Utilizing Transition Metal Catalysts in Living Cells

2024.04.27. Literature Seminar

M2 Takahiro Migita

Contents

1. **Introduction**
   ~transition metal applied in living cells

2. **Main Article**

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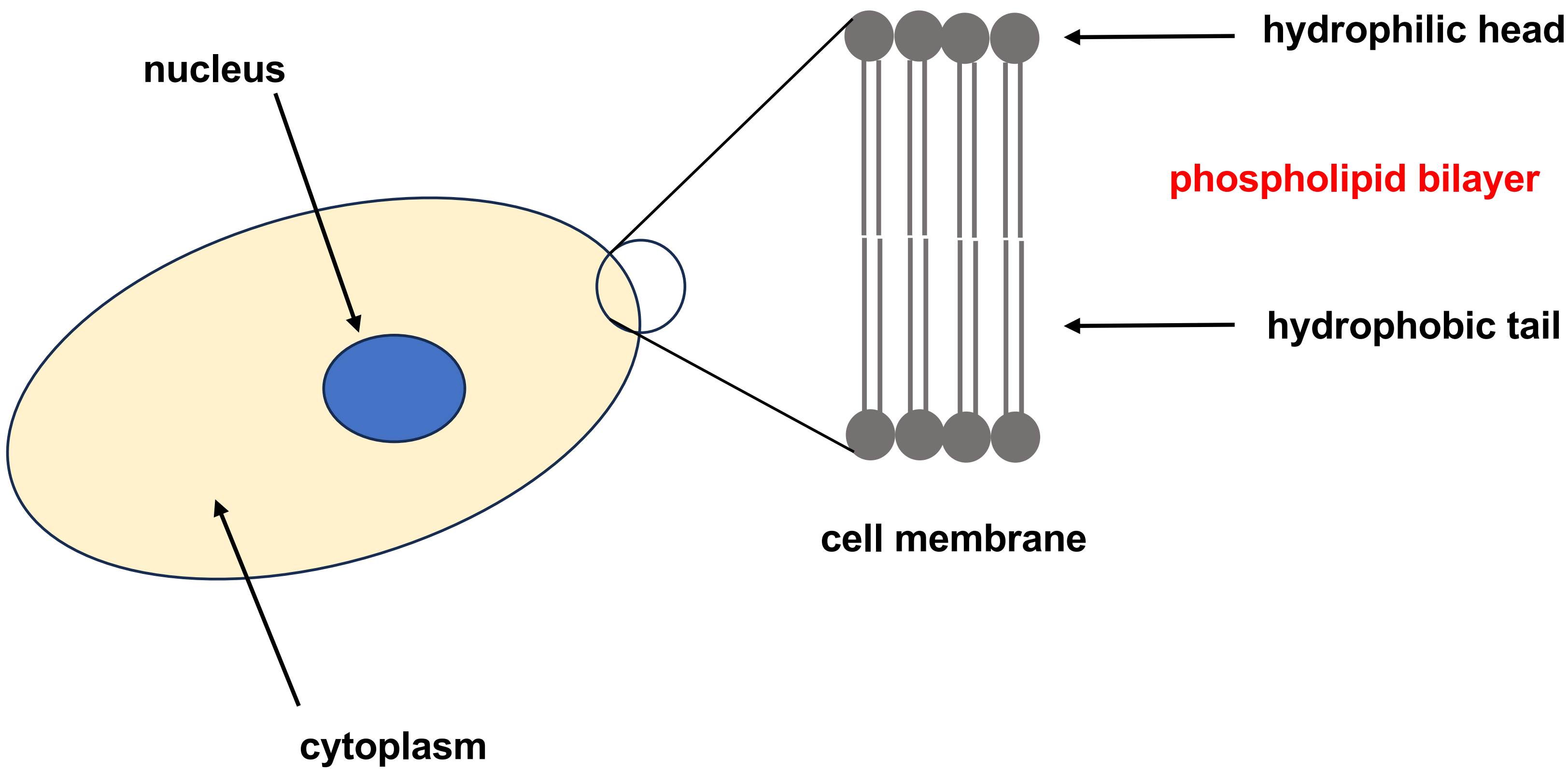
Article

A Transfer Hydrogenation Approach to Activity-Based Sensing of Formate in Living Cells

Steven W. M. Crossley,[#] Logan Tenney,[#] Vanha N. Pham, Xiao Xie, Michelle W. Zhao, and Christopher J. Chang*

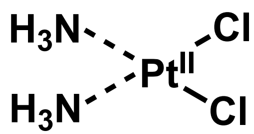
Eukaryotic Cellular Environment



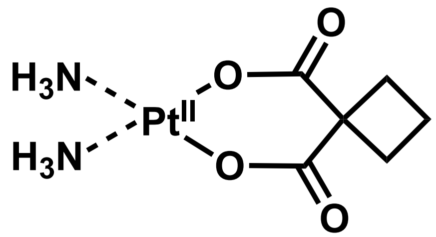
- Small neutral compounds can diffuse through the cell membrane.
- an aqueous aerobic environment (pH 7.0, 37 ℃)
- high salt concentrations and high quantities of thiols

James, C. C.; de Bruin, B.; Reek, J. N. *Angew. Chem., Int. Ed.* **2023**, *62*, e202306645.

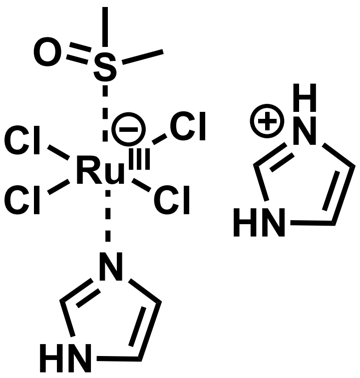
Transition Metal Drugs



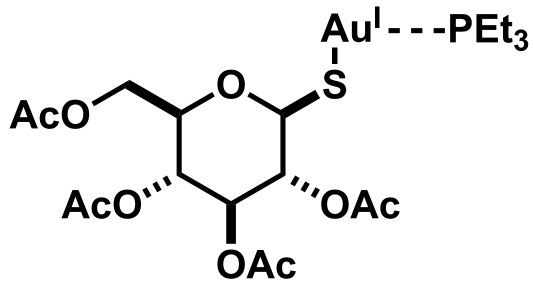
cisplatin
anti-cancer



carboplatin
anti-cancer



NAMI-A
anti-cancer, phase 2



auranofin
antirheumatic drug

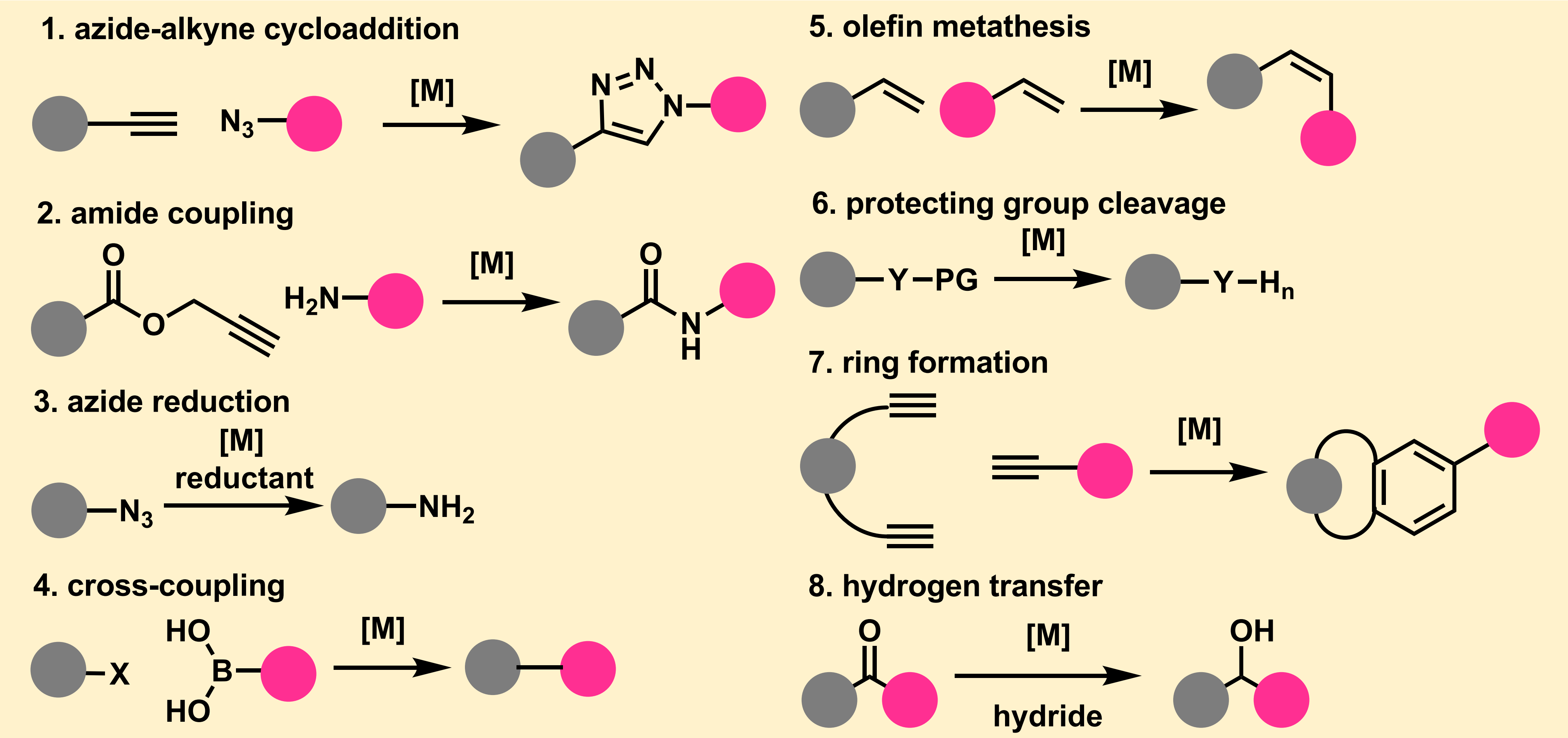
While highly active, all react only one time with their targets.

Soldevila-Barreda, J. J.; Metzler-Nolte, N. *Chem. Rev.* **2019**, *119*, 829-869.

Transition Metal Catalysts in Cells

Merits

- increasing the reaction and substrate scopes to **obtain non-natural reactivity**
- **tunable** metal center and ligands to achieve the desired reactivity

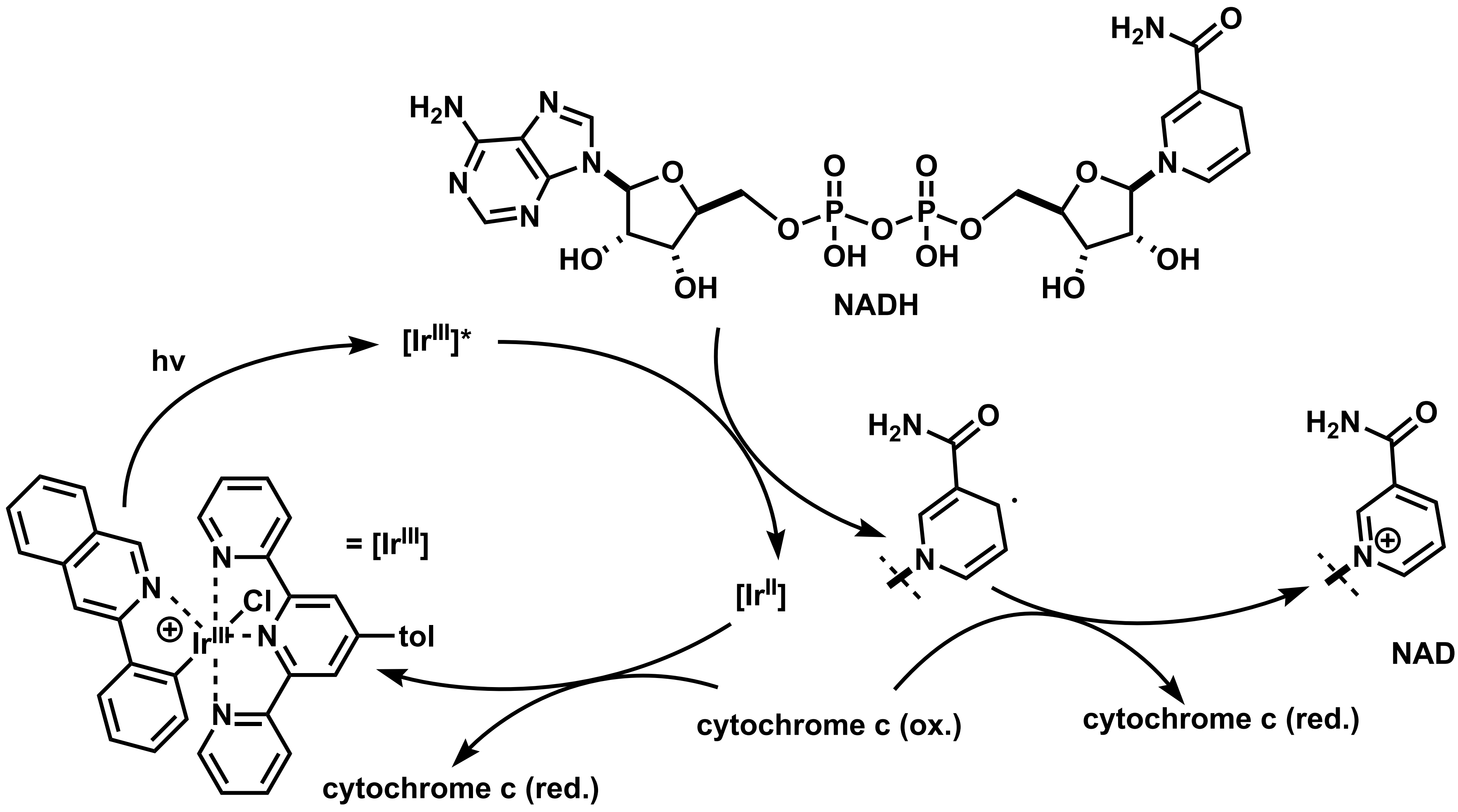


application: drug candidates (shown later), biomarker evaluation (this article)

1) James, C. C.; de Bruin, B.; Reek, J. N. *Angew. Chem., Int. Ed.* **2023**, *62*, e202306645. 2) Do, L. H. et. al. *ACS catalysis*, **2021**, *11*, 5148-5165.

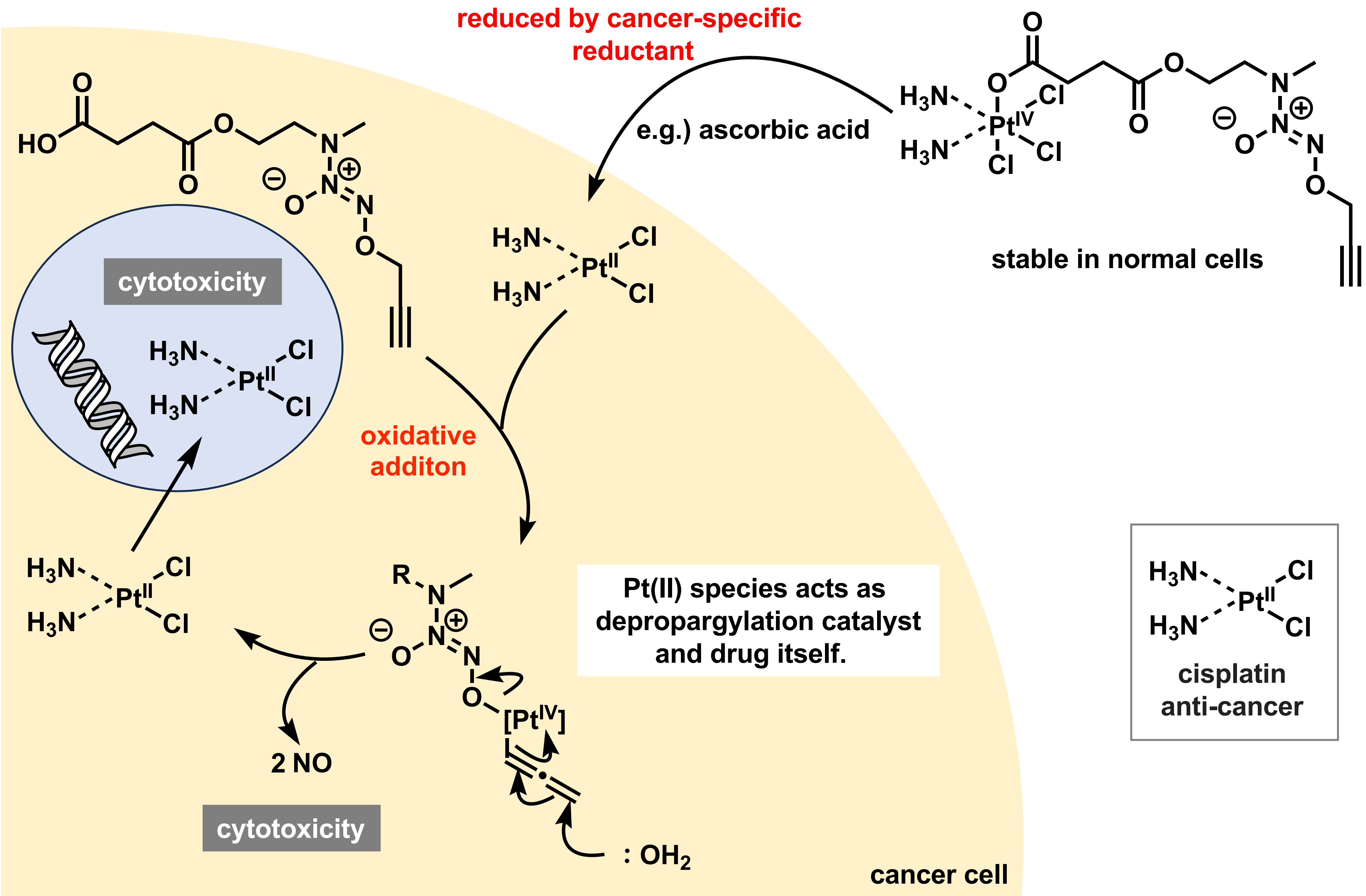
Oxygen-Independent Cytotoxic Photocatalyst

in cancer cell (hypoxic environment);



Cytotoxicity is expressed by the shortage in cytochrome c (ox.) and NADH.

Pt-Utilized Cancer-Specific Prodrug



Huang, Z. et. al. *J. Med. Chem.* **2020**, *63*, 13899-13912.

Contents

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Article

A Transfer Hydrogenation Approach to Activity-Based Sensing of Formate in Living Cells

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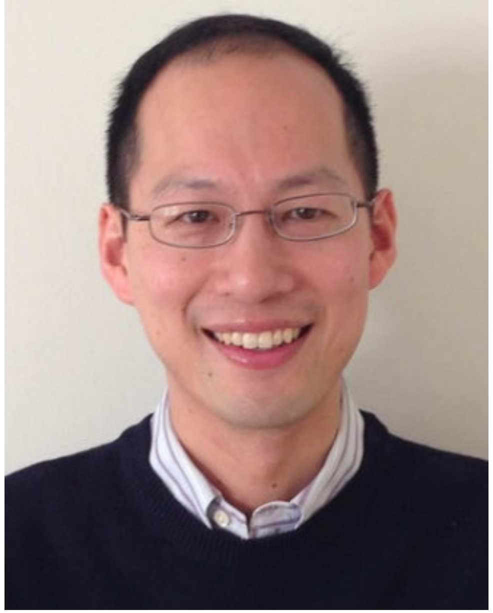
Prof. Christopher J. Chang

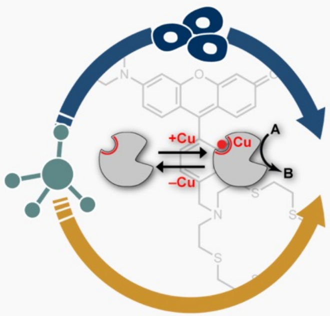


Career

1997 :M.S. @ California Institute of Technology (Prof. Harry B. Gray)
2002 :Ph.D. @ MIT (Prof. Daniel G. Nocera)
2002- :Postdoc. @ MIT (Prof. Stephen J. Lippard)
2004- :Assistant professor @ University of California, Berkeley
2009- :Associate Professor @ University of California, Berkeley
2012- :Full Professor @ University of California, Berkeley

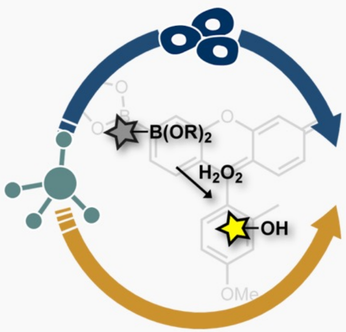
Research Field

1. Transition Metal Signaling and Metalloallostery
2. Activity-Based Sensing
3. Activity-Based Proteomics
4. Artificial Photosynthesis



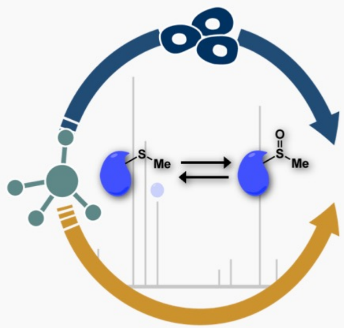
Transition Metal Signaling

Bioinorganic chemistry beyond active sites.



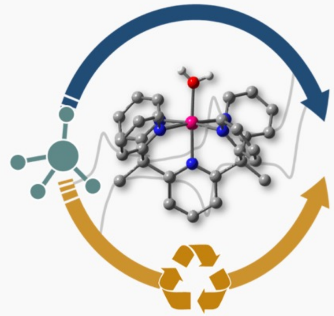
Activity-Based Sensing

Leveraging selective chemistry to decipher new redox and one-carbon biology.



Activity-Based Proteomics

Bioconjugation chemistry for single-atom signaling and redox drug discovery.

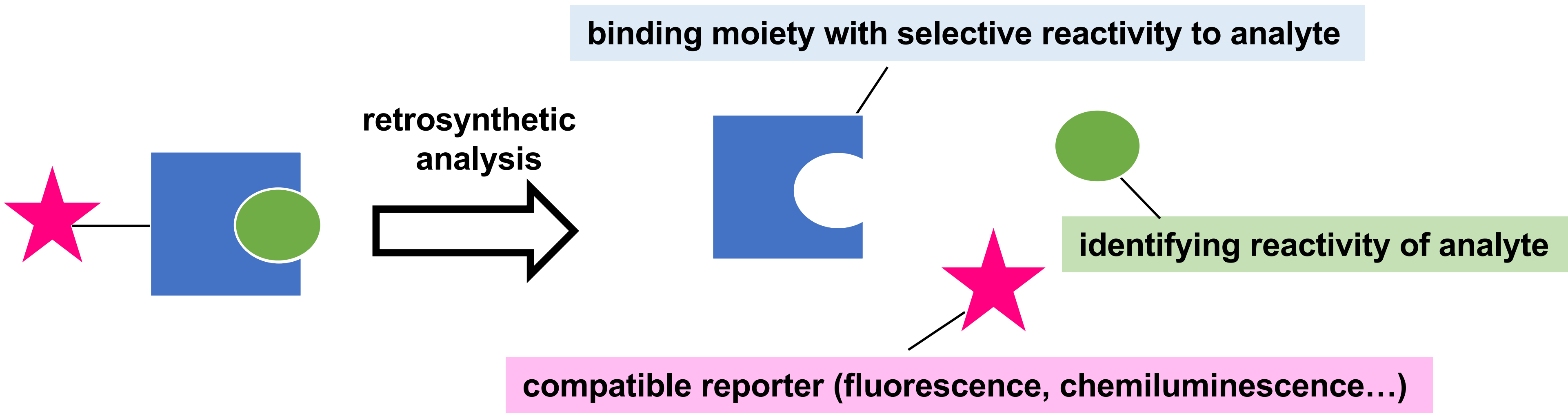


Artificial Photosynthesis

Catalyzing sustainable electrosynthesis.

Activity-Based Sensing

	traditional methods	activity-based sensing
analyte recognition	static (lock-and-key)	dynamic (chemospecific reaction)

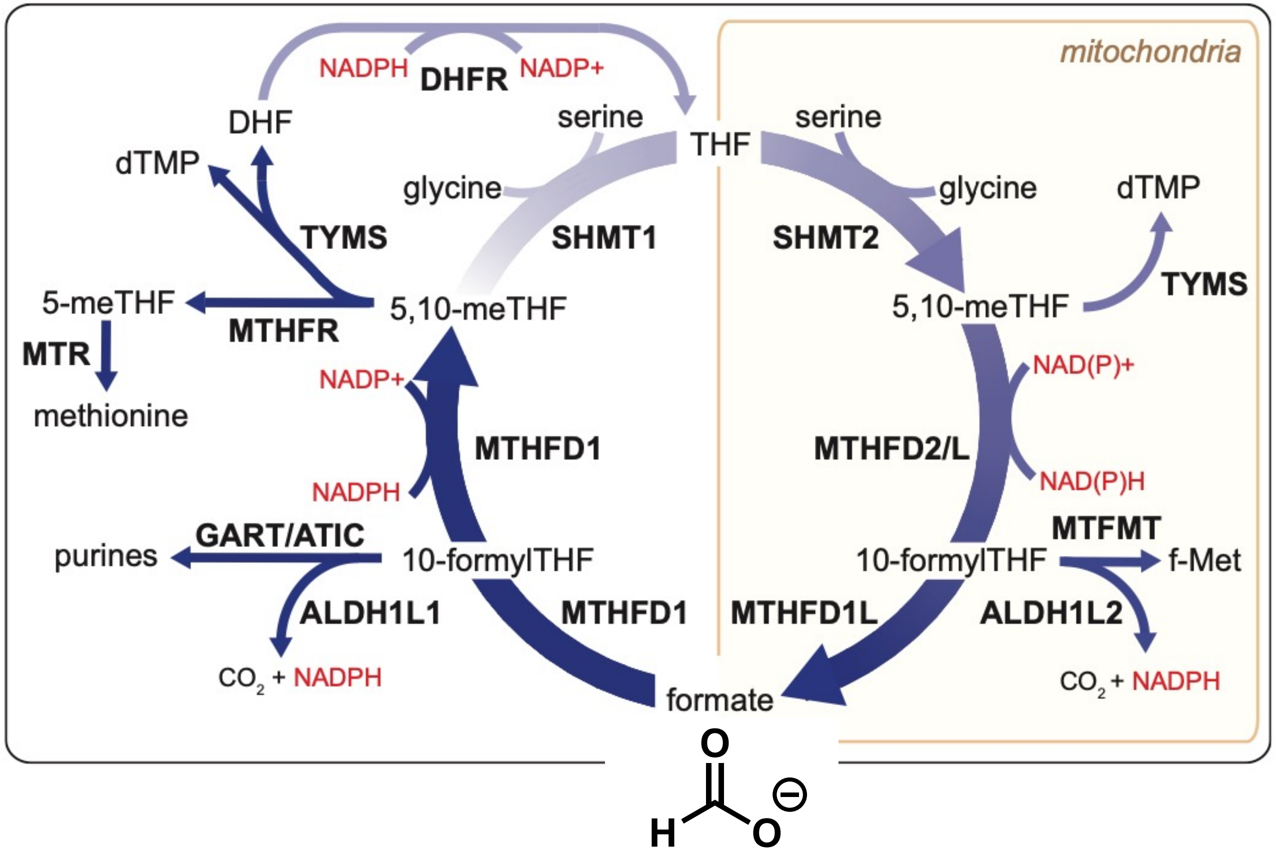


Controlling reactivity of each moiety is a central issue in ABS strategy.

1) Crossley, S. W.; Tenney, L.; Pham, V. N.; Xie, X.; Zhao, M. W.; Chang, C. J. *J. Am. Chem. Soc.* **2024**, *146*, 8865-8876.
2) Bruemmer, K. J.; Crossley, S. W. M.; Chang, C. J. *Angew. Chem., Int. Ed. Engl.* **2020**, *59*, 13734−13762.

Target is Formate

*THF means a kind of folate (葉酸) here.



Formate plays an important role in one-carbon metabolism, which controls homeostasis.
Formate is a potential biomarker in diagnosis in cancer and other serious disease.

However, current analysis methods are limited (LC-MS, NMR etc.).

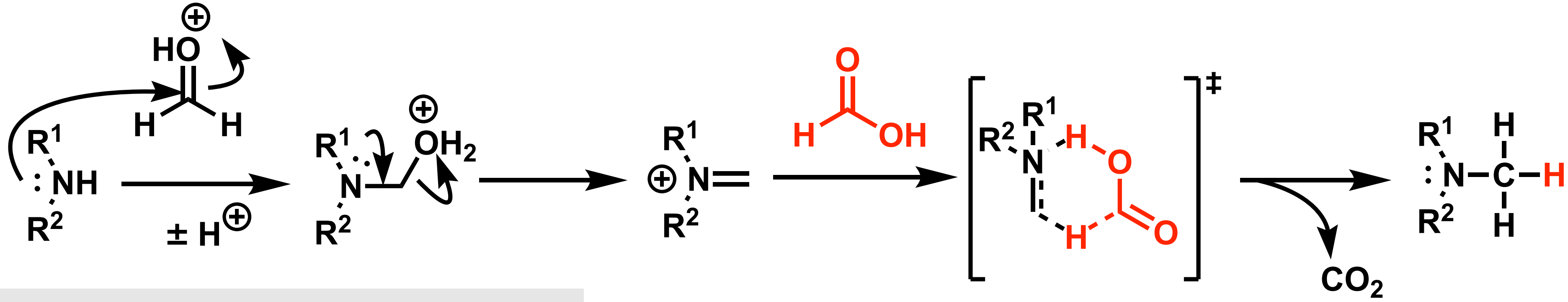
activity-based sensing

1) Crossley, S. W.; Tenney, L.; Pham, V. N.; Xie, X.; Zhao, M. W.; Chang, C. J. *J. Am. Chem. Soc.* **2024**, *146*, 8865-8876.
2) Ducker, G. S.; Rabinowitz, J. D. *Cell Metab.* **2017**, *25*, 27−42.

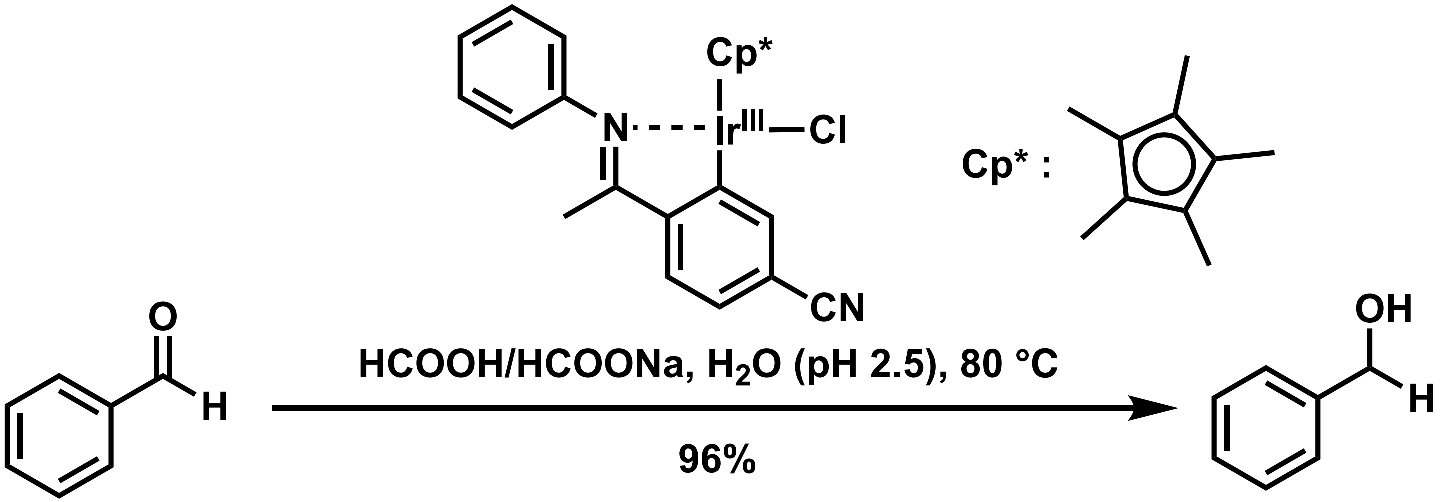
Formate as Hydride Donor

$$HCOO^{\ominus}_{(aq.)} \longrightarrow H^{\ominus}_{(aq.)} + CO_{2\,(g)} \quad 24.1\ \text{kcal/mol}$$

ex 1) Eschweiler-Clark reaction



ex 2) transfer hydrogenation catalysis → the authors chose the transfer reaction as the key reaction.

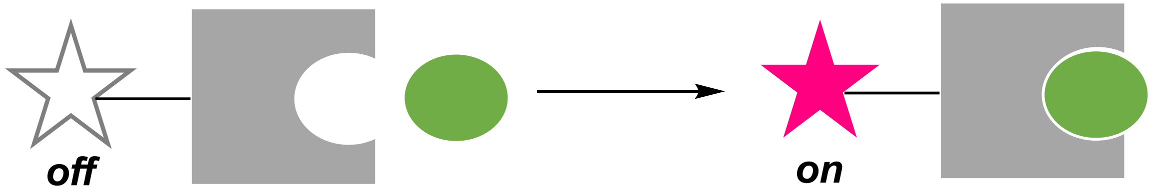


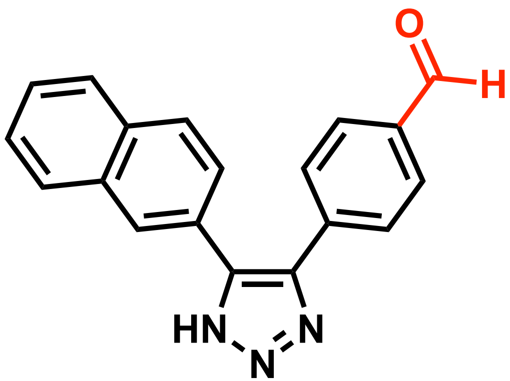
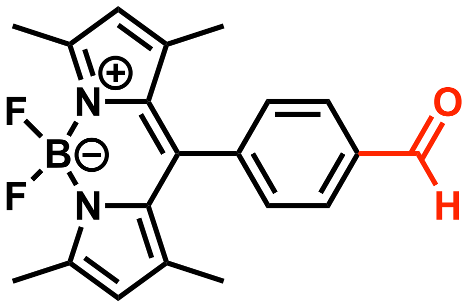
problems to tackle…
1. aldehyde-to-alcohol reduction to generate fluorophore
2. transition metal mediator working well in living cells
3. selective reactivity to formate

1) Chang, C. J. et. al. *J. Am. Chem. Soc.* **2024**, *146*, 8865-8876. 2) Xiao, J. et. al. *Green chemistry*, **2013**, *15*, 629-634.

Known Aldehyde-to-Alcohol Turn-on Fluorophores

*turn-on type fluorophore

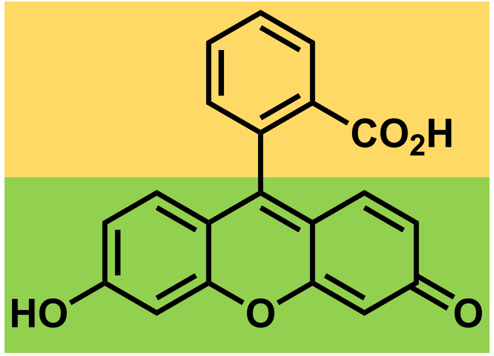


structure	$\lambda_{ex}/\lambda_{em}$ (nm)	photophysical changes
	300/359 high-energy ultraviolet ↓ *cell damages / autofluorescence*	Alcohol is 10-fold more emissive than aldehyde.
	480/415	Alcohol is only 5-fold more emissive than aldehyde.

Fluorescein-like scaffolds were investigated next.

1) Chang, C. J. et. al. *J. Am. Chem. Soc.* **2024**, *146*, 8865-8876. 2) Tanaka, F. et. al. *J. Org. Chem.* **2009**, *74*, 2417−2424.
3) Do, L. H. et. al. *J. Am. Chem. Soc.* **2017**, 139, 8792−8795.

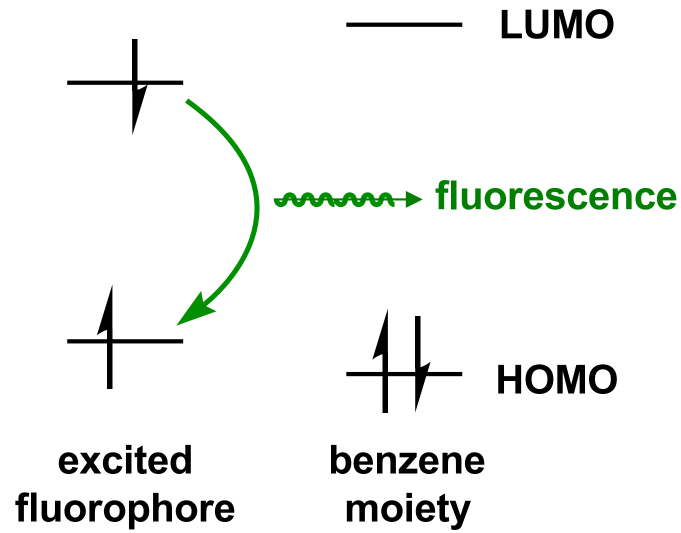
Fluorescein-like Scaffolds



benzene moiety

fluorophore

fluorescein

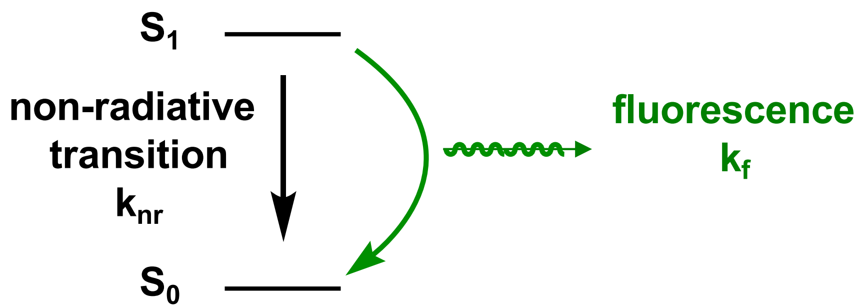


What is the merit of fluorescein-like scaffolds?

1. lower energy visible excitation wavelength
→ not damaging cells

2. high quantum yield in aqueous solvents
→ applicable in physiological condition

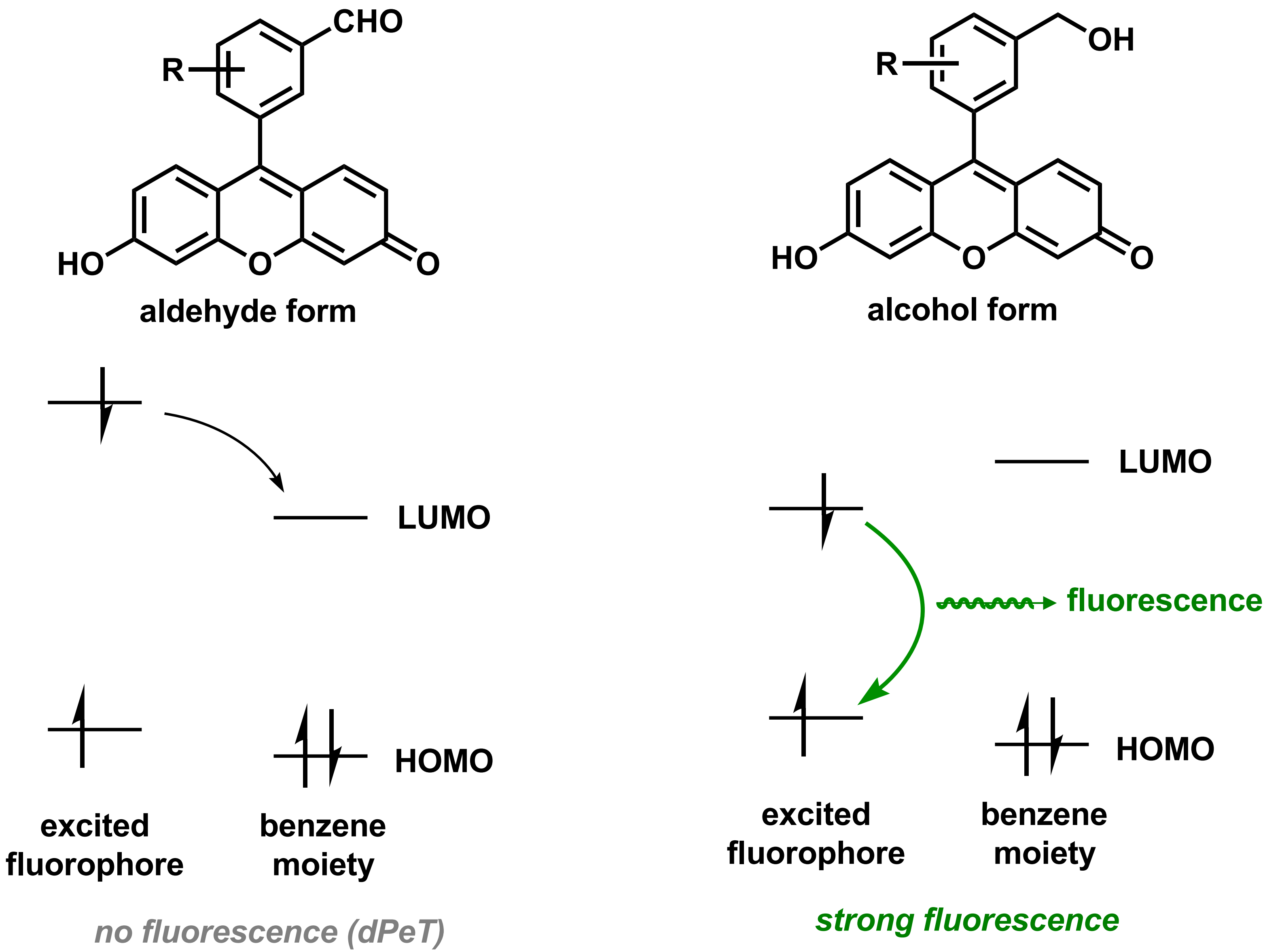
3. tunable fluorophore and benzene moiety
→ optimized to work well in specific condition



$$\text{quantum yield} = \frac{k_f}{k_f + k_{nr}}$$

1) Chang, C. J. et. al. *J. Am. Chem. Soc.* **2024**, *146*, 8865-8876. 2) Nagano, T. *Proc. Jpn. Acad. Ser. B, Phys.* **2010**, *86*, 837−847.
3) Nagano, T. et. al. *J. Am. Chem. Soc.* **2005**, *127*, 4888−4894.

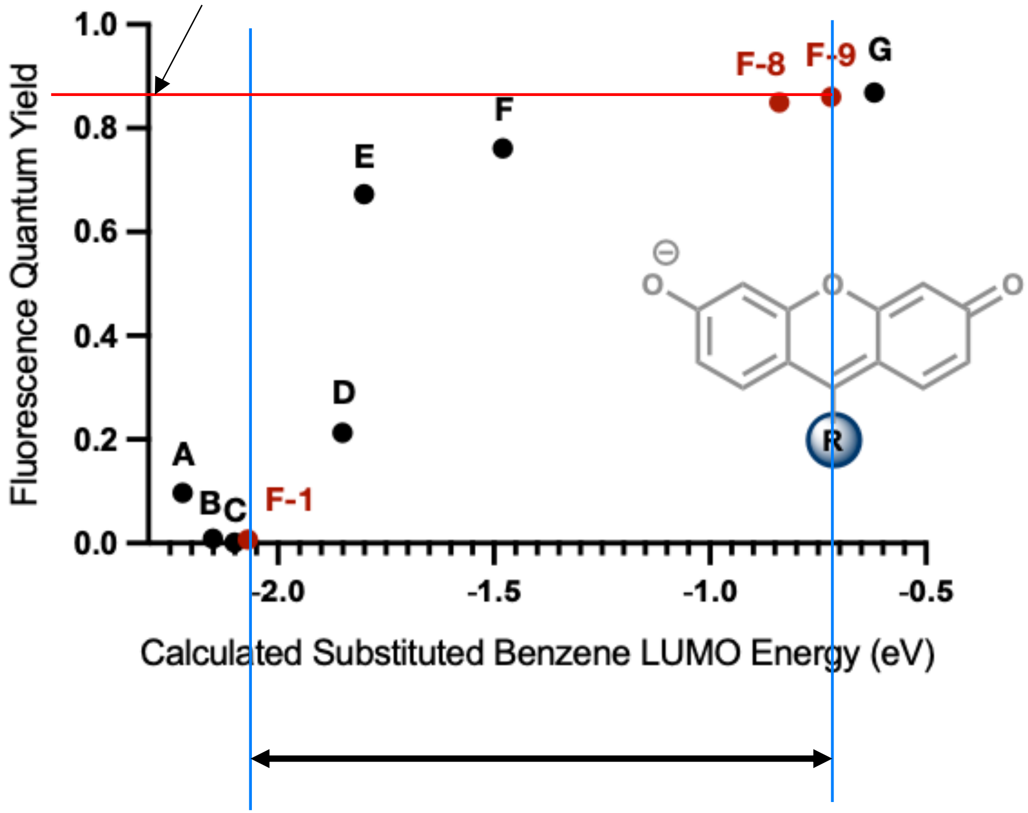
dPeT Strategy



1) Chang, C. J. et. al. *J. Am. Chem. Soc*. **2024**, *146*, 8865-8876. 2) Nagano, T. *Proc. Jpn. Acad. Ser. B, Phys.* **2010**, *86*, 837−847.
3) Nagano, T. et. al. *J. Am. Chem. Soc.* **2005**, *127*, 4888−4894.

Calculating LUMO of Benzene Moiety and Quantum Yield

high quantum yield

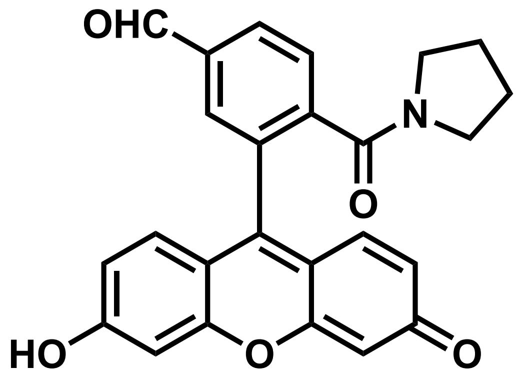


large gap in LUMO energy between aldehyde-form and alcohol-form

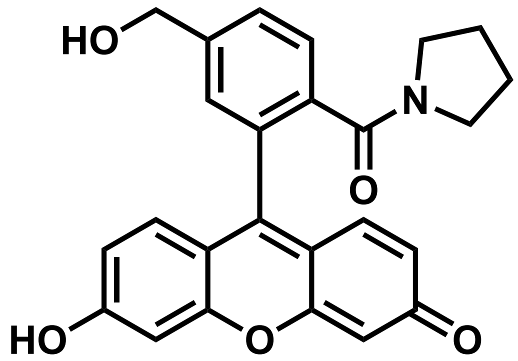
computed using the *Spartan '18* program from *Wavefunction, Inc*
submitted to an 'Equilibrium Geometry' calculation at 'Ground' state in 'Water' with 'Density Functional B3LYP method and a '6-311G*' basis set *post*-geometry minimization
a methyl group used as a surrogate for the fluoresceine moiety

A–G : references (experimental data)

F-1



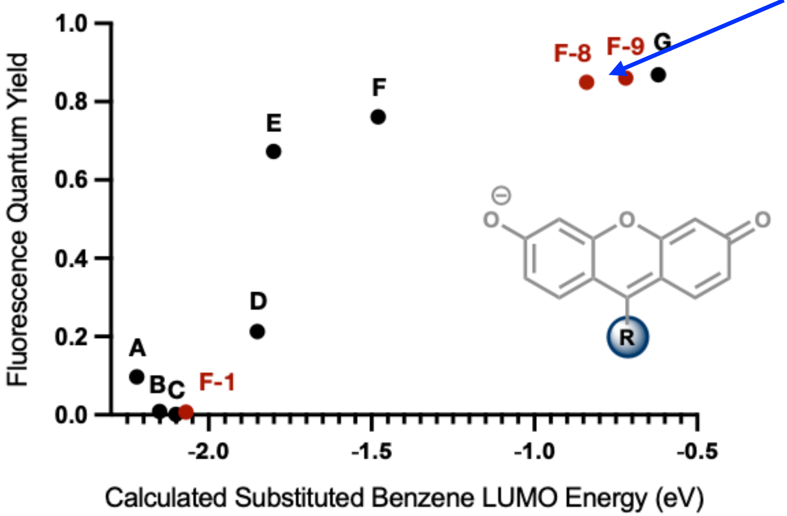
F-9

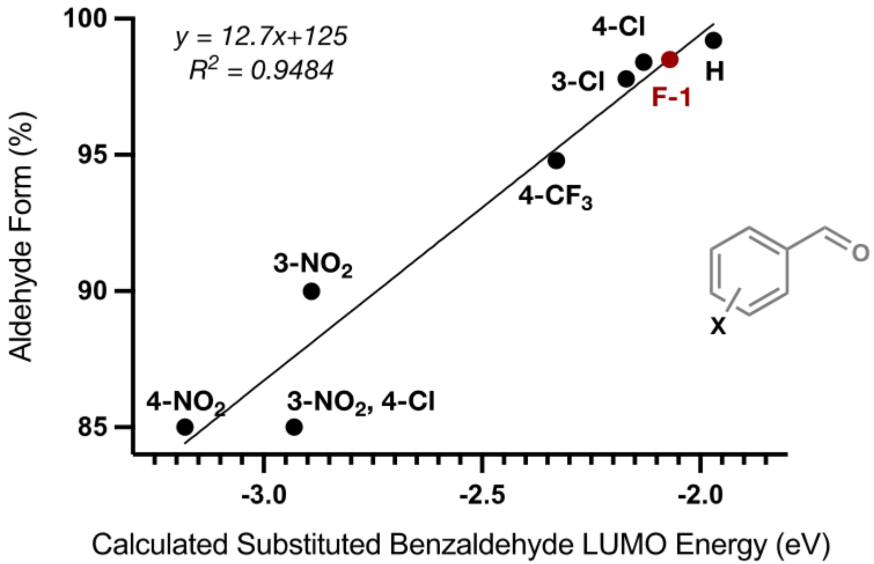


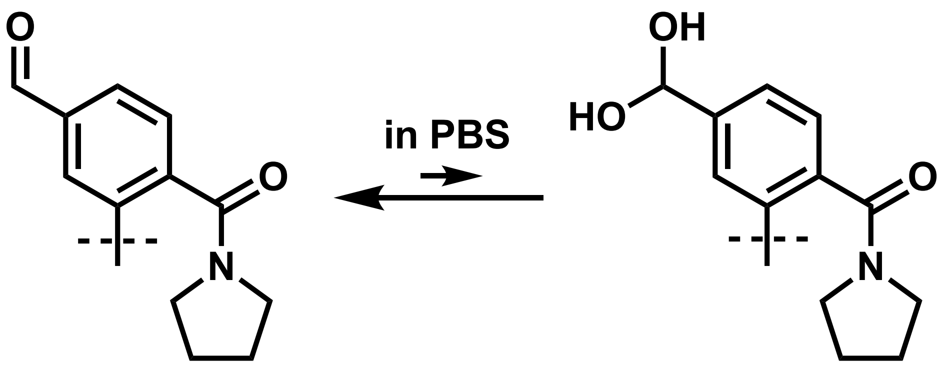
Crossley, S. W.; Tenney, L.; Pham, V. N.; Xie, X.; Zhao, M. W.; Chang, C. J. *J. Am. Chem. Soc.* **2024**, *146*, 8865-8876.

Predicted Effect of Hydration

Hydrate F-8 could be also bright.







F1 98.5%

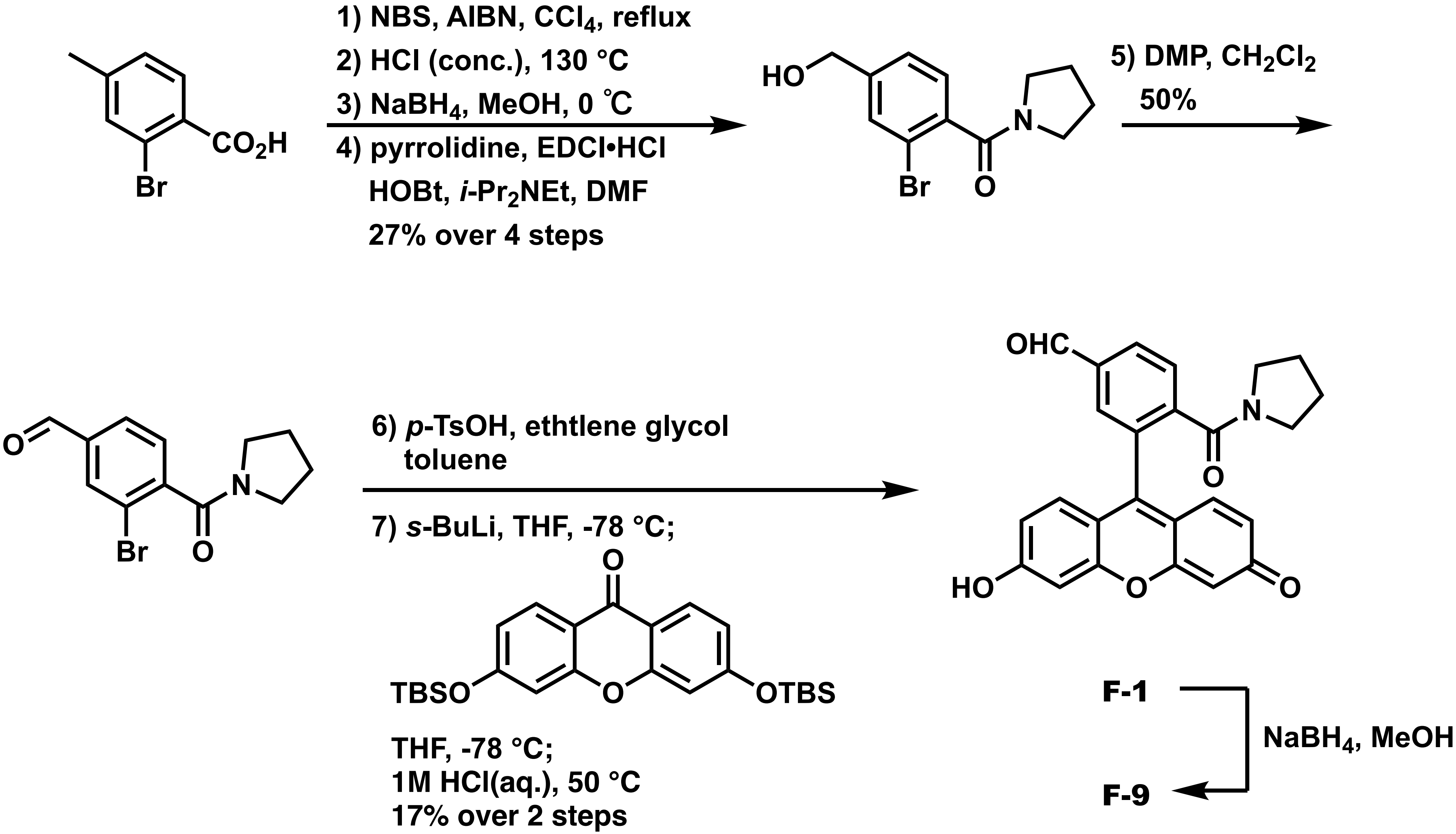
F8 1.5% ***negligible***

Theoretical design and analysis of probe was finished.

The fluorophore should be synthesized for further investigation.

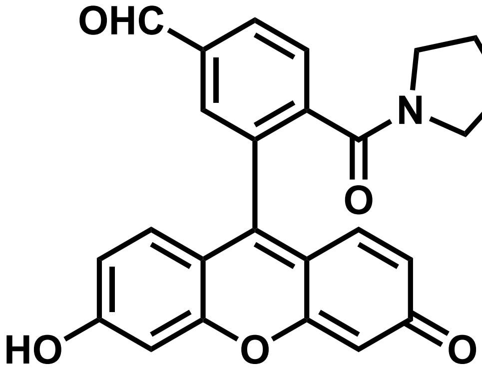
Crossley, S. W.; Tenney, L.; Pham, V. N.; Xie, X.; Zhao, M. W.; Chang, C. J. *J. Am. Chem. Soc.* **2024**, *146*, 8865-8876.

Synthesis of the Designed Fluorophore

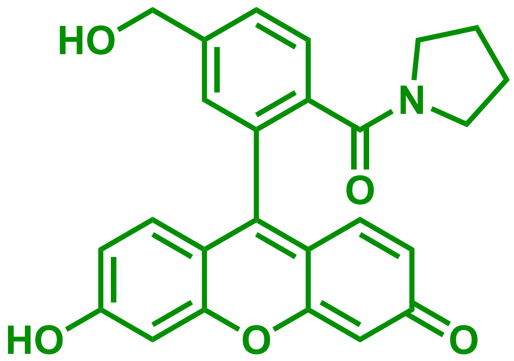


Crossley, S. W.; Tenney, L.; Pham, V. N.; Xie, X.; Zhao, M. W.; Chang, C. J. *J. Am. Chem. Soc*. **2024**, *146*, 8865-8876.

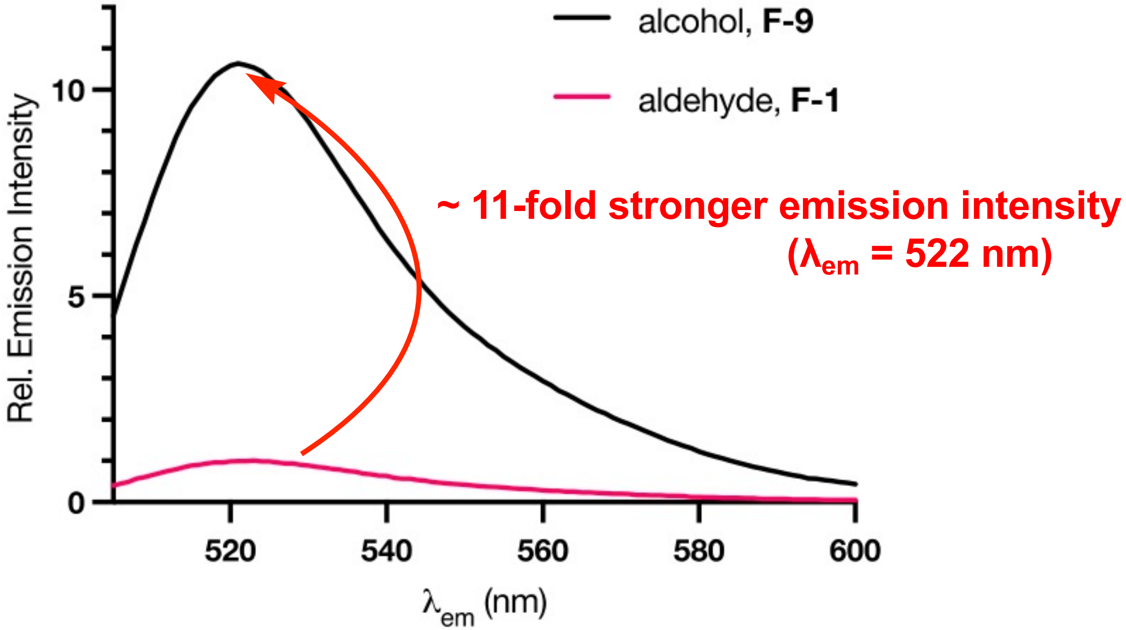
Emission Properties of F-1 and F-9



F-1



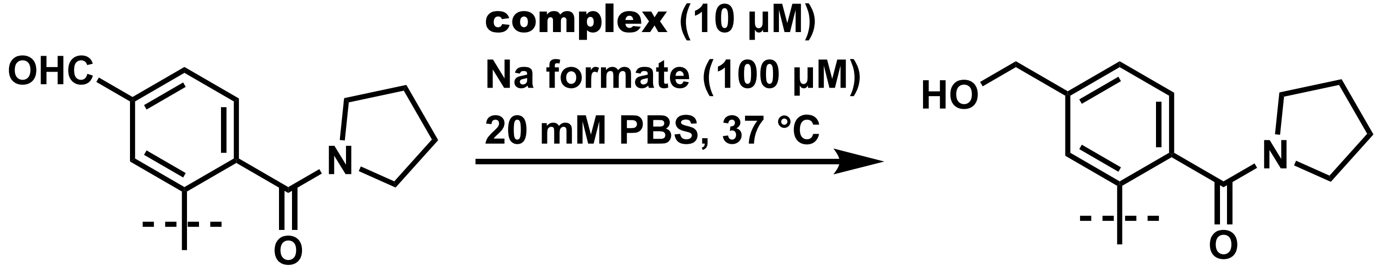
F-9

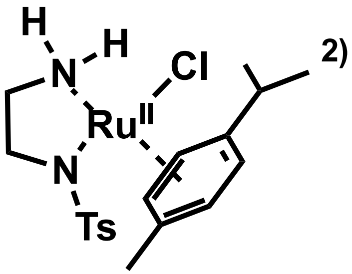
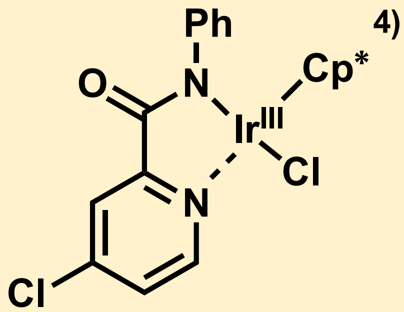
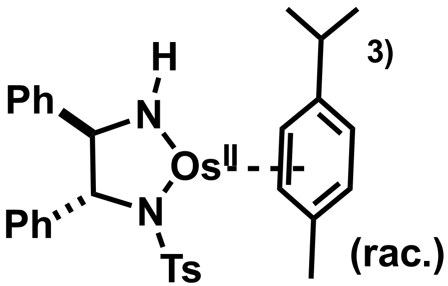
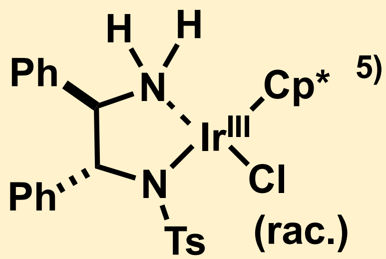
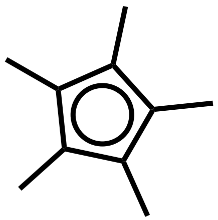
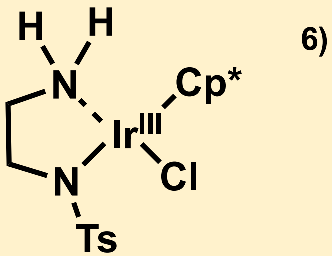


10 µM in 20 mM PBS, pH 7.4, λ_{ex} = 500 nm

Hydrogen transfer catalysts were investigated next.

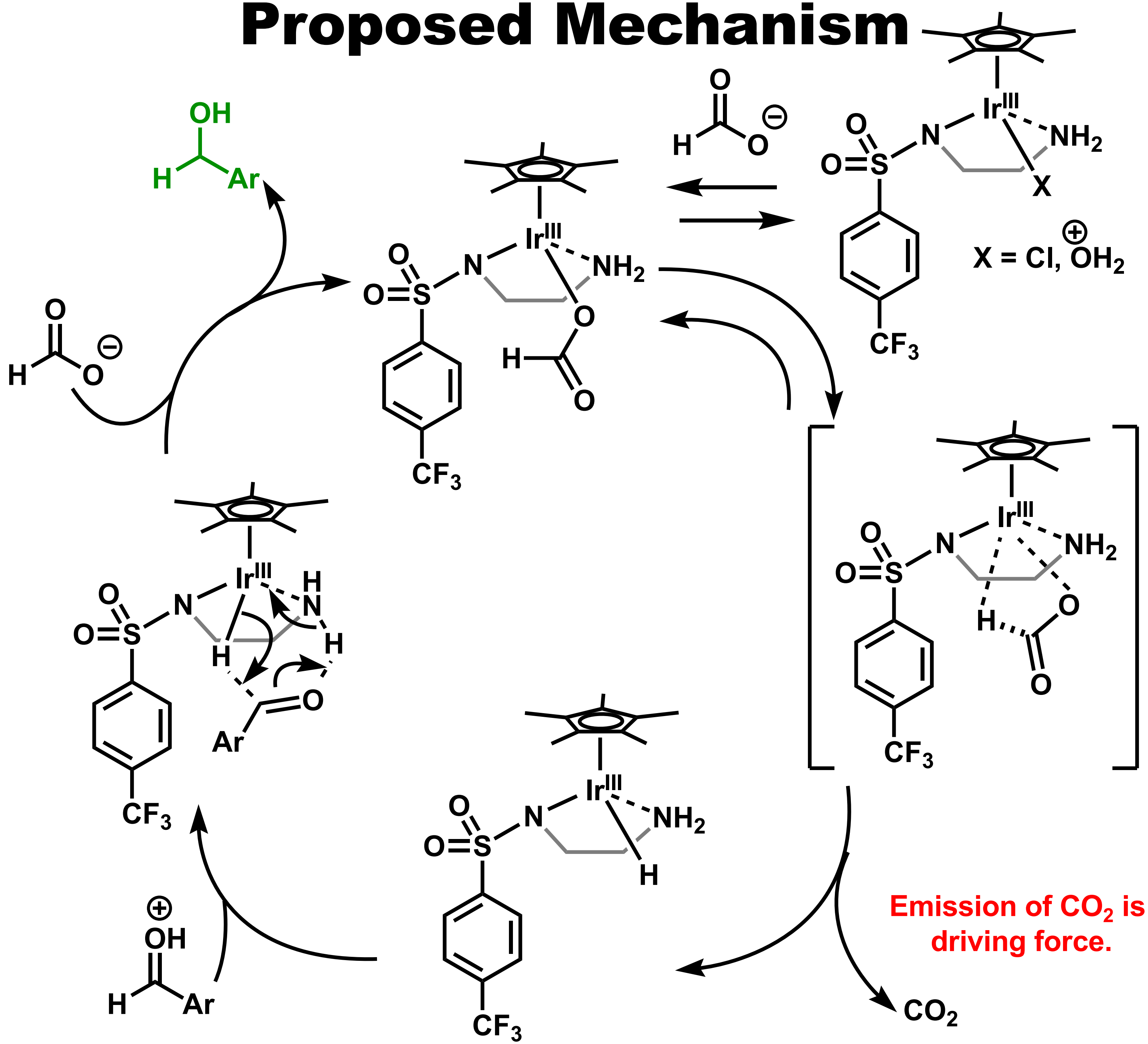
Metal Center



complex	relative initial rates	complex	relative initial rates
	1.0		2.8
	1.4		45.4
Cp* : 			30.5

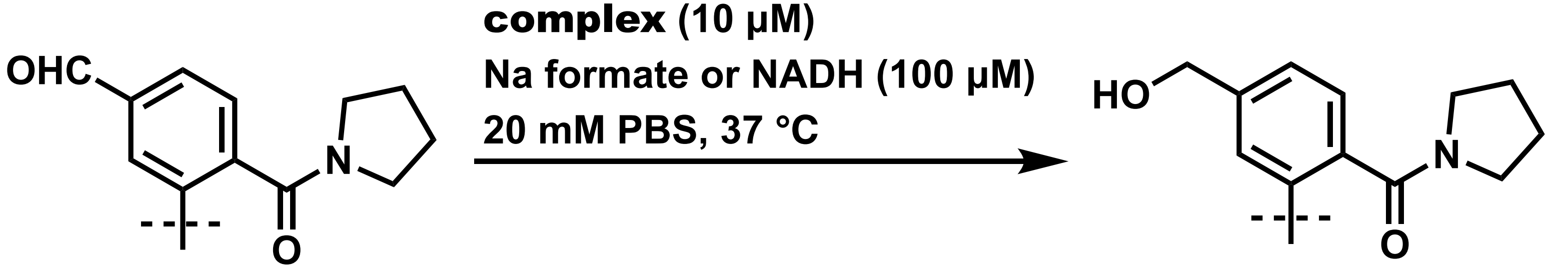
1) Chang, C. J. et. al. *J. Am. Chem. Soc*. **2024**, *146*, 8865-8876. 2) Sadler, P. J. et. al. *Dalton Transactions,* **2018**, *47*, 7178-7189.
3) Sadler, P. J. et. al. *Nat. Chem*. **2018**, *10*, 347−354. 4) Do, L. H. et. al. *J. Am. Chem. Soc*. **2017**, *139*, 8792−8795.
5) Rauchfuss, T. B. et. al. *Eur. J. Inorg. Chem*. **2009**, *33*, 4927−4930. 6) Xiao, J. et. al. *Angew. Chem., Int. Ed*. **2006**, *45*, 6718−6722.

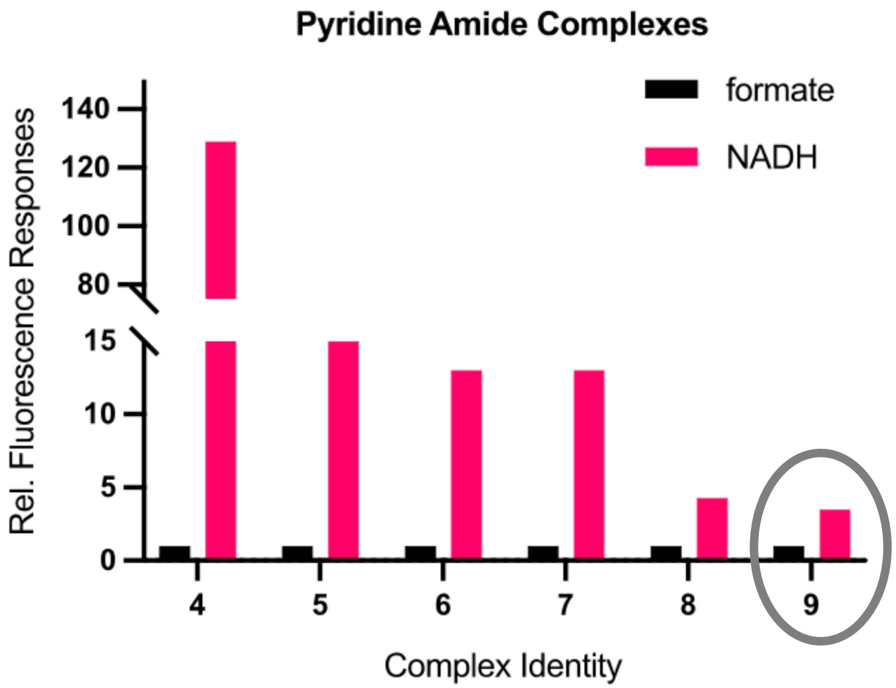
Proposed Mechanism

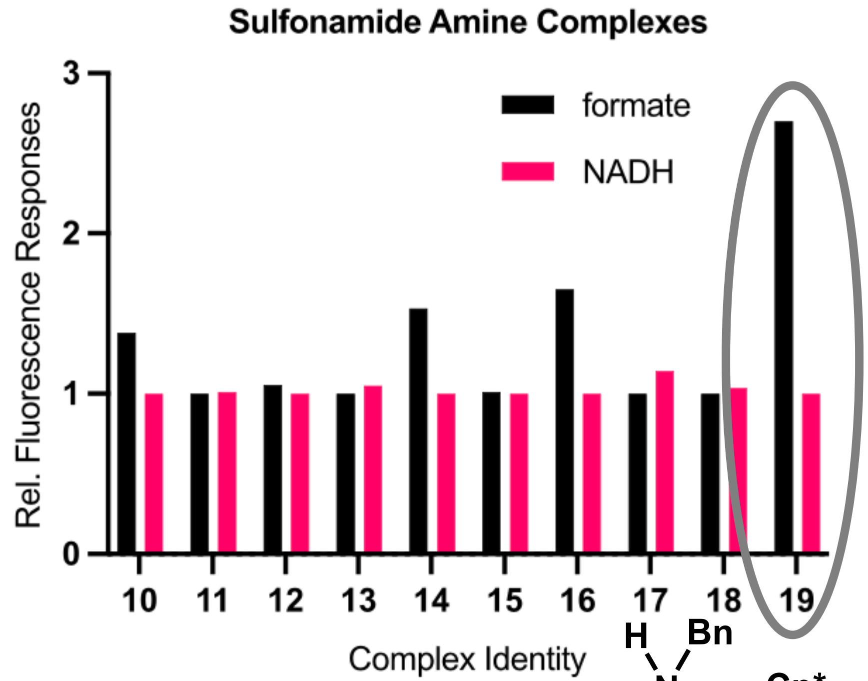


1) Chang, C. J. et. al. *J. Am. Chem. Soc*. **2024**, *146*, 8865-8876. 2) Page, M. I. et. al. *Catal. Sci. Technol*. **2020**, *10*, 590−612.

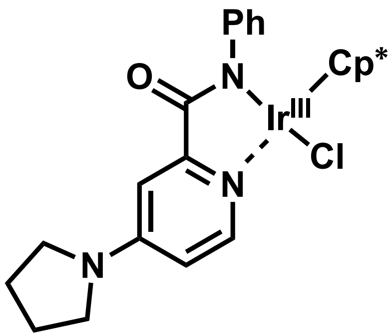
Screening ; Selectivity (Formate vs. NADH)



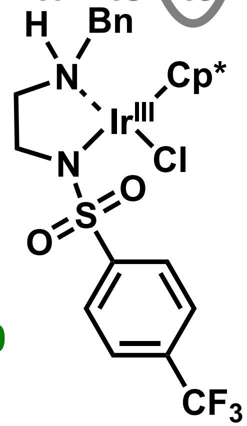




complex 9



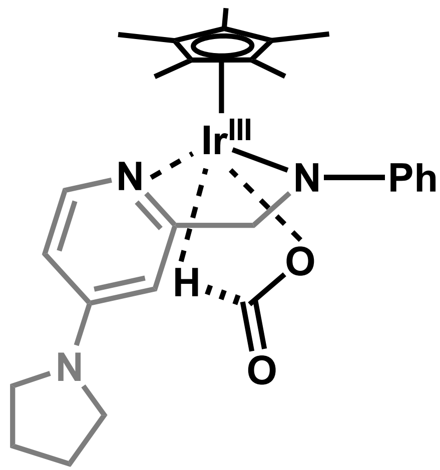
complex 19



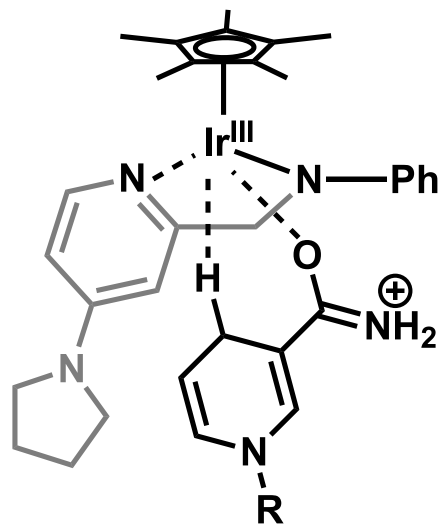
Crossley, S. W.; Tenney, L.; Pham, V. N.; Xie, X.; Zhao, M. W.; Chang, C. J. *J. Am. Chem. Soc.* **2024**, *146*, 8865-8876.

Rationale for Selectivity Observed on Complex 9

• formate; 4-membered ring transition state (unfavored)

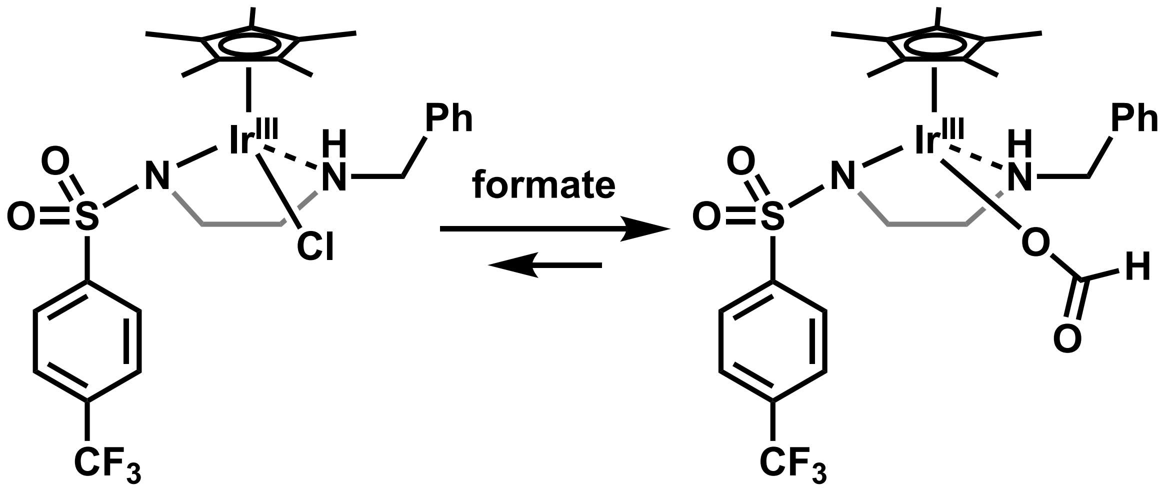


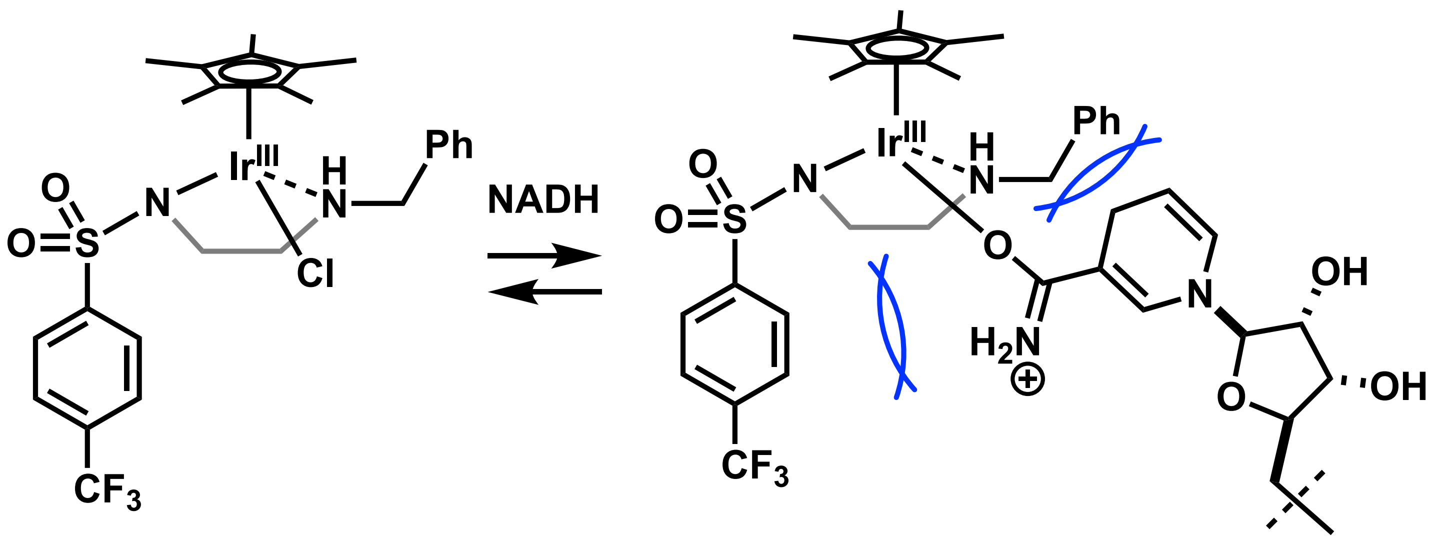
• NADH; 6-membered ring transition state (favored)→faster



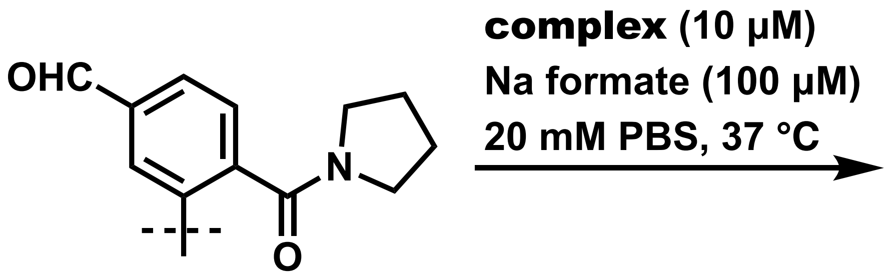
Crossley, S. W.; Tenney, L.; Pham, V. N.; Xie, X.; Zhao, M. W.; Chang, C. J. *J. Am. Chem. Soc*. **2024**, *146*, 8865-8876.

Rationale for Selectivity Observed on Complex 19



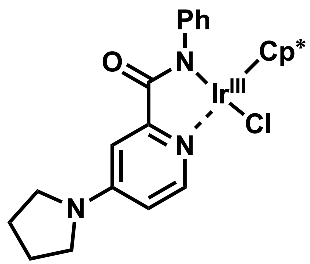


Comparing Kinetics of Catalysts

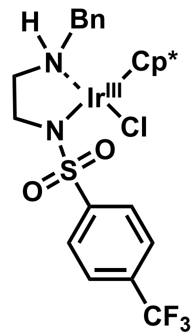


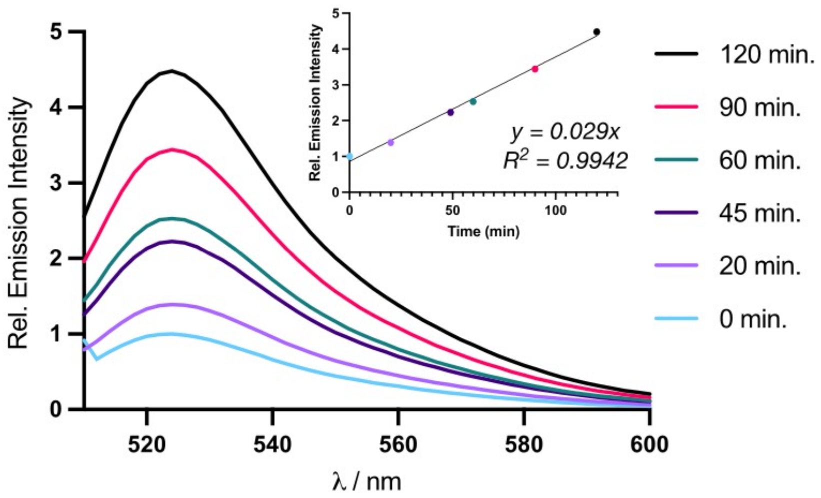
complex 9 showed 2.7-fold faster reduction rate than **complex 19**.

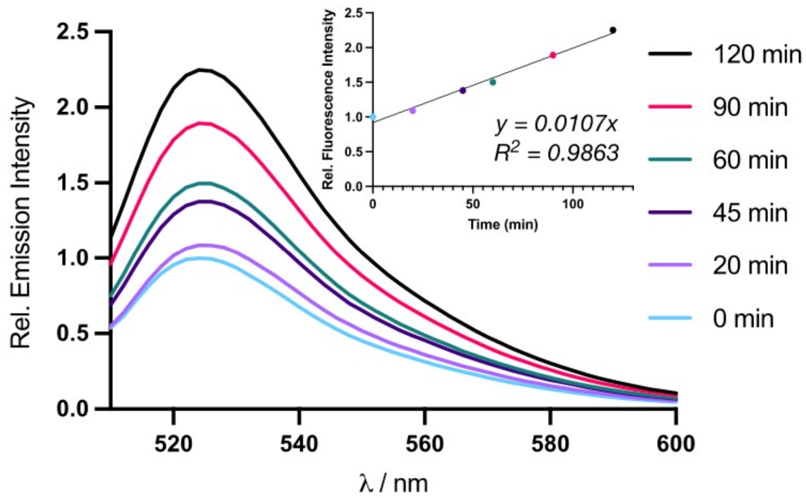
complex 9



complex 19

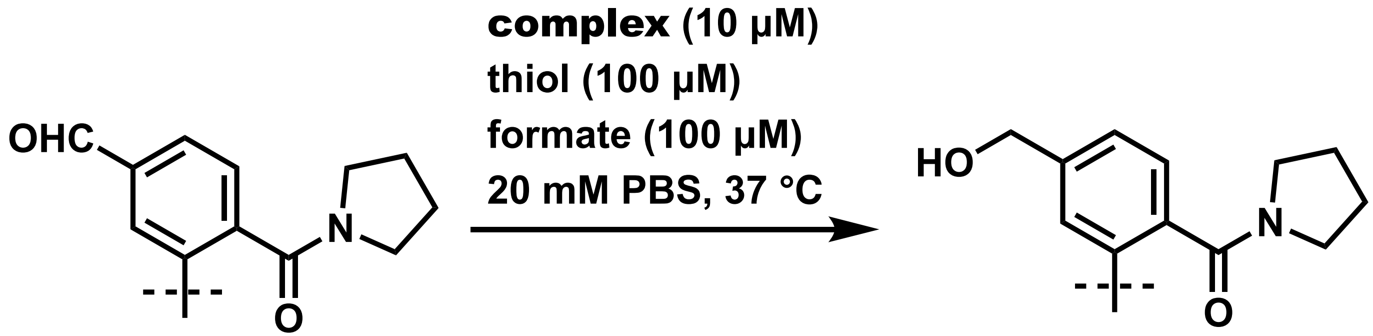






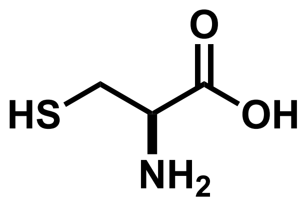
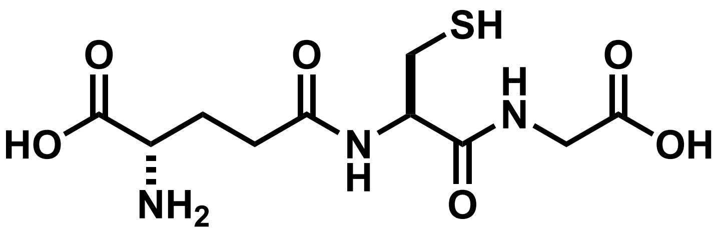
Crossley, S. W.; Tenney, L.; Pham, V. N.; Xie, X.; Zhao, M. W.; Chang, C. J. *J. Am. Chem. Soc*. **2024**, *146*, 8865-8876.

Thiols Inhibited the Reaction



(10 µM)

complex 9 was found to be appropriate catalyst.

thiol	normalized initial rate complex 9	normalized initial rate complex 19
none	1	1
 cystein	0.57	0
 glutathione	0.1	0

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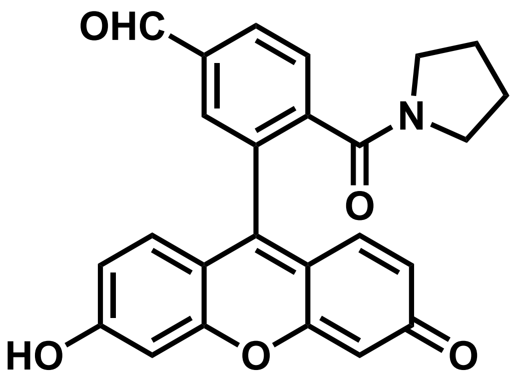
Failure in Turn-On Response and Redesigning Ratio-metric Response Fluorophore



F1

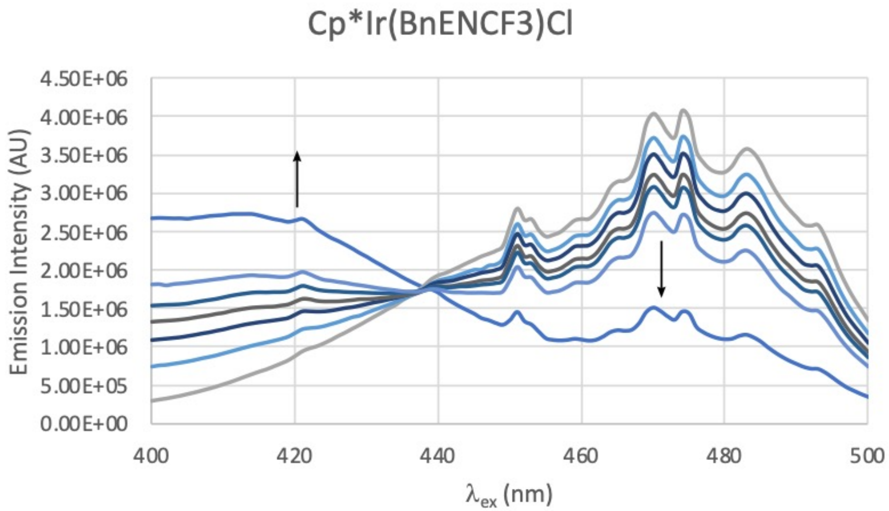
fail to work as turn-on fluorophore in living cells
---time/formate-dependent decreases in fluorescence intensity

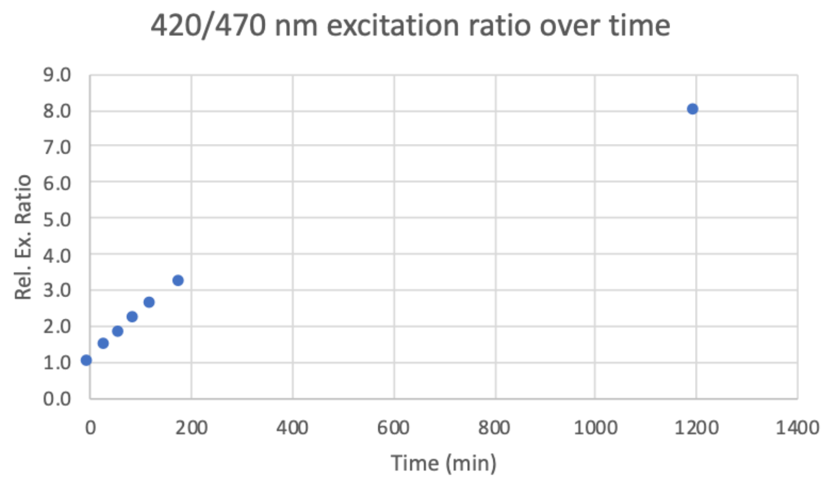
possible reasons
- fluorophore export ?
- degraded from formate-stimulated production of ROS ?

Ratio-metric Response Fluorophore : using intensity ratio, not intensity itself

Two or more wavelengths of an excitation or emission spectrum are measured.

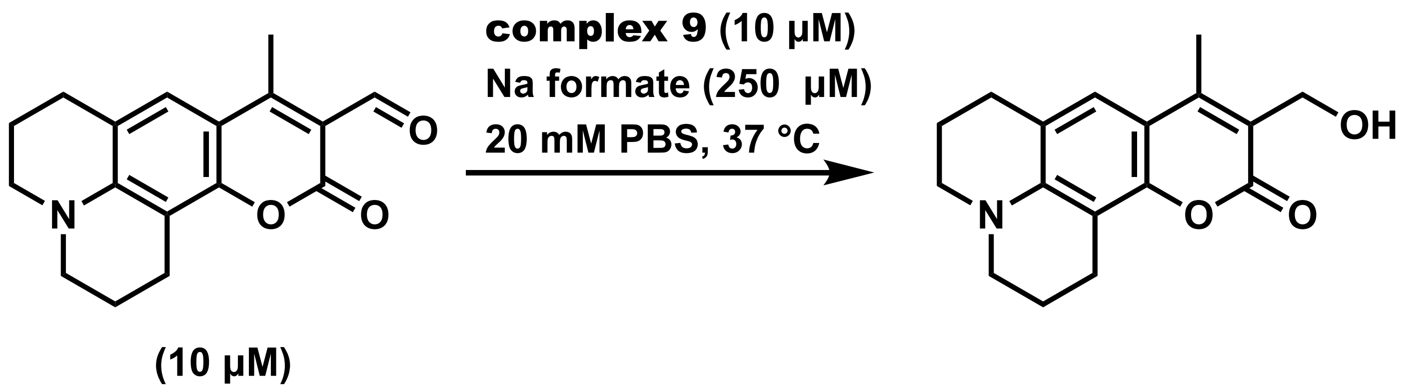
ex)
λ_{em} is fixed. A form: λ_{ex} = 420 nm, B form: λ_{ex} = 470 nm   A/B ratio is analyzed by intensity ratio.



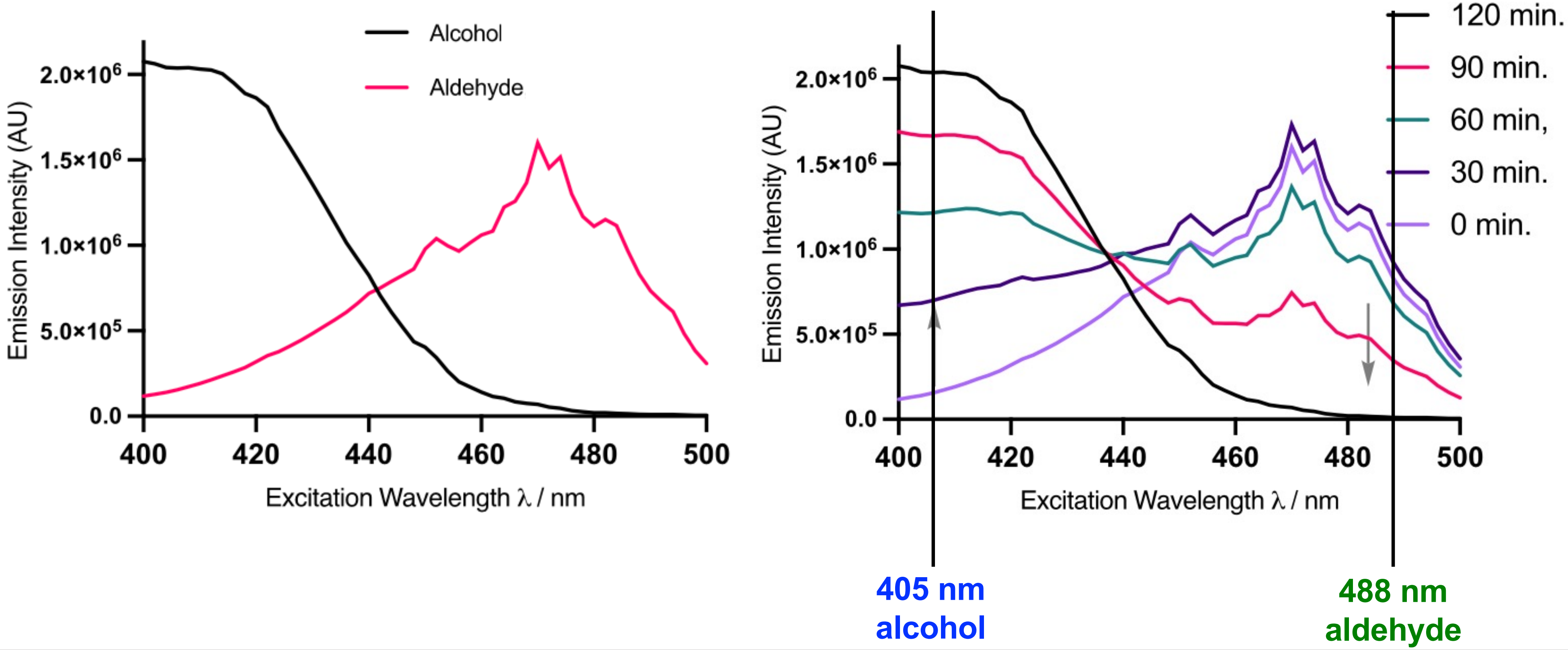


Crossley, S. W.; Tenney, L.; Pham, V. N.; Xie, X.; Zhao, M. W.; Chang, C. J. *J. Am. Chem. Soc.* **2024**, *146*, 8865-8876.

Profile of Ratio-metric Fluorophore



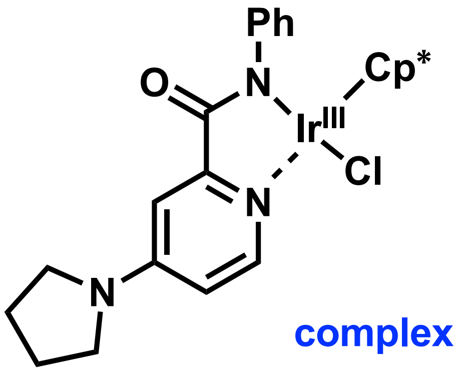
λ_{em} = 510 nm



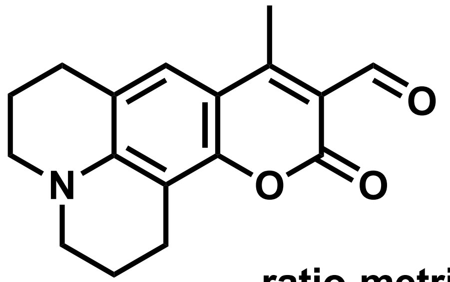
Crossley, S. W.; Tenney, L.; Pham, V. N.; Xie, X.; Zhao, M. W.; Chang, C. J. *J. Am. Chem. Soc.* **2024**, *146*, 8865-8876.

Applied in Cellular Environment

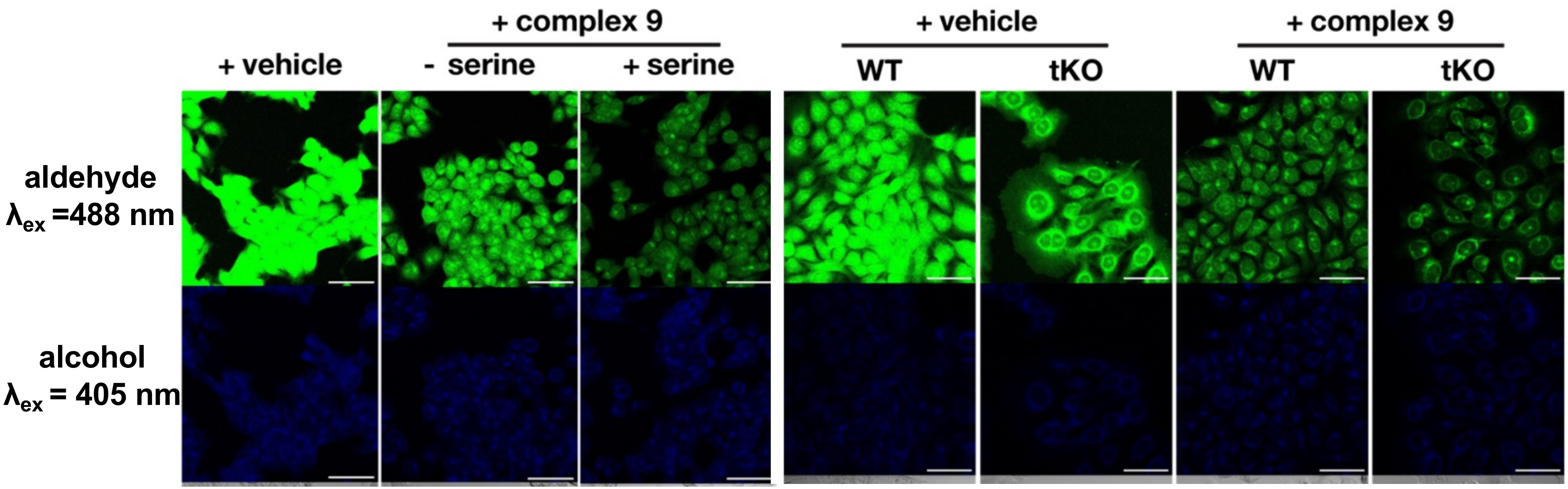
The probe successfully express the one-carbon metabolism in the living cells.



complex 9



ratio-metric fluorophore

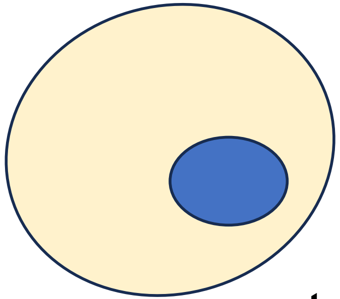


serine is one-carbon donor.
The more serine in the cells,
the more formate in the cells.

three enzymes controlling one-carbon metabolism are knocked out.
the fluorescence responded the deficient of formate.

Crossley, S. W.; Tenney, L.; Pham, V. N.; Xie, X.; Zhao, M. W.; Chang, C. J. *J. Am. Chem. Soc*. **2024**, *146*, 8865-8876.

Summary



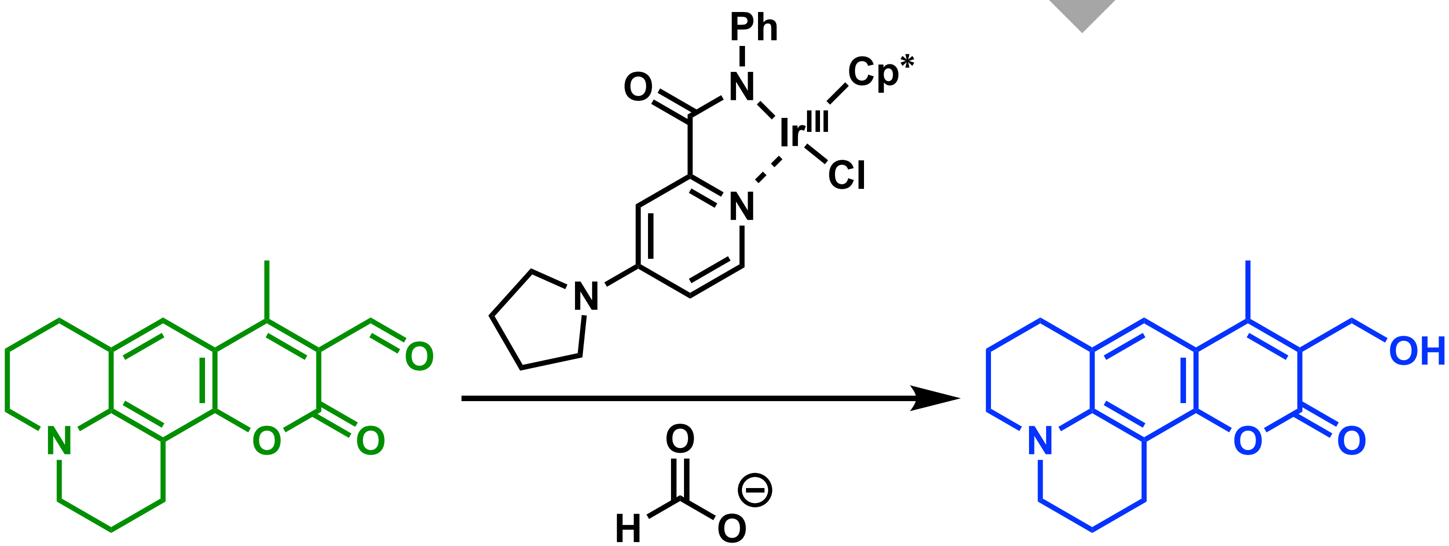
Cellular environment

- an **aqueous aerobic** environment (pH 7.0, 37 ℃)
- high quantities of **nucleophiles and thiols**

transition metal enables…

1. azide-alkyne cycloaddition
2. amide coupling
3. azide reduction
4. cross-coupling
5. olefin metathesis
6. protecting group cleavage
7. ring formation
8. **hydrogen transfer**

controlling reactivity of analyte, fluorophore, catalyst

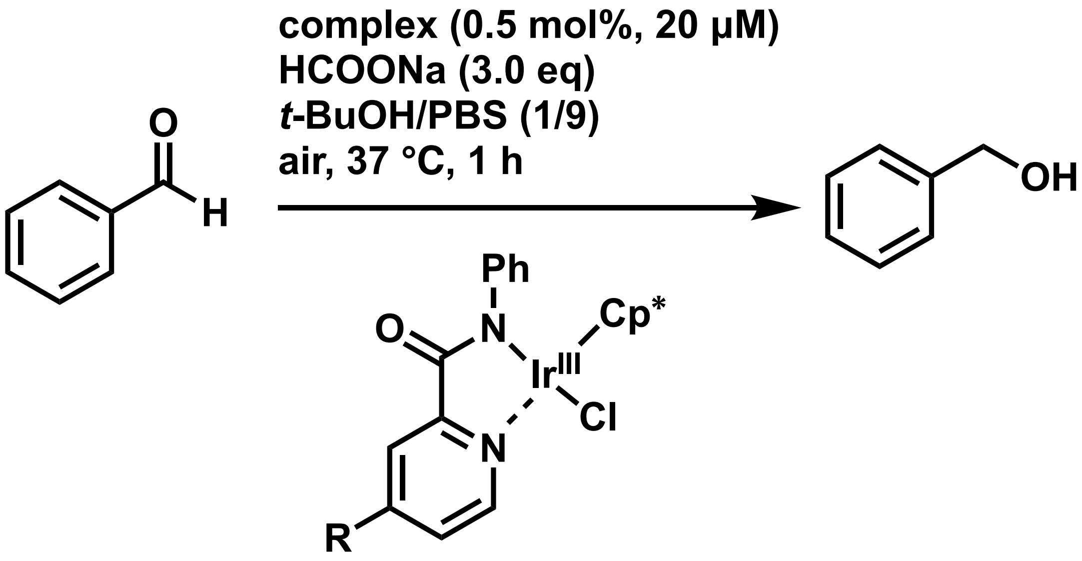


Transition metal enables us to visualize one-carbon metabolism!

Crossley, S. W.; Tenney, L.; Pham, V. N.; Xie, X.; Zhao, M. W.; Chang, C. J. *J. Am. Chem. Soc*. **2024**, *146*, 8865-8876.

Appendix

SAR of Ir Catalyst; Activity

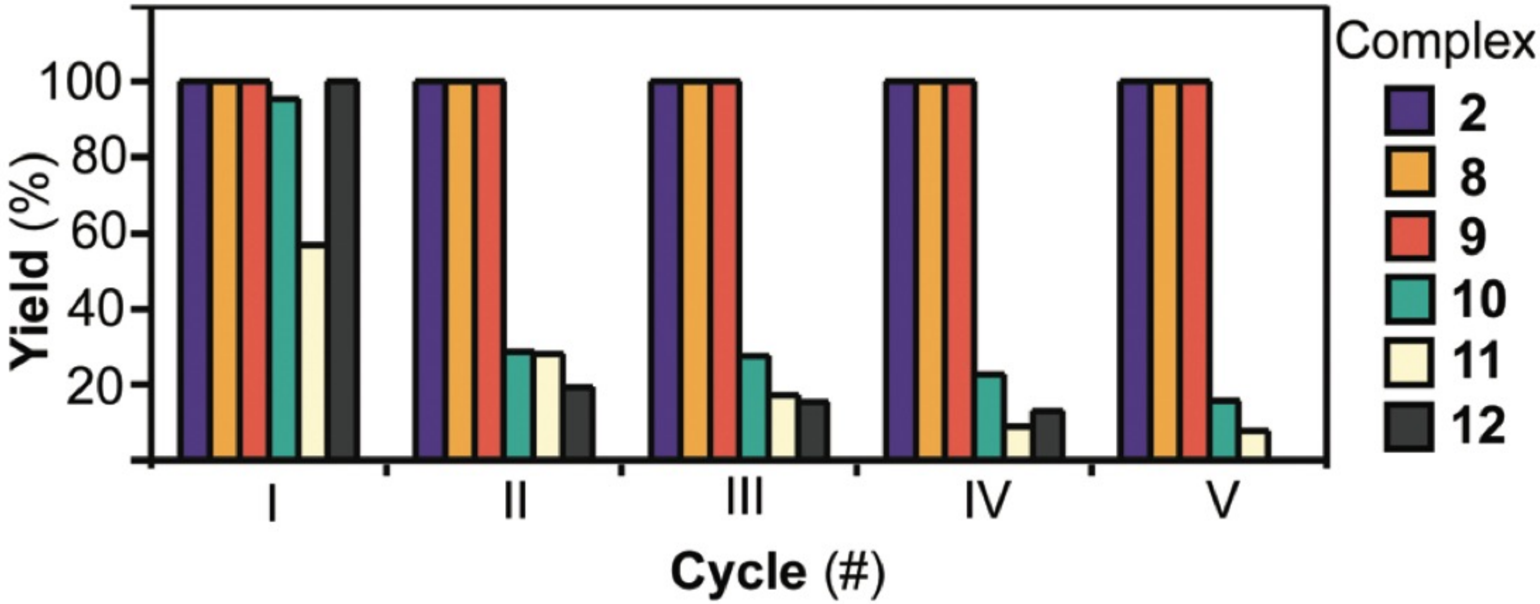


	R	yield (%)
electron donating	Cl	93
	NMe_2	97
	pyrrolidinyl (complex 9)	98
	H	45
electron withdrawing	CF_3	29
	CN	16

Electron donating groups enhance activity.

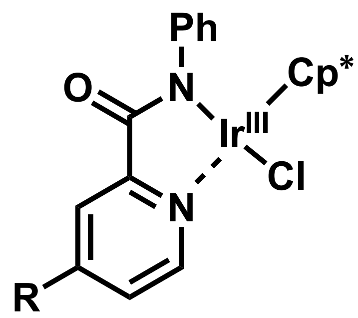
Ngo, A. H.; Do, L. H. *Inorg. Chem. Front.* **2020**, *7*, 583−591.

SAR of Ir Catalyst; Stability



multiple cycles of hydrogen transfer reaction

No.	R
8	NMe_2
9 (complex 9)	pyrrolidinyl
2	H
10	CF_3
11	CN

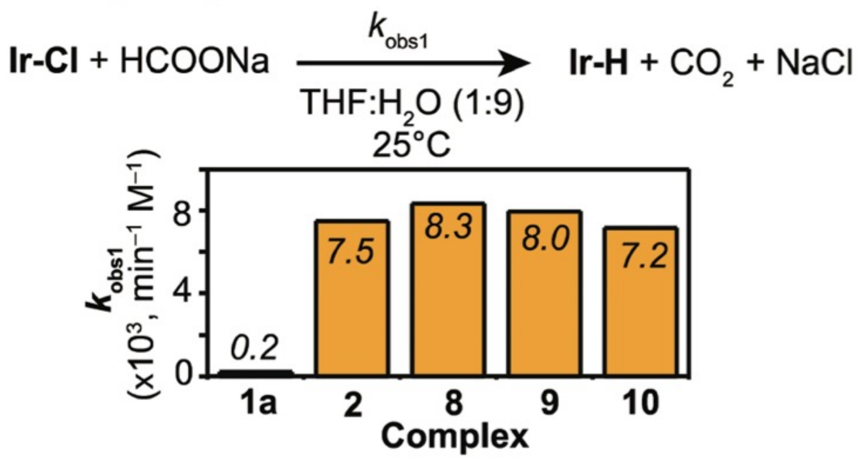


Electron donating groups enhance stability.

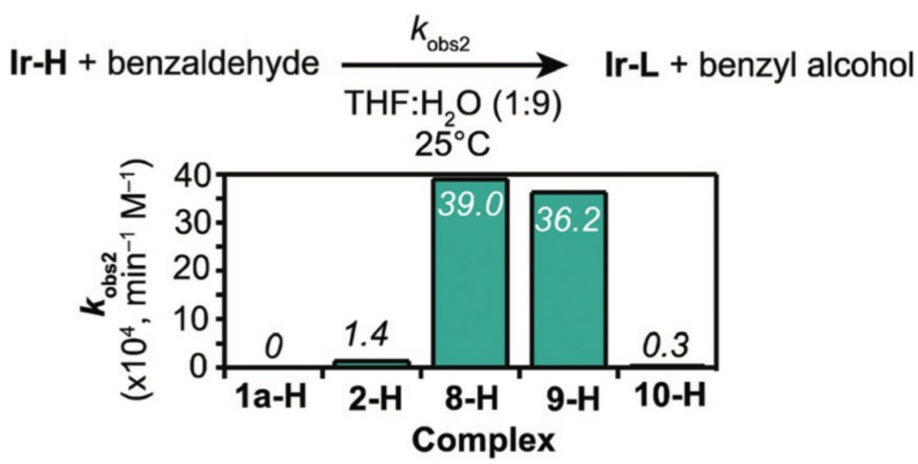
Ngo, A. H.; Do, L. H. *Inorg. Chem. Front.* **2020**, *7*, 583−591.

SAR of Ir Catalyst; Kinetics

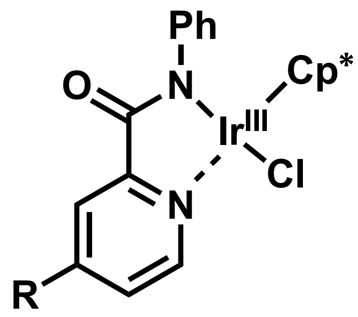
the rate of forming hydride



the rate of hydride transfer



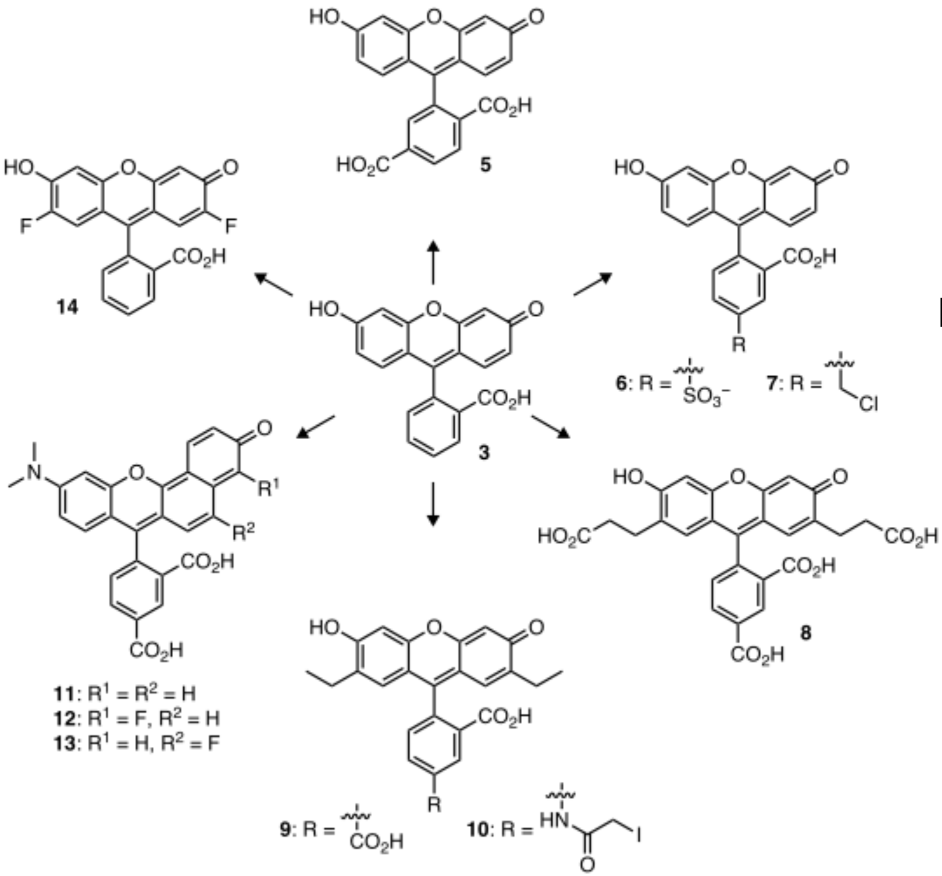
No.	R
8	NMe_2
9 (complex 9)	pyrrolidinyl
2	H
10	CF_3



Electron donating groups dramatically accelerate hydride transfer.

Ngo, A. H.; Do, L. H. *Inorg. Chem. Front.* **2020**, *7*, 583−591.

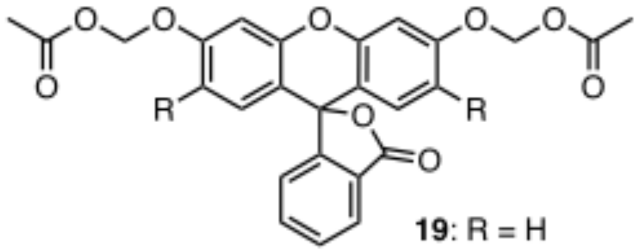
Fluorophore with Longer Retention Time in Cell



more anionic in cell

Masking anionic groups with ester to obtain membrane permeability

In cells, ester groups will be removed by esterase.



19: R = H
20: R = F

ex) acetoxymethyl group
(more stable than acetoxy group)

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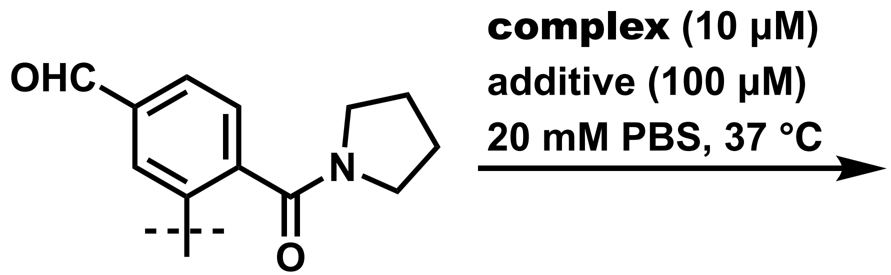
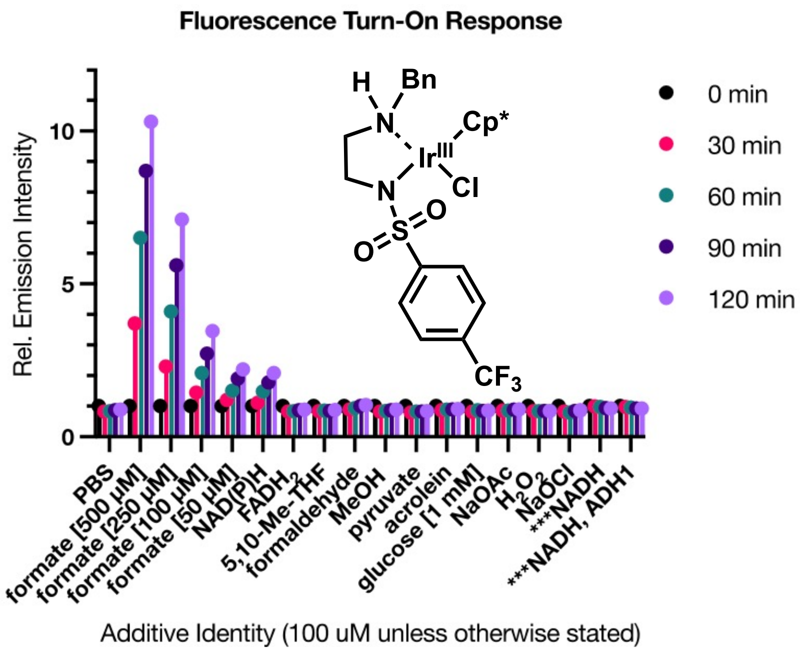
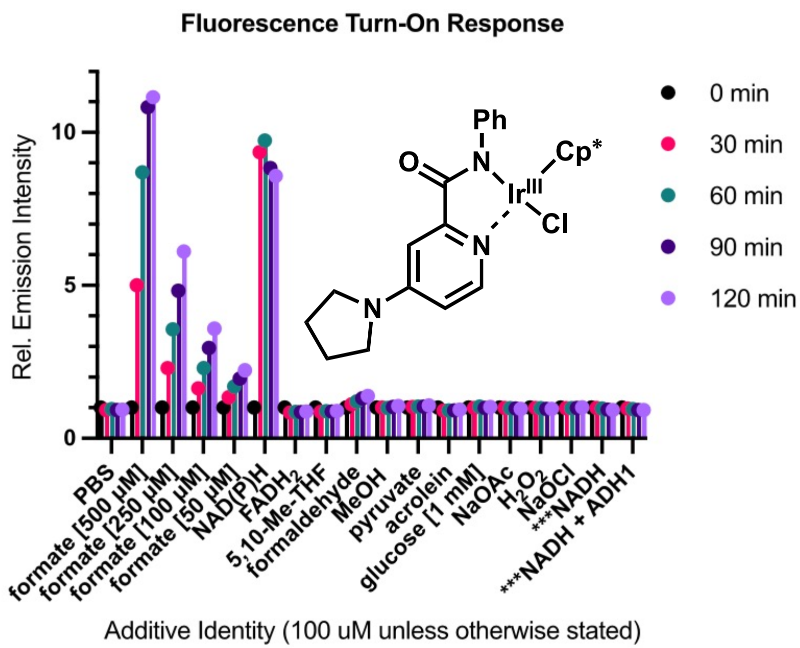
Comparing Selectivity of Catalysts with Additive



complex 19

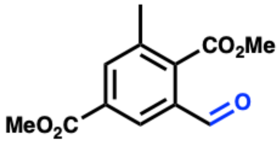


complex 9



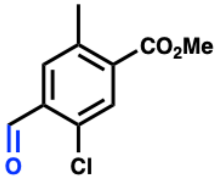
Crossley, S. W.; Tenney, L.; Pham, V. N.; Xie, X.; Zhao, M. W.; Chang, C. J. *J. Am. Chem. Soc.* **2024**, *146*, 8865-8876.

Other Calculated Candidates



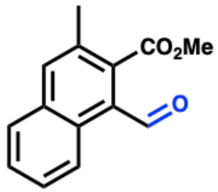
2b,3-CHO

eLUMO -2.36 eV
eHOMO -7.38 eV



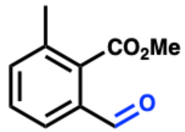
3b,4-CHO

eLUMO - 2.62 eV
eHOMO - 7.14 eV



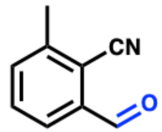
4b,3-CHO

eLUMO -2.41 eV
eHOMO - 6.42 eV



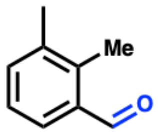
5b,3-CHO

eLUMO -2.13 eV
eHOMO -7.17 eV



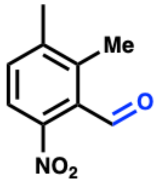
2-CN,3-CHO

eLUMO -2.53 eV
eHOMO -7.41 eV



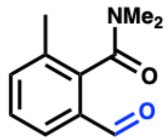
2-Me,3-CHO

eLUMO - 1.84 eV
eHOMO - 6.83 eV



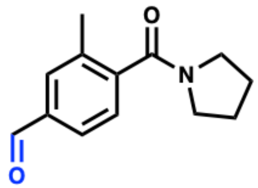
2-Me,3-CHO,4-NO$_2$

eLUMO - 2.86 eV
eHOMO - 7.31 eV



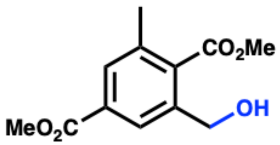
2-CONMe$_2$,3-CHO

eLUMO -2.05 eV
eHOMO -6.88 eV



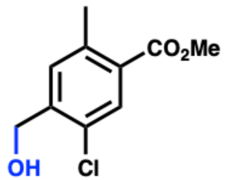
2-CONCy,5-CHO

eLUMO - 2.07 eV
eHOMO - 6.75 eV



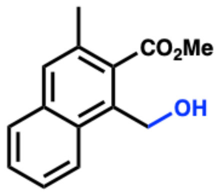
2b,3-CH$_2$OH

eLUMO -1.93 eV
eHOMO -7.04 eV



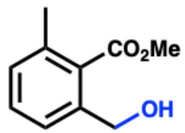
3b,4-CH$_2$OH

eLUMO -1.76 eV
eHOMO -6.96 eV



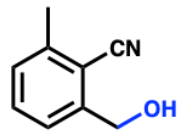
5b,4-CH$_2$OH

eLUMO -1.48 eV
eHOMO -6.17 eV



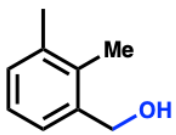
5b,3-CH$_2$OH

eLUMO -1.22 eV
eHOMO -6.83 eV



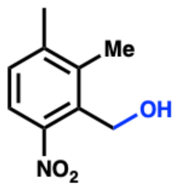
2-CN,3-CH$_2$OH

eLUMO -1.63 eV
eHOMO -7.15 eV



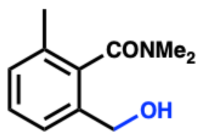
2-Me,3-CH$_2$OH

eLUMO -0.38 eV
eHOMO -6.56 eV



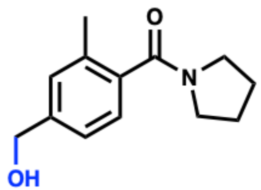
2-Me,3-CHO,4-NO$_2$

eLUMO - 2.54 eV
eHOMO - 7.00 eV



2-CONMe$_2$,3-CH$_2$OH

eLUMO - 0.51 eV
eHOMO - 6.60 eV

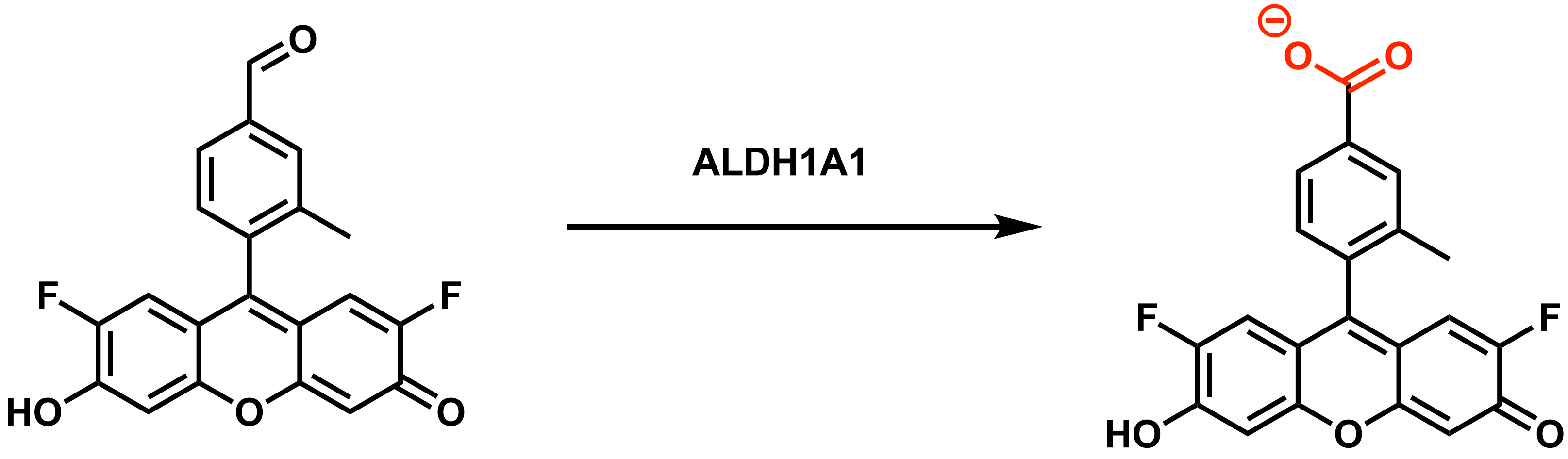


2-CONCy,5-CH$_2$OH

eLUMO - 0.72 eV
eHOMO - 6.58 eV

Avoiding Aldehyde on *p*-Position

there is an example that cellular enzyme oxidized *p*-aldehyde.



Pt-Utilized Cancer-specific Prodrug