

## Supplemental information

### **Structural and functional characterisation of the methionine adenosyltransferase from *Thermococcus kodakarensis***

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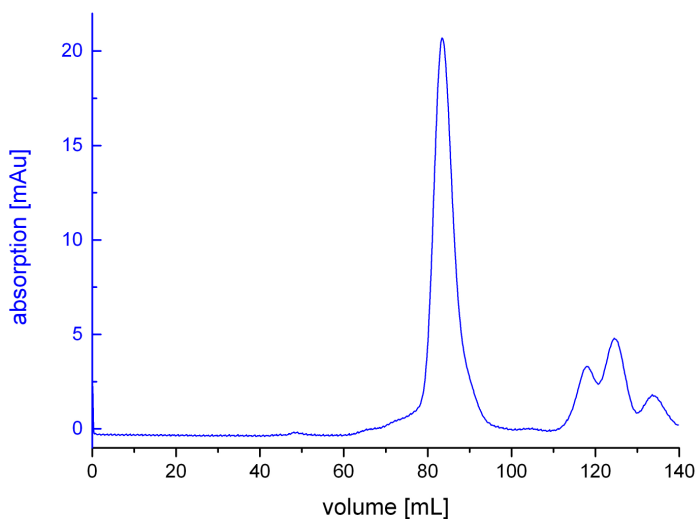
**Figure S1: Size exclusion chromatography of *TkMAT*.**

**Figure S2: Thermal denaturation of *TkMAT*.**

**Figure S3: Determination of kinetic parameters for *TkMAT*.**

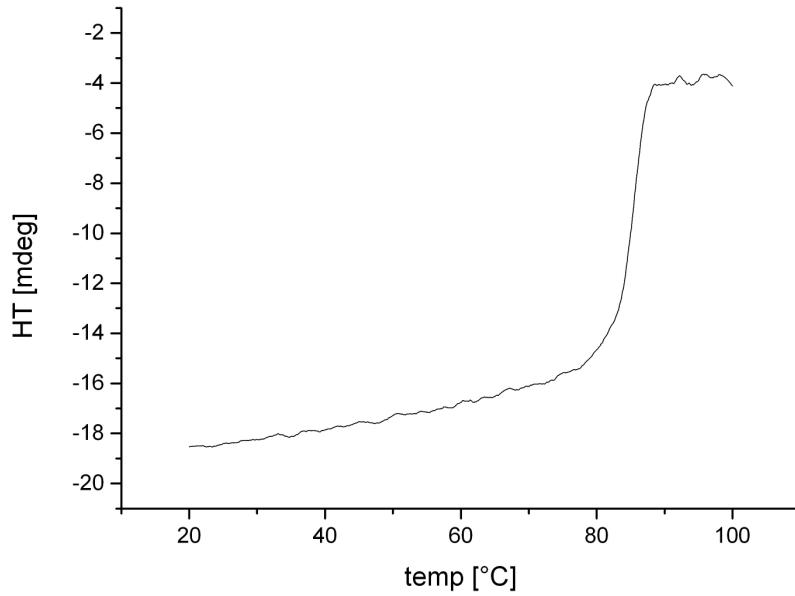
**Figure S4: Comparison of open and closed MAT structures.**

**Figure S5: Surface representation of MAT monomers.**

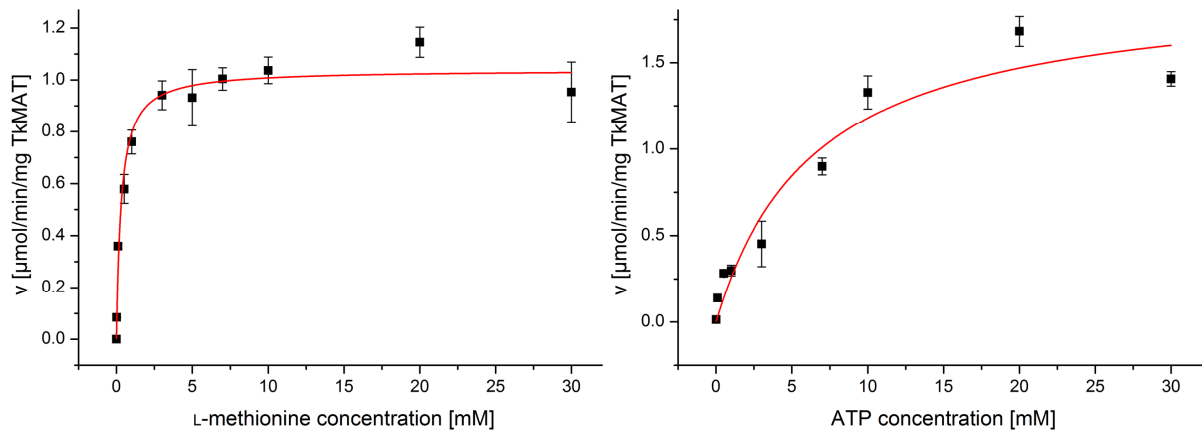


**Figure S1: Size exclusion chromatography of *TkMAT*.** *TkMAT* elutes in a single peak at a volume corresponding to a molecular mass of 81 kDa, clearly showing that the enzyme (monomer 46.8 kDa) is a dimer in solution. For calibration of the column a commercial protein standard mix (HMW calibration kit, GE Healthcare, consisting of ovalbumin [43 kDa], conalbumin [75 kDa], aldolase [158 kDa], ferritin [440 kDa] and thyroglobulin [669 kDa]) plus carboanhydrase (29 kDa) was used resulting in a calibration curve with the equation:

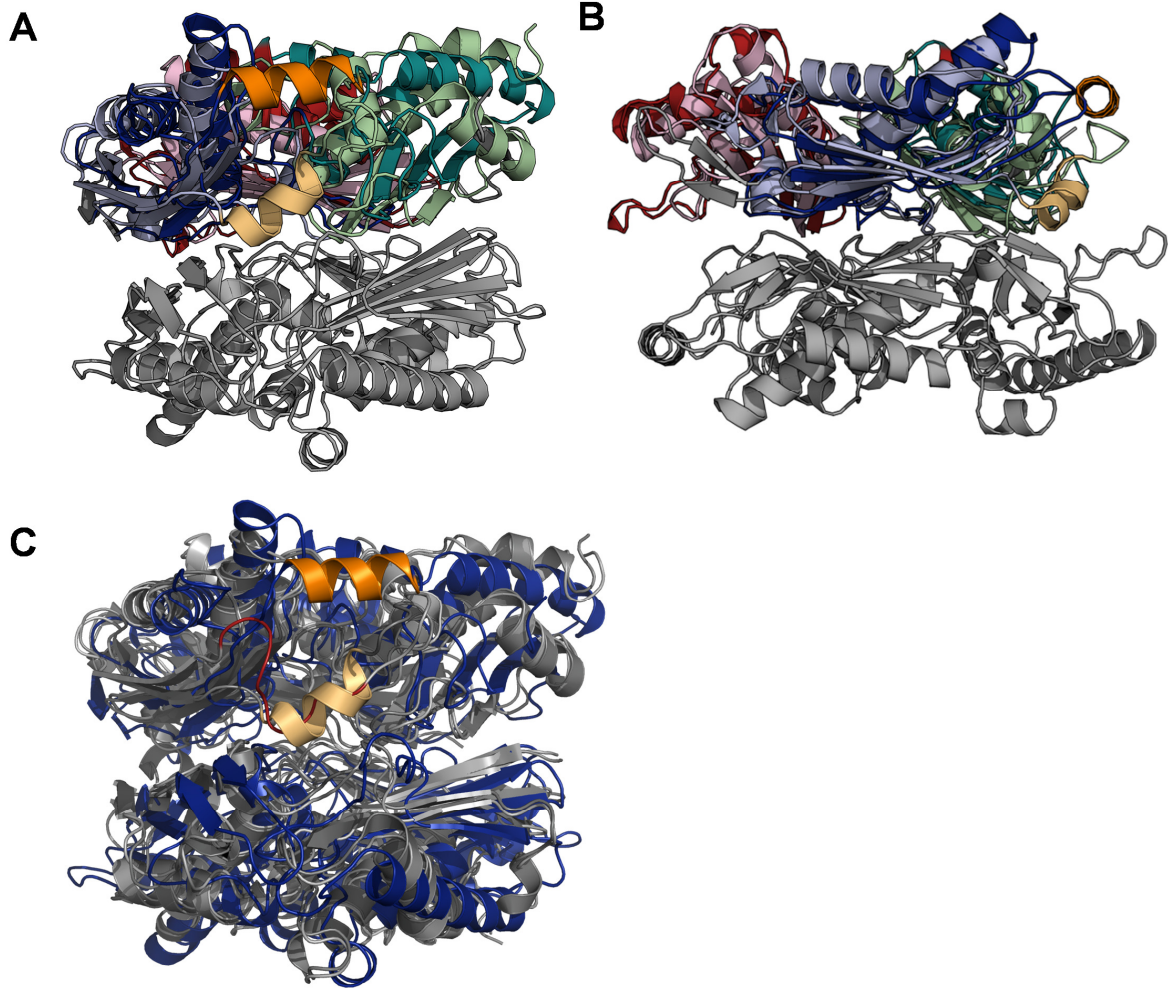
$$y = 10^{5.33 - 0.041 \cdot x}$$



**Figure S2: Thermal denaturation of *TkMAT*.** The inflection point of the graph (85 °C) corresponds to  $T_{50}$ .

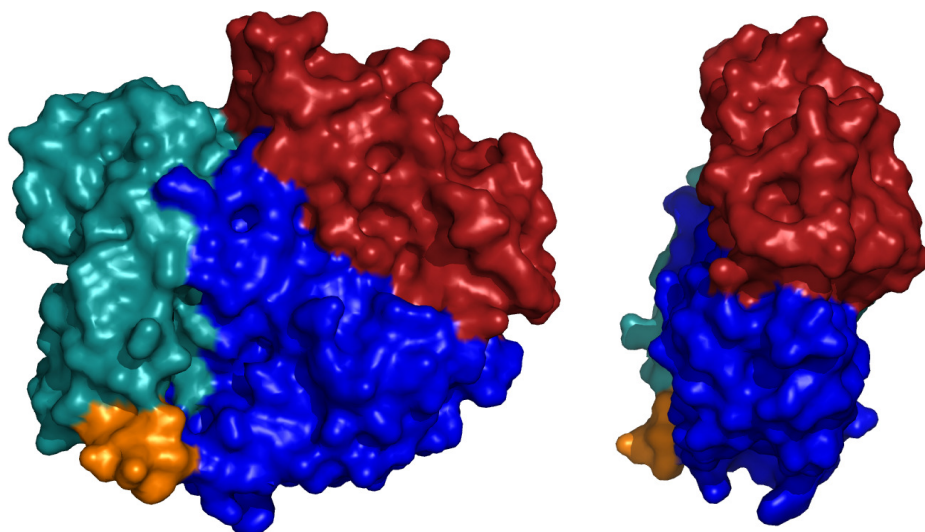


**Figure S3: Determination of kinetic parameters for *TkMAT*.** Left: variation of methionine concentration at constant ATP concentration (10 mM); right: variation of ATP concentration at constant methionine concentration (10 mM). Kinetic parameters were determined by non-linear fitting with the Origin software:  $K_M$  (Met) =  $0.31 \pm 0.07$  mM,  $K_M$  (ATP) =  $6.54 \pm 2.49$  mM,  $v_{max}$  =  $1.95 \pm 0.26$   $\mu\text{mol}/\text{min}/\text{mg}$ .

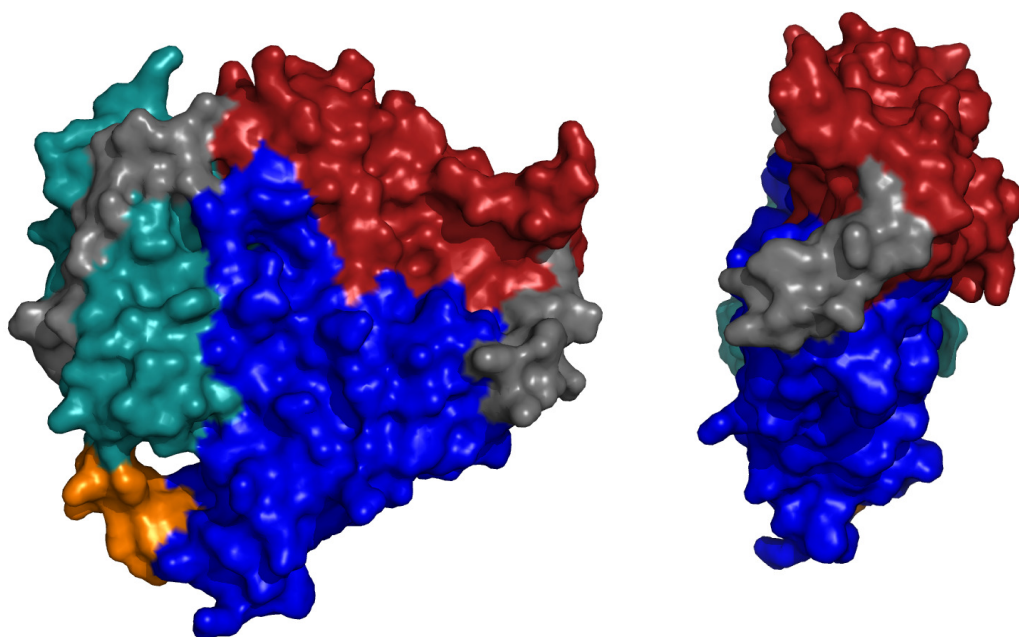


**Figure S4: Comparison of open and closed MAT structures.** A: overlay (upper subunit) of *TkMAT* in the open state and *EcMAT* in the closed state (1RG9). B: side view of A. C: overlay of *TkMAT* (open, orange helix) and two *EcMAT* structures: 1RG9 (closed, light orange helix) and 1FUG (quasi-closed/disordered loop, red loop).

**A: *EcMAT***



**B: *TkMAT***



**Figure S5: Surface representation of MAT monomers.** Left: dimer interface, right: side view. The additional  $\beta$ -strands present in *TkMAT* are highlighted in grey. The very first  $\beta$ -strand is part of the C-terminal domain (cyan), whereas the second additional element is part of the central domain (blue) and wraps around the loop region of the N-terminal domain (red), where it could be beneficial for the overall stability of the enzyme.