García-Linares *et al.*, 2016 ***Role of the Tryptophan Residues in the Specific Interaction of the Sea Anemone Stichodactyla helianthus's Actinoporin Sticholysin II with Biological Membranes***

SUPPLEMENTARY MATERIAL

Materials and Methods

Small scale protein production and solubility assessment

Freshly transformed RB791 *E. coli* colonies (W3110lac/qL8) containing the different plasmids constructed were grown in 5 ml of Luria-Bertani medium, containing 100μg/ml of ampicillin, up to an OD_{600} of 1.0. Then, isopropylthio-β-d-galactoside was added to 1.0 mM final concentration as inducer of the protein production and the culture was further incubated with vigorous shaking for 4 h at 37 ◦C. The cells were then pelleted at 5000×g for 30 min at room temperature. The cellular pellets were resuspended in 1.0 ml of water, exhaustively sonicated (seven pulses of 20 Kc for 1 min) in an ice bath, and centrifuged again at 14,000×g for 30 min at 4 ◦C. The supernatants thus obtained, containing the soluble recombinant proteins, as well as the corresponding cellular debris insoluble pellets, were then analyzed by means of 0.1% SDS – 15% PAGE [1].

Near-UV CD spectra

Near-UV circular dichroism (CD) spectra were obtained on a Jasco 715 spectropolarimeter at a 50 nm/min scanning speed. Optical path cells of 1.0 cm were employed. The proteins were dissolved in 15 mM MOPS buffer, pH 7.5, containing 100 mM NaCl (1.0 mg/mL protein concentration) [2]. At least four spectra were averaged to obtain the final spectrum.

Figures

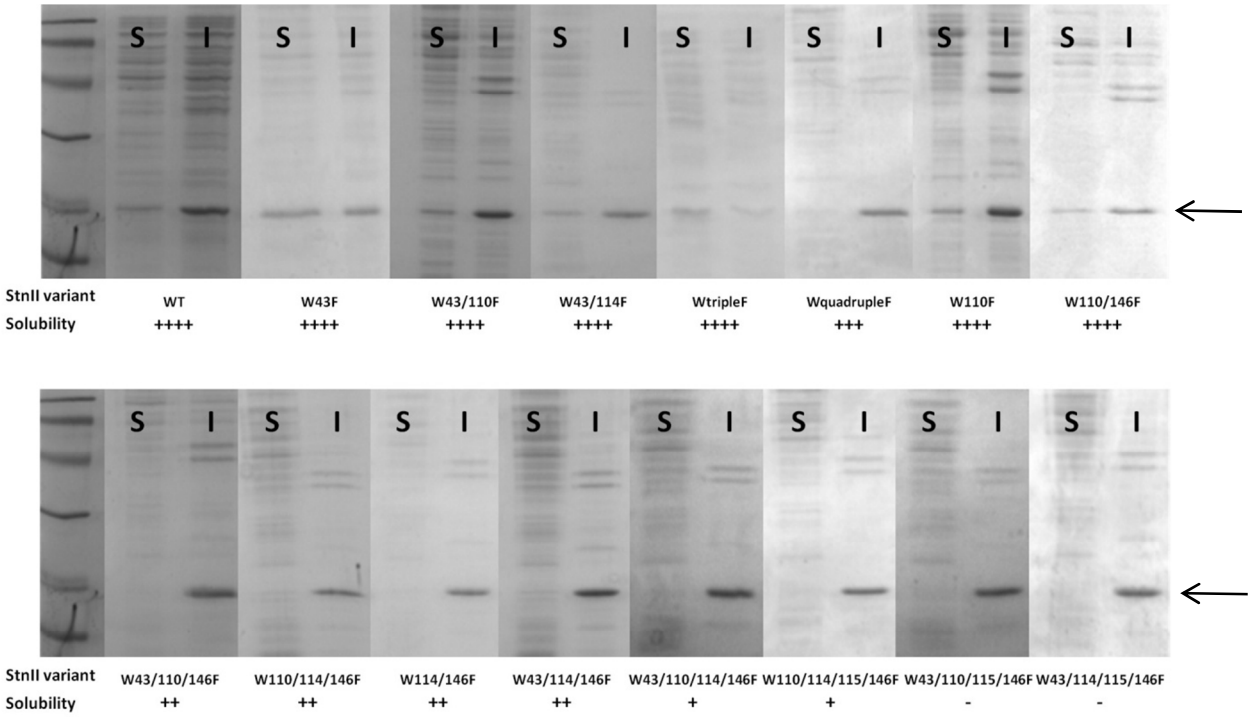

Figure S1.- 0.1% SDS – 15% PAGE analysis of small scale bacterial cultures harboring the plasmid responsible for the production of the wild-type StnII and the 15 different W mutants constructed. After induction with 1 mM IPTG, bacteria cells were collected by centrifugation, resuspended in water and lysed by exhaustive sonication. The soluble fraction (S) resulting from this treatment was recovered by centrifugation and the corresponding cellular debris insoluble pellet was again resuspended in water (I). The position of the StnII protein variants is indicated with arrows. BioRad Low Range Molecular weight marker (first row of both panels) values are 14.4, 21.5, 31.0, 45.0, 66.2, and 97.4 kDa. Relative solubility of all the proteins assayed by this procedure was qualitatively assessed using the wild-type protein as reference. This solubility is indicated by + or – symbols, where four ++++ indicates that solubility was estimated to be equal or larger than that of wild-type StnII. Identical notation is employed to denote solubility in Table 1 of the manuscript.

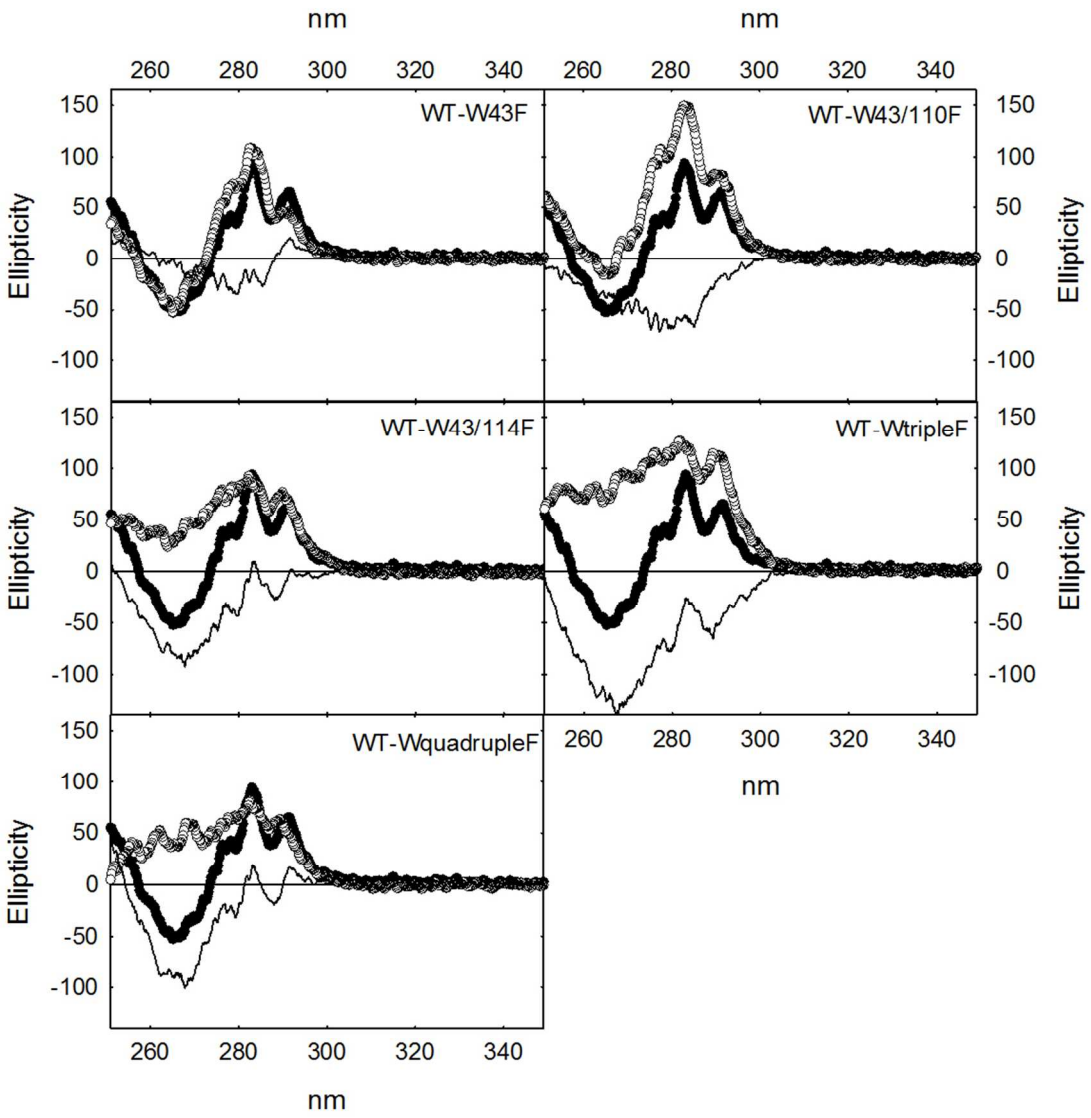

Figure S2. Near-UV circular dichroism spectra of wild-type StnII (black dots) and the various Trp mutants studied (white dots), as indicated in each panel. Black lines represent the calculated difference spectra for the wild-type minus the mutant. Mean residue weight ellipticity is expressed in units of degree x cm^2 x $dmol^{-1}$.

Table S1. Numbers corresponding to the position occupied by W residues along the sequences of the four best-characterized actinoporins: StnI, StnII, EqtII, and FraC.

StnI	StnII	EqtII	FraC
W44	W43	W45	W45
W111	W110	W112	W112
W115	W114	W116	W116
W116	W115	W117	W117
W147	W146	W149	W149

References

(1) Alegre-Cebollada, J., Clementi, G., Cunietti, M., Porres, C., Oñaderra, M., Gavilanes, J. G., and Martínez-del-Pozo, A. (2007) Silent mutations at the 5'-end of the cDNA of actinoporins from the sea anemone *Stichodactyla helianthus* allow their heterologous overproduction in *Escherichia coli*, *J. Biotechnol. 127*, 211-221.

(2) De Antonio, C., Martínez-del-Pozo, A., Mancheño, J. M., Oñaderra, M., Lacadena, J., Martínez-Ruiz, A., Pérez-Cañadillas, J. M., Bruix, M., and Gavilanes, J. G. (2000) Assignment of the contribution of the tryptophan residues to the spectroscopic and functional properties of the ribotoxin α-sarcin, *Proteins 41*, 350-361.