



PHARMASEA

Increasing Value and Flow in the Marine Biodiscovery Pipeline

Marcel Jaspars

Scientific Leader, PharmaSea Consortium

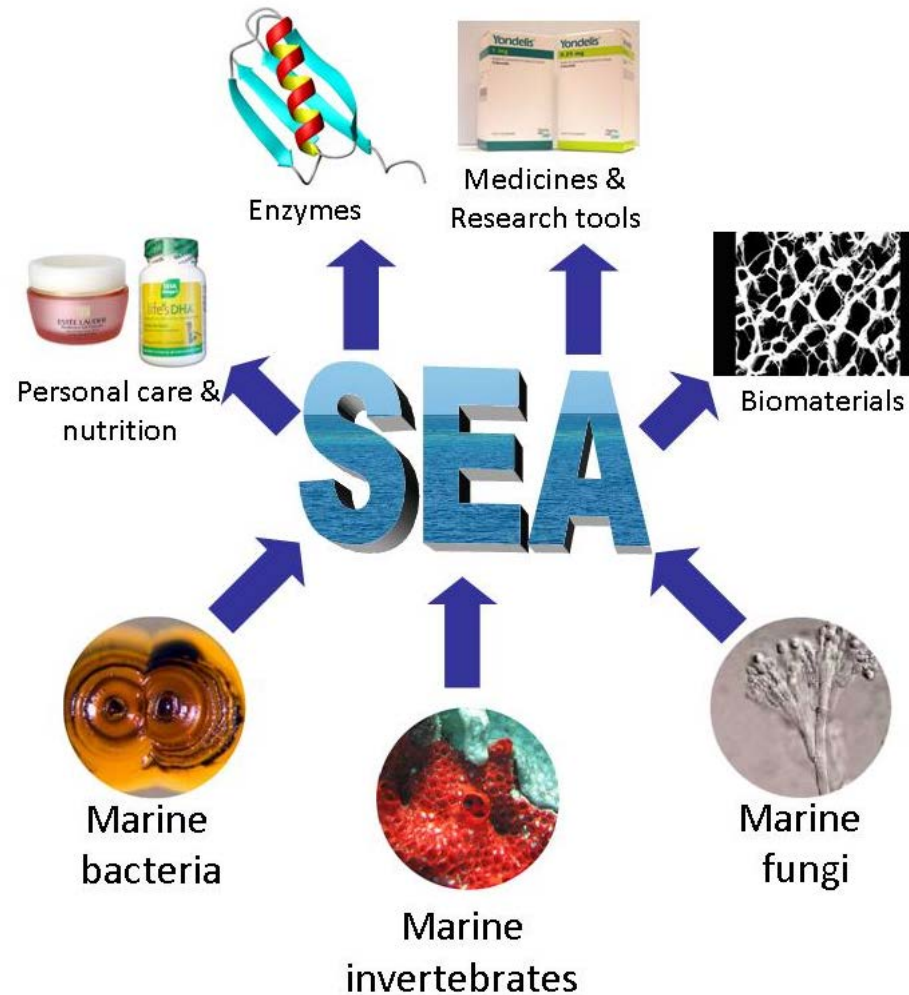
Director, Marine Biodiscovery Centre

University of Aberdeen

Scotland, UK

m.jaspars@abdn.ac.uk

Marine Biodiscovery



Why Use Marine Bioresources?

Offers advantage over comparable terrestrial resource:

- Superior performance

- Better economics

Unprecedented activity in particular application:

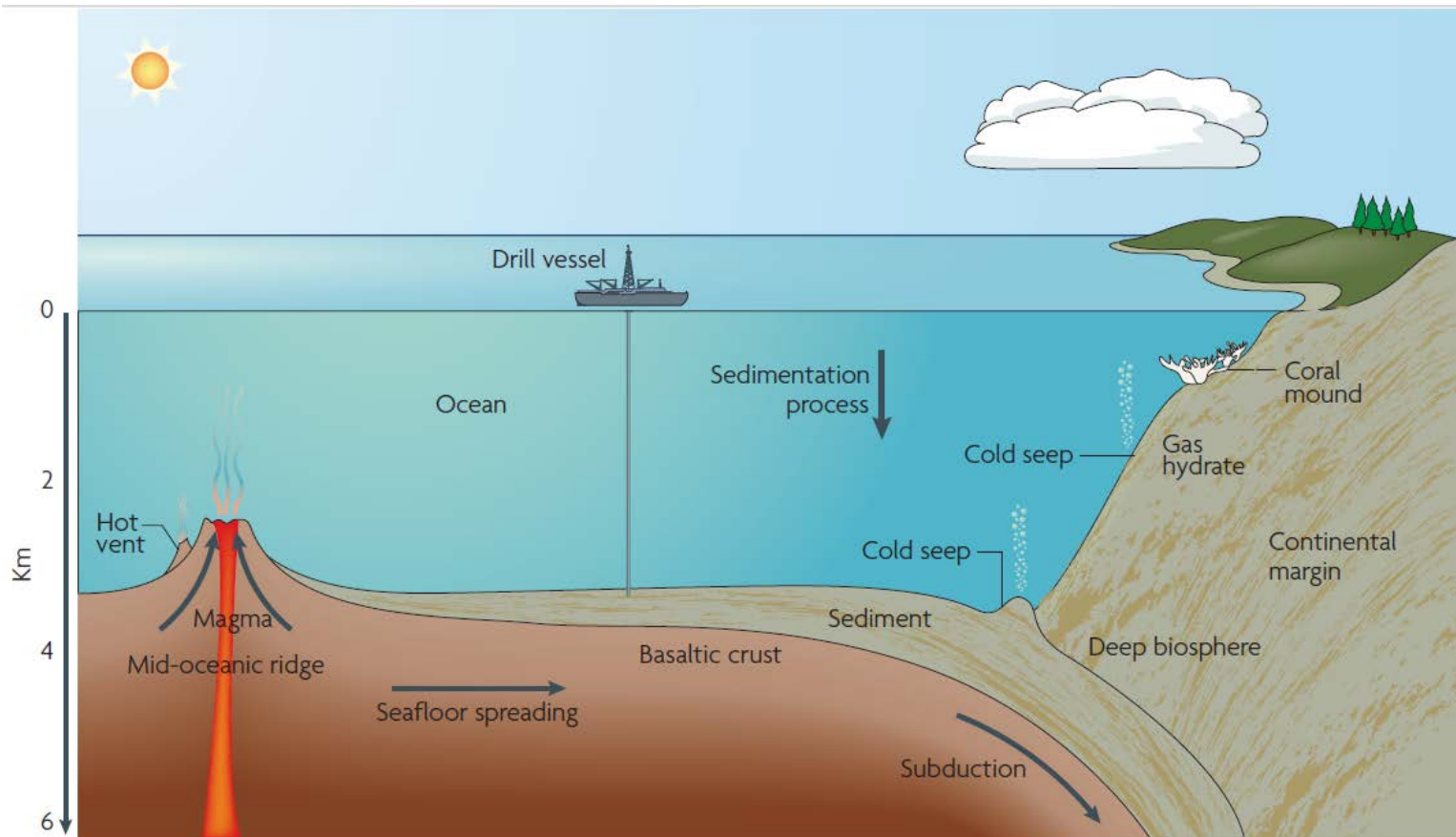
- Enzymes: new reactivity/new biotransformation

- Small molecules: new mechanism of action

- Materials: new properties

Why Marine?

Diversity of Habitat



Jørgensen *Nat Rev Microbiology*, **2007**, 5, 770

MGR Derived Pharmaceutical Products on the Market



Soft tissue carcinoma



Ecteinascidia turbinata



Chronic pain (analgesic)



Conus magus



Breast cancer



Halichondria okadai



Ara-C (cytarabine)
treatment of leukemia



Ara-A (vidarabine)
antiviral



Tethya crypta



Hodgkin's Lymphoma



Dolabella auricularia

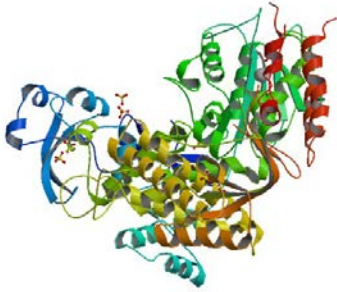


lowering very high
triglyceride levels



Purified
fish oil

Non-Pharma MGR Derived Products on the Market

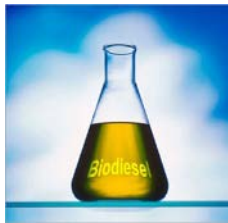


Vent Polymerase – for DNA amplification

Origin: Vent bacterium (Naples, Italy)

Production: Recombinant

Owner: New England Biolabs



Fuelzyme – Enzyme used in biodiesel production

Origin: Deep sea bacterium (location unknown)

Production: Recombinant

Owner: Verenium (BASF)



Cosmetic screening infra-red rays

Origin: Vent bacterium (location unknown)

Production: Bacterial culture

Owner: Sederma (Croda)



Anti biofilm agents

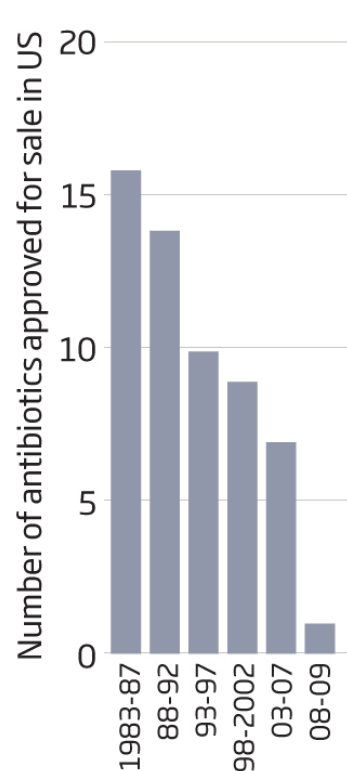
Origin: Red seaweed

Production: Chemical Synthesis

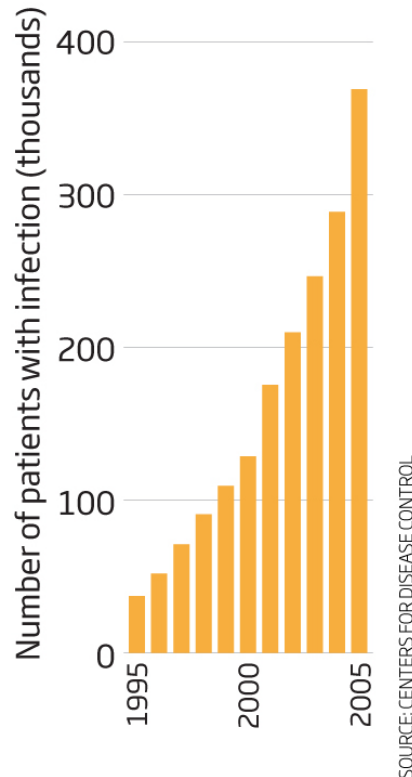
Owner: XXXXX

Why PharmaSea?

Decline in new approved antibiotics



Increase in hospital MRSA infections

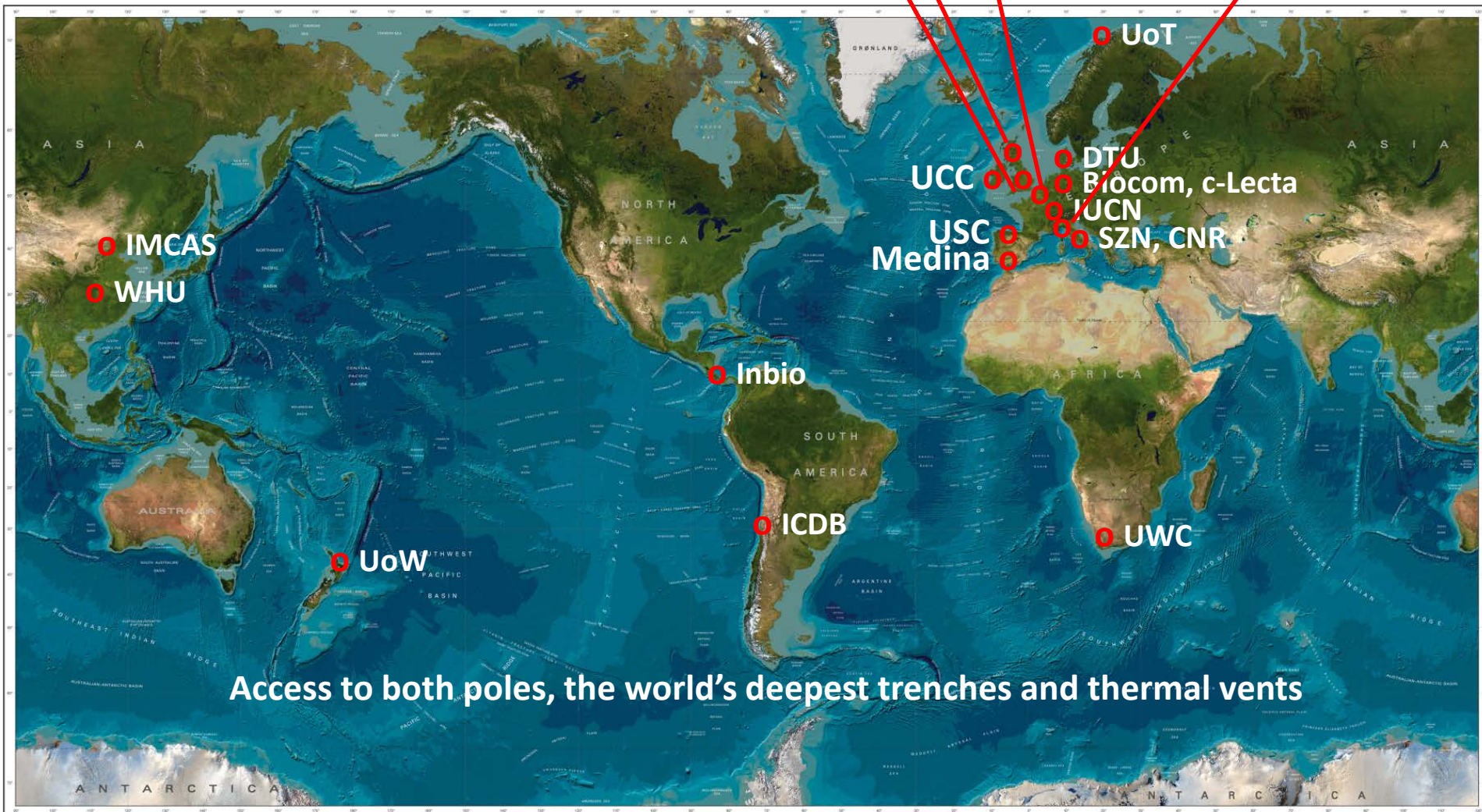


- New therapeutics for microbial infections and CNS diseases
- Widen bottlenecks in marine biodiscovery pipeline
- Develop mechanisms to transfer marine biotechnology to end users
- Make marine bioproducts more attractive to develop for industry

Source: New Scientist

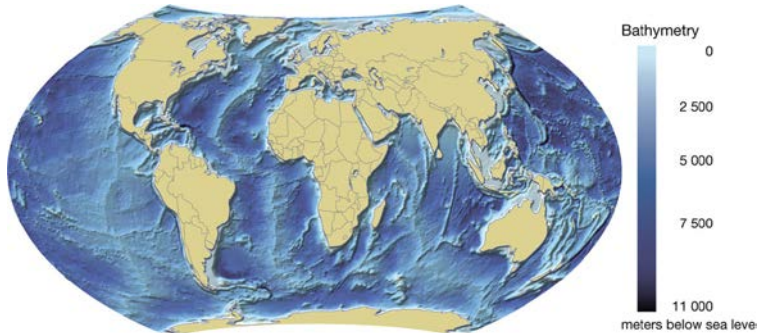
PHARMASEA

UniAbdn, DeepTek
ACDLabs, RSC, BioBridge
KULeuven, eCoast
SeaLife Pharma



- **Increasing Value and Flow in the Marine Biodiscovery Pipeline**
- EU Framework Programme 7 Consortium funded at EUR 9.5 million
- 24 Partners
- Norway, Denmark, UK, Belgium, Germany, Spain, Italy, Republic of Ireland, Chile, South Africa, China, New Zealand, Costa Rica
- *To improve the quality, volume and value of active agents discovered in the marine environment and increase the speed at which they can be delivered to the marketplace, by addressing bottlenecks and restrictions and adding technical booster-pumps*
- Start date 01/10/2012; Duration 48 months (& 6 extension)
- Project Coordinator Camila Esguerra/Peter de Witte, KU Leuven, Belgium

Extreme Marine Environments

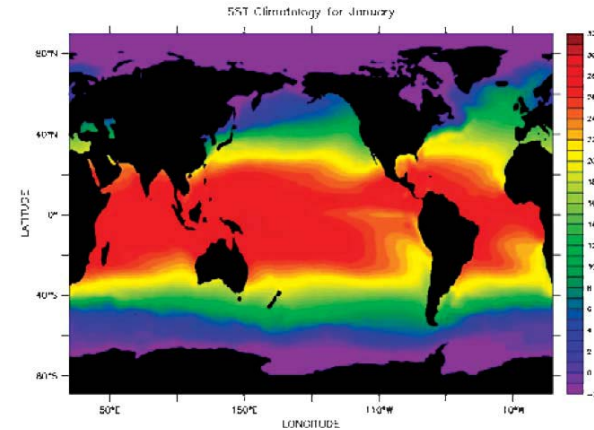


Deep Oceans

95 % > 1000 m deep

50 % > 3000 m deep

Average depth = 3790 m

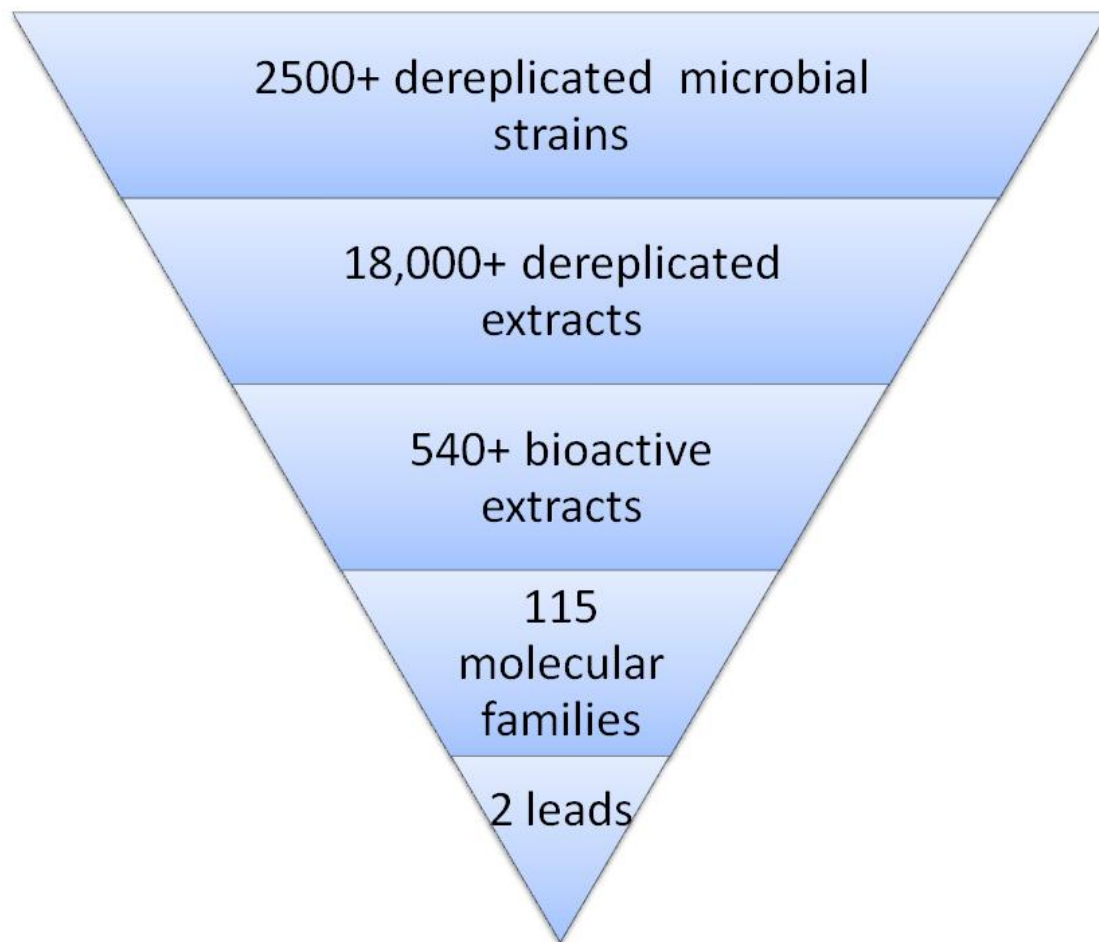


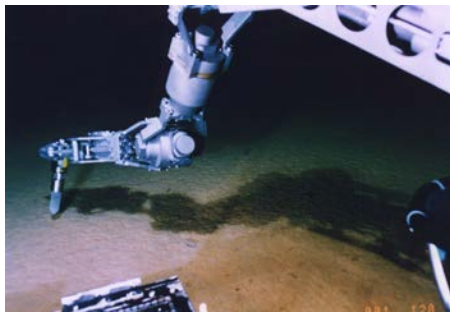
Cold Oceans



Thermal Vents

Discovery Funnel

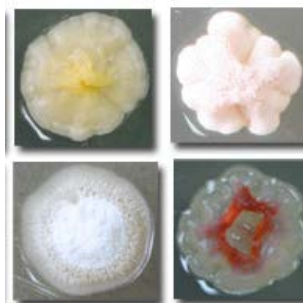




Extreme Environment

Legal Access

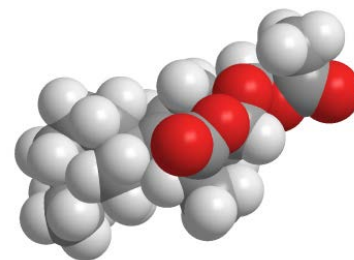
Physical Access



Novel Biology

Isolation/identification

Chemical talent



Novel Chemistry

Chemical novelty

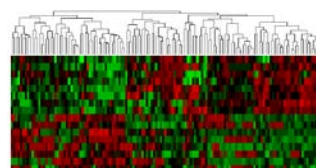
Datamining



Product

Scale-up

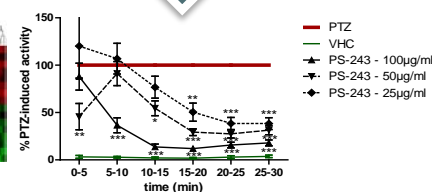
Knowledge transfer



Novel Activity

Mechanism of action

Toxicity



Create Science/Policy Interface

MGR
Practitioners
Research /
Industry



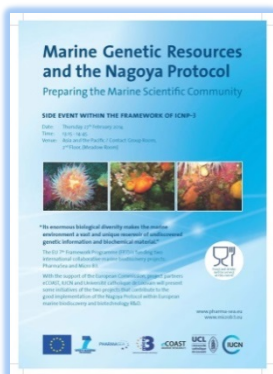
Legal Experts &
Policy Makers

EC (DG MARE & DG ENV),
UNDOALOS, CBD Secretariat,
CIESM, ISA, CMS Secretariat

Inform Policy

Awareness
Raising

Share best practice



WP1 Strain Collections (n = 13,689)

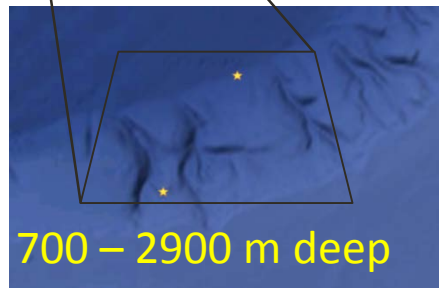


Legacy Collections: Arctic, Antarctic, Republic of Ireland, South Africa and Argentina

New Collections: Antarctic, South Africa

Scheduled Collections: South Shetland Trench (-5200 m)

WP1 Deep Sea Sampling



RV Celtic Explorer



ROV
Holland I



Live HD Video
of sampling



*Inflatella
pellicula*



*Lissodendoryx
diversichela*



*Stelletta
normani*



*Poecillastra
compressa*



Sediments
750 m – 2,900 m

750/2900 m

1,350 m

1,350 m

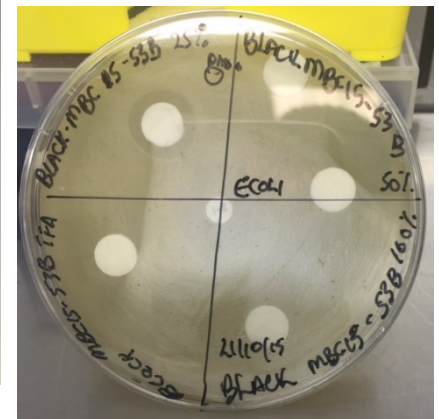
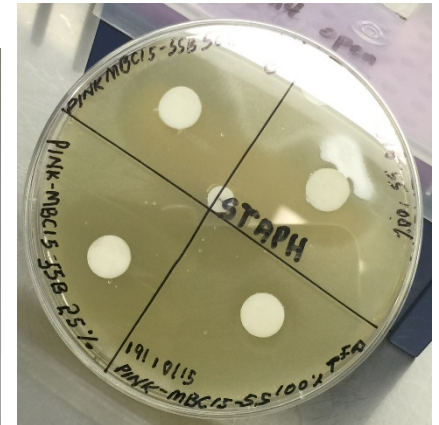
2,100 m



WP1 New Environments

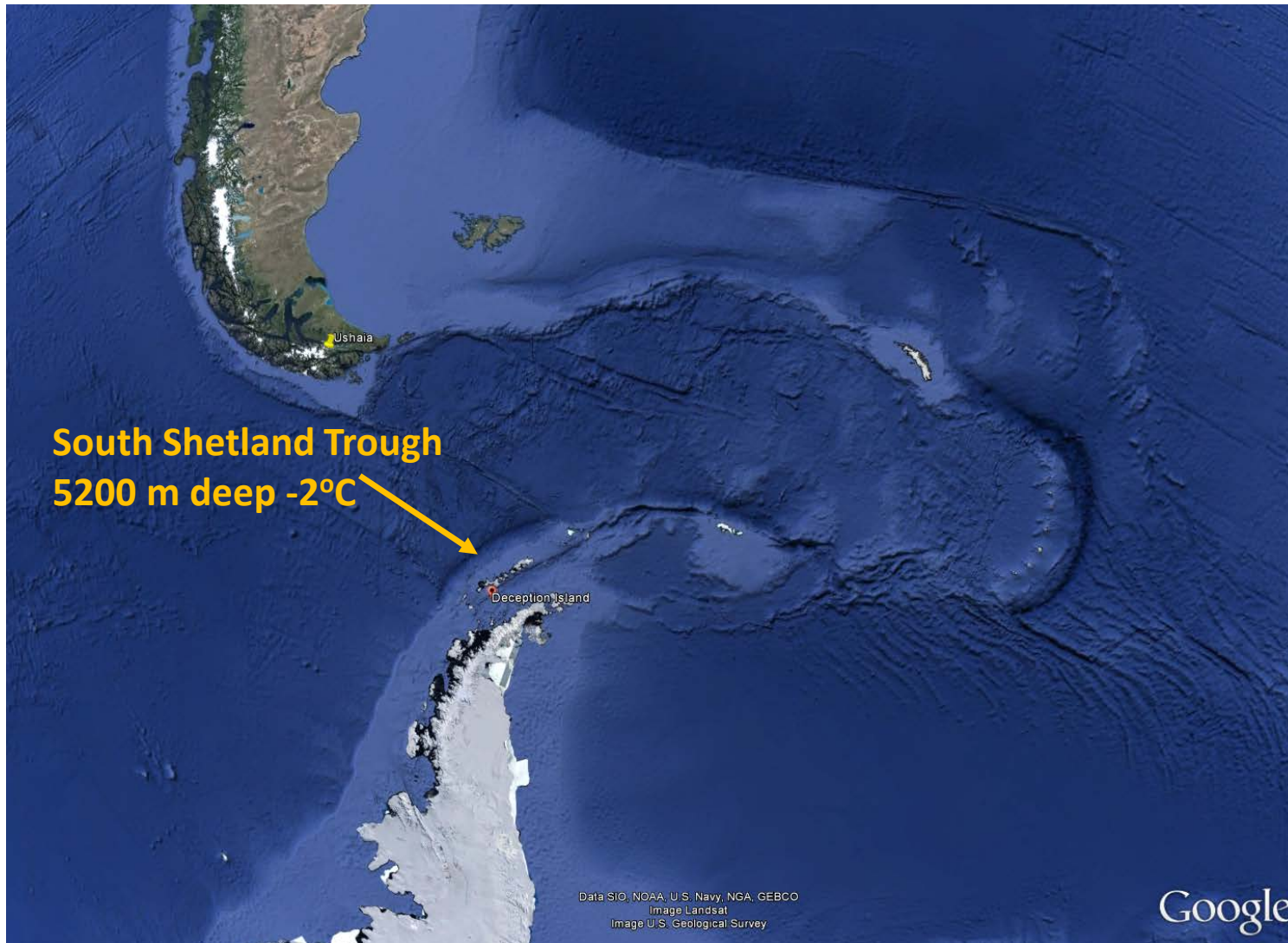


WP1 Recent Deep Sea Collections

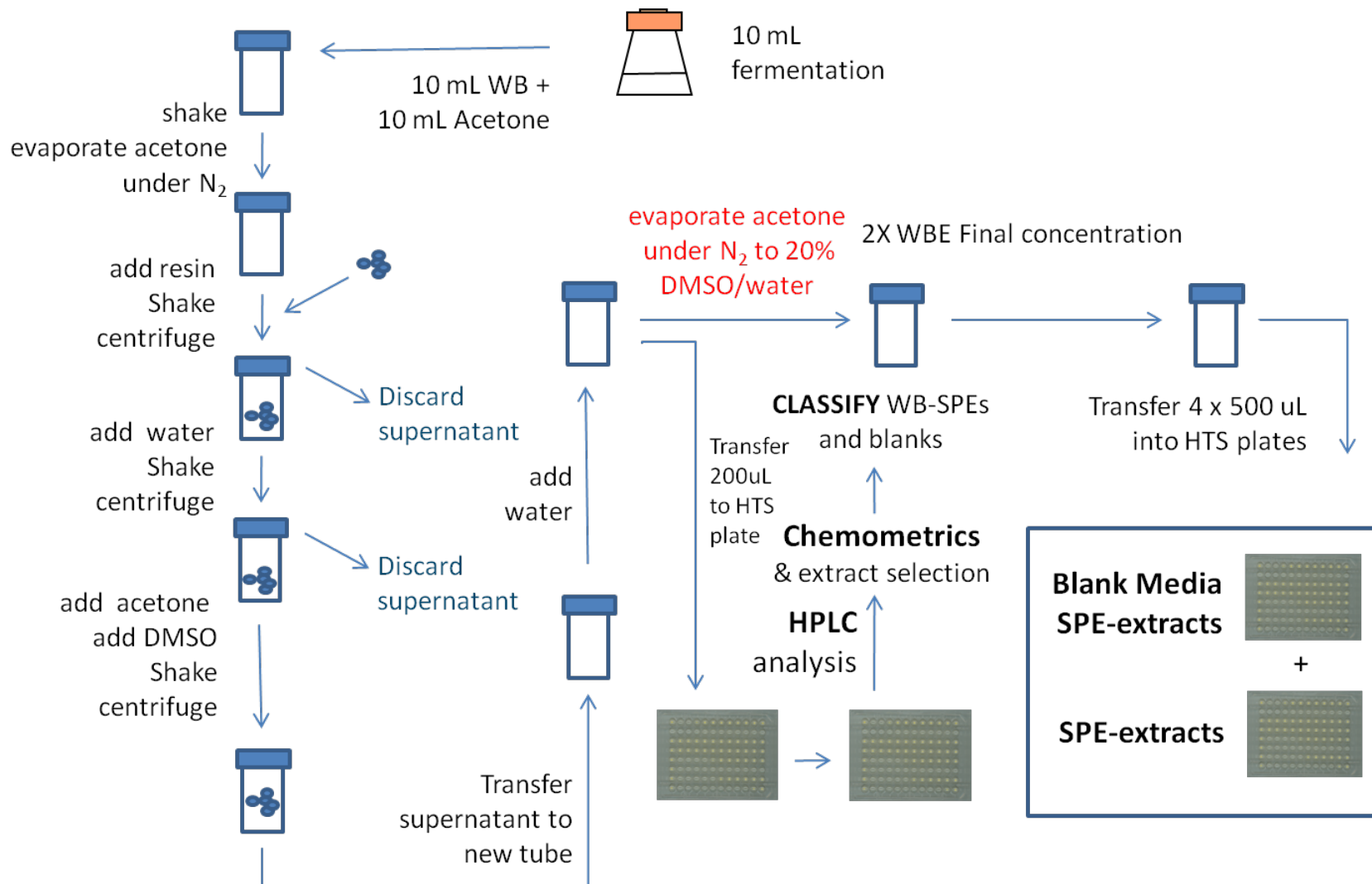


Alan Jamieson
Larry Mweetwa

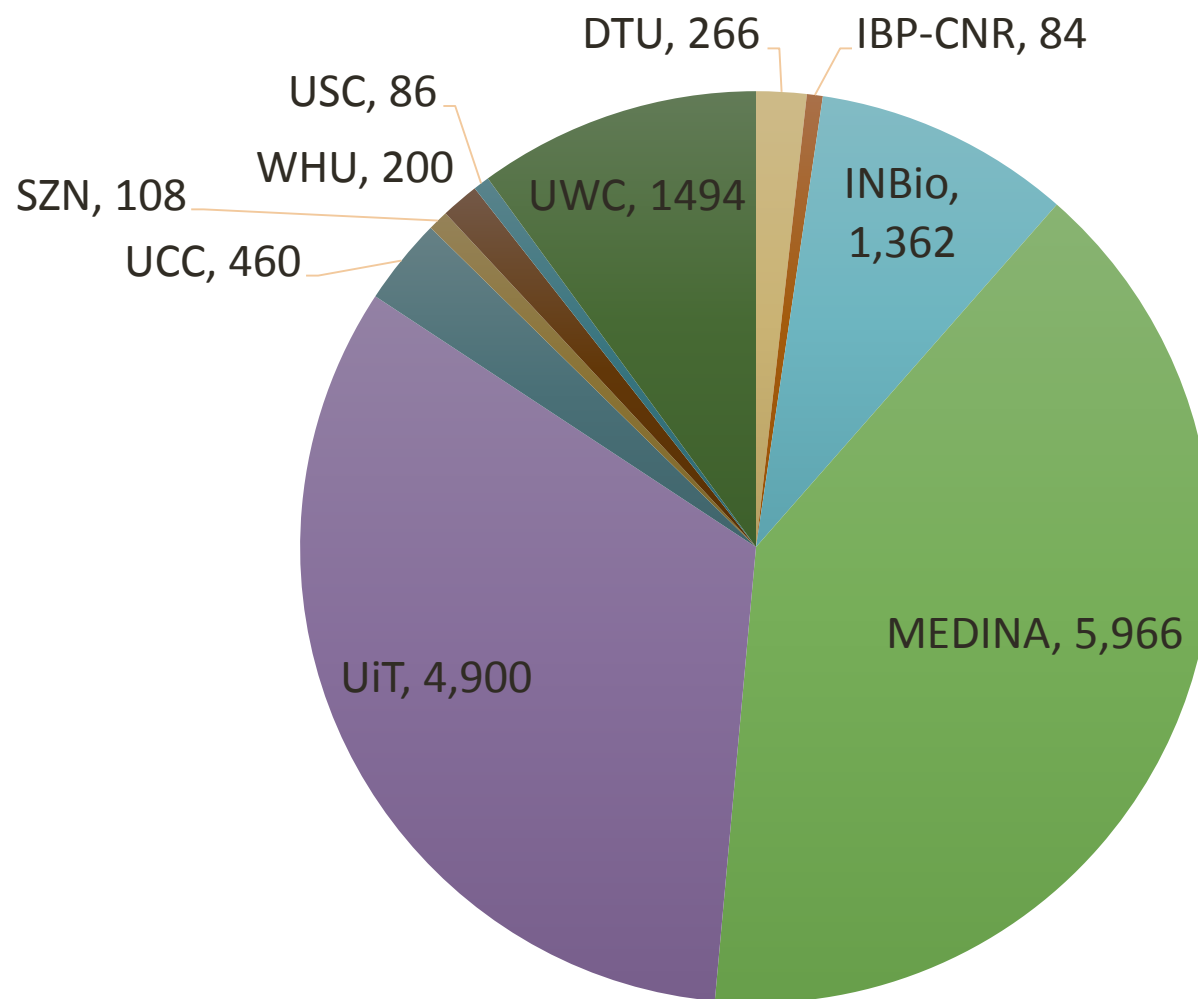
WP1 PharmaDeep Expedition December 2015



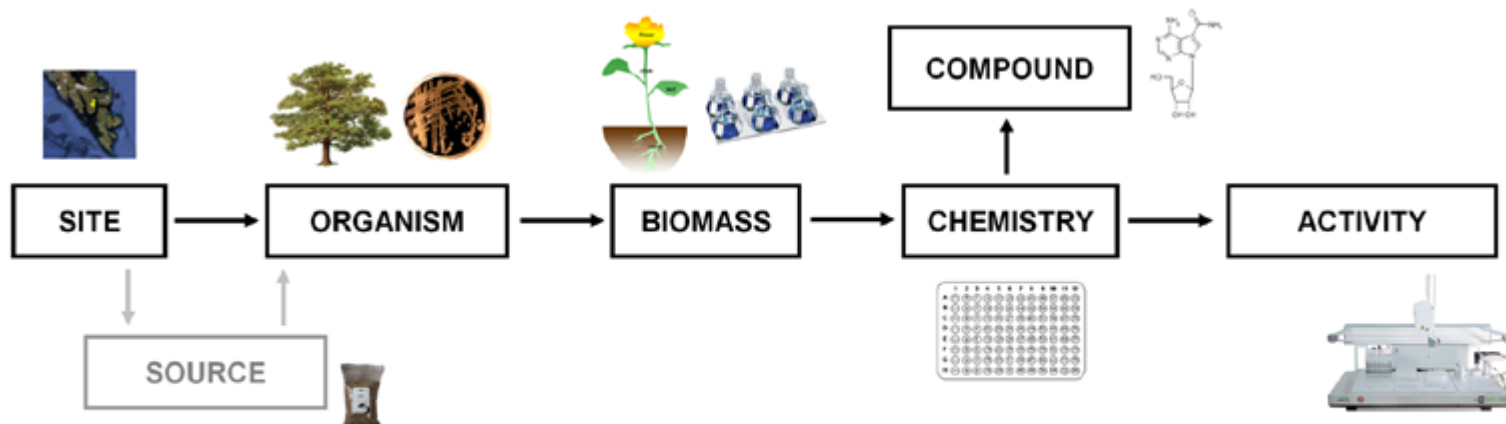
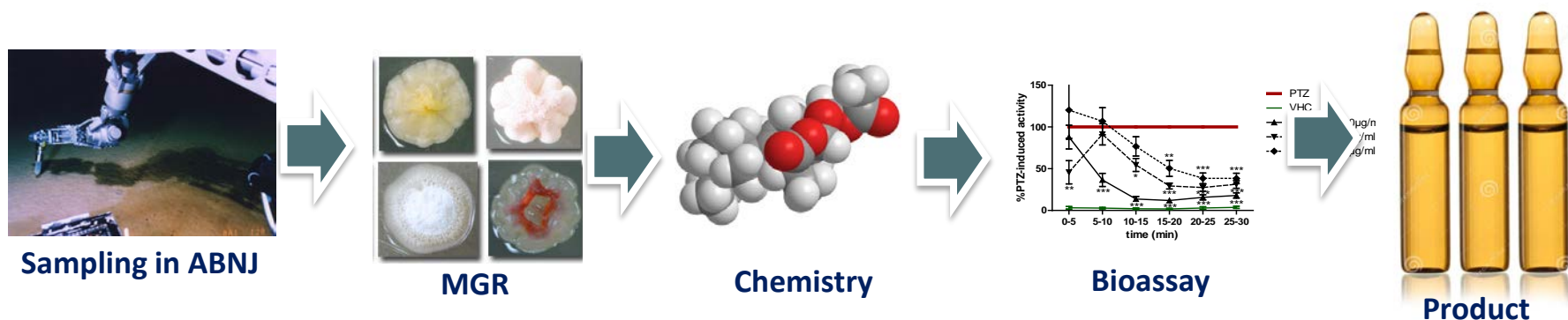
WP2 Standardised Fermentation and Extraction Protocols



WP2 – Extracts and Fractions (n=14,962; 83% of total)



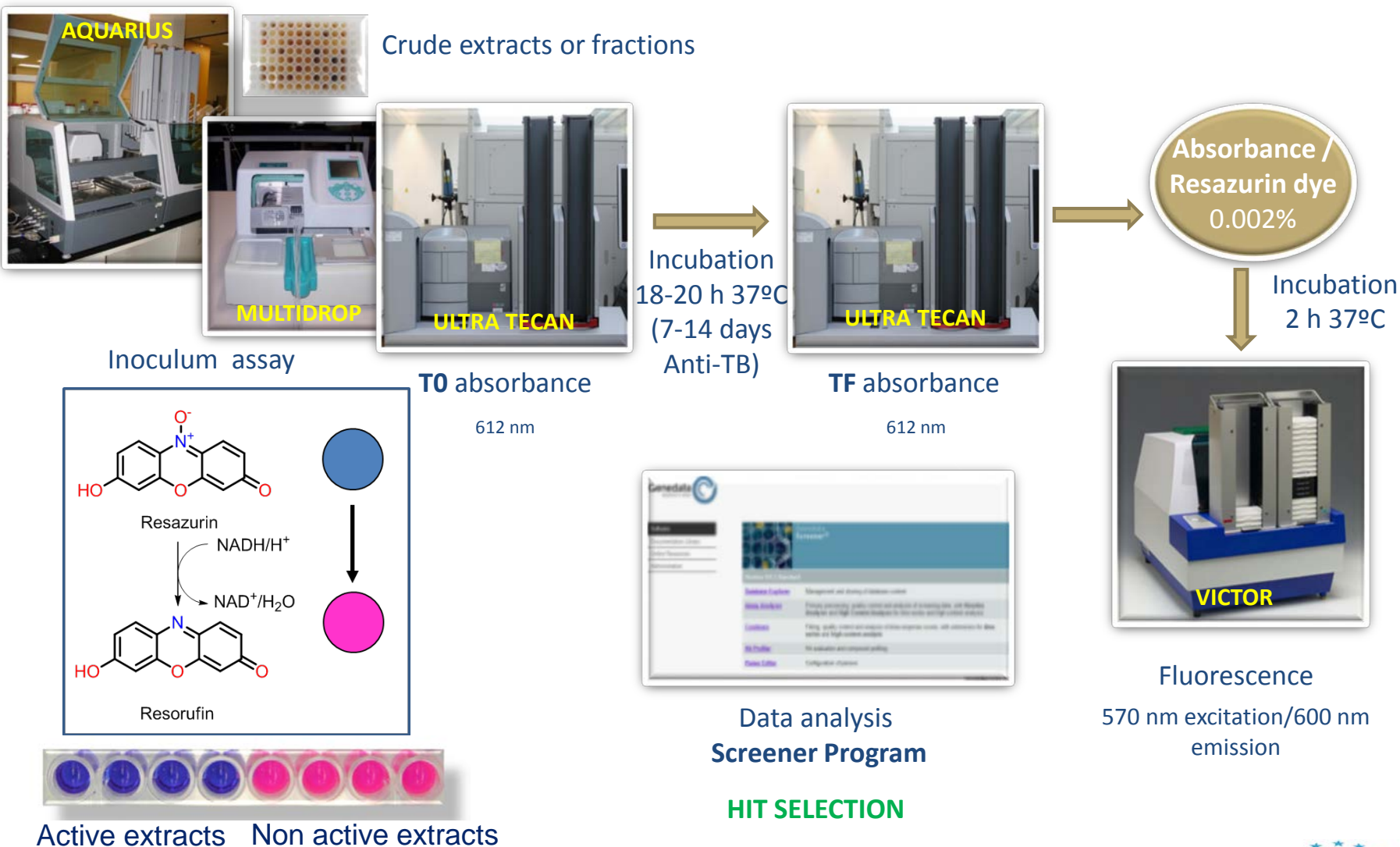
WP2/3 Data Management



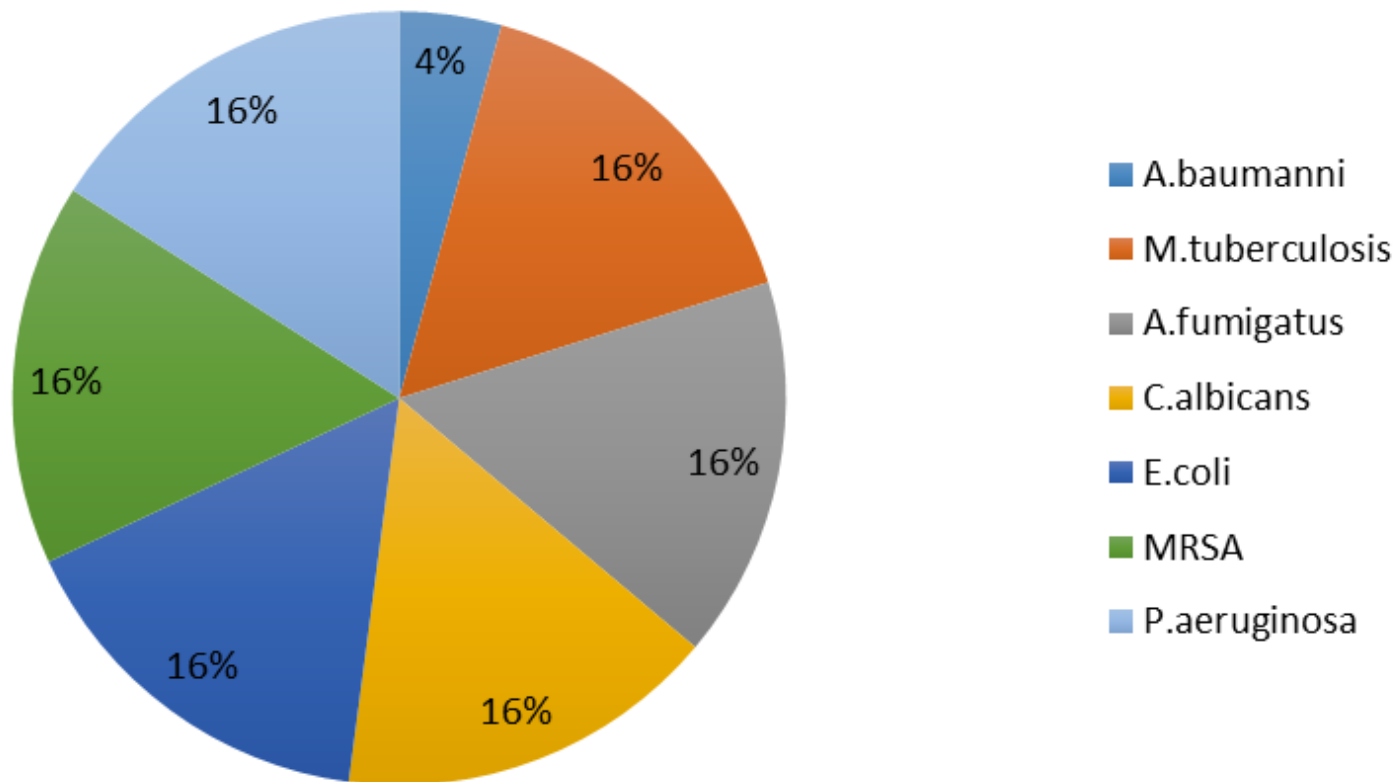
OpenNAPIS[™]
Functional Design

White Point Systems, Inc.
20100626

WP3 PharmaSea Anti-infective assays



WP3 – Anti-infective Screening Events (n = 87,356)



Over 15,000 Extracts and fractions tested

Antibacterial

- ✓ *Staphylococcus aureus* MRSA (3.29 % Active)
- ✓ *Acinetobacter baumannii* (0.67 % Active)
- ✓ *Escherichia coli* (0.98 % Active)
- ✓ *Pseudomonas aeruginosa* (0.48 % Active)

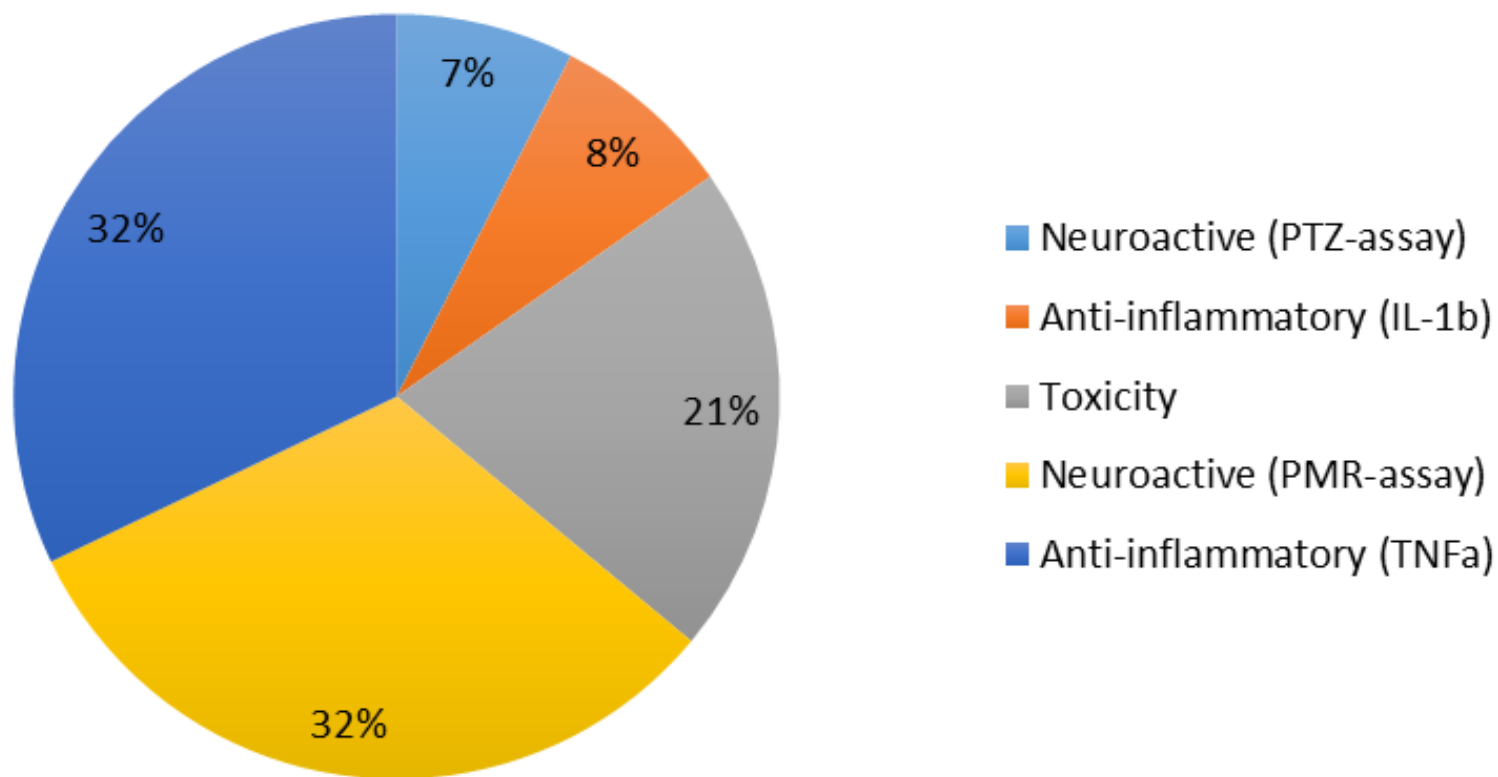
Antifungal

- ✓ *Candida albicans* (3.73 % Active)
- ✓ *Aspergillus fumigatus* (5.92 % Active)

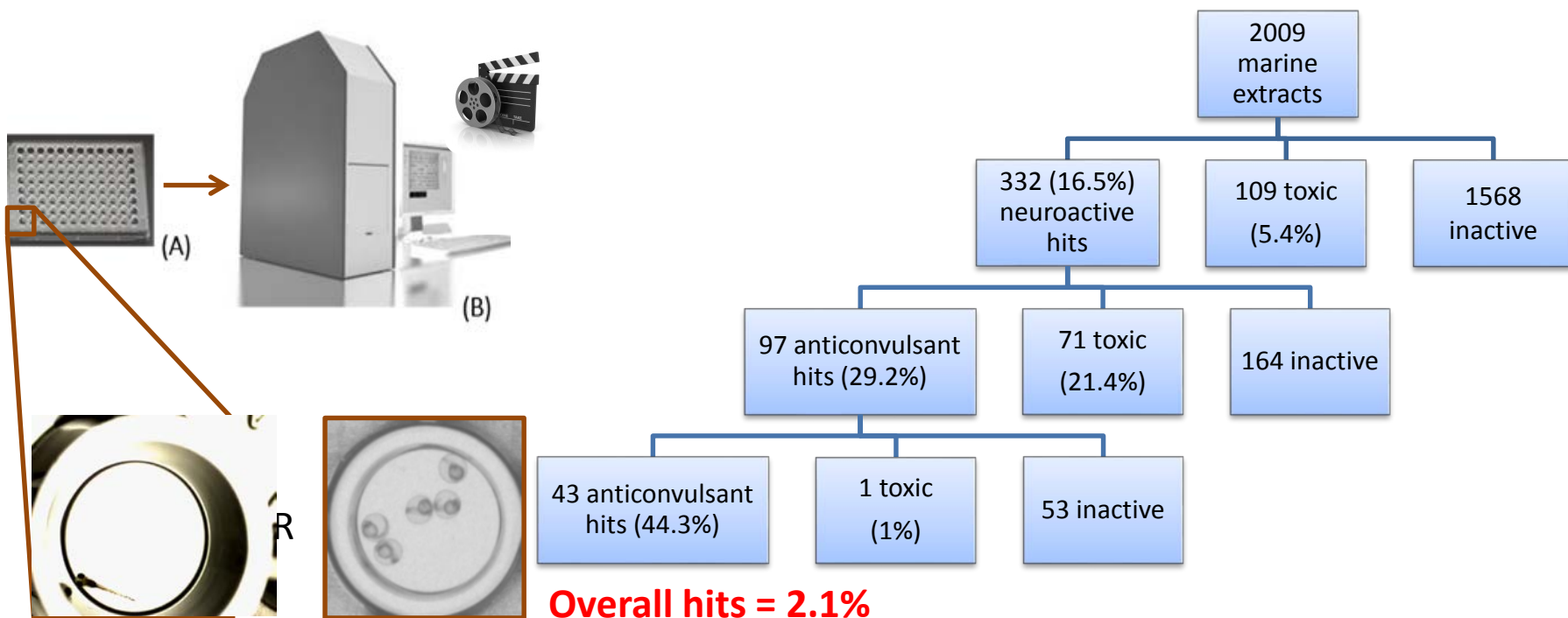
Anti-TB

- ✓ *Mycobacterium tuberculosis* H37Ra (3.86 % active)

WP 3 – Other Screening Events (n = 24,538)



WP4 CNS Assay Cascade



- **Primary Screen:** Photomotor response assay: neuroactive hits
- **Secondary Screens 1/2:** Epilepsy seizure model: anticonvulsant hits
- **Toxicity:** Maximum Tolerated Concentration (MTC) analysis

WP3 CNS Activity Traced to Active Principle

NAPIS®

Search: OSAR

Parameters

Results

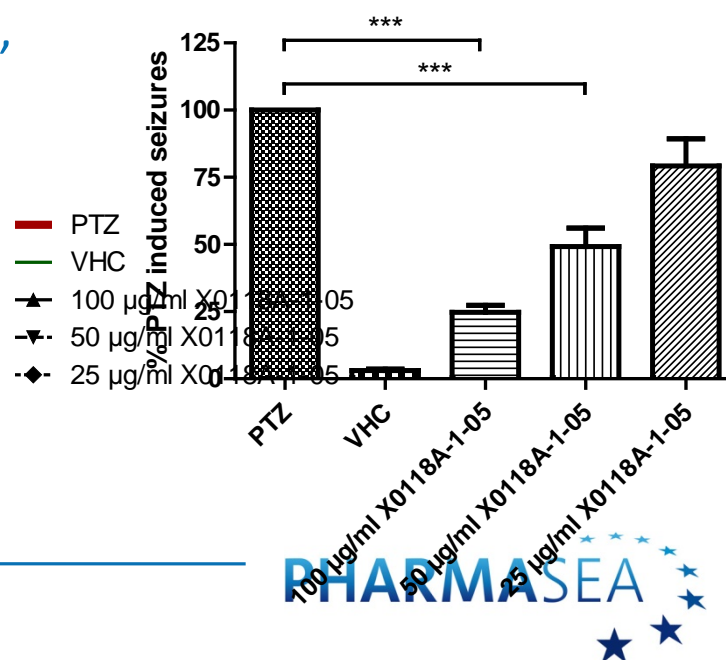
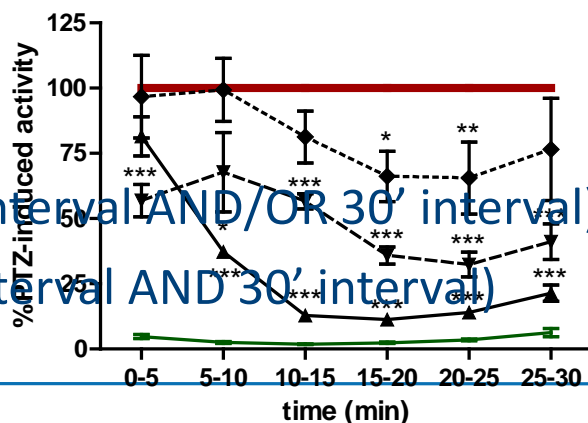
Results

Group Excel Map Chart Selectivity

chemistry_id	assay_category	assay_name	activity	units	assay_qual_result	concentration	concentration_units
SK0117	NEUROACTIVE	PTZ-ASSAY	144.8	% SEIZURE ACTIVITY	I	33	UG/ML
SK0117	NEUROACTIVE	PTZ-ASSAY	138.1	% SEIZURE ACTIVITY	I	11	UG/ML
SK0117	NEUROACTIVE	PTZ-ASSAY	104.7	% SEIZURE ACTIVITY	I	100	UG/ML
SK0010	NEUROACTIVE	PTZ-ASSAY	72.97	% SEIZURE ACTIVITY	I	11	UG/ML
SK0010	NEUROACTIVE	PTZ-ASSAY	3.279	% SEIZURE ACTIVITY	A	33	UG/ML
SK0010	NEUROACTIVE	PTZ-ASSAY	0.03664	% SEIZURE ACTIVITY	A	100	UG/ML

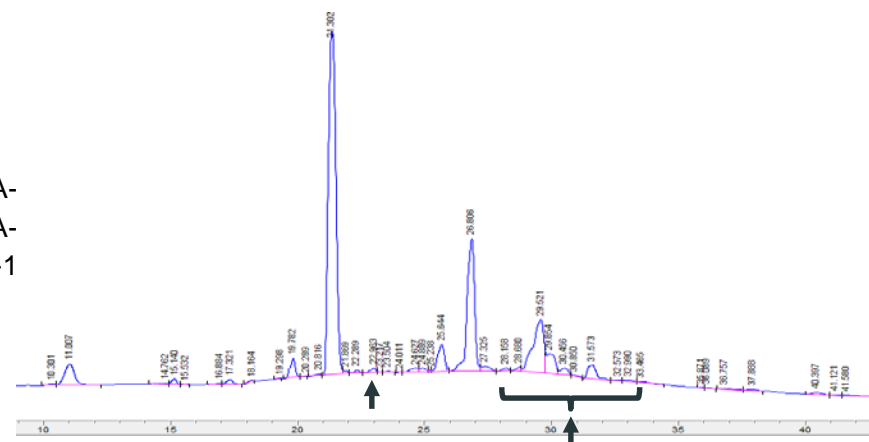
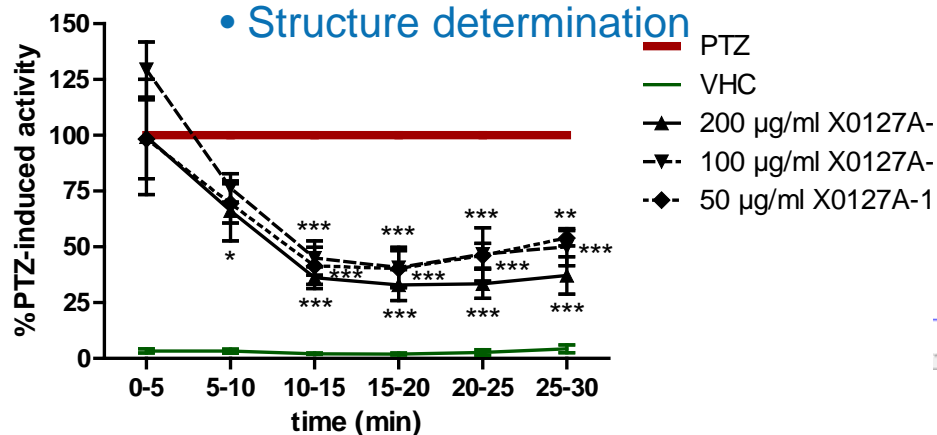
Normalized motion 5' interval = 6 dimensions,
not compatible with NAPIS
Normalized motion 30' interval = 1 dimension,
compatible

→ A: $p < 0.05$ (5' interval AND/OR 30' interval)
→ I: $p > 0.05$ (5' interval AND 30' interval)



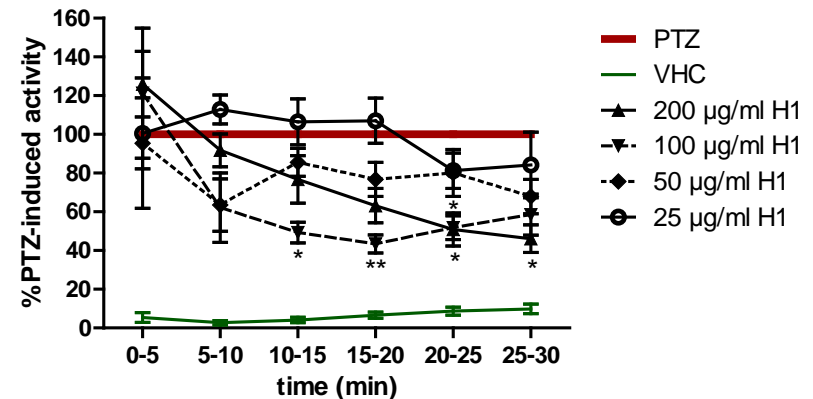
WP3/4/5 Identification of the Anticonvulsant Hit X0127A-1-04

- University of Tromsø
 - isolation of the marine microorganism
 - fermentation and extraction
 - pre-fractionation of the extract for bioactivity analysis
- KU Leuven
 - neuroactive and anticonvulsant screening
 - toxicity analysis
 - confirmation of anticonvulsant activity in three independent experiments
- University of Aberdeen
 - Purification step
 - Structure determination

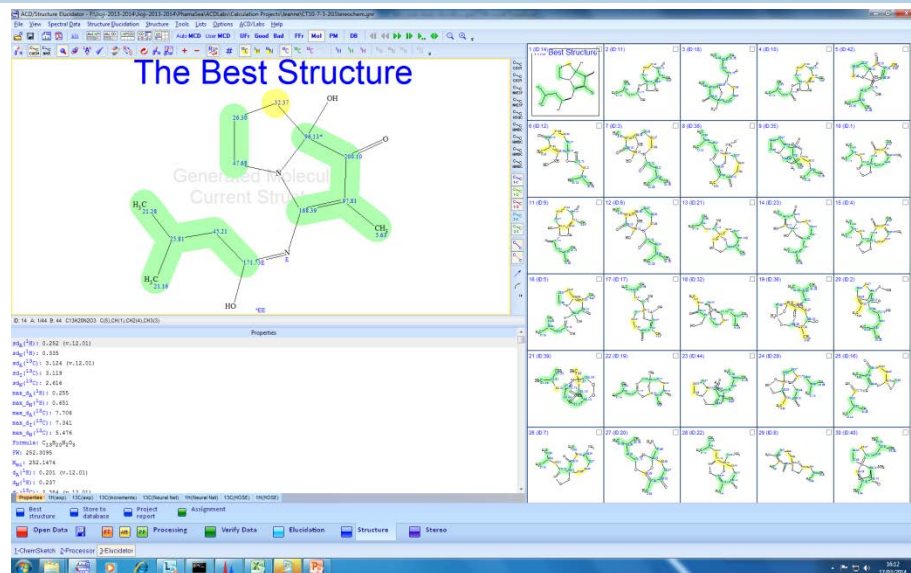
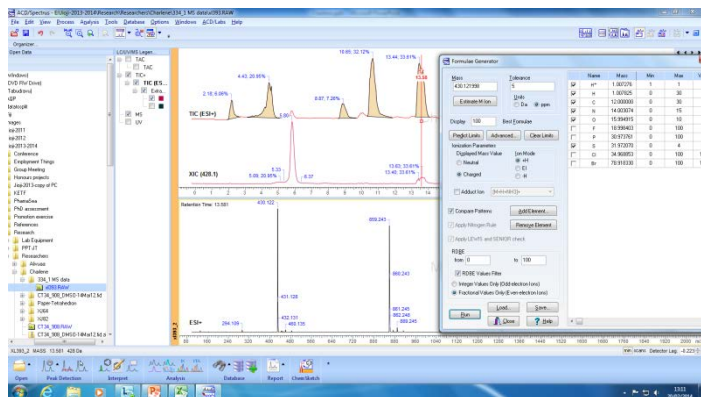


WP3/4/5 Function-based purification of X0127A-1-04

- SealifePharma
 - scale-up of X0127A-1-04
- University of Aberdeen
 - purification of final scale-up SPE100%
 - identification of one pure compound (novel small molecule)
 - purification of the peptides is ongoing
- KU Leuven
 - activity analysis
 - challenge: small molecule has anticonvulsant effect, but efficacy is lower than X0127A-1-04
 - analysis of the peptides will be initiated
 - next level analysis of anticonvulsant activity
 - investigate effect of active pure compound(s) also on other seizure markers than seizure behaviour



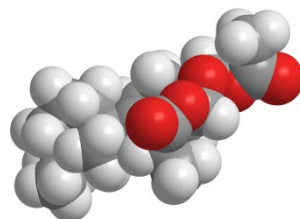
WP4 – Chemistry Tools and New Chemistry



Better chemical informatics

- Find known compounds & reduces wasted effort
- Pinpoints new compounds
- Automated processing of large volume of data

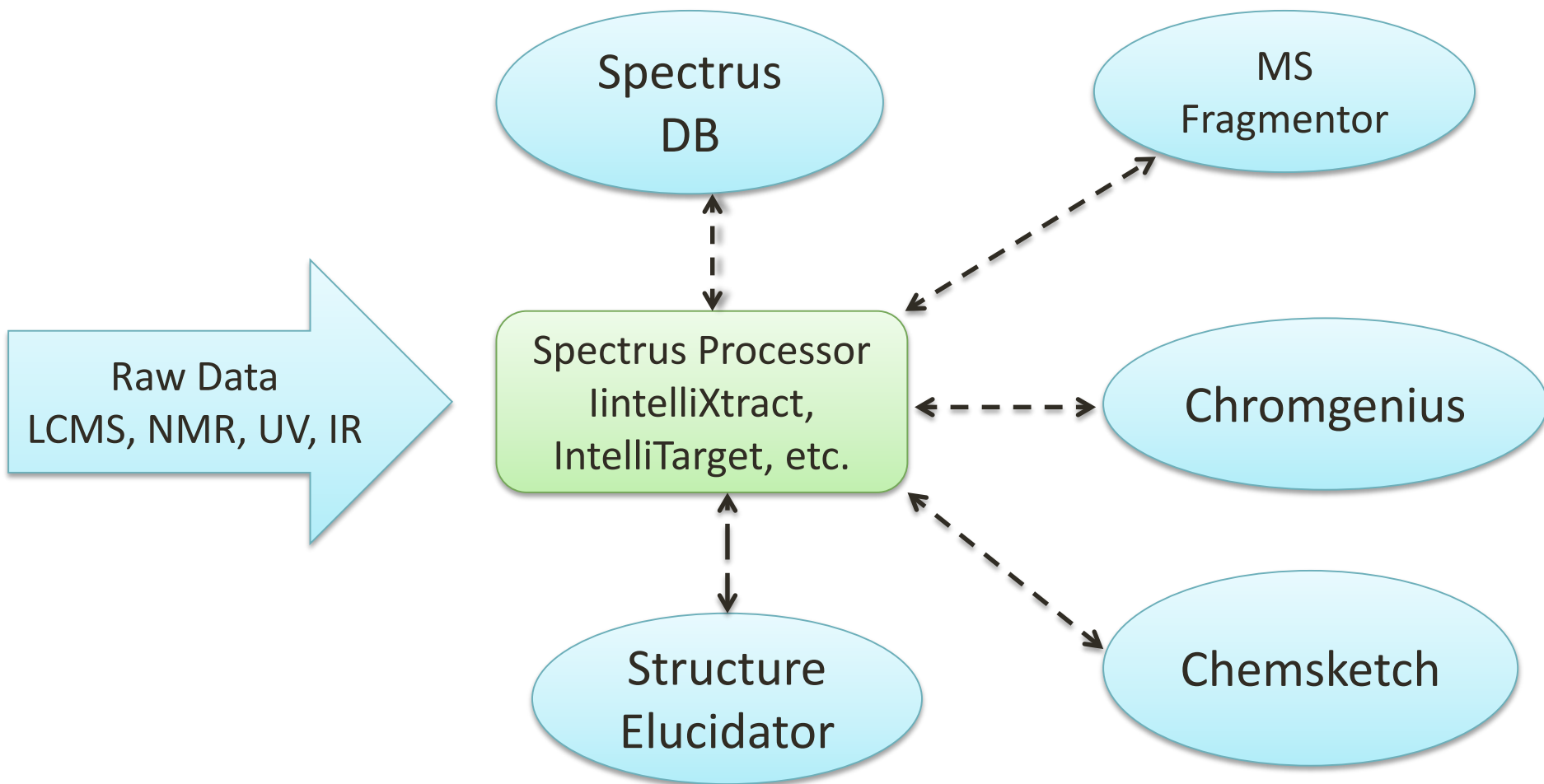
Automated structure determination workflow



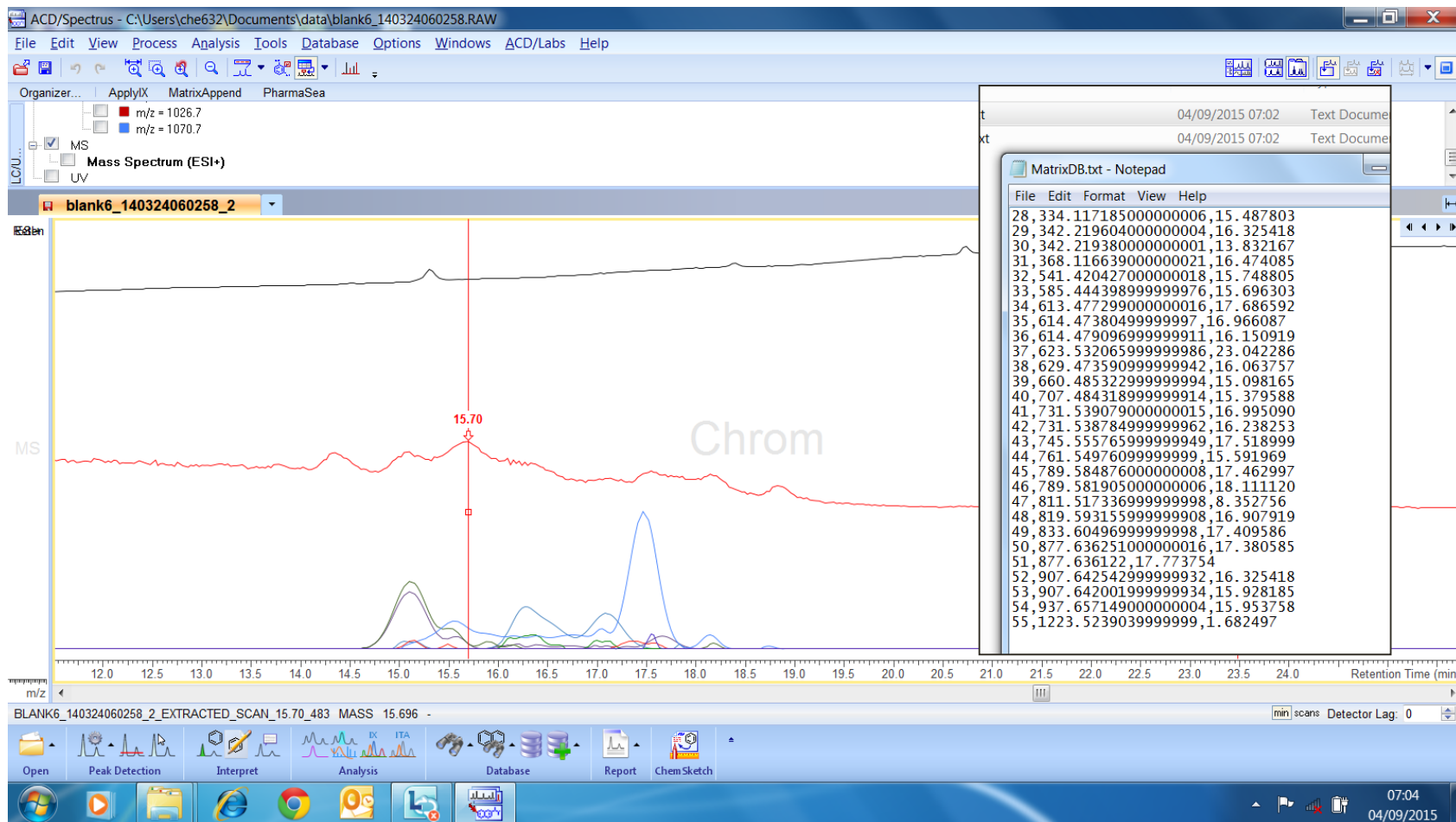
**Better drug candidates
With new intellectual property**

Compound	Kd (μM)	% inhibition
Standard Drug(0.2 mM)	6.8	100
Compound X(1 mM)	5.1	96

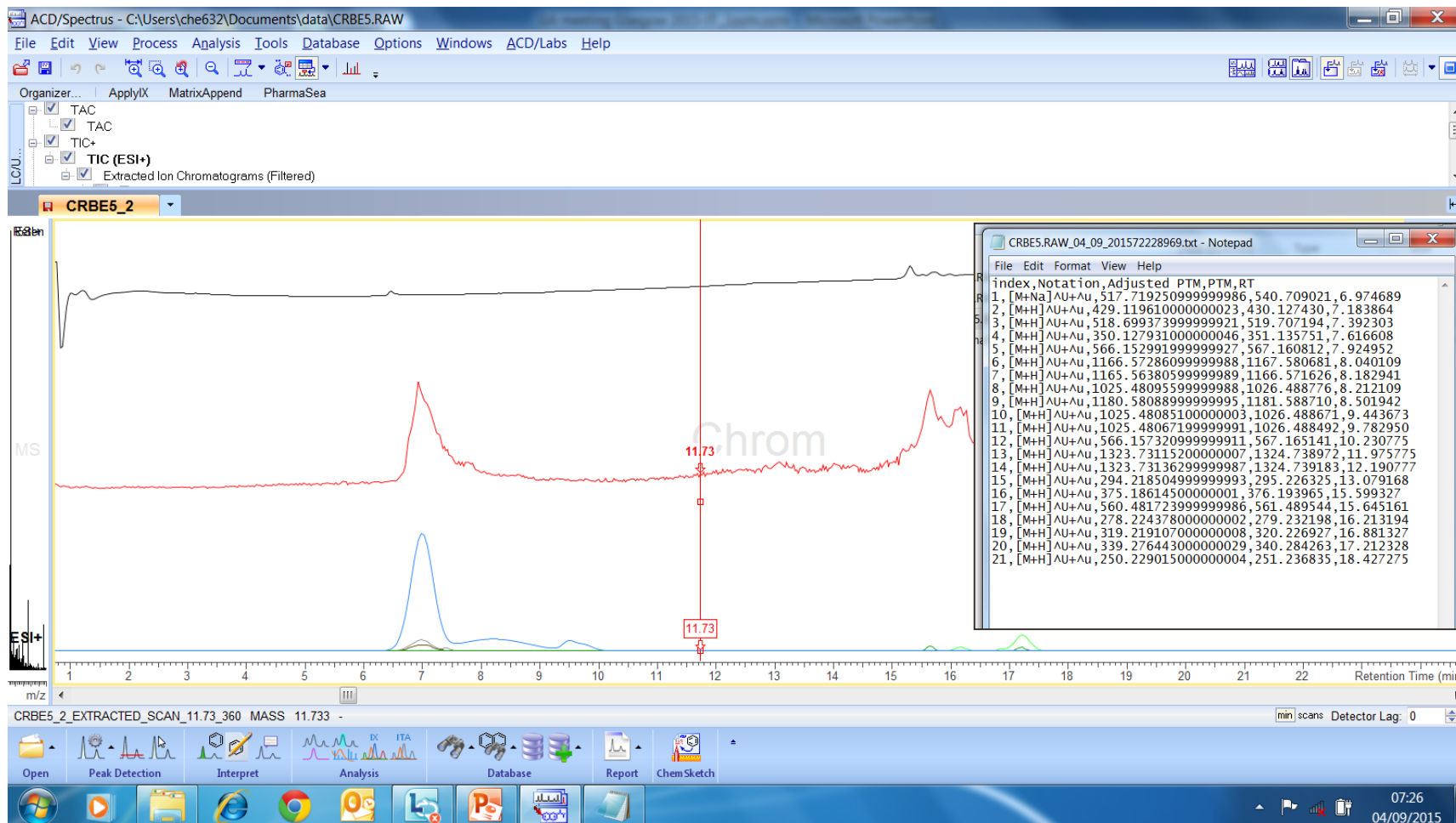
WP4 New dereplication tools by developing ACD/Labs software tools



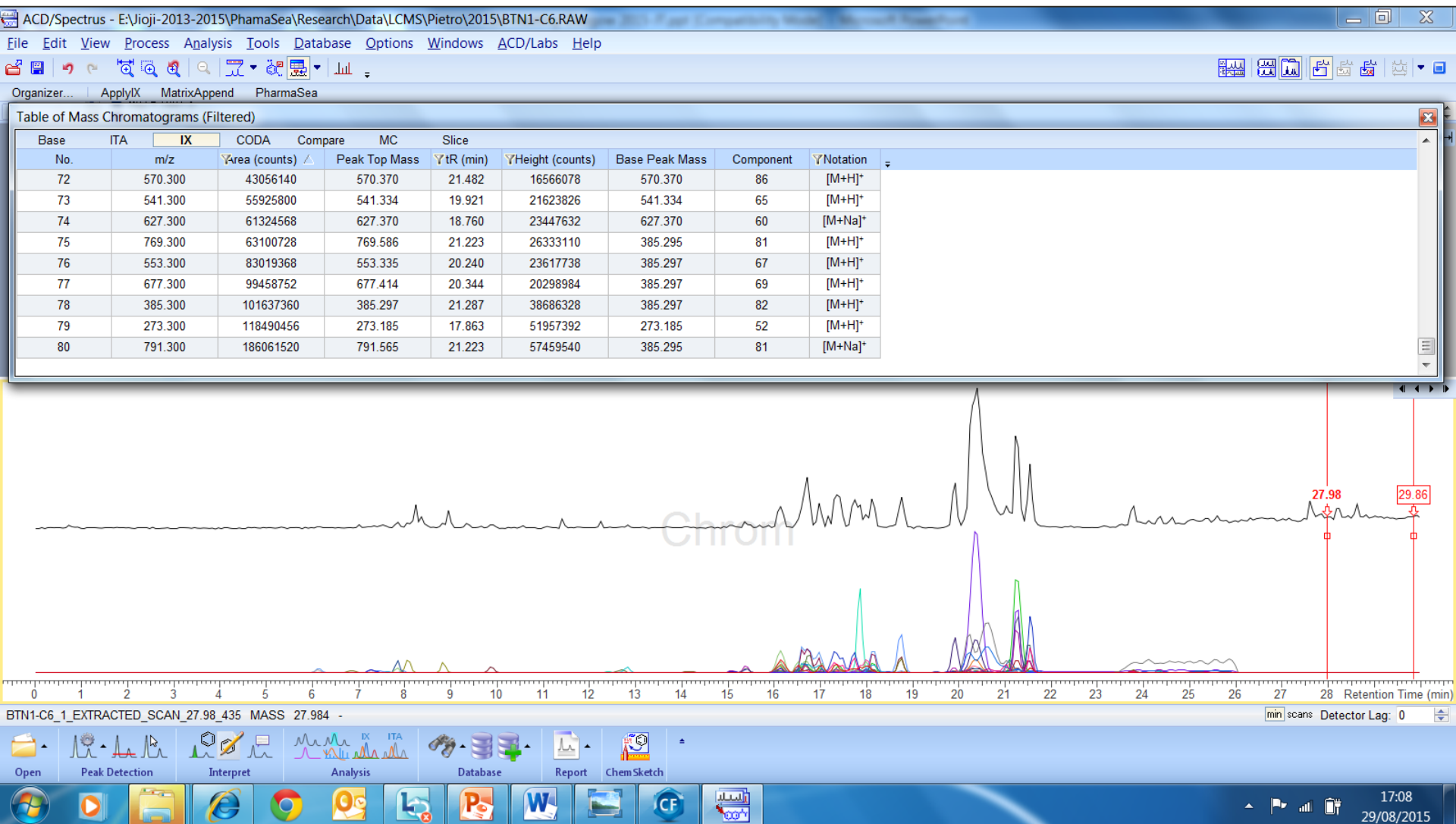
Untargeted Dereplication – Analysis of blank



Untargeted Dereplication – Blank and Medium Removed

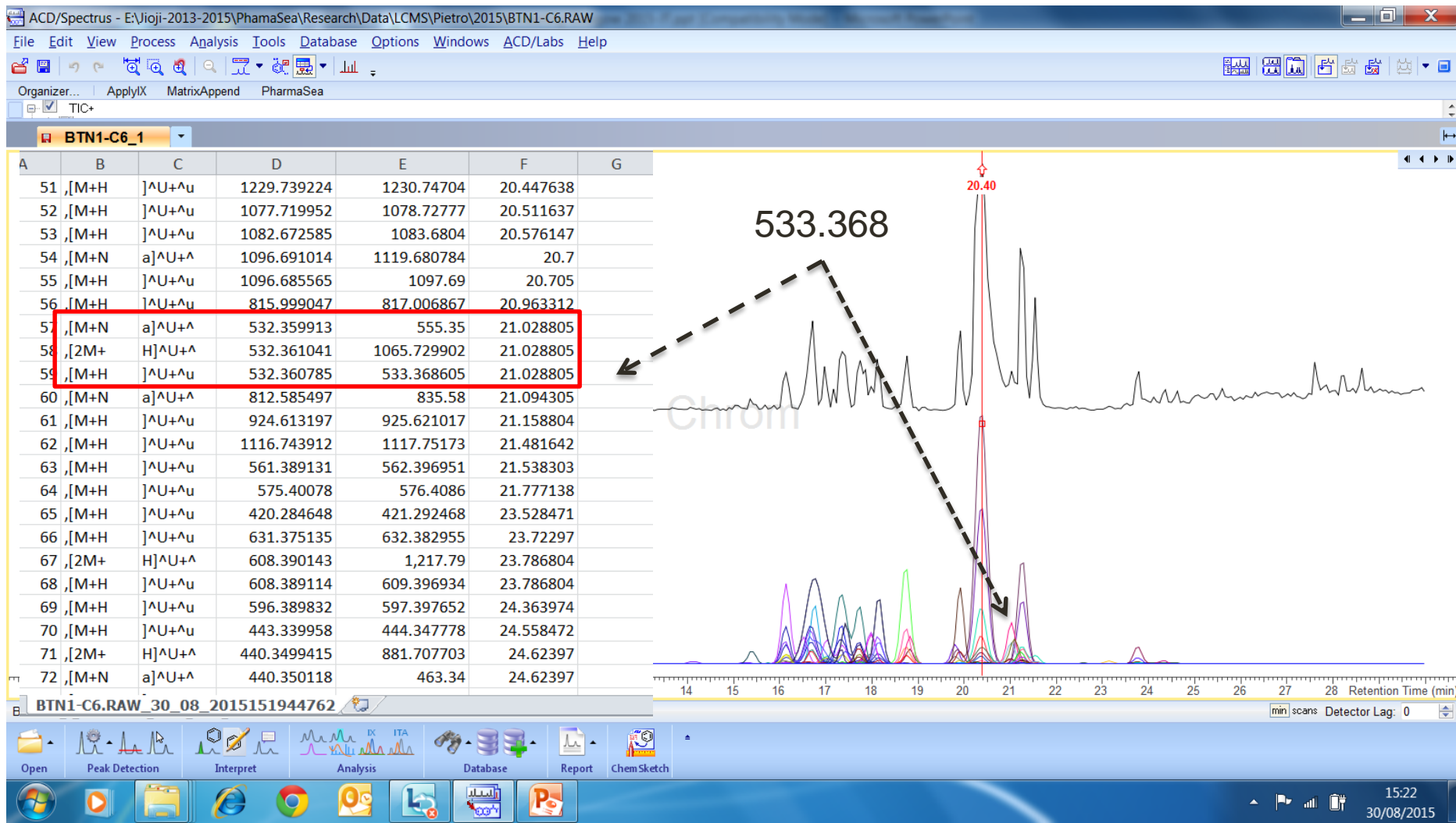


Untargeted Dereplication – Data Processing



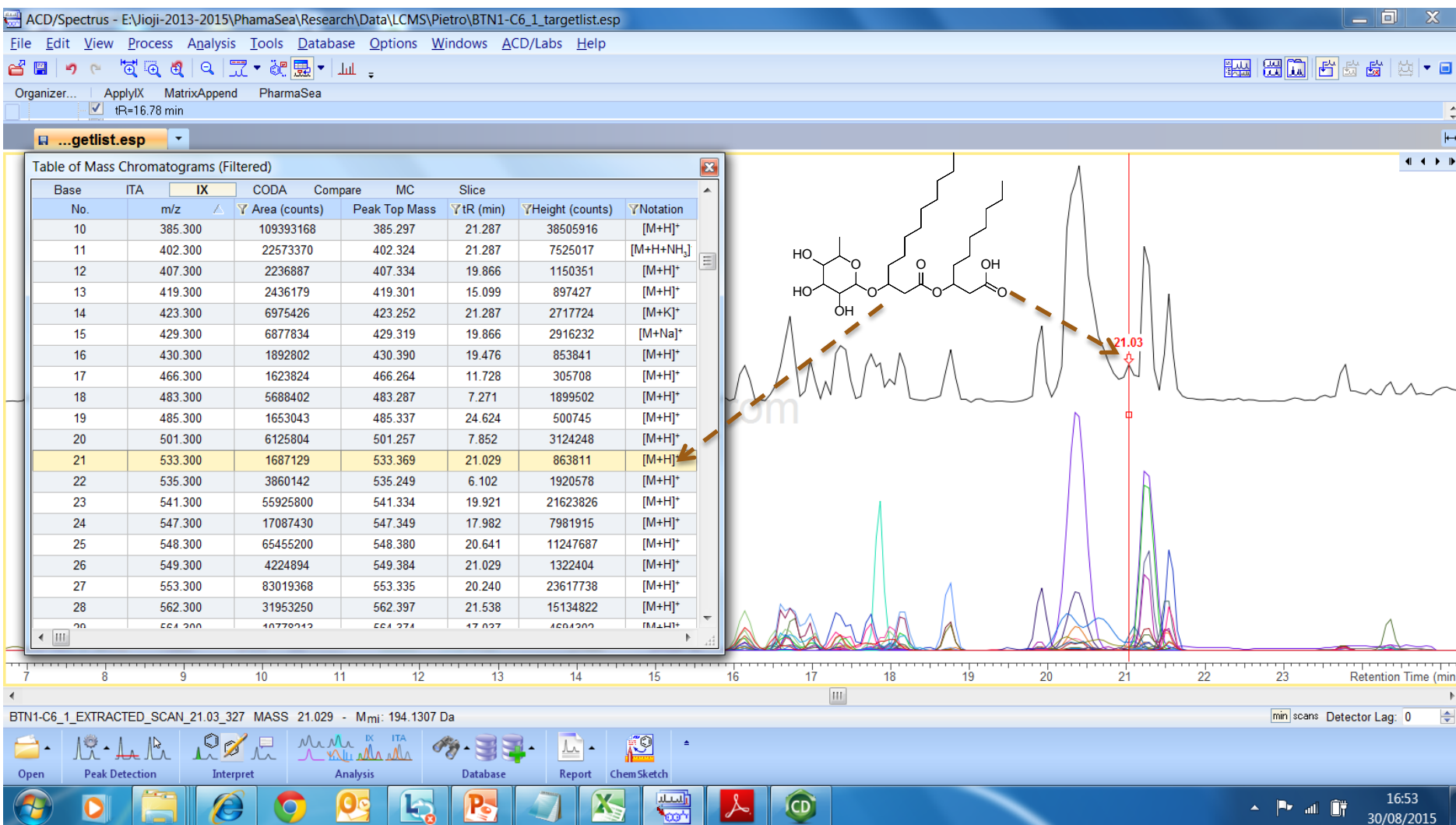
Untargeted Dereplication

Blank, Medium & PharmaSea DB Excluded



Untargeted Dereplication - Data Processing by IntelliXtract

Discover Unknowns



Targeted Dereplication

Calculation of tR by ACD/Labs ChromGenius

ACD/ChromGenius Batch: Database Window - [C:\Alberts-Cambridge July 2015\jioji chromgen latest.cgb]

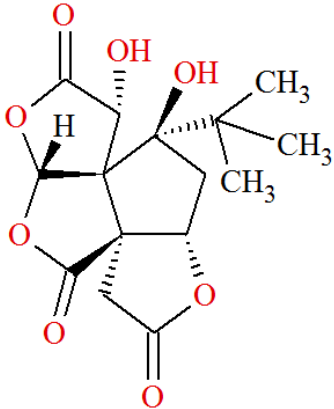
Database View Method Record Structure Search Lists Plates Options ACD/Labs Help

Tile Table Default Plates Screen Form 1

No.	ID	Name	tR	tR calc. ...	Differ. tR
1	7	Structure 7	6.400	7.344	

Method Parameters

Method Name: ABDN1
 Temperature: 25 deg.C
 Mobile Phase A: H2O; 0.03 M Formic Acid (pH 2)
 Mobile Phase B: Acetonitrile; 0.03 M Formic Acid (pH 2)
 Gradient: 100-0A 0-100B (20 min); 0-0A 100-100B (5 min)
 Flow Rate: 0.4 ml/min
 t0: 0.4 min at Flow Rate: 0.4 ml/min
 Plate Number: 10000
 Instrumental Name: Orbitrap Discovery
 Column Name: Agilent poroshell 120 EC-C18
 Diameter: 2.1 cm
 Length: 100 cm

Chemical Structure: 

LogD
 LogP
 BP
 MR
 MV
 MW
 NA
 ND
 PSA
 α
 β
 π
 EMR
 LogL₁₈

Formula: C₁₅H₁₈O₈
 FW: 326.2986
 M+: 326.0996
 [M+H]⁺: 327.1074
 [M-H]⁻: 325.0929
 <Double-click to enter new data item>

Graph

Retention time, min

Experimental

Calculated

304 MAE=1.6328 RMSE=2.3076 ☒ Show Bad Points log
 417 pts. R²= - StD= - ☒ Colored Points
 All Methods Mixed

ID: 7 A: 7/417 B: 417 02/06/2015 09:24:17 Single DB HPLC

1-ChemSketch 2-Results 3-History 4-Processor 5-CGDatabase

Windows Taskbar: 15:20 01/09/2015

Targeted Approach (HREIMS & tR)

Data processing by IntelliXtract

ACD/Spectrus - E:\ioji-2013-2015\PharmaSea\Research\Data\LCMS\Pietro\BTN1-C6_1_targetlist.esp

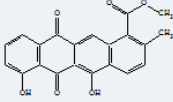
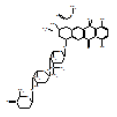
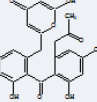
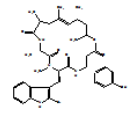
File Edit View Process Analysis Tools Database Options Windows ACD/Labs Help

IntelliXtract Options

Ion Presence

Modifications

Processing Options

	Reference Mass	Formula	Mass Delta	Structure	Ion Mode	Label	Mode	tR (Min)	tR Window
5548	362.079	C ₂₁ H ₁₄ O ₆	0		Auto	Resomycin C	Ref	16.4	4.0
5549	811.3415	C ₄₂ H ₅₃ NO ₁₅	0		Auto	Rhodirubin-G	Ref	11.2	4.0
5550	410.1002	C ₂₂ H ₁₈ O ₈	0		Auto	S-2617	Ref	9.34	4.0
5551	708.2522	C ₃₆ H ₄₅ BrN ₄ O ₆	0		Auto	Jasplakinolide	Ref	13.2	4.0

Load... Save... Import...

☐ Use Label Descriptor Only

☐ Overwrite Manually Created Labels

☐ Overwrite Automatically Created Labels

Add Delete Clear

Apply Compare IX Label Close ? Help

Open Peak Detection Interpret Analysis Database Report ChemSketch

16:33 30/08/2015

Targeted Dereplication

Find Matches

ACD/Spectrus - E:\Vioji-2013-2015\PharmaSea\Research\Data\LCMS\Pietro\BTN1-C6_1_targetlist.esp

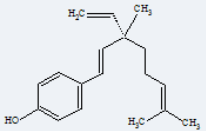
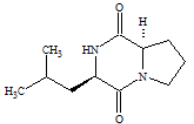
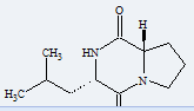
File Edit View Process Analysis Tools Database Options Windows ACD/Labs Help

Organizer... ApplyIX MatrixAppend PharmaSea

Table of Mass Chromatograms (Filtered)

Base No.	ITA m/z	IX Area (counts)	CODA Peak Top Mass	Compare MC	Slice YTR (min)	YHeight (counts)	YNotation
81	1169.300	920086	1169.641	18.112	427696	[2M+H] ⁺	
82	1174.300	1922386	1173.823	23.787	905409	[M+H] ⁺	
83	1186.300	880807	1186.594	18.177	348205	[M+H] ⁺	
84	1191.300	2882852	1190.852	23.787	1287528	[M+H+NH ₃] ⁺	
85	1354.300	7222414	1353.819	20.396	2549233	[2M+H] ⁺	
86	1505.300	558532	1504.838	8.046	230145	[2M+H] ⁺	
87	1876.300	2214634	1876.690	20.512	963517	[M+H] ⁺	

Retention Time (min)

No.	tR(min)	Mass(Ao)	[M+H] ⁺	M ⁺	M ⁻	MF	Structure	Name	MS Match
2	16.463	256.183	257.190	256.182	256.183	C ₁₈ H ₂₄ O		Bakuchiol	Excellent
3	5.518	210.137	211.144	210.136	210.137	C ₁₁ H ₁₈ N ₂ O ₂		(3R,7aR)-Cyclo(leuc)	Excellent
4	5.518	210.137	211.144	210.136	210.137	C ₁₁ H ₁₈ N ₂ O ₂		Gancidin-W	Excellent

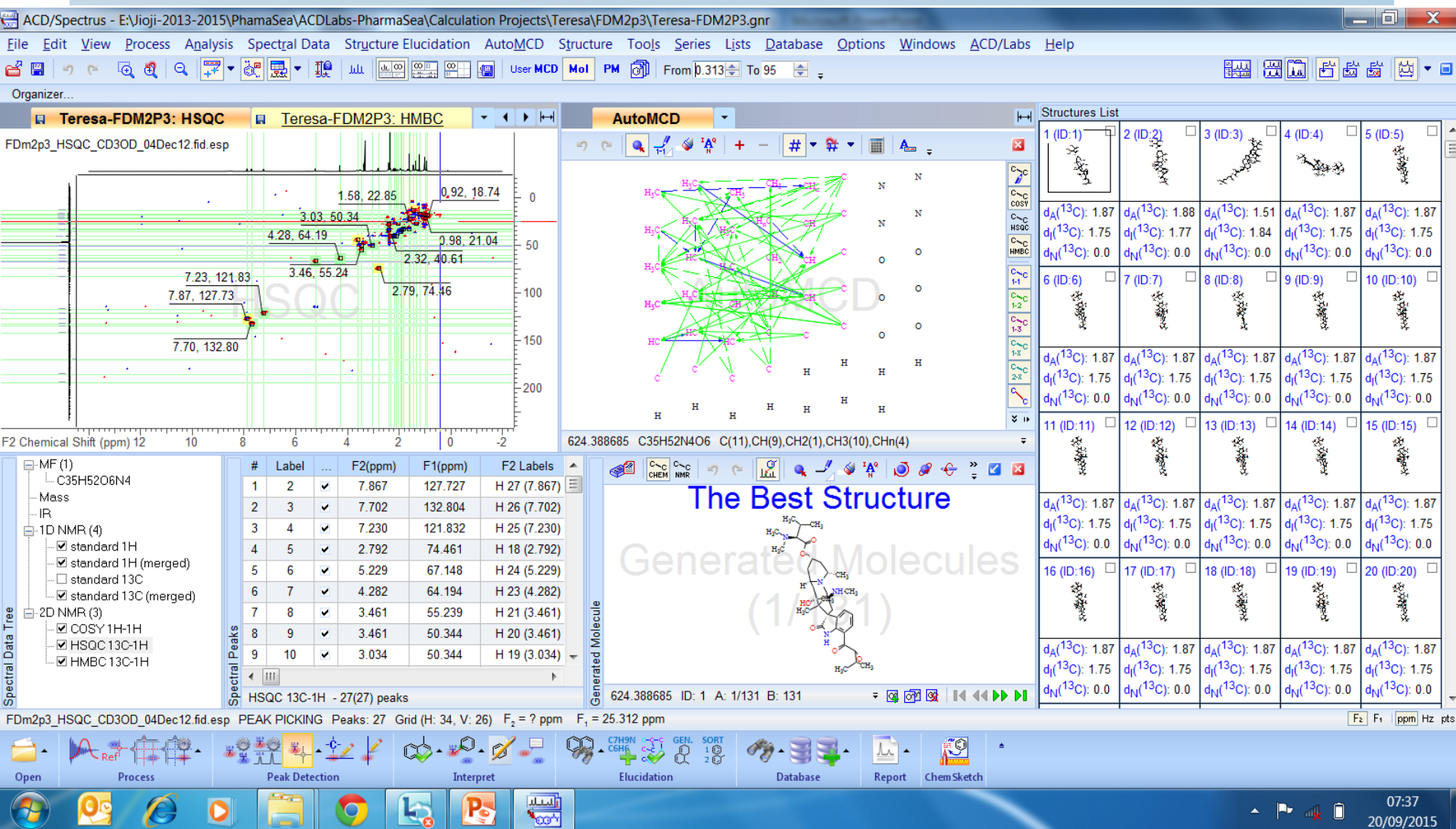
BTN1-C6_1_TARGETLIST_EXTRACTED_SCAN_5.45_85 MASS 5.454 - Mmi: 194.1307 Da

min scans Detector Lag: 0

Open Peak Detection Interpret Analysis Database Report Chem Sketch

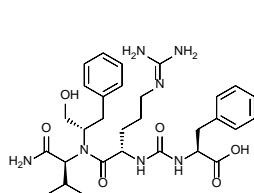
16:32 30/08/2015

Dereplication by NMR

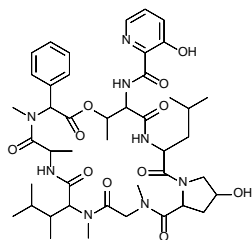


WP4 Some of the Chemical Diversity Isolated to Date

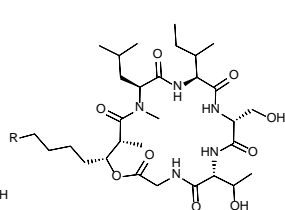
Several selected for scale up and further work



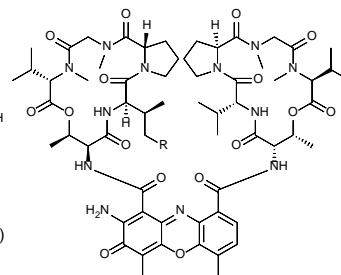
Mer-N 5075A
Family 1



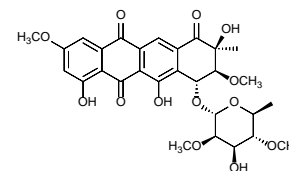
viridigrisein
Family 3



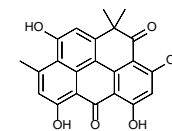
antibiotic SF 1902A1 (R = C₂H₅)
antibiotic SF 1902A2 (R = H)
Family 4



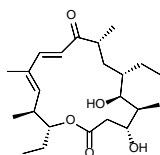
actinomycin D (R = H)
actinomycin C2 (R = CH₃)
Family 5



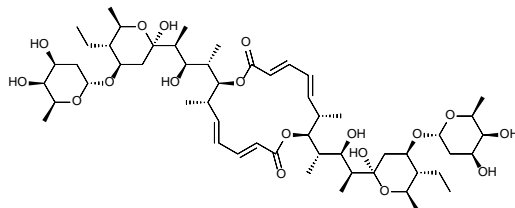
steffimycin B
Family 11



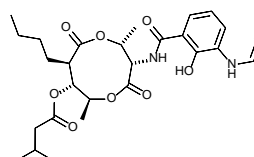
resistomycin
Family 12



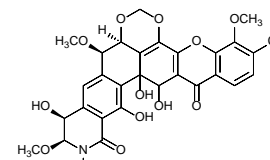
protylonolide
Family 6



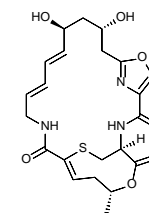
elaiophyllin (aka salbomycin)
Family 7



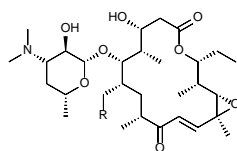
blastmycin
Family 21



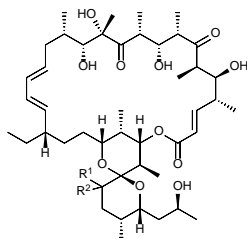
lysolipin X
Family 22



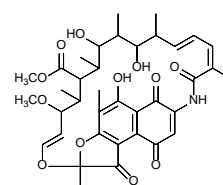
griseoviridin
Family 23



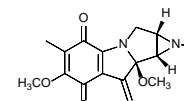
rosamicin (R = CHO)
juvenimicin (R = H, stereoisomer)
Family 8



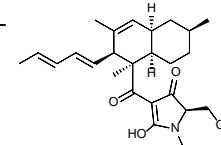
oligomycin A (R¹ = H, R² = H)
oligomycin B (R¹, R² = =O)
Family 9



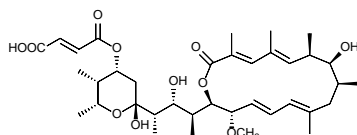
rifamycin S
Family 24



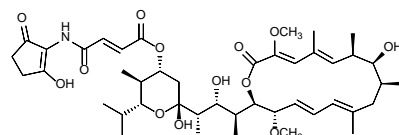
mitomycin K
Family 25



phomasetin
Family 26

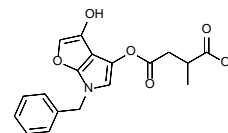


hygrolidin

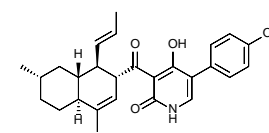


bafilomycin B₁

Family 10

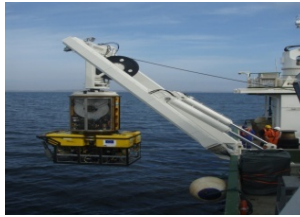


tensidol B
Family 27



ilicicolin H
Family 28

WP1-4 Assembling the Marine Biodiscovery Pipeline



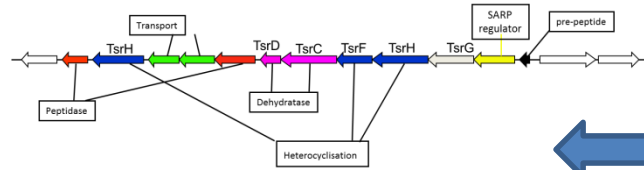
Stelletta normani
(1,300m)



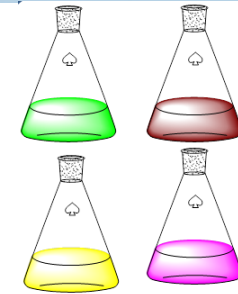
Lissodendoryx diversichela
(1,300m)



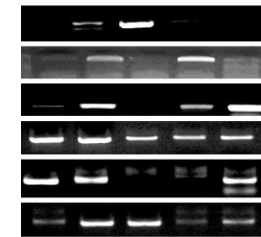
Inflatella pellicula
(2,900m)



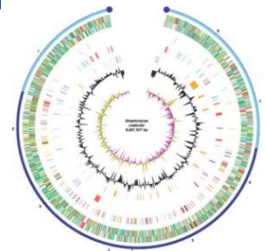
Discovery of new genes
giving new products



Fermentation under
different conditions



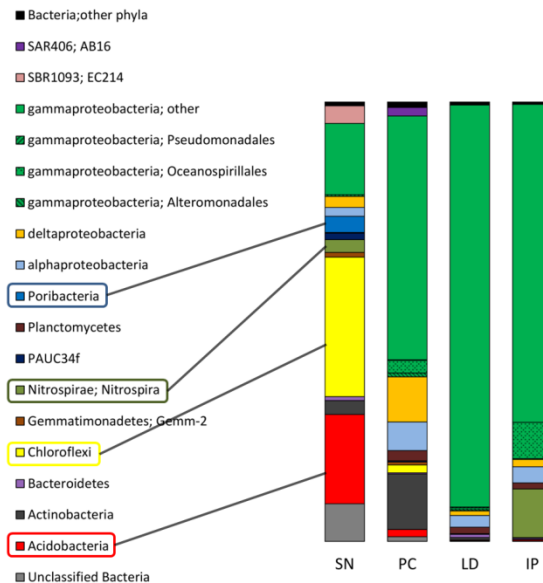
Gene expression
analysis



Genome
sequencing

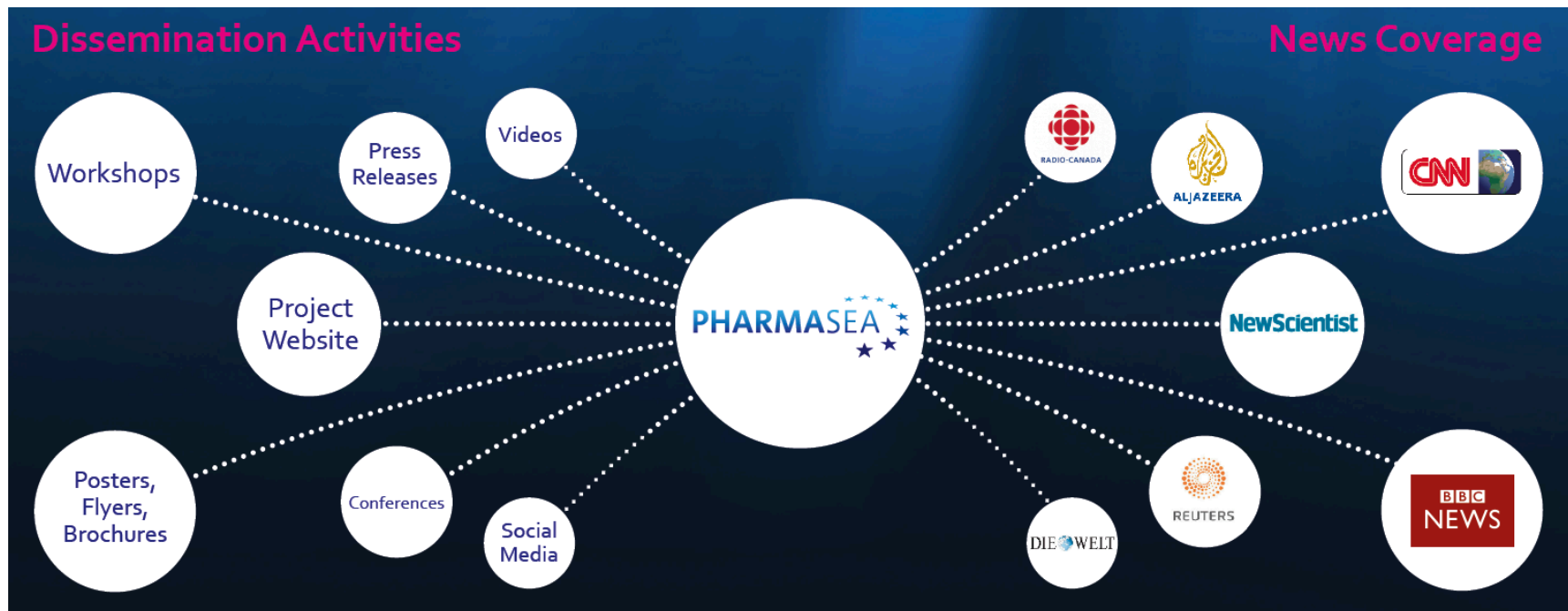


Isolation
of bacteria



Bacterial diversity in sponges

Communication and dissemination



WP7 Communication and dissemination: Radio and news articles



BBC: Drugs in dirt – Scientists appeal for help

US scientists are asking the public to join them in their quest to mine the Earth's soil for compounds that could be turned into vital new drugs...

» (read)

Copyright: <http://www.bbc.com>
20.01.2015



New Scientist: "Antibiotic abyss – the extreme quest for new medicines"

As antibiotic resistance increases, audacious expeditions are taking the quest to the ocean depths, and not a moment too soon...

» (read)

Copyright: www.newscientist.com



CNN: Arctic waters could hold secret to creating life-saving drugs

It is early afternoon on board the "Helmer Hanssen," and the Arctic sun is already starting to set. Near the back of the ship, two people dressed in orange rain slickers are anxiously waiting...

» (watch)

Copyright: <http://edition.cnn.com>



BBC Radio 4 Shared Planet: Medicinal Planet

Radio interview with PharmaSea's Project Leader Marcel Jaspars (Duration: 28 minutes)

In recent years some conventional medicines such as antibiotics have become less effective in treating diseases and infections. With an increasing human population worldwide, the need to discover new medicines for the benefit of human health will... [listen](#).



Reuters: Extreme medicine – The search for new antibiotics

Marcel Jaspars, a professor of organic chemistry at Britain's University of Aberdeen, is leading a dive deep into the unknown to search for bacteria that have, quite literally, never before seen the light of day...

» (read)

Copyright: www.reuters.com
17.08.2014



Welt Online: Neue Antibiotika schlummern in der Tiefsee

Antibiotika-Resistenzen breiten sich zunehmend aus. Um neue Wirkstoffe gegen die Superbakterien zu finden, starten Wissenschaftler jetzt eine Expedition zu den tiefsten Stellen der Tiefsee. ...» [read](#)

Copyright: Welt Online

WP7 Communication and dissemination: TV shows

“Vital Signs” on CNN



WP7 Communication and dissemination: TV shows

“The cure” on Al Jazeera

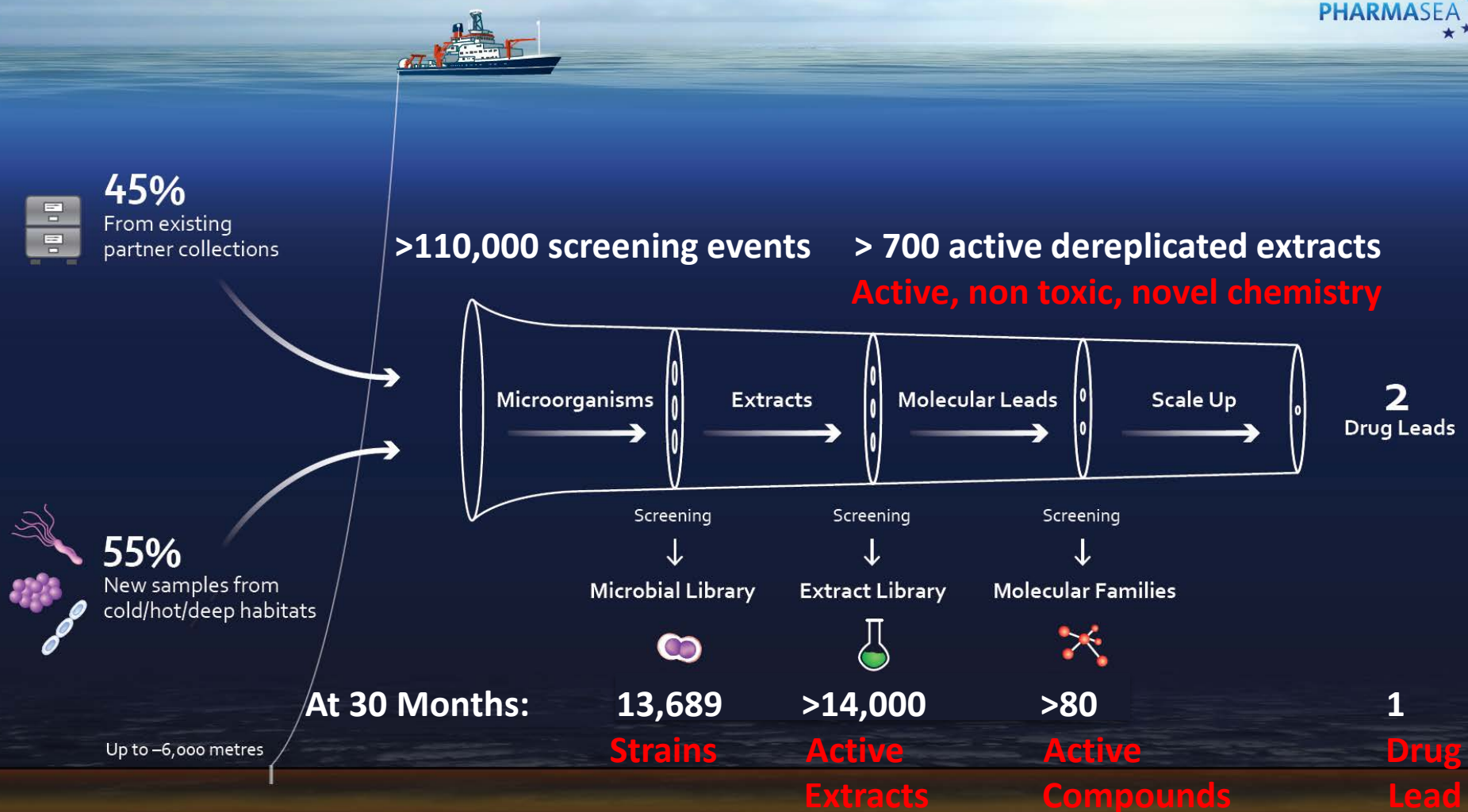


WP7 Communication and dissemination

PharmaSea was awarded at the **CommNet Impact Awards** in Brussels, Belgium on December 3rd, 2014 in the category "Engaging Citizens". The CommNet awards honour projects working across the bioeconomy, that have demonstrated excellence in communicating to European citizens, policy-makers, industry or young people.



PharmaSea Progress to Date



Conclusions

- PharmaSea will make marine biodiscovery more attractive for industry to adopt.
- PharmaSea is widening the bottlenecks
 - High quality biodiversity
 - Streamlined biodiscovery pipeline
 - New chemistry with new activity
- PharmaSea will provide mechanisms to transfer findings to end users whilst acknowledging:
 - Need for legal certainty over marine biodiversity collection.
 - Regulatory stress on companies.
 - Lack of risk taking by companies due to shareholder pressure.



<http://pharma-sea.eu/>



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