

## Supplementary Materials

# Glycation of host proteins increases pathogenic potential of *Porphyromonas gingivalis*

Michał Śmiga <sup>1</sup>, John W. Smalley <sup>2</sup>, Paulina Ślęzak <sup>1</sup>, Jason L. Brown <sup>2,†</sup>, Klaudia Siemińska <sup>1</sup>, Rosalind E. Jenkins <sup>3</sup>, Edwin A. Yates <sup>4</sup> and Teresa Olczak <sup>1,\*</sup>

<sup>1</sup> Laboratory of Medical Biology, Faculty of Biotechnology, University of Wrocław, 14A F. Joliot-Curie St., 50-383 Wrocław, Poland; [michal.smiga@uwr.edu.pl](mailto:michal.smiga@uwr.edu.pl) (M.Ś.); [paulina.stepien2@uwr.edu.pl](mailto:paulina.stepien2@uwr.edu.pl) (P.Ś.); [klaudia.sieminska@uwr.edu.pl](mailto:klaudia.sieminska@uwr.edu.pl) (K.S.)

<sup>2</sup> Institute of Life Course and Medical Sciences, School of Dentistry, The University of Liverpool, Pembroke Place, Liverpool L3 5PS, UK; e-mail [josmall@liv.ac.uk](mailto:josmall@liv.ac.uk)

<sup>3</sup> CDSS Bioanalytical Facility, Department of Pharmacology and Therapeutics, Institute of Systems, Molecular and Integrative Biology, Faculty of Health and Life Science, The University of Liverpool, Liverpool, L69 3GE, UK; [R.Jenkins@liv.ac.uk](mailto:R.Jenkins@liv.ac.uk)

<sup>4</sup> Institute of Systems, Molecular and Integrative Biology, Faculty of Health and Life Science, The University of Liverpool, Liverpool, L69 7ZB, UK; [e.a.yates@liv.ac.uk](mailto:e.a.yates@liv.ac.uk)

† Present address: Oral Sciences Research Group, Glasgow Dental School, School of Medicine, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, G2 3JZ, UK

\* Correspondence: [teresa.olczak@uwr.edu.pl](mailto:teresa.olczak@uwr.edu.pl) (T.O.)

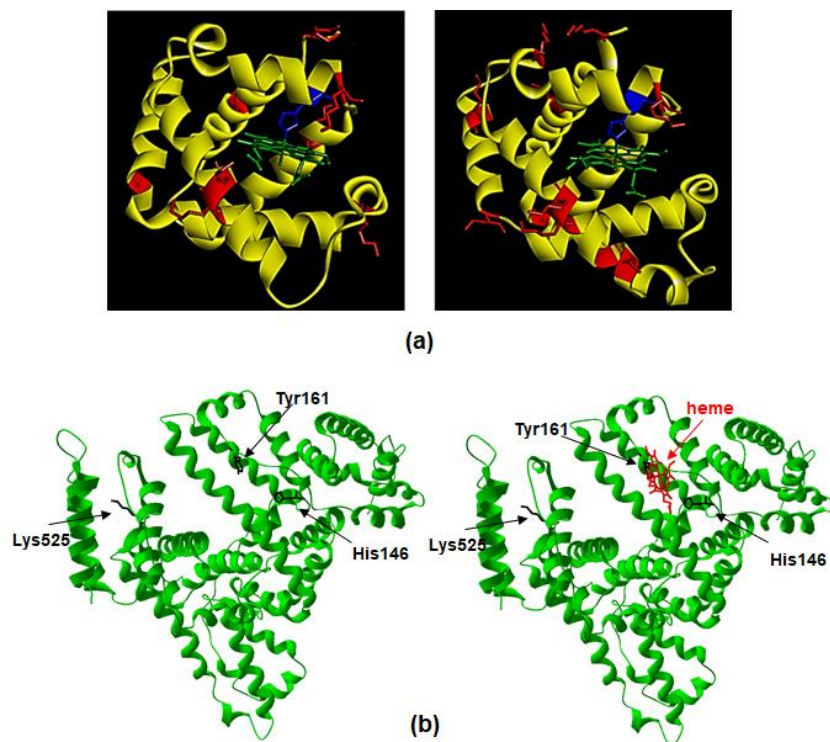
**Table S1 . Degree of glycation of haemoglobin as measured by assay for 5-hydroxymethylfurfural (5-HMF).** Values are expressed as the molar ratio of 5-HMF to haemoglobin subunit. Assays were carried out on three separate preparations of both glycated and un-glycated hemoglobin samples.

Sample number	Molar ratio (5-HMF:hemoglobin)	
	Glycated hemoglobin	Un-glycated hemoglobin
1	1.183	0.210
2	1.171	0.201
3	1.295	0.200
Average (mean±SD)	1.216±0.068	0.203±0.005

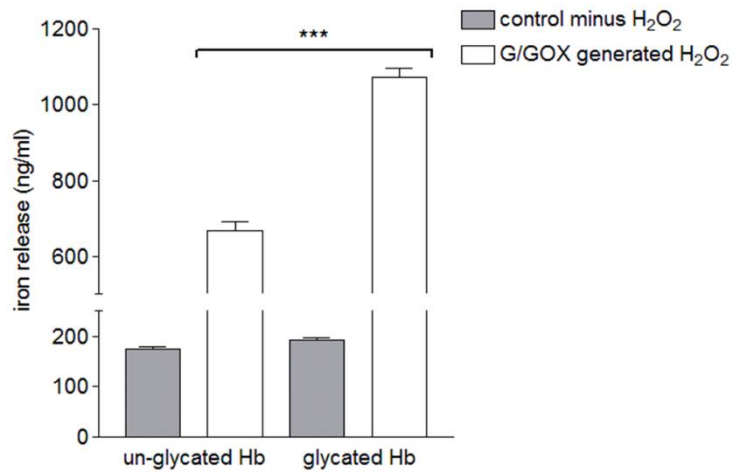
**Table S2. Comparison of hemoglobin glycation sites.**

Lysine residue	Glycation sites detected in this study	<i>In vivo</i> and <i>in vitro</i> glycation sites [1]	<i>In vivo</i> glycated sites [2]	<i>In vitro</i> glycated sites [3]	<i>In vitro</i> glycated sites [4]
7( $\alpha$ )	-	+	+	-	-
11( $\alpha$ )	-	-	-	-	-
16( $\alpha$ )	+	+	+	+	+
40( $\alpha$ )	+	+	+	-	-
56( $\alpha$ )	+	-	+	-	+
60( $\alpha$ )/61( $\alpha$ )	+	+	-	+	-
90( $\alpha$ )	+	-	-	-	-
99( $\alpha$ )	+	-	-	-	-
127( $\alpha$ )	+	-	+	-	-
139( $\alpha$ )	+	-	+	+	-
8( $\beta$ )	+	+	+	-	+
17( $\beta$ )	+	+	+	+	-
59( $\beta$ )	+	-	-	+	-
61( $\beta$ )	-	-	+	-	-
65( $\beta$ )/66( $\beta$ )	+	+	+	+	-
82( $\beta$ )	+	-	-	+	+
95( $\beta$ )	+	-	-	+	-
120( $\beta$ )	+	+	-	+	-
132( $\beta$ )	+	-	+	+	-
144( $\beta$ )	+	+	+	+	+

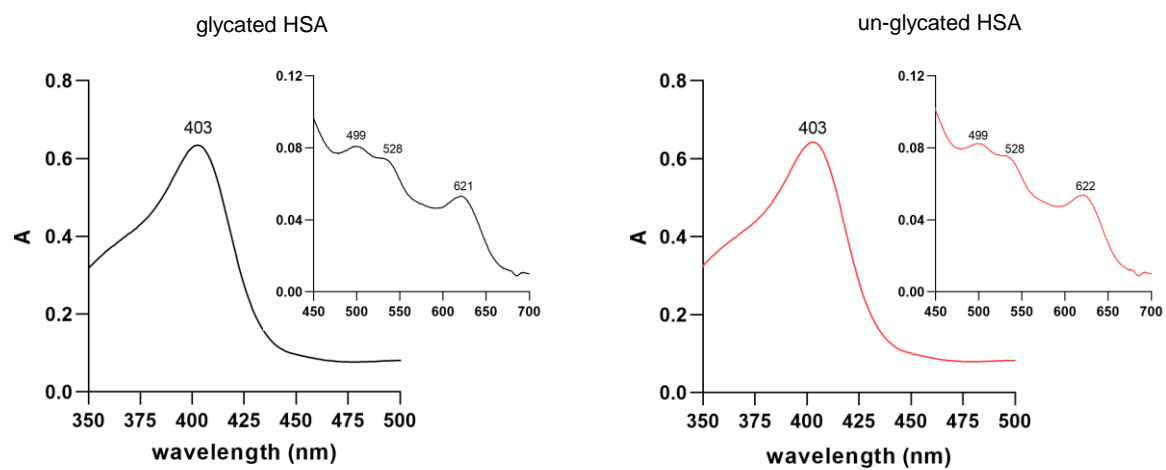
1. Shapiro, R.; McManus, M.J.; Zalut, C.; Bunn, H.F. Sites of nonenzymatic glycosylation of human hemoglobin A. *J Biol Chem* **1980**, *255*, 3120-3127.
2. Zhang, X.; Medzihradszky, K.F.; Cunningham, J.; Lee, P.D.; Rognerud, C.L.; Ou, C.N.; Harmatz, P.; Witkowska, H.E. Characterization of glycated hemoglobin in diabetic patients: usefulness of electrospray mass spectrometry in monitoring the extent and distribution of glycation. *J Chromatogr B Biomed Sci Appl* **2001**, *759*, 1-15.
3. Delpierre, G.; Vertommen, D.; Communi, D.; Rider, M.H.; Van Schaftingen, E. Identification of fructosamine residues deglycated by fructosamine-3-kinase in human hemoglobin. *J Biol Chem* **2004**, *279*, 27613-27620.
4. Ito, S.; Nakahari, T.; Yamamoto, D. The structural feature surrounding glycated lysine residues in human hemoglobin. *Biomed Res* **2011**, *32*, 217-223.



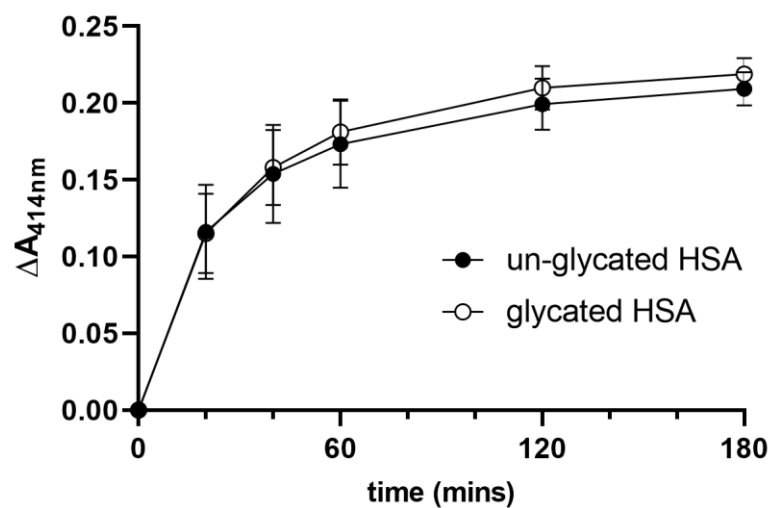
**Figure S1. Main glycation targets in hemoglobin and albumin.** (a) Glycated lysine residues in the  $\alpha$ -chain (left panel) and  $\beta$ -chain (right panel) of horse methemoglobin are shown. The protein structure was constructed using the structure deposited in the PDB data base (PDB ID: 2MHB). Glycated lysine residues (red), the heme molecule (green), and proximal histidine residues (His87 in  $\alpha$ -chain and His92 in  $\beta$ -chain) (blue) are indicated. (b) Location of heme-binding site (red) with amino acids coordinating heme iron (Tyr161 and His146; black) and main glycation lysine residue (Lys525) in human serum albumin are indicated.



**Figure S2. Quantification of iron (III) release from glycated and un-glycated hemoglobin.** Glycated and un-glycated hemoglobin (200  $\mu$ M with respect to tetramer) were exposed to a continuous flux of H<sub>2</sub>O<sub>2</sub> generated by glucose/glucose oxidase (G/GOX). \*\*\* $P$ <0.001



**Figure S3. Characterization of glycated human serum albumin.** The UV-visible spectra of the glycated (left panel) and un-glycated albumin (right panel) are shown. A, absorbance.



**Figure S4. Comparison of HmuY-Fe(III)heme complex formation from glycated and un-glycated albumin.** 5  $\mu\text{M}$  human serum albumin (HSA) was incubated with an equimolar concentration of HmuY. Absorbance values derived from difference spectra at 414 nm were used to create the curve demonstrating HmuY-Fe(III)heme complex formation.  $\Delta A$ , difference absorbance.