

# Bioinspired Sandcastle Worm-Derived Peptide-Based Hybrid Hydrogel for Promoting the Formation of Liver Spheroids

Yu-Hsu Chen <sup>1,†</sup>, Yuan-Hao Ku <sup>2,†</sup>, Kuo-Cheng Wang <sup>3,†</sup>, Hung-Chi Chiang <sup>4</sup>, Yu-Pao Hsu <sup>1</sup>, Ming-Te Cheng <sup>1</sup>,  
Ching-Shuen Wang <sup>5</sup> and Yinshen Wee <sup>6,\*</sup>

A

pc4

# of amino acids: 269  
M.W: 26670.86  
Theoretical pl: 9.58

MPTLYKKVGILAIIVTASVASA  
KYDTYSPSGGTHSGYN  
GPHGNVVKTYR  
GPYGAGAAKAWNHYGAGYTSVH  
HGPASTSWHTSWSNKKGGYGGL  
KNKKGYYGLKKVGYGVGLHAAGW  
HGVGPPYGAGY  
HGAGWNLGY  
HGAGYGV  
HGVGLHGAGYGL  
HGVGLHGVGYGL  
HGVGLHGAGYGL  
HGVGLHGVGYGI  
HGVGLHGAGYGI  
HGVGLHGVGYGL  
HGVGLHGAGFGL  
HGVGLHGAGYGI  
HGVGLHGAGCGI  
HKTACYGVGLHGHY

pc18

# of amino acids: 209  
M.W: 21297.16  
Theoretical pl: 9.33

LDGGCKPTGGFIKGSVGP  
GGYNHGHVVGPNGAHRGRV  
GYGPNKGYSQIYGNPGP  
GRYSHTVYPRVRPYGGYGFK  
GGYGGYHGVGYKG  
GYPGVGYHG  
GYHGVSYSG  
GYPGVGYHG  
GYHGVSYSG  
GYPGVGYHG  
GYHGVRYSG  
GYPGVGYHG  
GYHGVSYSG  
GYXGVGYHG  
GYHGVSYSG  
GYPGVGYHGGYH

pc21

# of amino acids: 252  
M.W: 27915.06  
Theoretical pl: 9.10

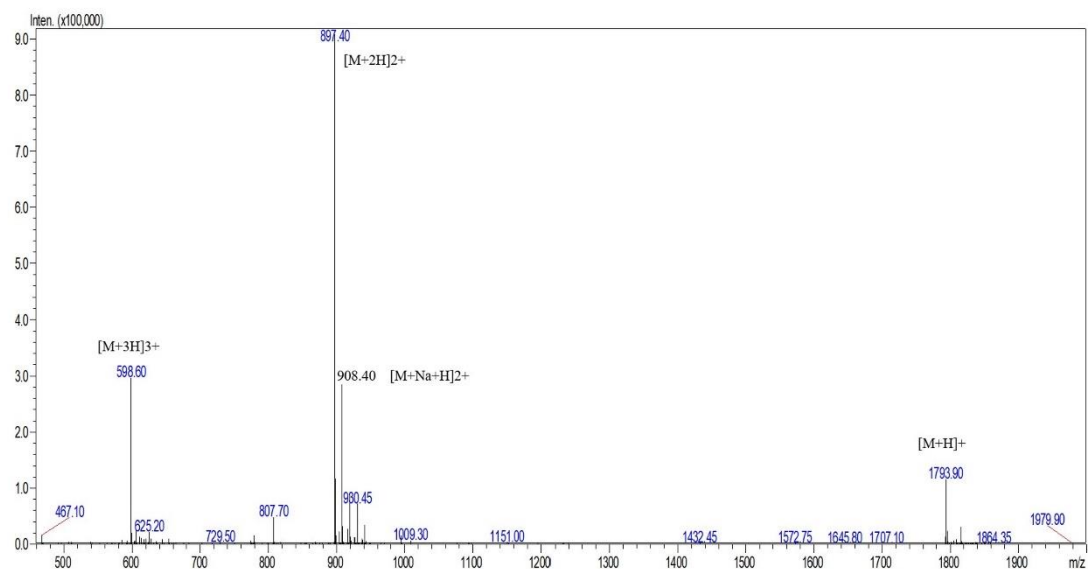
MKFFILAAALVYASGPV  
KDDDEYKETREYTKEYTVDRN  
KGYNRK  
GYGDDVSAKETFLRTTEYNDKD  
GYGSKNDGKQVKESYTREYIDKD  
GYGKDDVDTGKETRYRREYVDDD  
GYSRPRGYGRTDAY  
GLNDGYVRKDAY  
GLRRGP  
GLGVYGP  
GLGRKDVYPAT  
GLGRKDVYPV  
GLGRKDVYPAT  
GLGRKDVYPAT  
GLGRKDVYPAT  
GLGRKDVYPV  
GLGRKDVYPGP  
GLGRKGVYVGR  
GFGVPGPFW

B

Amino acid composition (mol %)

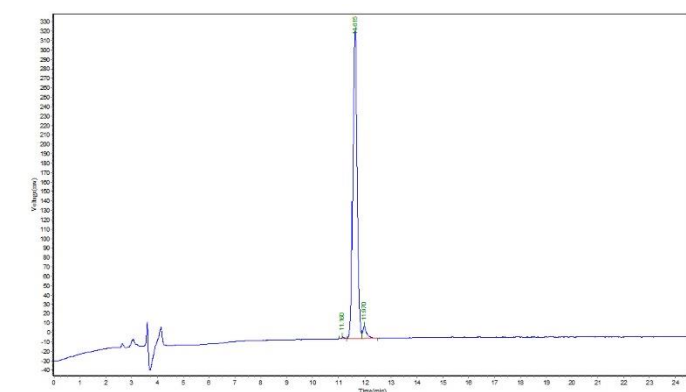
a.a	Pc4	Pc18	Pc21
Ala	8.2	0.5	4.4
Arg	0.4	2.9	8.7
Asn	2.2	1.9	2.0
Asp	0	0.5	10.7
Cys	0.7	1.0	0.4
Gln	0	1.0	0.4
Glu	0	0	4.4
Gly	31.2	35.9	16.7
His	11.5	9.1	0
Ile	2.6	1.0	0.8
Leu	7.8	0.5	6.0
Lys	4.5	2.4	9.1
Met	0.4	0	0.4
Phe	0.4	1.0	2.0
Pro	2.6	6.2	6.7
Ser	3.7	6.7	2.0
Thr	3.3	1.0	6.3
Trp	1.9	0	0.4
Tyr	9.7	18.2	10.3
Val	8.9	10	8.3

**Figure S1.** Amino acid composition of sandcastle worm's adhesive proteins.



Mw: 1792.94  
 Probe: ESI  
 Probe bias: +4.5kv  
 Nebulizer Gas Flow: 1.5L/min  
 Detector: 1.2kv  
 CDL: -20.0v

Figure S2. MS Spectrum of PC4 peptide.



Peak No.	Ret Time	Height	Area	Conc.
1	11.160	1688.932	12554.857	0.3317
2	11.615	326223.031	3636195.250	96.0547
3	11.970	12748.593	136797.188	3.6137
Total				100.00

Column :250\*4.6mm,Kromasil-C18-5um  
 Solvent A:0.1%TFA in 100%owater  
 Solvent B:0.1%TFA in 100%acetonitrile  
 Gradient :      A      B  
                  0.1min 79% 21%  
                  25.0min 54% 46%  
                  25.1min 0% 100%  
                  30.0min stop  
 Flow rate:1.0ml/min  
 Wavelength(nm):220  
 Volume :10ul

Figure S3. HPLC report of PC4 peptide.

**Table S1. Primer sequences.**

Gene	Forward (5'-3')	Reverse (5'-3')
<b>PCNA</b>	CCTGCTGGGATATTAGCTCCA	CAGCGGTAGGTGTCGAAGC
<b>c-Myc</b>	ATGGCCCATTACAAAGCCG	TTTCTGGAGTAGCAGCTCCTAA
<b>Ki67</b>	TTCGCAAGCGCATAACCCA	AACCGTGTACAGTGCCAAA
<b>bax</b>	CCCGAGAGGTCTTTTTCCGAG	CCAGCCCATGATGGTTCTGAT
<b>CYP3A4</b>	TTCAACAGATGATCGACTCCCA	TTGTGTCATAGGCAGCAAAAATG
<b>CK18</b>	TCGCAAATACTGTGGACAATGC	GCAGTCGTGTGATATTGGTGT
<b>CYP7A1</b>	AGAAGCATTGACCCGATGGAT	AGCGGTCTTTGAGTTAGAGGA
<b>A1AT</b>	CCTGAAGGCCCTGATAAGAA	GCTGGACTGATTGAGGGTGC
<b>CK7</b>	GTGGCAGCCGAAGTTAGGAAG	GCACTGTGGTGTGATGGGATA
<b>AFP</b>	CTTTGGGCTGCTCGCTATGA	GCATGTTGATTTAACAAGCTGCT
<b>E-cadherin</b>	CGAGAGCTACACGTTACGG	GGGTGTCGAGGGAAAAATAGG