

Hybrid methods guide structure based vaccine design for picornaviruses

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The physical properties of viral capsids are major determinants of vaccine efficacy for picornaviruses which impact on human and animal health. Current vaccines are produced from inactivated virus. Inactivation often reduces the stability of the virus capsid, causing a problem for Foot and Mouth Disease Virus (FMDV) where certain serotypes fall apart into pentameric assemblies below pH 6.5 or at temperatures above 37°C, destroying their effectiveness in eliciting a protective immune response. As a result, vaccines require a cold chain for storage and animals need to be frequently immunised.

Globally there are seven FMDV serotypes: O, A, Asia1, C and SAT-1, -2 and -3, contributing to a dynamic pool of antigenic variation. We sought to rationally engineer FMDV capsids either as infectious copy virus or recombinant empty capsids with improved thermo-stability for improved vaccines. Here we used in-silico MD simulations, molecular modelling, free energy calculations, X-ray crystallography, Cryo-electron microscopy (CryoEM) and various biochemical/biophysical techniques to design and help characterise the improved capsids. For the most unstable FMDV serotypes (O and SAT2), panels of stabilising mutants were characterised. Promising candidates were then engineered and shown to confer increased thermo- and pH-stability. Thus, in-silico predictions translate into marked stabilisation of both infectious and recombinant empty capsids.

An in-situ diffraction method was used to determine crystal structures to verify that no unanticipated structural changes have occurred as a consequence of the modifications made. Where it was difficult to obtain crystals/diffraction, structures were determined by high-resolution CryoEM (with the best electron density maps reaching 2.7Å resolution). The structures of the wildtype and two of the stabilised mutants for three different serotypes of FMDV showed the mutations made predicted interactions and the antigenic surfaces remained unchanged.

Animal trials showed stabilised particles can generate improved neutralising response compared to the traditional vaccines. Similar approach applied to the polio virus successfully produced antigenic VLPs using the plant based expression system. CryoEM reconstruction of polio VLPs produced 3.6Å resolution maps and the structure analysis suggested the plant based polio particles are identical to the native virus. We have successfully used a structure based rational engineering approach to increase the stability of viral capsids without affecting the antigenicity and demonstrated the direct application of structural biology and structure based design that has the potential to lead directly to a new generation of efficacious vaccines that can provide hope that the disease can be brought under control.

In addition, using CryoEM, CryoET and Focus Ion Beam milling of the infected cells, we are working towards understanding the picornavirus life cycle in molecular details. To this end, using localised reconstruction, we have determined the interaction between $\alpha\beta6$ and two FMDV strains at high resolution. In the preferred mode of engagement the fully open form of the integrin, hitherto unseen at high-resolution, attaches to an extended GH loop via interactions with the RGD motif plus downstream hydrophobic residues. In addition, an N-linked sugar of the integrin attaches to the previously identified HS binding site, suggesting a functional role.

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