Biochemical and structural characterization of a Schiff base in the radical-mediated biosynthesis of 4-demethylwyosine by TYW1

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SUPPLEMENTARY FIGURES

Figure S1. Double conformations of pyruvate-lysine adduct in TYW1. A. Two conformations of the pyruvate-lysine adduct, KAC41, are shown modeled into 2Fo-Fc electron density (blue) contoured at 1 σ. **B.** The same two conformations of KAC41 as in **A** are shown here modeled into 2Fo-Fc omit map density (cyan) contoured at 1 σ. The major conformation of the Schiff base is planar (gray), is contained in ~60% of the molecules in the crystal, and is observed to ligate the auxiliary cluster in a bidentate fashion. The minor conformation is not planar (salmon), is found in the remaining ~40 % of the molecules, and is observed with its carboxyl group rotated away from the cluster. **C**. Close-up of the two conformations of KAC41, showing that the carboxylate of the minor KAC41 conformation (salmon) is rotated 90° from the position of the carboxylate of the major KAC41 conformation (gray). **D**. Slight rearrangements of side chains are observed in response to the multiple conformations of KAC41. For example, Arg221, which is thought to be involved in binding of both m^1G37 (gray) and SAM (not shown), flips from its major conformation (gray) to an orientation that provides hydrogen bonds (~2.4 and 2.5 Å) to an oxygen of KAC41 in its minor conformation (salmon). Radical addition to C2 of the pyruvate moiety by m^1G37 is expected when the adduct is in the dominant planar conformation. The hydrogen bonds and ligation of Schiff base to the cluster is shown in black dashed lines. The purple dashed lines represent the hydrogen bonding-network formed by residues in the binding pocket.

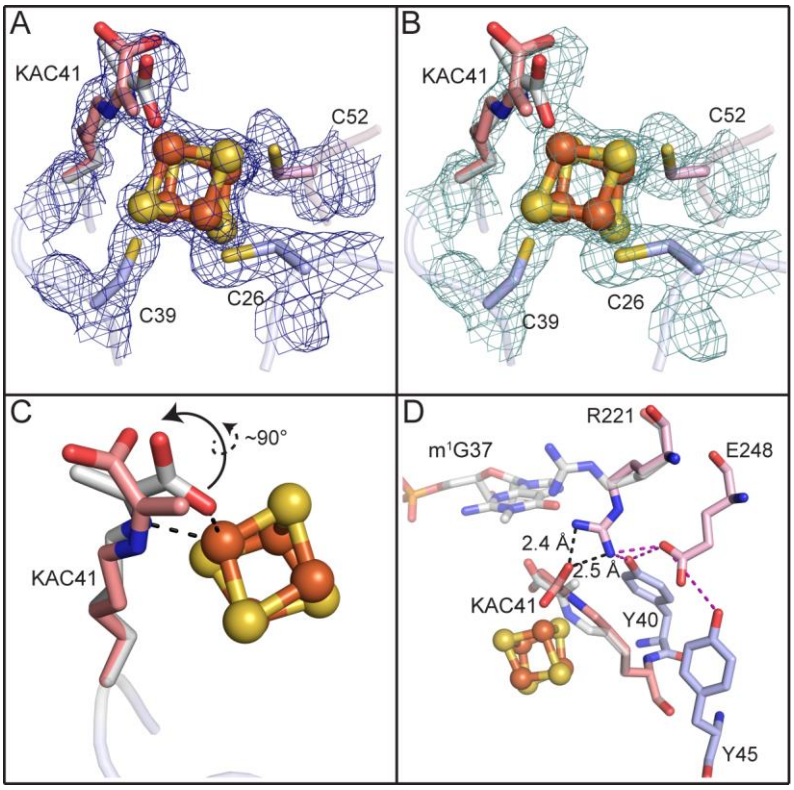

Figure S2. SAM binding motifs of MoaA and TYW1. A. MoaA (PDB 1TV8). **B.** Model of SAM binding to TYW1 using MoaA as a guide. Residues putatively involved in positioning of SAM are represented in sticks and colored based on their role/motif. The β6 motif (green), the GXIXGXXE motif (maroon) and the $CX_3CX\Phi C$ motif (light pink) have been previously shown to position the adenine moiety of SAM, whereas the ribose motif (tan) orients the ribose rings. The GGE motif (teal) and residues indicated in purple orient the amino group and the carboxylic group of SAM respectively, to ensure proper ligation to the radical SAM cluster, shown as balls-and-sticks (iron atoms in orange and sulfur atoms in yellow).

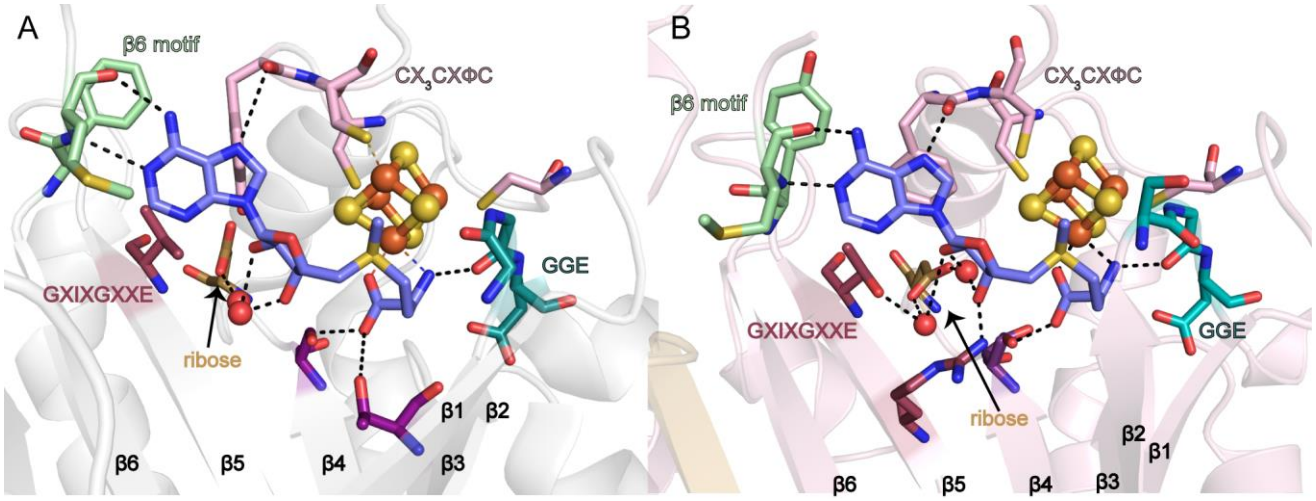

Figure S3. Extracted ion chromatograms of all observable tryptic peptides bearing Lys41. The extracted ion chromatograms at *m/z* 584.25 (N***C***YK), 656.27 (N***C***Y***K***), 799.37 N***C***YKSK), and 871.39 (N***C***Y***K***SK) ± 0.01.

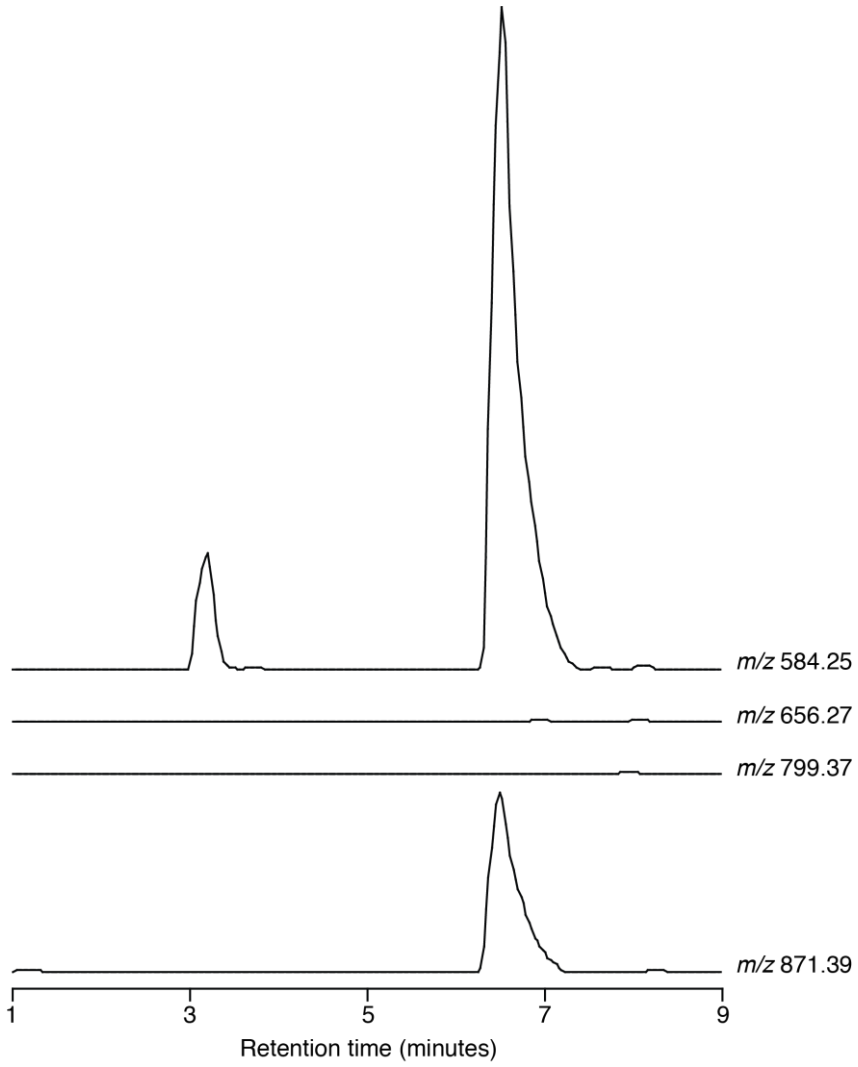

Figure S4. Extracted ion chromatogram of the Schiff base intermediate. The extracted ion chromatogram at *m/z* 874 and 871 showing the tryptic digest fragment containing the trapped Schiff base-lysine adduct when labelled and unlabelled pyruvate are used. The peak corresponding to isotopically enriched pyruvate adduct of the peptide at 7 min is only observed in the presence of labelled substrate.

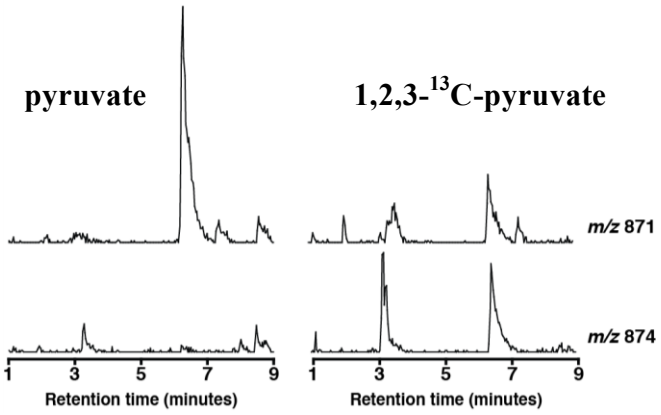

Figure S5. **Mass spectrum of the Schiff base intermediate**. The mass spectrum of a tryptic fragment of TYW1 incubated with 1,2,3-$^{13}C_3$ pyruvate in the absence of SAM, dithionite, or tRNA in the presence of either $NaCNBH_3$ or $NaCNB^2H_3$.

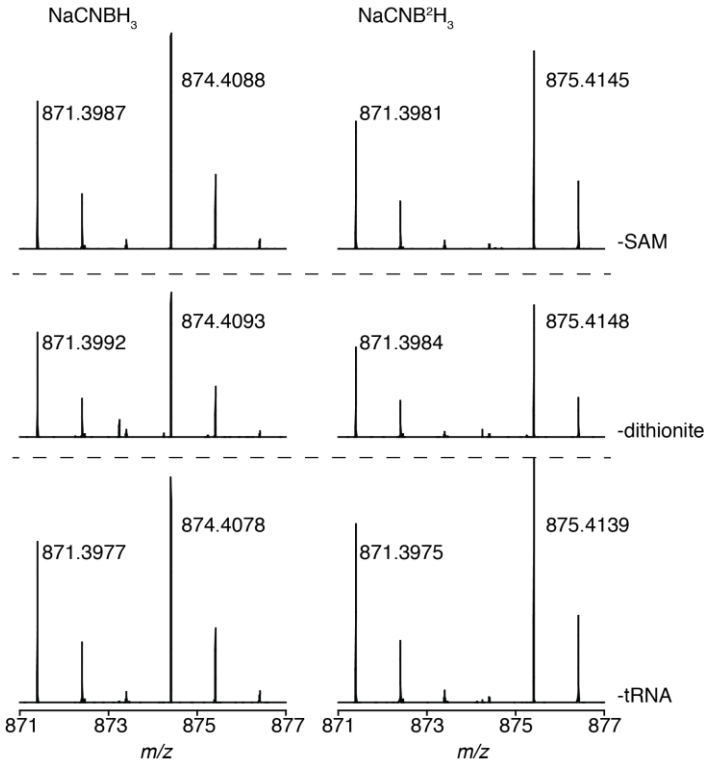

Table S1: TYW1 Data and Refinement Statistics

	TYW1
Beamline	APS[1] 24-ID-C
Wavelength (Å)	0.9792
Space group	$P2_12_12_1$
Cell dimensions (Å)	58.3, 80.2, 86.5
Resolution (Å)	50.0-1.64 (1.70-1.64)
Unique reflections	47,373
Completeness	94.1 (68.0)
Redundancy	6.5 (4.4)
R_{sym}	0.063 (0.689)
$CC_{1/2}$	(0.942)
I/σ(I)	30.2 (2.2)
Model Refinement	
Resolution limits (Å)	48.3-1.64 (1.67-1.64)
R_{work}/R_{free} [$]	0.1760/0.2024
Reflections	47,297
No molecules in asu	1
No atoms	
Protein	2586
Iron sulfur clusters	13
Adduct	28
Water	210
B-factors (Å^2)	
Protein	30.4
Iron sulfur clusters	31.4
Adduct	39.8
Water	47.9
R.M.S. deviations	
Bond Lengths, (Å)	0.007
Bond Angles (°)	0.889
Rotamer outliers (%)	1.45
Ramachandran Plot (%)	
Most Favored	96.9
Additionally allowed	3.1
Disallowed	0.0

Highest-resolution shell is shown in parentheses

[1]Advanced Photon Source, Argonne National Laboratory set.

$ 5% reflections used for test set