Figure Legends

Figure 1. *HIS4* promoter templates tethered to FeBABE support transcription

(a) 17 DNA-FeBABE probes were derived from the *HIS4* promoter with FeBABE attached to the non-template strand. 11 FeBABE-tethered probes were also derived from *HIS4* with FeBABE attached to the template strand (**Supplementary Fig. 1a**). Positions of FeBABE conjugation are indicated by the red dots. The major sites of transcription initiation at +1 and +12 are indicated by arrows, and the upstream and downstream bases are numbered with respect to +1. For comparison with the results of studies in the human system, the analogous numbering of the adenovirus major late promoter (AdMLP) is shown, based on alignment of TATA sequences and the site of transcription initiation in the human system. The TATA element is highlighted in blue and the position of the transcription bubble region in the human system is highlighted in red. **(b)** *In vitro* transcription activity using yeast nuclear extract from a subset of these promoters, in the absence (–) or presence (+) of tethered FeBABE, is shown.

Figure 2. Directed hydroxyl radical probing of RNA Polymerase II subunit Rpb1

(a) Cleavage fragments of Rpb1, triple Flag tagged at the C terminus (Rpb1-Flag) were visualized by Western Blotting with either anti-Flag antibody or **(b)** anti-Rpb1_N200 antibody that recognizes the first 200 residues of Rpb1. All 17 probes and a control probe containing no phosphorothioates (Control) were conjugated to FeBABE and used in PIC formation/ hydroxyl radical probing. Negative controls include control +FeBABE, probe 6 -FeBABE +H_2O_2, and probe 6 +FeBABE –H_2O_2. In (**a**) left hand blot, SDS-PAGE was carried out in MOPS NuPAGE buffer (Invitrogen) whereas for the 2 blots on the right hand side, SDS-PAGE was carried out in MES NuPAGE buffer (Invitrogen). In (**b**) cleavage products in the lower molecular weight range are shown with higher sensitivity due to their lower intensity (lower panel). The arrows point to full length Rpb1. Specific cleavage fragments are indicated by brackets and labeled to indicate the region of the protein cleaved. * Indicates non-specific polypeptide detected by antibodies. WT, wild type nuclear extract.

Figure 3. Directed hydroxyl radical probing of RNA Polymerase II subunit Rpb2
Cleavage fragments of Rpb2-Flag were visualized by Western Blotting with anti-Flag antibody as described in **Figure 2**. The arrow points to full length Rpb2. Specific cleavage fragments are indicated by brackets and labeled to indicate the region of the protein cleaved. P/L, Protrusion/Lobe domain. * Indicates non-specific polypeptides detected by antibodies. WT, wild type nuclear extract.

Figure 4. Model for the path of promoter DNA in the Preinitiation Complex
(a) Left hand panel: Hydroxyl radical cleavage sites of Rpb1 and Rpb2 were mapped to the surface of the complete Pol II crystal structure[12,13] from data in **Figures 2** and **3**. Right hand panel: Same as in left hand panel but rotated clockwise by 40^0 as indicated. (**b**) A model for the predicted path of *HIS4* promoter DNA within the Preinitiation Complex (two views, rotated by 40^0). TBP and TFIIBc were fitted into the complex based on the crystal structure of human TFIIBc-TBP-TATA box complex[33] , hydroxyl radical mapping of TFIIB to RNA Pol II[25], and based on data from this study (**Supplementary Fig. 4**). TFIIB and TBP are shown as yellow (magenta sphere, zinc) and green backbone models, respectively. The DNA non-template strand is colored pink, and the template stand is colored light blue. DNA base pair -12 (numbering based on the AdMLP), the site of DNA strand melting, is colored red for the template strand and purple for the non-template strand, respectively.

Figure 5. Hydroxyl radical probing of TFIIH subunit Rad25
(a) FeBABE-DNA cleavage assay in PICs using nuclear extract containing Rad25-Flag. Rad25 cleavage fragments were visualized by Western blotting with anti-Flag antibody. The arrow points to full length Rad25 and specific cleavage fragments are indicated by the brackets and labeled as to which region in the protein is cleaved. * Indicates non-specific polypeptides detected by antibodies. WT, wild type nuclear extract. **(b)** Schematic indicating Rad25 helicase domains based on sequence alignment (**Supplementary Fig. 6**) and summary of cleavage sites. DRD, damage recognition domain **(c)** Hydroxyl radical cleavage sites were mapped to a structure model of the Rad25 helicase domain. A nine-residue segment centered on the calculated site is colored

dark blue (strong/medium cleavage) and cyan (weak cleavage). The red arrow indicates the suggested path of promoter DNA, where DNA close to the transcription bubble is nearer the start of the arrow and downstream DNA (closer to the yeast transcription start site) is nearer the arrowhead.

Figure 6. Directed hydroxyl radical probing of TFIIF subunits Tfg1 and Tfg2
(a) Cleavage fragments of Tfg1-Flag were visualized by Western blotting with anti-Flag antibody. Arrow points to full length Tfg1 and specific cleavage fragments are indicated by the brackets and labeled as to indicate the region of the protein that is cleaved. C, control probe. * Indicates non-specific bands detected by antibodies. **(b)** Schematic indicating Tfg1 domains[39], arrows indicate cleavage sites. **(c)** Cleavage fragments of Tfg2-Flag were visualized by western blotting with anti-Flag antibodies, as in (a). **(d)** Schematic indicating Tfg2 domains[39] and arrows indicate the cleavage sites.

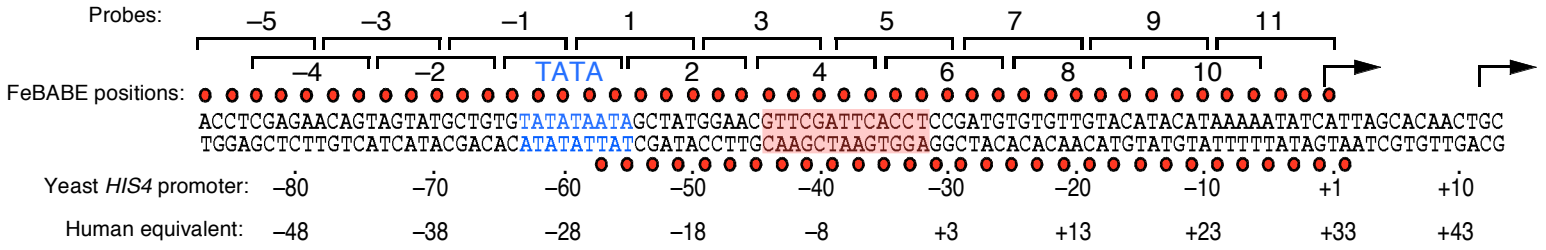
Figure 7. Directed hydroxyl radical probing of TFIIE subunits Tfa1 and Tfa2
(a) Cleavage fragments of Tfa1-Flag were visualized by Western blotting with anti-Flag antibody. Arrow points to full length Tfa1 and specific cleavage fragments are indicated by the brackets and labeled as to indicate the region of the protein that is cleaved. C, control probe. * Indicates non-specific bands detected by antibodies. **(b)** Schematic indicating Tfa1 domains[53] and arrows indicate cleavage sites. **(c)** Cleavage fragments of Tfa2-Flag were visualized by Western blotting with anti-Flag antibodies, as in (a). **(d)** Schematic indicating Tfa2 domains and arrows indicate the cleavage sites.

Figure 8. Summary of hydroxyl radical cleavage of Pol II and GTFs within the Preinitiation Complex
(a) Summary of the results of hydroxyl radical cleavage where FeBABE was tethered to the non-template strand of the *HIS4* promoter. Results of hydroxyl radical cleavage from FeBABE attached to the template strand are nearly identical. Pol II subunits Rpb1 and Rpb2 are highlighted in blue, TFIIF subunits Tfg1 and Tfg2 are highlighted in orange, TFIIE subunits Tfa1 and Tfa2 are highlighted in purple, TFIIH subunit Rad25 is highlighted in red and TFIIB is highlighted in yellow. **(b-d)** DNA probes resulting in

cleavage of TFIIF, TFIIE, and Rad25 are color-coded. **(b)** Left panel: Model of PIC structure (**Fig. 4a**) where DNA in proximity to Tfg1 is colored orange. Right panel: same as left panel but rotated by 40^0 as indicated **(c)** Same as (**b**) except DNA in proximity to Tfg2 is colored orange. **(d)** Same as (**b**) except DNA in proximity to Rad25 and TFIIE is colored red and purple respectively. DNA base pair –12 (numbering based on the AdMLP), the site of DNA strand melting, is colored red for the template strand and black for the non-template strand.

Figure 1 Miller and Hahn

a



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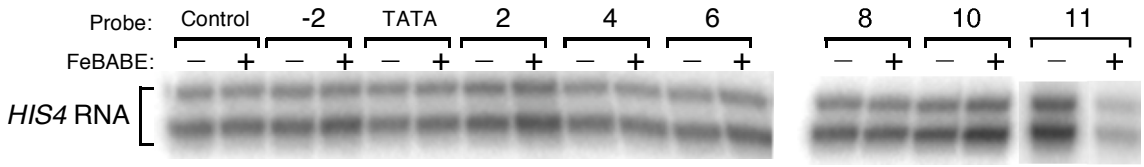


Figure 2 Miller and Hahn

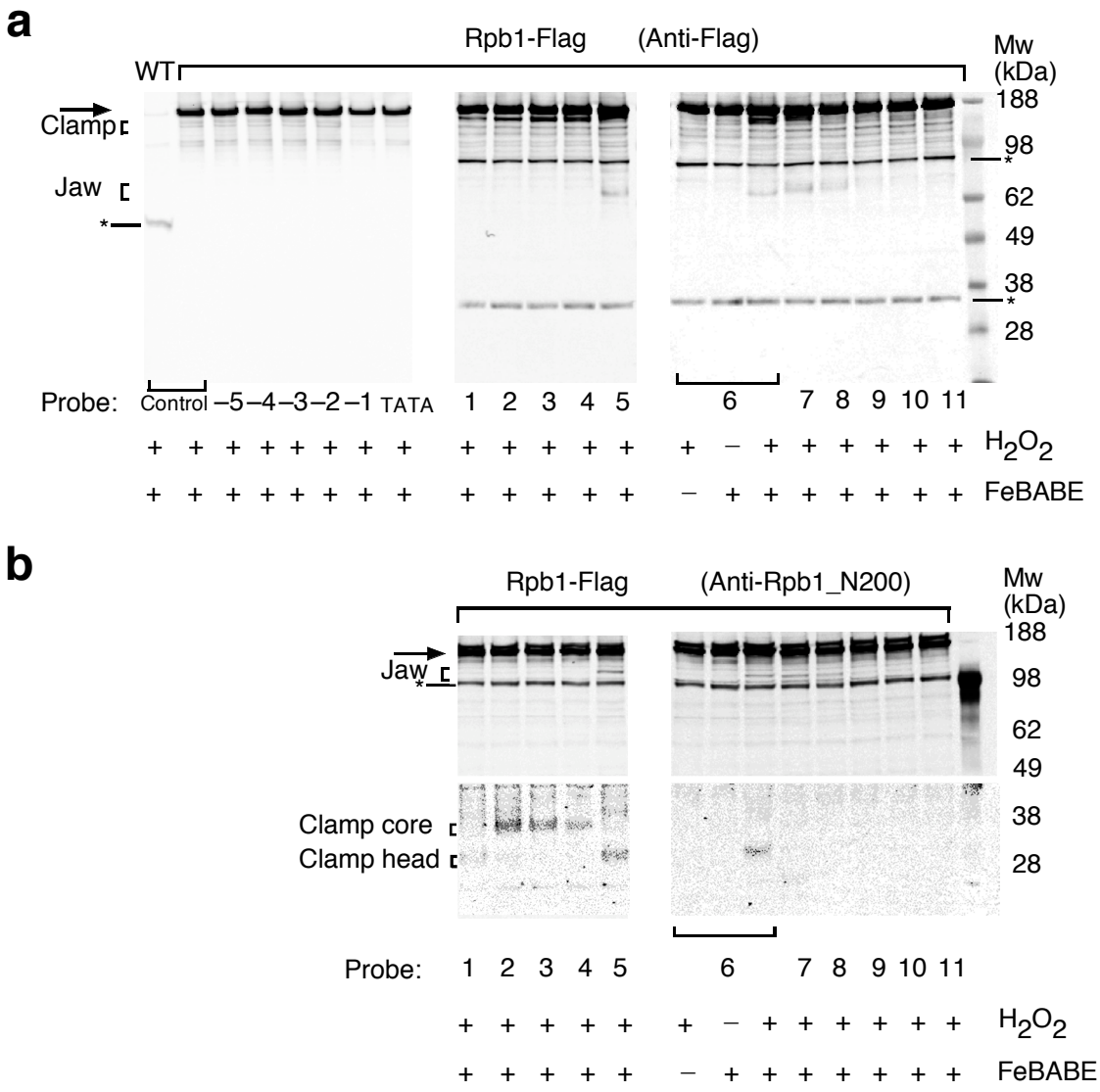


Figure 3 Miller and Hahn

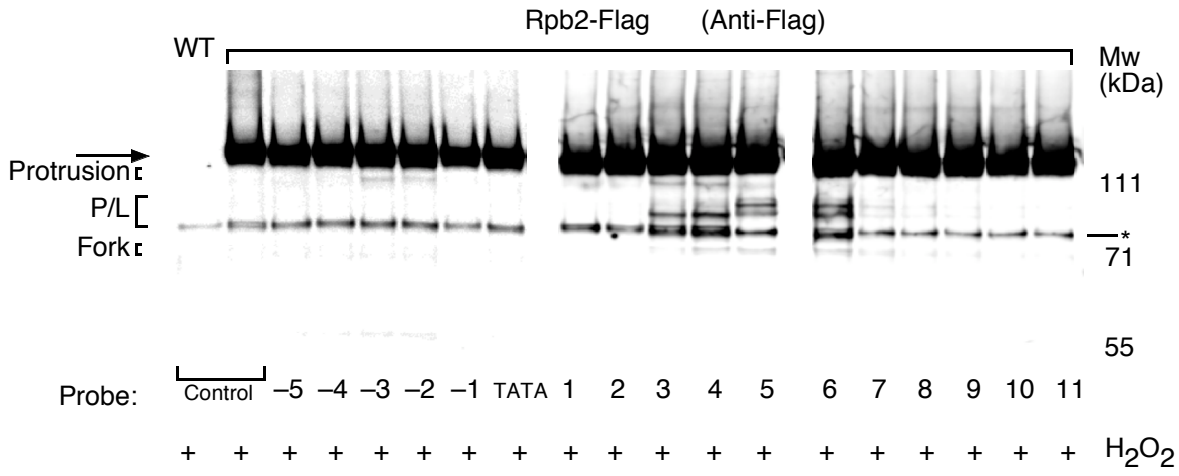


Figure 4 Miller and Hahn

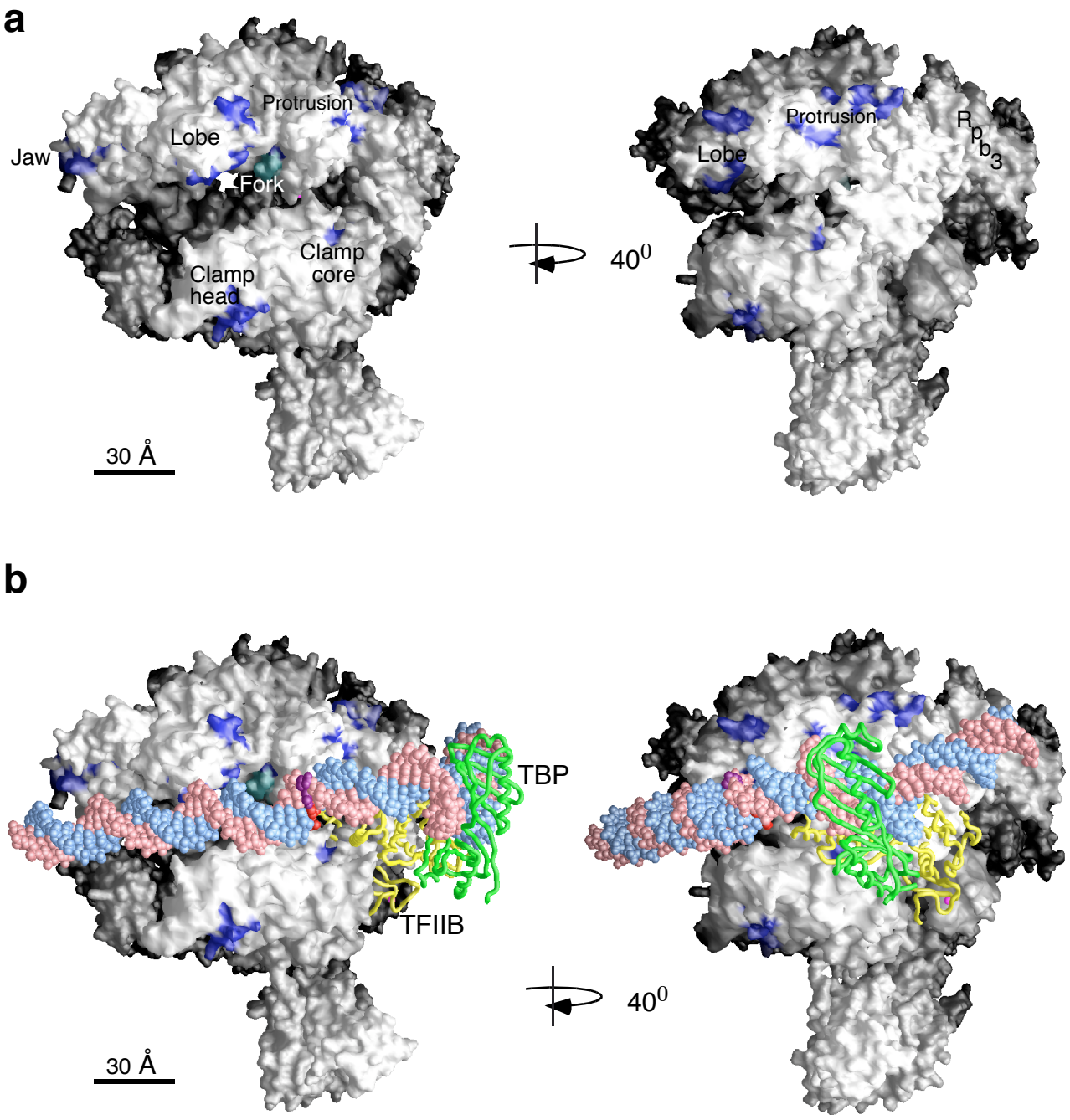
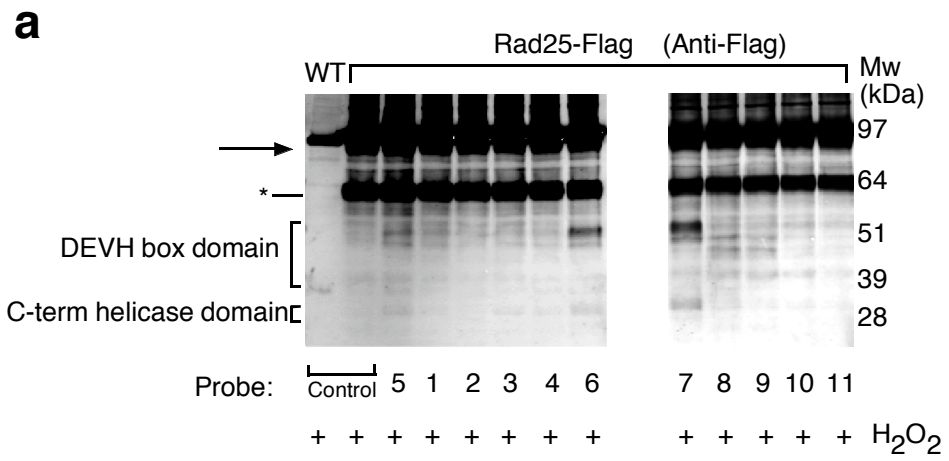
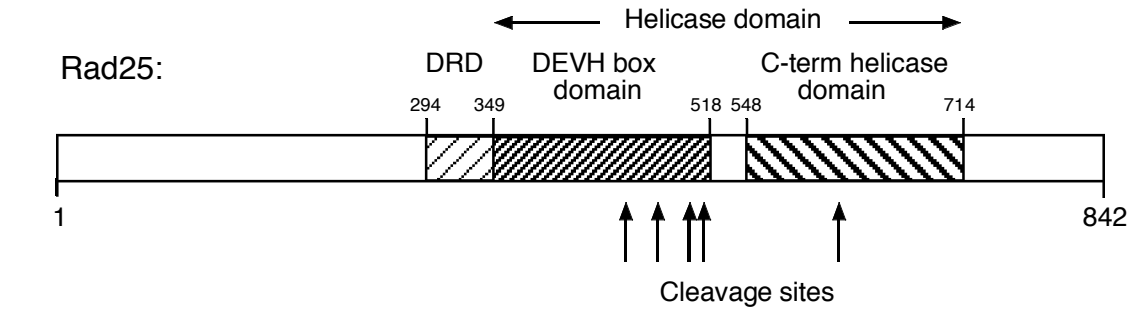


Figure 5 Miller and Hahn





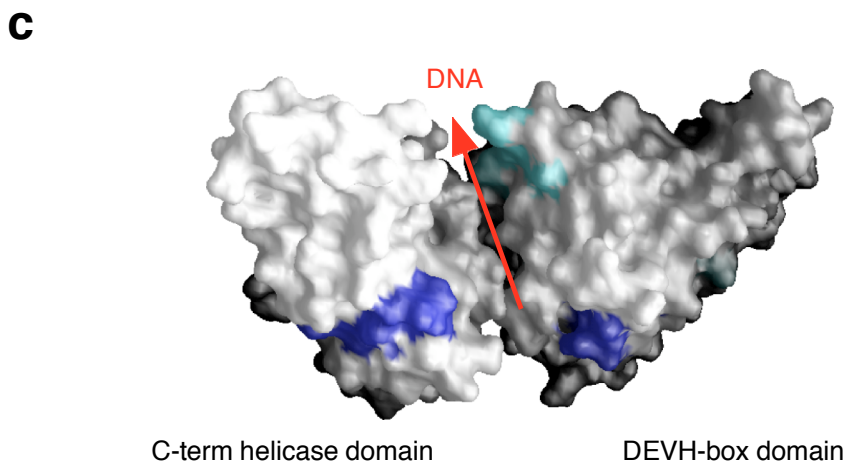


Figure 6 Miller and Hahn

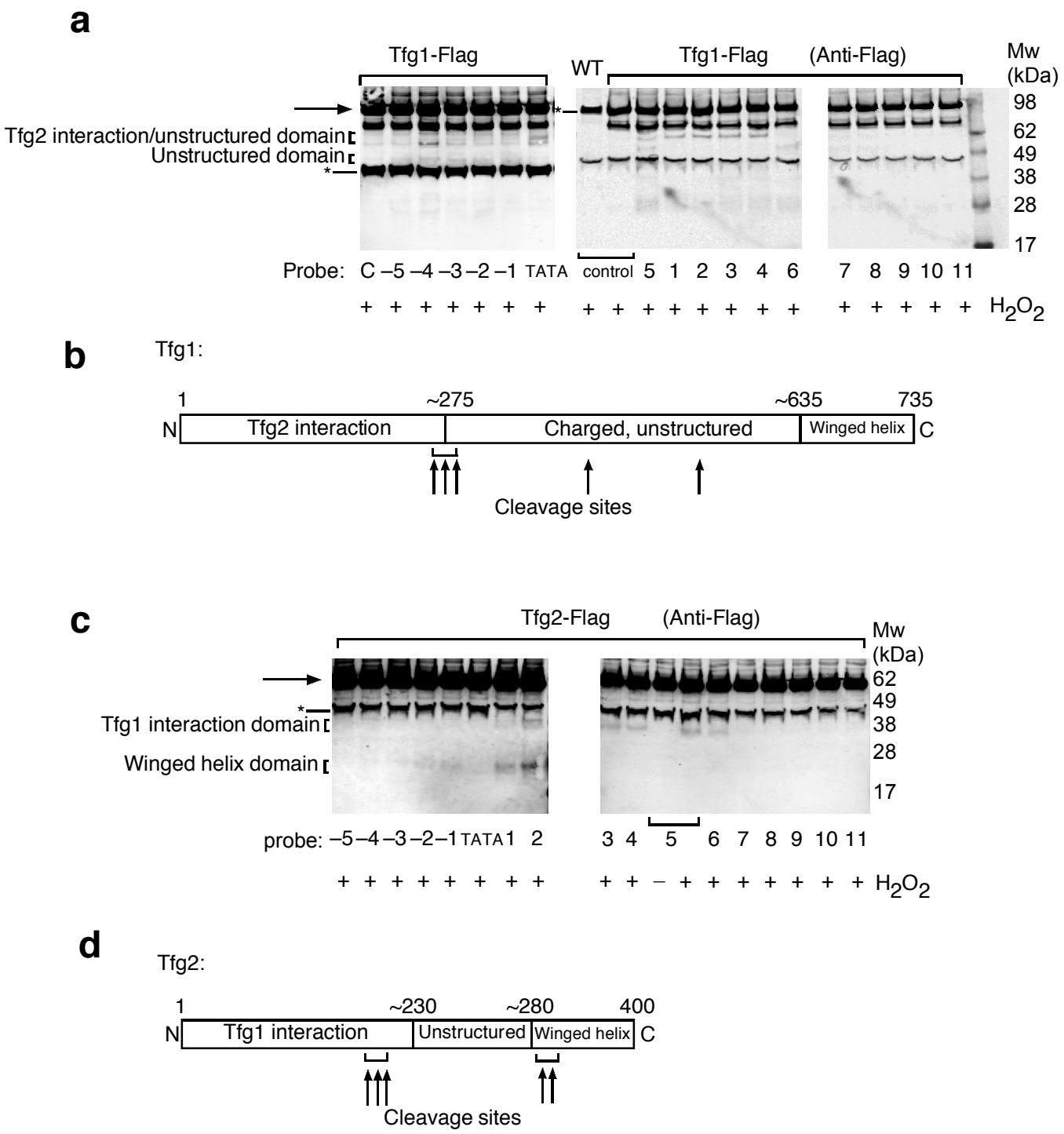


Figure 7 Miller and Hahn

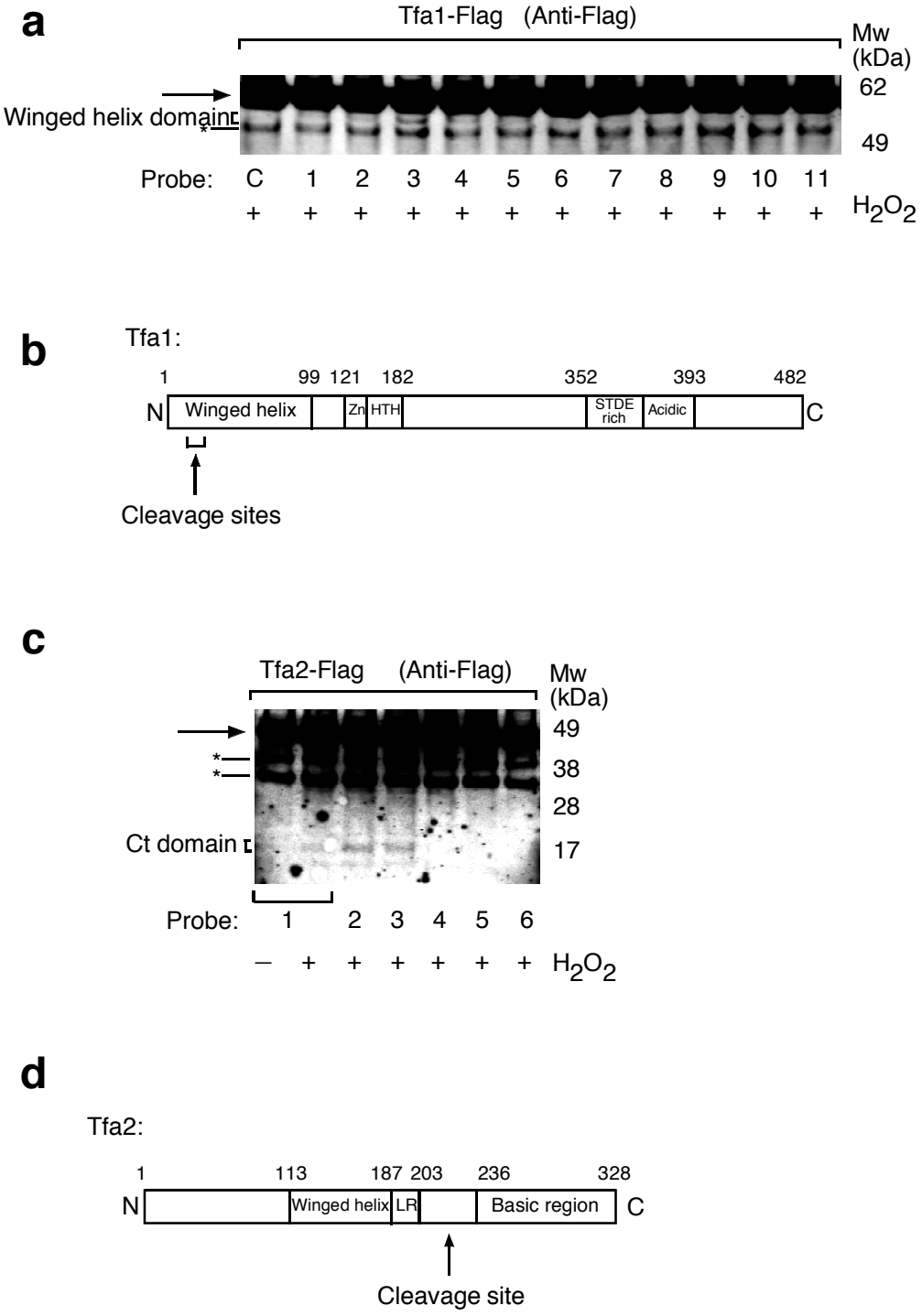


Figure 8 Miller and Hahn