

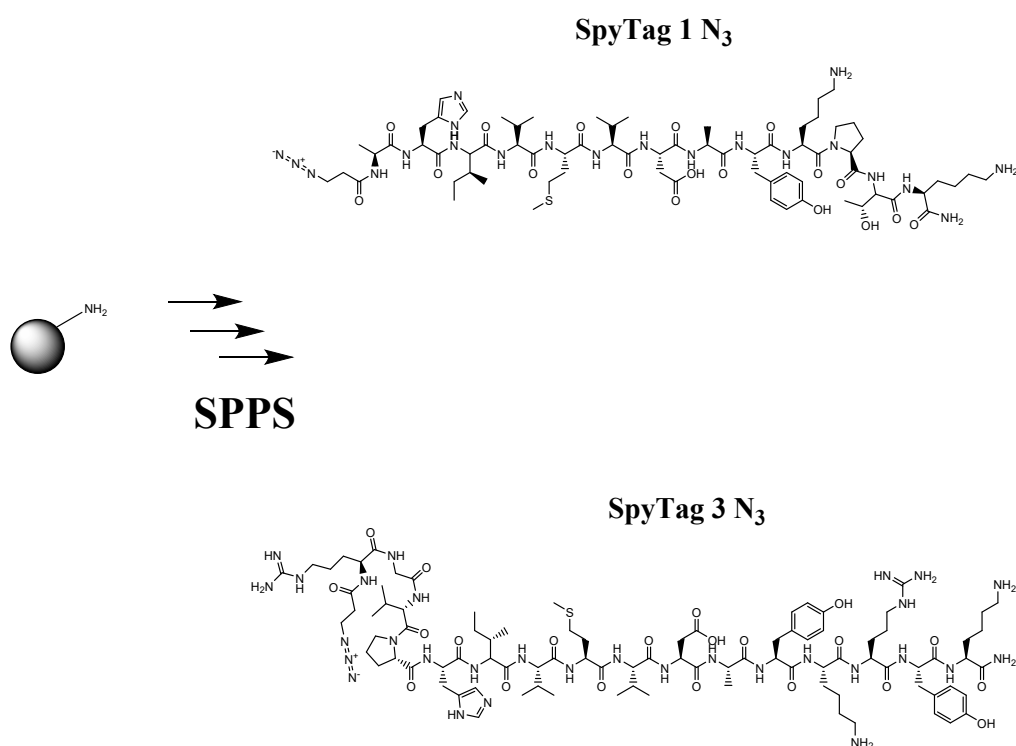
1 **Modification of Bacterial Microcompartments by Target Biomolecules via Post-Translational**

2 **SpyTagging.**

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4 Sarah Martin<sup>c</sup>, Andrew Morrell<sup>a</sup>, Emi Nemoto-Smith<sup>d</sup>, Joanne Roobol<sup>a</sup>, Maria Stanley<sup>a</sup>, C Mark Smales<sup>a,e†</sup>, Martin  
5 J Warren<sup>a,f,e†</sup>

6 **Supplementary**

7 **Figure 1 – Scheme showing the azido SpyTag 1 N<sub>3</sub> and SpyTag 3 N<sub>3</sub> synthesised by SPPS.**



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9 **SpyTag 1 N<sub>3</sub> amino acid sequence**

10 N<sub>3</sub> - AHIVMVDAYKPTK

11 **SpyTag 3 N<sub>3</sub> amino acid sequence**

12 N<sub>3</sub> - RGVPHIVMVDAYKRYK

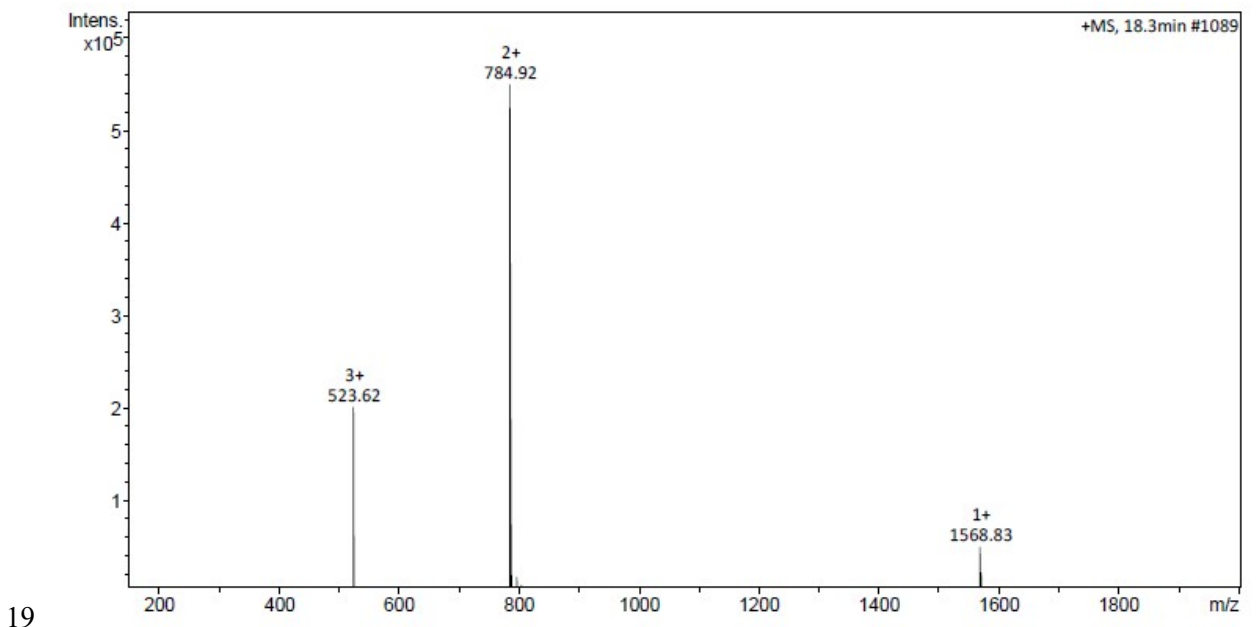
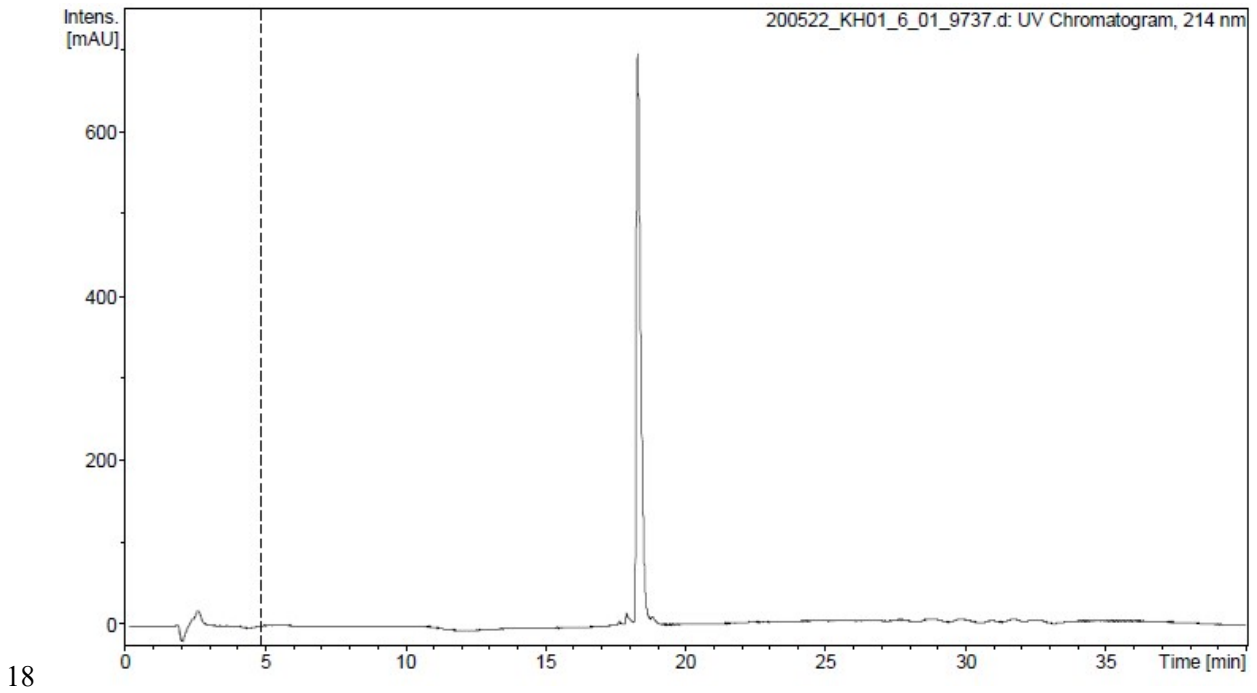
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15 **Figure 2 – LCMS analysis of SpyTag 1 N<sub>3</sub> (A) and SpyTag 3 N<sub>3</sub> (B)**

16 **A**

17 SpyTag 1 N<sub>3</sub> RMM = 1568.86 Da

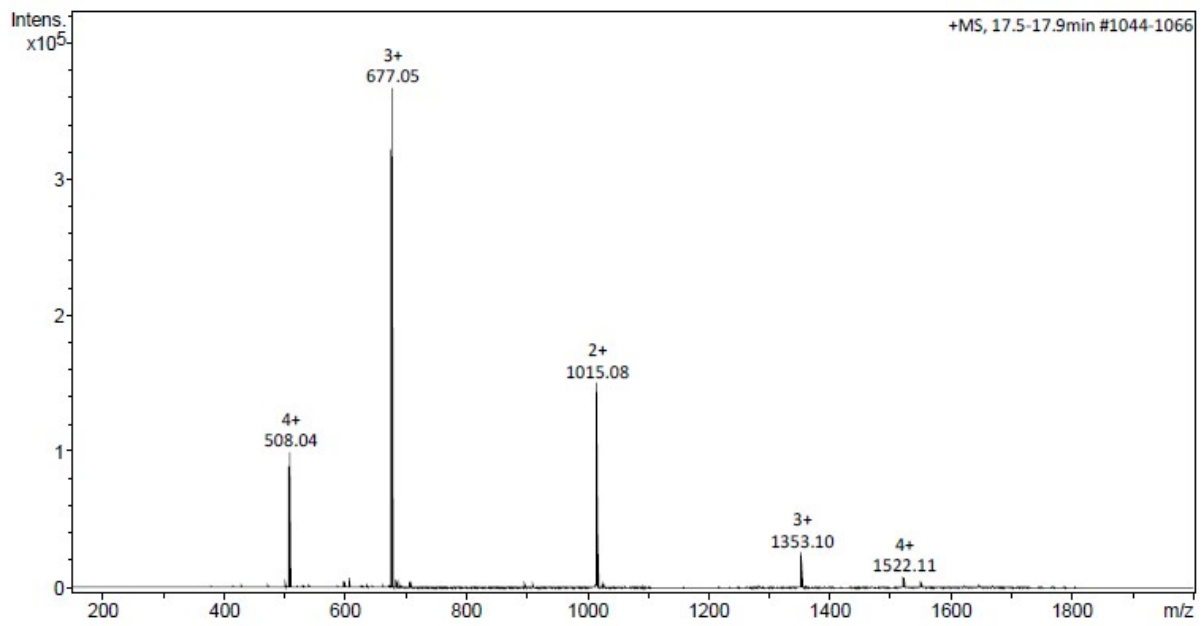
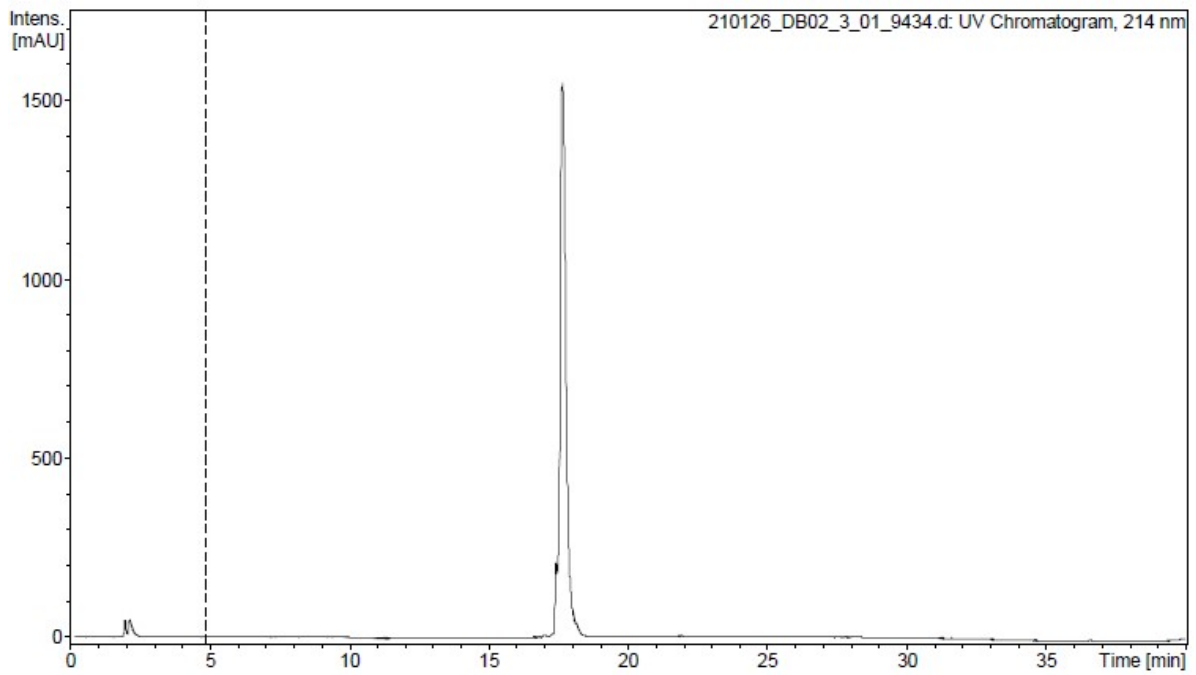


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22 **B**

23 SpyTag 3 N<sub>3</sub> RMM = 2028.42 Da



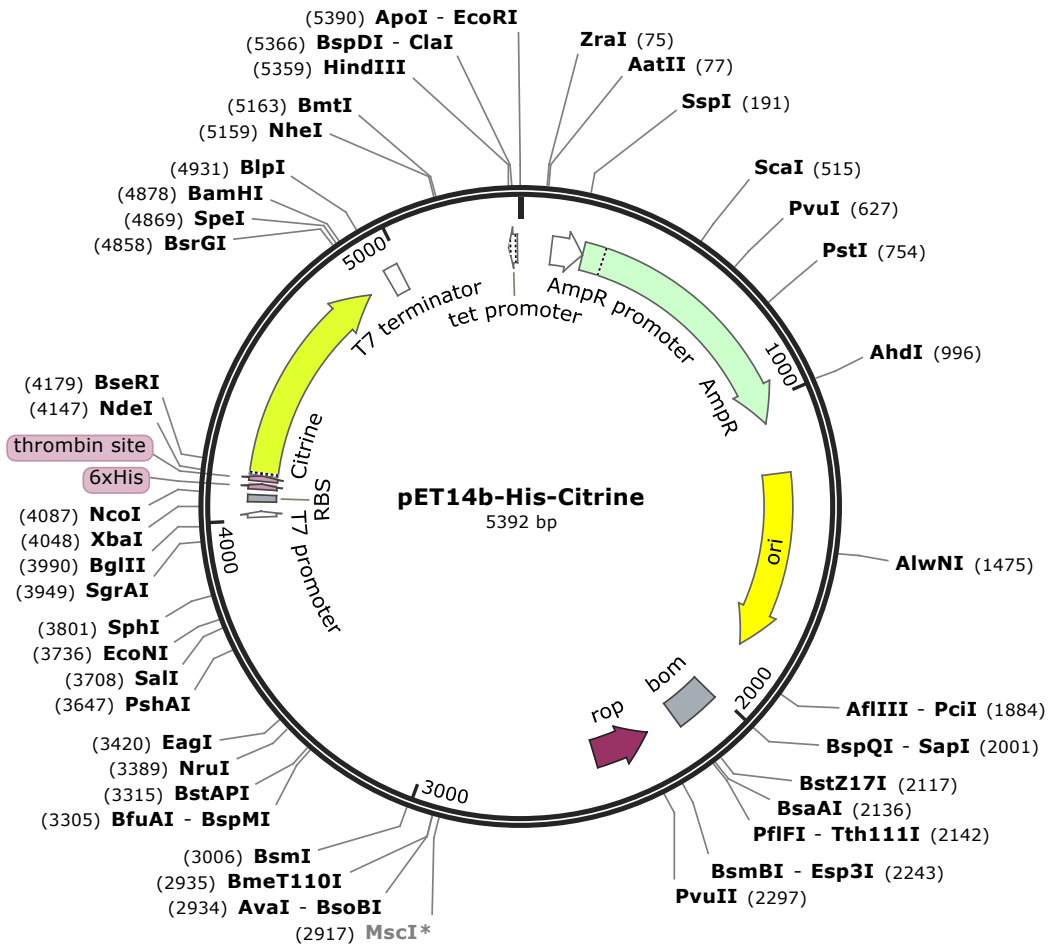
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29 **Figure 3. His tagged Citrine - plasmid map (A) and protein sequence (B)**

30 **A**



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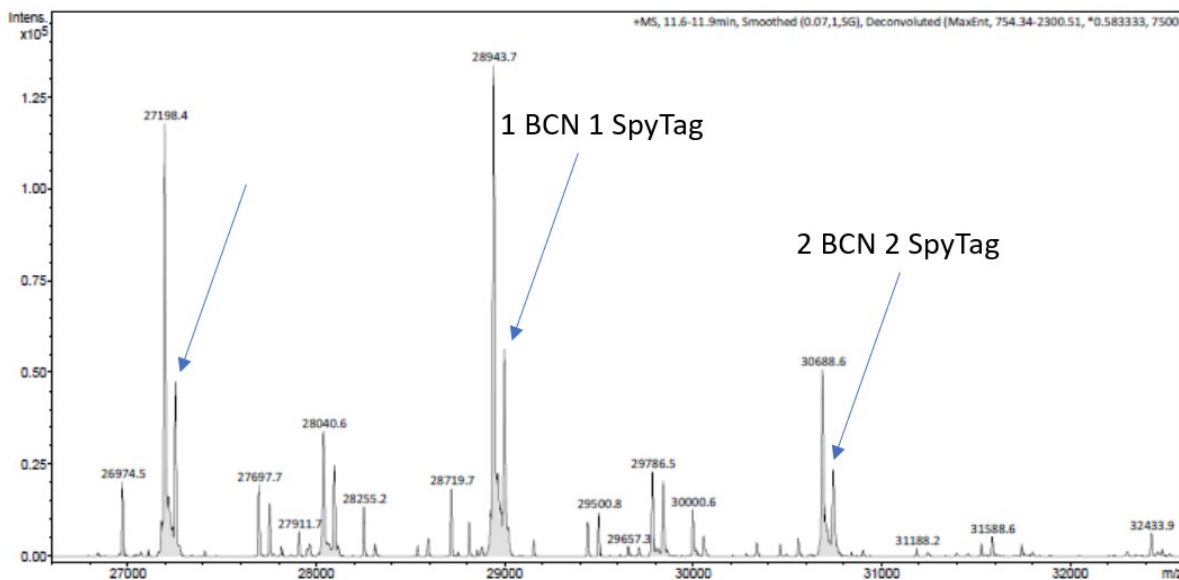
32 **B. Citrine amino acid sequence**

33 MGSSHHHHHHSSGLVPRGSHMVSKGEELFTGVVPILVELDGDVNGHKFSVSGEGEDATYGKLT LKFICTTGKLPV  
 34 PWPTLVTTFGYGLMCFARYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYKTRAEVKFEGDTLVNRIELKGIDF  
 35 KEDGNILGHKLEYNVSHNVYIMADKQKNGIKVNFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHLSYQS  
 36 ALSKDPNEKRDHMLLEFVTAAGITLGMDELYK

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39 Figure 4 ES MS analysis of the Citrine BCN SpyTag1 N<sub>3</sub> conjugate.

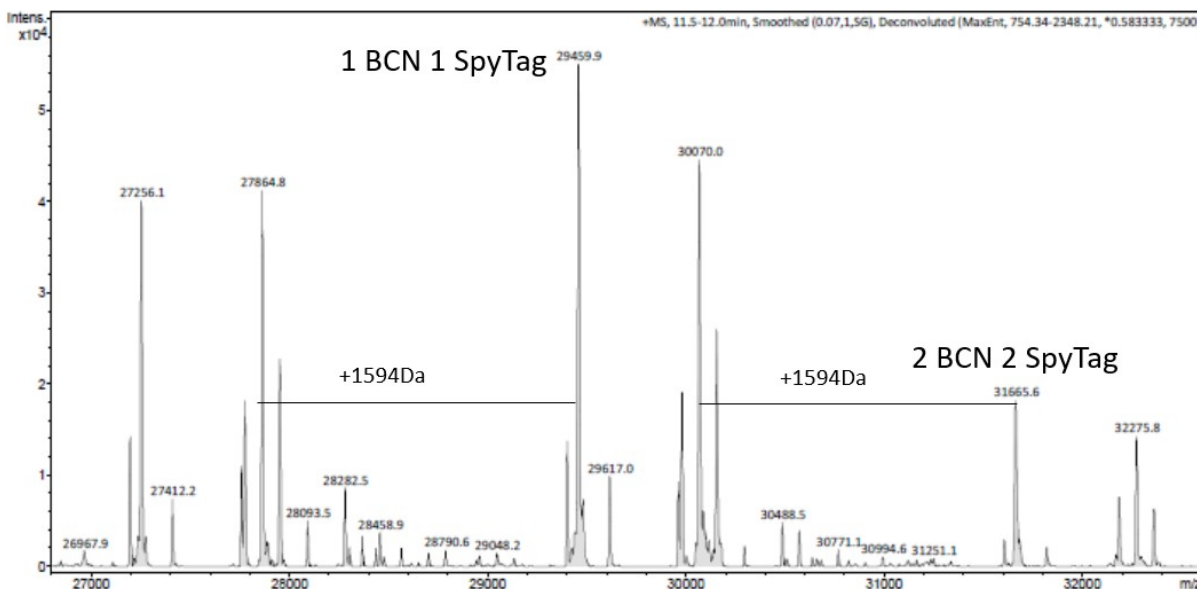


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43 Figure 5 ES MS analysis of the Citrine BCN SpyTag1 N<sub>3</sub> conjugate.



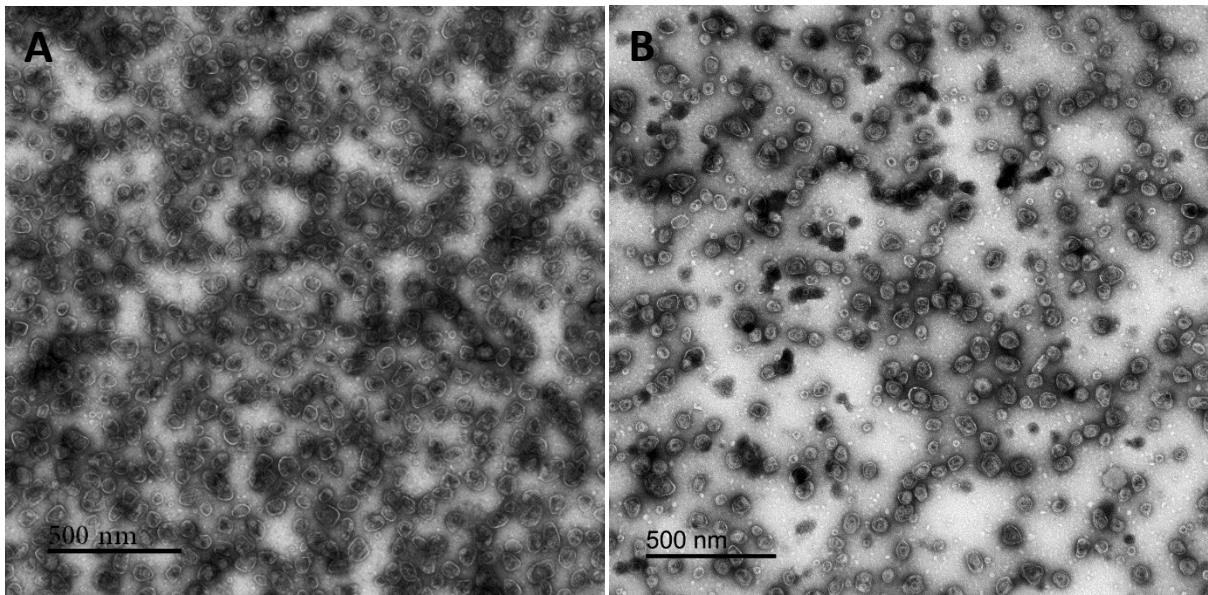
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48 **Figure 6** Electron micrographs of purified BMC. A) eBMC formed by three shell proteins. B) sc-  
49 eBMC form by three shell proteins with spycatcher sequence fused in one of the shell proteins,  
50 PduK.



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