Supporting Information

to

Self-adjuvanting polymer-peptide conjugates as therapeutic vaccine candidates against cervical

cancer.

Tzu-Yu Liu, [†] Waleed M. Hussein, [†] Zhongfan Jia, [‡] Zyta M. Ziora, [†] Nigel A. J. McMillan, ["] Michael J. Monteiro,[‡] Istvan Toth,^{†, §,} Mariusz Skwarczynski.^{†,*}

[†]The University of Queensland, School of Chemistry and Molecular Biosciences, Brisbane, QLD 4072, Australia, [‡]The University of Queensland, Australian Institute for Bioengineering and Nanotechnology, Brisbane, QLD 4072, Australia, [□]Cancer Research Centre, Griffith Health Institute and School of Medical Science, Griffith University, Gold Coast, QLD 4222, Australia, and [§]The University of Queensland, School of Pharmacy, Brisbane, QLD 4072, Australia.



Figure S1. HPLC trace of (a) $8Q_{min}$ azide peptide, (b) $8Q_{ser}$ azide peptide, and (c) $8Q_{Lys}$ azide peptide. (Background signal was marked with a cross).



Figure S2. Model "click" reaction between alkyne **1** and azide **2** in DMF at 50 °C using copper wires as a Cu (I) source. The reaction progress was monitored by HPLC and the products were detected by MS.

Cu source	Reducing	Solvent	T °C	Reaction	Major product
	agent			time (h)	
Cu wire		DMF	50 °C	20 h	Dimer of 3
Cu wire	Asc-H ¹	DMF	RT	28 h	Dimer of 2
CuSO ₄	$Asc-H^1$	DMF	RT	28 h	Dimer of 2
Cu wire		Solvent B ³	RT	28 h	Dimer of 2
CuI	$Asc-H^1$	Solvent B ³	RT	22 h	Dimer of 2
CuI	Asc-H ¹	DMF	RT	22 h	Dimer of 2
Cu wire	Added TCEP ²	DMF	50 °C	48 h	Unidentified mixture
	after 22 h				
CuSO ₄	Added TCEP ²	DMF	50 °C	48 h	Unidentified mixture
	after 22 h				

Asc-H: ascorbic acid
TCEP: tris(2-carboxyethyl)phosphine
Solvent B: acetonitrile/water/trifluoroacetic acid, 90/10/0.1

Figure S3. Summary of reaction conditions and results of CuAAC model studies.







Figure S4. Hydrodynamic diameter (volume-based particle size) as measured by DLS for (a) **S4-8Q**_{min}, (b) **S4-8Q**_{ser}, and (c) **S4-8Q**_{Lys} in water.



Figure S5. Confocal microscope images of (a) S4-8Q_{min}, (b) S4-8Q_{ser}, and (c) S4-8Q_{Lys} in PBS (scale bar: 20 μ m).



Figure S6. Development of TC-1 tumor (up to 21 days) in five mice treated with (a) S4- $8Q_{ser}$, (b) S4- $8Q_{Lys}$, or (c) 8Q + ISA51. Female C57BL/6 (6-8 weeks old) mice were challenged with TC-1 tumor on day 0. The mice received vaccination on day three.



Scheme S1. Isopeptide method for the synthesis of peptides that contain a difficult sequence $(8Q_{Ser})$.