BIOCHEMISTRY SEMINAR

Determine the mechanism by which the D630Y mutation constitutively activates PLCb4

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Uveal Melanoma (UM) is the most prevalent malignant intraocular tumor, making up 5% of all melanoma diagnoses. It is an extremely deadly disease, as 50% of patients develop liver metastases and fewer than 15% survive beyond 12 months. The majority UM is caused by activating mutations in either the heterotrimeric G protein subunit Gaq/11 or its downstream effector enzyme phospholipase Cb4 (PLCb4). While it is known how mutations in Ga q/11 render it constitutively active, the same is not true for PLCb4. The patient-derived D630Y mutation in PLCb4 results in the lipase having maximum activity, such that it no longer responds to regulation by Gaq/11. Based on other structures of PLCb enzymes, the mutation is located on a solvent-exposed loop in the catalytic domain, distant from known regulatory elements. This aspartic acid is highly conserved across PLC enzymes and in all cases to date, its mutation increases activity. To identify how D630Y activates PLCb4, I used cryo-electron microscopy to determine reconstructions of both PLCb4 and the PLCb4 D630Y mutant. These structures show unique features not previously observed in other PLCb enzymes, notably within established autoregulatory elements. I am now using in *cellulo* and *in vitro* functional assays to characterize the roles of the unique features in PLCb4 and how the D630Y mutation results in constitutive activation.

Monday, September 15, 2025 | 3:30 pm | BRWN 4102

PURDUE UNIVERSITY | James Tarpo Jr. and Margaret Tarpo Department of Chemistry

BIOCHEMISTRY SEMINAR

Using cryo-electron tomography to study in situ protein interactions in bacterial microcompartments

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α-Carboxysomes are bacterial microcompartments that enhance carbon fixation by encapsulating the enzyme Rubisco within a protein shell. Cryo-electron tomography (cryo-ET) has revealed that Rubisco molecules inside α-carboxysomes organize into lattices of self-polymerized fibrils. In this seminar, I will present my work on precisely mapping Rubisco particle locations and orientations using tomogram reconstruction and subtomogram averaging (STA), which enabled the calculation of an *in situ* Rubisco polymerization constant (K_{poly}). I will also discuss how these approaches are being extended to the propanediol utilization (Pdu) microcompartment of *Salmonella typhimurium*, to provide insight into how this compartment supports bacterial growth in the human gut.

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