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January 15, 2010

Dr. Colin R. Parrish  
Baker Institute for Animal Health  
College of Veterinary Medicine  
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Dear Colin,

This is to confirm my interest and ability to assist with your studies that involve the engineering and selection of altered versions of TfR ectodomains or antibody domains with increased or decreased affinities. One approach that you have proposed to use includes yeast expression and display using Aga2p fusions and selection of altered versions with high affinities after error-prone PCR. I have had considerable experience with these methods in my studies of integrins and antibodies. I have also been developing methods for expression of the mouse TfR ectodomain in the system for my own studies of blood-brain barrier dynamics, and for the development of isolated ICAM-1 domains for rhinovirus binding studies.

The tools for the assays are available in both our laboratories and can be readily adapted to the expression of the different antibody domains or the canine and feline TfRs. The post-doctoral fellows in our laboratories have been working on these projects for the past several months, and are making good progress, having prepared the candidate proteins in yeast expression systems. We are able to use either the fluorescence-based cell sorter or magnetic sorting of the yeast using either labeled parvoviruses or antibodies as the detection methods. I also have a Biacore 2000 analyzer in my laboratory which can be used for the assays of the binding affinities of the purified components.

I look forward to continuing collaborations, and expect that these studies will increase our understanding of the processes of virus binding to cells for infection, as well as antibody binding and neutralizations.

Best Regards,

A handwritten signature in black ink that reads "Moonsoo Jin".

Moonsoo Jin, Sc.D.  
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Dear Susan and Colin,

This letter is written in support of your application for an NIH grant studying the structures and functions of the parvovirus capsids. During the last few years, I have worked closely and collaborated on several projects with Dr. Hafenstein while she was at Purdue. These efforts have resulted in the publication of "Asymmetric binding of transferrin receptor to parvovirus capsids" in the *Proceedings of the Nation Academy of Sciences* and "The Interaction of Decay-accelerating Factor with Coxsackievirus B3" in the *Journal of Virology*. Dr. Hafenstein was first author on both of these publications.

The Structural Biology Group of Purdue's Biology Department has recently moved into their new building. The building is also the new home of the Biological Electron Microscopy Facility with three electron microscopes capable of performing high-resolution, cryo-electron microscopy of macromolecules such as viruses. This includes a recently acquired FEI Titan Krios instrument, a state of the art transmission electron microscope capable of achieving atomic resolution. This instrument is the most advanced electron microscope currently available. It has accessories to permit examination of frozen hydrated samples, a Gatan Tridiem energy filter to improve electron tomograms and an automatic loading device, eliminating the need for a technician to be present during each sample change. With automated image acquisition, optimized performance throughout the 80 to 300kV range and a Gatan 4k x 4k CCD camera, this instrument is highly versatile and designed for high throughput studies.

I am very enthusiastic about your project and would be happy to assist in the use of the microscopes for cryoEM, as well as in the data processing. I look forward to continuing to aid your research.

Best Regards,

A handwritten signature in blue ink, appearing to read "P. Chipman".

Paul Chipman,  
Director, Electron Microscopy Facility