Supplemental Data

Measurements of Backbone $^{15}$N Relaxation Parameters

The residue-specific backbone $^{15}$N longitudinal relaxation rate $R_1$, transversal relaxation rate $R_2$, and the heteronuclear $^{1H}$-$^{15}$N NOE values of Wzb were measured on a Bruker Avance 600 MHz spectrometer equipped with triple-resonance probe and three-axis pulsed-field gradients at 25 °C. To ensure an identical temperature for each experiment for $R_1$ and $R_2$ measurements, duty-cycle compensation schemes were employed. The $R_1$ and $R_2$ relaxation rates were measured at two protein concentrations (0.1 mM and 0.3 mM) in the same buffer conditions as that used for structure determinations. For $R_1$ measurements, the following delays were used: 10 ($\times2$), 110, 510, 750, 1010, 1210, 1510, 2010, 2510, 2990 ms at 0.3 mM sample concentration and 10, 110, 310, 750, 1010, 1510, 2010 and 2990 ms at 0.1 mM sample concentration. Sixteen and thirty-two transients were collected at 0.3 and 0.1 mM sample concentrations, respectively. For $R_2$ experiments, the following delays were used at both concentrations: 4 ($\times2$), 12, 20, 28, 44, 60, 68, 84, 124, 140, 180 and 220 ms. Sixteen and twenty-four transients were collected at 0.3 and 0.1 mM sample concentrations, respectively. Recycle delays of 2.7 s and 3 s were used for $R_1$ and $R_2$ experiments, respectively. Curve fittings were carried out using the program RELAXFIT (2). Three- and two-parameter exponential functions were used for determinations of $R_1$ and $R_2$, respectively. The uncertainties were estimated using 500 Monte-Carlo simulations. The $^{1H}$-$^{15}$N NOE experiments were carried out at 0.3 mM protein concentration in the presence or absence of a 3-second proton pre-saturation period. The recycle delay was set to 2 s and 64 scans were used. The spectral widths of 1490 Hz for $^{15}$N and 8389 Hz for $^1$H were used in the relaxation experiments.

Rotational Diffusion Tensor and Model-Free Analysis

The diffusion tensor was determined at each sample concentration using the program R2R1_diffusion (http://cpmcnet.columbia.edu/dept/gsas/biochem/labs/palmer/software.html). In the Model-Free framework, the NH bond motions can be described by the internal dynamic parameters, such as the generalized squared order parameter $S^2$, the internal correlation time $\tau_i$, and the chemical exchange contribution $R_{ex}$ (3, 4). In case that the internal motions occur on two different timescales, the Model-Free spectral density function can be extended and the squared order parameters $S_s^2$ and $S_f^2$ ($S^2 = S_s^2 * S_f^2$) are used to describe the amplitude of the internal motions on the slow and fast timescales, respectively (5). Five models of increasing complexity are usually tested to reproduce the experimentally determined relaxation data and analyzed using the F-test statistics (6). The program FAST-Modelfree (7, 8) was used during the analysis. In the first step, residues that could be fully described by model 1 were selected. The rotational diffusion tensor was subsequently optimized based on these residues. In the next step, all residues were included for
model selections using the diffusion tensor derived from the first step. In the last step, both diffusion tensor
and model selections were optimized for all residues. The $^{15}$N chemical shift anisotropy (CSA) was set to
-170 ppm during our analysis. The experimentally determined rotational diffusion tensor was compared to
that predicted by the program HYDRONMR (9) using the solution structure of Wzb. The radius for atomic
elements and the solvent viscosity were set to 3.3 Å and 0.009 poise, respectively.

**Construction of the phylogenetic tree**

The amino acid sequences of 23 LMW-PTPs were aligned as described in the text. For the construction of
the phylogenetic tree, two independent methods were used. First, the phylogenetic relationships were
inferred using the neighbor-joining (NJ) method available in the software package ClustalX (10). One
thousand bootstrap replicates were used to test the inferred phylogeny relationship. As shown on Figure III,
the orders of some branches were not statistically significant. For this reason, we assessed the stability of
the tree topology by using a completely different method based on maximum likelihood (ML) and
implemented online in PHYML (11, 12). Whatever the choice of the method used for tree inference and the
options in the respective algorithm, protein sequences from class I and class II were consistently classified
in different branches.

**Supplemental References**

1. Farrow, N. A., Muhandiram, R., Singer, A. U., Pascal, S. M., Kay, C. M., Gish, G., Shoelson, S. E.,
   Chem. Soc.* **112**, 4989-4991
    **23**, 403-405
Figure S1: $^{15}$N relaxation parameters ($R_1$, $R_2$ and NOE) of Wzb obtained at 0.1 mM (red circle) and at 0.3 mM protein concentration (black square).
Figure S2: Model-Free parameters ($S^2$, $\tau_e$, $R_{ex}$) of Wzb obtained at 0.1 mM (red circle) and at 0.3 mM protein concentrations (black square). No data were obtained for residue G99 at 0.1 mM concentration.
Figure S3: Unrooted neighbor-joining phylogenetic tree of 23 protein sequences of eukaryotic and prokaryotic LMW-PTPs. The number on branches represents the results of 1000 bootstrap replicates when this number is below 50%. The dashed line marks the separation between proteins sharing similar recognition mechanisms with the eukaryotic LMW-PTPs (class I LMW-PTPs) or with the prokaryotic LMW-PTPs like Wzb (class II LMW-PTPs).