A complex of methylthioadenosine/S-adenosylhomocysteine nucleosidase, transition state analogue and nucleophilic water identified by mass spectrometry

Shanzhi Wang[1], Jihyeon Lim[2], Keisha Thomas[1], Funing Yan[1], Ruth H. Angeletti[2] and Vern L. Schramm[1,*]

[1]Department of Biochemistry, Albert Einstein College of Medicine, 1300 Morris Park Avenue. Bronx, New York 10461

[2] The Laboratory for Macromolecular Analysis & Proteomics, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, New York 10461

* To whom correspondence should be addressed. Telephone: (718) 430-2813.

Email:vern.schramm@einstein.yu.edu.

Supplementary Materials

Methods

Reagents. BuT-DADMe-ImmA was synthesized by and was the generous gift of Industrial Research Ltd. (Lower Hutt, New Zealand).[1,2] Ammonium acetate solution (7.5 M) was purchased from Sigma-Aldrich Chemical Co. (Saint Louis, MO). All other chemicals or reagents were purchased with the highest purity commercially available and used without further purification.

*Protein purification. Ec*MTAN with a C-terminal His_6 tag was purified as follows: cells from three liters of BL21 (DE3) *E.coli* culture containing the appropriate plasmid was grown at 37 ^{0}C until O.D. reached 0.6, then IPTG was added to a final concentration of 1 mM and cells were grown for additional 16 hours at 20 ^{0}C. Cells were collected by centrifugation at 4,500 g for 30 min and refrigerated at -80 ^{0}C for at least 30 min. After thawing, the cells were re-suspended using 50 mL buffer containing 50 mM HEPES (pH 7.0), 200 μg/ml lysozyme, 10 μg/ml DNaseA and 3 tablets of EDTA-free protease inhibitor cocktail (Roche Diagnostics). The cells were disrupted with a French-press, which was followed by centrifugation at 36,000 g for 30 min. The supernatant was applied to a 10 mL Ni-NTA column, followed by extensive washing with 100 mL of 50 mM HEPES (pH 7.0) and 50 mM imidazole. The protein was eluted with 50 mL buffer containing 50 mM HEPES (pH 7.0) and 250 mM imidazole. Then the solution was concentrated to 10 mL using a centrifuge filter (Amicon). With 5 mM ammonium acetate (pH 6.8) as elution buffer, the protein was further purified using a Superdex200 26/60 gel-filtration column. The eluted protein was concentrated to 2 to 6 mM, followed by dialysis against 5 mM ammonium acetate with 3 buffer changes. After dialysis, the

protein was centrifuged at 30,000 g for 15 min and the supernatant was directly used for MS analysis. Glass containers were avoided during all possible procedures to minimize Na^+ adduct formation.

Mass Spectrometry (MS) analysis. All MS spectra were acquired on a Q-star XL™ instrument from AB Sciex with a nanospray source. The instrument was calibrated before data acquisition using clusters generated from $CsHCO_3$ and perfluoroheptanoic acid (Sigma) to cover the mass ranges up to m/z 10,000 as is described previously.[3]

The protein solution was diluted to 2 to 6 µM with 5 mM ammonium acetate and 10 µL solution was loaded to a conductively coated borosilicate glass tip (EconoTips™, New Objective, Inc., MA) for a static nanospray. Ionspray voltage, declustering potential (DP), focusing potential (FP), and collision gas was optimized using the dimeric apoprotein solution and directly applied to the protein inhibitor complex. Concentrated protein and inhibitor solution were diluted to 2 to 6 µM with 5 mM ammonium acetate separately and mixed immediately before MS acquisition. Different combinations of DP and FP were applied to observe the effect of water loss from the complex. MS spectrum of monomeric *Ec*MTAN was acquired under denaturing condition using 50% methanol and 0.1% formic acid in water. The inhibitor alone was analyzed on the same instrument for accurate mass measurement.

Acquired data were smoothed and deconvoluted using Bayseian protein reconstruction to establish the molecular weight of species.

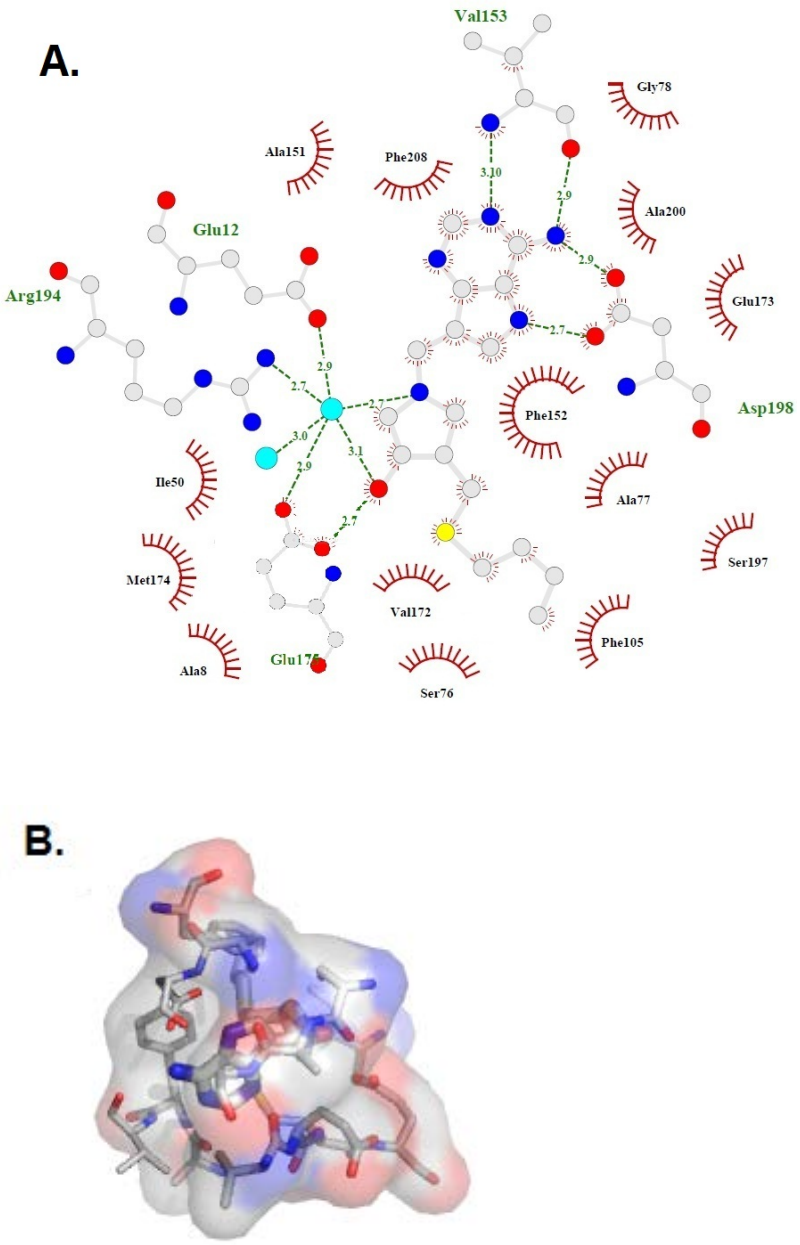

Figure S1: BuT-DADMe-ImmA interactions with *Vc*MTAN (pdb 3DP9) in two-dimensional (A) and surface representations (B). Polar contacts are shown in green. LIGPLUS and PYMOL were used to generate the graphs.

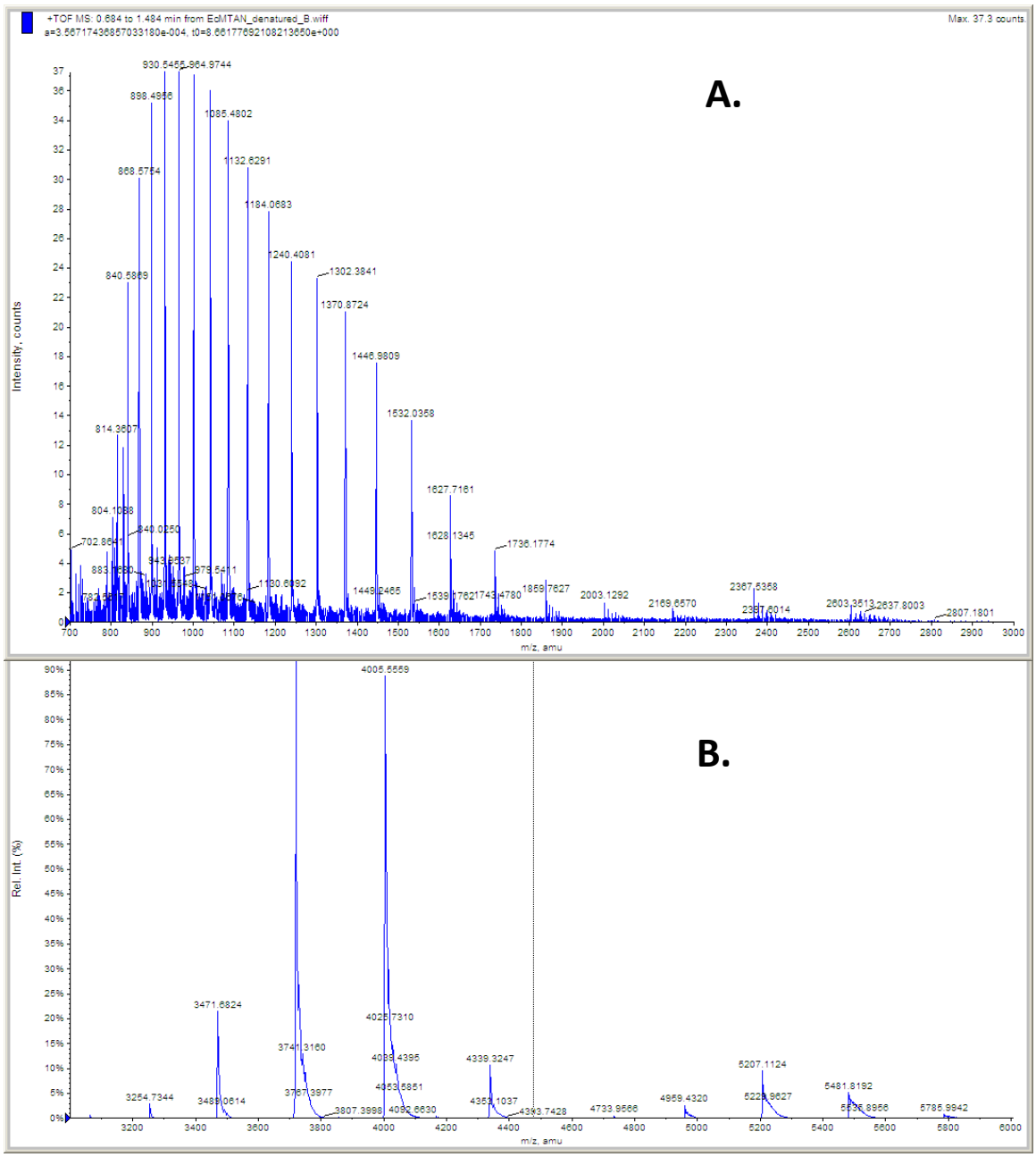

Figure S2: MS analysis of *Ec*MTAN under denaturing (A) and non-denaturing (B) condition.

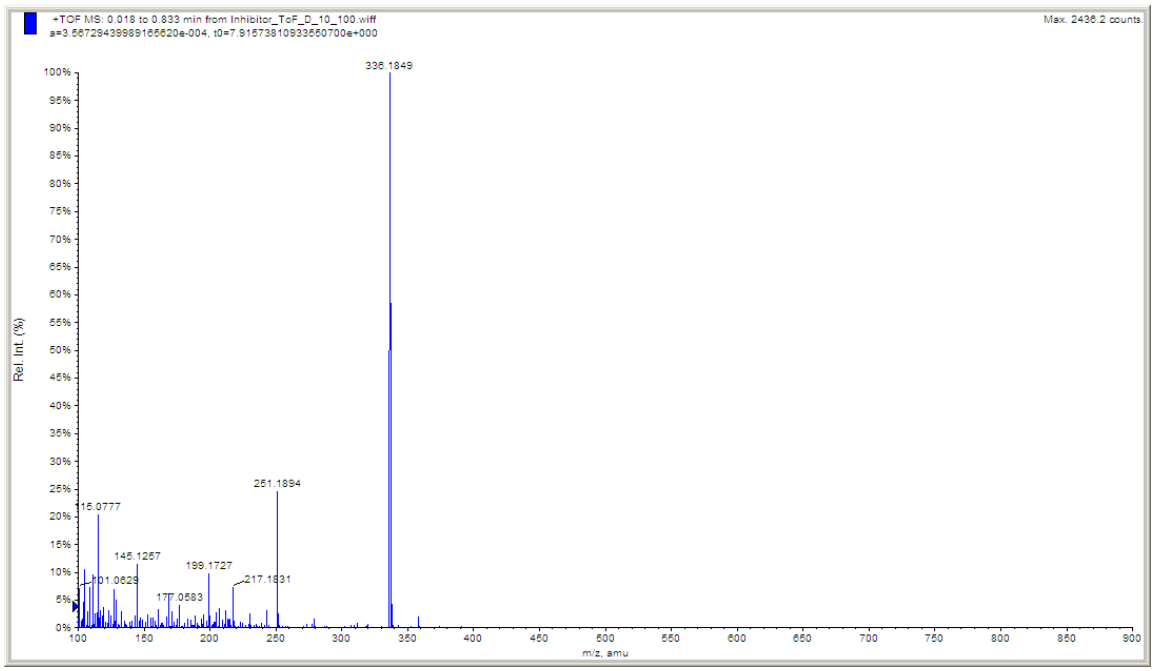

Figure S3: MS analysis of BuT-DADMe-ImmA. The dominant peak corresponds to the inhibitor, while smaller peaks are attributed to fragments of the inhibitor.

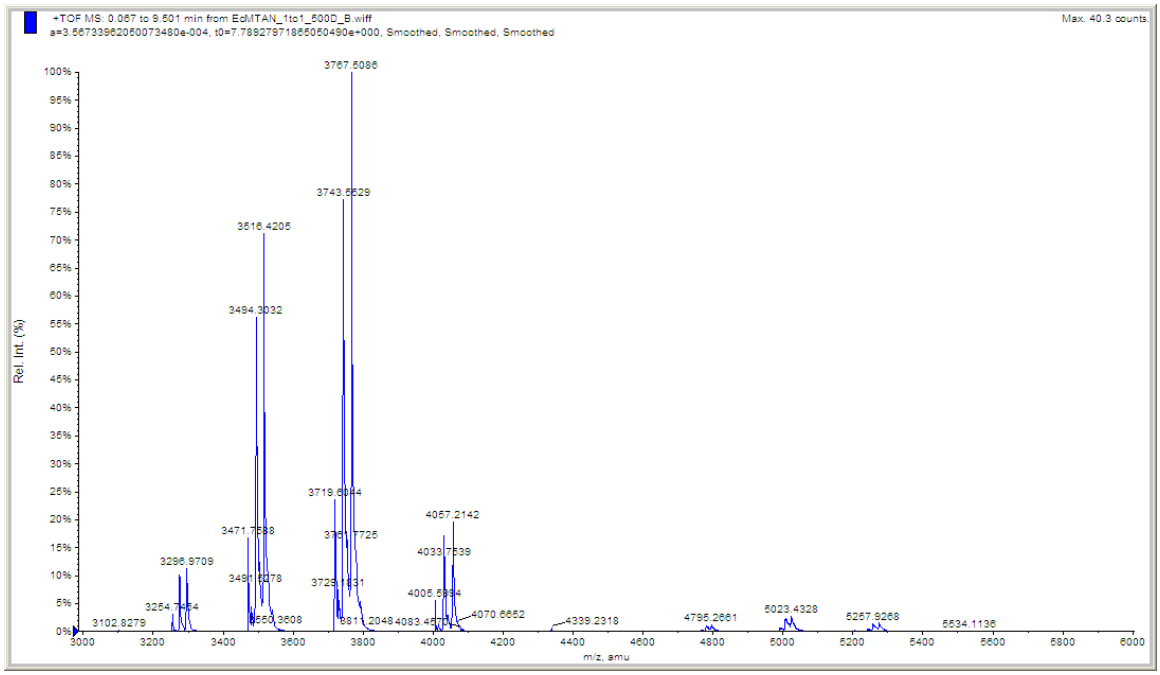

Figure S4: MS analysis of *Ec*MTAN in complex with sub-stoichiometric amounts of BuT-DADMe-ImmA.

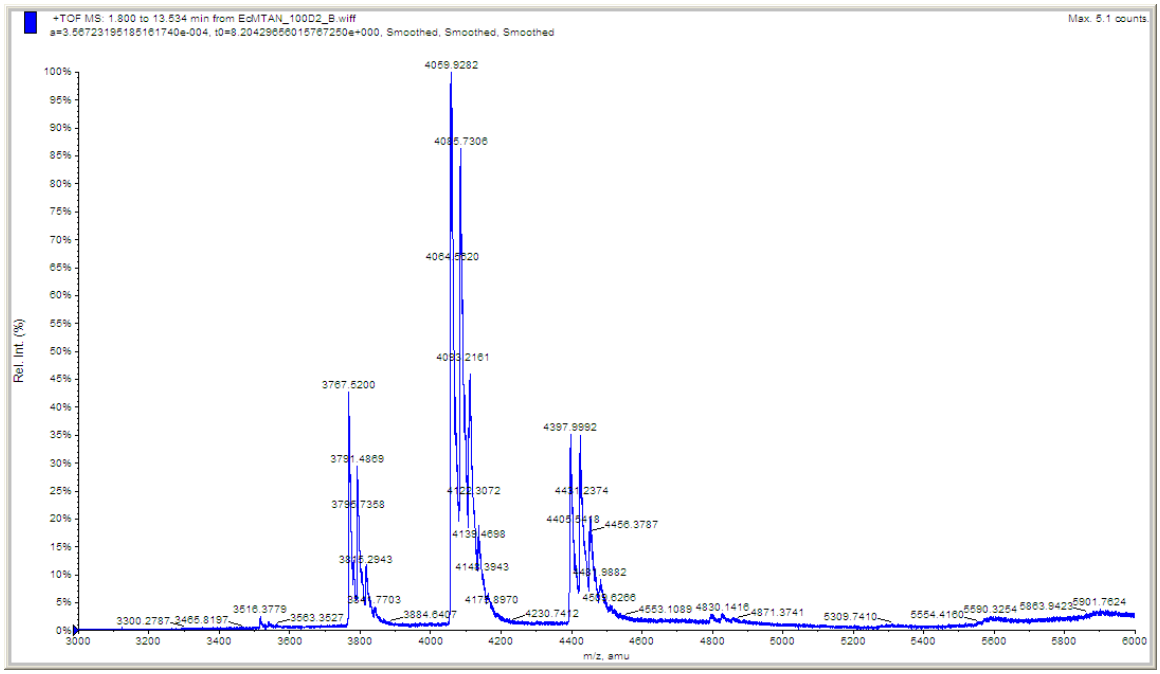

Figure S5: MS analysis of *Ec*MTAN in complex with super saturation amounts of BuT-DADMe-ImmA.

References:

(1) Evans, G. B.; Furneaux, R. H.; Schramm, V. L.; Singh, V.; Tyler, P. C. *J Med Chem* **2004**, *47*, 3275.

(2) Evans, G. B.; Furneaux, R. H.; Lenz, D. H.; Painter, G. F.; Schramm, V. L.; Singh, V.; Tyler, P. C. *J Med Chem* **2005**, *48*, 4679.

(3) Konig, S.; Fales, H. M. *J Am Soc Mass Spec* **1999**, *10*, 273.