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MBP Fusion FAQ

What are the advantages of using MBP as a fusion partner?

Very high yields of MBP fusion proteins can usually be obtained in the cytoplasm of *E. coli* because the juxtaposition of the ribosome binding site with the N-terminal MBP domain of the fusion protein results in efficient translation initiation. We have also observed that sometimes MBP protects its passenger proteins from proteolytic degradation *in vivo*. Additionally, because it is a natural affinity tag, MBP can be exploited to facilitate purification of the passenger protein. However, these attributes of MBP are also shared by other affinity tags. The most compelling reason to choose MBP as a fusion partner, rather than some other affinity tag, is its remarkable ability to enhance the solubility of its fusion partners [Kapust & Waugh, 1999]. Proteins that accumulate in an insoluble form when they are produced in an unfused state or fused to a different partner often can be recovered in a soluble and properly folded form as MBP fusion proteins.

How does MBP compare with other "solubility enhancers"

There is ample evidence in the literature to indicate that MBP is a very effective solubilizing agent, and it is the only thoroughly validated solubility enhancer that is also a natural affinity tag. Although other proteins such as thioredoxin, GST, NusA, DsbA, and gp5 have also been touted as solubility enhancers, in most cases there is relatively little data to support these claims. Our own experiments have demonstrated convincingly that despite the fact that it is highly soluble, GST has virtually no ability to enhance the solubility of its fusion partners [Kapust & Waugh, 1999; Fox *et al.*, 2003]. Not every highly soluble protein is capable of functioning as a solubilizing agent, and so such claims should be treated with skepticism until there is rigorous evidence to support them. Unfortunately, in most published side-by-side comparisons of solubility enhancers the length and amino acid sequence of the interdomain linkers were varied along with the identity of the solubility enhancing domain, and so it is not possible to distinguish between the impact of these two variables on solubility. This probably explains the inconsistencies that exist between such studies. Although we have not tried it ourselves, our colleagues in the Protein Expression Laboratory at SAIC-Frederick report that they have been able to corroborate the claim that *E. coli* NusA is also a potent solubility enhancer.

What can I do if my MBP fusion protein is poorly soluble?

Not every MBP fusion protein will be highly soluble. However, solubility usually can be increased by reducing the temperature of the culture from 37°C to 30°C or even lower during the time that the fusion protein is accumulating in the cells (*i.e.*, after the addition of IPTG). In many cases, the improvement can be quite dramatic. It may also be helpful to reduce the IPTG concentration to a level that will result in partial induction of the fusion protein. The appropriate IPTG concentration must be determined empirically, but is generally in the range of 10–20 μM. Under these conditions, longer induction times (18–24 h) are required to obtain a reasonable yield of fusion protein. Another possible solution is to use a maltodextrin-binding protein from a different organism [Fox *et al*., 2003], or a different solubility tag altogether, such as *E. coli* NusA.

How can I tell if the passenger protein is properly folded?

MBP is an excellent solubilizing agent, but some passenger proteins are unable to fold into their native conformations even after they have been rendered soluble by fusing them to MBP. These proteins evidently exist in a soluble but nonnative form that resists aggregation only as long as they remain fused to MBP. Consequently, it is difficult to assess the folding state of the passenger protein while it is still attached to MBP. In our lab, we have developed a simple method to rapidly ascertain whether a fusion protein will yield a soluble product after cleavage. For this purpose, we use another plasmid vector (pRK603) to coexpress TEV protease along with the fusion protein substrate [Kapust & Waugh, 2000]. First, IPTG is added to the log phase culture and the fusion protein is allowed to accumulate for a period of time. Then, we stimulate the production of TEV protease by adding anhydrotetracycline to the culture. This protocol must be performed in a strain of *E. coli* that produces the Tet repressor (*e.g.*, DH5alphaPRO or BL21PRO cells from Clontech); otherwise, the expression of TEV protease will be constitutive. The cells are harvested after the protease has had time to digest the fusion protein, and then samples of the total and soluble protein are prepared and analyzed by SDS-PAGE. If the passenger protein is soluble after intracellular processing, then it is also likely to be soluble after the fusion protein has been purified and processed *in vitro*. Occasionally, a passenger protein may accumulate in a soluble but biologically inactive form after intracellular processing of an MBP fusion protein. Exactly how and why this occurs is unclear, but we suspect that fusion to MBP somehow enables certain proteins to evolve into kinetically trapped, folding intermediates that are no longer susceptible to aggregation. Therefore, although solubility after intracellular processing is a useful indicator of a passenger protein's folding state in most cases, it is not absolutely trustworthy. For this reason, we strongly recommend that a biological assay be employed (if available) at an early stage to confirm that the passenger protein is in its native conformation. Biophysical methods like circular dichroism and dynamic light scattering can also be used to assess the folding state of the passenger protein.

What about proteins with disulfide bonds?

One of the advantages of using MBP as a fusion partner is that nature designed it to be secreted into the periplasmic space in *E. coli*, where the redox environment is favorable for the formation of disulfide bonds (the nucleotides encoding the natural N-terminal signal peptide have been deleted from the vectors designed for cytoplasmic expression of MBP fusion proteins). In most cases, MBP fusion proteins can also be successfully exported to the periplasm. However, one should bear in mind that the total yield of fusion protein is usually far lower than what can be obtained in the cytoplasm. It has been reported that certain mutant strains of *E. coli* (e.g., *trx/gor* mutants like AD194) will support the formation of disulfide bonds in the cytoplasm [Bessette *et al*., 1999], but our experience with this approach has not been very encouraging.

What types of MBP fusion vectors are available?

Vectors for producing *E. coli* MBP fusion proteins are available from New England Biolabs, Inc (http://www.neb.com). These include vectors for periplasmic as well as cytoplasmic expression and feature multiple cloning sites. Several Gateway© destination vectors for constructing *E. coli* MBP fusion proteins by recombinational cloning, either with or without supplemental affinity tags, are available from our lab [Fox & Waugh, 2003; Routzahn & Waugh, 2002], as are destination vectors for fusing passenger proteins to MBPs from other microorganisms [Fox *et al*., 2003].

Why doesn't my fusion protein bind to amylose resin?

About 20% of the MBP fusion proteins we have constructed do not bind efficiently to amylose resin. The reason for this is unclear, but it may be symptomatic of a fusion protein that exists in a soluble but aggregated state wherein the majority of the passenger protein is not properly folded. Sometimes better results can be obtained by performing amylose affinity chromatography at room temperature instead of 4 °C. This problem can also be circumvented by using vectors that are designed to produce MBP fusion proteins with additional accessory tags [Routzahn & Waugh, 2002].

What if my protein precipitates after it is released from MBP?

Then it is probably either improperly folded or prone to aggregate in its native state. Although painstaking experimentation may eventually lead to conditions that maintain solubility in such cases (*e.g.*, by varying the pH, buffer composition, metal ions or other additives), there is no guarantee that this will work. To avoid wasting time and effort, it is advisable to check the solubility of the passenger protein after intracellular processing of the fusion protein before proceeding with protein purification.

Can MBP fusion proteins be crytallized?

This has only worked in a small number of cases so far [Smith *et al.*, 2003]. In each instance, the fusion protein was engineered to have very few residues between the C-terminus of MBP and the N-terminus of the passenger protein to increase its rigidity. It is not clear how generally applicable this approach is, but our view is that it is definitely worth trying when more traditional approaches have failed to yield crystals.

References

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