

3rd International Conference on Nutrition and Food Science
(Nutritional Science-2014)
Track 1: Nutrition and Basic Science
14:10-14:30, September 23, 2014
Committee Room 1-2
Palacio de Congresos de Valencia, Spain

Recent Advances in Vitamin K Metabolism

Kobe Pharmaceutical University

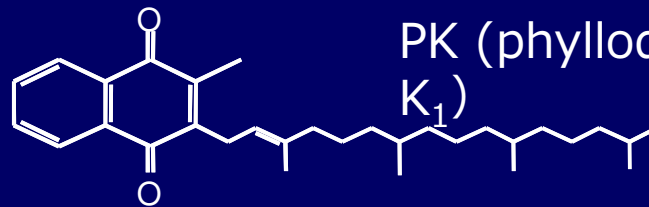
Toshio Okano

Kobe Pharmaceutical Univ.
Dept. Hygienic Sciences



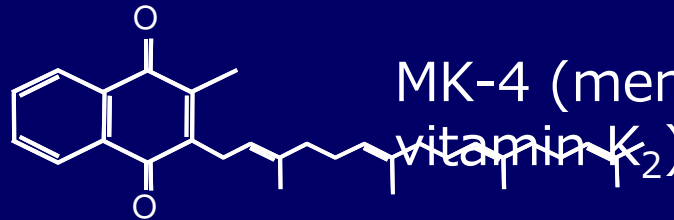
Naturally occurring vitamin K

Plant origin
(Major dietary source)



PK (phylloquinone: vitamin K₁)

Bacterial origin
(Minor dietary source)



MK-4 (menaquinone-4: vitamin K₂)



MK-7
(menaquinone-7)

Plasma vitamin K concentrations of healthy Japanese women

phylloquinone (ng/mL)	MK-4 (ng/mL)
1.74±1.29 (0.13~8.83)	0.10±0.19 (n.d.~1.44)

Tsugawa N, Okano T, et al, Am J Clin Nutr, 2006;83:380-386.

Amounts of dietary intake and plasma concentrations
phyllorquinone > > MK-4



Tissue concentrations of animals and humans
MK-4 > > phyllorquinone



Is phyllorquinone converted into MK-4 in the body ?



What is the physiological significance of this conversion and
what functions dose MK-4 have, and can we develop MK-4
analogues for clinical use ?

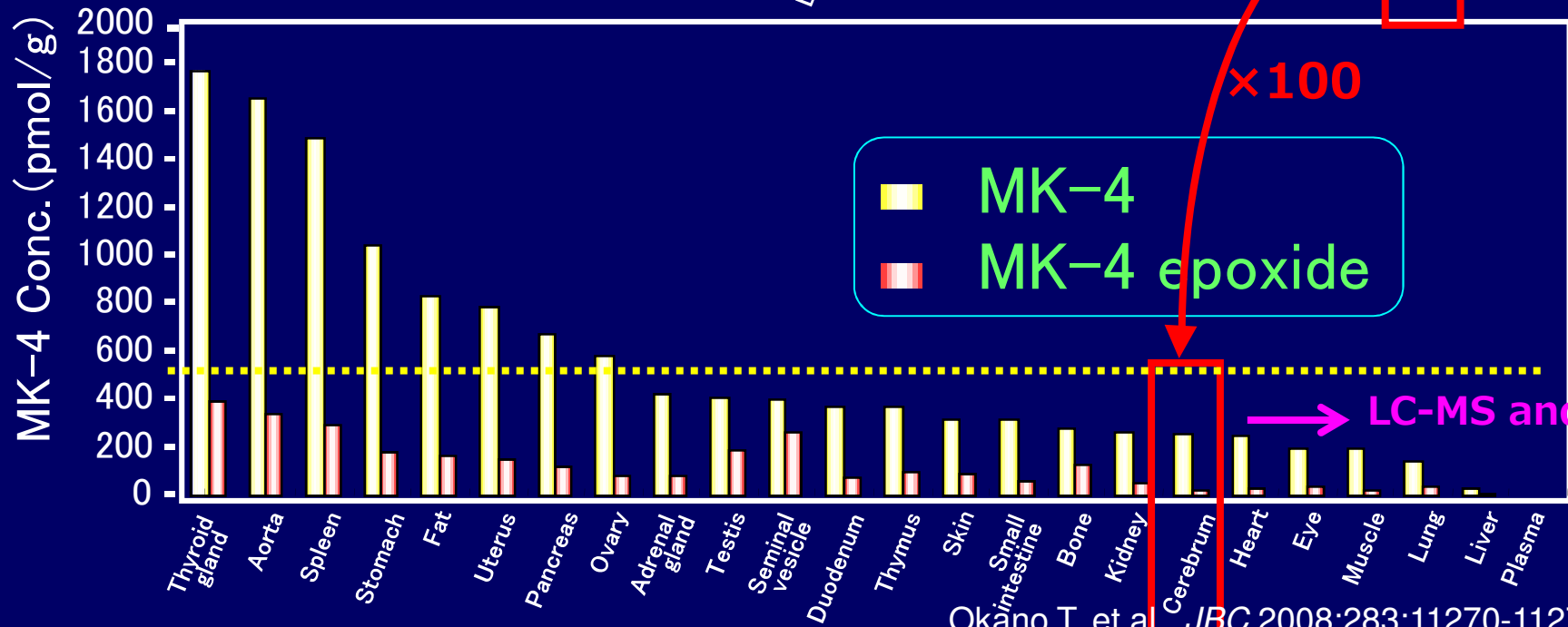
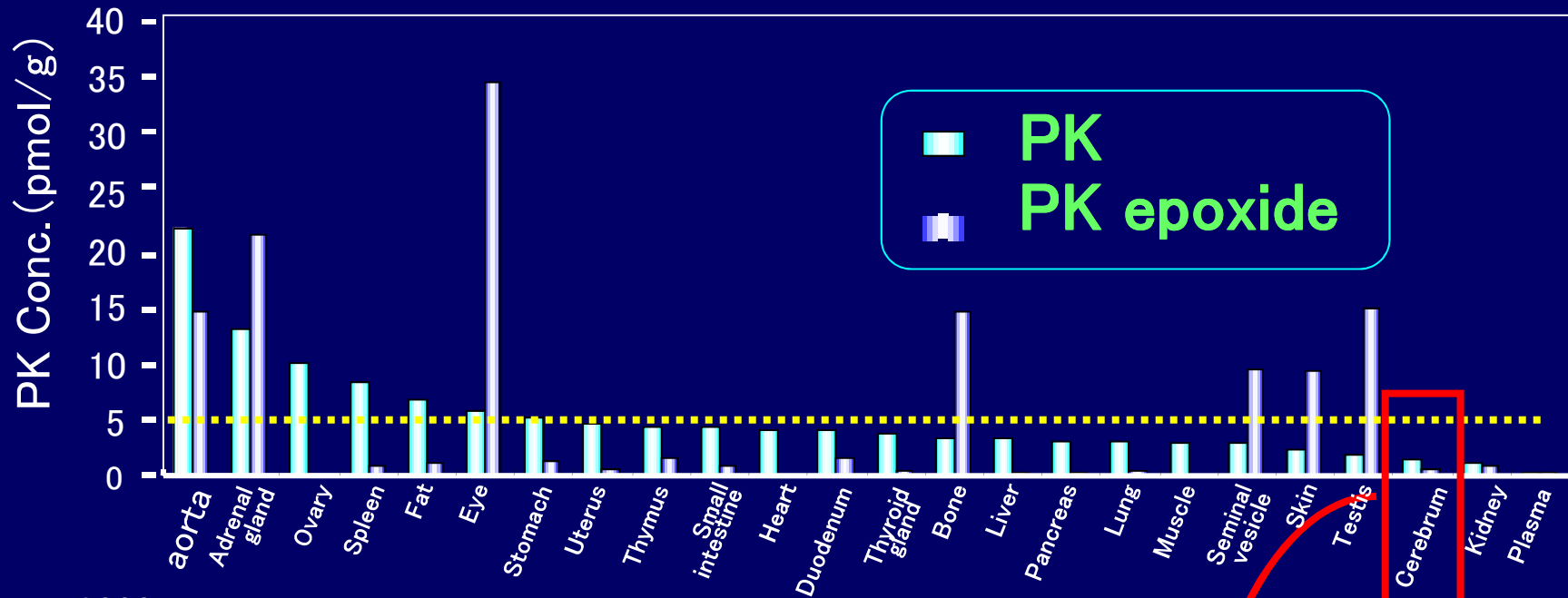
Suhara Y, Okano T, et al, *J Med Chem*, 2011;54:4269-4273

Suhara Y, Okano T, et al, *J Med Chem*, 2011;54:4918-4922

Suhara Y, Okano T, et al, *J Med Chem*, 2012;55:1553-1558

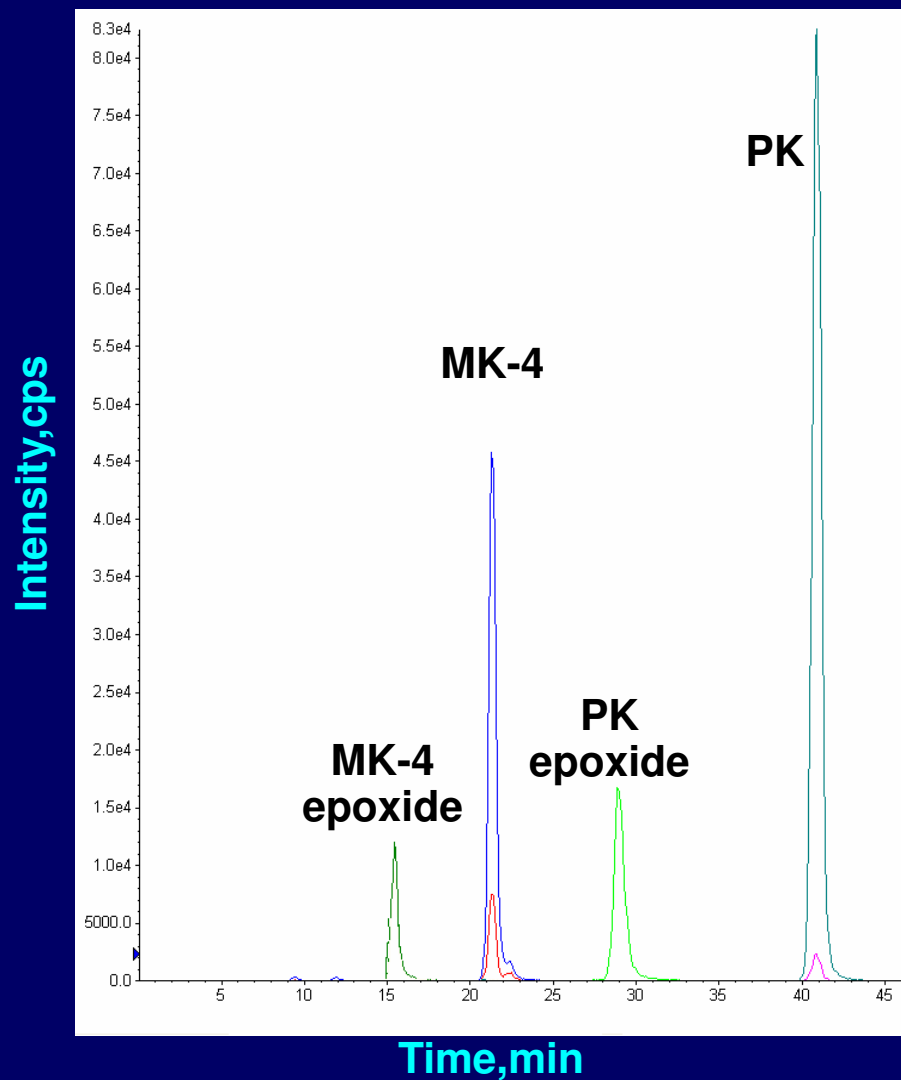
The aim of our study is to examine the
above issues in animals and humans.

Tissue concentrations of PK and MK-4 in mice fed a conventional laboratory chow diet

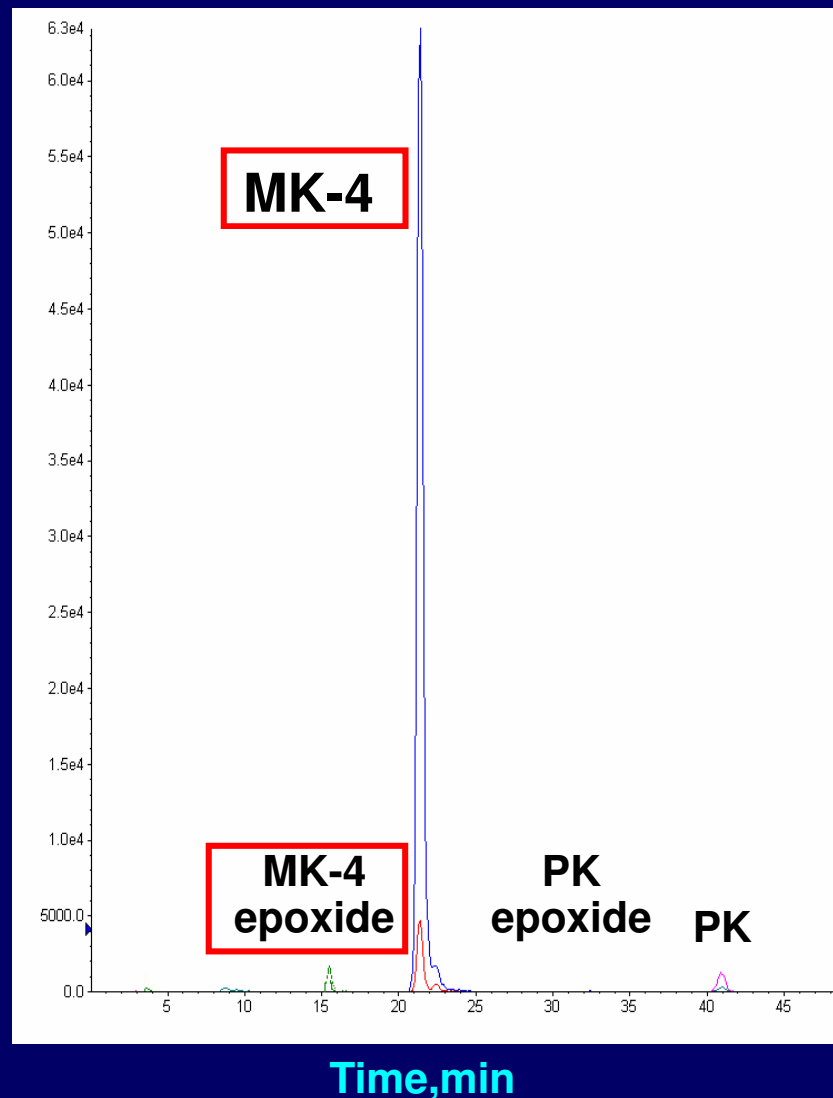


Identification of MK-4 from brain of mice by LC-MS/MS

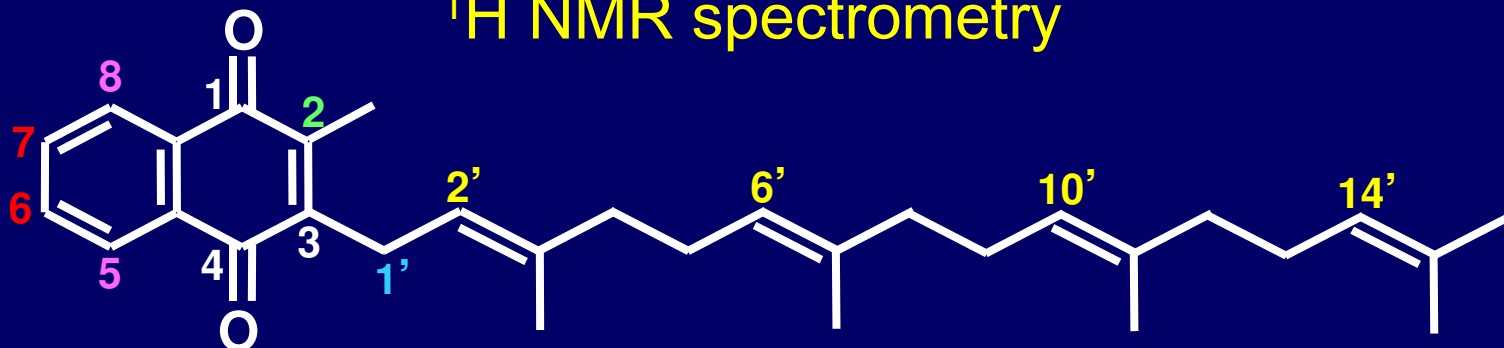
Authentic vitamin Ks



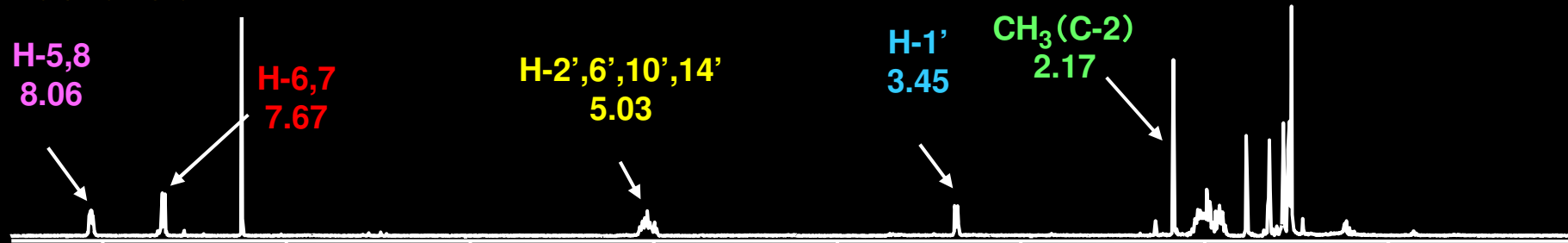
MK-4 fraction



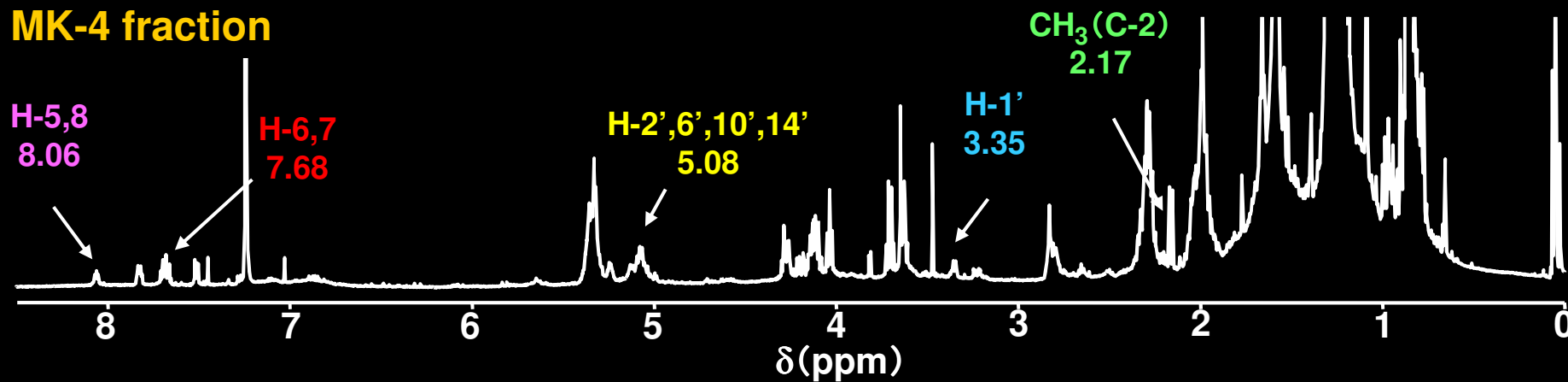
Identification of MK-4 from brain of mice by ^1H NMR spectrometry



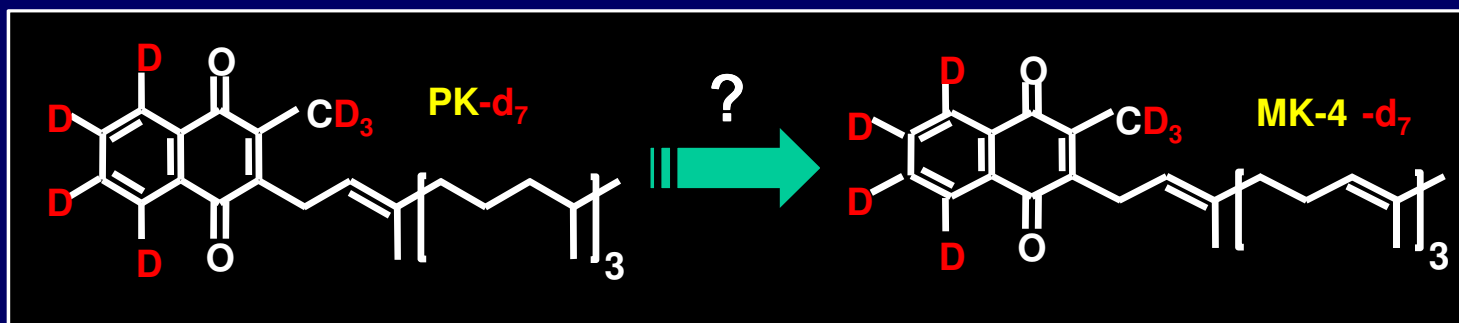
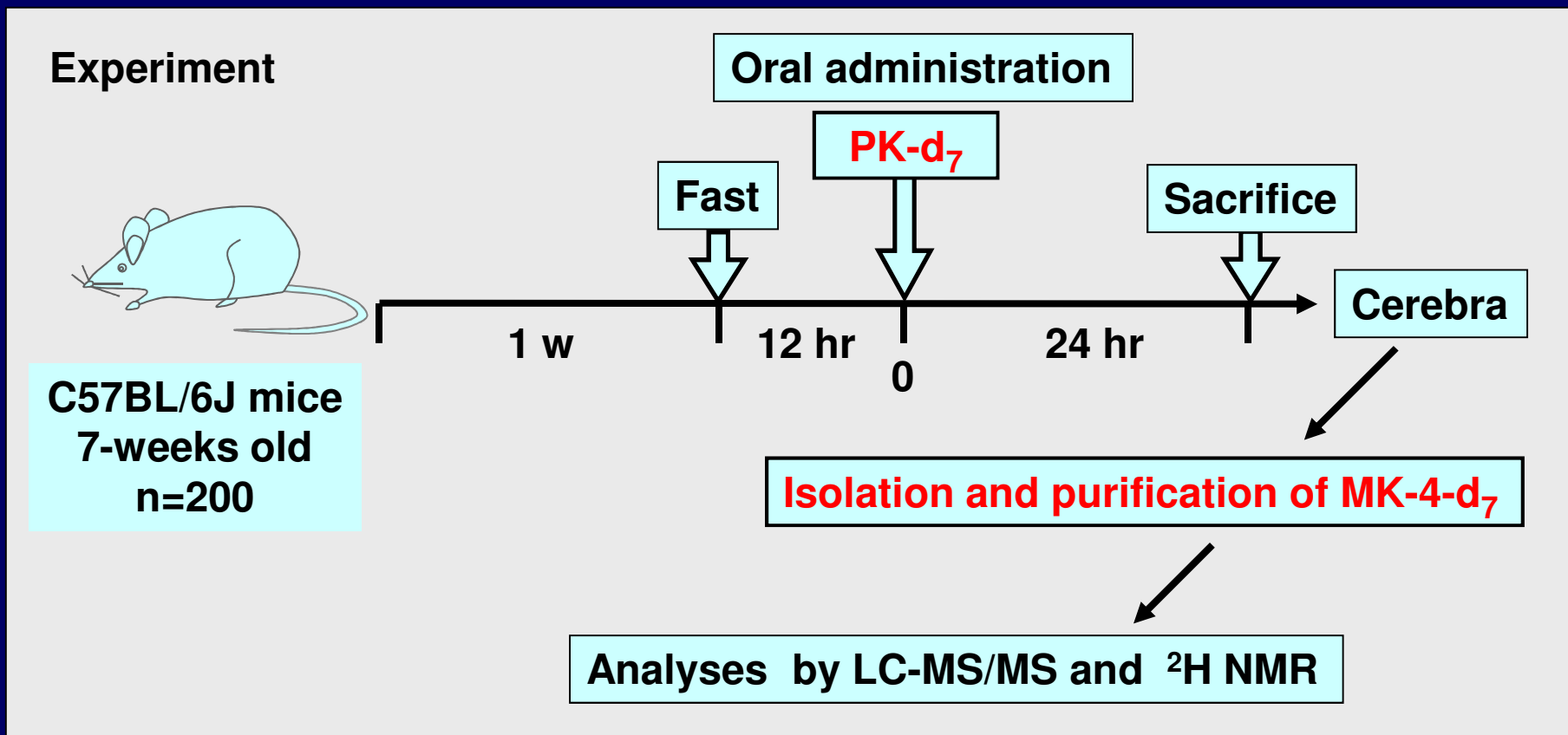
Authentic MK-4



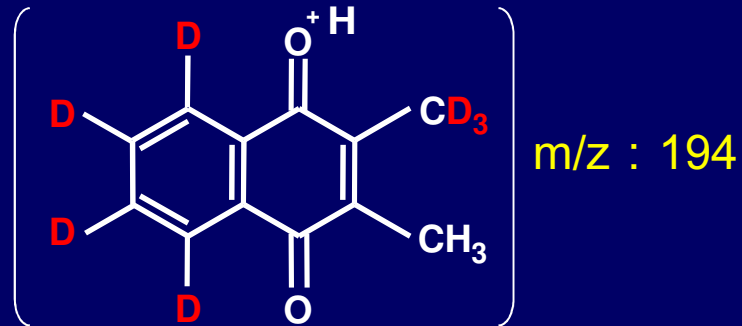
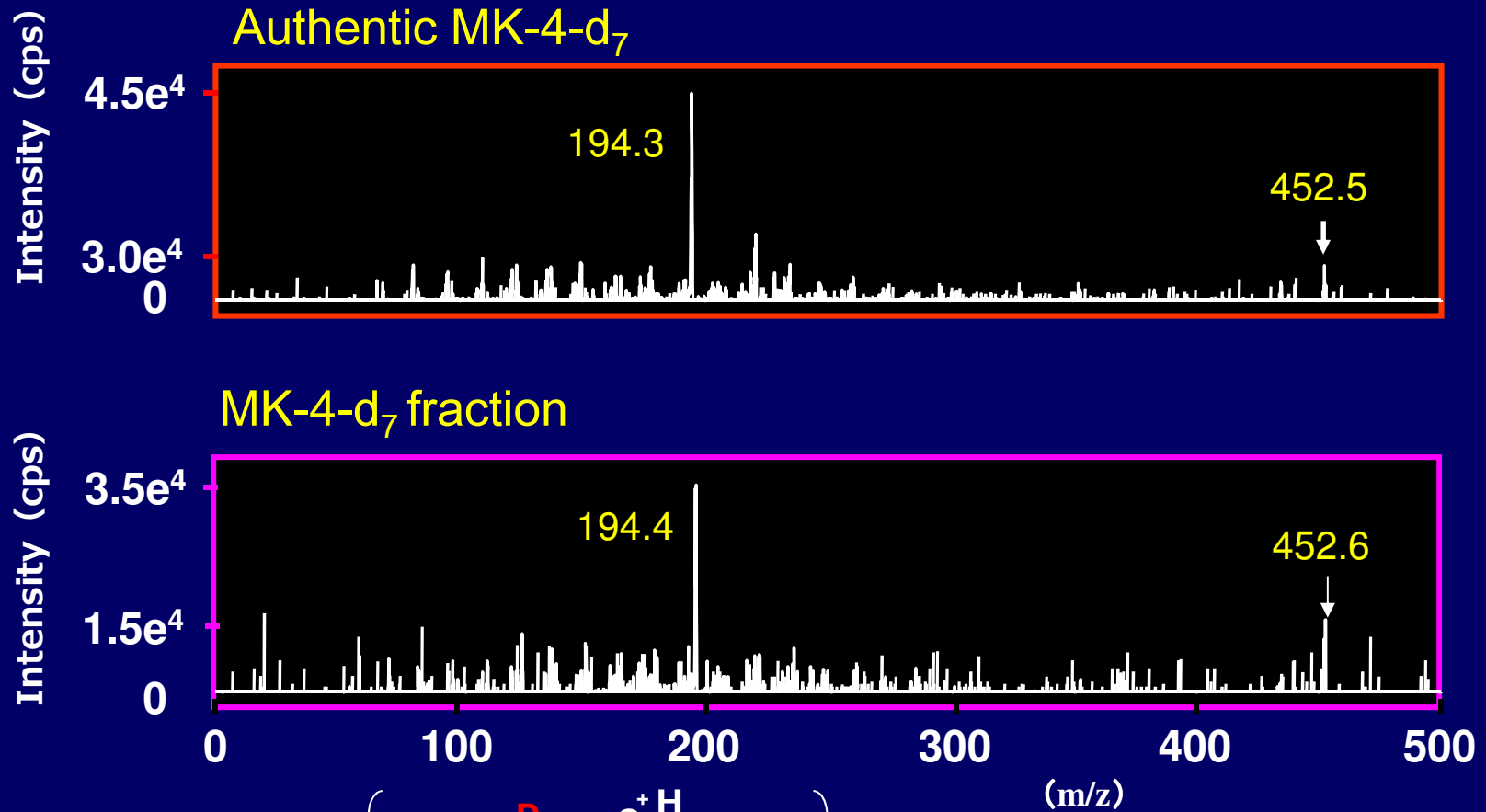
MK-4 fraction



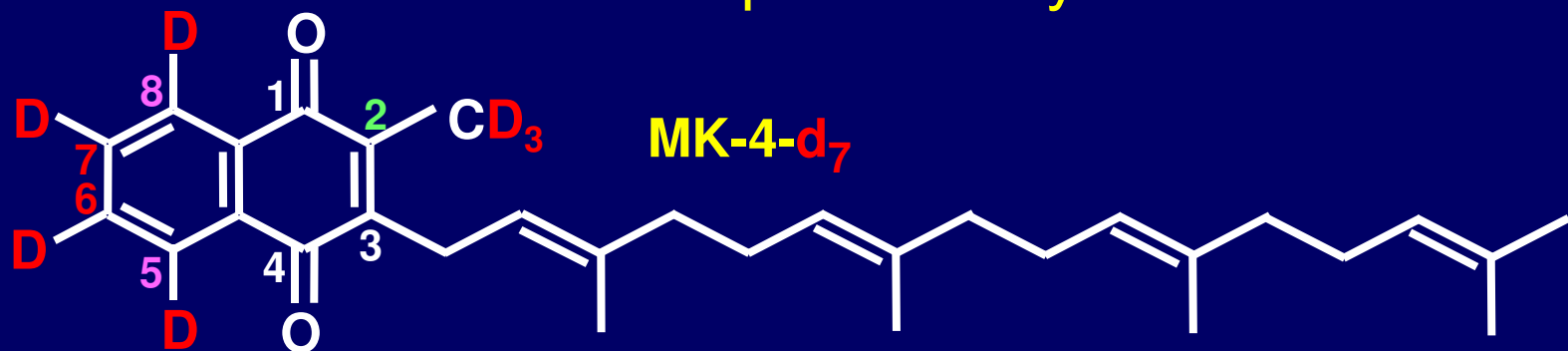
Experimental design



Identification of MK-4-d₇ from brain of mice by LC-MS/MS



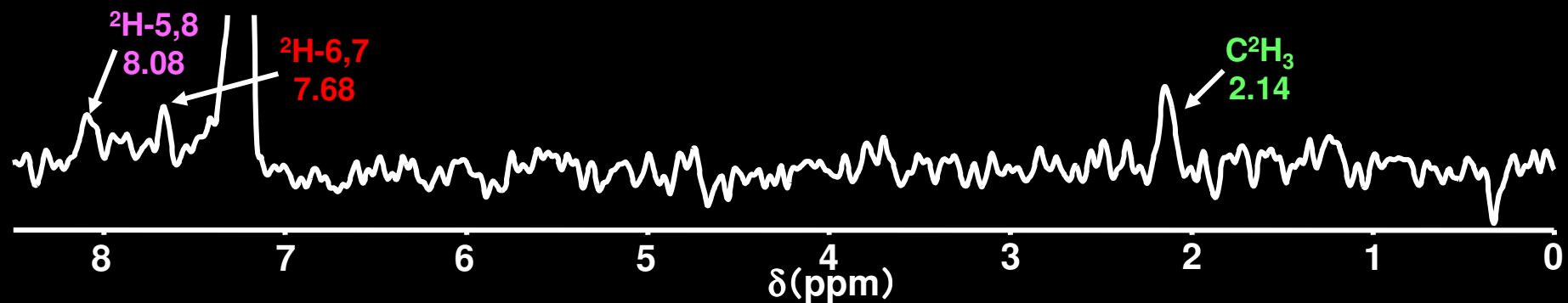
Identification of MK-4-d₇ from brain of mice by ²H NMR spectrometry



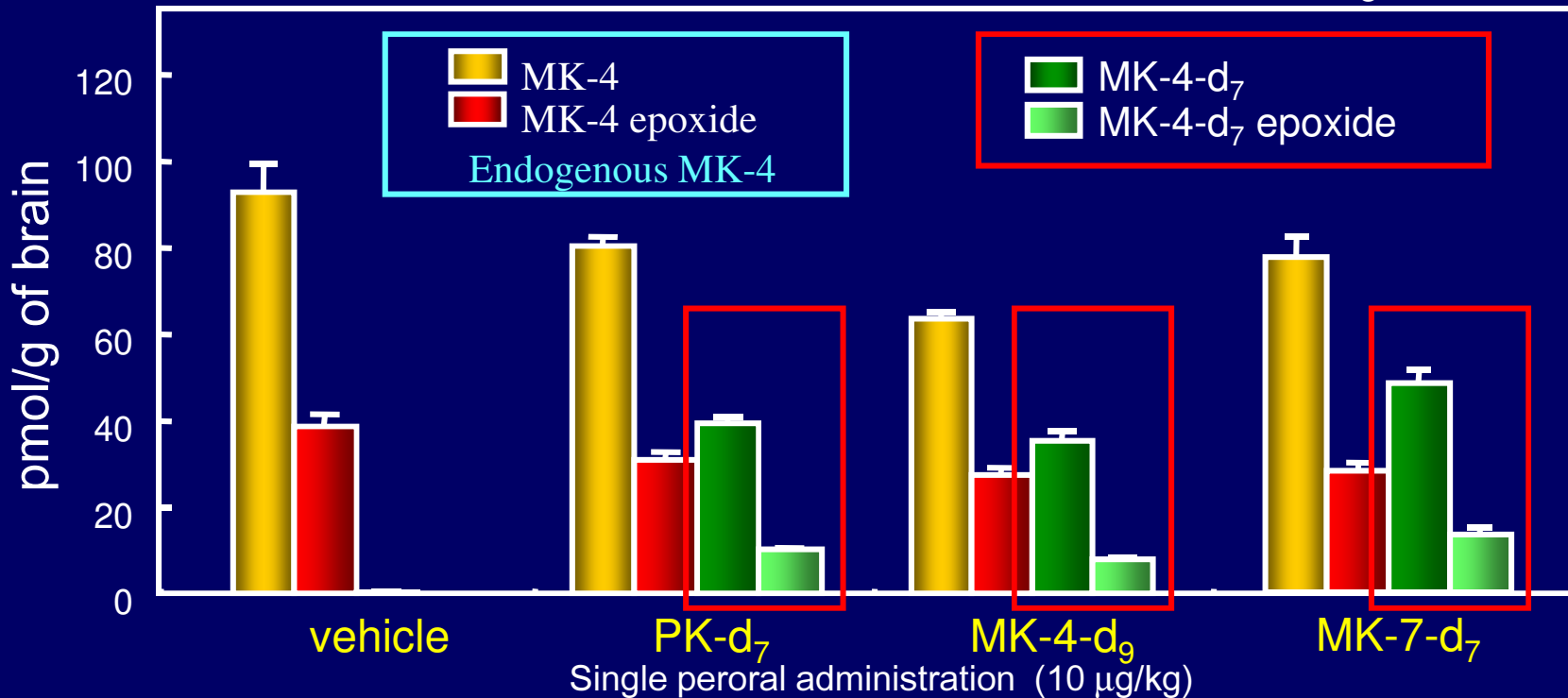
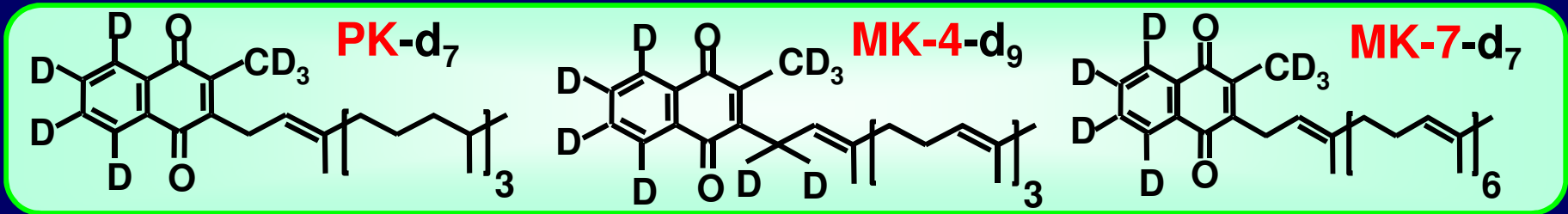
Authentic MK-4-d₇



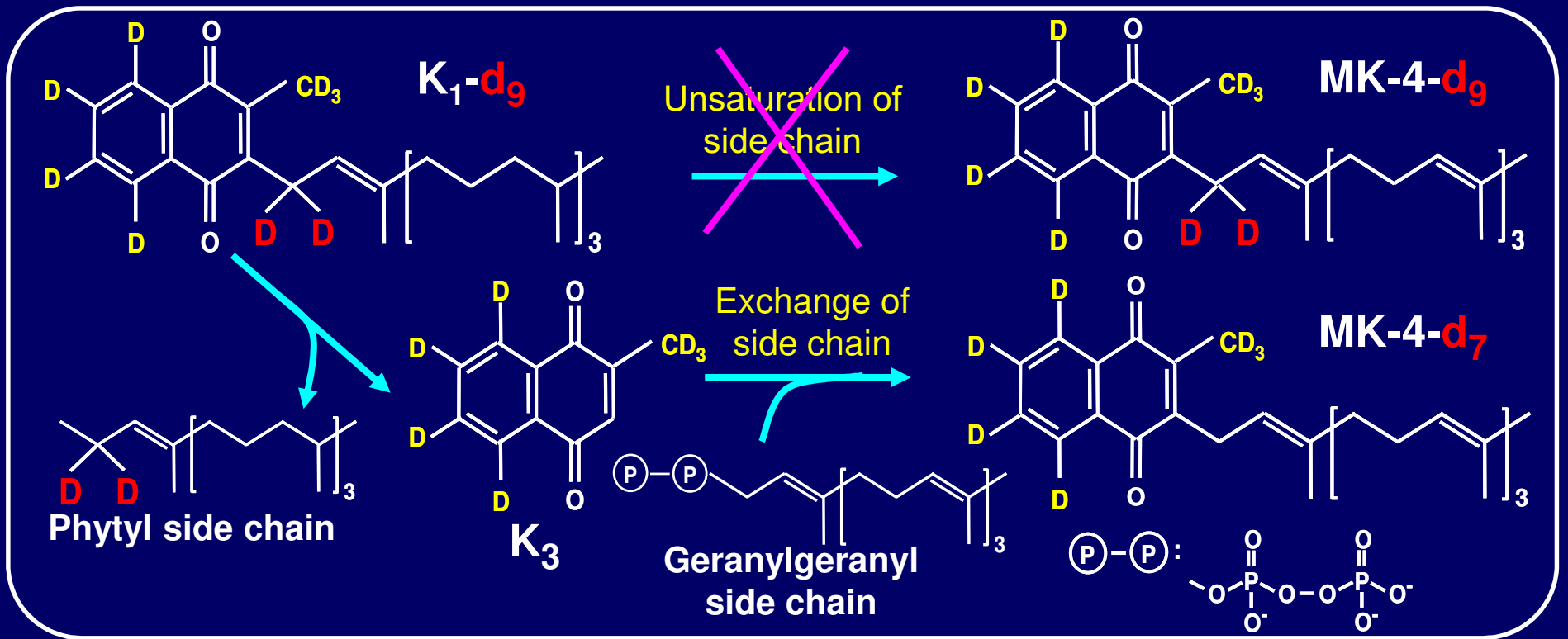
MK-4-d₇ fraction



Not only PK-d₇ but also MK-4-d₉ and MK-7-d₇ are converted into MK-4-d₇ and accumulate in brain of mice



Phylloquinone is converted into MK-4 via integral side-chain removal



K vitamins are converted into
MK-4 and accumulate in
tissues



Where does this conversion take
place?



Following four routes for the
conversion of PK or K₃ into MK-4,

1. Oral route
2. Enteral route
3. Intravenous route
4. Intra-cerebroventricular

route

were examined in mice.

Experimental design

Oral(1), enteral(2), intravenous(3) or intra-cerebroventricular(4) dose



C57BL/6J mice
7 weeks old

1 w

Fast

12 hr

0

24 hr

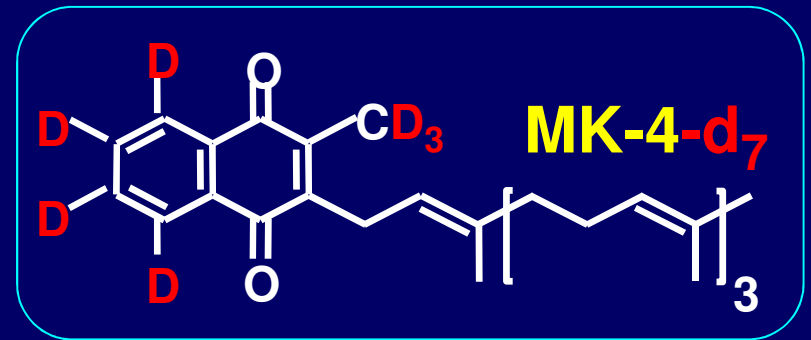
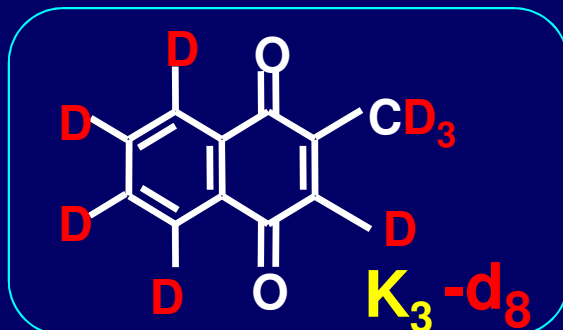
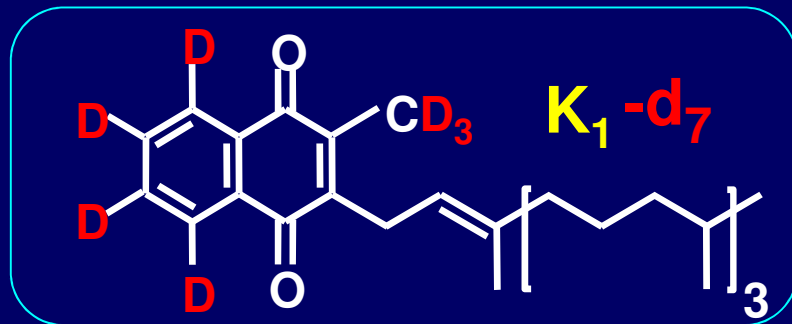
Sacrifice

Cerebra

LC-APCI//MS

A single dose of 10 $\mu\text{mol/kg}$ BW for (1), (2), (3)
At 0.1 $\mu\text{mol/Kg}$ BW for (4)

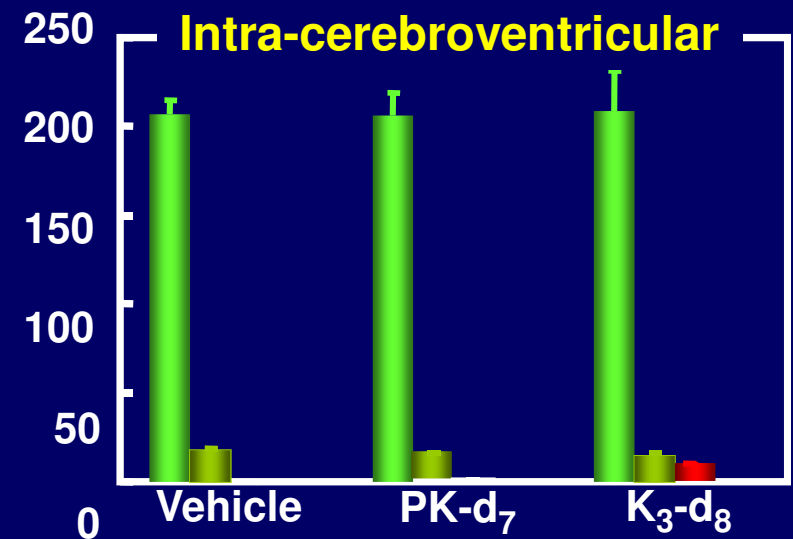
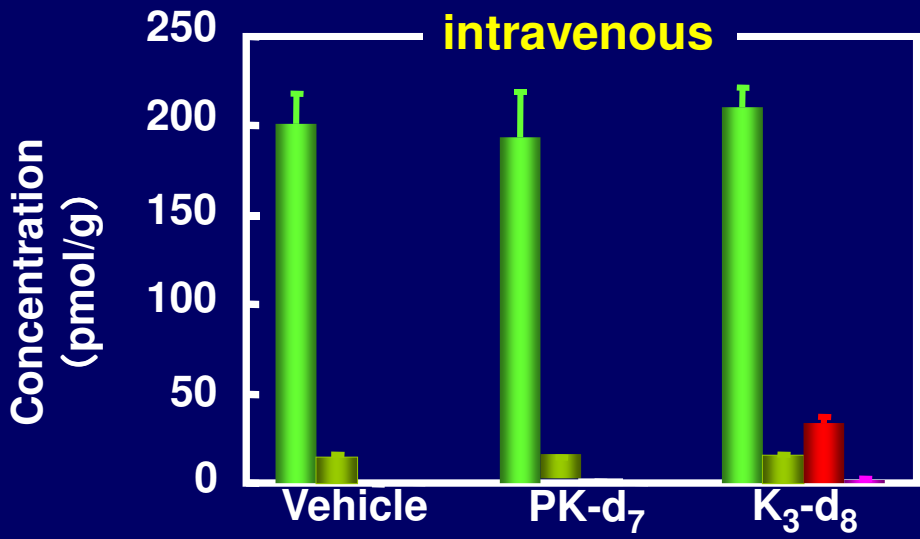
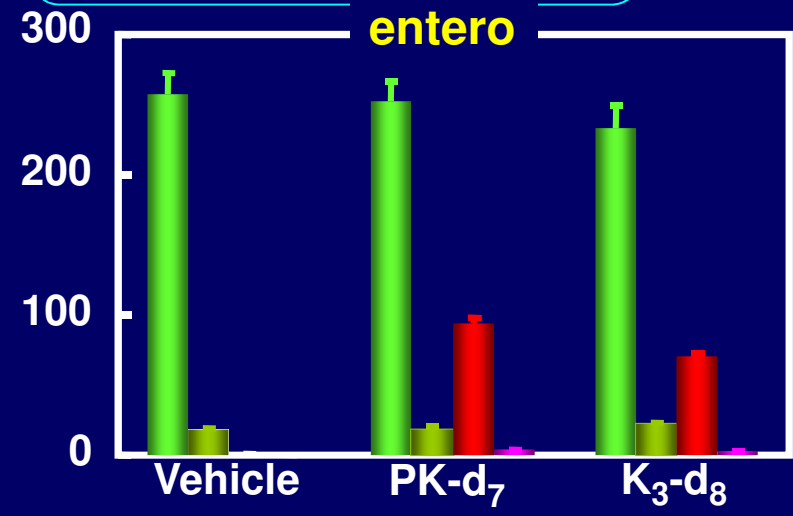
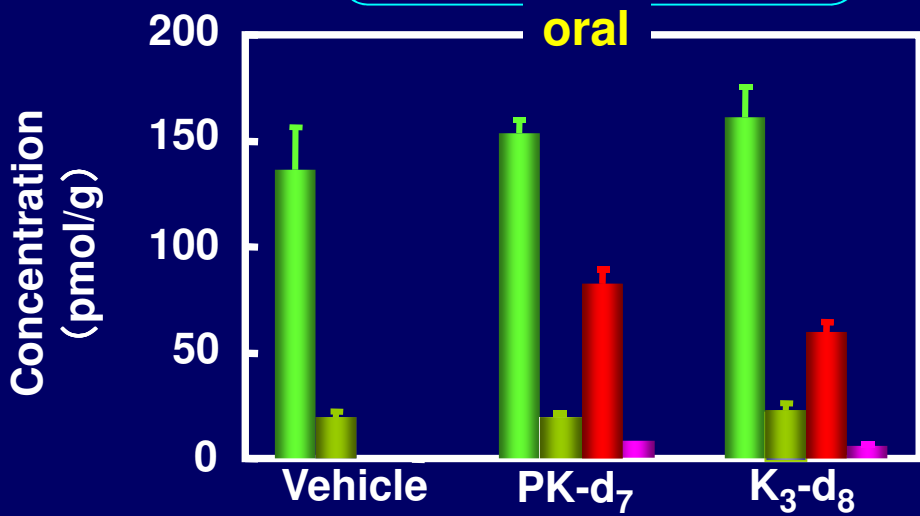
$K_1\text{-d}_7, K_3\text{-d}_8$



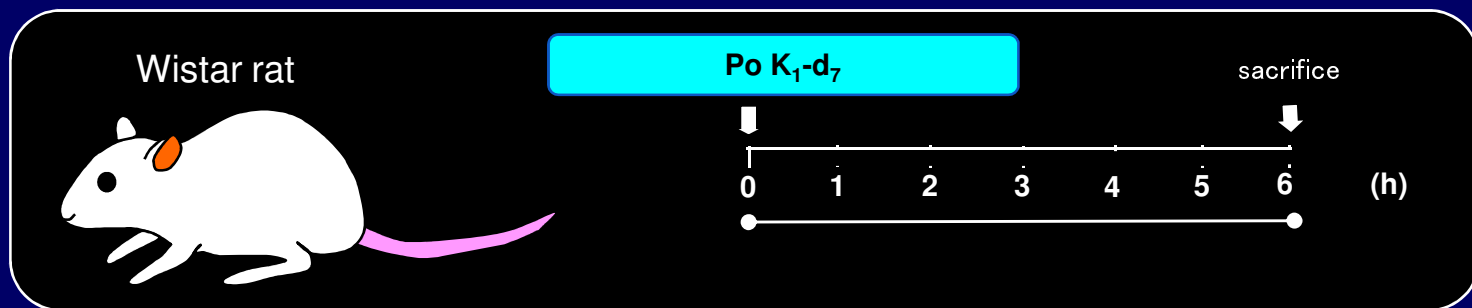
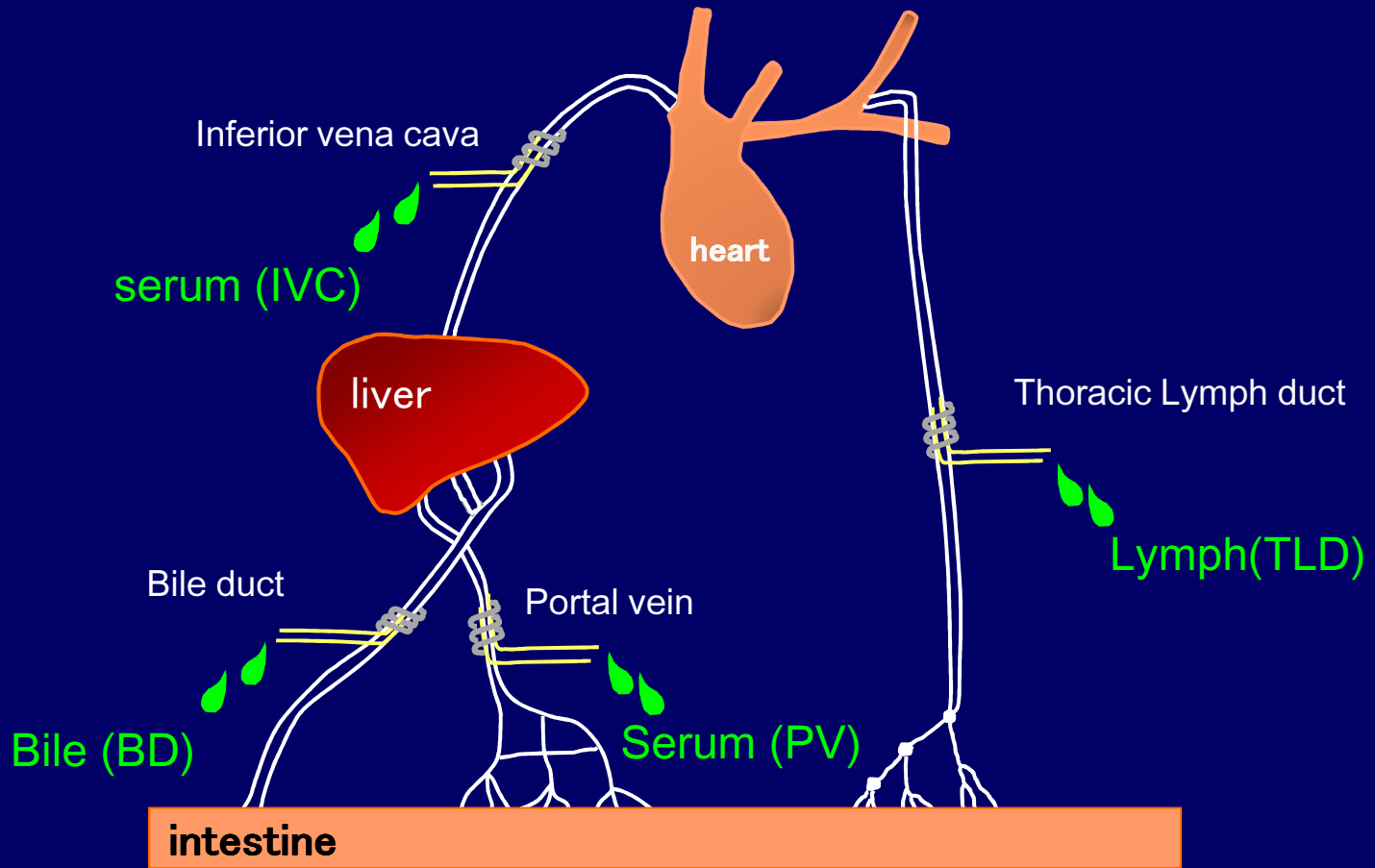
Concentrations of MK-4 in cerebra of mice

Endogenous MK-4
 ■ MK-4 ■ MK-4 epo

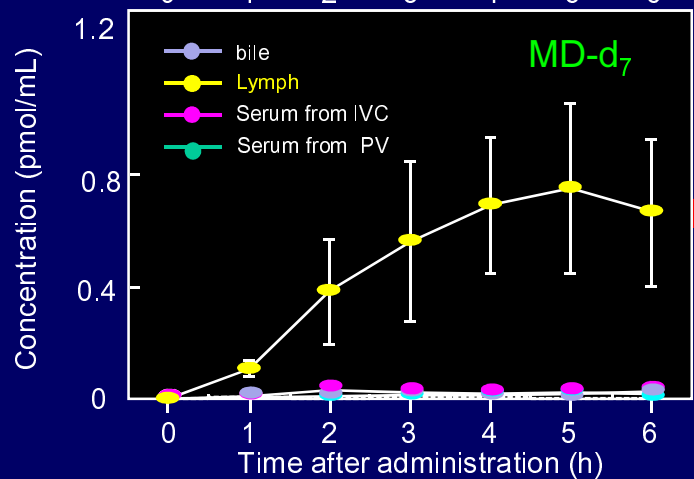
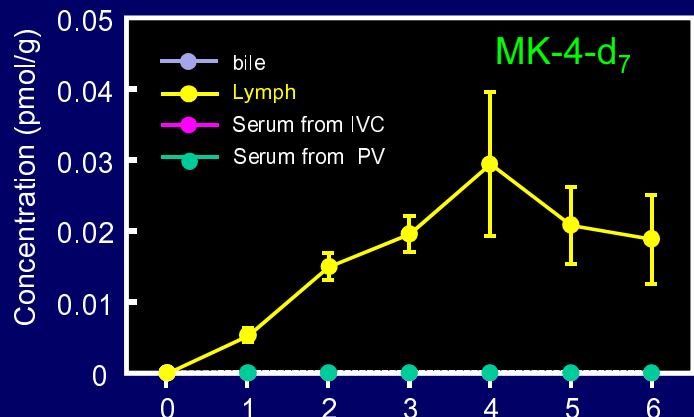
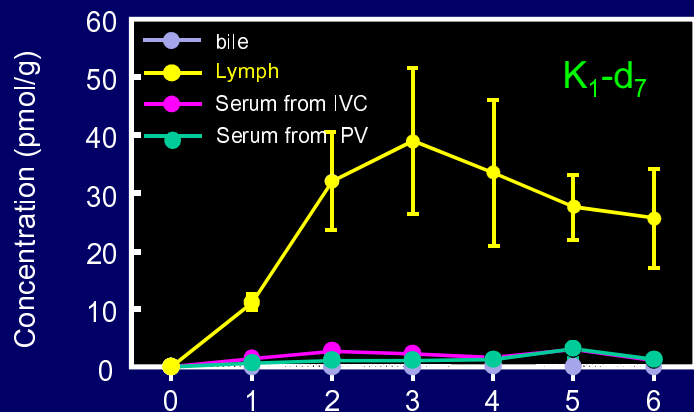
Converted MK-4
 ■ MK-4-d₇ ■ MK-4-d₇ epo



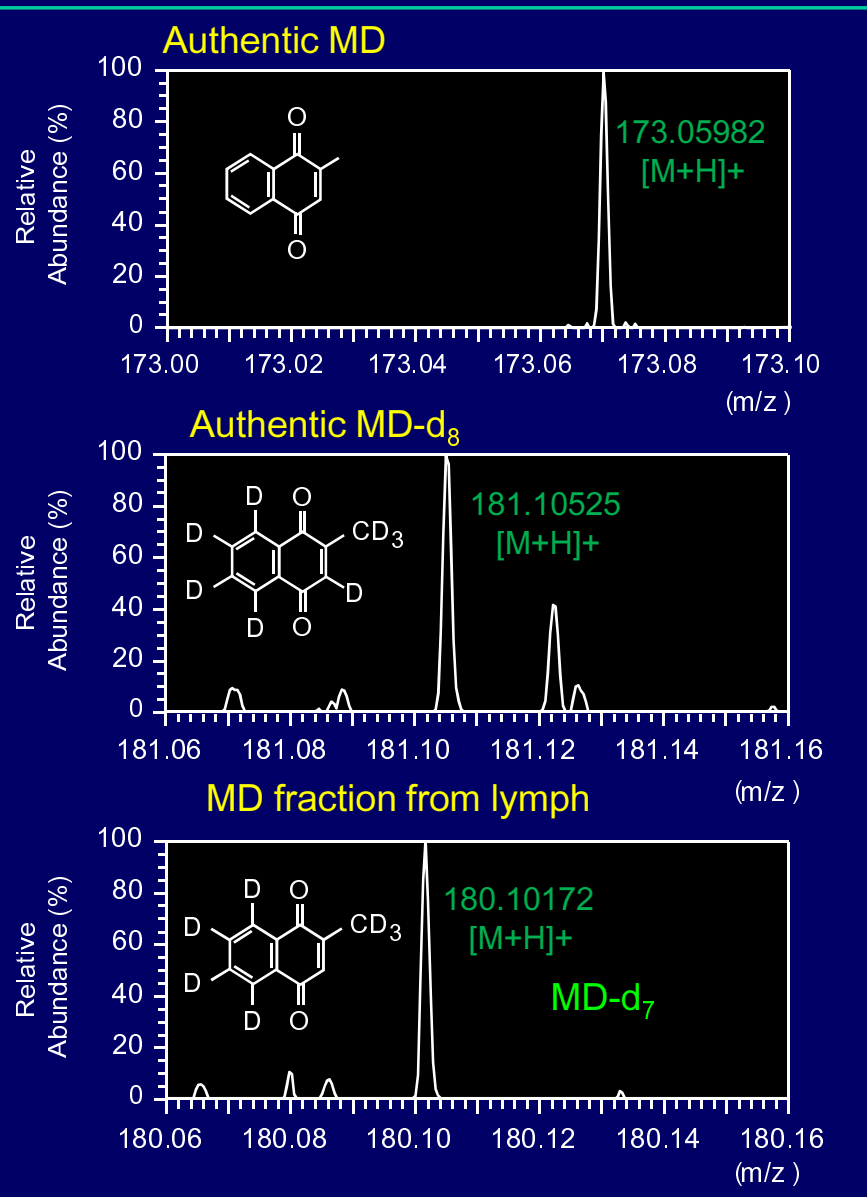
Four Sites Cannulation experiments



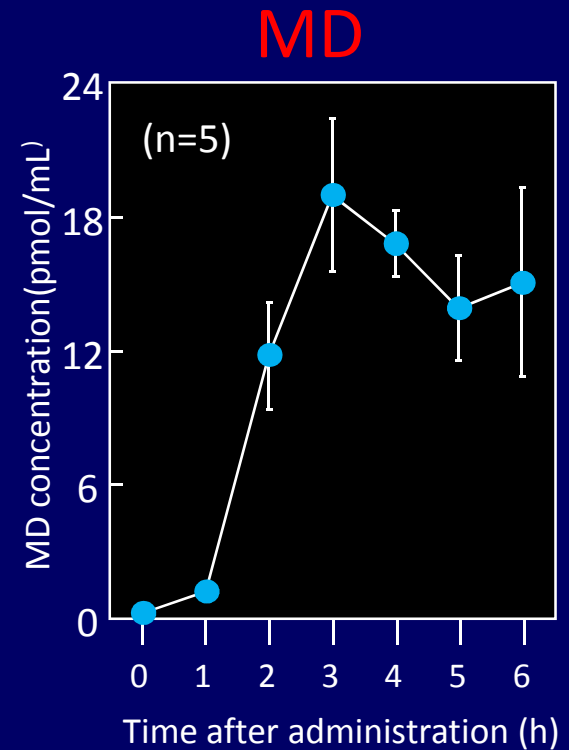
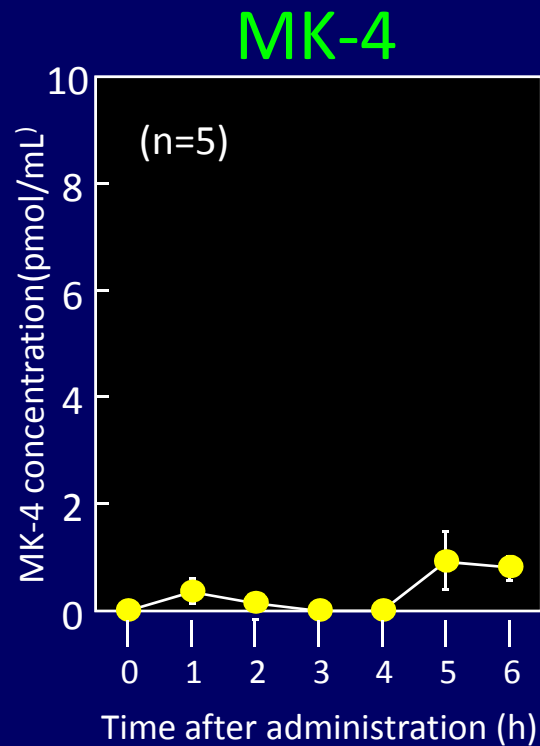
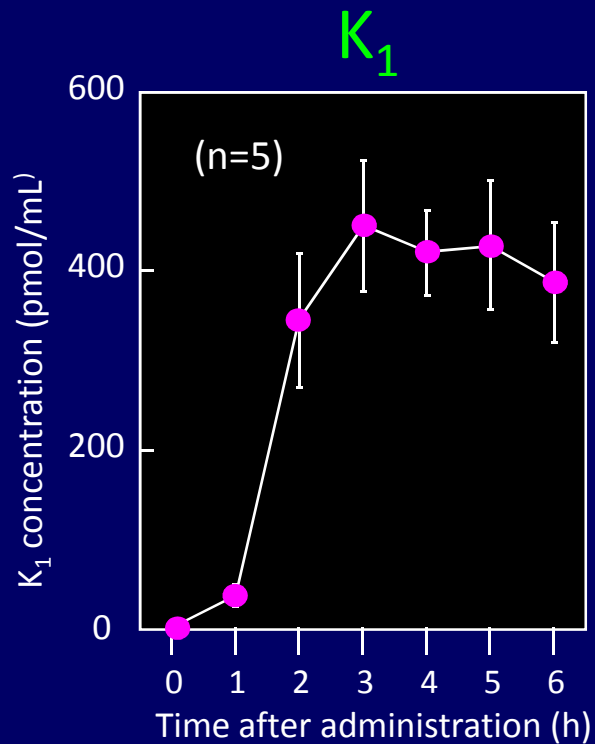
Concentrations of K₁-d₇, MK-4-d₇ and MD-d₇



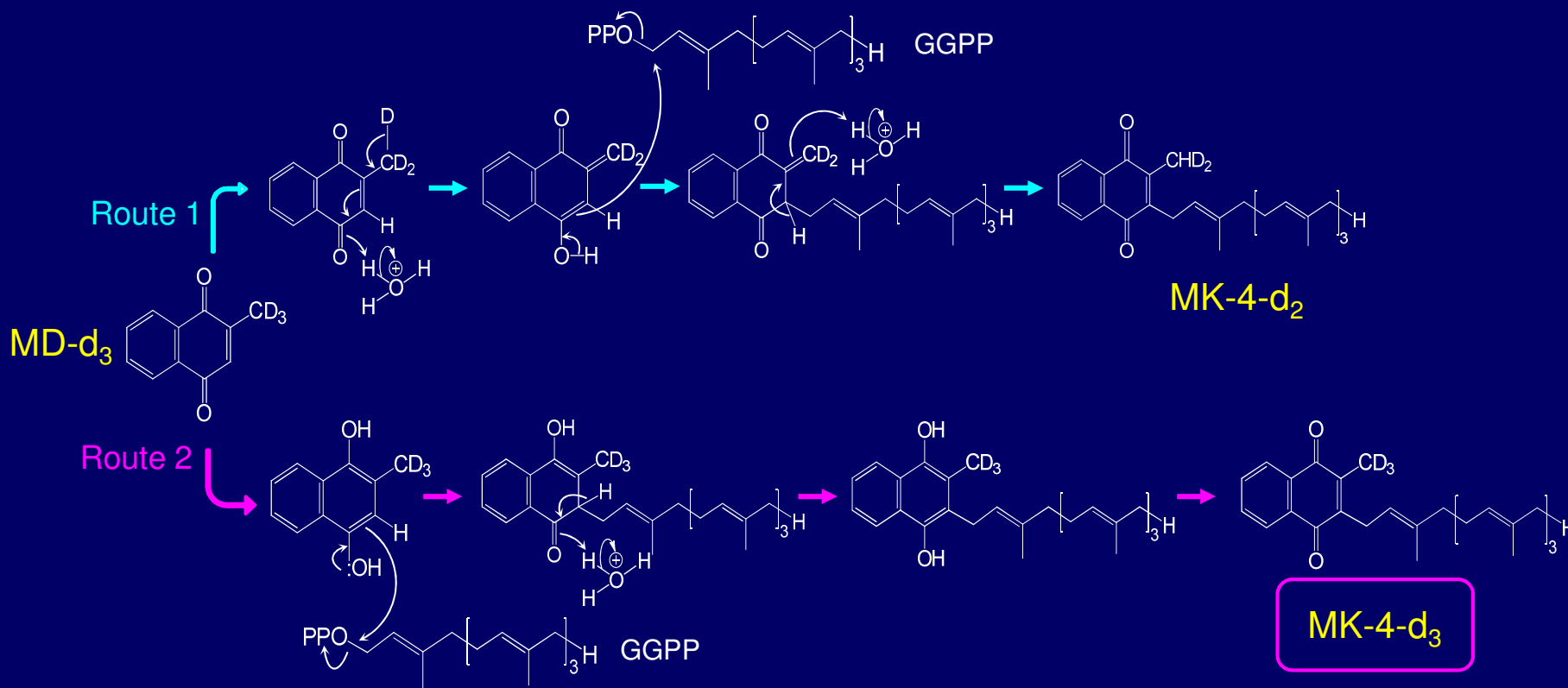
high resolution mass spectrometry (HR-MS)



Time course changes in serum concentrations of K_1 , MK-4 and MD in humans orally given K_1 capsules (40 mg)

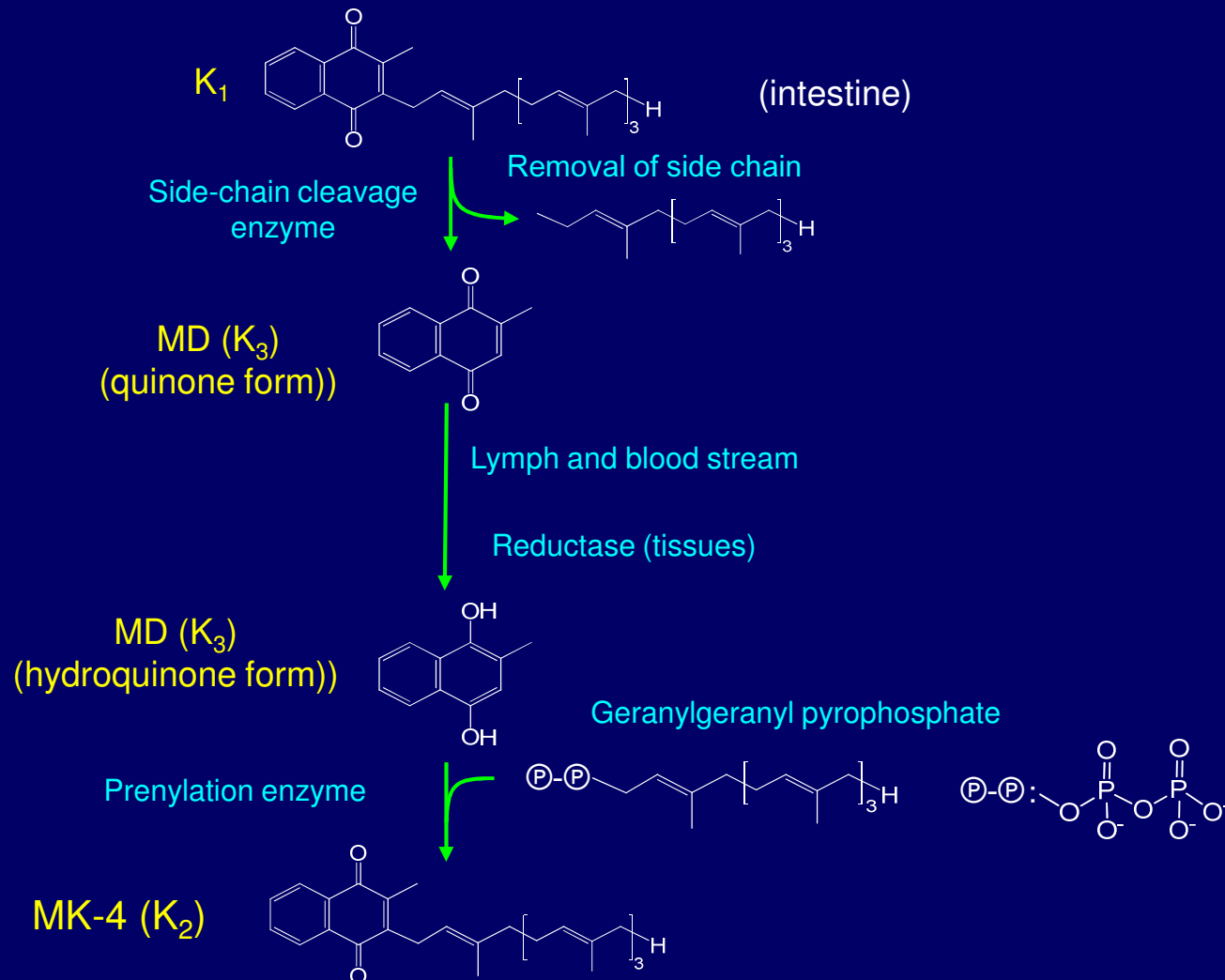


Conversion pathway of MD-d₃ to MK-4-d₃ demonstrated by HR-MS and
¹H NMR analyses (Route 2)



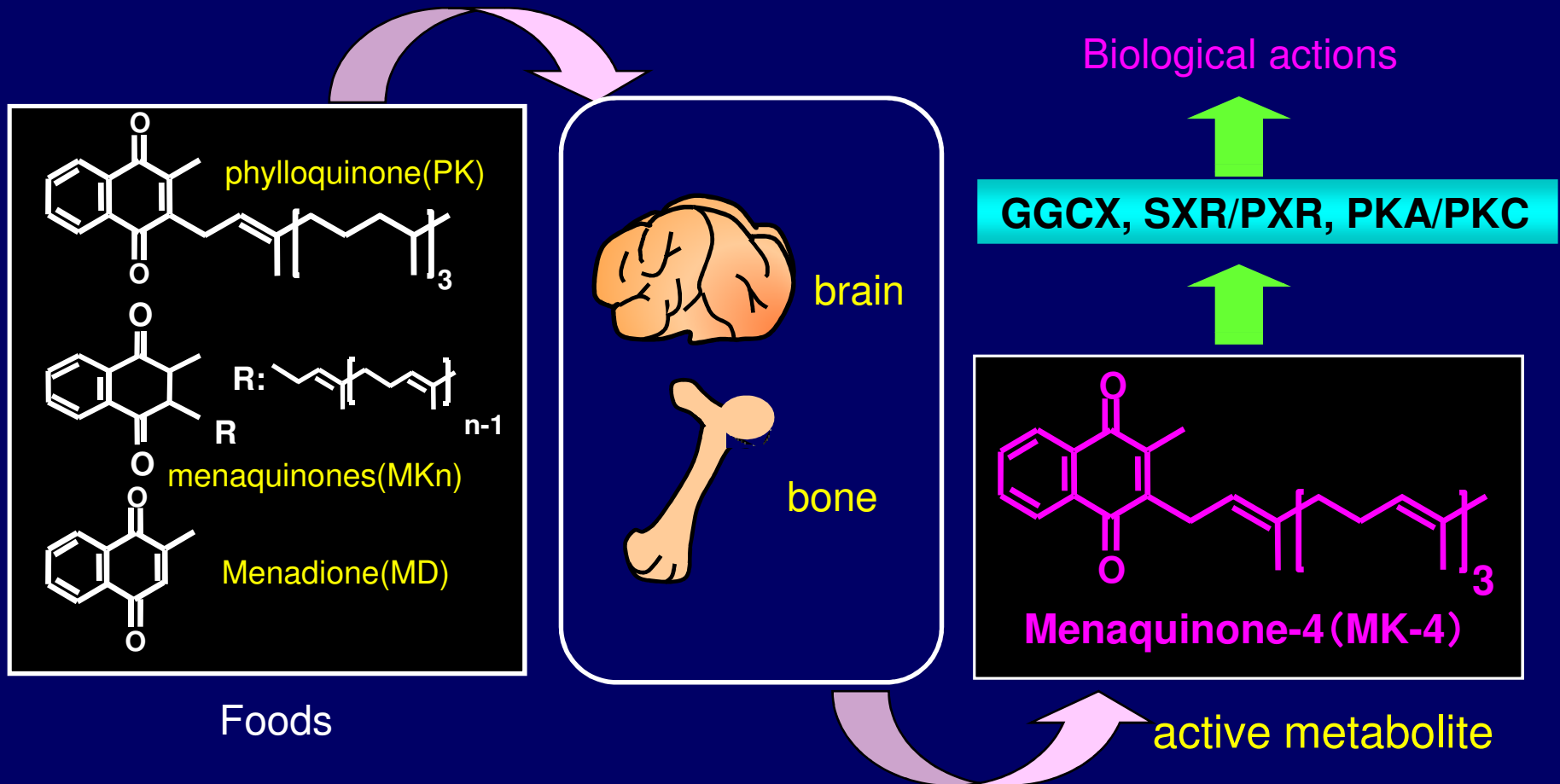
Hirota Y, Okano T, et al, J Biol Chem, 2013; 288:33071-33080

MD(K₃) is a catabolic product of oral phylloquinone (K₁) in the intestine and a circulating precursor of tissue MK-4 (K₂) in mammals

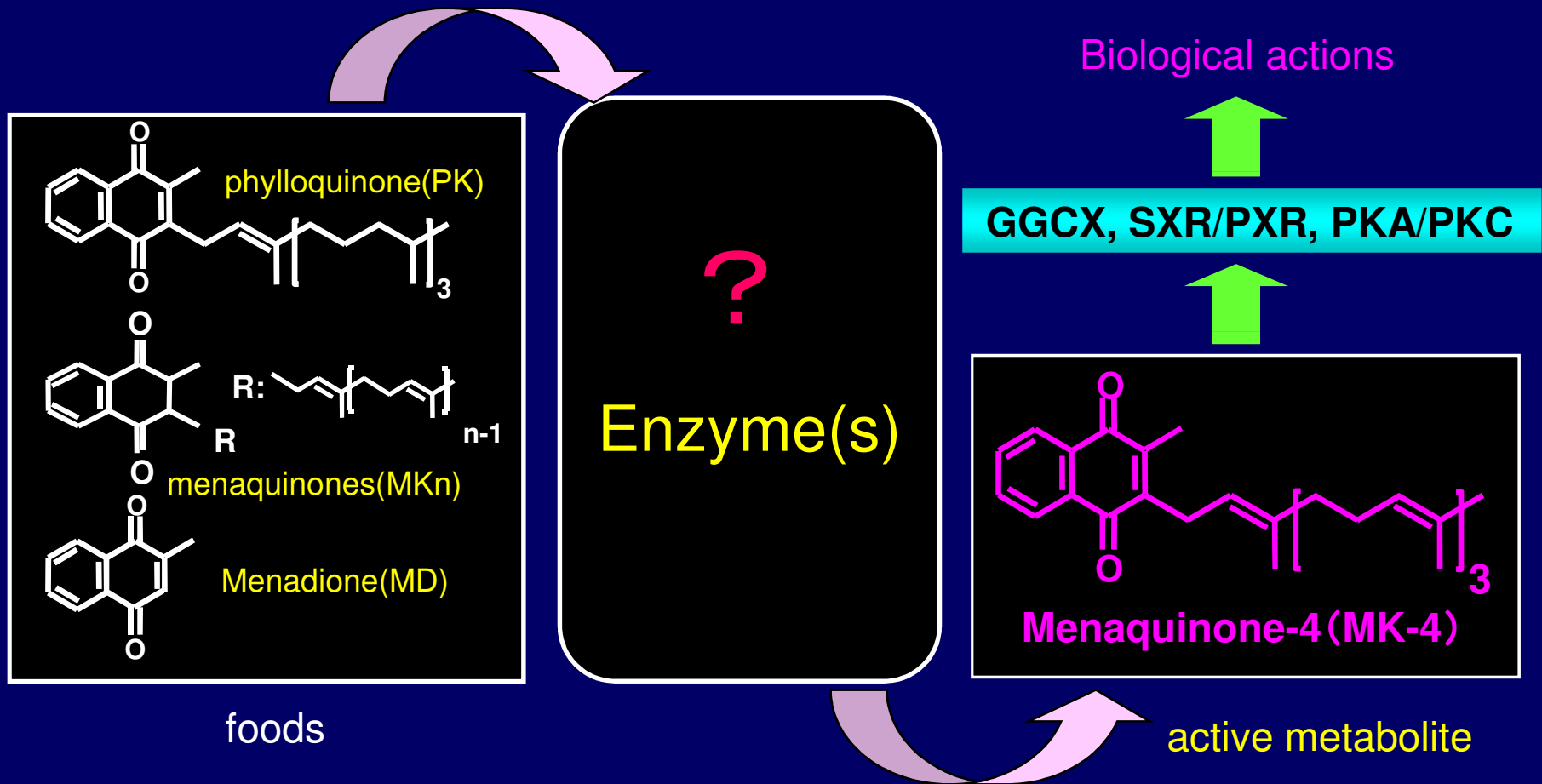


Hirota Y, Okano T, et al, J Biol Chem, 2013; 288:33071-33080

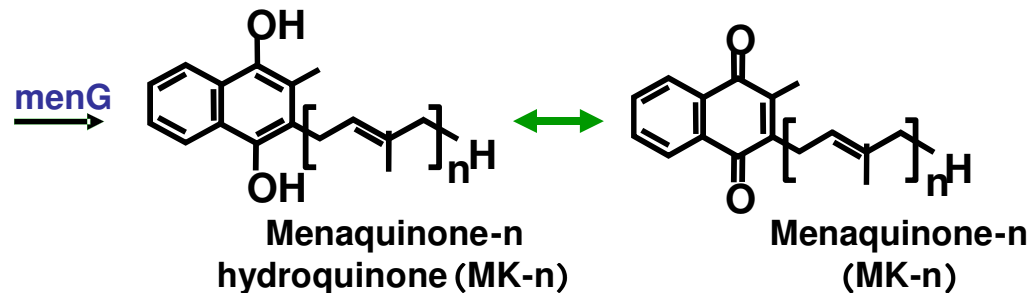
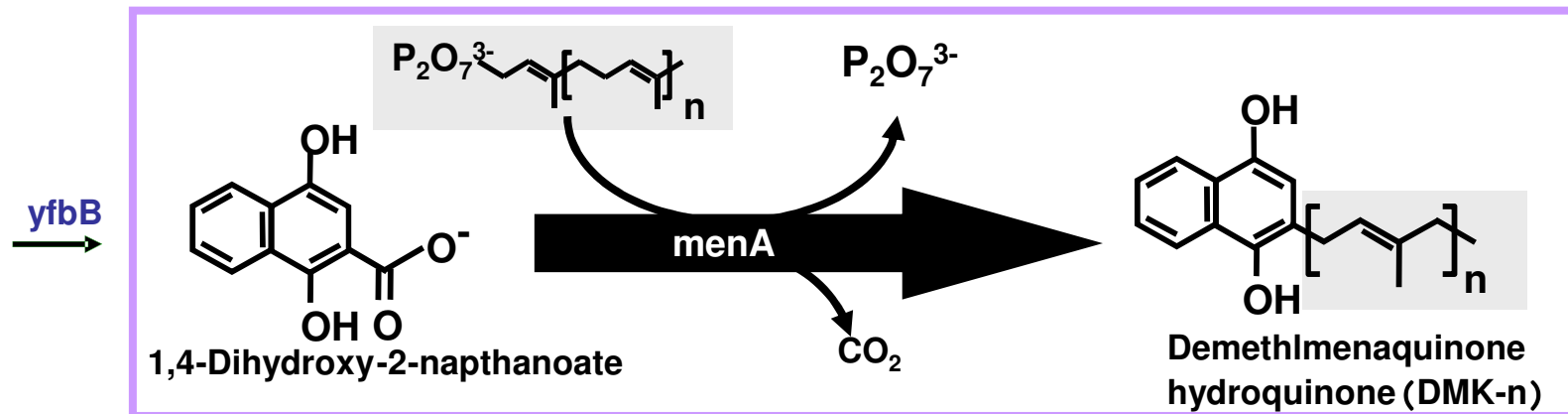
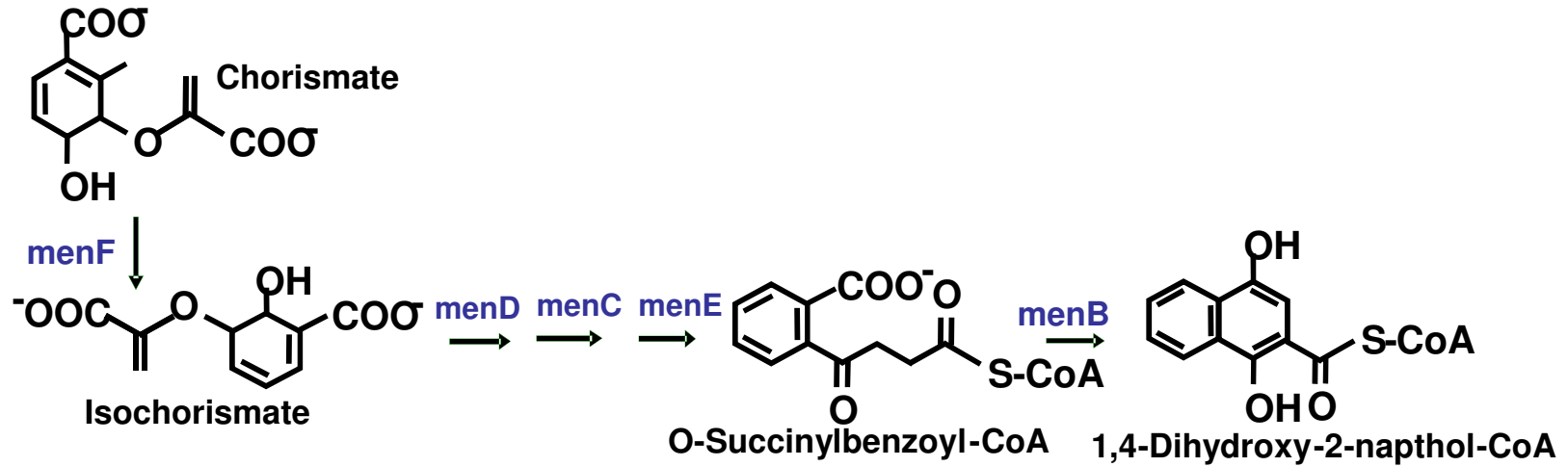
New paradigm of the metabolic activation of vitamin K in brain and bone



What is the enzyme(s) involved in MK-4 biosynthesis in mammals ?



Biosynthesis of menaquinones in *Escherichia coli*



Alignment of the amino acid sequence of Ubia(E.coli), COQ2(Homo-sapiens), Men A(E.coli) and UBIAD1(Homo-sapiens)

```

ubiA_[Escherichia_coli]          1  -----
COQ2_[Homo_sapiens]             1  MLGSRAAGFARGLRAVALAWLPGWRGRSFAALARAAGAPHGGDLQPPACPEPRGRQLSLSA
_menA_[Escherichia_coli]        1  -----MTEQQI
UBIAD1_[Homo_sapiens]           1  -----MAASQVLGKINILSGETVKAGDRDPLGNDCP

ubiA_[Escherichia_coli]          1  -MFWSLTQNKLLAFHRLMRTDKPIGALLLLWPTLWALWVATPG--VPQLWILAVFVAGVW
COQ2_[Homo_sapiens]             61  AAVVDSAPRPLQPYLRMLRDKPIGTWLLYLPCTWSIGLAAEPGCFPDWYMLSLFSGTGAI
_menA_[Escherichia_coli]        7   SRTQAWLESRLPKTLPLAFAAIIIVGTALAWWQGHFDPLVALLALITAGLLOILSNLANDY
UBIAD1_[Homo_sapiens]           33  EQDRLPQRSWRQKASYVLLALRPWSFSASLTPVALGSALAYRSHGVLDPRLLVGCVAVAL

ubiA_[Escherichia_coli]          58  LMRAGCQVNDYADRKFDPVHVKRTANRPLPSCAVTEKEARALFVVLVLIISFLLVLTNTM
COQ2_[Homo_sapiens]            121  LMRGAGCTINDMWDQDYDKKVTRTANRPLAAGDISTFQSFVFLGGQLTLALGVLLCLNY
_menA_[Escherichia_coli]        67  GDAVKGSDKPDRIQPLRGMQKGVITQOEMKRALIITVVLICLSGLALVAVACHTLADFGV
UBIAD1_[Homo_sapiens]           93  AVHGAGNLVNTYYDFSKGIDHKKSDDRITLVDRILEPQDVVRFVGFVLYTLGCVCAACLYYL

ubiA_[Escherichia_coli]          118 T-----ILLISIAALALAWVYPFMKR---YTHLPQVVLGAAFWSIPMAFAAVS-ESVPL
COQ2_[Homo_sapiens]            181  S-----IALGAGSLLLVITYPLMKR---ISYWPQLALGITFNWGLIGWSAIKGSCDPS
_menA_[Escherichia_coli]        127  F-----LILGGLSIIAAITYTVGNRPYGYIGLGDISVVLVFFGWLSVMGWSWYLAHTLIP
UBIAD1_[Homo_sapiens]           153  SPLKLEHLALIIYFGGLSGSFLYTGIGIKYVALGDLIILITFGPLAVMFAYAIQVGSIAI

ubiA_[Escherichia_coli]          168  SCWLMFLANILWAVAYDTQYAMVDRDDVKGIGIKSTAILFGQY-DKLIIGILQIGVLALM
COQ2_[Homo_sapiens]            232  VCLPLYFSGVMWTLIYDTIYAHQDKRDDVLIGLKSTALRFGEN-TKPWLSGFSVAMLGAL
_menA_[Escherichia_coli]        181  ALILPATACGLLATAVLNINNRDINSRENGKNTLVVRLGEVNRARRYHACLMLMGSIVCL
UBIAD1_[Homo_sapiens]           213  FPLVYAIPLALSTEAILHSNNTRDMESDREAGIVTLAILIGPT--FSYILYNTLLFLPYL

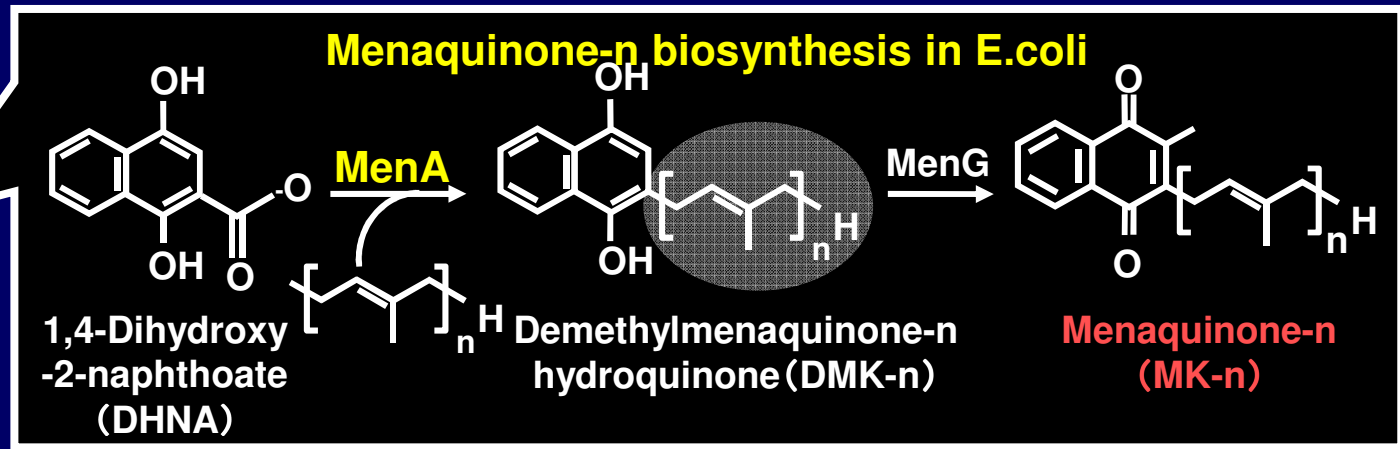
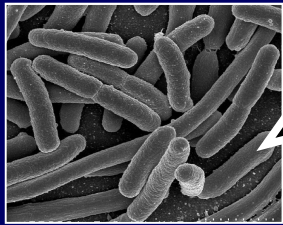
ubiA_[Escherichia_coli]          227  AIIIGELNGLGWGYYSILVAGALFVYQOKLIANREREACFKAFMNNNYVGLVFLGILAMS
COQ2_[Homo_sapiens]            291  SLVGVNSGQTAPYYAALGAVGAHLTHQIYTLDIRPEDCWNKELISNRTLGLIVFLGIVLG
_menA_[Escherichia_coli]        241  ALFNLFSLHSIWGLFLLAAPLLVKQARYVMREMPVAMRPMLERTVKGALLTNLLFVLG
UBIAD1_[Homo_sapiens]           271  VFSILATHCTIISLALPLLTIPMAFSLERQFRSQAFNKLPQRTAKLNLLGLFYVFGIILA

ubiA_[Escherichia_coli]          287  -YWHF-----
COQ2_[Homo_sapiens]            351  NLWKEKKTDKTKKGIENKIEN
_menA_[Escherichia_coli]        301  IFLSQWAA-----
UBIAD1_[Homo_sapiens]           331  PAGSLPKI-----






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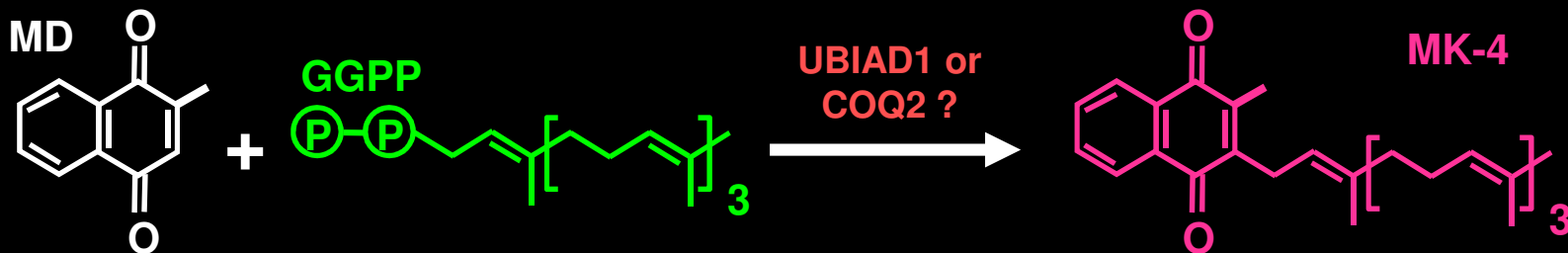
Search for the enzyme(s) responsible for the MK-4 synthesis

E.coli

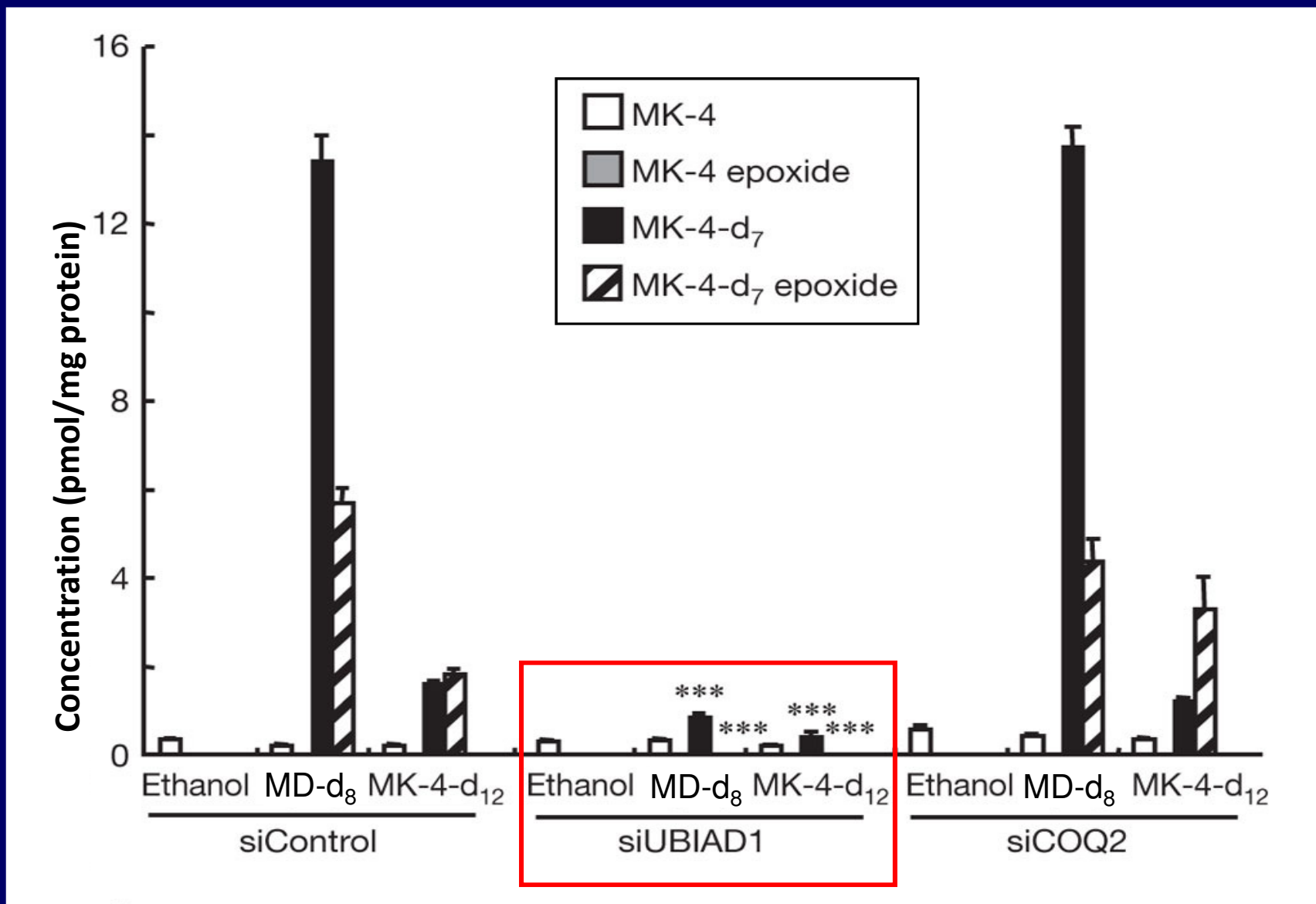


MenA human homologs

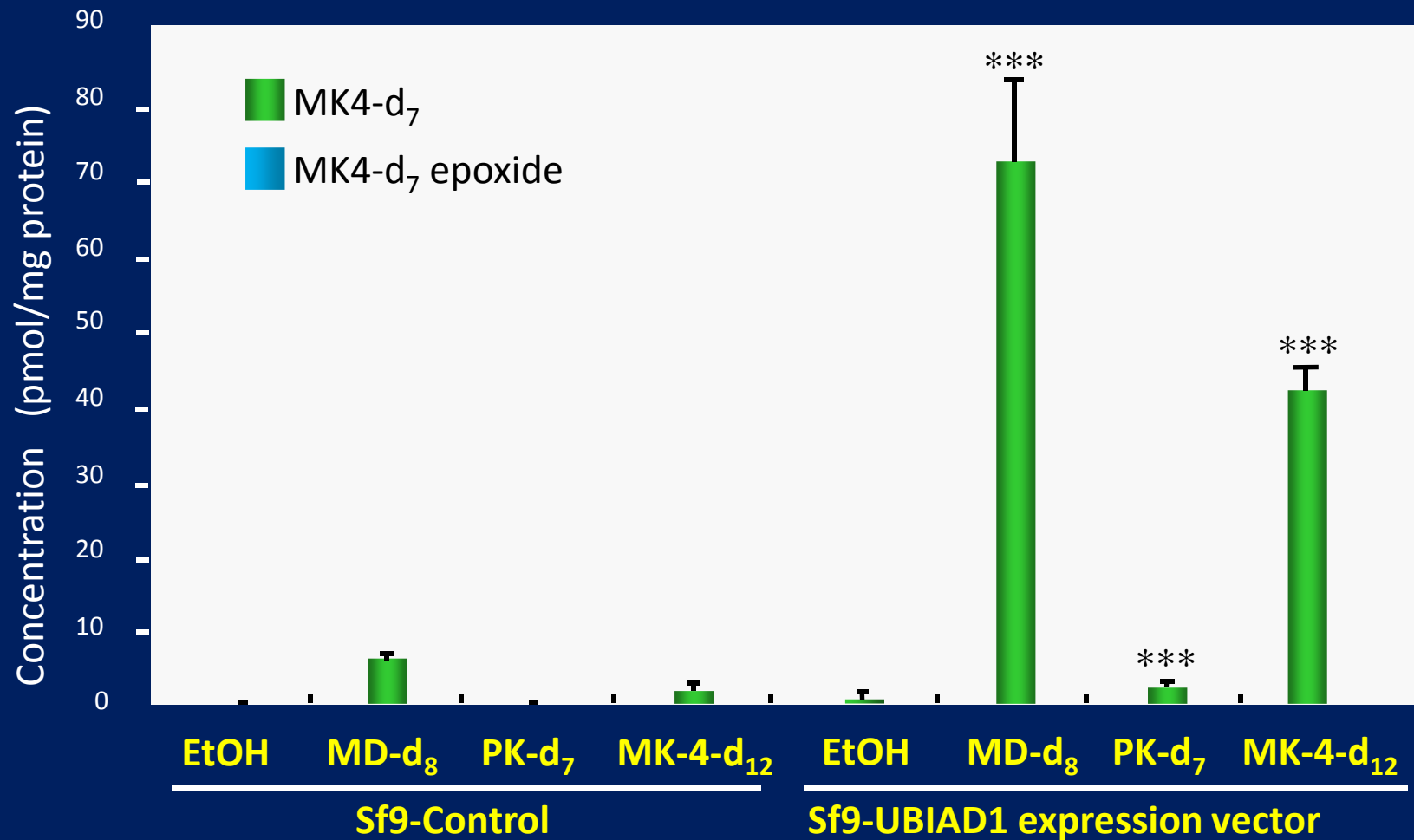
MenA		311 a.a	Chromosome 1 11.25 M
UbiA prenyltransferase domain containing 1 (UBIAD1)		338 a.a	
CoenzymeQ2 homolog, prenyltransferase (COQ2)		384 a.a	Chromosome 4 84.40 M 



Conversion of K₃-d₈ or MK-4-d₁₂ to MK-4-d₇ in siControl-, siUBIAD1- or siCOQ2-transfected MG-63 cells

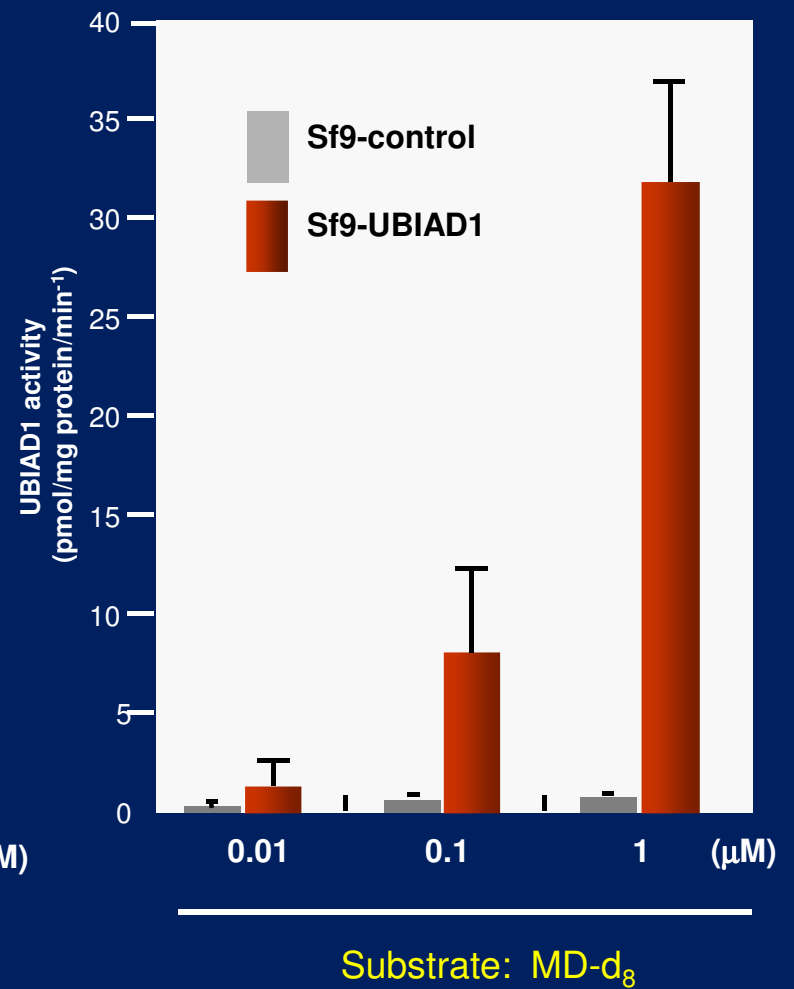
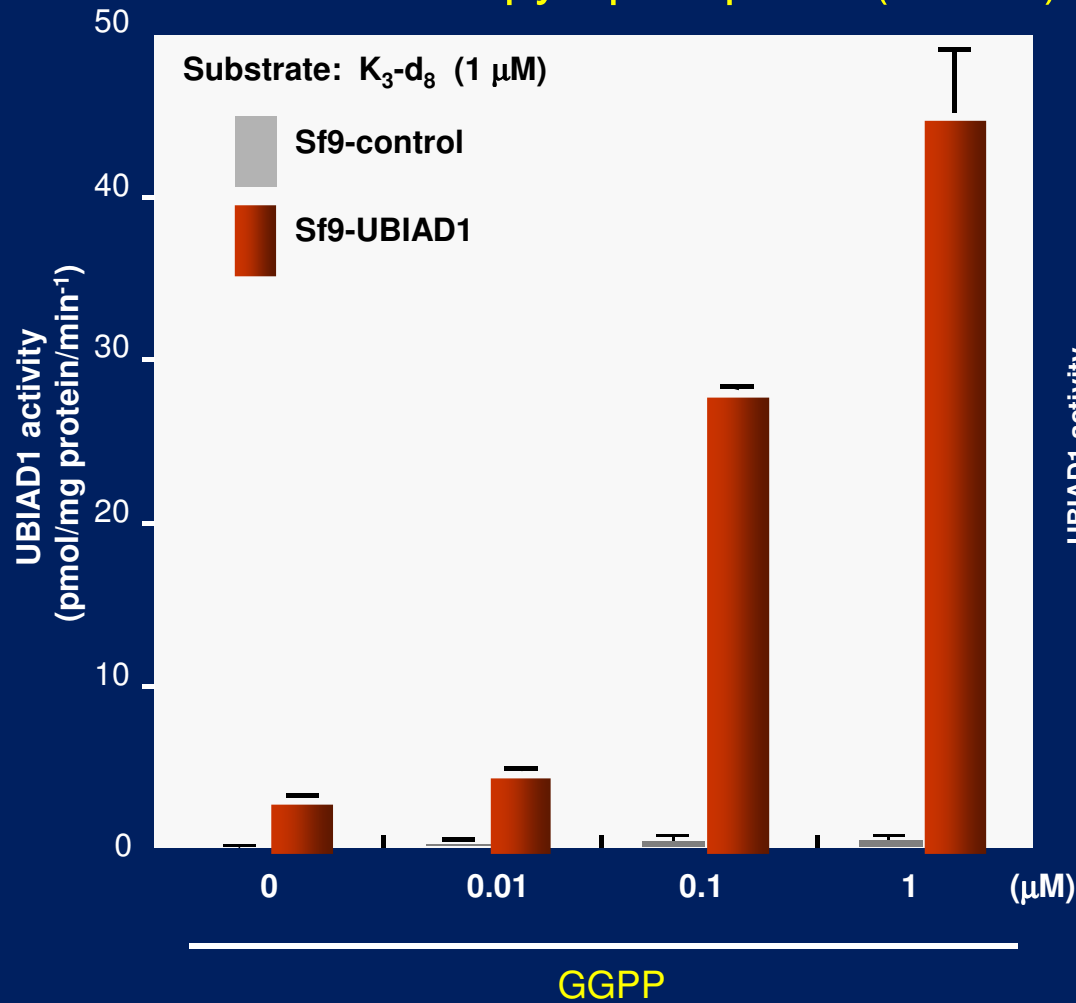


Conversion of PK-d₇ and MK-4-d₁₂ into MK-4-d₇ in Sf9 cells transfected with siControl or pcDNA3.3-UBIAD1(UBIAD1 expression vector)

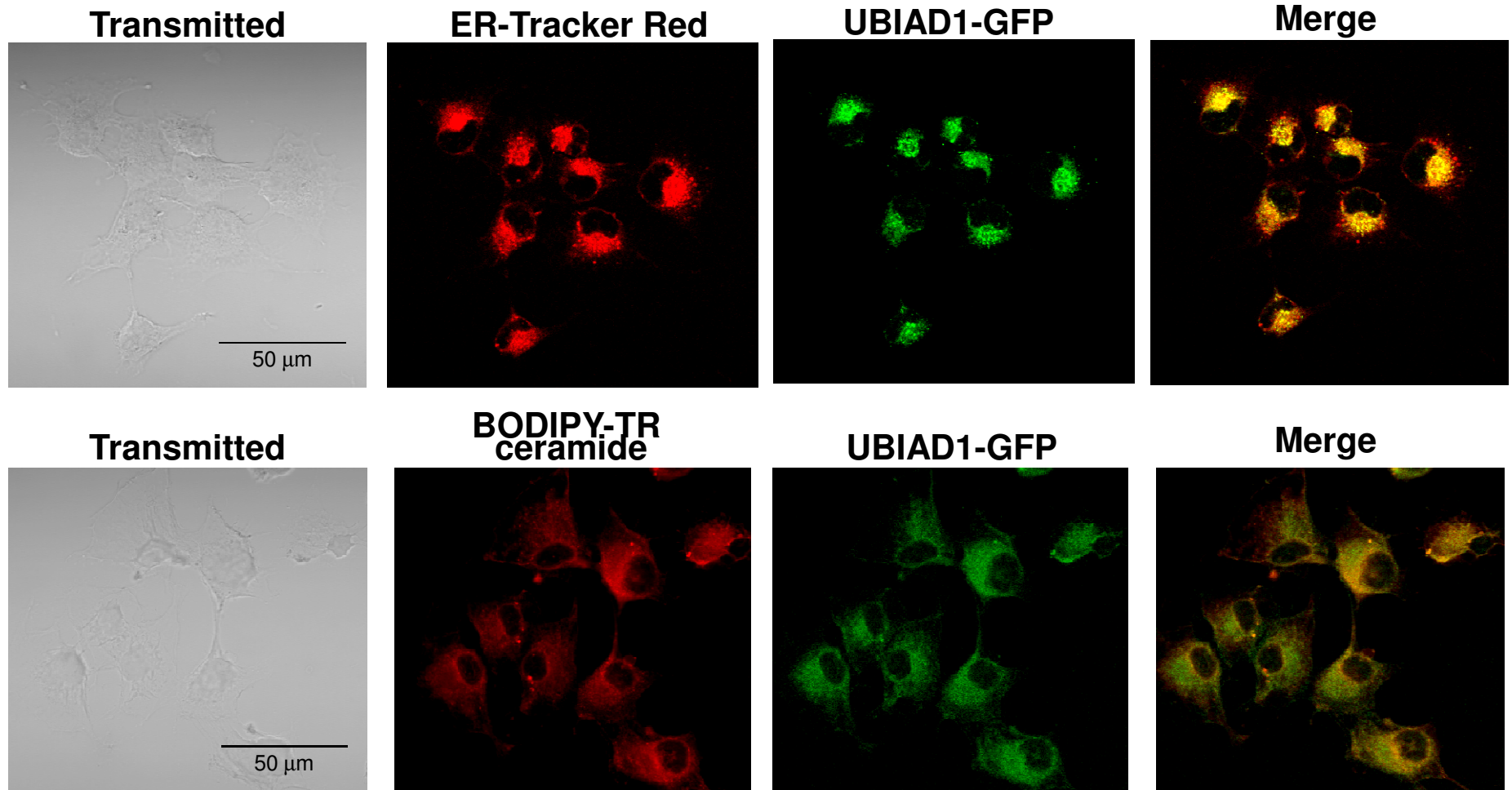


Values are means and s.e.m. ($n=6$). Three asterisks, $P < 0.001$ versus control-infected Sf9 cells with the same compound treatment.

MK-4 biosynthetic activity of UBIAD1 in microsomes prepared from *UBIAD1* baculovirus-infected Sf9 cells with geranylgeranyl pyrophosphate (GGPP) and MD



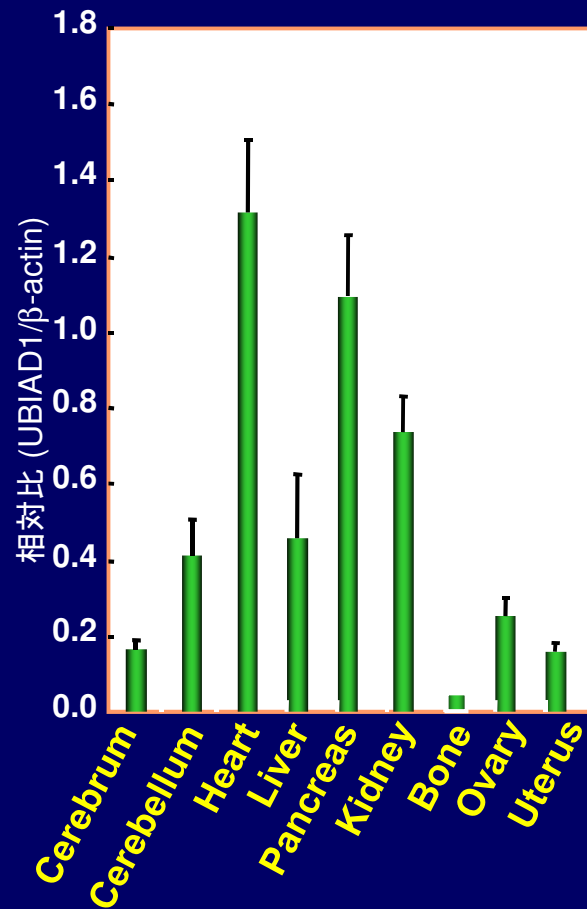
Subcellular localization of UBIAD1 in MG-63 cells



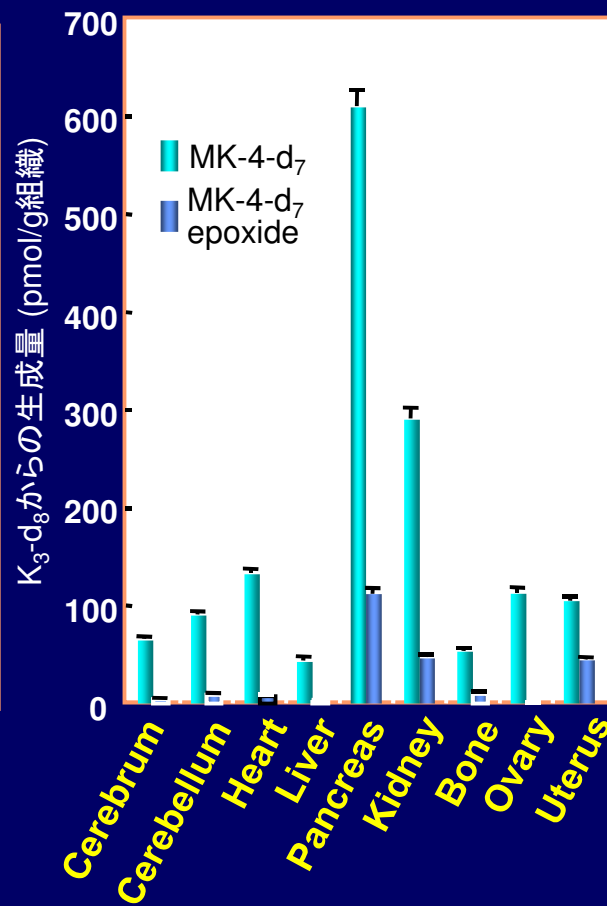
MG-63 cells stably transfected with a UBIAD1–GFP expression vector were stained with ER-tracker Red or BODIPY-TR ceramide (red) and were detected by GFP fluorescence (green). Merged images of GFP fluorescence and by ER-marker or Golgi-marker fluorescence are shown at the right. The control construct (mock-GFP) showed a diffuse fluorescence throughout the cytoplasm.

UBIAD1 mRNA expression, MK-4 biosynthetic activity, concentrations of MK-4 and its epoxide in tissues of female mice

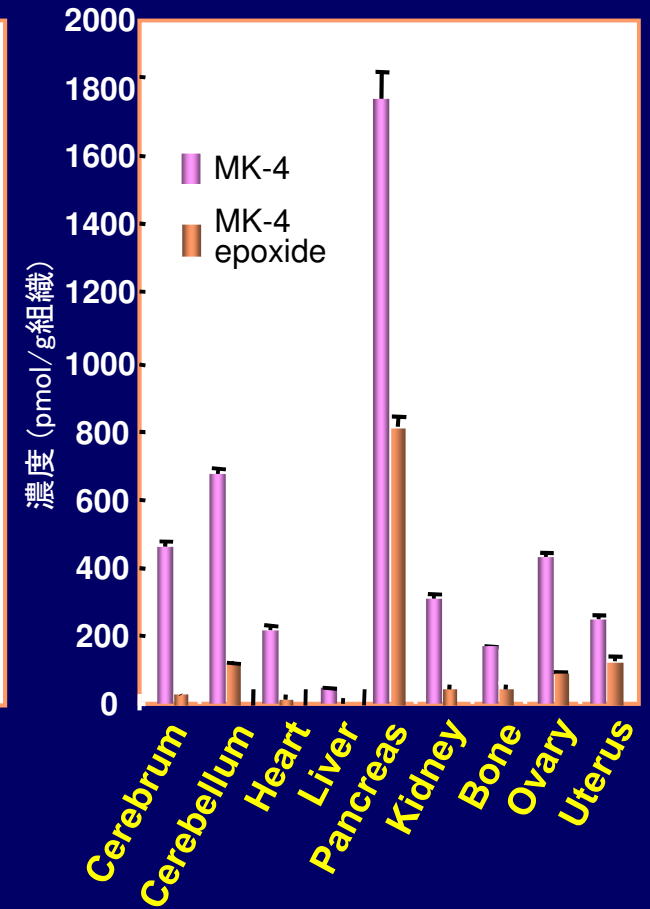
UBIAD1 mRNA



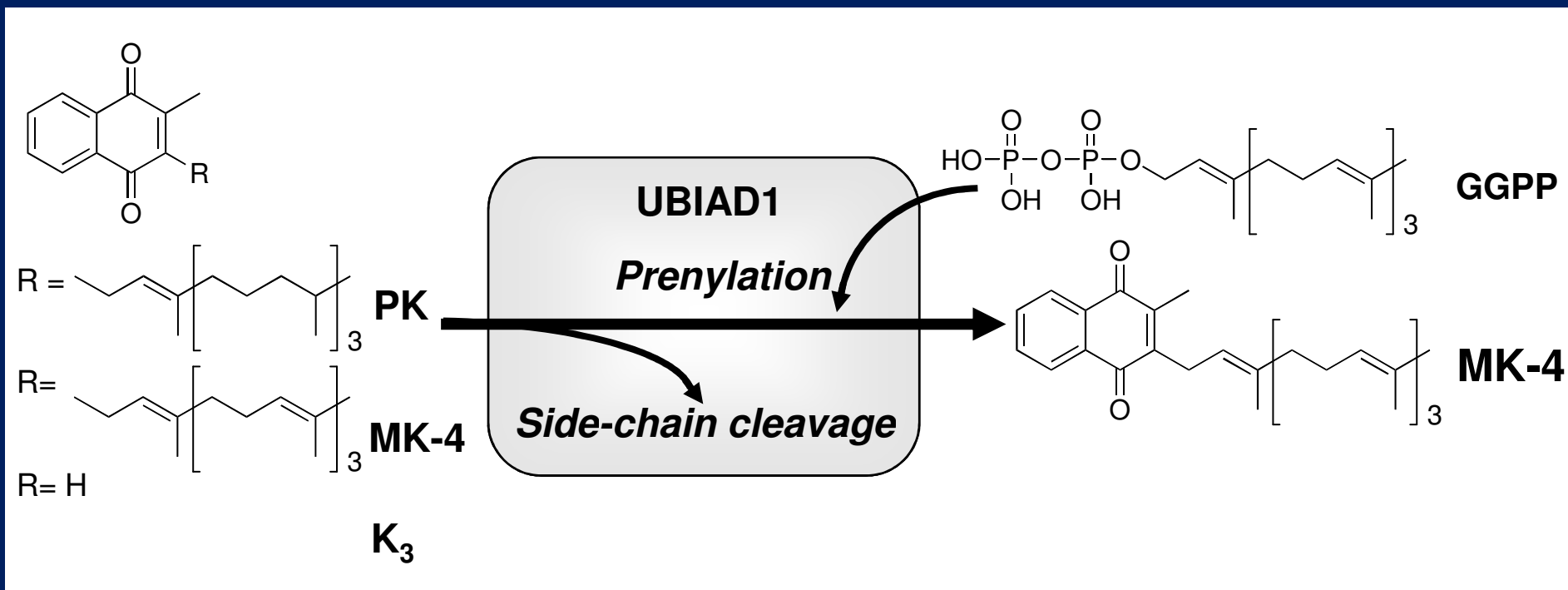
MK-4-d₇ biosynthetic activity



Amounts of MK-4 and its epoxide



UBIAD1 is a novel biosynthetic enzyme for MK-4 that may have both side-chain cleavage and prenylation activities



Identification of UBIAD1 as a novel human menaquinone-4 biosynthetic enzyme

Nature 2010; 468:117-121.

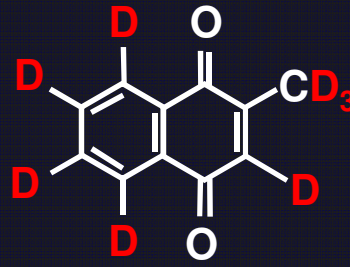
Kimie Nakagawa, Yoshihisa Hirota, Natsumi Sawada, Naohito Yuge, Masato Watanabe, Yuri Uchino, Naoko Okuda, Yuka Shimomura, Yoshitomo Suhara & Toshio Okano
Department of Hygienic Sciences, Kobe Pharmaceutical University,

MK-4 biosynthesis in tissues is decreased by the treatments with statins and bisphosphonates

Vitamin K homologues



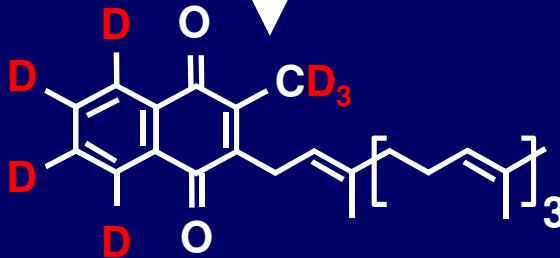
K₁-d₇



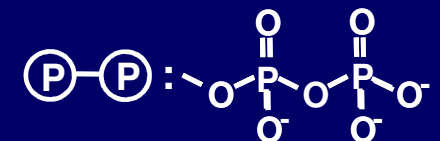
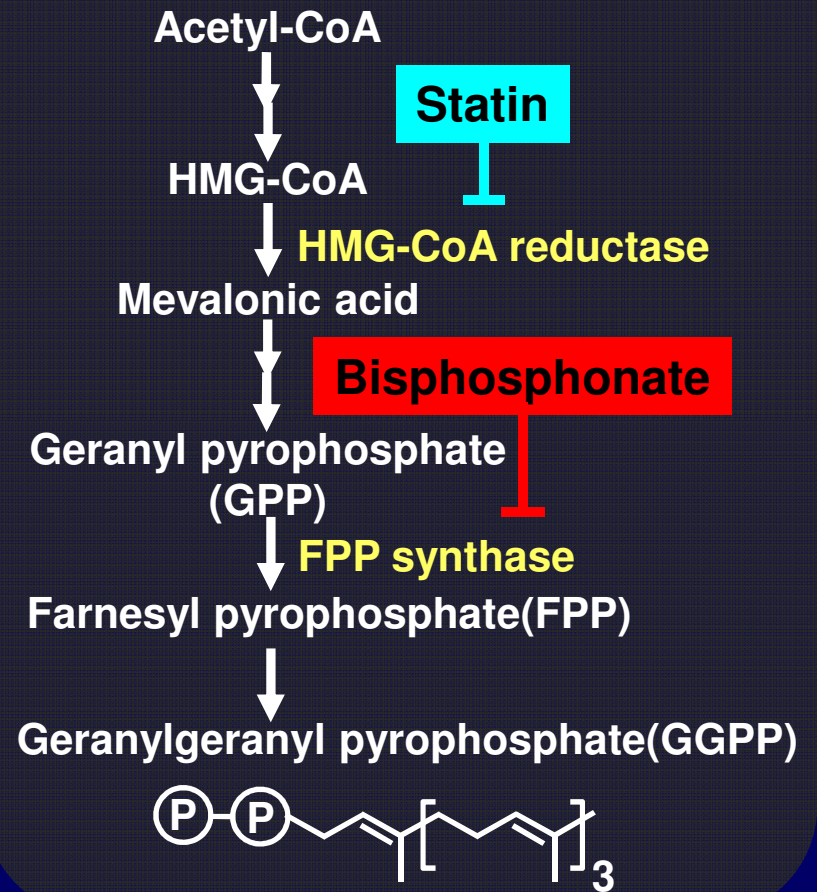
K₃-d₈

UBIAD1

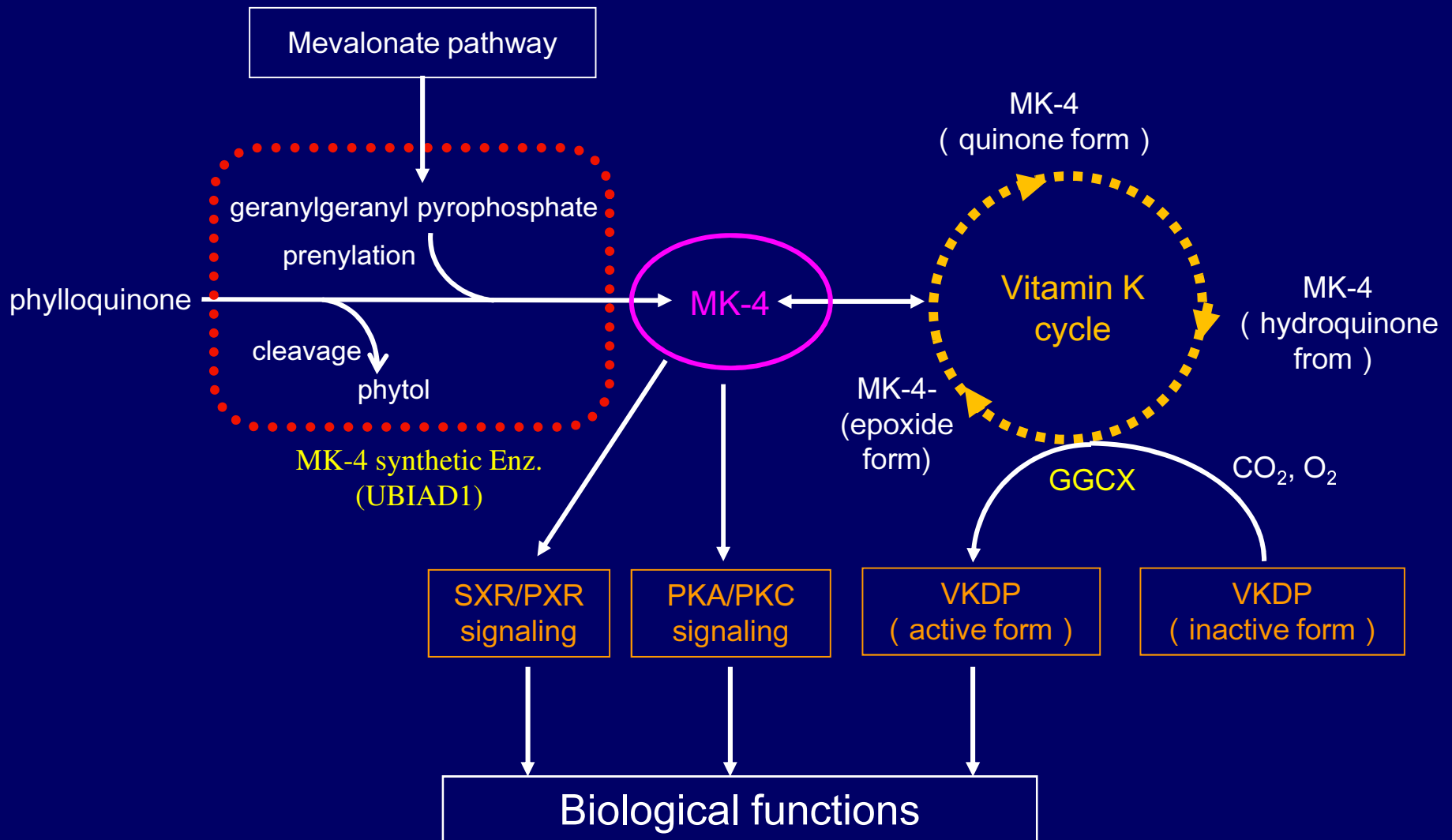
MK-4-d₇



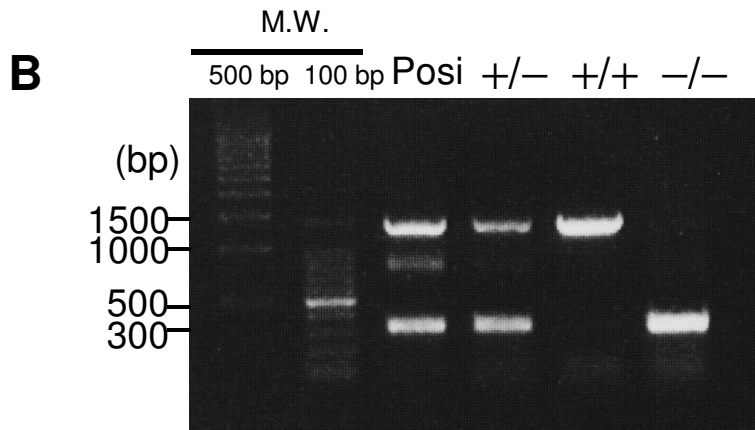
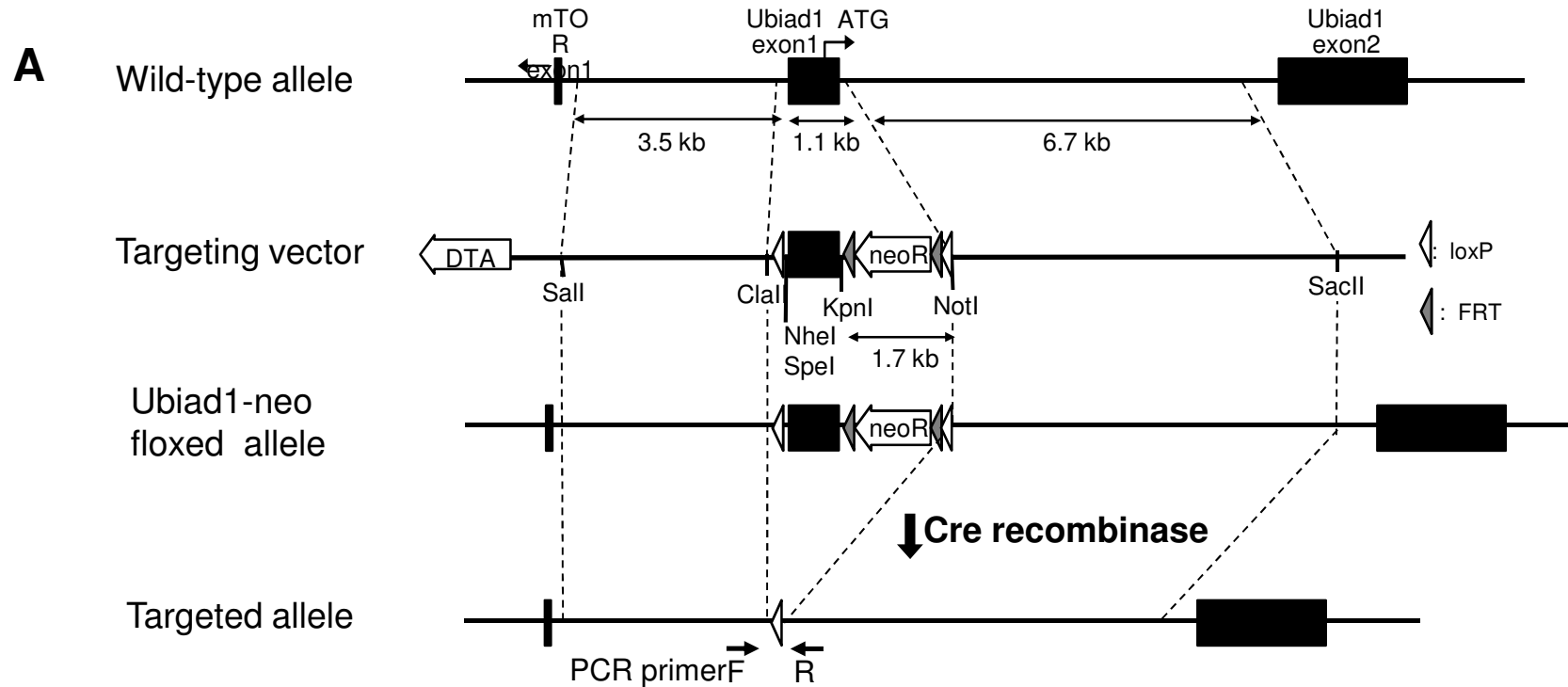
Mevalonate pathway



Possible interaction of MK-4 biosynthesis, vitamin K cycle and vitamin K action

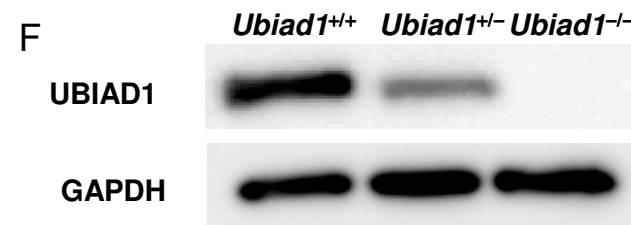
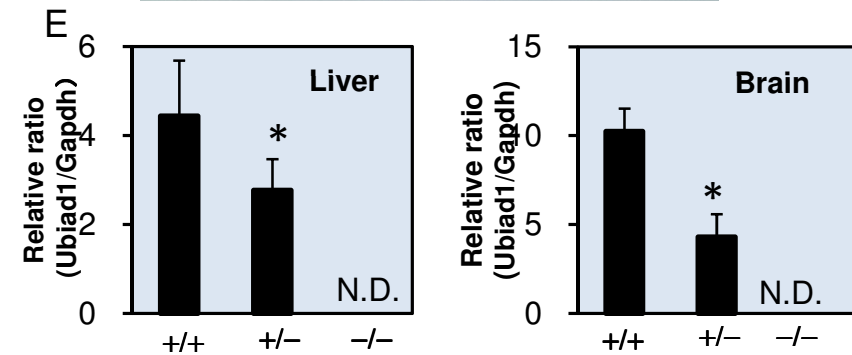
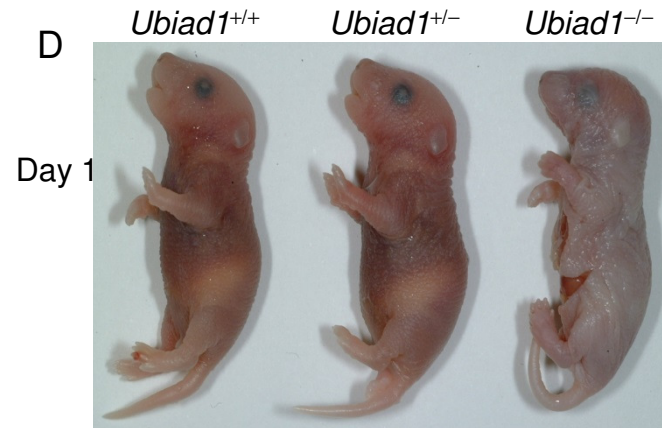
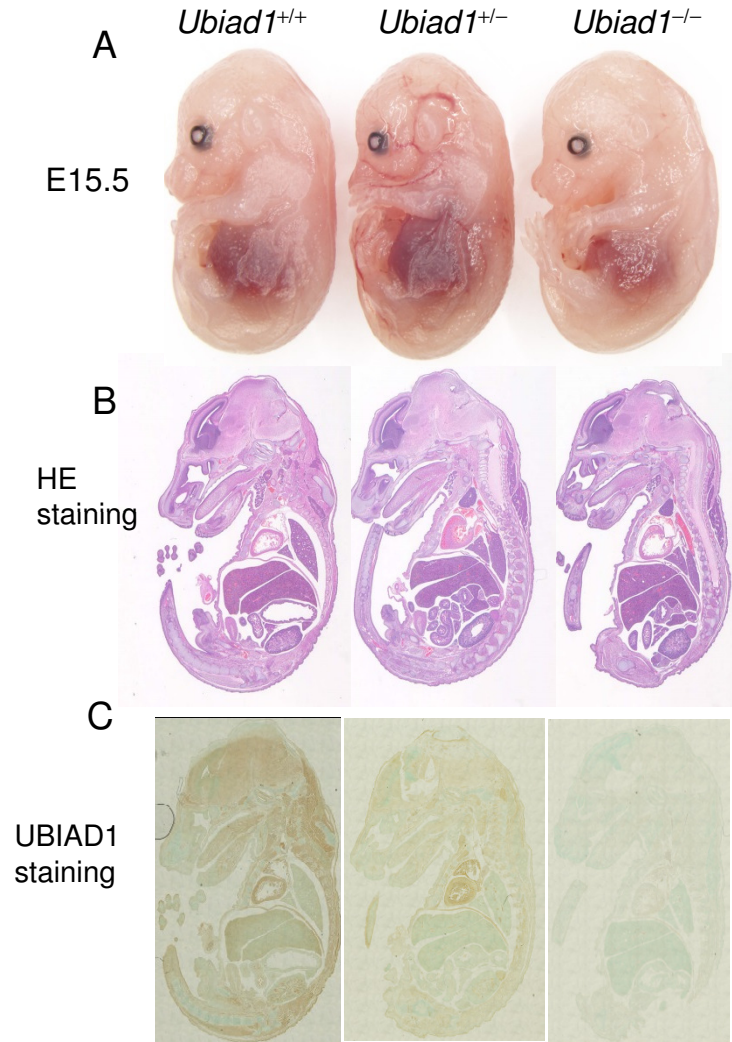


Generation of *Ubiad1* knockout mice



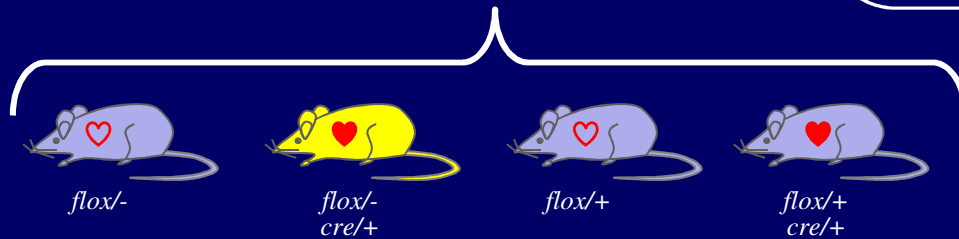
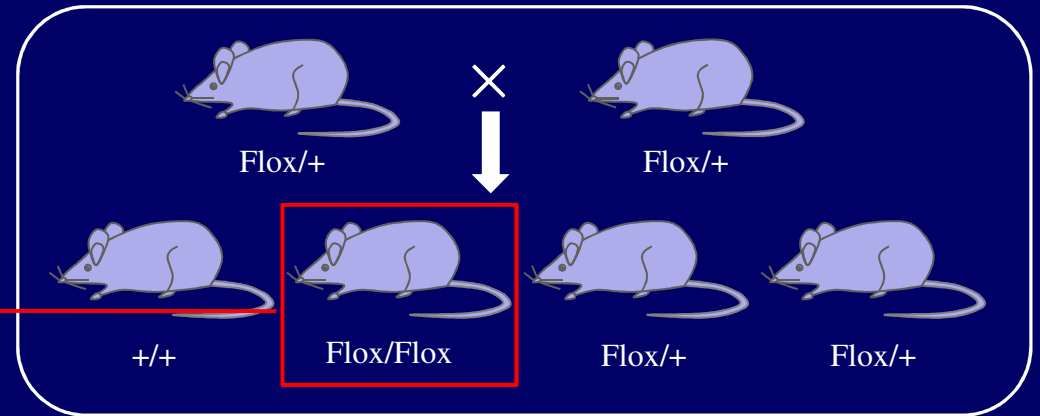
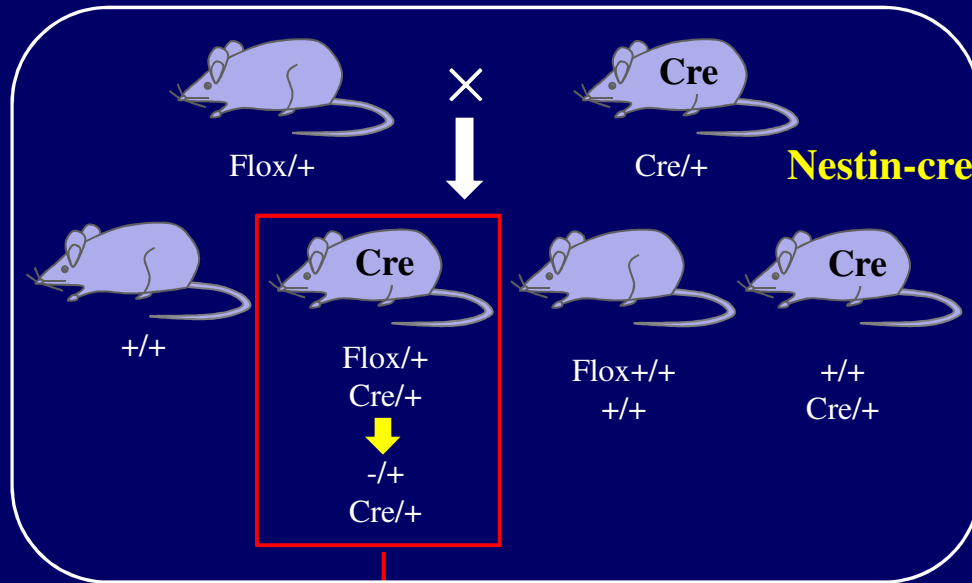
(A) Schematic presentation of *ubiad1* genome, targeting vector and disrupted *Ubiad1* genome. (B) PCR genotyping of *Ubiad1*^{+/+}, *Ubiad1*^{+/-} and *Ubiad1*^{-/-} embryos. PCR genotyping of tail DNA of *Ubiad1*^{+/+}, *Ubiad1*^{+/-} and *Ubiad1*^{-/-} embryos. Lane 1, positive controls for *Ubiad1*^{+/+} allele. Lane 2, PCR bands of *Ubiad1*^{+/-} embryos. Lane 3, PCR bands of *Ubiad1*^{+/+} embryos. Lane 4, PCR bands of *Ubiad1*^{-/-} embryos.

Morphological examination of *Ubiad1*^{+/+}, *Ubiad1*^{+/-} and *Ubiad1*^{-/-} embryos and weanling mice (postnatal day 1) from pregnant *Ubiad1*^{+/-} mice orally administered CoQ10



Ubiad1-deficient mouse embryos failed to survive beyond embryonic day 7.5 exhibiting small-sized body and gastrulation arrest !!!

Generation of a neural cell specific *Ubiad1*^{-/-} mouse



➔ Impairment of brain function



Thank you for your attention!