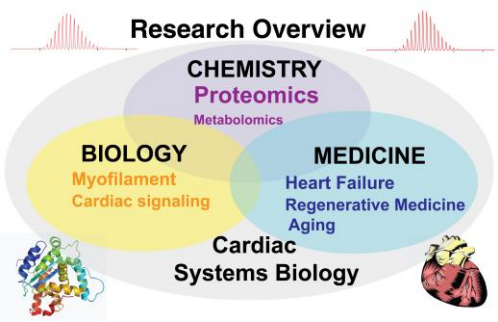
Top-down Proteomics in Cardiac Disease and Regeneration for Precision Medicine

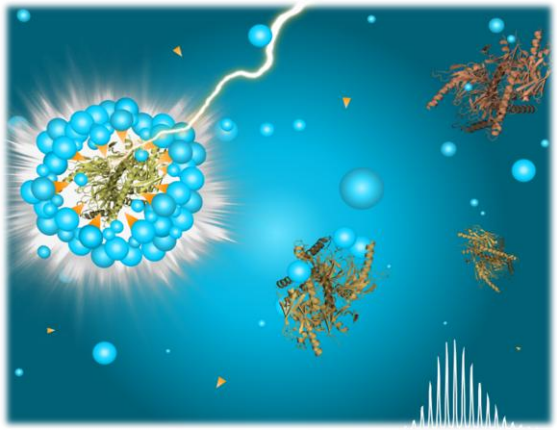
Ying Ge Research Group, University of Wisconsin-Madison



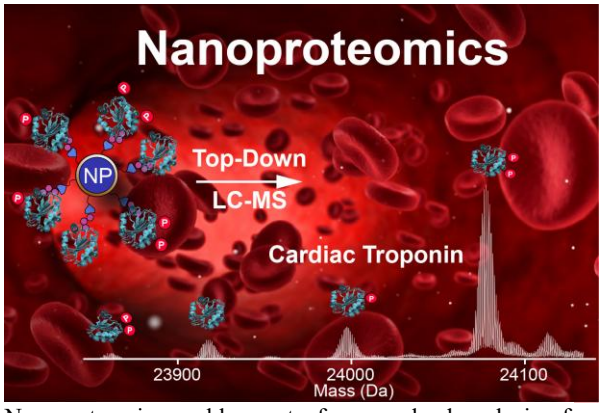
We have established an interdisciplinary research program that cuts across the traditional boundaries of chemistry, biology, and medicine. By creatively integrating our expertise in analytical chemistry/proteomics with cardiac biology/medicine, we aim to gain new molecular insights into the underlying mechanisms in cardiac disease and regeneration and to translate the bench discoveries to the clinic for precision medicine. Summarized below are current major research projects in our group from both technology development and biological/medical studies.

I. Novel Strategies to Address the Challenges in Top-down Proteomics (TDP)

Top-down mass spectrometry (MS)-based proteomics, which is based on analysis of intact proteins, is the most powerful method to comprehensively characterize proteoforms that arise from genetic variations, alternative splicing, and post-translational modifications (PTMs), but myriad challenges remain (Melby *et al.* JASMS 2021, Roberts *et al*., Nature Rev. Methods Primer, 2024). Herein, we are developing novel strategies to address the challenges in top-down proteomics in a multi-pronged approach: 1) *To address the protein* ***solubility*** *challenge*, we have recently identified a photo-cleavable anionic surfactant (referred to as "Azo") that can be rapidly degraded upon UV irradiation, for top-down proteomics. Azo is MS-compatible and can effectively solubilize proteins with performance comparable to SDS (the gold standard surfactant for protein solubilization but unfortunately is MS-incompatible). Importantly, Azo-aided TDP enables the solubilization of membrane proteins for comprehensive characterization of PTMs. Moreover, Azo is simple to synthesize and can be used as a general SDS replacement in SDS-PAGE (Brown *et al. Nature Methods, 2019*). Moreover, we have expanded the use of Azo-enabled bottom-up proteomics to extracellular matrix (ECM) proteomics (Knott et al., Anal Chem 2020, Towler et al., MCP, 2025) and exosome proteomics (Buck et al. Anal Chem 2022). The use of Azo has been successfully patented and licensed. *2) To address the proteome* ***complexity*** *challenge*, we have been developing new chromatography materials and novel strategies for multi-dimensional liquid chromatography (MDLC) to separate intact proteins. We developed novel hydrophobic interaction chromatography (HIC) materials for high-resolution separation of intact proteins under non-denaturing mode to advance TDP (Chen *et al*. Anal Chem 2016, 2018). Given the difficulty in detecting large proteins in top-down MS, importantly, we developed a novel serial size exclusion chromatography (sSEC) strategy for size-based protein separation that can be coupled with online reverse phase chromatography (RPC) and high-resolution MS which enabled the top–down MS analysis of large proteins (>200 kDa) (Cai *et al.* Anal Chem 2017). Importantly, we have developed a highly sensitive functionally integrated TDP method for the comprehensive analysis of proteoforms from single cells, highlighting the potential of TDP for uncovering the molecular underpinnings of cell-to-cell variation in complex systems (Melby *et al.* PNAS 2023). Recently, we have developed online native mixed-bed ion exchange chromatography (IEX) (Fischer *et al.* JPR 2024) and online MDLC method coupling SEC and mixed-IEX to enable separation of endogenous proteins and mixtures for native TDP, enabling high-throughput structural analysis of endogenous protein complexes (>350 kDa) (Fischer et al., Anal Chem. 2025). 3) *To address the proteome* ***dynamic range***, we have been developing novel nanomaterials that can bind low abundance proteins and PTMs with high specificity (In collaboration with Prof. Song Jin). First, we designed and synthesized novel superparamagnetic nanoparticles (NPs) whose surface is functionalized by multivalent ligand molecules that specifically bind

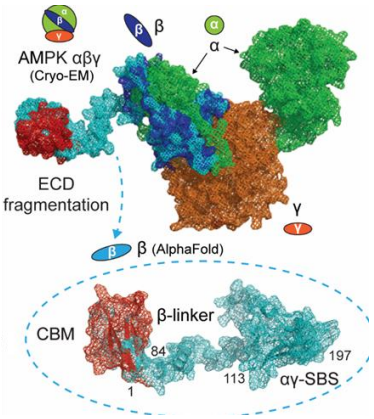


A novel photocleavable surfactant for top-down proteomics. Brown *et al*. Nature Methods, 2019 [**Article**] [**News Release**]



Nanoproteomics enables proteoform-resolved analysis of low-abundance proteins in human serum, Tiambeng *et al.* Nature Commun. *2020*, [**Article**][**News Release**]

to the phosphate groups on any phosphoproteins and demonstrated the high specificity for capturing phosphoproteins globally out of the human proteome for top-down phosphoproteomics (Hwang *et al*. JACS, 2015; Chen *et al. Chem Sci* 2017). Recently, we have developed an integrated nanoproteomics method coupling peptide-functionalized superparamagnetic nanoparticles (NPs) with top-down MS for the enrichment and comprehensive analysis of cardiac troponin I (cTnI), a gold-standard cardiac biomarker, directly from serum, providing high-resolution proteoform-resolved molecular fingerprints of diverse cTnI proteoforms to establish proteoform-pathophysiology relationships. This scalable and reproducible antibody-free strategy can generally enable the proteoform-resolved analysis of low-abundance proteins directly from serum to reveal previously unachievable molecular details for precision medicine. (Tiambeng et al., Nature Commun. 2020). Moreover, we have recently developed a "native nanoproteomics" method to allow direct enrichment and native top-down MS analysis of endogenous cardiac troponin complex from human heart tissue, providing insights into its structure and dynamics (Chapman *et al.,* Nature Commun.2023). Recently, by integrating native top-down electron capture dissociation (ECD) MS and AlphaFold, we elucidate a regulatory flexible region of protein kinase complex that was previously unresolvable with traditional structural biology tools (chan et al., JACS 2025) *4) To address the challenge in under-developed software*, we developed MASH Explorer, a comprehensive software tool for TDP including protein identification, quantitation, and characterization with visual validation and versatile user-friendly interface (Cai *et al.* MCP 2016). To streamline native TDP data analysis, we developed MASH Native, a unified software tool for processing native TDP datasets, offering an integrated solution for characterizing both protein complexes and proteoforms (Larson et al., Bioinformatics, 2023). We have been supporting over 4000 MASH users worldwide (http://prot.crb.wisc.edu/MASH/), to further advance TDP for its full potential in biomedical research. Moreover, our innovations native TDP offer new tools to investigate protein structure–function relationships and disease mechanisms at the proteoform level.

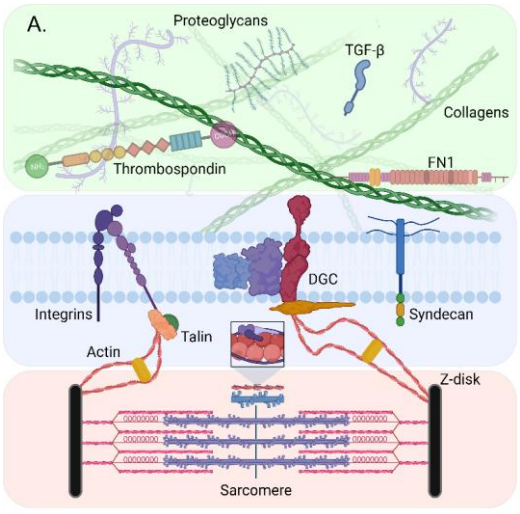


Native top-down ECD analysis and AlphaFold uncovers a previously unresolved flexible region in AMPK complex, Chan *et al.* JACS *2025*, Article.

II. The Role of Sarcomeric Modifications in Heart Failure (HF)

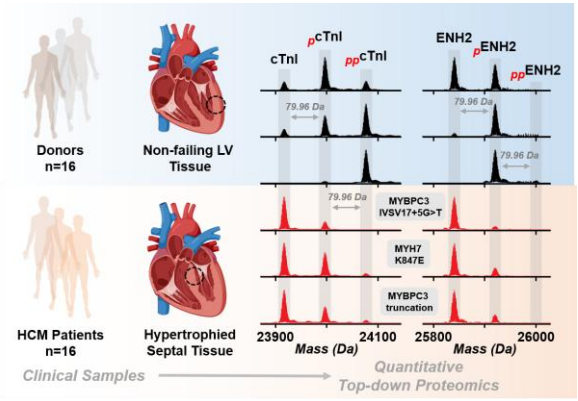
A major research objective in my lab is to understand how sarcomeric modifications regulate cardiac and skeletal muscle contractility in disease and aging using TDP in conjunction with *in vivo*, *ex vivo*, and *in vitro* functional measurements. The sarcomeres not only play essential roles in cardiac contractility but also are critical elements in signal reception and transduction during disease. The hypothesis is that both extrinsic and intrinsic stresses trigger molecular signaling processes that could result in altered modifications to sarcomere leading to contractile dysfunction. We established novel methods and made important contributions to cardiac proteomics and sarcomere/myofilament biology.

Importantly, we have linked altered sarcomeric PTMs to contractile dysfunction in HF using both animal models and human clinical samples. Enabled by our novel top-down LC-MS-based proteomics technology and a clinically relevant acute myocardial infarction (MI) swine model, we discovered a concerted reduction in the phosphorylation of three critical cardiac proteins: cardiac troponin I, a critical thin filament regulatory protein, myosin regulatory light chain (RLC) of the think myofilament, and, unexpectedly, enigma homolog isoform 2 (ENH2) of the Z-disc in acutely-infarcted swine myocardium. Our findings suggest that concerted regulation between myofilaments and the Z-disc may be an early molecular event that contributes to cardiac dysfunction post-MI and, ultimately, HF (Peng *et al*, MCP 2014). To determine if the ENH protein is required for the mechanical activity of cardiac muscle, we performed muscle mechanics analysis of isolated trabeculae from the hearts of $ENH^{+/+}$ and $ENH^{-/-}$ mice (In collaboration with Prof. Richard Moss). Our data suggest that the ENH protein influences tension redevelopment kinetics in mouse myocardium, possibly by affecting cross-bridge cycling kinetics (Gregorich *et al*, J Gen Physiol, 2019). Using top-down LC–MS proteomics, we uncovered widespread alterations in sarcomeric proteoforms including dysregulated phosphorylation and expression of key sarcomeric proteins in LV myocardium from end-stage ischemic cardiomyopathy (ICM) patients, revealing severe remodeling of the cardiac contractile apparatus (Chapman *et al*, JPR, 2023). Recently, we discovered a bidirectional communication between cardiomyocytes and ECM contributes to ICM (Buck *et al.* JCI insight, 2025).



Bidirectional communication between sarcomere and ECM contributes to ischemic cardiomyopathy

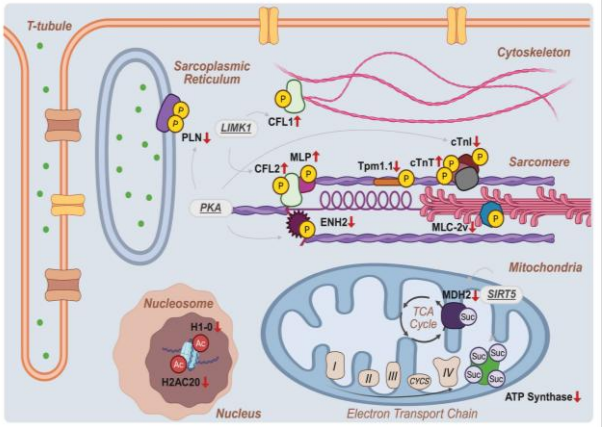
Moreover, we analyzed surgical heart tissue samples from hypertrophic cardiomyopathy (HCM) patients with severe outflow track obstruction which revealed a common pattern of altered sarcomeric proteoforms across HCM tissues compared to non-failing donor heart tissues. Our data suggest that common pathways are associated with clinical phenotypes in patients diagnosed with obstructive HCM, opening the door for the development of interventions that target the HCM phenotype rather than the individual sarcomeric gene mutation (Tucholski *et al.,* PNAS, 2020). Furthermore, a novel Azo-enabled serial extraction strategy enabled the first global TDP analysis in HCM, uncovering pathways beyond the sarcomere that contribute to disease pathophysiology and revealing potential targets for therapeutic intervention (Gao *et al.,* Circulation Heart Failure, 2025).



Distinct hypertrophic cardiomyopathy (HCM) genotypes result in convergent sarcomeric proteoform profiles revealed by top-down proteomics, Tucholski *et al*., *Proc Natl Acad Sci USA*, 2020, **Article.** [**News Release**]

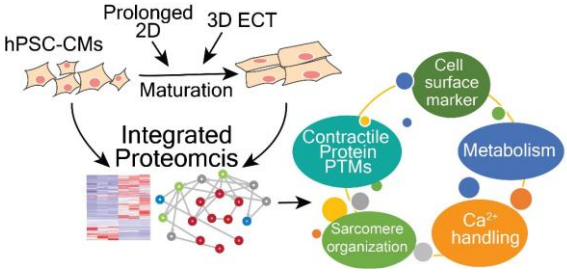
III. Stem Cell and Cardiac Regeneration

A new direction in my research program is to investigate the molecular mechanism in cardiac regeneration via systems biology approaches. The stem cell treatments have beneficial functional improvement for post-MI left ventricular (LV) remodeling, however, the underlying mechanisms remain poorly defined. Thus, we are undertaking a systems biology approach to comprehensively delineate the molecular signaling pathways underlying cardiac regeneration in response to stem cell transplantation (in collaboration with Prof. Jianyi Zhang and Prof. Wuqiang Zhu). Using a swine acute MI model with tri-lineage cardiovascular cell transplantation, we provided the direct evidence that the functionally beneficial effects of cell therapy is accompanied by changes in the protein expression profiles of the myocardial cells in the recipient myocardium—leading to the induction of beneficial signaling pathways (Ye *et al*., Cell Stem Cell 2014; Chang *et al*. Proteomics 2015). Moreover, we discovered that the MI-induced changes in sarcomeric proteins phosphorylation, particularly cTnI and ENH2, were reversed by cell transplantation of human cardiac muscle patches (hCMPs) derived from human induced-pluripotent stem cells (hiPSCs) in clinically relevant dimensions four weeks after MI injury (Gao *et al*., Circulation, 2018). Furthermore, we have identified alterations in sarcomere composition and developmental processes during postnatal swine heart development (Aballo et al. JMCC 2023). Recently, using integrated proteomics, we identified troponin I isoform switch as a regulator of a sarcomere-metabolism axis during cardiac regeneration (Aballo et al. Cardiovasc. Res. 2025).



Global proteoform alterations across multiple cellular compartments underlie obstructive hypertrophic cardiomyopathy (HCM), *Circulation Heart Failure* 2025, [**Article.**]

Moreover, we are harnessing the power of innovative TDP-based systems biology with patient specific hiPSC-derived cardiomyocytes (CMs) in engineered cardiac tissue to study hypertrophic cardiomyopathy (HCM) (in collaboration with Prof. Carter Raphe and Prof. Timothy Kamp). Since the immaturity of hPSC-CMs limit their clinical applications and there is an urgent need to establish an unbiased method for benchmarking hPSC-CM maturity, we developed an unbiased proteomics method to provide accurate and comprehensive assessment of hPSC-CMs maturation. It led to the discovery of new markers for specific benchmarking of hPSC-CM maturity and established a strong foundation for uncovering the molecular basis underlying cardiac disease using patient-specific hPSC-CMs (Cai *et al.* Circ. Res. 2019). Moreover, we reported the sequential assessment of functional properties and TDP from the same 3D hiPSC-engineered heart tissue (ECTs) (Melby *et al.,* JPR 2021). We also showed that lactate- and magnetic cell separation (MACS)-purified hiPSC-CMs generate 3D EHTs with comparable structural, functional, and proteomic features, suggesting lactate purification does not cause an irreversible change in hiPSC-CM phenotype (Rossler *et al.,* JCI insight, 2024). Furthermore, we generated HCM hiPSC-ECTs from patient-specific lines and CRISPR/Cas9-edited isogenic controls and discovered dysregulated proteoforms in HCM.



An unbiased method for benchmarking hPSC-CM maturity, Cai *et al., Circ. Res.* 2019, [**Article.**]