

Structure of arylamine *N*-acetyltransferase from *M. tuberculosis* determined by cross-seeding with homologous protein from *M. marinum*: Triumph over Adversity.

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Running title: TBNAT structure.

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Figure S1: Flowchart summarising the purification protocol used to prepare TBNAT for crystallisation trials in this work.

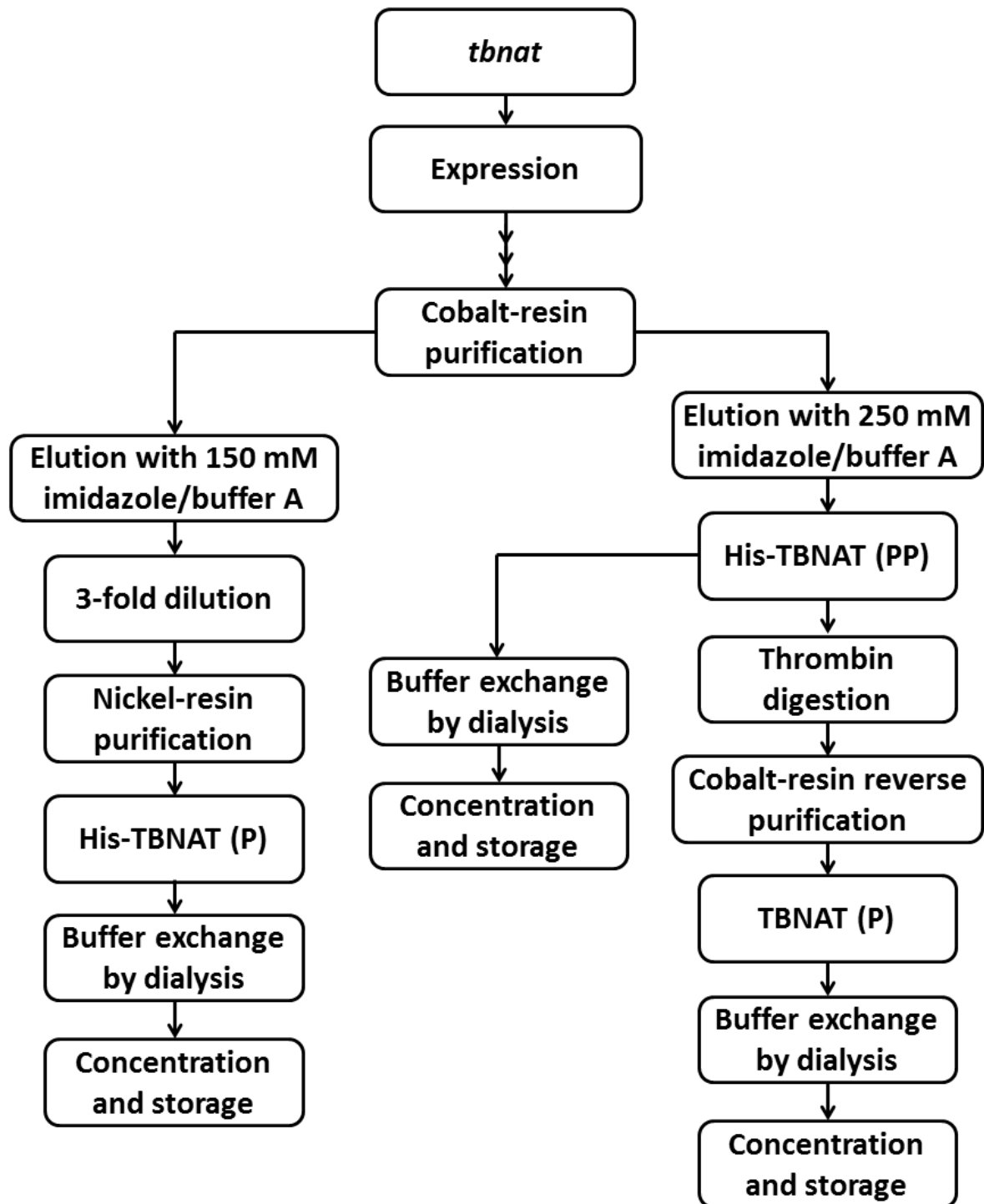


Figure S2: Purification of His-TBNAT.

(A) Partially purified His-TBNAT (arrow): Coomassie blue-stained 12 % SDS PAGE shows the whole cell lysate (lane 1), soluble fraction (lane 2), unbound fraction (lane 3), 25 mM imidazole (Imz)/buffer A wash (lane 4), 50 mM Imz/buffer A (lane 5), 150 mM Imz/buffer A (lane 6) and 250 mM Imz/buffer A (Lane 7). Bio-Rad low-range molecular mass protein standard (lane M). (B) Coomassie blue-stained 4-12 % SDS PAGE (NuPAGE Novex 4–12 % Bis-Tris Gel, Invitrogen) showing the purification of recombinant His-TBNAT (arrow) using the Ni-NTA resin. His-TBNAT eluted from the Cobalt-resin in 150 mM Imz/buffer A (lane 1), unbound fraction (lane 2), 50 mM Imz/buffer A (lane 3), 60 mM Imz/buffer A (lane 4), 75 mM Imz/buffer A (lane 5), 150 mM Imz/buffer A (lane 6), His-TBNAT after the initial dialysis and concentration as described in Section 4.2.4.2 (lane 7), and High-Range Rainbow marker (GE Healthcare) (lane M'). Buffer A: 20 mM phosphate buffer at pH 8, 75 mM NaCl. Diffuse bands that are likely to be due to co-purifying lipids are indicated within the red box.

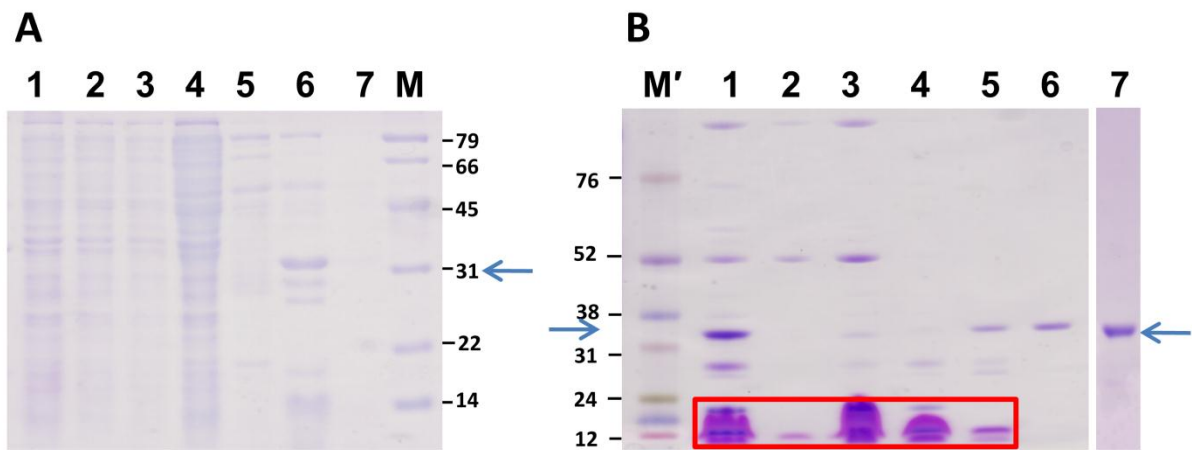


Figure S3. The melting curves of MMNAT and TBNAT.

The curves were obtained by plotting the relative fluorescence of SYPRO Orange against temperature for MMNAT and TBNAT in 100 mM HEPES, pH 7.0 and 150 mM NaCl. The melting temperature, T_m , on each curve is indicated by the red line.

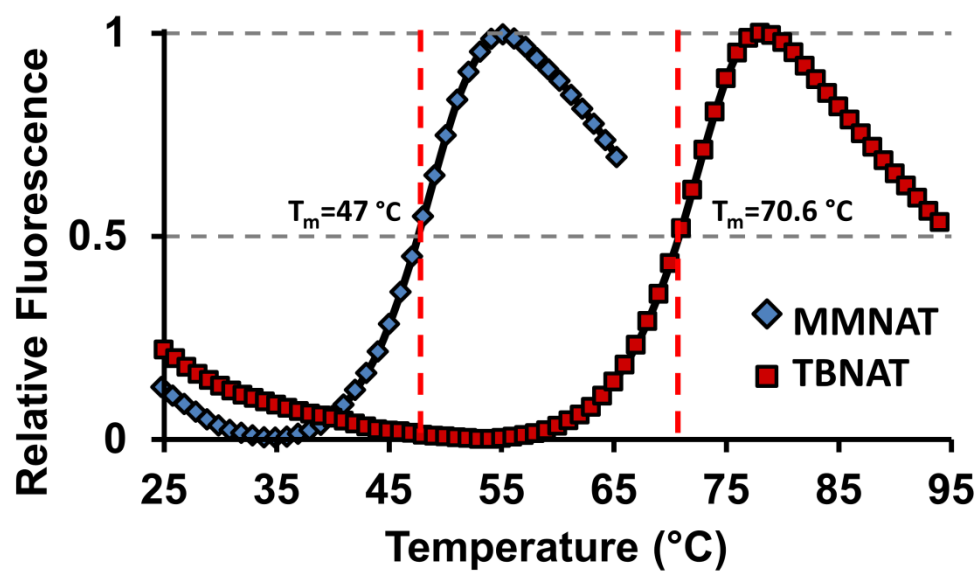
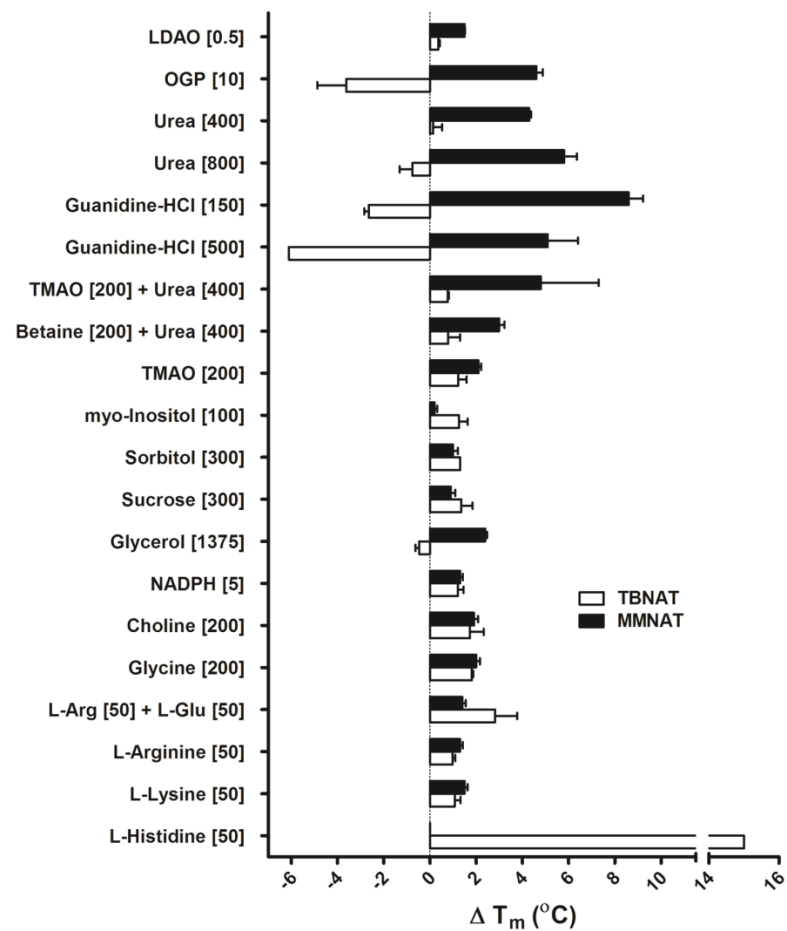


Figure S4: Changes in the TBNAT and MMNAT melting temperature in the presence of small-molecules from (A) the SCG-stability screen and (B) the NAT related ligands. Changes in the melting temperature (ΔT_m) were calculated from measurements on TBNAT/MMNAT in the presence of these additives from the SGC-stability screen which resulted in $\Delta T_m > 1$ °C using TBNAT/MMNAT in 100 mM HEPES, pH 7.5 and 150 mM NaCl as the reference. All solutions contained 100 mM HEPES buffer at pH 7.5. The numbers between square brackets represent the millimolar concentration of each additive. The columns represent the mean ΔT_m values \pm S.D of three independent measurements. A negative ΔT_m value signifies that the additive destabilises the protein, and a positive ΔT_m value indicates that the additive has a stabilising effect. TMAO: trimethylamine *N*-oxide, OGP: n-octyl glucopyranoside, LOAD: lauryldimethylamine *N*-oxide. IAA: iodoacetamide, AAc-CoA: acetoacetyl-CoA, Ar-CoA: arachidonoyl-CoA, Ma-CoA: malonyl-CoA, Hx-CoA: hexanoyl-CoA, De-CoA: decanoyl-CoA, Oc-CoA: octanoyl-CoA, HMG-CoA: hydroxymethylgluteryl-CoA, PABA: *p*-aminobenzoic acid, 5ASA: 5-aminosalicylic acid. Codes (A-G) correspond to inhibitors shown in Table S3.

A



B

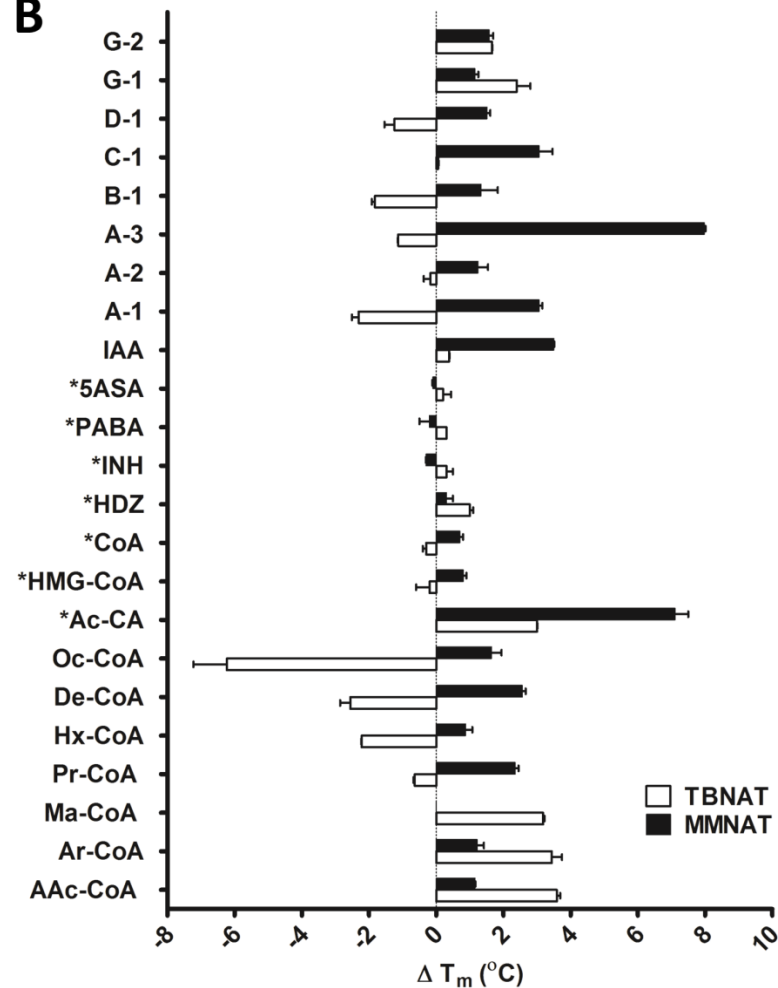


Figure S5. (A) The MMNAT crystal used for cross micro-seeding and the TBNAT crystal. MMNAT crystals grew in condition C10 of the PACT screen (see Methods). (B) A single TBNAT crystal grew in condition A4 of JCSG-plus screen after a week (0.02 M CaCl_2 , 0.1 M Na-acetate pH 4.6 and 30 % v/v MPD).

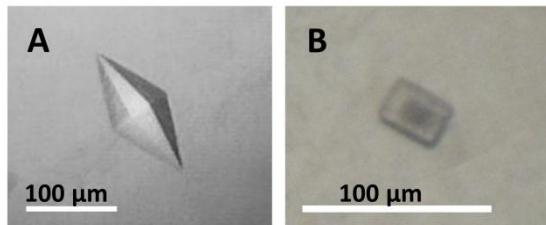


Figure S6: Amino acid sequence alignment of MMNAT and TBNAT.

The sequence alignment of NATs from *M. tuberculosis* (TBNAT) and *M. marinum* (MMNAT) was generated by using ClustalW2 (Thompson *et al.*, 1994). Residues strictly conserved have a red background, and similar residues are indicated by black bold letters with a yellow background according to a Risler matrix implemented in ESPript (Gouet *et al.*, 1999). The symbols above the sequence correspond to the secondary structure of TBNAT (PDB code 2VFB) and PL refers to the P-loop. The three domains of the MMNAT structure are indicated by the bars below the sequence alignment. Domain I is represented by the black bar, domain II is represented by the grey bar and domain III is represented by the light grey bar. The green box highlights the flexible loop observed in both structures. The figure was prepared using ESPript 2.2 (Gouet *et al.*, 1999).

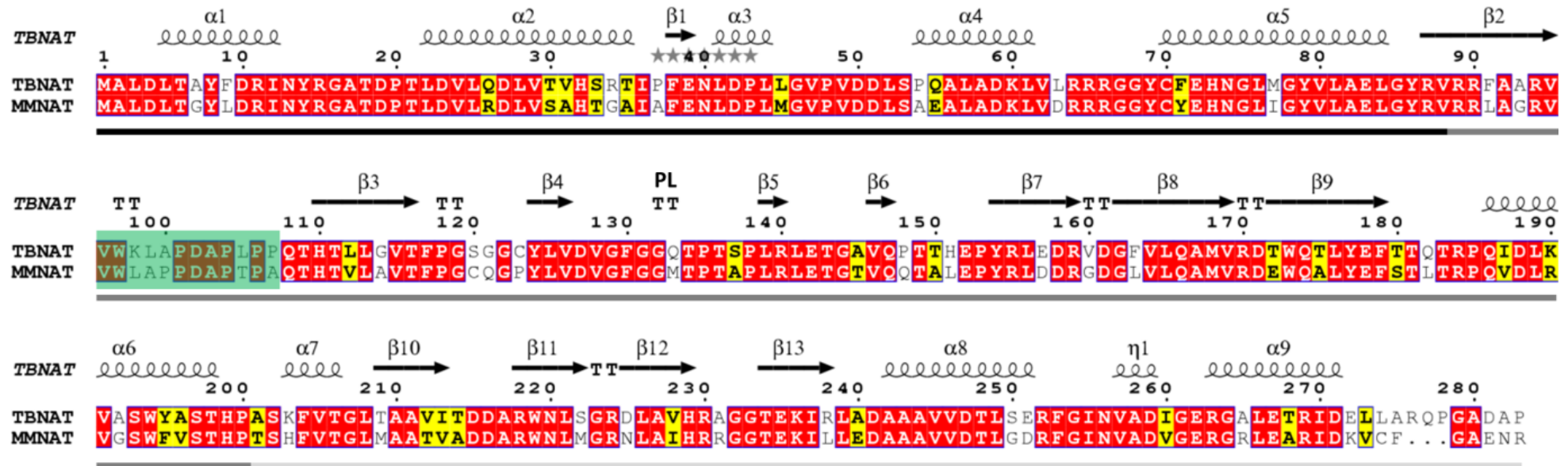


Table S1: List of commercially available crystallisation sparse matrix screens all used in this study.^a

Screen name	Supplier	Number of conditions	Description
Crystal screen I	Hampton Research	50	Broad-based sparse matrix
Crystal screen II	Hampton Research	48	Broad-based sparse matrix
Wizard I+II	Emerald Biostructures	96	Random sparse matrices
JCSG-plus	Molecular Dimensions	96	Very broad sparse matrix
Index	Hampton Research	96	Primary, diverse reagent
PEG/Ion II	Hampton Research	48	PEG, salt and pH matrix
Crystal Screen Lite	Hampton Research	48	Primary for membrane/limited solubility proteins
PACT premier	Molecular Dimensions	96	Systematic multi-component screen in three parts, screening PEG precipitants against: pH, cations and anions.
Morpheus	Molecular Dimensions	96	Low molecular weight ligands
Structure Screen I+II	Molecular Dimensions	96	Broad-based sparse matrix
PGA	Molecular Dimensions	96	Systematic screen based on the poly- γ -glutamic acid polymer
ProPlex	Molecular Dimensions	96	Medium-high MW PEG precipitants at relatively low concentrations
MIDAS	Molecular Dimensions	96	Based around alternative polymeric precipitants
Total number of conditions		1058	

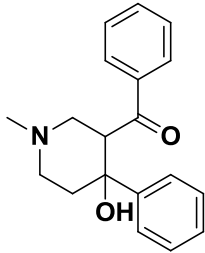
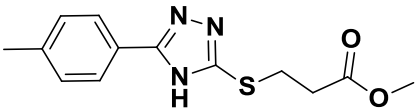
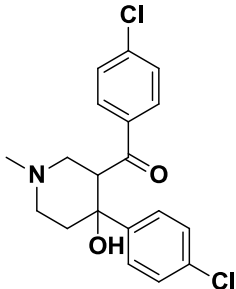
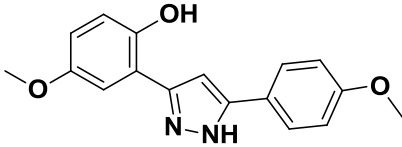
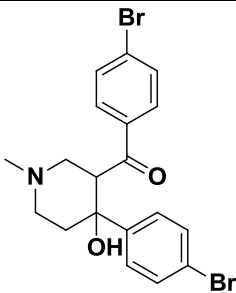
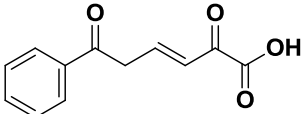
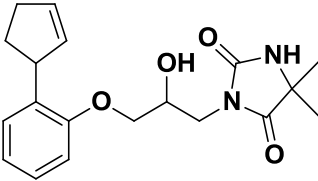
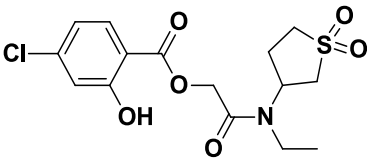
^a MW: molecular weight, PEG: polyethylene glycol.

Table S2: Summary of the initial conditions used to screen for suitable crystallisation conditions for TBNAT^a.

Screens Protein Solution	JCSG-plus	PACT	INDEX	PEG/Ion	St. S. I+II^b	Wizard I+II	Morpheus	Cr. S. I+II^c	Cr. S. Lite^d
5 mg/mL Tris-HCl pH 8	4 °C	---	---	---	---	---	---	---	---
	20 °C	---	---	---	---	---	---	---	---
8-11 mg/mL Tris-HCl pH 8	4 °C	4 °C	4 °C	---	---	---	---	---	---
	20 °C	20 °C	20 °C	20 °C	20 °C	20 °C	20 °C	20 °C	20 °C
19 mg/mL Tris-HCl pH 8	20 °C	---	20 °C	20 °C	---	---	---	---	---

^aCrystallisation trials using different protein concentration-sparse matrix screen-temperature combinations were performed by the sitting-drop vapour-diffusion technique. In each screen, an equal amount (100 nL) of freshly prepared TBNAT protein and precipitant were mixed and equilibrated against 100 µL reservoir at either 4 °C or 20 °C. ^bSt. S. I+II is Structure Screen I + II. ^cCr. S. I+II is Crystal Screen I + II. ^dCr. S lite is Crystal Screen Lite.

Table S3: Structural classes of NAT inhibitors used as additives in the TSAs and crystallisation trials.

Code	Structure	Code	Structure
A-1		C-1	
A-2		D-1	
A-3		G-1	
B-1		G-2	

(Abuhammad, D.Phil. thesis, University of Oxford, 2013)

Table S4: Additives added to the protein crystallisation solutions.

Class	Compounds	Concentration (mM)	Protein (10 mg/mL)
Substrates	HLZ	20	TBNAT
Inhibitor	A-1	5	TBNAT
	A-3	5	TBNAT
	G-1	5	TBNAT
	G-2	5	TBNAT
	IAA	5	TBNAT
	CoA	1	TBNAT
Cofactors	CoA	10	TBNAT
	CoA:HLZ (1:1)	20	His-TBNAT
	AAc-CoA	1	TBNAT
	Ma-CoA	1	TBNAT
	MO-CoA	20	His-TBNAT
	MN-CoA	20	His-TBNAT
	MMN-CoA	20	His-TBNAT
	OGP	1	TBNAT
	DTT	1	TBNAT/His-TBNAT
Stabilisers	NaN ₃	1	TBNAT/His-TBNAT
	MSG	50	His-TBNAT
	L-Histidine	50	His-TBNAT
	Arginien:glutamate (1:1)	50	His-TBNAT
Starting buffer	MES	20 / pH 6.5	His-TBNAT
	PIPES	20 / pH 7	His-TBNAT
	HEPES	20 / pH 7.5	His-TBNAT

MSG: 2-O-b-mannosylglycerate, MO-CoA: malonyl-oxo(dethia)-CoA, MN-CoA: malonyl-aza(dethia)-CoA, MMN-CoA: methylmalonyl-aza(dethia)-CoA. The codes of the inhibitors correspond to compounds in Table S3.