Kinetics and Mechanics of Pathogenic Virus Assembly on a Lipid Membrane.

The assembly of enveloped viruses such as HIV or influenza results from the self-association of a structural protein (Gag in the case of HIV, M1 for influenza) on a lipid bilayer (on the inner monolayer of the plasma membrane in the case of HIV and influenza) and leads to the formation of a spherical virion approximately 100 nm in diameter. The time required for this assembly is commonly accepted to be around 5 minutes, and we have been able to reproduce the experiment on a model system in the presence of the Gag protein and a supported lipid bilayer of controlled composition (1,2). In the literature, this same time has been established in the cell by different groups (3,4) and by ourselves (5), by measuring, using TIRF or SMLM microscopy, the evolution of the fluorescence of Gag clusters on the basal part, i.e., the part attached to the glass. However, in a recent article (6), using ion microscopy, the measurement of this time on the apical surface of the cell, i.e., the one facing the water, results in values of 10 to 20 seconds, e.g. 10 times faster, leading to controversy over the time required to assemble a virus in a cell. One possible explanation for this discrepancy is related to the difference in energy cost between virus formation (assembly and curvature of the membrane) against a rigid surface (glass) versus in water. Another possibility, related to the first, is that the deformation of the membrane around an assembling structure creates an attractive potential that promotes the growth of this structure, and that this potential is reduced by the presence of a rigid surface. To test this hypothesis, we propose to combine an experimental and theoretical approach to this phenomenon.

In the experimental approach, we will use an AFM microscope coupled with a STED. We will deposit lipid bilayers on substrates of varying rigidity calibrated a priori by AFM nanoindentation. We will then inject the labeled Gag protein and measure, in real time, the formation of assembly clusters, thus accessing the kinetics. At the end of the experiment, using AFM, we will image the topology of the assemblies and check for possible deformations of substrates of varying rigidity. All measurable parameters will be used to verify a model developed in collaboration with P. Sens at (Team leader Physical Approaches of Biological Problems, UMR168, Institut Curie), based on its expertise of this type of questions (7,8,9). The model will produce predictions regarding the effect of substrate rigidity on formation kinetics, which will be directly compared with the experimental results.

The intern will perform correlative microscopy experiments (AFM/STED) on substrates of varying rigidity developed in the laboratory. He/she will perform quantitative measurements of membrane tension, substrate rigidity and deformation (nano-indentation and AFM topological imaging), assembly kinetics (STED), and morphological characterization of assemblies (STED+AFM). In coordination with the theoretical physicist, he/she will enter these values into the models developed to test the validity of these models and, if necessary, adapt these predictive models to the experiment. At the end of this internship, we hope to obtain a model of the assembly that allows us to predict assembly rates as a function of membrane tension and the stiffness of the medium against which the assembly occurs. We will then test this model (or during, depending on progress) during the assembly of infectious viruses in a living cell. The intern is strongly encouraged to continue this project as part of a PhD program. This internship is funded for a maximum of 6 months and will take place in Montpellier.

Keywords: Modeling/Theory – Quantitative correlative microscopy (AFM/STED) – Mechanobiology – Kinetics/Dynamics – Nanometric molecular assemblies – Pathogenic viruses and cell membranes.

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