Supplementary Material

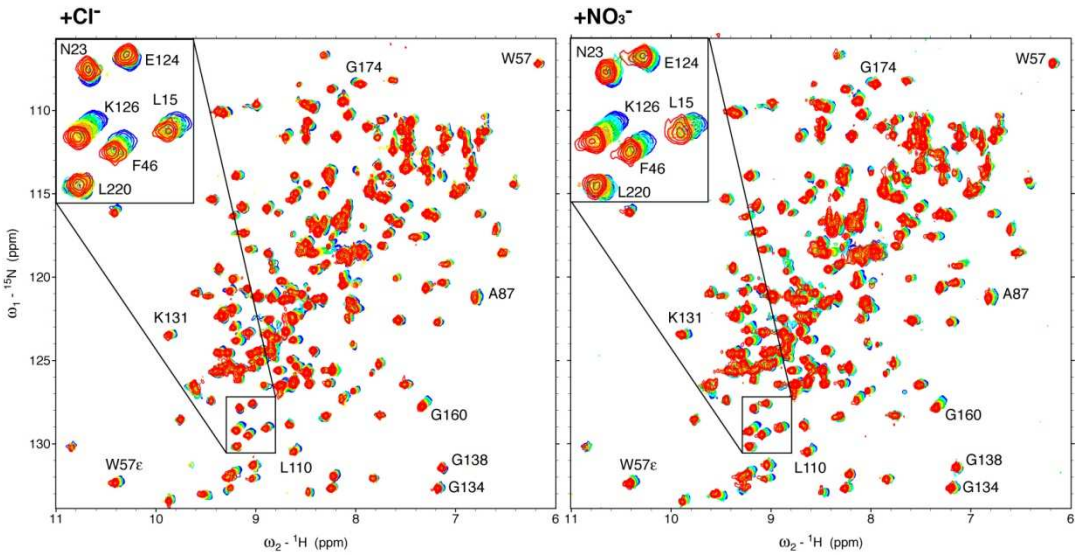

Figure S1. Venus-ion interactions mapped by NMR titration. Overlaid 700 MHz [^{15}N, ^{1}H] HSQC spectra of Venus in the presence of various amount of chloride ions (left) and nitrate ions (right) ranging from 0 to 400 mM. The spectra were recorded at pH 6.0 and at 298K. The spectra are colour-ramped from blue to red accordingly. The same representative regions of both titration series are enlarged with individual crosspeaks labelled with corresponding residue identities as insets. Other residues that exhibit significant chemical shift perturbations upon addition of ions are also labelled.

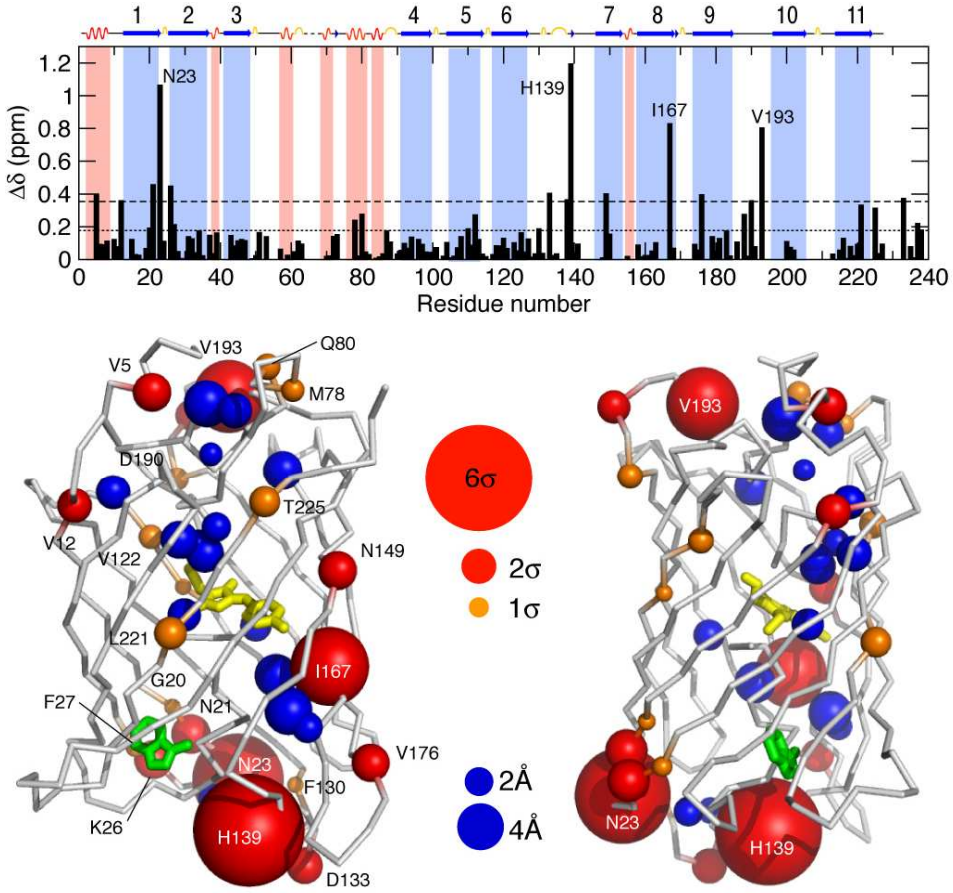

Figure S2. Structural mapping of pH effects on the chemical shifts of Venus. (Top panel) ^{1}H and ^{15}N weighted chemical shift differences (Δδ) of Venus between pH values of 6.0 and 9.6 are plotted as a function of residue number. The solid and dashed horizontal lines indicate one and two standard deviations (s) of the overall Δδ values. Residues that exhibit significant Δδ) are labeled with corresponding identities. (Bottom panel) Two orthogonal views of the crystal structure of Venus with structural mapping of the observed Δδ (pH 6.0-pH 9.6). The backbone nitrogen atoms of the residues that exhibit Δδ larger than 1s are shown in spheres and coloured in orange (<2s) and red (>2s). Cavities within the crystal structure of Venus are shown in blue spheres with varied radii as indicated in the middle. The cavities have been identified using the program Voronoia (http://bioinfomatics.charite.de/voronoia) (Rother *et al., (*2009)). These cavities are located along the central a-helix and are in close proximities with the top and bottom lids and around the chromophore (shown in yellow sticks). The side-chain of W56 is shown in green sticks.

Rother K, Hildebrand PW, Goede A, Gruening B, Preissner R. Voronoia: analyzing packing in protein structures. Nucleic Acids Res. 2009 37(Database issue), D393-5.

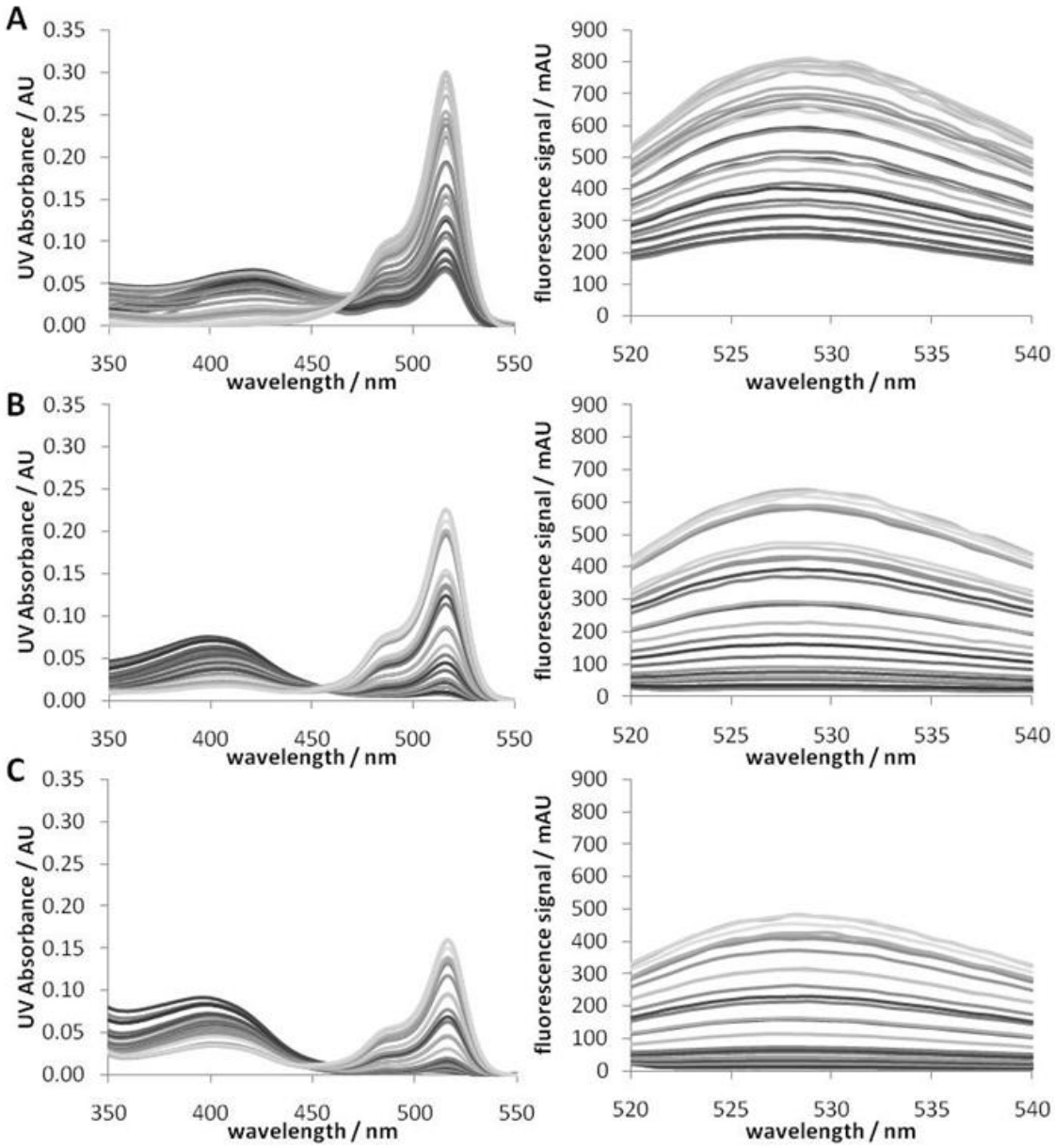

Figure S3. UV absorbance and fluorescence spectra of Venus as a function of pH. The panels show the UV absorbance (left half) and fluorescence (right half) of 10 µM Venus in 10 mM MES/MOPS at various pH (5.5-8.0, represented in shaded curves varying from black to light grey) in the absence (A), and presence of 0.4 M NaCl (B) or $NaNO_3$ (C) respectively.