Dendrimer mediated clustering of bacteria: Improved aggregation and evaluation of bacterial response and viability.

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Dendrimer Characterisation

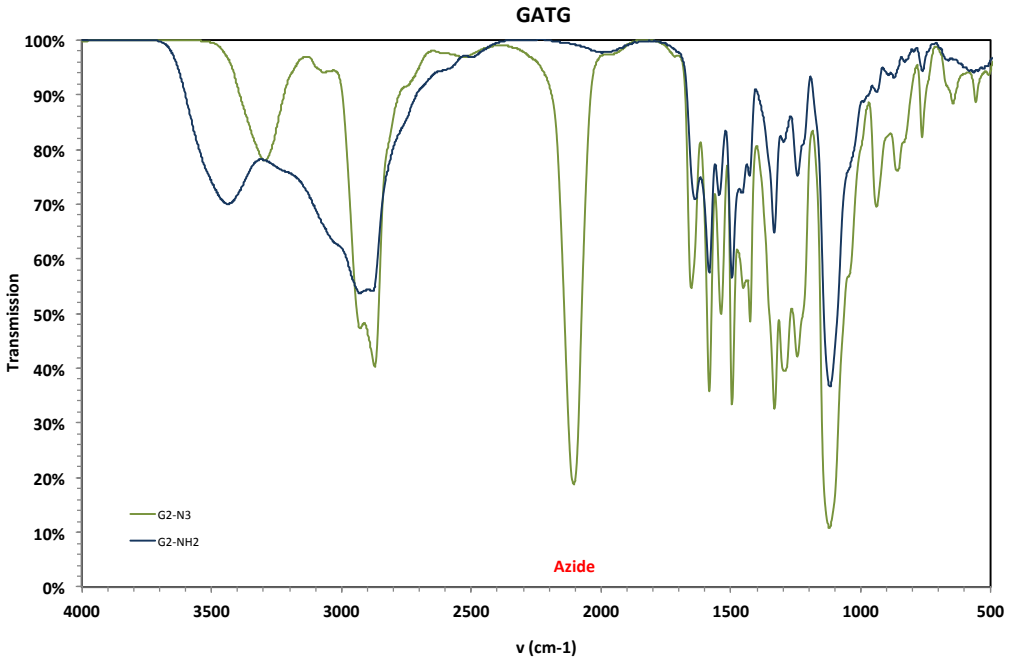

Figure S1: Representative monitoring of the synthesis of Gn-NH_2 and the disappearance of azide peak. IR spectra of G2-N_3 (green) and G2-NH_2 (blue).

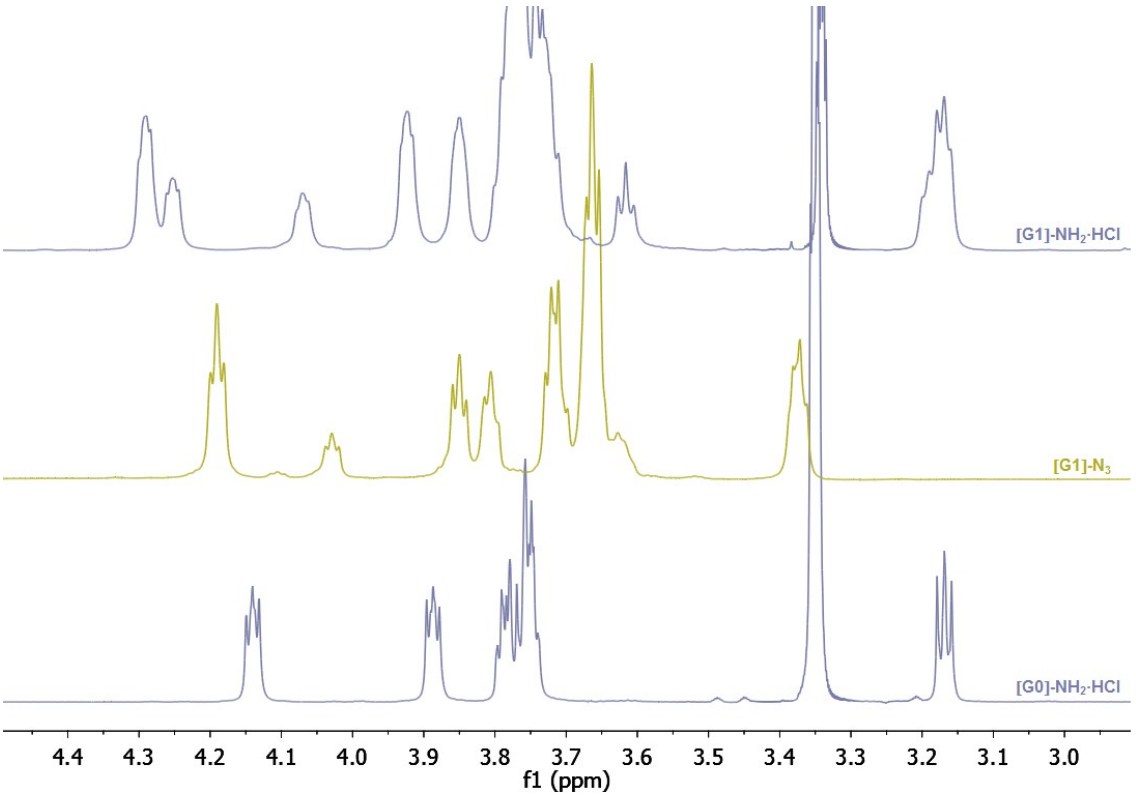

Figure S2: Representative monitoring of the synthesis of Gn-NH_2: ^{1}H-NMR spectra of 1,3,5-tri(2-(2-(2-aminoethoxy)ethoxy)ethoxy)benzene (G0-NH_2), G1-N_3 and G1-NH_2. ^{1}H-NMR spectra for the G1-N_3 was performed in $CDCl_3$ and G0-NH_2 and G1-NH_2 in CD_3OD.

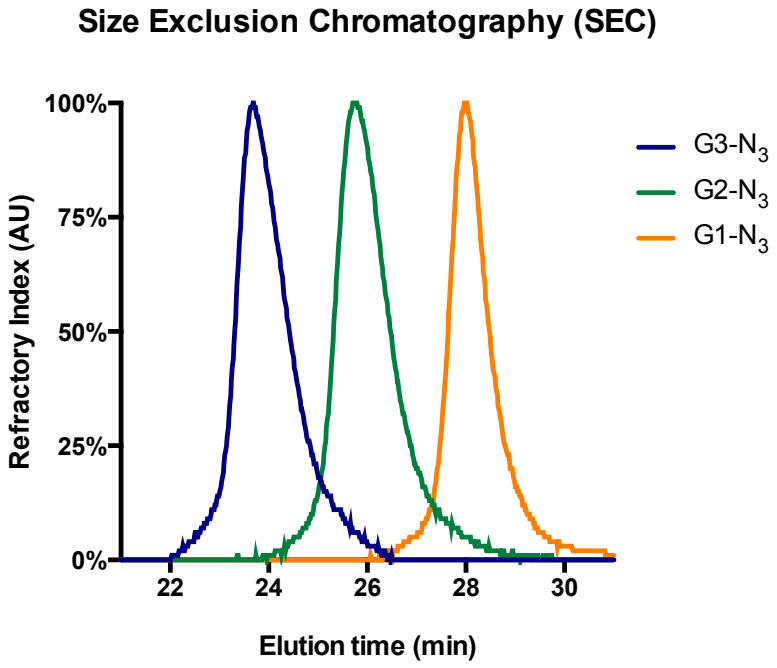

Figure S3: Normalised size exclusion chromatograms for GATG dendrimer G1-N_3 (orange), G2-N_3 (green) and G3-N_3 (blue). THF was used as an eluent at 1 mL/min. Samples were filtered through a 0.45 μm filter before injection.

Gn-NH_2	Amines	Mw / Da	Size / nm	ζ / mV
G1-NH_2	9	2389	-	-
G2-NH_2	27	7765	3.62 ± 0.42	30.6 ± 2.46
G3-NH_2	81	23891	5.68 ± 0.39	48.2 ± 4.75

Table S1: Number of surface amines, theoretical molecular weight, experimental size (as determined by DLS at 25 °C with hydrochloride amino dendrimers (1.5 mg/mL) in a 10 mM NaH_2PO_4 10% HCl 0.1M solution) and ζ-potential (as determined with hydrochloride amino dendrimers (10 mg/mL) in milliQ water).

Clustering of Vibrio harveyi

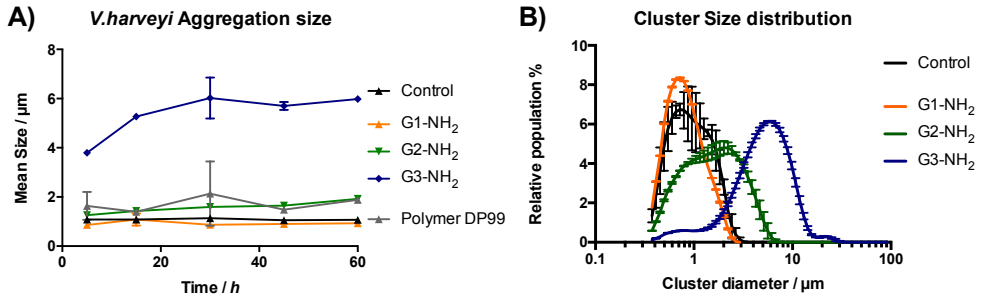

Figure S4: A) Mean cluster size of *V. harveyi* BB170 in the absence (black) and presence of G1-NH_2 (orange), G2-NH_2 (green), G3-NH_2 (blue) and p(DMAPMAm) DP 99 (grey). B) Size distribution of *V. harveyi*'s clusters in the absence (black) and presence of G1-NH_2 (orange), G2-NH_2 (green) and G3-NH_2 (blue). Initial OD_{600} = 1, [NH_2] = 0.33 mM in PBS at pH 7.4

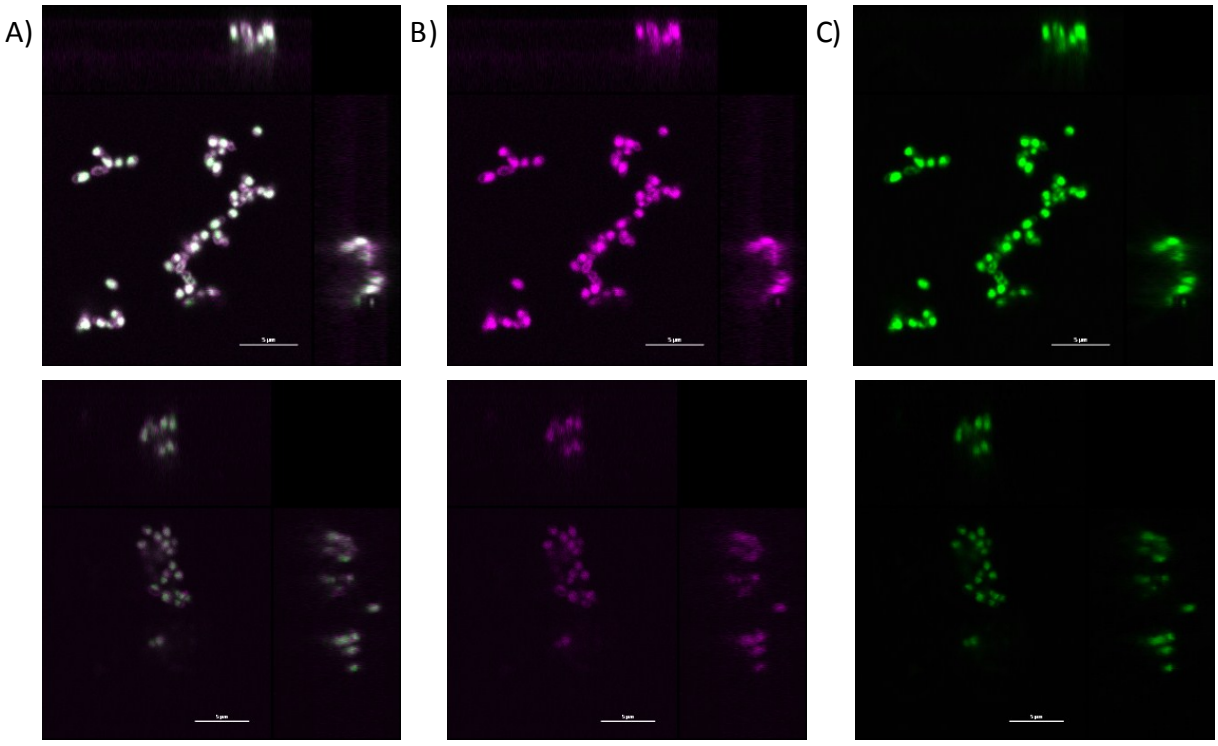

Figure S5: Confocal Laser Scanning Micrographs of *V. harveyi* BB170 (green) in the presence of MCCA labelled G3-NH_2 (magenta). A) Ortho projections from the overlaid magenta and green channels (white) including Z-stacks with transmission micrograph. B) Ortho projections for the blue channel and C) green channel with transmission micrograph. In all cases, the blue channel has been depicted magenta for clarity.

ζ-potential of *V. harveyi*

A single colony of *V. harveyi* from LB agar plates was used to inoculate 2 mL of LB medium containing 50 μg/mL kanamycin and incubated overnight at 30 °C. Bacteria were centrifuged at 9,000 rpm for 5 min at 4 °C, the supernatant was discarded and bacteria re-suspended in PBS. This washing step was repeated two times and bacteria were finally re-suspended at an OD_{600} = 1.0. Aliquots of the bacteria culture were mixed with known volumes of stock solutions of Gn-NH_2 in PBS pH 7.4. The mixture was incubated for 1 h at room temperature. The dendrimer-bacteria mixture was centrifuged at 9000 rpm for 5 min and sample was resuspended in water prior to measurement. The values of the concentrations reported corresponds to the final NH_2 concentration (mM) on dendrimers in the bacteria-suspension.

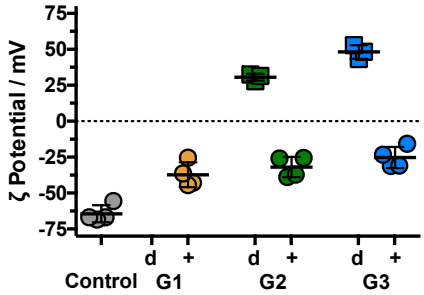

Figure S6: ζ-potential for Gn-NH_2 dendrimers (squares, [Gn-NH_2]=1.5 mg/mL); and for *V. harveyi BB170* (circles) in the absence (control) and presence of G1-NH_2 (orange), G2-NH_2 (green) and G3-NH_2 (blue). (Initial OD_{600} = 1, [NH_2] = 1.64 mM in water. ζ-potential of the dendrimers taken from Table S1.

Membrane permeability and viability

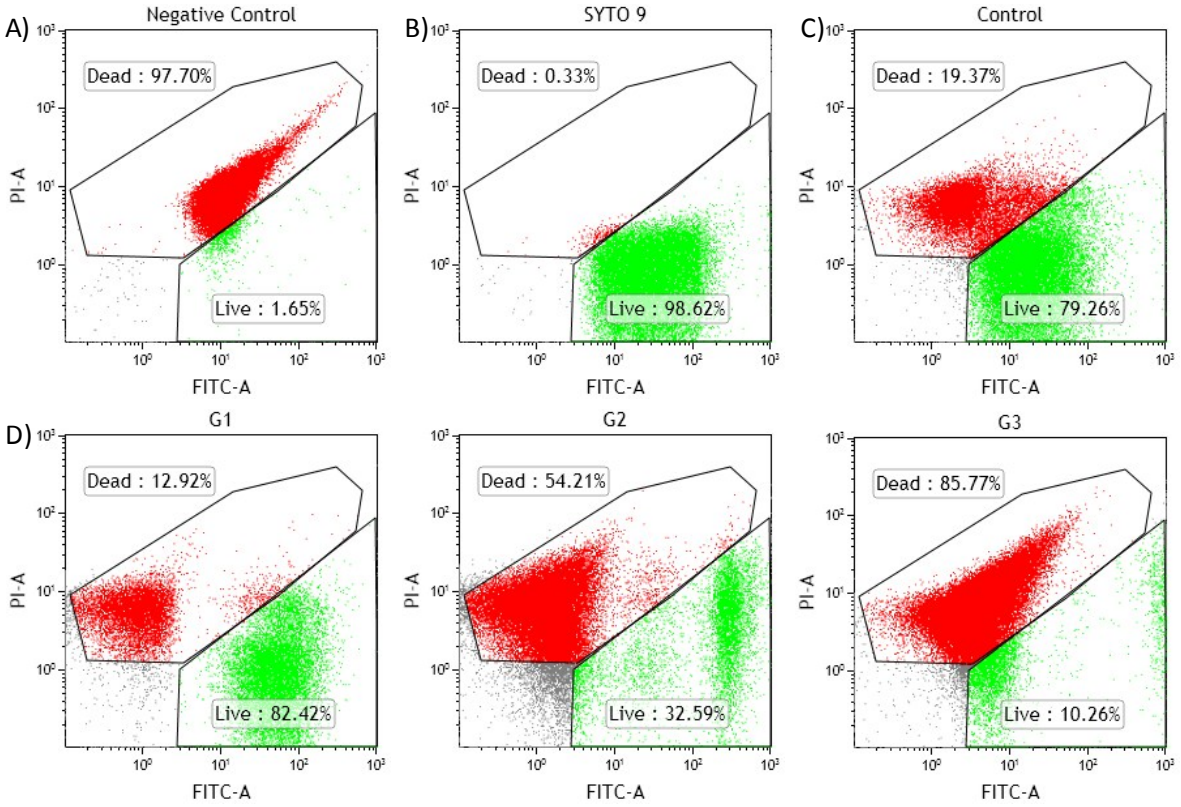

Figure S7: Flow cytometry of *V. harveyi* BB170 stained with propidium iodide (PI, red staining) and SYTO-9 (green staining). Red fluorescence was recorded on the PI channel (PI-A on y-axis) and green fluorescence on the FITC channel (FITC-A on x-axis). Gated population of *V. harveyi* BB170 based on calibration with A) bacteria treated with *i*-PrOH as a negative control

and stained with PI and SYTO-9 and B) non-treated bacteria stained with only SYTO-9 staining. C) Gated population of *V. harveyi* stained with PI and SYTO-9 in the absence (Control) and D) presence of Gn-NH_2 dendrimers for 1h at room temperature. Initial OD_{600} = 1, [NH_2] = 1.64 mM in PBS at pH 7.4. Values account for percentage of total number of counted cells.

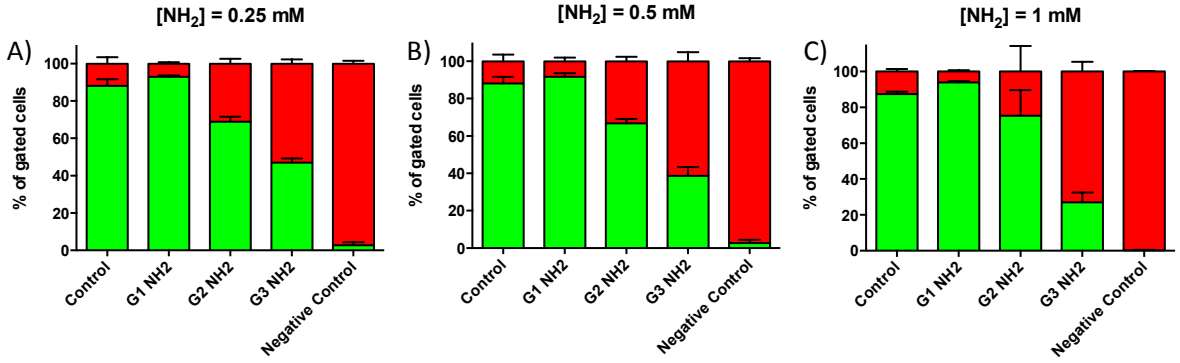

Figure S8: Normalized population of *V. harveyi* BB170 presented as the percentage of green and red cells. *V. harveyi* was incubated in the absence (control) and presence of Gn-NH_2. Bacteria were treated with *i*-PrOH as a negative control. Initial OD_{600} = 1. Bacteria diluted 5,000 fold in AB medium prior to incubation with Gn-NH_2. Final [NH_2] = 0.25 mM (A), 0.5 mM (B) and 1 mM (C).

Luminescence

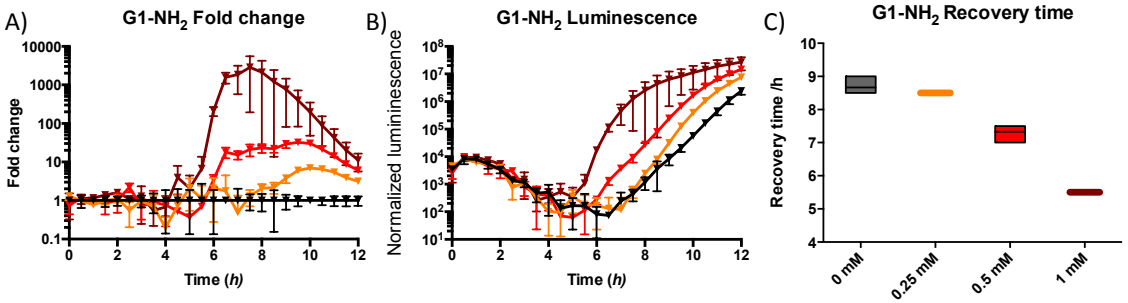

Figure S9: Luminescence of *V. harveyi* BB170 in the absence (black) and presence of G1-NH_2: [NH_2] = 0.25 mM (orange), 0.5 mM (red) and 1 mM (dark red). A) Fold increase of luminescence, B) normalised luminescence and C) recovery time.

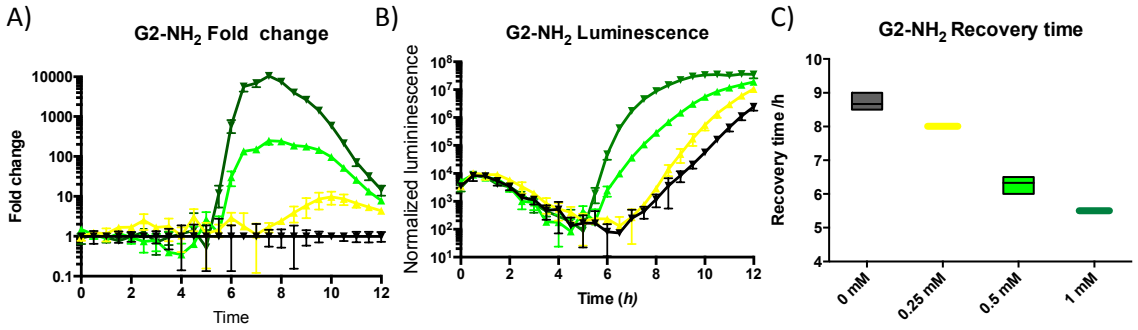

Figure S10: Luminescence of *V. harveyi* BB170 in the absence (black) and presence of G2-NH_2: [NH_2] = 0.25 mM (yellow), 0.5 mM (light green) and 1 mM (dark green). A) Fold increase of luminescence, B) normalised luminescence and C) recovery time.

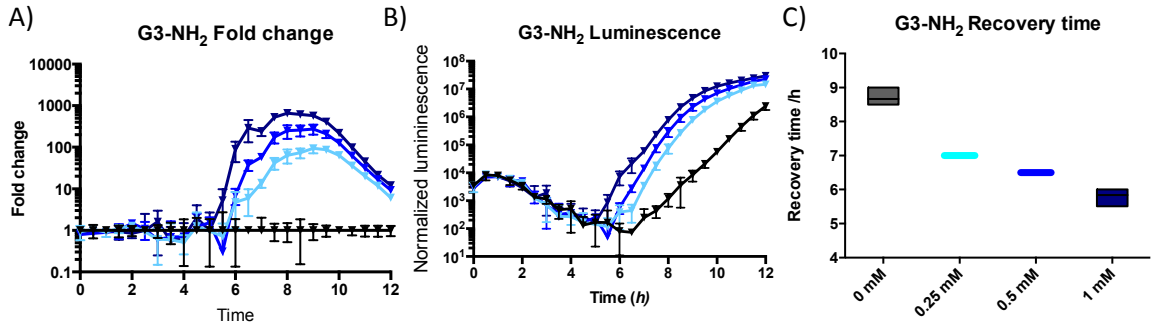

Figure S11: Luminescence of *V. harveyi* BB170 in the absence (black) and presence of G3-NH_2: [NH_2] = 0.25 mM (light blue), 0.5 mM (blue) and 1 mM (dark blue). A) Fold increase of luminescence, B) normalised luminescence and C) recovery time.

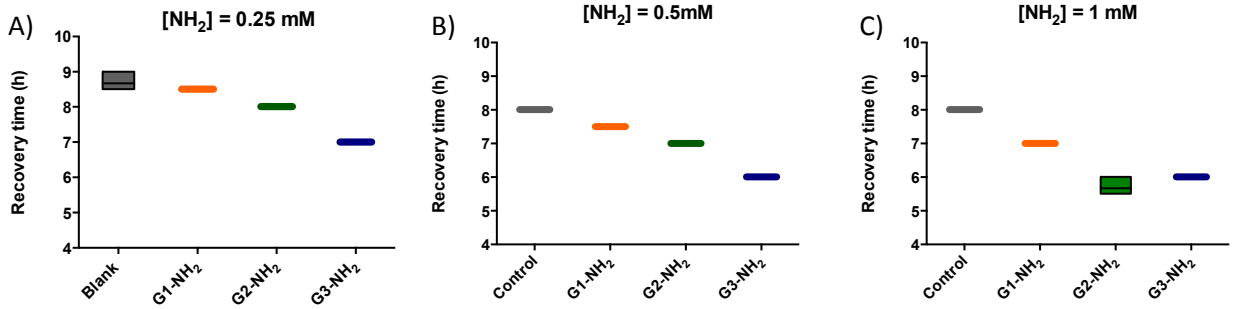

Figure S12: Representative generation dependent reduction (3 experiments) in the recovery time for Gn-NH_2 at 0.25 mM (A), 0.5 mM (B) and 1 mM (C).

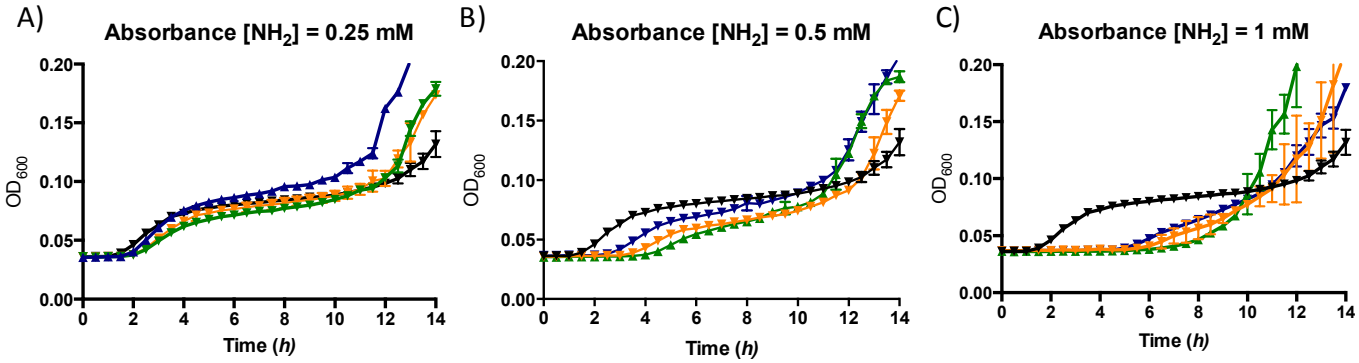

Figure S13: OD_{600} of *V. harveyi* BB170 in the absence (black) and presence of G1-NH_2 (orange), G2-NH_2 (green) and G3-NH_2 (blue) at [NH_2] = 0.25 mM (A), 0.5 mM (B) and 1 mM (C).

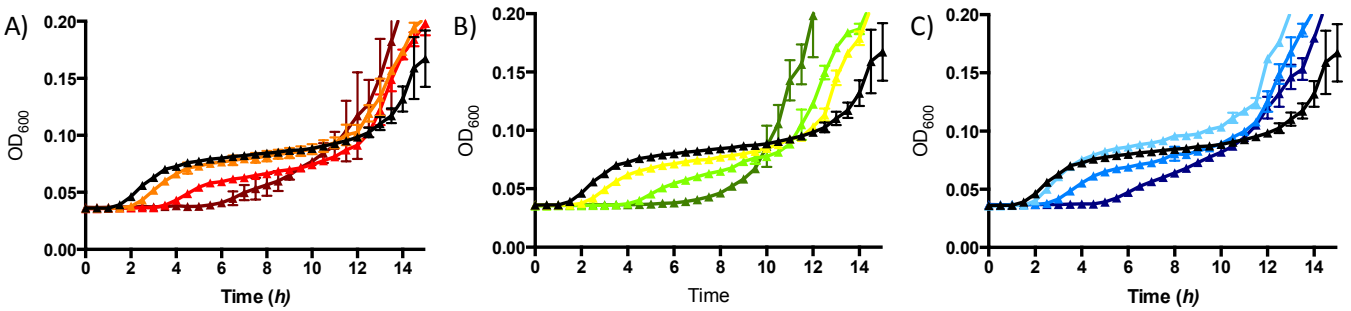

Figure S14: OD_{600} of *V. harveyi* BB170 in the absence (black) and presence of G1-NH_2 (A): [NH_2] = 0.25 mM (orange), 0.5 mM (red) and 1 mM (dark red); G2-NH_2 (B): [NH_2] = 0.25 mM (yellow), 0.5 mM (light green) and 1 mM (dark green); and G3-NH_2 (C): [NH_2] = 0.25 mM (light blue), 0.5 mM (blue) and 1 mM (dark blue).