



Development of analyzing pivotal metabolic precursor-pterins in the ocean



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Science with NO Boundary





Outline



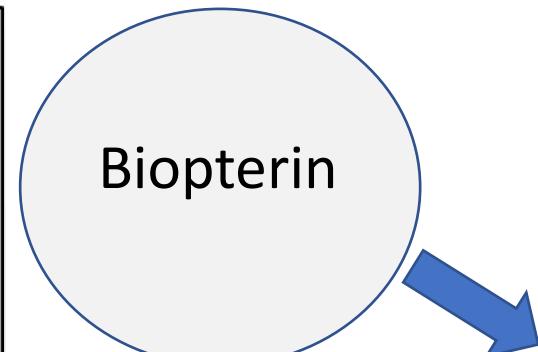
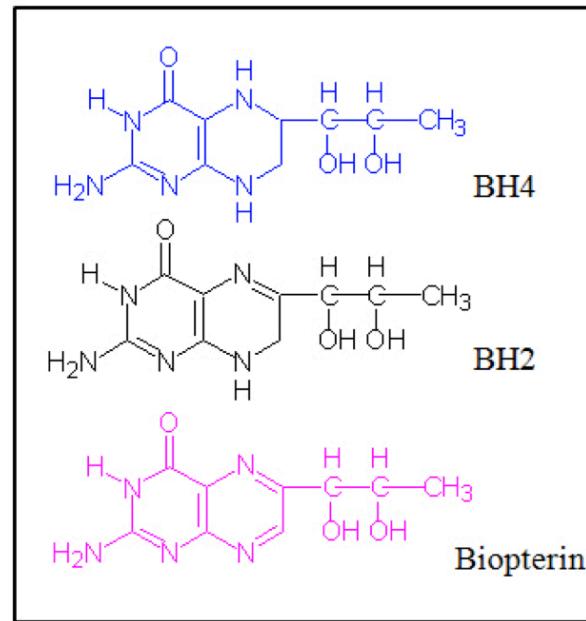
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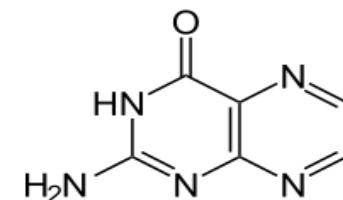


Pterins and derivatives

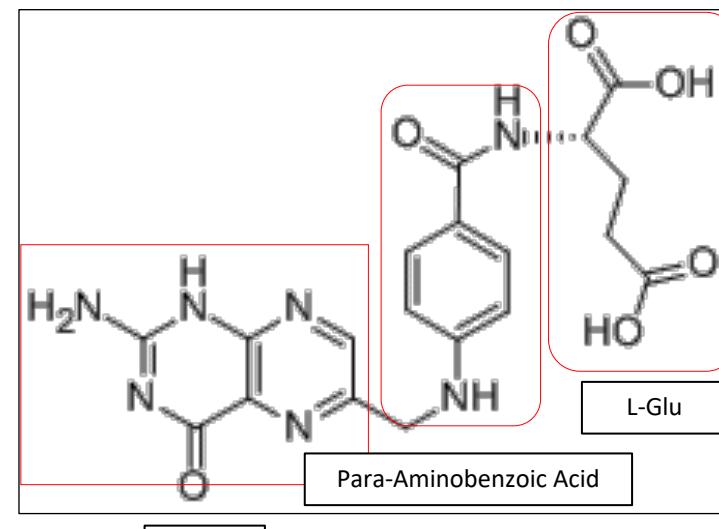
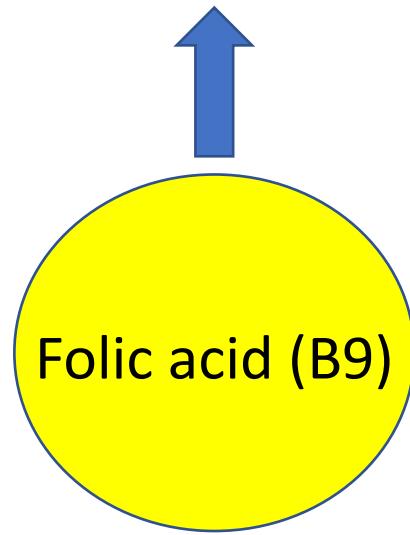
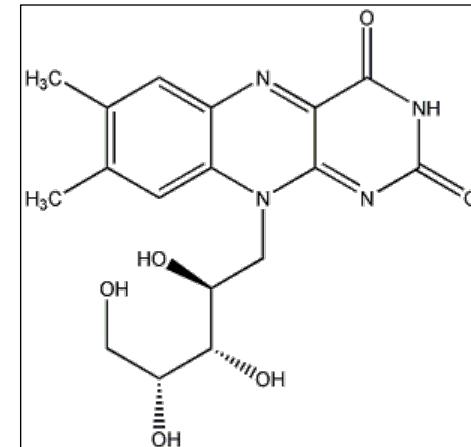
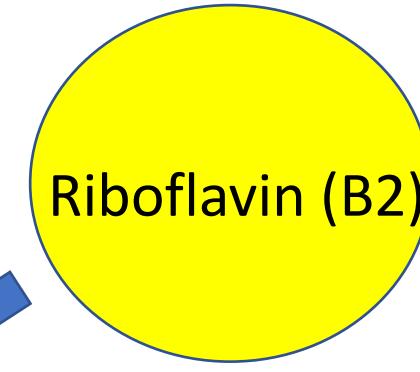
PART ONE



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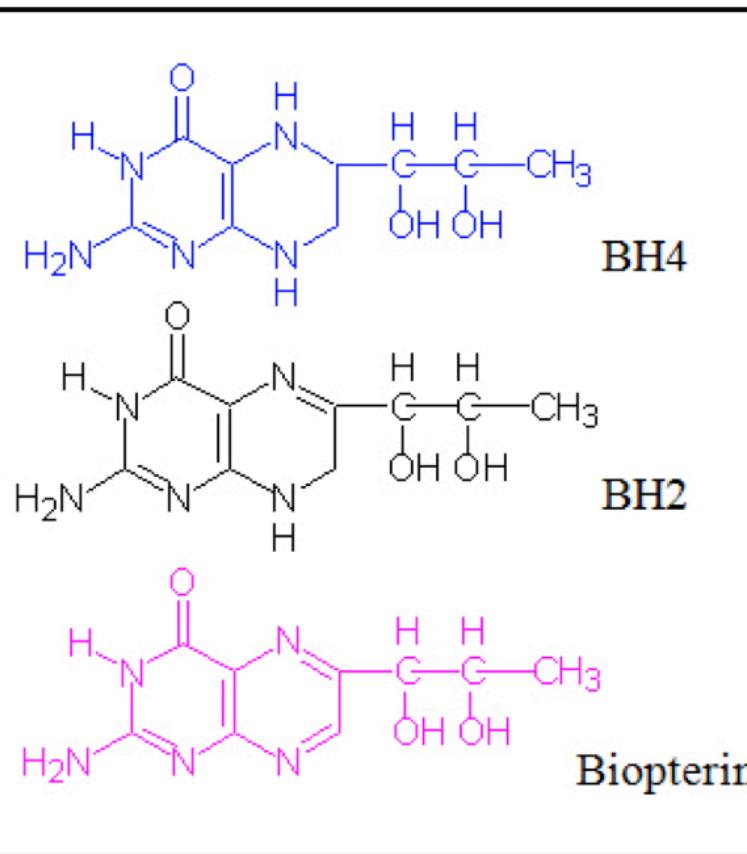


Pterin



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

Pterin



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.BP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

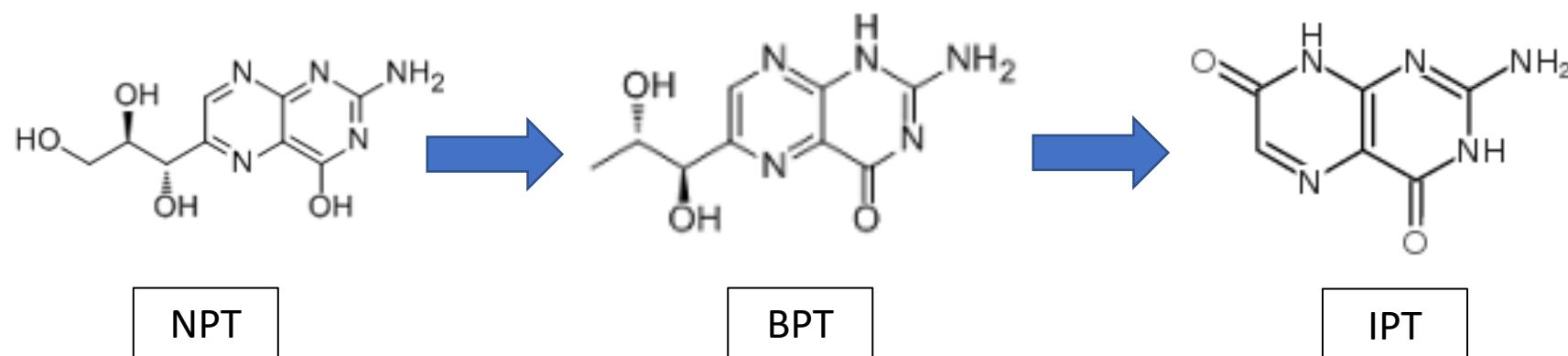
- 1.在生物体内充当内源性辅酶因子
2.作为基础前体物质 , 是叶酸、维生素B2的重要前体
3.积极参与C元素、N元素、S元素的合成代谢过程



Neopterin (NPT): 新蝶呤是四氢生物蝶呤合成途径中三磷酸鸟苷代谢的中间产物

Biopterin (BPT): 色素分子、芳族氨基酸的羟基化和一氧化氮合成、缓解 UV 辐射危害。

[Iso]xanthopterin (IPT): 二氢生物蝶呤和四氢生物蝶呤的最终代谢产物。





Major enzymes and organic molecules



MEL

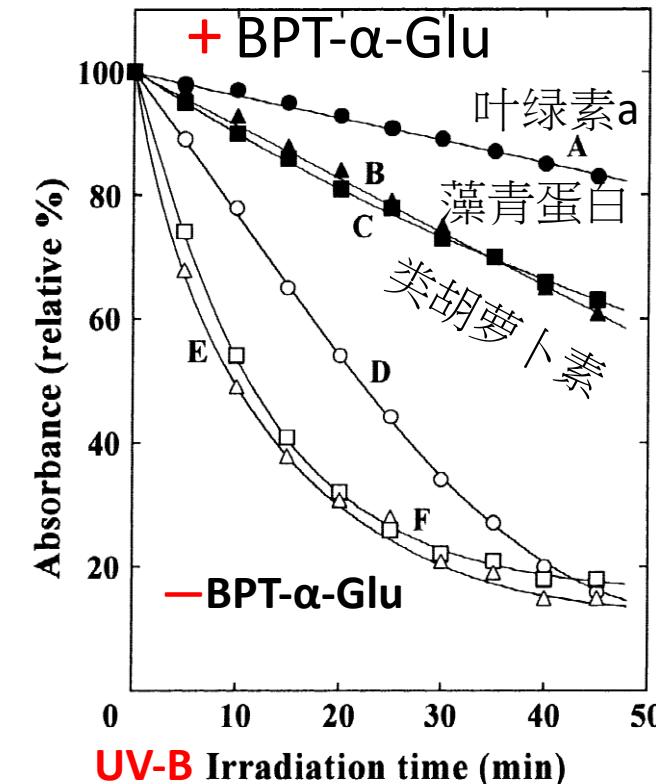
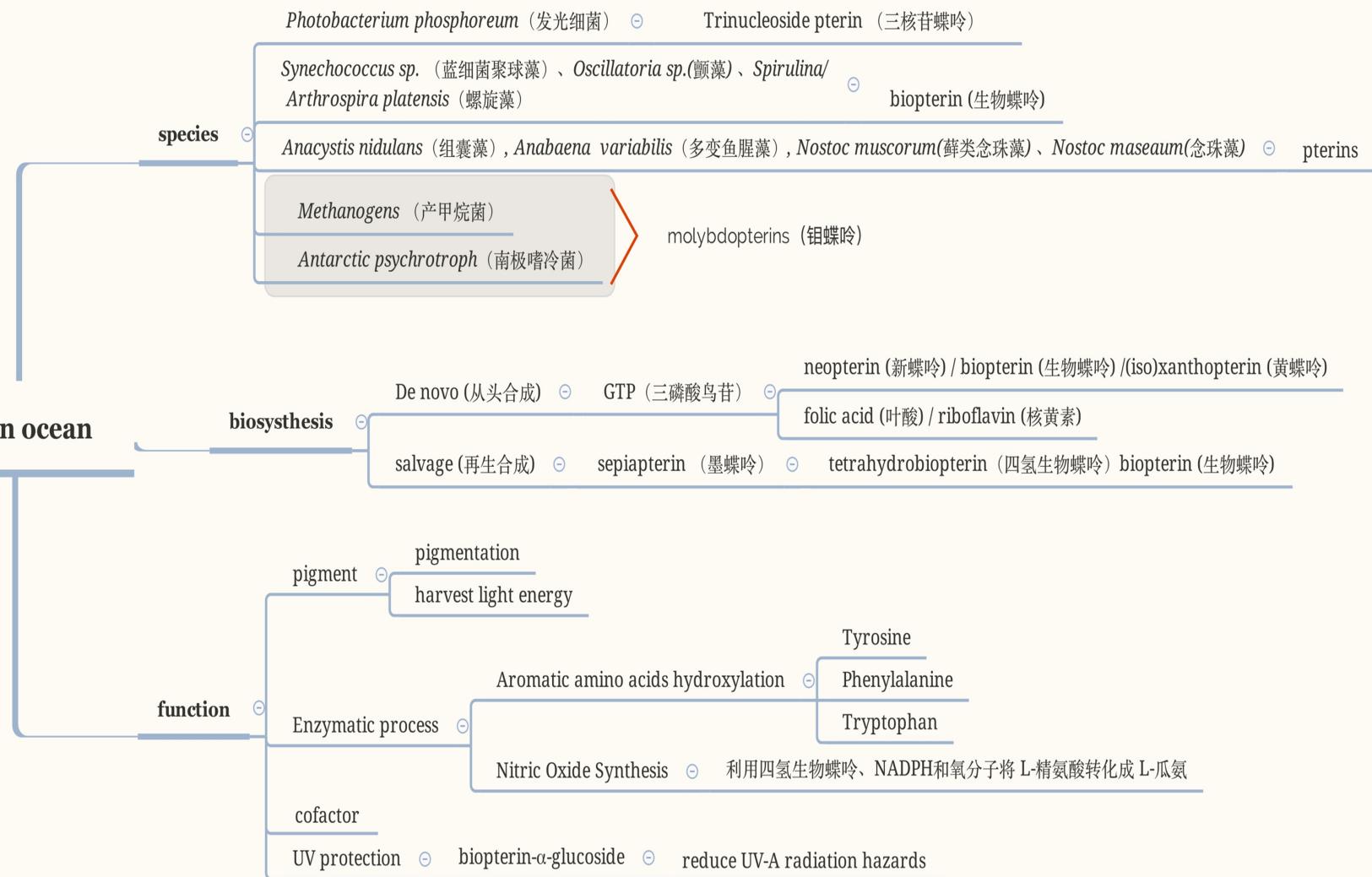
Key Laboratory of
Environmental Science

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

生物蝶呤控制或参与合成的酶类达**70**多种。



Pterins' study in the ocean



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

(Yukinori, 1999, Mar. Biotec.)

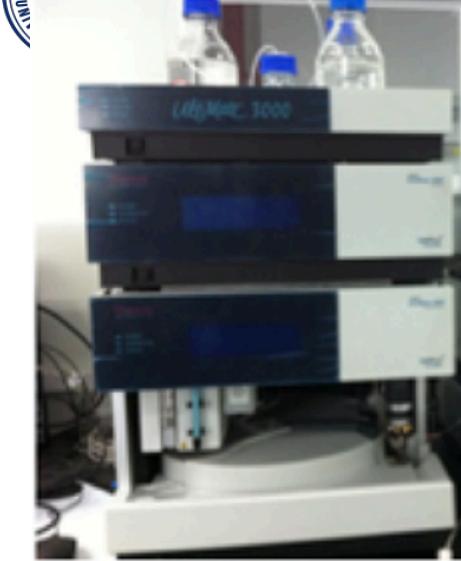
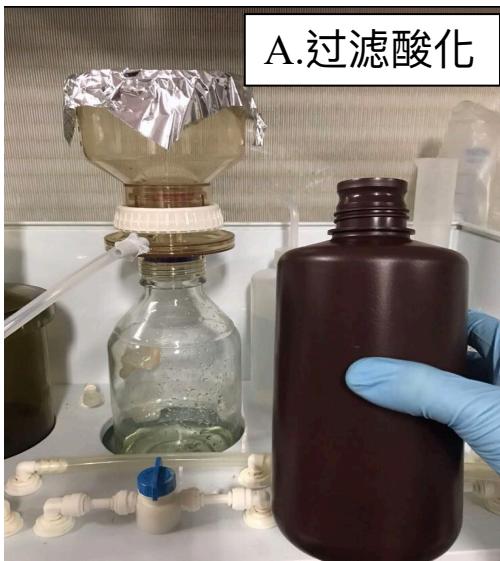


Dissolved pterins

PART TWO



E.上机测试



D.氮吹收集





Particulate pterins



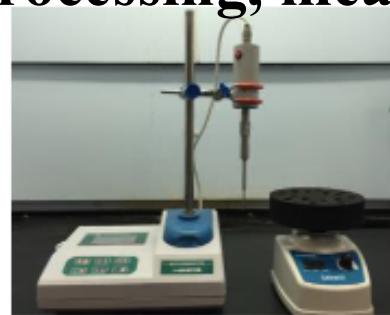
MEL

State Key Laboratory of
Marine Environmental Science

Sample processing, measurement and verification procedures

细胞内BPT
Methyl acid
膜样

①



超声细胞破碎仪—5min
Vortex涡旋混合器—30min

氯仿 (4ml)
提取疏水有机物

Chloroform

离心
提取水相

BPT存在于
上层水相



②



HPLC
fluorescent
detector

③

安捷伦液相色谱-
G6400 系列三重四极杆质谱联用仪

LC - MS



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网络交换机

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LAN

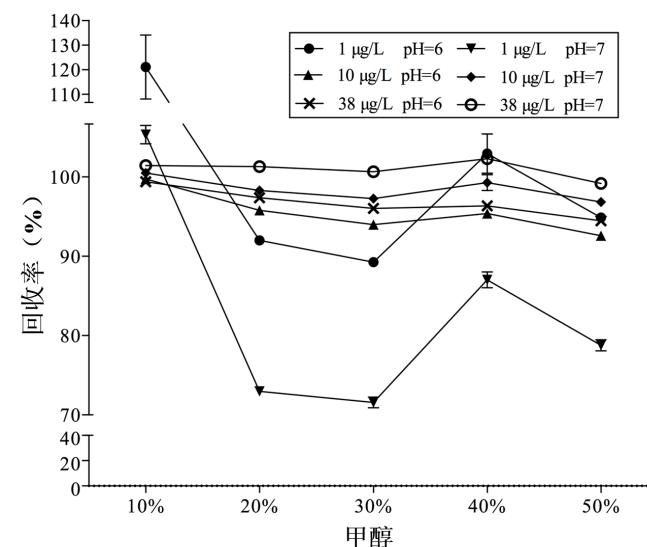
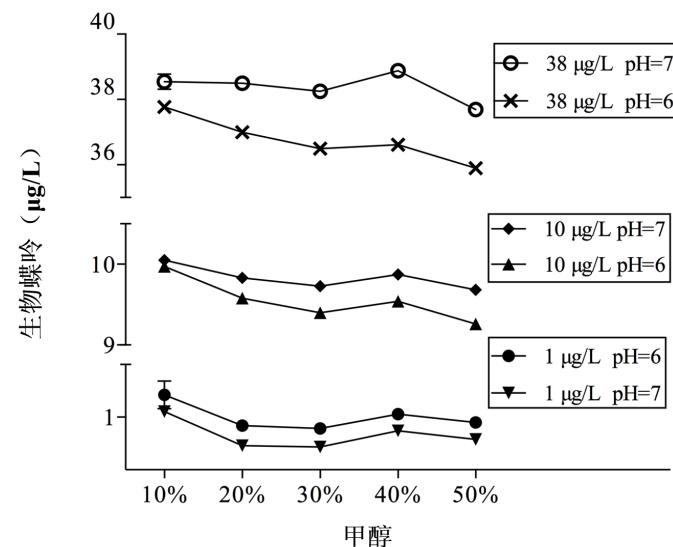
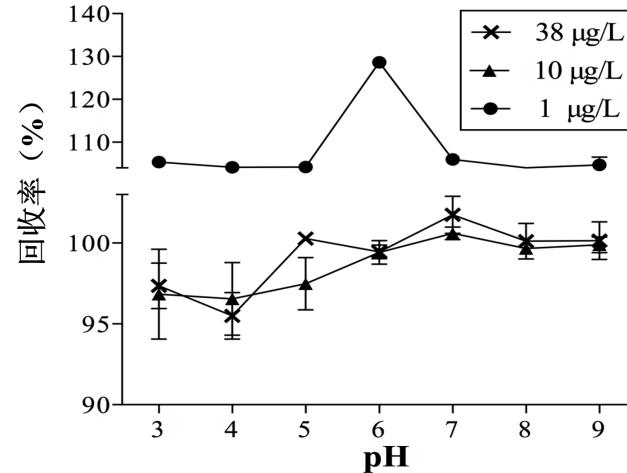
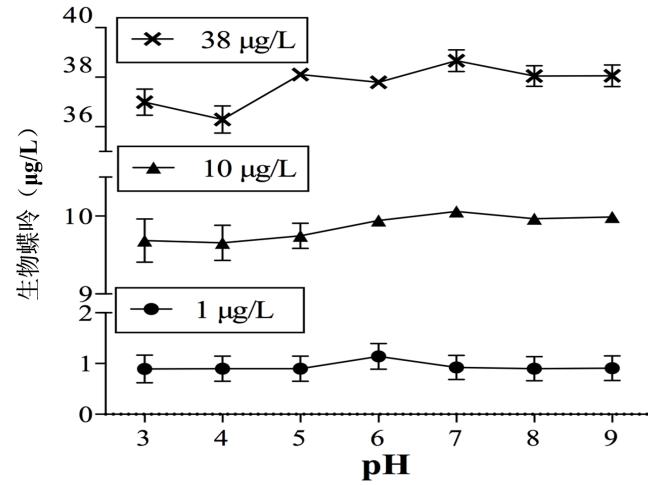
仪器：戴安ultimate 3000液相色谱

- 1、PH的选取：6.5左右最佳
- 2、流动相的配置：有机相为甲醇 体积比10% 水相为Milli-Q水 体积比90%
- 3、激发波长EX为280，发射波长EM为444
- 4、流动相流速：0.7ml/min
- 5、进样量 40μl

④



Method optimization



Phase A: MQ water

Phase B : MeOH

pH: 6 ~ 7

MeOH : 10%

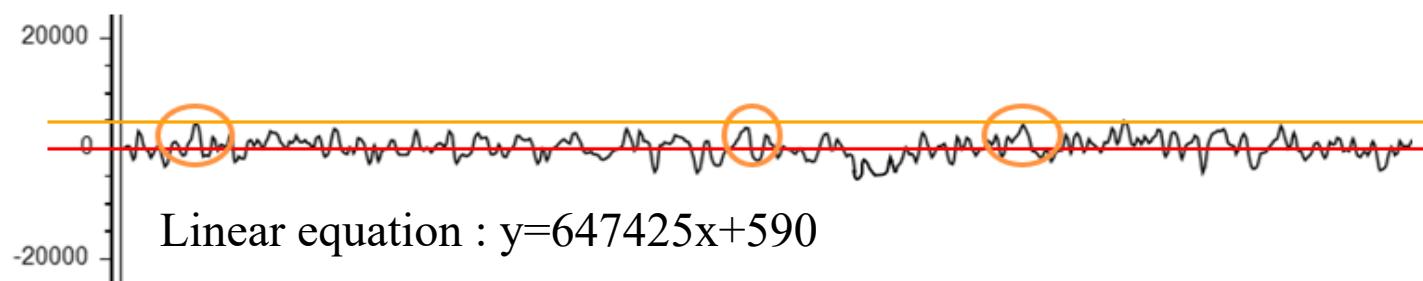
Flowrate : 1 ml/min



Detection limit



Number	SNR/20 ppb	SNR	Detection limit (mg/L)	Detection limit (ng/L)
NPT	64.4	440	0.388	120
BPT	44.5	390	0.562	170
IPT	179	410	0.140	40



Noise background chromatogram



Quality control



Recovery of the method



$m_{\text{background}} \text{ (}\mu\text{g/L)}$	$m_{\text{founded}} \text{ (}\mu\text{g/L)}$	Recovery/%	Average recovery/%	RSD/%
2.51	2.31	92.0	90.8	0.02
2.48	2.21	89.1		
2.51	2.29	91.2		
4.90	4.72	96.3	95.9	0.01
4.99	4.79	96.0		
4.91	4.69	95.5		

weighing method

20 $\mu\text{g/L}$ BTP
1.00ml

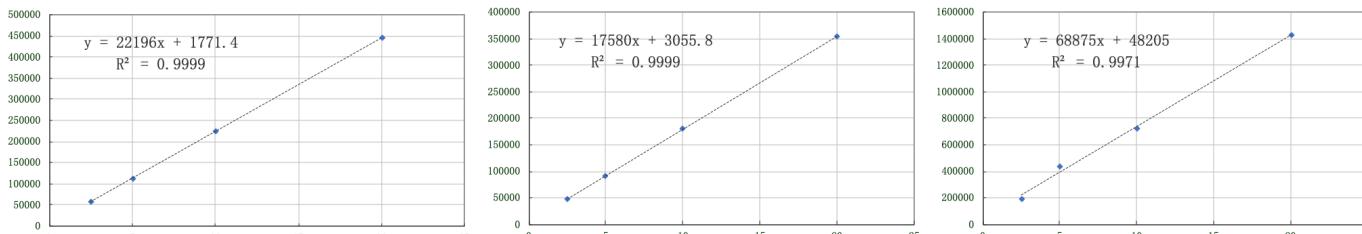
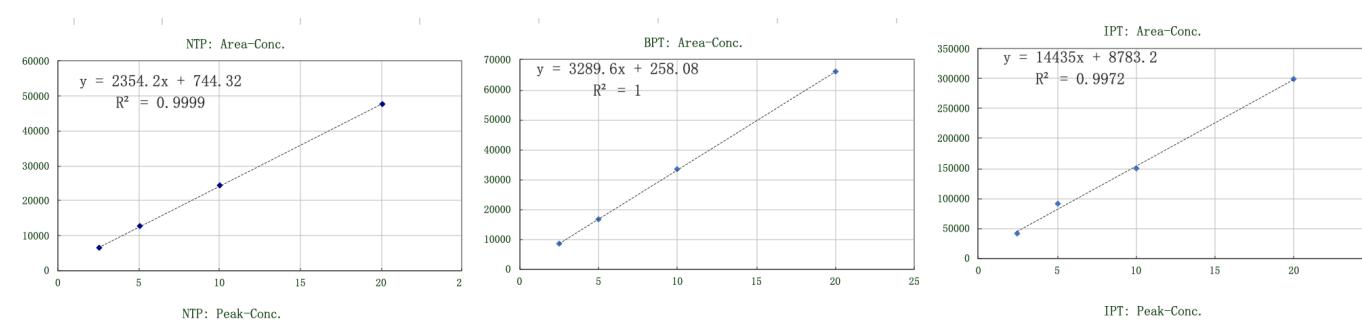
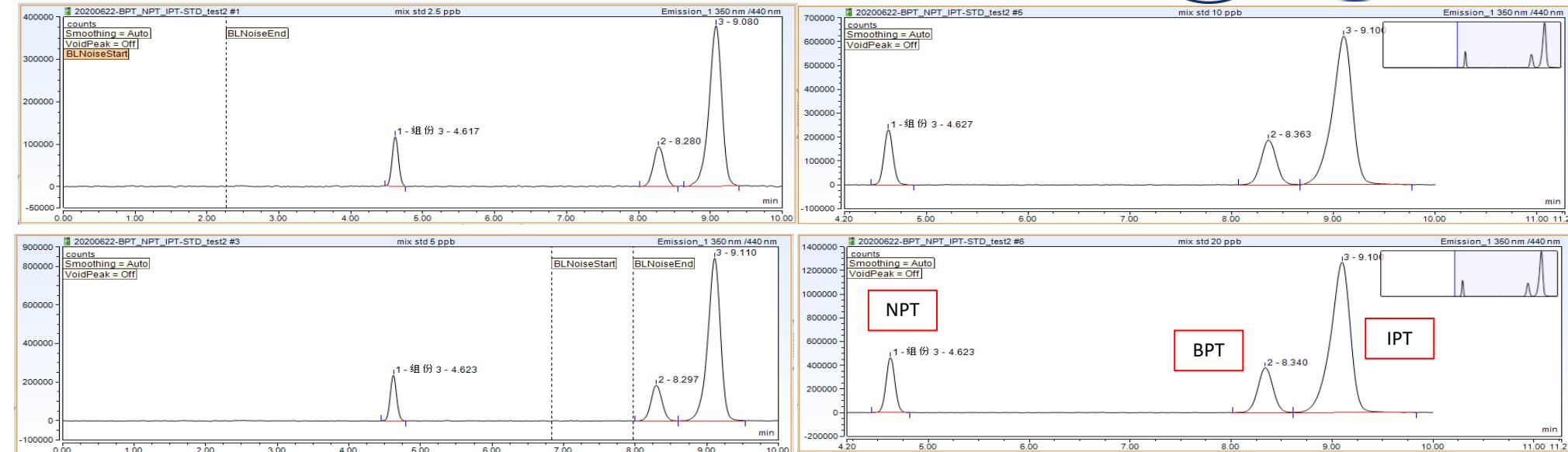
Cell lysis buffer
3.00ml

BTP : ~ 5 $\mu\text{g/L}$

Target concentration



Multi-pterins

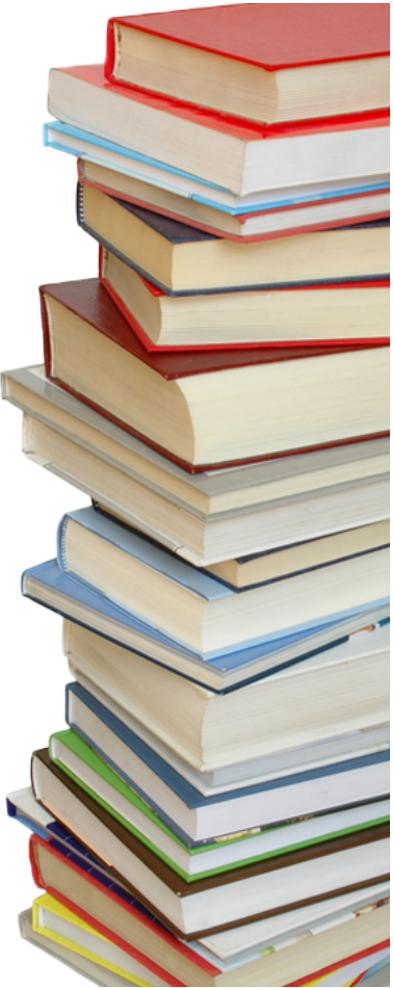


Phase A: MQ water
Phase B : MeOH



- 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]
- BPT was quantified via HPLC, and its compound was further confirmed by LC-MS;
- 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];
- We aim to obtain patent based on our newly developed technique; and further study its metabolic processes and potential implication in the ocean.

No technique, No method,
No paper, No future



**Especially appreciation for:
MEL, IME, OCG and its members
Prof. Nengwan Chen and his cruises
All the people who helped us.**

