Homework due at start of class on July 22

Student Discussion Leaders: Chris Choe, Tracy Ngo, Julia Olson (answers below by Greg)

Read: "*Toxoplasma gondii* calcium-dependent protein kinase 1 is a target for selective kinase inhibitors" (PubMed ID 20436472)

Suggestions/Notes:
Access the article published by *Nature Structural & Molecular Biology* by going through the UW electronic journals. The NIH Public Access Author Manuscript is also available. However, the figure numbers in this version do not line up with the text.

You *do not* have to understand Table 1. Table 1 concerns the crystallography data that Ojo et. al. obtained . Understanding this table is not entirely relevant for our discussion. If you do understand crystallography, you can interpret it at your leisure.

Background Information:
We've spent the quarter studying malaria, which begs the question: why would we suddenly read a paper about *Toxoplasma*? *Toxoplasma* and *Plasmodium* are phylogenetically related and share similarities amongst their calcium-dependent protein kinases (CDPKs).

CDPKs are essential in both species for regulating host cell invasion. As CDPKs only appear in certain plant species and Alveolates (the superphylum containing *Plasmodium* and *Toxoplasma*), studying CDPKs may yield drugs that can selectively target for *Toxoplasma* (and malaria) without affecting protein kinases necessary for human cell function. As *Toxoplasma* is more amenable to lab growth than *Plasmodium*, *Toxoplasma*, to an extent, can be used as a representative species for the study of certain aspects of malaria.

However, one fact that is not mentioned in the assigned article should be noted. Unlike *Toxoplasmdium*, *Plasmodium* CDPKs do not use a glycine gatekeeper, which may cause issues with using bumped kinase inhibitors (BKIs).

For further background understanding, you may refer to:
"*Toxoplasma* and *Plasmodium* protein kinases: Roles in invasion and host cell remodeling" (PMID 22154850) or "Transport and trafficking: *Toxoplasma* as a model for *Plasmodium*" (PMID 10645546).

Worksheet to hand in:

1. Generally speaking, what are BKIs and how do they work?

BKIs are compounds with a structural "bump" that prevents them from fitting into ATP binding pockets with large gatekeeper residues. Therefore BKIs are generally inactive against mammalian kinases.

2. What is role of CDPK1 in parasite invasion?

Ojo et al. cite reference 14, which says that CDPK1 probably regulates parasite motility and attachment to host cells by phosphorylating three substrate proteins. Reference 14 speculates that phosphorylation of these proteins may affect movement of micronemes (secretory organelles) into position for secretion and/or fusion of micronemes with the cell membrane. The details are currently unknown, however.

3. Why is it important that a glycine gatekeeper is used rather than, say, the gatekeeper amino acid gatekeepers (Phe, Met) typically used by humans and mammals?

Glycine is the smallest of the 20 major naturally occurring amino acids, meaning that it will take up minimal space in the ATP binding pocket, leaving more room for compounds such as BKIs to bind. Phenylalanine and methionine have larger R groups and do not accommodate BKIs nearly as well. The glycine gatekeeper occurs naturally in CDPK1, which is fortunate in allowing BKIs to be used against it.

4. Describe briefly the method by which the drug was validated.

The key question was whether the BKIs' inhibition of host-cell invasion is due primarily to its inhibition of CDPK1. 1-10 µM BKIs did not reduce invasion by parasites supplemented with BKI-insensitive CDPK1 (G128M), thus showing that CDPK1 was indeed the BKIs' primary target.

5. What issues still exist with using a GFP assay as described in "TgCDPK1 appears in the cytoplasm and nucleus of T. gondii cells"?

One can never be completely sure that adding a GFP tag to a protein does not affect its localization. Also, the apparent presence of CDPK1 in both the nucleus and cytoplasm leaves a lot of questions unanswered about what and where CDPK1's substrates are.

6. In what ways can Toxoplasma evolve resistance and how would co-administration of drugs reduce resistance?

A mutation of the gatekeeper residue would render Toxoplasma resistant to BKI-related drugs. However, co-administration of a drug with a different target would help eliminate all parasites from an individual before resistance could arise and spread.