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Supporting Information

Enzyme Selectivity Fine-Tuned through Dynamic Control of a Loop

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Supplementary MATERIALS AND METHODS

Protein and peptides. Unlabeled, ^{15}N-labeled and $^{15}N/^{13}C$-labeled full-length human cyclophilin was expressed as described below. The unlabeled peptides dansyl-AAPF and dansyl-$AAP_{(trans)}F$ (analogue proline in trans conformation) were bought from GL Biochem Shanghai, while $^{15}N/^{13}C$-labeled AAPF(Y), $AAP_{(trans)}F(Y)$ (analogue proline in trans conformation) and $AAP_{(cis)}FY$ (analogue proline in cis conformation) were bought from BACHEM, Switzerland (see supplementary Fig. 5).

Sample preparation. The gene of full-length human Cyclophilin was similar to that used in Ref (REF) and contained a MHHHHHHLVPRGS sequence tagged at the N-terminus or a LEHHHHHH sequence at the C-terminus (kind donation from Damien Morger from the University of Zurich, Switzerland). The His-tag was either cleaved off, leaving an extra GS-dipeptide at the N-terminus of the protein or experiments were done with in tag in tagged. The cDNA containing the cyclophilin gene was transformed into *E. coli* BL21 plyS cells and plated on an ampicillin/chloramphenicol-containing plate. The cells were grown over night at 37 ^{o}C and then used to inoculate a 10 ml pre-culture. The culture was grown at 37 ^{o}C for three hours and thereafter used to inoculate a 1-liter culture of LB medium for non-labeled protein or M9 medium for $^{15}N/^{13}C$- or ^{15}N-labeling. Cells were grown to an OD_{600} of 0.9. Protein expression was initiated by adding 1mM IPTG. Cells were then allowed to express over night at 30 ^{o}C (unlabeled) or 18 ^{o}C for labeled protein. Cells were harvested by spinning at 5,000 *g* for 20 minutes and re-suspended in purification buffer (10 mM Tris/HCl). Cells were lysed by French press and spun at 40,000 *g* for 20 minutes. The supernatant was filtered and loaded onto a nickel (II)-charged

chelating sepharose FF column (Amersham Biosciences), equilibrated with purification buffer as above and washed with 400 ml of the same buffer. The bound protein was eluted with 250 mM imidazole at pH 7.9, in aliquots of 12 ml. Fractions containing partially pure proteins were pooled, dialyzed for two hours and passed through a DEAE S-column equilibrated with purification buffer. Thrombin was then added to the flow-through containing pure uncleaved cyclophilin and incubated at room temperature for six hours. Pure cyclophilin was collected as flow-through by passing the digested fractions through a nickel (II)-charged chelating sepharose FF column equilibrated with purification buffer. The purity was checked on SDS PAGE stained with coomassie brilliant blue and its identity confirmed by Matrix Assisted Laser Desorption Ionization time-of-flight mass spectrometry (MALDI-TOF). Pure protein was concentrated to experimental concentration (0.15 to 1.5 mM) and the concentration determined by absorption measurement using the known molar absorption coefficient of cyclophilin.

Data collection and analysis. All NMR experiments were acquired on Bruker 600, 700, and 900 MHz spectrometers equipped with triple resonance cryogenic probes at 299 K (except stated otherwise) in 10 mM sodium phosphate, pH 6.5, 5 mM DTT and 0.01 % $NaNO_3$. Protein samples were dissolved in 3-10 % D_2O. For assignment purposes, HNCACB, ^{15}N-resolved NOESY-HSQC and ^{15}N-resolved HMQC-NOESY spectra [9] were recorded for free cyclophilin. R_1, $R_{1\rho}$, and R_2 relaxation rates, were determined for backbone ^{15}N [10]. Data processing and analysis were done with NMRpipe [11] and CCPnmr [12].

Cyclophilin-peptide interaction. Binding experiments were done at 4^{o}C (for FRET) and/or at 26 ^{o}C (for TROSY peak analysis). For NMR titration experiments, starting

with a fixed concentration of labeled/unlabeled protein (150-300 μM), increasing concentration of unlabeled/labeled peptide was added to achieve full saturation. For NMR titrations, full saturation was reached when the change in chemical shift ($(\partial H^2 + 0.14(\partial N^2))^{1/2}$) was close to zero. For equilibrium florescence experiments, binding was detected by FRET[13]. The FRET signal between the single tryptophan in cyclophilin and the dansylated peptide was determined with excitation at 295 nm and emission above 540 nm. The increase or decrease in FRET was measured with increasing concentration of peptide ligand. For estimation of the equilibrium dissociation constant, the chemical shift change/fluorescence signal change was fitted to the general equation describing the binding of two molecules at equilibrium as described[14]. The K_d of the cis-peptide was determined in an indirect manner[14]. Cyclophilin wild-type/mutant (10 μM) was pre mixed with dansylated peptide (40 μM) and increasing concentration of cis-peptide was added to chase out the bound dansylated peptide. The Kd was then estimated as described[14].

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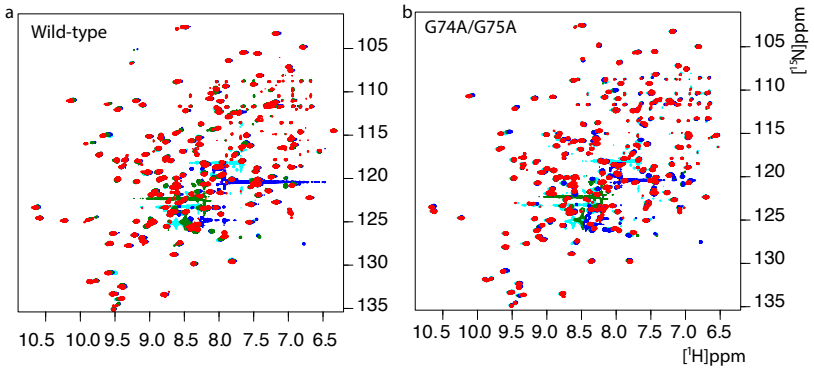
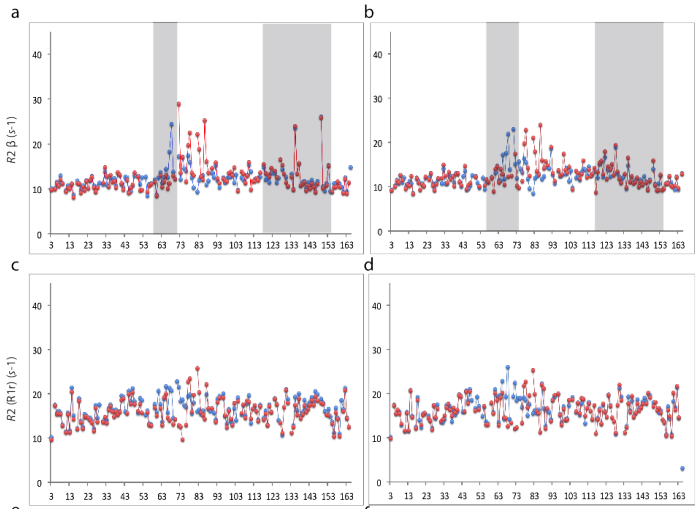
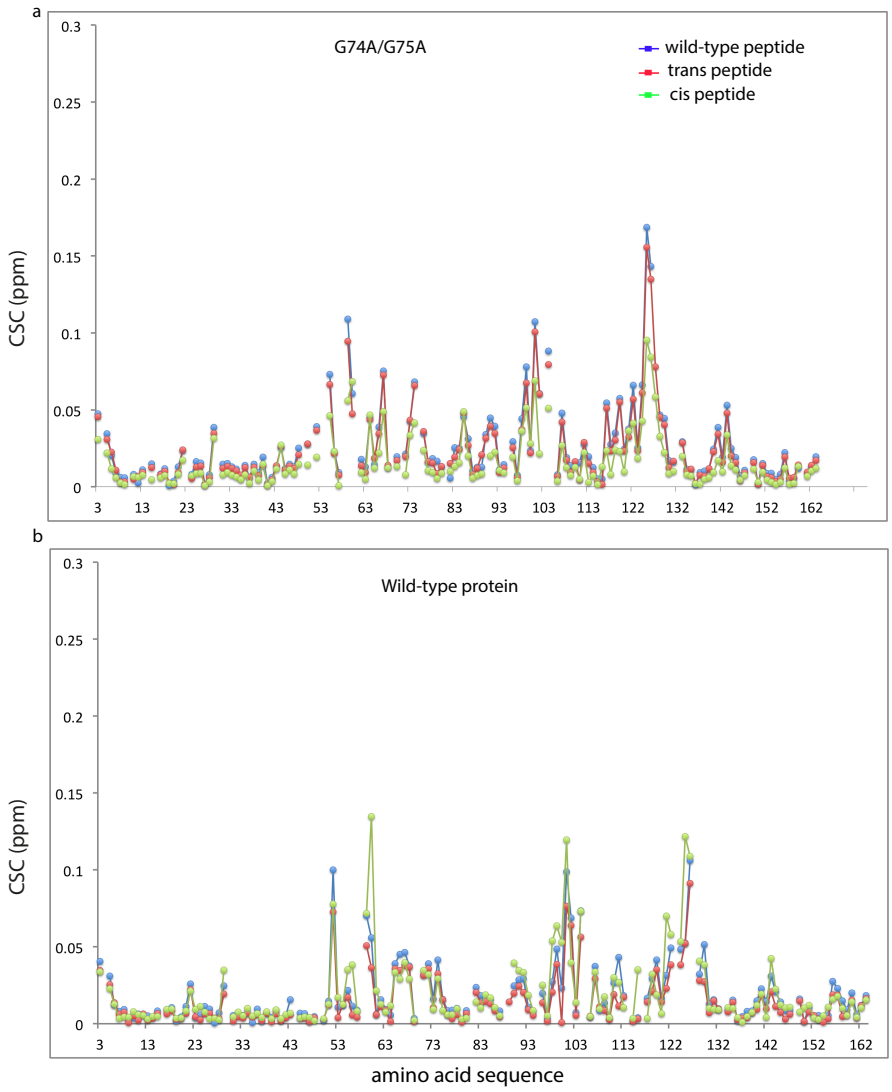
Supplementary figures



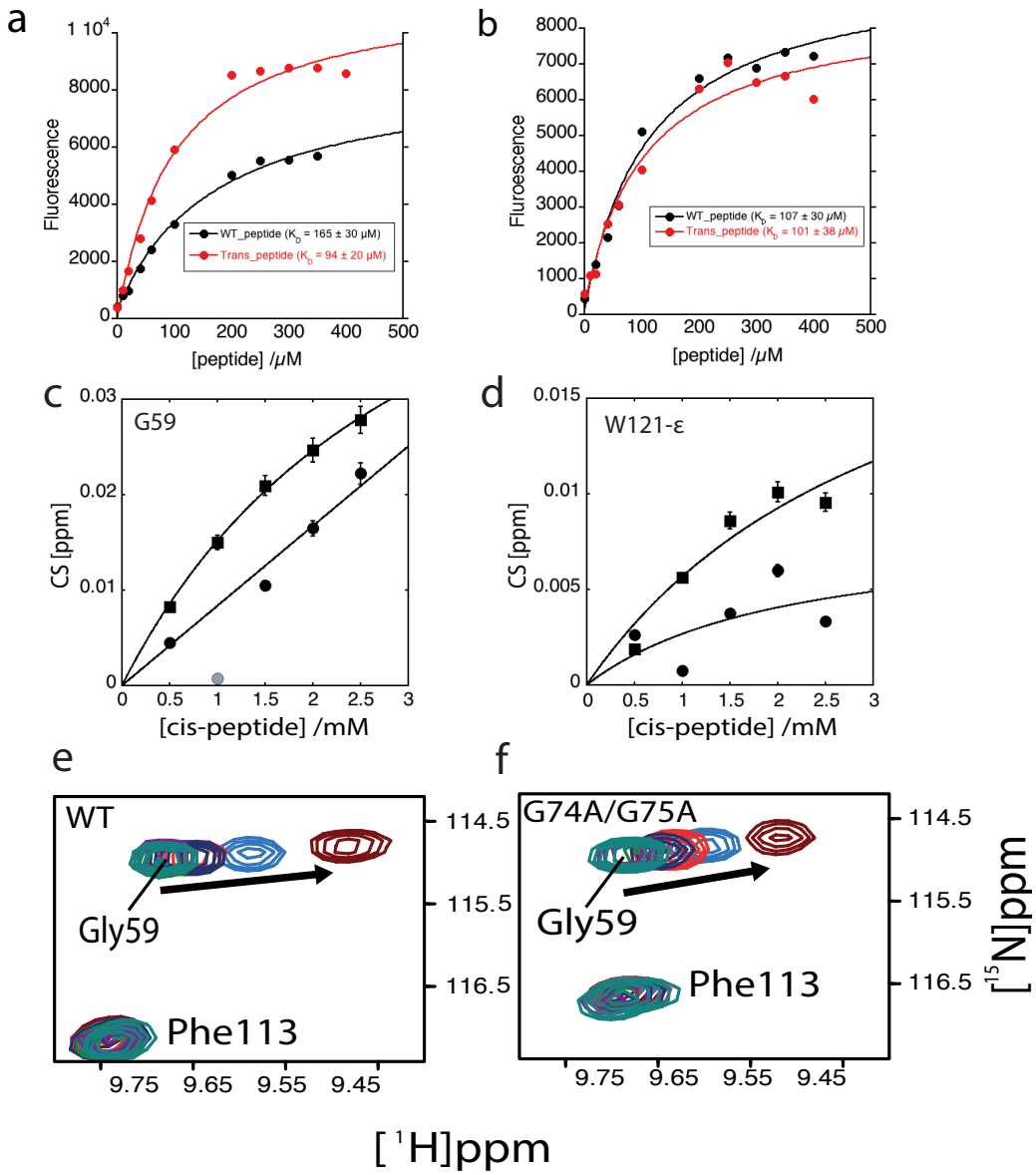
Supplementary Fig.1. [^{15}N,^{1}H]-TROSY spectra for a) wild-type cyclophilin and b) G74A/G75A mutant unbound (red), bound to wild-type peptide (blue), trans-peptide (green), and cis-peptide (cyan). Bound experiments were done at 1:11 protein to peptide ratio.



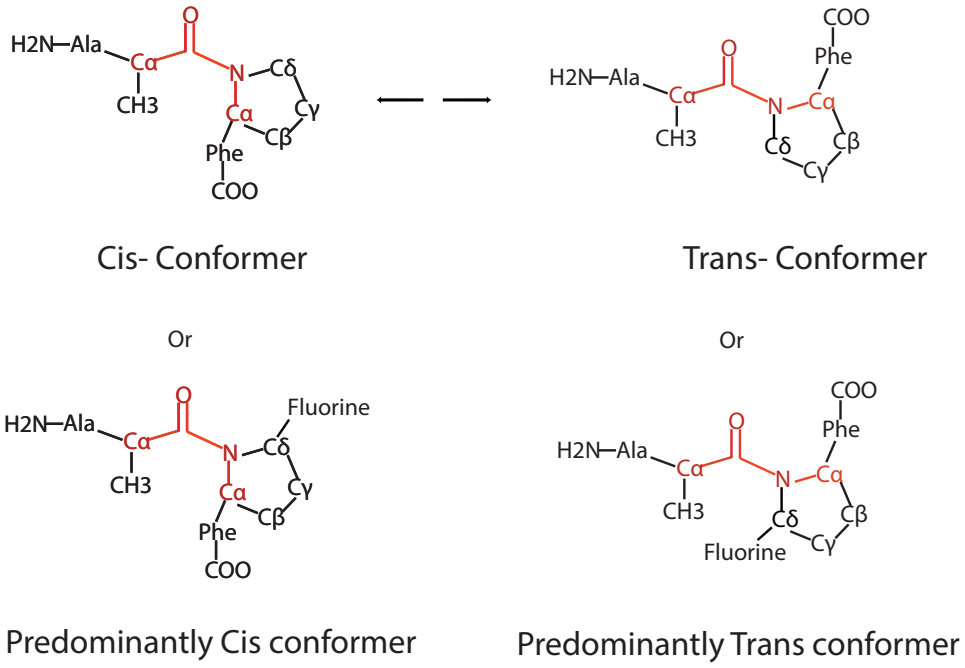
Supplementary Fig 2. **Relaxation data.** a, b) R_2 rates , c, d) R_{1rho} rates and (a, c) and complexed (b, d) wild-type (blue dots) and G74A/G75A mutant (red dots), respectively. All exchange contributions due to R_{ex} are absent in the R_{1rho} measurements indicating that they are caused by motions slower that 80 μs (500μs/2π).



Supplementary Fig. 3. **Chemical shift mapping analysis**. The difference in the [1H15N] TROSY peak position from the free wild-type or mutant was subtracted from that of the respective proteins bound to either of the wild-type peptide (blue), trans-peptide (red) or cis-peptide (green) and plotted the against amino acid sequence. a) the G74A/G75A double glycine mutant, b) the wild-type protein.



Supplementary Fig.4. **Ligand-binding upon mutation of cyclophilin**. a) wild-type (black) and trans peptide (red) to wild-type cyclophilin and b) to the G74A/G75A mutant (black) to the wild-type peptide and (red) to the trans peptide). c) CS from N-shift for glycine59, d) tryptophan121-Ɛ from the wild-type (round filled) and the G74A/G75A mutant (squared filled) plotted against increasing concentration of the cis-peptide. e) and f) overlay of TROSY spectra, showing the chemical shift change for G59 as the cis-peptide concentration is increased for the WT-cyclophlin and the G74A/G75A. For the determination of the binding constant, protein was mixed with the peptide ligands at various concentrations as described in the materials and methods. The ligand binding was measured by FRET was done at 4 ^{o}C while the chemical shift analysis was done at 26 ^{o}C. The affinity increased by almost one and half fold for the double mutant as compared to the wild-type cyclophilin for the trans peptides, while the affinity for the wild-type peptide remains constant. Error was determined from the signal to noise in the TROSY spectrum.



Supplementary Fig.5 Peptide conformers