Supplementary information

Probing cysteine reactivity in proteins by mass spectrometric EC-tagging

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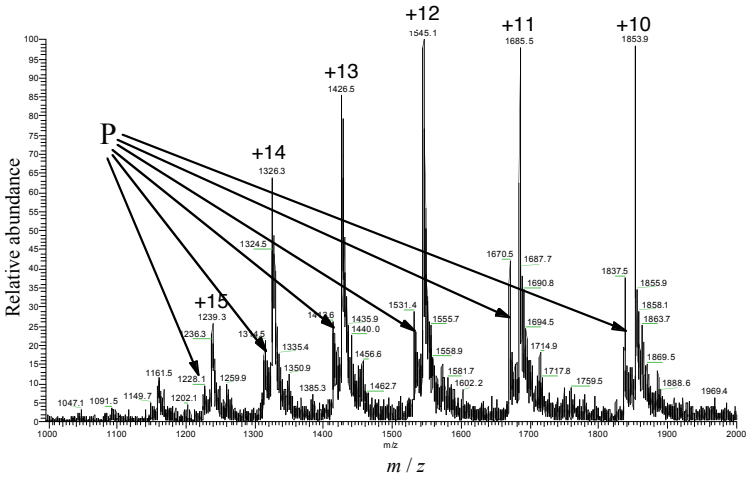
In following, the Maple programme to solve the kinetic equations for the tagging of a five-cysteine-containing protein is given.

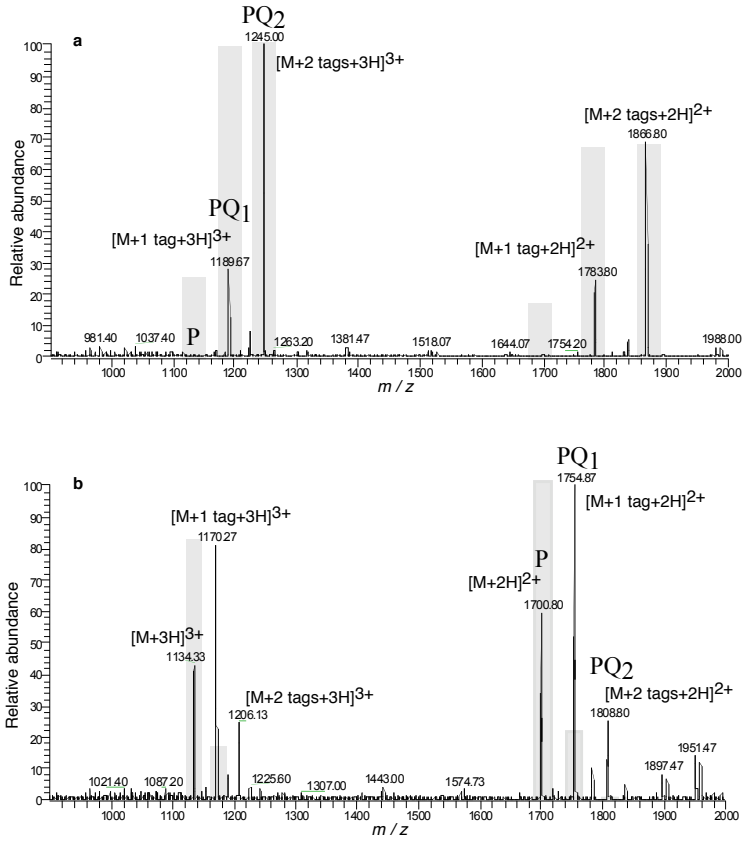
```
> restart:deq1:=diff(PQ1(t),t)=5*k*PP(t)*BQ(t)-4*k*PQ1(t)*BQ(t);
> deq2:=diff(PQ2(t),t)=4*k*PQ1(t)*BQ(t)-3*k*PQ2(t)*BQ(t);
> deq3:=diff(PQ3(t),t)=3*k*PQ2(t)*BQ(t)-2*k*PQ3(t)*BQ(t);
> deq4:=diff(PQ4(t),t)=2*k*PQ3(t)*BQ(t)-k*PQ4(t)*BQ(t);
> deq5:=diff(PQ5(t),t)=k*PQ4(t)*BQ(t);
> deq6:=diff(PP(t),t)=-5*k*PP(t)*BQ(t);
> deq7:=diff(BQ(t),t)=-5*k*PP(t)*BQ(t)-4*k*PQ1(t)*BQ(t)-3*k*PQ2(t)*BQ(t)-
2*k*PQ3(t)*BQ(t)-k*PQ4(t)*BQ(t);
> sys:={deq1,PQ1(0)=0,deq2,PQ2(0)=0,deq3,PQ3(0)=0,deq4,PQ4(0)=0,deq5,PQ5(0)=0,
deq6,PP(0)=P0,deq7,BQ(0)=BQ0};
> fcns:={PQ1(t),PQ2(t),PQ3(t),PQ4(t),PQ5(t),PP(t),BQ(t)};
> rep:=dsolve(sys,fcns);
> assign(rep);
> P0:=50e-6;
> BQ0:=93e-6;
> k:=5000;
> s1:=eval(BQ(t), t=4.7);
> evalf(s1);
> s2:=eval(PP(t), t=4.7);
> evalf(s2);
> s3:=eval(PQ5(t), t=4.7);
> evalf(s3);
```

Figure S1.



Microspray mass spectrum of a solution containing 50 M -lactoglobulin A and 25 M of methoxycarbonyl-1,4-benzoquinone after 30 minutes of reaction in the ESI medium. The main observed distribution (with indicated charge states) corresponds to the free-thiol tagged -lactoglobulin A (PQ_1). The distribution (*m*/*z* = 1413.6, 1531.4, 1670.5, 1837.5…) from the untagged -lactoglobulin A (P) represents around 30% of that of the tagged -lactoglobulin A.

Figure S2.



Microspray mass spectra of reduced insulin (50 M) infused with methoxycarbonyl-1,4-hydroquinone (2.5 mM) (**a**) and 1,4-hydroquinone (2.5 mM) (**b**). Only the B-chain was detected. Grey bars give predicted the distributions according to the analytical model. P, PQ_1 and PQ_2 indicate respectively the untagged, singly- and doubly-tagged proteins.