SUPPORTING INFORMATION

Single Molecule Detection of Transcription Factor Binding to DNA in Real Time: Specificity, Equilibrium and Kinetic Parameters

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CONTENTS:

SUPPORTING MATERIALS AND METHODS

Coincident event counting and cross-correlation analysis- In coincident event counting, the following were tabulated directly from the trace files:

RedOnly ≡ fraction of bins containing ≧ 1 photon count in the Red (DNA) channel but containing 0 in the Blue (protein) channel

BlueOnly ≡ fraction of bins containing ≧ 1 photon count in the Blue channel but containing 0 in the Red channel

Blank ≡ fraction of bins containing 0 in both Red and Blue channels

CoincidentBins ≡ number of bins containing ≧ 1 photon count in both Red and Blue channels

As described (*17*), the number of random coincident events (RandomCoinBins) was estimated from these values as:

RandomCoincidentBins = N × RedOnly × BlueOnly / Blank

where N represents the total number of bins in the trace files. The number of non-random coincident bins (Non-RandomCoincidentBins), which represents the number of protein-DNA complexes detected, was estimated as:

Non-RandomCoincidentBins = CoincidentBins – RandomCoincidentBins

Results were normalized for run time and presented as the number of bins per second (bins/s).

Spatial cross-correlation coefficients (Φ) of data trace files were determined using a standard formula for the normalized product:

$$\mathbf{\Phi(m)} \equiv \frac{\sum_{i}\left(x_i - \bar{x}\right)\left(y_{i+m} - \bar{y}\right)}{\sqrt{\sum_{i}\left(x_i - \bar{x}\right)^2 \sum_{i}\left(y_{i+m} - \bar{y}\right)^2}}$$

where x_i represents the fluorescence intensity in the ith bin of the Red channel, $\bar{x}$ the average, y_i the intensity in the ith bin of the Blue channel offset by m bins, and $\bar{y}$ the average.