Supplementary Information

for

Room temperature and selective triggering of supramolecular DNA assembly/disassembly by non-ionizing radiation

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Methods

Materials

Acrylamide, agarose, urea, and buffer salts were purchased from Bioshop Canada at the highest possible grade. Bromophenol blue, xylene cyanol, glycerine, and dithiothreitol were purchased from Sigma Aldrich. Unmodified DNA strands were purchased from idtDNA. Fluorophore- and maleimide- labeled strands were obtained from GeneLink. pDNA was obtained from ThermoFisher Scientific (Mississauga, Canada). T7 (10 $kU \cdot mL^{-1}$), Bal-31 (1 $kU \cdot mL^{-1}$), and exonuclease VII (10 $kU \cdot mL^{-1}$) were obtained from New England Biolabs (Whitby, Ontario), as were their corresponding buffers: NEB2 buffer (50 mM NaCl, 10 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl), 10 mM $MgCl_2$ 1 mM dithiothreitol, pH 7.9); BAL buffer (600 mM NaCl, 12 mM $CaCl_2$, 12 mM $MgCl_2$, 20 mM Tris-HCl, 1 mM EDTA, pH 8.0); Exonuclease reaction buffer (50 mM Tris-HCl, 50 mM sodium phosphate, 10 mM 2-mercaptoethanol, 8 mM EDTA, pH 8.0). TAMg buffer was made in-house (40 mM Tris, 12.5 mM $MgCl_2 \cdot 6H_2O$, 20 mM glacial acetic acid, pH 7.8), as was TBE buffer (90 mM Tris, 90 mM boric acid, 1.15 mM EDTA), phosphate buffer (8.98 $mg \cdot mL^{-1}$ potassium phosphate, pH 6.2), and Tris-$CaCl_2$ buffer (0.1 M Tris, 10 mM $CaCl_2$, pH = 8). Digestion buffer was used for antibody digestion (20 mM sodium acetate, pH 4.4, 0.05% sodium azide). Anion-exchange columns (HiTrap Q HP, 5 mL) were purchased from GE Healthcare. As a binding buffer for the anion-exchange purification, 0.02 M Tris, pH 8.9 was used. For elution, 1 M of NaCl was mixed with 0.02 M Tris, pH 8.9. Size-exclusion chromatography was performed using a HiPrep 16/60 Sephacryl™ S200 HR column from GE Healthcare. GelRed stain was purchased from VWR Canada (Ville Mont-Royal, Canada). Human AB serum was obtained from Mediatech (Manassas, VA). Lysozyme from chicken egg white was purchased in powder form from Sigma-Aldrich, as was α-chymotrypsin from bovine pancreas. Loading dye for agarose gels was made of 2 parts glycerol (1:1 glycerine:water) and 1 part blue dye stock (5 mg bromophenol blue, 5 mg xylene cyanole, 8 mL glycerine, and 2 mL water). Agar plates were made using LB agar from Bioshop Canada, and prepared according to manufacturer's directions. UV-transparent, ½ area microplates from Corning were purchased from Fisher Scientific and used in microplate experiments. Streptavidin-coated microplates for FLISA were made by Pierce and purchased through Thermofisher. Remicade (infliximab) IgG was obtained for research purposes from a commercial pharmacy. Immobilized pepsin was purchased from ProteoChem. Precast 4 – 15% PAGE gels and protein ladder were obtained from BioRad. For centrifugal dialysis, VivaSpin filters (30 kDa and 100 kDa MWCO) were purchased from GE Healthcare. NAb Protein A Plus spin columns (0.2 mL) were purchased from Fisher Scientific.

DNA Preparation

Strands were received from idtDNA and GeneLink in lyophilized form. 500 µL of deionized water was added to each vial, then vortexed to promote complete dissolution. Using a Nanodrop 2000 UV-vis spectrophotometer (Thermo Scientific, Wilmington, USA), each strand was quantified three times, using the absorbance at 260 nm as the optical density (OD/mL). Conversions for the number of nanomoles per OD were obtained from idtDNA's online oligoanalyzer tool.

Thermal annealing procedure

Thermally-annealed DNA nanostructures for exposure to microwave or THz radiation were prepared by combining equal volumes of 1 µM solutions (in TAMg buffer) of each of the three DNA strands in a PCR tube. A T-Professional Basic Gradient thermocycler (Biometra) was used to rapidly heat and maintain the samples at 95 ºC for 10 min, then slowly cool them to room temperature over the course of one hour.

Microwave source and exposure conditions

A Discovery™ single-mode microwave reactor from CEM (Matthews, NC, USA) with attached Coolmate® temperature control companion module employing perfluoropolyether (PFPE HT-55, Kurt J Lesker Company, Toronto, Canada) as the circulating coolant was used for all experiments involving microwaves. Sample temperature, pressure, and microwave power were controlled/recorded using the Synergy software (*vide infra*). Before each run, the temperature of the coolant was adjusted by addition of liquid nitrogen as appropriate for each experiment. These temperatures were selected to avoid sample freezing before the microwave source could be switched ON. For DNA experiments (both plasmid and nanostructures), a starting coolant temperature of –13 °C was found to be ideal. A 10-mL glass reaction vessel with ground glass joint (P/N 168302) was employed for all experiments with simultaneous cooling. Sample temperature was monitored *in situ* using a fibre optic probe (used without the quartz sheath provided by the manufacturer because of the small sample volumes) directly immersed into the sample. For proper immersion of the probe, a minimum of 180 µL of sample was required. All sample solutions were freshly prepared before exposure to microwave radiation. Of note, certain buffers used in this study absorbed microwave radiation more than others, which made it difficult

to control sample temperature at high microwave power. Problematic buffers were BAL buffer and TBE buffer. Non-problematic buffers were phosphate buffer, NEB2 buffer, and TAMg buffer.

Sample preparation for microwave anneals

DNA nanostructures: **95 W Anneal for the NINJA STAR system:** Equal volumes (60 µL) of 1 µM solutions of oligonucleotide strands 1, 2, and 3 in TAMg buffer were added to the reaction vessel at room temperature and mixed with gentle pipetting (total volume 180 µL). Using the 'Dynamic' method in the Synergy software, microwave power was programmed to a constant value of 95 W (exemplary value). For the first 5 min of the anneal, the maximum temperature was set to 30 ºC, so that the reactor more rapidly reached the programmed power of 95 W (Note: the temperature never reached 30 ºC during this period). For the remainder of the run, the maximum temperature was then decreased to 20 ºC. Once the program was started, the sample slowly heats to 20 ºC, over a 10 – 30 min period (depending on initial temperature and microwave power). Thereafter, the software automatically reduced the microwave power to maintain the set temperature, and to compensate for the slowly heating coolant (due to heat transfer from the sample). For all experiments, sample solutions were prepared at room temperature in the reaction vessel (i.e., outside of the reactor). Once the vessel was placed into the reactor, the microwave program was immediately engaged to avoid sample freezing due to the cold heat sink. The anneal was stopped when microwave power decreased to below 10 W. Following exposure, samples were transferred to PCR tubes and stored at 2 – 8 ºC pending analysis. For the *in-situ* monitoring experiment, shown in **Fig. 1C**, the aliquots for each time point were retrieved while maintaining microwave irradiation. The fibre optic probe and the stopper for the vial were briefly removed and the aliquots were retrieved using a pipette. **105 W Anneal for the DEDICATED NINJA STAR and TETRAHEDRON nanostructures:** Sample preparation was similar to the NINJA STAR system. 60 µL (1 µM) each of strands DNS1, DNS2, and DNS3 (for the DEDICATED NINJA STAR system) or 45 µL each of 1 µM Tet1, Tet2, Tet3, and Tet4 (for the TETRAHEDRON system) were used. Using the 'Dynamic' method in the Synergy software, a 5-step program was created: Step 1) Power = 105 W, Temperature = 30 °C, Time = 5 min; Step 2) Power = 105 W, Temperature = 28 °C, Time = 5 min; Step 3) Power = 105 W, Temperature = 26 °C, Time = 5 min; Step 4) Power = 105 W, Temperature = 24 °C, Time = 5 min; Step 5) Power = 105 W, Temperature = 20 °C, Time = 4 hours. The additional steps permitted greater control over the maximum power; raising the initial maximum temperature allows the microwave power to attain the set-point more rapidly. Slowly decreasing the

temperature keeps the sample from exceeding 25 °C. Although the time for Step 5 is set to 4 hours, the experiment was stopped manually when the microwave power fell below 10 W (~80 min.) Following exposure, samples were transferred to PCR tubes and stored at 2 – 8 °C pending PAGE analysis.

dsDNA strand exchange experiment: For each dsDNA length, two sets of complementary strands were ordered. One set was labeled with a 5'-Cy3 on one complement, and a 3'-BHQ-2 quencher on the other, such that when the duplex is assembled the fluorophore is quenched. The other set of strands has exactly the same sequence, but is unlabeled. For each dsDNA length, 900 µL of 1 µM labeled duplex and 180 µL of 10 µM unlabeled duplex, both in 1 × TAMg buffer, were prepared in separate vials. The labeled duplex solution was separated equally into 5 PCR tubes, which were then placed in a thermocycler and annealed as described above. After annealing, the 5 PCR tubes of labeled duplex were recombined and mixed gently with a pipette. Three aliquots of 30 µL labeled strands were set aside as blanks, to provide a reference for the minimum fluorescence. To the remaining 810 µL of labeled duplex solutions, sufficient unlabeled duplex was added to create a stock solution with a molar ratio of 1:2 labeled : unlabeled duplex (in this case, 162 µL of labeled duplex). Immediately, three aliquots of 30 µL were extracted and put aside to act as room temperature controls and ensure that any increase observed in fluorescence is due to the microwave and not ambient strand exchange. The remaining stock was wrapped in aluminum foil. Microwave experiments were performed with a starting volume of 180 µL. The 'Dynamic' method in the Synergy software was used to ensure that the temperature did not exceed the set point. For each sample, five different microwave powers were tested. Starting at the lowest (85 W for most duplexes), the Coolmate was chilled to approximately –11 °C. The timer was set to 5 min, and the cut-off temperature was set to 30 °C to encourage the microwave to rapidly achieve the desired power level. However, the actual sample temperature was manually maintained below 25 °C through addition of liquid nitrogen as necessary to the Coolmate accessory. After 5 min, the reaction vessel was removed from the microwave (to prevent it from freezing while the microwave was off) and a 30 µL aliquot was taken. The microwave power was raised by 10 W and the reaction vessel was placed back in the microwave for another 5 min. This process was repeated until five different microwave powers were tested. Each duplex was measured three times (n = 3). All samples were stored overnight at 4 – 8 °C. Fluorescence was measured using a Cytation 5 microplate reader from BioTek. Twenty-five microliters of each sample were used per well. The excitation and emission wavelengths were 540 nm and 570 nm, respectively.

Plasmids*:* A stock solution containing 2.5 µg of pDNA (5.04 µL of the 0.5 µg·µL^{-1} solution provided by the supplier) was diluted to 180 µL with NEB2 buffer at room temperature in the reaction vessel. Plasmids were microwaved at a constant power of either 80 or 95 W (depending on the experiment) for 20 min. The 'Fixed Power' method of microwave's Synergy software was used. This method fixes the power at a designated setting, and allows a temperature cut-off to be set. If the sample reaches the cut off temperature, the microwave will shut off, preventing the sample from overheating. For our purposes, the cut-off temperature was set to 30 °C. Temperature was controlled by addition of liquid nitrogen to the Coolmate as necessary. When the temperature climbed, liquid nitrogen was slowly added (to prevent the temperature from plummeting and freezing the sample). After microwaving, samples were stored in PCR tubes at 2–8 ºC pending analysis.

Bal-31: 3.42 µL of 1000 U·mL^{-1} Bal-31 stock solution, as supplied by the producer, was added to 256.6 µL of NEB2 buffer in the reaction vessel.

Lysozyme: In the reaction vessel, 240 µL of a 0.25 mg·mL^{-1} solution of lysozyme in phosphate buffer was prepared.

Chymotrypsin: Two hundred and forty-µL of a 2.5 mM solution of chymotrypsin was prepared in Tris-$CaCl_2$ buffer, in the reaction vessel.

Human serum: As-received serum was diluted to 0.25 % its initial concentration in TAMg buffer in the reaction vessel.

Microwave exposure of enzymes without simultaneous cooling

Enzymes solutions were prepared as described above. The Coolmate accessory for the Discover microwave was disconnected, and replaced with the Intellivent pressure module (CEM). Samples were placed in a 10-mL pyrex reaction vessel and covered with matching vessel caps (P/N 908035 and 909210 from CEM, respectively). For each run, a Fixed Power method was used, with the microwave power set to 95 W and the temperature cut-off set to maximum. During each run, the Intellivent pressure module was clamped to the top of the vessel, creating a sealed vessel. After 20 min of microwave exposure, the temperature was allowed to return to <50 °C before removing the pressure system.

Modified fluorescence-linked immunosorbent assay (FLISA)

Preparing the microplate

Each streptavidin-coated well was washed three times with 200 μL of wash buffer (BSA +0.05 % TWEEN 20), followed by addition of 100 μL of biotinylated antigen (10 μg•mL^{-1}, TNF-alpha). The plate was incubated at room temperature for two hours on a shaker. Excess biotinylated antigen was removed, and the wells were again washed three times with wash buffer, to remove any non-specifically bound antigen.

Sample preparation

A 3.6 mL stock solution was created by combining DNS1-fab', DNS2-Cy3, and DNS3-Cy5, to a final concentration of 0.18 μM (per DNA strand) in TAMg buffer. Three aliquots of 200 μL each were removed and assembled using the traditional thermal anneal (95 °C for 5 min followed by a slow cooling). 3x1 mL aliquots were subjected to a 105 W microwave anneal. Due to the increased volume of the samples (1 mL instead of 180 μL), a stir bar was added stirring was set to 'low.' From each of the six samples (3 for microwave, 3 for thermal anneal), 100 μL was taken and placed in a prepared well. The plate was incubated and shaken at room temperature for 3 hours. Each well was then washed three times with wash buffer to remove any unbound assemblies.

Fluorescent measurements were taken using a Cytation5 microplate reader (Biotek). Endpoint measurements were excited at 549 nm (slit width = 20 nm) and emission was collected at 665 nm (Cy5 emission, slit width = 20 nm, gain = 150).

THz source and exposure conditions

Intense THz pulses were generated at the Advanced Laser Light Source (INRS-EMT, Varennes, Canada) by optical rectification in a $LiNbO_3$ crystal and a pulse-front-tilt technique, as described elsewhere.[1] A scheme representing the optical layout is presented in **Supplementary Fig. S10**. The spectrum (peak at 0.9 THz) and temporal waveform are presented in **Fig 2A**. The radiated THz pulses have a 70 kV·cm^{-1} peak field at focus, an energy of 0.4 μJ, a pulse duration of 1 ps, and a repetition rate of 2.5 kHz. The peak intensity was 368 MW·cm^{-2} and the average intensity was 57 mW·cm^{-2}. Because the spot size of the THz pulse at the focus position was ~2 mm^3, the sample volume was limited (1.5 μL for DNA nanostructure, 0.7 μL for plasmids, and 0.49 μL for proteins). These small volumes further guarantee that the entire sample was exposed to THz radiation, though they needed to be more concentrated than for the experiments with microwaves, to satisfy the requirements for subsequent analysis. The required volume of sample was aspirated

into a 0.1 – 10 µL polypropylene (THz transparent) micropipette tip (Gilson brand from Mandel Scientific, Guelph, Canada), and then air was drawn into the tip by adjusting the pipette's volume setting. As such, the sample moved up the pipette tip, leaving an air gap at its extremity, which was sealed with Vaseline to prevent water evaporation during exposure. The pipette was then clamped to a retort stand on the optical table such that the sample was in the focal spot of the THz beam. The sample was exposed to the THz radiation for 10 min. For sample recovery, the pipette tip was cut in the region above the Vaseline and below the sample, then ejected into a PCR tube for storage.

Sample preparation for THz exposure

All samples solutions were freshly prepared before exposure to THz radiation.

DNA Nanostructures: **NINJA STAR System**: Stock solutions were made for each of the three DNA strands (114 µM in 1× TAMg). The stocks were split into two portions – one for pre-assembled nanostructures and one for unassembled nanostructures. For the pre-assembled nanostructures 1.75 µL of each stock was mixed together and annealed thermally. For each exposure, 0.5 µL of preassembled stock was exposed. For unassembled nanostructures, 0.17 µL of each strand solution were mixed in a PCR tube and aspirated into the sample holder (pipette tip; *vide supra*) for exposure to THz. Following exposure, the sample was recovered by cutting of the Vaseline-clogged portion of the tip (without disturbing the sample), and then using the pipette to expel the sample into a PCR tube for storage on ice until they could be transferred to a freezer (–20 ºC) until analyzed. **DEDICATED NINJA STAR and TETRAHEDRON**: Stock solutions were made by mixing 0.15 nmol of each strand (DNS1, DNS2, DNS3 or Tet1, Tet2, Tet3, and Tet4) and evaporating to dryness using a SpeedVac. Each mix was then re-suspended in 6 µL of 1× TAMg buffer and mixed thoroughly, prior to a thermal anneal. After annealing, the stock solution was briefly centrifuged to ensure all liquid was collected at the bottom of the tube, then separated into 6 × 1 µL samples. Three of the samples were put aside as controls. Each of the remaining samples was exposed to THz irradiation for 10 minutes. Once all samples were irradiated, 24 µL of 1× TAMg were added to each sample and control (giving a final concentration of 1 µM), and stored at 4 – 8 ºC until analyzed. ***Fluorescent dsDNA for strand exchange experiment:*** Five different duplex lengths were investigated: 10, 15, 20, 30, and 40 bp. In each duplex, one strand was labeled on the 5' end with a Cy3 fluorophore, and the other strand with a 3' BHQ2 quencher, such that when assembled into a duplex, the fluorophore and quencher are adjacent to each other. For each duplex, 0.225 nmol of each component strand was dried via SpeedVac. The strands were

then re-suspended in 18 µL of 1 × TAMg, and mixed thoroughly using a vortex mixer. Each vial was then briefly centrifuged prior to undergoing a thermal anneal. The thermal anneal served to assemble the duplexes into a 'quenched' form. After another brief centrifuge, each duplex was separated into 9 × 2 µL aliquots. Three were set aside as room temperature controls, three were exposed to 10 min of THz irradiation each, and three were kept as for use as kinetic controls. All samples were then diluted with 23 µL of 1× TAMg buffer to a final volume of 25 µL (1 µM final concentration).

Plasmids*:* 0.7 µL aliquots of as-delivered pUC18 or pBR322 (0.5 $\mu g \cdot \mu L^{-1}$) were aspirated into the sample holder for exposure to THz. After exposure, samples were transferred to a PCR tube and diluted to 25 µL with the appropriate buffer, depending on the experiment required (BAL buffer for Bal-31 digestions; Exonuclease Reaction buffer for exonuclease VII digestions; NEB2 buffer for T7 digestions and for gel electrophoresis). The diluted samples were stored at –20 °C before analysis, as were the controls not exposed to THz or microwave radiation.

Agarose gel electrophoresis

BioRad mini sub units were used for horizontal agarose gel electrophoresis. Native agarose gel electrophoresis was performed with 1% agarose slab gels prepared in Tris boric EDTA (TBE) buffer, pH 8.5. Five-µL of plasmid sample (in NEB2 buffer) was loaded into each lane, and the gel was run at 80 V for 3.25 h. GelRed in TBE buffer (8 µL in 150 mL) was used for post staining (min. 5 hours of staining), and gels were then imaged using a BioDoc-It™ imaging system from UVP, with UVP imaging software. Band intensity analyses were performed using ImageJ software.

For the antibody- and FRET-labeled DNS assemblies, a 1.5% agarose gel, run at 100 mA for 40 min was used. The gel was not stained, but instead imaged using a Typhoon imager to excite the Cy3 fluorophore.

Denaturing agarose gel electrophoresis was performed with 1% agarose gels prepared in TBE buffer containing 1 M urea as a denaturant. Prior to loading, 5 µL of each sample (in NEB2 buffer) were mixed with 5 µL of 8 M urea, and heated for 30 min at 80 °C to encourage denaturation. The gels were run at 80 V for 3.25 h then post-stained, imaged, and analyzed as above.

Poly(acrylamide) gel electrophoresis

A Hoeffer vertical electrophoresis unit was used for polyacrylamide gel electrophoresis, with which the DNA nanostructures were characterized. Polyacrylamide gels were prepared with 1× TAMg to a final acrylamide concentration of 6% (19:1 bis:acrylamide). Each THz-exposed sample was diluted to 20 µL with 1× TAMg. Ten-µL of this solution was combined with 2 µL of glycerol to create the final sample for gel analysis. The gel was run at 250 V for 2.5 hours, then stained, imaged, and analyzed as above.

Denaturing gels containing 12% acrylamide (19:1 bis:acrylamide) were prepared in TBE buffer containing 8 M urea. Using the remaining 10 µL of each sample from the native PAGE, 10 µL of 8 M urea was added to denature the DNA prior to loading. Gels were run at 250 V for 2.5 hours, in TBE running buffer, then stained, imaged, and analyzed as above.

Digestion of pDNA by T7 endonuclease

Each digestion was performed in triplicate using 350 ng of pDNA in 25 µL NEB2 buffer, and a stock solution of T7 endonuclease was made with 0.28 µL of as-received enzyme solution per 1 µL total volume (in NEB2 buffer). The first aliquot (5 µL, 0 min) was taken prior to the addition of T7 endonuclease. To the remaining 20 µL, 1 µL of the enzyme stock solution was added. Aliquots (5.25 µL) were taken at 1, 2, 3, and 4 h of digestion. Digestion in each aliquot was quenched by immediate addition of a loading dye/glycerol mix and storage at –20 ºC. Native agarose gel analysis was performed as described above to determine the percentage of supercoiled, linear, circular (etc.) pDNA at each time point by image analysis using ImageJ software. The decrease of supercoiled pDNA with time was fitted to a first order exponential decay, with the y offset set to zero on the assumption that given enough time, all supercoiled DNA would be digested. This yielded the half-life of supercoiled DNA digestion, a parameter that was used to qualitatively determine the change in the degree of supercoiling of pDNA before and after exposure to microwave/THz radiation. It was also used to compare the enzymatic activity of T7 endonuclease before and after exposure to microwave/THz radiation.

Digestion of pDNA by Exonuclease VII

Each digestion was performed in triplicate using 350 ng of pDNA in 25 µL in Exonuclease Reaction Buffer (pre-heated to 37 ºC using a heating block). Temperature was maintained at 37 ºC for the duration of the digestion. A stock solution of enzyme was made containing 0.28 µL of as-received enzyme solution per 1 µL in Exonuclease reaction buffer. The first aliquot (5 µL, 0 min) was taken prior to the addition of exonuclease VII. To the remaining 20 µL, 1 µL of enzyme

stock were added. Aliquots (5.25 µL) were taken at 1, 2, 3, and 4 h of digestion. Each aliquot was immediately quenched by the addition of 0.5 µL of the dye/glycerol and stored at –20 °C. The digestions were visualized using agarose gel electrophoresis (80 V, 3 h 15 min, 1% agarose), which were post-stained using GelRed and visualized using a transilluminator. ImageJ software was used to quantify the relative ratios of supercoiled, open circle, and linear DNA at each time point during the digestion. Using OriginLab, the decrease in supercoiled DNA over time was fitted using a first-order exponential decay curve, which yielded half-life data ($\tau_{1/2}$). Half-life was used to compare the effects of microwave and THz exposure to unexposed controls.

Digestion of pDNA by Bal-31

Each digestion was performed in triplicate using 350 ng of pDNA in 25 µL in BAL buffer. A stock enzyme solution was prepared containing 0.04 µL of enzyme per 1 µL of Bal buffer. The first aliquot (5 µL, 0 min) was taken prior to the addition of enzyme. To the remaining 20 µL, 1 µL of enzyme stock was added. Aliquots (5.25 µL) were taken at 0, 30, 60, 120, and 180 min of digestion. Aliquots were immediately quenched by the addition of glycerol/dye mix and frozen at –20 °C. Analysis was performed by native agarose gel electrophoresis, as described in the previous section.

NOTE: For experiments in which the Bal-31 itself was microwaved, it was necessary to use 1x TAMg in lieu of BAL buffer because the latter strongly absorbed microwaves, leading to excessive heating. In order to obtain digestion profiles similar to those obtained in BAL buffer, 8x as much Bal-31 was used (i.e., 0.32 U).

Digestion of pDNA by human serum

A stock solution of human serum was made by dilution to 5% its initial concentration using TAMg buffer. Three hundred and fifty-ng of pUC18 was diluted to 25 µL in TAMg buffer. A first aliquot of 5 µL was taken prior to the addition of human serum (t = 0 min), then 1 µL of serum stock solution was added to the remaining 20 µL of DNA, creating a final serum concentration of 0.25%. Remaining aliquots were taken at t = 30, 60, 120, 180 min.

Catalytic activity of lysozyme

For lysozyme, enzyme activity was determined via the digestion of the cell wall of *Micrococcus lysodeikticus* (Sigma Aldrich, Oakville, Canada). For each replicate, 480 µL of a 0.25 mg·mL^{-1} solution of *M. lysodeikticus* was mixed with 720 µL of phosphate buffer (66 mM, pH 6.24). Forty-µL of a lysozyme solution in the same buffer (0.25 mg·mL^{-1}) was added, and the absorbance of

the solution at 450 nm was monitored with time (every 2 s for 5 min) using a Nanodrop 2000 UV-Vis spectrophotometer. Catalytic activity was determined from the initial slope of the resulting curve, and was normalized to the slope of as-received lysozyme.

Catalytic activity of chymotrypsin

The catalytic activity of chymotrypsin was measured via its ability to hydrolyse *N*-succinyl-L-phenylalanine *p*-nitroanilide (Sigma Aldrich, Oakville, Canada). For each replicate, 760 µL of Tris-$CaCl_2$ buffer were mixed with 200 µL of substrate stock (2.5 mM in the same buffer). Forty-µL of chymotrypsin stock (212.4 µM in Tris-$CaCl_2$ buffer) was added, and absorbance at 405 nm was measured every two seconds for one minute in a Nanodrop 2000 spectrophotometer. Catalytic activity was determined from the initial slope of the resulting curve, and was normalized to the slope of as-received chymotrypsin.

Transformation of pDNA into *E. coli*

Competent *E. coli* cells were purchased from New England Biolabs (BL21 (DE3) Competent *E. coli,* Whitby, Ontario) or prepared in the laboratory (THz experiments). For the latter, *E. coli* cells were grown in 5 mL of LB broth for 16 h at 37 °C with shaking. The suspension was then diluted 100-fold in LB broth and incubated with shaking until the culture reached an optical density at 600 nm of 0.5 – 0.7. The suspension was then transferred to centrifugation tubes, put on ice for 15 min, and centrifuged at 8,000 rpm 15 min. The pellet was re-suspended in 33 mL of 100 mM KCl, 15% glycerol, 60 mM $CaCl_2$, and 30 mM potassium acetate, pH 5.8 (filter sterilized), and left on ice 1 h. The suspension was centrifuged again at 8,000 rpm for 15 min and the pellet re-suspended in 4 mL of cold 10 mM KCl, 15% glycerol, 75 mM $CaCl_2$ and 10 mM MOPS, pH 6.8, and left on ice for 15 min. Two hundred-µL aliquots were put on dry ice, frozen, and stored at –80 ºC.

In-house prepared competent cells were used for all THz-exposed samples, and purchased cells were used for microwaved samples. Approximately 45 min prior to experimentation, 100 µL of 100 $\mu g \cdot mL^{-1}$ ampicillin in water was spread evenly over each agar plate. Plates were put aside to allow the ampicillin time to penetrate the agar. pDNA samples (0.7 µL; 350 ng pDNA) emerging from THz exposure experiments were diluted to 0.14 $ng \cdot \mu L^{-1}$ with 1 M tris buffer containing 100 mM NaCl. For each transformation experiment, 2 µL of this solution were mixed with 30 µL of competent *E. coli* cells from a single tube, then set on ice for 30 min. Samples were then subjected to a heat shock for 45 s in a 42 ºC hot bath, then returned to the ice for 5 min. Three hundred-µL of SOC media (2% tryptone, 0.5% yeast extract, 10 mM NaCl,

2.5 mM KCl, 10 mM $MgCl_2$, 10 mM $MgSO_4$, and 20 mM glucose) were added to each sample, then placed on a shaker at room temperature for 30 min. One hundred-µL of each sample was spread on prepared ampicillin-laced agar plates, then incubated overnight. Colonies were counted the following morning.

Antibody Digestion

Prior to digestion, infliximab was reconstituted in 1 mL water to a concentration of 10 $mg \cdot mL^{-1}$, then concentrated using a 100 kDa MWCO dialysis filter, and rinsed once with digestion buffer (0.2 mM NaOAc, pH 4.5) to remove any salts that may have been present (15000 × g, until approx. 50 µL remained). Concentrated infliximab was quantified using A_{280} and a molar extinction coefficient of $\varepsilon^{0.1\%} = 1.3$, then diluted to 10 $mg \cdot mL^{-1}$ using digestion buffer. Infliximab was then digested to f(ab')$_2$ fragments using immobilized pepsin, as described in the manufacturer's protocol. All volumes were adjusted to accommodate the actual volume of infliximab that was used. Pepsin digestion was allowed to proceed for a maximum of 3 hours while shaking at 37 °C. The mix was centrifuged (1000 × g, 4 min), and the supernatant was recovered and stored overnight. The supernatant, containing a mix of undigested infliximab, f(ab')$_2$ fragments, and digested Fc fragments, was subsequently purified using NAb Protein-A Plus spin columns, as per the protocol supplied by the manufacturer. Protein-A is able to retain the undigested infliximab, while f(ab')$_2$ fragments can pass though the column and be collected. Following f(ab')$_2$ collection, the remaining infliximab was recovered and stored for future use. Collected f(ab')$_2$ was concentrated using a 30 kDa MWCO Vivaspin filter, and concentration was determined via UV/vis (A_{280}, $\varepsilon^{0.1\%} = 1.4$) and diluted to 1-3 mg/mL using digestion buffer. To digest f(ab')$_2$ fragments to fab' fragments, a 10 $mg \cdot mL^{-1}$ solution of f(ab')$_2$ and a 0.9375 mM solution of TCEP in 1× TAMg buffer were mixed in a 4:1 volumetric ratio. The reaction was left at room temperature for 90 min. Undigested f(ab')$_2$ and TCEP were separated from the fab' using an AKTA Start fast protein liquid chromatographer (FPLC) equipped with a HiPrep 16/60 Sephacryl™ S200 HR column. For purification, filtered 1× TAMg buffer passed through the column at a flow rate of 0.8 $mL \cdot min^{-1}$. The column was allowed to equilibrate for 0.2 column volumes (CV, 24 mL) before sample injection. Elution occurred over 120 mL (1 CV), while collecting fractions every 4 mL. The desired fab' fragments were found in fraction 16–19 (Supplementary **Fig. S12**). To prevent oxidation to f(ab')$_2$, the fab' fractions were kept dilute until ready for reaction with the DNA-maleimide (typically less than 3 days). At that time, the fractions containing fab' were concentrated using 30 kDa MWCO centrifugal filters.

DNA – Maleimide deprotection

Maleimide-labelled DNA purchased from Genelink arrives as a protected maleimido-2,5-dimethylfural cycloadduct. Maleimide deprotection was performed using a microwave deprotection strategy.[2] Protected maleimide-DNA was mixed with 1:1 MeOH:H_2O ratio to a final DNA concentration of 25 μM in 200 μL. This mix was placed in the microwave reactor, with the CoolMate peripheral replaced with the pressure clamp peripheral. A 'dynamic' method was prepared (T = 90 °C, Power = 300 W). With this method, the temperature was maintained at T = 90 °C by varying the power. The buildup of pressure kept the sample from boiling, and PowerMax was enabled to provide cooling via compressed air. The reaction was run for 90 minutes. Once the mixture had cooled, it was transferred to an Eppendorf tube and dry using a SpeedVac.

DNA-Maleimide to Fab' conjugation

Deprotected DNA-maleimide was re-suspended in 1× TAMg pH 7.0 to give a 50 μM stock solution. 2.5 equivalents of freshly concentrated fab' were added to the DNA, and sufficient buffer was added to bring the final DNA concentration to 20 μM. The reaction was left at room temperature for 2 hours, then stored in the fridge overnight. Purification was performed on an anion-exchange column (HiTrap Q HP, 5mL). Using a flow rate of 5 $mL \cdot min^{-1}$, the column was allowed to equilibrate for 1 min using the binding buffer (0.02 M Tris, pH 8.9) prior to sample injection. Gradient elution was used to elute the sample over the course of 6 column volumes, beginning with 100% binding buffer and ending with 100% elution buffer (0.02 M Tris, 1 M NaCl, pH 8.9. Fractions were collected every 1 mL. In all cases, the first peak was found to contain the DNA–fab' conjugate (see SI). The fractions were concentrated using a 30 kDa MWCO ultracentrifugation filter.

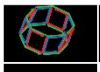
Surface Plasmon Resonance (SPR)

SPR was performed using a Biacore T100. To begin, anti-histidine antibodies were immobilized on a CM5 sensor chip using the *His* Capture Kit (GE Healthcare) as per the manufacturer's instructions. Next, the *his*-tagged antigen (TNF-alpha or EpCAM) was immobilized by passing 2 $\mu g \cdot mL^{-1}$ over the surface for 180 s, giving a response of 190 RU. This was followed by sample injection (100 nM, 60 $\mu L \cdot min^{-1}$ for 60 s) and then injection of buffer (HBS-EP) over the course of 4 min to observe dissociation. After each sample, the surface was regenerated by injecting 10 mM Glycine pH 1.5 for 30 s at a flow rate of 30 $\mu L \cdot min^{-1}$. Samples were prepared at a concentration of 0.5 μM per strand (DNS1-fab', DNS2, DNS3) in 1× TAMg buffer. Microwave

anneals (beginning at 105 W) and thermal anneals were compared. As a control, 0.5 μM of DNS1-fab' was prepared. All samples were diluted to 100 nM prior to injection.

	Control	85 W Start	95 W Start	105 W Start
Control	0	1	1	1
85 W Start	-	0	1	1
95W Start	-	-	0	1
105 W Start	-	-	-	0

	Control	85 W Start	95 W Start	105 W Start
Control	0	0	0	1
85 W Start	-	0	0	1
95W Start	-	-	0	1
105 W Start	-	-	-	0

	Control	85 W Start	95 W Start	105 W Start
Control	0	1	1	1
85 W Start	-	0	0	0
95W Start	-	-	0	0
105 W Start	-	-	-	0

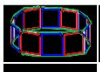
	Control	85 W Start	95 W Start	105 W Start
Control	0	1	1	0
85 W Start	-	0	0	1
95W Start	-	-	0	1
105 W Start	-	-	-	0

Table S1| Full comparison of all species following microwave anneals. The relevant species is indicated in the top left corner of each chart. An ANOVA Tukey analysis was used ($p = 0.05$). A '1' indicates that the average abundance of a particular structure is significantly different between two anneals.

NINJA STAR	5’ → 3’
Strand 1	TTGCGACCTTTTTGTGTGCCGTTTACGCCTGTCCTTTGTGTGCCGT
Strand 2	TGTGTGCCGTTTAAGGTCGCAATTTGTGTGCCGTTTGGACAGGCGT
Strand 3	ACGGCACACATTACGGCACACATTACGGCACACATTACGGCACACA
DEDICATED NINJA STAR	**5’ → 3’**
DNS1	CGGTCAGCGTTTATTCCCTATGTTCTACTTTACATTGATTGAGTTC
DNS2	TTCATCGCCCTTACGCTGACCGTTTTTGGCAGGTTTTGTAAAGTAG
DNS3	CATAGGGAATTTGGGCGATGAATTGAACTCAATCTTACCTGCCAAA
Unlabeled Duplexes (XU)	**5’ → 3’**
15U	TGTGATAAGCCTCGT
15U’	ACGAGGCTTATCACA
20U	TGTGATTCTATGCCAGTCGT
20U’	ACGACTGGCATAGAATCACA
30U	TGTGACTATCTTCGGAGGCTGGTAATGCGT
30U’	ACGCATTACCAGCCTCCGAAGATAGTCACA
40U	TGTGCTATTCCGCCGTGGCTGGTCCATTATGCGATGTCGT
40U’	ACGACATCGCATAATGGACCAGCCACGGCGGAATAGCACA
Labeled Duplexes (XL)	**5’ → 3’**
15L	Cy3-TGTGATAAGCCTCGT
15L’	ACGAGGCTTATCACA-BHQ2
20L	Cy3-TGTGATTCTATGCCAGTCGT
20L’	ACGACTGGCATAGAATCACA-BHQ2
30L	Cy3-TGTGACTATCTTCGGAGGCTGGTAATGCGT
30L’	ACGCATTACCAGCCTCCGAAGATAGTCACA-BHQ2
40L	Cy3-TGTGCTATTCCGCCGTGGCTGGTCCATTATGCGATGTCGT
40L’	ACGACATCGCATAATGGACCAGCCACGGCGGAATAGCACA-BHQ2
Labeled DEDICATED NINJA STAR	**5’ → 3’**
DNS1-mal	Maleimide-CGGTCAGCGTTTATTCCCTATGTTCTACTTTACATTGATTGAGTTC
DNS2-Cy3	TTCATCGCCCTTACGCTGACCGTTTTTGGCAGGTTTTGTAAAGTAG-Cy3
DNS3-BHQ2	BHQ2-CATAGGGAATTTGGGCGATGAATTGAACTCAATCTTACCTGCCAAA
DNS3-Cy5	Cy5-CATAGGGAATTTGGGCGATGAATTGAACTCAATCTTACCTGCCAAA
TETRAHEDRON	**5’ → 3’ (ref 32 from manuscript)**
Tet-A	ACATTCCTAAGTCTGAAACATTACAGCTTGCTACACGAGAAGAGCCGCCATAGTA
Tet-B	TATCACCAGGCAGTTGACAGTGTAGCAAGCTGTAATAGATGCGAGGGTCCAATAC
Tet-C	TCAACTGCCTGGTGATAAAACGACACTACGTGGGAATCTACTATGGCGGCTCTTC
Tet-D	TTCAGACTTAGGAATGTGCTTCCCACGTAGTGTCGTTTGTATTGGACCCTCGCAT

Table S2| DNA sequences. Abbreviations: Cy3 = cyanine 3 dye, Cy5 = cyanine 5 dye, BHQ2 = black hole quencher 2.

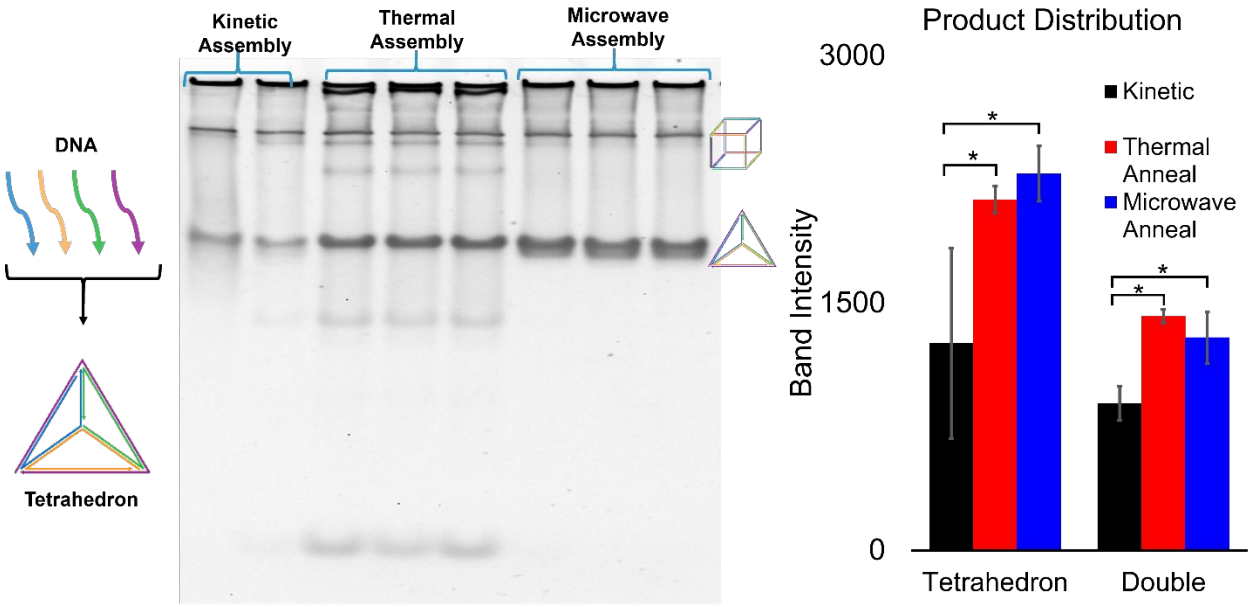


Figure S1| Assembly of the Turberfield tetrahedron using microwave anneal (105 W max.). (LEFT) Product distribution of the tetrahedron assembly products after various assembly methods. (RIGHT) Quantified band intensities for the thermodynamic products (TETRAHEDRON and DIMER).

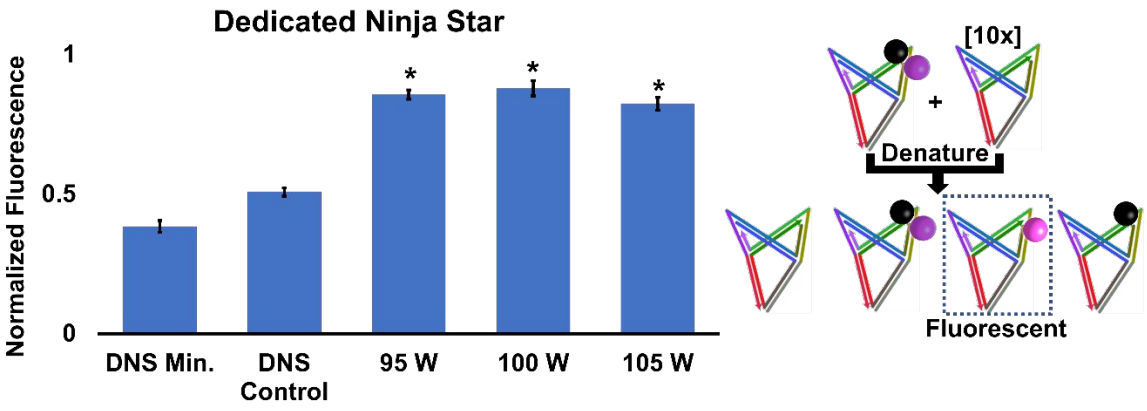


Figure S2 | Evaluation of microwave denaturation of the dedicated ninja star. At 95 W, fluorescent strand-exchange studies already demonstrate a significant increase in fluorescence over the control, suggesting that the denaturation power of the DEDICATED NINJA STAR ≤95 W. Increases in microwave power do not increase the fluorescence, implying that full denaturation has been achieved.

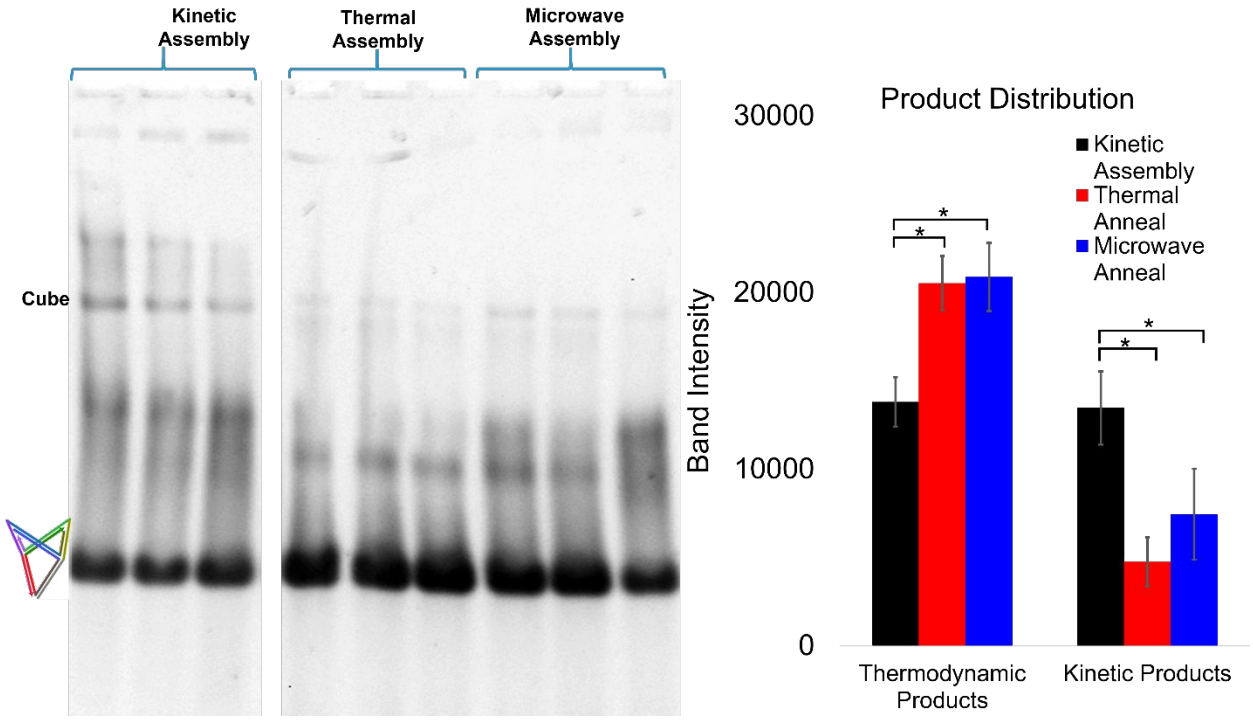


Figure S3| Assembly of the dedicated ninja star using microwave anneal (105 W max.). (LEFT) Product distribution of the DEDICATED NINJA STAR assembly products after various assembly methods. (RIGHT) Quantified band intensities for the thermodynamic products (NINJA STAR and CUBE).

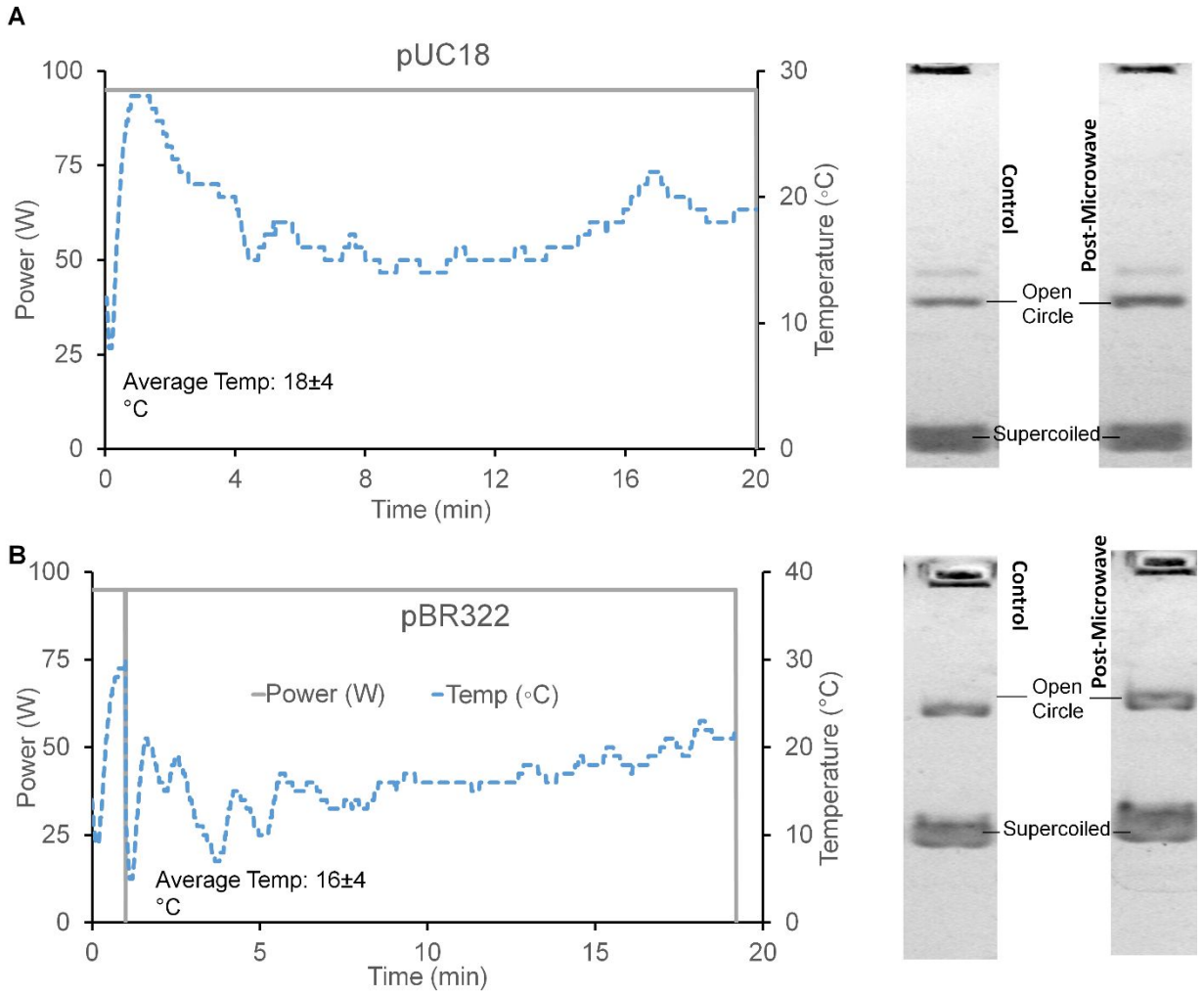


Figure S4| Exposure of pDNA to microwave radiation. (LEFT) Representative microwave power–temperature profiles observed during microwave exposure of pDNA. (RIGHT) Representative native agarose gels before and after exposure to microwave radiation. (A) pUC18 and (B) pBR322 (B).

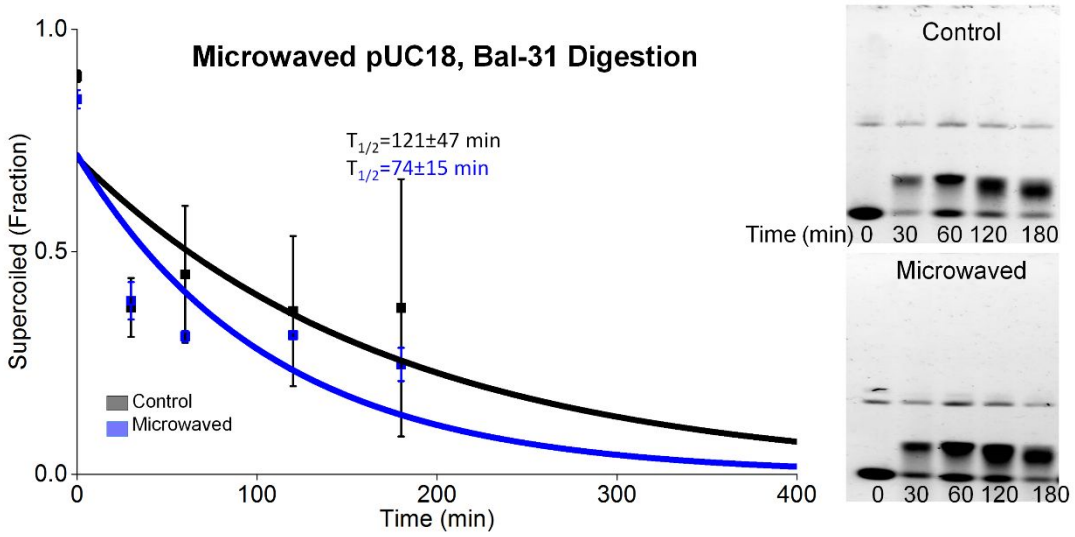


Figure S5| Digestion of microwave-exposed pUC18 by Bal-31. (LEFT) Representative digestion profiles obtained by image analysis of gels of native agarose electrophoresis (Representative image on RIGHT; At t = 0 (before Bal-31 was added), the majority of the DNA is present in supercoiled form. As digestion time increases, a shift from supercoiled (bottom band) to open circle (top band), and open circle to linear (middle band) was observed. Data presented as Mean ± SD (n = 3). Trend lines obtained by non-linear regression using a single-exponential function. Note that because Bal-31 is a mixture of enzymes, the function used may not best describe the profile, beyond the global trend. The difference between the half-lives of digestion were not significantly different (ANOVA, Tukey, p = 0.05).

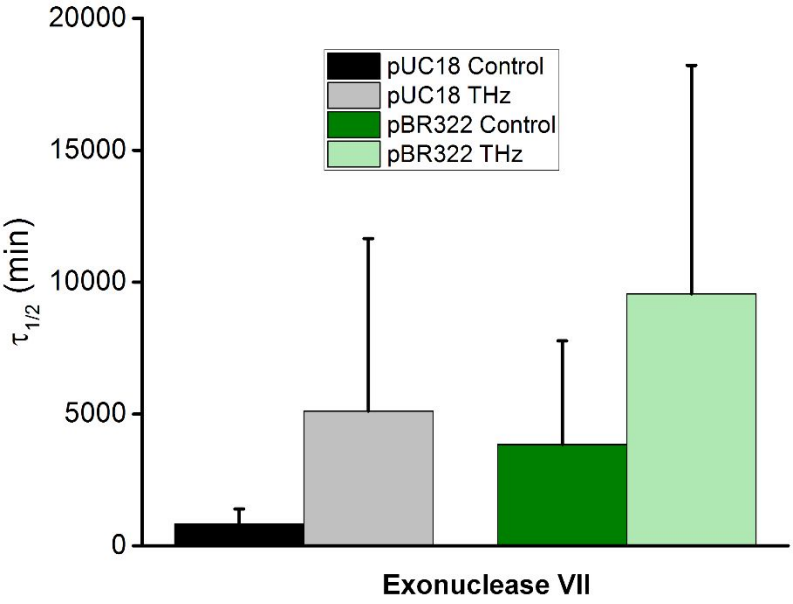


Figure S6| Digestion of THz-exposed pDNA by exonuclease VII. Exposure of pDNA to THz did not significantly alter their half-life of digestion by exonuclease VII (ANOVA, Tukey, p = 0.05). Large standard deviation results from the fact that pDNA are naturally closed, circular DNA and are only minimally digested by exonuclease VII within the time frame of the experiment. Data presented as Mean + SD (n = 3).

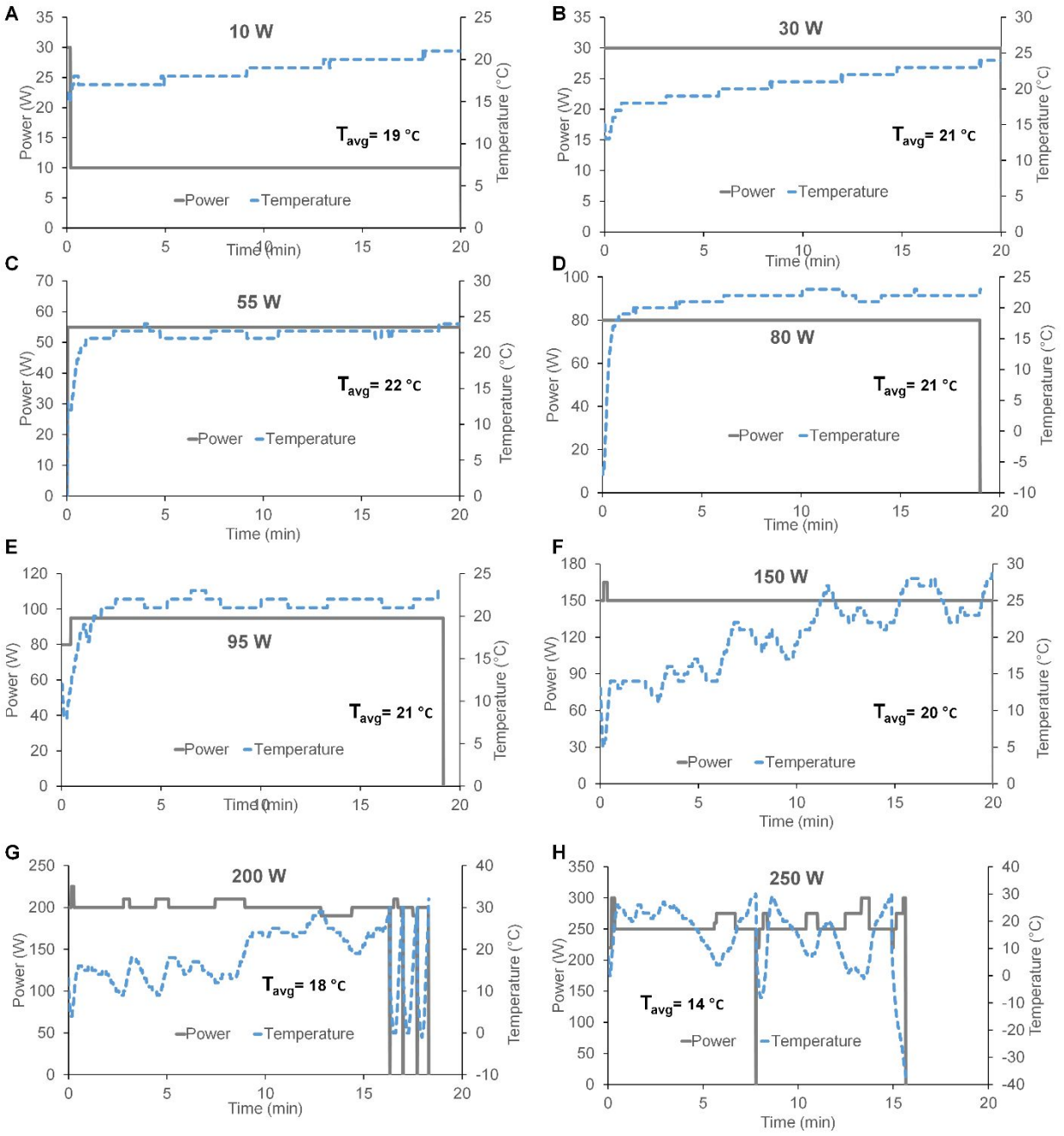


Figure S7| Power–Temperature profiles for exposure of ninja star to microwave power between 10 – 250 W. Representative profiles for microwave power fixed between 10 and 250 W. To avoid sample freezing at early time points, and to prevent overheating at high microwave powers, the initial coolant temperature was varied: 10 W = r.t.; 30 W = 18 ºC; 55 W = 7 ºC; 80 W = –3 ºC; 95 W = –10 ºC; 150 W = –36 ºC; 200 W = –56 ºC; 250 W = –67 ºC). It should be noted that high microwave power (and thus lower initial coolant temperatures) led to profiles that were more erratic. In these cases, maintaining sample temperature below 20 ºC required addition of liquid nitrogen to the cooling system during the experiment.

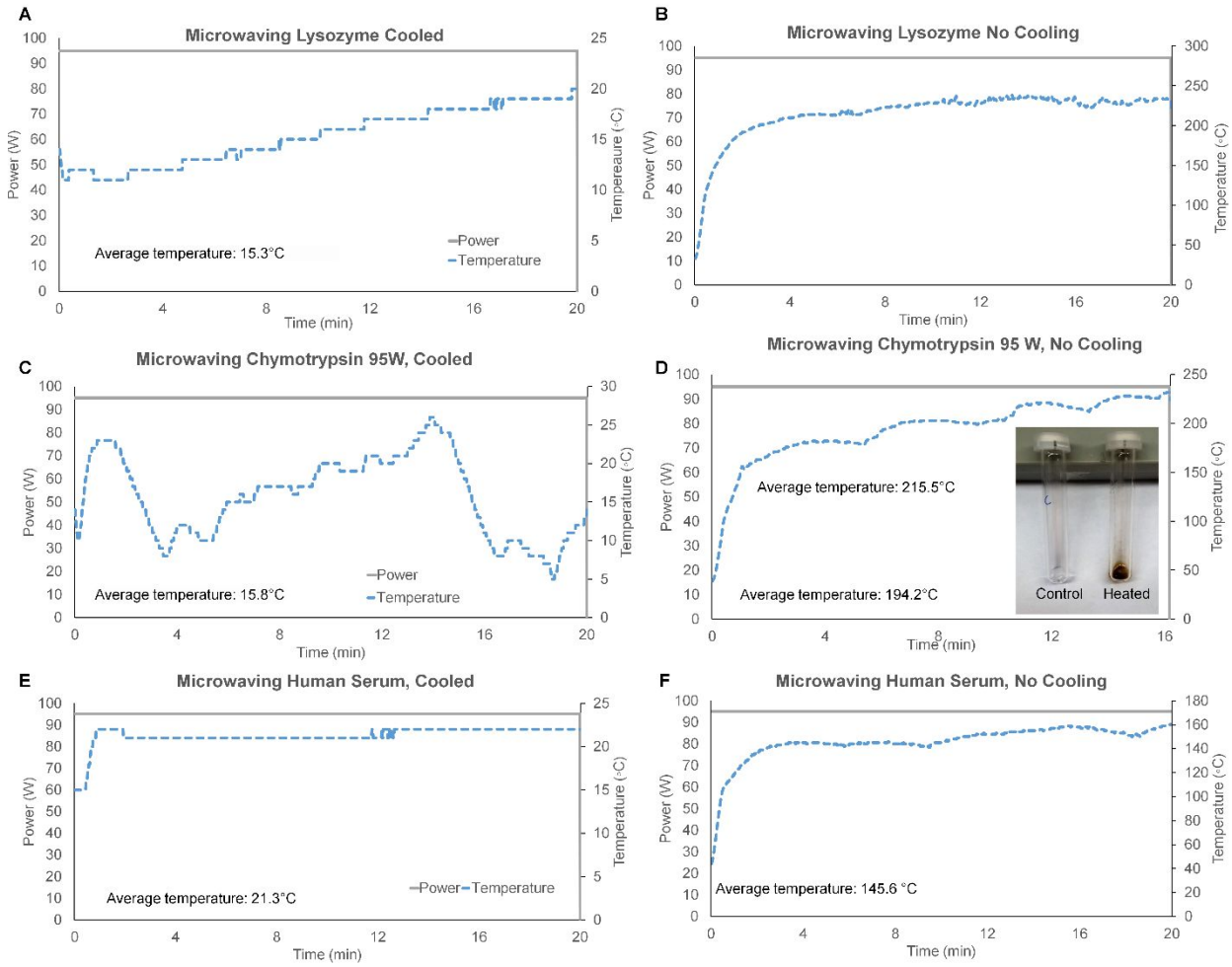


Figure S8| Exposure of proteins to microwave radiation. Representative microwave power–temperature profiles for proteins exposed to microwave radiation with active cooling, and without cooling. Inset: an example of 'browned' chymotrypsin following the microwave exposure.

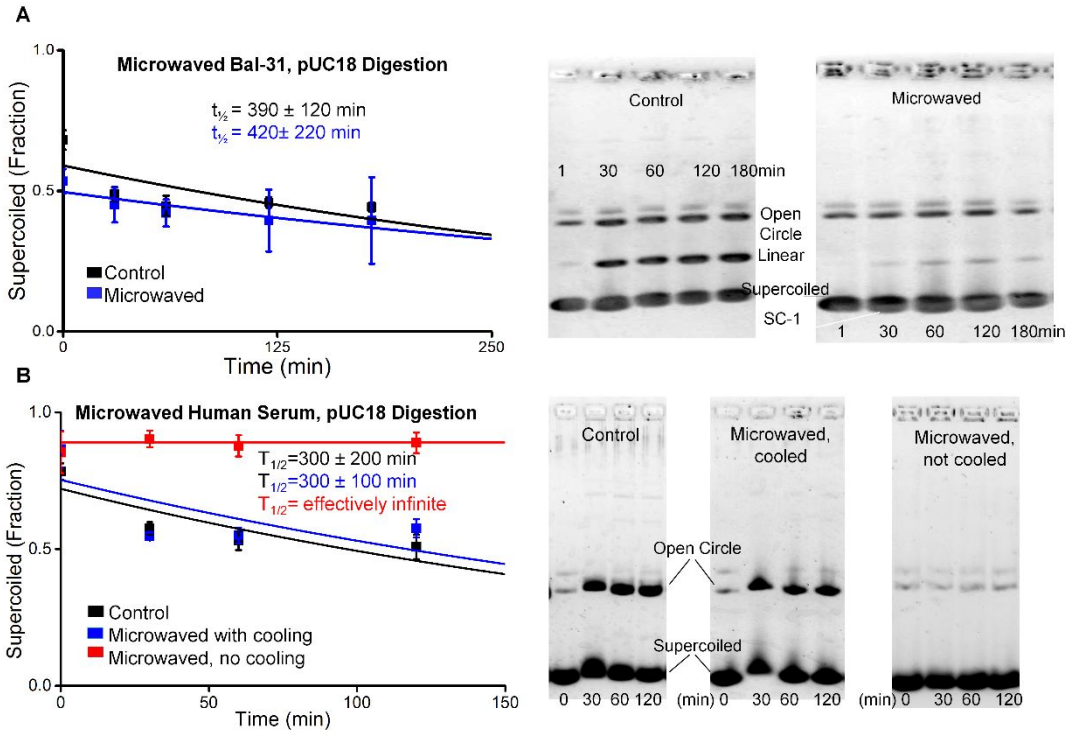


Figure S9| Digestion of pDNA by Bal-31 and human serum. LEFT: Digestion curves of supercoiled pUC18 pDNA, both before and after microwaving the serum. RIGHT: Representative native gel electrophoresis gels of the digestions. The fraction of DNA present as supercoiled in each lane was used to track overall digestion. The band labeled 'SC-1' is thought to be either a more mobile conformation of supercoiled, or a more compact version of one of the other conformations. (A) Bal-31, (B) Human Serum. Data presented as Mean ± SD (n = 3).

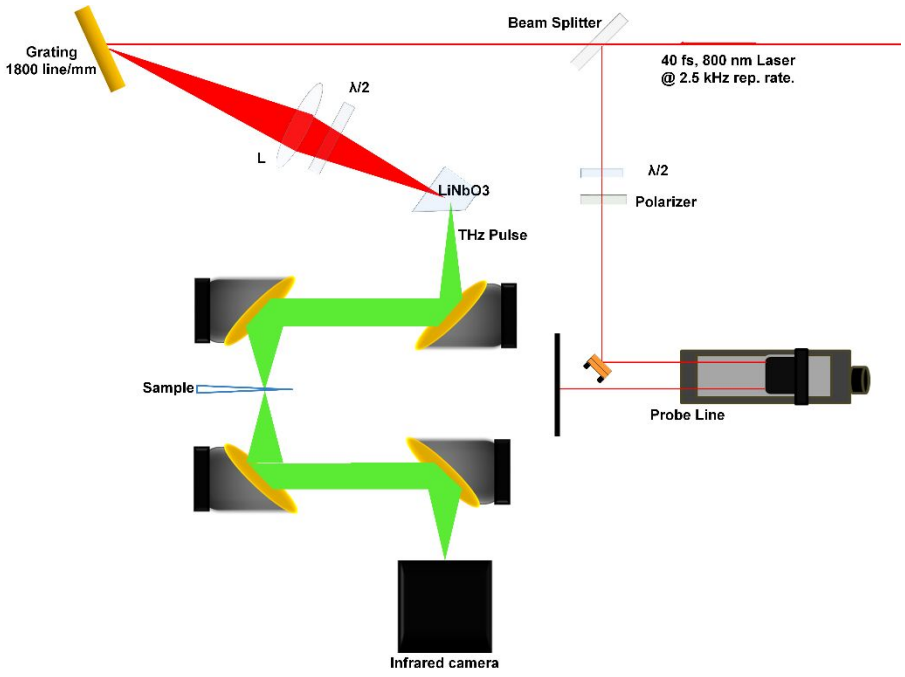


Figure S10| Scheme of optical layout for generating intense THz pulses. Intense THz pulses were generated at the Advanced Laser Light Source (INRS-EMT, Varennes, Canada) by optical rectification in a $LiNbO_3$ crystal and a pulse-front-tilt technique, as described in Blanchard et al., IEEE J. Sel. Top. Quantum Electron., 2011, 17, 5–1. The radiated THz pulses have a 70 $kV \cdot cm^{-1}$ peak field at focus, an energy of 0.4 µJ, a pulse duration of 1 ps and a repetition rate of 2.5 kHz. The peak intensity was 368 $MW \cdot cm^{-2}$ and average intensity was 57 $mW \cdot cm^{-2}$.

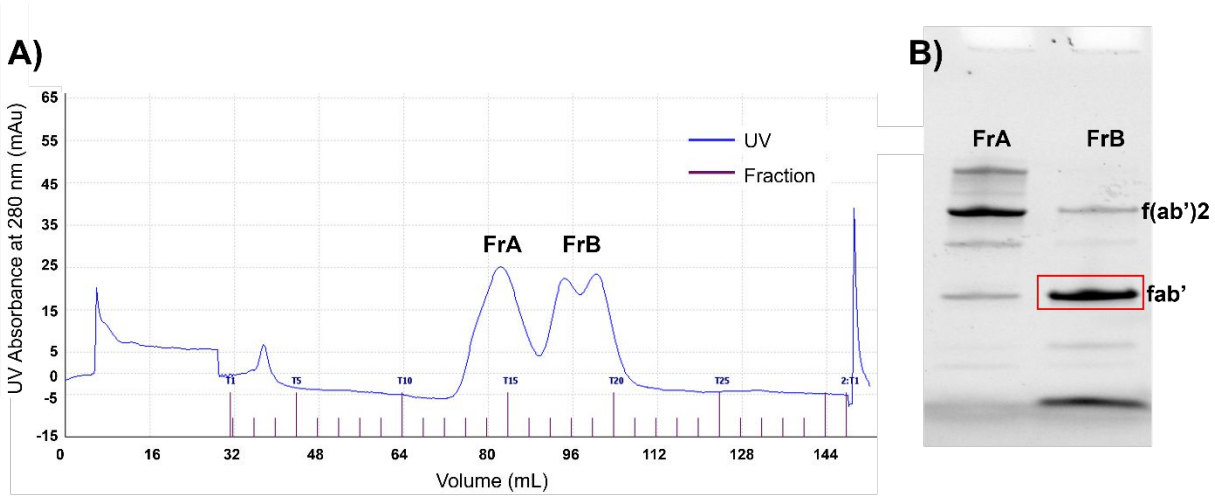


Figure S11| Size-exclusion purification of fab'. Following reduction of f(ab')2 using TCEP, size exclusion chromatography was used to purify the resulting fab'. A) Chromatogram for the purification. FrA and FrB were concentrated and identified via gel. B) Gel electrophoresis (4-15 % precast gel) indicates that FrA contained f(ab')2 and FrB contained primarily the desired product, fab' (red square).

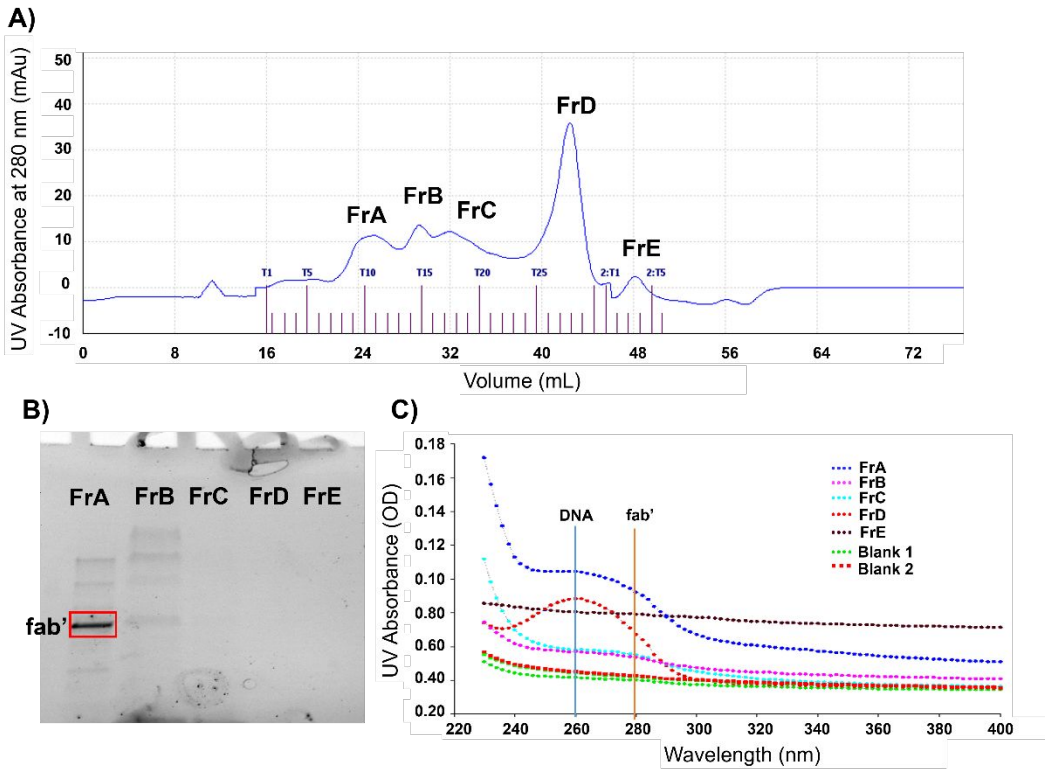


Figure S12| Anion-exchange purification of DNA-fab' conjugate. A) Chromatogram of anion-exchange purification following DNS1-mal and fab' coupling. Five fractions were collected and concentrated. B) Polyacrylamide gel electrophoresis (4–15% precast gel) indicates that DNS1-fab' elutes in fraction A. C) UV spectra of the various fractions. The absorbance of DNA and fab' are marked with blue and orange lines, respectively. Based on absorbance, fraction D contains unreacted DNA, and fraction A absorbs at both 260 and 280 nm, supporting the conclusion that fraction A contains the DNS1-fab' conjugate.

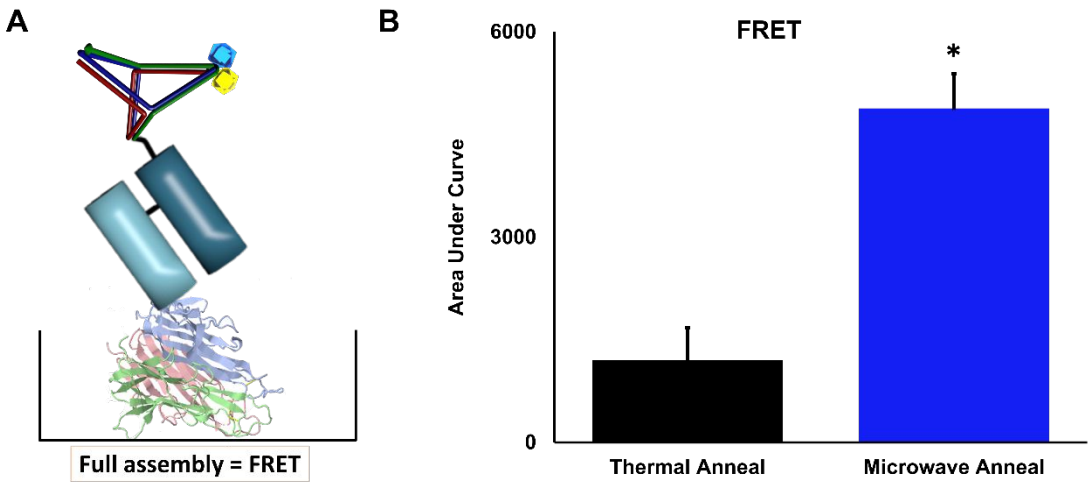


Figure S13| FLISA Experiment. Similar to an ELISA experiment, biotin-labeled TNF-α was immobilized onto the streptavidin-coated wells of a microplate. A) A schematic of a fully assembled DEDICATED NINJA STAR (labeled with Cy5, Cy3, and Fab') attached to a well. B) FRET measurements (Cy3 excitation, Cy5 emission) for the DNS assembled via thermal anneal and microwave anneal. For thermally-annealed structures, the Fab' has been denatured. Therefore, very little structure remains in the well after washing (non-specific binding). For microwave annealed structures, the significant increase in FRET signal indicates that the Fab' maintained binding ability, anchoring the structure to the antigen in the well. FRET also indicates that the Cy3 and Cy5 strands are present. Data presented as Mean + SD (n = 3)

References

1. Blanchard, F.; Sharma, G.; Razzari, L.; Ropagnol, X.; Bandulet, H.-C.; Vidal, F.; Morandotti, R.; Kieffer, J.-C.; Ozaki, T.; Tiedje, H., Generation of intense terahertz radiation via optical methods. *IEEE Journal of Selected Topics in Quantum Electronics* **2011,** *17* (1), 5-16.

2. Sánchez, A.; Pedroso, E.; Grandas, A., Maleimide-Dimethylfuran exo Adducts: Effective Maleimide Protection in the Synthesis of Oligonucleotide Conjugates. *Organic Letters* **2011,** *13* (16), 4364-4367.