# Linking structure to kinetics - Tau-Tubulin Kinase 1 and its interactions with inhibitors

- Introduction
- Structure of TTBK1
- Structure-kinetic relation of ligand interactions

Yafeng Xue, PSDI 2013-11-04

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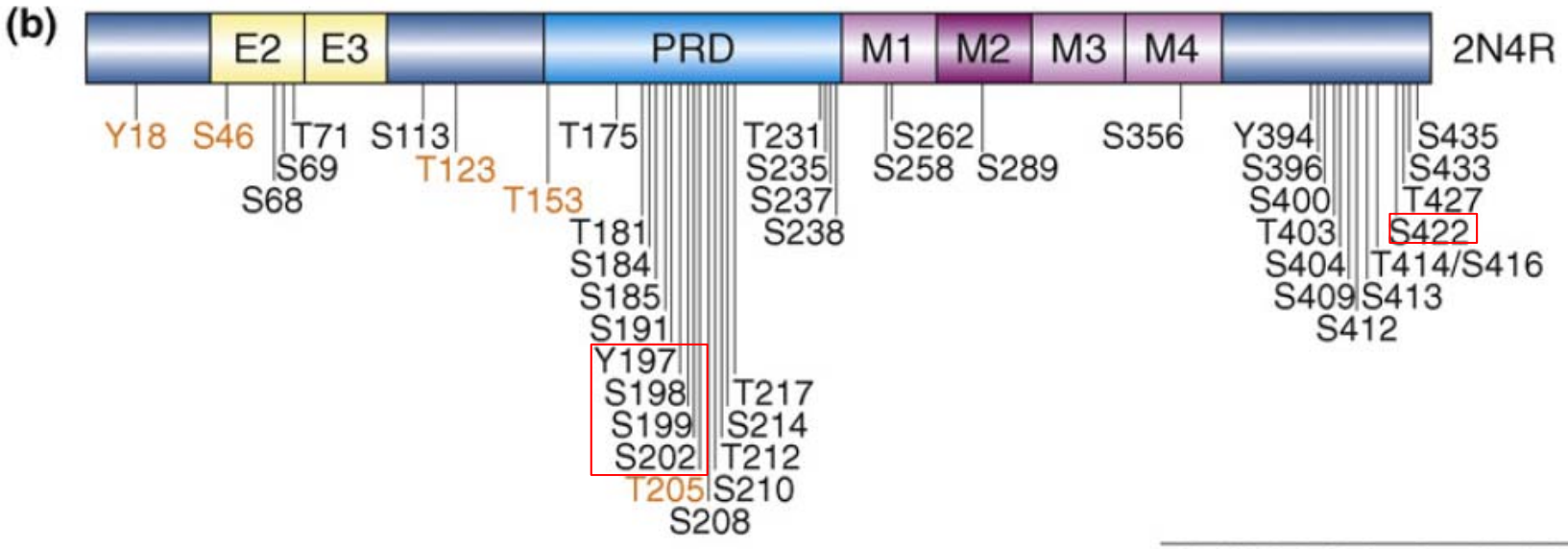
# Tau-tubulin kinase 1 (TTBK1) is a Ser/Thr/Tyr kinase

- TTBK1 expressed only in the brain
- TTBK1 can phosphorylate Tau at multiple sites
- Tau hyperphosphorylation is related to neurodegenerative disease

**Positioning of phosphorylation sites on tau from Alzheimer brain.**

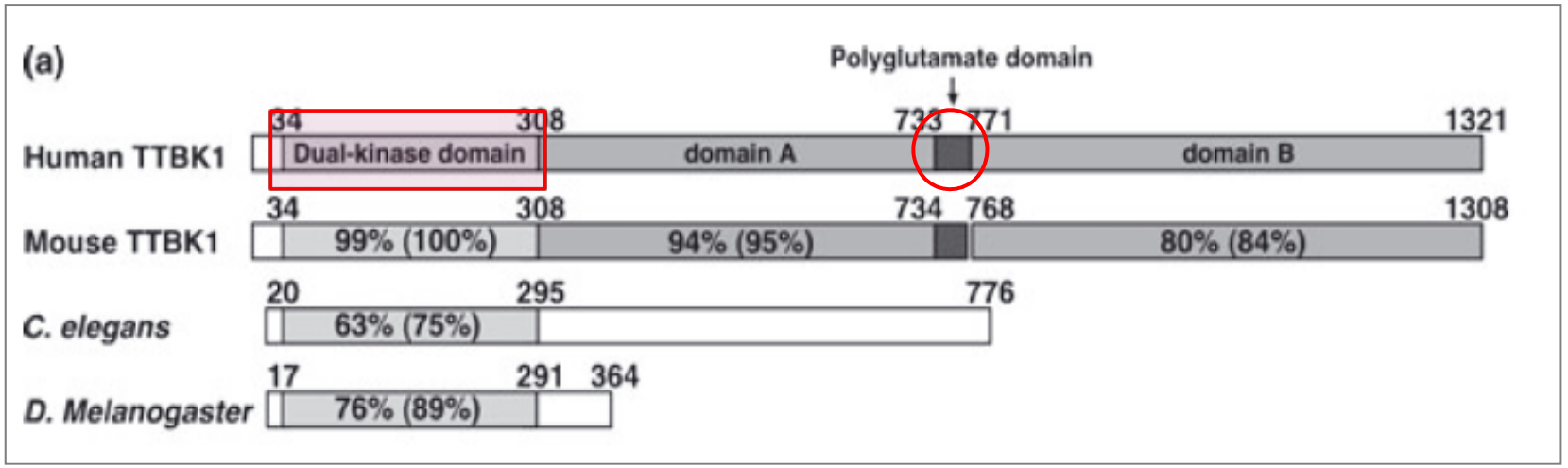



*TRENDS in Molecular Medicine*

•Hanger DP, Anderton BH, Noble W. Tau phosphorylation: the therapeutic challenge for neurodegenerative disease. Trends Mol Med. 2009 Mar;15(3):112-9..

# TTBK1 kinase domain: production of homogeneous protein

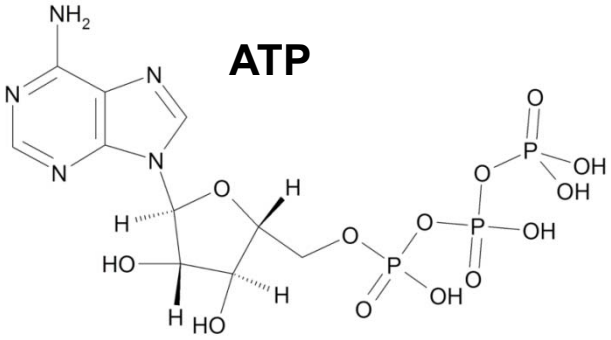
- 12 constructs screened for expression in *E. coli* and insect cells.
- Construct TTBK1 (1-313) and (14-313) produced multi-phosphorylated forms of soluble protein.
- Co-expressed TTBK1 with lambda Phosphatase resulted in completely unphosphorylated TTBK1



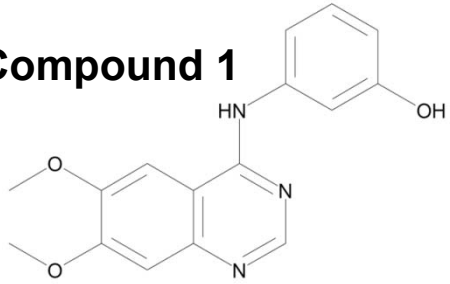
# TTBK1 Crystallization with ATP and inhibitors

## Inhibitors identified with biochemical assay (to develop tool compounds)

**ATP**



**Compound 1**



**Compound 2**

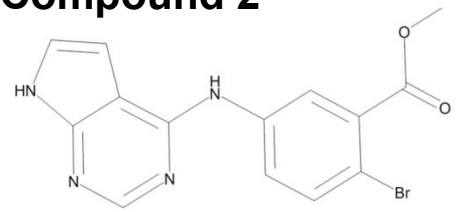
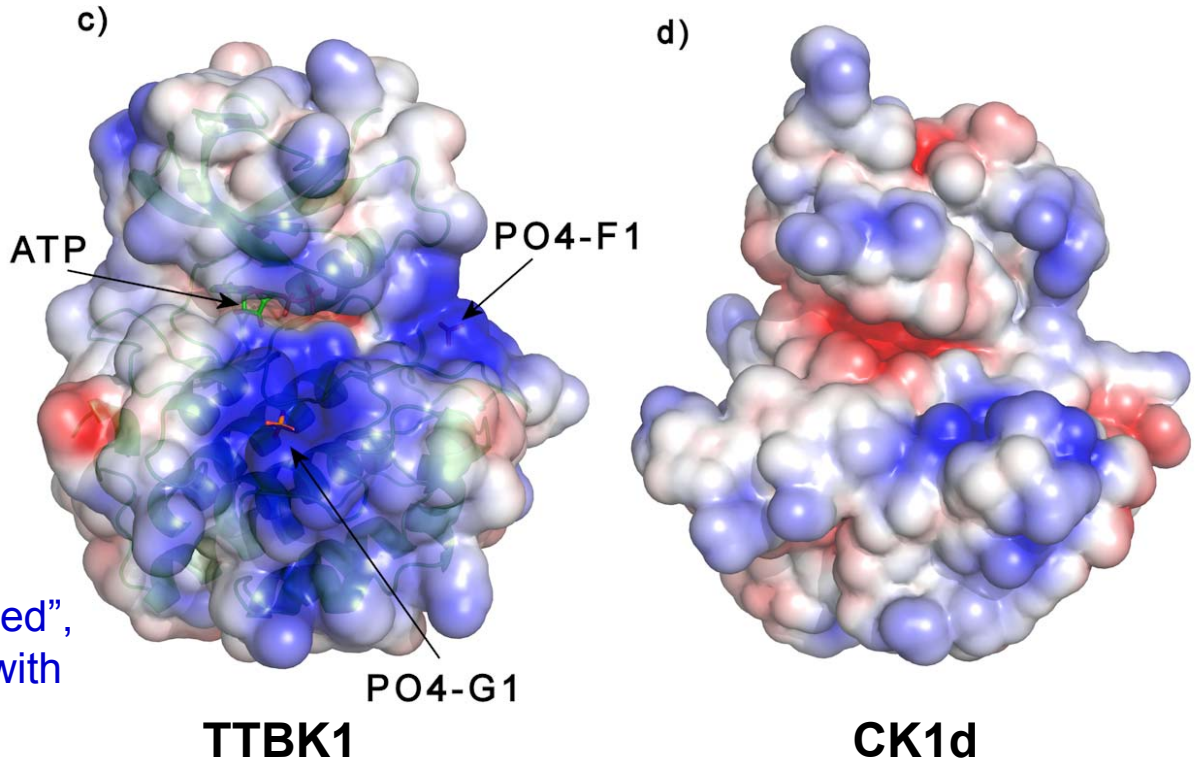


**Table S1. Data collection and refinement statistics**

| **Data set** | Apo-TTBK1 | TTBK1-ATP | TTBK1-compound **1** | TTBK1-compound **2** |
|---|---|---|---|---|
| Beam line | ID23-1/ESRF (λ=1.0723 Å) | IO4-1/Diamond (λ=0.9173 Å) | ID14-4 (λ=0.9322 Å) | IO4-1/Diamond (λ=0.9200 Å) |
| Space group | C2 | C2 | C2 | C2 |
| Cell parameter (Å/deg) | 170.16, 40.16, 49.88 / 90.00, 104.43, 90.00 | 125.98, 110.02, 110.55 / 90.00, 93.85, 90.00 | 170.89, 40.27, 50.25 / 90.00, 104.24, 90.00 | 127.59, 108.42, 110.76, / 90.00, 94.14, 90.00 |
| No of mol per ASU | 1 | 2 | 1 | 2 |
| Solvent content (%) | 51.4 | 78.8 | 52.7 | 78.8 |
| Resolution (last shell) (Å) | 1.85 (1.85-1.90) | 2.16 (2.16-2.22) | 2.00 (2.00-2.11) | 2.54 (2.54-2.61) |

# Two positive clusters identified on the surface → putative binding sites for the “primed” substrate

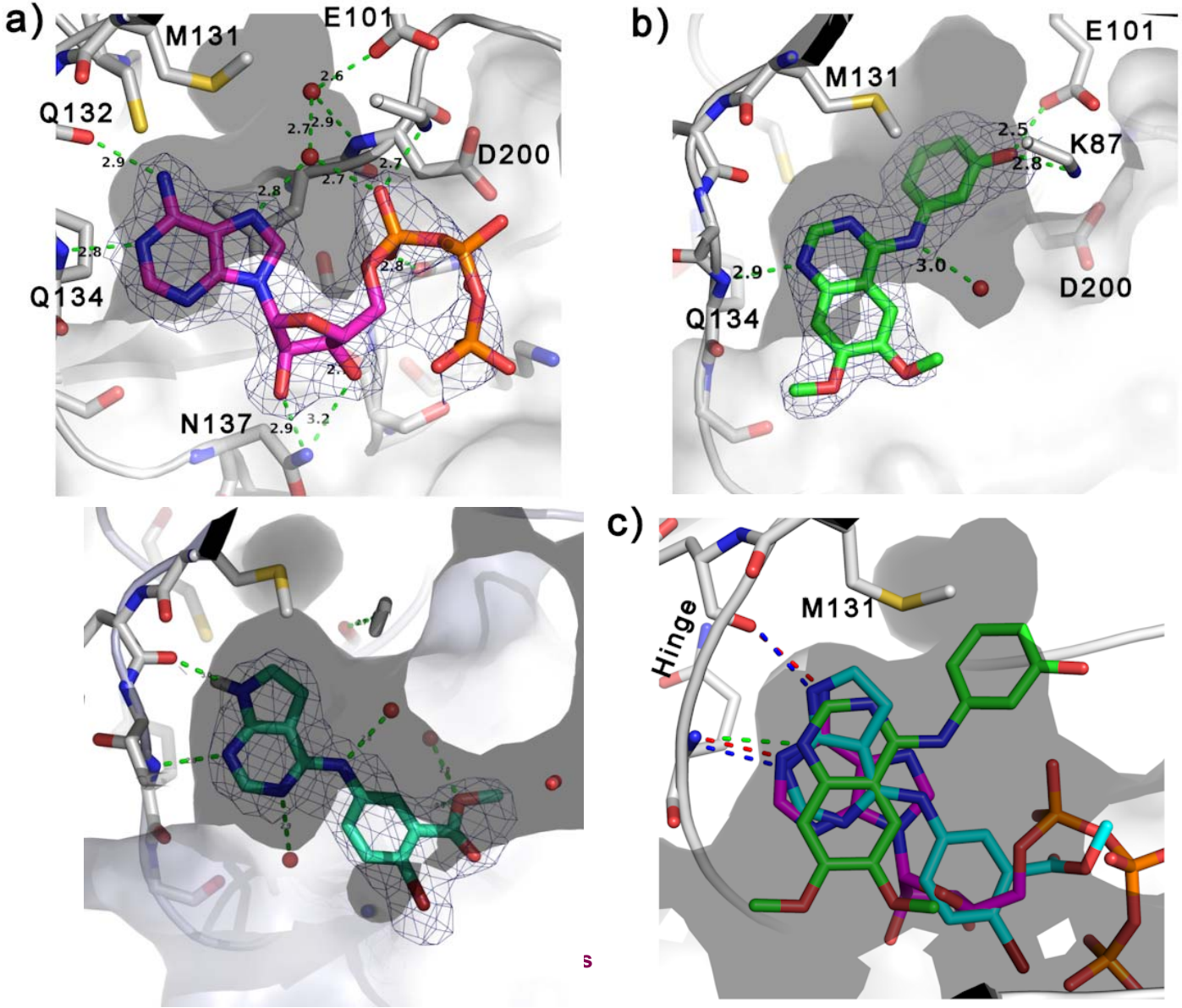
CK1 family kinases favour “primed”, pre-phosphorylated substrates with the motif pS/pT-X-X-S/T.




Also a plausible mechanism for the postulated auto-inhibition/regulation mediated by the Glu-rich region of TTBK1.

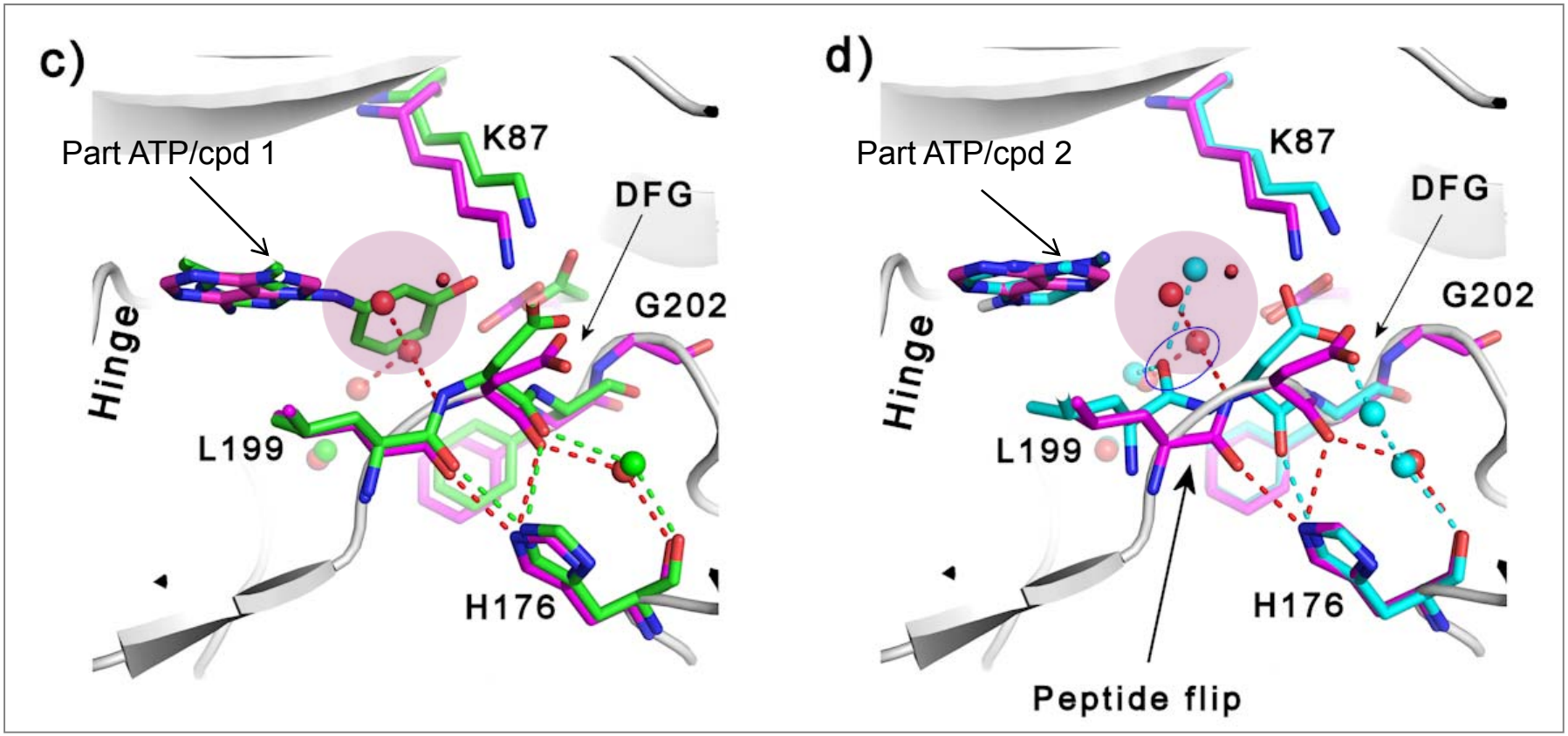
# TTBK1 Crystallization with ATP and inhibitors

## Electron density and the binding modes




- ATP
- Compound 1
- Compound 2

# Peptide flip of the DFG coincides with ligand binding

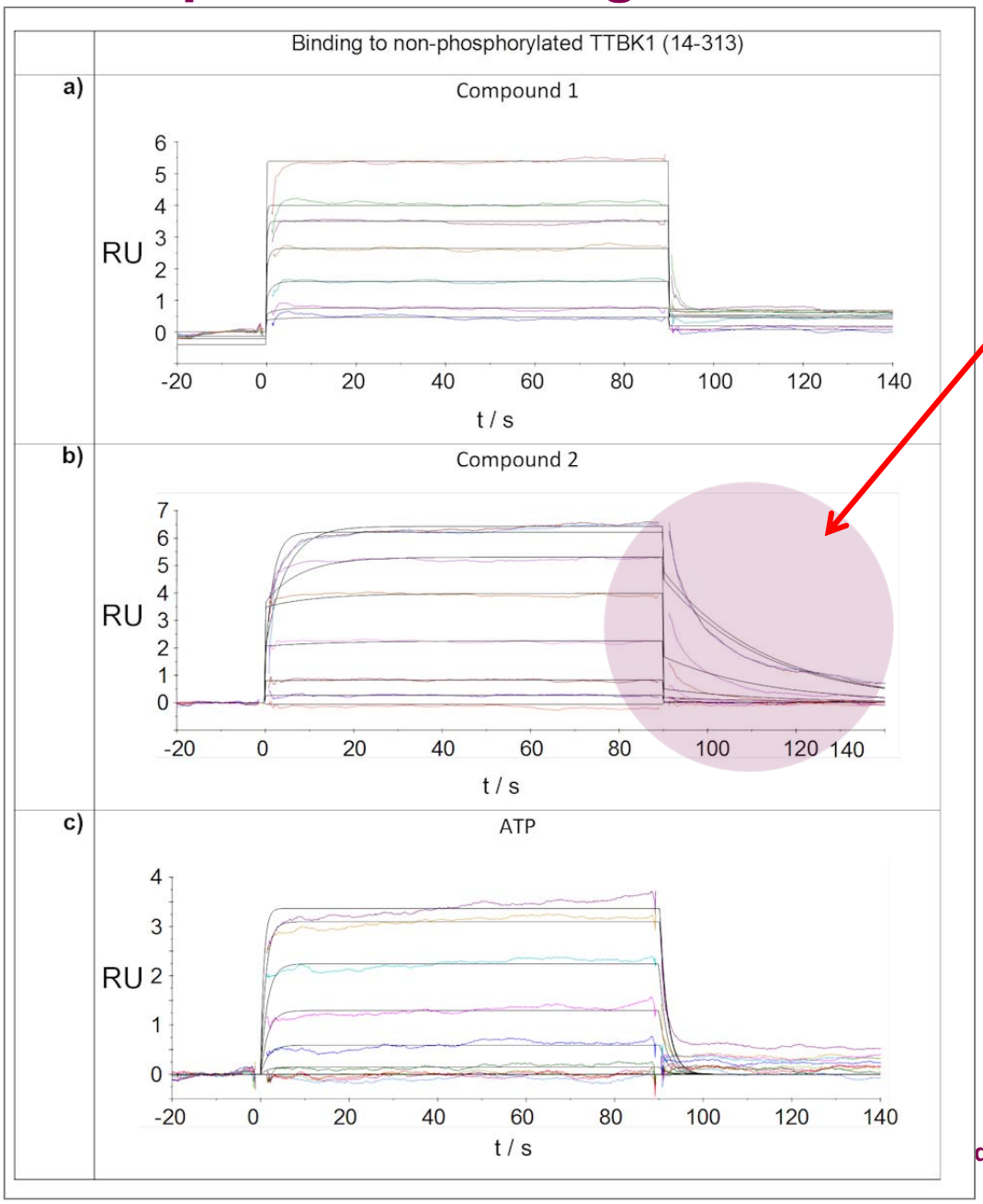



- Compound 1 enters the selectivity pocket, the DFG shows the same conformation as for ATP.

- Compound 2 does not reach the selectivity pocket, a peptide flip of the DFG led to different H-bond patterns

**ATP Compound 1 compound 2**

# Compound 2 binding shows slow kinetics




- The flipped peptide conformer has been observed in the MAP kinase family (e.g. both JNK3 and ERK2 but not P38 has this conformer as the "ground-state" conformer (PDB: 2exc, 1p38, 1wzy [21] ).


[21] F.C. Bernstein, T.F. Koetzle, G.J. Williams, E.F. Meyer Jr, M.D. Brice, J.R. Rodgers, O. Kennard, T. Shimanouchi, M. Tasumi, *J. Mol. Biol.* 1977, 112, 535-542.

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## Summary

- Structure of TTBK1 gave plausible explanation for its substrate specificity (“primed” substrate)
- Structure of ligand interaction is related to kinetic signature
- Distinct structural-kinetic behavior could be used for design of selective TTBK1 inhibitors.

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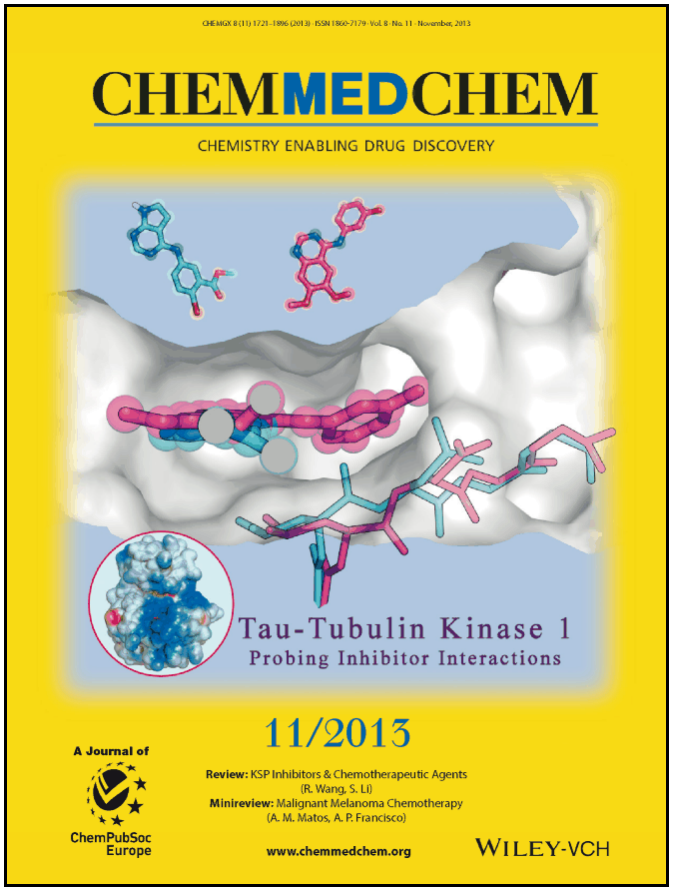
**Acknowledgement**

| | |
|---|---|
| Niek Dekker | (campaign lead/gene-to-protein) |
| Paul Wan | (protein) |
| Per Hillertz | (SPR) |
| Fritz Schweikart | (MS) |
| Lisa Wissler | (crystallization) |
| Yanlong Zhao* | (protein expression) |
| Mats Ormö | (assay dev.) |
| Fredrik Wågberg | (screening) |
| Ylva Gravenfors | (chemistry) |
| et al. | |

(* Viva Biotech, Shanghai)



Xue Y, Wan PT, Hillertz P, Schweikart F, Zhao Y, Wissler L, Dekker N. X-ray Structural Analysis of Tau-Tubulin Kinase 1 and Its Interactions with Small Molecular Inhibitors. ChemMedChem. 2013 Sep 13. doi: 10.1002/cmdc.201300274.

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