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Inserting a His Tag on GFP

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Many experiments involving proteins that rely on in vivo results are limited to observational and kinetic tools. We are interested in combining in vivo and in vitro experiments using a reactive oxygen specie's sensitive green fluorescent protein (royGFP). Isolated proteins can be used in specific kinetic assays without the complications of cellular proteins and molecules. To do these experiments, royGFP is to be isolated from all other cellular proteins. The easiest and well-established method is adding a histidine or 6 His tag to the N-terminus of royGFP. The His-tagged protein contains a positive charge and the entire contents of the cell can be passed over a negatively charged nickel column, allowing only proteins with the positive charge to be retained and separated from the rest of the cellular proteins. The expressed His-tagged protein's string of histidine binds to the metal ions in the nickel column, letting the protein to be purified and detected easily. Nickel provides good binding efficiency to the His-tagged protein, but it can also attract other positively charged materials as well. The amount of extra non-targeted material should be a small amount, thus not interfering much with the results. However, to ensure that the non-targeted material does not interfere, a method known as the Western Blot may be implicated. The method involves transferring the acquired protein and extra material onto a gel where they can be visualized specifically, thus allowing the His-tagged protein to be studied individually.