Radiolabeled Acridine Orange (AO) Derivatives as DNA-Targeted Probes for Auger Therapy

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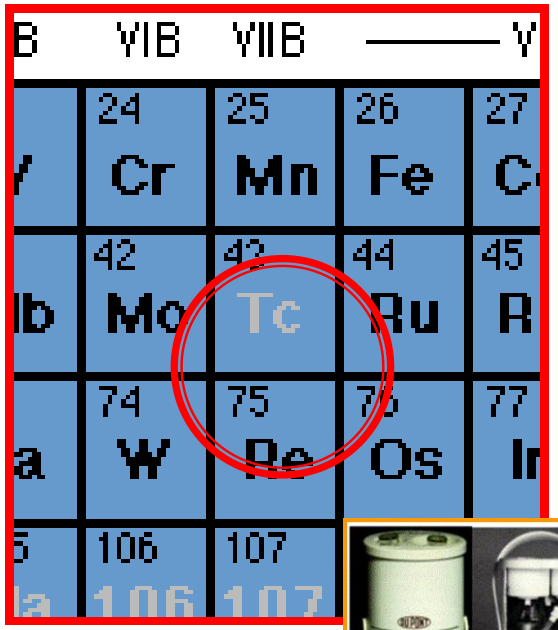
Technetium-99m

RICH CHEMISTRY



- The most important SPECT Radionuclide

- 85% Diagnostic Nuclear Medicine

  - ^{99m}Tc: γ 140 Kev; $T_{1/2}$= 6 h

  - $^{99}Mo/^{99m}Tc$ Generator

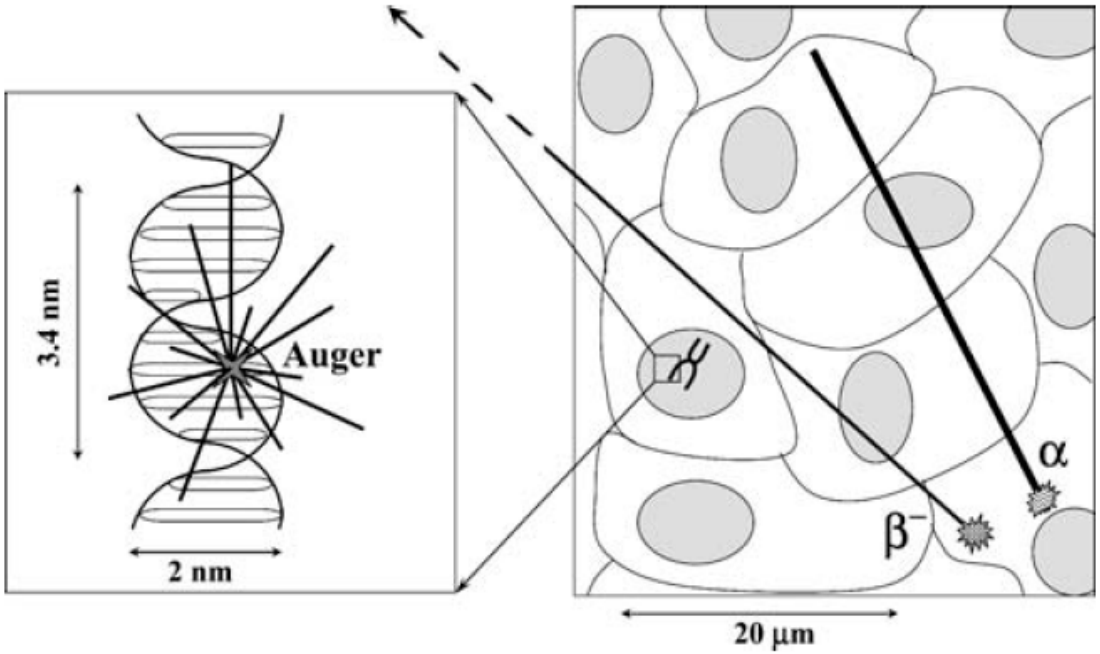
  - Variety of kits available

Technetium-99m

What about Auger therapy ?

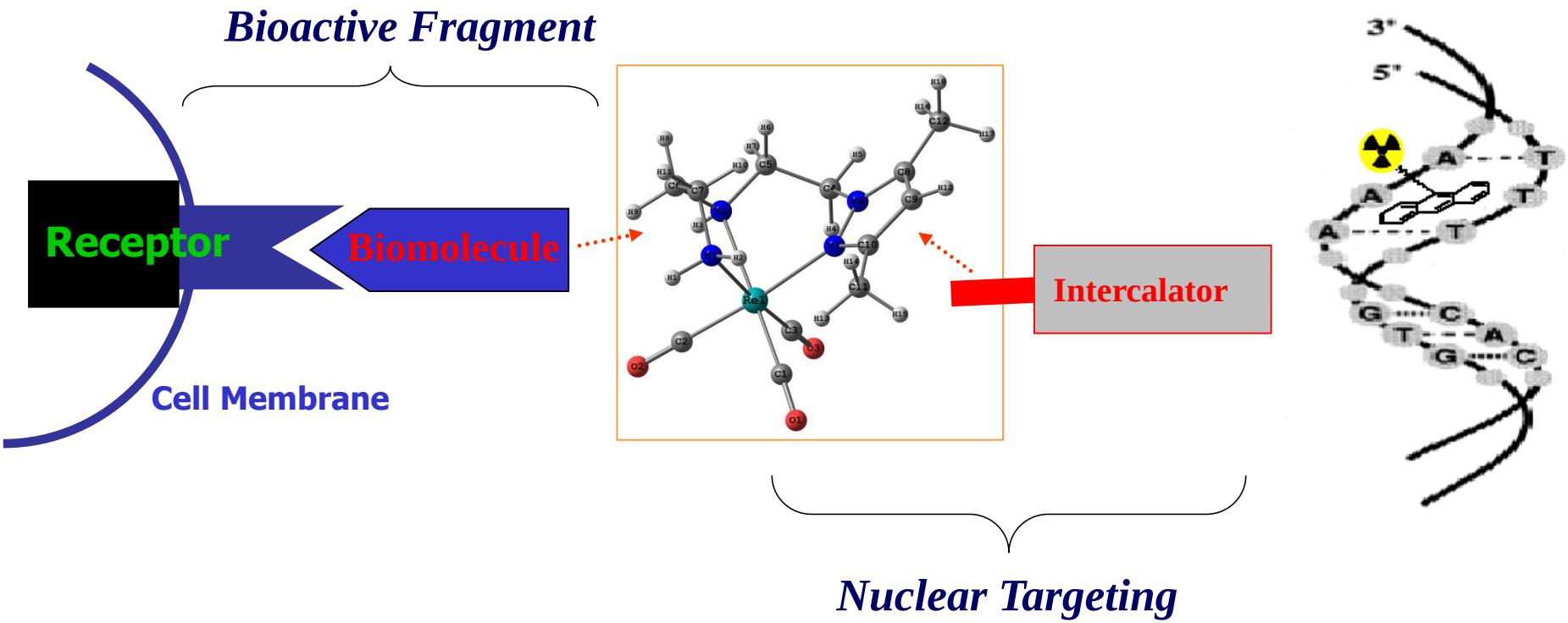
Auger emitter : 5e-/decay; 33-226 eV

e- Auger : short pathway but high LET!



Biological effects (e.g. induction of cell death) are highly dependent on subcellular localization and proximity towards DNA

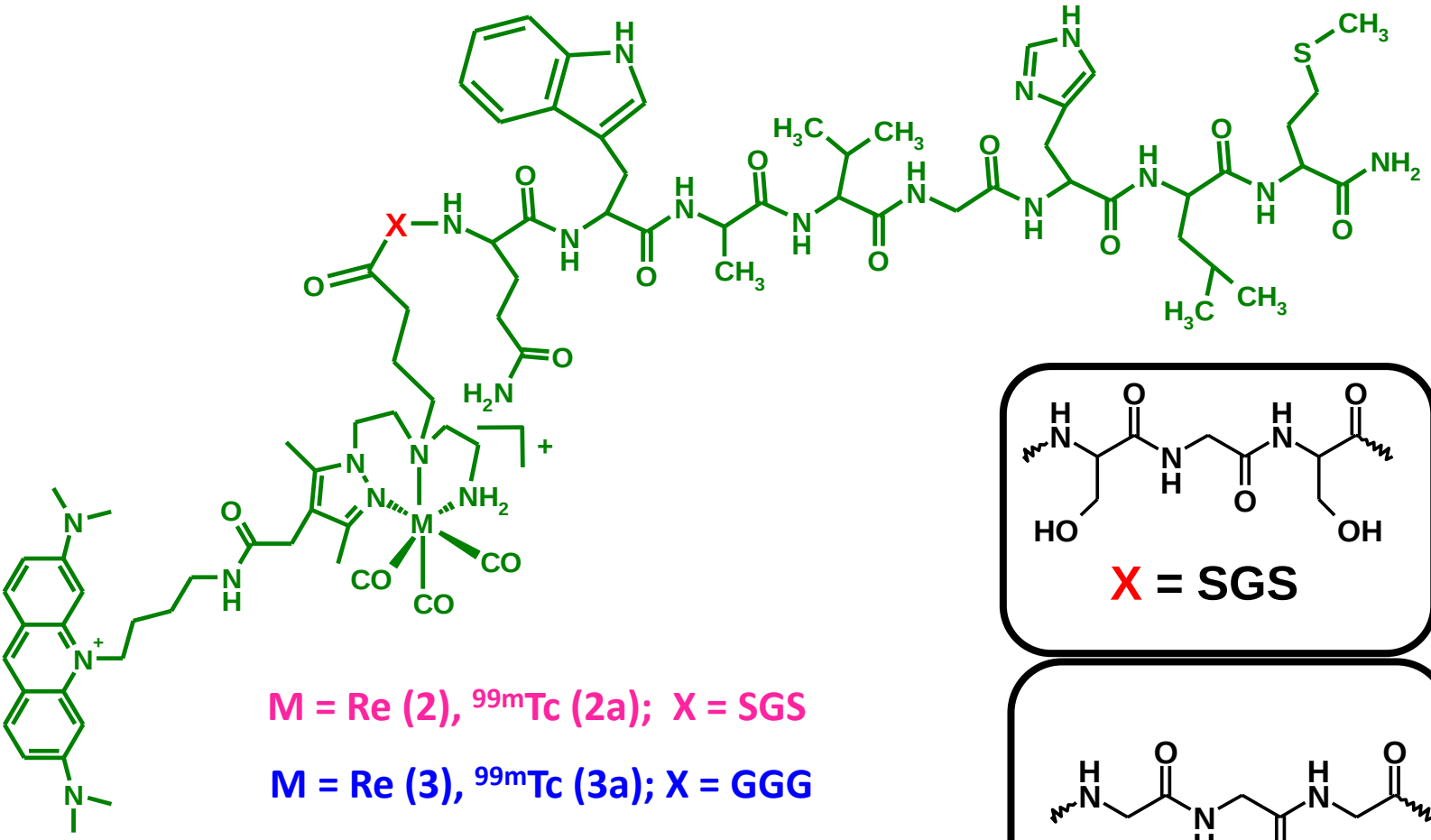
Multifunctional Compounds for Cell-Specific DNA Targeting



A. Paulo et al., *Org Biomol Chem* **2010**, *8*, 4104

A. Paulo et al., *J Biol Inorg Chem* **2011**, *16*, 1141

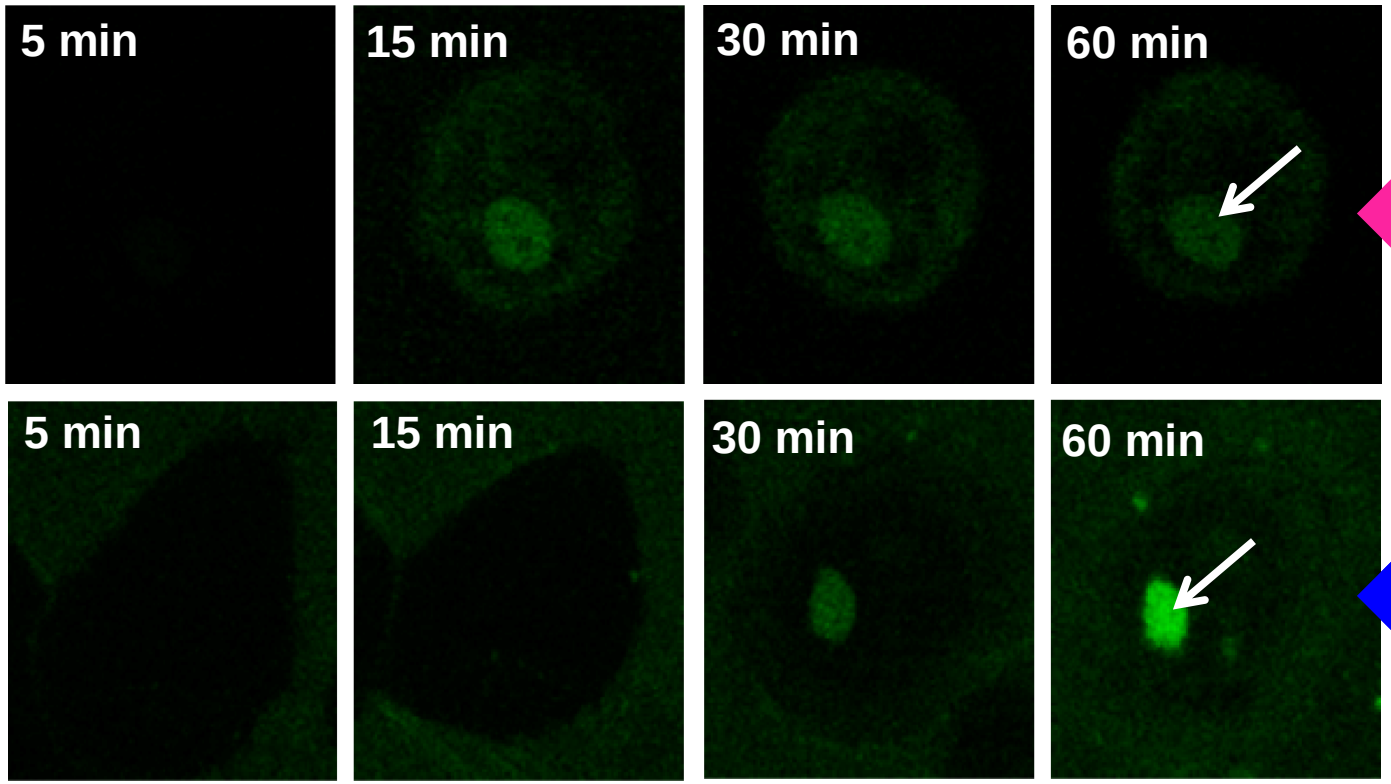
Re(I)/^{99m}Tc(I) Complexes with AO and BBN analogues



M = Re (2), ^{99m}Tc (2a); X = SGS

M = Re (3), ^{99m}Tc (3a); X = GGG

Re(I) Complexes: Cell Uptake

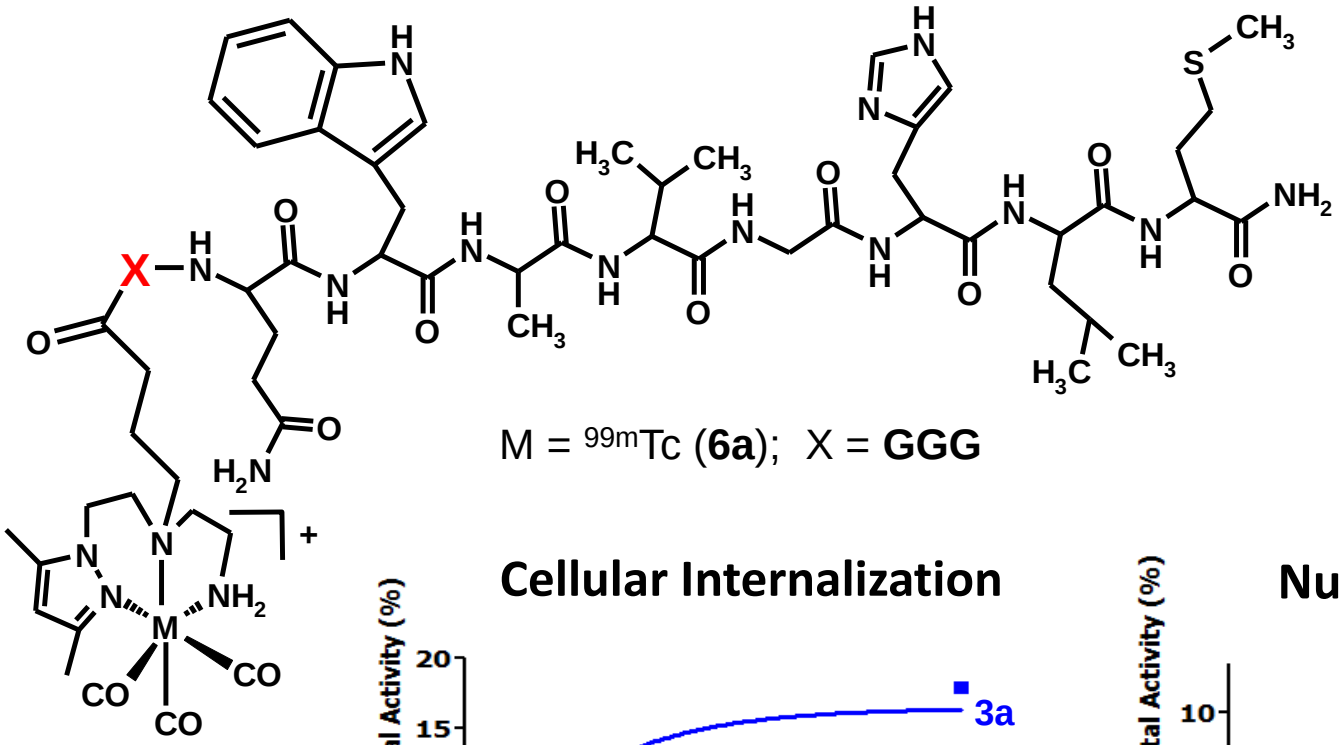


Complex 2

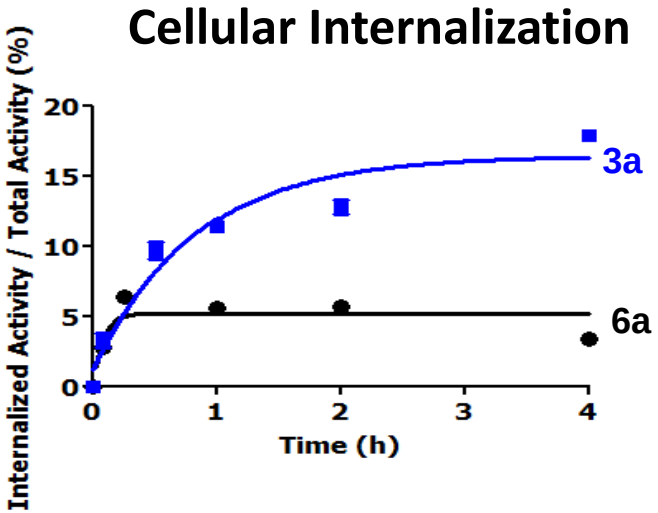
Complex 3

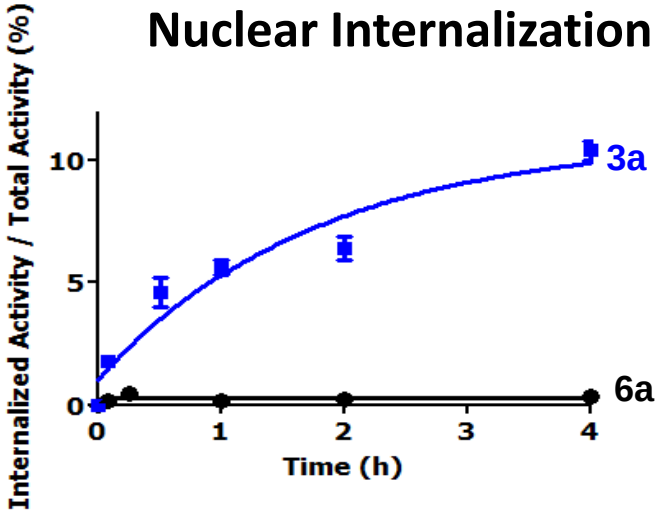
Fluorescence confocal microscopy pictures of PC-3 cells ($1{,}5 \times 10^{-5}$ M).

Influence of the Intercalator (AO)

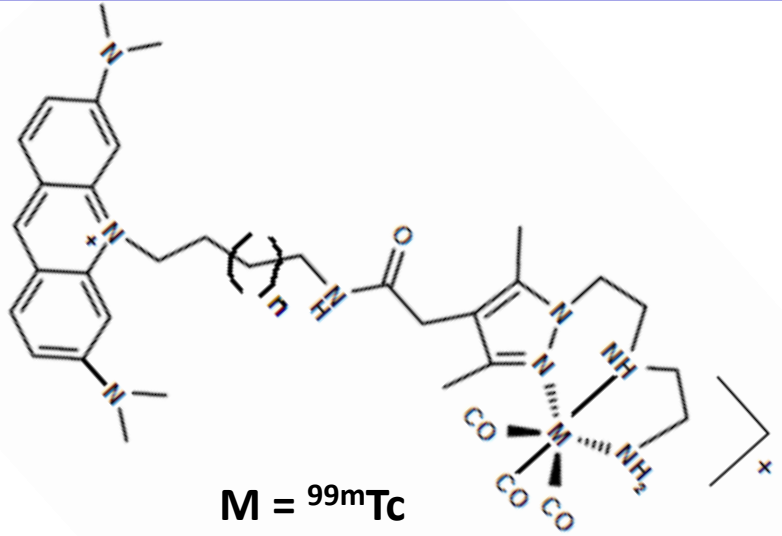


M = ^{99m}Tc (**6a**); X = **GGG**





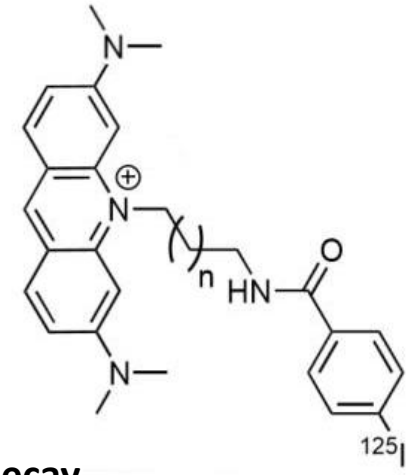
Comparison with ^{125}I-Congeners



M = ^{99m}Tc

5 e-/decay

Average energy (eV)	Yield/decay
15300	0,0126
17830	0,0047
42,9	0,0193
2050	0,0868
2320	0,0137
2660	0,0012
116	0,747
226	1,1
33,4	1,98



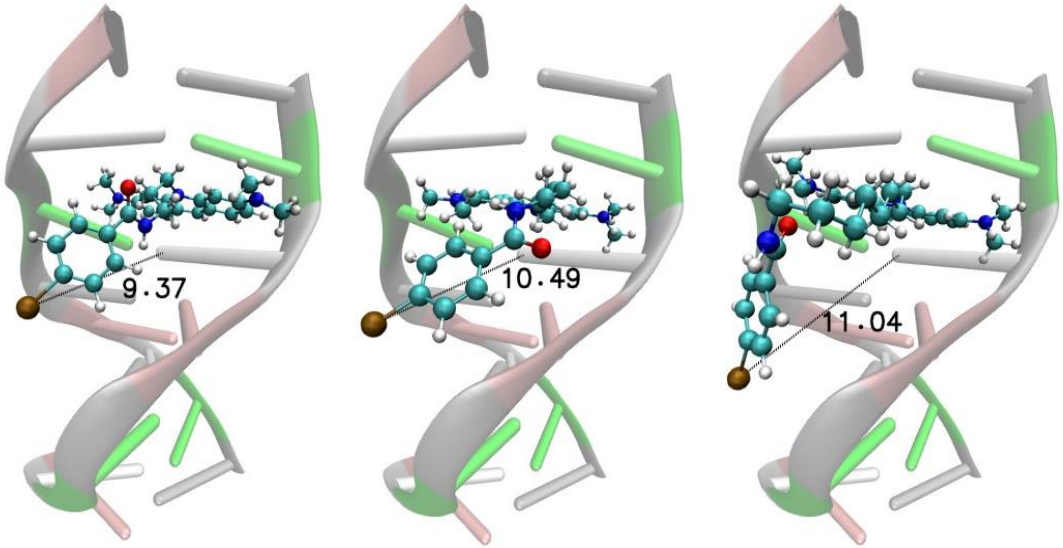
24 e-/decay

Average energy (eV)	Yield/decay
22400	0,138
26400	0,059
30200	0,0065
219	0,264
3050	1,25
3670	0,34
4340	0,0211
127	1,44
461	3,28
29,9	3,51
32,4	10,9

Auger spectrum data taken from Roger W. Howell, Medical Physics 1992

Molecular Docking

^{125}I-derivatives



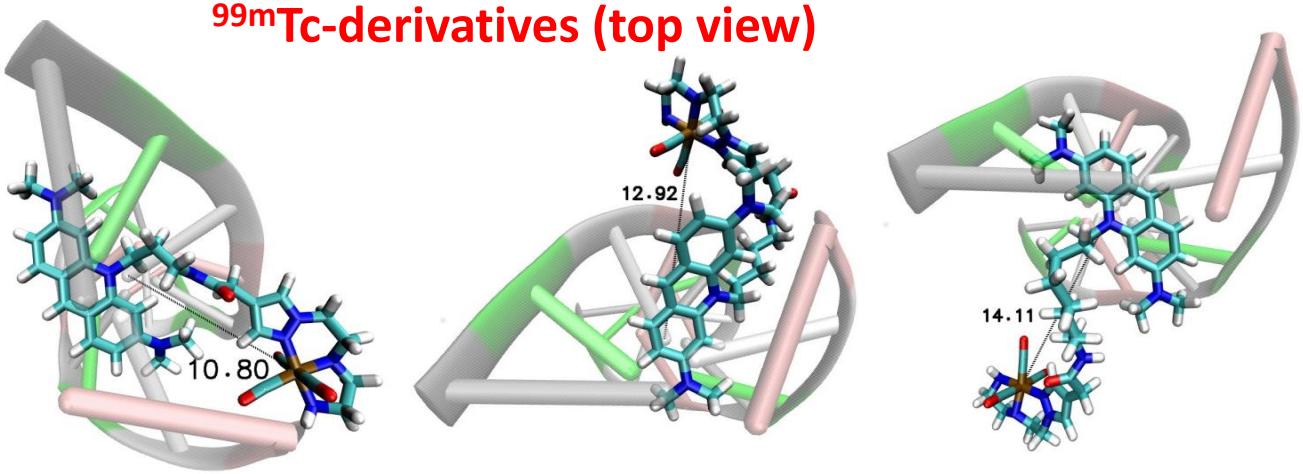
3C-Linker 5C-Linker 8C-Linker

Target DNA sequence: ds(ACGTACGT)$_2$

^{125}I-5C: 10.49 Å

^{99m}Tc-3C: 10.80 Å

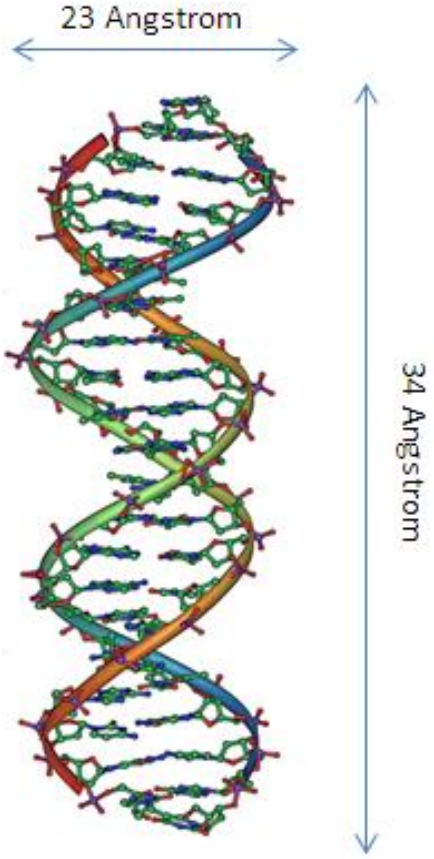
^{99m}Tc-derivatives (top view)

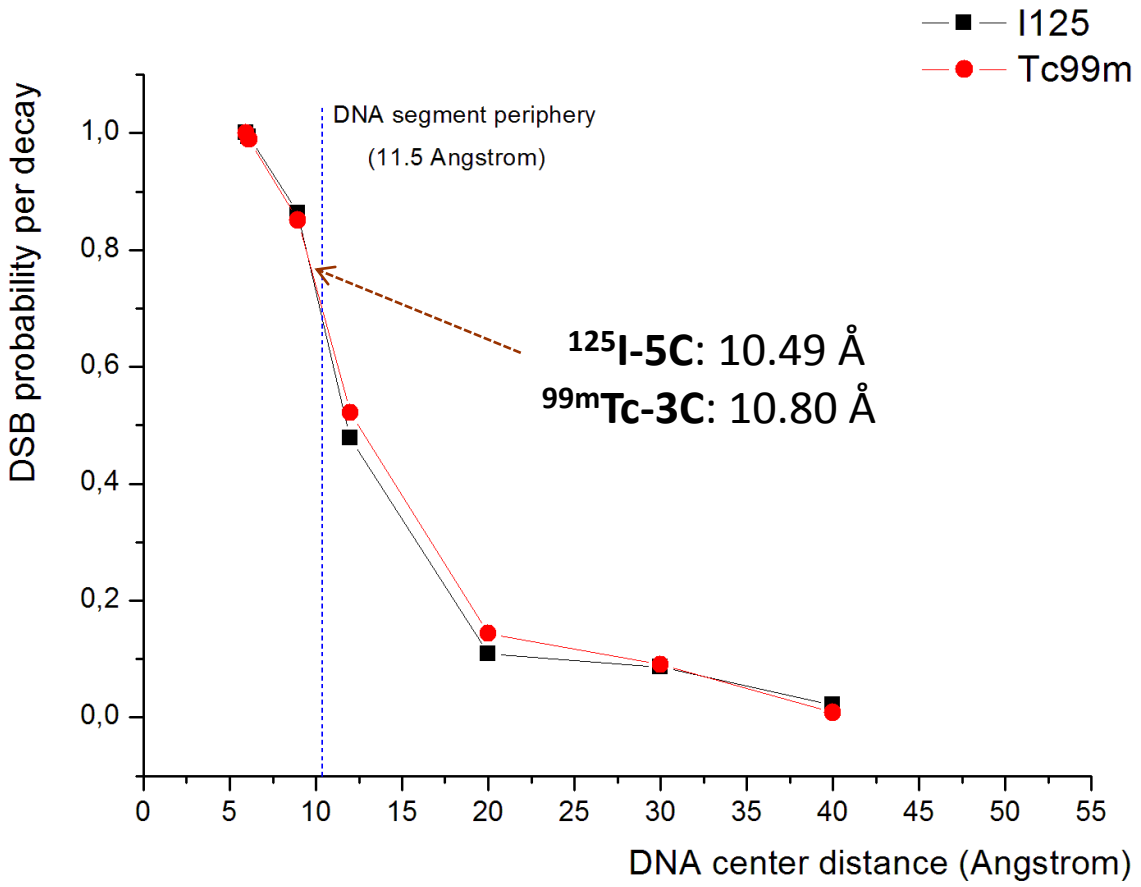


3C-Linker 5C-Linker 8C-Linker

Nanodosimetry

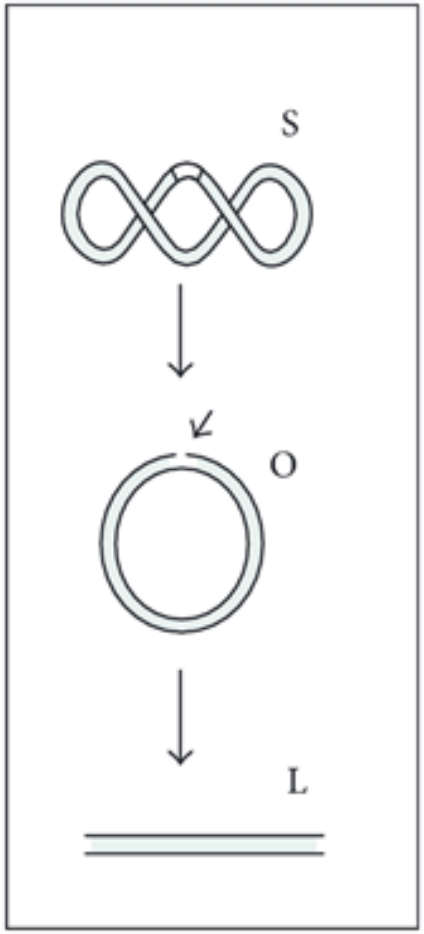
- DNA model = rigid rod
- MCNP6 Monte Carlo simulations; Simulated material = water
- Output of MC simulations= electron flux (1/cm^2) and dose (Gy)
- DSB probability linearly proportional to the segment dose

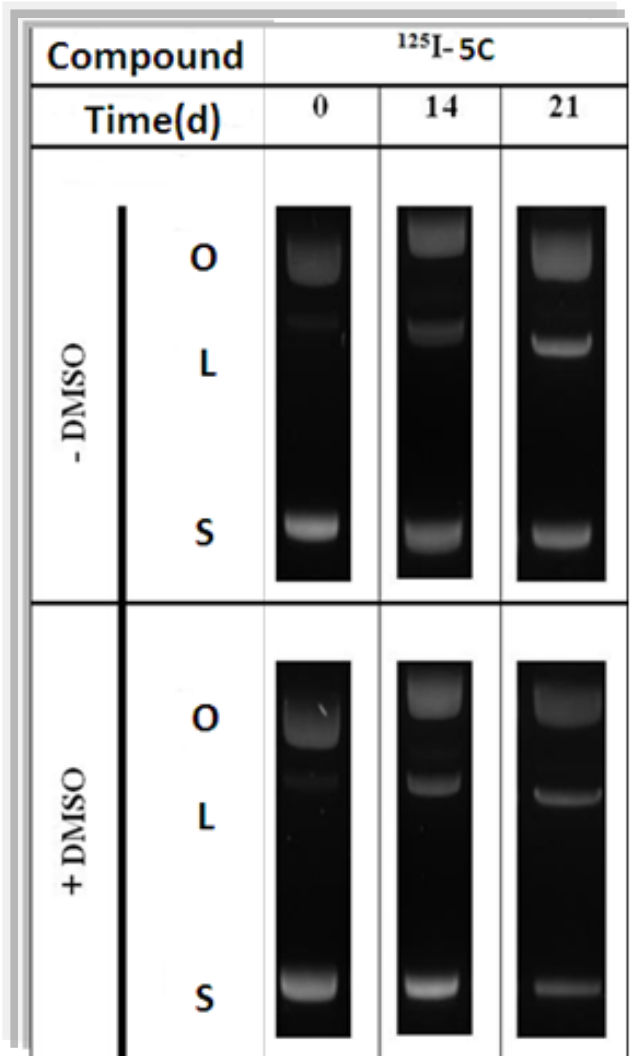


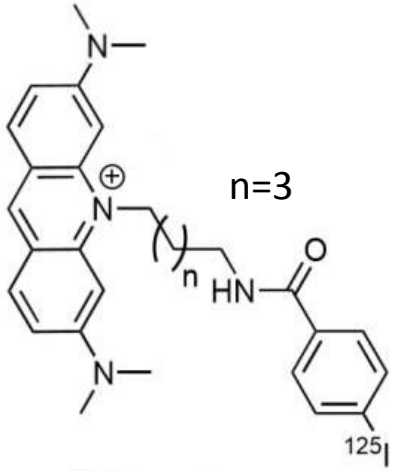


DNA damage: Plasmid Studies

Gel electrophoresis





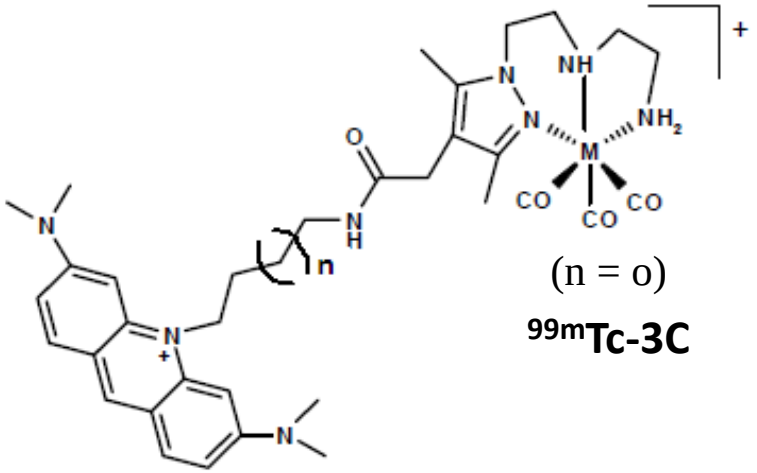


^{125}I-5C

(Cleavage of supercoiled ϕX174 DNA by **^{125}I-5C** (initial activity), after incubation at different intervals of time at 4°C in Tris.HCl buffer (pH 7.4) in the presence or absence of DMSO (0.2M))

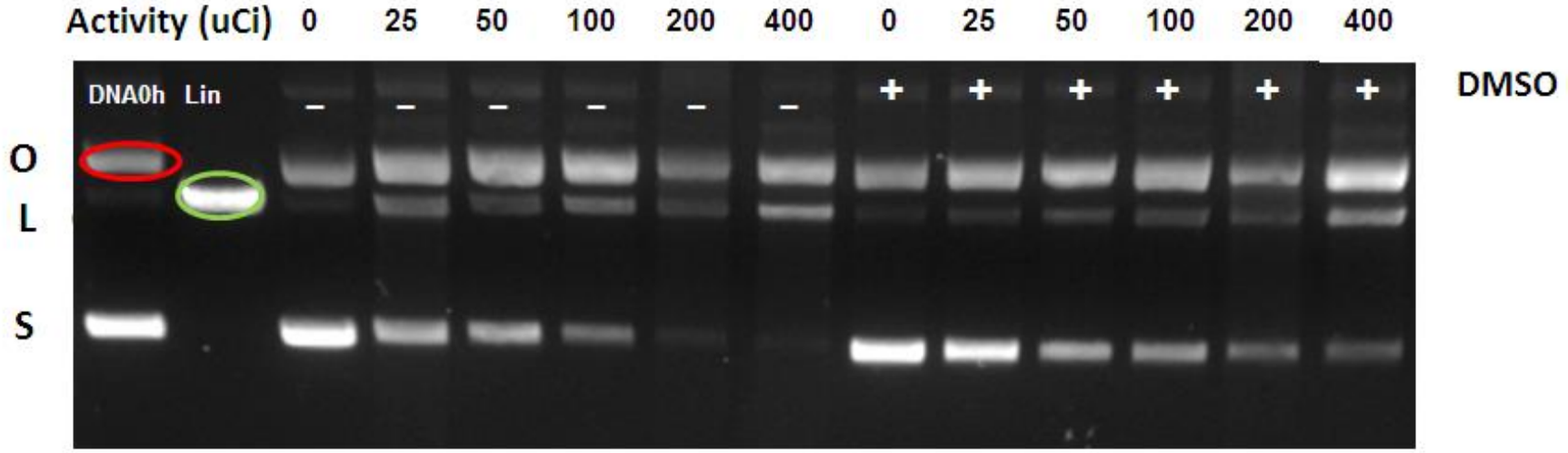
DNA damage: Plasmid Studies

Gel electrophoresis



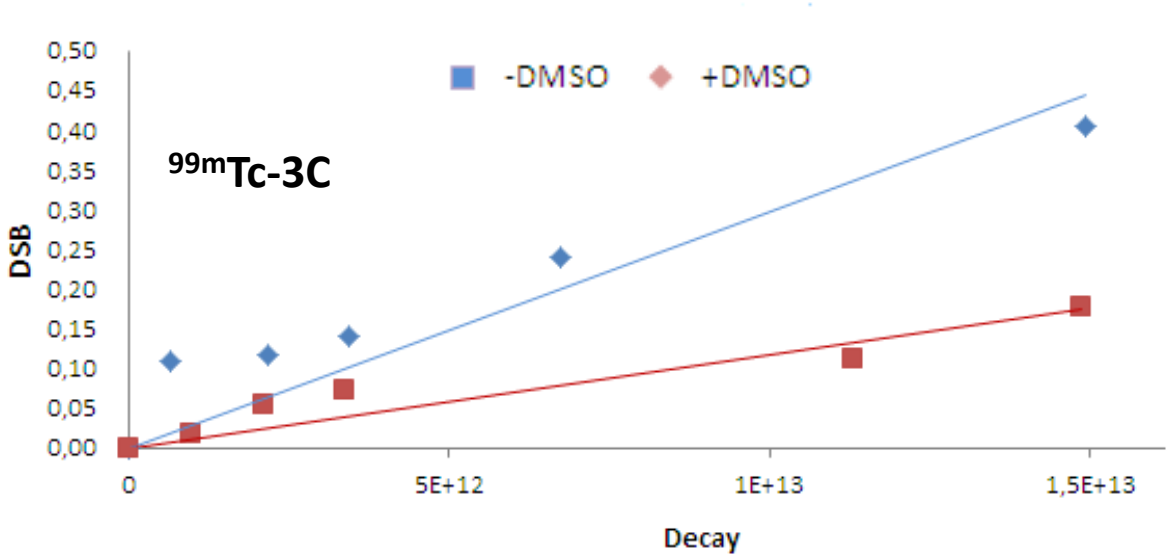
(n = o)

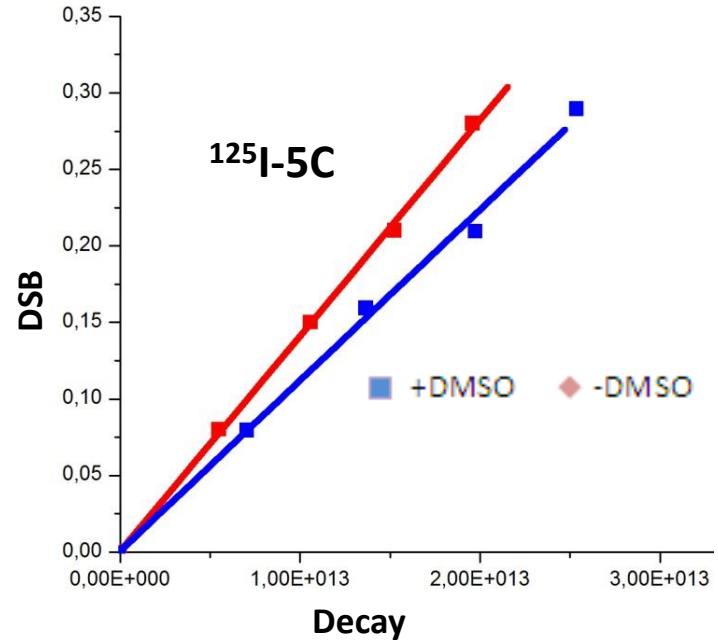
^{99m}Tc-3C



(Cleavage of supercoiled ϕX174 DNA by ^{99m}Tc-3c, after 24h of incubation at 4°C in Tris.HCl buffer (pH 7.4) in the presence or absence of DMSO (0.2M))

DNA damage: Plasmid Studies

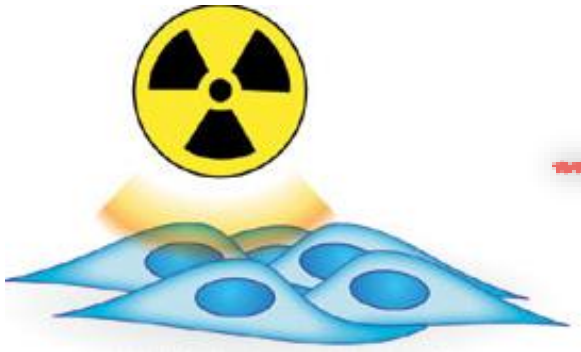


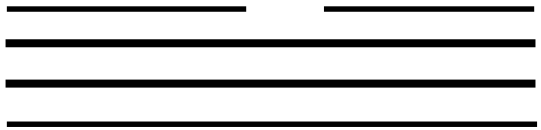


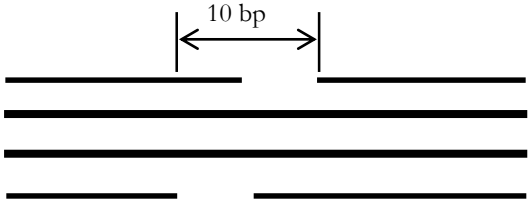
DSB/decay

Compound	- DMSO	+ DMSO
^{99m}Tc-3C	0.034	0.011
^{125}I-5C	0.050	0.040

DNA damage: Cell Studies

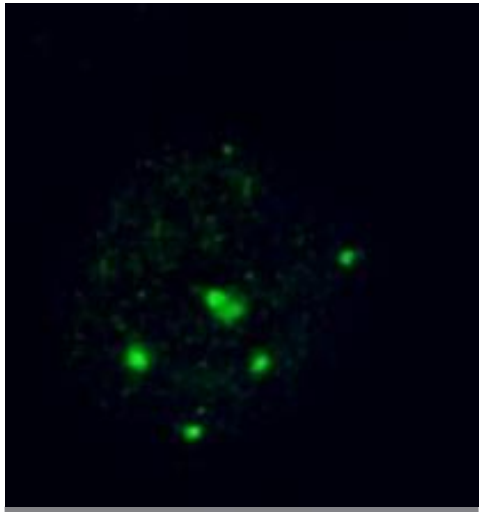






Types of cellular damage

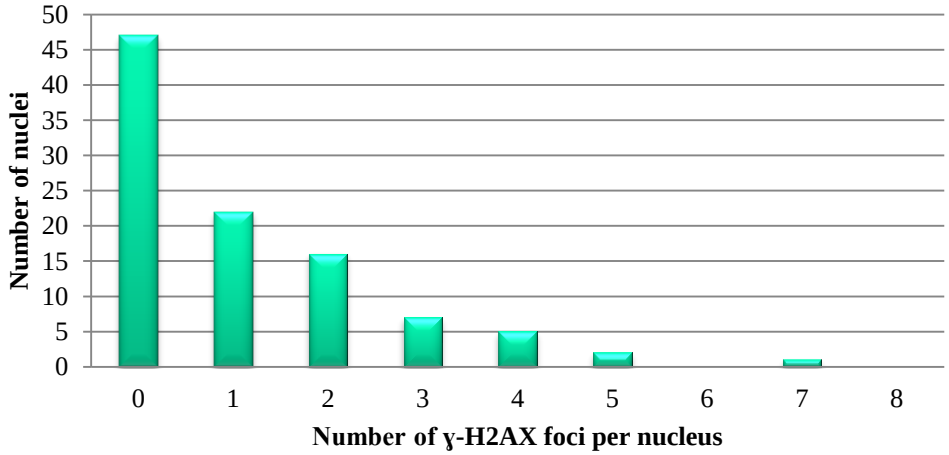
Histone proteins are modified around DNA (e.g. ɣ-H2AX foci)



Biological endpoint: Nuclear foci

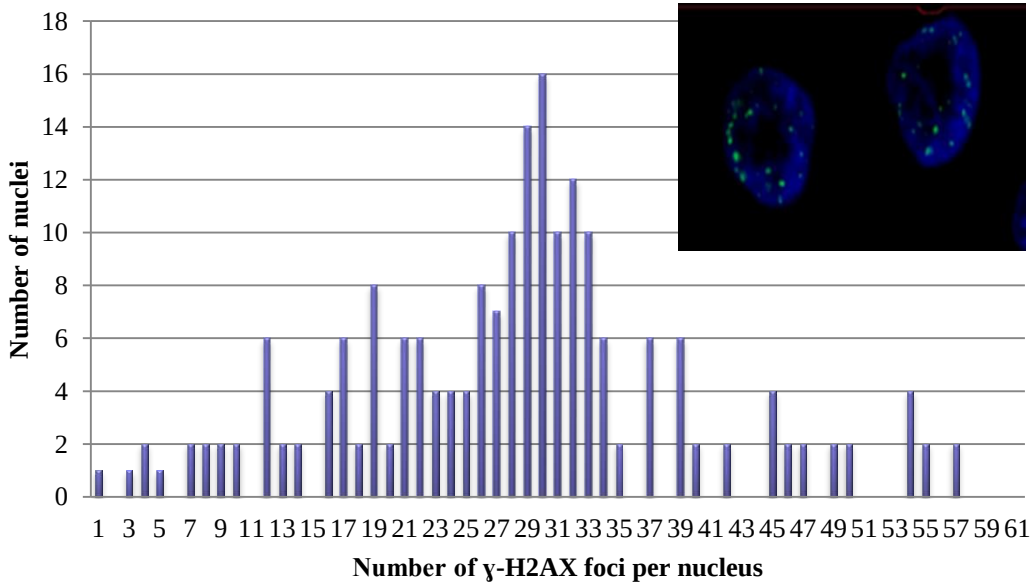
W. M. Bonner et al., *Nature Reviews Cancer* (**2008**), 8, 957-967

DNA damage: γ-H2AX assay



Control:

Average number of foci/nucleus=
1.12 ± 1.05



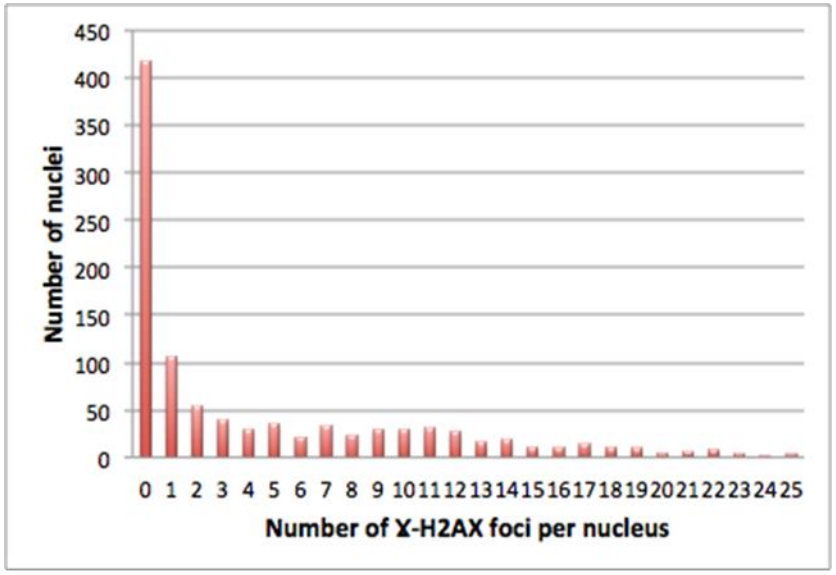
PC-3 cells incubated with ^{125}I-C3:

Average number of foci/nucleus=

26.13 ± 4.4

(8 uCi/2000 cells, 24 h)

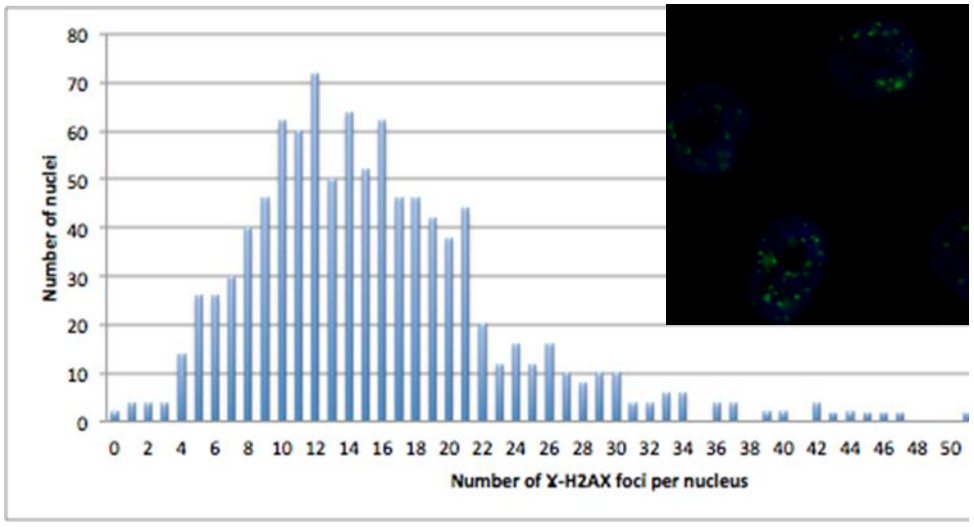
DNA damage: γ-H2AX assay



Control:

Av. number of foci/nucleus=

4.49 ± 2.12



PC-3 cells incubated with ^{99m}Tc-C5:

Av. number of foci/nucleus=

19.4 ± 4.2

(50 uCi/2000 cells, 24 h)

Conclusions

- Combining a DNA intercalator with a GPCR-targeting peptide can afford cell-specific targeting of nuclear DNA with ^{99m}Tc;

- Our results encourage the use of this approach (i.e. DNA intercalator combined with a target-specific vector) for other Auger emitters (e.g. ^{111}In, ^{155}Tb, ^{161}Tb, etc.);

- If placed in close proximity to DNA, ^{99m}Tc can induce DNA damage with an extent relatively similar to that induced by ^{125}I (either *in vitro* or *in vitro*);

- DNA damage due to indirect effects is more relevant for ^{99m}Tc than ^{125}I, which certainly reflects the higher energy of the γ-photons emitted by ^{99m}Tc.

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