Supplementary Information for Jose et al. gp32 cooperative binding ms (NAR ms 00785-M-2015)

Finite lattice calculations. The binding of overlapping (and cooperatively bound) ligands to relatively short (containing less than ~ 10 ligand binding sites at saturation) ssDNA lattices requires that the infinite lattice binding theory developed by McGhee and von Hippel (1) be modified to take explicit account of end effects in calculating the number of potential ligand binding sites per lattice available at each level of total ligand concentration added during a gp32 titration of the sort shown in Figs. 1, 4 and 5 of the present paper. A modified theory that deals with these issues was developed by Epstein (2), and played an important biological role in (for example) the development of a quantitative model to explain the auto-regulation of T4 gp32 synthesis by the competitive binding of gp32 to unstructured regulatory sites on its own mRNA (3). Here, as discussed in the attached paper in the Results section dealing with Fig. 1, we have used the Epstein finite lattice theory to calculate theoretical binding isotherms for comparison with the experimental results obtained with the 25-mer ssDNA lattices with spectroscopic probes in three different lattice positions shown in Fig. 1.

Of course, like the infinite-lattice binding theory (1), the finite lattice binding theory assumes that binding to all lattice positions is governed by the values of the K, n and ω parameters used, and does not take into account preferences for specific lattice binding positions that may be demonstrated by other approaches, such as the preferred binding of cooperatively bound gp32 clusters at the 5'-end of the lattice demonstrated in the present paper by spectroscopic approaches. However, if the binding free energy of cooperatively bound clusters is indeed equi-partitioned between the bound monomers of the cluster as discussed in connection with Fig. 1 (and that also, of course, underlies many other aspects of gp32 binding – e.g., see ref. 3), this should be apparent in the relevant finite lattice calculations plotted in Fig. S1.

In setting up these calculations the 132 unique arrangements of three or less gp32 molecules (excluded site size of 7 bases) on a 25-mer ssDNA lattice were tabulated by site number, assigned equal probabilities weighted by free ligand concentration, intrinsic binding constant (taken as 10^5 per molar in the low salt buffer) and cooperativity

factor (taken as 1000), using Epstein's lattice binding equations (2). The connection to total DNA oligomer and added gp32 concentrations was made iteratively using mass balance. The fluorescence intensities of the DNA oligomers were experimentally determined. The intensities of the DNA-gp32 complex at the three sets of probe sites were found by scaling the theoretical lines to the experimental data. Some variation was seen in the fluorescence intensities of the oligomers, as well as of the protein-oligomer complexes at the three sites (see Fig. 1). While Epstein theory treats the probe sites identically, some variation in fluorescence intensity at these sites might be expected due to polar binding of the asymmetric gp32 ligand protein to the polar lattice.

The results of these calculations are shown in Fig. S1, and demonstrate clearly that the fit of the finite lattice calculations to the experimental data of Fig. 1 at all three dimer probe sites are very good, and strongly support the assumption that the binding free energy of the bound 3-mer gp32 clusters is indeed equi-partitioned across all three gp32 monomers. As indicated above, the minor deviations of the experimental data from the theoretical fit doubtless reflect the minor differences in lattice occupancy at the three probe positions due to interactions that are not taken into account in the finite lattice calculations.

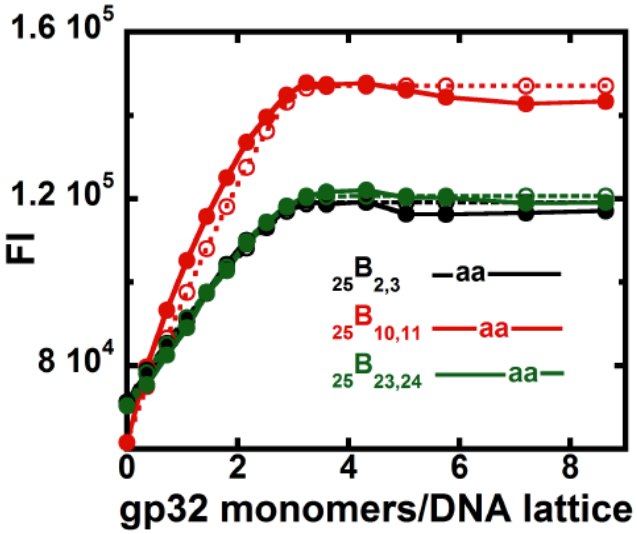

Figure S1. ***A comparison of theoretical (finite lattice calculations) and experimental fluorescence intensity changes observed for gp32 binding to 25-mer ssDNA constructs with 2-AP dimer probes located at different positions.*** The theoretical

curves were calculate using the Epstein finite lattice model (2), and both the theoretical and the experimental fluorescence intensity changes of the dimer probes for ssDNA constructs at 1μM concentration were plotted as a function of gp32 monomers present per ssDNA lattice. The plots are designated with the following colors and symbols: $_{25}B_{2,3}$ construct (experimental values: black, closed circles, solid line; theoretical values: black, open circles, dashed line); $_{25}B_{10,11}$ construct (experimental values: red, closed circles, solid line; theoretical values: red, open circles, dashed line) and $_{25}B_{23,24}$ construct (experimental values: green, closed circles, solid line; theoretical values: green, open circles, dashed line). The error bars for the experimental data (closed circles) are the same as in Figure 1B and are not included here to make it easier to visualize the close correspondence between the experimental and the theoretical values.

References

(1) McGhee, J.D. and von Hippel, P.H. (1974) Theoretical aspects of DNA-protein interactions: co-operative and non-co-operative binding of large ligands to a one-dimensional homogeneous lattice. *Journal of Molecular Biology*, **86**, 469-489.

(2) Epstein, I. R. (1978) Cooperative and non-cooperative binding of large ligands to a finite one-dimensional lattice. A model for ligand-oligonucleotide interactions. *Biophysical Chemistry* **8**, 327-339.

(3) von Hippel, P.H., Kowalczykowski, S.C., Lonberg, N., Newport, J.W., Paul, L.S., Stormo, G.D. and Gold, L. (1982) Autoregulation of gene expression. Quantitative evaluation of the expression and function of the bacteriophage T4 gene 32 (single-stranded DNA binding) protein system. *Journal of Molecular Biology*, **162**, 795-818.