Michael Givskov
Professor, Director
Costerton Biofilm Center
Faculty of Health and Medical Sciences
University of Copenhagen, Denmark

Research Director
Singapore Centre on Environmental Life Sciences Engineering (SCELSSE)
Nanyang Technological University
Singapore
• I will provide my view on “Research Quality in Microbiology”.

• It is based on my scientific experience and the discoveries I have made during my 35+ years as a Microbiologist.
1954 Born in London. Danish citizen
1975 Mat-Phys High School Graduate, Svendborg Gymnasium
1975 Enroll at Cell Biology, University of Southern Denmark
1983 MSc in Cell Biology, University of Southern Denmark
1988 PhD in Microbiology, University of Copenhagen
1998 Post-Doc, Microbiolog DTU
1996 Associate Prof. of Microbiology, DTU
2003 Head of Centre of Biomedical Microbiology, BioCentrum, DTU
2004 Professor of Biomedical Microbiology, BioCentrum, DTU
2006 Dr. Techn. in Microbiology, DTU
2008- Professor of Biomedical Microbiology, Department of Immunology and Microbiology, University of Copenhagen
2011- Research Director, Singapore Center of Environmental Life Science, Nanyang Technological University, Singapore
2013- Director, Costerton Biofilm Center, University of Copenhagen
• Microbiology research involves bench work.
• Microbiology unravels “the invisible life”
• State of the art experimental “tool box”.
• Molecular biology-genetic engineering.
• You can do a lot without looking at the microbes
• What is the outcome of your experiment?
• Prove or disprove your hypothesis?
• Something you perceived out of your creative mind.
• You didn’t clean up the mess on your lab-bench.
• After 2 weeks you saw something really striking.
• Flemings playing around with stacks of old agar plates on a dirty lab-bench (not allowed today) in combination with his creative mind resulted in an enormous discovery and subsequent development of antibiotics.
• As a kid I was struggling with biofouling in my aquariums.
• As a student, I often left cultures at the bench and paid attention to aggregation in *E. coli* cultures containing natural R1 plasmids.
• But I didn’t realize I was watching biofilm development.
Letters to Nature

*Nature* 412, 442-445 (26 July 2001) | doi:10.1038/35086581; Received 8 March 2001; Accepted 6 June 2001

Natural conjugative plasmids induce bacterial biofilm development

Jean-Marc Ghigo

1. Unité des Membranes Bactériennes Institut Pasteur (CNRS URA 2172), 25 rue du Dr Roux, 75724 Paris Cedex 15, France
Why didn’t I see this?

• I worked within a particular framework.
• I was too focused on genetic engineering.
• I focused so much on techniques that I didn’t see what was ahead of me.
• I didn’t use my imagination and creativity to unravel the phenomenon.
Here’s what to do:

• Realize that you are watching a certain biological phenomenon
• Use your imagination and creativity to put up a model.
• Unravel the phenomenon with clever empiric experiments.
• Don't think there is a special ”recipe”.
• A “true scientist” is a guy who breaks the line and neglects conformity.
But it’s not simple:

• A simple colony color change can’t be a final proof of your hypothesis unless your experiment is extremely clever designed.
• Hypotheses are mostly build on a level of complexity that would need many subsequent experiments.
• They will deliver circumstantial evidences to produce the verdict over your hypothesis.
> 20 years ago, Leo Eberl and I (both working in the Molin lab, DTU) provided evidence that bacteria could organize by means of cell-cell signaling as manifested in swarming motility.
The bacteria reach the quorum size (colony) before they start moving over the surface.
Colony expansion
1-10 mm/hrs (0.3-3 µm/sec)

Group motility
10-50 fold faster
Increasing cell density

Quorum Sensing

Cell

Signal

Products
Nature has developed chemistry against surface colonization-biofilm formation and fouling

NOTES

Eukaryotic Interference with Homoserine Lactone-Mediated Prokaryotic Signalling

MICHAEL GIVSKOV,1 ROCKY DE NYS,2 MICHAEL MANEFIELD,3 LONE GRAM,4 RIA MAXIMILIEN,2 LEO EBERL,1 SØREN MOLIN,1 PETER D. STEINBERG,2 AND STAFFAN KJELLEBERG2*

Department of Microbiology,1 and Danish Institute for Fisheries Research, Department of Seafood Research,3 The Technical University of Denmark, DK-2800 Lyngby, Denmark, and School of Biological Science2 and School of Microbiology and Immunology,3 University of New South Wales, Sydney 2052, Australia

Received 6 June 1996/Accepted 13 September 1996
**FIG. 1.** Structure of AHIAs produced by *A. liquefaciens* and two furans produced by *D. pulchra*.

**FIG. 2.** Effect of increasing concentrations (0, 30, 50, and 100 μg/ml) of *D. pulchra* furanone 2 on *S. liquefaciens* swarming motility. Agar plates were stab inoculated at the center from an exponentially growing culture (OD₆₅₀ of approximately 0.5) and incubated at 30°C. Swarming colonies were photographed 20 h after inoculation.
Inhibition of the QS controlled color reaction of *Chromobacterium*
• Total citations 470, still being cited

Annual citations
Delisea pulchra chemistry was developed into a functional antimicrobial principle...delivering the “proof of concept” that blockage of quorum sensing with an experimental drug could function as a biofilm antimicrobial in vivo.
Furanone C-30 was an almost perfect example of a chemical biology approach to block QS

Furanone C-30 targets the LasR and RhlR Q-sensors

Furanone C-30 reduces expression (> 5 fold) of 85% of the QS regulated *P. aeruginosa* genes.
QSI’s show synergy with tobramycin

A + Tobra

B Fur C-30 + Tobra
Furanone C-30 treatment (2 µg/g body weight)
Start inoculum

Saline treatment

Fur 30 treatments

CFU/lung

Mouse 1, 2, 3 .... etc
• Citations 750, still going strong

Anual citations

- 2002
- 2004
- 2006
- 2008
- 2010
- 2012
- 2014
- 2016

Citations: 750, still going strong
The “proof of concept” EMBO J., paper was evaluated 26 Aug 2003 and selected by the Faculty 1000. Eric Gilbert from the Georgia State University, United States wrote:

Read this paper and see the future of infectious disease control. This research is the most recent publication in a series of groundbreaking works by the authors applying their findings on the microbial ecology of bacterial cell-cell signaling, or quorum sensing, to the control of bacterial pathogens. The paper describes the effect of a synthetic quorum sensing inhibitor on gene expression by Pseudomonas aeruginosa (the aggressive pathogen lethal to many cystic fibrosis sufferers), but even more impressively demonstrates how this compound contributes to the clearance of biofilm-based infections in the lungs of infected mice. The work is the most significant demonstration to date of the potential for controlling disease by influencing bacterial quorum sensing.
Rapid necrotic killing of polymorphonuclear leukocytes is caused by quorum-sensing-controlled production of rhamnolipid by *Pseudomonas aeruginosa*

Peter Ø. Jensen,1,2 Thomas Bjarnsholt,2,3 Richard Phipps,2 Thomas B. Rasmussen,2 Henrik Calum,1 Lars Christoffersen,1 Claus Moser,1 Paul Williams,4 Tacjana Pressler,3 Michael Givskov2 and Niels Høiby1

1Department of Clinical Microbiology, Rigshospitalet, DK-2100 Copenhagen Ø, Denmark
2Centre for Biomedical Microbiology, BioCentrum, Technical University of Denmark
3Copenhagen CF Center, Rigshospitalet, DK-2100 Copenhagen Ø, Denmark
4Centre for Biomolecular Science

August 2012 Volume 80 Number 8

**Pseudomonas aeruginosa** recognizes and responds aggressively to the presence of polymorphonuclear leukocytes

Morten Alhede,1,2 Thomas Bjarnsholt,2,3 Peter Ø. Jensen,3 Richard Kerry Phipps,1 Claus Moser,3 Lars Christoffersen,3 Louise Dahl Christensen,2 Maria van Gennip,2 Matt Parsek,4 Niels Høiby,3 Thomas Bovbjerg Rasmussen2 and Michael Givskov2

1Department of Systems Biology, Technical University of Denmark, DK-2800 Lyngby, Denmark
2Department of International Health, Immunology and Microbiology, University of Copenhagen, DK-2200 Copenhagen, Denmark
3Department of Clinical Microbiology, Rigshospitalet, DK-2100 Copenhagen Ø, Denmark
4University of Washington School of Medicine Seattle, Seattle, WA 98195-7242, USA
5Department of Physiology, Chr. Hansen A/S, DK-2970 Hørsholm, Denmark

Inactivation of the prevents rhamnolipid against p

Maria van Gennip,a Louise Dahl Christensen,a Morten Alhede,a,c Klaus Qvortrup,b Peter Østrup Jensen,c Niels Høiby,a,c Michael Givskov,a,d and Thomas Bjarnsholt,a,c

aDepartment of International Health, Immunology and Microbiology, University of Copenhagen, Copenhagen, Denmark; bDepartment of Biomedical Sciences, University of Copenhagen, Copenhagen, Denmark; cDepartment of Clinical Microbiology, Rigshospitalet, Copenhagen, Denmark; and dSingapore Centre on Environmental Life Sciences Engineering, Nanyang Technological University, Singapore, Singapore

1Institute of International Health, Immunology and Microbiology, University of Copenhagen, Copenhagen;
2Department of Systems Biology, Technical University of Denmark, Lyngby; 3Department of Clinical Microbiology, Rigshospitalet, Copenhagen, Denmark; and 4Channing Laboratory, Department of Medicine, Brigham and Women’s Hospital, Harvard Medical School, Boston, MA, USA
QS inhibitors work as antimicrobials because they can reinstate proper anti-microbial action of the immune system.

van Gennip, Christensen, Bjarnsholt, Qvortrup
A characterization of DNA release in *Pseudomonas aeruginosa* cultures and biofilms

Marie Allesen-Holm, Kim Bundvig Barken, Liang Yang, Mikkel Klausen, Jeremy S. Wobb, Staffan Kjelleberg, Soren Molin, Michael Givskov and Tim Tolker-Nielsen

1Centre for Biomedical Microbiology, BioCentrum-DTU, Technical University of Denmark, DK-2800 Lyngby, Denmark.
2University of New South Wales, Sydney, NSW 2052, Australia.

Extracellular DNA Shields against Aminoglycosides in *Pseudomonas aeruginosa* Biofilms

Wen-Chi Chiang, Martin Nilsson, Peter Østrup Jensen, Niels Høiby, Thomas E. Nielsen, Michael Givskov, Tim Tolker-Nielsen

Department of International Health, Immunology and Microbiology, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark;
Department of Clinical Microbiology, University Hospital, Rigshospitalet, Copenhagen, Denmark; Department of Chemistry, Technical University of Denmark, Kgs. Lyngby, Denmark; Singapore Centre on Environmental Life Sciences Engineering, Nanyang Technological University, Singapore
“Drug hunting at the Great Barrier Reef”

August 2004, a DTU-JCU joint expedition based at Orpheus Island

400 Reef samples picked by SCUBA

Among corals and sponges 100 samples showed QS inhibition.
20 samples were active against our model bacterium *P. aeruginosa*
**QS inhibitors in natural products**

<table>
<thead>
<tr>
<th>Sample</th>
<th>QSI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beansprout</td>
<td>+</td>
</tr>
<tr>
<td>Blackberry</td>
<td>-</td>
</tr>
<tr>
<td>Brown onion</td>
<td>-</td>
</tr>
<tr>
<td>Camomille</td>
<td>+</td>
</tr>
<tr>
<td>Carrot</td>
<td>+</td>
</tr>
<tr>
<td>Coffee</td>
<td>-</td>
</tr>
<tr>
<td>Cranberry</td>
<td>-</td>
</tr>
<tr>
<td>Poison Ivy</td>
<td>-</td>
</tr>
<tr>
<td>Garlic</td>
<td>+</td>
</tr>
<tr>
<td>Gele Royal</td>
<td>-</td>
</tr>
<tr>
<td>Ginseng</td>
<td>-</td>
</tr>
<tr>
<td>Habanero</td>
<td>+</td>
</tr>
<tr>
<td>Honey (various sorts)</td>
<td>-</td>
</tr>
<tr>
<td>Clove</td>
<td>-</td>
</tr>
<tr>
<td>Leek</td>
<td>-</td>
</tr>
<tr>
<td>Mint-tea</td>
<td>-</td>
</tr>
<tr>
<td>Propolis</td>
<td>+</td>
</tr>
<tr>
<td>Raspberry</td>
<td>-</td>
</tr>
<tr>
<td>Red Chilli</td>
<td>-</td>
</tr>
<tr>
<td>Spring onion</td>
<td>-</td>
</tr>
<tr>
<td>Tea Tree Oil</td>
<td>-</td>
</tr>
<tr>
<td>Water lilly</td>
<td>-</td>
</tr>
<tr>
<td>Yellow pepper</td>
<td>+</td>
</tr>
<tr>
<td>Blood (plasma)</td>
<td>-</td>
</tr>
<tr>
<td>Stinging nettle</td>
<td>-</td>
</tr>
<tr>
<td>Anemone</td>
<td>-</td>
</tr>
<tr>
<td>Snowberry</td>
<td>-</td>
</tr>
</tbody>
</table>
Garlic blocks quorum sensing and promotes rapid clearing of pulmonary \textit{Pseudomonas aeruginosa} infections

Thomas Bjarnsholt,\textsuperscript{1} Peter Østrup Jensen,\textsuperscript{2} Thomas B. Rasmussen,\textsuperscript{1} Lars Christoffersen,\textsuperscript{2} Henrik Calum,\textsuperscript{2} Morten Hentzer,\textsuperscript{3} Hans-Petter Hougen,\textsuperscript{4} Jørgen Rygaard,\textsuperscript{5} Claus Moser,\textsuperscript{2} Leo Eberl,\textsuperscript{6} Niels Høiby\textsuperscript{2} and Michael Givskov\textsuperscript{1}

\textsuperscript{1}Centre for Biomedical Microbiology, BioCentrum, Technical University of Denmark, DK-2800 Lyngby, Denmark
\textsuperscript{2}Department of Clinical Microbiology, Rigshospitalet, DK-2100 Copenhagen Ø, Denmark
\textsuperscript{3}Carlsberg Research Center, Biosector, Gamle Carlsberg Vej 10, DK-2500 Valby, Denmark
\textsuperscript{4}Institute of Forensic Medicine, University of Copenhagen, DK-2100 Copenhagen Ø, Denmark
\textsuperscript{5}Bartholin Institutet, Kommune Hospitalet, Copenhagen, Denmark
\textsuperscript{6}Department of Microbiology, University of Zürich, CH-8008 Zürich, Switzerland
Ajoene – the major bioactive QSI compound

Ajoene content in garlic bulbs: 600-700 μg/g


Ajoene, a Sulfur-Rich Molecule from Garlic, Inhibits Genes Controlled by Quorum Sensing
Ajoene targets RsmY and RsmZ expression
Back on the small regulatory RNA’s again
(my first scientific paper)

Copy mutants of plasmid R1:
Effects of base pair substitutions in the copA gene
on the replication control system

Michael Givskov and Søren Molin
Department of Molecular Biology, Odense University, Campusvej 55, DK-5230 Odense M, Denmark

Fig. 2. The CopA RNA molecule. The nucleotide sequence and a possible secondary structure is shown. The mutations described in the text are indicated.
Our antimicrobial strategy:

• To force biofilm bacteria into an “accessible” mode with chemical signals.

• Bacteria are subsequently killed by the immune system and/or conventional antibiotics.

Cell-cell signaling
(Quorum Sensing)

Intracellular
c-di-GMP signaling
A) Antibiotic resistance

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Year deployed</th>
<th>Resistance observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulfonamides</td>
<td>1930s</td>
<td>1940s</td>
</tr>
<tr>
<td>Penicillin</td>
<td>1943</td>
<td>1946</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>1948</td>
<td>1953</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>1952</td>
<td>1988</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>1956</td>
<td>1988</td>
</tr>
<tr>
<td>Methicillin</td>
<td>1960</td>
<td>1961</td>
</tr>
<tr>
<td>Cephalosporins</td>
<td>1960s</td>
<td>1960s</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>1962</td>
<td>1962</td>
</tr>
<tr>
<td>Fluoroquinolones</td>
<td>1980s</td>
<td>1980s</td>
</tr>
<tr>
<td>Linezolid</td>
<td>1999</td>
<td>1999</td>
</tr>
<tr>
<td>Daptomycin</td>
<td>2003</td>
<td>2003</td>
</tr>
<tr>
<td>Fidaxomicin</td>
<td>2011</td>
<td>2011</td>
</tr>
<tr>
<td>Bedaquiline</td>
<td>2013</td>
<td>?</td>
</tr>
</tbody>
</table>

B) Timeline of antibacterial agents

- **Golden Age of Discovery** (1940-1960)
  - Sulfonamides
  - Penicillin
  - Tetracycline
  - Erythromycin
  - Vancomycin
  - Methicillin
  - Cephalosporins
  - Nalidixic acid
  - Fluoroquinolones
  - Linezolid
  - Daptomycin
  - Fidaxomicin
  - Bedaquiline

- **Golden Age of Med Chem** (1970-2010)
  - Quinolones
  - Macrolides
  - Tetracyclines
  - B-lactams
  - Sulfonamides
  - Oxazolidinones
  - Fidaxomicin
  - Lipopeptides

C) Number of new antibacterial drug approvals

- 1980-1984: 15
- 1985-1989: 10
- 1990-1994: 8
- 1995-1999: 6
- 2000-2004: 4
- 2005-2009: 2
- 2010-2012: 1

C) Examples of current antimicrobials

- **Levofloxacin**
  - Broad-spectrum
  - Market introduction: 1996
  - Resistance reported: 1996

- **Linezolid**
  - Gram-positive
  - Market introduction: 2000
  - Resistance reported: 2001

- **Tigecycline**
  - Broad-spectrum
  - Market introduction: 2005
  - Resistance reported: 2007

- **Daptomycin**
  - Gram-positive
  - Market introduction: 2003
  - Resistance reported: 2005
• Whether your work reflects “Quality in Microbiology” you will not know until one or two decades later, when you watch the growing number of citations of that particular scientific finding.
• What you published long time ago still holds.
• You and others continuously build on it.
Dr. Givskov is a natural leader in both groups, because of his intellectual gifts, and he is now in a position to lead the world-leading team in the determination of the scope of the bacterial activities that can be controlled by signals, and in the development of practical methods of manipulating these bacterial behaviours. I monitor the biofilm field at the world level, in the organization of conferences for the American Society for Microbiology (ASM), and my committee and I have named Dr. Givskov as the best laboratory-active biofilm/signal researcher in the world today. Note: I am usually a conservative reviewer, but this project will support a research program that I consider the most important and best in the world :: please forgive and understand my exuberance!!.

Bill Costerton in a review of on of my research proposals 2008
Citations

Total 20,000
Annual 2,100
H index 78
290 papers incl.
23 patents
• Like with everything, money has become the bottleneck for Microbiology research.
• But does big scale operations signify research quality because “big is better”? 
SCELSE

- Bioreactor engineering
- Bioengineering
- Analytical microbiology
- Molecular microbiology
- Experimental biofilms
- Metabolomics
- Sequencing and high performance compute cluster
- Biofilm Imaging Facility
- Animal facility

- Roche 454 Genome Sequencer FLX+
- Illumina MiSeq
- Liquid handling robotics
- Confocal microscope
- PacBio: RS II Single-Molecule Sequencing
- High performance compute cluster
- NeoPrep

Plate reader for small molecule screening assays (LSI chemical biology laboratory)
• Instead of spending the majority of your time designing smart experiments and pondering what the results may tell you, you spend more and more time running for grants.
• Not always a very fruitful and satisfactory experience for a scientist.
Don’t forget: Science is a social thing!

Networking plays a key role
Remember: Mentors and long time friendships are extremely important

And don’t forget: Playing is a must
The Indigo V expedition covered some of the most under-sampled and less characterized waters of the Indian Ocean.

Can meaningful oceanographic data be collected with the kind of narrowly focused, low-cost instrumentation that is easily mass produced and deployable?

*Plos Biology*
Don’t forget: Your family

Sydney, December 1994

My daughter once told me that there’re three words she’ll never forget: “Quorum Sensing” “Pseudomonas aeruginosa” “Biofilms”
All this leads me to my final remark: *Whether your science reflects quality in Microbiology research, you will know when it triggered the mind of a young undergraduate student to make the decision, “yes I want to become a microbiologist”*
You may sell Microbiology this way
第44回緑膿菌感染症研究会
平成22年2月12日（金）～13日（土）
会場
東邦大学医療センター大森病院
臨床講堂（5号館、地下1階）
会長
山口恵三（東邦大学医学部微生物・感染症学講座）