# Supporting Information

## Kondo et al. 10.1073/pnas.1121607109

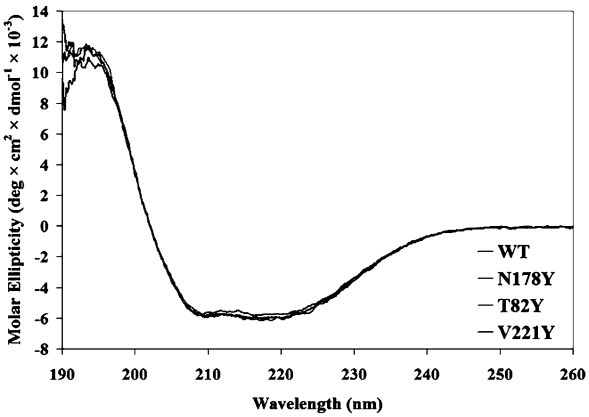


**Fig. S1.** CD spectra of wild-type *Tis*AFP and three mutants that were each tagged with GST. The four similar profiles show that their structural properties are well conserved and that the mutations did not alter the protein fold.

**Table S1. Data collection and refinement statistics for *Tis*AFP**

| Data collection | For SAD phasing | For refinement |
|---|---|---|
| Space group | *P*1 | |
| Unit-cell parameters | | |
| (*a*, *b*, *c*), Å | 38.79, 41.60, 70.32 | 38.89, 41.63, 70.25 |
| (α, β, γ), (deg) | 81.56, 83.08, 62.38 | 81.53, 83.13, 62.43 |
| No. molecules/asymmetric unit | 2 | |
| Beam line | SPring-8 BL44B2 | |
| Wavelength (Å) | 1.7000 | 0.7000 |
| Resolution range (Å)* | 20.0–2.2 (2.32–2.20) | 34.0–0.95 (1.00–0.95) |
| Total phi range (deg) | 1,800 | 1,800 |
| $R_{merge}$*,† | 0.074 (0.106) | 0.060 (0.366) |
| $R_{ano}$*,‡ | 0.052 (0.076) | |
| Observed reflections | 353,597 | 1,988,099 |
| Independent reflections | 18,126 | 232,289 |
| Completeness (%)* | 92.7 (74.9) | 95.5 (92.0) |
| Multiplicity* | 19.5 (19.0) | 8.6 (7.1) |
| $<I/\sigma(I)>$* | 11.2 (9.2) | 7.2 (2.0) |
| Refinement statistics | | |
| *R* factor*,§ | | 0.112 (0.165) |
| Free *R* factor*,§,¶ | | 0.129 (0.190) |
| rms bond lengths, Å | | 0.017 |
| rms bond angles (deg) | | 1.851 |
| Residues | | 223 × 2 |
| No. of non-H atoms | | |
| Protein | | 3,211 |
| Solvent | | 843 |
| Ramachandran plot, %‖ | | |
| Residues in favored regions | | 98.4 |
| Residues in allowed regions | | 1.6 |
| Residues in outlier regions | | 0.0 |
| Average B factor, Å$^2$ | | 9.5 |

The diffraction data were processed using HKL2000 (1) and CCP4 (2) program suite. In an attempt to prepare an iodine-labeled crystal, iodine solution was applied for 30 min, followed by additional $H_2O_2$ treatment for 30 min. Diffraction data from this crystal, which were collected at 100 K on BL44B2 at the SPring-8 using 2.0000-Å radiation, gave no interpretable solution for phase determination. However, this crystal was found to diffract up to the 0.95-Å resolution using 0.7000-Å radiation, which was applicable to the native dataset. After refinement of the structure, weak electron density due to monoiodination was identified at Y91. Both *Tis*AFP molecules in the asymmetric unit contain monoiodotyrosine at this position with low occupancy (0.2). For phase determination, we used the single-wavelength anomalous diffraction (SAD) method using HYPER-VIL procedures (3). The programs SOLVE and RESOLVE (4) were used for the phasing and model building, and CNS (5) and REFMAC5 (6) for structure refinement, respectively.

*Values in parentheses are for the highest resolution shell.

†$R_{merge} = \Sigma\Sigma_j|<I(h)> - I(h)_j|/\Sigma\Sigma_j<I(h)>$, where $<I(h)>$ is the mean intensity of a set of equivalent reflections.

‡$R_{ano} = \Sigma|I(+h) - I(-h)|/\Sigma[I(+h) + I(-h)]$.

§*R* factor $= \Sigma||F_{obs}(h)| - |F_{calc}(h)||/\Sigma|F_{obs}(h)|$, where $F_{obs}$ and $F_{calc}$ are the observed and calculated structure factors, respectively.

¶A randomly chosen 5.0% of the data were used to calculate the free *R* factor (7).

‖Statistics were obtained from RAMPAGE (8).


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