much interest in preparing nonviral vectors as lyophilized formulations [83, 85–90]. The lyophilization process subjects preparations to two distinct stresses, freezing and drying, which can alter DNA structure [91] and promote aggregation of nonviral vectors [69, 83]. Like other biomolecules (e.g., proteins [92–94]), previous work has demonstrated that sugars, especially disaccharides, have the ability to preserve lipid/DNA complexes [83, 86, 95], DNA [91, 96] as well as liposome formulations [97] when present above a critical mass ratio of stabilizer to nonviral particle during lyophilization. Particle size is considered a critical pharmaceutical quality attribute of the product that must be maintained during processing regardless of its effect on gene transfer [80, 98]. Although both freezing and drying can promote aggregation, earlier studies indicate that particle size increase is typically manifested during the freezing step of the lyophilization process [72, 83, 88, 99]. Even though the precise mechanism by which sugars stabilize nonviral vectors during freezing and drying remains unclear, it has been suggested that two mechanisms could potentially contribute to the observed particle stabilization during freezing: glass formation [100, 101] and particle isolation [102]; for further discussion read our previous review on the topic [80]. Furthermore, Allison and collaborators have demonstrated that an excipient to DNA (weight-to-weight ratio, *w/w*) ratio of around 1000 is sufficient to prevent aggregation of nonviral vectors during freezing [102], which is in good agreement with later studies on the stabilization of different types of lipid/DNA complexes [103]. In a further study, Armstrong and Anchordoquy [104] showed that particle aggregation in the frozen state is time and temperature dependent. More recently, a research study based on a theoretical modeling had suggested that both the initial sample viscosity and the residence time of the particle in the low-viscosity fluid state are predominant factors in the retention of particle size during freezing. This model has been applied only to polyplexes (DNA complexed with a cationic polymer [105]), and further studies are needed to better understand the relevant mechanism of stabilization of other systems that are known to aggregate during freezing (e.g., liposomes, proteins). More than two decades ago, Crowe and collaborators proposed the so-called water replacement hypothesis to explain how sugars preserve the integrity of liposomes during drying [106, 107]. This theory states that sugars function by replacing water that is bound to the lipid headgroup. To date, this hypothesis has also been implicated in the preservation of nonviral vectors in the dried state [86, 95, 102, 108]. It has recently been suggested that this theory may not explain the ability of sugars to protect lipoplexes (e.g., with polyethylene glycol (PEG)ylation-based components into the particle) for (e.g., with polyethylene glycol (PEG)-modified nonviral vector, PEGylation-based) that adopt a nonbilayer structure [98, 109]. Indeed, more studies are needed in order to investigate further the mechanism(s) involved in the protection of nonviral vectors during freezing and drying.

As mentioned above, it would be advantageous to develop dehydrated formulations that could be shipped and stored at ambient conditions, thus limiting the need for a dependable cold chain. Despite the growing body of research on nonviral vector dehydration, to date, lyophilization studies have mostly focused on the stability of vectors (e.g., naked DNA [91, 110–114], lipoplexes [83, 86, 115–117], polyplexes [85, 88, 99, 109, 118, 119], and lipopolyplexes [95, 120]) during acute