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Viruses as a tool in nanotechnology and target for conjugated polymers

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Summary

Viruses have been extensively investigated since decades. However, today they are not only being recognized for their clinical implications, but also as an inspiration and a powerful tool in multidisciplinary material science.

In Chapter 1 the main achievements in virus engineering were described. This was done with focus on formation of virus-like particles (VLPs) as nanocontainers. These objects consist of assembled capsid proteins that often lack viral genetic material. Instead they can be equipped with various cargos that exhibit diverse functionalities. Furthermore, the VLP exterior can be decorated with a vast variety of moieties, introduced either chemically or via genetic engineering. Such multifunctional bionanoparticles were formed around metal and semiconductor nanoparticles, synthetic polymers or imaging agents and successfully applied as in vivo imaging probes, targeted therapeutics or scaffolds to perform size constrained synthesis. Finally, the importance of viral oncotherapy, gene therapy and vaccine development were highlighted as they are the most prominent examples of virus engineering products applied in modern medicine.

In Chapter 2, we introduced new hybrids containing single walled carbon nanotubes (SWCNTs) as a new type of functional material and showed that these entities can be efficiently encapsulated in a protein shell. To control interactions of proteins with the surface of SWCNTs, DNA-guided assembly of viral coat proteins was performed. Afterwards, electron microscopy was employed to probe the assembly of two

filamentous and a spherical virus particle on SWCNT template in aqueous solution (tobacco mosaic virus (TMV), potato virus X (PVX) and cowpea chlorotic mottle virus (CCMV), respectively). It was revealed that rod-like virus candidates are not applicable as protein donors. In contrast, the CCMV coat protein yielded uniform and complete coverage along the carbon nanotube. Thereby, it was demonstrated that DNA-dispersed SWCNTs are well suited for templating despite their extreme shape anisotropy. Our approach did not involve covalent modifications, which is desired because it doesn't impair the functionality of SWCNTs or the protein structure. At the same time incorporation of the well-studied capsid proteins in such hybrids allows to benefit from a toolbox of genetically and chemically modified VLPs to tailor the system for future applications.

In Chapter 3, the influence of such a protein shell on electrical properties of the SWCNT was evaluated. Therefore, the method described in the previous chapter was adapted to obtain hybrids with defined electrical properties. At first, a specific DNA sequence was employed to isolate purely semiconducting SWCNTs. In a next step, the hybrids were used to form a virus-like SWCNT encapsulated system. It was revealed that the length of the oligonucleotide used as a dispersing agent plays an important role in the templating of the virus capsid. Sequences with more than 22 nucleotides represent a more suitable scaffold, and therefore, yield fully encapsulated SWCNTs. Furthermore, two electric circuits were constructed – one operating on a single object level, and another one assessing tube networks. The single tube measurements in a field effect transistor configuration showed that the hybrid material exhibits semiconducting behaviour originating from the presence of SWCNT. However, it was clearly demonstrated that the protein layer contributes to significant electrical insulation. In the next step, measurements on a SWCNTs network were performed. It was observed that the constructed hybrid allows for assembling of devices characterized by on/off ratios that are as high as 10^5 despite the presence of two layers of biological macromolecules. As such, we believe that this represents a system that can be easily adopted for any application in biosensing.

The study presented in Chapter 4 was focused on exploitation of a novel polymeric scaffold that enabled manipulation of the activity of Human Immunodeficiency Virus (HIV). Although a number of polymers were already employed in virology, the variety of investigated structures is

limited and more complex compounds remain unexplored. Hence we proposed two conjugated water soluble polyfluorenes – polycationic TMPF-P and polyanionic PPF-P, which were functionalized with quaternary ammonium salts and phosphonate group, respectively. Additionally, we included monomeric and dimeric structural analogues of the polymers, to compare their activity with the ones of the polymers. It was shown that the negatively charged PPF-P displays a virucidal character, while the cationic variant exhibits an opposite activity and promotes viral infections. At the same time, the low molecular weight analogues did not prove to be active. Furthermore both polymer candidates did not show any cytotoxicity. Although all performed experiments suggested that the polymers activity is most likely virus oriented as the cell-treatment did not show major changes, the mode of action of these agents remained unclear.

Therefore, in Chapter 5, we designed model membranes, which mimic the lipid composition of HIV and its host cell in respect to surface charge and fluidity. The biophysical studies presented in this chapter revealed that the PPF-P interacts with phospholipid membranes and to some extent destabilizes them in a charge-independent manner. This was in agreement with the outcome of the biological experiments. The viral membrane targeting mechanism and low cytotoxicity makes PPF-P a very promising virucidal agent. Next, it was shown that the activity of TMPF-P is driven by electrostatic interactions. The presence of this cationic agent lead to reduction of electrostatic barriers between negatively charged virions and cells. As a result, substantial aggregation occurred. Thus it is expected that such aggregation also happens to HIV virions, which therefore sediment faster on the cell surface, and increase the possibility of infection. It was pointed out that retroviruses are often used as a gene transfer tool in molecular biology as well as in gene therapy; therefore such infection enhancers can potentially improve efficiency of transfection in both fields *in vitro* and *in vivo*.

