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Electronic Supporting Information (ESI)

Self-assembled hybrid supraparticles that proteolytically degrade tumor necrosis factor-α

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Experimental Methods

Purification of pRgpA$_{CAT}$−Z$_E$

According to manufacturer's instructions (Qiagen), pRgpA$_{CAT}$−Z$_E$ was purified under native conditions. All prepared buffers contained 50 mM Na_2HPO_4, 300 mM NaCl, and 10 − 250 mM imidazole at pH 8.0. After cell lysis in buffer containing 10 mM imidazole by freeze-thaw and sonication, cell lysate was cleared by centrifugation. The cleared cell lysate was incubated with Ni-NTA resin, washed (20 mM imidazole), and eluted in the presence of 250 mM imidazole. Protein samples from purification steps were analyzed using tricine sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (Fig.2B).

Cloning of RgpA$_{CAT}$−Z$_E$

The plasmid pQE-60-RgpA$_{CAT}$−Z$_E$ was prepared, following the same protocols described for biosynthesis of pRgpA$_{CAT}$−Z$_E$ except using primers P5 and P2 (Table S2). Briefly, the DNA fragment for RgpA$_{CAT}$ was obtained by PCR of from the chromosomal DNA of *P. gingivalis* W50, using primers P5 and P2. The amplified DNA fragment was assembled with the Z$_E$-encoding DNA fragment, which was obtained by PCR using primers P3 and P4. Next, the assembled DNA fragment for RgpA$_{CAT}$−Z$_E$ was amplified by PCR with primers P5 and P4. After digestion using the *NcoI* and *BglII* restriction enzymes, the fragment for RgpA$_{CAT}$−Z$_E$ was inserted at the restriction sites of the pQE60 vector (Qiagen).

Expression and purification of $RgpA_{CAT}–Z_E$ and $RgpA_{CAT}$

The plasmid pJFQα1, which contains the gene fragment encoding $RgpA_{CAT}$ was a gift from Prof. M. Curtis and Dr. J. Aduse-Opoku (Queen Mary University of London). After transformation into the AFIQ-BL21 *E. coli* strain, cell cultures were grown at 37°C in 2X yeast extract and tryptone (YT) media containing ampicillin (200 mg/L) and chloramphenicol (34 mg/L). Protein expression was induced by 1.0 mM of isopropyl β-D-1-thiogalactopyranoside at an optical density at 600 nm of 0.8. Cells were harvested after 5 hours of expression at 37°C, and purified under denaturing conditions according to the manufacturer's instructions. Harvested cells were lysed by a freeze-thaw cycle and sonication in buffer containing 8 M urea, 10 mM Tris-Cl, and 100 mM Na_2HPO_4 at pH 8.0. After the cell lysate was cleared by centrifugation, it was incubated with Ni-NTA resin. The resin was washed using the same buffer at pH 6.3, and proteins were eluted using this buffer at pH 4.5.

Biocompatibility assay

Supraparticles with or without immobilized $pRgpA_{CAT}–Z_E$ were prepared as described in the manuscript. Following washing they were diluted into diluted in 100 µL of h Dulbecco's Modified Eagle's Medium (DMEM) medium supplemented with 10% FBS. J774A. 1 cells were seeded in 96 well plates at a concentration of 10^4 cells per well and were allowed to adhere overnight. The cell culture media was removed and the media containing particles was added directly to the cells and incubated for 24 hrs at 37°C. Supraparticles containing 0, 5, or 50 nM $pRgpA_{CAT}–Z_E$ were applied to the cells. Supraparticles containing 0 nM were dosed at a 6x higher concentration. Cell culture supernatants were collected and cytotoxicity measured by release of lactate dehydrogenase (LDH) according to manufacturer's instructions (Thermo Scientific). The negative control was cells with no added supraparticles or enzyme and the positive control (maximum cytotoxicity) cells were lysed with lysis reagent from the kit.

Protein and DNA Sequences

Table S1. Sequences of recombinant fusion proteins

Name	Sequence
pRgpA$_{CAT}$–Z$_E$	MGQQTELGRNPNVRLLESTQQSVTKVQFRMDNLKFTEVQTPKGMAQVPTYTEGVNLSEKGM PTLPILSRSLAVSDTREMKVEVVSSKFIEKKNVLIAPSKGMIMRNEDPKKIPYVYGKSYSQNKFFPG EIATLDDPFILRDVRGQVVNFAPLQYNPVTKTLRIYTEITVAVSETSEQGKNILNKKGTFAGFEDTY KRMFMNYEPGRYTPVEEKQNGRMIVIVAKKYEGDIKDFVDWKNQRGLRTEVKVAEDIASPVTA NAIQQFVKQEYEKEGNDLTYVLLIGDHKDIPAKITPGIKSDQVYGQIVGNDHYNEVFIGRFSCESKE DLKTQIDRTIHYERNITTEDKWLGQALCIASAEGGPSADNGESDIQHENVIANLLTQYGYTKIIKCY DPGVTPKNIIDAFNGGISLVNYTGHGSETAWGTSHFGTTHVKQLTNSNQLPFIFDVACVNGDFLF SMPCFAEALMRAQKDGKPTGTVAIIASTINQSWASPMRGQDEMNEILCEKHPNNIKRTFGGVT MNGMFAMVEKYKKDGEKMLDTWTVFGDPSLLVRTLVPTKMQVTAPAQINLTDASVNVSCDY NGAIATISANGKMFGSAVVENGTATINLTGLTNESTLTLTVVGYNKETVIKTINTNGEPNPYQPVS NLTATTQGQKVTLKWDAPSTKTNATTNTARSVDGIREMVLLSVSDAPELLRGSGSLEIEAAALEQ ENTALETEVAELEQEVQRLENIVSQYRTRYGPLRSHHHHHH
RgpA$_{CAT}$–Z$_E$	MGRYTPVEEKQNGRMIVIVAKKYEGDIKDFVDWKNQRGLRTEVKVAEDIASPVTANAIQQFVK QEYEKEGNDLTYVLLIGDHKDIPAKITPGIKSDQVYGQIVGNDHYNEVFIGRFSCESKEDLKTQIDR TIHYERNITTEDKWLGQALCIASAEGGPSADNGESDIQHENVIANLLTQYGYTKIIKCYDPGVTPK NIIDAFNGGISLANYTGHGSETAWGTSHFGTTHVEQLTNSNQLSFIFDVACVNGDFLFSMPCFAE ALMRTQKDGKPTGTVAIIASTINQSWASPMRGQDEMDEILCEKHPNNIKRTFGGVTMNGMFA MVEKYKKDGEEMLDTWTVFGDPSLLVRTLVPTKMQVTAPAQINLTDASVNVSCDYNGAIATISA NGKMFGSAVVENGTATINLTGLTNESTLTLTVVGYNKETVIKTINTNGEPNPYQPVSNLTATTQG QKVTLKWDAPSTKTNATTNTARSVDGIRELVLLSVSDAPELLRGSGSLEIEAAALEQENTALETEV AELEQEVQRLENIVSQYRTRYGPLRSHHHHHH
RgpA$_{CAT}$	MRGSHHHHHHGSACELRYTPVEEKQNGRMIVIVAKKYEGDIKDFVDWKNQRGLRTEVKVAEDI ASPVTANAIQQFVKQEYEKEGNDLTYVLLIGDHKDIPAKITPGIKSDQVYGQIVGNDHYNEVFIGR FSCESKEDLKTQIDRTIHYERNITTEDKWLGQALCIASAEGGPSADNGESDIQHENVIANLLTQYG YTKIIKCYDPGVTPKNIIDAFNGGISLANYTGHGSETAWGTSHFGTTHVEQLTNSNQLSFIFDVAC VNGDFLFSMPCFAEALMRTQKDGKPTGTVAIIASTINQSWASPMRGQDEMDEILCEKHPNNIK RTFGGVTMNGMFAMVEKYKKDGEEMLDTWTVFGDPSLLVRTLVPTKMQVTAPAQINLTDASV NVSCDYNGAIATISANGKMFGSAVVENGTATINLTGLTNESTLTLTVVGYNKETVIKTINTNGEPN PYQPVSNLTATTQGQKVTLKWDAPSTKTNATTNTARSVDGIRELVLLSVSDAPELLR

*The protein domains are colored: propetide (blue), RgpA$_{CAT}$ (purple), Z$_E$ (red), His$_6$ tag (gray).

Table S2. Sequences of primers used for PCR

Name	Sequence (5’ to 3’)
P1	TATCATCCATGGGTCAGCAGACAGAGTTGGGACGCAATC
P2	CAGGGATCCACTACCGCGAAGAAGTTCGGGGGCATC
P3	CTTCGCGGTAGTGGATCCCTGGAAATCGAAGC
P4	TATCATAGATCTCAGCGGACCGTAACGGGTAC
P5	TATCATCCATGGGTCGTTACACACCGGTAGAGGAAAAAC

Supplementary Data

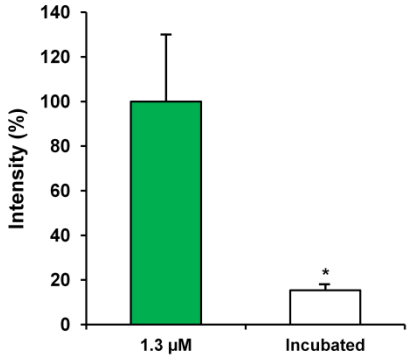

Figure S1. Quantification of EGFP−Z_E immobilization. Fluorescence intensities of 1.3 µM EGFP−Z_E solutions which were not incubated (1.3 µM) or incubated (incubated) with supraparticles. This concentration corresponds to the solution concentration used to make pRgpA$_{CAT}$−Z_E supraparticles labeled 50 nM in Fig. 6B. *p ≤ 0.05. Error bars represent the standard deviation (n = 3).

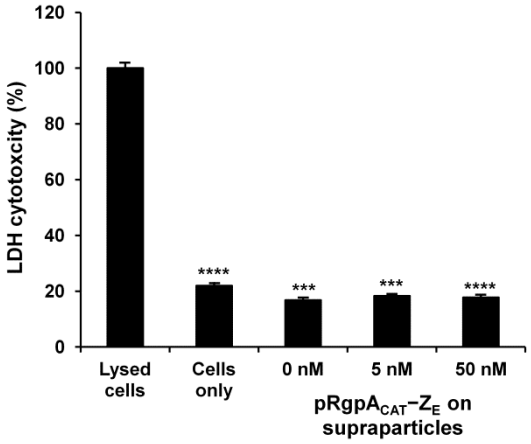

Figure S2. Biocompatibility assessment of pRgpA$_{CAT}$−Z_E supraparticles. LDH cytotoxicity of J774A.1 cells at different doses of pRgpA$_{CAT}$−Z_E immobilized on supraparticles. Sample labeled 0 nM contains 6-fold more supraparticles than 5 and 50 nM samples. ***p ≤ 0.001, ****p ≤ 0.0001. Error bars represent the standard deviation (n = 3).