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Conformational editing and intracellular delivery of intrinsically disordered protein domain

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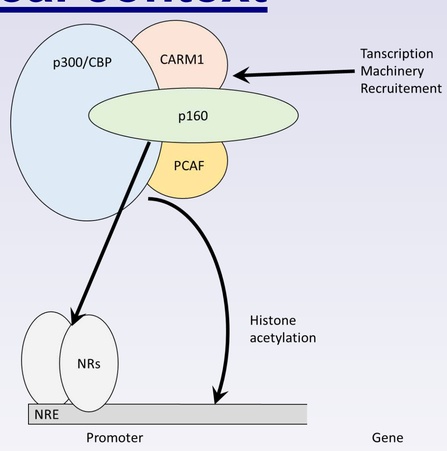
Introduction: In this study, we proposed an approach to target complex formation that involves IDPs that is based on conformational editing of the IDP domain by utilizing backbone conformational constraints (α-methylation) to facilitate binding and stabilize or destabilize helical structures. (1) Non-canonical modifications can also stabilize against proteolytic degradation—an advantage for intracellular activity of peptides.

Biological context



Our system of study:

NCBD of CBP/p300 (2066-2112)

H_2N-SALQDLLRTLKSPSSPQQQQQVLNILKSNPQL-Nle-AAFIKQRTAKYVAN-$CONH_2$

AD1-ACTR/p160 (1040-1086)

H_2N-EGQSDERALLDQLHTLLSNTDATGLEEIDRALGIPELVNQGQALEPK-$CONH_2$

Mutation site : X→α-methylation ; G→d-Ala

CBP/p300 interact with the promoter of **≈25%** of the human genome and more than **400** transcription factors.

Implication in numerous diseases such as estrogen-dependent (ER+) breast cancer, neurodegenerative diseases.(2)

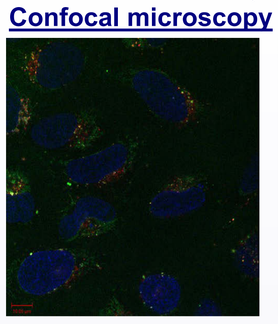
ACTR/p160 is implicated in the **estrogen production and response** and known to be overexpressed in **breast cancer ER+** and be involved in the **tamoxifen resistance.**(3)

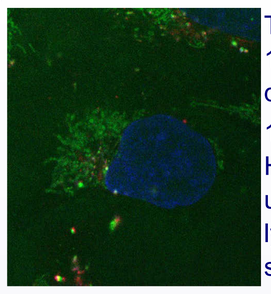
Cell culture

All experiments have been done on U2OS cells.

Variants used : Pen-cys(IAF)-peg5-aMex3

Confocal microscopy



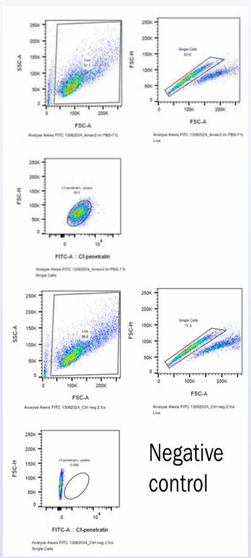


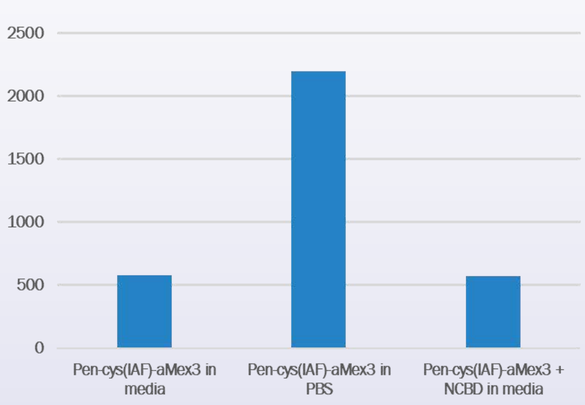
Three concentration tried : 10µM, 5µM, 2,5µM in media

1h incubation (Blue: Hoechst 33342 for nucleus, red: LysoTracker for lysosome and late endosome)

Endoplasmic reticulum? Golgi apparatus? Mitochondria ? Bind to CBP ? Aggregation ?

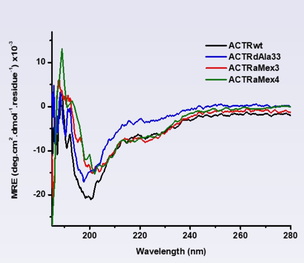
Flow cytometry





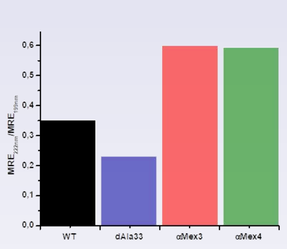
The use of PBS instead of media to make the incubation seems to increase the uptake of the peptide (interaction with media component ?).

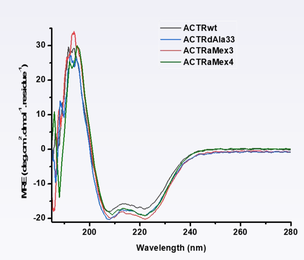
Circular dichroism characterization



Helical content

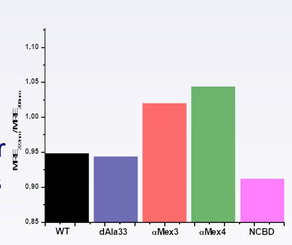
Enhancement for aMex3 and aMex4

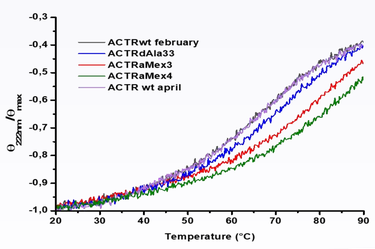




α-Helix content

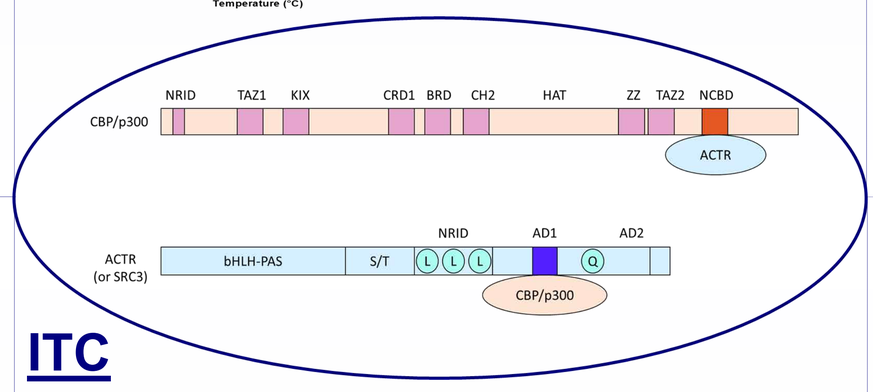
Enhancement for all complexes vs NCBD alone



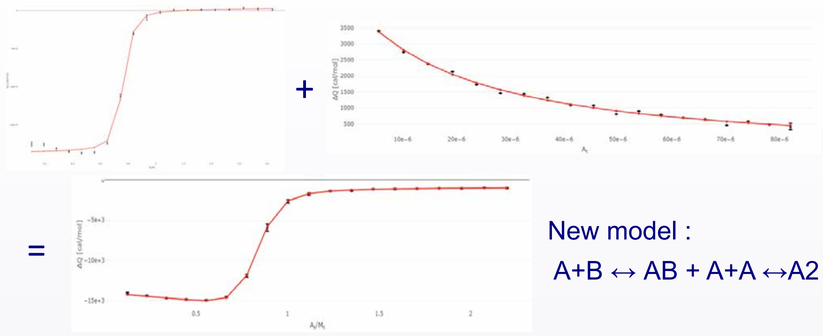


The thermal denaturation of complex NCBD/$ACTR_{Variants}$ shows that all the variants induce more stable complex with NCBD than WT.

Reproducibility of the data.



ITC



New model :

A+B ↔ AB + A+A ↔A2

Variant	Kd (nM)	ΔH (kcal/mol)	ΔG (kcal/mol)	-TΔS (kcal/mol)	ΔS (kcal/mol/deg)	ΔCp (kcal/deg,mol)
wt	260	-13,6	-9,16	4,48	-0,0147	
aMex4	119	-16,1	-9,63	6,44	-0,0212	
aMex3	97,7	-20,6	-9,75	10,9	-0,0358	-0,975
dAla33	188	-16,0	-9,36	6,64	-0,0218	-0,748

ΔG is nearly constant whereas ΔH and ΔS change a lot => **enthalpy-entropy compensation** (EEC).

ΔH = more contact or more tighter ones between both peptide.

New questions :

⇒ Does the EEC can be explain only by conformational entropy ?

⇒ Role of solvation ?

⇒ Role of hydrogen bonding ?

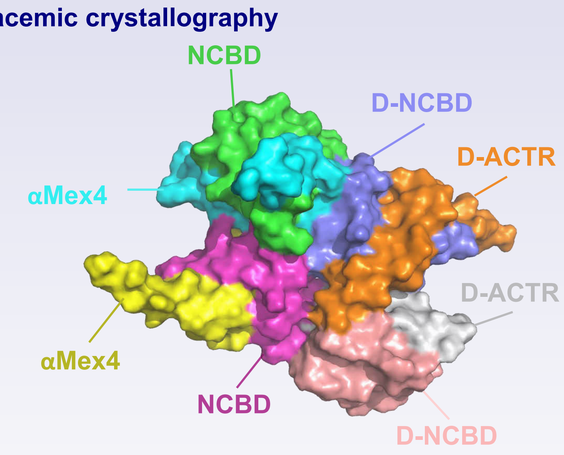
⇒ Role of ionic interactions ?

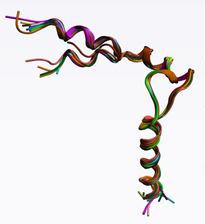
New experiments needed.

D_2O in place of water, different pH, different buffers...

Crystallography

Quasi-racemic crystallography



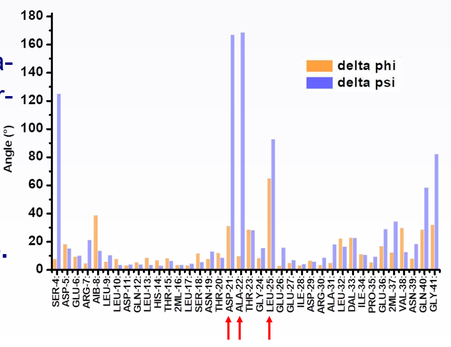


In each data set (n=8) we can see the two types of ACTR/NCBD complexes

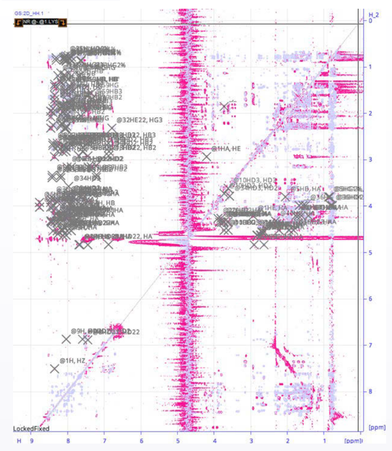
ACTRαMex4 adopts **two different conformations** in each crystal and it is very stable in a large pH range (4.5 to 9.0).

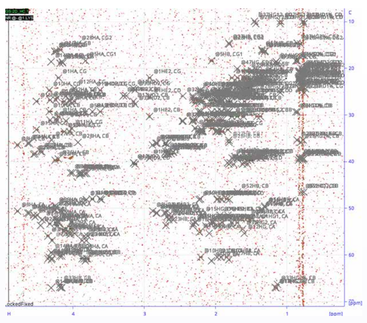
The backbone angle variability between two conformations.

Higher flexibility area between **Asp21** and **Leu25.**



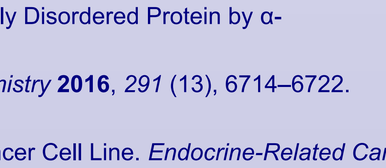
NMR





Amino-acid assignment, and sequential attribution of aMex3 done.

We recognized the signature of α-Helix in NOESY experiment.



Comparison of Cα/Hα correlation between ACTR WT and aMex3 in ^{13}C-HSQC spectra.

Conclusion: In this study, we enhanced helical content in free ACTR or in the complex with NCBD. We observed the presence of two distinct structures by X-ray crystallography. Their presence in the same crystal can indicate a dynamic interconversion between them. Furthermore, NMR on the three-methylated variant displayed a pattern of α-Helix which is coherent with the increase of the helical content observed in CD. In ongoing experiments, notably in NMR, the complex of NCBD with the three-methylated variant will be studied to characterize its solution dynamics. Another point that needs to be explored further is the energetic implication of solvation, hydrogen bonds and ionization during the complexation. We also started to explore the intracellular delivery of a construct to determine pathways it uses to go inside cells and if it can have an effect on breast cancer ER+ cell line.

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