

Imaging component of multifunctional pharmaceutical nanocarriers

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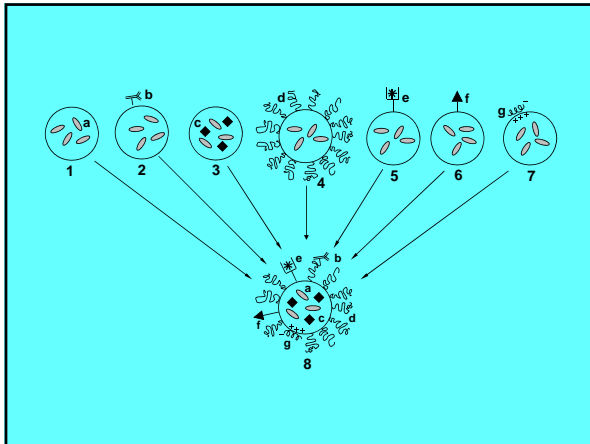


PHARMACEUTICAL CARRIERS

- soluble polymers
- microcapsules
- microparticles
- cells
- cell ghosts
- liposomes
- micelles
- niosomes
- metal particles
- solid lipid particles
- lipoproteins

INDIVIDUAL PROPERTIES OF PHARMACEUTICAL NANOCARRIERS THAT CAN BE COMBINED IF REQUIRED:

- size and size distribution
- charge
- stability at storage conditions and in vivo
- longevity in the circulation
- biodegradability
- targetability
- ability to carry a sufficient cargo
- ability to release a cargo
- ability to carry a reporter (contrast) moiety
- sensitivity towards magnetic field
- ability to complex DNA
- ability to escape from endosomes
- ability to penetrate inside cells



Different requirement for different agents

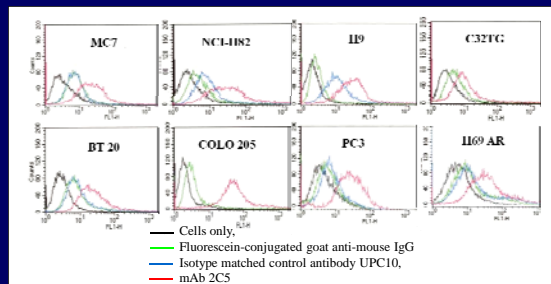
- ❖ Delivery of **imaging agents** requires good relative accumulation (high target-to-nontarget ratio)
- ❖ Delivery of **therapeutics** requires good absolute accumulation (maximum percent dose per gram of the target tissue)

Anti-nucleosome antibodies and cancer

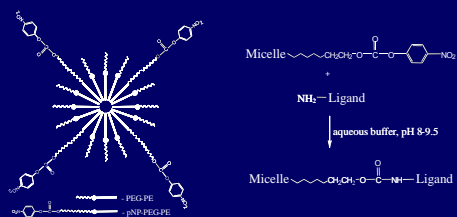
- The monoclonal non-pathogenic ANA 2C5, derived from healthy aged Balb/c mice, was shown to recognize the surface of numerous tumor, but not normal cells via their cell surface-bound nucleosomes.
- In addition to their anticancer effects, these antibodies can be used as targeting moieties for drug delivery systems.

(Gokhobor et al., Cancer Detect Prev, 1998; 22(5):479-8)

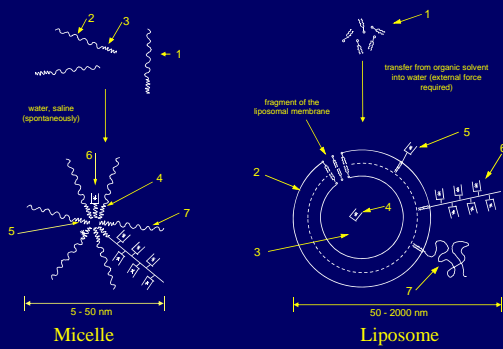
Surface binding of mAb 2C5 to different human tumor cell lines as shown by flow cytometry



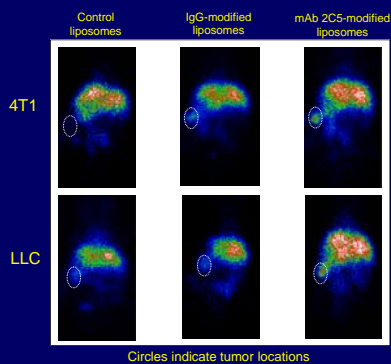
Preparation of immunomicelles



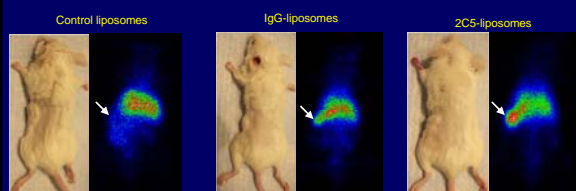
Schematic structures of a micelle and liposome: formation and loading with contrast agent



Whole body gamma-imaging of 4T1 and LLC tumor-bearing mice 12 hr after injection with ¹¹¹In-labeled liposomes



4T1 mouse model



Whole body imaging of 4T1 tumor-bearing mice, 4 hr after injection with ¹¹¹In-labeled liposomes: Arrows indicate tumor locations.



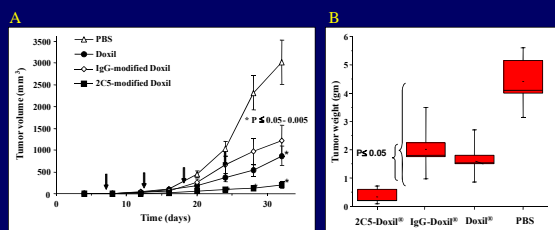
LLC mouse model



Whole body imaging of LLC tumor-bearing mice, 4 hr after injection with ¹¹¹In-labeled liposomes: Arrows indicate tumor locations.

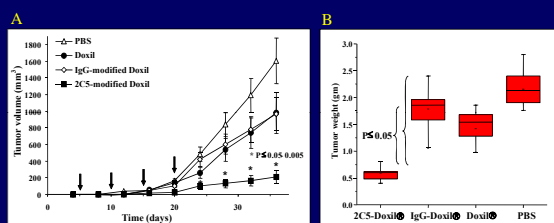


Therapeutic activity, expressed as tumor volumes (A) and tumor weight (B) of 2C5-modified Doxil® against control preparations in CT26 implanted mice



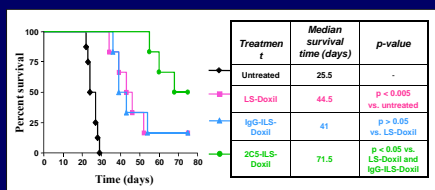
Arrows indicate treatment schedule, 2 mg/kg/q 5 days. (n= 8-10). * Two way ANOVA with Tukey's HSD Post-Hoc test (Results indicated ± SD)

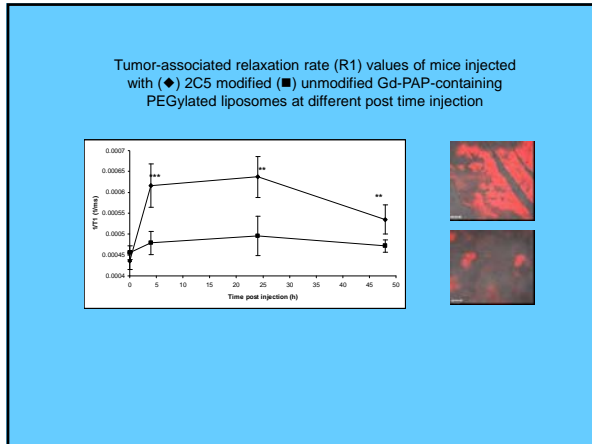
Therapeutic activity, expressed as tumor volumes (A) and tumor weight (B) of 2C5-modified Doxil® against control preparations in LLC implanted mice

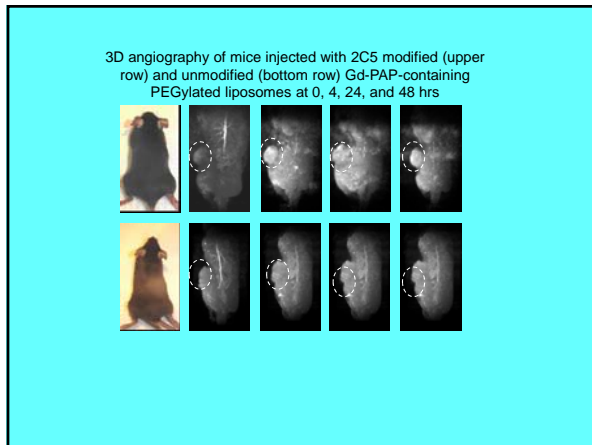


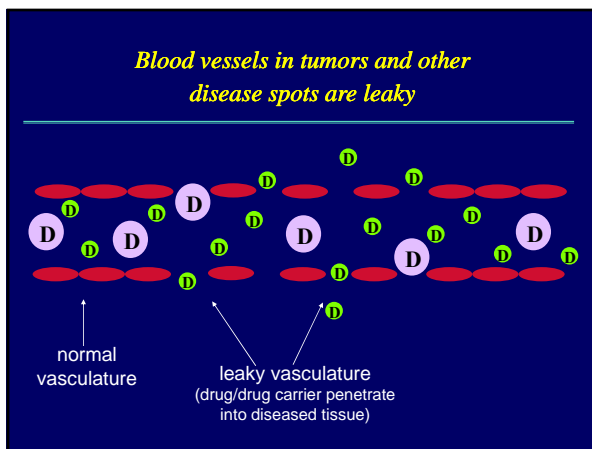
Arrows indicate treatment schedule, 2 mg/kg/q 5 days. (n= 8-10). * Two way ANOVA with Tukey's HSD Post-Hoc test (Results indicated ± SD)

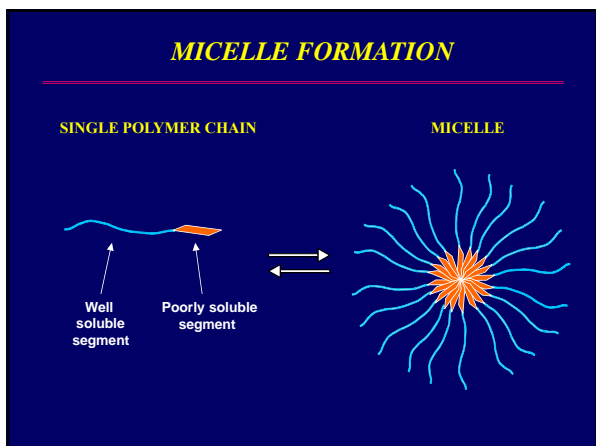
In vivo therapeutic efficacy of Doxil-loaded liposomes against intracranial U-87 MG astrocytoma xenograft in nude mice

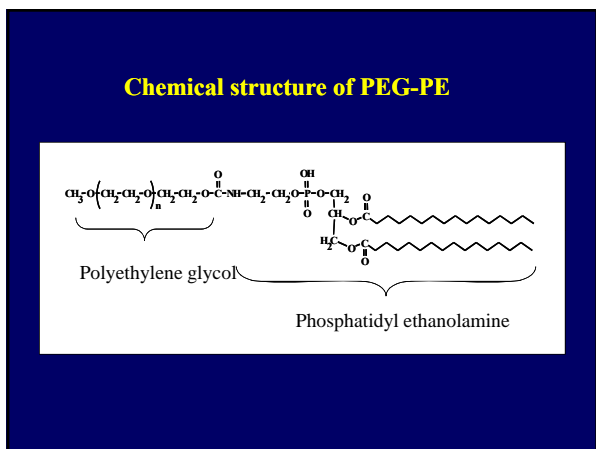


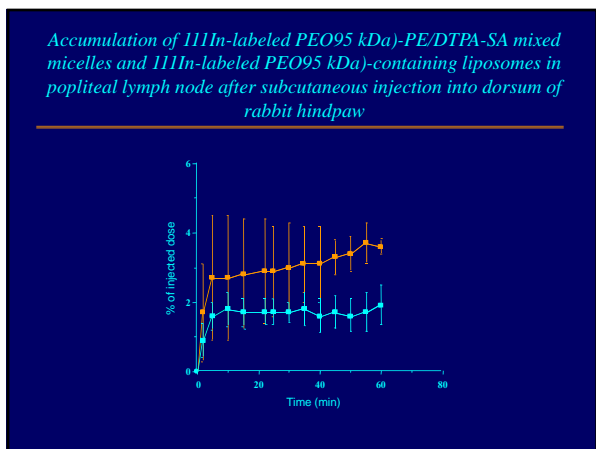


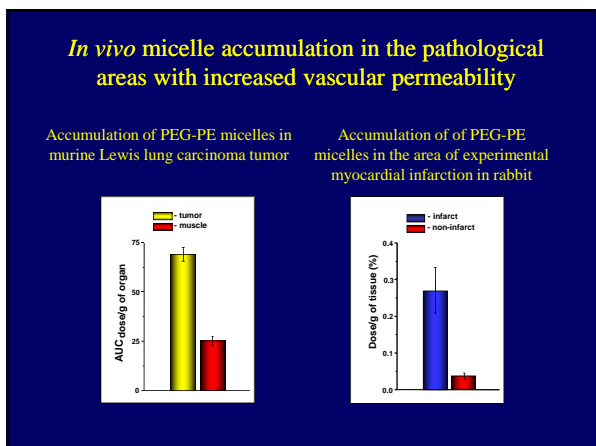


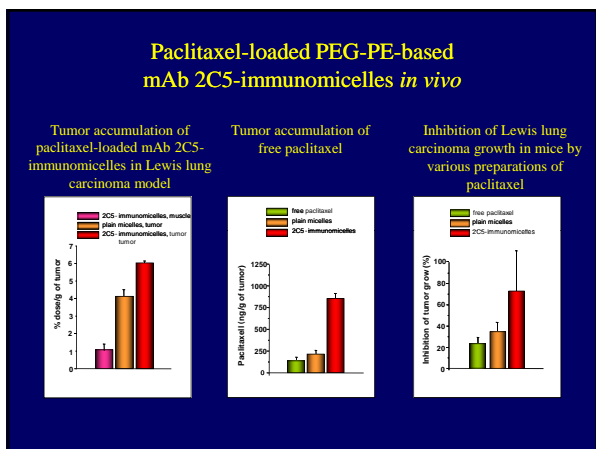


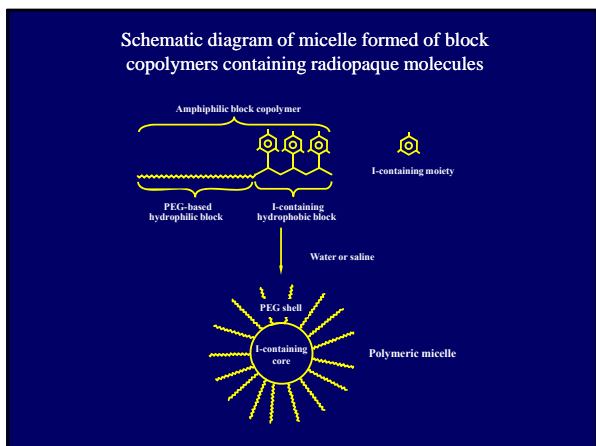


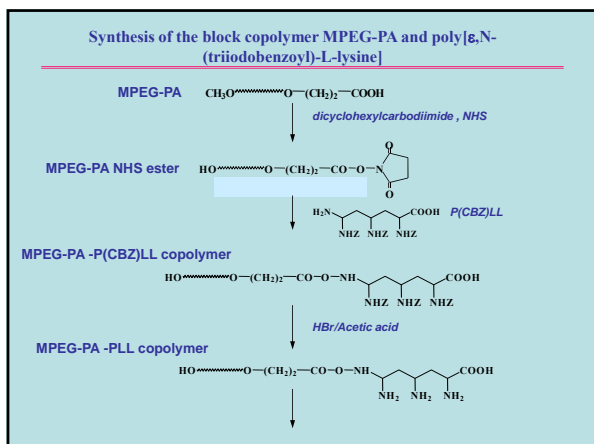


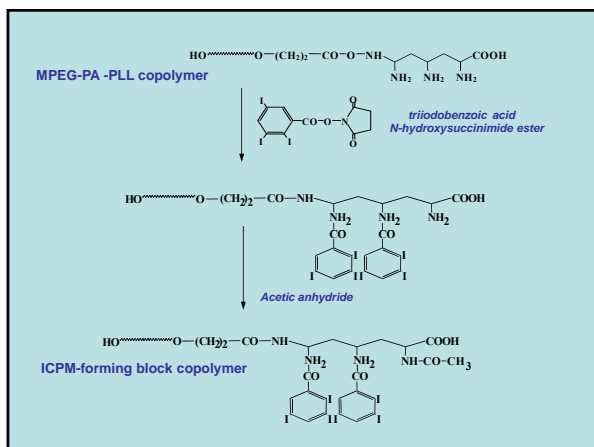


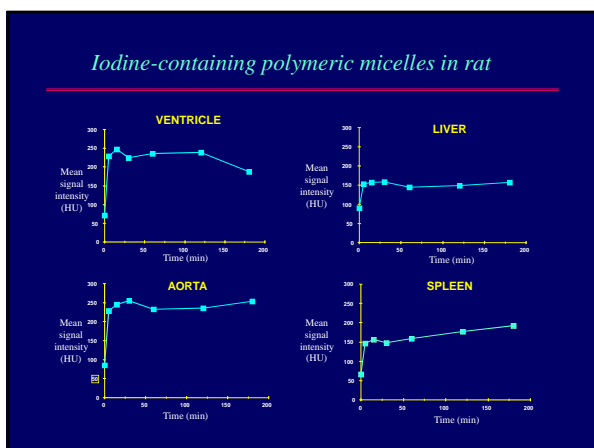


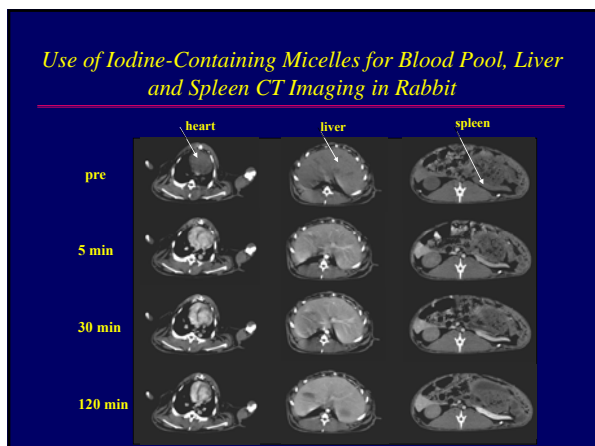












SPION-micelles

- Dry film of mPEG₂₀₀₀-PE and SPION was rehydrated using HEPES-buffered saline, pH 7.4 and vortexed for 10 min.
- Unincorporated SPION removed by external magnet.

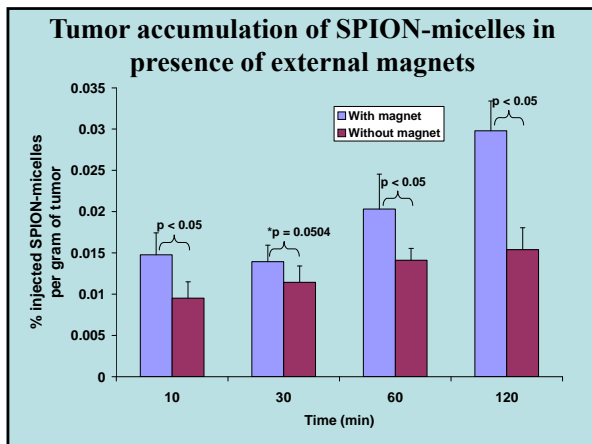
The diagram shows a central cluster of orange spheres representing SPION particles. This core is surrounded by a blue, wavy, brush-like structure representing the mPEG₂₀₀₀-PE polymer chains that form the micelle's shell. The entire structure is labeled 'SPION-micelles' in red text below it.

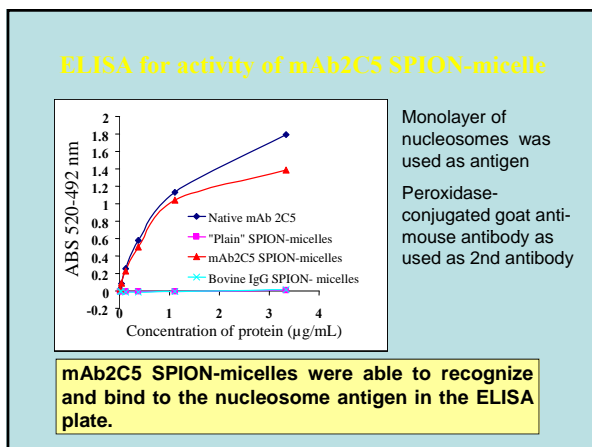
Magnetic susceptibility of SPION-micelles

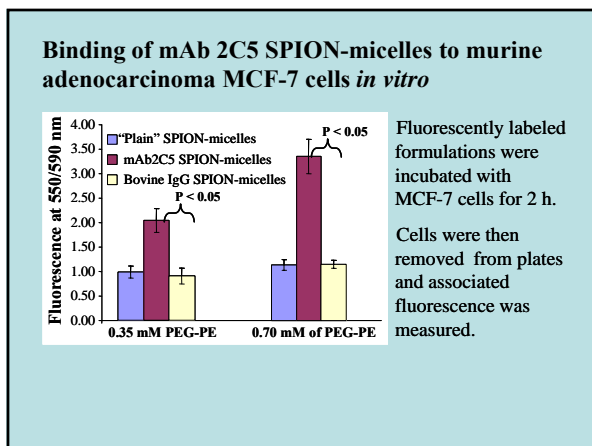
The figure includes two vials on the left, labeled '0 hr' and '2 hr', showing a color change from yellow to brown. To the right is a SQUID measurement graph. The y-axis is labeled 'M (A/m)' and ranges from -100000 to 100000. The x-axis is labeled 'H (A/m)' and ranges from -2.00E+06 to 2.00E+06. Two curves are shown: a red curve for 'SPION-micelles' and a black curve for 'SPIONs coated with oleic acid'. Both curves show a hysteresis loop with a slope of approximately 100000 A/m per 1000000 A/m, indicating superparamagnetic behavior. The temperature is noted as T = 260K.

No coercivity is seen indicating the super paramagnetic behavior of SPIONs.

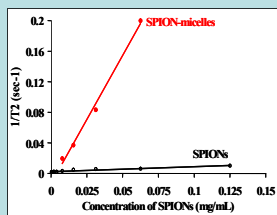
Entrapment of the SPIONs within the PEG-PE micelles does not affect the magnetic properties of the SPIONs







NMR relaxivity of SPION micelles



Measured using a bench top 5 mHz RADX NMR Proton Spin Analyzer in HBS, pH 7.4

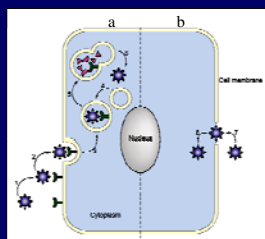
SPION-micelles offered improved T2 relaxivity signal at low concentrations when compared to plain SPIONs

Binding of mAb 2C5 SPION-micelles to murine adenocarcinoma MCF-7 cells *in vitro*

Formulations	Concentration of PEG-PE applied to MCF-7 cells.		
	0.35 mM	0.70 mM	
"Plain" SPION-micelles	4.3 ± 0.02 s ⁻¹	5.2 ± 0.05 s ⁻¹	} P < 0.05
mAb 2C5 SPION-micelles	5.6 ± 0.01 s ⁻¹	7.1 ± 0.02 s ⁻¹	
Bovine IgG SPION-micelles	4.3 ± 0.00 s ⁻¹	6.0 ± 0.02 s ⁻¹	

Formulations were incubated with MCF-7 cells for 2 h. Cells were then removed from plates and associated (1/T2) signal was measured using 500 MHz NMR machine.

Endocytosis (a) versus transduction (b) for intracellular drug delivery



Endocytosis:

- 1 - Binding of a drug delivery unit to a specific ligands
- 2,3 - Formation of endosomes
- 4 - Endosome-lysosome fusion
- 5 - Degradation of the endosomal content by lysosomal enzymes
- 6 - Possible endosomal escape, and delivery of drug to the cytoplasm

Transduction:

- 7,8 - Drug delivery units cross the cell membrane and enter the cytoplasm in the intact form and in a receptor-independent fashion

11-mer TAT-peptide:
TyrGlyArgLysLysArgArgGlyArgArgArg

NANOPARTICLES DELIVERED INTRACELLULARLY BY CPPs

- Paramagnetic iron oxide nanoparticles – 40 nm
- Liposomes – 100 nm, 200 nm
- Micelles – 20 nm
- Polymeric nanoparticles – 30-50 and 100-300 nm
- Quantum dots – ca. 5 nm

