Institution: University of York		
Unit of Assessment: 10 - Mathematical Sciences		
Title of case study: Discovery of a virus assembly mechanism using models of viral geometry: new opportunities for anti-viral therapy		
Period when the underpinning research was undertaken: 2005 - 2020		
Details of staff conducting the underpinning research from the submitting unit:		
Name(s):	**Role(s) (e.g. job title):**	**Period(s) employed by submitting HEI:**
Reidun Twarock	Professor	Feb 2005 - present
Eric Dykeman	Lecturer	Jul 2008 - present
Richard Bingham	Research associate	Feb 2014 - present
German Leonov	Research associate	Apr 2016 – Apr 2018
Period when the claimed impact occurred: 2014 – 2020		
Is this case study continued from a case study submitted in 2014? N		

1. Summary of the impact (indicative maximum 100 words)

Anti-viral therapies, drug delivery and vaccination have major public health importance. Novel opportunities in these vital areas of public health have been opened up by breakthrough discoveries made by Twarock and her group. They have pioneered mathematical approaches for the analysis of viral geometry and changed the existing paradigm, demonstrating that the long-standing assumption of a virus assembly mechanism driven by electrostatics is false. Together with experimental collaborators, the team has identified packaging signals and their use as drug targets for a number of viral families, including coronaviruses. The research has been exploited by the US National Institutes of Health (NIH) for exemplification of a new method for the identification of RNA binding drugs. Through numerous outreach projects, including collaboration with computer artists, the research has influenced public understanding of viruses and of the importance of mathematics in scientific discovery.

2. Underpinning research (indicative maximum 500 words)

A fundamental question in virology is how viruses achieve efficient assembly of their protein containers or capsids, and how this relates to achieving specific packaging of their viral genomes (which can either be DNA or RNA) in the face of competition from cellular DNA/RNA.

Twarock and her team have developed mathematical approaches to model the structures of the capsids that encapsulate viral genomes. Using group theory, graph theory and tiling theory, they developed techniques to predict the positions of, and interaction networks between, the protein building blocks. Until recently the prevailing paradigm was that a single packaging signal in the viral genome provided a contact point for the capsid protein to the genome. The lack of an obvious repeat of the packaging signal motif within viral genomes led scientists in the field to believe for decades that there are no other packaging signals.

However, Twarock's work on the geometry of viruses has shown that the traditional models of virus formation are inadequate for single-stranded RNA viruses such as Hepatitis C, HIV and the common cold. Using affine extended symmetry groups, derived by Twarock for the icosahedral group (the symmetry underpinning virus architecture), her group has characterised viruses via collective constraints on the structural organisation at different radii [R1]. One such level corresponds to the binding sites at the inner capsid surface, with relative positions corresponding to vertices of polyhedra that are defined by the constraints. Twarock and her team used these constraints in order to translate the viral genome packaging mechanism into a travelling salesman problem. Their approach is now known in virology as the Hamiltonian Paths Approach (HPA) [R2]. This showed that there are multiple dispersed sequence/structure motifs in viral genomes, termed packaging signals (PSs) that are important for virus assembly. These signals consist of sparse and often non-contiguous sequence motifs in the virus's own RNA [R3] that, when presented in secondary structure elements of a specific type, collectively promote capsid formation around the viral genome [R4]. This has overturned the existing paradigm of virus assembly, demonstrating that the long-standing assumption of assembly driven solely by electrostatics is false. The mathematical approach, combining models of capsid assembly via Gillespie algorithms with insights from Hamiltonian paths, explains why the strong variation of the packaging signal sequences around a core motif is important for function: it results in changes of the PS affinities for capsid protein in a way that is tuned by nature during evolutionary cycles so as to optimise virus assembly. This variation around a sparse core motif, often just two nucleotides long, had made it difficult to identify such signals via a traditional sequence search, but it was possible with the mathematics of viral geometry [R5]. Most recently, Twarock has derived a general mathematical theory for viruses that solves a number of open puzzles in structural virology [R6].

In collaboration with biochemists at the Astbury Centre for Structural Molecular Biology in Leeds, the team used this approach to identify PSs in a number of viruses, including major viral pathogens [R3]. They discovered the hidden virus assembly code in clinically relevant viruses such as Hepatitis C, Picornaviruses (such as Human Parechovirus) and Hepatitis B (HBV). Recent results also cover HIV, and viruses from the family containing the common cold and polio virus. Together with experimentalists in Leeds, Twarock's group collaborated with the US National Institutes of Health (NIH) to demonstrate the successful drug targeting of crucial contacts between PSs and capsid protein. Her group's modelling showed that such drugs are less likely to elicit therapy resistance than conventional forms of anti-viral therapy. They also showed that these PSs can be engineered to optimise the packaging of RNA molecules into viral capsids, a technology that has now been patented (WO2018/220371). As viral capsids display tags within their proteins, known as epitopes, that stimulate the immune system, this technique can be used to create stable virus-like particles that are not infectious but still elicit an immune response. This has important applications in vaccinology, where one would want to stimulate an immune response without conferring an active infection. Moreover, the fundamental understanding of the assembly mechanism enables the optimised use of viral capsids as delivery packaging for novel gene therapy.

3. References to the research (indicative maximum of six references)

[R1] *Keef, T., Wardman, J.P., Ranson, N.A., Stockley, P.G. and Twarock, R., 2013. Structural constraints on the three-dimensional geometry of simple viruses: case studies of a new predictive tool. *Acta Cryst. A*, 69(2),140-150.DOI:10.1107/S0108767312047150

[R2] *Twarock, R., Leonov, G. and Stockley, P.G., 2018. Hamiltonian path analysis of viral genomes. *Nature communications*, 9(1),1-3. DOI:10.1038/s41467-018-03713-y

[R3] *Twarock, R., Bingham, R.J., Dykeman, E.C. and Stockley, P.G., 2018. A modelling paradigm for RNA virus assembly. Current opinion in virology, 31, pp.74-81. DOI:10.1016/j.coviro.2018.07.003

[R4] *+Dykeman, E.C., Stockley, P.G. and Twarock, R., 2014. Solving a Levinthal's paradox for virus assembly identifies a unique antiviral strategy. *Proceedings of the National Academy of Sciences*, 111(14), 5361-5366. DOI:10.1073/pnas.1319479111

[R5] *Patel, N., Wroblewski, E., Leonov, G., Phillips, S. E., Tuma, R., Twarock, R., & Stockley, P. G. (2017). Rewriting nature's assembly manual for a ssRNA virus. Proceedings of the National Academy of Sciences, 114(46), 12255-12260. DOI:10.1073/pnas.1706951114

[R6] *+Twarock, R. and Luque, A., 2019. Structural puzzles in virology solved with an overarching icosahedral design principle. *Nature communications*, 10(1), pp.1-9. DOI: 10.1038/s41467-019-12367-3

*= peer reviewed publication
+=returned to REF2021

4. Details of the impact (indicative maximum 750 words)

Drug discovery and the pharmaceutical industry

The research of Twarock and her team has revolutionised thinking in virology, changing the existing paradigm and opening up novel opportunities in anti-viral therapies, drug delivery and vaccination.

The NIH in Washington has used the packaging signals for Hepatitis B virus to develop a novel drug screening platform. Traditionally, anti-viral drugs are developed against protein components, but increased understanding of the importance of RNA secondary structure elements in biological function suggests that they would also be important drug targets. The work of Twarock and collaborators demonstrates that the packaging signals of viruses are precisely such a target, unique in biology because of their multiply dispersed nature across the genome. Using the packaging signals in Hepatitis B virus identified by Twarock's team, the National Cancer Institute (NCI) in the USA was able to develop and test "novel antiviral strategies against a virus with major public health implications" [E1]. The chief of the RT Biochemistry Section at NCI emphasises that "there is currently no other high throughput platform to identify RNA-binding targets with an equivalent efficiency of scale and cost, our joint efforts therefore represent an important step in drug discovery" [E1].
Knowledge of the PSs in a viral genome not only allows inhibition of virus assembly by blocking these vital contacts, but can also be used in the manufacturing of virus-like particles (VLPs), a current bottleneck in vaccination, drug delivery and gene therapy. Such technologies rely on the use of viral capsids as protein nanocontainers, to either present viral epitopes or deliver a cargo into a host cell. However, if viral protein containers are assembled in isolation without the presence of the viral genome, they tend to be less stable. Moreover, as only some of the particles contain the cargo as intended, a costly sifting step is required to isolate the particles that have correctly packaged cargo. Modelling by Twarock and her team, together with experimental work in Leeds, has shown that the packaging signals in viral genomes make the capsid shells more stable, as well as being important for specific packaging of the correct cargoes [E2]. Therefore, constructs mimicking the packaging signal distribution are required, as laid out in the patent ("Virus-Like Particles"; (WO2018/220371) [E3]. GlaxoSmithKline have shown interest in this technology, in particular in relation to work on coronavirus packaging signals, recently patented ("Coronavirus PS"; GB2011609.1) [E4;E3].

Twarock's group has developed algorithms that allow the redundancy of the genetic code to be exploited in order to use packaging signals to enable more efficient packaging of RNA. In particular, higher yields of correctly packaged particles are achieved via these recoding algorithms, addressing the bottleneck in the manufacturing of VLPs for therapeutic purposes. The managing director of InnoSpective Ltd recognises the "unique knowledge and capabilities to develop novel therapeutic approaches in areas seen as very high value for investment by addressing clear unmet clinical need within and beyond viral infection" [E5].

Public understanding of STEM subjects

Twarock's "strong record of outreach and community service" is highlighted in the announcement of her 2018 Gold Medal Award from the Institute of Mathematics and its Applications (IMA) [E6], citing articles in international science magazines such as Quanta and SIAM News, as well as documentaries such as "Thinking space" [E7], commissioned by the London Mathematical Society as part of its 150th Anniversary Celebrations, and BBC4's "Is mathematics discovered or created?" with Hannah Fry.

Her explanation of long-standing open problems in structural virology via tiling theory has formed the basis of a collaboration with digital artists, who have used her models to render viruses in 3D, allowing the general public to experience viruses in a virtual reality (VR) environment. A VR presentation, designed in collaboration with artists from Goldsmiths, was shown after her IMA Gold Medal Award Lecture at the Royal Society in 2019. This led to contributions to national and international arts exhibitions: at the Lowry Gallery in Manchester ("Inside the HSV1, Herpes Simplex Virus in VR - Artistic variations", 9 November 2019 – 23 February 2020), and at the Centre Pompidou in Paris ("Dans le cadre de Mutation /Créations", opened 25 February –16 March 2020, closing early due to COVID19) [E8a]. One Goldsmiths' artist writes "Based on your ground-breaking article, 'Structural puzzles in virology solved with an overarching, icosahedral design principle' in the journal Nature Communications (2019), we designed the public exhibition 'The State of Us' at the Lowry Gallery… with exhibition visitors averaging 140,000 per annum and which has close links to the BBC" [E8b]. Feedback from attendees at the Lowry exhibition showed that 65% agreed that, after the visit, they had a greater understanding of the molecular structure of viruses. One participant said that they "didn't even know how important mathematics were in understanding viruses before I used VR", whilst another said they had learnt that "there are different structures for each virus" [E8a]. Twarock's work has inspired artists interested in geometric patterns as exemplified by the artist-in-residence at the University of Pennsylvania who wrote to say that the research "continues to spark new ways of seeing and interpreting in the development of my work" (see Figure 1 below) [E8c].

Figure 1. Artwork by the artist-in-residence at the University of Pennsylvania, inspired by the research of Twarock and colleagues.

Twarock is both an ambassador and an advisor to MathsWorldUK, formed in 2013 to advance the education of the public in mathematics. She is involved in the design of the Maths and Medicine zone of a forthcoming Mathematics Discovery Centre and has been working with MathsWorldUK to create an exhibition 'Mathematics of Viruses and Pandemics' [E9]. The CEO of MathsWorldUK says her contribution has "proved vital in the success of the early stages of this project", supporting MathsWorldUK at a Royal Society event at which they were able to "secure a pledge of £350K from a philanthropist" [E9].

Twarock's work has influenced public understanding of mathematics and science via many public talks, such as that at the National Concert Hall in Dublin for Maths Week Ireland in 2018 [E10], visited by over 100 school children and their teachers.

5. Sources to corroborate the impact (indicative maximum of 10 references)

[E1] Letter of support from the chief of the RT (reverse transcriptase) Biochemistry Section at the National Cancer Institute, USA.

[E2] Review article "Follow the Yellow Brick Road: A Paradigm Shift in Virus Assembly" by leading microbiologist Peter Prevelige on the importance of the Hamiltonian Paths Approach in our understanding of virus assembly: DOI:10.1016/j.jmb.2015.12.009.

[E3] Patents: WO2018/220371
https://patentscope.wipo.int/search/en/detail.jsf?docId=WO2018220371
GB2011609.1 bit.ly/PatentApplicationsFiled (Date Lodged: 27 July 2020).

[E4] E-mail from Expert Scientist, Vaccine Design, GlaxoSmithKline.

[E5] Letter of support from Managing Director of InnoSpective Ltd.

[E6] IMA announcement: https://ima.org.uk/10158/ima-gold-medal-2018-awarded-to-professor-reidun-twarock/

[E7] Frames of mind: extracts from the London Mathematical Society (LMS) commissioned film "Thinking space. https://www.lms.ac.uk/library/frames-of-mind

[E8a] Feedback from exhibition at the Lowry Gallery in Manchester: https://thelowry.com/whats-on/the-state-of-us/
[E8b] Letter of support from digital artist at Goldsmiths, University of London.
[E8c] Email from artist-in-residence in the College of Arts and Sciences, University of Pennsylvania.

[E9] Email from CEO MathsWorldUK.

[E10] Maths Week Ireland 2018: Tweet and information from website https://www.mathsweek.ie/2018/