

**Title:** Identification of the viral protein that mediates PRRSV attachment to the sialoadhesin receptor on primary macrophages, and determination of the minimal epitope on this protein needed for receptor interaction. **NPB Project #05-191.**

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### Abstract:

To identify potential PRRSV ligands that mediate virus binding with the macrophage-specific PRRSV receptor sialoadhesin, we constructed a soluble form of sialoadhesin. This soluble sialoadhesin consists of the first 4 IgG domains of Sn, fused to the human IgG1 Fc domain. The soluble sialoadhesin was excreted in the medium of transfected HEK-293T cells upon transfection of coding DNA. Further characterization revealed that the recombinant receptor had the same conformation as the native sialoadhesin and that it was processed similarly to the native sialoadhesin. The soluble sialoadhesin was also shown to be functional by red blood cell binding assays and in PRRSV infection blocking experiments. The soluble receptor was then used in a modified immunoprecipitation reaction. Soluble sialoadhesin was coated on protein A coated beads and mixed with a lysate of semi-purified virus. Afterwards, proteins bound to the soluble sialoadhesin were separated from non-bound proteins. Analysis of the obtained fractions on Western blot with PRRSV specific monoclonal antibodies revealed that the viral M-GP5 complex was bound to the soluble sialoadhesin, while GP4 and GP3 did not bind. Together, these results suggest that the M, or the GP5 protein mediate PRRSV attachment to sialoadhesin.

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