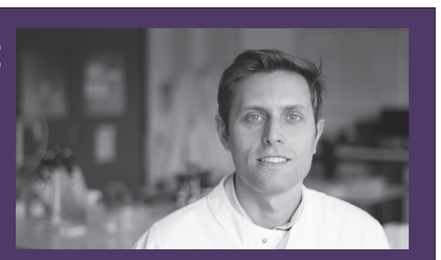
Investigating the role of insect vector glycosylation in African sleeping sickness transmission: Characterisation of procainamide-labelled tsetse fly saliva N-glycans

Radoslaw P. Kozak[1], Katherine Wongtrakul-Kish[1], Christopher Williams[2], Daryl L. Fernandes[1], Samirah Perally[2], Claire Rose[2], Daniel I.R. Spencer[1], and Alvaro Acosta-Serrano[2]

[1] Ludger Ltd, Culham Science Centre, Oxfordshire, UK; [2] Liverpool School of Tropical Medicine, Liverpool L3 5QA, UK



rad.kozak@ludger.com

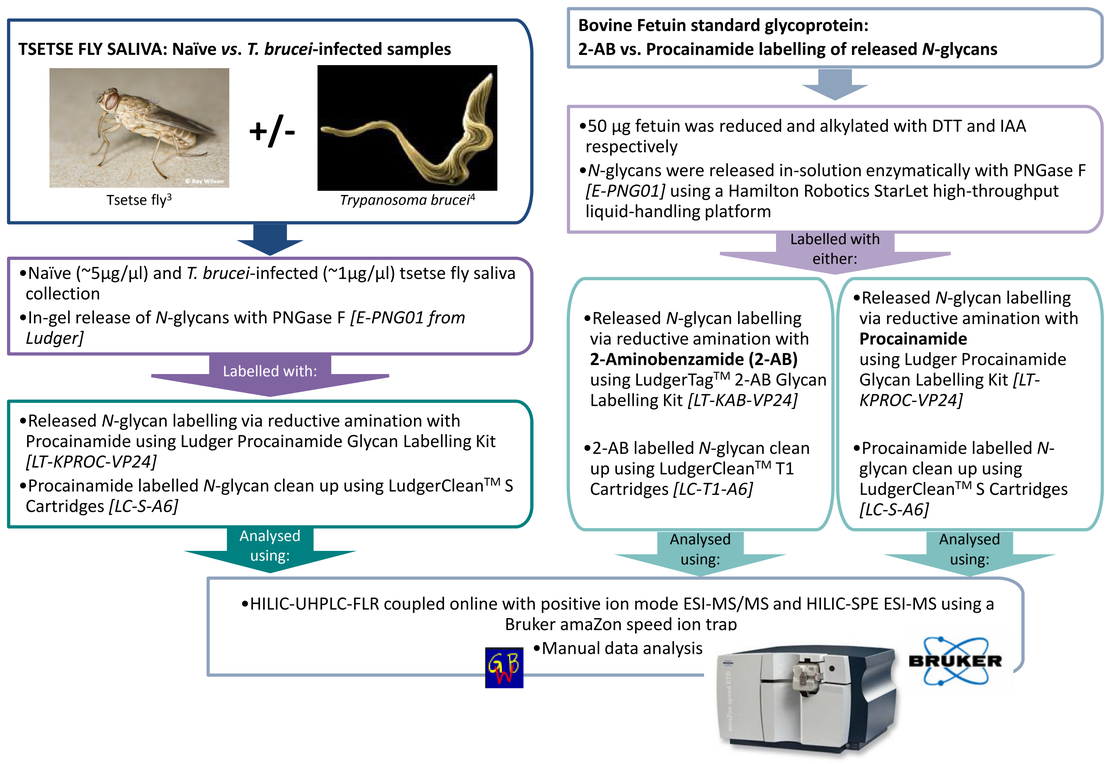
Introduction

African trypanosomiasis or sleeping sickness occurs in sub-Saharan African countries and is transmitted through the saliva of the haematophagus insect vector (tsetse fly) during feeding. The causative agents of this disease are trypanosome parasites of the species Trypanosoma brucei. Although sustained efforts to curb infection have resulted in a decrease of cases from 9,878 in 2009 to 7,216 in 2012[1], efforts to identify new drug targets to treat or prevent infection continue. Salivary glycoproteins have been reported to facilitate host infection through binding and transport of vector-borne diseases to host tissues, and also participate in host responses such as inflammation and immune response[2]. This role in infection presents new opportunities to identify the key mediators of transmission as well as to increase our understanding of the role of salivary glycans in haematophagus insects. The analysis of fly salivary glycoproteins however is challenging due to small sample volumes despite collection from several hundred flies, together with the need for high sensitivity.

Aims

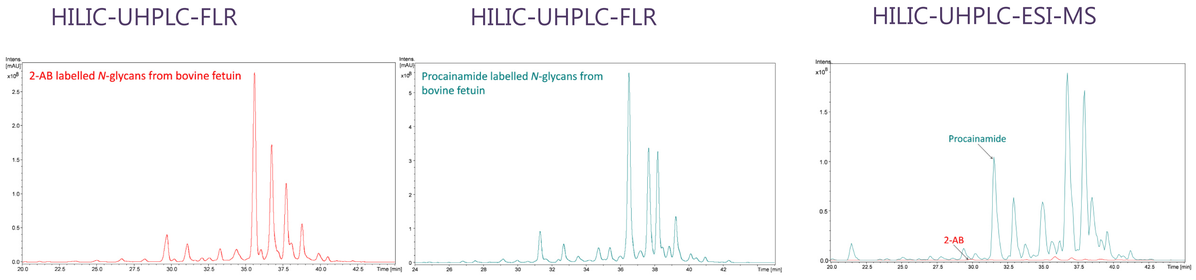
1. **To chose a glycomics workflow suitable for very small samples of complex biological fluids.** Specifically, we aimed to develop a suitable glycomics workflow coupling UHPLC with ESI mass spectrometry that would work well with very small samples of tsetse fly saliva. To this end we evaluated two candidate glyan labelling systems - 2-aminobenzamide (2-AB) and procainamide - using N-glycans enzymatically released from two standard glycoproteins, IgG[3] and bovine fetuin. Both labelling systems employed reductive amination with 2-picolineborane as the reductant. Our focus was on finding a method that worked in a highly sensitive and reliable way on with both mass spectrometric and fluorescence detection and that was suitable for future high throughput glycomics studies.
2. **To apply the chosen glycomics workflow to infected and non-infected tsetse fly saliva samples**. The emphasis was to investigate the role of N-glycosylation in vector based trypanosome infection by characterising the N-glycome of T. brucei-infected and naïve tsetse salivary glycoproteins.

Methods



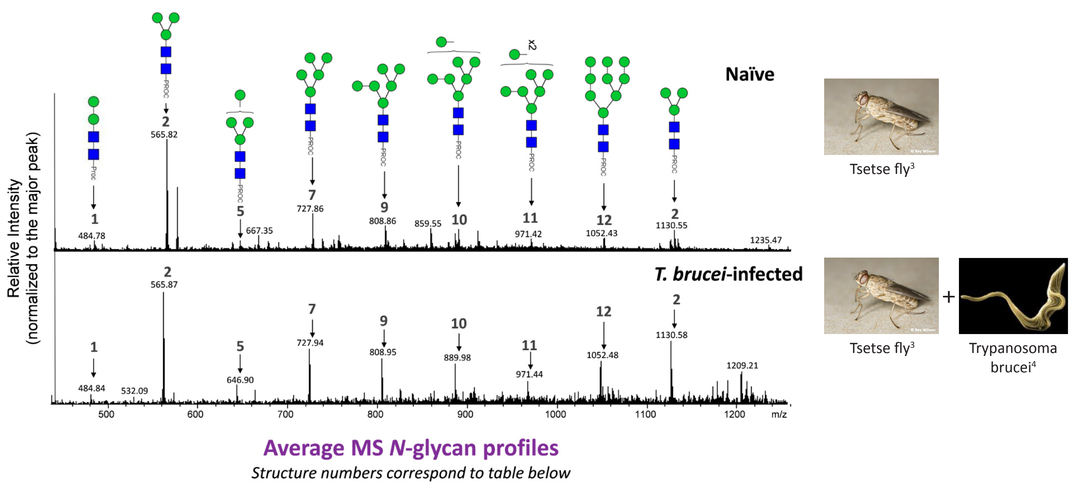
Results

1. Comparison of glycomics workflows for 2-AB and procainamide labelled N-glycans



- Procainamide gave higher fluorescence responses than 2-AB
- Procainamide labelled glycans and 2-AB labelled glycans showed comparable separation on the HILIC UHPLC
- 2-AB labelled glycans gave poor ESI-MS profiles with the amounts of samples tested
- Procainamide labelled glycans gave very good ESI-MS profiles with the amounts of samples tested
- MS/MS fragmentation of procainamide glycans (data not shown) contained diagnostic ions that aided detailed N-glycan structural characterisation

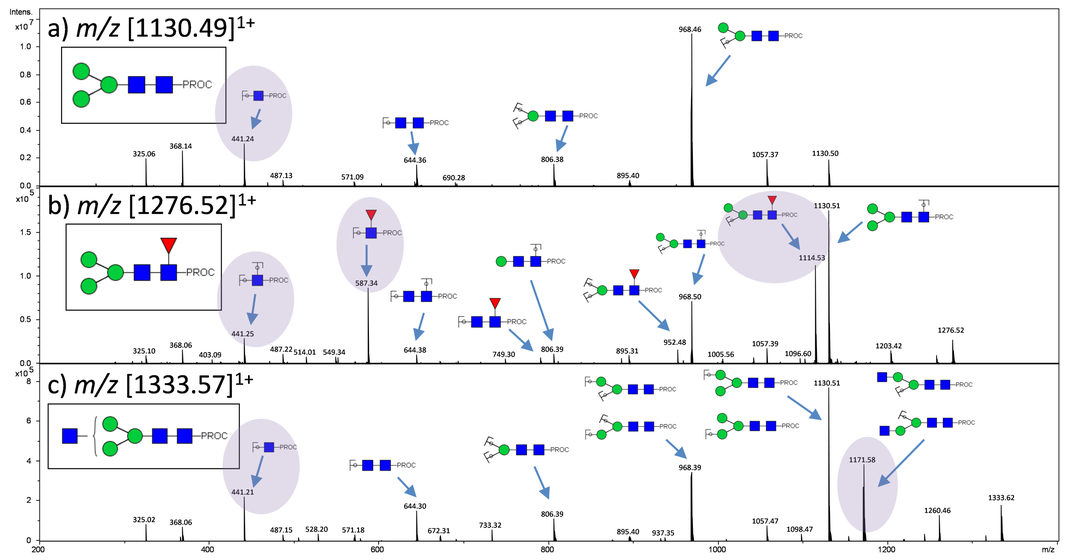
2. Comparison of procainamide labelled N-glycans from naïve and *T. brucei*-infected tsetse fly saliva glycoproteins by HILIC SPE-ESI-MS/MS glycomics workflow



Average MS *N*-glycan profiles

Structure numbers correspond to table below

- Pauci mannose and high mannose-type structures showed high expression the N-glycomes of both naïve and T. brucei-infected tsetse fly saliva glycoproteins with $(Man)_3(GlcNAc)_2$-Proc the dominant structure in both.
- Low abundance hybrid-type glycans were also detected in both samples (not visible in average MS profile) - they look the same



Examples of MS/MS fragmentation patterns of three of the N-glycans from the *T-brucei* infected tsetse fly saliva

3. N-glycan structures identified in tsetse saliva glycoproteins by MS/MS fragmentation of procainamide labelled oligosaccharides

HPLC Peak Id	Glucose Units	Tsetse Fly Saliva *N*-glycans Identified by HILIC-UHLPC ESI-MS/MS: Composition			$[m/z]^{1+}$ calculated	$[m/z]^{2+}$ calculated	$[m/z]^{1+}$ detected	$[m/z]^{2+}$ detected	Proposed Structure
		Hex	HexNAc	Fuc					
1	3.21	2	2	0	968.46	484.73	968.47	n.d.	
2	4.17	3	2	0	1130.51	565.76	1130.49	565.74	
3	4.62	3	2	1	1276.57	638.79	1276.52	638.77	
4	4.76	3	3	0	1333.59	667.30	1333.57	667.29	
5	5.00	4	2	0	1292.56	646.78	1292.54	646.74	
6	5.54	4	3	0	1495.64	748.32	1495.65	748.30	
7	6.00	5	2	0	1454.61	727.81	1454.57	727.79	
8	6.46	5	3	0	1657.69	829.35	1657.64	829.33	
9	6.87	6	2	0	1616.67	808.84	1616.61	808.81	
10	7.79	7	2	0	1778.72	889.86	1778.68	889.84	
11	8.53	8	2	0	1940.77	970.89	n.d.	970.87	
12	8.66	8	2	0	1940.77	970.89	n.d.	970.87	
13	9.35	9	2	0	2102.83	1051.92	n.d.	1051.90	

n.d. = not detected

Conclusions

- The procainamide system suitable for detailed glycomic analysis of insect saliva N-glycans small sample amounts showing high sensitivity
- These results suggest that upon colonisation and maturation of trypanosomes in the tsetse salivary glands, there are no detectable changes in the glycosylation of tsetse salivary glycoproteins.
- The presence of high levels of mannosylated structures may influence the half-life in blood of pharmacologically active salivary components.
- To our knowledge this is the first study to characterise saliva glycosylation from any insect vector. We are planning further studies on the glycobiology of parasitic infections using the procainamide-UHPLC-ESI-MS system. These will include investigation into changes in the glycosylation patterns of tissues from the human host, insect vector and the parasites throughout the disease process.

References

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Acknowledgements

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rad.kozak@ludger.com

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