Graphene Quantum Dots as Imaging, Sensing, and Delivery Agents

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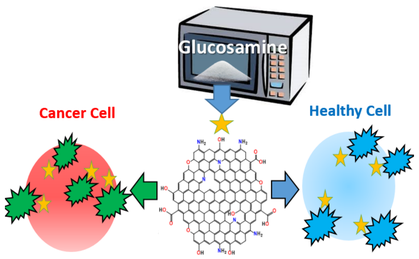
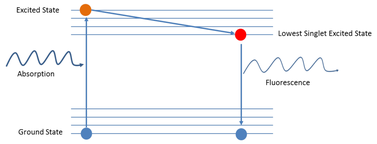
Abstract

Graphene quantum dots (GQDs) are novel materials with a number of unique properties that can be applied in electronics, sensing and biotechnology. GQDs possess physical properties that are critical for biomedical applications, including small size (3-5 nm), high quantum yield, and pH-dependent fluorescence emission in the visible/near-infrared, providing a possibility of molecular imaging, and pH-sensing. They also show very low cytotoxicity suggesting high potential for multiple biomedical applications. GQDs can also be doped to form nitrogen doped graphene quantum dots (N-GQDs), sulfur doped graphene quantum dots (NS-GQDs) and boron nitrogen doped graphene quantum dots (BN-GQDs), which allow these optical properties to be adjusted. We utilize and modify these properties to yield a multifunctional delivery/imaging/sensing platform geared toward the analysis of cancer therapeutics delivery *in vitro*. In our work, we outline how GQDs can serve as potential drug transport agents and as molecular markers for imaging the delivery pathways. Optimal emission and excitation are selected for each quantum dot to minimize the autofluorescence of cells, allowing them to be imaged *in vitro*. Emission in healthy (HEK-293) and cancer (HeLa and MCF-7) cells is quantified for a variety of pH environments to identify the ideal conditions for cellular internalization and pH-sensing of acidic cancerous environments. In addition, *in vitro* fluorescence microscopy analysis provides quantitative assessment for accumulation in cells. The results of this work suggest GQDs as innovative and effective highly biocompatible multifunctional platforms for cancer therapeutics.

Intro/Theory





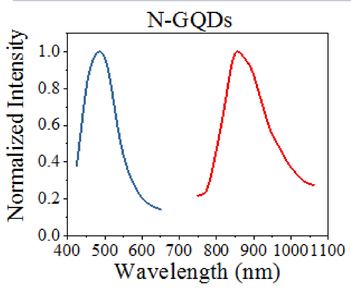
Graphene Quantum Dots:

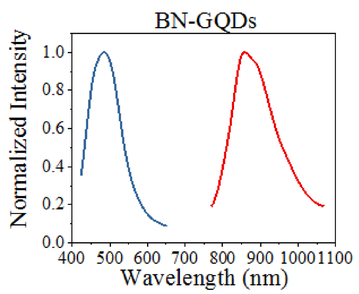
- Made in a microwave from glucosamine
- Possess graphene ordered structure
- Water soluble and emits fluorescence
- Can be used for:
  -Drug Delivery -Imaging -Sensing

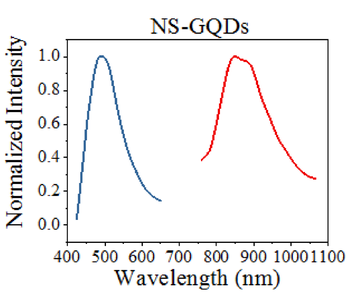
Purpose

The purpose of this experiment is to explore the feasibility of GQDs as multifunctional materials for drug delivery, imaging and biosensing for cancer therapeutics.

Effects of Doping

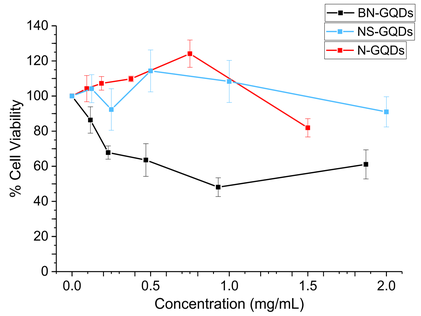


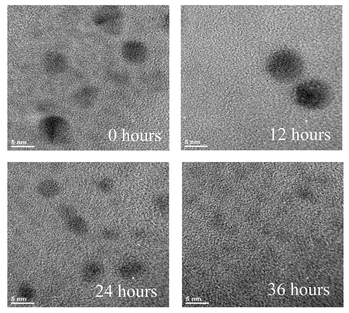




- Spectra in visible and NIR: allows imaging *in vitro* and *in vivo: NIR* light penetrates few cm of biological tissue (Smith et. al *Nat Nanotechnol* Vol. **4**) (low scattering and autofluorescence)
- Doping affects peak shape and intensity

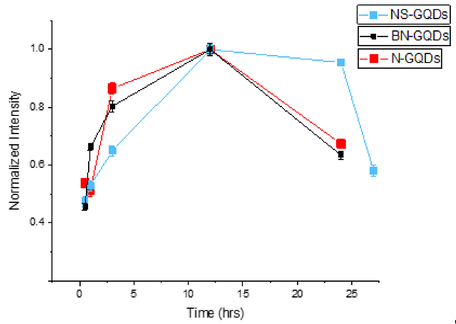
Cytotoxicity and Degradation





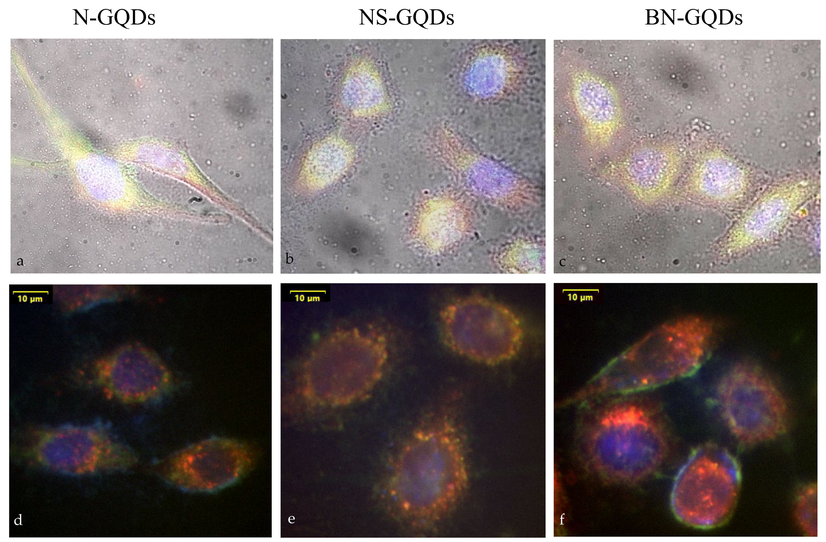
- NS-GQDs & N-GQDs show little toxicity up to high concentrations: 1.5 mg/mL!
- BN-GQDs show little toxicity at imaging concentrations, but more toxic overall
- Biodegradable: GQDs have circular shape up to 12 hrs; at 24 hrs become smaller and lose circular shape; at 36h - partially to fully degrade

Cellular Internalization



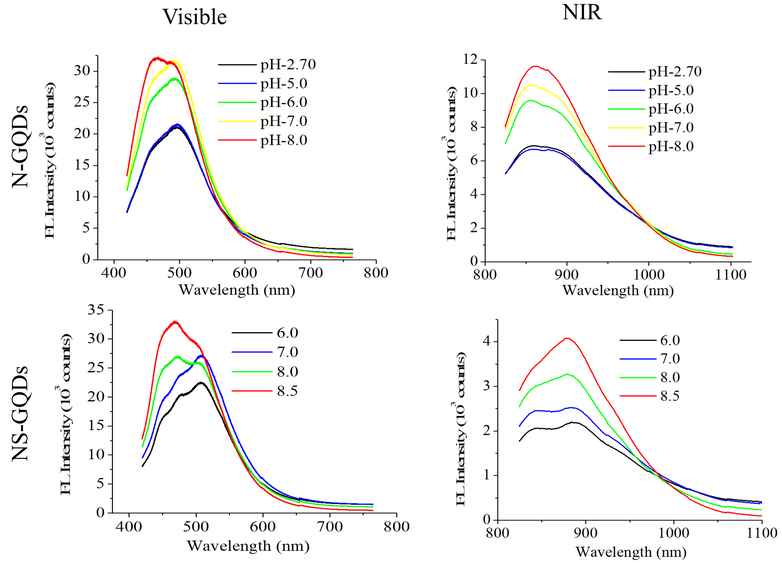
- Peak intensity: max internalization at 12 hrs.
- Then degradation or excretion

Colocalization and Confocal Fluorescence Imaging



- Regular and Confocal colocalization performed using dyes:
  - DAPI (Blue): Nucleus: excitation: 375 nm emission: 450 nm
  - Lysotracker Red: Lysosomes: excitation 540 nm emission 600 nm
  - Quantum Dots (Green): excitation 475 nm emission 535 nm
- **GQDs localize some with lysosomes, most are in cytoplasm with some in the nucleus**
- **GQDs can be utilized for drug delivery**

pH-sensing

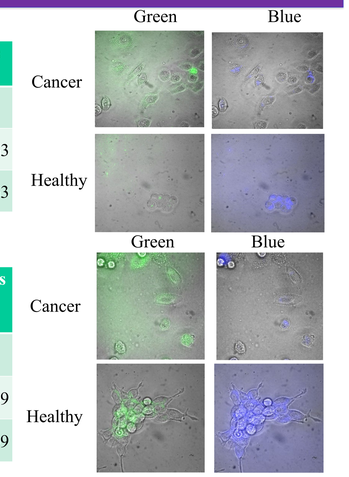


Both VIS and NIR fluorescence of N-GQD and NS-GQD are pH-dependent

Ratiometric pH-sensing Across Cell Lines

535/450 nm Intensity Ratios Across Cell Lines: N-GQDs			
	HEK-293	HELA	MCF-7
INTRACELLULAR	1.73 ± 0.49	2.56 ± 0.05	2.27 ± 0.63
EXTRACELLULAR	1.22 ± 0.01	8.27 ± 0.05	8.38 ± 0.63

535/450 nm Intensity Ratios Across Cell Lines: NS-GQDs			
	HEK-293	HELA	MCF-7
INTRACELLULAR	1.09 ± 0.01	2.18 ± 0.35	1.73 ± 0.09
EXTRACELLULAR	1.50 ± 0.01	4.96 ± 0.35	3.57 ± 0.09



Green/blue emission ratios are greater in cancer than healthy cells

Summary

N-GQDs and NS-GQDs can be used as imaging agent, drug delivery vehicle, and a pH biosensor: multifunctional material for bio applications.

- Simple and cost effective synthesis in a household microwave.
- Fluorescence in the VIS and NIR: potential for *in vitro & in vivo* imaging.
- N-GQDs and NS-GQDs are non-toxic at high concentration of 1.5 mg/mL and biodegradable at 36h.
- Best internalization occurs at 12h post transfection. GQDs localize in the cytoplasm, some in the nucleus: potential for drug or gene delivery
- pH sensing of acidic cancerous environments: green/blue N-GQD emission ratios vary by the factor of 7 between healthy and cancer cells.