BIOGRAPHICAL SKETCH

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NAME: Erbse, Annette Herta

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POSITION TITLE: Director of the Biochemistry Shared Instrument Pool and the Macromolecular X-ray Crystallography Core, Department of Biochemistry, University of Colorado, Boulder

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
Department of Chemistry/Biochemistry, University of Kaiserslautern (Germany)	Diplom-Chemistry	1993	Chemistry
Department of Chemistry/Biochemistry, University of Kaiserslautern (Germany)	Dr. rer. nat. (PhD)	1996	Chemistry/Biochemistry
Department of Biological Sciences, University of Birmingham (UK)	Postdoctoral	1996-1999	Biochemistry
Boyer Center of Molecular Medicine, Yale University and HHMI (USA)	Postdoctoral	1999-2002	Structural Biology

A. Personal Statement

I have strong expertise in biochemistry, protein chemistry and biophysics. In the past 9 years, I have established the Shared Instruments Pool (SIP) core facility in the Department of Biochemistry from the ground up and gained extensive experience managing a Shared Instrumentation facility. I have gained strong expertise with diverse instrumentation and in the training of undergraduate and graduate students and postdoctoral researchers in the rigorous and reproducible application of biophysical and biochemical methods. One aspect of the core SIP is to provide researchers with access to an array of basic research infrastructures like biomolecular imagers, centrifuges, autoclaves, and more. The second aspect is to provide access to state-of-the-art instruments and techniques, with a focus on biophysical methods for the characterization of biomolecular structures and interactions. Methods available in the core include fluorescence spectroscopy (steady state fluorimeters, fluorescence gel scanners and imagers), stopped-flow (for fluorescence, polarization, CD and absorption spectroscopy), circular dichroism, isothermal titration calorimetry, electron paramagnetic resonance spectroscopy, dynamic and multi-angle light scattering, and chemical quench-flow kinetics. In addition to my role as Director of the Shared Instrument Pool, I am the Director of the Macromolecular X-ray Crystallography Core since August 2016.

To maintain all instruments in both facilities in excellent working conditions, I have established a monthly and weekly maintenance schedule for all the instruments including regular testing, validation of function and calibration. I have developed facility websites that provide background information for the available instruments and methods as well as detailed step-by-step basic user protocols. I have implemented an online booking system that allows users to schedule instrument time and allows me to keep a record of user groups, individual users and user time. Together with the Biochemistry Department, we have established procedures and mechanisms to pay for service contracts and for repairs that I cannot perform myself.

Next to maintaining instruments, I train students and senior researchers in their proper use. I provide training and assistance from project planning, to sample preparation, data collection, data analysis, interpretation, and troubleshooting, to the evaluation of reproducibility, error assessment, data fitting and statistical analysis. I help

users to develop a strong understanding of the theoretical background of the methods they use and to understand the advantages and limitations of the methods. I provide training in hands-on, one-on-one sessions or small groups. I have developed training modules for most of the instruments to allow new users to gain hands-on experience with proven standard experiments, which can be used to benchmark the instrument and validate proper procedures. During in-depth training, this basic training is combined with training using user-provided samples. I have experience training students and researchers with a broad range of backgrounds from Biochemistry, to Biophysics, Cell Signaling, Chemical and Biological Engineering, Molecular Biology, and Physics.
In the past, I have developed an advanced undergraduate laboratory course in molecular biology: "Biochemistry of the Cell: Molecular Methods to Probe Protein Folding and Degradation *in vivo* and *in vitro.*" I taught this class in the summer semesters of 2003, 2004 and 2005 at the University of Heidelberg, Germany. Furthermore, I co-developed a graduate laboratory course in Biophysics with the topic "Mass Spectrometry in Bioanalytics" with Dr. Matthias Mayer. I team taught the course in the winter semesters of 2004 and 2005 at the University of Heidelberg, Germany. More recently, I have developed and taught the EPR section of the "Methods of Molecular Biophysics" course at CU Boulder in the fall semesters of 2008, 2010 and 2014. I collaborated with the lead PI of the Methods class in 2018 and 2020 to develop a module for the class exposing students to methods of data fitting and evaluation using real-life data collected in the instrument pool.

B. Positions, Scientific Appointments, and Honors

Positions and Employment

2016-present	Director, Macromolecular X-ray Crystallography Core, Department of Biochemistry, University of Colorado, Boulder
2012-present	Director, Shared Instruments Pool, Department of Biochemistry, University of Colorado, Boulder
2008-2012	Senior Research Associate, Department of Chemistry and Biochemistry, University of Colorado, Boulder
2006-2008	Research Associate, Department of Chemistry and Biochemistry, University of Colorado, Boulder
2002-2005	Oberassistent (Research Faculty), Center for Molecular Biology, University of Heidelberg (Germany)
1999-2002	Postdoctoral Fellow, Boyer Center for Molecular Medicine, Yale University / HHMI
1996-1999	Research Associate, Department of Biological Sciences, University of Birmingham, (UK)
1993-1996	Graduate Teaching and Research Assistant, Department of Chemistry/Biochemistry, University of Kaiserslautern (Germany)

Other Experience and Professional Memberships

since 2005	Member of the GdCh, Gemeinschaft deutscher Chemiker (German association of Chemists)
since 2005	Member of GMB, Gemeinschaft fuer Molekulare Biology (Association for Molecular Biology)
1994-1995	Representative and Ombudswoman for women in Chemistry/Biochemistry at the Department of Chemistry/Biochemistry, University of Kaiserslautern (Germany)

C. Contributions to Science

Bibliography: https://www.ncbi.nlm.nih.gov/myncbi/annette.erbse.1/bibliography/public/

1) Since becoming the Director of the Shared Instruments Pool in Biochemistry I was not only able to help and support many researchers of the wider CU research community, but I also contributed as a collaborator and co-author to several projects and publications from research groups from different departments and research fields. These publications highlight the importance of shared core facilities to enable the research of many, often very different disciples and to connect researchers from different disciplines to drive new approaches and advance research projects.

1. Leonard AC, Weinstein JJ, Steiner PJ, Erbse AH, Fleishman SJ, Whitehead TA. Stabilization of the SARS-CoV-2 receptor binding domain by protein core redesign and deep mutational scanning. Protein Eng Des Sel. 2022 Feb 17;35. doi: 10.1093/protein/gzac002. PubMed PMID: 35325236; PubMed Central PMCID: PMC9077414

2. Hannan JP, Swisher GH, Martyr JG, Cordaro NJ, Erbse AH, Falke JJ. HPLC method to resolve, identify and quantify guanine nucleotides bound to recombinant ras GTPase. Anal Biochem. 2021 Oct 15;631:114338. PMCID: PMC8511091
3. Wang X, Goodrich KJ, Conlon EG, Gao J, Erbse AH, Manley JL, Cech TR. C9orf72 and triplet repeat disorder RNAs: G-quadruplex formation, binding to PRC2 and implications for disease mechanisms. RNA. 2019 Aug;25(8):935-947. PMCID: PMC6633194.
4. Goodman SM, Levy M, Li FF, Ding Y, Courtney CM, Chowdhury PP, Erbse A, Chatterjee A, Nagpal P. Designing Superoxide-Generating Quantum Dots for Selective Light-Activated Nanotherapy. Front Chem. 2018;6:46. PMCID: PMC5861142.

2) Previously I served as the lead researcher in the development of a powerful one-sample FRET (OS-FRET) method. This novel method employs a non-fluorescent methanethiosulfonate-linked acceptor that can be reversibly coupled to a target sulfhydryl residue via a disulfide bond. After the quenched donor emission is quantitated, the acceptor is removed by reduction, allowing measurement of unquenched donor emission in the same sample. The new OS-FRET method is a generalizable spectrochemical approach that can be applied to macromolecular systems lacking essential disulfide bonds and eliminates the potential systematic errors of some earlier one-sample methods. In addition, OS-FRET enables quantitative FRET measurements in virtually any fluorescence spectrometer or detection device. Compared to conventional multi-sample FRET methods, OS-FRET conserves sample, increases the precision of data, and shortens the time per measurement. I illustrated the utility of the method by applying it to a protein complex of known structure formed by CheW and the P4-P5 fragment of CheA, both from *Thermotoga Maritima*. The findings confirmed the practicality and advantages of OS-FRET. Appropriate applications of OS-FRET include analysis of macromolecular structure, binding and conformational dynamics, and high-throughput screening for interactions and inhibitors.

1. Erbse AH, Berlinberg AJ, Cheung CY, Leung WY, Falke JJ. OS-FRET: a new one-sample method for improved FRET measurements. Biochemistry. 2011 Feb 1;50(4):451-7. PMCID: PMC3045706.

3) Earlier work focused on the molecular mechanism of the N-End rule pathway of protein degradation, which is an essential pathway in eukaryotes from mammals to fungi that links the half-lives of proteins to their N-terminal amino acids via the ubiquitin system. Different research groups speculated about a N-end rule in bacteria, but bacteria lack the ubiquitin system thus a bacterial N-end rule would require a different mechanism. Our research showed that bacteria possess an N-end pathway in which the ClpA adapter protein ClpS recognizes the target N-end motif in substrate proteins and directs them into the protease complex ClpA/ClpP complex. Furthermore we were able to characterize the bacterial N-End rule recognition motifs. This work established that N-End rule degradation extends into the realm of prokaryotes making it an important mechanism that regulates the timescales of cellular processes in all forms of life.

1. Erbse A, Schmidt R, Bornemann T, Schneider-Mergener J, Mogk A, Zahn R, Dougan DA, Bukau B. ClpS is an essential component of the N-end rule pathway in Escherichia coli. Nature. 2006 Feb 9;439(7077):753-6.
2. Erbse AH, Wagner JN, Truscott KN, Spall SK, Kirstein J, Zeth K, Turgay K, Mogk A, Bukau B, Dougan DA. Conserved residues in the N-domain of the AAA+ chaperone ClpA regulate substrate recognition and unfolding. FEBS J. 2008 Apr;275(7):1400-1410.

4) Other research focused on Hsp70 housekeeping chaperones central to protein folding events in all organisms from bacteria to mammals. These chaperones recognize both unfolded proteins and aggregated folded proteins. Crystal structures of the protein binding domain of DnaK, the Hsp70 of E.coli, in complex with a substrate peptide indicated that DnaK binds to substrates by enclosing a unfolded region of substrates tightly. This led to the speculation that natively folded substrates must have a unstructured region in order to interact with DnaK. Using a combination of disulfide crosslinking and SDSL EPR we were able to show that the "lid" region of the protein binding domain can adopt multiple conformations, closing firmly over unfolded peptides but leaving space to accommodate the binding of folded substrate. The structural flexibility displayed by this binding process is the key the involvement of Hsp70 in such an astonishingly broad spectrum of chaperone processes in living organisms.

1. Schlecht R, Erbse AH, Bukau B, Mayer MP. Mechanics of Hsp70 chaperones enables differential interaction with client proteins. Nat Struct Mol Biol. 2011 Mar;18(3):345-51
2. Erbse A, Mayer MP, Bukau B. Mechanism of substrate recognition by Hsp70 chaperones. Biochem Soc Trans. 2004 Aug;32(Pt 4):617-21.