# Mixed Micelles Composed of PEG-lipids and Cytotoxic Peptide Amphiphiles for Cancer Therapy

Matthew Black[1] and Matthew Tirrell[1,2]
1. Department of Chemical Engineering, University of California, Santa Barbara
2. Department of Bioengineering, University of California, Berkeley

**Statement of Purpose:** Peptides have enormous potential as therapeutic agents due to their ease of rational design and target specificity, but they are limited by low stability and their ability to reach their desired target. Peptide amphiphiles consist of a biofunctional peptide as the hydrophilic head group and either a single-chain fatty acid or a double-chain lipid as the hydrophobic group, often separated by a polyethylene glycol (PEG) or other spacer to drive self-assembly in aqueous solutions. The resulting micelles display a high density of functional peptides. Mixing different monomers leads to multifunctional mixed micelles with precise control over number and ratio of functionalities without the need for orthogonal chemical reactions. Therefore, multiple therapeutic, targeting, or internalizing peptides can be easily incorporated into the same structure. The work aims to incorporate therapeutic peptides into a micelle that can protect the peptide during circulation with the goal of targeting it specifically to cancer cells.

**Methods:** We constructed peptide amphiphiles using standard solid phase peptide synthesis. Carboxytetramethylrhodamine was incorporated where appropriate to create florescent peptide amphiphiles. The peptide amphiphile self assembly behavior was analyzed with dynamic light scattering and cryo-TEM. Micelle stability was measured by fluorescent quenching experiments. The ability of the peptide amphiphiles to cause cell death *in vitro* was quantified with standard cell viability assays and the mechanism of cell death and internalization was determined using confocal microscopy and flow cytometry.

**Results:** The membrane disrupting peptide D-$(KLAKLAK)_2$ was utilized as a therapeutic anti-cancer peptide and conjugated directly to a diC16 hydrophobic tail. The resulting peptide amphiphile self assembled into micelles and disrupted the outer membranes of cancer cells within minutes, resulting in necrotic cell death. The peptide amphiphile was mixed with PEG-lipids to form mixed micelles. The mixed micelles significantly decreased the cytotoxicity of the peptide amphiphile if the micelles were washed out over short time periods while the cytotoxicity of the peptide amphiphile alone was unaffected, indicating that the PEG was able to shield the anti-cancer peptide amphiphile. However, as the micelles slowly disassembled over 24 hours, the peptide amphiphile delivered in mixed micelles regained its cytotoxicity. The slow disassembly allowed the peptide amphiphile to enter cancer cells and initiate apoptosis by disrupting the mitochondrial membranes.

**Conclusions:** This work demonstrates that therapeutic peptides can be incorporated into self-assembled micelles with PEG-lipids. The mode of action of the cytotoxic peptide amphiphile was altered depending on how the peptide was displayed to the cells. This results from this work show that PEG-lipids will be able to protect therapeutic peptides during circulation. Previous work has demonstrated that micelles constructed from peptide-PEG-lipids can deliver imaging agents to tumors *in vivo*. Future work will include targeting peptides at the end of the PEG chains to specifically deliver these therapeutic peptides to cancer *in vivo*. This platform has the potential to deliver not only D-$(KLAKLAK)_2$ as a therapeutic to many types of cancers, but also provide a blueprint for an effective way to deliver a wide variety of peptide therapeutics to their disease targets.