Supplementary Information for

Molecular Shape of Palytoxin in Aqueous Solution

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Experimental Procedures

SAXS measurements and sample preparation. SAXS experiments were carried out at the BL45XU station at SPring-8 in Hyogo, Japan[1]. SAXS profiles were collected for 3 seconds with a C4880 camera[2]. The camera length was 335 mm. Palytoxin (PTX) was isolated from *Palythoa tuberculosa*[3]. The acetoamide of PTX (NAcPTX) was prepared from PTX by acetylation with *p*-nitrophenyl acetate[3]. All samples were dissolved in double-distilled water. To extrapolate to zero concentration, scattering was measured for at least four concentrations. The measured concentration ranges of PTX, NAcPTX and cytochrome C were from 0.51 to 1.5 mg/mL, from 2.9 to 5.8 mg/mL, and from 0.65 to 2.5 mg/mL, respectively. The extrapolation of PTX to zero concentration was performed using data for less than 1.5 mg/mL, where linear extrapolation was possible.

Data analyses. The radius of gyration, R_g, and the zero-angle scattering intensity *I(0)* were determined by the Guinier analysis based on the following approximation:

$$\ln(I(S)) = \ln(I(0)) - 4\pi^2 R_g^2 S^2/3$$

where $S = (2 \sin \theta)/\lambda$; 2θ is the scattering angle; λ is the wavelength of the incident X-ray; and $I(S)$ is the scattering intensity at S. The scattering profile of PTX from 50 to 220 $Å^{-2} \times 10^{-6}$ in S^2 and that of NAcPTX from 50 to 450 $Å^{-2} \times 10^{-6}$ in S^2 were selected as the linear fitting regions for the Guinier analyses. In this study, the maximum S value for the Guinier fitting region was determined by the criterion $2\pi R_g S < 1.6$. Scattering profiles were extrapolated to zero concentration and distribution functions were calculated using the *GNOM* package[4]. D_{max} was determined using a procedure described elsewhere[5]. The *S vs. S²I(S)* plots, Kratky plots, of PTX and NAcPTX are shown with open and closed circles in Fig. S1, respectively.

Low-resolution model simulations. Low-resolution model simulations for determining three-dimensional shapes from zero-concentration-extrapolated SAXS profiles were performed with the program *GASBOR*.[6] This program was originally designed to simulate the model structure of protein. An ensemble of dummy residues, *i.e.*, beads forming a chain-compatible model, represents the protein structure. The merit of using GASBOR instead of DAMMIN is that it can fit to a wider range of scattering angles.
The parameters used were EXPERT MODE = EXPERT; Bond length penalty weight = 0.02; Discontiguity penalty weight = 0.20; and Peripheral penalty weight = 0.0. Numbers of residues, *i.e.*, beads of PTX and NAcPTX, were presumed to be 25 and 50, respectively. These values were nearly equal to (total molecular weight)/(average molecular weight of a residue). The bond length

penalty weight and discontiguity penalty weight were varied so that the final beads were connected to each other. Simulations were carried out with more than 10 runs for different sets of parameters. The overall feature of the shape was conserved for all runs. The averaged models were calculated by the following procedure[7]. First, the difference in the orientation of models was calculated by the program *SUPCOMB*.[7] The direction was then aligned to the model that showed minimum deviation with respect to other models by the program *PDBAVER*. The model with minimum deviation was taken as a representative model. The typical mean value and variation of the normalized standard deviation, NSD, were *ca*. 0.7-0.8 and 0.02-0.05, respectively. The results of the simulations for PTX and NAcPTX are shown in Figs. 4 and 5. The representative models were drawn with opaque figures, while the other models are dotted. The scattering curves simulated from the representative models were plotted in Fig. S1. The residues of fitting for PTX and NAcPTX in Kratky plots were R_f=0.035 and R_f=0.021, respectively.

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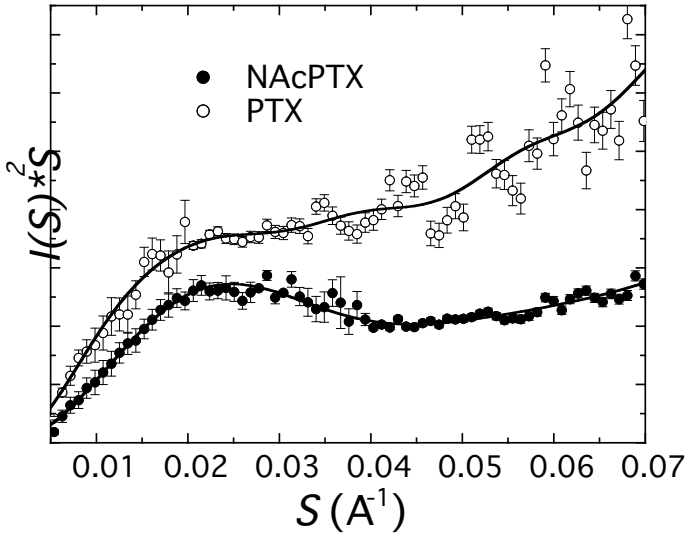

Figure S1. Kratky plots of PTX (●) and NAcPTX (○). The solid lines represent the simulation with representative models of PTX and NAcPTX, respectively.