Supplementary Data

Biochemical, thermodynamics and structural studies of recombinant homotetrameric adenylosuccinate lyase from *Leishmania braziliensis*

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Figure S1. SDS–PAGE (12%) analysis of recombinant *Lb*ASL (~ 51 kDa) using the three step purification protocol that yielded homogeneous protein. Lane 1: Molecular Weight Protein Marker; Lane 2 – Crude Extract; Lane 3 – Treated with ammonium sulfate 1.5 M; Lane 4 – Superdex 200 eluted; Lane 5 – Q Sepharose HP eluted and dialyzed against the 50 mM potassium phosphate buffer pH 7.0, containing 150 mM KCl, 1 mM EDTA, 1 mM DTT and 10 % glycerol.

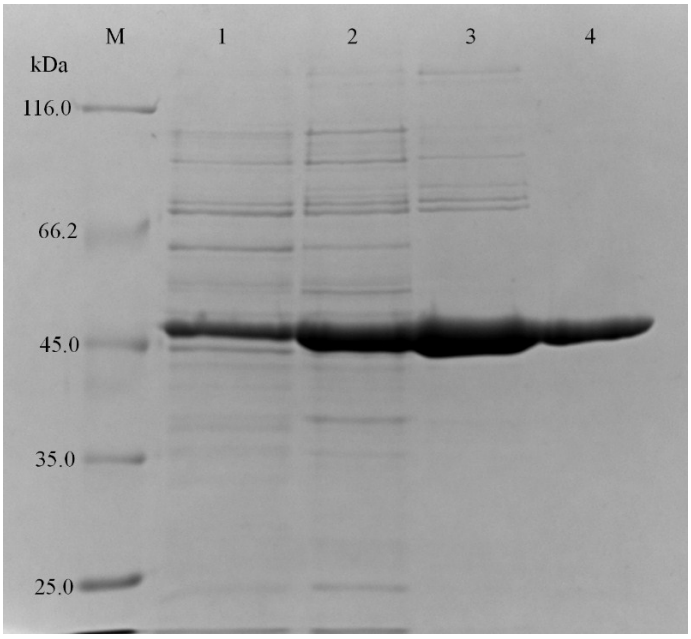

Table S1. Purification of recombinant *Lb*ASL expressed in *E. coli* BL21(DE3) cells. Typical purification protocol from 1.8 g wet cell paste.

Purification step	**Total protein (mg)**	**Total enzyme activity (U)**	**Specific activity (U mg^{-1})**	**Purification fold**	**Yield (%)**
Crude extract	723.96	228.06	0.32	1.00	100
Ammonium Sulfate	137.60	465.09	3.38	10.73	204
Superdex 200 eluate	43.20	1.30	0.03	0.09	1
Q Sepharose HP eluate	18.70	90.32	4.83	15.33	40

[a]One unit (U) of enzyme in this table is given as the amount of *Lb*ASL that converts 1 µmol of S-AMP into AMP and fumarate per second at pH 7.5 at 25 °C.

Figure S2. Representation of amino acid sequence coverage of recombinant *Lb*ASL. *Lb*ASL protein identify was confirmed by the identification of 189 unique peptides (yellow bars) covering 100% of the protein sequence (456 amino acid residues represented in blue).

Protein ID : tr|A4H3W5|A4H3W5_LEIBR Coverage : 456 / 456 = 1

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001 MISEDSPLFA LSPLDGRYKH STVPLRAYFS EYALFKYRVQ VEVLYFEALC KEVPTITQLR 060
061 GVTDAHLAQL RAATFENFTV DDATKIKDIE AVTKHDIKAV EYYLKDKMSA CGLETEKEFI 120
121 HFGLTSQDIN NTSIPMLLRD ALHHHYIPAL DQLIALLKSK LPEWDVPMLA RTHGQPASPT 180
181 NLAKEFMVWI ERLEEQRAML LSIPNTGKFG GATGNFNAHL CAYPGVDWRN FGDLFLSKYL 240
241 GLRRQRYTTQ IEHYDNLAAI CDACARLHTI LMDLAKDVWQ YISLGYFNQK VKAGEVGSSA 300
301 MPHKVNPIDF ENAEGNLGMS NAVLGFLSAK LPISRLQRDL TDSTVLRNLG VPLSHALIAF 360
361 ASLLRGVDKL LVNKPAIAAD LESNWAVVAE GIQTVLRREG YPKPYEALKD LTRGNAQVTK 420
421 ETVRTFIQQL EGITDEVRQE LLAITPFTYV GYTRCL
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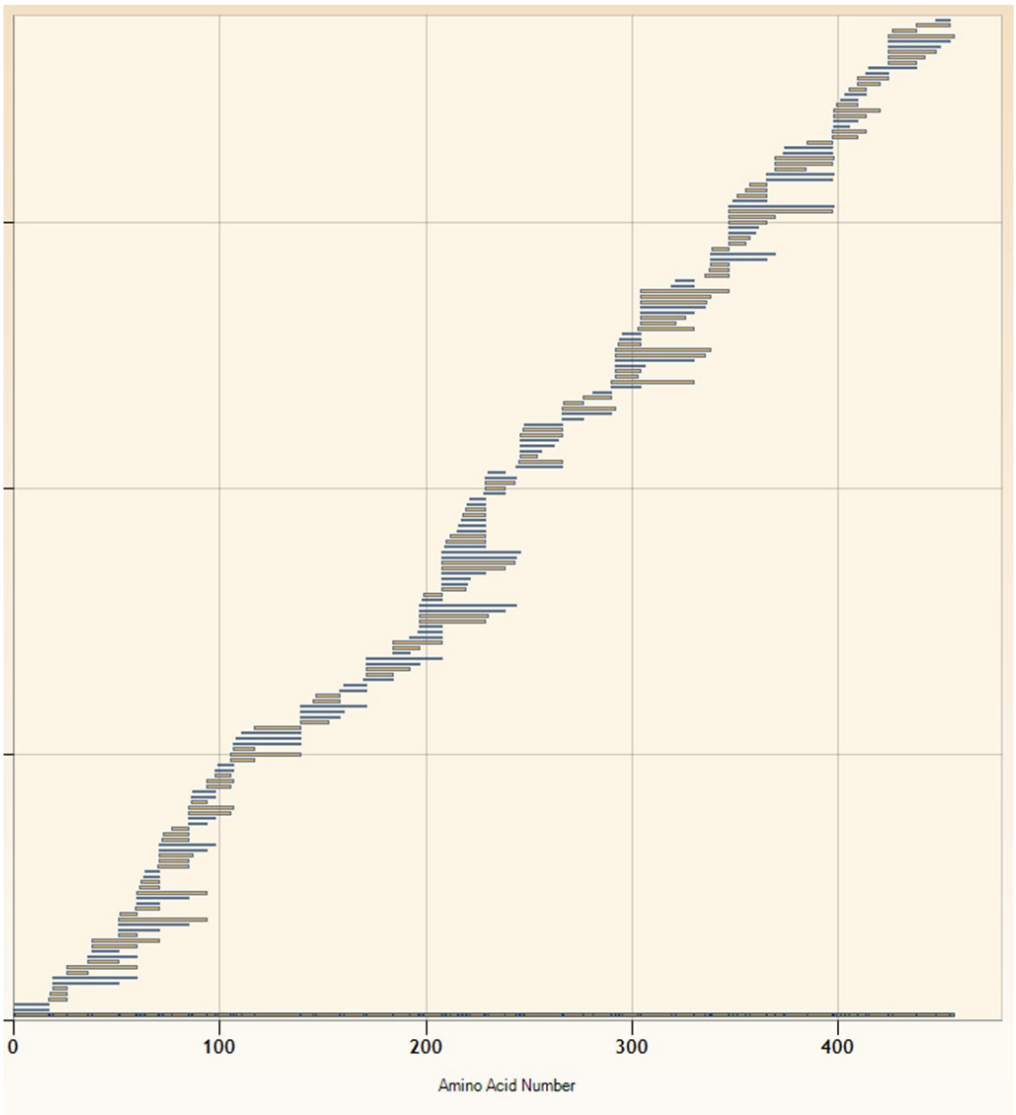

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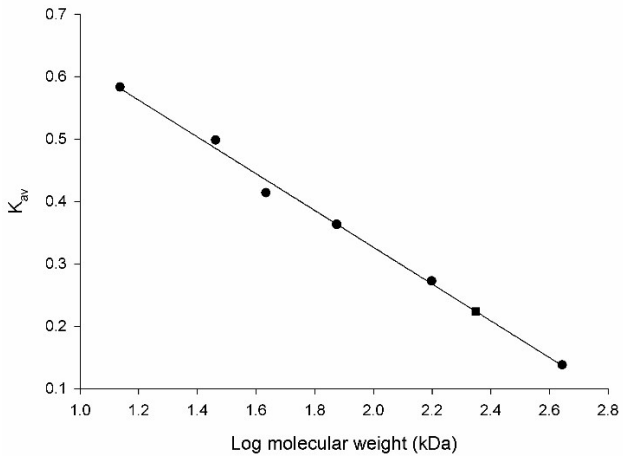

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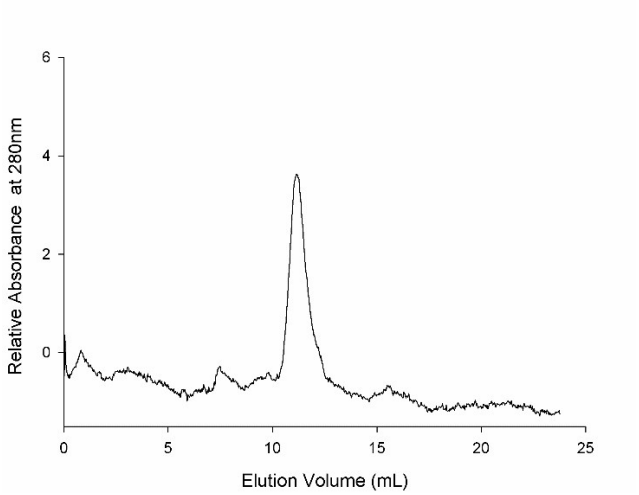

Figure S3. Determination of the oligomeric state of *Lb*ASL by gel filtration using a HighLoad 10/30 Superdex-200 column. (A) Protein standards (●) were: ferritin, aldolase, conalbumin, ovalbumin, ribonuclease A and carbonic anhydrase. Blue dextran 2000 was used to determine the void volume. K_{av} was determined using Eq. 1 (main text) for each standard, and these values were plotted against logarithm of the molecular mass of protein standards. A value of 223,357 for the apparent molecular mass of homogeneous recombinant protein (■) was estimated suggesting that *Lb*ASL is a tetramer in solution. (B) *Lb*ASL protein elution profile at 280 nm.

Figure S4. ITC analysis of *Lb*ASL titration with fumarate. The top panel shows raw data of the heat pulses resulting from titration of *Lb*ASL. The bottom panel shows the integrated heat pulses, normalized per mole of injection as a function of the molar ratio (ligand concentration/*Lb*ASL subunit concentration). Data were best fitted to one binding site model.