

# **Molecular evolution of Multi Copper Blue Proteins**

**Kensuke Nakamura**

# Metallome

いくつかの金属元素は生命現象に必須

**Fe, Zn, Cu, Mn, Co, V, Mo, W**

遷移金属

**Ca, Mg, Na, K**

アルカリ（土類）金属

銅イオンは活性酸素などのラジカル種を作り出すので有毒。  
だが、様々なタンパク質が銅イオンの酸化還元能力を利用している

シトクロム酸化酵素

活性酸素不活化酵素

チロシナーゼ

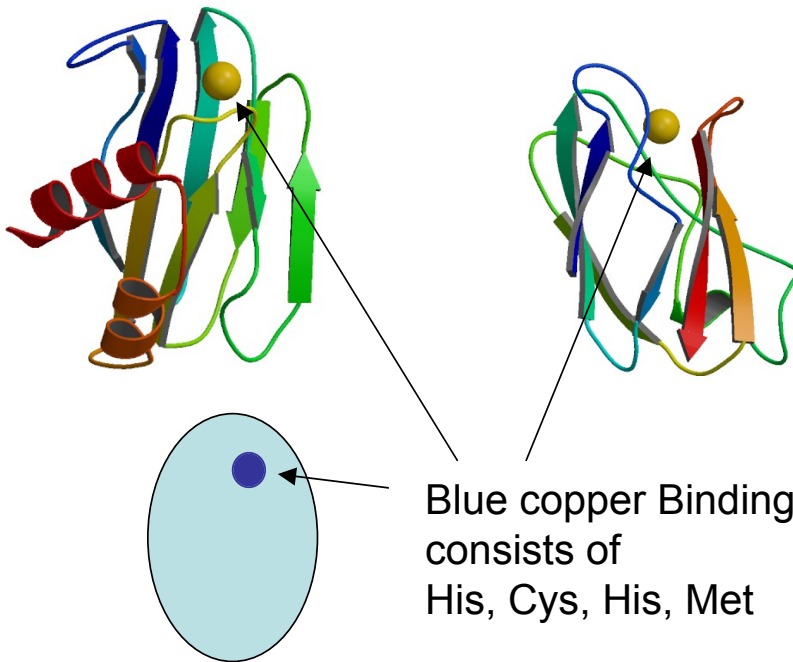
ヘモシアニン

など

# Blue Copper Proteins

Double Greek-Key eight  $\beta$ -strands

Blue-Copper center for Redox Function



**Cupredoxins** mono-domain

Azurin

Pseudoazurin

Amicyanin

Plantacyanin

(Basic Blue Protein)

Plastocyanin

Auracyanin

Rusticyanin

Stellacyanin

UCLAcyanin !!

Nodulin

**Cu-oxidases** multi-domain

Nitritereductase

Laccase

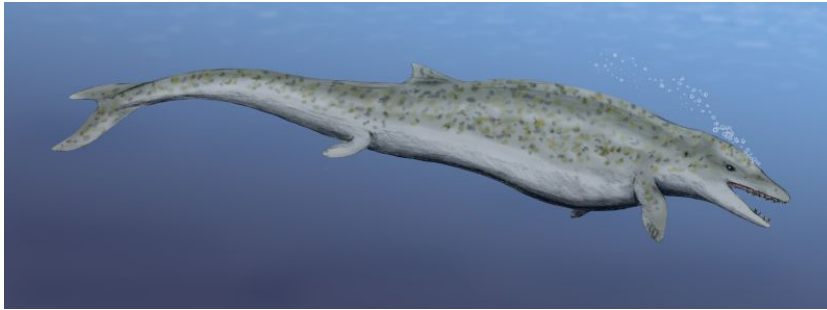
Ascorbate Oxidase

Ceruloplasmin

# Missing Link

博物学としての生物学からの例

## Dorudon

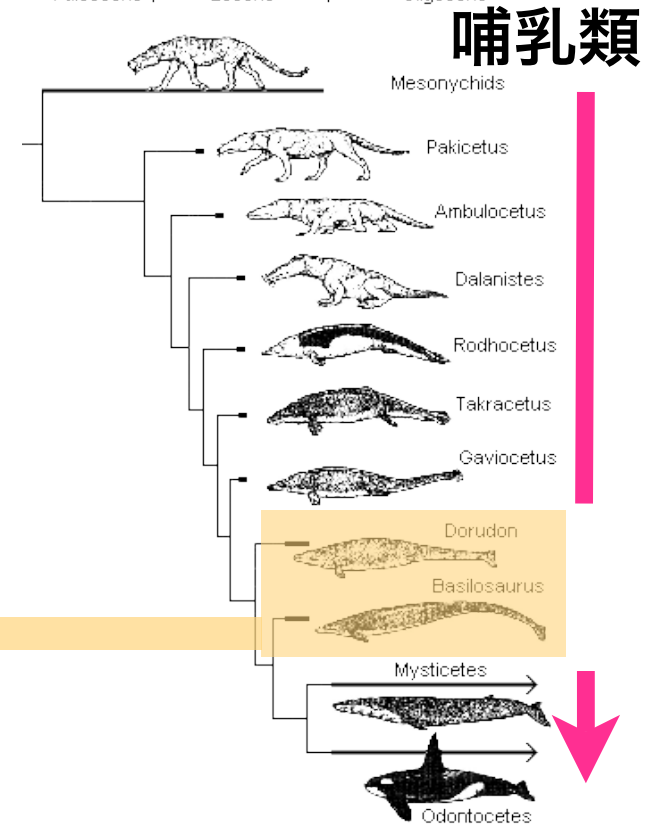
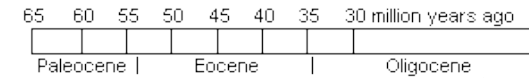


四脚哺乳類からクジラへの進化上の

ミッシングリンク



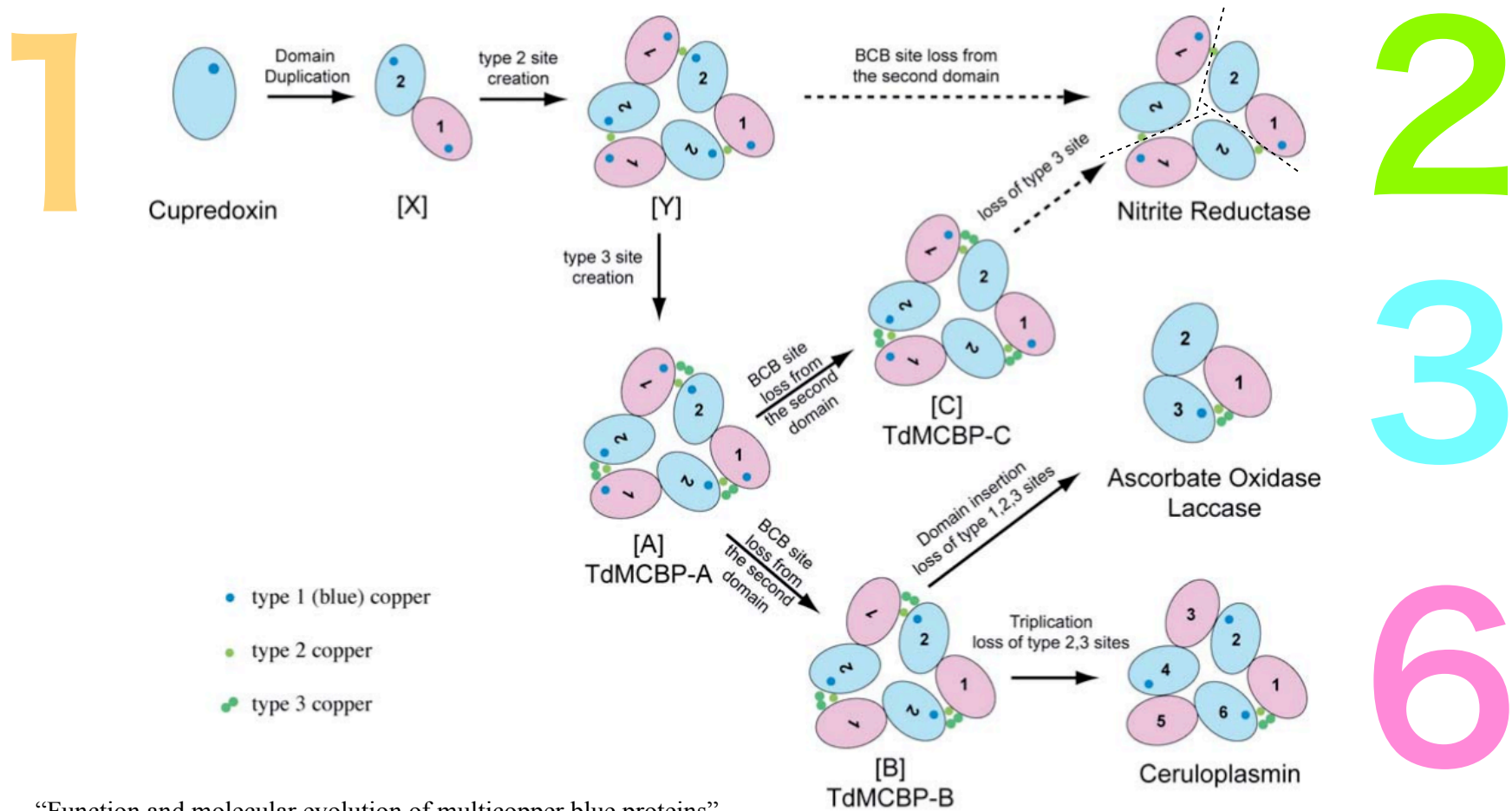
バイオインフォマティクスへ応用



哺乳類

クジラ

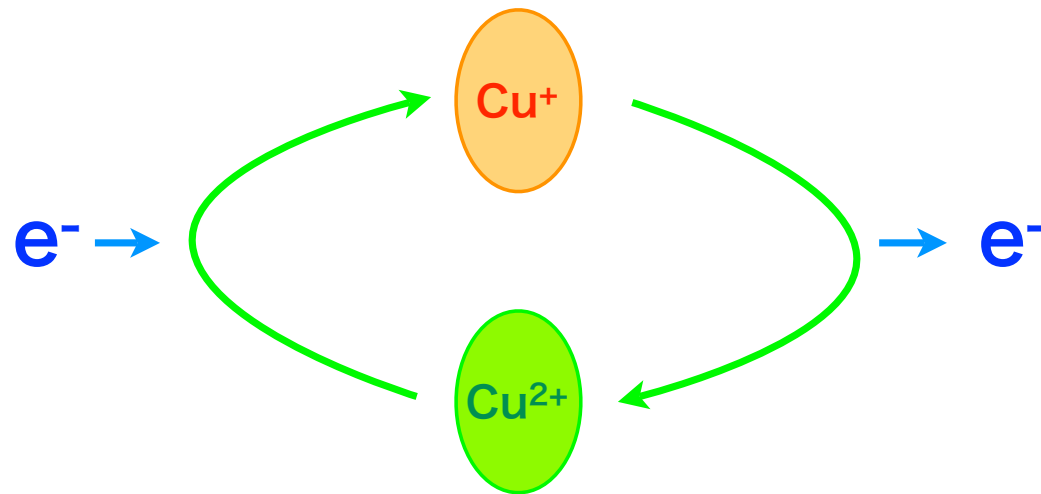
# 分子進化 マルチ銅ブルータンパク質



“Function and molecular evolution of multicopper blue proteins”  
K. Nakamura, N. Go CMLS 62, pp2050-2066 (2005)

プラストシアニン  
アズリン

電子輸送



1

植物

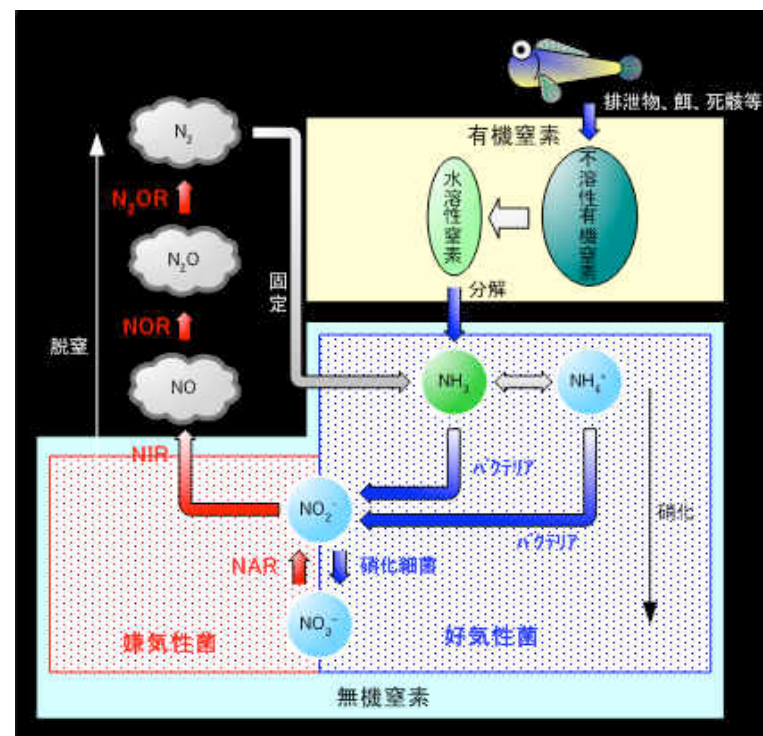
光合成系など

# 亞硝酸還元酵素

2  
NIR  
嫌氣性菌

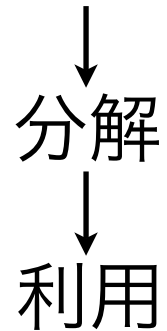


## 窒素循環



## ラッカーゼ

リグニン = 植物の細胞壁



バイオ燃料、洗剤、浄化槽、

## アスコルビン酸酸化酵素

ビタミン C 抗酸化作用

活性酸素への抵抗性・老化防止

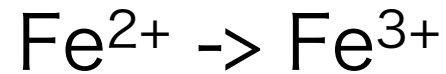
# 3

真菌  
植物  
バクテリア



# セルロプラスミン

ヘモグロビンに鉄を供給



6

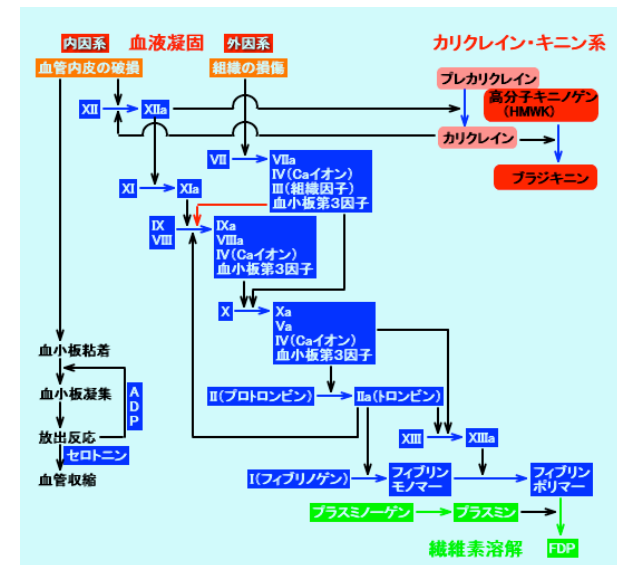
脊椎動物

## 血液凝固因子

BC5

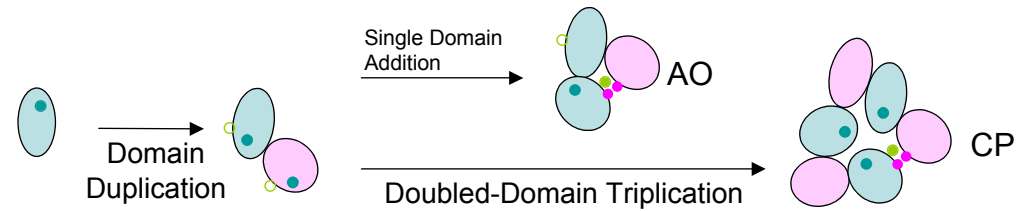
BC8

銅の必要性は不明

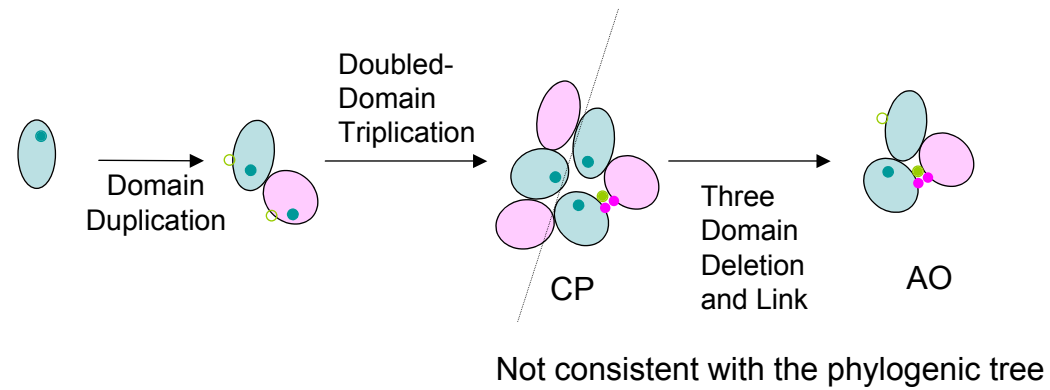


# Previously Inferred Paths of Evolution

## 1. Ryden and Hunt : based on Sequence Phylogeny

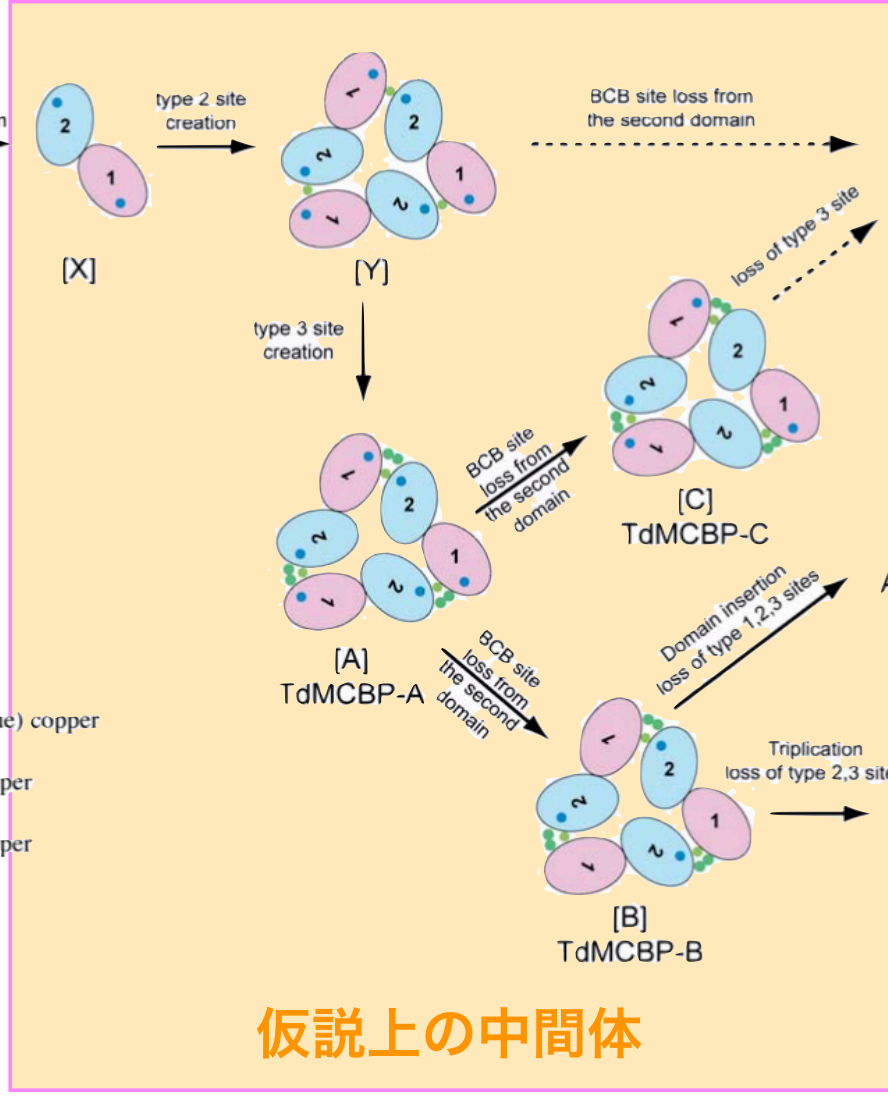
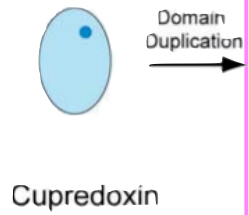


## 2. Murphy, Lindley Adman : based on Structural Similarity



# ここで提唱した進化経路

1



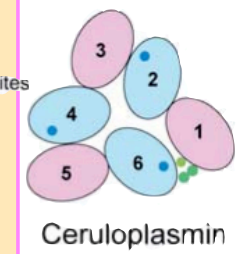
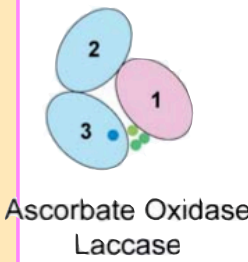
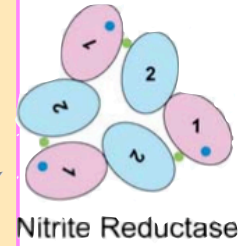
- type 1 (blue) copper
- type 2 copper
- type 3 copper

仮説上の中間体

2

3

6



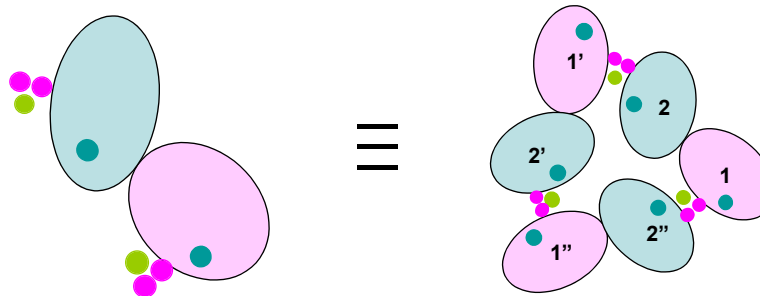
状況証拠 1：  
仮説上の中間体が配列データベースに見つかった

Found [A] *Halobacterium* NRC-1

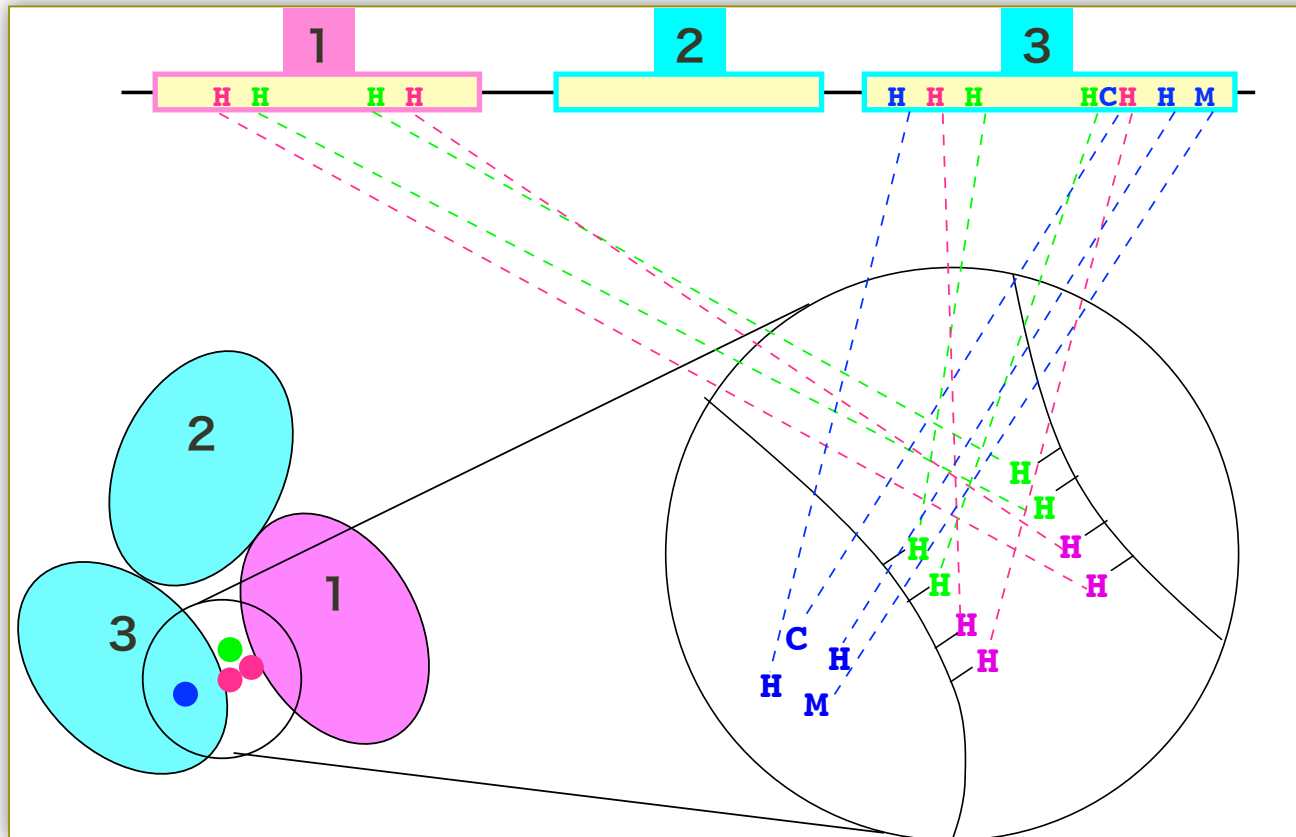
- Q9HQF4 : [A] type 2 domain MCO
  - 449 amino acids
    - Domain1 [77-203] : Blue copper, 4 interdomain Hs
    - Domain2 [206-352]: Blue copper, 4 interdomain Hs

Q9HQF4/ 77-203      DGKRPHTLHFHG----- ~~~ YHCHYQTRHIDM  
 Q9HQF4/206-352    GGYMNHPLHIHNHRFRM ~~~ MHCHK--VNHV-M

Possibly:



# 銅の配位残基の存在は配列情報だけである程度認識できる

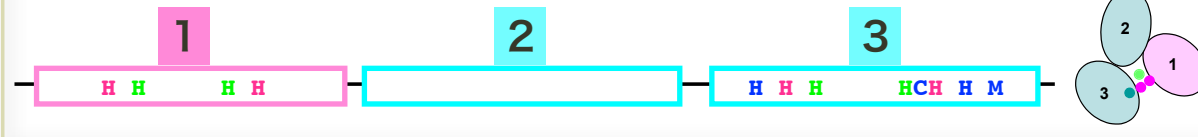


Copper-binding sites can be assigned in amino acid sequence

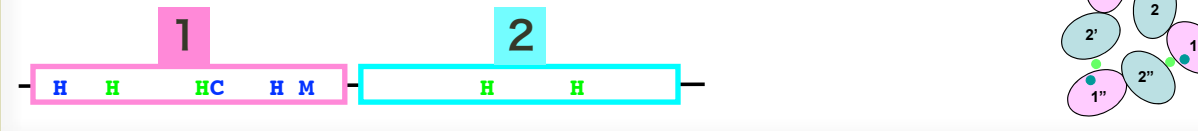
# ドメイン構成

Known

Laccase (3-domain)



Nitrite Reductase (2-domain)

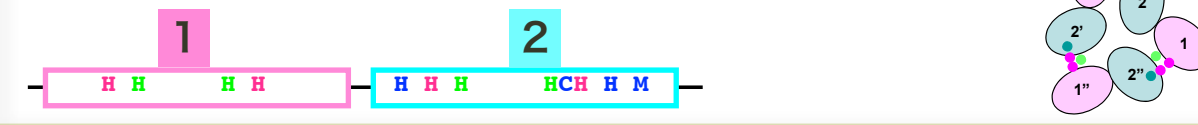


Hypothetical

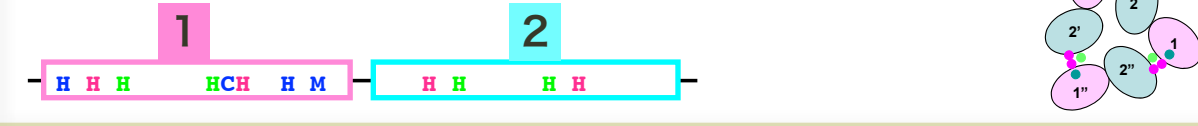
[A] (2-domain)



[B] (2-domain)



[C] (2-domain)



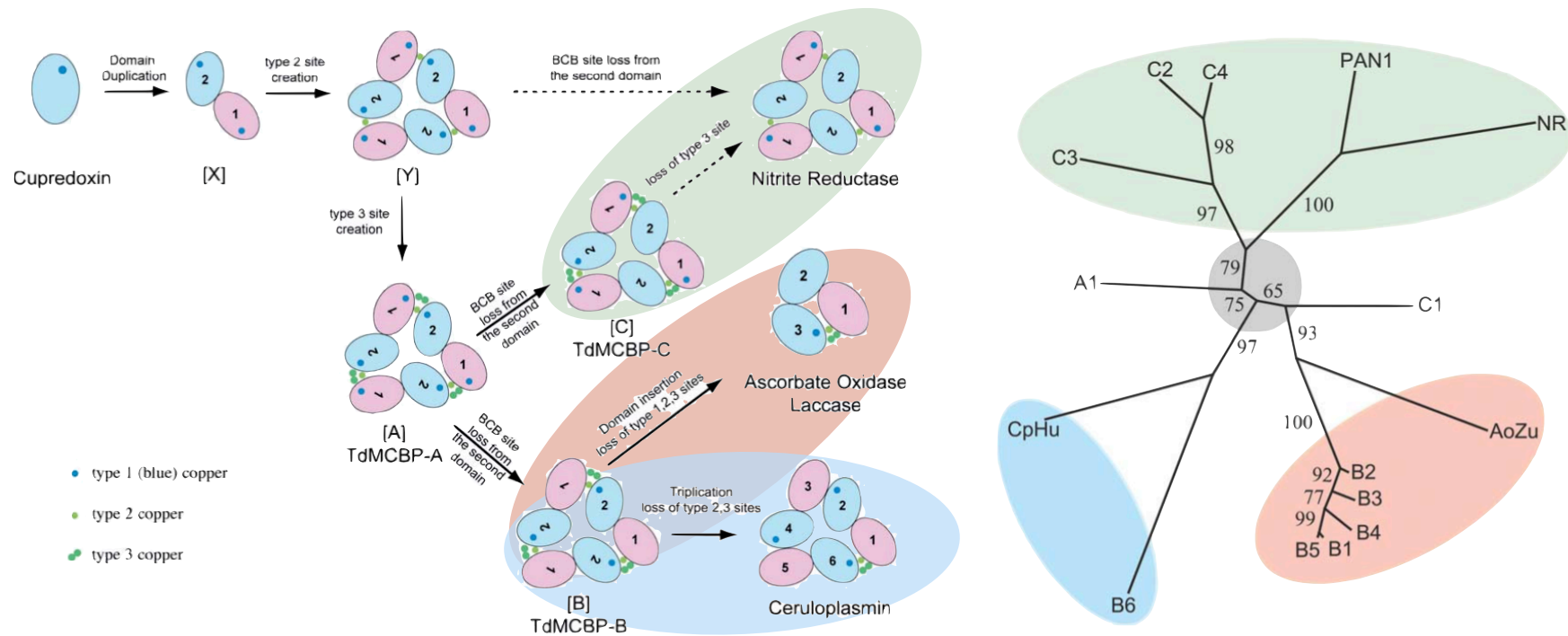
# 11 new types of two-domain MCO's

A	A1 : Q9HQF4	from Halobacterium strain NRC-1	(藍藻)
	B1 : Q92S43	from Shinorhizobium meliloti	(根粒菌)
	B2 : ZP_00029340	from burkholderia fungorum	(アニリン資化性グラム陰性菌)
B	B3 : NP_768850	from bradyrhizobium japonicum USDA 110	(根粒菌)
	B4 : ZP_00052601	from Magnetospirillum magnetotacticum	(磁性細菌)
	B5 : NP_356650	from Agrobacterium tumefaciens str C58	(根頭癌腫菌(薔薇))
	B6 : Q93HV5	from Streptomyces griseus	(ストレプトマイシン産生菌)
C	C1 : ZP_00002680	from Nitrosomonas Europaea	(硝化細菌)
	C2 : ZP_00073469	from Trichodesmium erythraeum IMS 101	(藍藻、紅色)
	C3 : NP_711736	from Leptospira interrogans serovar lai str	(家畜の病気)
	C4 : NP_487982	from Nostoc sp PCC7120	(藍藻)

		First domain					Last domain						
		1	2	3	312	1	1	1	2	3	312	1	1
MCBPs	3-domain	AoZu	57	VVIHWHGILQ	99	GTFPHYHGHLMQRSAGLYGSL	325	445	HPWHLEGHDF	501	GVWAFHCHIEPHLHMGMGVVF		
		LcAb	60	VSIHWHGFFQ	103	GTFWYHSHLSTQYCDGLRGAF	274	398	HPFHLEGHNF	446	GAWFLHCHIDWHLEAGLAIVF		
		MvBO	91	NSVHLHGSFS	128	RTLWYHDHAMHITAENAYRQQ	249	398	HPIHHLVDF	451	GVYMFHCHNLIHEDHDMMAAF		
		DHGO	114	TAVHWHGIRL	156	GTSWYHSHFSLQYSNGLYGPL	307	484	HPIHLEGHDF	538	GAWLLHCHLQYHASEGMALQY		
		YD56	97	TALHFHGVVP	140	GTFWYHSHSSVQYGDGMRGVL	291	452	HPWHMREGHDF	525	GKWWLHCHVEWHMMKGLGIVF		
		YAK8	82	TSLHSHGLFQ	124	GTWVHSHDMSQYPDGLRTPF	272	417	HPFHLEGHDF	475	GAWVIHCHIEWHMESGLLATF		
		CopA	96	TSIHWHGILL	136	GTWYHSHSGFQEQGVVYGP	365	522	HPIHLEGMWS	565	GRWAYHCHLLYHMEMGMFREV		
		CumA	102	TTIHWHGIRL	142	GSYWYHSPVSSSEELGRGLVP	235	398	HPIHLEGMWS	445	GTWVHCHVIDHMETGLMAAI		
		Fet3	78	TSMHFHGLFQ	121	GTWYHSHSTDGQYEDGMKGLF	271	413	HPFHLEGHAF	478	GVWFFHCHIEWHLLQQLGLVL		
		Fet5	76	TSLHFHGLFQ	123	GTFWYHSHMGAQYGDGMRGAF	274	418	HPFHLEGHNF	491	GVWYFHCHEVDWHLLQQLASGF		
6-domain	CpHu	98	YTFHSHGITY	156	VTRIYHSHIDAPKDIASGLIG	798	975	HTVHFHGHDF	1015	GIWLLHCHVTDHIHAGMETTY			
	NR	88	HNIDFHAATG	123	GVFVYHCAPEGMVPHVTSGM	101	245	TRPHLIGGHG	292	GVYAYVHNLIIEAFELGAAGH			
	PAN1	82	HNVDFAATG	117	GLYIYHCAVAPVGMHIANGMI	90	228	SSFHVGIEIF	270	GNVTLDVHSHIFRAFNGALGQ			
2-domain	NRMR	197	HSMDFHTAMV	231	GVFMYRCGTPRVLEHIASGMY	92	344	SSFHVVGAI	390	GAYVMVDHQFANASQGAAGVI			
	A1	135	HTLHFHGSQT	175	GTHLYHCHYQTRHIDMGMYG	86	282	HPLHINHRF	331	GIYLMHCHKVNVVMNGTFYFG			
	A2	139	HTVHFHAVQK	179	GTHLYHCHYQTRHIDMGMYG	86	286	HPLHINHRF	335	GIYLAHCHKVSHAMNGTAYFG			
B1	124	TTIHWHGMILL	164	GTFMYHSHSDEMVMAMGMMG	77	262	HPIHMHGYDF	250	GAWAICHKSHHTMNAAGHDI				
B2	125	TTVHWHGMILL	165	GTFMYHSHSDEMVMAMGMMG	77	263	HPIHMHGYDF	311	GDWAFHCHKSHHTMNAAGHQV				
B3	124	TTVHWHGMIV	164	GTFMYHSHSDEMVMAMGMMG	77	262	HPIHLEHSG	309	GDWAFHCHKSHHTMNAAGHEV				

4 [A]'s, 130 [B]'s, 36 [C]'s

## 状況証拠 2： 系統解析と進化仮説の一致



進化モデル上近い組み合わせが系統樹上でも近い  
位置に現れる



# 矛盾する実験例

## SLAC = B13

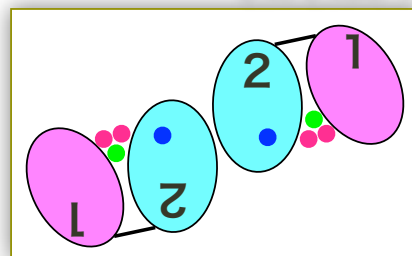
“Characterization of SLAC: A small laccase from *Streptomyces coelicolor* with unprecedented activity”, Machczynski MC, Vijgenboom E, Samyn B and Canters GW, Protein Science, 13, Aug, pp. 2388-97, (2004).

[SLAC = B13] リコンビナントに合成し物性を調べた

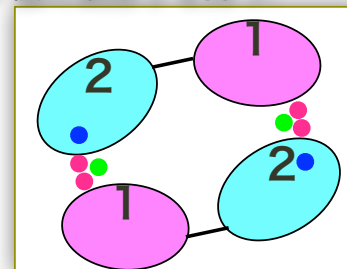
SLAC ラッカーゼ活性を示す  
短いタンパク質-ShortLACcaseと命名.

SLAC はホモダイマー.

SDS-PAGE 64kDa ダイマーのバンドが観測された.  
それぞれのサブユニットが 4 個の銅原子を持つ.

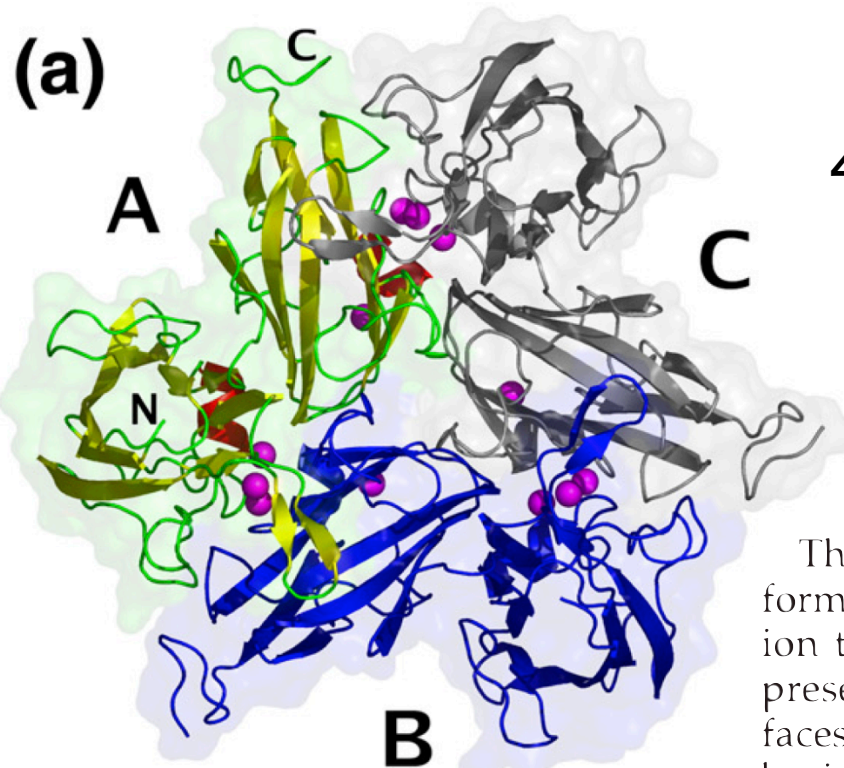


or



?

## X先結晶構造により我々の推定が検証された



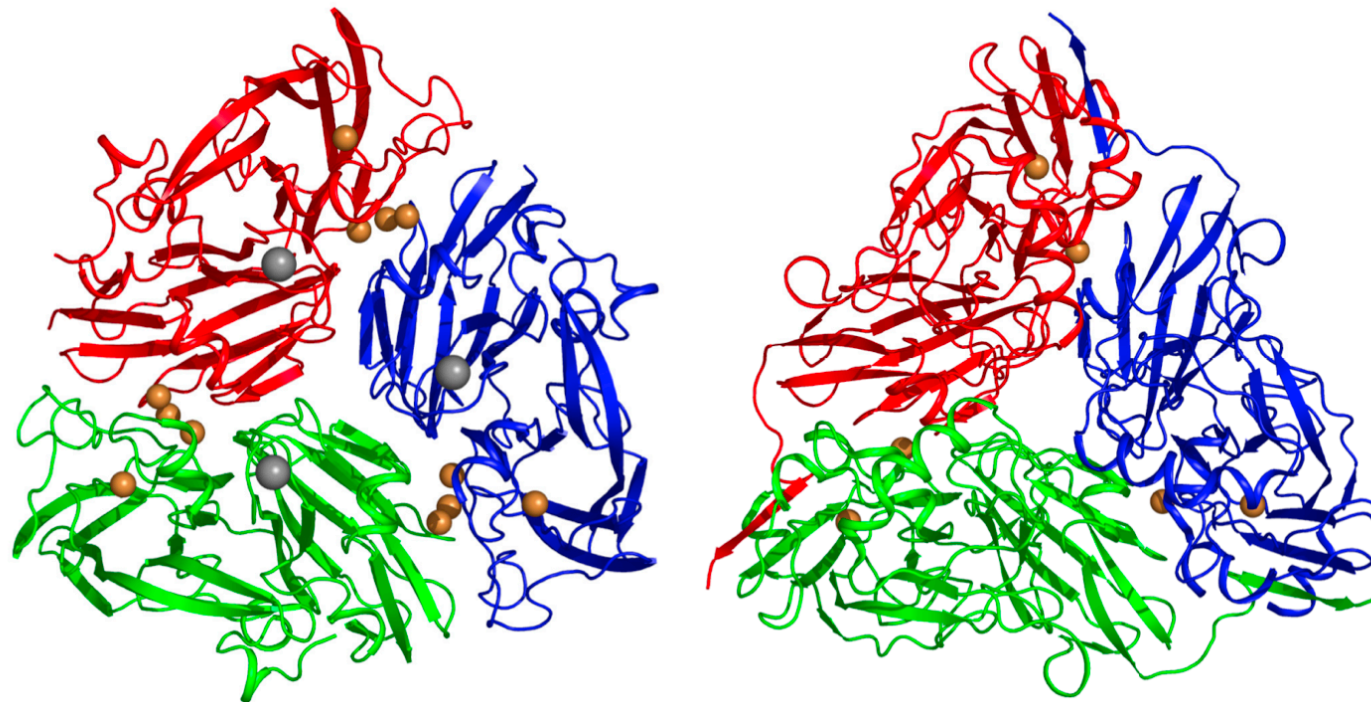
**SLAC**  
*Streptomyces coelicolor*  
**[B]**

X-ray 結晶構造により  
SLAC がホモトリマーを形成し  
4つの銅イオンを結合することが示された

The structure presented here confirms the trimeric form of SLAC. It also proves the presence of copper ion type 1 in each domain 2 of the trimer and the presence of the trinuclear cluster at all three inter-faces between domain 1 and domain 2 of the neighboring chains, as predicted by [Nakamura and Go.<sup>8</sup>](#)

“The structure of the Small Laccase from *Streptomyces coelicolor* Reveals Link Between Laccases and Nitrite Reductase” Tereza Skalova, J. Dohnalek, L.H. Ostergaard, P.R. Ostergaard, P. Kolenko, J. Duskova, A. Stepankova, J. Hasek, *JMB* 385, 1165-1178 (2009)

さらに、



AMMCO

[C]

*Anthrobacter* sp. FB24, NC\_008537

NIR

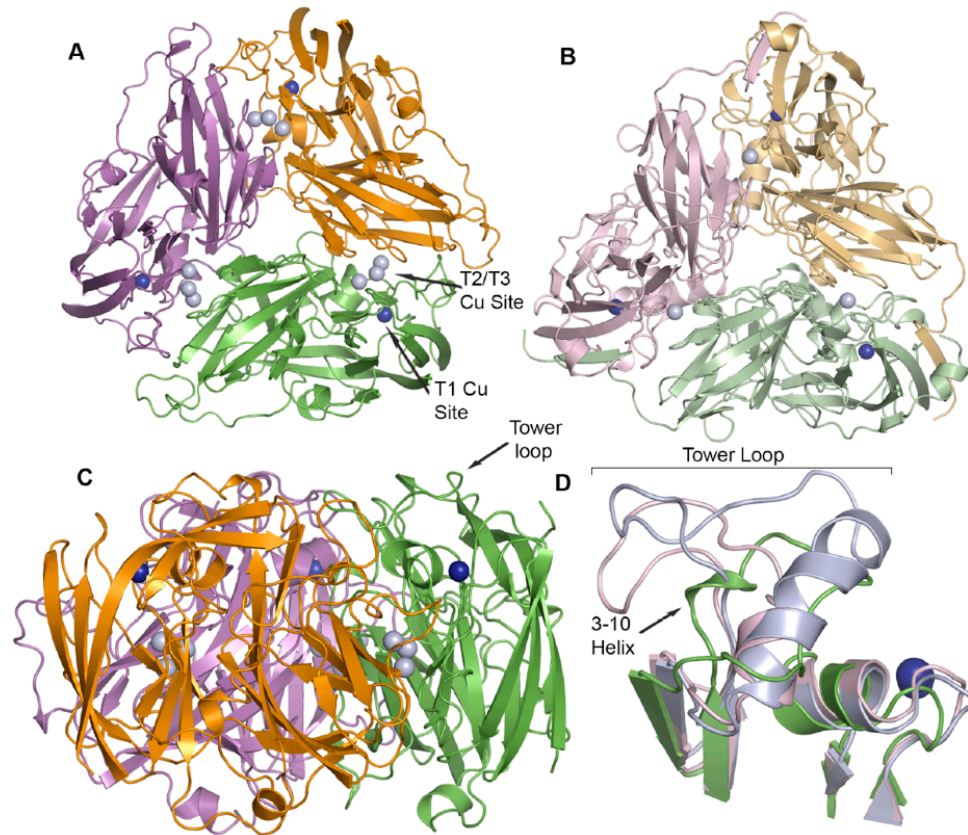
In particular, the evolutionary model of Nakamura and Go connecting NiR to three-domain MCOs is in need of experimental validation.

**[C]-タイプの AMMCO 結晶構造が NIR に類似**

“Evolution of Copper-Containing Nitrite Reductase” I. S. MacPherson, Doctoral Dissertation U. British Columbia (2008)

# もうひとつの[C]タイプ結晶構造

BCO  
[C]



NIR

*Nitrosomonas europaea*, NC\_008537

As of 2005, Nakamura and coworkers identified 21 different 2dMCOs in genome databases

“Crystal structure of a two-domain multicopper oxidase: implications for the evolution of multicopper blue proteins”, Lawton TJ, Sayavedra-Soto LA, Arp DJ, Rosenzweig AC, JBC (2009)

## **Summary**

**Series of recent X-ray structures of  
2-domain MCBP's supports our model**

**The evolutionary model is  
being widely accepted**

# **What are the function of these MCBP's in bacteria?**

**All 2-domain MCBP's are from Bacteria**

**Large number of 3-domain MCBP's are from Bacteria**

## Function of 3-domain MCBP's in Bacteria

<b>CotA</b>	<i>Bacillus subtilis</i>	Spore coat
<b>CueO</b>	<i>E. coli</i>	Oxidation of Cu(I)
<b>PcoA</b>	<i>E. coli</i>	Efflux of Cu
<b>Fet3p</b>	<i>Saccaromyces cerevisiae</i>	Oxidation of Fe(II)
<b>CumA, MofA, MnxG</b>	<i>Pseudomonas putida</i>	Oxidation of Mn(II)
<b>Phenoxasionone synhtase</b>	<i>Streptomyces lividans</i>	Biosynthesis of antibiotic

# MCBP's in Bacillus/Lactobacillus

## 3-domain

**Bacillus cereus; anthracis; thuringiensis; weihenstephanensis; halodurans;  
coagulans; pumilus; clausii;**

**Lactobacillus rhamnosus; casei; brevis; plantarum;**

**Lactobacillus lactis;**

**Pediococcus pentosaceus;**

**Leuconostoc citreum;**

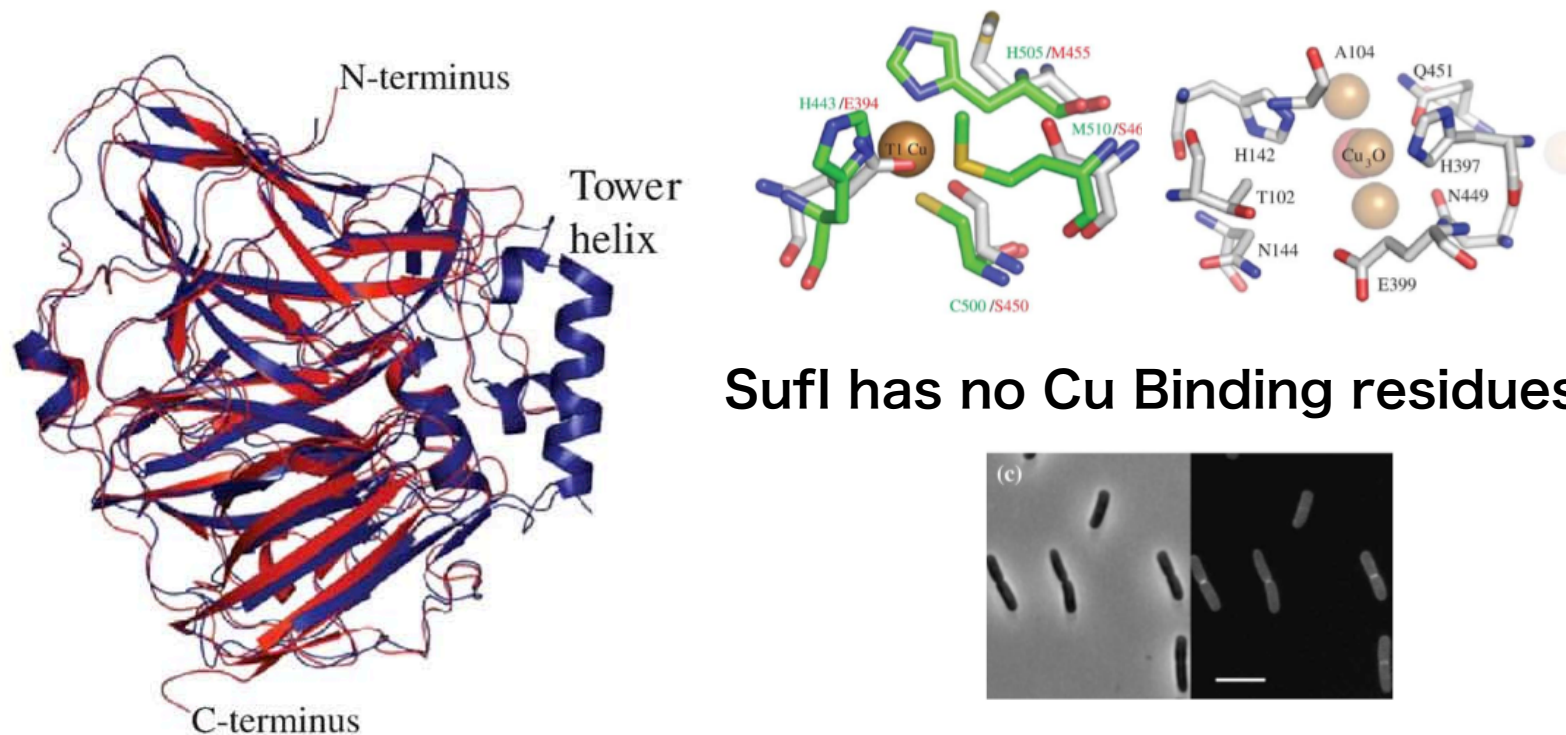
## 2-domain

**Bacillus sp. B14905;**

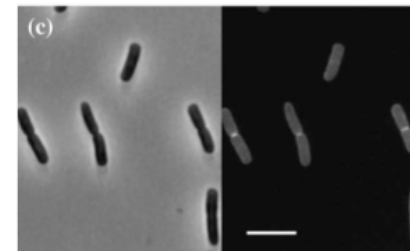
By homology, MCBP's in Bacteria are usually assigned as  
Copper resistance, Mn oxidation, Spore coat, or Cell division (SufI)



# SufI(FtsP) is a 3-domain MCBP form without Cu-binding sites



SufI has no Cu Binding residues



SufI (red) and CueO (blue)

SufI is involved in Cell Division

“The Escherichia coli Cell Division Protein and Model Tat Substrate SufI(FtsP) Localizes to the Saptal Ring and Has a Multicopper Oxidase-Like Structure” Tarry M., Ryan Arends SJ, Roversi P, Piette E, Sargent F, Berks BC, Weiss DS, Lea SM, JMB 386, 504-519 (2009)



## consensus is

once the whole genome is solved for one organism,  
homology analysis will provide the functional annotation for all genes

GP

once the X-ray structures for all possible folds are solved  
homology modeling can solve all the protein structures

SG

## in reality

**There are wide structural and functional variety  
in a group of homologous proteins**

**Careful inspection of each gene is important**

**And there is a plenty of room for Bioinformatics to contribute**

**Metagenome analysis should reveal  
number of characteristic genes with  
novel domain-organization, structure, and function**