

- A) Refolded, full-length A30 but not minimal A30 can assume alternative association or folding states which are less pronounced for 4-thioU substituted A30. Ethidium bromide staining of purified and refolded A30, A30^T, and mA30. Aptamers were boiled for 5 minutes in 50 mM Tris, 150mM NaCl, and allowed to refold at room temperature for 15 minutes. Samples were run on a 2.5% agarose gel in 1x TBE (90 mM Tris, 90 mM boric acid, 2 mM EDTA) at room temperature at 50V.
- **B)** Modified and unmodified aptamers show a comparable affinity to soluble ECD in gel shift studies. The percentage of 5' ³²P-labeled aptamers (50 pM) that are shifted in a native gel mobility shift assay and analyzed by densitometry is indicated as a function of the concentration of soluble ERBB3-ECD.
- **C)** A30 and A30^{BT} compete for the same binding site on ERBB3-ECD. The displacement of 4-thioU substituted and biotin carrying A30 (A30^{BT}) by unmodified A30 was measured by the efficiency of crosslinking at different concentrations of competing A30, shown previously to have a KD for soluble ECDs of 45nM (6). Crosslinked and Streptavidin-enriched ECDs were analyzed by Western blotting and quantitated by densitometry.
- **D)** A30 and A30^T show comparable potency in the inhibition of ERBB2/ERBB3 signaling in MCF7 cells. MCF7 parental cells were treated with 10nM of a recombinant thioredoxin neuregulin fusion construct for 10 minutes in the presence or absence of 100nM A30 (A) or A30^T (A^T), as indicated above lanes. The increase in tyrosine phosphorylation is shown as the readout.