## **Supplementary Information**

Interactions of the intact FsrC membrane histidine kinase with the tricyclic peptide inhibitor siamycin I revealed through synchrotron radiation circular dichroism

Mary K. Phillips-Jones<sup>\*+a</sup>, Simon G. Patching<sup>+b,c</sup>, Shalini Edara<sup>b</sup>, Jiro Nakayama<sup>d</sup>, Rohanah Hussain<sup>e</sup> and Giuliano Siligardi<sup>\*e</sup>

<sup>a</sup> School of Pharmacy and Biomedical Sciences, Faculty of Science and Technology, University of Central Lancashire, Preston, Lancashire, United Kingdom; Tel +44 (0)1772 895831; Fax: +44 (0) 1772 892929; E-mail:MPhillips-Jones@uclan.ac.uk

<sup>b</sup> Astbury Centre for Structural Molecular Biology, Faculty of Biological Sciences, University of Leeds, Leeds, United Kingdom. E-mail: S.G.Patching@leeds.ac.uk; Fax: +44 (0)113 343 3069; Tel: +44 (0)113 343 3172; and eshalini@hotmail.co.uk;

<sup>c</sup> School of Biomedical Sciences, University of Leeds, Leeds, United Kingdom.

<sup>d</sup> Department of Bioscience and Biotechnology, Faculty of Agriculture, Graduate School, Kyushu University, Fukuoka 812-8581, Japan. E-mail: nakayama@agr.kyushu-u.ac.jp

<sup>e</sup> Diamond Light Source Ltd, Harwell Science and Innovation Campus, Didcot, Oxfordshire, United Kingdom. E-mail: giuliano.siligardi@diamond.ac.uk.

\* co-corresponding authors

<sup>+</sup> These authors contributed equally to the work

\**Corresponding authors:* 

- (2) Dr Mary K. Phillips-Jones, School of Pharmacy and Biomedical Sciences, Faculty of Science and Technology, University of Central Lancashire, Preston, Lancashire, PR1 2HE, United Kingdom. Tel: +44 (0) 1772 895831.
- (2) Dr Giuliano Siligardi, Diamond Light Source Ltd, Harwell Science and Innovation Campus, Didcot, Oxfordshire, OX11 0DE, United Kingdom. Tel: +44 (0) 1235 778425;

Email addresses:

MPhillips-Jones@uclan.ac.uk; giuliano.siligardi@diamond.ac.uk

## 1. Figures in support of Supplementary Information

Fig. S1.



**Fig. S1.** Purity of preparations of intact FsrC used in the study. The intact membrane protein was prepared as described previously<sup>12</sup> using mixed *E. coli* membranes. M, molecular mass markers; Lanes 1 and 2 show purified protein from two separate preparations. The apparent mass is typical of that observed previously for the intact sequence-verified protein,<sup>5</sup> which runs anomalously in SDS-PAGE.



**Fig. S2.** Purity of purified siamycin I determined by mass spectrometry. Samples (20 pmol/ul) in methanol/formic acid mixtures were analysed by positive ionisation in a Q-tof electrospray ionisation instrument. The observed mass of siamycin I, which possesses two proton adducts,  $m/2H^+$ , is therefore 2161.84 and is in good agreement with the expected mass of 2164. Purity is > 90%.

## Fig. S2.





**Fig. S3.** Interaction between siamycin I and GBAP revealed through SRCD spectroscopy in the near-UV region. *Solid*) SRCD spectrum of GBAP (60  $\mu$ M) (dissolved in acetonitrile) in 10 mM potassium phosphate pH 7.5 containing 0.05% DDM, following subtraction of control spectrum (buffer plus equivalent concentration of acetonitrile); *Dash*): SRCD spectrum of siamycin I (100  $\mu$ M) (dissolved in methanol) in the above buffer , following subtraction of control spectrum (buffer plus equivalent concentration of methanol); SRCD spectrum of GBAP (60  $\mu$ M) (dissolved in acetonitrile) plus siamycin I (100  $\mu$ M) (dissolved in methanol), following subtraction of control spectrum (buffer plus equivalent concentration of methanol); SRCD spectrum of GBAP (60  $\mu$ M) (dissolved in acetonitrile) plus siamycin I (100  $\mu$ M) (dissolved in methanol), following subtraction of control spectrum (the above buffer containing equivalent concentrations of acetonitrile and methanol). Ten measurements (scans were obtained with integration time of 1 sec, bandwidth of 2 nm, 20 °C). Unsmoothed data are shown.