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Supporting Information

The Molecular Basis of the Interaction of Cyclophilin A with α-Synuclein

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Supplementary Figures

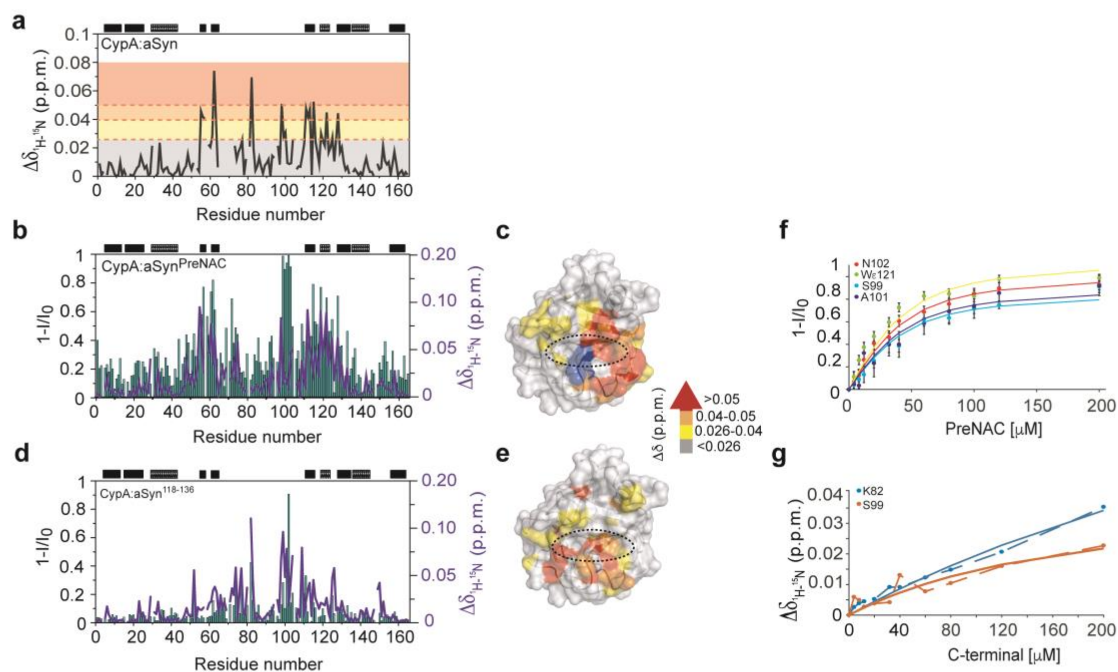

Figure S1. Residue-specific chemical shift perturbation in CypA upon binding to aSyn. **a)** Residue-specific chemical shift changes in CypA upon addition of a 5-fold excess of aSyn. Regions undergoing chemical shift changes are mapped in Fig. 2b onto the 3D structure of CypA from grey ($\Delta\delta_{1H\text{-}15N} < 0.026$ ppm) to red ($\Delta\delta_{1H\text{-}15N} > 0.05$ ppm). CypA secondary structure elements are shown on top. **b-e)** Intensity changes (green bars) and chemical shift changes $\Delta\delta_{1H\text{-}15N}$ (purple line) in ^{1}H-^{15}N HSQC spectra of CypA in presence of 8-fold molar excess of the peptides aSyn$_{PreNAC}$ (b,c) and aSyn$_{118\text{-}136}$ (d,e). CypA residues, which were broadened beyond detection in presence of aSyn$_{PreNAC}$, are shown in blue in (c). **f)** Changes in signal intensities of selected CypA residues at increasing concentrations of the peptide aSyn$_{PreNAC}$. **g)** Changes in chemical shifts of selected CypA residues at increasing concentrations of the peptide aSyn$_{118\text{-}136}$.

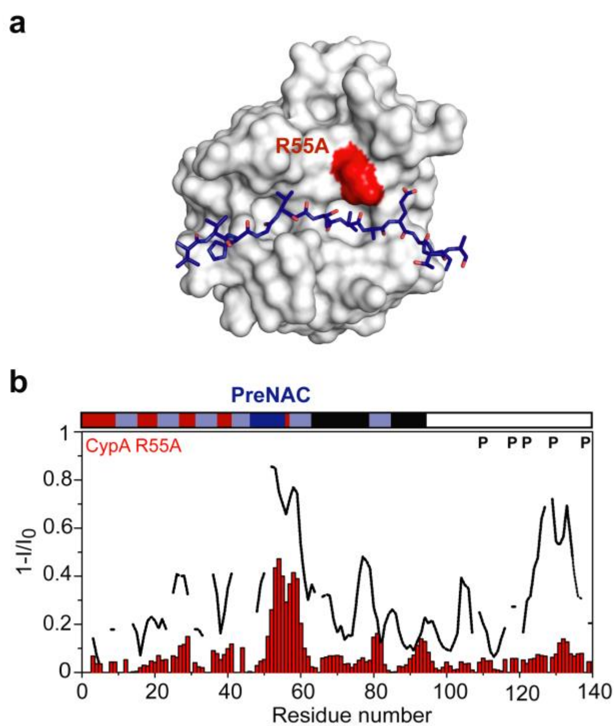

Figure S2. Mutation of the catalytic CypA residue R55 attenuates binding to aSyn. **a)** Location of the CypA mutation R55A in the 3D structure of the CypA/aSyn$_{PreNAC}$ complex. **b)** Residue-specific intensity changes in aSyn upon addition of a 5-fold excess of CypA$_{R55A}$ (red bars). I_0 and I are the intensities of ^{1}H-^{15}N HSQC cross-peaks in the absence and presence of CypA$_{R55A}$, respectively. For comparison, the black line displays the intensity broadening profile induced by wild-type CypA at the same molar ratio.

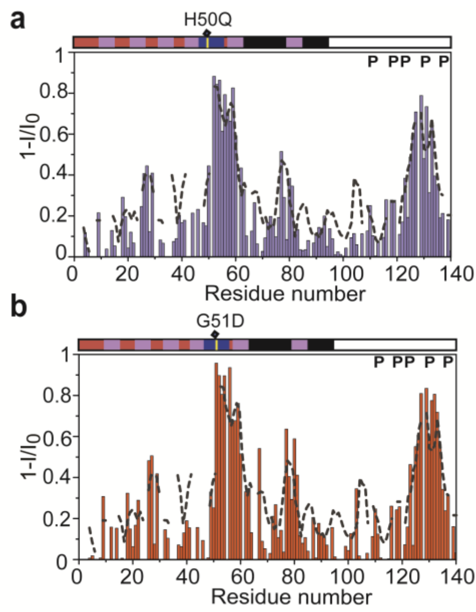

Figure S3. Binding of CypA to aSyn containing PD-associated mutations H50Q (a) and G51D (b). Residue-specific intensity changes upon addition of a 5-fold excess of CypA. The CypA-induced intensity broadening profile of wild-type aSyn is shown as dashed line.

Table S1. X-ray data collection statistics.

Data statistics	PreNac/CypA-complex
Wavelength	1.0 Å
Beamline	SLS-X10SA
Detector	PILATUS 6M
Space group	$P4_32_12$
a	61.034 Å
b	61.034 Å
c	129.152 Å
Resolution[a]	1.38 Å (1.40-1.38 Å)
Reflections measured	1,231,308
Unique reflections	51,144
Redundancy	24.01 (12.56)
Completeness(%)	99.7 (92.6)
Mean $I/\sigma(I)$	27.39 (1.16)
R_{rim} (%)[b]	2.92 (68.9)

[a] Values in parentheses are outer-resolution shell.

[b] $R_{rim} = \sum_{hkl}\left[N/(N-1)\right]^{1/2}\sum_i\left|I_i(hkl)-\langle I(hkl)\rangle\right| / \sum_{hkl}\sum_i I_i(hkl)$, where N is the redundancy and $I_i(hkl)$ is the ith observation of reflection hkl and ⟨I(hkl)⟩ is the weighted average intensity for all observations i of reflection hkl.

Table S2. X-ray structure refinement statistics CypA/αSyn$_{PreNAC}$-complex.

R-factor[a]	16.3%
R_{free}[b]	17.8%
Solvent	60.6%
Mean B-value (Å^2)	
chain A	24.26
chain B	32.01
waters	37.5
No. of protein residues	177
No. of water residues	248
Root mean square deviations from ideal geometry	
Bond lengths	0.019 Å
Bond angles	2.18°
Ramachandran plot (%)	
Favoured	95.1
Allowed	4.9
Outliers	0

[a] $R = \sum_{hkl} \left| |F_{obs}| - |F_{calc}| \right| / \sum_{hkl} |F_{obs}|$, where F_{obs} and F_{calc} are the observed and calculated structure factors, respectively.

[b] R_{free} was determined using 5% of the data [1].

Supplementary Experimental Section

Protein expression and purification of aSyn proteins was performed as described previously.[17] aSyn peptides were synthesized by solid-phase peptide synthesis. The gene of human CypA was cloned into a modified pET28a vector (Addgene) and site-directed mutagenesis was carried out using a QuikChange kit (Qiagen). CypA and its R55A variant were recombinantly expressed in *Escherichia coli* BL21(DE3) cells (Novagen) as described in [18]. Proteins were dialyzed against the NMR buffer containing 100 mM NaCl, 50 mM HEPES, 0.02 % NaN_3, pH 7.4.

NMR experiments were recorded on 600, 700, 800, 900, 950 MHz Bruker NMR spectrometers. For backbone resonance assignment of ^{15}N/^{13}C-labeled CypA, 3D HNCA, HNCACB, HNCO, HNCACO and ^{15}N-edited NOESY-HSQC (NOESY

mixing time: 120 ms) experiments were recorded. NMR-based interaction studies were acquired at 15 °C and the combined $^{1}H/^{15}N$ chemical shift perturbation $(((\delta_H)^2+(\delta_N/5)^2)/2)^{1/2}$ was calculated. The intensities were fitted assuming a simple two state exchange model and the K_d was calculated according to:

$$\left(1-\frac{I}{I_0}\right) = I_{max}\left[\frac{(P_0+x+K_d)-\sqrt{(P_0+x+K_d)^2-4P_0x}}{2P_0}\right]$$

where I is the intensity value along the titration, I_0 is the intensity value of the free state, P_0 is the total amount of protein, K_d is the dissociation constant and x the concentration of CypA in µM along the titration. Errors were estimated by evaluating the standard deviation of the intensity according to:

$$\sigma_I = \left(\frac{I}{I_0}\right)\sqrt{\left(\frac{\sigma I}{I}\right)^2 + \left(\frac{\sigma I_0}{I_0}\right)^2}$$

where σI and σI_0 are the standard deviations of the noise in the spectra. To identify the *cis*-proline conformers, aSyn (600 µM) was resuspended in 20 mM phosphate buffer (0.02% NaN_3, 2 mM DTT, pH 6.0).

For crystallization, the aSyn(E46-Q62) peptide was added in 4-fold molar excess to CypA. Total protein concentration was adjusted in NMR buffer to 20 mg/ml. Crystals were obtained at 20 °C by sitting drop vapor diffusion using 1.93 M tri-ammonium citrate, pH 7.0, as precipitant. For data collection crystals were soaked for 1 minute in 2.5 M tri-ammonium citrate, pH 7.0, as cryoprotectant. Data collection was performed at SLS Villigen, Switzerland (beamline PXII, Pilatus 6M detector [19]). Data were processed with XDS.[20] Space group determination and statistical analysis was performed with XPREP (Bruker AXS, Madison, Wisconsin, USA). The structure was solved by molecular replacement with PHASER [21] using the crystal structure of CypA (PDB code: 5KUL [22]) as search model. Refinement was performed with Refmac[23] alternating with manual model building in Coot.[24]