CRUK Cambridge Centre MRes rotation project

Rotation Project Title	Targeting the neuroblastoma oncoprotein, MYCN, using intracellular nanobodies.
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Project Outline	**Aims and objectives** As part of their normal immune response, camelids make heavy-chain only antibodies, of which the variable domain has been termed a 'nanobody.' Nanobodies have specificities and affinities comparable to conventional antibodies, and they preferentially bind to conformational epitopes. Their small size (15kD; 4nm x 2.5nm), excellent solubility and singular functionality make nanobodies effective at recognizing their antigen within cells, allowing for precise competition of endogenous protein-protein interactions in situ. MYCN protein is a key regulator of cell proliferation and stem cell pluripotency and is pathologically deregulated in neuroblastoma. MYCN is a transcriptional regulator that dimerizes with a partner Max on DNA binding sites, known as E boxes, to regulate gene expression. In neuroblastoma, higher levels of MYCN can result from gene amplification, and its expression levels are thought to dictate how densely canonical and variant promoter binding sites are occupied. Whether MYCN's transforming capacity is due to global transcriptional regulation or mis-regulation at specific gene targets, for example controlling differentiation, is still debated. We propose to use nanobodies to obstruct partner binding at the transactivation domain of MYCN and determine if this affects MYCN-driven apoptosis and cellular transformation.
Experimental plan	We will express our nanobodies that bind specifically to MYCN in neuroblastoma cell lines which depend on its expression. The effects of nanobody expression will depend on where they bind MYCN, and which co-factors can interact with it. We anticipate the expression of nanobodies that interfere with the binding of factors that enable MYCN's essential function(s) may cause cell death or reverse transformed phenotypes or change MYCN stability. We will determine the functional effect of nanobody expression within MYCN-dependent neuroblastoma cells, including on MYCN half-life, on its endogenous DNA binding sites and on cell viability. Our experiments will define which of MYCN's partners enable its functions in cells, and will validate specific target sites within the transactivation domain that can be investigated for rational drug design. The outcome of these experiments will be the identification of bioactive nanobodies that control MYCN-driven functions, and these studies will answer questions about the co-factor interactions and gene networks that enable MYCN's oncogenic activity *in vivo*.
Main Techniques	• IP mass spectrometry experiments to identify co-factors interacting with MYCN • immunoblotting for MYCN to assess its half-life • cell proliferation and apoptosis induction assays • ChIP-Seq analysis to measure changes in MYCN's DNA binding sites *in vivo*
Key References	• Muyldermans, S. Nanobodies: natural single-domain antibodies. Annu Rev Biochem, *82*: 775-797, 2013. • Dang, C. V. MYC on the path to cancer. Cell, *149*: 22-35, 2012. • Beltran, H. The N-myc Oncogene: Maximizing its Targets, Regulation, and Therapeutic Potential. Mol Cancer Res, *12*: 815-822, 2014. • Hann, S. R. MYC cofactors: molecular switches controlling diverse biological outcomes. Cold Spring Harb Perspect Med, *4*: a014399, 2014.