RECOGNITION OF L-□-HOMOMETHIONINE BY METHIONYL-tRNA SYNTHETASE

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Living cells commonly employ the 20 canonical □□amino acids for ribosomal protein synthesis, although a few other amino acids (such as selenocysteine or pyrrolysine) are also employed in rare cases. The natural repertoire of amino acids is therefore rather narrow. This limits the possibilities to engineer non-natural proteins with new properties, usable for therapeutic or industrial purpose. Therefore, a dominant trend in the field of protein engineering is to build up systems for incorporating non-natural amino acids during protein biosynthesis *in vivo* (reviewed in Xiao and Schultz, 2016). Although this strategy has been successful in several cases, all amino acids used to date were □ amino acids. Site-specific incorporation of a □ amino acid in a polypeptide would introduce unprecedented flexibility in the main chain, thereby enlarging the geometric possibilities for protein folding. It has recently been demonstrated, using an *in vitro* cell-free translation system, that the ribosome is able to efficiently incorporate □ amino acids at discrete sites in a polypeptide (Fujino et al, 2016). At this stage, a technological issue that needs to be resolved for *in vivo* synthesis of a protein containing □□amino acid residues is the design of aminoacyl-tRNA synthetases able to efficiently esterify the desired □□amino acids onto specific transfer RNAs. A recent study has shown that some *E. coli* aminoacyl-tRNA synthetases are indeed able to use □ amino acids as substrates (Melo Czekster et al, 2016).

Our final goal is to optimize incorporation of □ methionine in polypeptides. To this aim, we have first characterized the binding and activation of L-□-homomethionine by *E. coli* methionyl-tRNA synthetase. It is demonstrated that activation of the □ amino acid occurs but at a markedly lower rate than activation of L-□-methionine. A 1.45 Å crystal structure of methionyl-tRNA synthetase complexed with the □ amino acid will also be presented. We will discuss how this structure is used for optimizing aminoacylation of tRNA with L-□-homomethionine.

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