### Title

Association Mechanism of Leishmania major Peroxidase and cytochrome c revealed through Brownian and Molecular Dynamics




### Journal

Biophysical Journal, 110(3)

### ISSN

0006-3495



### Authors

Hollingsworth, Scott A
Fields, James B
Chreifi, Georges
et al.



### Publication Date

2016-02-01

### DOI

10.1016/j.bpj.2015.11.295

# Posters

## Protein Structure and Conformation I

**224-Pos Board B4**

**Association Mechanism of Leishmania major Peroxidase and cytochrome c revealed through Brownian and Molecular Dynamics**

**Scott A. Hollingsworth**[1], James B. Fields[2], Georges Chreifi[1], Matthias Heyden[2], Anton P. Arce[1], Hugo I. Magaña-Garcia[1], Douglas J. Tobias[2], Thomas L. Poulos[1].

Molecular Biology and Biochemistry, University of California-Irvine, Irvine, CA, USA, [2]Chemistry, University of California-Irvine, Irvine, CA, USA.

Leishmania major, the parasitic causative agent of leishmaniasis, produces L. major peroxidase (LmP) to protect itself from host-generated reactive oxygen species. LmP, a heme peroxidase, catalyzes the peroxidation of mitochondrial cytochrome c (LmCytc). The association of LmP and LmCytc, which is known from experimental measurements to be very fast (~ 10ˆ8 M-1 s-1), does not involve major conformational changes and has been suggested to be dominated by electrostatic interactions. To probe the association and formation of this complex, we employed Brownian dynamics to model the association of LmP and LmCytc. These simulations were able to confirm the importance of the negatively charged LmP active site residue D211 in a blind study with concurrent experimental mutagenesis and crystallographic experiments. The simulations also reveal the previously unrecognized role played by the A helix of LmP in the initial association of the complex. In silico mutations of helix A help identify the role of four negatively charged residues of helix A of LmP that act as the initial point of association to LmCytc. Brownian dynamics trajectories suggest that complex formation occurs via a ‘‘bind and crawl’’ mechanism wherein LmCytc first docks to a location on helix A of LmP that is far from the active site, forming an initial encounter complex, then moves along helix A to the active site. An atomistic molecular dynamics simulation confirms the helix A binding site while new steady state activity assays and stopped flow kinetics measurements confirm the role of the helix A charges in the proposed association mechanism.