Crystals 2020
The 2nd International Online Conference on Crystals
10–20 NOVEMBER 2020 | ONLINE
crystals MDPI

Polyproline-rich peptides organize 4 cholinesterase subunits into a tetramer

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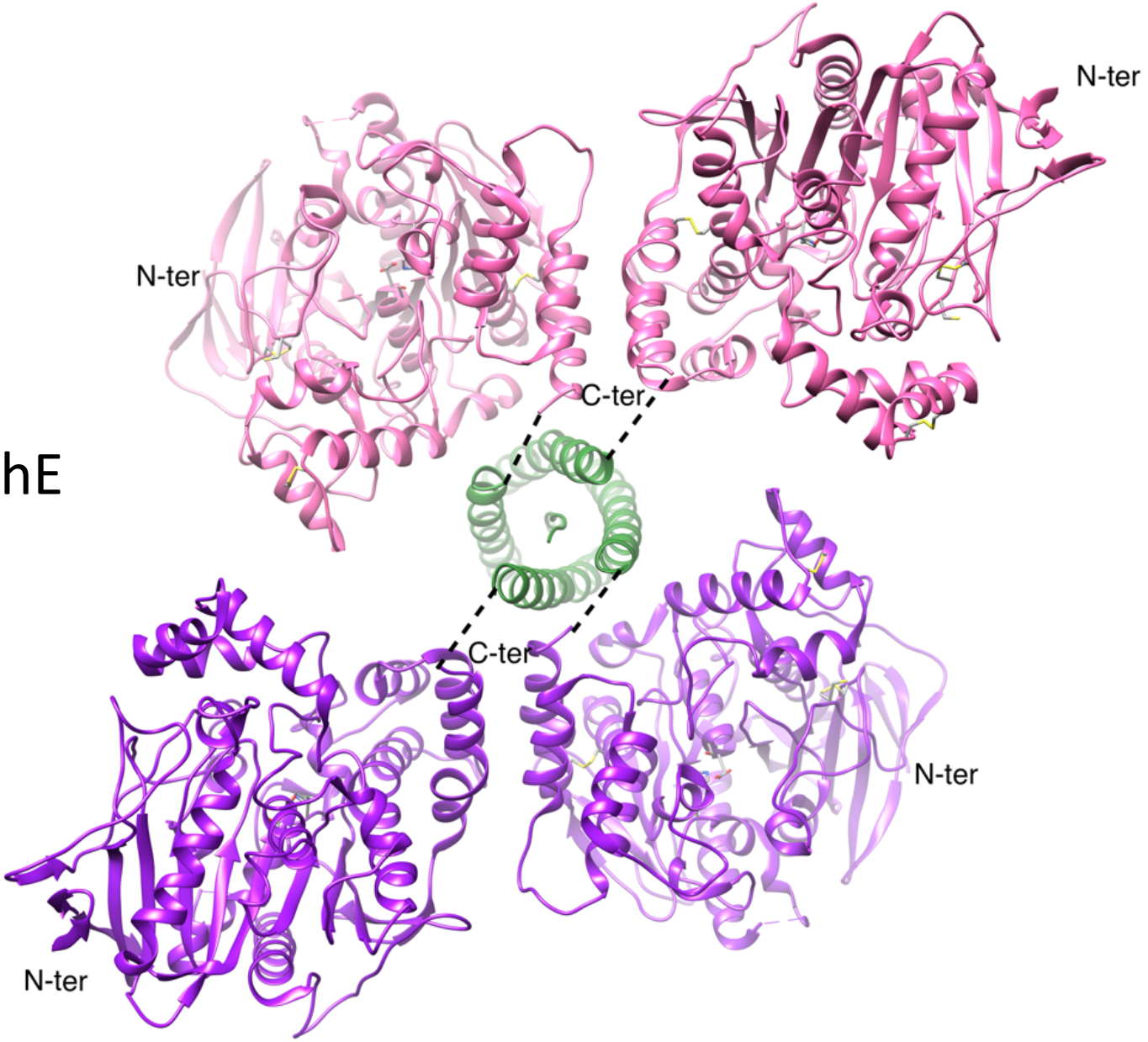
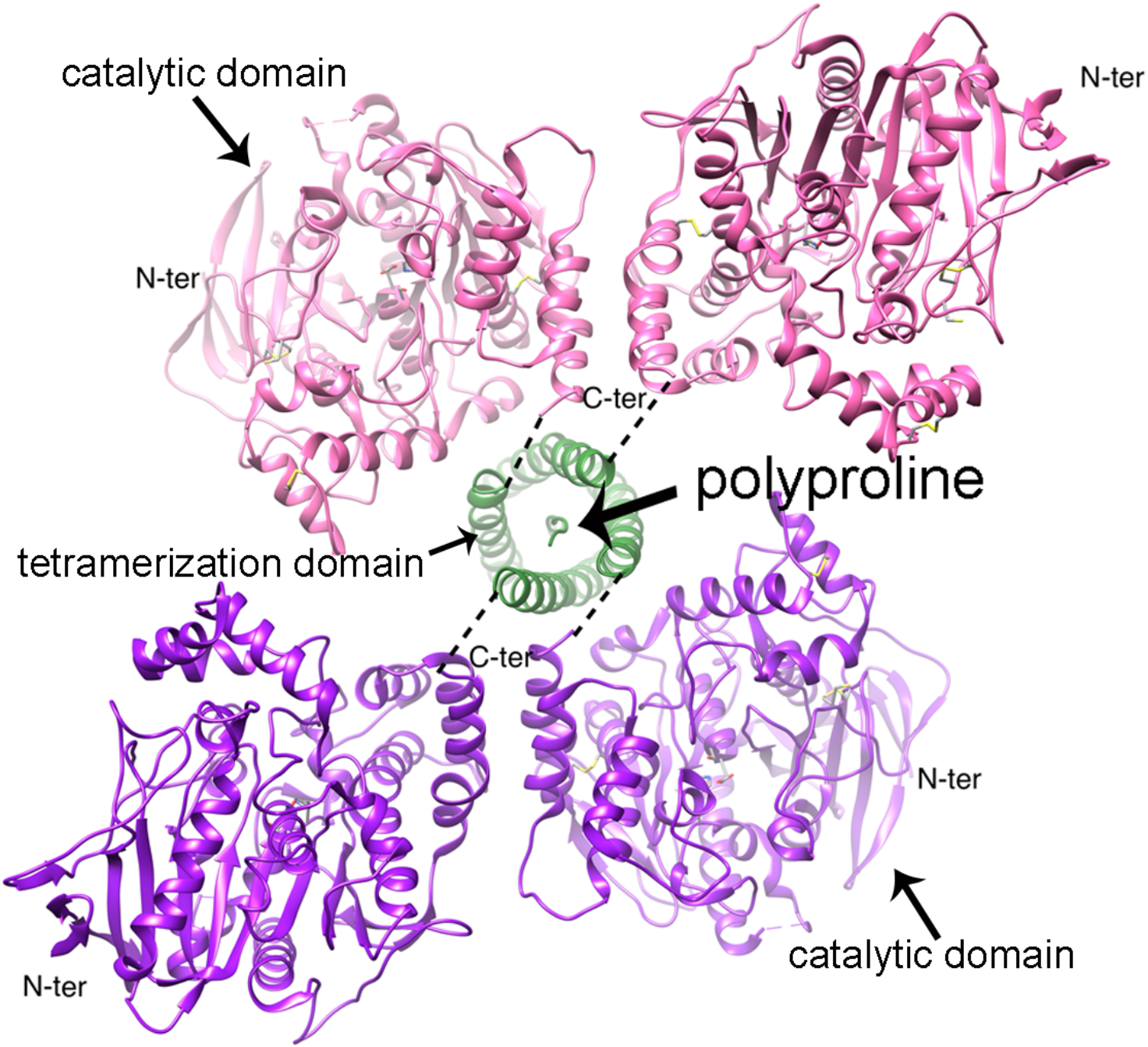
PDB 6i2t

Human BChE tetramer

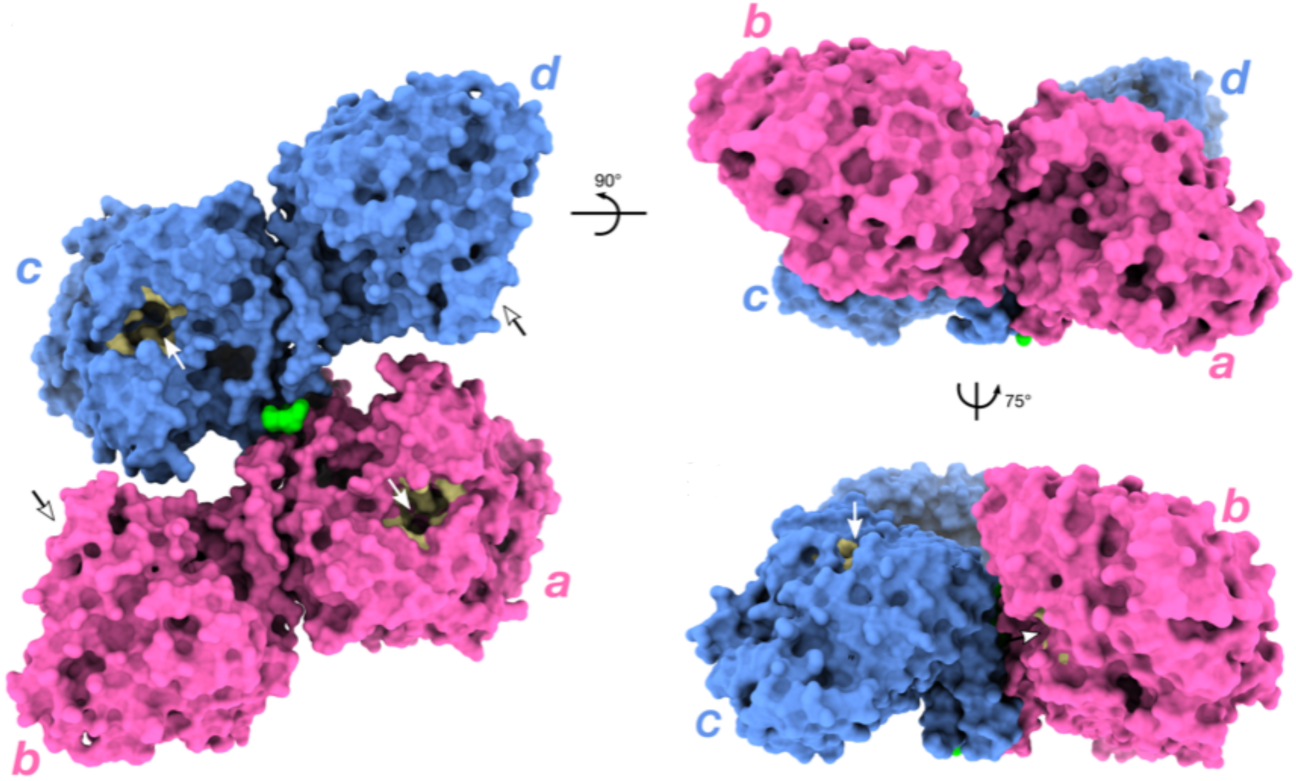


Leung MR, van Bezouwen LS, Schopfer LM, Sussman JL, Silman I, Lockridge O and Zeev-Ben-Mordehai T (2018) *Proc Natl Acad Sci U S A* **115**:13270-13275.


catalytic domain
N-ter
N-ter
C-ter
polyproline
tetramerization domain
C-ter
N-ter
N-ter
catalytic domain

- The tetramerization domain of BChE consists of 40 amino acids at the C-terminus.
- Four parallel alpha-helical tetramerization domains wrap around a single antiparallel polyproline-rich peptide helix.
- There are no covalent bonds between the tetramerization domain and polyproline.
- The structure is stabilized by hydrophobic contacts with the indole rings of 3 tryptophans from each BChE monomer and by a hydrogen-bond network.

4 catalytic domains do not lie on the same plane



Entrance to the active site gorge is indicated by arrows.

The soluble BChE tetramer is the product of more than 1 gene

- Human BChE (accession number P06276) is encoded by one gene located on chromosome 3q26.
- The BCHE gene makes monomers and disulfide linked dimers, but does not make BChE tetramers.
- Association of BChE into tetramers requires a polyproline-rich peptide.
- Polyproline-rich peptides embedded in BChE tetramers originate from a variety of proteins.

13 proteins donate polyproline-rich peptides to BChE tetramers in human plasma

1. Lamellipodin
2. UDP-N-acetylglucosamine transferase and deubiquitinase
3. Protein piccolo
4. Formin binding protein 4
5. Synaptopodin
6. Leimodin-2
7. Acetylcholinesterase membrane anchor PRiMA
8. Homeobox protein Hox-B4
9. Zinc finger CCCH domain-containing protein 4
10. Diaphanous 1
11. Zinc finger homeobox protein 4
12. Formin-like protein 1
13. Zinc finger protein Z1C5

Blue font indicates donor proteins for other BChE and AChE tetramers.

12 proteins donate polyproline-rich peptides to BChE tetramers in porcine milk

1. Acrosin
2. Homeobox protein HoxB4
3. Lysine-specific demethylase 6B
4. Zinc finger homeobox protein 4
5. Zinc finger CCCH type containing protein 4
6. Disabled homolog 2-interacting protein-like isoform 1
7. Protein FAM171A2
8. FH2 domain-containing protein 1
9. Proline-rich protein 12
10. WAS/WASL-interacting protein family member 2 isoform X1
11. Proline-rich protein 16
12. Proline-rich membrane anchor 1, PRIMA1

5 proteins donate polyproline-rich peptides to AChE tetramers in fetal bovine serum

1. Lamellipodin
2. UDP-N-acetyl glucosamine transferase ALG13 subunit homolog
3. Leiomodin-2
4. Protein piccolo
5. Zinc finger homeobox protein 4

60 proteins donate polyproline-rich peptides to BChE tetramers secreted by Chinese Hamster Ovary cells

Protein donors for recombinant human BChE, human plasma BChE and porcine milk BChE

1. Lamellipodin
2. Zinc finger homeobox protein 4
3. Leiomodin-2
4. Homeobox protein Hox-B4
5. Zinc finger CCCH domain-containing protein 4

Soluble plasma BChE and AChE tetramers do not derive from membrane-bound forms, anchored by ColQ or PRiMA

Polyproline peptides in soluble plasma BChE and AChE

PSPPL PPPPP PPPPP PPPPP PPPPP LP

human plasma BChE; lamellipodin

PPPPP PPPPP PPPPP PPPPP PPPPP

fetal bovine serum AChE; UDP-N-acetyl glucosamine transferase ALG13 subunit homology domain-containing protein 1

Polyproline peptides in membrane-bound BChE and AChE

LLTPP PPPLF PPPFF human ColQ

RPPPP LPPPPP PPPPP PRLLS human PRiMA

- Protein donors of polyproline-rich peptides are in the nucleus, cytoplasm, endoplasmic reticulum, and cell membrane.
- There is no consistent set of polyproline-rich peptides in BChE tetramers.
- For example, Lamellipodin is a source of polyproline-rich peptides for serum BChE, serum AChE, and BChE tetramers secreted by CHO cells, but is absent in porcine milk BChE tetramers.

Conclusion:
Soluble BChE and AChE subunits are polyproline peptide scavengers. Polyproline peptides become available during protein turnover. The scavenged polyproline peptides serve as the glue that joins 4 subunits into a stable tetramer.

Abstract: The genes for acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) encode the proteins responsible for enzyme activity. Additional gene products, PRiMA and ColQ, anchor AChE and BChE proteins into membranes. Soluble AChE and BChE tetramers are composed of 4 identical subunits plus one polyproline-rich peptide. Dilution does not release the polyproline-rich peptide from tetramers. However, protein denaturation, for example heating in a boiling water bath, dissociates the polyproline-rich peptide. Using mass spectrometry to sequence peptides released from soluble AChE and BChE tetramers, we find sequences that correspond to proline-rich regions from a variety of proteins. A typical peptide sequence contains 20 consecutive prolines in a 23-residue peptide LPPPPPPPPPPPPPPPPPPPLP. There is no single, common consensus sequence i.e., no specific gene appears to be responsible for the polyproline-rich peptides found in soluble AChE and BChE tetramers. We propose that during metabolic turnover, protein fragments containing polyproline-rich sequences are scavenged by AChE and BChE dimers, to make stable AChE and BChE tetramers. The 40-residue, alpha-helical C-terminus of AChE or BChE is the tetramerization domain that binds the polyproline-rich peptide. Four parallel alpha helices wrap around a single antiparallel polyproline peptide to lock the tetramer in place. This organization was established by classical X-ray crystallography for isolated C-termini in complex with a proline-rich peptide. The organization was confirmed for intact, tetrameric human BChE using cryoelectron microscopy.