

# ”A Systems Biology approach towards the understanding of Microbial Processes in Biofilms”

- the dependency between problem solving and basic research

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# Disposition

- The road from infectious problems in saturation diving to basic science in Biofilm
- Presentation of SINTEF collaborators in the SINTEF target programme on BIOFILM and Systems Biology
  - Local SINTEF/NTNU collaborators
- Presentation of Biofilm projects and collaborators outside SINTEF
- Biofilm and Systems Biology – potentials for future research and applications
  - invitation to cooperation

# SINTEF Health Research - Microbiology

## ■ A small unit



## ■ Broad microbiological competence

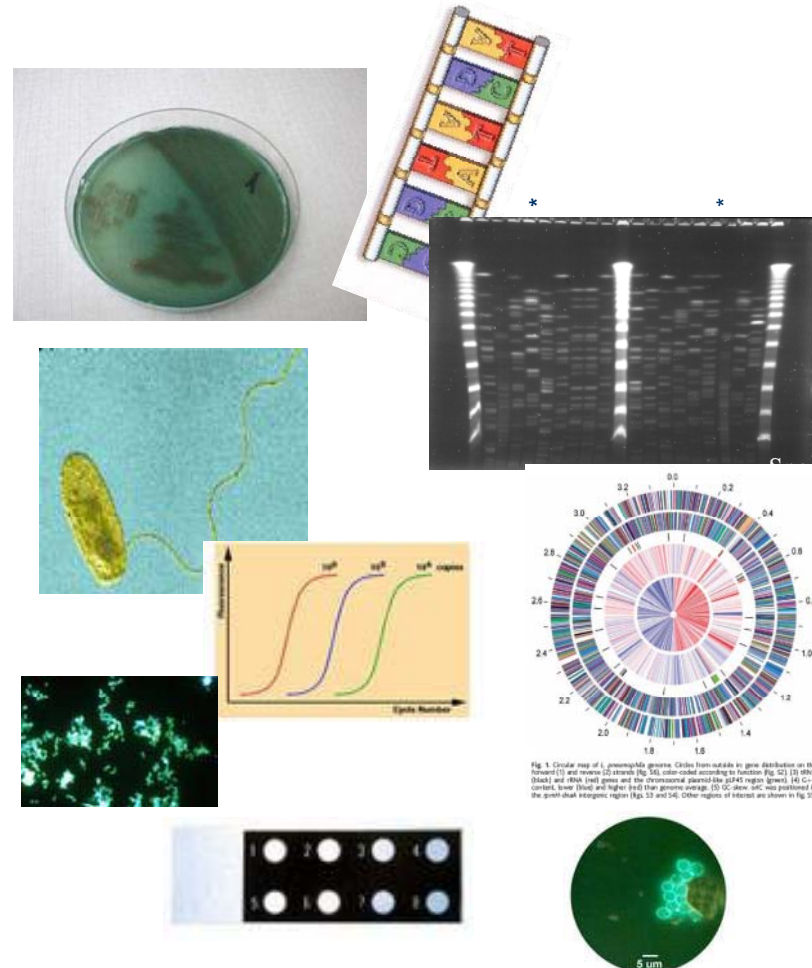
- Bacteriology
- Virology
- Mycologi
- Parasitology
- Water flora (fresh/sea)

## ■ Dpt Microbiology, St Olavs Hospital

## ■ Special skills

- *P. aeruginosa*
- *L. pneumophila*
- Immunologi

## ■ Water and infectious microbes





# In 1977, the world could first read about the specific role of *P. aeruginosa* in infection outbreaks in diving systems

- *Alcock SR. (1977). Acute otitis externa in divers in the North Sea. A microbiological survey of seven saturation dives. Journal of Hygiene (Camb) 78:395-409*
- The same serotype of *P. aeruginosa* (O12) in several divers made up the following hypothesis:
  - **person to person contagion**
  - **the divers "contaminate the environment"**
- The study concluded that infections are brought into the systems and are spread by – the divers

- and this was still common sense by the time for start of the systematic *R&D in Diving Medicine*\*  
1985

■ **HYGIENE**

- Personal hygiene
- Daily showering, antiseptic soap

■ **PREVENTION**

- Frequent control of divers
  - Ear samplings pre- and post saturation
- Profylaxis (ear drops)

■ **New information:**

*P. aeruginosa* only  
post saturation!

- \*R&D Diving programme: Statoil, Norsk Hydro, Saga, OD/Ptil, Esso



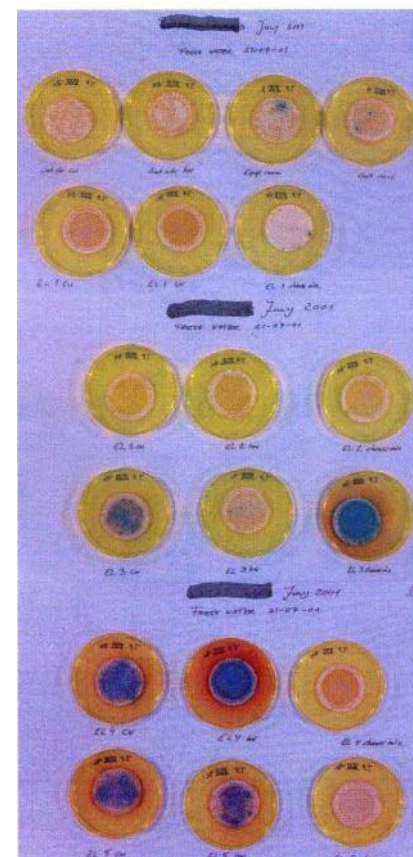
- The divers became sampling specialists

# This new information "demanded" environmental surveys – the first in 1986

## - For determination of relations to infections

- Water (freshwater)
  - Chamber external
  - Chamber internal
  - FW Tanks
- Environment
  - Shower equipment in chambers
  - Divers personal equipment
- The material is now the base plate in our unique biobank from the North Sea

## ■ Typical picture from initial water analyses



# *Pseudomonas aeruginosa* - "North Sea" Biobank (1)

- Serotyping 1985-1994 – same serotype in infections and water
  - Serotype O 11

- 1995 – a new tool - PFGE
  - Pulsed Field Gel Electrophoresis
  - Genetic finger-printing

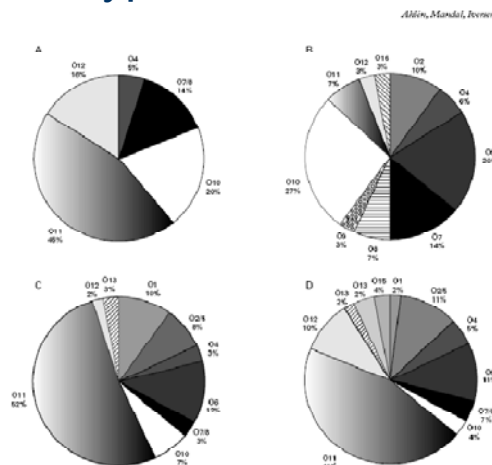
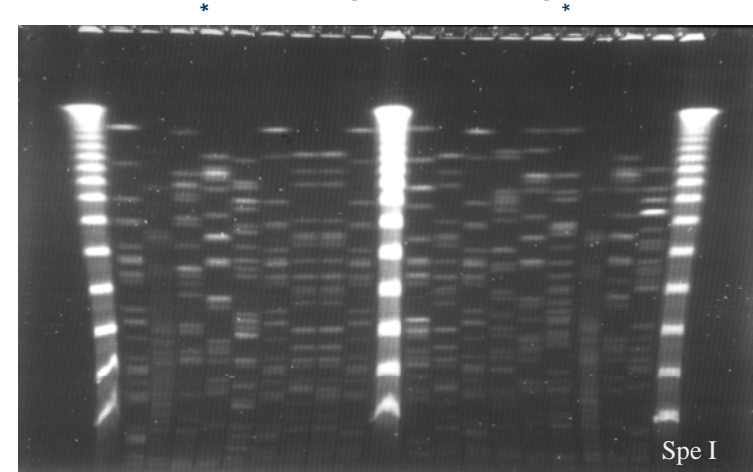


Figure 2. *Pseudomonas aeruginosa* O serotypes from (A) divers' skin infections (n=120), (B) from skin infections in non-divers (n=43), (C) from fresh water nano chambers (n=36), and (D) from the occupational saturation environment (n=150).

Ahlén C, Mandal LH, Iversen OJ. Identification of infectious strains of *Pseudomonas aeruginosa* in occupational saturation environment. *Occup Environ Med* 1998; 55:480-484

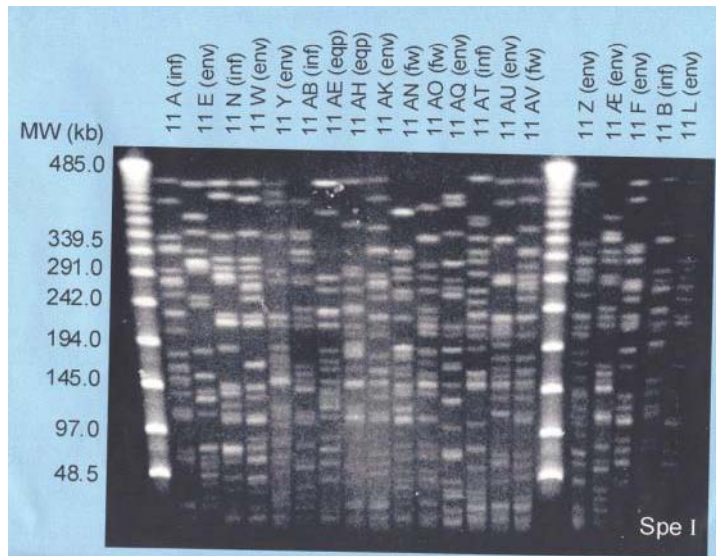


Ahlén C, Mandal LH, Johannessen, L, Iversen OJ. Survival of infectious *Pseudomonas aeruginosa* genotypes in occupational saturation environments and the significance of these genotypes for recurrent skin infections. 2000. *Am. J. Ind. Med.* 2000; 37 : 493-500

# *Pseudomonas aeruginosa*

## - "North Sea" Biobank (2)

- Serotyping versus PFGE
  - 20 Serotype O11 – 20 separate PFGE genotypes

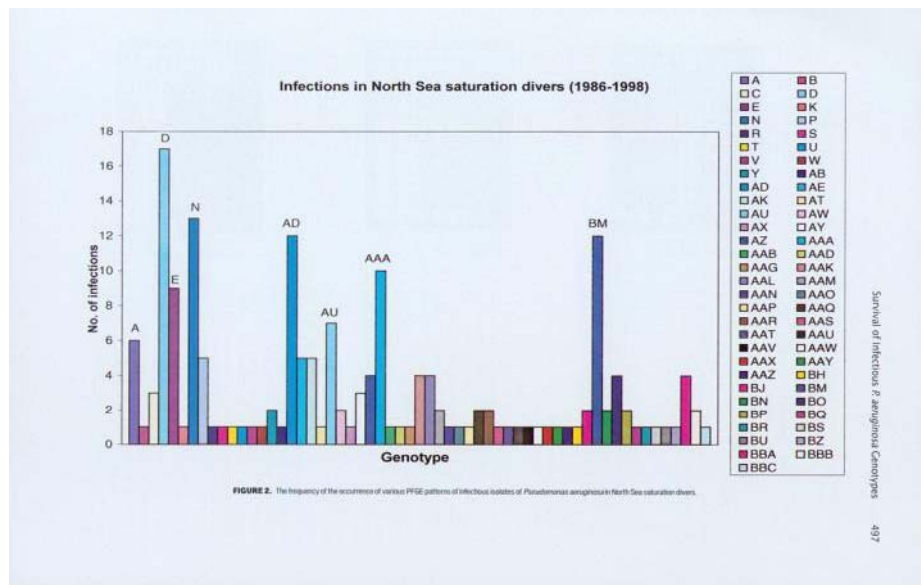


- Ahlén C, Mandal LH, Iversen OJ :The Impact of Environmental *Pseudomonas aeruginosa* Genotypes on Skin Infections in Occupational Saturation Diving Systems. *Scand.J Inf Dis.*33; 413-419, 2001

- Although serotyping was the gold standard for epidemiology of *P. aeruginosa* by that time
  - the tag "serotype" showed not to be useable for traceability of infection reservoirs within diving systems
    - and is most likely not usable for any source or reservoir detection
- Since 1995, PFGE has been our method for epidemiology of *P. aeruginosa*
  - and is today the gold standard for epidemiology of *P. aeruginosa* worldwide

# *Pseudomonas aeruginosa* - "North Sea" Biobank (3)

- Retrospective PFGE 1985 – 2000

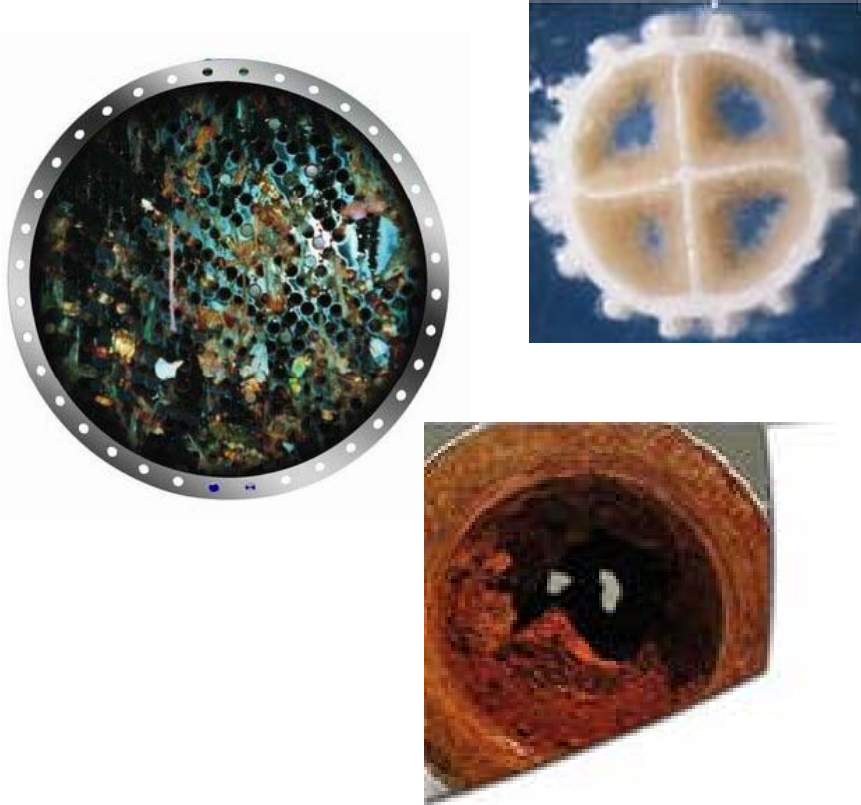


Ahlén C, Mandal LH, Johannessen, L, Iversen OJ. Survival of infectious *Pseudomonas aeruginosa* genotypes in occupational saturation environments and the significance of these genotypes for recurrent skin infections. 2000. *Am. J. Ind. Med.* 2000; 37:493-500

- Only a very few genotypes frequent in infections!
  - some of which seen for more than 15 years
    - despite regular disinfection regimes
  - some causes infections again and again
    - others are never seen in infections
- Documented presence prior to divers entrance
- Where do they hide?
  - and how?

# Long time persistence of infectious *P. aeruginosa* - the role of Biofilm?

- Biofilm is the microbiological society established within water systems



- Biofilm challenges

- 2006 Biofilm - training ground for pathogenic microbes

- *Maëlle Molmeret M., Horn M., Wagner M., Santic M. and Kwaik Y. (2005) Amoebae as Training Grounds for Intracellular Bacterial Pathogens. Appl. Environ. Micro. Vol. 71, No. 1. p. 20-28.*

- 2006 Biofilm generates genetic diversity!

- *Kolter R and Greenberg EP.: The superficial life of microbes. Nature Vol 441. May 18th, 2006.*

# R&D diving - 2007-2011

## - now also including Biofilm

- Elimination of infectious *P. aeruginosa* 2007 - 2011
  - Genetic sub-grouping - Biobank material (SINTEF/ R&D Diving)
  
- Biofilm establishment in water systems in new diving vessels
  - Field studies (SINTEF/R&D Diving)
    - Technip "Scandi Arctic" – 2008-2011
  
- Biofilm establishment and interventions
  - studies by experimental biofilm systems on medical and environmental biofilms (SINTEF/R&D Diving and UiB -Bio, MIC, Haukeland Micro)
    - Flow-cells and Confocal Laser Scanning Microscopy (CLSM) 2009 -2011



# 2007- SINTEF/NTNU Joint focus on Microbial Ecology: Biotechnology, Health, Environmental Microbiology and Aquaculture

## SINTEF

- T&S, Health, Medical technology – Microbiology
  - Senior Scientist Catrine Ahlén (project leader)
- M&C, Marine Environmental Technology
  - Senior Scientist Odd Gunnar Brakstad
- M&C, Biotechnology
  - Scientists Inga Marie Aasen and Kolbjørn Zahlsen
- Marine, Fisheries and Aquaculture, Marine resources Technology
  - Senior Scientist Jorunn Skjermo

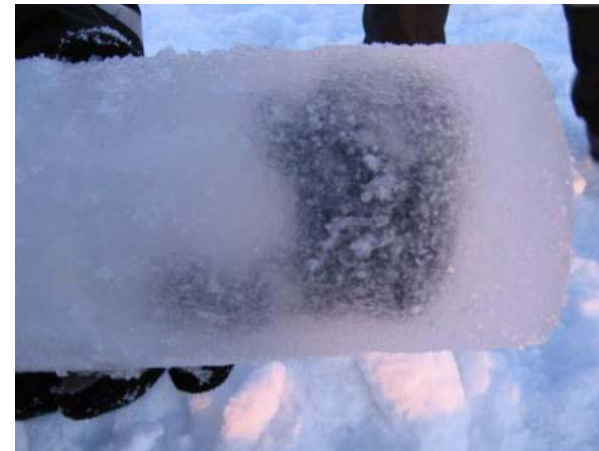
## NTNU

- Biotechnology
  - Professor Olav Vadstein

# SINTEF Materials and Chemistry

## Marine Environmental Technology

- Studies of biofilm communities and processes on oil surfaces  
1988 –
  - Offshore oil reservoirs
  - Oil spills in cold environments (Seawater, seabed sediments, marine ice)



# SINTEF Materials and Chemistry

## Marine Environmental Technology

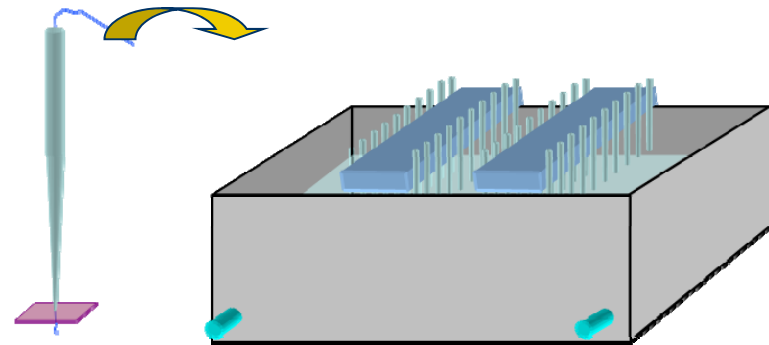
- Fouling studies 1993 -
  - Detection and characterization of biofilms in marine installations
  - Prevention and treatment of biofilms



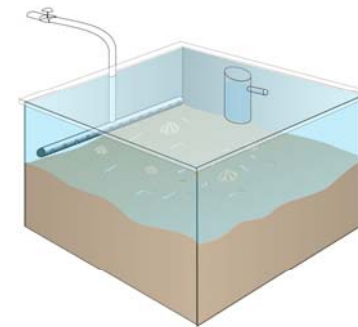
# Experimental systems – laboratory

## SINTEF Marine Environmental Technology

- Seawater flow-through surface systems for biofilm generation
- Flow-through systems for studies of biofilms in marine porous sediment systems



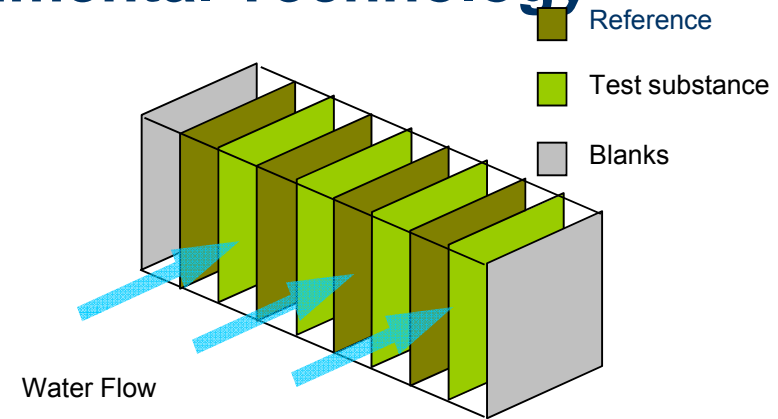
*Seawater flow-through system for generation of biofilms growing on immobilised oil films*



# Experimental systems – Field

## SINTEF Marine Environmental Technology

- Field testing panels for and nets for studies and prevention of biofouling on steel steel surfaces
- Field testing of marine growth on fish farm nets



*Steel panels for biofilm studies*



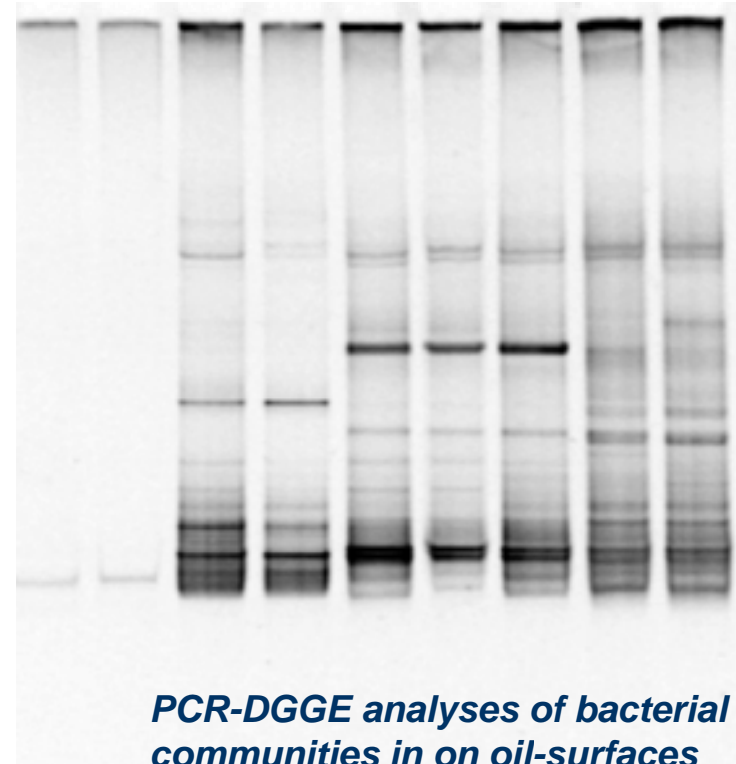
*Field test systems for marine growth on fish farm nets (Photo T. Nordtug)*

# Analyses of biofilms

## SINTEF Marine Environmental Technology

### ■ Analyses

- *Fluorescence microscopy*
- *Changes in microbial communities (PCR-DGGE; cloning-sequencing analyses)*
- *Expression systems in biofilms*
  - *qPCR*
  - *mRNA-based suppression-subtractive hybridization (SSH) libraries*
- *Quorum sensing analyses with luminescent bacterial cultures*
- *Chemical analyses of microbial degradation products*
  - *GC-FID, GC-MS, LC-MS*



*PCR-DGGE analyses of bacterial communities in on oil-surfaces (Photo: O.G. Brakstad, SINTEF)*

# SINTEF Materials and Chemistry

## Department of Biotechnology

### ■ Microbial biotechnology

- Cultivation of free-living cells
  - Micro-well plates
  - Shake flasks
  - Fermentors
- On-line analyses
  - pH, dissolved oxygen, exhaust gas composition
- Systems biology
  - Alginate synthesis in *Pseudomonas fluorescense*



### ■ Biofilms

- Inhibition of medical relevant biofilms
- Cultivation in micro-well plates and biofilm reactors



# SINTEF Materials and Chemistry

## Department of Biotechnology

### ■ Analyses of microbial metabolites

- GC-MS1 (MCF derivatization; org. acids, amino acids; database: >100 compound peaks)
- GC-MS2 (silylation; e.g. sugars, sugar alcohols, org. acids, phospho-metabolites; database: >1000 compounds)
- LC-MS1 (phospho-metabolites, org. acids; database: 21 compounds)



### ■ Planned future developments:

- Expansion of databases
- **Metabolic footprint analysis (exo-metabolome)**
- **Metabolic fingerprint analysis (endo-metabolome)**

**Biofilms**

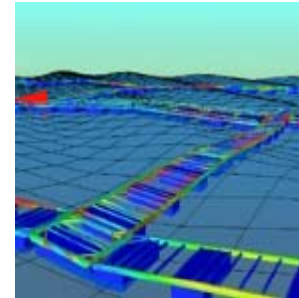
### ■ Instruments:

5xLC-MS-SQ, LC-MS-TOF, LC-MS-QTOF, 2xLC-MS-QQQ, CE, LC-MS-Trap, 3xGC-MS-SQ, GCxGC-TOF(2010)

underlined: most relevant for metabolite analyses

# SINTEF Fisheries and Aquaculture

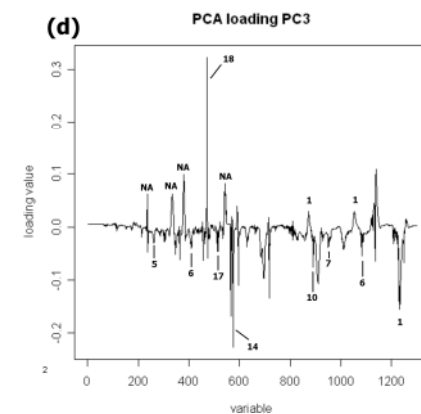
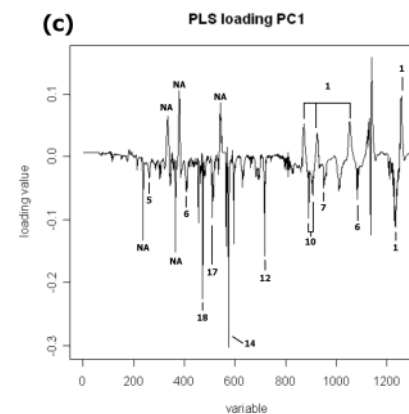
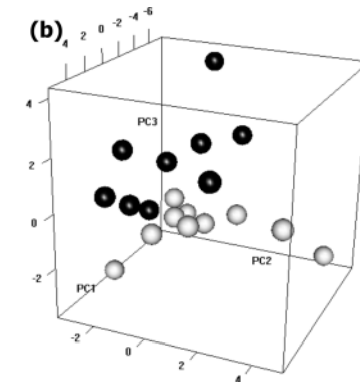
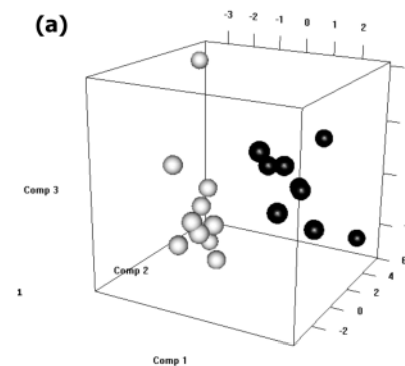
- Aquaculture Technology
- Fisheries Technology
  
- Marine Resources Technology
  - Live feed
  - Microbial control
  - Water treatment
  - First feeding technology
  - Microalgae
  - Probiotic bacteria
  - Immunostimulants
  - Seaweeds
  - Integrated aquaculture
  
- Processing Technology



# SINTEF Fisheries and Aquaculture - NMR Metabolomics



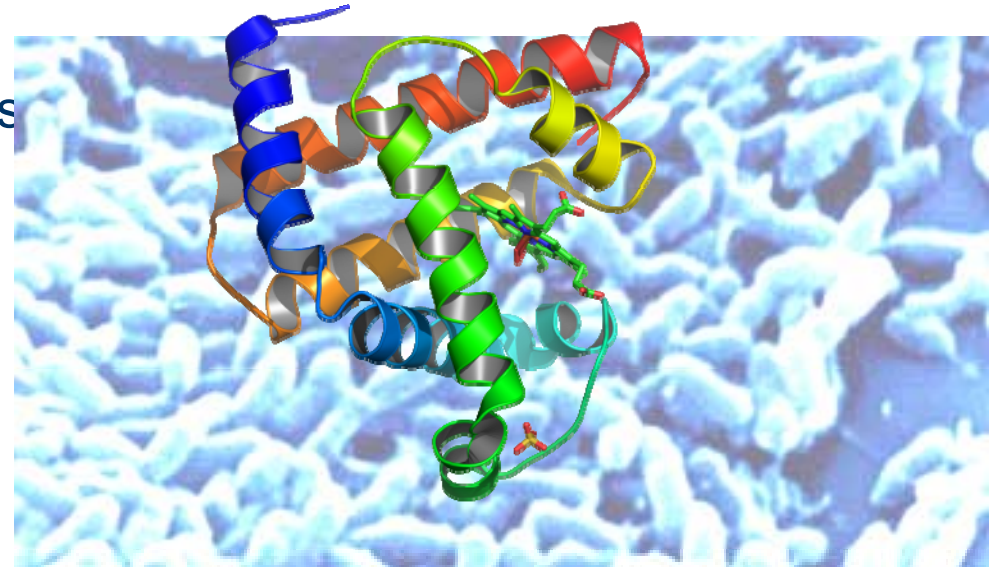
- **Metabolic fingerprints** are recorded using State of the art high field NMR
- **Multivariate analysis** is used to find differences in fingerprints between groups
- Differences are identified to give metabolites
- Gives changes in phenotype.
- Example is from SINTEF project *Calanomics*. *Calanus finmarchicus* exposed to chemical DEA. (○) control, (●) exposed.



# BIOFILM AND SYSTEMS BIOLOGY: A STRATEGIC PROJECT in SINTEF

- 2008-2011: SINTEF Target project on Biofilm:  
"A Systems Biology approach towards the understanding of Microbial Processes in Biofilms"

- Experimental biofilm systems
- Analytical systems:
  - Genomics
  - Transcriptomics
  - Metabolomics
- Modelling systems



- Model organisms: *Pseudomonas aeruginosa*, *Vibrio anguillarum* and *Desulfovibrio vulgaris*

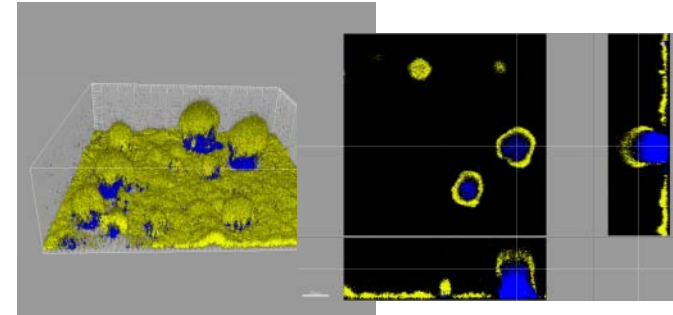
# Internal cooperation (SINTEF/NTNU)

## ■ SINTEF

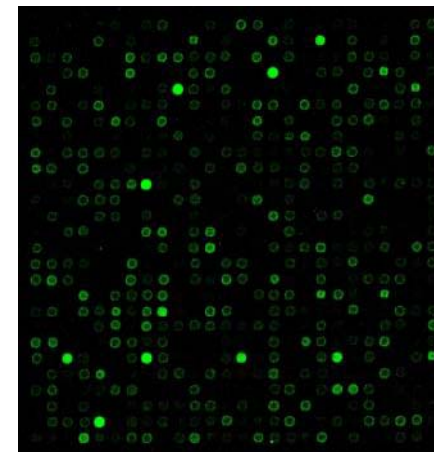
- Chemical expertise
  - *Synthetic anti-bacterial molecules*
  - *Molecule binding technology to surfaces*
- Material surface expertise
  - *Polymer and surface coating technology*
  - *Corrosion mechanisms and analyses*
- "Geminisenter - Medical Microbiology" (SINTEF/NTNU/St Olav's)
  - Biofilm
  - Genomics
  - Large scale serology

## ■ NTNU

- FUGE Node
  - Microarray Technologies
- Microbial ecology
- Microscope technology
- Master and PhD students



*Flow-cell grown Biofilm analysed by CLSM  
(Photo C Ahlén, SINTEF)*



*Microarray analyses of bacterial oil reservoir community  
(Photo Vidar Beisvåg, NTNU)*

# Present external collaborators

- Statoil (Norsk Hydro, Saga) Norske Esso, Ptil (R&D Diving)
  - Since 1988 – (2011)
- Acergy (1985- ) Technip (1992- ) Subsea7 (1991- )
- University of Bergen 2008 -
  - Med. Odont faculty
    - Bio – Rune E Skjåstad
  - Dept of Clin Dentistry (V. Bakken)
  - Molecular Imaging Center MIC
- University of Stravanger / IRIS
  - Studies on oil-related biofilms (R. Kommedal /G.K. Eilertsen)
  - Respirometric analyses in biofilms
- University of Oslo
  - Quorum sensing analyses (A. A. Scheie)
- Swedish Institute for Infectious Disease Control 2004 -
  - Parasitology (J Krusnell)
- DTU, Dpt of Systems Biology (S Molin) 2009 -

Exploring the Biofilm secrets requires a great  
specter of disciplines and expertise  
- there's no way to combate them but  
through cooperation 😊

