Electronic Supplementary Material (ESI) for Analytical Methods

Engineering Liposomes as Detection Reagents for CD4+ T-Cells

Katie A. Edwards, Katherine Meyers, Barbara Leonard, John T. Connelly, Yang Wang, Tara Holter, Antje Baeumner^

Cornell University,
Department of Biological and Environmental Engineering,
145 Riley-Robb Hall,
Ithaca, NY 14853

Relationship between mol% N-glutaryl DPPE and non-specific binding to monocytes and CD4 cells

High mol% concentrations of N-glutaryl DPPE yielded high non-specific binding to CD4 cells (Fig. S1, as detailed further in the main text) and monocytes (Fig. S2). Briefly, liposomes prepared with the lipid compositions altered as listed on the x-axes were incubated with either CD4+ cells (Fig. S1) or monocytes (Fig. S2) captured by anti-CD3 or anti-CD-14 magnetic beads, respectively. As the detection antibody was not present, any interaction of the liposomes with the captured cells was non-specific. For both cell types, these interactions increased with the mol% N-glutaryl DPPE in the lipid formulation.

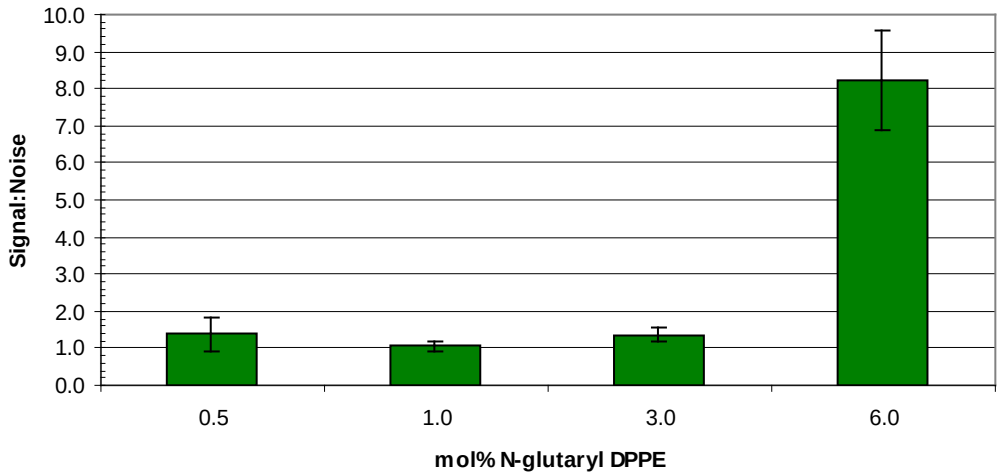


Fig. S1 Relationship between liposomal mol% N-glutaryl DPPE and non-specific binding to CD4+ cells. Liposomes with 0.5-6.0 mol% N-glutaryl DPPE at 50 µM phospholipid were incubated with negatively isolated $CD4^+$ cells captured by anti-CD3 magnetic beads. Signal resulted from lysis of the non-specifically bound liposomes with 30 mM n-octyl-β-D-glucopyranoside. Noise levels were from the above procedure, but with buffer only in place of the CD4+ cells. Each bar is the average of triplicate determinations with error bars representing one standard deviation of the measurements with cells.

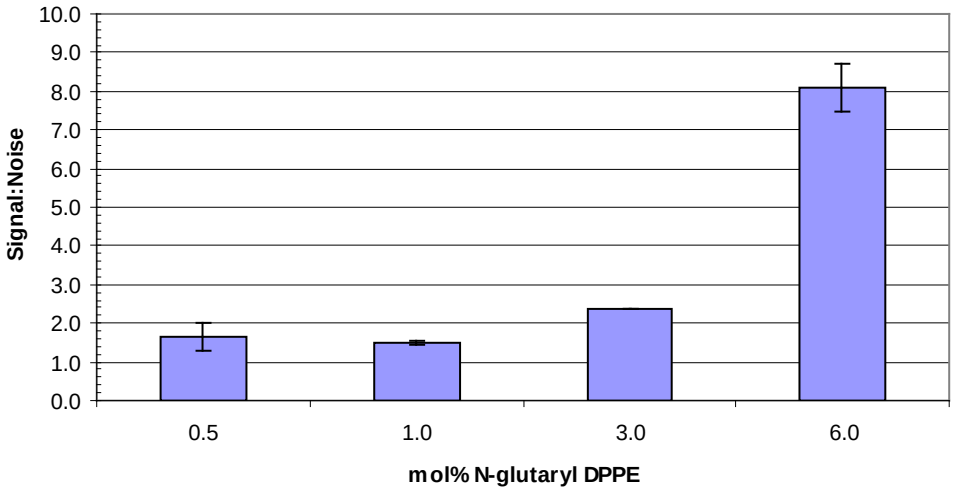


Fig. S2 Relationship between mol% N-glutaryl DPPE and non-specific binding to monocytes. Liposomes with 0.5-6.0 mol% N-glutaryl DPPE at 50 µM phospholipid were incubated with monocytes isolated by Ficoll-Paque separation, then captured by anti-CD14 magnetic beads. Signal resulted from lysis of the non-specifically bound liposomes with 30 mM n-octyl-β-D-glucopyranoside. Noise levels were from the above procedure, but with buffer only in place of monocytes. Each bar is the average of triplicate determinations with error bars representing one standard deviation of the measurements with cells.

High mol% of PEGylated lipid and binding to CD4 cells

In the full sandwich complex (Fig. 1), such liposomes prepared with 6 mol% N-glutaryl DPPE yielded poor discrimination in the detection of 180 CD4+ cells/µL versus buffer (Fig. S3). In fact, they yielded stronger discrimination when not conjugated to streptavidin, which highlighted further their problematic non-specific interactions with cells (Fig. S3).

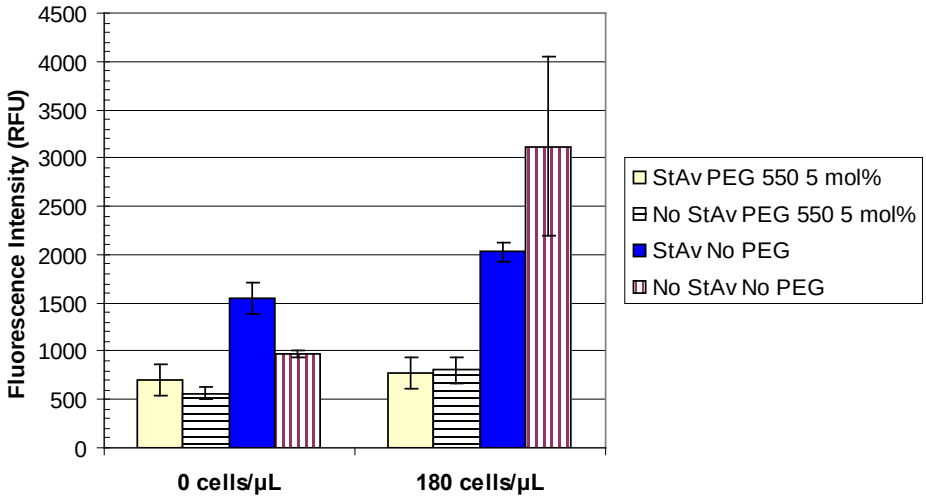


Fig. S3 Effect of high PEG550 concentration on CD4 cell binding. Sandwich complex formation between immobilized anti-CD45, CD4 cells (buffer only or 180 cells/µL), biotinylated anti-CD3 and either unconjugated or streptavidin conjugated liposomes with 5 mol% PEG550 or the standard formulation only. Biotin-binding and CD4 cell assays were carried out as described in materials and methods. Each point is the average of triplicate determinations with error bars representing their standard deviation

Relationship between mol% N-glutaryl DPPE and liposome zeta potential

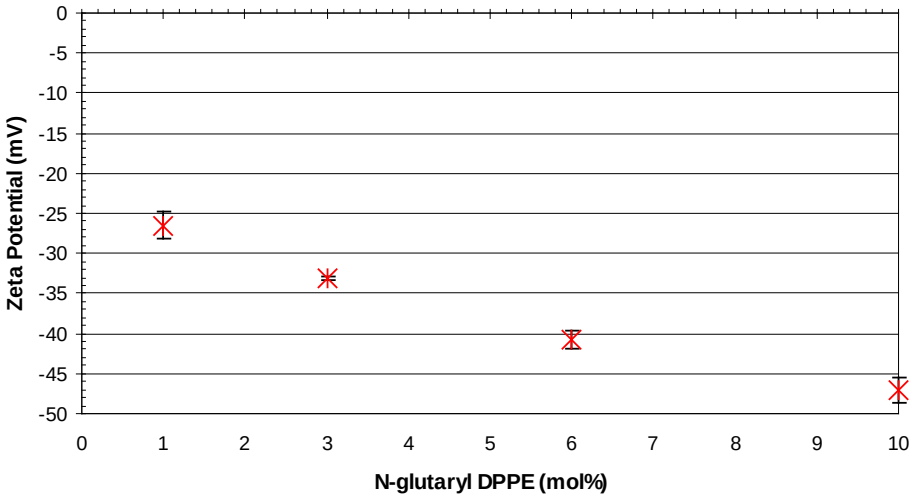


Fig. S4 Relationship between liposome zeta potential and N-glutaryl DPPE mol%. Liposomes prepared with 1 to 10 mol% N-glutaryl DPPE were diluted to 10 µM phospholipid in HSS for zeta potential determinations. Each point is the average of triplicate determinations with error bars representing their standard deviation

Assay optimization

- Several anti-CD3 antibodies were evaluated as capture reagents in this system. Of those compared, Abcam catalog #ab8090-100 and Biodesign P86616M, were found to yield a response proportional to CD4 cell concentration.
- In terms of blocking the high binding polystyrene microtiter plates, 0.01% (w/v) casein, BSA, and Tween-20 were compared and the latter yielded the best signal to noise ratio over a range of CD4 cell concentrations.
- The following biotinylated anti-CD4 antibodies, BD catalog #555345 and Biodesign catalog #P01221B, were found to yield a response proportional to CD4 cell concentration in this format.
- When tested from 3.1 to 397 µM, the 1 mol% PEG750 liposomes did not show a notable difference in signal to noise ratio with varying phospholipid concentration.
- When the PEG750 liposomes were conjugated to 0.05, 0.1, 0.15, or 0.2 mol% streptavidin, 0.05 mol% yielded a markedly enhanced signal to noise ratio over a range of CD4 cell concentrations (Figure S5). Streptavidin was found to cause some non-specific binding to CD4 cells, hence reducing its concentration while retaining strong binding was desired.

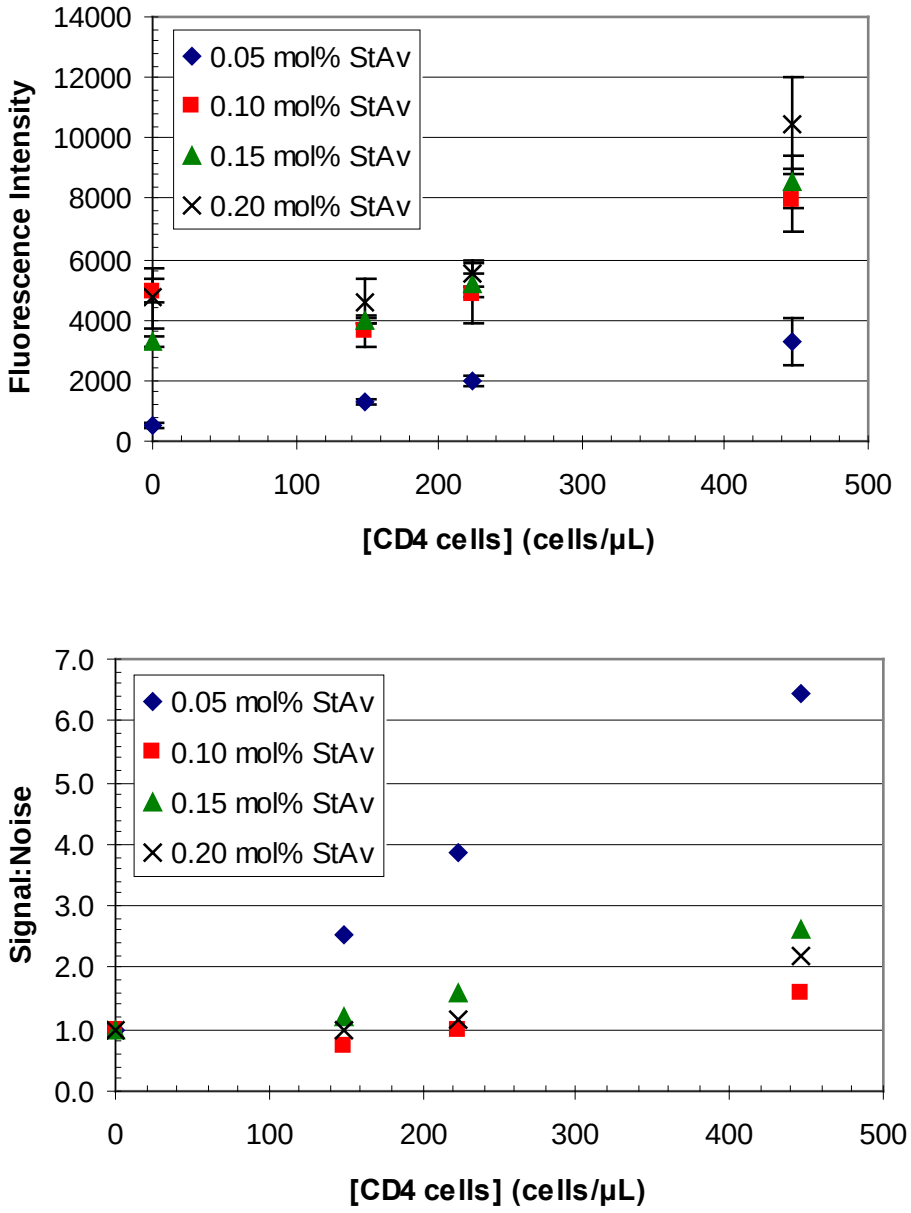


Fig. S5 Response between increasing CD4 cell concentration and fluorescence intensity for liposomes prepared with 6 mol% N-glutaryl DPPE and 1 mol% PEG750 and conjugated to 0.05-0.2 mol% streptavidin. Negatively isolated CD4 cells (0-450 cells/µL) were added to a high binding microtiter plate coated with anti-CD3 antibody. Sandwich complex formation with biotinylated anti-CD4 and streptavidin conjugated liposomes as outlined in materials and methods was carried out. (top) Each point is the average of triplicate determinations with error bars representing their standard deviation. (bottom) Signal to noise ratios for the same data

Relationship between PEGylated lipid mol% and chain length to non-specific binding to monocytes

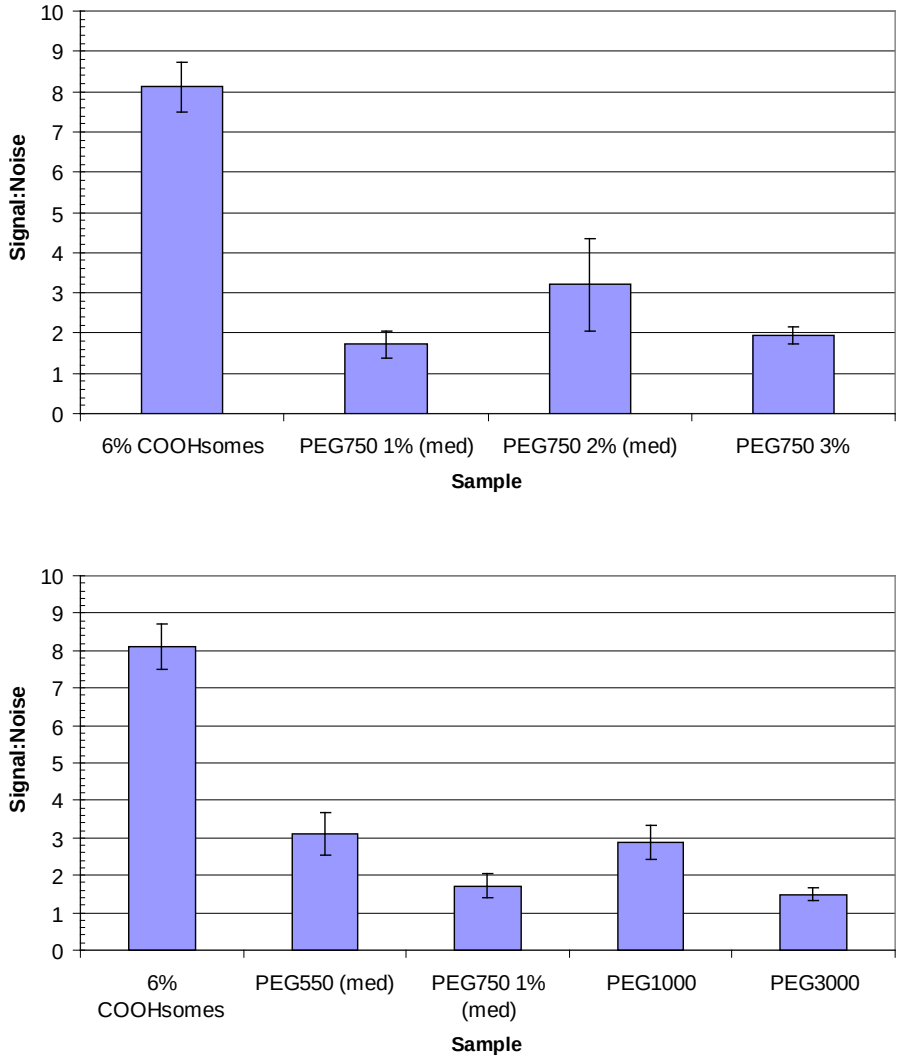
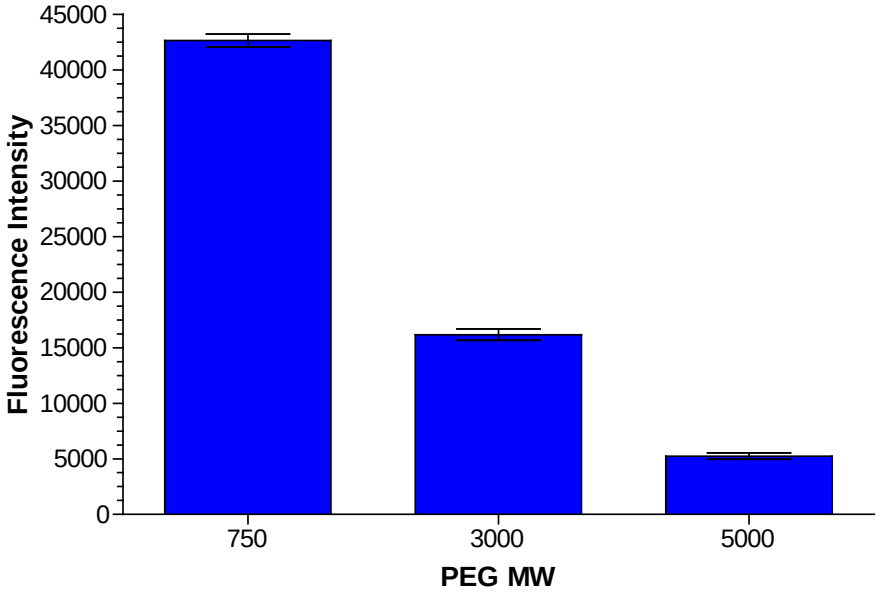
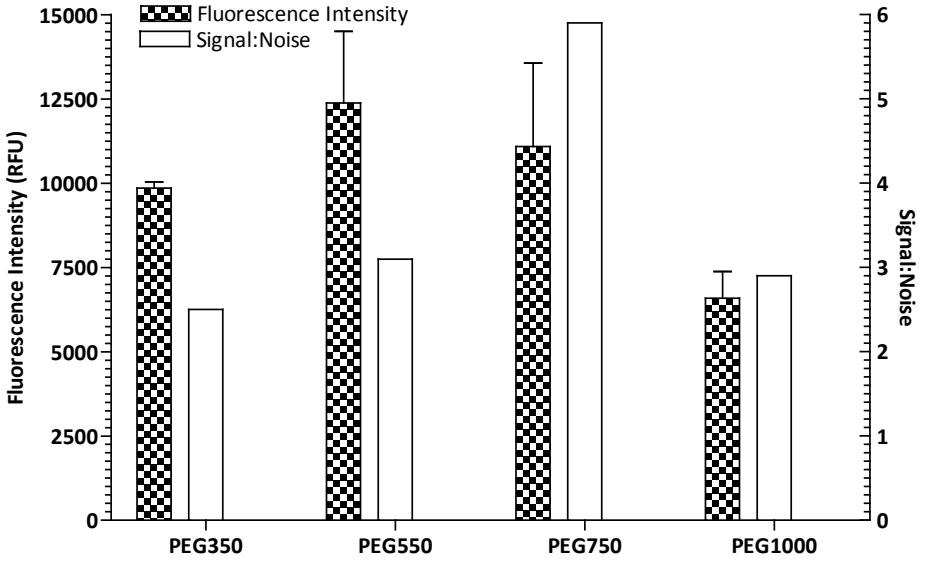


Fig. S6 Relationship between top.) mol% PEG750-DPPE or bottom.) PEG chain length and non-specific binding to monocytes. Liposomes with 1.0-3.0 mol% PEG750 or 1.0% PEG550, 750, 1000, or 3000 (in addition to 6 mol% N-glutaryl DPPE) at 50 µM phospholipid were incubated with monocytes isolated by Ficoll-Paque separation, then captured by anti-CD14 magnetic beads. Signal resulted from lysis of the non-specifically bound liposomes with 30 mM n-octyl-β-D-glucopyranoside. Noise levels were from the above procedure, but with buffer only in place of monocytes. Each bar is the average of triplicate determinations with error bars representing one standard deviation

Relationship between PEGylated lipid molecular weight and binding efficiency

a.





b.

Fig. S7 Relationship between PEG-DPPE MW and binding efficiency. a.) SRB-encapsulating liposomes with 1.0 mol% PEG750, PEG3000, or PEG5000 were conjugated to 0.05 mol% streptavidin then normalized to 10 µM phospholipid and introduced to a biotin coated microtiter plate. b.) sandwich complex formation between immobilized anti-CD45, CD4 cells (639 cells/µL), biotinylated anti-CD3 and streptavidin conjugated liposomes with 1 mol% PEG350-PEG1000. Biotin-binding and CD4 cell assays were carried out as described in materials and

methods. Each point is the average of triplicate determinations with error bars representing their standard deviation

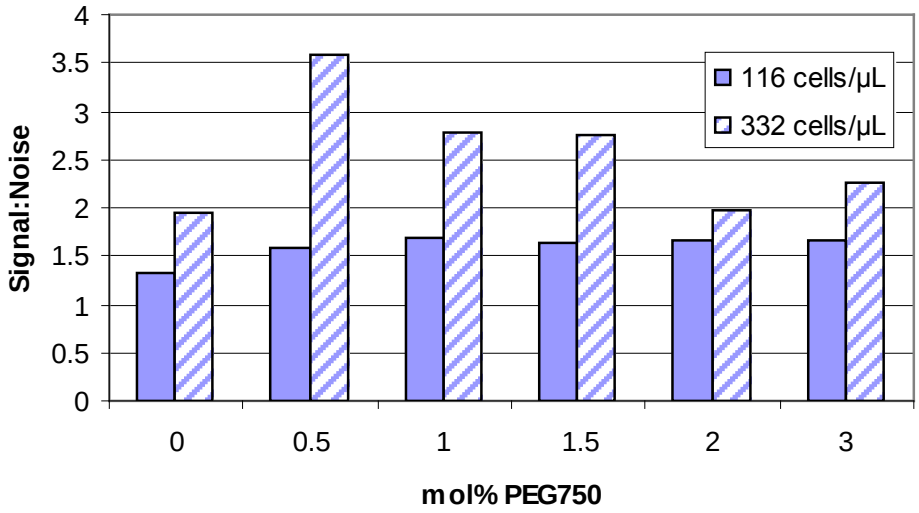


Fig. S8 Response between increasing CD4 cell concentration and fluorescence intensity for liposomes prepared with 6 mol% N-glutaryl DPPE and 0.5-3 mol% PEG750. Negatively isolated CD4 cells were used at 100% concentration or a 50% dilution PBS and added to a high binding microtiter plate coated with anti-CD3 antibody. Sandwich complex formation with biotinylated anti-CD4 and streptavidin conjugated liposomes as outlined in materials and methods was carried out. Each point is the average of triplicate determinations and is plotted in terms of signal resulting from lysis of specifically bound liposomes with 30 mM n-octyl-β-D-glucopyranoside in the presence of cells at the concentrations indicated versus noise with buffer only in place of the CD4+ cells.