Clickable Methyltetrazine-Indocarbocyanine Lipids: A Novel Toolkit for Efficient Modifications of Cell Membranes

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INTRODUCTION

- Cell based therapeutics are one of the most promising and exciting breakthroughs in modern medicine.
- Erythrocytes can be efficiently painted with phospholipid-conjugated antibodies.
- Indocarbocynine lipids have been used for efficient labelling of cell membrane in vitro and in Vivo.
- Inverse Electron Demand Diels-Alder (IEDDA) bioorthogonal click chemistry involving strained trans-cyclooctene (TCO) and methyl tetrazine (MTz) rather than Michael (thiol-maleimide) addition is a efficient way to conjugate antibodies to lipids.

Indocarbocynine Lipid conjugates

Figure 1. A) Structure of DiI and DiR and the corresponding Cy3 and Cy7 distearoyl phosphatidylcholine; B) Lipid painting of human RBCs shows more efficient membrane incorporation of indocyanine lipids; C) Structure of DOCy7-NH₂ and DOCy7-PEG3400-MTz; D) Painting of human RBCs with different DOCy7 derivatives; E) Painting of human RBCs with DOCy7-PEG3400-MTz at various concentrations. Percent labeling was determined by flow cytometry.

Figure 2. A) Bioorthogonal click chemistry reaction between IgG labeled with trans cyclooctene (TCO) and DOCy7-PEG3400-MTz; B) SDS-PAGE analysis of DOCy7-PEG3400-MTz conjugation with IgG-TCO antibody. NIR fluorescence was detected by Li-COR Odyssey scanner at 800 nm.

RESULTS

Human RBC painting with DOCy7-PEG3400-IgG and DiI-PEG3400-IgG.

Figure 3. A) Schematic representation of erythrocyte painting using 1-step or 2-step method; B) IgG on human RBCs painted with 1-step or 2-step method was detected with AlexaFluor 488 goat anti-human IgG (green channel); C) NIR fluorescent microscopy shows DOCy7-PEG-MTz and IgG on RBCs; D) Number of IgG per erythrocyte was determined by dot blot assay using secondary anti-human IgG-IRDye 680.

Jurkat and T cell painting with DOCy7-PEG3400-IgG and DiI-PEG3400-MTz.

Figure 5. A) Lipid painting of Jurkat cells with DOCy7-PEG3400-MTz and DiIPEG3400-MTz; B) Comparison of IgG levels after painting with DOCy7-PEG3400-IgG and DiI-PEG3400-IgG (1-step). IgG was detected with AlexaFluor 488 goat anti-human IgG (green channel); C) Comparison of 1-step and 2-step method using DOCy7 anchor; D) Confocal microscopy image of Jurkat cells painted with DOCy7-PEG3400-IgG (detected with AF488 secondary antibody); E) The number of IgG per Jurkat cell was detected by dot blot assay using secondary anti-human IgG-IRDye 680.

Figure 4. A) Biorthogonal conjugate of IgG labelled with TCO and DiI-PEG3400-MTz; B) Human RBCs were first painted with DiI-PEG3400-MTz and then conjugated with human IgG-TCO. IgG was detected with AlexaFluor 488 goat anti-human IgG (green channel) and DiI fluorescence was detected in the yellow channel. C) Fluorescent microscopy of painted RBCs; D) RBCs were painted with DiI-PEG3400-MTz or DOCy7-PEG3400-MTz and then conjugated with IgG-TCO-IRDye 680. For flow analysis, the antibody was detected with AlexaFluor 488 goat anti-human IgG; E) IgG/RBC ratio was determined by direct dot blot assay of IRDye680-labeled antibody.

Figure 6. A) T-cells were first painted with DOCy7-PEG3400-MTz (10µM) and then conjugated with human IgG-TCO (3µM). IgG was detected with AlexaFluor 488 secondary antibody; B) IgG/cell ratio was determined by dot blot assay using secondary anti-human IgG-IRDye680; C) T-cells were painted with 10µM DOCy7-PEG3400-MTz or 10µM DiI-PEG3400-MTz and then conjugated to polyclonal human Cy5-IgG-TCO (left, center) or Cy5-rituximab-TCO (right). Cy5 fluorescence was detected in red channel; D) Confocal microscopy of T-cells painted with DiI-PEG-MTz/Cy5-IgG-TCO.

CONCLUSIONS

- We systematically compared click derivatives of two different dioctadecyl indocyanines for decoration of cell surface with antibodies.
- DiI (DOCy3) and DOCy7 conjugates have similar painting efficiencies (number of IgG per cell).
- Indocyanine lipids can paint different cell types, including human and mouse RBCs, Jurkat cells, and primary T cells.
- Painting of RBCs results in ~80,000 IgG per cell, whereas painting of Jurkat and T-cells results in over 1 million and 7 million IgG per cell.
- The next critical milestone would be to use indocyanine anchors for therapy, imaging and drug delivery.

ACKNOWLEDGEMENTS: We appreciate the funding EB022040 and CA194058 from NIH