Project Details	
Project Code	IIAR27Ba Preston
Title	Do respiratory cilia sense and signal infection?
Research Theme	Infection, Immunity & Antimicrobial Resistance
Project Type	Wet lab
Summary	Respiratory cilia are largely regarded as purely mechanical, sweeping particles trapped in mucus out of the respiratory tract. However, they contain key signalling molecules, suggesting sensory and response functions. We will investigate this uncharacterised aspect of cilia biology. We will exploit the exquisite specificity of Bordetella bacteria for adherence to respiratory cilia that involves several different protein adhesins that engage currently unknown cilia receptors to alter ciliary beat patterns. We will characterise adhesin-receptor interactions, intra-cilia signalling induced by receptor engagement and the results of this signalling. This will redefine our understanding of sensing and responses at the respiratory epithelium surface.
Description	The mucociliary escalator is a primary defence of the mammalian respiratory tract that entraps and expels particulate matter inhaled into the airways, including a vast number of virus particles and bacteria. Respiratory pathogens must overcome this defence to colonise the airways to enable subsequent disease. For decades, respiratory cilia have been considered much like the bristles on a brush, with a purely mechanical role of sweeping mucus with trapped particles out of the airways by coordinated beating in a lungs to larynx direction. Recently, it has been discovered that respiratory cilia contain key signalling molecules such as Hedgehog, Smoothened, T2R, Tlrs and SRF suggesting that cilia are active in intracellular signalling pathways. This suggests that respiratory cilia play a role in sensing and responding to stimuli on the surface of the respiratory epithelium as part of homeostasis in the airways. However, the stimuli for this signalling and the subsequent effectors are unknown. Several species of the bacterial genus Bordetella colonise the mammalian respiratory tract. Some cause disease, such as B. pertussis the cause of the globally important disease whooping cough, while others such as B. bronchiseptica can be carried asymptomatically for long periods. These bacteria display exquisite specificity for adherence to the cilia of the respiratory tract, representing the primary site of colonisaton for these bacteria. In cases of disease, adherence can cause cessation of ciliary beat (ciliostasis) while we and others have shown the adherence can also disorganise rather than stop ciliary beat. Several bacterial adhesins (factors mediating adherence) have been identified that each play different roles in binding to the cilia. Genetic knock out of different combinations of adhesins affects the binding of the bacteria to the cilia and thus the degree of interference in ciliary beat. Thus the Bordetella represent a novel tool to probe ciliary dynamics and function in a cilia-specific manner. In this project we ask: ‘what is the role of cilia-specific signalling in sensing and responding to bacterial adherence?’ Our objectives are: 1. Define the ciliary receptors for binding to Bordetella adhesins. Preliminary evidence suggests cilia glycosphingolipids (GSLs) are bound

	directly by adhesins. We will purify and characterise cilia GSLs by mass spectrometry, identify receptor-adhesin combinations by TLC overlay experiments and blocking of adhesin binding by specific lectins. 2. Identify intracellular signalling initiated in response to bacterial adherence, including mutants deficient for specific adhesins, and engagement of receptors by specific, purified adhesins bound to microsphere beads. Preliminary evidence implicates Ca2+ signalling as critical to control of ciliary beat, but other key secondary messengers will be investigated along with the effect of antagonists of the signalling pathways mentioned above. 3. Identify the intercellular effects of signalling by analysing the secretion of cytokines from epithelial cultures in response to ciliary signalling. Blocking specific signalling pathways with specific antagonists will identify signal-cytokine pathways. 4. Model the effect of altered ciliary beat of fluid flow dynamics. Ciliary beat will be modulated using bacteria and adhesin binding, and the use of signalling pathway antagonists. The clearance function of cilia involves the movement of the overlying fluid phase by ciliary beat, but the control of this by coordinated ciliary beat, and the biophysics of this are poorly understood. To do this we will use organ culture and differentiated respiratory epithelial cell culture models derived from ovine respiratory cells. Tracheal tissue will be obtained from the University of Bristol abattoir. This will be used as tissue for high-speed imaging and modelling of cilia-driven fluid flows, work that will take place in the Wan lab at Exeter. Differentiated airway epithelial cultures, differentiated at the air-liquid interface will be used for analysis of signalling using high resolution confocal microscopy and cytokine responses using ELISA analysis of basal compartment fluids. The ovine lineage of B. parapertussis will be used to manipulate ciliary beat and responses, providing a natural host-pathogen interaction. Defined single and combination knock outs of key adhesins for this lineage are available to the student in the Preston lab. There are clear opportunities for the student to direct this project. Each of the different areas could become a major focus, depending on the interests of the student. There will be several key decision points dependent on initial results where the student could devise their own lines of investigation. For example, the study of fluid flow dynamics offers the chance to develop models that explain the effect of ciliary beat frequency, waveform, the viscosity of the surrounding fluid, the coordination of ciliary beat on flow generation function.
Can be completed part time?	No
Supervisory Team	
Lead Supervisor	
Name	Professor Andrew Preston
Affiliation	Bath
College/Faculty	Faculty of Science
Department/School	Department of Life Sciences
Email Address	ap753@bath.ac.uk
Co-Supervisor 1	
Name	Professor Kirsty Wan

Affiliation	Exeter
College/Faculty	Faculty of Health Sciences
Department/School	Living Systems Institute
Co-Supervisor 2	
Name	
Affiliation	
College/Faculty	
Department/School	
Co-Supervisor 3	
Name	
Affiliation	
College/Faculty	
Department/School	