Assembly of natural compounds with micro-particles to improve the targeting of human topoisomerase I in cancer therapy

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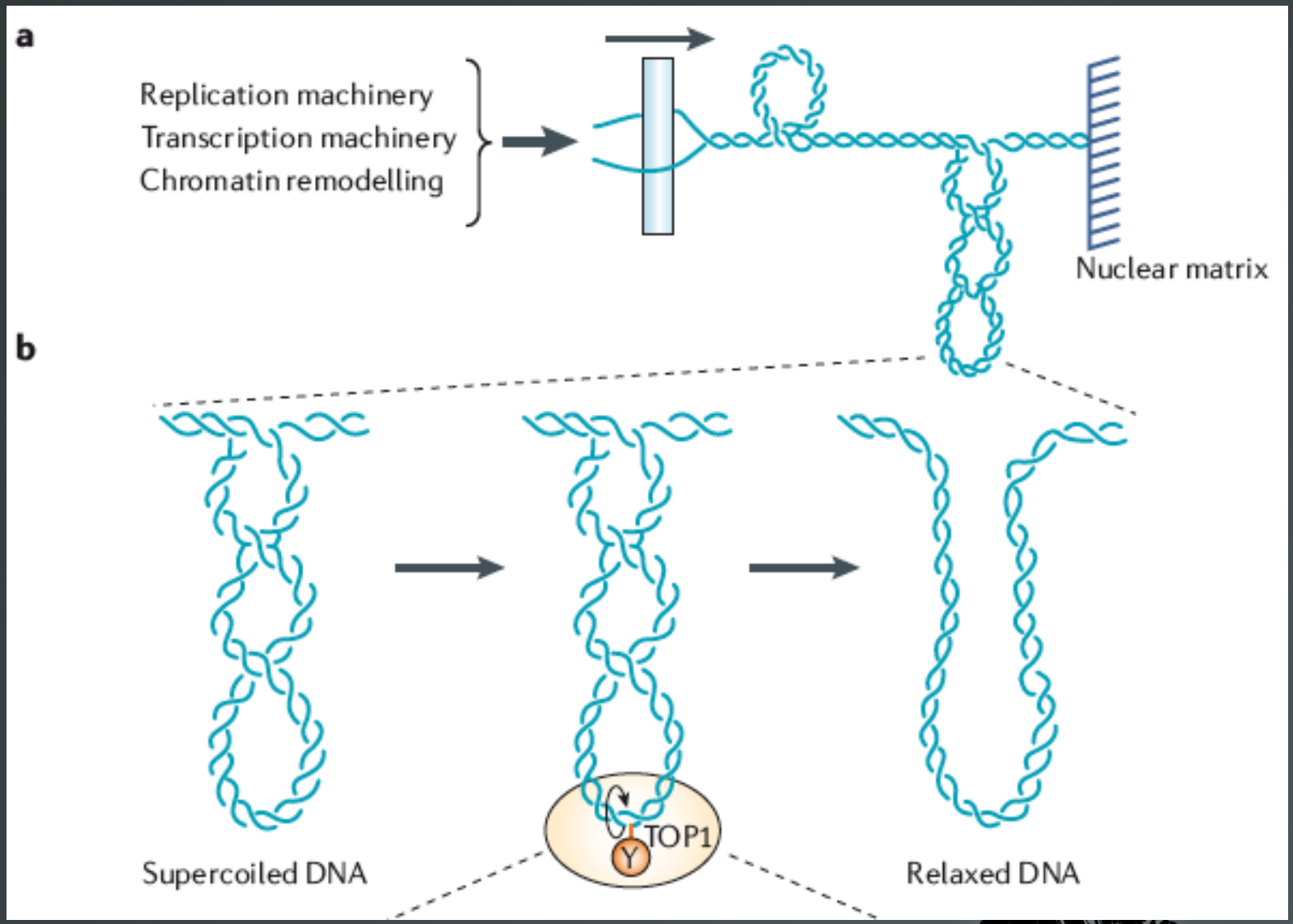
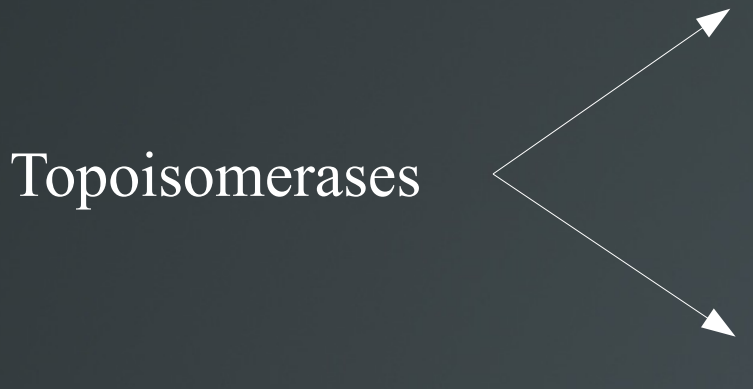

a
Replication machinery
Transcription machinery
Chromatin remodelling
Nuclear matrix
b
TOP1
Y
Supercoiled DNA
Relaxed DNA

Topoisomerases are classified as type I and type II



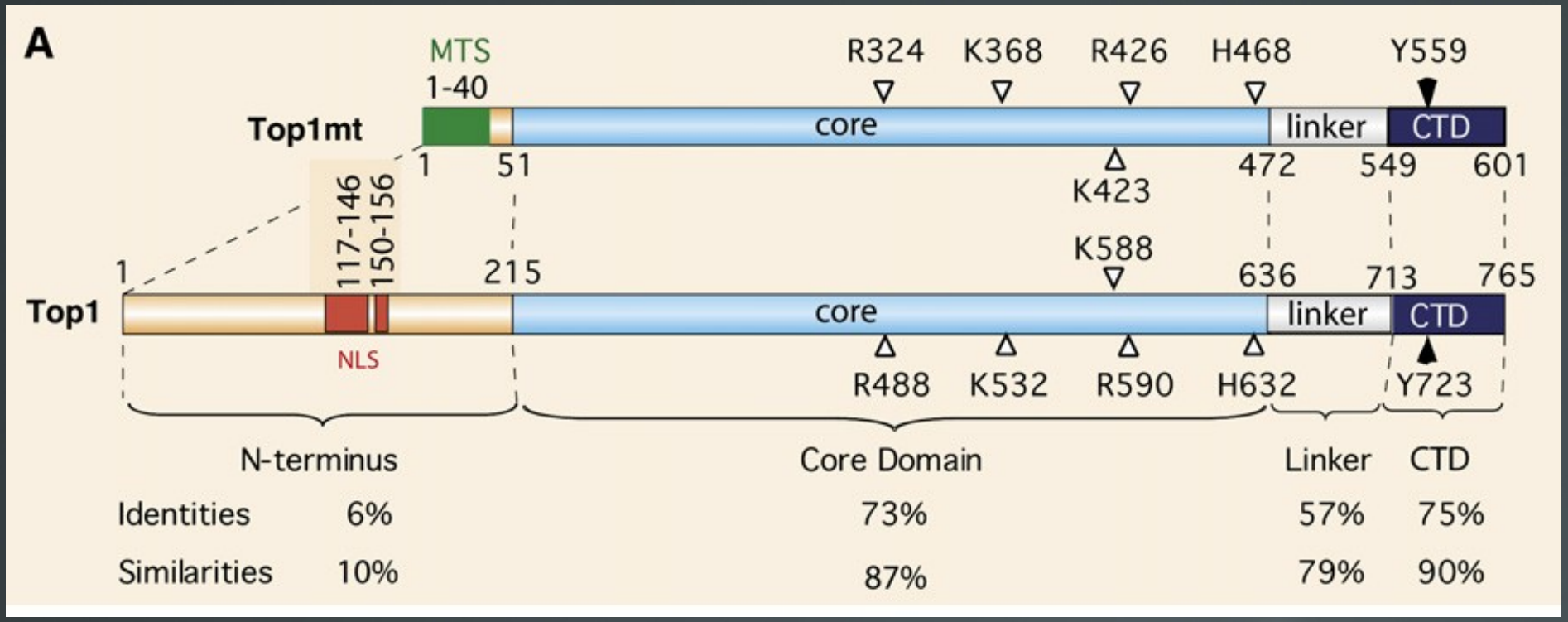
Monomeric. Cleave one strand forming a covalent protein-DNA intermediate

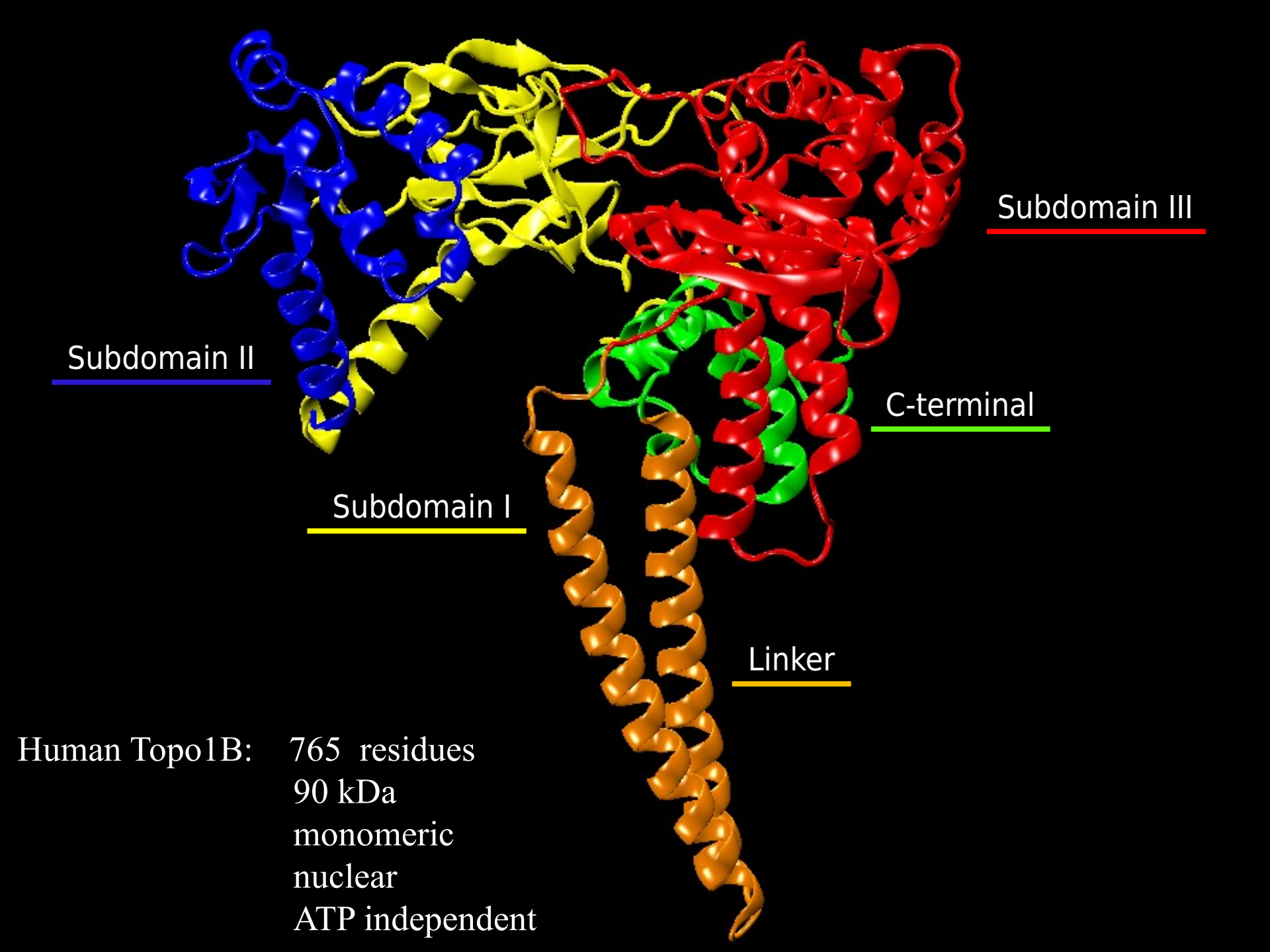
Dimeric. Cleave both strands forming a covalent protein-DNA intermediate for each monomer

They cleave the DNA phosphodiester backbone by nucleophilic attack from a catalytic tyrosine residue which becomes linked to the phosphate end (P-Y) of the DNA break.

The reaction is highly reversible and leaves the DNA sequence unchanged following topoisomerization.

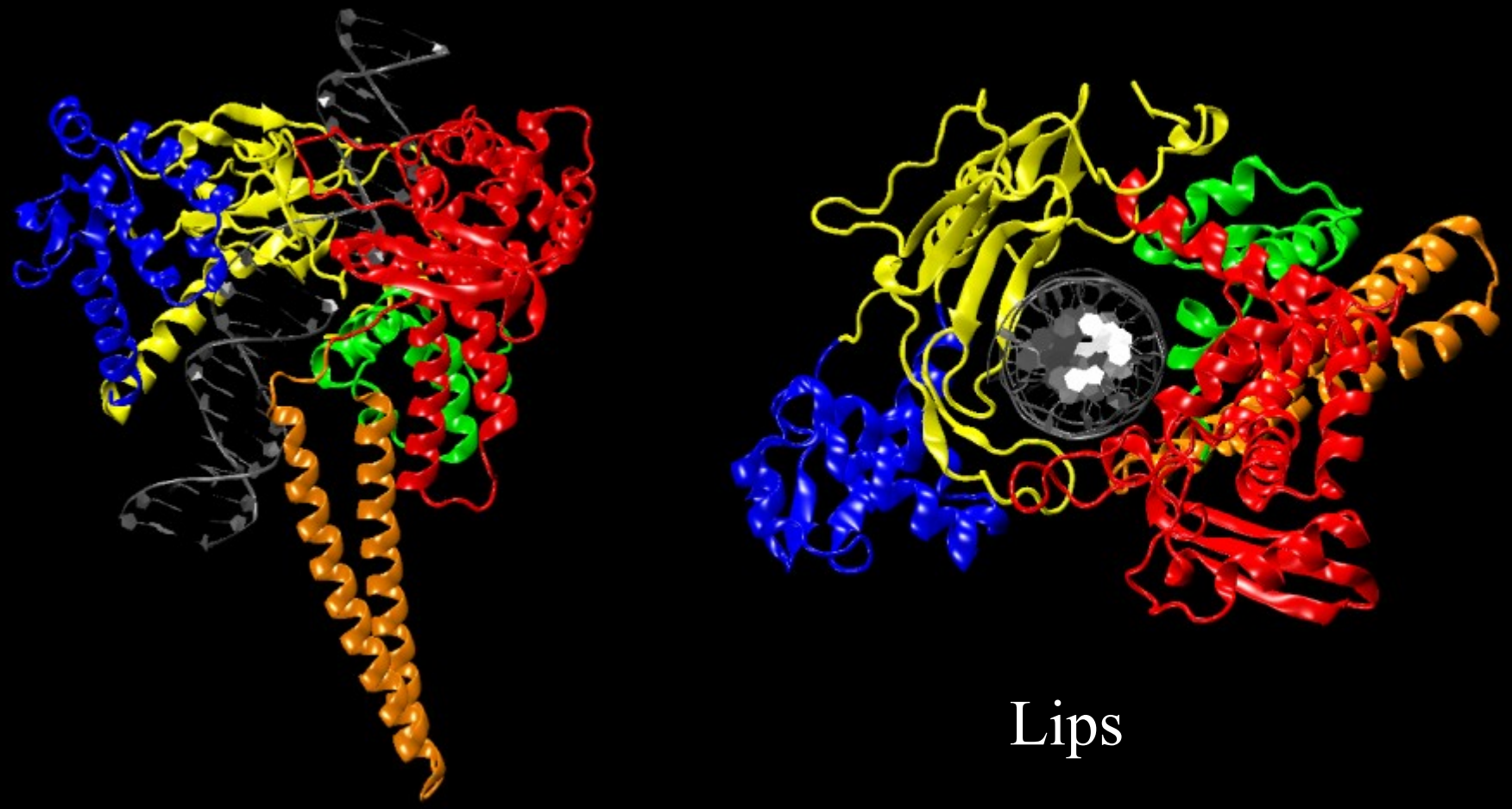
Human topoisomerase IB





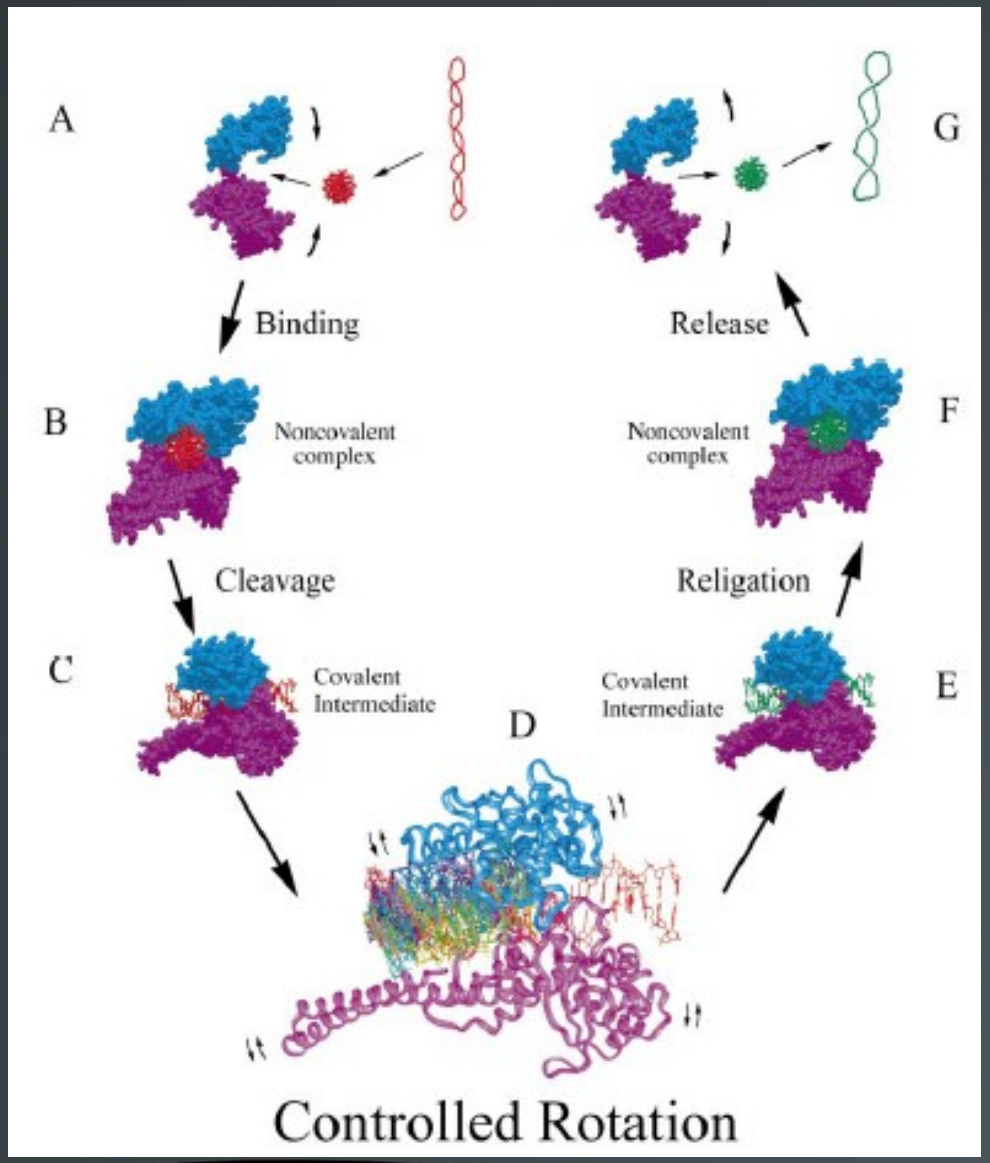
Human Topo1B: 765 residues
90 kDa
monomeric
nuclear
ATP independent

The protein has a bilobed shape and completely clamps around the DNA



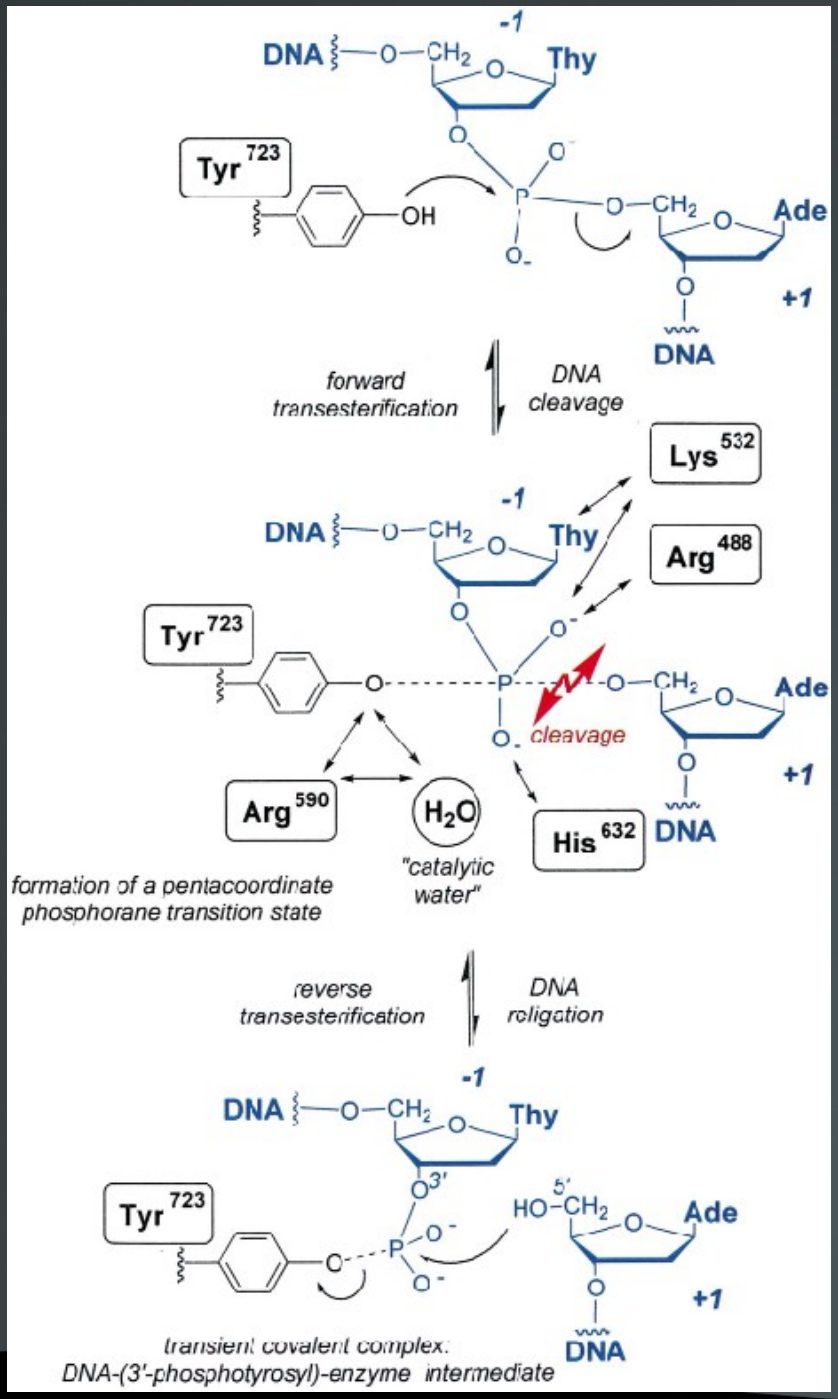
Lips

Mechanism of action: five steps



The linker domain and the nose cone helices are responsible for the *Controlled Rotation* mechanism.

Stewart *et al.*, 1998



Nucleophilic attack

DNA topoisomerase I inhibitor classes

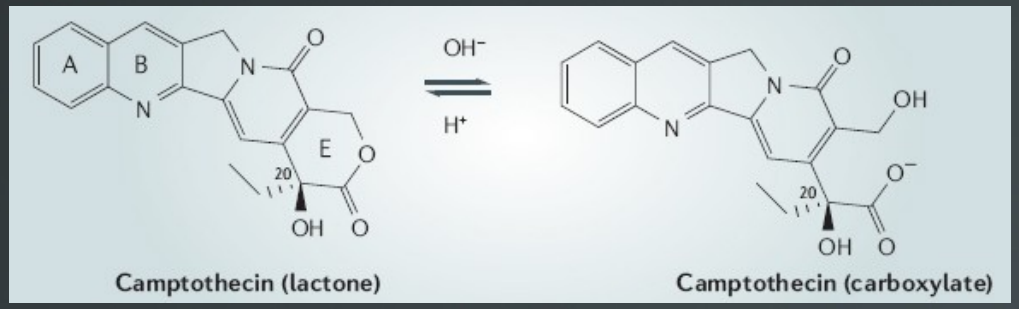
Class I

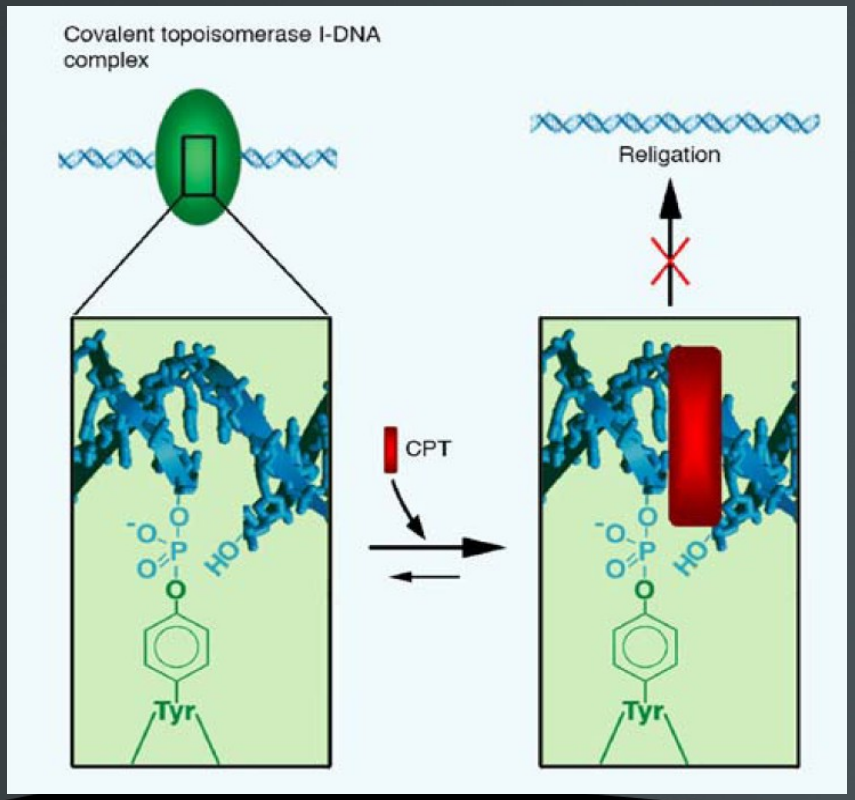
- Cellular poisons
- Bind reversibly the cleaved complex
- Diminish the “religation” speed

Class II

- Catalytic Inhibitors
- Interfere with DNA-topoisomerase I binding
- Interact with the protein active site

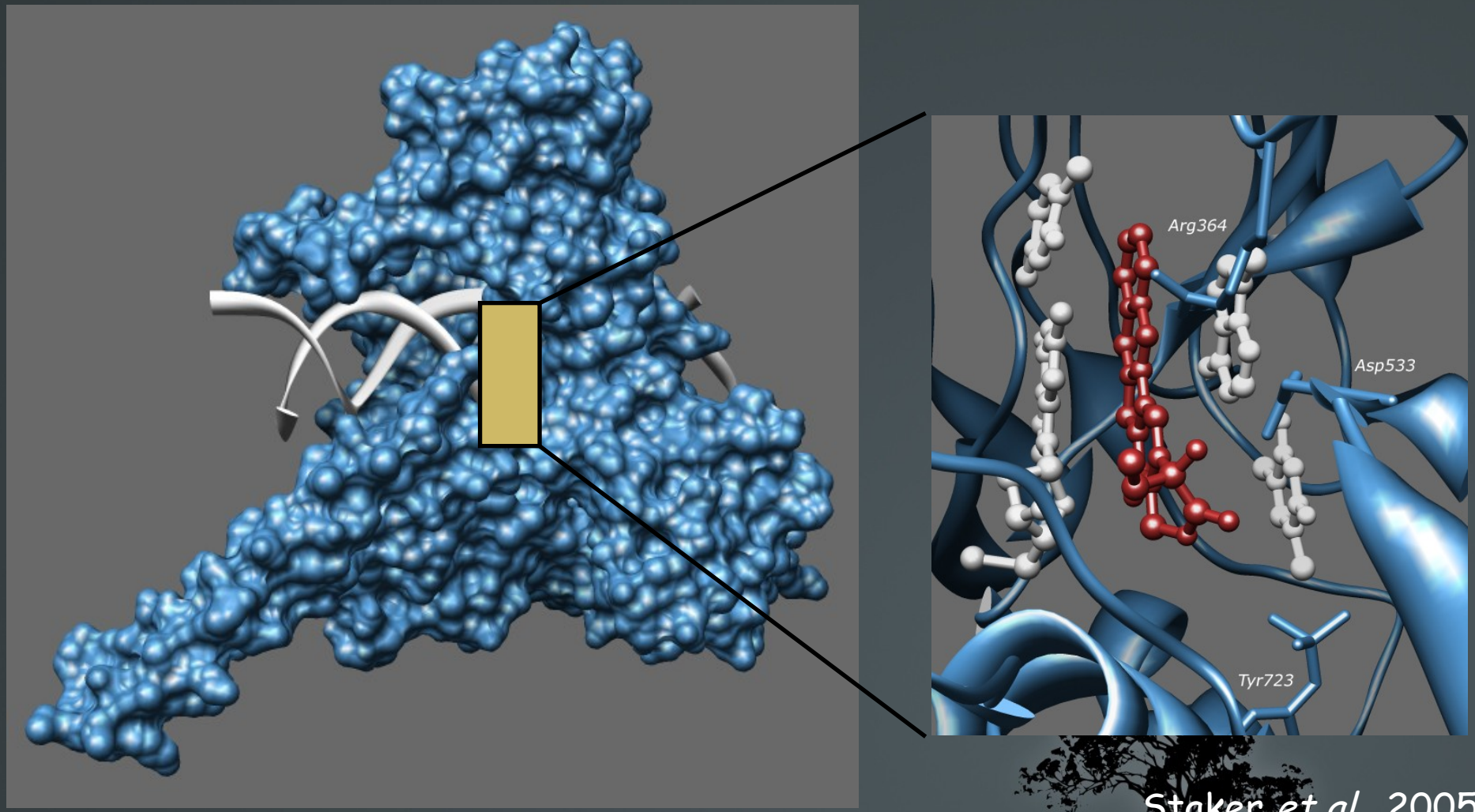
Human Topoisomerase IB is the unique target of the antitumor drug camptothecin





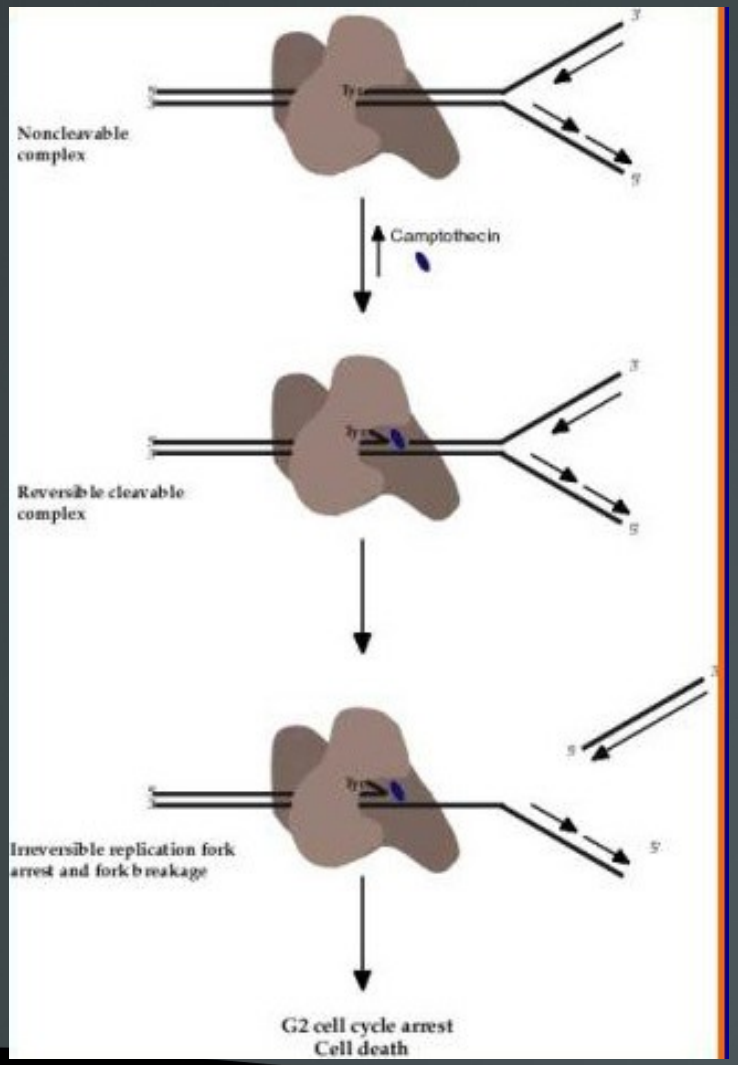
The anticancer drug camptothecin (CPT) specifically binds to the covalent human topoisomerase I–DNA complex stabilizing it and then inducing cell death.

The drug intercalates between the DNA base pairs interacting both with protein and the DNA.

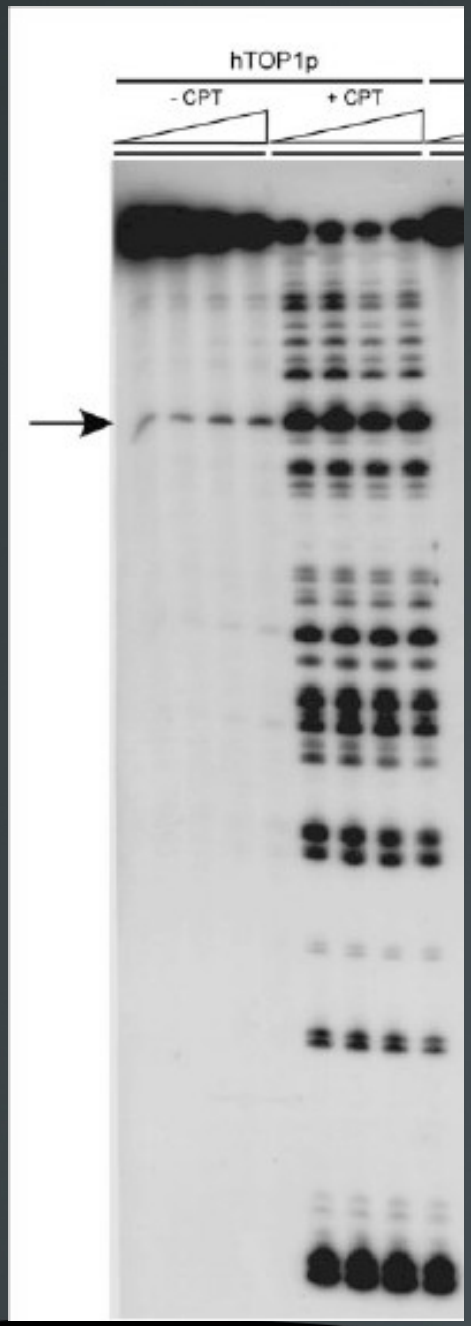


Staker *et al.*, 2005

The binding of the drug to the covalent complex is reversible, but when the complex collides with the replication fork it induces an irreversible effect. The inhibition is S-phase specific.

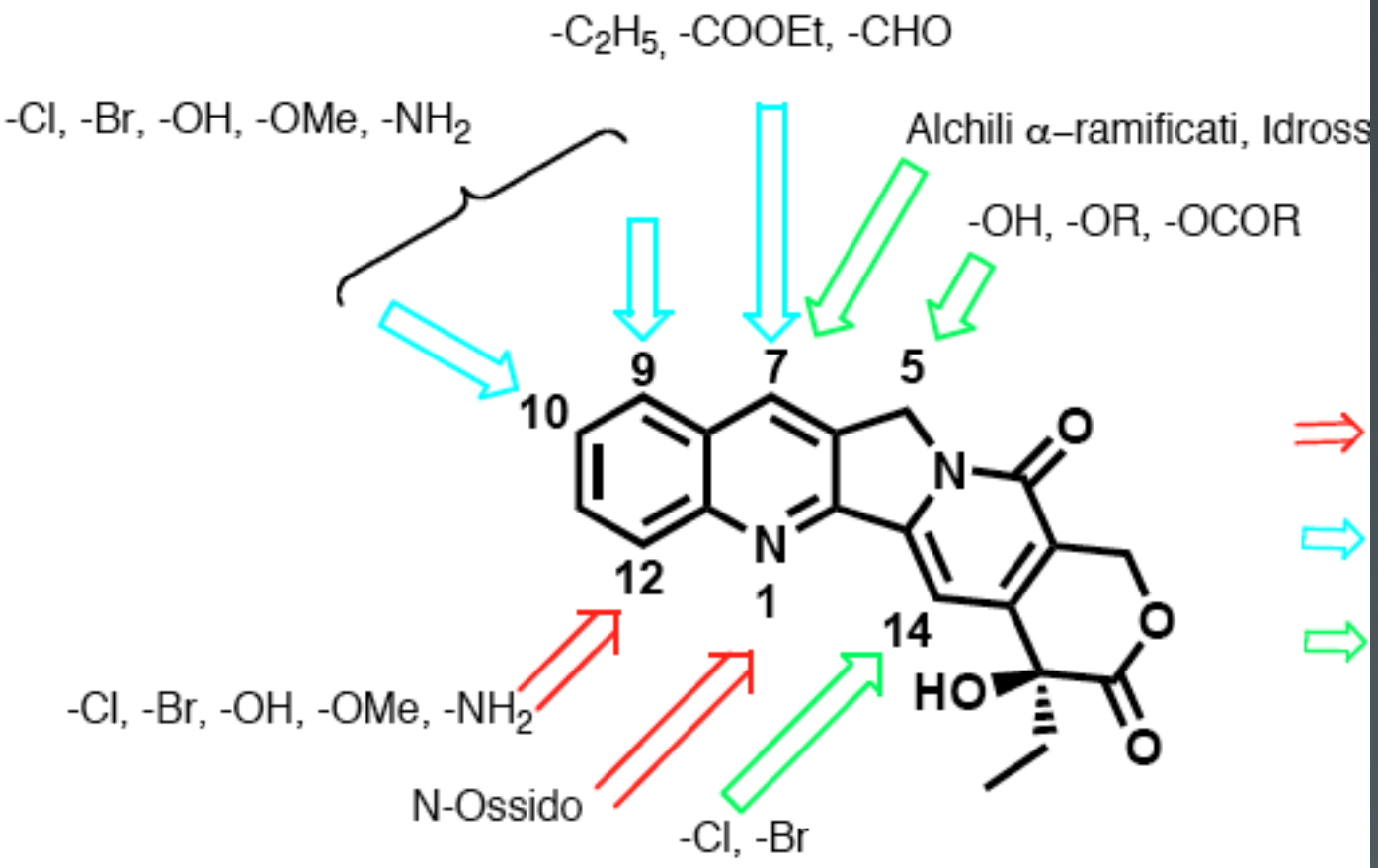


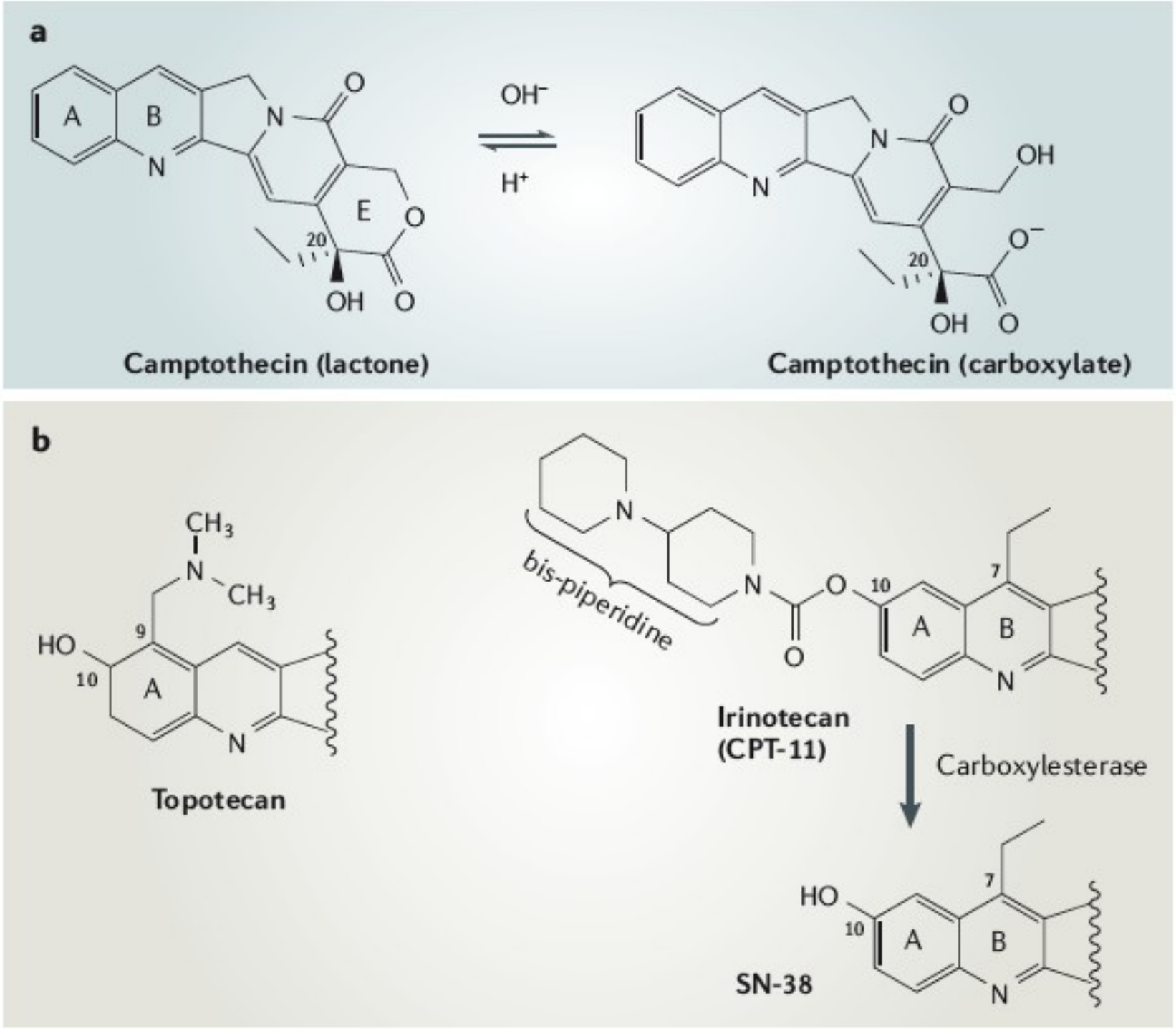
Schneider, Hsiang & Liu, 1990



Cleavage/Religation equilibrium in absence and presence of CPT using a 900 bp dsDNA as a substrate.

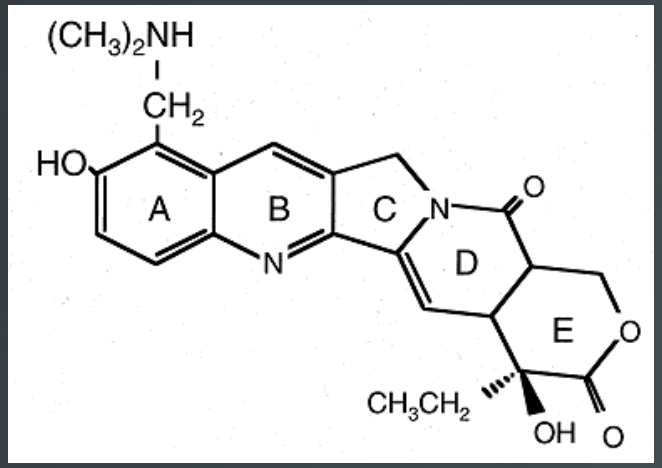
$$K_{eq} = k_{cl}/k_r$$


-C2H5, -COOEt, -CHO
-Cl, -Br, -OH, -OMe, -NH2
Alchili α–ramificati, Idross
-OH, -OR, -OCOR
9
7
5
10
12
1
14
N
O
HO
-Cl, -Br, -OH, -OMe, -NH2
N-Ossido
-Cl, -Br
Decrease activity
Improve activity
Eliminate activity


a
A
B
N
O
N
E
O
20
OH
O
OH⁻
H⁺
N
O
OH
N
O⁻
20
OH
O
Camptothecin (lactone)
Camptothecin (carboxylate)
b
CH3
N
CH3
HO
9
10
A
N
Topotecan
N
bis-piperidine
N
O
O
10
7
A
B
N
Irinotecan
(CPT-11)
Carboxylesterase
HO
7
10
A
B
N
SN-38

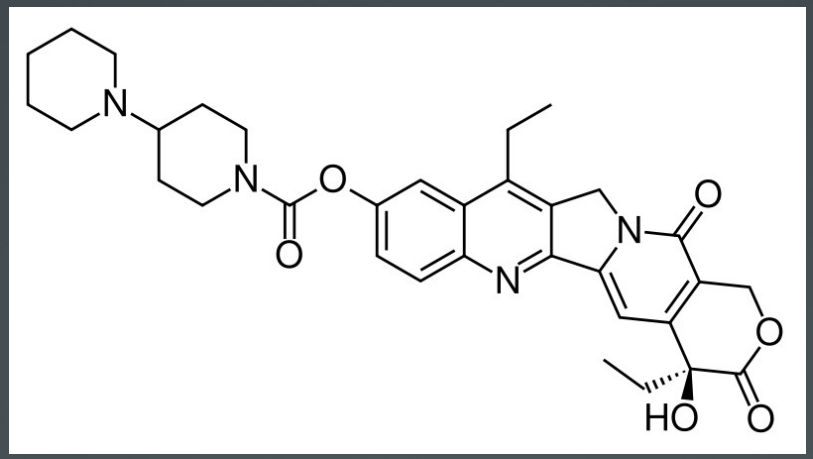
Clinically approved Camptothecin Derivatives

Topotecan (Hycamptyn)



Ovarian Carcinoma

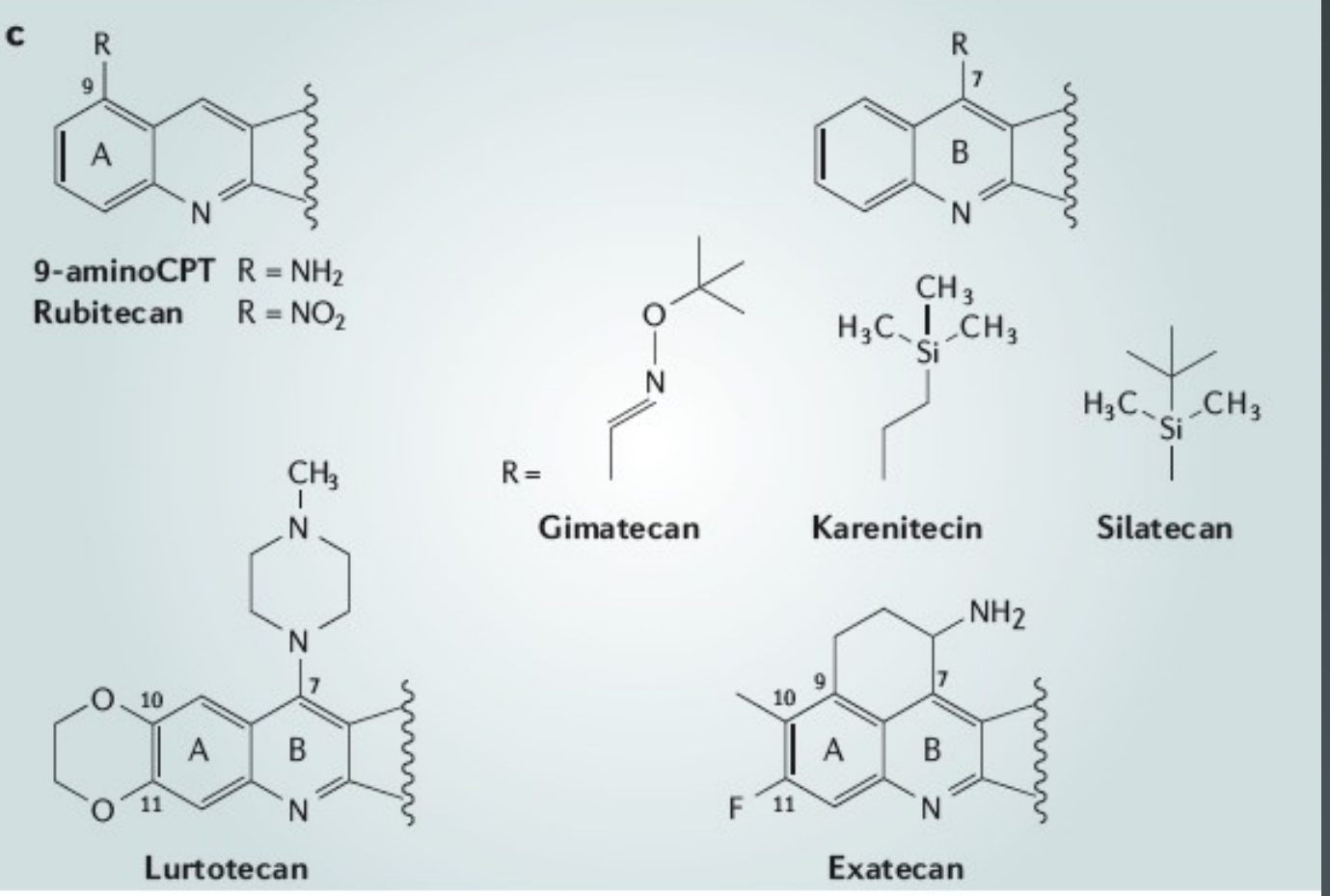
Irinotecan (CPT-11)

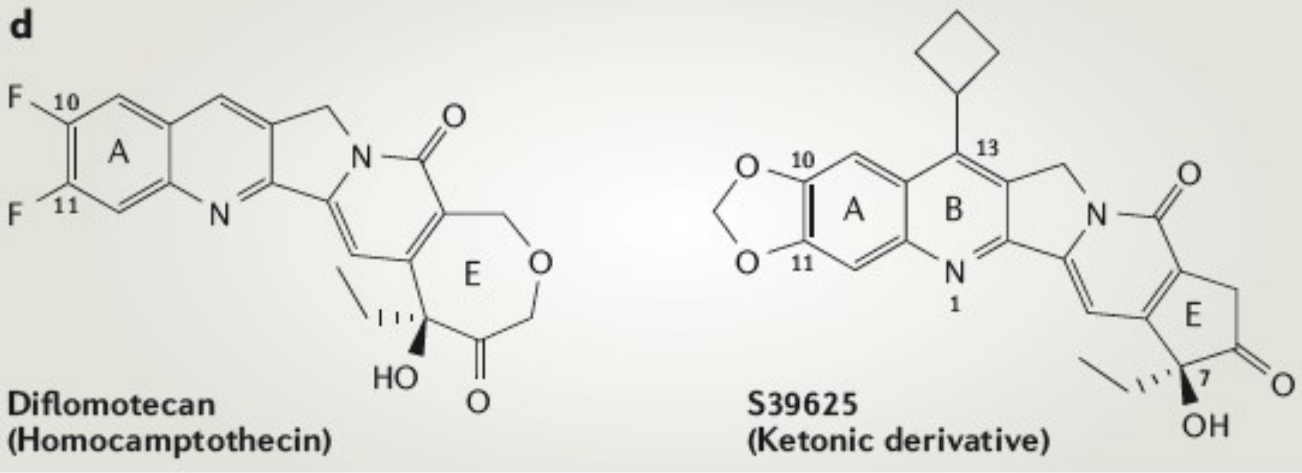


Metastatic colo-rectal cancer

Renal expulsion

glucuronidation


c
R
9
A
N
9-aminoCPT R = NH2
Rubitecan R = NO2
R
7
B
N
R =
O
N
CH3
H3C
Si
CH3
H3C
Si
CH3
Gimatecan
Karenitecin
Silatecan
CH3
N
N
O
10
7
A
B
O
11
N
Lurtotecan
NH2
10
9
7
A
B
F
11
N
Exatecan

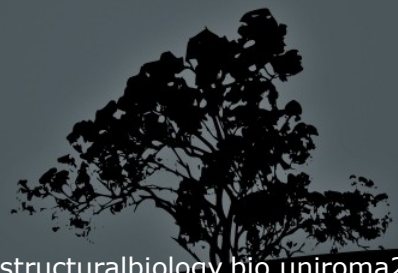

d
F
10
A
F
11
N
N
O
E
O
HO
O
Diflomotecan
(Homocamptothecin)
O
10
13
A
B
O
11
N
1
N
O
E
7
OH
O
S39625
(Ketonic derivative)

ADVANTAGES

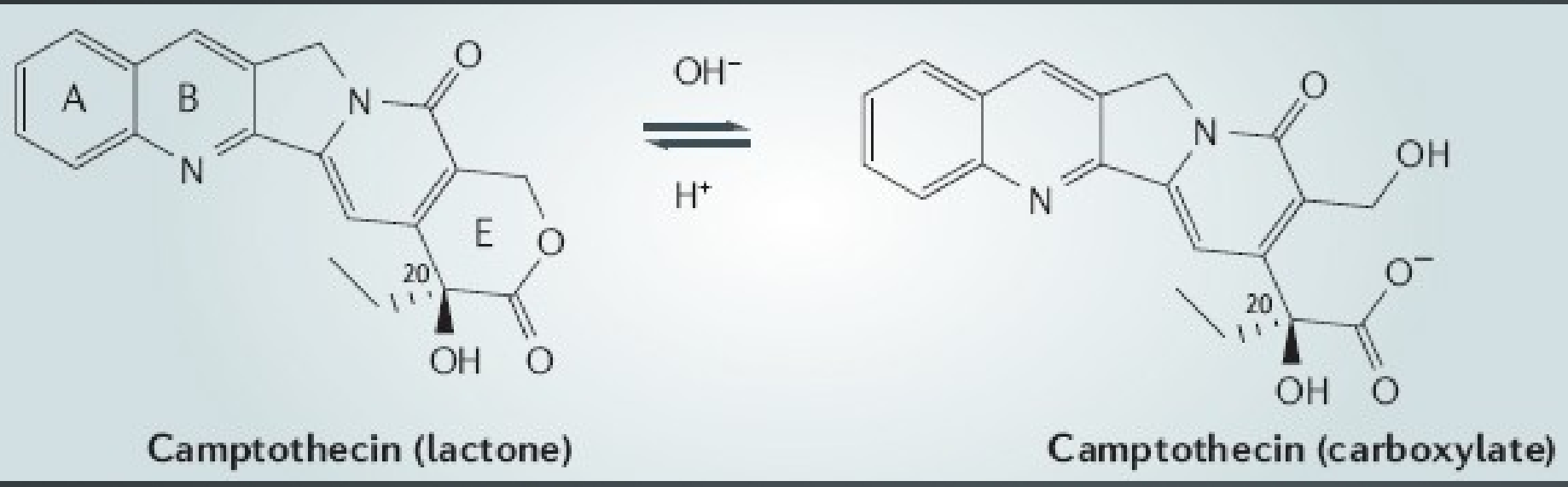
TOP1ccs is the only target

Camptothecins penetrate vertebrate cells readily and target TOP1 within minutes of exposure.

CPTand its derivatives have a relatively low affinity for TOP1ccs, micromolar drug concentrations are required to detectably trap TOP1ccs which indicates that camptothecin was naturally selected for on the basis of its selectivity rather than its potency.



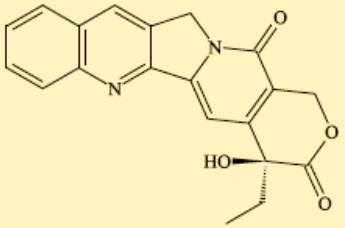
MAIN LIMITATIONS



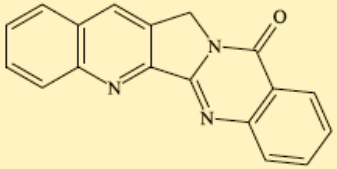
The α-hydroxylactone E-ring of camptothecins is readily converted into a carboxylate which is inactive against TOP1 and binds tightly to serum albumin

Accumulation of CPT induces side effects

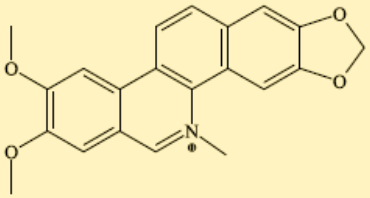
Camptothecin



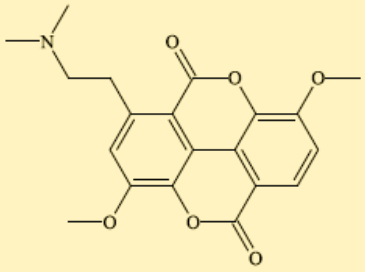
Luothonin



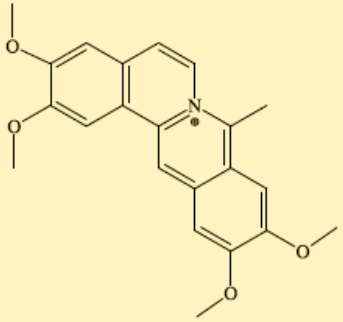
Nitidine



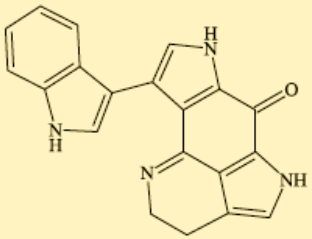
Thaspine



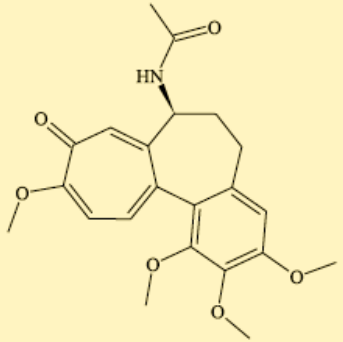
Coralyne



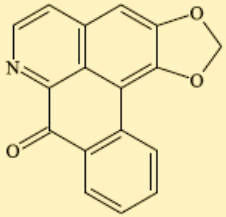
Wakayin



Colchicine



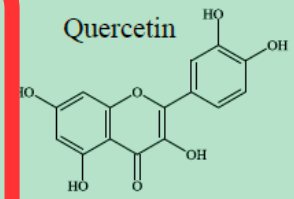
Liriodenin



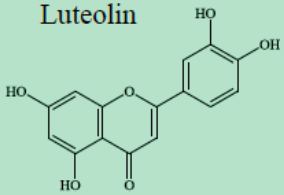
Alkaloids

Flavones

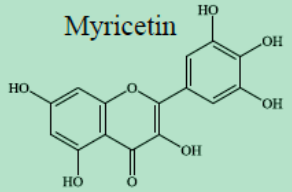
Quercetin



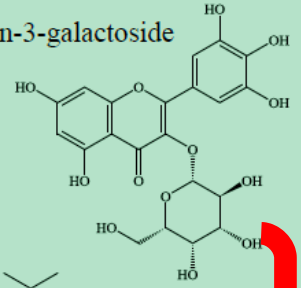
Luteolin



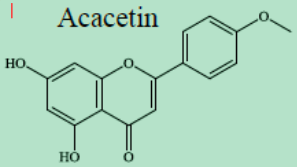
Myricetin



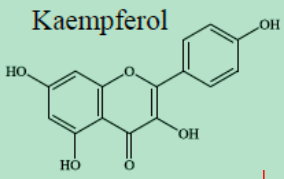
Myricetin-3-galactoside



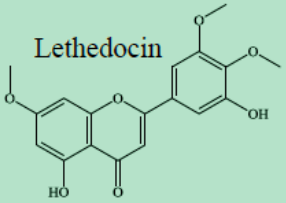
Acacetin



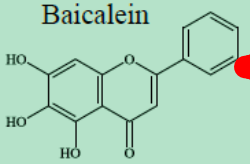
Kaempferol



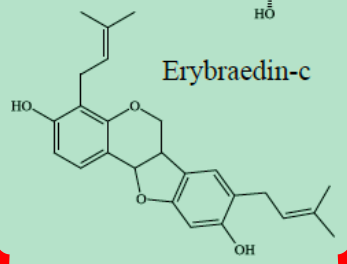
Lethedocin

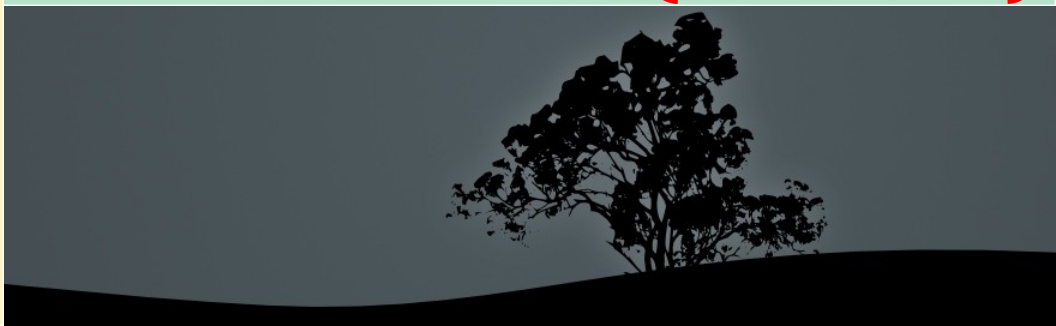


Baicalein

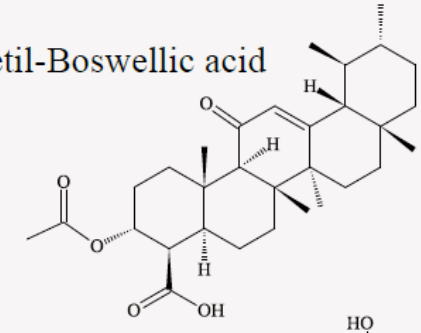


Erybraedin-c

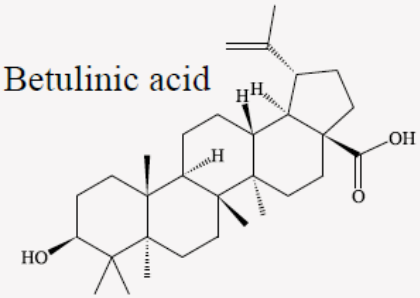




Acetil-Boswellic acid



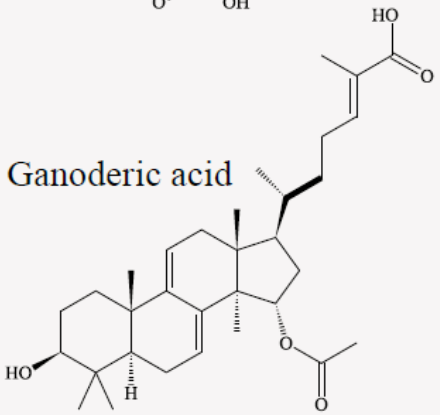
Betulinic acid



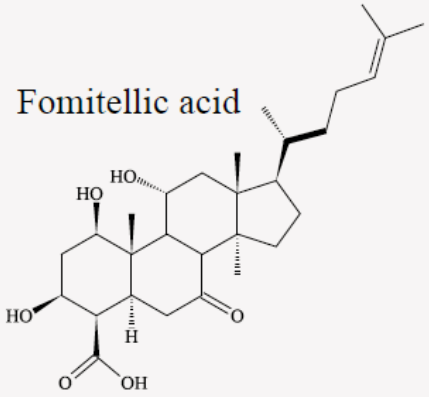
Triterpens

Quinones

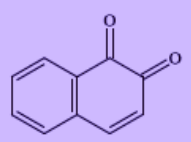
Ganoderic acid



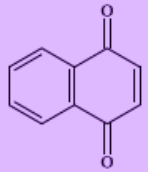
Fomitellic acid



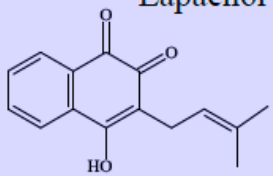
1,2-Naphtoquinone



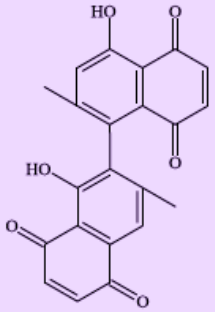
1,4-Naphtoquinone



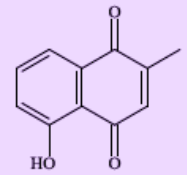
Lapachol



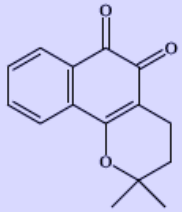
Isodiospyrin



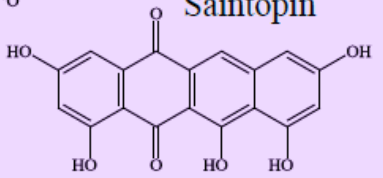
Plumbagin



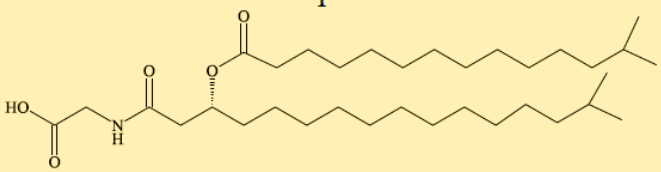
β-Lapachone



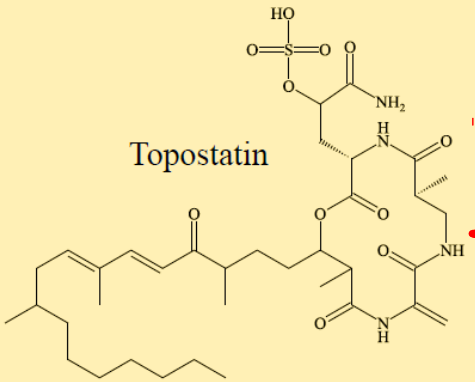
Saintopin



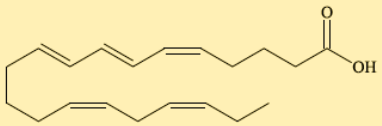
Topostin



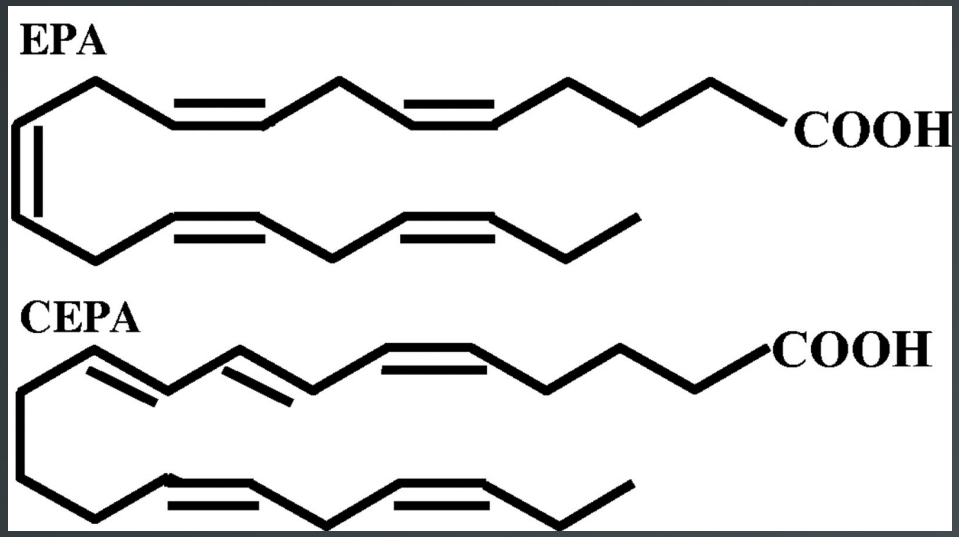
Topostatin



Conjugated eicosapentanoic acid



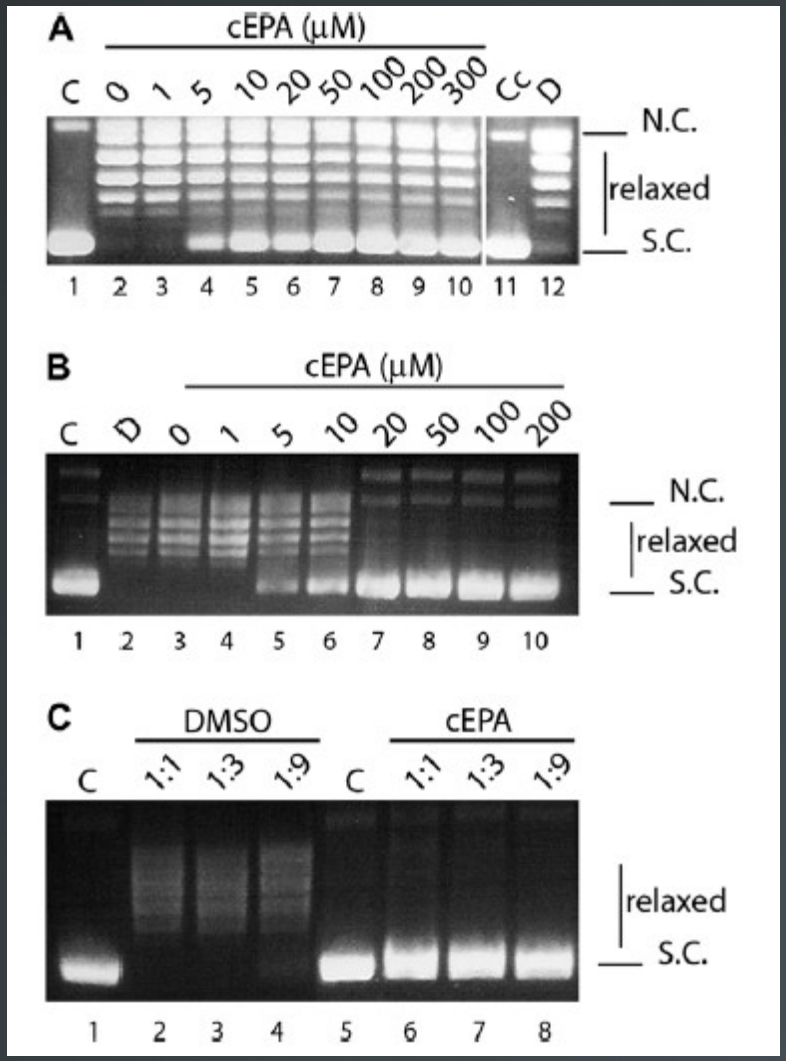
Fatty acids

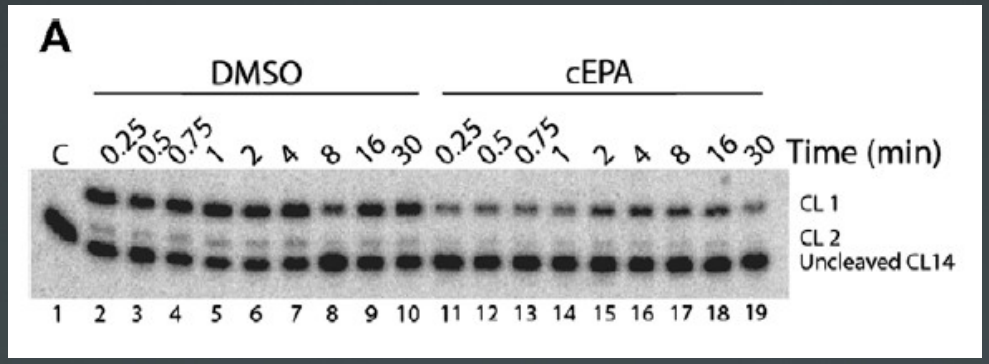


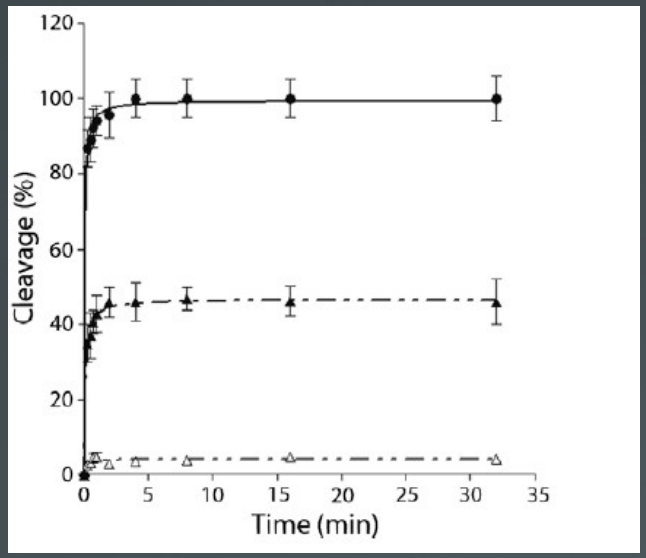
cEPA

- cEPA, conjugated eicosapentaenoic acid

  is found in seaweeds such as red and green algae
- It was found to have an inhibitory effect on human cancer cells, inducing cell apoptosis through both p53-dependent and p53-independent pathways in cell lines NALM-6 and HL-60 (human leukemia).
- Int. J. Oncol., 30 (2007), pp. 1197–1204

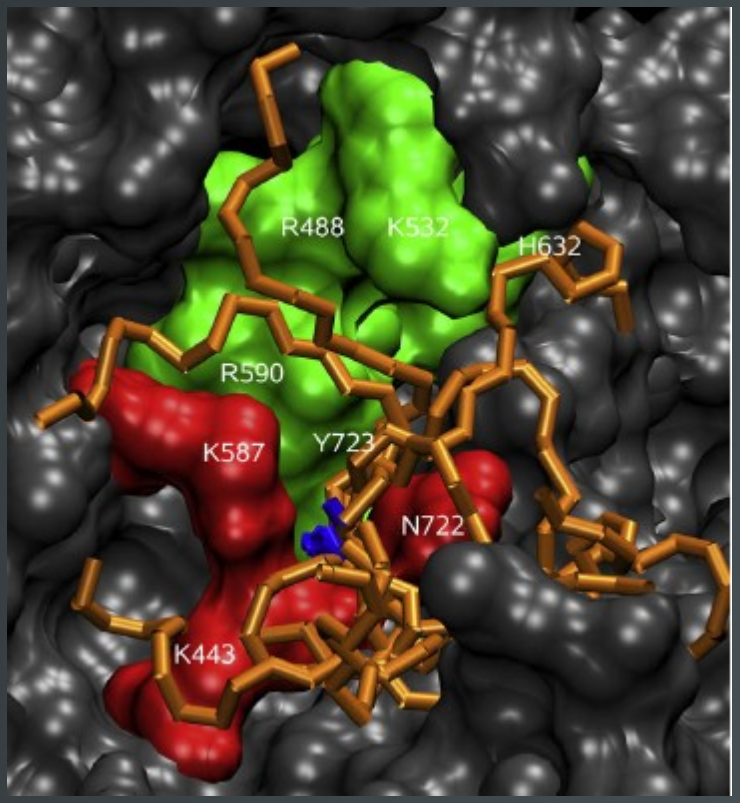


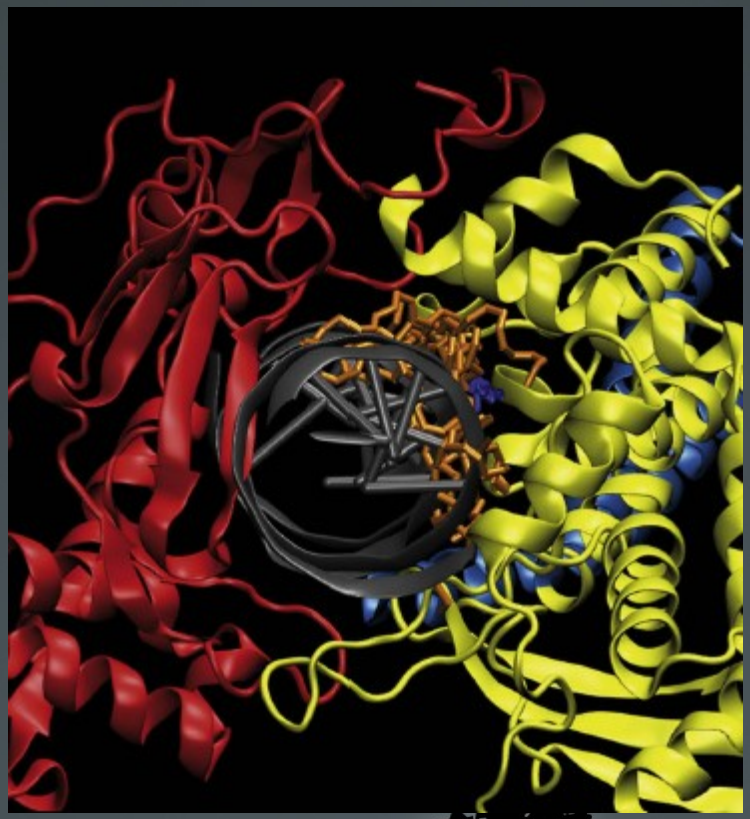




Inhibits relaxation, completely and irreversibly with preincubation, inhibits cleavage, but not binding nor religation.

cEPA Docking on protein and binary complex





Arch Biochem Biophys. 2009 486(2):103

BJ www.biochemj.org

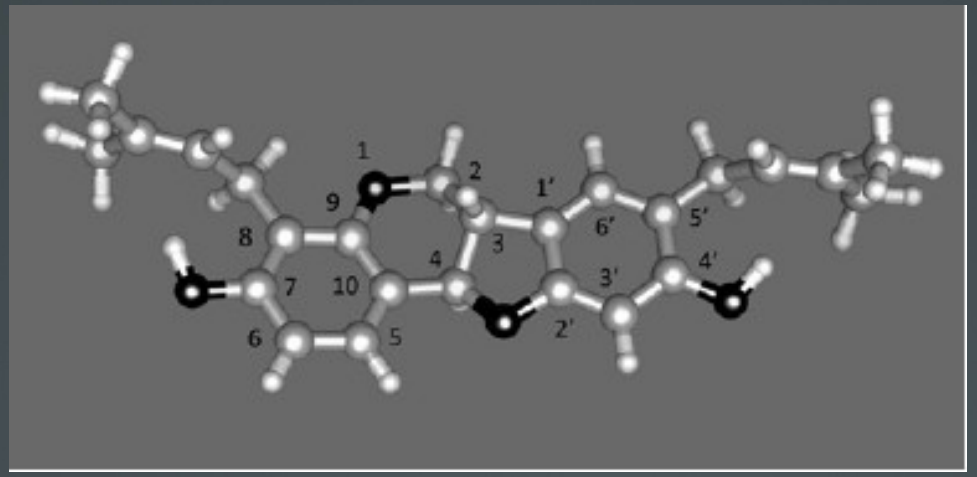
Biochem. J. (2010) **425**, 531–539 (Printed in Great Britain) doi:10.1042/BJ20091127

online data

531

Erybraedin C, a natural compound from the plant *Bituminaria bituminosa*, inhibits both the cleavage and religation activities of human topoisomerase I

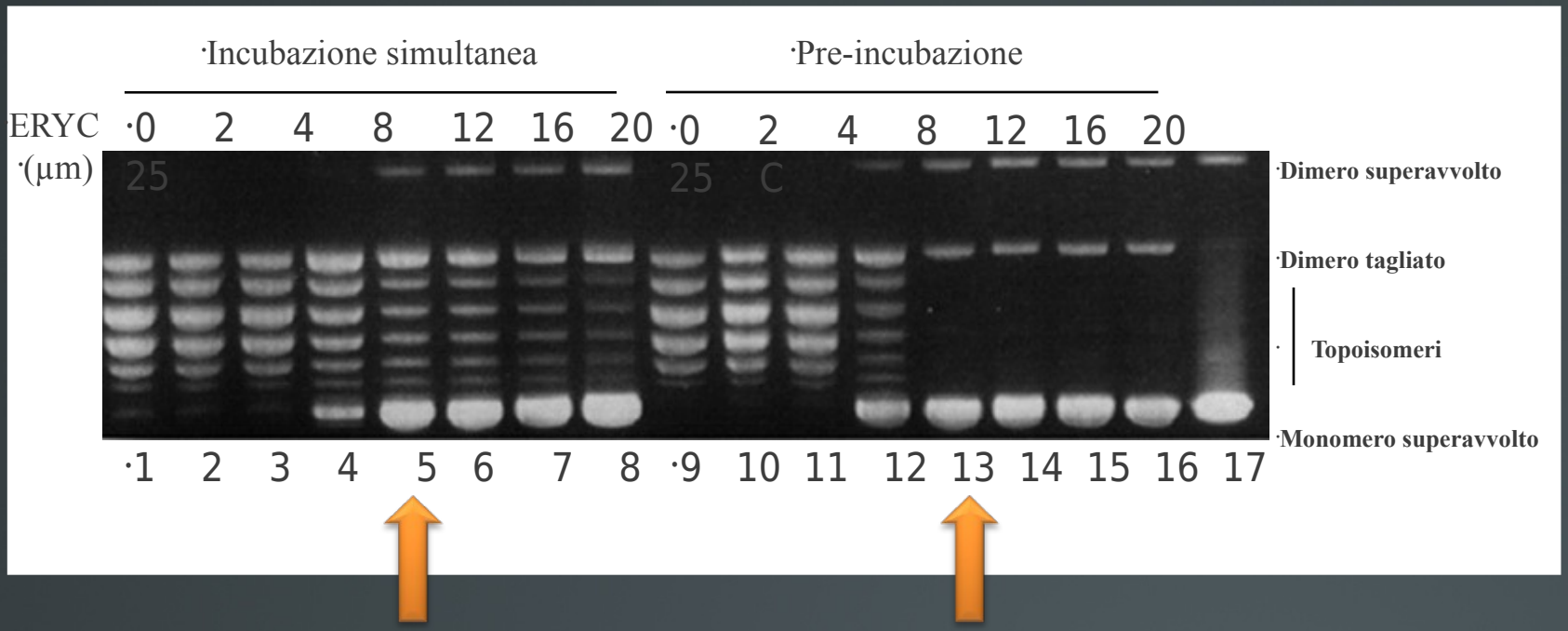
Cinzia TESAURO*, Paola FIORANI*, Ilda D'ANNESSA†, Giovanni CHILLEMI†, Gino TURCHI‡ and Alessandro DESIDERI*[1]



The pterocarpan Erybraedin C (ERYC) from *Bituminaria bituminosa*

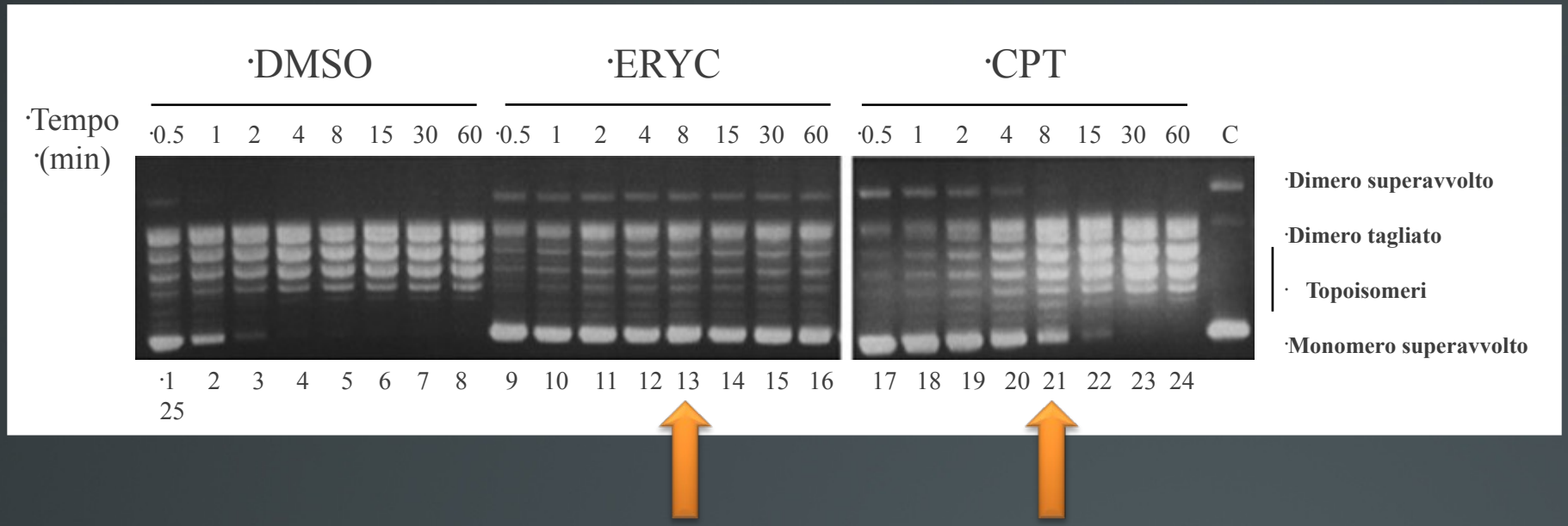
It contains a tetracyclic ring system characterized by the presence of two hydroxy groups, located, respectively, on the 7 and 4 position, and two prenyl groups (*γ ,γ -dimethylallyl)* on the 8 and 5 position.

Relaxation assay with ERYC



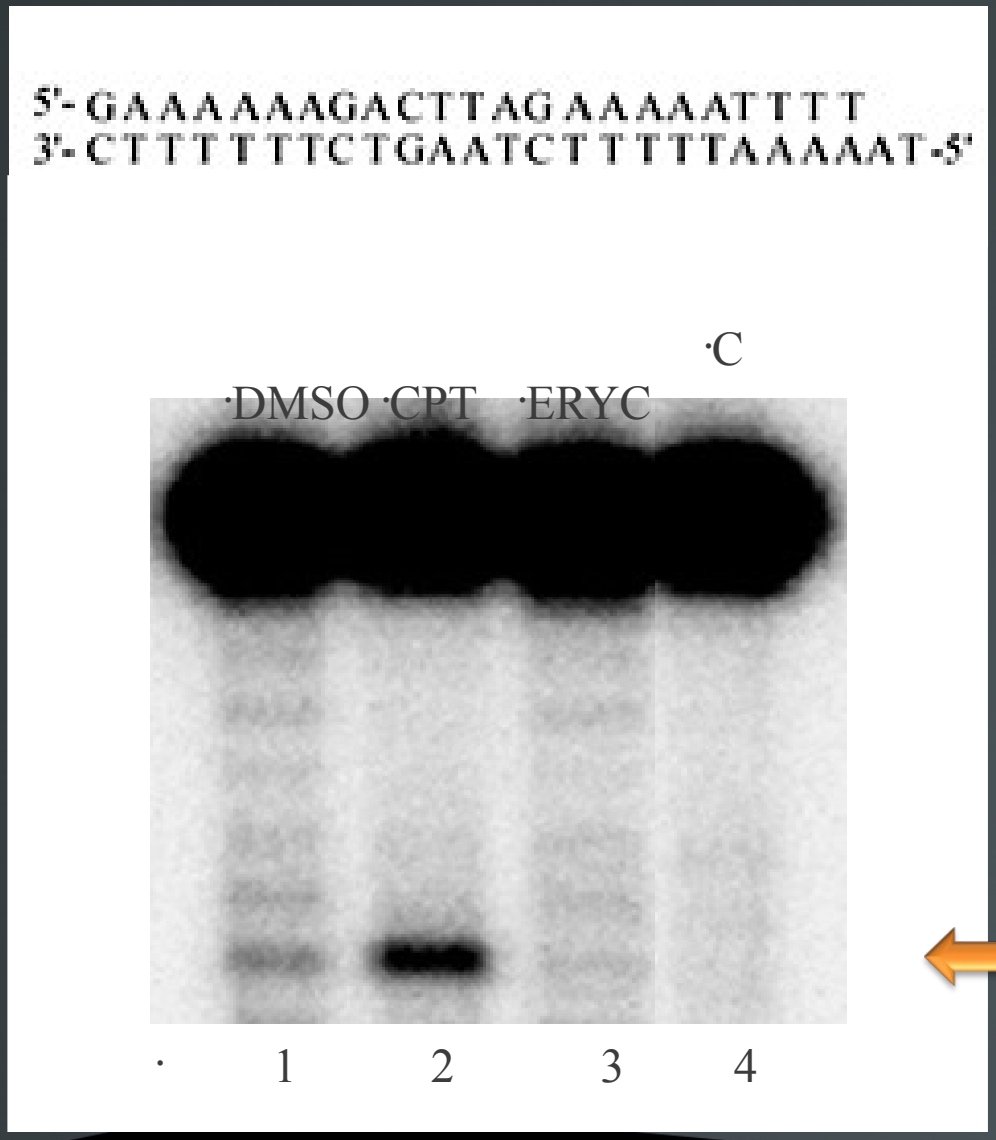
ERYC inhibits topoisomerase I relaxation activity in a dose dependant manner and is enhanced by pre-incubation with the enzyme

Relaxation kinetics with ERYC



ERYC is an irreversible inhibitor of topoisomerase I

“Cleavage-religation” equilibrium



·ERYC does not stabilize the covalent complex

Three cases:

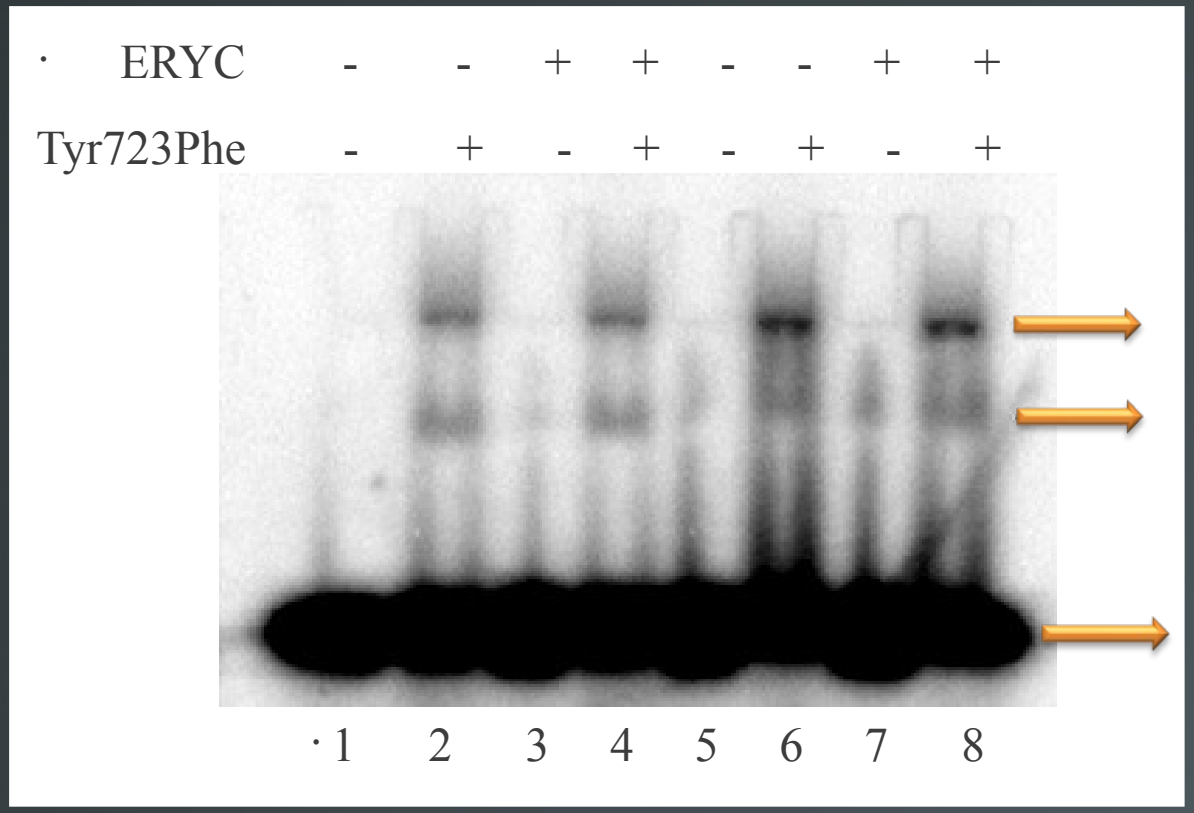
“binding” inhibition

“cleavage”inhibition

Acceleration of “religation”

Covalent complex

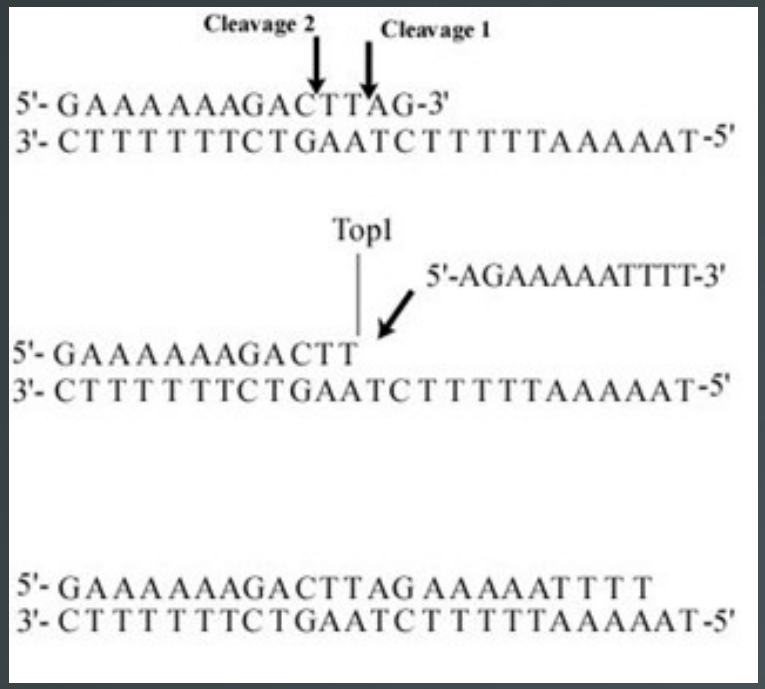
Electrophoretic mobility-shift assay (EMSA)



- Complex 1 Topo I-DNA
- Complex 2 Topo I-DNA
- DNA

ERYC does not inhibit the binding of topoisomerase I to DNA

Religation kinetics with ERYC



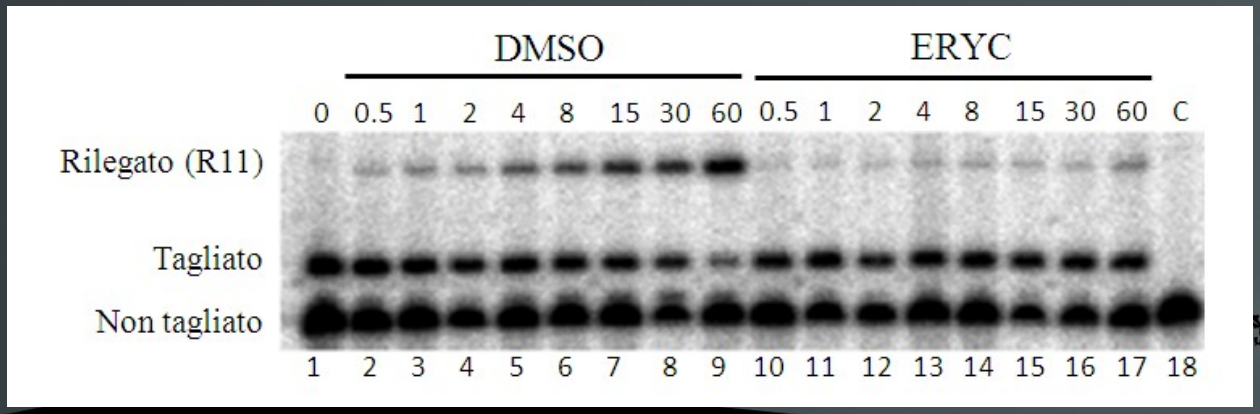
Suicide Substrate

"Cleavage"

ERYC

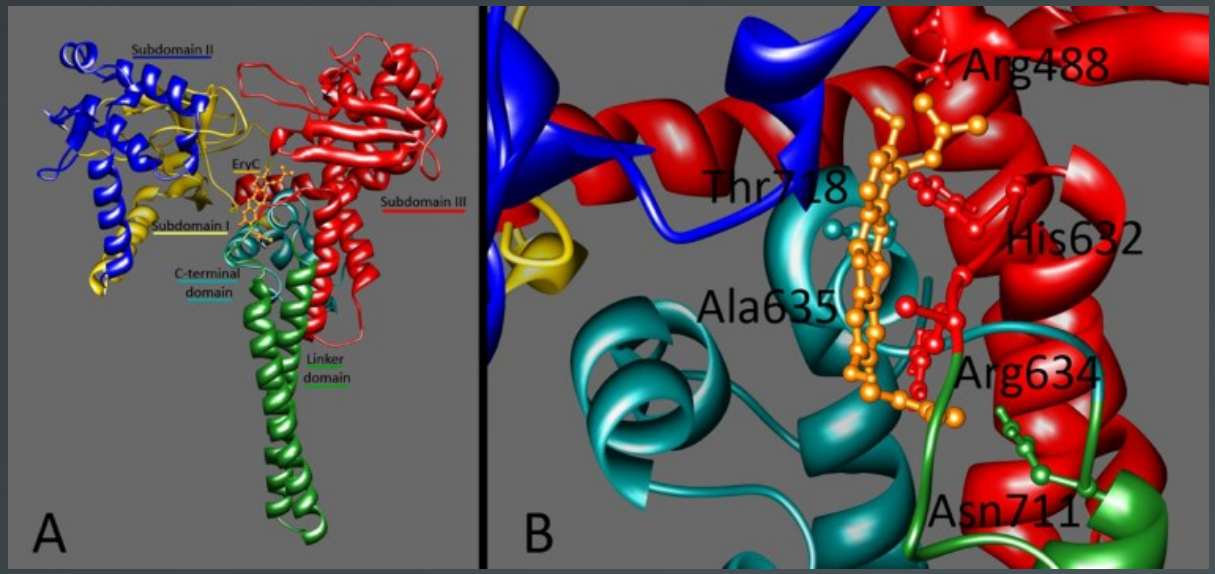
Oligonucleotide R11

"Religation"



ERYC inhibits "religation"

Molecular Docking



(Dott.ssa Ilda D'Annessa)

Prenyl group in position 8 interacts with Arg488 and His 632

The compound bind both the free protein and the binary complex topoisomerase I- DNA

EryC summary

ERYC acts with a different mechanism from CPT and can be classified as a catalytic inhibitor of topoisomerasi I

Blocks both “cleavage” and “religation” steps

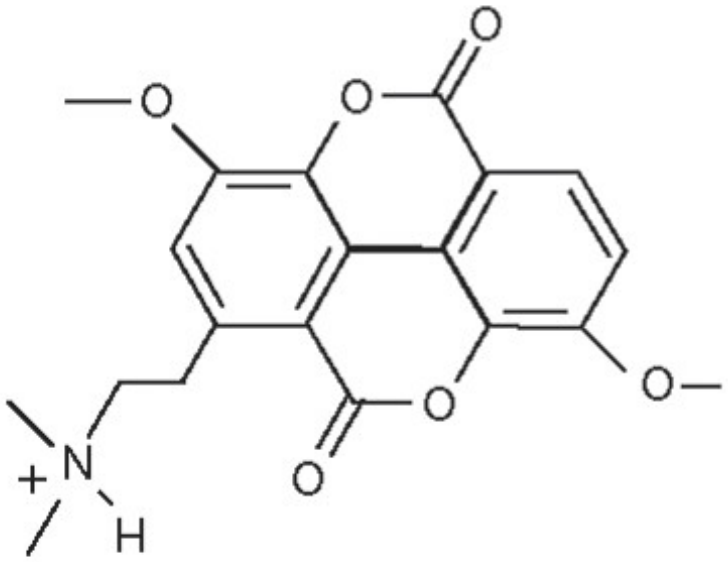
No effect on DNA binding

Anti-Cancer Agents in Medicinal Chemistry, 2013, *13*, 000-000

A Natural Anticancer Agent Thaspine Targets Human Topoisomerase IB

Silvia Castelli[1], Prafulla Katkar[1], Oscar Vassallo[1], Mattia Falconi[1], Stig Linder[3] and Alessandro Desideri[1,2,*]

THASPINE (NSC76022)

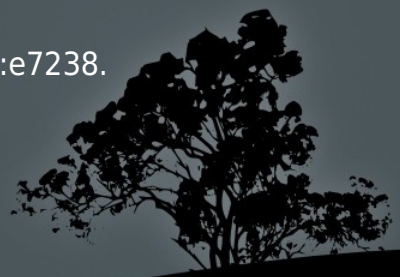


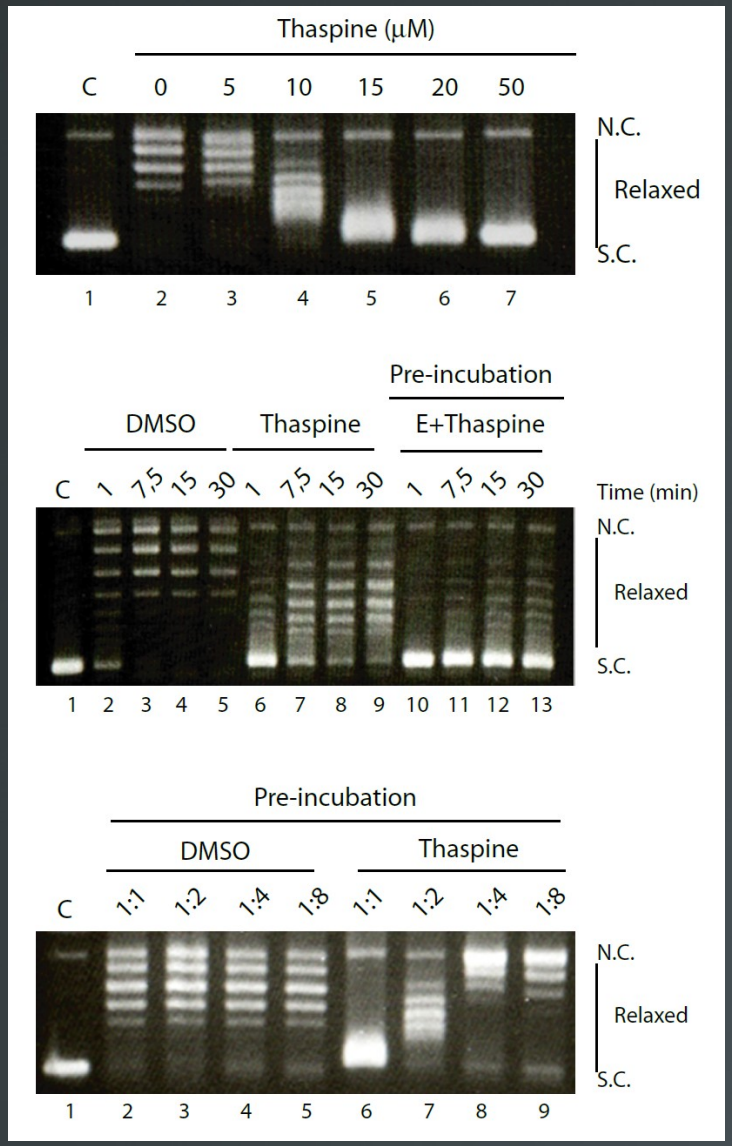
An alkaloid from the South American tree *Croton lechleri*.

Thaspine was found to induce conformational activation of the pro-apoptotic proteins Bak and Bax, mitochondrial cytochrome c release and mitochondrial membrane permeabilization in HCT116 cells.

The gene expression signature of thaspine is similar to that shown by camptothecin

PLoS One. 2009 Oct 2;4(10):e7238.

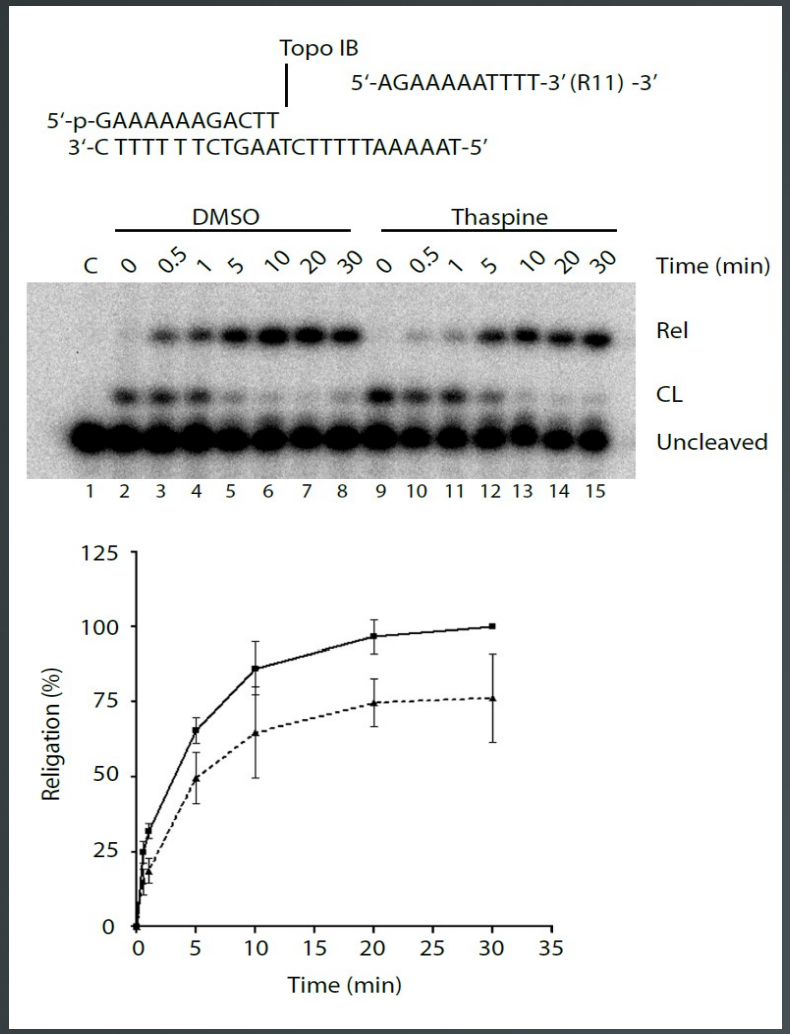




thaspine inhibits topoisomerase I activity in a dose-dependent manner

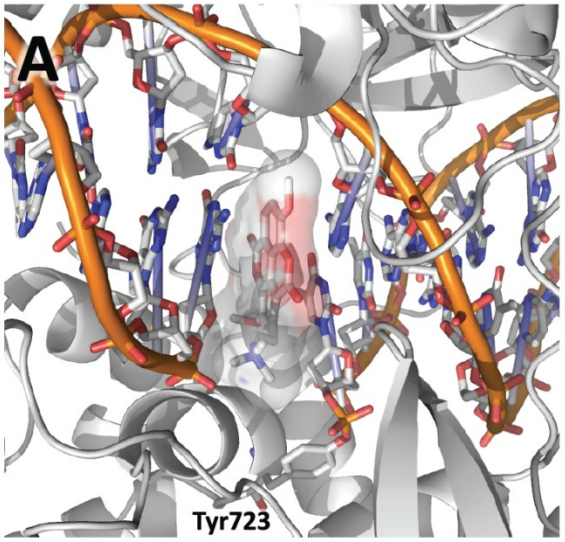
Pre-incubation increases inhibition

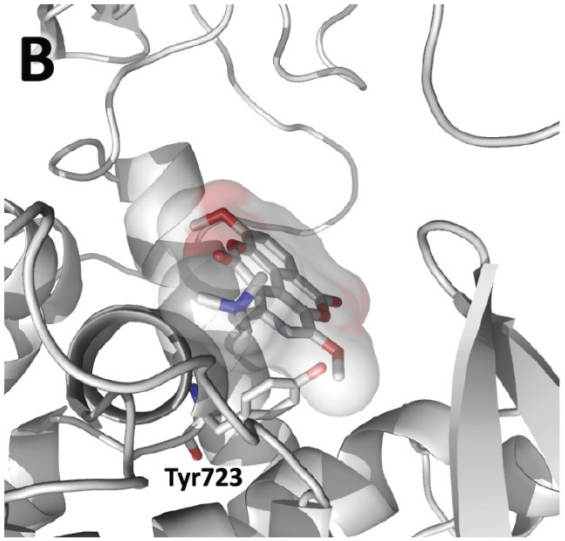
Inhibition is reversible



Inhibition of the cleavage

Inhibition of the religation





In the presence of the DNA duplex, thaspine docks in the DNA nick with a good interaction energy that explains the ability of the drug to inhibit the reaction of religation

In the absence of DNA duplex in all the docking runs thaspine is flattened over the active site surface, in the proximity of Tyr723 explaining the ability of the drug to inhibit the cleavage reaction

CLOSED STATE

Thaspine conclusion

Thaspine targets human topoisomerase IB

It acts as a poison but also as a catalytic inhibitor

Chemical modifications of the thaspine molecule may confer specificity toward one of the two characteristics

Anticancer Agents Med Chem. 2013 Feb;13(2):356-63.

How can we improve the targeting of the drug, maximizing its effect and minimizing the side effects on healthy tissue or on accumulation sites?

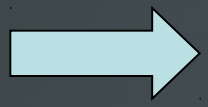
Camptothecin loaded Chitosan-Folate Microcapsules specifically target HeLa tumor cells

PVA=Poly(vinyl alcohol)

Is water soluble

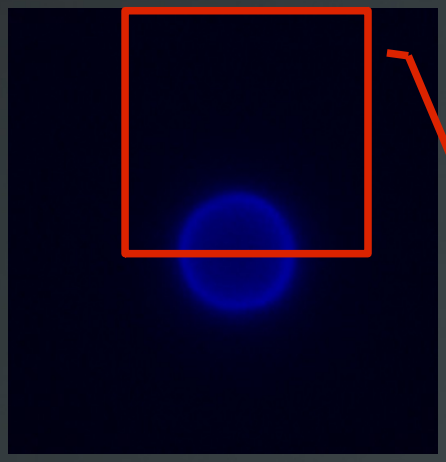
Biocompatible

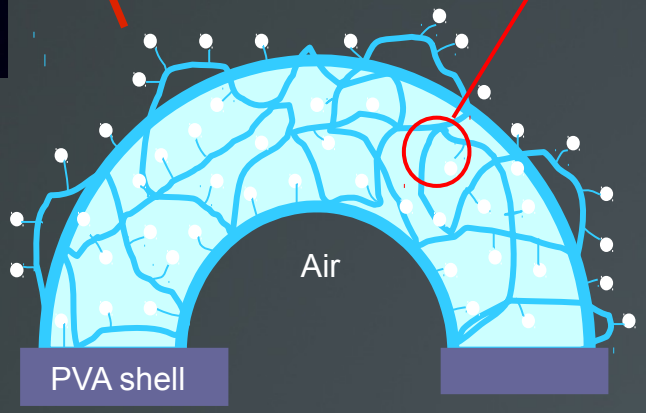
Injectable



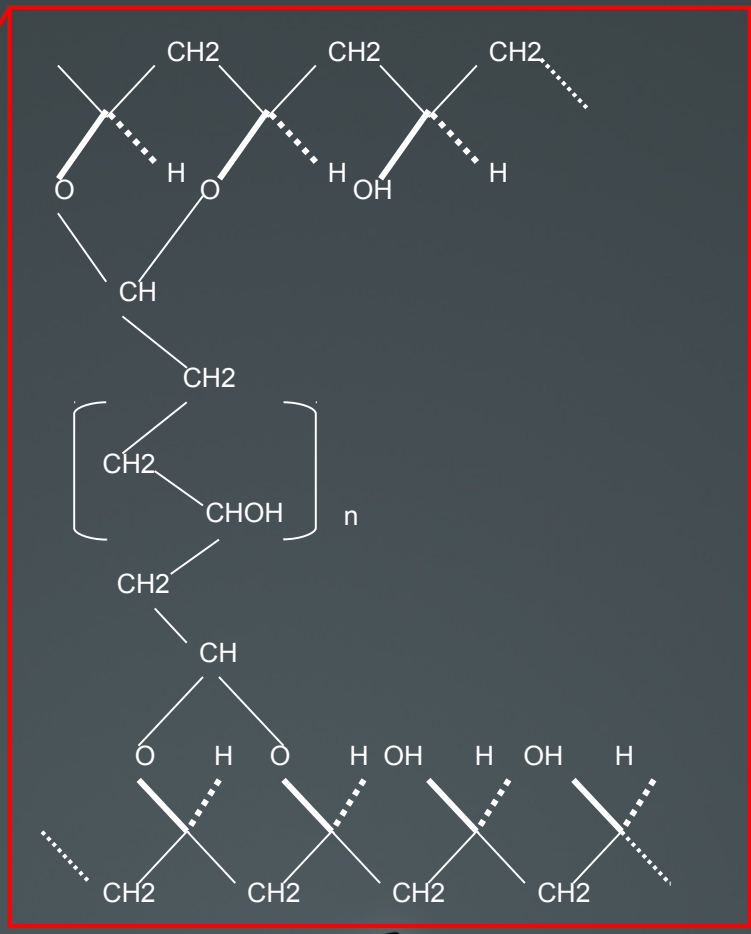
It can be used for biomedical applications and to design new micro-(nano) structured devices

- **Microballoons**
- **Hydrogel microparticles**
- **Films**

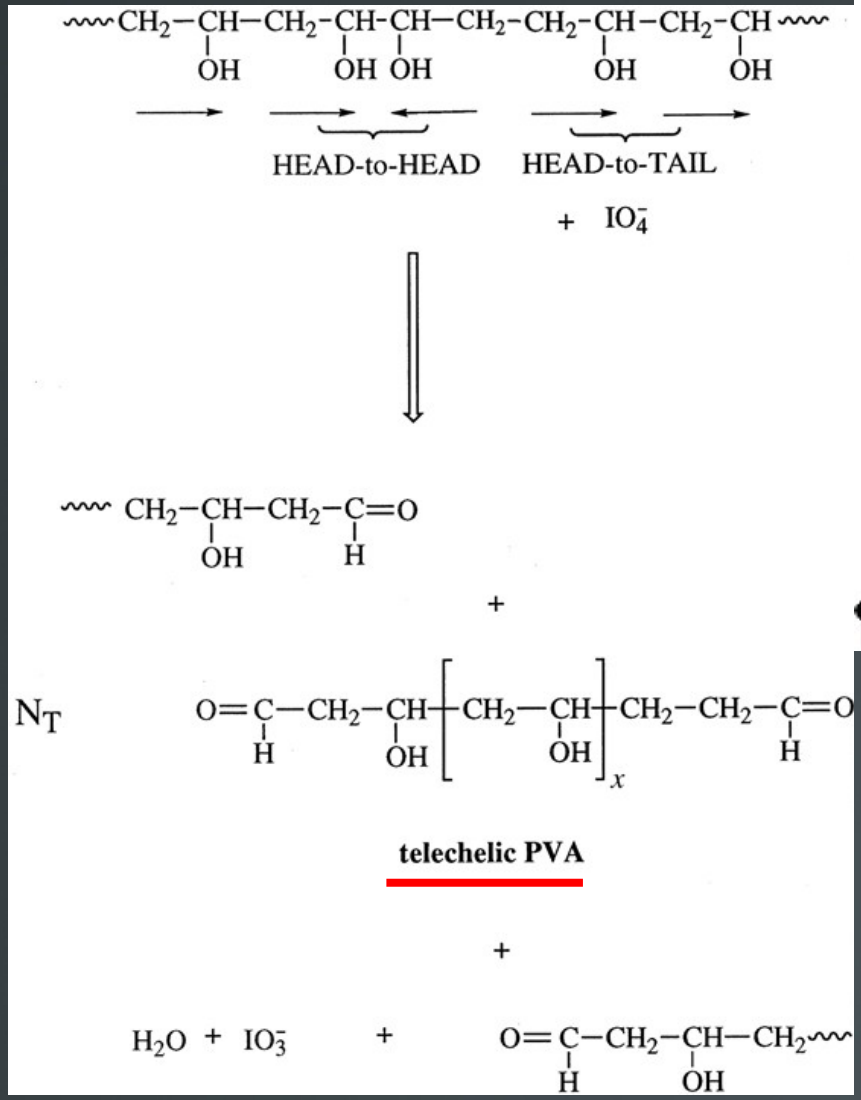


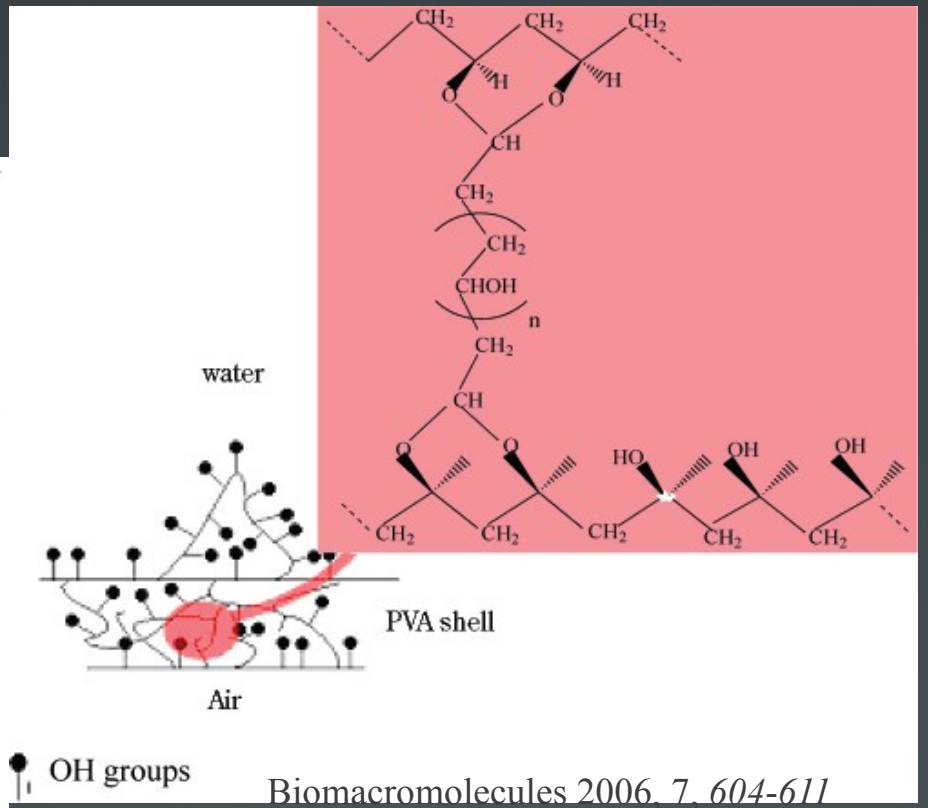

Water
Air
PVA shell

OH groups


CH2
CH2
CH2
H
H
H
O
O
OH
CH
CH2
CH2
CHOH
n
CH2
CH
O
H
O
H
OH
H
OH
H
CH2
CH2
CH2
CH2

Atactic commercial PVA





Biomacromolecules 2006, 7, 604-611

Diameter 4.2μm

Shell thickness 0.9μm

Z-potential -4.7±0.6mV

MB → MC

EtOH

STRATEGY

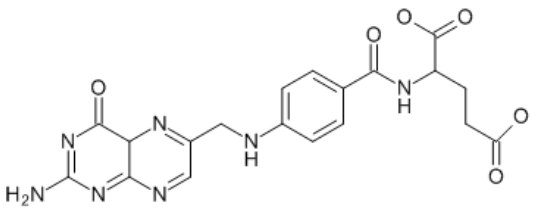
Exploit the structure of the microcapsules to target camptothecin toward tumor cells. PVA is the “sponge” for CPT

Exploit the Folic Acid receptor overexpression profile of a number of tumors. Folic acid is the key recognition element

Use a spacer arm of chitosan, in order to maintain the flexibility needed to interact with the receptor

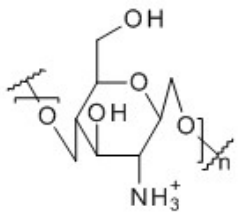
Test on two cell lines: immortalized NIH3T3 fibroblasts, not expressing folic acid receptor, and Hela cells of cervical cancer who have high folic acid receptor levels.

Chemical synthesis



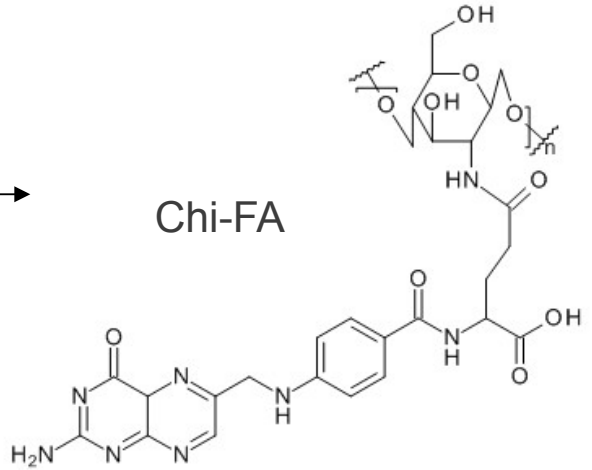
Folic acid

+



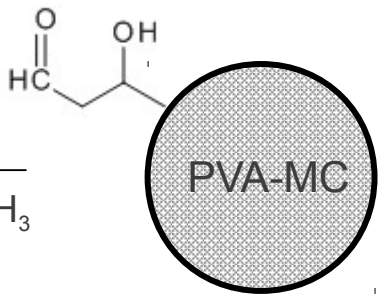
Chitosan

EDC
DMSO



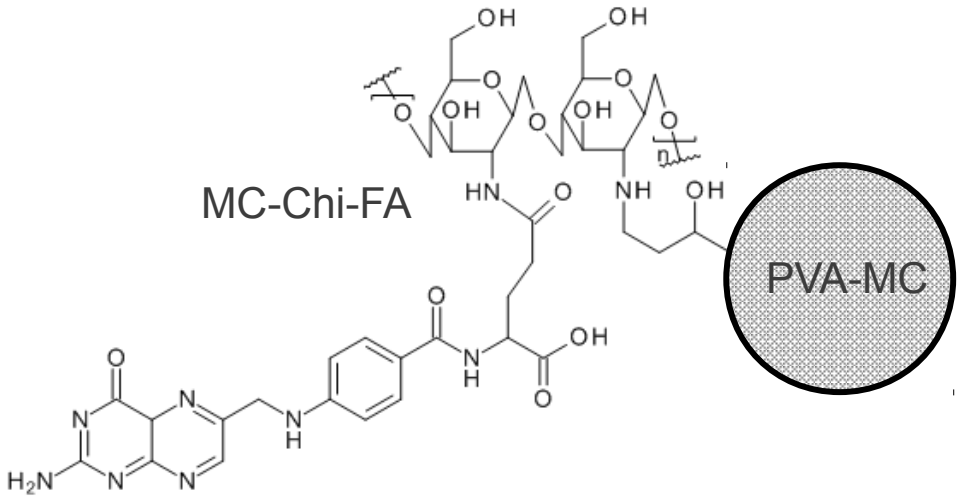
Chi-FA

+



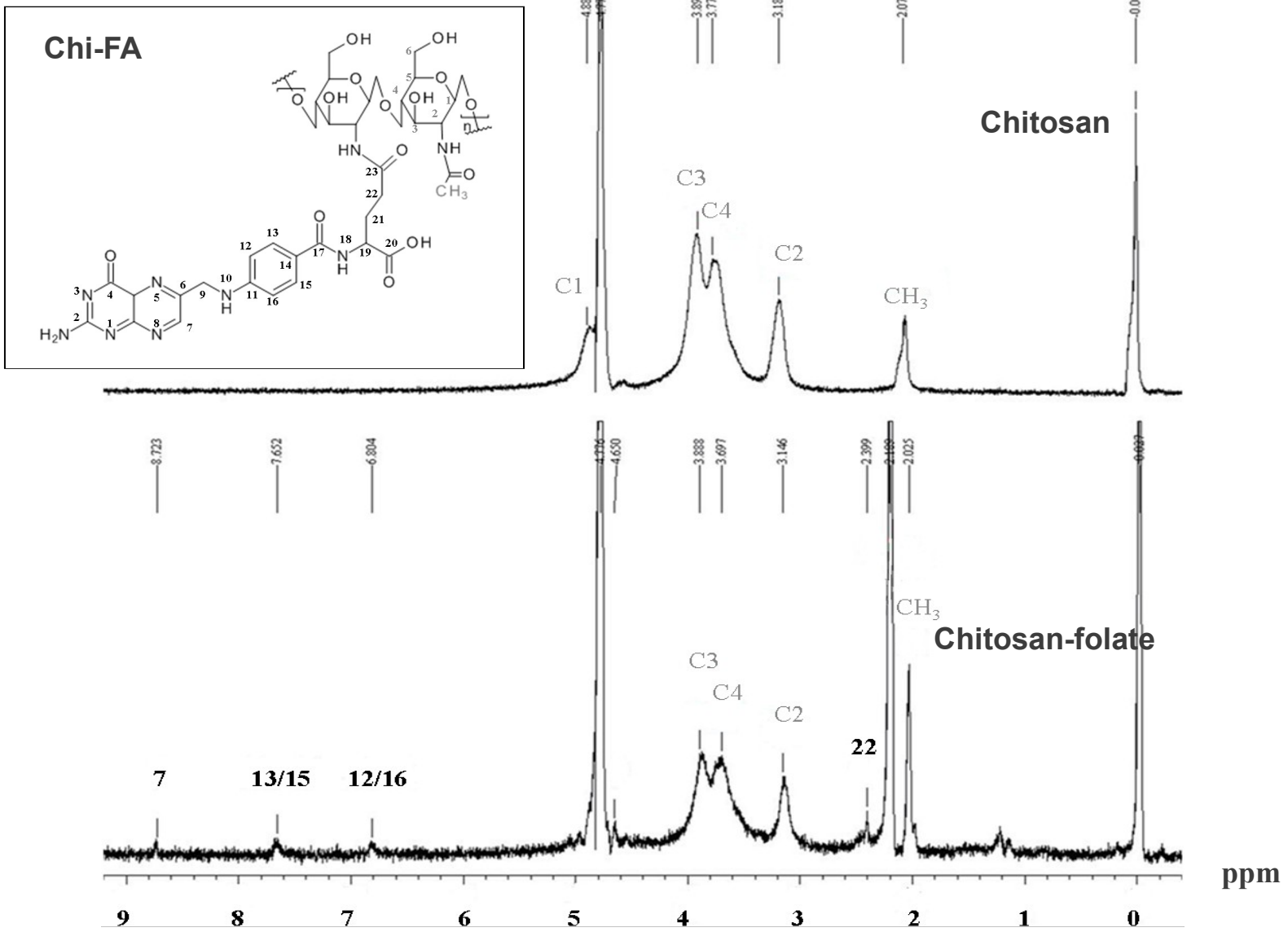
PVA-MC

$Na(CN)BH_3$
water



MC-Chi-FA

PVA-MC



H-NMR spectra in D2O 3%CD3COOD

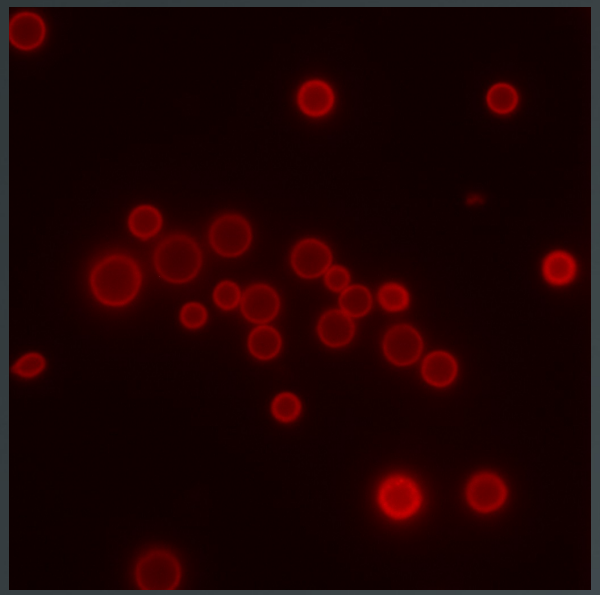
Chitosan: C3, C4, C2 carbon atoms of the sugar and to the methyl protons, falling at 3.90, 3.77, 3.18 and 2.07 ppm respectively.

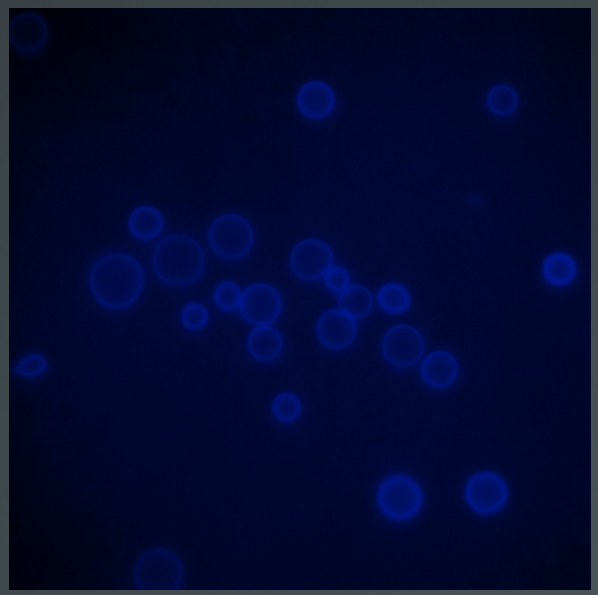
Chitosan-folate: additional peaks at 8.73, 7.66 and 6.83 ppm due to the folate aromatic rings and one at 2.40 ppm due to the protons linked to C22.

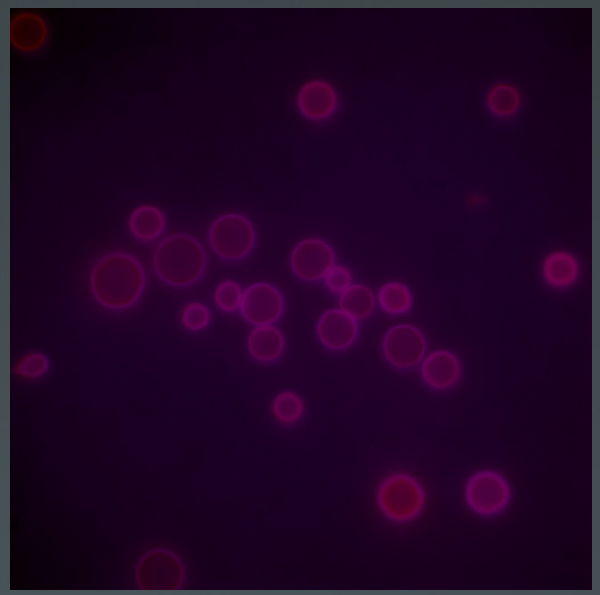
Chi-FA-Rhod

CPT

merge





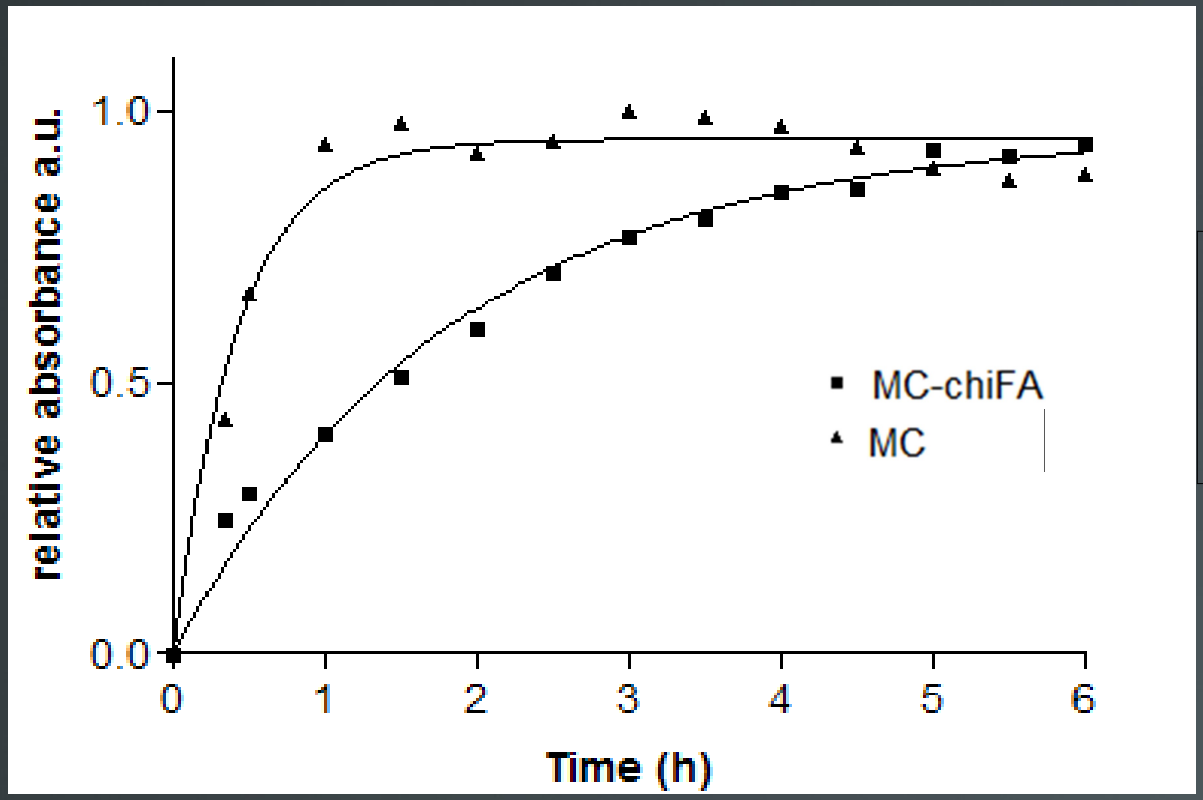


MC can be functionalized with the chitosan-folate and absorbed with CPT on PVA Shell.

The absorbed CPT is indirectly quantified following the spectrophotometer signal at λ=370nm in the external solution to the MC

Functionalization with Chi or Chi-FA allows the absorption of larger amounts of CPT (78% of the concentration of external solution 100um), compared to 50% absorbed by nude MC.

Release kinetics of CPT from MC and MC-Chi-FA in D-MEM at 37°C



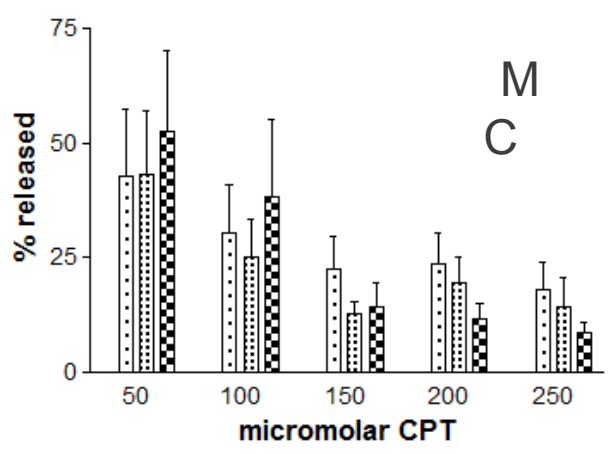
Rate of release

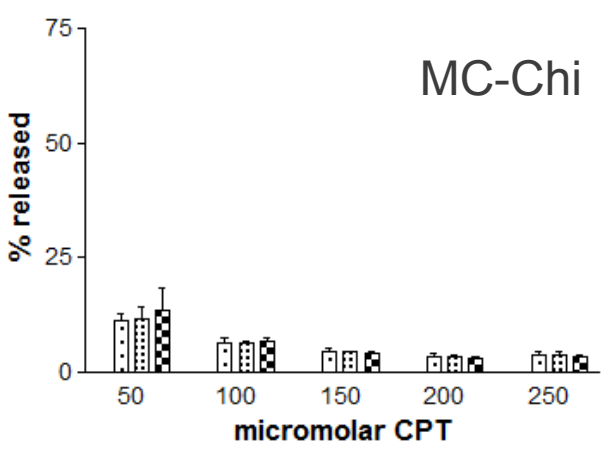
MC: 2.4 ± 0.2 h-1

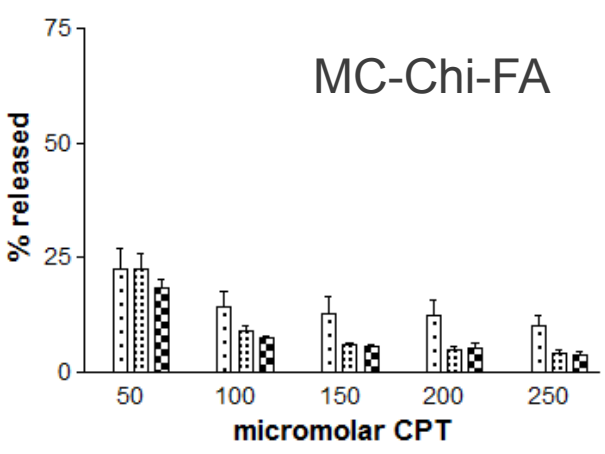
MC-Chi-FA: 0.52 ± 0.02 h-1

Data are reported as percentage of release normalized to the maximum of released drug. In both cases the maximum quantity of released drug corresponds to about 20-25% of the total adsorbed CPT.

CPT released in H2O RT,
changing the media every 3 days.

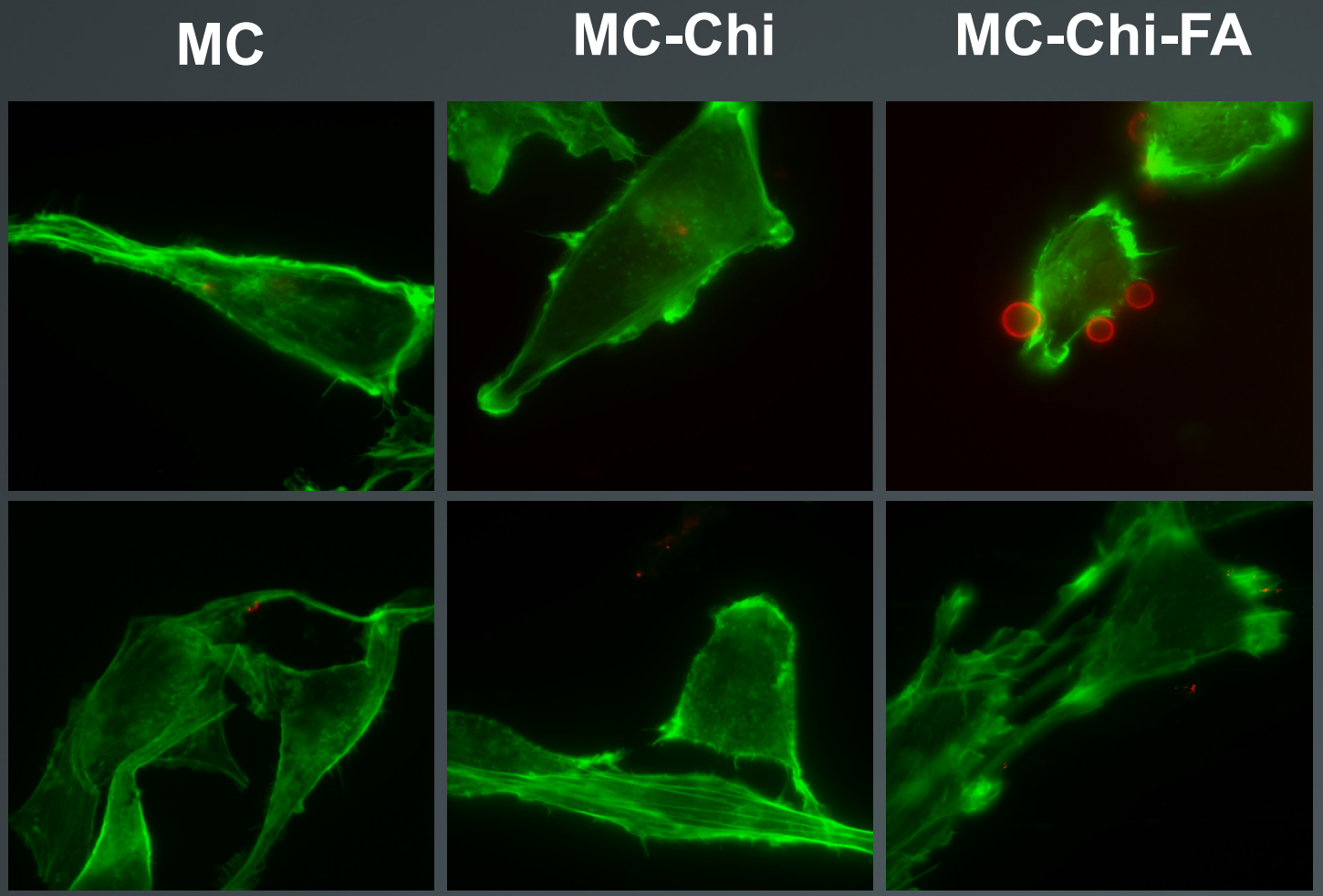






MC release more drug, and earlier than the functionalized capsules

The percentages are always calculated based on the amount of CPT that is still linked to the structure after the change of the medium



MC-Chi-FA selectively target HeLa tumour cell

$3*10^{-10}$moles Chi-FA/mg MC

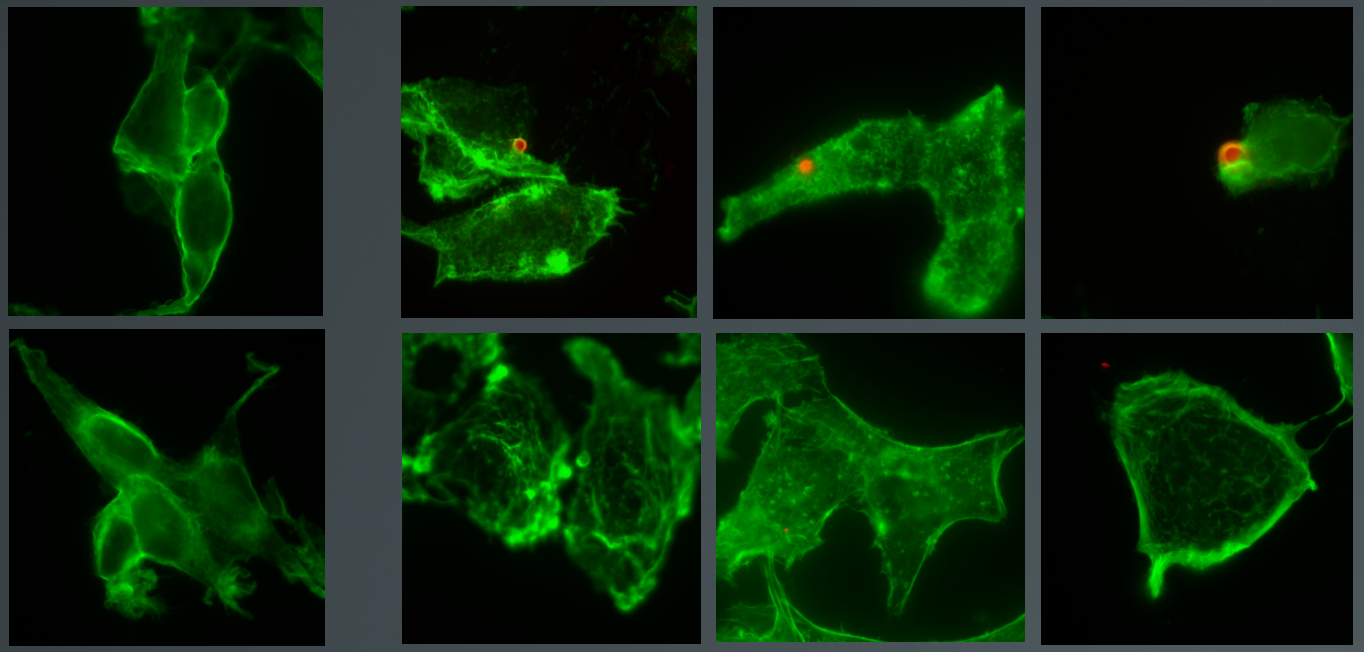
Green:Phalloidin-FITC

Red:MC-Chi-FA-Rhod

Competition experiments

with 4mg/L of folate excess in D-MEM

NIH3t3 HeLa



-FA

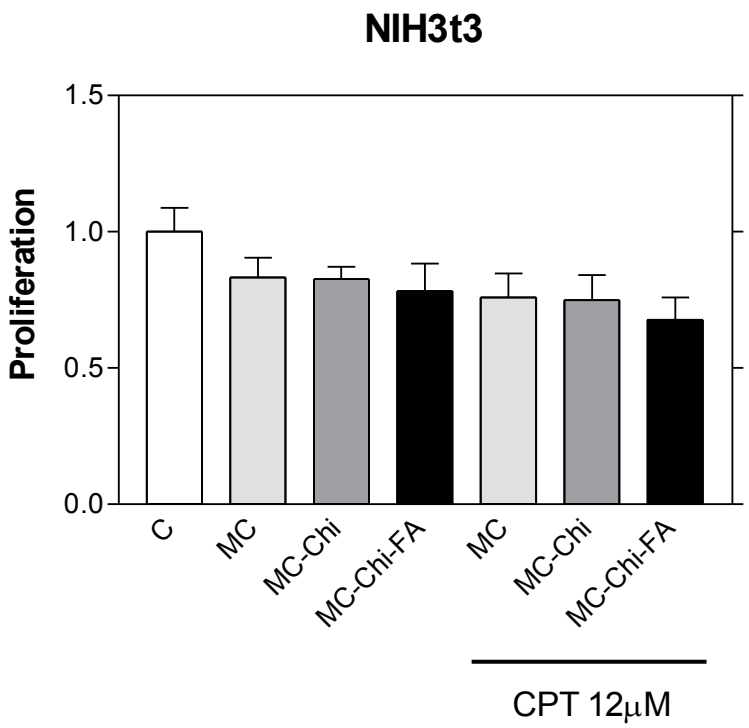
+FA

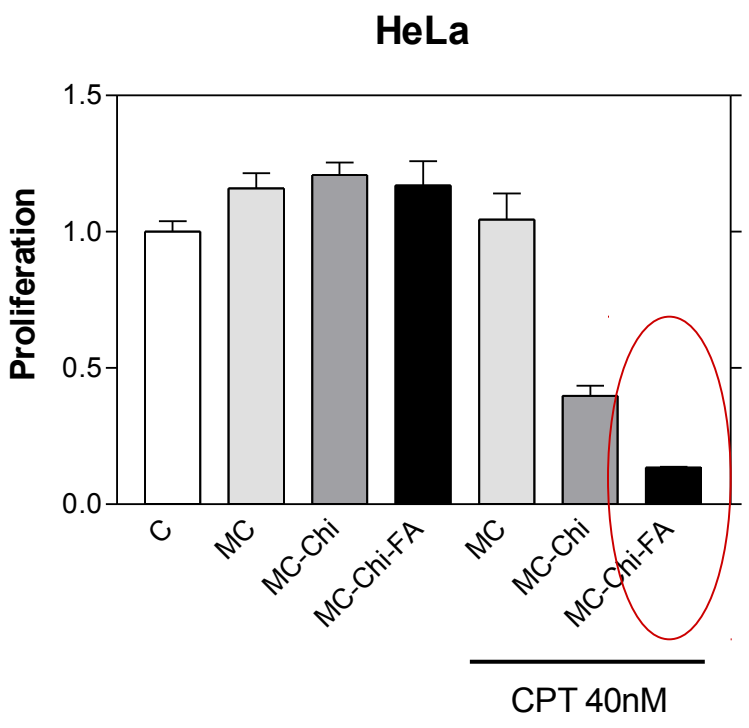
Green:Phalloidin-FITC

Red:MC-Chi-FA-Rhod

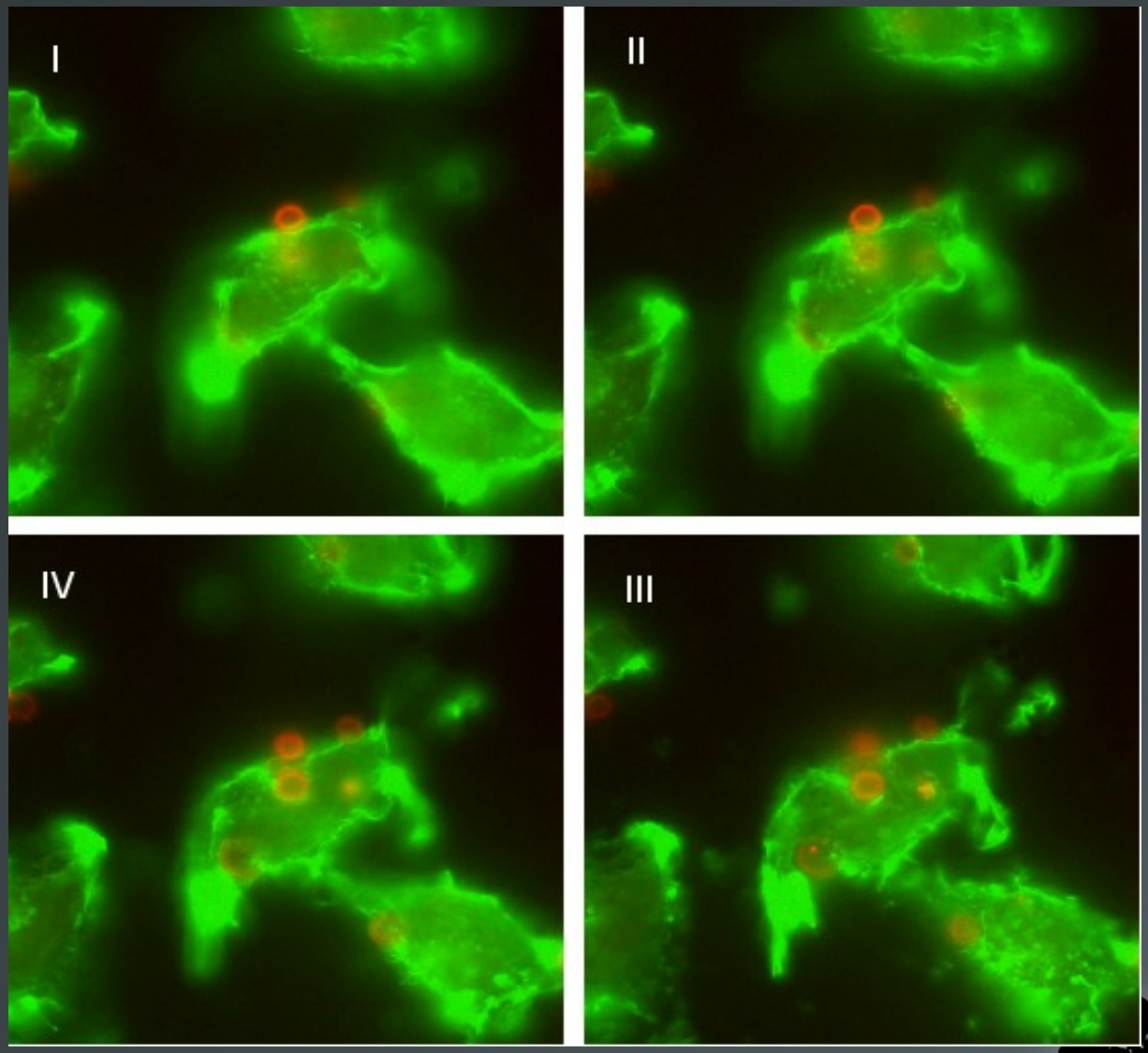
The interaction between MC-Chi-FA and HeLa cells is due to the presence of folate on the microstructure surface

Proliferation: cells have been incubated with MC for 48 hours and allowed to grow on fresh medium for additional 48 hours before being assayed on their proliferation





MC-Chi-FA impact the proliferation of HeLa tumor cells, while do not perturb in significant way, the growth of NIH3t3 cells.



Green:Phalloidin-FITC

Red:MC-Chi-FA-Rhod

MC-Chi-FA are internalized by HeLa cells

SUMMARY

Chitosan-folate on MC leads to greater absorption of the drug.

Lower rate of drug release from functionalized MC

MC-Chi-FA are able to bind and to be internalized by HeLa cells

MC-Chi-FA, interacting with HeLa cells, impact their proliferation

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...And you for your Kind Attention!