# SUPPORTING INFORMATION

# The effects of acid hydrolysis on protein biosurfactant molecular, interfacial, and foam properties: pH responsive protein hydrolysates

*Mirjana Dimitrijev-Dwyer,*[a,b] *Lizhong He,*[#a] *Michael James,*[c] *Andrew Nelson,*[c] *Liguang Wang,*[b] *Anton P. J. Middelberg**[a,b]

[a] Centre for Biomolecular Engineering, Australian Institute for Bioengineering and Nanotechnology

[b] School of Chemical Engineering, the University of Queensland, St Lucia, QLD 4072, Australia

[c] Bragg Institute, Building 87, Australian Nuclear Science and Technology Organisation, Locked Bag 2001, Kirrawee DC, NSW 2232, Australia

[#] current address: Department of Chemical Engineering, Monash University, Clayton, VIC 3800, Australia

**Identifying the R1 and R2 regions in Figure 2A by ESI mass spectrometry**

To identify the R1 and R2 regions in Figure 2A, a Waters Quattro Micro API quadrupole mass spectrometer (Waters, UK) with electrospray ionization (ESI) was used in positive ion mode. The R1 and R2 fractions were manually collected into eppendorf tubes after elution from the HPLC column, frozen at -80°C overnight, and lyophilized to remove solvents. The lyophilized R1 and R2 fractions were then resuspended in a minimal amount of Milli-Q water to ensure maximum signal, and directly injected into the mass spectrometer at a flowrate of 10µL $min^{-1}$. Control and data collection was performed by Waters MassLynx software, and manually analysed.

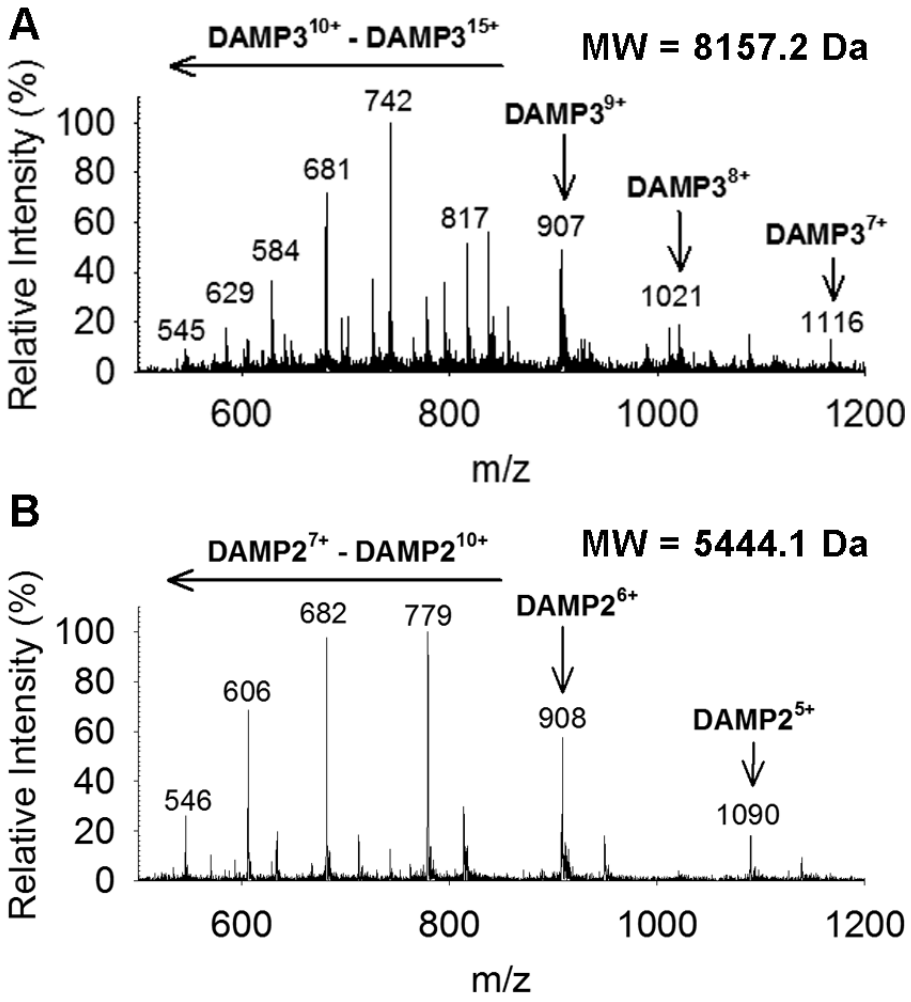


**Figure S1.** ESI mass spectrum of DAMP4 cleavage intermediates, from regions shown in the Figure 2A, 6 h HPLC trace. A) Mass spectrum of R1, showing the presence of DAMP3 (8157.2 Da). The other peaks are due to incomplete separation from R2 and the DAMP4 peak, as well as other reaction intermediates such as MD-DAMP3 (DAMP3 before cleavage of the N-terminal MD motif). B) Mass spectrum of R2, showing the presence of DAMP2 (5444.1 Da). The other peaks are mainly due to the presence of MD-DAMP2 (DAMP3 before cleavage of the N-terminal MD motif).