Research Statement:

Proteins are crucial components in the molecular machinery of cells, responsible for transporting molecules or catalyzing biochemical reactions. Despite remarkable progress in experimental techniques for producing and characterizing proteins, a detailed understanding of folding and interaction of proteins is still lacking. While simulations can complement experiments in probing folding, aggregation, binding, and other fundamental processes in cells, they are computationally challenging for realistic protein models. This is because most of the above processes happens on time scales (seconds to days) that exceed the ones available in atomistic molecular dynamics simulations. One focus of research in my group focuses is the development of sampling techniques that can overcome (or at least narrow) this gap. The second focus area is the application of these techniques to investigate folding, association and aggregation of proteins. In past research we studied mainly t the folding mechanism in small globular proteins of up to ≈ 60 − 70 residues, but applications over the last few years have shifted toward the oligomerization and growth of fibrils of amyloid forming peptides. A **c**omplete list of my scientific publications (a total of over 240 papers; **H-index: 36** on Web of Science; **48** on Google Scholar), can be found at the group web-page. In the following, I describe the three main areas of research in my lab, before sketching some ongoing and planed work.

1) Generalized Ensemble and Replica Exchange Sampling

I am most recognized for pioneering the generalized-ensemble approach and replica-exchange techniques, methods now routinely used to tackle the numerical difficulties which often hinder the use of molecular dynamics or Monte Carlo in molecular biophysics. These techniques, including multicanonical sampling, replica-exchange-molecular dynamics or energy landscape paving, allow sampling of low-energy configurations without that the simulations gets trapped in a local minimum, enabling in this way a more exhaustive sampling of the energy landscape of the biomolecular systems.

a) U.H.E. Hansmann and Y. Okamoto, *Prediction of Peptide Conformation by Multicanonical Algorithm: A new Approach to the Multiple-Minima Problem*, Journal of Computational Chemistry,**14**, 1333 (1993)
b) U.H.E. Hansmann, *Parallel Tempering Algorithm for Conformational Studies of Biological Molecules*, Chemical Physics Letters, **281**, 140 (1997).

While use of these techniques has become ubiquitous, their utility is still often limited in certain applications; and the continuing improvement of simulation methods has stayed a main area of research in our lab. An example is the Replica-Exchange-with Tunneling approach recently introduced by us, which is the start point for some of the techniques that we plan to develop over the next years.

a) F. Yasar, N.A. Bernhardt and U.H.E. Hansmann, *Replica-Exchange-with-Tunneling for fast Exploration of Protein Landscapes*, Journal of Chemical Physics, **143** 224102 (2015).
b) F. Yasar, A.J. Ray and U.H.E. Hansmann, *Resolution Exchange with Tunneling for Enhanced Sampling of Protein Landscapes*, Physical Review E, **106**, 015302 (2022).

Many of our algorithms are implemented in the freeware program package SMMP available from either the program library of *Computer Physics Communications* or directly from the authors (*www.hansmann-lab.com/cbpc/smmp/smmp.php*); and increasingly also as add-ons to the popular molecular dynamics program GROMACS. This software is made available on Github (*github.com/hansmann-lab*).

2) Protein Folding and Protein Fold Switching

My work is characterized by a close connection between development of algorithms and their application to protein physics. I have early on studied the helix-coil transition in homopolymers and proteins and explored how far this transition can be described as a finite-system analog of a phase transition. These investigations were later extended to the folding mechanism of small proteins. For instance, we could show the C-fragment of TOP7 folds by a novel mechanism which utilizes the Chameleon behavior of a certain segment to inhibit miss-folding and aggregation. This demonstrates that the folding landscape of proteins is often not trivial. Instead it may allow for switching between different protein structures depending on evolutionary history, environment, interaction with other molecules, or kinetic needs.

a) N.A. Alves and U.H.E. Hansmann, *Partition Function Zeros and Finite Size Scaling of Helix-Coil Transitions in a Polypeptide*, Physical Review Letters, **84**, 1836 (2000).
b) S. Mohanty, J.H. Meinke, O. Zimmermann and U.H.E. Hansmann, *Simulation of Top7-CFr: a transient helix extension guides folding*, Proceeding of the National Academy of Science (USA), **105** 8004 (2008).

Recent work has extended these folding investigations to proteins that can take more than a single fold, with the long-term goal of deriving a general multi-funnel theory of folding and association of proteins. For instance, the transcription factor RfaH changes biological function by switching be- tween distinct three-dimensional folds. RfaH regulates transcription if the C-terminal domain folds into a double helix bundle, and promotes translation when this domain assumes a β- barrel form. We have studied this fold-switching in RfAH using an enhanced sampling method introduced by us, replica-exchange-with-tunneling (RET), to calculate the free-energy landscape of the protein. Analyzing this landscape, we could already propose a mechanism for TfAH and another switching protein, Lymphotactin, a conversion process that is consistent with experimental measurements.

a) N.A. Bernhardt and U.H.E. Hansmann, *Multi-Funnel Landscape of the Fold-Switching Protein RfaH-CTD*, Journal of Physical Chemistry B, **122**, 1600 (2018).
b) P. Khatua, A.J. Ray and U.H.E. Hansmann, *Bifurcated Hydrogen Bonds and the Fold Switching of Lymphotactin*, Journal of Physical Chemistry B, **124** 6555 (2020).

As extension of this research we are looking into the early steps of the conversion between native PrPP and the toxic scrapie PrPSc form of prion proteins, and how this process can be triggered by interaction with RNA.

a) E.A. Lubecka and U.H.E. Hansmann, *Early Stages of RNA-Mediated Conversion of Human Prions*, Journal of Physical Chemistry B, **126**, 6221 (2022).

3) Protein Aggregation

I have a long-standing interest in the phenomena of aggregation and amyloid formation, examples for self-assembly of proteins. For instance, we have studied self-assembly of phenylalanine into nanotubes that are build out of rings of four molecules. The size of these aggregates is consistent with experimental measurements of fibrils obtained from mice with phenylketonuria. However, most research in the lab is concerned with the β-amyloid peptide implied in Alzheimer's disease, where I have studied the folding and the formation of toxic oligomers and fibrils.

a) P. Anand, N.S. Nandel and U.H.E. Hansmann, *The Alzheimer's β-amyloid Aβ(1-39) monomer in an implicit solvent*, Journal of Chemical Physics,**128**, 165102 (2008),
b) W.M. Berhanu and U.H.E. Hansmann, *Structure and dynamics of amyloid-β segmental polymorphism*, PlosONE, **7**, e41479 (2012).
c) H.W. German, S. Uyaver and U.H.E. Hansmann, *Self-Assembly of Phenylalanine-Based Molecules*, Journal of Physical Chemistry A, **119,** 1609 (2015).

The β-amyloid aggregates exhibit i*n vitro* a remarkable polymorphism that is missing in patient-derived Aβ aggregates. We could show that the lack of polymorphism in the brain-derived fibrils is likely due to the different environmental condition by which the fibrils grow in vitro and in the brain, and should be considered in the design of drug candidates that bind to amyloids. Of special interest are the transitions between polymorphs; exploring how they can be modulated by small molecules as drug candidates, and whether some of these aggregates are "infectious". For instance, the mutation D23N leads to higher neurotoxicity that is correlated with the presence of meta-stable anti-parallel fibrils. Utilizing our RET sampling approach, we could show that wild type and mutant form disparate salt bridges that stabilize different fibril organizations. The conversion between the two fibril forms releases of small aggregates that in the Iowa mutant may shift the equilibrium from fibrils to more toxic oligomers. The polymorphism of amyloids is not restricted to Aβ

aggregates, and is studied by us also for other proteins, as amylin, Serum Amyloid A or αSynuclein, each connected with a certain amyloid disease.

a) E.J. Alred, M. Phillips, W.M. Berhanu and U.H.E. Hansmann, *On the lack of polymorphism in Aβ-peptide aggregates derived from patient brains*, Protein Science, **24**, 923 (2015)
b) W. Xi and U.H.E. Hansmann, *Conversion between parallel and antiparallel β-sheets in wild type and Iowa mutant Aβ40 fibrils*, Journal of Chemical Physics, **148**, 045103 (2018).
c) W. Wang, P. Khatua and U.H.E. Hansmann, *Cleavage, down-regulation and aggregation of serum amyloid A*, Journal of Physical Chemistry B, **125,** 1009 (2020).

Given the complexity of amyloid formation, conversion and propagation, we expect that our above studies will inspire further development of methods and algorithms, directed toward the long-term goal of simulating cells on all time and length scales relevant for medical and technological applications.

4) Ongoing and Future Work

Building on previous work, we will study in the next years the modulation of amyloid formation by proteins from viruses and other pathogens. The microbial protection hypothesis assumes that amyloids are formed as an immune response, entrapping and neutralizing pathogens. While intriguing, neither validity nor scope of this hypothesis have been established. Acknowledging the complexity of the problem, our research limits itself to the question whether viral proteins can trigger amyloid formation. Impelled by the COVOD2019 pandemic focused on the potential for amyloid formation triggered by exposure to SARS-COV-2 proteins. Preliminary data are published in:

a) A.K. Jana, A.B. Greenwood and U.H.E. Hansmann, *Presence of a SARS-COV-2 protein enhances Amyloid Formation of Serum Amyloid A*, Journal of Physical Chemistry B, **125,** 9155 (2021).
b) A.K. Jana, C.W. Lander, A.D. Chesney and U.H.E. Hansmann, *Effect of an amyloidogenic SARS-COV-2 protein fragment on α-synuclein monomers and fibrils*, Journal of Physical Chemistry B, **126**, 3648 (2022).
c) A.D. Chesney, B.Maiti, U.H.E. Hansmann, *Human Amylin in the Presence of SARS-COV-2 Protein Fragments*, ACS Omega **8**, 12501(2023).

Complementary to our research on virus-induced amyloid formation are ongoing investigations in the effect of *D-Retro Inverso (DRI)* peptide on fibril stability. DRI-proteins are built from D-isomers of the respective residues, but with the sequence reversed, leading to close similarity in structure and function with the L-parent, while at the same time more resistant to proteolytic digestion. I have committed to help Dr. Maria Cimini (Temple University) with the design of DRI-based inhibitors to SAA amyloids for her project "*Role of Podoplanin Positive Cell-Exosomes in Cardiac Inflammation and Amyloidosis*". The computational studies will complement her *in vitro* and *in vivo* investigations and build on a recent publication from my lab:

a) A.K. Jana, A.B. Greenwood and U.H.E. Hansmann, *Small Peptides for Inhibiting Serum Amyloid A Aggregation*, ACS Medicinal Chemistry Letters, **12,** 1613 (2021).