PI Name: Christina M. Woo PI email (if different): cwoo@chemistry.harvard.edu

Intracellular protein targets of the opioids

Nandini Vallavoju[1], Mark Anthony Leon-Duque[1], Christina M. Woo[1]

[1]Department of Chemistry and Chemical Biology, Harvard University

The synthetic opioids possess analgesic properties, yet unlike the natural opioid peptides, have dangerous effects on breathing and heart rate, which combined with their addictive properties can lead to death. These properties may arise from the potential for a broader array of targets that the synthetic opioids, like morphine and heroin, engage beyond the natural opioid peptides. To directly investigate the cellular targets of the opioids, we developed two probes with specific chemical functionalization to enable the unbiased detection and visualization of these protein interactions, termed photo-morphine and dialkynylacetyl morphine (DAAM). Photo-morphine was identified following systematic structure–activity relationship studies for use as a binding site probe that retains the activity of the parent compound. In parallel, we developed DAAM for profiling acetylation sites that arise specifically from opioid treatment in the cell. We employed these two probes within a chemical proteomics platform to characterize protein binding partners of the opioids throughout the cell. This platform involves: (1) treatment of cells or an animal model with the opioid probe, (2) chemical enrichment of the protein binding partners or acetylation sites, and (3) unbiased identification of the protein target and modification site. Application of this chemical proteomics platform to SH-SY-5S cells with DAAM revealed the engagement and acetylation of a novel target, the mitochondrial phosphate carrier protein PiC (SLC25A3). PiC is a ubiquitously expressed and essential transporter of phosphate and copper ions from the cytosol to the mitochondrial matrix. The physiological role of PiC in regulating oxidative phosphorylation and pH is necessary for normal cardiac and muscular function and is associated with roles in other tissues. The effect of synthetic opioid binding and acetylation of PiC on mitochondrial function in neuronal and cardiac cells will be presented. These findings provide novel targets that may have a relationship to the most dangerous side effects of these compounds using chemical probes that expand the toolbox for studying the mechanisms of these molecules.