O-35

Statistical, Evolutionary, and Multiplex Methods to Map Phenotypic Variation onto Rigid Viral Structures

Luk H. Vandenberghe

Department of Ophthalmology, Harvard Medical School, Boston, MA, USA; The Broad Institute of Harvard and MIT, Cambridge MA, USA; Grousbeck Gene Therapy Center, Schepens Eye Research Institute and Mass Eye and Ear Infirmary, Boston MA, USA

The icosahedral particle architecture of parvoviruses is structurally conserved across its subfamilies and generae. Variable domains on that rigid architecture are thought to provide the wide diversity in viral functions within the subfamilies, generae, species and isolates. Our laboratory studies within the Dependovirus genera how this- at the same time both rigid and variable- structure is able to impose such degree of phenotypic variation. It is this variation that is exploited in the field of gene therapy in which immunological, tropism, viral yield, stability, and many other differences determine the feasibility, safety, and/or efficacy of a therapeutic approach. Methods to systematically study this structure-function landscape are limited, primarily due to the tertiary and quaternary interactions that need to be preserved even to just achieve assembly. Here, we present several novel methods toward these questions with illustrations of how they allow to map and provide insight in AAV mechanisms. A first method is phenotype-to-phylogeny mapping which seeks to define branchpoints in which phenotypic switches occur. Previous work on ancestral sequence reconstruction for primate AAV isolates established functional reagents of a putative AAV phylogeny (AncAAVs). Interrogating AncAAV function, as we have done for viral assembly and receptor usage, allows the mapping along branches, clades, and nodes where within a small genetic window a property was lost or gained. Second, we have developed an analytical method based on defined AAV structures to visualize intra- and intermolecular interactions and networks. Third, we have developed a quantitative high-throughput assay for parallel assessment of combinatorial variation on the capsid. Specifically, leveraging data from a maximum-likelihood analysis, 11 residues were identified as selective drivers and for which we interrogated their combinatorial impact on assembly, packaging, and tissue tropism. These methods may be potent tools to disentangle epistatic functional networks from the constraints the icosahedral capsid symmetry imposes.