Lipid-Oligonucleotide Conjugates Forming G-Quadruplex (Lipoquads) as Potent Inhibitors of HIV Entry

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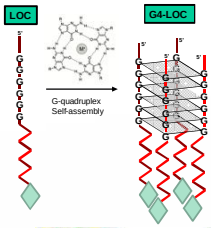
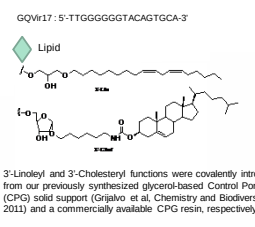
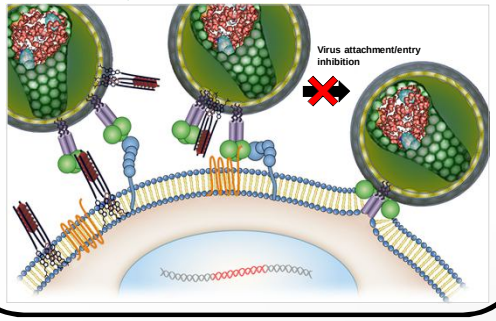
OVERVIEW

Molecules that block virus entry by interfering with the actions of the viral fusion proteins are of primary concern in the search of antiviral drugs. We present here the synthesis and the antiviral activities of lipid-oligonucleotide conjugates (Lipoquads) forming a highly stable tetramolecular parallel G-quadruplex. We show that these molecules block HIV-1 and HIV-2 entry with submicromolar activities, demonstrating the great advantage of targeting both viral Envelope glycoprotein and lipids rafts, key platform in virus entry.



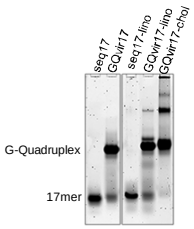


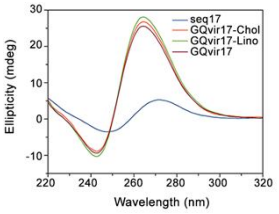
3'-Linoleyl and 3'-Cholesteryl functions were covalently introduced from our previously synthesized glycerol-based Control Pore Glass (CPG) solid support (Grijalvo et al, Chemistry and Biodiversity, 8, 2011) and a commercially available CPG resin, respectively.

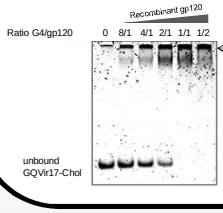


Name	Sequence 5'-3'	G-Quadruplex
Seq17	TTGGAAAGTACAGTGCA	No (control)
Seq17-lino	TTGGAAAGTACAGTGCA-lino	No (control)
GQvir17	TT**GGGGGG**TACAGTGCA	Tetramolecular parallel
GQvir17-lino	TT**GGGGGG**TACAGTGCA-lino	Tetramolecular parallel
GQvir17-Chol	TT**GGGGGG**TACAGTGCA-chol	Tetramolecular parallel

Native PAGE separation and circular dichroism assess G4 formation



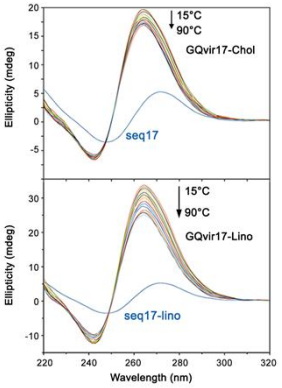


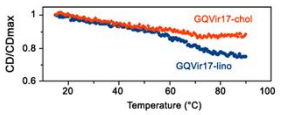


Electromobility-shift assay demonstrates that our G4-LOC is bound by recombinant gp120 in vitro

GQvir17-Chol (0.2µM) was titrated by 25, 50, 100, 200 and 400 nM gp120 for 15 minutes at 37 ° C. Bound/unbound DNA were separated on a non-denaturing 10% Acrylamide gel and stained with SybrGold.

The G4-LOC are highly stable molecules





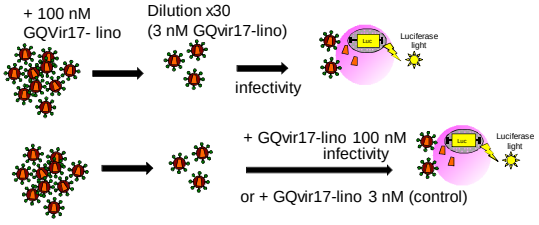
G4-LOC are potent inhibitors of HIV-1 in cell cultures

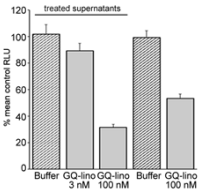
	IC50 viral infectivity (nM)		
	HIV-1 NL4-3	HIV-1 Lai	HIV-2 Rod
Seq17	NA	NA	
Seq17-lino	>40000	37000	
GQvir17	1100 ± 80	2360 ± 64	
GQvir17-lino	90 ± 20	375 ± 27	1180 ± 30
GQvir17-chol	50 ± 18	106 ± 15	

The infectivity assay of HIVNL4-3 was performed by infecting SupT1 cells with the NL4-3REN vector in presence of test compounds. HIV replication was evaluated 72 hours post-infection following Renilla luciferase fluorescence. For HIVLAI and HIV2Rod, TZM-bl cells were incubated for 1 h with increasing concentrations of test compounds and next cells were infected with viruses and 48 h after infection cells were assayed for Tat-dependent luciferase expression.

G4-LOC neutralize HIV-1 particles

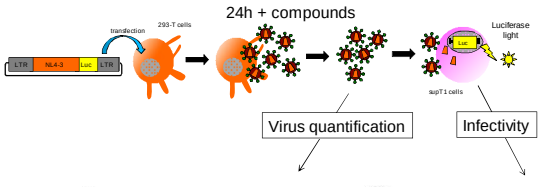
Pre-treated supernatant

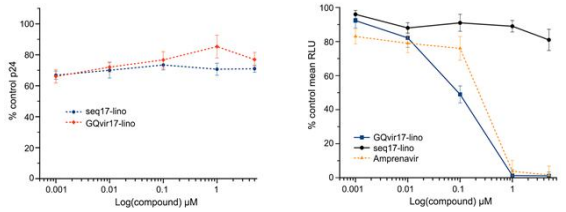




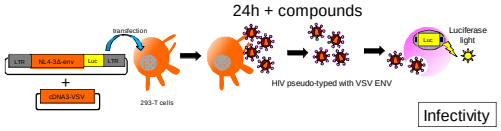
Recombinant NL4-3-ren supernatants were incubated with 100nM of GQVir17-lino for 3h and next diluted 30 times to infect SupT1 cells, which therefore diluted the compounds below EC50 levels (e.g. 3nM). SupT1 cells were infected in parallel with the dilution control (3nM) and direct inhibition of infection was performed as a positive control with 100nM of G4-LOC.
This assay produced 50% of neutralized viruses when incubated with the compounds, suggesting their direct and persistent binding to the virus.

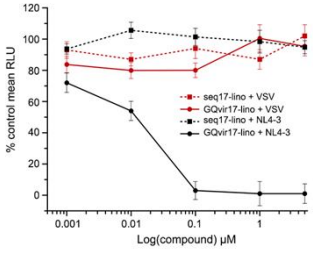
The G4-LOC do neither target virus assembly nor virus production





G4-LOC are entry inhibitors

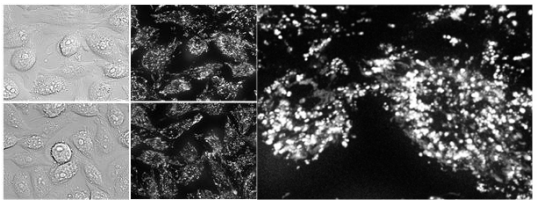




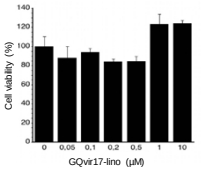
Viral entry inhibition is demonstrated by infecting SupT1 cells with HIV recombinant virus pseudo-typed with the G protein of VSV (NL4.3-Δenv-VSV-Luc), which enters host cells independently of cell receptors. VSV-pseudo-typed virus infection was not affected by the presence of neither GQvir17-lino nor the control seq17-lino, while 100 nM of GQvir17-lino inhibited HIV NL4.3 infection with an efficiency of around 70%.

Cy5-G4-LOC bind to plasma membranes

HeLa cells treated 4h with 1µM of Cy5-GQVir17-Lino show plasma membrane-associated fluorescence



Our G4-LOC do not affect HeLa cells viability at 20µM, as also seen with TZB-bl, SupT1, ACH-2 and 293T cells



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