

Research Interests – Prof. Dr. Brenda Schulman

Proteins are the workhorses of cells. Some proteins play structural roles, for example serving as architectural frameworks underlying cell shape; other proteins are enzymes that synthesise and break down sugars, fats, and other types of molecules needed for cellular metabolism; some proteins have motor domains to achieve movement, for example untangling complicated macromolecular assemblies or transporting cargoes to precise locations within cells. The staggering array of protein functions vastly exceeds the number of different proteins encoded by an organism's genome (e.g., roughly 20,000 different proteins can be made from scratch in human cells). To meet cellular demands and establish regulation, the structures and functions of pre-existing proteins can be transformed in innumerable ways by post-translational modification. A predominant set of modifications involves covalent linkage of ubiquitin (UB) and members of the family of ubiquitin-like proteins (UBL). We study molecular principles, cellular pathways, and biological roles of regulation by UB and UBL. This is of broad importance for understanding numerous biological processes, and for deciphering signalling pathways and their roles in diseases.

The UB/UBL system is truly remarkable in that it involves the physical linkage of entire proteins onto other proteins. With more than 1000 atoms, each UB and UBL is a relatively massive modification that can profoundly affect the chemical and physical properties of its targets. There is great diversity in the system, with more than a dozen different UBL in human cells. Furthermore, UB and some UBL become linked to each other in "chains" with different linkages defined by the amino acids connecting one UB (or UBL) and the next in the chain. The different types of UB chains and UBL modifications impart an astonishing variety of new fates to their targets. As examples, some UB chains regulate protein stability, by directing modified targets for degradation by the Proteasome. Other types of UB chains regulate protein trafficking and localisation within cells, while yet others control multiprotein or DNA-protein complex formation. Meanwhile, UBL have distinct effects, such as LC3 bridging interactions between cytosolic proteins and autophagosomal membranes, or NEDD8 altering the conformations and activities of hundreds of ubiquitylating enzymes.

To investigate this regulation, we combine structure (cryo EM, X-ray crystallography, and NMR), biochemistry, organic chemistry, enzymology, biophysics, protein design, gene editing, genetics and cell biology in an integrated and circular process, with continuous flow of

information between methodologies. This strategy enables identification of novel regulatory pathways as well as defining structural mechanisms by which large, transient macromolecular assemblies mediate dynamic regulation.

Our research encompasses three broad areas. One goal is to use structural biology, particularly cryo EM, to define fundamental mechanisms. We want to understand how dynamic, multiprotein assemblies of E1 activating enzymes, E2 conjugating enzymes, E3 ligase enzymes and DUB deconjugating enzymes match particular forms of UB with their specific cellular targets, and how UB and UBL switch the functions of their targets. E1s, E2s, E3s, DUBs, and UB/UBL binding proteins account for about 5 percent of all proteins in an organism, with roughly 1000 proteins dedicated to the UB/UBL system in humans. We study representative members of key enzyme families regulating cell division, protein trafficking, metabolic signalling and autophagy to determine the basic molecular principles used in many pathways. Our early work defined structures initiating UBL conjugation, including how E1s recognise specific UBL and promote conjugation to a cognate E2. However, despite great importance, our knowledge of substrate modification and polyubiquitylation by E3 enzymes remains rudimentary. Thus, we are employing cryo EM and other structural methods to visualise E3 ligases trapped as if in the act of handing UB off to its recipient, and to understand how ubiquitylation is activated by E3 assembly, relief from autoinhibition, dissociation of cellular inhibitors and/or stimulation by post-translational modifications and binding of activating subunits.

A second major focus is to discover new UB and UBL pathways mediating biological regulation. We previously focussed on pathways associated with cancers, but we are expanding efforts to also identify and understand roles of ubiquitylation in the hematopoietic system.

Our third major focus is to develop innovative chemical and biologic tools for probing the UB system. We are currently implementing our novel chemical and affinity probes to study structural mechanisms and cellular roles of ubiquitination. However, a long-term goal is for our basic research is to provide new avenues for diagnostics and therapeutics for diseases such as cancers. Our basic research into UB conjugation mechanisms has explained how mutations in a particular E3 ligase disrupt ubiquitylation in many prostate cancers; shown how disease-associated mutations in a cellular E3 ligase inhibitor would lead to wayward ubiquitylation in certain vascular lesions; revealed that some pathogenic bacteria thwart an enormous family of E3 ligases during infections; and demonstrated that E2-E3 interactions can be selectively and effectively disrupted by small molecules. We hope that by building new tools

based on our deep mechanistic knowledge, we can provide new ways of tracking, mobilising, or inhibiting UB and UBL systems to fight diseases.