IroT/MavN is a *Legionella* transmembrane Fe(II) transporter: metal selectivity and translocation kinetics revealed by *in-vitro* real-time transport

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**Introduction**

Legionella pneumophila is an intravascular pathogen which is the causative agent for Legionnaires’ disease. Survival of *Legionella pneumophila* within macrophages requires the formation of a specialized intracellular compartment known as the Legionella containing vacuole (LCV). *Legionella* utilizes the type IV-B Dot/Icm secretion system to inject effector proteins into the host cells and the vacuolar membrane at the host-pathogen interface. In such intracellular pathogens, iron is essential for growth and virulence. In *Legionella pneumophila*, a putative transmembrane protein inserted in the surface of the host pathogen-containing vacuole, IroT/MavN, facilitate intravacuolar iron acquisition from the host by an unknown mechanism, bypassing the problem of Fe(III) insolvability and mobilization.

**Critical steps involved in the growth and replication cycle of *Legionella pneumophila* containing macrophage. Figure adapted from reference 3.**

In this study we developed a platform for purification and reconstitution of IroT in artificial lipid bilayer vesicles (proteoliposomes) to investigate metal functions.

**Purification of IroT protein and setup of the real time transport assay**

LpIroT was purified by immobilized metal affinity chromatography (IMAC) and size exclusion chromatography (SEC) to > 95% purity.

**Vesicle characterization of LpIroT incorporated in proteoliposomes**

SOS-PAGE analysis of incorporation of LpIroT which reveals >90% incorporation into proteoliposomes.

**Selective Fe(II) transport by wtLpIroT incorporated proteoliposomes**

Metal tritons of M10 with 15 mM Fluozin-3 in the transport assay buffer in the presence of control liposomes.

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**Effect of mutations in the transmembrane region for Fe(II) translocation**

Concentration dependent LpIroT-mediated Fe(II) transport in proteoliposomes, containing mutations in the transmembrane region.

**Effect of mutations in potential residues in soluble domains**

C-term truncation mutant

C-term truncation mutant exhibited Fe(II) translocation below wtLpIroT and minimal for higher concentrations.

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**Conclusions**

We developed a platform for purification and reconstitution of IroT in artificial lipid bilayer vesicles (proteoliposomes). By encapsulating the fluorescent reporter probe Fluozin-3, we reveal, by real-time metal transport assays, that IroT is a high affinity secondary active iron transporter selective for Fe(II) over other essential transition metals. Mutational analysis reveals important residues in the transmembrane helices, soluble domains and loops important for substrate recognition and translocation.

**Acknowledgement**