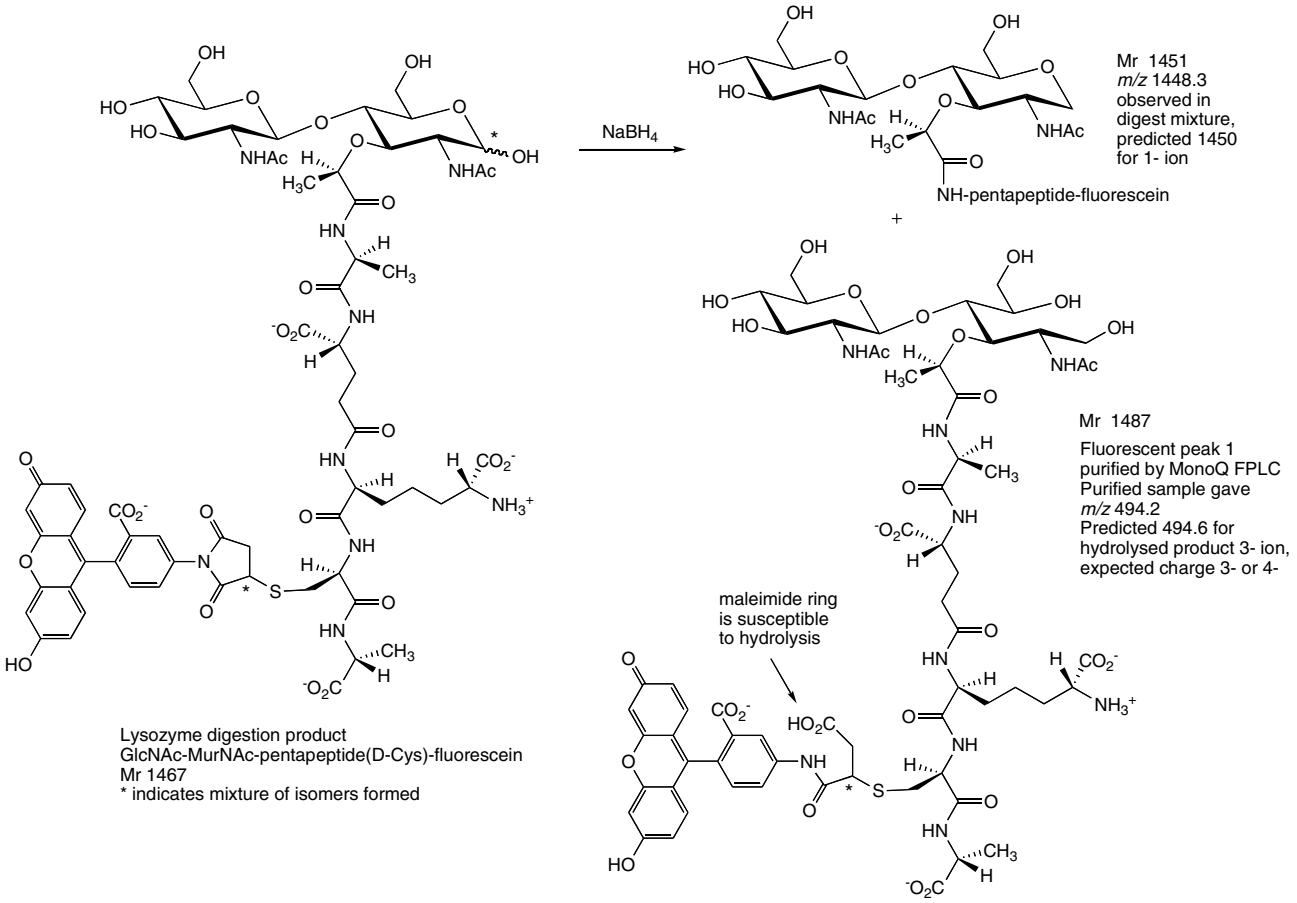
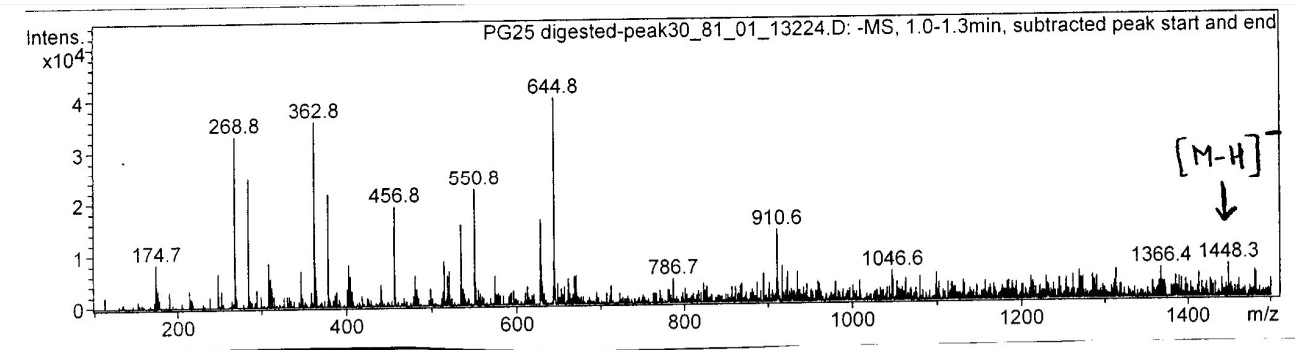
Supporting Information for Vinatier *et al*. "*In vitro* biosynthesis of bacterial peptidoglycan using D-Cys-containing precursors: fluorescent detection of transglycosylation and transpeptidation"

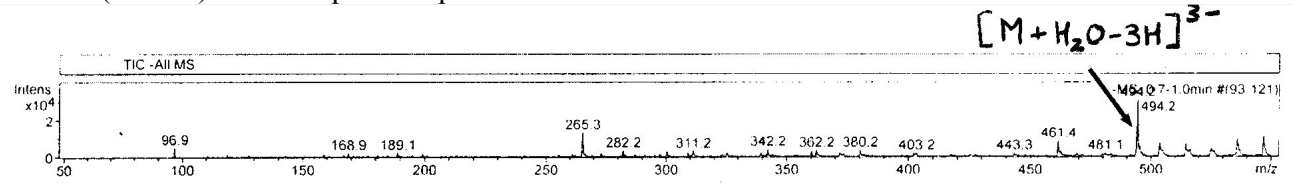
Fluorescent peak 1 – GlcNAc-MurNAc-pentapeptide(D-Cys)-fluorescein



ES-MS (-ve ion) of lysozyme digest after $NaBH_4$ treatment

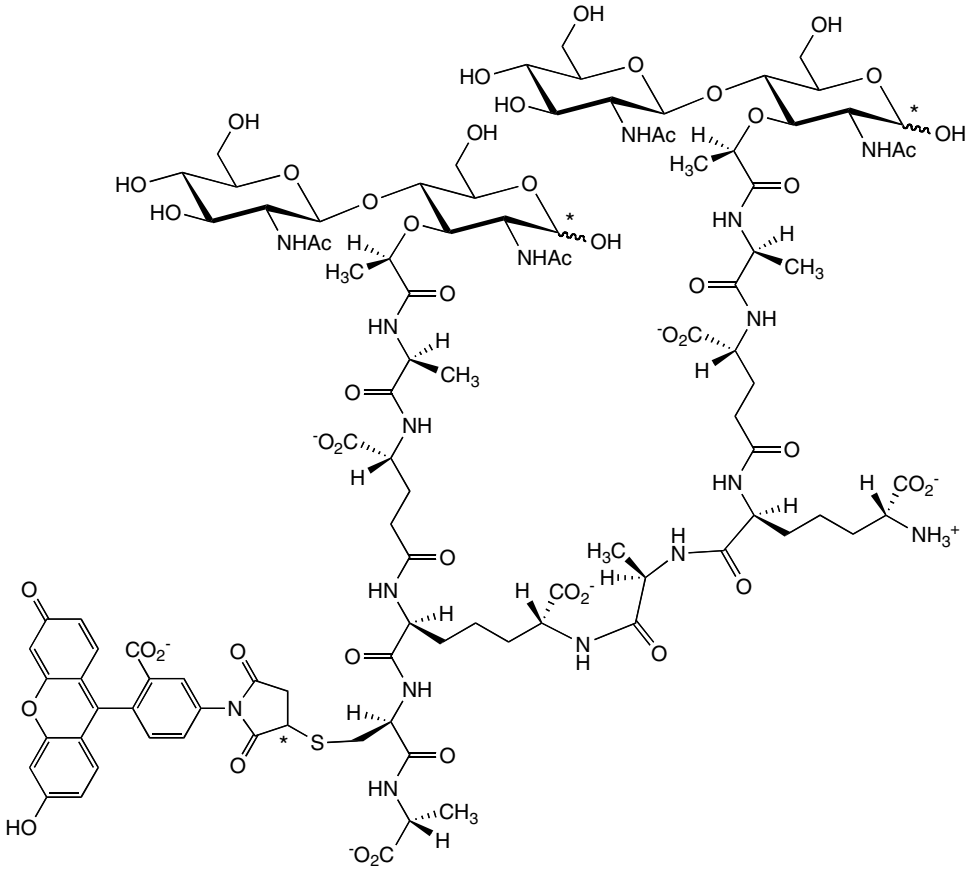


ES-MS (-ve ion) of FPLC purified peak



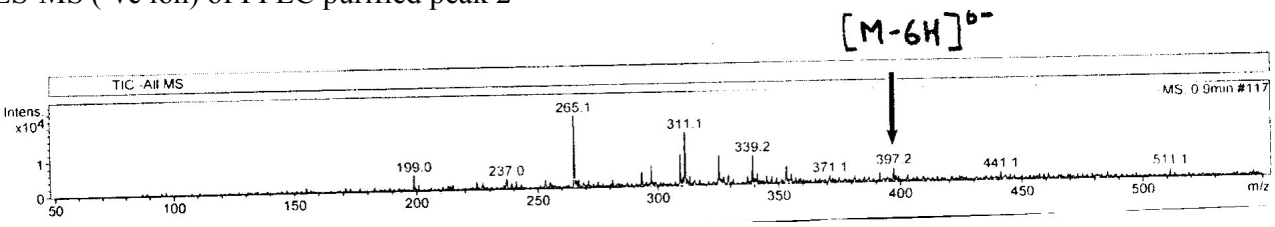
Fluorescent peak 2 – GlcNAc-MurNAc-pentapeptide dimer-fluorescein

Lysozyme digestion product
GlcNAc-MurNAc-pentapeptide dimer-fluorescein
Mr 2389
* indicates mixture of isomers formed

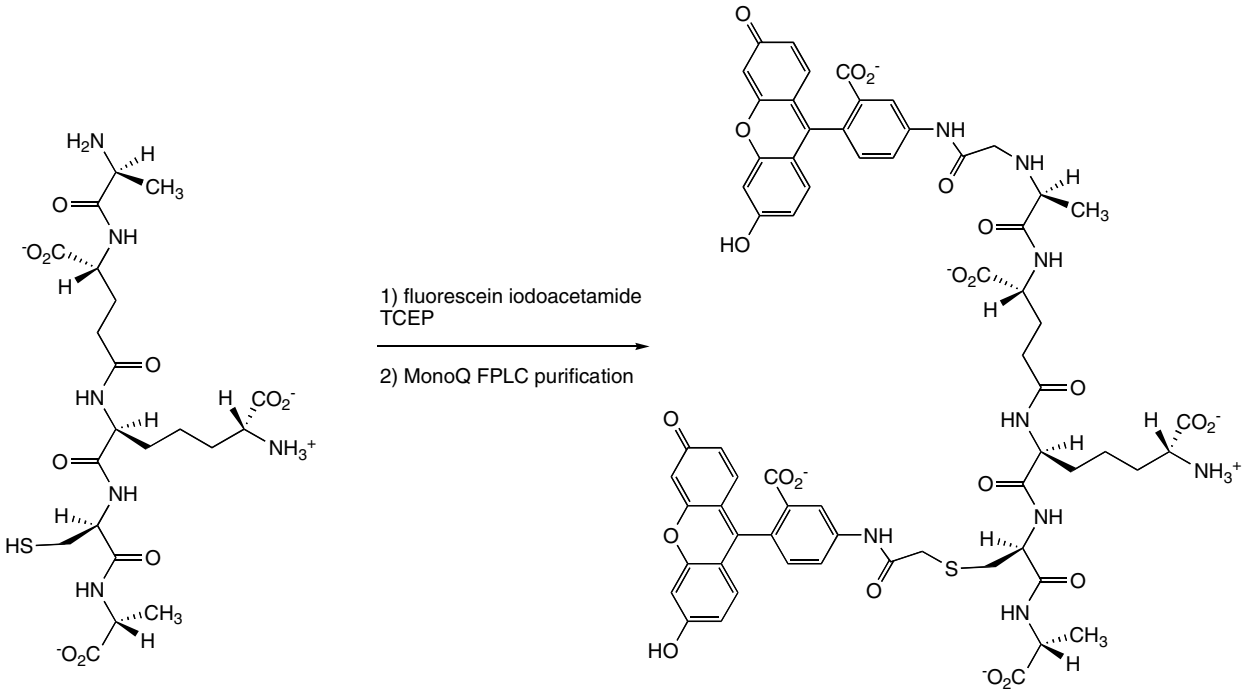


Fluorescent peak 2
(prior to $NaBH_4$ treatment)
after MonoQ FPLC purification
gave *m/z* 397.2
Predicted *m/z* 397.2 for 6- ion
Expected charge 5- or 6-

ES-MS (-ve ion) of FPLC purified peak 2



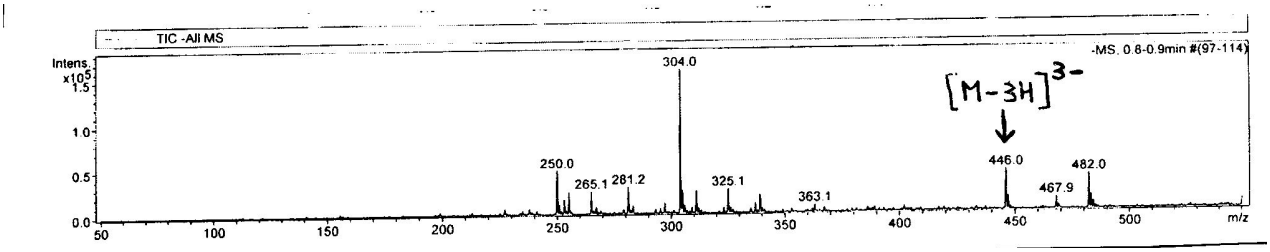
Digestion of D-Cys-labelled peptidoglycan with MurNAc-L-Ala amidase, followed by labelling with fluorescein iodoacetamide, and purification of fluorescently labelled products by MonoQ FPLC, gave signals corresponding to the pentapeptide product, labelled with two IAF fluorophores:



MurNAc-L-Ala amidase digestion product

Mr 1340.7
FPLC purified sample gave *m/z* 446.0
Predicted 445.9 for 3- ion,
expected charge 3- or 4-.

ES-MS (-ve ion) of FPLC-purified amidase digest



Surface plasmon resonance protocol

SPR measurements were performed using a custom built SPR instrument based on a Kretschmann configuration, using a 5mW HeNe laser (λ = 632.8 nm). SPR spectra were simulated using a four layer model representing prism, gold, organic layer, and subphase with refractive indices of *n* 1.84, 0.25-3.1, 1.45, and 1.33 respectively. Spectra were taken over a 16° range to include the critical angle and the minima for accurate modelling. All kinetic data were taken one degree, external angle, below the initially observed minima to give a maximum change in signal. SPR measurements were undertaken on Schott TiH53 high refractive index right angle prisms (Galvoptics, Essex, U.K.) with a 48 nm gold layer on the hypotenuse. The gold layer was deposited by electrothermal evaporation (rate 0.1-0.2 nm s^{-1}, pressure 8 x 10^{-7} mbar) directly onto the prisms. A 1 mM solution of self assembled monolayer forming thiols was obtained by mixing the respective EO3-cholesterol component (L.M. Williams et al., *Langmuir* **1997**, *13*, 751-757) and the mercaptoethanol component to the desired ratio in propan-2-ol. EO3-cholesterol was used at 3 mol% in solution, which approximates a surface area of 30%. The gold coated prisms were incubated in the mixed thiol solution for 1 h, after which they were rinsed in MilliQ deionized water and propanol and then stored under nitrogen until required.

The mixed SAM activated prism was incubated with the relevant vesicle solution and the kinetics of adsorption followed in real time, by monitoring the change in reflectivity at a fixed angle below the resonance minimum. Upon reaching steady state conditions, the flow cell, which had a volume of 100 µL, was flushed with 8 times its volume to remove all vesicles from the solution as well as any nonspecifically surface bound vesicles. Peptidoglycan precursors were then injected into the flow cell and the kinetics of binding and polymerisation followed. Once the kinetic data collection was completed, a reflectivity curve was taken and compared to the initial curve, thus giving a quantification of adsorbed layer thickness by modelling of the curves and the respective shift in the resonance minima.