**Proposal**: Designing and testing the efficacy of a RSV phosphoprotein mimetic on the inhibition of RSV replication and infection

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**Research Objectives**:

To design a stable and efficient peptide mimetic of Respiratory Syncytial Virus' phosphoprotein and to attach it to a human carrier protein

## Background

Human respiratory syncytial virus (RSV) is a pathogen associated with acute lower respiratory infection (ALRI), which is the leading cause of childhood hospitalization [1, 2]. RSV is the most important cause of lower respiratory tract infection in infants less than one years of age and almost all children have been infected with RSV at least once by two years of age [3, 4, 5]. Infection in young children is mainly characterized by wheezing, bronchiolitis or pneumonia while infections in older children and adults clinically manifest as low grade fever and upper respiratory tract complications, such as sinusitis and otitis media [3, 5]. RSV can also repeatedly infect individuals throughout their life, which makes people with compromised immune, pulmonary or cardiac systems, and the elderly highly susceptible to infection [3]. In 2005 alone, it was estimated that there were at least 33.8 million worldwide cases of RSV-associated ALRI in children below the age of five [1]. At least 3.4 million of these cases required hospitalization and it is estimated that between 66 000 to 199 000 lives were lost due to RSV-associated ALRI complications with the majority of the cases occurring in developing countries [1]. However, these statistics are most likely underestimations because they were obtained only from children who reached a hospital [6]. Just in the year 2000, in the United States alone, it was estimated that the annual medical cost for all RSV-infection related hospitalizations and medical encounters totaled to approximately $652 million USD [7]. From 1997 to 2000 in the USA, total annual RSV costs for infants were estimated to be $2.6billion for hospitalizations and exceeding $1billion for treating elderly patients.

Despite the large economic burden posed by RSV, there is currently no vaccine available to prevent RSV infection. Ribavirin is the only commercially approved antiviral drug against RSV. However, it is not widely used due to its high costs and low clinical benefits [8]. There are two RSV prophylactic treatments in development: palivizumab and motavizumab [8]. Early clinical trials have shown that palivizumab is effective in protecting premature infants, with chronic lung disease and congenital heart disease, against RSV hospitalization [8]. It has been approved by the United States Food and Drug Administration (FDA), but is restricted only for infants at high risk of developing severe RSV due to cost considerations [8, 9]. Motavizumab has shown comparative success with palivizumab, but it is not approved by the FDA as a result of mild side effects [8]. As of the moment, infants hospitalized with RSV are given supportive treatments, which involve maintaining adequate oxygen and hydration status [8]. Since there is no effective vaccine or therapeutic treatment against RSV, there is a growing need for the development of novel anti-RSV drugs.

RSV is classified within the *Paramyxoviridae* family and consists of an enveloped, non-segmented negative-strand RNA virus [10]. Its genome is composed of 10 genes, which encode for 11 different proteins [11]. There are 8 structural proteins present and 2 non-structural proteins, which interfere with the host's innate immune response [12]. Of these viral structural proteins, the nucleoprotein (N), the phosphoprotein (P), and the large polymerase (L) form a RNA-dependent RNA polymerase complex, which is responsible for viral RNA replication [12, 13]. Furthermore, there is a well characterized interaction between RSV's P and N protein. It has been shown in scientific literature that the P protein forms a soluble complex with the N protein, which allows for a strong, specific, and possibly irreversible binding to viral RNA [13]. It has also been shown that the last nine C-terminal amino acids on the P protein is sufficient and necessary to mediate efficient binding to the N protein [14]. This interaction also serves as a potential therapeutic drug target for inhibiting RSV replication and infection.

Peptide mimetics are a novel means to exploit this interaction. A peptide mimetic, also referred to as a dominant negative mutant, refers to a gene product that can bind to the same target as a naturally occurring enzyme, but it lacks the ability to catalyze reactions [15]. This concept can also be applied to disrupt essential protein-protein interactions by using truncated versions of a peptide to outcompete its native full-length form for specific protein binding. The laboratory that I am working in has already designed and tested a RSV phosphoprotein mimetic peptide (RSVP), which has been successful in inhibiting RSV replication and infection *in vitro*. The current RSVP construct contains the final 21 C-terminal amino acids of the RSV P protein ($P_{220-241}$), which has been fused to an *Escherichia coli* protein, Maltose Binding Protein (MBP), and a cell penetrating peptide derived from the HIV-1 Tat protein. MBP is a carrier protein that increases the solubility and stability of the construct while the nuclear localisation signal segment of the HIV-1 Tat protein (NLS) allows for the RSVP mimetic to enter into the cell and disrupt the essential RSV N-P interaction. It also does not significantly affect the viability and replication ability of cells, which makes this recombinant protein a promising therapeutic drug.

However, MBP is an *E. coli* protein, which will cause the MBP-RSVP construct will elicit an immune response in downstream animal and human trials. The purpose of my project will be to find a suitable human carrier protein to replace MBP and to test its efficacy. A candidate for this are Designed Ankyrin Repeat Proteins (DARPins). They are based off of naturally occurring ankyrin repeats, which are one of the most abundant proteins found in the human genome and across all three superkingdoms [16, 17]. They possess many favourable biophysical properties such as their ease of production and purification, solubility, thermall stability, and resistance to proteolytic degradation [16, 18]. All of these properties make it a suitable candidate to fuse the $P_{220-241}$ and cell penetrating peptide to.

According to its crystallographic data, both the N-terminal and C-terminal ends of DARPins are exposed to the extracellular environment and can serve as potential sites for attaching the $P_{220-241}$ and NLS to. The E3_5 DARPin construct from GenBank AY195853 (Binz et al. 2003) with three repeating subunits was used chosen as the carrier protein, since three repeat subunits is the most common found within naturally occurring ankyrin repeat proteins [17, 18]. Thus, the final genetic construct chosen was E3_5 DARPin-NLS-$P_{220-241}$. I hypothesize that this DARPin-NLS-RSVP construct will exhibit the same level of inhibition on RSV replication and infection, which will be assessed using a challenge test with RSV.

## References


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