Designing multi-tasking protein nanoparticles from scratch

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Background

Over the last few years, computationally designed de novo protein nanoparticles have seen a dramatic increase in their therapeutic potential. Specifically, numerous nanoparticles have been created enabling the multivalent display of protein antigens and encapsulation of genetic material. These achievements have been accomplished by modifying the exterior and interior of the been shown to display antigens and encapsulate their own genome just by modifying the exterior and interior of the protein nanoparticle scaffolds, revealing a multitude of potential immunological and drug delivery applications. A recently designed set of protein nanoparticles incorporating the unmodified Fc domain of antibodies have been used to help target specific immune cells. Recent data has shown that these antibody nanoparticles robustly promote receptor agonism, as compared to free antibody obtained from the same clone. This platform benefits from its 'plug-and-play' design philosophy, which enables the simple swapping of the antibody-of-interest (or Fc-fused targeting domain) during nanoparticle assembly, thereby removing the need for any computational redesign when changing cellular targets. Despite their usefulness, these protein nanoparticle scaffolds currently contain a large pore located on their 3-fold symmetry axes. This pore provides an opportunity for protein design to 'plug' this region to allow for geometric-control of multivalent displayed protein antigens alongside the Fc-fused receptor targeting domains. Finally, successful plugging of this pore will help enhance encapsulation and retention of small molecules, and will pave the way for a variety of targeted drug-delivery applications.

Methods

We aimed to develop a computational method that would plug the pore in the antibody nanoparticle scaffolds with a functional protein. Among these potential proteins are scaffolds that can display viral antigens or proteins that are sensitive to cellular environments. Our computational method samples the rotational and translation degrees of freedom and designs a hydrophobic protein-protein interface between the plug and the pore. We also examine the assembly dynamics and validate the structure of the designed plugged antibody nanoparticles in vitro utilizing a diverse array of biochemical, biophysical, and structural methods.

Results

We have developed a computational method that orients the plug and pore and designs a protein-protein interface between them. The resulting protein nanoparticle has two designed protein-protein interfaces—one between the plug and the nanoparticle

assembler and another between the nanoparticle assembler and the Fc. Assembling the three components in vitro require cooperativity between all three components. We have also assembled the protein components in vitro and have successfully validated a three-component plugged antibody nanoparticles with low- and high-resolution techniques (Figure 1).

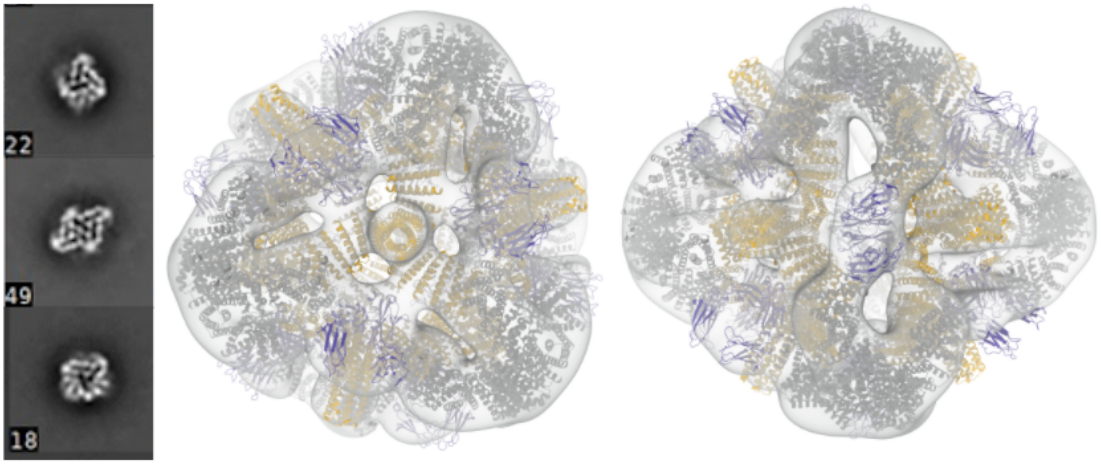

Figure 1: Two-dimensional class averages and three-dimensional reconstructions of plugged antibody nanoparticle. In vitro assembly of a trimeric plug, tetrameric nanoparticle assembler, and Antibody Fc fragment analyzed for assembly by negative stain electron microscopy. A 10.6Å reconstruction shows the nanoparticle assembler (gray) assembling in an octahedral nanoparticle and making interfaces with a trimeric plug (gray) and Fc fragment (purple).

Conclusion

We have demonstrated that it is possible to computationally design and in vitro assemble three-component nanoparticles with two designed protein-protein interfaces. A generalizable computational plugging method enables the design of nanoparticles that can perform multiple functions simultaneously. The computational design of plugged antibody nanoparticles will have great impacts on modulating immune responses and in targeted drug delivery.

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