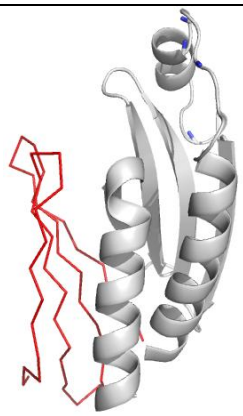
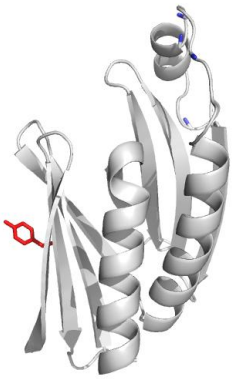
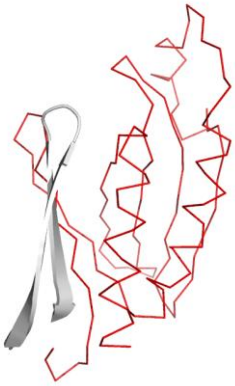
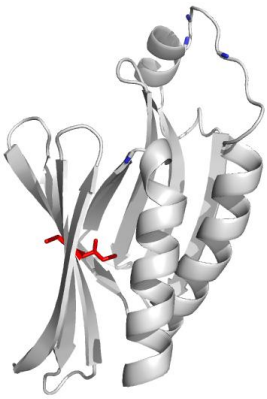
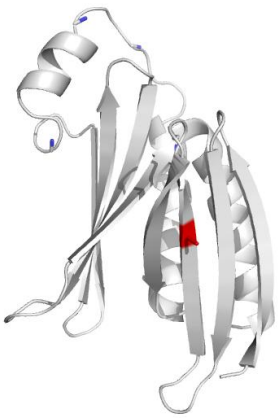
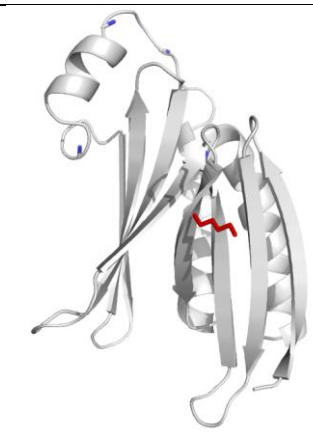
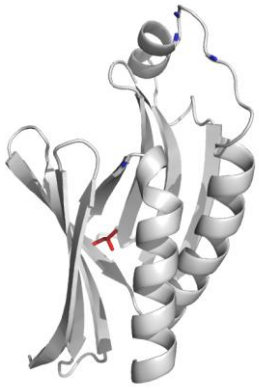
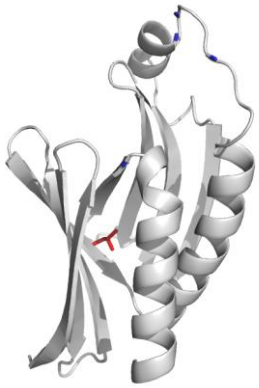
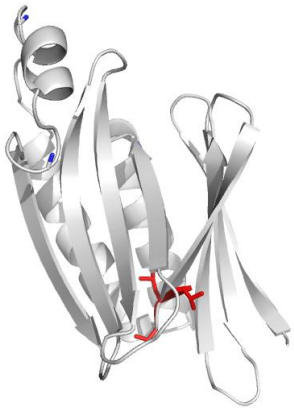
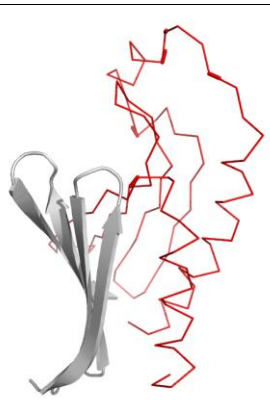
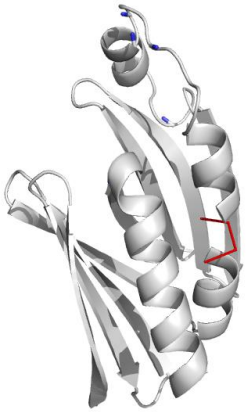
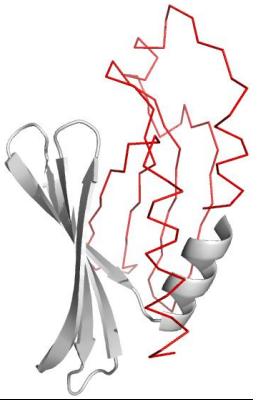
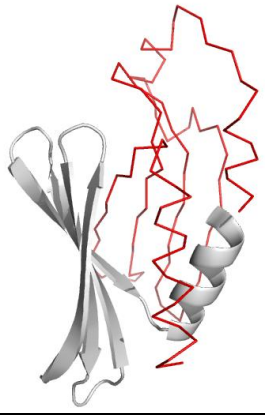
Mutations in unstructured region at N-terminus

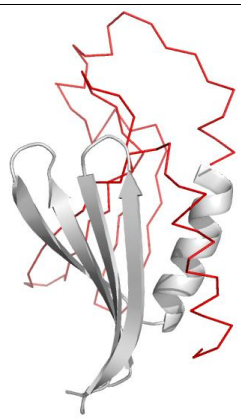
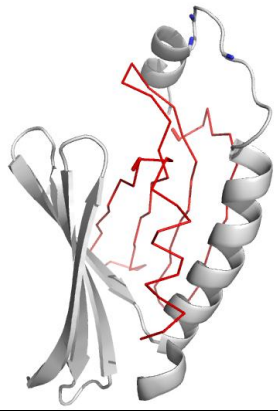
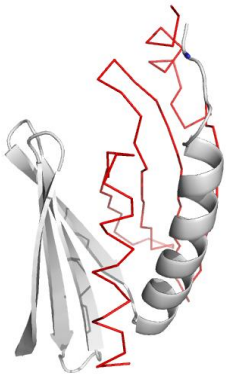
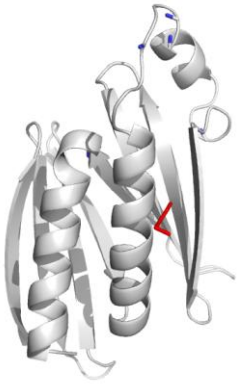
Nucleotide / Protein	Implications on protein folding and function	Structural presentation
c.1A>T p.Met1 *Tanaka # 3*	Start codon mutated, second ATG starts protein in position Met104. N-terminus and N-terminal part of N-terminal PUR domain is missing, the C-terminal PUR domain is not affected. Class: E	
c.4_8delGCGGA p.Ala2Profs*197 *Tanaka # 5*	Frame-shift destroys the entire protein sequence. All PUR domains are destroyed. Class A1	**No PUR domains**
c.25G>T p.Glu9* *Individual 7*	All PUR domains are destroyed. Class A1	**No PUR domains**
c.127-130delAGTG p.Ser43Alafs*34 *Individual 22*	All PUR domains are destroyed. Class A1	**No PUR domains**
c.135_138dupCGGC p.Gly47Argfs*155 *Individual 29*	All PUR domains are destroyed. Class A1	**No PUR domains**
c.158_159delGG p.Gly53Alafs*147 *Individual 13*	All PUR domains are destroyed. Class A1	**No PUR domains**
c.153delA p.Leu54Cysfs*24 *Individual 31*	All PUR domains are destroyed. Class A1	**No PUR domains**

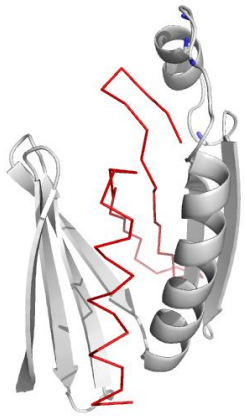
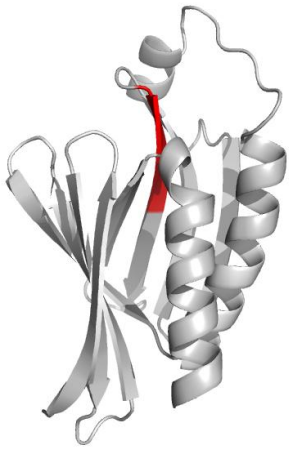
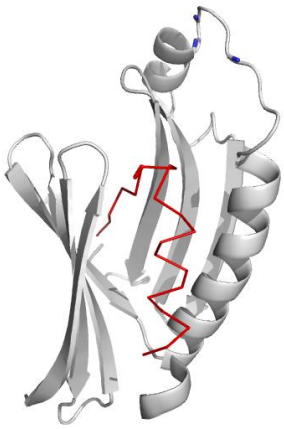
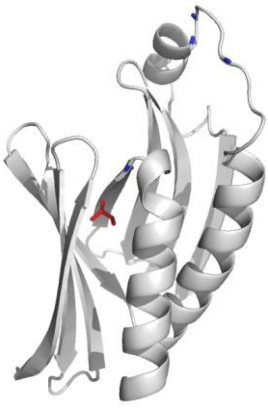
Mutations in N-terminal PUR domain (repeats I-II)

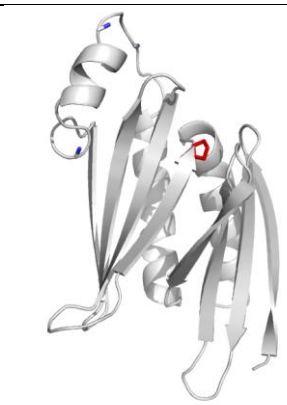
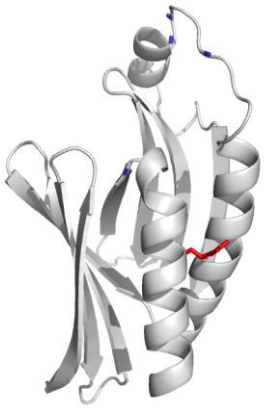
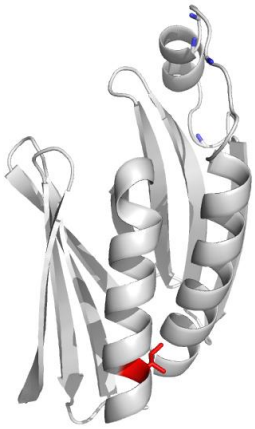
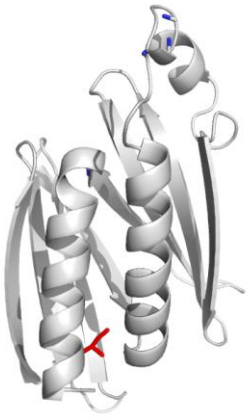
Nucleotide / Protein	Implications on protein folding and function	Structural presentation
c.220T>C p.Tyr74His *Individual 3*	Folding not affected. Instead DNA/RNA binding by N-terminal PUR-domain is likely to be impaired. The C-terminal PUR-domain is not affected. Class C	
c.235C>T p.Gln79* *Individual 2*	Most of N-terminal PUR-domain and entire C-terminal PUR-domain are deleted. Class A1	
c.263_265delTCG p.Ile88_Ala89delinsThr *Lalani # 10*	Changes register of beta-sheet, which has two potential effects: 1) Moderately affecting the hydrophobic core (i.e. stability) of the domain. 2) Reorienting the negatively charged Glu90 towards the solvent. This likely affects nucleic acid binding. The C-terminal PUR domain is not affected. Class B	
c.265G>C p.Ala89Pro *Lalani # 9*	Likely impairs folding of the beta-sheet in the N-terminal PUR-domain. The C-terminal PUR-domain is not affected. This mutation may affect DNA/RNA binding Class B	

c.289A>G p.Lys97Glu *Individual 20* *Lalani # 4*	Mutation of basic amino acid (Lys) into acidic amino acid (Glu) on RNA/DNA-binding surface. The domain folding is not affected, DNA/RNA binding is likely impaired. The C-terminal PUR-domain is not affected. Class C	
c.299T>C p.Leu100Pro *Lalani # 5*	Impairs folding of N-terminal PUR-domain. The C-terminal PUR-domain is not affected. Class B	
c.299T>G p.Leu100Arg *Individual 21*	Likely impairs folding of N-terminal PUR-domain by introducing positively charged bulky side chain into the hydrophobic core of this domain. The C-terminal PUR-domain is not affected. Class B	
c.302_310delCTCTCTCCA p.Thr101_Ser103del *Tanaka # 6*	Deletion of three amino acids likely impairs folding of N-terminal PUR-domain. The C-terminal PUR-domain is not affected. Class B	

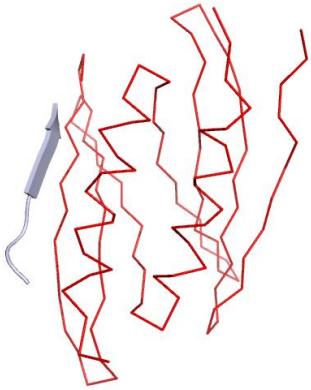
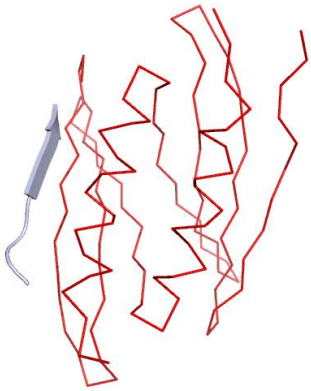
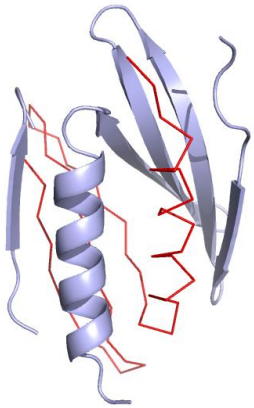
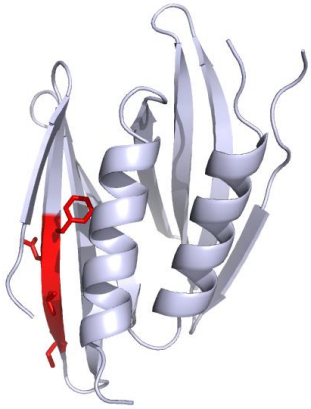
c.307_308delTC p.Ser103Hisfs*97 *Lalani # 2*	Frame-shift deletes most of N-terminal PUR-domain and entire C-terminal PUR-domain. Class A1	
c.331_342del p.Arg111_Leu114del *Okamoto # 1*	Deletion of four amino acids in alpha-helix likely impairs folding of N-terminal PUR-domain. The C-terminal PUR-domain is not affected. Class B	
c.340delC p.Leu114Trpfs*111 *Individual 18*	Frame-shift deletes most of N-terminal PUR-domain and entire C-terminal PUR-domain. Class A1	
c.338_341dupACCT p.Gly115Profs*87 *Individual 11*	Frameshift deletes most of N-terminal PUR-domain and entire C-terminal PUR-domain. Class A1	

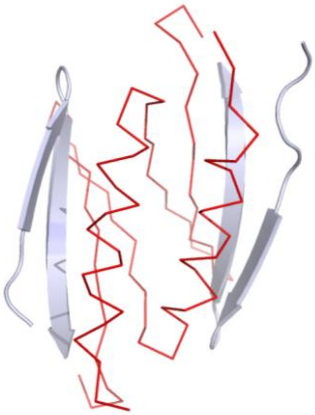
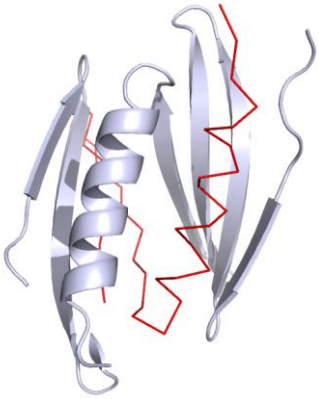
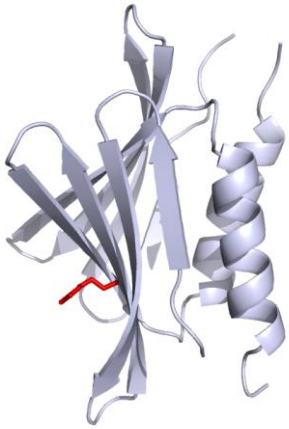
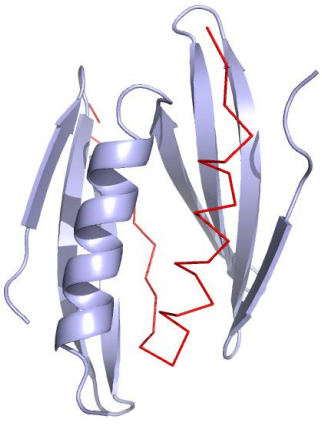
c.351dupC p.Ile118Hisfs*83 *Individual 16*	Frameshift deletes most of N-terminal PUR-domain and entire C-terminal PUR-domain. Class A1	
c.363C>G p.Tyr121* *Lalani # 6*	Frameshift deletes most of N-terminal PUR-domain and entire C-terminal PUR-domain. Class A1	
c.382C>T p.Gln128* *Individual 23*	Stop codon deletes most of N-terminal PUR-domain and entire C-terminal PUR-domain. Class A1	
c.470 T>A p.Met157Lys *Lalani # 8*	Point mutation impairs (but most likely does not destroy) hydrophobic core of the N-terminal PUR-domain. The C-terminal PUR-domain is not affected. Class B	

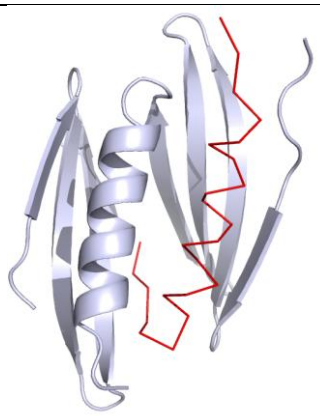
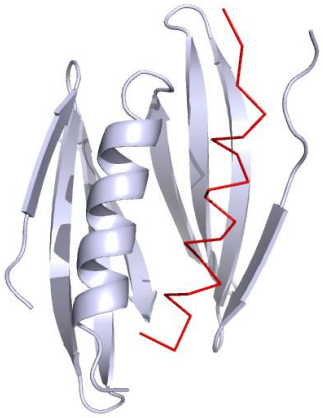
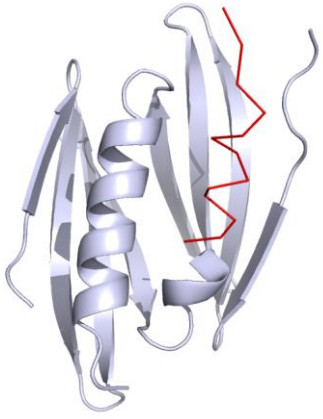
c.478A>T p.Leu160* *Individual 26*	Frameshift deletes part of N-terminal PUR-domain and entire C-terminal PUR-domain. Class A1	
c.488_489insGCGCGGCCGCTTCCT p.Gly165_Arg169dup *Individual 19*	Insertion of five amino acids in loop region that is involved in RNA/DNA binding. No folding defect expected but likely impairment of nucleic-acid binding. Class C	
c.556C>T p.Gln186* *Lalani # 3*	Frameshift deletes part of N-terminal PUR-domain and entire C-terminal PUR-domain. Class A1	
c.563 T>C p.Ile188Thr *Tanaka # 1*	Point mutation creates local defect on hydrophobic core. Hydrophobic amino acid is exchanged against polar residue. This should have a modest effect on folding of N-terminal PUR domain. A negative influence on RNA/DNA binding is possible. The C-terminal PUR-domain is not affected. Class B	

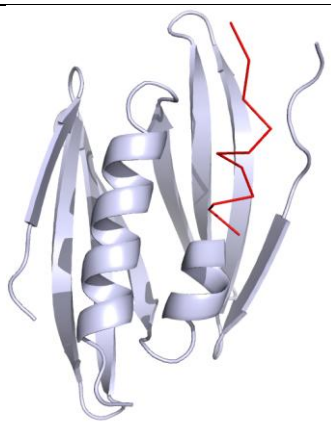
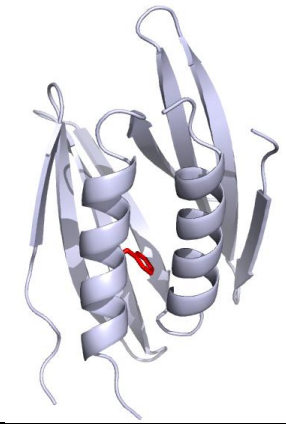
c.572C >T p.Pro191Leu *Individual 9*	Point mutation in a short loop region between beta-sheet and alpha-helix. This likely causes folding defects in the N-terminal PUR-domain. The C-terminal PUR-domain is not affected. Class B	
c.596 G>C p.Arg199Pro *Lalani # 11*	Mutation into Proline impairs alpha-helix of N-terminal PUR-domain and likely causes local structural defect. The C-terminal PUR domain is not affected. Class B	
c.616_618delATC p.Ile206del *Individual 25*	Deletes a single amino acid at the C-terminus of N-terminal PUR domain, likely resulting in local destabilization of the C-terminus of its alpha-helix. The C-terminal PUR-domain is not affected. Class B	
C616A>T p.Ile206Phe *Hunt # 3*	Point mutation in alpha-helix introduces steric clash with the second alpha-helix of N-terminal PUR-domain. This likely causes folding defects. The C-terminal PUR-domain is not affected. Class B	

Mutations in C-terminal PUR domain (2x repeat III of two Pur-alpha molecules)

Nucleotide / Protein	Implications on protein folding and function	Structural presentation
c.675_676insA p.Val226Serfs*68 *Individual 6*	Mutation destroys repeat III and thus the C-terminal PUR-domain. Class A2	
c.677_678del p.Val226Glyfs*67 *Individual 10*	Mutation destroys repeat III and thus the C-terminal PUR-domain. Class A2	
c. 685A>T p.Lys229* *Individual 32*	Mutation destroys repeat III and thus the C-terminal PUR-domain. Class A2	
c.697_699delTTC p.Phe233del *Individual 4,5 and 14* *Tanaka # 4* *Hunt # 4*	Mutation impairs the beta-sheet of repeat III and folding of the C-terminal PUR-domain. Class B	

C711dupC pAsn238Glnfs*56 *Individual 27*	Mutation destroys part of repeat III and thus the C-terminal PUR-domain. Class A2	
c.726_727delGT p.Phe243Tyrfs*50 *Hunt # 1*	Mutation destroys part of repeat III and thus the C-terminal PUR-domain. Class A2	
c.734G>C p.Arg245Pro *Individual 1 and 15*	Mutation locally distorts the beta-sheet and impairs folding of the C-terminal PUR-domain. An impaired nucleic-acid interaction is also possible. Class B (and perhaps class D)	
c.746_749dupTGAA p.Lys250Asnfs*45 *Individual 12*	Mutation destroys repeat III and thus the C-terminal PUR-domain. Class A2	

c.768dupC p.Ile257Hisfs*37 *Tanaka # 2*	Mutation destroys repeat III and thus the C-terminal PUR-domain. Class A2	
c.771_776del p.Ile257_Val259delins Met *Individual 17*	Mutation impairs local folding of beta-sheet. Class B	
c.783C>G p.Tyr261* *Lalani # 7*	Mutation destroys repeat III and thus the C-terminal PUR-domain. Class A2	
c.802G>T p.Gly268* *Individual 8*	Mutation destroys repeat III and thus the C-terminal PUR-domain. Class A2	

c.808_809delAC p.Thr270Leufs*23 *Individual 30*	Mutation destroys repeat III and thus the C-terminal PUR-domain. Class A2	
c.812_814delTCT p.Phe271del *Individual 28* *Lalani # 1*	Mutation changes register of C-terminal alpha-helix and should destroy domain fold. Class B	

Mutations in C-terminal tail (C-terminal of repeat III)

Nucleotide / Protein	Implications on protein folding and function	Structural presentation
c.847delG p.Glu283Arg fs*45 *Hunt # 2*	Only very C-terminus affected. All PUR domains fold correctly. Class D	No domain affected

Supplementary Table 3: Structural interpretation of identified mutations.
Interpretations are based on homology models generated from crystal structure of *Drosophila* Pur-alpha. Classification of mutations:
Class A1: No functional domains made, protein should have lost all functions.
Class A2: No functional C-terminal PUR-domain is made. Thus mainly dimerization should be affected.
Class B: Local impairment of folding in either the N-terminal or the C-terminal PUR-domain.
Class C: RNA/DNA binding of N-terminal PUR-domain likely affected.
Class D: amino acids in regions without known function. Possibly protein-interactions are impaired.
Amino acids affected by mutations are shown in red. Mutations that alter the identity of a given residue are shown with side chain. Deleted regions are shown as thin red wire frame. Mutations in the third repeat are shown as heterodimer with a wild-type copy of PUR repeat III as it best reflects the heterozygous genetic background of PURA-syndrome patients.