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Analysis of the role of VP35 in Ebola virus infection, focusing on the importance of protein structure in interferon response inhibition and RNA synthesis and the potential of these functions as drug targets

A report submitted as the examined component of the
Project Module SXL390 (H route)

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Emerging infectious disease

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Abstract

Since the first recorded outbreaks of Ebola virus in 1976, thousands of people in Africa have died of Ebola virus disease in regular outbreaks. While much is already understood regarding the transmission and virulence factors of this virus, few preventive or therapeutic drugs are available or in development. Could a better understanding of its virulence factors be the key to developing effective and affordable drugs?

While the overall structure of viral protein 35 (VP35), which is considered to be one of the key virulence factors of Ebola virus, is quite well documented, insights continue to be gained into how the protein folds and the number of subunits which combine, which could relate to protein function. Similarly, while much research has already been carried out into how VP35 and in particular its interferon inhibitory domain (IID) inhibit the interferon response in humans, new mechanisms continue to be discovered. VP35 is also known to be involved in RNA binding and RNA synthesis, but recent research has located the precise area of the protein required for this. Finally, with only limited – expensive – treatments for Ebola virus disease having been approved, computer-based insights into how compounds can bind to areas of VP35 and block its mechanisms are a promising avenue in finding new affordable treatments.

This literature review examines the current status of research into the structure and role of VP35, focusing specifically on the importance of protein structure in RNA synthesis and inhibition of the host interferon response. It then considers how insights gained are contributing to research into development of effective, inexpensive therapeutic treatments, including *in silico* and *in vitro* studies of compounds such as flavonoids.

[274 words]

List of abbreviations

CC	Coiled coil
dsRNA	Double-stranded RNA
EMSA	Electrophoretic mobility shift assay
GP	Glycoprotein
¹H NMR	Proton nuclear magnetic resonance
IC50	Half maximal inhibitory concentration
IFN	Interferon
IID	Interferon inhibitory domain
IRF	Interferon regulatory factor
ITC	Isothermal titration calorimetry
NP	Nucleoprotein
NPBP	Nucleoprotein-binding peptide
RIG-I	Retinoic acid-inducible gene I
RNA	Ribonucleic acid
RNAi	RNA interference
SEC-MALS	Size-exclusion chromatography and multi-light angle scattering
siRNA	Small interfering RNA
VP24	Viral protein 24
VP30	Viral protein 30
VP35	Viral protein 35
VP40	Viral protein 40

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1. Introduction

1.1 Background

Ebola virus is a highly contagious RNA virus with a high death rate per infected population (**mortality**) averaging 50% (WHO, 2021) that causes **haemorrhagic fever** (viral infection causing high fever and haemorrhage) in humans (Cantoni and Rossman, 2018). It has to date largely remained confined to Africa, with the few cases outside the continent being 'imported' through travel and resulting in little or no spread (Zinzula *et al.*, 2019; WHO, 2021), meaning that it may often be considered only a remote threat to health in most parts of the world. However, the recent COVID-19 pandemic has demonstrated viruses are not confined by national borders and our global lifestyles facilitate their spread. Given its virulence, there have also been concerns about its potential weaponisation (Maras and Miranda, 2016) in addition to the threat it represents to and the burden it places on public health.

Research to date has found that **viral protein 35 (VP35)**, which aids immune response evasion, is one of the main Ebola virus **virulence factors**, i.e. one of the molecules enabling it to successfully infect host cells. VP35 has been found to inhibit production of **interferon (IFN) α/β** (signalling proteins in the immune response) in a number of ways, including preventing activation of human immune signalling pathways, binding dsRNA and blocking phosphorylation of interferon-promoting transcription factors, and it also plays an important role in viral RNA synthesis (Leung *et al.*, 2015; Bharaj *et al.*, 2017; Dilley *et al.*, 2017; Di Palma *et al.*, 2019).

Despite extensive research into the mechanisms of infection of Ebola virus, few vaccines or treatments are in development or approved to date, including two relatively expensive monoclonal antibodies (EMA, 2021; WHO, 2021). However, development of further cost-effective vaccine and/or drug-based options is vital to prevent or respond to outbreaks due to the 'least developed country' status (UN, 2021) of many areas affected by Ebola virus and the ability of viruses to mutate. A better understanding of the virus' mechanisms is needed to achieve these advances in treatment/prevention.

1.2 Scope and objectives

Considering that VP35 is a major multifunctional virulence factor of Ebola virus and constitutes a potentially powerful target in tackling the virus, this report investigates how the structure of Ebola virus VP35 contributes to inhibiting the interferon response and its role in RNA synthesis. It also looks at how this knowledge could be exploited as a drug development target.

The objectives of the report are as follows:

1. Outline the emergence, geographic distribution and outbreaks of Ebola virus.
2. Discuss current understanding of Ebola virus transmission.
3. Describe the biology of Ebola virus and the mechanisms for its success as a viral pathogen. Introduce the VP35 protein.
4. Analyse the role of VP35 structure in inhibiting the interferon response.
5. Analyse the role of VP35 structure in RNA synthesis.
6. Evaluate the use of knowledge of the role of VP35 in inhibiting the interferon response and RNA synthesis as a potential target for preventive and/or therapeutic Ebola virus treatments.

1.3 Methodology

Literature searches were performed in PubMed, Web of Science and Google Scholar using Boolean keyword combinations (Table 1.1), focusing on peer-reviewed journals. Searches were largely limited to the past five years. Citations of papers of interest were also checked, and their references pursued until no further papers of interest were found.

Table 1.1 Examples of Boolean searches carried out

Ebola OR ebolavirus	AND VP35	AND interferon OR IFN
Ebola OR ebolavirus	AND virulence factors	
Ebola OR ebolavirus	AND VP35	AND RNA
Ebola OR ebolavirus	AND VP35	AND pathogenesis
Ebola OR ebolavirus	AND transmission	NOT COVID NOT SARS-COV-2
Ebola OR ebolavirus	AND VP35	AND flavonoids

2. Ebola virus

2.1 A brief overview of Ebola virus outbreaks

Ebola virus is a **zoonotic disease**, i.e. one that can transfer from animals to humans, that gets its name from the Ebola River in Democratic Republic of Congo where one of the first two recorded outbreaks of the Zaire subtype occurred in 1976 (Zawilińska and Kosz-Vnenchak, 2014). There have since been significant outbreaks in a number of African countries (Figure 2.1). While many outbreaks have been relatively small, the largest to date was in 2014-2016, affecting Guinea, Liberia and Sierra Leone, with total cases in the tens of thousands. The second-largest hit Democratic Republic of Congo in 2018-2020 with close to 4,000 cases (WHO, 2021). Mortality in previous outbreaks has ranged between approximately 25% and 90% (EMA, 2021; WHO, 2021), as shown in Figure 2.2. While there are six known subtypes of Ebola virus – Bombali, Bundibugyo, Reston, Sudan, Tai Forest and Zaire – the majority of outbreaks have involved the Zaire subtype, with the Sudan subtype being second-most common. The Zaire subtype is responsible for the large outbreaks in recent years (WHO, 2021). Of these six subtypes, only Bombali Ebola virus does not infect humans (Yamaoka and Ebihara, 2021).



Figure 2.1 Map of African countries experiencing significant Ebola virus outbreaks between 1976 and 2021 (created by the author, using an image from Andreas 06, 2006; data from WHO, 2021)

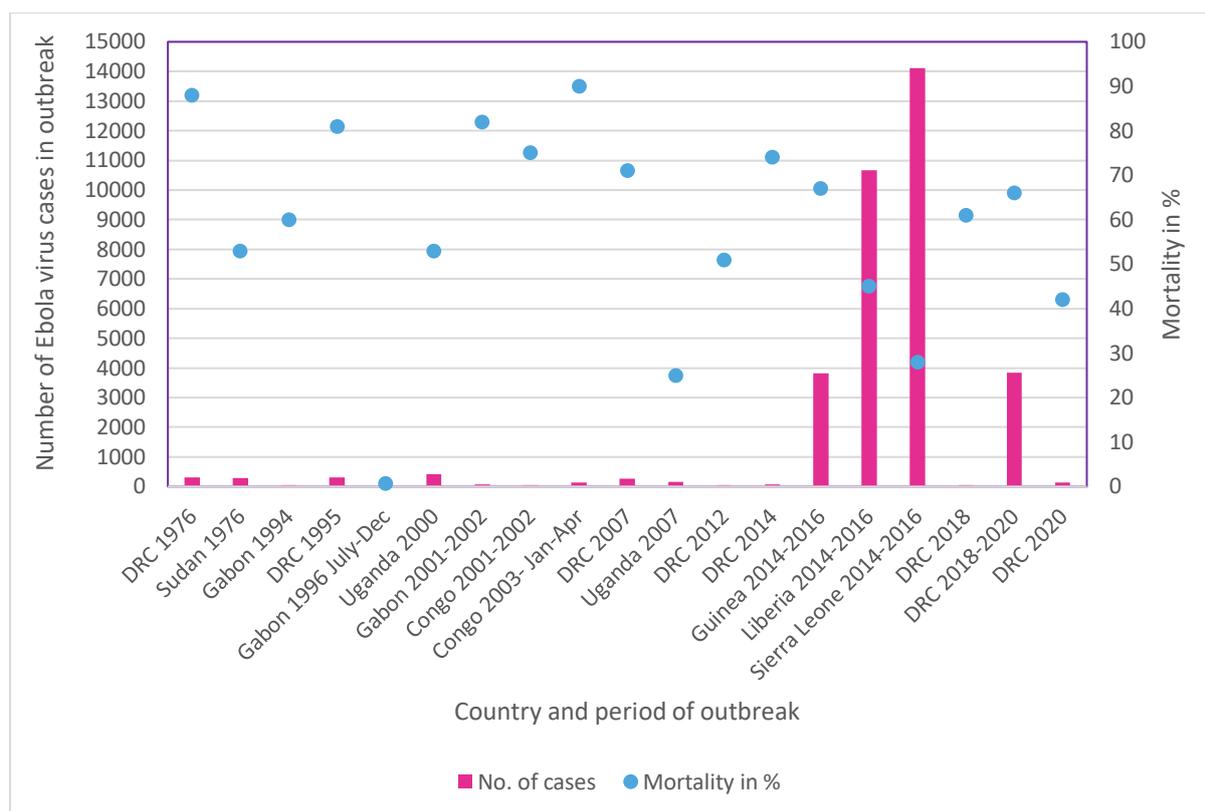


Figure 2.2 Overview of number of cases and mortality rates in percent in Ebola virus outbreaks exceeding 50 cases in African countries for the period 1976-2020 (minimum of 50 cases chosen for display purposes; data from Nanclares et al., 2016; WHO, 2021)

2.2 Ebola virus disease – infection and symptoms

Initial transmission of Ebola virus is thought to be through ‘**spillover events**’, i.e. contact with infected animals such as non-human primates or fruit bats, including via bushmeat, while human-human transmission is via contact with infected bodily fluids (blood, breastmilk, saliva and semen) or objects contaminated with infected bodily fluids, with virus entry via the mucous membranes (CDC, 2021b; Qazi, Khanam and Raza, 2021; WHO, 2021). Infection causes Ebola haemorrhagic fever. Symptoms of the disease (Figure 2.3) develop within three weeks of exposure and include fever and haemorrhage (CDC, 2021a). Individuals are infectious from when symptoms appear and for as long as the virus is in their blood (WHO, 2021). The virus can persist in ‘**immune-privileged**’ parts of the body, such as the eye and central nervous system, where there is generally no immune response to foreign antigens (Mak *et al.*, 2014, pp.237-8).

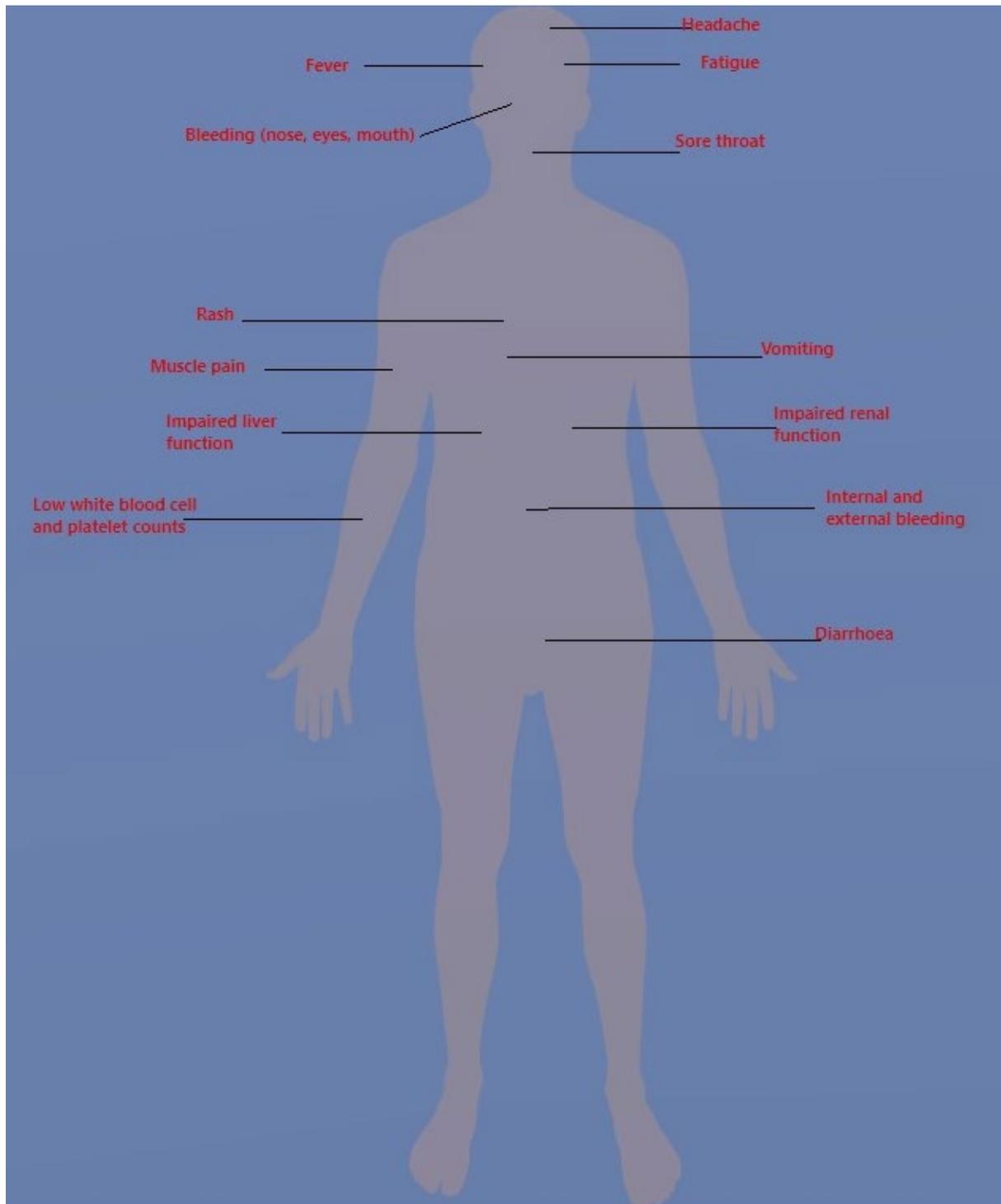


Figure 2.3 Symptoms of Ebola virus disease (created by the author, using an image by Häggström, 2009; information from CDC, 2021a; WHO, 2021)

2.3 Overview of the biology of Ebola virus

Ebola virus is a member of the *Filoviridae* (filovirus) family and is a non-segmented, negative-sense single-stranded RNA virus (Feldmann, Sprecher and Geisbert, 2020; Qazi, Khanam and Raza, 2021). The size of the Ebola virus genome is approximately 19kb, i.e. 19,000 base pairs (Leung *et al.*, 2015). The genomic structure of Ebola virus features seven genes, which encode nine proteins (Leung *et al.*, 2015; Yamaoka and Ebihara, 2021), as shown in Figure 2.4. The main virulence factors of Ebola virus are considered to be VP24 and VP35, which both hamper the IFN response (Cantoni and Rossman, 2018; Yamaoka and Ebihara, 2021), and glycoprotein (GP), which attaches to host cells and enables the virus to enter them (Cantoni and Rossman, 2018) as well as activating pro-inflammatory responses through interaction with toll-like receptor 4 (Yamaoka and Ebihara, 2021). The viral genome is encapsulated in a nucleocapsid, formed by VP30, VP35, RNA polymerase and nucleoprotein, surrounded by a viral matrix (VP24 and VP40), and the virus as a whole is covered by a viral envelope studded with transmembrane glycoproteins (Zawilińska and Kosz-Vnenchak, 2014; Yamaoka and Ebihara, 2021) as illustrated in Figure 2.5.



Figure 2.4 Genomic organisation of Ebola virus (based on Yamaoka and Ebihara, 2021, Figure 1)

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Figure 2.5 Structure of a filovirus (Feldmann, Sprecher and Geisbert, 2020, Figure 1A)

3. VP35 as a virulence factor

3.1 Structure of VP35

VP35 is considered one of the main virulence factors of Ebola virus and is known to consist of 340 amino acid residues (Fanunza *et al.*, 2019). The protein comprises two portions (Figures 3.1 and 3.2): an N and a C terminal. The coiled-coil (CC) N terminal consisting of **α helices** (corkscrew arrangement of amino acids, green in Figure 3.2), which is involved in replication, **chaperoning** i.e. guiding correct assembly and binding of nucleoprotein (Kirchdoerfer *et al.*, 2015), and **oligomerisation**, in which several polypeptide chains of a molecule interact to form a larger structure. The C terminal portion, known as the **interferon inhibitory domain (IID)** (Figure 3.1, residues 221-340), comprises an N-terminal subdomain of α helices and a C-terminal subdomain of **β sheet** (pleated sheet arrangement of amino acids, blue arrows in Figure 3.2) and is involved in IFN inhibition and binding dsRNA (Leung *et al.*, 2009; Brown *et al.*, 2014; Dapiaggi, Pieraccini and Sironi, 2015; Seesuy *et al.*, 2018; Di Palma *et al.*, 2019). The IID in particular is highly conserved in Ebola virus (Glanzer *et al.*, 2016), meaning it is relatively unchanged over the history of the virus, making it a good potential target in drug development.

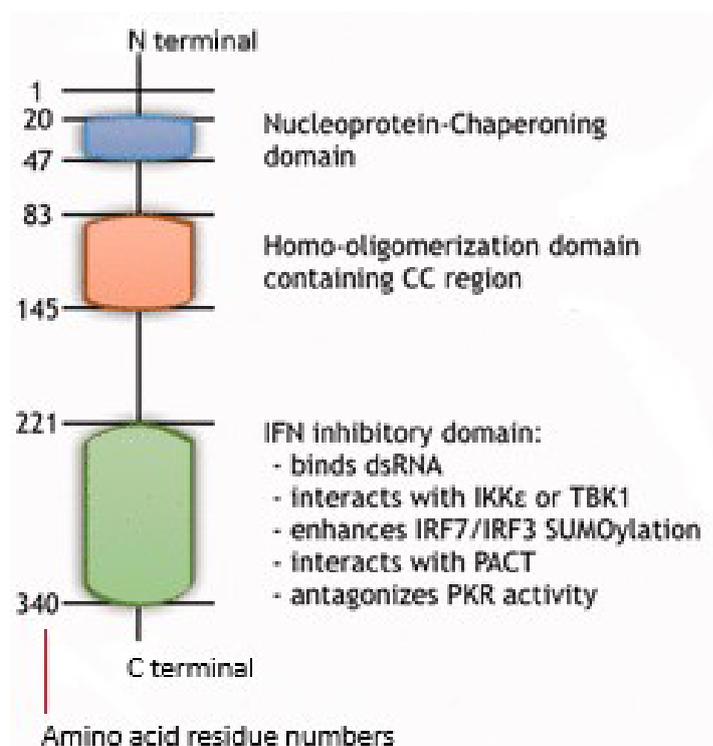


Figure 3.1 Ebola virus VP35 overview (adapted from Di Palma et al., 2019 Figure 1a)

IMAGE REMOVED FOR COPYRIGHT REASONS

Figure 3.2 Ribbon diagram of VP35 in complex with a ligand (orange), showing α helices (green) and β sheets (blue arrows; Dapiaggi, Pieraccini and Sironi, 2015, Figure 1)

VP35 suppresses the host immune response in a number of ways. Past research has found that VP35 suppresses **RNA interference (RNAi)**, meaning regulation of gene expression by RNA molecules (Haasnoot *et al.*, 2007). Here, VP35 sequesters **small interfering RNAs (siRNAs)**, which are non-coding RNA molecules involved in RNA interference produced from cleavage of RNA. It effectively hides them, preventing the action of host cell enzymes that would destroy them (Haasnoot *et al.*, 2007; Alberts *et al.*, 2008, pp.1534-5). It also blocks phosphorylation of interferon regulatory factors (**IRFs**) such as the kinases **TBK1** and **IKK ϵ** (Dilley *et al.*, 2017; Di Palma *et al.*, 2019), which is required to promote production of IFN α and β . In addition, it inhibits the action of the RNA-dependent protein kinase **PKR**, which is involved in the translation of mRNA into amino acids during protein synthesis. VP35 is also a recognised type I interferon (IFN) antagonist, meaning that it prevents the type I interferon response. It decreases production of type I IFNs α and β by inhibiting activation of the **RIG-I** immune signalling pathway in several ways: by binding to the RNA-binding domain of RIG-I and preventing dsRNA recognition, as

well as interacting with the PKR activating protein PACT, and binding with dsRNA, preventing activation of **interferon regulatory factor 3 (IRF-3)** (Figure 3.3) (Cárdenas *et al.*, 2006; Luthra *et al.*, 2013; Zinzula and Tramontano, 2013; Bharaj *et al.*, 2017).

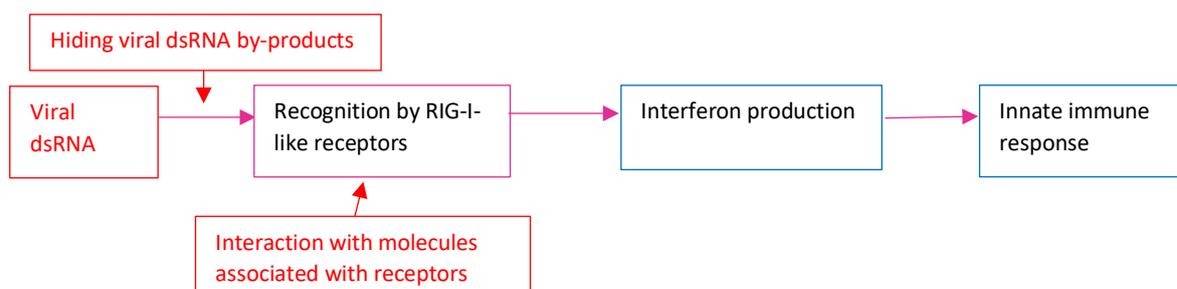


Figure 3.3 Ways in which Ebola virus VP35 inhibits the RIG-I pathway (based on Zinzula and Tramontano, 2013, Figures 1, 4 & 5)

3.2 VP35 structure and inhibition of the interferon response

Much research has already been carried out into the role of VP35 in inhibiting the interferon response, with a great deal focusing on its interventions at various points in the RIG-I pathway and blocking phosphorylation of IRF-3 (Zinzula and Tramontano, 2013; Cantoni and Rossman, 2018; Di Palma *et al.*, 2019). Previous research has been built on by investigating the structure of the protein overall and the CC region of its N terminal region based on their involvement in IFN inhibition. The findings both advance understanding of how VP35 inhibits the interferon response and could be applied in targeting this area in drug development. Studies have looked in particular at the CC region's involvement in the protein's ability to form **dimers** (complex formed by two protein monomers) and **oligomers** (complex formed by three or more protein monomers), a process which ultimately inhibits production of IFN by inhibiting IRF-3 (Möller *et al.* 2005 in Ramaswamy *et al.*, 2018). Approaches to investigating dimerisation and oligomerisation have varied. Di Palma *et al.* (2019) adopted an *in silico* approach using a proprietary software and standard settings to reveal mutations at key residues (L90, L93, L107A, creating VP35-3m). They found this affected the ability of the protein to form a stable dimer or CC structure versus wild-type VP35 (VP35-wt; Figure 3.4).

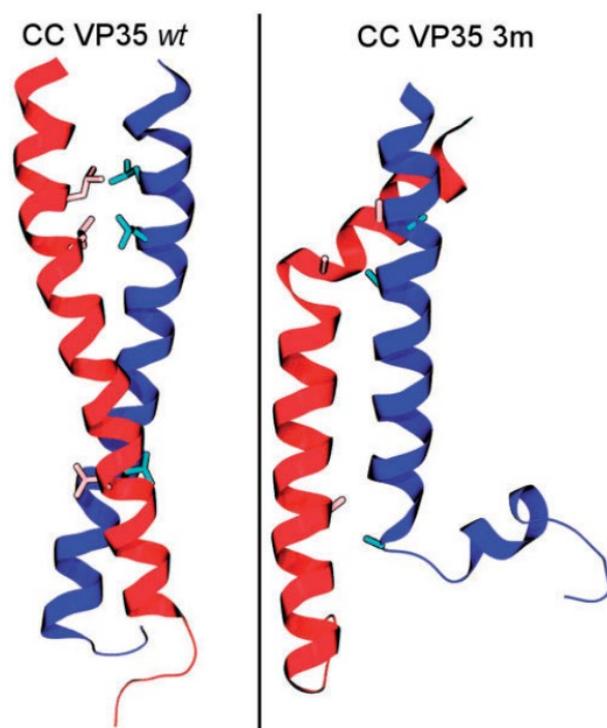


Figure 3.4 Coiled-coil conformation and dimerisation of VP35-wt (left) versus disordered structure of VP35-3m (right; Di Palma *et al.*, 2019, Figure 4)

In contrast to this purely *in silico* approach, several groups investigated the oligomerisation of VP35 using **size-exclusion chromatography and multi-angle light scattering (SEC-MALS)**, which is used to assess conformation based on molar mass and size. Chanthamontri *et al.* (2019) analysed three residue sequences (20-150, 50-150 and 70-150) using SEC-MALS, small-angle X-ray scattering and crosslinking with mass spectrometry and found VP35 primarily forms a four-subunit compound or **tetramer**, with some smaller oligomers (Figure 3.5). However, research by Zinzula *et al.* (2019) does not wholly support this conclusion. They focused on a narrower residue range (82-145), also using SEC-MALS and mass spectrometry in addition to far UV circular dichroism spectroscopy analysis to determine secondary structure (three-dimensional arrangement of polypeptides; Loughlin, 2014). They found that this region can in fact form tetramers or **trimers** (three-subunit compound) in solution (Figure 3.6), and suggest that the tetramer form is functionally dominant. Like Di Palma *et al.* (2019), Ramaswamy *et al.* (2018) also used *in silico* modelling to investigate structure, but in a narrow stretch of residues (83-120) in the CC region. Simulated oligomerisation predicted dimerisation of the CC region in line with Di Palma *et al.* (2019) and – similarly to Chanthamontri *et al.* (2019) – trimers and tetramers

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Figure 3.5 Graph showing the relative abundance in percent of monomers and oligomers of three residue sequences of VP35, as established by mass spectrometry (from Chanthamontri *et al.*, 2019, Figure 1d)

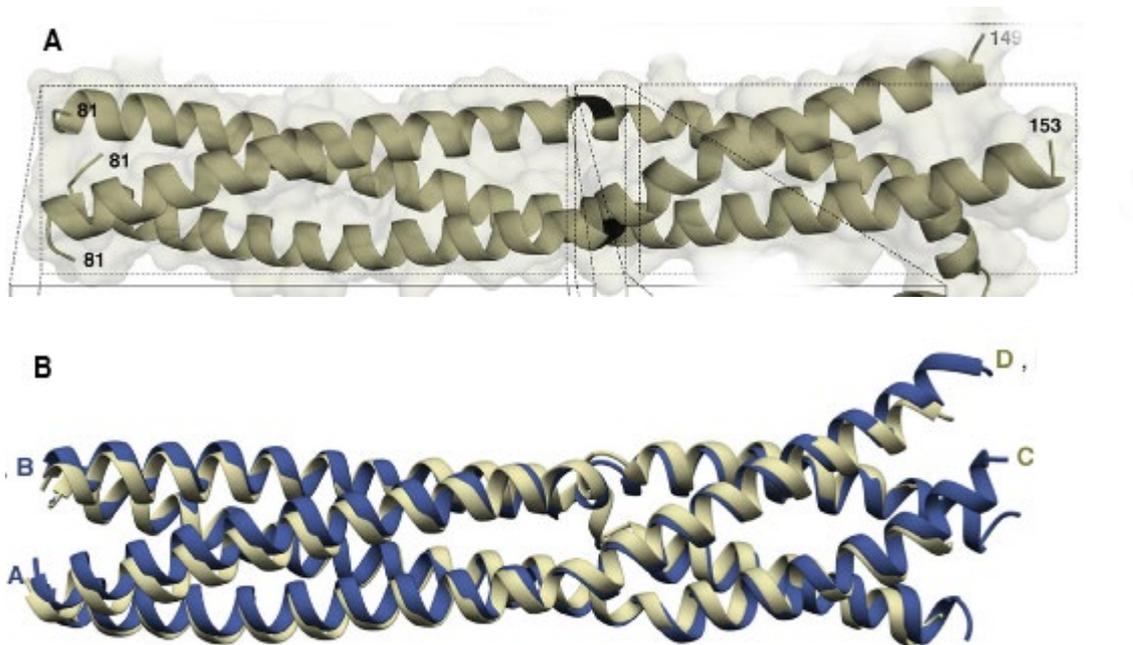


Figure 3.6 VP35 as a trimer (A) and tetramer (B) (adapted from (Zinzula *et al.*, 2019, Figures 2c and 7a)

Unlike other groups, Di Palma *et al.* (2019) moved their research into *in vitro* testing to assess the effects of changing protein conformation on VP35's ability to inhibit IFN β production. An *in vitro* luciferase reporter gene assay in cancerous lung epithelial cells (A549 cells) transfected with IFN β and 100-250ng VP35-m or VP35-wt or nothing (control) assessed inhibition of IFN production. Influenza A RNA was added after 24 hours to assess the IFN response, and while VP35-3m was still found to inhibit IFN β production, this inhibition was significantly reduced versus VP35-wt (Figure 3.7).

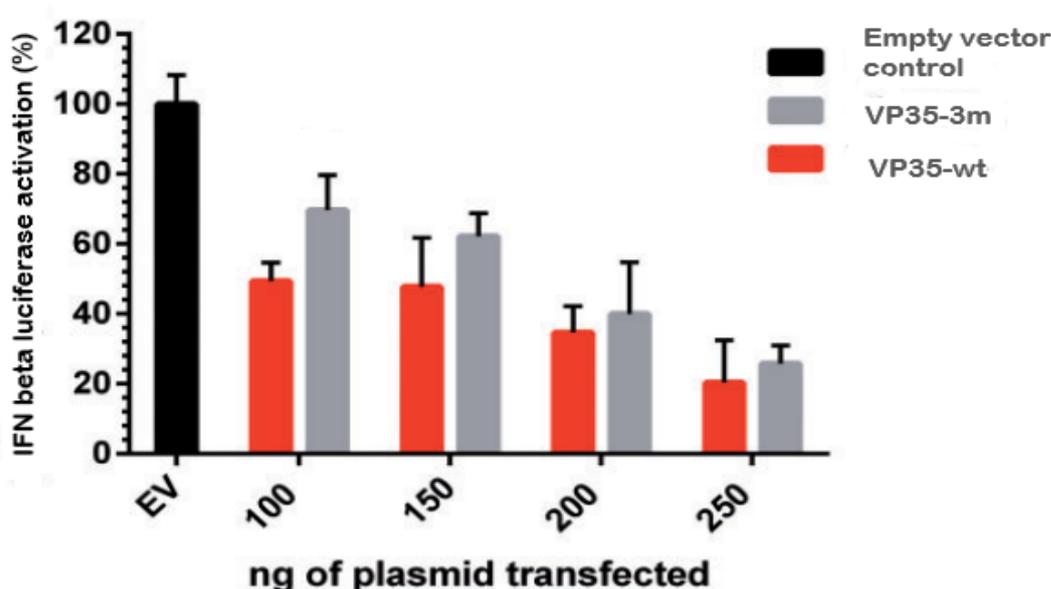


Figure 3.7 Inhibition of IFN β production by mutated VP35 (VP35-3m) versus wild type VP35 (VP35-wt) and control (adapted from Di Palma *et al.*, 2019, Figure 6b

3.3 VP35 structure and RNA synthesis

While the overall structure of Ebola virus has been well described in the literature, better understanding of VP35's structure and how it interacts with RNA could make a major contribution to drug development by enabling more accurate drug-target modelling. Along with L protein, VP30 and nucleoprotein, VP35 is part of the RNA-dependent RNA polymerase complex, which catalyses RNA replication and protein synthesis (Leung *et al.*, 2015). This makes VP35 essential for virus replication and viral protein synthesis.

VP35's IID has proven to be of significant research interest. Prins *et al.* (2010) previously found a group of residues known as the 'first basic patch' (Figure 3.8) in the IID to be crucial in nucleoprotein binding, meaning it is required for viral RNA synthesis given the involvement of VP35 and nucleoprotein in the RNA polymerase complex. This finding has since been confirmed by Leung *et al.* (2015) and Miyake *et al.* (2020). Leung *et al.* (2015) used a technique known as **isothermal titration calorimetry (ITC)**, which measures how much heat is absorbed or released when molecules bind (Malvern Panalytical, no date). The technique was applied to precisely locate the 'nucleoprotein-binding peptide' (NPBP), i.e. the area of the IID that binds to nucleoprotein, based on binding affinity. The study concluded residues 20-48 (NPBP), were crucial to high-affinity binding (Table 3.1; a lower binding constant (K_D) indicates greater binding affinity), which fit with the findings of Prins *et al.* Leung *et al.* (2015) further confirmed in tests using a shortened version of the viral genome or '**minigenome**' that deletion of the NPBP and the 19 residues preceding it resulted in almost total loss of minigenome activity as a model of viral replication. Meanwhile, Miyake *et al.* (2020) used **pulldown assays** to determine the effect of key residues in the IID on nucleoprotein binding. This technique is used to determine interactions between proteins and detect levels of protein activation (ThermoFisher Scientific, no date b). The study found that mutations to two key residues in the first basic patch prevented binding to the NPBP while mutations to two key residues from the 'central basic patch' area of the IID did not affect binding, thereby supporting the findings of Prins *et al.* (2010).

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Figure 3.8 VP35 IID showing residues (pink) from the first basic patch (from Prins et al., 2010, Figure 1A)

Table 3.1 Binding affinity of sections of VP35 to the nucleoprotein N-terminal domain (adapted from Leung et al., 2015 Table S2C)

VP35 residues	Equilibrium dissociation constant (K_D in nM)
20→48	28.9 ± 6.4
26→48	237 ± 49
27→48	162 ± 25
28→48	215 ± 62
29→48	Not determined
30→48	Not determined

3.4 Discussion

The varying results concerning VP35 oligomerisation is an area which could warrant further investigation. Ramaswamy *et al.*'s (2018) *in silico* research would seem to confirm the accuracy of computer-based modelling, as the results align with the *in vitro* research of Chanthamontri *et al.* (2019). However, more evidence of *in silico* modelling being confirmed by *in vitro* results would be valuable. It is interesting that overall similar approaches based around SEC-MALS by Chanthamontri *et al.* (2019) and Zinzula *et al.* (2019) resulted in different conclusions as regards predominant formation of trimers or tetramers. However, the slight differences in residue ranges may have played a role here. Additionally, while this area of VP35 is known to be involved in IFN inhibition, neither group tackled conformation in the context of function, with Chanthamontri *et al.* (2019) noting specifically that the effect of structural difference on function was unknown and Ramaswamy *et al.* (2018) expressing a need for research here. Only Di Palma *et al.* (2019) assessed function, and the inhibition of IFN production found would appear to confirm and demonstrate the importance of protein conformation on at least this function.

Although different approaches were used, the findings of research into VP35 and nucleoprotein binding from the various groups largely corresponded, indicating that all techniques used were appropriate. It is also indicative of the findings being sound, in that certain key residues and residue ranges are important in VP35 binding to nucleoprotein and therefore in RNA synthesis, as without formation of the RNA polymerase complex which includes these two proteins, RNA synthesis and thus virus proliferation cannot occur.

4. Development of Ebola virus treatments

4.1 VP35 as a potential treatment target

Given the limited number of approved Ebola virus disease treatments (CDC, 2021c) and the possibility of virus mutations making treatments ineffective, finding new and particularly cost-effective drugs is essential. Affordability is also vital in light of the 'least developed country' status (UN, 2021) of the nations affected by outbreaks. Development of anything other than affordable drugs would mean they were out of reach for the majority in the countries concerned, would remain largely unused and would therefore not help to prevent deaths and disease spread. The role of VP35 as a key virulence factor of the virus makes it an obvious potential target in drug development, particularly given that mutations preventing its proper function have been found to impact on virulence (Fanunza *et al.*, 2019). Greater insight into VP35 gained from research above, in particular as regards its structure, could therefore contribute significantly to drug development.

4.2 VP35 and *in silico* modelling in drug design

Research into the highly conserved VP35 C terminal IID domain has proven it is a good potential target for drug development. Much of the research to date has focused on *in silico* (computer-based) structure-based screening of compounds. This involves modelling how – and how well – compounds of interest would dock to sections of the IID based on the types of chemical bonds such as hydrogen bonds and hydrophobic interactions the software predicts will form (Figure 4.1) and the binding energy at these sites (Raj and Varadwaj, 2016; Setlur *et al.*, 2017).

IMAGE REMOVED FOR COPYRIGHT REASONS

Figure 4.1 Bonding and interaction of myricetin with residues of the VP35 IID predicted by *in silico* docking (adapted from Daino et al. (2018), Figure 5b)

Several research groups have modelled natural or other small compounds as possible treatments targeting VP35. Choices of compounds of interest have varied, with investigations of wide ranges of compounds from compound libraries (Glanzer *et al.*, 2016; Raj and Varadwaj, 2016), selections of more limited numbers of compounds from the literature (Ren *et al.*, 2016; Setlur *et al.*, 2017) and focuses on specific compounds of interest such as curcumin and derivative compounds (Baikerikar, 2017) and the flavonoid myricetin (Daino *et al.*, 2018).

Docking approaches have also varied, with Daino et al (2016) opting for a 'consensus approach', combining three to four algorithms (Figure 4.2), and Raj and Varadwaj (2016) taking a similar approach by applying three phases of screening with different levels of precision to find the optimum docking position of compounds. By contrast other research groups have used a single algorithm approach (Ren *et al.*, 2016; Baikerikar, 2017; Setlur *et al.*, 2017), which may not provide as precise a solution to the actual docking of compounds. Glanzer *et al.* (2016) also adopted a slightly different approach, running *in silico* docking of compounds against both a single static model of the IID and a flexible model of the IID based on its 26 known crystal structures to improve the accuracy of the predicted docking affinity. All approaches were able to find potential compounds of interest that could potentially be developed further.

IMAGE REMOVED FOR COPYRIGHT REASONS

Figure 4.2 ‘Consensus approach’ to docking showing overlapping binding of myricetin to VP35 (adapted from Daino et al. (2018), Figure 5a)

Despite research such as the above finding numerous promising compounds that could be effective – and affordable – Ebola virus treatments, little of the research has been applied beyond the *in silico* phase to date. Glanzer et al (2016) and Daino et al (2016) are two of the few examples where *in vitro* testing has confirmed successful inhibition of VP35-dsRNA binding as predicted by *in silico* modelling. Both research groups ultimately aimed to find the **IC50** (concentration at which the compound produces half of its maximum inhibitory effect; AAT Bioquest, 2019) of their compounds of interest. However, they applied different methods to show protein-protein interactions first. Glanzer et al (2016) used an **electrophoretic mobility shift assay (EMSA)** to test inhibition of VP35-RNA binding. EMSA is a technique that can be used to determine protein-protein interactions with RNA by rate of movement of substances through agarose gel based on size and charge (ThermoFisher Scientific, no date a). Compounds of interest were identified using used proton nuclear magnetic resonance (H NMR), a form of spectroscopy, to determine the compounds

in the most successful extracts analysed. By contrast, Daino *et al.* (2018) developed a novel assay to assess VP35-RNA binding. They adapted existing techniques by using fluorescence rather than radiolabelling of the RNA for cost and safety reasons, then investigated RNA-VP35 interaction in a pulldown assay, and measured the fluorescence signal from RNA bound to VP35 using a plate reader set up to read the relevant wavelengths.

Both groups also tested the inhibition of VP35-RNA binding of their compounds of interest through serial dilutions to determine the IC₅₀ as shown in Table 4.1. The *in silico* findings of Glanzer *et al.* (2016) for the compound ZINC05328460 were shown to be predictive of their *in vitro* results. Meanwhile, Daino *et al.* (2018) found myricetin to have the lowest IC₅₀ of their two main compounds of interest (myricetin and EGCG; Table 4.1). The results of their *in silico* and *in vitro* testing therefore suggest the mechanism of action behind the effects of myricetin *in vitro*.

Table 4.1 Inhibition of VP35-RNA binding (data from Daino *et al.*, 2018, Table 2 and Glanzer *et al.*, 2016)

Compound	IC ₅₀ (µg/mL)
Epigallocatechin 3-Ogallate (EGCG) (Daino <i>et al.</i> , 2018)	2.7 ± 0.9
Myricetin (Daino <i>et al.</i> , 2018)	43.5 ± 4.2
ZINC05328460 (Glanzer <i>et al.</i> , 2016).	approx. 4 (no SD given)

4.3 Discussion

The various approaches to *in silico* screening are interesting. It could be argued that a more standardised approach to modelling docking should be adopted in research as the slight differences in method could result in varying levels of accuracy. For example, while the single algorithms applied by Ren *et al.* (2016) and Baikerikar (2017) may well be quite accurate, the application of a ‘consensus approach’ of three to four algorithms by (Daino *et al.*, 2018) seems likely to give far greater accuracy. However, it could equally be argued that (Ren *et al.*, 2016) aim to overcome any potential shortcomings in individual steps of their method by applying three phases – high-throughput screening, standard precision and extra-precision – to achieve more accurate compound-protein docking results. While the methodologies are similar, the

slight differences in approach do make it difficult to assess them, as there is seemingly no 'standard'.

It is also notable that Daino *et al.* (2018) opted to screen compounds *in vitro* first, before using docking to determine the mechanism of action of their ultimate compound of interest (myricetin) – a decision which is not clearly explained. This seems slightly 'backwards' and does not fit with the approach that seems standard among other research groups (e.g. Glanzer *et al.*, 2016), where compounds are first screened *in silico* before moving into *in vitro* and potentially *in vivo* testing. In addition, this is potentially a more expensive approach given what should be lower costs of computer modelling of a large number of compounds versus testing a smaller number of compounds *in vitro*.

Given the promising results with *in silico* testing of these more affordable compounds, it is disappointing that there has not been further *in vitro* or even *in vivo* testing of these potential treatments. At some point, the purpose of all of this research has to be questioned if it does not move into more meaningful testing and ultimately – hopefully – application. Yet several years after research by these groups, there are no published results of studies that have moved these findings forward.

5. Conclusion

This literature review has given a clear background to Ebola virus, including its history and biology. It has discussed the role of VP35 as a viral virulence factor, and given a more detailed insight into current research into the role of its structure in RNA synthesis and inhibiting the IFN response. It has also looked in some detail at how this knowledge is being applied to target VP35 in preventive and therapeutic Ebola virus disease treatment. While the review has necessarily been limited by the wordcount, the objectives have been met.

Overall, VP35 has been shown to be a promising target for drug development given its highly conserved nature between strains and over time, and as one of the primary virulence factors of the virus. While it has been shown that research is ongoing into targeting this viral protein, it is disappointing that little has moved beyond the *in silico* phase. It is equally discouraging from an affordability perspective that the only two therapeutic drugs approved to date are monoclonal antibodies.

Going forward, it would seem rational to continue to focus on VP35 as a target. Standardising *in silico* approaches to a flexible docking approach, perhaps also with a consensus base, would seem to be wise for good accuracy. It would also be prudent to begin *in vitro* and later *in vivo* testing of more of the compounds that have shown promise in *in silico* testing – particularly those that are lower cost, as more affordable options for the countries affected. This could ultimately mean an effective and – just as importantly – inexpensive treatment option for this devastating virus.

References

- AAT Bioquest (2019) *What is the ic50 value?* Available at: <https://www.aatbio.com/resources/faq-frequently-asked-questions/What-is-the-ic50-value> (Accessed: 15 August 2021).
- Alberts, B. *et al.* (2008) 'Pathogen', in *Molecular Biology of the Cell*. Fifth Edit. New York: Garland Science, pp. 1485–1538.
- Andreas 06 (2006) 'Blank Map – Africa', *Wikimedia Commons*. Available at: https://commons.wikimedia.org/wiki/File:Blank_Map-Africa.svg (Accessed: 3 July 2021).
- Baikerikar, S. (2017) 'Curcumin and natural derivatives inhibit Ebola viral proteins: An In silico approach', *Pharmacognosy Research*, 9 (Suppl.), pp. S15–S22. doi: 10.4103/pr.pr_30_17.
- Bharaj, P. *et al.* (2017) 'The Host E3-Ubiquitin Ligase TRIM6 Ubiquitinates the Ebola Virus VP35 Protein and Promotes Virus Replication', *Journal of Virology*, 91(18). doi: 10.1128/jvi.00833-17.
- Brown, C. S. *et al.* (2014) 'In Silico Derived Small Molecules Bind the Filovirus VP35 Protein and Inhibit Its Polymerase Cofactor Activity', *Journal of Molecular Biology*, 426(10), pp. 2045–2058. doi: 10.1016/j.jmb.2014.01.010.
- Cantoni, D. and Rossman, J. S. (2018) 'Ebolaviruses: New roles for old proteins', *PLoS Neglected Tropical Diseases*, 12(5), article no. e0006349. doi: 10.1371/journal.pntd.0006349.
- Cárdenas, W. B. *et al.* (2006) 'Ebola Virus VP35 Protein Binds Double-Stranded RNA and Inhibits Alpha/Beta Interferon Production Induced by RIG-I Signaling', *Journal of Virology*. doi: 10.1128/jvi.02199-05.
- CDC (2021a) *Ebola (Ebola Virus Disease): Signs and Symptoms*. Available at: <https://www.cdc.gov/vhf/ebola/symptoms/index.html> (Accessed: 26 June 2021).
- CDC (2021b) *Ebola (Ebola Virus Disease): Transmission*. Available at: <http://www.cdc.gov/vhf/ebola/transmission/> (Accessed: 26 June 2021).
- CDC (2021c) *Ebola (Ebola Virus Disease): Treatment*. Available at: <https://www.cdc.gov/vhf/ebola/treatment/index.html> (Accessed: 8 May 2021).
- Chanthamontri, C. K. *et al.* (2019) 'The Ebola Viral Protein 35 N-Terminus Is a Parallel Tetramer', *Biochemistry*, 58(6), pp. 657–664. doi: 10.1021/acs.biochem.8b01154.
- Daino, G. L. *et al.* (2018) 'Identification of Myricetin as an Ebola Virus VP35–Double-Stranded RNA Interaction Inhibitor through a Novel Fluorescence-Based Assay', *Biochemistry*, 57(44), pp. 6367–6378. doi: 10.1021/acs.biochem.8b00892.
- Dapiaggi, F., Pieraccini, S. and Sironi, M. (2015) 'In silico study of VP35 inhibitors: From computational alanine scanning to essential dynamics', *Molecular BioSystems*, 11(8), pp. 2152–2157. doi: 10.1039/c5mb00348b.
- Dilley, K. A. *et al.* (2017) 'The Ebola virus VP35 protein binds viral

immunostimulatory and host RNAs identified through deep sequencing', *PLOS ONE*. Edited by R. Lu, 12(6), article no. e0178717. doi: 10.1371/journal.pone.0178717.

EMA (2021) *Ebola*. Available at: <https://www.ema.europa.eu/en/human-regulatory/overview/public-health-threats/ebola> (Accessed: 16 March 2021).

Fanunza, E. *et al.* (2019) 'Insights into Ebola Virus VP35 and VP24 Interferon Inhibitory Functions and their Initial Exploitation as Drug Targets', *Infectious Disorders - Drug Targets*, 19(4), pp. 362–374. doi: 10.2174/1871526519666181123145540.

Feldmann, H., Sprecher, A. and Geisbert, T. W. (2020) 'Ebola', *New England Journal of Medicine*. Edited by E. W. Campion, 382(19), pp. 1832–1842. doi: 10.1056/NEJMra1901594.

Glanzer, J. G. *et al.* (2016) 'In silico and in vitro methods to identify ebola virus VP35-dsRNA inhibitors', *Bioorganic & Medicinal Chemistry*, 24(21), pp. 5388–5392. doi: 10.1016/j.bmc.2016.08.065.

Haasnoot, J. *et al.* (2007) 'The ebola virus VP35 protein is a suppressor of RNA silencing', *PLoS Pathogens*. doi: 10.1371/journal.ppat.0030086.

Häggström, M. (2009) 'Human body silhouette', *Wikimedia Commons*. Available at: https://commons.wikimedia.org/wiki/File:Human_body_silhouette.svg (Accessed: 3 July 2021).

Kirchdoerfer, R. N. *et al.* (2015) 'Assembly of the Ebola virus nucleoprotein from a chaperoned VP35 complex', *Cell reports*. NIH Public Access, 12(1), p. 140. doi: 10.1016/J.CELREP.2015.06.003.

Leung, D. W. *et al.* (2009) 'Structure of the Ebola VP35 interferon inhibitory domain', *Proceedings of the National Academy of Sciences*, 106(2), pp. 411–416. doi: 10.1073/pnas.0807854106.

Leung, D. W. *et al.* (2015) 'An Intrinsically Disordered Peptide from Ebola Virus VP35 Controls Viral RNA Synthesis by Modulating Nucleoprotein-RNA Interactions', *Cell Reports*, 11(3), pp. 376–389. doi: 10.1016/j.celrep.2015.03.034.

Loughlin, J. (2014) 'Proteins', in *S294 Book 2: Working Cells*. Milton Keynes: The Open University, pp. 1–48.

Luthra, P. *et al.* (2013) 'Mutual antagonism between the ebola virus VP35 protein and the RIG-I activator PACT determines infection outcome', *Cell Host and Microbe*. doi: 10.1016/j.chom.2013.06.010.

Mak, T. W. *et al.* (2014) 'Regulation of Immune Responses in the Periphery', in *Primer to the Immune Response*, pp. 227–246. doi: 10.1016/b978-0-12-385245-8.00010-8.

Malvern Panalytical (no date) *Isothermal Titration Calorimetry*. Available at: <https://www.malvernpanalytical.com/en/products/technology/microcalorimetry/isothermal-titration-calorimetry> (Accessed: 6 July 2021).

Maras, M. H. and Miranda, M. D. (2016) 'The weaponization of Ebola: A new risk in the wake of an outbreak?', *Comparative Strategy*, 35(1), pp. 72–79. doi: 10.1080/01495933.2016.1134000.

Miyake, T. *et al.* (2020) 'Ebola Virus Inclusion Body Formation and RNA Synthesis Are Controlled by a Novel Domain of Nucleoprotein Interacting with VP35', *Journal of Virology*. Edited by M. T. Heise, 94(16). doi: 10.1128/JVI.02100-19.

Nanclares, C. *et al.* (2016) 'Ebola virus disease, democratic republic of the Congo, 2014', *Emerging Infectious Diseases*, 22(9), pp. 1579–1586. doi: 10.3201/eid2209.160354.

Di Palma, F. *et al.* (2019) 'Relevance of Ebola virus VP35 homo-dimerization on the type I interferon cascade inhibition', *Antiviral Chemistry and Chemotherapy*, 27, pp. 1–13. doi: 10.1177/2040206619889220.

Prins, K. C. *et al.* (2010) 'Basic Residues within the Ebolavirus VP35 Protein Are Required for Its Viral Polymerase Cofactor Function', *Journal of Virology*, 84(20), pp. 10581–10591. doi: 10.1128/JVI.00925-10.

Qazi, S., Khanam, A. and Raza, K. (2021) 'Ebola Virus: Overview, Genome Analysis and Its Antagonists', in *Human Viruses: Diseases, Treatments and Vaccines*. Cham: Springer International Publishing, pp. 123–142. doi: 10.1007/978-3-030-71165-8_6.

Raj, U. and Varadwaj, P. K. (2016) 'Flavonoids as Multi-target Inhibitors for Proteins Associated with Ebola Virus: In Silico Discovery Using Virtual Screening and Molecular Docking Studies', *Interdisciplinary Sciences: Computational Life Sciences*, 8(2), pp. 132–141. doi: 10.1007/s12539-015-0109-8.

Ramaswamy, V. K. *et al.* (2018) 'Insights into the homo-oligomerization properties of N-terminal coiled-coil domain of Ebola virus VP35 protein', *Virus Research*, 247, pp. 61–70. doi: 10.1016/j.virusres.2018.02.003.

Ren, J. X. *et al.* (2016) 'Identification of novel VP35 inhibitors: Virtual screening driven new scaffolds', *Biomedicine and Pharmacotherapy*, 84, pp. 199–207. doi: 10.1016/j.biopha.2016.09.034.

Seesuay, W. *et al.* (2018) 'Human transbodies that interfere with the functions of Ebola virus VP35 protein in genome replication and transcription and innate immune antagonism', *Emerging Microbes & Infections*, 7(1), pp. 1–15. doi: 10.1038/s41426-018-0031-3.

Setlur, A. S. *et al.* (2017) 'Herbal Lead as Ideal Bioactive Compounds Against Probable Drug Targets of Ebola Virus in Comparison with Known Chemical Analogue: A Computational Drug Discovery Perspective', *Interdisciplinary Sciences: Computational Life Sciences*, 9, pp. 254–277. doi: 10.1007/s12539-016-0149-8.

ThermoFisher Scientific (no date a) *Gel Shift Assays (EMSA)*. Available at: [//www.thermofisher.com/uk/en/home/life-science/protein-biology/protein-biology-learning-center/protein-biology-resource-library/pierce-protein-methods/gel-shift-assays-ems.html](https://www.thermofisher.com/uk/en/home/life-science/protein-biology/protein-biology-learning-center/protein-biology-resource-library/pierce-protein-methods/gel-shift-assays-ems.html) (Accessed: 13 August 2021).

ThermoFisher Scientific (no date b) *Pull-Down Assays*. Available at: [//www.thermofisher.com/uk/en/home/life-science/protein-biology/protein-biology-learning-center/protein-biology-resource-library/pierce-protein-methods/pull-down-assays.html](https://www.thermofisher.com/uk/en/home/life-science/protein-biology/protein-biology-learning-center/protein-biology-resource-library/pierce-protein-methods/pull-down-assays.html) (Accessed: 6 July 2021).

UN (2021) *UN list of least developed countries* | UNCTAD. Available at: <https://unctad.org/topic/least-developed-countries/list> (Accessed: 16 March 2021).

WHO (2021) *Ebola virus disease*. Available at: <https://www.who.int/news-room/fact-sheets/detail/ebola-virus-disease> (Accessed: 16 March 2021).

Yamaoka, S. and Ebihara, H. (2021) 'Pathogenicity and Virulence of Ebolaviruses with Species- and Variant-specificity', *Virulence*, pp. 885–901. doi: 10.1080/21505594.2021.1898169.

Zawilińska, B. and Kosz-Vnenchak, M. (2014) 'Introduction into the Ebola virus biology and disease', *Folia Medica Cracoviensia General*, LIV(3), pp. 57–65. Available at: http://www.fmc.cm-uj.krakow.pl/pdf/54_3_57.pdf (Accessed: 27 June 2021).

Zinzula, L. *et al.* (2019) 'Structures of Ebola and Reston Virus VP35 Oligomerization Domains and Comparative Biophysical Characterization in All Ebolavirus Species', *Structure*, 27(1), pp. 39–54. doi: 10.1016/j.str.2018.09.009.

Zinzula, L. and Tramontano, E. (2013) 'Strategies of highly pathogenic RNA viruses to block dsRNA detection by RIG-I-like receptors: Hide, mask, hit', *Antiviral Research*, 100(3), pp. 615–635. doi: 10.1016/j.antiviral.2013.10.002.

Glossary

α helix	A type of secondary structure in proteins; the amino acid residues are arranged in a corkscrew
β sheet	A type of secondary structure in proteins; the amino acid residues are arranged in a pleated sheet
Dimer	A complex formed by two protein monomers)
Electrophoretic mobility shift assay (EMSA)	A technique which determines protein interactions with DNA/RNA using agarose gel electrophoresis. It is based on DNA/RNA moving more slowly through the gel when bound to a protein.
Haemorrhagic fever	A serious viral infection causing high fever and bleeding from the organs and/or mouth, nose; examples include Ebola virus disease
IC50	Concentration at which a drug produces half of its maximum inhibitory effect
IKKϵ	Inhibitor- κ B kinase ϵ ; an enzyme involved in the innate immune response; induces type I interferons
Immune-privileged	Refers to parts of the body where there is generally no immune response to foreign antigens
Interferons / IFNs	Signalling proteins that are part of the innate immune response
Interferon inhibitory domain / IID	Section of the VP35 protein involved in dsRNA binding and interferon inhibition
Interferon regulatory factor 3 / IRF-3	A transcription factor involved in the activation of IFN α and β production
Isothermal titration calorimetry	A technique which measures how much heat is absorbed or released when molecules bind; can be used to determine how the molecules are interacting

Minigenome	A shortened version of a virus's genome that can be used to study replication and transcription in dangerous viruses safely. The viral genes are removed and replaced with a non-viral reporter gene.
Mortality	Number of deaths caused by a disease or condition per specified population (may be e.g. total population or infected population)
Nucleoprotein chaperoning	Guiding correct assembling and binding of nucleoprotein
Oligomer	A complex formed by three or more protein monomers
Oligomerisation	Interaction between several polypeptide chains of a molecule to form a larger structure
PKR	Protein kinase R; a protein involved in the innate immune response
Pulldown assay	A method used to determine interactions between proteins and detect levels of protein activation
RIG-I	Retinoic acid-inducible gene I; a type of receptor involved in the type-I interferon response
RNA interference / RNAi	Regulation of gene expression by RNA molecules
Size-exclusion chromatography and multi-angle light scattering /SEC-MALS	A technique used to assess conformation based on molar mass and size
Small interfering RNA / siRNA	Non-coding RNA molecules involved in RNA interference
Spillover event	Transmission of a virus between species

TBK1	TANK-binding kinase 1; an enzyme involved in the innate immune response; involved in inducing type-I interferons
Tetramer	A complex formed of four protein monomers
Trimer	A complex formed of three protein monomers
Viral protein 35 / VP35	A virulence factor of Ebola virus that aids the virus in evading the host immune response through a range of mechanisms
Virulence factor	A molecule enabling a virus or bacterium to successfully infect host cells
Zoonotic disease	A disease that has transferred from animals to humans

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