Bioengineered norovirus S_{60} nanoparticles as a vaccine platform

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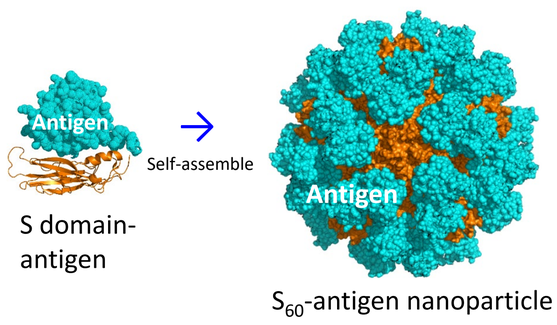
Abstract

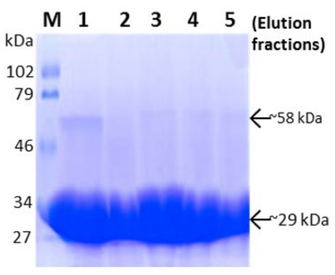
Introduction. Homotypic interactions of major viral capsid proteins are common, driving viral capsid self-formation in nature. It is known that repeated patterns of antigens displayed by a viral capsid-based nanoparticle enhance their immune responses, making such a nanoparticle an ideal platform for antigen presentation for vaccine development. **Aims.** This study is to develop a new norovirus (NoV) shell (S)-based nanoparticle as a platform to display antigens for improved immunogenicity for novel vaccine development. **Methods.** A 60-valent, icosahedral S_{60} nanoparticle was developed by introduction of modifications to NoV S domain. The self-assembled S_{60} nanoparticles are produced by expression of the modified S domain proteins in the *E. coli* system. **Results.** The S_{60} nanoparticle with 60 exposed S domain C-termini offers an ideal platform for antigen presentation, leading to enhanced immunogenicity toward the displayed antigens. This was proven by constructing a chimeric S_{60} nanoparticle displaying 60 rotavirus (RV) VP8* proteins, the major RV neutralizing antigen. The S_{60}-VP8* particles are easily produced, highly stable, and highly immunogenic, eliciting high antibody titers toward the VP8* antigens in mice. The resulted mouse antisera exhibited high neutralizing activities against RV replication in culture cells. The S_{60}-VP8* nanoparticle vaccine also protected immunized mice from murine RV infection. Furthermore, the S_{60} nanoparticle can display other antigens, supporting the notion that the S_{60} nanoparticle is a multifunctional vaccine platform. **Discussion.** This S_{60} nanoparticle offers a new polyvalent platform to turn the small, low valent, and low immunogenic antigens into large, polyvalent, and highly immunogenic antigens for vaccine development. Our S_{60} nanoparticle platform may be applied to many antigens for novel vaccine development.



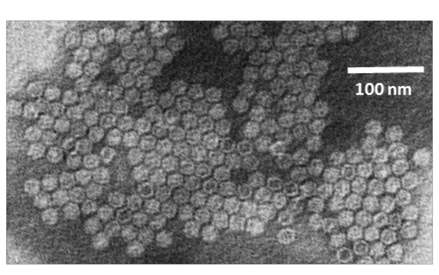
Creation of the NoV S_{60} nanoparticle

The inner shell of NoV capsid is made by the shell (S) domains of NoV capsid protein VP1. We invented a technology to efficiently produce uniform 60-valent, icosahedral S_{60} nanoparticles. This is achieved by introducing modifications to the S domain, including an $R_{69}A$ mutation to destruct the exposed R_{69}-N_{70} proteinase site that causes degradation of the S protein. SDS PAGE gel showed the high yields and stability of S proteins production. EM observation revealed large amount of uniform S_{60} particles. Gel-filtration chromatography showed that majority of the S proteins forms S particles as shown by the first peak. Native mass spectrometry analysis detected only 60 valent, but not 180 valent S particles

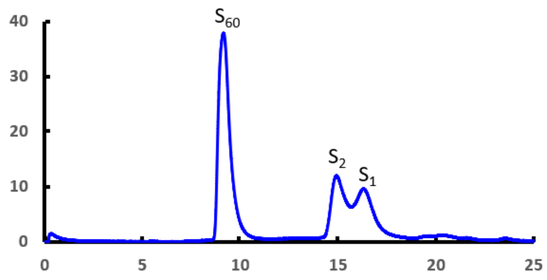
Expression construct



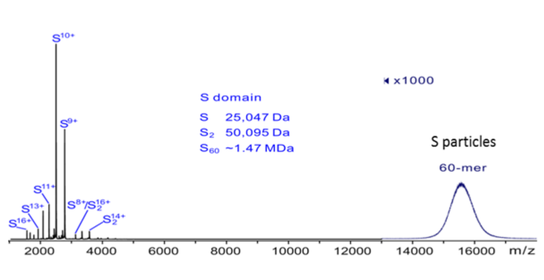
SDS PAGE



EM observation



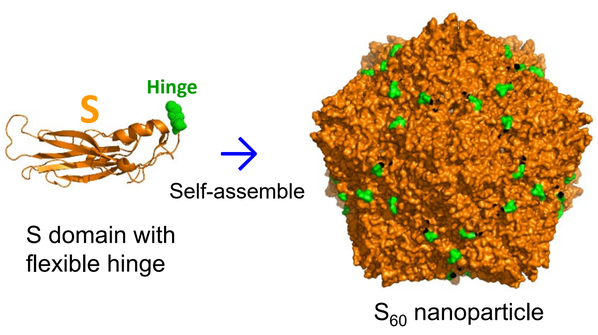
Gel-filtration chromatography



Native mass spectrometry

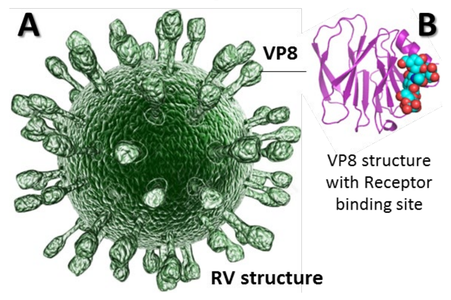
Structural Analyses of the NoV S_{60} nanoparticles

Based on the known 60 valent feline calicivirus (FCV) capsid, we modeled the structure of the S_{60} nanoparticle and found that each S_{60} nanoparticle has 60 freely exposed S domain C-termini that serve as excellent fusion sites for foreign antigen presentation, making the S_{60} nanoparticle a potent platform to display antigens for improved immune responses. When antigens are fused to the S domain C-termini via recombinant DNA technology and the S-antigen fusion proteins are produced via the *E. coli* system, chimeric S_{60}-antigen nanoparticle self-assembles with antigens being displayed on the surface. For example, when His-tag is fused to the hinge, the exposed His-tags enable purification of the S_{60} nano-particles.



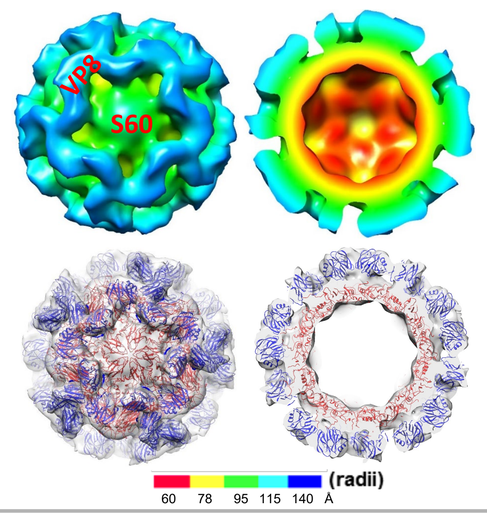
Rotavirus (RV) VP8* as an excellent vaccine target

VP8* is the distal head of RV spike protein VP4 that determines RV P types. VP8* binds host glycan attachment factors or receptors to initiate RV infections, and thus is a key vaccine target. Recent studies showed that VP8* play key roles in RV immune response as shown by 1) antibodies elicited by nature RV infections are mostly (75%) VP8*-specific; 2) vast majority (92%) of VP8*-directed monoclonal antibodies (mAbs) neutralized RV infections; 3) the VP8*-directed mAbs protect sucking mice from human RV challenges; 4) a nanoparticle-based VP8* vaccine protected immunized Gn pigs from RV infection and disease; and 5) most isolated VP8*-directed mAbs reacted with more than one VP8* type. These new data support the idea to develop P type-based RV vaccines targeting the VP8* antigens, leading to the development of our S_{60}-VP8* nanoparticle vaccine.



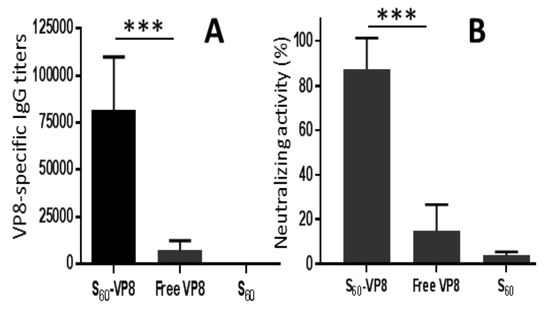
Development of the S_{60}-VP8* nanoparticle

A P[8] VP8* was used to construct the first S_{60}-VP8* nanoparticle. Our data showed that **1)** production of the S-VP8* fusion protein had high yields of 30-40 mg soluble protein per liter bacterial culture; **2)** ~95% of the purified fusion protein self-assembled into uniform S_{60}-VP8* nano-particles, **3)** the Hisx6-tagged S_{60}-VP8* nanoparticles can be purified by His-tag binding resin; **4)** the purified S_{60}-VP8* nanoparticles are highly stable; and **5)** the S_{60}-VP8* nanoparticle is highly immunogenic. The structure of the S_{60}-VP8* nanoparticle has been solved by cryo-EM and verified by fitting of the related crystal structures of an FCV 60-valent shell (4BP6) and P[8] VP8*s (2DWR) to the corresponding regions of the electron density maps.



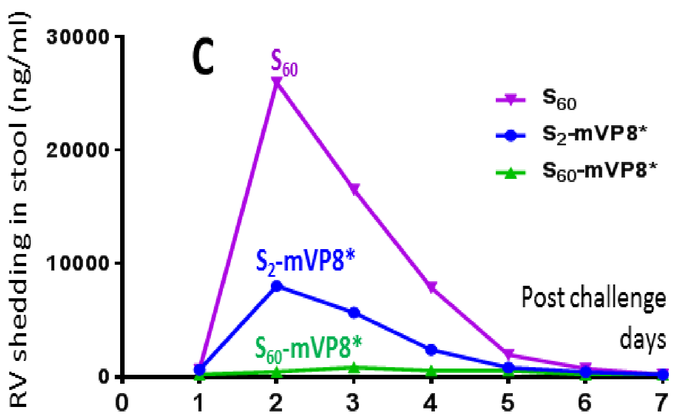
The S_{60}-VP8* nanoparticle elicited strong VP8*-specific immune response

Mouse immunization study showed that the S_{60}-VP8* nanoparticle elicited 11.6-fold higher VP8*-specific IgG titer than that induced by the free VP8* antigens (P=0.0004). The mouse sera after immunization with the nanoparticle showed 22.8-fold higher 50% blocking titer (BT_{50}) against RV VP8*-glycan receptor attachment than that of the mouse sera after immunization with the free VP8* antigens (P=0.0003). In addition, the mouse sera after immunization with the S_{60}-VP8* nanoparticle exhibited 5.28-fold higher neutralizing activity against RV replication in culture cells than that of the mouse sera after immunization with free VP8* antigens (P=0.0001). Thus, the S_{60}-VP8* nano-particles are a promising RV vaccine candidate.



The S_{60}-VP8* nanoparticles protected immunized mice against infection by homologous murine RVs.

A mouse RV challenge study using mRV (EDIM strain) was conducted to assess the protective efficacy of the S_{60}-VP8* nanoparticles. We found that the S_{60}-VP8* nanoparticles containing the mRV VP8* at 5 μg/dose for three doses protected the nanoparticles containing the mRV VP8* at 5 μg/dose for three doses protected the immunized mice against mRV infection at a high efficacy of 97% in mRV shedding reduction compared with the mock control mice immunized with the S_{60} nanoparticles without VP8*. These data further testify the S_{60}-VP8 nanoparticles as a potent RV vaccine.



Mouse RV shedding dynamics after vaccination and mRV challenge

Conclusions and discussions

The S_{60} nanoparticle serves as potent platform to display foreign antigens for improved immunogenicity.

This S_{60} nanoparticle offers a new versatile platform to turn the small, low valent, and low immunogenic antigens into highly immunogenic ones for vaccine development.

Our S_{60} nanoparticle platform may be applied to many antigens for novel vaccine development.