正構及異構調控藥設計之清華開發平台: 從結合位偵測、分子設計、作用力量測到藥物遞送

A NTHU-based platform for allosteric drug development - from allosteric site identification, drug design, affinity measurement to drug delivery

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Two years ago we proposed to identify allosteric site, design the drug against it, measure the affinity and formulate drug delivery carriers for the designed drugs when needed. Two years later, we successfully refined our tdLRT theories (https://doi.org/10.1101/677617) to discover a new allosteric site on ATG4B (Fig 1F), design drugs against it (Fig 1G), validate the efficacy *in vitro* (biochemically and in three cancer cell lines; Fig 1H) and *in vivo* (Fig 1B and 1C) and eventually demonstrate a synergistic drug efficacy (data included in recent US provisional patent application) when co-used with our published orthosteric drug Tc (Liu et al., *Theranostics*, 2018). An electrification-based, self-powered biosensor has been developed by Prof Z.H. Lin to find surface voltage growing with the drug-protein affinity (Fig 1I,J). Also, with this 深耕 input, we also developed enzyme-substrate interface blocker Vb which had demonstrated good synergistic effect when co-used with Tc (Fig 1B,C,D). This joined research has brought external funding of 1.4 million TWD for Prof Yang and Chen (SPARC program, MOST), nomination (to be announced within a month) for a 20 million Industrial Value Creation Program (IVCP; 價創二:育新創) fund for our spin-off CADD/ CDMO/CRO start-up company, which just attended the Merck KGaA Inc. deep-dive workshop and won the 50K € support to develop a scRNAseq-based strategy against drug-resistant cancer cell lines. Leading a result of 14 publications of >10 impact factors in 2020-2021 in addition to 6 patents, two member PIs in our team were promoted to their full professorship with winning the Ta-You Wu Memorial Awards (吳大猷獎) at the same time. We plan to apply external funding further after this term period with our current results.

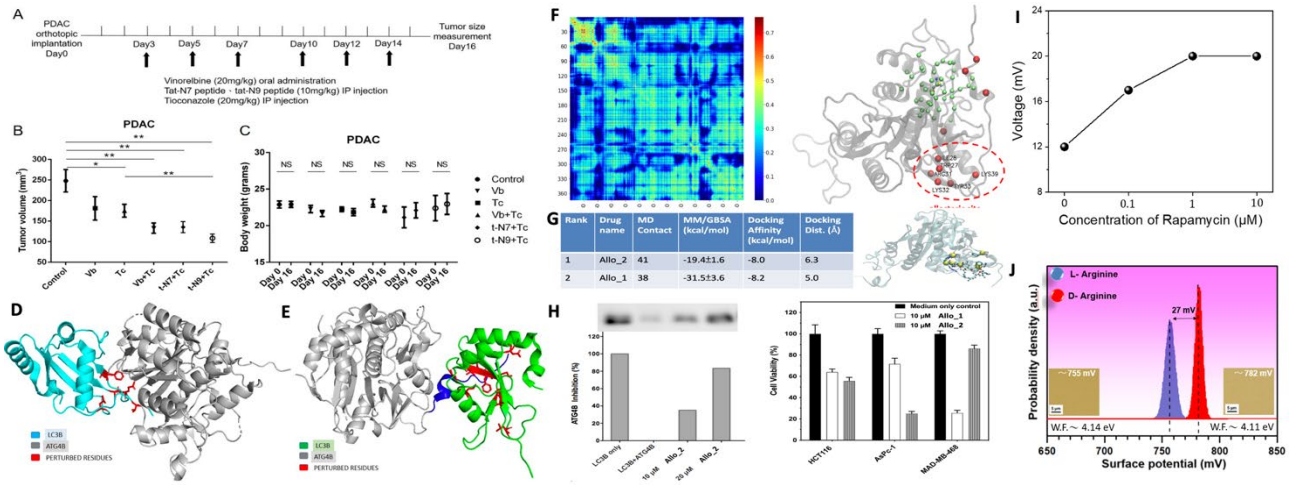

Figure 1. ATG4B can be suppressed through protein-protein interface, inter-molecular allosteric site and intra-molecular allosteric site. In animal experiments **(A,B)**, this drug can effectively inhibit tumor growth in mouse orthotopic pancreatic cancer cell models **(B)** and does not significantly affect the mice's body weight **(C)**. Furthermore, we have also confirmed that the allosteric regulatory peptides tat-N7 and tat-N9 peptides designed by Yang's lab can effectively inhibit tumor growth in mouse especially when co-used with Tc **(D,E)** Professor Yu's laboratory has confirmed, by using NMR HSQC spectroscopy, that Vb **(D)** and tat-N7 **(E)** are indeed bound to the interface of LC3-ATG4B and the N-terminus binding site of LC3, respectively. In addition,

Yang's lab found another allosteric site **(F)** on ATG4B based on the linear response theory, and the top two FDA-approved drugs screened by our DRDOCK pipeline (Fig 1) were found to bind with this site with favorable contact and energy **(G)**. We also found their tumor suppression capability on colorectal, pancreatic, and breast cancer cell lines **(H)**. In 2020-2021, with collaborating with us, Prof Zong-Hong Lin developed a new self-powered nanosensor using solid/liquid friction electrification to render electrical signals as functions of the affinity between target proteins and a number of tested drugs, which is a big step forward to our low-cost but high-throughput drug-affinity measurements. This is definitely important for our FF optimization and training of a practical AI model. In panel **(I)**, the team showcased the IC50 of Rapamycin to FKBP protein (20 μM) is in tens of nanomoles, which is close to the literature results (<10 nM). The Lin laboratory team also realized the difference in the surface potential of L- and D-form amino acids measured by KPFM **(J)**.

Team publication list with impact factor > 10 (2020 – 2021):

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5. Y.-J. Fan et al. **Zong-Hong Lin** (2020) Enhancing the sensitivity of portable biosensors based on self-powered ion concentration polarization, electrical kinetic trapping. *Nano Energy*, 69, 104407.
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