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Novel Method to Express Functional Epitope-Tagged GPCRs

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Epitope tagging of G-protein coupled receptors (GPCRs) typically involves the addition of a specific antigenic amino acid sequence (e.g., hemagglutinin) to the N-terminus, a region not involved in receptor ligand binding or signal transduction functions. However, with certain GPCRs this method fails due to post-translational cleavage of the N-terminal tag or inhibition of membrane isertion. To overcome this, we modified a cloning vector so that it would insert an artificial cleavage site at the N-terminus of the protein before the epitope. This ensures that a functional receptor will be expressed and properly inserted into the membrane. Clones of a □ opiod and calcitonin-like receptor (the tagged versions of which are subject to improper post-translational processing) were tagged using this new vector. The modified clones were expressed in a mammalian cell line and visualized using immunoflorescence. This method offers an efficient means to create immunologically identifiable constructs of human GPCRs.

Amanda Sutterer is a sophomore in BioChemical Engineering from Jackson Missouri who is active in RHA and plans to either go to graduate school or enter the field of research and development.