Abstract Submitted
for the MAR08 Meeting of
The American Physical Society

The study of viral assembly with fluorescence fluctuation spectroscopy JOACHIM MUELLER, BIN WU, YAN CHEN, University of Minnesota — Enveloped viruses contain an encapsulating membrane that the virus acquires from the host cell during the budding process. The presence of the enveloping lipid membrane complicates the physical characterization of the proteins assembled within the virus considerably. Here we present a method based on fluorescence fluctuations that quantifies the copy number of proteins within an enveloped viral particles. We choose the viral protein Gag of the human immunodeficiency virus (HIV) type 1 as a model system, because Gag expressed in cells is sufficient to produce viral-like particles (VLPs) of the same size as authentic virions. VLPs harvested from cells that express fluorescently labeled Gag were investigated by two-photon fluorescence fluctuation spectroscopy. The autocorrelation functions of the fluctuations revealed a hydrodynamic size of the fluorescent VLPs consistent with previous results based on electron microscopy. Further analysis of the fluctuations revealed a copy number of Gag per virion that is inconsistent with the prevailing model of HIV assembly. We will discuss the implications of our experimental results for the assembly process of VLPs.

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Date submitted: 27 Nov 2007 Electronic form version 1.4