Supplement

Modulation of a pre-existing conformational equilibrium tunes adenylate kinase activity

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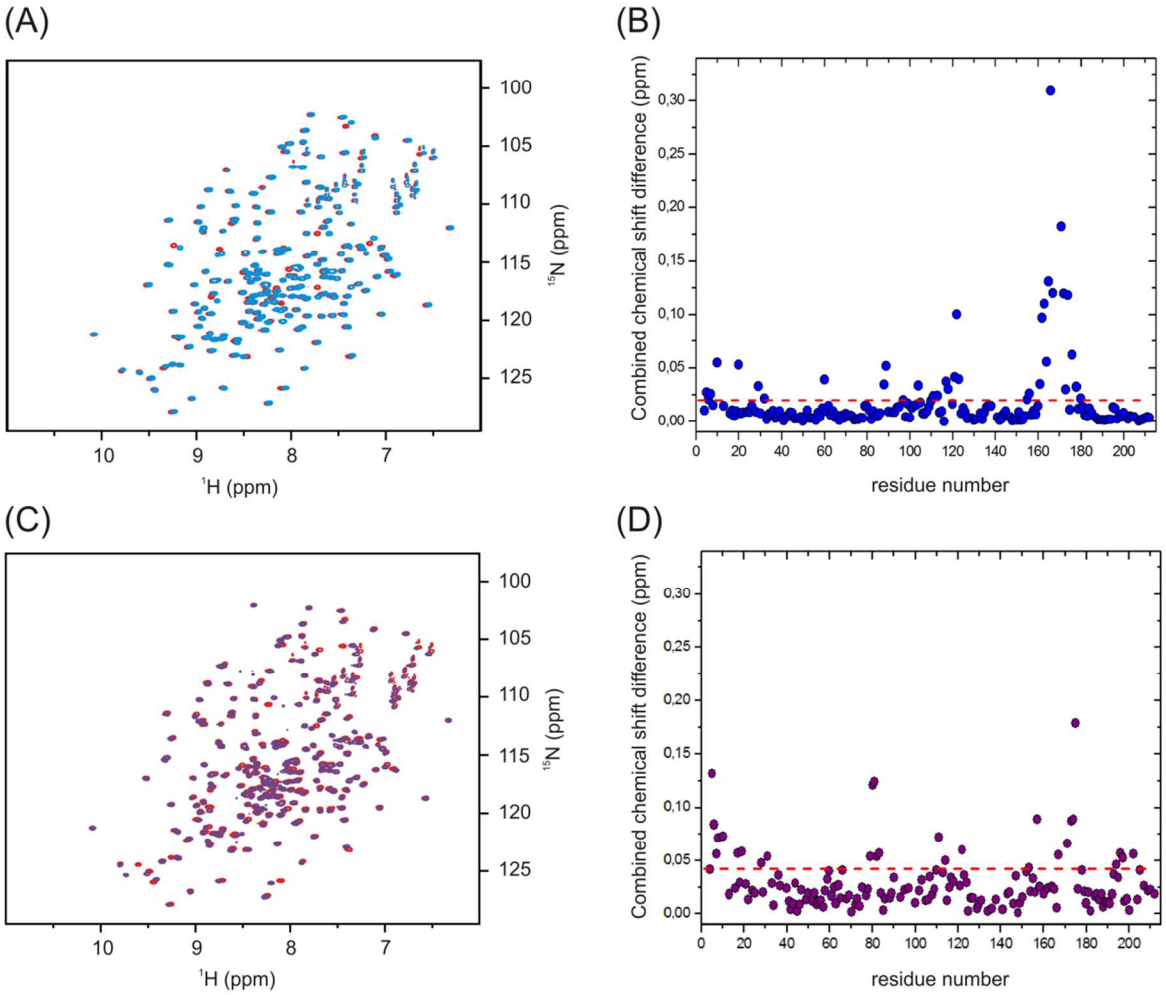

Figure S1. NMR spectra of mutational- and osmolyte perturbed AK_{eco}. (**A**) Overlay of ^{1}H-^{15}N HSQC spectra corresponding to wild type AK_{eco} (red contours) and E170A (blue contours). (**B**) Combined chemical shift perturbations (referenced to wild type AK_{eco}) resulting from the E170A mutation quantified according to: $\delta\omega=0.2\cdot|\delta^{15}N| + |\delta^{1}H|$ (ppm). (**C**) Overlay of ^{1}H-^{15}N HSQC spectra corresponding to wild type AK_{eco} (red contours) and AK_{eco} with 0.35M TMAO (purple contours). (**D**) Combined chemical shift perturbations of AK_{eco} in 0.35 M TMAO (referenced to AK_{eco} in regular buffer) mutation. The dashed lines in (B) and (D) indicates the threshold value used in Fig. 3.

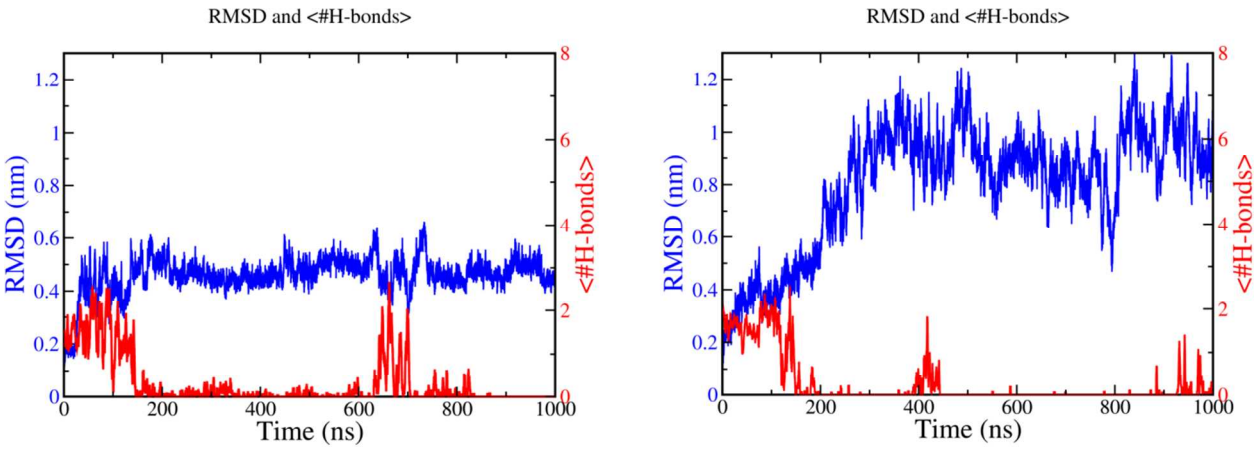

Figure S2. Molecular dynamics simulations of the closed to open transition in AK_{eco}. RMSD with respect to the initial WT closed state (blue) and average number of hydrogen bonds connecting L58 and E170 (red) are shown for two simulations.

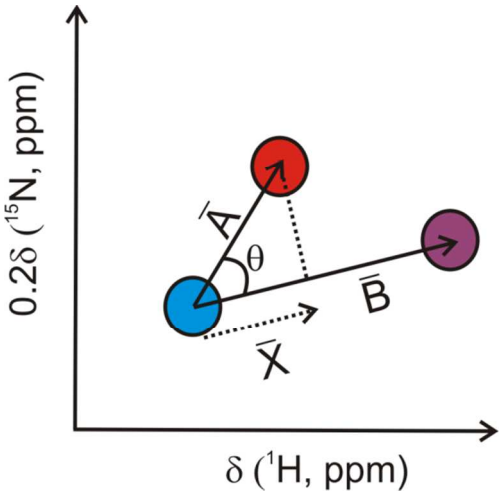

Figure S3. Definition of vectors and the projection angle in the chemical shift projection analysis. Chemical shifts are colored according to; blue, E170A; red, AK_{eco} and purple AK_{eco} with 0.35 M TMAO. The amplitude of the AK_{eco} chemical shift vector (A) projected onto the "activation" vector (B) is defined as "X". The projection angle is defined as "θ".

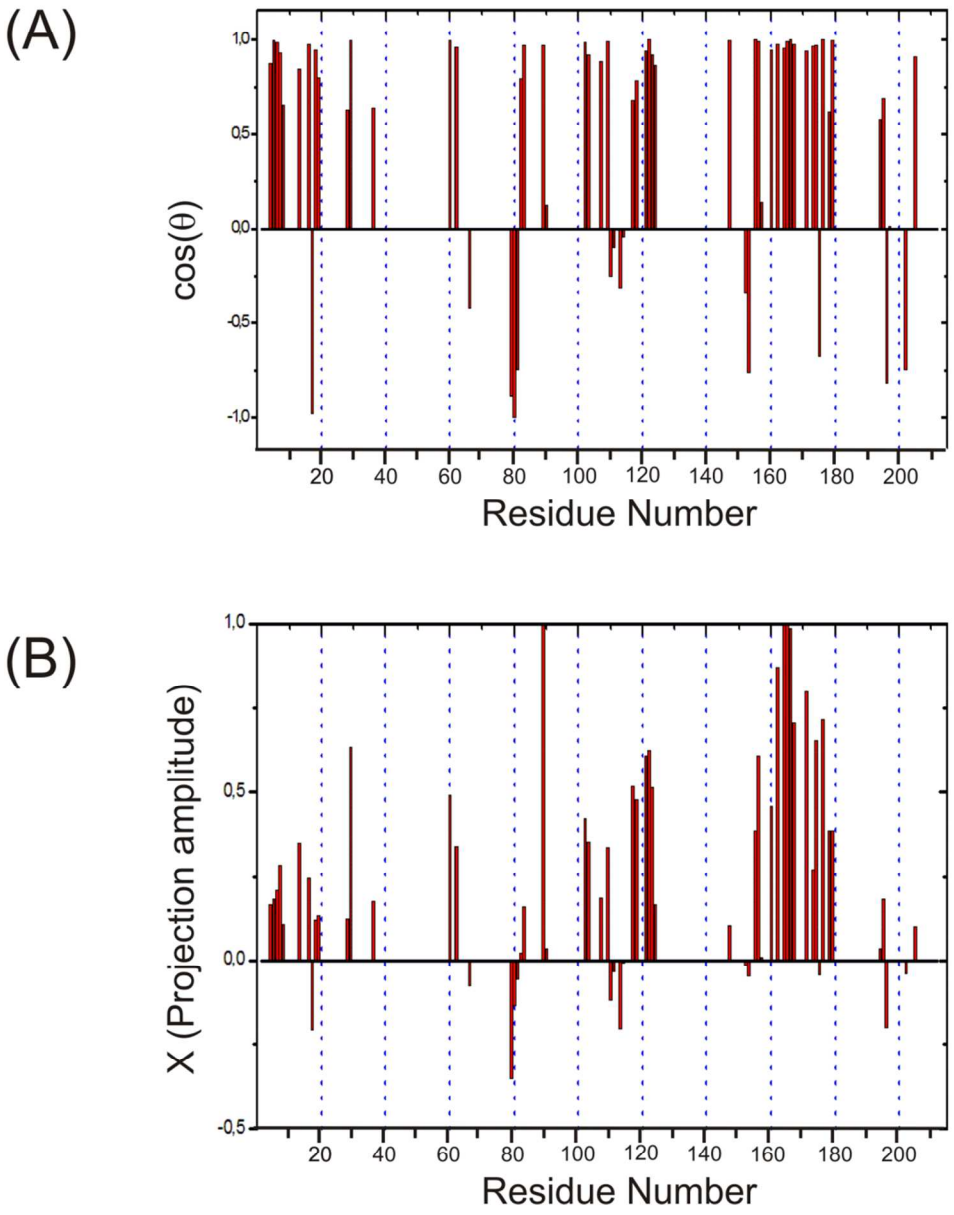

Figure S4. Residue specific projection angles (cosθ) and amplitudes (X) from the definition in Figure S3. Residues with a compounded chemical shift difference ($\delta\omega=0.2\cdot|\delta^{15}N| + |\delta^{1}H|$) between E170A and AK_{eco} with 0.35 M TMAO larger than 0.3 ppm were included in the analysis. (**A**) Projection angle reported as cosθ. (**B**) Projection amplitude (X).

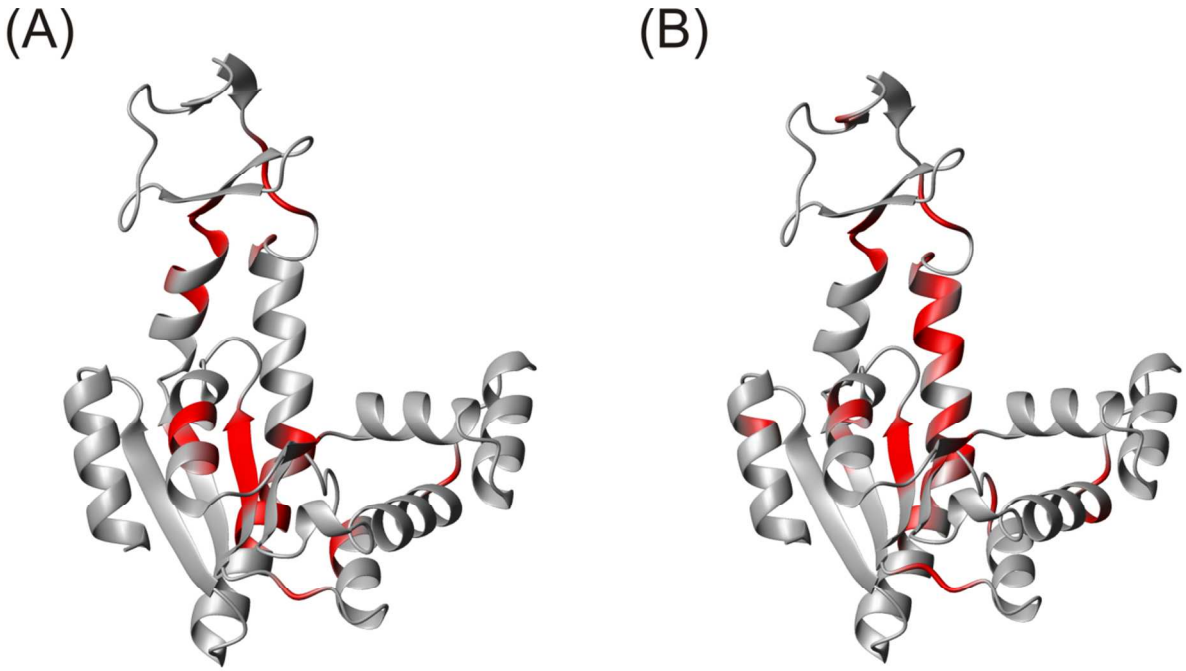

Figure S5. Structural distribution of residues identified in the linear correlation- and projection analysis. (**A**) Residues identified form the linear correlation analysis are shown in red on the open AK_{eco} structure. (**B**) Residues with a projection angle, cosθ, larger than 0.9 are shown in red on the open AK_{eco} structure.

Ligand binding in coupled equilibrium reactions

The dissociation constant (K_d) for a one to one binding reaction is given by equation (**1**).

$$K_d = \frac{[E][S]}{[ES]} \quad \textbf{(1)}$$

In (**1**) E corresponds to enzyme, S to substrate and ES to the enzyme-substrate complex.

For AK_{eco} the minimal ligand binding mechanism is described by **Fig. 1D**, where the initial open equilibrium complex (ES^O) isomerizes to a closed complex (ES^C). For this mechanism the apparent binding affinity (K_d^{app}) is described by equation (**2**).

$$K_d^{app} = \frac{[E][S]}{[ES]^O + [ES]^C} \quad \textbf{(2)}$$

The expression in **(2)** can be simplified into **(5)** by insertion of **(3)** and **(4)** that are definitions of the equilibrium constants for association of substrate (K_d) and the subsequent conformational change (K_{conf}).

$$K_d = \frac{[E][S]}{[ES]^O} \quad \textbf{(3)}$$

$$K_{conf} = \frac{[ES]_C}{[ES]_O} \quad \textbf{(4)}$$

$$K_d^{app} = \frac{K_d}{1 + K_{conf}} \quad \textbf{(5)}$$

From equation **(5)** it is evident that K_d^{app} is modulated by K_{conf}.

Hydrogen to deuterium exchange kinetics for L58

The time points are defined as the middle of each ^{1}H-^{15}N HSQC spectrum (acquisition time equal to 26 minutes). The first experiment was started after a dead-time of 11 minutes after sample preparation. Intensities are normalized to the intensity of the first experiment.

Time (min)	Intensity
24.2	1
50.5	0.7
76.8	0.4397
103.1	0.3404
129.5	0.2245
155.8	0.1558
190.4	0.1121
216.7	0.1182
243.0	0.0457
269.3	0.1075
295.6	0.0239
321.9	0.0728
348.3	0.0646
374.6	0.0671
400.9	0.0693
427.2	0.0673
453.5	0.0794
479.8	0.0511
506.1	0.0009
532.5	0.0635
558.8	0.0545
585.1	0.0347
611.5	0.0465
637.8	0.0558
664.1	0.0572
690.4	0.0342
716.7	0.0702
743.0	0.0019
769.3	0.0231
795.6	0.0539
822.0	0.0208
848.3	0.0224
874.6	0.0497
900.9	0.0629
927.2	0.0633
953.5	0.0634

979.8	0.0569
1006.1	0.0578
1032.4	0.0741
1058.8	0.0742
1085.2	0.0668
1111.5	0.0086
1137.8	0.0481
1164.1	0.0538
1190.4	0.0475
1216.7	0.0294
1243.0	0.0714
1269.3	0.0379
1295.7	0.0631
1322.0	0.016
1348.3	0.0262
1374.6	0.0476
1400.9	0.0604
1427.2	0.0589
1453.5	0.0685
1479.8	0.0279
1506.2	0.0432
1532.5	0.0608
1558.8	0.0637
1585.1	0.0656
1611.4	0.0542
1637.7	0.0642
1664.0	0.0143
1690.4	0.0767
1785.1	0.0459
1811.9	0.0635
1838.2	0.0424
1864.6	0.037
1890.9	0.0342
1917.2	0.0572
1943.5	0.0504
1969.8	0.0499
1996.2	0.0612
2022.5	0.0522
2048.8	0.0373
2075.1	0.0822
2101.4	0.0276
2127.7	0.0408
2154.0	0.0322

2180.3	0.0538
2206.7	0.0603
2233.0	0.0637
2259.3	0.0522
2285.6	0.0231
2311.9	0.0433
2338.2	0.0562
2364.5	0.0234
2390.8	0.0727
2417.2	0.025
2443.5	0.0463
2469.8	0.0714
2496.1	0.0701
2522.4	0.048
2548.7	0.0465
2575.0	0.0464
2601.3	0.0428
2627.6	0.0429