**_Trypanosoma brucei_ 20S proteasome homology modeling and validation of compound interaction assists in designing novel proteasome inhibitors**

**Srinivasa Rao, Li Tian, Hazel Koh, Vanessa Manoharan, Chen Yen-Liang and Jan Jiricek**

Phenotypic high through put screening resulted in identification of triazolopyrimidine (TP) class of inhibitors that are active against all kinetoplastids. Whole genome sequencing of resistant *T. cruzi* mutants against these inhibitors showed single nucleotide polymorophism (F24L and I29M) in β4 sub-unit of 20S proteasome. The proteasome sequence is very well conserved across 3 different kinetoplastids. Ectopic expression of β4 F24L and I29M mutations in *T. brucei* resulted in significant shift in $IC_{50}$ against TP series compounds, thus validating proteasome as the target. In the present study, we developed *T. brucei* 20 S proteasome homology model by using human 20S proteasome template (PDB: 4R67). The differential $IC_{50}$ data for multiple compounds generated using proteasome mutants helped in visualizing binding pocket between β4 and β5. Further, the model showed a few possible new interactions namely, S96 with flourophenyl group, Y113 and G129 form hydrogen bonds with amide group and one of the 2 nitrogens in the TP core probably interacting with the T1 of β5 subunit. The TP compounds have low solubility and low brain penetration. The homology model is helping medicinal chemists to design new compounds. In parallel, β4 mutants (F24L and I29M) are assisting in “on target lead optimization” of compounds having core and side-chain modifications in order to optimize for better pharmacological properties. Further validation of probable β5 interactions is being investigated.