Supplementary Information for:

Analysis of protein glycation using fluorescent phenylboronate gel electrophoresis

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Supplementary Table S1

Contents of Supplementary Information, Pereira Morais *et al.*,:

Analysis of protein glycation using fluorescent phenylboronate gel electrophoresis

Supplementary Figures

Supplementary Table

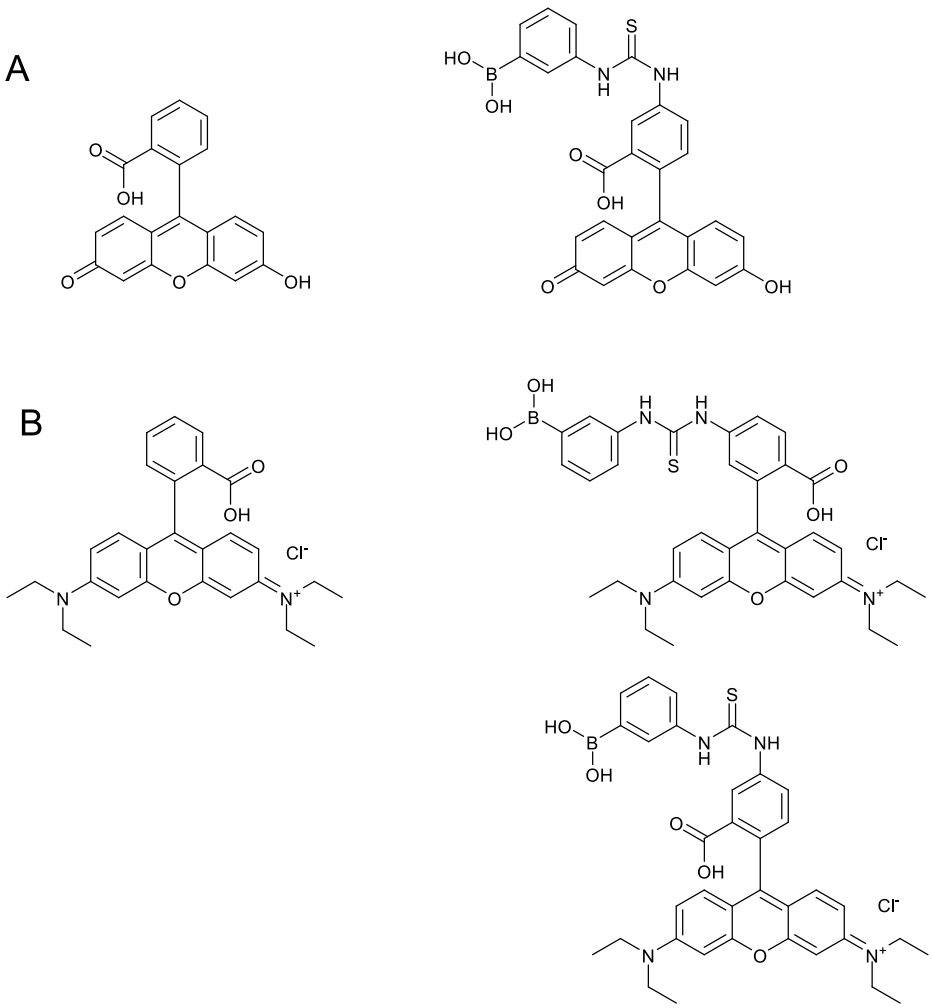

Figure S1. Chemical structures of the fluorescent labels. (A) Fluorescein (left) and fluorescein-boronic acid (right), **(B)** rhodamine (left) and rhodamine-boronic acid (mixed isomers; top right, 5-isomer; bottom right, 6-isomer) structures generated using ChemDraw (CambridgeSoft).

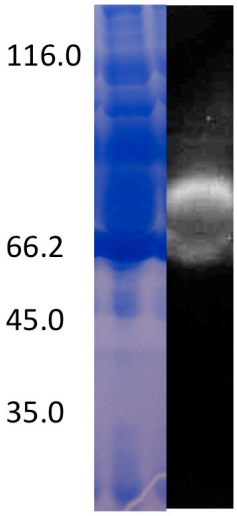

Figure S2. Fluorescein-boronic acid labelled serum analysed by mP-AGE. mP-AGE gel profile of human serum labelled with fluorescein-boronic acid when imaged with UV prior to protein staining (right) and after Coomassie stain (left).

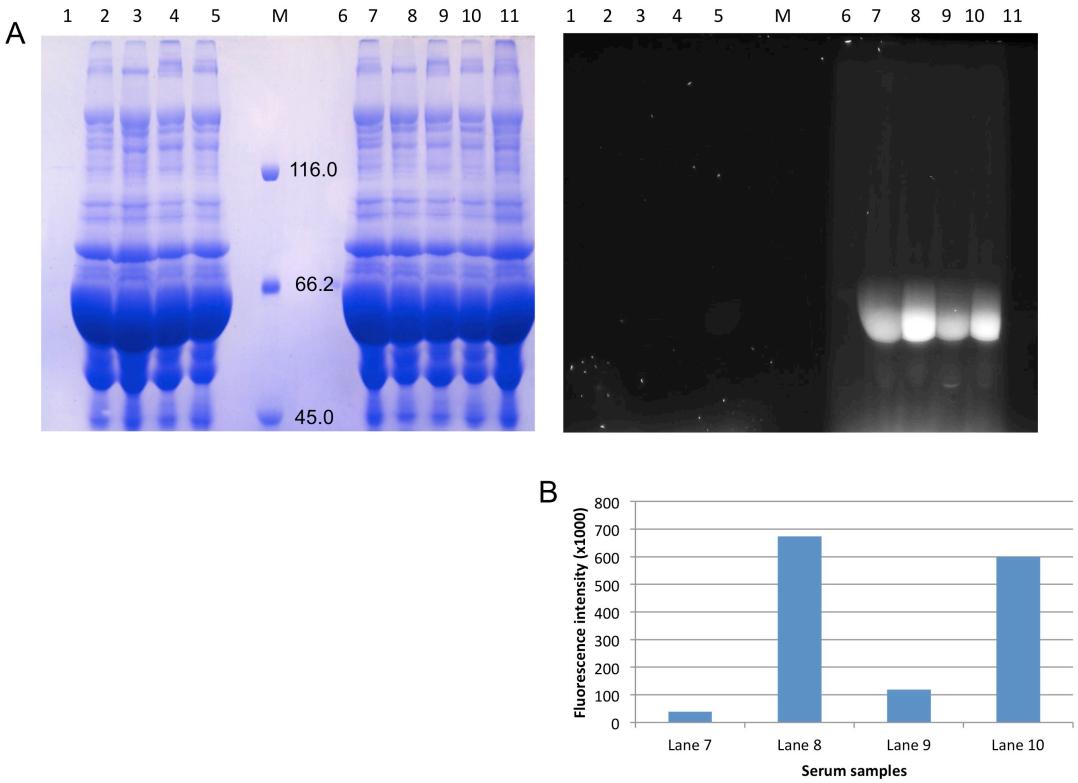

Figure S3. Flu-PAGE analysis of diabetic serum. **(A)** 8 % SDS-PAGE of fluorescein (lanes 2-5) and fluorescein boronic acid labelled samples (lanes 7-11), control serum (lanes 2 and 7) and type 1 diabetes human sera of three individuals (lanes 3-5 and 8-10). Lanes 1 and 6 show the labels of fluorescein and fluorescein-boronic acid respectively. Lane 11 shows unlabelled control human serum. Gel was visualised and imaged with UV (365 nm and green filter 537 nm) on AlphaImager (right), and Coomassie stained (left). **(B)** shows relative fluorescence intensity of the HSA band in serum samples labelled with fluorescein-boronic acid. The fluorescence intensity values are corrected for protein concentration as determined by Coomassie stain, using TotalLab Quant.

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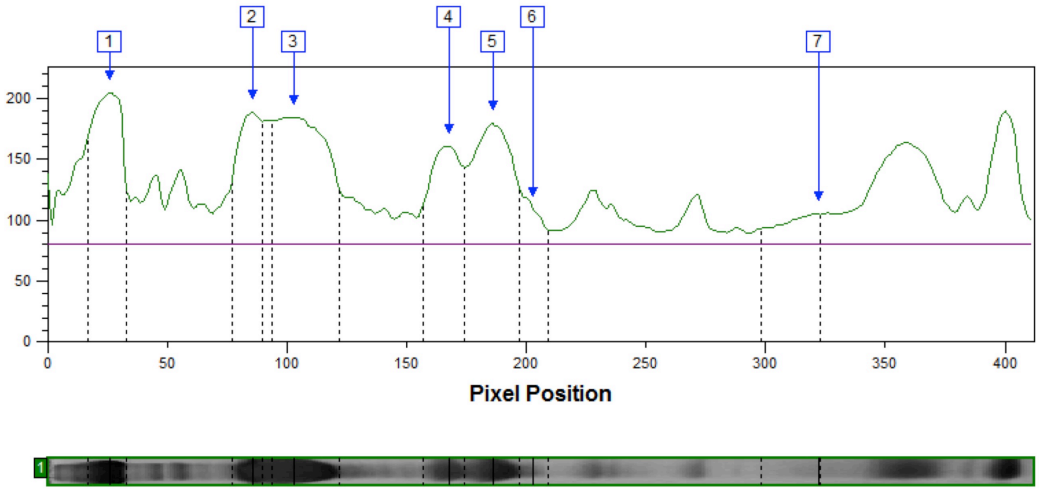
Pixel Position

B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C

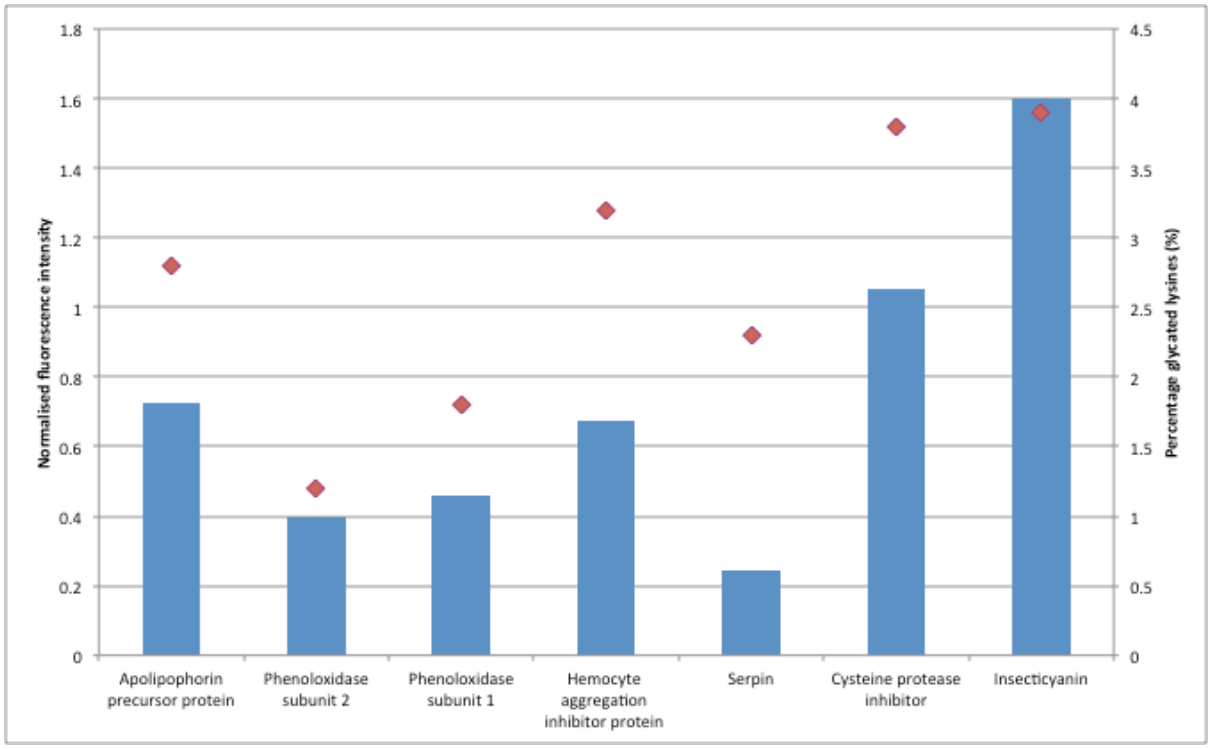

Figure S4. Quantitative fluorescence intensity analysis of *Manduca sexta* hemolymph. Comparative analysis of protein band intensities of hemolymph SDS-PAGE profiles in Flu-PAGE **(A)** and subsequent Coomassie stained gel **(B)**. The gel intensity profiles shown were produced using TotalLab Quant. The identified intensity peaks correspond to **1)** *apolipophorin precursor protein*, **2)** *pro-phenoloxidase subunit 2*, **3)** *pro-phenoloxidase subunit 1*, **4)** *hemocyte aggregation inhibitor protein precursor*, **5)** *serpin 1*, **6)** *putative C1A cysteine protease precursor* and **7)** *insecticyanin*.

Graph **C** shows the fluorescence intensity of glycated protein bands (blue bars) that have been normalised with respect to their corresponding Coomassie stain intensity. The percentages of glycated lysines (see **Supplementary Table S1**) in the respective proteins are shown as diamonds.

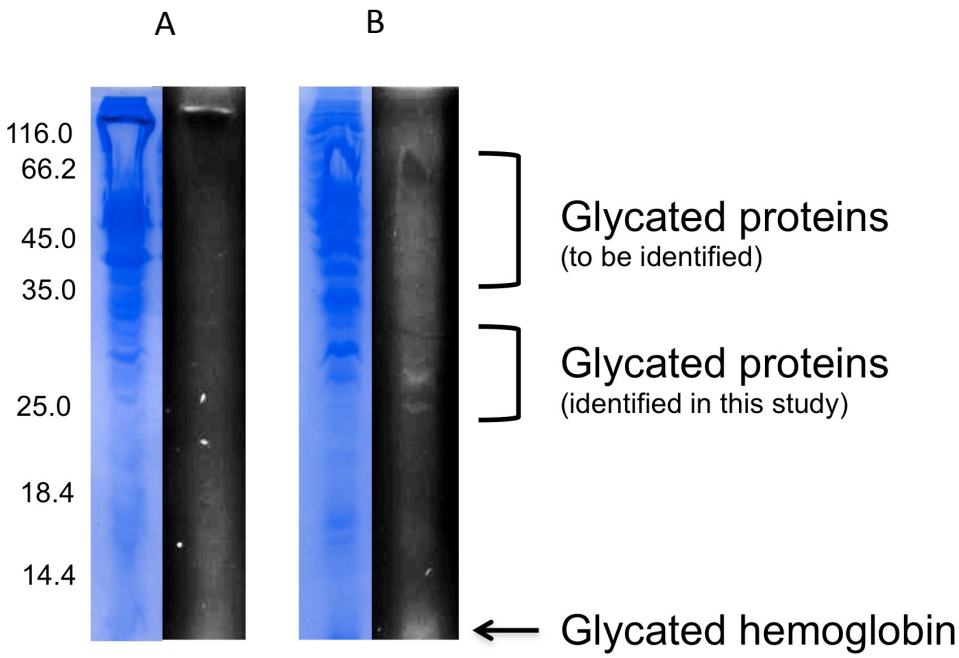

Figure S5. Flu-PAGE analysis of TASTPM and wildtype mouse brain homogenates. Flu-PAGE analysis of wild type **(A)** and TASTPM **(B)** mouse cortex homogenates. The gel was visualised with UV (left) prior to Coomassie staining (right). There are many more fluorescent protein bands in the TASTPM affected mouse sample compared to the age-matched control. Proteins that were analysed by MS were indicated.

Table S1. Glycated proteins identified by Flu-PAGE.

Protein	*Database number*	*MW (kDa)*	*Number of amino acids*	*Number of K/glycated K**	*Number of glycated K/total amino acids (%)*
	***Manduca sexta* hemolymph**				
apolipophorin precursor protein	AAB53254.1	367	3305	282/92 (33%)	2.8
pro-phenoloxidase subunit 2	Q25519.3	80	695	31/8 (26%)	1.2
pro-phenoloxidase subunit 1	O44249	79	685	27/12 (44%)	1.8
hemocyte aggregation inhibitor protein precursor	ACW82749.1	48	434	28/14 (50%)	3.2
serpin 1	AAC47343.1	43	392	34/9 (26%)	2.3
putative C1A cysteine protease precursor	CAX16635.1	38	342	29/13 (45%)	3.8
insecticyanin	CAA45969.1	23	206	19/9 (47%)	4.4
	TASTPM cortex homogenates				
14-3-3 ε	P62259	29	255	18/3 (17%)	1.2
14-3-3 ζ/δ	P63101	28	245	20/11 (55%)	4.5
Triose phosphate isomerase	CAA37420.1	27	249	20/5 (25%)	2.0
glutathione S-transferase Mu1	P10649	26	218	18/4 (22%)	1.8
glutathione S-transferase P1	P19157	24	210	12/6 (50%)	2.9
Hemoglobin α β	P01942.2 P02088.2	15 16	142 147	22/11 (50%)	7.7
	Human serum				
serotransferrin	P02787.3	77	698	58/18 (31%)	2.6
serum albumin	P02768	69	609	60/24 (40%)	3.9
IgG heavy chain	AAA02914.1	52	476	32/18 (56%)	3.8
apolipoprotein A-I	P02647.1	31	267	22/9 (41%)	3.4

***Analysis and prediction of mammalian protein glycation.** *Johansen MB, Kiemer L and Brunak S,* **Glycobiology**, 16:844-853, **2006**.