Nanoparticles as Selective Anti-Bacterial Compounds?

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OIST

A graduate university

2005: Research institute with 5 Principal Investigators (PI)

2007: Research institute with 10 PI

2011: University accreditation
25 faculty members (professor)

2012: 35 graduate students
40 professors

2013: 58 graduate students
50 professors

Okinawa Institute of Science and Technology
www.oist.jp
A Tiny University trying to build bridges with institutions worldwide.

Each year, around 40 students come for an internship for 2 to 6 months.

https://groups.oist.jp/grad/research-interns

<table>
<thead>
<tr>
<th>Starting Date</th>
<th>Jan 4 – Mar 31</th>
<th>Apr 1 – Jun 30</th>
<th>Jul 1 – Sep 30</th>
<th>Oct 1 – Dec 28</th>
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<td>Application Deadline</td>
<td>Aug 30</td>
<td>Nov 29</td>
<td>Feb 28</td>
<td>May 31</td>
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Okinawa Institute of Science and Technology

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Okinawa Institute of Science and Technology
JAPAN

Windfall for Tiny University With Outsized Ambitions

TOKYO—Japan is doubling its bet on a young graduate university, based on a remote island, that has aspirations of becoming a research powerhouse. If approved by the Diet, the annual budget of the Okinawa Institute of Science and Technology (OIST) will jump from $110 million this year to $204 million in 2014. OIST’s governors met last week with Japanese Prime Minister Shinzo Abe to outline expansion plans. “We said we admire and congratulate the government for being willing to try to realize the vision for this university,” says neuroscientist Torsten Wiesel, a 1981 Nobel laureate who chairs OIST’s board.

OIST opened for research in 2005 and began taking students last year. Now it intends to double its faculty roll to 100 within 7 years, and ultimately up it to 300—roughly the size of the California Institute of Technology (Caltech). Enrollment

To chart a course for building a formidable institution from scratch, Omi assembled a Nobel-studded team of advisers. They recommended that OIST enroll only graduate students, recruit half its faculty from abroad, and emphasize interdisciplinary research and education. Government planners gave OIST greater autonomy than other universities by placing it directly under the prime minister’s office rather than the education ministry. That privileged treatment riled Dorfan, a physicist and former director of what is now the SLAC National Accelerator Laboratory in Menlo Park, California. One element, he says, is top-flight faculty members like Keshav Dani, a 34-year-old physicist who studied at Caltech and the University of California, Berkeley. Dani accepted a position at OIST over several tenure-track offers from top U.S. and European universities. When he first visited the campus, he says, “they laid out a fantastic vision and I was blown away.” His startup package included an expensive laser setup for femtosecond spectroscopy.

OIST also has a mandate to spur economic development in Okinawa. Toward that end, Dorfan says, the university in April will establish its first startup company, which will commercialize a technique that determines the molecular structure of proteins developed by OIST biologist Ulf Skoglund. The technique is expected to be used for drug discovery.

—DENNIS NORMILE

11 OCTOBER 2013 VOL 342 SCIENCE www.sciencemag.org

Published by AAAS
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Presentation of the bacterial flagellum

What is the “type III secretion system” (T3SS)

Why do we work on
  the flagellar “type III secretion system”

Inhibition of the “T3SS”
Electron micrograph of *Salmonella*

(Namba & Vonderviszt, *Q.Rev.Biophys.* 1997)
Growth of a bacterial flagellum
The Bacterial Flagellum

Type 3 Secretion System

- 5 essential membrane proteins:
- Cytoplasmic components

Dedicated to motility
**Animal pathogens:**
*Bordetella bronchiseptica* (whooping cough)
*Yersinia* (plague)
*Salmonella* (food poisoning & typhoid fever)
*Shigella* (dysentery)
*Enteropathogenic E. coli* (diarrhea)
*Enterohemorrhagic E.coli* (Bloody diarrhea)
*Chlamydia* (respiratory, ocular disease)

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**Type 3 Secretion System**
- 5 essential membrane proteins
  - Cytoplasmic components:

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**Toxins delivery into host cells**

Flagellum

FlhA (75 kDa)
FlhB (42 kDa)
FliP (27 kDa)
FliQ (10 kDa)
FliR (29 kDa)

Injectosome

Analogy between the bacterial flagellum and the needle complex
Why do we work on the flagellar T3SS system instead of the injectosome T3SS?
An easy functional assay: swimming

After 8 hours incubation

Agar - Trypton
Inhibition of the Type III secretion System
Salmonella typhimurium FlhB (42.4 Kda)

MAEESDDDDKT EAPTPHRLEK AREEGQIPRS RELTSSLILL VGVCIIWFGG ESLARQLAGM LSAGLHFHDHR MVNDPNLILG QIILLKAAM MALLPLIAGV VLVALISPVM LGGLIFSGKS LQPKFSKLNP LPGIKRMFSA QTGAELLKAV LKSTLVGCVT GFYLWHHWPQ MMRLMAESPI VAMGNALDLV GLCALLVVLG VIPMVGFVDVF FQIFSHLKKL RMSRQDIRDE FKESEGDPHV KGKIRQMQRA AAQRRMMEDV PKADVIVTNP THYSVALQYD ENKMSAPKVV AKGAGLIALR IREIGAEHRV PTLLEAPPLAR ALYRHAEGGQ QIPGQLYAAY AELWQWVWQL KRWRLAGGQR PPQPENLPVP EALDFMNEKN TDG

(Fraser et al., Mol.Micro., 2003)
(Minamino & Macnab, J.Bact., 2000)
Salmonella typhimurium FlhBc

MKLRMSRQDI RDEFEKSEGDRHVKGKIRQMQRAAAQRMMEDVPKADVIVTNPTHYSVALQYDENKMSAPKVVAKGAGLIALRIREIGAERHRVPTLEAPP
LARALYRHAEIGQQIPGQLYA AveVLAWVWQLKRWRLAGGQRPPQPENLPVPEALDFMNFKNTDA

166 a.a., 18.8 KDa

(Meshcheryakov et al., Acta Cryst. D, 2013)
**Functional assay with FlhBc**

“281-285” loop: Glu-Asn-Lys-Met-Ser

1. FlhB knock out

2. Wild-type Salmonella

3. Δ281-285 (loop deletion)

4. “AAAAA” (loop Mutation)

5. “PPPPP” (loop Mutation)

After 8 hours incubation

(Meshcheryakov et al., Acta Cryst. D, 2013)
Salmonella typhimurium FlhBc

SpaS (*Salmonella* injectosome)

EscU (*E. coli* injectosome)

YscU (*Yersinia* injectosome)

Spa40 (*Shigella* injectosome)

FlhB (*Salmonella* flagellum)

Effect of the loop deletion/mutation in Salmonella

Salmonella_SpaS ... SRLIVANPHTITIGIYFKPELMPIMPIMISVYETNQRALA 289
Shigella_Spa40 ... SKLVVMNPTHIAIGIYFNPEIAAPPFISLIEITNQCALA 288
Ecoli_EscU ... STVIVKNPHTIAICLYYKLGETPLPLVIEKTGKDAKLQ 293
Yersinia_YscU ... SSVVANPHTIAIGIYKRGETPLPLVTFKYTDATQVQT 294
Salmonella_FlhB ... ADVIVTNPHTYSVALQYDENKMSAPKVVAKGAGLIALR 300

(Meshcheryakov et al., Acta Cryst. D, 2013)
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FlhB & paralogs YscU, EscU, SpaS, Spa40

Salmonella_SpaS ... SRLIVANPHTHITIGIYFKPELMPIPMISVYETNQRALA 289
Shigella_Spa40  ... SKLVVMNPTHIAIGIYFNPEIAAPFISLIETNQCALA 288
Ecoli_EscU      ... STVIVKNPTHIAICLYYKLGETPLPLVIEIETGKDAKALQ 293
Yersinia_YscU   ... SSVVVANPHTHIAIGILYKRGETPLPLVTFKYTDAAQVQQT 294
Salmonella_FlhB ... ADVIVTNPTHYSVALQYDENKMSAPKVVAKGAGLIALR 300
Acknowledgment

Clive Barker
Young-Ho Yoon
Hideyuki Matsunami (from OIST)
Vladimir Meshcheryakov

Prof. Akio Kitao from the University of Tokyo