

## References

- Abrahams, G., & Newman, J. (2019). *BLASTing away preconceptions in crystallisation trials*. 75(3), 184–192. <https://doi.org/10.1107/s2053230x19000141>
- Batool, M., Ahmad, B., & Choi, S. (2019). A Structure-Based Drug Discovery Paradigm. *International Journal of Molecular Sciences*, 20(11), 2783. <https://doi.org/10.3390/ijms20112783>
- Beelman, R. B., Kalaras, M. D., & Richie, J. P. (2019). Micronutrients and Bioactive Compounds in Mushrooms. *Nutrition Today*, 54(1), 16–22. <https://doi.org/10.1097/nt.0000000000000315>
- Bolanos-Garcia, V. M., & Davies, O. R. (2006). Structural analysis and classification of native proteins from *E. coli* commonly co-purified by immobilised metal affinity chromatography. *Biochimica et Biophysica Acta (BBA) - General Subjects*, 1760(9), 1304–1313. <https://doi.org/10.1016/j.bbagen.2006.03.027>
- Clark, D. P., & Pazdernik, N. J. (2013). Regulation of Transcription in Prokaryotes. *Molecular Biology*, e338–e343. <https://doi.org/10.1016/b978-0-12-378594-7.00050-0>
- Cong, W., Xu, H., Liu, Y., Li, Q., Li, W., & Zhou, X. (2014). Production and functional characterisation of a novel fungal immunomodulatory protein FIP-SN15 shuffled from two genes of *Ganoderma* species. *Applied Microbiology and Biotechnology*, 98(13), 5967–5975. <https://doi.org/10.1007/s00253-014-5539-4>
- Dessau, M. A., & Modis, Y. (2011). Protein Crystallisation for X-ray Crystallography. *Journal of Visualized Experiments*, 47. <https://doi.org/10.3791/2285>
- Ding, Y., Seow, S. V., Huang, C. H., Liew, L. M., Lim, Y. C., Kuo, I. C., & Chua, K. Y. (2009). Coadministration of the fungal immunomodulatory protein FIP-Fve and a tumour-associated antigen enhanced anti-tumour immunity. *Immunology*, 128(1pt2), e881–e894. <https://doi.org/10.1111/j.1365-2567.2009.03099.x>
- Duong-Ly, K. C., & Gabelli, S. B. (2014). Salting out of Proteins Using Ammonium Sulfate Precipitation. *Methods in Enzymology*, 541, 85–94. <https://doi.org/10.1016/b978-0-12-420119-4.00007-0>

- Ejike, U. C., Chan, C. J., Lim, C. S. Y., & Lim, R. L. H. (2021). Functional evaluation of a recombinant fungal immunomodulatory protein from *L. rhinocerus* produced in *P. pastoris* and *E. coli* host expression systems. *Applied Microbiology and Biotechnology*, *105*(7), 2799–2813. <https://doi.org/10.1007/s00253-021-11225-x>
- Ejike, U. C., Chan, C. J., Okechukwu, P. N., & Lim, R. L. H. (2020). New advances and potentials of fungal immunomodulatory proteins for therapeutic purposes. *Critical Reviews in Biotechnology*, *40*(8), 1172–1190. <https://doi.org/10.1080/07388551.2020.1808581>
- Fekete, S., Beck, A., Veuthey, J.-L., & Guilleme, D. (2014). Theory and practice of size exclusion chromatography for the analysis of protein aggregates. *Journal of Pharmaceutical and Biomedical Analysis*, *101*, 161–173. <https://doi.org/10.1016/j.jpba.2014.04.011>
- Flick, A. C., Leverett, C. A., Ding, H. X., McInturff, E., Fink, S. J., Mahapatra, S., Carney, D. W., Lindsey, E. A., DeForest, J. C., France, S. P., Berritt, S., Bigi-Botterill, S. V., Gibson, T. S., Liu, Y., & O'Donnell, C. J. (2021). Synthetic Approaches to the New Drugs Approved during 2019. *Journal of Medicinal Chemistry*, *64*(7), 3604–3657. <https://doi.org/10.1021/acs.jmedchem.1c00208>
- Gligorijević, V., Renfrew, P. D., Kosciolk, T., Leman, J. K., Berenberg, D., Vatanen, T., Chandler, C., Taylor, B. C., Fisk, I. M., Vlamakis, H., Xavier, R. J., Knight, R., Cho, K., & Bonneau, R. (2021). Structure-based protein function prediction using graph convolutional networks. *Nature Communications*, *12*(1). <https://doi.org/10.1038/s41467-021-23303-9>
- Gomes, L., Monteiro, G., & Mergulhão, F. (2020). The Impact of IPTG Induction on Plasmid Stability and Heterologous Protein Expression by *Escherichia coli* Biofilms. *International Journal of Molecular Sciences*, *21*(2), 576. <https://doi.org/10.3390/ijms21020576>
- Guillamón, E., García-Lafuente, A., Lozano, M., D'Arrigo, M., Rostagno, M. A., Villares, A., & Martínez, J. A. (2010). Edible mushrooms: Role in the prevention of cardiovascular diseases. *Fitoterapia*, *81*(7), 715–723. <https://doi.org/10.1016/j.fitote.2010.06.005>

- Hamilton, S., Odili, J., Pacifico, M. D., Wilson, G. D., & Kupsch, Jörg-M. (2003). Effect of imidazole on the solubility of a his-tagged antibody fragment. *Hybridoma and Hybridomics*, 22(6), 347–355. <https://doi.org/10.1089/153685903771797048>
- Hicks, M., Bartha, I., di Iulio, J., Venter, J. C., & Telenti, A. (2019). Functional characterisation of 3D protein structures informed by human genetic diversity. *Proceedings of the National Academy of Sciences*, 116(18), 8960–8965. <https://doi.org/10.1073/pnas.1820813116>
- HSU, H.-C., HSU, C.-I., LIN, R.-H., KAO, C.-L., & LIN, J.-Y. (1997). Fip-vvo, a new fungal immunomodulatory protein isolated from *Volvariella volvacea*. *Biochemical Journal*, 323(2), 557–565. <https://doi.org/10.1042/bj3230557>
- Hsu, M. F., Wang, A. H. J., Yang, C. S., Huang, C. T., Hseu, R. S., Lin, C. W., Wu, M. Y., Huang, C. S., & Fu, H. Y. (2010). *Crystal structure of Ganoderma fungal immunomodulatory protein, GMI*. <https://doi.org/10.2210/pdb3kcw/pdb>
- Huang, L., Sun, F., Liang, C., He, Y.-X., Bao, R., Liu, L., & Zhou, C.-Z. (2009). Crystal structure of LZ-8 from the medicinal fungus *Ganoderma lucidum*. *Proteins: Structure, Function, and Bioinformatics*, 75(2), 524–527. <https://doi.org/10.1002/prot.22346>
- Jeong, H., Kim, H. J., & Lee, S. J. (2015). Complete Genome Sequence of *Escherichia coli* Strain BL21. *Genome Announcements*, 3(2). <https://doi.org/10.1128/genomea.00134-15>
- Karbalaei, M., Rezaee, S. A., & Farsiani, H. (2020). *Pichia pastoris*: A highly successful expression system for optimal synthesis of heterologous proteins. *Journal of Cellular Physiology*, 235(9), 5867–5881. <https://doi.org/10.1002/jcp.29583>
- Kazemipoor, M., Radzi, C. W. J. W. M., Cordell, G. A., & Yaze, I. (2012). Safety, Efficacy and Metabolism of Traditional Medicinal Plants in the Management of Obesity: A Review. *International Journal of Chemical Engineering and Applications*, 288–292. <https://doi.org/10.7763/ijcea.2012.v3.201>
- Kittimongkolsuk, P., Roxo, M., Li, H., Chuchawankul, S., Wink, M., & Tencomnao, T. (2021). Extracts of the Tiger Milk Mushroom (*Lignosus rhinocerus*) Enhance Stress Resistance and Extend Lifespan

- in *Caenorhabditis elegans* via the DAF-16/FoxO Signaling Pathway. *Pharmaceuticals*, *14*(2), 93.  
<https://doi.org/10.3390/ph14020093>
- Kim, J. S., Valencia, C. A., Liu, R., & Lin, W. (2007). Highly-Efficient Purification of Native Polyhistidine-Tagged Proteins by Multivalent NTA-Modified Magnetic Nanoparticles. *Bioconjugate Chemistry*, *18*(2), 333–341. <https://doi.org/10.1021/bc060195l>
- Kong, X., Zhang, J., Han, X., Zhang, P., Dai, X., Liu, J., Zhang, X., Lee, I., & Liu, S. (2013). High-Yield Production in *Escherichia coli* of Fungal Immunomodulatory Protein Isolated from *Flammulina velutipes* and Its Bioactivity Assay in Vivo. *International Journal of Molecular Sciences*, *14*(2), 2230–2241. <https://doi.org/10.3390/ijms14022230>
- Lai, C. K. M., Wong, K.-H., & Cheung, P. C. K. (2008). Antiproliferative Effects of Sclerotial Polysaccharides from *Polyporus rhinocerus* Cooke (Aphyllophoromycetidae) on Different Kinds of Leukemic Cells. *International Journal of Medicinal Mushrooms*, *10*(3), 255–264. <https://doi.org/10.1615/intjmedmushr.v10.i3.60>
- Lau, B. F., Abdullah, N., & Aminudin, N. (2013). Chemical Composition of the Tiger's Milk Mushroom, *Lignosus rhinocerotis*(Cooke) Ryvarden, from Different Developmental Stages. *Journal of Agricultural and Food Chemistry*, *61*(20), 4890–4897. <https://doi.org/10.1021/jf4002507>
- Lee, M. L., Tan, N. H., Fung, S. Y., Tan, C. S., & Ng, S. T. (2012). The Antiproliferative Activity of Sclerotia of *Lignosus rhinocerus*(Tiger Milk Mushroom). *Evidence-Based Complementary and Alternative Medicine*, *2012*, 1–5. <https://doi.org/10.1155/2012/697603>
- Li, L.-D., Mao, P.-W., Shao, K.-D., Bai, X.-H., & Zhou, X.-W. (2019). Ganoderma proteins and their potential applications in cosmetics. *Applied Microbiology and Biotechnology*, *103*(23-24), 9239–9250. <https://doi.org/10.1007/s00253-019-10171-z>
- Li, S., Nie, Y., Ding, Y., Shi, L., & Tang, X. (2014). Recombinant Expression of a Novel Fungal Immunomodulatory Protein with Human Tumor Cell Antiproliferative Activity from *Nectria*

- haematococca. *International Journal of Molecular Sciences*, 15(10), 17751–17764.  
<https://doi.org/10.3390/ijms151017751>
- Li, Q., Wang, X., Bao, T., Ran, L., Lin, J., & Zhou, X. (2010). In vitro Synthesis of a Recombinant Fungal Immunomodulatory Protein from Lingzhi or Reishi Medicinal Mushroom, *Ganoderma lucidum* (W.Curt.:Fr.) P.Karst. (Aphyllophoromycetidae) and Analysis of Its Immunomodulatory Activity. *International Journal of Medicinal Mushrooms*, 12(4), 347–358.  
<https://doi.org/10.1615/intjmedmushr.v12.i4.20>
- Lin, H., Yu Shan Chang, Lin, L., Chiung Fang Haung, Wu, C.-Y., & Keng Liang Ou. (2014). *An Immunomodulatory Protein (Ling Zhi-8) from a Ganoderma lucidum Induced Acceleration of Wound Healing in Rat Liver Tissues after Monopolar Electrosurgery*. 2014, 1–12.  
<https://doi.org/10.1155/2014/916531>
- Lin, J., Guan, S., Duan, Z., Shen, Y., Fan, W., Chen, L., Zhang, L., Zhang, L., & Li, T. (2016). Gene cloning of a novel fungal immunomodulatory protein from *Chroogomphis rutilus* and its expression in *Pichia pastoris*. *Journal of Chemical Technology & Biotechnology*, 91(11), 2761–2768.  
<https://doi.org/10.1002/jctb.4881>
- Lin, J.-W., Jia, J., Shen, Y.-H., Zhong, M., Chen, L.-J., Li, H.-G., Ma, H., Guo, Z.-F., Qi, M.-F., Liu, L.-X., & Li, T.-L. (2013). Functional expression of FIP-fve, a fungal immunomodulatory protein from the edible mushroom *Flammulina velutipes* in *Pichia pastoris* GS115. *Journal of Biotechnology*, 168(4), 527–533. <https://doi.org/10.1016/j.jbiotec.2013.09.013>
- Lindequist, U., Kim, H. W., Tiralongo, E., & Van Griensven, L. (2014). Medicinal Mushrooms. *Evidence-Based Complementary and Alternative Medicine*, 2014, 1–2.  
<https://doi.org/10.1155/2014/806180>
- Liu, Y., Bastiaan-Net, S., & Wichers, H. J. (2020). Current Understanding of the Structure and Function of Fungal Immunomodulatory Proteins. *Frontiers in Nutrition*, 7.  
<https://doi.org/10.3389/fnut.2020.00132>

- Liu, Y., Bastiaan-Net, S., Toke Hoppenbrouwers, Li, Z., Xie, Y., Wang, Y.-L., Wei, X., Du, G., Zhang, H., Shariff, K., Xin, F., & Wichers, H. J. (2021). *Linking the thermostability of FIP-nha (Nectria haematococca) to its structural properties.* 213, 555–564. <https://doi.org/10.1016/j.ijbiomac.2022.05.136>
- Liu, Y., Bastiaan-Net, S., Toke Hoppenbrouwers, Li, Z., Xie, Y., Wang, Y.-L., Wei, X., Du, G., Zhang, H., Shariff, K., Xin, F., & Wichers, H. J. (2022). *Fungal immunomodulatory protein FIP-gmi.* 213, 555–564. <https://doi.org/10.2210/pdb7WDM/pdb>
- Lopez-Gartner, G. A., & Uribe-Echeverry, P. T. (2017). Fungal immunomodulatory proteins in the context of biomedicine. *Frontiers in Bioscience*, 9(2), 286–306. <https://doi.org/10.2741/e803>
- Luo, S., McNeill, M., Myers, T. G., Hohman, R. J., & Levine, R. L. (2007). Lon protease promotes survival of *Escherichia coli* during anaerobic glucose starvation. *Archives of Microbiology*, 189(2), 181–185. <https://doi.org/10.1007/s00203-007-0304-z>
- McPherson, A., & Gavira, J. A. (2013). Introduction to protein crystallisation. *Acta Crystallographica Section F Structural Biology Communications*, 70(1), 2–20. <https://doi.org/10.1107/s2053230x13033141>
- Mesters, J. R., & Hilgenfeld, R. (2007). Protein Glycosylation, Sweet to Crystal Growth? *Crystal Growth & Design*, 7(11), 2251–2253. <https://doi.org/10.1021/cg7006843>
- Nallathamby, N., Phan, C.-W., Seow, S. L.-S., Baskaran, A., Lakshmanan, H., Abd Malek, S. N., & Sabaratnam, V. (2018). A Status Review of the Bioactive Activities of Tiger Milk Mushroom *Lignosus rhinocerotis* (Cooke) Ryvarden. *Frontiers in Pharmacology*, 8. <https://doi.org/10.3389/fphar.2017.00998>
- Novagen. (n.d.). *pET-28a-c(+)* Vectors. [https://www.helmholtz-muenchen.de/fileadmin/PEPF/pET\\_vectors/pET-28a-c\\_map.pdf](https://www.helmholtz-muenchen.de/fileadmin/PEPF/pET_vectors/pET-28a-c_map.pdf)
- Paaventhana, P., Joseph, J. S., Seow, S. V., Vaday, S., Robinson, H., Chua, K. Y., & Kolatkar, P. R. (2003). A 1.7Å Structure of Fve, a Member of the New Fungal Immunomodulatory Protein Family.

*Journal of Molecular Biology*, 332(2), 461–470. [https://doi.org/10.1016/s0022-2836\(03\)00923-](https://doi.org/10.1016/s0022-2836(03)00923-9)

9

Parker, M. W. (2003). Protein Structure from X-Ray Diffraction. *Journal of Biological Physics*, 29(4), 341–362. <https://doi.org/10.1023/a:1027310719146>

Piešová, M., Czán, A., Šajgalík, M., Czánová, T., & Čep, R. (2017). Experimental Quantification of the Austenitic Phase in Steels Using the Average Peak Method of x-ray Diffractometry. *Procedia Engineering*, 192, 689–694. <https://doi.org/10.1016/j.proeng.2017.06.119>

Pushparajah, V., Fatima, A., Chong, C. H., Gambule, T. Z., Chan, C. J., Ng, S. T., Tan, C. S., Fung, S. Y., Lee, S. S., Tan, N. H., & Lim, R. L. H. (2016). Characterisation of a New Fungal Immunomodulatory Protein from Tiger Milk mushroom, *Lignosus rhinocerotis*. *Scientific Reports*, 6. <https://doi.org/10.1038/srep30010>

Rabiee, M., Namaei Ghasemnia, N., Rabiee, N., & Bagherzadeh, M. (2021). Microfluidic devices and drug delivery systems. *Biomedical Applications of Microfluidic Devices*, 153–186. <https://doi.org/10.1016/b978-0-12-818791-3.00013-9>

Rafieian-Kopaei, M. (2012). Medicinal plants and the human needs. *Journal of HerbMed Pharmacology Journal Homepage J HerbMed Pharmacol*, 1(1), 1–2. <https://core.ac.uk/download/pdf/143844776.pdf>

Rafieian-Kopaei, M., Baradaran, A., & Rafieian, M. (2013). Oxidative stress and the paradoxical effects of antioxidants. *Journal of Research in Medical Sciences: The Official Journal of Isfahan University of Medical Sciences*, 18(7), 629. <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3897036/>

Ray, W. J., & Bracker, C. E. (1986). Polyethylene glycol: Catalytic effect on the crystallisation of phosphoglucosmutase at high salt concentration. *Journal of Crystal Growth*, 76(3), 562–576. [https://doi.org/10.1016/0022-0248\(86\)90173-9](https://doi.org/10.1016/0022-0248(86)90173-9)

- Sarris, J., Kavanagh, D. J., & Byrne, G. (2010). Adjuvant use of nutritional and herbal medicines with antidepressants, mood stabilisers and benzodiazepines. *Journal of Psychiatric Research*, *44*(1), 32–41. <https://doi.org/10.1016/j.jpsychires.2009.06.003>
- Schmidt, T., Bergner, A., & Schwede, T. (2014). Modelling three-dimensional protein structures for applications in drug design. *Drug Discovery Today*, *19*(7), 890–897. <https://doi.org/10.1016/j.drudis.2013.10.027>
- Seow, S. V., Kuo, I-Chun., Paaventhana, P., Kolatkar, P. R., & Chua, K. Y. (2003). Crystallisation and preliminary X-ray crystallographic studies on the fungal immunomodulatory protein Fve from the golden needle mushroom (*Flammulina velutipes*). *Acta Crystallographica Section D Biological Crystallography*, *59*(8), 1487–1489. <https://doi.org/10.1107/s0907444903011879>
- Shilling, P. J., Mirzadeh, K., Cumming, A. J., Widesheim, M., Köck, Z., & Daley, D. O. (2020). Improved designs for pET expression plasmids increase protein production yield in *Escherichia coli*. *Communications Biology*, *3*(1). <https://doi.org/10.1038/s42003-020-0939-8>
- Shire, S. J. (2015). Formulation of proteins and monoclonal antibodies (mAbs). *Monoclonal Antibodies*, 93–120. <https://doi.org/10.1016/b978-0-08-100296-4.00004-x>
- Smyth, M. S., & Martin, J. H. J. (2000). X Ray Crystallography. *Molecular Pathology*, *53*(1), 8–14. <https://doi.org/10.1136/mp.53.1.8>
- Tan, E. S. S., Leo, T. K., & Tan, C. K. (2021). Effect of tiger milk mushroom (*Lignosus rhinocerus*) supplementation on respiratory health, immunity and antioxidant status: an open-label prospective study. *Scientific Reports*, *11*(1). <https://doi.org/10.1038/s41598-021-91256-6>
- Valverde, M. E., Hernández-Pérez, T., & Paredes-López, O. (2015). Edible Mushrooms: Improving Human Health and Promoting Quality Life. *International Journal of Microbiology*, *2015*, 1–14. <https://doi.org/10.1155/2015/376387>
- Wang, P.-H., Hsu, C.-I., Tang, S.-C., Huang, Y.-L., Lin, J.-Y., & Ko, J.-L. (2004). Fungal Immunomodulatory Protein from *Flammulina velutipes* Induces Interferon- $\gamma$  Production through p38 Mitogen-



- Activated Protein Kinase Signaling Pathway. *Journal of Agricultural and Food Chemistry*, 52(9), 2721–2725. <https://doi.org/10.1021/jf034556s>
- Wang, X., Su, K., Bao, T., Cong, W., Chen, Y., Li, Q., & Zhou, X. (2011). IMMUNOMODULATORY EFFECTS of FUNGAL PROTEINS. *CURRENT TOPICS in NUTRACEUTICAL RESEARCH*, 10(1).
- Xu, X., Yan, H., Chen, J., & Zhang, X. (2011). Bioactive proteins from mushrooms. *Biotechnology Advances*, 29(6), 667–674. <https://doi.org/10.1016/j.biotechadv.2011.05.003>
- Yadav, D., & Negi, P. S. (2021). Bioactive components of mushrooms: Processing effects and health benefits. *Food Research International*, 148, 110599. <https://doi.org/10.1016/j.foodres.2021.110599>
- YU, C.-C. (2018, April 10). *Use of immunomodulatory protein from ganoderma in inhibiting cancer stem cells*. <https://patents.google.com/patent/US9937226B2/en>
- Zhang, C.-Y., Wu, Z.-Q., Yin, D.-C., Zhou, B.-R., Guo, Y.-Z., Lu, H.-M., Zhou, R.-B., & Shang, P. (2013). A strategy for selecting the pH of protein solutions to enhance crystallisation. *Acta Crystallographica Section F Structural Biology and Crystallization Communications*, 69(7), 821–826. <https://doi.org/10.1107/s1744309113013651>
- Zhou, R., Liu, Z. K., Zhang, Y. N., Wong, J. H., Ng, T. B., & Liu, F. (2019). Research Progress of Bioactive Proteins from the Edible and Medicinal Mushrooms. *Current Protein & Peptide Science*, 20(3), 196–219. <https://doi.org/10.2174/1389203719666180613090710>