

The molecular mechanism of three novel peptides from C-phycoyanin alleviate MPTP-induced Parkinson's disease-like pathology in zebrafish

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Supplementary Information

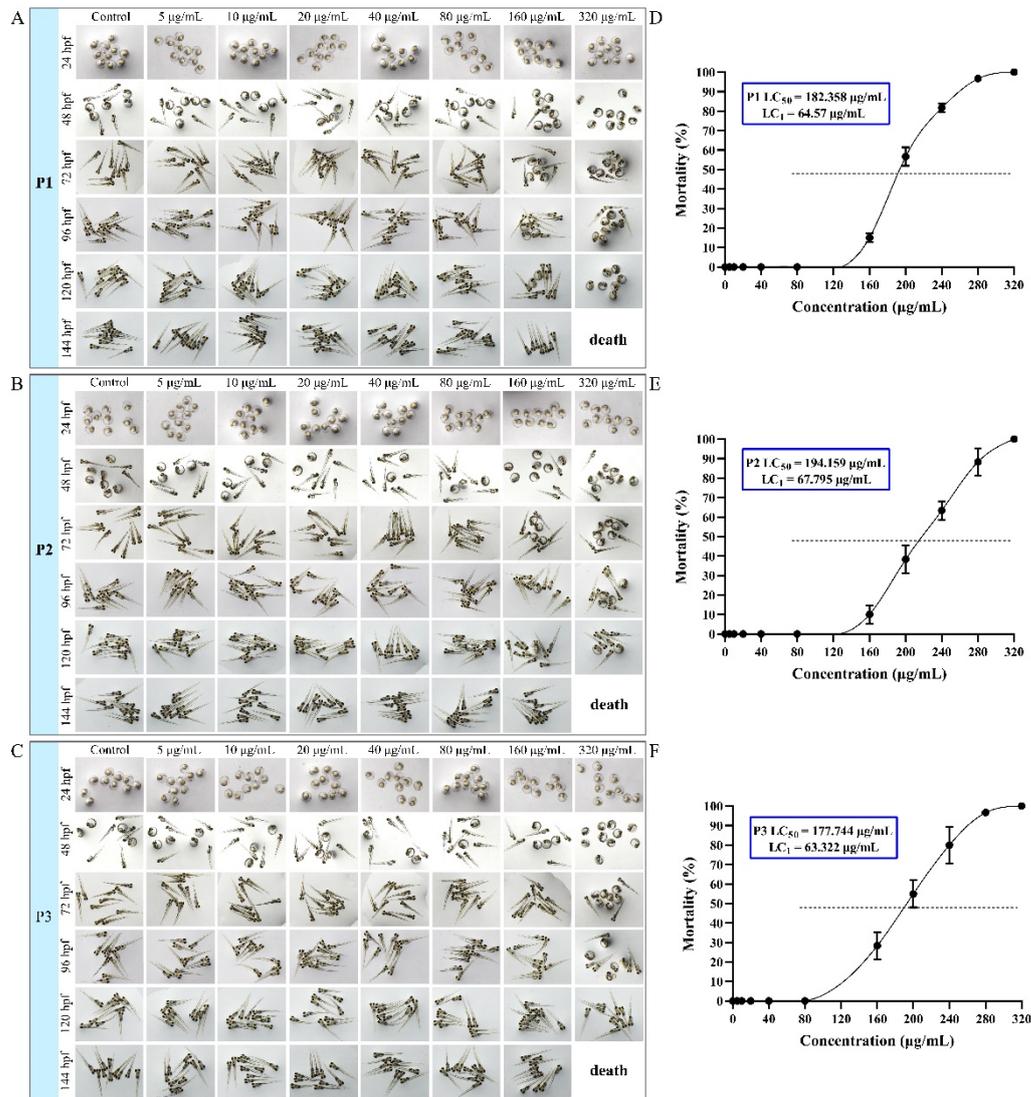
Supplementary Table 1.

The gene primers for quantitative real-time PCR

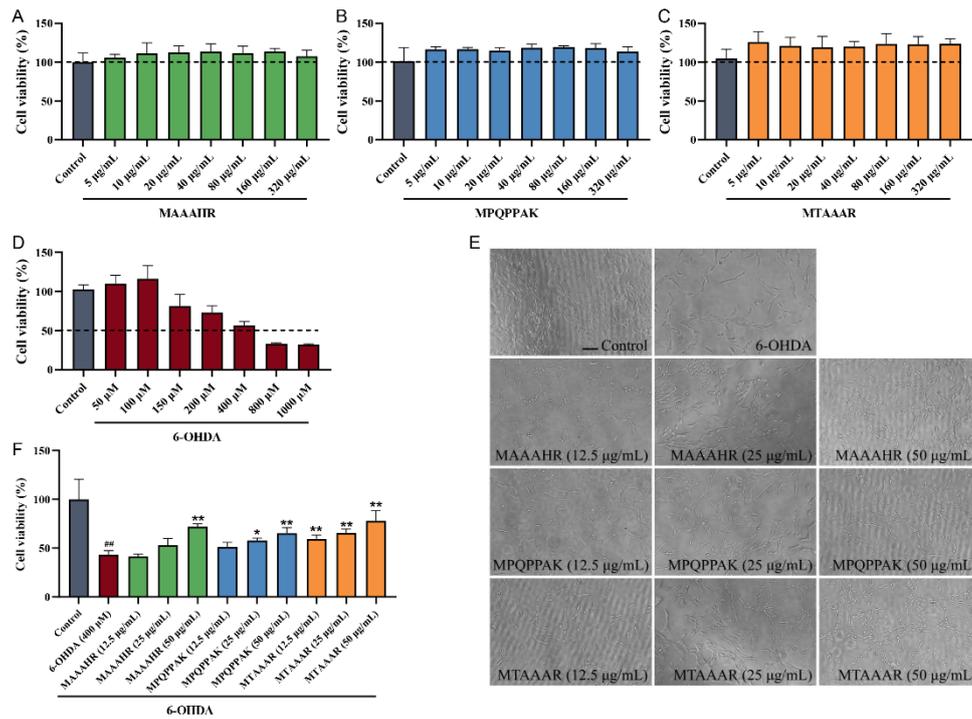
| Primer | | Sequence | Length (nucleotide) |
|--------------|---------|-------------------------|---------------------|
| <i>rps18</i> | Forward | ATACAGCCAGGTCCTTGCTAATG | 23 |
| | Reverse | GTGACGGAGACCACGGTGAG | 20 |
| <i>keap1</i> | Forward | ATACCAACCAGACACCAACAC | 21 |
| | Reverse | GGTTTGCCATCATAGCCTCC | 21 |
| <i>nrf2</i> | Forward | ATGTCTAAAATGCAGCCAAGCC | 22 |

| | | | |
|--------------------------------|---------|--------------------------|----|
| | Reverse | CGGTAGCTGAAGTCGAACAC | 20 |
| <i>gclc</i> | Forward | CGGATGGAGAGTGGAGTTCA | 20 |
| | Reverse | TTCGCTTCTGGGCTACCTT | 19 |
| <i>gclm</i> | Forward | ATCCATCAGAAGTGCGGTAG | 20 |
| | Reverse | TGCAGGTGTGTCAGTGTCT | 19 |
| <i>ho-1</i> | Forward | AAGCAAAGCGGCAGAGAAC | 19 |
| | Reverse | TGGAGCAGTCAGATGAAGTGT | 21 |
| <i>nqo1</i> | Forward | CTGGGTGGTGTGTTTGAAGAA | 21 |
| | Reverse | GCTGTGGTAATGCCGTAGG | 19 |
| <i>α-syn</i> | Forward | ATGGATGTTTTTATGAAGGGGC | 22 |
| | Reverse | ACGCTGTCTTTGGTCTTGCT | 20 |
| <i>parkin</i> | Forward | GGCAATGAAGATGATGTGGAAC | 22 |
| | Reverse | ATCACGTTGGGATGAGCACT | 20 |
| <i>beclin1</i> | Forward | AGAGCATTGAGACAAAGCGTGAA | 23 |
| | Reverse | TCTGCCAAGGCGGAAGTTATT | 21 |
| <i>atg5</i> | Forward | AGGGGATAACAGCACAAACG | 20 |
| | Reverse | CTTCTTATGCAGCGTGCCA | 20 |
| <i>map1lc3b</i> | Forward | AAAGGAGGACATTTGAGCAG | 20 |
| | Reverse | AATGTCTCCTGGGAAGCGTA | 20 |
| <i>atg3</i> | Forward | GGCTGTTGGATATGATGAG | 20 |
| | Reverse | AGCAGGTGGAGGGAGATTAG | 20 |
| <i>caspase-1</i> | Forward | TCAGCAAAGGAAATGGAT | 18 |
| | Reverse | TTAGACGGCGGTAGACAT | 18 |
| <i>caspase-3</i> | Forward | TCAGTCACGGCGATGAGGG | 19 |
| | Reverse | CCTCGACAAGCCTGAATAAAGAAC | 24 |
| <i>caspase-8</i> | Forward | AAGACCTGATTCTGCGACTG | 20 |
| | Reverse | TAGGCTGAGACACCTTTACG | 20 |
| <i>caspase-9</i> | Forward | GAGACCAACCAGGCCAAGAC | 20 |
| | Reverse | TCTCGTGCCTTATGCGTTTAGAT | 23 |
| <i>bax</i> | Forward | GGCTATTTCAACCAGGGTTCC | 21 |
| | Reverse | TGCGAATCACCAATGCTGT | 19 |

| | | | |
|--------------|---------|-----------------------|----|
| <i>bcl-2</i> | Forward | TCACTCAGTTCAGACCCTCAT | 21 |
| | Reverse | ACGCTTTCACGCACAT | 17 |

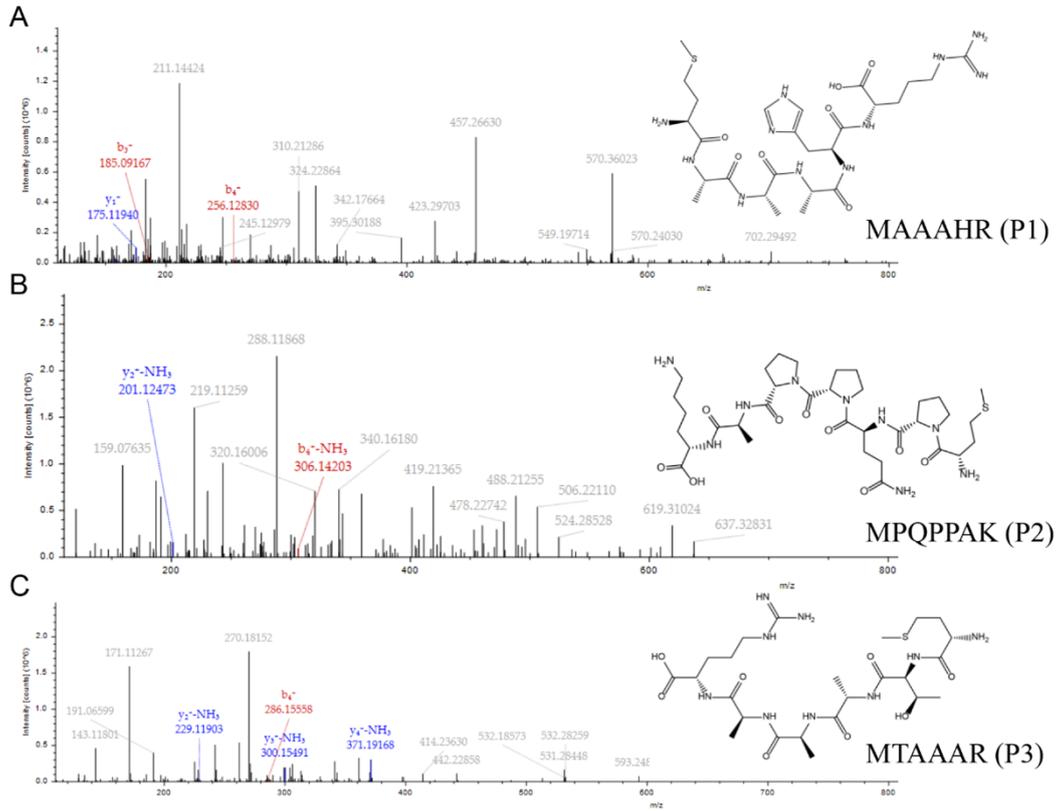


Supplementary Fig. 1. The effects of three peptides on the morphology and mortality of zebrafish embryonic/larval. (A)-(C) The overall developmental morphology of zebrafish at 24–144 hpf. (D)-(F) The mortality of zebrafish larvae at 144 hpf.



Supplementary Fig. 2. Effects of three novel peptides on 6-OHDA-induced PC12 cell death.

(A) Cells were treated for 6 h with or without different concentrations of MAAAHR. (B) Cells were treated for 6 h with or without different concentrations of MPQPPAK. (C) Cells were treated for 6 h with or without different concentrations of MTAAAR. (D) PC12 cells were incubated for 12 h with different concentrations of 6-OHDA. (E) Morphological changes in PC12 cells were visualized under an inverted microscope. (F) Cells were pretreated for 6 h with or without different concentrations of C-PC peptides. All groups were exposed to 400 μM 6-OHDA for another 12 h after the pretreatment. In A-F, cell viability was measured by the CCK8 assay and the results are expressed as a percentage of that of the control group (without treatment with 6-OHDA). ^{##}*p* < 0.01 vs. Control; **p* < 0.05, ***p* < 0.01 vs. 6-OHDA group.



Supplementary Fig. 3. MS/MS spectra and structures of the three peptides from C-Phycocyanin (C-PC). (A) MAAHR (P1). (B) MPQPPAK (P2). (C) MTAAAR (P3).