Supramolecular Enzyme-Peptide Gels for Localized Therapeutic Biocatalysis

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Enzyme therapeutics are often compromised by poor accumulation within target tissues, necessitating high doses that can exacerbate off-target effects. We report an injectable supramolecular enzyme-peptide gel platform for prolonged local enzyme retention *in vivo*. The gel is based on CATCH(+/-) (Co-Assembling Tags based on CHarge-complementarity), cationic and anionic peptide pairs that form β-sheet fibrils upon mixing[1]. Enzymes recombinantly fused to CATCH(-) peptide integrate into CATCH(+/-) β-sheet fibrils during assembly, resulting in an enzyme-peptide gel. Catalytically-active gels were fabricated with four disparate enzymes: CATCH(-)-NanoLuc luciferase, CATCH(-)-cutinase, CATCH(-)-uricase, and CATCH(-)-adenosine synthase A. CATCH(-)-cutinase gels demonstrated tunability of the platform, while CATCH(-)-NanoLuc gels demonstrated prolonged tissue retention relative to soluble enzyme. CATCH(-)-uricase gels resolved localized inflammation in a gout model, while CATCH(-)-adenosine synthase A gels suppressed localized lipopolysaccharide-induced inflammation. Modular and tunable enzyme content, coupled with prolonged tissue retention, establish CATCH enzyme-peptide gels as a generalizable vehicle for effective local therapeutic biocatalysis.

Harnessing enzymes as therapeutics has faced significant challenges since the mid-twentieth century. Currently there are a modest ~40 FDA-approved enzyme-based therapeutics, which pales in comparison to the ~120 approved antibody-based therapeutics[2,3]. To be effective, an enzyme must persist long enough in the body to exert its catalytic effector functions. Effectiveness of enzymes delivered systemically without chemical modification (e.g., PEGylation, Fc-fusion, or similar) is challenged due to rapid clearance and limited accumulation within the target tissue[2,4,5]. However, prolonged systemic delivery increases the likelihood of off-target accumulation and unintended side effects. Inspired by multi-cellular organisms, wherein tissue-specific enzyme expression is tightly regulated spatially and temporally to govern biological processes[6,7], an ideal enzyme-based therapy should transiently persist locally within the target tissue over a therapeutically effective window.

We previously reported pairs of cationic and anionic β-sheet fibrillizing peptides known as CATCH(+/-) (Co-Assembly Tags based on CHarge complementarity) to create supramolecular protein-peptide gels[1]. Separately, CATCH(+) and CATCH(-) peptides do not self-assemble due to electrostatic repulsion, but once combined, they co-assemble into β-sheet fibrils. Either CATCH peptide can be recombinantly fused onto a larger folded protein domain, and this CATCH-tagged fusion protein can be expressed and recovered from living hosts in the soluble phase[1]. CATCH-tagged fusion proteins, such as CATCH-GFP, integrate into the β-sheet fibrils formed by

CATCH(+) and CATCH(-) mixtures, resulting in supramolecular protein-peptide gels endowed with the activity of the folded protein domain[1,8]. CATCH gels are injectable, induce weak rapidly resolving inflammation, and are not immunogenic in pre-clinical models[9], suggesting their potential as localized protein delivery vehicles.

Here four different CATCH(-)-enzyme fusion proteins with disparate physical and catalytic properties were employed to demonstrate that CATCH(+/-) enzyme-peptide gels enable localized therapeutic biocatalysis. First, CATCH(-)-NanoLuc® luciferase (NL) was installed as a bioluminescence reporter to demonstrate prolonged active enzyme retention at an *in vivo* injection site (Figure 1). NL with a CATCH(-) peptide tag fused to the N-terminus (CATCH-NL) was expressed and recovered from *E. coli* with comparable activity to unmodified NL (Extended Data Fig. 1). The ternary mixture of CATCH(+), CATCH(-), and CATCH-NL formed gels that emitted blue bioluminescence in the presence of furimazine, while gels formulated without enzyme (empty gels) did not (Fig 1a-c). CATCH-NL was retained within the gels *in vitro* (Fig 1d). Within minutes after subcutaneous injection, greater local bioluminescence, indicative of a higher local NL dose, was observed from CATCH-NL immobilized in gels when compared to soluble CATCH-NL (Fig. 1e, 1f, 1g), likely because the gel reduced the convective forces acting on soluble enzyme that would push it through the tissue. Enzymatically active CATCH-NL gels were detectable at a subcutaneous injection site for up to 21 days, whereas soluble CATCH-NL injected into the same site was cleared within a day (Fig. 1e-h).

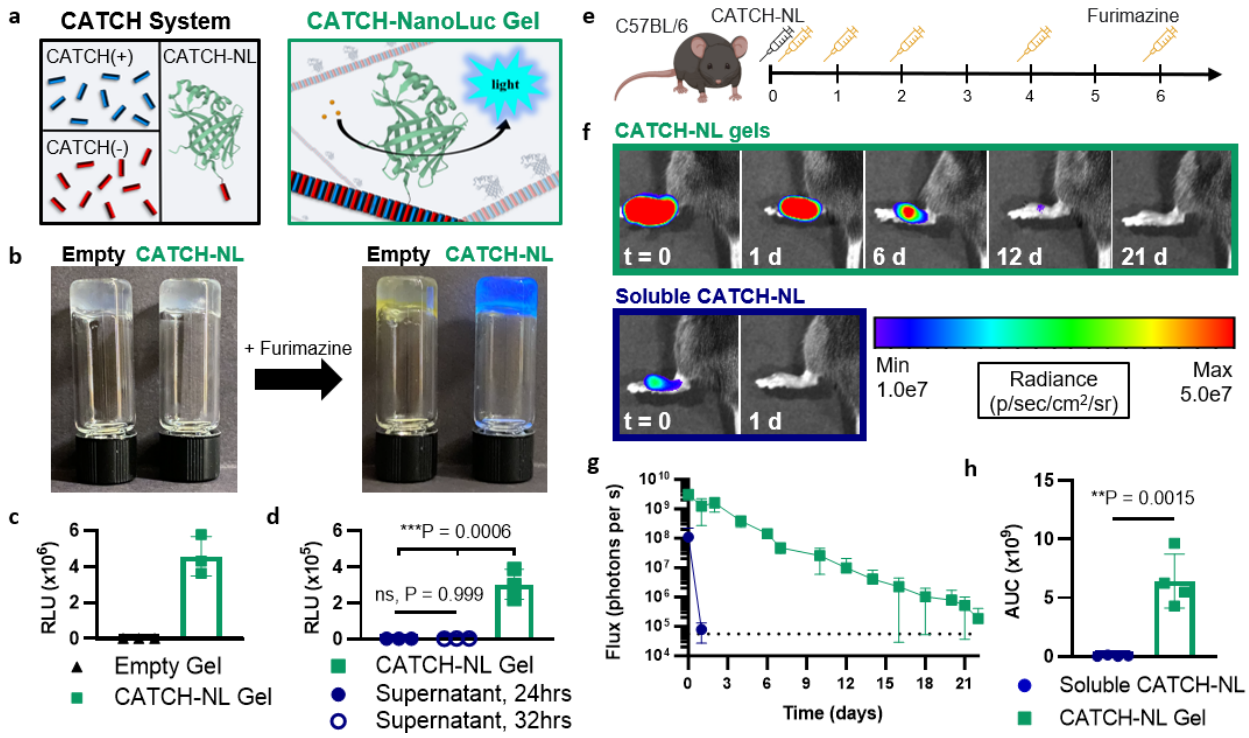

Figure 1: CATCH-NL gels are active and prolong injection site retention. (a) Schematic of CATCH peptide fibers with integrated CATCH-NL and catalysis of furimazine substrate (orange circles). (b) Digital images of empty and CATCH-NL gels before and after the addition of furimazine. (c) Luminescence output from empty and CATCH-NL gels. (d) Luminescence output

of PBS supernatants collected 24 and 32 h after incubation with CATCH-NL gels compared to luminescence output from the washed gels. N = 3, ***P ≤ 0.001, ANOVA with Tukey's post hoc. (e) Schedule to evaluate retention of CATCH-NL subcutaneously injected into the top of the hind paw (black syringe) at day 0 and subsequent administration of furimazine over time (orange syringe), with IVIS measurements following each injection. (f) Representative longitudinal IVIS images, (g) quantitative analysis of NL activity, and (h) resultant area under the curve (AUC) following furimazine injection in mice that received soluble CATCH-NL (dark blue circles) or CATCH-NL gels (green squares) (dashed line represents average background), N = 4, **P ≤ 0.01, t-test. All data are presented as mean ± s.d.

To demonstrate that the catalytic activity of CATCH enzyme-peptides gels can be tuned, CATCH-cutinase was incorporated as a model colorimetric reporter (Figure 2). Cutinase with a CATCH(-) peptide tag fused to the N-terminus (CATCH-cutinase) was expressed and recovered from *E. coli* (Extended Data Fig. 2). Ternary mixtures of CATCH(+), CATCH(-), and CATCH-cutinase formed gels that converted colorless *p*-nitrophenyl butyrate (pNPB) to yellow *p*-nitrophenol. CATCH-cutinase gels demonstrated Michaelis-Menten kinetics (Figure 2b), with reaction velocity that can be titrated by varying the concentration of CATCH-cutinase assembled into the gel (Figure 2c). This was consistent with prior work which showed the amount of protein immobilized in β-sheet fibrillar biomaterials corresponded to the peptide:fusion protein ratio in the pre-assembled mixture[10], establishing that tunable activity is a general feature of supramolecular peptide:fusion protein biomaterials. Gel mechanics were unaffected by cutinase cargo (Figures 2d-e).

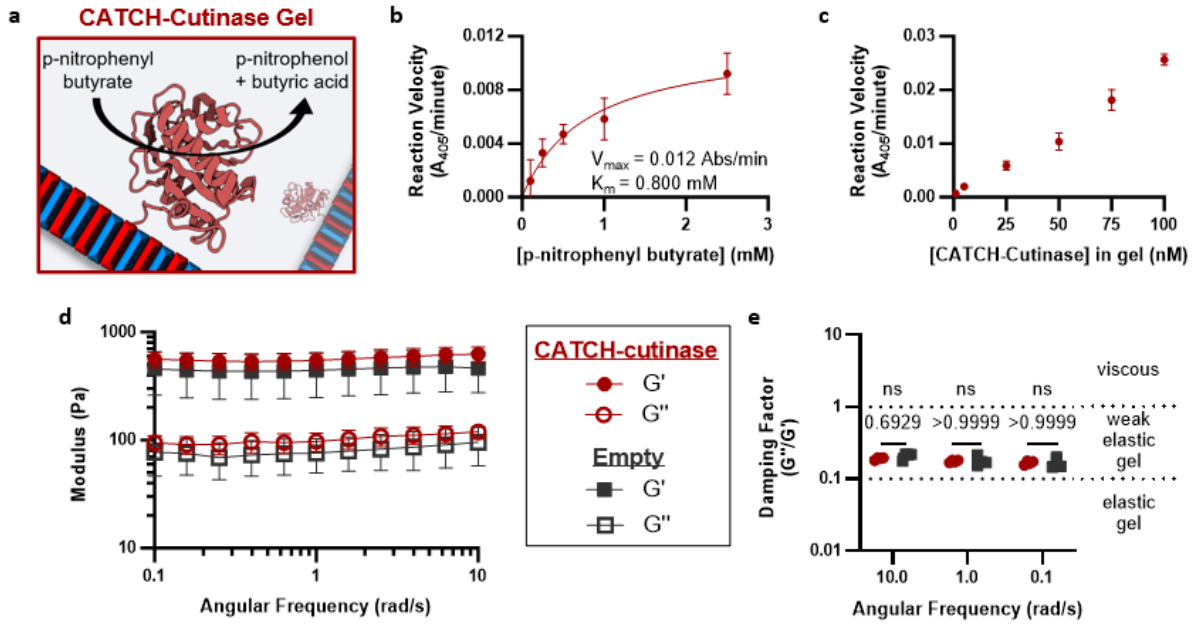

Figure 2: Activity titration and rheology of CATCH-cutinase gels. (a) Schematic of CATCH-cutinase bearing β-sheet fibrils. Reaction velocity of CATCH-cutinase gels as a function of (b) substrate concentration in the supernatant or (c) immobilized enzyme concentration in a range of gel formulations. Solid line in (b) denotes Michaelis-Menten fit of enzyme activity of CATCH-cutinase gels, R^2 = 0.89. (d) Storage (G') and loss (G'') moduli, and (e) damping factor of CATCH-cutinase gels (red circles) and empty gels (gray squares) at 37 °C after mock injection through a 27G needle. N = 4, ns denotes P > 0.05, ANOVA with Bonferonni post hoc. All data are presented as mean ± s.d.

To demonstrate therapeutic effectiveness using a clinically-established enzyme, we created CATCH-uricase (CATCH-U) gels (Figure 3). In humans, elevated uric acid levels can seed formation of monosodium urate (MSU) crystals that induce gouty inflammation, particularly within digits[11]. Although systemically administered uricase variants find clinical use as second-line therapies for gout, their effectiveness is variable because they fail to rapidly dissolve localized MSU crystals[12,13]. Here we used a preclinical murine model, in which MSU crystals are injected directly into the subcutaneous space[14,15], to evaluate localized CATCH-U gels. To prepare CATCH-U gels, uricase with a CATCH(-) peptide tag fused to the N-terminus was expressed and recovered from *E. coli* (Fig. 3a, Extended Data Fig. 3a-c). Uricase was stable during the gel fabrication process and CATCH-U was retained within the gel (Extended Data Fig. 3-4). CATCH-U gels degraded MSU crystals and uric acid in vitro, whereas empty gels did not (Figure 3b-c). CATCH-U did not alter the rheological properties of the gels (Extended Data Fig. 5). Based on *in vivo* fluorimetric imaging, Cy5.5-labeled CATCH-U gels had a half-life of ~6.9 days at a subcutaneous injection site, compared to <1 day for soluble Cy5.5-labeled uricase (Extended Data Fig. 6). At the subcutaneous MSU challenge site, CATCH-U gels spared paw distention and provided more rapid resolution of paw swelling than soluble uricase or empty gels (Figs 4d-f). CATCH-U gels also alleviated leukocyte infiltration, neutrophil infiltration, and neutrophil degranulation. (Fig 4g-h, Extended Data Fig.7).

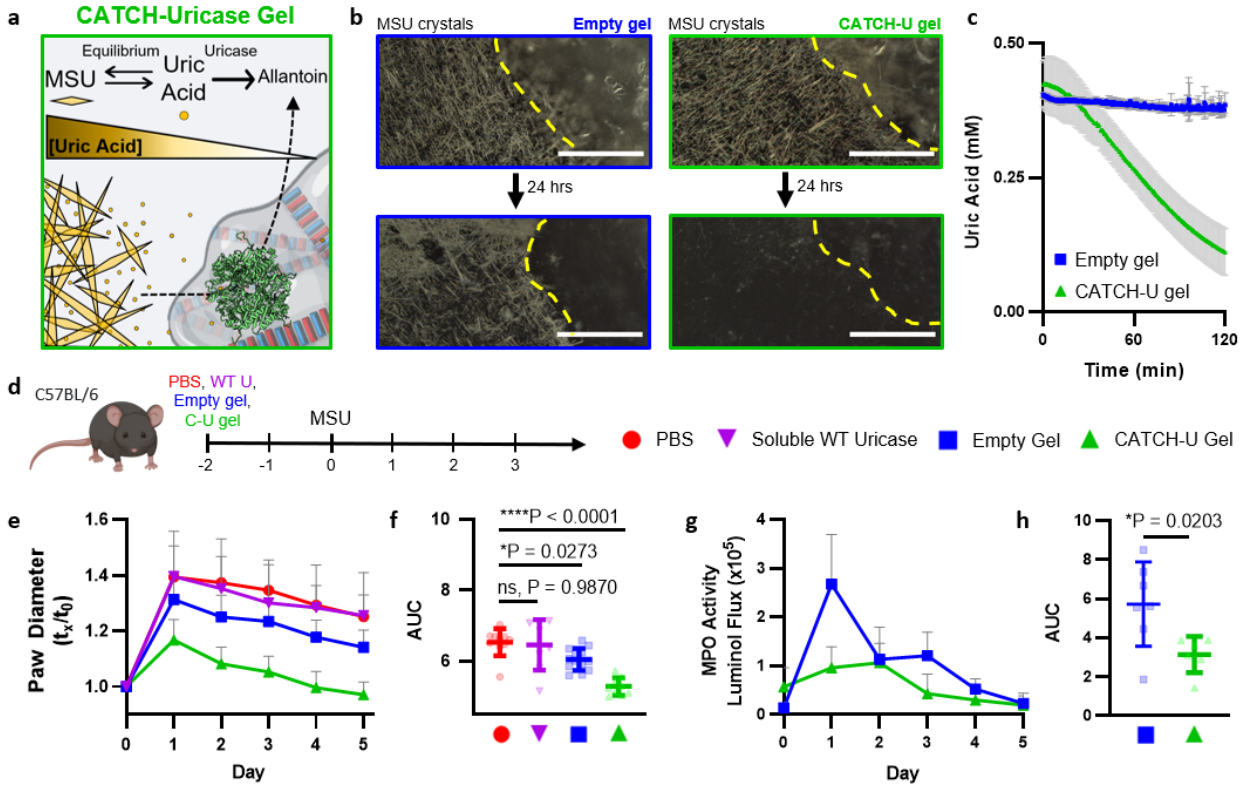

Figure 3: CATCH-uricase gels deplete MSU crystals and suppress gouty inflammation. (a) Schematic of MSU crystal depletion by CATCH-U gel catalyzed by local enzymatic conversion of uric acid to allantoin. (b) Micrographs of empty and CATCH-U gels (gel edge indicated by dashed yellow line) incubated with MSU crystals at t = 0 and 24 h. Scale bar = 200 μm. (c) Level of uric acid present in solution containing empty versus CATCH-U gels. (d) Schedule to evaluate inflammation following subcutaneous MSU challenge in animals pretreated with PBS (red circle, positive control), soluble wild-type (WT) uricase (purple inverted triangle), empty gel (blue square,

vehicle control), or CATCH-U gel (green triangle, experimental group). (e) Fold change in paw diameter relative to day 0, and (f) AUC of each trace in (e). N = 8-13, ****P ≤ 0.0001, *P ≤ 0.05, ANOVA with Dunnett's multiple comparisons test to PBS group. (g) Neutrophil degranulation, reported as myeloperoxidase (MPO) activity, as measured by IVIS luminol bioluminescence in mice treated with empty or CATCH-U gels, and (h) AUC of each trace in (g). N = 6-8, *P ≤ 0.05, t-test. All data are presented as mean ± s.d.

To investigate therapeutic effectiveness of a new candidate enzyme, we created CATCH-Adenosine synthase A (CATCH-AdsA) gels (Fig. 4). AdsA is a *Staphylococcus aureus* enzyme that catalyzes dephosphorylation of extracellular ATP, a pro-inflammatory signal, into adenosine, an anti-inflammatory signal[16–18]. AdsA acts as an immune evasion factor in *S. aureus* infections by mimicking the actions of the mammalian membrane-anchored enzymes CD39/CD73[18]. To fabricate CATCH-AdsA gels, AdsA with a CATCH(-) peptide tag fused to the N-terminus was expressed and recovered from *E. coli* (Extended Data Fig. 8). Recombinant CATCH-AdsA was active over a wide pH and temperature range (Extended Data Fig. 8). *In vitro*, CATCH-AdsA gels depleted ATP from bulk media in the gel state (Fig 4a-b, Extended Data Fig. 9) and *in vitro* suppressed secretion of IL-1β by PMA-differentiated THP-1 cells (Figure 4c), which is induced by extracellular ATP after LPS priming[19]. At a subcutaneous LPS challenge site, CATCH-AdsA gels blunted neutrophil infiltration and degranulation more effectively than empty gels (Fig 4d-h, Extended Data Fig. 10).

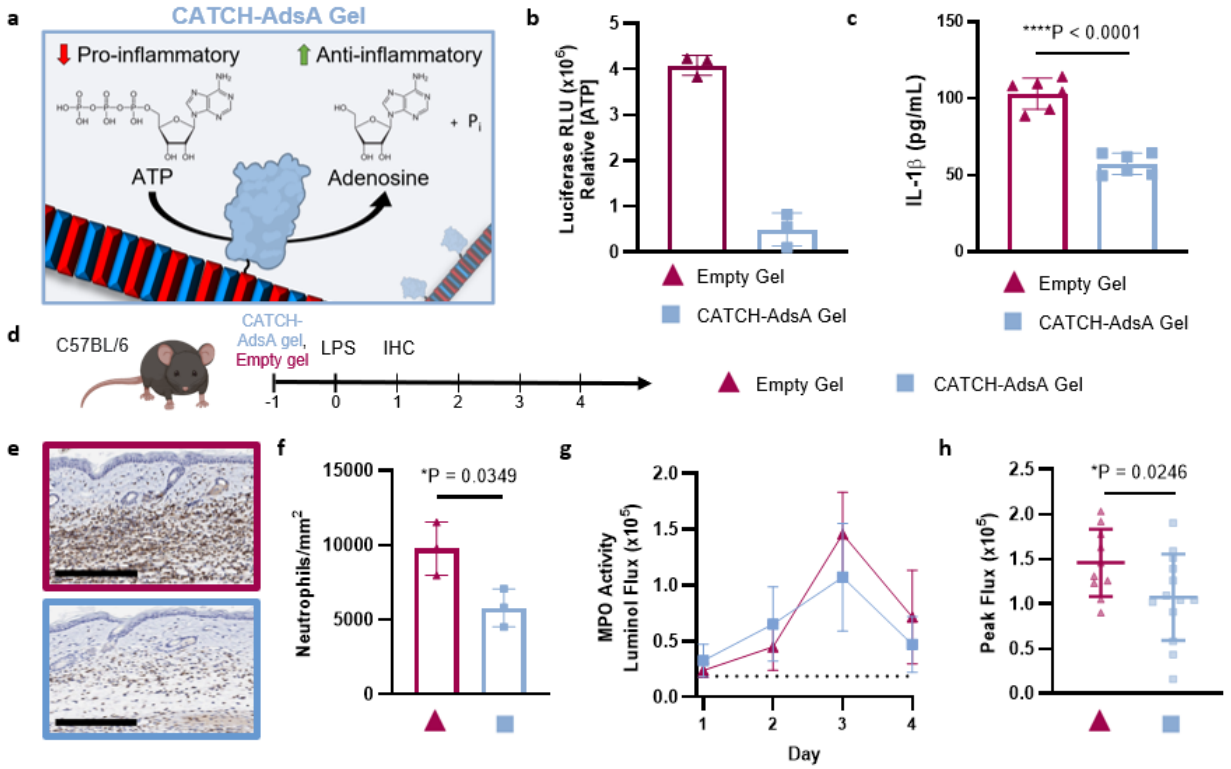

Figure 4: CATCH-AdsA gels deplete ATP and suppress local LPS-induced inflammation. (a) Schematic of CATCH-AdsA β-sheet fibrils. (b) Relative ATP concentration, as measured via *in vitro* luciferase bioluminescence, after empty and CATCH-AdsA gels were incubated with ATP for one hour. (c) IL-1β secreted from PMA-treated THP-1 cells stimulated in vitro with LPS

overnight followed by ATP-supplemented media that was pre-incubated with either empty or CATCH-AdsA gels. N = 6, ****P ≤ 0.0001, t-test. (d) Schedule to evaluate subcutaneous LPS challenge via daily luminol measurements or immunohistochemistry (IHC) at t = 1 d. Animals received empty gels (pink triangle) or CATCH-AdsA gels (light blue square) on day -1. (e) Representative anti-Ly6G IHC images and (f) neutrophil number per image area, N = 3, *P ≤ 0.05, t-test. (g) MPO activity and (h) peak flux as measured by IVIS luminol bioluminescence in mice that received empty or CATCH-AdsA gels, N = 10 or 13, * P ≤ 0.05, t-test. All data are presented as mean ± s.d.

In summary, this report demonstrates the successful realization of CATCH enzyme-peptide gels for localized therapeutic biocatalysis. CATCH-tagged fusion enzymes non-covalently integrate into β-sheet fibrils that are formed by CATCH(+/-) peptide pairs. At millimolar concentrations, these enzyme-bearing fibrils entangle into physically crosslinked gels[9]. Integration of four distinct functional CATCH fusion enzymes into gels showcased the modularity of this platform. Non-covalent incorporation of enzymes into CATCH(+/-) gels enabled prolonged local tissue retention. Enzyme activity was titratable by varying the fusion enzyme concentration in the pre-gel mixture. By providing modular enzyme content and prolonged tissue retention, CATCH enzyme-peptide gels afforded a generalizable vehicle to revitalize a systemically-ineffective therapeutic enzyme in uricase, as well as advance a new candidate therapeutic enzyme in AdsA.

This work establishes supramolecular biocatalytic gels for *in vivo* use. Non-therapeutic biocatalytic gels have been developed for various industrial applications, such as chemical synthesis, food processing, biosensing, and environmental remediation[20–22]. Prior to the last decade, enzyme immobilization was dominated by simple adsorption and covalent conjugation strategies that are often difficult to control and result in inconsistent active enzyme concentration. Non-covalent immobilization of enzymes within gels via supramolecular assembly can circumvent these challenges[10]. The electrostatically controlled selective co-assembly of CATCH(+) and CATCH(-) peptides at physiologic pH, ionic strength, and temperature avoids denaturing stimuli, crosslinkers, or chemical modifications that can compromise enzyme catalytic activity. Supramolecular peptide gels, such as CATCH, are also well positioned for *in vivo* applications because they are made of natural, biodegradable amino acids and are generally well tolerated[9,23]. Finally, the shear thinning and recovery properties of CATCH gels[9], which are common among supramolecular peptide biomaterials[24], enable focal delivery through a fine gauge needle at the desired tissue site, as shown by CATCH-NL and CATCH-uricase gels.

Direct local injection of a biodegradable enzyme-gel depot affords the advantage of better mimicking natural transient local enzyme expression than systemic or targeted delivery approaches. Depots that prolong local enzyme retention can be achieved via fusion to anchoring domains with affinity for tissue ligands[25,26] or by encapsulation in controlled release vehicles that leverage porosity or enzyme-carrier interactions for extended release[27–32]. The CATCH gel system simplifies localized delivery by providing non-covalent enzyme immobilization in a carrier that is agnostic to tissue interactions and cargo properties. The 11 amino acid CATCH tag is amenable to proteins of disparate shapes, sizes, charges, and quaternary structures, as demonstrated with GFP (monomer, 26.6kDa, negative)[1], NanoLuc® (monomer, 19.1kDa, negative), cutinase (monomer, 22.6kDa, near neutral), uricase (tetramer, 136.9 kDa, negative), and AdsA (probable dimer, 87.4kDa, positive). Installation of the fusion enzyme directly into the β-sheet fibrils of the CATCH gel can provide up to weeks of localized biocatalysis *in vivo* (Fig. 1f). By significantly

extending localized enzyme pharmacokinetics, CATCH-enzyme gels are likely to reduce injection frequency or obviate repeated dosing entirely, thereby alleviating a common limiter of clinical compliance.

The CATCH enzyme-peptide gel platform meets common criteria for effective clinical translation[33], including precise chemical definition, reproducible and tunable bioactivity, and scalable manufacturability. Once considered cost prohibitive, advances in peptide manufacturing capabilities can now support large-scale production of good manufacturing practice (GMP)-grade products[23,33]. This has facilitated the emergence and approval of supramolecular peptide biomaterials for clinical applications[23], opening the door for more to follow. Coupled with existing infrastructure for recombinant protein production, there is a foundation for the scale-up and clinical translation of CATCH enzyme-peptide gels as a generalized vehicle for localized therapeutic biocatalysis.

Conflicts of Interest

Patents are filed by the University of Florida for the CATCH peptide platform (US10906939B2) that names D.T.S. and G.A.H. as co-investors, and CATCH-uricase (WO2022081774) that names M.J.F., D.T.S., B.G.K., and G.A.H. as co-inventors.

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Methods

Expression and Purification of Recombinant Proteins

(i) Recombinant protein expression and lysis. All proteins were recombinantly expressed in an *E. coli* host system. Proteins included: CATCH-NL, WT NL, CATCH-cutinase, CATCH-uricase, CATCH-AdsA. The fusion proteins consist of the enzyme covalently fused to the CATCH(-) peptide on the N terminus using a serine-glycine linker, and a 6-histidine tag on the C terminus for purification. The genes for these proteins were designed and ordered through GenScript in pET-21(+) vectors. Plasmid was transformed into One Shot™ TOP10 Chemically Competent *E. coli* (ThermoFisher) and plated on 100 µg/mL ampicillin LB/agar plates overnight at 37 ˚C. Isolated colonies were selected and cultured in 5 mL of LB media with 100 µg/ mL ampicillin overnight in an orbital shaker at 225 rpm and 37 ˚C. Recombinant DNA was recovered with a Plasma Miniprep Kit (Qiagen) and sequenced using the Sanger method (GeneWiz). Positive DNA sequences were then transformed into Origami B (DE3) *E. coli* (Novagen) and plated on ampicillin (100 µg/mL) and kanamycin (50 µg/mL) LB/agar plates overnight at 37 ˚C. Positive clones were picked and used to inoculate 5 mL of LB or 2xTy media with ampicillin (100 µg/mL) and kanamycin (50 µg/mL). The 5 mL of solution was cultured overnight at 37 ˚C on an orbital shaker (225 rpm) and then sub-cultured into 1 L of 2xTY media, consisting of 16 g tryptone, 10 g yeast extract, and 5 g NaCl, with ampicillin (100 µg/mL) and kanamycin (50 µg/mL) at 37 ˚C and in an orbital shaker

until an optical density of 0.8 or greater at 600 nm was reached. To induce protein expression, the cultures were then supplemented with 0.5 mM isopropyl β-D-1-thiogalactopyranoside and incubated at 18 ˚C in an orbital shaker (225 rpm) for 18 h. Bacteria was then pelleted via centrifugation at 4 ˚C and 11,300 x g for 10 min using a Sorvall RC 6 Plus Superspeed Centrifuge. For CATCH-AdsA, bacteria pellet was resuspended in 20mL 50 mM HEPES and 100 mM NaCl, while the other protein-expressing bacteria was resuspended in 20mL 1x PBS. The bacteria were then centrifuged again using the same settings and the supernatant was discarded. Bacteria cell pellets were mechanically resuspended using a spatula in lysis buffer containing B-PER™ bacterial protein extraction reagent (ThermoFisher Scientific, 4 mL per gram of bacteria), lysozyme (50 mg/mL), DNAse I (2400 units/mL), and Pierce protease inhibitor. Suspension was incubated on a rocker for 20 minutes at room temperature. Lysates were then centrifuged at 42600 x g and 4˚C for 15 minutes. The supernatants were collected by decanting for further purification.

(ii) Cloning WT uricase. Template DNA was extracted from the CATCH-uricase TOP10 *E. coli* using a Plasma Miniprep Kit (Qiagen). WT uricase was cloned by deletion of the CATCH(-) tag using the Phusion™ Site-Directed Mutagenesis Kit (Thermo Scientific), according to the manufacturer's instructions. The new plasmid was transformed into Origami B (DE3) *E. coli*, expressed, and purified as described above and below.

(iiia) Purification of CATCH-AdsA. The His-tagged protein was purified by immobilized metal affinity chromatography (IMAC) using HisTrap FF Crude Prepacked Columns (Cytiva) connected to an ÄKTA Pure FPLC system (GE Healthcare). Fusion protein was eluted from the column using 20-250 mM imidazole gradient in buffer containing 50 mM HEPES and 100 mM NaCl. The imidazole in the solution was removed using Amicon Ultra Centrifugal Filters with a 10kDa cutoff (MilliporeSigma). IMAC and centrifugal filtration were repeated following the same conditions. The protein was purified through size exclusion chromatography (SEC) on the FPLC using a HiLoad™ 26/600 Superdex 200 pg column. Using absorbance at 280nm, the protein was eluted and collected in 50 mM HEPES and 100 mM NaCl buffer.

(iiib) Purification of CATCH-NL, WT NL, CATCH-cutinase, CATCH-uricase, and WT uricase. Proteins were purified following the same IMAC methods and centrifugal outlined above but with the imidazole gradient in 1x PBS. One round of IMAC was done for each protein. The imidazole solution was removed using 1x PBS and Amicon Centrifugal Filters with a 10kDa cutoff.

(*iv) Endotoxin control.* Endotoxin content of the recombinant proteins was reduced below 1.0 EU/mL using cloud point precipitation of Triton X-114 or Endotoxin Removal Solution (Sigma). For CATCH-AdsA, $MnCl_2$ was added to the protein for a final concentration of 0.5 mM $MnCl_2$ in 50 mM HEPES and 100 mM NaCl buffer. All other proteins were formulated in 1x PBS. Final endotoxin content was confirmed below 1.0 EU/mL, determined with Pierce LAL Chromogenic Endotoxin Quantitation Kit (ThermoFisher) or Chromo-LAL Endotoxin Quantification (Cape Cod Inc.), according to the manufacturer's instructions.

CATCH Peptide Stock Solutions

A library of CATCH peptide variations exists based on the identity and number of the charged residues[1,9,34]. This work focuses on: CATCH(+) [Ac-QQKFKFKFKQQ-Am] and CATCH(-) [Ac-E QEFEFEFEQE-Am]. The complementary peptides were synthesized and purified by GenScript. Lyophilized peptides were dissolved separately in endotoxin free water at a concentration of

24mM determined by weight. Sodium hydroxide was added to CATCH(-) to a pH ~6.8 to aid dissolution. Both peptides were sonicated to ensure complete dissolution. Phenylalanine absorbance (258nm) was measured by NanoDrop (ThermoFisher) to determine peptide concentration. For *in vivo* applications, endotoxin was confirmed below 1.0 EU/mL, determined with Pierce LAL Chromogenic Endotoxin Quantitation Kit (ThermoFisher) or Chromo-LAL Endotoxin Quantification (Cape Cod Inc.), according to the manufacturer's instructions.

Gel Preparation

CATCH(+) was diluted with buffer to 12mM, while CATCH(-) was diluted with buffer and CATCH-fusion protein to 12mM. For CATCH-AdsA gelsl, 50 mM HEPES, 100 mM NaCl, 0.5 mM $MnCl_2$ buffer was used, while 1x PBS was used for gel formation for all other proteins. Final concentrations of protein within the gel varied based on experiment. Gels were formulated to 12mM total peptide either by pipetting equal volumes of each stock solution on a surface (i.e. 96-well plate) or by drawing up equal volumes of each stock solution into the barrel of a syringe. In either case, the gels cured for 1 hour at room temperature before proceeding. For formation in the syringe, the 12mM peptide solutions were heated for 5 minutes before being sequentially drawn into the syringe to allow for more thorough mixing within the confines of the barrel. CATCH-AdsA was heated at 68°C and CATCH-NL, CATCH-cutinase, and CATCH-uricase were heated at 55°C. Allergy syringes with 27G needles (BD) were used.

CATCH-NanoLuc® Enzyme Activity

(i) Effect of CATCH-tag on NL activity. NanoLuc® is an engineered deep sea shrimp luciferase variant developed by Promega[35]. The activity of CATCH-NL and WT NL were compared at concentrations of 0-10nM. 50uL of protein at each concentration was added to separate wells of white 96-well plate. Then 50uL Nano-Glo furimazine (Promega) (diluted 1:50 with 1x PBS) was added and the luminescence was immediately measured using the SpectraMax M3 plate reader (Molecular Devices).

(ii) Activity of CATCH-NL gels. 10uL CATCH gels with and without 100nM CATCH-NL were formed in triplicate in a white 96-well plate, cured for 1 hour, and washed 3 times with 200uL 1x PBS. 90uL 1x PBS was added to each gel for a total well volume of 100 µL. 50uL Nano-Glo furimazine (diluted 1:50 with 1x PBS) was added to the wells and the luminescence was measured overtime. Peak luminescence is reported for the empty gels and the CATCH-NL gels.

(iii) CATCH-NL retention in CATCH gels. CATCH-NL was immobilized within 10uL gels to a final concentration of 5nM in a white 96-well plate and incubated for 24 hours. 90uL of 1x PBS was added to each gel and incubated for another 24 hours. 80uL of the supernatant was transferred to a separate well. An additional 80uL 1x PBS was added to each gel, and 8 hours later 80uL of supernatant was again removed and added to a separate well. Finally, 80uL 1x PBS was again added to each gel and the luminescence of the gels, as well as the supernatants from 24- and 32-hour timepoints, were immediately measured after addition of 50uL Nano-Glo furimazine (diluted 1:50 with 1x PBS).

Animal Study Conditions

Animal protocols were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Florida. Procedures were performed following the Guidelines for Care and Use of Laboratory of the University of Florida and USDA guidelines. Mice were housed 5 to a cage,

provided reverse osmosis water and standard diet, 14hr:10hr dark:light cycle, 68-79°F room temperature and 30-70% relative humidity. Female 8- to 15-week-old C57BL/6J mice (The Jackson Laboratory) were used for all experiments.

CATCH-NL *in vivo* imaging

Previously published methods were adapted to measure *in vivo* bioluminescence of CATCH-NL[25]. Mice were anesthetized with 2-3% isoflurane. While anesthetized, mice received a single 20uL injection of 50nM CATCH-NL formulated in CATCH gels (N = 4) or soluble 50nM CATCH-NL in sterile PBS (N = 4) subcutaneously into the top of the hind paw. Five minutes later, 20uL Nano-Glo furimazine (diluted 1:50 with 1x PBS) was injected subcutaneously into the same paw. Immediately after, mice were anatomically positioned with the hind paw facing the camera of an IVIS Spectrum In Vivo Imaging System (PerkinElmer) and bioluminescence images were taken. The Nano-Glo furimazine injections and IVIS imaging were repeated under anesthesia every 1-3 days over a 22-day period or until no signal above baseline was detected. Bioluminescent images were captured using an open emission filter, 1s exposure time, subject size 1cm, field-of-view B (6.6cm), medium binning (factor of 8) resolution, and a 1 F/stop aperture. Signal was quantified as photon flux (photons/s) using a round region of interest (ROI) in the Living Image analysis software. The ROI captured the whole hind paw, stopping at the ankle. The same ROI was used for each animal and each time point. Light intensities for each time point and animal were normalized to the same scale (min = 1.0e7 and max = 5e7) and set as pseudo color— where purple and red represented the least and most intense light respectively. To determine the average background signal, the naive contralateral paw of each mouse received one subcutaneous injection of 20uL Nano-Glo furimazine (diluted 1:50 with 1x PBS) and was imaged and analyzed as described above. Data were graphed as photon/s versus day, with the average background signal graphed as a dotted line. The area under the curve (AUC) for each mouse was determined in GraphPad Prism software, using the average background signal as baseline.

CATCH-Cutinase Activity

(i) CATCH-cutinase gel kinetics. 10uL gels with 5nM CATCH-cutinase were formed in 96-well plates. Then 100uL of solution containing varying concentrations of pNPB (0-2.5mM) in 1x PBS was added to each well. Absorbance was read at 405nm for 60 minutes. The slopes of the linear region of the kinetic measurements were determined and plotted against pNPB concentration. The Michaelis-Menten model in GraphPad was used to determine Michaelis constant, K_m, and maximum velocity, V_{max}.

(ii) CATCH-cutinase gel activity titration. 10uL gels with varying concentrations of CATCH-cutinase (0-100nM) were formed in 96-well plates. Then 90uL of 1mM pNPB in 1x PBS was added to each well. Absorbance was read at 405nm for 60 minutes. The slopes of the linear region of the kinetic measurements were determined and plotted against CATCH-cutinase concentration in GraphPad Prism.

Oscillating Rheology

Samples of CATCH gels were prepared in syringes as described above. Samples included empty gels (no enzyme), gels with 100nM CATCH-cutinase, and gels with 16.5uM CATCH-uricase. Gel

rheological properties were analyzed using an Anton Paar rheometer (MCR 302). The gels were injected onto the instrument's rough-coated plate, and the 8mm rough-coated parallel plate was lowered to the gel at 0.5mm. The linear viscoelastic region (LVR) was determined at 37°C using amplitude sweeps. Frequency sweeps (10-0.1 rad/s) were run in the LVR at 0.3% strain to determine the storage modulus (G') and loss modulus (G''). Damping factor was calculated as G'/G'' at angular frequencies 0.1, 1.0, and 10 rad/s.

CATCH-Uricase Enzyme Activity and Retention

(i) CATCH-U gel activity with MSU crystals. 20uL gels were formed with or without 16.5uM CATCH-U in syringes. After the gels were cured at room temperature for an hour, they were injected into a 48-well plate and allowed to rest for 10 minutes at 4°C. The gels were washed three times with 200uL 1x PBS. Then 200uL 1x PBS with 0.625 g/L MSU and 2250 U/mL catalase was added to each well. The gels were imaged immediately using a Zeiss Axio inverted microscope using the Ph3 condenser annulus. The gels were incubated for 24 hours at 37°C with gentle rocking and imaged again.

(ii) CATCH-U gel activity with uric acid. 20uL gels were formulated with or without 16.5uM CATCH-U in syringes. After the gels were cured at room temperature for an hour, they were injected into a UV-transparent 96-well plate (Corning) and allowed to rest for 15 minutes at 4°C. The gels were incubated with 200uL 1x PBS for 1 hour at 37°C to remove any unincorporated enzyme. Then the PBS wash was removed, 200uL 0.4mM uric acid was added to each well, and the absorbance of uric acid (293nm) was read immediately at 37°C for one hour. A linear regression of a uric acid standard curve was used to convert absorbance into uric acid concentration.

MSU inflammation studies with CATCH-uricase

(i) Paw caliper measurements. Prior to injection, mice were anesthetized with isoflurane. While anesthetized, mice received a 20uL injection subcutaneously into the top of the hind paw of one of the following treatments: 1x PBS (N = 10), 16.5uM WT uricase in 1x PBS (N = 8), empty CATCH gel (N = 15), or CATCH gel with 16.5uM CATCH-uricase (N = 15). Two days later, the mice were challenged with 0.5mg MSU crystals suspended in 20uL 1x PBS, a common model of gouty inflammation[14,15]. Paw diameter was measured with digital calipers prior to treatment and then everyday thereafter until the end of the study. Change in paw diameter as a marker for inflammation was determined by normalizing each measurement to the paw diameter of that animal immediately before MSU injection. Animals that responded poorly to injections were defined as those that had a significant paw diameter change following the treatment injection, prior to MSU challenge. The threshold was set as a change of 1.45 using the measurement 24 hours after treatment injection (day -1) over the naive measurement before treatment injection (day -2). These animals were excluded from the study.

(iii) Luminol measurements. Luminol was used to assess neutrophil infiltration in the injection site according to[36]. The animals received either 20uL 16.5uM WT uricase in 1x PBS (N = 8), empty CATCH gel (N = 7), or CATCH gel with 16.5uM CATCH-U (N = 6) injected subcutaneously into the top of the hind paw. Two days later, the animals received a 100uL intraperitoneal (i.p.) injection of luminol solution (20.2 mg/mL in 0.9% NaCl saline). 10 minutes after, the mice were individually

anatomically positioned with the hind paw facing the camera of the IVIS Spectrum In Vivo Imaging System (PerkinElmer) and bioluminescence images were taken. Then the mice were challenged with 0.5mg MSU crystals suspended in 20uL PBS. The luminol imaging was repeated every 24 hours for 5 consecutive days to assess for acute inflammation by measuring neutrophil MPO activity. Bioluminescent images were captured using an open emission filter, 1 minute exposure time, subject size 1cm, field-of-view B (6.6cm), large binning resolution, and a 1 F/stop aperture. Luminol emission signal was quantified as total flux using a round region of interest (ROI) using the Living Image analysis software. The ROI used captured the whole hind paw, stopping at the ankle. The same ROI was used for each animal and each time point.

CATCH-AdsA Enzyme Activity

The ability of CATCH-AdsA gels to dephosphorylate ATP was tested by measuring ATP concentration using the ATP Bioluminescence Assay Kit CLS II (Roche). Empty and 9uM CATCH-AdsA gels were prepared at 40uL in syringes as described previously. Gels were injected into a white 96-well plate and incubated with 40uL 2000 μM ATP in HEPES buffer for one hour. Approximately 15 minutes before the end of the hour, fresh luciferase reagent was prepared following the manufacturer's instructions. Luciferase was added at 80uL/well, and luminescence readings were taken, with an integration time of 1000 ms.

IL-1β Production by PMA-treated THP-1 Cells

THP-1 cells were seeded into a 6-well plate at a density of 1 million cells per well and incubated at 37ºC and 5% CO_2. Cells were treated with 50 ng/mL phorobol-12-myristate-13-acetate (PMA) for 24 hours. Media was replaced and cells were incubated for another 24. Cells were harvested and reseeded into a 96-well plate at a density of 300,000 cells per well. Cells were cultured overnight before being stimulated with 10 μg/mL lipopolysaccharide (LPS) for 18 hours. Culture media was replaced, and the cells were treated with 100uL of the following: PBS, 2mM ATP, 2mM ATP reacted for 2 hours with empty gels, or 2mM ATP reacted for 2 hours with 2uM CATCH-AdsA functionalized gels. Cultures were incubated for 1 hour before the media was collected for analysis. IL-1β production was quantified using a human IL-1β ELISA kit (BD OptEIA) according to manufacturer's protocol.

LPS Inflammation Studies with CATCH-AdsA

(i) Luminol measurements. Cohorts of female mice received a 40uL subcutaneous injection of either empty gel (N = 10) or gel with 9uM AdsA (N = 13) 24 h before 40 μL of LPS (1 mg/mL) injection at day 0. Luminol was used to assess neutrophil infiltration in the injection site as described previously with CATCH-U above.

(ii) Immunohistochemistry. Paws were harvested from a cohort of animals 24 hours after LPS injection. These animals were treated with either CATCH-AdsA gels (N = 3) or empty gels (N = 3) 24 hours prior to the LPS injection. Neutrophil infiltration into the subcutaneous space of the hind paw was assessed with immunohistochemistry (IHC). The whole paws were fixed with 10% formalin and decalcified then cut bilaterally and embedded in paraffin. The blocks were sectioned onto slides and stained with anti-neutrophil Ly6B.2 antibody (Abcam, ab53457). Histological images were analyzed by a blinded individual. The observer created four rectangular regions of interest 400um long equally spaced along the length of the paw. The number of Ly6B.2+ stained

cells were manually counted in each region and normalized by the area. The average across the four sections was calculated for each animal.

Statistical Analysis

Statistical analyses were performed using GraphPad prism. Study-specific analyses are reported in figure captions using the following: non-significant (ns) $P > 0.05$, ****$P \leq 0.0001$, all others as reported in the figure.

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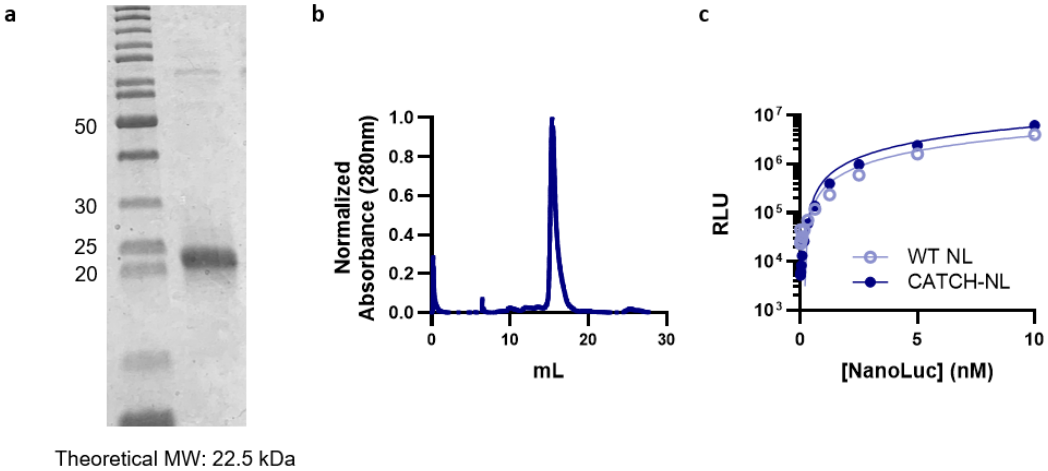
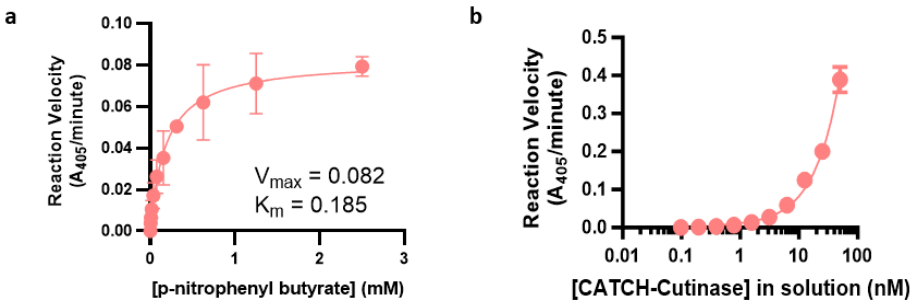
Extended Data



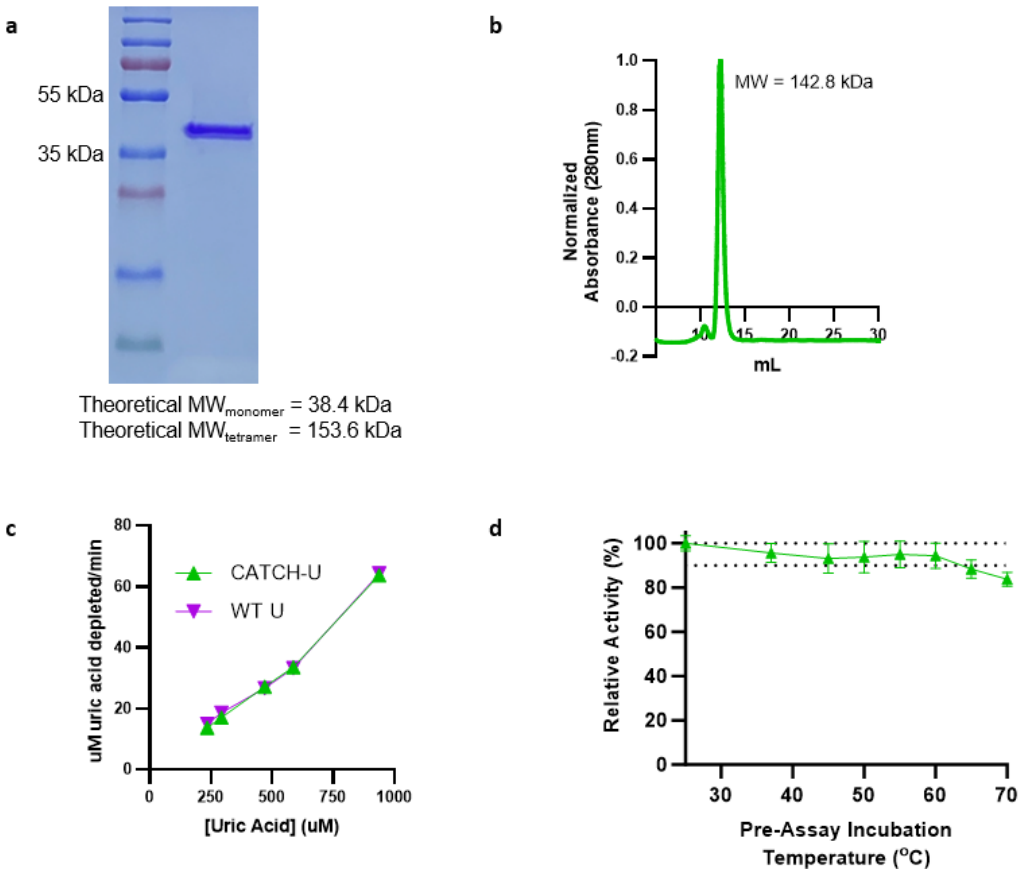
Extended Data Fig. 1: Expression and activity of CATCH-NL

(a) SDS-PAGE and (b) SEC of CATCH-NL purified from *E. coli.* (c) Luminescence output of wild-type (WT) and CATCH-tagged NL. Data are presented as mean ± s.d.



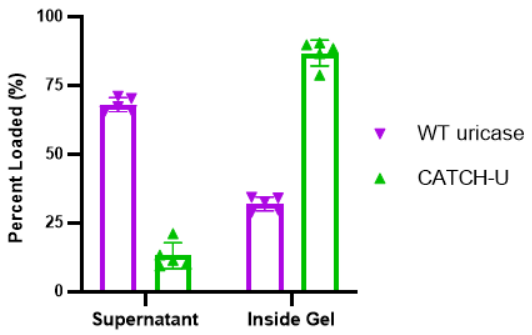
Extended Data Fig. 2: Enzymatic activity of CATCH-cutinase

(a) Reaction velocity of soluble CATCH-cutinase over a range of substrate concentrations. Solid line denotes Michaelis-Menten fit, $R^2 = 0.94$. (b) Reaction velocity of CATCH-cutinase at varying enzyme concentrations. All data points are presented as mean ± s.d.



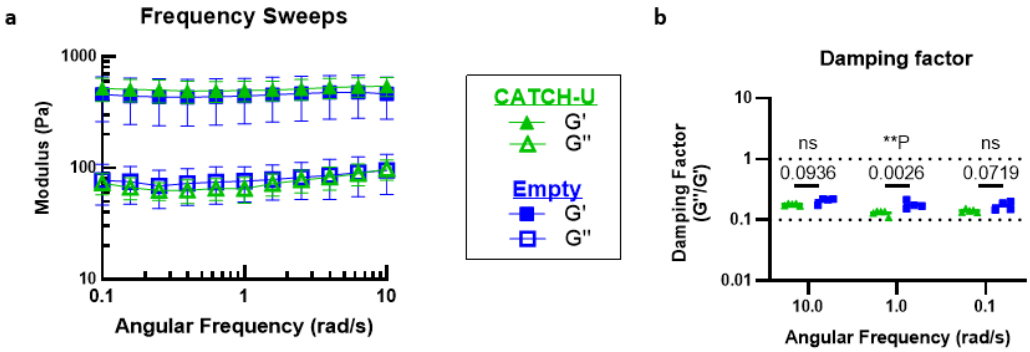
Extended Data Fig. 3: Expression and activity of CATCH-U

(a) SDS-PAGE and (b) analytical SEC of CATCH-U purified from *E. coli.* (c) Velocity of uric acid depletion by WT and CATCH-U over a range of substrate concentrations. Data are presented as mean ± s.d. (d) Relative activity of soluble CATCH-U after a 5-minute incubation at various temperatures, as measured by velocity of uric acid depletion normalized to velocity after room temperature incubation. Dashed lines indicate 100% and 90% activity from room temperature. Data are presented as mean ± s.d.



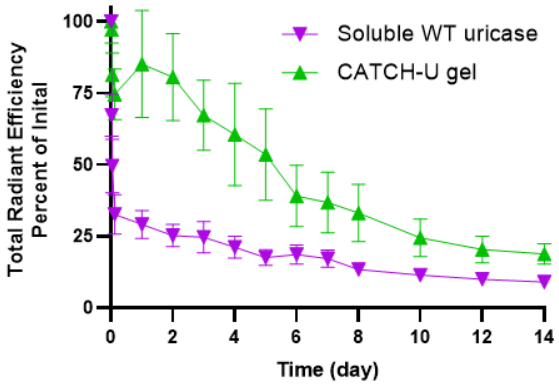
Extended Data Fig. 4: Retention of CATCH-U versus WT Uricase in gels

Percent of WT uricase (inverted purple triangle) or CATCH-U (green triangle) retained within CATCH gels vs released into the supernatant after a 48-hour incubation at room temperature, normalized to total measured protein. Data are presented as mean ± s.d.



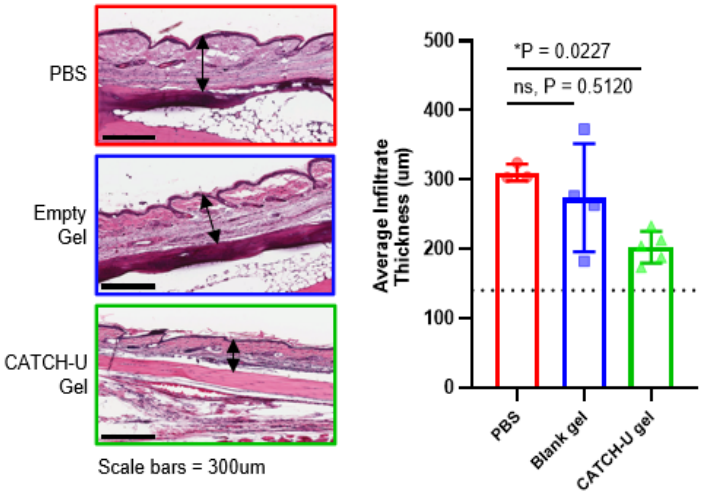
Extended Data Fig. 5: Gel mechanics are unaffected by incorporated CATCH-U

(a) Storage (G') and loss (G'') moduli, and (b) damping factor of CATCH-U and empty gels at 37 ºC after mock injection through a 27G needle. N = 3, ns denotes P > 0.05, **P < 0.01, ANOVA with Bonferroni post hoc. All data are presented as mean ± s.d.



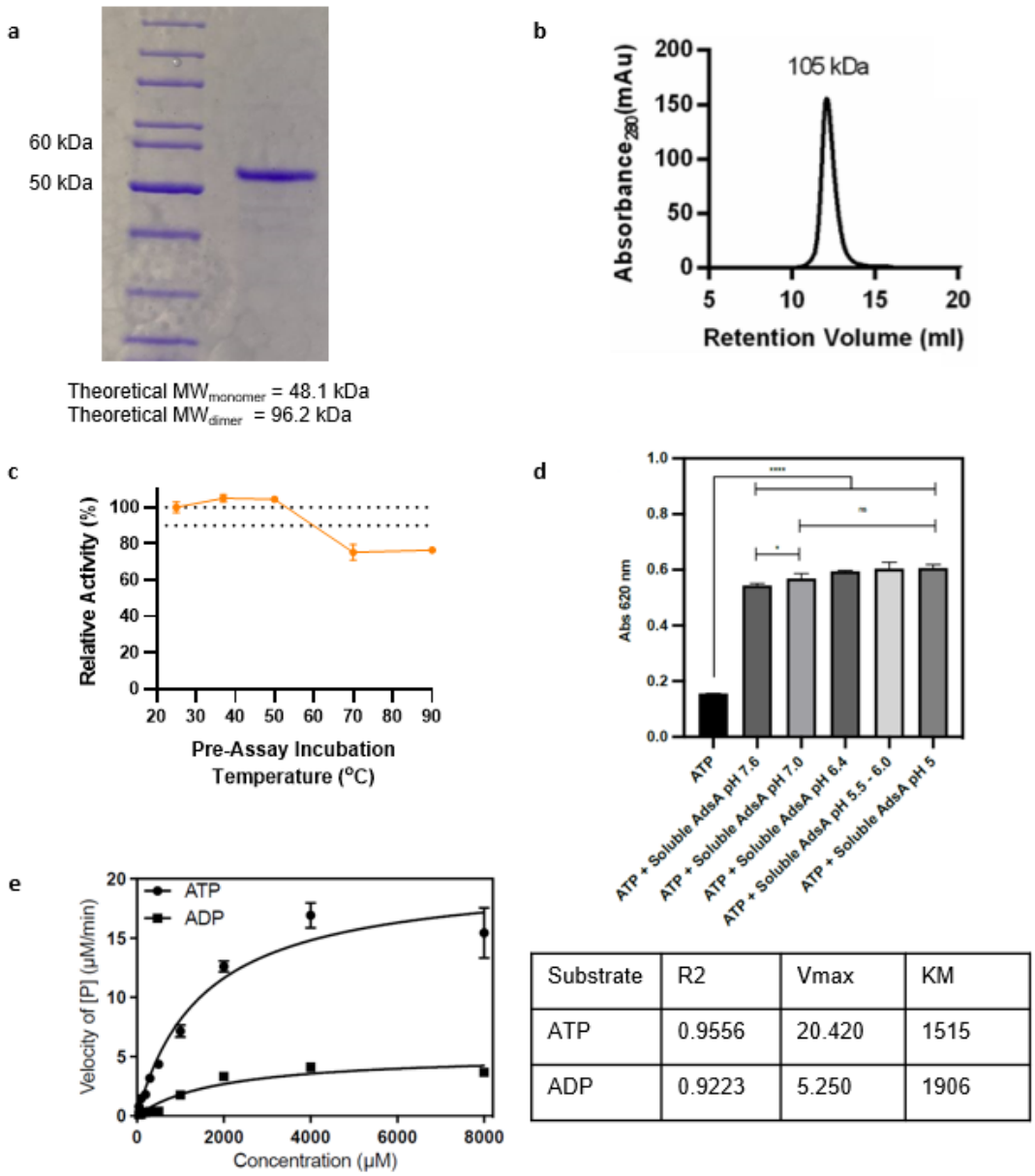
Extended Data Fig. 6: CATCH gel prolongs CATCH-U residence time *in vivo*

Relative *in vivo* fluorescence of Cy5.5-labeled WT uricase (inverted purple triangle) in PBS vehicle or Cy5.5-labeled CATCH-U immobilized in CATCH gels (green triangle) subcutaneously injected into the top of the hind paw. Soluble WT uricase half-life = 0.03 d, R^2 = 0.915, CATCH-U half-life = 6.9 days, R^2 = 0.8309. Data are presented as mean ± s.d.



Extended Data Fig. 7: CATCH-U gel reduces immune cell infiltration in a mouse model of gouty inflammation

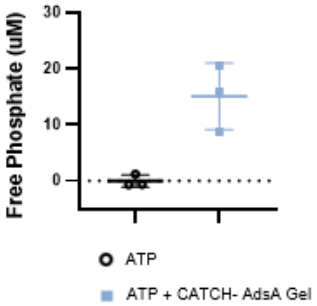
H&E histology of mouse paws 144 hours post MSU challenge. Mice received PBS (red), blank gels (blue), or CATCH-U gels (green) 48 hours prior to MSU crystal injection. um = microns. Arrows denote cutaneous infiltrate thickness. Dashed line represents average paw thickness from N = 1 untreated control mouse. N = 3-5, *P ≤ 0.05, ANOVA with Dunnett's multiple comparison test to PBS group. Data are presented as mean ± s.d.



Substrate	R2	Vmax	KM
ATP	0.9556	20.420	1515
ADP	0.9223	5.250	1906

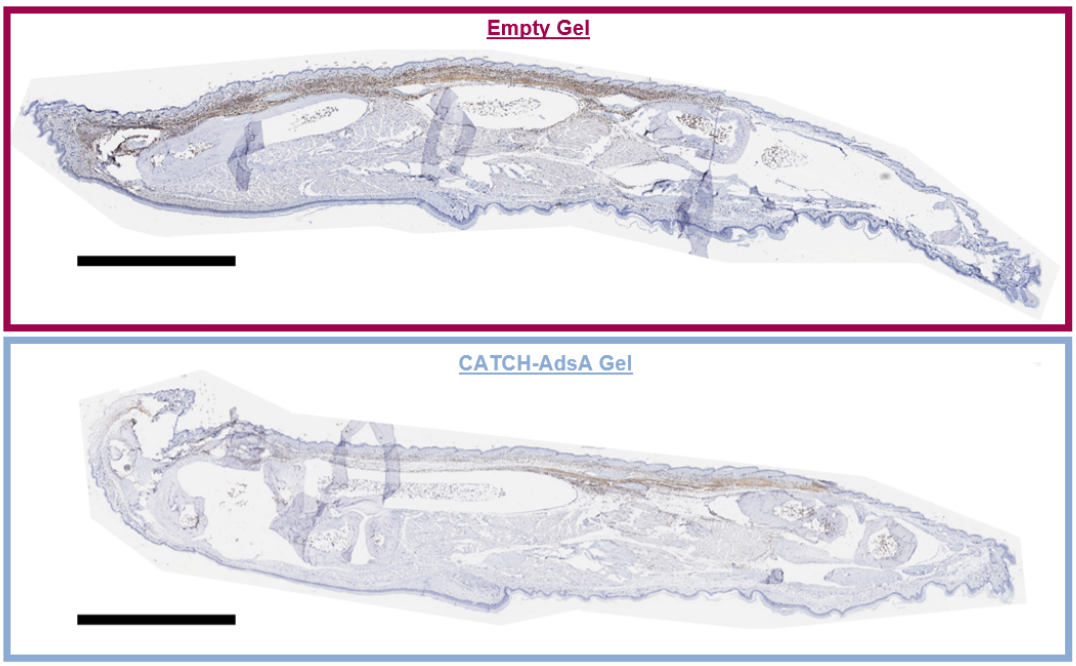
Extended Data Fig. 8: Expression and activity of soluble CATCH-AdsA

(a) SDS-PAGE and (b) analytical SEC of CATCH-AdsA purified from *E. coli.* (c) Relative activity of soluble CATCH-AdsA after a 5-minute incubation at the designated temperature, as measured by velocity of free phosphate generation normalized to velocity at room temperature. Dashed lines indicate 100% and 90% activity from room temperature. (d) Relative activity of soluble CATCH-AdsA over a pH range as measured by free phosphate generation (λ = 620 nm). Data presented as mean ± s.d. (N = 3). *$p<0.05$, ****$p<0.0001$, ANOVA with Tukey's post hoc. (e) Reaction velocity of soluble CATCH-AdsA acting on ATP or ADP as substrates (N = 3). Solid line denotes Michaelis-Menten fit. Table of Michaelis-Menten parameters. All data are presented as mean ± s.d.



Extended Data Fig. 9: CATCH-AdsA gels generate free phosphates when incubated with ATP

Free phosphates generated by ATP incubated alone or with CATCH-AdsA gels for 1 hour, as measured with a commercial malachite green assay kit. Data are presented as mean ± s.d.



Extended Data Fig. 10: CATCH-AdsA gels decrease LPS-induced neutrophil infiltration

Anti-Ly6G IHC of mouse hind paws at t = 1 d after subcutaneous LPS challenge in mice that received an empty gel (top, pink) or CATCH-AdsA gel (bottom, light blue) at t = -1 d. Scale bar = 2 mm.

Supplementary Methods

CATCH-NanoLuc amino acid sequence: MAEQEFEFEFEQEGSGGGSGGSGGGGSGGSGEF
MAVFTLEDFVGDWRQTAGYNLDQVLEQGGVSSLFQNLGVSVTPIQRIVLSGENGLKIDIHVIIPY
EGLSGDQMGQIEKIFKVVYPVDDHHFKVILHYGTLVIDGVTPNMIDYFGRPYEGIAVFDGKKITV
TGTLWNGNKIIDERLINPDGSLLFRVTINGVTGWRLCERILALEHHHHHH

CATCH-cutinase amino acid sequence: MAEQEFEFEFEQEGSGGGSGGSGGGGSGGSGEFM
RSGLPTSNPAQELEARQLGRTTRDDLINGNSASCADVIFIYARGSTETGNLGTLGPSIASNLESA
FGKDGVWIQGVGGAYRATLGDNALPRGTSSAAIREMLGLFQQANTKCPDATLIAGGYSQGAAL
AAASIEDLDSAIRDKIAGTVLFGYTKNLQNRGRIPNYPADRTKVFCNTGDLVCTGSLIVAAPHLA
YGPDARGPAPEFLIEKVRAVRGSALEHHHHHH

CATCH-Uricase amino acid sequence: MAEQEFEFEFEQEGSGGGSGGSGGGGSGGSGEFMS
AVKAARYGKDNVRVYKVHKDEKTGVQTVYEMTVCVLLEGEIETSYTKADNSVIVATDSIKNTIYI
TAKQNPVTPPELFGSILGTHFIEKYNHIHAAHVNIVCHRWTRMDIDGKPHPHSFIRDSEEKRNVQ
VDVVEGKGIDIKSSLSGLTVLKSTNSQFWGFLRDEYTTLKETWDRILSTDVDATWQWKNFSGL
QEVRSHVPKFDATWATAREVTLKTFAEDNSASVQATMYKMAEQILARQQLIETVEYSLPNKHY
FEIDLSWHKGLQNTGKNAEVFAPQSDPNGLIKCTVGRSSLKSKLLEHHHHHH

CATCH-AdsA amino acid sequence: MAEQEFEFEFEQEGSGGGSGGSGGGGSGGSGGGQH
TPMKAHAVTTIDKATTDKQQVPPTKEAAHHSGKEAATNVSASAQGTADDTNSKVTSNAPSNKP
STVVSTKVNETRDVDTQQASTQKPTHTATFKLSNAKTASLSPRMFAANAPQTTTHKILHTNDIH
GRLAEEKGRVIGMAKLKTVKEQEKPDLMLDAGDAFQGLPLSNQSKGEEMAKAMNAVGYDAM
AVGNHEFDFGYDQLKKLEGMLDFPMLSTNVYKDGKRAFKPSTIVTKNGIRYGIIGVTTPETKTKT
RPEGIKGVEFRDPLQSVTAEMMRIYKDVDTFVVISHLGIDPSTQETWRGDYLVKQLSQNPQLKK
RITVIDGHSHTVLQNGQIYNNDALAQTGTALANIGKITFNYRNGEVSNIKPSLINVKDVENVTPNK
ALAEQINQADQTFRAGGGGSGGGGSHHHHHH

Analytical size exclusion chromatography. 500 µL solution of protein (CATCH-NL, CATCH-uricase, or CATCH-AdsA) was loaded to Superdex Increase 75 or 200 10/300 GL column (Cytiva) connected to an ÄKTA Pure FPLC system. Using absorbance of 280nm and protein standard markers, the fusion protein molecular weight was determined.

Soluble CATCH-cutinase activity. In 1 96-well plate, 5nM CATCH-cutinase was incubated with varying concentrations of 100uL *p*-nitrophenyl butyrate (pNPB ; 0-2.5mM) in 1x PBS and reaction velocity was measured at 405nm, the absorbance wavelength of the enzymatic product p-nitrophenol. The slopes of the linear region of the kinetic measurements were determined and plotted against pNPB concentration. The Michaelis-Menten model in GraphPad was used to determine Michaelis constant (K_m) and maximum velocity (V_{max}). Additionally, 100uL 1mM pNPB was incubated with varying concentrations of CATCH-cutinase (0.1 - 50nM) and reaction velocity was measured at 405nm.

Preparation of monosodium urate (MSU) crystals. MSU crystals were prepared as previously described[1]. 20mM uric acid (Alfa Aesar) was dissolved in boiling water containing 20mM NaOH. The solution was cooled to 60°C, adjusted to pH 9 with NaOH, and sterile filtered. The solution was left under mild agitation at room temperature for 24-72 hours to allow crystal formation. The crystals were then harvested on a sterile filter, washed with 70% ethanol, and dried overnight at 60°C. The crystals were stored dry at 4°C or resuspended in 1x PBS and stored at -20°C. Solutions of crystals were sonicated for 3-5 minutes to reduce crystal size to allow injection

through a 27G needle. Crystals were analyzed by microscopy to ensure correct formation of their needle-like structure. Endotoxin content was confirmed to be below 1.0 EU/mL, determined with Chromo-LAL Endotoxin Quantification (Cape Cod Inc.), according to the manufacturer's instructions.

Temperature sensitivity of CATCH-uricase activity. The ability of the pre-heated uricase to deplete uric acid was assessed by measuring the absorbance of uric acid at 293nm over time. CATCH-U at 16.5uM was incubated for 5 minutes at room temperature, 37°C, 45°C, 50°C, 55°C, 60°C, 65°C, and 70°C. 20uL pre-heated CATCH-U was added in triplicate to a UV-transparent 96-well plate with 200uL of 0.4mM uric acid and absorbance was read for 30 minutes. To get relative activities, the reaction velocity was determined for each condition and normalized to reaction velocity at RT incubation.

Effect of CATCH-tag on uricase activity. The activity of CATCH-U was compared to the activity of WT uricase. 5uL solution containing 12.6ug of each protein was pipetted into a UV-transparent 96-well plate. Then 75uL of uric acid at various concentrations (250-1000uM) was added to each well and absorbance was immediately read at 293nm for 10 minutes at 37°C. The slopes of the linear region of the kinetic measurements were determined and converted to reaction velocity (uric acid depletion per minute) using the slope of a uric acid standard curve. These activities were then plotted against initial uric acid concentration and the slopes of the CATCH-uricase and WT uricase were compared in GraphPad Prism.

CATCH-uricase retention in CATCH gels. 20uL gels were formulated with 16.5uM CATCH-U or with 16.5uM WT uricase in syringes. The gels were injected into a 96-well plate, 200uL 1x PBS was added to each well, and the plate was left at room temperature. A standard curve of CATCH-U and WT protein (200uL/well) was also plated. 48 hours later 175uL of the PBS supernatant was removed from each gel and plated in a black 96-well plate, along with 175uL of each standard. The remaining 25uL 1x PBS supernatant on the gels was discarded. 200uL of fresh 1x PBS was added to each gel and rapidly pipetted to dislodge and homogenize the gel. 175uL of the gel homogenate was plated in the black 96-well plate. Then tryptophan fluorescence (ex/em 280/345) was measured to quantify the protein content. A linear regression of the standard curves was used to convert fluorescence into protein content. Then percentages of each fraction, inside the gel vs in the supernatant, were calculated using the total quantified protein signal of the sample; i.e. percentage in the supernatant equals signal from supernatant divided by signal from supernatant plus signal from gel.

CATCH-U-cy5.5 In vivo imaging. CATCH-U and wild type uricase was fluorescently labeled with cyanine-5.5 (cy5.5) through maleimide-cysteine chemistry. The reactions were carried out with a 20x molar excess sulfo-cy5.5 maleimide (Lumiprobe) at 4°C overnight according to the manufacturing guidelines. Unreacted dye was removed by buffer exchange and filter centrifugation. Cohorts of mice were injected with 20uL into the subcutaneous space on the top of the hind paw with either 16.5uM WT uricase-Cy5.5 in 1x PBS (N=4) or CATCH gels with 16.5uM CATCH-U-cy5.5 (N=5). The mice were individually anatomically positioned with the hind paw facing the camera of the IVIS and fluorescent images were taken immediately after injection. Images were taken again 30 minutes, 1 hour, and 3 hours later, then once per day for a 10-day period. Fluorescent images were captured using ex/em 675/720nm, 1 second exposure time, subject size 1cm, field-of-view B (6.6cm), small binning resolution, and a 2 F/stop aperture. Cy5.5 signal was quantified as radiant efficiency using a round ROI in the Living Image analysis

software. The ROI used captured the whole hind paw, stopping at the ankle. The same ROI was used for each animal and each time point. Half-life was calculated in GraphPad prism.

Paw histology. Paw tissue was harvested from a cohort of animals 144 hours after MSU crystal injection. These animals were treated with either gels with 16.5uM CATCH-U (N=5), empty gels (N=4), or PBS (N=3) 48 hours prior to MSU injection. Cell infiltration into the paw was assessed with histology. The whole paws were fixed with 10% formalin and decalcified then cut bilaterally and embedded in paraffin. The blocks were sectioned and stained with hematoxylin and eosin (H&E). Histology images were analyzed by a blinded individual. The observer measured the thickness of immune infiltrate in the subcutaneous space of the hind paw. Measurements were taken at 0.4mm intervals across the length of the paw and averaged per animal. One contralateral, naïve paw was used as a baseline control, shown as a dashed line on the graph.

CATCH-AdsA gel activity. The activity of CATCH-AdsA gels to dephosphorylate ATP was assessed by measuring free phosphate generation using the malachite green assay kit (Sigma). Gels were formed with 2uM CATCH-AdsA in a 96-well plate and incubated with 30uM ATP for 1 hour. 80uL 30uM ATP alone was incubated for 1 hour as a control. 20uL of the working reagent was added to each well. After 15 minutes, absorbance was measured at 620nm and compared to a standard curve.

Temperature sensitivity of CATCH-AdsA activity. The activity of the pre-heated AdsA to dephosphorylate ATP was assessed by measuring free phosphate generation using the malachite green assay kit (Sigma) in a 96-well plate. CATCH-AdsA at 2uM was heated for 5 minutes at room temperature, 37°C, 50°C, 70°C, or 90°C. 80uL of the pre-heated CATCH-AdsA and 30uM ATP was incubated in triplicate for one hour. 20 µL of the working reagent from the kit was added. After 15 minutes, absorbance was measured at 620nm. Relative activity was calculated as the amount of free phosphates generated normalized to the amount of free phosphates generated at RT.

pH sensitivity of CATCH-AdsA activity. The activity of CATCH-AdsA gels to dephosphorylate ATP at varying pHs was assessed by measuring free phosphate generation using the malachite green assay kit (Sigma) in a 96-well plate. Sodium hydroxide was added to CATCH-AdsA to a final pH of 5, 5.5-6.0, 6.4, 7.0, and 7.6. Then 80uL of each CATCH-AdsA was added to 30uM ATP. After a 1-hour incubation, 20uL of the working reagent from the malachite green assay kit was added, and 15 minutes later, absorbance was measured at 620nm.

Soluble enzyme kinetics. Soluble CATCH-AdsA protein kinetics was assessed using malachite green assay kit (Sigma) in a 96-well plate. To analyze the substrate-dependent activity, 2 µM AdsA was incubated with ATP and ADP at 50 µM – 8 mM. After 5 minutes of incubation, the working reagent was added, and 5 min later absorbance was measured at 620 nm. This step was repeated, until each concentration of ATP was incubated with AdsA for 30 minutes (for a total of 6 time points). Using a linear regression, the Michaelis-Menten model in GraphPad prism was used to determine Michaelis constant (K_M) and maximum velocity (V_{max}).

References

1. Alberts A, Klingberg A, Wessig AK, et al. C-reactive protein (CRP) recognizes uric acid crystals and recruits proteases C1 and MASP1. *Sci Reports 2020 101*. 2020;10(1):1-13. doi:10.1038/s41598-020-63318-8