NDnano
Center for Nano Science and Technology

NDnano Summer Undergraduate Research 2017 Project Summary

1. Student name & university: William McCarthy,
Trinity College Dublin.

2. ND faculty name & department: Dr. Matt Webber,
Department of Chemical and Biomolecular Engineering.

3. Project title: **Synthesis of Peptidic Derivatives of 1,3,5-benzenetricarboxamide and Investigation of their Supramolecular-Assembly**

4. Briefly describe new skills you acquired during your summer research:

- I furthered my synthetic proficiency, carrying out a broad range of reactions, including both manual and automated peptide synthesis, Curtius rearrangement, Sonogashira coupling and Huisgen 1,3-dipolar cycloadditions.
- I broadened my purification and analytical skillset by performing HPLC/LC-MS.
- I gained valuable experience in communicating scientific data through both a 10 minute oral presentation and poster presentation.

5. Briefly share a practical application/end use of your research:

The field of supramolecular biomaterials is a topical and rapidly expanding area of research. Supramolecular biomaterials, including hydrogels which are the focus point of this project, are being developed for a number of applications from drug-delivery to regenerative medicine. Hydrogels can act as delivery systems by encapsulating a desired cargo (drugs/cells) in a controllable supramolecular matrix. Hydrogel delivery-systems offer control over the site and mechanism of delivery as well as the rate of release of the cargo. Hydrogels can be engineered to present a specific peptide/oligosaccharide/nucleotide sequence that drives localization of the system at a certain site in the body. Similarly, enzyme-responsive moieties, such as ester bonds cleavable by esterases, can be presented by the hydrogel to stimulate release of cargo from the surface of the gel, which remains intact, or disintegration of the gel to release the cargo. Utilizing these properties, allows hydrogel delivery-systems to be designed that increase efficacy and reduce systemic toxicity.

6. Abstract of your project:

Supramolecular self-assembly describes a process of molecular recognition and organization into defined structures using specific, directional, non-covalent interactions. Supramolecular structures can lead to materials with dynamic, responsive and highly tuneable properties that have application in drug delivery, tissue engineering and regenerative medicine among many others. We are specifically interested in illustrating the utility of creating supramolecular biomaterials arising from the 1,3,5-benzenetricarboxamide (**BTA**) hydrogen bonding motif. Specifically, through appendage of peptides to the BTA core, it is our objective to create hydrogels which may be useful as biomaterials.

7. References for papers, posters, or presentations of your research:

1. M. J. Webber, E. A. Appel, E. W. Meijer and R. Langer, *Nature Materials*, 2016, **15**, 13-26.
2. R. C. T. Howe, A. P. Smalley, A. P. M. Guttenplan, M. W. R. Doggett, M. D. Eddleston, J. C. Tanb and G. O. Lloyd, *Chem. Commun.,* 2013, **49**, 4268.
3. A. Desmarchelier, M. Raynal, P. Brocorens, N. Vanthuyned and L. Bouteiller, *Chem. Commun.,* 2015, **51**, 7397-7400.
4. M. M. J. Smulders, A. P. H. J. Schenning, and E. W. Meijer, *J. Am. Chem. Soc*., 2008, **130**, 606-611.

One-page project summary that describes problem, project goal and your activities/results:

My project was centred around the development of proof-of-concept materials based on the 1,3,5-benzenetricarboxamide (**BTA**) core. **BTA** is a well-studied supramolecular motif that is proven to form long, helical fibers as a result of hydrogen-bonding between the amide functionalities of adjacent molecules. The directionality and strength of these interactions makes **BTA** a very reliable core for supramolecular-assembly. **BTA** has been widely studied as the core motif of a number of supramolecular hydrogelators. However, the appendage of peptide sequences to **BTA** has not been previously undertaken. These peptidic biomaterials provide a number of advantages over traditional metallic/liposomal biomaterials in that enzyme-responsive linkers or cell-specific binding sequences can be incorporated to provide greater control over and predictability of the hydrogels behaviour *in-vivo*. My efforts were focused on utilizing **BTA** supramolecular interactions to template assembly of biocompatible peptide materials.

The specific sequence of the incorporated peptides can be controlled, with the intention of altering both molecular stacking and interfiber crosslinking. The first generation of peptides synthesised were simple dipeptides consisting of a non-natural N-terminal amino-acid, either 4-amino-benzoic acid (Benz) or 6-amino-hexanoic acid (Hex), and a glutamic acid (E) C-terminal amino acid. The difference between these two peptides being the rigidity of the N-terminal amino acid which connects the peptide sequence to the **BTA** core. The peptidic **BTA** derivatives were formed by standard amide bond formation between 1,3,5-benzenetricarbonyl trichlroide and the desired peptide. It was found that **BTA**-$(HexE)_3$ did not form hydrogels while **BTA**-$(BenzE)_3$ did, as shown in the figures below. The **BTA**-$(BenzE)_3$ hydrogels could not be formed under physiological conditions on a useful timescale (Figure A). However, using excess salt or lowering the pH did generate hydrogels instantaneously (Figures B and C, respectively). These gels were not outstanding but provided a starting-point for optimization of a biocompatible **BTA** hydrogel.

As the **BTA**-$(BenzE)_3$ system showed greater potential, the second generation of peptides focused on more rigid N-terminal amino acid linkers. Benz-L-L-E and G-L-L-E sequences were synthesised and appended to the **BTA** core. Here, the leucine-leucine (L-L) dipeptide between the N-terminal and C-terminal amino acids was introduced to increase the amphiphilicity of the peptide sequences, promoting improved molecular stacking as a result of "hydrophobic collapse". Further to the amide-amide hydrogen-bonding of adjacent **BTA** moieties, the iso-butyl side-chains of the leucine residues, being extremely hydrophobic, drive molecular stacking of **BTA** units by their propensity to be internalised in water solvent. However, **BTA**-$(BenzLLE)_3$ did not yield hydrogels like the simpler **BTA**-$(BenzE)_3$ system. This may have been a result of steric hindrance introduced by the bulky leucine side-chains. To mitigate this problem, synthesis of the Benz-V-V-E sequence will be attempted by the group in the future. The iso-propyl side-chains of the valine (V) residues are hydrophobic and so maintain the idea of "hydrophobic collapse" but are less bulky than the leucine side-chains.

The glutamic acid C-terminal amino acid is common to each peptide sequence explored. This design feature utilizes the carboxylic acid side-chains to crosslink **BTA** fibers *via* secondary interactions between glutamic acid residues to form physically entangled hydrogels. The glutamic acid residues of different fibers are linked by the formation of salt bridges using divalent cations, such as Ca^{2+}. This mechanism of crosslinking is biocompatible and very reliable.

In addition to modification of the peptide sequences, alterations to the basic supramolecular motif were probed by inclusion of urea (twice the hydrogen-bonding capacity)(**BTU**) and triazole (no hydrogen-bonding capacity)(**BTT**) in place of the amide in the core motif. The peptidic **BTU** derivatives were synthesised via Curtius rearrangement. 1,3,5-benzenetricarbonyl trichlroide was treated with sodium azide to form the triacyl azide, which was heated under reflux to initiate the Curtius rearrangement, forming the triisocyanate intermediate, which was reacted with the desired peptide *in situ*. The peptidic **BTT** derivatives were formed by amide bond formation between the desired peptide and 1,3,5-tris(1,2,3-triazol-4-yl)benzene functionalized in the triazole 1 position with an acetic acid moiety. Although attempts at formation of **BTU** and **BTT** were attempted, time constraints meant that peptidic derivatives were not achieved. Nevertheless, peptidic **BTU** and **BTT** derivatives will be further pursued by the group based on synthetic strategies implemented during my project.

Although the desired standard of supramolecular biomaterials was not achieved, through this bottom-up molecular design and logical modification, significant progress has been made towards the realization of dynamic and tuneable hydrogels.

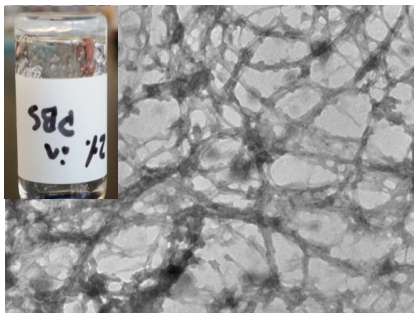

Figure A

Benz-E, 2wt% PBS, CaCl2, Gelation - 4 days

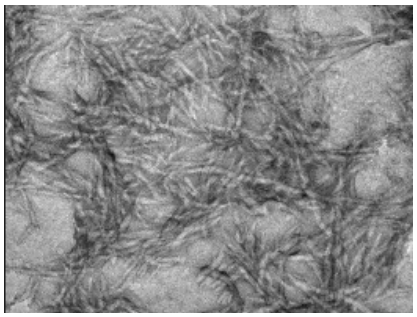

Figure B

Benz-E, 2wt% H2O, CaCl2 (8x excess) Gelation - Instant

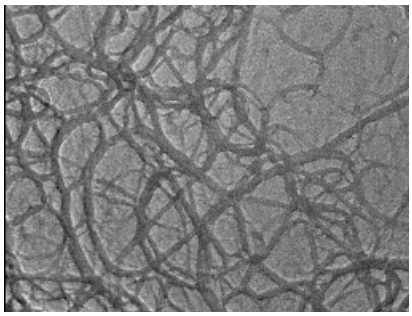

Figure C

Benz-E, 1wt% PBS, CaCl2, pH4.25, Gelation - Instant

BenzE samples prepared by Michael VandenBerg and TEM images captured by Dr. Jugal Sahoo.