

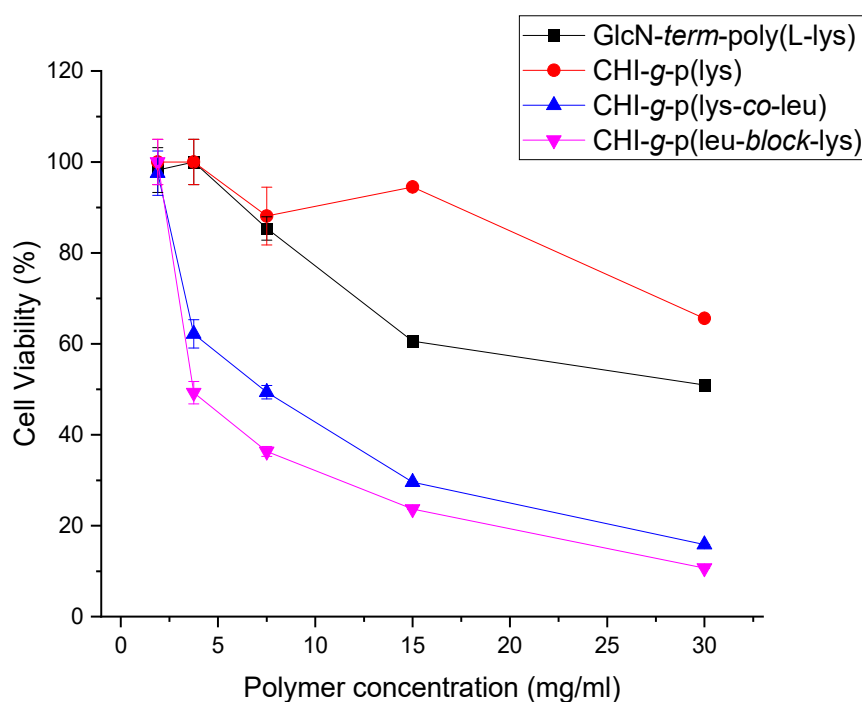
## Supplementary Information

# Synthesis and antibiotic activity of Chitosan-based comb-like co-polypeptides

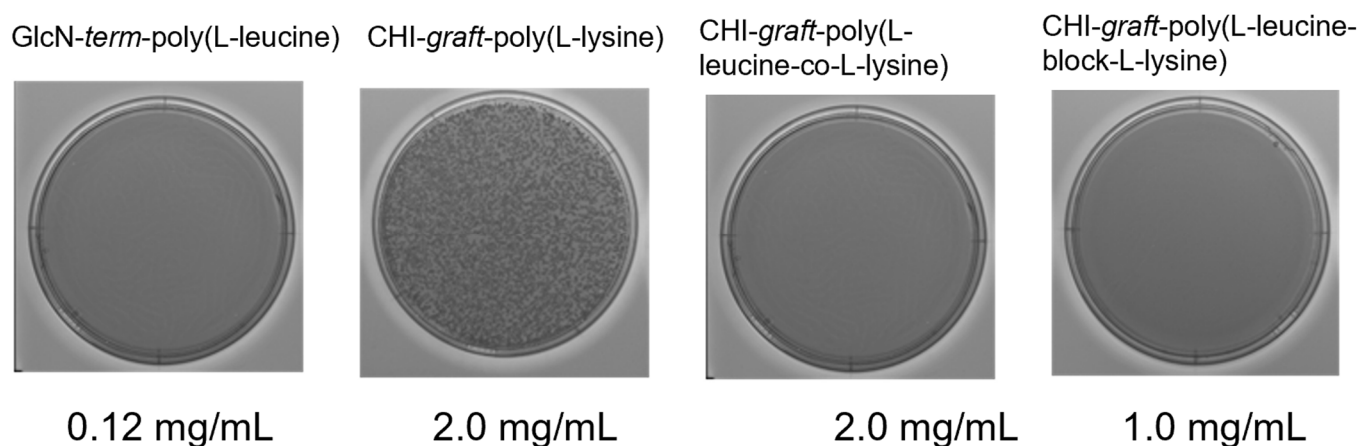
Timothy Enright<sup>1</sup>, Dominic Garcia<sup>1</sup>, Gia Storti<sup>1</sup>, Jason Heindl<sup>2</sup>, and Alexander Sidorenko<sup>1,\*</sup>

### MTT assay for cell viability

The cytotoxicity of CHI-g-Polypeptides was evaluated against Human Dermal Fibroblasts (HDF) cells using a MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay. MTT is reduced in the mitochondria of metabolically active cells by succinate dehydrogenase to yield formazan, a water-insoluble crystal which is strongly colored purple. The amount of formazan is proportional to the number of viable cells and is quantified spectroscopically. Following a method adopted from Riss *et al.* [1]. Cells were grown in Sigma-Aldrich's Fibroblast Growth Medium and seeded to a concentration  $10^5$  cells/well in a microtiter plate and incubated for 24 h at 37 °C in 5 % CO<sub>2</sub>. Next, increasing amounts of test compounds were added to each well and again incubated for 24 h. MTT was then added to a concentration of 0.45 mg/ml and incubated. After 3 h DMSO was added to dissolve any formazan crystals and absorbance was measured at 570 nm.



**Figure S1.** MTT cell viability assay results. Human Dermal Fibroblast (HDF) cells were exposed to the indicated concentrations of glucosamine-terminated poly(lysine) (black), CHI-graft-poly(lysine) (red), Chi-graft-poly(lysine-co-leucine) (blue), and Chi-graft-poly (leucine-block-lysine) (magenta) for 24hrs. Data are presented as the mean  $\pm$  standard deviation,  $n=3$ .



**Figure S2.** MBC Plates. *E. coli* cultures with the lowest concentration of graft copolymer that exhibited no change in growth were subcultured to determine minimum bactericidal concentrations. Each image displays bacteria growth on Mueller-Hinton agar plates after 24 h incubation. Note 2.0 mg/mL was the highest concentration tested and is below the MIC of CHI-*graft*-poly(L-lysine), i.e. MIC of CHI-*graft*-poly(L-lysine) is not reached at the concentration as high as 2.0 mg/mL.

## Reference

- S1. Riss, T.L.; Moravec, R.; Niles, A.; Duellman, S.; Benink, H.; Worzella, T.; Minor, L. Cell viability assays assay guidance manual. *Assay Guid. Man.* **2004**, 1–23.