

**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: Carolyn M. Teschke

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

POSITION TITLE: Professor and Associate Department Head

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 Wisconsin-Eau Claire	BS	05/83	Chemistry
Washington State University	PhD	09/90	Biochemistry
Massachusetts Institute of Technology	Post-doc	08/94	Biochemistry/Biophysics

**A. Personal Statement**

The goal of my lab's work is to investigate how protein interactions support the high-fidelity assembly of an icosahedral virus. Specifically, we are determining how different domains in bacteriophage P22's coat protein are involved on the determination of size and morphology of the capsid. We are also working to understand how scaffolding protein, the protein catalyst for the assembly reaction, is involved in nucleation and elongation of procapsids. Scaffolding protein is also involved in the incorporation of the portal protein complex at a unique vertex in the capsid. The portal complex is involved in DNA packaging and genome delivery. We are working to understand the mechanisms by which all these proteins interact to achieve the proper active assembly product.

I have worked with P22 since I was a post-doc, so I have the extensive experience in the field. In addition, I have worked on protein folding, and protein interactions since I was a graduate student. For instance, we characterized the chaperone-independent folding of P22 coat protein, and how GroEL/S interacts and assists in the folding of variant coat proteins. My laboratory established that procapsid assembly is a reversible reaction. This discovery was significant because it allowed the first application of rigorous, thermodynamic analysis to a two-protein assembly system, when coat and scaffolding protein assemble into virus-like procapsid particles. Implementation of rigorous thermodynamic analysis led to generalizable knowledge about virus assembly. For example, we found that the individual subunit interactions of a procapsid are weak but because of the additive nature of the interactions, the association energy of procapsids is very large. We have established methods that allow us to screen for interesting mutants and through these we have found coat variants that assemble into particles with different morphologies, including helical arrays and petite heads. My lab played a major role in a collaborative effort to solve two pseudoatomic resolution models of coat protein, which were a boon to our research. More recently we completed a solution NMR structure of an inserted domain in P22 coat protein and determined it is crucial for folding and stability of coat protein, which led to investigation of the structure and function of I-domains in P22-like phages that have only 15-25% sequence identity to P22's coat protein. We are actively pursuing the process by which capsid nucleation occurs through a detailed understanding of the interactions of coat, scaffolding and portal proteins, and mRNA, as well as the mechanisms involved in DNA packaging. I have assembled an excellent team of collaborators, including cryoEM and NMR experts, with whom I have worked for many years.

Ongoing projects that I would like to highlight include:

R01GM076661

Teschke (PI)

09/23/2019 – 06/30/2023

Understanding the Protein: Protein Interactions Required for Virus Assembly

R21AI156838

Simon White (PI), Teschke (col), Michael Lynes (col)

12/1/2020 – 11/30/2022

Characterization of long-circulating phages isolated from in vivo mouse studies

R01AI149727 (no cost extension)

Miraim Braunstein (PI), Teschke (PI) subcontract

01/03/2020 – 12/31/2021

A Novel Protein Export Chaperone of Mycobacterium Tuberculosis

Defense Health Agency: funded, pending

Robin Bogner (CoPI), Teschke (CoPI), in consortium with Physical Sciences, Inc.

Stabilized, Freeze Dried Bacteriophage for use in Austere Environments

## **B. Positions, Scientific Appointments, and Honors**

### Positions and Employment

2022-2027 Department Head, Dept. of Molecular and Cell Biology, University of Connecticut

2021 fall semester, Interim Department Head for MCB, University of Connecticut

2016-2021, Associate Department Head for MCB, University of Connecticut

2008-present, Professor, Depts. of Molecular and Cell Biology, and Chemistry, University of Connecticut

2000-2008, Associate Professor, Dept. of Molecular and Cell Biology, University of Connecticut

1994-2000, Assistant Professor, Dept. of Molecular and Cell Biology, University of Connecticut

1991-1994, Post-doctoral Associate, MIT; Advisor: Dr. Jonathan A. King

1983-1990, Doctoral Graduate Student, Washington State University; Advisor: Dr. Linda L. Randall

### Additional Professional Education

HERS Bryn Mawr Summer Institute for Women in Higher Education Administration, Summer 2013

COACh training for career building workshops, Summer 2016

### Honors

Connecticut Academy of Science and Engineering, elected member 2022

Fulbright U.S. Scholar, University of York, 1/2022-5/2022

AAAS Council Delegate, Section on Biological Sciences, nationally elected, 2020-2023

Editorial Board, Journal of Virology, 2020-2023

Associate Editor, Science Advances, AAAS, 2018-

Committee on the Status of Women in Microbiology, American Society of Microbiology, 2018-2024

Alice C. Evans Award, American Society of Microbiology, 2018

Basic Science Awards Nominating Committee, American Society of Microbiology, 2016-2019

Jefferson Science Fellow, serving at the U.S. State Dept., 2015-16

Embassy Science Fellow, 2016

Fellow of the AAAS, elected, 2015

Co-organizer for FASEB summer conference on Virus Structure and Assembly, 2014

Co-vice organizer for FASEB summer conference on Virus Structure and Assembly, 2012

Co-organizer for the New England Structure Symposium, 2006, 2017

Nominating committee for the Protein Society, elected, 2005-2009

### Other Experience and Professional Memberships

Stage 2 Editorial Board reviewer for the NIH Director's New Innovator Award, 2022

AAAS Kavli Science Journalism Awards screener, 2020, 2021, 2022

NSF ad hoc reviewer, 2017, 2019, 2020

NIH Ad Hoc reviewer Prokaryotic Molecular and Cell Biology study section, 2008, 2009, 2010, 2016

Ad hoc reviewer for NIH F31 fellowship in Molecular Genetics, 2007

University of Kansas Cobre grants, 2007, 2016

Ad hoc Grant Reviewer for United States-Israel Binational Science Foundation, 1998, 2004, 2006

Memberships: ASM, ASV, ASBMB, AAAS

### **C. Contribution to Science**

We investigate the ability of viral proteins to assemble into complexes. Specifically, we study the assembly of viruses using bacteriophage P22 as a model. Our goal is to understand how viruses assemble, which will allow the design of inhibitors of the assembly process. My lab has also studied chaperoned protein. Below I describe in broad strokes five major projects my lab has worked on and the significant contributions arising from each:

#### **1. Viral capsid assembly—investigations of capsids:**

Assembly of a virus is a highly coordinated process involving sequential addition of multiple proteins, ultimately leading to an infectious virion. How do interactions between individual proteins support assembly of a whole virus? Our work addresses how viruses assemble precisely into the proper size and shape, given their capsid proteins are pliable by design. We use bacteriophage P22 as a model dsDNA virus. In bacteriophage P22, herpesviruses, adenoviruses, and many other dsDNA viruses, the initial product of assembly is a precursor capsid (the protein shell of a virus) known as the procapsid, which undergoes a coordinated series of interactions to become the final mature capsid.

Assembly of phage P22 is a particularly tractable model where we can apply phage genetics with rigorous biochemical, biophysical and structural analyses of the reaction and assembly products. For instance in collaboration with Martin Jarrold's lab, we have determined the mass of entire phages. My lab played a major role in a collaborative effort to solve two near atomic resolution models of coat protein. In addition, my laboratory established procapsid assembly is a reversible reaction. This discovery was significant because it allowed the first application of rigorous, thermodynamic analysis to a complex assembly system. We found that the individual coat protein subunit interactions in a procapsid are weak but because of the additive nature of the interactions, the association energy of procapsids is very large.

1. Leavitt, J.C., Gilcrease, E.B., Woodbury, B.M., Teschke, C.M.\*, Casjens, S.R.\* (2021) Intravirion DNA can access the space occupied by the bacteriophage P22 ejection proteins. *Viruses*. 13:1504. doi: 10.3390/v13081504. \*co-corresponding authors
2. Asija, K., Teschke, C.M. (2019) Of capsid structure and stability: the partnership between charged residues of E-loop and P-domain of the bacteriophage P22 coat protein. *Virology*. 534:45-53. doi: 10.1016/j.virol.2019.05.021.
3. Newcomer, R.L., Schrad, J.R., Gilcrease, E.B., Casjens, S.R., Feig, M., Teschke, C.M.\*, Alexandrescu, A.T., and Parent, K.N.\* (2019) The phage L capsid decoration protein has a novel OB-fold and an unusual capsid binding strategy. *Elife*. 8:e45345. doi: 10.7554/eLife.45345. \*co-corresponding authors
4. Parent, K.N., Khayat, R., Tu, L.H., Suhanovsky, M.M., Cortines, J.R., Teschke, C.M., Johnson, J.E., Baker, T.S. (2010) P22 coat protein structures reveal a novel mechanism for capsid maturation: stability without auxiliary proteins or chemical crosslinks. *Structure*, 18, 390-401.

#### **2. Viral capsid assembly—studies of scaffolding protein:**

A scaffolding protein directs assembly of coat protein to form procapsids. We found a simple interaction between coat and scaffolding protein drives the proper assembly of the complex procapsids. Thus, in recent years my group has discovered that the protein:protein interactions required to generate a capsid should be disrupted readily, suggesting capsid assembly is a good drug target. We have recently developed a novel method to use NMR to investigate disorder inside a procapsid.

1. Whitehead III, R.D., Teschke, C.M.\*, Alexandrescu, A.T.\* (2019) NMR Mapping of Disordered Segments from a Viral Scaffolding Protein Encapsulated in a 23 MDa Procapsid Complex, *Biophys. J.*, 17(8):1387-1392. doi: 10.1016/j.bpj.2019.08.038. \*co-corresponding authors
2. Cortines, J.R., Motwani, T., Vyas, A.A., Teschke, C.M. (2014) Highly specific salt bridges govern bacteriophage P22 icosahedral capsid assembly: identification of the site in coat protein responsible for interaction with scaffolding protein. *J. Virol.*, 88, 5287-97.

3. Padilla-Meier, G.P., Teschke, C.M. (2011) Conformational changes in bacteriophage P22 scaffolding protein induced by interaction with coat protein, *J. Mol. Biol.*, 410, 226-40.
4. Cortines, J.R., Weigele P.R., Gilcrease, E.B., Casjens, S.R., Teschke, C.M. (2011) Decoding bacteriophage P22 assembly: Identification of two charged residues in scaffolding protein responsible for coat protein interaction. *Virology*, 421, 1-11.

### **3. Viral capsid assembly—studies of portal protein**

Portal proteins of dsDNA phages and viruses are essential for both DNA packaging and ejection into host cells. As such, portal proteins are excellent targets for anti-viral compounds. When these viruses assemble, a portal protein complex is incorporated at only one vertex of the capsid. This specificity of assembly is not understood. We are characterizing the assembly of P22 procapsid with the portal ring. We have discovered that scaffolding protein catalyzes portal ring assembly and that portal nucleates assembly of procapsids.

1. Woodbury, B.M., Motwani, T., Leroux, M.N., Barnes, L.F., Lykтей, N.A., Banerjee, S., Dedeo, C.L. Jarrold, M.F., Teschke, C.M. (2022). Tryptophan residues are critical for portal protein assembly and incorporation in bacteriophage P22. *Viruses* (special issue in memory of Lindsay Black), 14:1400. doi: 10.3390/v14071400
2. Motwani, T. Lokareddy, R.K., Dunbar, C.A., Cortines, J.R., Jarrold, M.F., Cingolani, G., Teschke, C.M. (2017) A viral scaffolding protein triggers portal ring oligomerization and incorporation during procapsid assembly. *Sci. Adv.* 3, e1700423. doi: 10.1126/sciadv.1700423. eCollection 2017 July.
3. Lokareddy, R., Sankhala, R., Roy, A., Afonine, P., Motwani, T., Teschke, C.M., Parent, K.N., and Cingolani, G. (2017) Portal protein functions akin to a DNA-sensor that couples genome-packaging to icosahedral capsid maturation. *Nature Communications*, 8:14310. doi: 10.1038/ncomms14310.
4. Motwani, T., Teschke, C.M. (2019). The architect of virus assembly: portal protein nucleates procapsid assembly in bacteriophage P22. *J. Virol.*, 93:e00187-19. doi: 0.1128/JVI.00187-19.

### **4. Phage P22 coat protein folding and assembly:**

The cytoplasm of a cell is a crowded environment with DNA, RNA and proteins, all found at very high concentrations, which can lead to misfolding and misassembly of new polypeptide chains. In bacteria there are many molecular chaperones, including GroEL and GroES, to assist in the folding and assembly of substrate polypeptides. but how chaperones recognize substrates remains unclear. The folding of coat protein of phage P22 is a particularly appropriate model for understanding how GroEL/S interact with substrate polypeptides because single amino acid substitutions, which lead to a temperature-sensitive-folding phenotype (*tsf*), cause coat protein folding intermediates to become substrates for the chaperones. WT coat protein, in contrast, does not require GroEL/S for folding. By investigating the folding of the *tsf* coat proteins my laboratory defined some of the mechanisms that target proteins to be substrates for chaperones.

1. Asija, K, Teschke, C.M. (2019) A hydrophobic network: Intersubunit and intercapsomer interactions stabilizing the bacteriophage P22 capsid. *J. Virol.*, 93, pii: JVI.00727-19. doi: 10.1128/JVI.00727-19.
2. D'Lima, N.G., Teschke, C.M. (2015) A molecular staple: D-loops in the I-domain of bacteriophage P22 coat protein make important inter-capsomer contacts required for procapsid assembly. *Journal of Virology*, 89, 10569-79.
3. Parent, K.N., Teschke, C.M. (2007) GroEL/S substrate specificity based on substrate unfolding propensity. *Cell Stress and Chaperones*, 12, 20-32.
4. Doyle, S.M., Anderson, E., Zhu, D., Braswell, E.H., Teschke, C.M. (2003) Rapid unfolding of a domain populates an aggregation-prone intermediate that can be recognized by GroEL. *J. Mol. Biol.*, 332, 937-951.

### **5. Folding and NMR studies of P22 coat protein I-domain:**

Recently, we completed a solution NMR structure of an inserted domain in P22 coat protein and determined this small domain is crucial for folding and stability of the entire coat protein. The I-domain folds much faster than the remainder of the protein, and provides a folding nucleus for the unusual HK97 fold. We were the first group to ascribe a function for an accessory domain inserted into the HK97 fold.

1. Tripler, T.N. Kaplan, A.R., Alexandrescu, A.T.\*, and Teschke, C.M.\* (2019) Conservation and divergence of the I-domain inserted into the ubiquitous HK97 coat protein fold in the P22-like bacteriophages. *J. Virol.*, 93:e00007-19. doi:10.1128/JVI.00007-19. \*co-corresponding authors

2. Harprecht, C, Okifo, O, Robbins, K.J., Motwani, T., Alexandrescu, A.T., Teschke, C.M. (2016) Contextual Role of a Salt-Bridge in the Phage P22 Coat Protein I-Domain. *J Biol Chem.*; 291,11359-72. doi: 10.1074/jbc.M116.716910.
3. Rizzo, A.A., Suhanovsky, M.M., Baker, M.L., Fraser, L.C.R., Jones, L.M., Rempel, D.L., Gross, M.L., Chiu, W., Alexandrescu, A.A., Teschke, C.M (2014) Multiple functional roles of the accessory I-domain of bacteriophage P22 coat protein revealed by NMR structure and cryoEM imaging. *Structure*, 22, 830-41.
4. Suhanovsky, M.M. and Teschke, C.M. (2013) An intramolecular chaperone inserted in bacteriophage P22 coat protein mediates its chaperonin-independent folding, *J. Biol. Chem.*, 288, 33772-83.

### **Summary:**

The research done in my lab has shown capsid assembly is a viable target for development of antiviral drugs because of the specific interactions required for the reaction. We have found regions of coat protein critical for assembly and discovered how simple interactions can control assembly. We have developed methods for the incorporation of the dodecameric portal complex into assembly procapsids, a first for phage P22. We have also investigated the mechanisms that proteins use to fold via interaction with cellular chaperones.

### **NCBI My bibliography URL**

[https://www.ncbi.nlm.nih.gov/sites/myncbi/1fA\\_Czh9bi2k7/bibliography/45934037/public/?sortby=pubDate&sdirection=descending](https://www.ncbi.nlm.nih.gov/sites/myncbi/1fA_Czh9bi2k7/bibliography/45934037/public/?sortby=pubDate&sdirection=descending)

## 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: **Gino Cingolani, Ph.D.**

eRA COMMONS USER NAME (agency login): **cingolag**

POSITION TITLE: **Professor**

EDUCATION/TRAINING

INSTITUTION AND LOCATION	DEGREE (if applicable)	Completion Date MM/YYYY	FIELD OF STUDY
University of Bari, Italy	B.S./M.S.	07/1995	Biochemistry
University Joseph Fourier, Grenoble, France	D.E.A.	06/1996	X-ray Crystallography
European Molecular Biology Laboratory	Ph.D.	07/1999	Structural Biology
The Scripps Research Institute, CA, USA	Post-doc	12/2003	Virus Crystallography/Cell Biology

### A. Personal Statement

My research interests and expertise lie in the field of Structural Biology. I trained in Biological Chemistry and Macromolecular Crystallography at different European Universities in the nineties. I moved to the United States in 1999 to continue my training at The Scripps Research Institute in the laboratories of Dr. Larry Gerace (Cell Biology) and Dr. Jack Johnson (Structural Virology). I have been a principal investigator since 2004, first at SUNY Upstate Medical University, and since 2009, at Thomas Jefferson University (TJU). For the last fifteen years, my laboratory has been continuously supported by multiple grants from NIH. Consistent with my multi-disciplinary training, my lab uses biophysical, biochemical, and cellular techniques to study macromolecules' structure and function. Our long-term goal is to employ rigorous chemical and physical methodologies to study medically-relevant problems that help decipher the most fundamental mechanisms of life and improve human health. Faithful to the idea that 'seeing is believing,' we investigate the structure of signaling and viral macromolecules as a starting point to probe their function and engineer their activity using small-molecule compounds. We are particularly interested in solving the atomic structures of 'druggable' biological targets linked to human diseases.

Currently, the main focus of my laboratory's research is to understand the molecular mechanisms of protein nuclear import and viral genome-delivery into gram-negative bacteria. Much of the work on nuclear import spearheaded from the pioneered structure of human importin  $\beta$  solved in complex with a classical (Cingolani et al., *Nature*, 1999) and non-classical (Cingolani et al., *Mol Cell*, 2002) import cargo, which I determined as a trainee. Over the last two decades, we have expanded the analysis of the structure/function of importins to importin  $\alpha$  isoforms, with emphasis on the aberrant nuclear translocation of disease-associated transcription factors (STATs, NF- $\kappa$ B, RCC1, etc) and viral proteins. As far as the second research area, we study the mechanisms used by bacterial viruses to package genomes into empty capsid as well as eject genomes into Gram-negative. Both events occur through a dodecameric protein channel known as the portal protein (Olia et al., *NSMB* 2011; Lokareddy et al., *Nat Comms* 2017), which occupies a unique vertex of the icosahedral capsid breaking the icosahedral symmetry. We are particularly interested in bacteriophages used in phage therapy that eradicates human pathogens such as *Pseudomonas aeruginosa* and *Salmonella enterica*.

Throughout my career, I have maintained a strong commitment to graduate education. I have mentored ten Ph.D. students and four MS students, and I am currently mentoring three Ph.D. students in addition to three post-docs. I have developed and a graduate course (PR613) focused on biophysical methods in structural biology, and I teach a full graduate course in Biochemistry (PHRM 510) for Pharmacy students (a class of ~65 students). My service to the community includes serving as director of the X-ray Crystallography & Molecular Characterization Facility, one of the eight NCI-supported shared resources in the Sidney Kimmel Cancer Center at TJU. I also founded and developed the Jefferson Cryo-Electron Microscopy Core (JEMiCo), which is operational since March 2021 under my leadership. This state-of-the-art cryo-EM core boasts a new Glacios 200 kV cryo-transmission electron microscope equipped with a Falcon 4 direct electron detector, a Vitrobot, a glow discharger, and three multi-GPUs workstations for advanced cryo-EM image-processing.

I serve as Vice-Chair for Research of the Dept of Biochemistry and Mol Biology, and chair of the NIH Prokaryotic Cell and Molecular Biology (PCMB) study section panel. I have been the main PI on three shared instrumentation (S10) grants, all successfully funded, to acquire a hybrid diffractometer, a crystallization robot, a 200 kV Glacios cryo-electron microscope and associated equipment.

**Cingolani, G.**, Petosa, C., Weis, K., and Mueller, C.W. (1999) Structure of importin  $\beta$  bound to the IBB domain of importin  $\alpha$ . *Nature*, 399(6733): 221-229. PMID: 10353244.

**Cingolani, G.**, Bednenko, J., Gillespie, M., and Gerace, L. (2002) Molecular basis for the recognition of a *non-classical* nuclear localization signal by importin  $\beta$ . *Molecular Cell*, 10: 1345-1353. PMID: 12504010.

Olia, A.S, Prevelige Jr., P.E., Johnson, J.E. and **Cingolani, G.** (2011) Three-dimensional structure of a viral genome-delivery portal vertex. *Nature Struc Mol Biol.* 18(5):597-603. PMCID: PMC3087855.

Lokareddy, R.K., Sankhala, R.S., Roy, A., Afonine, P.V., Motwani, T., Teschke, C.M., Parent, K.N. and **Cingolani, G.** (2017) Portal protein functions akin to a DNA-sensor that couples genome-packaging to icosahedral capsid maturation. *Nature Commun.* 8:14310. PMCID: PMC5290284.

Ongoing and recently completed projects that I would like to highlight include:

R35 GM140733

Cingolani (PI)

06/01/2021 – 05/31/2026

***Mechanism of Viral Genome Delivery into Cells***

R01GM122844

Cingolani (PI)

03/01/2018 – 12/31/2021

***Regulation of Nuclear Import through Importin Alpha Isoforms***

R01 AI137338

Niederweis, Cingolani (mPI)

04/01/2019 – 03/31/2024

***Hemoglobin utilization by Mycobacterium tuberculosis***

P01 NS097197

Minassian (PI), Role co-project lead

06/01/2016 – 05/31/2021

***Genome Editing, mRNA suppression, and Glycogen Chain Termination to Inhibit Glycogen Storage as Therapy for Lafora Disease***

P30 CA056036

Knudsen (PI), Role: director of the X-ray Crystallography Shared Resource

06/01/2018 – 5/31/2021

***Translational Research in Cancer***

1S10 OD030457-01

Cingolani (PI)

07/1/2022 – 06/30/2023

***A new Cryo-Transmission Electron Microscope at Thomas Jefferson University***

## **B. Positions and Honors**

### **Positions**

04/2017-present	Vice-Chair, Dept. of Biochemistry and Mol. Biology, Thomas Jefferson University, PA
06/2015-present	Director, X-ray Crystallography Facility, Sidney Kimmel Cancer Center
09/2015-12/2015	Visiting Professor, Dept. of Biochemistry and Mol. Biology, University of Bari, Italy
01/2015-present	Professor, Dept. of Biochemistry and Mol. Biology, Thomas Jefferson University, PA
06/2009-12/2014	Associate Professor with Tenure, Dept. of Biochemistry and Mol. Biology, Thomas Jefferson University, PA, USA
01/2004-05/2009	Assistant Professor, Dept. of Biochemistry and Mol. Biology, SUNY Upstate, NY, USA

### **Scientific Appointments**

2021-2023      National Institute of Health (NIH) PCMB study section, chair

2022	Antiviral Drug Discovery (AViDD) Centers for Pathogens of Pandemic (U19), reviewer
2018-2023	National Institute of Health (NIH) PCMB study section, permanent member
2018-present	European Union, Horizon 2020, reviewer
2017	National Institute of Health (NIH) ZRG1 F04B-D (20) L study section, reviewer
2016	National Institute of Health (NIH) ZRG1 F04B-D (20) L study section, reviewer
2015	Israel Science Foundation (ISF), ad hoc reviewer
2014	National Institute of Health (NIH) ZRG1 F04B-D (20) L study section, reviewer
2013	Italian Ministry of Health (MoH), Research Proposals, study section member
2013	National Institute of Health (NIH) ZRG1 F04-W(20) L study section, reviewer
2013	Advanced Photon Source (APS) beamlines NE-CAT's 24-ID-C and 24-ID-E, reviewer
2013	National Institute of Health (NIH) MFSC study section, ad hoc reviewer
2012	Italian Ministry of Health (MoH), Research Proposals, study section member
2012	National Institute of Health (NIH) ZRG1 F04-D (20)/F04-K (09) L study section, reviewer
2012-present	Italian Scientists and Scholars in North America Foundation, member
2011	National Institute of Health (NIH) MFSC study section, ad hoc reviewer
2011	Research Grants Council (RGC) of Hong Kong, reviewer
2010	National Institute of Health (NIH) ZRG1 F04B-B(20) study section, reviewer
2010	National Institute of Health (NIH) ZGM1 CBB-0 (BC) U01 for Structural Biology, reviewer
2010	National Institute of Health (NIH) ZRG1 F05-C(20) L study section, reviewer
2009-2014	National Synchrotron Light Source (NSLS) Users' Committee beamline X6a, member
2008	National Science Foundation (NSF), ad hoc grant reviewer
2007-2009	American Society for Cell Biology (ASCB), member
2007-2008	Cornell High Energy Synchrotron Source (CHESS) Executive Committee, member
2007	National Science Foundation (NSF), panelist
2006-2007	Cornell High Energy Synchrotron Source (CHESS) Executive Committee, member
2006	Medical Research Council (MRC), UK, ad hoc grant reviewer
2004-2005	Cornell High Energy Synchrotron Source (CHESS) Express Mode proposal reviewer

### Honors

2021	Vice-Chair, Jefferson Committee on Research (JCoR)
2019	Provost Award for Basic Research
2018	Fredric Rieders Faculty Prize in Graduate Education for Academic Year 2018-19
2017-present	Acta crystallographica D, co-Editor
2013	Jefferson Medical College Early Career Investigator Award
2004	Leukemia Research Foundation Young Investigator award
2000	Human Frontier Science Program (HFSP) post-doctoral fellowship
2000	Award for the best presentation at the TSRI Society of Fellows 4th symposium
1999	Award for the best presentation at the TSRI Society of Fellows 3rd symposium
1996	European Molecular Biology Laboratory (EMBL) pre-doctoral fellowship

## **C. Contribution to Science**

**1. Mechanisms of viral genome delivery.** The long-term goal of our work is to understand how double-stranded DNA viruses such as herpesviruses and tailed bacteriophages package their large genomes (~40-250 kb) inside empty precursor capsids ('procapsids'), as well as eject the genomes inside infected cells. Both processes are mediated by complex, multi-subunit machines that assemble transiently during the virus life-cycle. We have investigated the architecture, composition, and assembly of bacterial virus packaging motors for nearly two decades using hybrid structural methods. We determined the portal protein changes conformation during genome-packaging and determined the structure of the mature (Olia et al., *NSMB*, 2011) and immature portal assembly (Lokareddy et al., *Nature Commun*, 2017) from the *Salmonella*-phage P22. We also dissected the architecture of the small (TerS) and large (TerL) terminase subunits implicated in viral genome recognition and packaging (Niazi et al., *Nucleic Acid Res* 2020). Our more recent work focuses on the DNA-ejectosome and *Pseudomonas*-phages used in phage therapy. Using cryo-EM single-particle analysis, we provide high-resolution architectural insights into the machines used by *Pseudomonas* phages to attach the bacterium cell envelope, eject



their genome into the host and package newly replicated genomes into precursor capsids. Key publications in this field include:

- a. Olia, A.S, Prevelige Jr., P.E., Johnson, J.E. and **Cingolani, G.** (2011) Three-dimensional structure of a viral genome-delivery portal vertex. *Nature Struc Mol Biol.* 18(5):597-603. PMID: PMC3087855.
- b. Lokareddy, R.K., Sankhala, R.S., Roy, A., Afonine, P.V., Motwani, T., Teschke, C.M., Parent, K.N. and **Cingolani, G.** (2017) Portal protein functions akin to a DNA-sensor that couples genome-packaging to icosahedral capsid maturation. *Nature Commun.* 8:14310. PMID: PMC5290284.
- c. Niazi, M., Florio, T.J., Yang, R., Lokareddy, R.K., Swanson, N.A. Gillilan, R.E. and **Cingolani, G.** (2020) Biophysical analysis of Pseudomonas-phage PaP3 small terminase suggests a mechanism for sequence-specific DNA-binding by lateral interdigitation. *Nucleic Acid Res.* 48(20):11721-11736. PMID: PMC7672466
- d. Swanson, N.A., Lokareddy, R.K., Li, F., David, Hou C-F, Leptihn, S., Pavlenok, M., Niederweis, M., Pumroy, R.A., Moiseenkova-Bell, V.Y., **Cingolani, G.** (2021) Cryo-EM Structure of the Periplasmic Tunnel of T7 DNA-Ejectosome at 2.7 Å resolution. *Molecular Cell.* 81(15):3145-3159. PMID: PMC8349896

**2. Mechanisms and regulation of nucleocytoplasmic transport.** Nucleocytoplasmic transport is central to the functioning of eukaryotic cells and is an integral part of the processes that lead to most human diseases. Nuclear availability of essential molecules such as transcription factors, DNA replication factors, and oncogenes is emerging as a powerful way to control gene expression, cellular differentiation, and transformation, as well as a novel and promising target for *pharmacological intervention*. In the first part of my career, I elucidated the molecular basis for the recognition of *classical* (Cingolani et al., *Nature*, 1999) and *non-classical* (Cingolani et al., *Mol Cell*, 2002) import substrates by the transport factor importin  $\beta$ . As an independent investigator (2004-present), I studied the molecular basis for nuclear import of critical signaling molecules such as the phospholipid scramblase 1 (Chen et al., *J Biol Chem*, 2005) and 4 (Lott et al., *J Biol Chem*, 2010), the small nuclear ribonucleoprotein transporter snurportin (Mitrousis et al., *J Biol Chem*, 2008), the transcription factor STAT1 (Nardozi et al., *J Mol Biol*, 2011) and the mechanisms of membrane protein translocation to the Inner Nuclear Membrane (Lokareddy et al., *structure*, 2015). More recently, my lab became interested in understanding the regulation of nuclear transport by importin  $\alpha$  isoforms. We delineated the molecular basis for nuclear import of Influenza polymerase subunit Pb2 (Pumroy et al., *structure*, 2015) and RCC1 (Sankhala et al., *Nature Commun*, 2017) by the isoform importin  $\alpha 3$ . We recently determined the heterodimeric transcription factor NF- $\kappa$ B (p65:p50) is imported by the isoform importin  $\alpha 3$  (Florio et al., *submitted*), and we are pursuing basic and translational studies on the ALS-related RNA-binding protein TDP-43. Overall, the long-term goal of our work is to decipher the mechanisms governing the nuclear entry of critical signaling molecules and to devise new small molecule inhibitors that could reduce aberrant nuclear translocation of signaling factors linked to cancer (Liao et al., *Mol Cancer Ther*, 2015; De Dominici et al., *Blood*, 2020). Key publications include:

- a. **Cingolani, G.**, Petosa, C., Weis, K., and Mueller, C.W. (1999) Structure of importin  $\beta$  bound to the IBB domain of importin  $\alpha$ . *Nature*, 399(6733): 221-229. PMID: 10353244.
- b. **Cingolani, G.**, Bednenko, J., Gillespie, M., and Gerace, L. (2002) Molecular basis for the recognition of a *non-classical* nuclear localization signal by importin  $\beta$ . *Molecular Cell*, 10: 1345-1353. PMID: 12504010.
- c. Sankhala, R.S., Lokareddy, R.K., Begum S., Pumroy, A.R., Gillilan, E.R. and **Cingolani, G.** (2017) Three-dimensional context rather than NLS amino acid sequence determines importin  $\alpha$  subtype specificity for RCC1. *Nature Commun.* 8(1):979. PMID: PMC5645467
- d. Florio, T.J., Lokareddy, R.K., Yeggoni, D.P., Sankhala, R.S., Ott, C.A., Gillilan, E.R. and **Cingolani, G.** (2022) Differential recognition of canonical NF- $\kappa$ B dimers by Importin  $\alpha 3$ . *Nature Commun.* 13(1):1207. PMID: PMC8904830

**3. Structure and inhibition of disease-related dual-specificity phosphatases.** Dual specificity phosphatases (DSPs) are essential signaling enzymes whose misregulation is intimately linked to human diseases such as cancer, diabetes, inflammation, and Alzheimer's disease. The human genome encodes 38 DSPs (known as VH1-like phosphatases), which regulate critical aspects of the cell cycle. In my laboratory, we study the structure and function of disease-related DSPs. We have determined the atomic structure of the prototypical Vaccinia virus VH1 (Koksal et al., *J Biol Chem*, 2009; Koksal and Cingolani, *J Biol Chem*, 2011), the p53-phosphatase DUSP26 (Lokareddy et al., *Biochemistry*, 2013), the 5'-RNA-phosphatase PIR1 (Sankhala et al., *Biochemistry*, 2014) and the glycogen phosphatase laforin (Sankhala et al., *J Biol Chem*, 2015). The long-term work of this work is to decipher the molecular determinants that make DSPs substrate-specific *in vivo*. In the case of multi-domain phosphatases like laforin, we seek to understand how phosphatase activity is regulated in the context of

the full-length enzyme. This is essential to develop new 'smart' drugs that selectively interfere with substrate recognition, as opposed to catalytic activity. Key publications in this field include:

- e. Koksai, A., Nardozi, J., and **Cingolani, G.** (2009) Dimeric quaternary structure of the prototypical dual-specificity phosphatase VH1. *J. Biol. Chem.* 284(15):10129-37. PMCID: PMC2665067.
- f. Lokareddy, K.R., Bhardwaj, A. and **Cingolani, G.** (2013) Atomic structure of DUSP26, a novel p53 phosphatase. *Biochemistry*, 52(5):938-48. PMCID: PMC3619938.
- g. Sankhala, S.R., Koksai, C.A., Ho, L., Nitschke, F., Minassian, A.B. and **Cingolani, G.** (2015) Dimeric quaternary structure of human laforin. *J Biol. Chem.* 290(8):4552-559. PMCID: PMC4335197.
- h. Florio T., Lokareddy R.K., Gillilan R. and **Cingolani, G.** (2019) Molecular architecture of the inositol phosphatase Siw14. *Biochemistry*. 58(6):534-545. PMCID: PMC6526948

**4. Structure and inhibition of bacterial virulence factors.** We are interested in the structure and enzymatic mechanisms of pathogenesis-related bacterial proteins as novel antibacterial targets. We determined the structure of the Escherichia coli F<sub>1</sub> ATPase core auto-inhibited by the epsilon subunit (Cingolani and Duncan, Nature Struc Mol Biol. 2011), which revealed a novel mode of intramolecular regulation of rotary catalysis. In collaboration with Dr. Michael Niederweis at the University of Alabama, we determined the crystal structure of the Mycobacterium tuberculosis Necrotizing Toxin (TNT) in complex with the Immunity factor IFT, at 1.1 Å resolution (Sun et al. Nature Struc Mol Biol. 2011). We also demonstrated that TNT hydrolyzes the essential co-enzyme nicotinamide adenine dinucleotide (NAD<sup>+</sup>) in the cytosol of Mtb-infected macrophages. In collaboration with Dr. Paumet at Thomas Jefferson University, we have studied the Chlamydia trachomatis inclusion protein IncA, which is the first example of a bacterial SNARE-like protein mediating homotypic fusion of intracellular inclusions (Cingolani et al., Nature Commun 2019). Our current work focuses on the structural analysis of Mycobacterium tuberculosis outer membrane proteins that harbor catalytic domains and that represent virulence factors. Key publications in this field include:

- i. **Cingolani, G.\*** and Duncan, T.M. (2011) Structure of the ATP synthase catalytic complex (F<sub>1</sub>) from Escherichia coli in an auto-inhibited conformation. *Nature Struc Mol Biol.* 18(6):701-7. (\* corresponding authors) PMCID: PMC3109198
- j. Sun, J., Siroy, A., Lokareddy, K.R., Speer, A., Doornbos, K.S., **Cingolani, G.\*** and Niederweis, M.\* (2015) The tuberculosis necrotizing toxin kills macrophages by hydrolyzing NAD<sup>+</sup>. *Nature Struc Mol Biol.* 22(9):672-8. (\* corresponding authors) PMCID: PMC4560639
- k. **Cingolani, G.\***, McCauley, M., Loble, A., Bryer, A.J., Wesolowski, J., Greco, D.L., Lokareddy, R.K., Ronzone, E., Perilla, J.R. and Paumet, F.\* (2019) Structural basis for the homotypic fusion of chlamydial inclusions by the SNARE-like protein IncA. *Nature Commun.* 10(1):2747. (\* corresponding authors) PMCID: PMC6588587
- l. Mitra, A., Ko, Y.H., **Cingolani, G.\*** and Niederweis, M.\* (2019) Heme and hemoglobin utilization by Mycobacterium tuberculosis. *Nature Commun.* 10(1):4260. (\* corresponding authors) PMCID: PMC6751184

**Complete List of Published Work in MyBibliography (98 publications, >5,920 citations, H-index=39):**

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**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: White, Simon

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

POSITION TITLE: Assistant 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)	END DATE MM/YYYY	FIELD OF STUDY
University of Hertfordshire, Hatfield, Hertfordshire	BS	07/2005	Applied Biology
University of Leeds, Leeds, West Yorkshire	MS	07/2006	Biosciences
University of Leeds, Leeds, West Yorkshire	PHD	01/2011	Bionanotechnology

**A. Personal Statement**

I am well placed to pursue the work laid out in this application. I have extensive experience working with different bacteriophages (various actinobacteriophages, see Podgorski et al. 2020, and M13 and MS2 bacteriophages). I have the experience with cryo-electron microscopy needed for success and have six structures deposited in the electron microscopy databank, and I am currently collaborating with others at the University of Connecticut on cryo-electron microscopy data. In the past year my lab has built de novo models using actinobacteriophage cryoEM maps for sixteen bacteriophages (to be deposited). I have the experience needed with cryoSPARC, Relion and with Phenix for model building.

I am currently in my fifth year at the University of Connecticut as an assistant Professor and I supervise five graduate students (three PhD and two masters) and mentored thirteen undergraduate students (five of whom are still in my lab). We have monthly lab meetings where they present their work in a friendly and supportive environment. These lab meetings are an important tool for trouble shooting problems they may have and developing new avenues of research. They are an excellent team and we are well placed complete the stated aims.

1. Luque A, Benler S, Lee D, Brown C, White S. The Missing Tailed Phages: Prediction of Small Capsid Candidates. *Microorganisms*. 2020 December 08; 8(12):1944-. Available from: <https://www.mdpi.com/2076-2607/8/12/1944> DOI: 10.3390/microorganisms8121944
2. Podgorski J, Calabrese J, Alexandrescu L, Jacobs-Sera D, Pope W, Hatfull G, White S. Structures of Three Actinobacteriophage Capsids: Roles of Symmetry and Accessory Proteins. *Viruses*. 2020 March 08; 12(3):294. Available from: <https://www.mdpi.com/1999-4915/12/3/294/htm> DOI: 10.3390/v12030294
3. Shakeel S, Dykeman EC, White SJ, Ora A, Cockburn JJB, Butcher SJ, Stockley PG, Twarock R. Genomic RNA folding mediates assembly of human parechovirus. *Nat Commun*. 2017 Feb 23;8(1):5. PubMed Central PMCID: PMC5431903.
4. White SJ, Johnson S, Szymonik M, Wardingley RA, Pye D, Davies AG, Wälti C, Stockley PG. Directed surface attachment of nanomaterials via coiled-coil-driven self-assembly. *Nanotechnology*. 2012 Dec 14;23(49):495304. PubMed Central PMCID: PMC4785676.

**B. Positions, Scientific Appointments and Honors****Positions and Scientific Appointments**

2017 - Assistant Professor, UNIVERSITY OF CONNECTICUT  
2010 - 2017 Post doctoral research assistant, UNIVERSITY OF LEEDS

## **Honors**

2016	Best Poster Award at FASEB: Virus Structure and Assembly, FASEB
2016	Best Poster Award at the Astbury Conversation Symposium, University of Leeds
2016	The Dean's Vacation Research Scholarship, University of Leeds
2015	Award for Outstanding Contribution (Oral Presentation) at the XXIV Biennial Conference on Phage/Virus Assembly, Phage and Virus Assembly
2006	Award for Best Result in Year Group, University of Leeds
2005	Award for Best Result in Year Group, University of Hertfordshire

## **C. Contribution to Science**

1. Over the past two years I have obtained sixteen cryo-EM maps of actinobacteriophages, the majority of which are under 3 Angstrom resolutions. This represents an approximately 50% increase in the number of the high resolution HK97-fold bacteriophage capsid models for the field. I have used these maps, along with AlphaFold predictions, to create a structural phylogenetic tree of the 3000 actinobacteriophage major capsid proteins. I have shown that they can be classified into twelve structural groups. I have analysed one of these groups in depth and showed that although they have a highly similar fold, they use subtly different capsid stabilisation mechanisms. Importantly I have shown that the isopeptide bond that was first discovered in the HK97 bacteriophage is not an evolutionary "dead-end" for these bacteriophages and that they can adapt to remove the isopeptide bond. I have also identified a number of other unique capsid stabilisation mechanisms in the actinobacteriophages that have not been identified in other HK97-fold bacteriophages. For example, the use of disulphide bonds in the major capsid protein, as well as a fused "minor capsid protein" domain within a major capsid protein that may explain how minor capsid proteins evolved in these bacteriophages.
  - a. Podgorski J, Calabrese J, Alexandrescu L, Jacobs-Sera D, Pope W, Hatfull G, White S. Structures of Three Actinobacteriophage Capsids: Roles of Symmetry and Accessory Proteins. *Viruses*. 2020 March 08; 12(3):294. Available from: <https://www.mdpi.com/1999-4915/12/3/294/htm> DOI: 10.3390/v12030294
2. I was a PDRA at Leeds University for 5 years, working with Professor Peter Stockley, investigating the assembly mechanisms of positive sense-single stranded RNA viruses. My PDRA work has been instrumental in the discovery, characterisation and validation of a completely unsuspected aspect of the assembly mechanisms in this class of RNA viruses. It appears that they all use an evolutionarily conserved mechanism to ensure survival of the virion in the challenging environment of the infected cell. Our recent discovery of this mechanism in a picornavirus forces a re-examination of decades of research that showed that they did not exist in this family of viruses. These discoveries lead naturally to potential real world applications, including the development of novel antiviral therapies and the creation of artificial and completely safe viral vaccines.

The work to identify packaging signals in human pathogens (human parechovirus, hepatitis B and C) had a major collaborative association with Prof. Reidun Twarock at the University of York (Department of Mathematics, Centre for Complex Systems Analysis). It features in high impact papers, which have been published in *Nature Communications* (Shakeel, Dykeman and White [joint first authors], et al.) and *Nature Microbiology* (Patel and White [joint first authors], et al.). It was an important corner-stone of a patent on a novel anti-viral strategy (jointly held by the Universities of York, Leeds and Helsinki, US20160326529), for which I am a co-inventor, and a Wellcome Trust Joint Senior Investigator Grant to Profs. Stockley and Twarock as well as a grant from the Medical Research Council for which I was named post-doc. The *Nature Communications* paper received widespread media coverage and resulted in 32 news articles, including articles in CNN and ABC. Likewise, the *Nature Microbiology* paper resulted in 7 news articles, a "Behind the paper" article in *Nature Microbiology*, a specific article in *Nature Reviews Microbiology* discussing the work (York, A. (2017). *Viral Infection: Packing to Leave*. *Nature Reviews Microbiology*. 15: 450-451) and three cryo-electron microscopy structures (in which I did the reconstructions) deposited in the electron microscopy data bank (EMDB-3714, EMDB-3715 and EMDB-3716).

My other work on packaging signals has focused on one of the model systems for viral assembly: the bacteriophage MS2. Working with Prof. Reidun Twarock (York University) and Prof. Cheng Kao (Indiana University), I was part of the team that identified the 60 coat protein:RNA contacts in MS2, the first time that all of the packaging signals have been identified within a virus (see Direct Evidence for Packaging Signal-Mediated Assembly of Bacteriophage in JMB). Our work was the focus of a review by Peter Prevelige (Follow the Yellow Brick Road: A paradigm Shift in Virus Assembly, 2016, Journal of Molecular Biology, 428: 416-418) which highlights the importance of my work. The entire field of (+)ssRNA viruses must now revisit existing mechanisms of assembly in the light of our work and I expect many groups to start exploring packing signals in other (+)ssRNA viruses.

- a. Patel N, White SJ, Thompson RF, Bingham R, Weiß EU, Maskell DP, Zlotnick A, Dykeman E, Tuma R, Twarock R, Ranson NA, Stockley PG. HBV RNA pre-genome encodes specific motifs that mediate interactions with the viral core protein that promote nucleocapsid assembly. *Nat Microbiol.* 2017 Jun 19;2:17098. PubMed Central PMCID: PMC5495169.
  - b. Shakeel S, Dykeman EC, White SJ, Ora A, Cockburn JJB, Butcher SJ, Stockley PG, Twarock R. Genomic RNA folding mediates assembly of human parechovirus. *Nat Commun.* 2017 Feb 23;8(1):5. PubMed Central PMCID: PMC5431903.
  - c. Stewart H, Bingham RJ, White SJ, Dykeman EC, Zothner C, Tuplin AK, Stockley PG, Twarock R, Harris M. Identification of novel RNA secondary structures within the hepatitis C virus genome reveals a cooperative involvement in genome packaging. *Sci Rep.* 2016 Mar 14;6:22952. PubMed Central PMCID: PMC4789732.
  - d. Rolfsson Ó, Middleton S, Manfield IW, White SJ, Fan B, Vaughan R, Ranson NA, Dykeman E, Twarock R, Ford J, Kao CC, Stockley PG. Direct Evidence for Packaging Signal-Mediated Assembly of Bacteriophage MS2. *J Mol Biol.* 2016 Jan 29;428(2 Pt B):431-48. PubMed Central PMCID: PMC4751978.
3. I have made contributions to the field of bio-nanoscience, exploring bio-templated device construction in collaboration with Profs. Giles Davies and Christoph Wälti at the University of Leeds and Dr. Steve Johnson at the University of York. This utilized the M13 bacteriophage displaying coiled-coil motifs to specifically assemble the phage particle between two electrodes. This work culminated in the publication in the high impact journal of *Angewandte Chemie* studying the structure and assembly of coiled-coils on a 2-D surface. I showed that the assembly of coiled-coils on a surface results in molecular crowding and results in the properties of the coiled-coil changing, i.e. the pH at which they assemble/disassemble is radically changed when crowded onto a 2D surface, as compared to solution. This work has important implications for various 2-D surface techniques, e.g. surface plasmon resonance, and bio-nano diagnostic devices. It has been cited 9 times. The work is continuing using the B23 (circular dichroism) beamline at the diamond light source to study the control of coiled-coil interactions using voltage controlled electrodes with the aim of making a switchable surface for the capture and release of various biological molecules, e.g. specific cell types in cell sorting applications.
- a. White SJ, Johnson SD, Sellick MA, Bronowska A, Stockley PG, Wälti C. The influence of two-dimensional organization on peptide conformation. *Angew Chem Int Ed Engl.* 2015 Jan 12;54(3):974-8. PubMed Central PMCID: PMC4506555.
  - b. White SJ, Johnson S, Szymonik M, Wardingley RA, Pye D, Davies AG, Wälti C, Stockley PG. Directed surface attachment of nanomaterials via coiled-coil-driven self-assembly. *Nanotechnology.* 2012 Dec 14;23(49):495304. PubMed Central PMCID: PMC4785676.
  - c. White SJ, Morton DW, Cheah BC, Bronowska A, Davies AG, Stockley PG, Wälti C, Johnson S. On-surface assembly of coiled-coil heterodimers. *Langmuir.* 2012 Oct 2;28(39):13877-82. PubMed Central PMCID: PMC4820041.
4. I have used SELEX to identify many novel RNA aptamers which have played an important role in the development of biosensors. The first set of aptamers was involved in the detection of the aminoglycoside antibiotics in collaboration with FERA (Food and Environment Research Agency) in the U.K. who wanted to develop a cheap and quick biosensor for the detection of aminoglycoside antibiotics in milk. Aptamers were discovered that could be used in an assay and detect the antibiotics in the nM range. The second set of

RNA aptamers that I developed was for the differentiation between different conformations of amyloid precursors. I successfully identified aptamers that could achieve this.

- a. Sarell CJ, Karamanos TK, White SJ, Bunka DHJ, Kalverda AP, Thompson GS, Barker AM, Stockley PG, Radford SE. Distinguishing closely related amyloid precursors using an RNA aptamer. *J Biol Chem*. 2014 Sep 26;289(39):26859-26871. PubMed Central PMCID: PMC4175327.
  - b. Derbyshire N, White SJ, Bunka DH, Song L, Stead S, Tarbin J, Sharman M, Zhou D, Stockley PG. Toggled RNA aptamers against aminoglycosides allowing facile detection of antibiotics using gold nanoparticle assays. *Anal Chem*. 2012 Aug 7;84(15):6595-602. PubMed Central PMCID: PMC3413241.
5. I have developed a novel class of small molecule inhibitors of the Picornavirus family that target the viral 2C helicase. These molecules inhibit the 2C:capsid protein interaction and I am currently developing these as probe molecules to understand the critical interaction during the assembly of the capsid