

## Supporting Information

### Mechanism of dimerisation of vascular endothelial growth factor C

Joyce Chiu<sup>†</sup>, Jason W. H. Wong<sup>†</sup>, Michael Gerometta<sup>‡</sup> and Philip J. Hogg<sup>†,\*</sup>

<sup>†</sup>Lowy Cancer Research Centre and Prince of Wales Clinical School, University of New South Wales, Sydney, NSW 2052, Australia

<sup>‡</sup>Circadian Technologies Limited, Vegenics Pty Ltd, 650 Chapel Street, South Yarra, VIC 3141, Australia

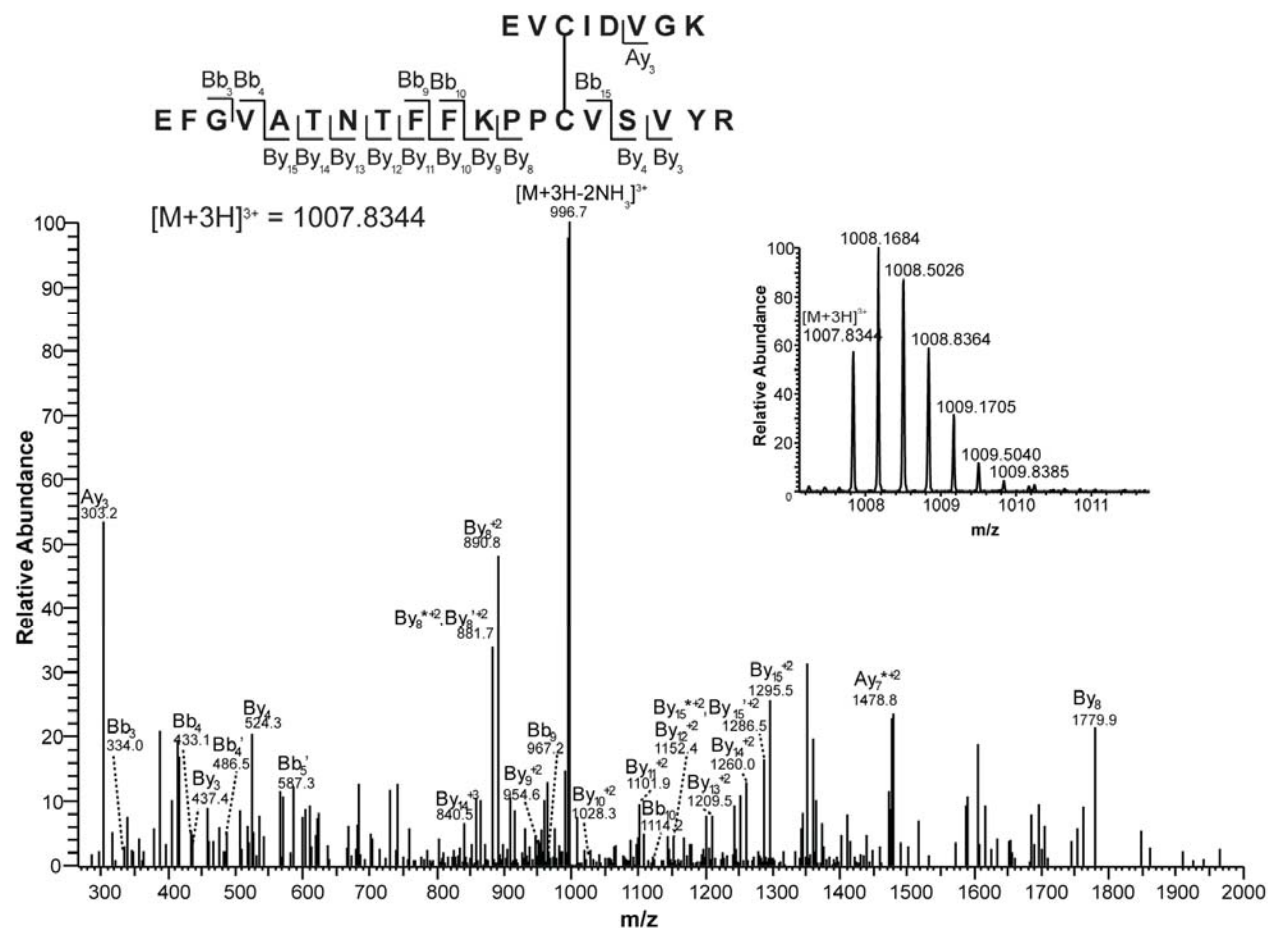
#### Production of recombinant VEGF-C

There are various forms of mature VEGF-C in nature because the N- and C-terminal propeptides can be cleaved from the central domain at different locations. We have produced a human VEGF-C encompassing residues 112-227, which mimics a mature form of the growth factor<sup>(1)</sup>. Our expression vector incorporated an IL-3 signal sequence followed by a FLAG sequence and then VEGF-C residues 112-227. Cleavage of the IL-3 signal peptide resulted in an N-terminus of ASIS, followed by the FLAG residues DYKDDDDK, then VEGF-C residues 112-227. The N-terminus was confirmed by Edman sequencing.

1. Joukov, V., Pajusola, K., Kaipainen, A., Chilov, D., Lahtinen, I., Kukk, E., Saksela, O., Kalkkinen, N., and Alitalo, K. (1996) A novel vascular endothelial growth factor, VEGF-C, is a ligand for the Flt4 (VEGFR-3) and KDR (VEGFR-2) receptor tyrosine kinases, *EMBO J.* 15, 290-298.

**Table 1.** Quantification of the Cys137-Cys156 disulfide linked peptide in VEGF-C monomer and dimer.

Experiment	Peptide	Cys residues	VEGF-C monomer		VEGF-C dimer	
			abundance	relative abundance	abundance	relative abundance
1	EVCIDVGK	137-165	51779290	6.44	1503181	0.96
	EFGVATNTFFKPPCVSVYR					
	SIDNEWK	control	804294435		155958793	
2	EVCIDVGK	137-165	15822918	2.32	4206646	0.80
	EFGVATNTFFKPPCVSVYR					
	SIDNEWK	control	682773735		524863548	
3	EVCIDVGK	137-165	19291111	2.56	1994156	0.76
	EFGVATNTFFKPPCVSVYR					
	SIDNEWK	control	752525957		262815032	



**Figure S1.** Cys137 forms a disulfide bond with Cys156 in VEGF-C dimer. Tandem mass spectrum of the peptide linked by a disulfide bond between Cys137 and Cys156 in VEGF-C dimer. The accurate mass spectrum of the peptide is shown in the inset (observed  $[M+3H]^{3+} = 1007.8344$  m/z; expected  $[M+3H]^{3+} = 1007.8331$  m/z). Ions with neutral loss of small molecules are labeled by asterisk (\*, loss of ammonia) and prime (', loss of water).