KINASES, PROTEIN PHOSPHORYLATION AND CANCER JUNIOR GROUP

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OVERVIEW

Rational and precise targeting of oncogene-driven signalling is a crucial but still pending challenge in current cancer research. Understanding the structural and molecular bases of oncogene activation and signalling is key for the design and development of better therapeutics. Our research focuses on the structural and molecular understanding of protein kinase function: how protein kinases are activated and regulated by posttranslational modifications and allosteric inputs, and how they assemble into macromolecular protein complexes to transmit signals inside the cell. We place special emphasis on how these mechanisms are corrupted in cancer due to oncogenic mutations and other oncogenic insults. Crucially, such atomic and molecular information can be translated into the design and development of more potent and specific protein kinase inhibitors, leading eventually to more effective drugs for the treatment of cancer patients.

We apply an integrated and multidisciplinary approach combining molecular biology for the generation of suitable constructs, protein biochemistry and biophysics for protein purification, quality assessment and functional evaluation, mass spectrometry (MS) for the quantification of posttranslational modifications, X-ray crystallography, and *in vivo* validation using *Drosophila* models. Furthermore, we use structure-guided drug discovery and MD simulation approaches to exploit structural and functional vulnerabilities for the design, development, and optimisation of optimal protein kinase inhibitors.

Graduate Students
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RESEARCH HIGHLIGHTS

In 2020, we made significant progress on the research lines initially established in the laboratory:

1. We elucidated important structural and molecular details about the precise mechanism of the catalytic activation and auto-regulation of the c-Src oncogene. By applying a systemic phospho-proteomic approach, we identified new c-Src autophosphorylation sites and revealed that "canonical" activating and repressive tyrosine residues actually play other important roles and functions not previously envisioned.
2. Another research line was directed at dissecting the function of CCDC6-RET, a RET oncogenic fusion and driver in NSCLC. We successfully purified this challenging protein in different isoforms and length-variants and, by applying an integrated approach, demonstrated that the full-length construct behaves as an active dimer in solution. Auto-phosphorylation assays demonstrated fast kinetics compared to RET wild-type constructs. Further phospho-site mapping by MS and dissection of the activation mechanism highlighted important roles for catalytic activity and substrate specificity through unexpected elements.
3. A third research line focused on the exploitation of structural and functional vulnerabilities in RET for the rational design and development of highly specific inhibitors. Our current paradigm is based on recently developed second generation RET inhibitors LOXO-292 and BLU-667 that showed excellent results in both preclinical models and early clinical trials, resulting in their timely FDA approval for the treatment of RET-rearranged or -mutated cancers. We are applying an integrated approach combining structural data, molecular docking, structure-guided molecular dynamics simulations, screening with both virtual and chemical libraries, together with biophysical and biochemical tools for functional validation. Following this multidisciplinary approach, we identified an allosteric interface in RET with a good druggability score that can potentially be targeted with allosteric inhibitors. Furthermore, we uncovered a new sub-pocket within the ATP-binding site that is exploited by highly specific second-generation type I RET inhibitors (FIGURE). This information will be crucial for the design and development of highly specific, clinically successful third generation RET inhibitors able to overcome refractory RET mutations. ■

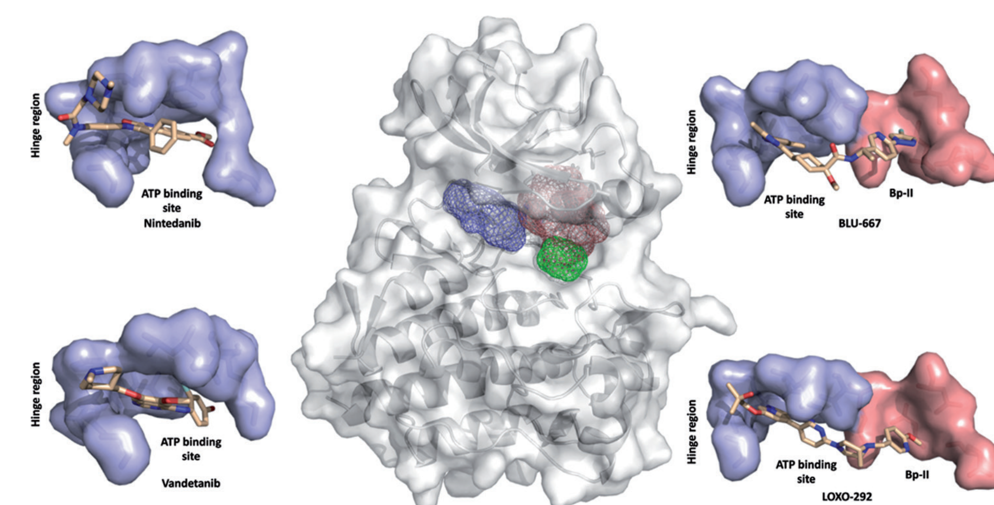

FIGURE Structure-based drug-discovery for new druggable pockets in RET. Cartoon representation of RET kinase domain with mapped druggable pockets within the active site depicted in mesh representation. The pockets are ranked from highest to lowest based on the colour code: red, green, and blue. We identified a new pocket within the ATP-binding site that is exploited by highly potent and specific type I RET inhibitors (Shehata *et al.*, submitted).