A Novel Therapeutic Approach For Triple-Negative Breast cancer



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Nothing to Disclose

Breast cancer is classified into clinical subtypes based upon receptor expression

These subtypes dictate possible therapeutic options and vary in their prognosis

Luminal: derived from the luminal cells

ER^+, PR^+, HER^+

Can use hormonal therapy

Basal: derived from myoepithelial cells

ER^-, PR^-, HER^-

No specific target for therapies

TNBC accounts for 10-20% of all breast cancer, but much higher proportion of all breast cancer mortality.

Younger age at diagnosis, high grade, large tumor size, aggressive relapse.

High proliferation, poor differentiation, and aggressive clinical course with early relapse and decreased survival.

There is no specific therapeutic target for TNBC, and therefore must use cytotoxic chemotherapeutics, surgery, radiation.

Current Options for TNBC

Standard course of treatment is very aggressive: surgery and radiation therapy combined with adjuvant and neo-adjuvant chemotherapy.

Chemotherapy typically includes combinations of taxanes, anthracyclines, and oxazophorines.

The search is on for specific therapeutic targets!!!

It has previously been reported that because of the anti-apoptotic activity of glucocorticoid receptor (GR) in estrogen receptor (ER)-negative breast epithelial and cancer cells, high GR expression/activity in early-stage TNBC significantly correlates with chemotherapy resistance and increased recurrence.

A recent study has shown that pre-treatment with mifepristone/RU486, a GR antagonist, potentiates the efficacy of chemotherapy in TNBC by inhibiting the anti-apoptotic signaling pathways of GR and increasing the cytotoxic efficiency of chemotherapy.

These results suggest that blocking GR activity could be a useful strategy for increasing tumor cell apoptosis in TNBC.

Clin. Cancer Res. 19, 6163–72, 2013

Another recent study showed that a peptide containing LXXLL motif of steroid receptor coactivator-1 (SRC-1) can severely reduce the viability and proliferation of hormone-unresponsive breast cancer MDA-MB-231 cells. (*Int. J. Mol. Sci.* 2014, *15*, 5680-5698)

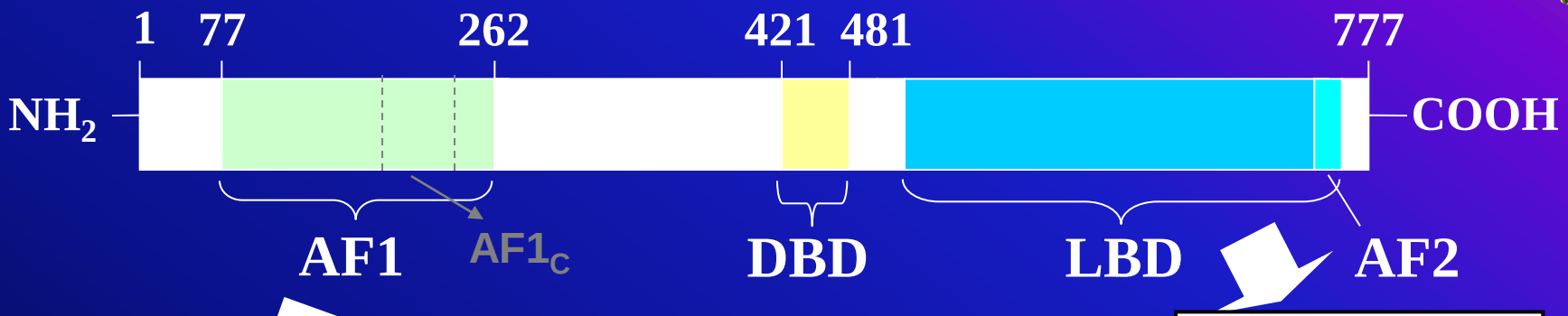
SRC-1 is known to function as a coactivator for steroid hormone receptors including GR.

These results suggest that blocking GR/SRC-1 interaction may be an effective drug candidate in the treatment of TNBC.

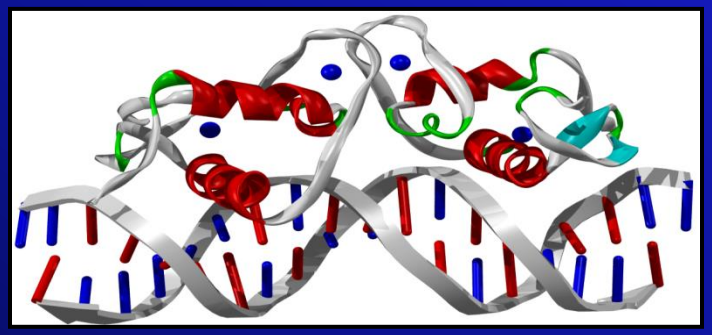
When viewing steroid receptors (e.g., GR) as therapeutic targets, the challenge is how to selectively control cell/tissue and gene specificity in a manner that affects only deleterious actions of receptor in diseased tissues without altering essential normal functions.

One attractive, but limited, approach is the development of selective steroid receptor modulators (SRMs) that regulate a subset of the normal gene repertoire. SRMs have the clinically useful but enigmatic property of evoking anywhere from full agonist to full antagonist activity in a gene/tissue-dependent manner. Thus SRMs can display between 100% and 0% efficacy.

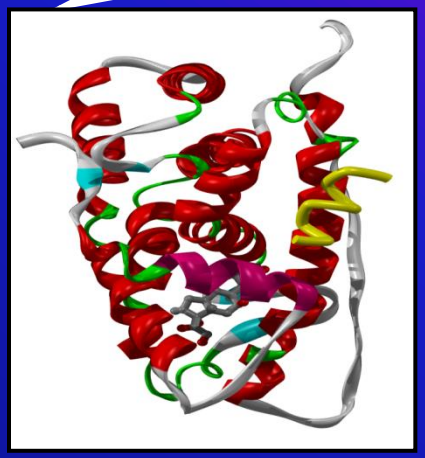
Human Glucocorticoid Receptor (GR)







Nature 352 (1991) 497-505.

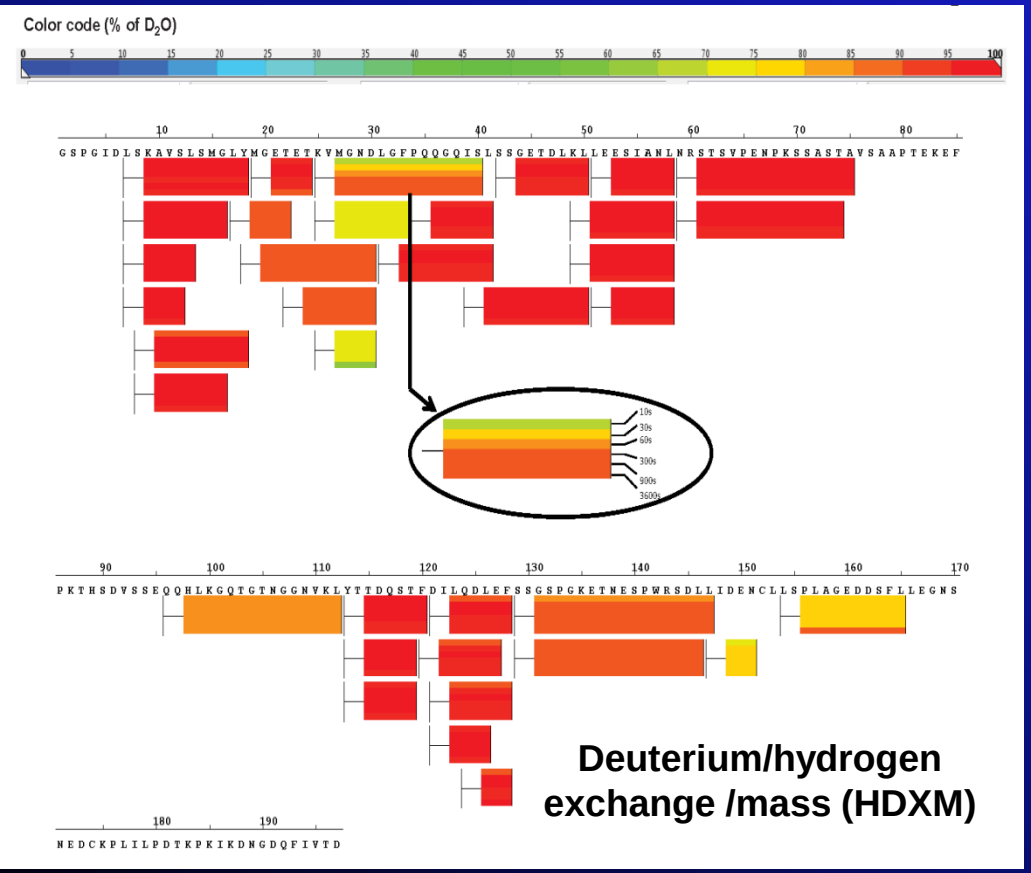


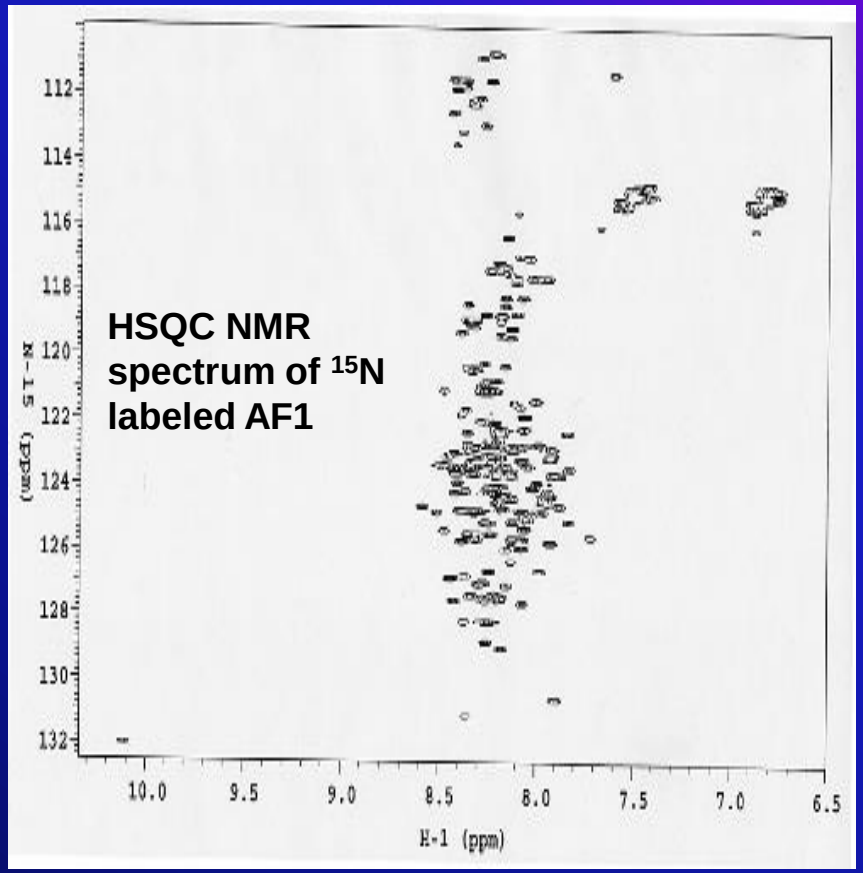
Cell 110 (2002) 93-105

The "Holy Grail" for hormone therapies is to target full SR signaling in a receptor- and tissue/gene-selective manner to maximize therapeutic benefits and to minimize effects on other targets. However, due to available LBD/AF2 crystal structures, the current design of small molecule selective receptor modulators (SRMs) for clinical uses is primarily based on their modulation of AF2 activity, which often fail to inactivate AF1, and thereby missing the whole SR spectrum during endocrine-based therapies. This is despite the fact the most of cell/tissue-specific SRM activities are AF1-dpependent.

The AF1/NTD of all the SHRs studied so far have been found to exit in an intrinsically disordered (ID) conformation.

Experiments showing intrinsically disordered AF1 conformation





Similar results are also obtained using several other biophysical methods such as CD and FTIR

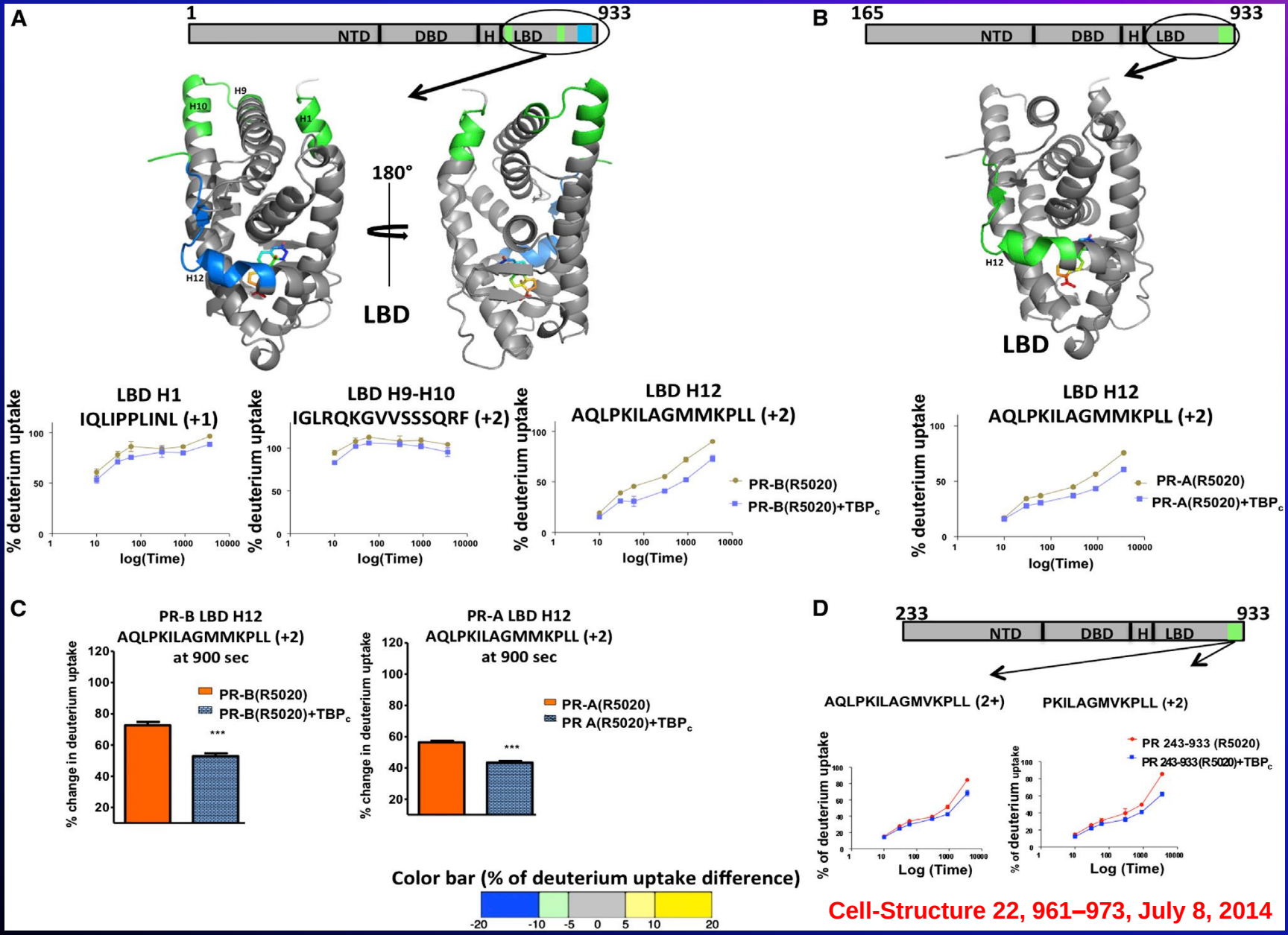
Conformational flexibility in IDs allows it to interact with binding partners with high specificity and low affinity that means on one hand these provide specific interactions at the same time allow them to be easily dispersed when need arises, an important function of steroid receptors to turn on or turn off signals of target genes as required.

ID proteins normally undergo “disorder-to-order” transition upon interactions with their target molecule(s).

Like C-terminal AF-2, AF1 interacts with several specific cofactor proteins including several coactivators

Question: Does GR AF1 adopt a functionally active ordered structure when bound to a binding partner protein such that its interaction with SRC-1 is facilitated?

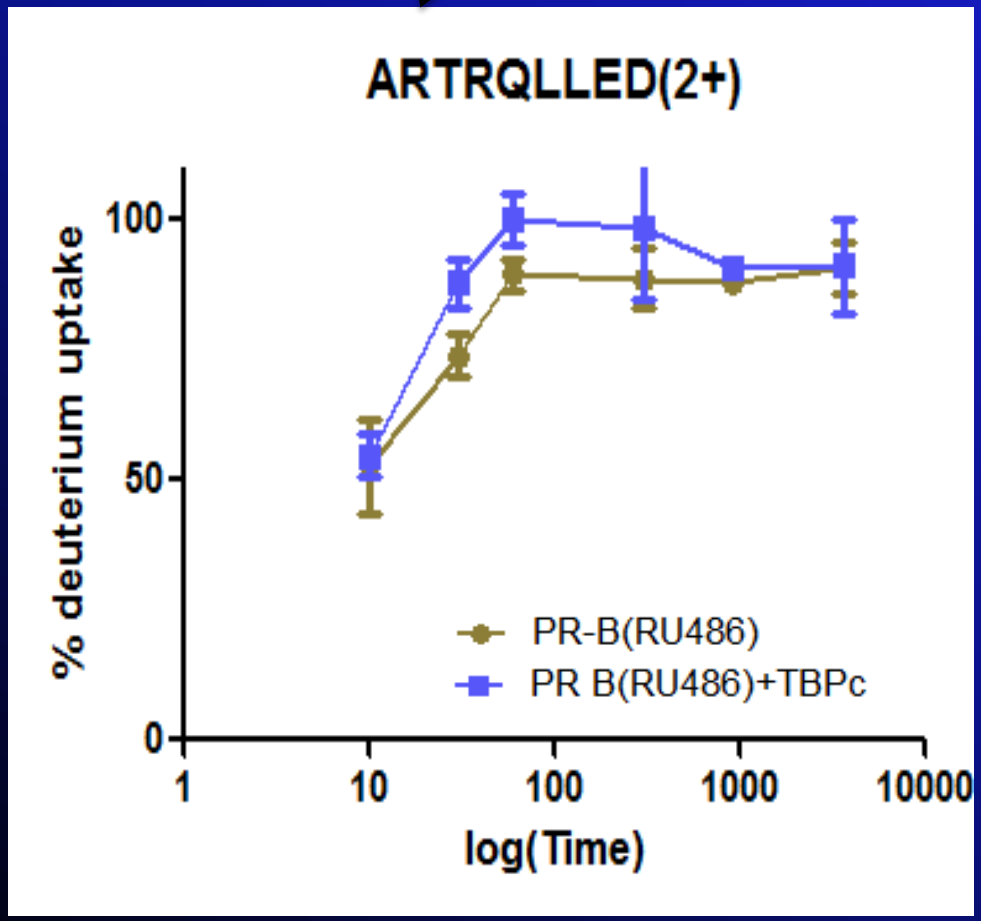
Interaction of TBP to agonist-bound PR alters LBD conformation

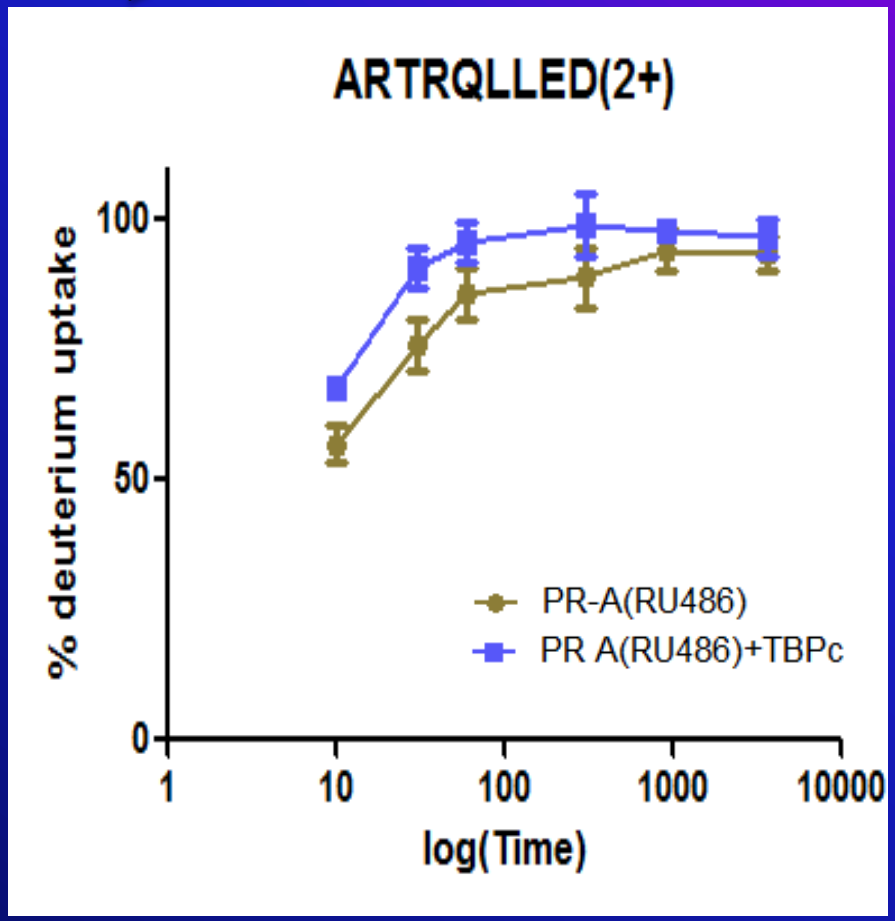


Cell-Structure 22, 961–973, July 8, 2014

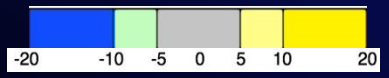
Interaction of TBP to antagonist-bound PR alters NTD/AF1 conformation





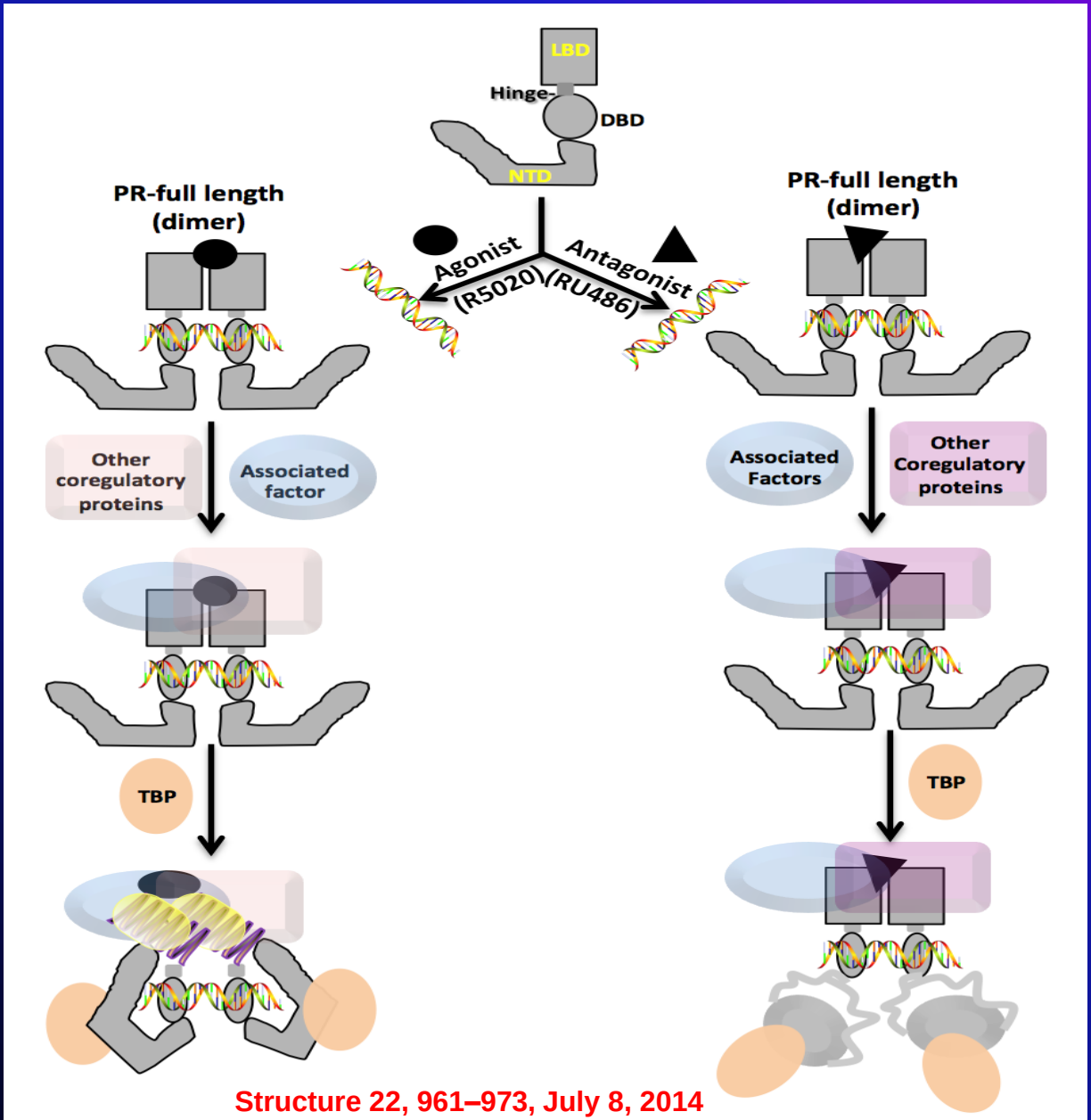


Color bar (% of deuterium uptake difference)

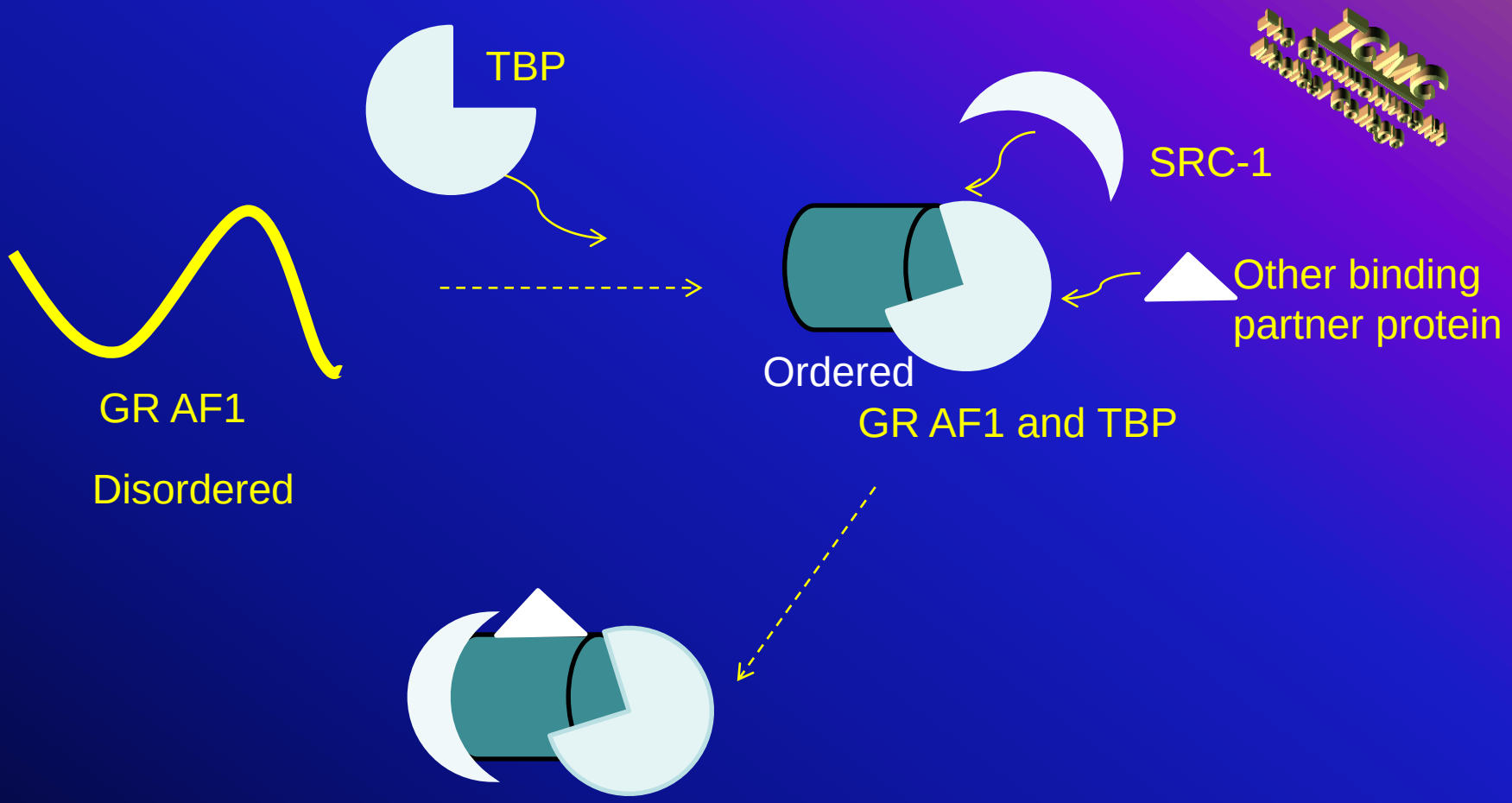


Cell- Structure 22, 961–973, July 8, 2014

A therapeutic Model for Endocrine-related cancers

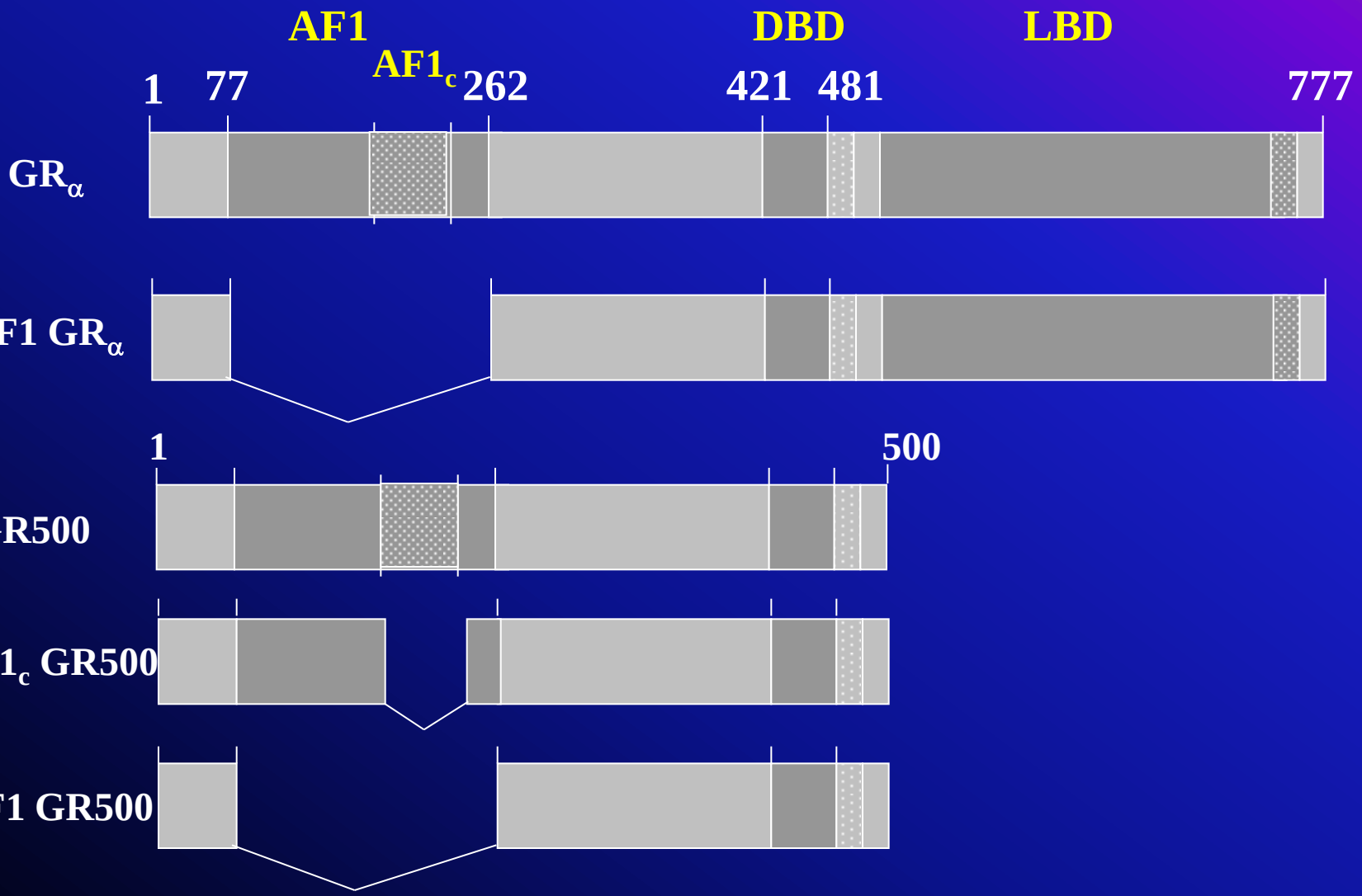


Structure 22, 961–973, July 8, 2014



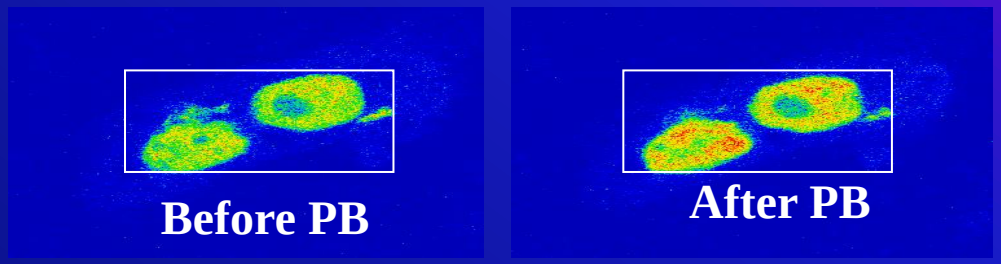
Multi-protein assembly involved in GR-mediated gene regulation

Constructs of GR

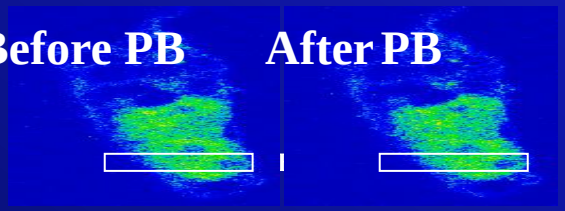


FRET analysis showing the GR AF1:TBP interaction in CV-1 cells

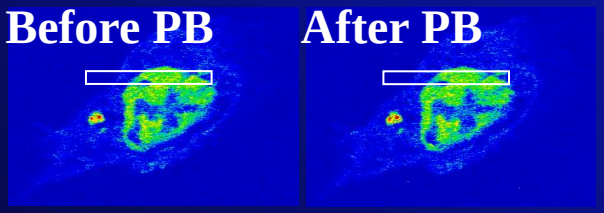
CFP-GR500 + YFP-TBP



CFP-ΔAF1_GR500 YFP-TBP

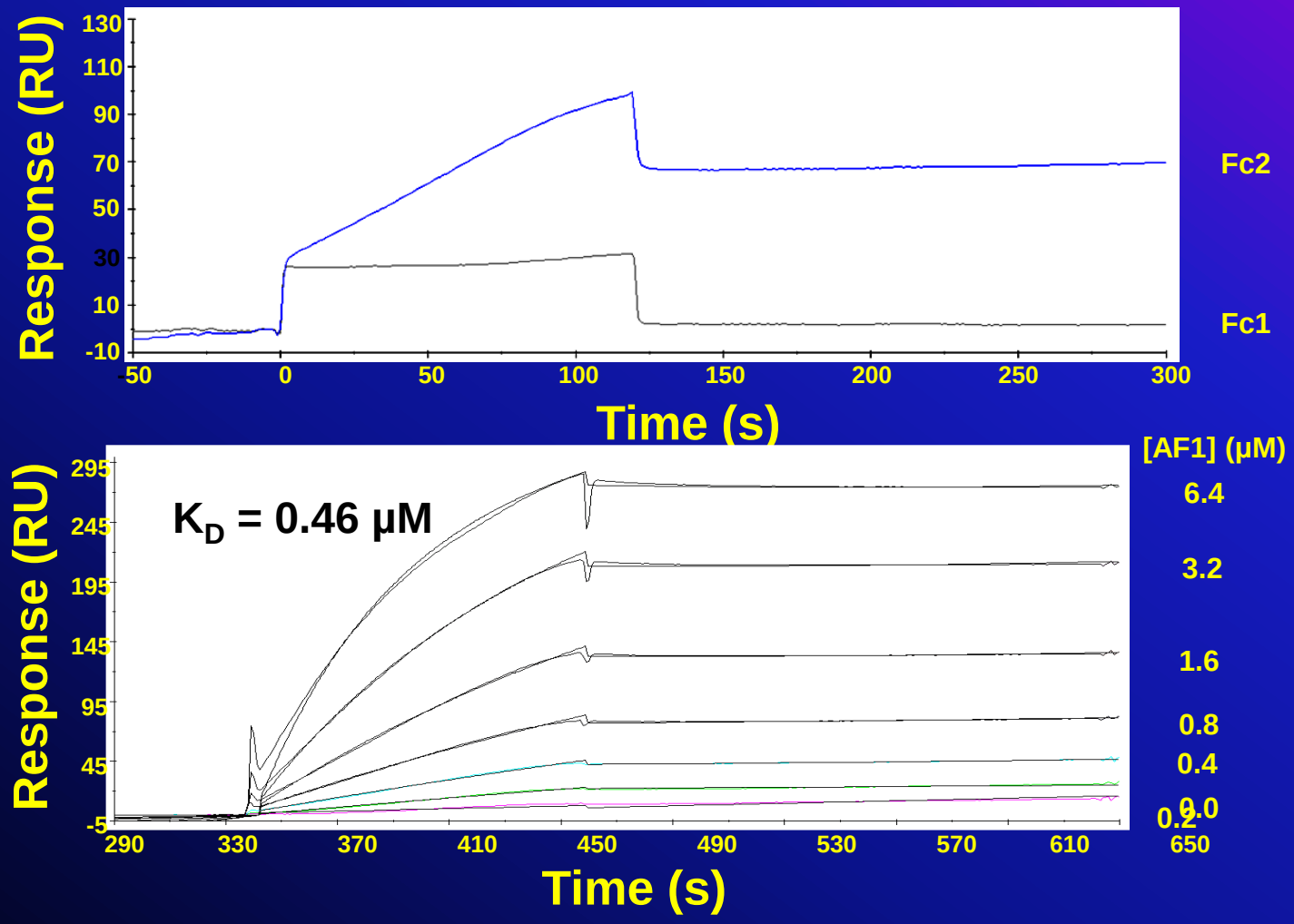


CFP-GR500 + YFP-TBP+pTAL

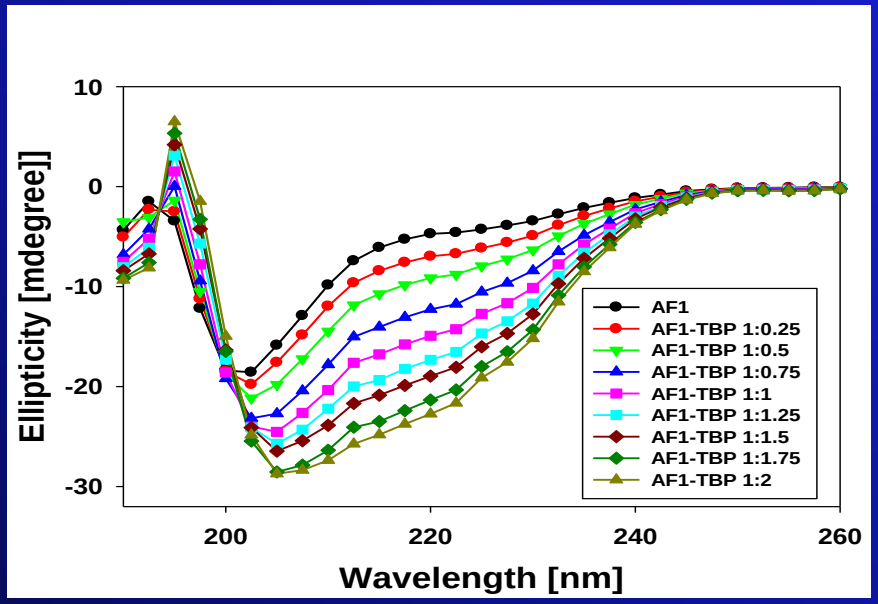


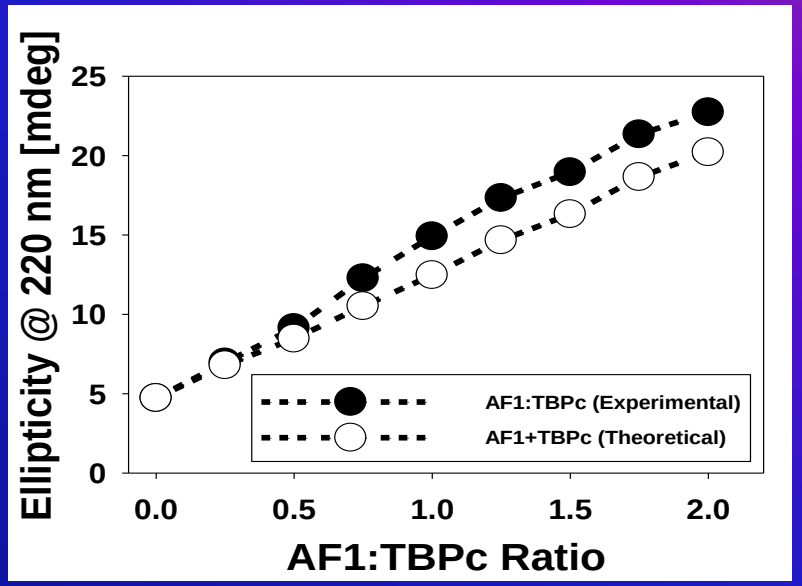
Copik et al., Mol. Endocrinol. 2006

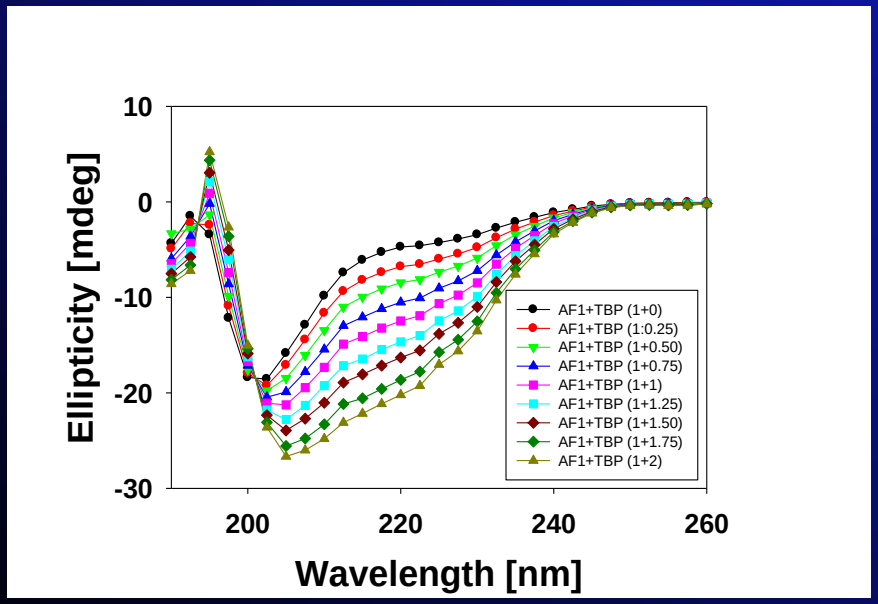
Kinetics of binding between GR AF1 and TBP by Surface Plasmon resonance (SPR) method using BIACORE

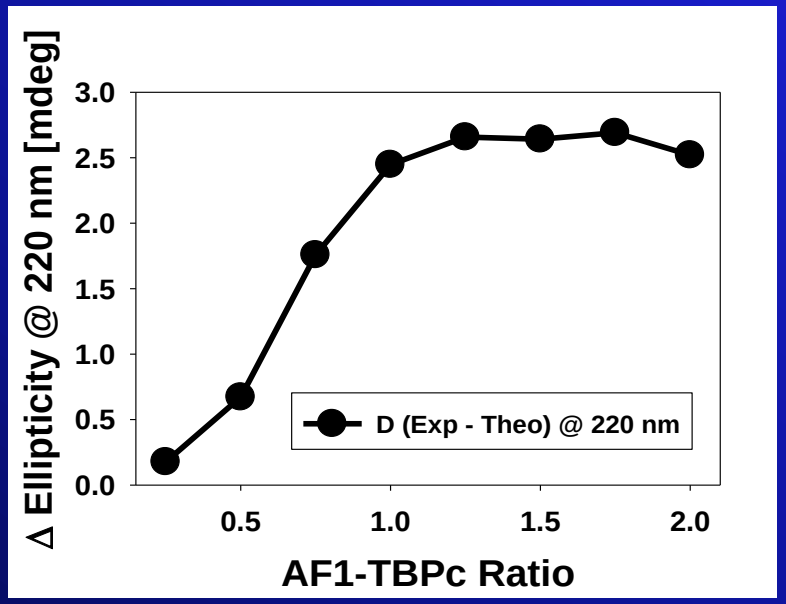


Far-UV CD spectra of AF1:TBP complex shows that AF1 adopts ordered structure when bound to TBP

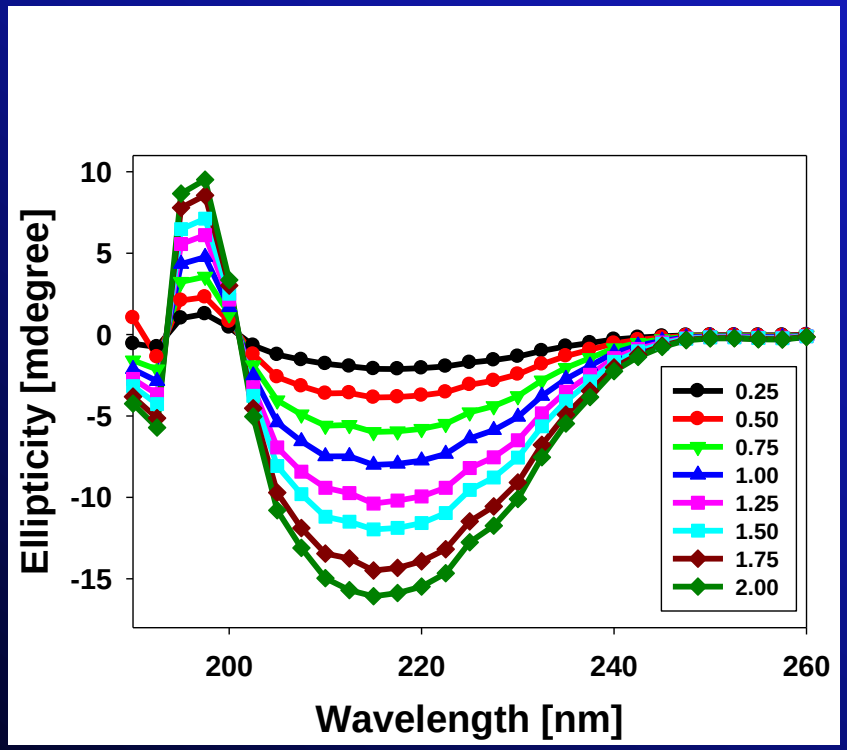


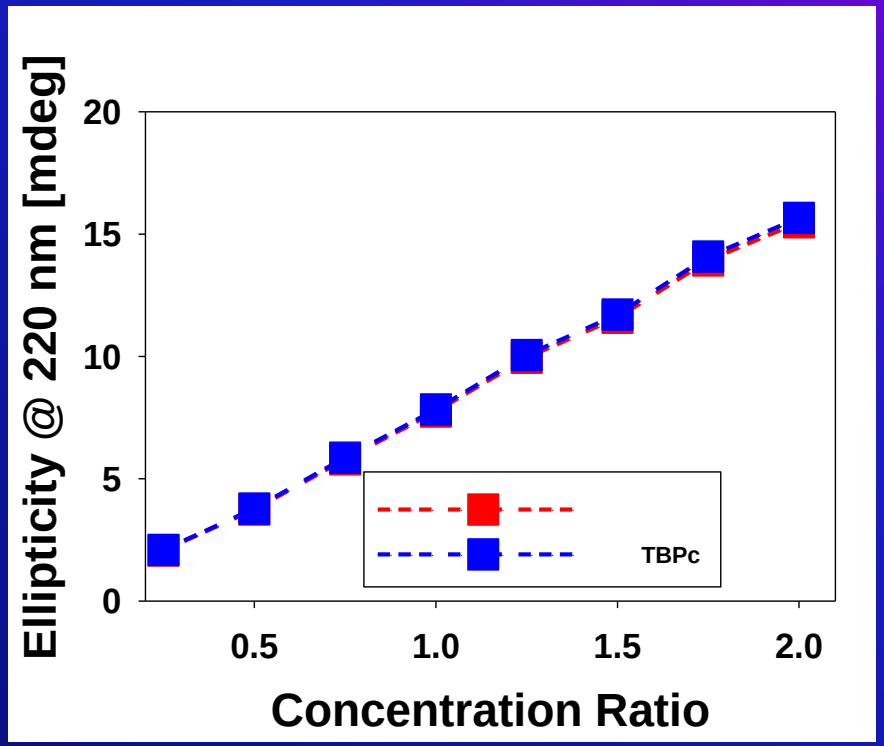




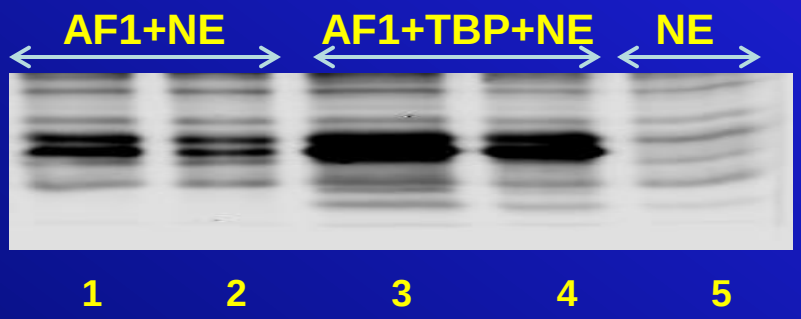


Far-UV CD spectra of TBP at various concentrations showing no structural changing happening in TBP

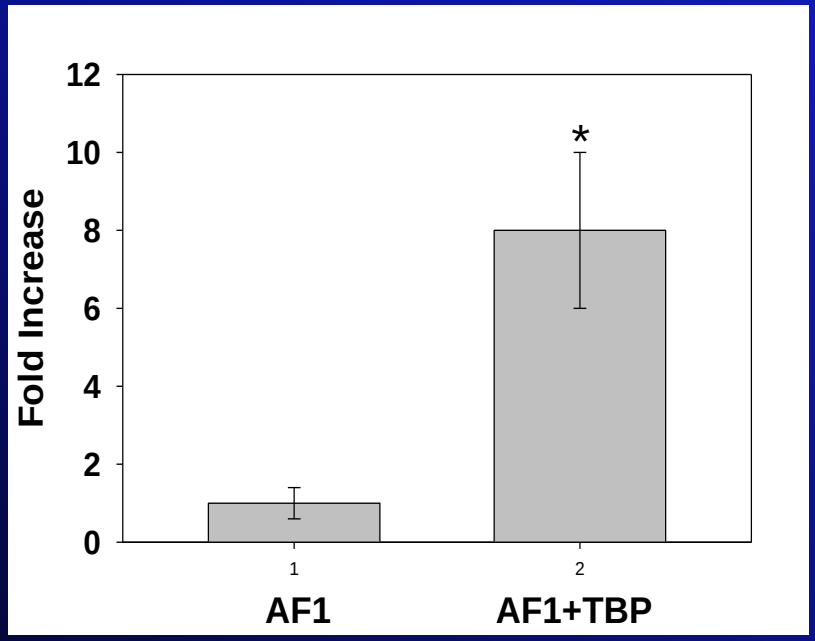




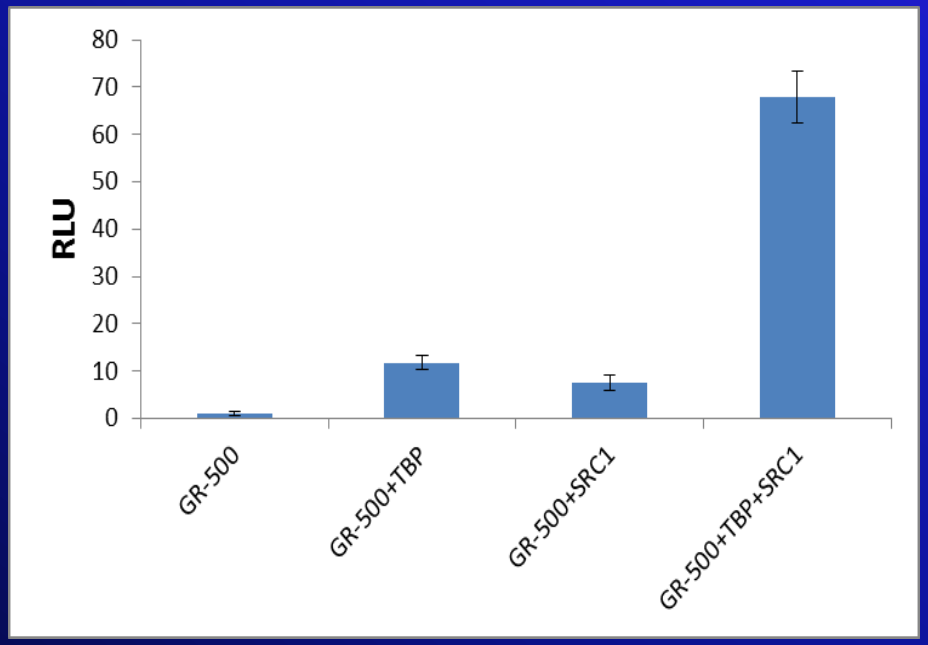
TBP-binding/folding induced conformation in AF1 facilitates AF1's interaction with SRC-1



IP = SRC-1 and blot = AF1 antibody

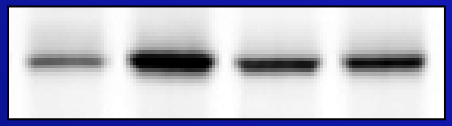


Synergistic effects of TBP and SRC-1 on GR AF1 activity. GRE-SEAP



Western blots showing higher levels of TBP and SRC-1 expression in MDA-MB-231 cells compared to MCF-7 cells.

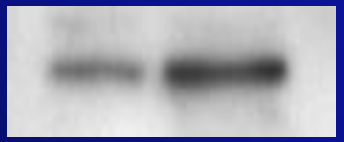
MCF-7 MDA-MB-231



Nuclear GR

C Dex C Dex

MCF-7 MDA-MB-231

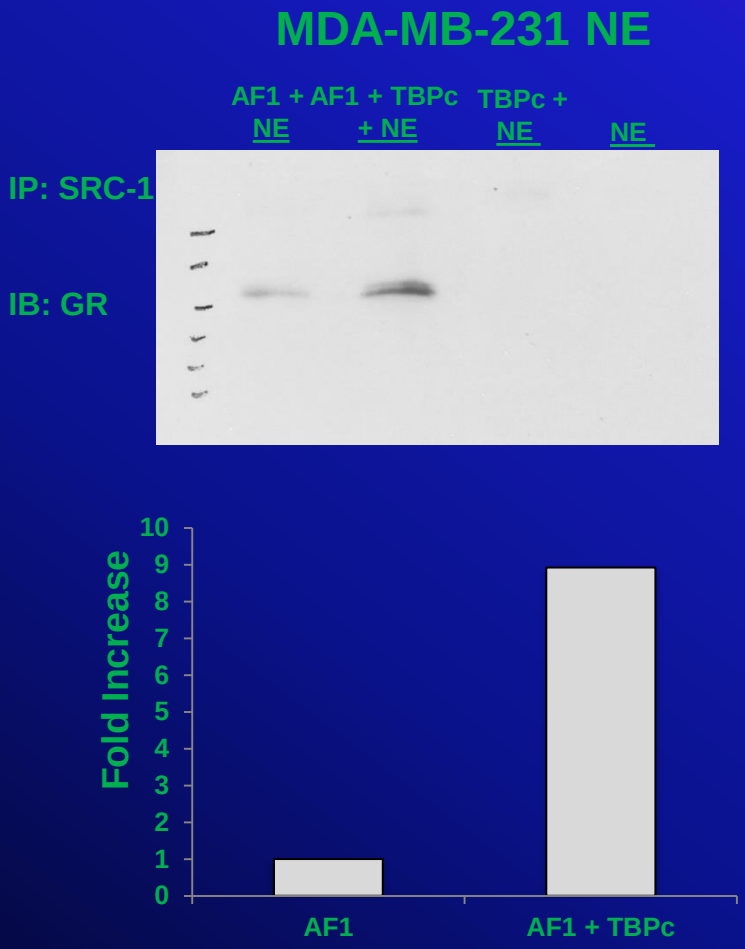


TBP

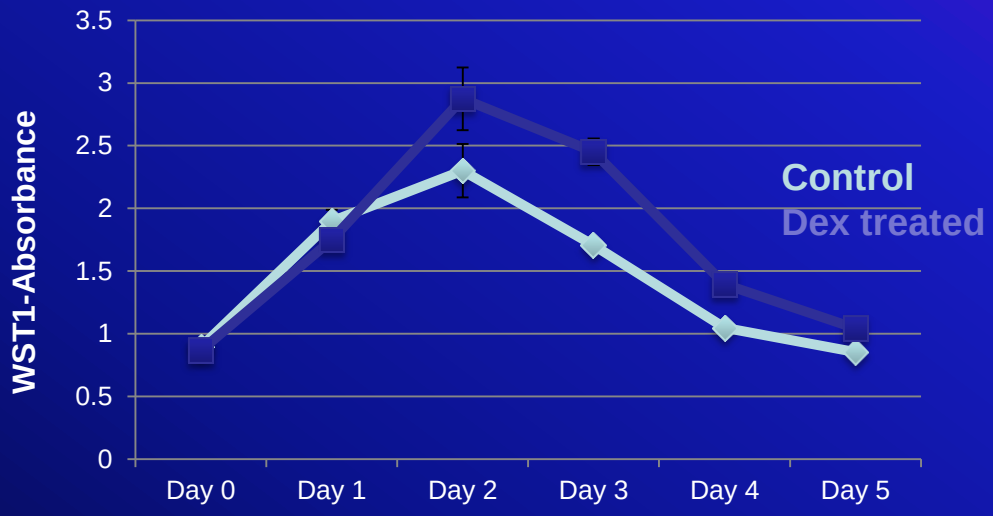


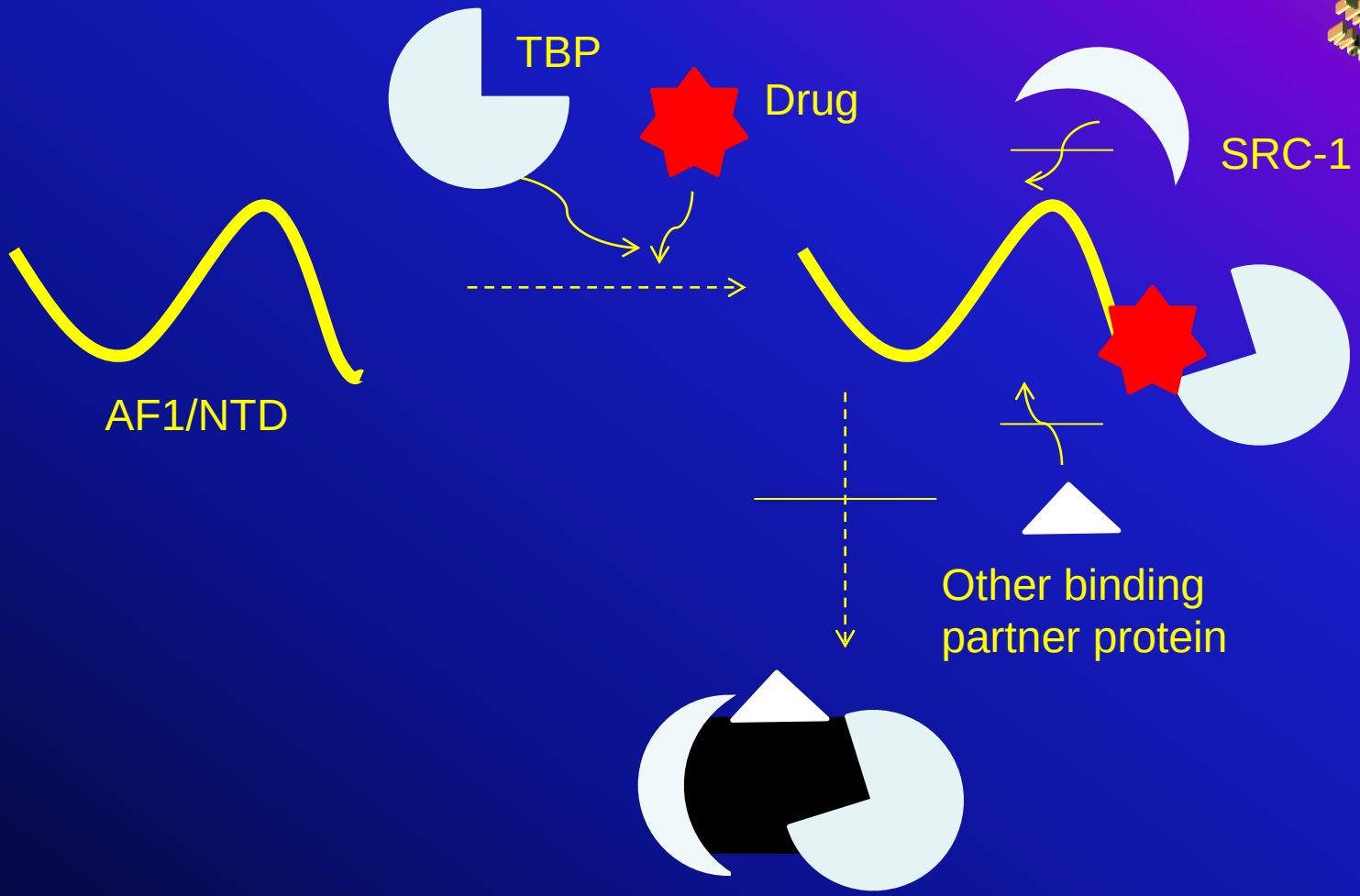
SRC-1

TBP-binding to GR AF1 enhances its interaction with SRC-1 in NDA MB 231 cell nuclear extracts (NE)

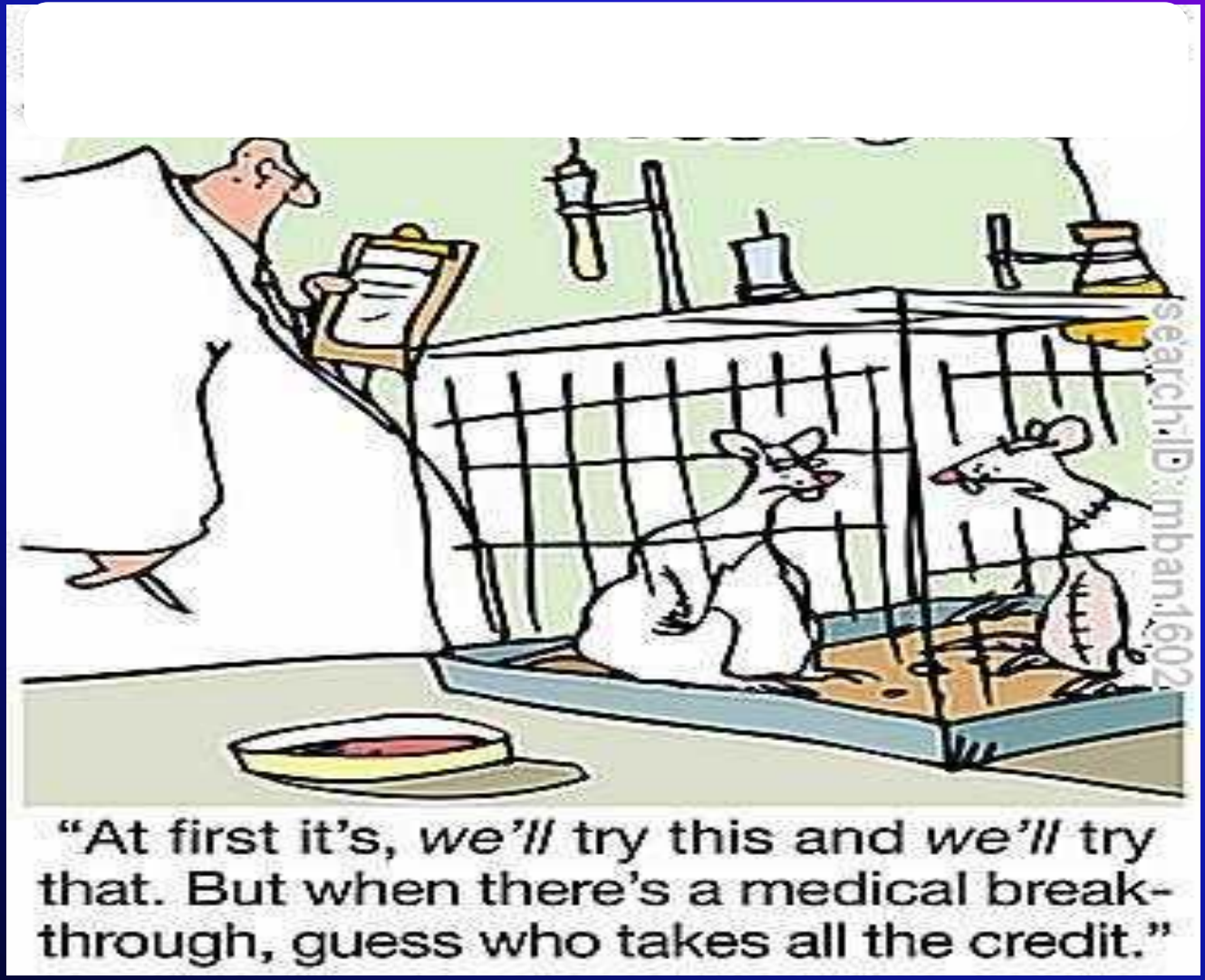


Dex treatment results into higher cell viability of MDA MB-231 cells





Tissue specific gene regulation and a drug target for TNBC


"At first it's, *we'll* try this and *we'll* try that. But when there's a medical breakthrough, guess who takes all the credit."

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