Bisubstrate Analogues as Structural Tools to Investigate m^{6}A Methyltransferase Active Sites

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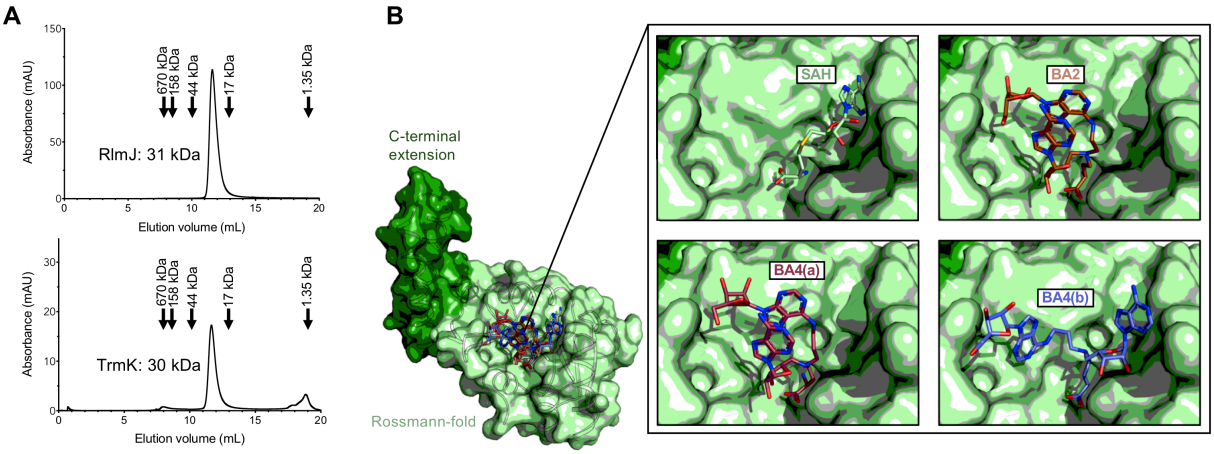
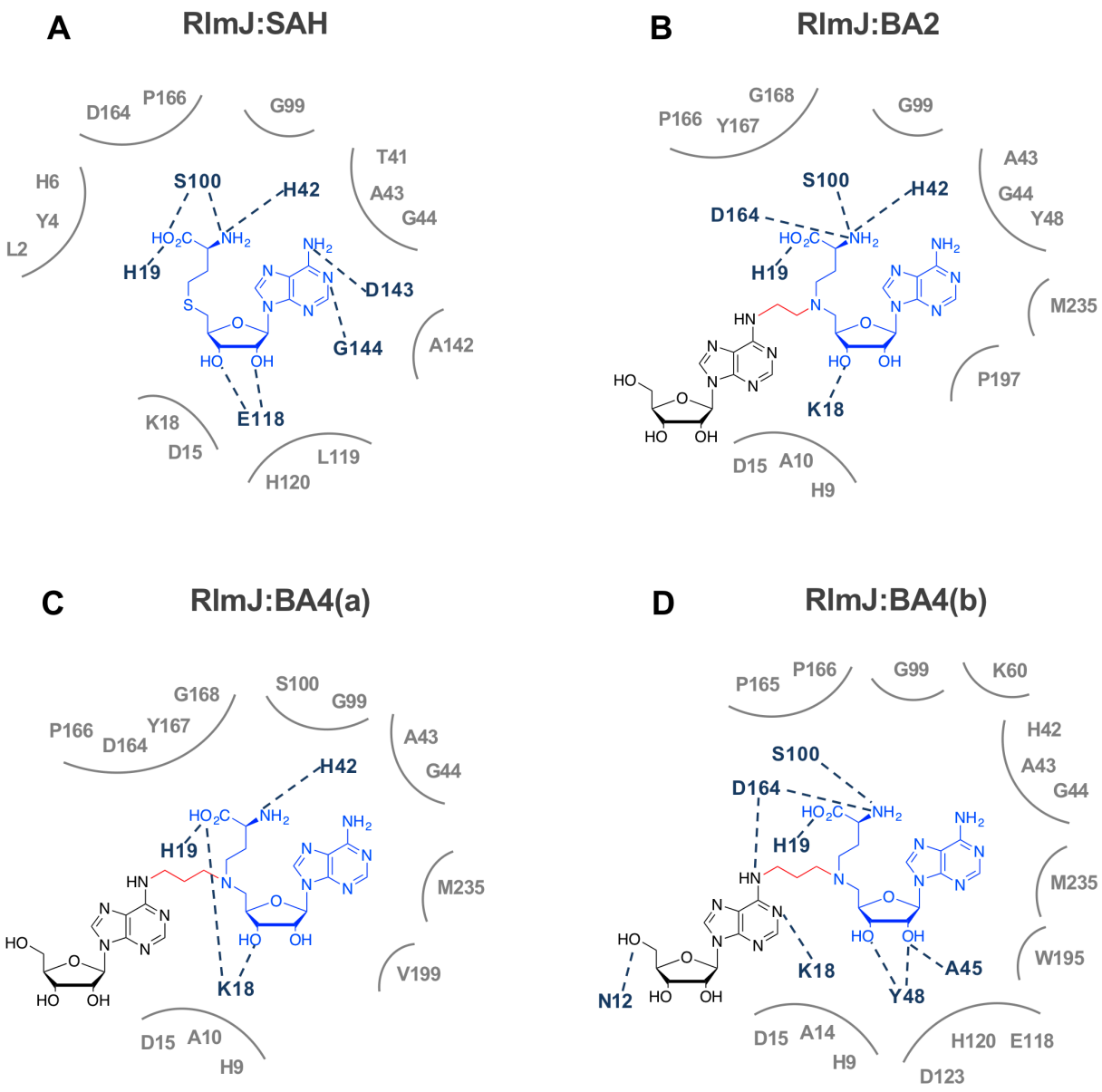
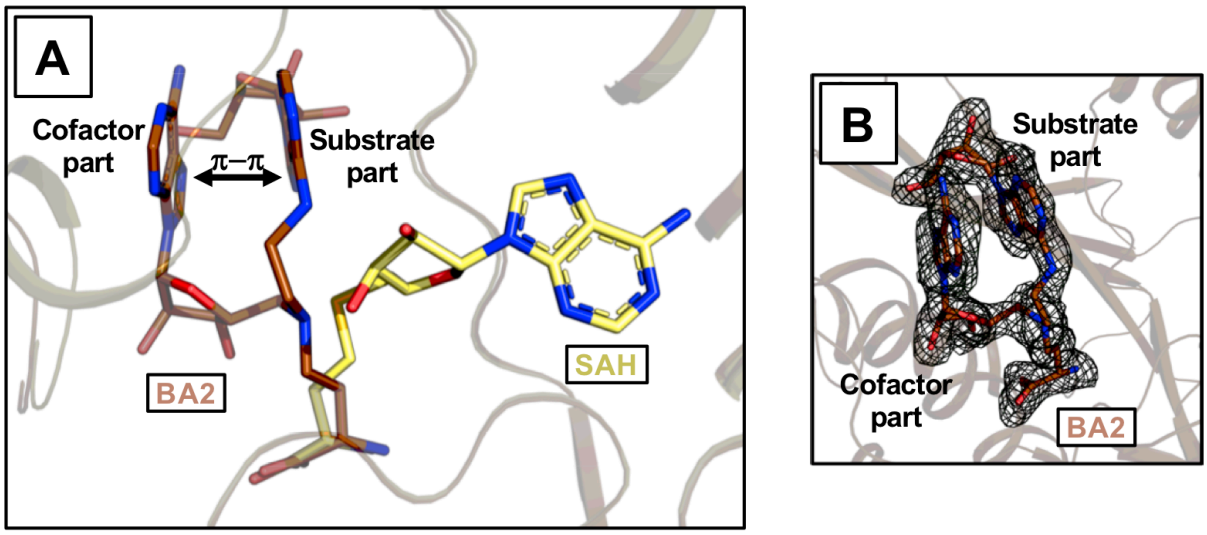


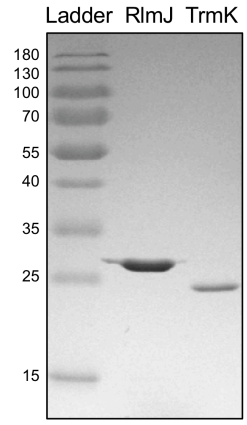
Supplementary Figure S1. Protein oligomeric state and compound modelling for TrmK (**A**) Chromatogram from Analytical SEC analysis of RlmJ (top) and TrmK (bottom). The elution volumes of standard marker proteins are indicated with arrows and their respective sizes are shown above the chromatogram. The size corresponding to the elution volume of RlmJ and TrmK is indicated next to their elution peak. MW_{SEC}: RlmJ = 31 kDa, TrmK = 30 kDa; calculated $MW_{monomer}$: RlmJ = 32 kDa, TrmK = 28 kDa. (**B**) Surface representation of TrmK:SAH (green) with co-crystallised SAH (green) and modelled BA2 (brown), BA4(a) (red), and BA4(b) (blue). All ligands are shown in stick representation. Positioning of the bisubstrate analogues is based on superpositioning of SAH from RlmJ:SAH with SAH from TrmK:SAH, and next alignment of RlmJ from RlmJ:SAH with RlmJ from RlmJ:BA2 or RlmJ:BA4 chain A (BA4(a)) or B (BA4(b)). The insert presents a close-up view of the binding pocket with the co-crystallised SAH and modelled bisubstrate analogues.



Supplementary Figure S2. Protein-ligand interaction analysis of RlmJ with SAH, BA2 or BA4. Outline of protein-ligand interactions from a LigPlot analysis of (**A**) SAH in RlmJ:SAH, (**B**) BA2 in RlmJ:BA2, (**C**) BA4(a) in chain A of RlmJ:BA4, (**D**) BA4(b) in chain B of RlmJ:BA4. All figures show the residues in RlmJ (dark blue) that form hydrogen bonds (dashed lines) with the ligand (black/red/blue). Residues involved in formation of the binding pocket environment are also shown (grey).



Supplementary Figure S3. Binding of BA2 to RlmJ (**A**) Binding of BA2 to RlmJ (brown), compared with RlmJ bound to SAH (yellow). Ligands are shown as stick representation. The π-π stacking bases in BA2 are indicated along with the parts of BA2 corresponding to the cofactor and substrate (**B**) Fo-Fc of BA2 in RlmJ contoured at 2.0 σ. The substrate -and cofactor parts are indicated for clarity.



Supplementary Figure S4. SDS-PAGE (14%) analysis of recombinant, purified RlmJ protein from *E. coli* (first lane, 1.3 μg load) and TrmK from *M. capricolum* (second lane, 1 μg load) used throughout experiments. Protein standard ladder is shown (left) and protein sizes are indicated in kilo Dalton (kDa). Calculated molecular weight for RlmJ = 32 kDa and TrmK = 28 kDa.