Supplementary Data

Thermodynamics, Functional and Structural Characterization of Inosine-Uridine Nucleoside Hydrolase from *Leishmania braziliensis*

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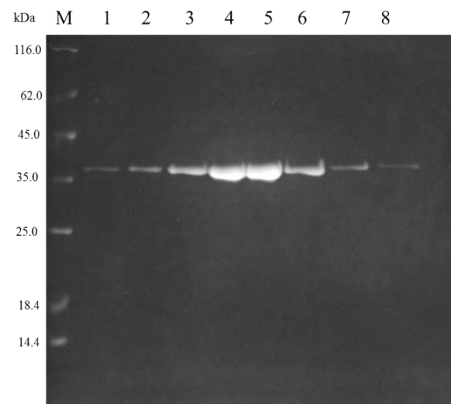
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Figure S1. SDS–PAGE (12%) analysis of recombinant *Lb*IU-NH (~ 34 kDa) using the three-step purification protocol that yielded homogeneous protein. Lane 1: Molecular Weight Protein Marker; Lane 1 – 8 *Lb*IU-NH homogenous protein.
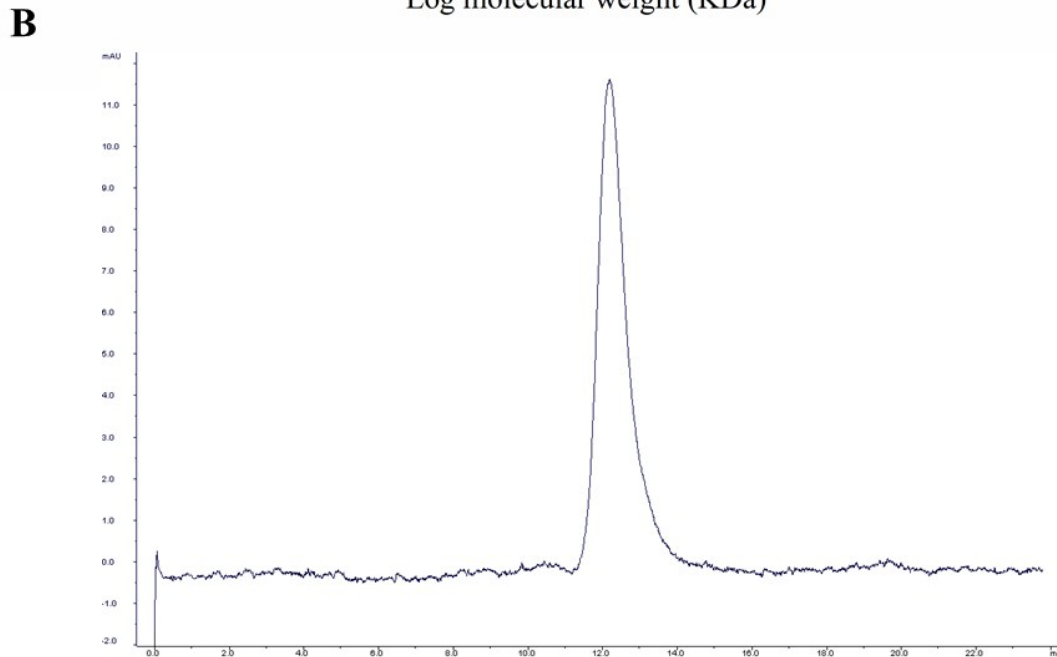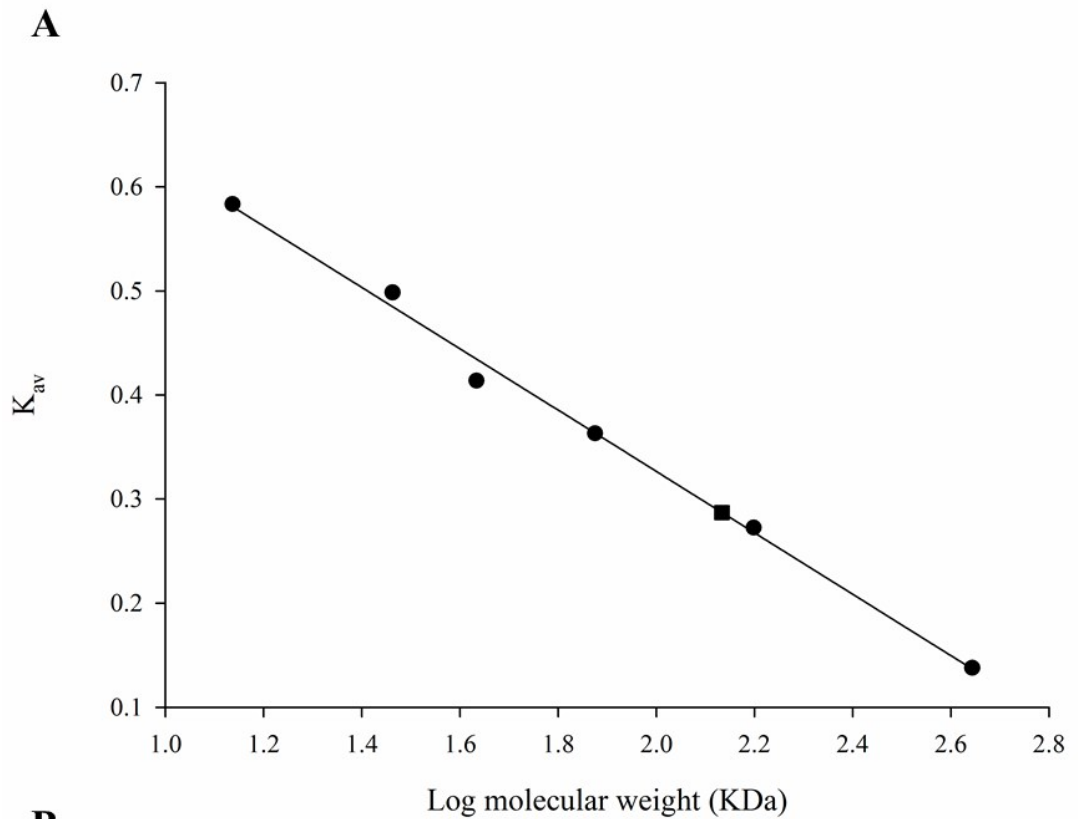
A

B

Figure S2. Determination of the oligomeric state of *Lb*IU-NH 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 of protein standards. A value of 136,019 for the apparent molecular mass of homogeneous recombinant protein (■) was estimated suggesting that *Lb*IU-NH is a tetramer in solution. B- Protein elution profile at 280 nm.
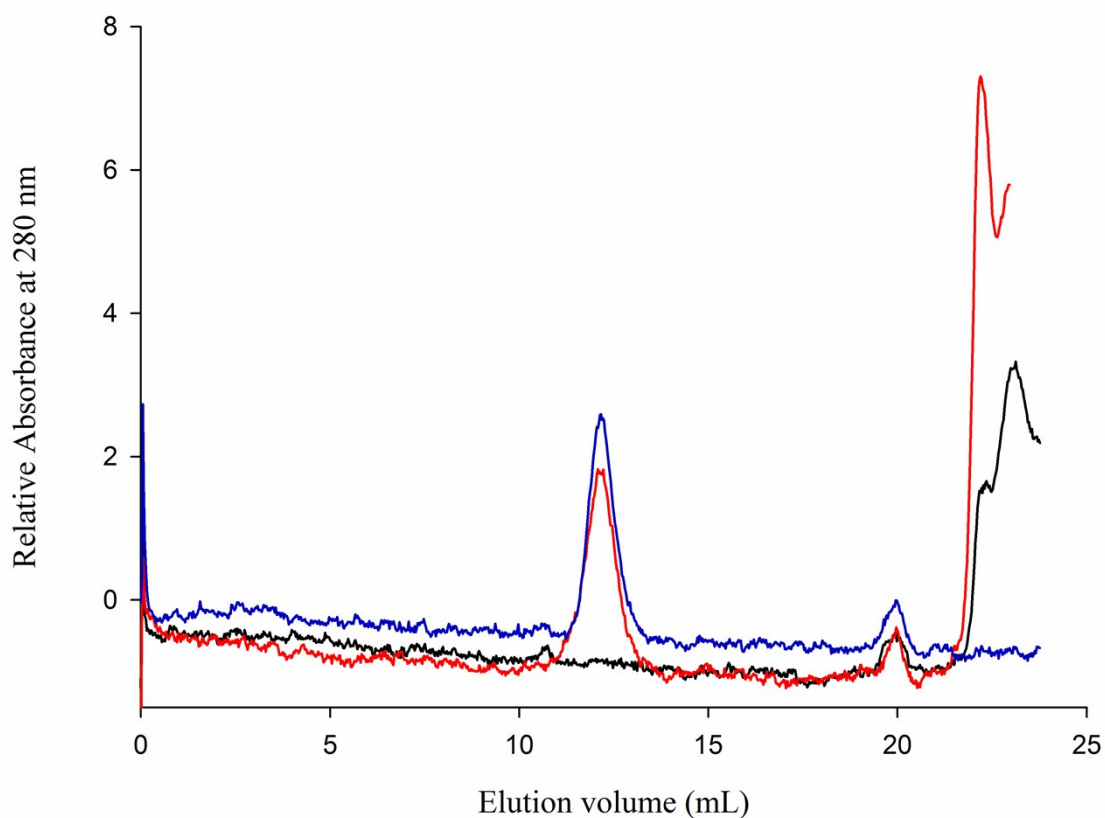
Figure S3. Evaluation by gel filtration chromatography of DTT effects on *Lb*IU-NH quaternary structure. *Lb*IU-NH (7 μM) was incubated in either the presence (red line) or absence (blue line) of 100 mM DTT in Tris HCl 50 mM pH 7.5 for 2 hours at room temperature. A profile for DTT 100 mM in Tris HCl 50mM pH 7.5 (black line) was used as chemical background control. The same protein elution volume (12.178 mL corresponding to 136,019 Da) was observed for both samples (blue and red lines), suggesting that disulfide bonds play no role in maintaining the tetrameric structure of *Lb*IU-NH.
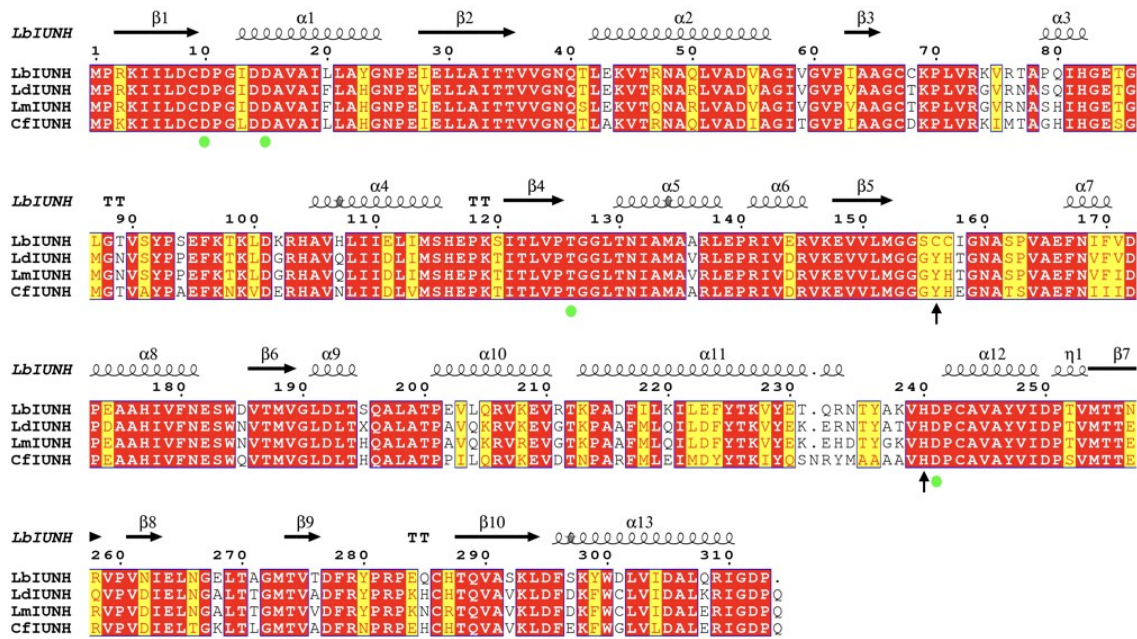
Figure S4. Primary sequence alignment of protozoan IU-NHs. The green circles are highlighting the residues responsible for anchoring the calcium ion and the arrows show the Cys157 that is involved in the disulfide bond and the residue His240. Image generated with ESPript.
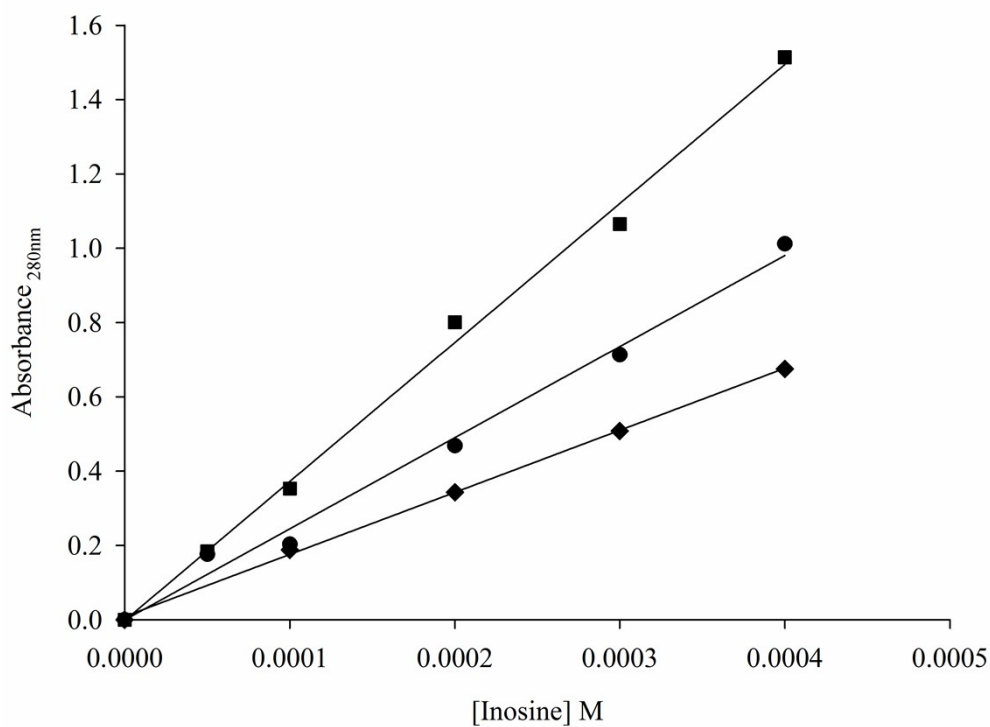
Figure S5. Molar absorption values at 280 nm (ε_{280}) for inosine in solvents with different dielectric constants (D). Absorbance at 280 nm were measured as a function of varying concentrations of inosine (25μM-400μM) in 50 mM Tris HCl pH 7.5 (♦), methanol (MeOH) (●) or dimethyl sulfoxide (DMSO) (■). The values for ε_{280} were: 1.7 ($\pm$ 0.3) x 10^3 M^{-1} cm^{-1} for Tris HCl 50 mM (D_{water} = 78.74), 2.4 ($\pm$ 0.1) x 10^3 M^{-1} cm^{-1} for MeOH (D_{MeOH} = 32.6) and 3.7 ($\pm$ 0.1) x 10^3 M^{-1} cm^{-1} for DMSO (D_{DMSO} = 49).
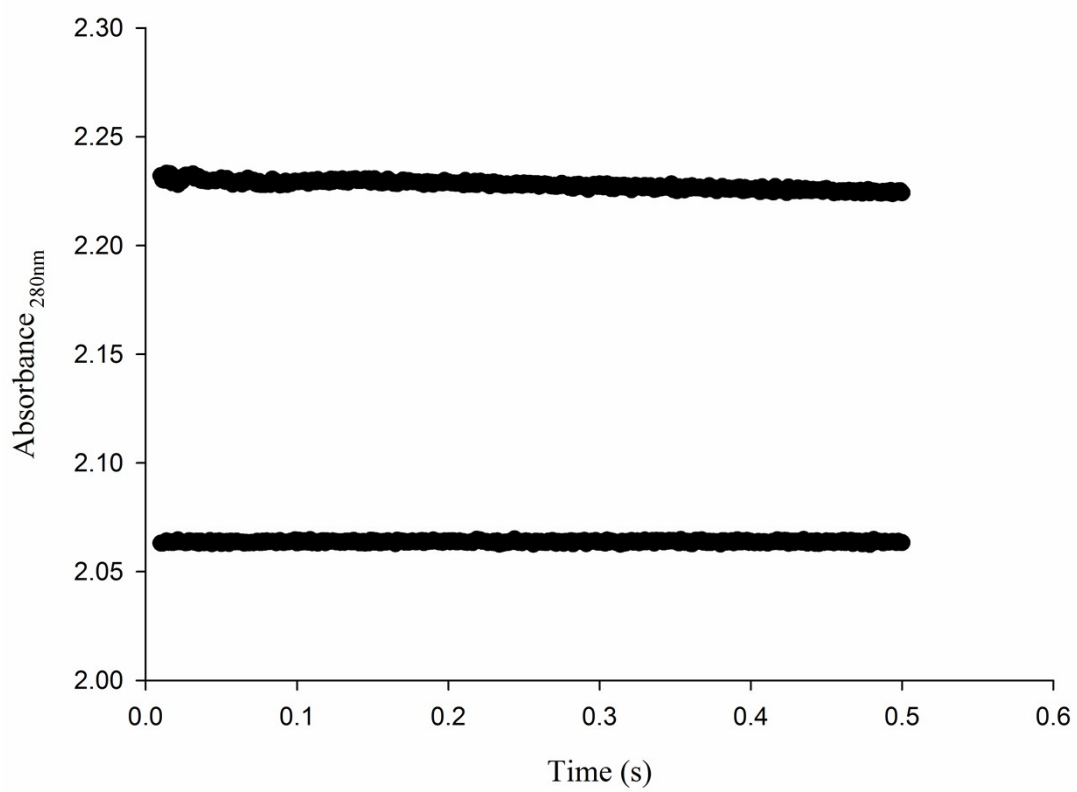
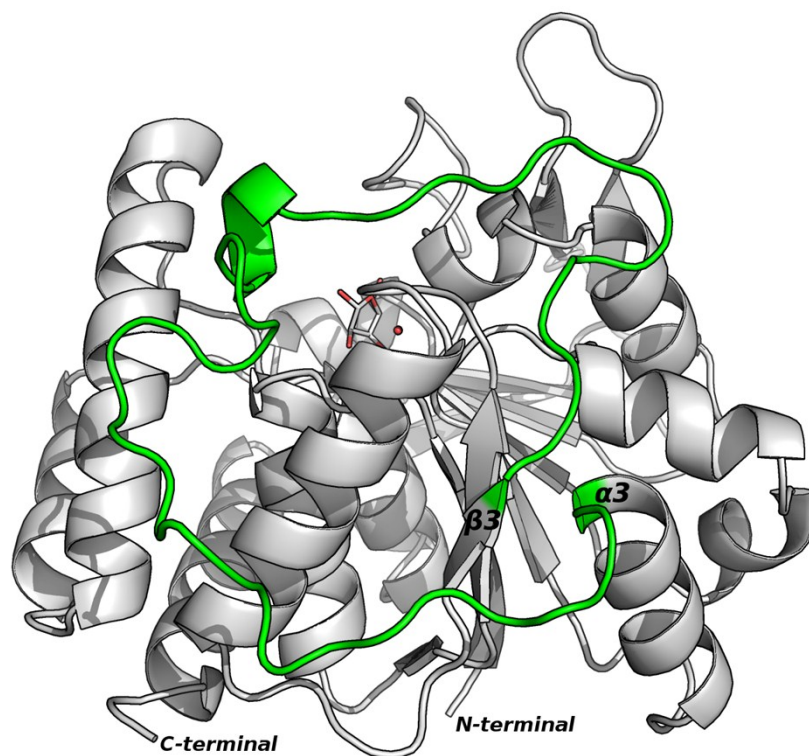**Figure S6.** Stopped-flow trace for product formation.

Figure S7. Overall structure of *Lb*IU-NH. The loop between β3-α3 influenced by the ligand binding is highlighted in green. Image generated with PyMOL.
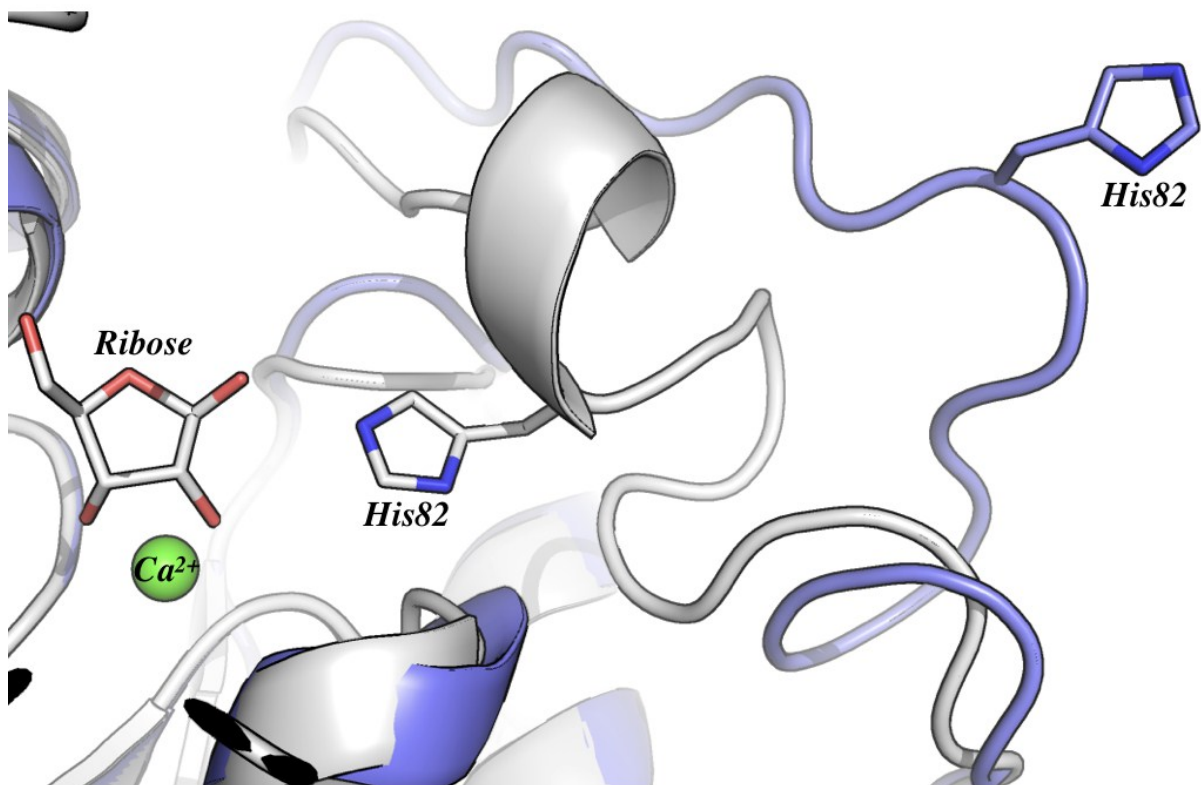
Figure S8. Structural superposition of IU-NH from *L. major* (PDB ID 1EZR) and *L. braziliensis*. The structures are drawn as cartoon, residues His82 are shown as sticks, and the Ca^{2+} ion is represented according to van der Waals radii. *Lb*IU-NH and *Lm*IU-NH are colored in gray and blue, respectively. The loop between β3-α3 is found closed when a ligand is associated and open in the apo form. The image was generated by PyMOL.