

Supplementary Materials

Figure S1 – Structures for each substrate tested, with specific activity shown below ($\text{nmol}\cdot\text{min}^{-1}\cdot\text{mg}^{-1}$).

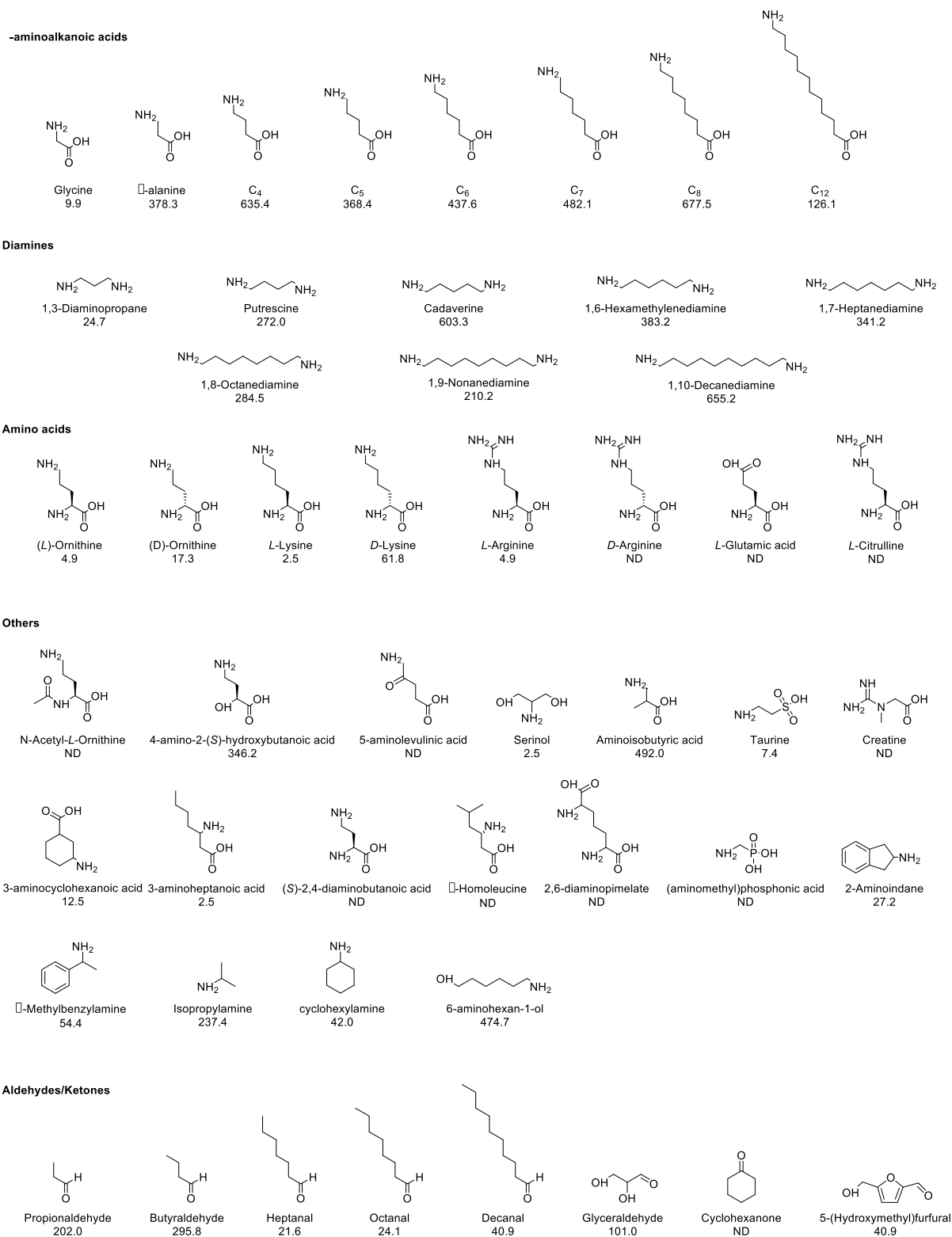


Figure S2 – Chain length vs. Specific Activity. SD shown as error bars.

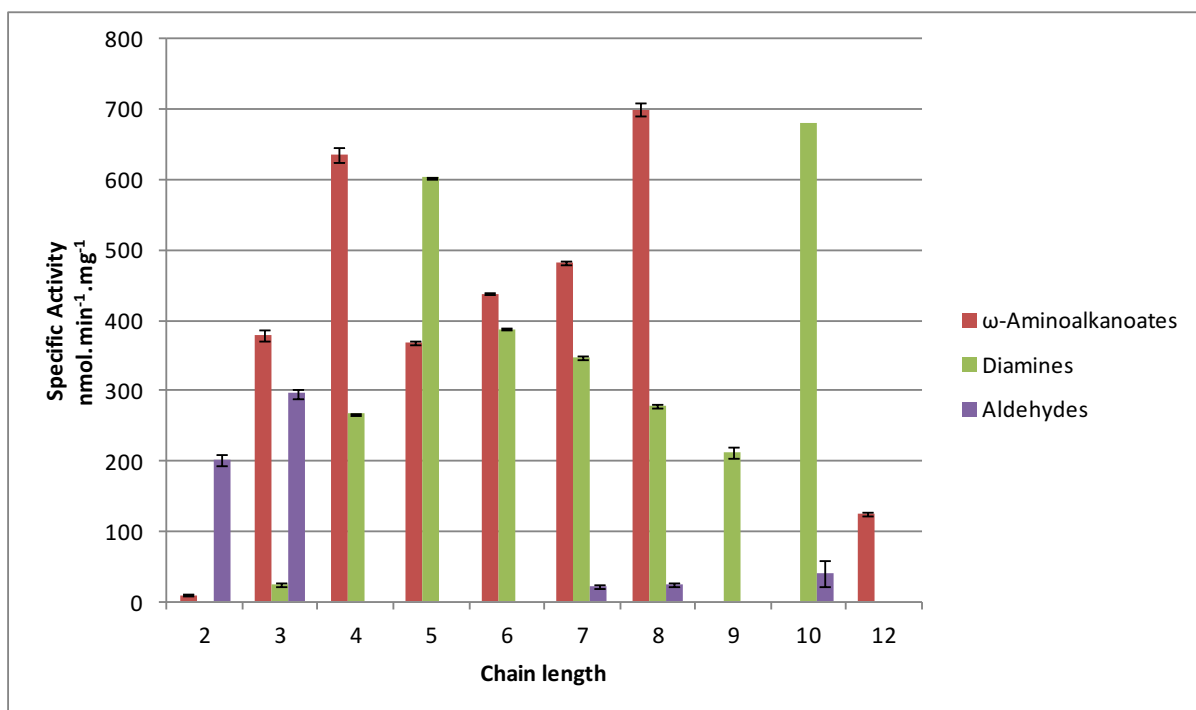


Figure S3 – KES23458 Gel Filtration Trace

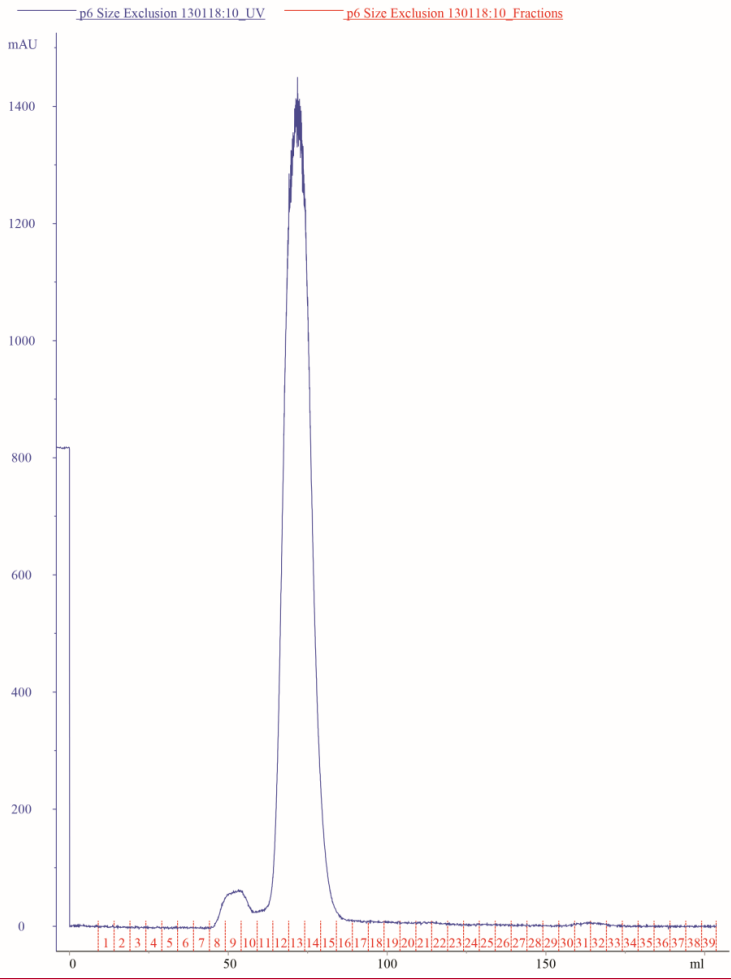


Figure S4 (a) – KES23458 thermotolerance by residual activity. Activity was measured using a standard assay as described in the Experimental section and the data was fitted to a sigmoidal curve with equation $f(T) = 100/(1 + e^{(T-T_{50})})$ where T is the temperature and $f(T)$ is the residual activity (%) at a given T.

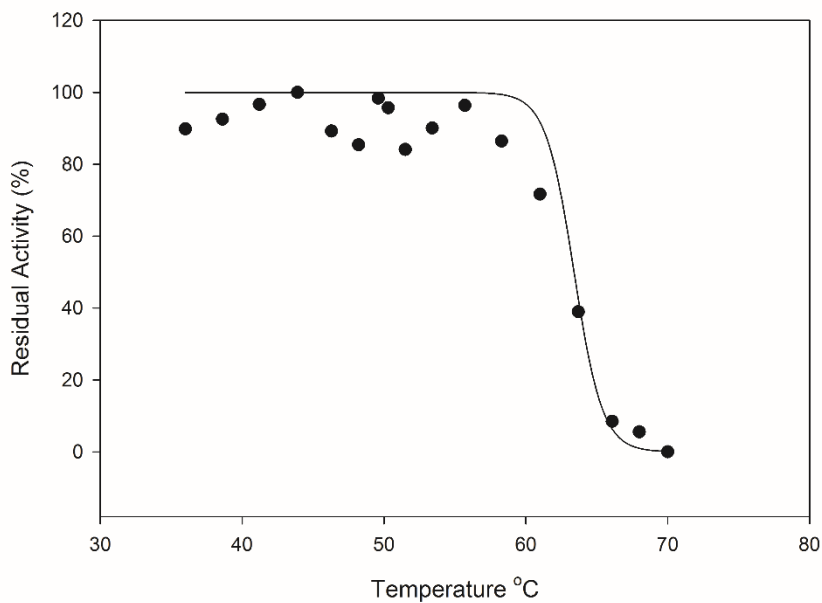


Figure S4 (b) – Triplicate replicates of a Differential Scanning Fluorimetry assay of KES23458 in 10 mM phosphate buffer. T_m is defined as the inflection point of the sigmoidal transition, and was measured to be 58.1 °C.

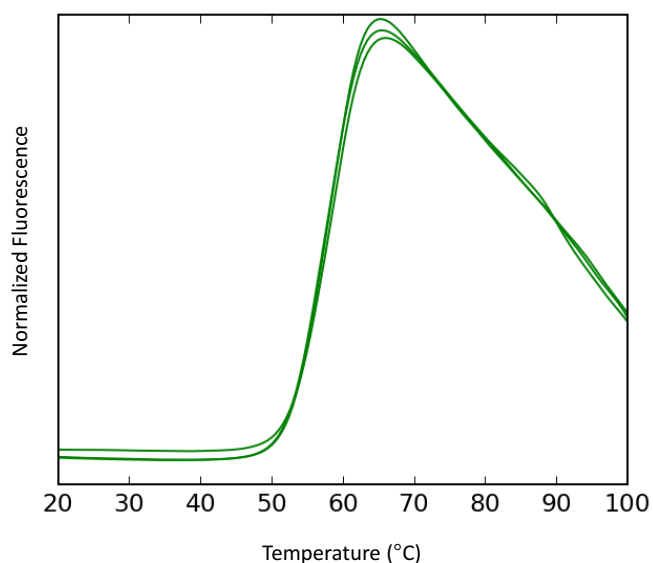


Figure S4 (c) – Triplicate replicates of a Differential Scanning Fluorimetry assay of KES23458 in a range of buffers, salts and pHs. The red dashed line is at 58 degrees (T_m in the original phosphate buffer).

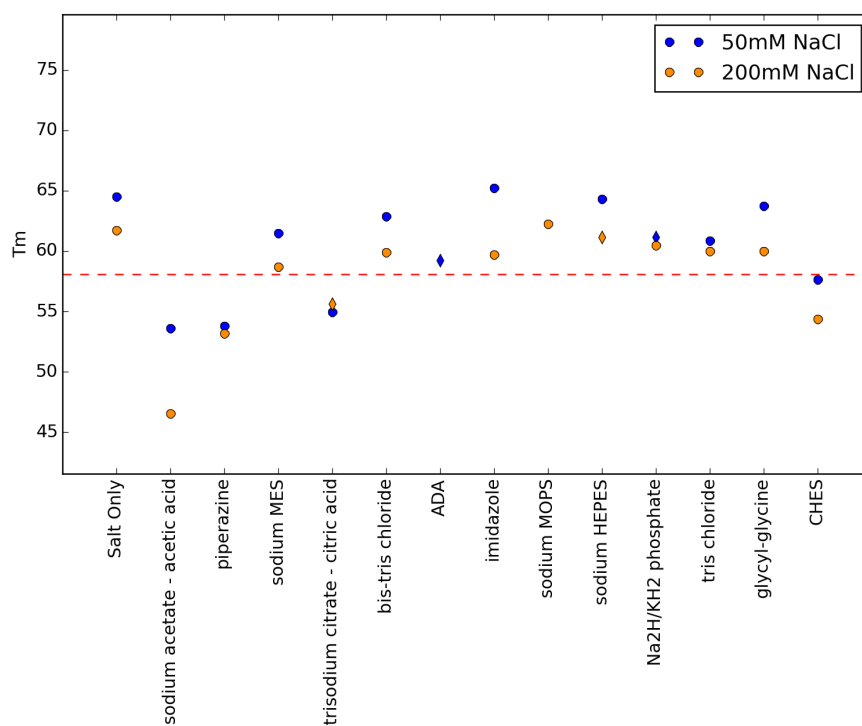


Figure S5– TA Crystal

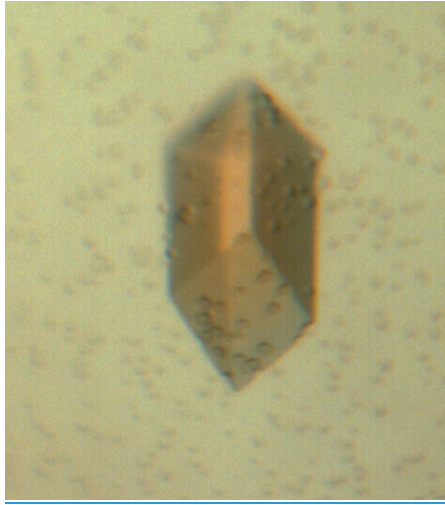
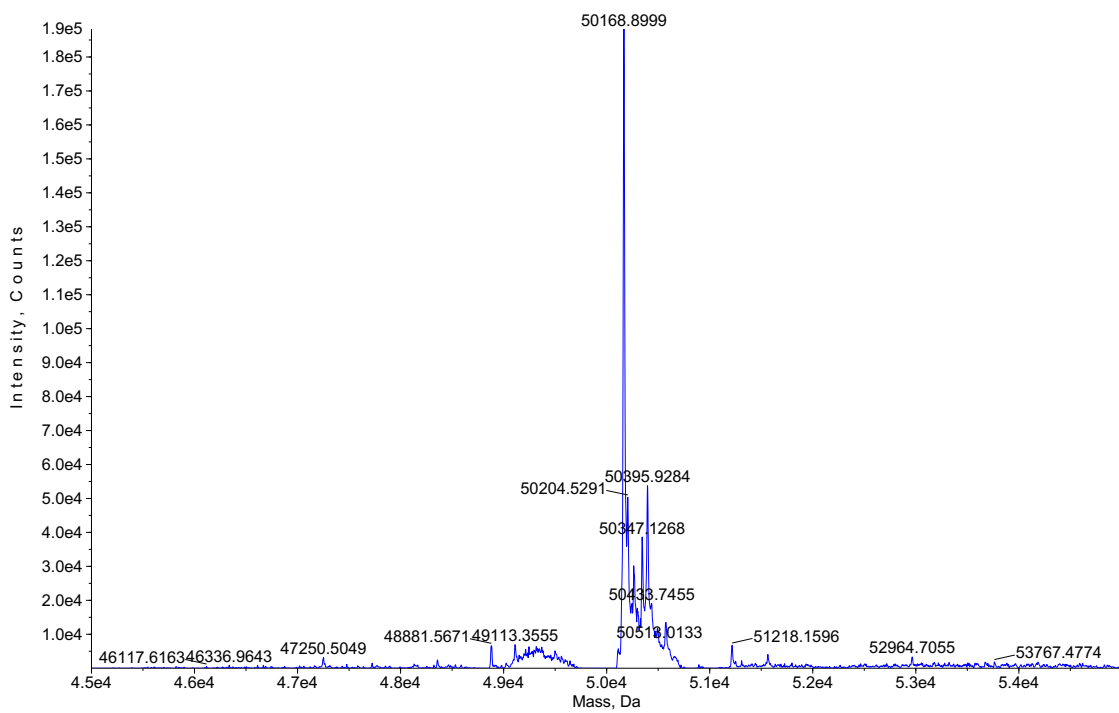


Figure S6 – Mass spectrometry data. Top: Untreated KES23458. Bottom: KES23458 treated with NaBH₄. m/z – 50168 – [apoprotein]⁺. 50397 – [Holoprotein]⁺. 50399 – [Holoprotein +2H]⁺.



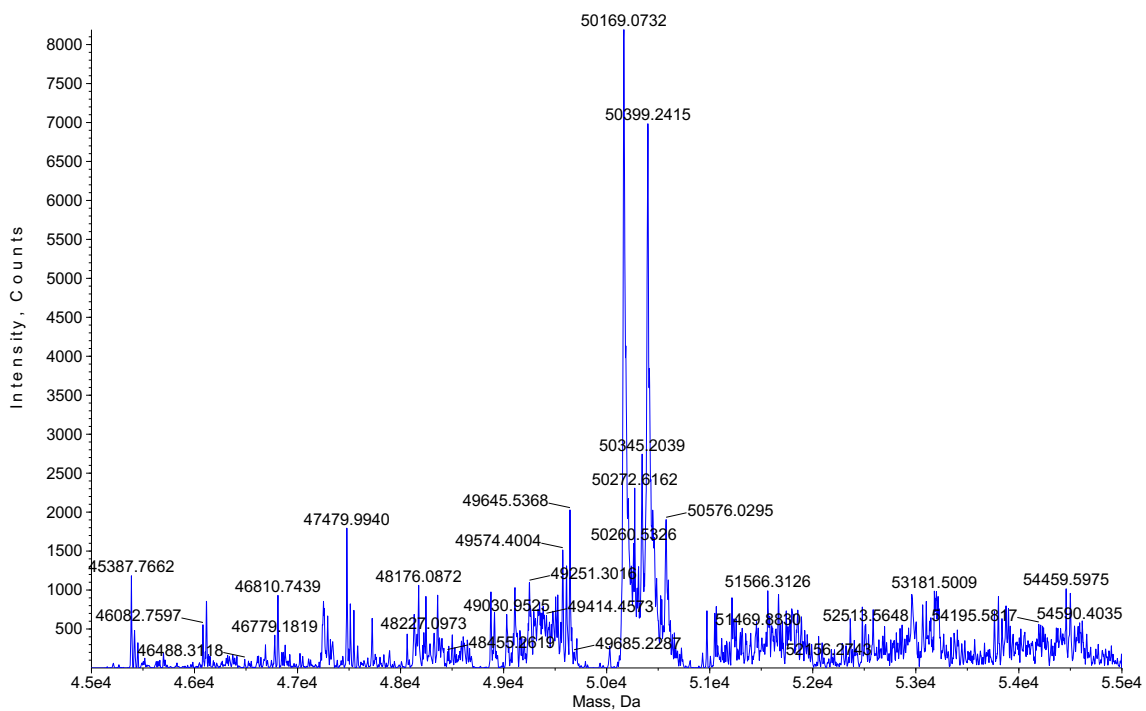


Figure S7 – RMSD analysis on KES23458 with β -alanine and 12-aminododecanoic acid docked.

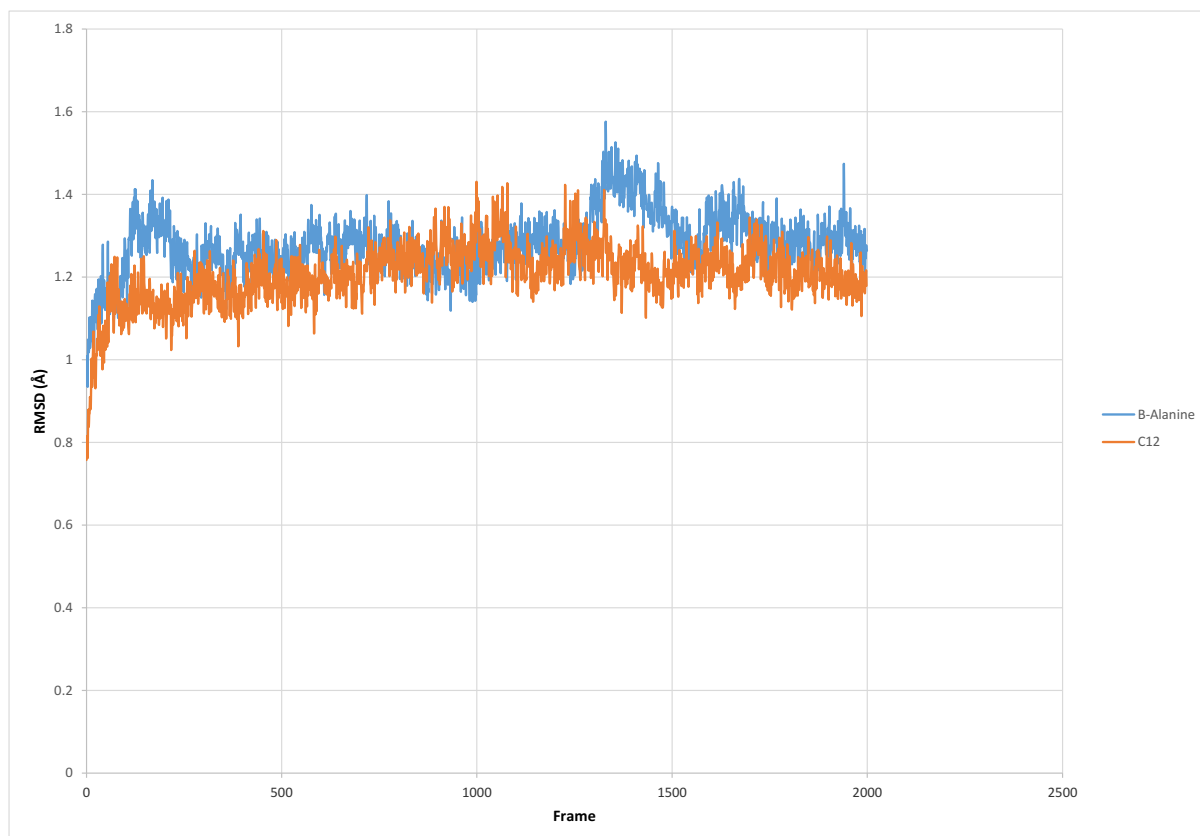


Table S1

Specific activities and relative substrate activities for KES23460.

Substrate	SA (nmol min ⁻¹ mg ⁻¹)	Relative Activity (%)
Glycine	N.D.	N.D.
B-Alanine	535.0 ± 31.3	100
D/L-Aminoisobutyric acid	15.8 ± 0.1	3.0
4-Aminobutyric acid	33.2 ± 1.0	6.1
5-Aminopentanoic acid	5.2 ± 0.3	0.7
6-Aminohexanoic acid	4.7 ± 0.6	0.6
1,3-Diaminopropane	1.4 ± 1.7	0.2
1,6-Hexamethylenediamine	4.9 ± 0.1	1.0
1,7-Heptanediamine	4.5 ± 0.4	0.8
1,8-Octanediamine	3.5 ± 0.6	0.6
1,9-Nonanediamine	4.2 ± 0.4	0.7
1,10-Decanediamine	N.D.	N.D.

Note that in each case the acceptor used was pyruvate. Assay conditions as described in Experimental section in potassium phosphate buffer (100 mM, pH 9). ND – Activity not determined

KES23458 Peptide sequence including N-terminal His₆-Tag

MGSSHHHHHSSGLVPRGSHMNQQVNVAPSAADLNLKAHWMPFSANRNFHKDPRIIVAAEGSWL
VDDKGRRIYDSLGLWTCGAGHSRKEIADAVAKQIGTLDYSPGFQYGHPLSFQLAEKIAQMTPGTLT
HVFFTGSSECADTSIKMARAYWRIKQQAQKTKLIGRARGYHGVNVAGTSLGGIGGNRKMFGPLMD
VDHLPHTLQPGMAFTKGAETGGVELANELLKLIELHDASNIAAVIVEPMSGSGAGVIVPPKGYLQRLRE
ICDANDILLIFDEVITAFGRMGKATGAEYFGVTPDIMNVAKQVTNGAVPMGAVIASSEIYDTFMNQNL
EYAVEFGHGYTSAHPVACAAGIAALDLLQKENLIQQSAELAPHFEKALHGLKGTKNVIDIRNCGLAGA
IQIAARDGDAIVRPFEASMKLWKEGFYVRFGGDTLQFGPTFNAKPEDLDRLFDAVGEALNGVA

KES23460 Peptide sequence including N-terminal His₆-Tag

MRGSHHHHHHGMASMTGGQQMGRDLYDDDDKGSMTLLIKHLIGGELIADTGRADVFNPSTGEAV
RKVPLADRETMQQAIDAAKAAFPWRNTPPAKRAQVLFKQKLLANEERIVKLISEEHGKTIEDAAG
ELKRGIEVYATAAPEILKGEYSRNVGPNIDAWSDFQPIGVVAGITPFNFPAMVPLWMYPLAIACGNT
FILKPSERDPSSTLLIAELFHEAGLPKGVNLVHGDKGAVDALIEAPEVKALSFVGSSTPIAEYIYSEGTK

RGKRVQALGGAKNHAVLMPDADLDNAVSAALMGAAYGSCGERCMAISVAVCVGDQIADALVQKLVPO
IKGLKIGAGTSCGLDMGPLVTGAARDKVTGYIDTGVAQGAELVVDGRGYKVAGHENGFFLGGTLFDR
VTPEMTIYKEEIFGPVLCIVRVNSLEEAMQLINDHEYGNGTICIFTRDGEAARLFCDEIEVGMVGVNVPL
PVPVAYHSFGGWKRSLFGDLHAYGPDGVRFYTKRKAITQRWPQRKSHEAAQFAFPSNS