

## **ALESSANDRA ASTEGNO**

### **Associate Professor of Biochemistry**

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### **Current position**

2022-present: Associate Professor of Biochemistry, Dept of Biotechnology, University of Verona

### **Previous positions**

- 2011-2022: Assistant Professor of Biochemistry, University of Verona.
- 2019: Visiting researcher, CIC bioGUNE Research Center (Spain).
- 2010-2011: Postdoctoral Fellow in Biochemistry, University of Verona
- 2007-2010: PhD Fellow, Laboratory of Biochemistry, Dept of Biotechnology, University of Verona.
- 2004: Visiting scholar, University of Manchester, UK.

### **Education**

- 2010: PhD in Applied Biotechnologies, University of Verona
- 2010: Master degree cum laude in Agri-Food Biotechnology, University of Verona.
- 2004: Master Degree cum laude in Agro-industrial Biotechnology, University of Verona.

### **Career breaks**

- January 2015- June 2015: Maternity Leave
- October 2020- February 2021: Maternity Leave

### **Awards and qualifications**

- 29 September 2023: Qualification as Full Professor of Biochemistry in Italian Universities.
- 24 September 2018: Qualification as Associate Professor of Biochemistry in Italian Universities.
- 2019: Winner, International Mobility Grant, University of Verona, for research at CIC bioGUNE Research Center, Spain
- 2017: winner of an Italian FFABR ("Fondo per il Finanziamento delle Attività Base di Ricerca", Italian Law 232/2016) research fund. Amount: € 3000.

### **Current teaching activities in degree programs at the University of Verona**

- Course of Biochemistry, degree program in Pharmacy.
- Course of Molecular mechanisms of diseases, international Master's degree program in Molecular and Medical Biotechnology.
- Course of Nutritional Biochemistry, degree program in Innovation and Sustainability in Industrial Food Production.
- Course of Enzymology in Medical Biotechnology, international Master's degree program in Molecular and Medical Biotechnology.

### **Current institutional roles at the University of Verona**

- Member of the Academic Board of the PhD Program in Molecular, Industrial, and Environmental Biotechnologies, University of Verona.
- Member of the Quality Assurance Committee for the Master's degree program in Molecular and Medical Biotechnology, and for the degree program in Pharmacy.
- Member of the Quality Assurance Committee for Research, Dep of Biotechnology.
- Member of the Quality Assurance Committee for the PhD Program in Molecular, Industrial, and Environmental Biotechnologies.
- Member of the Teaching Committee for the degree program in Pharmacy and for the Master's degree program in Molecular and Medical Biotechnology.
- Member of the selection committee for the awarding of scholarships to foreign students for the Master's degree program in Molecular and Medical Biotechnology.

- Member of the Committee for the evaluation of applications for external teaching positions, Department of Biotechnology.

### Supervision activities

- Supervisor of > 40 experimental theses for the Bachelor's Degree in Biotechnology and the Master's Degree in Molecular and Medical Biotechnology, University of Verona.
- Supervisor of 3 PhD students and co-supervisor of 4 PhD students in biochemistry at the University of Verona.
- Supervisor of > 10 research fellows and postdoctoral researchers in biochemistry, University of Verona.

### Scientific interests

My scientific career focused, as a general topic, on the structure-function relationships in enzymatic proteins, both in eukaryotes and bacteria. These enzymes have been investigated by using a multidisciplinary methodological approach, including kinetic studies, structural studies and protein-ligand interaction studies.

My group has wide expertise in recombinant protein production and protein engineering, enzymology, biochemistry and biophysics applied to characterize the following protein families in different organisms:

- **Metabolic enzymes involved in cysteine biosynthesis in eukaryotes and bacteria.** My laboratory is investigating the reaction mechanisms of catalysis and regulation of enzymes involved in H<sub>2</sub>S biogenesis in eukaryotic and bacterial cells. H<sub>2</sub>S has emerged as a multifunctional signaling molecule in human pathogens and targeting the enzymes involved in its metabolism represent an optimal strategy for versatile antibiotic/drug therapies.
- **Calcium sensors (calmodulin and centrin) and the mechanisms by which these proteins regulate complex molecular calcium dependent processes in different organisms.** My lab has been systematically cloning and studying the structural, metal and target-peptide binding properties of different member of calmodulin and centrin proteins in Arabidopsis and in human pathogens using complementary biophysical and structural approaches. This has allowed significant steps forward in the understanding if these proteins act as true Ca<sup>2+</sup> sensors and how they contribute to Ca<sup>2+</sup> signalling in distinct ways.
- **Cyclophilins and their interaction with cyclosporin A.** My research activity focused also on the characterization of enzymes with a peptidyl-prolyl isomerases (PPIases) activity from pathogens. These enzymes, also known as cyclophilins, are crucial for different biological processes and represent attractive drug candidates for the development of new molecules with antimicrobial activity.

### Funded Projects

- 2023: Project funded by the Veneto Region under the European Social Fund. "Identification of Bioactive Molecules in Nutraceuticals and Bee Products and Study of Their Potential Neuroprotective Effect." Co-supervisor.
- 2022: PRIN 2022 project (MUR; European Union – NextGenerationEU). "Hydrogen sulfide metabolism in Pseudomonas aeruginosa (patho)physiology." Unit Coordinator.
- 2022: Project funded by the Spanish Ministry of Science and Innovation (MICINN). "Structural Basis of CBS Domain-Mediated Regulation of the Enzyme Cystathionine Beta-Synthase and the Magnesium Transporter CNNM1 (CBS-CNNM1)." Participant.
- 2021: CIFRE PhD program (ANRT, French Ministry of Higher Education and Research). Partners: University of Montpellier, University of Verona, Oenobrand. "Identification and characterization of C-S lyase enzymes for aroma release in beer and wine." Supervisor of the PhD research activity (Luigi Clerat) at the University of Verona.
- 2020: Joint Project, University of Verona. "Exploring the natural biodiversity of C-S lyase enzymes in lactic acid bacteria for food and food-related applications." Co-supervisor.
- 2019: Research contract with Oenobrand. "Production of the recombinant enzyme C-S lyase from Lactobacillus delbrueckii subsp. bulgaricus." Principal Investigator.
- 2019: CooperInt grant (international academic mobility), University of Verona / CIC bioGUNE (Spain). "Structure-function-activity studies on Toxoplasma gondii transsulfuration enzymes." Principal investigator.
- 2019: Joint Project, University of Verona. "Green fluorescent protein-based biosensor for multiplexed detection of heavy metals in natural waters." Principal Investigator.
- 2018: PRIN 2017 project. "Regulatory signals and redox systems in plant growth-defence trade-off." Unit Coordinator.
- 2017-2020: Project "3S-4H - Safe, Smart, Sustainable Food for Health." Participant.
- 2016: Joint Project, University of Verona. "Engineering a fusion enzyme for determination of bioactive amines." Principal Investigator.
- 2016: Joint Project, University of Verona. "Improving wine aroma complexity by directed evolution of non-Saccharomyces yeast enzymes." Participant.

- 2014: Joint Project, University of Verona. “Engineering volatile thiol release for improved wine aroma: directed evolution of yeast cystathionine beta-lyase.” Participant.

#### **Invited lectures/seminars and organization of meetings (last ten years)**

- 2026: chair of the session “Proteins in Health and Disease” at the Decoding Protein Function Through Structure and Engineering: a joint symposium by the INPEC network and the SIB Proteins Groups. Rome, September 16-18, 2026.
- 2026: Member of the Organizing Committee, Italian Congress of Biotechnology (Biotech 2026), University of Verona, June 24-26, 2026.
- 2026: Invited seminar, “Structural and Regulatory Diversity of Cystathionine- $\beta$ -Synthase: Insights from Pathogen Enzymes.” ITACA.SB Workshop Crystallographic Quality and Beyond: Structural Biology at the Core of Rational Drug Design, Rome (Italy), February 11-13, 2026.
- 2026: Invited short course (2 hours), “Application of Targeted Protein Degradation in Infectious Diseases.” PhD Program in Molecular, Industrial and Environmental Biotechnologies, University of Verona, January 30, 2026.
- 2024: Invited seminar, “The Large Repertoire of Calmodulin-Related Proteins in Plants and Parasites.” 17th International Meeting of the European Calcium Society (ECS2024), Cambridge (UK), September 1-4, 2024.
- 2023: Invited seminar, “Targeting H<sub>2</sub>S-Producing Enzymes as an Antimicrobial Strategy.” Manchester Institute of Biotechnology, Manchester (UK), September 27, 2023.
- 2022: Invited short course, “Enzyme Therapy: Current Challenges and Future Perspectives.” PhD Program in Biotechnology, University of Verona.
- 2022: Session Chair, “Decoding Calcium Signals: Sensors and Effectors.” Plant Calcium Signaling Conference (PCS 2022), Milan (Italy), July 11-13, 2022.
- 2021: Selected oral presentation, “Unique Conformation of Cystathionine Beta-Synthase from *Toxoplasma gondii*.” 61st Congress of the Italian Society of Biochemistry and Molecular Biology (SIB), virtual meeting, September 23-24, 2021.
- 2018: Organizer, workshop “Protein Structure-Function Relationship: New Challenges and Advancements.” Verona, October 19, 2018.
- 2018: Organizer, National Symposium “Proteine 2018” (Italian Society of Biochemistry and Molecular Biology), Verona, May 28-30, 2018.
- 2018: Organizer, Winter School in Applied Bioinformatics, Alba di Canazei (Italy), January 21-25, 2018.
- 2018: Invited seminar, “Plant Calmodulin-Like Proteins: Calcium Binding and Target Interactions.” Proteine 2018, Verona, May 28-30, 2018.
- 2018: Invited seminar, “Towards Understanding Plant Calcium Signaling through Calmodulin-Like Proteins: A Biochemical and Structural Perspective.” PhD Program in Biochemistry, Sapienza University of Rome (Italy), March 16, 2018.
- 2018: Invited seminar, “Characterization of Cystathionine  $\gamma$ -Lyase from *Toxoplasma gondii*: A Target for Drug Development?” 3rd International Conference on Enzymology and Molecular Biology, London (UK), March 5-7, 2018.
- 2018: Invited seminar, “Advances in Biochemical and Structural Understanding of Plant Calmodulin-Like Proteins: The Case of Arabidopsis CML19.” University of Milan (Italy), February 15, 2018.
- 2017: Invited seminar, “A Biophysical and Structural Approach to Investigate Calcium Sensor Properties of Plant Calmodulin-Like Proteins.” 9th International Conference on Structural Biology, Zurich (Switzerland), September 18–20, 2017.
- 2016: Invited seminar, “Calcium Signaling in Plants through Calmodulin-Like Proteins: Biochemical and Biophysical Characterization of Arabidopsis CML36.” National Meeting of the Division of Biological Systems Chemistry, Verona (Italy), September 21-23, 2016.

#### **Public Engagement**

- 2026: PLS (National Plan for Scientific Degrees): “*Biotechnology in the Laboratory: From DNA to Recombinant Proteins.*” Outreach activity for high school students, University of Verona.
- 2025: Initiative within “*BIOTECH WEEK 2025: BIOTECNOLOGIE A VERONA: IERI PIONIERI, OGGI PROTAGONISTI DELL’INNOVAZIONE.*” Mini-symposium, “*H<sub>2</sub>S in Pseudomonas aeruginosa: Pathophysiological Mechanisms and Novel Therapeutic Targets.*” University of Verona. Initiative for the promotion, dissemination, and public engagement of research activities.
- 2025: Round Table, Workshop: “*Beneficial Effects on Health of Biomolecules from Food and Nutraceuticals.*” University of Verona.

- 2025: PLS Biotechnology - Biotechnology for the Planet and Human Health (PCTO Laboratory): “*Proteins as Therapeutic Targets: Cystathionine  $\gamma$ -Lyase from Pseudomonas aeruginosa.*” Outreach activity for high school students, University of Verona.
- 2024: PCTO Program: “*Proteins as Therapeutic Targets: Cystathionine  $\gamma$ -Lyase from Pseudomonas aeruginosa.*” Outreach activity for high school students, University of Verona.
- 2017: Outreach seminar, “From Gene to Protein.” National Plan for Scientific Degrees (PLS), University of Verona, March 24, 2017.

#### Memberships:

- 2017-present: Member of the Italian Society of Biochemistry and Molecular Biology (SIB)
- 2016-present: Member of the European Calcium Society (ECS)

#### Citation metrics and publications

My scientific activity is documented by 54 peer-reviewed publications, and I serve as corresponding author in 26 of them.

GOOGLE SCHOLAR, h-index=22; i10-index 36, citations 1531 (may 2026)

[https://scholar.google.it/citations?hl=it&user=1KWW\\_hoAAAAJ](https://scholar.google.it/citations?hl=it&user=1KWW_hoAAAAJ)

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1. Favretto F, Jiménez-Faraco E, Fruncillo S, Masè N, Dominici P, Hermoso JA, **Astegno A\***. Structural and Kinetic Variability of Pathogen Cyclophilins: Functional Diversification and Therapeutic Potential. *ACS Infect Dis.* 2026 May 8;12(5):1538-1554. doi: 10.1021/acsinfecdis.6c00016.
2. Massa N, Catalano F, Fruncillo S, Troilo F, Mellini M, Favretto F, Leoni L, Rampioni G, Giuffrè A, di Matteo A, **Astegno A\***. Distinct contributions of O-acetylserine sulfhydrylases to cysteine biosynthesis in *Pseudomonas aeruginosa*. *Protein Sci.* 2026 Mar;35(3):e70498. doi: 10.1002/pro.70498.
3. Lauriola A, Enriquè Steinberg JH, Sarubo M, Maspero E, Rossi FA, Mouri Y, Pedretti M, Hajisadeghian M, Taibi V, Vettori A, Vitulo N, Assfalg M, D'Onofrio M, Rossi M, Yasue A, **Astegno A**, Polo S, Santi S, Kudo Y, Guardavaccaro D. The E3 ligase RNF32 controls the I $\kappa$ B kinase complex and NF- $\kappa$ B signaling in intestinal stem cells. *Mol Cell.* 2025 Nov 20;85(22):4254-4267.e9. doi:10.1016/j.molcel.2025.10.005. Epub 2025 Oct 29. PMID: 41167191.
4. Favretto F, Fruncillo S, Jiménez-Faraco E, Masè N, Dominici P, de José UP, Muñoz IG, Hermoso JA, **Astegno A\***. Redox-dependent activity and thioredoxin interaction of cyclophilin TgCyp21 from *Toxoplasma gondii*. *Int J Biol Macromol.* 2025 Nov;330(Pt 1):148019. doi: 10.1016/j.ijbiomac.2025.148019. Epub 2025 Sep 30. PMID: 41038464.
5. Clérat L, Conter C, **Astegno A**, Schneider R, Cavalier F, Vivès E. Exploring Carbon-Sulfur (CS) Lyase Enzymes across Microbial Diversity for Enhanced Thiol Release in Beer and Wine. *J Agric Food Chem.* 2025 Sep 8. doi:10.1021/acs.jafc.5c07932.
6. Li Z, Kim HJ, Luoni L, Conter C, Masè N, Resentini F, Xie P, **Astegno A\***, Bonza MC, Hua J. Evolutionarily conserved BON1 regulates the basal cytosolic Ca<sup>2+</sup> level by calmodulin-independent activation of Ca<sup>2+</sup> pumps in *Arabidopsis*. *Proc Natl Acad Sci U S A.* 2025 Jun 10;122(23):e2504457122. doi: 10.1073/pnas.2504457122.
7. Conter C, Núñez-Franco R, Al-Sadeq DW, Fernández-Rodríguez C, Goikoetxea-Usandizaga N, Nasrallah GK, Nomikos M, Martínez-Chantar ML, **Astegno A**, Jiménez-Osés G, Martínez-Cruz LA. The disease-linked R336C mutation in cystathionine  $\beta$ -synthase disrupts communication with the PLP cofactor, yet maintains the enzyme's overall structural integrity. *FEBS J.* 2025 May 6. doi: 10.1111/febs.70116.
8. Favretto F, Jiménez-Faraco E, Catucci G, Di Matteo A, Travaglini-Allocatelli C, Sadeghi SJ, Dominici P, Hermoso JA, **Astegno A\***. Evaluating the potential of non-immunosuppressive cyclosporin analogs for targeting *Toxoplasma gondii* cyclophilin: Insights from structural studies. *Protein Sci.* 2024 Oct;33(10):e5157.
9. Pedretti M, Fernández-Rodríguez C, Conter C, Oyenarte I, Favretto F, di Matteo A, Dominici P, Petrosino M, Martínez-Chantar ML, Majtan T, **Astegno A\***, Martínez-Cruz LA. Catalytic specificity and crystal structure of cystathionine  $\gamma$ -lyase from *Pseudomonas aeruginosa*. *Sci Rep.* 2024 Apr 23;14(1):9364.
10. Al-Sadeq D, Conter C, Thanassoulas A, Al-Dewik N, Safieh-Garabedian B, Luis Alfonso Martínez-Cruz LA, Nasrallah G, **Astegno A\***, Nomikos M. Biochemical and structural impact of two novel missense mutations in cystathionine beta-synthase gene associated with homocystinuria. *Biochem J.* 2024 Apr 2:BCJ20240012.

11. Caruso L, Mellini M, Catalano Gonzaga O, **Astegno A**, Forte E, Di Matteo A, Giuffrè A, Visca P, Imperi F, Leoni L, Rampioni G. Hydrogen sulfide production does not affect antibiotic resistance in *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother*. 2024 Apr 3;68(4):e0007524.
12. Catalano F, Santorelli D, **Astegno A**, Favretto F, D'Abramo M, Del Giudice A, De Sciscio ML, Troilo F, Giardina G, Di Matteo A, Travaglini-Allocatelli C. Conformational and dynamic properties of the KH1 domain of FMRP and its fragile X syndrome linked G266E variant. *Biochim Biophys Acta Proteins Proteom*. 2024 Apr 17;1872(4):141019.
13. Pedretti M, Favretto F, Troilo F, Giovannoni M, Conter C, Mattei B, Dominici P, Travaglini-Allocatelli C, Di Matteo A, **Astegno A**. Role of myristoylation in modulating PCaP1 interaction with calmodulin. *Plant Physiol Biochem*. 2023 Oct;203:108003. doi: 10.1016/j.plaphy.2023.108003.
14. Conter C, Favretto F, Dominici P, Martinez-Cruz LA, **Astegno A\***. Key substrate recognition residues in the active site of cystathionine beta-synthase from *Toxoplasma gondii*. *Proteins*. 2023 Oct;91(10):1383-1393.
15. Fernández-Rodríguez C, Conter C, Oyenarte I, Favretto F, Quintana I, Martínez-Chantar ML, **Astegno A\***, Martínez-Cruz LA. Structural basis of the inhibition of cystathionine  $\gamma$ -lyase from *Toxoplasma gondii* by propargylglycine and cysteine. *Protein Sci*. 2023 Apr;32(4):e4619.
16. Favretto F, Jiménez-Faraco E, Conter C, Dominici P, Hermoso JA, **Astegno A\***. Structural Basis for Cyclosporin Isoform-Specific Inhibition of Cyclophilins from *Toxoplasma gondii*. *ACS Infect Dis*. 2023 Feb 10;9(2):365-377.
17. Bombardi L, Favretto F, Pedretti M, Conter C, Dominici P, **Astegno A\***. Conformational Plasticity of Centrin 1 from *Toxoplasma gondii* in Binding to the Centrosomal Protein SFI1. *Biomolecules*. 2022 Aug 13;12(8):1115.
18. Conter C, Fruncillo S, Favretto F, Fernández-Rodríguez C, Dominici P, Martínez-Cruz LA, **Astegno A\***. Insights into Domain Organization and Regulatory Mechanism of Cystathionine Beta-Synthase from *Toxoplasma gondii*. *Int J Mol Sci*. 2022 Jul 25;23(15):8169.
19. Troilo F, Pedretti M, Travaglini-Allocatelli C, **Astegno A\***, Di Matteo A. Rapid kinetics of calcium dissociation from plant calmodulin and calmodulin-like proteins and effect of target peptides. *Biochem Biophys Res Commun*. 2022 Jan 29;590:103-108.
20. Pedretti M, Bombardi L, Conter C, Favretto F, Dominici P, **Astegno A\***. Structural Basis for the Functional Diversity of Centrin: A Focus on Calcium Sensing Properties and Target Recognition. *Int J Mol Sci*. 2021 Nov 10;22(22):12173.
21. Fernández-Rodríguez C, Oyenarte I, Conter C, González-Recio I, Núñez-Franco R, Gil-Pitarch C, Quintana I, Jiménez-Osés G, Dominici P, Martínez-Chantar ML, **Astegno A\***, Martínez-Cruz LA. Structural insight into the unique conformation of cystathionine  $\beta$ -synthase from *Toxoplasma gondii*. *Comput Struct Biotechnol J*. 2021 Jun 6;19:3542-3555.
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23. **Astegno A**, Conter C, Bertoldi M, Dominici P. Structural Insights into the Heme Pocket and Oligomeric State of Non-Symbiotic Hemoglobins from *Arabidopsis thaliana*. *Biomolecules*. 2020 Nov 29;10(12):1615.
24. Conter C, Fruncillo S, Fernández-Rodríguez C, Martínez-Cruz LA, Dominici P, **Astegno A\***. Cystathionine  $\beta$ -synthase is involved in cysteine biosynthesis and H<sub>2</sub>S generation in *Toxoplasma gondii*. *Sci Rep*. 2020 Sep 4;10(1):14657.
25. Bombardi L, Pedretti M, Conter C, Dominici P, **Astegno A\***. Distinct Calcium Binding and Structural Properties of Two Centrin Isoforms from *Toxoplasma gondii*. *Biomolecules*. 2020 Aug 4;10(8):1142.
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31. La Verde V, Dominici P, **Astegno A\***. Towards Understanding Plant Calcium Signaling through Calmodulin-Like Proteins: A Biochemical and Structural Perspective. *Int J Mol Sci*. 2018 Apr 30;19(5):1331.

32. Rossignoli G, Phillips RS, **Astegno A**, Menegazzi M, Voltattorni CB, Bertoldi M. Phosphorylation of pyridoxal 5'-phosphate enzymes: an intriguing and neglected topic. *Amino Acids*. 2018 Feb;50(2):205-215.
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34. **Astegno A\***, Bonza MC, Vallone R, La Verde V, D'Onofrio M, Luoni L, Molesini B, Dominici P. *Arabidopsis* calmodulin-like protein CML36 is a calcium sensor that interacts with the plasma membrane Ca<sup>2+</sup>-ATPase isoform ACA8 and stimulates its activity. *J Biol Chem*. 2017 Sep 8;292(36):15049-15061.
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**Protein Data Bank submissions:**

| <b>ID</b> | <b>TITLE</b>   | <b>RELEASE DATE</b> |
|-----------|--|---------------------|
| 8R7S      | Crystal Structure of Cyclophilin TgCyp23 from Toxoplasma gondii in complex with NIM811 (N-methyl-4-isoleucine cyclosporin) | 2024-10-30          |
| 8B58      | Crystal Structure of Cyclophilin TgCyp23 from Toxoplasma gondii in complex with Cyclosporin A                              | 2023-02-01          |
| 8BIS      | Crystal structure of cystathionine gamma-lyase from Toxoplasma gondii in complex with DL-propargylglycine                  | 2023-04-12          |
| 7NL1      | Crystal structure of cystathionine gamma-lyase from Toxoplasma gondii  | 2022-03-02          |
| 6Y21      | Crystal structure of delta466-491 cystathionine beta-synthase from Toxoplasma gondii with L-Cystathionine                  | 2021-02-24          |
| 6XYL      | Crystal structure of delta466-491 cystathionine beta-synthase from Toxoplasma gondii with L-serine                         | 2021-02-10          |
| 6Z3S      | Crystal structure of delta466-491 cystathionine beta-synthase from Toxoplasma gondii with O-Acetylserine                   | 2021-06-02          |
| 6ZS7      | Crystal structure of delta466-491 cystathionine beta-synthase from Toxoplasma gondii with L-cysteine                       | 2021-07-28          |
| 7BA4      | Structure of Cystathionine gamma-lyase from Pseudomonas aeruginosa   | 2021-09-29          |
| 6XWL      | Cystathionine beta-synthase from Toxoplasma gondii   | 2021-02-03          |
| 3HBX      | Crystal structure of GAD1 from Arabidopsis thaliana  | 2009-07-28          |