Supporting Information

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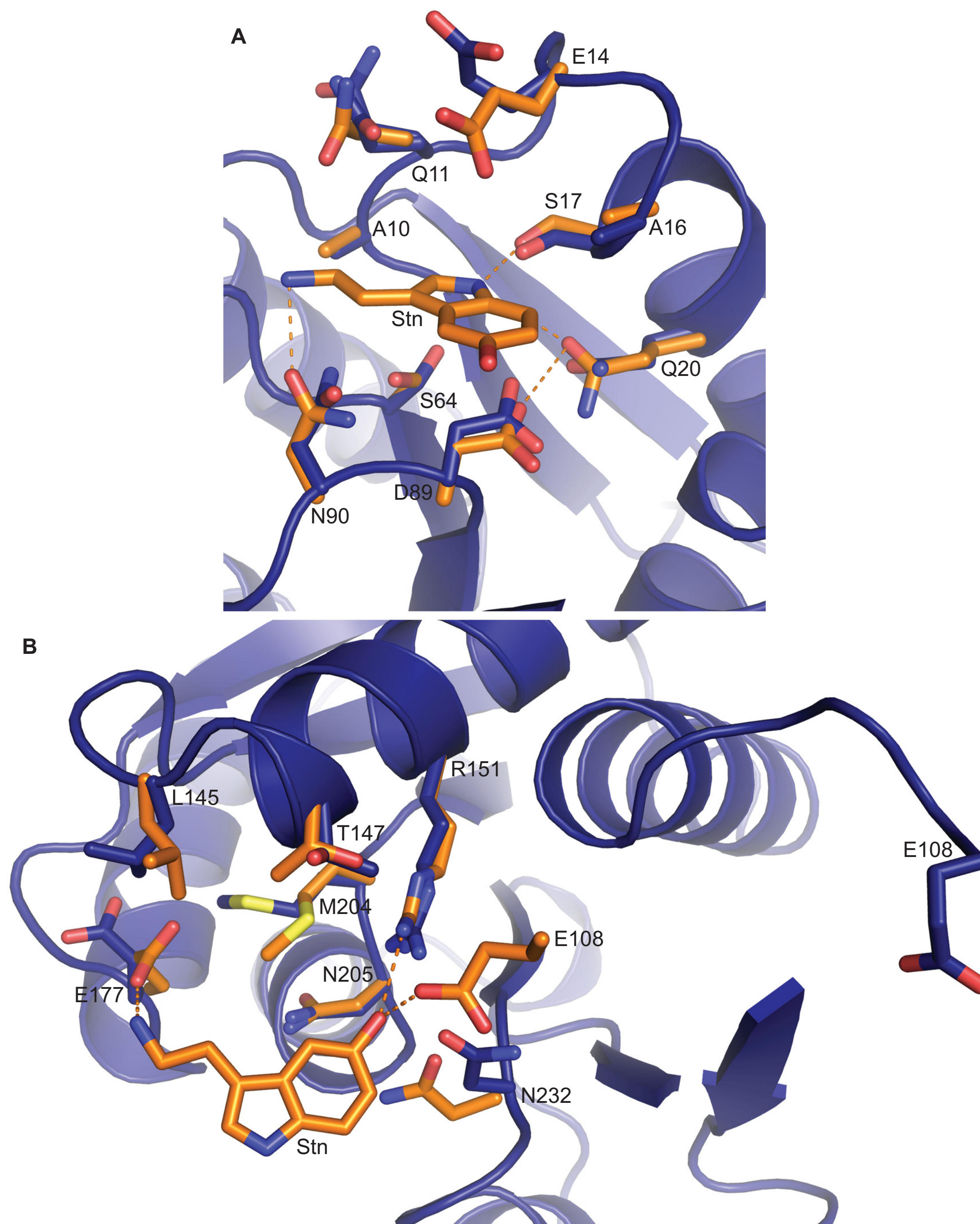

Fig. S1. Comparison of the binding pocket residues in the Stn.A2 design prediction (orange) with the ones in the crystal structure (molecule B, dark blue). (*A*) Superimposition of the binding pocket residues in the N-terminal domain (rmsd of 1.17 over all atoms). (*B*) Superimposition of the binding pocket residues (excluding E108) in the C-terminal domain (rmsd of 1.06 over all atoms).

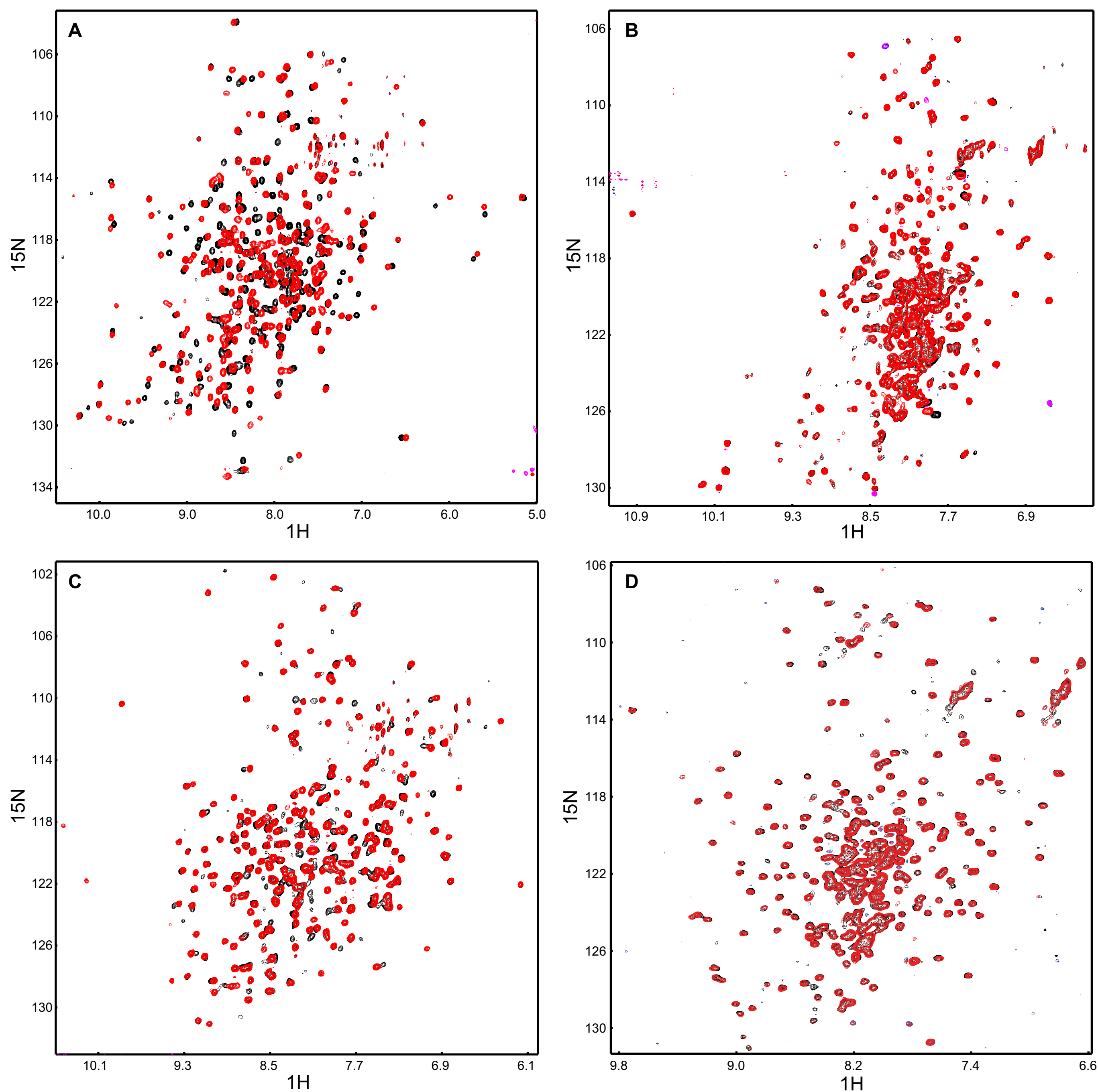

Fig. S2. End-point chemical shift titrations of wild-type PBPs and PBP-based receptors using 2D NMR spectroscopy. (*A*) GBP without ligand (black) and 20-fold excess of glucose (red). Large changes in the spectra occur because of ligand binding and ligand-induced conformational changes. (*B*) PMPA.G12 without ligand (black) and 20-fold excess of PMPA (red). Because of aggregation or partial folding, the spectra show unresolved areas. No significant chemical shift changes upon addition of ligand could be detected. (*C*) RBP without ligand (black) and 20-fold excess of glucose (red). Without ligand, RBP exists in equilibrium between open and closed states. Addition of ligand shifts the spectrum to the bound and closed state. (*D*) TNT.R1 without ligand (black) and twofold excess of TNT (red). The solvent acetonitrile destabilizes the protein, thus to analyze the effect of TNT on its own, acetonitrile was added in equal amounts to both measurements. Because of aggregation at high protein concentrations or partial folding, the spectra show unresolved areas. No significant changes upon ligand binding could be detected. Aliased resonances are shown in blue and magenta for protein without and with ligand, respectively.

Table S1. Overview of conditions used for NMR measurements

Protein	Temperature [°C]	Protein concentration [mM]	Ligand concentration [mM]	Ligand excess
ABP (C64A L253C)	30	0.44	8.6	20×
Stn.A2	30	0.54	5.4	10×
Lac.A1	20	0.25	24.7	100×
GBP (S112C)	30	0.87	17.5	20×
PMPA.G12	30	0.13	2.5	20×
RBP (A234C)	30	0.41	8.3	20×
TNT.R1	30	0.17	0.34	2×

Table S2. Data and refinement statistics for the Stn.A2 dataset

Method detail	Results
Experimental procedures	
Crystallization condition	20% PEG 3350, 0.2M (NH_4)citrate, 2 μmol/ml serotonin
Protein solution	0.46 μmol/ml ABPsero (15.7 mg/ml) in 30 mM Tris pH 8.0
Cryo protection	+ 10% PEG 400
Data collection	
Detector distance (nm)	180
Wavelength (Å)	0.978
Spacegroup	$P2_12_12_1$
Cell dimensions	a = 79.9Å, b = 86.3Å, c = 116.9Å, $\alpha = \beta = \gamma = 90.0°$
Data range (Å)	40.0–2.20 (2.33–2.20)
Unique reflections	41,597 (6,539)
Redundancy	7.28 (7.14)
Completeness (%)	99.7 (98.7)
Rmerge (%)*	17.1 (89.1)
I/σ	13.30 (2.33)
Refinement statistics	
Rfact (%)	21.0
Rfree (%)	27.2
rmsd of bond lengths (Å)	0.012
rmsd of bond angles (Å)	1.25
No. of water molecules	429
No. of monomers/asymmetric unit	2
Structure and model quality	
Missing amino acids	Molecule A: C-terminus (300–308) + His-tag
	Molecule B: C-terminus (296–308) + His-tag
Residues in most favored region	594 (97.9%)
Residues in allowed region	606 (99.8%)
Residues in outlier region	1 (0.17%)

*Rmerge is relatively high, particularly in the highest resolution shell. Still, this shell was included because I/σ, completeness, and redundancy are reasonable.