



JSPS 「日本におけるケミカルバイオロジー研究の新展開」
に関する研究開発専門委員会第7回委員会

2013. 11. 6. 東京

ケミカルバイオロジー研究のための プローブ分子の開発

理化学研究所
袖岡有機合成化学研究室
環境資源科学研究センター

袖岡 幹子



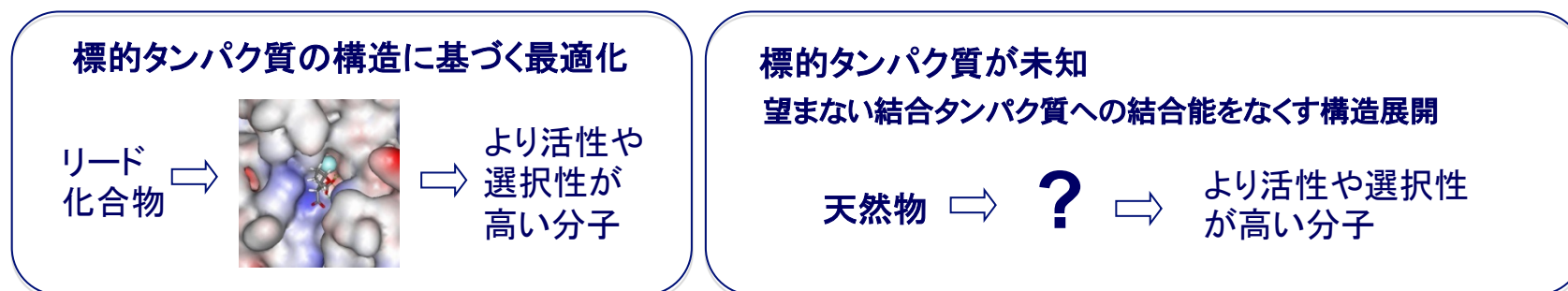
ERATO 袖岡生細胞分子化学プロジェクト



ケミカルバイオロジー研究のための新しいプローブ分子の開発

プローブ分子(探針分子):新しい生物学的発見に役立つ分子

- ・ 新しい生物活性分子:シャープな活性と選択性

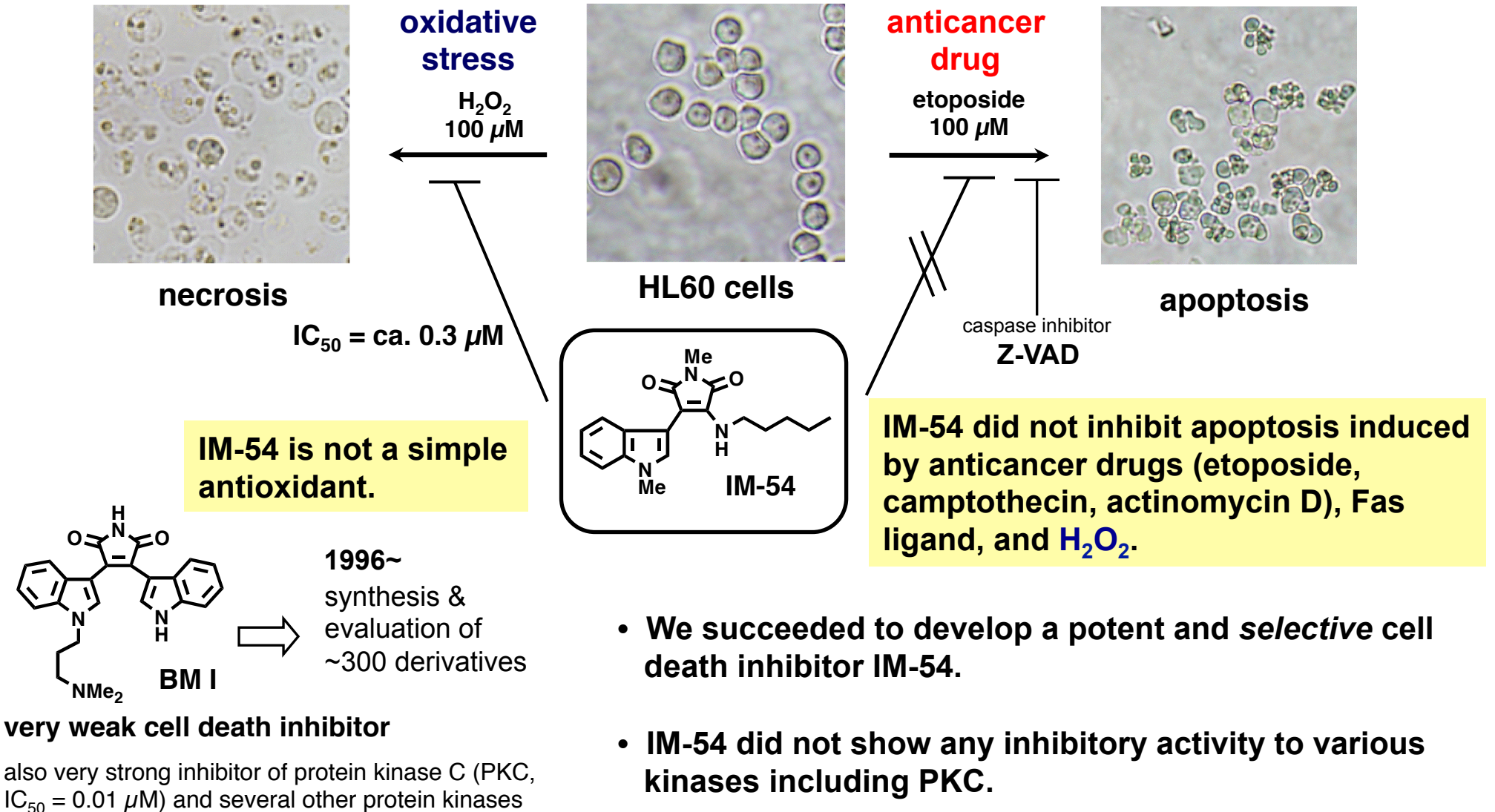


ネクローシスの選択的阻害剤の開発と作用機序研究

ケミカルバイオロジー研究のための新しい化学的手法の開発

ラマン分光のケミカルバイオロジー研究への利用

Example 1: Development of a Cell Death Inhibitor IM-54.

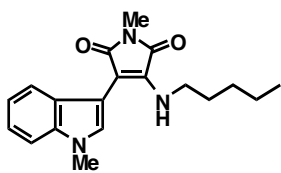


R. Asakai *et al. Neurosci Res.* 2002

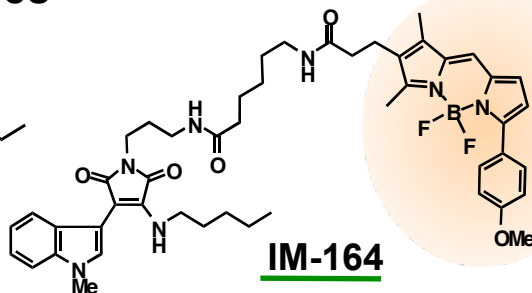
Dodo, K.; Katoh, M. Shimizu, T.; Takahashi, M.; Sodeoka, M.
Bioorg. Med. Chem. Lett. **2005**, *15*, 3114.

Example 2: New Imaging Method for Small Molecules

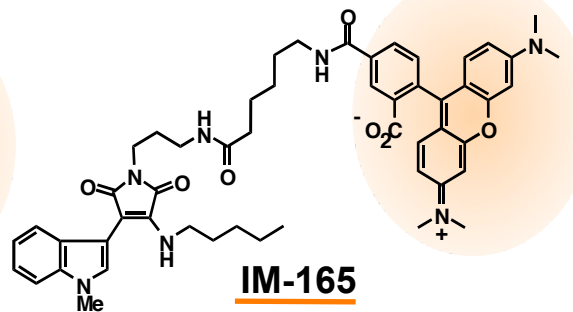
IM derivatives



IM-54



IM-164



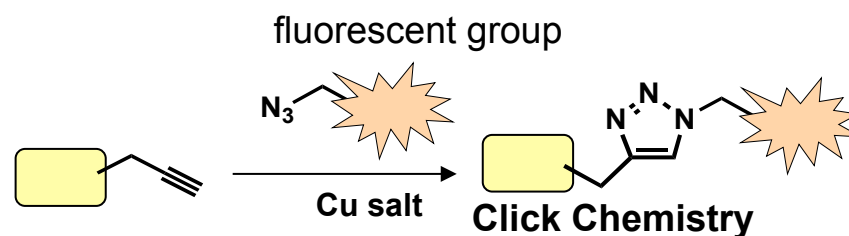
IM-165

Problems of Large Fluorescent Group

- Introduction of large fluorescent group diminished its biological activity.
lower binding affinity to the target protein/poor cell permeability
- Introduction of a fluorescent group changes localization of small molecule.

Existing Solution: Click Chemistry

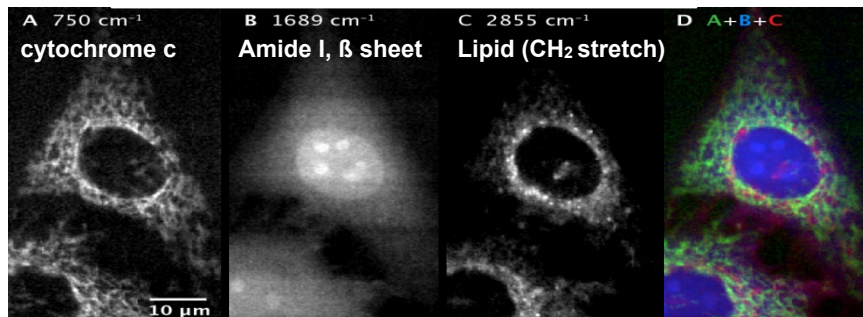
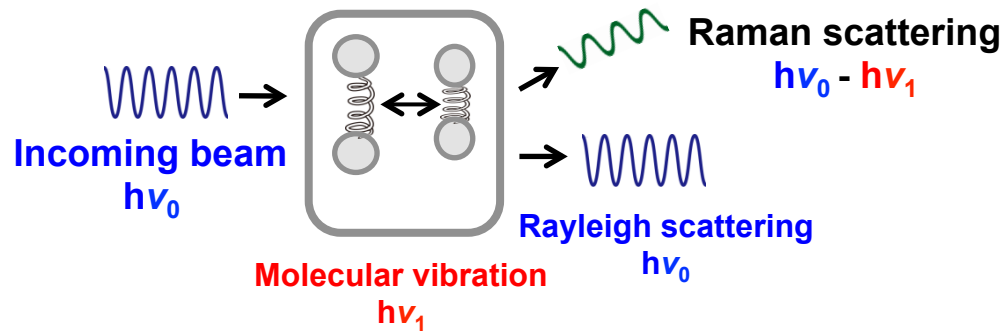
Alkyne Tag = Very Small and Bioorthogonal!



Normally toxic Cu salt is required and difficult to apply live cell imaging.

Laser Raman Microscope is a powerful tool for live cell imaging of non-stained cells.

Raman spectroscopy



Slit-scanning Laser Raman Microscope

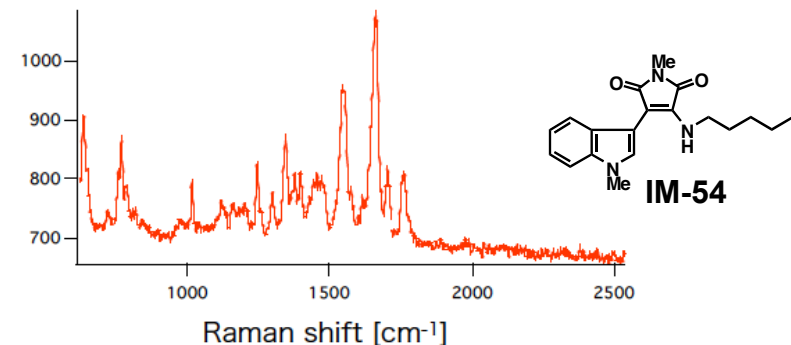
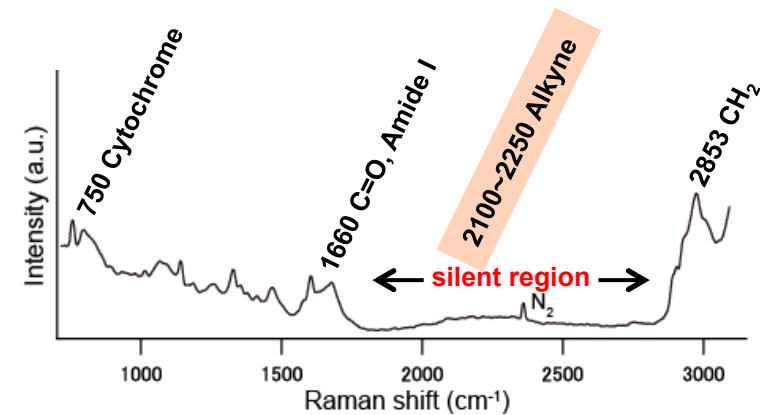
Prof. Katsumasa Fujita
Prof. Satoshi Kawata (Osaka University)

Alkyne Tag

tag =

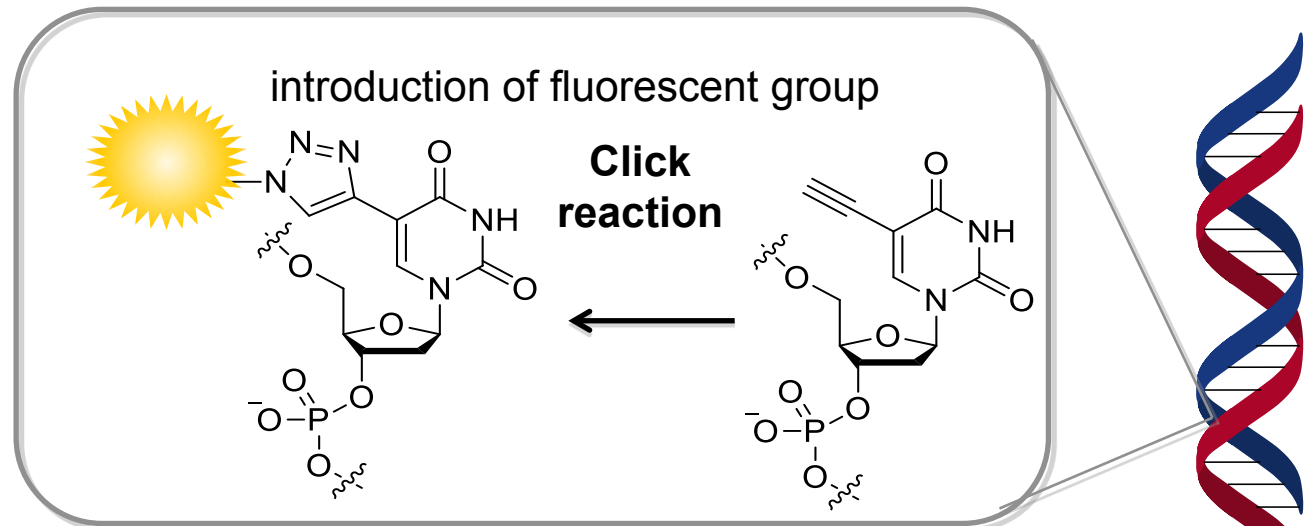
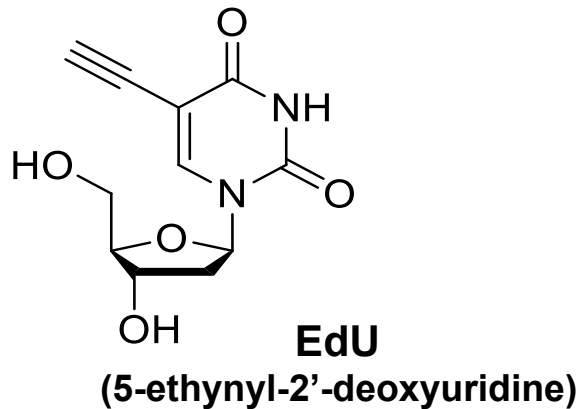


Raman spectrum of living HeLa cell



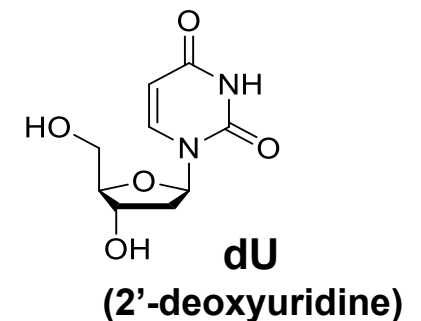
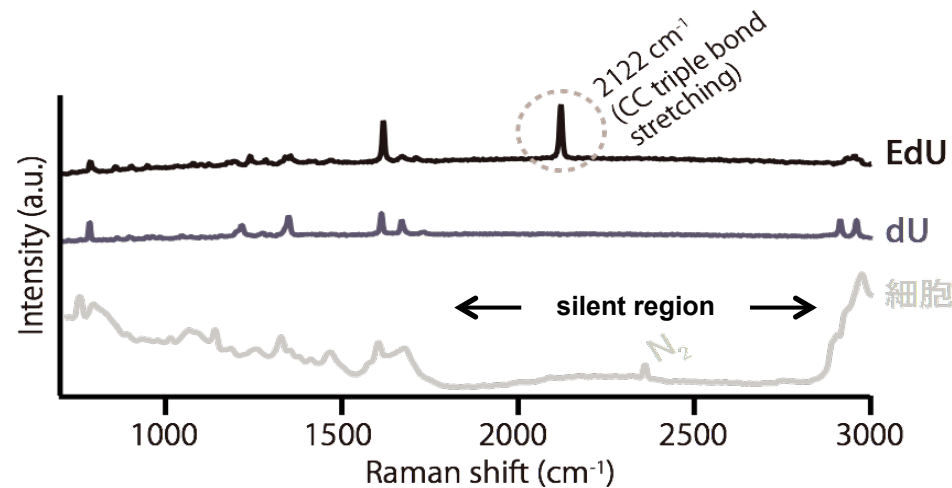
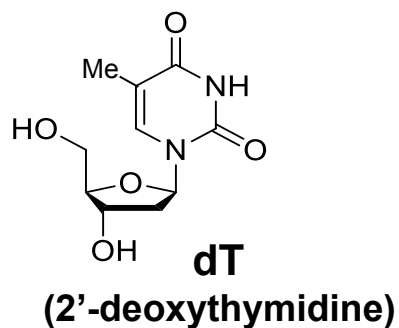
Direct imaging of alkyne tag by Raman microscope is possible?

EdU, an alkyne-tagged cell proliferation probe

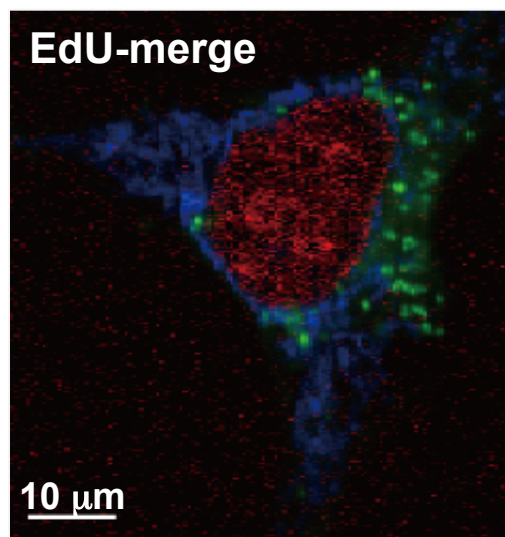


- Nucleoside analogue of thymidine
- A alternative for cell proliferation probe BrdU
- EdU is incorporated into DNA during active DNA synthesis
- Click chemistry-based detection **after fixation or lysis**

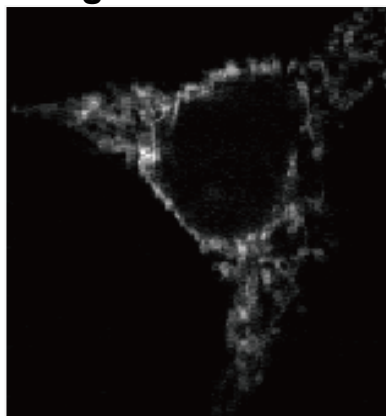
A. Salic et al. *Proc. Natl. Acad. Sci. USA* **2008**, 105, 2415.



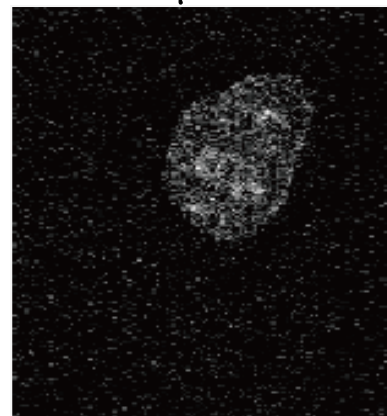
Click-free Live Cell Imaging of EdU by Raman Microscopy



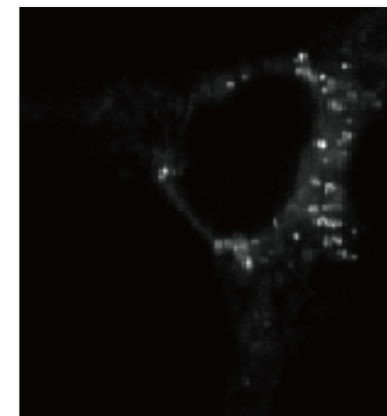
Living HeLa cells treated with 20 μM EdU for 6 hr 532 nm irradiation



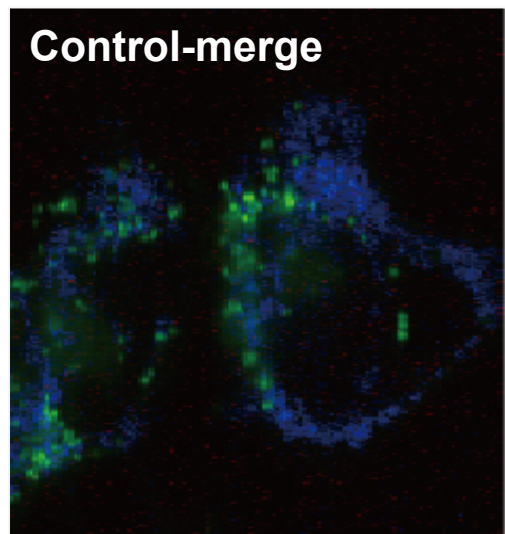
Blue: 749 cm^{-1}
Cytochrome



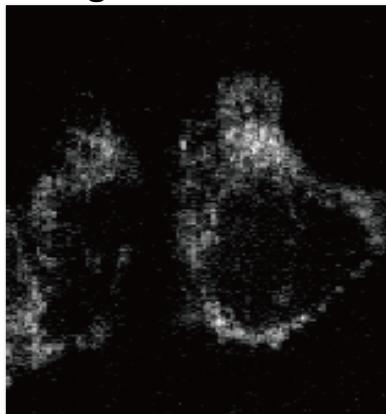
Red: 2123 cm^{-1}
EdU



Green: 2849 cm^{-1}
Lipid



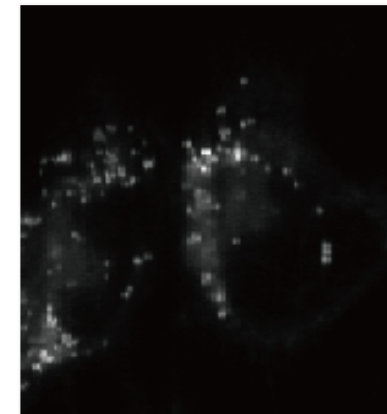
Living HeLa cells without EdU



Blue: 749 cm^{-1}
Cytochrome



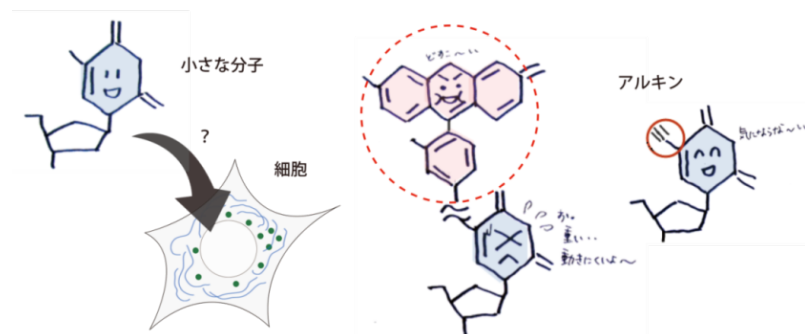
Red: 2123 cm^{-1}
EdU



Green: 2849 cm^{-1}
Lipid

Yamakoshi, H.; Dodo, K.; Okada, M.; Ando, J.; Palonpon, A.; Fujita, K.; Kawata, S.; and Sodeoka, M. *J. Am. Chem. Soc.* 2011, 133, 6102.

Proof of concept of Alkyne-Tag Raman Imaging (ATRI) has been achieved.



Next challenges of ATRI

- Imaging of mobile non-covalent-bond-forming molecules
- Multi-color imaging of small molecules in live cells



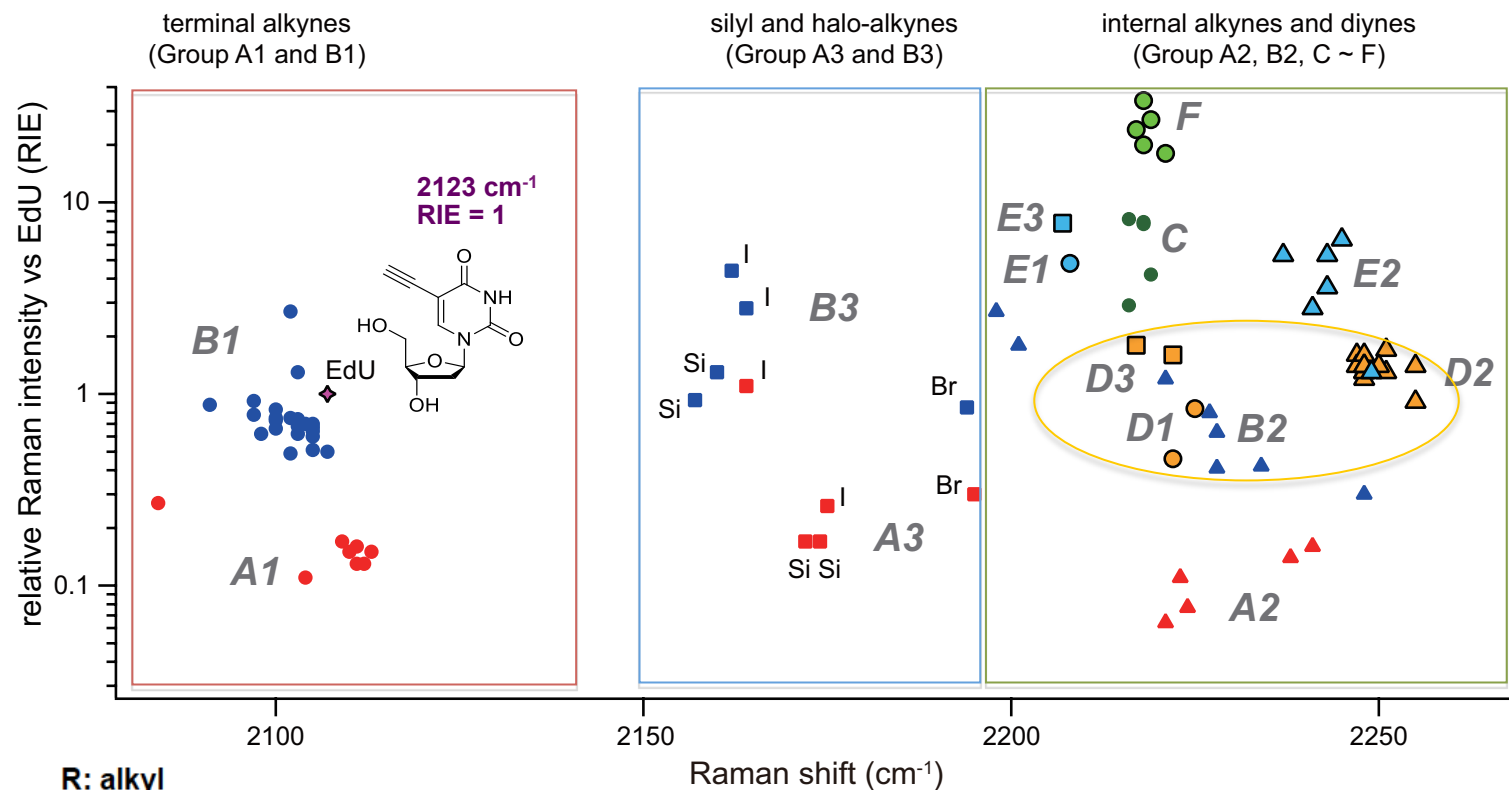
· **Structure-Raman Shift/Intensity Relationship Study**

Guideline for molecular design

Yamakoshi, H.; Dodo, K.; Palonpon, A.; Ando, J.; Fujita, K.; Kawata, S.; Sodeoka, M.
J. Am. Chem. Soc. **2012**, *34*, 20681.

Palonpon, A.; Ando, J.; Yamakoshi, H.; Dodo, Sodeoka, M ; Kawata, S.; Fujita, K.
Nature Protocol **2013**, *8*, 677.

Structure-Raman Shift/Intensity Relationship of Alkynes



R: alkyl

Ar: aromatic

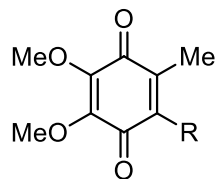
X: Br, I, Si

Average RIE (Raman shift cm⁻¹)

Average RIE (Raman shift cm⁻¹)

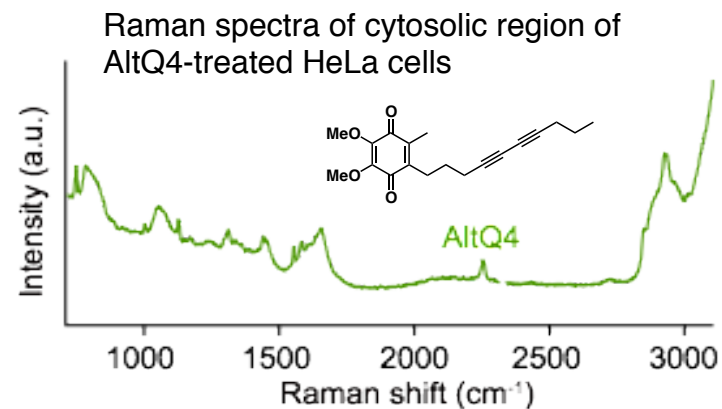
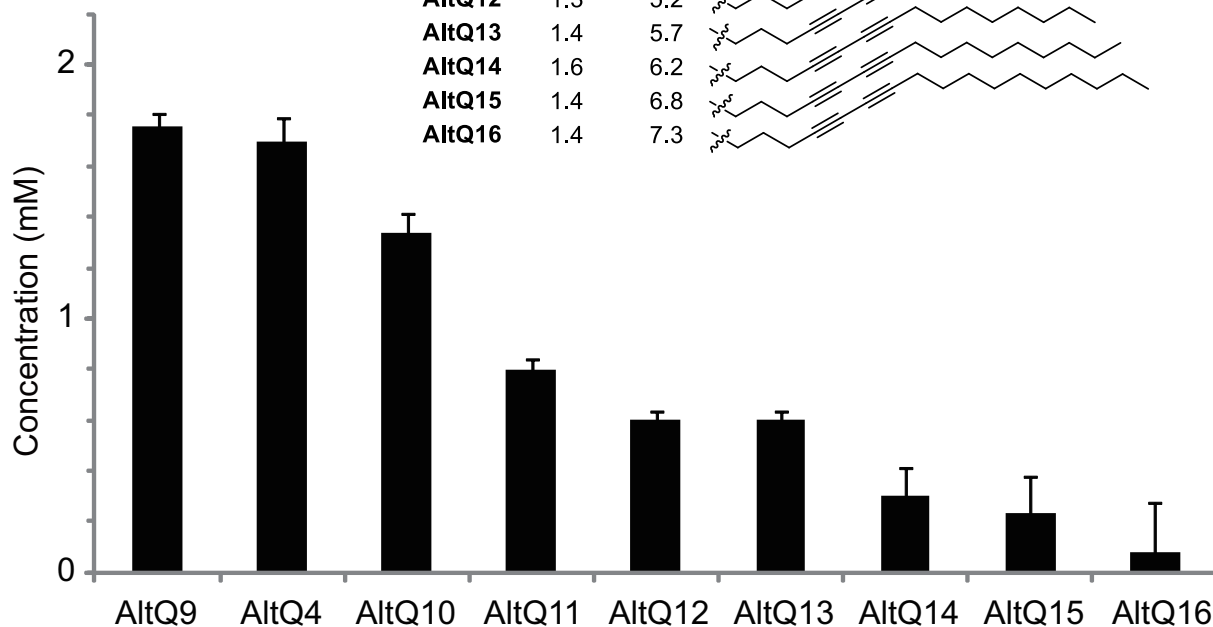
● A1	R≡H	0.16 (2084 ~2113)	● D1	R≡≡H	0.65 (2222 ~2225)
▲ A2	R≡R	0.11 (2221 ~2241)	▲ D2	R≡≡R	1.4 (2247 ~2255)
■ A3	R≡X	0.40 (2164 ~2195)	■ D3	R≡≡X	1.7 (2217 ~2222)
● B1	Ar≡H	0.81 (2091 ~2107)	● E1	Ar≡≡H	4.8 (2208)
▲ B2	Ar≡R	1.0 (2198 ~2248)	▲ E2	Ar≡≡R	4.1 (2237 ~2249)
■ B3	Ar≡X	2.1 (2157 ~2194)	■ E3	Ar≡≡X	7.8 (2207)
● C	Ar≡Ar	6.2 (2216 ~2219)	● F	Ar≡≡Ar	25 (2217 ~2221)

Estimation of Intracellular Concentration of Ubiquinone Analogs

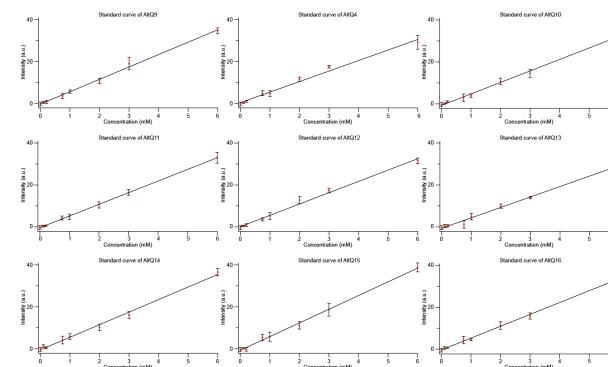


CoQ₁₀	CLogP: >12	R =
decylubiquinone	CLogP: 5.4	R =
idebenone	CLogP: 3.4	R =

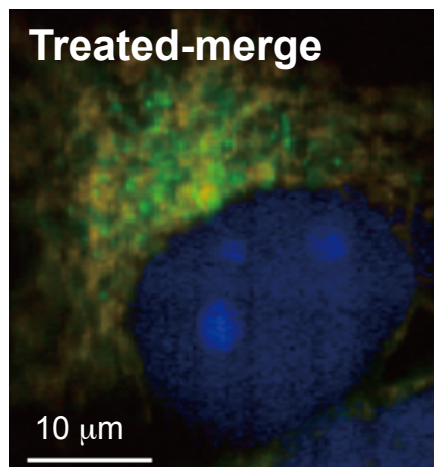
compound	RIE	CLogP	R
AltQ9	1.6	3.1	
AltQ4	1.4	3.6	
AltQ10	1.3	4.1	
AltQ11	1.2	4.7	
AltQ12	1.3	5.2	
AltQ13	1.4	5.7	
AltQ14	1.6	6.2	
AltQ15	1.4	6.8	
AltQ16	1.4	7.3	



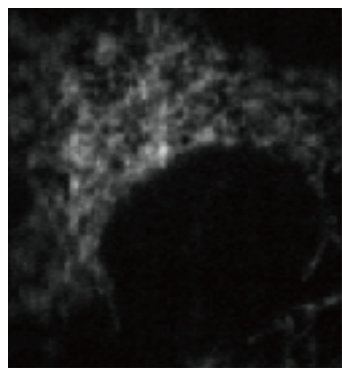
Relative Raman intensity of diyne-tagged analogues in live HeLa cells. Averaged Raman spectra of cytoplasmic region (3.6 μm x 3.6 μm) of 21 cells cultured with AltQs. Sample concentration was 20 μM . Incubation time was 60 min. The light intensity at the sample plane was 3 mW/mm^2 , and the exposure time for each line was 10 sec.



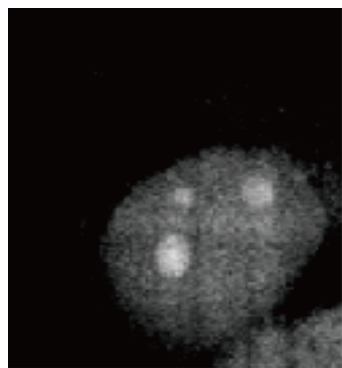
Two-color alkyne tag Raman imaging



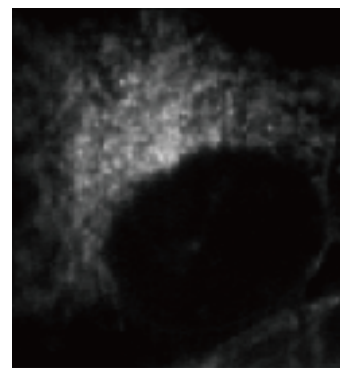
2 μM AltQ2 and 40 μM EdU, HeLa cell



Red: 747 cm^{-1}
Cytochrome c

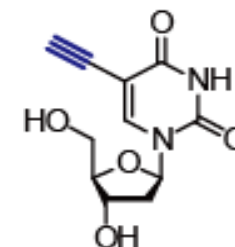


Blue: 2122 cm^{-1}
EdU

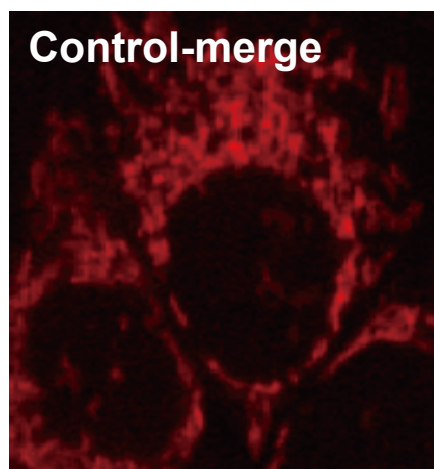


Green: 2248 cm^{-1}
AltQ2

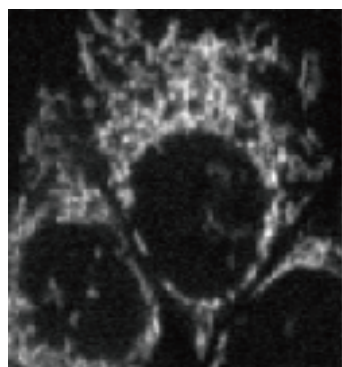
532 nm irradiation



EdU



Control (without treatment), HeLa cell



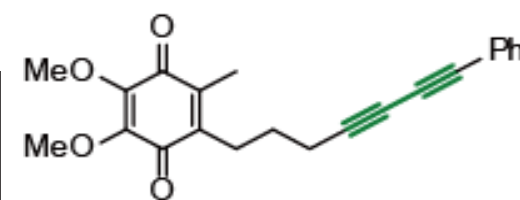
Red: 747 cm^{-1}
Cytochrome c



Blue: 2122 cm^{-1}
EdU



Green: 2248 cm^{-1}
AltQ2



AltQ2

532nm, 3 $\text{mW}/\mu\text{m}^2$, 10
sec/line, 170 lines, image
acquisition time: 38min.

Simultaneous detection is possible by combinations of alkyne tags!