

Short communication

Crystal structure determination and analysis of 11S coconut allergen: Cocosin[☆]S. Vajravijayan, N. Nandhagopal^{*}, K. Gunasekaran^{*}

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ABSTRACT

Allergy is an abnormal immune response against an innocuous target. Food allergy is an adverse reaction caused by common foods most well-known being those involving peanuts. Apart from mono sensitized food allergy, cross-reactivity with other food allergens is also commonly observed. To understand the phenomenon of cross-reactivity related to immune response, three dimensional structures of the allergens and their antigenic epitopes has to be analysed in detail. The X-ray crystal structure of Cocosin, a common 11S food allergen from coconut, has been determined at 2.2 Å resolution using molecular replacement technique. The monomer of 52 kDa is composed of two β-jelly roll domains, one with acidic and the other with basic character. The structure shows hexameric association with two trimers facing each other. Though the overall structure of Cocosin is similar to other 11S allergens, the occurrence of experimentally determined epitopes of the peanut allergen Ara h 3 at flexible as well as variable regions could be the reason for the clinically reported result of cross-reactivity that the peanut allergic patients are not sensitized with coconut allergen.

1. Introduction

Coconut (*Cocos nucifera* Linn.) is a monocotyledonous plant belonging to Arecaceae family whereas most of the tree nuts belong to the phylogenetically distant dicots. Coconut is widely distributed and is a primary source of drink, food, shelter and animal feed in many tropical countries (Seow and Gwee, 1997). In general, allergy is an abnormal response of the body that causes an immune reaction (Sampson, 2004), symptoms of which can range from local itching or swelling to generalized urticaria, pharyngeal or laryngeal edema, gastrointestinal involvement, bronchospasm, or even cardiovascular collapse. The peanut and tree nut registry of Food Allergy and Anaphylaxis Network (FAAN) report the severity of peanut and other food allergies. Common sources of food allergens are soybean, peanut, kidney bean, pea, lentil, and tree nuts (Barre et al., 2008; Mills et al., 2002; Willison et al., 2008). Earlier reports suggest differences in tree nut allergy severity and in the frequency of sensitization to the various nuts (Prescott et al., 2013). The possibility for potential cross-reactivity between coconut and sesame allergy and the recent proposal to list coconut as a tree nut have increased interest in the basis for coconut allergic response. (Beausoleil and Spergel, 2006; Thompson et al., 2006).

The major coconut allergens are identified as 7S and 11S globulins. In their apoform, the 7S globulins have molecular mass of

150–200 kDa, composed of three subunits of 40–70 kDa. The 11S globulins have molecular mass of 300–400 kDa, composed of six subunits of 50–60 kDa (Derbyshire et al., 1976). 11S globulins possess intra- and inter-chain disulphide bridges (Withana-Gamage and Wanasundara, 2012) whereas 7S globulins are devoid of disulphide bonds. The acidic and basic polypeptides of the 11S globulin are linked by a disulphide bridge and cleavage between asparagine and glycine residues resulting in mature hexameric protein. This hexameric 11S globulin is commonly called cocosin and causes coconut food allergy which remains rare, with only a small number of reported cases (Nguyen et al., 2004).

Immediate hypersensitivity to coconut is a relatively new phenomenon; there is a case report of atopic dermatitis in an infant attributed to formula prepared with coconut (Stutius et al., 2010). Moreover, coconut food allergy has been reported as a secondary phenomenon in two patients with severe tree nut allergy to walnut. In one of the earlier reports, walnut directed immunoglobulin E (IgE) was shown to be cross-reactive with coconut (Teuber and Peterson, 1999). Previously, cross-reactivity between coconut and hazelnut is reported using inhibition immunoblot assay (Nguyen et al., 2004). The recent clinical study performed at Children's Hospital Boston using skin prick testing (SPT) on relationship between sesame, coconut and nut allergy in children reported that allergy to peanuts or tree nuts were not more likely to be sensitized to coconut (Stutius et al., 2010).

[☆] This paper is dedicated to the memory of Professor S.S. Rajan who passed away 9th December 2016.

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From immunological studies, epitopes responsible for immune reaction (antigenic) in peanut and almond allergens were identified (de Leon et al., 2003). Also, from structure analysis, such epitope identification could be achieved as attempted in various allergens (11S) (Jenkins et al., 2005). Since cross-reactivity of cocosin is observed with nut allergy, identification of epitopes becomes necessary for further understanding. Thus determination of the three dimensional structure of cocosin becomes essential. Though preliminary reports are available on crystallization (Carr et al., 1990; Balasundaresan et al., 2002), structure of cocosin has not been determined till date. In the present study, we substantially refined purification and crystallization enabling structural analysis and subsequent comparison with other 11S allergens.

2. Materials and methods

2.1. Protein extraction and purification

Mature coconuts were harvested from cultivatable field near Chennai (13°02'27.31"N 79°48'08.64"E) during December 2016. A sample containing 550 g of endosperm was isolated from testa and endocarp, thoroughly washed with distilled water, and macerated in 1600 ml of 20 mM sodium phosphate buffer (6.5) supplemented with 32 mM NaCl using a blender. The resulting fine paste was suspended in toluene in order to remove fatty oil by continuous stirring at 500 rpm at 37 °C for 24 h. Debris was then removed by passing through a muslin cloth and residual oily material was decanted using a separatory funnel. The resulting supernatant was fractionated with (NH₄)₂SO₄ in relative saturation stages, i.e., 0–30, 30–60 and 60–90%. After centrifugation at 15,000 g for 50 min, precipitates were dissolved in minimum volume of 10 mM sodium phosphate buffer (6.5), 25 mM NaCl and dialyzed against the same buffer using 10 kDa membranes (Sigma chemicals) for 12 h. Fraction containing high cocosin content (30–60%) was subjected to ion exchange chromatography using DEAE-cellulose (Pharmacia) on FPLC (Akta purifier, GE). Fractions corresponding to the major peak (Fig. S1) were pooled together, concentrated to 12 mg/ml as estimated by Lowry Method.

2.2. Crystallization and data collection

Crystallization was performed over 0.2 ml reservoir solutions in multi-well trays. Crystals were obtained with 2 µl cocosin solution when mixed with 2 µl reservoir solution of 0.1 M HEPES (pH 7.5), 0.2 M MgCl₂ and 10% PEG 4000. Single prismatic crystals that grew in 2 days at 293 K were found suitable for X-ray diffraction experiments. Crystals were cryo protected with the mother liquor containing 45% (v/v) glycerol. The diffraction data collected on MAR345dtb detector at PX-BL21 beamline of Indus-2 synchrotron, India (Kumar et al., 2016). Data up to 2.2 Å resolution were collected at wavelength of 0.9794 Å at 100 K.

2.3. Structure solution and refinement

Data were processed by XDS (Kabsch, 2010). Molecular replacement strategies with several 11S globulin structures from PDB were carried out. The cocosin structure was solved by molecular replacement using program BALBES (Long et al., 2008) with the final search model based on procruciferin, an 11S globulin from *Brassica napus* (wwPDB: 3KGL). Using this preliminary model, a second monomer was found using MOLREP (Vagin and Teplyakov, 2010) from the CCP4 program suite (Winn et al., 2011). The sequence cocosin isoform1 (UniProt: A0A0R7UCU5) was modelled into the electron density. Refinement was carried out using Phenix program (Adams et al., 2010). Twinning was detected by Phenix.xtirage and the corresponding twin operator for space group H3 (k, h, –l) was used in the refinement. Model building and refinement was done iteratively using COOT (Emsley et al., 2010)

Table 1

Data collection and refinement statistics.

Data collection	
Diffraction source	PX-BL21, Indus-2, India
Wavelength (Å)	0.9794
Temperature (K)	100
Detector	Mar345dtb Image plate
Space group	H3
Cell dimensions a, b and c (Å)	128.347, 128.347, 66.701
Resolution range (Å)	31.87–2.2 (2.279–2.2)
Measured reflections	173936 (33917)
I/σ (I)	8.5(0.7)
No. of unique reflections	33993 (3338)
Completeness (%)	99.7(97.45)
Multiplicity	2.5 (2.3)
R _{sym} ^b	0.082 (1.064)
Refinement	
Resolution range (Å)	31.87–2.2 (2.279–2.2)
Used reflections	33979 (3329)
Residues/waters	716/158
Average B factor (Å ²)	53.05
Bond length r.m.s. (Å)	0.011
Bond angle r.m.s. (°)	1.17
R factor (%)	0.2084 (0.3302)
R _{free} (%)	0.2977 (0.3226)
Ramachandran plot (%) ^a	
Favoured	85.55
Outliers	3.18

Values in parentheses are for the highest resolution shell.

^a Calculated using PROCHECK.

^b $R_{sym} = \sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle| / \sum_{hkl} \sum_i I_i(hkl)$, where $I_i(hkl)$ is the observed intensity in the i th data set and $\langle I(hkl) \rangle$ mean intensity of reflection hkl over all measurements of $I(hkl)$.

and Phenix. The final R-work and R-free are 20.84% and 29.77% respectively. Data collection and refinement parameters are summarized in Table 1.

2.4. Structural analysis

The refined model was validated by Ramachandran plot (Ramachandran and Sasisekharan, 1968) calculated using PROCHECK (Laskowski et al., 1993). Superposition of structures was carried out using Chimera (Pettersen et al., 2004). The accessible surface area were calculated using AREAIMOL (Lee and Richards, 1971). Electrostatic potential was calculated using ABPS tools (Baker et al., 2001). The molecular surfaces are calculated and displayed using PyMol. The sequences of 11S allergens available for reported structures from wwPDB were used for sequence analysis. Sequence alignment was done using Clustal Omega (Li et al., 2015) and formatting was done using ESPript 3.0 (Robert and Gouet, 2014).

3. Results and discussion

3.1. Overall structure

The crystal structure revealed the presence of two protomers in an asymmetric unit related by a pseudo two-fold axis. Arrangement of two protomers around a crystallographic three-fold axis resulted in a Hexameric structure formed by two trimers. The trimeric structure is similar to previously reported 11S globulin structures. The trimeric structure is formed by head to tail arrangement (Fig. 1A) of three protomers.

The monomer is composed of two similar domains, acidic and basic domains, each consisted of β-jelly roll structure with an additional helix (Fig. 1A). Two domains are related with a pseudo diad. Several regions are found to be disordered based on electron density. These regions are different in each monomer A and B. In general, we could observe four

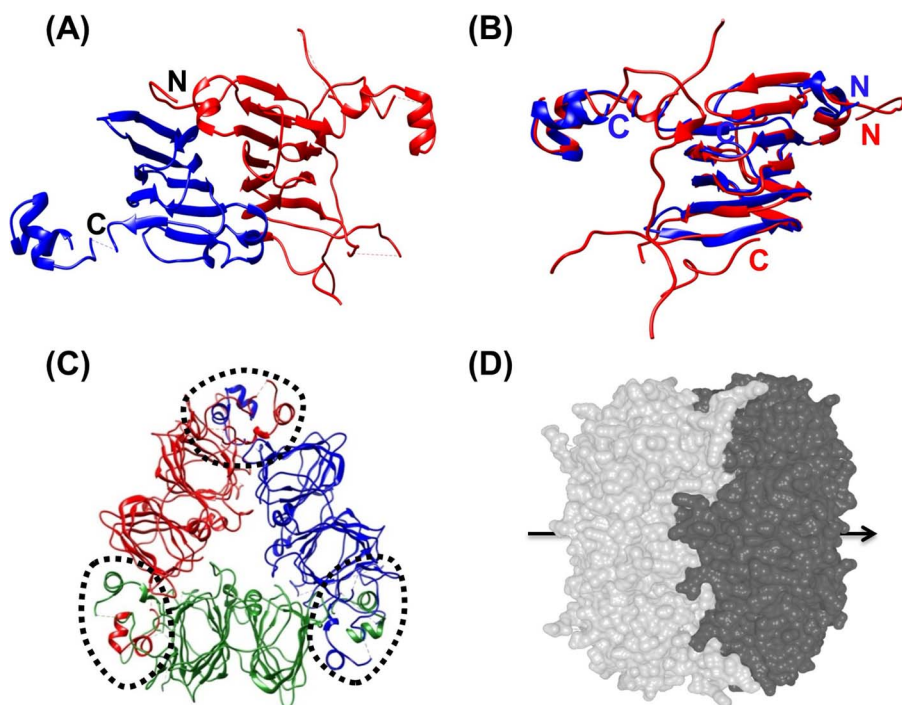


Fig. 1. Coccosin monomer and hexamer: (A) The monomer is composed of two continuous similar domains acidic (red) and basic (blue) domains, each forms β -jelly roll structure with an additional helix. (B) Superposition of acidic and basic domains (C) Trimeric structure is formed by crossing over of head to tail arrangement with three monomers, highlighted with dotted circles. (D) Hexamer is formed by the tight interactions from same face of each trimer. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

disordered regions at N and C termini of the polypeptide chain. Regions 126–139, 203–215, 271–283 and 419–422 in monomer A and regions 129–139, 203–218, 268–281 and 416–420 in monomer B are not modelled. These disordered regions are comparable to the reported disordered regions in other 11S structures. The two similar domains of a protomer can be superimposed with rmsd of 0.975 Å of (Fig. 1B). This similarity can be attributed to the gene duplication in the course of evolution related to the functions of 11S. There are two disulphide bonds in each monomer and they are conserved across 11S globulins.

The two monomers in the asymmetric unit related by pseudo two-fold axis can be superimposed with rmsd of 0.524 Å. The buried surface area between two monomers is 10738 Å² which is about one third of the total surface area 36582 Å² comprising two monomers.

3.2. Trimeric and hexameric structures

Three protomers are held tightly together by a number of hydrogen bonds and charged interactions. Total buried surface area of the trimer is 14,064 Å² and this large surface shows tight cooperation. In each monomer, a helix-loop structure at C-termini of both domains extends into the neighbouring monomer structure leading to head to tail arrangement (Fig. 1C). In each trimer the acidic domain faces the basic domain of the monomer and vice versa. The interacting residues between monomers of a trimer are well conserved among all the reported 11S allergens. Two sets of trimers formed around the crystallographic three-fold axis stack on one another as two doughnuts (Fig. 1D). The hexamer is formed by the interactions resulting from same face of each trimer, consistent with the reported structures (Fig. S3). Two of the disordered regions are buried at the interface of the trimers. Interestingly, no space is available to model those disordered regions which are termed as variable regions and hence it can be hypothesized that these regions are cleaved to facilitate the hexamer formation. The electrostatic potential surfaces of the faces buried between two trimers are shown in (Fig. S2A). Though, overall charge distribution is similar for two trimer surfaces facing each other, the hexamer formation is attributed to the alternative arrangement of acidic and basic domains complementing each other (Fig. S2B and C). Interacting faces are almost flat when compared to outer surfaces having relatively larger curvature. The total buried surface area between two trimers is

24,107 Å². The interacting residues in the trimer are well conserved among available 11S structures from wwPDB (Fig. S3). They are marked with stars in sequence alignment (Fig. S4).

3.3. Comparison with other 11S allergens

The sequence alignment of the available structures of 11S allergens are shown in Fig. S4. Regions corresponding to the reported epitopes of Ara h 3 are marked as boxes (green). Sequence comparison of epitope regions of Ara h 3 with other 11S allergens clearly shows that they are variable with inclusion of structurally conserved residues. Since the experimentally determined epitopes of Ara h 3 occur at variable and structurally flexible regions (Fig. 2), antigenic epitopes of coccosin must be different. This may explain why children allergic to oeanuts, treenuts or both are not at higher risk for coconut sensitivity. (Stutius et al., 2010).

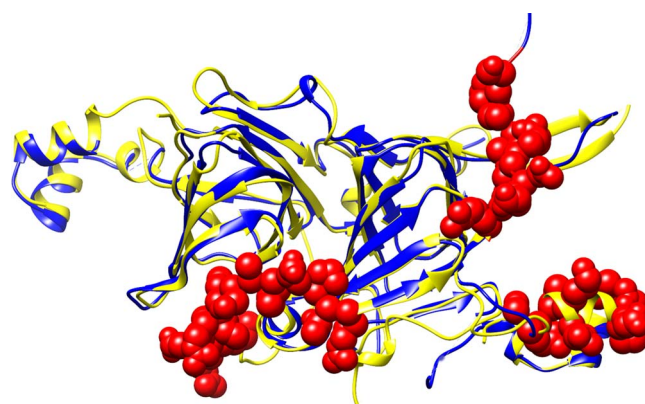


Fig. 2. Superposition of Coccosin and Ara h 3: Monomer of coccosin is shown in blue and Ara h 3 in yellow. The rmsd is 0.830 Å. All experimentally determined epitopes of Ara h 3 present at flexible region are shown as spheres in red. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

4. Conclusion

Coconut is one of the common foods in many populations and though the coconut allergy is very rare, cross-reactivity between coconut and some of the tree nut allergens and sesame seeds made us to study the 3D structure of cocosin which belongs to 11S globulins. Though crystal quality and diffraction data quality were good, only after resolving space group twinning issues with the X-ray crystallographic data, a well refined model could be obtained. The overall structure and hexameric arrangement are similar to other allergens. The trimer-trimer association through charge complementarity is clearly explained. A possible reason for the absence of cross-reactivity of cocosin with peanut allergen was inferred from the comparison of sequence and structural regions of the epitope of Ara h 3.

Accession number

The accession number for the atomic coordinates of cocosin deposited in wwPDB is 5XTY.

Disclosure

The authors have declared that no conflict of interest exists.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.molimm.2017.10.018>.

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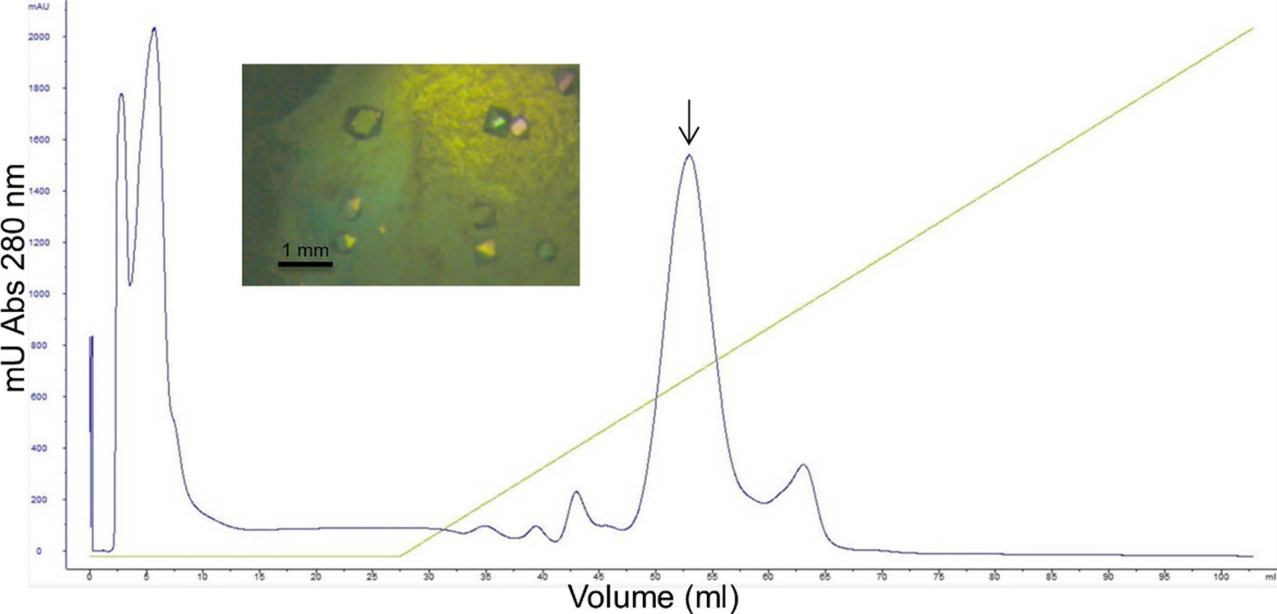
SUPPLEMENTARY FIGURES

Fig. S1. Cocosin purification: Ion Exchange chromatogram of 30–60% Ammonium sulphate fraction using DEAE-Cellulose. The major peak corresponds to purified cocosin. Insert shows prismatic crystals grown using hanging drop method with 0.1M HEPES (pH 7.5), 0.2M MgCl₂ and 10% PEG 4000 as reservoir solution.

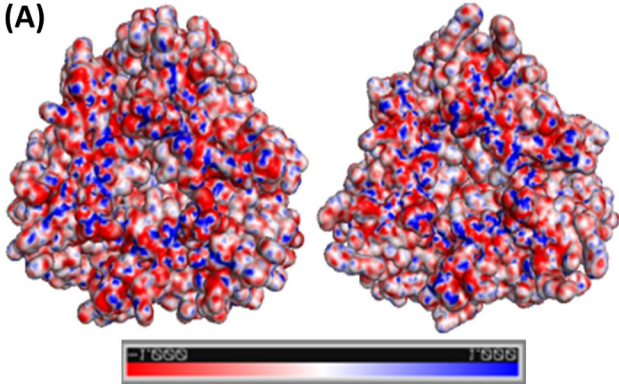
Fig. S2. Assembly of cocosin hexamer. (A) The electrostatic surface representation of faces buried perpendicular to three-fold axis. (B) Surface representation Acidic and Basic domain arrangement of hexamer (c) Schematic diagram showing complementary arrangement of acidic and basic domains of hexamer.

Fig. S3. Superposition of 11S allergens viewed down three-fold axis: The superposition done using the program Chimera shows that all the reported structures are well conserved. Structures are retrieved from wwPDB (Codes are given in figure 2).

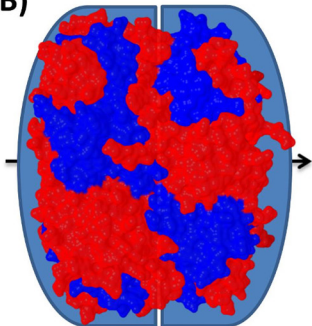
Fig. S4. Sequence alignment of reported 11S allergens: Experimentally found epitopes of peanut 11S Ara h 3 is shown in green boxes. The interacting residues of the trimer are shown with blue stars and are well conserved among available 11S structures.



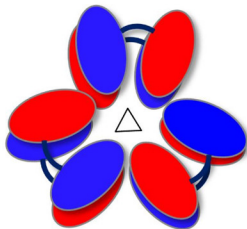
(A)

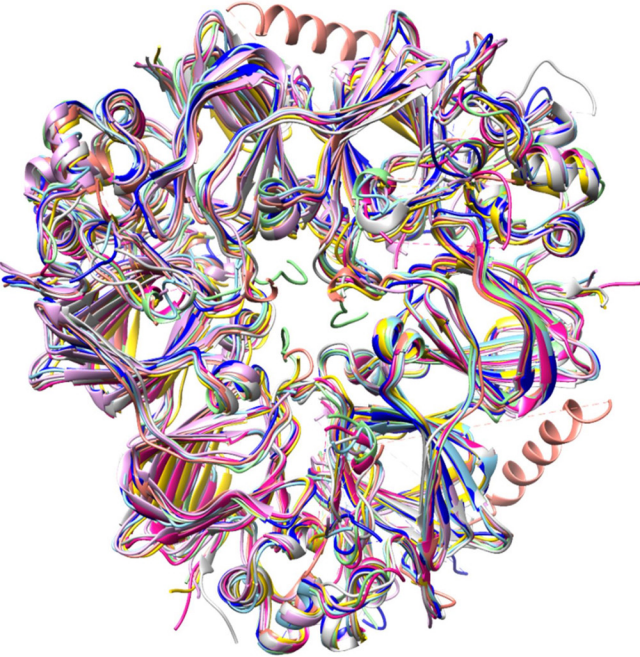


(B)



(C)





COCO 1 10 20 30 40 50 60 70 80 90
 MASSLSLSFSCLLLCLHLCHLSQAQFGSSQGESPFQSP...RRVSFR^{β1}ECRTET^{β2}LNAL^{β3}ET^{β4}TR^{β5}VRS^{β6}AGV^{β7}TV^{β8}FD^{β9}EDNEQ^{β10}FCAG^{β11}GV^{β12}STIR^{β13}RV^{β14}EPR^{β15}GL
 2EVX MARSSLSFTFLCLAVFINGCLSQIEQQSPWEFQGSSEVWQGHRY^{β1}QSP^{β2}FRAC^{β3}LEN^{β4}LR^{β5}AD^{β6}VVR^{β7}RAE^{β8}NA^{β9}FT^{β10}EV^{β11}WD^{β12}QND^{β13}DF^{β14}CA^{β15}GV^{β16}NM^{β17}IR^{β18}HT^{β19}IR^{β20}PK^{β21}GL
 3KGL QQQQFFRLQDRHOKTRIRIRE^{β1}GD^{β2}LV^{β3}VP^{β4}AC^{β5}VS^{β6}HW^{β7}YNR^{β8}QSD^{β9}LV^{β10}IV^{β11}FA^{β12}DT^{β13}RV^{β14}AN^{β15}QND^{β16}DP^{β17}YL^{β18}RK^{β19}FY^{β20}LAG^{β21}RP^{β22}...
 3FZ3 QQQQFFRLQDRHOKTRIRIRE^{β1}GD^{β2}LV^{β3}VP^{β4}AC^{β5}VS^{β6}HW^{β7}YNR^{β8}QSD^{β9}LV^{β10}IV^{β11}FA^{β12}DT^{β13}RV^{β14}AN^{β15}QND^{β16}DP^{β17}YL^{β18}RK^{β19}FY^{β20}LAG^{β21}RP^{β22}...
 3QAC QQQQFFRLQDRHOKTRIRIRE^{β1}GD^{β2}LV^{β3}VP^{β4}AC^{β5}VS^{β6}HW^{β7}YNR^{β8}QSD^{β9}LV^{β10}IV^{β11}FA^{β12}DT^{β13}RV^{β14}AN^{β15}QND^{β16}DP^{β17}YL^{β18}RK^{β19}FY^{β20}LAG^{β21}RP^{β22}...
 3C3V QQQQFFRLQDRHOKTRIRIRE^{β1}GD^{β2}LV^{β3}VP^{β4}AC^{β5}VS^{β6}HW^{β7}YNR^{β8}QSD^{β9}LV^{β10}IV^{β11}FA^{β12}DT^{β13}RV^{β14}AN^{β15}QND^{β16}DP^{β17}YL^{β18}RK^{β19}FY^{β20}LAG^{β21}RP^{β22}...
 3KSC QQQQFFRLQDRHOKTRIRIRE^{β1}GD^{β2}LV^{β3}VP^{β4}AC^{β5}VS^{β6}HW^{β7}YNR^{β8}QSD^{β9}LV^{β10}IV^{β11}FA^{β12}DT^{β13}RV^{β14}AN^{β15}QND^{β16}DP^{β17}YL^{β18}RK^{β19}FY^{β20}LAG^{β21}RP^{β22}...
 1OD5 QQQQFFRLQDRHOKTRIRIRE^{β1}GD^{β2}LV^{β3}VP^{β4}AC^{β5}VS^{β6}HW^{β7}YNR^{β8}QSD^{β9}LV^{β10}IV^{β11}FA^{β12}DT^{β13}RV^{β14}AN^{β15}QND^{β16}DP^{β17}YL^{β18}RK^{β19}FY^{β20}LAG^{β21}RP^{β22}...

COCO 100 110 120 130
 COCO LPSMSNAP^{β1}RLVYIV^{β2}GR^{β3}GV^{β4}LV^{β5}ME^{β6}GC^{β7}PET^{β8}QSF^{β9}Q...RSEREE...RRSQSA...
 2EVX LPSMSNAP^{β1}RLVYIV^{β2}GR^{β3}GV^{β4}LV^{β5}ME^{β6}GC^{β7}PET^{β8}QSF^{β9}Q...RSEREE...RRSQSA...
 3KGL LPSMSNAP^{β1}RLVYIV^{β2}GR^{β3}GV^{β4}LV^{β5}ME^{β6}GC^{β7}PET^{β8}QSF^{β9}Q...RSEREE...RRSQSA...
 3FZ3 LPSMSNAP^{β1}RLVYIV^{β2}GR^{β3}GV^{β4}LV^{β5}ME^{β6}GC^{β7}PET^{β8}QSF^{β9}Q...RSEREE...RRSQSA...
 3QAC LPSMSNAP^{β1}RLVYIV^{β2}GR^{β3}GV^{β4}LV^{β5}ME^{β6}GC^{β7}PET^{β8}QSF^{β9}Q...RSEREE...RRSQSA...
 3C3V LPSMSNAP^{β1}RLVYIV^{β2}GR^{β3}GV^{β4}LV^{β5}ME^{β6}GC^{β7}PET^{β8}QSF^{β9}Q...RSEREE...RRSQSA...
 3KSC LPSMSNAP^{β1}RLVYIV^{β2}GR^{β3}GV^{β4}LV^{β5}ME^{β6}GC^{β7}PET^{β8}QSF^{β9}Q...RSEREE...RRSQSA...
 1OD5 LPSMSNAP^{β1}RLVYIV^{β2}GR^{β3}GV^{β4}LV^{β5}ME^{β6}GC^{β7}PET^{β8}QSF^{β9}Q...RSEREE...RRSQSA...

COCO 140 150 160 170 180 190 200 210
 COCO GERHRWSR^{β1}DE^{β2}HQ^{β3}RV^{β4}YF^{β5}GD^{β6}LV^{β7}VP^{β8}NG^{β9}FA^{β10}W^{β11}CY^{β12}YN^{β13}GEN^{β14}GV^{β15}VA^{β16}IT^{β17}LD^{β18}TS^{β19}ND^{β20}AN^{β21}QND^{β22}DR^{β23}SH^{β24}RO^{β25}F^{β26}LAG^{β27}RQ^{β28}...EQGRQ...EQVERGVEEWEERSRKGS...
 2EVX GERHRWSR^{β1}DE^{β2}HQ^{β3}RV^{β4}YF^{β5}GD^{β6}LV^{β7}VP^{β8}NG^{β9}FA^{β10}W^{β11}CY^{β12}YN^{β13}GEN^{β14}GV^{β15}VA^{β16}IT^{β17}LD^{β18}TS^{β19}ND^{β20}AN^{β21}QND^{β22}DR^{β23}SH^{β24}RO^{β25}F^{β26}LAG^{β27}RQ^{β28}...EQGRQ...EQVERGVEEWEERSRKGS...
 3KGL GERHRWSR^{β1}DE^{β2}HQ^{β3}RV^{β4}YF^{β5}GD^{β6}LV^{β7}VP^{β8}NG^{β9}FA^{β10}W^{β11}CY^{β12}YN^{β13}GEN^{β14}GV^{β15}VA^{β16}IT^{β17}LD^{β18}TS^{β19}ND^{β20}AN^{β21}QND^{β22}DR^{β23}SH^{β24}RO^{β25}F^{β26}LAG^{β27}RQ^{β28}...EQGRQ...EQVERGVEEWEERSRKGS...
 3FZ3 GERHRWSR^{β1}DE^{β2}HQ^{β3}RV^{β4}YF^{β5}GD^{β6}LV^{β7}VP^{β8}NG^{β9}FA^{β10}W^{β11}CY^{β12}YN^{β13}GEN^{β14}GV^{β15}VA^{β16}IT^{β17}LD^{β18}TS^{β19}ND^{β20}AN^{β21}QND^{β22}DR^{β23}SH^{β24}RO^{β25}F^{β26}LAG^{β27}RQ^{β28}...EQGRQ...EQVERGVEEWEERSRKGS...
 3QAC GERHRWSR^{β1}DE^{β2}HQ^{β3}RV^{β4}YF^{β5}GD^{β6}LV^{β7}VP^{β8}NG^{β9}FA^{β10}W^{β11}CY^{β12}YN^{β13}GEN^{β14}GV^{β15}VA^{β16}IT^{β17}LD^{β18}TS^{β19}ND^{β20}AN^{β21}QND^{β22}DR^{β23}SH^{β24}RO^{β25}F^{β26}LAG^{β27}RQ^{β28}...EQGRQ...EQVERGVEEWEERSRKGS...
 3C3V GERHRWSR^{β1}DE^{β2}HQ^{β3}RV^{β4}YF^{β5}GD^{β6}LV^{β7}VP^{β8}NG^{β9}FA^{β10}W^{β11}CY^{β12}YN^{β13}GEN^{β14}GV^{β15}VA^{β16}IT^{β17}LD^{β18}TS^{β19}ND^{β20}AN^{β21}QND^{β22}DR^{β23}SH^{β24}RO^{β25}F^{β26}LAG^{β27}RQ^{β28}...EQGRQ...EQVERGVEEWEERSRKGS...
 3KSC GERHRWSR^{β1}DE^{β2}HQ^{β3}RV^{β4}YF^{β5}GD^{β6}LV^{β7}VP^{β8}NG^{β9}FA^{β10}W^{β11}CY^{β12}YN^{β13}GEN^{β14}GV^{β15}VA^{β16}IT^{β17}LD^{β18}TS^{β19}ND^{β20}AN^{β21}QND^{β22}DR^{β23}SH^{β24}RO^{β25}F^{β26}LAG^{β27}RQ^{β28}...EQGRQ...EQVERGVEEWEERSRKGS...
 1OD5 GERHRWSR^{β1}DE^{β2}HQ^{β3}RV^{β4}YF^{β5}GD^{β6}LV^{β7}VP^{β8}NG^{β9}FA^{β10}W^{β11}CY^{β12}YN^{β13}GEN^{β14}GV^{β15}VA^{β16}IT^{β17}LD^{β18}TS^{β19}ND^{β20}AN^{β21}QND^{β22}DR^{β23}SH^{β24}RO^{β25}F^{β26}LAG^{β27}RQ^{β28}...EQGRQ...EQVERGVEEWEERSRKGS...

COCO 220 230 240 250 260 270
 COCO EGS...TKEN^{β1}IL^{β2}RG^{β3}FT^{β4}EL^{β5}MA^{β6}AF^{β7}GV^{β8}N^{β9}MEL^{β10}ARK^{β11}LQ^{β12}CR^{β13}DT^{β14}RG^{β15}IV^{β16}RA^{β17}EN^{β18}GL^{β19}QV^{β20}IR^{β21}PS^{β22}GM^{β23}...
 2EVX EGS...TKEN^{β1}IL^{β2}RG^{β3}FT^{β4}EL^{β5}MA^{β6}AF^{β7}GV^{β8}N^{β9}MEL^{β10}ARK^{β11}LQ^{β12}CR^{β13}DT^{β14}RG^{β15}IV^{β16}RA^{β17}EN^{β18}GL^{β19}QV^{β20}IR^{β21}PS^{β22}GM^{β23}...
 3KGL EGS...TKEN^{β1}IL^{β2}RG^{β3}FT^{β4}EL^{β5}MA^{β6}AF^{β7}GV^{β8}N^{β9}MEL^{β10}ARK^{β11}LQ^{β12}CR^{β13}DT^{β14}RG^{β15}IV^{β16}RA^{β17}EN^{β18}GL^{β19}QV^{β20}IR^{β21}PS^{β22}GM^{β23}...
 3FZ3 EGS...TKEN^{β1}IL^{β2}RG^{β3}FT^{β4}EL^{β5}MA^{β6}AF^{β7}GV^{β8}N^{β9}MEL^{β10}ARK^{β11}LQ^{β12}CR^{β13}DT^{β14}RG^{β15}IV^{β16}RA^{β17}EN^{β18}GL^{β19}QV^{β20}IR^{β21}PS^{β22}GM^{β23}...
 3QAC EGS...TKEN^{β1}IL^{β2}RG^{β3}FT^{β4}EL^{β5}MA^{β6}AF^{β7}GV^{β8}N^{β9}MEL^{β10}ARK^{β11}LQ^{β12}CR^{β13}DT^{β14}RG^{β15}IV^{β16}RA^{β17}EN^{β18}GL^{β19}QV^{β20}IR^{β21}PS^{β22}GM^{β23}...
 3C3V EGS...TKEN^{β1}IL^{β2}RG^{β3}FT^{β4}EL^{β5}MA^{β6}AF^{β7}GV^{β8}N^{β9}MEL^{β10}ARK^{β11}LQ^{β12}CR^{β13}DT^{β14}RG^{β15}IV^{β16}RA^{β17}EN^{β18}GL^{β19}QV^{β20}IR^{β21}PS^{β22}GM^{β23}...
 3KSC EGS...TKEN^{β1}IL^{β2}RG^{β3}FT^{β4}EL^{β5}MA^{β6}AF^{β7}GV^{β8}N^{β9}MEL^{β10}ARK^{β11}LQ^{β12}CR^{β13}DT^{β14}RG^{β15}IV^{β16}RA^{β17}EN^{β18}GL^{β19}QV^{β20}IR^{β21}PS^{β22}GM^{β23}...
 1OD5 EGS...TKEN^{β1}IL^{β2}RG^{β3}FT^{β4}EL^{β5}MA^{β6}AF^{β7}GV^{β8}N^{β9}MEL^{β10}ARK^{β11}LQ^{β12}CR^{β13}DT^{β14}RG^{β15}IV^{β16}RA^{β17}EN^{β18}GL^{β19}QV^{β20}IR^{β21}PS^{β22}GM^{β23}...

COCO 280 290 300 310
 COCO ...EEE...REEGRS^{β1}IN^{β2}GF^{β3}EET^{β4}Y^{β5}CS^{β6}MK^{β7}IK^{β8}QNT^{β9}GD^{β10}PRR^{β11}AD^{β12}VFN^{β13}PR^{β14}GR^{β15}GRIT^{β16}T...
 2EVX ...EEE...REEGRS^{β1}IN^{β2}GF^{β3}EET^{β4}Y^{β5}CS^{β6}MK^{β7}IK^{β8}QNT^{β9}GD^{β10}PRR^{β11}AD^{β12}VFN^{β13}PR^{β14}GR^{β15}GRIT^{β16}T...
 3KGL ...EEE...REEGRS^{β1}IN^{β2}GF^{β3}EET^{β4}Y^{β5}CS^{β6}MK^{β7}IK^{β8}QNT^{β9}GD^{β10}PRR^{β11}AD^{β12}VFN^{β13}PR^{β14}GR^{β15}GRIT^{β16}T...
 3FZ3 ...EEE...REEGRS^{β1}IN^{β2}GF^{β3}EET^{β4}Y^{β5}CS^{β6}MK^{β7}IK^{β8}QNT^{β9}GD^{β10}PRR^{β11}AD^{β12}VFN^{β13}PR^{β14}GR^{β15}GRIT^{β16}T...
 3QAC ...EEE...REEGRS^{β1}IN^{β2}GF^{β3}EET^{β4}Y^{β5}CS^{β6}MK^{β7}IK^{β8}QNT^{β9}GD^{β10}PRR^{β11}AD^{β12}VFN^{β13}PR^{β14}GR^{β15}GRIT^{β16}T...
 3C3V ...EEE...REEGRS^{β1}IN^{β2}GF^{β3}EET^{β4}Y^{β5}CS^{β6}MK^{β7}IK^{β8}QNT^{β9}GD^{β10}PRR^{β11}AD^{β12}VFN^{β13}PR^{β14}GR^{β15}GRIT^{β16}T...
 3KSC ...EEE...REEGRS^{β1}IN^{β2}GF^{β3}EET^{β4}Y^{β5}CS^{β6}MK^{β7}IK^{β8}QNT^{β9}GD^{β10}PRR^{β11}AD^{β12}VFN^{β13}PR^{β14}GR^{β15}GRIT^{β16}T...
 1OD5 ...EEE...REEGRS^{β1}IN^{β2}GF^{β3}EET^{β4}Y^{β5}CS^{β6}MK^{β7}IK^{β8}QNT^{β9}GD^{β10}PRR^{β11}AD^{β12}VFN^{β13}PR^{β14}GR^{β15}GRIT^{β16}T...

COCO 320 330 340 350 360 370 380 390 400 410
 COCO LNSEK^{β1}TFIL^{β2}RT^{β3}MSA^{β4}ER^{β5}VY^{β6}LY^{β7}RNA^{β8}MV^{β9}SP^{β10}HN^{β11}NI^{β12}NA^{β13}HS^{β14}IM^{β15}YQ^{β16}TG^{β17}RG^{β18}RV^{β19}EA^{β20}DD^{β21}RG^{β22}ET^{β23}V^{β24}DE^{β25}GL^{β26}IR^{β27}Q^{β28}GL^{β29}LIV^{β30}P^{β31}ON^{β32}FAM^{β33}I^{β34}ER^{β35}AG^{β36}SAG^{β37}F^{β38}OL^{β39}VS^{β40}TK^{β41}SD^{β42}RAM...
 2EVX LNSEK^{β1}TFIL^{β2}RT^{β3}MSA^{β4}ER^{β5}VY^{β6}LY^{β7}RNA^{β8}MV^{β9}SP^{β10}HN^{β11}NI^{β12}NA^{β13}HS^{β14}IM^{β15}YQ^{β16}TG^{β17}RG^{β18}RV^{β19}EA^{β20}DD^{β21}RG^{β22}ET^{β23}V^{β24}DE^{β25}GL^{β26}IR^{β27}Q^{β28}GL^{β29}LIV^{β30}P^{β31}ON^{β32}FAM^{β33}I^{β34}ER^{β35}AG^{β36}SAG^{β37}F^{β38}OL^{β39}VS^{β40}TK^{β41}SD^{β42}RAM...
 3KGL LNSEK^{β1}TFIL^{β2}RT^{β3}MSA^{β4}ER^{β5}VY^{β6}LY^{β7}RNA^{β8}MV^{β9}SP^{β10}HN^{β11}NI^{β12}NA^{β13}HS^{β14}IM^{β15}YQ^{β16}TG^{β17}RG^{β18}RV^{β19}EA^{β20}DD^{β21}RG^{β22}ET^{β23}V^{β24}DE^{β25}GL^{β26}IR^{β27}Q^{β28}GL^{β29}LIV^{β30}P^{β31}ON^{β32}FAM^{β33}I^{β34}ER^{β35}AG^{β36}SAG^{β37}F^{β38}OL^{β39}VS^{β40}TK^{β41}SD^{β42}RAM...
 3FZ3 LNSEK^{β1}TFIL^{β2}RT^{β3}MSA^{β4}ER^{β5}VY^{β6}LY^{β7}RNA^{β8}MV^{β9}SP^{β10}HN^{β11}NI^{β12}NA^{β13}HS^{β14}IM^{β15}YQ^{β16}TG^{β17}RG^{β18}RV^{β19}EA^{β20}DD^{β21}RG^{β22}ET^{β23}V^{β24}DE^{β25}GL^{β26}IR^{β27}Q^{β28}GL^{β29}LIV^{β30}P^{β31}ON^{β32}FAM^{β33}I^{β34}ER^{β35}AG^{β36}SAG^{β37}F^{β38}OL^{β39}VS^{β40}TK^{β41}SD^{β42}RAM...
 3QAC LNSEK^{β1}TFIL^{β2}RT^{β3}MSA^{β4}ER^{β5}VY^{β6}LY^{β7}RNA^{β8}MV^{β9}SP^{β10}HN^{β11}NI^{β12}NA^{β13}HS^{β14}IM^{β15}YQ^{β16}TG^{β17}RG^{β18}RV^{β19}EA^{β20}DD^{β21}RG^{β22}ET^{β23}V^{β24}DE^{β25}GL^{β26}IR^{β27}Q^{β28}GL^{β29}LIV^{β30}P^{β31}ON^{β32}FAM^{β33}I^{β34}ER^{β35}AG^{β36}SAG^{β37}F^{β38}OL^{β39}VS^{β40}TK^{β41}SD^{β42}RAM...
 3C3V LNSEK^{β1}TFIL^{β2}RT^{β3}MSA^{β4}ER^{β5}VY^{β6}LY^{β7}RNA^{β8}MV^{β9}SP^{β10}HN^{β11}NI^{β12}NA^{β13}HS^{β14}IM^{β15}YQ^{β16}TG^{β17}RG^{β18}RV^{β19}EA^{β20}DD^{β21}RG^{β22}ET^{β23}V^{β24}DE^{β25}GL^{β26}IR^{β27}Q^{β28}GL^{β29}LIV^{β30}P^{β31}ON^{β32}FAM^{β33}I^{β34}ER^{β35}AG^{β36}SAG^{β37}F^{β38}OL^{β39}VS^{β40}TK^{β41}SD^{β42}RAM...
 3KSC LNSEK^{β1}TFIL^{β2}RT^{β3}MSA^{β4}ER^{β5}VY^{β6}LY^{β7}RNA^{β8}MV^{β9}SP^{β10}HN^{β11}NI^{β12}NA^{β13}HS^{β14}IM^{β15}YQ^{β16}TG^{β17}RG^{β18}RV^{β19}EA^{β20}DD^{β21}RG^{β22}ET^{β23}V^{β24}DE^{β25}GL^{β26}IR^{β27}Q^{β28}GL^{β29}LIV^{β30}P^{β31}ON^{β32}FAM^{β33}I^{β34}ER^{β35}AG^{β36}SAG^{β37}F^{β38}OL^{β39}VS^{β40}TK^{β41}SD^{β42}RAM...
 1OD5 LNSEK^{β1}TFIL^{β2}RT^{β3}MSA^{β4}ER^{β5}VY^{β6}LY^{β7}RNA^{β8}MV^{β9}SP^{β10}HN^{β11}NI^{β12}NA^{β13}HS^{β14}IM^{β15}YQ^{β16}TG^{β17}RG^{β18}RV^{β19}EA^{β20}DD^{β21}RG^{β22}ET^{β23}V^{β24}DE^{β25}GL^{β26}IR^{β27}Q^{β28}GL^{β29}LIV^{β30}P^{β31}ON^{β32}FAM^{β33}I^{β34}ER^{β35}AG^{β36}SAG^{β37}F^{β38}OL^{β39}VS^{β40}TK^{β41}SD^{β42}RAM...

COCO 420 430 440 450 460
 COCO VST^{β1}V^{β2}V^{β3}CK^{β4}T^{β5}AL^{β6}RG^{β7}MP^{β8}EV^{β9}LV^{β10}MS^{β11}SY^{β12}LR^{β13}SD^{β14}RE^{β15}ARR^{β16}V^{β17}KL^{β18}RG^{β19}DEV^{β20}AT^{β21}TP^{β22}RR^{β23}ES