Exploitation of marine rest raw materials

Lisbon, December 14th, 2011
Inge W. Nilsen,
Research Director, Marine Biotechnology

Situation in Norway

Seafood export value: € 7 billions

Three big-volume species: Cod, herring and salmon (aquaculture)

Marine raw rest materials: 950,000,000 kg

- 15 % not exploited (/dumped at sea – i.e. cod)

Commercial products from “Norwegian” rest material: € 600 million

- Dominant market: Animal feed
- Main product: Omega-3 oils; >50% of raw oil imported

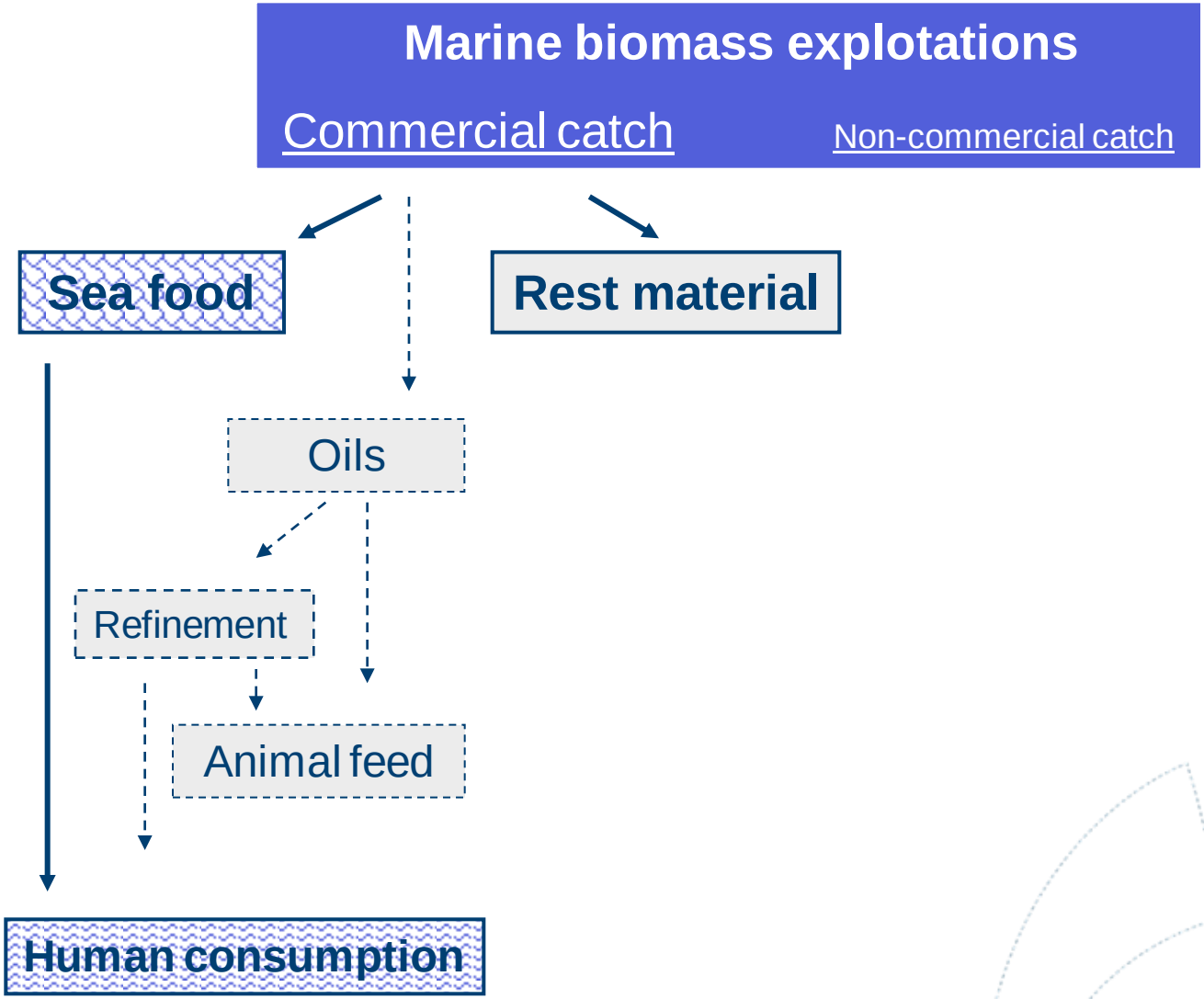
+ products such as alginate from macro algae: € 150 million

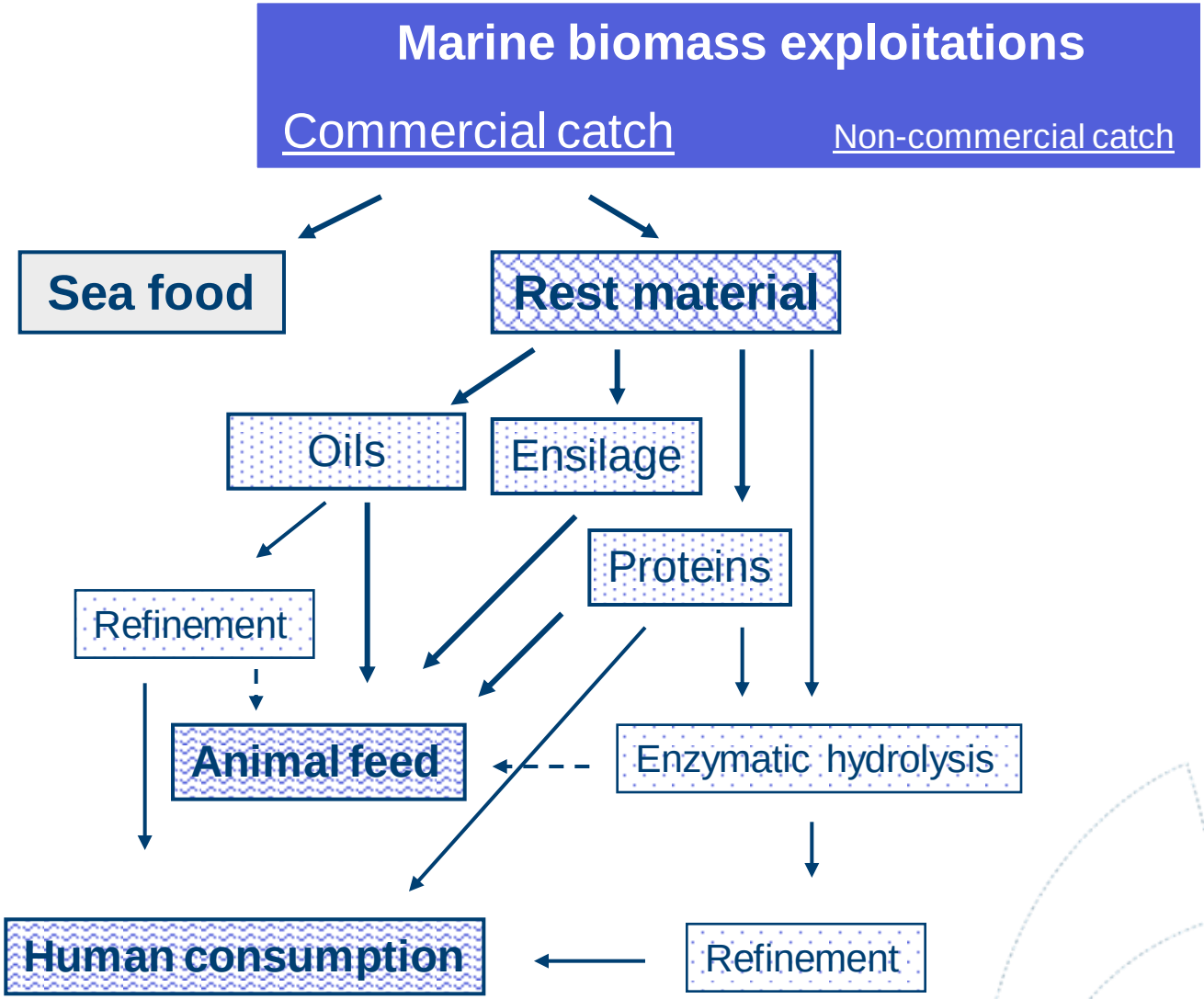
Marine rest materials – ”Norwegian challenges”

Large proportion of rest materials already exploited (although at low values)

→ Higher values should come from better exploitation of existing volumes:

- secure optimal quality of rest material
- optimized process lines
- increase product range and product quality from rest materials


Marine biomass explotations
Commercial catch
Non-commercial catch
Sea food
Rest material
Oils
Refinement
Animal feed
Human consumption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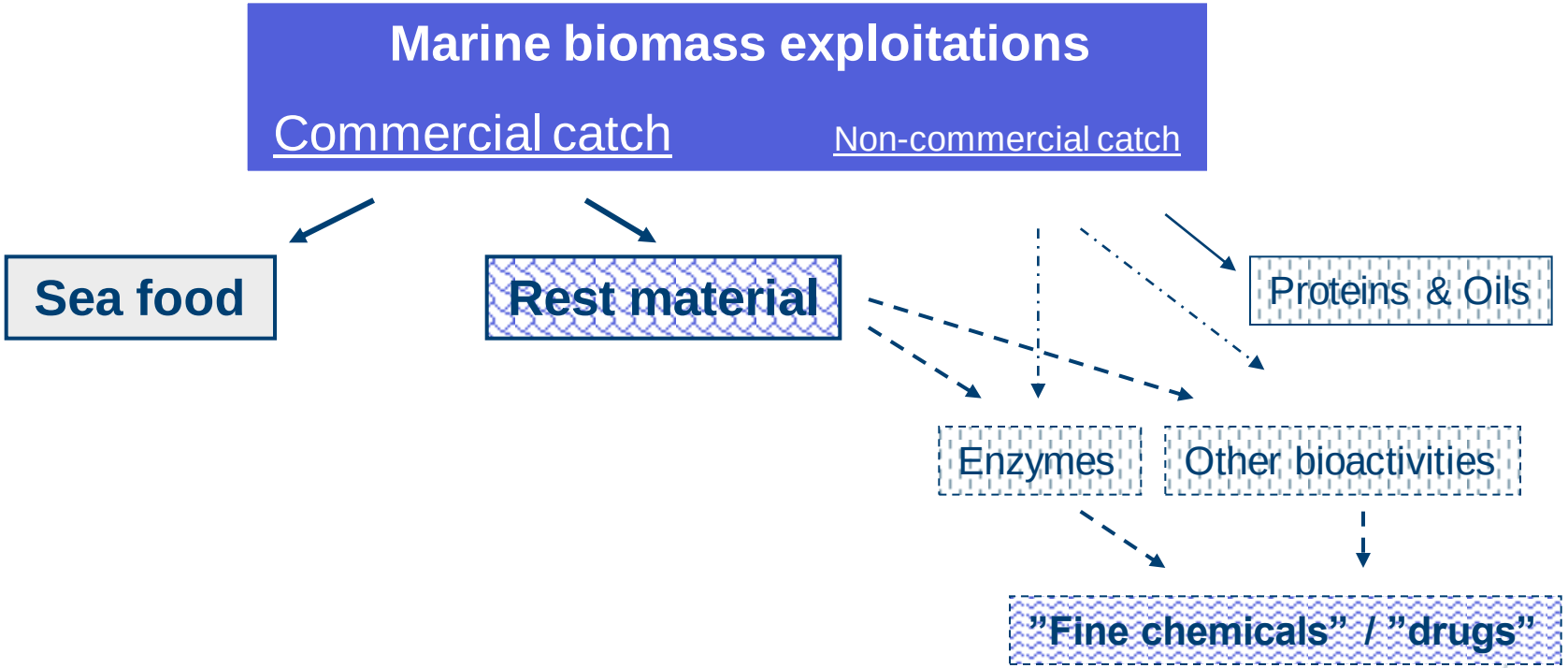
Animal feed
Enzymatic hydrolysis
Human consumption
Refinement


Marine biomass exploitations
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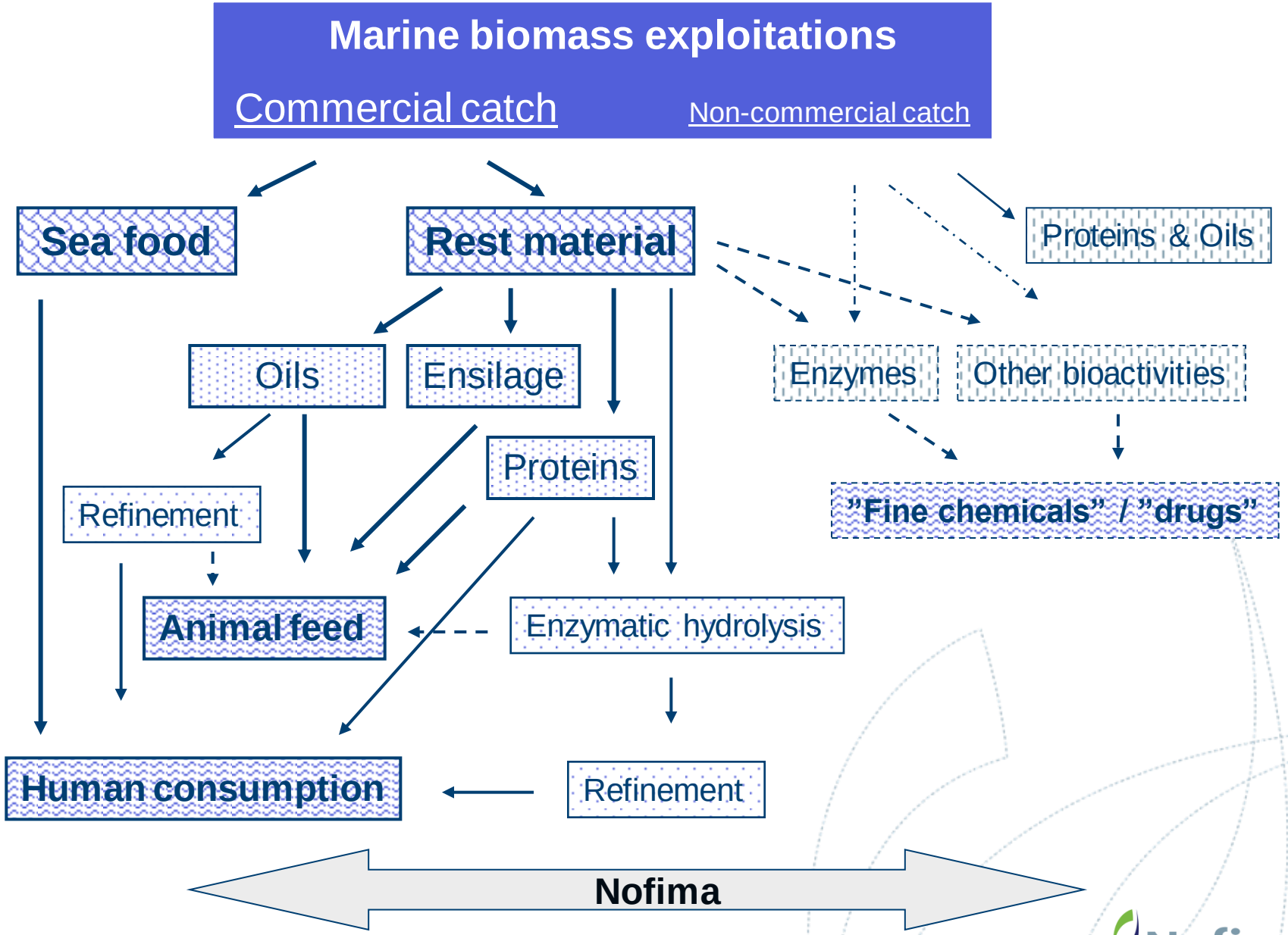
Proteins & Oils
Enzymes
Other bioactivities
"Fine chemicals" / "drugs"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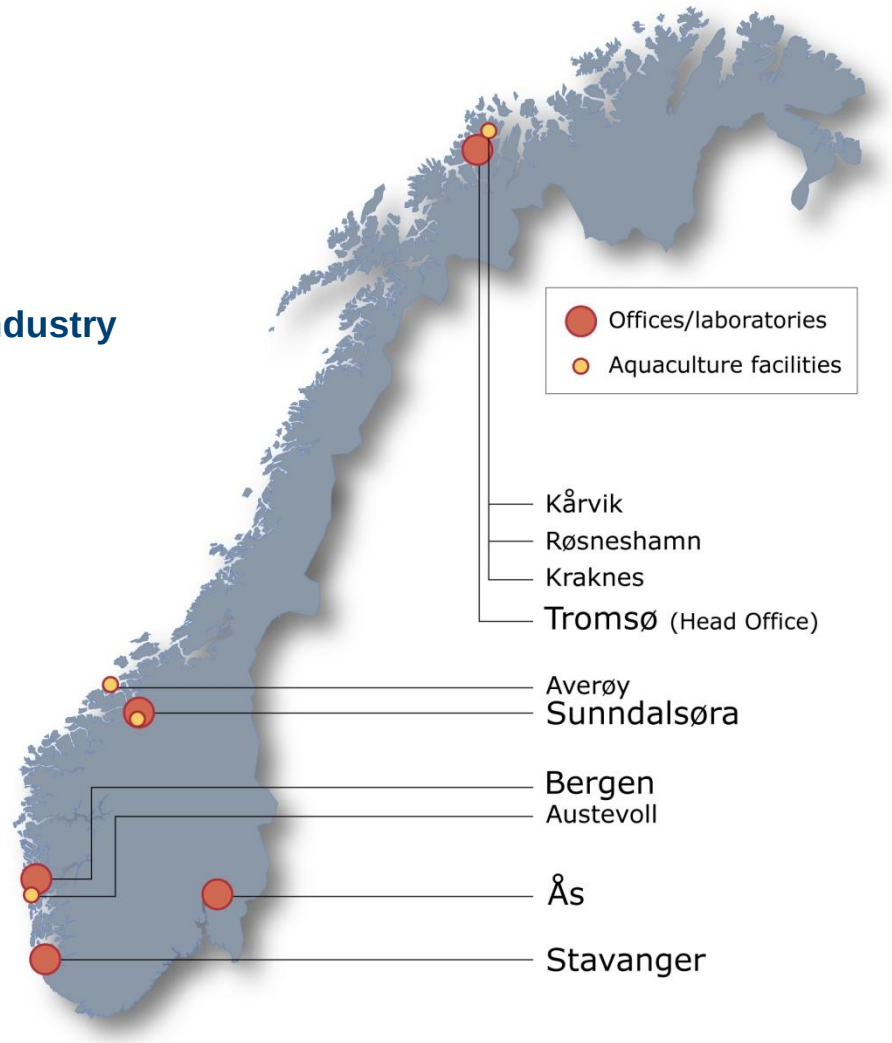
Nofima

NOFIMA AS – A merge between 4 sector institutes

- **Target sectors: Aquaculture, Food & Seafood Industry**
- **Employees: 450 (app. 230 holding a Ph.D)**
- **Turnover 2010: 470 mill NOK (~ € 60 mill)**
- **Head office in Tromsø**



2012 - Nofima opens

National Pilot Facility for Marine Bioprocessing

- Investments hardware: € 3 millions
- Module-based process line (*roll in/out* - connect/disconnect units)
- ”Complete” process from raw material to product
- Small industrial scale processes (~ 1,000 liters batches)
- Approved for food supplement / ingredient production

National Pilot Facility for Marine Bioprocessing

- Multi-purpose pilot facility for premarketing and product testing
- To facilitate process development programs
- To handle and process different types of marine raw materials
- Production of dried components from extracted or hydrolyzed products
- Unrefined marine oil (side-) products

Prospecting and industrial processing – the challenge: from bench to industrial scale

- Lab-scale bioactivity screening → isolation of compounds
- Up-scaling to industrial processes

Ex.: evaporation on bench and in pilot scale

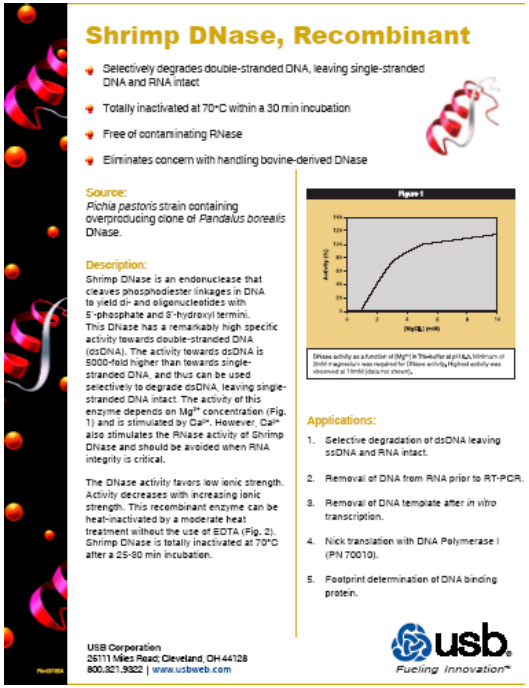
”Bioprospecting” at Nofima:

Why rest materials?

- **Biomass is sustainable - ”waste” from sea food production**
- **Huge volumes available**
  - sufficient amount of material from ”hit” to identification
  - reliable taxonomy
  - source for yielding native bioactivities
- **Known but underexplored species**
  - bioactivities largely unknown – interesting activities revealed

”Bioprospecting” at Nofima:

Commercialised enzymes from shrimp processing (”waste”) water



Shrimp DNase, Recombinant

- Selectively degrades double-stranded DNA, leaving single-stranded DNA and RNA intact
- Totally inactivated at 70°C within a 30 min incubation
- Free of contaminating RNase
- Eliminates concern with handling bovine-derived DNase

Source:
Pichia pastoris strain containing overproducing clone of *Pandalus borealis* DNase.

Description:
Shrimp DNase is an endonuclease that cleaves phosphodiester linkages in DNA to yield di- and oligonucleotides with 5'-phosphate and 3'-hydroxyl termini. This DNase has a remarkably high specific activity towards double-stranded DNA (dsDNA). The activity towards dsDNA is 5000-fold higher than towards single-stranded DNA, and thus can be used selectively to degrade dsDNA, leaving single-stranded DNA intact. The activity of this enzyme depends on Mg^{2+} concentration (Fig. 1) and is stimulated by Ca^{2+}. However, Ca^{2+} also stimulates the RNase activity of Shrimp DNase and should be avoided when RNA integrity is critical.

The DNase activity favors low ionic strength. Activity decreases with increasing ionic strength. This recombinant enzyme can be heat-inactivated by a moderate heat treatment without the use of EDTA (Fig. 2). Shrimp DNase is totally inactivated at 70°C after a 25-30 min incubation.

Figure 1

DNase activity as a function of (Mg^{2+}) in 10mM tris pH 8.0. A minimum of 2mM magnesium was required for DNase activity. Highest activity was observed at 10mM (data not shown).

Applications:
1. Selective degradation of dsDNA leaving ssDNA and RNA intact.
2. Removal of DNA from RNA prior to RT-PCR.
3. Removal of DNA template after *in vitro* transcription.
4. Nick translation with DNA Polymerase I (PN 70010).
5. Footprint determination of DNA binding protein.

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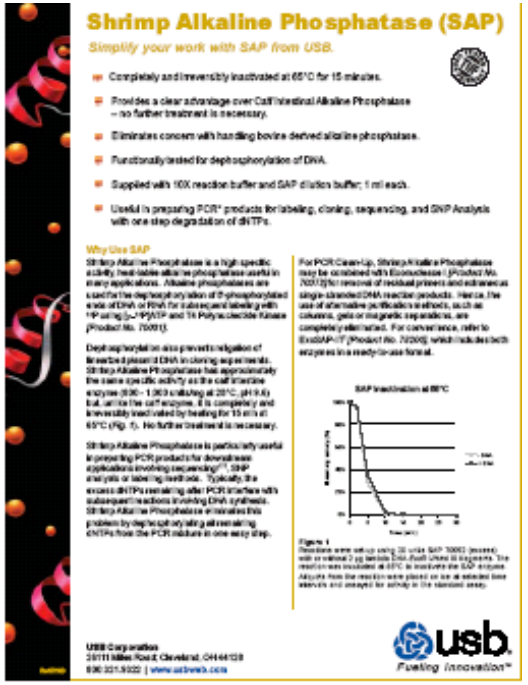
26111 Miles Road, Cleveland, OH 44128
800.321.9922 | www.usbweb.com

usb
Fueling Innovation™

Nilsen I.W., Sandsdalen E. and Stenberg E. (2003) A method of removing nucleic acid contamination in amplification reactions. US patent 6,541,204.



Shrimp Alkaline Phosphatase (SAP)

Simplify your work with SAP from USB.

- Completely and irreversibly inactivated at 65°C for 15 minutes.
- Provides a clear advantage over Calf Intestinal Alkaline Phosphatase – no further treatment is necessary.
- Eliminates concern with handling bovine-derived alkaline phosphatase.
- Functionally tested for dephosphorylation of DNA.
- Supplied with 10X reaction buffer and SAP dilution buffer, 1 ml each.
- Useful in preparing PCR* products for labeling, cloning, sequencing, and SNP Analysis with one step degradation of dNTPs.

Why Use SAP

Shrimp Alkaline Phosphatase is a high specific activity, heat-labile alkaline phosphatase useful in many applications. Alkaline phosphatases are used for the dephosphorylation of 5'-phosphorylated ends of DNA or RNA for subsequent labeling with ^{32}P using [γ-^{32}P]ATP and T4 Polynucleotide Kinase (Product No. 70031).

Dephosphorylation also prevents religation of linearized plasmid DNA in cloning experiments. Shrimp Alkaline Phosphatase has approximately the same specific activity as the calf intestine enzyme (830 - 1,300 U/mg at 25°C, pH 9.0) but, unlike the calf enzyme, it is completely and irreversibly inactivated by heating for 15 min at 65°C (Fig. 1). No further treatment is necessary.

Shrimp Alkaline Phosphatase is particularly useful in preparing PCR products for downstream applications involving sequencing[1], SNP analysis or labeling methods. Typically, the excess dNTPs remaining after PCR interfere with subsequent reactions involving DNA synthesis. Shrimp Alkaline Phosphatase eliminates this problem by dephosphorylating all remaining dNTPs from the PCR mixture in one easy step.

For PCR Clean-Up, Shrimp Alkaline Phosphatase may be combined with Exonuclease I (Product No. 70073) for removal of residual primers and extraneous single-stranded DNA reaction products. Hence, the use of alternative purification methods, such as columns, gels or magnetic separations, are completely eliminated. For convenience, refer to ExoSAP-IT® (Product No. 70200), which includes both enzymes in a ready-to-use format.

SAP Inactivation at 65°C

Figure 1

USB Corporation

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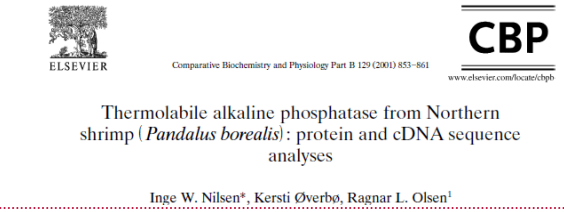
Fueling Innovation™

Nilsen I.W., Øverbø K., and Lanes O. (2007) Shrimp alkaline phosphatase. European Patent EP1326890.

Nofima

Published studies on marine enzymes - Nofima



ELSEVIER

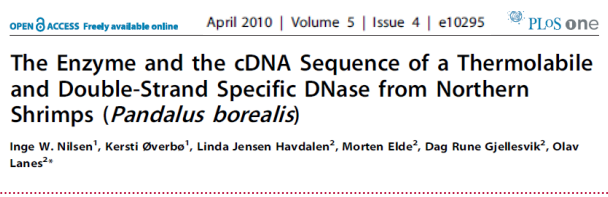
Comparative Biochemistry and Physiology Part B 129 (2001) 853–861

CBP

www.elsevier.com/locate/cbpb

Thermolabile alkaline phosphatase from Northern shrimp (*Pandalus borealis*): protein and cDNA sequence analyses

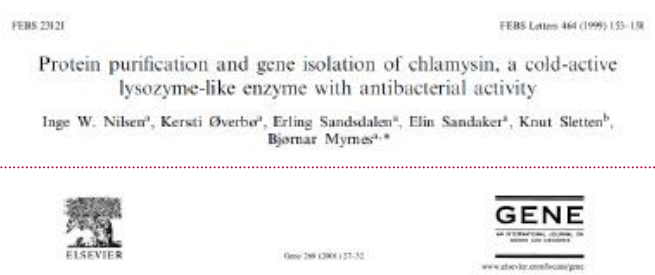
Inge W. Nilsen*, Kersti Øverbø, Ragnar L. Olsen[1]



OPEN ACCESS Freely available online April 2010 | Volume 5 | Issue 4 | e10295 PLoS one

The Enzyme and the cDNA Sequence of a Thermolabile and Double-Strand Specific DNase from Northern Shrimps (*Pandalus borealis*)

Inge W. Nilsen[1], Kersti Øverbø[1], Linda Jensen Havdalen[2], Morten Elde[2], Dag Rune Gjellesvik[2], Olav Lanes[2]*



FEBS 2121

FEBS Letters 464 (1999) 133–138

Protein purification and gene isolation of chlamysin, a cold-active lysozyme-like enzyme with antibacterial activity

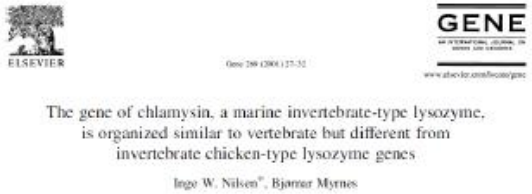
Inge W. Nilsen[a], Kersti Øverbø[a], Erling Sandsdalen[a], Elin Sandaker[a], Knut Sletten[b], Bjørnar Myrnes[a,*]

Research Article

Cell. Mol. Life Sci. 64 (2007) 2841 – 2847
1420-682X/07/212841-7
DOI 10.1007/s00018-007-7372-8
© Birkhäuser Verlag, Basel, 2007

A cold-active salmon goose-type lysozyme with high heat tolerance

P. Kyomuhendo, B. Myrnes and I. W. Nilsen*



ELSEVIER

Gene 269 (2001) 27–32

GENE

www.elsevier.com/locate/gene

The gene of chlamysin, a marine invertebrate-type lysozyme, is organized similar to vertebrate but different from invertebrate chicken-type lysozyme genes

Inge W. Nilsen*, Bjørnar Myrnes

Comparative Biochemistry and Physiology, Part B

Thermodynamics and structure of a salmon cold active goose-type lysozyme

Peter Kyomuhendo[a,b,1], Bjørnar Myrnes[a,1], Bjørn-Olav Brandsdal[b,c], Arne O. Smalås[b], Inge W. Nilsen[a,c], Ronny Helland[b,c]

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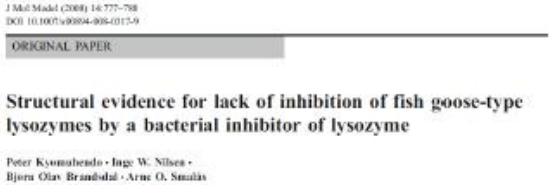
Comparative Biochemistry and Physiology Part B 136 (2003) 103–115

CBP

Multiple invertebrate lysozymes in blue mussel (*Mytilus edulis*)

Ørjan M. Olsen[a], Inge Waller Nilsen[a], Knut Sletten[b], Bjørnar Myrnes[a,*]

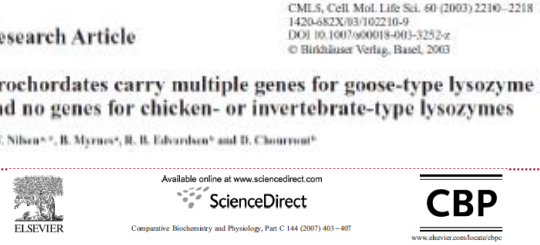


J Mol Model (2008) 14:777–788
DOI 10.1007/s00894-008-0317-9

ORIGINAL PAPER

Structural evidence for lack of inhibition of fish goose-type lysozymes by a bacterial inhibitor of lysozyme

Peter Kyomuhendo · Inge W. Nilsen · Bjørn Olav Brandsdal · Arne O. Smalås



Research Article

CMLS, Cell. Mol. Life Sci. 60 (2003) 2210–2218
1420-682X/03/102210-9
DOI 10.1007/s00018-003-3252-z
© Birkhäuser Verlag, Basel, 2003

Urochordates carry multiple genes for goose-type lysozyme and no genes for chicken- or invertebrate-type lysozymes

I.W. Nilsen[a,*], B. Myrnes[a], R.B. Edvardsen[b] and D. Chourrout[b]

Cell. Mol. Life Sci.
DOI 10.1007/s00018-010-0507-3

Cellular and Molecular Life Sciences

RESEARCH ARTICLE

Identification of a bacterial inhibitor against g-type lysozyme

L. Vanderkelen · J. M. Van Herreweghe · K. G. A. Vanmarcke · G. Baggerman · B. Myrnes · P. J. Declerck · I. W. Nilsen · C. W. Michiels · L. Callewaert

ELSEVIER

Available online at www.sciencedirect.com

ScienceDirect

Comparative Biochemistry and Physiology, Part C 144 (2007) 403–407

CBP

www.elsevier.com/locate/cbpc

Glutathione *S*-transferase from the Icelandic scallop (*Chlamys islandica*): Isolation and partial characterization

Bjørnar Myrnes*, Inge W. Nilsen

ELSEVIER

Available online at www.sciencedirect.com

SCIENCE DIRECT

Comparative Biochemistry and Physiology, Part B 143 (2006) 315–318

CBP

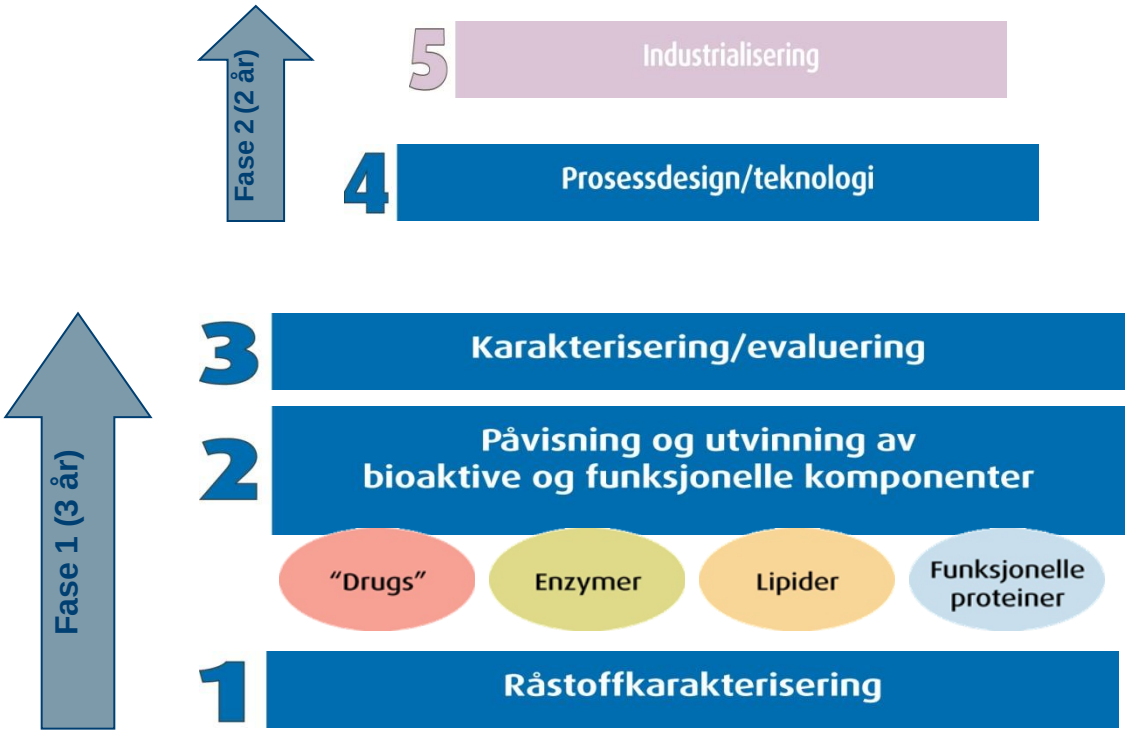
www.elsevier.com/locate/cbpb

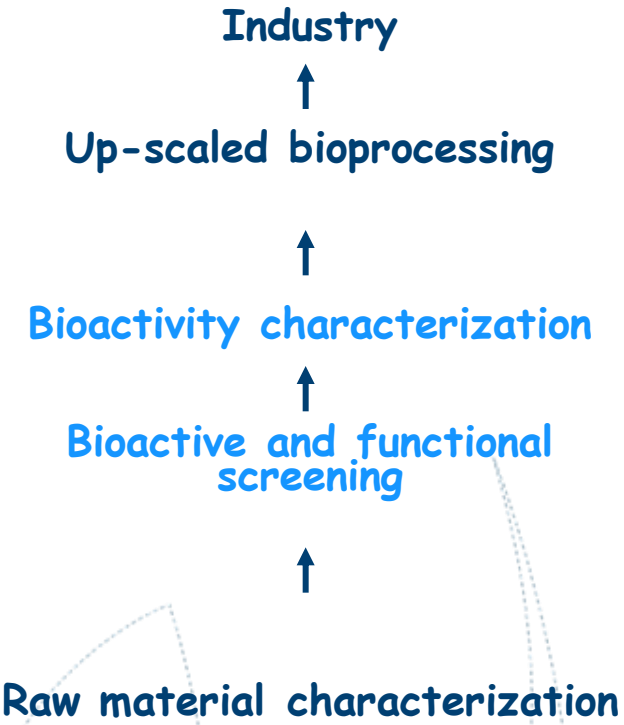
Deoxyribonuclease II from the Icelandic scallop (*Chlamys islandica*): Isolation and partial characterization

Kersti Øverbø, Bjørnar Myrnes*

Current project example:

Gold from the silvery sea





Unique and broad mapping of bioactivities in one type biomass

Gold from the silvery sea

- Project in its second year
- Approximately 50 enzymes / classes under investigation
- Inhibitor-activities against 9 drug targets screened for / under investigation

- Alternative source of known enzyme activities ?
- Source of new or special feature enzyme activities ?
- Can large-scale processing be used for industrial production ?
- Templates for novel drugs ?

Nofima

Other bioprospecting projects

- Focusing on ”drug”-like (small molecule) marine bioactivities
- Includes international collaborations
  - Instituto Superior Técnico, Lisboa – PhD student research visit at Nofima:

    ”Drugs” to enhance efficiency of delivery systems for DNA in non-viral gene therapy or vaccination

- Coordinated proposal to EU (KBBE2012)

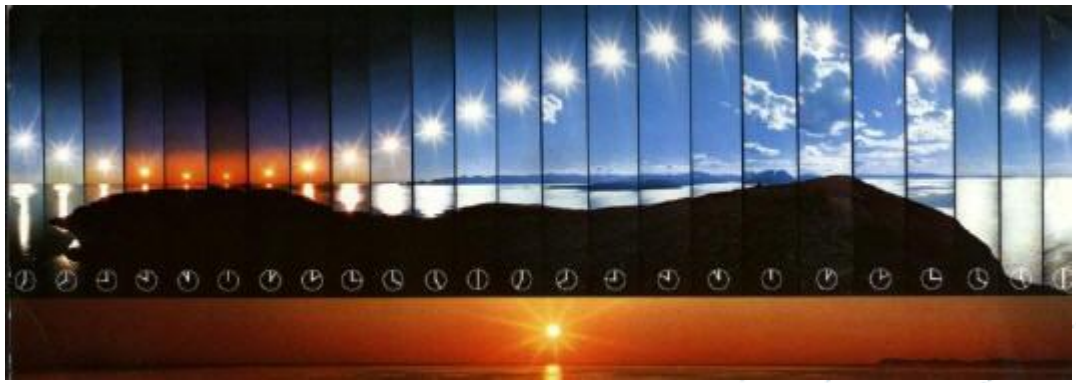
  SmallBlue – Small molecules with big opportunities for blue biotech industry

  *(*new technologies for discovering drugs against 35 targets related to cancer, CNS and viral / bacterial diseases*)*

  14 partners involved, including:
  - Instituto Superior Técnico
  - Instituto Politécnico de Leiria

With greetings from Tromsø - thank you !

Aurora borealis (January)



Sun position (24 h - June)

Nofima