



The nucleotide specificity of succinyl-CoA synthetase of *Plasmodium falciparum* is not determined by charged gatekeeper residues alone

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Substrate specificity of an enzyme is an important characteristic of its mechanism of action. Investigation of the nucleotide specificity of Plasmodium falciparum succinyl-CoA synthetase (SCS; PfSCS) would provide crucial insights of its substrate recognition. Charged gatekeeper residues have been shown to alter the substrate specificity via electrostatic interactions with approaching substrates. The enzyme kinetics of recombinant PfSCS (wild-type), generated by refolding of the individual P. falciparum SCSB and Blastocystis SCSa subunits, demonstrated ADP-forming activity $(K_{\text{mATP}} = 48 \ \mu\text{M})$. Further, the introduction of charged gatekeeper residues, either positive (Lys and Lys) or negative (Glu and Asp), resulted in significant reductions in the ATP affinity of PfSCS. It is interesting to note that the recombinant P/SCS β subunit can be refolded to a functional enzyme conformation using Blastocystis SCSa, indicating the possibility of subunits swapping among different organisms. These results concluded that electrostatic interactions at the gatekeeper region alone are insufficient to alter the substrate specificity of PfSCS, and further structural analysis with a particular focus on binding site architecture is required.

In any biological system, the substrate specificity is a characteristic property of the enzymes. There are two landmark models to describe substrate specificity of an enzyme: 'lock-and-key model' [1] proposes a rigid fit, whereas the 'induced fit model' [2] suggests a flexible nature of the enzyme to fit the substrate. At the molecular level, the substrate specificity is best described by the molecular interactions of a protein and its substrates. The free energies of the hydrogen bonds between a protein–substrate and the propensity of specific amino acids around the substrate play a critical role in determining the substrate specificity of an enzyme [3]. In addition, weak interactions, such as van der Waals and electrostatic interactions [4], between

the protein and its substrate also have significant contribution in the substrate specificity of an enzyme, especially when the proteins have to discriminate between two similar substrates, e.g. adenine and guanine, in the case of nucleotide-binding proteins. Basu *et al.* [4] reported that a strong ligand-free electrostatic potential could discriminate between A/G binding sites, and hence established the role of an electrostatic component in the molecular discrimination of adenine and guanine. Previously, the electrostatic potential arising from the charged amino acids inside the active site of the subtilisin enzyme had been shown to be functionally significant [5]. However, the role of other charged amino acids near or outside the active site has

Abbreviations

IB, inclusion body; MSA, multiple sequence alignment; PDB, Protein Data Bank; *Pf*SCS, *Plasmodium falciparum* succinyl-CoA synthetase; SCS, succinyl-CoA synthetase; TCA, tricarboxylic acid; WT, wild-type.

not been investigated thoroughly. In 2008, Hamblin et al. [6] proposed an electrostatic gatekeeper effect, in which the nucleotide access was controlled by the charged amino acids (gatekeeper residues) outside the binding site of the succinyl-CoA synthetase (SCS) of Blastocystis, a human intestinal parasite. Recently, we have experimentally demonstrated the 'electrostatic gatekeeper effect', where the gatekeeper residues were found to be critical for nucleotide specificity in Blastocystis SCS [7]. Interestingly, this study also established a novel enzyme engineering approach, where the switching of the charge of the gatekeeper residues from positive to negative demonstrated that the ADP-forming SCS could also utilize GTP. Surprisingly, two binding site modifications in addition to the charge switching resulted in a complete reversal of an ADPforming SCS to GDP-forming SCS.

To further signify the role of gatekeeper residues in determining the nucleotide specificity, we explored another model enzyme, SCS of Plasmodium falciparum. P. falciparum is an important human parasite that causes malaria, a significant infectious disease, with ~219 million clinical cases and ~0.43 million deaths worldwide (https://www.mmv.org/newsroom/ publications/world-malaria-report-2018). The first line of defense for P. falciparum malaria is artemisinin combination therapies. However, the emergence of resistance against artemisinin combination therapies is a matter of great concern, as was with the previous generation of antimalarials, such as chloroquine, sulfadoxine and pyrimethamine. Therefore, a considerable amount of effort is currently being devoted to identify novel drug targets for malaria, simultaneously expanding the fundamental understanding of Plasmodium biology. SCS is a crucial enzyme of the tricarboxylic acid (TCA) cycle, for its unique capability of generating ATP via substrate-level phosphorylation. In P. falciparum, however, the TCA cycle has been suggested to be of limited importance [8], yet the parasite synthesizes all the TCA cycle enzymes [9]. During the asexual growth of the malaria parasite, the absence of any specific phenotypes in $\Delta KDH/\Delta SCS$ and $\Delta SCS/\Delta SCS$ ΔSDH knockout lines (KOs), indicated metabolic plasticity in the TCA cycle (where KDH represents α ketoglutarate dehydrogenase, SCSa subunit, and SDH represents SDH flavoprotein subunit) [10]. Unlike the asexual stages of P. falciparum, the SCS is significant in terms of maintaining the reserves of succinyl-CoA, as an initial substrate for heme biosynthesis along with glycine for its sexual stages [11]. This study explored the alteration of the charge of the gatekeeper residues and its subsequent effect on the substrate specificity of PfSCS.

Materials and methods

Computational analysis of the SCS subunits

SCS is composed of two subunits. SCS α and SCS β . whereas the SCS β subunit carries the only nucleotide binding site. The amino acid sequences of the SCSB subunits from phylogenetically diverse organisms were retrieved from UniProtKB, and respective details are summarized in Table 1. A multiple sequence alignment (MSA) of these sequences was performed using ClustalO. The alignment output representation was performed by Boxshade server. Weblogos were also generated from the respective alignments of the ADP-forming and GDP-forming SCS to identify the most frequently present gatekeeper residues. After identification of the gatekeeper residues from the MSA, various mutants were designed in an attempt to alter the charge of the gatekeeper residues; details are summarized in Table 2. Structure models were generated for the wild-type (WT) and various mutant PfSCSB subunits by using MODELER 9v13 (University of California San Francisco, CA, USA), with the following templates, E. coli SCS [Protein Data Bank (PDB): 1COI] [12] and pig SCS (PDB: 2FP4) [13]. The models were further analyzed by Ramachandran scatterplots and DOPE

Table 1. List of ADP-forming and GDP-forming SCS β subunits from various organisms with their UniProt IDs. The gatekeeper residues in bold and shaded rows have been used for comparison in previous studies also [7].

S. No.	Organism	UniProt IDs of SCSβ	Gatekeeper residues	
1 2 3 4 5 6 7 8 9	P. falciparum Blastocystis E. coli Toxoplasma gondii Leishmania major Homo sapiens Arabidopsis thaliana Bos Taurus (bovine) C. elegans	Q8ILE9 B3FHPO B7M5P1 Q1KSE5 Q4Q1C4 Q9P2R7 O82662 Q148D5 P53588 D45124	DY KK PD DF KG DY ES DY DF	ADP-
10 11 12 13 14 15 16 17	H. Influenzae Mus musculus (mouse) Mycobacteriaceae Oryza (rice) S. cerevisiae Sus scrofa (pig) Drosophila melanogaster Rattus norvegicus (rat)	A3Q5P5 Q6K9N6 P53312 Q97580 Q9VHJ8 F1LM47	KD DY PD ES KD DY NF DY	forming
18 19 20 21	Homo sapiens Sus scrofa (pig) Bos taurus (bovine) Columba livia (pigeon)	Q96I99 P53590 Q3MHX5 Q9YI36	ED ED ED EN	← GDP- forming

KK (GM-2 KK)

DE (GM-3 DE)

ED (GM-4 ED)

Gatekeeper mutant-3

Gatekeeper mutant-4

nucleotides (bold and italics) in the respective mutants. FP, forward primer; RP, reverse primer.						
Gatekeeper residues of <i>Pf</i> SCSβ (bold and italics)	Primers					
<i>Pf</i> SCSβ WT <i>DY</i> (WT-DY)	No mutation	FP: 5'-TAT <u>GGATCCA</u> TGGCCCGTTTTAAGAGCC-3' [BamHI]				
		RP: 5'-A'I'I' <u>GICGAC</u> ITAAACGAGATGICTATG-3' [<u>Sall]</u>				
Gatekeeper mutant-1	$D \rightarrow K$ at 95 position	FP: 5'-TGGTGATAAT <u>aag</u> TTAGTAATAAAAGCTC-3'				
KY (GM-1 KY)		RP: 5'-CAAACGTTTTGTAATAATAAAGC-3'				
Gatekeeper mutant-2	$Y \rightarrow K$ at 164 position	FP: 5'-GAACGTTTTT <i>aag</i> TAAGAAAAGAAAGATATATTGC-3'				

Table 2. Nomenclature of the WT and various gatekeeper mutants of *Pf*SCSβ subunits and the respective primer sequences used for cloning. Restriction enzymes are italicized and underlined for the WT *Pf*SCSβ subunit. Mutations are represented by underlined lowercase nucleotides (bold and italics) in the respective mutants. FP, forward primer; RP, reverse primer.

scores. The electrostatic surfaces of the gatekeeper regions were also constructed using eF-surf server and visualized using PDBjViewer [14].

 $\boldsymbol{Y} \rightarrow \boldsymbol{E}$ at 164 position

positions, respectively

 $\boldsymbol{D} \rightarrow \boldsymbol{E}$ at 95 and $\boldsymbol{Y} \rightarrow \boldsymbol{D}$ at 164

Determination of the nucleotide specificity of native *Pf*SCS enzyme

The nucleotide specificity of the native *Pf*SCS enzyme was determined from the lysate of the cultured *P. falciparum* strain 3D7, as described earlier [15]. In brief, the parasites were grown in human erythrocytes using 2% hematocrit in RPMI-1640 supplemented with 10% human serum. The lysate was prepared by saponin lysis and ultrasonication of the cultured parasites, centrifuged at 25,000 *g* for 15 min at 4 °C. The supernatant was collected, and enzymatic assays were performed as described earlier [6]. In brief, the supernatant containing the native *P. falciparum* SCS was added to the assay buffer [129 μ M CoA, 10 mM sodium succinate, 50 mM KCl, 10 mM MgCl₂ and 50 mM Tris–HCl (pH 7.4)] with respective nucleotide substrates (ATP and GTP, 150 μ M each). The assay recorded the formation of a thioester bond in succinyl-CoA at 232 nm.

Cloning, recombinant protein expression and refolding of *Pf*SCS

The *Pf*SCS β WT subunit was amplified using the primer sequences given in Table 2. The amplified *Pf*SCS β gene was ligated in expression vector pET28a vector (Novagen, Merck KGaA, Darmstadt, Germany) with 6X His-tag, using appropriate restriction sites and transformed into *E. coli* (DH5 α cells). For recombinant protein expression, the *Pf*SCS β + pET28a construct was transformed into *E. coli* BL21-CodonPlus® competent cells. The *Pf*SCS β gatekeeper mutants were generated by a commercially available Q5 site-directed mutagenesis kit (New England Biolabs, MA, USA) and confirmed by sequencing of the constructs for desired mutations at respective positions. The respective primer sequences for substituting the codons are mentioned in Table 2. Despite multiple efforts, it was not possible to clone the $P/SCS\alpha$ subunit; hence the *Blastocystis* SCS α subunit (having >60% identity with $P/SCS\alpha$) was chosen to generate the refolded P/SCS enzyme.

RP: 5'-ACATATAAATACAGTATTACATTTTTTTC-3'

RP: 5'-ACATATAAATACAGTATTACATTTTTTTC-3'

FP: 5'-TGGTGATAATgagTTAGTAATAAAAG-3'

RP: 5'-CAAACGTTTTGTAATAATAAAGC-3'

FP: 5'-GAACGTTTTTgagTAAGAAAAGAAAGATATATTGCTTTTC-3'

The protein expression was carried out using standard protocols, optimized in the laboratory [7]. In brief, the overexpression of the cloned PfSCSB subunit was induced by the addition of 1 mM IPTG after the A values reached 0.4-0.6, and was grown for 4 h postinduction. The bacterial cell pellets were reconstituted in lysis buffer [50 mM NaH₂PO₄, 10 mM Tris, 500 mM NaCl, 10 mM imidazole (pH 8.0)] and sonicated. Centrifugation at 25,000 g for 30 min at 4 °C yielded the supernatant and cell debris pellet. The pellet was further processed for isolation of inclusion bodies (IBs) containing the 6X-His-tagged PfSCSB subunits, washed twice with 1M urea and 1% Triton X-100, and finally with 1 M urea alone. The IBs were solubilized in solubilization buffer containing 6 м guanidine hydrochloride and 10 mM Tris-HCl (pH 8.0) overnight. The purification of $PfSCS\beta$ was carried out by a custompacked column with Ni-NTA resin (Nucleopore; Genetix Biotech Asia, Delhi, India) using a fast-process liquid chromatography system, AKTA Prime, FPLC (GE Life Sciences, MA, USA). The elutions were collected from the 200 mM imidazole fractions and analyzed by SDS/PAGE. The P/SCS β subunit was confirmed by western blotting using a commercially available mouse monoclonal antibody raised against 6X-His-tag (Sigma-Aldrich, Merck KGaA, Darmstadt, Germany). As mentioned previously, the Blastocystis SCSa subunit was used at the time of refolding with $PfSCS\beta$ subunit [7]. Both the subunits were again denatured in the solubilization buffer and concentrated using 10 kDa cutoff Centricons (Vivaspin). Optimized refolding was performed in buffer [50 mM Tris-HCl, 25% glycerol, 25 mM DTT and 100 µM MgCl₂ (pH 7.2)] with rapid dilution (100-fold) of the respective subunits in 1:1 ratio and incubated overnight at 4 °C. The refolded PfSCS

enzymes were again concentrated with a 10 kDa cutoff Amicon stirred-cell (Millipore, Merck KGaA, Darmstadt, Germany) and centrifuged at 14 500 r.p.m. for 15 min at 4 °C, to remove precipitated/misfolded proteins, before performing the enzymatic assays.

Enzyme kinetics of the *Pf*SCS (WT and various gatekeeper mutants)

Enzymatic assays were performed with optimized conditions in buffer [10 mM sodium succinate, 50 mM KCl, 10 mM MgCl₂ and 50 mM Tris–HCl (pH 7.4)]. One hundred twenty-nine micromolar CoA and ~30 nM refolded *PfSCS* enzymes (WT and various gatekeeper mutants) were added in each reaction mix. Varying concentrations of ATP and GTP were used to carry out the enzymatic reaction. The product formation was followed for 10 min with 1-min intervals. A UV-absorbance at 232 nm was recorded in the quartz cuvette of 10-mm path length corresponding to the formation of a thioester bond in succinyl-CoA. The enzyme kinetics results were analyzed to calculate the Michaelis– Menten constant (K_m) by using (GRAPHPAD PRISM, CA, USA) 5.0 software.

Results

Sequence and molecular modeling analysis of the various SCSβ subunits

The MSA of SCS β subunit sequences from various organisms is presented in Fig. 1A, and the respective gatekeeper residues are shaded. Among the ADPforming SCS β subunits, the gatekeeper residues are listed in Table 1. Human intestinal parasite Blastocystis SCS has Lys and Lys (positively charged) gatekeeper residues, whereas PfSCS has Asp and Tyr (negatively charged and hydrophobic) gatekeeper residues. Another apicomplexan parasite. Toxoplasma gondii, also has the negatively charged and hydrophobic gatekeeper residues (Asp and Phe), but Leishmania major has positive and nonpolar (Lys and Gly) gatekeeper residues. Two representative plant species, Arabidopsis and Oryza, have negatively charged and polar/uncharged gatekeeper residues (Glu and Ser, respectively). The ADP-forming SCSB subunits of Homo sapiens, Bos taurus, Mus musculus and Sus scrofa have the similar gatekeeper residues as P. falciparum (Asp and Tyr); however, the GDP-forming SCSB subunits of H. sapiens, B. taurus and S. scrofa have the negatively charged gatekeeper residues, Glu and Asp. The weblogos demonstrated that the most common gatekeeper residues among the ADP-forming SCS β subunits are Asp and Tyr (Fig. 1B), whereas in the GDP-forming SCS β subunits,

the most frequently present gatekeeper residues are Glu and Asp (Fig. 1C). From the MSA, we have designed various gatekeeper mutants of the $PfSCS\beta$ subunit, particularly to alter the charge at the gatekeeper region (Table 2).

The molecular models of PfSCSß subunits from WT and various gatekeeper mutants were generated, and further electrostatic surfaces were constructed for all the models. The snapshots of the gatekeeper region of the $PfSCS\beta$ subunits are represented in Fig. 2. The PfSCSB WT-DY carried the negatively charged and polar gatekeeper residues (Asp and Tyr), and hence the corresponding gatekeeper region represents the negative and polar character (Fig. 2A). E. coli SCSB subunit displayed the gatekeeper region as negative and nonpolar as a result of Pro and Asp residues at the gatekeeper region (Fig. 2F). GM-1 KY and GM-2 KK were constructed by sequential substitutions of Asp \rightarrow Lys and Tyr \rightarrow Lys, respectively, which are indicated by the presence of positive charge at the gatekeeper region (Fig. 2B,C). Other gatekeeper mutants, GM-3 DE and GM-4 ED, both carried the negative gatekeeper residues, whereas it is only the latter that emulated the negatively charged Glu and Asp from the pig SCSβ subunit (Fig. 2D,E). Interestingly, the gatekeeper region did not show the negatively charged gatekeeper region as intense as it did in pig SCSB (Fig. 2G) [6].

Determination of the nucleotide specificity of native and recombinant *Pf*SCS enzymes

The nucleotide specificity of P/SCS was determined from the crude lysate of *in vitro*-cultured *P. falciparum* using the enzymatic assay, as described by Hamblin *et al.* [6]. In accordance with the previous assumption, because of the presence of negative and hydrophobic gatekeeper residues of the *E. coli* SCS β subunit, the *Pf*SCS enzyme should use both nucleotides (ATP and GTP). However, the native *Pf*SCS enzyme was found to be predominantly ADP forming, having some insignificant activity with the GTP (Fig. 3).

Recombinant protein expression was carried out in *E. coli* (BL21DE3) cells for all the *Pf*SCS β subunits, including the WT and its various gatekeeper mutants. The affinity chromatography-purified fractions of *Pf*SCS β subunits from the IBs were analyzed by SDS/PAGE (Fig. 4B–E), and as mentioned previously, the 6X-His-tagged *Blastocystis* SCS α was purified separately in native conditions by affinity chromatography (Fig. 4A). The *Pf*SCS β WT-DY and the *Blastocystis* SCS α subunits were confirmed by western blot

ADP_Toxoplasma 105 EAATFLESESPSCHEEPUETVUKAQULAGGRAGUGFFENDUGGGUQUCESPEVUETVAEMM ADP_Leishmani S3 ACAK	ADP_	P.falciparu 83	KALLLQNVCGDNDLVIKAQVLSGGRGVGYFKENNFEGGVHVCRNSMEVKEIATKM
ADP_Leishmania 63 ACAKIKTEKKVVKSQILAGGRAGUFEF-SGLKGGVHVDBAAAAVEAAAVEAAKHM ADP_Labidopsis 60 AlQDVFPOSKUVVIKAQVLAGGRAGTEF-SGLKGGVHVDFPDEARAFASSGM ADP_Labidopsis 60 AlQDVFPOSKUVVIKAQVLAGGRAGTEF-SGLKGGVHVDFPDEARAFASSGM ADP_Basscorysi 40 IAKK-LGSKUVVIKAQVLAGGRAGTEF-SGLKGGVHVDFSPDEARAFASSGM ADP_C.elegans 57 EAKRIGSKUVVIKAQVLAGGRAGTEF-SGLKGGVKVVFSPDEARAFASSGM ADP_C.elegans 57 EAKRIGSKUVVIKAQVLAGGRAGTEF-SGLKGGVKVVFSPDEARAFASSGM ADP_K.influenza 34 VLAQLSGCKWAAKCQVHAGGRAGTFF-SGLKGGVKVVFSPDEARAFASSGM ADP_Ms 86 IAKK-LGSKUVVIKAQVLAGGRAGTFF-SGLKGGVKVVFSPDEARAFASSGM ADP_Ms 86 IAKK-L	ADP	Toxoplasma 105	EAATFLSESPSGDGEPVDFVVKAQVLAGGRGLGFFRENGYQGGVQVCESPREVGIVAEKM
ADP_ruman %6 IAKKLOSKUVVIKAQVLAGGRGKGFFF-SGLKGGVKIVFSPEEAKAVSSQM ADP_slastocysti 48 IAKK-MOFFPGGKEVVKAQVLAGGRGKGFF-SGLKGGVKIVFSPEEAKAVSSQM ADP_slastocysti 48 IAKK-MOFFGSKUVVIKAQVLAGGRGKGFF-SGLKGGVKINFSPEEAKAVSSQM ADP_c.elegans 57 EAKRIGSKUVVIKAQVLAGGRGKGFFS-SGLGGVVIVFSPEEAKAVSSQM ADP_t.olluena 34 VLQLSGCKAAKGVLAGGRGKGFFS-SGLGGVVIVFSPEEAKAVSSQM ADP_t.olluena 34 VLQLSGCKAAKGVLAGGRGKGFFS-SGLGGVVIVFSPEEAKAVSSQM ADP_Mis 66 IAKKLGSKUVVIKAQVLAGGRGKGFFS-SGLGGVKIVFSPEEAKAVSSQM ADP_Mis 66 IAKKLGSKUVVIKAQVLAGGRGKGFFS-SGLGGVKIVFSPEEAKAVSSQM ADP_Mis 66 IAKKLGSKUVVIKAQVLAGGRGKGFFS-SGLGGVKIVFSPEEAKAVSSQM ADP_Mis 66 IAKK-LGSKUVVIKAQVLAGGRGKGFFS-SGLGGVKIVFSPEEAKAVSSQM ADP_Sis 64 AAKK-LGSKUVVIKAQVLAGGRGKGFFS-SGLGGVKIVFSPEEAKAVSSQM ADP_Sis 64 IAKK-LGSKUVVIKAQVLAGGRGKGFFS-SGLGGVKIVFSPEEAKAVSSQM ADP_Sis 64 IAKK-LGSKUVVIKAQVLAGGRGKGFFS-SGLGGVKIVFSPEEAKAVSSQM ADP_Sis 72 AAKK-LGSKUVVIKAQVLAGGRGKGFFS-SGLGGVKIVFSPEEAKAVSSQM ADP_Sis 72 AAKK-LSKUVVIKAQVLAGGRGKGFFS-SGLKGGVKIVFSPEEAKAVSSQM GDP_Human 71 AAKR-LNAKUVIKAQILAGGRGKGFFS-SGLKGGVHITKDPHVVQLAKQM GDP_Sis 72 AAKR-LNAKUVIKAQILAGGRGKGFFS-SGLKGGVHITKDPHVVQLAKQM GDP_Sis 71 AAKR-LNAKUVIKAQILAGGRGKGFFS-SGLKGGVHITKDPHVVQLAKQM GDP_Sis 71 AAKR-LNAKUVIKAQILAGGRGKGFFS-SGLKGGVHITKDPHVVQLAKQM GDP_Columba 30 AAQR-LNAKUVIKAQILAGGRGKGFFS-SGLKGGVHITKDPHVVQLAKQM GDP_Sis 71 AAKR-LNAKUVIKAQILAGGRGKGFFS-SGLKGGVHITKDPHVVQLAKQM GDP_Sis 71 AAKR-LNAKUVIKAQILAGGRGKGFFS-SGLKGGVHITKDPHVVQLAKQM GDP_Sis 71 AAKR-L	ADP	Leishmania 53	ACAKIKTEKKVVKSQILAGGRGKGVFK-DGFQGGVHVCDSAAAAVEAAKHM
ADP_Arabidopsis 60 ATODVPPPGCKFUVKSQLLAGGRGKGTHF-SGLKGGVKIVKR-DEAEEIAGKM ADP_Bos 60 IAKKLGCKFUVVKAQULAGGRGKGTHF-SGLKGGVKIVKFSPEAKAVSSQM ADP_C.elegans 57 EARKLGCKGUVVKAQULAGGRGKGTF-SGLKGGVKIVFSPEAKAVSSQM ADP_E.coll 34 AASKLGCKGUVVKAQULAGGRGKGFFSGLKGGVKIVFSPEAKAVSSQM ADP_MIN 84 IAKKLGCKGUVVKAQULAGGRGKGFFAGGVKLWQDUEEARFAEKW ADP_MIN 84 IAKKLGSKGUVAACGAGKAGGVKIVGVNHSESDAGADV ADP_MIN 84 IAKKLGSKGUVAACGAGKAGGVKIVFSPEEAKAVSSQM ADP_Grysa 61 TLKNVFPGSKGUVVKAQUKAGGRGKFFF-SGLKGGVKIVFSPEEAKAVSSQM ADP_Grysa 61 TLKNVFPSKFIVVKSQLLAGGRGKGFFF-SGLKGGVKIVFSPEEAKAVSSQM ADP_Grysa 61 TLKNVFPGSKGUVIKAQULAGGRGKGFFF-SGLKGGVKIVFSPEEAKAVSSQM ADP_Grysa 61 TLKNVFPGSKGUVIKAQULAGGRGKGFFF-SGLKGGVKIVFSPEEAKAVSSQM ADP_Grysa 61 TLKNVFPGSKGUVIKAQULAGGRGKGFFF-SGLKGGVKIVFSPEEAKAVSSQM ADP_Grysa 71 IAKKLGSKGUVIKAQULAGGRGKGFFF-SGLKGGVKIVFSPEEAKAVSSQM GDP_SUN 71 AAKKLNAFEIVLKAQULAGGRGKGFFF-SGLKGGVKIVFSPEEAKAVSSQM GDP_SUN 71 AAKKLNAFEIVLKAQULAGGRGKGVFN-SGLKGGVKIFNGEKKVVGQLAKQM GDP_SUN 71 AAKKLNAFEIVLKAQULAGGRGKGVFN-SGLKGGVKIFKDEVVGQLAKQM GDP_Columba 30 AAQRLNAFEIVLKAQULAGGRGKGVFN-SGLKGGVKIFKDEVVGQLAKQM GDP_Columba 136 LUNILITKQSGPEGKKCNTVFICERF-YIRKEKYVAILMDRGAGGPILIGSARGGTSIED ADP_Leishnania 136 LUNILITKQSGPEGKKCNTVFICERF-YIRKEKYVAILMDRGAGGPILIGSARGGTSIED ADP_Arabidopsis 111 LOQUVKTGGFEGKGLNVKUVCERK-YFRREYYFAITMERSFQGPVLIGSSGGVNIED ADP_SN 136 IGKKLFTKQTGEKGRICNQVUVCERK-YFRREYYFAITMERSFQGPVLIGSSGGVNIED ADP_SN 136 IGKKLFTKQTGEKGRICNQVUVCERK-YFRREYYFAITMERSFQGPVLIGSSGGVNIED ADP_SN 136 IGKKLFTKQTGEKGRICNQVUVCERK-YFRREYYFAITMERSFQGPVLIASSGGVNIED ADP_SN 136 IGKKLFTKQTGEKGRICNQVUVCERK-YFRREYYFAITMERSFQGPVLIASSGGVNIED ADP_SN 136 IGKKLFTKQTGEKGRICNQVUVCERK-YFRREYYFAITMERSFQGPVLIASSGGVNIED ADP_SN 136 IGKKLFTKQTGEKGRICNQVUVCERK-YFRREYYFAITMERSFQGPVLIASSGGVNIED ADP_SN 136 IGKKLFTKQTGEKGRICNQVUVCERK-YFRREYYFAITMERSFQGPVLIASSGGVNIED ADP_SN 136 IGKKLFTKQTGEKGRICNQVUVCERK-YFRREYYFAITMERSFQGPVLIASSGGVNIED ADP_SN 136 IGKKLFTKQTGEKG	ADP	Human 86	IAKKLGSKDVVIKAQVLAGGRGKGTFE-SGLKGGVKIVFSPEEAKAVSSQM
ADP_stastocysti 48 IAKLNNOFOCKFUVKAQULAGGRGKGH-HONOGUNLAKTPEEVYEIANEM ADP_sos 66 IAKKCGKUVVKAQULAGGRGKGTFS-SGLQGGVQIVFTPDEVKQKAGGM ADP_C.elegans 57 EAKRIGGKUVVKAQULAGGRGKGFS-SGLQGGVQIVFTPDEVKQKAGGM ADP_E.coli 34 AASKIGGKUVVKAQULAGGRGKGFAGGVKUVNSKEDIRAFAENM ADP_Mis 86 IAKKLSGGKAAACQUHAGGRGKAGGVKUVQNEEDRAFAENM ADP_Mis 86 IAKKLSGGKUVKAQULAGGRGKGFAGGVKUVQNEEDRAFAENM ADP_Mis 86 IAKKLSGKUVKAQULAGGRGKGFSGLKGGVKIVGYBEEAKAVSSQM ADP_S.cerevisia 64 AAKKLGKUVVKAQUVLAGGRGKGFFSGLKGGVKIVFSPEEAKAVSSQM ADP_Sus 61 IAKKLGSKUVVIKAQULAGGRGKGFF-SGLKGGVKIVFSPEEAKAVSSQM ADP_Sus 61 IAKKLGSKUVVIKAQULAGGRGKGFFF-SGLKGGVKIVFSPEEAKAVSSQM ADP_Sus 61 IAKKLGSKUVVIKAQULAGGRGKGFFF-SGLKGGVKIVFSPEEAKAVSSQM ADP_Sus 61 IAKKLGSKUVVIKAQULAGGRGKGFFF-SGLKGGVKIVFSPEEAKAVSSQM ADP_Dorsophila 71 IATKLGSKUVVIKAQULAGGRGKGFFF-SGLKGGVKIVFSPEEAKAVSSQM GDP_Bus 71 AAKRLMAFEIVLKAQILAGGRGKGFFF-SGLKGGVKIVFSPEEAKAVSSQM GDP_Sus 72 AAKRLMAFEIVLKAQILAGGRGKGFFF-SGLKGGVHLFKDPKVVGQLAKQM GDP_Sus 71 AAKRLMAFEIVLKAQILAGGRGKGVFS-SGLKGGVHLKDPKVVGQLAKQM GDP_Columba 30 AAQRLMAFEIVLKAQILAGGRGKGVFS-SGLKGGVHLKDPKVVGQLAKQM GDP_Columba 103 LONILVIKQTGFGGKUNVICGXKIKEFYIJAILDBRAAGGPIILGSAGGMSIEE ADP_Leishmania 103 LONILVIKQTGFGGKUNVICCKL-SUNGHYJFILDRISAGPHFIGSAEGGMSIEE ADP_Leishmania 103 LONILVIKQTGFGGKUNVICCKL-SUNGHYJFILDRISAGPHFIGSAEGMSIEE ADP_Leishmania 104 LONILVIKQTGFGGKUNVICCKL-SUNGHYJFITMERSFQGPVLIGSSHGGVNIED ADP_C.elegans 107 IGAKLFKQTGEKGRINQUVICCKR-YFRREYYFAITMERSFQGPVLIGSSHGGVNIED ADP_C.elegans 107 IGAKLFKQTGEKGRINQUVICCKR-YFRREYYFAITMERSFQGPVLIGSSHGGVNIED ADP_Leishmania 104 LONILVIKQTGFQGVNICCKL-SUNGHYFYFAITMERSFQGPVLIGSSHGGVNIED ADP_S.ceii 77 LGKRLFFKQTGEKGRINQUVICCKR-YFRREYYFAITMERSFQGPVLIGSSHGGVNIED ADP_S.ceii 77 LGKRLFFKQTGEKGRINQUVICCKR-YFRREYYFAITMERSFQGPVLIGSSHGGVNIED ADP_S.ceii 114 LUQUVIKQTGFQGKNYSVICKEN-TFREYFYFAITMERSFQGPVLIGSSHGGVNIED ADP_S.se 96 IGKKLFFKQTGEKGRINQUVICCKR-YFRREYYFAITMERSFQGPVLIGSSQGWNIED ADP_S.se 96	ADP	Arabidopsis 60	AIQDVFPNESELVVKSQILAGGRGLGTFK-SGLKGGVHIVKR-DEAEEIAGKM
ADD Bos 66 IAKK-LGSKDVVIKAQULAGGRGKDES-SGLGGVVIVTSPEEAKAVSSQM ADD C.elegars 57 EAKRIGSKDVVIKAQUAGGRGKDES-SGLGGVVIVTSPEEAKAVSSQM ADP E.coli 34 AASKIGSGKWAVKCQVHAGGRGKAGGVKVVNSKEDIRAFAENW ADP H.influenza 34 VLAQLSGGKWAAKCQVHAGGRGKOTTGGKGVVIVTSPEEAKAVSSQM ADD Mycobacteri 34 IAEEGSKDVVIKAQUAGGRGKOTTGGKGVVIVTSPEEAKAVSSQM ADD Mycobacteri 34 IAEEGSKDVVIKAQUAGGRGKOTTGGKGVVIVTSPEEAKAVSSQM ADD Mycobacteri 34 IAEEGSKDVVIKAQUAGGRGKOTFGGLGGVVIVTSPEEAKAVSSQM ADD Dysos 61 TLKNVFPSGKDVVIKAQUAGGRGKOTFGGLGGVVIVTSPEEAKAVSSQM ADP Drosophi 71 IATKLSGKDVVIKAQUAGGRGKOTFSGLGGVVIVTSPEEAKAVSSQM ADP Drosophi 71 IATKLSGKDVVIKAQUAGGRGKOTFSGLKGGVVIVTSPEEAKAVSSQM ADP Bactus 88 IAKKLGSKDVVIKAQUAGGRGKOTF-SGLKGGVVIVTSPEEAKAVSSQM GDD Human 71 AAKRLNAKEIVLKAQUAGGRGKOFF-SGLKGGVVILTSPEEAKAVSSQM GDP_Sus 72 AAKRLNAKEIVLKAQILAGGRGKOFF-SGLKGGVHLTKDPEVVGQLAKQM GDP_Sus 72 AAKRLNAKEIVLKAQILAGGRGKOFF-SGLKGGVHLTKDPEVVGQLAKQM GDP_Cclumba 30 AAQRLKAKEIVLKAQILAGGRGKOFF-SGLKGGVHLTKDPEVVGQLAKQM GDP_Cclumba 136 LOKILVIKQGFEGKKCNTVFICERF-FIRKEXYIAFLLDRNSDGILLGSSIGGSSIED ADP_Toxoplasma 145 LOKILVIKQGFEGKKCNTVFICERF-FIRKEXYIAFLLDRNSDGILLGSSIGGSSIED ADP_Dsishmania 136 IGKKJFTKQTGKEGKGINONVVUCEK-YPREYYFATIMERSFQGFVLIGSSHGGVNIE ADP_Blastocysti 102 IGHKLITKQTGEKGRINONVVUCEK-YPREYYFATIMERSFQGFVLIGSSHGGVNIE ADP_Blastocysti 102 IGHKLITKQTGEKGINONVVUCER-YPREYYFATIMERSFQGFVLIGSSHGGVNIE ADP_Scererisa 146 IGKKJFTKQTGEKGRINONVVUCER-YPREYYFATIMERSFQGFVLIGSSHGGVNIE ADP_Scererisa 147 LGQUVUFGTPGCVVVVUCER-YPREYYFATIMERSFQGFVLIGSSHGGVNIE ADP_Scererisa 146 IGKKJFTKQTGEKGRINONVVUCER-YPREYYFATIMERSFQGFVLIGSSHGGVNIE ADP_Scererisa 147 LGQUVIKGTPGCKVVYNVUCER-YPREYYFATIMERSFQGFVLIGSSHGGVNIE ADP_Scererisa 146 IGKKJFTKQTGEKGRINONVVUCER-YPREYYFATIMERSFQGFVLIGSSHGGVNIE ADP_Scererisa 146 IGKKJFTKQTGEKGRINONVVUCER-YPREYYFATIMERSFQGFVLIGSSQGGNNIE ADP_Nycobacteri 76 LGCDIKHVVKKLLVAEAS-DIAEFYTISTLDDAWRTYMATLACSVCGGVUIEE ADP_Scereris	ADP	Blastocysti 48	IAKKLWNQFPGCKFVVKAQVLAGGRGKGHWE-HGMQGGVKLAKTPEEVYEIANEM
ADP C.elegans 57 EARR-IGGCD VUVEAQULAGGRGKGAGGVKUVKSUKAGK ADP E.eoli 34 AASK-IGGCDVVVKCQUAGGRGKAGGVKUVKSUKEIDAFAFENW ADP Mus 6 TARK-LGSKDVVIKQULAGGRGKGAGGVKUVKSUFENEN ADP Mus 6 TARK-LGSKDVVIKQULAGGRGKGTAGGVKLVQUVEEARAFAEKW ADP Mus 6 TARK-LGSKDVVIKQULAGGRGKGTAGGVKLVATADDAFTHAQNI ADP S.erevisia 64 AAKK-LGSKDVVIKQULAGGRGKGTF-SGLKGGVKIVTSPEEARAVSSQM ADP Dryza 61 TLKNVFPSEKEVVKSQLAGGRGGTFF-SGLKGGVKIVTSPEEARAVSSQM ADP Drosophila 71 TARK-LGSKDVVIKAQVLAGGRGKGTFF-SGLKGGVKIVTSPEEARAVSSQM ADP Drosophila 71 TARK-LGSKDVVIKAQVLAGGRGKGTFF-SGLKGGVKIVTSPEEARAVSSQM GDP Muman 71 AAKR-LGSKDVVIKAQVLAGGRGKGTFF-SGLKGGVKIVTSPEEARAVSSQM GDP Muman 71 AAKR-LNAREIVLKAQILAGGRGKGTF-SGLKGGVHIKDPNVVQLAKQM GDD Bos 71 AAKR-LNAREIVLKAQILAGGRGKOVFS-SGLKGGVHIKDPNVVQLAKQM GDD Bos 71 AAKR-LNAREIVLKAQILAGGRGKOVFS-SGLKGGVHIKDPNVVQLAKQM GDP Columba 30 AAQR-LNAREIVLKAQILAGGRGKOVFS-SGLKGGVHIKDPNVVQLAKQM GDP Leishmani 103 LUNTLITKQSGPEGKKCNTVFICERF-YIRKERYTAFLLDRNSDGIILLGSSIGGSSIED ADP Trooplasma 165 LGKTUVTQTGKEGKGLCNRVUVDEFF PIRKEKYTAFLLDRNSDGIILGSSIGGSSIED ADP Arabidopsis 111 LQUVINGTGFEGKKCNTVFICERF-YIRKERYTAFLLDRNSDGIILGSSAGGWIEE ADP Muman 136 IGKKLFTKQTGEKGRICNQVUVCERA-YPREYYFAITMERSFQGPVLIGSSHGGVNIED ADP Arabidopsis 111 LQUVINGTGAGGNGVVNVCUCAR-YPREYYFAITMERSFQGPVLIGSSHGGVNIED ADP SLOOI 77 LGKNLTKQTGEKGRICNQVUVCERA-YPREYYFAITMERSFQGPVLIGSSHGGVNIED ADP SLOOI 77 LGKNLTKQTGEKGRICNQVUVCERA-YPREYYFAITMERSFQGPVLIGSSQGVNIED ADP SLOOI 76 LGKLTKQTGEKGRICNQVUVCERA-YPREYYFAITMERSFQGPVLIGSSQGVNIED ADP SLOOI 76 LGKLTKQTGEKGRICNQVUVCERA-YPREYYFAITMERSFQGPVLIGSSQGVNIED ADP SLOOI 76 LGKLTKQTGEKGRICNQVUVCERA-YPREYYFAITMERSFQGPVLIGSSQGVNIED ADP SLOS 98 IGKLFFKQTGEKGRICNQVUVCERA-Y	ADP	Bos 86	IAKKLGSKDVVIKAQVLAGGRGKGTFE-SGLKGGVKIVFSPEEAKAVSSQM
ADP_E.coli 34 AASKIGAGWWVKCQUHAGGRGKAGGVKUVDSKEDIRAFAENW ADP_H.influenza 34 VLAQLGSKDVVIKAQUKAGGRGKAGGVKUVDUEERAFAESKW ADP_Mycobacteri 34 IAEEGSKDVVIKAQUKAGGRGKGTFT-SGLKGGVKIVFSPEAKAVSSQM ADP_Mycobacteri 34 IAEEGSKDVVIKAQUKAGGRGKGTF-SGLKGGVKIVFSPEAKAVSSQM ADP_Oryza 61 TLKNVFPSEKBIVVKSQILAGGRGKGFF-SGLAGGVKIVFSPEAKAVSSQM ADP_Sus 40 IAKKLSEKBIVVKSQILAGGRGKGTFF-SGLAGGVKIVFSPEAKAVSSQM ADP_Dosophila 11 IATKLSKDVVIKAQULAGGRGKGTFF-SGLAGGVKIVFSPEAKAVSSQM ADP_Battus 88 IAKKLSKDVVIKAQULAGGRGKGTF-SGLAGGVKIVFSPEAKAVSSQM GDP_Muman 71 IAKKLSKDVVIKAQULAGGRGKGTF-SGLAGGVKIVFSPEAKAVSSQM GDP_Sus 72 AAKRLNAKBIVLKAQULAGGRGKGVFS-SGLKGGVHLTKDPEVVQGLAKQM GDP_Sus 72 AAKRLNAKBIVLKAQILAGGRGKGVFS-SGLKGGVHLTKDPEVVQGLAKQM GDP_Columba 30 AAQRLNAKBIVLKAQILAGGRGKGVFS-SGLKGGVHLTKDPEVVQGLAKQM GDP_Columba 105 LGKTLVTKQTGEGRGKCVFV-SGLKGGVHLTKDPKVUQGLAKQM GDP_Columba 106 LGKTLVTKQTGEGRGKUVFI-SGLKGGVHLTKDPKIVEQLAKQM GDP_Leishmania 103 LGKNLTVKQTGEGRGKUVFS-SGLKGGVHLTKDPKIVEQLAKQM ADP_Noroplasma 165 IGKKLFTKQTGERGRCUVVLVTERF-FIRKEKYVAILMDRGAGGPLLIGSSIGGSSIED ADP_Leishmania 103 LGKNLTVKQTGEGRGKUVVLVEEK-YPRITMELSTGGVULGSARGGTSIED ADP_Blastocysti 102 IGHKLITKQTGERGRCUVVLVCERF-YERKEYVAILMERSFGGVLIGSARGGTSIED ADP_Sis 111 LGQULVTKQTGERGRCUVVLVCERF-YERKEYYRAITMERSFGGVLIGSARGGMSIEE ADD_Blastocysti 102 IGHKLITKQTGARGINGUVUCERF-YERKEYYRAITMERSFGGVLIGSARGGMSIED ADP_Sis 116 GGKLITKQTGARGINGUVUCERF-YERREYYRAITMERSFGGVLIGSARGGMSIED ADP_Sis 116 IGKKLFTKQTGERGRICUQULVCERF-YERREYYRAITMERSFGGVLIGSARGGMSIED ADP_Sis 116 IGGKLITKQTGERGRICUQULVCERF-YERREYYRAITMERSFGGVLIGSAGGMSIED ADP_Sis 116 IGGKLITKQTGERGRICUQULVCERF-YERREYYRAITMERSFGGVLIGSAGGMIED ADP_Sis 116 IGGKLITKQTGERGRICUQULVCERF-YERREYYRAITMERSFGGVLIGSAGGMIED ADP_Mis 116 IGGKLITKQTGERGRICUQULVCERF-YERREYYRAITMERSFGGVLIGSAGGWIED ADP_Mis 116 IGGKLITKQTGERGRICUQULVCERF-YERREYYRAITMERSFGGVLIGSAGGWIED ADP_Mis 116 IGGKLITKQTGERGRICUQULVCERF-YERREYYRAITMERSFGGVLIGSAGGWIED ADP_Sis 31 IGGKLITKQTGERGRICUQULVCERF-YERREYFANMER	ADP	C.elegans 57	EAKRIGKDYVVKAQVLAGGRGKGRFS-SGLQGGVQIVFTPDEVKQKAGMM
ADD Mus 34 VLAQLSGCKWAARCQVHAGGRGKAGGVKLUQDVEEDARFAERW ADD Mus 86 IAKKLSGCKWAARCQVHAGGRGKGTFT-SGLKGGVKLVFSPEEARAVSSQM ADD Mycobacteri 34 IAEESGCWWARQVHGGRGKGTFT-SGLKGGVKLVFSPEEARAVSSQM ADD S.cerevisia 64 AAKKLSGCWWIKAQULAGGRGKGTFD-TGYKSGVHMIESPQQAEDVAREM ADD S.cerevisia 64 AAKKLSGCWUYKAQULAGGRGKGTFD-TGYKSGVHMIESPQQAEDVAREM ADD S.cerevisia 64 IAKKLSGSKDVVIKAQULAGGRGKGTFD-TGYKSGUMHIESPQQAEDVAREM ADD Stas 48 IAKK-LSGSKDVVIKAQULAGGRGKGTFD-TGYKSGURVIFSPEEARAVSSQM ADD Dcosophila 71 IAKKLSGSKDVVIKAQULAGGRGKGTF-SGLKGGVHLTKDPKVVGQLAKQM GDD Human 71 AAKRLNAKBIVLKAQILAGGRGKGVFN-SGLKGGVHLTKDPKVVGQLAKQM GDD Sus 72 AAKRLNAKBIVLKAQILAGGRGKGVFN-SGLKGGVHLTKDPKVVGQLAKQM GDD Columba 30 AAQRLNAKBIVLKAQILAGGRGKGVFN-SGLKGGVHLTKDPKVVGQLAKQM GDD Columba 30 AAQRLNAKBIVLKAQILAGGRGKGVFN-SGLKGGVHLTKDPKVVQLAKQM ADD Leishmania 103 LCHTUTNGTGRGKGKCNVVLVTERF-FIRKEYYAILDRNSDGIILGSARGGTSIED ADD Human 136 IGKKLTRVGTGRKGKUCNVVLVTERF-FIRKEYYAILMENSGAGFUIGSARGGNIED ADD Human 136 IGKKLTRVGTGRKGKICNVVLVCERK-YPREYYFAITMESFQGVLIGSSHGGWNIED ADD Human 136 IGKKLTRVGTGRKGKICNVVLVCERK-YPREYYFAITMESFQGVLIGSSHGGWNIED ADD Mus 106 IGKKLTRVGTGRKGKICNV	ADP_	E.coli 34	AASKIGAGPWVVKCQVHAGGRGKAGGVKVVNSKEDIRAFAENW
ADP_Mus 06 IAKKLGSKÜVVIKAQULAGGRGKOFFT-SGLKGGVKIVFSPERAKAVSSQM ADP_Mycobacteri 34 IAEEGKKÜVYKAQULAGGRGKGFFT-SGLQGGVKIVFSPERAKAVSSQM ADP_Cryra 61 TLKNVFPSEKKIVVKSQILAGGRGKGFFD-TGYKSGVHMIESPQQAEDVAKEM ADP_Oryra 61 TLKNVFPSEKKIVVKSQILAGGRGKGFFF-SGLKGGVKIVFSPERAKAVSSQM ADP_Drosophila 71 IATKLKIKKIVLAGQULAGGRGKGFFF-SGLKGGVKIVFSPERAKAVSSQM ADP_Ratus 08 IAKKLRIKKIVLKAQULAGGRGKGFFF-SGLKGGVKIVFSPERAKAVSSQM GDD_Ruman 71 AAKKLRIKKIVLKAQULAGGRGKGFFF-SGLKGGVKIVFSPERAKAVSSQM GDD_Sus 72 AAKKLNAKKIVLKAQILAGGRGKGVFF-SGLKGGVLIKDPEVVQLAKQM GDD_Sus 72 AAKKLNAKKIVLKAQILAGGRGKGVFF-SGLKGGVLIKDPEVVQLAKQM GDD_Columba 30 AAQRLKAKKIVLKAQILAGGRGKGVFF-SGLKGGVLIKDPEVVQLAKQM GDD_Leishmania 165 LGKLIVKQTGEGGKLCNVVITEEF-FIKKEKYIAFLLDRNSDGIILLGSSIGGSSIED ADP_Leishmania 165 LGKLIVKQTGEGGKLCNVLVTEEF-FIKKEKYIAFLLDRNSDGIILLGSSIGGSSIED ADP_Leishmania 165 IGKKLIVKQTGEGGKLCNVVVVEEF-FIKKEKYIAFLLDRNSDGIILLGSSIGGSSIED ADP_Leishmania 166 IGKKLFTKQTGEGGKLCNVUVCEEF-YIRKERYIAFLLDRNSDGIILLGSSIGGSSIED ADP_Leishmania 166 IGKKLFTKQTGEGGKLCNVUVCEFF-FIKKEYYIAFLLDRNSDGIILLGSSIGGVNIED ADP_Leishmania 166 IGKKLFTKQTGEGGKCNVVVVEEK-YEREYYFAITMERSFQGPVLIGSSHGGVNIED ADP_Leishmania 166 IGKKLFTKQTGEGGKCNVVVCEK-YEREYYFAITMERSFQGPVLIGSSHGGVNIED ADP_Lasidopsis 11 LGQVUTKQTGPKGQLVNCEK-YEREYYFAITMERSFQGPVLIGSSHGGVNIED ADP_Bos 166 IGKKLFTKQTGEGGRICNQVLVCERK-YEREYYFAITMERSFQGPVLIGSSHGGVNIED ADP_Bos 166 IGKKLFTKQTGERGRICNQVLVCERK-YEREYYFAITMERSFQGPVLIGSSHGGVNIED ADP_Lc.elegans 107 IGAKLITKQTGERGRICNQVLVCERK-YEREYYFAITMERSFQGPVLIGSSQGGVNIEE ADP_LS.corisia 114 LINNUTGTARGFVSAVVUKKL-FINNEYYSILDDRNAKVFVMSTEGGVEIEK ADP_LS.CORVISIA 716 LGCLIVKQTGERGRICNQVLVCERK-YEREYYFAITMERSFQGPVLIGSSQGGVNIEE ADP_LS.CORVISIA 716 LGCLIVKQTGERGRICNQVLVCERK-YEREYYFFAITMERSFQGPVLIGSSQGGVNIED ADP_Mus 161 IGQULIVKQTGARGRICNQVLVCERK-YEREYYFFAITMERSFQGPVLIGSSQGGVNIEE ADP_LS.CORVISIA 114 LINNUTKQTGARGRICNQVLVCERK-YEREYYFFAITMERSFQGPVLIGSSQGGVNIEE ADP_SS 91 IKKLFKTGTGEGGRICNQVLVCERK-YEREYYFFILDERNTACPLIACSSQGTSIE	ADP	H.influenza 34	VLAQLSGCKWAAKCQVHAGGRGKAGGVKLVQDVEEARAFAEKW
ADD_Mycobacteri 34 IAEEIGKWUYKAQUKVGGKGKAGGUKYAATADDAFTHAQNI ADP_S.cerevisia 64 AAKKLNTNKLVIKAQALTGGRGKGHFD-TGYKSGVHNIKA-EEAESLAAKM ADD_Oryra 61 TLKNVFPSEKKUVKKQULAGGRGKGTFT-SGLQGGVHVKA-EEAESLAAKM ADD_Sus 48 IAKKLSKKWVIKAQULAGGRGKGTFT-SGLKGGVHVKA-EEAESLAAKM ADD_Ratus 88 IAKKLGSKWVIKAQULAGGRGKGTFT-SGLKGGVHVVSDPGTAEELSSKM ADP_Ratus 88 IAKKLGSKWVIKAQULAGGRGKGTFT-SGLKGGVKVDSPEEAKAVSSQM GDD_Ruman 71 AAKKLNAKKIVLKAQILAGGRGKGVFT-SGLKGGVLIKDPNVVQDQLAKQM GDD_Sus 72 AAKKLNAKKIVLKAQILAGGRGKGVFT-SGLKGGVLIKDPNVVQQLAKQM GDD_Columba 30 AAQRLNAKKIVLKAQILAGGRGKGVFS-SGLKGGVLIKDPNVVQQLAKQM GDD_Columba 30 AAQRLNAKKIVLKAQILAGGRGKGVFS-SGLKGGVLIKDPNVVQQLAKQM GDD_Columba 30 AAQRLKAKKIVLKAQILAGGRGKGVFS-SGLKGGVLIKDPNVVQQLAKQM GDD_Columba 105 LGKILVIKOTGKEGKLCNNVVTECEF-VIRKERVIAFLLDRNSDGILLGSSIGGSSIED ADD_Toxoplasma 165 LGKILVIKOTGKEGKLCNNVVTECEF-VIRKERVIAFLLDRNSDGUILLGSSIGGSSIED ADD_Leishmania 103 LGNILVIKOTGFKGGLVNILVITER-PINKERVVAILMDRGAGGPILIGSARGGTSIED ADD_Human 165 IGKLFIKOTGEKGRICNQVUVCERK-YPRREYVFAILMERSFQGVILGSSMGGNIED ADD_Arabidopsis 111 LGQVLVIKQTGFQGGKVVSKVYLCEKL-SLVNEMYFSILLDRNSAGPILIACKKGGTSIED ADD_Bastocysti 102 IGHKLIFKOTGEKGRICNQVUVCERK-YPRREYVFAILMERSFQGVUIGSSMGGVNIEE ADD_Bos 136 IGKKLFKOTGEKGRICNQVUVCER-YPRREYVFSILLDRNSGVVIIASSQGVNIEE ADD_LS.Coli 77 LGKRLVYGTDANGQFVNQILVERAT-DIAKELYLGAVDRSRRVVFNASIEGGVSIEE ADD_Ms 136 IGKKLFKOTGEKGRICNQVUVCERA-PINKEYVFSILDRNNGFVIIASSQGVNIEE ADD_Ms 136 IGKKLFKOTGEKGRICNQVUVCERA-PINKEYVFSILDRNNGFVIIASSQGCVNIEE ADD_Ms 136 IGKLIFKOTGEKGRICNQVUVCERA-PINKEYVFSILDRNNNGFVIIASSQGCVNIEE ADD_Ms 136 IGKLIFKOTGEKGRICNQVUVCERA-PINKEYVFSILDRNNNGFVIIASSQGCVNIEE ADD_Ms 136 IGQLIFKOTGEKGRICNQVUVCERA-PINKEYVFSILDRNNNGFVIIASSQGCVNIEE ADD_Ms 136 IGQLIFKOTGEKGRICNQVUVCERA-PINKFYFSILDDRNNTAFUIAKSSQGCNIEE ADD_NSSQGSNIEE ADD_NS 98 IGKLKFKOTGEKGRICNQVUVCERA-PINKFYFSILDDRNTKMENTAFSQGCNIEE ADD_SSS 98 IGKKLFKTGTGEKGRICNQVUVCERA-PINKFYFSILDDRNTKMENTAFSQGCNIEE ADD_SSQGSNIEE 138 IGQLIFKOTGAKGRICNQVUVCERA-PINKEYFAITHERSFQGPVLIGSSQGCNIEE	ADP	Mus 86	IAKKLGSKDVVIKAQVLAGGRGKGTFT-SGLKGGVKIVFSPEEAKAVSSQM
ADP_S.cerevisia64AAKKLNINELVIKAQALTGGRGKGHFD-TGYKSGVHNIESPQQAEDVAKEMADP_Oryza61TLKNVFPSEKIVVIKAQIAGGRGKGTFE-SGLGGGVHVKA-EAAESLAAKMADP_Sus46IAKKLGSKUVVIKAQIAGGRGKGTFE-SGLKGGVKIVFSPELAAKAVSSQMADP_Rattus88IAKKLGSKUVVIKAQIAGGRGKGTFE-SGLKGGVKIVFSPELAKAVSSQMADP_Rattus88IAKKLNAKSIVLKAQILAGGRGKGTFF-SGLKGGVHLTKDPFNVGQLAKQMGDP_Sus72AAKRLNAKSIVLKAQILAGGRGKGVFS-SGLKGGVHLTKDPFNVGQLAKQMGDP_Sus72AAKRLNAKSIVLKAQILAGGRGKGVFS-SGLKGGVHLTKDPFVVGQLAKQMGDP_Columba30AAQRLNAKSIVLKAQILAGGRGKGVFS-SGLKGGVHLTKDPFVVGQLAKQMGDP_Columba33AAQRLNAKSIVLKAQILAGGRGKGVFS-SGLKGGVHLTKDPFVVGQLAKQMGDP_Columba30AAQRLNAKSIVLKAQILAGGRGKGVFS-SGLKGGVHLTKDPFVVGQLAKQMADP_Leishmania138LNNTLITKQSGPEGKKCNTVFICERF-YIRKERYVALMDRGRAGGFILLGSSIGGSSIEDADP_Leishmania138LONITVRQTGPKGQLVNLVVTRF-FIRKERYVALMDRGRAGGFILLGSSAGGVNIEDADP_Human136IGKKLFTKQTGEKGRICNQVLVCERK-YPRREYYFAITMERSFQGPVLIGSSHGGVNIEDADP_Arabidopsis111LQVUTNRQTGPQGVVVSKVLCEKL-SUNPMYFSILLDRAKGCFUILATSGGMGIEEADP_So136IGKKLFTKQTGEKGRICNQVVCERR-YPRREYYFAITMERSFQGPVLIGSSGGVNIEDADP_So136IGKKLFTKQTGEKGRICNQVVCERR-YPRREYFAITMERSFGGPVLIGSSGGVNIEDADP_So136IGKKLFTKQTGEKGRICNQVVCERR-YPRREYFAITMERSFGGPVLIGSSGGVNIEDADP_So136IGKKLFTKQTGEKGRICNQVVCERR-YPRREYFAITMERSFGGPVLIGSSGGVNIEDADP_So136IGKKLFTKQTGEKGRICNQVVCERR-YPRREYFAITMERSFGGPVLIGSSGGVNIEDADP_So	ADP	Mycobacteri 34	IAEEIGKPVMVKAQVKVGGRGKAGGVKYAATADDAFTHAQNI
ADP_Oryra 61 TLKNVFPSEKETVVKSQTLAGGGKGUFFK-SGLGGGVHIVKA-EEAESLAAKM ADP_Sus 46 TAKKLSKEVVVKAQVLAGGGKGGFFE-SGLKGGVKTVFSPEAKAVSSQM ADP_Drosophila 1 TAKKLGSKEVVIKAQVLAGGGKGGFFE-NGLKGGVKTVFSPEAKAVSSQM GDP_Kuus 88 TAKKLSKEVVIKAQVLAGGGKGGVFF-SGLKGGVKTVFSPEAKAVSSQM GDP_Sus 71 AAKRLNAKETVLKAQTLAGGRGKGVFS-SGLKGGVHLTKDPVVGQLAKQM GDP_Sos 71 AAKRLNAKETVLKAQTLAGGRGKGVFS-SGLKGGVHLTKDPKVVGQLAKQM GDP_Columba 30 AAQRLNAKETVLKAQTLAGGRGKGVFS-SGLKGGVHLTKDPKVVGQLAKQM GDP_Columba 30 AAQRLKAKETVLKAQTLAGGRGKGVFS-SGLKGGVHLTKDPKVVGQLAKQM GDP_Columba 30 AAQRLKAKETVLKAQTLAGGRGKGVFS-SGLKGGVHLTKDPKVVGQLAKQM GDP_Doso 71 AAKRLKAKETVLKAQTLAGGRGKGVFN-SGLKGGVHLTKDPKVVGQLAKQM GDP_Columba 30 AAQRLKAKETVLKAQTLAGGRGKGVFN-SGLKGGVHLTKDPKVVGQLAKQM ADP_Leishmania 103 LGNTLVTKQTGREGKLCKVLVTERF-PIRKERVYAFLDRNSGGTGLGSAFGGNSTED ADP_Leishmania 103 LGNTLVTKQTGREGKLCKVLVTERF-PIRKERVYAFLMDRGAGGPLIGSAFGGNSTED ADP_Arabidopsis 111 LQQUVVTKQTGPGKQUVNTLVVEAVAGTKKEYYFATTHERSFGGFVLIGSAFGGNTED ADP_Arabidopsis 111 LQQUVTKQTGPGGKVVSKVLCKKL-YDRREYYFATTHERSFGGFVLIGSAFGGNTED ADP_Bastocysti 102 IGHKLITKQTGAKGRICNQUVCCRA-YRREYYFATTHERSFGGFVLIGSAFGGNTED ADP_Leishman 136 IGKLFTKQTGEKGRICNQUVCCRA-YRREYYFATTHERSFGGFVLIGSAFGGNTED ADP_Bastocysti 107 IGANLITKQTGAKGTICNQUVCRA-YRREYYFATTHERSFGGFVLIGSAFGGNTED ADP_Locli 77 LGKRLVYQTDANGQFVNQUVCRA-YRREYYFATTHERSFGGFVLIGSAFGGVNTED ADP_Locli 77 LGKRLVYQTDANGQFVNQUVCRK-PRREYYFATTHERSFGGFVLIGSAFGGVNTED ADP_S.cerevisi 14 LNHNLITKQTGIAGKFVSAVYIVKRV-DTKHEAYLSILMDRQTKKPMIASSGGMNTED ADP_Ms 136 IGGKLTRQTGEKGRICNQUVCCRK-PRREYFFATTHERSFGGFVLIGSAGGVNTED ADP_S.cerevisi 14 LNHNLITKQTGIAGKFVSAVYIVKRV-DTKHEAYLSILMDRQTKKPMIASSGGGNNTED ADP_SUS 91 IGKLITKQTGEKGRICNQUVCCRK-PRREYFFATTHERSFGGFVLIGSAGGGNNTED ADP_SUS 91 IGKLITKQTGFAGRICNQUVCRK-PRREYFFATTHERSFGGFVLIGSSGGGNNTED ADP_SUS 91 IGKLITKQTFAGARGRICNQUVCRK-PRREYFATTHERSFGGFVLIGSSGGGNNTED ADP_SUS 91 IGKLITKQTFAGARGRICNQUVCRK-PRREYFATTHERSFGGFVLIGSSGGGNNTED ADP_SUS 91 IGKLITKQTFAGARGRICNQUVCRK-PRREYFATT	ADP	S.cerevisia 64	AAKKLNINKLVIKAQALIGGRGKGHFD-IGYKSGVHMIESPQQAEDVAKEM
ADP_Sus 46 IAKKLGSKDVUIKAQULAGGRGKGTFI-SGLKGGVKIVFSPEEAKAVSSOM ADP_Drosophila 71 IATKLGSKDVUIKAQULAGGRGKGTFI-SGLKGGVKIVFSPEEAKAVSSOM ADP_Ratus 88 IAKKLGSKDVUIKAQULAGGRGKGTFI-SGLKGGVKIVFSPEEAKAVSSOM GDP_Ruman 71 AAKRLOSKDVUIKAQULAGGRGKGVFI-SGLKGGVHLTKDPNVVGQLAKOM GDP_Sus 72 AAKRLNAKBIVLKAQILAGGRGKGVFI-SGLKGGVHLTKDPEVVGQLAKOM GDP_Columba 30 AAQRLNAKBIVLKAQILAGGRGKGVFI-SGLKGGVHLTKDPEVVGQLAKOM GDP_Columba 30 AAQRLKAKBIVLKAQILAGGRGKGVFI-SGLKGGVHLTKDPKIVGQLAKOM ADP_Talciparu 138 LNNTLITKOSGPEGKKCNTVFICERF-YIRKERYIAFLLDRNSDGIILLGSSIGGSSIED ADP_Leishmania 103 LONTUVRQTGFKGGLUNVLVVERF-PIRKEKYVALHDDRGAGGPILIGSARGGTSIED ADP_Leishmania 103 LONTUVRQTGFKGGLUNVLVUTERF-PIRKEKYVALHDDRGAGGPILIGSARGGTSIED ADP_Arabidopsis 111 LGQVLVKQTGFKGRICNQUVCERK-YPREYYFAITHERSFQGPVLIGSSHGGVNIED ADP_Arabidopsis 111 LGQVLVKQTGFKGRICNQUVCERK-YPREYYFAITMERSFQGPVLIGSSHGGVNIED ADP_Sos 136 IGKKLFTKQTGEKGRICNQUVCERK-YPREYYFAITMERSFQGPVLIGSSHGGVNIED ADP_C.elegans 107 IGANLITKQTGFKGRICNQUVCERR-YPREYYFAITMERSFQGPVLIGSSHGGVNIED ADP_L.influenza 77 LGCRLVTQTDANGQFVNQLVCERA-YPREYYFAITMERSFQGPVLIGSSHGGVNIED ADP_S.cerevisia 114 LNNLITKQTGAKGRICNQUVCERR-YPREYYFAITMERSFQGPVLIGSSHGGVNIED ADP_Kus 136 IGCKLIFKQTGEKGRICNQUVCERR-YPREYYFAITMERSFQGPVLIGSSHGGVNIED ADP_S.cerevisia 114 LNNLITKQTGAKGRICNQVVCKEL-FIREYYFSILDRNINGPIVIASSQGGVNIED ADP_Mus 136 IGCKLIFKQTGEKGRICNQVVCERK-PREYYFAITMERSFQGPVLIGSAGGVNIED ADP_S.cerevisia 114 LNNLITKQTGIAGKPVSAVVIVKRU-TIKEAYLSILDRANGGVVFIASEGGVEE ADP_Mus 136 IGCKLIFKQTGEKGRICNQVVVCERK-PREYYFSILDRNINGPIVIASSQGGVNIED ADP_S.cerevisia 114 LNNLITKQTGIAGKPVSAVVIVERL-TKREYVFAITLESFQGPVLIESAGGVNIED ADP_SUS 98 IGKKLFFKQTGEKGRICNQVVVCERK-PREYYFAITMERSFQGPVLIESAGGVNIED ADP_SUS 98 IGKKLFFKQTGEKGRICNQVVVCERK-PREYFFAITMERSFQGPVLIESAGGVNIED ADP_SUS 98 IGKKLFFKQTGEKGRICNQVVVCERA-PREYFFAITMERSFQGPVLIESAGGVNIED ADP_SUS 98 IGKKLFFKQTEGEKGRICNQVVCERA-PREFYFFAITMERSFQGPVLIESAGGVNIED ADP_SUS 98 IGKKLFFKQTEGEKGRICNQVVCERA-PREFYFFAITMERSFQGPVLIESAGGVNIED ADP_SUS 98 IGKKLFFKQTE	ADP	Oryza 61	TLKNVFPSEKEIVVKSQILAGGRGLGTFK-SGLQGGVHIVKA-EEAESLAAKM
ADPDrosophila71IATKLKICNUVLKAQVLAGGRGKGTFK-NGLKGVKUVVDPQTAELSSKMADP_Rattus88IAKKLNAKSTVLKAQVLAGGRGKGTFT-SGLKGVVHITKDPNVVGQLAKQMGDP_Sus72AAKRLNAKSTVLKAQILAGGRGKGVFN-SGLKGVHLTKDPEVVGQLAKQMGDP_Sos71AAKRLNAKSTVLKAQILAGGRGKGVFN-SGLKGVHLTKDPEVVGQLAKQMGDP_Columba30AAQRLNAKSTVLKAQILAGGRGKGVFN-SGLKGVHLTKDPEVVGQLAKQMGDP_Columba30AAQRLNAKSTVLKAQILAGGRGKGVFN-SGLKGVHLTKDPKVVGQLAKQMADP_Columba30AAQRLNAKSTVLKAQILAGGRGKGVFN-SGLKGVHLTKDPKVVGQLAKQMADP_Columba30AAQRLKAKSTVLKAQILAGGRGKGVFN-SGLKGVHLTKDPKVVGQLAKQMADP_Columba30AAQRL	ADP	Sus 48	IAKKLGSKDVVIKAQVLAGGRGKGTFE-SGLKGGVKIVFSPEEAKAVSSQM
ADP_Ratus 86 IARKLORKIVUIKAQULAGGGKGTFT-SGLKGGVHITKDPENKAVSGM GDP_Human 71 AAKRLNAKBIVLKAQILAGGGKGKUFS-SGLKGGVHITKDPENVGQLAKQM GDP_Sus 72 AAKRLNAKBIVLKAQILAGGRGKGVFS-SGLKGGVHITKDPENVGQLAKQM GDP_Bos 71 AAKRLNAKBIVLKAQILAGGRGKGVFS-SGLKGGVHITKDPENVGQLAKQM GDP_Columba 30 AAQRLKAKBIVLKAQILAGGRGKGVFS-SGLKGGVHITKDPENVGQLAKQM ADP_D.falciparu 138 LNNTLITKQSGPEGKKCNTVFICERF-YIRKERYIAFLLDRNSDGIILLGSSIGGSSIED ADP_Ioxoplasma 165 LGKTLVTKQTGKEGKLCNKVLVTERF-PIRKEKYVAILMDRGAGGPILIGSARGGTSIED ADP_Leishmania 103 LONTLVTKQTGFKGGLUNTLVTEAVAGIKKELYLSLILDRKSASPMETIGAEGGNSIED ADP_Husm 136 IGKKLFTKQTGEKGRICNQULVCEKL-SLUNEMYFSIILDRKSAGPLILACKKGGTSIED ADP_Arabidopsis 111 LQUVIKQTGPQGKVVSKVLCEKL-SLUNEMYFSILLDRKSAGPLILACKKGGTSIED ADP_Bastocysti 102 IGHKLITKQTGKGRICNQULVCERL-YDRREYTFAITHERSFGGVLGSGGNNIED ADP_Bisstocysti 102 IGHKLITKQTGKGRGKICRQUVCRL-TREYTFSITLDRAMGCPVIIATSQGGMIEE ADP_Locia 107 IGAKLITKQTGKGRGKCEVWUCRL-TREYTFSITLERAMGCPVILASSGGMNIED ADP_Sistocysti 102 IGHKLITKQTGKGRGKCEVWUCRL-TREYTFSITLERAMGCPVILASSGGMNIEE ADP_Sistocysti 102 IGHKLITKQTGKGRGKCEVW	ADP	Drosophila 71	IATKLKTDNLVLKAQVLAGGRGKGTFK-NGLKGGVRVVYDPQTAEELSSKM
GDP_Human 71 AAKRLNAKBIVLKAQILAGGRGKGVFN-SGLKGGVHLTKDPNVVGQLAKQM GDP_Sus 72 AAKRLNAKBIVLKAQILAGGRGKGVFS-SGLKGGVHLTKDPEVVGQLAKQM GDP_Bos 71 AAKRLNAKBIVLKAQILAGGRGKGVFS-SGLKGGVHLTKDPEVVGQLAKQM GDP_Columba 30 AAQRLKAKBIVLKAQILAGGRGKGVFN-SGLKGGVHLTKDPKIVEQLAKQM ADP_Columba 138 LNNTLITKOSGPEGKKCNTVFICERF-YIRKERYIAFLLDRNSDGIILLGSSIGGSSIED ADP_Toxoplasma 165 LGKTLVTKQTGKEGKLCNKVLVTERF-FIRKEKYVAILMDRGAGGPILIGSARGGTSIED ADP_Leishmania 103 LONTLVTKQTGFKGQLVNTLVTEAVAGIKREKYVSLIDRKSASPMFIGSAEGGMSIEE ADP_Maman 136 IGKKLFTKQTGEKGRICNQULVCERK-YPREYYFAITMERSFQGPVLIGSSHGGMSIED ADP_Arabidopsis 11 LGQVLVTKQTGFQGKVASVVLCEKL-SUNNEMYFSILDRKSAGPLIIACKKGTSIED ADP_Bastocysti 10 IGKKLFTKQTGEKGRICNQULVCERK-YPREYYFSILDRNKGDEVILATSQGGMSIED ADP_Celegans 107 IGANLITKQTAKGINCNKVMVCGAV-KILKERYISILDRXMGDFVILASSQGGMSIED ADP_C.elegans 107 IGANLITKQTDAKGINCNKVMVCGAV-KILKERYISILDRXMGPVILASSQGGMSIED ADP_Mus 136 IGQKLITKQTGEKGRICNQVUVCERK-YPREYYFSITMERSFQGPVLIGSSGGMNIED ADP_Mus 136 IGQKLITKQTGEKGRICNQVVVCERK-YPREYYFSILDRNMGPVLIASSQGGNNIED ADP_Mobacteri 16 LGQLUVFGYTDAKQPVNUVVCERK-	ADP	Rattus 88	IAKKLGSKDVVIKAQVLAGGRGKGTFT-SGLKGGVKIVFSPEEAKAVSSQM
GDP_Sus 72 AAKRLNAKBIVLKAQILAGGRGKGVFS-SGLKGCVHLTKDPEVVGQLAKQM GDP_Bos 71 AAKRLNAKBIVLKAQILAGGRGKGVFS-SGLKGCVHLTKDPEVVGQLAKQM GDP_Columba 30 AAQRLNAKBIVLKAQILAGGRGKGVFN-SGLKGCVHLTKDPEVVGQLAKQM ADP_Columba 30 AAQRL	GDP	Human 71	AAKRLNAKEIVLKAQILAGGRGKGVFN-SGLKGGVHLTKDPNVVGQLAKQM
GDP_Bos 71 AARRLNAKBIVLKAQILAGGROKGVFS-SGLKGGVHLTKDPKVVGQLAKQM GDP_Columba 30 AAQRLKAKBIVLKAQILAGGROKGVFS-SGLKGGVHLTKDPKVVGQLAKQM ADP_Columba 30 AAQRLKAKBIVLKAQILAGGROKGVFN-SGLKGGVHLTKDPKIVEQLAKQM ADP_Locoplasma 165 LGKTLVTKQTGKEGKLCNKVLVTERF-FIRKEKYVAILMDRGAGGPILIGSARGGTSIED ADP_Leishmania 103 LONTLVTKQTGPKGQLVNTLVTEAVAGIKKEYLSLILDRKSASPMFIGSAEGGNSIED ADP_Huishnania 103 LONTLVTKQTGPKGQLVNTLVTEAVAGIKKEYLSLILDRKSASPMFIGSAEGGNSIED ADP_Brabidopsis 111 LGQVLVTKQTGPQGKVVSKVLCEKL-SLVHEMYFSILLDRKSAGPLILACKKGGTSIED ADP_Bastocysti 102 IGHKLITKQTGAKGINONKVMVCGAV-KLIKEYLSLLDRAGGPVILGSSGGWNIED ADP_Bos 136 IGKKLFTKQTGEKGRICNQUVCERL-YPRREYYFAITHERSFQGPVLIGSSGGWNIED ADP_C.elegans 107 IGANLITKQTDRGKKCEEVMVCKRL-TRREYYFSITLDRNTNGFUVLASSGGWNIED ADP_C.elegans 107 IGANLITKQTDRGKKCEEVMVCKRL-TRREYYFSITLDRNTNGFUVLASSGGWNIED ADP_C.elegans 107 IGANLITKQTGEKGRICNQUVCERL-YPRREYYFAITHERSFQGFVLIGSAGGWNIED ADP_Kus 136 IGQKLITKQTGEKGRICNQUVCERL-YPRREYYFAITHESFSQGFVLIGSAGGWNIED ADP_Mus 136 IGQKLITKQTGEKGRICNQUVCERL-YPRREYYFAITHERSFQGFVLIGSAGGWNIED ADP_Ncobacteri 76 LGLDIKGPVLSAVUDRTSILDDRNTAGPLILACSKGGSWNIED ADP_Nus 136 IGQKLITKQTGEKGRICNQUVCERL-YPRREYFAITHERSFQGFVLIASSGGGWNIED	GDP	Sus 72	AAKRLNAKEIVLKAQILAGGRGKGVFS-SGLKGGVHLTKDPEVVGQLAKQM
GDP_columba 30 AAQRLKAKBIVLKAQILAGGRGKGVFN-SGLKGGVHLIKDPKIVEQLAKQM ADP_falciparu 138 LNNTLITKQSGPEGKKCNTVFICERF-YIRKERYIAFLLDRNSDGIILLGSSIGGSSIED ADP_Toxoplasma 165 LGKTLVTKQTGKEGKLCNKVLVTERF-FIRKEKYIAFLLDRNSDGIILLGSSIGGSSIED ADP_Leishmania 103 LCNTLVTKQTGFKGQLVNTLVTERVAGIKKEYVAILMDRGAGGPILIGSAGGTSIED ADP_Maman 136 IGKKLFTKQTGEKGRLCNKVLVTERF-FIRKEKYIAFLLDRSSQGPVLIGSSAGGNSIED ADP_Arabidopsis 111 LGQVLVTKQTGPKGQLVNTLVTERVAGIKKEYVSLIDRKSASPMFIGSAEGGNSIED ADP_Arabidopsis 111 LGQVLVTKQTGPKGKUNKVVLCEK-YPRREYYFAITMERSPQGPVLIGSSHGGMNIED ADP_Arabidopsis 111 LGQVLVTKQTGEKGRICNQVLVCERK-YPRREYYFAITMERSPQGPVLIGSSHGGMNIED ADP_Bastocrysti 107 IGKNLFTKQTGEKGRICNQVLVCERR-YPRREYYFAITMERSPQGPVLIGSSHGGMNIED ADP_C.elegans 107 IGANLITKQTGHKGKNCEV/MVLVERA-TDIKKEYLGAVUDRSSKVVFMASTEGGVEIEK ADP_S.influenza 77 LGCRLVTQTDANGQPVNQIVVERAT-DIKKEYLGAVUDRSSKVVFMASTEGGVEIEK ADP_Mus 136 IGQVLITKQTGEKGRICNQVLVCERK-YPRREYYFAITMERSFQGPVLIGSAGGNNIED ADP_Mus 136 IGQVLITKQTGEKGRICNQVLVCERK-YPRREYYFAITMERSFQGPVLIGSAGGNNIED ADP_S.cerevisia 114 LNNNLITKQTGIAGKPVSAVVIVKRV-DTKEAYLSILDNNGQTKKPMILASSGGGNNIED ADP_S.s 98 IGKKLFTKQTGEKGRICNQVLVCERR-YPRREYYFAITLESFCGPVLIGSSGGSNIED ADP_Sus 98 IGKLFTKQTGEKGRICNQVLVCERR-YPRREYFFAITMERSFGGPVLIESSGGSGNNIED D	GDP	Bos 71	AAKRLNAKEIVLKAQILAGGRGKGVFS-SGLKGGVHLTKDPKVVGQLAKQM
ADP_P.falciparu138LNNTLITKQSGPEGKKCNTVFICERF-YIRKERYIAFLLDRNSDGIILLGSSIGGSSIEDADPIOxoplasma165LGKTLVTKQTGKEGKLCNKUVUTERF-IRKEYVAILMDRGAGGPILLGSARGGTSIEDADP_Leishmania103LGNTLVTKQTGKEGKLCNKUVUTERF-IRKEYVAILMDRGAGGPILLGSARGGTSIEDADP_Buman136IGKLFTKQTGFKGGLCNQULVCERK-YPRREYYFAITHERSFQGPVLIGSSHGGVNIEDADP_Arbidopsis111LQQUVTKQTGPQGKVVSKVVLCKL-SUNEWYFSILLDRKSASPLIIACKKGGTSIEDADP_Blastocysti102IGKKLFTKQTGEKGRICNQULVCERK-YPRREYYFAITHERSFQGPVLIGSSHGGVNIEDADP_C.elegans107IGANLITKQTDRKGKKCEEVMVCKL-TREYYFSITLDRNINGFVIASSQGSWNIEDADP_L.influenza77LGQRLVTYQTDANGQPVNQILVERA-TPIAKEYYFSITLDRNINGFVIASSGGSWNIEDADP_Ms136IGQKLITKQTGEKGRICNQULVCERK-YPRREYYFAITHERSFQGPVLIGSAGGSWNIEDADP_Ms136IGQKLITKQTGEKGRICNQULVCERK-YPRREYYFAITHERSFGGPVLIGSAGGSWNIEDADP_Ms136IGQKLITKQTGEKGRICNQULVCERK-YPRREYYFAITHERSFGGPVLIGSAGGSWNIEDADP_Ms136IGQKLITKQTGEKGRICNQULVCERK-YPRREYYFAITHERSFGGPVLIGSAGGSWNIEDADP_S.cerevisia114LNHNLITKQTGIAGKPVSAVYIVKRV-DTKHEAYLSILMDRQTKKPMIASSGGGWNIEDADP_Sus98IGKKLFKQTGEKGRICNQULVCERR-YPRREYYFAITHERSFGPVLLASSGGSWNIEDADP_Sus98IGKKLFKQTGEKGRICNQULVCERR-YPRREYYFAITHERSFGPVLLASSGGSWNIEDADP_Sus98IGKKLFKQTGEKGRICNQULVCERR-YPRREYFAITHERSFGGPVLLGSSGGWNIEDADP_Sus98IGKKLFKQTGEKGRICNQULVCERR-YPRREYFAITHERSFGGPVLLGSSGGWNIEDADP_Sus98IGKLKFKQTGEKGRICNQUVUCER-YPRREYFAITHERSFGGPVLLGSSGGWNIEDADP_Sus98IGKLKFKQTGEKGRAGGNCNCQUVUCERR-YPRREYFAITHERSFGGPVLLSSEGGVLED <th>GDP</th> <th>Columba 30</th> <th>AAQRLKAKEIVLKAQILAGGRGKGVFN-SGLKGGVHLTKDPKIVEQLAKQM</th>	GDP	Columba 30	AAQRLKAKEIVLKAQILAGGRGKGVFN-SGLKGGVHLTKDPKIVEQLAKQM
ADP_Toxoplasma 165 LGKTLVTKQTGKEGKLCNKVLVTERF-PIRKEKYVAILMDRGAGGPILIGSARGGTSIED ADP_Leishmania 165 LGKTLVTKQTGFKGQLVNTLVTERVAGIKKEYVLSLILDDRSASPMFTGSAEGGMSIED ADP_Human 136 IGKKLFTKQTGEKGRLCNQVLVCEKK-YPRREYYFATTMERSFQGPVLIGSAGGNSIED ADP_Arabidopsis 111 LGQVLVTKQTGPQGKVVSKVYLCEKL-SLVNEMYFSILLDRKSAGPLIACKKGGTSIED ADP_Bastocysti 102 IGKKLTTKQTGKGRLCNQVLVCERK-YPRREYYFATTMERSFQGFVLIGSAGGNNIED ADP_Bastocysti 103 IGKKLFTKQTGEKGRLCNQVLVCERR-YPRREYYFATTMERSFQGFVLIGSAGGNNIED ADP_C.elegans 107 IGANLITKQTDRGKKCEEVVVCKLP-TRREYYFSITLDRNTNGFIVLASSGGGNNIED ADP_E.coli 77 LGKRLVTQTDANGQPVNQILVEAAT-DIAKELYLGAVVDRSSRVVFMASEGGVNIEE ADP_E.coli 77 LGKRLVTQTDANGQPVNQILVEAAT-DIAKELYLGAVVDRSSRVVFMASEGGNNIEE ADP_Kisfluenza 71 LGQKLTTKQTGEKGRLCNQVLVCERK-YPREYYFSITLDRNTNGFIVLASSGGGNNIEE ADP_Mis 136 IGQKLTKQTGEKGRLCNQVLVCERK-YPREYYFSITLDRSFQGFVLIGSAGGNNIEE ADP_Mis 136 IGQKLTKQTGEKGRLCNQVLVCERK-YPREYYFATTMERSFQGFVLIGSAGGNNIEE ADP_S.cerevisia 114 LNHNLITKQTGIAGKPVSAVVIVKRV-DTKHEAYLSILMDRQTKKPMIIASSGGGNNIEE ADP_Oryza 112 LQQILVTKQTGPQGKIVSKVVLCEKL-SLVMEMFAITMERSFQGFVLIGSAGGGNNIED ADP_Disophila 121 IDQLLVTKQTGAAGRICKVVVARKV-PRREYYFAITMERSFQGFVLIGSAGGVNIED ADP_Atus 138 IGQKLITKQTGKGRKGKNCNQVLVCERK-PRREYFAITMERSFQGFVLIGSAGGVNIED ADP_Atus 138 IGQKLITKQTGKGARGICNQVLVCERK-PRREYFAITMERSFQGFVLIGSAGGVNIED ADP_Atus 121 IDQLLVTKQTGAAGRICKVVVLXCER-PRREYFAITMERSFQGFVLIGSAGGVNIED ADP_Atus 121 IGYNLATKQTFKGVKVNKVAAL-DISSET		D falciparu 138	INNTLITKOSGDEGKKONTVETCEDE-WIDKEDVILELINDNSDGITLLGSSIGGSSIED
ADPLeishmania103LGNTLVTKQTGPKGQLVNTLVVTEAVAGIKRELYLSLILDRKSASPMFIGSAEGGMSIEEADPHuman136IGKKLFTKQTGPKGRLCNQUVUCERK-YPRREYYFAITMERSFQGPVLIGSSHGGVNIEDADPArabidopsis111LGQULVTKQTGPQGKVVSKVVLCEKL-SLVNMYYFSIILDRKSAGPLIIACKKGGTSIEDADPBlastocysti102IGKKLFTKQTGEKGRLCNQUVUCERA-YPRREYYFAITMERSFQGPVLIGSSHGGVNIEDADPBos136IGKKLFTKQTGEKGRLCNQUVUCERA-YPRREYYFAITMERSFQGPVLIGSSHGGVNIEDADPC.elegans107IGANLIKQTDHRGKKCEEVMUCKRL-PTRREYYFAITMERSFQGPVLIGSSHGGVNIEDADPADP106IGQKLITKQTGEKGRLCNQUVUCERA-YPRREYYFAITMERSFQGPVLIGSSHGGVNIEDADPADP136IGQKLITKQTGEKGRLCNQUVUCERK-YPRREYYFAITMERSFQGPVLIGSSHGGVNIEDADPMus136IGQKLITKQTGEKGRLCNQUVUCERK-YPRREYYFAITMERSFQGPVLIGSAGGVNIEDADPSerevisia114LNHNLITKQTGIAGKPVSAVVIVKRV-DTKHEAYLSILDRNARTILAKSVEGGVEEDADP_Sus96IGKKLFTKQTGEKGRICNQUVUCER-YPRREYYFAITMERSFQGPVLIGSSGGGVNIEDADP_Sus96IGKKLFTKQTGERGRICNQUVUCER-YPRREYFFAITMERSFQGPVLIASSGGGVNIEDADP_Sus96IGKKLFTKQTGAGRGICKNVLECKL-SLVNMYFAITLENTAGPULIASSGGGVNIEDADP_Sus96IGKKLFTKQTGEKGRICNQUVUCER-YPRREYFFAITMERSFQGPVLIASSGGGVNIEDADP_Sus136IGQKLITKQTGAGRGICKNVVLEKL-YPRREYFFAITMERSFQGPVLIASSGGGVNIEDADP_Sus136IGQKLITKQTGAGRICKNVVLEK-YPRREYFFAITMERSFQGPVLIASSGGGVNIEDADP_Rattus138IGQKLITKQTGAKGRICNQUVUCER-YPRREYFFAITMERSFQGPVLIASSQGGVNIEDADP_Rattus138IGQKLITKQTGAKGRICNQUVUCERK-YPRREYFFAITMERSFQGPVLIASSQGGVNIEDADP_Rattus	ADP	Toxoplasma 165	LGKTLUTKOTGKEGKLCNVULVERE-EIRKEKYVALLMDRGAGGPLLIGSARGGTSIED
ADP_Human 136 IGKKLFTKQTGEKGRICNQVLVCERK-YPRREYYFAITMERSFQGPVLIGSSHGGVNIED ADP_Arabidopsis 111 LQQVLVTRQTGPQGKVVSKVVLCEKL-SUNEYYFAITMERSFQGPVLIGSSHGGVNIED ADP_Blastocysti 102 IGKKLFTKQTGEKGRICNQVLVCERL-SUNEYYFAITMERSFQGPVLIGSSHGGVNIED ADP_Bos 136 IGKKLFTKQTGEKGRICNQVLVCERR-YPRREYYFAITMERSFQGPVLIGSSHGGVNIED ADP_C.elegans 107 IGANLITKQTDARGKKCEEVMVCKRL-TRREYYFSITLDRNTNGFIVLASSQGGVNIED ADP_L.einfluenza 77 LGKRLVTYQTDANGQPVNQILVEARAT-DIAKEVLYGAVDRSSRKVVFMASTEGGVEIEK ADP_H.influenza 77 LGQRLVTYQTDANGQPVNQILVERAT-DIAKEVLYGAVDRSSRKVVFMASTEGGVEIEK ADP_Mus 136 IGQKLITKQTGEKGRICNQVLVCERK-YPRREYYFAITMERSFQGPVLIGSAGGVNIED ADP_Mus 136 IGQKLITKQTGEKGRICNQVLVCERK-YPRREYYFAITMERSFQGPVLIGSAGGVNIED ADP_S.cerevisia 114 LNHNLITKQTGIAGKPVSAVYIVKRV-DTKHEAYLSILMDRQTKKPMIIASSQGGNNIED ADP_S.cerevisia 114 LNHNLITKQTGIAGKPVSAVYIVKRV-DTKHEAYLSILMDRQTKKPMIIASSQGGNNIED ADP_Sus 98 IGKKLFTKQTGEKGRICNQUVCERR-YPRREYYFAITMERSFQGPVLIGSSQGGVNIED ADP_Sus 98 IGKKLFTKQTGEKGRICNQUVCERR-YPRREYFAITMERSFQGPVLIACSGGVDIED ADP_Sus 98 IGKKLFTKQTGEKGRICNQUVCERR-YPRREYFAITMERSFQGPVLIASSGGVNIED ADP_Sus 98 IGKKLFTKQTGEKGRIC	ADP	Leishmania 103	LGNTLVTKOTGPKGOLUNTLVVTEBVBGIKRELVLSLILDRKSBSPMEIGSBEGGMSIEF
ADP_Arabidopsis 111 LGQVLVTKQTGPQGKVVSKVVLCEKL-SLVNEMYFSIILDRKSAGPLIIACKKGGTSIED ADP_Blastocysti 102 IGHKLITKQTGAKGINOKKVVCGAV-KLKEFYLSILLDRAMGCPVIIATSQGGMGIEE ADP_Bos 136 IGKKLFTKQTGEKGRICNQVLVCERR-YPRREYYFSIILDRAMGCPVIIATSQGGMGIEE ADP_C.elegans 107 IGKLFTKQTGEKGRICNQVLVCERR-YPRREYYFSIILDRNNGPIVIASSQGGVNIED ADP_S.coli 77 LGKRLVTYQTDANGQPVNQILVEAAT-DIAKELYLGAVUDRSSRKVVFMASTEGGVEIEK ADP_H.influenza 77 LGQRLVTFQTDKLGQPVNQILVEAAT-DIAKELYLGAVUDRSSRKVVFMASTEGGVEIEK ADP_Ms 136 IGQKLITKQTGEKGRICNQVLVCERK-YPRREYYFAITMERSFQGPVLIGSAGGGVNIED ADP_Mycobacteri 76 LGLDIKGHVVKKLLVAEAS-DIAEEYYISILDRANGTVLASSQGGVNIED ADP_S.cerevisia 114 LNHNNITKQTGIAGKFVSAVVIVKRV-DIAKEAYLSILMDRQTKKPMIIASSQGGVNIED ADP_Sus 98 IGKKLFTKQTGEKGRICNQVLVCERK-YPRREYYFAITMERSFQGPVLIGSSQGGVNIED ADP_Sus 98 IGKKLFTKQTGAKGRICNQVLVCERK-YPRREYYFAITMERSFQGPVLIGSSQGGVNIED ADP_Sus 98 IGKKLFTKQTGAKGRICNQVLVCERK-YPRREYYFAITMERSFQGPVLIGSSQGGVNIED ADP_Sus 121 IOQLVTKQTGAGRICNQVLVCERK-YPRREYYFAITMERSFQGPVLIASKEGVDIEE ADP_Rattus 138 IGQKLITKQTGAKGRICNQVLVCERK-YPRREYYFAITMERSFQGPVLIASKGGSQGVNIED ADP_Rattus 138 IGQKLITKQTGAKGRIC	ADP	Human 136	IGKKLFTKOTGEKGRICNOVL/CERK-YPRREYYFAITMERSFOGPVLIGSSHGGVNIED
ADP_Blastocysti 102 IGHKLITKQTGAKGINCNKVMVCGAV-KILKEFYLSILLDRAMGCPVIIATSQGGMGIEE ADP_Bos 136 IGKKLFTKQTGEKGRICNQVLVCERA-YPRREYYFAITMERSFQGPVIIATSQGGMGIEE ADP_C.elegans 107 IGANLITKQTDHRGKKCEEVMVCKRL-PTRREYYFAITMERSFQGPVIIATSQGGMGIEE ADP_E.coli 77 LGKRLVTQTDANGQPVNQLVCERA-YPRREYYFAITMERSFQGPVIIASSEGGWNIEE ADP_H.influenza 77 LGGRLVTFQTDENLGQPVNQLVCERA-YPRREYYFAITMERSFQGPVLIGSAGGWNIEE ADP_Mus 136 IGQKLITKQTGEKGRICNQVLVCERK-YPRREYYFAITMERSFQGPVLIGSAQGGWNIED ADP_Mus 136 IGQKLITKQTGEKGRICNQVLVCERK-YPRREYYFSILDRANGTVLAKSVEGGVEIEE ADP_S.cerevisia 114 LNHNNLTKQTGIAGKPVSAVVIVKRV-DIAES-VIAEAS-UNAMTYLAKSVEGGVEIEE ADP_Oryza 112 LGQLLVTKQTGPQGKIVSKVVLCEKL-SLVNMYFAITLDRNTAGPLIIACSKGGTSIED ADP_Sus 96 IGKKLFTKQTGAGRGICNQVLVCERA-YPRREYYFAITMERSFQGPVLIASSQGGWNIEE ADP_Sus 10 IGKLFTKQTGAKGRICNQVLVCERA-YPRREYFFAITMERSFQGPVLIASKGGVDIEE ADP_Sus 10 IGKLFTKQTGAKGRICNQVLVCERA-YPRREYFFAITMERSFQGPVLIASKGGVDIEE ADP_Sus 10 IGKLFTKQTGAKGRICNQVLVCERA-YPRREYFFAITMERSFQGPVLIASKGGVDIEE ADP_Cotyza 112 IGQLLVTKQTGAAGRICKNVVAERA-PRREYFFAITMERSFQGPVLIASKGGVDIEE ADP_DEncesophila 121 IGQLLTKQTGAAGRICNQVVVCERA-YPRR	ADD	Arabidoneis 111	LGOVLUTKOTGPOGKUVSKUVLCEKI, SUNNEMYESIILDPKSAGPLIIACKKGGTSIED
ADP_Bos 136 IGKKLFTKQTGEKGRICNQVLVCERR-YPRREYYFAITMERSFQGPVLIGSSHGGVNIED ADP_C.elegans 107 IGANLITKQTDERGKKCEEVMVCKRL-TRREYYFSITLDRNTNGFIVLASSQGGVNIEE ADP_L.ool 77 LGKRLVTYQTDANGOPVNQILVERAAT-DIAKELYLGAVUDRSSRVVFNASEGGWEIEE ADP_H.influenza 77 LGQRLVTFQTDKLGQPVNQIYFEETC-DIDKEFYLSAVVDRTSQKVVFIASSEGGWNIEE ADP_Mus 136 IGQKLITKQTGEKGRICNQVLVCERK-YPRREYYFAITMERSFQGPVLIGSAQGGVNIEE ADP_Mus 136 LGQLDIKGFVVKLVLAEAS-DIAEEYISILDRANFTLAMCSVEGGWEIEE ADP_S.cerevisia 114 LNHNLITKQTGIAGKPVSAVYIVKRV-DTKHEAYLSILMDRQTKKPMIIASSQGGNNIEE ADP_Oryza 112 LQQILVTKQTGPQGKIVSKVVLCEKL-SUNHYYFAITMERSFQGPVLIGSGGGVNIED ADP_Dsus 98 IGKKEFKQTGEKGRICNQUVCERA-YPRREYYFAITMERSFQGPVLIGSGGGVNIED ADP_Drosophila 121 IDQLLVTKQTGAAGRICKNVVLAERK-PRREFYFAITMERSFQGPVLIGSSGGVNIED ADP_Ratus 138 IGQKLITKQTGAGRICKNVVLVERK-PRREFYFAITMERSFQGPVLIGSSQGGVNIED ADP_RATUS 138 IQQKLITKQTGAGRICKNVVLVERK-PRREFYFAITMERSFQGPVLIGSSQGGVNIED ADP_RATUS 138 IGQKLITKQTGAGRICKNVVLVERK-PRREFYFAITMERSFQGPVLIGSSQGGVNIED	ADP	Blastocysti 102	IGHKLITKOTGAKGINCNKUMUCGAU-KILKEFYLSILLDRAMGCPUTTATSOGGMGIEF
ADP_C.elegans 107 IGANLITKQTDHRGKKCEEVMVCKRL-PTRREYYFSITLDRNINGPIVIASSQGGVNIEE ADP_E.coli 77 LGKRLVTYQTDANGQFVNQILVEAAT-DIRKELYLGAVVDRSSRVVFMASTEGGVEIEK ADP_H.influenza 77 LGQRLVTQTDANGQFVNQILVEAAT-DIRKELYLGAVVDRSSRVVFMASTEGGVEIEK ADP_Ms 136 IQQRLVTQTFQTDKLGQFVNQILVEAAT-DIRKELYLGAVVDRSSRVVFMASTEGGVEIEK ADP_Mus 136 IQQRLVTRQTEKCRICNQULVCERK-YPRREYYFAITMERSFQGFVLIGSSQGGVNIED ADP_Mycobacteri 76 LGLDIKGHVVKKLLVAEAS-DIAEEYYISFLLDRANRTYLAMCSVEGGVEIEE ADP_S.cerevisia 114 LNNNLTKQTGIACKFVSAVVIVKRV-DIKLENYLSILLDRANRTYLAMCSVEGGVEIEE ADP_Oryra 112 LQGILVTKQTGACKFVSAVVIVKRV-DIKLENYLSILLDRANGPLIIASSGGGNNIED ADP_Sus 98 IGKKLFTKQTGEKGRICNQVLVCERL-SUNMEWYFAITLDRNTAFDLIIASSGGGSUNIED ADP_Sus 98 IGKKLFTKQTGAKGRICNQVLVCERR-YPRREYYFAITMERSFQGPVLIGSSQGGVNIED ADP_Cattus 121 IQQLVTKQTGAAGRICKKVWAERK-PRREYYFAITMERSFQGPVLIGSSQGGVNIED ADP_Rattus 138 IGQKLTKQTGAKGRICNQVUVCERK-YPRREYYFAITMERSFQGPVLIGSSQGGVNIED ADP_Rattus 121 IGYLLAKQTFAKGGVKUVVCERK-YPRREYYFAITMERSFQGPVLIGSSQGGVNIED ADP_Rattus 121 IGYLLAKQTFAKEGVKUVKKWAEAL-DISREYYLAILMDRSCNGPVLJGSSQGGVDIEE	ADP	Bos 136	IGKKLETKOTGEKGRICNOVI//CERE-YPREYYFAITMERSFOGPVLIGSSHGGVNIED
ADP_E.coli 77 LGKRLVTYQTDANGQPVNQILVEAAT-DIAKELYLGAVVDRSSRRVVFMASTEGGVEIEK ADP_H.influenca 77 LGQRLVTFQTDKLGQPVNQILVEAAT-DIAKELYLGAVVDRSSKRVVFMASTEGGVEIEK ADP_Mus 136 IGQKLITKQTGEKGRICNQVLVCERK-YPRREYYFAITMERSFQGPVLIGSAQGGVNIED ADP_Mycobacteri 76 LGLDIKGHVVKKLVABAS-DIAEEYYISILDRANRTYLAMCSVEGGVEIEE ADP_S.cerevisia 114 LMNNLITKQTGIAGKPVSAVVIVKRV-DIKKEAVLSILMDRQTKKPMILASSQGGVNIED ADP_Oryza 112 LGQILVTKQTGPQGKIVSKVYLCEKL-SLVNEMYFAITLDRNTAGPLIIACSKGGTSIED ADP_Sus 96 IGKKFFTKQTGEKGRICNQVLVCERA-YPRREYYFAITMERSFQGPVLIGSSQGGVNIED ADP_Drosophila 121 IDQLLVTKQTGAGKGICNQVLVCERA-YPRREYYFAITMERSFQGPVLIGSSQGGVNIED ADP_Rattus 138 IGQKLITKQTGAKGRICNQVLVCERK-YPRREYYFAITMERSFQGPVLIGSSQGGVNIED GDP_Euman 121 IGYNLATKQTFKEGVKNNKVMVABAL-DISRETYLAILMDRSCNGPVLVGSSQGGVDIEE	ADP	C.elegans 107	IGANLITKOTDHRGKKCEEVMVCKRL-FTRREYYFSITLDRNTNGPIVIASSOGGVNIEE
ADP_H.influenza 77 LGQRLVTFQTDKLGQPVNQIYFEETC-DIDKEFYLSAVVDRTSQKVVFIASSEGGMNIEE ADP_Mus 136 IGQKLITKQTGEKGRICNQULVCERK-YPRREYYFAITMERSFQGPVLIGSAQGGWNIED ADP_Mycobactei 76 LGLDIKGKVVKLVLARAS-DIAEEYVISILDRANFYTIAMCSVEGGVEIEE ADP_S.cerevisia 114 LNHNLITKQTGIAGKPVSAVYIVKRV-DTKHEAYLSILMDRQTKKPMIIASSQGGMNIEE ADP_Oryra 112 LQQILVTKQTGFQGKIVSKVVLCEKL-SLVNEHYFAITLDRNTAGPLIIACSKGGTSIED ADP_Sus 98 IGKKFTKQTGEKGRICNQULVCERL-YPRREYYFAITMERSFQGFVLIGSSQGGWNIED ADP_Drosophila 121 IDQLLVTKQTGAGKGICNQVLVCERK-YPRREYYFAITMERSFQGPVLIGSSQGGVNIED ADP_Rattus 138 IQQKLITKQTGAGKGICNQULVCERK-YPRREYYFAITMERSFQGPVLIGSSQGGVNIED GDP_Human 121 IGYNLAKQTFKEGVKVNKVMVAERL-DISRETYLAILMDRSCHOFVLIGSSQGGVDIEE	ADP	E.coli 77	LGKRLVTYOTDANGOPVNOILVEAAT-DIAKELYLGAVVDRSSRRVVFMASTEGGVEIEK
ADP_Mus 136 IGQKLITKQTGEKGRICNQVLVCERK-YPRREYYFAITMERSFQGPVLIGSAQGGVNIED ADP_Mycobacteri 76 LGLDIKGRVVKKLVAEAS-DIAEEYYISFLDRANRTVLANCSVEGGVEIEE ADP_S.cerevisia 114 LNHNLITKQTGIAGKPVSAVYIVKRV-DIKHEAYLSILMDRQTKKPMIIASSQGGNNIED ADP_Oryra 112 LOGILVTKQTGFQGVGKUVSKVVLCKKL-SLVNEWYFAITLDRNTAGPLIACSKGGTSIED ADP_Sus 98 IGKKLFTKQTGEKGRICNQVLVCERR-YPRREYYFAITMERSFQGPVLIGSSQGGVNIED ADP_Drosophila 121 IOQLUTKQTGAAGRICKKVNVAERK-PPRREYYFAITMERSFQGPVLIGSSQGGVNIED ADP_Rattus 138 IGQKLITKQTGAKGRICNQVLVCERK-YPRREYYFAITMERSFQGPVLIGSSQGGVNIED ADP_Rattus 121 IGYLATKQTGAKGRICNQVLVCERK-YPRREYYFAITMERSFQGPVLIGSSQGGVNIED GDP_Human 121 IGYLATKQTFKEOVKKVMKUNAEAL-DISREYYLAILMDRSCNGPVLJQSSQGGVDIEE	ADP	H.influenza 77	LGORLVIFOIDKLGOPVNOIYFEEIC-DIDKEFYLSAVVDRISOKVVFIASSEGGMNIEE
ADP_Mycobacteri 76 LGLDIKGHVVKKLLVAEAS-DIAEEYYISFLLDRANRTYLAMCSVEGGVEIEE ADP_S.cerevisia 114 LMHNLITKQTGIAGKPVSAVVIVKRV-DIKKEMYLSILMDRQTKKPMIIASSQGGMNIEE ADP_Oryra 112 LGQILVTKQTGPGGKIVSKVVLCEKL-SLVNEWYFAITLDRNTAGPLIIACSKGGTSIED ADP_Sus 98 IGKKLFTKQTGEKGRICNQVLVCERR-YPRREYYFAITMERSFQGPVLIGSSQGGVNIED ADP_Drosophila 121 IDQLLVTKQTGAAGRICKKVMVAERK-PPRREYYFAITMERSFQGPVLIGSSQGGVNIED ADP_Rattus 138 IGQKLITKQTGAKGRICNQVLVCERK-YPRREYYFAITMERSFQGPVLIGSSQGGVNIED GDP_Human 121 IGYNLATKQTPKEGVKVMKVMVAEAL-DISRETYIAILMDRSCNGPVLVGSSQGGVDIEE	ADP	Mus 136	IGOKLITKOTGEKGRICNOVLVCERK-YPRREYYFAITMERSFOGPVLIGSAOGGVNIED
ADP_S.cerevisia 114 LNHNLITKQTGIAGKPVSAVYIVKRV-DTKHEAYLSILMDRQTKKPMIIASSQGGMNIEE ADP_Oryra 112 LQQILVTKQTGFQGKIVSKVYLCEKL-SLUNEHYFAITLDRNTAGFLIIACSKGGTSIED ADP_Sus 98 IGKKFTRQTGEKGRICKQVLVCERR-YPRREYYFAITMERSFQGFVLIGSSQGGVNIED ADP_Drosophila 121 IDQLLVTKQTGAAGRICKKVHVAERK-PPRREFYFAITMERSFQGPVLIGSSQGGVNIED ADP_Rattus 138 IGQKLITKQTGAKGRICKQVLVCERK-YPRREYYFAITMERSFQGPVLIGSSQGGVNIED GDP_Human 121 IGYNLATKQTFKEGVKVNKVHVAERL-DISRETYLAILMDRSCHOFVLJGSSQGGVDIEE	ADP	Mycobacteri 76	LGLDIKGHVVKKLLVAEAS-DIAEEYYISFLLDRANRTYLAMCSVEGGVEIEE
ADP ⁻ Oryza 112 LGQILVTKOTGPQGKIVSKVYLCEKL-SLVNEMYFAITLDRNTAGPLIIACSKGGTSIED ADP ⁻ Sus 98 IGKKLFTKOTGEKGRICNQULUCERA-YPRREYYFAITLERSFQGPVLIGSSQGGVNIED ADP ⁻ Drosophila 121 IDQLLVTKOTGAAGRICKKVMVAERK-PPRREYFAVMMERAFNGPVLIASKEGGVDIEE ADP ⁻ Rattus 138 IGGKLITKOTGAKGRICNQVLUCERK-YPRREYFAITMERSFQGPVLIGSSQGGVNIED GDP ⁻ Human 121 IGYNLATKOTPKEGVKVMKVMVAEAL-DISRETYLAILMDRSCNGPVLUGSSQGGVDIEE	ADP	S.cerevisia 114	LNHNLITKOTGIAGKPVSAVYIVKRV-DTKHEAYLSILMDROTKKPMIIASSOGGMNIEE
ADP_Sus 98 IGKKLFTKQTGEKGRICNQVLVCERR-YPRREYYFAITMERSFQGPVLIGSSQGGVNIED ADP_Drosophila 121 IDQLUTKQTGAAGRICKKVMVAERK-PPRREYFAVMMERAFNGPVLIASKEGGVDIEE ADP_Rattus 138 IGQKLITKQTGAKGRICNQVLVCERK-YPRREYYFAITMERSFQGPVLIGSSQGGVNIED GDP_Human 121 IGYNLATKQTPKEGVKVMKVMVAEAL-DISRETYLAILMDRSCNGPVLVGSSQGGVDIEE	ADP	Oryza 112	LGQILVTKQTGPQGKIVSKVYLCEKL-SLVNEMYFAITLDRNTAGPLIIACSKGGTSIED
ADP_Drosophila 121 IOQLLVTKOTGAAGRICKKVNVAERK-PPRREFYFAVMMERAFNGPVLIASKEGGVDIEE ADP_Rattus 188 IGQKLITKOTGAKGRICNQVLVCERK-YPRREFYFAVMMERAFNGPVLIGSSQGGVNIED GDP_Human 121 IGYNLATKOTPKEGVKVNKVNVAERAL-DISRETYLAILMDRSCNGPVLVGSPQGVDIEE	ADP	Sus 98	IGKKLFTKQTGEKGRICNQVLVCERR-YPRREYYFAITMERSFQGPVLIGSSQGGVNIED
ADP_Rattus 138 IGQKLITKQTGAKGRICNQVLVCERK-YPRREYYFAITMERSFQGPVLIGSSQGGVNIED GDP_Human 121 IGYNLATKQTPKEGVKVNKVMVAEAL-DISRETYLAILMDRSCNGPVLVGSPQGGVDIEE	ADP	Drosophila 121	IDQLLVTKOTGAAGRICKKVMVAERK-FPRREFYFAVMMERAFNGPVLIASKEGGVDIEE
GDP_Human 121 IGYNLATKQTPKEGVKVNKVMVAEAL-DISRETYLAILMDRSCNGPVLVGSPQGGVDIEE	ADP	Rattus 138	IGQKLITKQTGAKGRICNQVLVCERK-YPRREYYFAITMERSFQGPVLIGSSQGGVNIED
	GDP	Human 121	IGYNLATKOTPKEGVKVNKVMVAEAL-DISRETYLAILMDRSCNGPVLVGSPOGGVDIEE
GDP_Sus 122 IGYNLATKQTPKEGVKVNKVMVAEAL-DISRETYLAILMDRSCNGPVLVGSPQGGVDIEE	GDP	Sus 122	IGYNLATKQTPKEGVKVNKVMVAEAL-DISRETYLAILMDRSCNGPVLVGSPQGGVDIEE
GDP_Bos 121 IGYNLATKQTPKEGVKVKKVMVAEAL-DISRETYLAILMDRSCNGPVLVGSPQGGVDIEE	GDP	Bos 121	IGYNLATKQTPKEGVKVKKVMVAEAL-DISRETYLAILMDRSCNGPVLVGSPQGGVDIEE
GDP_Columba 80 IGYNLSTKQTPKDGVTVKKVMVAEAL-NISRETYFAILMDRACNGPVMVGSPQGGVDIEE	GDP	Columba 80	IGYNLSTKQTPKDGVTVKKVMVAEAL-NISRETYFAILMDRACNGPVMVGSPQGGVDIEE
	-		—
ADP-forming SCS subunits (beta)			ADP-forming SCS subunits (beta)



Fig. 1. (A) MSA of various SCS β subunits from phylogenetically diverse organisms (gatekeeper residues are shaded in gray). Weblogo representing the gatekeeper residues in (B) ADP-forming SCS β subunits and (C) GDP-forming SCS β subunits, indicating the most common residues from the representative organisms aligned in the previous figure (highlighted by red arrows). The height of the amino acid letter indicates its prevalence in the number of sequences available.

Determining nucleotide specificity of P. falciparum SCS



Fig. 2. Snapshots of the electrostatic surface models of the *Pf*SCSβ gatekeeper region. Electrostatic surfaces of the gatekeeper region of *Pf*SCSβ subunits indicated by a black oval. (A) Gatekeeper region of WT-DY. (B) Gatekeeper region of the gatekeeper mutant GM-1 KY. (C) Gatekeeper region of GM-2 KK. (D) Gatekeeper region of GM-3 DE. (E) Gatekeeper region of GM-4 ED. The electrostatic surface of the gatekeeper region shown in red indicates an overall negative charge, blue indicates positive charge and purple indicates the polar character of the residue. The electrostatic surfaces were prepared by using Modeller9V1032 and eF-surf server and visualized in PDBjViewer. For reference, the SCSβ subunits from *E. coli*, Pig and *Blastocystis* are also represented here (F–H) [7].

showing the presence of two expected size bands by mouse monoclonal anti-His antibody (Fig. 4F). Before proceeding for the enzymatic analysis of the recombinant P/SCS, the WT and gatekeeper mutants were refolded as described in Materials and methods.

It is interesting to note that the $PfSCS\beta$ and *Blastocystis* SCS α subunits were separately denatured and refolded into active enzyme confirmations, as per optimized protocols. Because the nucleotide-binding site lies in the SCS β subunit, this unique approach was followed after failed attempts to clone the $PfSCS\alpha$ subunit. Interestingly, the *Blastocystis* SCS α subunit did provide the CoA binding site essential for the enzyme activity. The refolded WT and gatekeeper

mutant PfSCS enzymes were subjected to enzyme kinetics studies. The PfSCS native enzyme was found to be ADP forming (0.36 μ M min⁻¹), while a moderate GDP-forming activity $(0.10 \ \mu \text{M} \ \text{min}^{-1})$ was also observed. However, the enzyme kinetics analysis of the recombinantly expressed P/SCS WT-DY enzyme demonstrated specifically ATP affinity with $K_{\text{mATP}} = 48 \ \mu\text{M}$ (Fig. 5A) and no activity with the GTP. The positively charged gatekeeper region of the mutant (GM-2 KK) emulated the Blastocystis SCS WT enzyme in terms of its gatekeeper residues (Lys and Lys). The GM-2 KK mutant showed a mild decrease in the ATP affinity with $K_{mATP} = 61 \ \mu M$ (Fig. 5B). To create a negative gatekeeper region,



Fig. 3. Initial rates of reaction for native *Pf*SCS enzyme. *Pf*SCS enzyme activity with both nucleotides (ATP and GTP) at 150 μ m concentration (Conc.). The error bars represent the standard error of the mean from duplicate experiments.

 $(Tyr \rightarrow Glu)$ mutant, GM-3 DE was constructed, and the enzyme kinetics analysis was carried out. The $K_{\text{mATP}} = 84 \ \mu\text{M}$ (Fig. 5C) values again demonstrated the enzyme to be ADP forming exclusively, contrary to the case in Blastocystis SCS, where the negative gatekeeper region demonstrated dual-nucleotide specificity with the introduction of negative gatekeeper residues (Glu and Asp) [7]. To further emulate the sequence-matched gatekeeper residues from pig SCS, we constructed another mutant GM-4 ED with Glu and Asp. A similar observation with $K_{mATP} = 119 \ \mu M$ (Fig. 5D) demonstrated only ATP using the potential of the enzyme. However, we have recorded some insignificant activity with GTP in the case of GM-3 DE and GM-4 ED PfSCS enzymes, and thus the $K_{\rm m}$ values could not be calculated for GTP.

Discussion

In the absence of any biochemical studies on *PfSCS* enzyme with particular focus on its nucleotide specificity, this study stands right with following novel aspects: (a) identifying the corresponding gatekeeper residues from phylogenetically diverse organisms, (b) assessing the substrate specificity of native *PfSCS*, (c) refolding of recombinantly expressed SCS β subunits of *P. falciparum* (WT and gatekeeper mutants) and successful refolding in the presence of the *Blastocystis* SCS α subunit, (d) performing enzyme kinetics studies of the refolded enzymes with both nucleotides (ATP and GTP), and (e) determining the effect of the charged gatekeeper residues on the nucleotide

specificity. However, it is worth mentioning that with the two separate model systems (*Blastocystis* and *P. falciparum* SCS), where in the case of *Blastocystis* SCS charged gatekeeper residues were able to discriminate between ATP and GTP, the charged gatekeeper residues of *P. falciparum* altered only the binding affinity of ATP, implying that charged gatekeeper residues might be a way of nucleotide discrimination by proteins, but not a general mechanism for ATP versus GTP discrimination.

In an attempt to identify the gatekeeper residues among the phylogenetically diverse organisms using MSA tools, we observed that the most common gatekeeper residues in the ADP-forming SCS enzymes were Asp and Tyr (P. falciparum, H. sapiens, B. taurus, M. musculus and S. scrofa), while the GDP-forming enzymes possessed Glu and Asp residues (H. sapiens, S. scrofa and B. taurus) (Table 1). Interestingly, our previous study [7] has shown that the ADP-forming Blastocystis SCS is unique in having exclusively positively charged gatekeeper residues (Lys and Lys), where alteration of the charges of the gatekeeper region profoundly altered the substrate specificity. However, the PfSCS has distinct gatekeeper residues (Asp and Tyr) matching with others, such as H. sapiens, B. taurus, M. musculus and S. scrofa. A peculiar characteristic of the SCS enzyme to have two isoforms in one organism (ADP/GDP-forming) is worth investigating, with particular focus on the gatekeeper residues. As evident by the MSA analysis, the ADPforming SCS enzymes have Asp and Tyr residues. deviating from the GDP-forming SCS in having Glu and Asp, as gatekeeper residues from the same source. This observation strongly points toward an important role of gatekeeper residues in determining the substrate specificity of the SCS enzyme. However, the analysis of gatekeeper residues in other organisms is beyond the scope of this study.

Enzyme activity of native PfSCS demonstrated the predominantly ADP-forming activity; however, a moderate GDP-forming activity was also observed (Fig. 3). It is important to note that the assessment of nucleotide specificity from crude *P. falciparum* lysate is not reliable due to the presence of other parasite proteins, DNA/RNA and nucleotides, metabolites, a variety of other ionic components, etc. Hence we performed the enzyme kinetics analysis with the recombinantly expressed and refolded *Pf*SCS and its mutants. To explore a unique aspect in the refolding process of *Pf*SCS, we used the *Blastocystis* SCS α subunit to refold along with the *Pf*SCS β subunit. Refolding of the chimeric subunits (SCS α from *Blastocystis* and SCS β from *P. falciparum*) to a functional enzyme



Fig. 4. SDS/PAGE analysis of the *Blastocystis* SCSα and *Pf*SCSβ subunits. (A) *Blastocystis* SCSα, lanes 2 and 3 containing purified fractions at size 33 kDa. (B) *Pf*SCSβ WT-DY containing purified fractions in lanes 4–6 at size 52 kDa. (C) Gatekeeper mutant GM-2 KK containing purified fractions in lanes 3 and 4 at size 52 kDa. (D) GM-3 DE containing purified fractions in lanes 3 and 4 at size 52 kDa. (E) GM-4 ED containing purified fractions in lane 3 at size 52 kDa. (F) Western blot of the *Pf*SCSβ WT-DY and *Blastocystis* SCSα subunits detected by anti-His antibody (protein marker is represented by kDa).

successfully validated that swapping of SCS subunits among different organisms is feasible. Because of the nucleotide binding site in the $PfSCS\beta$ subunit, it was possible to investigate the nucleotide specificity of the PfSCS by the chimeric refolded enzyme. The enzyme kinetics studies have demonstrated that in PfSCS, the alteration of the electrostatic properties of the gatekeeper residues did not affect the nucleotide specificity, as it did in our previous serendipitous model enzyme, *Blastocystis* SCS. Surprisingly, the *Blastocystis* SCS enzyme with the positively charged gatekeeper residues favored ATP, whereas with the negatively



Fig. 5. Enzyme kinetics of *Pf*SCS recombinantly expressed and refolded enzymes with variable concentrations of ATP and GTP. Graphs are showing the initial rates (μ M·min⁻¹) versus ATP and GTP concentrations (μ M). Graph of *Pf*SCS WT-DY (A), GM-2 KK (B), GM-3 DE (C) and (D) GM-4 ED. The *K*_m values were calculated by GRAPHPAD PRISM 5.0. The error bars represent the standard error of the mean from duplicate experiments.

charged gatekeeper residues, it could use GTP as well, particularly because of the electrostatic interactions with the approaching substrate. This led us to hypothesize that it could be a general mechanism for determining the substrate specificity in other enzymes as well, and it can be further exploited as a novel enzyme engineering approach to alter the substrate specificity. However, in the case of PfSCS, the distinct gatekeeper region as depicted in the electrostatic surfaces models of the WT and various mutants of SCSB subunits, as compared with the Blastocystis SCSß subunit, was observed. The electrostatic interactions of SCS protein with its approaching substrates (nucleotides) could be masked by other neighboring amino acids and hence could be responsible for a moderate reduction in the ATP affinity of the PfSCS enzyme. However, a detailed structural analysis via molecular modeling and simulation studies could provide a clearer picture of the molecular interactions of the gatekeeper region and the approaching nucleotides in PfSCS. A thorough comparison of the ADP/GDP- forming isoforms of SCS from the same organism would also be a fruitful attempt in understanding the molecular basis of substrate specificity for enzymes, which can bind to similar substrates, such as ATP/GTP.

Conclusions

This study concluded that the P_fSCS is an ADP-forming isoform of the SCS enzyme and possesses the gatekeeper residues, which are similar for the ADPforming SCS of human, bovine and murine representative organisms. Contrary to our initial assumption that charged gatekeeper residues 'alone' could alter the substrate specificity of nucleotide-binding enzymes such as P_fSCS , our experimental data demonstrated only a mere reduction in ATP affinity across all the mutants of P_fSCS enzyme. Thus, our study again points out the unanswered question to pinpoint the molecular interactions required for discrimination of similar substrates by the proteins.

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Conflict of interest

The authors declare no conflict of interest.

Data Accessibility

All the data generated from this study are presented in the manuscript.

Author contributions

KCP and KV conceived and designed the experiments. KV, PS and SV performed the experiments. KCP, KV, RD and NM analyzed the data and wrote the manuscript. All authors reviewed the final version of the manuscript.

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