Parallel G-quadruplexes, small nanostructures with enhanced cellular uptake properties

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Nucleic acid Therapies(CSHL, virtual)

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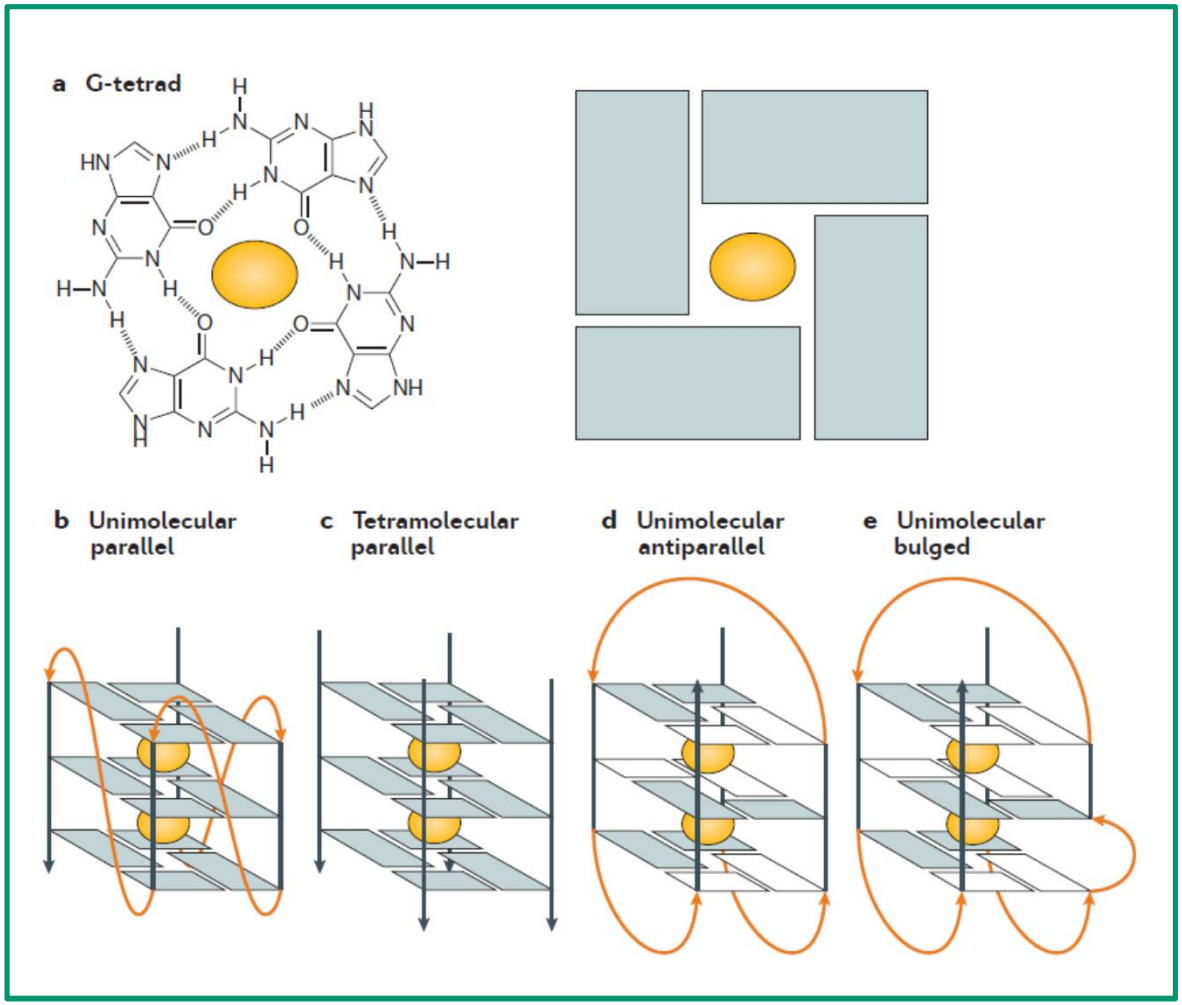
G-quadruplex



Balasubramanian *et al*. *Nat. Rev. Mol. Cell. Biol*. **2017**, *18*, 279

Single-stranded guanine rich DNA can fold into stable intra- and intermolecular four-stranded structures

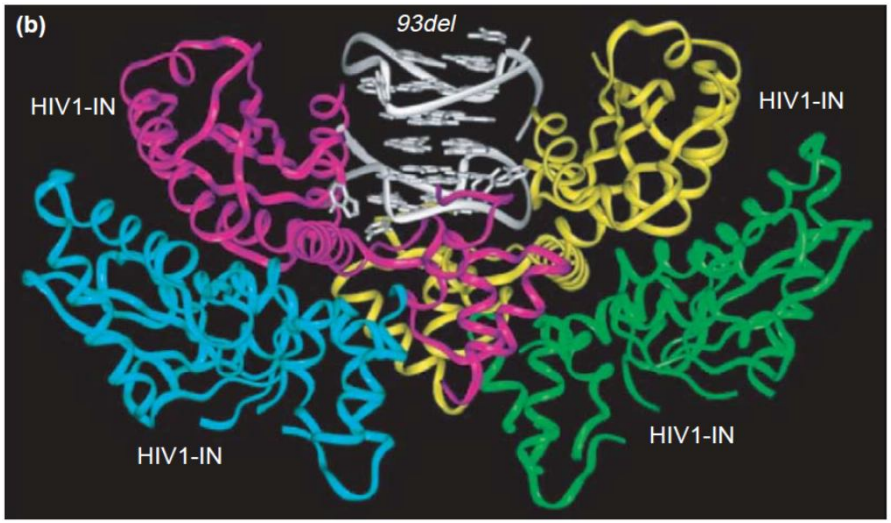
G4s arise from Hoogsteen hydrogen bonding of four guanines that are arranged within a planar quartet (G-quartet)

Self-stacking of two or more G-quartests affords a G4 structures which is further stabilized by monovalent cations ($K^+ > Na^+ > NH_4^+ > Li^+$)

G4 formation has been observed in synthetic ODNs derived from the human genome (gene promoters and telomers)

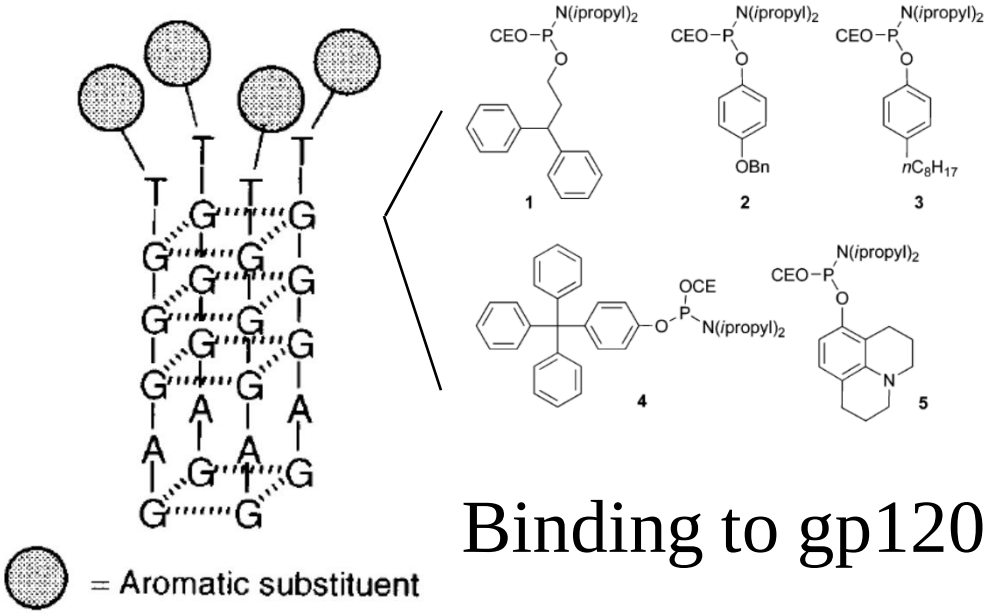
Applications may range from supramolecular chemistry to medicinal chemistry

Antiviral G-quadruplexes



Aptamer 93del is positioned into the channel formed by the HIV-IN tetramer to block the HIV1-IN catalytic site

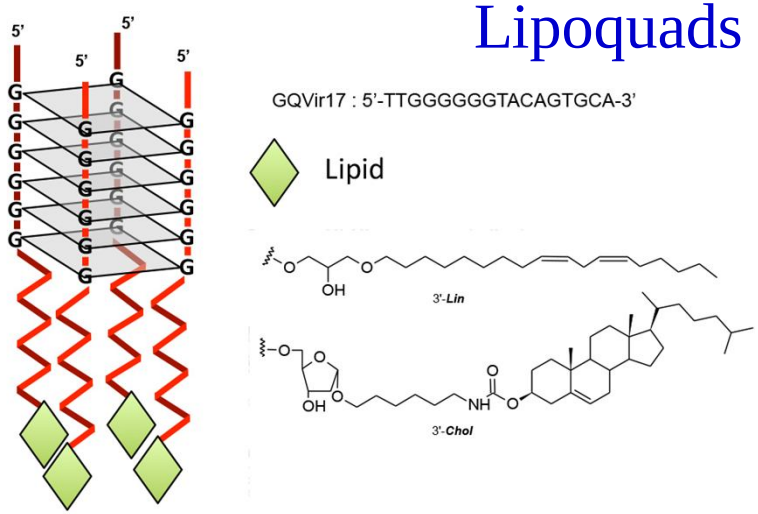
Chou; S-H. *et al. TRENDS in Biochem. Sci*. **2005**, *30*, 231-234



Binding to gp120

Hotoda, H. *et al. J. Med. Chem*. **1998**, *41*, 3655-3663
Di Fabio, G. *et al. Chem. Commun*. **2011**, *47*, 2363-2365
D'Onofrio, J. *et al. Bioconjug. Chem*. **2008**, *19*, 607-616

Lipoquads

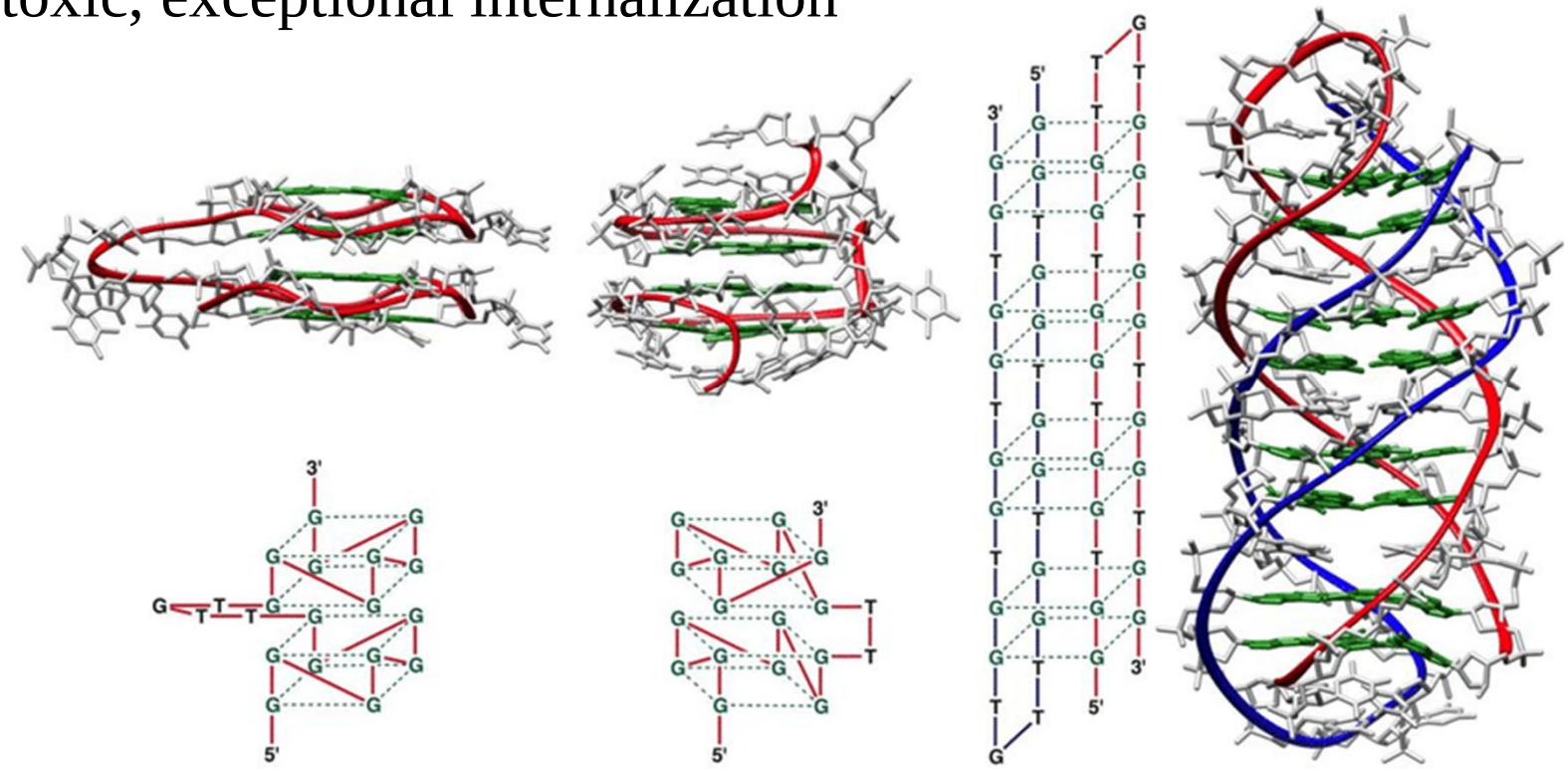


Binding to gp120

Koutsoudakis et al, *Antimicr. Agents Chemother*., **2017**, 61, e02354-16-

Antiproliferative G-quadruplexes

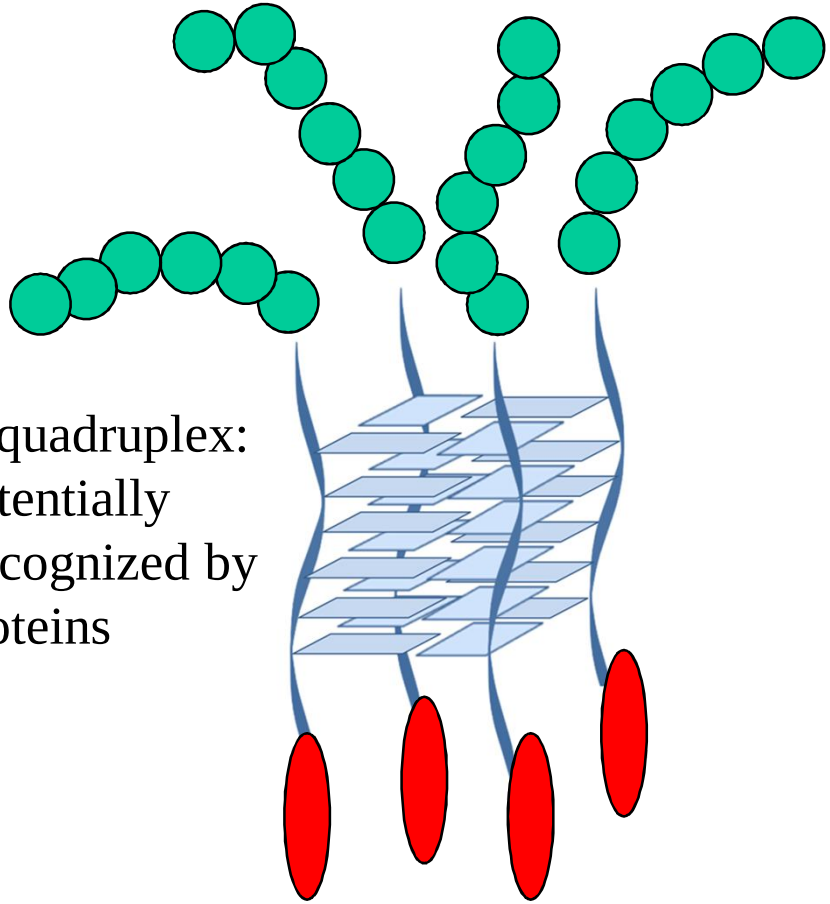
AS1411 (AGRO100) aptamer (binding to nucleolin), cytotoxic, exceptional internalization



Mechanism of action unclear. Anti-HIV. Potential toxicity by GMP formation

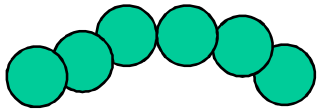
Bates P.B. et al. *Biochimica et Biophysica Acta*, **2017**, 1861, 1414-1428.
Perone et al. *Int. J Antimicrob Agents*, **2016**, 47, 311-316.
Métifiot M. et al. *Biochimie*, **2015**,118, 173–175.
Zhang et al. *Chem Sci*, **2015**, 6, 3831-3838

Aim: Parallel G-quadruplexes to enhance cellular uptake

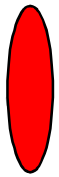


G-quadruplex: Potentially Recognized by proteins

Cargo: Therapeutic Oligonucleotides: Antisense, FdU oligomers



Delivery Enhancer: Lipid, peptide, GalNAc



Simple self-assembly process:
Tetramerization of single stranded oligonucleotides

Potential advantages /disadvantages

Pro's

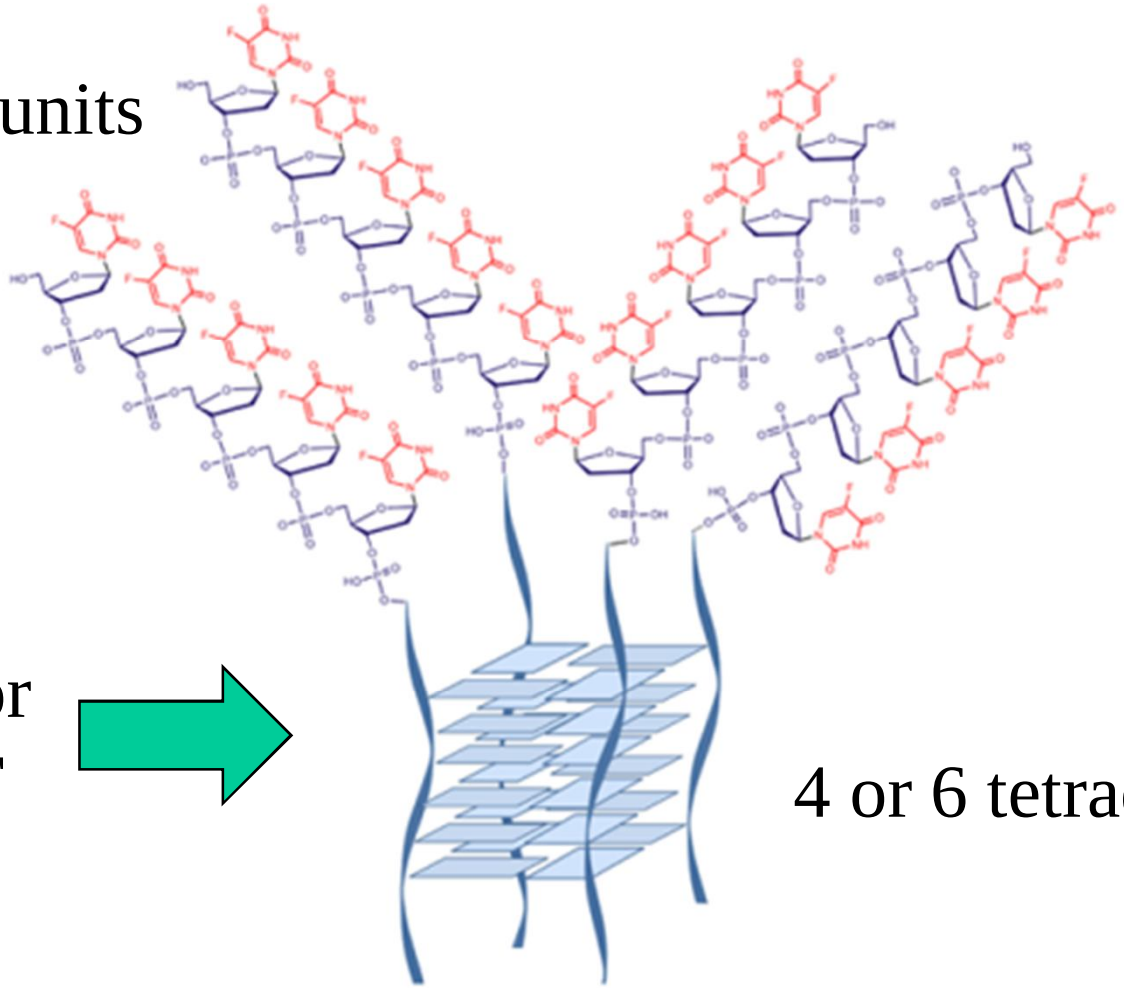
- Simplicity. The smaller nanostructure accesible by tetramerization of a single oligonucleotide
- Easy to make although tetramerization is a slow process
- Two ends for functionalization
- Tetramerization allows 4 ligands in one end without branching
- Existence of proteins with affinity to G-quadruplex specially in tumor cells

Contra's

- Dissociation is slow but can happen by dilution
- Potential toxicity to cells by production dGMP
- Not compatible with the presence of C's as duplexes are more stable

Parallel G-quadruplex structures increase cellular uptake and cytotoxicity of 5-fluoro-2’-deoxyuridine

5 or 10 FdU units

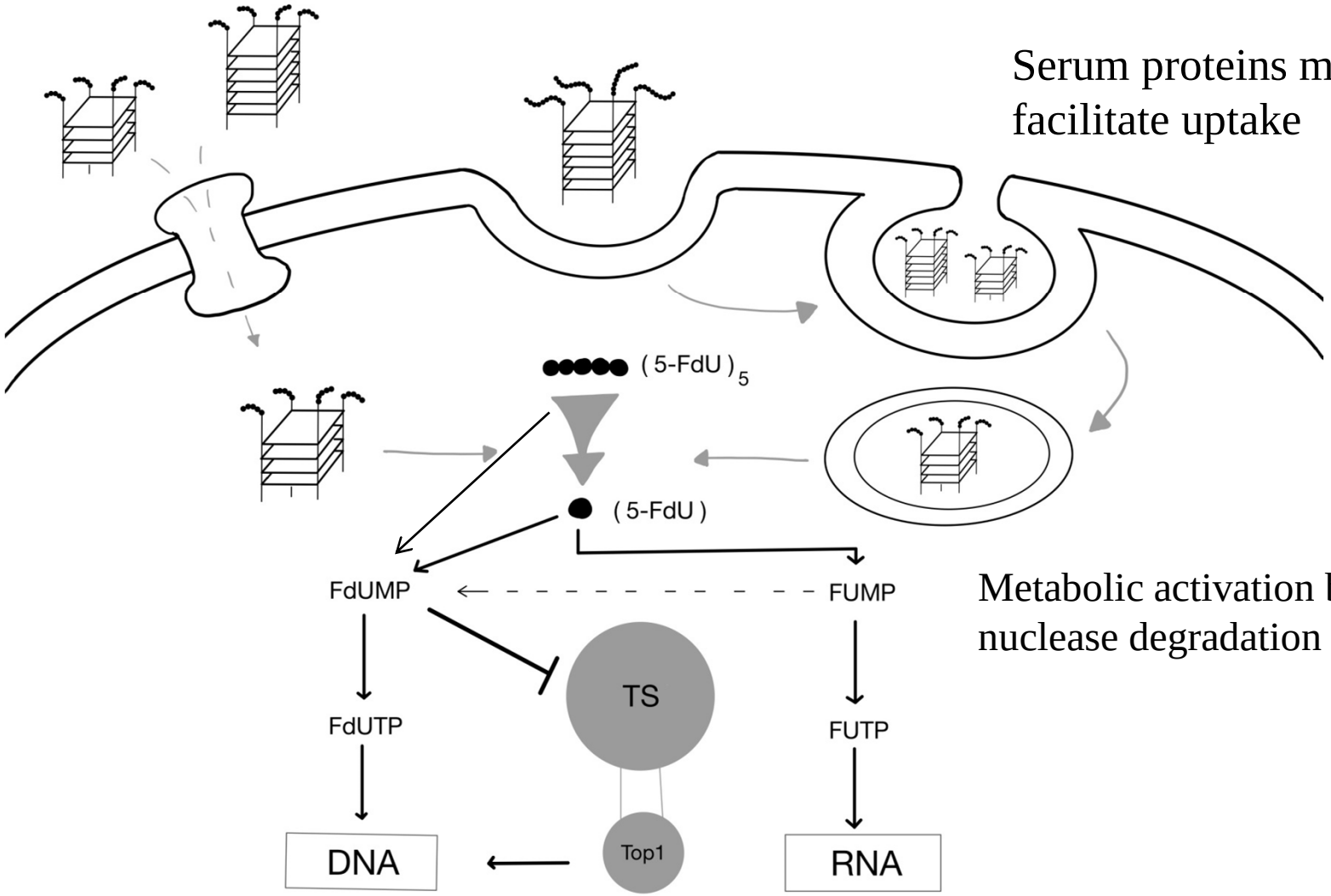


TGGGGT or
TGGGGGT

4 or 6 tetrads

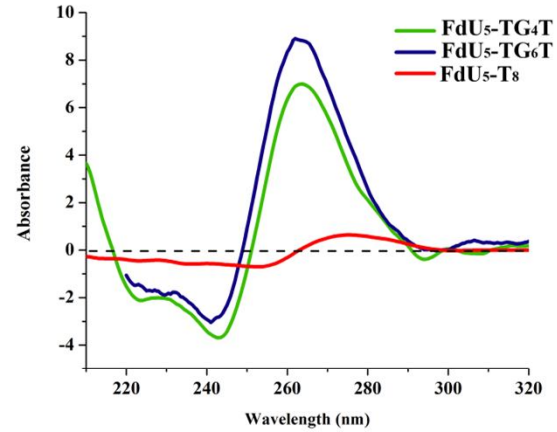
Control sequence TTTTTTTT

Parallel G-quadruplex structures increase cellular uptake and cytotoxicity of 5-fluoro-2’-deoxyuridine

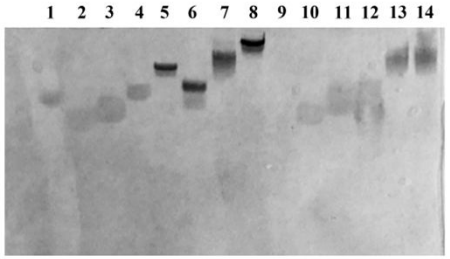


Clua A. et al. *Molecules* **2021**, 26, 1741

CD

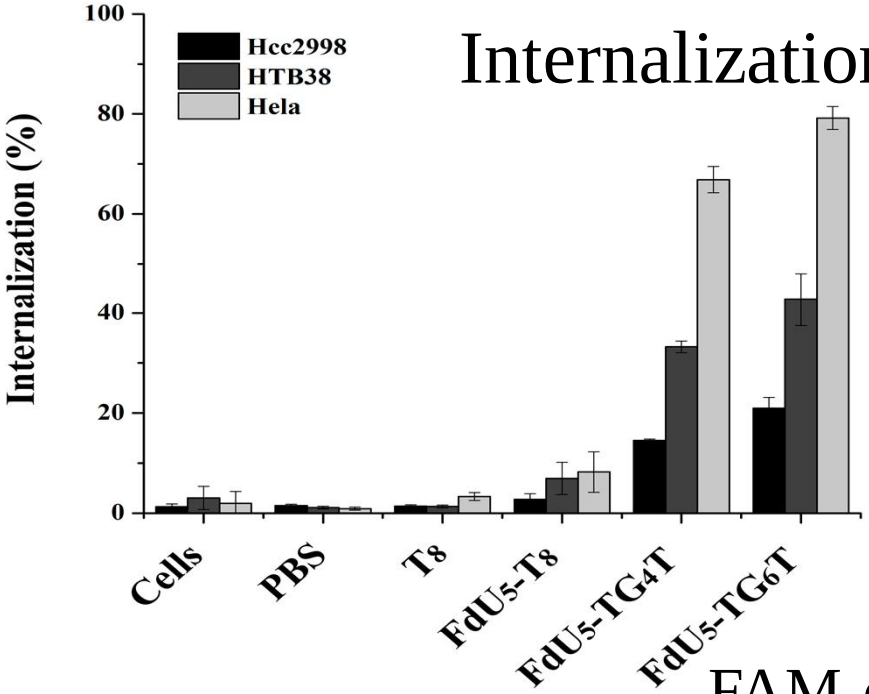


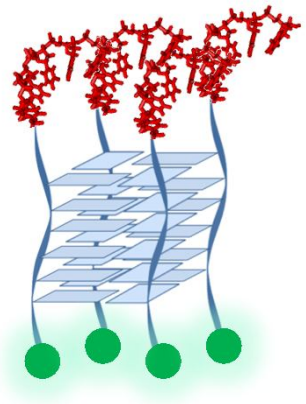
Native PAGE

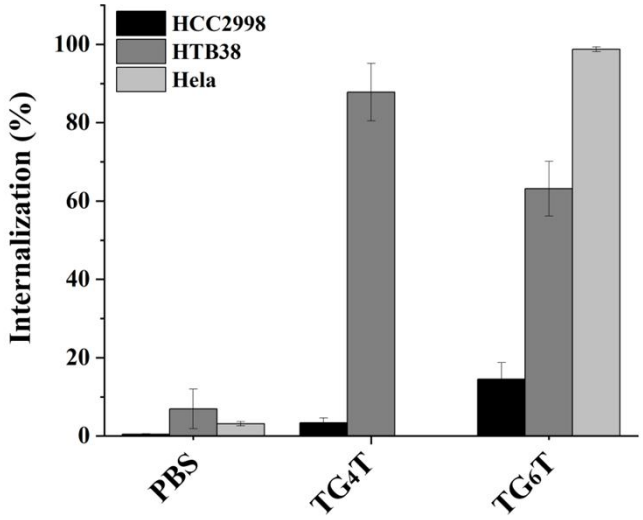


Stains-all

Internalization

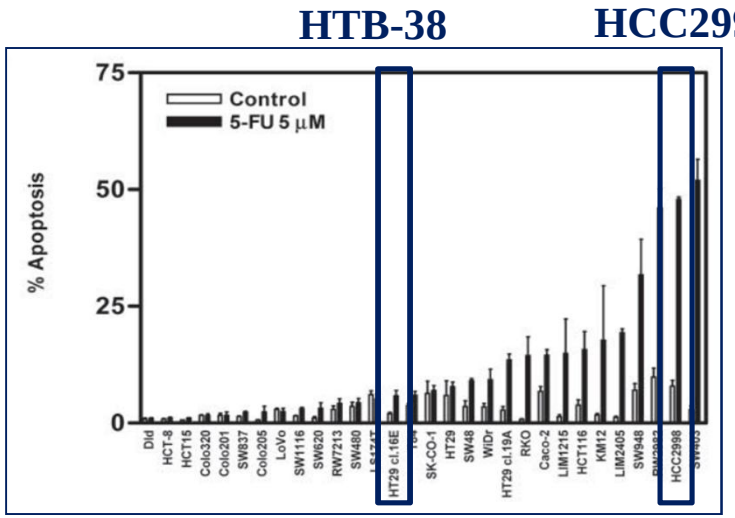




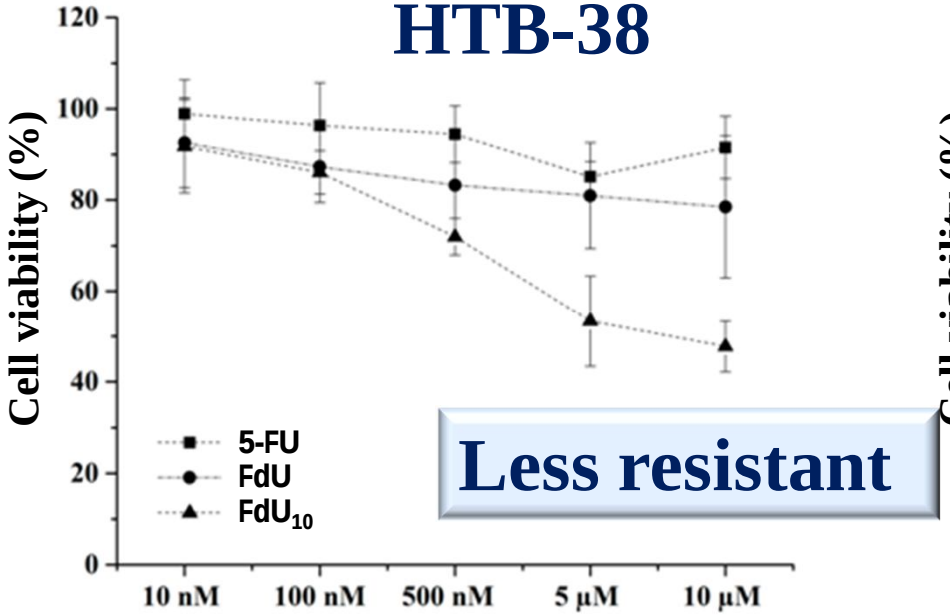


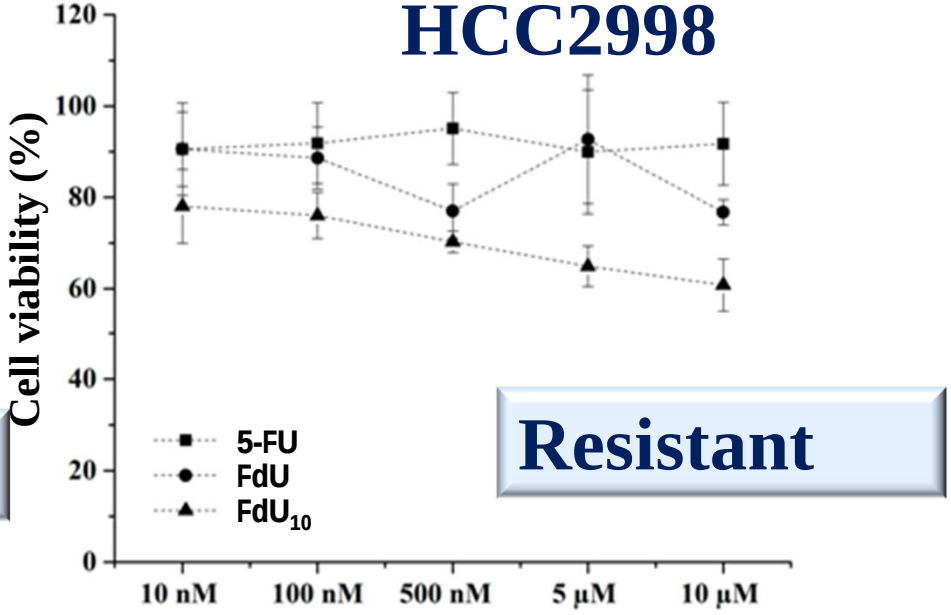
FAM-quadruplexes up to 80% of cells, after 24 hr

FU resistant colorectal cancer cell lines

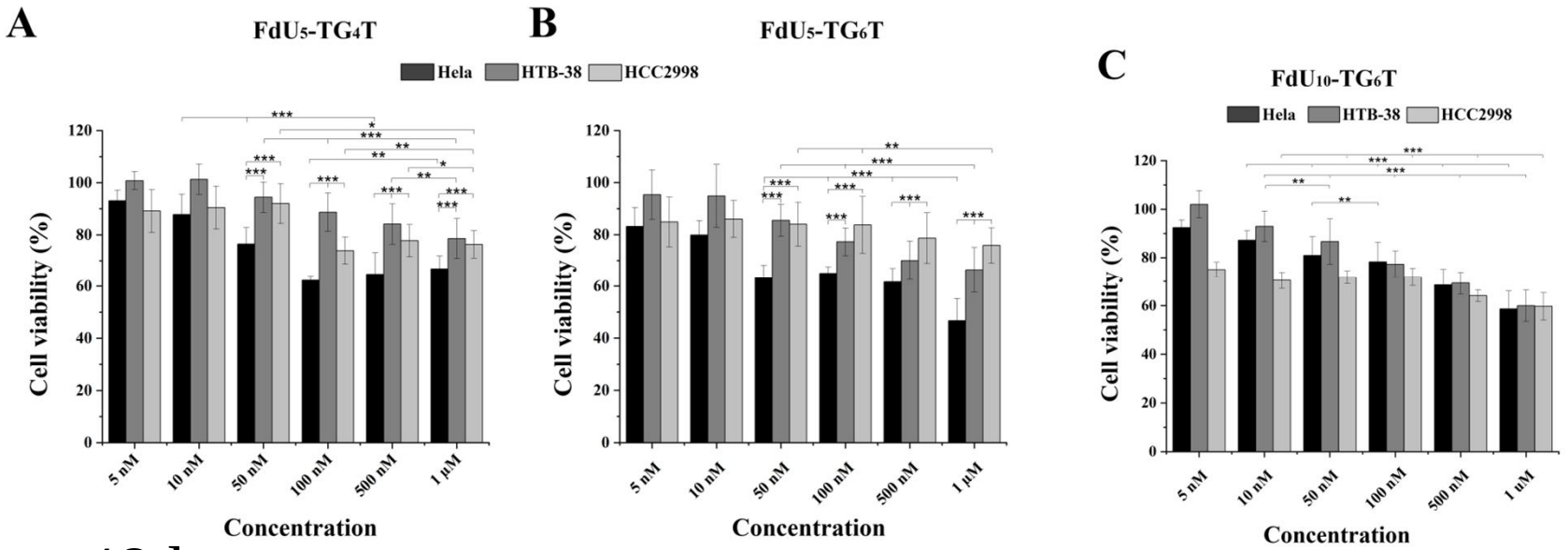


J. Mariadason et al. cancer research, 63, 8791-8812, 2003



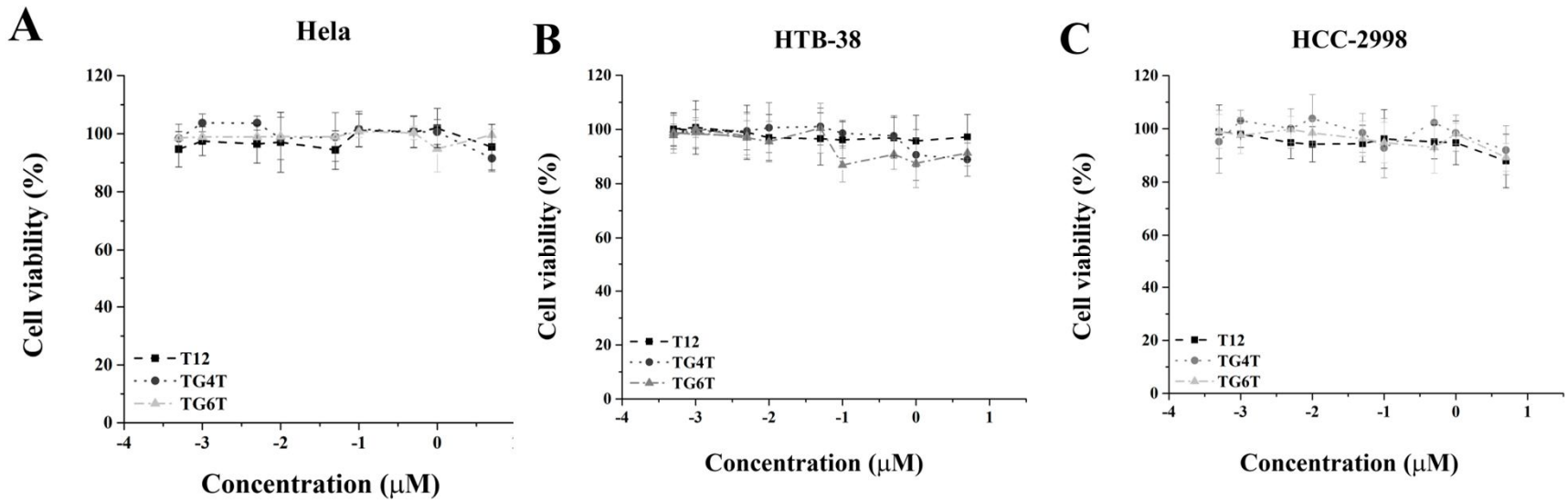


MTT results



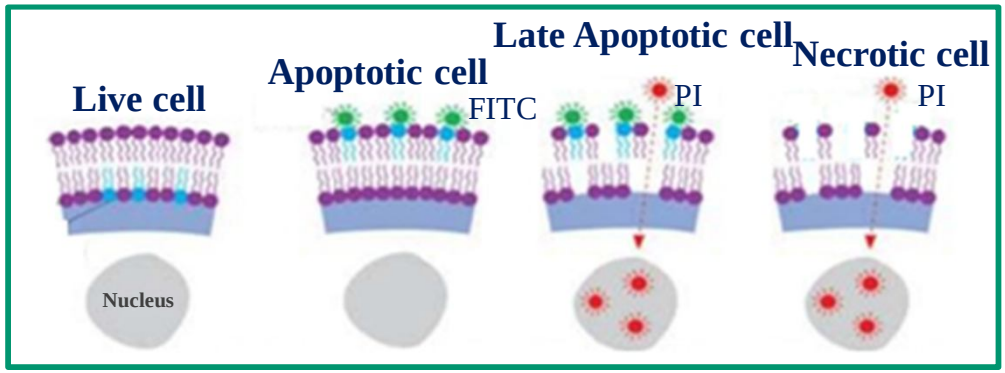
After 48 hrs

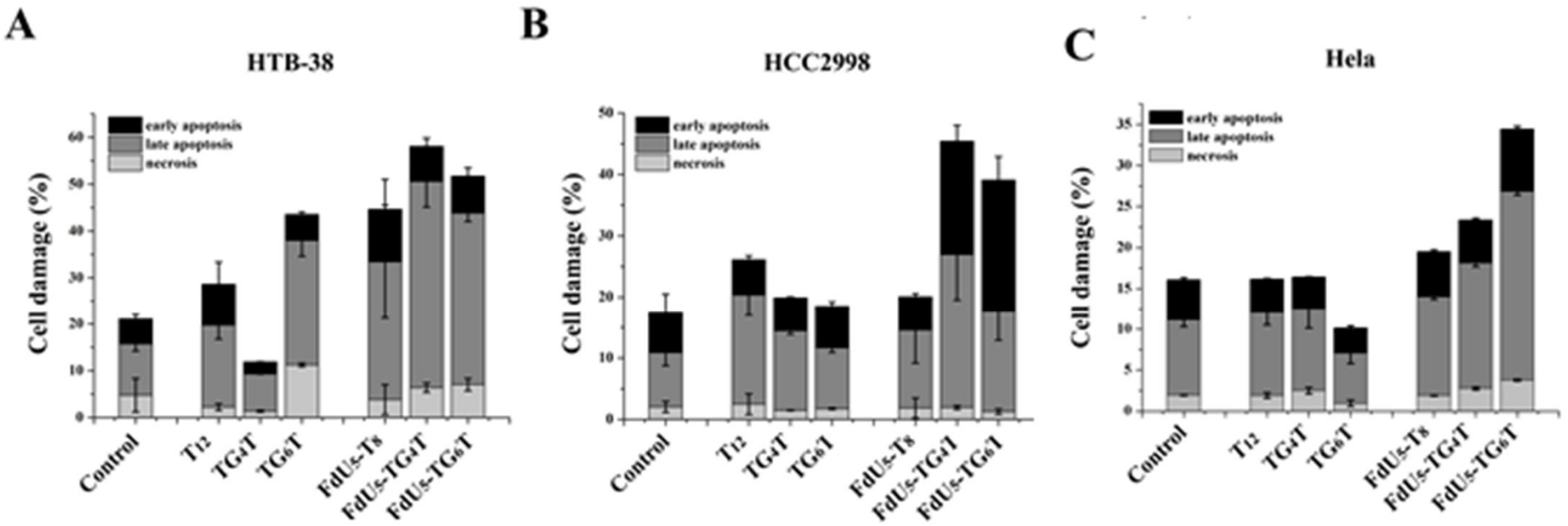
T_{12}
TG_4T
TG_6T



In absence of FdU there is no toxicity to cells (> 85% viability)

Cell death induction





After 48 hrs

Conclusions

- Internalization studies confirmed the ability of such G-quadruplex nanostructures to facilitate the transport of the FdU pentamer and increase its cytotoxic effect relative to conventional FU drug in FU-resistant colorectal cancer cells.
- These results suggest that FdU oligomers linked to G-quadruplex parallel sequences may be a promising strategy to deliver fluoropyrimidines to cancer cells.


Thank
You
Merci
Gracias
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aDank
BarakAllahuFiik
Grazie
Chokrane
Kiitos
Dziakuju
GratiasAgimus
GratiasAgo
Gracie
KopKhunKha
Motashakkeram
Tak
Sagolun
Dankie
Ačiū
TänanVäga
Chnorakaloutioun
CámOn
Multumesc
Saha
Salamat
Obrigado
Bayarlalaa
Nirringrazzjak
NajisTuke
Efharisto
Faleminderit
Gràcies
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Taiku
Mercé
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Blagodaram
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Buznyg
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Köszönöm
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Grazzi
Mauruuru
Tenki
Tanemirt
Aabhar
Grandmercé
Gratzias
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Mamnun
aNiKié
Shukriya
TapadhLeibh
FaafetaiLava
Ngiyabonga
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Bedankt
Zikomo
Nouari
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Welalin
TangioTumas
Dhanyavad
Sukriya
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Dhanyavadalu
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Merkzi
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ÇoxSagOlun
Meharbani
Mahalo
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DankJuWel
Wado
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Díky
TakkFyri
Miigwetch
Asante
Spas
EskerrikAsko Dankewol
Diolch
Dankje
Paldies
DjiereDieuf
ANiKié
Rahmet
MaloTeOfa
Trugéré
Misaotra
Akpé
Milesker
Tanmirt
Akiba
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GoRaibhMaithAgat
Mochchakkeram