Sources and exposure to aerosolized marine biomolecules in Norwegian seafood industry

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Background:

Respiratory health among seafood industry workers

- Numerous studies report that seafood-processing workers exhibit impaired respiratory health status compared to controls. 2- 36 % occupational asthma is reported.

(reviewed in *Jeebhay & Cartier 2010).*

- Impaired respiratory health status is confirmed in Norwegian white fish, salmon, shrimp, king crab and brown crab industry as well as among fishermen on Russian trawlers

(*Bang et al 2005, Shiryaeva et al. 2010 and 2011, Thomassen et al 2017*).

- A **healthy worker** effect is likely: Those with most symptoms will need to leave the industry

Seafood workers are exposed to **“bioaerosols”**

defined as airborne droplets or particles, consisting of, or originating from, living organisms

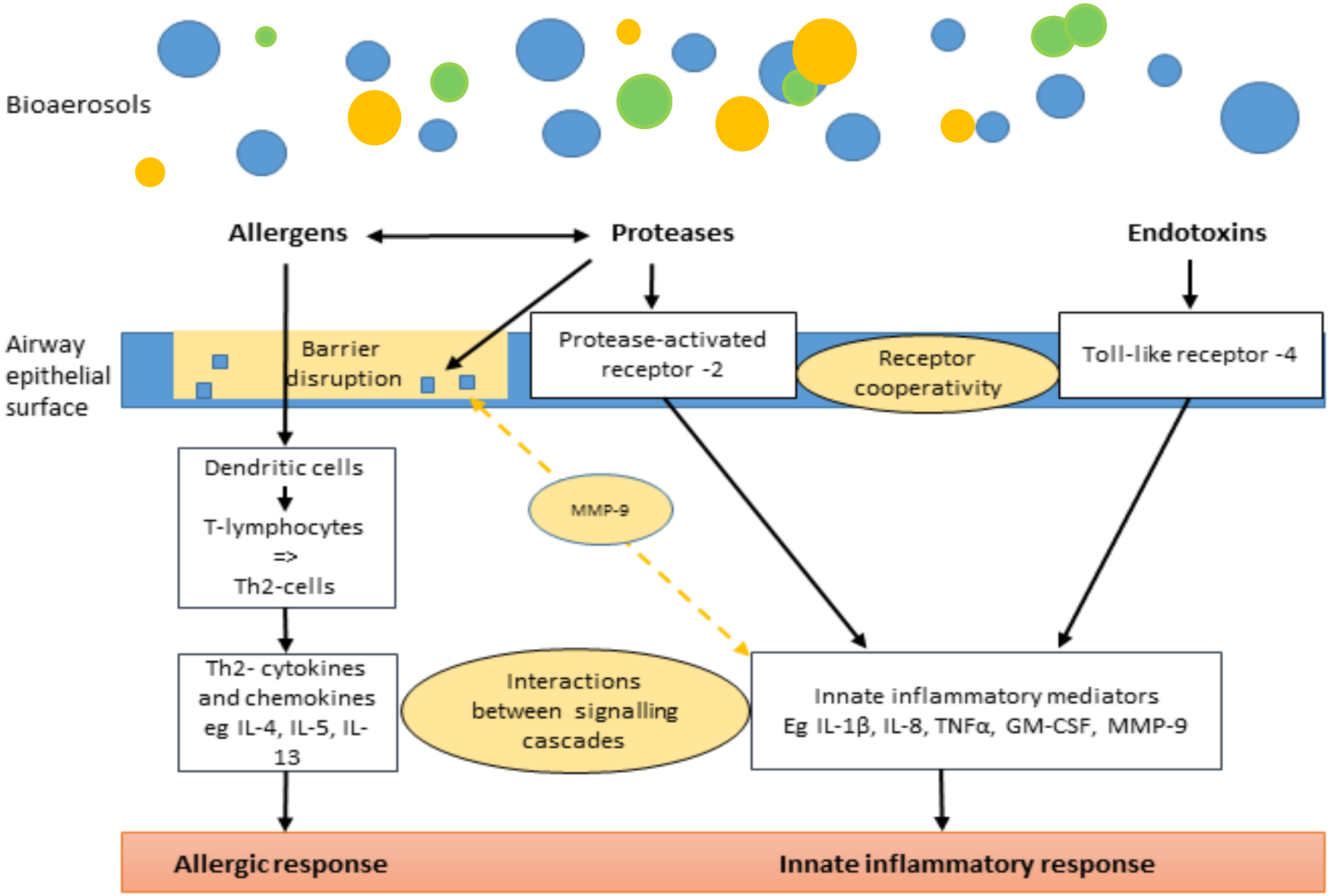






Sources of bioaerosols in seafood industry workplaces are cleaning operations where water beams hit surfaces containing organic materials, mechanical and manual processing operations, waste treatment, and airflows, eg. in shrimp industry for removal of shell remains.

Bioaerosols are mixtures of bioactive molecules



Bang 2018

Assessing bioaerosol exposure

- Crude exposure parameters
- Specific bioactive agents

Crude exposure parameters:

- Particle counts, size selective (counts/m^3)
- Gravimetry (mg/m^3)
- Total protein (µg/m^3)
  - Measure the organic component of the aerosol





Exposure to **total proteins** in different types of Norwegian seafood workplaces

	Fish			Crustaceans	
	Salmon (216 samples) µg/m³	Herring* (130 samples) µg/m³	Trawlers (252 samples) µg/m³	King crab (43 samples) µg/m³	Brown crab (24 samples) µg/m³
Mean(geom)	2.7	*	*	3.6	12.5
Range	0.8 -13	*	*	0.3 – 48	2.4 - 98

Significant associations with acute symptoms and cross shift fall in FEV_1 in the beginning of the workweek

Full shift personal exposure measurements

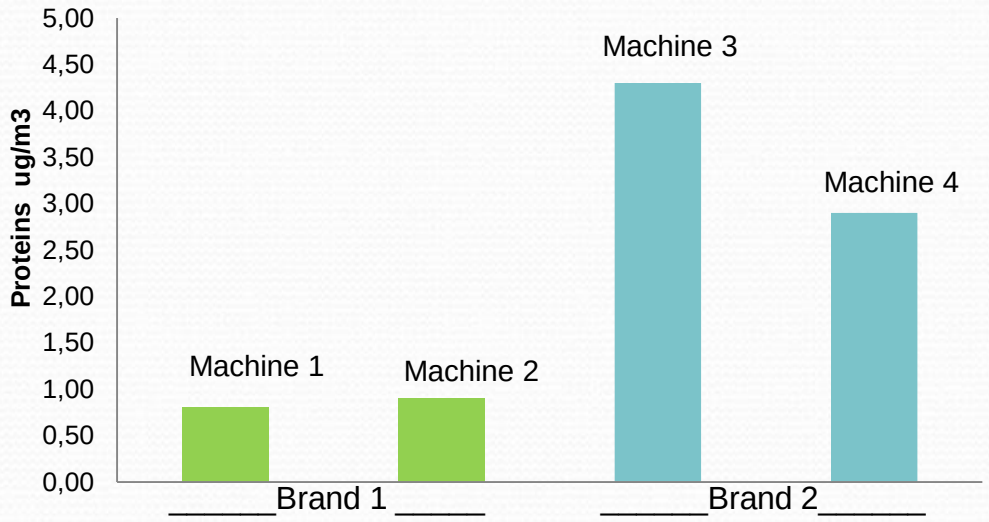
(*Shiryaeva et al. 2011, Thomassen et al 2017,* *unpublished data)

Assessment of total proteins is useful to identify sources of bioaerosol exposure

Use of high pressure water against surfaces with organic matter is a significant determinant of exposure to proteins (total fraction)

Shiryaeva 2011





Comparing two different brands of fillet machines

Unpublished data

Basis for recommendations of preventive actions

Assessment of specific bioactive agents

Measurement of specific bioactive molecules is needed to get a deeper understanding of cause and effect and suggest more specific / individual preventive and treatment strategies

- Allergens (ng/m^3)
- Endotoxins (endotoxin units (EU) or ng/m^3)
- Proteases (enzyme activity units, or ng/m^3)

Personal exposure to allergens in Norwegian seafood workplaces

	Fish	Shellfish	
	Salmon Parvalbumin (227 samples) ng/m³	King crab Tropomyosin (54 samples) ng/m³	Brown crab Tropomyosin (24 samples) ng/m³
Mean (geom)	7	2	23
Min - max	0.2 – 358	0.1 – 76	0.4 – 96

Higher in filet departments than slaughtery departments

- Higher in brown crab than king crab industry
- Higher in cooked crab dpt. than raw crab dpt

Correlation* between allergen levels and total protein levels:

Salmon: r = 0.2
King crab: r = 0.6
Brown crab: r = 0.4

* Pearsons's correlation coefficient

Allergy

Allergic sensitization is more prevalent in shellfish industry than in fish industry

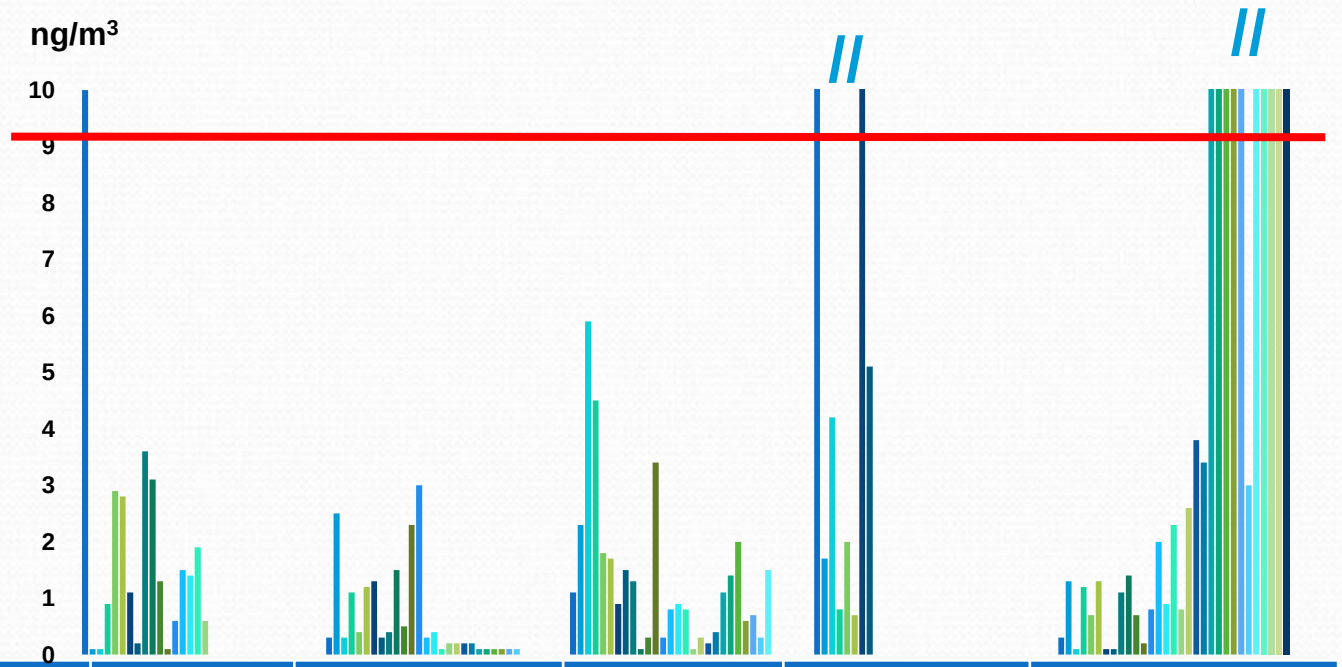
Industry		% of workers IgE positive	% of workers skin prick test positive
Shellfish	King crab (n=113)	**9**	**18**
	Brown crab (n=83)	**12**	**18**
	Shrimp (n=59)	**20**	
Fish	Salmon (n=89)	**2**	
	White fish (n=73)	**3**	
	Herring (n= 54)		*

(*Bang et al 2005, Shiryaeva et al. 2010, Thomassen et al 2017,* *unpublished data).

Exposure to endotoxins in different types of Norwegian seafood industries

Mean personal exposure to endotoxin during a work shift

Proposed no adverse effect level (NOAEL) for occupational exposure
(90EU/m^3 ≈ 9ng/m^3)

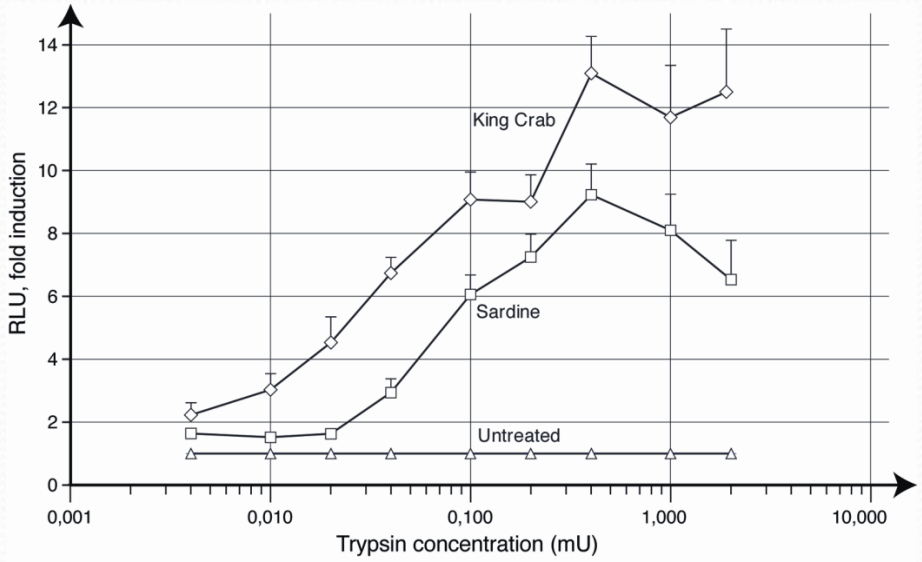


	Shrimp n =17	Salmon n=26	White fish n=27	Brown crab n=8	King crab n=31
Mean (ng/m^3) (geom)	0,94	0,35	0,84	3,49	*
Max (ng/m^3)	10	3	5,9	34	*

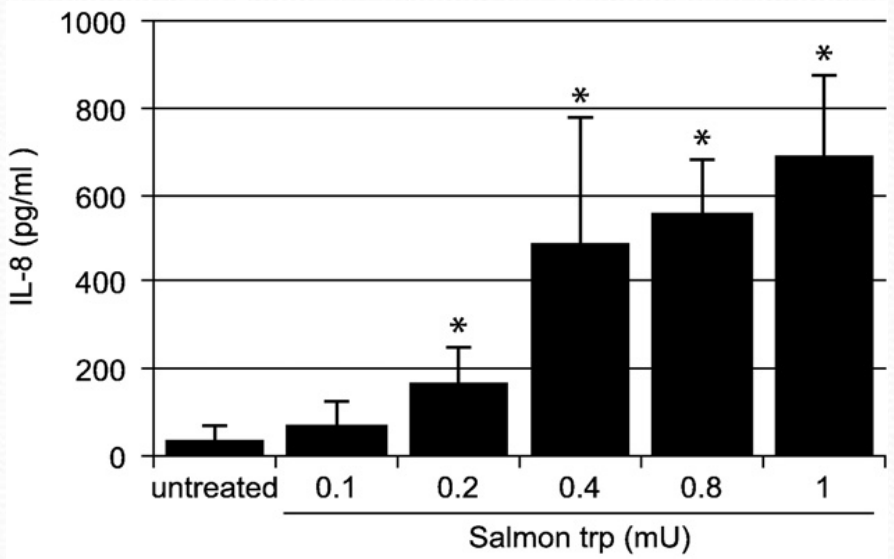
(*Bang et al 2005, Thomassen et al 2017, * unpublished data*).

In vitro studies of seafood trypsin in airways and skin cell models support a role in inflammation:

NFκB



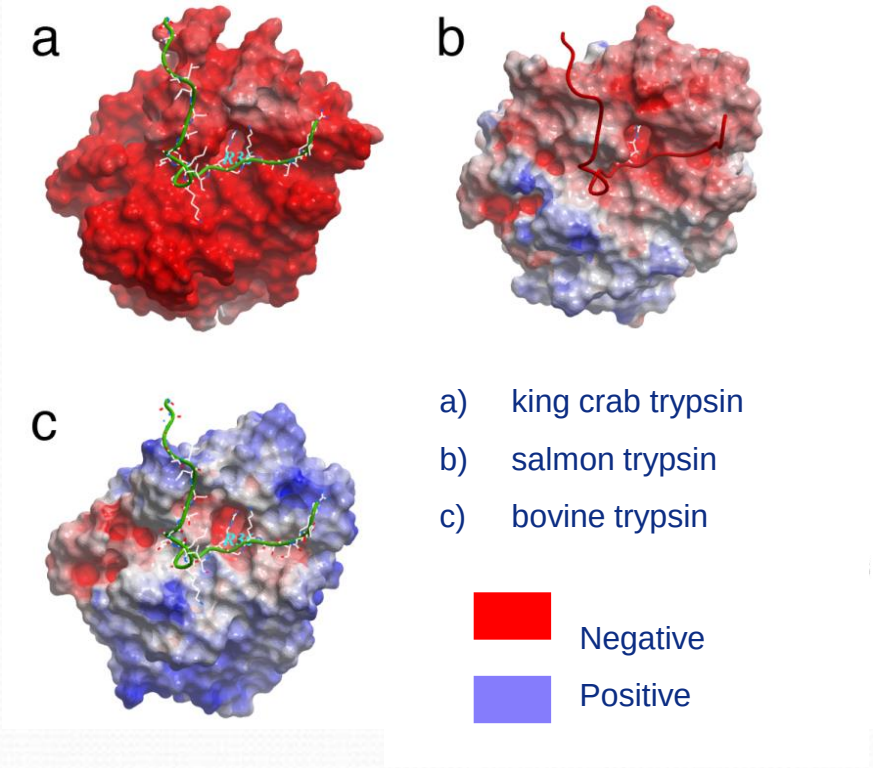
Interleukin-8

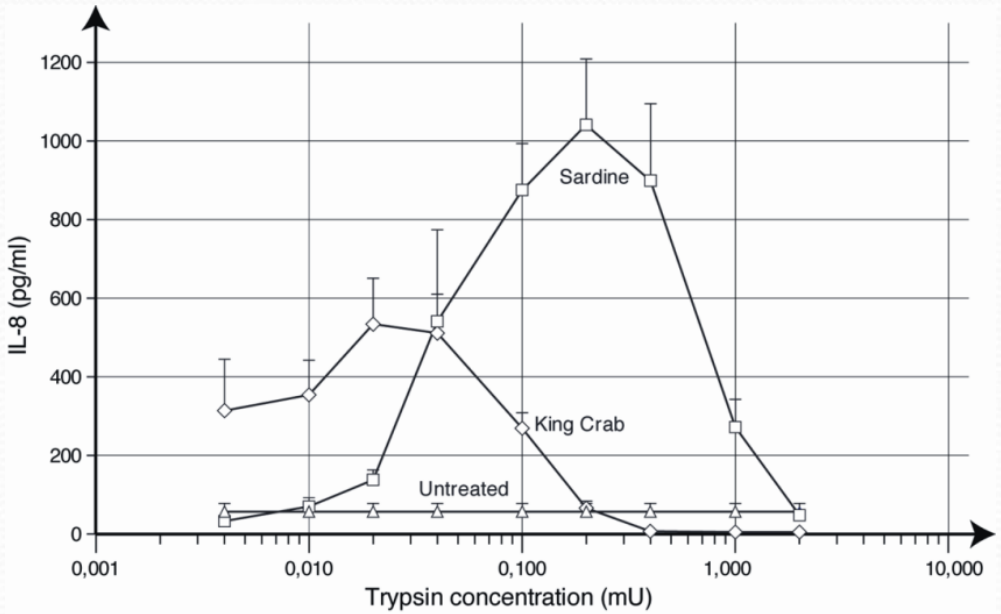


Trypsin purified from salmon, pilchard and king crab increase the secretion of inflammatory mediators in cell models in a concentration dependent manner, effects shown to be dependent on proteolytic activity and the activation of protease activated receptor (PAR-2)

Larsen et al. 2008 & 2013, Bhagwat et al. 2014

Seafood trypsins are different in different species and show differences in ability to activate inflammatory signalling





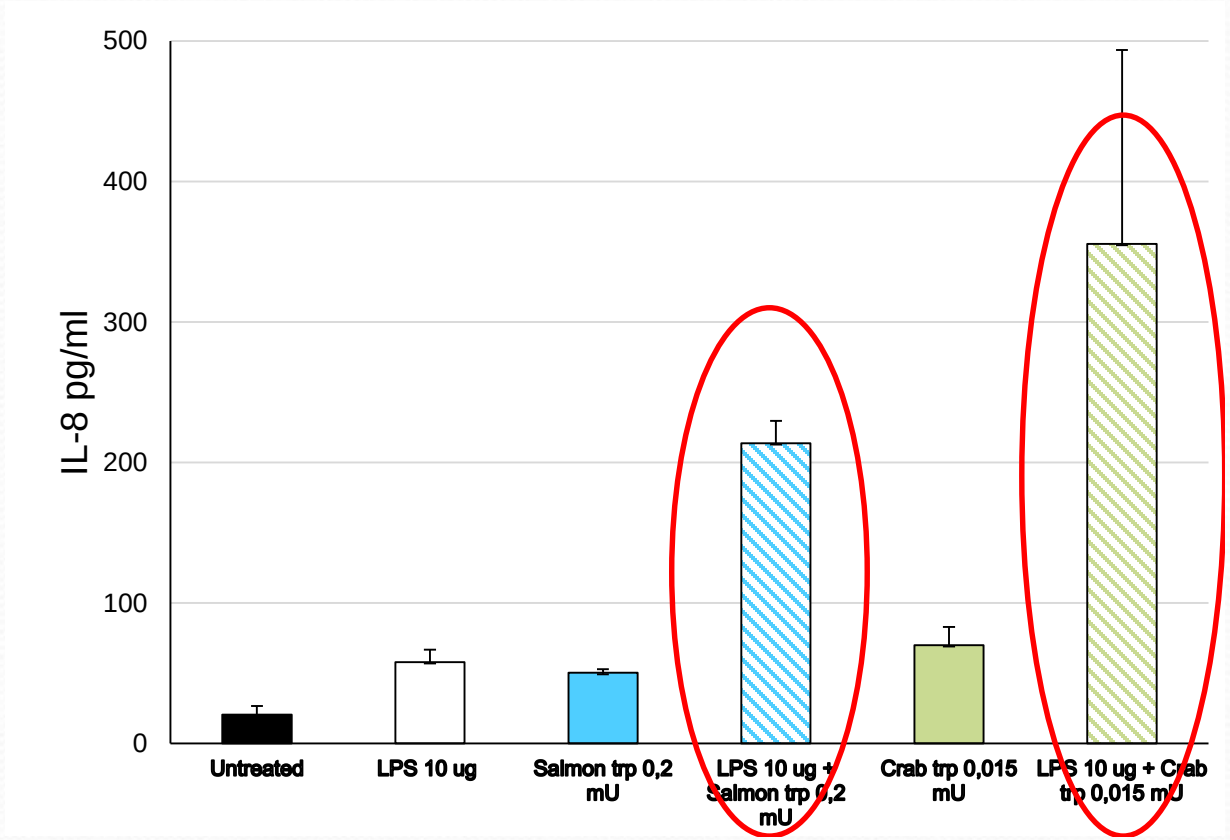
Models by Kurt Kristiansen

Larsen et al 2013

Effects of proteases in bioaerosols is an under-researched field

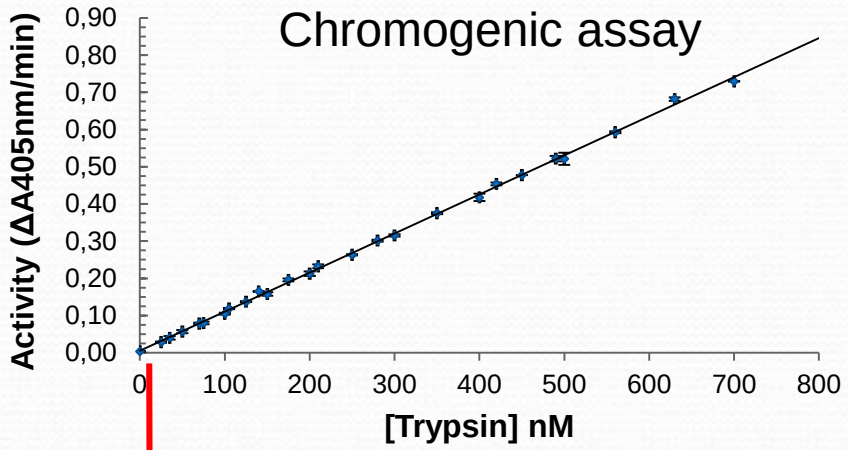
Combined exposure – seafood trypsins and LPS (endotoxin)

- Combined exposure to low concentrations of seafood trypsins and low concentrations of LPS (endotoxin) produces a **synergistic** effect *in vitro* (1 + 1 = 3)



Bhagwat et al 2016

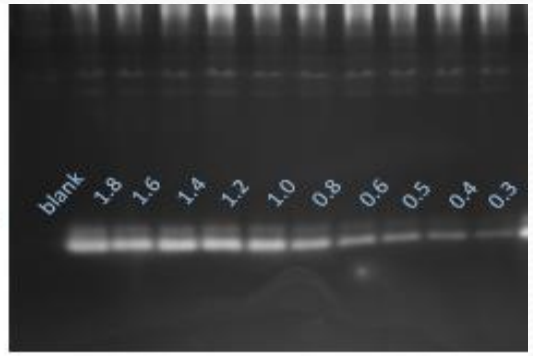
=> Need of sensitive assay for proteases in air samples



[E]: 0 – 1 nM

Chromogenic (or fluorescence based) assays are not sensitive enough for most bioaerosol samples

Zymography assay

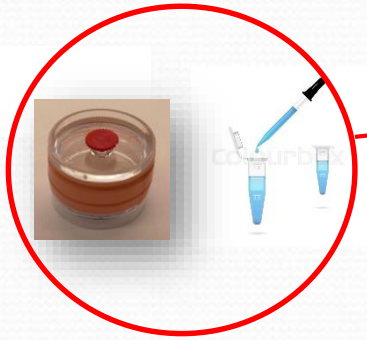


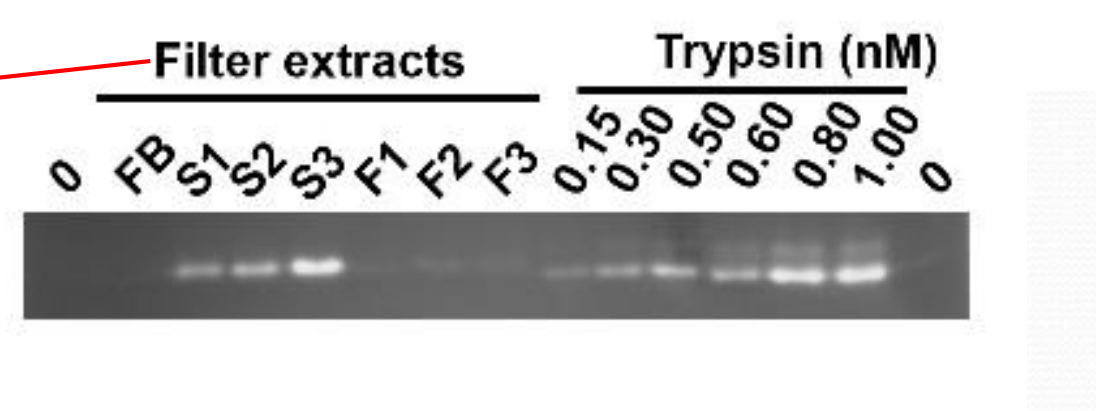
Separate proteins (SDS PAGE added gelatin as substrate)

Proteases are visible as unstained bands where the substrate has been digested

Trypsin standard curve with increasing band intensity

Quantification of trypsin in air samples





- Standards and extract samples are run on a zymography gel
- The intensity (transparency) of the enzyme band is read by an image analyse software
- Detection limit 0.056 nM

Bang BE, Malla N, Bhagwat SS, Aasmoe L, Winberg J-O. A sensitive assay for proteases in bioaerosol samples. Characterization and quantification of airborne proteases in salmon industry work environments. Annals of Work Exposure and Health. 2018.

Examples of protease exposure levels in salmon industry







Bang et al 2018

Take-home messages

- Bioaerosols are mixtures of bioactive molecules
- Measurement of total protein in filter samples is useful as a crude measure to assess the exposure to organic components of aerosols
- Measurement of specific bioactive molecules is necessary to get a deeper understanding of cause and effect
- *In vitro* data suggest airway effects of environmental exposure to seafood proteases => assessment of protease exposure should be included in occupational studies
- A new sensitive assay for proteases in air samples is suggested

Contributors

Lisbeth Aasmoe, Anett Kristin Larsen, Olga Shiryaeva, Marte Thomassen, Sampada Bhagwat, Cecilie Thon Heidelberg, Merethe Larsen, Eva Kramvik, Arild Øvrum, Ann Helen Olsen, Marit N. Hegseth, Randi Olsen, Gerd Sissel Andorsen, Anne Renstrøm, Anne-Sophie Merritt, Ole-Morten Seternes, Bjørn Straume, Nabin Malla, Kurt Kristiansen, Ingebrigt Sylte, Jan-Olof Winberg, Wijnand Eduard, Andreas Lopata, Sandip Kamath, Anne-Mette Madsen, Imin Wushur