Differentiation of opioid drug effects by hierarchical multi-site phosphorylation

Sascha Just, Susann Illing, Michelle Trester-Zedlitz, Elaine K. Lau, Sarah J. Kotowski, Elke Miess, Anika Mann, Christian Doll, Jonathan C. Trinidad, Alma L. Burlingame, Mark von Zastrow, Stefan Schulz

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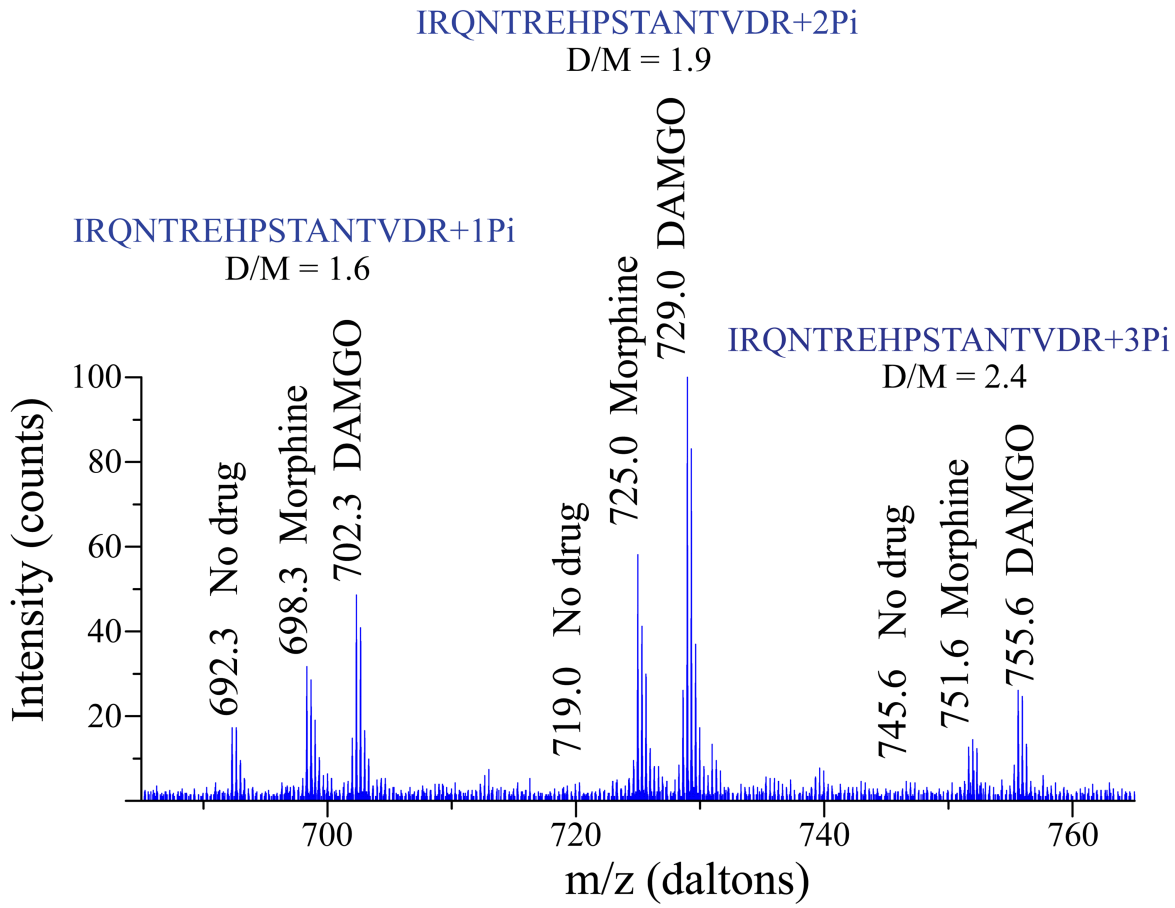
Supplemental Figure S1



Supplemental Figure S1: The μ-opioid receptor cytoplasmic tail undergoes agonist-selective multi-site phosphorylation within 2 minutes at physiological temperature. Receptors were purified by anti-Flag affinity chromatography after exposure of isotope-labeled cells to 10 μM DAMGO or 10 μM morphine for 2 minutes at 37˚C. The indicated phosphopeptides corresponding to the middle portion of the μ-opioid receptor cytoplasmic tail representing single (+1P), double (+2P) and triple (+3P) phosphorylation in this region. Adjusted isotope ratios were calculated by summing signal count areas for 2-3 mass isotope peaks, adjusted for isotope overlap, and calibrated using an internal MOR reference peptide. No signal above noise was observed for the No drug condition at m/z= 745.6. The adjusted isotope ratio indicating the relative abundance of each species between DAMGO and morphine conditions (D/M) is indicated over each set of peaks.