

SUPPLEMENTARY MATERIAL

Role of Electrostatic Interactions in Transient Encounter Complexes in Protein-Protein Association Investigated by Paramagnetic Relaxation Enhancement

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Sample preparation

Protein expression, purification, and conjugation of paramagnetic labels were carried out as described previously.^{S1} All NMR spectra were recorded on a Bruker DRX-600 spectrometer equipped with a cryogenic z -gradient, triple resonance probe.

Isothermal Titration Calorimetry (ITC) data on the binding of HPr to EIN

ITC was performed using a high-precision VP-ITC calorimetry system (Microcal Inc). Analysis of the data was performed using the Origin software provided with the instrument.

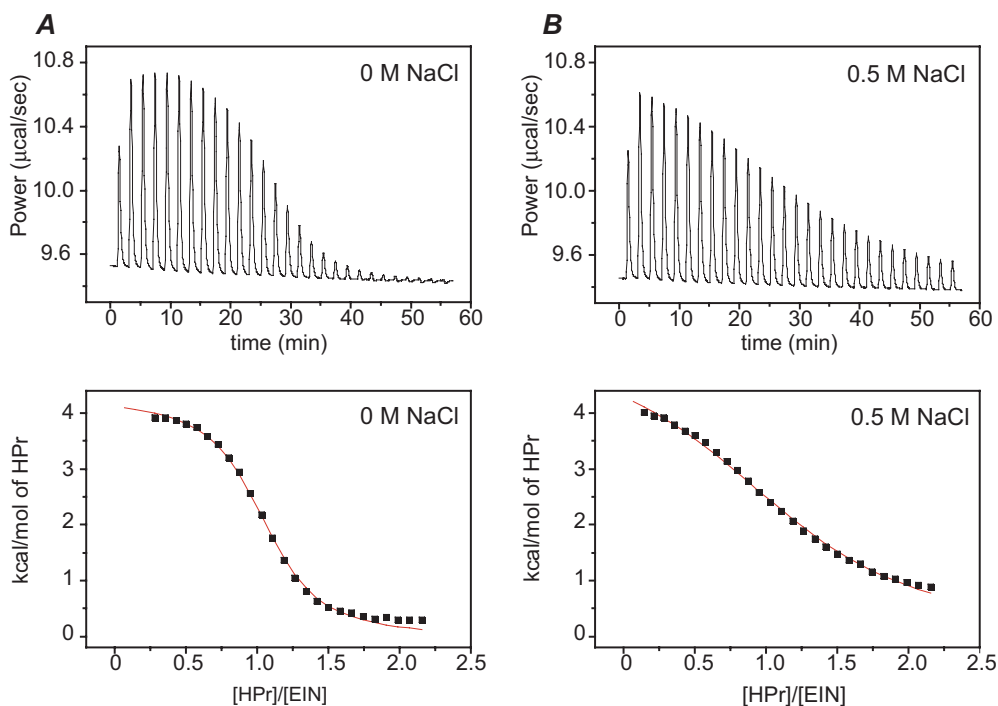


Fig. S1. Raw ITC data (top panels) and integrated heats of injection (bottom panels) for the HPr/EIN titration at (A) 0 M NaCl and (B) 0.5 M NaCl. 28 injections of 10 ml each of a 1 mM stock HPr solution into a solution of 0.1 mM EIN was carried out at 40°C in 20 mM Tris-HCl buffer pH 7.4. The square circles in the bottom panels are the experimental data, and the red lines represent the least-squares best-fit curves derived for a one-site binding model. The parameters for the fits are given in Table S1.

Table S1. Optimized values of the equilibrium dissociation constant (K_D), ΔH and ΔS obtained from least-squares fitting of single-site binding model to the ITC data for the interaction of HPr with EIN.^a

| | NaCl Concentration | | | | |
|--|--------------------|-----------------|-----------------|-----------------|------------------|
| | 0 M | 0.15 M | 0.3 M | 0.5 M | 2.0 M |
| K_D (μM) | 3.7 \pm 0.3 | 7.3 \pm 0.4 | 15.7 \pm 1.1 | 26.2 \pm 1.8 | 131.4 \pm 12.1 |
| ΔH (kcal.mol ⁻¹) | 4.25 \pm 0.05 | 5.82 \pm 0.08 | 5.76 \pm 0.12 | 5.21 \pm 0.11 | 4.94 \pm 2.19 |
| ΔS (cal.mol ⁻¹ .K ⁻¹) | 38.4 | 42.1 | 40.4 | 37.6 | 33.5 |

^aThe experiments were carried out at 40°C in 20 mM Tris-HCl buffer, pH 7.4, and with the NaCl concentration as indicated in the table

References

S1. Tang, C., Iwahara, J.; Clore, G. M. *Nature* **206**, 444, 383-386.