Outlook

For me the work on this thesis has certainly raised further questions. I hope to be able to pursue research on the topic of viral fusion, not only because it is such an interesting topic. I want to understand how viruses infect humans and animals, and most importantly how we can prevent viral infection. This may enable us to prevent illness and save lives. Beyond the need to survive and to live and travel in risk areas for viral diseases, the understanding of viral infection mechanisms may also lead to new technologies. We might for example develop drug delivery systems that reach targets inside patient cells in similar ways as viruses when they infect the host cell.

I intend to run more simulations of the dengue viral E protein to test the new hypotheses put forward in this thesis. The plan is to add one or two membranes, i.e. lipid bilayers, to the simulation system and to simulate the interactions between the protein and the membranes. The effects that the proteins might have on the membranes may offer new insights into how the E protein mediates membrane fusion. In fact, for many viral proteins the mechanisms by which they mediate fusion are not known and difficult to investigate experimentally, mainly because membranes are difficult to study. Membrane structure and dynamics are good examples of topics that can be studied more easily with computer simulations than in real experiments. What we learn from simulations can in turn be used to generate new hypotheses for experiments, in other words, simulations can give clues on what to look for in experiments. In the case of the dengue viral E protein, the model for fusion proposed in this thesis could be tested by attaching the two subunits of the protein with a flexible linker that allows some conformational change, but not the separation into individual subunits. If the protein mediates fusion in the dimeric form, i.e. consisting of two subunits, then fusion will occur despite the linker, but no trimers will form, as this would require three separate subunits.

Further questions related to viral fusion are: Given the mixture of lipids in viral membranes, how are the different lipid species distributed, homogeneously or do they form clusters? How do the transmembrane helices of the envelope proteins affect the viral membrane?
So far I have simulated the ectodomain of the E protein, which is truncated from the membrane domain. The membrane domain is made up of transmembrane helices that run through the viral membrane and anchor the ectodomain to the membrane. The M protein is another, smaller envelope protein. It also has a membrane domain that contains transmembrane helices. I suspect that the transmembrane helices of both the E and the M protein organise the structure of the viral membrane, and I would like to investigate this in simulations of the membrane domains with a membrane. The membrane domains might even affect the ectodomain of the E protein, which was proposed in the hypothesis on the flaviviral fusion mechanism in the final chapter of this thesis. This could be tested by simulating a system that combines all the components of the viral envelope: the E and the M protein and the viral membrane.

Eventually any simulation result needs to be verified experimentally. New techniques like fluorescent imaging are being developed for membrane experiments, that may offer insights into the effects of protein/membrane interactions on membrane structure and dynamics.