

PLENARY LECTURE P3

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From Actin-bundling Proteins to Coiled Coils and, eventually, Peptide Nanoparticles

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Cortexillins are actin-bundling proteins that dimerize via an 18 heptad-repeat oligomerization domain which forms a parallel 2-stranded α -helical coiled coil. By solving the crystal structure of this 19 nm long dimeric rod, we have found that heptads 13 and 14 exhibit a distinct network of intra- and interhelical salt bridges that, in combination with the hydrophobic interactions occurring along the dimer interface, render this 14-residue segment an effective ‘coiled-coil trigger site’. Based on this structural insight, we *de novo* designed, optimized and characterized a series of two heptad-repeat long peptides by systematically varying their complex network of intra- and interhelical salt bridges. As expected, in the absence of any interhelical salt bridge, while still being α -helical, the corresponding peptides no longer formed stable coiled coils. In one case a highly regular, octameric nanoparticle with a 1 nm- diameter central cavity formed that was completely devoid of coiled-coil interactions. Next, we designed segmented heptad-repeat peptides aimed to self-assemble into regular polyhedra similar in size and shape to small virus capsids. More specifically, a *de novo* designed trimeric coiled-coil forming domain was joined by a short linker segment to the pentameric coiled-coil forming domain of the cartilage protein COMP. The linker was designed such that the two α -helical segments were inclined by the same angle relative to each other as are the 3- and 5-fold axes of symmetry within a regular icosahedron. Indeed, the resulting peptide self-assembled into globular, 16 nm-diameter nanoparticles harboring a 6.5 nm-diameter central cavity.

These ‘synthetic capsids’ made of 60 peptides each, can be produced recombinantly in *E. coli*. Hence, they represent a novel type of nanoparticles that are optimally suited for synthetic vaccine design, diagnostic imaging, and drug delivery and targeting. Since they are proteinaceous, they are fully biocompatible and biodegradable. Moreover, the modular peptide design allows for almost unlimited customization of these nanoparticles in terms of their size, shape, stability, and functionalization. In particular, they represent a most versatile design platform for a drug targeting toolbox: different drugs (e.g., radioisotopes, doxorubicin, etc.) may be combined with different targeting moieties (e.g., somatostatin, bombesin, etc.) in an easy and effective manner.

Most significantly, similar to virus capsids, such ‘crystalline’ nanoparticles exhibiting polyhedral symmetry represent an ideal ‘rig’ for repetitive antigen display. Surface proteins of pathogens or fragments thereof can easily be grafted onto the peptide building block so that they are optimally displayed on the nanoparticle’s surface. Notably, the

surface proteins of enveloped viruses contain a trimeric coiled-coil sequence crucial for their cell penetration mechanism. For example, by extending the peptide building block's trimeric coiled-coil segment by the respective coiled-coil sequences of HIV, influenza, Ebola, or another enveloped virus, a subunit vaccine against these viruses can readily be designed. Most importantly, such nanoparticle-based vaccines can be produced in a single manufacturing process without the need for any additional chemical modifications, and, as opposed to most virus-like particles, are stable without the need of packaging DNA inside the particles.