第七届生物-有机地球化学研讨会·北京

Analysis of pivotal metabolic precursor-pterins in marine phytoplankton and bacteria

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Outline

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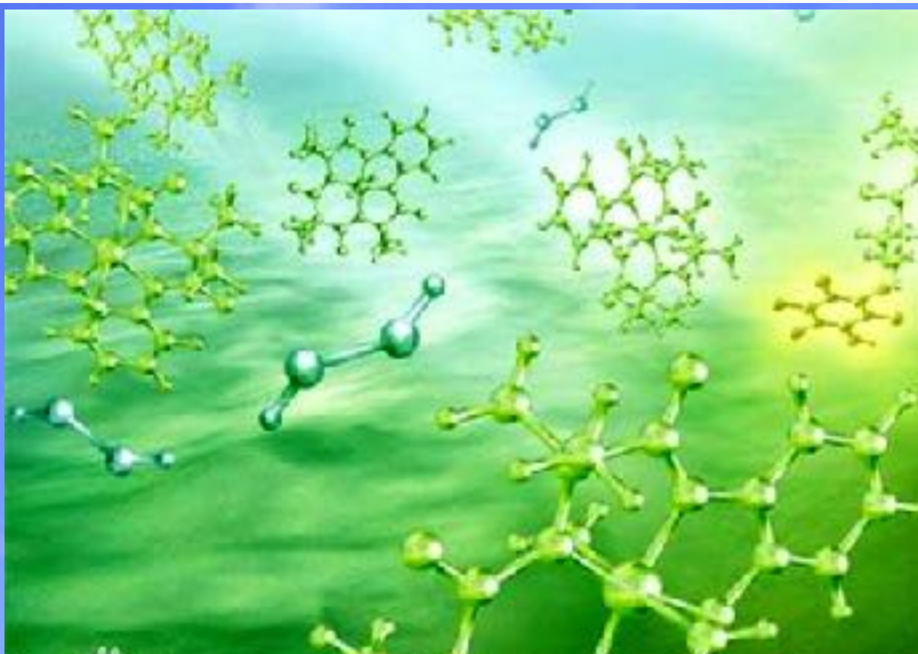
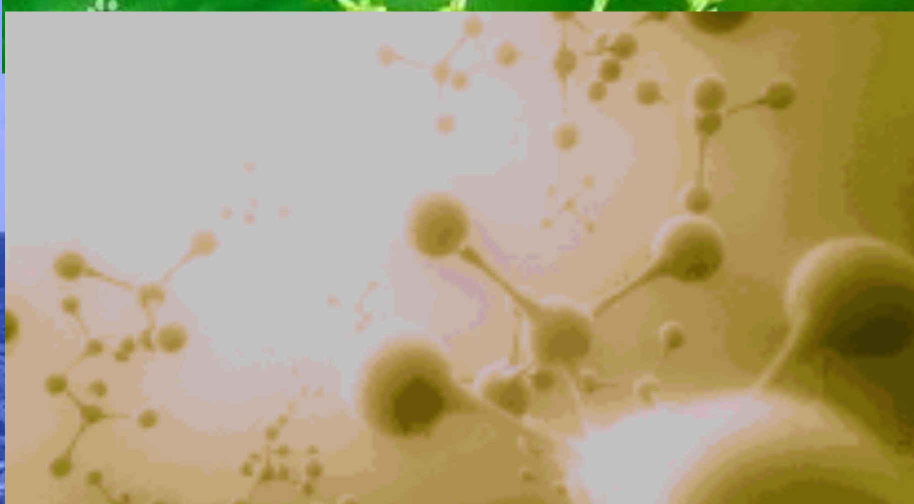


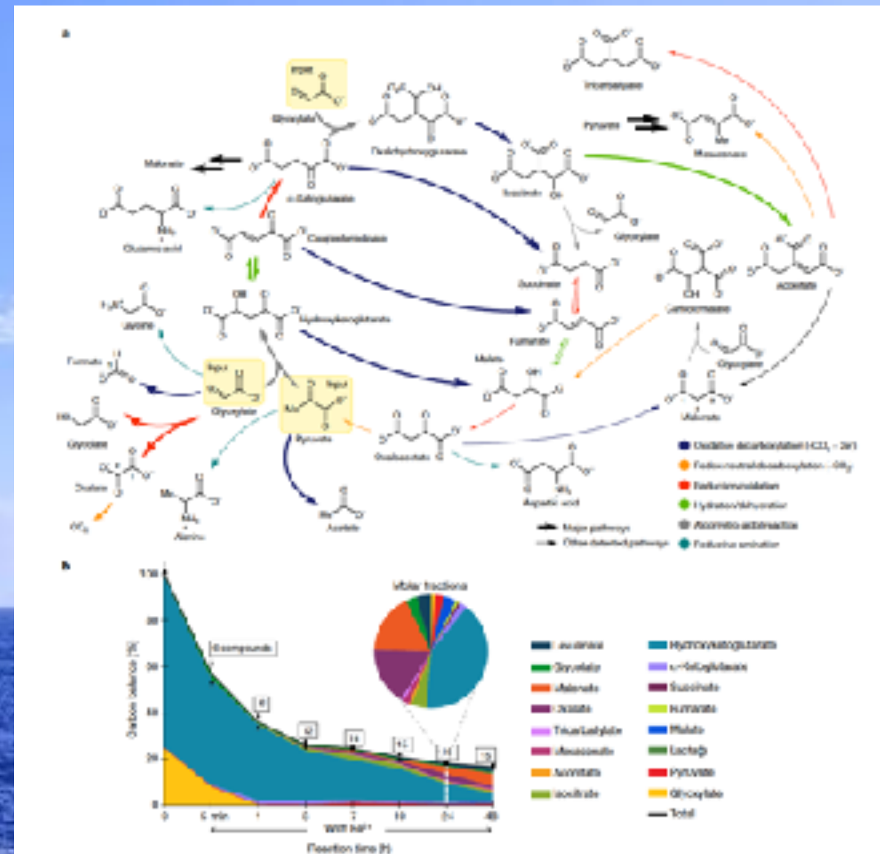


LETTER

https://doi.org/10.1038/s41586-019-1151-1

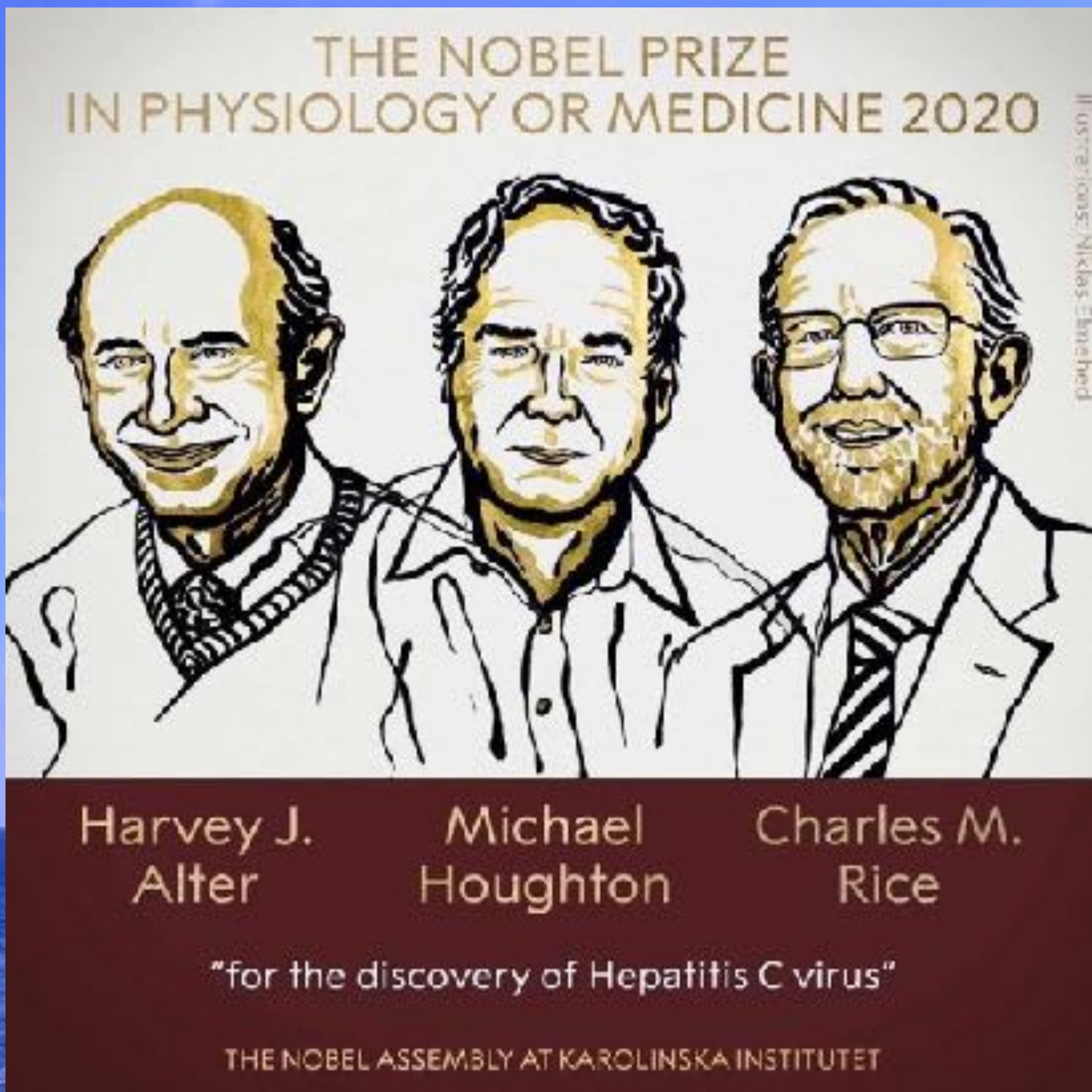
Synthesis and breakdown of universal metabolic precursors promoted by iron

Kamila B. Muchowska, Sreejith J. Varma & Joseph Moran



Kamila, Nature, 2019

2020 Nobel Prize



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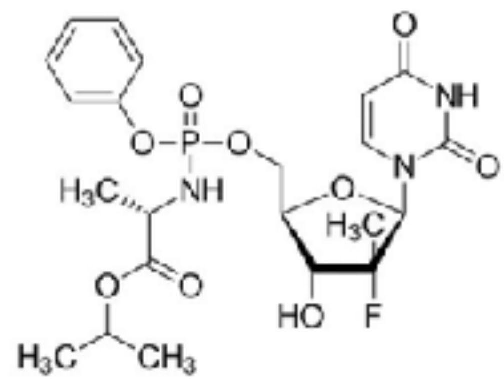

索非布韦

Pterins in medicine

PROTOCOL

Analysis of human cerebrospinal fluid monoamines and their cofactors by HPLC

Marta Batllori[1-3], Marta Molero-Luis[1-3,9], Aida Ormazabal[1-3], Mercedes Casado[1], Cristina Sierra[1], Angels García-Cazorla[1-3], Manju Kurian[4-7], Simon Pope[8], Simon J Heales[4-8] & Rafael Artuch[1-3]

Published online 19 October 2017; doi:10.1038/nprot.2017.138

The presence of monoamines and their cofactors (the pterins and vitamin B_6 (pyridoxal phosphate (PLP))) in human cerebrospinal fluid (CSF) can be used as indicators of the biosynthesis and turnover of dopamine and serotonin in the brain. In addition, abnormalities in the CSF levels of these molecules are associated with various neurological diseases, including genetic diseases leading to dopamine and serotonin deficiency. Here, we provide a set of quantitative high-performance liquid-chromatography (HPLC) approaches to determine CSF levels of monoamines and their cofactors. This protocol describes step-by-step procedures for CSF sample preparation for the analysis of different molecules, HPLC calibration and analysis, and data quantification and interpretation. Unlike plasma/tissue/blood samples, CSF requires minimal sample preparation; in this protocol, only the analysis of PLP requires mixing with trichloroacetic acid to release the protein-bound vitamin, centrifugation, and mixing of the supernatant with phosphate buffer and sodium cyanide for derivatization in alkaline conditions. Monoamines are analyzed by HPLC with coulometric electrochemical detection (ED), pterins are analyzed by HPLC with coupled coulometric electrochemical and fluorescence detection, and PLP is analyzed by HPLC with fluorescence detection. The quantification of all compounds is achieved by external calibration procedures, and internal quality control and standards are analyzed in each run. We anticipate that investigation of dopamine and serotonin disturbances will be facilitated by measurements of these compounds in human CSF and other biological samples. The estimated time for the different procedures primarily depends on the electrochemical detector stabilization. Overnight stabilization of this detector is advised, and, after that step, preanalytical equilibration rarely exceeds 3 h.

Current Microbiology
https://doi.org/10.1007/s00284-018-1433-0

Evaluation of Pterin, a Promising Drug Candidate from Cyanide Degrading Bacteria

Ramasamy Mahendran[1] · Murugesan Thandeeswaran[1] · Gopikrishnan Kiran[1] · Mani Arulkumar[1] · K. A. Ayub Nawaz[1] · Jayamanoharan Jabastin[2] · Balraj Janani · Thomas Anto Thomas[3] · Jayaraman Angayarkanni[1]

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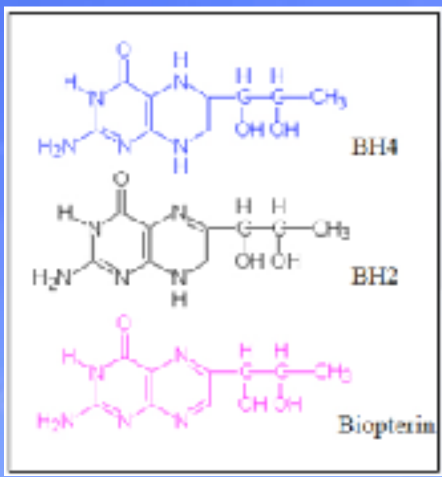
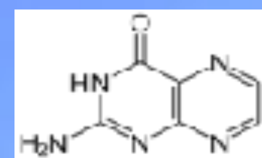
Abstract
Pterin is a member of the compounds known as pteridines. They have the same nucleus of 2-amino-4-hydroxypteridine (pterin). However, the side-chain is different at the position 6, and the state of oxidation of the ring may exist in different form viz. tetrahydro, dihydro, or a fully oxidized form. In the present study, the microorganisms able to utilize cyanide, and heavy metals have been used for the efficient production of pterin compound. The soil samples contaminated with cyanide and heavy metals were collected from Salem steel industries, Tamil Nadu, India. Out of 77 isolated strains, 40 isolates were found to utilize sodium cyanate as nitrogen source at different concentrations. However, only 13 isolates were able to tolerate maximum concentration (50 mM) of sodium cyanate and were screened for pterin production. Among the 13 isolates, only 1 organism showed maximum production of pterin, and the same was identified as *Bacillus pumilus* SVD06. The compound was extracted and purified by preparative high-performance liquid chromatography and analyzed by UV-visible, FTIR, and fluorescent spectrum. The antioxidant property of the purified pterin compound was determined by cyclic voltammetry. In addition, antimicrobial activity of pterin was also studied which was substantiated by antagonistic activity against *Escherichia coli*, and *Pseudomonas aeruginosa*. Besides that the pterin compound was proved to inhibit the formation of biofilm. The extracted pterin compounds could be proposed further not only for antioxidant and antimicrobial but also for its potency to aid as anticancer and psychotic drugs in future.

- ➢ As a biomarker in genetic diseases or cancer
- ➢ As drug： Antifolate antitumor drugs
- ➢ biopterin deficiency : phenylketonuria (苯丙酮尿症)

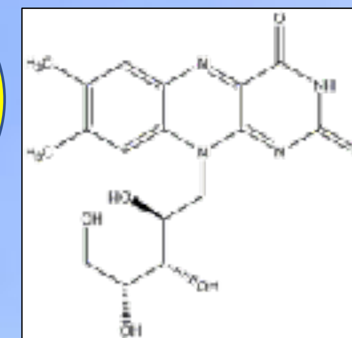
Pterins and derivatives



Biopterin



Riboflavin (B2)



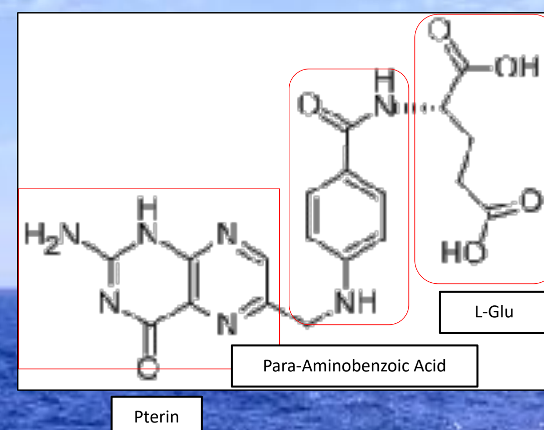
Pterin

Neopterin

(iso)xanthopterin

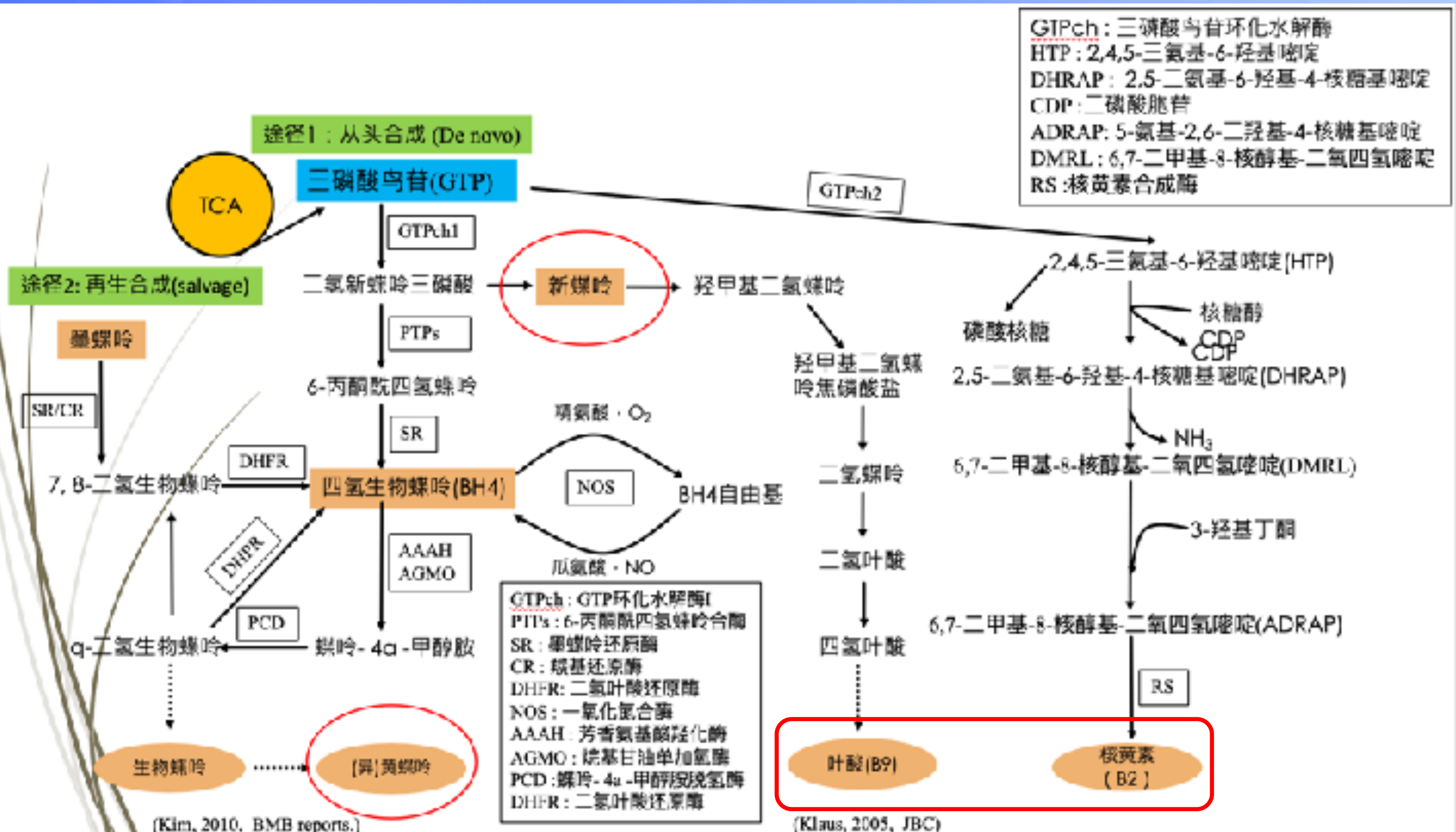
…

Folic acid (B9)

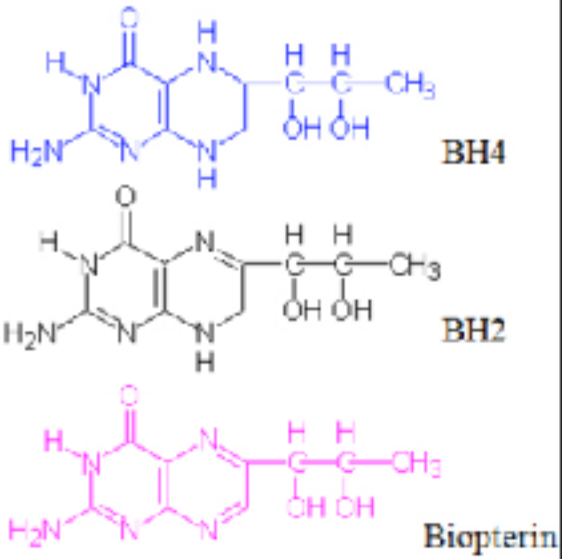


- N-containing 6-membered pteridine double-ring
- Synthetic substrates （B2; B9）
- Pigment , Precursor & Cofactor

Pterin metabolism



Biopterins (生物蝶呤)



性质

1.**蝶呤类化合物属于蝶啶的衍生物**，生物蝶呤分子式为：$C_9H_{11}N_5O_3$,化学分子量为237.22，水溶解度为1:7左右，弱碱性。

2.Biopterin是**三磷酸鸟苷（GTP）**一种代谢产物，控制GTP转化成生物蝶呤的酶（GTP环水解酶I）在**原核生物和真核生物**中均有发现。

分布

1.除昆虫以外广泛分布于**细菌到高等动植物**

2.种类繁**多**，含量极**低**

合成

1.一是**从头合成**（de novo）： GTP为底物

2. 二是**补救途径**（salvage）：墨蝶呤为底物

3. 都是在细胞的胞浆内合成的(Kim et al., 2010)

功能

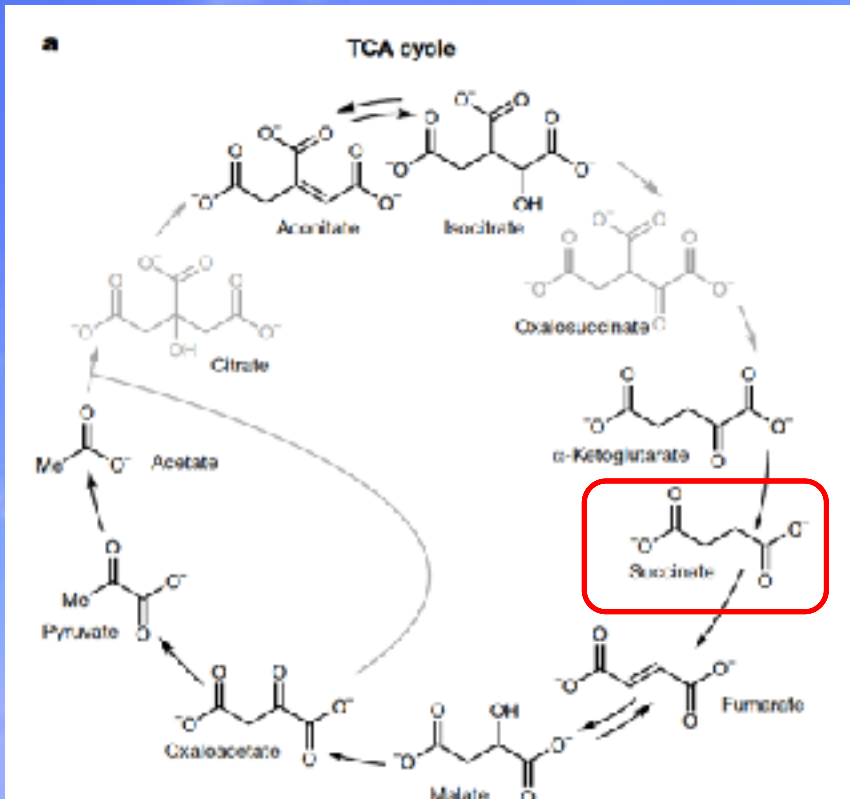
1.在生物体内充当内源性辅酶因子

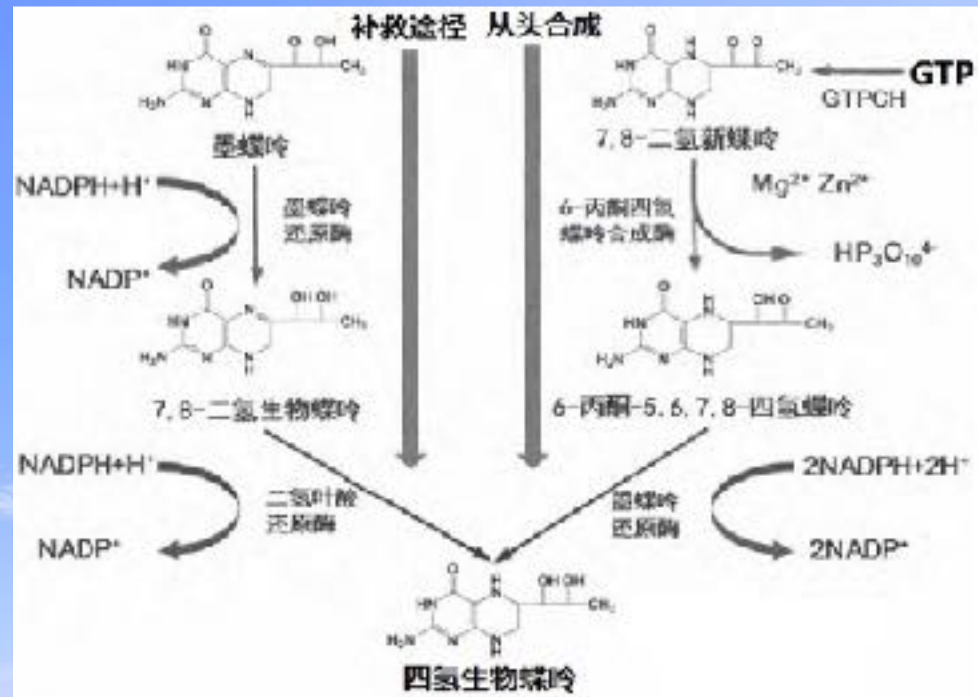
2.作为基础前体物质，是**叶酸、维生素B2**的重要前体

3.积极参与C元素、N元素、S元素的合成代谢过程

生物蝶呤 生物蝶呤是三磷酸鸟苷的代谢产物，细胞主要存在形式四氢生物蝶呤（tetrahydrobiopterin，BH4）非共轭蝶呤，广泛存在于蓝细菌中，主要作为辅因子参与芳族化合物的羟基化和一氧化氮的合成，充当色素以及与细胞生长密切相关。在医学上作为癌症和肿瘤的标志物。

Biopterin biosynthesis





Kamila, Nature, 2019

Chemical book

$$\text{乙酰CoA} + GDP + Pi + 3NAD^{+} + FAD + 2H_2O \longrightarrow 2CO_2 + CoA + GTP + 3NADH + H^{+} + FADH_2$$

Major enzymes and organic molecules

Substance	Category	Metal	Functioning
Folic acid(叶酸)	辅因子	-	参与氨基酸代谢，并与维生素B12共同促进细胞的生成和成熟，是制造红血球不可缺少的物质;
Riboflavin (维生素B2/核黄素)	辅因子	-	在生物氧化还原中发挥递氢作用，既可作氢供体，又可作氢递体，是机体中一些重要的氧化还原酶的辅基;
Tyrosine hydroxylase (酪氨酸羟化酶)	C	Cu	负责催化L-酪氨酸转变为二羟基苯丙氨酸（多巴）的酶;
Phenylalanine hydroxylase (苯丙氨酸羟化酶)	C	Fe	一种由苯丙氨酸形成的酪氨酸加氧酶，如苯丙酮尿症是由于缺乏此酶引起的;
Xanthine oxidase (黄嘌呤氧化酶类)	C/N	Mo/Fe	能催化次黄嘌呤生成黄嘌呤，进而生成尿酸，又能直接催化黄嘌呤生成尿酸;
Nitrate reductase (硝酸盐还原酶)	N	Mo/Fe	膜结合硝酸盐还原酶介导的硝酸盐还原为细菌提供氮源和能量;
Nitric oxide synthase (一氧化氮合酶)	N	Fe/Zn	促进细胞组织内产生NO，并且协助细胞通讯及与原生膜联合
Sulfite oxidase (亚硫酸盐氧化酶类)	S	Mo	催化亚硫酸盐气化成硫酸盐的酶（钼酶），参与哺乳动物硫化物的脱毒、嘌呤代谢等过程

There are more than 70 enzymes involved in bio-metabolic synthesis of biopterin.

Pterins' study in the ocean

Pterins' study in the ocean

Mar. Biotechnol. 1, 207–210, 1999

MARINE BIOTECHNOLOGY

Short Communications

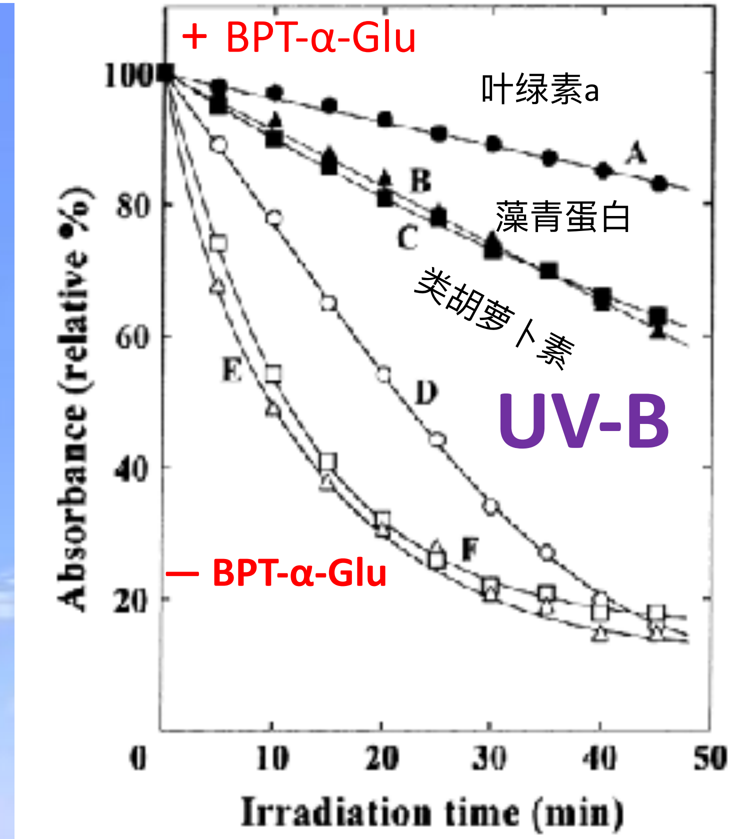
Isolation of Biopterin-α-glucoside from *Spirulina* (*Arthrospira*) *platensis* and Its Physiologic Function

Yukinori Noguchi, Asako Ishii, Ayako Matsushima, Daisuke Haishi, Ken-ichi Yasumuro, Tomohisa Moriguchi, Takeshi Wada, Yoji Kodera, Misao Hiramoto, Hiroyuki Nishimura, Mitsuo Sekine, and Yuji Inada

Abstract: A fluorescent substance was isolated from the cyanobacterium with a yield of 4.5 mg per 10 g of dried *Spirulina* (*Arthrospira*) *platensis* cells by gentle extraction and ethanol fractionation followed by column chromatography. The fluorescent substance, which has absorption maxima at 256 nm and 362 nm (pH 8.4), was identified as biopterin-α-glucoside by spectrophotometry and nuclear magnetic resonance spectroscopy. Biopterin-α-glucoside prevented decolorization of the photosynthetic pigments, chlorophyll a, phycocyanin, and carotenoids in photosynthetic vesicles of *Spirulina platensis* cells, by ultraviolet irradiation.

Key words: biopterin-α-glucoside, Spirulina platensis, cyanobacteria, chlorophyll a, phycocyanin, carotenoids

"sunscreen"



Yukinori, 1999

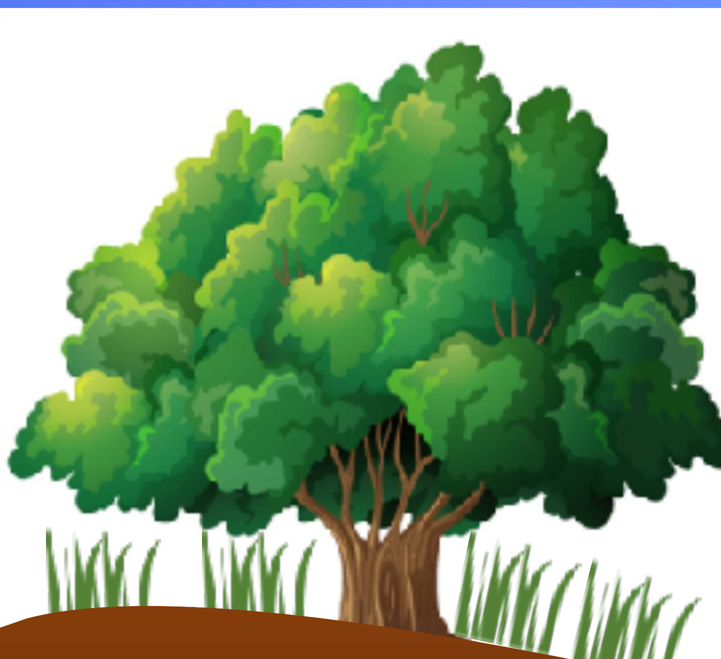
➢ Some biopterin-glycosides are a case in point: detected in marine cyanobacteria, a sunscreen role was assigned to them on the basis of their absorption spectrum and because their cellular levels increased under UV-A (320～400nm) radiation (Gao et al., Nature, 2011).

➢BPT-α-Glu can alleviate the hazards of UV-B(280-320nm) radiation and improve productivity.

➢10g *spirulina lyophilized* extracted 4.5mg of blue fluorescent substance biopterin glucoside

Land and ocean ecosystem

Data : SeaWiFS for Global Biosphere



Terrestrial Ecosystem

Biomass : 1837×10^9 t

Total Primary production
: 115×10^9 t /yr

Tree & Grass

Marine Ecosystem

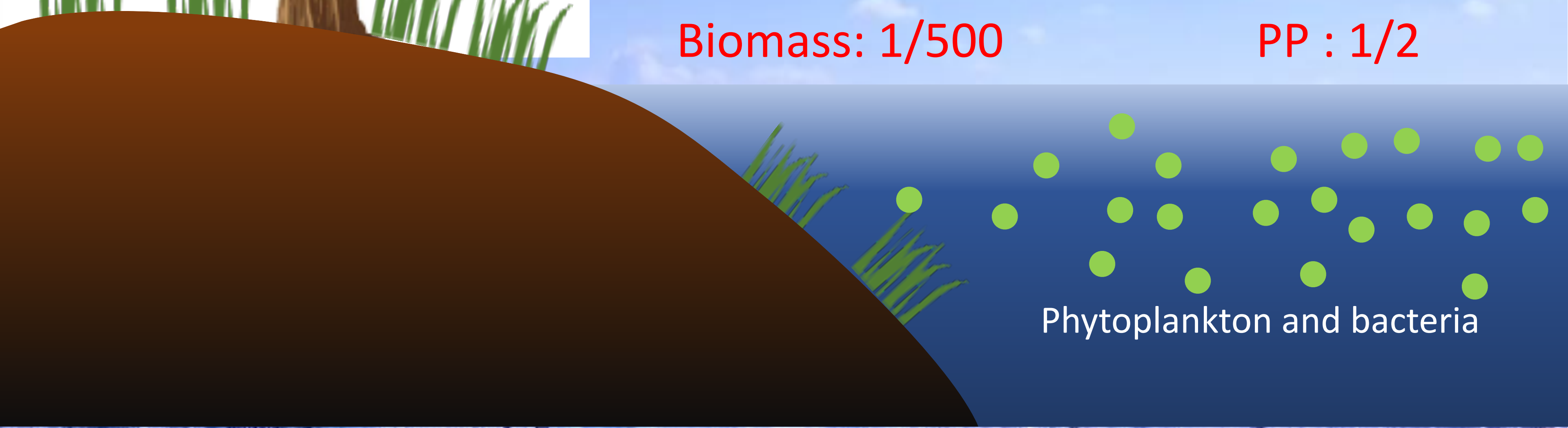
Biomass : 3.9×10^9 t

Total Primary production
: 55×10^9 t /yr

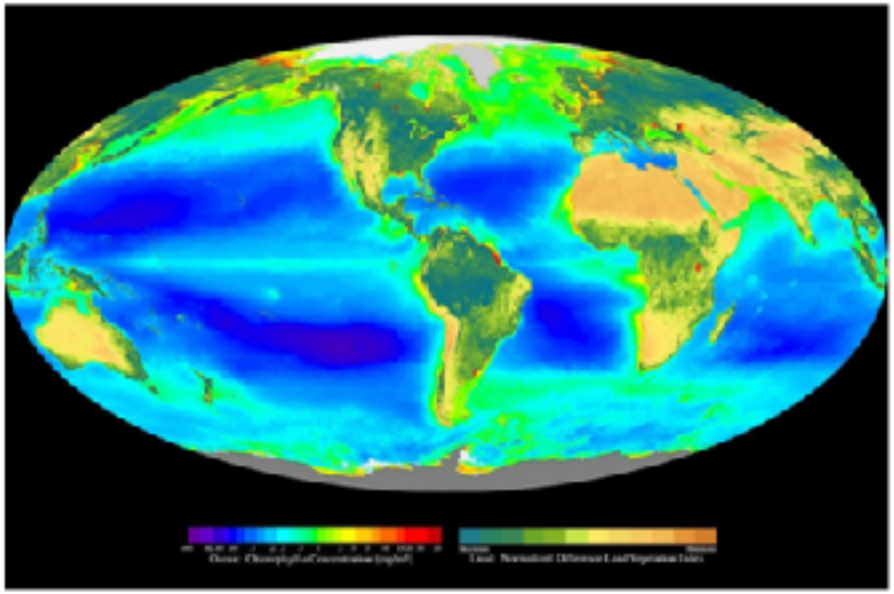
Photoautotroph

Biomass: 1/500

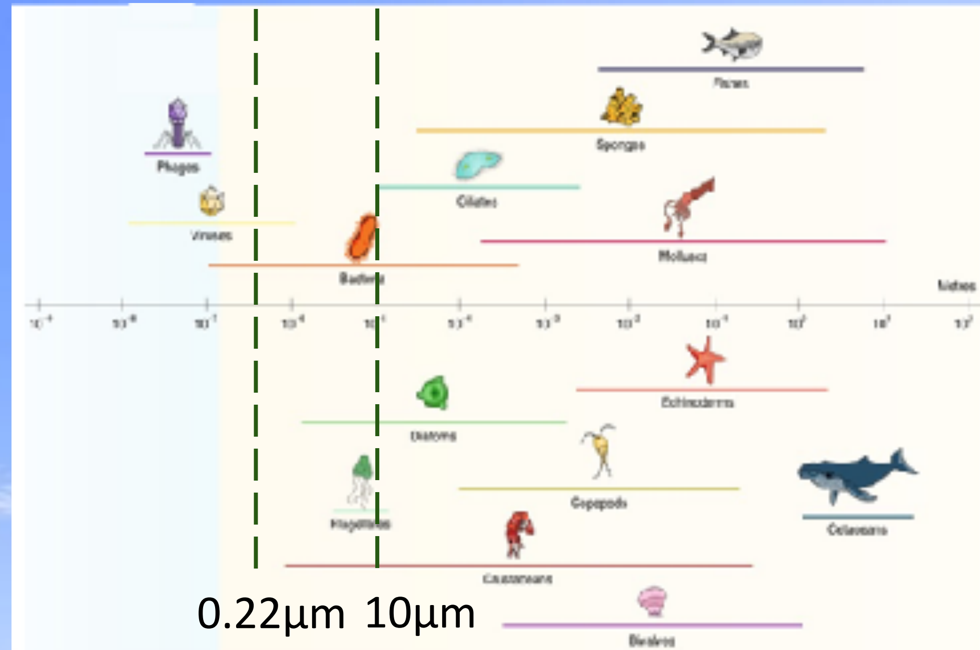
PP : 1/2



Marine microbes



全球海洋与陆地光和自养生物分布（来自：NASA）



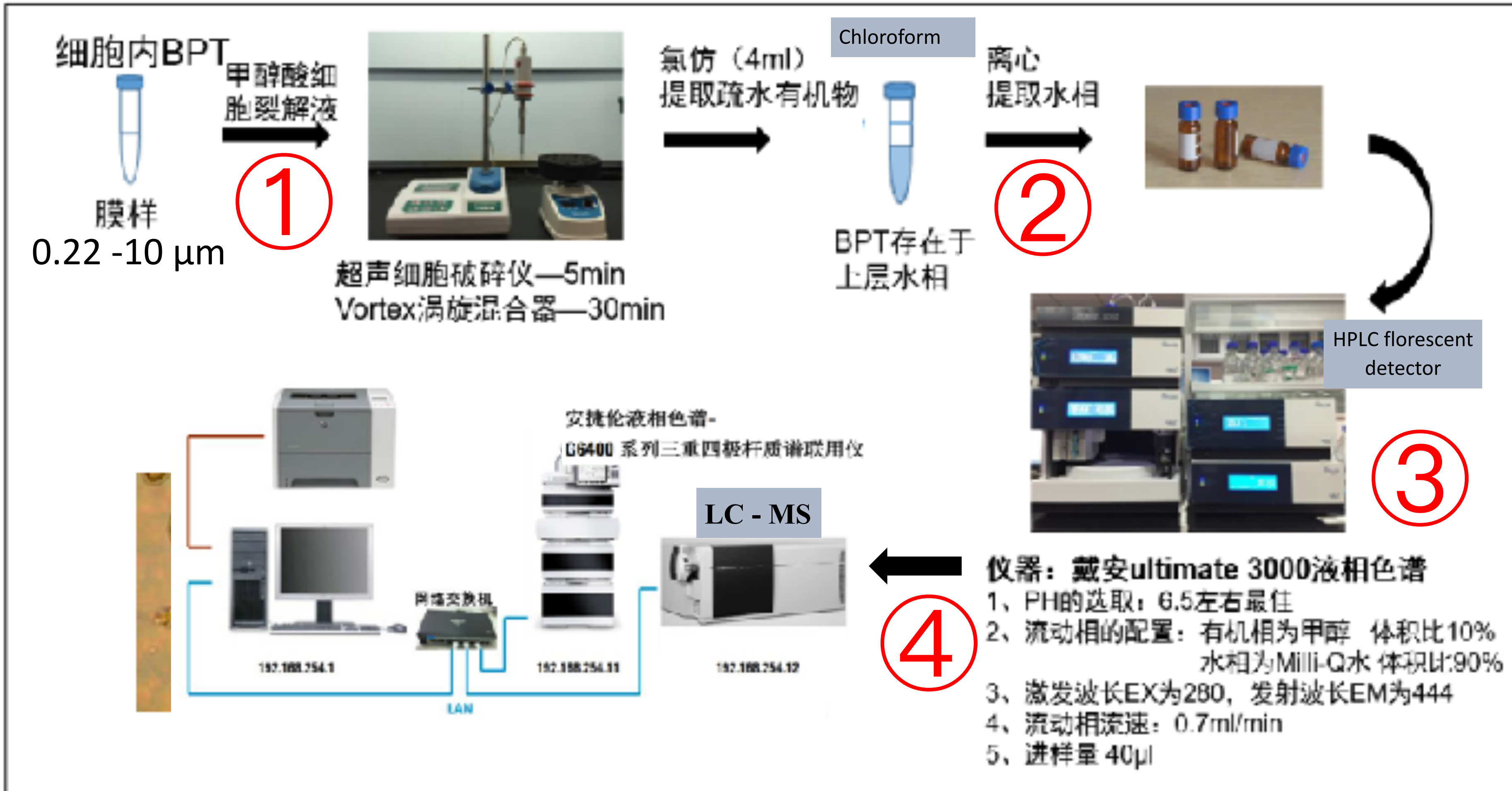
Yawei Luo et al.

➢0.22-10 um microorganisms are the main contributor to primary production

Question: The contribution and role of pterins in the carbon & nitrogen cycle of marine phytoplankton and bacteria?

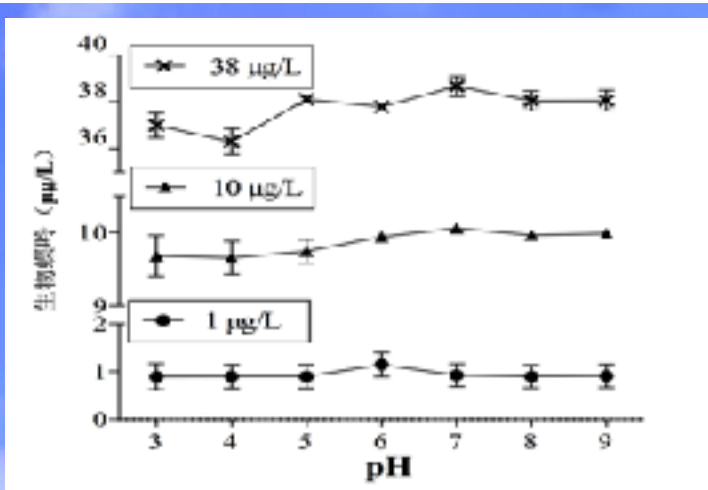
Part II Particulate pterins

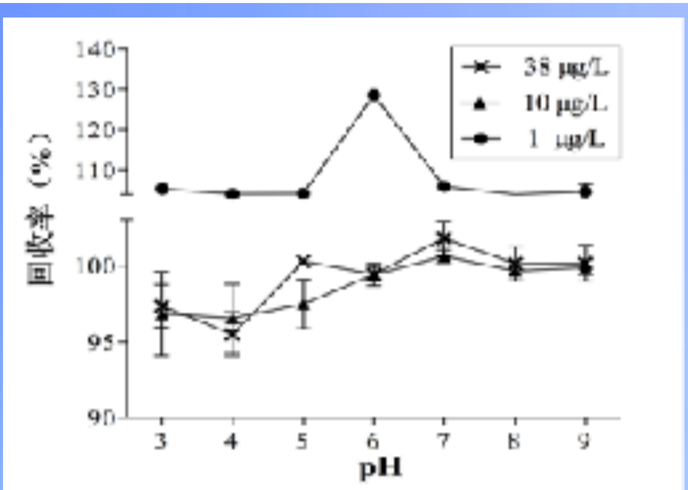
Sample processing, measurement and verification procedures

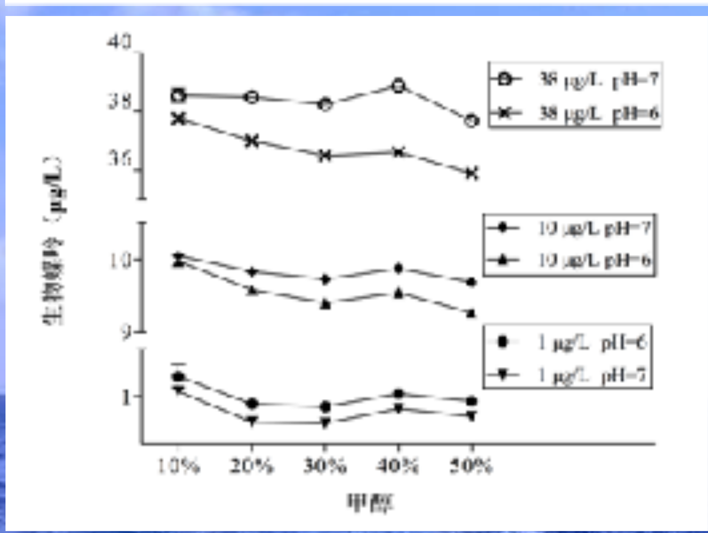


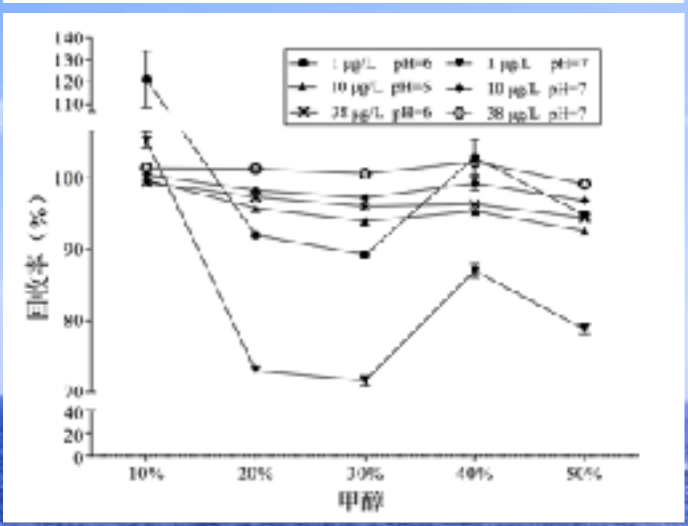
Modified from Mengqiu Shi, 2019

Method optimization









Phase A: MQ water

Phase B： MeOH

pH: 6 ~ 7

MeOH : 10%

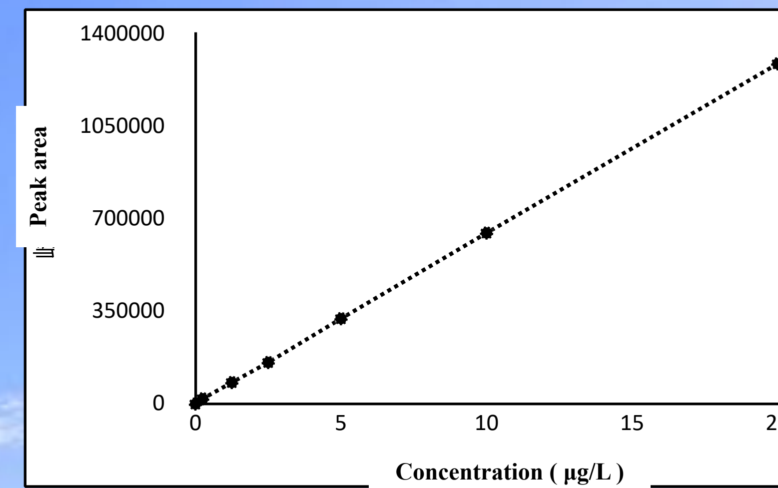
Flowrate： 1 ml/min

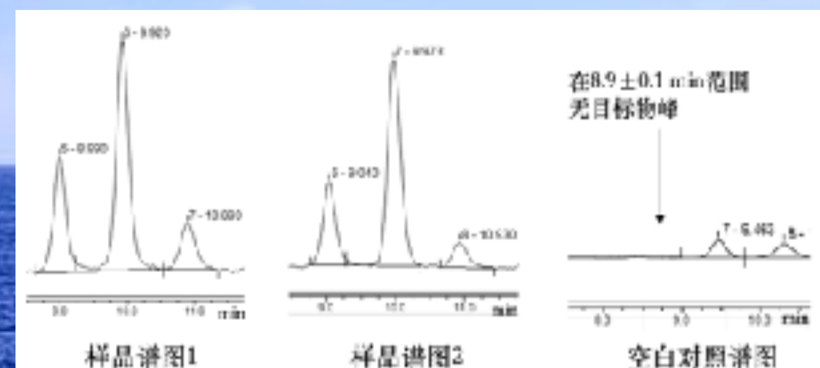
Standard curve of BPT

Chromatographic signal data for several BPT concentrations

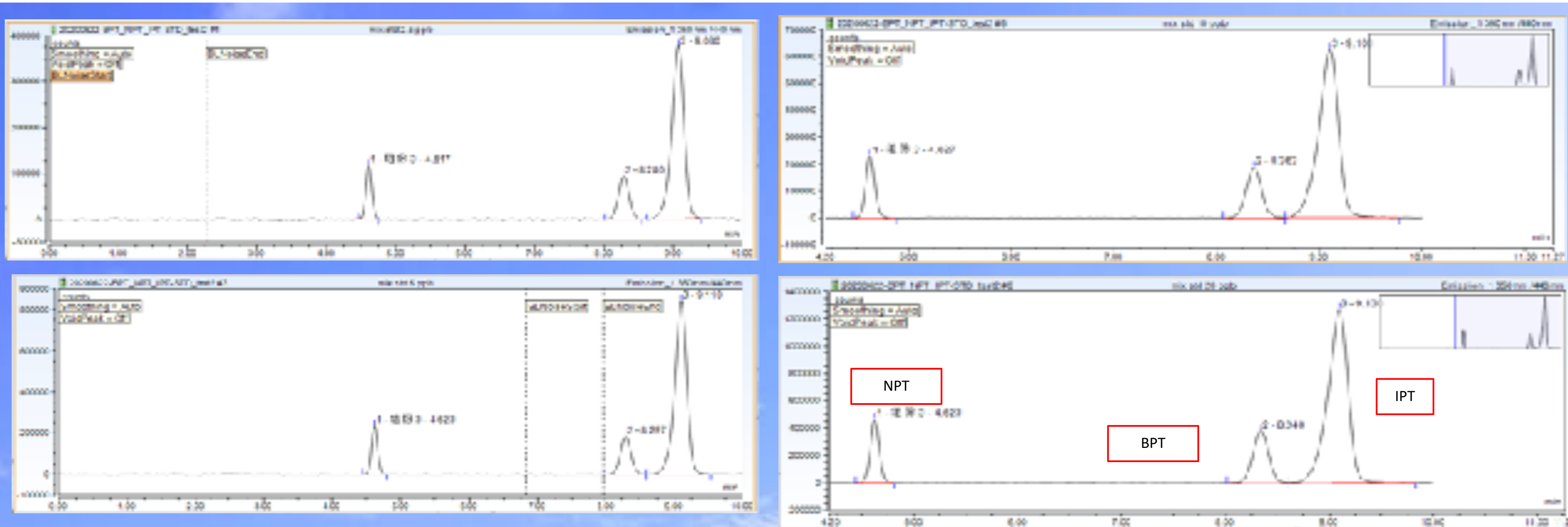
Level	Conc. (μg/L)	The peak height	The peak area
1	20.00	6882258	1288003
2	10.00	3442005	647398
3	5.00	1705578	323117
4	2.50	829292	157214
5	1.25	421981	81374
6	0.25	93191	19884
7	0.00	413	---

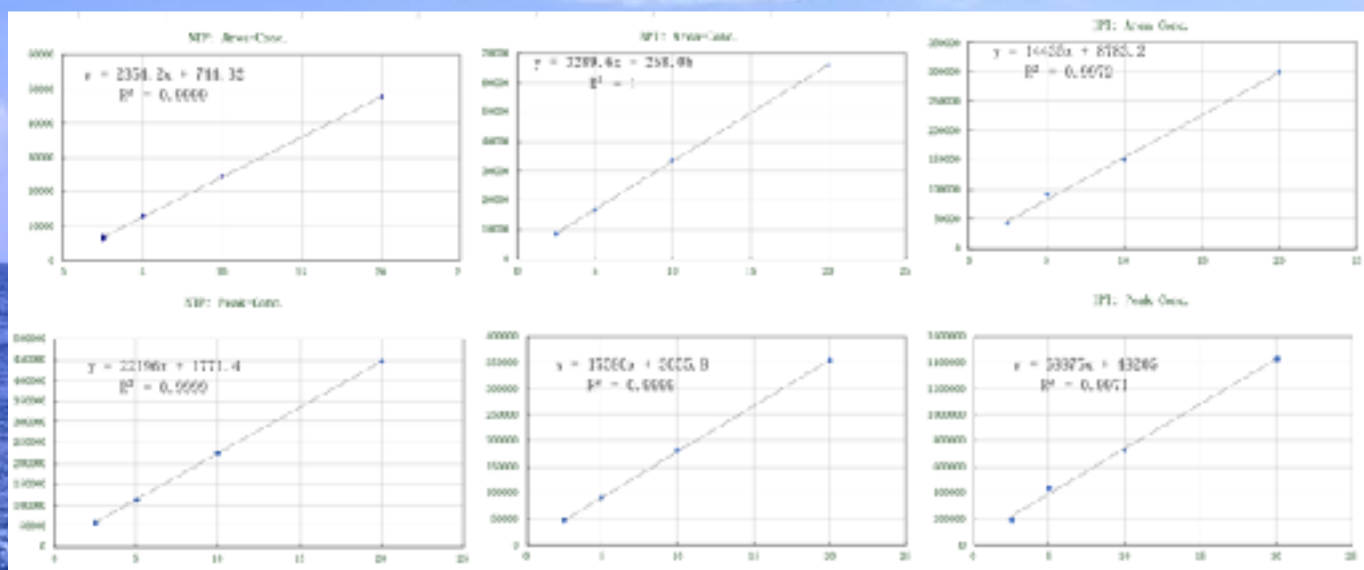
BTP retention time: 8.97±0.02min



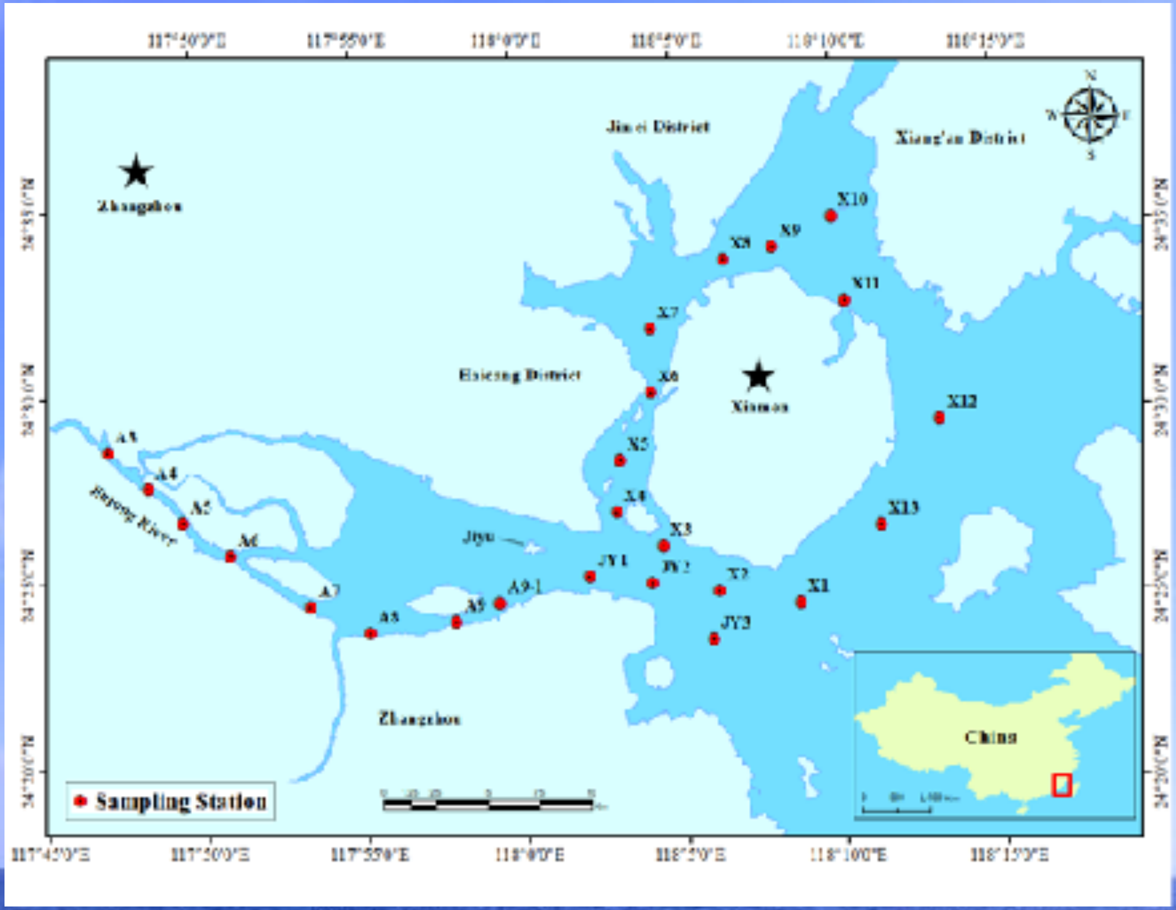


Multi-pterins





Part III Case study and results

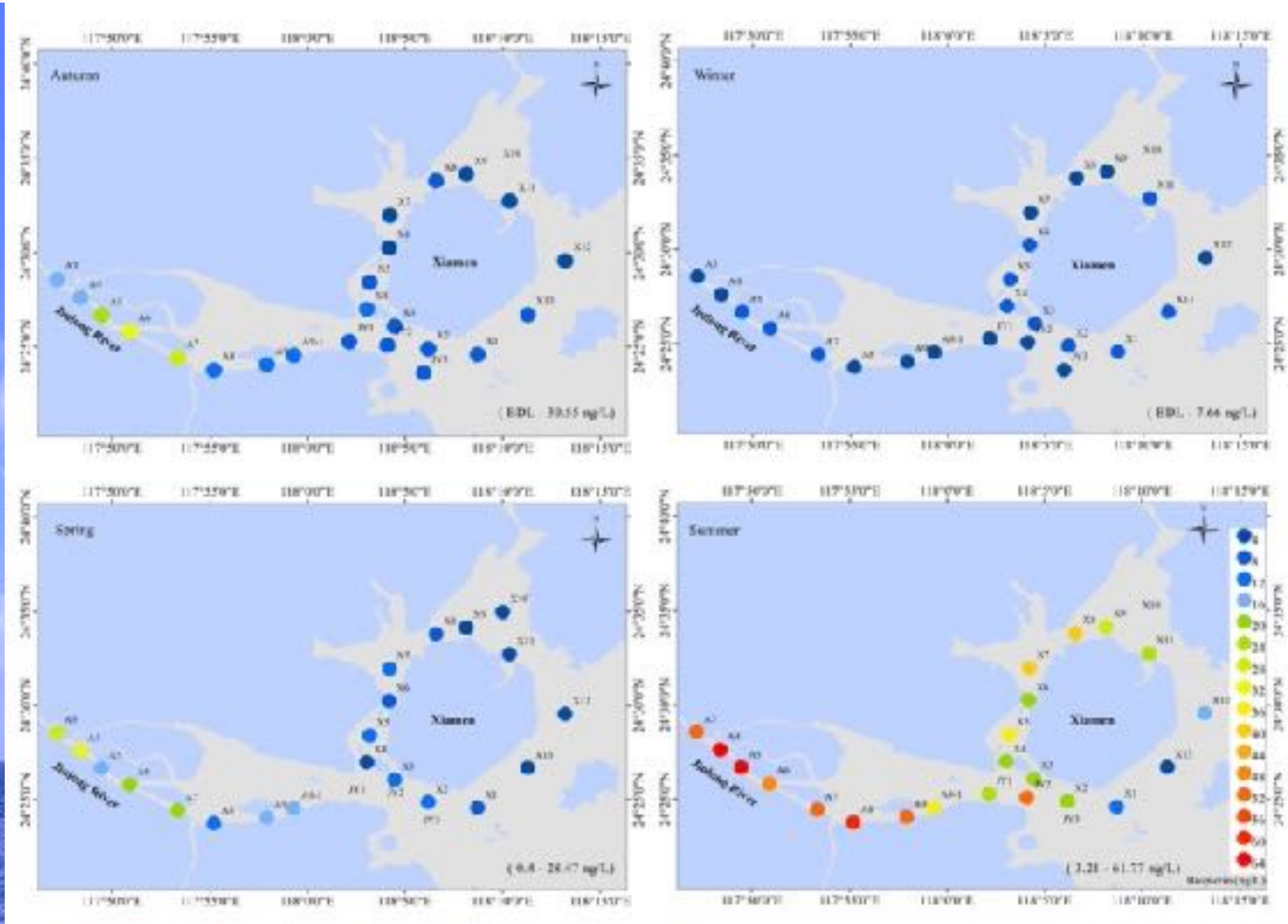


Sampling stations in Xiamen coastal sea

➢ 0.22-10 um microorganisms, 150 mL for particulates

➢4 seasons: Jan.; Apr.; Jul.; Nov. (2018)

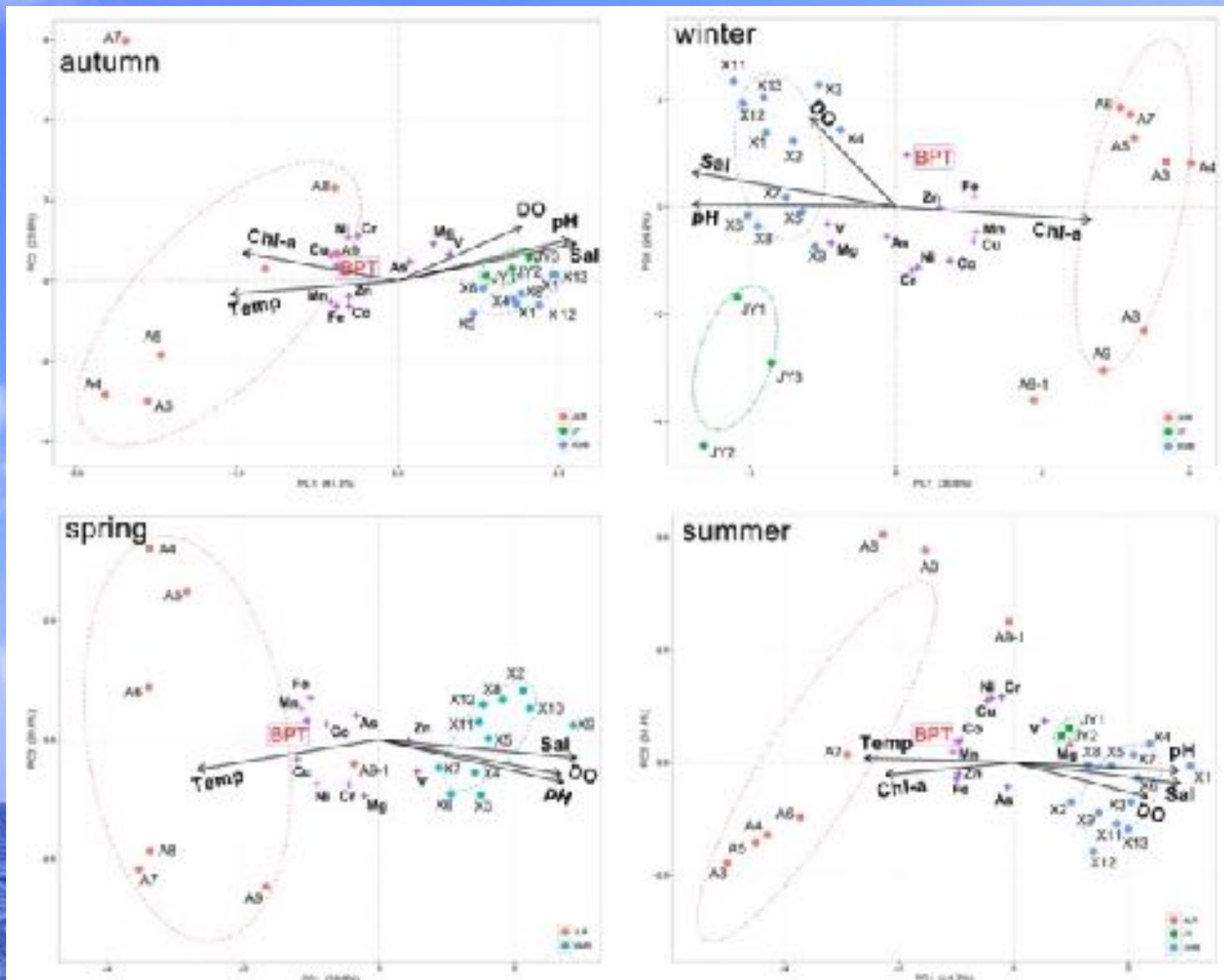
Data arranging



Seasonal distribution of particulate biopterin

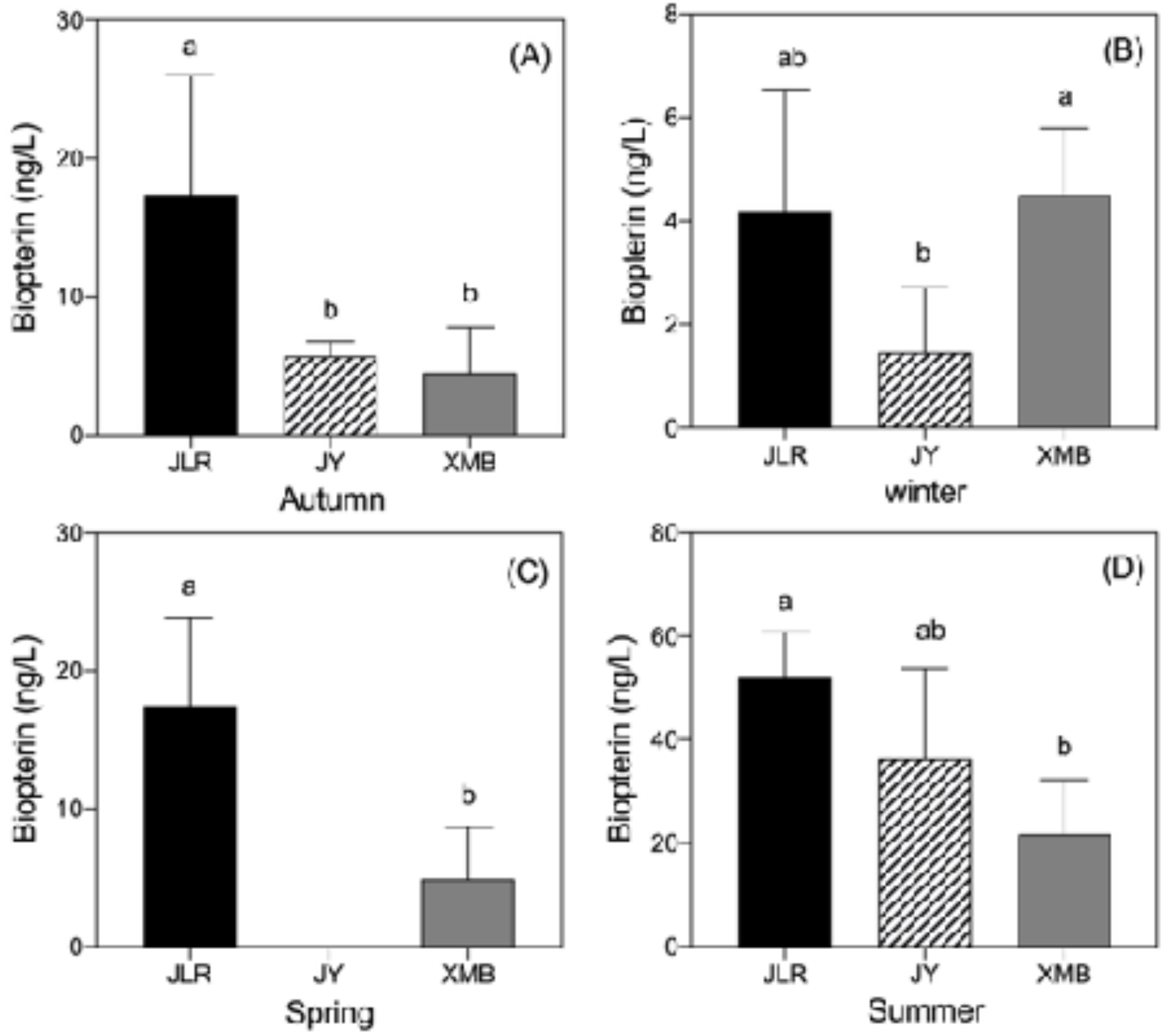
Principle Component and Factor analysis

Data: Nengwan Chen and Bangqin Huang



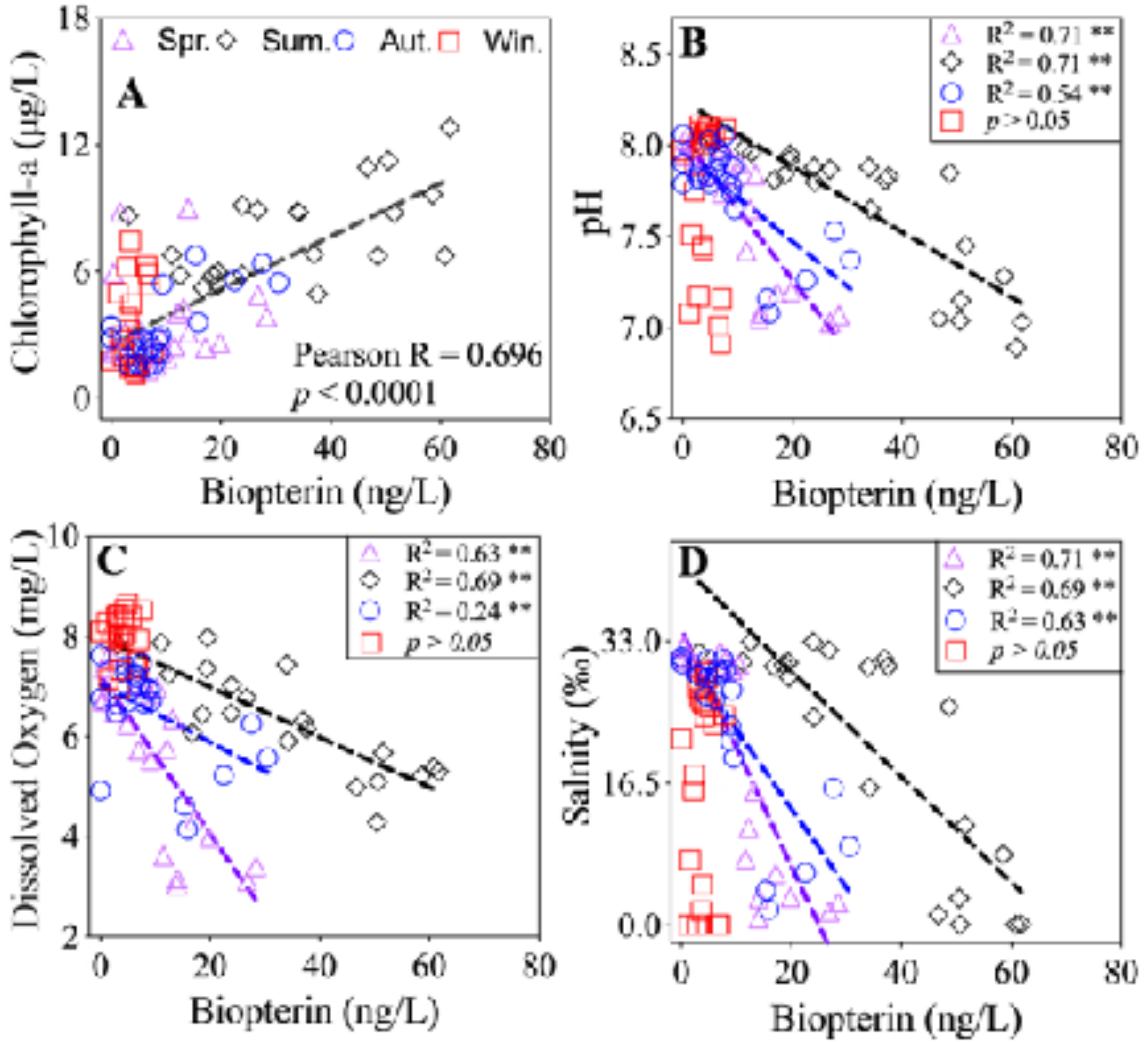
➢ PCA divides the sampling stations into three groups

Distribution pattern



- River > Bay
- Sum. > Aut.; Spr. > Win.

Parameters correlation



➢ There was a significant positive correlation between biopterin and chlorophyll, however, biopterin significantly negative correlated with pH, dissolved oxygen and salinity.

Part IV Summary

- For the first time, we developed a new method measuring pterins in **phytoplankton** and **bacteria [DL:** NPT- 120 ng/L, BPT: 170 ng/L, IPT： 40 ng/L]
- We identified BPT in phytoplankton and bacteria ranging from 2.3 to tens of ng/L in natural waters (Jiulong River and Xiamen Bay) [converted as in cells per volume];
- Spatial and seasonal distribution of particulate biopterin are as the following: Jiulong River > Jiyu > Xiamen Bay; Summer > Spring, Autumn > Winter.
- We aim to obtain patent based on our newly developed technique; and further study its metabolic processes and potential implication (marine biomedical development) in the ocean.

四氢生物蝶呤合成代谢图
三磷酸鸟苷（GTP）
GTPch
P35
新蝶呤
(N)
三磷酸二氢新蝶呤
PTPS
-
+
6-丙酮酰四氢蝶呤
SR
苯丙氨酸
酪氨酸
色氨酸
四氢生物蝶呤(BH4)
DHPR
PAH
TH
TPH
PCD
二氢生物蝶呤(BH2)
蝶呤-4a-甲醇胺
酪氨酸
左旋多巴
5-羟色氨
生物蝶呤(B)
多巴胺
5-羟色胺

Many thanks!

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