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Supporting information for article:

Crystal structure of fungal tannase from *Aspergillus niger*

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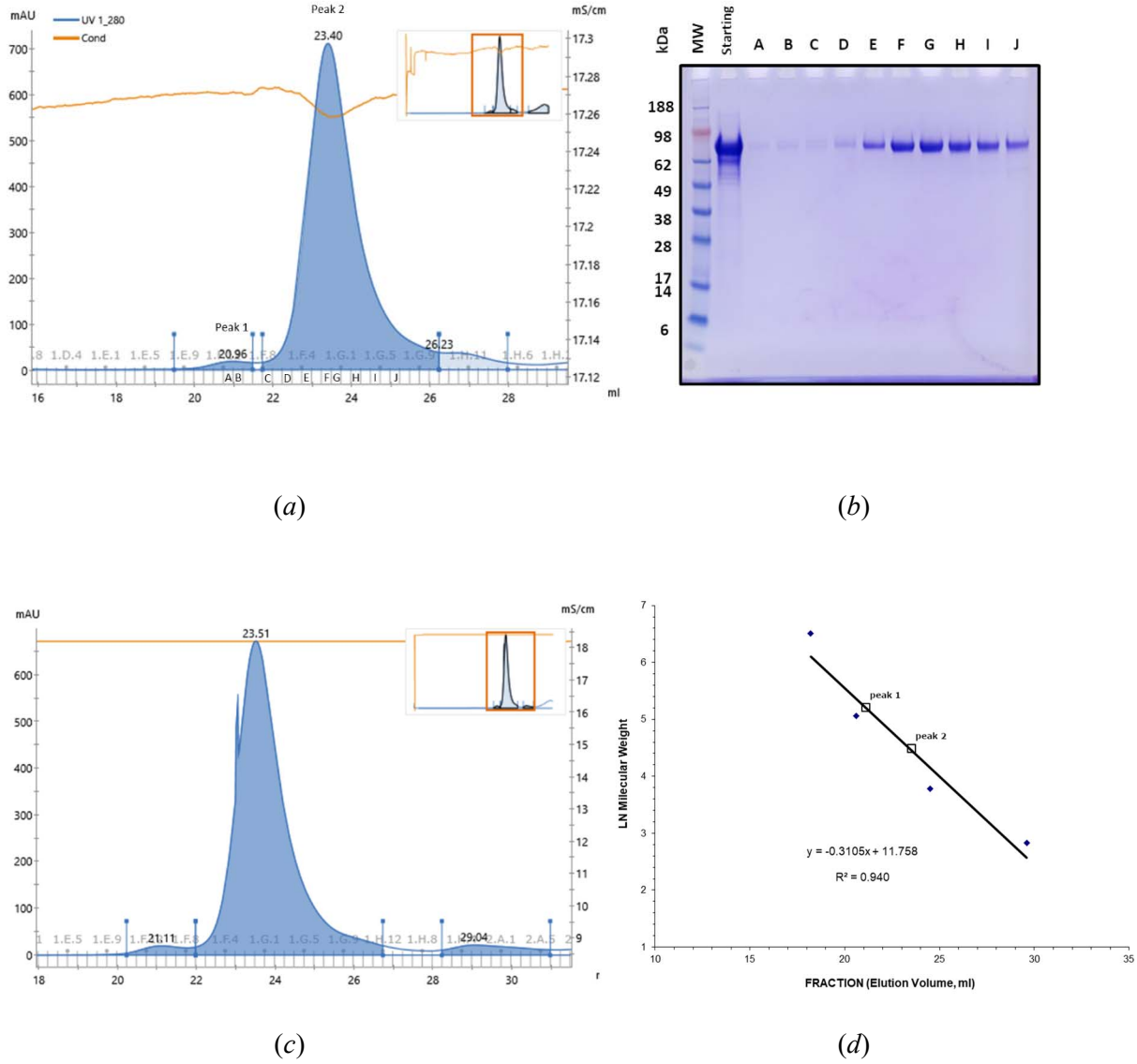
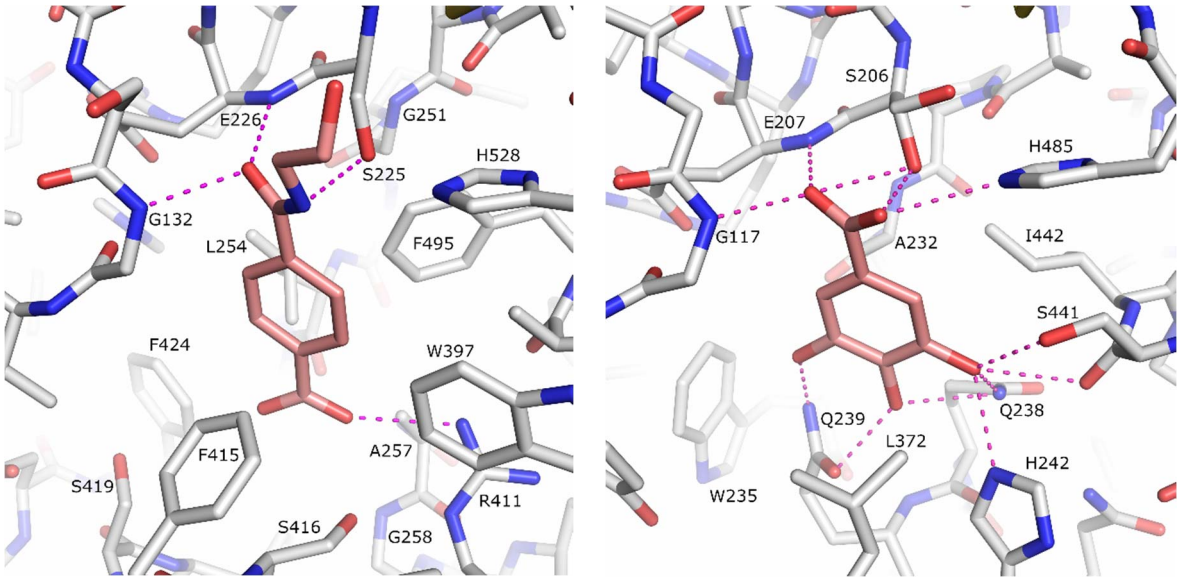


(*a*) (*b*)

(*c*) (*d*)

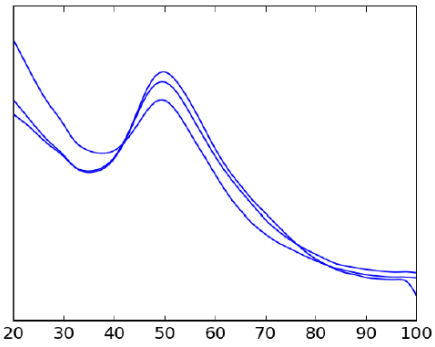
Figure S1 Size exclusion chromatography analysis of An-Tan. (*a*) The UV chromatogram of An-Tan with PBS buffer at pH 7.4. (*b*) SDS-PAGE analysis of the samples in (*a*). (*c*) The UV chromatogram of An-Tan with PIPES-NaOH buffer at pH 5.8. (*d*) Calibration curve of the column in (*a*). The molecular-mass markers are depicted as filled squares, including bovine thyroglobulin (670 kDa), bovine-globulin Supporting information (158 kDa), chicken ovalbumin (44 kDa) and horse myoglobin (17 kDa). The positions corresponding to peaks 1 and 2 in (*a*) are shown as open squares, indicating the existence of both monomeric and dimeric form in solution.



(*a*) (*b*)

Figure S2 **S**ubstrate binding site comparison of (*a*) *Ideonella sakaiensis* MHETase (Palm *et al.*, 2019) and (*b*) An-Tan. The carbon atoms of the substrate analogue **(**4-(2-hydroxyethylcarbamoyl) benzoic acid) in (*a*) and gallic acid in (*b*) are coloured in brown. Hydrogen bonding interactions are shown as broken lines.

Supplementary DSF analysis S1. MELTDOWN (Liu *et al.*, 2017) analysis of the formulation buffer screening on crude An-Tan.



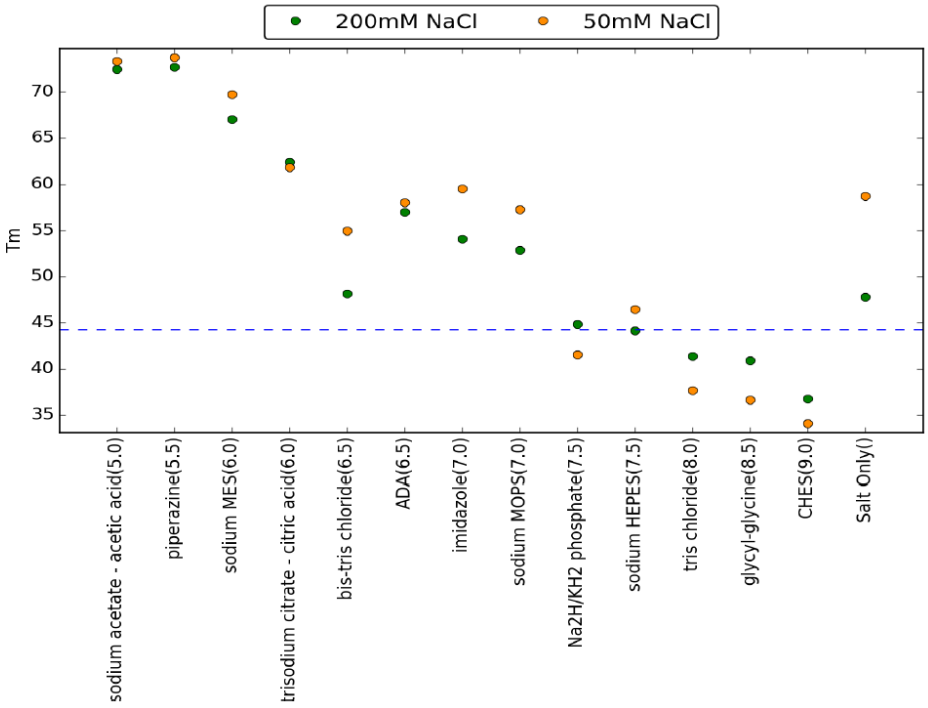
Full interpretation of the results requires you to look at the individual melt curves.

Curves used in Tm estimations (ideally 100%): **92%**

Average estimation error: **1.2°C**

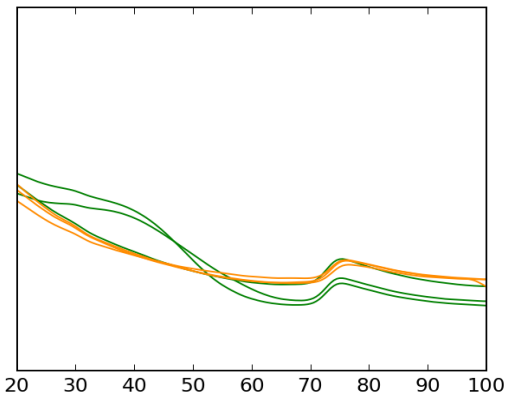
Protein as supplied:
Tm = 44.29(+/-0.52)

Lysozyme Control: Passed
No Dye Control: Passed
No Protein Control: Passed



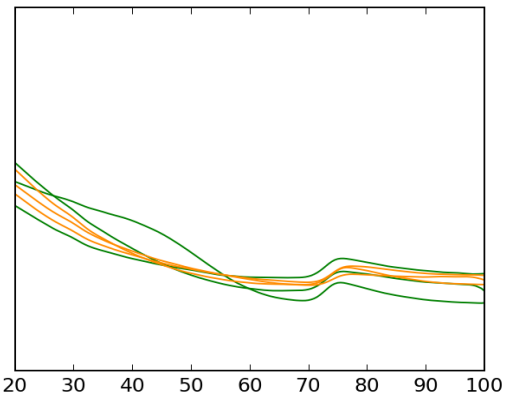
Highest Tm = 73.7 +/- 0.03
(piperazine / 50mM NaCl)

Sample supplied by customer, results apply to sample as received.



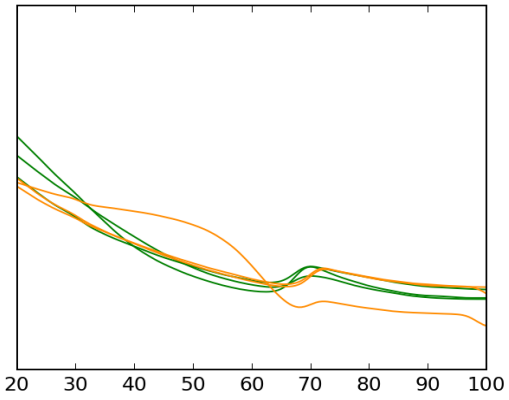
sodium acetate - acetic acid (5.0)

Grouped by	Tm
200mM NaCl	72.44 (+/-0.02)
50mM NaCl	73.31 (+/-0.1)



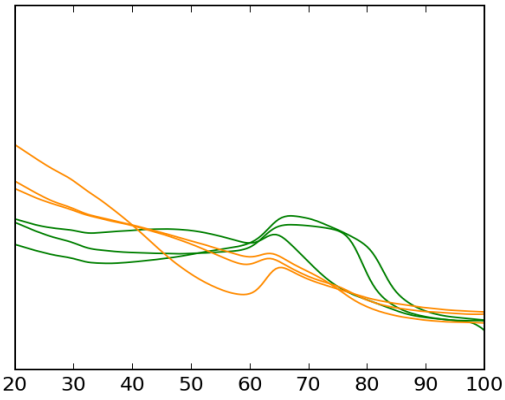
piperazine (5.5)

Grouped by	Tm	Adjusted pH at Tm
200mM NaCl	72.67 (+/-0.03)	4.54
50mM NaCl	73.7 (+/-0.03)	4.52



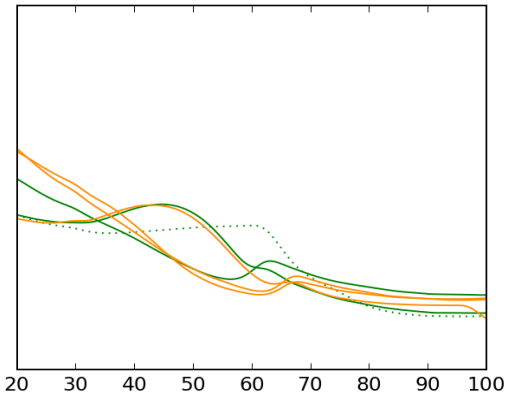
sodium MES (6.0)

Grouped by	Tm	Adjusted pH at Tm
200mM NaCl	67.03 (+/-0.08)	5.64
50mM NaCl	69.75 (+/-0.09)	5.61



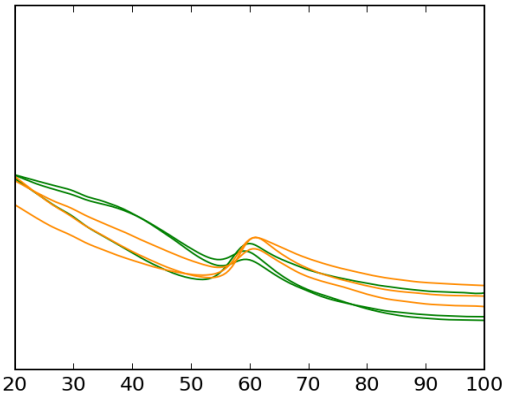
trisodium citrate - citric acid (6.0)

Grouped by	Tm	Adjusted pH at Tm
200mM NaCl	62.42 (+/-0.37)	6.17
50mM NaCl	61.82 (+/-0.49)	6.16



bis-tris chloride (6.5)

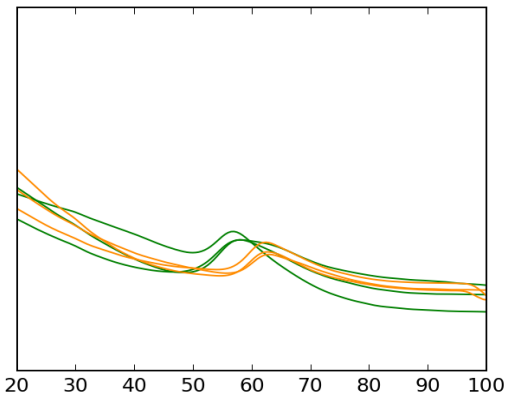
Grouped by	Tm	Adjusted pH at Tm
200mM NaCl	48.13 (+/-12.11)	6.13
50mM NaCl	54.93 (+/-14.12)	6.05



ADA (6.5)

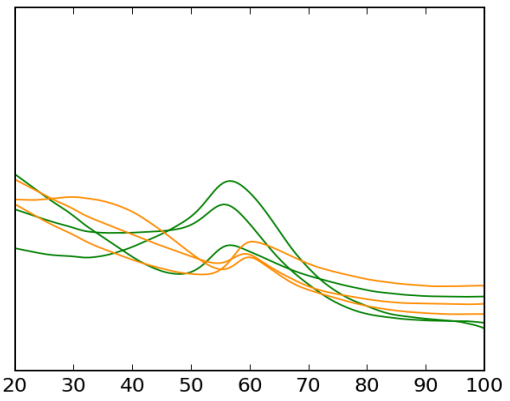
Grouped by	Tm	Adjusted pH at Tm
200mM NaCl	56.97 (+/-0.16)	6.37
50mM NaCl	58.0 (+/-0.08)	6.37

Monotonic, saturated, in the noise, and outlier curves are dotted, and excluded from Tm calculations
Curves drawn with dashed lines have unreliable Tm estimates



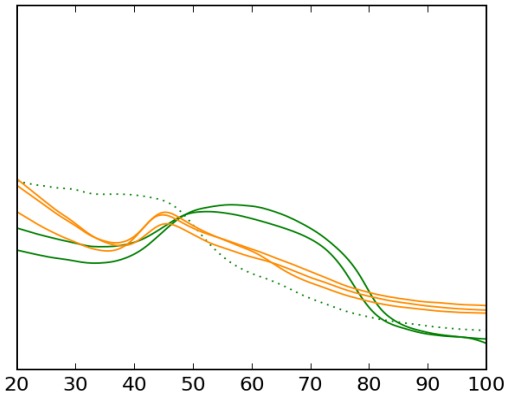
imidazole (7.0)

Grouped by	Tm	Adjusted pH at Tm
200mM NaCl	54.06 (+/-0.18)	6.42
50mM NaCl	59.53 (+/-0.26)	6.33



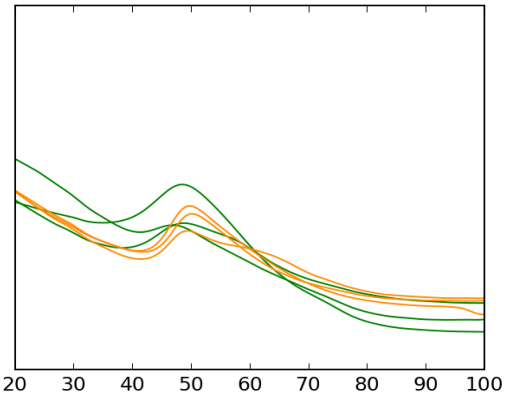
sodium MOPS (7.0)

Grouped by	Tm	Adjusted pH at Tm
200mM NaCl	52.84 (+/-0.31)	6.73
50mM NaCl	57.25 (+/-0.29)	6.69



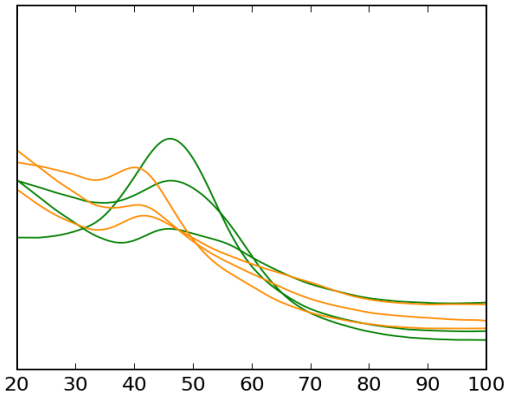
Na2H/KH2 phosphate (7.5)

Grouped by	Tm	Adjusted pH at Tm
200mM NaCl	44.86 (+/-0.38)	7.64
50mM NaCl	41.55 (+/-0.39)	7.62



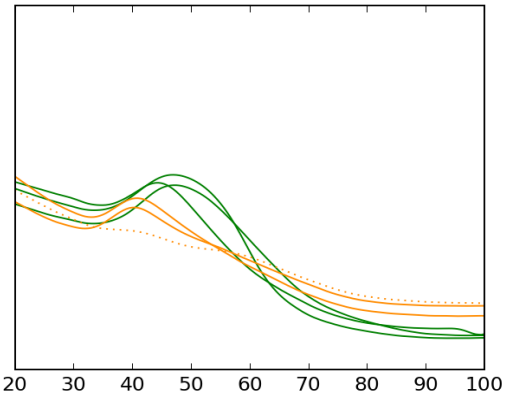
sodium HEPES (7.5)

Grouped by	Tm	Adjusted pH at Tm
200mM NaCl	44.14 (+/-0.13)	7.36
50mM NaCl	46.47 (+/-0.28)	7.34



tris chloride (8.0)

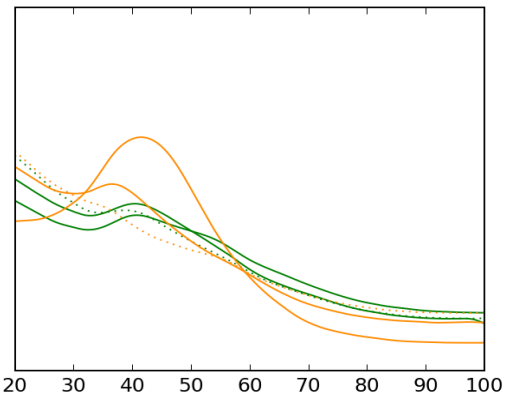
Grouped by	Tm	Adjusted pH at Tm
200mM NaCl	41.39 (+/-0.64)	7.57
50mM NaCl	37.7 (+/-0.63)	7.64

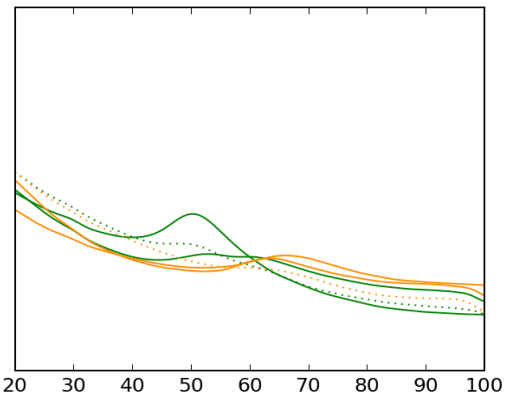


glycyl-glycine (8.5)

Grouped by	Tm	Adjusted pH at Tm
200mM NaCl	40.91 (+/-0.33)	8.15
50mM NaCl	36.64 (+/-0.27)	8.22

Monotonic, saturated, in the noise, and outlier curves are dotted, and excluded from Tm calculations
Curves drawn with dashed lines have unreliable Tm estimates





CHES (9.0)

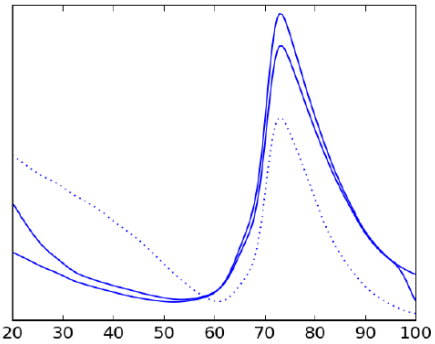
Grouped by	Tm	Adjusted pH at Tm
200mM NaCl	36.75 (+/-0.13)	8.8
50mM NaCl	34.12 (+/-0.51)	8.83

Salt Only ()

Grouped by	Tm
200mM NaCl	47.78 (+/-1.27)
50mM NaCl	58.67 (+/-0.75)

Monotonic, saturated, in the noise, and outlier curves are dotted, and excluded from Tm calculations
Curves drawn with dashed lines have unreliable Tm estimates

Supplementary DSF analysis S2. MELTDOWN (Liu *et al.*, 2017) analysis of the formulation buffer screening on purified An-Tan.



Full interpretation of the results requires you to look at the individual melt curves.

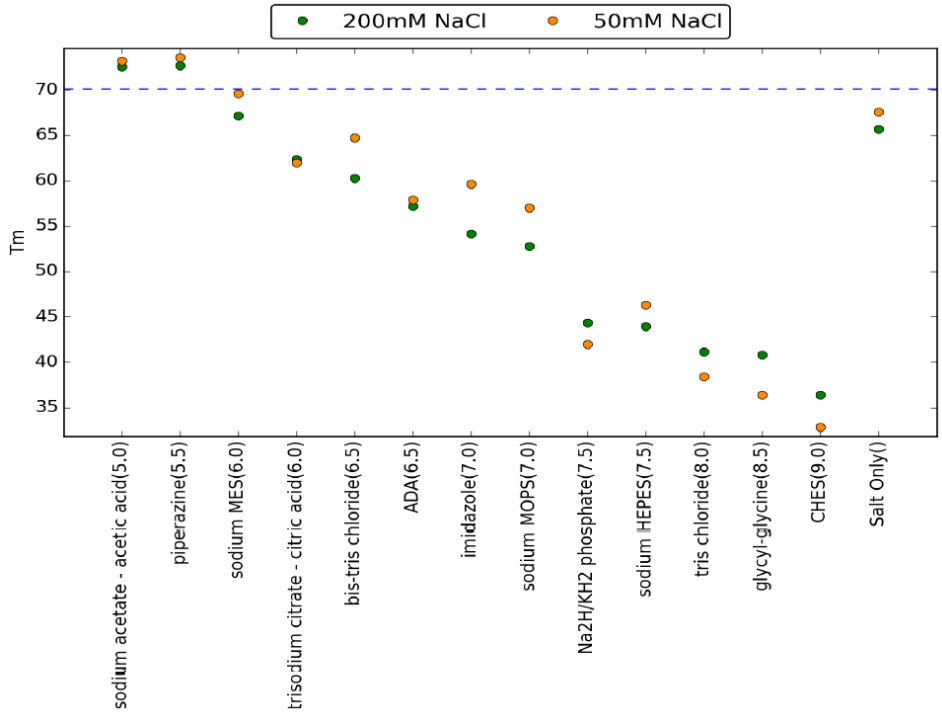
The summary graph appears to be unreliable

Curves used in Tm estimations (ideally 100%): **96%**

Average estimation error: **0.2°C**

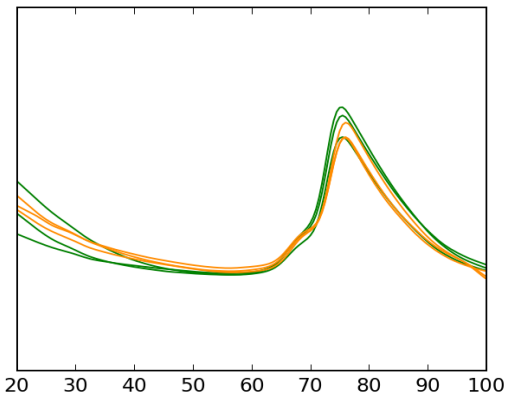
Protein as supplied:
Tm = 70.14(+/-0.02)

Lysozyme Control: Passed
No Dye Control: Passed
No Protein Control: Passed



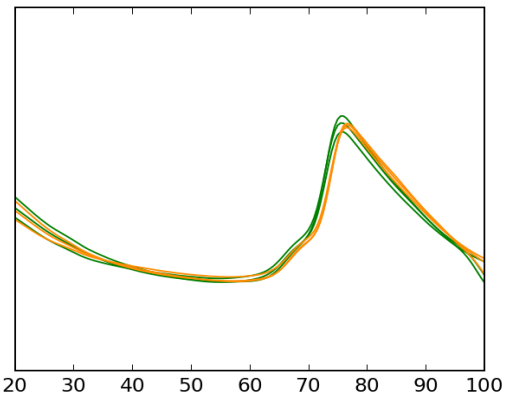
Highest Tm = 73.58 +/- 0.08
(piperazine / 50mM NaCl)

Sample supplied by customer, results apply to sample as received.



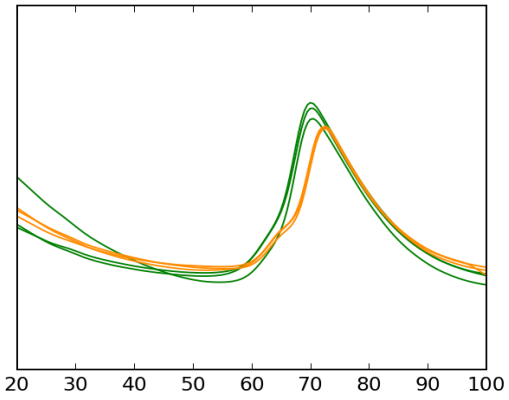
sodium acetate - acetic acid (5.0)

Grouped by	Tm
200mM NaCl	72.55 (+/-0.07)
50mM NaCl	73.23 (+/-0.02)



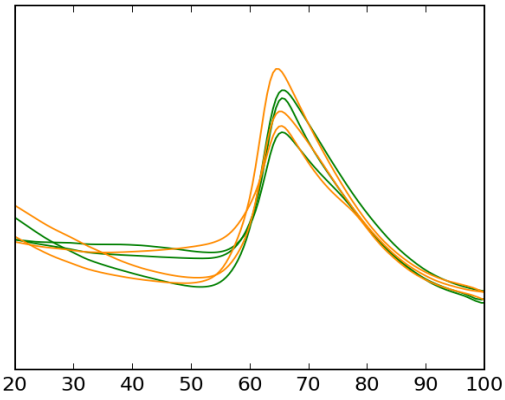
piperazine (5.5)

Grouped by	Tm	Adjusted pH at Tm
200mM NaCl	72.67 (+/-0.05)	4.54
50mM NaCl	73.58 (+/-0.08)	4.52



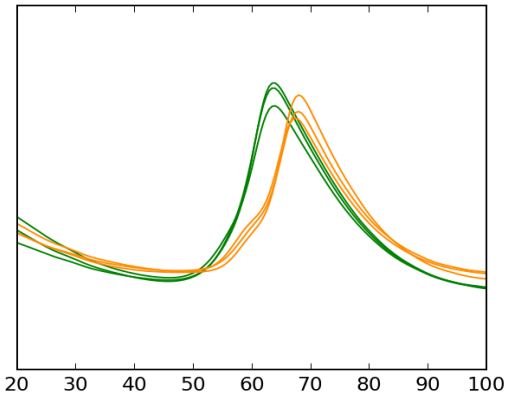
sodium MES (6.0)

Grouped by	Tm	Adjusted pH at Tm
200mM NaCl	67.14 (+/-0.09)	5.63
50mM NaCl	69.57 (+/-0.13)	5.62



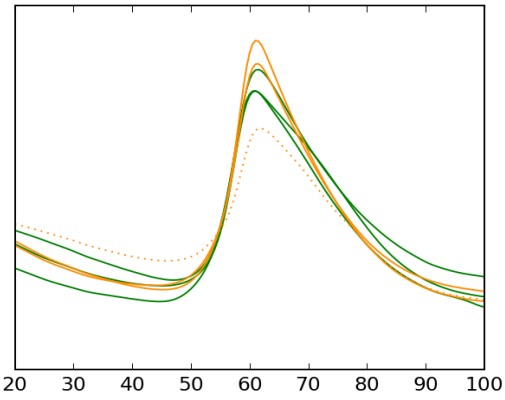
trisodium citrate - citric acid (6.0)

Grouped by	Tm	Adjusted pH at Tm
200mM NaCl	62.35 (+/-0.2)	6.17
50mM NaCl	61.96 (+/-0.37)	6.16



bis-tris chloride (6.5)

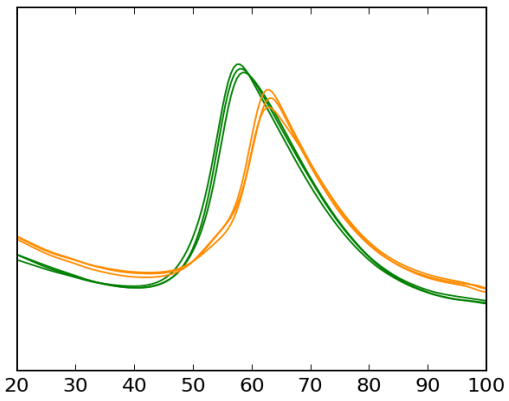
Grouped by	Tm	Adjusted pH at Tm
200mM NaCl	60.26 (+/-0.06)	5.98
50mM NaCl	64.73 (+/-0.21)	5.92



ADA (6.5)

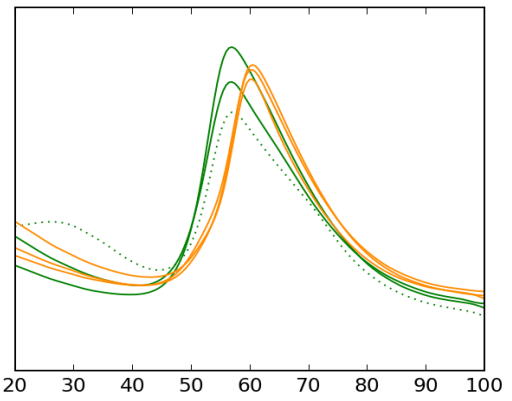
Grouped by	Tm	Adjusted pH at Tm
200mM NaCl	57.15 (+/-0.13)	6.37
50mM NaCl	57.91 (+/-0.0)	6.37

Monotonic, saturated, in the noise, and outlier curves are dotted, and excluded from Tm calculations
Curves drawn with dashed lines have unreliable Tm estimates



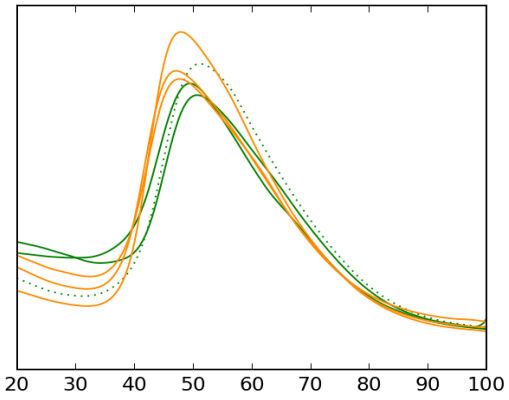
imidazole (7.0)

Grouped by	Tm	Adjusted pH at Tm
200mM NaCl	54.13 (+/-0.28)	6.42
50mM NaCl	59.64 (+/-0.24)	6.33



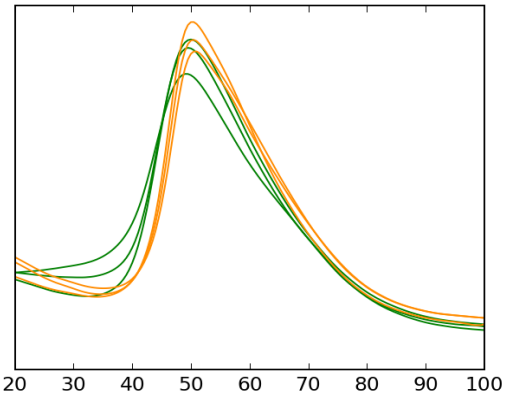
sodium MOPS (7.0)

Grouped by	Tm	Adjusted pH at Tm
200mM NaCl	52.78 (+/-0.01)	6.73
50mM NaCl	57.0 (+/-0.03)	6.69



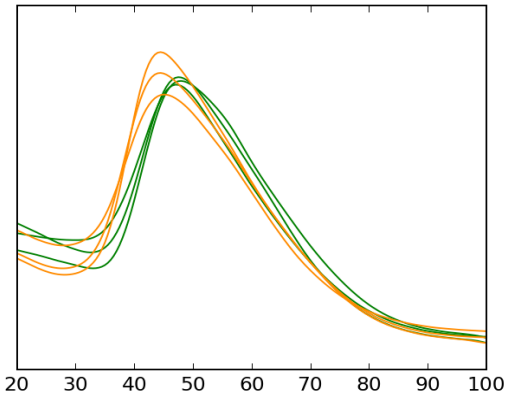
Na2H/KH2 phosphate (7.5)

Grouped by	Tm	Adjusted pH at Tm
200mM NaCl	44.35 (+/-0.53)	7.63
50mM NaCl	41.99 (+/-0.37)	7.62



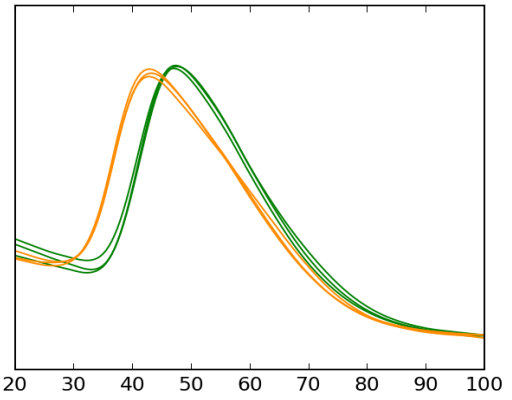
sodium HEPES (7.5)

Grouped by	Tm	Adjusted pH at Tm
200mM NaCl	43.93 (+/-0.21)	7.36
50mM NaCl	46.3 (+/-0.23)	7.34



tris chloride (8.0)

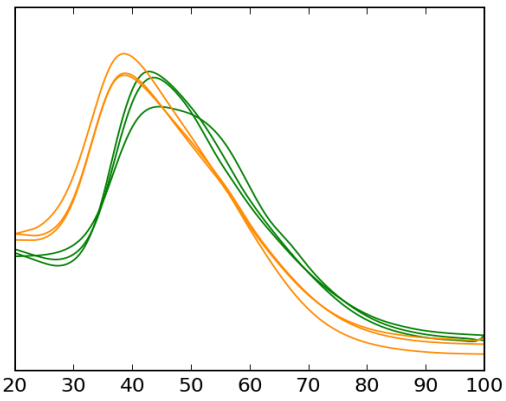
Grouped by	Tm	Adjusted pH at Tm
200mM NaCl	41.17 (+/-0.1)	7.57
50mM NaCl	38.44 (+/-0.25)	7.63

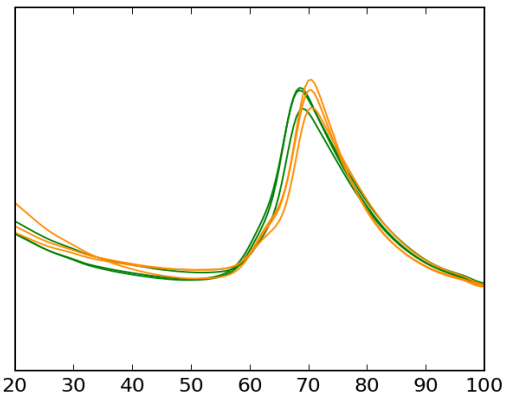


glycyl-glycine (8.5)

Grouped by	Tm	Adjusted pH at Tm
200mM NaCl	40.81 (+/-0.18)	8.15
50mM NaCl	36.39 (+/-0.06)	8.23

Monotonic, saturated, in the noise, and outlier curves are dotted, and excluded from Tm calculations
Curves drawn with dashed lines have unreliable Tm estimates





CHES (9.0)

Grouped by	Tm	Adjusted pH at Tm
200mM NaCl	36.37 (+/-0.16)	8.8
50mM NaCl	32.83 (+/-0.04)	8.85

Salt Only ()

Grouped by	Tm
200mM NaCl	65.65 (+/-0.24)
50mM NaCl	67.58 (+/-0.06)

Monotonic, saturated, in the noise, and outlier curves are dotted, and excluded from Tm calculations
Curves drawn with dashed lines have unreliable Tm estimates