stem region of the H1 HA glycoprotein, yet was capable of evoking broadly cross-reactive antibodies that, in contrast to plain nanoparticles, protected mice and ferrets against lethal doses of heterosubtypic H5N1 virus [94].

Another similar study was also reported by the Nabel team, wherein the conserved receptor-binding domain (a site of vulnerability) of the gp350 antigen from Epstein–Barr virus was presented in a structurally optimized orientation on nanoparticles of ferritin (24 subunits) or encapsulin (60 subunits). In preclinical studies, the chimeric nanoparticle-gp350 antigens elicited 10- to 100-fold more potent virus-neutralizing antibody titers than the soluble gp350 antigens alone [95]. Importantly, in addition to their immuno-focusing ability to generate high-quality antibody responses, the recombinant nature of these nanoparticle antigens has the benefit of high purity, safety, and tolerability, further strengthening the appropriateness of this vaccination strategy (Table 2).

Also, very recent is the work by He et al., describing *in silico* studies to optimize molecular scaffolds for epitope presentation and leading to the generation of recombinant ferritin nanoparticles displaying epitope-scaffolds harboring E1 or E2 epitopes from hepatitis C virus, promising candidates for preclinical studies in the quest for an HCV vaccine [96]. This recent example applied to HCV builds on the epitope-scaffold rational design strategy that emerged in previous attempts to graft HIV epitopes onto heterologous protein scaffolds [97], and effectively combines this approach with the multivalent nanoparticle format.

Ferritin has also been used as antigen support in the search for potent and safe vaccine tools against HIV [98], which despite its identification more than 30 years ago remains as one of the most devastating pathogens afflicting the human population. In order to circumvent the fact that Abs others than the so-called broadly neutralizing antibodies (bNAbs) might occlude highly vulnerable HIV sites, Kwong and co-workers grafted a series of these HIV target motifs into different protein templates and the resultant chimeras were named 'supersite transplants'. Transplants bearing a glycopeptide from the variable region 3 on gp120 were recognized by neutralizing antibodies from three different donors, and binding was enhanced by presentation of the transplants on ferritin nanoparticles.

Lumazine synthase (LS) represents another example of the inclusion of a bacterial particulate base for the optimization of vaccine candidates, as reported recently by Jardine et al. in their attempts to enhance the immunoreactivity of recombinant gp120 against HIV infection [99]. As mentioned before, HIV represents a major health problem worldwide. With 35 million people carrying the virus worldwide and a yearly morbidity of 1.7 million people (AVERT, http://www.avert.org/worldwide-hiv-aids-statistics.htm), the lack of a vaccine is an enormous unmet medical need. A key challenge in designing an anti-HIV vaccine is the high mutagenic capability of the virus and the unfeasibility of administering attenuated or killed virus because of safety issues. An additional hurdle is the negligible recognition potential of germline precursors of bNAbs, such as VRC01, against the wild-type gp120, the major immunogenic component of the HIV virus envelope. One way to overcome this obstacle was recently reported by Schief and co-workers [99], who boosted the affinity of the germline antibodies for the viral gp120 glycoprotein by displaying multiple copies of an engineered form of the antigen on a lumazine synthase (LS) nanoparticle.

LS, which is responsible for the penultimate catalytic step in the biosynthesis of riboflavin, is an enzyme present in a broad variety of organisms, including archaea, bacteria, fungi, plants, and eubacteria [100]. The LS monomer is 150 amino acids long, and consists of beta-sheets along with tandem alpha-helices flanking its sides. A number of different quaternary structures have been reported for LS, illustrating its morphological versatility: from homopentamers up to symmetrical assemblies of 12 pentamers forming capsids of 150 Å diameter. Even LS cages of more than 100 subunits have been described [101].

Using LS from the thermophilic bacterium *Aquifex aeolicus* as a nanoparticle platform for epitope display, Jardine et al. succeeded in increasing the potency of the immune response and breadth of coverage against HIV. The envelope (Env) glycoprotein is the only HIV surface protein targeted by neutralizing antibodies; it is made of three gp160 precursors that trimerize and are each then cleaved into gp120 and gp41 subunits. Jardine et al. engineered LS to display an optimized sub-component (termed, eOD-GT6) of the wild-type gp120 antigen from the Env trimer [99]. This approach overcame the issue that germline precursors of VRC01 bNAbs show undetectable affinity for wild-type Env. With additional structural stabilization of the trimer provided by an N-terminal coiled-coil GCN4 domain, the eOD-GT6 immunogen was fused to the C-terminus of the LS gene construct. The resulting recombinant nanoparticle antigens were efficiently obtained from mammalian cells, in stable and homogeneous self-assemblies of 60 LS monomers each presenting a glycosylated eOD-GT6. In contrast with the monomeric eOD-GT6 that did not stimulate B-cell activation, the LS-eOD-GT6 nanoparticles remarkably activated both germline and mature B cells. In accordance with related studies discussed above, Jardine et al. also hypothesize that the ability of the nanoparticles to induce cross-linking with the B-cell receptors was important to promote a successful immune response.

2.3. Micellar nanoparticles

A method to obtain protein micelles from full-length amphiphilic membrane proteins was developed and used to prepare viral surface proteins as water-soluble particles with a hydrophobic interior and a polar exterior, of relatively homogeneous size: approximately 20–30 nm diameter, depending on the protein [102]. Therein, Simons et al. predicted several possible applications of the approach, including the opportunity to make virus glycoprotein micelle vaccines. Indeed, a similar approach has been adapted for the preparation of protein nanoparticles comprised of amphiphilic antigens, where the protein micelles are prepared by extraction with non-ionic detergents from Sf9 insect cells expressing the recombinant antigen. In a compelling example, a slightly genetically modified full-length form of the RSV fusion (F) surface glycoprotein was extracted and purified from insect cell membranes and used to create protein nanoparticle micelles of ~40 nm diameter, where the trimeric F protein assembled into rosettes exposing conformational epitopes similar to those of the post-fusion F conformation and able to raise neutralizing Abs [103]. In very recent clinical trials, these RSV F antigen nanoparticles appeared safe, promoted immunogenicity, and reduced RSV infections [104], raising high expectations for a nanoparticle vaccine against RSV.

Table 2
A table listing nanoparticle platforms of bacterial nature, with their composition, production method, and stage of (pre)clinical development.

Platform	Antigen	Target	Expression system	Stage	Ref.
Ferritin	GP350 CR2-binding domain	Epstein–Barr virus	Mammalian cells	Preclinical	[94]
Ferritin	E1/ E2 envelope proteins	Hepatitis C virus	Mammalian cells	Preclinical	[95]
Ferritin	Variable region 3 on gp120	HIV	Mammalian cells	Preclinical	[97]
Ferritin	HA	Influenza	Mammalian cells	Preclinical	[92]
Encapsulin	GP350 CR2-binding domain	Epstein–Barr virus	Mammalian cells	Preclinical	[94]
Lumazine Synthase	Engineered gp120	HIV	Mammalian cells	Preclinical	[98]

Abbreviations: HIV, human immunodeficiency virus; HA, hemagglutinin.