BIOGRAPHICAL SKETCH

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NAME: Keerthi Gottipati, PhD

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

POSITION TITLE: Research Scientist-II

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
University of Texas Medical Branch, Galveston, Texas	PhD	01/2009-08/2012	Biochemistry and Molecular Biology
University of Texas Medical Branch, Galveston, Texas	MS	08/2005-12/2008	Biochemistry and Molecular Biology
A. C. College of Technology, Anna University, Chennai, India	B.Tech.	07/2001-05/2005	Industrial Biotechnology

A. Personal Statement

My long term goal is to develop a seamless template for rational drug design based on macromolecular structures of RNA and RNA-protein complexes involved in viral disease pathogenesis. I use Cryo-EM and X-ray crystallography in conjunction with RNA engineering to study viral RNA, protein, and RNA-protein complex structures. I recently determined the first reported high-resolution structure of the Enterovirus cloverleaf RNA, a functionally critical RNA structure involved in the regulation of enteroviral replication which was recognized by a US National Science Foundation conference award to support training and innovation in RNA science at the 2021 RNA society meetings in May of 2021. I was instrumental in the initial design and development of the tRNA scaffold approach that our lab successfully employed to determine the molecular structures of several viral RNAs, including Dengue and Zika virus Stem-loop A RNA.

My previous research involved the structural and biochemical studies of viral proteins involved in replication and immune evasion of positive strand RNA viruses. This work provided me a strong background specifically in structural virology and viral biochemistry. I received extensive training at the cryo-electron microscopy laboratory at The University of Texas Medical Branch in cryo-EM sample preparation, handling and imaging. My ability to bridge the biological and technical aspects of RNA structure solution is an asset as it allows for the development of a collaborative pipeline for the structural studies of large viral RNA-protein complexes using a combination of scientific expertise in X-ray crystallography, solution biophysics and cryo-electron microscopy. To this end, my current project targets one of the extensively studied viral RNA-protein complexes, between the HIV-1 Rev Response Element (RRE) RNA and the HIV-1 Rev protein, for which structural data is still lacking. A reconstruction of the HIV RRE-Rev multimer unit that is critical for HIV replication would define possible therapeutic targets for HIV.

B. Positions, Scientific Appointments, and Honors

Positions and Employment

10/2014 - Present Research Scientist-I, Dept. of Biochemistry and Molecular Biology, UTMB, Galveston, TX

09/2012-10/2014 Postdoctoral researcher, Dept. of Biochemistry and Molecular Biology, Sealy Center for Structural Biology, UTMB, Galveston, TX

Professional Memberships

2021 – Present Member, RNA Society
2011-2015 Member, American Society for Virology
2007-08; 2011-12 Member, Biophysical Society

Awards

2021 : IHII Data Acquisition Award, UTMB ($20,000)
2021 : Annual RNA Society Meetings: NSF Conference Award ($100)
2014 : Poster award; Sealy Center for Structure Biology Symposium ($200)
2011-2013 : McLaughlin predoctoral fellowship for research in Infection and Immunity ($42,000)
2013 : American Society for Virology postdoctoral travel grant ($500)
2012 : American Society for Virology student travel grant ($500)
2011 : American Society for Virology student travel grant ($500)
2011 : Ann and John Hamilton Endowed Scholarship for Academic Excellence ($1000)

C. Contributions to Science

Publications

Gottipati K, McNeme, SC., White, MA., and Choi KH. A Unique A-C-U base triple stabilizes the H-shaped Architecture of Enterovirus Cloverleaf RNA. (Submitted)

Lee E, Bujalowski PJ, Teramoto T, **Gottipati K**, Scott SD, Padmanabhan R, Choi KH. Structures of flavivirus RNA promoters suggest two binding modes with NS5 polymerase. Nat Commun. 2021 May 5;12(1):2530. doi: 10.1038/s41467-021-22846-1. PMID: 33953197; PMCID: PMC8100141.

Gottipati K, Woodson M, Choi KH. Membrane binding and rearrangement by chikungunya virus capping enzyme nsP1. Virology. 2020 May; 544:31-41. doi: 10.1016/j.virol.2020.02.006. Epub 2020 Feb 24. PMID: 32174512; PMCID: PMC7103501.

Gottipati, K, Hothauzen, LMF, Ruggli, N, Choi, KH. **2016**. Pestivirus N^{pro} directly interacts with interferon regulatory factor 3 monomer and dimer. J. Virol90: 7740 –7747. doi:10.1128/JVI.00318-16. *('Spotlight' feature for the issue*)

Klema, V, Ye, M, Hindupur, A, Teramoto, T, **Gottipati, K**, Padmanabhan, R, Choi, KH. **2016**. Dengue virus nonstructural protein 5 (NS5) assembles into a dimer with a unique methyltransferase and polymerase interface. PLoS Pathog 12(2): e1005451. doi:10.1371/journal.ppat.1005451

Gottipati, K, Acholi, S, Ruggli, N, Choi, KH. **2014**. Autocatalytic activity and substrate specificity of the pestivirus N-terminal protease N(pro.). Virology 452-453, 303-309.

Gottipati, K, Ruggli, N, Gerber, M, Tratschin, JD, Benning, M, Bellamy, H, Choi, KH, **2013**. The structure of classical swine fever virus N(pro): a novel cysteine Autoprotease and zinc-binding protein involved in

subversion of type I interferon induction. PLoS Pathog 9(10): e1003704. doi:10.1371/journal.ppat.1003704

Szymanski, MR, Fiebach, AR, Tratschin, JD, Gut, M, Ramanujam, VM, **Gottipati, K,** Patel, P, Ye, M, Ruggli, N, Choi, KH. **2009**. Zinc binding in pestivirus N(pro) is required for interferon regulatory factor 3 interaction and degradation. J. of Mol. Biol. 391, 438-449.

Gottipati, K, Dodson, ML, Beasley WCD, Barrett, DTA, Lee JC. Dynamic Motions of Flaviviral Envelope Protein Domain-3 Modulate Their Interactions with Monoclonal Antibody: A potential Mechanism for Antibody Resistance (In preparation).

Abstracts

Oral Presentations

Gottipati K, McNeme, SC, Choi KH, A Unique A-C-U base triple stabilizes the H-shaped Architecture of Enterovirus Cloverleaf RNA, RNA2021: Annual Meeting of the RNA Society, Virtual, May 25-June 5, 2021.

Gottipati, K, Hothauzen, LMF, Ruggli, N, Choi, KH. Pestivirus N^{pro} directly interacts with interferon regulatory factor 3 (IRF3) monomer and dimer. 35^{th} Annual meeting of the American Society for Virology, Virginia Polytechnic Institute and State University, Blacksburg, Virginia, June 18-22, 2016

Gottipati, K, Spiedel, TJ, Acholi, S, Jay RK, Choi, KH, Biochemical Basis of Interaction of Pestivirus N-terminal Protease (Npro) with Interferon Regulatory factor-3 (IRF3). 32nd Annual meeting of the American Society for Virology, Penn State's University Park Campus, State College, Pennsylvania, July 20–24, 2013

Gottipati, K, Ruggli, N, Gerber, M, Tratschin, J, Bellamy, H, Choi, KH. Crystal Structure of Npro of Classical Swine Fever Virus.: Novel fold of a Viral Leader Protease. 31st annual meeting of the American Society for Virology, University of Wisconsin-Madison, Madison, Wisconsin. July 21-25, 2012

Gottipati, K, Fiebach, A, Ruggli, N, & Choi, KH. Crystal Structure of Npro: A Novel Cysteine Auto-protease of Classical Swine Fever Virus. 30th annual meeting of the American Society for Virology, University of Minnesota, Minneapolis, July 16-20, 2011

BIOGRAPHICAL SKETCH

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NAME: Choi, Kyung H.

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

POSITION TITLE: 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
Ewha Womans University, Seoul, Korea	B.S. summa cum laude	02/93	Chemistry
Boston University, Boston	M.S./Ph.D.	08/98	Biochem/Structural Biol.
Boston University School of Medicine, Boston	Post-doc	11/01	Structural Biology
Purdue University		12/06	Structural Biology

A. Personal Statement

The goal of this project is to determine the structure of human immunodeficiency virus (HIV) Rev response element (RRE) and exploit the structural information for the design of small molecule inhibitors. My educational and professional experiences are uniquely suited to carry out this project. For my Ph.D. (with Drs. Richard Laursen and Karen Allen) and a post-doctoral (with late Dr. Michael Rossmann, NAS) work, X-ray crystallography, cryo-electron microscopy (cryo-EM), and biochemistry were used to investigate problems in the mechanistic enzymology and structural virology. In my laboratory at the University of Texas Medical Branch (UTMB), I investigate the structure and mechanism of positive-strand RNA virus replication and infection machinery using biochemical and structural methods. We are interested in how viral RNA genome structure influences viral replication processes and how viral replication enzymes coordinate negative- and positive-strand RNA synthesis. Clearly, these processes are key steps in viral replication that can be targeted for antiviral development.

HIV uses the highly structured viral RNA, RRE to export viral RNA from the nucleus to the cytoplasm. RRE interacts with the viral protein, Rev, and forms the functional RRE-Rev complex containing 6-8 Rev proteins. However, neither the tertiary structure of RRE nor the quaternary architecture of the functional Rev-RRE multimeric complex are unknown. Structure determination of medium-sized (50-200 nt) RNAs is challenging due to large size and dynamic nature of RNAs. We thus have developed RNA scaffold approaches to determine viral RNA structures. In this proposal, we will use complementary approaches of RNA-scaffold and antibody-assisted crystallization, X-ray crystallography and cryo-EM to determine the RRE structure and identify small molecules that bind to RRE. Small molecules that bind structured essential viral RNA is a new frontier for antiviral development. Similar to antibiotics that bind ribosomal RNA, small molecules that bind viral RNA structures may provide a new avenue for antiviral therapeutics. My expertise in viral replication, years of experience in cutting-edge structural biology, and record of successful and productive research will enable me to lead the proposed project.

1. **Choi KH** (2021) The role of the stem-loop A RNA promoter in flavivirus replication. *Viruses* 13(6), 1107
2. Knyazhanskaya E, Morais MC, **Choi KH** (2021) Flavivirus enzymes and their inhibitors. *Enzymes* 49, 265-303
3. Klema VJ, Padmanabhan R, **Choi KH** (2015) Flaviviral Replication Complex: Coordination between RNA Synthesis and 5′-RNA Capping. *Viruses* 7, 4640-4656
4. **Choi, KH**. High-throughput crystallography (2010). International tables for crystallography vol. F: Crystallography of biological macromolecules. (Rossmann MG and Arnold E. ed), published for International Union of Crystallography. Kluwer academic publishers, Dordrecht/Boston/London

B. Positions, Scientific Appointments, and Honors

Positions and employment:

2020 -	Professor, Biochemistry and Molecular Biology, University of Texas Medical Branch, Galveston, TX
2013 - 2020	Associate Professor, Biochemistry and Molecular Biology, University of Texas Medical Branch, Galveston, TX (Tenured)
2007 - 2013	Assistant Professor, Biochemistry and Molecular Biology, University of Texas Medical Branch, Galveston, TX (Tenure Track)
2002 - 2006	Research Assistant Scientist, Purdue University, West Lafayette, IN (Mentor; Michael G. Rossmann, Ph.D., NAS)
1999 - 2001	Post-doctoral Research Associate, Boston University School of Medicine, Boston, MA (Mentor: Karen N. Allen, Ph.D.)

Professional Memberships:

2004 –	American Society of Virology
2016 –	American Society for Microbiology
2020 –	RNA society
1999 – 2002	Protein Society

Honors and Awards:

2020	Tramonte Faculty Award, UTMB
2011	AAMC Early Career Woman Faculty Professional Development Seminar travel award
2009	NIH Western regional center of excellence career development award
2008	7th ESVV Pestivirus Symposium Travel award
2001	American Crystallography Association Summer Course Tuition Scholarship
1993 – 1998	Boston University Teaching Fellowship
1993	Ewha Chemistry Award for graduating first in the class
1991 – 1992	Chemistry Alumni Award (Gold medal recipient) from Ewha Womans University for top 1% undergraduate
1990 – 1993	Ewha Womans University Honor Prize
1990	ELS (English Language Service) International Seoul Scholarship

Other Experience:

2018 – 2022	NIH/NIAID Scientific Review Panel, Virology A, member
2017	NIH/NIAID Scientific Review Panel, Virology A, ad hoc reviewer (February and October)
	NIH/NIAID Scientific Review Panel, Rapid assessment of Zika virus complications
2016	NIH/NIAID Scientific Review Panel, Infectious Diseases and Microbiology special emphasis panel, ad hoc reviewer
	NIH NIDCR Zika Review Panel
	NSF PBI grant reviewer
2015	NIH/NIAID Scientific Review Panel, Virology A, ad hoc reviewer (June & October)
2014	NIH/NIAID Scientific Review Panel, F13 fellowships, Infectious disease and microbiology (June & October)
	Reviewer, National Medical Research Council (NMRC), Ministry of Health, Singapore
2013 –	American Heart Association Peer Review Study Group
2013	NIH/NIAID Scientific Review Panel, Center for Influenza Research and Surveillance
	Reviewer, L'Agence nationale de la recherche (ANR) Programme Blanc, France
2012	Reviewer, Medical Research Council (MRC) research grant, United Kingdom.
2011	NIH/NIAID Scientific Review Panel, Biological Chemistry and Macromolecular Biophysics (BCMB) program

C. Contributions to Science

1. Coordination between RNA synthesis and 5’ RNA capping in flavivirus

Flavivirus genome replication requires both RNA synthesis and 5’ RNA capping. The viral polymerase

NS5 (nonstructural protein 5) consists of the N-terminal methyltransferase (MTase) involved in 5' RNA capping and the C-terminal RNA-dependent RNA polymerase (RdRp) involved in RNA synthesis. The physical link between MTase and RdRp within in a single NS5 protein suggests that RNA synthesis and 5' RNA capping is likely coupled, but the mechanism of coupling is not known. In collaboration with R. Padmanabhan (Georgetown University), we have determined the significance of NS5 domain-domain interactions and NS5-RNA interactions in dengue virus replication. We also determined the crystal structure of dengue virus NS5 dimer. The structure explained why mutations on the protein surface, which are not involved in the enzymatic activities, diminish viral replication. We proposed a structure-based mechanism for NS5, in which RNA synthesis and 5' RNA capping can occur in coordinated fashion across the NS5 dimer, i.e., RdRp of a NS5 molecule coordinates with MTase of the neighboring NS5 within the dimer.

a. Lee E, Bujalowski PJ, Teramoto T, Gottipati K, Scott SD, Padmanabhan R, **Choi KH** (2021) Structure of flavivirus RNA promoters suggest two binding modes with NS5 polymerase. *Nat Commun* 12, 2530

b. Klema VJ, Ye M, Hindupur A, Teramoto T, Gottipati K, Padmanabhan R and **Choi KH**. (2016) Dengue virus nonstructural protein 5 (NS5) assembles into a dimer with a unique methyltransferase and polymerase interface. *PLoS Pathogens*. 12(2), e1005451

c. Bujalowski PJ, Bujalowski W, **Choi KH**. (2017) Interactions between the Dengue Virus Polymerase NS5 and Stem-Loop A. *J Virol*. 91(11). pii: e00047-17.

d. Bujalowski PJ, Bujalowski W, **Choi KH**. (2020) Identification of the viral RNA promoter stem-loop A (SLA)-binding site on Zika virus polymerase NS5. *Sci Rep*. 10:13306

2. Mechanism of positive-strand RNA virus replication

Positive strand virus RNA-dependent RNA polymerases initiate RNA synthesis either via protein-primed or *de novo* (i.e., primer-independent) mechanism. Enterovirus polymerase 3D uses a protein (VPg)-primed RNA synthesis, while pestivirus and alphavirus use a *de novo* mechanism. We have been investigating these virus-specific RNA synthesis mechanisms and exploring them as targets of antiviral inhibitor development. For example, the X-ray crystal structures of a pestivirus polymerase trapped in various catalytic states led us to propose a *de novo* RNA initiation mechanism that is now widely accepted for *Flaviviridae* polymerases. We have recently determined the cloverleaf RNA structure that functions as an RNA primer for negative strand RNA synthesis (submitted). We believe our new structure will help understand how enteroviruses use viral RNA to switch from viral translation to replication and initiate RNA synthesis.

a. Schein CH, Rowold D, **Choi KH**. (2016) Allosteric inhibitors of Coxsackie virus A24 RNA polymerase. Bioorg Med Chem. 2016 Feb 15;24(4):570-7.

b. Gottipati K, Woodson M, **Choi KH** (2020) Membrane binding and rearrangement by chikungunya virus capping enzyme nsP1. *Virology*. 544:31-41.

c. **Choi KH** and Rossmann MG (2009) RNA-dependent RNA polymerases from *Flaviviridae*. *Curr. Opin. Struct .Biol*.

a. **Choi KH**, Groarke JM, Young DC, Kuhn RJ, Smith JL, Pevear DC, Rossmann MG. (2004) The structure of the RNA-dependent RNA polymerase from bovine viral diarrhea virus establishes the role of GTP in *de novo* initiation. *Proc Natl Acad Sci U S A*. 101, 4425-30. PMCID: PMC38476

3. Virus and capsid assembly

We use cryo-electron microscopy (cryo-EM) image reconstruction to study virus and capsid assembly. The cryo-EM structures of bacteriophages N4 and ϕ29 allowed us to identify positions of individual virion proteins (such as virion polymerase, the major capsid protein, a decorating protein, and a tail appendage protein) in combination with genetic manipulation. In particular, the locations of N4 virion proteins could not be assigned prior to our efforts. N4 packages a virally encoded RNA polymerase into its capsid along with the viral genome. The structures further allowed us to propose how the encapsidated virion polymerase could be transferred from inside of the capsid into the cell upon infection. Using our knowledge of virus structure and assembly, we are currently developing methods to determine the structure of medium-sized RNA (< 200 nt), which otherwise preclude any cryo-EM studies.

a. Xia H, Xie X, Zou J, Noble CG, Russell WK, Holthauzen LMF, **Choi KH**, White MA, Shi PY (2020) A cocrystal structure of dengue capsid protein in complex of inhibitor. *Proc Natl Acad Sci U S A*. 117(30):17992-18001

b. Morais MC, **Choi KH**, Koti JS, Chipman PR, Anderson DL and Rossmann MG. (2005) Conservation of the capsid structure in tailed dsDNA bacteriophages: the pseudoatomic structure of phi29. *Mol Cell.* **18**(2): 149-59. (Cover illustration of Molecular Cell).

c. **Choi KH**, Morais MC, Anderson DL, Rossmann MG. (2006) Determinants of bacteriophage ø29 head morphology. *Structure* **14**, 1723-7

d. **Choi KH**, McPartland J, Kaganman I, Bowman VD, Rothman-Denes LB, Rossmann MG (2008) Insight into DNA and protein transport in double-stranded DNA viruses: The structure of bacteriophage N4. *J. Mol. Biol.* 378, 726-36.

4. Subversion of innate immune response by viral antagonists

Most viruses subvert host's innate immune response to establish viral infection. However, how viral proteins mediate such functions are not well understood. Pestivirus N^{pro} blocks interferon induction by targeting interferon regulatory factor 3 (IRF3, a transcription factor for interferon-α/β genes) for proteasomal degradation. N^{pro} self-activates using its autocatalytic protease activity and then induces degradation of IRF3 in the proteasome. We have determined the crystal structure of N^{pro}, and identified key features in N^{pro} and IRF3 that are required for N^{pro}-mediated IRF3 degradation in collaboration with Nicolas Ruggli (IHII, Switzerland). The unique structure of N^{pro} suggests the mechanism of autocatalysis at its C-terminus and subsequent auto-inhibition, and provides insight regarding potential interaction sites with IRF3.

a. Gottipati K, Ruggli N, Gerber M, Tratschin J-D, Benning M, Bellamy H, **Choi KH** (2013) The structure of classical swine fever virus N^{pro}: a novel cysteine autoprotease and zinc-binding protein involved subversion of Type I interferon induction. *PLOS Pathogens* 9, e1003704.

b. Gottipati K, Holthauzen LM, Ruggli N, **Choi KH** (2016). Pestivirus Npro directly interacts with interferon regulatory factor 3 monomer and dimer. J. Virol. 90, 7740-7

c. Szymanski MR, Fiebach AR, Tratschin J-D, Gut M, Ramanujam VMS, Patel P, Ye M, Ruggli N, **Choi KH** (2009) Zinc-binding in pestivirus N^{pro} is required for interferon regulatory factor 3 (IRF3) interaction and degradation. *J. Mol. Biol.* 391, 438-49. PMCID: 19540847

d. Ruggli N, Summerfield A. Fiebach AR, Guzylack L, Bauhofer O, Lamm CG, Waltersperger S, Matsuno K, Liu L, Gerber M, **Choi KH**, Hofmann MA, Sakoda Y, Tratschin JD. (2009) Classical swine fever virus can remain virulent after specific elimination of the interferon regulatory factor 3 degrading function of N^{pro}. *J. Virol.* 83, 817-29.

5. Mechanistic enzymology

My earlier work was focused in the mechanistic enzymology of cysteine proteases and fructose 1,6-bisphosphate aldolase (FBA) using X-ray crystallography, mass spectrometry, and enzyme kinetics. In particular, FBA catalyzes the cleavage of fructose 1,6-bisphosphate through a covalent Schiff base intermediate, but assigning catalytic roles to the various enzyme active site residues were difficult due to lack of a structure for enzyme-substrate complex. I determined the crystal structures of native, covalent intermediate, and product-bound forms of FBA, representing snapshots of catalysis. This work provided detailed reaction mechanism for covalent Schiff base intermediate.

a. **Choi KH**, Mazurkie AS, Morris AJ, Utheza D, Tolan DR, Allen KN. (1999) Structure of a fructose-1,6-bis(phosphate) aldolase liganded to its natural substrate in a cleavage-defective mutant at 2.3 Å. *Biochemistry*. **38**, 12655-64.

b. **Choi KH**, Shi J, Hopkins CE, Tolan DR, Allen KN. (2001) Snapshots of catalysis: the structure of fructose-1,6-(bis)phosphate aldolase covalently bound to the substrate dihydroxyacetone phosphate. *Biochemistry*. **40**, 13868-75.

c. **Choi KH**, Tolan DR. (2004) Presteady-state kinetic evidence for a ring-opening activity in fructose-1,6-(bis)phosphate aldolase. *J Am Chem Soc*. **126**, 3402-3.

d. **Choi KH**, Laursen RA, Allen KN. (1999) The 2.1 Å structure of a cysteine protease with proline specificity from ginger rhizome, *Zingiber officinale*. *Biochemistry*. **38**, 11624-33.https://www.ncbi.nlm.nih.gov/myncbi/kyung.choi.1/bibliography/public/