# On the contribution of substrate flexibility to define Methionine adenosyltransferase specificity

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Methionine adenosyltransferase (MAT) is an essential enzyme, found in all three kingdoms of life. MAT catalyzes the synthesis of S-adenosyl-L-methionine (AdoMet) from ATP and L-methionine. Aiming at the biosynthesis of an unnatural AdoMet, we tested the specificity of adenosine binding pockets of MAT for different nucleotides from E. coli and Human orthologous enzymes.

E. coli and human MATs are similar at sequence level (59 % identity) and have identical adenosine binding pocket. Surprisingly, catalytic assay of human MAT shows promiscuities towards nucleotides while E. coli MAT is specific for ATP binding. A detailed structural and dynamic analysis of the MAT enzymes reveals that a combination of structural flexibility of protein and substrate play a major role in defining substrate specificity. Furthermore, a metabolomic analysis of human cells allowed to detect a new cofactor synthesized by MAT enzyme in cancer cells.

Ultimately, our findings suggest that (i) structural flexibility of substrate can play a major role in defining enzyme specificity; (ii) promiscuities can be relevant in vivo; (iii) substrate specificity might have been evolutionary driven by metabolites concentration in different organisms.