The micellar nanoparticle approach has also been exploited in the search for vaccines against the Coronaviridae virus (CoV) family, which represents an important group of emerging human pathogens, as witnessed in the severe acute respiratory syndrome SARS-CoV and Middle East respiratory syndrome MERS-CoV outbreaks of 2003 and 2012, respectively. Recombinant full-length forms of the major immunodominant CoV antigen—the amphiphilic spike glycoproteins—both from SARS-CoV and MERS-CoV were successfully obtained via non-ionic detergent-extraction from Sf9 cells. The purified spike proteins assembled into nanoparticles of ~25 nm diameter that, in adjuvanted formulations tested in mice, were capable of raising high-titer neutralizing antibody responses against the homologous virus [105]. These preclinical examples suggest that this protein nanoparticle approach may be suitable for rapid production of relatively simple but effective vaccines in response to emerging pathogens (Table 3).

2.4. New protein platforms

Proposals to perform molecular manipulations that, by exploiting chemical forces in a repetitious fashion, could lead to the production of interesting materials that date back at least to the 1960s [14,15]. Indeed, in addition to the naturally occurring self-assembling proteins described above, several groups have explored ways to design and produce nanoparticle materials based on non-native polypeptides. For example, Burkhard and co-workers produced chimeric polypeptides capable of self-assembling into regular polyhedral nanoparticles [106]. The polypeptide consisted of an N-terminal pentamer-forming subunit derived from the cartilage oligomeric matrix protein (COMP), followed by a *de novo*-designed trimeric subunit domain. Both subunits present oligomeric coiled-coil conformations and importantly, the resultant synthetic molecule was shown to refold and self-assemble into nanoparticles with polyhedral symmetry. Alternative oligomerization motifs such as the trimeric foldon domain from fibritin have also been used in such designs [107]. The assembly of such nanoparticles gives rise to a multivalent molecular architecture that allows diverse immunogenic epitopes to be repeatedly displayed on the surface of the nanoparticles in a strictly arranged manner, a strategy that appears to be broadly applicable.

Indeed, using the polypeptide approach, the Burkhard team fused the C-terminal heptad repeat (HRC) region of the SARS-CoV spike protein in its pre-fusogenic state in frame with the nanoparticle scaffold [108]. This strategy allowed conservation of the trimeric coiled-coil conformation of the spike epitope. Immunization of mice with these SARS-nanoparticles successfully elicited neutralizing antibodies specific for the trimeric coiled-coil epitope of the pre-fusogenic HRC. Additional applications of this system targeted an HIV vaccine, by using a nanoparticle made of two covalently linked coiled-coil domains designed to incorporate the membrane proximal external region (MPER) of HIV-1 gp41 [109]. However, while high MPER-specific titers were raised by this nanoparticle, none of the sera displayed detectable neutralizing activity against HIV-1. More promisingly, similarly designed polypeptide nanoparticles displaying multiple copies of a rodent malaria epitope from the circumsporozoite protein of *Plasmodium berghei* elicited a long-lasting immune response [110]. Collectively, this preclinical research suggests that the self-assembling protein nanoparticle (SAPN) approach can generate safe non-native polypeptide antigens approximating the size and multivalent scenario of a virus and thus facilitate the recognition of the antigen by immune receptors.

Early in the 21st century, Yeates and co-workers developed the nanohedra protein-design method, which was subsequently extended by Noble and co-workers [111,112]. The Yeates team rationally designed genetic fusions of the trimeric bromoperoxidase and the dimeric M1 matrix protein of influenza virus, such that the combination of the two naturally oligomeric proteins generated self-assembling nanostructures, including a 15-nm-wide molecular cage and a 4-nm-wide filamentous superstructure [111,112]. Later, a well-ordered tetrahedral cage with 12 subunits was designed and its crystal structure was determined and revealed to closely match the intended design, validating this approach [113]. The Noble team used proteins with higher symmetry, allowing design of fusions with two or more connections, generating regular molecular arrays that formed protein lattices, but not closed nanohedral particles [114]. Several subsequent *in silico* and crystallographic studies have further developed nanostructure design strategies, including the generation of particles over 22 nm in diameter [115]. Therefore, with the development of these more powerful computational approaches for the *ab initio* design of new protein-protein interfaces with defined symmetry, geometry, and complementary packing arrangements, and the increasing number of protein structures in the PDB, it is speculated that additional achievements in the field of self-assembling protein design will be possible [96,115–122]. It will be interesting to see if such scaffolds can be fully exploited to display antigenic epitopes suitable for full development into clinically efficacious vaccines.

3. Conclusions

Despite many successes in the field of vaccinology, new breakthroughs are still needed to protect humans from several important life-threatening diseases. Here, we have reviewed how a variety of non-infectious biological nanoparticles can offer solutions. For example, some plain nanoparticles (e.g. HBsAg or the HPV L1 protein) are simple molecular self-assemblies that are safe and efficacious vaccine antigens licensed for human use. Or, more complex chimeric nanoparticles can be platforms on which pathogen-derived immunogenic motifs can be presented to the host immune system. These biological scaffolds range from synthetic polypeptides to native macromolecules such as ferritin, lumazine synthase or VLP-forming antigens or lipid-enveloped VLPs. For some chimeric nanoparticles, there is evidence that the immunogenicity of the platform carrier itself is negligible or low compared to that of the mounted immunogen being presented [93]. Because these nanoparticles display an ordered matrix of immunogens, they enable more

Table 3
A table listing nanoparticle platforms of diverse nature, with their composition, production method, and stage of (pre)clinical development.

Platform	Antigen	Target	Expression system	Stage	Ref.
Micellar protein	Glycoprotein F	RSV	Insect cells	Phase II	[103]
Micellar protein	Spike glycoprotein S	SARS and MERS coronavirus	Insect cells	Preclinical	[104]
Virosome	Her-2 peptides of Her-2/neu	Breast cancer	Cell free	Phase I	[130]
Virosome	Aspartyl proteinase-2	*Candida albicans*	Cell free	Phase I	[131]
Virosome	P1 and recombinant gp41	HIV	Cell free	Phase I	[132]
Virosome	HA, NA	Influenza	Cell free	One license	[133]
Ty p1	p17/p24	HIV	Yeast	Phase II	[134]
Synthetic polypeptide	Heptad repeat of CoV spike protein (pre-fusogenic state)	SARS	Bacteria	Preclinical	[107]
Synthetic polypeptide	Membrane proximal gp41	HIV	Bacteria	Preclinical	[108]
Synthetic polypeptide	*Plasmodium berghei* CSP B-cell epitope	Malaria	Bacteria	Preclinical	[109]

Abbreviations: CSP, circumsporozoite; RSV, respiratory syncytial virus; HA, hemagglutinin; NA, neuraminidase; SARS, severe acute respiratory syndrome; MERS, Middle East respiratory syndrome; HIV, human immunodeficiency virus.