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SUPPORTING INFORMATION

Structural and Spectroscopic Insight into Metal Binding Properties of *o*-Aminophenol-*N,N,O*-Triacetic Acid (APTRA) Chelator: Implications for Design of Metal Indicators

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S1. Supporting Figures and Tables

Table S1. Crystal intensity, collection and refinement data for APTRA complexes of Mg^{2+}, Ca^{2+}, Zn^{2+} and K^{+}.

	$[Mg(OH_2)_6][(APTRA)Mg]_2 \cdot 6(H_2O)(dioxane)$	$[Ca(OH_2)_3]_2\{[(APTRA)Ca\text{-}(OH_2)]_2(\mu\text{-}H_2O)_2\}Cl \cdot 2(H_2O)$	$[Zn(OH_2)_6][(APTRA)Zn]_2 \cdot 6(H_2O)(dioxane)$
Crystal Lattice	Monoclinic	Triclinic	Monoclinic
Formula	$C_{28}H_{52}Mg_3N_2O_{28}$	$C_{12}H_{22}Ca_2ClNO_{13}$	$C_{28}H_{52}N_2O_{28}Zn_3$
Formula weight	937.64	524.0	1060.89
Space group	$C2/c$	$P\bar{1}$	$P2_1/n$
a/Å	24.2955(12)	8.4079(5)	9.2960(3)
b/Å	9.4801(4)	11.2214(7)	18.4231(7)
c/Å	18.5395(9)	12.0301(8)	24.4820(9)
α/°	90	108.3734(10)	90
β/°	103.9470(10)	104.9899(10)	90.0021(6)
γ/°	90	96.6152(11)	90
V/Å^3	4144.2(3)	1016.26(11)	4192.8(3)
Z	4	2	4
Temperature (K)	100(2)	100(2)	100(2)
Radiation (λ, Å)	0.71073	0.71073	0.71073
ρ (calcd.), g cm^{-3}	1.503	1.647	1.681
μ (Mo Kα), mm^{-1}	0.174	0.758	1.802
θ max, deg.	28.287	28.283	28.305
Completeness to θ (%)	100	100	100
No. of data	9928	15949	10445
No. of parameters	314	298	612
No. of restraints	19	13	88
R_1 [I > 2σ(I)]	0.0399	0.0435	0.0428
wR_2[all refs]	0.0953	0.1057	0.1241
GOF [I > 2σ(I)]	1.025	1.020	1.053

Table S1 (contd). Crystal intensity, collection and refinement data for APTRA complexes of Mg^{2+}, Ca^{2+}, Zn^{2+} and K^{+}.

	$(H_2APTRA)K$
Crystal Lattice	Monoclinic
Formula	$C_{12}H_{12}NO_7K$
Formula weight	321.33
Space group	$P2_1/c$
a/Å	12.6083(7)
b/Å	12.3265(7)
c/Å	8.9860(5)
α/°	90
β/°	104.5060(10)
γ/°	90
V/Å^3	1352.05(13)
Z	4
Temperature (K)	100(2)
Radiation (λ, Å)	0.71073
ρ (calcd.), g cm^{-3}	1.579
μ (Mo Kα), mm^{-1}	0.427
θ max, deg.	28.39
Completeness to θ (%)	100
No. of data	3381
No. of parameters	201
No. of restraints	1
R_1 [I > 2σ(I)]	0.0309
wR_2 [all refs]	0.0748
GOF [I > 2σ(I)]	1.038

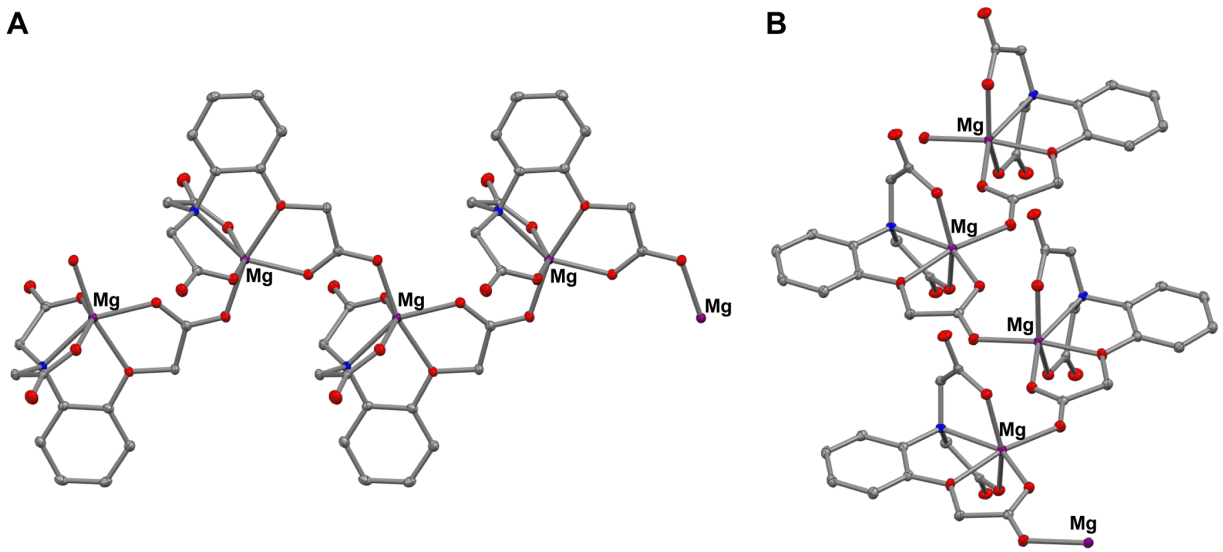

Figure S1. Crystal structure of $[Mg(OH_2)_6][(APTRA)Mg]_2$, showing the extended chain formed by carboxylate bridging of $[(APTRA)Mg]^-$ units in the solid state. Hydrogen atoms omitted for clarity. Thermal ellipsoids shown at 50% probability. (A) View down crystallographic *a* axis. (B) Alternate view.

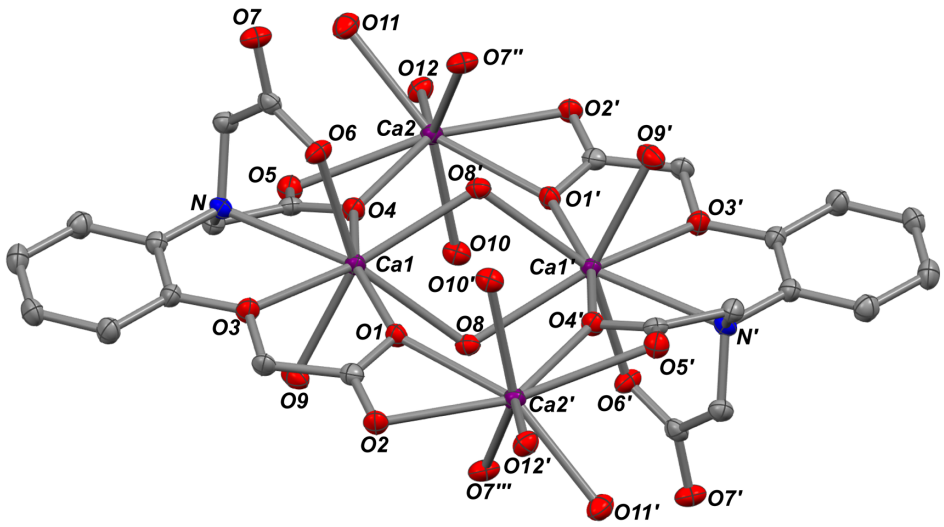

Figure S2. Crystal structure of $\{[Ca(OH_2)_3]_2\{[(APTRA)Ca(OH_2)]_2(\mu\text{-}H_2O)_2\}\}^{2-}$ anion. Hydrogen atoms omitted for clarity. Thermal ellipsoids shown at 50% probability.

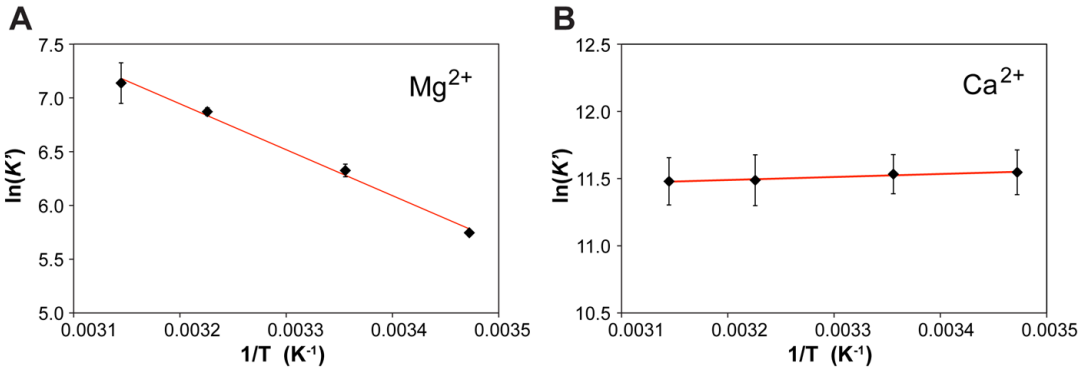

Figure S3. Van't Hoff plots for the binding of APTRA to (A) Mg^{2+}, and (B) Ca^{2+} at pH 7.0

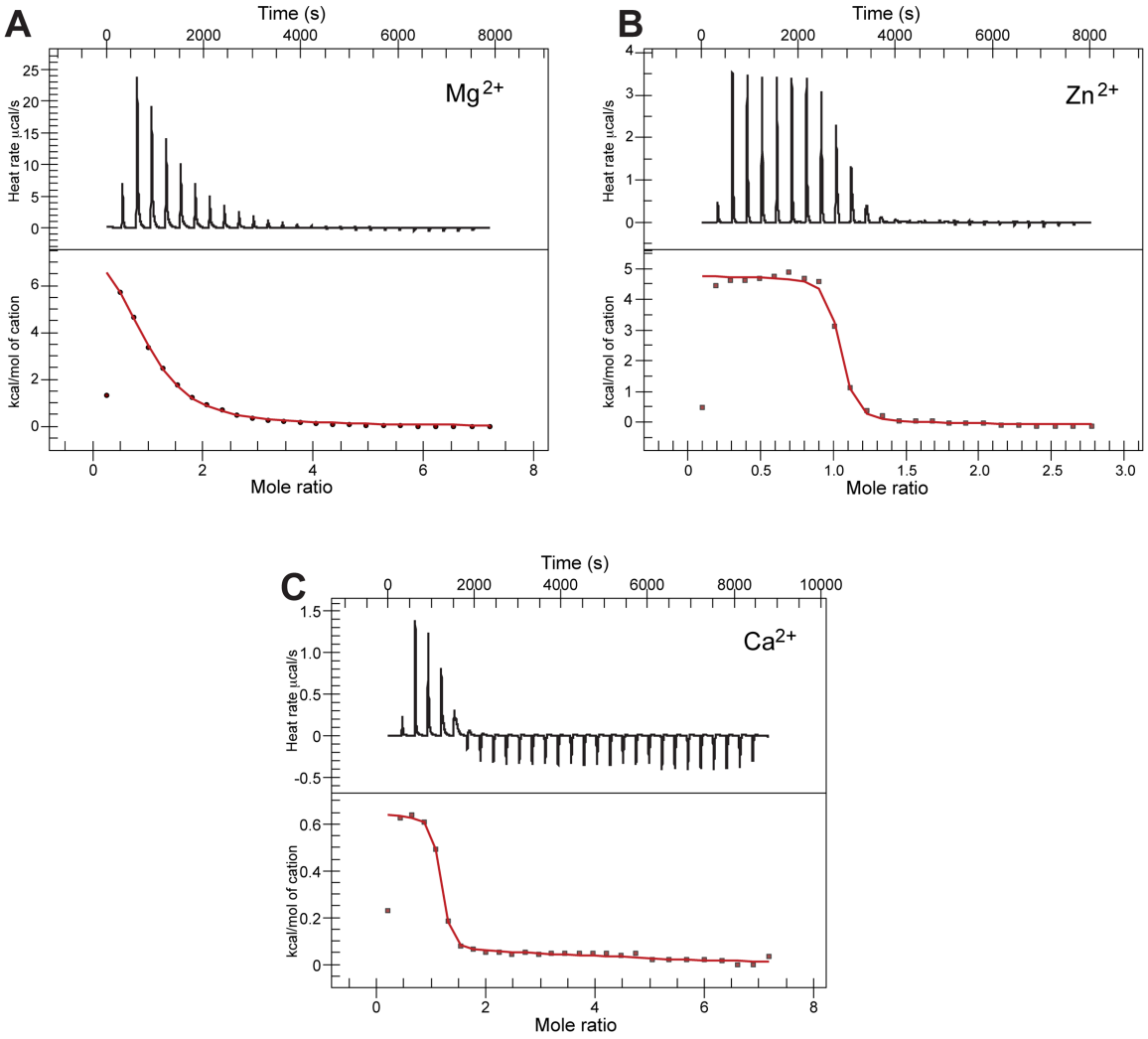

Figure S4. Representative ITC curves of APTRA treated with (A) $MgCl_2$, (B) $ZnCl_2$ or (C) $CaCl_2$. Values of ΔH for the binding reported in the manuscript correspond to averages of three measurements.

S2. Space Group Determination of $[Zn(OH_2)_6][(APTRA)Zn]_2$

1) 318 reflections violate the systematic absence in the space group of *Pnma*, which indicates the *a* glide plane is a pseudo symmetry. The correct space group is $P2_1/n$ in monoclinic. Some violating reflections are listed below.

h	k	l	Fo^2	Sigma	Why rejected
3	3	0	0.60	0.13	observed but should be systematically absent
3	3	0	0.44	0.11	observed but should be systematically absent
3	3	0	0.60	0.09	observed but should be systematically absent
3	3	0	0.48	0.09	observed but should be systematically absent
3	3	0	0.41	0.08	observed but should be systematically absent
3	3	0	0.32	0.08	observed but should be systematically absent
3	3	0	0.34	0.08	observed but should be systematically absent
3	3	0	0.34	0.08	observed but should be systematically absent
3	4	0	0.57	0.09	observed but should be systematically absent
3	4	0	0.61	0.10	observed but should be systematically absent
3	4	0	0.70	0.14	observed but should be systematically absent
3	4	0	0.73	0.14	observed but should be systematically absent
3	4	0	0.61	0.10	observed but should be systematically absent
3	4	0	0.43	0.10	observed but should be systematically absent
9	4	0	1.04	0.24	observed but should be systematically absent
9	4	0	0.98	0.17	observed but should be systematically absent
9	4	0	0.76	0.17	observed but should be systematically absent
9	4	0	0.90	0.22	observed but should be systematically absent
9	4	0	0.89	0.19	observed but should be systematically absent
5	5	0	0.61	0.11	observed but should be systematically absent
5	5	0	0.67	0.12	observed but should be systematically absent
5	5	0	0.55	0.12	observed but should be systematically absent
5	5	0	0.58	0.12	observed but should be systematically absent
1	6	0	0.93	0.19	observed but should be systematically absent
11	6	0	1.18	0.24	observed but should be systematically absent
11	6	0	1.15	0.24	observed but should be systematically absent
11	6	0	1.18	0.20	observed but should be systematically absent
11	6	0	1.32	0.20	observed but should be systematically absent
11	6	0	1.03	0.20	observed but should be systematically absent
11	6	0	1.73	0.30	observed but should be systematically absent
11	6	0	0.88	0.19	observed but should be systematically absent
11	6	0	1.52	0.30	observed but should be systematically absent
1	7	0	0.75	0.15	observed but should be systematically absent
1	7	0	0.65	0.15	observed but should be systematically absent
1	7	0	0.86	0.17	observed but should be systematically absent
1	7	0	0.69	0.14	observed but should be systematically absent
1	7	0	0.67	0.14	observed but should be systematically absent
1	7	0	0.90	0.17	observed but should be systematically absent

```
  1   7   0       0.63      0.15     observed but should be systematically absent
  3   7   0       2.74      0.20     observed but should be systematically absent
  3   7   0       2.24      0.19     observed but should be systematically absent
  3   7   0       2.53      0.20     observed but should be systematically absent
  3   7   0       3.02      0.27     observed but should be systematically absent
  3   7   0       2.87      0.20     observed but should be systematically absent
  3   7   0       2.98      0.19     observed but should be systematically absent
  3   7   0       4.30      0.30     observed but should be systematically absent
  3   7   0       3.11      0.25     observed but should be systematically absent
  3   7   0       2.89      0.25     observed but should be systematically absent
  7   7   0       1.95      0.19     observed but should be systematically absent
  7   7   0       1.73      0.19     observed but should be systematically absent
** etc. **
  66051  Reflections read, of which  3081  rejected
-24 =< h =< 24,    -32 =< k =< 32,    -12 =< l =< 12,   Max. 2-theta =   56.59
    318  Systematic absence violations
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2) As an example the reflection 3 3 0 was found in the diffraction images, which verifies the incorrectness of the orthorhombic space group of *Pnma*.

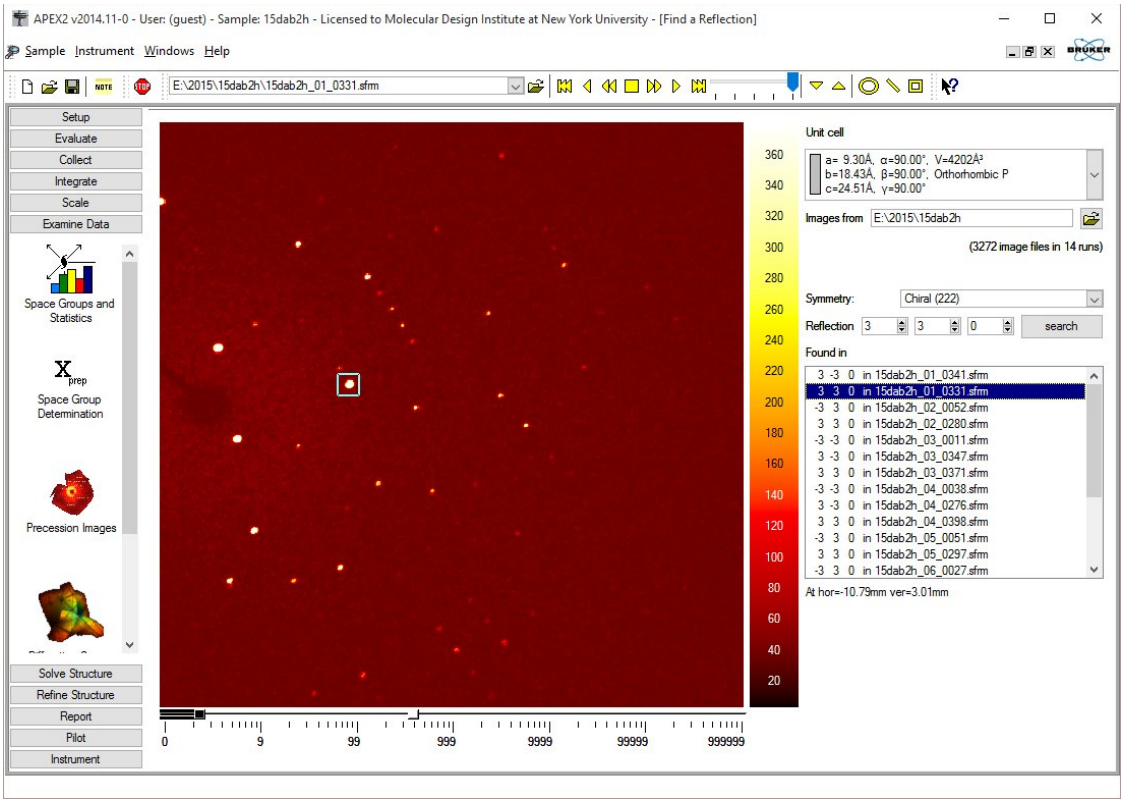

Figure S5. Reflection 3 3 0 found in the diffraction image

S3. Spectrophotometric Determination of Metal Binding Constants

S3.1 Spectrophotometric Determination of Mg^{2+} and Ca^{2+} Apparent Binding Constants

Apparent affinity constants *K'* for 1:1 stoichiometry metal complexes of APTRA were obtained from non-linear fit of absorbance values at 287 nm, A, as a function of metal concentration. Data fitting was performed with OriginPro 9.0 software according to equation S1, where A_{max} and A_{min} represent the absorbance values for the metal-saturated chelator and metal-free chelator at the beginning and the end of the titration, respectively.

$$A = \frac{A_{min} + A_{max} K'[M^{2+}]}{1 + K'[M^{2+}]} \quad \text{(S1)}$$

For Mg^{2+} titrations, the approximation $[Mg^{2+}] \approx [Mg^{2+}]_t$ was made. For Ca^{2+} titrations, the free metal concentration at each point was obtained from S2[1]

$$K'[Ca^{2+}]^2 + (1 + [APTRA]_t K' - [Ca^{2+}]_t K')[Ca^{2+}] - [Ca^{2+}]_t = 0 \quad \text{(S2)}$$

S3.2 Spectrophotometric Determination of Zn^{2+} Apparent Binding Constant

Zinc(II) titrations were conducted using an EGTA buffer system. The free metal ion concentration at each point was calculated from the total Zn^{2+} concentration, $[Zn^{2+}]_t$, solving equation S3 with an apparent binding constant for Zn^{2+}-EGTA of $\log K'_{ZnEGTA} = 8.24$ at pH 7.0.

$$K'_{ZnEGTA}[Zn^{2+}]^2 + (1 + [EGTA]_t K'_{ZnEGTA} - [Zn^{2+}]_t K'_{ZnEGTA})[Zn^{2+}] - [Zn^{2+}]_t = 0 \quad \text{(S3)}$$

The apparent binding constant was calculated from the absolute stability constant $\log K_{ZnEGTA} = 12.6$ (25 °C, μ= 0.1) listed in Martell and Smith,[2] using Schwarzenbach's α coefficient method, according to the following equation:

$$K'_{ZnEGTA} = \alpha_{EGTA} K_{ZnEGTA} \quad \text{(S4)}$$

where α_{EGTA} corresponds to the fraction of fully deprotonated EGTA at the pH of interest. The pK_a values for EGTA used to calculate α_{EGTA} were corrected upwards by 0.11 units to account for the fact that the tabulated pK_a values in Martell and Smith[2] are determined using concentration and not activity of the hydrogen ion. According to the National Bureau of

Standards, pH is defined as -log(a_H), where a_H is 0.78[H^+] at 0.1 M ionic strength. As a result, the pK_a values for EGTA employed in our calculations were: pK_{a1}= 9.51, pK_{a2}= 8.89, pK_{a3}= 2.77, pK_{a4}= 2.11.

S4. HPLC data for hydrolysis of APTRA trimethyl ester

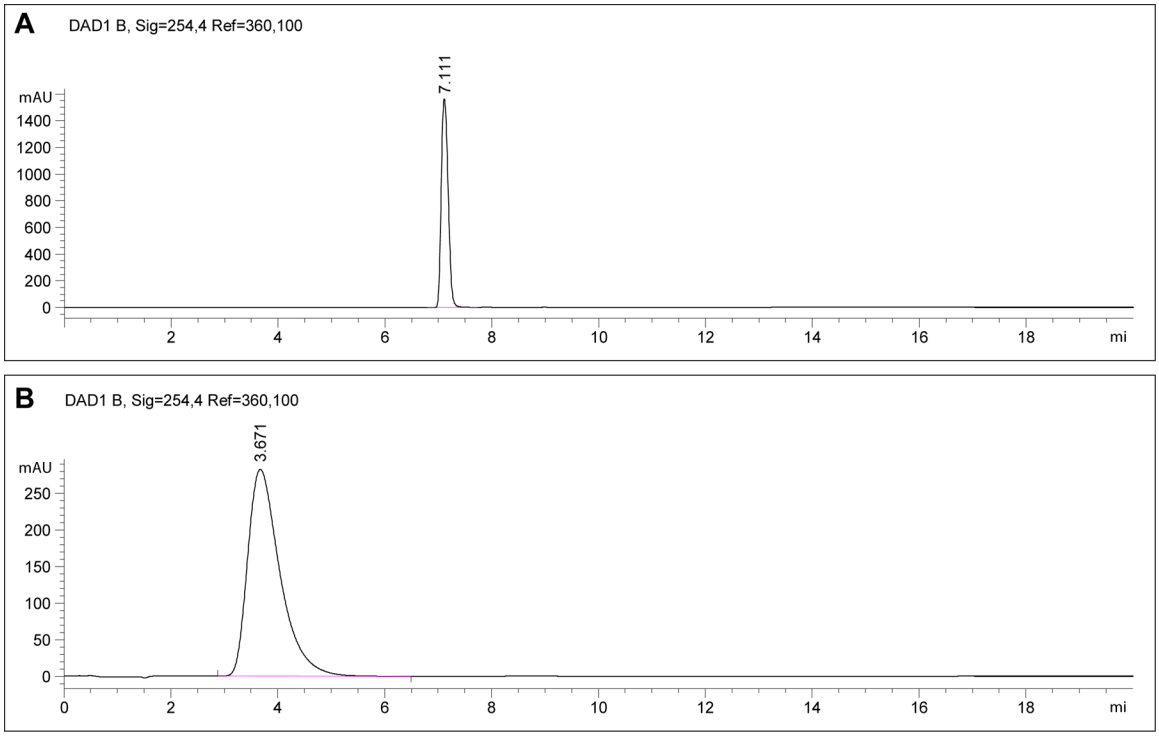

Figure S6. HPLC trace of (A) APTRA trimethyl ester and (B) APTRA free acid product of hydrolysis.

S5. References

1. A. E. Hargrove, Z. Zhong, J. L. Sessler and E. V. Anslyn, *New J. Chem.*, 2010, **34**, 348-354.
2. A. Martell and R. M. Smith, *Critical Stability Constants. First Supplement*, Plenum Press, New York, 1982.