Medicines & Healthcare products Regulatory Agency

PhD studentship – Division of Biotherapeutics

Position: PhD Student
Division: Biotherapeutics, NIBSC
Location: South Mimms, Potters Bar, Hertfordshire
Reference number: SCI03Z
Grade and salary range: PhD Student - £18,500 annual stipend
Contract Type: 3-year Fixed Term Contract
Closing Date: 12 noon UK time (midday) on Friday 09 March 2018

Title: Optimisation of cross-neutralising nanobodies to influenza haemagglutinin using *in vitro* molecular evolution

A 3-year full-time studentship is available at the National Institute for Biological Standards and Control, Division of Virology, in collaboration with the Division of Infection and Immunity, University College, London (UCL). The studentship is expected to start on 1st October 2018.

Purpose of the studentship

Influenza A virus remains a persistent threat to public health resulting in 200,000-500,000 deaths worldwide every year. Vaccination is the main public health treatment option to reduce the impact of influenza, however, the rapid evolution of the virus remains a constant challenge for effective vaccine production. The pre-dominant host immune response is directed against the globular head of the main viral glycoprotein, haemagglutinin (HA), and this selective pressure drives the continuous antigenic changes of the influenza virus. The structure of the membrane proximal stem region is significantly more resistant to antigenic change and this feature has led to the discovery of stem binding human monoclonal antibodies which have broad viral neutralisation activity. The discovery of these monoclonal antibodies has led to renewed interest in passive immunotherapy as an additional treatment option to vaccination. An interesting feature of these monoclonal antibodies is that they only use their heavy chain for recognition of a conserved epitope in the HA stem, with the light chain (LC) being superfluous to requirements. This suggests that 'heavy chain only' may be a preferred mode of binding to the influenza HA stem suggesting naturally occurring 'heavy chain only' antibodies, such as nanobodies (Nbs), may be well equipped to access similar epitopes. Our laboratories have recently described cross-neutralising Nbs which share the high selectivity, cross-reactivity and affinity of these human monoclonal antibodies, however we believe their well-documented small size, high stability, and cleft binding properties offers distinct advantages as immunotherapeutics.

In order to understand nanobody (Nb) cross-reactivity, mechanism of action and the relative resistance to antigenic escape, their epitopes have been mapped on the structure of HA. We have used an experimental system based on yeast display and mutational scanning which can be used to explore the co-evolution of influenza HA and recombinant Nbs. By displaying randomly mutated HA molecules on the surface of yeast and selection using highly controllable flow cytometric cell sorting, it has been possible to analyse the functional significance of millions of mutations in parallel on a massive scale. This project will build on previous work from our laboratories and explore the structure/function of cross-neutralising nanobodies, so contributing to their development as immunotherapeutics.

Project hypothesis: Introduction of mutational changes into the antigen binding loops of anti-influenza specific nanobodies to generate libraries of nanobody sequences and their subsequent selection will give new and improved Nbs that can mitigate the risk of viral escape, have improved potency and enhanced cross-reactivity.

Objective 1: Generate a database of mutations with the potential to impact binding of nanobodies (Nbs) to influenza hemagglutinin (HA). The student will select a site-saturated yeast displayed HA library for the loss of binding to HA specific nanobodies. Deep sequence and bioinformatics analysis will be used to evaluate the enrichment or depletion of amino acid mutations in

HA in parallel on a massive scale. The effects of individual mutations on Nb binding will then be experimentally confirmed. Correlation of the total mutational landscape with databases of naturally occurring mutations will predict mutations which represent a functionally viable path of viral escape. The student will experimentally test predicted HA escape mutations and produce recombinant HA protein variants for selection of yeast displayed nanobody libraries.

Objective 2: Optimise nanobody binding to HA to (i) overcome viral escape (ii) improve nanobody affinity (affinity maturation) (iii) optimise viral cross reactivity. The student will build libraries of nanobodies carrying mutations within the antigen binding loops. These libraries will then be displayed on yeast and selected using fluorescent activated cell sorting (FACS) to identify mutated nanobodies which overcome viral escape, have improved affinity and broad cross-reactivity. The student will then characterise selected Nbs and experimentally evaluate the functional significance of the mutations identified in the nanobody sequences. This will involve expression and purification of nanobodies for testing in biochemical and viral neutralisation assays.

Additional reading:

Hufton SE, Risley P, Ball CR, Major D, Engelhardt OG, Poole S (2014) The breadth of cross sub-type neutralisation activity of a single domain antibody to influenza hemagglutinin can be increased by antibody valency. PLoS One 9: e103294. 10.1371/journal.pone.0103294 [doi];PONE-D-14-18443 [pii].

Gaiotto T and Hufton S.E. Cross-neutralising nanobodies bind to a conserved pocket in the hemagglutinin stem region identified using yeast display and deep mutational scanning. PLoS One.11(10):e0164296. doi: 10.1371/journal.pone. (2016)

Key responsibilities:

- to undertake the research project in line with the project aims
- to communicate effectively, orally and through written media, undertake presentations at scientific meetings and maintain excellent records.
- to interact regularly and effectively with your supervisors and interact appropriately and effectively with other staff.
- to fulfil the requirements of the University PhD programme and to undertake specific training as required by the host institutions.

About NIBSC

NIBSC is a centre of the Medicines and Healthcare products Regulatory Agency which enhances and improves the health of millions of people every day through the effective regulation of medicines and medical devices, underpinned by science and research.

NIBSC is a global leader in the characterisation, standardisation and control of biological medicines and has a major role in protecting and improving public health globally. NIBSC is the leading WHO International Laboratory for Biological Standardisation and is responsible for producing and distributing over 90% of all WHO International Standards introduced for the quality assurance of biological medicines. Our scientists also test products, carry out valuable research and provide advice on a routine basis and in response to emergencies. The importance of the Institute's work is well recognised nationally and internationally.

About the UCL

UCL is London's leading multidisciplinary university, with approximately 11,000 staff and 38,000 students from 150 different countries. Founded in 1826 in the heart of London, UCL was the first university in England to welcome students of any class, religion, and the first to welcome women on equal terms with men. The UCL Division of Infection & Immunity (I&I) is part of the Faculty of Medical Sciences. In the last Research Assessment Exercise (RAE 2014), the Infection and Immunology panel graded 80% of outputs from the Division as 4* (world leading) or 3* (internationally excellent). I&I has been very successful in training PhD students. Over 60% of our students carry on with their career in academia at world leading Institutions, and another 22% enter the academic medical profession.

The project will be supervised by Dr. Simon E. Hufton, Dr. Othmar Engelhardt and Dr. Mark Preston (NIBSC) and Dr. Yasu Takeuchi (UCL). The student will be based primarily at NIBSC with the opportunity for attendance at the University for additional training.

Person Requirements

In addition to meeting all the academic, security and residency requirements, you will have -

- an academic background in molecular biology, biochemistry or related life science discipline
- a demonstrated aptitude in a laboratory setting and motivation to undertake research
- a demonstrated ability to work accurately and precisely
- excellent, demonstrated oral and written communication
- a demonstrated interest in the field of study
- some previous experience in molecular and biochemical techniques
- an interest in molecular immunology/virology
- an interest in bioinformatics

Qualification requirements for UCL, Infection and Immunity Division

A first-class UK Bachelor's degree from any university or a minimum of an upper second-class UK Bachelor's degree in a relevant discipline from a Russell Group University, or an overseas qualification of an equivalent standard, or an appropriate Master's degree.

Funding

Tuition fees and consumables are covered and there is an £18,500 annual stipend. Please note funding is available for UK and European Economic Area (EEA) nationals only.

English language requirements

Applicants whose first language is not English are normally expected to meet the minimum University requirements (e.g. 6.5 IELTS). For further information click here

How to apply

Please submit:

(i) a CV (including the name and contact details of two academic referees) and,

(ii) a personal statement of no more than 1000 words explaining your interest in this project and aspirations for undertaking a PhD to studentship@nibsc.org by **12 noon UK time (midday) on Friday 09 March 2018.**

Please ensure the studentship reference number is included in the subject line of the email and your personal statement.

We are an equal opportunities employer and welcome applications from suitably qualified people regardless of age, gender, sexual orientation, marital status, race, religion, politics or disability. The Medicines and Healthcare products Regulatory Agency commits itself to the Guaranteed Interview Scheme (GIS). This means that it guarantees to interview all disabled candidates (as defined by the Disability Discrimination Act 1995), who satisfy the minimum essential criteria for the advertised post. If a candidate wishes to apply for consideration under this scheme, please include this in your covering letter.

Any offer of a studentship is conditional upon successful background screening which includes, but is not limited to, checks on identity, qualifications and right to study in the UK.