

BioJapan2019, 2019年10月10日, パシフィコ横浜

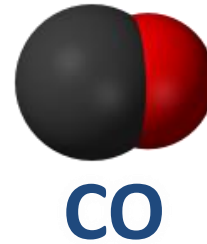
有毒ガスでありながら生体内シグナルガスとしても注目される一酸化炭素の生体内除去およびデリバリーツール



北岸 宏亮

Doshisha University, Kyoto, Japan

一酸化炭素

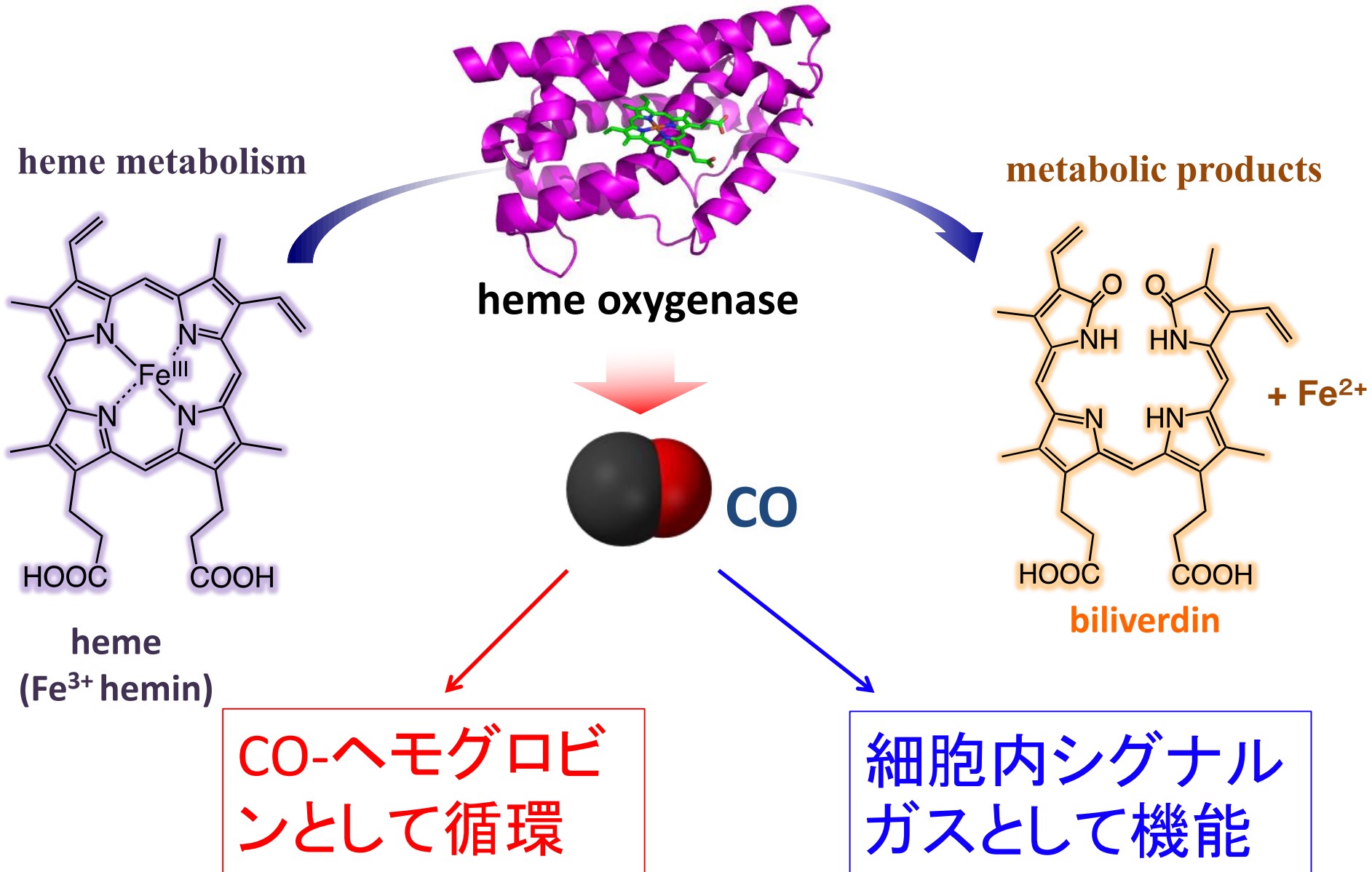


有毒ガス，火災の死亡原因，排気ガス，タバコ，練炭自殺...etc.

生理活性ガス，ヘムタンパク質へのシグナル，抗炎症作用，臓器保存効果...etc.

有毒ガス = 生体内で作用する
= 微量は生物に必要

内因性の一酸化炭素の産生について



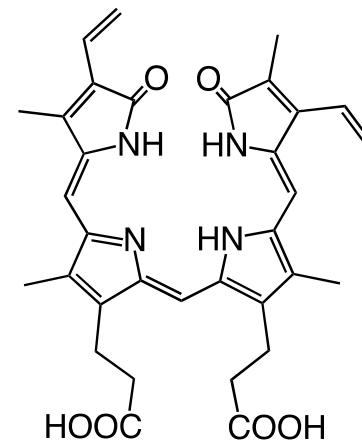
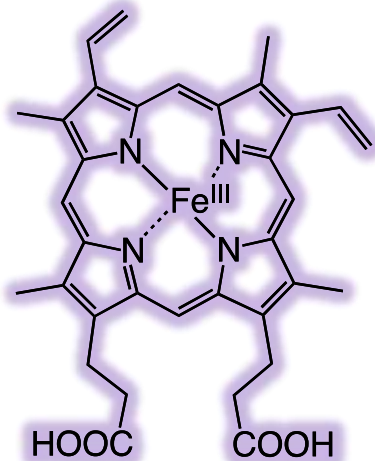
Gaseous signaling molecules

NO

H₂S

Knockout/knockdown methods
are available.

CO



+ Fe²⁺ + CO

Knockout/knockdown cannot be available for CO.

COの生理機能には不明な点が多く、注目ターゲット

今回のトピック

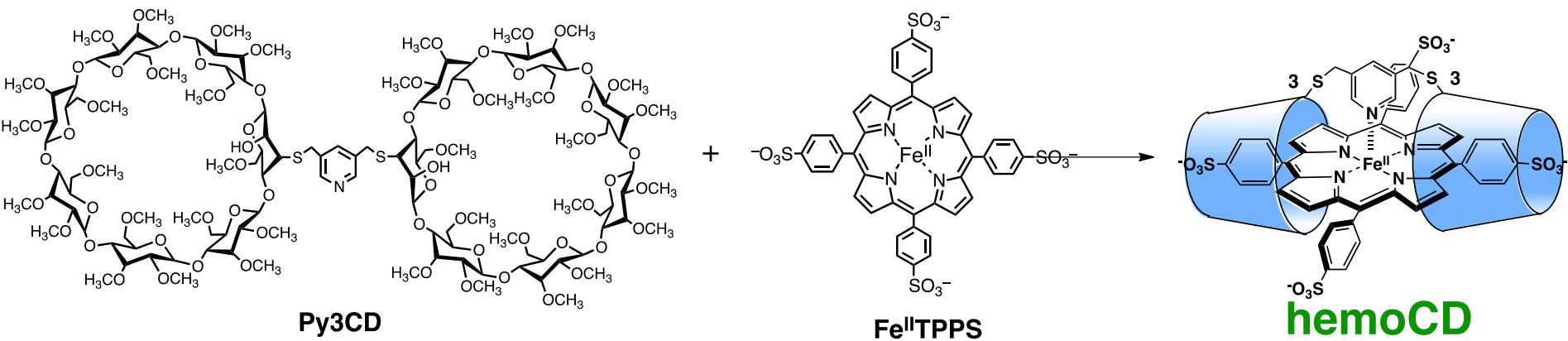
生体内から一酸化炭素を除去する物質hemoCD

- ✓ 内因性COの生理機能を追求する
- ✓ 過剰のCOを体内から除去できる

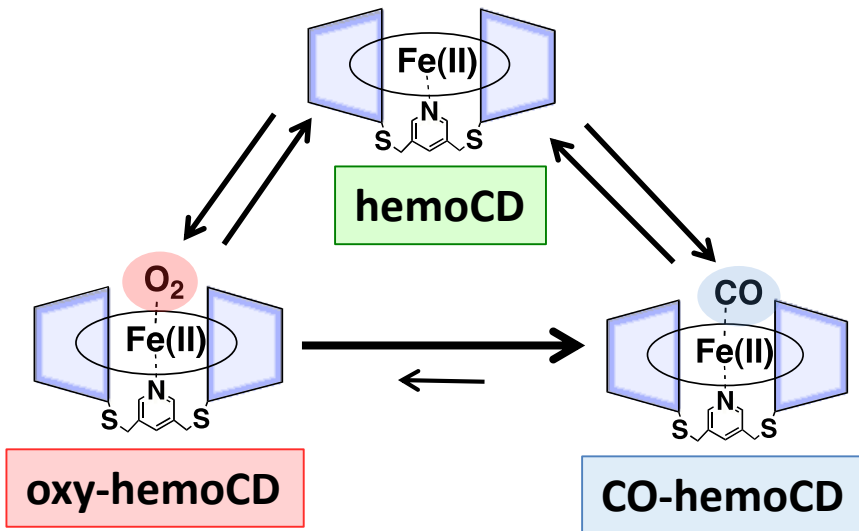
生体内に一酸化炭素をとどける物質CORM

- ✓ COを除放する固体粉末試薬CORM
- ✓ 細胞膜透過性の高いCORMの開発

一酸化炭素の捕捉物質hemoCDについて



An Hb/Mb functional model

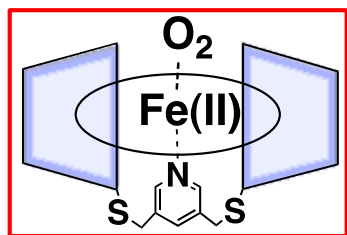


	$P_{50}(\text{O}_2) / \text{Torr}$	$P_{50}(\text{CO}) / \text{Torr}$
Hb (T-state)	22	-
Hb (R-state)	0.22	0.0013
hemoCD	10	0.000015

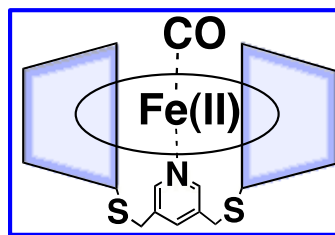
K. Kano, H. Kitagishi et al *Inorg. Chem.* 2006, 45, 4448–4460.

HemoCD is a useful CO receptor in aqueous media.

動物体内のCOを尿中へと強制的に追い出す



oxy-hemoCD

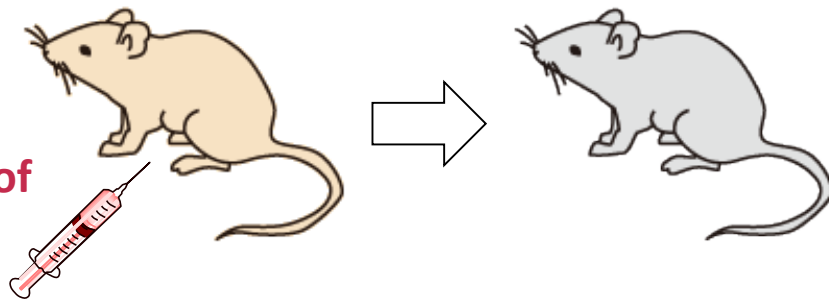


CO-hemoCD (in urine)

**A CO-removal agent
in the living organisms**

H. Kitagishi et al., *Angew. Chem. Int. Ed.* 2010, 49, 1312–1315.

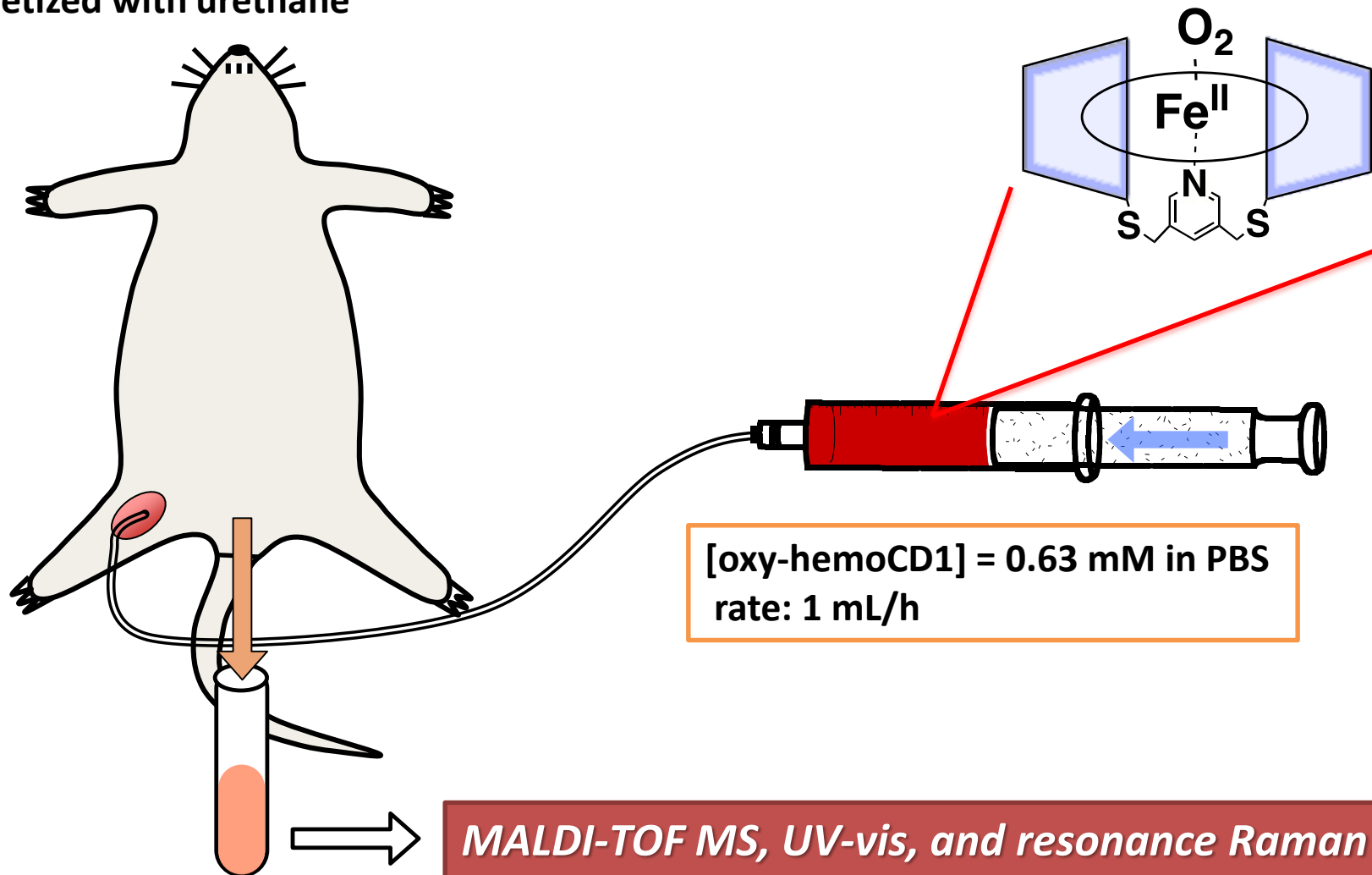
**Administration of
hemoCD**

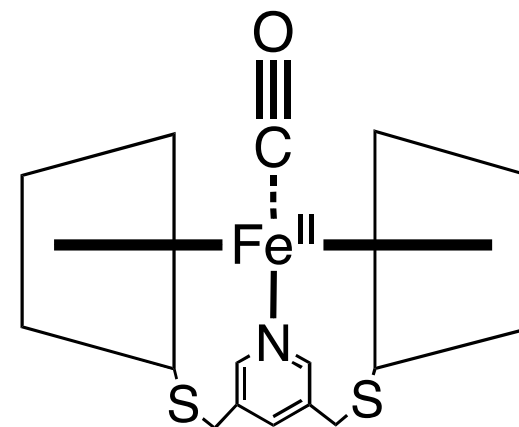
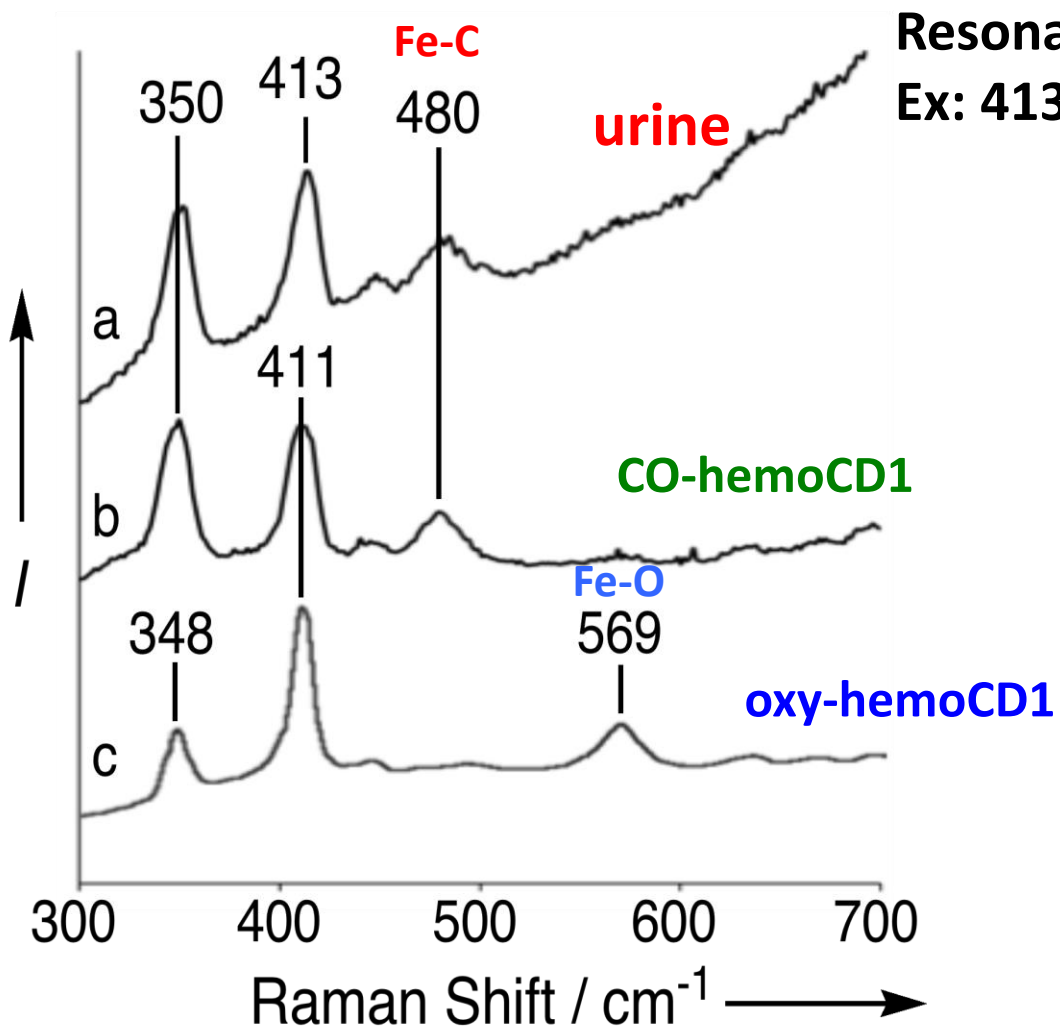


**Pseudo-knockdown
of CO in mice**

Endogenous CO deleted state

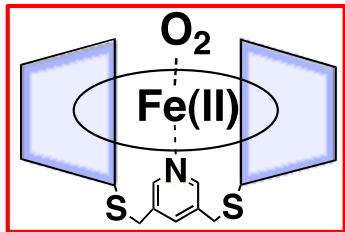
Animal: Wistar male rat; 250–350 g;
anesthetized with urethane



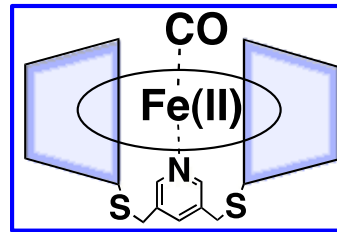


480 cm⁻¹ : Fe-C stretching mode

動物体内のCOを尿中へと強制的に追い出す



oxy-hemoCD

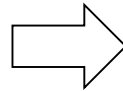


CO-hemoCD (in urine)

A CO-removal agent
in the living organisms

Angew. Chem. Int. Ed. 2010, 49, 1312–1315.

Administration of
hemoCD

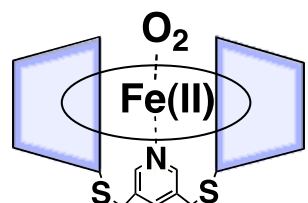


Endogenous CO-depleted state

Pseudo-knockdown
of CO in mice

内在性COを欠乏した疑ノックダウンマウス

血中COヘモグロビンの定量

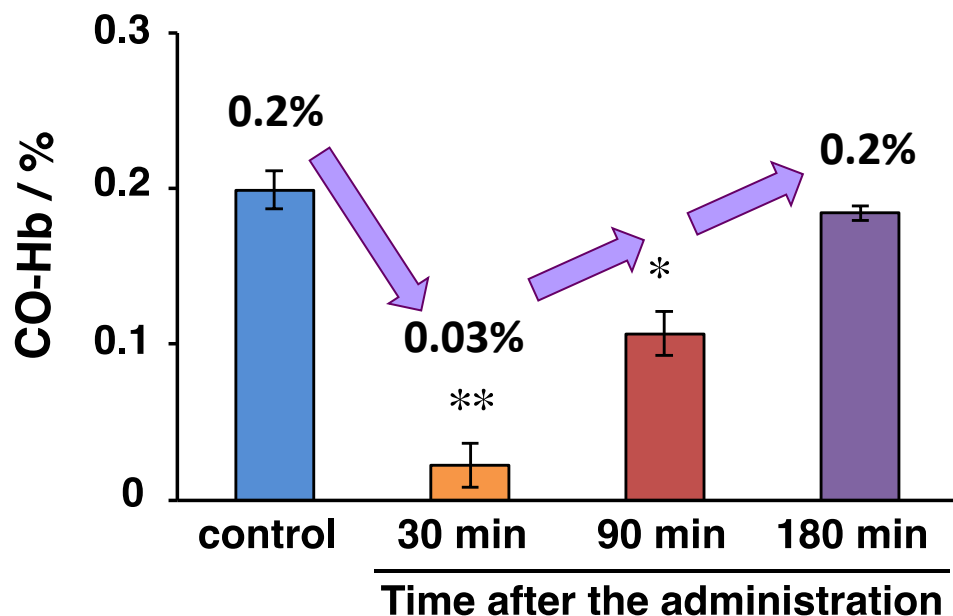


oxy-hemoCD
(1 mM, 0.15 mL)



→ **blood sampling**

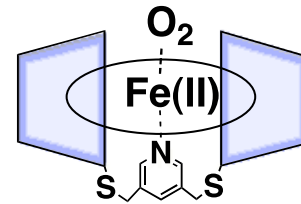
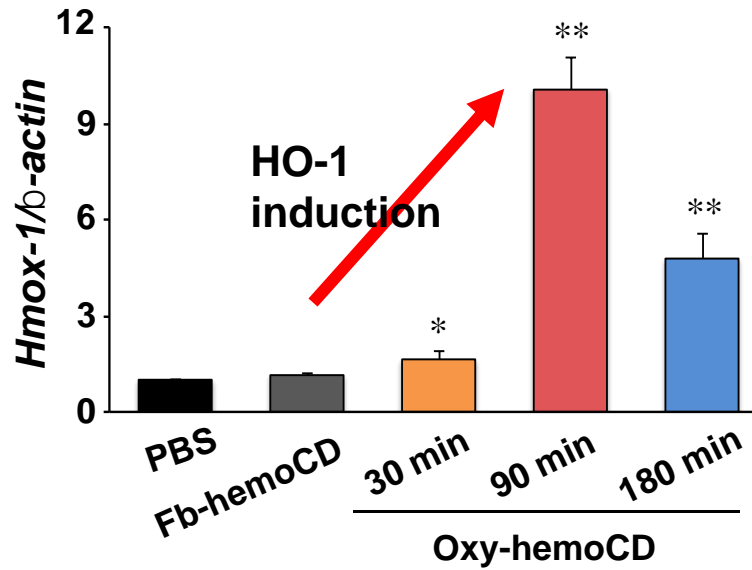
The CO-Hb content (%) in the blood was measured by gas chromatography.



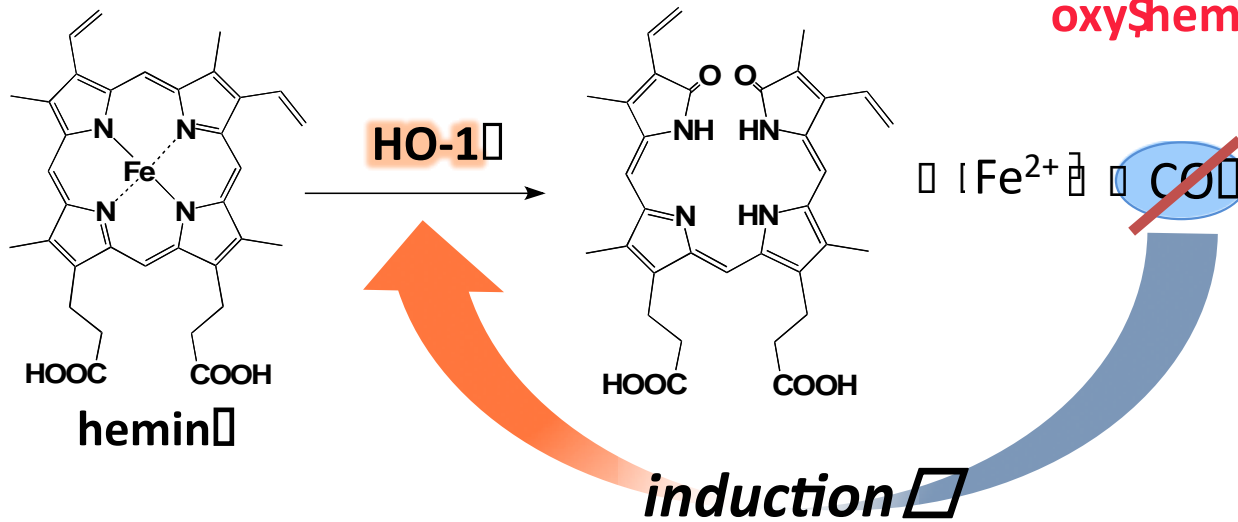
CO-ヘモグロビンの量がほとんどゼロになった

すぐに定常値へと回復した。すなわちCOの産生が亢進された？

The expression of HO-1 strongly increased in the hemoCD-treated mice.



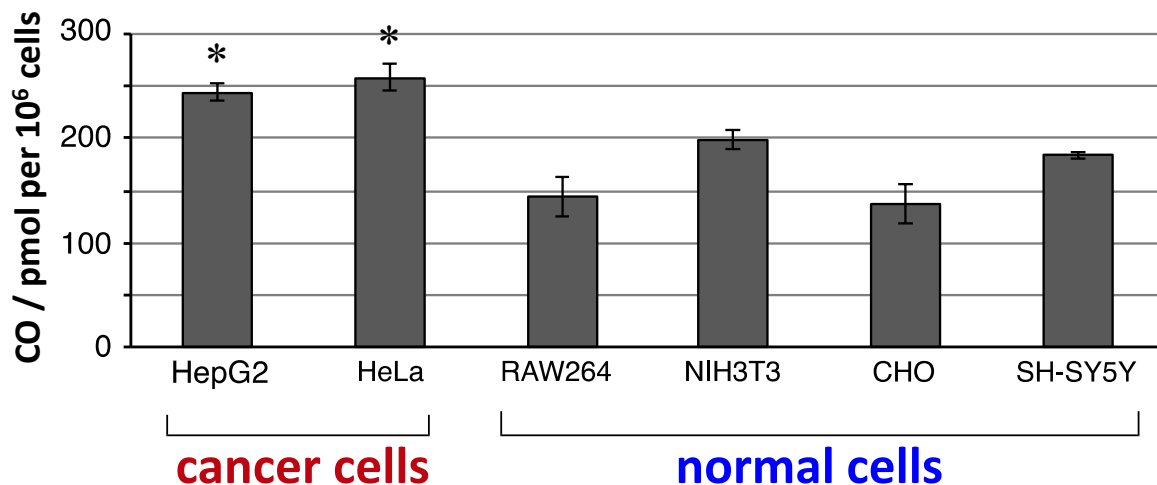
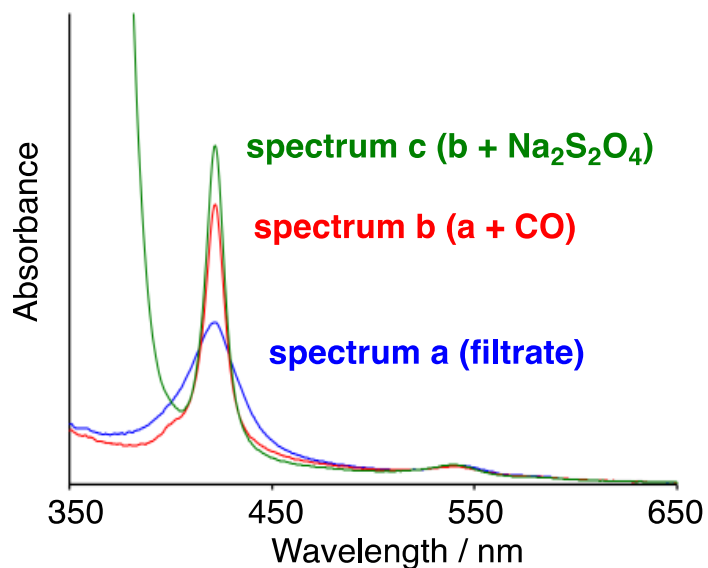
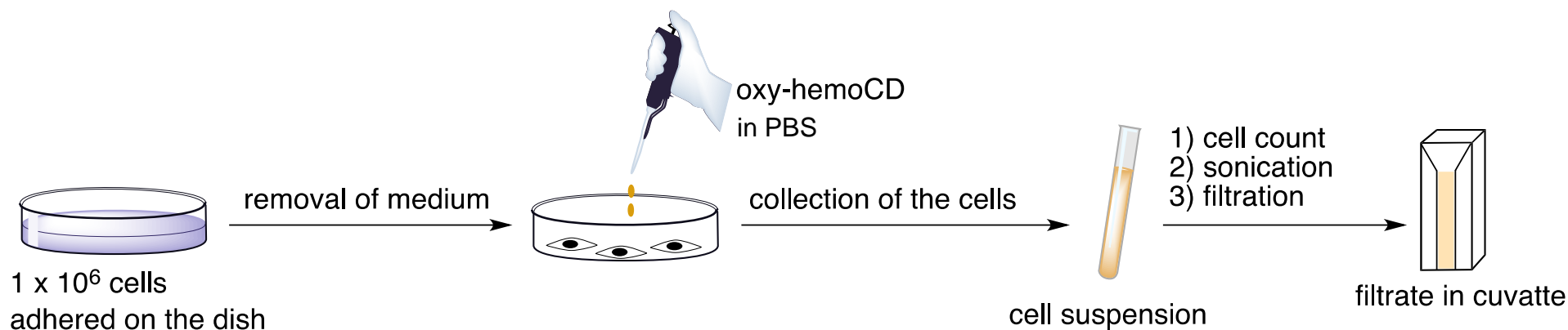
oxyhemoCD



Removal of endogenous CO.

CO恒常性維持のためのフィードバックシステムの発見！

HemoCDは微量COの検出試薬としても使える



細胞内にある内在性COの定量に成功！

Measurement for biological CO

1) ガスセンサー/ガスクロマトグラフィー法

ヒトの場合：呼気のCOを測定し，血中CO量を推定する

血液/組織：COの結合したヘムを酸化し，遊離COの気相分析を行う

2) 赤外レーザー分光法

細胞内COを感度良くリアルタイム計測可能だが，特殊装置が必要
(Y. Morimoto, et al., *Am. J. Physiol.* **2001**, 280, H483)

3) ケミカルプローブの利用

CO選択的な蛍光プローブ (BODIPY-Pd錯体) 定量性には乏しい
(C. J. Chang, et al., *J. Am. Chem. Soc.* **2012**, 134, 15668)

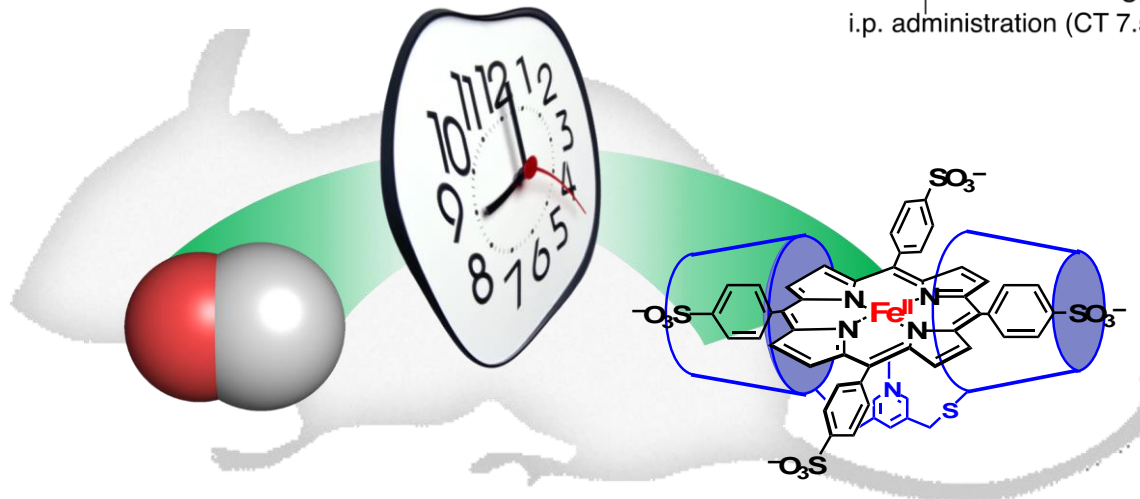
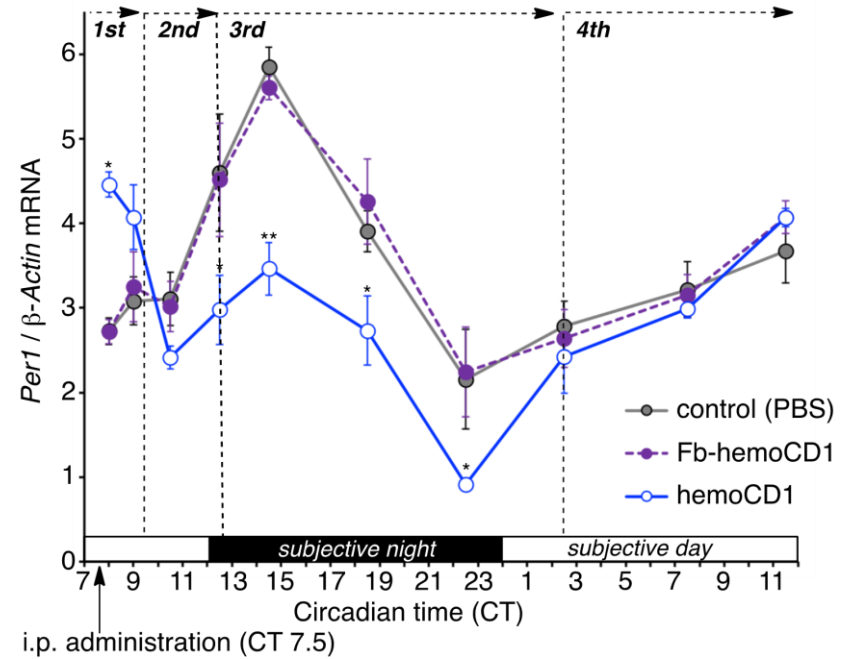
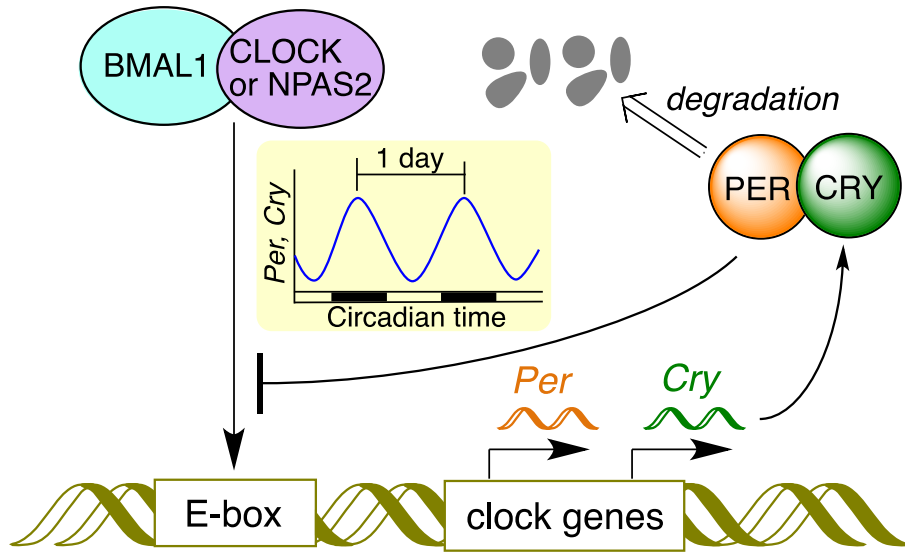
HemoCD アッセイ

NEW!

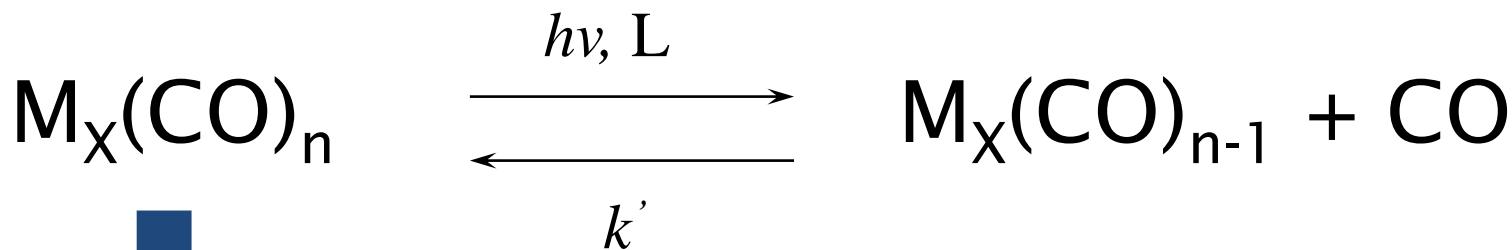
Hbなどの生体内のあらゆるヘムタンパク質からCOを奪い，吸光度測定により簡単かつ正確にCOを定量可能

外来性COの臓器分布計測
新生児黄疸の早期診断法への応用etc.

内在性COの欠乏は、体内時計のリズムを乱す



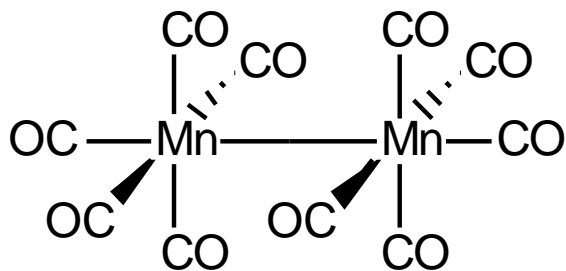
一酸化炭素徐放試薬CORMについて



TRANSITION METAL CARBONYLS

M = Ru, Fe, Mn, Mo, Ni, Co, Rh

金属カルボニル錯体
COを放出, 意外に低毒性



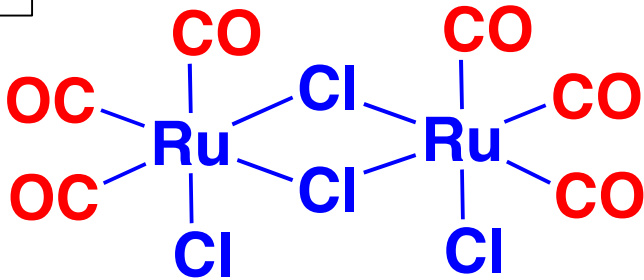
Dimanganese decacarbonyl

$[\text{Mn}_2(\text{CO})_{10}]$

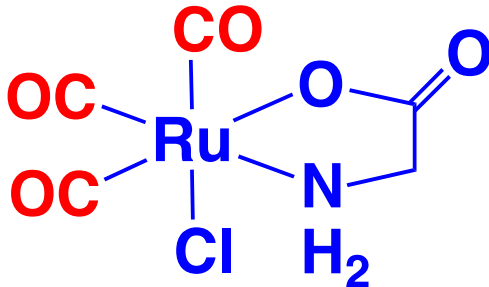
CORM-1

CO-releasing molecules as therapeutic agents

CO-RM2



CO-RM3

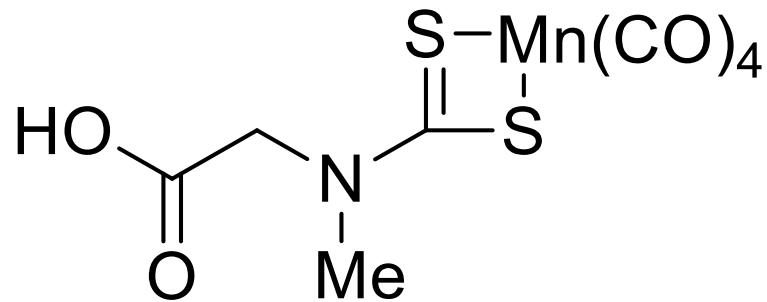


effective against...

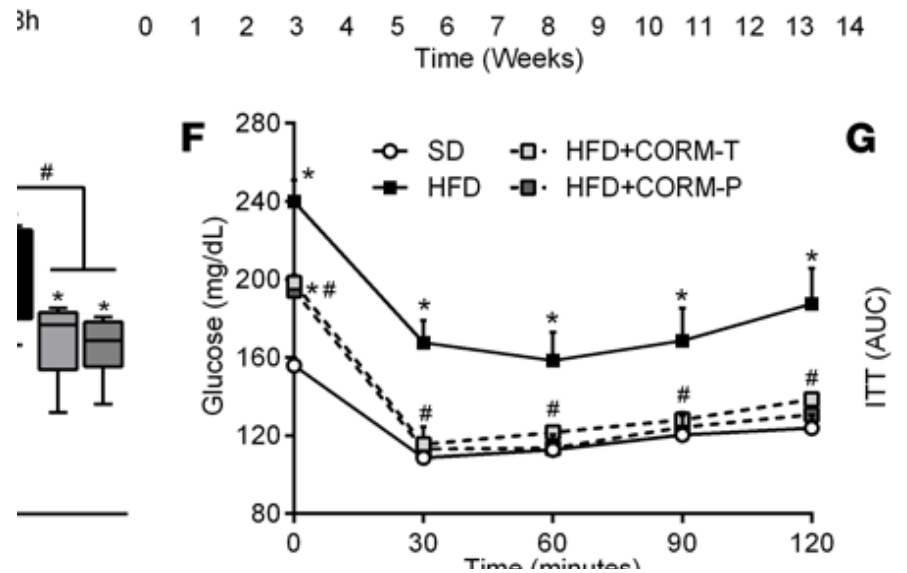
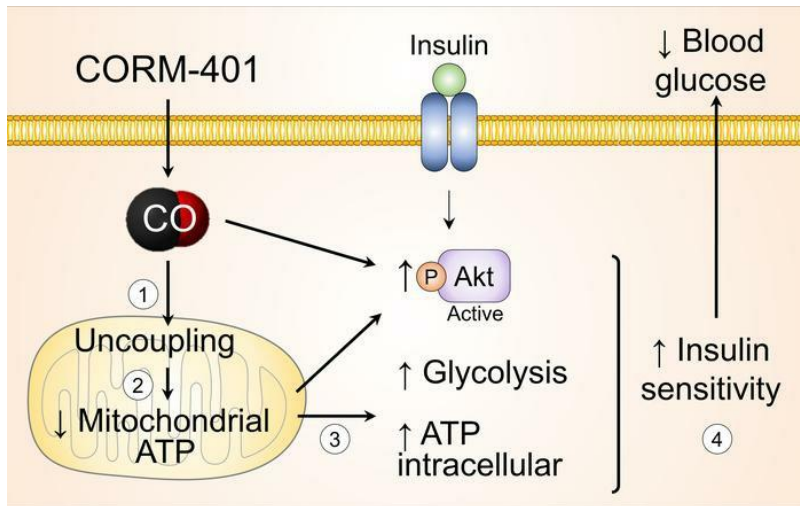
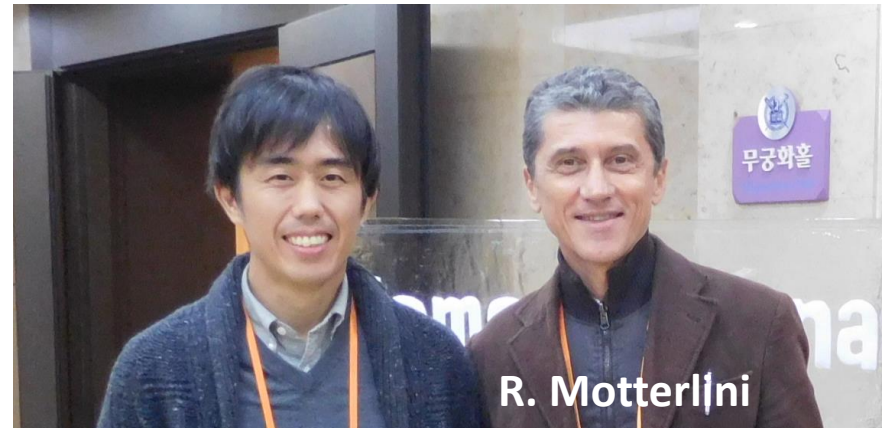
- やけど
 - 失血性ショック
 - 感染症
 - 臓器移植の副症状
 - 抗がん剤副作用
- ... and more

R. Motterlini, L. E. Otterbein, *Nat. Rev. Drug Discov.* **2010**, *9*, 728.

CORM-401



水溶性, 3CO/molecule放出



COの継続投与は, 体重増加を効果的に抑制する
=ダイエットピル?

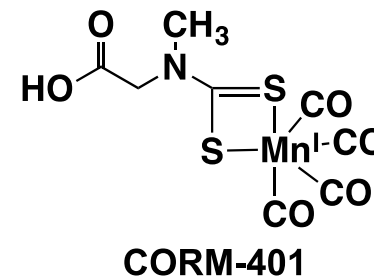
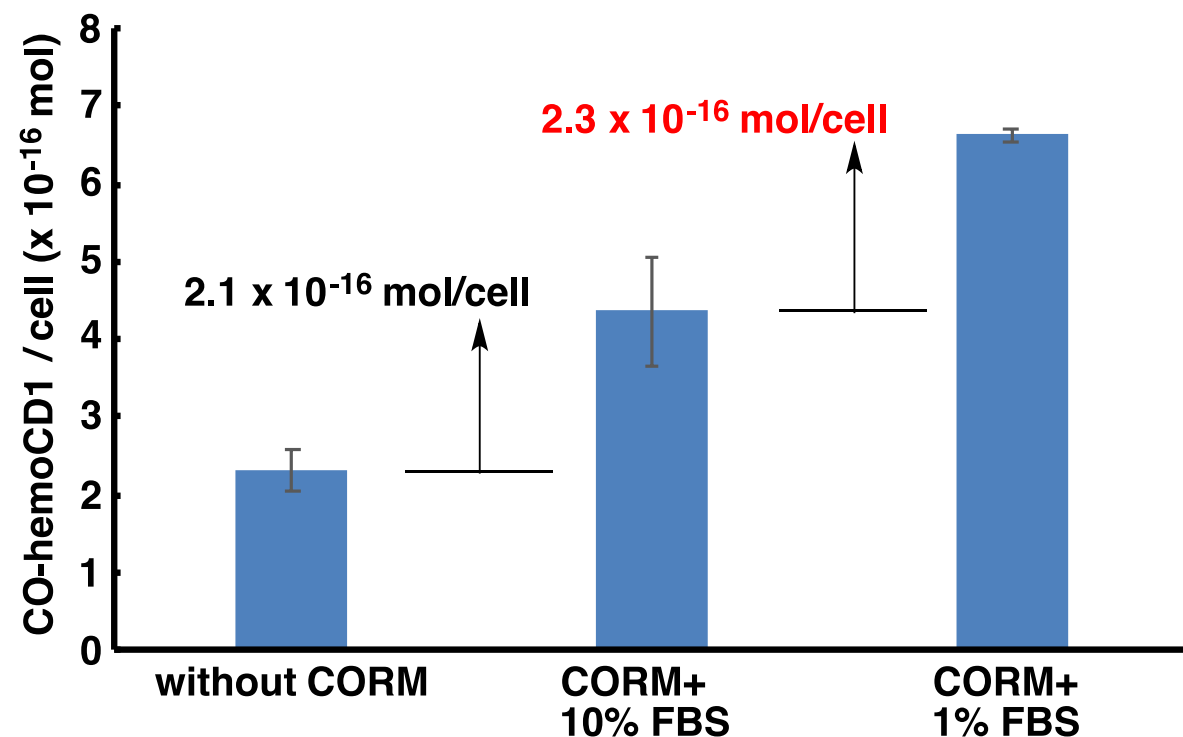
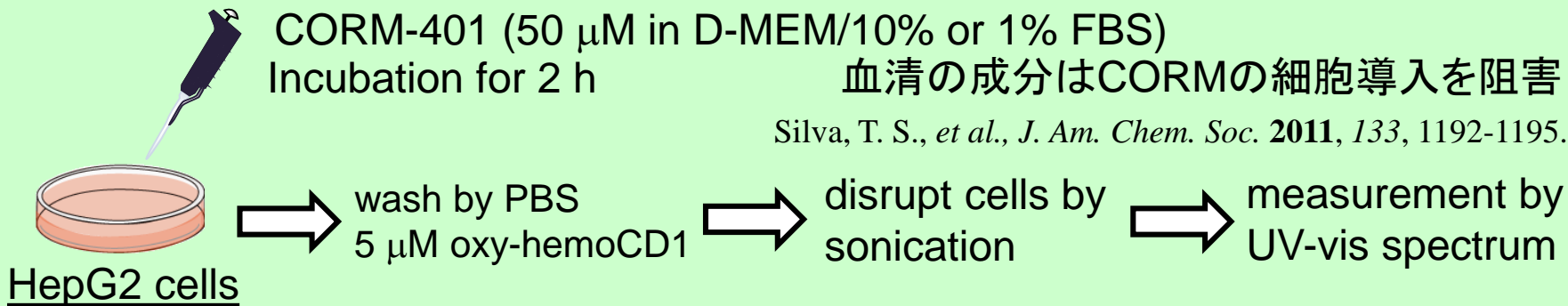
JCI Insight, 3, e123485 (2018).

Cellular uptake of CORM-401

CORM-401 (50 μ M in D-MEM/10% or 1% FBS)
Incubation for 2 h

血清の成分はCORMの細胞導入を阻害

Silva, T. S., *et al.*, *J. Am. Chem. Soc.* **2011**, *133*, 1192-1195.



血清を減らすことで、細胞へのCO送達量が2.1倍増加した

細胞導入率

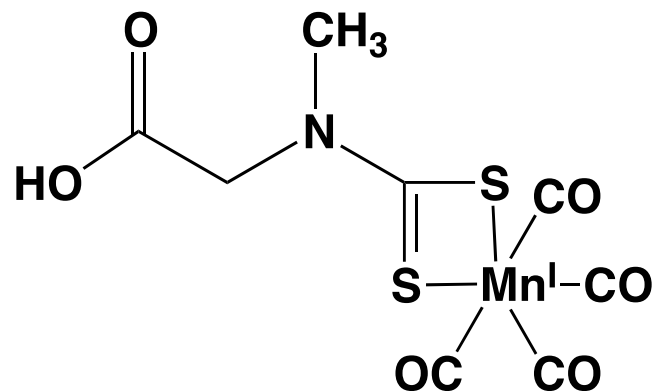
10% FBS : 0.4%

1% FBS : 0.8%

投与量に対して非常に少ない

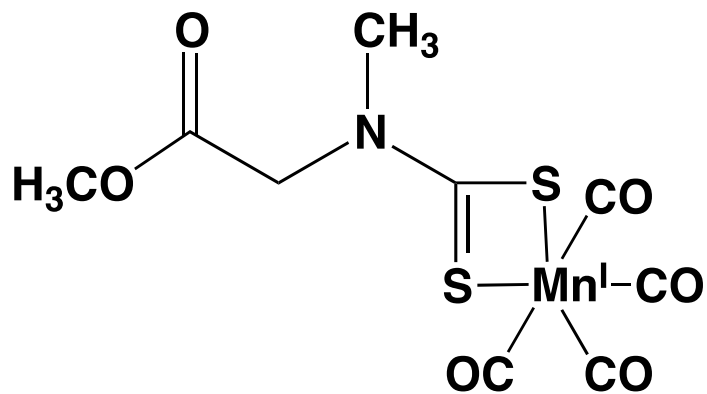
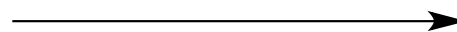
Figure 2. Quantification of endogenous CO in cells after the addition of 50 μ M CORM-401 by hemoCD. Cells were incubated in D-MEM/10% or 1% FBS.

CORMの細胞内取り込みを向上させる

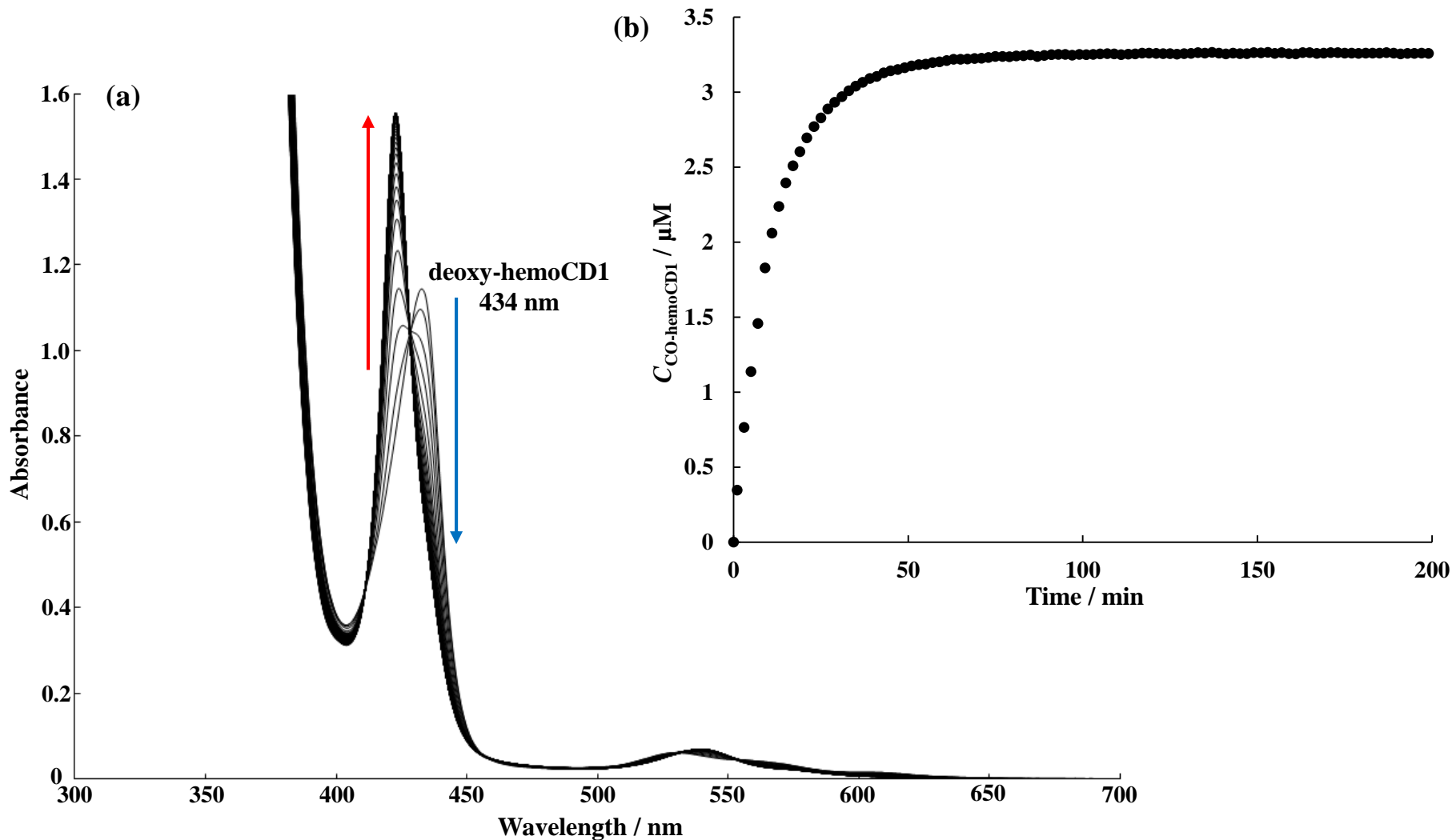


CORM401

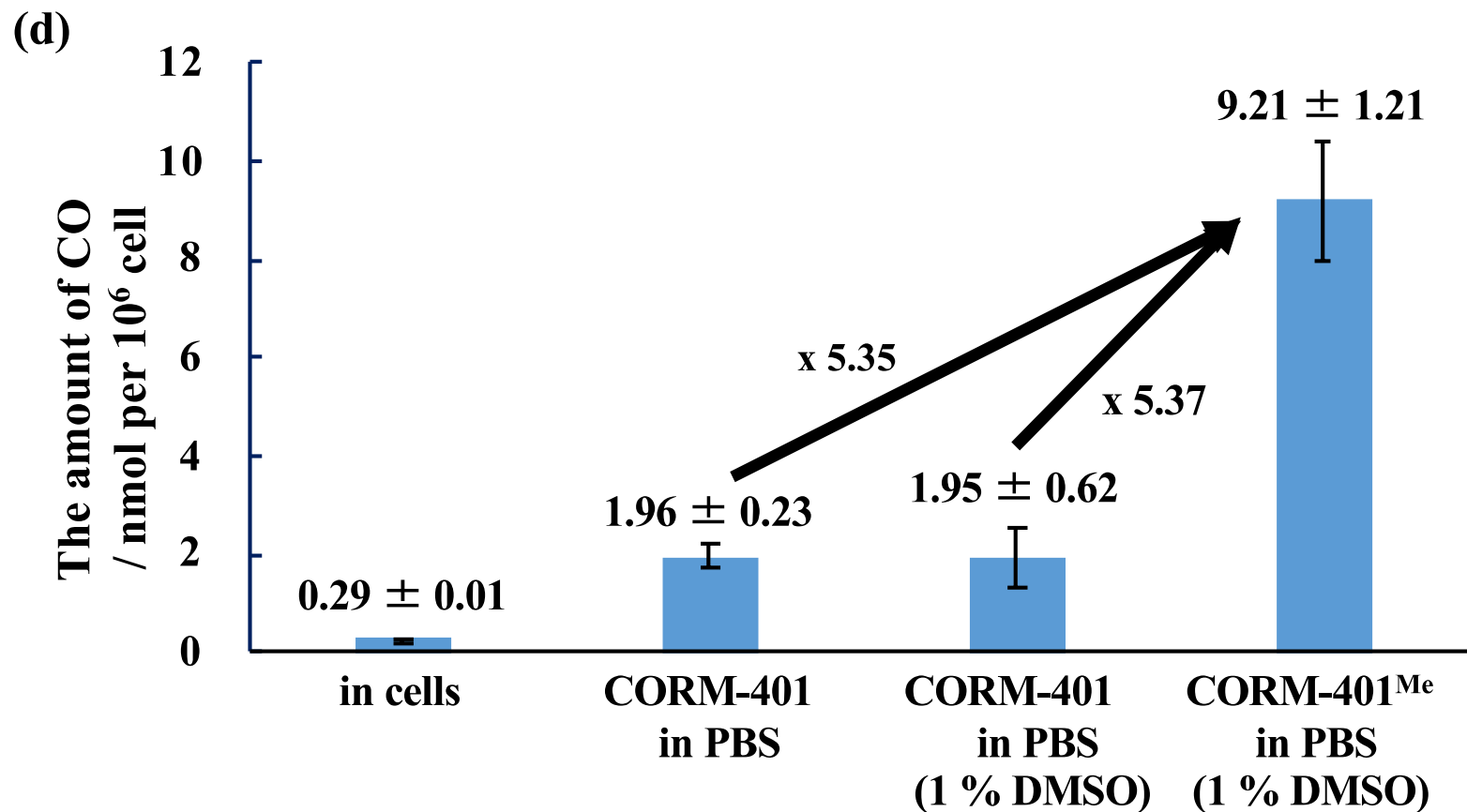
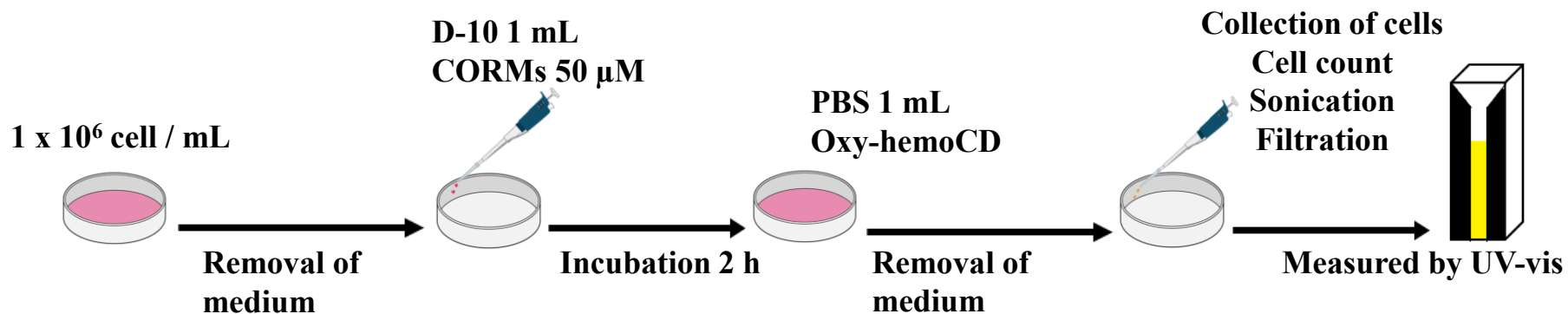
TMS-diazomethane

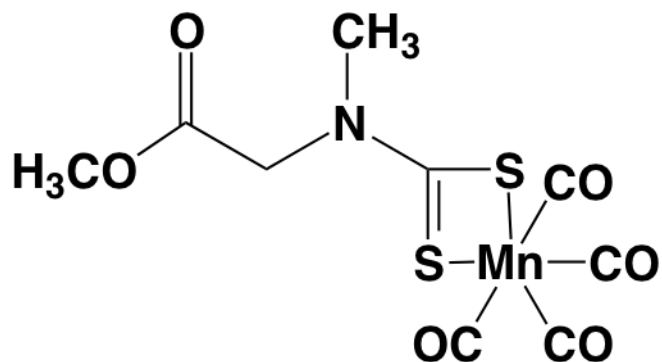


CORM401-E



CORM401-Eも, 3CO/moleculeのCO放出能を示した。





CORM401-E

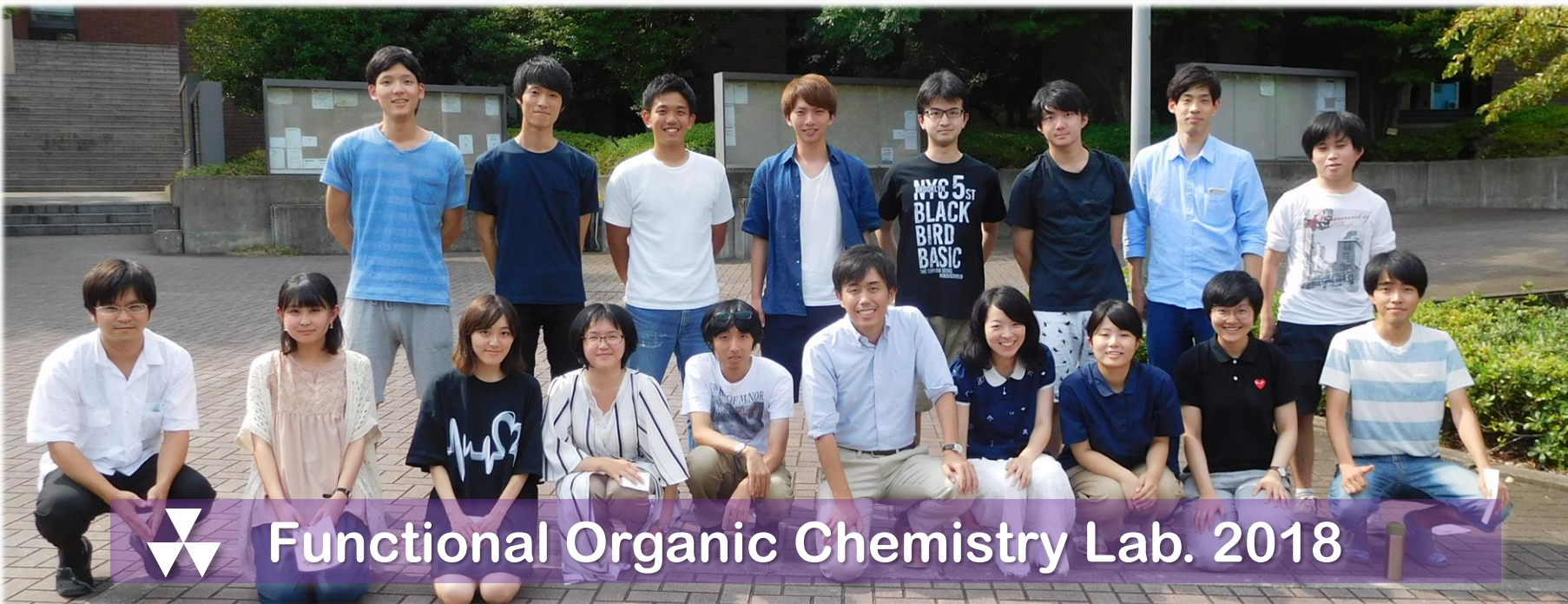
大量合成できるCORM401から、簡便で後処理ほとんどなくメチルエステル化に成功

従来のCORM401よりも5倍以上の高い胞内取り込み効率を示した

COの研究をするための研究用試薬としての需要は高い

北岸宏亮, 高山実花子, R. Motterlini, 特許出願中

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Dr. Roberta Foresti

Prof. Hidetoshi Arima

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Prof. Shigeru Taketani

Prof. Takashi Hayashi

...and more!

Thank you for your kind attention!