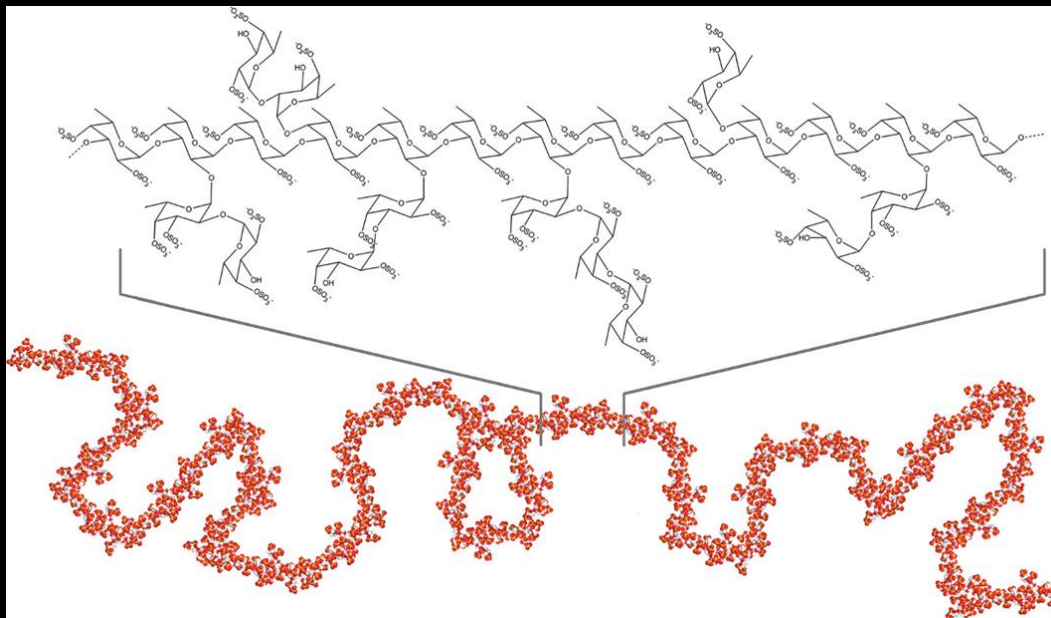


Cladosiphon okamuranus



Structural challenges

- Standard protocols for characterizing and detecting fucoidan are not established
- Lower molecular weight fragments needed – native fucoidan is too complex

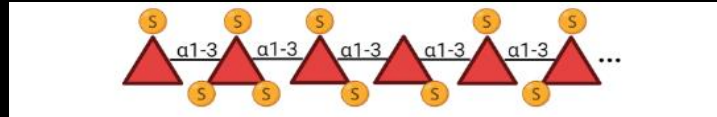




Introduction to enzymes

- Degrading fucoidan to lower molecular weight fragments without destroying important structural features

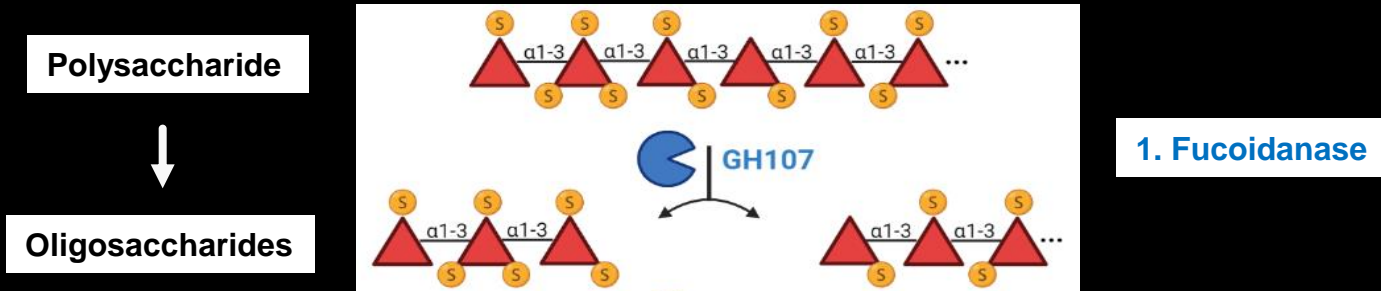
Polysaccharide





Introduction to enzymes

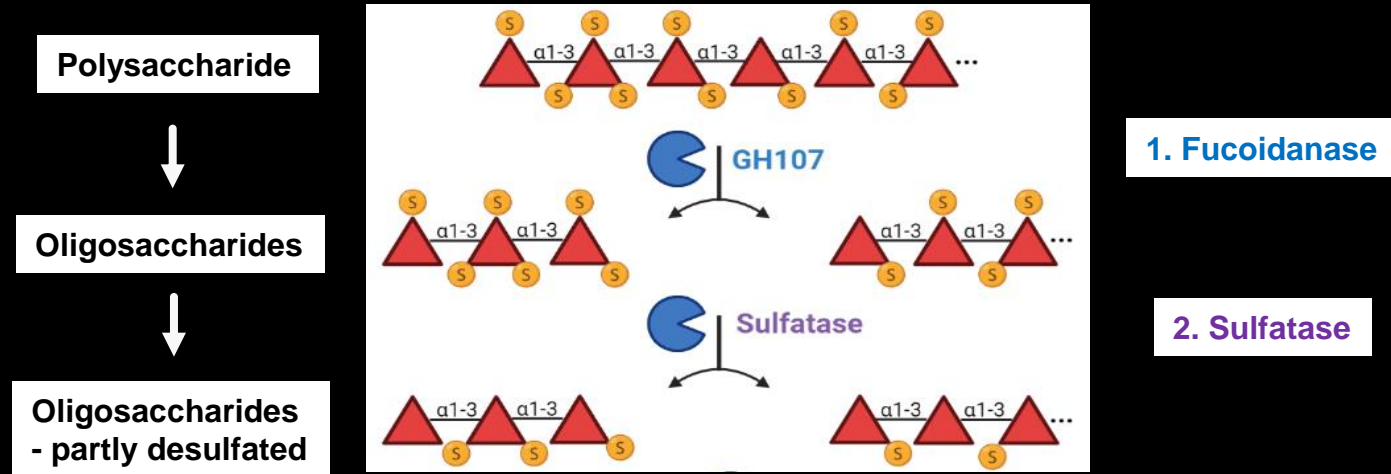
- Degrading fucoidan to lower molecular weight fragments without destroying important structural features





Introduction to enzymes

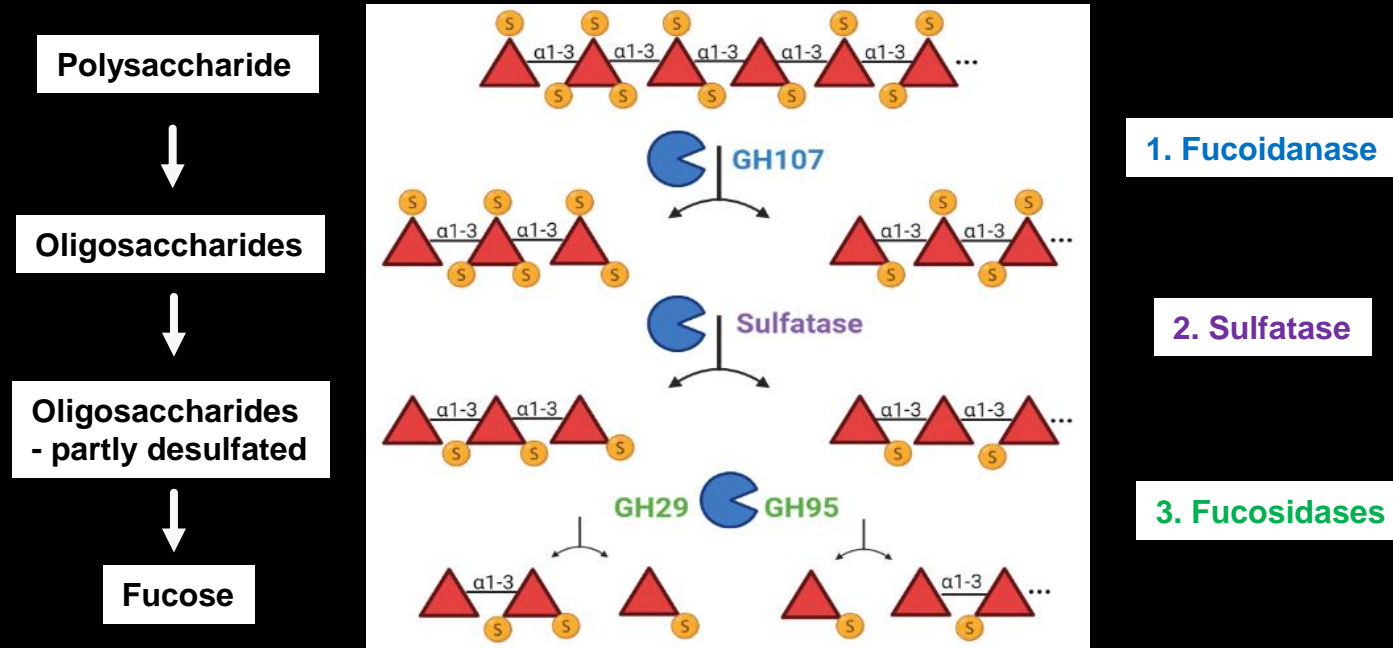
- Degrading fucoidan to lower molecular weight fragments without destroying important structural features





Introduction to enzymes

- Degrading fucoidan to lower molecular weight fragments without destroying important structural features

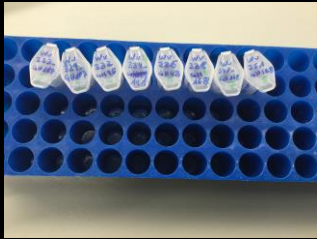




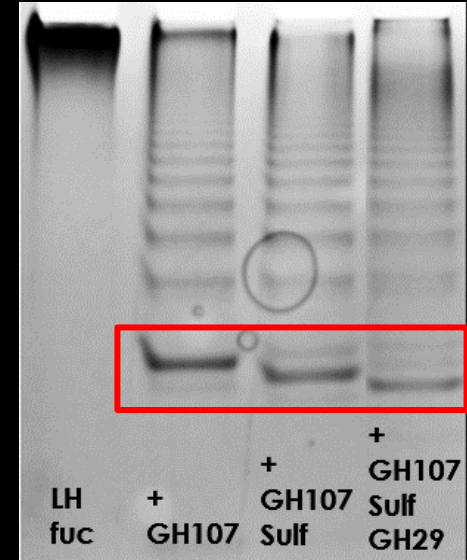
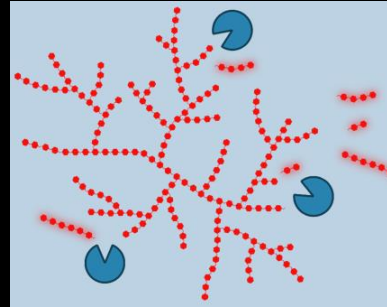
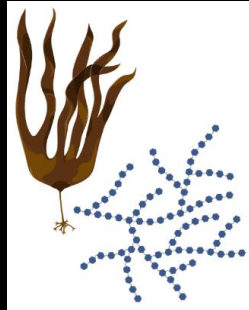
New tools for characterization

- C-PAGE shows progressive degradation of fucoidan is achieved with fucoidanase, sulfatase and fucosidase

Production of recombinant enzymes in *E. coli*



Enzymatic hydrolysis of fucoidan

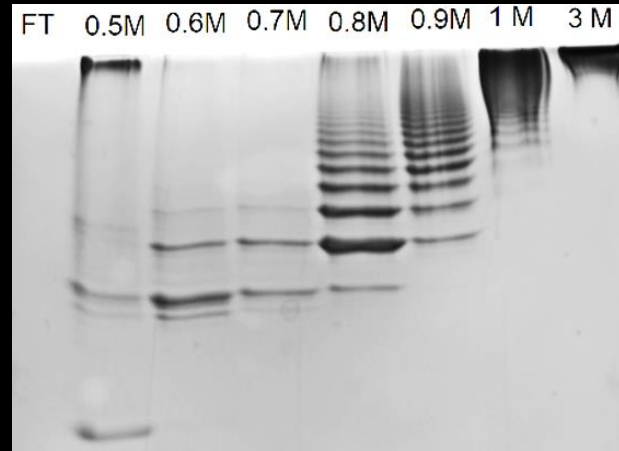


Oligosaccharides migrate in C-PAGE gel based on size + charge

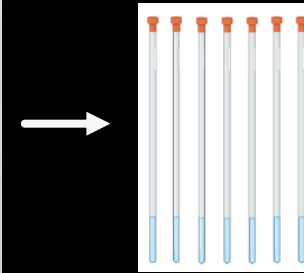


New tools for characterization

- Oligosaccharides are prepared for analysis with NMR to investigate enzyme cleaving patterns and elucidating oligosaccharide structures



Oligos separated by ion exchange chromatography with NaCl gradient

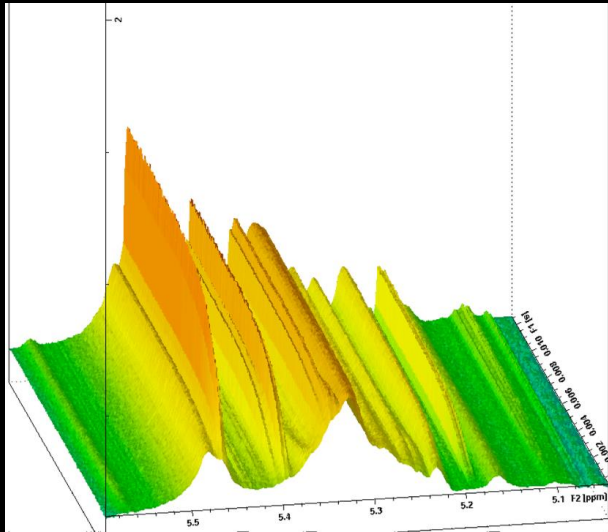


Oligo samples ready for NMR analysis



New tools for characterization

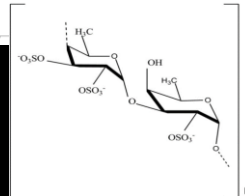
- ^1H - ^{13}C HSQC NMR spectra confirm enzymatic hydrolysis of fucoidan yields defined oligomer fractions



Well-defined signals for sulfated, fucose-containing oligosaccharides

Methyl signal

Anomeric region



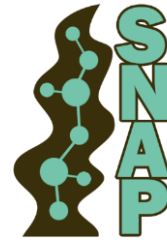
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Biopolymers and Biomaterials group



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