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Characterization of Aurora-A Activation and Allosteric Regulation by TACC-3 Peptidomimetics

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TACC3 / Aur A Protein-Protein Interaction

Aurora-A is a Ser/Thr protein kinase that regulates key mitotic events by recruiting and phosphorylating a range of different intrinsically disordered proteins. Among them, the Aurora-A interaction with TACC-3 (Transforming Acidic Coiled-Coil Containing Protein 3) is instrumental for spindle assembly and chromosome segregation,[2,3] and is frequently up-regulated in many different types of cancer.[4]

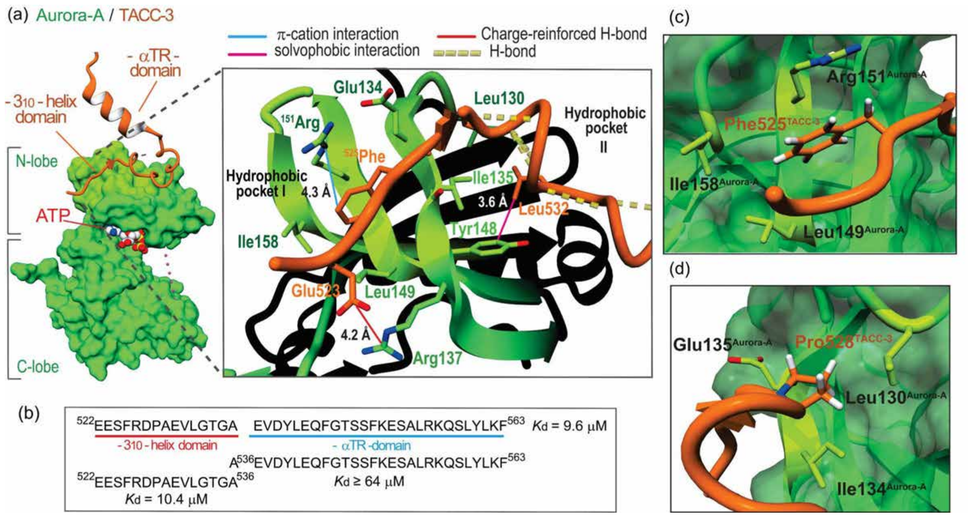


Figure 1. Key features of the Aurora-A/TACC-3 complex. (a) Crystal structure of the Aurora-A catalytic domain (green) in complex with TACC-$3_{522–563}$ (orange; PDB: 5ODT). **(b)** Sequence of TACC-3 docking region to Aurora-A, TACC-$3_{522-563}$, and individual FA dissociation constants (K_d) for each domain. **(c-d)** MD calculated energy minimum structure of WT TACC-$3_{522-536}$ in the presence of Aurora-A, showing the key Phe525^{TACC-3} and Pro528^{TACC-3} *exo*-pucker conformation.

TACC3 / Aur A Protein-Protein Interaction

We used sequence truncation and fluorescence anisotropy (FA) to optimize the TACC3 docking segment.

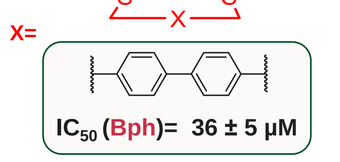
TACC3 522-536 **EESFRDPAEVLGTGA** IC_{50}= 163 ± 37 μM

TACC3 518-532 **LELK EESFRDPAEVL** **IC_{50}= 124 ± 33 μM**

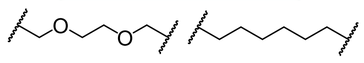
Stapled TACC3 518-532 L/R

LECKEESFCDPAEVL

X=



IC_{50} (Bph)= 36 ± 5 μM



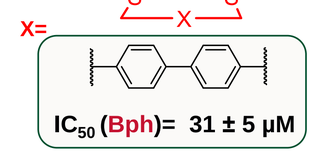
IC_{50} (PEG)= 380 ± 13 μM

IC_{50} (Oct)= 65 ± 6 μM

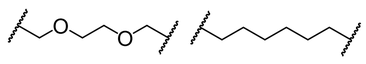
Stapled TACC3 518-532 S/E

LELKEECFRDPACVL

X=



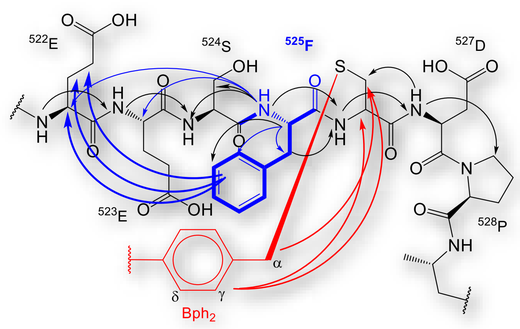
IC_{50} (Bph)= 31 ± 5 μM



IC_{50} (PEG)= 320 ± 52 μM

IC_{50} (Oct)= N/D

NMR structure analysis

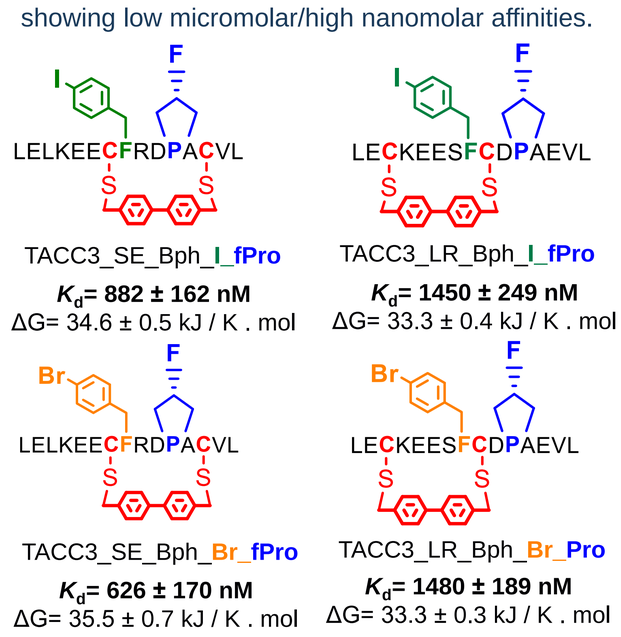


Schematic showing the observed NMR nOE contacts in stapled TACC3 518-532 L/R at the 525F region.

Bph constraints restrict the conformational landscape and orientates the key 525Phe predisposing the peptide towards Aur A binding

Optimization of Phe525 and Pro528 interaction with Aur-A

By using unnatural phenylalanine analogs and exploiting stereo-electronic effects (i.e. gauche effect) twelve-fold improved Kd values were measured for new constrained peptides when compared to the linear sequences, with all variants showing low micromolar/high nanomolar affinities.



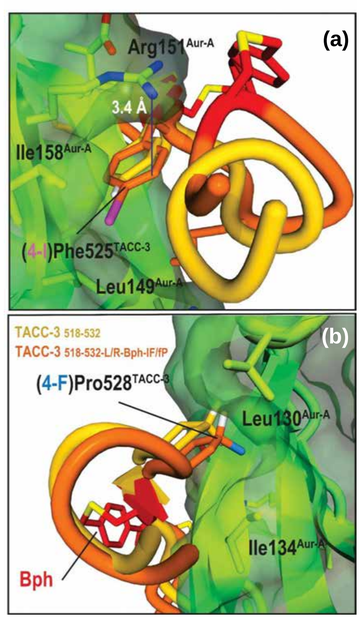
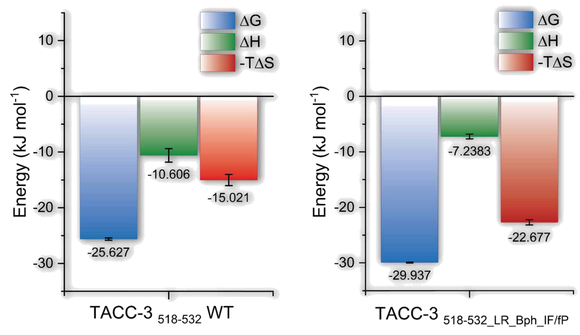


Figure 2. Key features of TACC-3 constrained peptidomimetics. (a) Iodinated (4-I)-Phe525^{TACC-3} in its binding pocket. **(b)** Exo-pucker conformation of fluorinated *trans*-(4-F)Pro528^{TACC-3} in its binding pocket.

Enhanced binding of peptidomimetics to Aur-A is entropically driven

To explore how restricting the accessible conformations influences the thermodynamics of binding, we carried out isothermal titration calorimetry (ITC) and variable-temperature FA experiments. Constrained variants exhibited a more favorable entropy of binding in comparison to the linear variants.



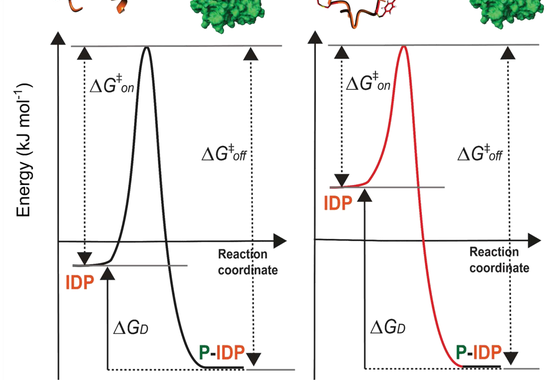
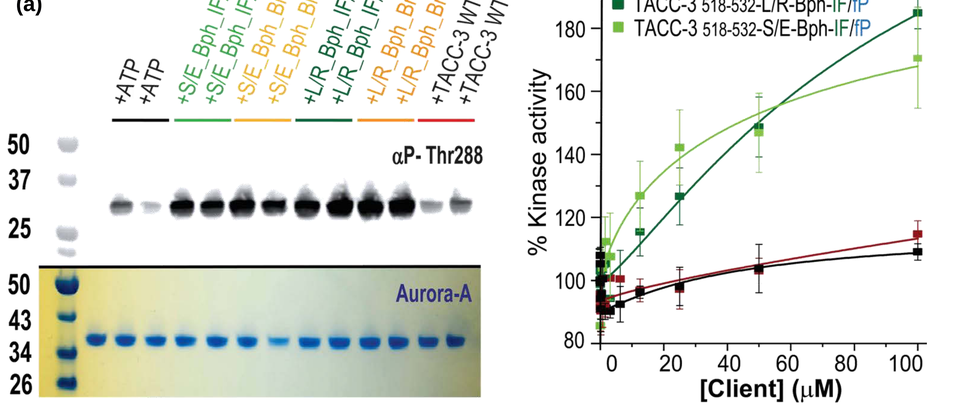


Figure 3. Thermodynamics of binding in the presence of Aurora-A. (a) ITC thermodynamic signatures of linear TACC-$3_{518-532}$ and **(b)** constrained TACC-$3_{518-532-S/E-Bph-I/fP}$ binding to Aurora-$A_{122-403-C290A/C393A}$; **(c)** Diagram schematically illustrating the hypothetical free energy profile of a one-step/one-barrier peptide-protein binding event for a linear and a constrained peptide.

TACC-$3_{518-532}$ peptidomimetics stimulate Aur-A

Linear WT variants are relatively poor activators of the kinase, whereas the constrained peptidomimetics stimulate Aurora-A and promote substrate phosphorylation in a dose-dependent manner by up to 160-190%.



TACC-$3_{518-532-L/R-Bph-I/fP}$ as an allosteric inhibitor of the N-MYC/Aur-A interaction

Constrained peptidomimetics exhibited limited evidence of TPX2 displacement, indicating promising specificity for the TACC-3 binding site (IC50 >> 200 μM). Surprisingly, the constrained variants were observed to out-compete N-MYC.

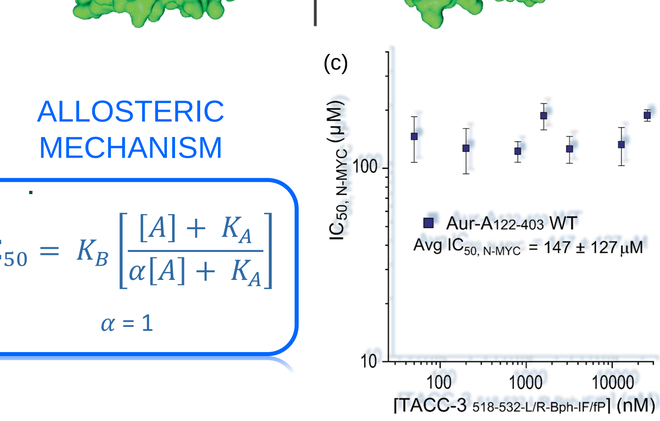


Figure 7. Constrained TACC-$3_{518-532\_Bph\_LR\_IF/fP}$ as an allosteric inhibitor of N-MYC/Aurora-A PPI: (a) Aligned x-ray crystal structures of TACC-3/Aurora-A complex (PDB: 5ODT), TPX2 (PDB: 1OL5), and N-MYC (PDB: 5G1X); **(b)** FA competition assay of control N-MYC_{61-89} (black line) and constrained TACC-$3_{518-532\_Bph\_LR\_IF/fP}$ (forest green) against FAM-Ahx-N-MYC_{61-89} (200 nM), and reverse assay in the presence of Aurora-A; **(c)** Competition FA N-MYC IC50's values for N-MYC_{61-89} at increased TACC-$3_{518-532\_Bph\_LR\_IF/fP}$ concentrations; **(d)** X-ray crystal structures of TACC-3/Aurora-A and N-MYC/Aurora-A complexes..

Conclusions

We have developed a series of **constrained peptides that inhibit TACC3/Aurora A PPI with up to 12-fold enhanced IC_{50}/K_d values**. NMR and MD analysis of the peptides show that improved affinities are achieved due to the combined effect of **restricting the conformational landscape of the peptide** (Bph constraints), **fine-tunning the orientational preference of the key Phe525** (4I and 4Br-Phe substitutions) and **conformational pucker-ring selection at the Pro528 level** (4F-Pro). Our minimal peptidomimetics are sufficient to induce the conformational changes needed to **activate the kinase by binding only the "F" pocket on the N-lobe, and to allosterically displace N-MYC from its binding site**. Knocking-out the TACC-3/Aurora-A interaction without affecting kinase activity represents a promising alternative to active-site kinase inhibition. Similarly, inhibition of the Aurora-A/N-MYC interaction without downregulating other Aurora-A essential functions also represents a major target for anticancer drug-development.

References

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Acknowledgements

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