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online data

SUPPLEMENTARY ONLINE DATA

Fluorescence detection of GDP in real time with the reagentless biosensor rhodamine–ParM

Simone KUNZELMANN and Martin R. WEBB[1]

MRC National Institute for Medical Research, Mill Hill, London NW7 1AA, U.K.

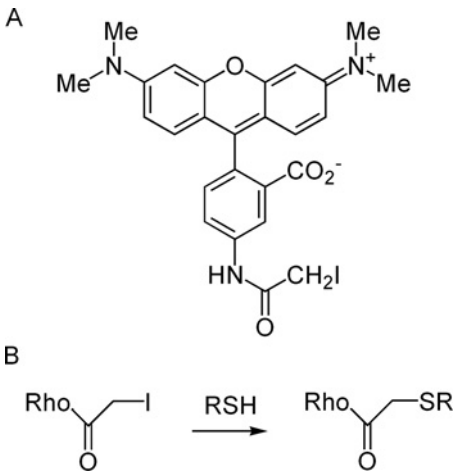

Figure S1 Structure of 5-IATR and the substitution reaction

(**A**) Structure of 5-IATR. (**B**) Iodoacetamide reaction with thiol-groups of proteins (present in cysteine residues) to form a thioether [1].

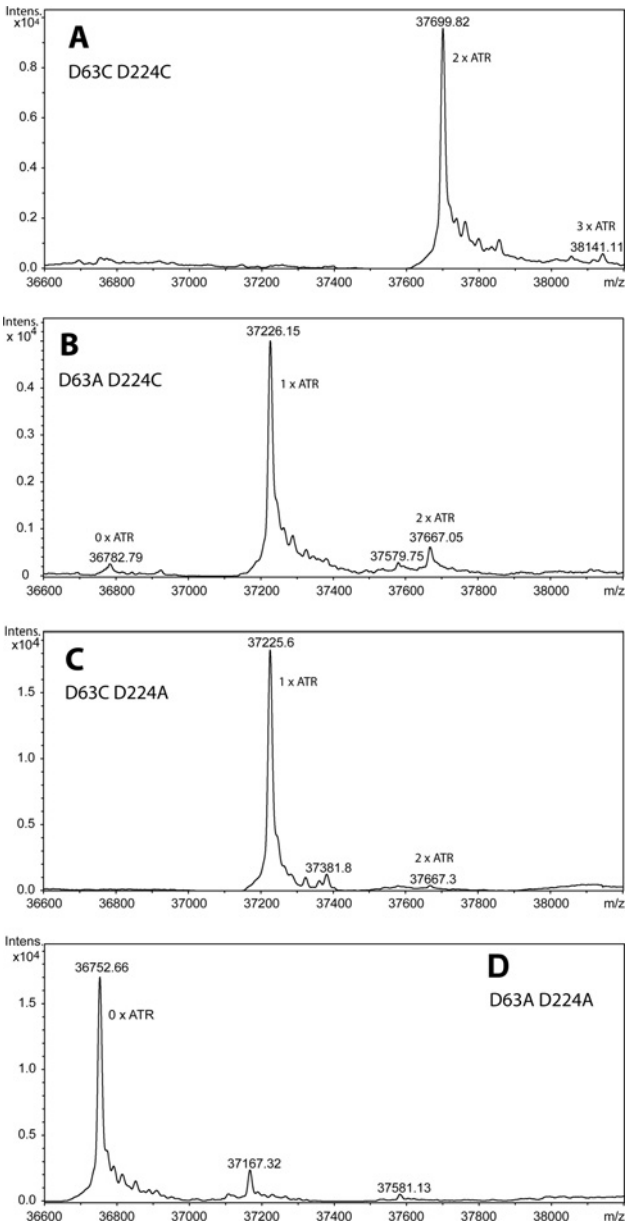

Figure S2 Analysis of different 5-IATR-labelled ParM constructs using ESI–MS

Mass spectra of ParM (His_6/K33A/T174A/T175N/C287A) with the additional mutations D63C/D224C (**A**), D63A/D224C (**B**), D63C/D224A (**C**) and D63A/D224A (**D**) after a reaction with 5-IATR. The spectra each show one main peak which indicates the labelling stoichiometry for the different mutants (Table S1). There are also peaks with much lower intensity, some of them corresponding to masses due to more or fewer rhodamines attached, as indicated on the spectra. The mass change due to each rhodamine is 441 Da. These spectra indicate a low extent of labelling amino acids other than the exposed cysteine residues.

[1] To whom correspondence should be addressed (email mwebb@nimr.mrc.ac.uk).

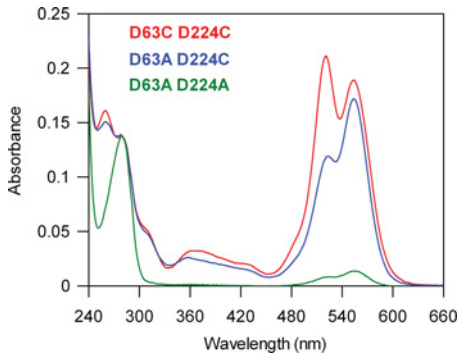

Figure S3 Absorbance spectra of different ParM constructs labelled with 5-IATR

The spectra are scaled so they have identical absorbance at 280 nm to illustrate approximately the different content of rhodamine labels, indicated by the absorbance in the visible range. Tetramethylrhodamine has two absorbance peaks in the visible range, whose maxima are around 520 and 550 nm. Their relative height depends on the stacking interaction of the rhodamines [2–4]. Although the shapes of the spectra of the D63A D224C and D63A D224A mutants indicate that the rhodamine does not stack with a second fluorophore, the spectra of D63C D224C suggest that the attached rhodamines form a stacking interaction (as expected for this doubly labelled species). Because of the large absorbance of rhodamine at 280 nm and the strong dependence of the spectra on rhodamine stacking and interaction with the protein, the ratio of label to protein cannot be calculated from the absorbance spectrum with high accuracy, especially for the doubly rhodamine labelled mutant. However, an estimate of 4% labelling can be calculated for the D63A D224A mutant, using the molar absorption coefficient of small thiol adducts of 5-IATR ($\varepsilon^{528} = 52\,000\ M^{-1} \cdot cm^{-1}$ [5] and $\varepsilon^{280} = 0.6 \times \varepsilon^{528}$) and the molar absorption coefficient of ParM ($\varepsilon^{280} = 34380\ M^{-1} \cdot cm^{-1}$).

Table S1 Labelling stoichiometry of different ParM constructs as determined by MS (Figure S2)

Differences between the contructs are indicated in bold.

ParM construct	Number of 5-IATR labels	Theoretical mass (Da)	Measured mass (Da)
(A) K33A **D63C** T174A T175A **D224C** C287A	2	37699.5	37699.8
(B) K33A **D63A** T174A T175A **D224C** C287A	1	37226.5	37226.2
(C) K33A **D63C** T174A T175A **D224A** C287A	1	37226.5	37225.6
(D) K33A **D63A** T174A T175A **D224A** C287A	0	36753.4	36752.7

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