4-6-2022

Rapid iterative design of tandem-core virus-like particles using Escherichia Coli-based cell-free protein synthesis

Beatrice Melinek

Jaime Teneb-Lobos

Beatrice Melinek

Stefanie Frank

Alex Ramirez

See next page for additional authors

Authors

Beatrice Melinek, Jaime Teneb-Lobos, Beatrice Melinek, Stefanie Frank, Alex Ramirez, William Rosenberg, and Daniel G. Bracewell

RAPID ITERATIVE DESIGN OF TANDEM-CORE VIRUS-LIKE PARTICLES USING *ESCHERICHIA COLI*-BASED CELL-FREE PROTEIN SYNTHESIS

Noelle Colant, UCL, United Kingdom
noelle.colant.17@ucl.ac.uk
Jaime Teneb-Lobos, UCL, United Kingdom
Beatrice Melinek, UCL, United Kingdom
Stefanie Frank, UCL, United Kingdom
Alex Ramirez, iQur, United Kingdom
William Rosenberg, iQur, United Kingdom
Daniel G. Bracewell, UCL, United Kingdom

Key words: cell-free protein synthesis; virus-like particles; distributed manufacturing

Virus-like particles (VLPs) are self-assembling conformations of viral structural proteins. These particles resemble viruses in many ways, but unlike viruses, they do not contain genetic material and therefore lack pathogenicity as well as replicative abilities. Due to their similarity in structure to their corresponding viruses, VLPs can invoke strong B cell and T cell responses even when delivered in low doses, making them valuable vaccine candidates. Not only can VLPs be administered as vaccines to their corresponding virus, but some can be used as scaffolds to display other antigens. These antigens are either fused genetically to VLP subunit proteins or attached by covalent or noncovalent mechanisms to the VLP surface. This allows for the design of modular VLPs where different antigens can be inserted for different applications.

In order to rapidly design, build, and test these new VLP candidates, we need a production system that generates high titers in short timescales. Cell-free protein synthesis (CFPS) has emerged as a viable platform for VLP production having been previously used to produce hepatitis B core antigen (HBcAg) VLPs at concentrations (445 mg/L) never before achieved in vivo in only a few hours (Bundy, Franciszkowicz, & Swartz, 2008).

We have developed our own E. coli-based CFPS platform and used it to produce tandem-core HBcAg VLPs. In tandem-core HBcAg VLPs, two HBcAg monomers are joined together by a poly-glycine-serine linker protein. This allows for two different antigens to be displayed on the VLP surface. Using our CFPS platform, we have tested a variety of constructs for tandem-core VLPs including single antigen and multi-antigen displaying VLPs and VLPs where the arginine-rich region typically found in wild-type HBcAg (which is used to assist in encapsulation of the viral RNA pre-genome) is removed from one or both monomers. We've also examined the affects that different plasmid vectors have on VLP titers. By using this CFPS system to rapidly iterate on our designs, we have identified modifications that inhibit VLP assembly and modifications that increase yield over six-fold.

Using this same CFPS platform offers potential for scalable manufacture where reagent production can be separated from the CFPS reaction and product synthesis. This would enable distributed manufacture which may be critical to a rapid response in a pandemic or other global health emergency.

References:
Bundy, B. C., Franciszkowicz, M. J., & Swartz, J. R. (2008). Escherichia coli-Based Cell-Free Synthesis ofVirus-Like Particles. Biotechnology and Bioengineering, 100(1), 28-37.