Reactive Oxygen Species (ROS) - Development of Innovative Drugs and Analytical Methods -

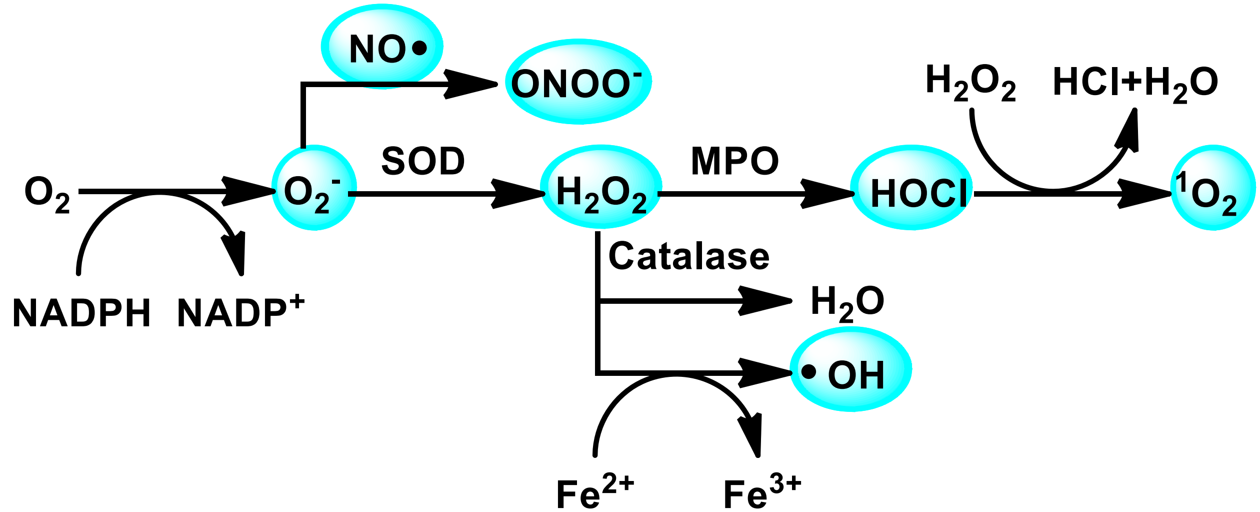
Literature Seminar 2014.9.6

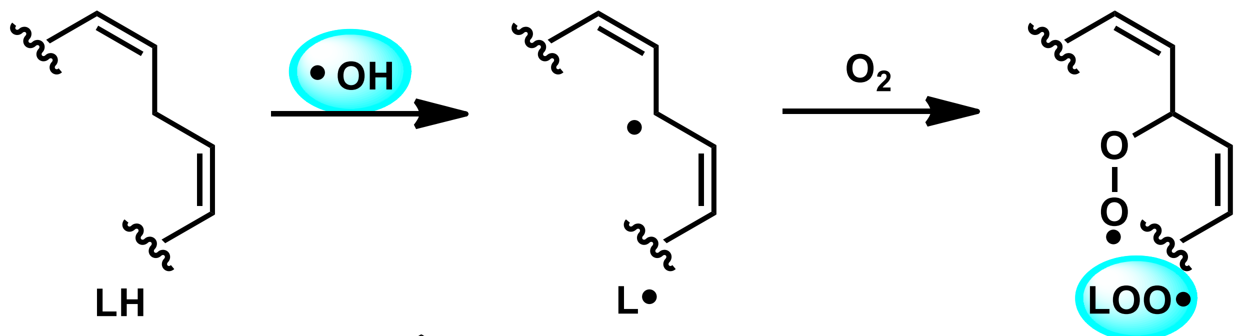
D1 Yohei Seki

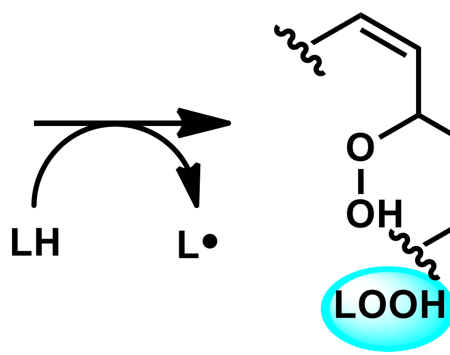
Contents

Contents

1-1. What is a Reactive Oxygen Species (ROS)



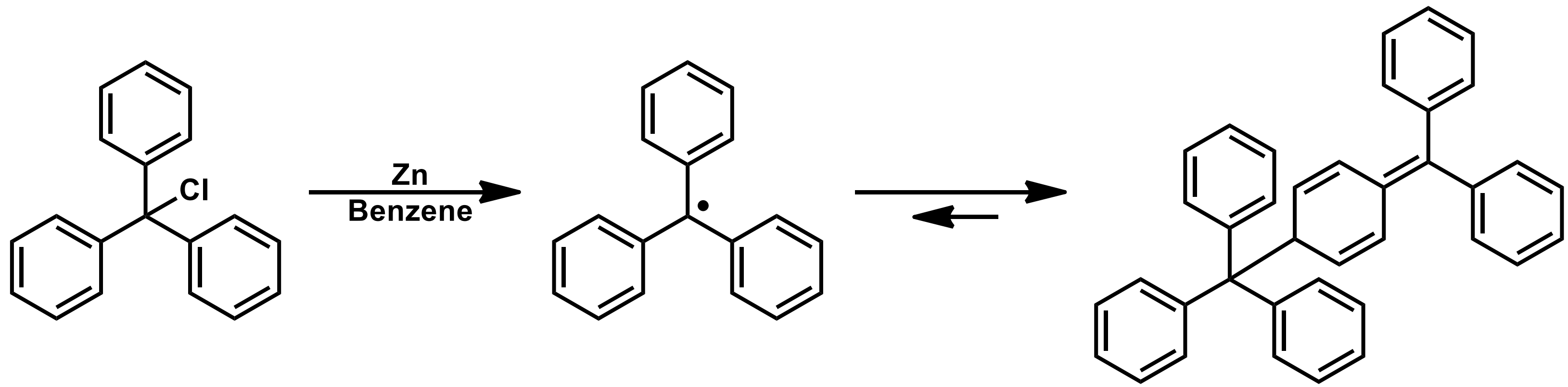




Free radicals
and
non-radical ROS

1-2. History of free radicals

1. Triphenylmethyl radical was discovered in 1900.



Gomberg, M. *J. Am. Chem. Soc.* **1900**, *22*, 757.

2. "Free radical theory" was articulated of aging in 1957 speculating endogenous oxygen radicals were generated in cells and resulted in a pattern of cumulative damage.

Harman, D. *J. Gerontol.* **1957**, *2*, 298.

3. Superoxide dismutase was discovered in 1969.

McCord, J. M.; Fridovich, I. *J. Biol. Chem.* **1969**, *244*, 6049.

1-2. History of free radicals

4. Superoxide was produced from leukocytes in 1973.

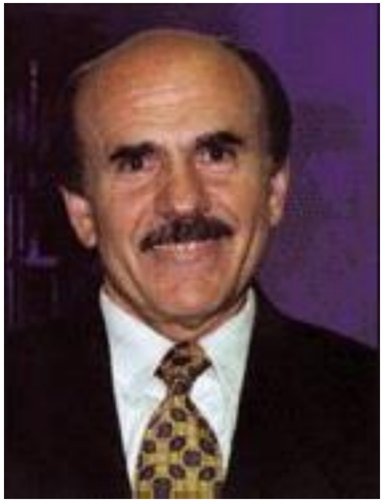
Babior, B. M. *et al*. *J. Clin. Invest.* **1973**, *52*, 741.

5. Endothelium-derived relaxing factor (EDRF) was NO in 1987.

Furchgott, R. F. *et al*. *Nature* **1980**, *288*, 373.

Moncada, S. *et al*. *Nature* **1987**, *327*, 524.

Furchgott, R. F.

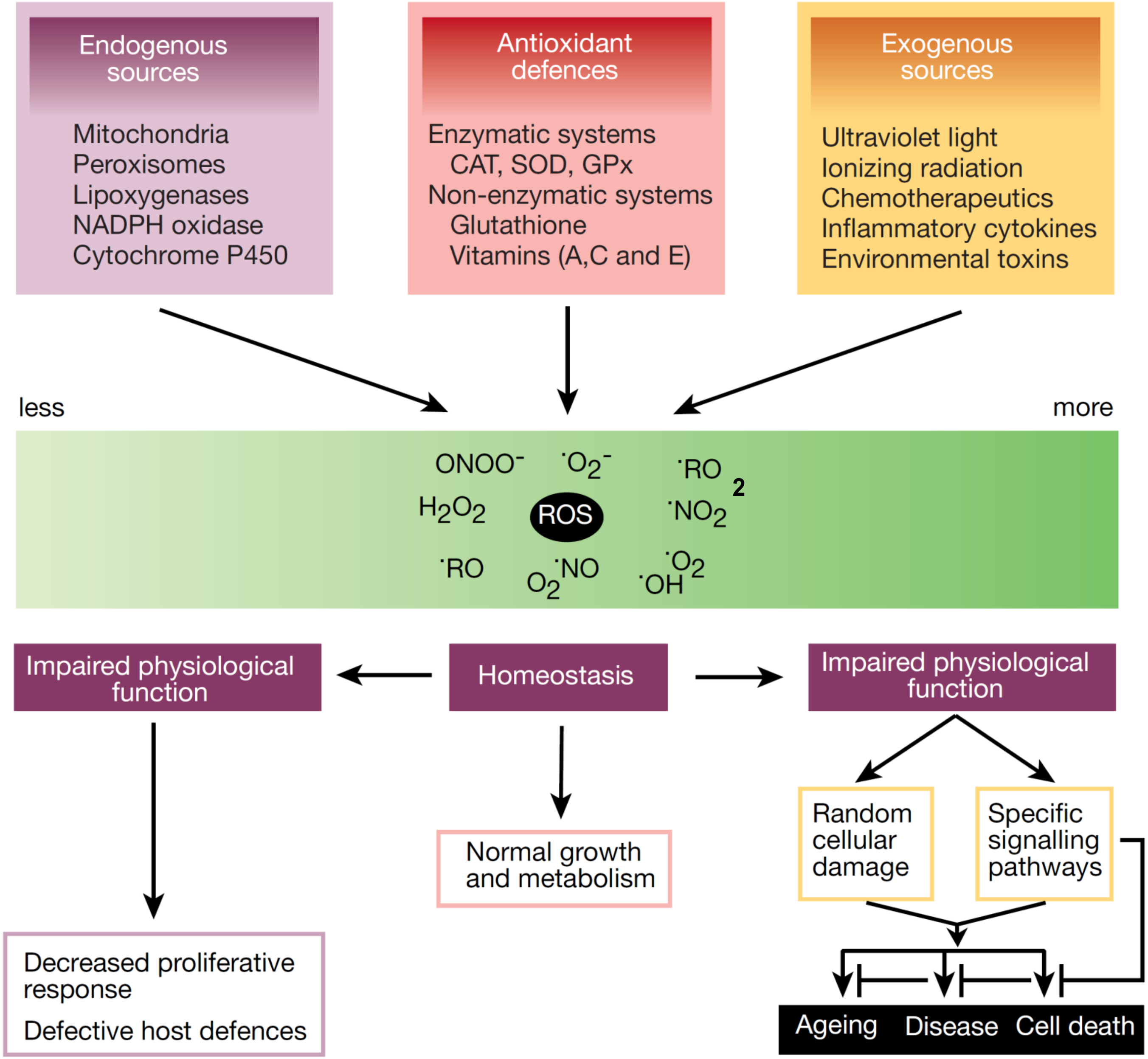


Ignarro, L. J.

Murad, F.

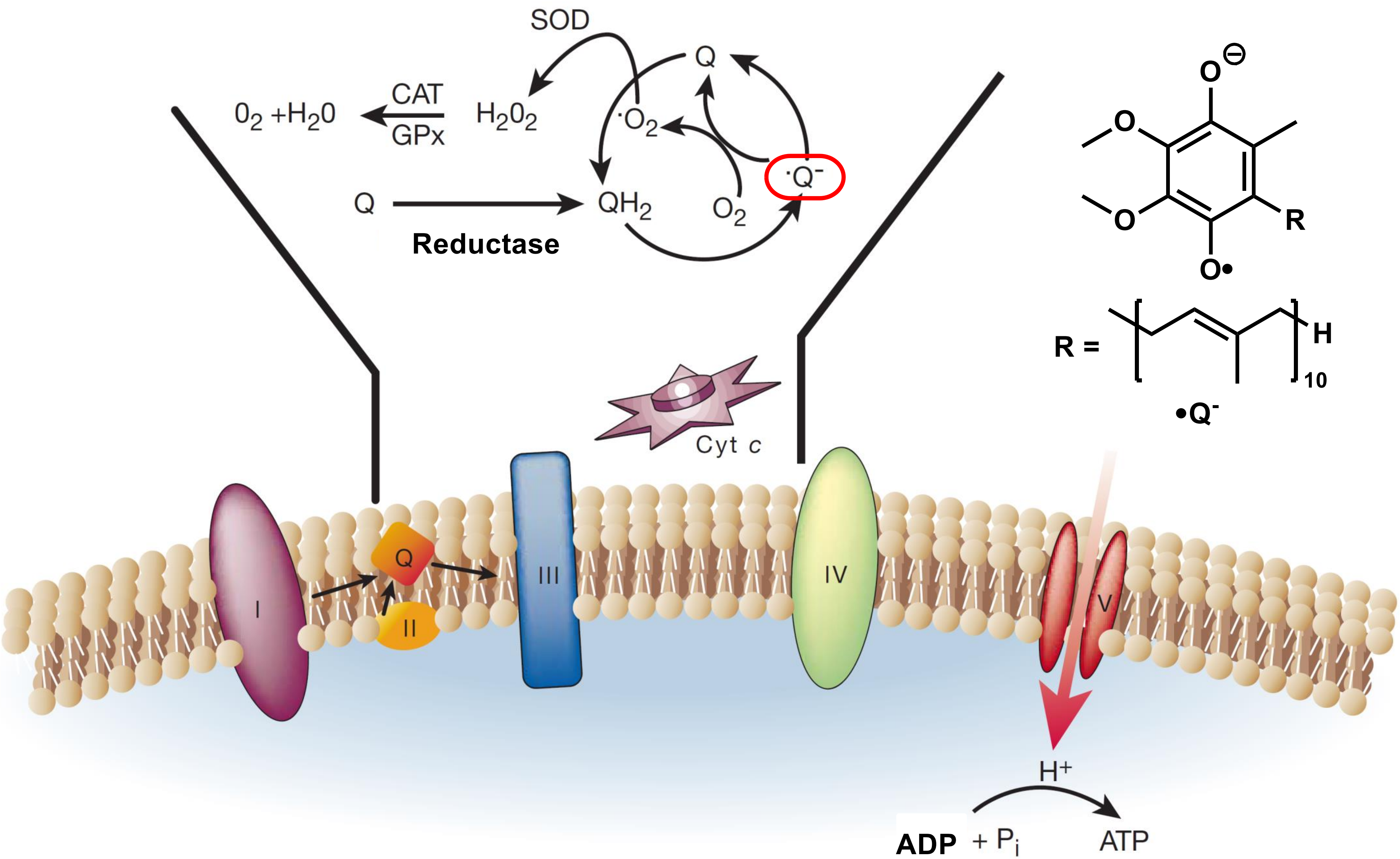
The nobel prize in physiology or medicine in 1998

1-3. The sources and cellular resposes to ROS



Finkel, T. *et al*. *Nature* **2000**, *408*, 239.

1-4. Mitochondrial ROS production

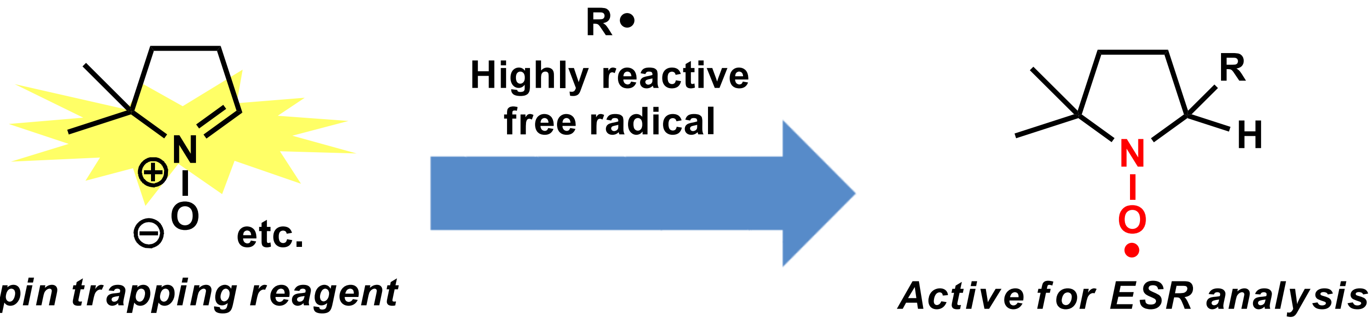


Finkel, T. *et al*. *Nature* **2000**, *408*, 239.

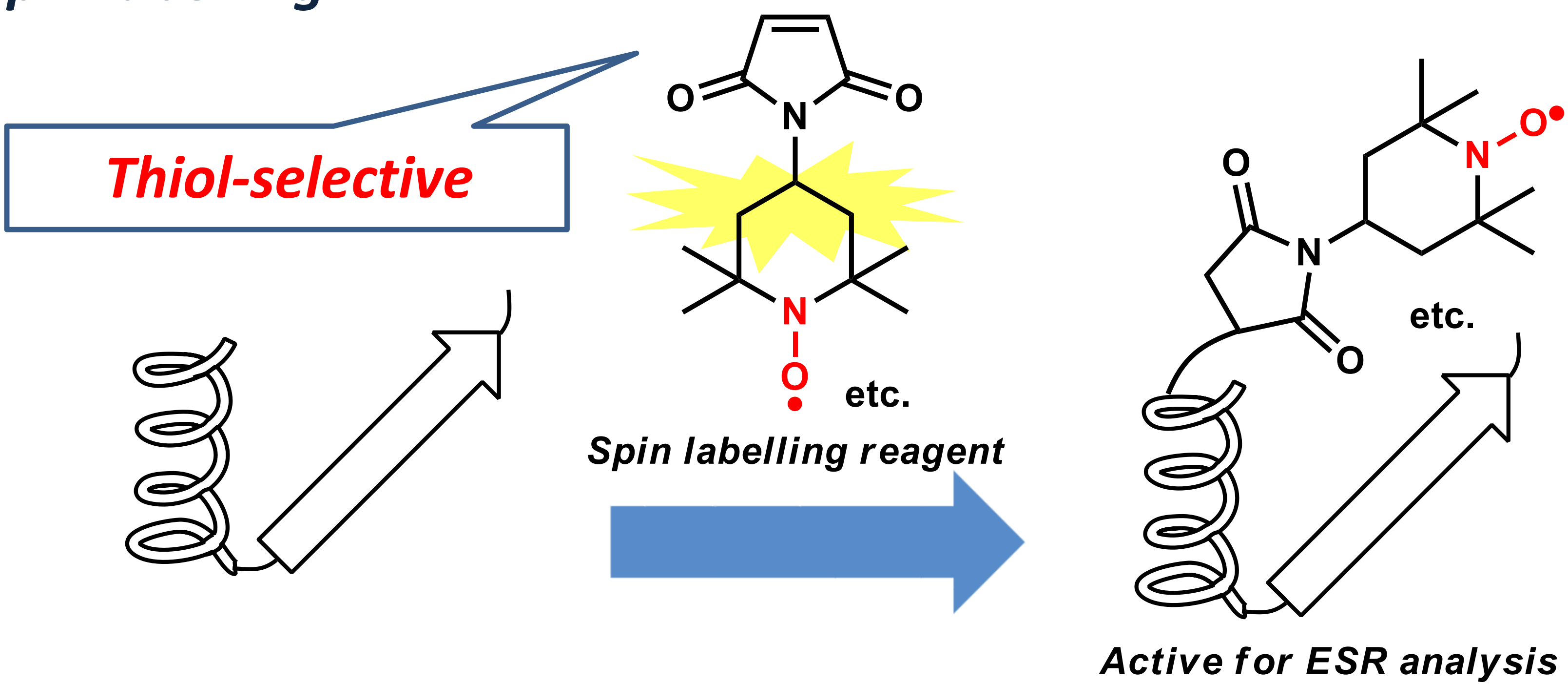
Contents

2-1. Spin trapping and labelling using ESR

Spin trapping



Spin labelling



2-1. Spin trapping and labelling using ESR

東京化成工業株式会社
Moving Your Chemistry Forward

Japan

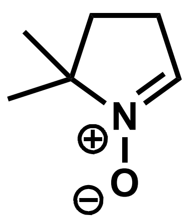
スピントラップ剤 & スピンラベル / ESR Spectrometry

http://www.tcichemicals.com/eshop/ja/jp/category_index/00424/

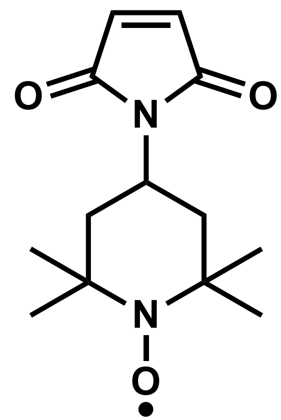
funakoshi
FRONTIERS IN LIFE SCIENCE

HOME > 試薬 > タンパク質／酵素 > タンパク質解析 > スピンラベル試薬

http://www.funakoshi.co.jp/contents/3227



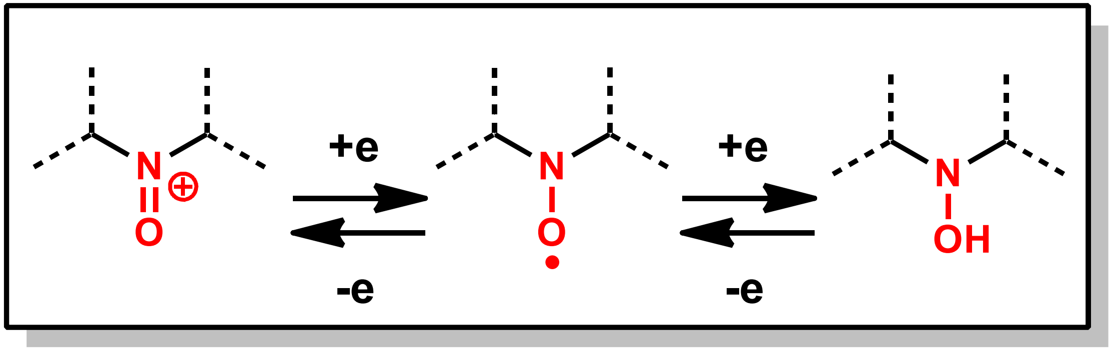
5,5-Dimethyl-1-pyrroline N-Oxide
1 g/21000 yen

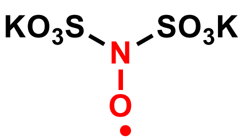


N-(1-Oxyl-2,2,6,6-Tetramethyl-4-Piperidinyl)Maleimide
10 mg/19000 yen

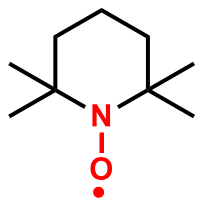
2-2. Redox-sensitive probe

N-oxylradical moiety

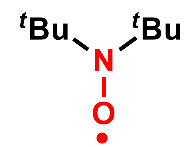




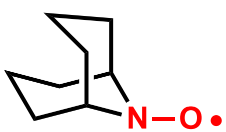
Fremy's salt
(1845, E. Fremy)



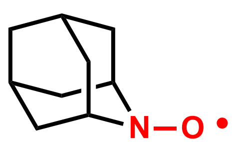
TEMPO
(1960, O. L. Lebeder; S. N. Kazarnovskii)



Di-*tert*-butylnitroxyl radical
(1961, A. K. Hoffmann; A. T. Henderson)



ABNO
(1973, K. U. Ingold)



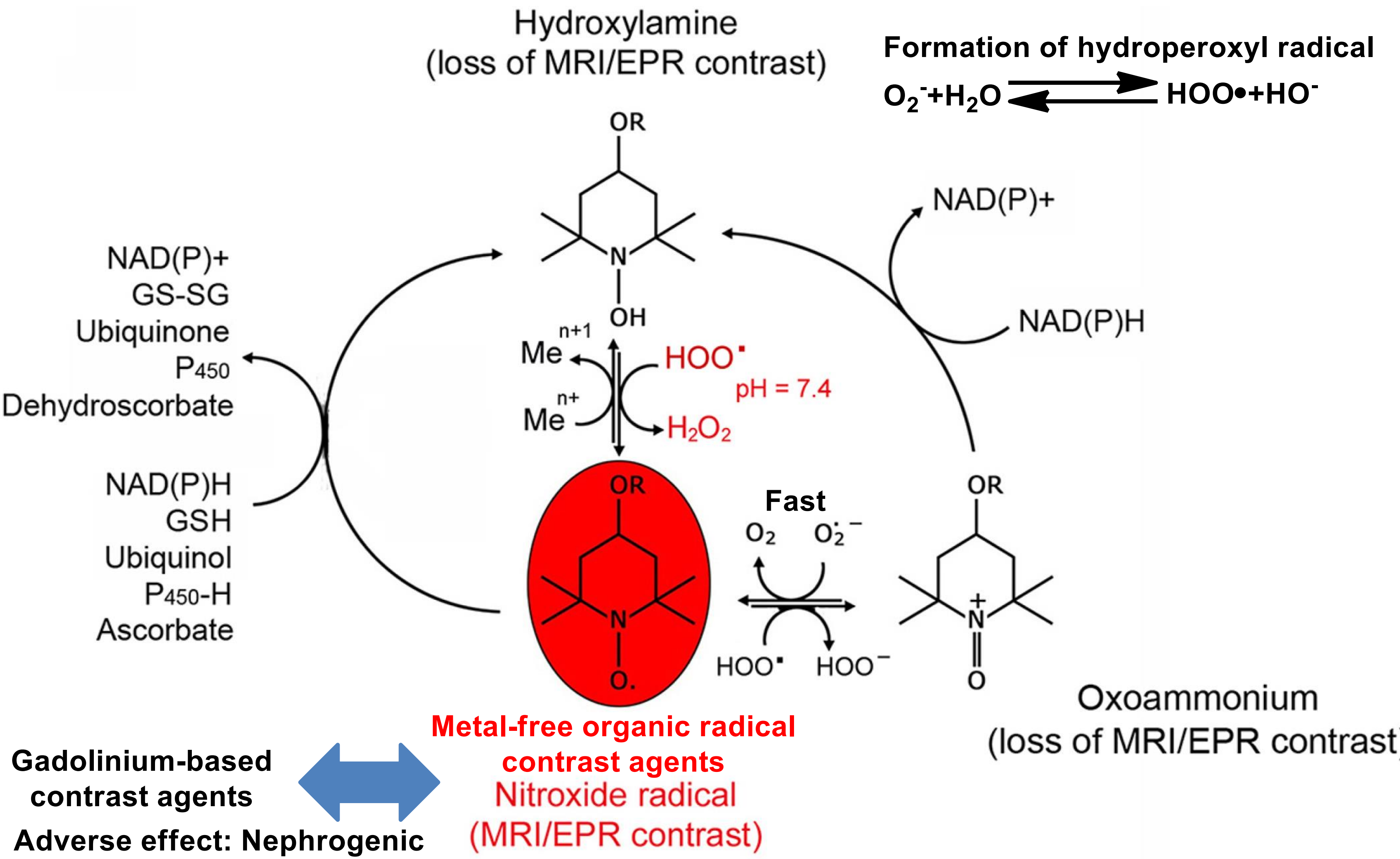
AZADO
(1978, R. M. Dupeyre; A. Rassat)

cf. Dr.Tanaka's literature seminar (5/19/2010)
Mr.Hashizume's literature seminar (7/2/2012)
Seki, Y.; Oisaki, K.; Kanai, M. *Tetrahedron Lett.* **2014**, *55*, 3738.

2-2. Redox-sensitive probe

Formation of hydroperoxyl radical

$$O_2^- + H_2O \rightleftharpoons HOO\bullet + HO^-$$



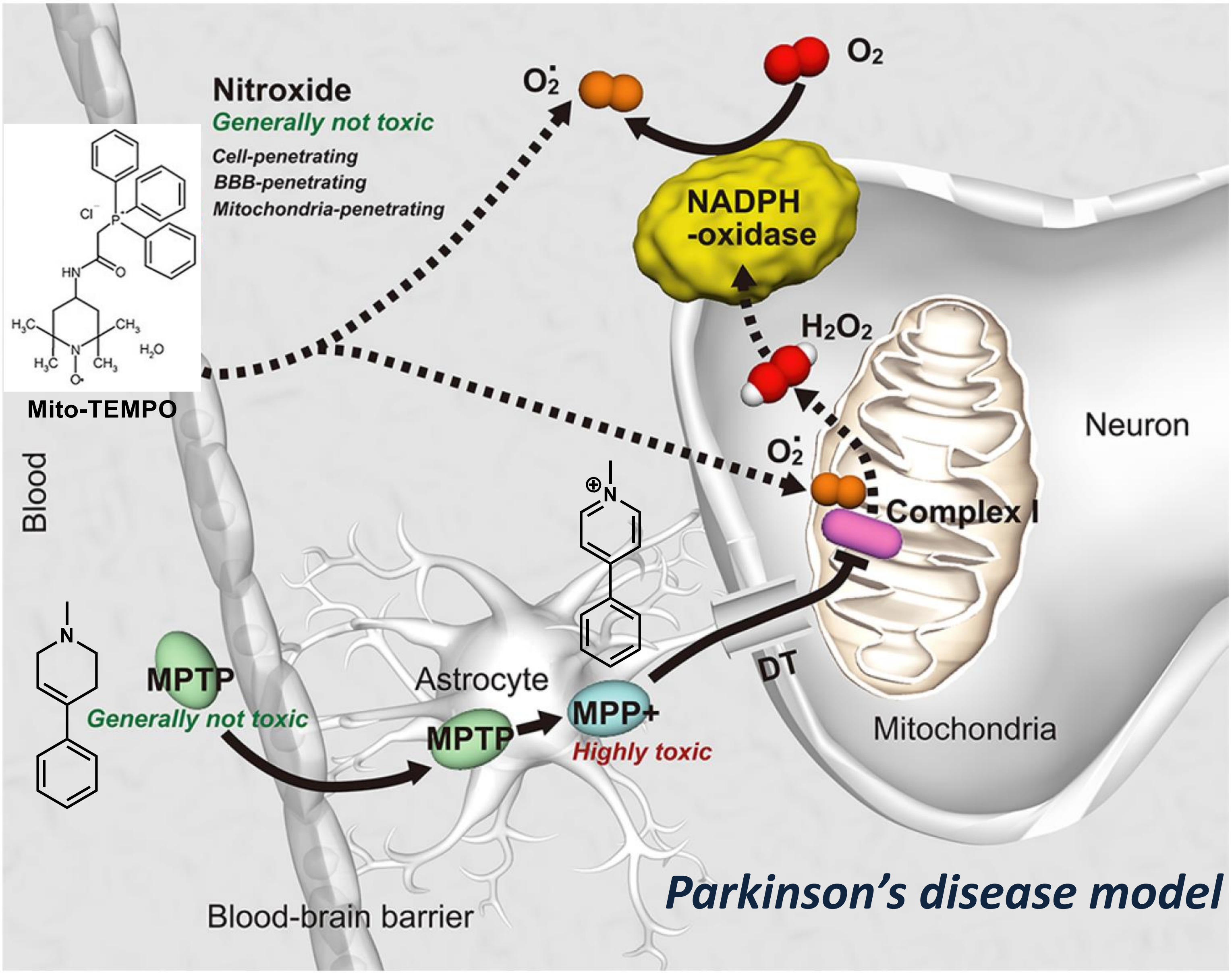
Metal-free organic radical contrast agents

Gadolinium-based contrast agents

Adverse effect: Nephrogenic Systemic Fibrosis (NSF)

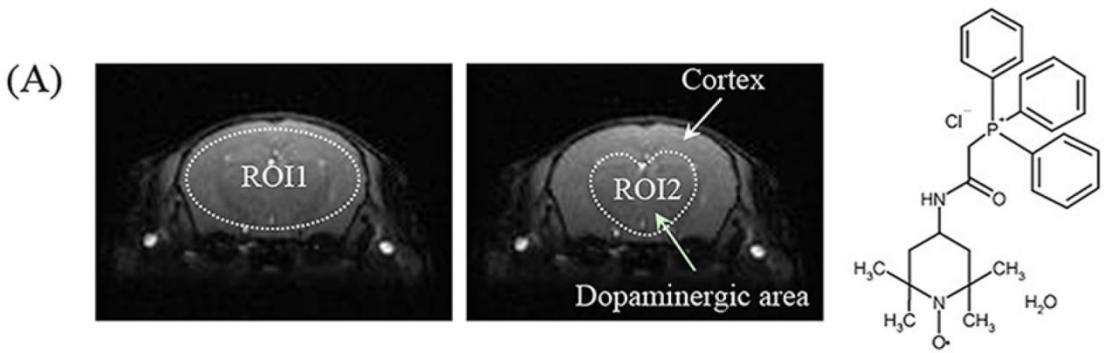
Bakalova, R. *et al*. *ACS Chem. Neurosci.* **2013**, *4*, 1439.

2-2. Redox-sensitive probe

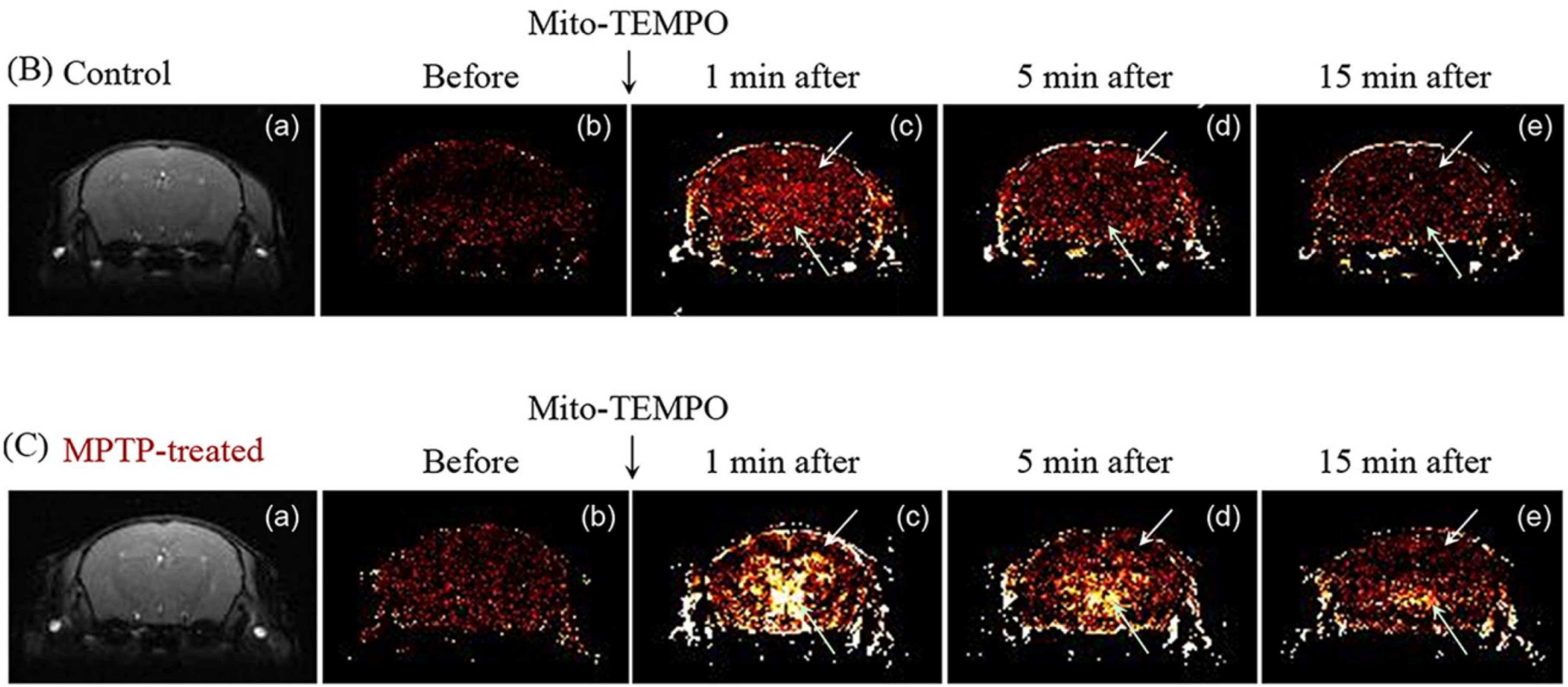


Bakalova, R. *et al*. *ACS Chem. Neurosci*. **2013**, *4*, 1439.

2-2. Redox-sensitive probe



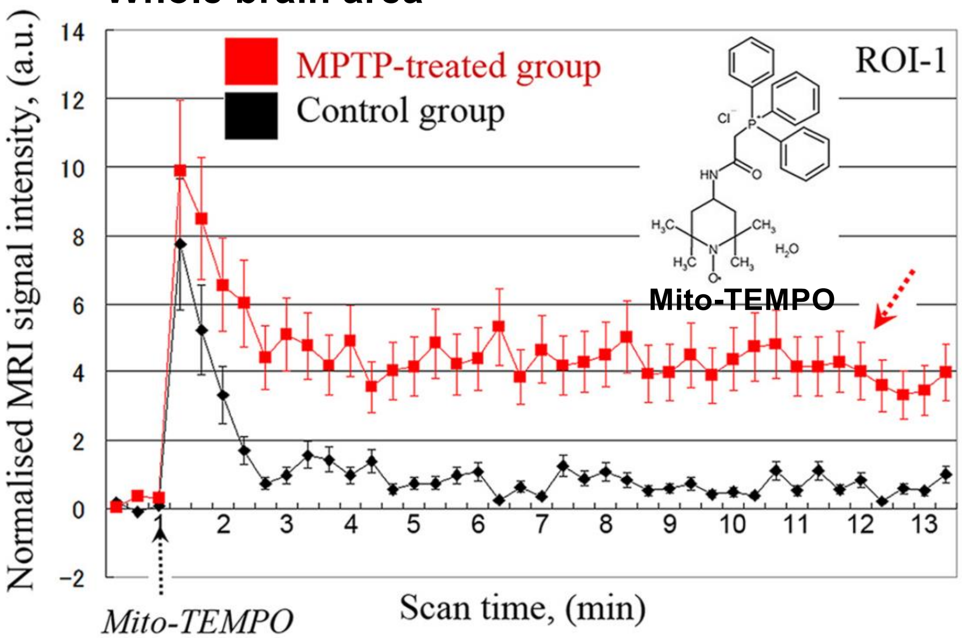
N-oxylradical-enhanced MRI



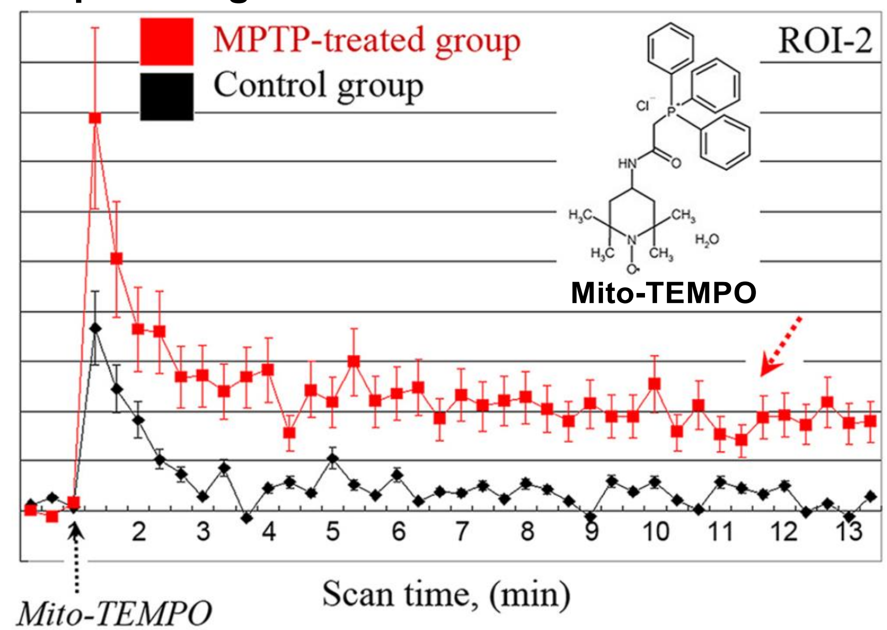
Bakalova, R. *et al*. *ACS Chem. Neurosci*. **2013**, *4*, 1439.

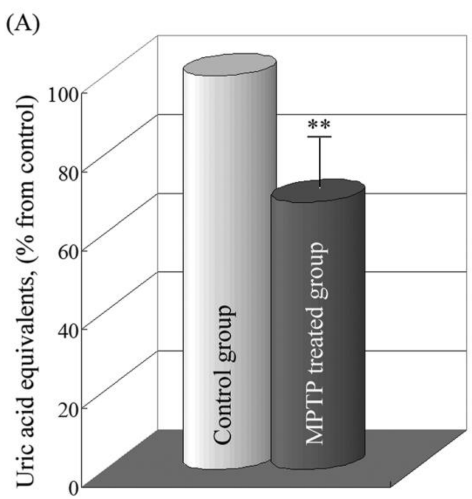
2-2. Redox-sensitive probe

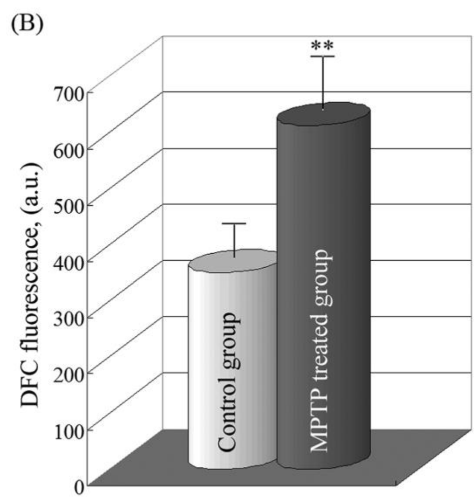
Whole brain area



Dopaminergic area







ROS marker: DCF

A: Total antioxidant capacity

B: ROS level

Bakalova, R. *et al*. *ACS Chem. Neurosci*. **2013**, *4*, 1439.

2-2. Redox-sensitive probe

1. Fast reduction and 2. low r_1 of N-oxylradical were problematic.

Modification of N-oxylradical

Signal intensity formula of SE method

$S = f(v)\ p\ (1-\exp(-Tr/T_1))\ \exp(-Te/T_2)\ \exp(-bD)$

T_1: 縦緩和時間

r_1 and SBM formula

$$r_1[\mathrm{Gd}] = \frac{1}{(T_1)_{\text{inner sphere}}} + \frac{1}{(T_1)_{\text{outer sphere}}}$$

$$\frac{1}{(T_1)_{\text{inner sphere}}} = \frac{P_m q}{(T_{1\mathrm{m}} + \tau_\mathrm{m})}$$

$$\frac{1}{T_{1\mathrm{m}}} = \frac{2}{15}\frac{\gamma_\mathrm{H}^2 g_\mathrm{e}^2 \mu_\mathrm{B}^2 S(S+1)}{r_{\mathrm{GdH}}^6}\left[\frac{7\tau_{\mathrm{c}2}}{1+\omega_S^2\tau_{\mathrm{c}2}^2} + \frac{3\tau_{\mathrm{c}1}}{1+\omega_\mathrm{H}^2\tau_{\mathrm{c}1}^2}\right]$$

$$\frac{1}{\tau_{\mathrm{c}i}} = \frac{1}{\tau_\mathrm{m}} + \frac{1}{\tau_\mathrm{R}} + \frac{1}{T_{ie}};\ i=1,2$$

r_1: 造影剤分子の緩和能

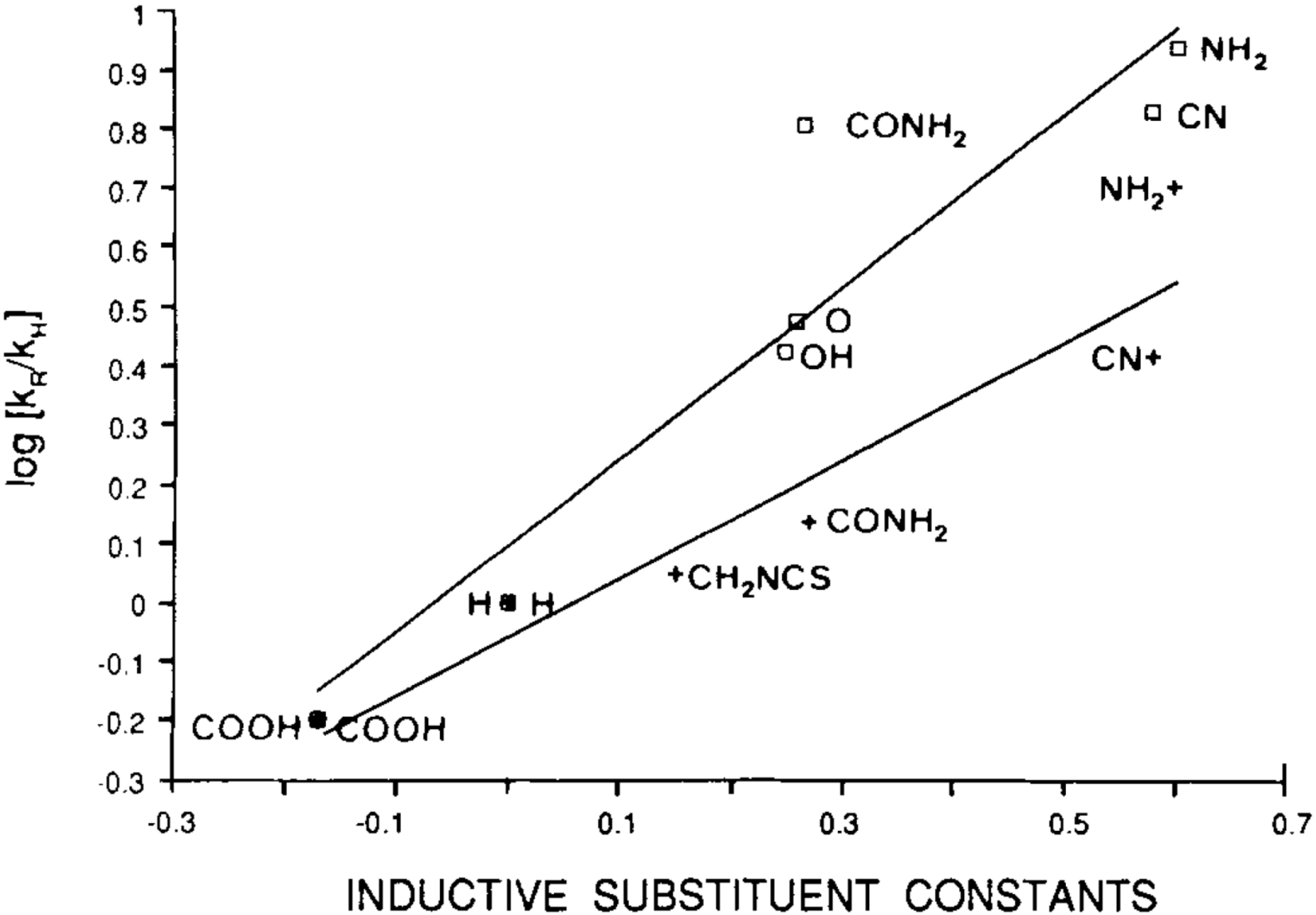
τ_R: 造影剤分子の回転速度

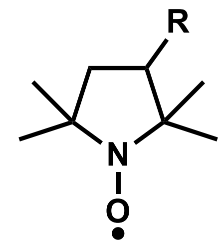
r_1↑, T_1↓, and τ_R↓

Caravan, P. *Chem. Soc. Rev.*, **2006**, *35*, 512.

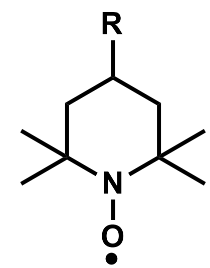
2-2. Redox-sensitive probe

Modification of N-oxylradicals for long in vivo lifetime
Inductive effects on rates of reduction of ascorbate





5: +



6: □

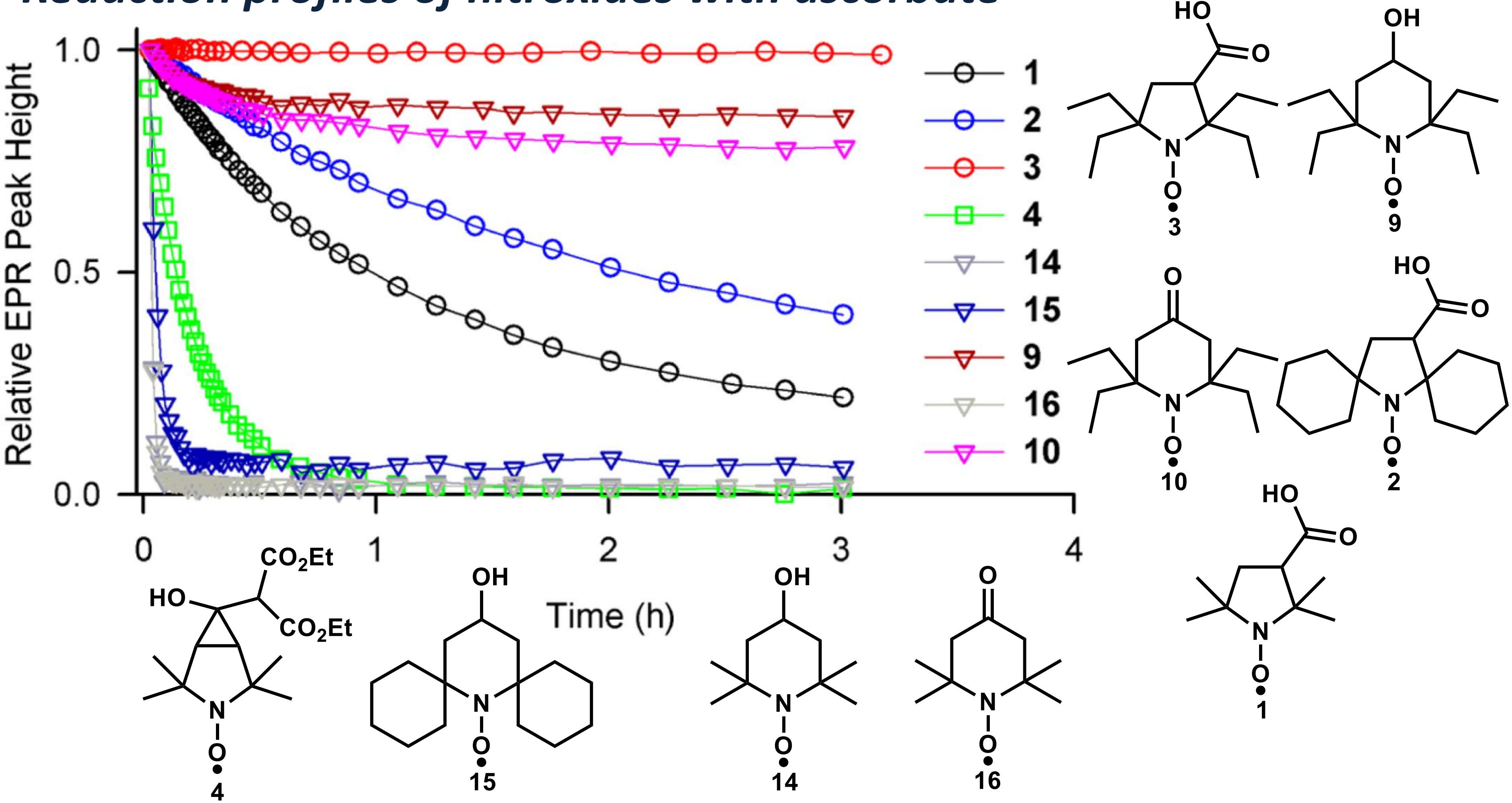
Six-membered N-oxylradical > Five-membered N-oxylradical

Morris, S, Swartz, H. M. *et al*., *J. Pharm. Sci.* **1991**, *80*, 149.

2-2. Redox-sensitive probe

Modification of N-oxylradicals for long in vivo lifetime

Reduction profiles of nitroxides with ascorbate

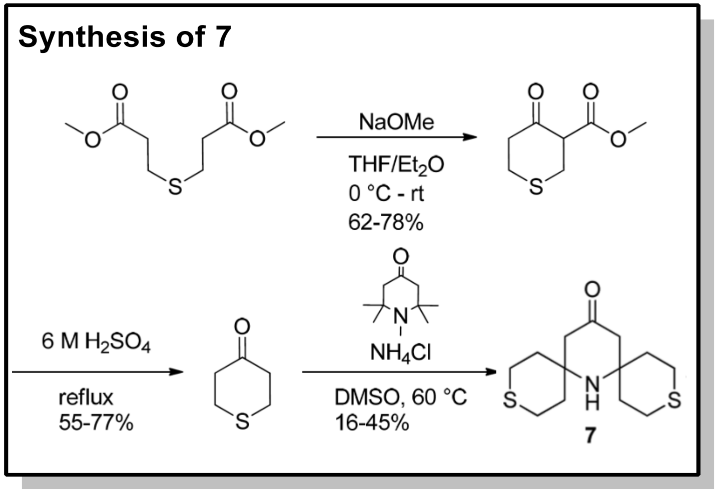


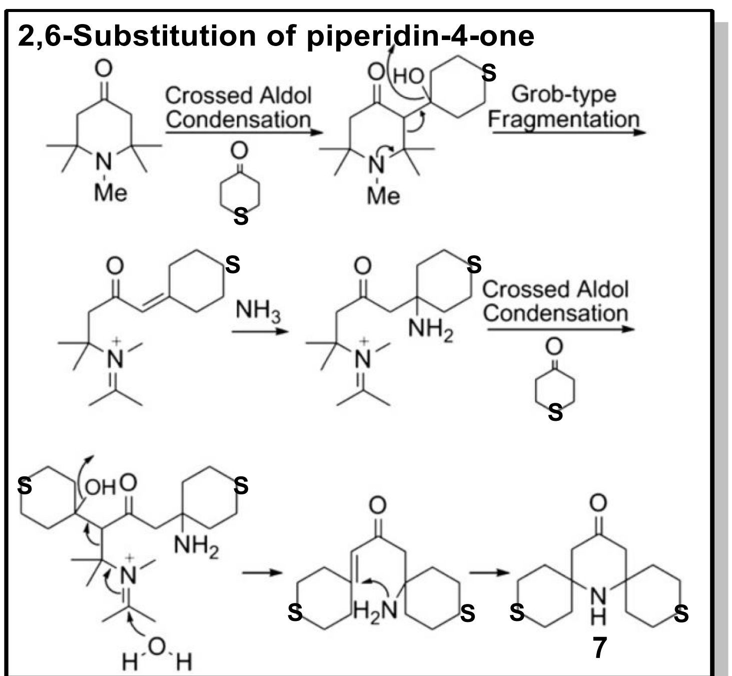
Sterically shielded pyrrolidine N-oxylradical 3 was slowest.

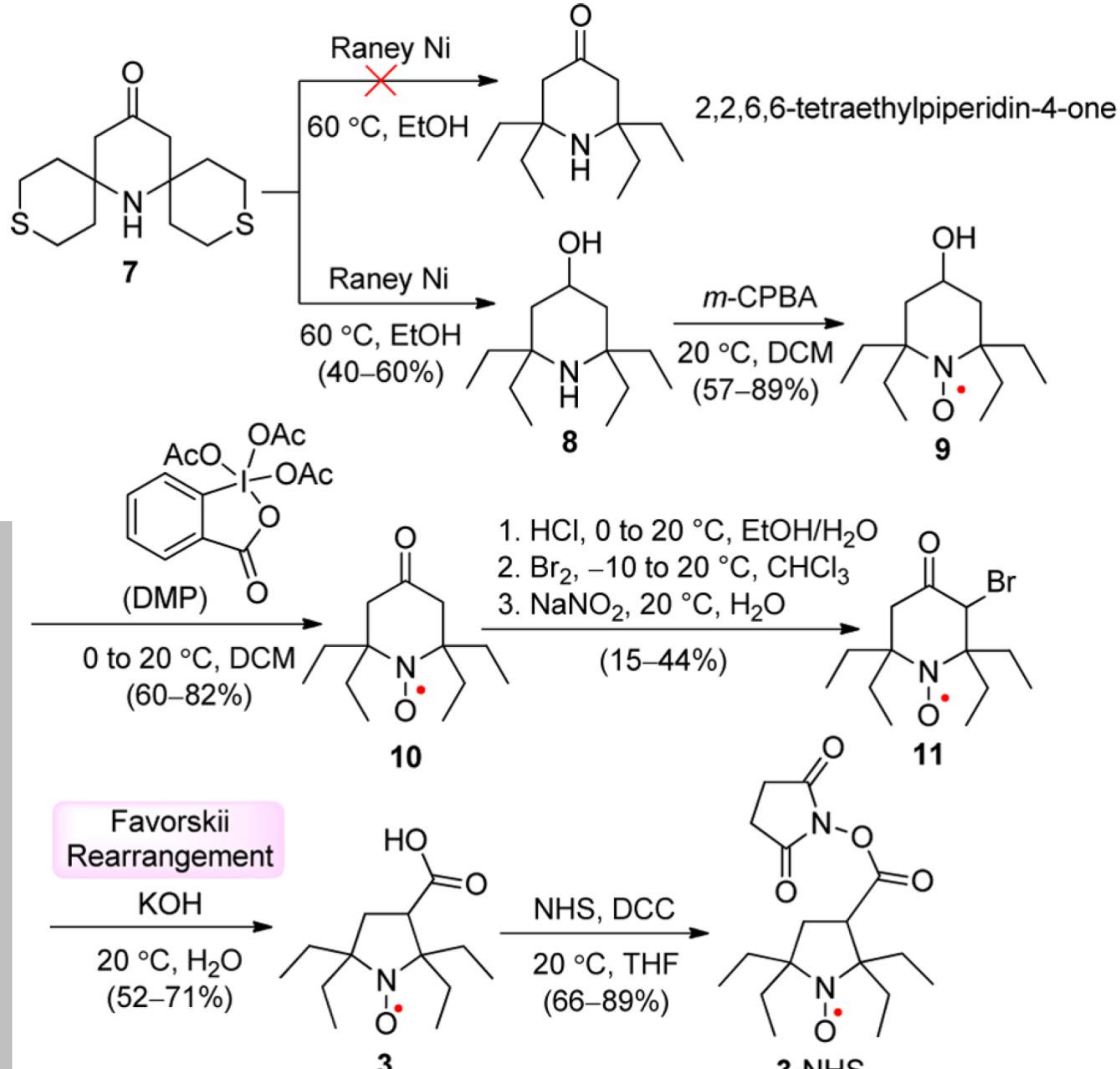
Rajca, A. *et al*., *Org. Lett.* **2012**, *14*, 5322.

2-2. Redox-sensitive probe

Synthesis of sterically shielded pyrrolidine N-oxylradical 3



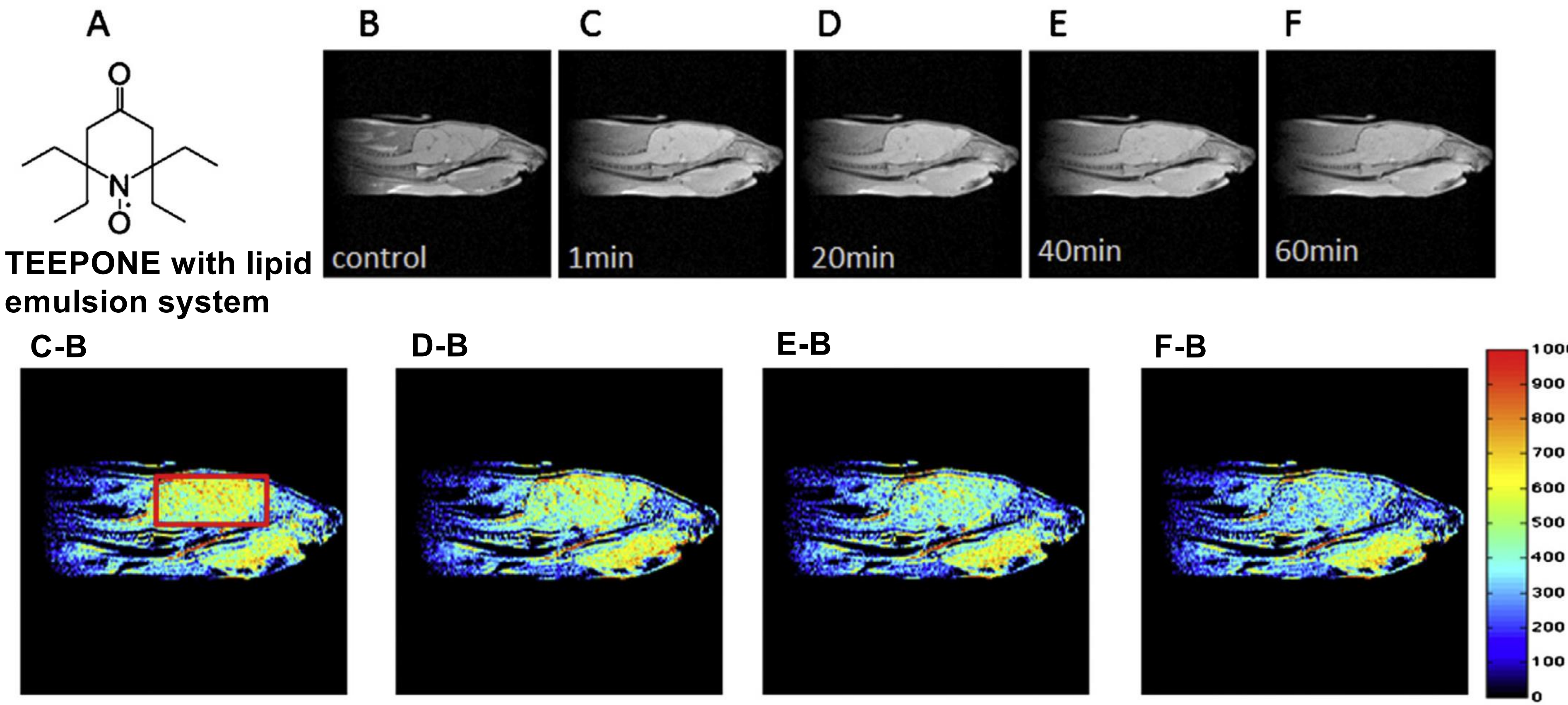




Utsumi, H. *et al.*, *Tetrahedron* **2010**, *66*, 2311.
Rajca, A. *et al.*, *Org. Lett.* **2012**, *14*, 5322.

2-2. Redox-sensitive probe

N-oxylradical-enhanced MRI

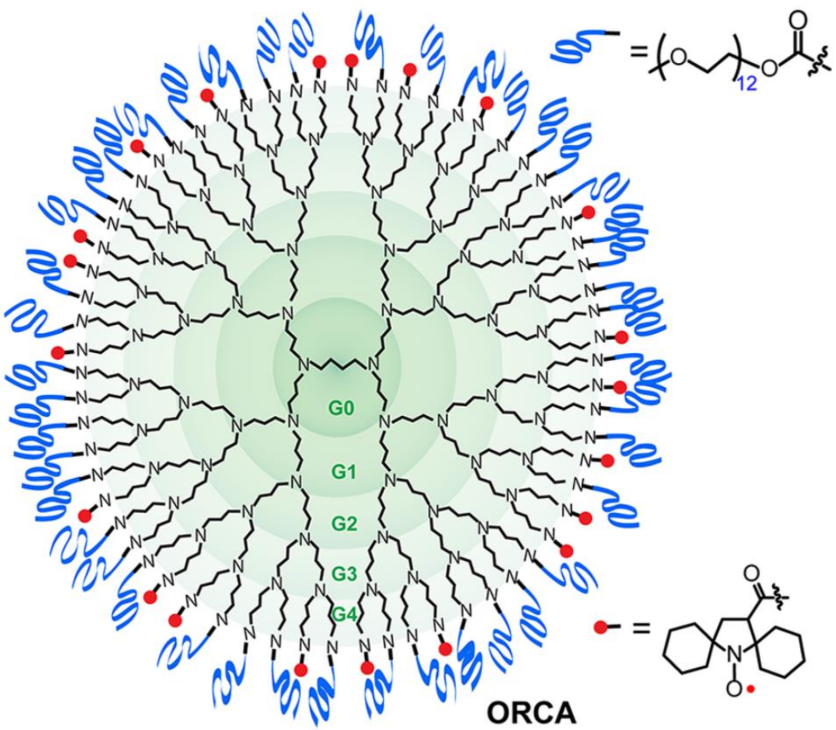


TEEPONE was found to have high resistivity against bio-reduction.

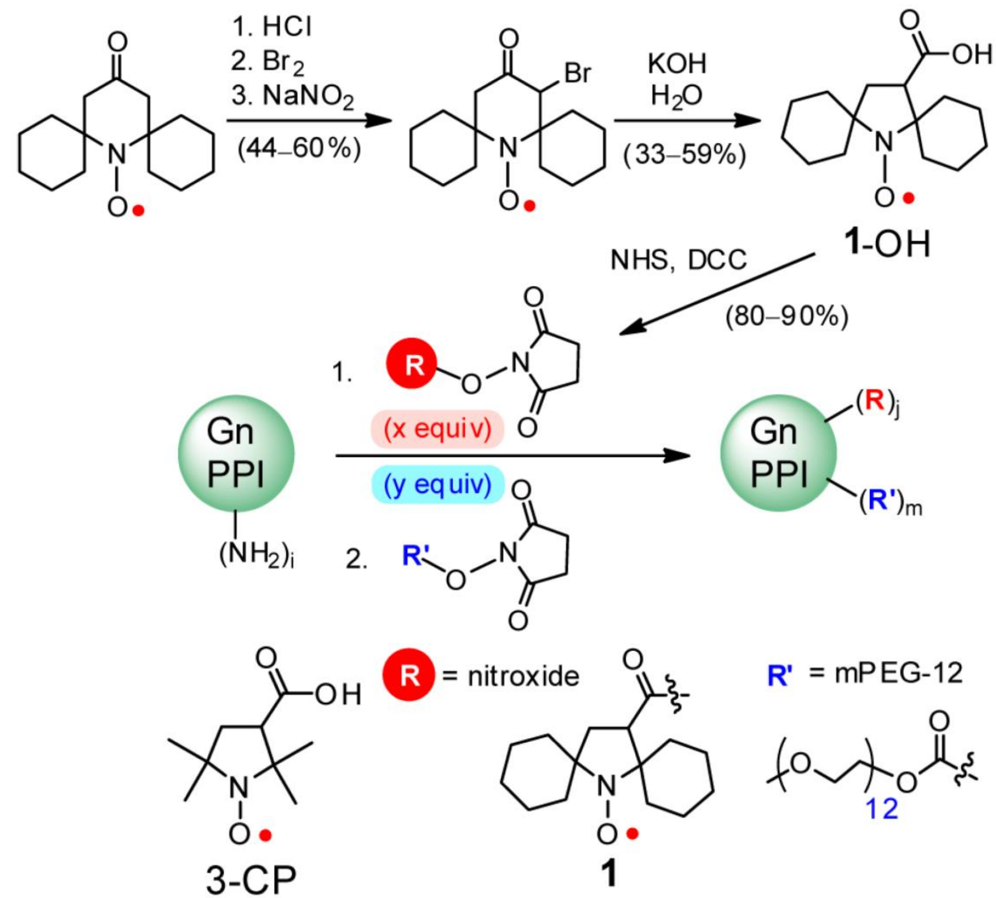
Fujii, H. G. *et al.*, *Neurosci. Lett.* **2013**, *546*, 11.

2-2. Redox-sensitive probe

Modification of N-oxylradicals for long in vivo lifetime and high r₁



^{1}H water relaxiviy = ca. 5 $mM^{-1}s^{-1}$
(3-CP's one = ca. 0.14 $mM^{-1}s^{-1}$)

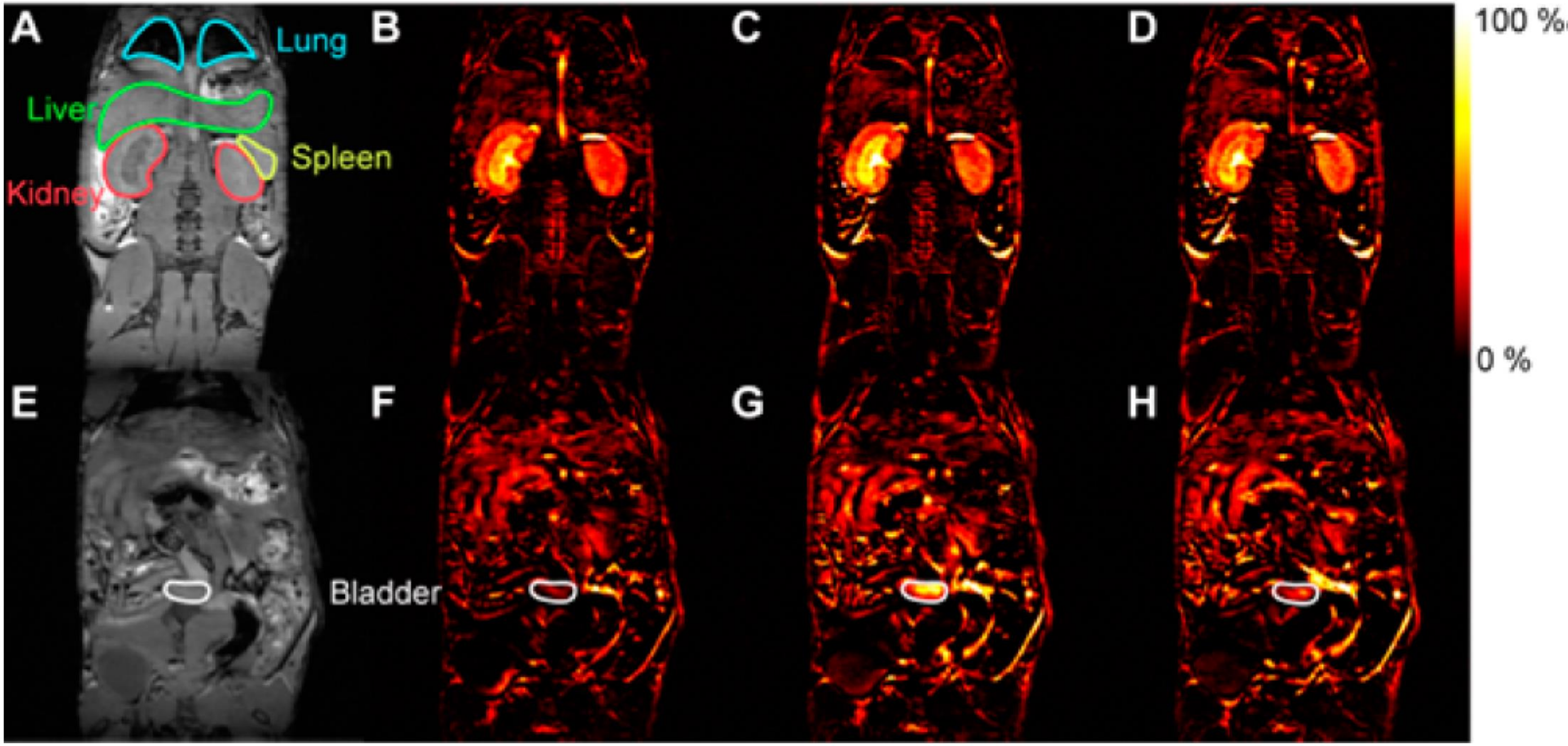


Spirocyclohexyl N-oxylradicals and PEG chains conjugated to dendrimer scaffold were effective.

Rajca, A. *et al*., *J. Am. Chem. Soc.* **2012**, *134*, 15724.

2-2. Redox-sensitive probe

N-oxylradical-enhanced MRI



Before injection **0-30 min** **30-60 min** **60-90 min**

Spirocyclohexyl N-oxylradicals and PEG chains conjugated to dendrimer scaffold enhanced MRI in mice for over 1 h.

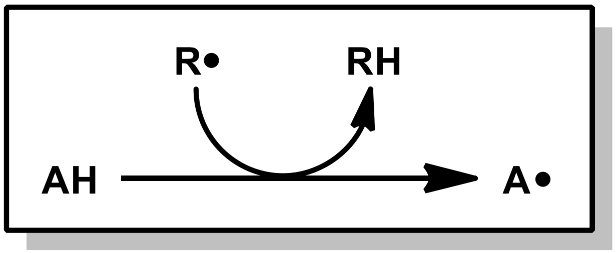
Rajca, A. *et al.*, *J. Am. Chem. Soc.* **2012**, *134*, 15724.

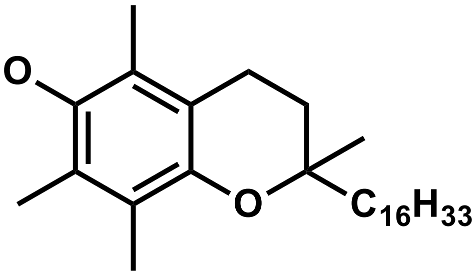
Contents

3-1. What is an antioxidant

Native antioxidants

Mechanism 1

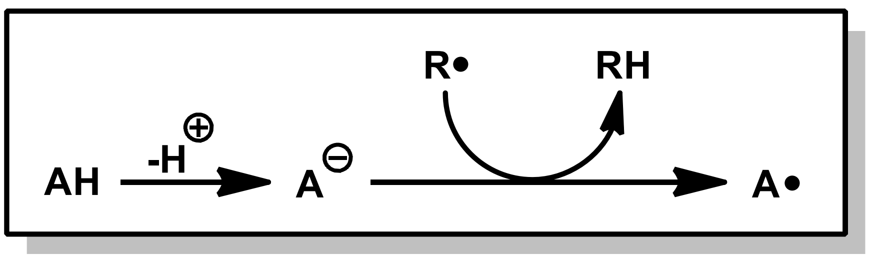


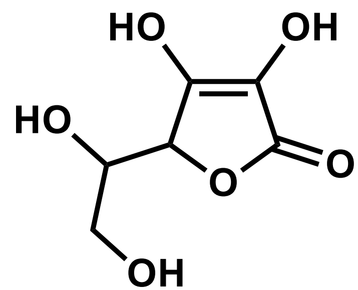


α-Tocopherol

etc.

Mechanism 2

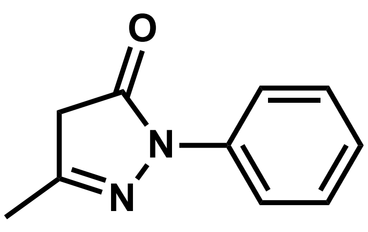




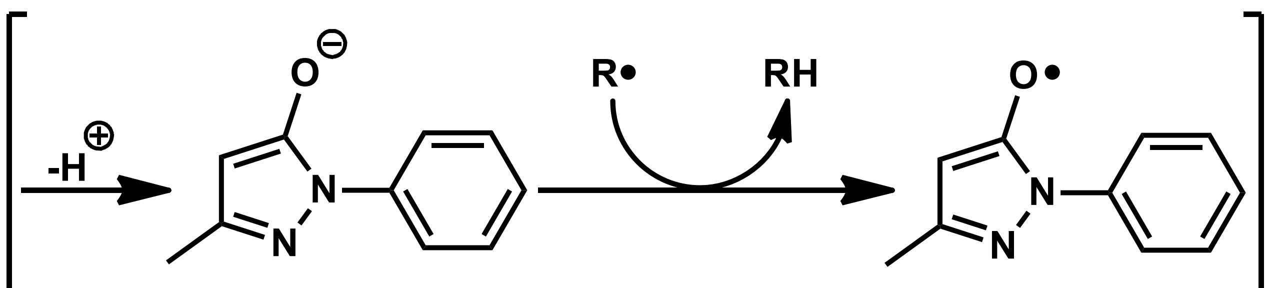
Ascorbic acid

etc.

Sole drug which has antioxidant activity as its main effect

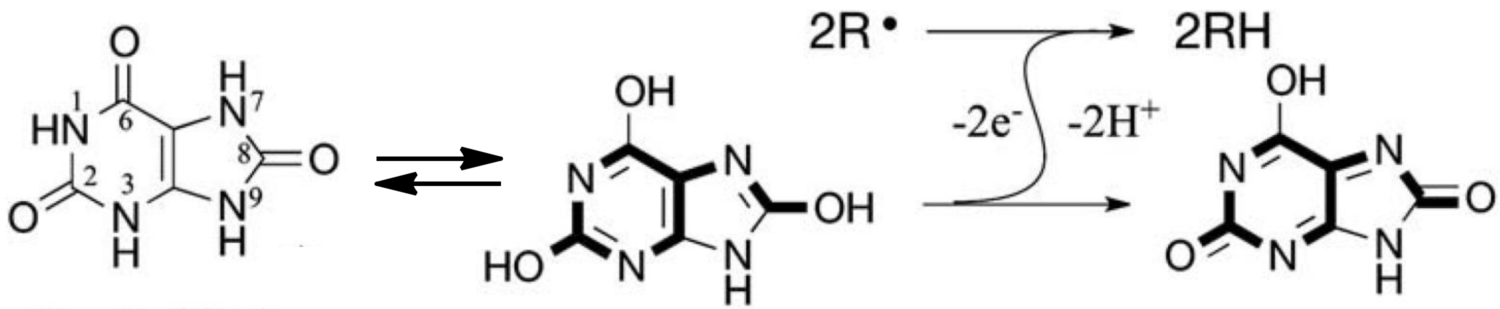


Edaravone



3-1. What is an antioxidant

Proposed radical scavenging pathway of uric acid

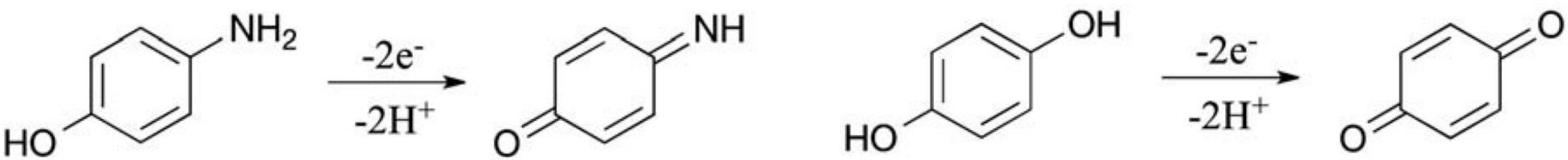


uric acid (UA)

low solubility

↓

hyperuricemia
gouty arthritis



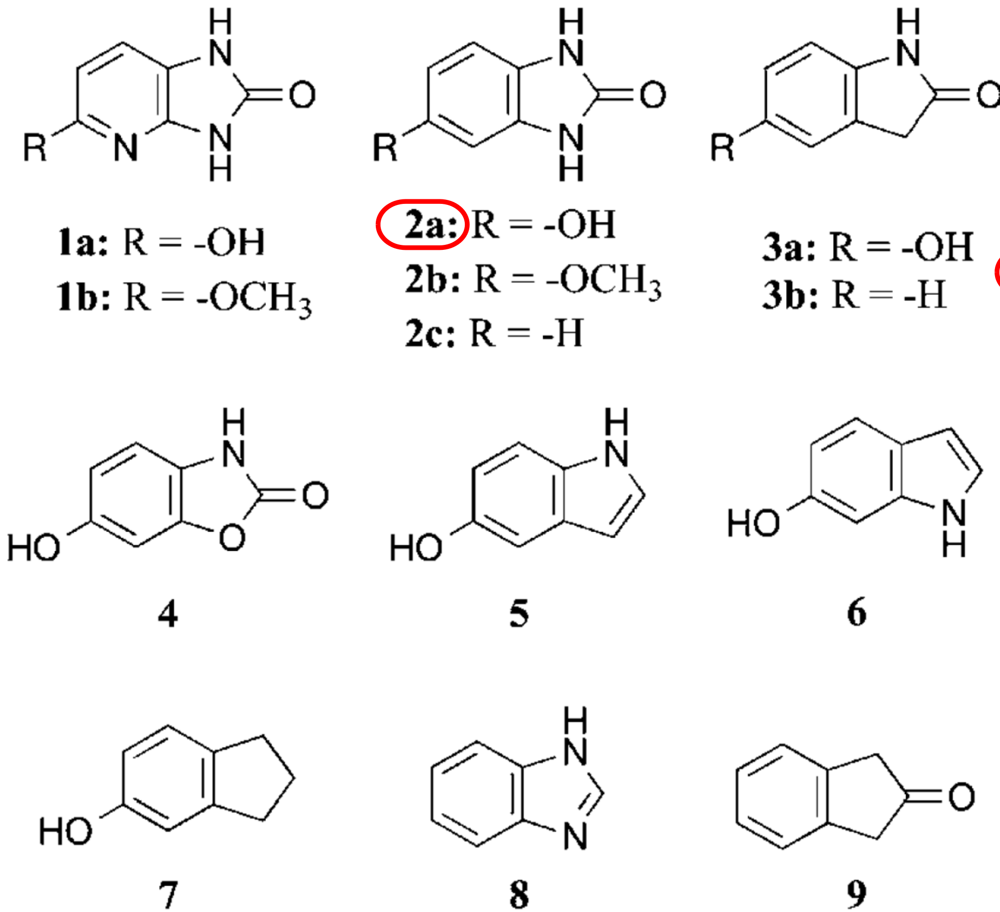
p-aminophenol **10** benzoquinoimine hydroquinone **11** benzoquinone

UA can be equivalent to p-aminophenol or hydroquinone.

Mashino, T. *et al*., *Med. Chem. Commun.* **2013**, *4*, 527.

3-1. What is an antioxidant

Radical scavenging activity of uric acid analogs



Compounds		DPPH radical scavenging rate constants ($\times 10^3\ M^{-1}\ s^{-1}$)
Uric acid		0.7
1a		71
2a	**No cytotoxicity**	110
3a		11
4		8.7
5		2.6
6		11
10	**Cytotoxicity**	310
11		4.4
Edaravone		72

5-hydroxyindolinones (2a) showed sufficient activity with high solubility and low cytotoxity.

Mashino, T. *et al.*, *Med. Chem. Commun.* **2013**, *4*, 527.

3-2. Recent therapeutic antioxidant

Characteristics of H_2

H_2 is a colorless, odorless, tasteless and combustible diatomic gas.

H_2 is expected as a promising energy carrier in future energy system.

cf. Dr. Saga's literature seminar (7/10/2010)

H_2 potentials for antioxidants

H_2 reduces the ・OH that is produced by radiolysis or photolysis of water.

Ross, A. B. *et al*., *J. Phys. Chem. Ref. Data,* **1988**, *17*, 513.

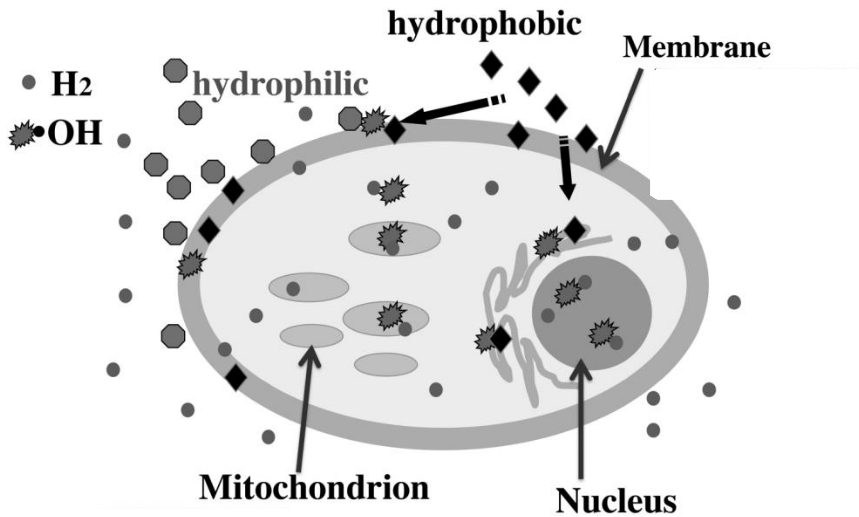
H_2 behaves as an inert gas in the absence of catalysts or at body temperature.

H_2 can be dissolved in water up to 0.8 mM under atmospheric pressure.

Ohta, S. *et al*., *Nat. Med.* **2007**, *13*, 688.
Ohta, S. *et al*., *Curr. Pharm. Des.* **2011**, *17*, 2241.

3-2. Recent therapeutic antioxidant

H_2 has rapid diffusion.



<u>Formation of highly ROS</u>

Fenton reaction

$$H_2O_2 + Cu^+ \longrightarrow \bullet OH + OH^- + Cu^{2+}$$

Weise reaction

$$\bullet O_2^- + H_2O_2 \longrightarrow \bullet OH + OH^- + O_2$$

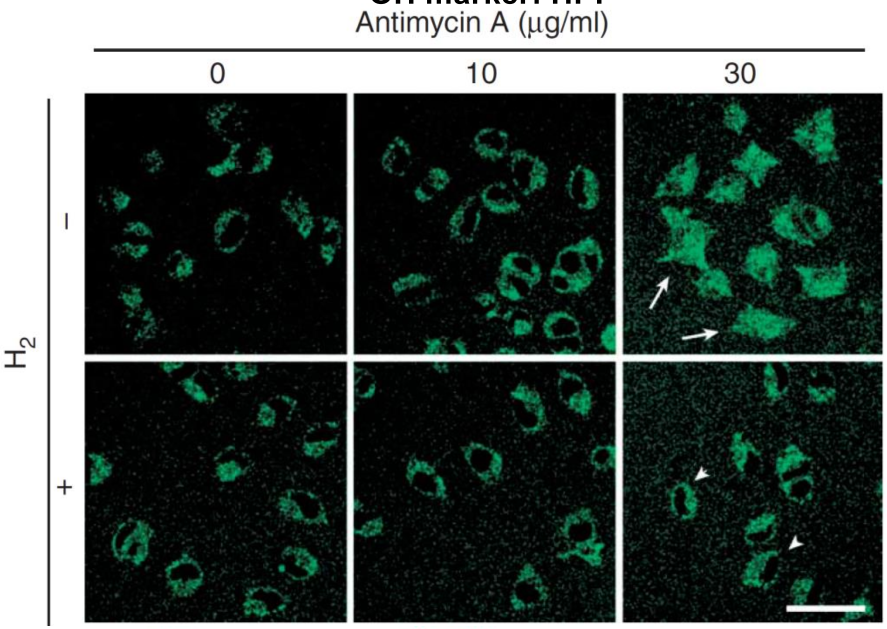
• OH is the strongest free radical.

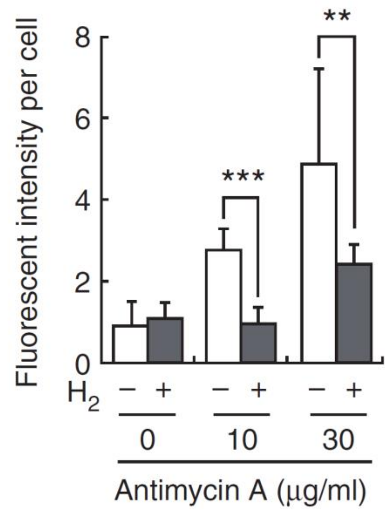
Ohta, S. *et al*., *Nat. Med.* **2007**, *13*, 688.
Ohta, S. *et al*., *Curr. Pharm. Des.* **2011**, *17*, 2241.

3-2. Recent therapeutic antioxidant

H_2 dissolved in medium reduced •OH in cultured cell.

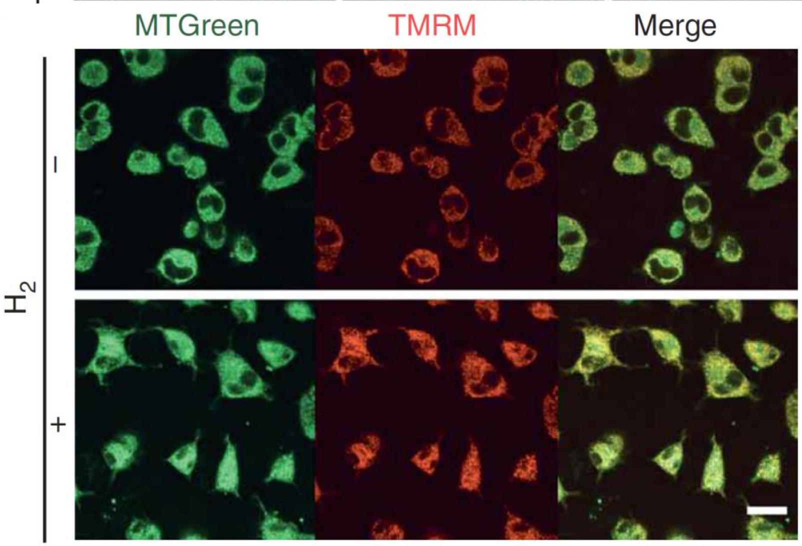
•OH marker: HPF



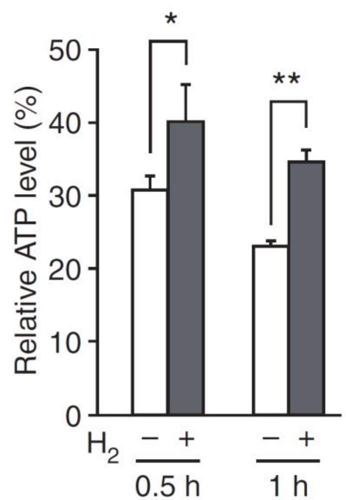


H_2 decreased

•OH levels.



Antimycin (10 μg/ml)
Mitochondria membrane potential marker: TMRM



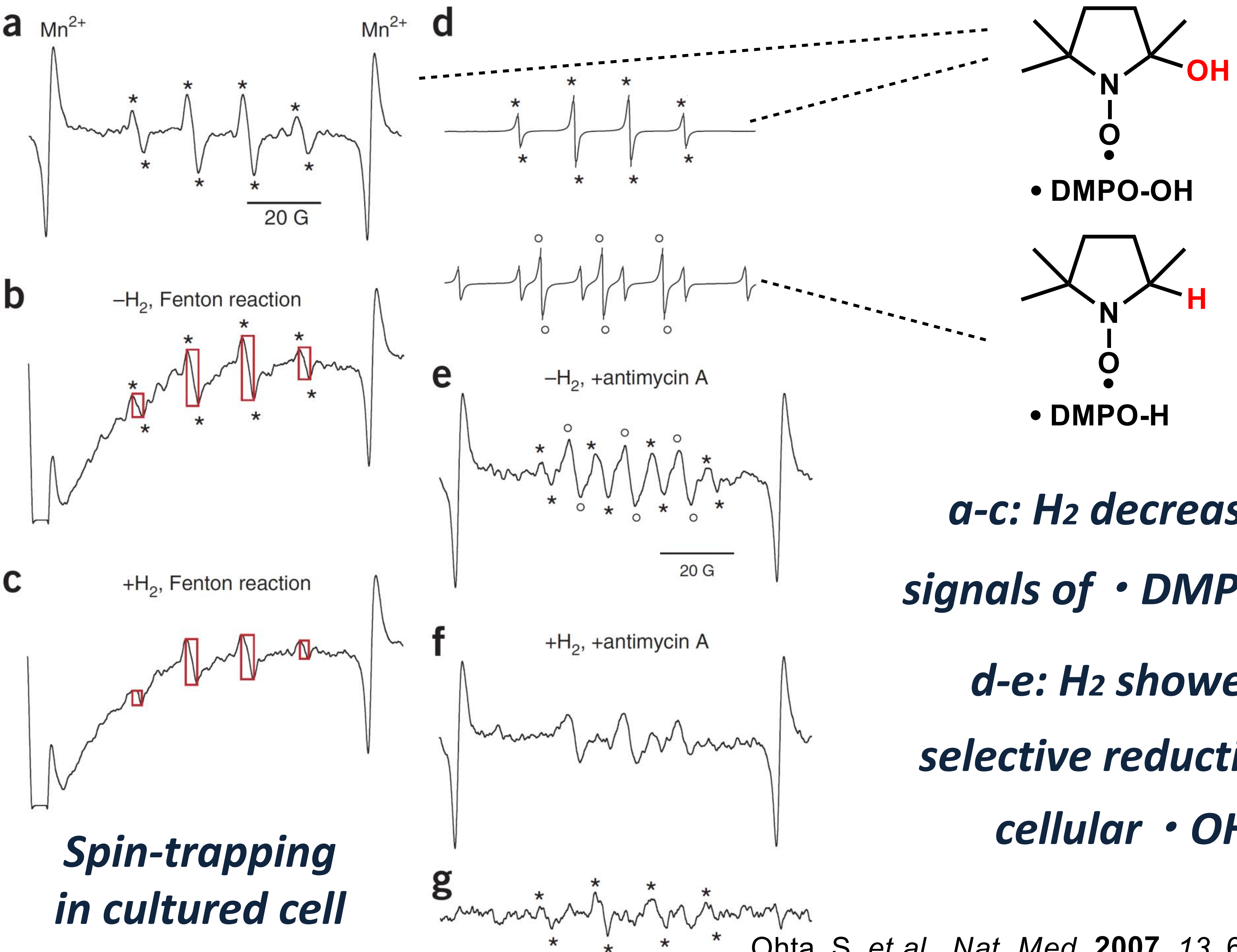
Antimycin (30 μg/ml)

H_2 protected

mitochondria.

Ohta, S. *et al*., *Nat. Med*. **2007**, *13*, 688.

3-2. Recent therapeutic antioxidant



Spin-trapping in cultured cell

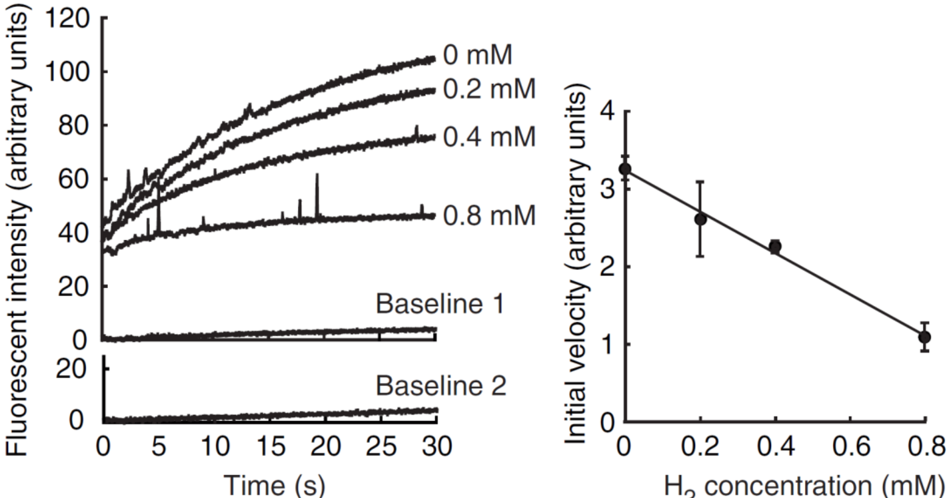
a-c: H_2 decreased signals of ・DMPO-OH.

d-e: H_2 showed selective reduction of cellular ・OH

Ohta, S. *et al.*, *Nat. Med.* **2007**, *13*, 688.

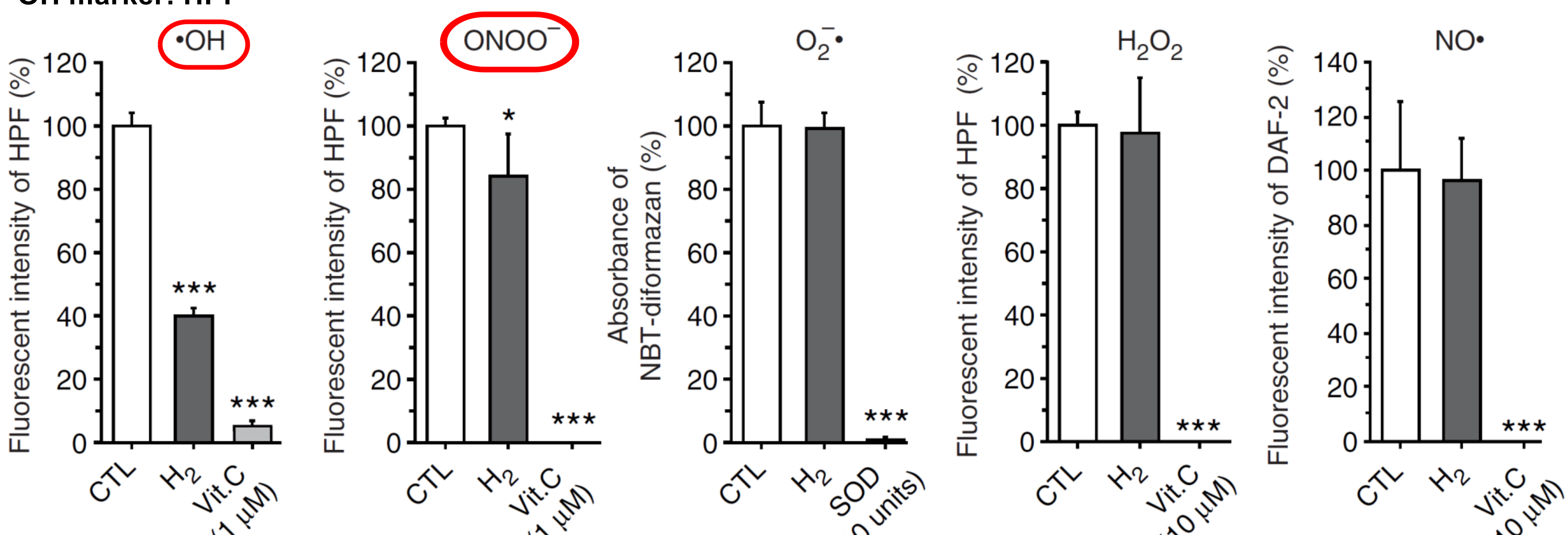
3-2. Recent therapeutic antioxidant

H_2 dissolved in solution reduced ・OH in cell-free systems.



H_2 reduced ・OH and $ONOO^-$ somewhat.

• OH was generated by Fenton reaction.
• OH marker: HPF

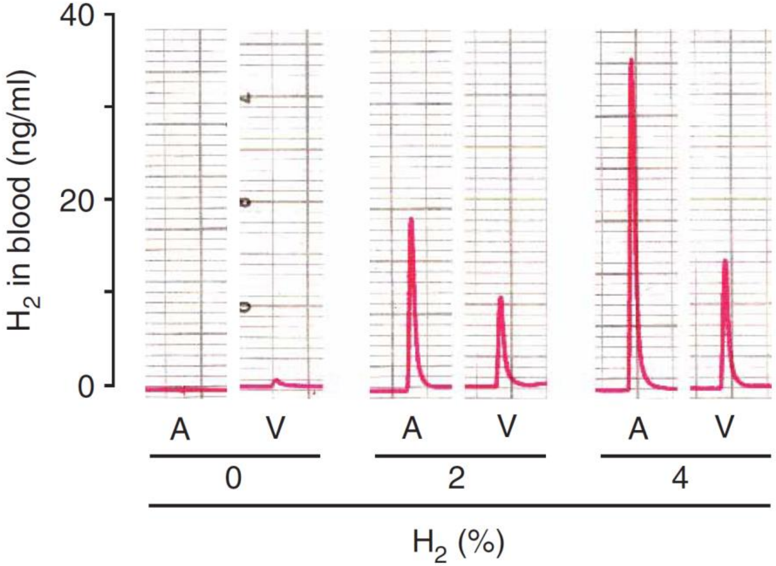


H_2 (0.6 mM)

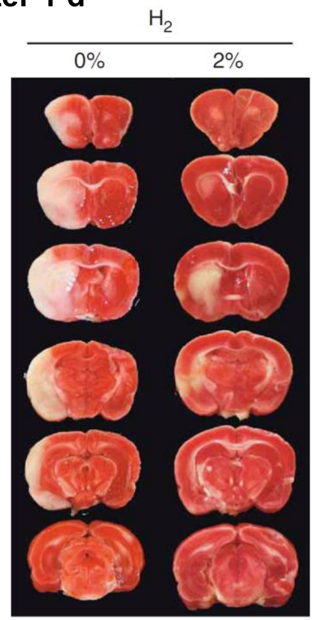
Ohta, S. *et al*., *Nat. Med.* **2007**, *13*, 688.

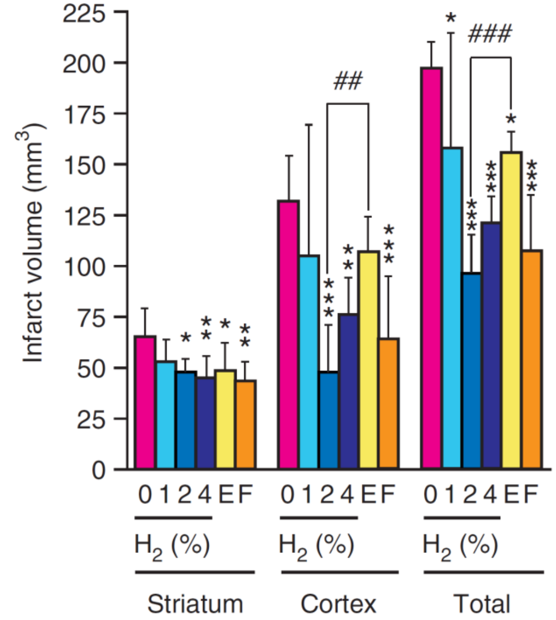
3-2. Recent therapeutic antioxidant

Inhalation of H_2 protected against ischemia-reperfusion injury.

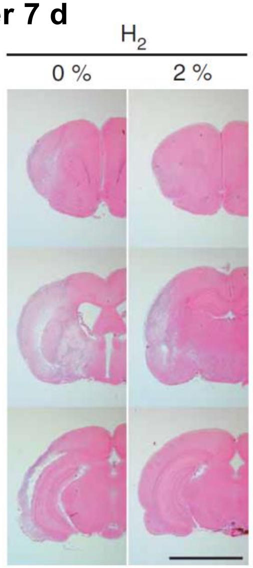


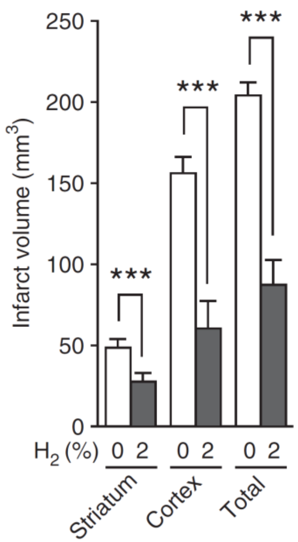
After 1 d





After 7 d





H_2 suppressed not only the initial brain injury but also its progressive damage.

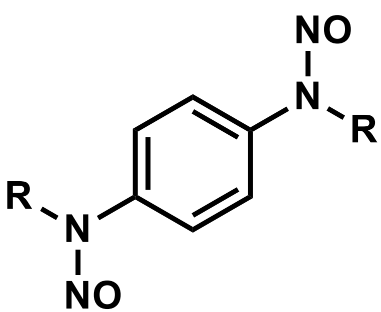
Ohta, S. *et al*., *Nat. Med.* **2007**, *13*, 688.

Contents

4-1. NO releaser

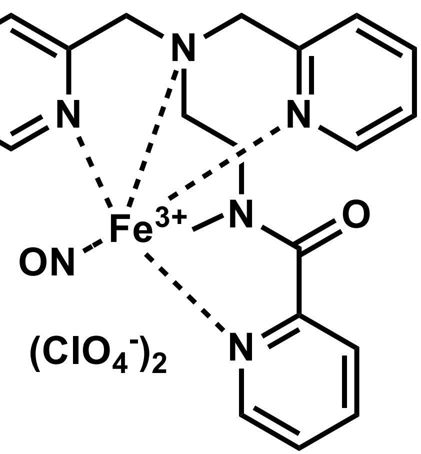
NO is a colorless gas that is unstable under physiological conditions ($t_{1/2}$ = 0.1-5 s).

NO releasers, which can store and release NO, have been developed for biological research on the roles of NO or as candidates of chemotherapeutic agents.



BNNs
R=CH_3, CH_2COOH

UV light



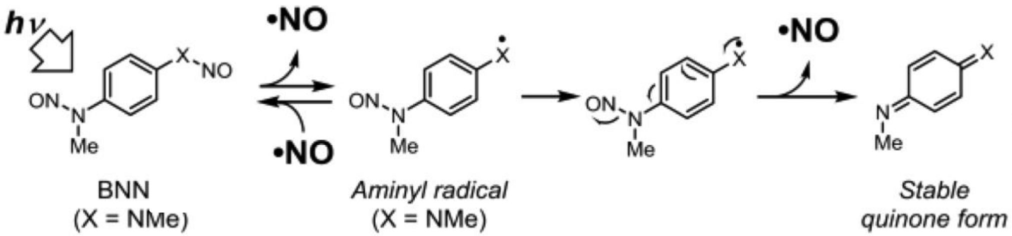
Fe (III) complex

Transition metal

Fujimori, K. *et al*., *J. Am. Chem. Soc.* **1997**, *119*, 3840.
Mascharak, P. K. *et al*., *Angew. Chem. Int. Ed.* **2002**, *41*, 2512.

4-2. Blue-light-controllable NO releaser

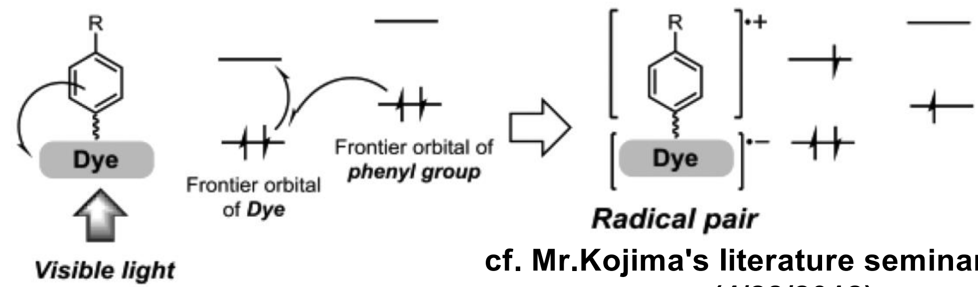
Design a visible-light-controllable NO releaser



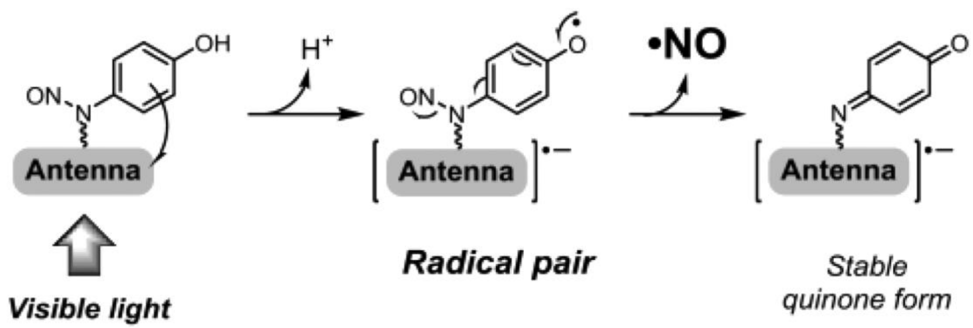
No cytotoxicity

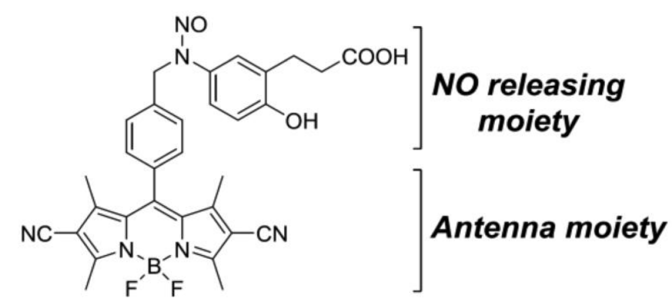
NO release from BNN

Photoinduced electron transfer



cf. Mr.Kojima's literature seminar (4/28/2012)



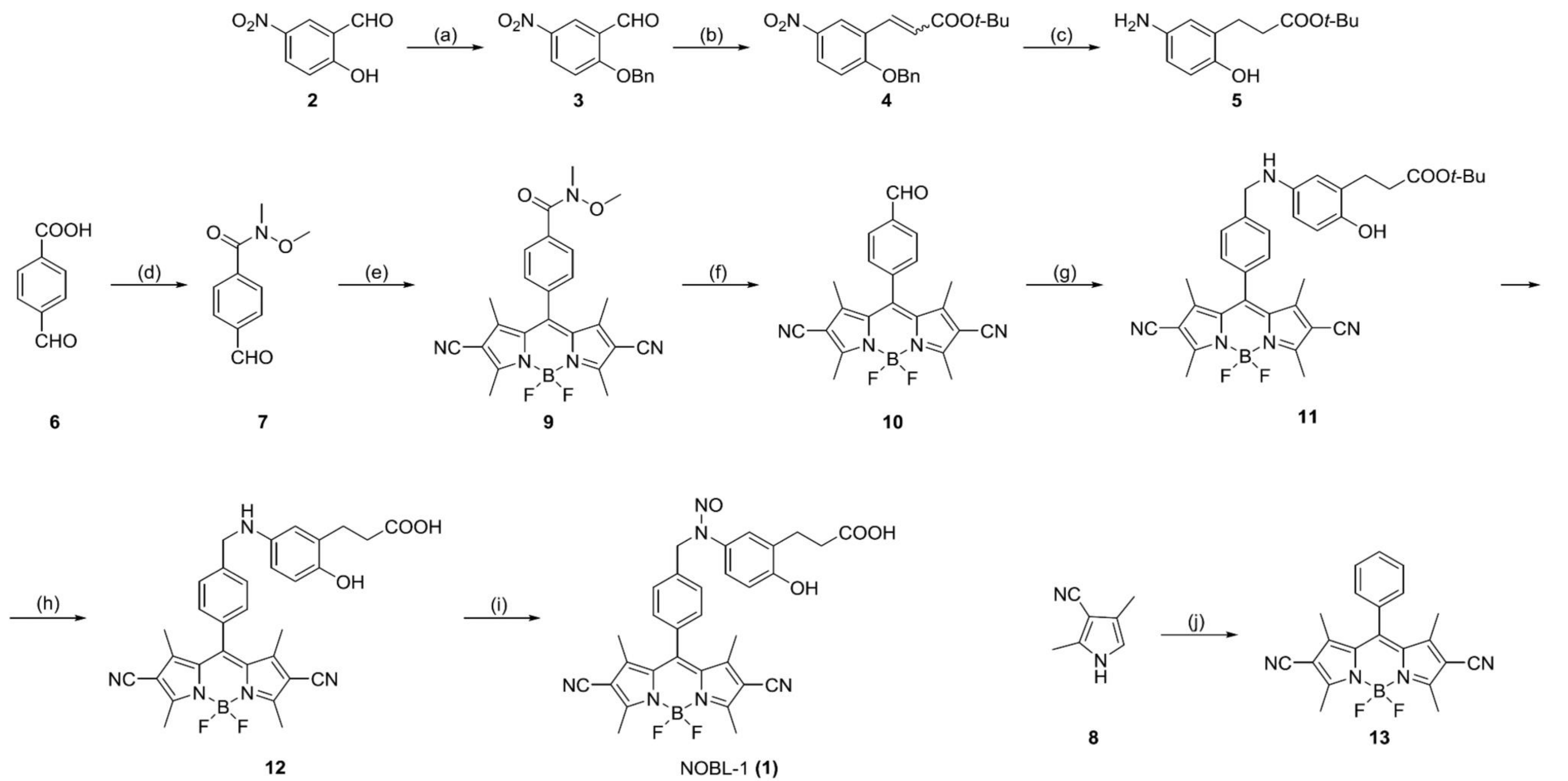


NOBL-1 (1)
(***NO*** releaser triggered by ***B***lue ***L***ight)

Nakagawa, H. *et al*., *J. Am. Chem. Soc.* **2014**, *136*, 7085.

4-2. Blue-light-controllable NO releaser

Synthesis of NOBL-1 (1)



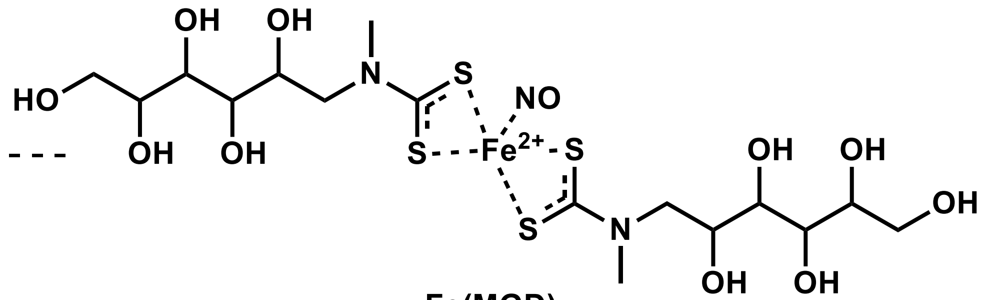
[a]Reagents and conditions: (a) BnBr, K_2CO_3, DMF, rt, 90%; (b) $BrCH_2COO$*t*-Bu, PPh_3, sat. $NaHCO_3$, THF, rt, quant.; (c) Pd–C, MeOH, H_2, rt, 79%; (d) NHMeOMe·HCl, EDCI·HCl, *N*-methylmorpholine, CH_2Cl_2, rt, 67%; (e) 8, TFA, CH_2Cl_2, rt; then, DDQ; then, $BF_3·OEt_2$, DIPEA, 0 °C, 64%; (f) Cp_2ZrHCl, THF, rt, 70%; (g) **5**, AcOH, CH_2Cl_2, rt; then, $NaBH(OAc)_3$, rt, 83%; (h) HCl, AcOEt, rt; (i) $NaNO_2$, AcOH, H_2O, 0 °C, 44% (2 steps); (j) benzaldehyde, TFA, CH_2Cl_2, rt; then, DDQ, rt; then, $BF_3·OEt_2$, DIPEA, rt, 48%.

Nakagawa, H. *et al*., *J. Am. Chem. Soc.* **2014**, *136*, 7085.

4-2. Blue-light-controllable NO releaser

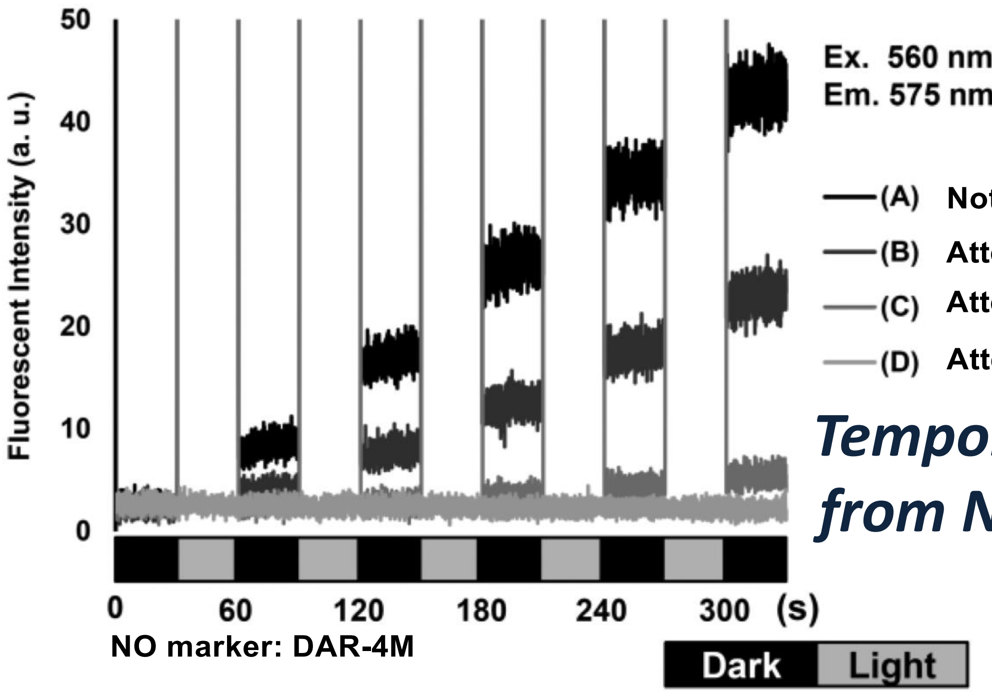
Spin-trappingin cell-free systems

Blue-light irradiation (470-500nm, 200 mW/cm^2)



$Fe(MGD)_2$

Detection of NO generation from NOBL-1 in cell-free systems



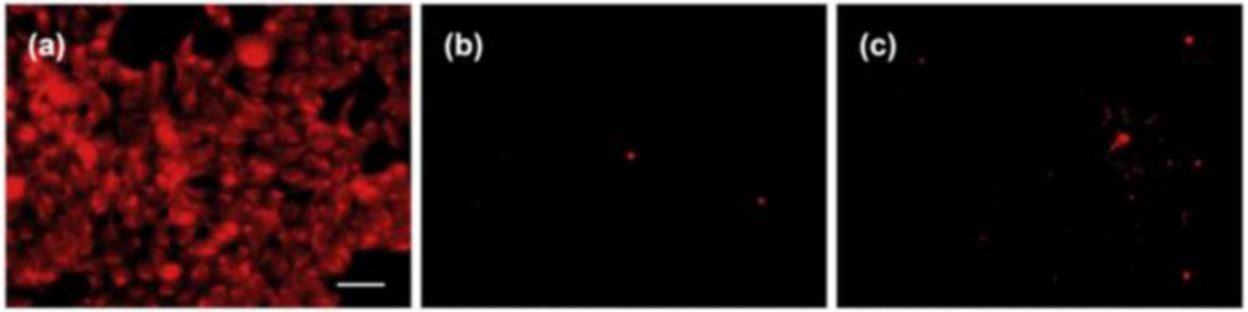
NO marker: DAR-4M

Temporally precise control of NO release from NOBL-1 could be achieved.

Nakagawa, H. *et al*., *J. Am. Chem. Soc.* **2014**, *136*, 7085.

4-2. Blue-light-controllable NO releaser

Fluorescence imaging of NO release from NOBL-1 in cultured cell.



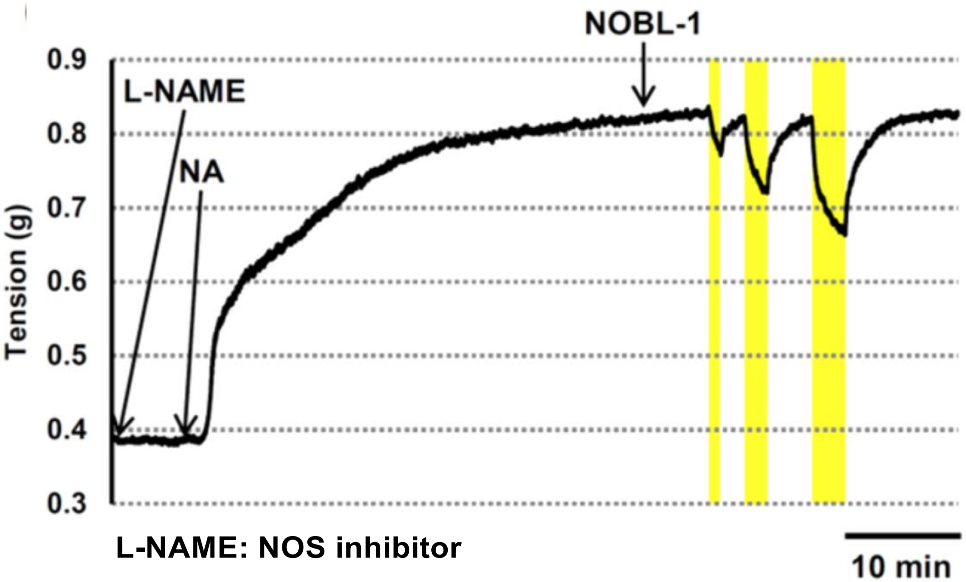
After photoirradiation with NOBL-1

After incubation with NOBL-1 in the dark.

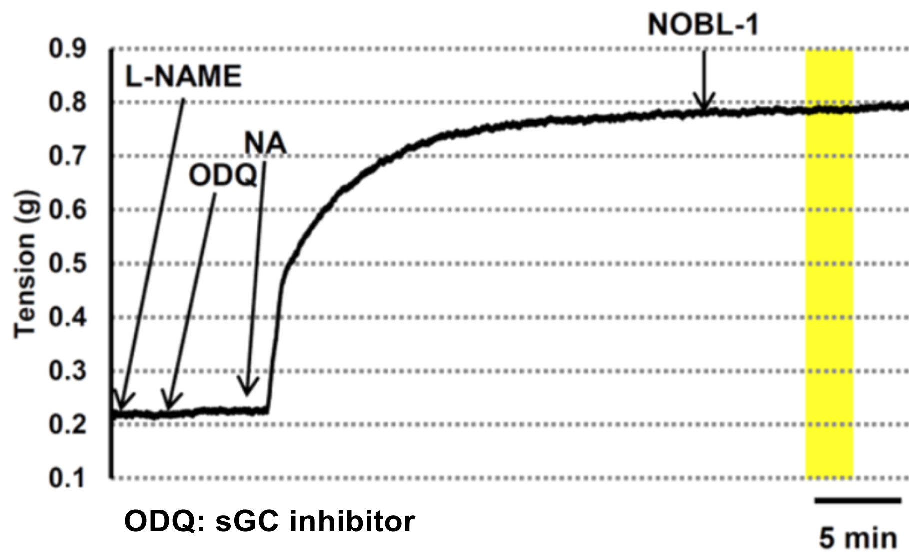
After photoirradiation without NOBL-1.

Blue-light irradiation (470-500nm, 25 mW/cm^2)
NO marker: DAR-4M AM

Changes in tension of rat aorta ex vivo



L-NAME: NOS inhibitor

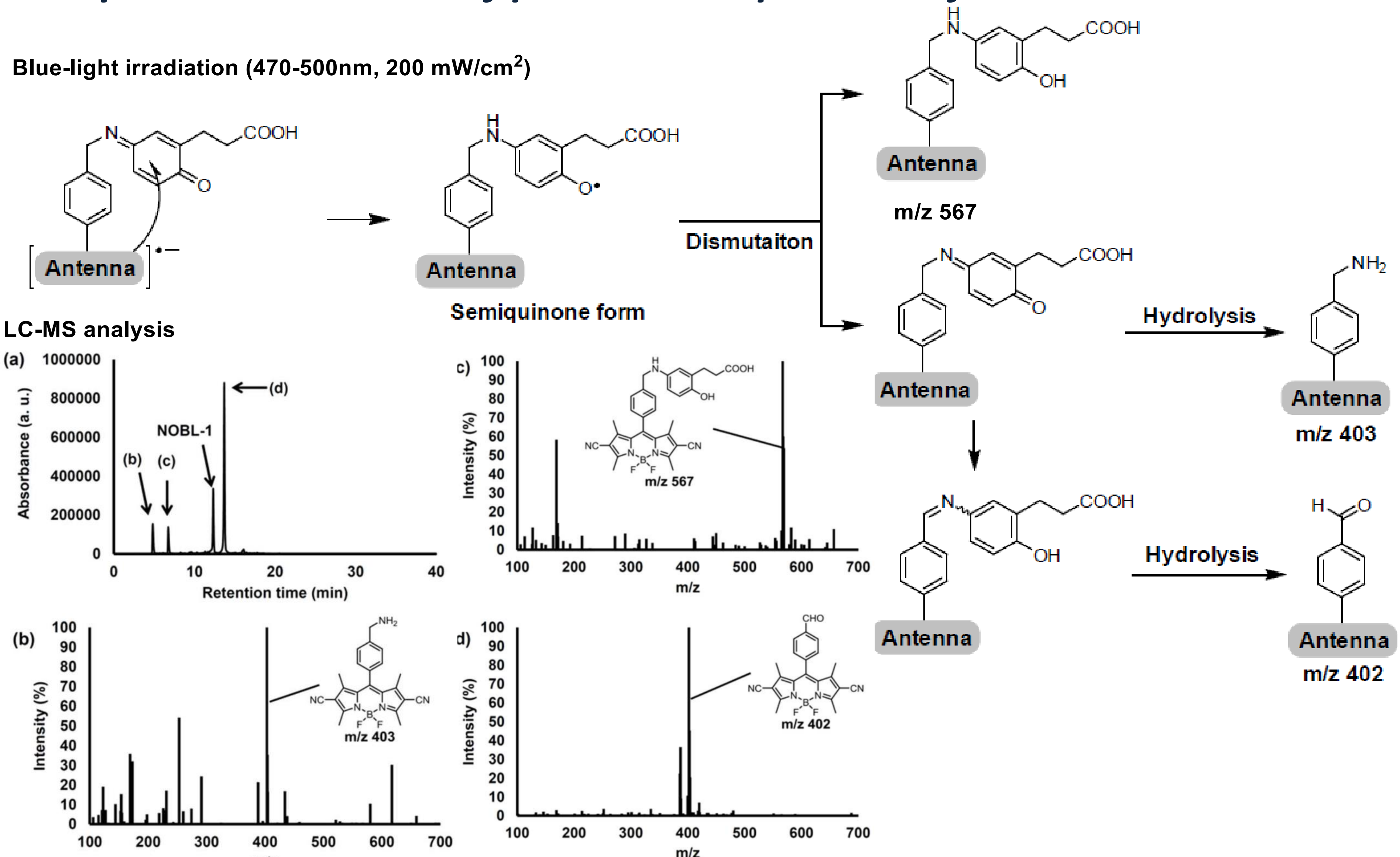


ODQ: sGC inhibitor

Nakagawa, H. *et al*., *J. Am. Chem. Soc.* **2014**, *136*, 7085.

4-2. Blue-light-controllable NO releaser

Proposed mechanism of photodecomposition of NOBL-1

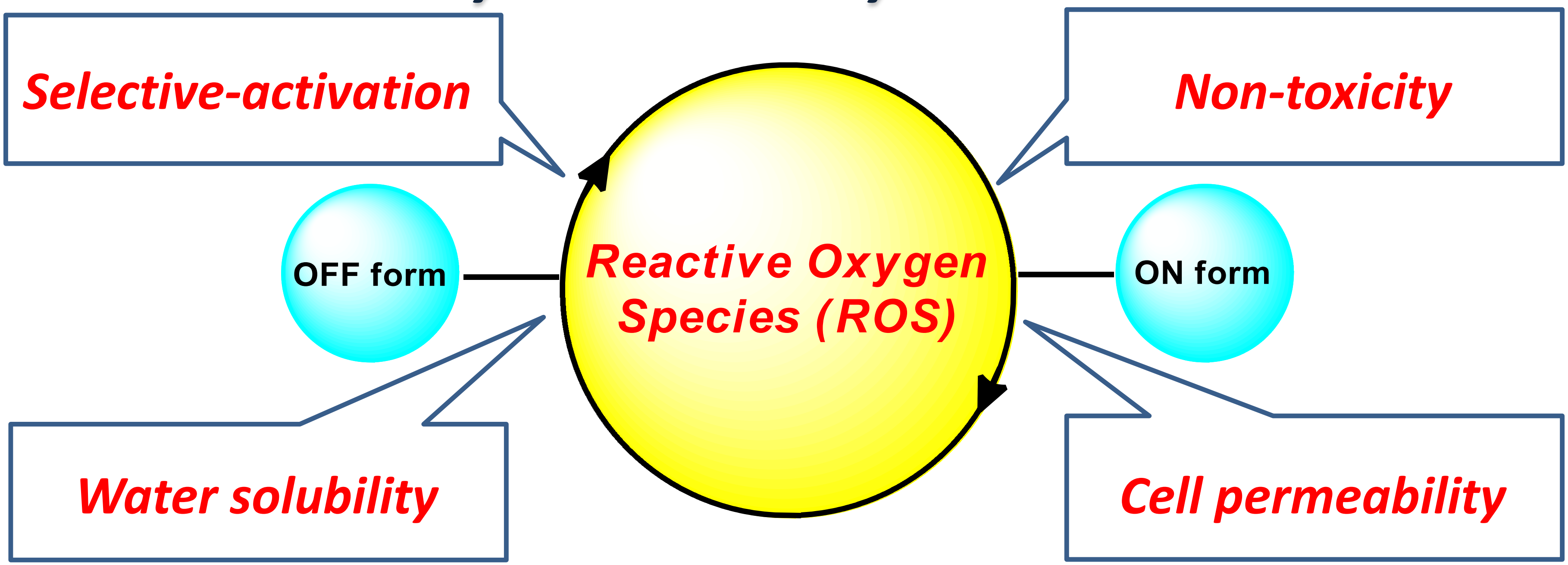


Nakagawa, H. *et al.*, *J. Am. Chem. Soc.* **2014**, *136*, 7085.

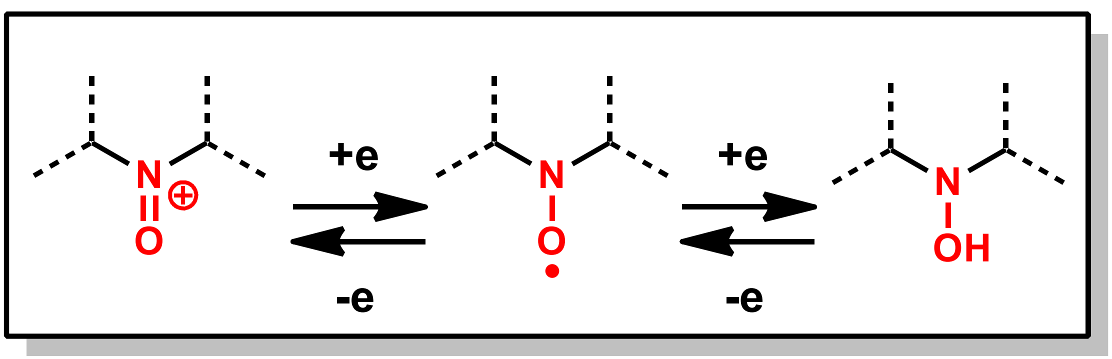
Contents

5. Application to ROS-dependent chemical reaction

Redox active catalyst mediated by ROS

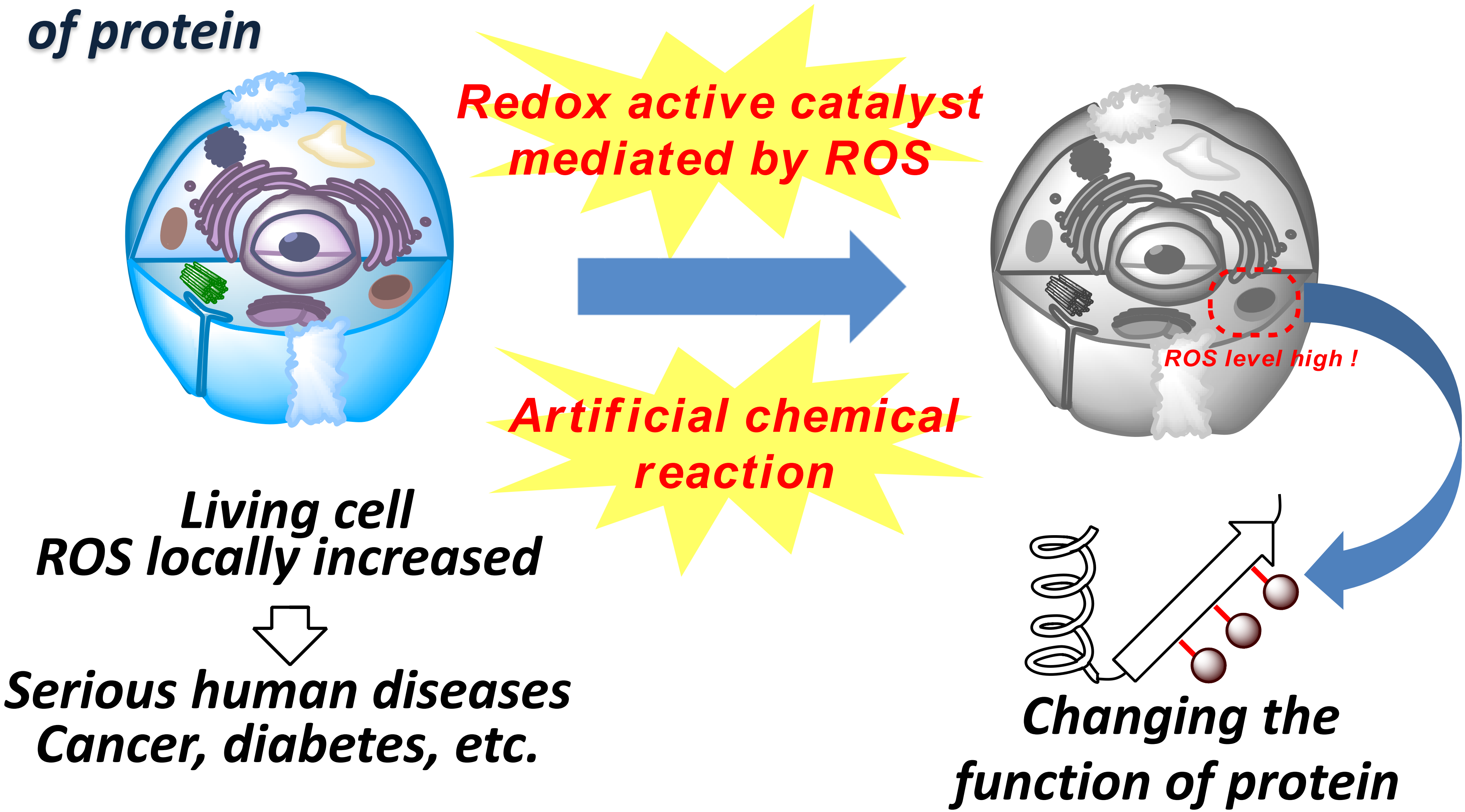


Today's seminar: N-oxylradical moiety



5. Application to ROS-dependent chemical reaction

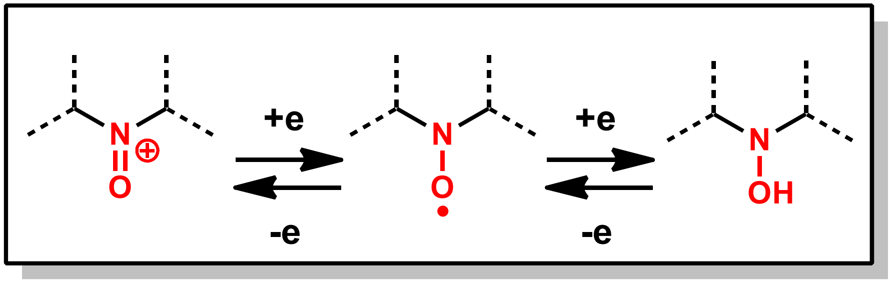
Ros-dependent chemical reaction and changing the function of protein



Is it possible to apply this system to controling the ROS ?

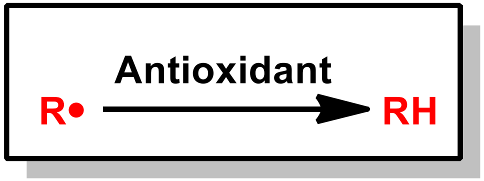
5. Summary

Section 2
Probing for the redox status using N-oxylradicals



- ***Various kinds of N-oxylradicals for probing have been developed.***
- ***Slow reduction and high r_1 of N-oxylradical were important.***

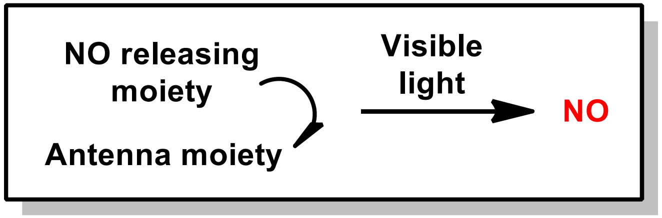
Section 3
Developments of small molecules for antioxidants



- ***Drug which has antioxidant activity as its main effect is only edarabone.***
- ***H_2 has potential as recent therapeutic antioxidant.***

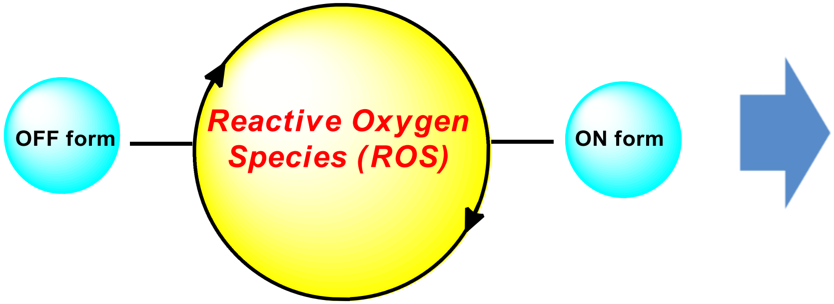
5. Summary

Section 4
Visible-light-controllable NO releaser



- *NOBL-1 which bears NO releasing moiety tethered to antenna moiety released NO on blue-light irradiation.*
- *NOBL-1 can be a useful tool for understanding NO action in tissues and may also have potential for phototherapy.*

Section 5
ROS-dependent chemical reaction



Chemical biology tool

Medicinal application

Appendix 2-2. Redox-sensitive probe

SE method

$S = f(v)\, p\, (1-\exp(-Tr/T_1))\, \exp(-Te/T_2)\, \exp(-bD)$

S: 信号強度　f(v): 流速　p: プロトン強度　T_1: 縦緩和時間　T_2: 横緩和時間
b: b値　D: 拡散定数　Tr: 繰り返し時間　Te: エコー時間

r_1 and SBM formula

$$r_1[\mathrm{Gd}] = \frac{1}{(T_1)_{\text{inner sphere}}} + \frac{1}{(T_1)_{\text{outer sphere}}}$$

$$\frac{1}{(T_1)_{\text{inner sphere}}} = \frac{P_m q}{(T_{1\mathrm{m}} + \tau_\mathrm{m})}$$

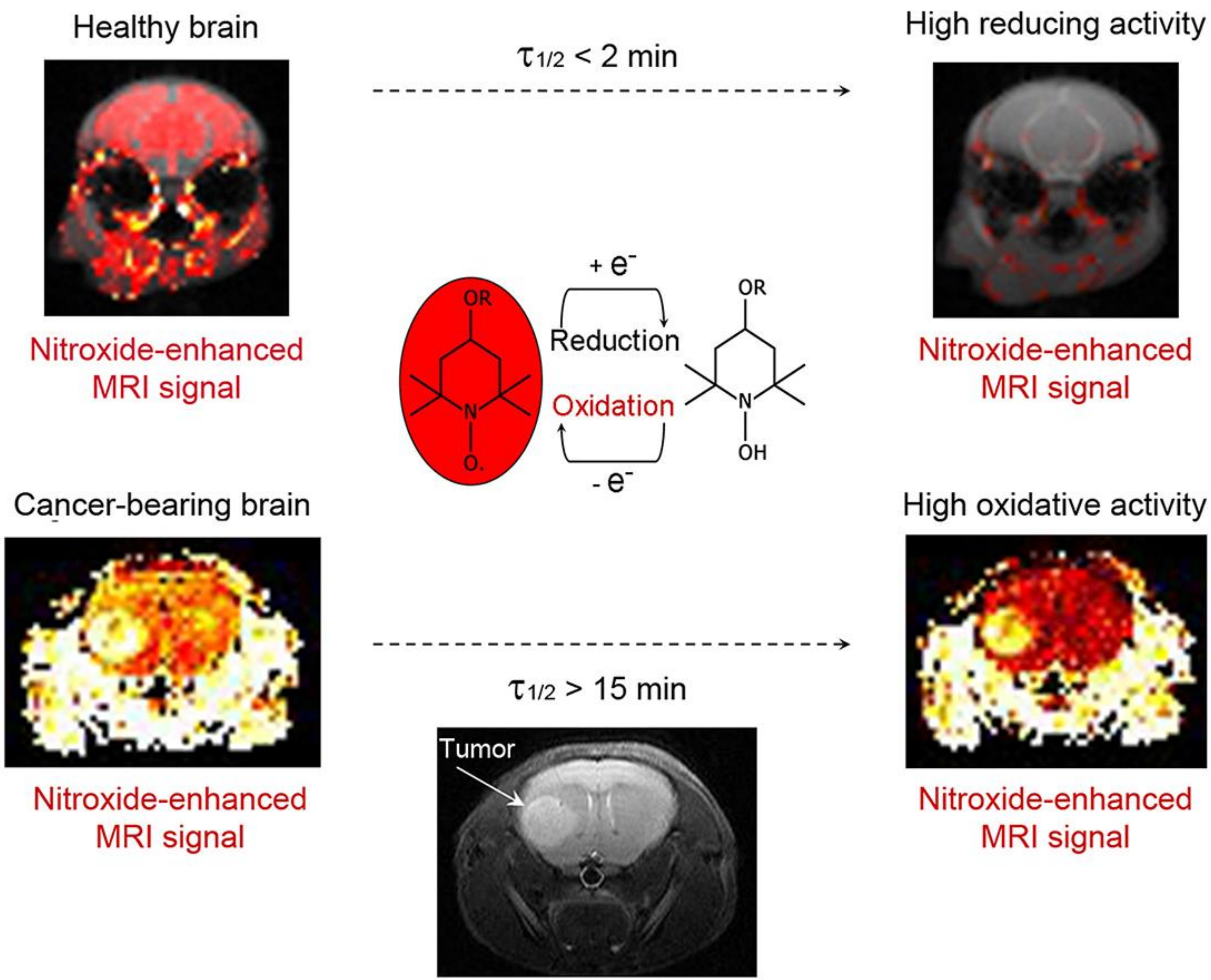
$$\frac{1}{T_{1\mathrm{m}}} = \frac{2}{15} \frac{\gamma_\mathrm{H}^2 g_\mathrm{e}^2 \mu_\mathrm{B}^2 S(S+1)}{r_\mathrm{GdH}^6} \left[\frac{7\tau_{\mathrm{c}2}}{1+\omega_S^2 \tau_{\mathrm{c}2}^2} + \frac{3\tau_{\mathrm{c}1}}{1+\omega_\mathrm{H}^2 \tau_{\mathrm{c}1}^2} \right]$$

$$\frac{1}{\tau_{\mathrm{c}i}} = \frac{1}{\tau_\mathrm{m}} + \frac{1}{\tau_\mathrm{R}} + \frac{1}{T_{ie}}; i = 1,2$$

P_m: Gd^{3+}原子のモル分率　q: Gd^{3+}に配位した水分子数　τ_m: 結合状態にある水分子の平均存在時間　T_{1m}: 結合状態の水分子の緩和時間　$(T_1)_{inner}$: inner sphereに存在する水分子の緩和時間　$(T_1)_{outer}$: outer sphereに存在する水分子の緩和時間　γ_H: 磁気回転比　g_e: 電子の因子　g_e: ボーア磁子　S: Gd^{3+}の合計スピン数　r_{GdH}: Gd^{3+}-^1H間の距離　ω_e, ω_H: 電子及びプロトンのラーモア周波数　τ_{c1}, τ_{c2}: 双極子-双極子相互作用による相関時間　τ_R: 造影剤分子の回転速度　T_{1e}, T_{2e}: 電子緩和時間

Caravan, P. *Chem. Soc. Rev.*, **2006**, *35*, 512.

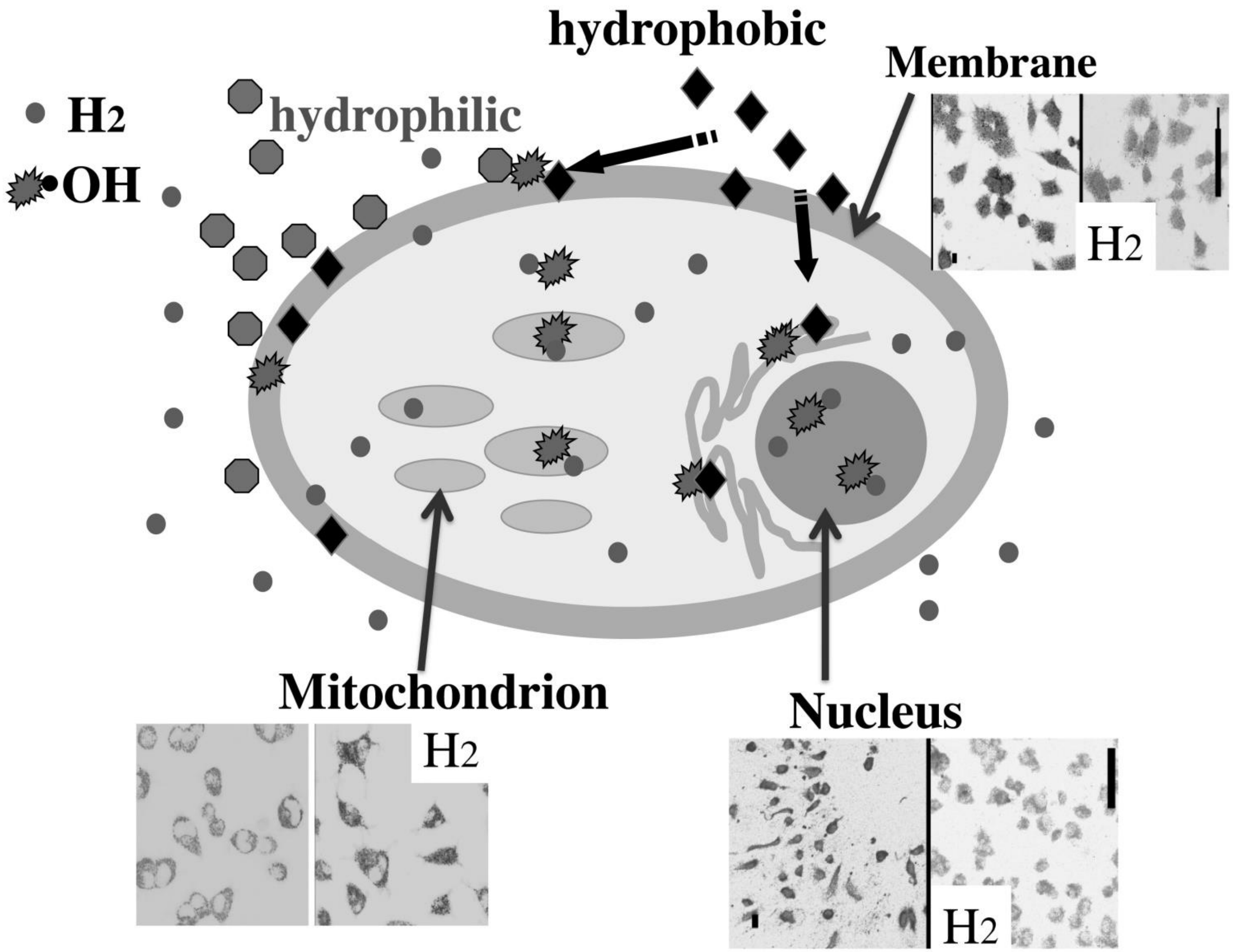
2-2. Redox-sensitive probe



Bakalova, R. *et al*. *ACS Chem. Neurosci*. **2013**, *4*, 1439.

3-2. Recent therapeutic antioxidant

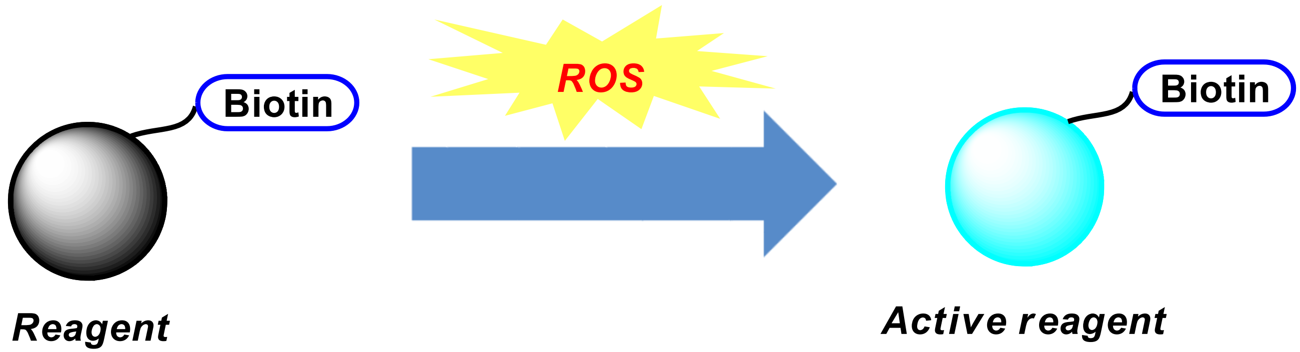
Diffusion of H_2 in cell



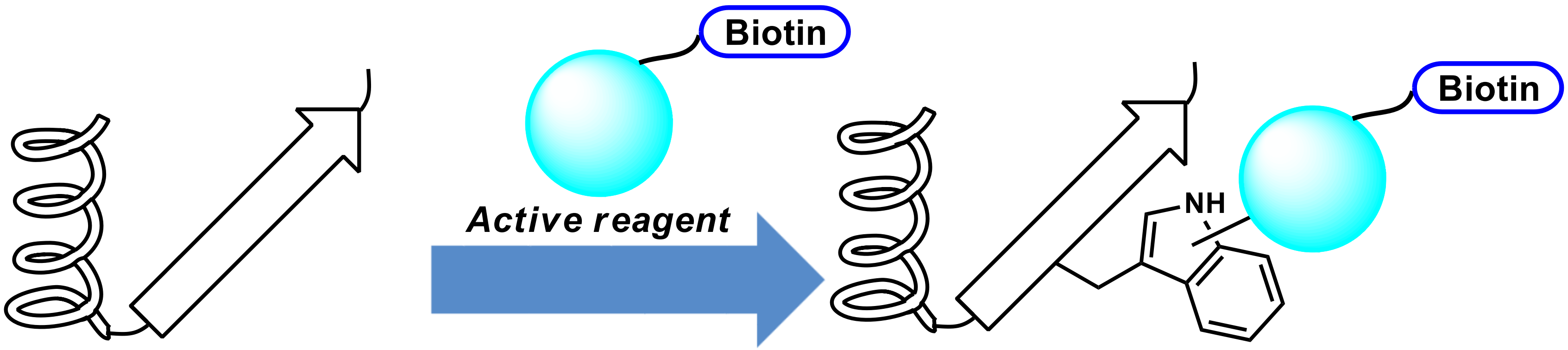
Ohta, S. *et al*., *Nat. Med.* **2007**, *13*, 688.
Ohta, S. *et al*., *Curr. Pharm. Des.* **2011**, *17*, 2241.

5. Strategy for detection of ROS and related protein

First step: ROS-dependent activation of catalyst



Second step: Trptophan-selective conjugation



Challenge:

Development of reagent activated ROS-dependently and conjugating selectively to tryptophan

2-2. Redox-sensitive probe