BIOGRAPHICAL SKETCH

NAME: Hayer-Hartl, Manajit

POSITION TITLE: Group Leader

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Ministry of Education, Singapore	General Teacher Certificate	1996	Mathematics and Science
University of Stirling, Scotland, UK	BS	1981	Biology & Chemistry
University of Stirling, Scotland, UK	PHD	1984	Chemistry
University of Oxford, England, UK	Postdoctoral	1986	Biochemistry/Biophysics
Louis Pasteur Institute, Strasbourg, France	Postdoctoral	1987	Chemistry/Biophysics
Institute für Physikalische Biochemie, Germany	Postdoctoral	1989	Biophysics
Jules Stein Eye Institute, Los Angeles, U.S.A.	Postdoctoral	1990	Chemistry/Biophysics

A. Personal Statement

I am a Group Leader at the Max Planck Institute of Biochemistry, Martinsried, Germany. My research is focused on the structural and mechanistic analysis of molecular chaperones involved in protein folding, assembly and conformational maintenance. After my postdoctoral training, I joined the research group of my husband, F. Ulrich Hartl, as Research Associate at Sloan-Kettering Institute in New York and moved with him to the Max Planck Institute of Biochemistry in Germany. During this time I became familiar with many different biochemical assays and together with my previous knowledge of biophysical methods, I investigated the mechanism of the bacterial chaperonin system, GroEL and its co-factor GroES. I showed that the GroEL/ES system, besides providing a nano-cage for protein folding to occur unimpaired by aggregation, profoundly influences the energy landscape and the pathway of folding, resulting in accelerated folding for some proteins. In 2006, I was externally reviewed and promoted to Principal Investigator. Since then I have developed my own research centered around the enzyme ribulose-bisphosphate carboxylase-oxygenase (Rubisco). Rubisco is a key enzyme of photosynthesis. It mediates the first step in the fixation of atmospheric CO_2, resulting in the production of sugars and amino acids, the building blocks of life. This project has led to the discovery and understanding of the mechanisms of the chaperone machineries required for Rubisco biogenesis, comprising the chloroplast chaperonin system and several specific assembly chaperones (RbcX, Raf1, Raf2, BSD2), culminating in the successful expression of plant Rubisco in *E. coli*. My group also solved another long-standing question in the Rubisco field, that is, the mechanism of repair of inhibited Rubisco by the AAA+ chaperone Rubisco activase (Rca). More recently, we have made discoveries providing insight into the mechanism β-carboxysome formation in cyanobacteria, membraneless microcompartments that sequester Rubisco and carbonic anhydrase to generate a high CO_2 environment for carbon fixation. My long-term goal is to identify variants of Rubisco with improved enzymatic properties, either by directed evolution or rational design (artificial intelligence), for introduction into plants to increase crop yields.

Recent publications that I would like to highlight here include:

1. Bhat, J.Y., Miličić, G., Thieulin-Pardo, G., Bracher, A., Maxwell, A., Ciniawsky, S., Mueller-Cajar, O., Engen, J.R., Hartl, F.U., Wendler, P. & **Hayer-Hartl, M.** (2017). Mechanism of enzyme repair by the AAA+ chaperone Rubisco activase. Mol. Cell 67(5), 744-756. (doi: 10.1016/j.molcel.2017.07.004).
2. Aigner, H., Wilson, H.R., Bracher, A., Calisse, L., Bhat, J.Y., Hartl, F.U. & **Hayer-Hartl, M.** (2017). Plant Rubisco assembly in *E. coli* with five chloroplast chaperones including BSD2. Science 358(6368), 1272-1278. (doi: 10.1126/science.aap9221).
3. Yan, X., Shi, Q., Bracher, A., Miličić, G., Singh, A.K., Hartl, F.U. & **Hayer-Hartl, M.** (2018). GroEL ring separation and exchange in the chaperonin reaction. Cell 172, 605-617. (doi: 10.1016/j.cell.2017.12.010).
4. Wang, H., Yan, X., Aigner, H., Bracher, A., 1, Nguyen, N.D., Hee, W.Y., Long, B.M., Price, G.D., Hartl F.U. & **Hayer-Hartl, M.** (2019). Rubisco condensate formation by CcmM in β-carboxysome biogenesis. Nature 566(7742), 131-135. (doi: 10.1038/s41586-019-0880-5).
5. Flecken, M., Wang, H., Popilka, L., Hartl, F.U., Bracher, A. & **Hayer-Hartl, M.** (2020). Dual role of a Rubisco activase in metabolic repair and carboxysome organization. Cell 183(2), 457-473. (doi: 10.1016/j.cell.2020.09.010). bioRxiv: https://doi.org/10.1101/2020.05.16.099382.
6. Zang, K., Wang, H., Hartl, F.U. & **Hayer-Hartl, M.** (2021). Scaffolding protein CcmM directs multiprotein phase separation in b-carboxysome biogenesis. Nat. Struct. Mol. Biol. 28(11), 909-922. (doi: 10.1038/s41594-021-00676-5).

B. Positions, Scientific Appointments, and Honors

Positions and Scientific Appointments

2023-Present	Member of the committee for the Paul Ehrlich and Ludwig Darmstaedter Early Career Award.
2022-Present	Advisory board member of Trends in Biochemical Sciences (TIBS)
2022	Chair of FASEB conference “Protein Folding in the Cell” Malahide, Ireland (postponed from 2020). Co-chaired the meeting in 2018.
2018	Elected member, German Academy of Sciences Leopoldina.
2018-Present	Member, Cell Stress Society International (CSSI)
2016-Present	Member, International Society of Photosynthesis Research (ISPR)
2016-2020	Editorial Board eLIFE.
2016	Elected Member, European Molecular Biology Organization (EMBO).
2014-Present	Member, Protein Society
2014	Ad Hoc reviewer for Ministry of Education, Singapore – Academic Research Fund Tier 2 grant applications.
2012-Present	Member, American Society for Biochemistry & Molecular Biology (ASBMB)
2011-Present	Lecturer at the biannual course on “Molecular Organization, Function and Dynamics of Biomembranes”, held in Cargèse, Corsica, France from 2011-2018, and since 2020 in Spetses, Greece.
2006-Present	Research Group Leader and Principal Investigator, Department of Cellular Biochemistry, Max Planck Institute of Biochemistry, Martinsried, Germany.
1999-Present	Member, Gesellschaft für Biochemie und Molekularbiologie (GBM)
1997-2005	Research Group Leader, Department of Cellular Biochemistry, Max Planck Institute of Biochemistry, Martinsried, Germany.
1991-1996	Research Associate, Department of Cellular Biochemistry and Biophysics, Sloan-Kettering Institute, New York, U.S.A.
1976-1977	Science and Mathematics Teacher, Jurong Secondary School, Singapore.

Honors

2023	Plenary Speaker at the workshop on “Phase separation regulated life, in and outside of cells”, Nanyang Technological University, Singapore.
2022	Lawrence Bogorad Award for Excellence in Plant Biology Research from the American Society of Plant Biologists (ASPB).
2022	Plenary Speaker at the Satellite meeting of the 18th International Congress on Photosynthesis Research (ICPR) in Christchurch, New Zealand.
2022	Plenary Speaker at the 18th International Congress on Photosynthesis Research (ICPR) in Dunedin, New Zealand.

2022	Plenary Speaker at the 10th International Symposium on Inorganic Carbon Utilization by Aquatic Photosynthetic Organisms (CCM10), Princeton University, New Jersey, U.S.A.
2021	Merck Award of the American Society for Biochemistry & Molecular Biology (ASBMB).
2019	Plenary Speaker in the President's Symposium at the Annual Meeting of the American Society of Plant Biologists (ASPB).
2018	Charles F. Kettering Award for Excellence in the field of Photosynthesis from the American Society of Plant Biologists (ASPB).
2017	Dorothy Crowfoot Hodgkin Award of the Protein Society.
2017	Reinhold-von-Sengbusch Lecture at the 30th Conference on Molecular Biology of Plants, Dabringhausen, Germany.
2016	Nominated to join AcademiaNet.
2016	Plenary speaker at the 17th International Congress on Photosynthesis, Maastricht, Netherlands.
2013	Keynote Speaker at the 28th European Crystallographic Meeting, Warwick, England, U.K.
2010	The Fifth Rebeiz Foundation for Basic Research (RFFBR) Paper Award (http://www.vlpbp.org/).
1989-1990	Postdoctoral Fellowship RPB Manpower Award, U.S.A.
1987-1989	Postdoctoral Fellowship from Deutsche Forschungsgemeinschaft, Germany.
1986-1987	Postdoctoral Fellowship from CNRS, France.
1984-1986	Postdoctoral Fellowship from The Multiple Sclerosis Society, U.K.
1976	JAL Scholarship, a 6 week Cultural Course at Tokyo University, Japan.

C. Contributions to Science

1. My early publications, in collaboration with F. Ulrich Hartl, addressed the mechanism of the bacterial chaperonin system, GroEL and its co-factor GroES. GroEL/ES functions by enclosing a single non-native protein molecule in a nano-cage for folding to occur unimpaired by aggregation. This body of work led to the insight that the GroEL/ES cage, in addition to preventing aggregation, profoundly influences the energy landscapes and the pathways of folding, resulting in accelerated folding for some proteins. This novel mechanism is based on the entropic destabilization of unfolded states in the confining environment of the folding cage (Brinker et al, Cell 2001; Tang et al., Cell 2006; Sharma et al., Cell 2008; Chakraborty et al., Cell 2010; Georgescauld et al., Cell 2014; Singh et al., JMB 2020). How exactly this is achieved is still an open question, however, and a detailed comparison of folding in bulk solution with folding in the nano-cage is currently of central interest. More recently, we showed that the two rings of GroEL undergo transient separation in the chaperonin cycle, a step that is important to ensure efficient substrate binding and folding (Yan et al., Cell 2018). Another question of current interest concerns the mechanistic differences between the bacterial and eukaryotic chaperonin systems in facilitating the folding of their respective substrate proteins (Balchin et al., Cell 2018).

   Recent reviews that I would like to highlight here include:
   a. **Hayer-Hartl, M.**, Bracher, B. & Hartl, F.U. (2016). The GroEL-GroES chaperonin machine – a nano-cage for protein folding. Trends Biochem. Sci. 41(1), 62-76 (doi: 10.1016/j.tibs.2015.07.009).
   b. Balchin, D, **Hayer-Hartl, M.** & Hartl, F.U. (2020). Recent advances in understanding catalysis of protein folding by molecular chaperones. FEBS Lett. 594, 2770-2781 (doi: 10.1002/1873-3468.13844)..

2. In 2005, research on the folding of the hetero-oligomeric form I Rubisco led me into the field of protein assembly. Form I Rubisco is a complex consisting of 8 large (RbcL; ~55 kDa) and 8 small (RbcS; ~14 kDa) subunits. Assembly, unlike folding, is an intermolecular process in which folded subunits interact to form oligomeric complexes. Based on previous findings, we knew that the folding of the RbcL subunits of Rubisco are absolutely dependent on the chaperonin system, however, all attempts to obtain the form I Rubisco with the chaperonin system alone failed. In this project, we discovered that several assembly chaperones are specifically required for the assembly of form I Rubisco. In the case of cyanobacterial form I Rubisco, we identified RbcX and Raf1 to bind to the RbcL subunits immediately after their folding by the chaperonin system, acting in parallel or in tandem (Saschenbrecker et al., Cell 2007; Liu et al., Nature 2010; Bracher et al., Nat. Struct. Mol. Biol. 2011; Hauser et al., Nat. Struct. Mol. Biol. 2015). In the case of the form I Rubisco

from plants we showed that two other proteins, Raf2 and BSD2, were necessary in addition for recombinant production in *E. coli* (Aigner et al., Science 2017; Wilson et al., FEBS Lett. 2019). Interestingly, we find that the factors RbcX, Raf1 and BSD2 function in stabilizing the anti-parallel $RbcL_2$ units and facilitate formation of the $RbcL_8$ core complex of Rubisco – displaying functional mimicry. Our breakthrough in producing functional plant Rubisco in *E. coli* will now facilitate efforts to improve Rubisco's catalytic properties either by directed evolution (Durao et al., Nature Chem. Biol. 2015) or rational design. I have made the Rubisco-producing *E. coli* strain freely available to literally dozens of groups world-wide.

Recent reviews that highlight these findings include:

a. Hauser, T., Popilka, L., Hartl, F.U. & **Hayer-Hartl, M.** (2015). Role of auxiliary proteins in Rubisco biogenesis and function. Nature Plants 1:15065. (doi: 10.1038/nplants.2015.65).
b. Wilson, R.H. & **Hayer-Hartl, M.** (2018). The complex chaperone dependence of Rubisco biogenesis. Biochemistry 57(23), 3210-3216. (doi: 10.1021/acs.biochem.8b00132).
c. **Hayer-Hartl, M.** & Hartl, F.U. (2020). Chaperone machineries of Rubisco – the most abundant enzyme. Trends Biochem. Sci. 45(9), 748-763. (doi: 10.1016/j.tibs.2020.05.001).

3. A related area of Rubisco research is elucidating the structure and mechanism of the AAA+ protein Rubisco activase (Rca), which is required for maintenance of Rubisco in a functional state. During the complex multistep catalytic reactions of Rubisco, misfire products are generated which inhibit the function of Rubisco during photosynthesis, limiting the growth of crop plants. To the rescue comes Rca. By combining crystallography, cryo-electron microscopy and biochemistry we showed that photosynthetic organisms have evolved different mechanisms to solve the problem of enzyme repair. For example, Rca of red-type Rubisco (found in red algae and certain photosynthetic bacteria) remodels the inhibited Rubisco by pulling the extended C-terminus of the RbcL subunit into the central pore of its hexameric ring structure, while the Rca of green-type form IB Rubisco proteins, which lack an extended RbcL C-terminus, have adapted to binding and pulling on the flexible N-terminus of the RbcL subunit (Mueller-Cajar et al., Nature 2011; Stotz et al., Nat. Struct. Mol. Biol. 2011; Bhat et al, Mol. Cell 2017; Flecken et al., Cell 2020). Besides understanding the mechanism of these AAA+ ATPase enzymes, we also discovered the phosphatase that is required to hydrolyze the potent sugar inhibitor of Rubisco, xylulose-1,5-bisphosphate (XuBP) (Bracher et al., Nature Plants 2015). Structural analysis revealed the remarkable specificity of the phosphatase for XuBP (Km ~30 μM) over RuBP (the proper substrate of Rubisco) (Km ~3 mM), although these sugar phosphates differ only by the stereochemistry at the C3 position. Plant Rca proteins tend to be thermally labile, which poses a problem as climate extremes become more frequent. Detailed knowledge of the structure and mechanism of activase is thus important in efforts to engineer a more thermostable activase.

Recent reviews that highlight these findings include:

a. Bracher, A., Whitney S.M., Hartl, F.U. & **Hayer-Hartl, M.** (2017). Biogenesis and metabolic maintenance of Rubisco. Annu. Rev. Plant Biol. 68, 29-60 (doi: 10.1146/annurev -arplant-043015-111633).
b. Bhat, J.Y., Thieulin-Pardo, G., Hartl, F.U. & **Hayer-Hartl, M.** (2017). Rubisco activases: AAA+ chaperones adapted to enzyme repair. Front. Mol. Biosci. 4(20). (doi: 10.3389/fmolb.2017.00020).
c. **Hayer-Hartl, M.** (2017). From chaperonins to Rubisco assembly and metabolic repair. Protein Sci. 26(12), 2324-x2333. (doi: 10.1002/pro.3309).
d. **Hayer-Hartl, M.** & Hartl, F.U. (2020). Chaperone machineries of Rubisco – the most abundant enzyme. Trends Biochem. Sci. 45(9), 748-763. (doi: 10.1016/j.tibs.2020.05.001).

4. Unlike plants, cyanobacteria have evolved a CO_2 concentrating mechanism (CCM) by sequestering Rubisco together with the enzyme carbonic anhydrase (CA) into microcompartments called carboxysomes. This serves to generate high levels of CO_2 in the vicinity of Rubisco, thus allowing cyanobacteria to use less selective Rubiscos that are ~10 times faster than the enzymes of land plants. Carboxysomes resemble intracellular viruses with a highly symmetric proteinaceous shell, and have been classified into α and β based on the type of Rubisco they encapsulate. During β-carboxysome biogenesis Rubisco first aggregates in a process mediated by the scaffolding protein CcmM, followed by shell formation. There are two isoforms of CcmM in the cyanobacterium *Synechococcus elongatus* PCC 7942. The full-length protein (also called M58) contains a CA-like domain (CAL), followed by three small subunit-like (SSUL) modules that have 25% sequence identity to RbcS of Rubisco. A smaller isoform, called M35, consists only of the three SSUL

modules. It had been speculated that these domains might replace RbcS subunits, thereby linking Rubisco complexes into a 3D network. Using cryo-EM and single particle analysis (Wang et al., Nature 2019), we showed that, contrary to expectation, the SSUL modules do not replace RbcS, but bind close to the equatorial region of Rubisco between RbcL dimers, and thereby link Rubisco molecules. This results in the phase separation of Rubisco into a condensed, liquid-like matrix. Moreover, we found that the cyanobacterial Rca also uses similar SSUL modules to ensure its packaging into carboxysomes together with Rubisco (Flecken et al. Cell 2020). Thus, these modules can serve as a tool to include proteins of interest into carboxysomes. More recently, we solved the crystal structure of the conserved C-terminal sequence of the carbonic anhydrase, CcaA, in complex with the CAL domain of the full-length CcmM, M58. We found that multivalent interactions of the trimeric M58 and tetrameric CcaA result in phase separation and condensate formation (Zang et al., Nat. Struct. Mol. Biol. 2021). Our structural and biochemical analyses suggest that M58 is the central organizer of pro-carboxysome biogenesis and, in cooperation with M35, ensures that Rubisco and CcaA are co-packaged into the same condensate. These findings provide insights for future attempts to introduce a carboxysome-like CO_2 concentrating mechanism into plant chloroplasts, thus allowing plants to utilize the faster cyanobacterial Rubisco or certain re-engineered forms of plant Rubisco, thereby enhancing growth rates and improve crop yields.

Recent publications:

a. Wang, H., Yan, X., Aigner, H., Bracher, A., 1, Nguyen, N.D., Hee, W.Y., Long, B.M., Price, G.D., Hartl F.U. & **Hayer-Hartl, M.** (2019). Rubisco condensate formation by CcmM in β-carboxysome biogenesis. Nature 566(7742), 131-135. (doi: 10.1038/s41586-019-0880-5).
b. Zang, K., Wang, H., Hartl, F.U. & **Hayer-Hartl, M.** (2021). Scaffolding protein CcmM directs multiprotein phase separation in β-carboxysome biogenesis. Nat. Struct. Mol. Biol. 28(11), 909-922. (doi: 10.1038/s41594-021-00676-5).
c. Gionfriddo M., Zang, K. & **Hayer-Hartl, M.** (2023). Graphical Review “The challenge of engineering Rubisco for improving photosynthesis”. FEBS Lett. 597(13), 1679-1680. (doi: 10.1002/1873-3468.14678).

In summary, my ongoing research to understand the machineries of Rubisco folding and assembly facilitates engineering efforts with the goal to improve the catalytic properties of this very abundant but inefficient enzyme. In an initial study we used a Rubisco-dependent *E. coli* strain (from S. Whitney) to explore how the RbcX assembly chaperone affects Rubisco evolvability (Durao et al., Nature Chem. Biol. 2015). While these investigations are of general interest in the field of enzyme evolution, our long-term goal is to identify variants of Rubisco with improved enzymatic properties.

Complete List of Published Work in MyBibliography:
https://www.ncbi.nlm.nih.gov/myncbi/1pytN7vBrEZkj/bibliography/public/

Total citations April 2023: ~26000; H-index 59 (source Google Scholar:
https://scholar.google.de/citations?user=MsIdmfQAAAAJ&hl=en&oi=ao)