

Supplemental Information

## Problems and Solutions in Click Chemistry Applied to Drug Probes

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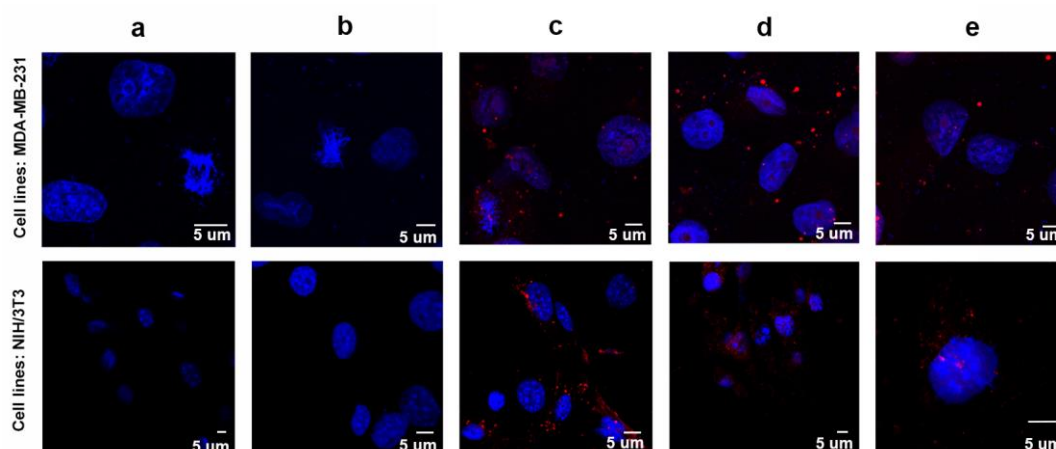
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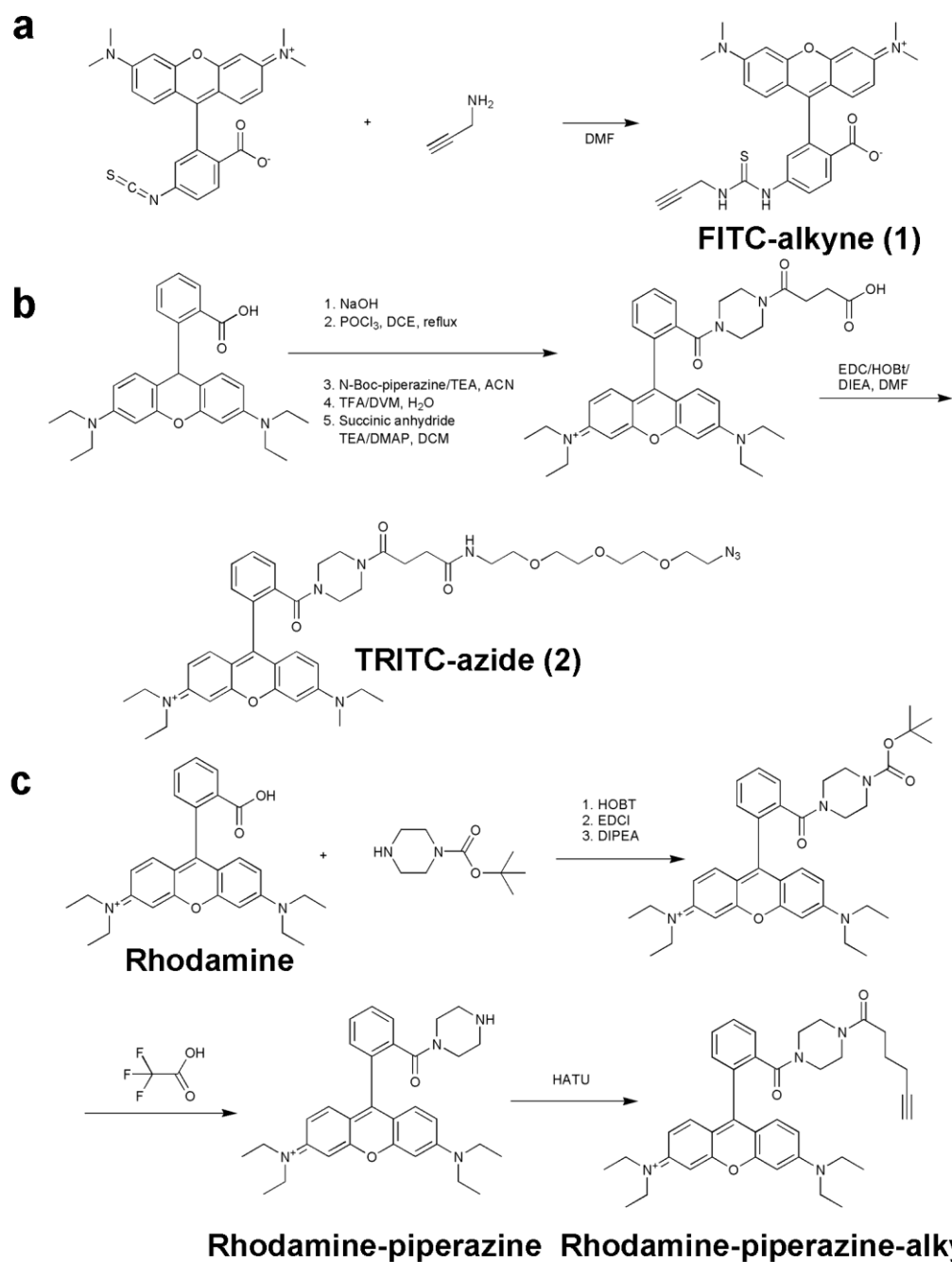
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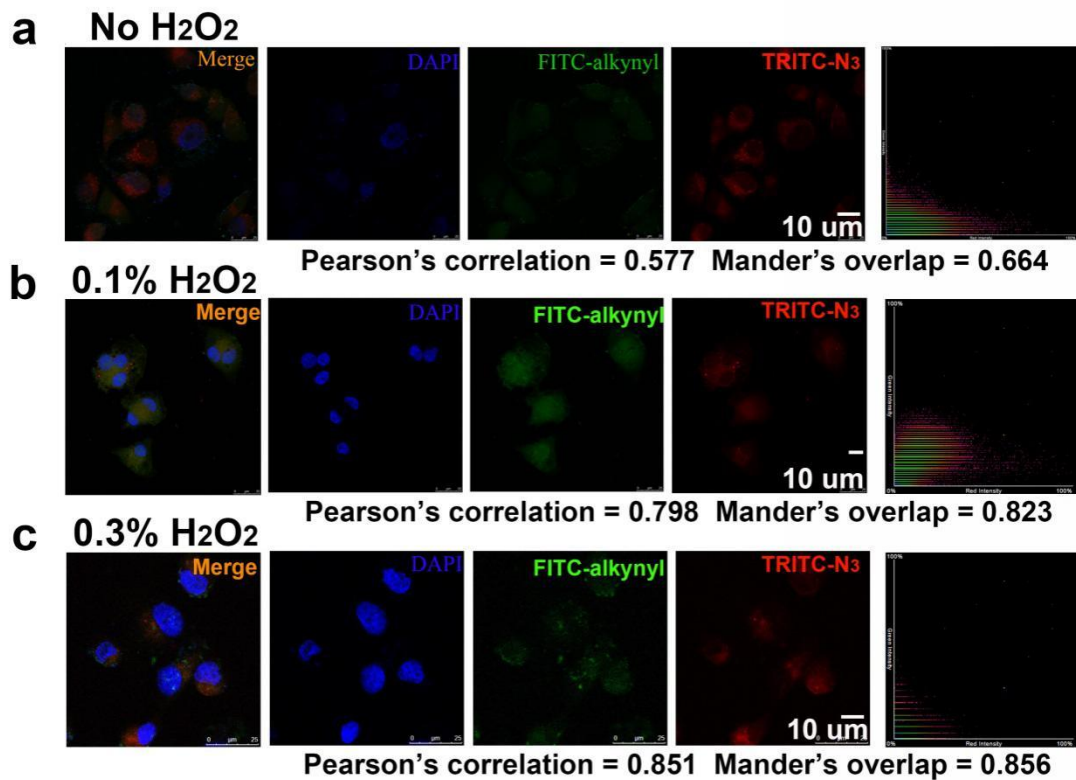
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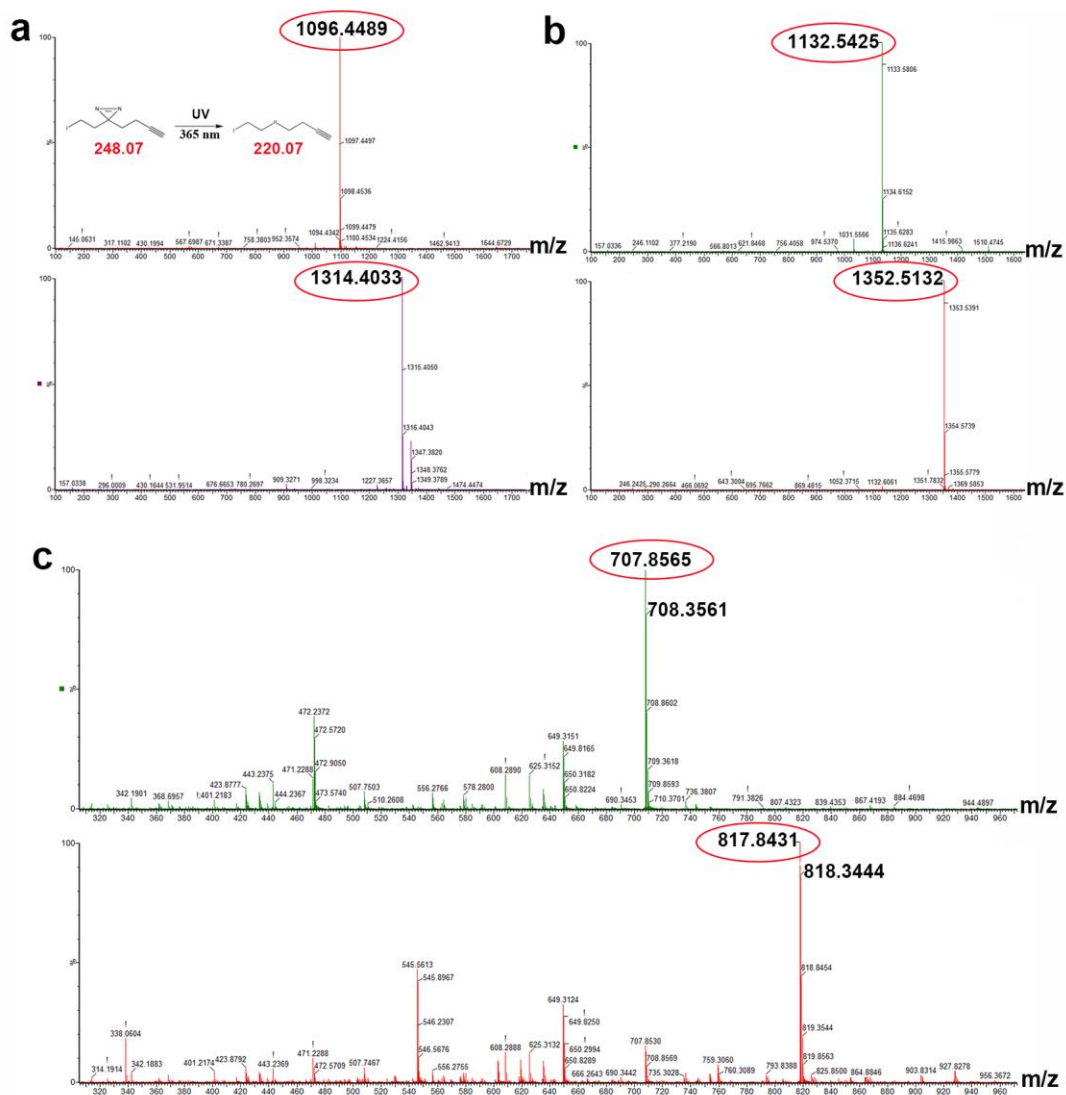
**Figure S1. Related to Figure 1. The click reaction results in catalyzed copper ions with or without copper ion catalysis.** Confocal fluorescence results for **a**, rhodamine. **b**, rhodamine-piperazine. **c**, rhodamine-piperazine-alkyne. **d**, rhodamine-piperazine-alkyne treatment with EDTA. **e**, rhodamine-piperazine-alkyne without catalysts.



**Figure S2.** Related to Figure 1. Synthesis of FITC-alkyne, TRITC-azide and rhodamine-piperazine-alkyne. **a**, Synthesis of FITC-alkyl. **b**, Synthesis of TRITC-azide. **c**, Synthesis of rhodamine-piperazine-alkyne.



**Figure S3. Related to Figure 1. Co-localization efficiency at different hydrogen peroxide concentrations. a,** Cells without hydrogen peroxide treatment. Effects of **b,** 0.1% hydrogen peroxide treatment and **c,** 0.3% hydrogen peroxide treatment. Hydrogen peroxide eliminates the interference of cysteine and improves the co-localization efficiency of FITC-alkyne and TRITC-azide in living cells.



**Figure S4. Related to Figure 2. Molecular weight changes of peptide 1-3 after reaction with alkyl diazirine under 365 nm UV irradiation.**

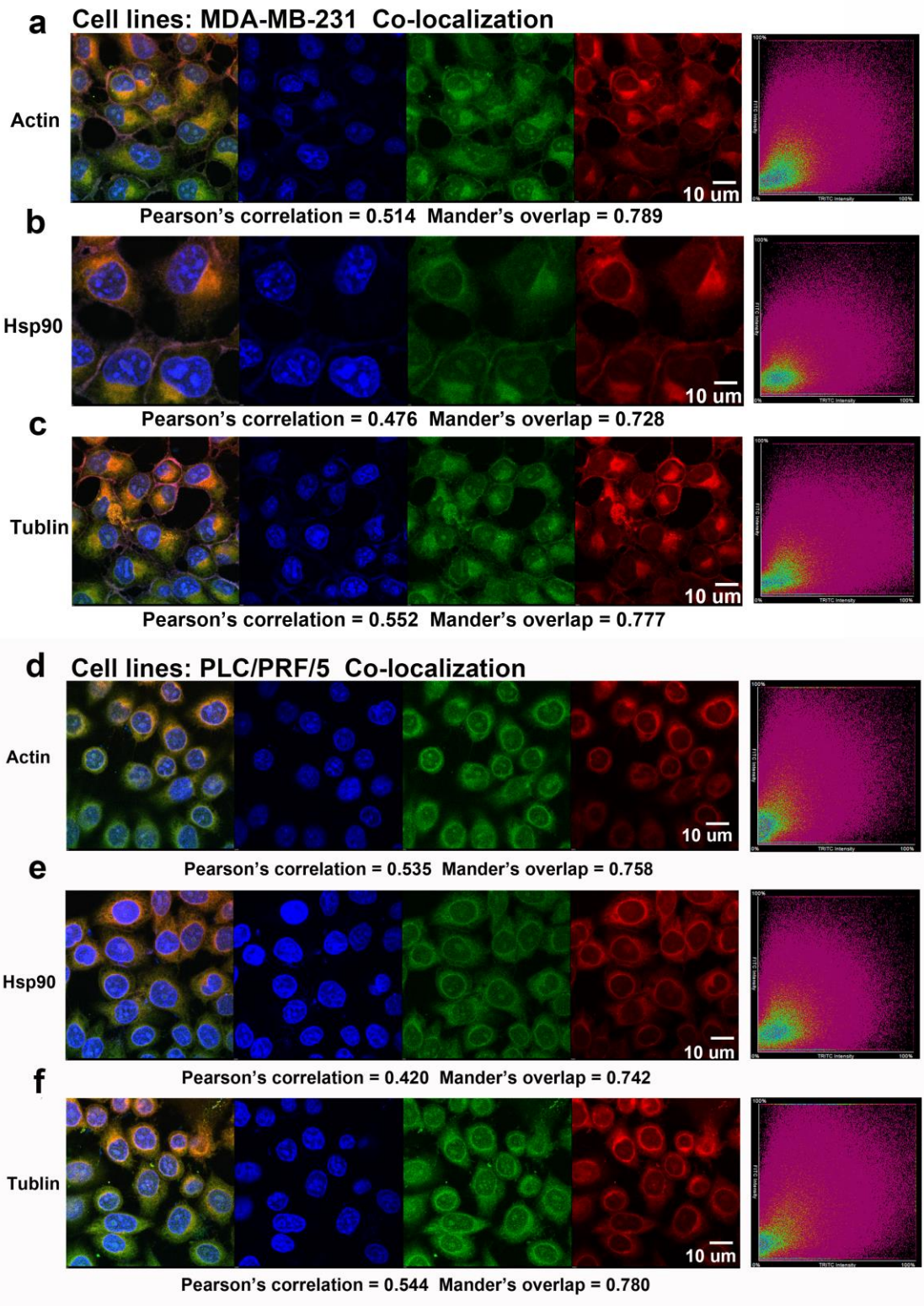


Figure S5. Related to Figure 3. Co-localization of alkyl diazirine (AD) and tubulin, actin and HSP90 in 231 and PLC cells.

**Table S1. The reaction efficiencies of four peptides reacting with benzophenone and alkyl diazirine (AD).**

Peptide	365 nm				350 nm			
	P1	P2	P3	P4	P1	P2	P3	P4
Benzophenone	0.539	0.444	1.943	1.965	NA	NA	NA	15.403
Alkyl diazirine	275.899	919.674	177.186	26.195	NA	121.66	70.738	1366.7
	2326.055	2532.69	2746.684	2.891	NA	3474.202	6637.455	NA
	1573.198	3840.942	1497.542	261.512				

Note:

P1: SGGDILQSGCCG; P2: INTQWLLSGT; P3: ARNEHKMFPWV; P4: ARDCEHKMFPYV.

Red values represent the peak area of the products, and blue values represent the peak area of the reactants. The results show that alkyl diazirine readily reacts with the peptide under 365 nm UV irradiation. In contrast, the benzophenone reaction efficiency was low under both 350 nm and 365 nm UV irradiation.