

## De novo glycomics of the parasite *Cryptosporidium parvum*

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### Introduction

*Cryptosporidium parvum*, an obligate intracellular parasite, causes severe gastroenteritis. It poses a serious threat to public drinking water supplies and has been identified as one of the leading causes of infant mortality in underdeveloped countries. Several glycoproteins produced by *C. parvum* are critical components throughout its complex life cycle, and during host-parasite interaction. For a large mucin-like protein, GP900, the isoforms GP40/GP15 are important during host-cell invasion. While in the infective stage, the oocyst wall proteins (OWPS) (Chatterjee et al., 2010), play a role during transmission to a new host. A novel scheme of nano-flow-HPLCs coupled to high resolution MS/MS has, for the first time, allowed for confident identification of glycopeptides, glycoforms, and glycosites in *C. parvum*.

### Methods

*Cryptosporidium parvum* oocysts passaged through Holstein calves were purchased from a commercial source (BunchGrass Farms, Deary, ID). Cleaned oocysts were broken using sonication and a bead beater. Soluble proteins were purified, and in-solution tryptic digestion was performed on biological replicates. Fritted, polyimide-coated fused silica tubing was packed in house with either SeQuant® ZIC®-HILIC (MerckMillipore) 5- $\mu$ m resin (normal phase), or Michrom Magic C18 3- $\mu$ m/5- $\mu$ m resin (reversed phase) to make nanoflow HPLC columns. Each sample was analyzed in replicate on separate normal or reversed phase columns using a nanoAcquity HPLC coupled to a LTQ-Orbitrap, with ACN/ 0.1% FA for the mobile phase. High resolution precursor ion spectra with MS2 spectra acquired, in the ITMS after CID, or in the orbitrap after HCD.

### Preliminary Results/Abstract

Many abundant glycopeptides were observed in the sample, without the requirement for a pre-enrichment step. Running paired samples on reversed phase and normal phase nano-flow chromatograph systems expanded peptide coverage for the proteins in the sample. Each chromatography system complemented deficit(s) in the other. Furthermore, combining the results from paired CID/HCD spectra produced a very informative glycomics profile, interpreted *de novo*. Prominent oxonium ions (B-series) allowed for effective screening of the MS/MS spectra for potential glycopeptides, and the presence of the corresponding Y-series fragments in both the CID and HCD spectra allowed for glycoform assignment; finally, the peptide backbone b/y series in the HCD MS<sup>2</sup> spectra provided definition of the glycosites.

The combined fragmentation techniques, together with the different chromatographic methods, enabled mapping multiple glycoproteins. Prior to this study, the glycoproteins of *C. parvum* had only been inferred from indirect observations. Traditional glycan profiling approaches were not feasible due to inherent difficulties with this parasite. In a truly *de novo* glycomics system, one must be absolutely certain the data belongs to the organism of interest. The only way this can be accomplished is to retain the peptide information with the glycan.

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### Novel Aspect

Combination of normal and reversed phase nanoflow-HPLC-MS/MS with different fragmentation techniques enabled *de novo* glycomics profiling of *C. parvum*.