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The transcription bubble of the RNA polymerase-promoter open complex exhibits conformational heterogeneity and millisecond-scale dynamics: implications for transcription start-site selection

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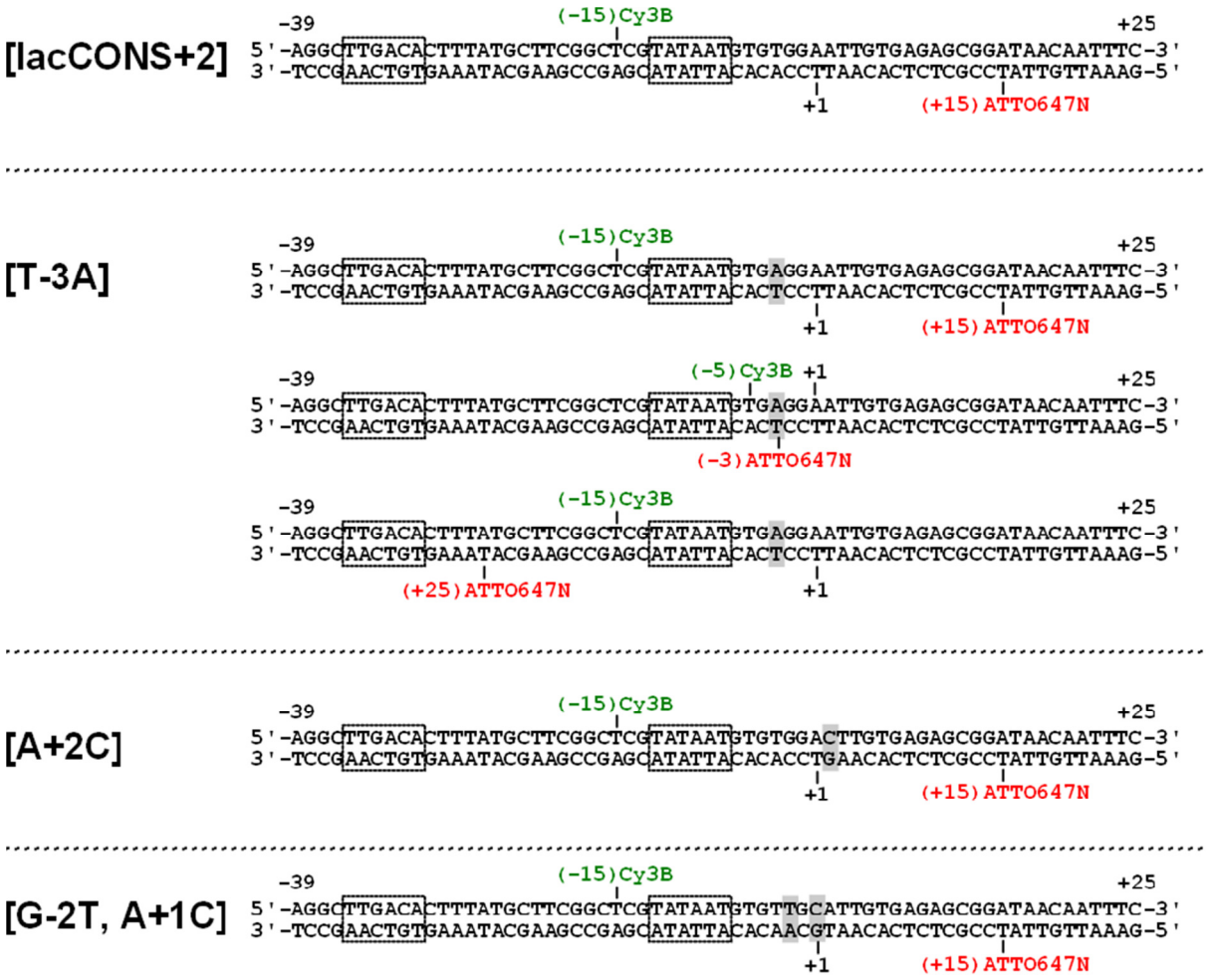

Figure S1. List of the DNAs and the fluorescent dye labelling schemes used. The donor dye Cy3B is shown in green and the acceptor dye ATTO647N is shown in red. Base pair substitutions changed from the *lacCONS+2* sequence are highlighted in grey.

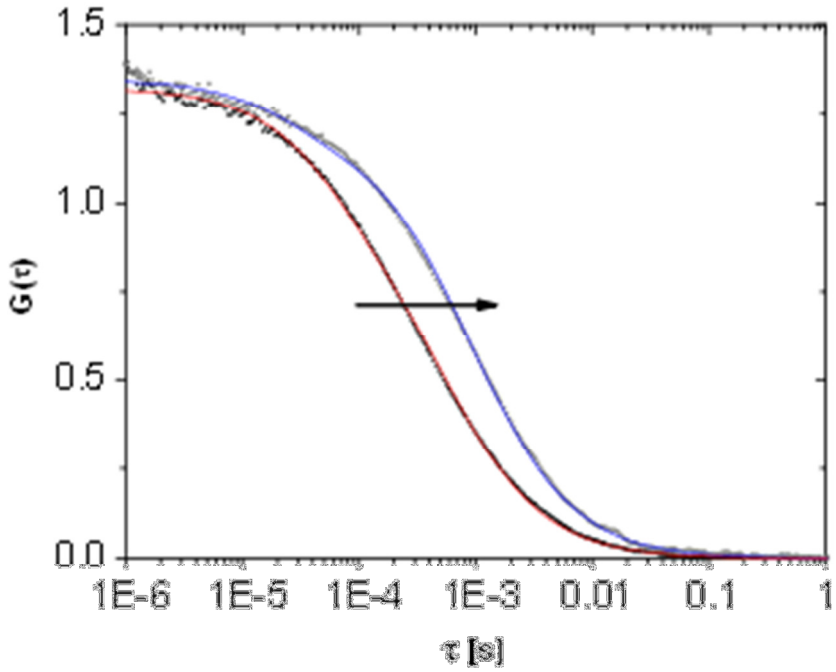

Figure S2. Fluorescence correlation spectroscopy (FCS) verifying that the FRET histogram for RP_o with dsDNA labelled at positions -15 and -25 represents a substantial amount of RNAP complex formation. FCS curve of the *lacCONS+2*(T-3A) dsDNA promoter alone (red line) was compared to that of RP_o (blue line).

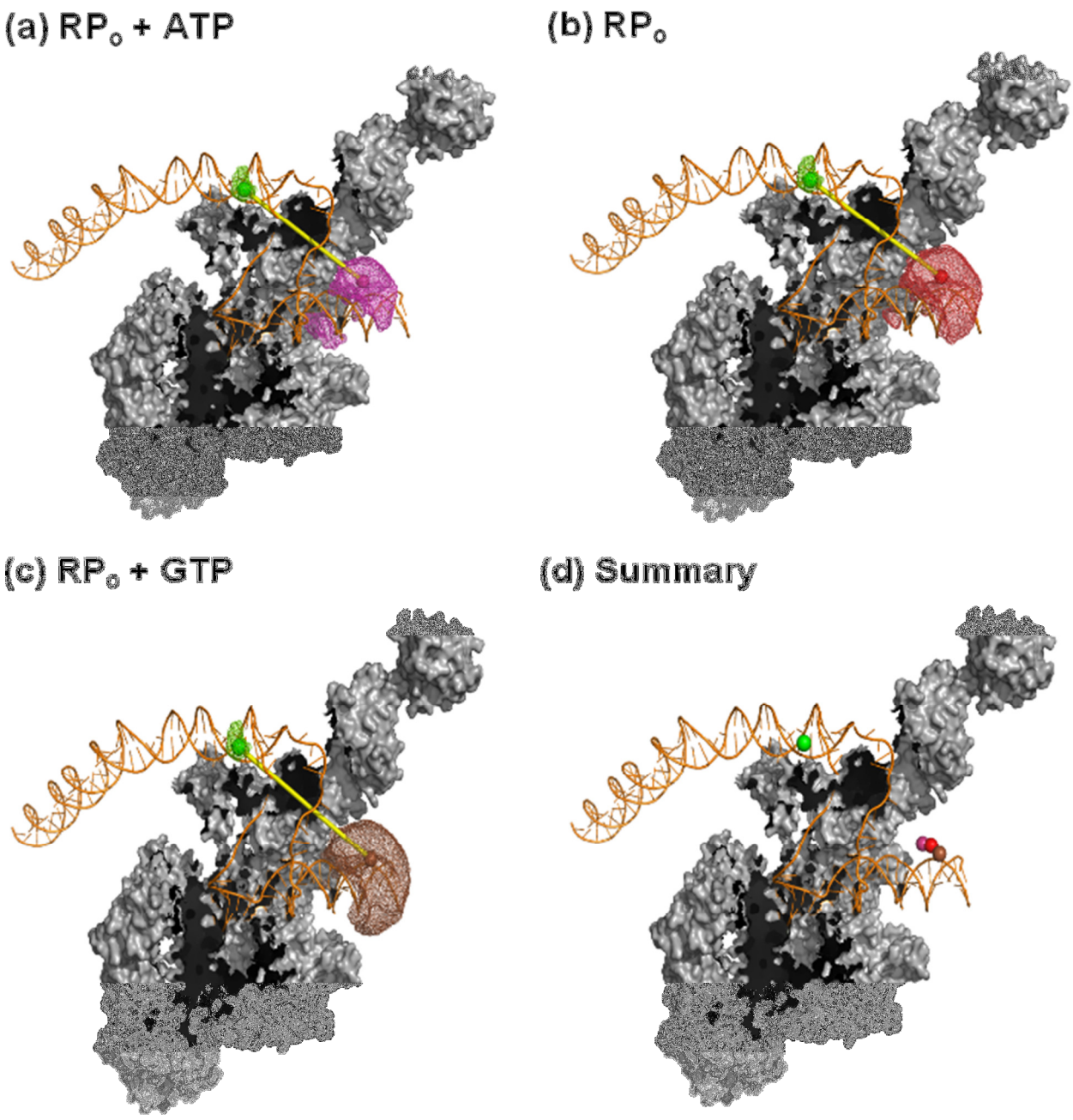

Figure S3. Distances between dye positions for Figure 4(a). The distances were measured using an RP_o structure[36; 37] modeled with downstream DNA. (a) Accessible volumes of the donor dye at position -15 on the DNA (green mesh) and the estimated acceptor dye position (purple mesh) when ATP was added were calculated[38]. The accessible volumes were then approximated by a 3D Guassian, the centre of which was taken as the average position of the dyes (green and purple spheres). The distance (represented by the yellow line) between the two average dye positions was measured to be 71.6 Å. (b) Accessible volumes and average positions of the donor dye at -15 on the DNA (green sphere and mesh) and the acceptor dye at position +15 on the DNA (red sphere and mesh) in RP_o. The distance between the two average dye positions was measured to be 73.3 Å. (c) Accessible volumes and average positions of the donor dye at position -15 on the DNA (green sphere and mesh) and the estimated acceptor dye position (brown sphere and mesh) when GTP was added. The distance between the two average dye positions was measured to be 76.0 Å. (d) Model showing all three relative acceptor dye positions (RP_o+ATP – purple sphere, RP_o – red sphere, RP_o+GTP – brown sphere).