Supplementary Information for

The role of alanine 163 in solute permeability of *Leishmania major* aquaglyceroporin LmAQP1

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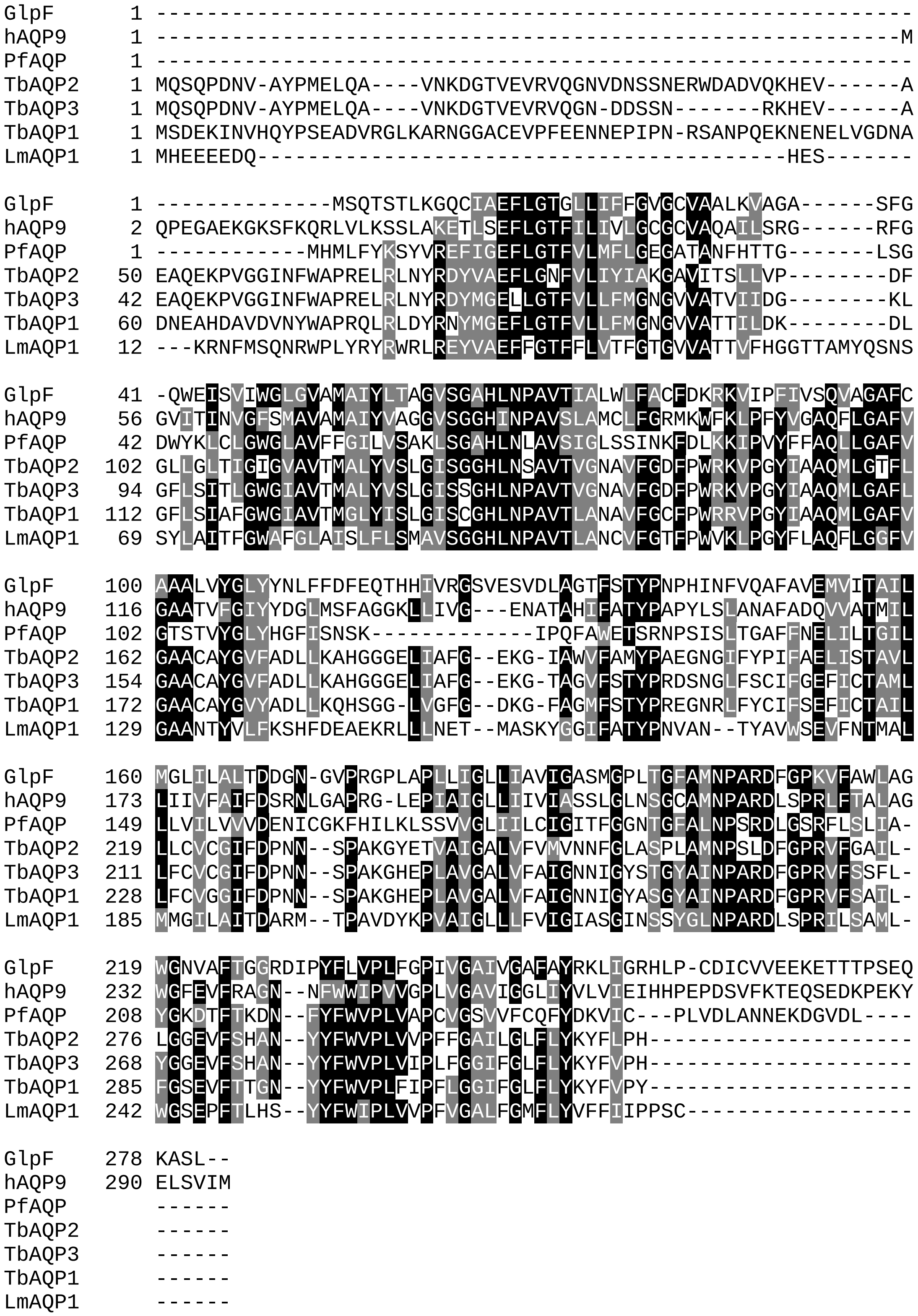

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GlpF       1 ------------------------------------------------------------
hAQP9      1 -----------------------------------------------------------M
PfAQP      1 ------------------------------------------------------------
TbAQP2     1 MQSQPDNV-AYPMELQA----VNKDGTVEVRVQGNVDNSSNERWDADVQKHEV------A
TbAQP3     1 MQSQPDNV-AYPMELQA----VNKDGTVEVRVQGN-DDSSN-------RKHEV------A
TbAQP1     1 MSDEKINVHQYPSEADVRGLKARNGGACEVPFEENNEPIPN-RSANPQEKNENELVGDNA
LmAQP1     1 MHEEEEDQ------------------------------------------HES-------

GlpF       1 --------------MSQTSTLKGQCIAEFLGTGLLIFFGVGCVAALKVAGA------SFG
hAQP9      2 QPEGAEKGKSFKQRLVLKSSLAKETLSEFLGTFILIVLGCGCVAQAILSRG------RFG
PfAQP      1 ------------MHMLFYKSYVREFIGEFLGTFVLMFLGEGATANFHTTG-------LSG
TbAQP2    50 EAQEKPVGGINFWAPRELRLNYRDYVAEFLGNFVLIYIAKGAVITSLLVP--------DF
TbAQP3    42 EAQEKPVGGINFWAPRELRLNYRDYMGELLGTFVLLFMGNGVVATVIIDG--------KL
TbAQP1    60 DNEAHDAVDVNYWAPRQLRLDYRNYMGEFLGTFVLLFMGNGVVATTILDK--------DL
LmAQP1    12 ---KRNFMSQNRWPLYRYRWRLREYVAEFFGTFFLVTFGTGVVATTVFHGGTTAMYQSNS

GlpF      41 -QWEISVIWGLGVAMAIYLTAGVSGAHLNPAVTIALWLFACFDKRKVIPFIVSQVAGAFC
hAQP9     56 GVITINVGFSMAVAMAIYVAGGVSGGHINPAVSLAMCLFGRMKWFKLPFYVGAQFLGAFV
PfAQP     42 DWYKLCLGWGLAVFFGILVSAKLSGAHLNLAVSIGLSSINKFDLKKIPVYFFAQLLGAFV
TbAQP2   102 GLLGLTIGIGVAVTMALYVSLGISGGHLNSAVTVGNAVFGDFPWRKVPGYIAAQMLGTFL
TbAQP3    94 GFLSITLGWGIAVTMALYVSLGISSGHLNPAVTVGNAVFGDFPWRKVPGYIAAQMLGAFL
TbAQP1   112 GFLSIAFGWGIAVTMGLYISLGISCGHLNPAVTLANAVFGCFPWRRVPGYIAAQMLGAFV
LmAQP1    69 SYLAITFGWAFGLAISLFLSMAVSGGHLNPAVTLANCVFGTFPWVKLPGYFLAQFLGGFV

GlpF     100 AAALVYGLYYNLFFDFEQTHHIVRGSVESVDLAGTFSTYPNPHINFVQAFAVEMVITAIL
hAQP9    116 GAATVFGIYYDGLMSFAGGKLLIVG---ENATAHIFATYPAPYLSLANAFADQVVATMIL
PfAQP    102 GTSTVYGLYHGFISNSK-------------IPQFAWETSRNPSISLTGAFFNELILTGIL
TbAQP2   162 GAACAYGVFADLLKAHGGGELIAFG--EKG-IAWVFAMYPAEGNGIFYPIFAELISTAVL
TbAQP3   154 GAACAYGVFADLLKAHGGGELIAFG--EKG-TAGVFSTYPRDSNGLFSCIFGEFICTAML
TbAQP1   172 GAACAYGVYADLLKQHSGG-LVGFG--DKG-FAGMFSTYPREGNRLFYCIFSEFICTAIL
LmAQP1   129 GAANTYVLFKSHFDEAEKRLLLNET--MASKYGGIFATYPNVAN--TYAVWSEVFNTMAL

GlpF     160 MGLILALTDDGN-GVPRGPLAPLLIGLLIAVIGASMGPLTGFAMNPARDFGPKVFAWLAG
hAQP9    173 LIIVFAIFDSRNLGAPRG-LEPIAIGLLIIVIASSLGLNSGCAMNPARDLSPRLFTALAG
PfAQP    149 LLVILVVVDENICGKFHILKLSSVVGLIILCIGITFGGNTGFALNPSRDLGSRFLSLIA-
TbAQP2   219 LLCVCGIFDPNN--SPAKGYETVAIGALVFVMVNNFGLASPLAMNPSLDFGPRVFGAIL-
TbAQP3   211 LFCVCGIFDPNN--SPAKGHEPLAVGALVFAIGNNIGYSTGYAINPARDFGPRVFSSFL-
TbAQP1   228 LFCVGGIFDPNN--SPAKGHEPLAVGALVFAIGNNIGYASGYAINPARDFGPRVFSAIL-
LmAQP1   185 MMGILAITDARM--TPAVDYKPVAIGLLLFVIGIASGINSSYGLNPARDLSPRILSAML-

GlpF     219 WGNVAFTGGRDIPYFLVPLFGPIVGAIVGAFAYRKLIGRHLP-CDICVVEEKETTTPSEQ
hAQP9    232 WGFEVFRAGN--NFWWIPVVGPLVGAVIGGLIYVLVIEIHHPEPDSVFKTEQSEDKPEKY
PfAQP    208 YGKDTFTKDN--FYFWVPLVAPCVGSVVFCQFYDKVIC---PLVDLANNEKDGVDL----
TbAQP2   276 LGGEVFSHAN--YYFWVPLVVPFFGAILGLFLYKYFLPH---------------------
TbAQP3   268 YGGEVFSHAN--YYFWVPLVIPLFGGIFGLFLYKYFVPH---------------------
TbAQP1   285 FGSEVFTTGN--YYFWVPLFIPFLGGIFGLFLYKYFVPY---------------------
LmAQP1   242 WGSEPFTLHS--YYFWIPLVVPFVGALFGMFLYVFFIIPPSC------------------

GlpF     278 KASL--
hAQP9    290 ELSVIM
PfAQP        ------
TbAQP2       ------
TbAQP3       ------
TbAQP1       ------
LmAQP1       ------
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Fig. S1. Comparison of amino acid sequence of *E. coli*, human, and protozoan aquaglyceroporins. Abbreviations: H, *Homo sapiens*; Lm, *Leishmania major*; Pf, *Plasmodium* *falciparum*; Tb, *Trypanosoma brucei*. The protein source and GenBank accession numbers of the aligned sequences are *E. coli* GlpF (1FX8_A); hAQP9 (NP_066190); PfAQP (3C02_A); TbAQP1 (CAG27020); TbAQP2 (CAG27021); TbAQP3 (CAG27022); and LmAQP1 (AAS73184). Multiple sequence alignments was performed with ClustalW and shading by BOXSHADE. The dashes indicate the gaps introduced to maximize sequence alignment. The black and grey regions indicate sequence identity and sequence similarity, respectively.

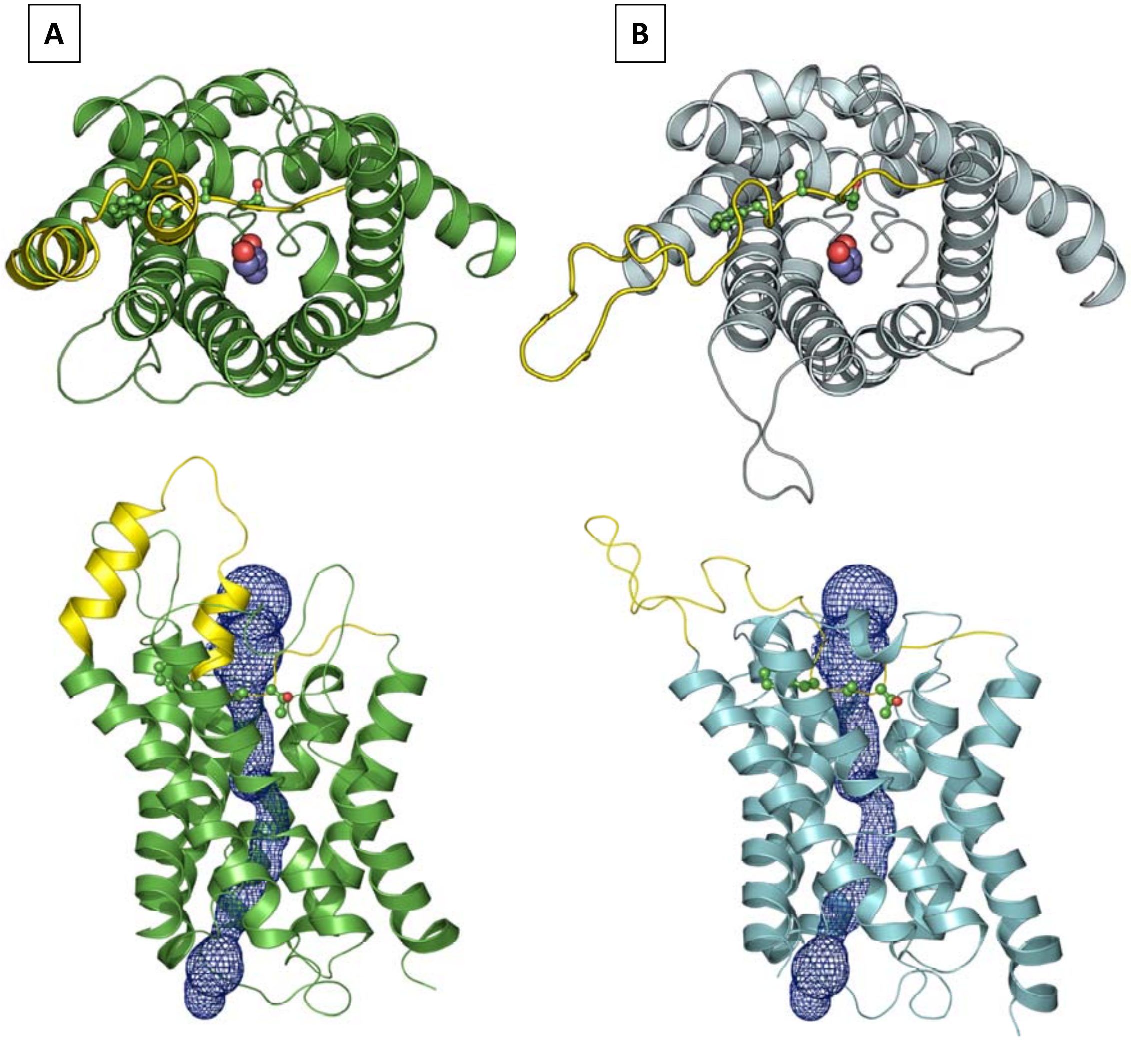

Fig. S2. Molecular modeling of LmAQP1 based on the crystal structures of GlpF and PfAQP. A. Ribbon representation of a model of LmAQP1 monomer (green) based on the structure of GlpF (PDB ID: 1LDF). *Top*, viewed from the periplasmic side, and *Bottom*, viewed parallel to the membrane. B. Ribbon representation of a model of LmAQP1 monomer (cyan) based on the structure of PfAQP (PDB ID: 3C02). *Top*, viewed from the periplasmic side, and *Bottom*, viewed parallel to the membrane. The C-loop is highlighted in yellow and the amino acid triad Phe_{162}-Ala_{163}-Thr_{164} is shown as ball and stick model with the carbon and oxygen atoms colored as green and red, respectively. A glycerol molecule is shown at the centre of the channel as a spherical model with the oxygen and carbon atoms colored as red and magenta, respectively. The channel is depicted as blue mesh.

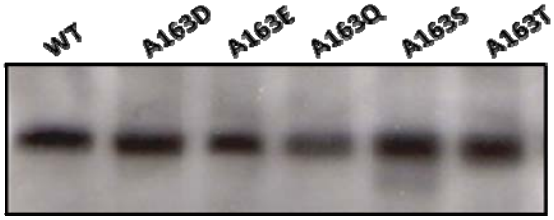

Fig. S3. Expression and localization of wild-type and altered LmAQP1 in *L. donovani* promastigotes. Western blot analysis of flagellar membrane fractions of *L. donovani* promastigotes expressing wild type and altered LmAQP1 were probed with the LmAQP1 antipeptide antibody as described in Materials and Methods.

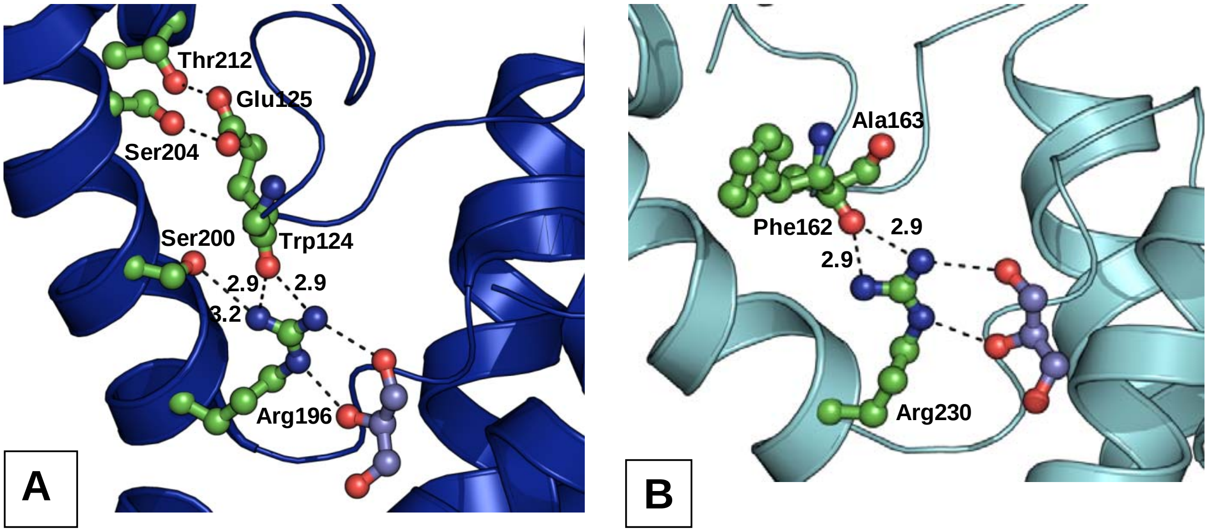

Fig. S4. Selectivity filter region site of LmAQP1 modeled on PfAQP. Ribbon representations of the active site of (A) PfAQP (blue); (B) LmAQP1 based on PfAQP (cyan). Residues involved in the selectivity filter of the channel are shown as ball-and-stick model with the carbon, oxygen, and nitrogen atoms colored as green, red, and blue, respectively. Glycerol is drawn as ball-and-stick model with the carbon and oxygen colored as magenta and red, respectively. The hydrogen bond network around the arginine residue is shown as dotted lines with measurements in angstroms. Transmembrane helices 1, 4, and 8 have been removed for improving the clarity of the selectivity filter region.