Supporting Information

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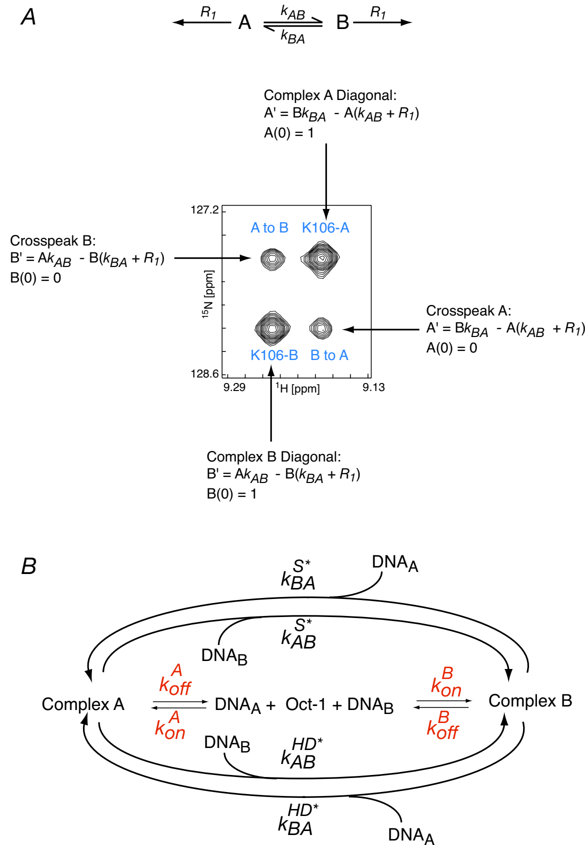

Fig. S1. Model and equations for fitting exchange data. (*A*) Auto-(diagonal) and cross-peak intensities for individual residues were fit to a simple exchange scheme with the first-order exchange equations and initial conditions shown. A and B represent the magnetizations for the two Oct-1·*HoxB1* complexes. For each residue, four parameters were optimized: the apparent exchange rates k_{AB}^{app} and k_{BA}^{app}; the longitudinal ^{15}N relaxation rate R_1 (assuming the same values for the A and B complexes); and the scaling factor S_A for complex A (with the scale factor S_B for complex B given by $S_B = S_A k_{AB}^{app}/k_{BA}^{app}$). (*B*) The combined jumping (full dissociation) and intersegment transfer scheme used to fit the DNA-dependent $^{15}N_z$-exchange data. The data for both domains (S60 for POU_S and K106 for POU_{HD}) at five DNA concentrations (308 data points) were fit simultaneously using the differential equations below and optimizing 16 parameters: the global association constant k_{on}^A; domain-specific second-order rate constants k_{AB}^{S*}, k_{BA}^{S*}, and k_{AB}^{HD*} the longitudinal ^{15}N relaxation rates for POU_S (R_1^S) and POU_{HD} (R_1^{HD}); and scaling factors at each DNA concentration, $S_{A,1}^S$ to $S_{A,5}^S$ and $S_{A,1}^{HD}$ to $S_{A,5}^{HD}$ (with $S_{B,1}^S = S_{A,1}^S k_{AB}^{S*}[DNA_B^{free}]/k_{BA}^{S*}[DNA_B^{free}]$ and $S_{B,1}^{HD} = S_{A,1}^{HD} k_{AB}^{HD*}[DNA_B^{free}]/k_{BA}^{HD*}[DNA_A^{free}]$). In the equations below A_S and B_S refer to the magnetizations of POU_S in complexes A and B, respectively; A_{HD} and B_{HD} to the magnetizations of POU_{HD} in complexes A and B, respectively; and P_S and P_{HD} to the magnetizations of the POU_S and POU_{HD} domains, respectively, in free Oct-1 (i.e., fully dissociated from DNA). The differential equations describing the evolution of magnetization are as follows:

$$dA_S/dt = k_{on}^A[DNA_A^{free}]P_S + k_{BA}^{S*}[DNA_A^{free}]B_S - A_S(k_{AB}^{S*}[DNA_B^{free}] + k_{off}^A + R_1^S) \quad [S1]$$

$$dB_S/dt = k_{on}^B[DNA_B^{free}]P_S + k_{AB}^{S*}[DNA_B^{free}]A_S - B_S(k_{BA}^{S*}[DNA_A^{free}] + k_{off}^B + R_1^S) \quad [S2]$$

$$dA_{HD}/dt = k_{on}^A[DNA_A^{free}]P_{HD} + k_{BA}^{HD*}[DNA_A^{free}]B_{HD} - A_{HD}(k_{AB}^{HD*}[DNA_B^{free}] + k_{off}^A + R_1^{HD}) \quad [S3]$$

$$dB_{HD}/dt = k_{on}^B[DNA_B^{free}]P_{HD} + k_{AB}^{HD*}[DNA_B^{free}]A_{HD} - B_{HD}(k_{BA}^{HD*}[DNA_A^{free}] + k_{off}^B + R_1^{HD}) \quad [S4]$$

$$dP_S/dt = k_{off}^A A_S + k_{off}^B B_S - P_S(k_{on}^A[DNA_A^{free}] + k_{on}^B[DNA_B^{free}] + R_1^S) \quad [S5]$$

$$dP_{HD}/dt = k_{off}^A A_{HD} + k_{off}^B B_{HD} - P_{HD}(k_{on}^A[DNA_A^{free}] + k_{on}^B[DNA_B^{free}] + R_1^{HD}) \quad [S6]$$

with the magnetizations of the autopeaks set to 1 and cross-peaks set to 0 at $t = 0$. Note that no auto- or cross-peaks corresponding to P_S or P_{HD} are observed in the ^{1}H–^{15}N TROSY-based $^{15}N_z$-exchange spectra, and the maximal magnetization of the calculated P_S and P_{HD} cross-peaks do not exceed 1.6×10^{-4} relative to the autopeak intensity of 1 at $t = 0$. In addition, the following relationships apply: $k_{BA}^{HD*} = k_{AB}^{HD*}k_{BA}^{S*}/k_{AB}^{S*}$; $K_{diss}^B = K_{diss}^A k_{AB}^{S*}/k_{BA}^{S*}$ with $K_{diss}^A = 16.3$ nM (determined from the equilibrium binding isotherm shown in Fig. S3*A*); $k_{off}^A = k_{on}^A/K_{diss}^A$; $k_{off}^B = k_{on}^B/K_{diss}^B$; and $k_{on}^A = k_{on}^B$ (assumed). χ^2 per degree of freedom for the overall fit is 0.9. The values of the rate constants are as follows: $k_{on}^A = k_{on}^B = 2.34(\pm0.12) \times 10^8$ M^{-1} s^{-1}; $k_{off}^A = 3.73 \pm 0.19$ s^{-1}; $k_{off}^B = 5.09 \pm 0.26$ s^{-1}; $k_{AB}^{S*} = 2.88(\pm0.10) \times 10^4$ M^{-1} s^{-1}; $k_{BA}^{S*} = 3.92(\pm0.13) \times 10^4$ M^{-1} s^{-1}; $k_{AB}^{HD*} = 1.85(\pm0.08) \times 10^4$ M^{-1} s^{-1}; $k_{BA}^{HD*} = 2.52(\pm0.11) \times 10^4$ M^{-1} s^{-1}; $R_1^S = 1.60 \pm 0.03$ s^{-1}; $R_1^{HD} = 1.53 \pm 0.03$ s^{-1}.

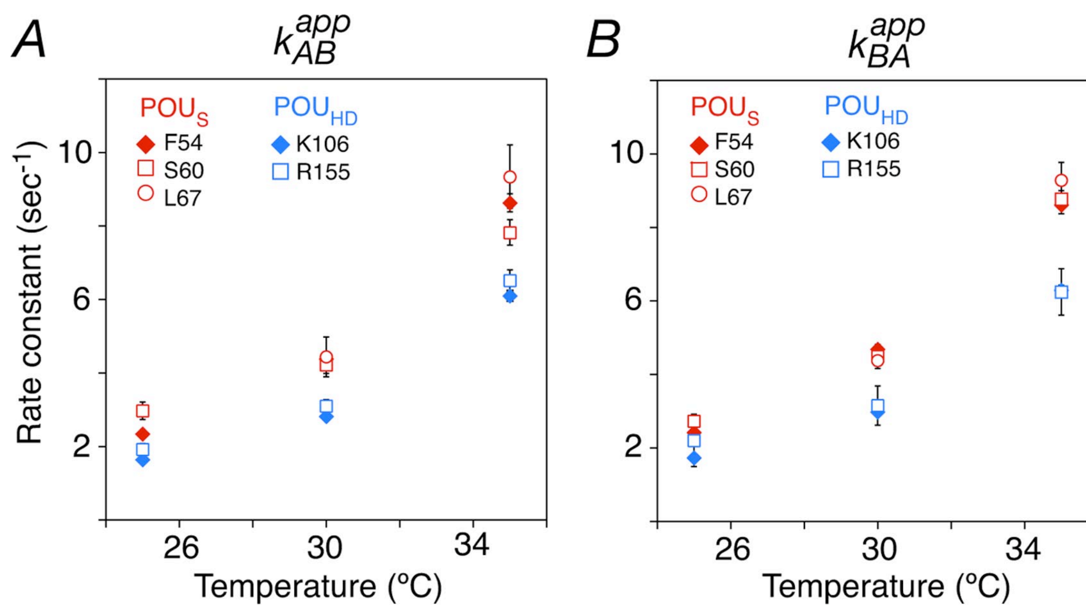

Fig. S2. Plot of temperature vs. apparent intermolecular exchange rate for individual residues of POU$_S$ (red) and POU$_{HD}$ (blue). Exchange data for each residue were fit separately to the first-order exchange model shown in Fig. S1*A*. Exchange rates from complex A to complex B (k_{AB}^{app}) and vice versa (k_{BA}^{app}) are shown in *A* and *B*, respectively. Error bars represent 1 SD.

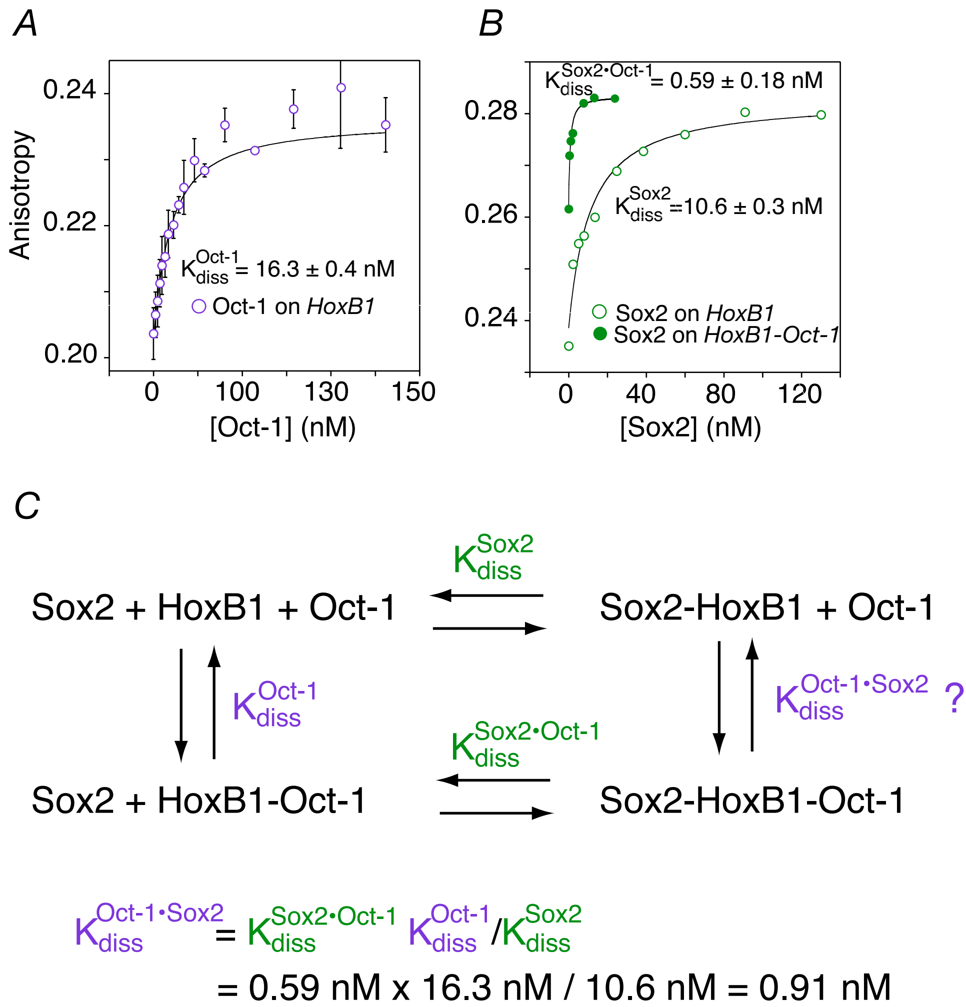

Fig. S3. Sox2 increases the affinity of Oct-1 for the *HoxB1* DNA site ≈20-fold. (*A* and *B*) Change in fluorescence anisotropy of dye-labeled *HoxB1* fragment upon titration (*A*) with Oct-1 alone, and (*B*) with Sox2: alone (open circles) and in the presence of Oct-1 (closed circles). The dye used was fluorescein in *A* and rhodamine in *B*. (*C*) The equilibrium dissociation constant for Oct-1 in the presence of Sox2 was calculated by using the thermodynamic cycle and the three experimental equilibrium dissociation constants shown.

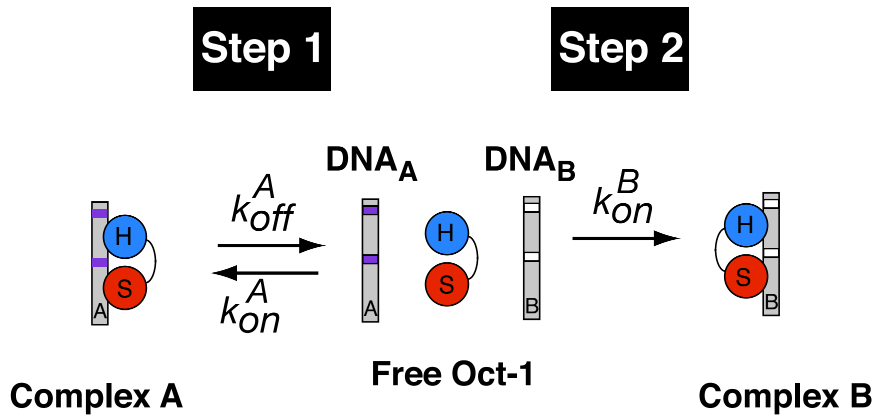

Fig. S4. The apparent rate of exchange from complex A to complex B through a fully dissociative or jumping mechanism is independent of DNA concentration under our experimental conditions. For the mechanism shown, the apparent rate from complex A to B is in general the product of the forward rate of the partial reaction labeled Step 1, and the net forward rate of the partial reaction is labeled Step 2. The forward rate of Step 1 is the dissociation rate of Oct-1 from complex A (k_{off}^{A}); the net forward rate of Step 2 is the association rate of Oct-1 with free DNA$_B$ ($k_{on}^{B}[DNA_B^{free}]$) weighted by the sum of the association rate of Oct-1 with free DNA$_B$ and the association rate of Oct-1 with free DNA$_A$ ($k_{on}^{B}[DNA_B^{free}] + k_{on}^{A}[DNA_A^{free}]$). Thus, the apparent exchange rate from complex A to B is: $k_{AB}^{app} = k_{off}^{A}k_{on}^{B}[DNA_B^{free}]/(k_{on}^{B}[DNA_B^{free}] + k_{on}^{A}[DNA_A^{free}])$. For the experimental conditions used, $k_{on}^{A} \approx k_{on}^{B}$ and $[DNA_A^{free}] = [DNA_B^{free}]$, reducing the apparent exchange rate to: $k_{AB}^{app} = k_{off}^{A}/2$.