The Marine Biodiscovery Pipeline

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• PhD in Synthetic Organic Chemistry
• Post-doc with Prof Phil Crews, University of California, Santa Cruz on Marine Natural Products
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• Project Leader, PharmaSea Consortium
• Co-Author of ESF Marine Board Position Paper “Marine Biotechnology – A New Vision and Strategy for Europe”
• Visiting Professor (20%) at University of Tromsø
• Scientific advisor to MabCent, Tromsø
• Member of the Industrial Biotechnology Sector Group of the Biosciences Knowledge Transfer Network
• Unpaid advisor to Aquapharm Biodiscovery Ltd and Glycomar Ltd, UK.
Why Use Marine Bioresources?

Offers advantage over comparable terrestrial resource:
  Superior performance
  Better economics

Unprecedented activity in particular application:
  Enzymes: new reactivity/new biotransformation
  Small molecules: new mechanism of action
  Materials: new properties
Biodiscovery is the discovery of compounds and associated ideas from natural sources to develop novel biomedicines.

Biodiscovery generates chemical diversity that is used to find initial biological activity in disease focused screens.

Biodiscovery also includes the development of biomedical research tools, antifoulants, catalysts, nutraceuticals and cosmeceuticals.
Why Marine?

Diversity of Habitat

Jørgensen Nat Rev Microbiology, 2007, 5, 770
Extreme Marine Environments

Deep Oceans
95% > 1000 m deep
50% > 3000 m deep
Average depth = 3790 m
1-3% trench ecosystems

Cold Oceans

Thermal Vents
Marine Microbial Diversity

**Taxonomic ‘space’**

Marine and terrestrial species clearly separated.
Biological Diversity = Chemical Diversity

Small Molecules

Biomolecules
Marine and Terrestrial Chemical Diversity are Different

- 71% of scaffolds are exclusively marine
- These cover only 30% of marine natural products
- Many marine natural products scaffolds appear only once

Kong, *Drug Discovery Today*, 2010, 15, 884
Marine Natural Products on the Market

Vent Polymerase

$\omega$-3 polyunsaturated fatty acids for heart disease

Prialt for pain

Halaven for cancer
The Marine Biodiscovery Process

Sampling → Curation → Biomass → Extraction → Assay → Purification → Active NCE → Development
Little Sampling Done Beyond 3000 m
Research Vessels

- High daily rate
- Limited number of vessels globally
- Access competitive
- Long time between bid for time and actual cruise
- Many different types of science accommodated may lead to compromises
- Shared resources and bartering systems operate to optimise usage

RRS Discovery (UK)

Chikyu (Japan)
Submersibles

ROV Isis (UK)  
(6500 m)

Shinkai (Japan)  
(6500 m)

ROV Nereus (US)  
(11000 m)

Deepsea challenger (US)  
(11000 m)
Data Logging for Research Cruises
Sampling Devices
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## Sample Data and Storage

### Metadata may include
- Location
- Depth
- Temperature
- Salinity
- pH
- Oxygen content
- Seafloor conditions

### Sample storage
- Ambient temperature
- Cooler (4°C)
- Freezer (-20°C)
- -80°C Freezer
- Liquid nitrogen (-196°C)
- Formaldehyde
- Ethanol
- DNA/RNA preservation liquids
Further sampling is essential for the following reasons:

• Origin may be difficult to ascertain (e.g., location, depth, collector, date, ownership etc). A minimal data set is imperative.
• IP status not clear
• May not have been collected in a way consistent with proposed use
• May not have been stored correctly to ensure sufficient quality for proposed use.
• The amount of material may not be sufficient for proposed multiple uses.
• Very few locations have been sampled so repository may not be representative of ABNJs.
If previous points can be addressed then such a repository might be viable

- The rules for terrestrial biorepositories may not apply for their marine equivalent.
- Much information on biorepositories is based on situation with respect to plants where samples can be propagated.
- A deepsea core sample or a marine macroorganism collected on one sampling expedition is finite.
- Microbes can be cultured but again culturing a microbe from a hydrothermal vent is quite different from culturing a microbe from a terrestrial habitat.
The Marine Biodiscovery Process

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Biomass
The Marine Biodiscovery Process

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Extraction and Purification

- Solvent-solvent partition
- Size-exclusion chromatography
- High performance Liquid chromatography
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Assay

Crude extract
Active

S1 Inactive
S2 Inactive
S3 Inactive
S4 Inactive
S5 Active
S6 Inactive

S6F1 Inactive
S6F2 Inactive
S6F3 Inactive
S6F4 Active

S6F4H1 Inactive
S6F4H2 Inactive
Pure Compound Active

Cell based
Enzyme based
The Marine Biodiscovery Process

1. Sampling
2. Curation
3. Biomass
4. Extraction
5. Assay
6. Purification
7. Active NCE
8. Development
Structure Determination

Spectroscopic data

2D Structure

3D Structure
Metagenomic Approach

Genome sequencing

Search for all permutations

gDNA

Shotgun cloning

E. coli clone library

Identification of biosynthetic pathway

Cloning and heterologous expression of pathway

Identification of productive clones

LC-MS deconvolution of clone library

Positive plate

Positive column

Positive well

Sequencing

Product
Bioinformatic Databases and the Metagenomic Approach

- Who acquired/deposited the data and with what authority?
- Who has access to the data?
- Is genome and metagenome information sufficient?
- Many genes found in marine species are not in the current bioinformatic databases
- The function of many of these genes cannot be determined without laboratory work
- Difficulty in cloning genes of marine origin
- Lack of suitable tools (vectors/host)
Gene Synthesis

GATTACAGGACGCTT
ATTTTTTCGACGATGC
TTGGGGAAATGCAAA
GATTCAGCTAAAGTC

Gene sequence

DNA

Molecule

Protein