**Supplementary Material for:**

# Discovery of catalytic phages by biocatalytic self-assembly

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Materials and Methods

Figures S1, S3, S4, S5

Tables S1 and S2

## SI 1: Optimization of phage elution.

When the target phages are separated from the library by centrifugation, the Fmoc-TL-OMe/$NH_2$ aggregate is expected to cover the end of the phage where the catalytic peptides are displayed (the minor protein pIII). To recover its infection ability for the following amplification step, the hydrogel must be removed and pIII exposed. For this purpose, treatment with subtilisin from *Bacillus licheniformis* (Sigma-Aldrich) was used to digest Fmoc-TL-OMe (Das *et al*., Small, 2008). To determine the optimum concentration where the hydrogel is effectively digested but phage's infection ability is maintained, different concentrations of subtilisin were applied to the phage, followed by titration experiments to determine the survival ratio of the treated phages. This experiment revealed that the phage can tolerate up to 1 μg $mL^{-1}$ subtilisin (Figure S1a).

The Fmoc-TL-$NH_2$ hydrogelator is not readily hydrolysed by subtilisin, but it was found that 50% acetonitrile (v/v in water) could dissolve the gel. The phages can tolerate 50% acetonitrile (Fig. S1b)

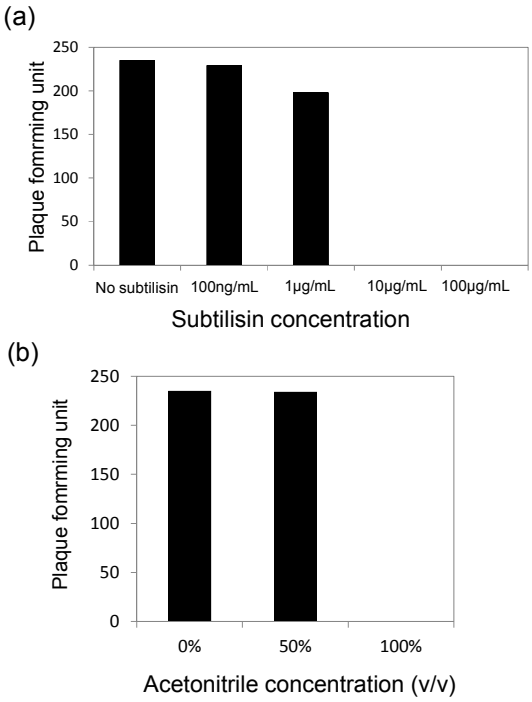


**Figure S1:** Survival rate of bacteriophage M13 in the treatment of subtilisin (a) and acetonitrile (b). Phages were incubated in subtilisin solution or acetonitrile overnight and their infection ability analysed by titration experiments.

## SI 2: Selection of catalytic phages.

The Fmoc-T/L-OMe biopanning process revealed 18 peptides (listed in Table S1) in the second round of panning. Four peptides (CP1-4) were selected for further study.

**Table S1:** Sequence of catalytic peptide candidates identified after 2 rounds of panning using Fmoc-T/L-OMe.

| Name | Sequence |
|---|---|
| CP1 | T D H T H N K G Y A N K |
| CP2 | T S H P S Y Y L T G S N |
| CP3 | S H Q A L Q E M K L P M |
| CP4 | S M E S L S K T H H Y R |
| | K L H I S K D H I Y P T |
| | N R P D S A Q F W L H H |
| | D P Q N H N W T N K P A |
| | Y L P H M L V H G S R H |
| | T Y P V V G H Q Q N V M |
| | D I M P K L R D D V H N |
| | N A H T S N N V V A F P |
| | Y G T S M T Q S N W R H |
| | S Y G S L Q T R F G H I |
| | K F F N N T E A T T R P |
| | N Y A L R D P V G Q R Y |
| | L P S V T E I L G S N F |
| | T S A V T L T S D P T L |
| | Q N F S Q M M S I P R K |

**Table S2:** Sequence of catalytic peptide candidates identified after 2 rounds of panning using Fmoc-T/L-$NH_2$.

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| Name | Sequence | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CPN1 | **A** | **M** | **H** | **S** | **L** | **V** | **G** | **P** | **A** | **F** | **N** | **R** |
| CPN2 | **H** | **D** | **T** | **S** | **E** | **Q** | **L** | **L** | **V** | **A** | **P** | **S** |
| CPN3 | **D** | **L** | **R** | **S** | **C** | **T** | **A** | **C** | **A** | **V** | **N** | **A** |
| | **A** | **T** | **T** | **W** | **T** | **V** | **A** | **H** | **G** | **V** | **S** | **R** |
| | **S** | **T** | **D** | **D** | **D** | **H** | **L** | **L** | **A** | **A** | **T** | **T** |
| | **H** | **P** | **T** | **G** | **S** | **K** | **S** | **T** | **T** | **S** | **T** | **Y** |
| | **H** | **T** | **D** | **S** | **D** | **P** | **L** | **L** | **A** | **A** | **P** | **S** |
| | **P** | **T** | **S** | **E** | **V** | **Y** | **L** | **F** | **S** | **G** | **N** | **F** |

## SI 3: Catalytic self-assembly by selected phage displaying catalytic peptides

For analysis by TEM, the eluted phage at each round ($10^{10}$ pfu) was mixed with Fmoc-T and L-OMe under the same conditions used in the screening process. After incubation during varying times at room temperature, a drop of the phage solution (3 µL) was applied to a carbon-coated copper TEM grid (Electron Microscopy Sciences, PA) and dried. The sample was stained by using 2% uranyl acetate, and examined by TEM (JEOL 2100) at 200 kV. Globular aggregates were observed at the end of phage M13 platform (Fig.2c in main text). This suggested localised generation of Fmoc-TL-OMe aggregates around the peptides displayed on pIII minor coat protein, which are located at one end of bacteriophage M13. Although nanofibrous self-assembly of Fmoc-TL-OMe has been observed in enzymatically triggered assembly of hydrogels,[1] we observed the formation of spherical aggregates that did not further assemble to fibers. This limited self-assembly is thought to be related to the lower catalytic activity of CPs compared to enzymes, combined with the assembled material blocking access for further substrates and a possible role for Fmoc-T co-assembly with the reaction product.

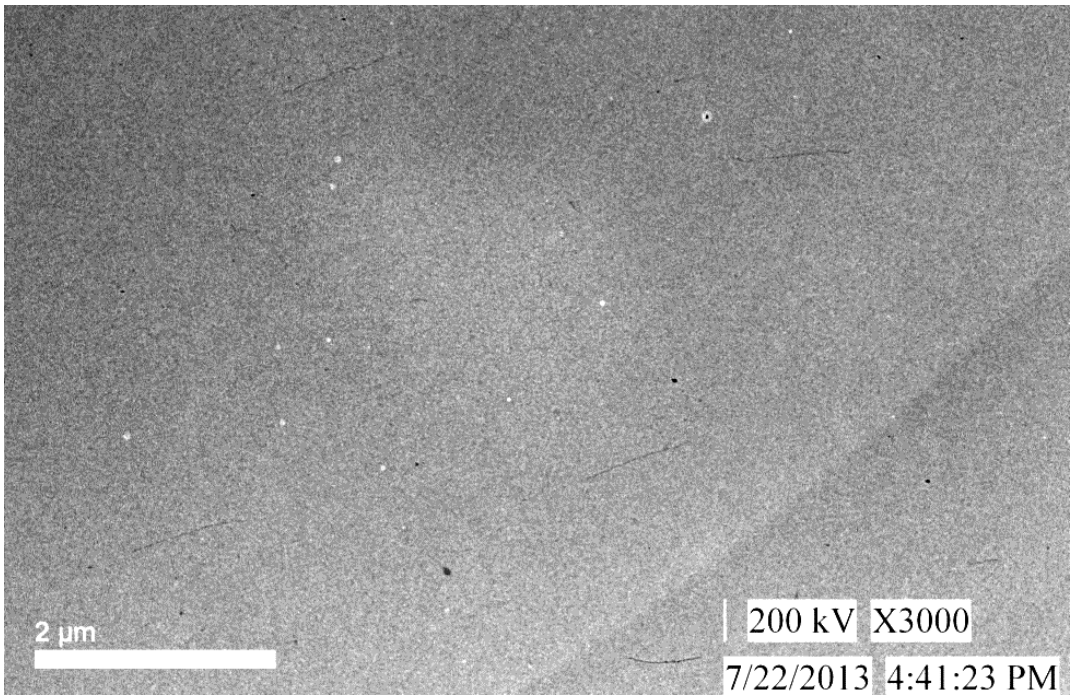



**Figure S3.1:** TEM image of CP4 phage in the absence of Fmoc-T/L-OMe substrate, after 30 minutes incubation.

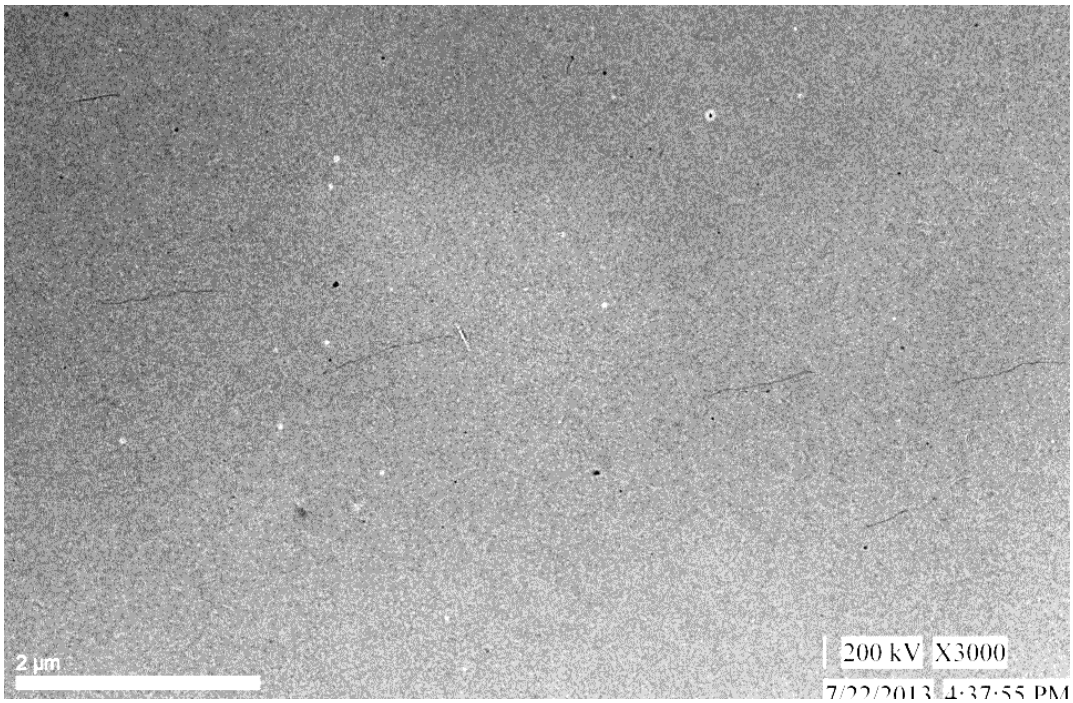



**Figure S3.2:** TEM image of CP4 phage in the absence of Fmoc-T/L-OMe substrate, after 60 minutes incubation.

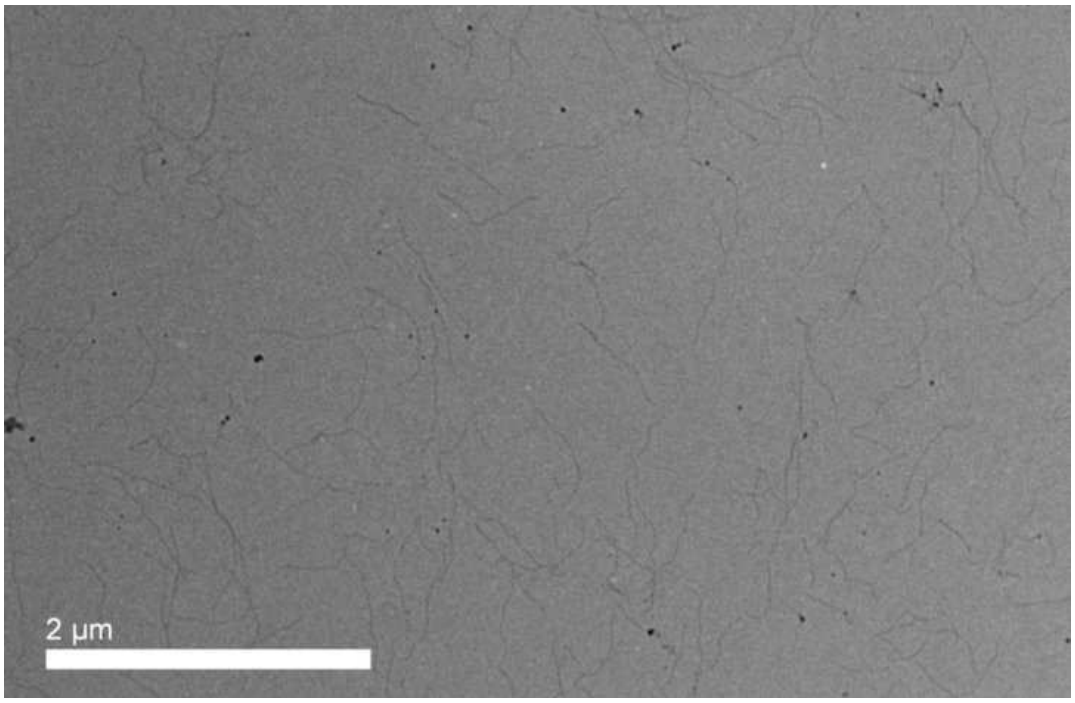



**Figure S3.3:** TEM image of CP4 phage in the presence of Fmoc-T/L-OMe substrate, after 10 minutes incubation.

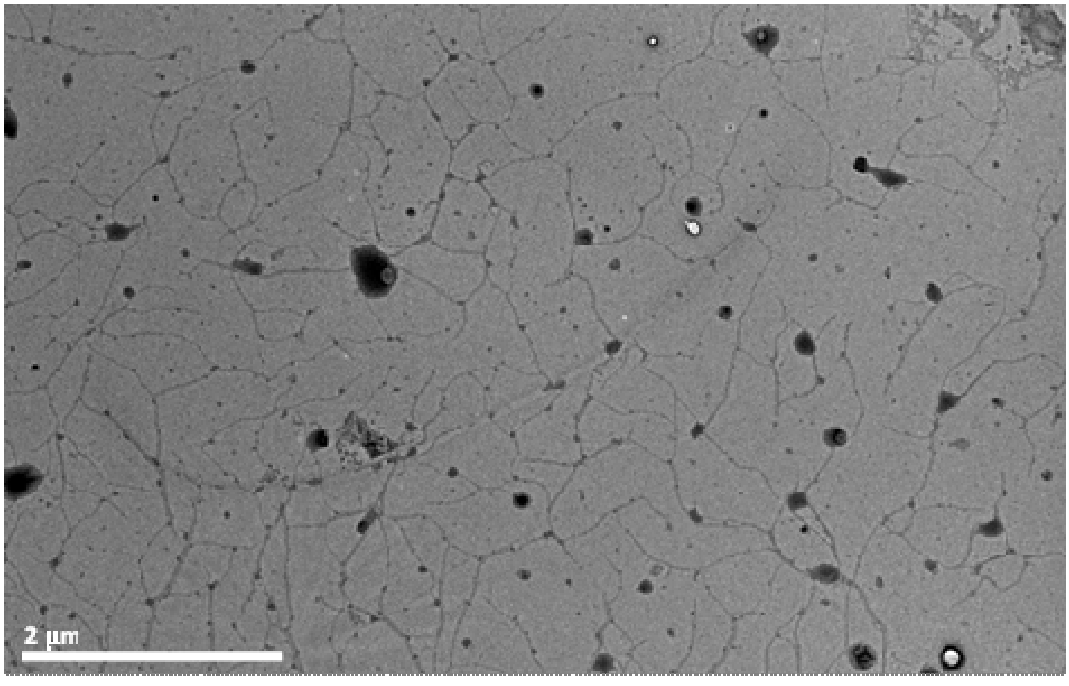



**Figure S3.4:** TEM image of CP4 phage in the presence of Fmoc-T/L-OMe substrate, after 30 minutes incubation.

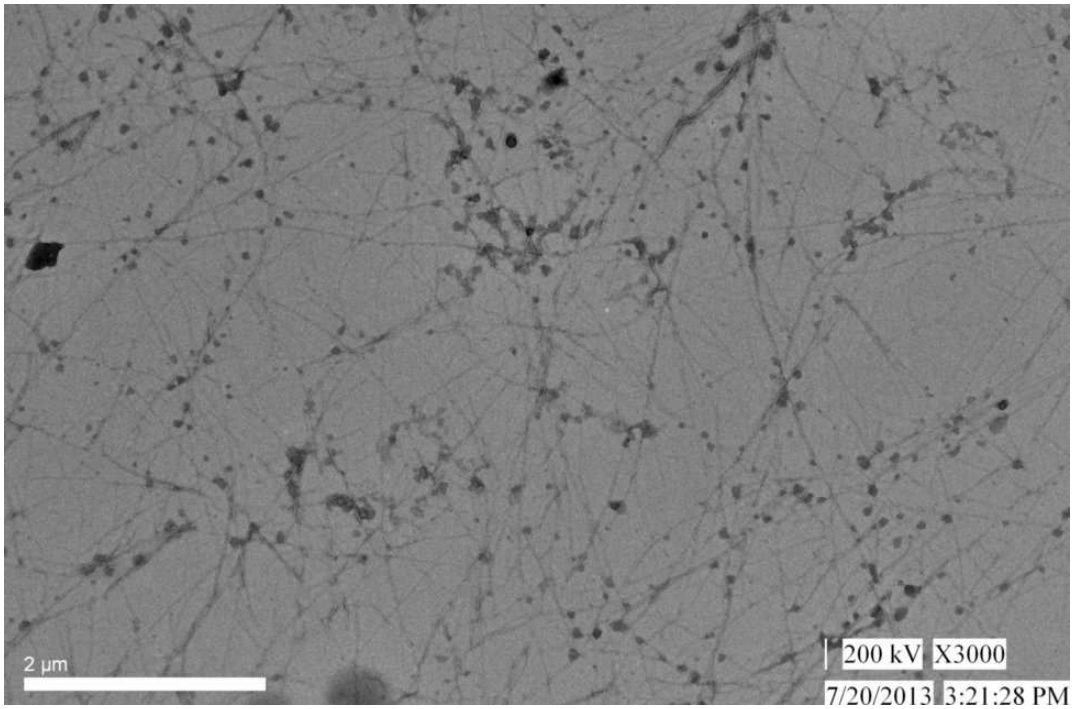



**Figure S3.5:** TEM image of CP4 phage in the presence of Fmoc-T/L-OMe substrate, after 60 minutes incubation.

## SI 4: Amide Hydrolysis by Phages

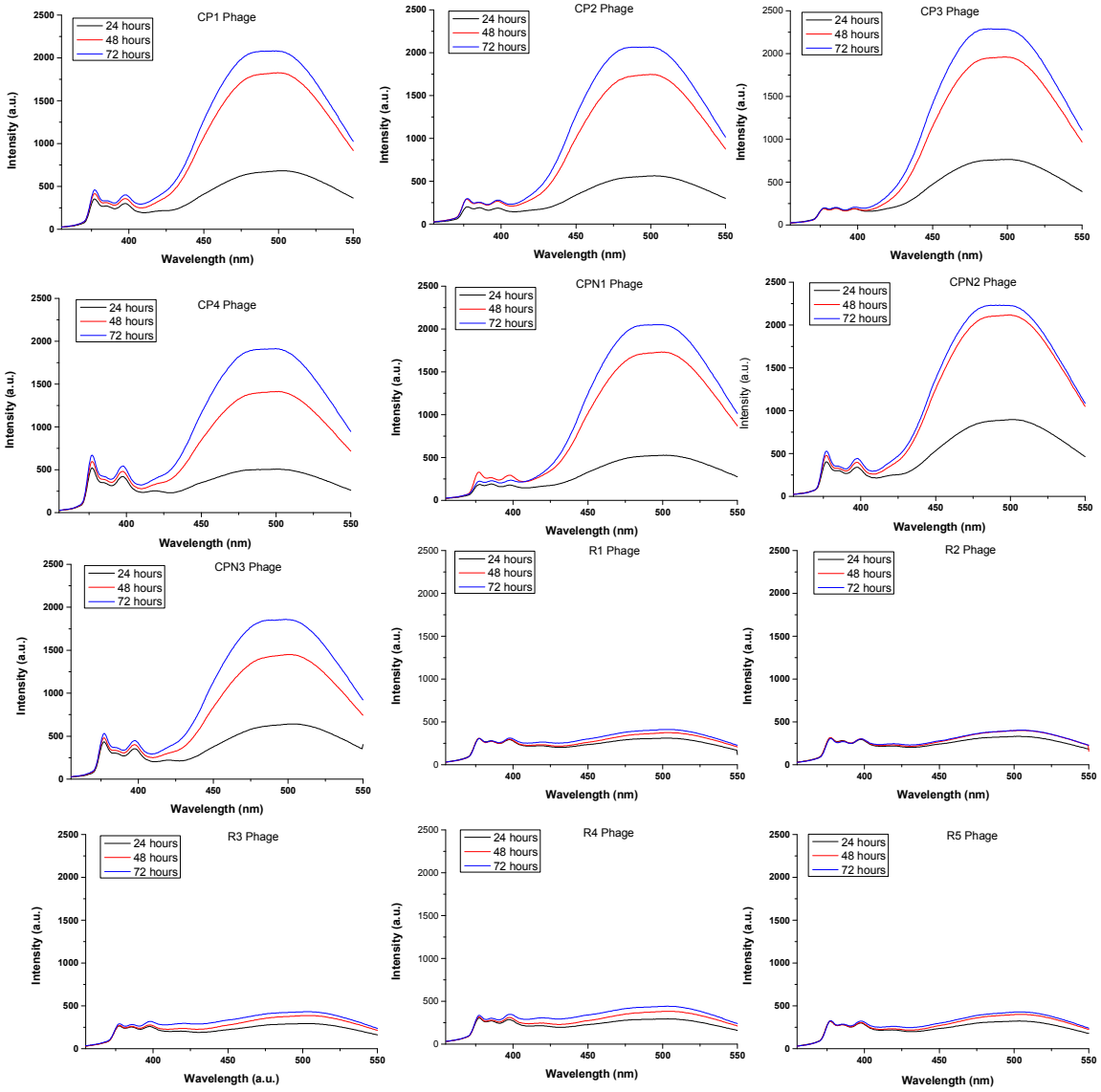



**Figure S4.1:** Amidase activity (hydrolysis of FRET peptide) of phages analyzed by fluorescence spectroscopy. Phage concentration is 0.0167μM, concentration of FRET peptide is 2 μM.

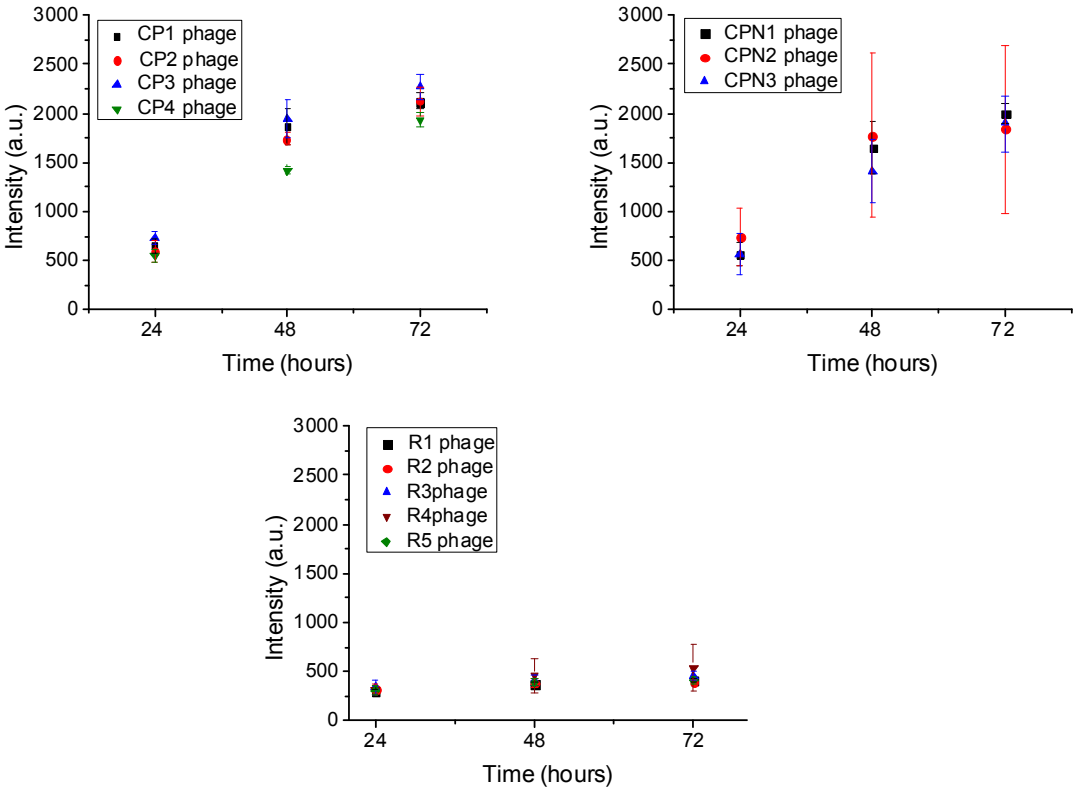
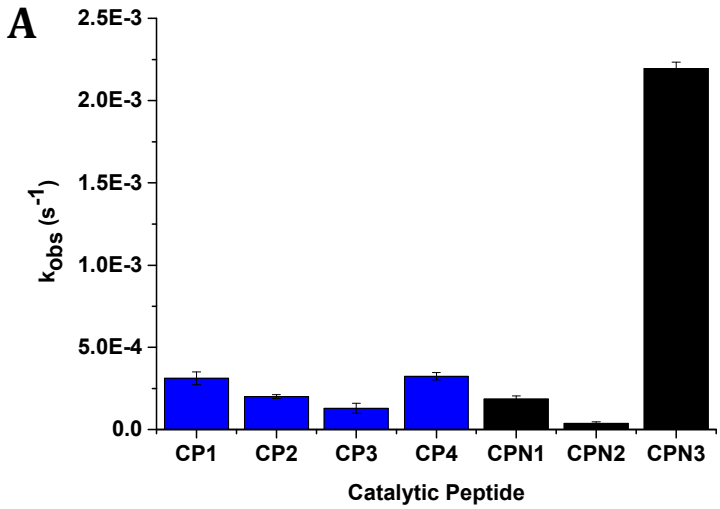



**Figure S4.2:** Time courses observed (hydrolysis of FRET peptide) of phages analyzed by fluorescence spectroscopy.

## SI 5: Esterase actvitiy of free peptides



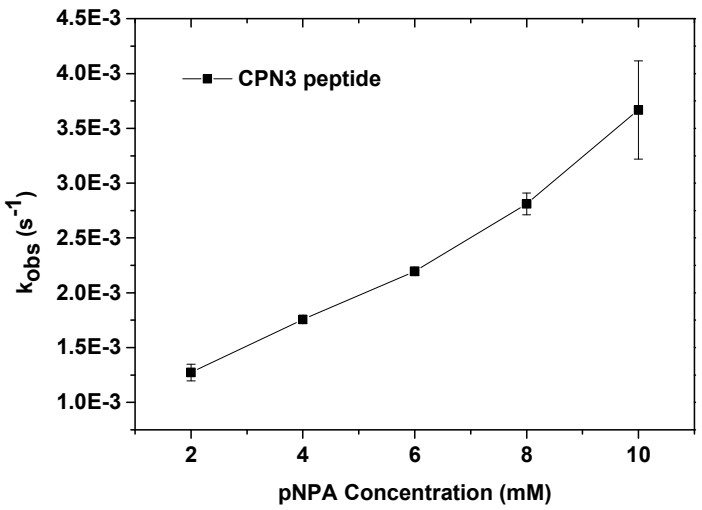




**Figure S5:** Hydrolysis of para-nitrophenyl acetate catalyzed by free peptides and analyzed by UV/Visible spectroscopy. The peptide concentration was 500 μM and the concentration of pNPA is 6 mM. (a) $K_{obs}$ values of the ester hydrolysis by various peptides. (b) Catalytic activity of the CPN3 peptide at substrate concentrations of 2 – 10mM.

## SI 6: Materials and Methods

### Materials and Methods

Peptides were purchased from Almac Sciences (East Lothian, Scotland) and GenScript (NJ, USA). Fmoc-T, L-$NH_2$ and subtilisin were purchased from Sigma-Aldrich and L-OMe·HCl from Fluka.

**Combinatorial peptide display.** The Ph.D.-12 phage display peptide library kit (New England BioLabs, Beverly, MA) was used to select catalytic peptides. For the amide-gel biopanning, the phage library ($10^{11}$ plaque forming unit, pfu) was mixed with Fmoc-T and L-OMe/L-$NH_2$ (10 mM and 40 mM at a final concentration, respectively) in 200 µL of phosphate buffer (0.94 g $L^{-1}$ monosodium phosphate monohydrate, 25 g $L^{-1}$ disodium phosphate heptahydrate, pH 8.0) and incubated for 3-4 days at room temperature. The phage particles were recovered by centrifugation (18000 x *g*, 30 min), and then washed with phosphate buffer. Fmoc-peptide aggregates associated with the phages was removed by digestion with 200 µL of 1 µg $mL^{-1}$ subtilisin (Sigma-Aldrich) or dilution with acetonitrile (see SI 2) during overnight incubation. The eluted phages were amplified by infection into *E. coli* strain ER2738, followed by purification by precipitation with polyethylene glycol. The amplified and purified phages were titered on Lysogeny Broth plates containing tetracycline, X-gal (5-bromo-4-chloro-3-indolyl-b-d-galactoside) and IPTG (isopropylb-d-thiogalactopyranoside). The DNA products were then amplified by polymerase chain reaction and sequenced on ABI Prism 3730xl DNA sequencers (SeqWright, Houston, TX).

**Transfer of Amplified phages from LB medium to buffer for activity assays.** The amplified phage samples in LB medium were centrifuged for 10 minutes at 12,000 rpm to pellet any *E. Coli* cells present in the samples. The supernatant was transferred to a fresh tube, re-spun and the pellet discarded. The top 80% of the supernatant was transferred to a fresh tube and 1/6 volume of 20% PEG/2.5 M NaCl was added and left overnight at 4°C to precipitate the phages. The PEG precipitated phages were spun down at 12,000 rpm for 15 minutes at 4°C. The supernatant was removed to leave a white smear on the tube. The pellet was re-suspended in 1 mL of buffer and micro- centrifuged at 14,000 rpm for 5 minutes at 4°C to remove any residual *E. Coli* cells. The supernatant was transferred to a fresh tube and re-precipitated by again adding 1/6 volume of PEG/NaCl and incubated on ice for 1 hour. The solution was micro centrifuged at 14,000 rpm for 15 minutes at 4°C,

the supernatant discarded and the tube spun briefly to obtain the phage smear on the side of the tube. The phages were suspended in buffer, micro centrifuged for 1 minute to precipitate any insoluble material left in the sample and then transferred the supernatant to a fresh tube and used in buffer in activity assays.

**UV Esterase Assay.** ***Phages.*** The UV substrate, p-nitrophenol acetate, was purchased from Sigma Aldrich. 300 µl of catalytic phage in PBS buffer pH 7.4 was added to 600 µl PBS buffer. 40 µl methanol was added to the phage/buffer mix. Finally 60 µl of 100 mM p-NPA in methanol was added to the sample. ***Free peptides.*** 500 µl of 1 mM catalytic peptide in PBS buffer pH 7.4 was added to 400 µl PBS buffer. 40 µl methanol was added to the peptide in buffer. Finally 60 µl of 100 mM p-NPA in methanol was added to the sample. Samples were run on a Jasco V-660 spectrophotometer at 400 nm over a course of 10 minutes, 1 scan per minute.

**FRET Assay.** The FRET substrate, Glu(EDANS)-Gly-Thr-Leu-Gly-Lys(DABCYL), was purchased from Almac. Catalytic phage (300 µl, 2.5 mM, PBS buffer 100 mM pH 8.0) was added to the FRET substrate (6 µl, 500 µM) and phosphate buffer (1194 µl PBS buffer 100 mM pH 8.0) and incubated at 37 ˚C over a period of three days. Final concentrations where 0.0167 µM phage/500 µM peptide and 2 µM substrate. The emission spectrum for each sample was recorded on a Jasco-FP-8500 spectrophotometer at 24 hour intervals. The FRET fluorescence parameters are excitation at 340 nm and emission between 355 and 550 nm. The emission peak is at 493 nm.

**FITC-Casein Assay.** The FITC–Casein kit was purchased from Sigma Aldrich, which included all substrate and buffer required for this assay. 10 µl of catalytic phage was added to 20 µl of the FITC substrate and 20 µl of incubation buffer. This was incubated at 37 ˚C for 24 hours resulting in the cleavage of the FITC-labelled casein substrate into smaller fragments. After incubation the sample was acidified with trichloroacetic acid and centrifuged so that any undigested substrate formed a pellet and any small acid soluble fragments remained in solution. 10 µl of supernatant was transferred into 1 mL of assay buffer so that the fluorescence could be recorded. The emission spectrum for each sample was recorded on a Jasco-FP-6500 spectrophotometer. The excitation wavelength was 485 nm and the emission wavelength was 535 nm. The spectra were recorded over a range of 500-600 nm.

**Amide Condensation by Catalytic Phages.** The precursors Fmoc-T (10 mM) and L-$NH_2$ (40 mM) were mixed (at 1:4 ratio) in a glass vial. The mixture was suspended in 950 µL of 100 mM phosphate buffer (pH 8) with the addition of 50 µL of the phage solution ($10^{11}$ p.f.u.). The mixture was vortex mixed for 1 min to ensure dissolution. Samples were incubated at room temperature and samples (25 µL of gel/viscous solution was dissolved in 975 µL of 50:50 acetonitrile water mixture having 0.1 % TFA) were taken after 24, 48, 72 and 216 hours for reverse phase HPLC analysis to determine the percentage conversion.