THE DEVELOPMENT OF AN *IN VITRO* PROTEIN INTERACTION ASSAY FOR ARF GTPASE ACTIVITY

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The discovery and the development of druggable cancer targets has been revolutionalized over the last decade, most notably the shift from a one size-fits-all-approach that emphasized cytotoxic chemotherapy to a personalized medicine strategy that focusses on the exploitation of particular genetic variants, dependencies and the vulnerabilities of cancer cells. Arf GTPases are key regulators of the secretory and endocytic pathways. Based on existing evidence gained from gene manipulation and cell biological investigations, Arf1 and Arf6 are fundamentally important for cancer cell proliferation and metastasis and may be promising targets for the development of anti-cancer drugs. To confirm their status as cancer drug targets using chemical validation experiments, novel Arf GTPase inhibitory compounds are needed. This requires the development of complex in vitro protein- protein interaction assays that can be used to screen chemical libraries for Arf GTPase inhibitors.

Arf GTPases are activated by exchanging bound GDP for GTP, a reaction which is facilitated by accessory ArfGEFs (guanine nucleotide exchange factors). One conceptually promising approach to detect Arf GTPase activation is to immobilize His-tagged Arf1 and Arf6 on nickel-coated plates and measure their ability to recruit GST-GGA3 to the plate using a colorimetric GST enzyme assay (GGA3 is an effector protein that only binds to active, GTP-bound Arf). Inclusion of an ArfGEF in the assay would allow for the screening for compounds that inhibit the GEF-catalyzed activation of the Arf GTPases. To facilitate development of the assay, the cloning of the human GGA3 GAT domain into a GST expression plasmid followed by expression and purification of the GST-GGA3 fusion protein from *E. coli* is required, as well as the expression and purification of the ARNO Sec7 domain (ARNO is an ArfGEF for Arf1 and Arf6). The coding sequence of the GGA3 GAT domain has been obtained by PCR amplification and plasmid cloning is currently in progress. The ARNO Sec7 domain has been successfully expressed and purified as a His-tagged protein from recombinant *E. coli*.