Research and technological quality, including any interdisciplinary and multidisciplinary aspects of the proposal

I. Objective

Influenza virus critically depends on cellular RNA Polymerase II (Pol II) functions to progress through its life cycle. Pol II transcripts are snatched to provide capped primers for viral transcription, and Pol II is also known to be involved in coordinating splicing, mRNP packaging and nuclear export. The overall objective of this project is to understand the biological significance of the interaction between influenza virus polymerase and the cellular Pol II transcriptional machinery. Although research in the host group has shown that the viral polymerase interacts with the C-terminal domain of the large subunit of Pol II, whether this is a direct interaction or if other cellular factors are involved remains unclear. This project will address this question and identify mediating factors if the interaction is not direct. We will map the Pol II binding domain in the viral polymerase and test the hypothesis that the interaction with Pol II is essential for viral mRNA synthesis, as well as identify genome-wide binding sites of the viral polymerase. This project will provide a novel strategy for the design of specific antivirals that would inhibit influenza virus replication by disrupting its interaction with Pol II.

II. Background

Social and economic impact of influenza

Influenza is an acute viral infection that represents a severe global public health problem, causing annual epidemics that can seriously affect all age groups. Worldwide, these yearly epidemics result in about 3 to 5 million cases of severe illness, and about 250,000 to 500,000 deaths. Although most people recover without requiring medical attention, influenza can take its toll on the high-risk population (young children, the elderly and people with medical conditions or weakened immune systems).

Epidemics also cause economic problems in terms of reduced work productivity and increased burden to health services. In developed countries, epidemics can result in high levels of absence from work and productivity losses. Also, clinics and hospitals can be overwhelmed when large numbers of sick people require treatment and hospitalization during peak illness periods.

In addition to seasonal epidemics, influenza A viruses can also cause unpredictable pandemics. This occurs when a virus of animal origin acquires the ability to infect and transmit between humans, and encounters a population with little or no pre-existing immunity to the emergent virus. Influenza pandemics can originate when an avian virus is directly transmitted to humans or by the emergence of reassortant viruses containing genes from mammalian and avian origin. Wild birds are a natural reservoir for influenza A viruses and this represents a threat of novel pandemics because highly pathogenic strains currently prevalent amongst birds (H5N1, H7N7 and H9N2) could acquire the ability to infect humans directly through adaptation or reassortment (*2;3*). In this scenario, vaccines against seasonal influenza would not confer protection and a matching vaccine would not be immediately available, while the effectiveness of the limited number of antiviral treatments that are currently available could be reduced by the proven ability of the virus to develop drug resistance. In all, because we would be almost defenceless in such a situation new control measures for influenza need to be urgently developed.

Influenza virus polymerase complex

Influenza viruses possess a negative-sense segmented genome that is transcribed (vRNA → mRNA) and replicated (vRNA → cRNA → vRNA) by the viral RNA polymerase in the nucleus of infected cells (*4-6*). The viral ribonucleoprotein (vRNP) is the functional unit for transcription and replication, and consists of the RNA-dependent RNA polymerase (RdRp) bound to a vRNA segment coated by multiple copies of nucleoprotein (NP). The influenza RdRp is a complex of three proteins: polymerase basic protein 1 (PB1), polymerase basic protein 2 (PB2), and polymerase acidic protein (PA) (*7*). PB1 contains the catalytic site (*8*), and PB2 binds the cap on host pre-mRNA molecules (*9;10*). PA has recently been shown to contain an endonuclease domain responsible for the generation of 5' capped RNA primers by cleaving host pre-mRNAs (*11;12*). Initiation of viral transcription is dependent on these primers, and transcription results in an incomplete copy of the vRNA template which is similar to host mRNAs in that it is capped and polyadenylated. Replication is primer-independent and occurs via a cRNA intermediate, an exact positive-sense copy of the vRNA for which it also serves as a template. Although the regulation of these two processes is poorly understood, both viral and cellular factors have been proposed to play a role (*13*).

Interplay of the viral polymerase complex with the host cell

Genome-wide screens and proteomic approaches have identified host factors that interact with influenza virus at virtually every step of its life cycle, but few of these factors have been studied in detail (*14*). In addition, many studies indicate that the viral polymerase is a crucial determinant of host range specificity and pathogenicity (*15-18*), demonstrating that its activity is regulated by interactions with components of the host cell. Among them,

BAT1 (UAP56), Hsp90, the minichromosome maintenance (MCM) complex, Tat-SF1 and DNA-dependent RNA polymerase II (Pol II) have been shown to stimulate viral RNA synthesis (*19-25*).

The active form of Pol II, which catalyzes the synthesis of mRNA precursors and most snRNA and microRNAs, is required for viral transcription (mRNA synthesis) (*24;25*). In the current model, the viral polymerase hijacks Pol II to gain access to host factors required for viral mRNA synthesis (*26*). These include not only the capped RNA fragments derived from Pol II transcripts used as primers by the viral RNA polymerase, but possibly also factors required for splicing, packaging into mRNPs and nuclear export and translation of viral mRNPs (*26;27*). Intriguingly, it has also been shown that influenza virus infection induces the degradation of the large subunit of Pol II (*28*). This ability to degrade Pol II has been linked to virulence (*29*), and could also provide an explanation for host shut-off and the sharp decline in viral mRNA synthesis late in infection. However, many aspects of this interaction remain unclear, and the functional significance for viral mRNA synthesis is not known. Indeed, whether the interaction between the viral polymerase and Pol II is direct or indirect is currently a matter of controversy, and there is no published data addressing whether viral RNAs are also associated with the viral polymerase-Pol II complex. If the RdRp interaction with Pol II were to facilitate viral mRNA synthesis, RdRp in the context of vRNP would be expected to bind Pol II. My current work is addressing this question, and since joining the host group in November 2011 I have been able to show that viral RNAs co-immunoprecipitate with Pol II from infected cells (*Figure 1*). The viral polymerase has also been reported to associate with promoter DNA of three Pol II genes (DHFR, β-actin, and GAPDH) while no association with Pol I (45S rRNA) and Pol III (7SK RNA) genes was observed (*24*). Further research is needed to determine whether a particular subset of host genes is targeted by the viral polymerase, and how their expression is affected by the binding of the viral polymerase. However, no genome-wide search for these associations has been conducted to date.

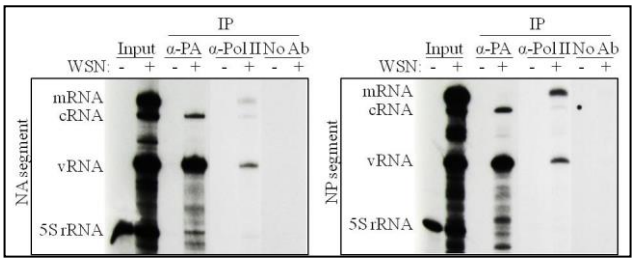

Figure 1. *Co-immunoprecipitation of viral RNAs with viral polymerase (PA) and cellular Pol II analyzed by primer extension. NA (left) and NP (right) segments have been used as templates for RNA synthesis.*
(Martínez Alonso and Fodor, unpublished data).

On this background, we propose the following ***Aims and objectives:***

The general aim of this project is to understand how interactions of the viral RdRp with cellular factors regulate its function at the molecular level.

The hypothesis that will be tested is that the interaction between the viral RNA polymerase and the CTD of Pol II is essential for the transcriptional activity (mRNA synthesis) of the viral RNA polymerase *in vivo*.

There are two Specific Aims that will be completed during the fellowship, which are in turn divided into specific and measurable objectives:

Aim 1: Characterize the interaction between the viral RNA polymerase and Pol II.

Objective 1.1. Compare the *in vitro* binding of purified RdRp heterotrimer and vRNP to synthetic CTD mimic peptides.

Objective 1.2.a. Map the Pol II interaction binding domain of the viral polymerase.

Objective 1.2.b. Identify factors mediating the interaction between the viral polymerase and Pol II.

(Objectives 1.2.a. and 1.2.b. are mutually exclusive; see below)

Objective 1.3. Analyze the transcriptional and replicative activity of viral polymerase mutants that do not interact with Pol II.

Aim 2: Conduct a genome-wide search for associations of influenza virus RNA polymerase.

Objective 2.1. Develop a method to analyze interactions between influenza virus proteins and DNA on a genome-wide scale.

Objective 2.2. Identify sites in the human genome targeted by the viral RNA polymerase.

Success of the project will only be achieved by effective integration of multidisciplinary aspects, including virology, cellular biology, proteomics and bioinformatics, and will involve the combined expertise of two laboratories. The first aim will clarify the current controversy regarding the interaction between Pol II and the viral polymerase, while the second aim will identify host genes that are targeted by influenza virus polymerase upon infection. *Overall, this project will characterize in detail the molecular interactions between the viral RdRp and Pol II and shed light on its role in viral transcription.*

Appropriateness of research methodology and approach

Aim 1: Characterize the interaction between the viral RNA polymerase and Pol II.

Although previous research in the host laboratory has shown that the viral RdRp interacts with the CTD of the large subunit of cellular RNA polymerase II (*25*), these experiments did not exclude the possibility that the interaction between the CTD of Pol II and the viral polymerase is indirect. Indeed, although preliminary results in the host laboratory suggest that the interaction between the viral RNA polymerase and the CTD is direct and Tafforeau *et al* also suggest that the binding is direct and occurs through PA (*30*), others have suggested that the interaction might be mediated by host factors (*31;32*). This Aim will explore in detail this interaction and map the Pol II binding domain of the viral RdRp. More importantly, these experiments will also generate a set of viral polymerase mutants unable to interact with Pol II that will enable us to test our hypothesis – i.e. that the interaction between viral RdRp and Pol II is required for viral mRNA synthesis.

Objective 1.1. Compare the *in vitro* binding of purified RdRp heterotrimer and vRNP to synthetic CTD mimic peptides.

To determine if the interaction of the viral RdRp with the CTD of Pol II occurs via direct or indirect binding we will carry out *in vitro* binding assays with biotinylated CTD mimic peptides and purified RdRp heterotrimer (PB1, PB2 and PA). The RdRp heterotrimer will be purified from 293T cells co-transfected with PB1, PB2 and PA expression plasmids already available in the host laboratory. PB2 contains a C-terminal TAP tag that allows tandem affinity purification of the complex. To address if RNA plays a role in the binding of RdRp to the CTD of Pol II, as my preliminary results suggest (*see Figure 1*), the RdRp heterotrimer will also be purified bound to a short-length vRNA whose expression will be driven from a pPOL-I plasmid (*33*) cotransfected into 293T cells together with the PB1, PB2 and PA expression plasmids (*Figure 2*). Biotinylated CTD mimic peptides will be chemically synthesized and designed to contain 4 copies of the conserved heptapeptide repeat (YSPTSPS) of Pol II CTD. Although the full-length human CTD consists of 52 heptad repeats, 4 repeats are predicted to be sufficient for the interaction based on structural studies of other CTD-binding proteins (*34*). We will supply CTD mimic peptides either unphosphorylated or phosphorylated at Ser2 or Ser5. The viral polymerase has been shown to interact with the initiating form of Pol II, which is phosphorylated at Ser5 in the CTD, but not with the elongating form, phosphorylated at Ser2 (*25*). Therefore, only CTD peptides phosphorylated at Ser5 will be expected to interact with RdRp. CTD peptides will be incubated with the purified RdRp (+/- vRNA) and complexes formed will be captured with streptavidin-coated beads. Bound complexes will be released by boiling and will be loaded directly onto SDS-PAGE gels. Binding will be monitored by Western blot using specific antibodies against the three RdRp subunits, whose presence will indicate direct binding to Pol II CTD.

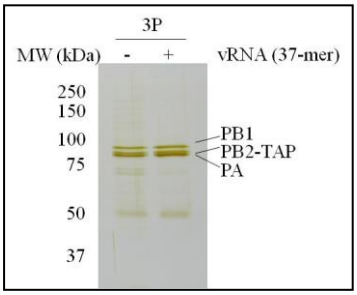

Figure 2. *Silver stained gel of RdRp heterotrimer purified in presence (+) or absence (-) of a short vRNA (37-mer).*
(Sharps, Martínez Alonso and Fodor, unpublished data).

In the event that no binding is observed with purified RdRp heterotrimer, the CTD-mimic peptides will be incubated with lysates of cells infected with A/WSN/33. A positive binding observed in these conditions would indicate that a mediating factor(s) might be facilitating the interaction between the viral polymerase and Pol II CTD. This scenario would be addressed with the experiments described in Objective 1.2.b.

Although Ser2 and Ser5 phosphorylation are the best characterized modifications of Pol II CTD, all the amino acids of the consensus repeat (YSPTSPS) can be potentially modified independently of one another generating a wide range of distinct combinations. Other well studied modifications include phosphorylation of Ser7 and isomerisation of the two proline residues in the consensus repeat. Non-consensus repeats can also be modified. Arginine can be methylated, while lysine is a potential substrate for acetylation, methylation, sumoylation and ubiquitylation (*35*). The effect of these modifications on the binding of the viral polymerase to Pol II CTD is currently unknown. To analyze in further detail if some of these modifications can play a role in the binding of influenza polymerase to Pol II CTD we have set up a collaboration with Dr Stephen Fuchs (Tufts University, USA), an expert in peptide microarray technology development (*36*). He is currently synthesizing a peptide library to study how protein factors interact with post-translationally modified repeats of the CTD of Pol II. This library will initially comprise a total of 256 unique peptides, including all combinations of phosphorylation at Ser2 and Ser5, and either proline or 5,5-dimethylproline (an analog that favors *cis*- peptide bond conformation) at positions 3 and 6. Eventually this library will grow to include Ser7 phosphorylation. Peptides will be biotinylated at the N-terminus, which will allow them to be arrayed onto streptavidin-coated slides, and contain a fluorescein moiety at the C-terminus for downstream normalization. Dr Fuchs has agreed to provide this novel technology to further characterize the interaction of Pol II CTD and influenza virus polymerase. The RdRp heterotrimer will be purified as described above and bound to the arrays, which will be then incubated with specific primary antibodies against one (e.g. PA) of the three RdRp subunits, followed by incubation with a fluorochrome-conjugated secondary antibody (e.g. Alexa Fluor 647).

Alternatively, GFP- or mOrange-tagged RdRp, available in the host laboratory, will be used to facilitate direct detection. Arrays will then be scanned using a Typhoon imager with appropriate filter sets for fluorescein and the secondary antibody (or GFP or mOrange), and interactions will be quantified with ImageQuant array software. Relevant interactions will be further validated using Surface Plasmon Resonance (e.g. BIAcore), and their affinity and kinetic constants determined.

We will also investigate if RdRp can interact with the CTD of Pol II in the context of vRNPs in virus-infected cells. To do this, we will take advantage of a new technology developed at the host laboratory which allows selective and specific purification of vRNP and cRNP complexes from infected cells. Briefly, 293T cells will be infected with a double recombinant influenza virus (already available at the host laboratory) that contains an RNA tag and Strep-tagged PB2. The RNA tag consists of a hairpin stem loop that is bound by the *Pseudomonas aeruginosa* phage 7 (PP7) coat protein (*37*), which has been introduced into the NA or NS segments of recombinant influenza viruses. Therefore, upon co-expression of His-tagged PP7 protein RNA-tagged vRNP/cRNP complexes can be by purified specifically by Ni-agarose chromatography. This is followed by a second step consisting of a One-Strep purification. *(Figure 3).* The One-Strep affinity purification tag is a short peptide which binds specifically to a derivative of streptavidin called Strep-tactin (*38*). Therefore, the Ni-agarose eluted complexes are bound to Strep-tactin beads (*39;40*), and this second step improves specificity at the protein level. Bound complexes are finally eluted with desthiobiotin. This method will be used to selectively purify vRNPs, which will be included in the CTD binding assays described above.

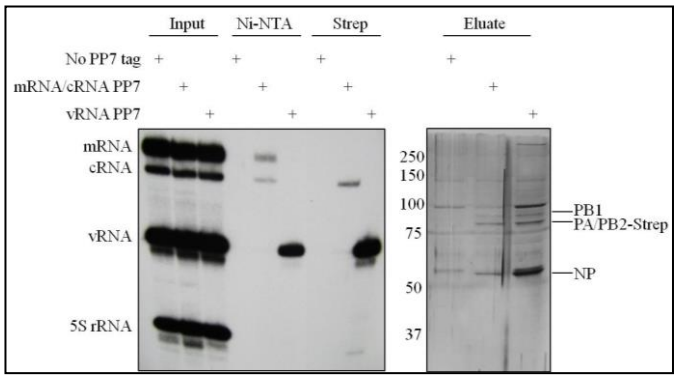

Figure 3. *Purification of PP7/PB2-Strep-tagged mRNA/cRNA and vRNA from infected cells analyzed by primer extension (left) and silver stain (right). (York and Fodor, unpublished data).*

The outcome of this objective will determine the next step of the project. If the interaction of the viral polymerase and Pol II is confirmed as direct, we will map the Pol II interaction domain of the viral polymerase (Objective 1.2.a). However, if the interaction between the viral RNA polymerase and the CTD is indirect, we will search for the factor(s) that mediate the interaction (Objective 1.2.b).

Objective 1.2.a. Map the Pol II interaction binding domain of the viral polymerase.

As a first approach we will construct deletion mutants of the individual polymerase subunits, avoiding regions known to interact with other polymerase subunits for heterotrimeric complex formation (*Figure 4*). Although the binding of the viral polymerase to the CTD of Pol II was originally believed to require interaction with the heterotrimeric complex (*25*), more recent data suggest that the binding site might be located in PB2 (*41*). In this study, a polymerase complex was reconstituted with a PB2 protein containing mutation F363A, known to block cap-binding activity. The PB2-F363A mutation renders the polymerase deficient in cap-dependent transcription, but not replicative activity (*42*). Unexpectedly, the heterotrimer containing PB2-F363A displayed a markedly reduced binding to Pol II. On this background, we will pay special attention to PB2 and will construct truncated versions where each of its known functional domains (nuclear import, 627 domain and cap binding) will be sequentially deleted. The ability of these PB2 mutants to interact with the CTD will be assessed *in vitro* in experiments similar to those described in Objective 1.1. In addition, because *Tafforeau et al* suggest that PA mediates a direct binding (*30*) PA will be considered in parallel to PB2. Similar deletion mutants for PA will be constructed, starting deletions from the N-terminus (endonuclease domain). These mutants should still interact with PB1 and form a trimeric RdRp. If the CTD binding region cannot be identified in PB2 or PA, we will extend the analysis of deletion mutants to PB1. In a second approach the polymerase complex will be cross-linked to biotinylated synthetic CTD mimic peptides and subjected to limited proteolysis with trypsin. As the CTD mimic peptides contain no basic residues they would not be digested by trypsin, and therefore regions of the polymerase subunit(s) involved in the binding would be protected from proteolysis. Polymerase-derived peptides that remain cross-linked to the CTD-mimic peptides will be analyzed by liquid chromatography and tandem mass spectrometry (LC-MS/MS) after cross-link reversal. The combined use of

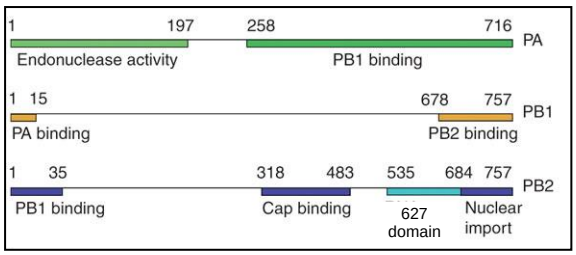

Figure 4. *Linear representation of the viral polymerase. The segments that have structural information are represented as coloured bars. Adapted from (1).*

these two approaches will allow us to identify the location of the CTD binding region. Alanine scanning mutagenesis will be then performed to map the interaction domain on the viral polymerase.

Objective 1.2.b. Identify factors mediating the interaction between the viral polymerase and Pol II.
If our experiments do not confirm the interaction between the viral RNA polymerase and the CTD as direct, we will search for the factor(s) mediating the interaction. Others have suggested that this interaction might be mediated by host factors (*31;32*). We will use proteomic approaches to carry out a broad search for unknown factors that associate both with the viral polymerase and the Pol II machinery. If binding is observed for CTD-mimic peptides incubated with lysates of infected cells (Objective 1.1), complexes bound to the peptides will be analyzed by liquid chromatography and tandem mass spectrometry (LC-MS/MS) to identify the factor(s) mediating the interaction. Alternatively, to enrich for complexes containing both Pol II and viral RdRp, we will initially perform PB2-Strep purification of the viral polymerase followed by immunoprecipitation of Pol II. Briefly, 293T cells will be infected with a recombinant virus expressing PB2-Strep (available in the host laboratory) and the viral RdRp purified as described (Objective 1.1). Bound complexes will be eluted with desthiobiotin and immunoprecipitated with Pol II specific antibodies. The reverse experiment (initial Pol II immunoprecipitation followed by Strep-purification of the RdRp) will also be performed. Immunoprecipitates will be analyzed by LC-MS/MS, and novel factors identified through both purification strategies will be selected for further analysis. To determine if they play a role in the viral life cycle, these factors will be knocked down by siRNA in experiments conducted in infected cells. A scrambled sequence of the siRNA target sequence will be used as a negative control. Any relevant factors identified at this stage will be assessed for their ability to bind directly to the viral polymerase (or individual polymerase subunits) in experiments similar to those described in Objective 1.1, but where TAP-tagged purified polymerase subunits will be used instead of the CTD mimic peptides. When a direct binding is identified, we will map the binding domain as described in Objective 1.2.a.

Objective 1.3. Analyze the transcriptional and replicative activity of viral polymerase mutants that do not interact with Pol II.
The RdRp mutants that do not interact with the CTD of Pol II (or the mediating factor) constructed in Objective 1.2 will allow us to investigate the biological role of the interaction between the viral polymerase and Pol II. If our hypothesis is correct and this interaction is crucial for viral transcription, we would expect these mutants to show impaired mRNA synthesis while possibly retaining their ability to replicate. To test the function of the mutant polymerases, we will first use an *in vivo* transcription/replication assay (developed and already established in the host laboratory, (*43*)) that is based on *in vivo* reconstitution of viral ribonucleoprotein complexes. PB1, PB2 and PA containing the mutation(s) identified in Objective 1.2 will be coexpressed in 293T cells with influenza NP protein (this forms the minimal set of influenza viral proteins required for transcription and replication of vRNA) and a vRNA template (e.g. the NA segment). *In vivo* synthesis of mRNA, cRNA and vRNA will be evaluated by primer extension of the extracted RNA.

We will also test the compatibility of these mutations with viral viability by using established reverse-genetics techniques (developed by the supervisor, (*33*)). Briefly, rescue of recombinant viruses will be performed co-transfecting 293T cells with eight pPOL-I plasmids encoding the eight vRNA segments of influenza A/WSN/33 virus and four pcDNA plasmids encoding mRNAs for PB1, PB2, PA and NP. The mutations identified in Aim 1 will be subcloned into the pPOL-I plasmids to express mutant vRNA, while pcDNA plasmids will encode wild type subunits of the polymerase. The presence of infectious virus in the supernatant of 293T transfected cells will be tested by plaque assay on Madin-Darby Bovine Kidney (MDBK) cells. If infectious virus is recovered, virus growth kinetics will also be determined. However, if Pol II binding is essential for viral mRNA synthesis we do not expect to be able to recover infectious virus from the disabled mutants.

Aim 2: Conduct a genome-wide search for associations of influenza virus RNA polymerase.
Previous research in the host group identified a specific interaction of the viral polymerase with the promoter region of Pol II genes (*24*). This is likely to be mediated by the interaction of the viral polymerase with the CTD of initiating Pol II (Ser5 phosphorylated form), and it was proposed that this interaction could trigger premature Pol II degradation therefore contributing to inhibition of host protein synthesis, which is known as cell shut-off. Aim 2 will test the hypothesis that the viral polymerase exclusively targets Pol II genes, and in particular a specific subset of genes leading to the inhibition of the expression of a subset of host proteins (e.g. antiviral host factors).
To achieve this goal, we will first develop a further improvement of the currently established ChIP-sequencing technology that will allow us to explore the genome-wide interactions of the viral polymerase.

Objective 2.1. Develop a method to analyze interactions between influenza virus proteins and DNA on a genome-wide scale.
Although chromatin immunoprecipitation combined with second generation DNA-sequencing (ChIP-seq) is now a well established standard method for genome-wide association studies, one of its major limitations is the need for highly specific antibodies that can precipitate efficiently the protein of interest (*44*). Indeed, preliminary data from

our lab indicates that the efficiency of currently available antibodies against influenza virus polymerase is limiting to obtain the minimal amounts of DNA required for sequencing. For this reason, we will use a modification of the standard ChIP-seq method where a Strep-tagged PB2 will be used to bypass the need for antibodies. Briefly, cells will be infected with the recombinant virus and protein-DNA complexes crosslinked with formaldehyde. Chromatin will be sheared by sonication and polymerase-bound complexes will be purified by One-Strep purification on Strep-tactin beads as described (Objective 1.1). Uninfected cells will be used as a negative control. The method will be validated using qPCR to monitor the presence of specific DNA fragments (e.g. Pol II genes: β-actin, DHFR - positive control) and other genomic DNA fragments that are not expected to be present in the purified samples (e.g. Pol III genes: 7SK RNA - negative control).
Alternatively, we will use DamID (*45*) to localize DNA binding sites of the viral polymerase. For this approach reverse genetics (*33*) will be used to rescue a recombinant virus expressing PB2 as a fusion protein of adenine methyltransferase (Dam), which would result in methylation of adenines near sites where influenza virus polymerase interacts with the DNA. Methylated sequences can then be amplified by methylation-specific PCR and identified by hybridization to microarrays. In the case that a PB2-Dam fusion protein is not compatible with virus viability, PB2-Dam will be co-transfected with PB1 and PA into 293T cells (as described for RdRp purification in Objective 1.1) and DamID carried out as described.

Objective 2.2. Identify sites in the human genome targeted by the viral RNA polymerase.
DNA samples obtained in Objective 2.1 will be submitted to the Wellcome Trust Centre for Human Genetics (University of Oxford) for high-throughput sequencing. Data processing and analysis will be performed by Dr Martínez Alonso with the help of Dr Martin Dienstbier, a colleague in the department with extensive experience in analysis of ChIP-seq datasets. Reads will be aligned to the human genome using Geneious (Biomatters Ltd) and analyzed with SeqMonk (The Babraham Institute), which allows visualization of mapped datasets against an annotated genome and quantitation of the data. Identified sites will be validated by independent ChIP analysis followed by qPCR.

Completion of this objective will identify host genes targeted by influenza RNA polymerase upon virus infection, providing information on whether particular genes are exclusively targeted and how their expression is regulated by the binding of the viral polymerase.

Originality and innovative nature of the project, and relationship to the 'state of the art' of research in the field
Detailed characterization of the association between the influenza virus RNA polymerase and the host cell at the molecular level is expected to uncover new targets for drug development. More specifically, the information that this project will deliver by mapping the Pol II interaction domain in the viral polymerase could pave the way for the development of specific antivirals that would prevent this interaction. By underpinning the development of new antiviral treatments this project also has the potential to impact public health and the economy in the long term. New antivirals could contribute to reduced illness and costs associated with hospitalization and absence from work. The availability of novel drugs would broaden our line of defence against an influenza pandemic. This would have significant potential for commercial exploitation, and may lead to patents and the creation of spin-off companies.
The project is highly innovative with regards to the approaches and methods that will be employed. The use of state-of-the-art peptide microarrays will speed up the advance of the project by allowing high-throughput screens to be conducted in a single experiment. Novel vRNP purification methods recently developed at the host laboratory will not only facilitate the search for host factors interacting with the viral polymerase, but also provide the basis for further development of existing technologies (ChIP-seq) that will enable a genome-wide search for binding sites of the viral polymerase. Overall, each objective of this project will contribute to the state of the art in the influenza research field.

Timeliness and relevance of the project
Influenza viruses pose not only a major threat to human health, but also a large economic burden to healthcare systems. In addition to yearly epidemics, occasional pandemics can also occur, and the threat of a novel pandemic influenza virus emerging is of concern because it is likely that there would be no pre-existing immunity in the human population. Indeed, existing vaccines may not confer protection against a novel pandemic virus, and development of resistance against the limited number of antivirals available is also of concern. In terms of economical consequences, influenza results in enormous costs associated with hospitalizations and reduced productivity due to absence from work. On this background, it is clear that new control measures are urgently needed but to be able to develop new vaccines or antiviral drugs targeting influenza we first need a much deeper understanding of the molecular interactions between the virus and the host cell. The project that we propose addresses this need.