Engineering Neonatal Fc Receptor (FcRn)-mediated Recycling and Transcytosis in Recombinant Proteins by Short Terminal Peptide Extensions
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Purpose
The importance of therapeutic recombinant proteins in medicine has led to a variety of tactics to increase their circulation time or to enable routes of administration other than injection. One clinically successful tactic to improve both protein circulation and delivery is to fuse the Fc-domain of immunoglobulin G (IgG) to therapeutic proteins so that the resulting fusion proteins interact with the human neonatal Fc receptor (FcRn). Although successful, Fc-fusion proteins significantly increase molecular weight thereby restricting tissue penetration, decrease protein function, and are limited to mammalian expression systems due to their complex disulfide structure. As an alternative to grafting the high molecular weight Fc-domain to therapeutic proteins, we have modified their N- and/or C-terminus with a short peptide sequence that interacts with FcRn.

Methods
FcRn binding peptide (FcBP) sequences were genetically fused to the N- and/or C-terminus of model proteins and expressed in E. coli. The interaction between FcBP fusion proteins and FcRn was characterized in vitro through a number of molecular and cell-based assays including surface plasmon resonance, FACS, fluorescence microscopy, and cellular recycling and transcytosis.

Results
The small size and simple structure of the FcBP allows for expression of FcBP fusion proteins in E. coli and results in their pH-dependent binding to FcRn with an affinity comparable to that of IgG. The FcBP fusion proteins are internalized, recycled and transcytosed across cell monolayers that express FcRn. Modification of FcBP sequence results in FcBP fusion proteins with differential FcRn binding properties and altered transport across cell monolayers.

Conclusion
We demonstrate that proteins can be engineered to interact with FcRn by recombinant fusion of a short FcBP sequence at a proteins N- and/or C-terminus. The FcBP fusion approach may overcome limitations associated with alternative strategies to improve protein half-life, be easy to manufacture, and provide a solution to both the rapid elimination and limited protein delivery routes that may translate to improved therapeutic activity and compliance in the clinic.