FLAG EPITOPE TAG TECHNOLOGY TO ACCELERATE PROGRESS IN PROTEIN CRYSTALLOGRAPHY

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Why is FLAG the most commonly used epitope tag?

AspTyrLysAspAspAspAspLys
D Y K D D D D K
GATTACAAGGACGACGATGACAAG

Figure 1. The FLAG epitope tag. The eight-amino acid sequence of the FLAG tag (1) is shown in three-letter amino acid code, one-letter amino acid code, and in the DNA code that specifies it. Due to its compact nature, FLAG is readily incorporated into target genes using easily synthesized short oligonucleotides. It has found widespread use in recombinant protein production and purification. It has been incorporated into transgenic plants, animals, and microbes. Recently it has been employed in CRISPR mutagenesis. Because purification can be achieved under physiological conditions using anti-FLAG monoclonal antibodies, even complex multi-subunit proteins have been studied with its help.

Because FLAG is the most hydrophilic epitope tag

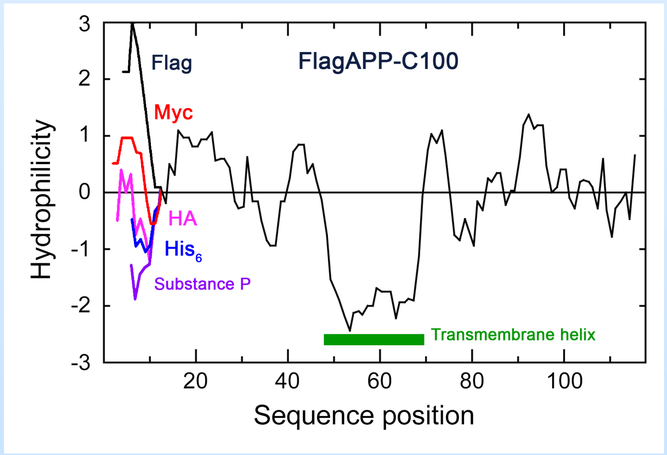

Figure 2. Hydrophilicity plots for epitope-tagged forms of the Alzheimer's Plaque Polypeptide (APP) by the Hopp & Woods method (2). The black plot represents APP with an N-terminal FLAG epitope tag. The red, magenta, blue, and purple plots show Myc, HA, His_6, and Substance P fusions. The FLAG segment yields a peak of maximum hydrophilicity while the others are much less hydrophilic. Substance P was the earliest epitope tag published but has since been abandoned due to insolubility of its fusion proteins. The data suggest hydrophilicity is correlated with success in epitope tagging experiments.

FLAG fusions can be critically important in protein 3D work

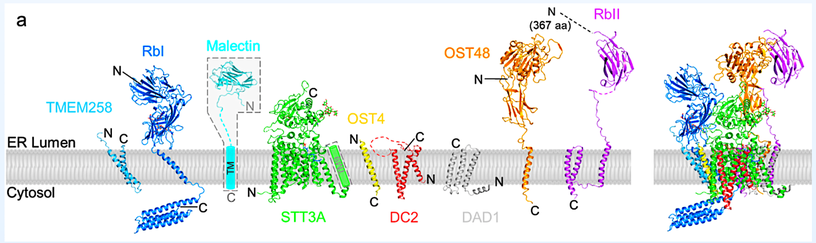

Figure 3. The 3D structure of human OST-A oligosaccharyltransferase multi-enzyme complex determined by cryo-electron microscopy (3). Integral to the success of this ground-breaking experiment was FLAG tagging of the DC2 subunit, followed by mild purification of the entire 9-subunit transmembrane protein using anti-FLAG affinity chromatography. The anti-FLAG beads were washed with detergent solutions to exchange membrane lipids to create a more homogeneous micellar form.

Polypeptide fusions can enhance protein crystallizability

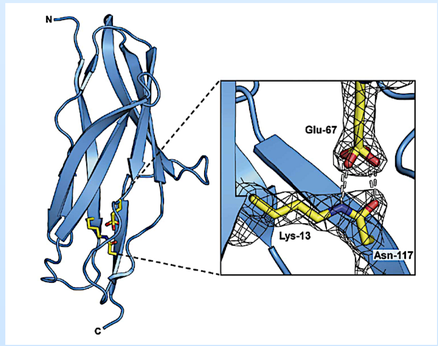

Figure 4. The 3D structure of pilin FctB of *Streptomyces pyogenes* determined by x-ray crystallography (4). Although FctB would not crystallize by itself, fusion to a readily crystallizable protein, maltose binding protein, allowed co-crystallization followed by determination of a high-resolution 3D structure.

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Working toward FLAG-enhanced protein crystallography

We found that anti-FLAG M1 antibody binds Samarium, useful for anomalous dispersion

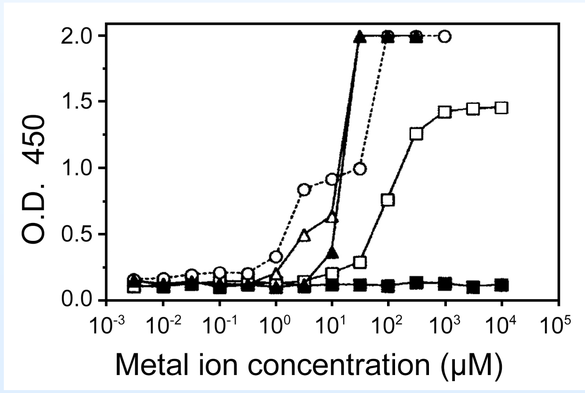

Figure 5. ELISA test of metal binding by FLAG M1 antibody. Color development depended on antibody binding to plates with FLAG peptide attached. Metals were: lanthanum (○), samarium (△), terbium (▲), calcium (□), and none (■). Sigmoid curves, metal-dependent binding; values above 2.0 were due to metal precipitation.

Next steps: Engineer M1 to increase crystallizability of Sm-FLAG complexes

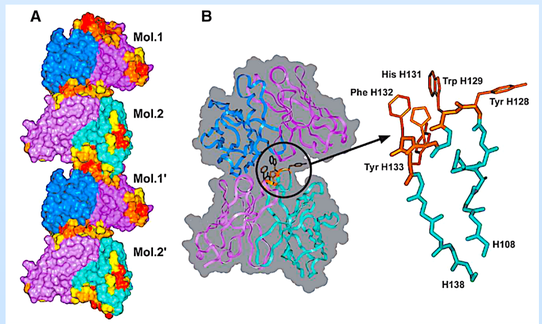

Figure 6. Mutation of crystal contact amino acids can alter the quality of protein crystals, improving clarity of the 3D structure (5). This monoclonal antibody was mutated to block bad crystal contacts allowing an alternative crystal lattice to form.

References

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