

# Conformational Changes And flexibility In Cobalamin-Dependent Methionine Synthase (Meth) Studied by SAXS And Cryo-EM

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Cobalamin-dependent methionine synthase (MetH) catalyzes the B12-mediated synthesis of methionine from homocysteine and 5-methyltetrahydrofolate, providing the link between the S-adenosylmethionine cycle and folate cycle in one-carbon metabolism. Decades of biochemical and structural studies on *E. coli* MetH have indicated that this system is a flexible, modular enzyme that adopts two major conformations to prevent a futile cycle of methionine production and consumption. However, as MetH is a dynamic protein as well as both an oxygen-sensitive and photosensitive metalloenzyme, it has posed a significant challenges for structural studies, and so existing structural information has necessarily come from a “divide and conquer” approach. We investigated *E. coli* MetH and a thermophilic homolog from *Thermus filiformis* using primarily small-angle X-ray scattering (SAXS) and single-particle cryo-electron microscopy (cryo-EM), complemented with comprehensive analysis of the AlphaFold2 database to present for the first time a structural description of MetH in its entirety. By combining a range of SAXS experiments with a 3.6-Å cryo-EM structure of the *T. filiformis* enzyme, we show that the system adopts a stable resting-state conformation and that conformational changes during turnover are driven by the folate substrate. Finally, by combining AlphaFold2-guided sequence analysis and our experimental findings, we propose a general model for functional switching in MetH.