Clustered, Not Scattered: Understanding *T. gondii*'s Clustered Invasion Behaviour

The apicomplexan protozoan parasite *Toxoplasma gondii* has a unique ability to infect any nucleated cell within warm blooded mammals. As an obligate intracellular parasite, *T. gondii* must scavenge nutrients from their host for survival and replication. *In vitro* culture of *T. gondii* tachyzoites shows an intriguing phenomenon whereby multiple tachyzoites will invade the same host cell, within a homogeneous cell population, while neighbouring cells remain uninfected. Biologically, this must surely be detrimental to parasite survival, replication as sequestered nutrients and amino acids are limited within the host cell. This raises the question, what factors within a single cell population drive tachyzoites to choose to invade the same host cell and leave neighbouring cells with low or no infection?

This study investigated whether multiple invasions of host cells was a consequence of low parasite load. Multiplicity of infection (MOI) assays spanning a 1:8 to 8:1 tachyzoite-to-host-cell ratio, in HFF, SH-SY5Y, and U2OS host cell lines demonstrated that increasing MOI correspondingly increased the frequency of multiple tachyzoites invading a single cell, while neighbouring cells remained uninfected. These results were followed up by performing sequential invasion assays using mNeonGreen and tdTomato expressing RH tachyzoites. Results indicated that, upon secondary inoculation tachyzoites preferentially invaded host cells that were already infected rather than uninfected neighbouring cells.

From these results, four working hypotheses are under experimental investigation; (1) is the phenomenon of multiple cell invasion an artefact of *in vitro* 2-dimensional cell culture? (2) Are changes in the cell surface proteome, or changes in membrane tension, during the host cell cycle making them more permissible for tachyzoite invasion? (3) Do changes in host cell membrane tension following primary tachyzoite invasion provide a thermodynamic advantage for subsequent invasions? (4) Can changes in the host cell or parasite secretome following initial invasions increase specific cell tropism or host cell permissibility?