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Industrial Postdoc/Postmaster : กำลังคนคุณภาพสูงเพื่อสนับสนุนอุตสาหกรรมยุทธศาสตร์ของประเทศ

30 มีนาคม 2567

สำนักงานพัฒนาวิทยาศาสตร์และเทคโนโลยีแห่งชาติ

Identification and Validation of potential *Plasmodium* E3 Ligases for PROTAC Platform

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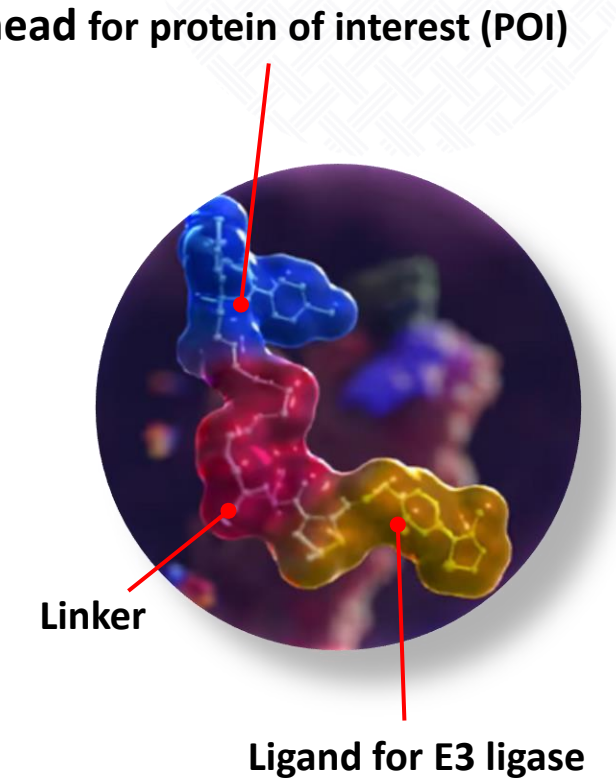
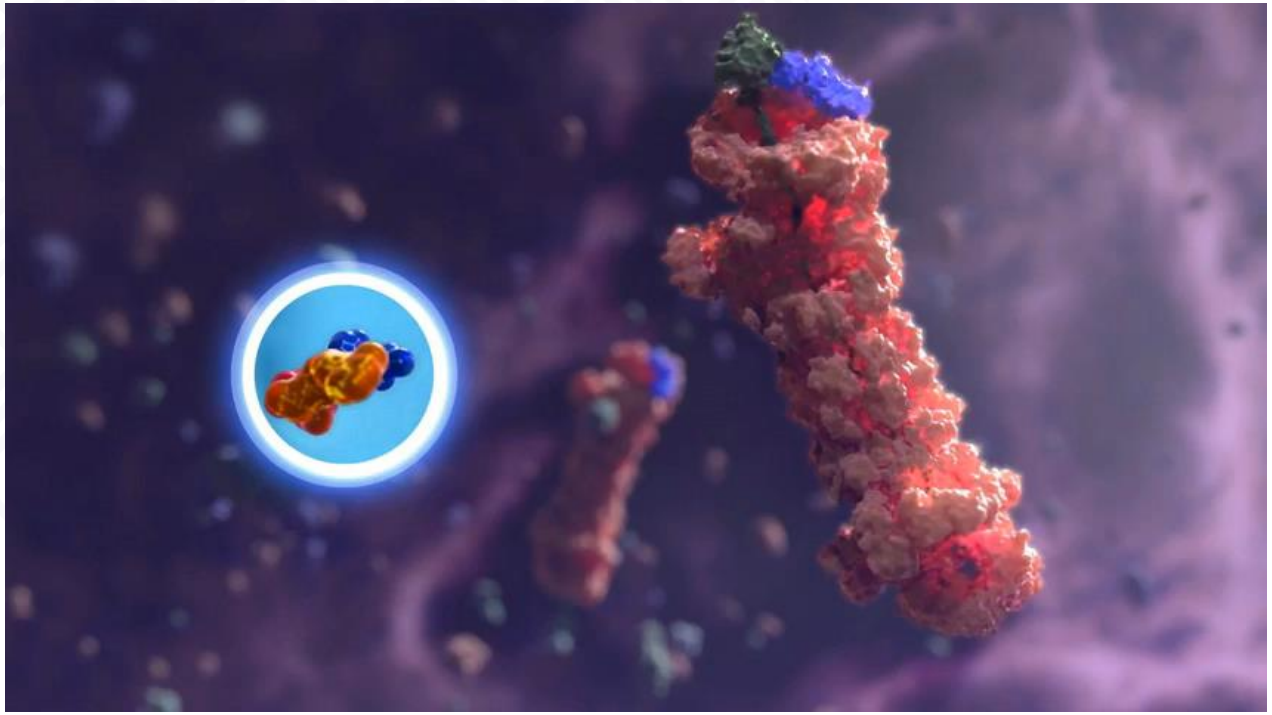
สวทช.

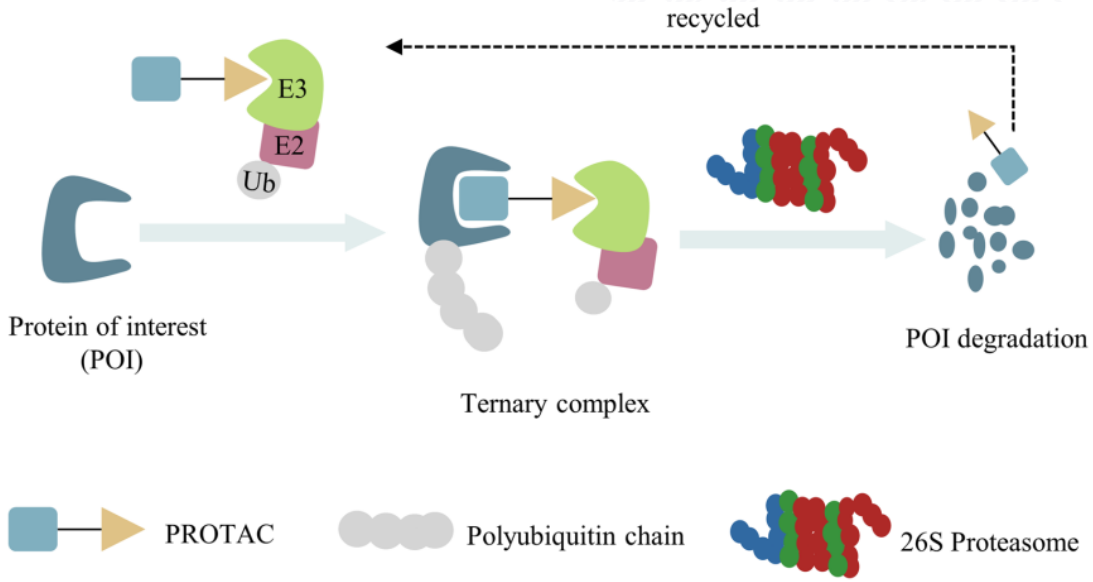
PROteolysis-TArgeting Chimeras (PROTACs)

- A rapidly developing engineering technology for targeted protein degradation using the ubiquitin–proteasome system (UPS).
- Potential clinical treatments for diseases such as cancer, immune disorders, viral infections, and neurodegenerative diseases.
- Heterobifunctional small molecules that degrade target proteins by hijacking the ubiquitin–proteasome system (UPS).
- Consisting of two ligands joined by a linker:
  - A warhead binds to a protein of interest (POI).
  - E3 ligase ligand recruits and binds to an E3 ubiquitin ligase.
  - Forming POI-PROTAC-E3 ubiquitin ligase ternary complex.
- PROTAC induces the ubiquitination of the POI and its subsequent degradation by the 26S proteasome.



PROTACs: How it works?





Liu et al., 2022

- PROTAC molecule can bind to E3 ligase and the target protein to form POI-PROTAC-E3 ligase ternary.
- Simultaneous binding of the POI and E3 ligase by the PROTAC induces ubiquitylation of the POI and its subsequent degradation by the ubiquitin–proteasome system (UPS).
- PROTAC is then recycled to target another copy of the POI.

No reports on PROTAC-type drug for infectious disease such as Malaria.

Credits: https://xvivo.com/blog/illustrating-the-unconventional-protac-technology-for-disease-therapy/

Malaria

Malaria is a life-threatening disease.

Caused by ***Plasmodium* parasites** that are transmitted to people through the bites of infected female *Anopheles* mosquitoes.

The first symptoms: **fever**, **headache** and **chills** (10–15 days after infected)

Left untreated, *P. falciparum* malaria can **progress to severe illness and death** within a period of 24 hours.

Preventable and curable.

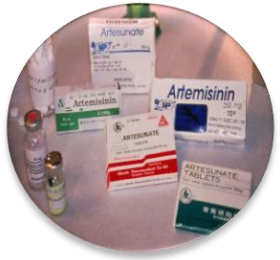
Thailand Incidence of Malaria

In Jul–Sep 2023, Greater Mekong subregion (GMS) countries reported **52,956** malaria cases.[1]

In Thailand, **5,206 cases**, a 48% increase compared to the same time period in 2022. *P. falciparum* + mixed cases and *P. vivax* constituted 4% and 95% of cases, respectively.[1]

Cause: the surge of cases in Myanmar spilled over to Thailand, as people sought health care across the border.

Treatment: Artemisinin-based combination therapies (ACTs)



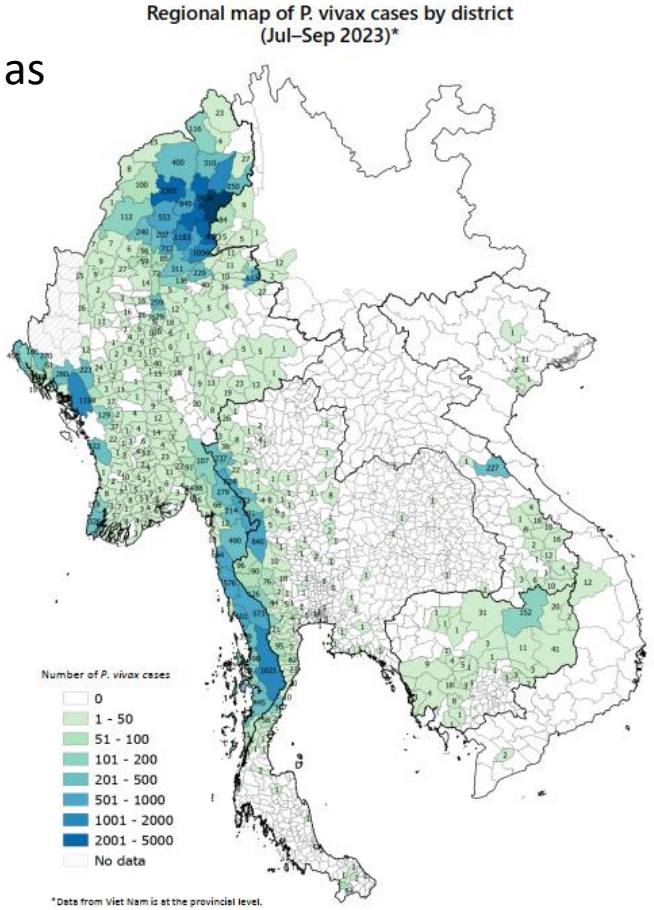
PROBLEM

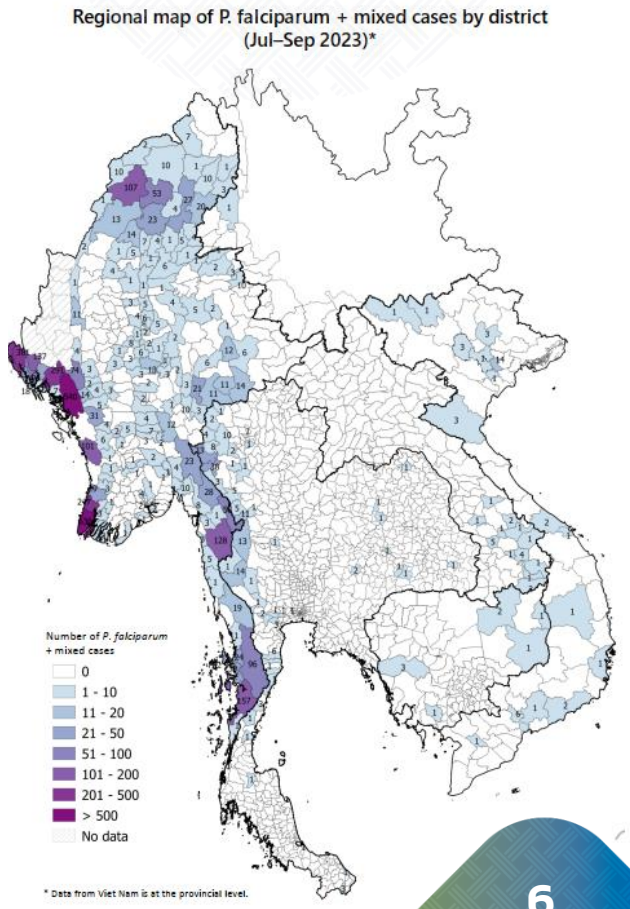
Drug-resistance parasites

CHALLENGE

New drug development: PROTAC

- No structure is available for *Plasmodium* parasite E3 ligases.
- The function of parasite E3 ligases should be characterized for PROTAC development.





[1]WHO-Mekong malaria elimination: epidemiology summary, volume 23, July–September 2023

Plasmodium E3 ligase candidates

Murine Double Minute 2 (MDM2)

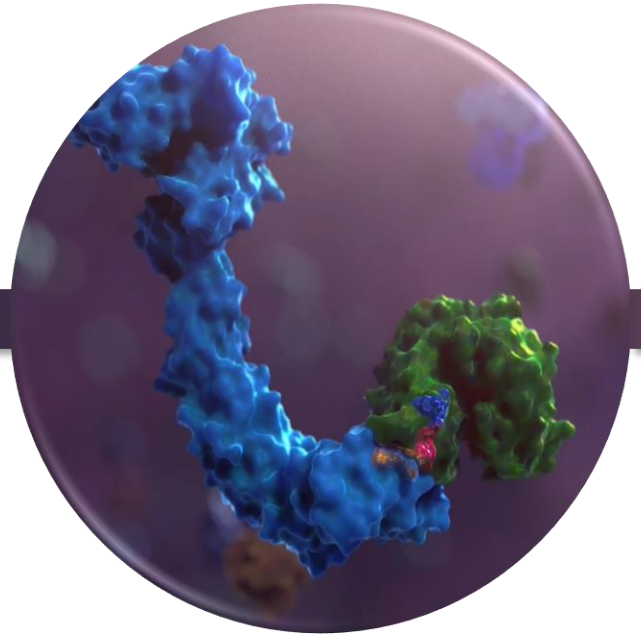
- Gene ID: PF3D7-0518200 SWIB/MDM2 domain-containing protein from Plasmo-DB (Vieira and Coetzer, 2016).
- MDM2 binds and ubiquitinates p53, facilitating it for degradation by the ubiquitin–proteasome pathway and represses p53 transcriptional activity.
- Idasanutlin-based chimeric compounds show target degradation activity in *P. falciparum.*
- Nutlin-based PROTACs recruit human MDM2-type E3 ligases (Hines et al 2020).

NOT4 NEW

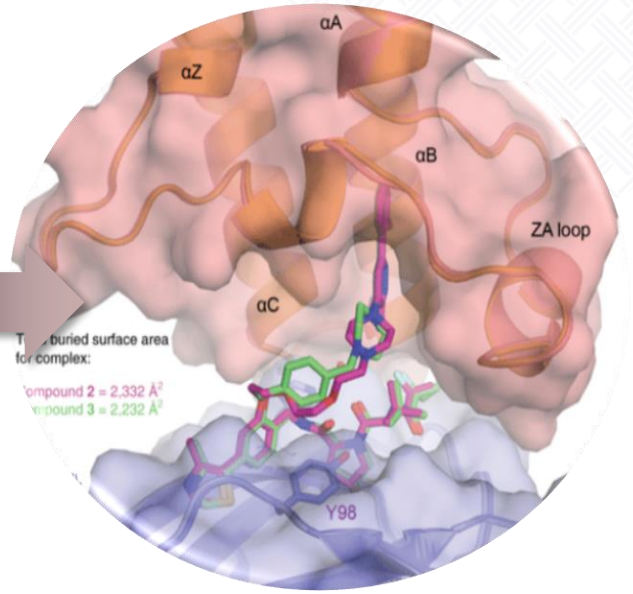
- Gene ID: PF3D7_1235300 CCR4-NOT transcription complex subunit 4.
- Recruited by co-immunoprecipitation from *P. falciparum* parasites expressing HA tagged-DHFR-TS protein briefly treated with PROTACs.
- A component of the CCR4-NOT (carbon catabolite repressor 4–negative on TATA) complex for co-translational quality control. (Albert et al., 2002; Panasenko, 2014; Buschauer et al., 2020).
- An E3 ligase of the RING family type that catalyzes protein ubiquitination (Panasenko, 2014).

Overall experiments

Cloning, expression and purification of potential *Plasmodium* E3 ligase



Characterizations
(Enzyme activity and binding assay)



Protein Crystallization and structure analysis

- E3 ligase activity assay (Auto-ubiquitylation Assay)
- Binding assay using Differential Scanning Fluorimetry (DSF)

Results

E3 ligase candidate: Murine Double Minute 2 (MDM2)

>PF3D7_0518200.1| Plasmodium falciparum 3D7 | SWIB/MDM2 domain-containing protein | protein | length=131 (PfMDM2 protein)
MKLLRTNIFSAQNFLFRNNYAHVFNSLQNKKNFTTDNGKHDNTKKKRPNGLQI
DCEIKSPLKEFLNADTASRVFVLKYAWKYIKDNNLQNPNMKRKIIPDDKLKQV
LDKDEVDILEVPKLLFKHMSSIRKE*
(Vieira and Coetzer, 2016)

codon optimized for expression in *Escherichia coli*

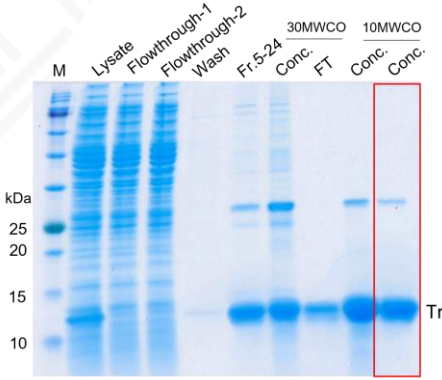
ATGAAATTACTAAGGACAAATATATTTTCAGCGCAGAACTTTTTGTTTCGTA
ATAACTATGCGCATGTGTTCAACAGCCTGCAAAACAAAAAGAACTTCACCAC
GGACAACGGCAAACACGACAATACCAAAAAGAAGCGCCCAAATGGTCTGCAG
ATTGATTGCGAAATTAAGTCCCCGCTGAAGGAGTTCTTGAATGCAGATACCG
CTTCTCGTGTTTTTGTTTTAAAGTACGCCTGGAAATACATCAAGGACAACAA
CCTGCAAAACCCGAATATGAAACGCAAAATCATCCCGGATGATAAGCTCAAG
CAGGTCCTGGATAAAGACGAGGTGGACATCCTGGAAGTTCCGAAATTGCTGT
TCAAGCACATGAGCAGCATTCGTAAAGAGTAA

Mitochondrial localization peptide

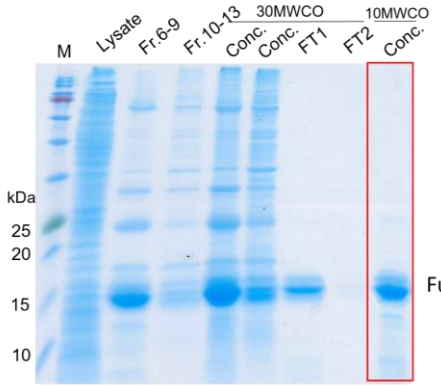
Full length *Pf*MDM2 (396 bp)

Truncated *Pf*MDM2 (306 bp)

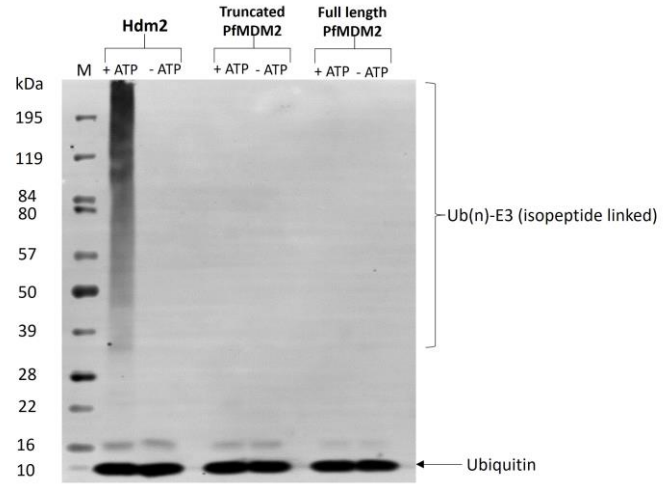
Expression vectors containing C-terminus His-tag for purification

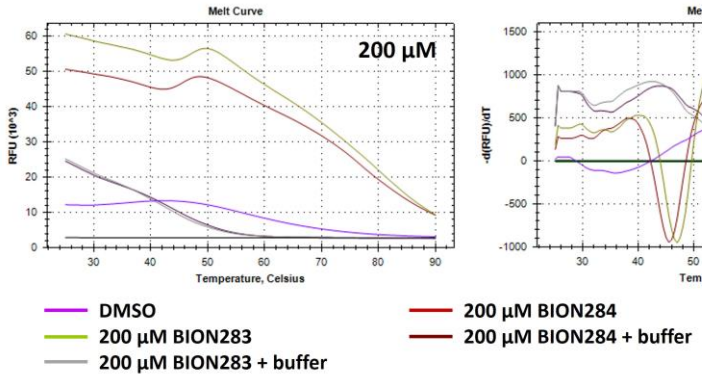


Truncated *Pf*MDM2
(~12 kDa)
(28.64 mg/ml)

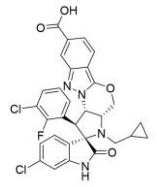


Full length *Pf*MDM2
(~19 kDa)
(11 mg/ml)

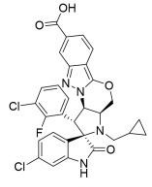




Differential Scanning Fluorimetry (DSF)



BION-283



BION-284

Cloning

Expression and Purification

Characterization

Summary

- PROTAC could be a promising technology for development of new drug for Malaria treatment to overcome drug resistance.
- Identifying *Plasmodium* E3 ubiquitin ligases is important for PROTAC development including new drug discovery.
- At present, *Plasmodium* MDM2 (PF3D7-0518200) was selected for the identification and validation of E3 ligase function using recombinant protein by cloning and expression in *Escherichia coli* system.
- Unfortunately, the E3 ligase activity (Auto-ubiquitylation Assay) could not be observed for *Pf*MDM2 comparing to control protein Hdm2 under this assay condition.
- However, an increases in melting temperature (ΔTm = +10 °C) have been observed between *Pf*MDM2 and idasanutlin derivatives (BION283 and BION284) by DSF assay suggesting some interaction between compounds for E3 ligase ligands and E3 ligase.
- New E3 ligase candidate *Pf*NOT4 has been identified from co-immunoprecipitation and currently being cloned and expressed for functional study.

ขอขอบคุณผู้ให้การสนับสนุน Industrial Postdoc

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โดยหน่วยบริหารและจัดการทุน ด้านการพัฒนากำลังคน และทุนด้านการพัฒนาสถาบันอุดมศึกษา
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thank you

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