between the full length (TFAM) and the CTD deletion (TFAM-dCT). TFAM-d10CT was able to support transcription in our *in vitro* assays. The Last 10 amino acids contribute to DNA binding by 3-fold, while the whole CTD contributes 30-fold.

**TC-TFAM-DNA Binding Characterization:** As depicted in Figure 2b, to study the determinants of binding specificity within the DNA, it is useful to incorporate the fluorescent label into the protein. To do so, we introduced a tetra cystein (TC) motif in the N-terminal region of TFAM. The TC motif can then conjugate the fluorescent dye, FlAsH. It was important to confirm that TFAM function has not been affected by this modification. This was also determined using FP in which TC-TFAM was titrated.

TC-TFAM binds with relatively tight affinity with a *Kd* of 3 nM (Figure 6). This result is comparable to the one obtained with wild-type TFAM and indicates that the addition of the TC motif does not impact DNA binding. This finding is in agreement with our results, showing that TC-TFAM functions as TFAM in transcription assays *in vitro*.

**Discussion**

The present work illustrates the use of FP to study TFAM binding to its DNA cognate. Here we showed that the carboxy-terminal domain has a critical role in DNA binding, explaining previous observations that TFAMdCT does not support