BIOGRAPHICAL SKETCH

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NAME: Nayak, Digant

eRA COMMONS USER NAME (credential, e.g., agency login): NAYAKDI

POSITION TITLE: Research Fellow

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
Birla Institute of Technology (Ranchi, India)	B.Tech	05/2009	Biotechnology
Indian Institute of Technology (Kharagpur, India)	M.Tech.	05/2011	Biotechnology and Biochemical Engineering
National University of Singapore (Singapore)	PhD	12/2016	Biochemistry, Structural Biology
National University of Singapore (Singapore)	Postdoc	04/2019	Biochemistry, Structural Biology
Medical University of South Carolina (Charleston, SC)	Postdoc	05/2020	Biochemistry, Structural Biology
University of Texas Health Science Centre (San Antonio, Texas)	Postdoc	Ongoing	Biochemistry, Structural Biology

A. Personal Statement

My future aspiration is to run an independent lab that utilizes a unique combination of molecular, biochemical and structural techniques to study the relationship between posttranslational modification by ubiquitin, ubiquitin-like proteins and cancer. As an undergraduate I was really attracted to subjects like biochemistry and molecular biology and would spend hours reading through textbooks trying to understand biochemical processes like glycosylation and the krebs cycle. This piqued my scientific curiosity and inspired me to pursue the path of scientific research and knowledge.

It was not until I started my masters that I first got the opportunity to join a lab for my thesis. I worked with a PhD student and helped him in a small-scale bacterial purification of a kinase protein. I later used this protein for antibody generation in a rabbit and tested its efficacy using biochemical assays, establishing a meaningful and enjoyable connection with protein biochemistry. I continued my path for scientific discovery by pursuing PhD at National University of Singapore. During my PhD, I first came across ubiquitin signaling and was really fascinated by how a cascade of three enzymes are not only responsible for protein degradation, but also a seemingly endless number of cellular processes. I took up the challenge as the first lab member to study

ubiquitin biology, determined to uncover the mechanism of an E3 ligase enzyme, coupling biochemical assays and X-ray cystallography. During this period, I quickly became independent, developing many protocols that I shared with my fellow lab members, and published two single author papers. Towards the end of my PhD, I was motivated to use my training to address how misregulation of the ubiquitin pathway is implicated in human health and diseases – in particular cancer – which led me to do my postdoctoral training at Dr. Shaun Olsen's lab at UT Health San Antonio.

Dr. Olsen is one of the leading structural biologists in the field of ubiquitin and ubiquitin-like signaling and shares my enthusiasm in understanding the role of posttranslational modifications and cancer. His work on Cdc34 and SUMO E1 proteins inspired me to join his lab and develop my biochemical, structural and biological toolkit to gain a deeper understanding about how structural mechanism impacts at cellular level. One of my primary projects in the lab focuses on PROTAC mediated protein degradation. PROTACs are small molecules, consisting of two warheads connected via linker molecule that redirect E3 ligases for targeted protein ubiquitination. In order to understand the molecular mechanism of one particular PROTAC found to be efficacious in cancer cells by our collaborator, I am using cryoEM to resolve the structure of a biochemically reconstituted 9 subunit E3 ligase complex consisting of cofactors and substrate. My TP1 training at NCCAT will be critical for me to become proficient in cryoEM and solve this and other related E3 complexes, ultimately allowing me to establish an independent research group and do impactful science in the field of human health.

B. Positions and Honors

Positions and Employments

2016-2017 Research Assistant, National University of Singapore, Singapore

Honors

2008 Selected to be a summer research fellow in May-July 2008 jointly sponsored by Indian Academy of Sciences (Bangalore), Indian National Science Academy (New Delhi) and National Academy of Sciences (Allahabad).

2009-2011 Received Ministry of Human Resource Development, Government of India Scholarship for graduate studies at Indian Institute of Technology, Kharagpur from 2009-2011.

2011-2016 Received NUS Research Scholarship for graduate research from 2011-2016.

2013 Poster presentation at 7th International Conference on Structural Biology and Functional Genomics, Singapore 2013

2014 Oral presentation at 18th Biological Sciences Graduate Congress, Singapore January 2014.

2015 Selected for the EMBO practical Course in France, 2015 and awarded with travel grant to participate in it based on Singapore-EMBO cooperation agreement which brings together Agency for Science, Technology and Research (A*STAR), National University of Singapore (NUS) and Nanyang Technological University (NTU).

2015 Selected for the "3rd CCP4-OIST School: Collaborative computational project No.4 Software for Macromolecular X-Ray Crystallography" in Okinawa, Japan from 2nd-7th November 2015.

2017 Selected for oral presentation and awarded with Young participant IUCr award for AIC school "Bridging the gap between cryo-EM and crystallography" to cover travel and accommodation costs.

2018 Selected for the EMBO practical Course on "CEM3DIP 2018: of macromolecular assemblies and cellular tomography." in New Delhi, India from 18th March- 29th March 2018.

C. Contribution to Science

1. Early Career: While working on my Masters thesis I was assisting a PhD student who working on elucidating the encystation mechanism in *Entamoeba invadens.* Based on RNA profiling, he had identified few target genes, which were upregulated during encystation. My job was to express one of these proteins through recombinant means and develop antibodies against it, which would be utilized for subsequent studies. Towards this end I cloned, expressed and purified the protein. I further processed the purified protein with Freund adjuvant and injected it into a rabbit and keep collecting samples at regular intervals to assess antibody generation. I also gave booster doses to increase the yield of antibodies and after final collection of serum from animal I performed a protein-A sepharose based purification of IgGs. To assess the efficacy of the antibodies, I performed ELISA based titration assay. The antibodies were of good quality and specificity as we also tested various controls. The training exposed me to protein biochemistry and purification techniques, which led me to path of structural biology.

a) A novel encystation specific protein kinase regulates chitin synthesis in *Entamoeba invadens.*
Samanta, Sintu Kumar, Sneha Susan Varghese, Deepak Krishnan, Mithu Baidya, **Digant Nayak**, Sumanta Mukherjee, and Sudip K. Ghosh. **Molecular and biochemical parasitology** 220 (2018): 19-27.

2. Graduate Career: Ubiquitination is a well-studied posttranslational modification, which not only controls key cellular processes but also has been implicated in various diseases including cancer. Ubiquitination involves interplay of three enzymes: E1-activating, E2-conjugating and E3-ligase enzymes. My work was focused on an understudied family of E3 ligases termed as LNX (Ligand of Numb protein-X). I crystallized and solved the structure of the RING or active domain in LNX1 and LNX2. The structures were unique since in addition to RING domain there two additional zinc-finger motifs (Znf), which were not predicted based on sequence information. This form of domain architecture where a RING domain is sandwiched between two Znfs has never been reported. Moreover, the N-terminal Znf lacked any secondary structure and adopted an open circle conformation. Based on biochemical and mutational assays, I found that N-terminal Znf was crucial for the activity of LNX2, while both N- and C-terminal Znfs were indispensable for the LNX1 activity. I also identified corresponding E2`s for LNX1 i.e. Ubc13 and UbcH5b. In order to further understand the role of these Znfs at atomic level we solved the structure of LNX1 in complex with Ubc13 conjugated to ubiquitin. This structure was interesting as it clearly justified the need for C-terminal Znf, which contacts the ubiquitin in trans to stabilize the E2-Ub "close" conformation. I further validated these observations using mutational analysis using full-length proteins. This was a unique finding since for similar complexes in literature, one or two residues maintain the close conformation but in our case it was maintained by a whole motif. The impact of this research can be judged from the fact that this work was featured in the Research Highlights of FEBS journal. During my graduate studies I was also involved in other collaborative projects, which led to publications.

a) Structure of LNX1: Ubc13~Ubiquitin Complex Reveals the Role of Additional Motifs for the E3 Ligase Activity of LNX1.
Digant Nayak and J. Sivaraman.
Journal of Molecular Biology, Volume 430, Issue 8, 2018, Pages 1173-1188. This was also featured in the **Research Highlights in FEBS journal** (Volume 285, Issue 8, 23 April 2018, Page-1378).

b) Structural basis of the indispensible role of a unique zinc finger motif in LNX2 ubiquitination
Digant Nayak and J. Sivaraman.
Oncotarget. 2015 Oct 27; 6(33): 34342-57. doi: 10.18632/oncotarget.5326

c) Structure of ScpC, a virulence protease from Streptococcus pyogenes, reveals the functional domains and maturation mechanism.
Chacko Jobichen, TanYing Chong, MahalakshmiTirumuru Prabhakar, **Digant Nayak**, Debabrata Biswas, Navraj Singh Pannu, Emanuel Hanski, J Sivaraman. **Biochemical Journal** Jul 2018,BCJ20180145; DOI: 10.1042/BCJ20180145

3. Postdoctoral Career:

PROteolysis-TArgeting Chimeras (PROTAC) are heterobifunctional small molecules, which binds both an E3 ligase and protein of interest (POI) and brings them in close proximity followed by degradation of POI via the ubiquitin proteasome system. PROTACs provide many advantages compared to the conventional small molecule inhibitors (SMI) like ability to target "undruggable" proteins, required in sub-stoichiometric amounts for POI degradation, is "event-drive" i.e. it doesn't require to inhibit the POI, etc. These properties of PROTAC really intrigued me and fortunately I got the opportunity to work with PROTACs against proteins like Bcl-2, Bcl-XL and KRAS. To develop more efficient PROTACs through structure based drug design, we collaborate with a group specializes in PROTAC synthesis, while I focus on structure determination of ternary complexes through X-ray crystallography ternary complexes, and more recently, cryoEM, as well as design of biochemical assays to evaluate effectiveness of these novel compounds *in vitro.*

a) Lv D, Pal P, Liu X, Jia Y, Thummuri D, Zhang P, Hu W, Pei J, Zhang Q, Zhou S, Khan S, Zhang X, Hua N, Yang Q, Arango S, Zhang W, **Nayak D**, Olsen SK, Weintraub ST, Hromas R, Konopleva M, Yuan Y, Zheng G, Zhou D. Development of a BCL-xL and BCL-2 dual degrader with improved anti-leukemic activity. *Nature Communications* **12**, 6896 (***2021***)

COVID-19 is a highly infectious disease caused by SARS-CoV-2, which was responsible for a number of deaths since 2019. There are many viral co-factors, which are responsible for survival and propagation inside the host. Our lab was interested in one of these co-factors called PLpro, which is a deubiquitinases. PLpro is an interesting target since compared to PLpro 1 (from SARS-CoV-1), it catalyzes the removal of ISG15 from host protein more efficiently as compared to removal of ubiquitin. Our group has been successful in solving various co-complex structures of PLpro with small molecule covalent inhibitors which helped in explaining the mode of action of this enzyme.

a) Rut W, Lv Z, Zmudzinski M, Patchett S, **Nayak D**, Snipas SJ, El Oualid F, Huang TT, Bekes M, Drag M, Olsen SK. Activity profiling and crystal structures of inhibitor-bound SARS-CoV-2 papain-like protease: A framework for anti-COVID-19 drug design**.** *Science Advances* **6**,eabd4596 (***2020***)

Complete list of published work in mybibliography:

https://www.ncbi.nlm.nih.gov/myncbi/12GDbuXZRma9jc/bibliography/public/

D. Research Support

None

E. Work under progress

1. **Nayak D**, Bury PDS, Drag M, Njkerk AN, El Oualid F, Olsen SK**.** Characterization of the Ubiquitin and ISG15 Deconjugase Activity of SARS-CoV-1 and 2. *Methods in Molecular Biology IN REVISION* (***2022***)

2. Yuan L, Gao F, Lv Z, **Nayak D**, Nayak A, Bury PDS, Cano KE, Jia L, Atligan FC, Oleinik N, Ogretmen B, El Oualid F, Wasmuth EV, **Olsen SK**. Structures of the bispecific Ubl activating enzyme Uba6 reveal novel catalytic and regulatory mechanisms. *IN REVIEW* (***2022***)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FIVE PAGES.**

NAME: ***Olsen, Shaun K.***

eRA COMMONS USER NAME (credential, e.g., agency login): ***olsensk***

POSITION TITLE: ***Associate Professor***

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
Rutgers University, New Brunswick, NJ	B.A.	05/2000	Biology
New York University, New York, NY	Ph.D.	05/2006	Biophysics/Biochemistry
Sloan Kettering Institute, New York, NY	Postdoctoral	07/2013	Biophysics/Biochemistry

A. Personal Statement

In broad terms, the research in my laboratory focuses on mechanisms of molecular recognition in protein-protein interactions and on the structural enzymology of proteins essential for cellular function. The majority of our efforts are focused on understanding how enzymes in the ubiquitin (Ub) conjugation cascade (E1, E2, and E3) function together to conjugate Ub to cellular proteins. Post-translational modification of proteins by Ub is a means of regulating fundamental cellular processes including cell cycle control, DNA repair, signal transduction, and immunity. The importance of understanding how the Ub system works is underscored by the fact that dysregulation of Ub signaling is implicated in a number of human disorders and that the pathway is a validated target for therapeutic intervention in cancer. To achieve our goals, we employ a multidisciplinary approach that includes X-ray crystallography, enzymology, a variety of biochemical and biophysical techniques, and cell-based functional assays in various eukaryotic systems to assess the biological importance of our structural, biochemical, and biophysical findings. More recently, my lab has begun expanding our structural toolkit to include single particle cryo-electron microscopy (cryo-EM) as we prepare for our Glacios microscope to come online in early summer. As Digant's mentor and Director of Structural Biology Core Facilities at University of Texas Health at San Antonio (UTHSA), I can attest that the TP1 training Digant receives at NCCAT will allow him to become a driving force in disseminating cryo-EM knowledge within my lab, the Biochemistry and Structural Biology department, and the larger scientific community at UTHSA.

B. Positions, Scientific Appointments, and Honors

Positions

2006-2008	Research Associate, Protein Research Group, RIKEN Genomic Sciences Center, Yokohama, Kanagawa, Japan
2008-2010	Adjunct Assistant Professor, Department of Chemistry & Biochemistry, Queens College (City University of New York, New York, NY
2008-2013	Postdoctoral Fellow, Structural Biology Program, Sloan-Kettering Institute, New York, NY
2013-2019	Assistant Professor, Department of Biochemistry & Molecular Biology, Medical University of South Carolina, Charleston, SC
2015-2020	Director, X-ray Crystallography Core, Medical University of South Carolina, Charleston, SC
2020	Associate Professor, Department of Biochemistry & Molecular Biology, Medical University of South Carolina, Charleston, SC

2020-pres Director, Structural Biology Core Facilities, University of Texas Health at San Antonio, San Antonio, TX

2020-pres Co-Director, Drug Discovery and Structural Biology Shared Resource, Mays Cancer Center University of Texas Health at San Antonio, San Antonio, TX

2020-pres Associate Professor (tenured), Department of Biochemistry & Structural Biology, University of Texas Health at San Antonio, San Antonio, TX

Honors

1999 Phi Beta Kappa inductee
2000 Degree with Honors in the Liberal Arts, Rutgers University
2010-2012 Charles H. Revson Fellow, Sloan Kettering Institute
2010 Memorial Sloan Kettering Postdoctoral Researcher Award
2011 Blavatnik Award for Young Scientists (Finalist)
2017 MUSC High Impact Publication Award
2017 MUSC College of Graduate Studies Teacher of the Year Award
2018 MUSC College of Graduate Studies Teacher of the Year Award
2019 MUSC Developing Scholar Award
2020 Cancer Prevention & Research Institute of Texas- Rising Star Award
2021 UT STARs Faculty Award

C. Contributions to Science

Post-translational modification of proteins by ubiquitin, Nedd8, and SUMO (collectively termed Ubls) is a means of regulating fundamental cellular processes such as cell cycle control, signal transduction, and differentiation. Ubls are conjugated to proteins through the activity of an enzymatic cascade comprised of an E1 activating enzyme, an E2 conjugating enzyme, and in most instances, an E3 ligase. E1s function as gatekeepers of the conjugation cascades by specifically activating their cognate Ubl in two half-reactions involving adenylation and thioester bond formation. Despite a wealth of biochemical and structural information representing decades of research on E1s, the molecular mechanisms of E1-catalyzed adenylation and thioesterification of Ubls were unknown when I began working in this field, partially due to the unstable nature of key intermediates generated during catalysis. To solve this problem, I was involved in the development and application of semisynthetic protein-based inhibitors of Ubl E1s that enabled us to trap and determine structures of unstable intermediates formed in the two half-reactions catalyzed by E1s. These studies revealed that SUMO E1 has a single active site that is reconfigured for catalysis of adenylation or thioester bond formation via a series of complementary conformational changes in several regions of the E1. After adenylation, contacts to ATP/Mg are released, facilitating a dramatic 130 degree rotation of the E1 Cys domain and remodeling of several other structural elements that comprise the catalytic machinery of the adenylation active site. As a result, more than half of the residues that promote adenylation are replaced with residues that promote thioester bond formation, thereby toggling the catalytic competency of the active site. More recently, we obtained the first crystallographic snapshot of Ub E1 in which domain alternation and active site remodeling were observed. This study suggests that domain alternation and active site remodeling are intrinsic and interconnected structural features of Ub E1 and that the salient features of adenylation and thioester bond formation by Ub E1 is conserved across canonical Ubl E1s.

a. **Olsen SK,** Capili AD, Lu X, Tan DS, Lima CD. Active site remodeling accompanies thioester bond formation in SUMO E1. *Nature* **463**, 906-12 (2010) *[PMCID: 2866016]*

b. Lv Z, Yuan L, Atkison JH, Aldana-Masangkay G, Chen Y, **Olsen SK**. Domain Alternation and Active Site Remodeling Are Conserved Structural Features of Ubiquitin E1. *J. Biol. Chem*. **292**, 12089-12099 (2017) *[PMCID: 5519361]*

c. Lv Z, Yuan L, Atkison JH, Williams KM, Vega R, Sessions EH, Divlianska DB, Davies C, Chen Y, **Olsen SK**. Molecular mechanism of a covalent allosteric inhibitor of SUMO E1 activating enzyme. *Nat. Commun.* **9**,5145 (2018) *[PMCID: 6279746]*

d. Lv Z, Williams KM, Yuan L, Atkison JH, **Olsen SK**. Crystal structure of a human ubiquitin E1-ubiquitin complex reveals conserved functional elements essential for activity. *J. Biol. Chem*. **293**, 18337-18352 (2018) *[PMCID: 6254350]*

II. Once the E1~Ubl thioester intermediate has been generated, the next step in the Ubl conjugation cascade involves recruitment of an E2 enzyme followed by transfer of the Ubl from the E1 catalytic cysteine to the E2 catalytic cysteine. Although the structure of Nedd8 E1 in complex with its E2 had been determined prior to my entering this area of research, the E1 and E2 active sites were situated more than 20 Å away from each other and thus the conformational changes and contacts involved in bringing the E1and E2 active sites were unknown. Another unresolved issue was the basis by which a particular Ubl E1 achieves selectivity for its cognate E2(s) as opposed to E2s from other Ubl pathways. To gain insights into this process I developed a strategy for trapping E1 and E2 in a catalytically relevant conformation by specifically cross-linking their catalytic cysteine residues, a breakthrough that led to the first crystal structure of a Ub E1-E2 complex. The structure revealed a combinatorial mechanism for Ub E2 recruitment to Ub E1 that involves E2 contacts to two distinct domains of the E1 as well as conformational changes within the E1 that are required for the E1 and E2 active sites to come together during thioester transfer. More recently, we uncovered a new E1 binding mode for the E2 Ubc15 and determined that the intrinsically low level of E1-E2 thioester transfer activity of Ubc15 largely results from the presence of an acidic residue at its N-terminus that electrostatically clashes with an acidic patch on E1. Notably, the N-termini of many other Ub E2s are serine/threonine rich and we demonstrated that phosphorylation of these sites could serve as novel regulatory mechanism of Ub E2 activity.

a) **Olsen SK,** Lima CD. Structure of a ubiquitin E1-E2 complex: insights to E1-E2 thioester transfer. *Mol Cell.* **49**, 884-96 (2013) *[PMCID:* 3625138*]*

b) Lv Z, Rickman KA, Yuan L, Williams K, Selvam SP, Woosley AN, Howe, PH, Ogretmen B, Smogorzewska A, **Olsen SK**. *S. pombe* Uba1-Ubc15 structure reveals a novel regulatory mechanism of ubiquitin E2 activity. *Mol Cell.* **65**, 699-714 (2017) *[PMCID: 5319395]*

c) Williams KM, Que S, Atkison JH, Salazar-Arango S, Diehl JA, **Olsen SK**. Structural insights into E1 recognition and the ubiquitin-conjugating activity of the E2 enzyme Cdc34. *Nat. Commun.* **10**, 3296 (2019) *[PMCID: 6656757]*

d) Yuan L, Lv Z, Adams MJ, **Olsen SK**. Crystal structures of an E1-E2-ubiquitin thioester mimetic reveal molecular mechanisms of transthioesterification. *Nat. Commun.* **12**, 2370 (2021) *[PMCID: 8062481]*

III. The E2~Ub intermediate resulting from E1-E2 thioester transfer interacts with members of three different families of Ub E3 ligases (RING, HECT, and RING-in-between-RING (RBR)) that catalyze Ub conjugation to target proteins by distinct mechanisms. RBR E3s are a distinct class of Ub E3 ligases that function through a RING/HECT hybrid mechanism in which the RBR RING1 domain initially recruits the E2~Ub thioester intermediate, similar to canonical RING E3s. However, rather than facilitating Ub discharge from E2~Ub onto target protein lysine residues directly like a canonical RING E3, E2~Ub binding to the RING1 domain of RBR E2s is followed by thioester transfer of Ub to a catalytic cysteine residue in the RING2 domain similar to HECT E3s. A fundamental question that arises from these observations is how RBRs prevent Ub discharge to lysine residues upon E2~Ub to the RING1 domain, despite RING1 strongly resembling a canonical RING E3. We recently determined the crystal structure of the RBR E3, HHARI, in complex with a UbcH7~Ub thioester mimetic. The structure revealed mechanistically important UbcH7~Ub-induced conformational changes in the RING1 and UBA-like domains of HHARI that play a key role in determining the specificity of this E2/E3 pair, as well as in promoting recruitment of UbcH7~Ub in the 'open' conformation. Overall, our structural and biochemical studies indicate that HHARI ensures transfer of Ub from E2 to the RING2 catalytic cysteine as opposed to discharge from E2 directly to lysine residues upon RING1 binding in at least three ways: 1) by evolving a mechanism to specifically recruit an E2 that solely performs transthiolation (UbcH7), 2) by harboring a loop insertion in the RING1 domain (unique to RBR E3s) that is involved in determining specificity of HHARI for UbcH7 and is incompatible with the UbcH7~Ub binding in the 'closed' conformation primed for Ub discharge to lysine residues, and 3) contacts between Ub and the UBA-like domain that promote recruitment of UbcH7~Ub in the inactive 'open' conformation.

a) Yuan L, Lv Z, Atkison JH, **Olsen SK**. Structural insights into the mechanism and E2 specificity of the RBR E3 ubiquitin ligase HHARI. *Nat Commun* **8**, 211 (2017) *[PMCID: 5548887]*

IV. The emergence of severe acute respiratory syndrome (SARS-CoV-2) in 2019 marked the third occurrence of a highly pathogenic coronavirus in the human population since 2003. As the death toll surpasses 4 million globally and economic losses continue, designing potential drugs that could curtail infection and disease

progression is critical. Viral papain-like cysteine protease (PLpro, NSP3) is essential for SARS-CoV-2 replication and represents a promising target for the development of antiviral drugs. To address the need for COVID-19 antiviral therapies, we used a combinatorial substrate library and performed comprehensive activity profiling of SARS-CoV-2 PLpro. On the scaffold of the best hits from positional scanning, we designed optimal fluorogenic substrates and irreversible inhibitors with a high degree of selectivity for SARS PLpro. We determined crystal structures of two of these inhibitors in complex with SARS-CoV-2 PLpro that reveals their inhibitory mechanisms and provides a molecular basis for the observed substrate specificity profiles. We demonstrate that SARS-CoV-2 PLpro harbors deISGylating activity similar to SARS-CoV-1 PLpro but its ability to hydrolyze K48-linked Ub chains is diminished, which our sequence and structure analysis provides a basis for. Together, this work has revealed the molecular rules governing PLpro substrate specificity and provides a framework for development of inhibitors with potential therapeutic value or drug repurposing. In later work, we identified a molecular sensor within the S1 Ub-binding site of PLpro that serves as a key determinant of substrate specificity. Amino-acid variations within the S1 sensor specifically alter cleavage of Ub substrates but not of the Ubl ISG15. Significantly, a variant of concern associated with immune evasion carries a mutation in the S1 sensor and this mutation enhances PLpro activity on Ub substrates. Collectively, our data identify the S1 molecular sensor as a potential hotspot of variability that could dramatically alter host antiviral immune responses to newly emerging SARS-CoV-2 lineages.

a) Rut W, Lv Z, Zmudzinski M, Patchett S, Nayak D, Snipas SJ, El Oualid F, Huang TT, Bekes M, Drag M, **Olsen SK**. Activity profiling and crystal structures of inhibitor-bound SARS-CoV-2 papain-like protease: A framework for anti-COVID-19 drug design. *Science Advances* **6**,eabd4596 (2020) *[PMCID: 7567588]*

b) Patchett S, Lv Z, Rut W, Bekes M, **Olsen SK***, Huang TT. A molecular sensor determines the ubiquitin substrate specificity of SARS-CoV-2 papain-like protease. *Cell Reports* **36**, 109754 (2021) *[PMCID: 8423903]*
*co-corresponding author

c) Lv Z, Cano KE, Jia L, Drag M, Huang TT, Olsen SK. Targeting SARS-CoV-2 proteases for COVID-19 antiviral development. *Frontiers in Chemistry* **9**, 819165 (2022) *[PMCID: 8850931]*

V. Fibroblast growth factors play an essential role in human biology as regulators of embryonic development, homeostasis and regenerative processes. The twenty-two mammalian FGFs elicit their distinct biological effects by binding to and activating a unique subset of FGF receptor tyrosine kinases (FGFR1-4) at the cell surface. Alternative splicing of FGFs and FGFRs generates an additional level of sequence diversity, which in turn ultimately determines specificity in the FGF-FGFR system. A tissue-specific alternative splicing event in Ig domain 3 of FGFR1-3 results in the expression of epithelial FGFRb isoforms and mesenchymal FGFRc isoforms, and allows for directional epithelial-mesenchymal signaling which is required for organogenesis. To better understand the basis for molecular recognition in FGF-FGFR interactions and how alternative splicing governs specificity in these interactions, I structurally and biochemically characterized a selected set of FGF-FGFR pairs. The results of these studies revealed the basis by which FGF8 achieves exquisite specificity for the ‘c’ isoforms of FGFR and for how alternative splicing at the N-terminus of FGF8 regulates the organizing potential of this protein in the brain. I also determined the molecular basis by which FGF1 functions as a ‘pan’ FGFR ligand due to its ability to activate all FGFRs. These studies revealed that FGF1 exhibits a remarkable degree of structural plasticity that equips this protein with the adaptability required to engage in contacts with the distinct interaction surfaces presented to it by the repertoire of FGFRs present in cells. Together, this work answered a number of longstanding questions in the FGF field and contributed to the transformation of FGF signaling into one of the foremost structurally understood ligand-receptor systems among receptor tyrosine kinases.

a. **Olsen SK**, Bromleigh C, Ibrahimi OA, Eliseenkova AV, Zhang F, Linhardt RJ, Joyner AL, Mohammadi, M. Structural Basis By Which Alternative Splicing Modulates the Biological Activity of FGF8 in the Brain. Genes Dev. **20**, 185-98. (2006) *[PMCID: 1356110]*

b. **Olsen SK**, Ibrahimi OA, Raucci A, Zhang F, Eliseenkova AV, Yayon A, Basilico C, Linhardt RJ, Schlessinger J, Mohammadi M. Insights into the molecular basis for fibroblast growth factor receptor autoinhibition and ligand-binding promiscuity. Proc. Natl. Acad. Sci. U S A. **101**, 935-40 (2004) *[PMCID: 327120]*

c. **Olsen SK**, Garbi M, Zampieri N, Eliseenkova AV, Ornitz DM, Goldfarb M, Mohammadi, M. Fibroblast growth factor (FGF) homologous factors share structural but not functional homology with FGFs. J. Biol. Chem. **278**, 34226-36 (2003) *[PMCID:12815063]*

Complete List of Published Work in My Bibliography:

https://www.ncbi.nlm.nih.gov/myncbi/shaun.olsen.1/bibliography/public/

D. Additional Information: Research Support and/or Scholastic Performance

Ongoing

1R01GM115568 09/01/2020 – 08/31/2024 Olsen (PI)

Structural Biology of the Ubiquitin Conjugation System- The research in this proposal aims to establish the rules governing molecular recognition and promiscuity across Ub E1-E2 and E2-RING-in-between-RING E3 ligase interactions.

Role: PI

1R01GM128731 04/15/2019 – 02/28/2023 Olsen (PI)

Structure and function of the essential cell cycle regulator Cdc34- The goal of this project is to elucidate the molecular mechanisms by which the three enzymes E1, Cdc34, and SCF E3 function together as essential regulators of the cell cycle by specifically assembling Lys48 polyubiquitin chains on target proteins.

Role: PI

RR200030 09/01/2020 – 08/31/2025 Olsen (PI)

Recruitment of Rising Stars Investigator- This faculty recruitment award is a startup package that provides general support to the Olsen laboratory over a 5-year period, including salaries for laboratory personnel, supplies, equipment purchase, equipment maintenance and repair, facility renovations, secretarial support, core facility access fees, and travel.

Role: PI

P30 CA054174 08/01/2020-7/31/2025 Mesa (PI)

Mays Cancer Center at UT Health San Antonio. This cancer center support grant provides research core and program infrastructure support to members of the cancer center for the conduct of their cancer research.

Role: co-Leader, DDSBSR, CCSG Core 005

SA PPT 2021 01/01/2021-12/31/2023 Bohmann (PI)

HSC-SA PARTNERSHIP PRECISION THERAPEUTIC $25,000

Development of Therapies for the Henipaviruses Hendra and Nipah- The goal of this project is to develop new antiviral small-molecules by targeting the attachment glycoprotein G
of Nipah and Hendra virus.

Role: co-investigator

PRMRP Ref# PR211252 01/01/2022 – 01/01/2024 (Bohmann, PI)

DoD $50,000

Development of Antiviral Therapies against Nipah and Hendra Viruses- The goal of this project is to determine whether novel compounds from a custom library of small molecules affect Nipah and/or Hendra virus infection in vitro, by targeting the viral F Fusion protein.

Role: co-investigator