

Supplementary Materials: Gemini Cationic Lipid-Type Nanovectors Suitable for the Transfection of Therapeutic Plasmid DNA Encoding for Pro-Inflammatory Cytokine Interleukin-12

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1. Physicochemical Characterization

Table S1. Effective charges of GCLs and pDNA and the values of electroneutrality ratio $(m_L/m_{pDNA})_\phi$ for the lipoplexes studied in this work.

System	GCL1/DOPE-pDNA	GCL2/DOPE-pDNA	GCL3/DOPE-pDNA
$q_{\text{eff,GCL}}^+$	1.9 ^a	1.7 ^b	1.8 ^c
$q_{\text{eff,pDNA}}^-/\text{bp}$	-1.5	-1.3	-1.4
$(m_L/m_{pDNA})_\phi$	4.5	4.5	1.6

Values estimated with a 5% error. The $q_{\text{eff,GCL}}^+$ values were obtained in previous studies [1–3], considering $q_{\text{nom,ctDNA}}^- = -2.0/\text{bp}$. The $q_{\text{eff,pDNA}}^-/\text{bp}$ values were obtained from plot of ζ -potential as a function of m_L/m_{pDNA} (Figure 1) and working with these $q_{\text{eff,GCL}}^+$ values. ^a Ref. [1], ^b Ref. [2], and ^c Ref. [3].

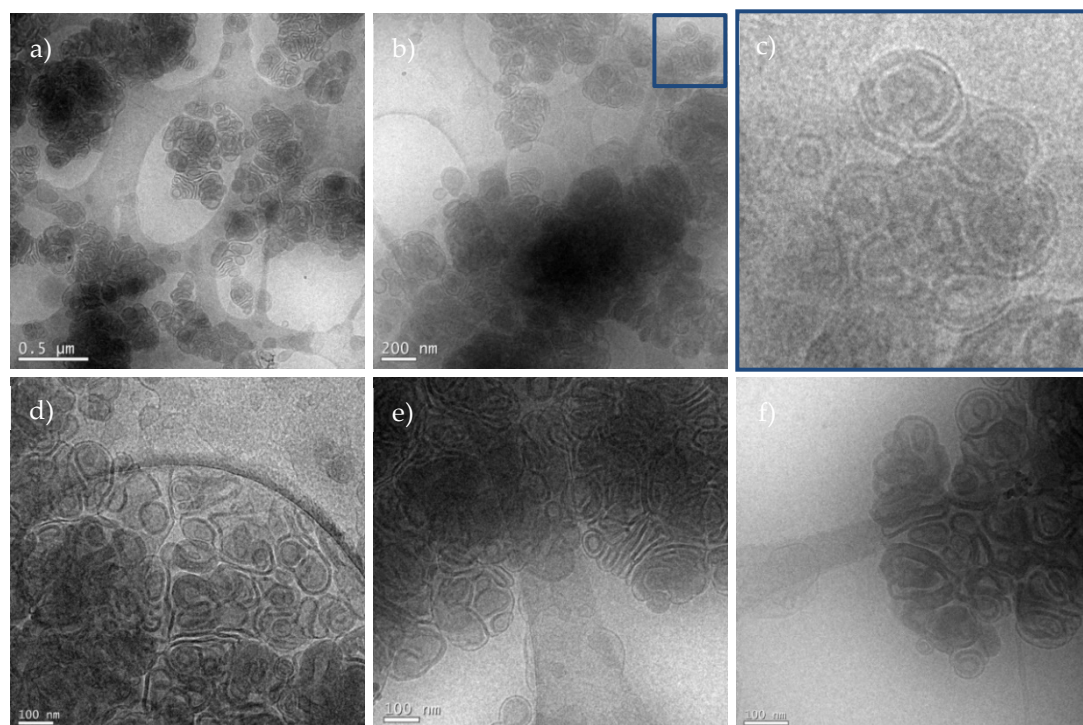
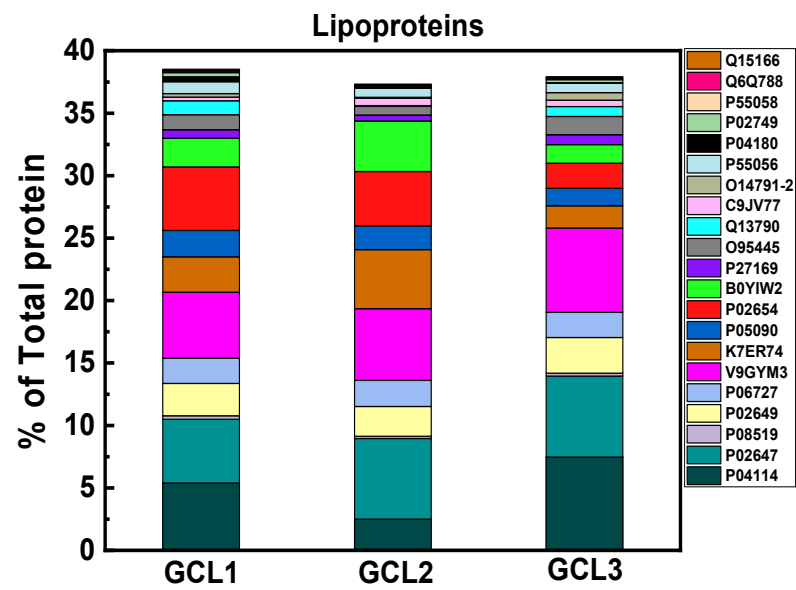


Figure S1. Cryo-TEM micrographs of GCL2/DOPE-pCMV-Luc lipoplexes at $\rho_{\text{eff}} = 5$ and $\alpha = 0.2$. Scale bars of micrographs are 500 (a), 200 (b) and 100 (d–f) nm. Panel (c) shows a zoom view of the blue square in panel (b).

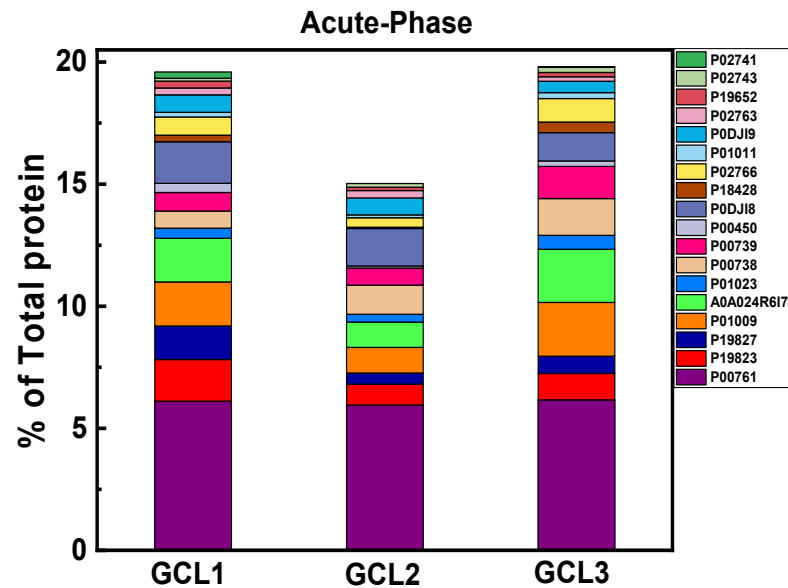
2. Protein Corona Characterization

Table S2. Relative percentage of proteins surrounding the surfaces of GCL/DOPE-pCMV-Luc lipoplexes, classified by their physiological function, by their molecular weight (MW) in kDa and by their isoelectric point (pI) related with Figure 4.

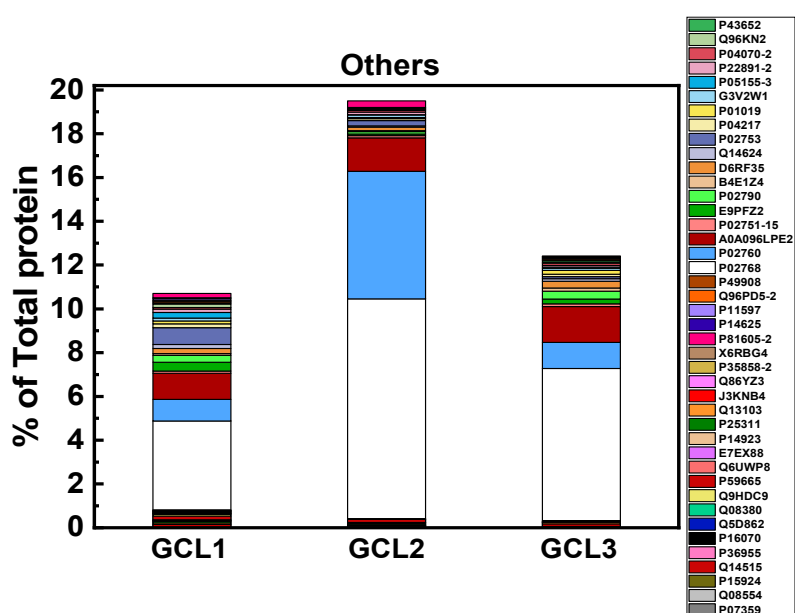
MW				pI				Physiological Function			
%				%				%			
	GCL1	GCL2	GCL3		GCL1	GCL2	GCL3		GCL1	GCL2	GCL3
>300	5.7	2.7	7.7	>9	0.9	0.7	0.7	Lipoproteins	38.5	37.3	37.9
150–300	3.6	2.6	3.2	08_09	16.8	18.3	14.6	Acute-phase	19.6	15.0	19.8
100–150	4.4	1.7	2.7	07_08	21.4	16.0	22.1	Immuno-globulins	12.4	14.2	16.5
80–100	1.3	0.5	1.0	06_07	32.8	39.7	34.2	Tissue Leak-age	2.9	3.2	3.1
70–80	3.2	2.6	2.7	05_06	27.8	24.9	28.2	Coagulation	6.9	4.7	6.0
60–70	10.1	13.5	9.1	<5	0.3	0.2	0.1	Complement	8.0	5.4	4.0
50–60	7.1	6.2	7.6					Others	11.7	20.0	12.8
40–50	11.3	10.6	14.5								
30–40	12.3	17.0	14.8								
20–30	17.0	17.4	14.7								
<20	24.1	25.1	22.0								



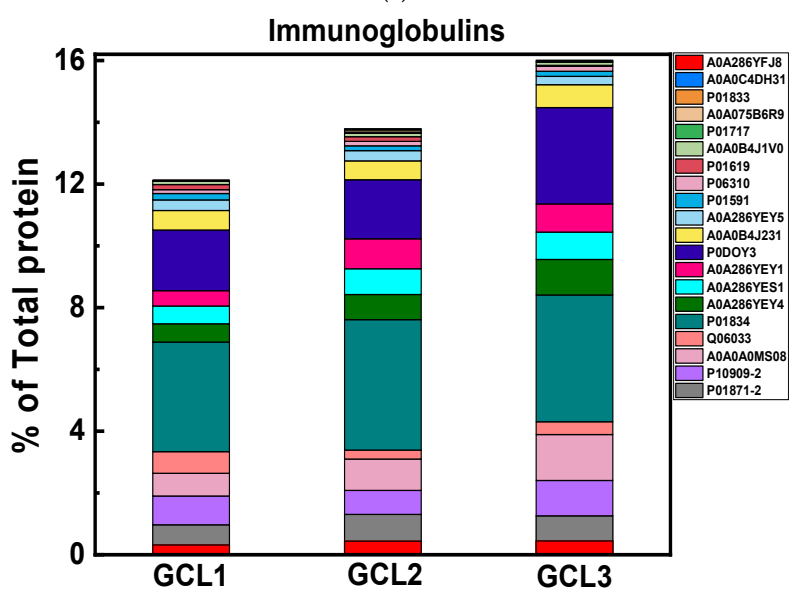
(a)



(b)

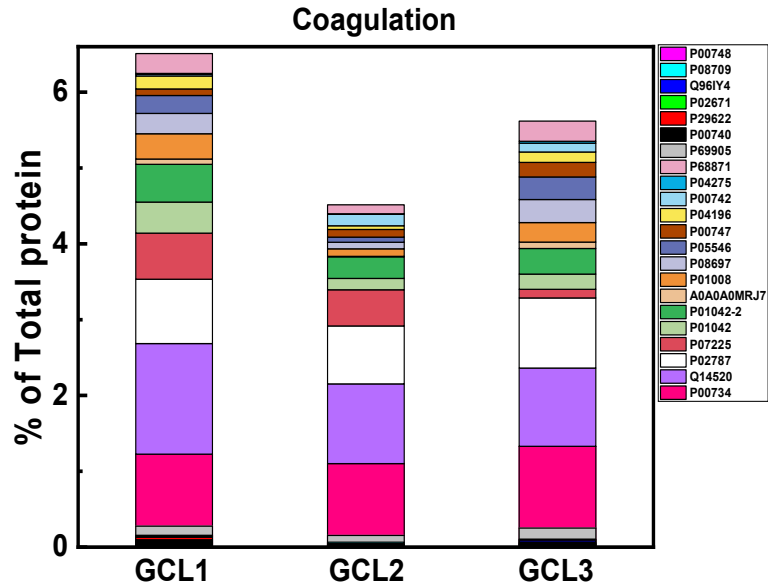
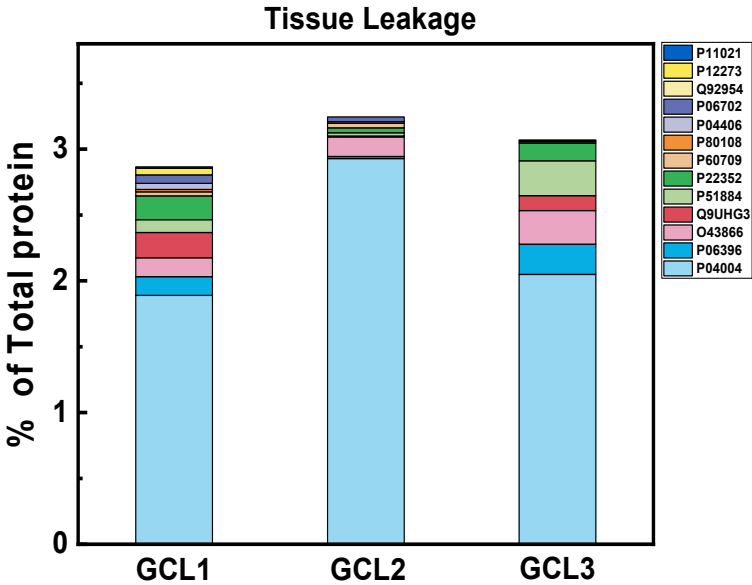


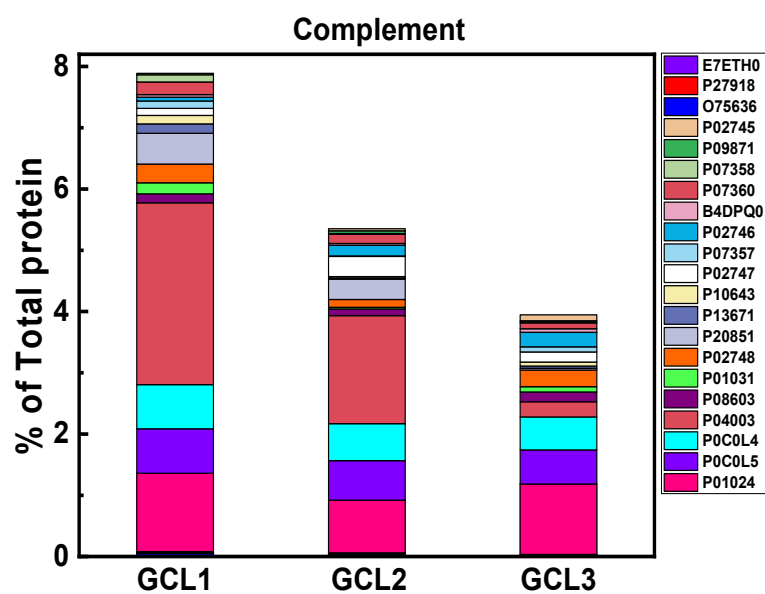
(c)



(d)

Figure S2. Classification of the most abundant proteins ((a) lipoproteins, (b) acute-phase, (c) others and (d) immunoglobulins proteins) found in the protein corona surrounding the surfaces of GCL/DOPE-pCMV-Luc lipoplexes.





(c)

Figure S3. Classification of the proteins that constitute a minor fraction ((a) tissue leakage, (b) coagulation, and (c) complement proteins) present in the protein corona surrounding the surfaces of GCL/DOPE-pCMV-Luc lipoplexes.

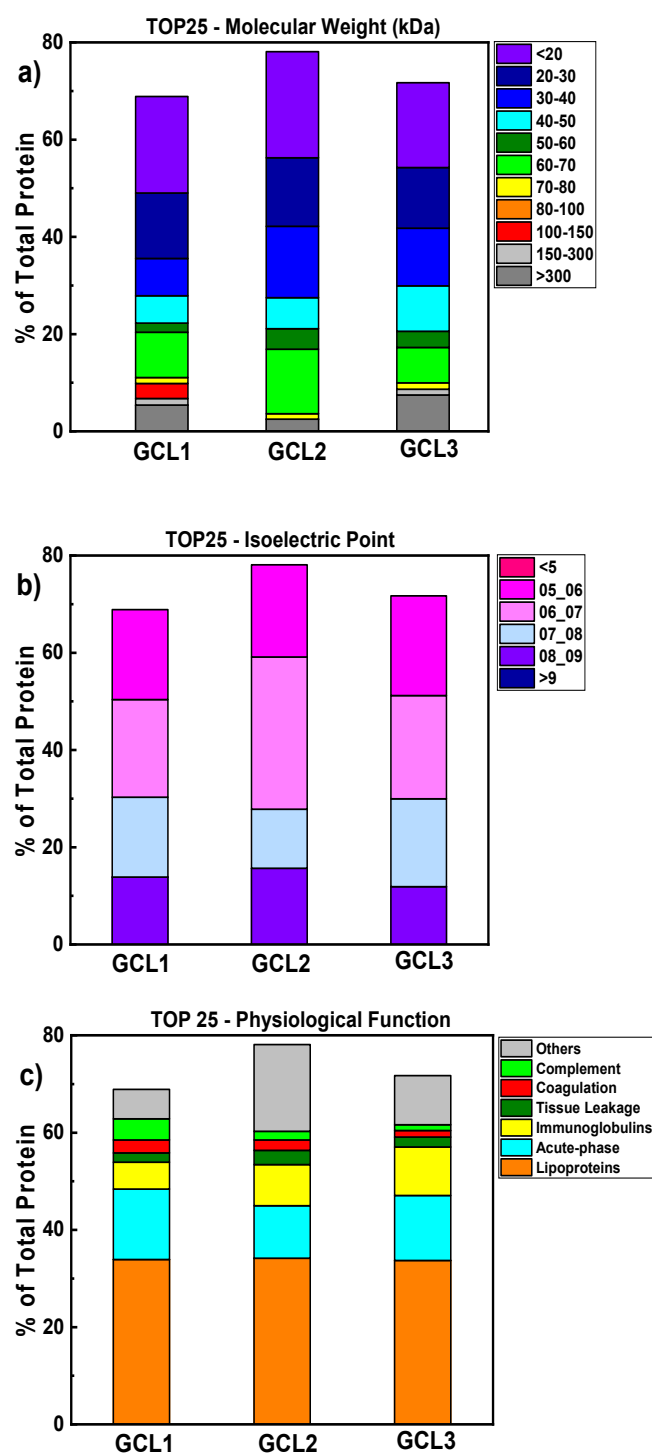


Figure S4. Relative percentage of the TOP 25 proteins found within the protein corona (PC) of GCL/DOPE-pCMV-Luc lipoplexes formed at $\rho_{\text{eff}} = 5$, classified by their molecular weight, MW in kDa (**a**), by their isoelectric point, pI (**b**) and by their physiological function (**c**).

3. In Vitro Studies

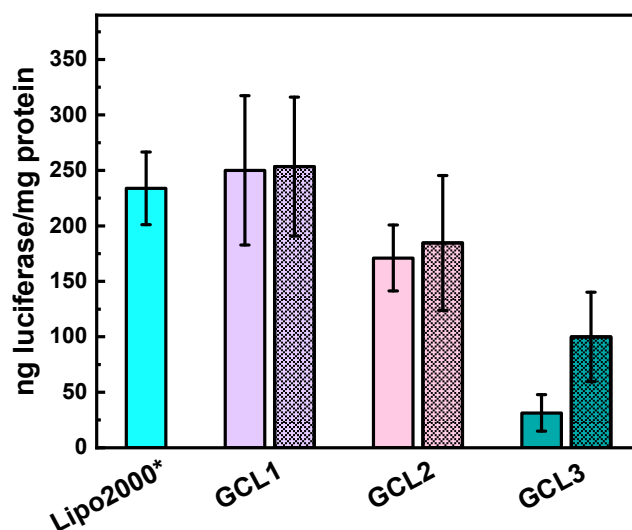


Figure S5. Transfection efficiency levels expressed as ng luciferase/mg protein in COS-7 cell line in presence of GCL/DOPE-pCMV-Luc lipoplexes formed at $\rho_{eff} = 5$ (unstriped bars) and 10 (striped bars). Lipoplexes were incubated with cells during 48 h. Blue bar corresponds to commercial control Lipo2000*, used as positive control. Data represent the mean of three wells and errors are within $\pm 5\%$.

References

1. Kumar, K.; Barrán-Berdón, A.L.; Datta, S.; Muñoz-Úbeda, M.; Aicart-Ramos, C.; Kondaiah, P.; Junquera, E.; Bhattacharya, S.; Aicart, E. A delocalizable cationic headgroup together with an oligo-oxyethylene spacer in gemini cationic lipids improves their biological activity as vectors of plasmid DNA. *J. Mat. Chem. B* **2015**, *3*, 1495–1506, doi:10.1039/c4tb01948b.
2. Misra, S.K.; Muñoz-Úbeda, M.; Datta, S.; Barrán-Berdón, A.L.; Aicart-Ramos, C.; Castro-Hartmann, P.; Kondaiah, P.; Junquera, E.; Bhattacharya, S.; Aicart, E. Effects of a delocalizable cation on the headgroup of gemini lipids on the lipoplex-type nano-aggregates directly formed from plasmid DNA. *Biomacromolecules* **2013**, *14*, 3951–3963, doi:10.1021/bm401079h.
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