Egyptian Blue Nanosheets as a Novel Bioimaging Agent

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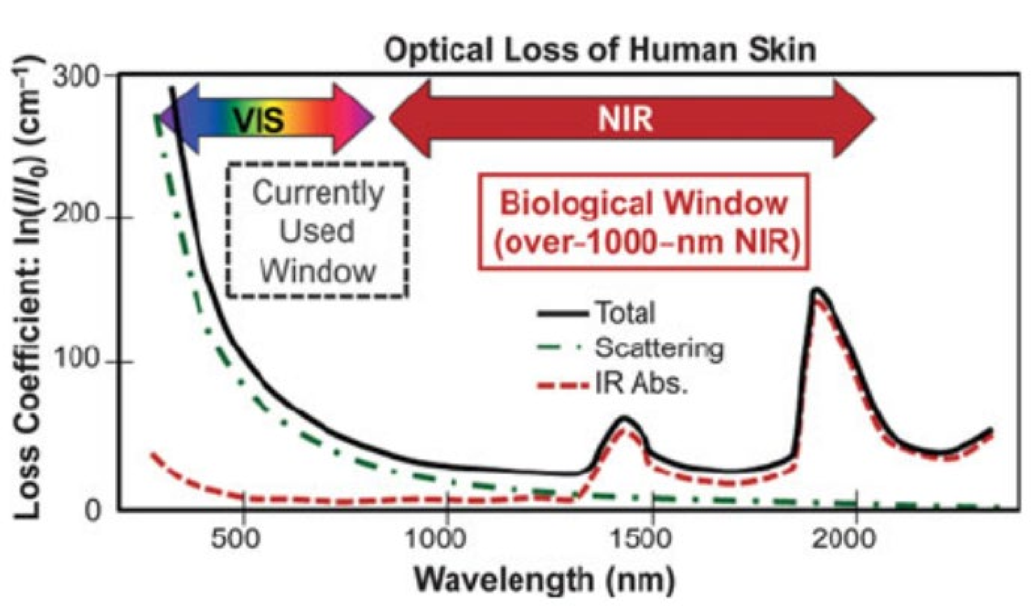
Abstract

Since the ancient times, a common pigment used for expression in clothes and art was Egyptian blue (EB). Today, instead of using this cuprous silicate as a way for one's personal expression, we will provide reasons why this pigment can be used as a novel bioimaging agent for cell work. Finding another bioimaging agent for cell-use is always an advantage because each agent supplies their own advantages when working in cells. So the more agents we have in our possession, the more angles we can take on a problem. To be considered a bioimaging agent, it needs to dissolve in polar solvents (mainly water), be non-toxic, and display fluorescence in the near-infrared range of the optical spectrum. EB has all three of these properties with the right preparation. Sonicating EB reduces their size to become extremely small sheets, which increases interaction with water molecules to ultimately allow the sheets to dissolve within the water solvent. These sheets are on the nanoscale, so they will be referred to as EB nanosheets (EBNS). EBNS fluoresce in the near infrared and have no history of being toxic. EBNS have the capability of emitting more photons per photons absorbed compared to most materials (high quantum efficiency). This novel material also does not quench fluorescently as easily as other agents due to its copper atoms. We want to highlight why EBNS can be an effective platform for future bioimaging applications and ultimately, cancer imaging/treatment applications.

Motivation

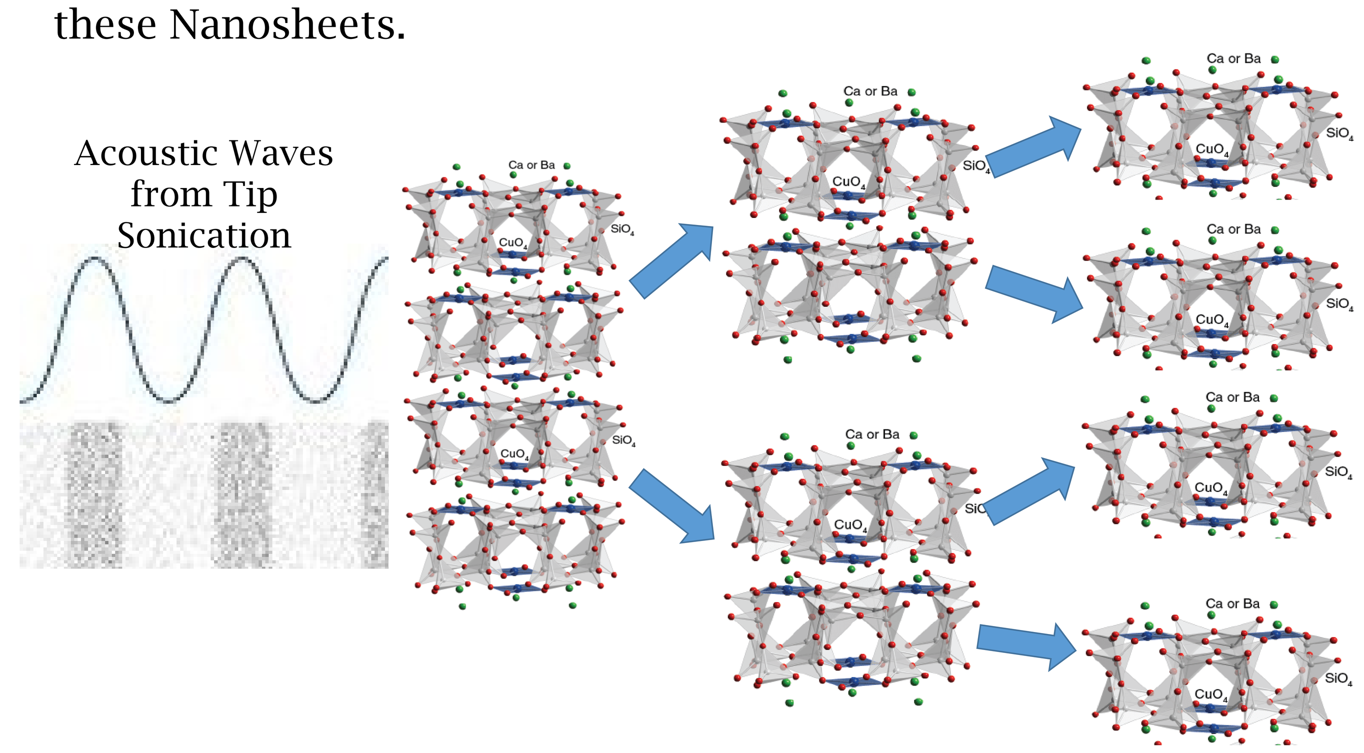
- **Bioimaging:** utilizing near-infrared light (NIR) is more beneficial



- Less tissue scattering and absorbance in the biological windows (900-1000 nm)
- Less likely to photo bleaching due to their luminous centers and high quantum efficiency. Other commonly used dyes photo bleach quickly and is a problem.
- Egyptian blue is a common dye used on clothing for thousands of years, there is no background of it being toxic to humans.
- **Photothermal Therapy:** EBNS's property of converting NIR radiation into heat while being photo stable gives high potential as a Photo Thermal Therapy agent.
- **Goal:** Create a new versatile fluorophore that has multiple applications in the body.

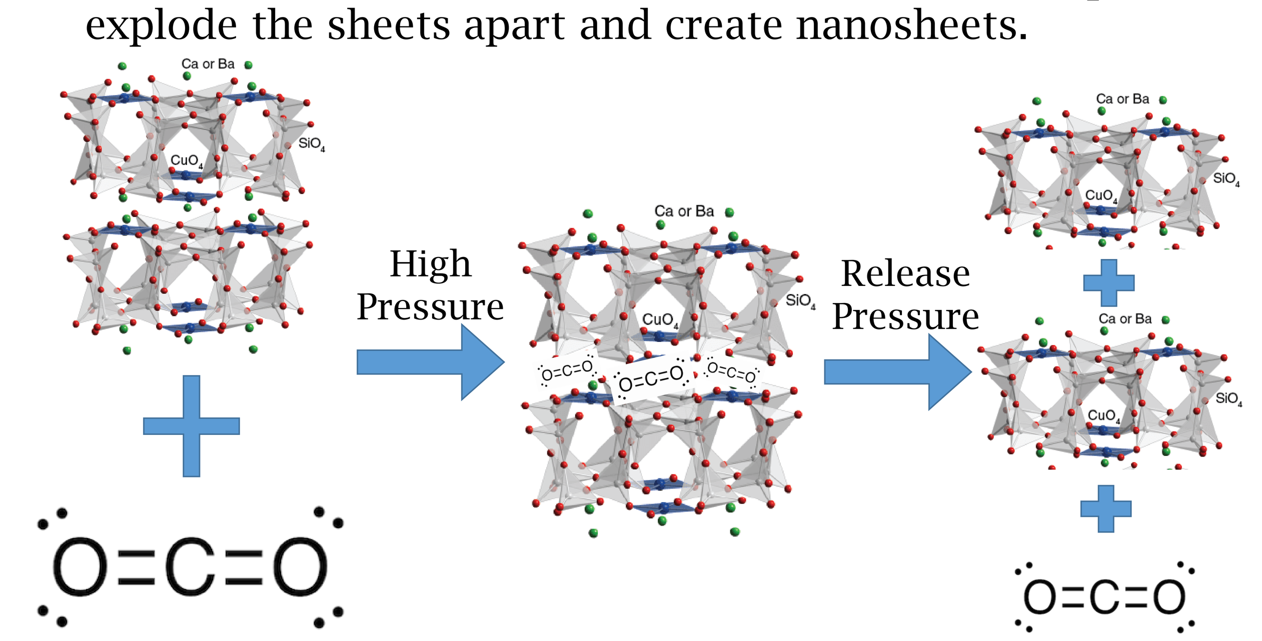
Synthesis 1 – Tip Sonication

- Nanosheets are made through a tip sonication of the original dye and extensive mortar and pestling.
- There are different methods to exfoliation, but we have found great success with tip sonication.
- Tip sonication provides acoustic waves that break the weaker bonds of the material, allowing them to split into "sheets."
- After six hours of tip sonication treatment, the originally micron sized dye turns into a nanomaterial with a sheet structure. We call these Nanosheets.

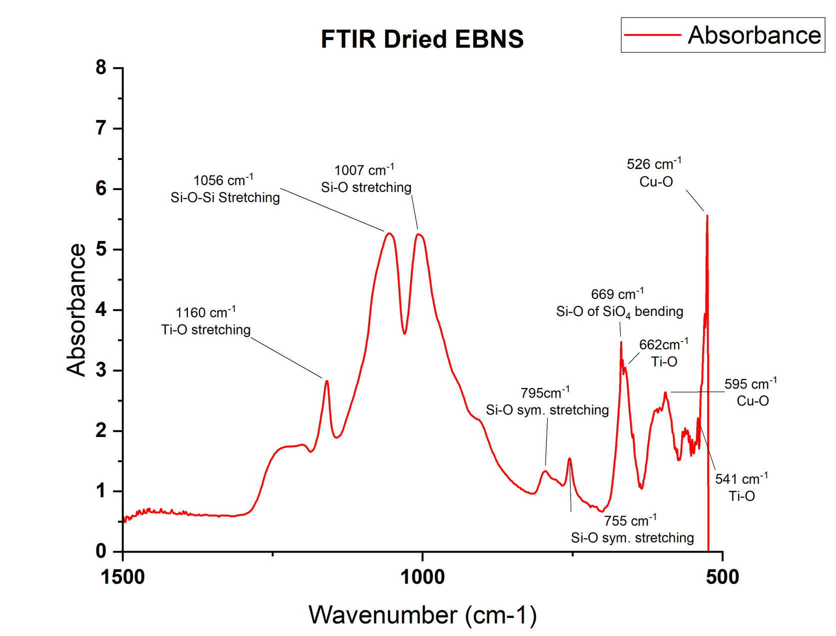


Synthesis 2 – High Pressure CO_2

- Another synthesis that we tried was using high pressure CO_2 in an attempt to "explode" the layers apart
- After grinding the original precursor down with a mortar and pestle, we mix the grinded precursor in DI water and put it into an autoclave. We also add dry ice to the autoclave and heat the sample up to 200 Celsius.
- After the sample was allowed to reach extreme pressures, some of the CO_2 should find itself in between the layers of the sheets.
- Finally, we release the pressure quickly and the CO_2 should explode the sheets apart and create nanosheets.

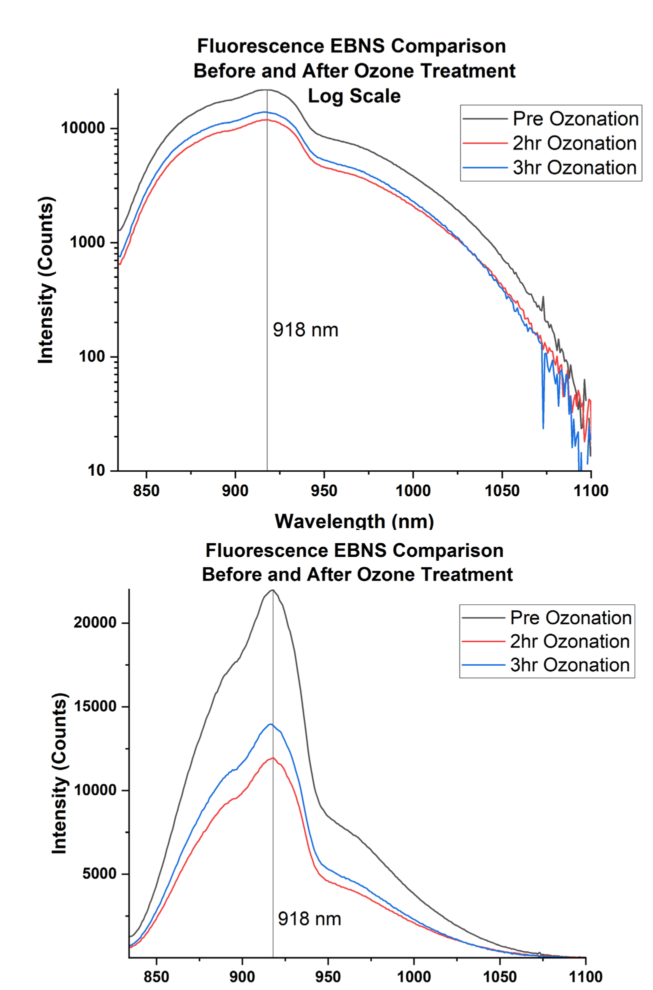


Fourier Transform Infrared Spectroscopy:



- This was the first of many steps to ultimately characterize what was made.
- FTIR is a study of vibrational modes activated through IR electromagnetic waves.
- Each bond in a material has a specific frequency it will vibrate at and these peaks tell us this information.
- After research, we can figure out which peaks are actually which bond vibration.
- With our FTIR, we have given another data point to what is inside our material.

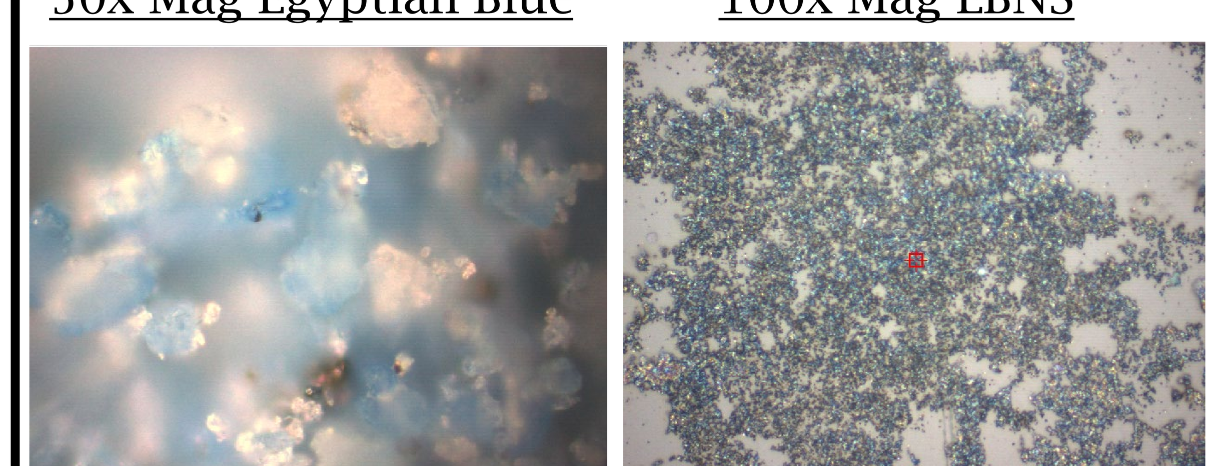
EBNS Fluorescence and the Effects of Ozonation:



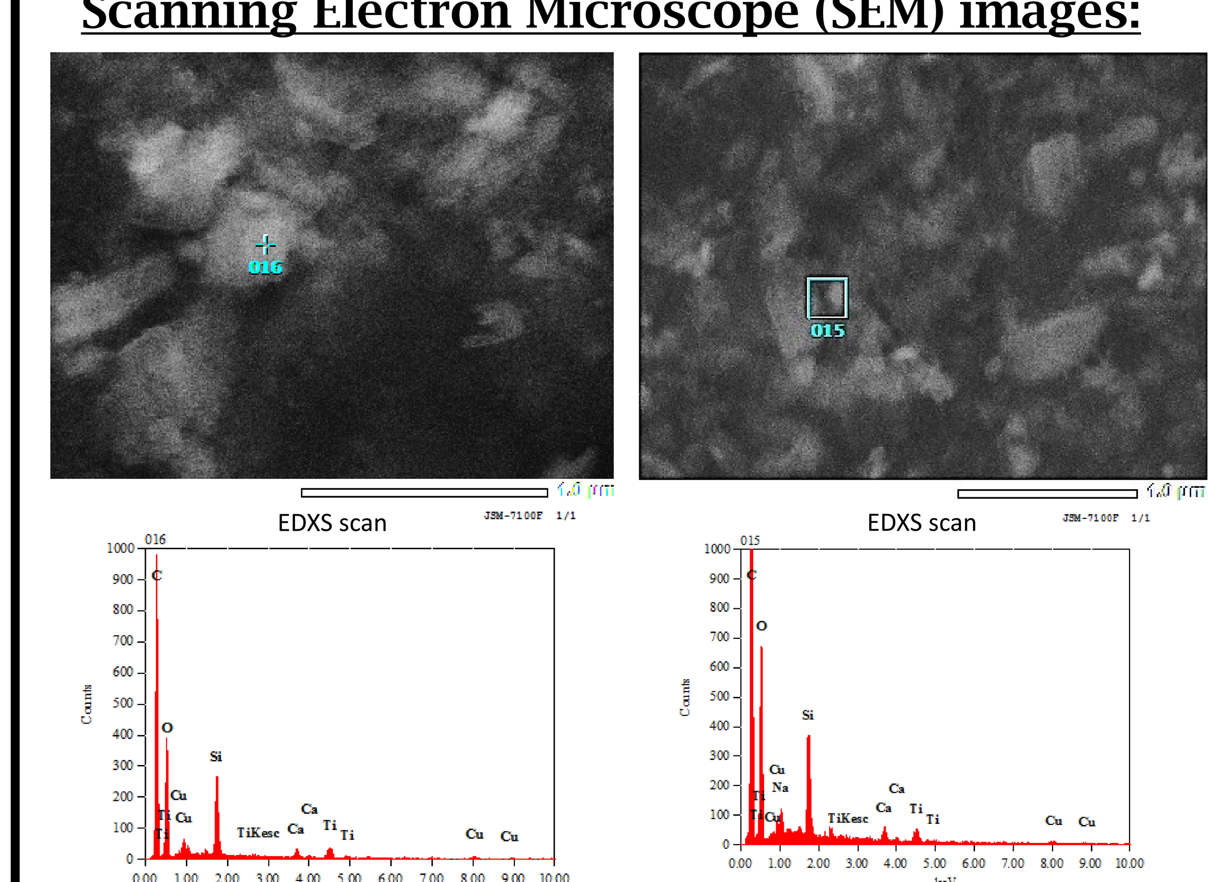
- 808nm Excitation
- Fluorescence of EBNS is in biological window (918nm).
- Ozonation does not produce any new luminescent spectral features, just changes overall intensity.

SEM/Photo Images of EBNS:

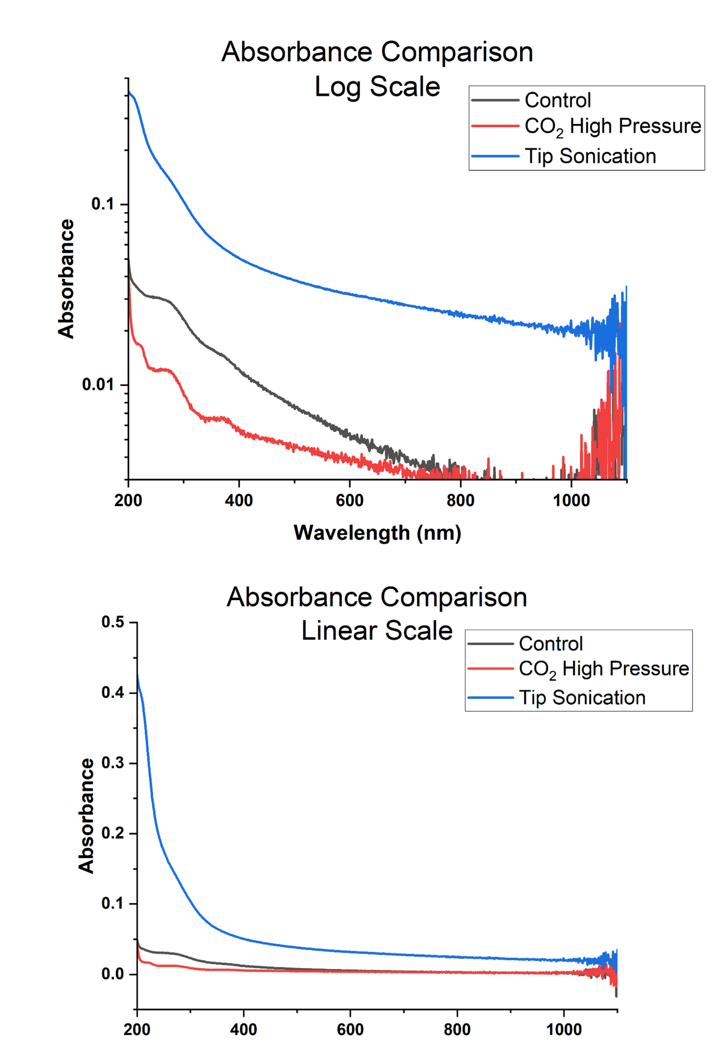
50x Mag Egyptian Blue   100x Mag EBNS



Scanning Electron Microscope (SEM) images:



Absorption Between 1 & 2:



- These absorbances were collected from aqueous solutions of each respective synthesis
- Control is Egyptian Blue in DI water
- The point of absorbance measurements is the more EB dissolved, the larger the absorbance.

Conclusion

So far, we have been able to successfully characterize what our material is made out of along with its contaminations. We have confirmed fluorescence within the preferred window (918nm). To eliminate contamination, we plan to use a filter that does not allow big titanium and aluminum pieces through.

As for the high-pressure CO_2 method, it does not have the properties that we seek due to its low solubility in DI water. Also, according to the absorption curves, we seem to have introduced a new peak with this method. This implies that a new structure may have been made in our material.

Following promising papers describing the material and proving its effectiveness of NIR imaging in plants and fly embryos, we hope to continue the project toward the goal of using it to image and treat cancer cells.

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Egyptian Blue (EB) pigment has potential to be used as a novel bioimaging agent for cell/cancer work. EB is nontoxic, displays fluorescence that penetrates cell walls, and can be soluble in water. However, EB is too big for cell work. We reduce the structure by tip sonication to turn them into nanosheets (EBNS). EBNS have the capability of emitting a surplus of photons without losing this intensity over long periods of time. Current bioimaging dyes don't have this property, making EBNS useful for long-term imaging. Eventually, we want to prove that EBNS can be used in cancer imaging/treatment applications.