* IF EC, FC
* IF EC, FC 🡪 Correlation Coefficient and Colocalization
* Binding Competition (FC:EC)
* Binding Competition (FC: FCCHT)
* Infection Competition (FC:EC)

**Conclusions**

* FC are preferentially bound compared to EC.
* EC do not interefere with genome delivery to the nucleus.
* EC have lower affinity to sialic acid
* FC / EC Pufification from CsCl gradients
* Binding saturation curve (Moi 20) saturated
* Neuraminidase treatment kinetics

Aim:

Roles of N-VP2 in the life cycle of MVM

* Entry
* Maturation
* Egress

**EC can be separated from FC by CsCl gradient centrifugation**

Due to their lack of DNA, EC band at lower density in a CsCl gradient compared to FC. While FC entered the gradient to a density of 1.46 g/cm3, EC already banded at 1.32 g/cm3. Analysis by quantitative PCR (qPCR) confirmed that EC were depleted from viral DNA containing particles by almost 3 logs.

**EC and FC exhibit morphological differences**

The flexible, unordered N-terminus of the major structural protein, VP2, shows distinct conformation in either capsid population. N-VP2 is accessible to proteolytic digestion or specific antibodies only in FC, whereas it remains inaccessible in EC.

**Both FC and EC bind specifically to SA residues on the cell surface**

In order to characterize the binding specificity of FC and EC, both capsid types were allowed to bind to susceptible, restrictive murine fibroblasts at 4 °C. At such low temperature, cell-mediated uptake through endocytosis is prohibited. Unbound viruses were removed by washing the adherent cells several times with PBS. Both capsid species restrictively bind to SA residues since they can be completely depleted from the cell surface by treatment with neuraminidase, an enzyme that specifically hydrolyzes glycosidic linkages of neuraminic acids. In order to guarantee a complete removal of viral particles from the cellular surface, a minimal dose of 25 U/mL is required.

**FC are preferentially bound to the SA residues on the cell surface**

When FC and EC capsids were bound to cells at equal stoichiometry, FC preferentially bound to the cell surface, indicating a higher binding affinity for FC compared to EC. Even under non-saturated conditions, EC were detected rarely when applied as mixed populations. Only when FC were added subsequently to the EC a slight increase in bound EC was observed. Nevertheless, EC did not represent 50% of the bound population.

Additional Results Structure:

* EC also can be purified from SN of infected cultures
* Role of EC?
  + Display morphological differences
  + Bind specifically to SA residues on cell surface
  + Do NOT compete with FC in terms of binding and infection
* Role of N-VP2..? Binding? Stabilization? Internalization..?

Study of infectious virus populations by FPLC

* *In vivo*: AEX: Native, bound, internalized 🡪 Characterization of virus populations
* *In vitro*: Chromatofocusing: CSL project (sensitive to changes on the very surface

FPLC

* Our lab: trafficking: Rearrangements (Mani, Nico,…)
* Nico 🡪 B19V in vivo aquires phosphorylations and undergoes structural rearrangements
* FPLC: chromatography surface features lead to separation
  + AEX: surface charges, Cl- gradient
  + Chromatofocusing: IP, pH-Gradient