

## **Supplemental material for:**

# Decorating the Anammox House: Sialic Acids and Sulfated Glycosaminoglycans in the Extracellular Polymeric Substances of Anammox Granular Sludge

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Supplementary Tables S3 and S4 are provided in one separate excel file

Table S3. Alignment table *Campylobacter jejuni*

Table S4. Alignment table *Halorubrum sp. PV6*

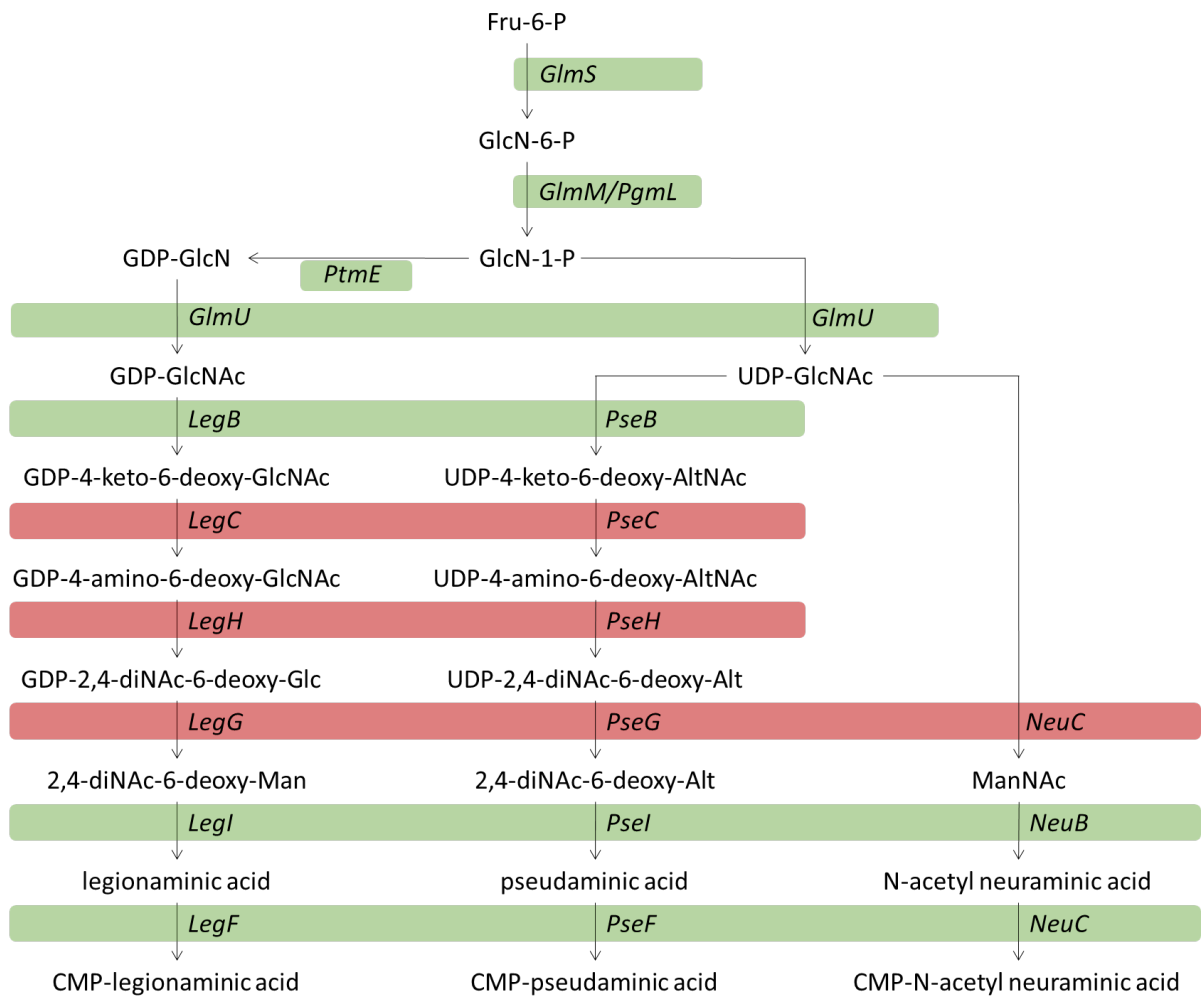


Figure S1: Pathways for the biosynthesis of legionaminic acid, pseudaminic acid and N-acetyl neuraminic acid in *Campylobacter jejuni*. Reference pathways were taken from Schoenhofen et al. (2009). The green have a match with *Ca. Brocadiales* (Table S1)

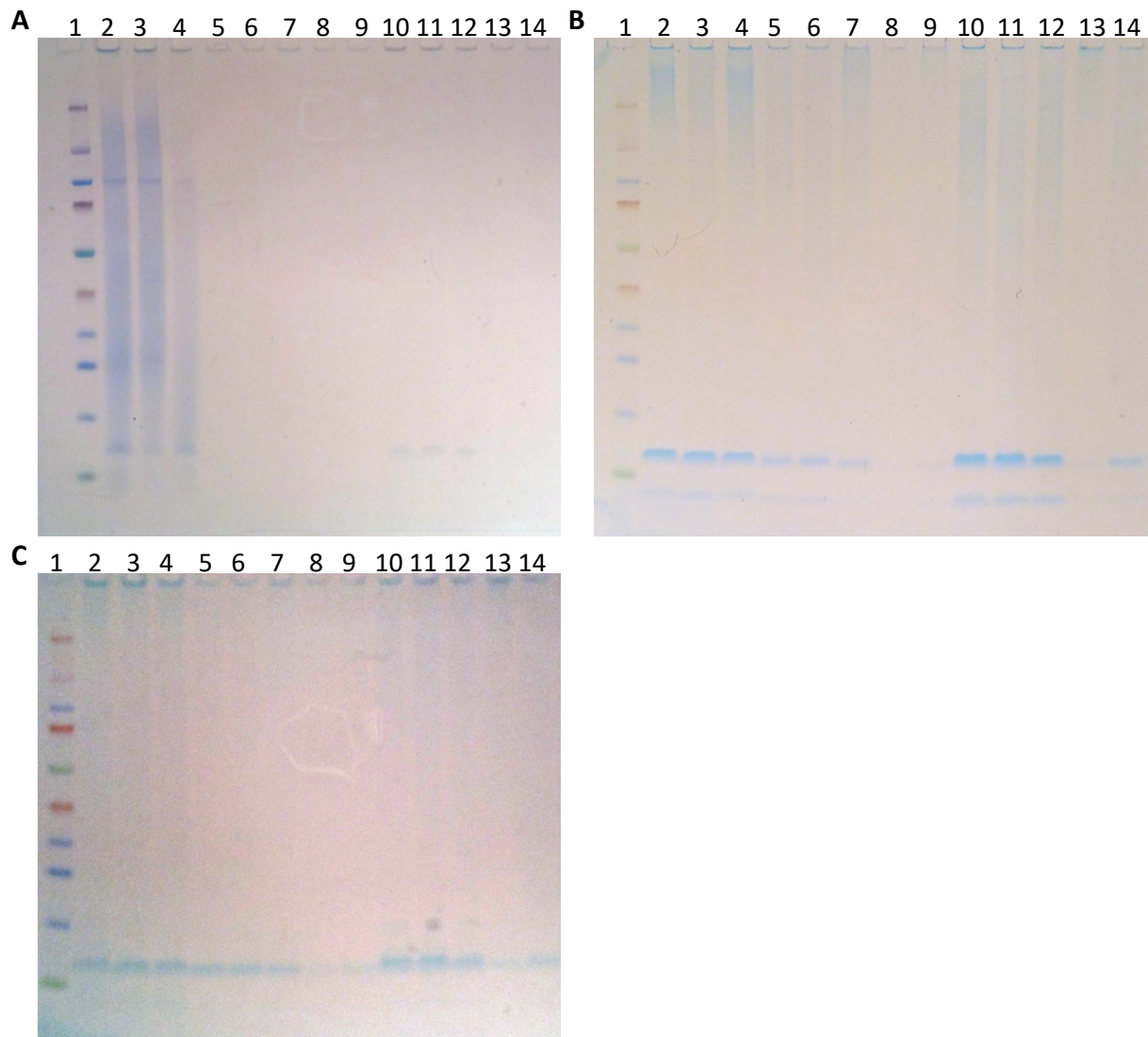


Figure S2: Images of SDS-PAGE gels. Gels were stained with A) Coomassie Blue, B) Alcian blue at pH 2.5 and C) Alcian blue at pH 1.0. The samples in the lanes are as follows: 1: MW ladder, 2: Extract 1 (pH 5), 3: Extract 2 (pH 2.5), 4: Extract 3 (dialyzed), 5: Extract 1 (pH 5) – after denaturation and enzyme treatment, 6: Extract 2 (pH 2.5) – after denaturation and enzyme treatment, 7: Extract 3 (dialyzed) – after denaturation and enzyme treatment, 8: Pellet – after denaturation and enzyme treatment, 9: Granules – after denaturation and enzyme treatment, 10: Extract 1 (pH 5) – after Blyscan, 11: Extract 2 (pH 2.5) – after Blyscan, 12: Extract 3 (dialyzed) – after Blyscan, 13: Pellet – after Blyscan, 14: Granules – after Blyscan

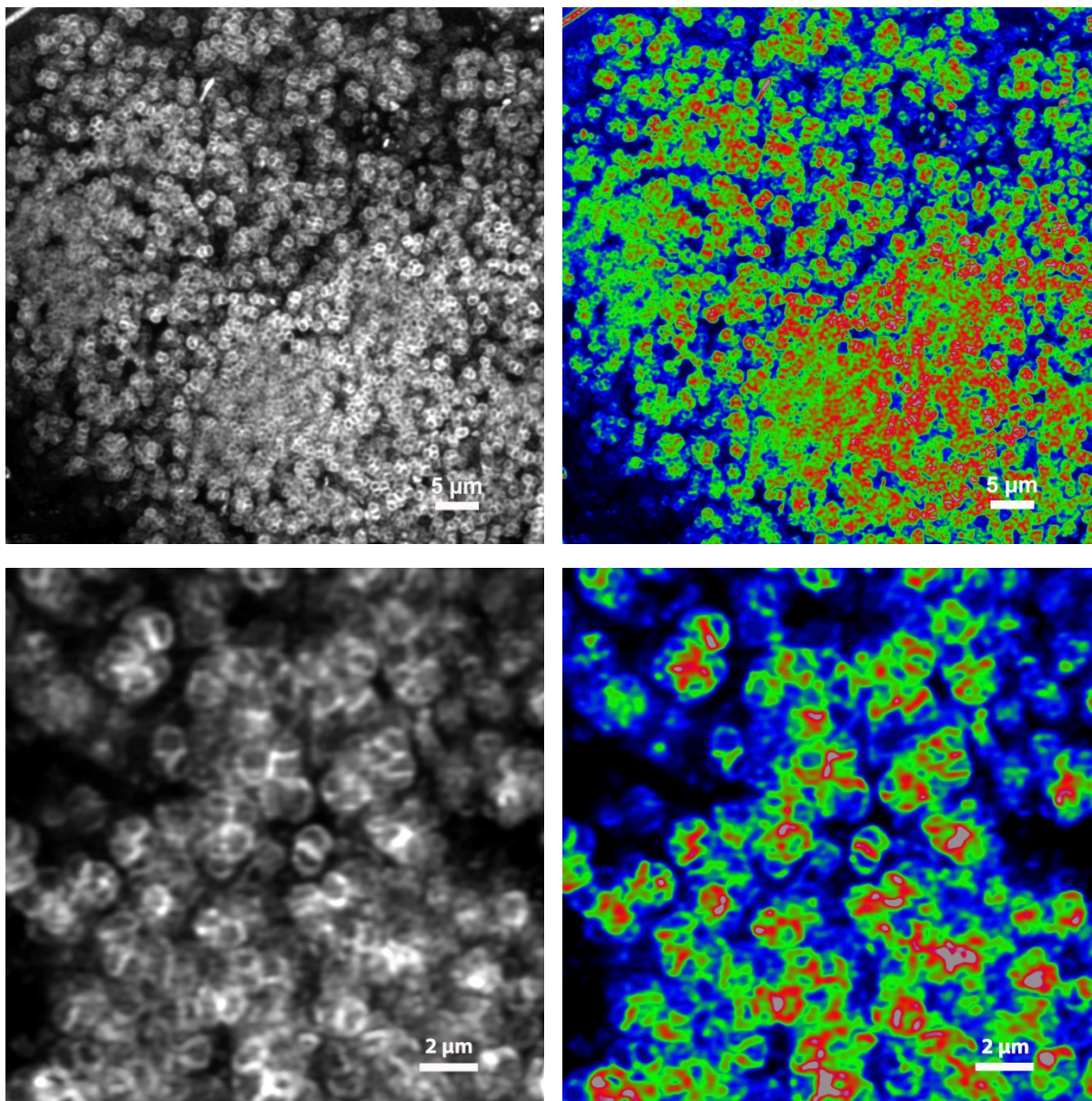


Figure S3: Confocal laser scanning microscopy of Heparin Red, bound to components in the fully hydrated, intact anammox granule. A) and C) are greyscale images of the fluorescent signal. A) and B) represents an image series of 45 optical sections at  $0.17\ \mu\text{m}$  step size shown as maximum intensity projection, C) and D) represents a single image. B) and D) are the images of A and C, respectively, converted to color images with three pixel intensities: red = binding of Heparin Red, green = quenched signal, implying high negative charge density, and blue = no binding of Heparin Red. These images are the same as presented in Figure 3 of the manuscript, but with deconvolution and therefore appear sharper. The shape of the cells is therefore more clear.

*Table S1: Mass spectrometric sialic acid analysis. The obtained (DMB-labelled) masses matched with; Pse/Leg (Pseudaminic acid/legionaminic acid), KDN (deaminated neuraminic acid), and NeuAc (N-acetyl neuraminic acid).*

Structure	Sum formula	monoisotopic mass	mass delta (av, ppm)	run 1	run2
Pse/Leg	C <sub>20</sub> H <sub>26</sub> N <sub>4</sub> O <sub>8</sub>	450.1750638	-0.130837989	x	x
KDN	C <sub>16</sub> H <sub>20</sub> N <sub>2</sub> O <sub>9</sub>	384.1168802	2.31439972	x	x
NeuAc	C <sub>18</sub> H <sub>23</sub> N <sub>3</sub> O <sub>9</sub>	425.1434293	-0.705173782	x	x

Table S2: Enzymes from the pathway (depicted in Figure S1) were BLASTed against the *Ca. Brocadiiales* database (NCBI). The best match is shown in this table. For the complete alignment tables see SI Table S3 (excel file).

Gene C. jejuni	Enzyme	Accession nr C. jejuni	Best match Ca. Brocadiiales	Name	e-value	ID (%)
PseB	dehydratase/epimerase	CAL35407.1	WP_034409924.1	UDP-N-acetylglucosamine 4,6-dehydratase (inverting) [Candidatus Brocadiaceae bacterium S225]	4E-131	56.6
PseC	aminotransferase	CAL35408.1	WP_034409923.1	UDP-4-amino-4,6-dideoxy-N-acetyl-beta-L-altrosamine transaminase [Candidatus Brocadiaceae bacterium S225]	5E-73	37.6
PseH	N-acetyl transferase	CAL35427.1	-	-	-	-
PseG	UPD-sugar hydrolase	CAL35426.1	-	-	-	-
PseI	pseudaminic acid synthase	CAL35431.1	TVL99718.1	pseudaminic acid synthase [Candidatus Brocadia sp. WS118]	4E-92	44.4
PseF	CMP-pseudaminic acid synthetase	CAL35425.1	RIK01952.1	acylneuraminate cytidyltransferase family protein [Candidatus Brocadia sp.]	8E-44	37.2
LegB	dehydratase	CAL35433.1	TLD41235.1	UDP-glucose 4-epimerase [Candidatus Jettenia ecosi]	2E-136	59.4
LegC	aminotransferase	CAL35434.1	OQY98965.1	aminotransferase DegT [Candidatus Brocadia sp. UTAMX2]	1E-138	48.7
LegH	N-acetyl transferase	CAL35412.1	-	-	-	-
LegG	Hydrolyzing 2-epimerase	CAL35441.1	-	-	-	-
LegI	legionaminic acid synthase	CAL35440.1	TVL99724.1	N-acetylneuraminate synthase [Candidatus Brocadia sp. WS118]	4E-120	54.6
LegF	CMP-legionaminic acid synthetase	CAL35444.1	TVL99729.1	acylneuraminate cytidyltransferase family protein [Candidatus Brocadia sp. WS118]	3E-35	35.6
NeuC	Hydrolyzing 2-epimerase	CAL35259.1	-	-	-	-
NeuB	sialic acid synthase	CAL35258.1	TVL99718.1	pseudaminic acid synthase [Candidatus Brocadia sp. WS118]	3E-54	33.0
NeuA	CMP- sialic acid synthetase	CAL35260.1	TVL99729.1	acylneuraminate cytidyltransferase family protein [Candidatus Brocadia sp. WS118]	4E-32	38.3