Vaccine xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

# Simultaneous surface display and cargo loading of encapsulin nanocompartments and their use for rational vaccine design

Priscillia Lagoutte [a], Charlotte Mignon [a], Gustavo Stadthagen [a], Supanee Potisopon [a], Stéphanie Donnat [a], Jan Mast [b], Adrien Lugari [a,*], Bettina Werle [a,*]

[a] BIOASTER, Protein and Expression System Engineering unit, 40 avenue Tony Garnier, 69007 Lyon, France
[b] Service Trace Elements and Nanomaterias, Sciensano, Groeselenbergstraat 99, B-1180 Brussels, Belgium

ARTICLE INFO

*Article history:*
Received 15 February 2018
Received in revised form 30 April 2018
Accepted 5 May 2018
Available online xxxx

*Keywords:*
Encapsulin nanocompartments
Surface display
Cargo encapsulation
Protein-based nanoparticle carrier
Rational vaccine design

ABSTRACT

In the past decades protein nanoparticles have successfully been used for vaccine applications. Their particulate nature and dense repetitive subunit organization makes them perfect carriers for antigen surface display and confers high immunogenicity. Nanoparticles have emerged as excellent candidates for vectorization of biological and immunostimulating molecules. Nanoparticles and biomolecular nanostructures such as ferritins or virus like particles have been used as diagnostic and therapeutic delivery systems, in vaccine development, as nanoreactors, etc. Recently, a new class of bacterial protein compartment has been discovered referred to as encapsulin nanocompartment. These compartments have been used for targeted diagnostics, as therapeutic delivery systems and as nanoreactors. Their biological origin makes them conveniently biocompatible and allows genetic functionalization. The aim of our study was to implement encapsulin nanocompartements for simultaneous epitope surface display and heterologous protein loading for rational vaccine design. For this proof-of-concept-study, we produced *Thermotoga maritima* encapsulin nanoparticles in *E. coli*. We demonstrated the ability of simultaneous display in our system by inserting Matrix protein 2 ectodomain (M2e) of influenza A virus at the nanoparticle surface and by packaging of a fluorescent reporter protein (GFP) into the internal cavity. Characterization of the nanoparticles by electronic microscopy confirmed homogenously shaped particles of 24 nm diameter in average. The results further show that engineering of the particle surface improved the loading capacity of the heterologous reporter protein suggesting that surface display may induce a critical elastic deformation resulting in improved stiffness. In Balb/c mice, nanoparticle immunization elicited antibody responses against both the surface epitope and the loaded cargo protein. These results confirm the potential of encapsulin nanocompartments for customized vaccine design and antigen delivery.



## 1. Introduction

For several decades, nanoparticles, and in particular Virus like Particles (VLPs) based on subunits of viral surface proteins have been commercialized as vaccines in human health against human hepatitis B virus, hepatitis E virus and Human Papilloma Virus [1], and in animal health against porcine circovirus type II [2]. These vaccine delivery systems attempt to mimic various properties of pathogens and thereby increase immunogenicity.

Diverse delivery systems are currently under development. Examples include nanoparticles which have dimensions that are similar to those of microbial pathogens. Such particulate delivery systems constitute a heterogeneous category of carriers including protein cage based [3–6], liposomes [7,8] and inorganic and polymeric nanoparticles [9–11]. In contrast to lipid based, polymer-based or inorganic-based, protein based nanoparticles are biocompatible systems composed of multiple copies of one or few subunits leading to repetitive structures that auto-assemble having highly uniform size and symmetry [12,13]. This particulate nature and high repetitive surfaces makes them ideal carriers for antigen and epitope surface display (unrelated to the nanoparticles itself) and confers high immunogenicity [13]. The most frequently used

*Abbreviations: T. maritima*, *Thermotoga maritima*; *E. coli*, *Escherichia coli*; GFP, Green Fluorescence Protein; M2e, Matrix2 protein ectodomain; VLP, Virus Like Particle; *R. erytropolis*, *Rhodococcus erytropolis*; *P. furiosus*, *Pyrococcus furiosus*; *Myxococcus xanthus*, *B. linens, Brevibacterium linens*; TEM, Transmission Electron Microscopy; RFU, Relative Fluorescence Unit; NP, Nanoparticle.

* Corresponding authors.
*E-mail addresses:* alugari@gmail.com (A. Lugari), bettina.werle@bioaster.org (B. Werle).

https://doi.org/10.1016/j.vaccine.2018.05.034
0264-410X/

nanoparticles carriers are derived from viral coat proteins, in particular from bacteriophages (MS2, Qβ, P22), from hepatitis B virus (core and surface protein) or from plant viruses (Cowpea chlorotic mottle virus and Cowpea mosaic virus) [14]. Antigens and epitopes are displayed on the surface by genetic fusion or by conjugation systems [3,13–21]. Besides surface display nanoparticles have emerged as excellent candidates for vectorization systems of biological molecules as nucleic acid, CpGs, proteins or other immunostimulation molecules [13,14,22,23].

In the present study we engineered recently discovered virus capsid-like nanocompartments called encapsulin as nanocarrier particle for customized epitope display and cargo protein vectorization. Encapsulin nanocompartments have been discovered in different bacteria and archaea including medically important species such as *Mycobacterium tuberculosis* [24–27]. Even if their biological functions are not completely elucidated, McHugh et al., [28] have recently shown that these nanocompartments could store iron and protect bacteria from oxidative stress. *Thermotoga maritima* [24], *Mycobacterium tuberculosis* [29] and *Rhodococcus erytropolis/josti* [30] nanocompartments consist of 60 copies of identical subunits that form a T = 1 icosohedral capsid-like particles of 20–24 nm. In contrast, *Pyrococcus furiosus* [25] and *Myxococcus xanthus* [28] nanocompartments contain 180 protein subunits that form a T = 3 icosahedral particle with a diameter of 30 to 32 nm. In bacteria, encapsulin compartments have the ability to package functional enzymes such as ferritin-like proteins and Dyp-type peroxidases [24,28,29]. These encapsulated proteins have a specific C-terminus sequence that leads them to bind to the interior surface of encapsulin [24]. This specific cargo-loading capacity has been used to package non-native cargo proteins [31,32]. Various applications of encapsulin-based nanocompartments have been nicely reviewed by T. Giessen [6]. Among these, Moon et al., demonstrated the use of the engineered encapsulins from the thermophilic *T. maritima* for targeted diagnostics and targeted therapeutic delivery [33]. More recently, specific loading of heterologous cargo proteins as eGFP and luciferase inside the encapsulin compartment from *R. erythropolis* N771 and *Brevibacterium linens* has been described. However the packaging efficacy of both guest proteins was either low [31,32,34], or loading destabilized the correct assembly of the nanocompartment [35].

In the present study we describe the engineering of thermophilic encapsulin *T. maritima* to generate a multifunctional nanoplatform for simultaneous heterologous protein loading and epitope surface display. We engineered *T. maritima* eGFP loaded encapsulin nanoparticles that display the Matrix protein 2 ectodomain epitope of the influenza A virus [36] at their surface. After purification, the potential of the packaging efficiency was demonstrated. Immunogenicity studies revealed antibody responses against both the surface epitope and the loaded cargo protein after immunization of Balb/c mice. These new findings confirm the potential of encapsulin nanocompartments as a versatile nanoparticle carrier for rational vaccine design and antigen delivery [6,37].

## 2. Materials and methods

### 2.1. Construction of the plasmids

Four encapsulin constructs (NC000853; GI:1564277) carrying the previously described C200S mutation [33] were generated as shown in Fig. 1. The first construct (NP) encodes the encapsulin gene under the control of the T7 promoter. The second and third constructs contain bicistronic cassettes encoding both: eGFP fused to the C-terminal extension sequence of the ferritin-like protein (IEEETSGGSENTGGDLGIRKL; GI:15642775) annotated as flp tag and the encapsulin gene without (NPGFP) and with an insertion of the M2e epitope GGEVETPIRNEGG from *Influenza A* virus H1N1 (GI:21693176) between amino acid positions 138 and 139 ($NP_{M2e}$-GFP). The fourth construct is identical to NPGFP with an additional Hexahistidine peptide ($NP_{His}GFP$) inserted between amino acid positions 42 and 43 which was previously described to improve particles heat stability [33]. As a positive control of M2e display,

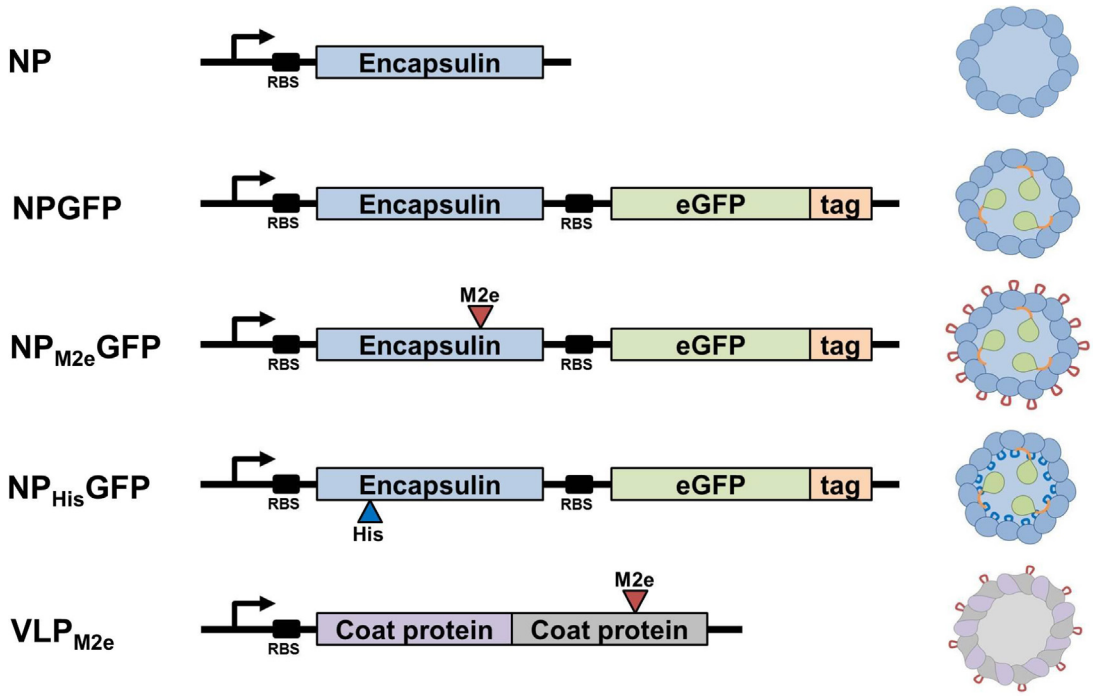


**Fig. 1.** Schematic representation of genetic constructs designed for nanoparticle generation. Color code : encapsulin in light blue; eGFP in green; flp tag in orange; M2e (ectodomain of Matrix protein) in red; HexaHis (HexaHistidine peptide) in dark blue; coat protein dimer (MS2 bacteriophage) in purple and grey respectively; RBS (ribosome binding protein) in black. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Please cite this article in press as: Lagoutte P et al. Simultaneous surface display and cargo loading of encapsulin nanocompartments and their use for rational vaccine design. Vaccine (2018), https://doi.org/10.1016/j.vaccine.2018.05.034