

***Figure 1. Comparison of bioassay readouts between the rhodamine substrate and casein-FITC.***

*Different concentrations of crude snake venoms (33, 1, 4, 1 and 0.4 μg/mL) were tested against three concentrations of rhodamine substrate (;;). A comparison was then made with the optimized casein-FITC (Column 4: 10ug/mL) bioassay. Row (****a****) Echis carinatus Row (****b****) Echis carinatus and Row (****c****) Echis pyramidum leakeyi.*



***Figure 2. Comparison of casein-FITC and rhodamine substrate bioassay readout.*** *Red chromatogram: E. ocellatus + casein-FITC substrate; blue chromatogram: E. ocellatus + rhodamine substrate; X-axis: time (min).**E. ocellatus venom was subjected to at-line nanofractionation after which the casein-FITC and rhodamine substrate bioassays were performed.**Top: Fluorescence bioassay trace of nanofractionated E. ocellatus venom with casein-FITC substrate [10 g/mL]. Bottom: Fluorescence bioassay trace of nanofractionated E. ocellatus venom with rhodamine substrate [500 nM].*



***Figure 3. Overlay of bioassay, UV and MS data.*** *Fluorescence bioassay signal obtained with rhodamine substrate (blue), eXtracted Ion Chromatogram (XIC of m/z 2325,54+14 with an exact mass of 30233 Da; orange), UV trace (green; plotted only for the relevant time frame in which bioactivity was observed), and Total Ion Chromatogram (TIC; dotted black) traces of nanofractionated venom from Lachesis muta.*

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***Figure 4.*** *Eight examples of time correlated MS, UV, and protease activity traces of nanofractionated venoms from different viperid species. Top left: Echis ocellatus; top right: Lachesis muta; second from top left: Echis carinatus; second from top right: Echis coloratus; second from bottom left: Echis pyramidium leakeyi; second from bottom right: Daboia russelii; bottom left: Macrovipera lebetina; bottom right: Calloselasma rhodostoma. The TIC and UV traces, top/black and middle/green respectively, were obtained concurrently during the fractionation of the same sample of venom which was subsequently subjected to the rhodamine bioassay to produce the fluorescence bioactivity trace shown in blue at the bottom of each figure. Traces were adjusted (aligned) according to the delay time between system components.*