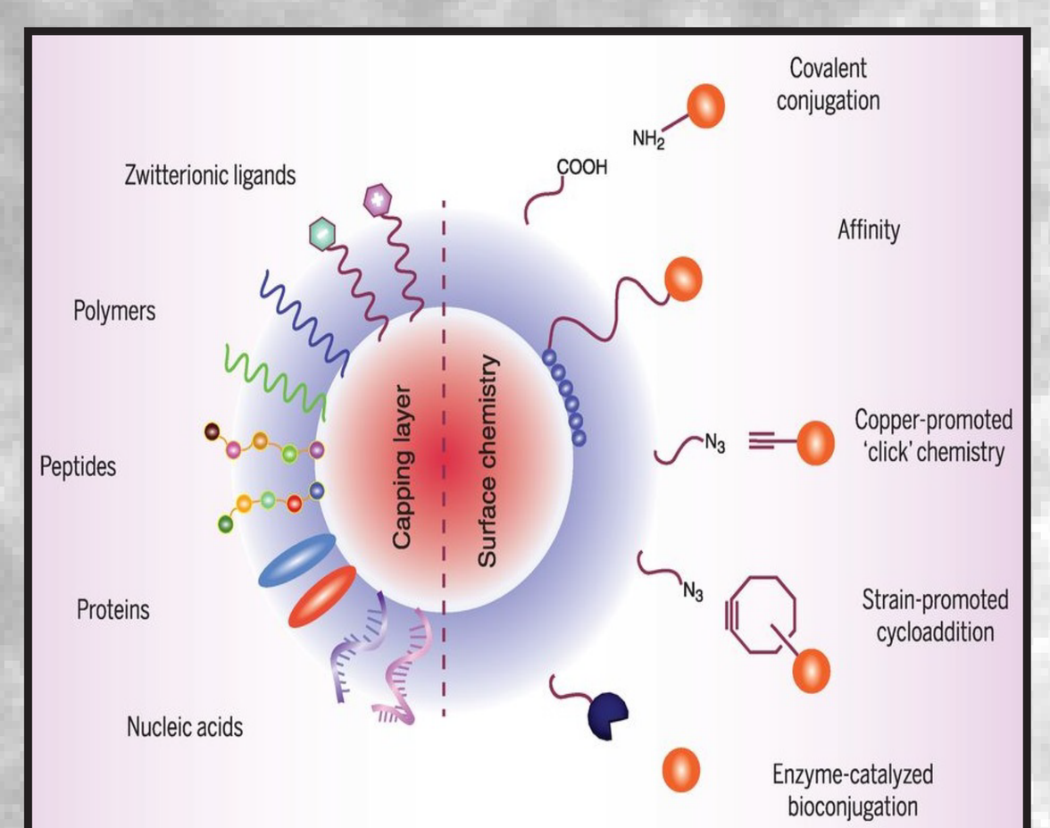
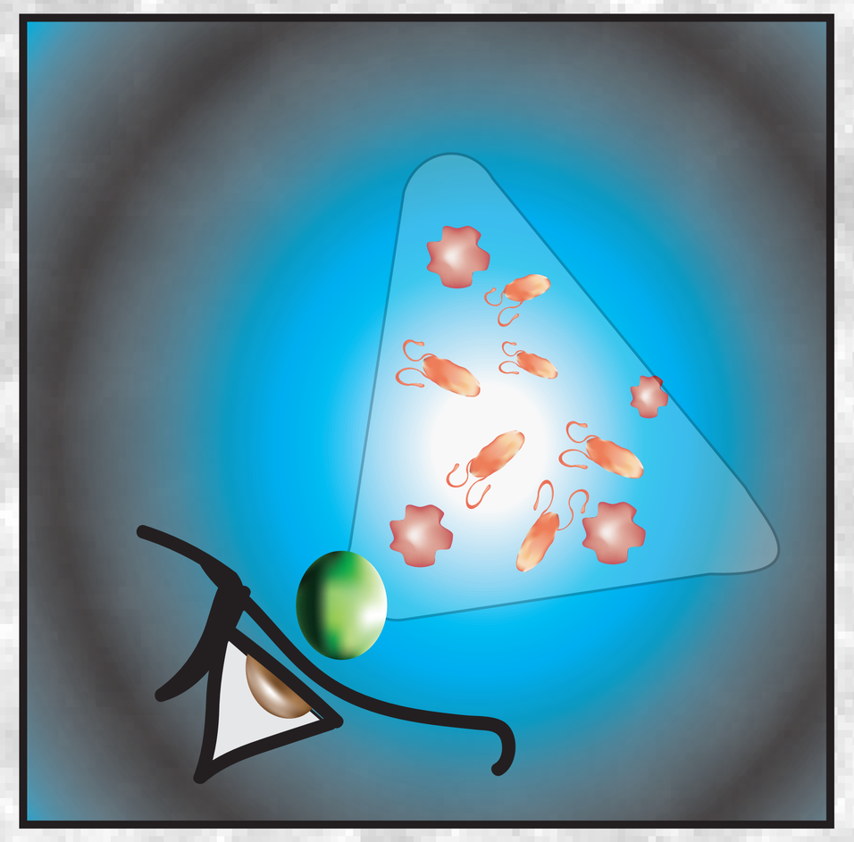
Cu_2S Nanocrystals as Signal Amplifiers for Biomolecule Detection

Louis K. Apraku-Boadi[1], Evan H. Robinson[2], and Janet E. Macdonald[2]
[1]Department of Chemistry, University of West Georgia, Carrollton, GA 30118
[2]Department of Chemistry, Vanderbilt University, Nashville, TN 37235
VANDERBILT
UNIVERSITY of West Georgia
College of Science and Mathematics

Molecular Telescopes

Due to their unique chemical properties, nanocrystals have gained increasing attention and application in molecular and cellular biology. Through chemical manipulation, they allow investigators to observe nano and micro scale environments.



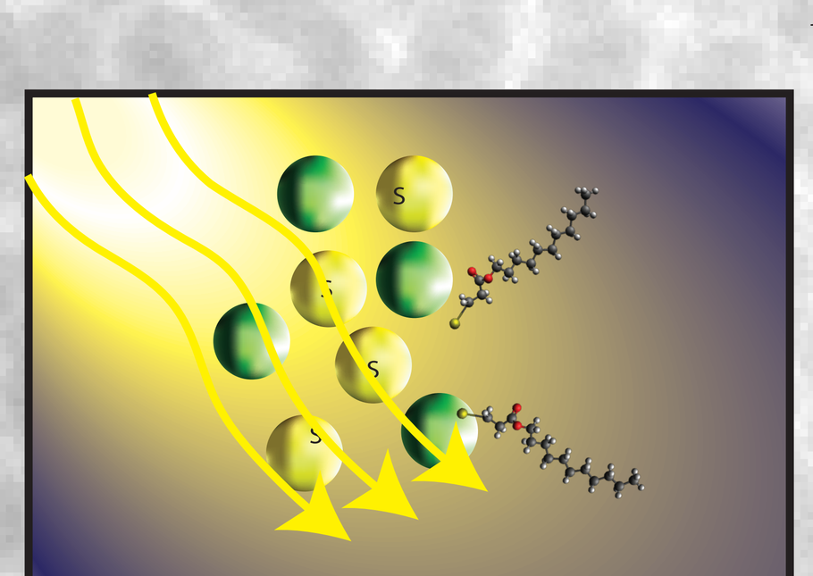


Bioprobes

By immobilizing a specific protein, enzyme, nucleic acid or other substance onto its surface, a nanocrystal can be tuned to recognize a wide range of molecules. This allows on-site, "instrument free" detection, making nanocrystals valuable diagnostic tools.[1]

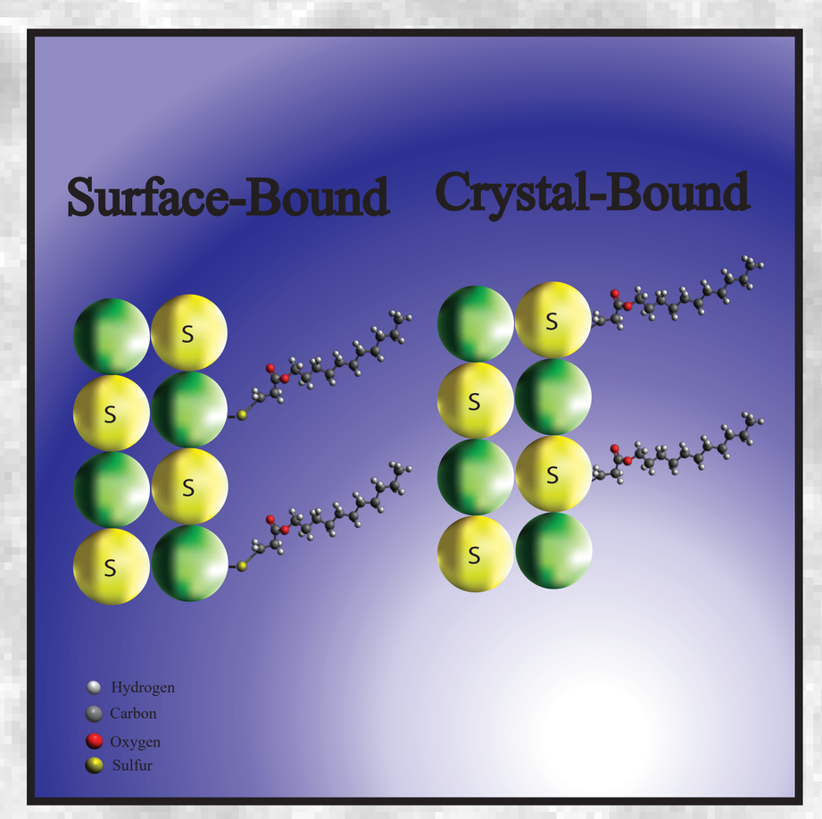
Ligand Chemistry

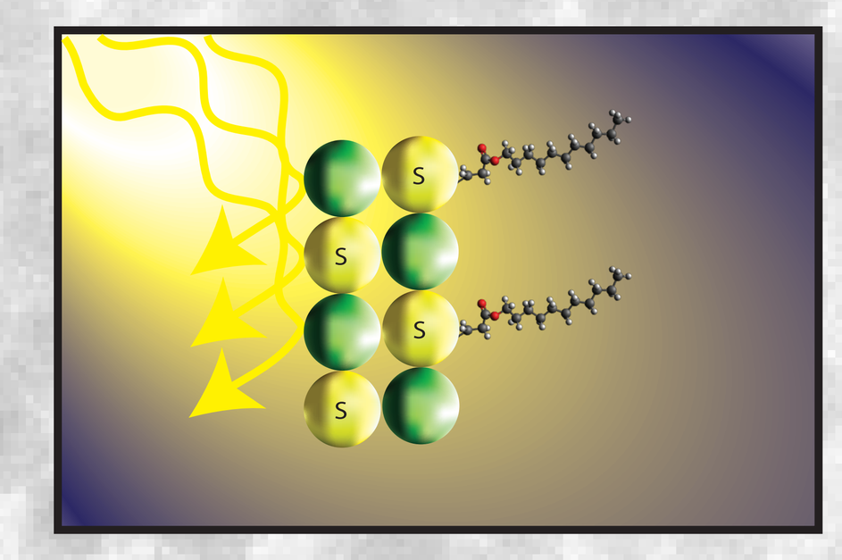
The properties and stability of nanoparticles are influenced to a great extent by the ligands attached to their surface.



Surface-Bound Nanocrystals

Surface-bound ligands weakly interact with crystal surfaces. This makes them susceptible to degradation by light, air, and ligand exchange.

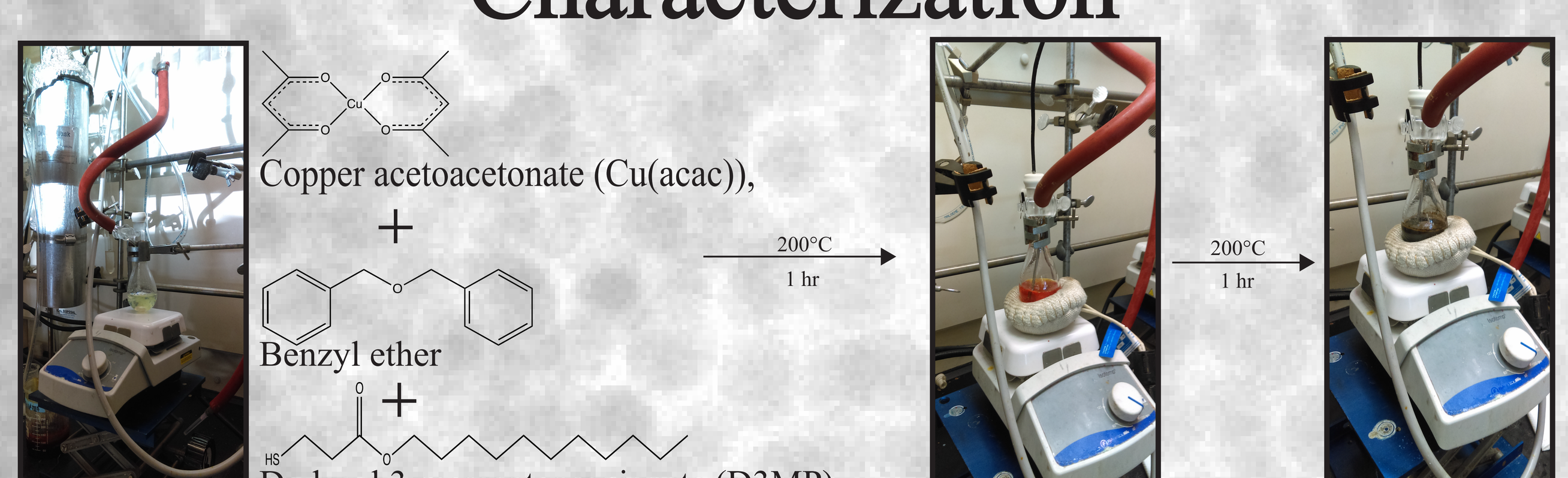




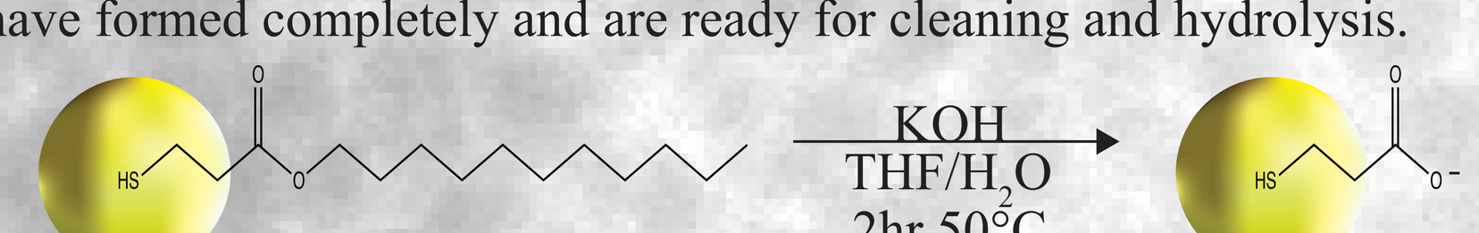
Crystal-Bound Nanocrystals

By directly inserting ligands into the structure of nanocrystals, surface reactivity is reduced thereby increasing structural stability.

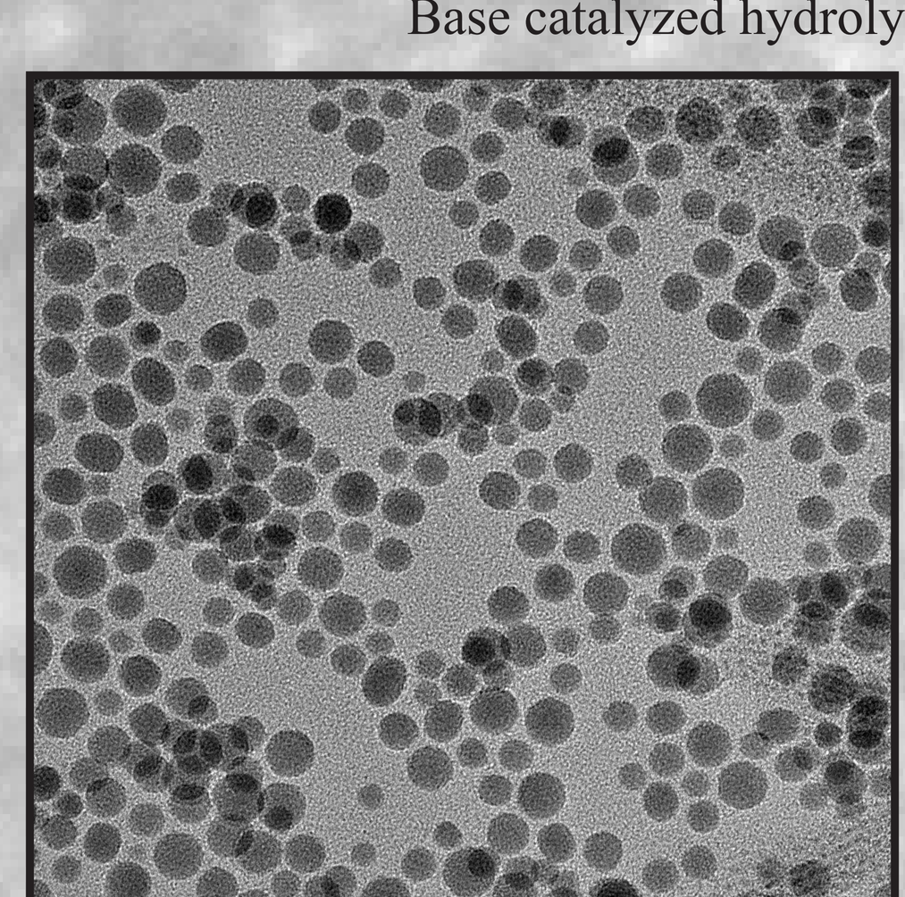
Nanocrystal Synthesis, Hydrolysis and Characterization



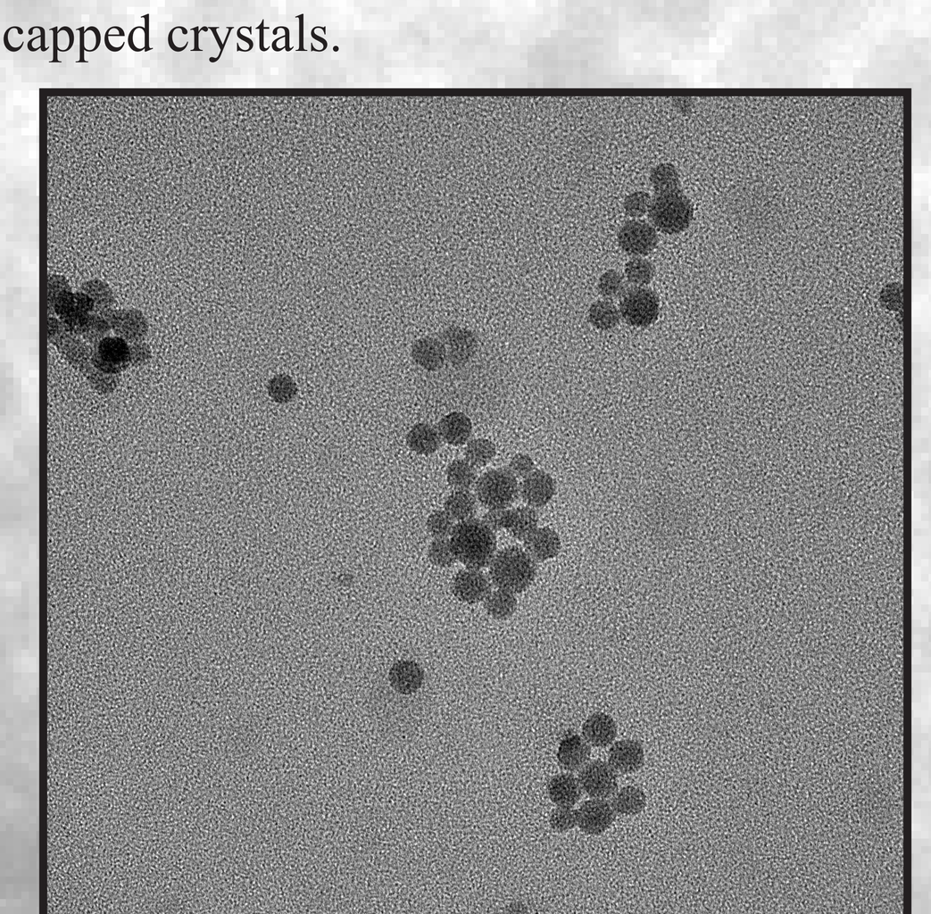
D3MP is the ligand, Cu(acac) serves as a copper source, and benzyl ether is a non-coordinating solvent that offers control of nanocrystal size. After one hour, the solution turns a bright orange, signifying the initial stages of nanocrystal formation. Once the solution obtains a black color, the nanocrystals have formed completely and are ready for cleaning and hydrolysis.



Base catalyzed hydrolysis of D3MP capped crystals.

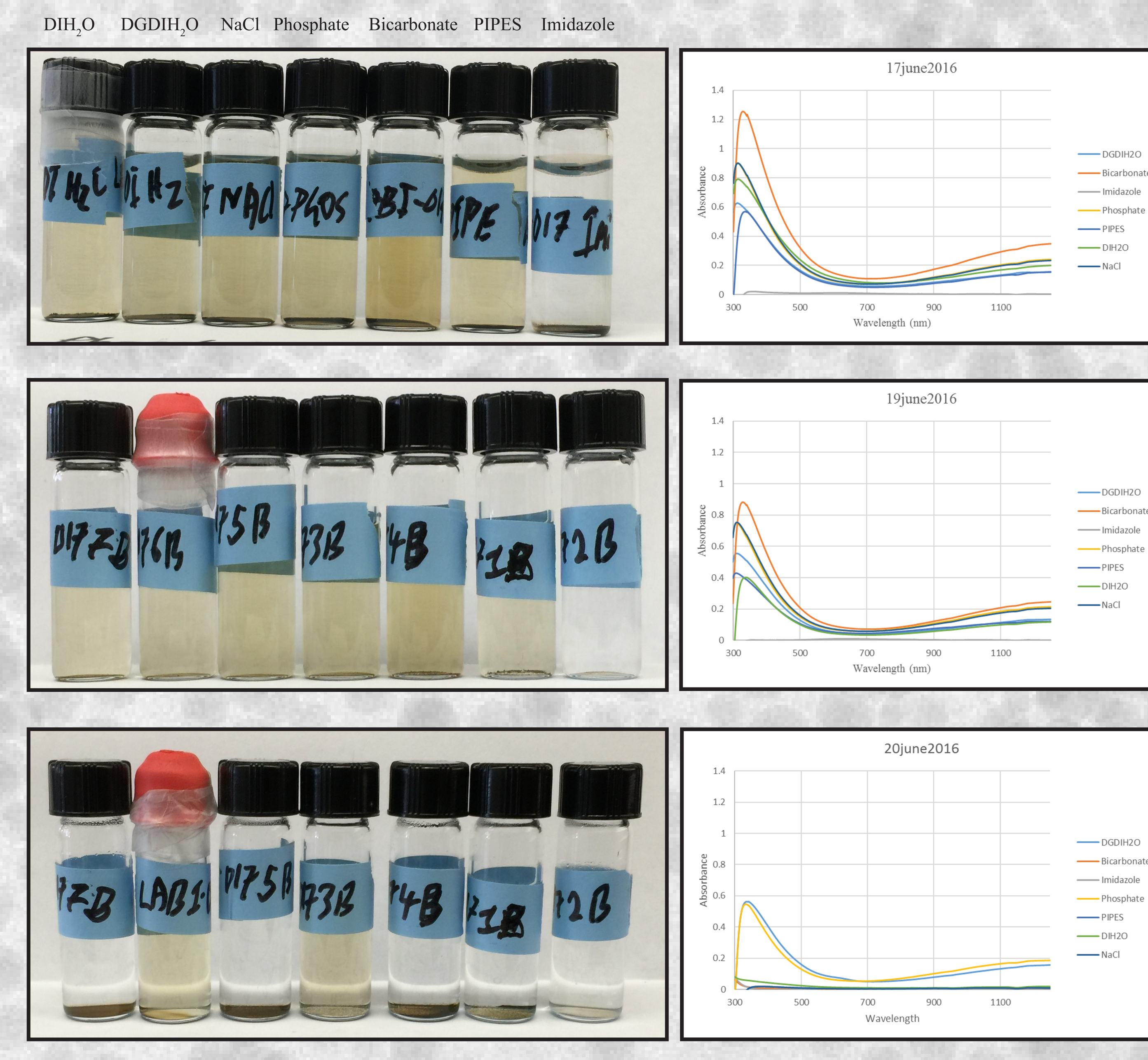


TEM image of post synthetic nanocrystals (d=11±1.2, n=150)



TEM image of post hydrolysis nanocrystals (d=9±2, n=150)

Nanocrystal Stability

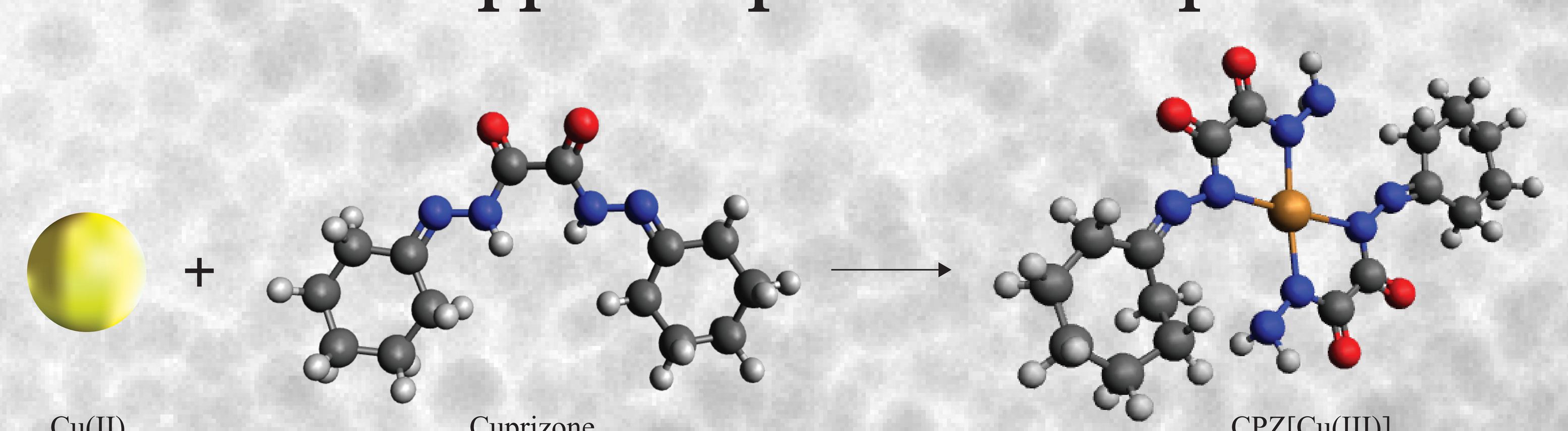


To test the stability of crystal-bound nanocrystals, the particles were immersed in 10 mM buffers at pH 7. The progressive loss in absorbance of the solutions was correlated to NC stability in a particular solution.

Buffer	Concentration	Stability (days)	pH
Degassed DI H_2O ($DGDIH_2O$)	N/A	31	5.5
Bicarbonate	10 mM	3	7
Imidazole	10 mM	0	7
Phosphate	10 mM	6	7
PIPES	10 mM	3	7
DI H_2O	N/A	3	5.5
NaCl	10 mM	3	5.5

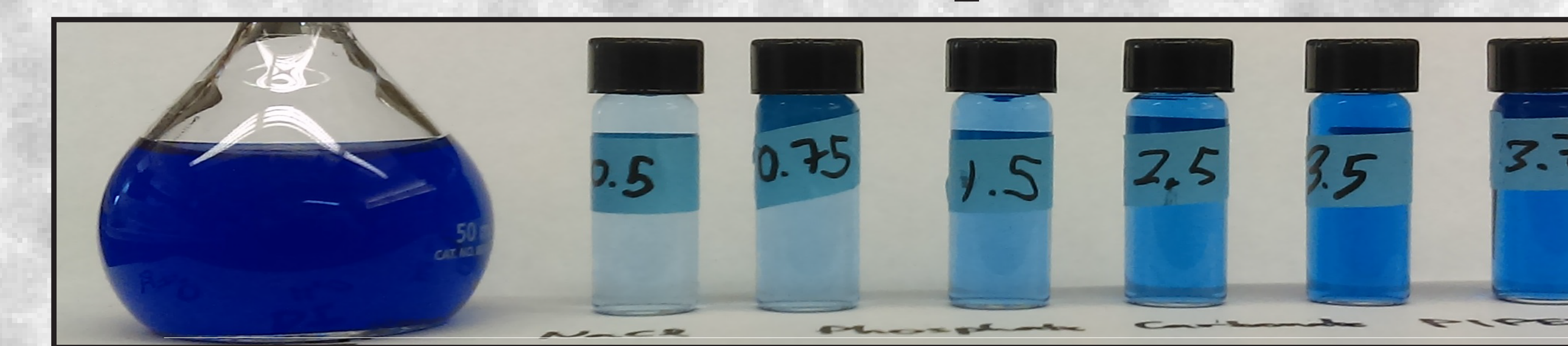
The $DGDIH_2O$ flask was evacuated, bubbled through with argon,and left under argon overnight on a daily basis. Hypothetically, this prevents the surfaces of the particles from being oxidized by oxygen in the air. No other solution was given the same treatment.

The Copper-Cuprizone Complex

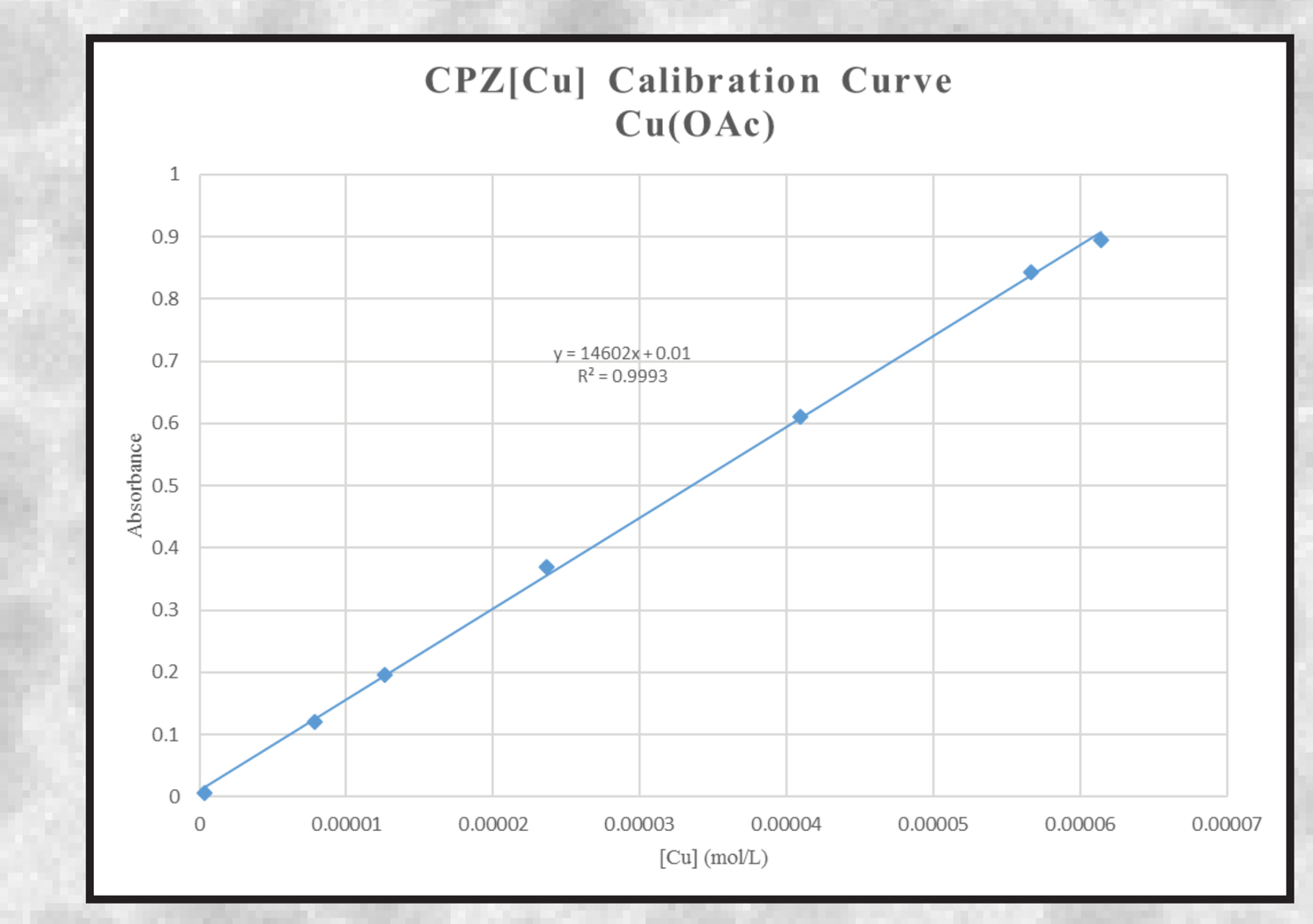


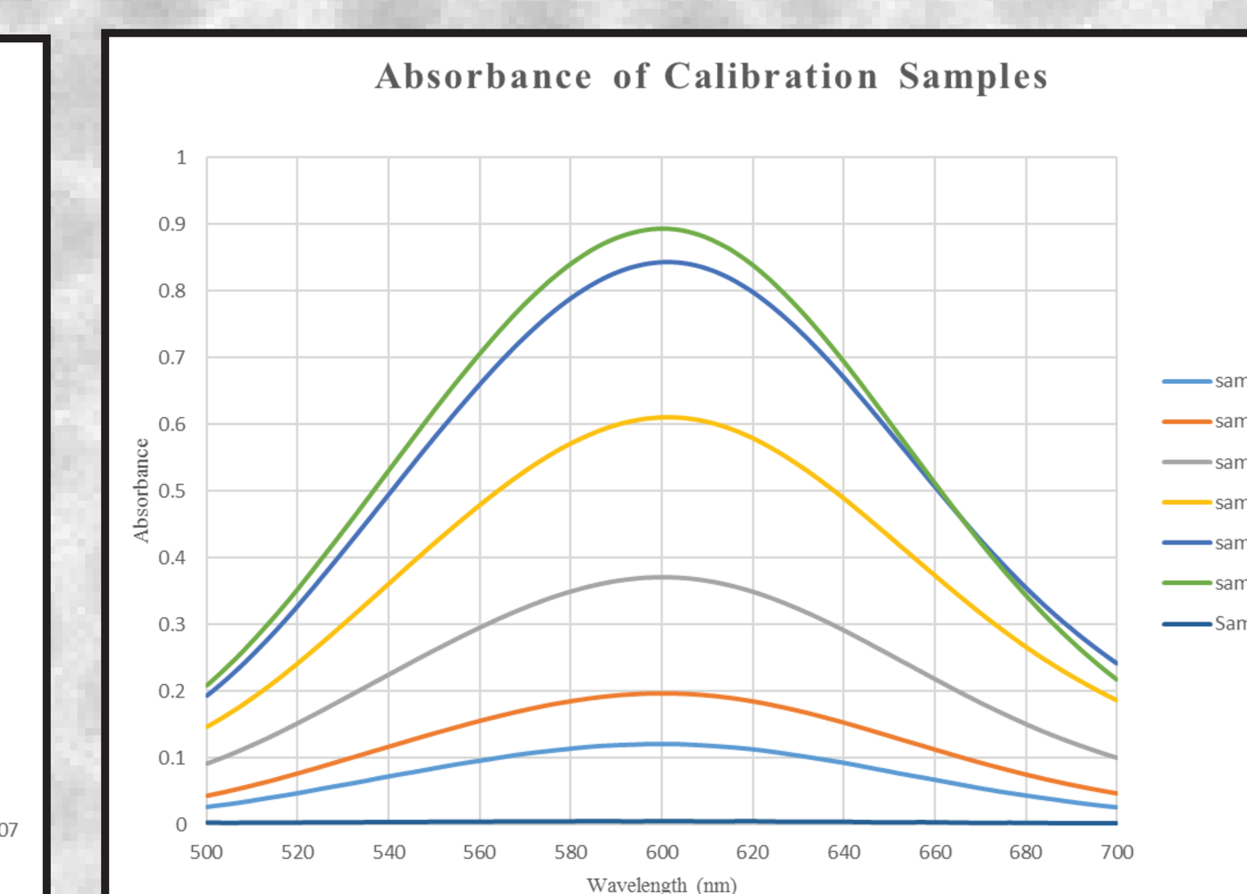
Cuprizone (Bis(cyclohexanone)oxaldihydrazone) forms a chromogenic bidentate complex with copper. This fact is often exploited for the accurate quantification of copper. The complex has an intense and characteristic absorption band centered at 600 nm.

Molecular Reporter

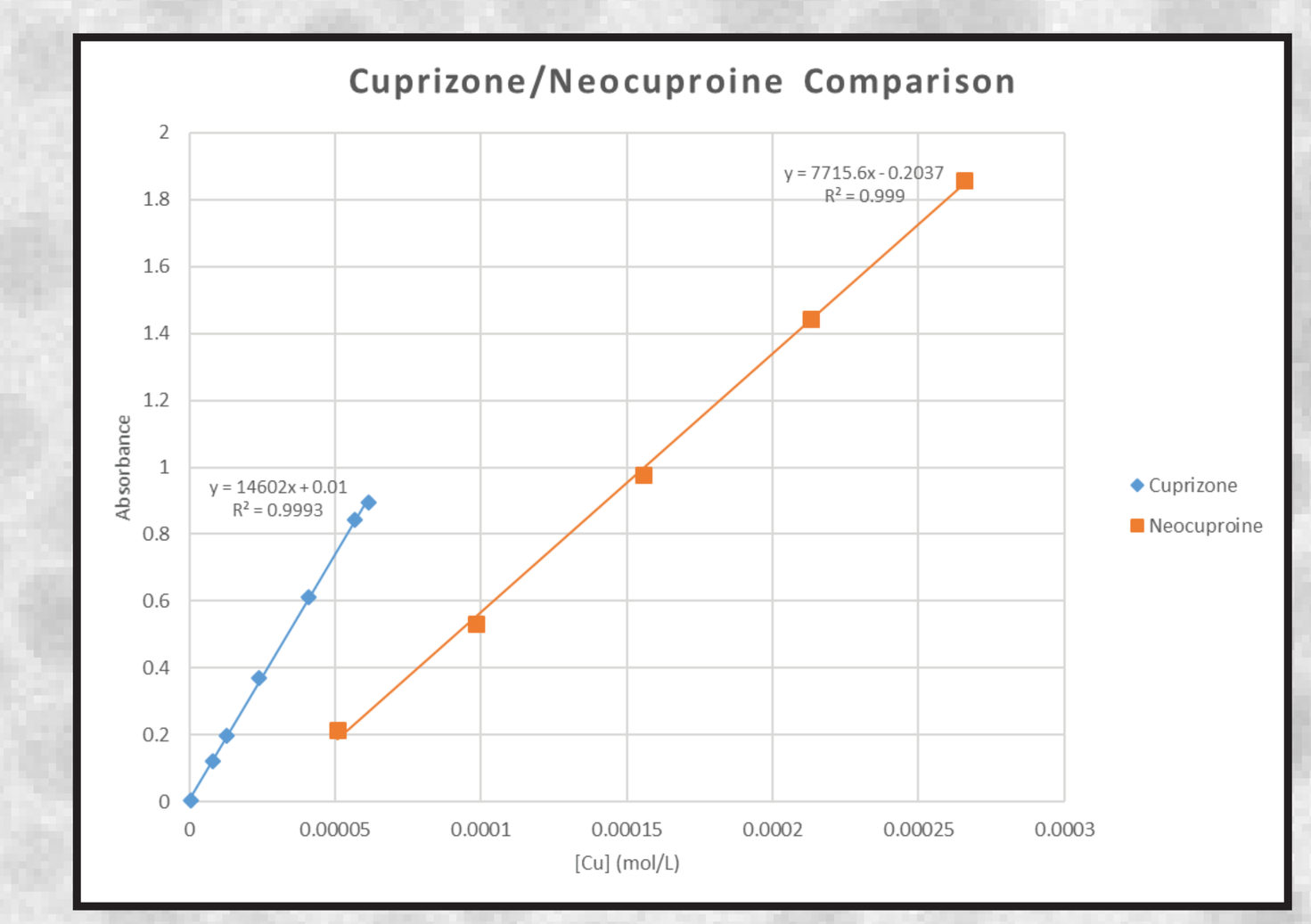


A solution containing copper turns a bright blue color once cuprizone is introduced to it.





To accurately determine unknown quantities of copper, a calibration curve was created. Solution concentrations of 0.311, 7.87, 12.6, 23.7, 40.9, 56.6, and 61.4 μM Cu were created and chelated with appropriate amounts of cuprizone. As can be observed, cuprizone allows the determination of copper in micro molar amounts with a detection limit of ~ 0.3 μM Cu.



Our lab has so far used neocuproine to determine Cu concentrations. However, cuprizone is far more sensitive and would be an acceptable replacement.

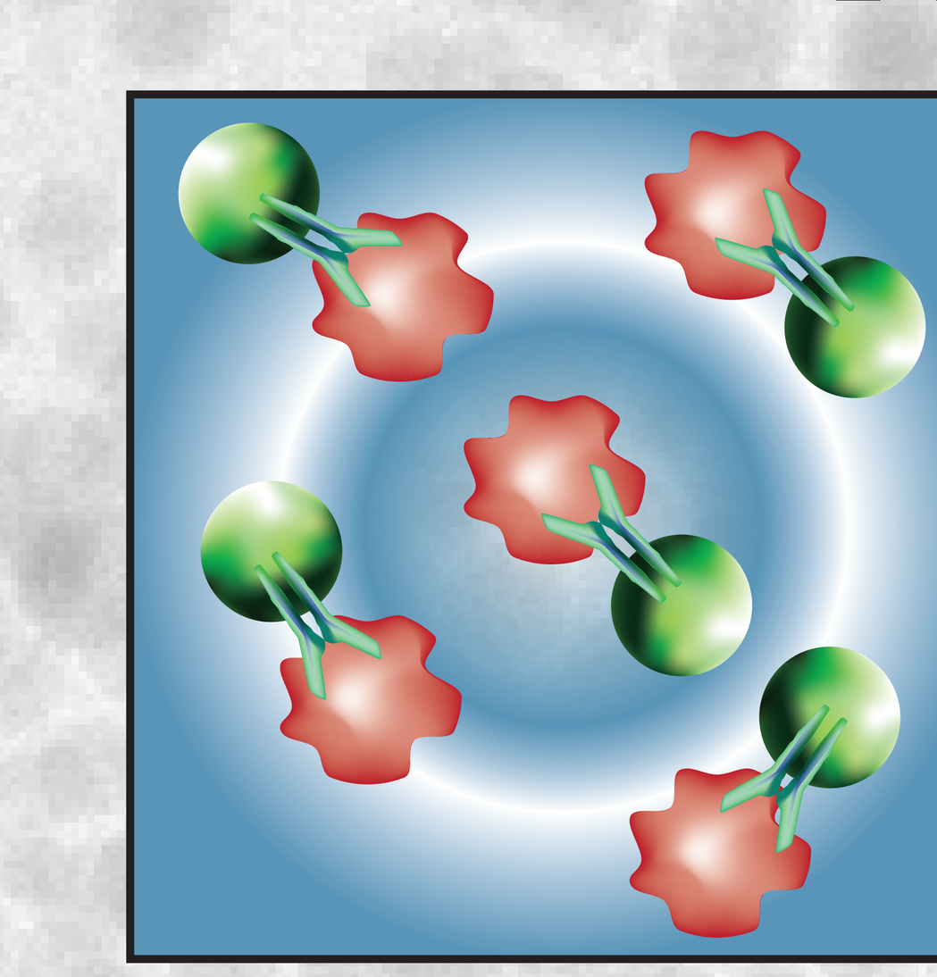
Conclusions

Hydrolyzed nanocrystals are stable in inorganic buffers for days and are stable in degassed deionized H_2O for weeks.

Crystal-bound nanocrystal degradation is not strongly dependent on pH but by reaction with dissolved species from the air or photodegradation.

The copper-cuprizone complex is a highly effective means of detecting micro molar amounts of copper. This provides a strong argument for the use of Cu_2S nanocrystals in biochemical and biological assays.

Future Work



Subsequent research will focus on the functionalization of Cu_2S species with biomolecular recognition elements and determine their utility for in-vivo and in-vitro assays. Specifically, the crystals will be functionalized with an antibody and then used in a sandwich assay similar to that depicted on the graphic to the left. Furthermore, the other solutions used in the buffer experiment will be degassed in a process similar to that conducted on $DGDIH_2O$. This will provide further data on the process by which crystal-bound nanocrystals degrade.

Acknowledgments

The author thanks Evan H. Robinson for his help and patience and Dr. Janet E. Macdonald for the opportunity to work in her lab. The author is also grateful for the help and advice of Lauren Gibson and all members of the Macdonald lab. Funding for this project was provided by VINSE NSF REU grant number DMR-1263182.

NSF
ViNSE