# Graphene and amyloid peptide binding and its implications in Alzheimer's disease

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## Introduction

Alzheimer's disease is a neurodegenerative disease caused by the incorrect cleaving of the transmembrane Amyloid Precursor Protein into the neurotoxic $A\beta_{40}$ and $A\beta_{42}$ fragments[2]. These fragments are soluble oligomers with a random coil conformation that can impair synapses or neurotransmission; they may also aggregate into parallel and antiparallel beta sheets to form amyloid plaques, which can block or distort signaling between neuronal pathways[7].

Aβ fibrils self-assemble into parallel and antiparallel beta sheets on hydrophobic graphite, but not on hydrophilic mica[5,6]. Aβ fibrils also assemble on graphene, which irreversibly captures fibrils[3], suggesting graphene might have a role in the study of Alzheimer's amyloid plaque.

These studies characterize binding between amyloid beta peptide fibrils and graphene using Raman spectroscopy, scanning electron microscopy (SEM), and circular dichroism (CD). The goal is to provide evidence that graphene can attract free floating Aβ fibrils and Aβ plaque. Both studies currently use diphenylalanine peptide, a self-assembling model peptide for Aβ fibrils.

## Methods

### Experiment 1

-For the SEM, a stock solution of diphenylalanine dissolved in 1,1,1,3,3,3-hexafluouro-2-propanol (HFIP) (100 mg/mL) was added to graphene dispersed in 7x TE (1 mg/mL). All samples were dried on aluminum stubs coated with gold. A total of fourteen samples were analyzed.
-Samples 1 and 2 were the stock graphene and diphenylalanine solutions
-Samples 3-14 were three sets of four samples each. The 1st of a set was not shaken; the 2nd was hand shaken; the 3rd was vortex shaken; the 4th was sonicated. -Samples 2-6 had a 1:1 ratio of graphene and diphenylalanine; samples 7-10 had a 2:1 ratio; samples 11-14 had a 1:2 ratio.

### Experiment 2

-For the CD spectra, a stock solution of diphenylalanine was dissolved in HFIP (100mg/mL) was diluted into $ddH_20$ to a 2 mg/mL concentration, and then immediately diluted again into $ddH_20$ to a 0.04 mg/mL concentration. A control solution of dialanine was made under the same conditions. Controls of $ddH_20$ and HFIP were analyzed.

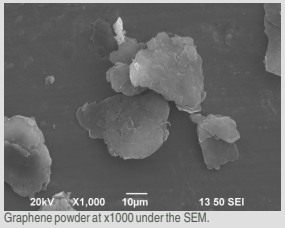
-For the SEM, a stock solution of diphenylalanine was dissolved in HFIP (100mg/mL), then diluted into $ddH_20$ to a 2 mg/mL concentration. A control solution of dialanine was made under the same conditions. A solution of graphene in 7x TE (1 mg/mL) was added to the stock solution of diphenylalanine in a 1:1 and 2:1 (gr:diphe) ratio.

Note: All Experiment 2 solutions were vortexed before and after every combination.

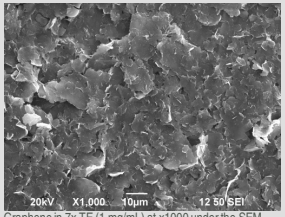
## Results

### Experiment 1

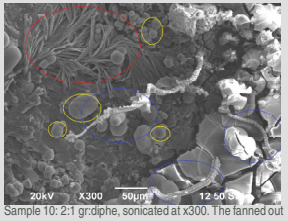
Signs of binding include fanned out or twisted cylinders with graphene "scales" or "ridges" on them. All samples were prevalently graphene with no observable effect from diphenylalanine.



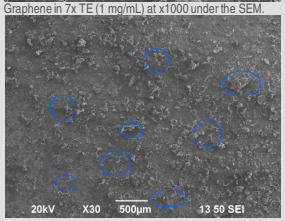
Graphene powder at x1000 under the SEM.



Graphene in 7x TE (1 mg/mL) at x1000 under the SEM.



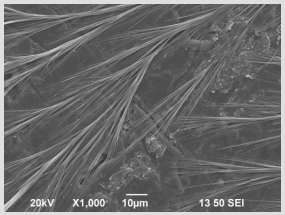
Sample 10: 2:1 gr:diphe, sonicated at x300. The fanned out cylinders are circled in red. The twisted cylinders are circled in blue. Some droplets of gold left by SEM preparation are circled in yellow. The rest is graphene.



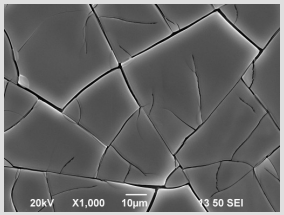
Sample 8: 2:1 gr:diphe, hand shaken at x30. There are twisted cylinders all over the surface, some circled in blue.

### Experiment 2

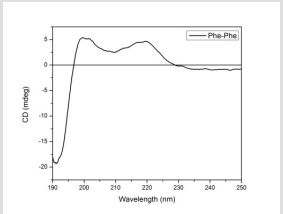
The diphenylalanine samples self-assembled into nanotubes, while the dialanine control did not. The diphenylalanine-graphene solutions did not show signs of binding.



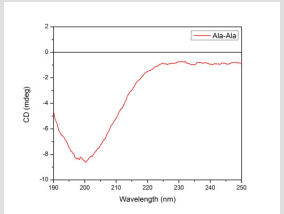
Diphenylalanine and HFIP diluted in $ddH_2O$ (2 mg/mL).



Dialanine and HFIP diluted in $ddH_2O$ (2 mg/mL).



CD spectra of diphenylalanine and HFIP diluted in $ddH_2O$ (0.04 mg/mL). The dual peaks at 200 and 220 nm are indicative of self-assembled binding.



CD spectra of dialanine and HFIP diluted in $ddH_2O$ (0.04 mg/mL). The downward slope as the spectra Approaches 200 nm implies no binding in this sample.

## Conclusions

Experiment 1 explored if diphenylalanine easily bound to graphene dispersed in 7xTE, which is ideal for keeping graphene powder in an even suspension within a solution. Graphene did bind to diphenylalanine, with a higher binding rate in a 2:1 gr:diphe ratio, but otherwise the binding efficacy was seemingly random and unreliable. Furthermore, the reference article[4] found ideal binding occurred in the 3.7-5.4 pH range. This study's samples had a 7.3 pH to mimic cerebrospinal fluid.

Experiment 2 successfully created the self-assembling diphenylalanine nanotubes in $ddH_20$ from the reference article[1]. The nanotubes were confirmed in both SEM and CD analysis. The control did not self-assemble nor did the diphenylalanine/HFIP stock solution, which may further explain why there was less than ideal binding in Experiment 1 and no binding between the stock diphenylalanine/HFIP solution in Experiment 2.

These experiments have demonstrated that there are binding capabilities between graphene and diphenylalanine, even in less than ideal situations. Diphenylalanine was chosen because its two phenylalanines mimic those in amyloid beta peptides. There results are sufficient to continue experiments using amyloid beta peptide instead of diphenylalanine with a few small changes in methodology.

## Future Directions

The next step is to recreate an aqueous graphene dispersion using a modified Hummers method[4] and determine if it is a better medium for diphenylalanine binding than 7x TE, since 7x TE is a possible deterrent of binding, and $ddH_20$ is a possible enabler. Once the more productive dispersion solution is determined, we will experiment the conditions of binding between graphene and amyloid beta peptide, whether in random coil or beta sheet conformation. The pH will remain comparable to that of cerebrospinal fluid.

Afterwards, experimentation of amyloid beta peptide to graphene will be done in artificial cerebrospinal fluid to determine how the extracellular fluid in the brain may change the reactions between Aβ and graphene. It is possible hydrophilic mica may be present to imitate hydrophilic cell membranes of neurons in the brain.

## Citations

1. Adler-Abramovic, L, Reches M, Sedman VL, Allen S, Tendler SJB, Gazit, E. **Thermal and Chemical Stability of Diphenylalanine Peptide Nanotubes: Implications for Nanotechnological Applications.** *Langmuir*; 2006;22:1313-1320
2. Buchet R, Pikula S. **Alzheimer's disease: its origin at the membrane, evidence and questions.** *Acta Biochim. Pol.* 2000;47:725–733.
3. Cheng Z, Zhou Q, Wang C, Li Q, Wang C, Fang Y. **Toward Intrinsic Graphene Surfaces: A Systematic Study on Thermal Annealing and Wet-Chemical Treatment of $SiO_2$-Supported Graphene Devices.** *Nano Letters.* 2011;11:767-771.
4. Han TH, Lee WJ, Lee DH, Kim JE, Choi EY, Kim SO. **Peptide/Graphene Hybrid Assembly into Core/Shell Nanowires.** *Advanced Materials.* 2010;22:2060-2064.
5. Kowalewski, T and Holtzman, D. ***In situ* atomic force microscopy study of Alzheimer's β-amyloid peptide on different substrates: New insights into mechanism of β-sheet formation.** *Proc. Natl. Acad. Sci. USA.* 1999;96:3688-3693.
6. Losic D, Martin LL, Aguilar MI, Small DH. **β-Amyloid Fibril Formation id Promoted by Step Edges of Highly Oriented Pyrolytic Graphene.** *Biopolymers (Peptide Science).* 2006;84:519-526.
7. Morris, R. **A needle from the haystack**. *Nature;* 2006:440.

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