Discovering the Maltose Binding Protein epitope tag for *Escherichia coli* expressed proteins

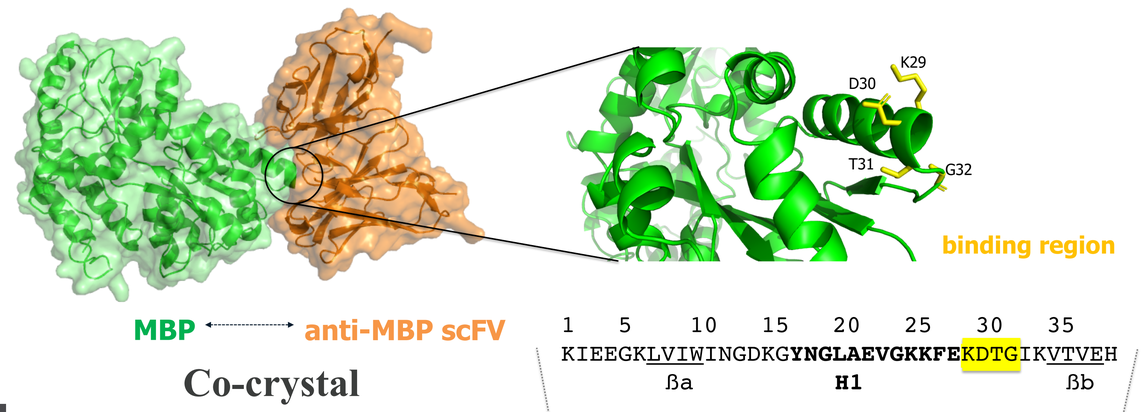
Marine Lénon[1], Patrick Loll[2], Paul Riggs[1] and Mehmet Berkmen[1]
[1]New England Biolabs, Ipswich, MA 01938, USA; [2] Drexel University College of Medicine, Philadelphia, PA.

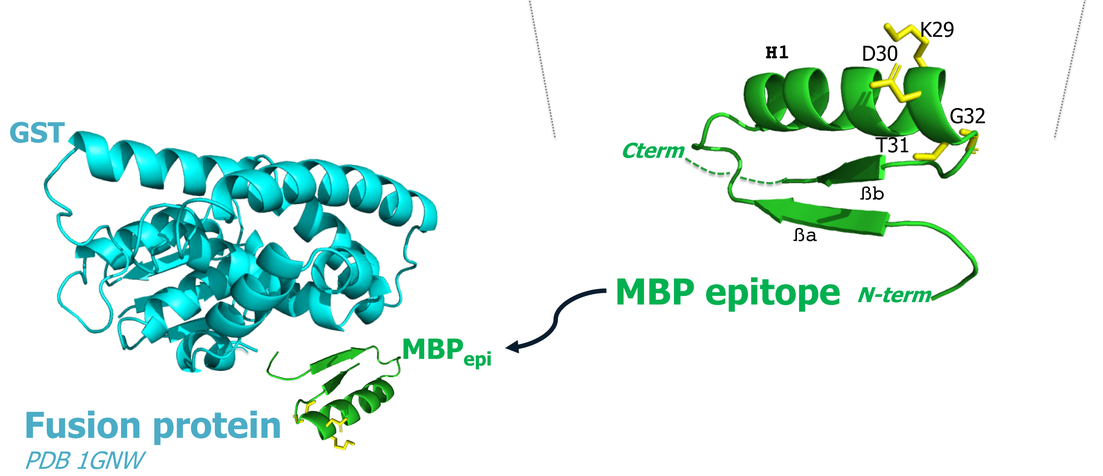
INTRODUCTION

Maltose Binding Protein (MBP) is used in recombinant protein expression as an affinity and solubility tag. The anti-MBP monoclonal antibody B48 **binds tightly** and has **no cross-reactivity** to other proteins in an *E. coli* lysate. For all these criteria, the MBP tag provides a useful epitope for fusion proteins expressed in *E. coli*.

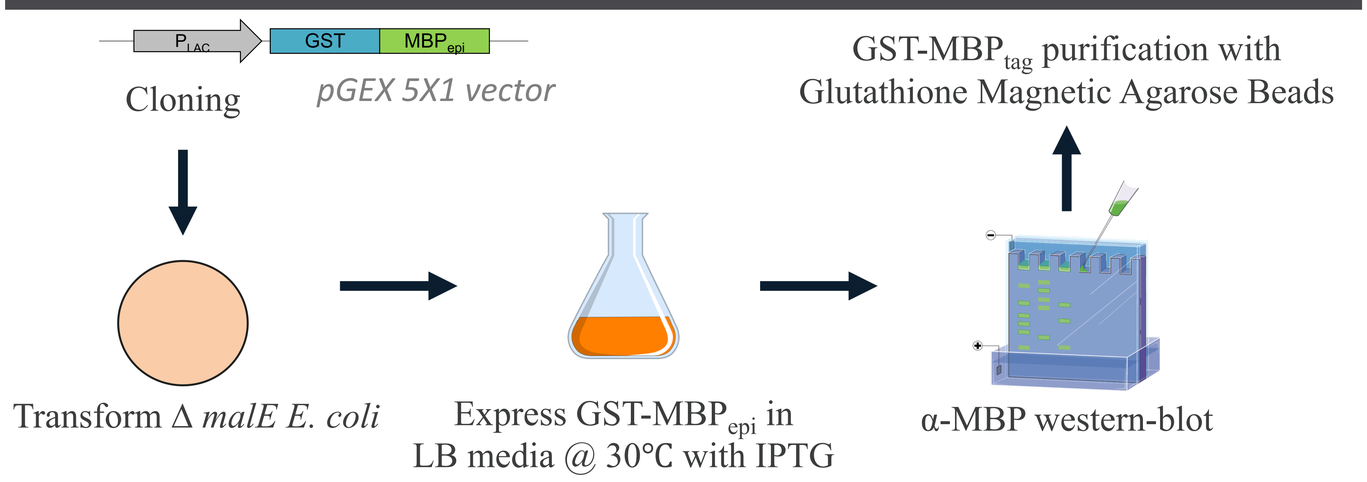
The co-crystal structure of MBP bound to its antibody was solved and four amino acids of MBP were found to define the binding interaction. This epitope is the turn of an alpha helix packed by two beta strands. The failure to find a linear epitope by phage display suggests the **helix-turn-sheet** is important in defining the smallest MBP epitope. Fusion of various fragments of MBP to the glutathione S-transferase protein was engineered in order to identify the smallest fragment, still recognized by the anti-MBP antibody. Further engineering of the epitope to stabilize and minimize the tag is in progress.

Discovery of the MBP epitope





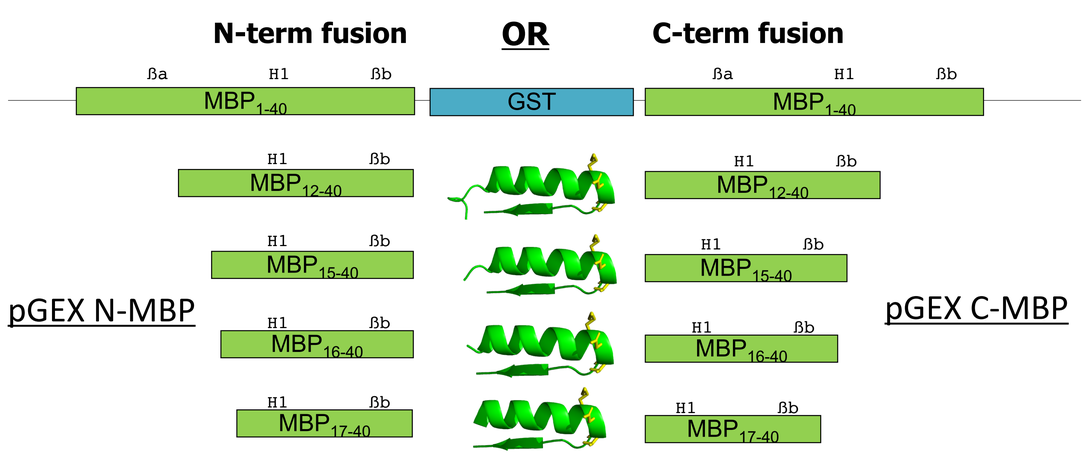
METHODS



RESULTS

The MBP epitope was fused to GST as a C-term or N-term fusion:

- fusion protein detected with α-MBP antibody ✓
- molecular weights of the native fusion proteins confirmed by MS ✓
- detection of mutated MBP epitope ✓

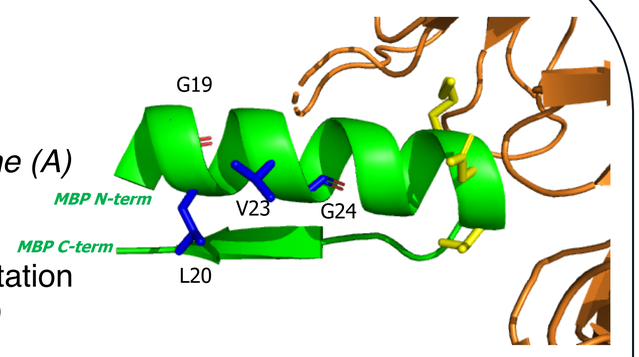


Can we make the MBP epitope stronger?

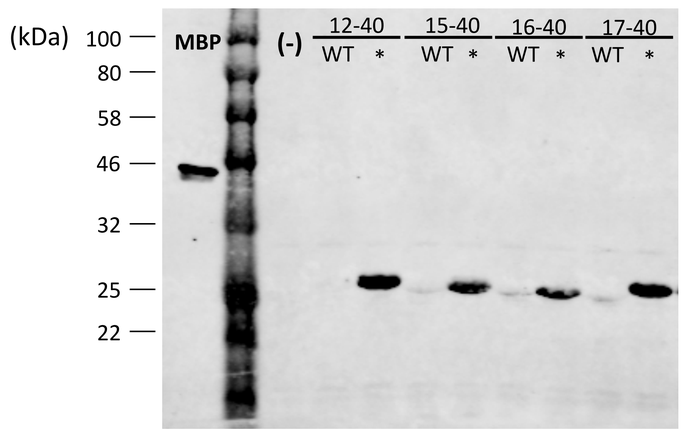
MBP* mutations

1) Helix promoting mutations
   glycine (G19, G24) to alanine (A)
   valine (V23) to leucine (L)

2) Hydrophobicity promoting mutation
   leucine (L20) to arginine (R)



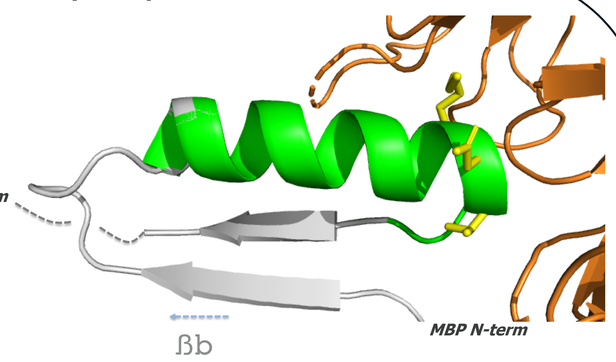
ßa H1 ßb
KIEEGKLVIWINGDKGYNGLAEVGKKFEKDTGIKVTVEH
AR LA
** **



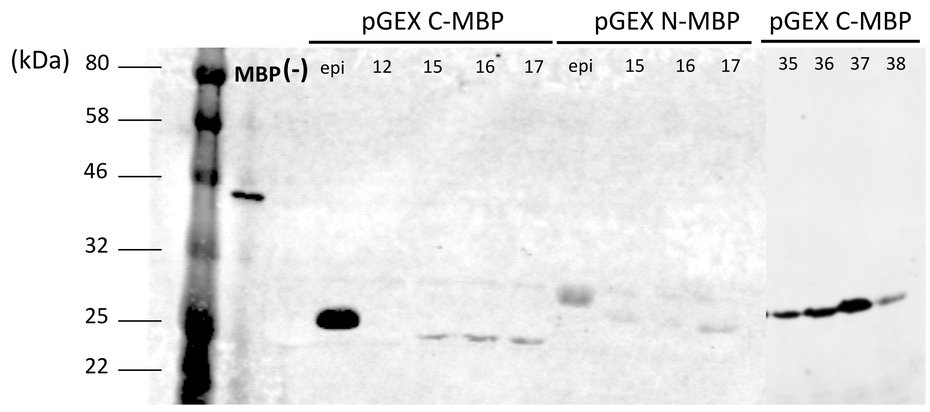
Can we make the MBP epitope smaller?

Deletion of MBP fragment:

1) N-term deletions ✓
2) C-term deletions ✓
3) N-term and C-term deletions …



ßa H1 ßb
N-term KIEEGKLVIWINGDKGYNGLAEVGKKFEKDTGIKVTVEH C-term
17 35



CONCLUSIONS

- The GST C-term fusions of MBP_{epi} work better
- The minimal epitope is $MBP^*_{17\text{-}40}$
- The helix-turn-sheet will be promoted by disulfide bonds, as redox sensor