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METABOLIC **D**EUTERIUM OXIDE (**D**₂O) LABELING IN STUDYING ENVIRONMENTAL DISEASES

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Dec 16, 2023

The 97th Annual Meeting of the Japanese Pharmacological Society

COI開示

- ☑ 発表内容に関連し，過去３年間，開示すべき
COI関係にある企業などはありません

筆頭発表者： 金泰咏 (Kim, Tae-Young)

責任発表者： 金泰咏 (Kim, Tae-Young)

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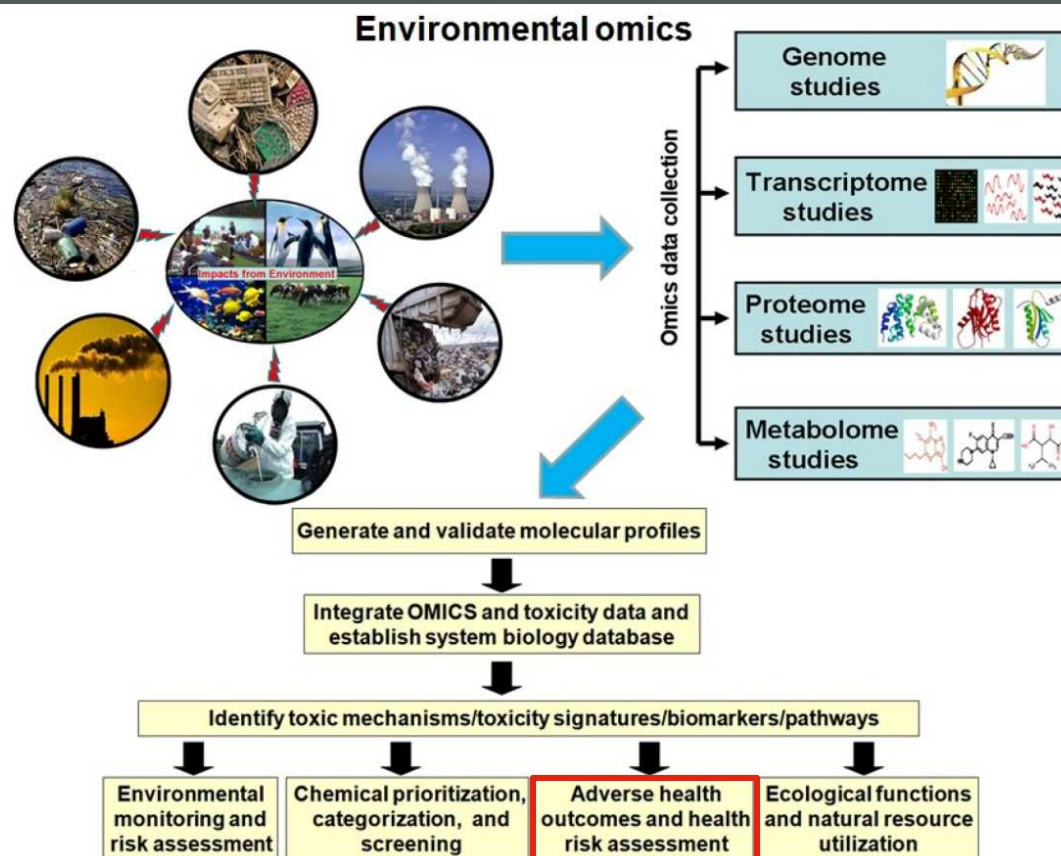
ENVIRONMENTAL OMICS

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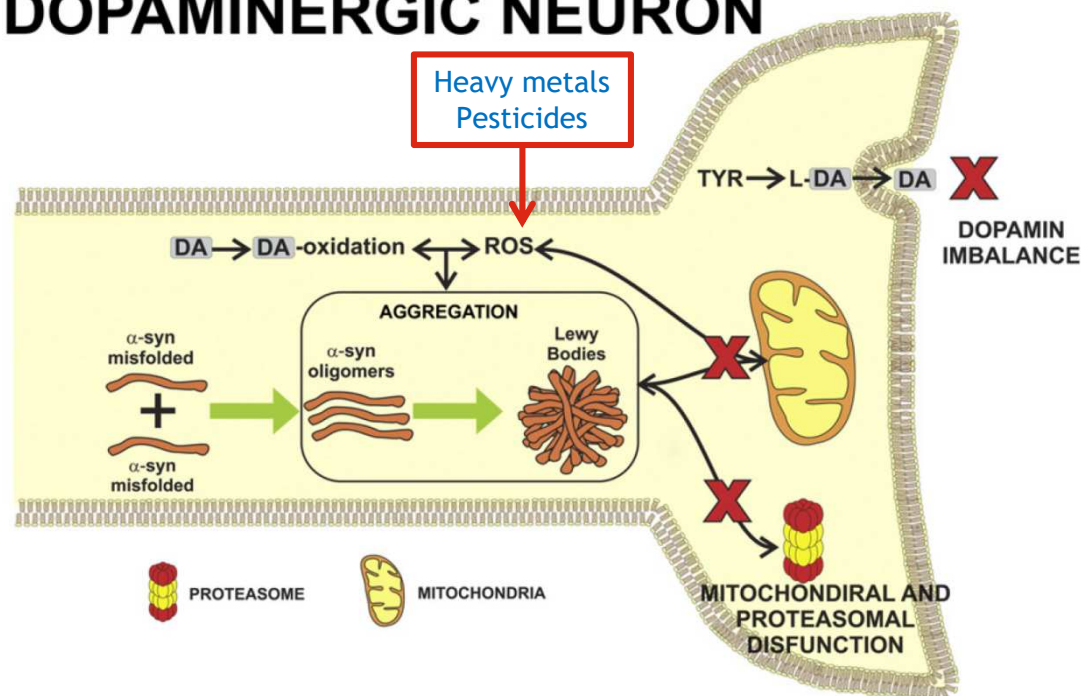
Environmental omics: health risk assessment

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J. Integr. OMICS, 3, 75-87 (2013)

DOPAMINERGIC NEURON





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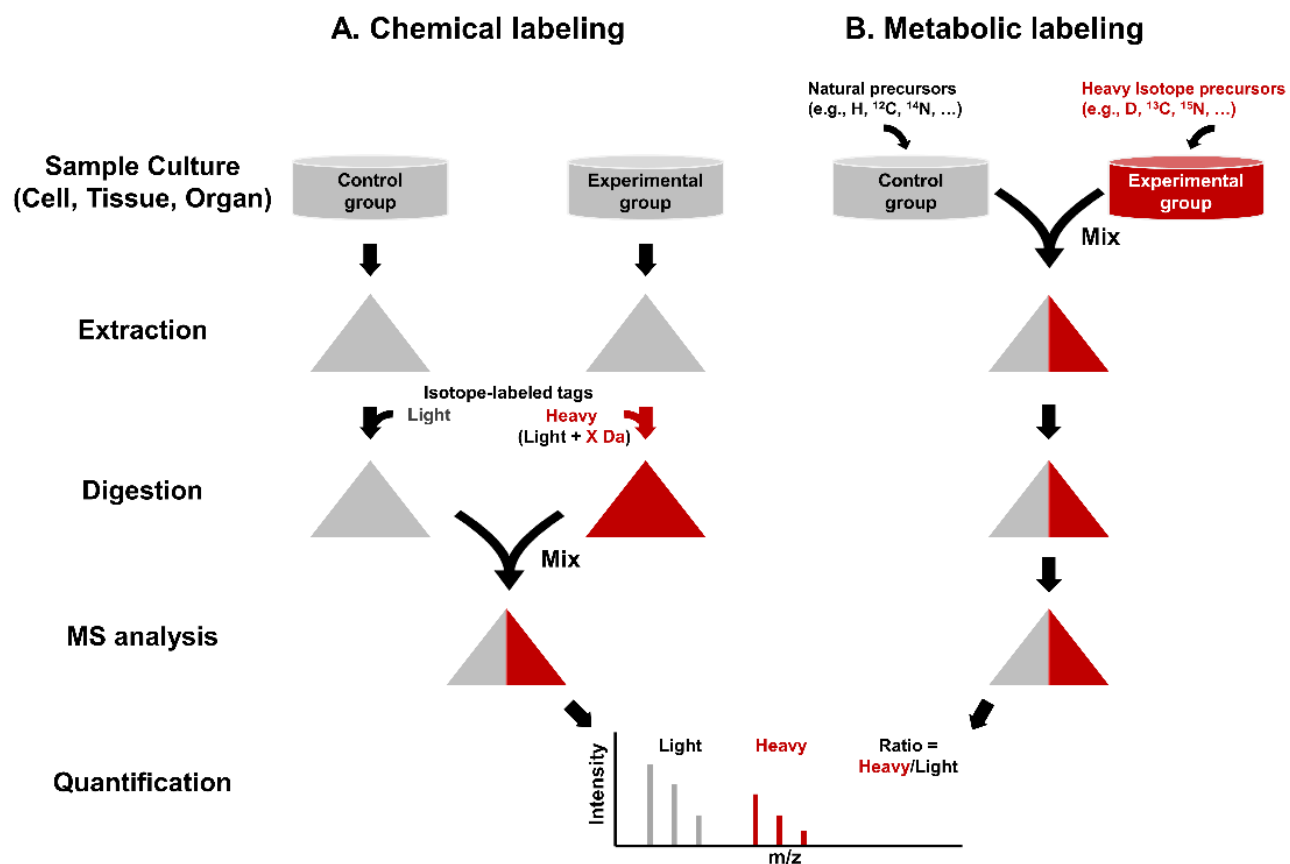
METABOLIC **D**EUTERIUM OXIDE (**D**₂O) LABELING

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Strategies for stable isotope labeling in quantitative MS

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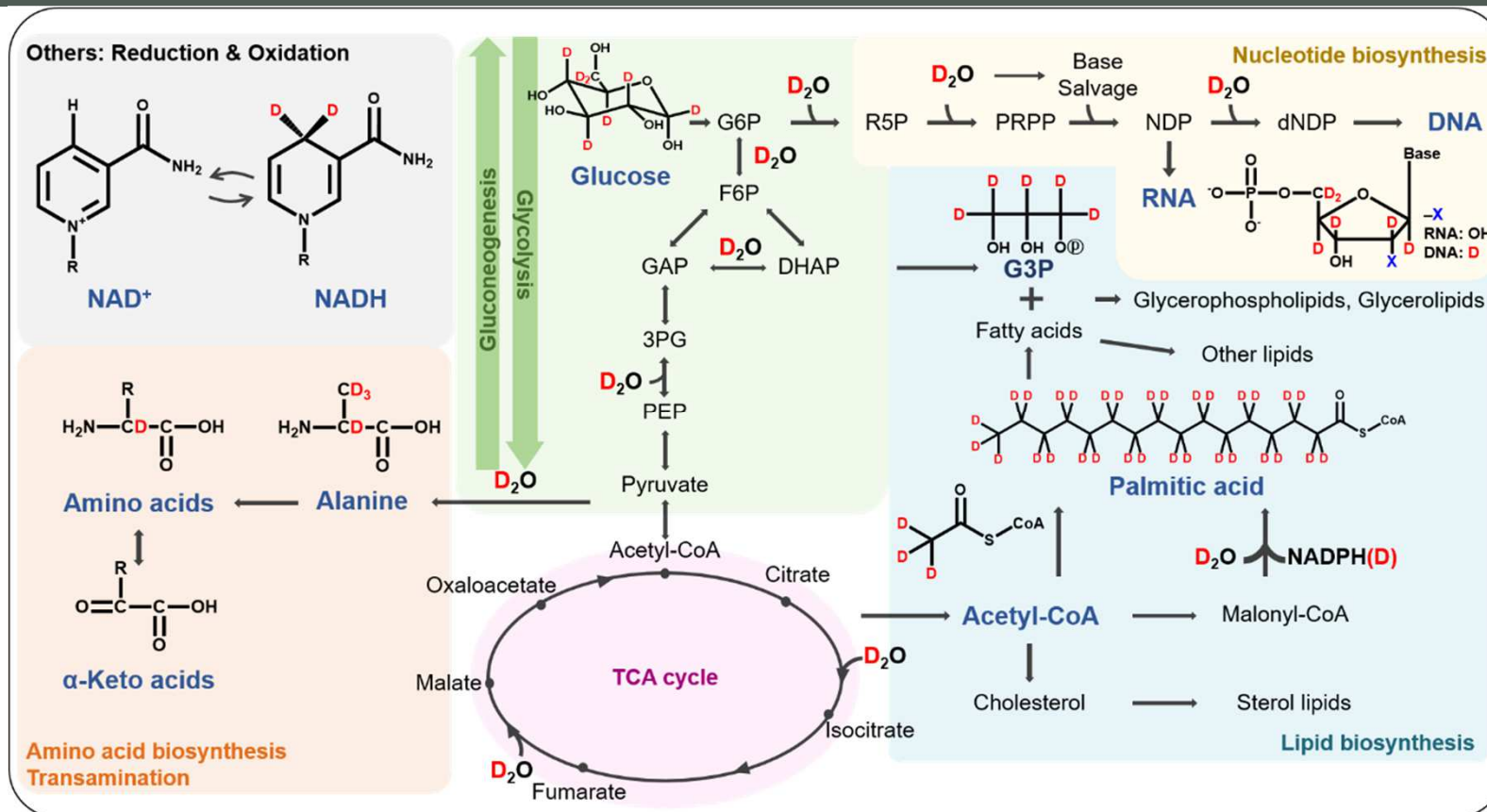
Properties of metabolic D_2O labeling

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Metabolic D-labeling pathways by D₂O

10

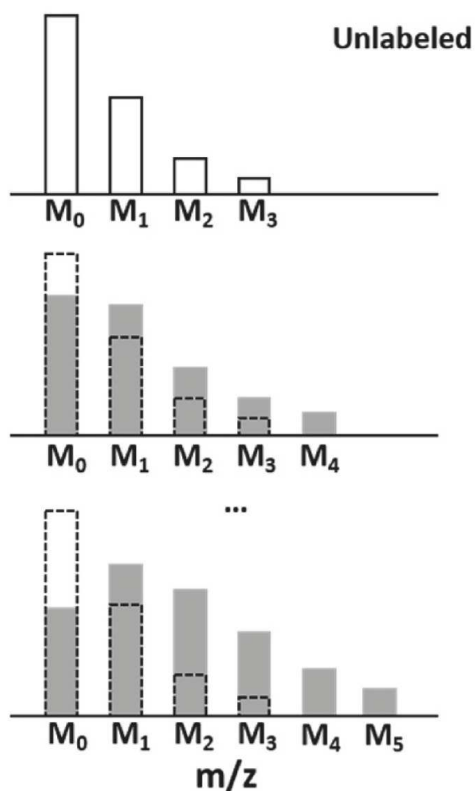


Anal. Chim. Acta 1242, 340722 (2023)

Metabolic partial D-labeling & factors affecting D-labeling

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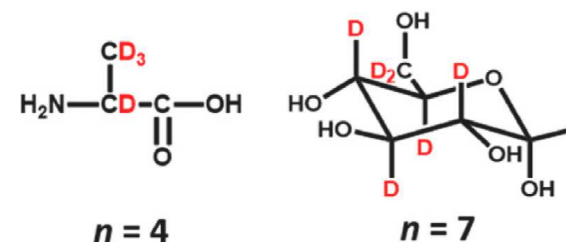
Isotopic distribution change



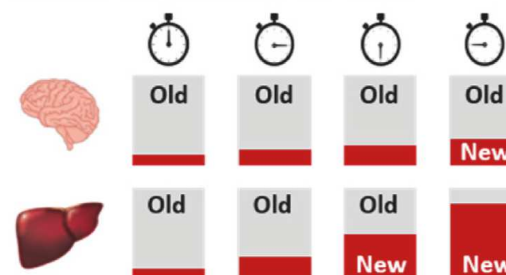
• Precursor enrichment



• Number of labeling sites



• Intrinsic turnover rate



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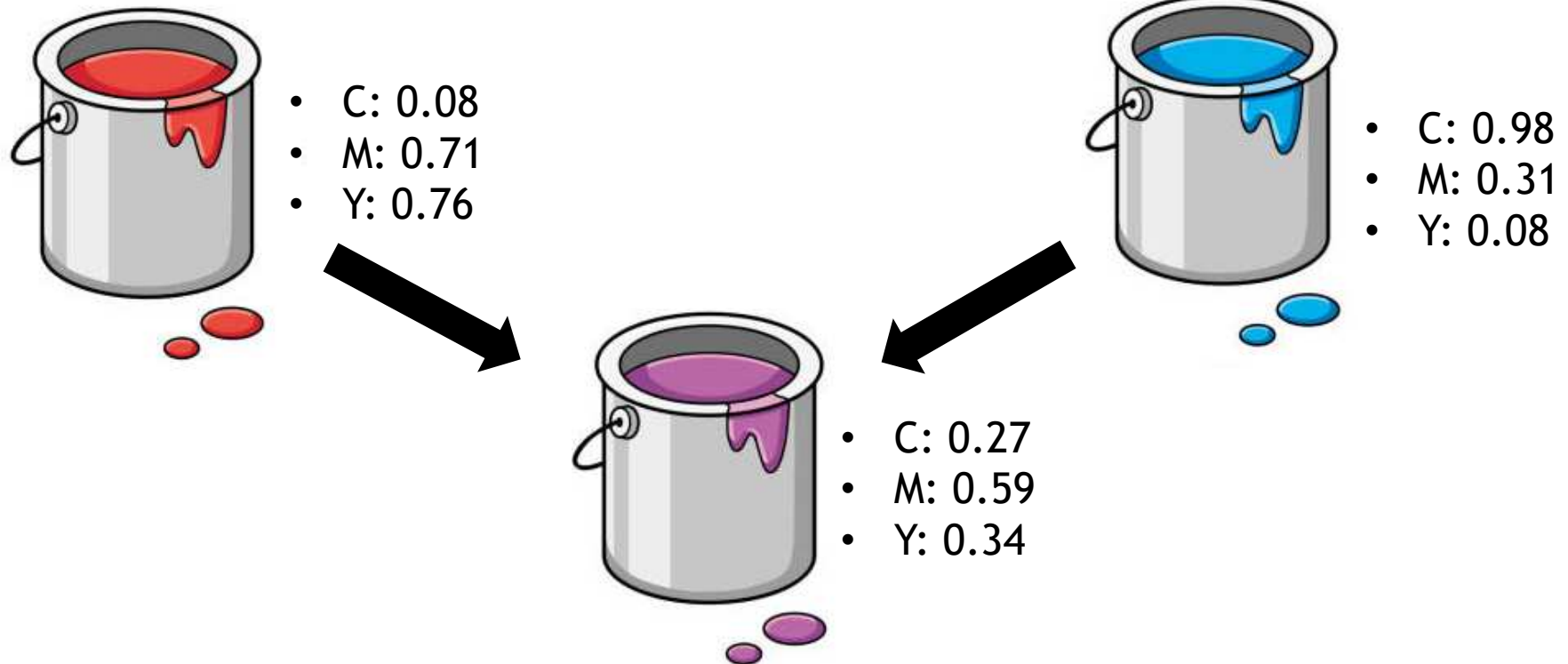
DEUTERIUM OXIDE LABELING FOR GLOBAL OMICS RELATIVE QUANTIFICATION (DOLGOREQ)

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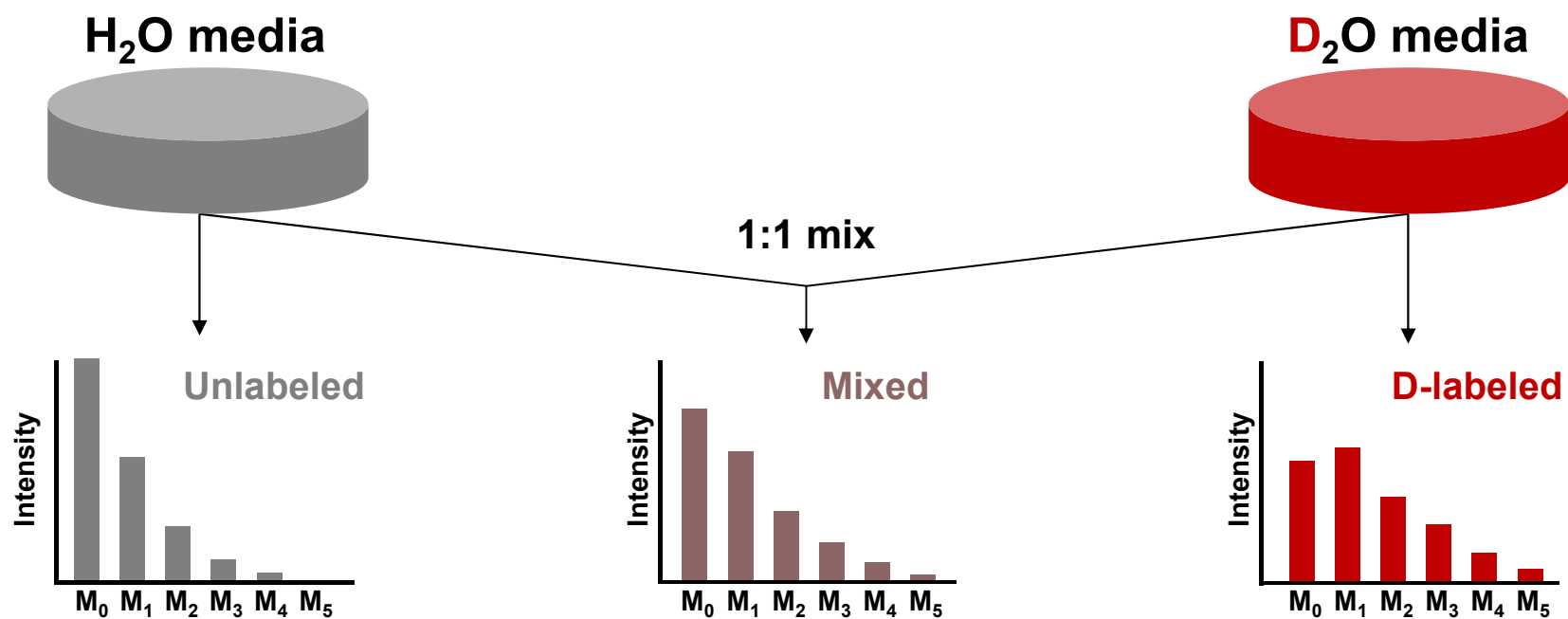
Idea of the DOLGOREQ

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Metabolic partial D-labeling & MS

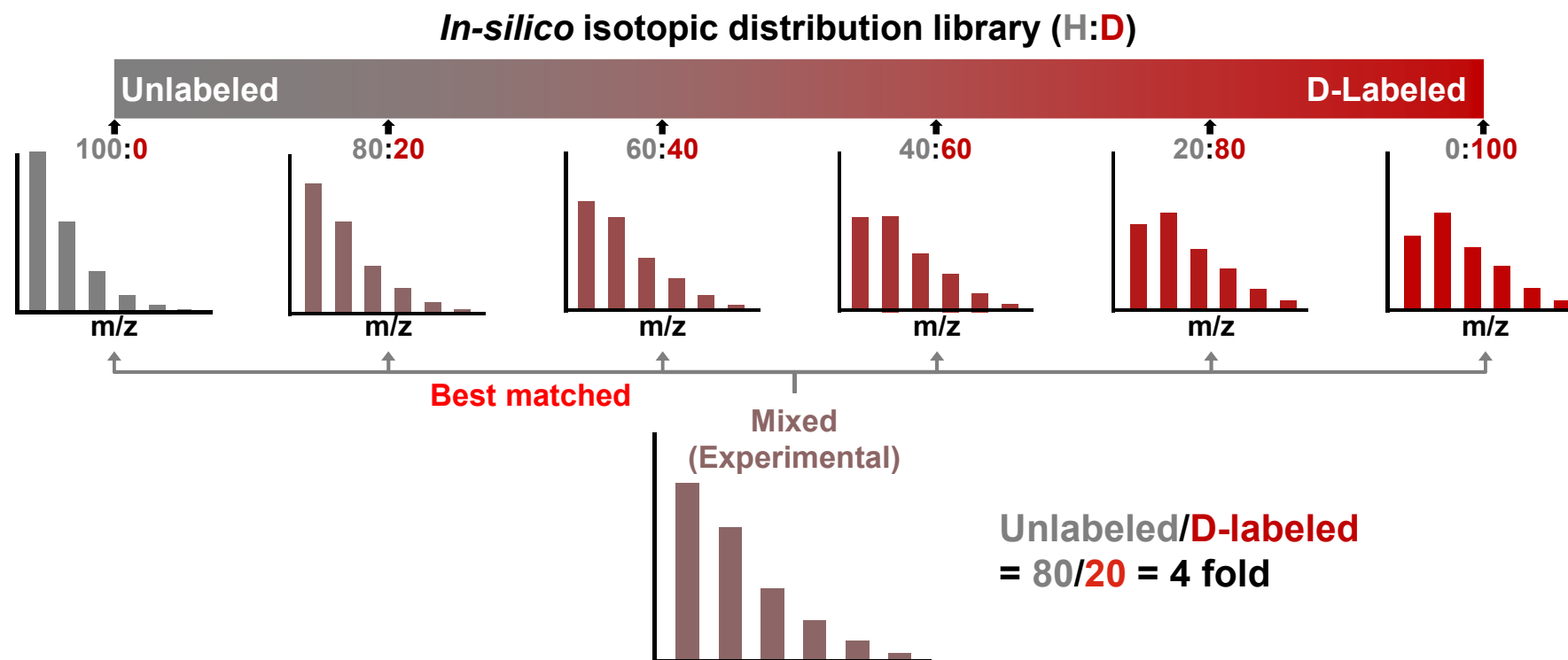
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Relative quantification

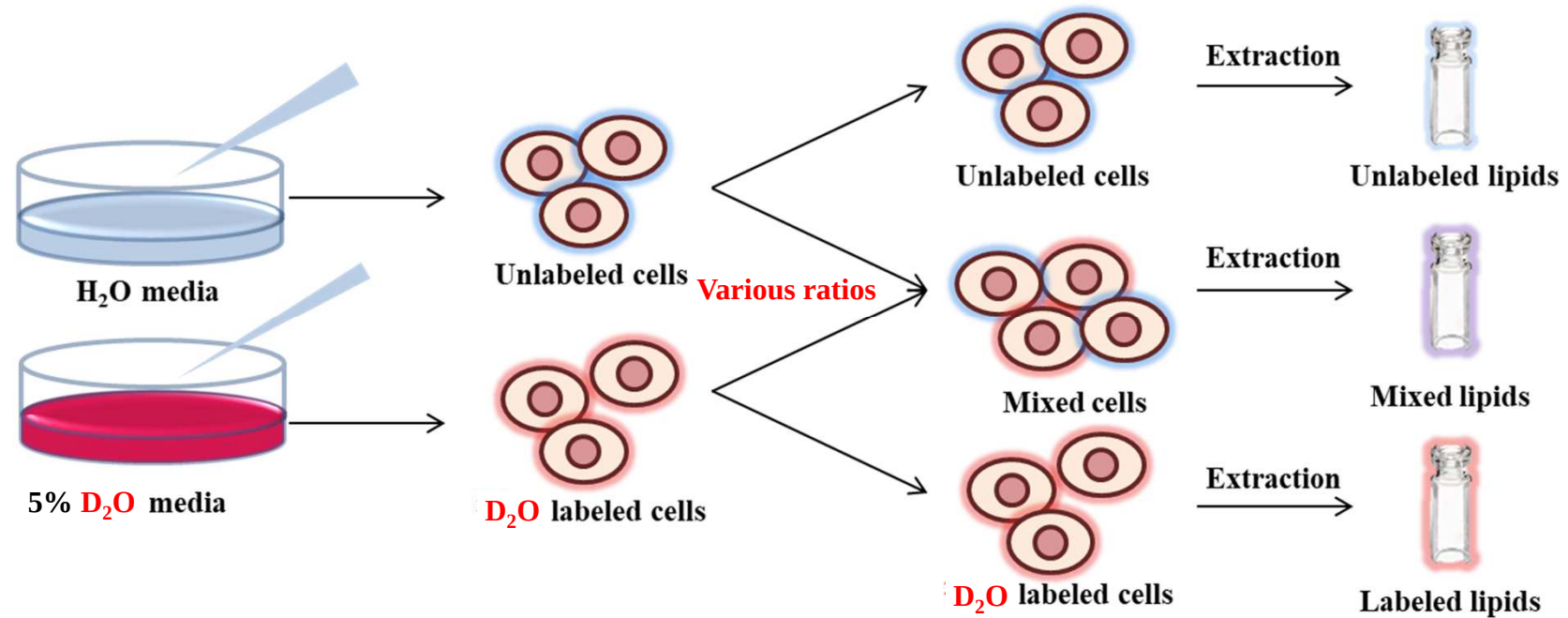
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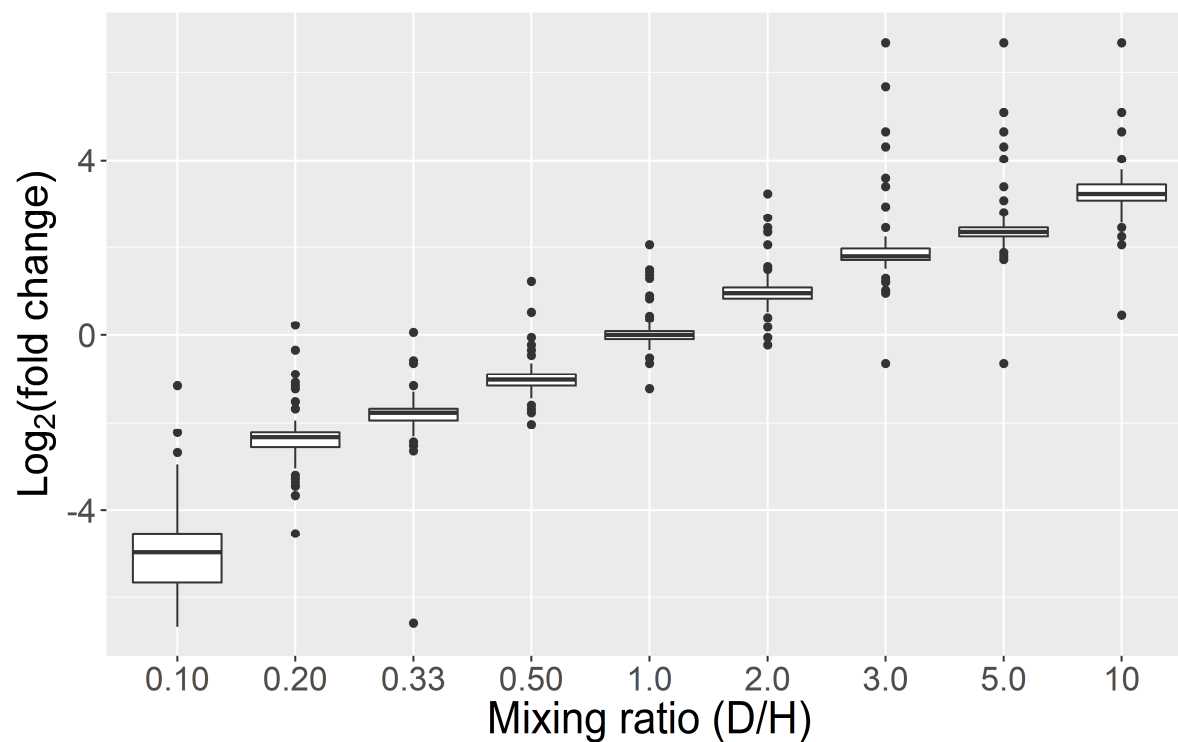
Quantification performance test: HeLa cell lipids

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Quantification dynamic range: various mixing ratios

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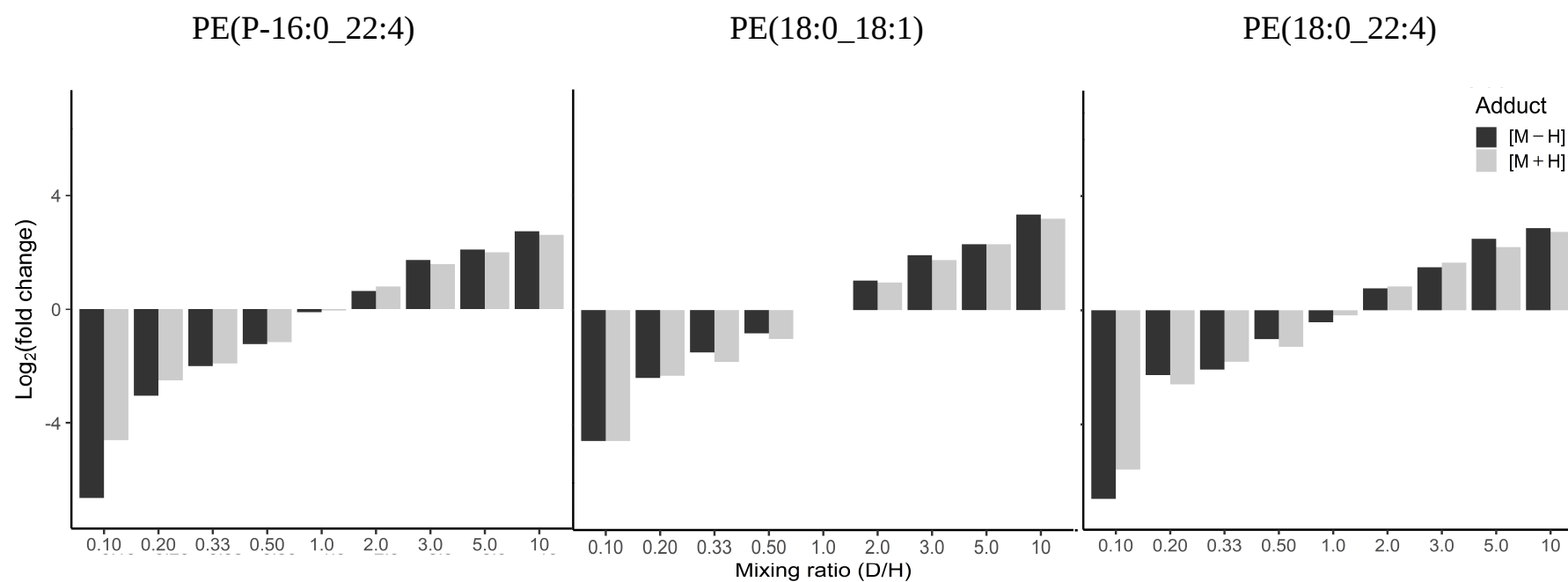


Expected fold change	Measured median fold change
0.100	0.0322
0.200	0.198
0.333	0.294
0.500	0.490
1.00	1.00
2.00	1.93
3.00	3.48
5.00	5.08
10.0	9.37

Anal. Chem. **91**, 8853 (2021)

Quantification precision: different ion forms

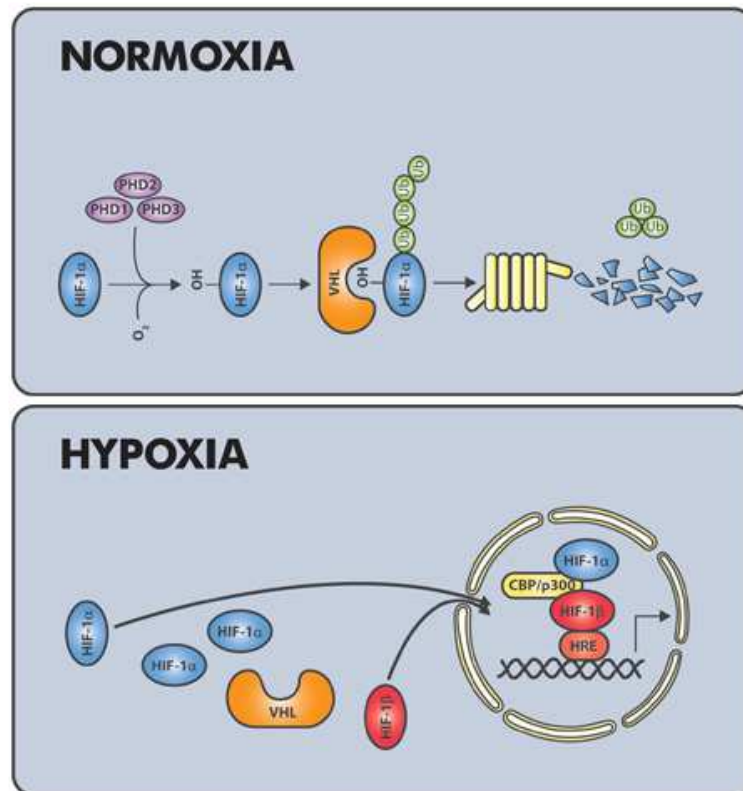
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Application of DOLGOREQ: CoCl_2 induced hypoxia

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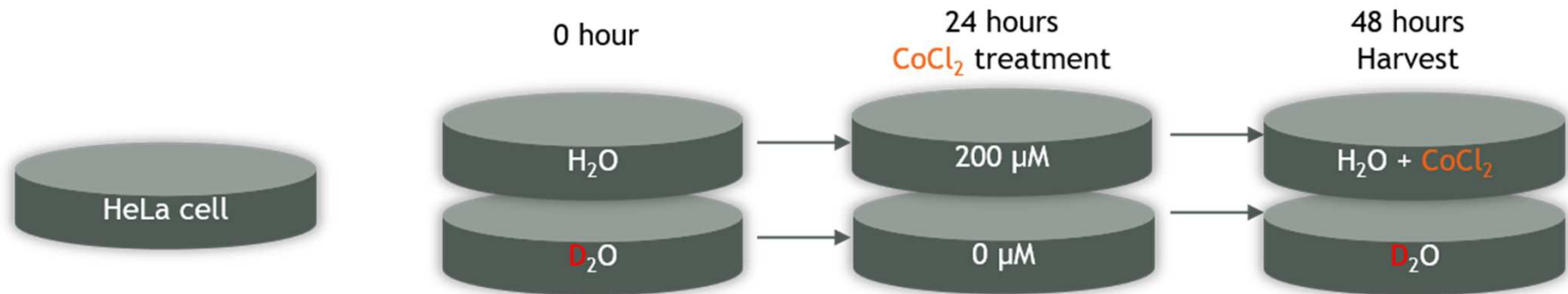
CoCl_2 inhibits PHD enzymes (the oxygen sensors) through replacement of Fe with Co making these enzymes unable to mark HIF-1 α for degradation.

- Hypoxia inducible factor-1 (HIF-1)
- Prolyl hydroxylase-domain enzymes (PHDs)
- von Hippel Lindau (VHL) protein
- Hypoxia response element (HRE; 5'-RCGTG-3')

<https://www.novusbio.com/support/hypoxia-and-hif-faqs>

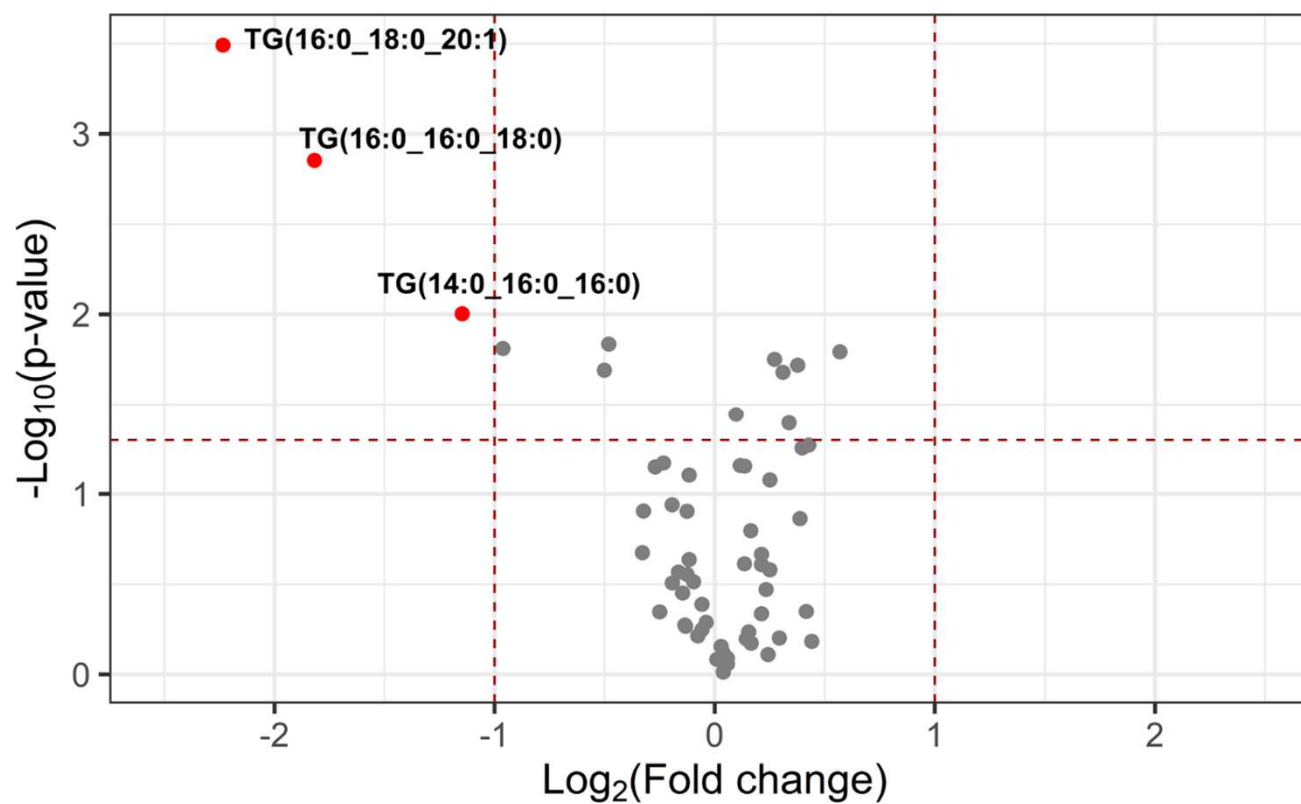
CoCl₂ induced hypoxia: experimental scheme

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Lipid fold-change between normoxia & hypoxia HeLa cells

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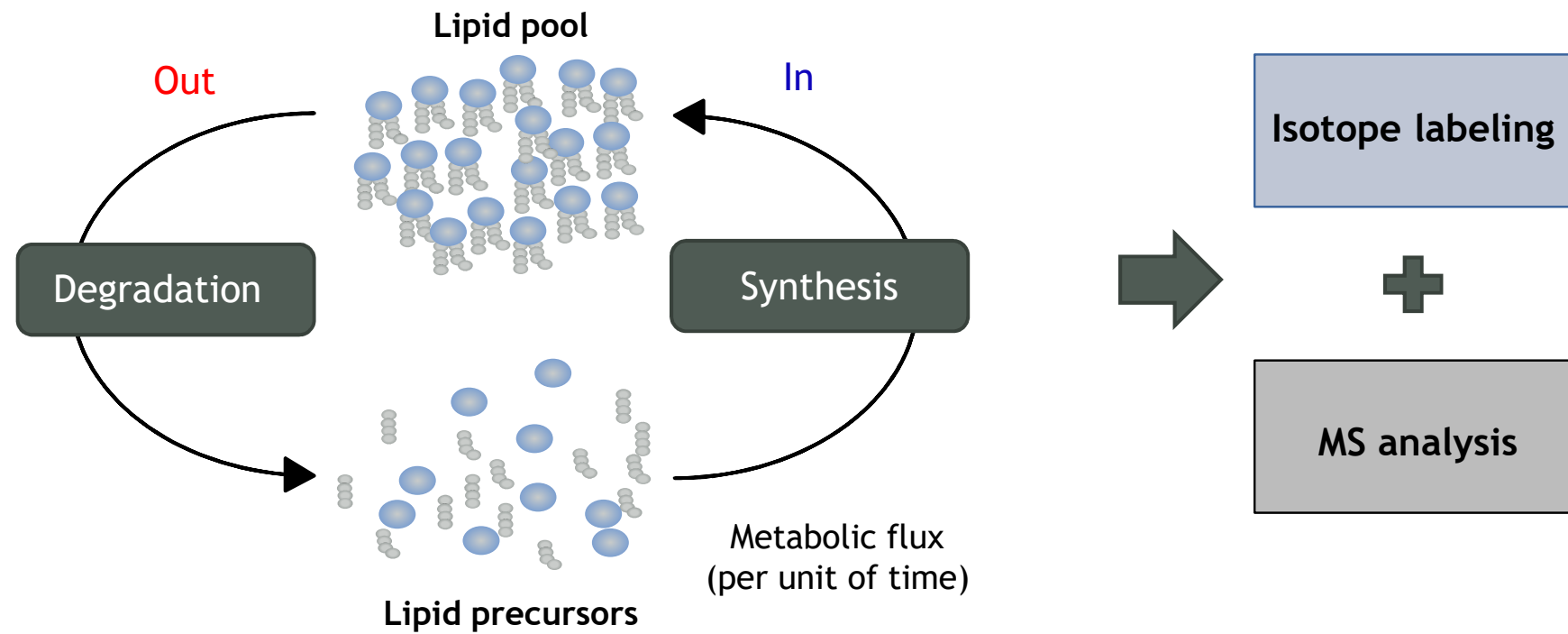
D_2O LABELING FOR LIPID TURNOVER MEASUREMENT

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Lipid turnover

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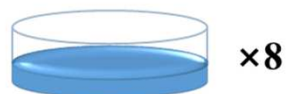


Lipid turnover measurement: experimental schemes

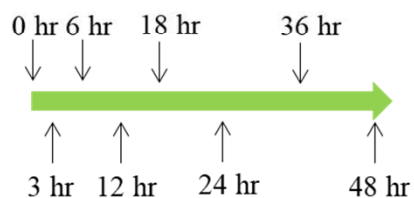
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A Metabolic D_2O labeling of HeLa cell

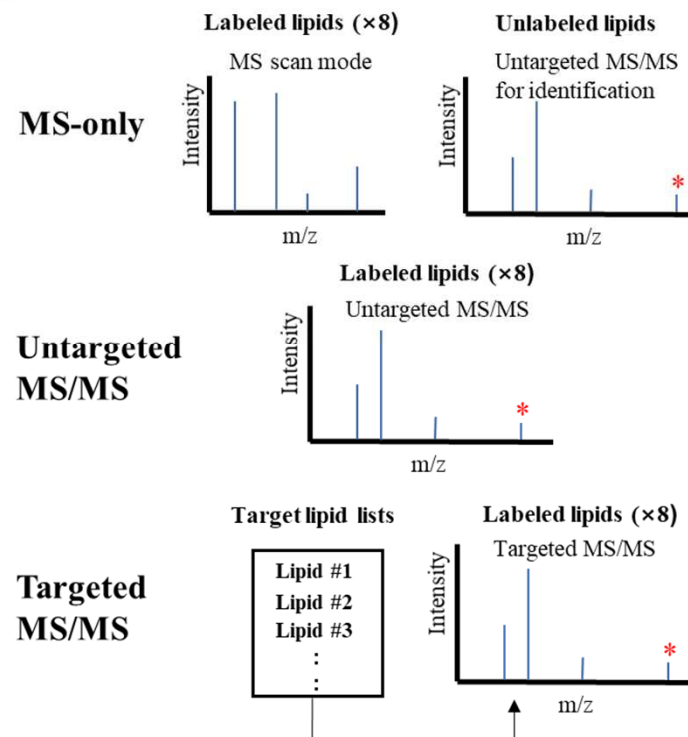
5% 2H_2O enriched media



Sampling



B LC-MS and LC-MS/MS

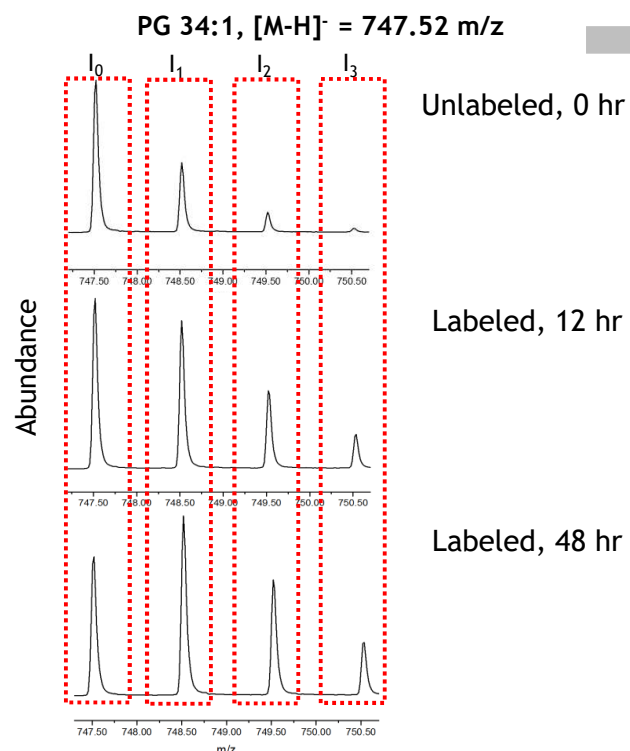


Anal. Chem. **90**, 6509 (2018)

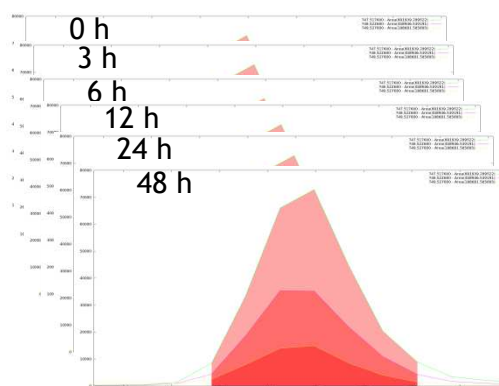
Lipid turnover measurement: MS data-processing

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□ D-labeled MS spectra

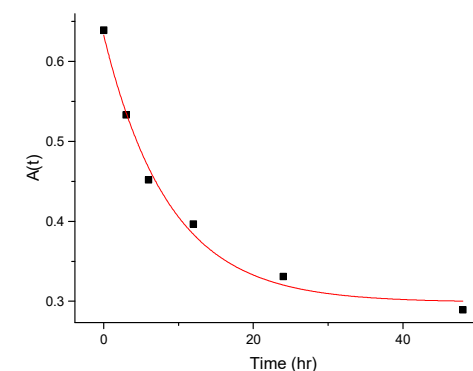


□ Extract Chromatogram & Area Integration



- Perform peak area integration of XIC for each identified lipid.
- Repeat the same procedure for each time point of labeling.

□ Graph fitting for turnover rate



$$A_i(t) = I_i / \sum_j^N I_j$$

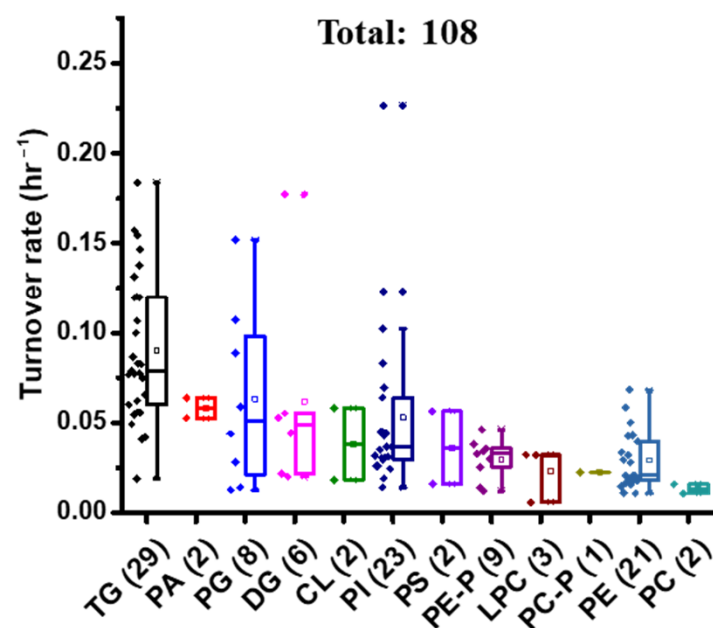
$$A(t) = A(0) + \{A(\infty) - A(0)\} \times (1 - e^{-kt})$$

I_i = peak intensity of the mass isotopomer i ,
 t = time (hr), k = turnover rate

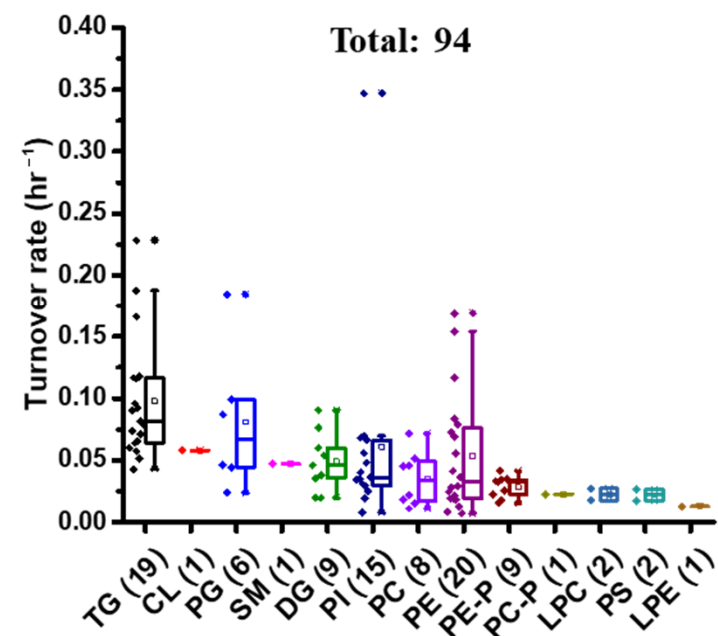
Lipid turnover measurement: turnover rates

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A MS-only



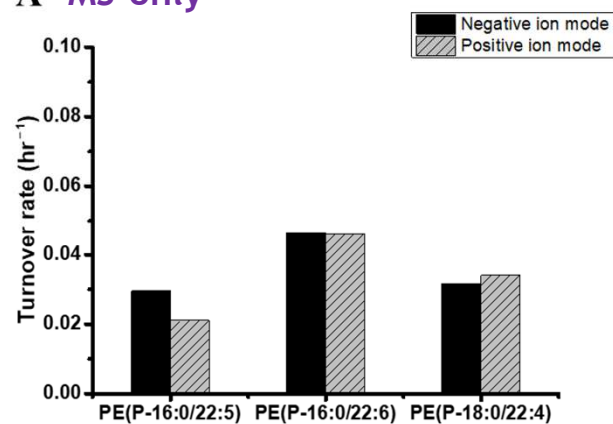
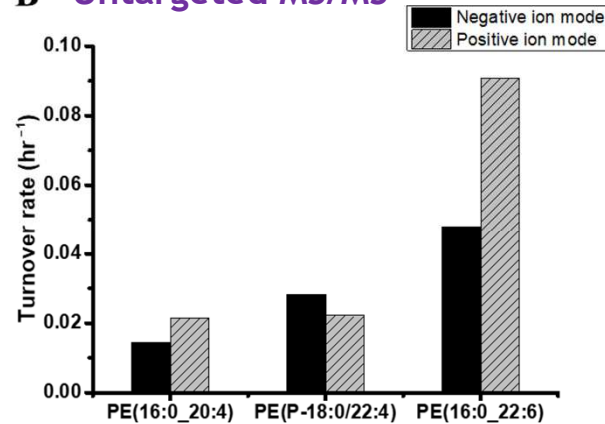
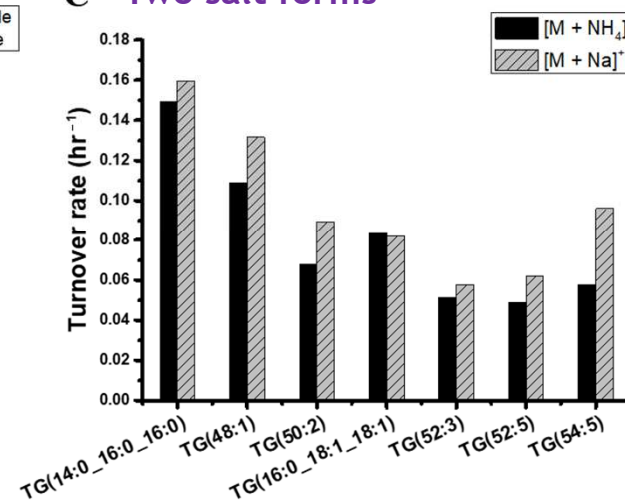
B Untargeted MS/MS



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Lipid turnover measurement: reproducibility

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A MS-only**B Untargeted MS/MS****C Two salt forms**

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Conclusion

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- New **analytical platforms** for **relative quantification** and **measurement of *in vivo* turnover rate** of lipids on a global scale based on partial metabolic D_2O labeling has been developed.
- **DOLGOREQ** is effective in a linear dynamic range over **two orders of magnitude**.
- Turnover rates of more than **100 HeLa lipids at species-level** were determined with good reproducibility based on metabolic D_2O labeling..
- Because of its **universality**, **DOLGOREQ** can be exploited in **multi-omics studies** covering lipidomics, proteomics, and glycomics.
- The **economy** of D_2O labeling makes it possible to apply our methodology to a **long-term kinetics study for higher organisms** including human.