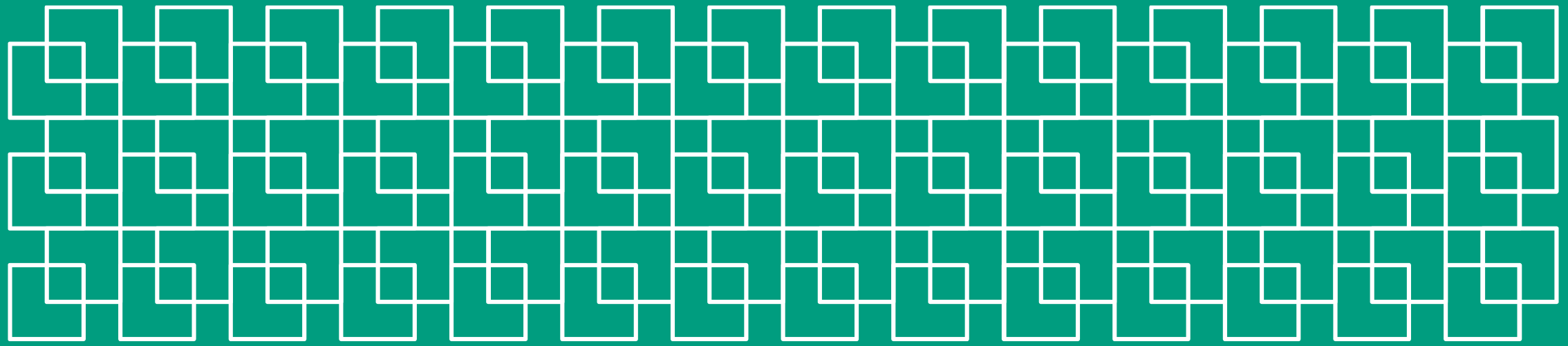
Enzymatic fucoidan extraction from brown seaweeds

Nanna Rhein-Knudsen



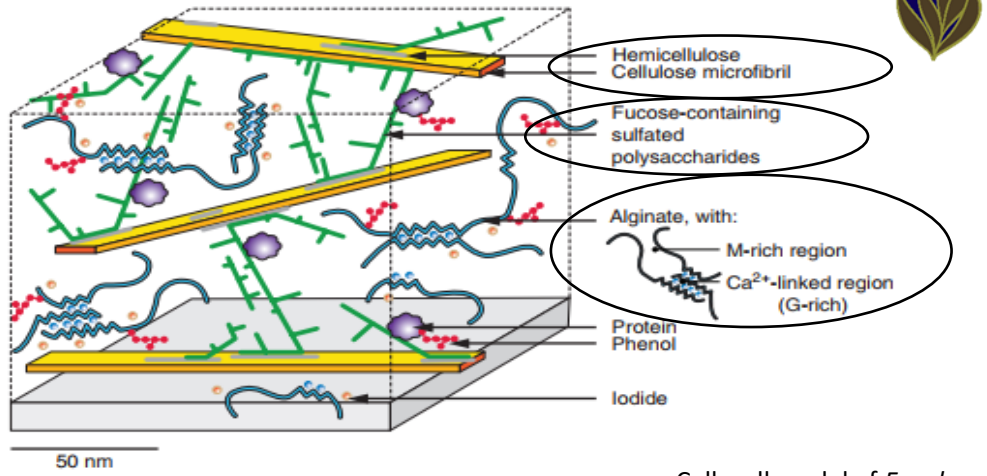
- PostDoc at the Bioprocess Technology and Biorefining (BioRef) group at NMBU

Norwegian University of Life Sciences

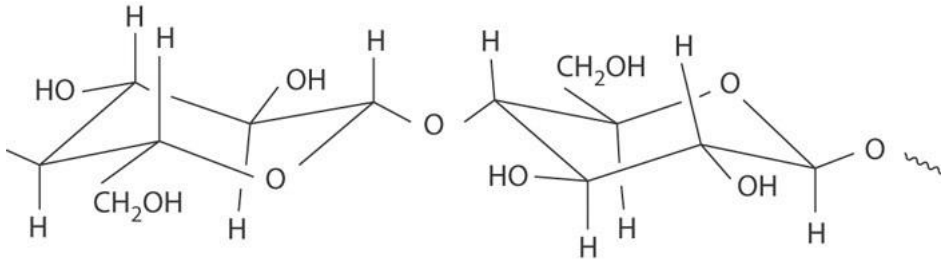
- Working in the Norwegian Seaweed Biorefinery Platform

- Focus on enzyme discovery and enzymatic proccessing of seaweeds and seaweed carbohydrates

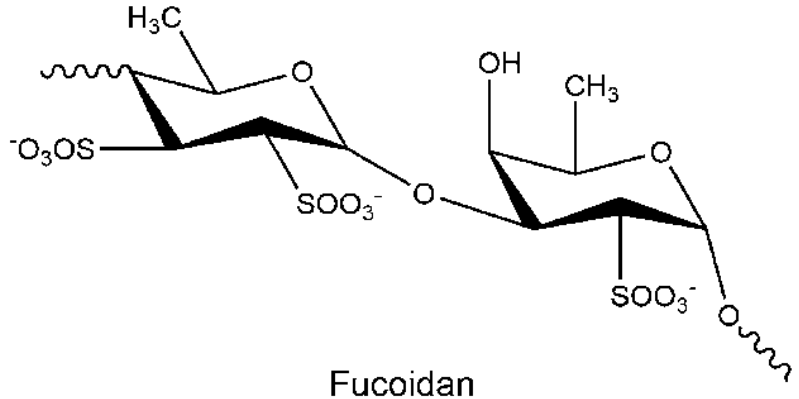
Norwegian Seaweed Biorefinery Platform



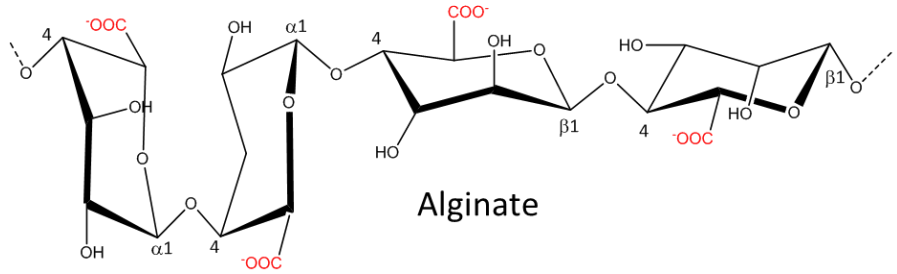
Cell wall model of *Fucales*
Deniaud-Bouët et al. (2014)



Cellulose

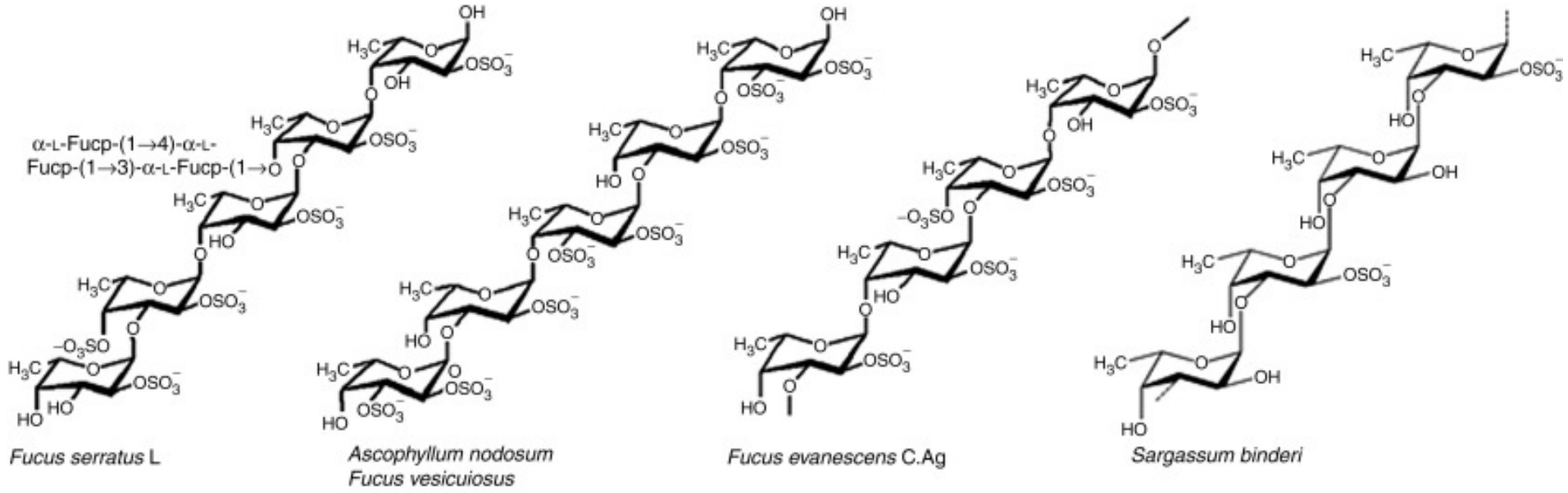


Fucoidan



Alginate

Fucoidans



Fucus serratus L

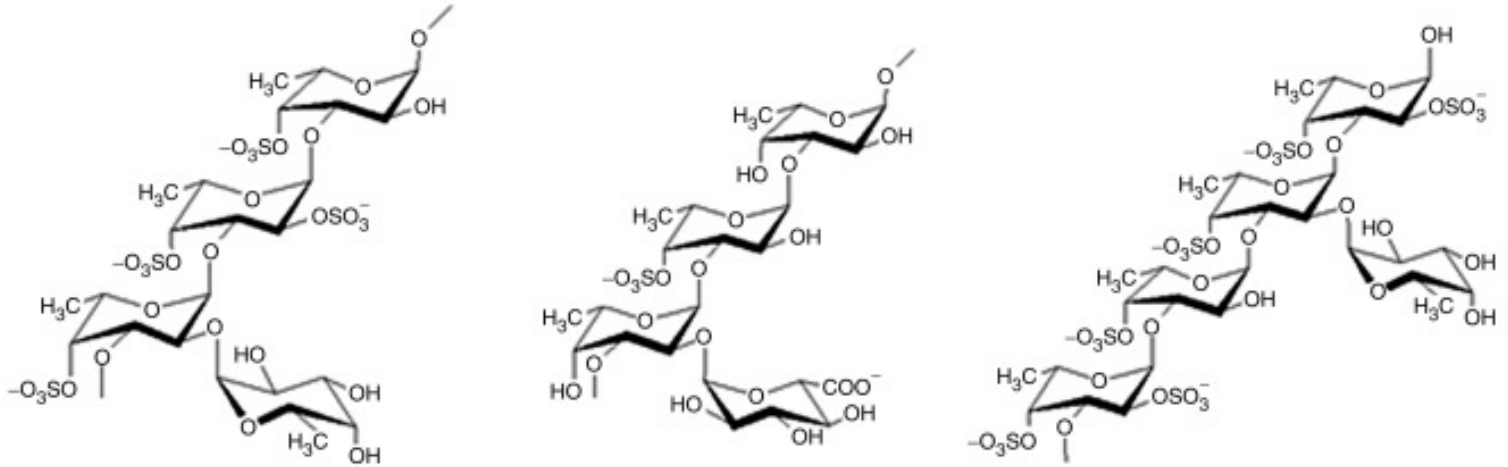
Ascophyllum nodosum
Fucus vesicuiosus

Fucus evanescens C.Ag

Sargassum binderi

Many useful bioactivities:

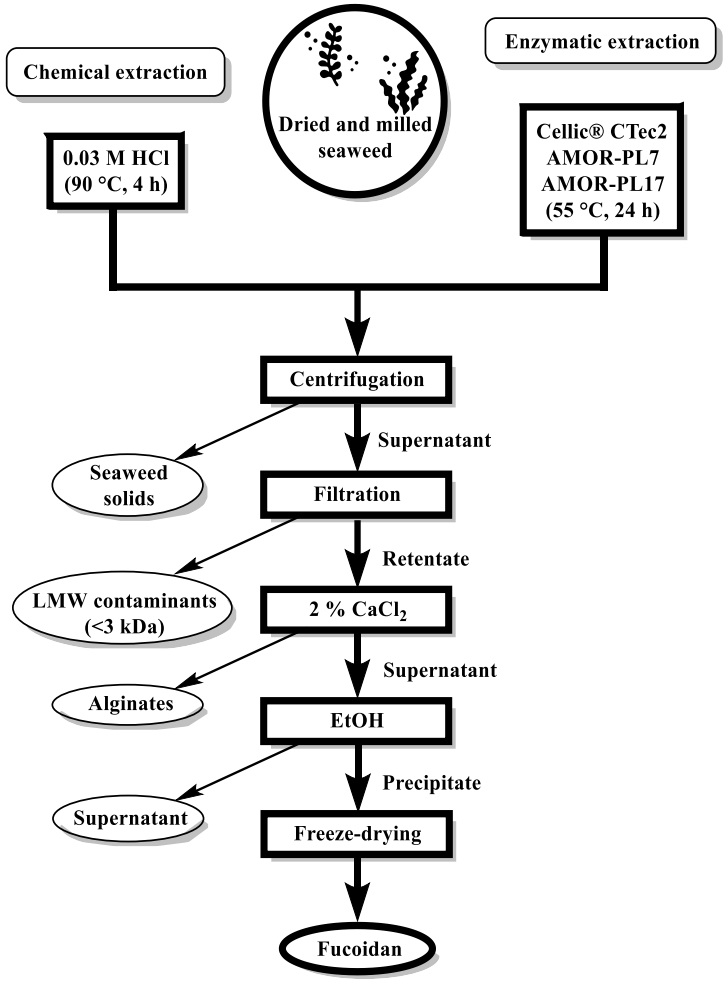
- Anti-oxidant
- Anti-tumor
- Anti-inflammatory
- Anti-coagulant
- Neuroprotective

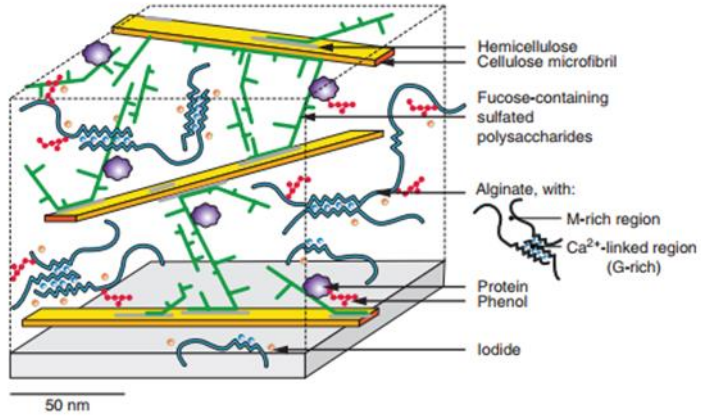


Laminaria saccharina

Cladosiphon okamuranus

Chorda filum





JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

Cite This: J. Agric. Food Chem. 2019, 67, 2936–2945

pubs.acs.org/JAFC

Production, Characterization, and Application of an Alginate Lyase, AMOR_PL7A, from Hot Vents in the Arctic Mid-Ocean Ridge

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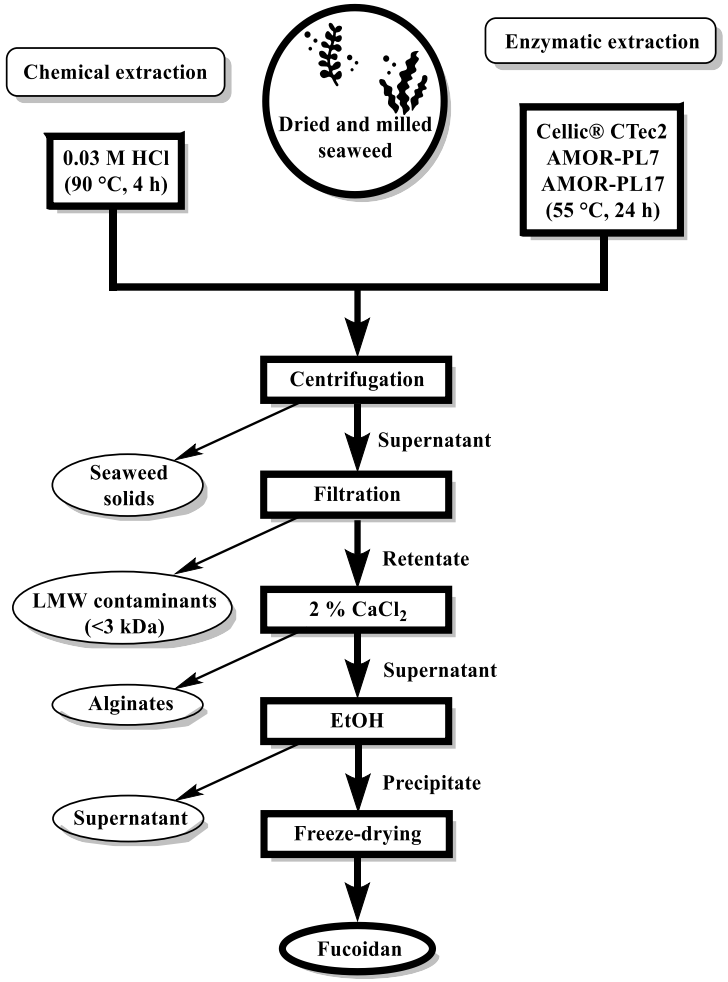
ENZYMOLOGY AND PROTEIN ENGINEERING

Alginate Degradation: Insights Obtained through Characterization of a Thermophilic Exolytic Alginate Lyase

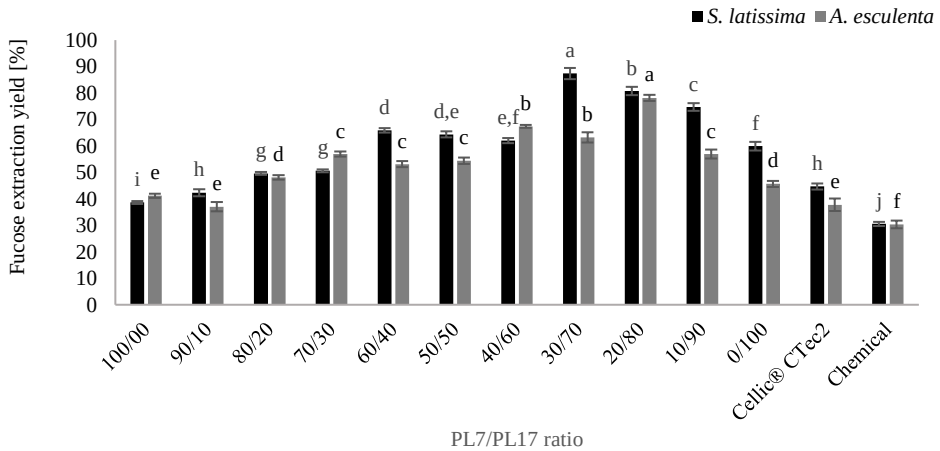
Magnus Ø. Arntzen,[a] Bjørn Pedersen,[a] Leesa J. Klau,[c] Runar Stokke,[b] Maren Oftebro,[a] Simen G. Antonsen,[a] Lasse Fredriksen,[a] Håvard Sletta,[d] Olav A. Aarstad,[c] Finn L. Aachmann,[c] Svein J. Horn,[a] Vincent G. H. Eijsink[a]



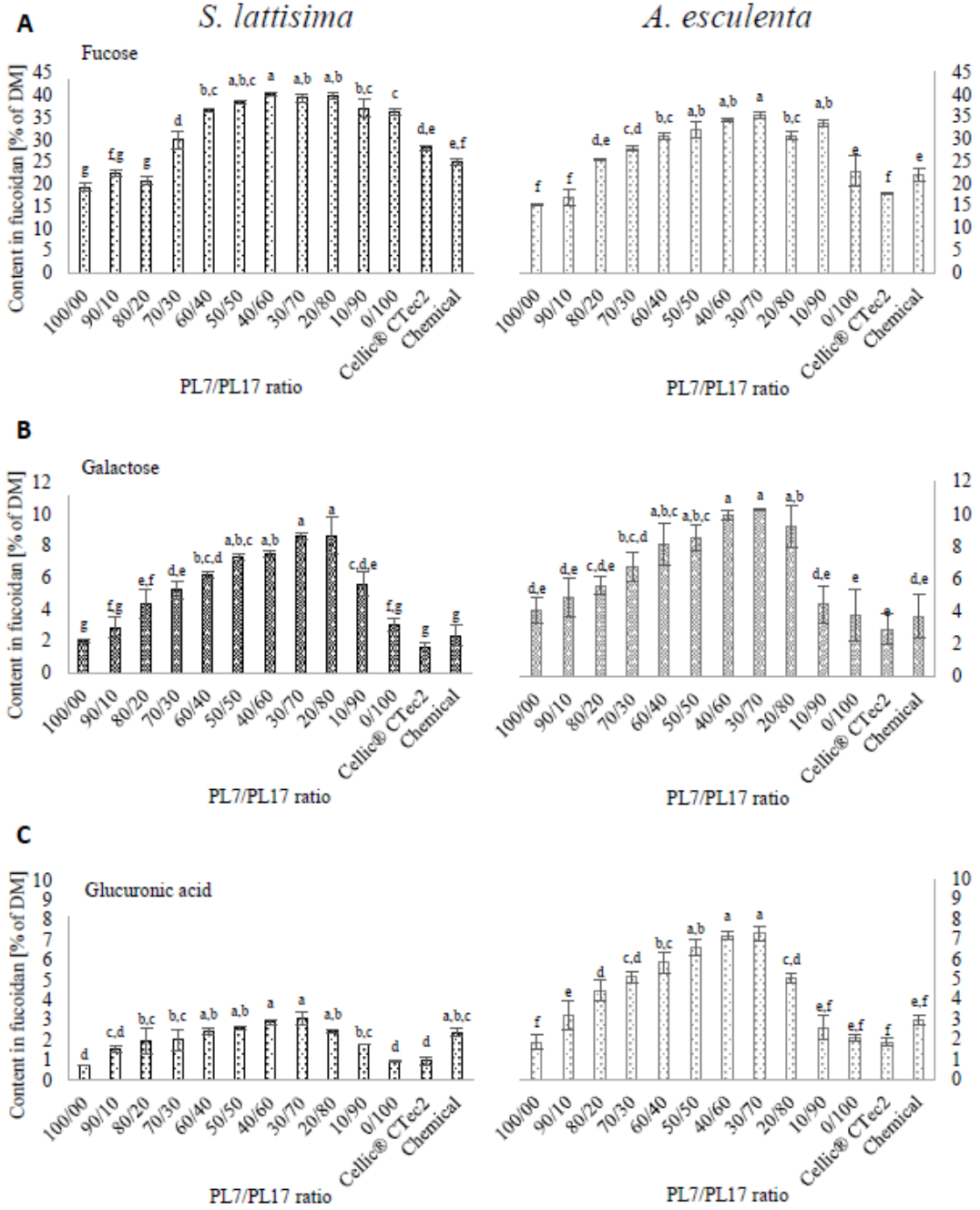
Saccharina latissima

Alaria esculenta

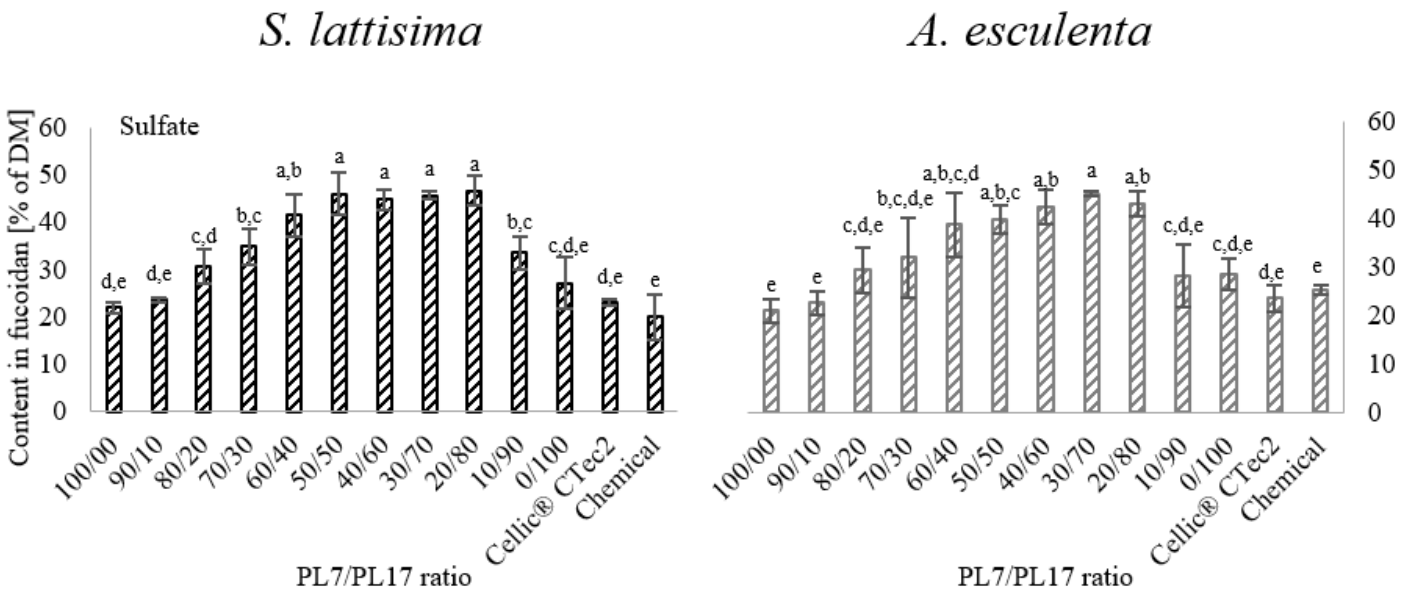
Extraction yields ranged from 8-12 % (w/w) DM for *S. latissima* and from 4-15 % (w/w) DM for *A. esculenta*, respectively.



87 % and 78 % of the fucose present in the seaweeds were extracted by enzymes using a ratio of 30/70

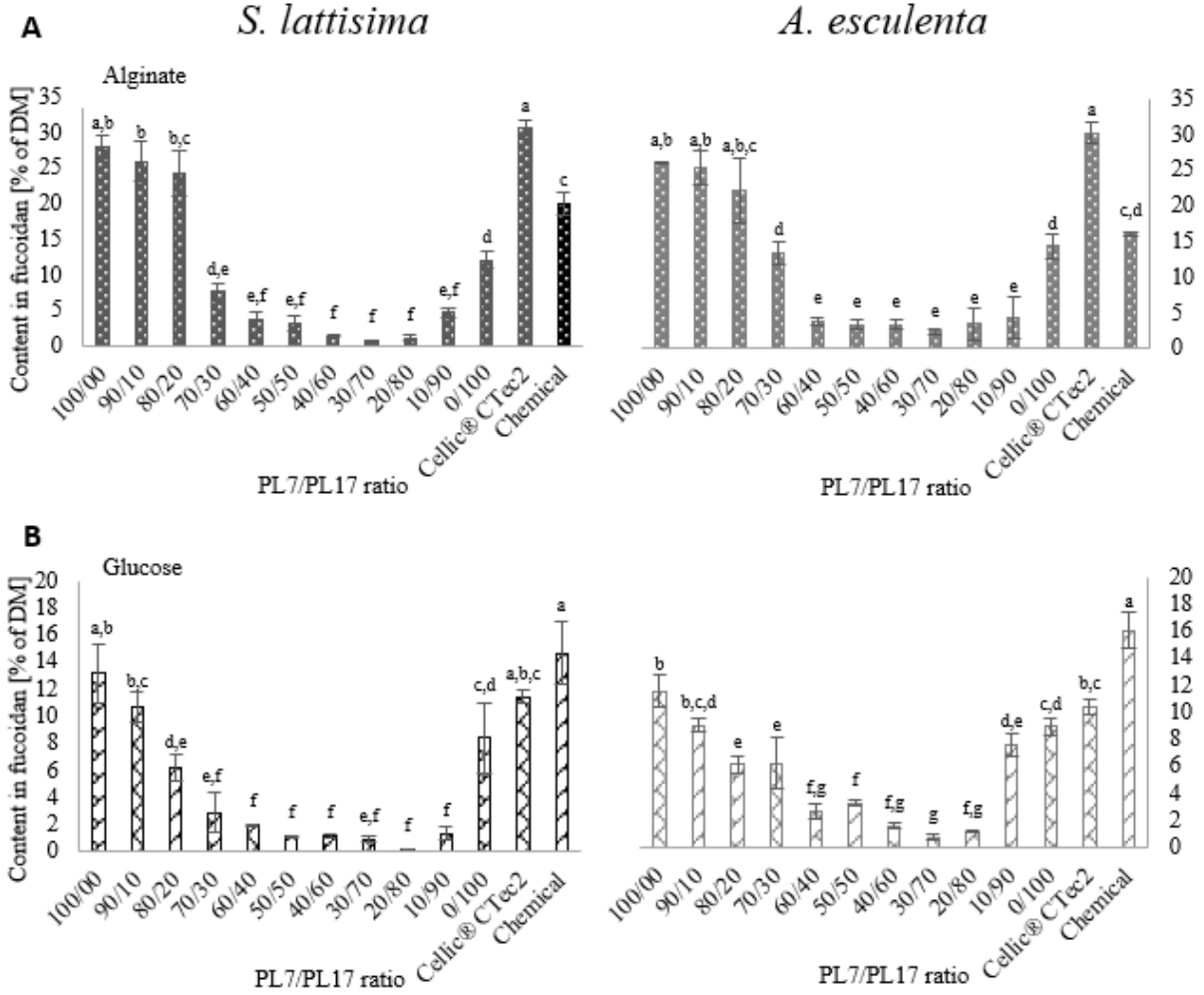


- Maximum fucose contents were around 40 % using PL7/PL17 ratios of 40/60 and 30/70
- 60 % higher fucose content using enzymes than achieved by the chemical extraction technique
- Galactose were contents around 10 % and followed the same trend as fucose
- Glucuronic acids were presents from 1-11 % and are assumed to originate from modifications



PL7/PL17 ratio	*S. latissima*	*A. esculenta*
100/00	$1.7 \pm 0.0^{a,b,c,d}$	2.1 ± 0.2^{a}
90/10	$1.6 \pm 0.1^{b,c,d}$	2.1 ± 0.4^{a}
80/20	2.2 ± 0.3^{a}	$1.8 \pm 0.3^{a,b}$
70/30	$1.8 \pm 0.3^{a,b,c}$	$1.7 \pm 0.5^{a,b}$
60/40	$1.7 \pm 0.2^{a,b,c}$	$1.9 \pm 0.4^{a,b}$
50/50	$1.8 \pm 0.2^{a,b,c}$	$1.9 \pm 0.2^{a,b}$
40/60	$1.7 \pm 0.1^{b,c}$	$1.9 \pm 0.2^{a,b}$
30/70	$1.8 \pm 0.0^{a,b,c}$	$1.9 \pm 0.0^{a,b}$
20/80	$1.8 \pm 0.1^{a,b,c}$	2.1 ± 0.1^{a}
10/90	$1.4 \pm 0.2^{c,d}$	1.3 ± 0.3^{b}
0/100	1.1 ± 0.2^{d}	$1.9 \pm 0.2^{a,b}$
Cellic® CTec2	0.5 ± 0.0^{e}	2.3 ± 0.2^{a}
Chemical	$2.0 \pm 0.2^{a,b}$	2.5 ± 0.2^{a}

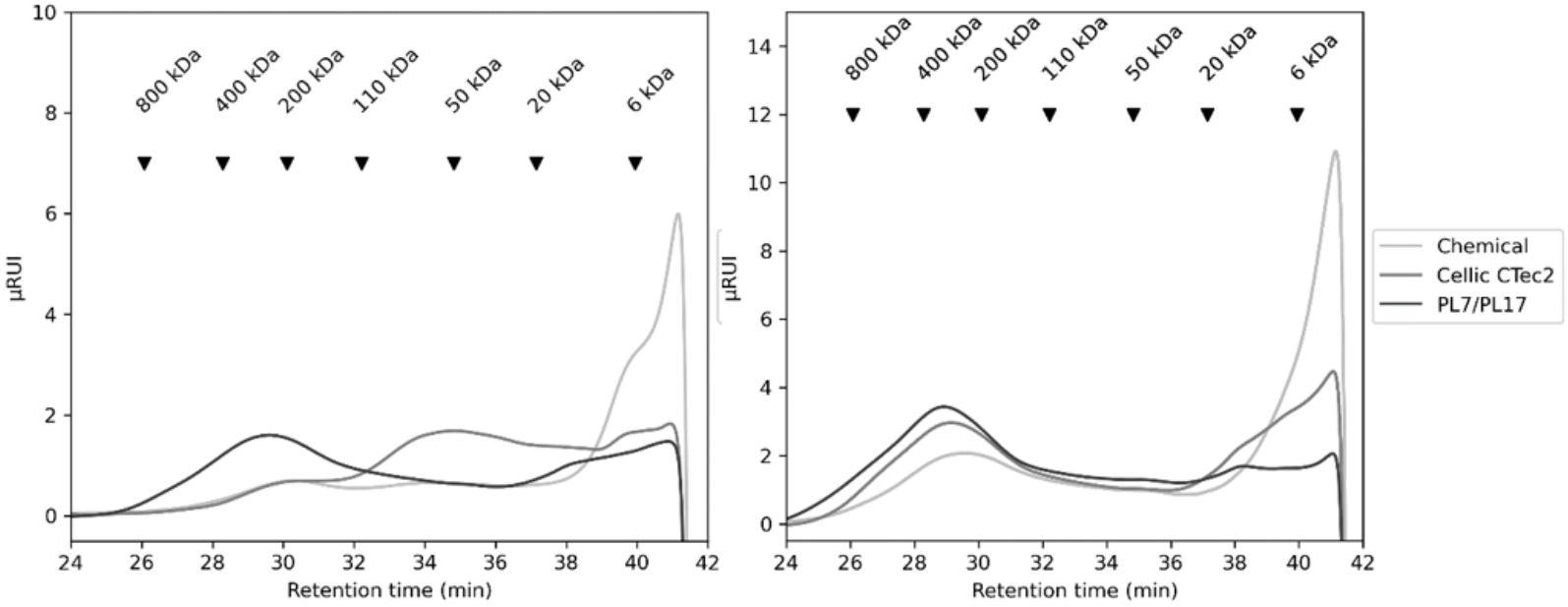
- The sulfate content increased with increasing amount of fucose
- Highest sulfate contents were acchived using lyases in a ratio of 50/50 to 20/80
- Degree of sulfation gave results between 0 and 3



- Lowest alginate content was on 1 % and 3 % and was achhieved using a PL7/PL17 ratio of 30/70
- 20 % and 16 % alginate was present in the chemical extracted fucoidans
- The alginate lyases act in synergi and endo-enzymes are needed to provide attack sites for the exo-enzymes
- Glucose is assumed to originate from cellulose and laminarin contaminations and were lowest using a PL7/PL17 ratio of 20/80 and 30/70
- Using lyases in combination with cellulases gave glucose contents 98 and 95 % lower compared to chemical extraction
- The cellulases have to be combined with lyases for maximum effectiveness

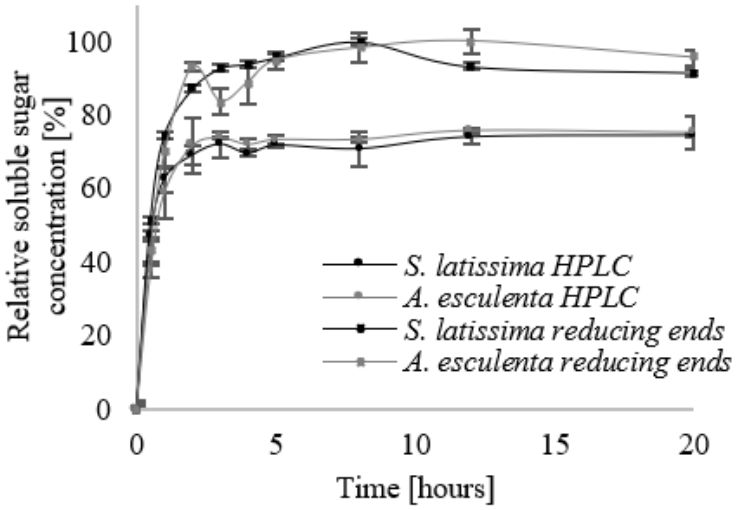
S. latissima

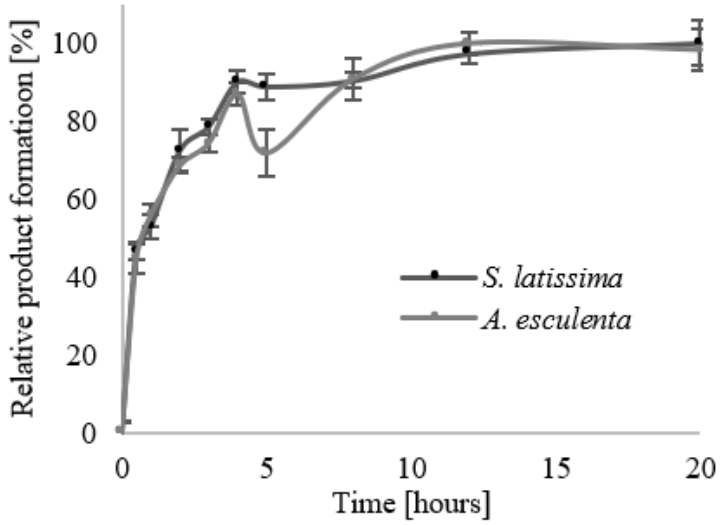
A. esculenta



Enzymatic assisted extraction gives more intact fucoidans

Optimization of reaction

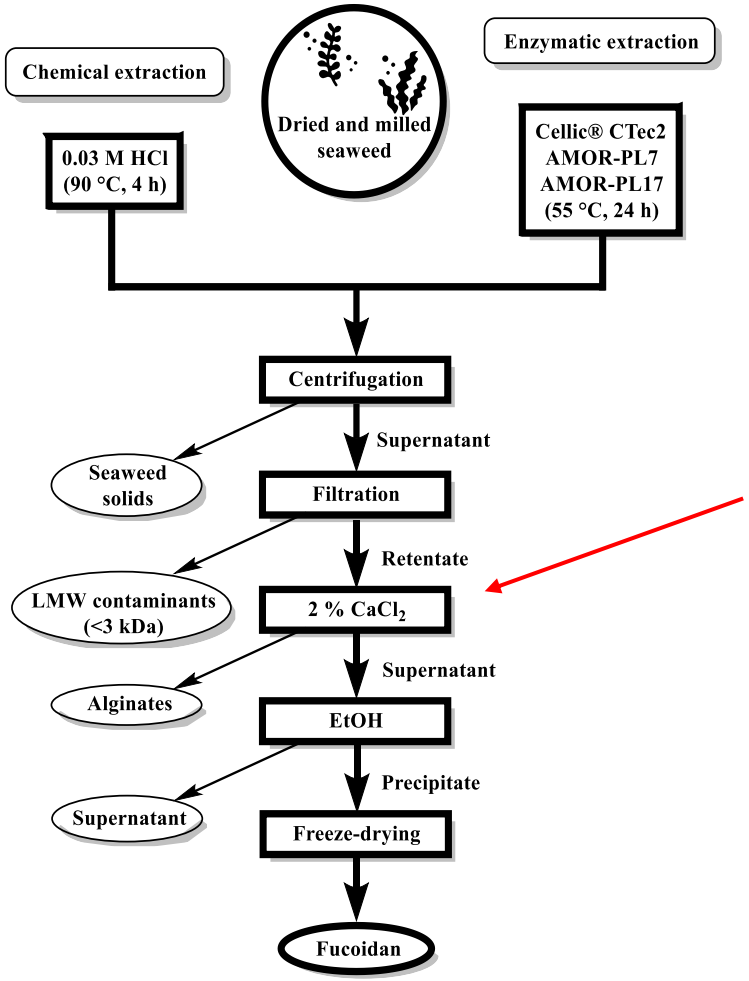




12 hours reaction for fucoidan extraction

- 63 % and 49 % of released glucose was detected after 30 min and 97 % after 3 hours.
- Reaction was faster on *S. Latissima*, most likely due to the different compositions of the alginates and the specificity of the lyases.
- Reducing ends includes the alginates and was at maximum after 8 and 12 hours.
- Double bond formation was the highest after 10 hours of reaction.

Optimization of reaction



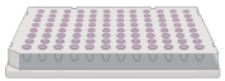
Fucoidans extracted without Calcium precipitation contained 195 and 162 % more alginate than those extracted with

Further studies - Bioactivities

WORK FLOW

4 Atlantic salmon:
- anesthesia overdose (MS 222)
- bled completely (caudal vein + gill cut)

Leukocyte isolation: spleen and head kidney (HK)
- Erythrocyte lysis
- Antibiotic treatment



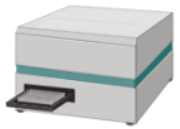
Seeding and 24h cell rest
(culture media with antibiotics)

Incubation with fucoidan fractions:
- *S. latissima*
- *A. esculenta*

M1: traditional method (chemical)
M2: cellulases (CTEC)
M3: enzymatic

100 µg/mL
500 µg/mL

6h
24h
48h
72h



Viability assay: fluorescence-based

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Further studies - Bioactivities

Evaluation of immuno modulatory properties

Phase 1: all fractions at 100 µg/mL

Spleen and head kidney

- Determination of which fucoidan fraction is the most bioactive (for further experiments)
- Evaluation of the response over time: 6h and 24h
- Evaluation of gene modulation by qPCR

Phase 2: selected fractions

Specific *in vitro* analysis

- Gene profiling
- Functional immune-assays

Phase 3: *In vivo* experiments (salmon)

Immuno-nutritional approach

- Nutritional evaluation
- Effects on immunity in fresh/sea water

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Conclusion

- Alginate lyases and cellulases can be used for fucoidan extraction
  - High fucose extraction yield
  - Low alginate contamination
  - Low glucose contamination
  - No depolymerisation

Under review: International Journal of Biological Macromolecules

- Synergy effect of the alginate lyases are important
- Specificity of the alginate lyases are important

- Bioactivities ???

Acknowlegement

- Svein Jarle Horn
- Diego Reyes
- The BioRef group at NMBU

- Ruth Tamara Montero
- Liv Torunn Mydland
- Margereth Øverland

Norwegian University of Life Sciences

Norwegian Seaweed Biorefinery Platform

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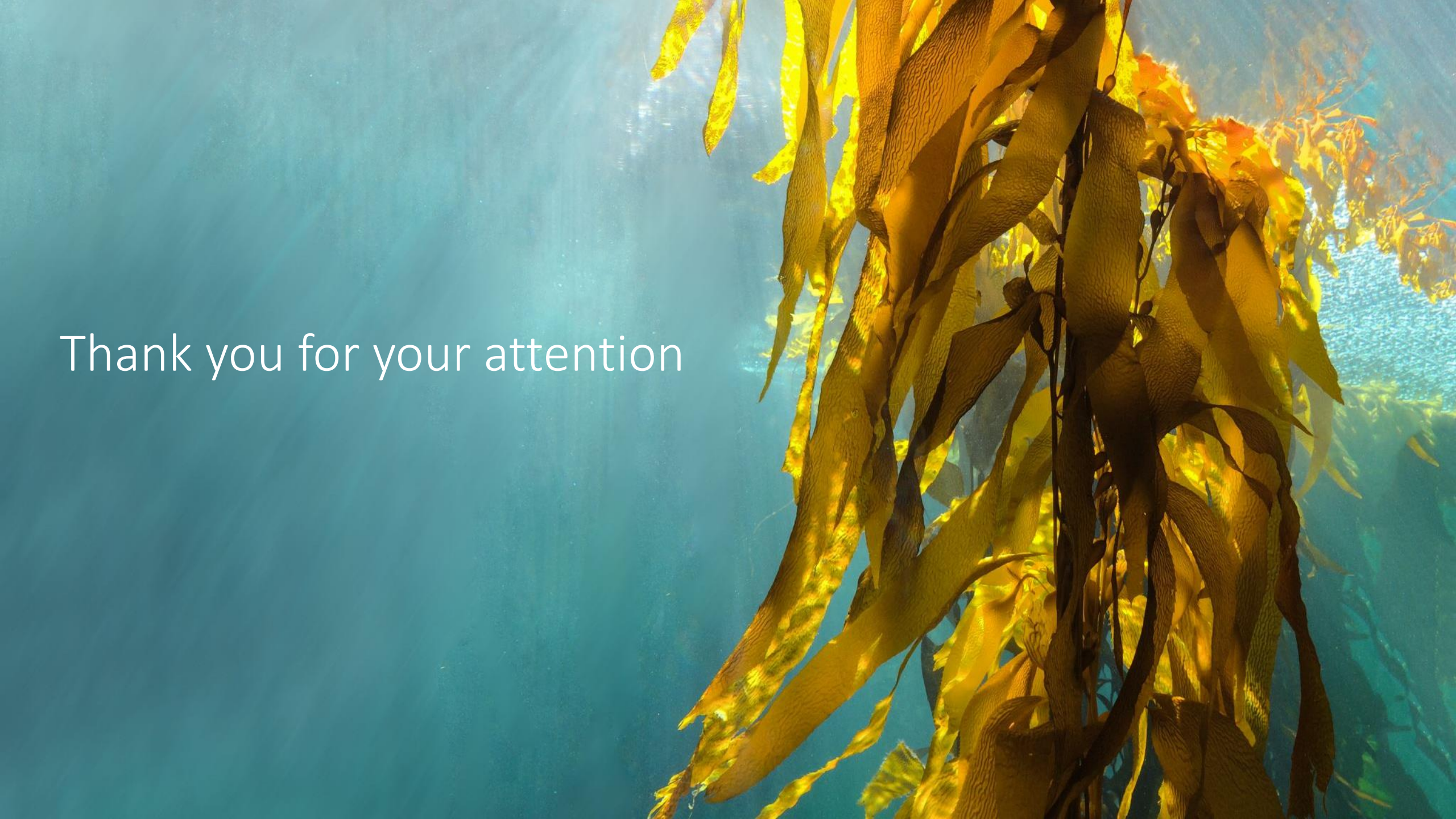
Thank you for your attention