DATA SUPPLEMENT

Antibody–targeted chromatin enables effective intracellular delivery and functionality of CRISPR/Cas9 expression plasmids

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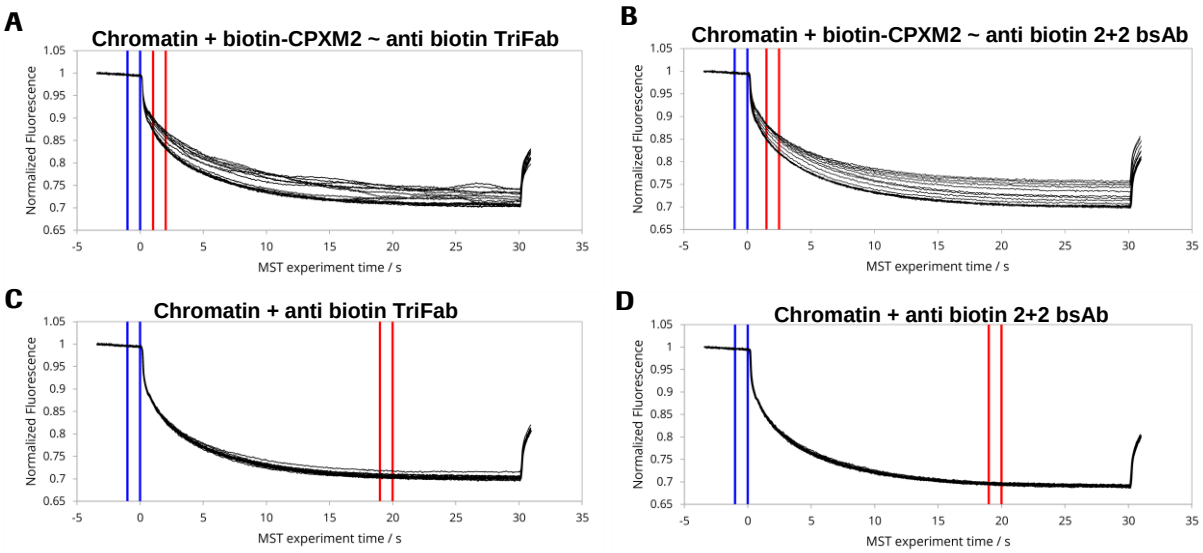

Figure S1 MST traces of monovalent anti biotin TriFab and bivalent anti biotin 2+2 bsAb constructs and controls. A *Chromatin + biotin-CPXM2 ~ anti biotin TriFab interaction.* ***B*** *Chromatin + biotin-CPXM2 ~ anti biotin 2+2 bsAb interaction.* ***C-D*** *Antibody only control measurements without biotin-CPXM2 peptide but with anti biotin TriFab (C) and anti biotin 2+2 bsAb (D); MST traces show no aggregation or precipitation effects with anti biotin 2+2 bsAb and just minor aggregation with anti biotin TriFab. Area within blue lines determines the fluorescence before activation of the infrared laser and area within blue lines determines average fluorescence after activation of the IR-laser. Average values were subsequently used for fluorescence normalization.*

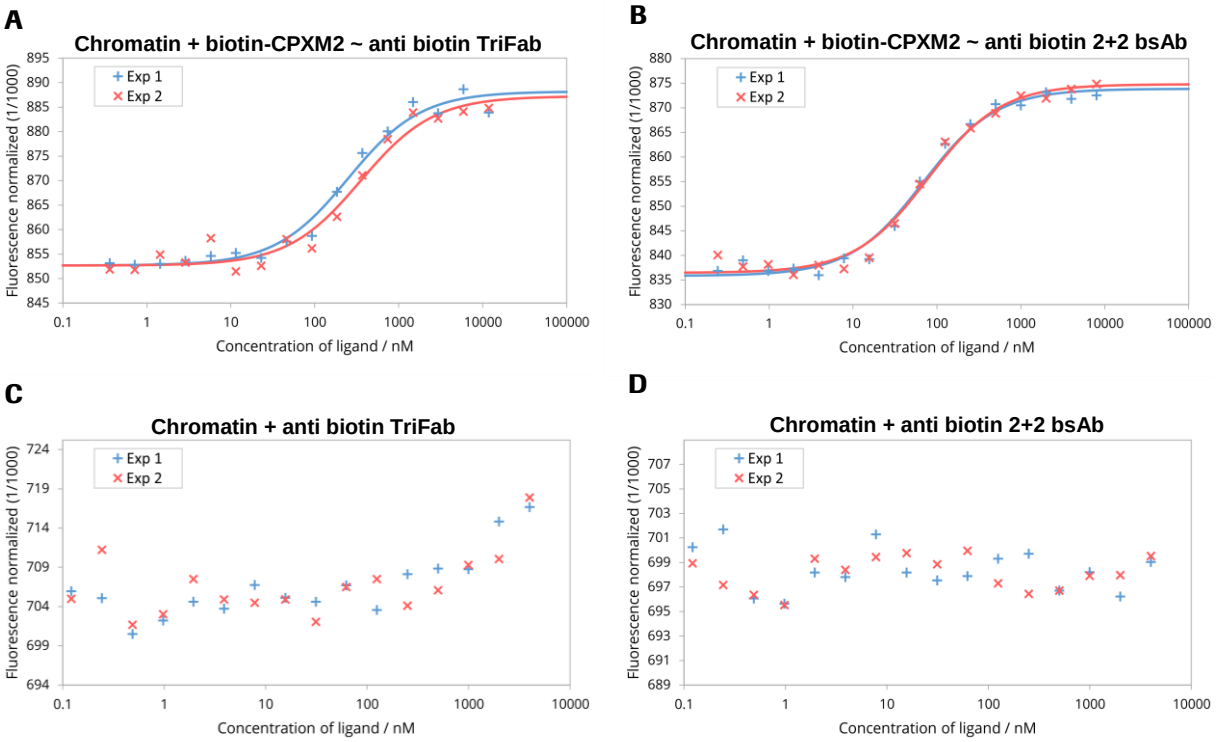

Figure S2 MST runs of monovalent anti biotin TriFab and bivalent anti biotin 2+2 bsAb constructs and controls. ***A*** *Chromatin + biotin-CPXM2 ~ anti biotinTriFab interaction; Ligand concentration refers to biotin-CPXM2 peptide (twice as much as the respective anti biotin 2+2 bsAb concentration).* ***B*** *Chromatin + biotin-CPXM2 ~ anti biotin 2+2 bsAb interaction; Ligand concentration refers to biotin-CPXM2 peptide, but is equal to the respective anti biotin TriFab concentration.* ***C-D*** *Antibody only control measurements without biotin-CPXM2 peptide but with anti biotin TriFab (C) and anti biotin 2+2 bsAb (D); In contrast to peptide containing constructs, runs with antibody only controls did not show a concentration dependent increase in fluorescence, demonstrating that no unspecific interaction between antibody and chromatin occurs. Exp 1 (blue) and Exp 2 (red) are independent experiments of the same construct with the respective curve fit for K_D determination if applicable.*

METHODS: Microscale thermophoresis experiments, data processing and determination of K_D values was performed by 2bind GmbH (Regensburg). Antibody and peptide were diluted in PBS and pre-incubated for 30 mins at RT with a 1 : 1 or 1 : 2 molar ratio for TriFab : peptide or 2 + 2 bsAb : peptide, respectively. A serial dilution of the ligand was prepared in a way to match the final buffer conditions in the reaction mix (1x PBS, 0.05 % Tween-20). 5 µl of each dilution step were mixed with 5 µl of fluorescent labelled plasmid chromatin. The final reaction mixture, which was filled in capillaries, contained a respective amount of ligand and constant 0.25 nM fluorescent molecule. The samples were analysed on a Monolith NT.115 Pico at 25 °C, with 10 % LED power and 60 % Laser power. Fluorescence values were normalized and data were displayed according the analysed peptide concentration (1). K_D values were determined, if normalized fluorescence values allowed a proper curve fit.

(1) Jerabek-Willemsen, M., André, T., Wanner, R., Roth, H.M., Duhr, S., Baaske, P. and Breitsprecher, D. (2014) MicroScale Thermophoresis: Interaction analysis and beyond. Journal of Molecular Structure, 1077, 101-113.