# A Multifunctional Solution Composed of TMPyP, 1,5-DHN, and Fe(III) Ions Produces One or More Reactive Oxygen Species (ROSs) in Aerobic, Anaerobic, and $H_2O_2$ Rich Environments

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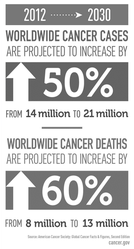
## ABSTRACT

Here, we report a multifunctional solutions which is comprised of Fe(III) ions, cationic meso-tetra(N-methyl-4-pyridyl)porphine tetrachloride (TMPyP), and 1,5-dihydroxynapthalene (1,5-DHN) at a mole ratios of 1.0:20:17, respectively in aqueous solution. The multifunctional solution provides methods for producing *in situ* one or more reactive oxygen species (ROSs) such as, singlet oxygen ($^1O_2$), hydroxyl radical (ȮH), and a napthoquinone derivative, 5-hydroxy-1,4-naphthalenedione (Juglone) in both aerobic, anaerobic, and $H_2O_2$ rich conditions in presence of or absence of visible light. The investigation of photodynamic properties of the multifunctional solution revealed that it produces $^1O_2$, ȮH, and Juglone in aerobic conditions and it forms ȮH, and Juglone in anaerobic conditions under visible light irradiation. Furthermore, the multifunctional solution was found to generate ȮH, and Juglone in $H_2O_2$ rich environment in the absence of visible light. A 20-fold increase in the rate of photooxidation of 1,5-DHN by Fe(III)/TMPyP in aerobic conditions and a 2.0 fold increase in the rate of photooxidation of 1,5-DHN by Fe(III)/TMPyP in anaerobic conditions were observed when compared to the rate of 1,5-DHN photooxidation by Fe(III)TMPyP in the presence of visible light. Further experimentations revealed that the multifunctional solution with an optimum Fe(III) concertation of 25 µM reacted with 400 µM $H_2O_2$ and produced ȮH, Juglone, and oxygen gas ($O_2$) in the absence of light without forming $Fe(OH)_3$ sludge while Fe(III)TMPyP under identical conditions formed a sludge of $Fe(OH)_3$ and became ineffective. Finally, the high fluorescence quantum yield (0.120) suggest that the multifunctional solution is a good diagnostic agent in parallel to be a potential cancer therapeutic agent.

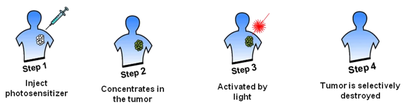
## INTRODUCTION




- Common Methods for Cancer Treatment.
- Radiation, chemotherapy, surgery found to be very successful but have serious life-threatening side-effects
- Photodynamic Therapy (PDT), an effective and noninvasive cancer treatment method

HOW DOES PDT WORK?

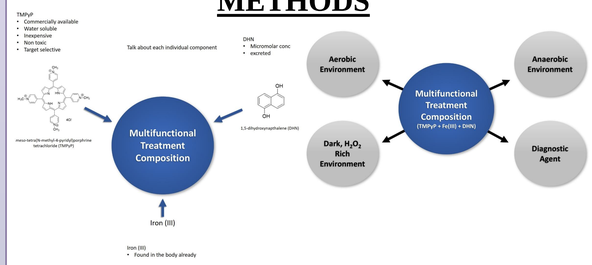
Photosensitizer + $O_2$ + Visible Light → Singlet Oxygen ($^1O_2$)




PDT LIMITATIONS

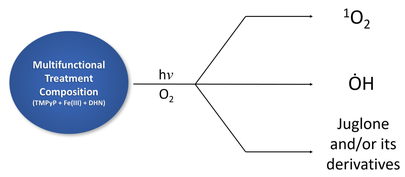
- Poor water solubility, involves complex organic/inorganic synthesis
- Tumor hypoxia, ineffective in absence of visible light

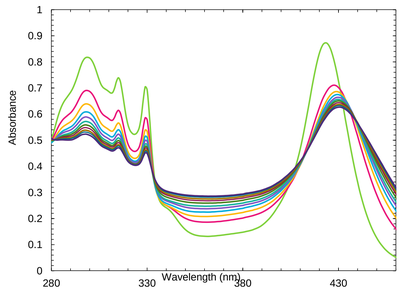
## MULTIFUNCTIONAL SOLUTION & METHODS

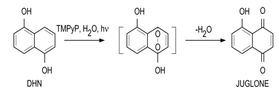



## RESULTS

## AEROBIC ENVIRONMENT:

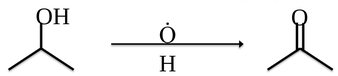


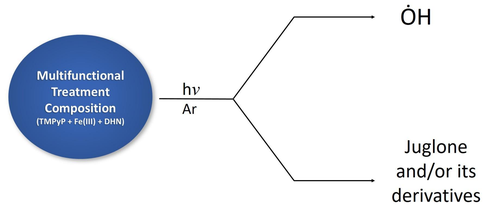




| Solution of DHN and TMPyP with | Rate constant, $k_{obs}$ ($s^{-1}$) | $R^2$ |
|---|---|---|
| No Fe (III) ions | $6.58 \times 10^{-4}$ | 0.8951 |
| $2.0 \times 10^{-6}$ M Fe (III) | $3.90 \times 10^{-4}$ | 0.8321 |
| $4.0 \times 10^{-6}$ M Fe (III) | $5.27 \times 10^{-4}$ | 0.8507 |
| $3.0 \times 10^{-5}$ M Fe (III) | $7.15 \times 10^{-4}$ | 0.9505 |
| $5.0 \times 10^{-5}$ M Fe (III) | $7.98 \times 10^{-4}$ | 0.9495 |
| $1.0 \times 10^{-4}$ M Fe (III) | $9.43 \times 10^{-4}$ | 0.9422 |
| $1.5 \times 10^{-4}$ M Fe (III) | $5.68 \times 10^{-4}$ | 0.9018 |

- Hydroxyl radical production by multifunctional solution was chemically detected by 2-propanol



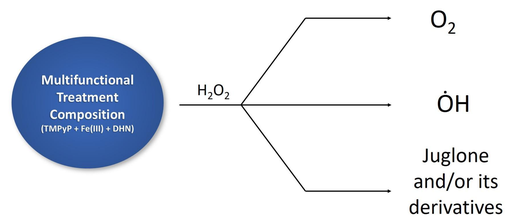

## ANAEROBIC ENVIRONMENT:



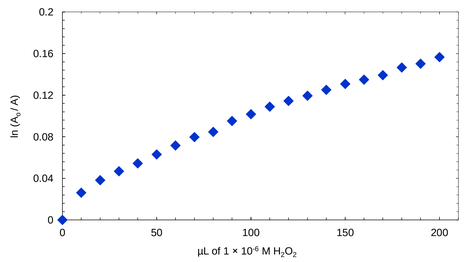

TMPyP + DHN + [ $Fe^{3+}$] + light → Juglone

| Solution of DHN and TMPyP with | Rate constant, $k_{obs}$ ($s^{-1}$) | $R^2$ |
|---|---|---|
| No Fe (III) ions | $1.80 \times 10^{-5}$ | 0.8642 |
| $2.0 \times 10^{-6}$ M Fe (III) | $2.80 \times 10^{-5}$ | 0.9783 |
| $4.0 \times 10^{-6}$ M Fe (III) | $2.70 \times 10^{-5}$ | 0.8177 |
| $3.0 \times 10^{-5}$ M Fe (III) | $5.80 \times 10^{-5}$ | 0.9221 |
| $5.0 \times 10^{-5}$ M Fe (III) | $7.0 \times 10^{-5}$ | 0.9169 |
| $1.0 \times 10^{-4}$ M Fe (III) | $1.9 \times 10^{-4}$ | 0.9434 |
| $1.5 \times 10^{-4}$ M Fe (III) | $1.13 \times 10^{-4}$ | 0.9579 |

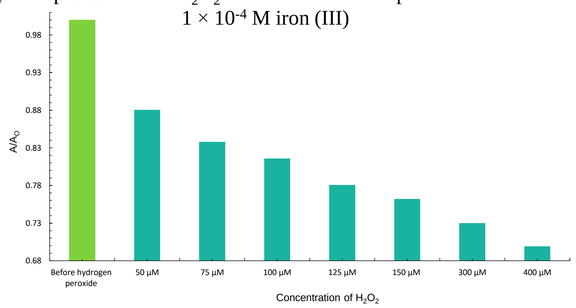
## DARK HYDROGEN PEROXIDE ENVIRONMENT:




Fe (III) + TMPyP + $H_2O_2$ → ȮH → Juglone (DHN)




Optimization of $H_2O_2$ concentration in the presence of $1 \times 10^{-4}$ M iron (III)




## FLUORESCENCE PROPERTIES :

| Solution | Fluorescence Quantum Yield |
|---|---|
| TMPyP | 0.125 |
| TMPyP w/ Fe(III) | 0.120 |

- Fluorescence quantum yields over 0.01 are sufficient enough to facilitate measurements for tissue distribution

## CONCLUSIONS

- Cheap and simple to synthesize
- Soluble in biological media
- Strong photochemical activity
- Effective in hypoxic conditions
- Effective in absence of visible light
- High singlet oxygen quantum yield
- Local accumulation in tumor tissue

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## REFERENCES

International Application (PCT) titled “Multifunctional Treatment and Diagnostic Compositions and Methods” in the US patent and trademark office before the US receiving office, International Application Number PCT/US18/24338, March 28, 2018. PCT application published on 10/3/2019 in WIPO PatentScope data base (International Application No.: PCT/US2018/024338)