An economic approach to efficient isotope labeling in insect cells using homemade 15N-, 13C- and 2H-labeled yeast extracts

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Heterologous expression of proteins in insect cells is frequently used for crystallographic structural studies due to the high yields even for challenging proteins requiring the eukaryotic protein processing capabilities of the host. However for NMR studies, the need for isotope labeling poses extreme challenges in eukaryotic hosts. Here, we describe a robust method to achieve uniform protein (15)N and (13)C labeling of up to 90 % in baculovirus-infected insect cells. The approach is based on the production of labeled yeast extract, which is subsequently supplemented to insect cell growth media. The method also allows deuteration at levels of >60 % without decrease in expression yield. The economic implementation of the labeling procedures into a standard structural biology laboratory environment is described in a step-by-step protocol. Applications are demonstrated for a variety of NMR experiments using the Abelson kinase domain, GFP, and the beta-1 adrenergic receptor as examples. Deuterated expression of the latter provides spectra of very high quality of a eukaryotic G-protein coupled receptor.

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