**Investigating the potential of the ErpY-like protein of *Leptospira* in regulating hemostasis**

**Saswat Hota and Manish Kumar\***

Department of Biosciences and Bioengineering, Indian Institute of Technology Guwahati, Guwahati, Assam, 781039

E-mail: [*saswat.hota@iitg.ac.in*](mailto:saswat.hota@iitg.ac.in)

The onset of leptospirosis caused by the pathogenic *Leptospira* depends on its ability to survive in the host by evading and countering the immune response and spreading to different host tissues [1, 4]. Like other pathogens, *Leptospira* expresses multiple virulence factors factors that interfere with immune signaling pathways, inhibiting the host's defense mechanisms to facilitate infection and pathogenesis [3, 4]. In a recent study, an outer membrane surface-exposed ErpY-like lipoprotein (LIC11966) has been proposed to be expressed exclusively by pathogenic *Leptospira* and helps increase the virulence capacity. Our research group has shown that the ErpY-like protein is a supramolecule with moonlighting effect [3]. ErpY-like protein contributes to the bacterium's virulence by binding to different extracellular matrix components in mammalian hosts. The protein's interaction with complement regulators factor H (FH) and factor I (FI) enables *Leptospira* to evade the host complement system, further enhancing its pathogenicity [3,4]. Here we show that the recombinant form of this protein inhibits the clotting of platelet-poor citrated bovine plasma in a concentration-dependent manner. In fact, adding 2 µM of rErpY-like protein to 20% diluted bovine plasma completely halts the clotting process. Furthermore, rErpY-like protein at a concentration of 2 µM reduces fibrin clot formation in diluted bovine plasma by approximately 95%. The recombinant ErpY-like protein is capable of prolonging the clotting time of bovine plasma by more than 1.5-fold, by targeting both the extrinsic and common pathways of coagulation. These findings demonstrate the multifaceted function of ErpY-like lipoprotein in leptospiral infection, including its ability to impact coagulation rates in host organisms.

**Keywords:** *Leptospira*, Host-pathogen interaction, ErpY-like protein, anti-coagulation

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| Figure 1. The results of a time-dependent absorbance assay investigating the inhibitory effect of the recombinant ErpY-like protein on bovine plasma coagulation. Citrated bovine plasma (80 µL) was incubated with increasing concentrations (0.5 – 5 µM) of rErpY-like (A) or rClpP1 (negative control; B) for 15 min at 37°C in a microtiter plate. Following incubation, CaCl2 (15 mM) was added to the plasma, which was then mixed by pipetting after adjusting the volume to 100 µL with Tris-HCl buffer (pH 8.0). The absorbance at 320 nm was recorded at 37°C over time, and reactions were performed in duplicates, thrice independently. ClpP1 instead of ErpY-like was used as a control. |