Madhumati (Madhu) Sevvana, Ph.D.

Structural Immunologist and Virologist

Assistant Research Scientist
Hockmeyer Hall of Structural Biology
Department of Biological Sciences
Purdue University, 240 S. Martin Jischke Dr.
West Lafayette, IN 47907
Email: **msevvana@purdue.edu**

EDUCATION

PhD, Protein crystallography 04/2003 - 06/2006
George-August University, Germany
Faculty mentors: George M. Sheldrick and Isabel Uson

M. Sc, Molecular biology 09/2001 - 03/2003
Max Planck Research School, Germany
Faculty mentors: George M. Sheldrick and Isabel Uson

M. Sc, Biochemistry 06/1999 - 03/2001
University of Hyderabad, India
Faculty mentor: Sivakumar Nadimpalli

B. Sc, Chemistry, Biochemistry, Biotechnology 07/1996 - 05/1999
Andhra University, India

RESEARCH EXPERIENCE

Research scientist 06/2019 – current
The Kuhn and the Rossmann Labs, Purdue University, USA
Postdoctoral research associate 07/2016 – 05/2019
The Rossmann Lab, Purdue University, USA
Faculty mentors: **Richard J. Kuhn, Michael G. Rossmann**
Number of publications/MS submitted/preparation*:* **8/3/2**
Research areas*:* Zika virus neutralization mechanism by antibodies;[1-3,5-7,36-38]
Structure guided HCV vaccine design;[35]
Anti-virals against chikungunya virus and alphaviruses;
Capsid assembly in Pacmanvirus, a 250 nm giant virus;[8,31,39]
Near-atomic resolution maps using cryo electron microscopy;[6,39]
Nuclear egress in HCMV and structure-based inhibitor design;

Senior scientist 12/2010 - 06/2016
The Muller Lab, FAU Erlangen-Nuernberg, Germany
Faculty mentors: **Yves Muller, Manfred Marschall**
Number of publications*:* **5**
Research areas*:* Nitrogen metabolism in Corynebacteria;[9,16]
HCMV infection mechanism;[10-12] Crystallographic computing;

Postdoctoral research associate 12/2006 - 10/2010
The Muller Lab, FAU Erlangen-Nuernberg, Germany
Faculty mentors: **Yves Muller, Bjoern Dahlbaeck** (Lund university)
Number of publications: **6**
Research areas: Human apolipoprotein M in Atherosclerosis;[14,15,17]
Molecular mechanisms of antibiotic resistance;[13,18,22]

Graduate student 10/2002 - 11/2006
The Sheldrick Lab, George-August University, Germany
Faculty mentor: **George M. Sheldrick, Isabel Uson**
Number of publications: **13**

Research areas: Crystallographic analysis of twinned crystals;[4]
Transmembrane signalling in sensor kinases;[21,32]
Substrate binding in PDI-related chaperones;[19,23,27]
Crystallographic methods development;[20]
Structures of therapeutic natural compounds; [24-26,28,29,30]

#publications from each research area as superscript (please see list of publications)

KEYWORDS/AREAS OF INTEREST

Structural virology; Structural immunology; virology; host-pathogen interactions; anti-viral therapeutics; antibody therapeutics; structure-based protein/vaccine design; cryo-EM; cryo-ET; X-ray crystallography; methods development; Molecular modeling;

SCHOLARSHIPS/AWARDS

Fakultaetsfrauenpreis 10/2009 - 10/2010
(Women in science academic award with research funding)
FAU Erlangen-Nuernberg, Germany
Fellowship (Funding for research on antibiotic resistance to YM) 12/2006 - 09/2009
Volkswagen-Stiftung, Germany
Scholarship (Among 20 students of 500 world wide applicants) 09/2001 - 08/2002
Max Planck Research School, Germany
Merit Scholarship (University topper for two consecutive years) 06/1999 - 03/2001
University of Hyderabad, India

TEACHING AND RESEARCH MENTORING EXPERIENCE

Institute of Biotechnology **2006 – /2016**
FAU Erlangen-Nuernberg, Germany
(class size of 45 undergraduate/graduate students)

Molecular Science, Integrated life sciences students
Course design, Lectures, Lab: "Structural biology and crystallography"
Course design and Lectures: "Crystal structure analysis"

Graduate students
Course design and advanced lectures on
"Protein crystallography"

Institute of Structural Chemistry, *George-August University and International Max-Planck Research School, Germany* **2003 – 2006**
(class size of 10-60 undergraduate/graduate students)

Teaching Assistant (Tutor for lectures)
"Protein crystallography" and "Structural chemistry"

Institute of Biotechnology **2003 - 2016**
FAU Erlangen-Nuernberg, Germany
Research mentoring**:** 14 graduate students (M.Sc and Phd)

The Kuhn lab, Purdue Unviersity, Indiana, USA **2019 - current**
Research mentoring: Graduate students, lab managers, and colleagues

Certificate in foundations for college teaching at *Purdue University* **2019**

PROFESSIONAL SERVICE AND OUTREACH

1. Special issue guest editor for Pathogens: *"Molecular Mechanisms of Immune Response to Viral Infections"* 2020-2021
2. Reviewer for journals: Virology; Pathogens; Biomolecules Diagnostics; Vaccines; Antibiotics; Viruses; PLOS Pathogens; 2019-current
3. Reviewer for Indiana CTSI Postdoc challenge 2019, 2020, 2021
4. Judge for *Purdue University* UG Research conference 2019, 2020
5. Research mentor for high school and undergraduate students (*India, Germany and USA*) 2014-current

Scientific communication events for diverse communities
Organizer, group lead for the following events:

- Long night of science in Nuernberg metropolitan area 2009- 2013
- STEM events at Waldorf Montessori Middle School 2014
- Indo-German international womens club, Germany 2010-2016

Topics: *Structural biology;*
Structure-guided drug discovery; 3D modeling;

STEM events for middle school and high school students 2009-2014
Organizer and team lead
Girls day and Boys day, FAU Erlangen-Nuernberg, Germany
Topics: *Scientific method; Experimentation; 3D modeling;*
Structural biology; Drug discovery;

Parenting in Academia conference, Purdue University, USA 2021
Panelist for "Postdocing with parenting session"

Cambridge Science Festival, Virtual event, MA, USA 2021
Panelist on behalf of "The Social Scientist" for
1. Science education for kids
2. Science communication

ACADEMIC LEADERSHIP ROLES

1. **Science and Research Opportunities in India** 2021
   Volunteer
- Co-ordinate academic outreach in Europe
- STEM engagement via webinars and interviews

2. **Mentor at "The Social Scientist"**
- Co-ordinate science communication outreach 2021
- Online one-on-one mentorship in STEM

3. **Inventory and Integrations management** 2020
   Purdue university, USA
- Kuhn/Rossmann lab integration
- Streamline, track and manage lab inventory

4. **Collaborative research center coordinator** (SFB796) 2009-2014
   FAU Erlangen-Nuernberg, Germany
- SFB796 on mechanisms of host-pathogen interactions
- managed and coordinated structural-biology projects

5. **Computational resource management** 2007-2014
   FAU Erlangen-Nuernberg, Germany
- crystallographic software infrastructure and management
- group/user management and administration

6. ***In house*** **and synchrotron crystallography team lead** 2007-2014
   FAU Erlangen-Nuernberg, Germany
- Research mentoring undergraduate and graduate students
- Overseeing data processing and curation
- Organizing and leading synchrotron data collection

- User training in crystallographic data processing

PUBLICATIONS

1. **Sevvana, M.,** Rogers, TR., Miller, AS., Long, F., Beutler, N., Lai, YC., Parren, M., Walker, LM., Buda, G., Klose, T., Burton, DR., Rossmann, MG., Kuhn, R. J. *Structural perspectives on Zika virus specific neutralization in subsequent flavivirus infections.* (2020). Viruses. "*In memory of Michael Rossmann*"
2. Nicholls, CMR, **Sevvana, M** and Kuhn, RJ. *Structure-guided paradigm shifts in flavivirus assembly and maturation mechanisms.* (2020). AVR. doi: 10.1016/bs.aivir.2020.08.003
3. **Sevvana, M.** and Kuhn. R. J. *The diverse structural landscape of antibody repertoire against flaviviruses.* (2020). Current Opinions in Virology. https://doi.org/10.1016/j.coviro.2020.07.006
4. **Sevvana, M.,** Ruf, M., Uson, I., Sheldrick, GM., Herbst-Irmer, R. *Non-merohedral Twinning: from Minerals to Proteins.* (2019). Acta Cryst D. 75(Pt 12): 1040–1050.

- One of the most read articles as acclaimed by Acta D.
- http://5.196.90.133/d/services/mostread.html
- Methods development paper

5. Long, F., Doyle, M., Fernandez, E., Miller, AS., Klose, T., **Sevvana, M.,** Bryan, A., Davidson, E., Doranz, BJ., Kuhn, RJ., Diamond, MS., Crowe, JE Jr., Rossmann, MG. *Structural basis of a potent human monoclonal antibody against Zika virus targeting a quaternary epitope.* (2019). Proc Natl Acad Sci U S A. 116(5):1591-1596.
6. **Sevvana, M.,** Long, F., Miller, AS., Klose, T., Buda, G., Sun, L., Kuhn, RJ., Rossmann, MG. *Refinement and Analysis of the Mature Zika Virus Cryo-EM Structure at 3.1 Å Resolution.* (2018). Structure. 26(9):1169-1177.

- https://www.purdue.edu/newsroom/releases/2018/Q4/2018s-top-10-research-news-stories-from-purdue.html
- https://mobile.nytimes.com/2018/06/26/health/zika-virus-image.html
- https://www.pbs.org/newshour/science/what-happened-to-zika

7. Hasan, SS., **Sevvana, M.,** Kuhn, RJ., Rossmann, MG. *Structural biology of Zika virus and other flaviviruses.* (2018). Nat Struct Mol Biol. 25(1):13-20.
8. Andreani, J., Khalil, JYB., **Sevvana, M.,** Benamar, S., Di Pinto, F., Bitam, I., Colson, P., Klose, T., Rossmann, MG., Raoult, D., La Scola, B. *Pacmanvirus, a New Giant Icosahedral Virus at the Crossroads between Asfarviridae and Faustoviruses.* (2017). J Virol. 91(14).

- 'Spotlight' section of Journal of Virology highlighted my paper

9. **Sevvana, M.,** Hasselt, K., Grau, FC., Burkovski, A., Muller, YA. *Similarities in the structure of the transcriptional repressor AmtR in two different space groups suggest a model for the interaction with GlnK.* (2017). Acta Crystallogr F Struct Biol Commun. 73(Pt 3):146-151.
10. Walzer, SA., Egerer-Siebe,r C., Sticht, H., **Sevvana, M.,** Hohl, K., Milbradt, J., Muller, YA., Marschall, M. *Crystal Structure of the Human Cytomegalovirus pUL50-pUL53 Core Nuclear Egress Complex Provides Insight into a Unique Assembly Scaffold for Virus-Host Protein Interactions.*(2015). J Biol Chem. 290(46):27452-8.

- Paper of the week by JBC
- Lead the project for two years and played crucial role in deciphering the structure

11. Scherer, M., Klingl, S., **Sevvana, M.,** Otto, V., Schilling, EM., Stump, JD., Müller, R., Reuter, N., Sticht, H., Muller, YA., Stamminger, T. *Crystal structure of cytomegalovirus IE1 protein reveals targeting of TRIM family member PML via coiled-coil interactions.* (2014). PLoS Pathog. 10 (11):e1004512. doi: 10.1371/journal.ppat.1004512.
12. Milbradt, J., Auerochs, S., **Sevvana, M.,** Muller, YA., Sticht, H., Marschall M. *Specific residues of a conserved domain in the N-terminus of the human cytomegalovirus pUL50 protein determine its intranuclear interaction with pUL53.* (2012). J Biol Chem. 287(28):24004-16.
13. **Sevvana, M.,** Goetz, C., Goeke, D., Wimmer, C., Berens, C., Hillen, W., Muller YA. *An exclusive α/β code directs allostery in TetR-peptide complexes.* (2012). J Mol Biol. 416(1), 46-56.
14. Christofferson, C., Obinata, H., Kumaraswamy, SB., Galvani, S., Ahnström, J., **Sevvana, M.,** Egerer- Sieber, C., Muller, YA., Hla, T., Nielsen, LB., Dahlbäck, B. *Endothelium-protective sphingosine 1- phosphate provided by HDL-associated apolipoprotein M.* (2011). Proc Natl Acad Sci U S A. 108(23), 9613-8.
15. **Sevvana, M.,** Kassler, K., Ahnström, J., Weiler, S., Dahlbäck, B., Sticht, H and Muller, YA. *Mouse apoM displays an unprecedented 7-stranded lipocalin fold - Folding decoy or alternative native fold?* (2010). J Mol Biol. 404(3), 363-71.
16. Hasselt, K., **Sevvana, M.,** Burkovski, A., Muller, YA. *Crystallization and preliminary crystallographic analysis of the global nitrogen regulator AmtR from Corynebacterium glutamicum.* (2009). Acta Cryst. F65, 1123-1127.
17. **Sevvana, M.,** Ahnström, J., Egerer-Sieber, C., Dahlbäck, B and Muller, YA. *Serendipitous fatty acid binding reveals the structural determinants for ligand recognition in apoM.* (2009). J Mol Biol. 393(4), 920-36.
18. Resch, M., Roth, HM., Kottmair, M., **Sevvana, M.,** Bertram, R., Titgemeyer, F., Muller, YA. *Cloning, expression, purification, crystallization and preliminary X-ray diffraction analysis of YvoA from Bacillus subtilis.* (2009). Acta Cryst. F65, 410-414.
19. Barak, NN., Neumann, P., **Sevvana, M.,** Schutkowski, M., Naumann, K., Malesević, M., Reichardt, H., Fischer, G., Stubbs, MT, Ferrari DM. *Crystal structure and functional analysis of the protein disulfide isomerase-related protein ERp29.* (2009). J Mol Biol. 385(5), 1630-42.
20. Pal, A., Debreczeni, J. É., **Sevvana, M.,** Grüne, T., Kahle, B., Zeeck, A. & Sheldrick, G. M. *Structures of viscotoxins A1 and B2 from European mistletoe solved using native data alone.* (2008). Acta Cryst. D64, 985-992.

- Methods development.

21. **Sevvana, M.,** Vijayan, V., Zweckstetter, M., Reinelt, S., Madden, DR., Herbst-Irmer, R., Sheldrick, GM., Bott, M., Griesinger, C., Becker, S. *A ligand-induced switch in the periplasmic domain of sensor histidine kinase CitA.* (2008). J Mol Biol. 377(2), 512-23.
22. Resch, M., Striegl, H., Henssler, EM., **Sevvana, M.,** Egerer-Sieber, C., Schiltz, E., Hillen, W., Muller, YA. *A protein functional leap: how a single mutation reverses the function of the transcription regulator TetR.* (2008). Nucleic Acids Res. 36 (13), 4390-401.
23. **Sevvana, M.,** Biadene, M., Ma, Q., Guo, C., Söling, H. D., Sheldrick, G, M and Ferrari, D. M. *Structural elucidation of the PDI-related chaperone Wind with the help of mutants.* (2006). Acta Cryst. D62, 589- 594.
24. Maskey, R. P., Serge, F., **Sevvana, M.,** Uson, I., Grun-Wollny, I and Laatsch H. *Kettapeptin: Isolation, Structure elucidation and Activity of a new Hexadepsipeptide Antibiotic from a Terrestrial Streptomyces sp.* (2006). J Antibiot (Tokyo). 59(5), 309-14.

25. Jin, Y., Serge, F., Yongtang, Z., **Sevvana, M.,** Laatsch, H and Zhang, W. *Halichondria sulfonic acid, a new HIV-1 inhibitory guanidino-sulfonic acid, and halistanol sulfate isolated from the marine sponge Halichondria rugosa Ridley & Dendy*. (2006) Nat Prod Res. 20(12), 1125-39.
26. Dalai, S., Limbach, M., Zhao, L., Tamm, M., **Sevvana, M.,** Sokolov, V and de Meijere, A. *Highly Diastereoselective Sequential Michael-Aldol reactions of Methyl 2-Chloro-2-cyclopropylideneacetate with Grignard Reagents and Aldehydes*. (2006). Synthesis. 471-479.
27. Barnewitz, K., Guo, C., **Sevvana, M.,** Ma, Q., Sheldrick, G. M., Söling, H. D and Ferrari, D. M. *Mapping of a substrate binding site in the Protein disulfide isomerase-related chaperone Wind based on protein function and crystal structure*. (2004). J Biol Chem. 279(38), 39829-39837.
28. Than, N. N., Serge, F., **Sevvana, M.,** Sheldrick, G. M., Fiebig, H. H., Kelter, G and Laatsch, H., *Sesquiterpene Lactones from Elephantopus Scaber*. (2004). Z. Naturforsch. 60(b), 200-204.
29. Maskey, R. P., **Sevvana, M.,** Uson, I., Helmke, E and Laatsch, H. *Gutingimycin: a highly complex metabolite from marine Streptomycete*. (2004). Angew Chem Int Ed Engl. 43(10), 1281-1283.
30. Magyar, A., Wolfling, J., Kubas, M., Cuesta-Seijo, J. A., **Sevvana, M.,** Herbst-Irmer, R., Forgo, P and Schneider, G. *Synthesis of novel steroid-tetrahydroquinoline hybrid molecules and D-homosteroids by intramolecular cyclization reactions*. (2004). Steroids. 69(5), 301-12.

BOOK CHAPTERS/THESIS

31. **Sevvana, M.,** Klose, T and Rossmann, M. G. (2020) *Principles of virus structure*. Encyclopedia of Virology, 4th Edition, Elsevier Inc.
32. Kneuper, H., Scheu, P., Etzkorn, M., **Sevvana, M.,** Duennwald, P., Becker, S., Baldus, M., Griesinger, C., Unden, G. (2010) *Sensing ligands by periplasmic sensing histidine kinases with sensory PAS domains. In: Sensory Mechanisms in Bacteria*. S. Spiro, R. Dixon, eds. Horizon Press.
33. **PhD thesis:** *Crystallographic Analysis of Pathological Crystals, Periplasmic Domain of Ligand Free CitA Sensor Kinase and PDI-related Chaperones*. https://ediss.uni-goettingen.de/handle/11858/00-1735- 0000-0006-AC86-6
34. **Masters thesis:** *Crystal structure analysis of biologically active natural products*.

M/S SUBMITTED/IN PREPARATION/ACCEPTED

35. **Sevvana, M.,** Foung, S. F. H., Kuhn, R. J. Molecular Atlas of Humoral Immune Evasion Mechanisms by Hepatitis C Virus. Accepted at Current Opinions in Virology.
36. Dowd, K. A., Sirohi, D., Speer, S., Mukherjee, S., Govero, J., Aleshnick, M., Larman, B., Sukupolvi-Petty, S., **Sevvana, M.,** Miller, A. S., Klose, T., Zheng, A., Kielian, M., Kuhn, R. J., Diamond, M. S. and Pierson, T. C. prM-reactive Antibodies Reveal a Role for Partially Mature Virions in Dengue Virus Pathogenesis. To be submitted to Cell Host and Microbe in February 2021.
37. Lai, YC., Long, F., Shrihari, S., Ricketts, J., **Sevvana, M.,** Whitener, B., Beutler, N., Miller, A. S., Thackray, L., Parren, M., Klose, T., Diamond, M. S., Kuhn, R. J. and Roger, T. Identification

of a novel broadly neutralizing class of Zika virus Domain I monoclonal antibodies. To be submitted to Nature Immunology (February, 2021).

38. Miller, AS., **Sevvana, M.,** Klose, T., Rossmann, MG., Kuhn, R. J. Cryo-EM structure of Yellow fever virus vaccine strain. Submitted to Viruses "*In memory of Michael Rossmann*" in February 2021.
39. **Sevvana, M.,** Andreani, J., Fang, Q., Klose, T., La Scola, B., Rossmann, MG. *The 2.8 Å Resolution Cryo-EM structure of Pacmanvirus, a 250 nm Giant Virus and Perspectives on Capsid Assembly Mechanism*. (2020). M/S in preparation.

ABSTRACTS

SELECTED POSTER PRESENTATIONS

40. Sevvana, M., et al., *Structures of Mature Zika Virus and its Complex with a Potent Virus-Specific Antibody*. Purdue Cryo-EM symposium, Purdue Univeristy, 2018.
41. Sevvana, M., et al., *Refinement and analysis of Zika virus structure at 3.1A resolution*. 6th Pan-American Dengue Research Network Meeting, Galveston, TX, USA, 2018.
42. Marschall, M., Sticht, H., Sonntag, E., Walzer, S., Sevvana, M., et al., Egerer-Sieber, C., Socher, E., Lamm, C., Sonnewald, U., Bahsi, H., Steingruber, M., Hutterer, C., Lischka, P., Muller, Y., Milbradt, J. *Novel clues in anti-cytomegalovirus drug targeting based on phosphorylation-triggered nuclear host cell egress*. Congress congenital CMV, ECCI 2016.
43. Sevvana, M., Goeke, D., Goetz, C., Gruess, F., Hillen, W., Muller, Y. *Novel agonists and antagonists question molecular recognition and information transmission in TetR*. European Crystallography Meeting at Darmstadt, Germany, 2010.
44. Sevvana, M., Muller, YA. *Unravelling Information Transmission Pathways in Tetracycline Repressor*. DGK Meeting, Erlangen, Germany, 2008.
45. Sevvana, M., Ma, Q., Barnewitz, K., Guo, C., Söling, H. -D., Ferrari, D. M and Sheldrick, G. M. *Structural and functional analysis of PDI-related proteins*. (2005). Acta Cryst . A61, C250. Poster at XX Congress of the International Union of Crystallography, Florence, 2005.
46. Dix, I., Sevvana, M., Bunkoczi, G., Debreczeni, J and Sheldrick, G. M. *The EU BIOXHIT standard test crystal*. (2005). Acta Cryst . A61, C147. XX Congress of the International Union of Crystallography, Florence, 2005.
47. Sevvana, M., Herbst-Irmer, R and Sheldrick, G. M. *Solving non-merohedrally twinned crystals using single wavelength anomalous scattering*. 37th course of International school of Crystallography at Erice, Sicily, 2005.

SELECTED TALKS

1. Sevvana M. *The quaternary epitope landscape of Zika virus neutralizing antibodies. Insights for structure-based therapeutics and vaccine design*. M&M, 2021.
2. Sevvana M. *The quaternary epitope landscape of Zika virus neutralizing antibodies*. ASV, 2021.
3. Sevvana M. *The quaternary epitope landscape of antibody response against Zika virus*. NIH Future Research Leaders Conference, 2021.
4. Sevvana M. *The diverse structural landscape of the flavivirus antibody repertoire*. Tackling Global Viral Epidemics. WSV, 2021.
5. Sevvana M. *Zika virus specific neutralization in subsequent flavivirus infections*. Purdue SBBC inaugural symposium, Purdue University, 2021.
6. Sevvana M. *Mapping the diverse landscape of Flavivirus Antibody Repertoire for structure-based vaccine design*. Invited virtual talk at Friedrich Alexander-University, Erlangen-Nuernberg, 2020.

7. Sevvana M. *ZIKV-Fab complexes with asymmetric and partial occupancy: Single particle Cryo-EM dat processing challenges*. S2C2 cryoEM Image processing workshop, 2020.
8. Sevvana, M. *3D structures and human diseases. A high resolution picture of underlying pathological and molecular mechanisms*. Virology institute, FAU Erlangen-Nuernberg, 2015.
9. Sevvana, M. *Structures of human and mouse apoM - serendipitous fatty acid in the binding pocket and refolding gone wrong*. 12th HEC-Biocrystallography meeting, Czech Republic, 2009.
10. Sevvana, M. *An orthogonal and not inverse mechanism is responsible for the phenotype switch in reverse TetR*. 10th HEC-Biocrystallography meeting, Poland, 2007.
11. Sevvana, M. *Data Processing, Structure-Solution and Refinement of non-merohedrally twinned macromolecular crystals*. HEC-Biocrystallography meeting, Teistungenburg, Germany, 2006.
12. Sevvana, M. *Examples of non-merohedral twins*. SHELX/MAXINF Workshop, Göttingen, Germany, 2006.
13. Sevvana, M. *Towards a novel covalent DNA-Intercalator from the structure of antimetabolite, Gutingimycin*. 6th HEC-Biocrystallography meeting at Wittenberg, Germany, Sep 2003.
14. Sevvana, M., Herbst-Irmer, R and Sheldrick, G. M. *Solving non-merohedrally twinned crystals using single wavelength anomalous scattering*. 37th course of International school of Crystallography at Erice, Sicily, 2005.

CURRENT RESEARCH

(1) Structures of Zika virus and its complexes with neutralizing antibodies

Richard Kuhn (late Michael Rossmann), Dennis Burton, James Crowe

***Aims and Results*:** High resolution cryo-EM structures of Zika virus (ZIKV) and its complexes with antibodies to decipher the structural components of ZIKV infection mechanism and its neutralization. Using structural data, we mapped the epitopes of diverse human monoclonal antibodies against ZIKV. This data is being used for structure based design of vaccines and therapeutic antibodies. *(See CV/Publications: 1, 2, 3, 5, 6, 7, 36, 37, 38)*

Approach: Vero-furin cells to prepare homogenous virus samples; Single particle Cryo-EM; We are optimizing methods, for gaining structural information using Cryo-ET for asymmetrically bound Fabs/ligands; Preparation of some of the Fab-virus complexes causes aggregation. Therefore, we are working on creating affinity grids to minimize/eliminate aggregation.

(2) Near atomic resolution structure of Pacmanvirus, a 250 nm dsDNA giant virus

Richard Kuhn (late Michael Rossmann), Thomas Klose, Bernard La Scola

***Aims and Results*:** High resolution structure of Pacmanvirus to identify major and minor capsid proteins and understand capsid assembly in giant viruses. Single particle cryo-EM produced an icosahedrally averaged map at a resolution of 4 Å. The map could be further improved to a resolution of 2.8 Å using localized 3D reconstruction methods. *(See CV/Publications: 8, 39)*

Approach: Prepare homogenous virus samples using amoeba cultures; Single particle Cryo-EM to calculate an icosahedrally averaged map; Localized reconstruction to improve local resolution. Mass spectrometric analysis of structural proteins;

(3) Structure based design of a universal HCV vaccine

Richard Kuhn, Steven Foung, Michael Houghton

Aims: The overall objective of the proposed research is to determine the structure of HCV and the arrangement of the E1E2 heterodimers on the virus surface. We will also examine the structure of virus – Fab complexes to understand epitope organization and mechanisms of antibody-mediated neutralizations. Our preliminary data shows that the virus can be purified and optimization is under progeress. We are also expressing and purifying E1E2 heterodimers to obtain high resolution 3D structure of the complex, which can be used to interpret the Cryo-ET maps.

Approach and anticipated results: Structure-based design will be accomplished using an integrated platform of five complementary methods: (1) X-ray crystallography, (2) Cryo-electron microscopy (Cryo-EM), (3) Cryo-electron tomography (Cryo-ET), (4) Molecular modeling, and (5) Molecular virology, to decode E1E2 interaction networks with diverse neutralizing antibodies (nAbs) and receptor molecules. The gained structural information will be utilized to design novel antigens as part of a recently funded U19.