Assessment of Antioxidant Copolymers for Superoxide Scavenging

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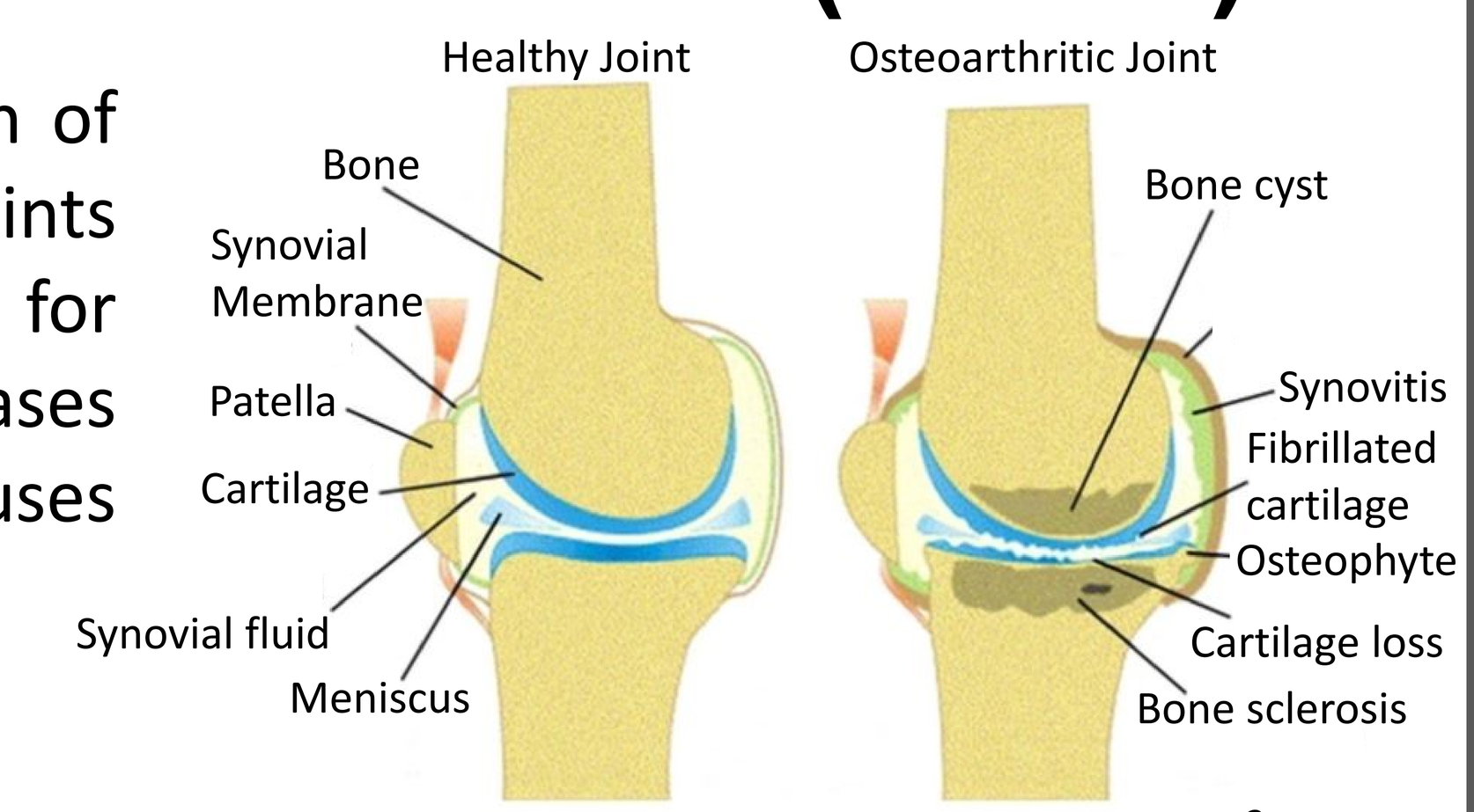
Introduction

Post Traumatic Osteoarthritis (PTOA)

PTOA is identified by the degradation of cartilage and surrounding tissue to joints following injury. PTOA accounts for around 5.6 million osteoarthritis cases per year and is one of the leading causes for mobility related diseases.[1,2]



Healthy joint vs. osteoarthritic joint[2]

Reactive Oxygen Species (ROS)

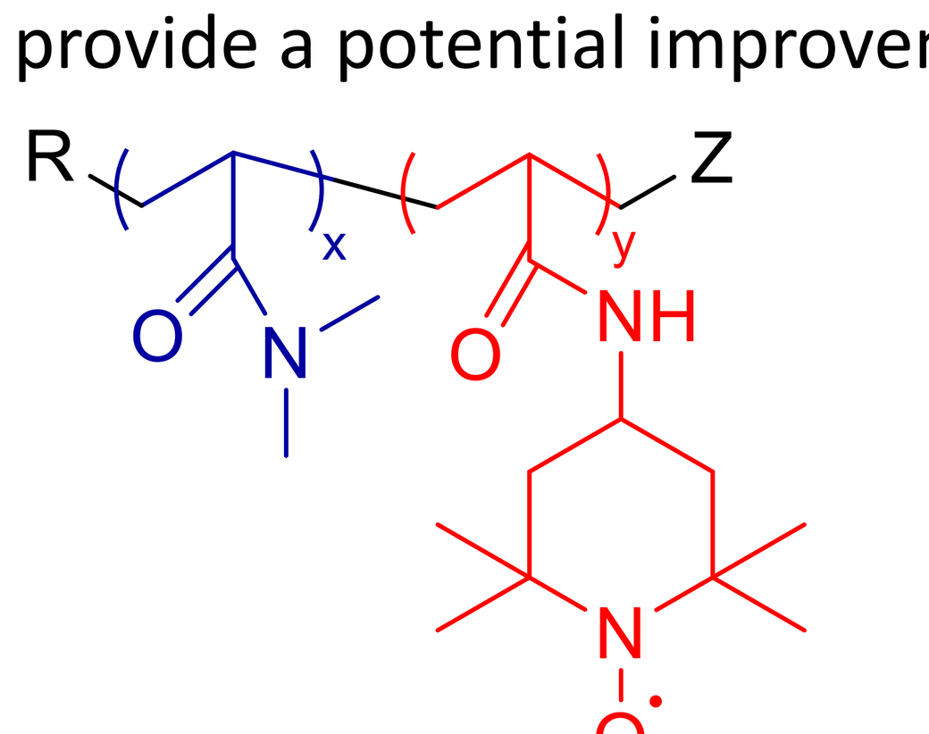
Therapeutic Obstacles

The overproduction of ROS such as superoxide anions ($^{\cdot}O_2^-$) occur immediately after traumatic injury to joints, leading to cellular damage. TEMPO, an antioxidant, has the potential to scavenge superoxide anions ($^{\cdot}O_2^-$). The hydrophobic properties of the drug cause poor retention time within joints due to synovial clearing. Therefore, an improved delivery system for TEMPO is needed.

DMA-co-TEMPO

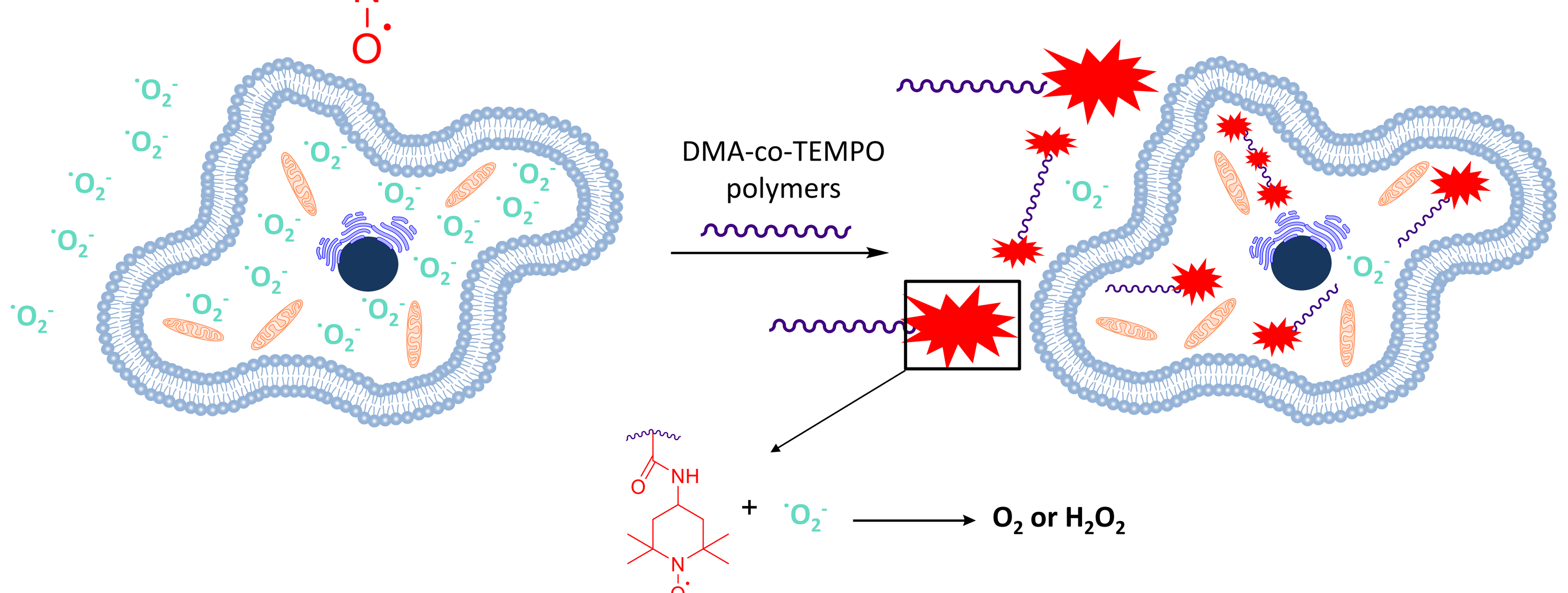
Improved Superoxide Scavenging

Previously formed dimethylacrylamide (DMA) and TEMPO grafted copolymers provide a potential improvement to TEMPO bioactivity.



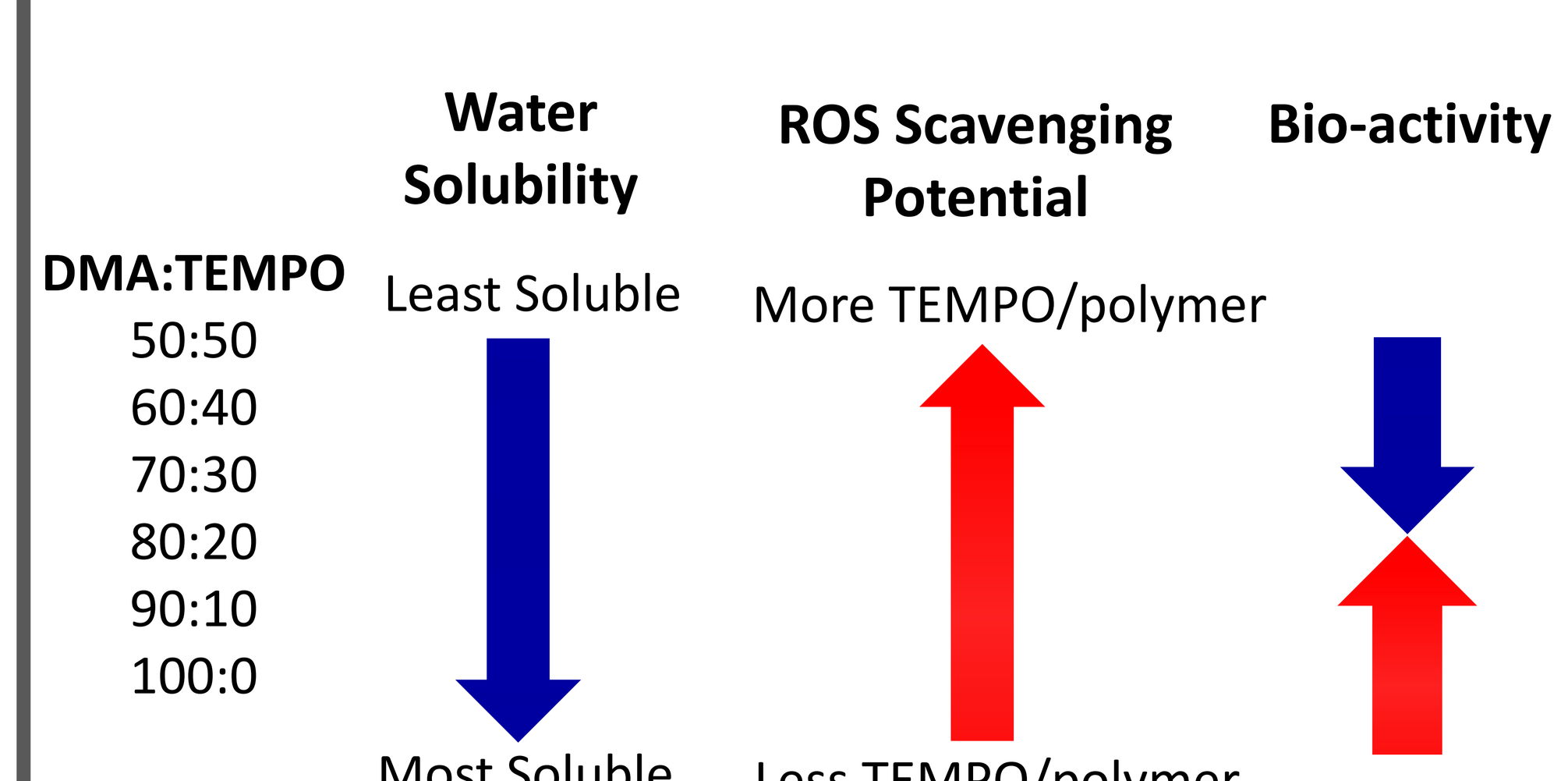
Dimethylacrylamide: Increases polymer hydrophilicity, improving retention time.

TEMPO: Provides superoxide scavenging.



Schematic of cellular DMA-co-TEMPO delivery

Superoxide Scavenging Assessment



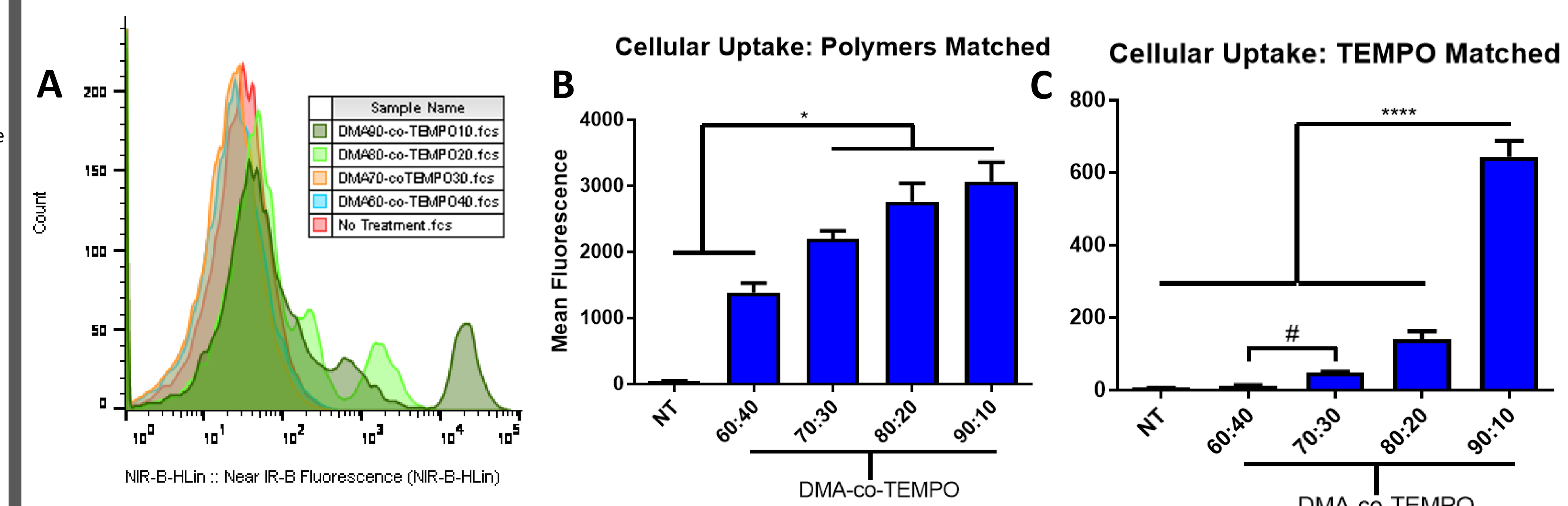
DMA-co-TEMPO libraries were synthesized at various ratios as previously described.

Cellular uptake, cell free, and *in vivo* superoxide scavenging experiments were utilized to assess the bioactivity of each polymer.

Results

Hydrophilic DMA-co-TEMPO Provides Maximum Cellular Uptake

Polymers fluorescently labeled at a 1:1 ratio were used to measure uptake via flow cytometry.

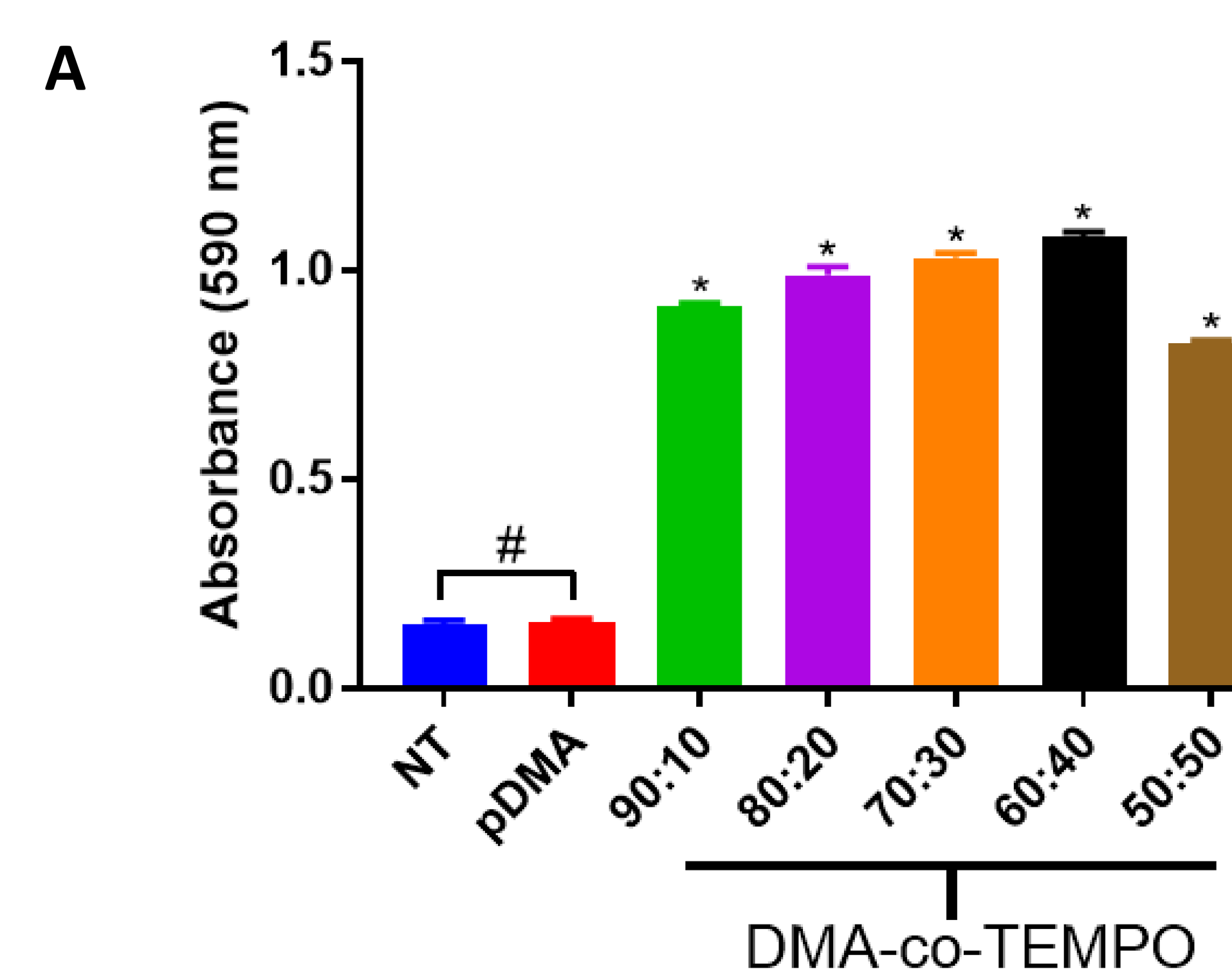


Uptake of DMA-co-TEMPO within RAW 246.7 cells. A.) Representative fluorescence histogram for flow cytometry data. **B.)** Hydrophilic polymers present higher cellular uptake with matched polymer doses, *p<0.05. **C.)** 90:10 DMA-co-TEMPO provides maximum cell uptake upon matching TEMPO dosage, *p<0.0001.

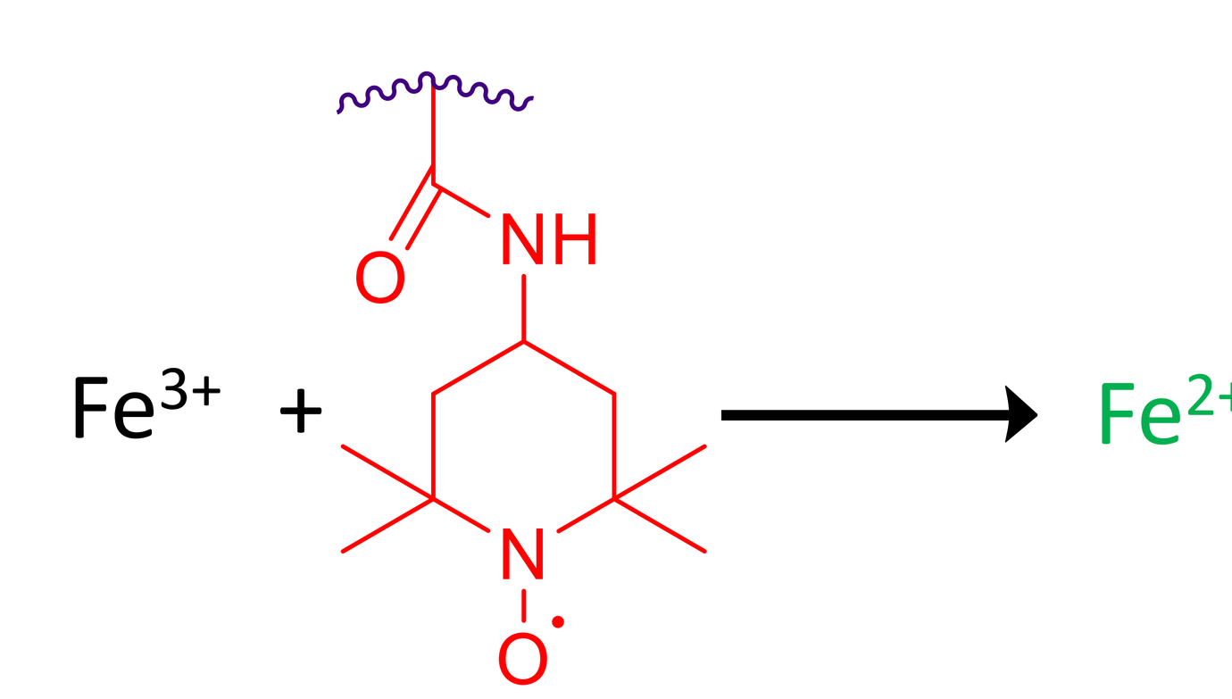
DMA-co-TEMPO Polymers Show Antioxidant Efficacy

Ferric Reducing Antioxidant Power (FRAP) assay was utilized to assess the effectiveness of the polymer libraries at stabilizing free radicals by monitoring iron reduction via UV-Vis.

DMA-co-TEMPO Reducing Potential: Mass Matched

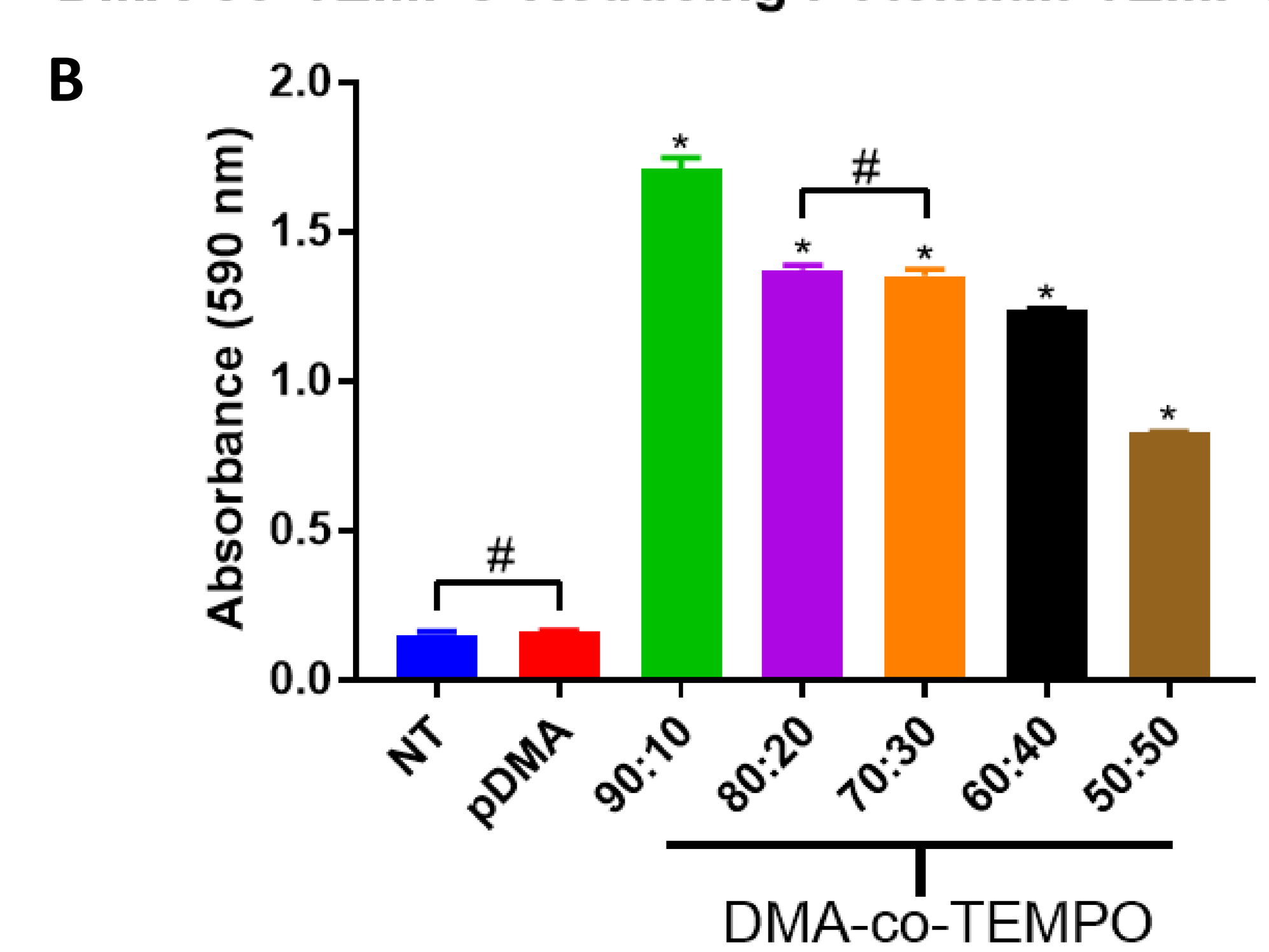


A.) 60:40 DMA-co-TEMPO polymers provide maximum antioxidant potential when matching polymer mass, *p<0.05.



FRAP mechanism. Reduced iron absorbs at 590 nm.

DMA-co-TEMPO Reducing Potential: TEMPO Matched

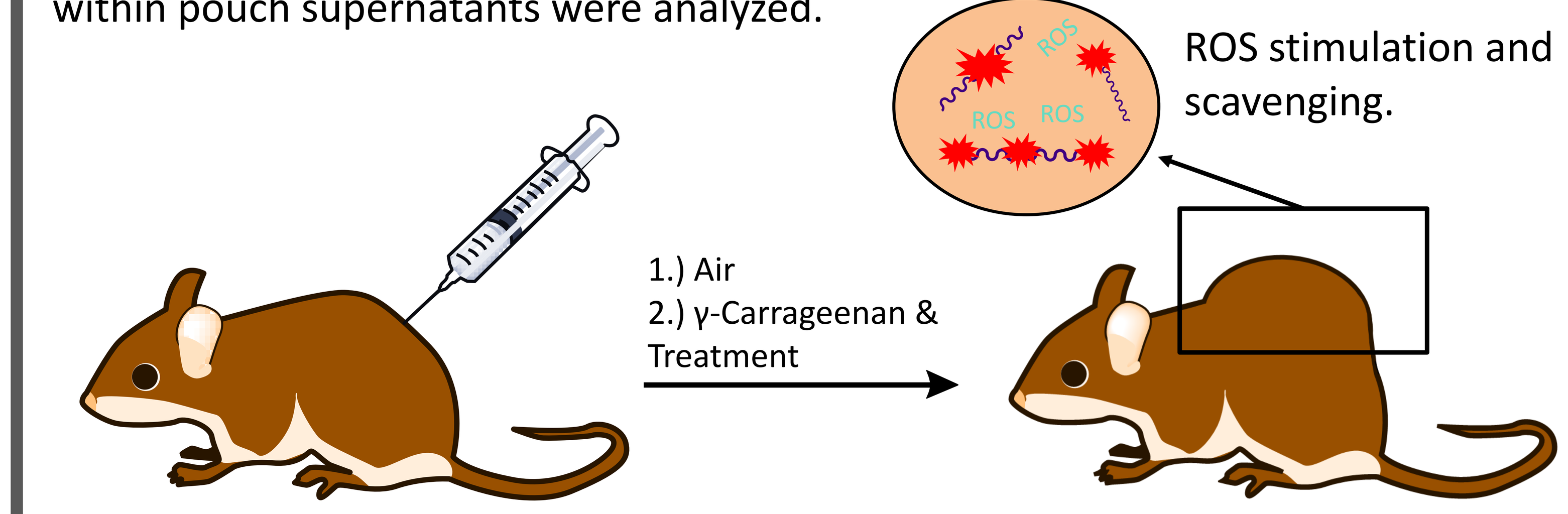


B.) 90:10 DMA-co-TEMPO shows maximum antioxidant potential for matching TEMPO dosage, indicating that 90:10 DMA-co-TEMPO provides optimum scavenging, *p<0.05.

Results

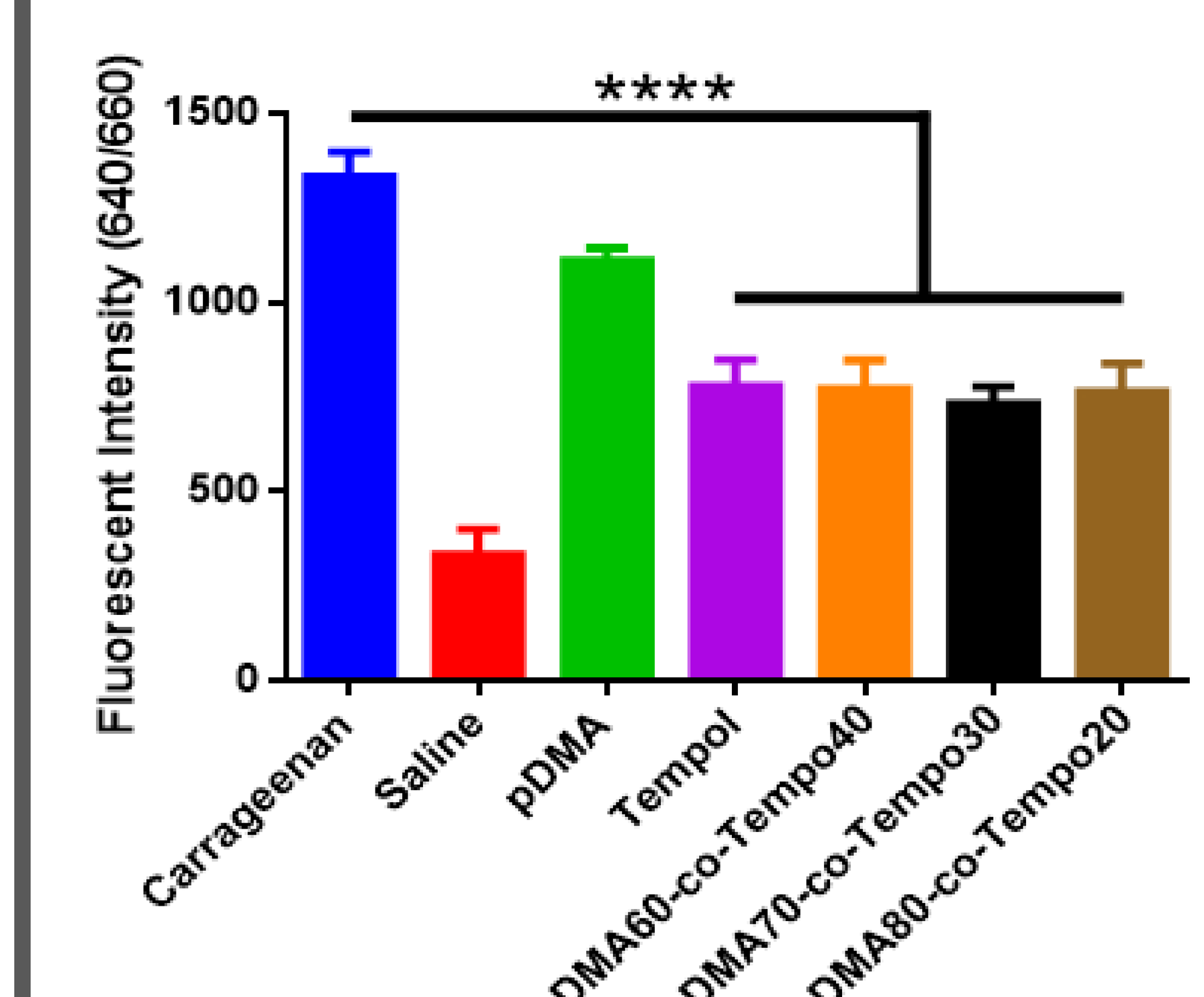
DMA-co-TEMPO Administers *in vivo ROS* Scavenging

Air pouches were inserted into mice and filled with a γ-carrageen solution to stimulate inflammation and ROS. Following DMA-co-TEMPO treatment, ROS levels within pouch supernatants were analyzed.



Air pouch formation and subsequent ROS activity

ROS Content Measured by ROSStar



***In vivo* scavenging via DMA-co-TEMPO polymers.** All treatments appear to provide significant scavenging potential compared to untreated mice and mice treated with polyDMA, *p<0.0001.

Conclusions and Future Work

DMA-co-TEMPO polymers provide cell free and *in vivo* scavenging. Results indicate that 90:10 DMA-co-TEMPO provides maximum TEMPO delivery in aqueous environments as well as maximum cellular uptake.

Further optimization of in vivo assays to distinguish responses of libraries.

In vivo PTOA experiments.

In vitro assays to specifically assess extracellular and intracellular bioactivity.

PPS-DMA-co-TEMPO microspheres to provide maximum ROS scavenging.

References

1 . Punzi, L., *et. al.* Post-traumatic arthritis: overview on pathogenic mechanisms and role of inflammation. *RMD Open* **2016,** *2* (2), 279-288.
2. Parthiban Periyasamy, *et. al.* Nanomaterials for the Local and Targeted Delivery of Osteoarthritis Drugs. *Journal of Nanomaterials* **2012,** *2012*,1- 13.

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