

## Supplementary Material

### Early Stage Monitoring of Enzyme-Assisted Self Assembling Peptide Hydrogels

Richard J. Williams,<sup>A,B</sup> James Gardiner,<sup>A,C</sup> Anders B. Sorensen,<sup>A</sup> Silvia Marchesan,<sup>A</sup> Roger J. Mulder,<sup>A</sup> Keith M McLean<sup>A</sup> and Patrick G. Hartley<sup>A</sup>.

<sup>A</sup>CSIRO Materials Science and Engineering, Clayton, VIC 3168, Australia

<sup>B</sup>School of life and Enviromental Sciences, Deakin University, Waurn Ponds, VIC 3026, Australia

<sup>C</sup>Corresponding author. Email: james.gardiner@csiro.au

### Supplementary Material Table of Contents

#### 1. Effect of enzyme concentration on self assembly

**Figure 1.** Formation of Fmoc-Leu<sub>3</sub> from 20/40mmol Fmoc-Leu/Leu<sub>2</sub> with 0.33, 0.67 and 1mg.mL of enzyme at pH7.2.

**Figure 2.** Timecourse CD spectra of the self assembly of FmocLeu<sub>3</sub> from 20/40mmol Fmoc-Leu/Leu<sub>2</sub> at pH7.2 with 0.33, 0.67 and 1mg/mL of enzyme.

#### 2. Effect of substrate concentration on self assembly

**Figure 3.** Formation of Fmoc-Leu<sub>3</sub> at pH7.2 with 0.33mg/mL of enzyme at 40/80mmol, 20/40mmol, and 10/20mmol concentrations of Fmoc-Leu/Leu<sub>2</sub>.

**Figure 4.** Rate of Fmoc-Leu consumption in first 20minutes at 10/20mmol, 20/40mmol and 40/80mmol concentrations of Fmoc-Leu/Leu<sub>2</sub> at pH7.2 with 0.33mg/mL of enzyme

### 3. Effect of pH on self assembly

**Figure 5.** Formation of Fmoc-Leu<sub>3</sub> from 20/40mmol Fmoc-Leu/Leu<sub>2</sub> at pH8.0 with 0.33, 0.67 and 1mg/mL of enzyme.

**Figure 6.** Formation of Fmoc-Leu<sub>3</sub> from 20/40mmol Fmoc-Leu/Leu<sub>2</sub> at pH8.4 with 0.33, 0.67 and 1mg/mL of enzyme.

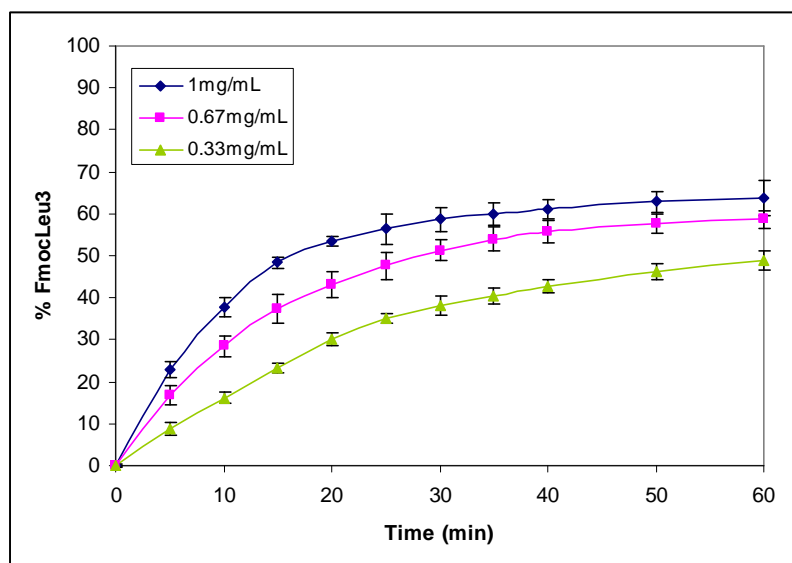
### 4. Effect of substrate ratio on self assembly

**Figure 7.** Formation of Fmoc-Leu<sub>3</sub> at pH 7.2 with 0.33mg/mL of enzyme with 20/20mmol and 20/10mmol concentrations of Fmoc-Leu/Leu<sub>2</sub>.

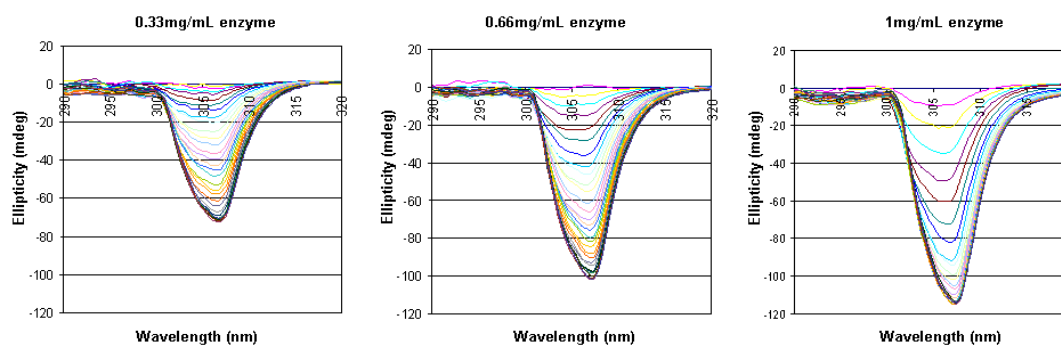
### 5. Monitoring of Self Assembly by <sup>1</sup>H NMR

**Figure 8.** Log plot of the rate of proton integral decay over time during the enzyme catalysed self assembly of FmocLeu<sub>3</sub>

### 1. Effect of enzyme concentration on self assembly

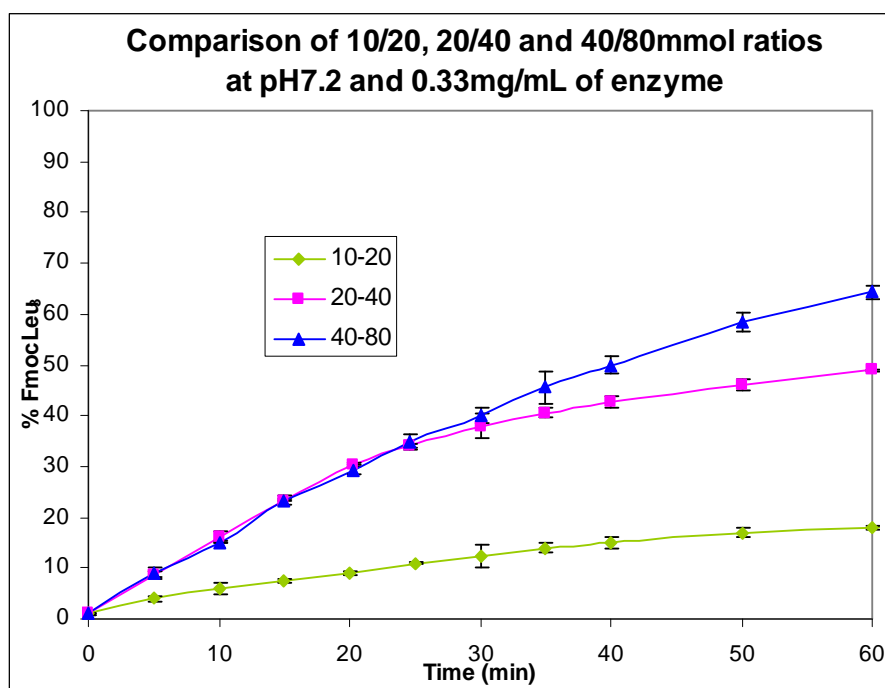


**Figure 1.** Formation of Fmoc-Leu<sub>3</sub> (in % yield) from 20/40mmol Fmoc-Leu/Leu<sub>2</sub> with 0.33, 0.67 and 1mg.mL of enzyme at pH7.2 (derived from an average of 3 data sets per sample).

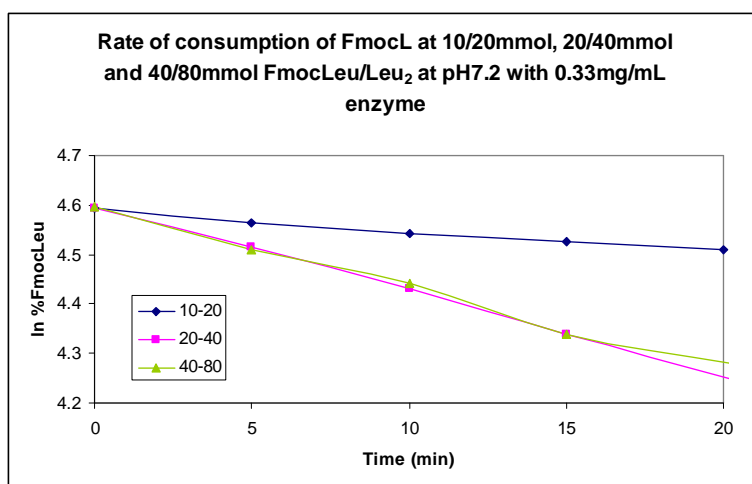


**Figure 2.** Timecourse CD spectra of the self assembly of 20/40mmol Fmoc-Leu/Leu<sub>2</sub> at pH7.2 with 0.33, 0.67 and 1mg/mL of enzyme.

2. Effect of substrate concentration on self-assembly

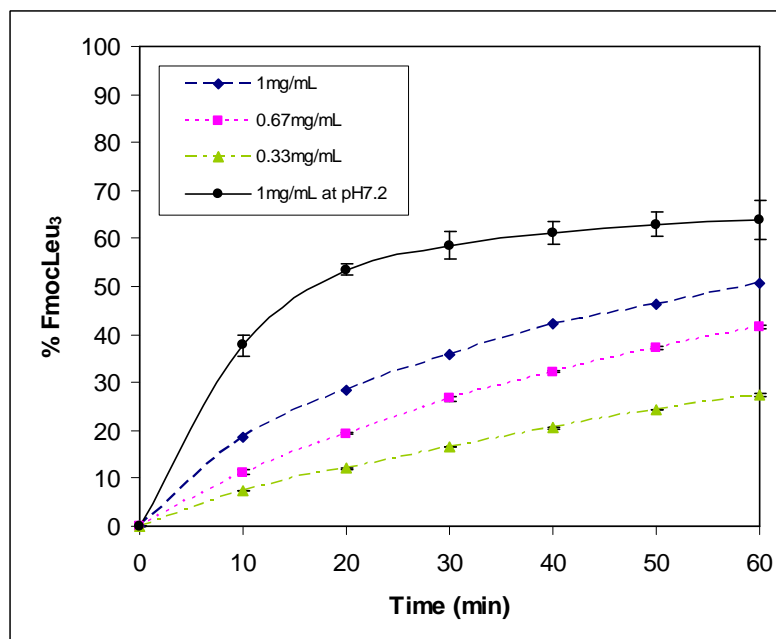


**Figure 3.** Formation of Fmoc-Leu<sub>3</sub> (in % yield) at pH7.2 with 0.33mg/mL of enzyme:at 40/80mmol, 20/40mmol, and 10/20mmol concentrations of Fmoc-Leu/Leu<sub>2</sub> (derived from an average of 3 data sets per sample).

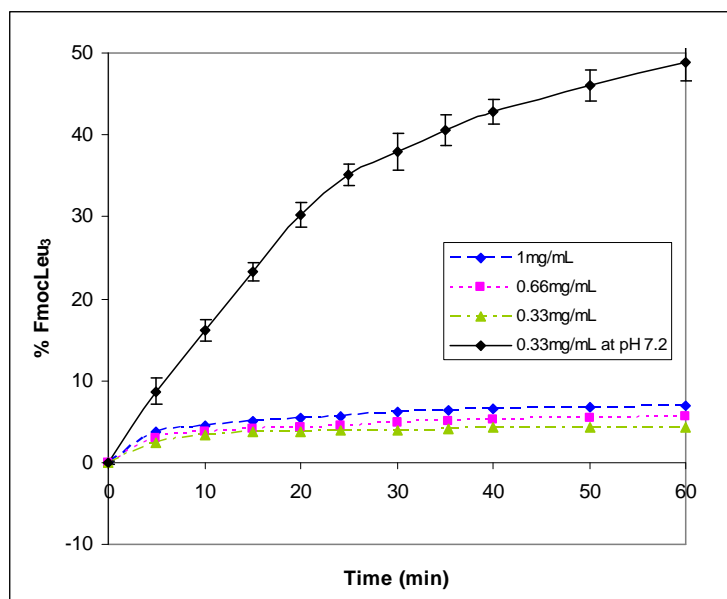


**Figure 4.** Rate of Fmoc-Leu consumption in first 20minutes at 10/20mmol, 20/40mmol and 40/80mmol concentrations of Fmoc-Leu/Leu<sub>2</sub> at pH7.2 with 0.33mg/mL of enzyme (derived from an average of 3 data sets per sample).

### 3. Effect of pH on Self-Assembly

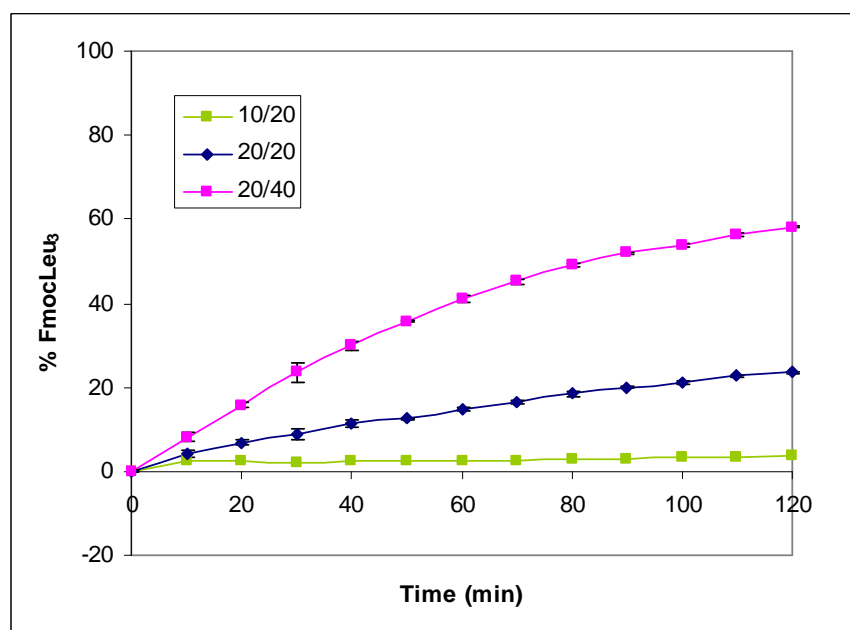


**Figure 5.** Formation of Fmoc-Leu<sub>3</sub> (in % yield) from 20/40mmol Fmoc-Leu/Leu<sub>2</sub> at pH8.0 with 0.33, 0.67 and 1mg/mL of enzyme (derived from an average of 3 data sets per sample). Formation of Fmoc-Leu<sub>3</sub> is significantly slowed at pH8.0 as compared to pH7.2 (Figure 1).



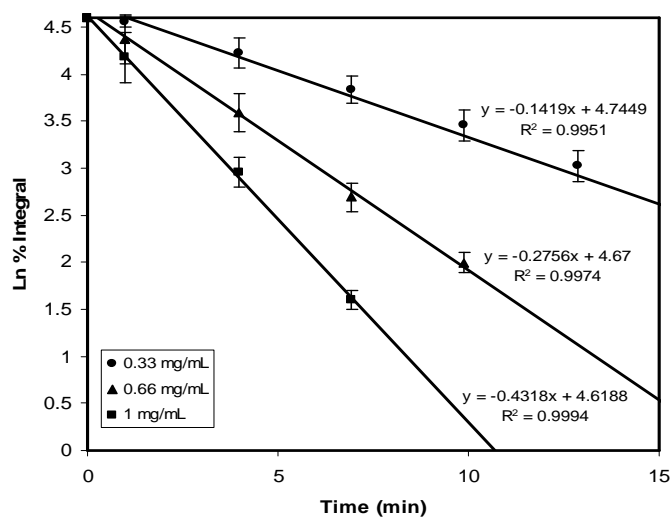
**Figure 6.** Formation of Fmoc-Leu<sub>3</sub> (in % yield) from 20/40mmol Fmoc-Leu/Leu<sub>2</sub> at pH8.4 with 0.33, 0.67 and 1mg/mL of enzyme (derived from an average of 3 data sets per sample). Formation of Fmoc-Leu<sub>3</sub> is almost halted at pH8.4 as compared to pH7.2..

#### 4. Effect of substrate ratio on self-assembly



**Figure 7.** Formation of Fmoc-Leu<sub>3</sub> (in % yield) at pH 7.2 with 0.33mg/mL of enzyme with 20/20mmol and 20/10mmol concentrations of Fmoc-Leu/Leu<sub>2</sub>. (derived from an average of 3 data sets per sample). .

#### 5. Monitoring of FmocLeu<sub>3</sub> Self Assembly by <sup>1</sup>H NMR



**Figure 8.** Log plot of the rate of proton integral decay over time during the enzyme catalysed self assembly of FmocLeu<sub>3</sub> from FmocLeu and Leu<sub>2</sub>. At time zero the proton integral is plotted as ln 100%. For the first 5-15 minutes of the process linear rates are observed. Addition of increasing amounts of enzyme leads to a proportional increase in the rate of integral decay in a manner similar to that observed by HPLC and CD analysis.