

**Antifouling compounds from
deep-sea
Streptomyces strains**

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Biofouling

Biofouling: undesired buildup of marine organisms, such as bacteria, marine invertebrates, macroalgae and etc.
widespread phenomenon

Adverse effects: Up to 40% increase in fuel consumption in ships; Cost about 6 billion US\$ worldwide every year



Ship in dry dock with fouled hull.



40cm

Polychaete worms that clogged
the pipelines in HKUST



Bryozoans that clogged
the aquaculture net

Three major fouling organisms in HK

Barnacle



0 h



6 h



12 h



Adults

Polychaete



0 h



0.5 h



2 h



6 h



9 h



Adult

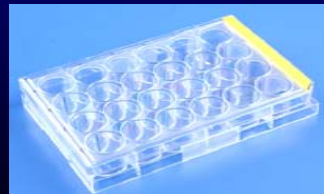
Bryozoan



0 h



Adults



bacteria in deep-sea

- Distribution: 90% of the ocean volume
- Extreme living environments
 - high or low temperature
 - elevated pressure
 - no available natural light

biological deserts



rainforests

bacteria in deep-sea

- ❖ Highly diverse
 - ✓ biodiversity → chemical diversity
- ❖ Metabolic pathways
 - ✓ different from terrestrial bacteria
 - ✓ unusual metabolites
 - ✓ new source for natural products

Deep-sea bacteria and biotechnology

❖ Industrial enzymes

- ✓ Heat stable enzymes, cold-adaptive enzymes

❖ Biomedication and bioremediation

- ✓ a deep-sea *Vibrio* isolate secreted a novel exopolysaccharide of medical interest
- ✓ a *Pseudomonas species* could accumulate very high concentration of cadmium onto its cell wall

Natural products as antifoulants

Advantages of natural products:
environmentally safe, easy to be degraded

- ❖ Many antifouling compounds have been isolated from sponges due to their chemical defense
- ❖ Problems: these compounds are usually very complex and limited reserves of sponges

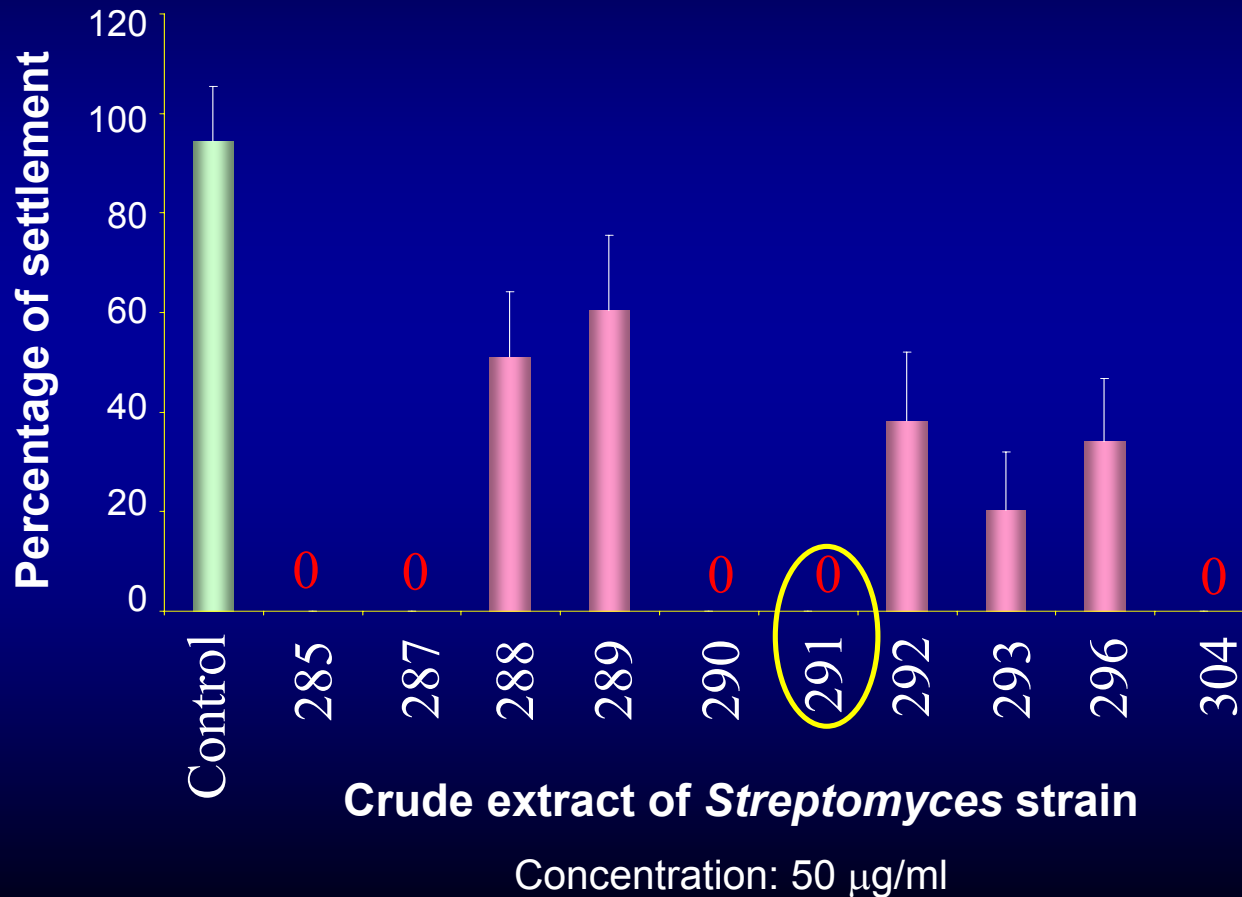
Microorganisms: unlimited proliferation

- ❖ *Streptomyces*: Gram-Positive bacteria with the largest genome size in Kingdom Bacteria
- ❖ Excellent source for bioactive compounds, such as antibiotics and enzymes
- ❖ Deep-sea *Streptomyces* has not been well explored

Objectives

- ❖ Isolate & identify bioactive compounds from *Streptomyces* isolated from deep-sea sediments
- ❖ Optimize the culture conditions on the yield of target bioactive compounds in bacteria

Larval settlement assay against *Balanus amphitrite* larvae



Isolation and purification of bioactive compounds

Mass culture of active *Streptomyces* strain 291

↓ Centrifugation

Bacterial
broth

↓ EA extraction

Crude extract

↓ Suspend in H₂O, partition
with hexane, EA, butanol

Hexane fraction

EA fraction

Butanol fraction

Water soluble fraction

↓ bioassay

Active fraction

↓ bioassay

Active sub-fractions or peaks

↓ Column Chromatography separation

↓ HPLC purification

Purify enough compounds

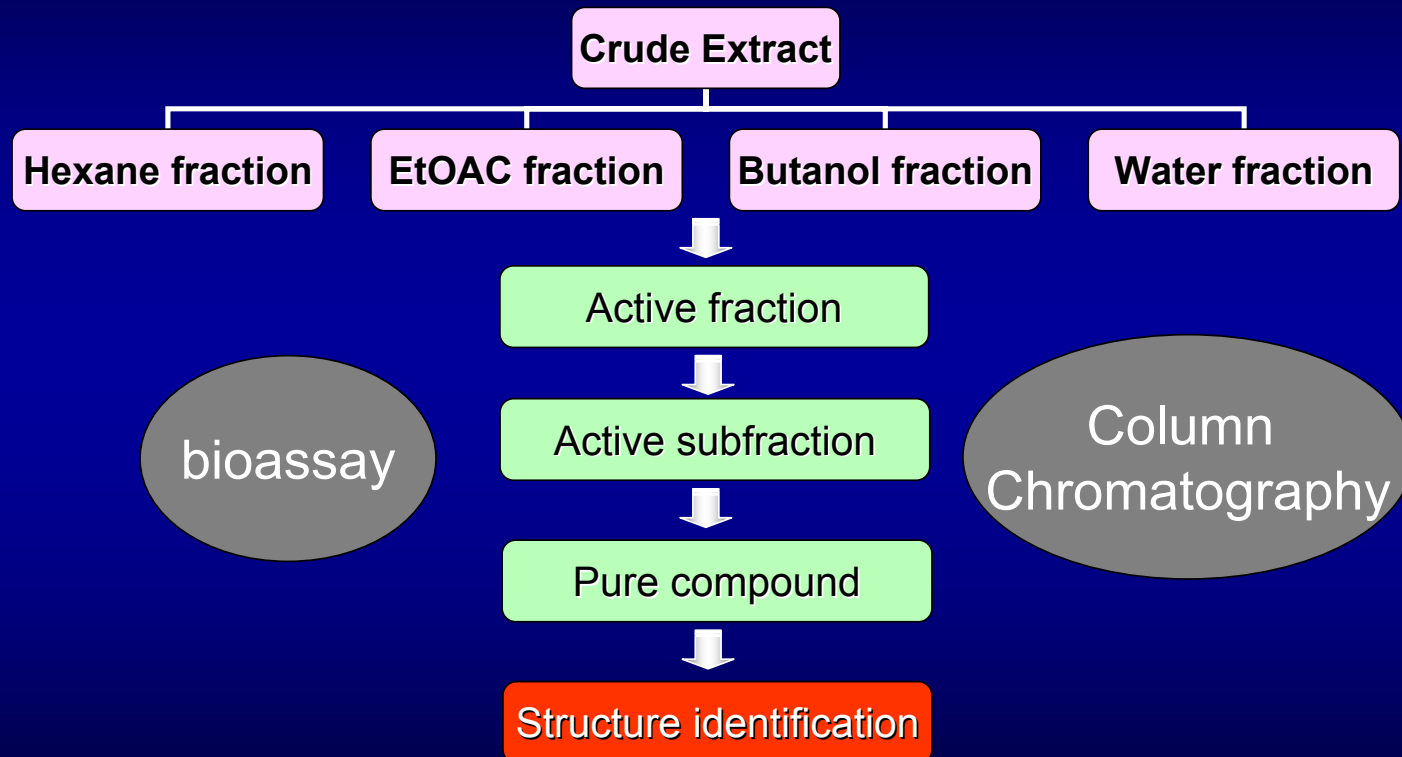
↓
Identify pure compounds using
NMR, GC-MS

Extraction or
partition process






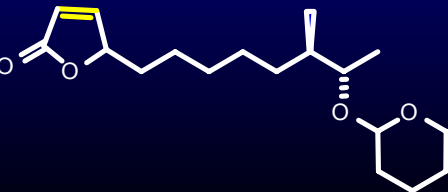


HPLC analysis and separation

Mass culture → Bacterial broth



Butenolides isolated and chemically synthesized

Compound	EC ₅₀ (LC ₅₀) against barnacle larvae(μg/ml)	
	16.9 (>100)	
	11.4 (>100)	<ul style="list-style-type: none"> The 2-furanone substructure is responsible for the activity?
	11.8 (>100)	
	120 (>140)	
	NA	<ul style="list-style-type: none"> The lipophilicity affects the activity?
	3.6 (>80)	
	NA: not active at 100μg/ml	

Structure-activity-relationship (SAR)



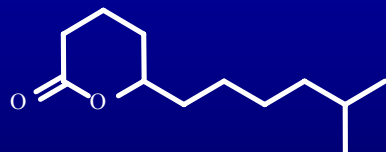
4 (>50)



2 (>50)



NA



NA

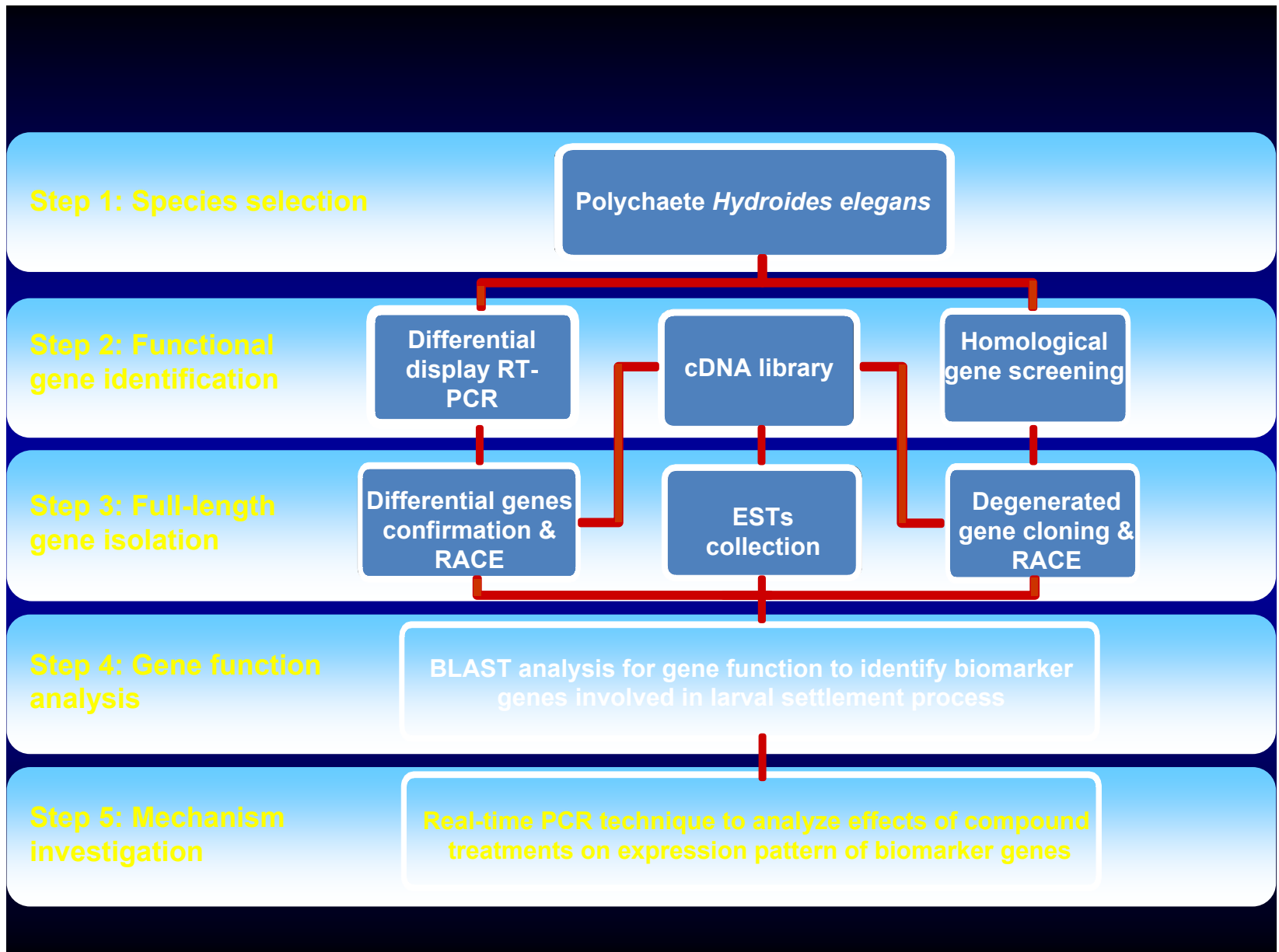


NA

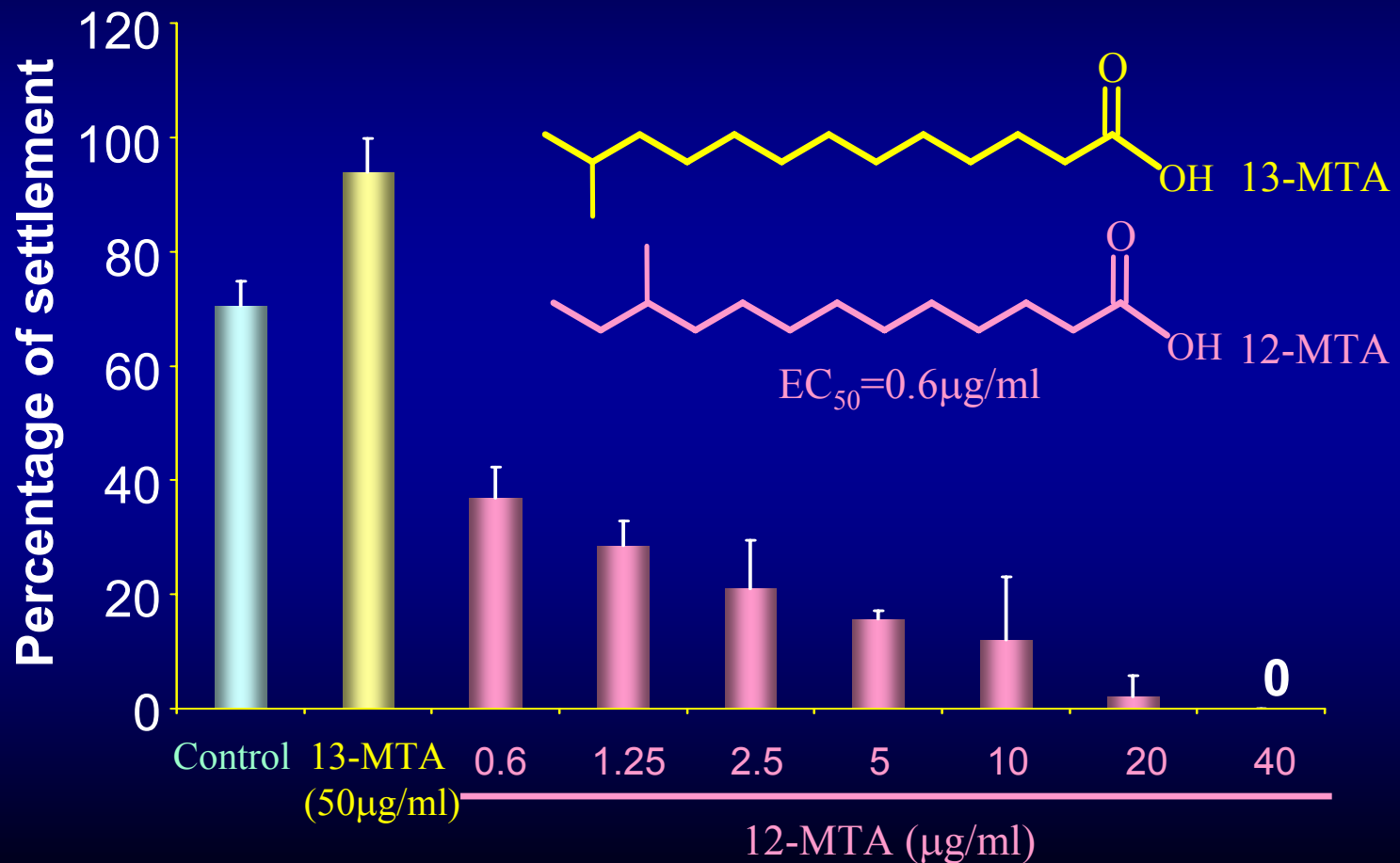
NA: not active at 100μg/ml

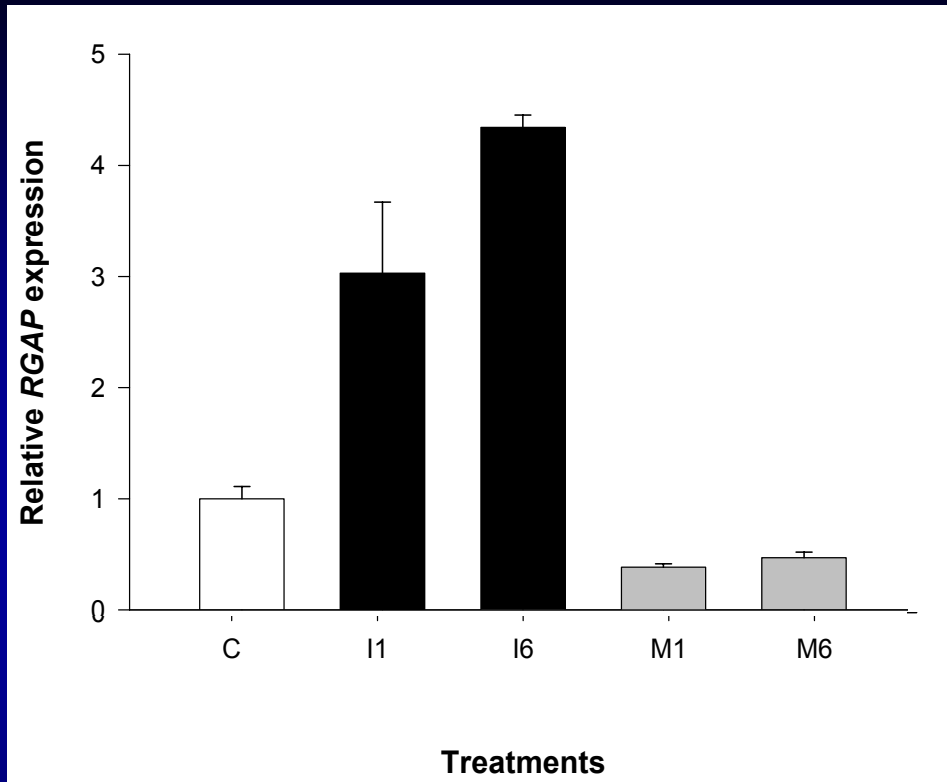
Summarization of SAR

- ❖ The furanone substructure
- ❖ The position of the double bond
- ❖ The lipophilicity



12-MTA inhibits larval settlement of *Hydroides elegans*





RGAP: Ran GTPase
activating protein

C: Competent larvae (Control)

I: IBMX (artificial larval settlement inducer)

M: 12-MTA (larval settlement inhibitor)

Future plan

- Further optimize the culture conditions for bioactive compound production
- Investigate the mode-of-action of bioactive compounds in larvae

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Lab mates and friends

Thank you!