Modular Effect on Click Chemicals for Bioorthogonal Ligation

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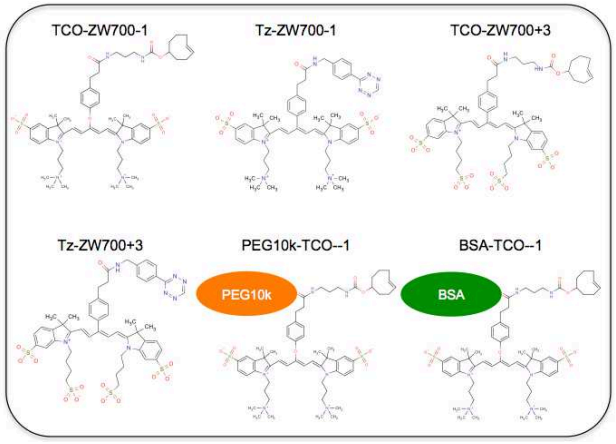
Statement of Purpose: Bioorthogonal click chemistry is attracting increasing interest in advanced biomedical research through selective labeling of organelles in living systems. To be used *in vivo*, the click reaction must fulfill a number of requirements, such as selectivity, reaction rate, biocompatibility, biological inertness, and *in vivo* kinetics. Particularly, *in vivo* behavior of click molecules is a critical factor to enhance the reactivity. The click molecules are often consisted of functional cues and imaging agents with different chemical composition and formulation, which could alter biodistribution, blood half-life, and extravasation rate. This study was made use of modular design strategy of each chemical domain to monitor *in vivo* performance, in variety of logD, surface charge, and molecular size.

Methods and Materials: For click reaction between tetrazine (Tz) and trans-cyclooctene (TCO), human lung carcinoma cells (A549) were engineered to present either Tz or TCO on the surface, and introduced into the lung in normal CD-1 mice. After short period time (i.e., 30 - 60 min), 6 complementray probes (10 nmol/100 μL) were administrated to the body, and real-time intraoperative near-infrared (NIR) imaging were performed with FLARE system. Kinetic response of each molecules over 4 h post-injection were measured, and accordingly click reaction was confirmed using fluorescent intenisty from the excised lungs.



Results and Discussion: Physicochemical properties of the modulated probes were ranged diversely, such as logD -2.65 to 0.84, charge -3 to 1, and molecular weight 1,031 to 66,000 Da. The reactivity of click ligation was verified by *in vitro* cell assay, which resulted in the same high affinity and specificity among the probes, although stablity was different (data not shown).

We previously reported the intraoperative imaging in the NIR window, utilizing FLARE system and various fluorophores, so we are conferred a benefit on practical monitoring of biodistribution parameters. To make trackable cells, we stained A549 cells by PS126, which is strong binder with cytosolic compartments as well as NIR imaging tracer, before administration to the mice. As shown in Figure 1A, despite molecular similarity, each probes behaved differntly in terms of distribution and clearance. In addition, the circualtion time was prolonged as size increase, which is highly related with extravasation rate. To find bioorthogonal response, we quantified uptake rate from dissected organs including the lung (Fig 1B). Particularly, Tz+3 showed lowest non-specific organ uptake with high *in vivo* click reactivity.

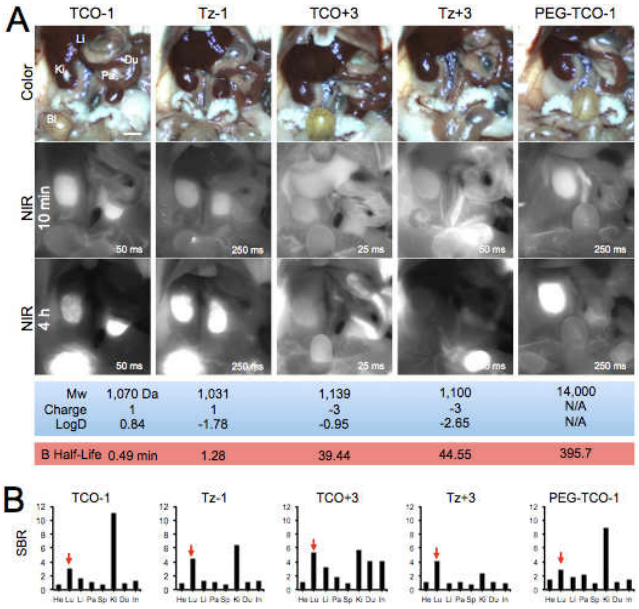


Fig 1. A) Intraoperative NIR imaging using click probes in the mice pre-loaded with cellular substrates. 2x10^6 cells was intravenously injected into normal CD-1 mice 5-6 h prior to imaging, followed by injection of counter probes 4 h prior to imaging. B) SBR quantification of dissected organs from each mouse. Scale bars = 3 mm.

Conclusions: Biodistribution, circulation time, and other physiological criteria were demonstrated to be important for the study of *in vivo* click chemistry, and could be optimized by simple chemical modulation.

References:

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