nature biotechnology

ARTICLES

https://doi.org/10.1038/s41587-019-0242-8

Multi-input chemical control of protein dimerization for programming graded cellular responses

Glenna Wink Foight[1,2,8], Zhizhi Wang[2,3,8], Cindy T. Wei[1], Per Jr Greisen[2,4,6], Katrina M. Warner[1], Daniel Cunningham-Bryant[1], Keunwan Park[2,4,7], T. J. Brunette[2,4], William Sheffler[2,4], David Baker[2,4,5] and Dustin J. Maly[1,4*]

Chemical and optogenetic methods for post-translationally controlling protein function have enabled modulation and engineering of cellular functions. However, most of these methods only confer single-input, single-output control. To increase the diversity of post-translational behaviors that can be programmed, we built a system based on a single protein receiver that can integrate multiple drug inputs, including approved therapeutics. Our system translates drug inputs into diverse outputs using a suite of engineered reader proteins to provide variable dimerization states of the receiver protein. We show that our single receiver protein architecture can be used to program a variety of cellular responses, including graded and proportional dual-output control of transcription and mammalian cell signaling. We apply our tools to titrate the competing activities of the Rac and Rho GTPases to control cell morphology. Our versatile tool set will enable researchers to post-translationally program mammalian cellular processes and to engineer cell therapies.

Cells exhibit proportional, graded, digital and temporal behaviors in sensing and responding to multiple environmental or autologous inputs[1–3]. Biologists seeking to reproduce natural functions, or create new ones, need tools that can program a similar range of behaviors. Most reported synthetic biology tools are based on transcriptional circuits that can enable a wide variety of quantitative control modes[4,5]. However, methods for rapid, protein-level manipulation of cellular processes have lagged behind due to the difficulty of engineering complex post-translational control schemes.

For mammalian synthetic biology applications, post-translational control systems that use small molecules as extrinsic inputs are desirable for many applications because they are easy to use in vitro, ex vivo and in vivo to confer temporal modulation[6]. Chemically controlled proteases and degradation domains have been applied for post-translational control[7–9]. Two recently developed, chemically controlled systems that use catalytically active hepatitis C virus protease NS3a as a cleavage-based modulator of mammalian cellular processes are particularly attractive because they use orally available, clinically approved drugs that are orthogonal to mammalian systems as extrinsic inputs[10,11]. Chemically induced dimerization (CID) systems, which modulate cellular processes through small molecule-induced protein proximity, are advantageous for applications that require more rapid cellular responses, such as cellular signaling, than protease- or degradation-based systems[12–14]. Although there has been recent success in expanding the diversity of small molecules that can be used in CID systems, no system that uses a clinically approved drug that lacks an endogenous mammalian target has been described to date[15].

A limitation of current chemically controlled systems is that they rely on single small molecule inputs that are translated into single outputs, which limits the types of cellular responses that can be programmed. There has been success in combining orthogonal CID systems to achieve digital logic control of cell signaling and transcription[14,16]. In addition, combining composable, single-input/single-output protease-based systems has allowed the assembly of a diversity of digital circuits[17]. While digital logic is useful, current post-translational control systems lack robust analog outputs, such as graded and proportional control, that are needed to fully mimic natural cellular processes.

Here, we present a post-translational control system that utilizes the NS3a protease as a central receiver protein that can be targeted by multiple clinically approved drug inputs. To translate different drug-bound states of NS3a into diverse outputs, we engineer computationally designed 'reader' proteins that recognize specific inhibitor-bound states of NS3a and use a genetically encoded peptide that selectively recognizes the apo form of this protease (Fig. 1a). Our system, named Pleiotropic Response Outputs from a Chemically Inducible Single Receiver (PROCISiR), can be used to program diverse cellular responses owing to its single receiver protein architecture.

Results

Computational design of NS3a readers. Rosetta interface design allowed us to develop protein readers that selectively recognize a binding surface centered on NS3a-bound inhibitors (Fig. 1b)[18]. First, we used a set of stable, de novo-designed proteins as scaffolds on which to design an interface with the danoprevir/NS3a

[1]Department of Chemistry, University of Washington, Seattle, WA, USA. [2]Institute for Protein Design, University of Washington, Seattle, WA, USA. [3]Department of Biological Structure, University of Washington, Seattle, WA, USA. [4]Department of Biochemistry, University of Washington, Seattle, WA, USA. [5]Howard Hughes Medical Institute, University of Washington, Seattle, WA, USA. [6]Present address: Global Research, Novo Nordisk A/S, Måløv, Denmark. [7]Present address: Systems Biotechnology Research Center, Korea Institute of Science and Technology, Gangneung, Republic of Korea. [8]These authors contributed equally: Glenna Wink Foight, Zhizhi Wang. *e-mail: djmaly@uw.edu

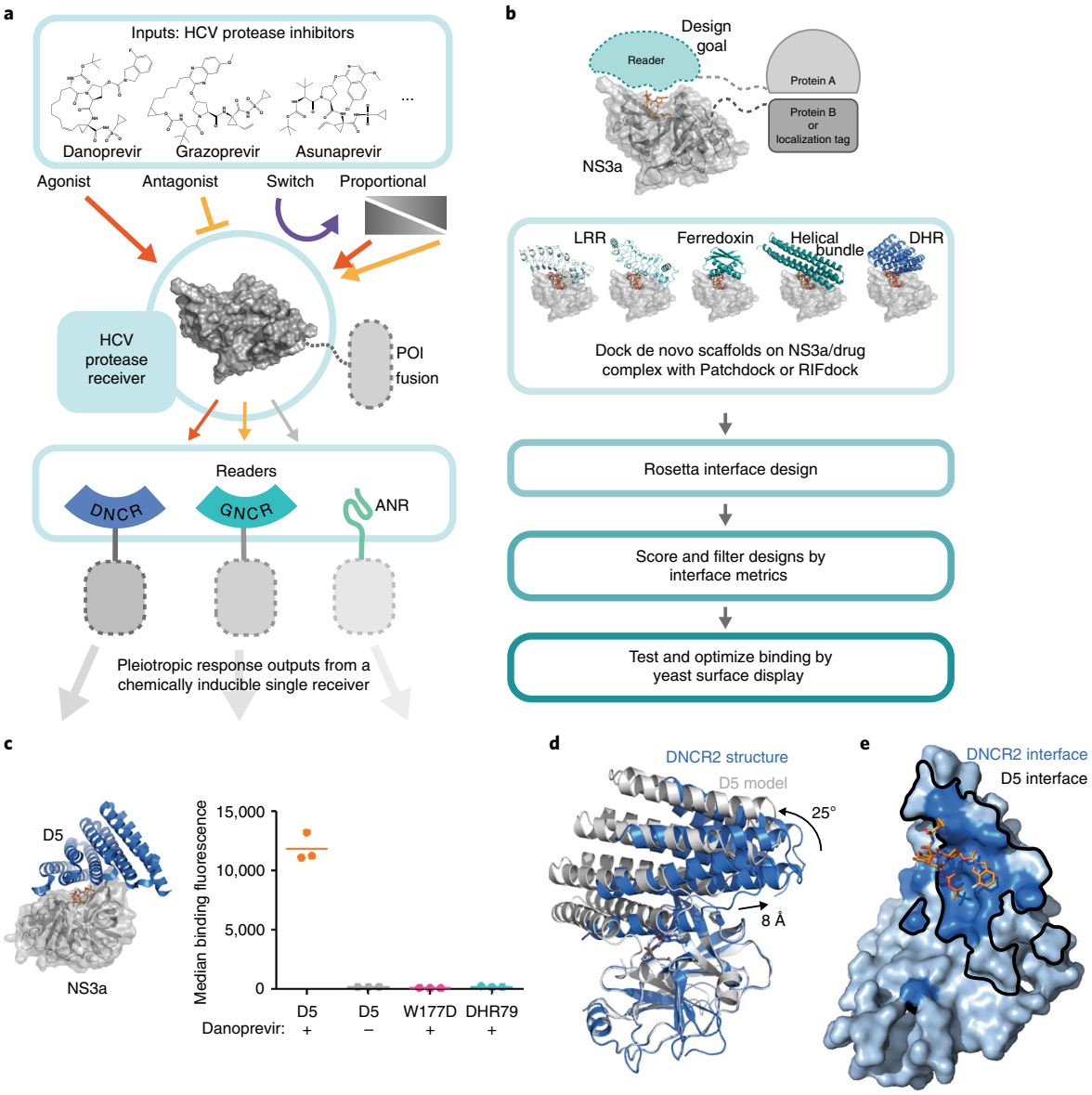


Fig. 1 | Design of a danoprevir/NS3a complex reader. a, Schematic of the PROCISiR system. Multiple NS3a-targeting drugs are used as inputs that are interpreted by designed readers to generate multiple outputs. **b**, Goal and process for designing and optimizing drug/NS3a complex readers, starting from docking of several scaffold classes on a drug/NS3a complex, Rosetta design of the reader interface, filtering based on Rosetta interface scoring metrics and finally testing and optimization via yeast surface display. **c**, Rosetta model of D5 (left) and binding of 1 μM NS3a with avidity to yeast-displayed D5 in the presence or absence of 10 μM danoprevir. A D5 interface mutant, W177D, and the original DHR79 scaffold show no binding. Technical triplicates and means from one experiment. **d**, A cocrystal structure of the DNCR2/danoprevir/NS3a complex aligned with the D5/danoprevir/NS3a model via NS3a. Measurements indicate shifts of DNCR2 relative to D5. **e**, Residues within 4 Å of NS3a/danoprevir are highlighted in dark blue on the surface of DNCR2. Residues at the interface in the D5 model are outlined in black. HCV, hepatitis C virus.

complex, including leucine-rich repeat proteins (LRRs), designed helical repeat proteins (DHRs), ferredoxins and helical bundles[19–21]. As a starting point, PatchDock was used to center each scaffold over NS3a-bound danoprevir, followed by RosettaDesign on the scaffold surface that forms the binding interface[22]. DHR design D5, 1 of 31 designs selected for testing via yeast surface display based on interface scoring metrics, showed modest, drug-dependent binding to NS3a, which required designed interface residues (Fig. 1c). To improve D5's affinity for the NS3a/danoprevir complex, we used two sequential yeast surface display libraries (Supplementary Fig. 1 and Supplementary Notes). Our final variant of the danoprevir/NS3 complex reader (DNCR), DNCR2, had an apparent affinity for the NS3a/danoprevir complex of 36 pM, no detectable binding to apo NS3a and >20,000-fold selectivity over NS3a bound to the drugs grazoprevir or asunaprevir (Supplementary Table 1 and Supplementary Fig. 2a). Further biochemical analysis confirmed that DNCR2 does not bind substantially to free danoprevir and that DNCR2/danoprevir/NS3a form a 1:1:1 complex (Supplementary Fig. 2b,e and Supplementary Notes). A 2.3 Å resolution structure of the DNCR2/danoprevir/NS3a complex revealed a modest shift for DNCR2 relative to the D5 model, but with both interfaces sharing an overlapping set of positions (Fig. 1d,e, Supplementary Fig. 2c,d and Supplementary Table 2). The structural basis for the selective binding of DNCR2 to the NS3a/danoprevir complex is clearly apparent when structures of asunaprevir- or grazoprevir-bound NS3a are aligned to the DNCR2/danoprevir/NS3a complex (Supplementary Fig. 2f)[23,24].

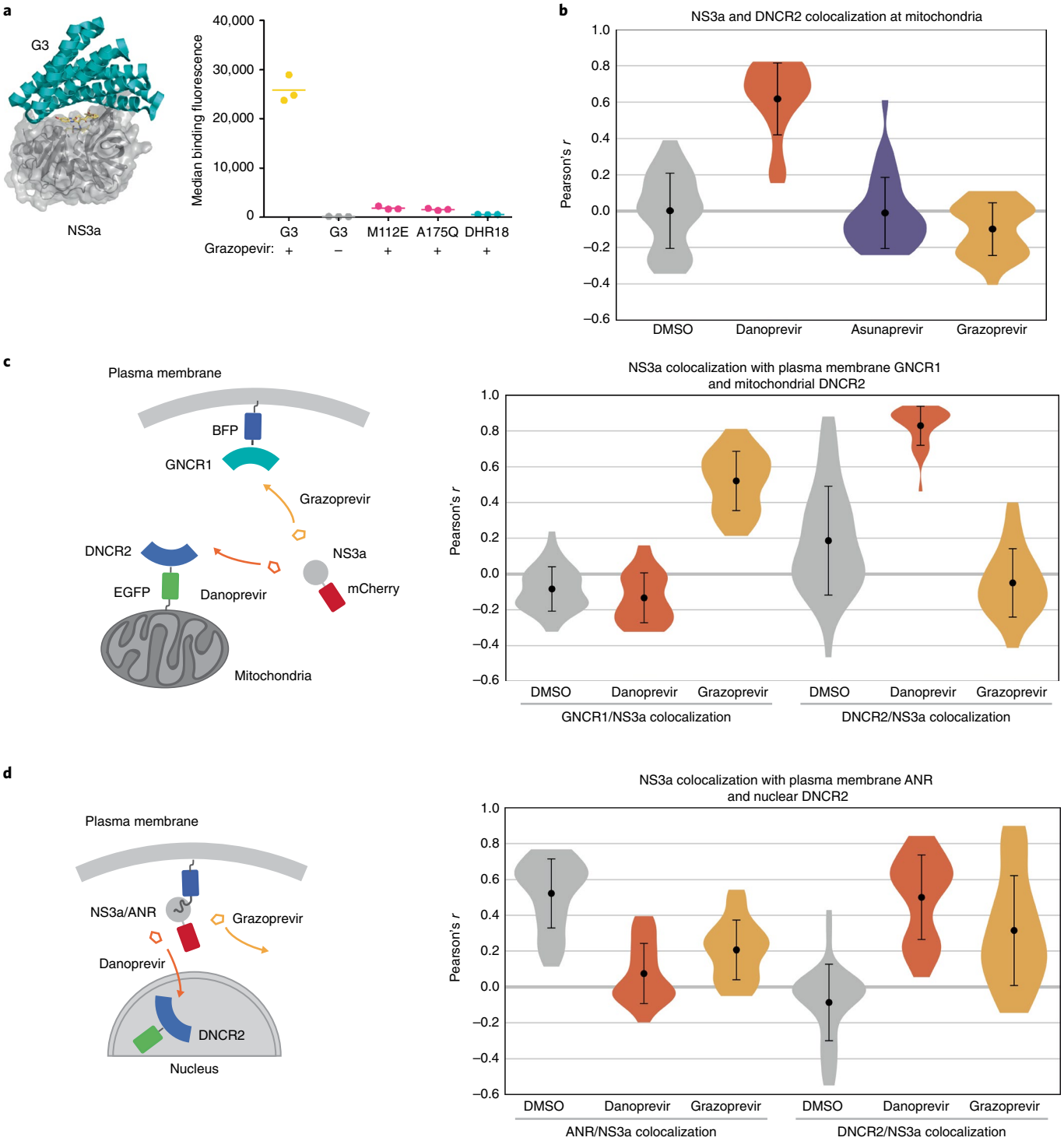


Fig. 2 | Design and testing of a grazoprevir/NS3a complex reader. a, Rosetta model (left) and binding (right) of 1 μM NS3a with avidity to yeast-displayed G3 in the presence or absence of 10 μM grazoprevir. Point mutants at the G3 interface, M112E and A175Q, and the original DHR18 scaffold show no binding. Technical triplicates and means from one experiment. **b**, Colocalization of DNCR2-EGFP with Tom20-mCherry-NS3a after treatment with the drug indicated or DMSO. **c**, Colocalization of NS3a-mCherry with GNCR1-BFP-CAAX or Tom20-DNCR2-EGFP after treatment with danoprevir, grazoprevir or DMSO. **d**, Colocalization of NS3a-mCherry with ANR-BFP-CAAX or NLS-DNCR2-EGFP after treatment with danoprevir, grazoprevir or DMSO. The mean (marked by dot) and standard deviation (error bars) of the Pearson's *r* of red/blue or red/green pixel intensities for the number of cells stated in Supplementary Table 3 are given for each condition in **b**–**d**, along with the distributions of Pearson's *r*. See Supplementary Table 3 for sample sizes and *P* values.

The high specificity of DNCR2 provided confidence that we could design additional readers that selectively recognize other NS3a/drug complexes. We computationally designed a reader of the grazoprevir/NS3a complex by applying a similar methodology, this time focusing exclusively on DHR scaffolds due to the success of D5 as a reader of the danoprevir/NS3a complex (see Supplementary

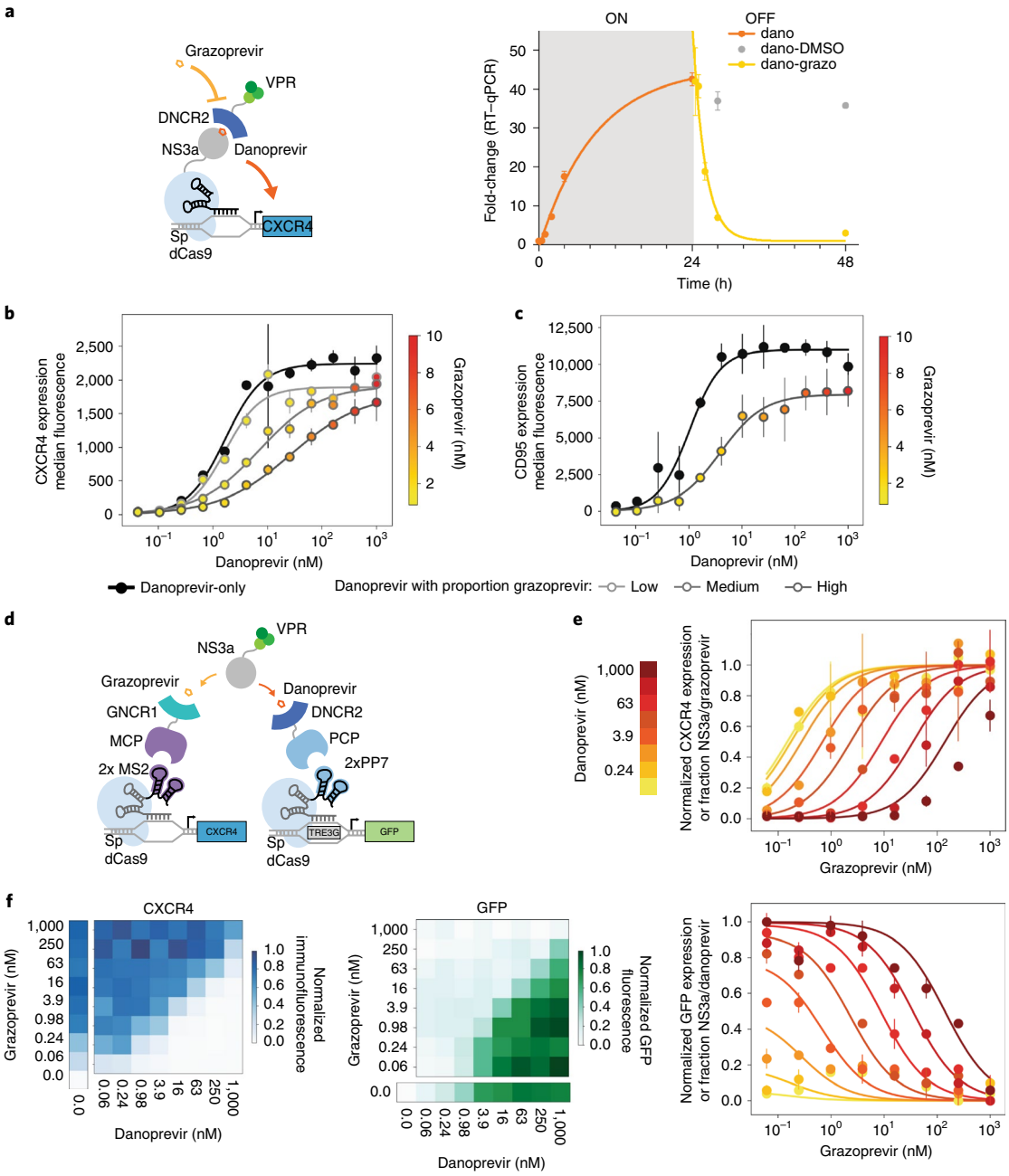


Fig. 3 | Temporal, graded and proportional transcriptional control using PROCISiR. a, Reversibility of CXCR4 induction from danoprevir-promoted recruitment of DNCR2-VPR to NS3a-dCas9. 'OFF' conditions indicate replacement of danoprevir-containing medium with DMSO- (gray) or grazoprevir-containing medium (yellow). **b**,**c**, Cotitrating grazoprevir as a competitor in the presence of a uniform titration of danoprevir inducer in cells expressing the constructs shown in **a** extends the linear range of the CXCR4 (**b**) or CD95 (**c**) expression response resulting from DNCR2-VPR recruitment. The curves with higher proportions of the competitive inhibitor grazoprevir (darker gray) were created by performing lower fold-dilutions of grazoprevir ('low' (light gray), 2-fold; 'medium' (medium gray), 1.5-fold; and 'high' (dark gray), 1.25-fold serial dilutions). See Supplementary Table 4 for the exact drug concentrations used for each condition. **a**–**c**, Mean and standard deviation of three biological replicates relative to DMSO—baseline subtracted values. **d**, Schematic of the transcriptional activation system used in **e** and **f** to simultaneously modulate expression of CXCR4 and GFP in cells coexpressing an MS2 scRNA targeting CXCR4, a PP7 scRNA targeting a GFP reporter, GNCR1-MCP, DNCR2-PCP, NS3a-VPR and dCas9. **e**, Data from **f** (points, mean, standard deviation) overlaid with modeling (lines) of the fraction of NS3a bound to grazoprevir (top) or danoprevir (bottom) at the drug concentrations used in **f**, as described in the Supplementary Notes. **f**, Expression of CXCR4 and GFP after cotreatment with danoprevir and grazoprevir. Matrices are means of two biological replicates; single-drug titrations are means of three biological replicates. Raw median fluorescence values for **e** and **f** are shown in Supplementary Fig. 8e,f. RT–qPCR, quantitative PCR with reverse transcription.

Notes for more design details). We also employed a reverse rotamer docking protocol, rotamer interaction field docking (RIFdock), in addition to the more coarse-grained PatchDock method[25]. One design of the 29 tested, G3, showed modest, grazoprevir-dependent binding, which was not observed for the original scaffold or interface mutants (Fig. 2a). Screening a single library for improved

affinity yielded grazoprevir/NS3a complex reader 1 (GNCR1) (Supplementary Fig. 3a,b and Supplementary Notes). GNCR1 demonstrated an apparent affinity for the grazoprevir/NS3a complex of 140 nM and little to no affinity for apo, danoprevir-bound or asunaprevir-bound NS3a (Supplementary Fig. 3c and Supplementary Table 1). To provide a third reader, we used a previously described, genetically encoded peptide that binds to the active site of apo NS3a, here called apo NS3a reader (ANR)[26,27]. ANR forms a basal complex with NS3a with an affinity of 10 nM, which is disrupted by NS3a-targeting drugs (Fig. 1a).

Validation of PROCISiR in mammalian cells. We next sought to evaluate the unique, multi-input/multi-output behaviors that PROCISiR could achieve in mammalian cells. First, we verified that danoprevir was capable of rapidly colocalizing DNCR2 with membrane-targeted NS3a ($t_{1/2}$ of colocalization, 76 ± 27 s (mean ± s.d.)), which was capable of activating PI3K-Akt signaling when DNCR2 was fused to the inter-SH2 domain from the p85 regulatory subunit of PI3K (Supplementary Fig. 4)[28]. The drug specificity of DNCR2 was maintained in cells, as neither grazoprevir nor asunaprevir promoted DNCR2-EGFP colocalization with mitochondrially targeted NS3a (Fig. 2b). We then combined DNCR2 with GNCR1 or ANR to divergently localize mCherry-NS3a to different subcellular spaces. We observed that grazoprevir exclusively colocalized NS3a-mCherry with membrane-targeted GNCR1, while only danoprevir led to colocalization with mitochondrially targeted DNCR2 (Fig. 2c and Supplementary Fig. 5a). Likewise, membrane-targeted ANR prelocalized NS3a-mCherry to the plasma membrane, and danoprevir treatment recruited NS3a to the nucleus with nuclear-targeted DNCR2 (Fig. 2d and Supplementary Fig. 5b). These and additional colocalization experiments (Supplementary Figs. 6 and 7 and Supplementary Notes) validated that the DNCR2, GNCR1 and ANR readers are selective for their targeted state of NS3a and can be used in concert.

PROCISiR enables programmable transcriptional control. The ability of our readers to discriminate between different states of NS3a allows complex control modes to be achieved by combining inputs and/or readers, a capability not shared by chemically inducible systems for which there is only one input and one subsequent protein complex formed. First, we used danoprevir as an agonist and grazoprevir as an antagonist to temporally and proportionally control transcription of one endogenous gene in HEK293 cells using DNCR2-VPR (a transcriptional activator) and an NS3a-dCas9 fusion (*Streptococcus pyogenes*)[29,30]. By chasing with grazoprevir after pulsing transcriptional activation with DNCR2-VPR and danoprevir, we were able to rapidly reverse CXCR4 expression (Fig. 3a). Next, to achieve graded control of the steady-state transcriptional output from DNCR2-VPR, we cotreated cells with danoprevir and increasing grazoprevir proportions, producing a linear output from endogenous CXCR4 and CD95 promoters over three orders of magnitude of danoprevir input (Fig. 3b,c). We found that this precise titration of gene expression on a single-cell level could also be produced from an exogenous promoter (Supplementary Fig. 8a). Commonly used methods for mammalian gene induction, such as highly cooperative TetR-based systems, are ineffective at achieving intermediate levels of gene expression[31].

We also combined two inputs with two readers to achieve proportional and graded control of two transcriptional outputs at once. Titration of each drug alone in the two-gene transcriptional activation system shown in Fig. 3d, which utilizes orthogonal RNA hairpin/RNA-binding proteins to target two different promoters, demonstrated that observed half-maximum effective concentration (EC_{50}) values, 0.16 ± 0.03 nM and 0.79 ± 0.15 nM (mean ± s.d.) for the grazoprevir/NS3a and danoprevir/NS3a readers, respectively, are in close agreement with NS3a's inhibition constant (K_i) value for each drug (Supplementary Fig. 8c,d)[23,32]. Hence, the transcriptional output from these systems depends on the binding affinity of the drugs to NS3a, not the affinity of the readers for their drug/NS3a complex. The dependence of DNCR2- and GNCR1-promoted transcription levels on their inducer's K_i value allowed us to model the steady-state output from each reader/drug/NS3a complex at mixed danoprevir and grazoprevir concentrations (Fig. 3e, Supplementary Fig. 9 and Supplementary Notes). Proportional expression output of CXCR4 and GFP across a matrix of grazoprevir and danoprevir concentrations demonstrated close concordance with predicted NS3a/drug complex levels (Fig. 3e,f). See Supplementary Notes and Supplementary Fig. 10 for a description of additional transcriptional control modes that can be achieved, including three-gene control and switchable repression/overexpression. The responsive nature of the PROCISiR architecture enables diverse modes of temporal, graded or proportional, and multi-state transcriptional control, all realized at the post-translational level.

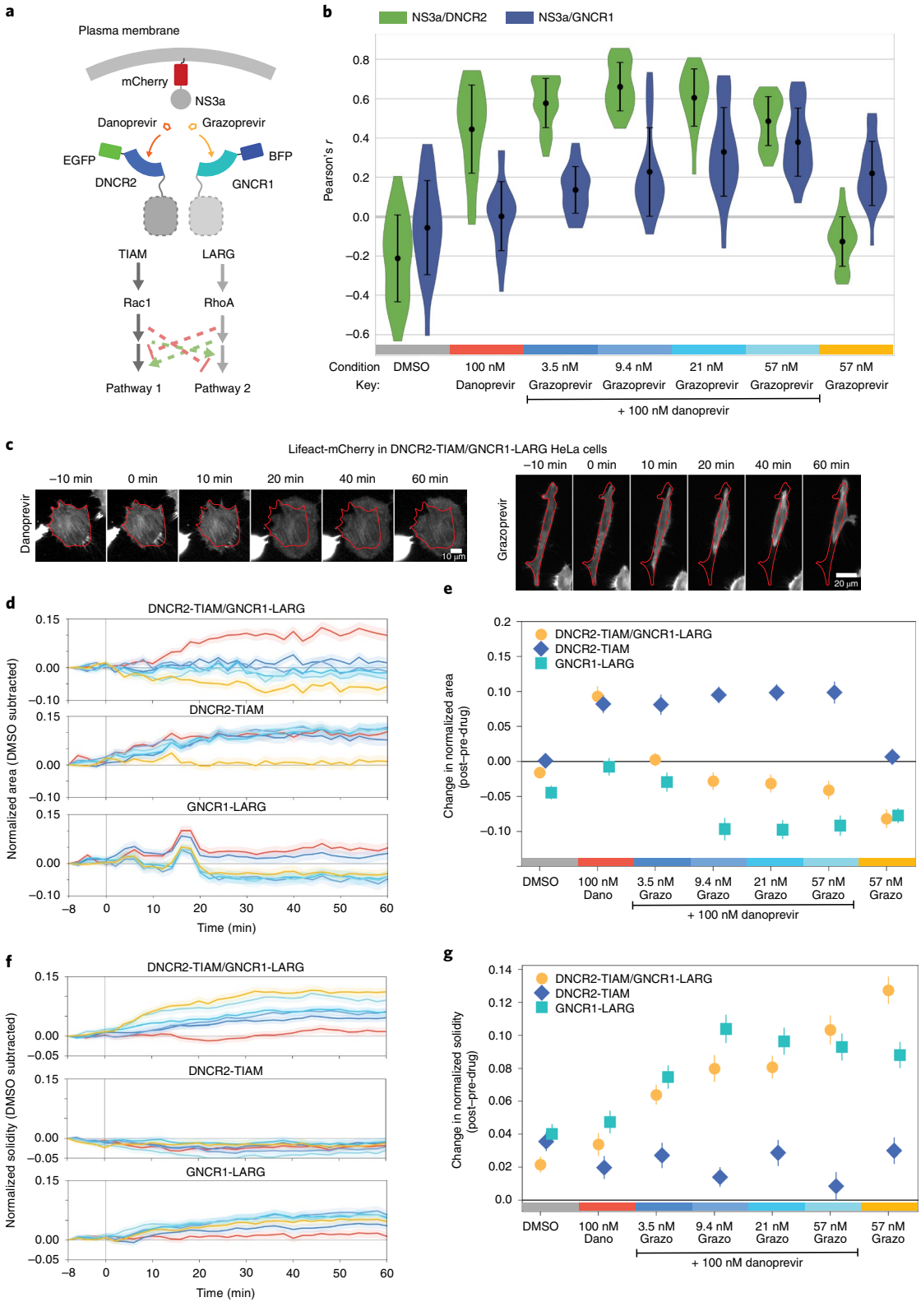
Proportional control of cell signaling with PROCISiR. Finally, we used PROCISiR to interrogate signaling pathway cross-talk by colocalizing signaling effector domains fused to DNCR2 or GNCR1 with membrane-localized NS3a in HeLa and NIH3T3 cells (Fig. 4a). We predicted danoprevir and grazoprevir concentrations that would provide graded and proportional regimes of DNCR2 and GNCR1 colocalization with NS3a by modeling NS3a/drug complex levels (Supplementary Fig. 9 and Supplementary Notes). Confocal microscopy of EGFP-DNCR2 and BFP-GNCR1 with membrane-localized NS3a validated that these drug combinations provided the desired colocalization regimes (Fig. 4b and Supplementary Fig. 11c). These datasets based on fluorescent protein colocalization at the membrane provide a useful model for the competition between DNCR2 and GNCR1 for binding to membrane-bound NS3a at different danoprevir and grazoprevir concentrations.

We next used these same danoprevir/grazoprevir drug combinations with DNCR2-TIAM (Rac1 guanine nucleotide exchange factor) and GNCR1-LARG (RhoA/B/C guanine nucleotide exchange factor) fusions to provide proportional and graded control over the activation of Rac and Rho GTPases, which have opposing effects

Fig. 4 | Graded and proportional control of GTPase-driven signaling pathways. a, Schematic of the colocalization system used in **c**–**g** for GTPase-driven signaling activation. Combinations of danoprevir and grazoprevir were used to control the proportions of DNCR2 and GNCR1 colocalizing with NS3a at the plasma membrane. **b**, Colocalization of EGFP-DNCR2 (green) or BFP-GNCR1 (blue) with mCherry-NS3a-CAAX quantified by Pearson's *r* from confocal images of HeLa cells. The mean (marked by dot) and standard deviation (error bars) of the Pearson's *r* of red/blue or red/green pixel intensities for the number of cells stated in Supplementary Table 3 are given for each condition, along with the distributions of Pearson's *r*. **c**, Representative images from two experiments of HeLa cells coexpressing EGFP-DNCR2-TIAM, BFP-GNCR1-LARG, NS3a-CAAX and Lifeact-mCherry treated with danoprevir (left) or grazoprevir (right) for the times indicated. Fluorescent signal for Lifeact-mCherry, which stains F-actin, is shown. A red outline illustrating the cell boundary at −10 min is overlaid on each panel to illustrate the change in cell size. **d**,**f**, Change in normalized area (**d**) or solidity (**f**) (DMSO baseline subtracted) over time (drug addition at 0 min) in HeLa cells expressing NS3a-CAAX with either DNCR2-TIAM and GNCR1-LARG (top), DNCR2-TIAM alone (middle) or GNCR1-LARG alone (bottom). Line colors correspond to the drug conditions in **b**. **e**,**g**, Change in normalized area (**e**) or solidity (**g**) (average last 10 min − first 10 min). **d**–**g**, Mean and s.e.m. of the number of cells per condition listed in Supplementary Table 3 from four independent wells. Dano, danoprevir; grazo, grazoprevir.

on the actin cytoskeleton and cell morphology[33]. In cells coexpressing DNCR2-TIAM and GNCR1-LARG with membrane-localized NS3a, with F-actin visualized by Lifeact-mCherry, danoprevir treatment resulted in cell expansion due to the activity of Rac in promoting actin polymerization and formation of lamellipodia (Fig. 4c, left panel). Grazoprevir treatment caused cell contraction and stress fibers (visualized by bright actin staining along the cell periphery), which is consistent with Rho activation (Fig. 4c, right panel).

We then used time-lapse, wide-field microscopy to track the morphological changes of hundreds of cells coexpressing DNCR2-TIAM and GNCR1-LARG (dual effector) under the drug treatment conditions shown in Fig. 4b, which were compared with responses in cells expressing a single effector, DNCR2-TIAM or GNCR1-LARG (Fig. 4d–g and Supplementary Fig. 12). Changes to cell size measured by total cell area (Fig. 4d,e) and perimeter (Supplementary Fig. 12a,b) show that Rac activity induced by danoprevir treatment



caused modest increases in cell size in cell lines with DNCR2-TIAM, while grazoprevir-mediated Rho activity caused cells to contract in cells with GNCR1-LARG. We also tracked changes to cell shape measured by solidity (defined as the cell's area divided by the area of an ellipse drawn around the cell, such that a cell with more membrane protrusions will have lower solidity) and circularity (0–1, where 1 is a perfect circle, and 0 a line) (Fig. 4f,g and Supplementary Fig. 12c,d). Rho activity promoted by grazoprevir treatment in cells with GNCR1-LARG caused cells to increase in solidity and circularity, indicative of contraction of protrusions and cell length.

A notable difference between dual-effector and single-effector lines was that titration of a single effector resulted in only a few gradations in morphology change, which plateaued at a maximum with at most one intermediate level (blue and teal mean values in Fig. 4e,g, and see Supplementary Fig. 12b,d). In contrast, dual-effector cells showed more graded population-level changes in cell morphology as a result of different ratios of Rac and Rho activity. However, concurrent variation in Rho and Rac activity does not result in an average phenotype, but one closer to the Rho-only (GNCR1-LARG line) condition, indicating that for this pair of opposing signaling effectors, Rho has the dominant effect on cell morphology. Single-cell distributions of the changes in cell morphology statistics show that the majority of cells in each treatment population responded to GTPase activation (Supplementary Fig. 12e–g). See the Supplementary Notes for more discussion of single-cell-level responses. Coactivation of Rac and Rho also resulted in graded population-level solidity and circularity changes in NIH3T3 cells coexpressing DNCR2-TIAM and GNCR1-LARG (Supplementary Fig. 11 and Supplementary Notes). However, the increase in area and perimeter from danoprevir treatment was lower compared with that observed in HeLa cells, likely due to general morphological differences between the more adherent and flat, fibroblast NIH3T3 cells and the more rounded, epithelial HeLa cell lines. Thus, PROCISiR's ability to proportionally coactivate multiple signaling pathways provides basic insight into competing cellular processes and the achievement of finer gradations in phenotypic output than is observable with the titration of single effectors.

Discussion

We report here that PROCISiR can control switching behavior and provide graded, proportional control over two cellular outputs at once. The control modalities in PROCISiR can be used to manipulate mammalian cellular processes and potentially engineer drug-regulated cell therapies.

The main challenge in developing PROCISiR was the generation of two CID systems, which we accomplished through the de novo design of protein readers that selectively recognize distinct inhibitor-bound states of NS3a. To address the challenge of generating proteins that selectively recognize protein–small molecule complexes, we applied Rosetta protein design to highly stable de novo scaffolds. The specificity achieved by the design and engineering process relied only on the chemical differences between inhibitors, as there is little-to-no rearrangement of NS3a in different inhibitor complexes[23]. Thus, our design strategy for generating protein-based readers should be scalable to other clinically approved NS3a inhibitors, allowing the development of additional orthogonal PROCISiR components[34]. Our computational approach for generating protein-based readers is complementary to a phage display-based selection strategy that was recently used to identify an antibody fragment that selectively binds to a complex of the anti-apoptotic protein Bcl-x_L bound to the small molecule ABT-737 (ref. [15]). While both strategies are capable of generating high-affinity readers of protein–small molecule complexes, the highly stable DHRs used in our computational approach are better suited than antibody fragments for intracellular synthetic biology applications[20].

CID systems have been used in numerous applications and are the first post-translational control module to enter the clinic in the form of inducible caspase 9 suicide switches for cell therapies[35,36]. All current CID systems use small molecules that either have off-target effects in mammalian systems or do not behave well in vivo[12–15,37]. Thus, the ability to modulate PROCISiR with clinically approved drugs that are orthogonal to mammalian systems makes it a promising replacement for single-input/single-output CID systems that are currently being explored for ex vivo and in vivo cellular therapies, including improving the safety of therapeutic chimeric antigen receptor T-cells. One limitation of PROCISiR and any CID system is that the constitutive presence of a regulated protein activity in the cell may lead to higher background than desired for some applications. The availability of ANR to basally localize a protein activity fused to NS3a away from a site of action, or to act as an intramolecular regulatory switch, provides an appealing option for reducing background, but protease-based NS3a control systems offer the possibility of obtaining the complete absence of a protein activity in the cell before the addition of a small molecule input[10,11,27,38]. However, the reduced background that can be obtained with protease-based systems comes at the expense of speed, as protein accumulation is required for activation. Furthermore, we have found that catalytically dead NS3a is an equally effective component of PROCISiR as the active protease, which is advantageous for applications in which off-target proteolytic activity may be a concern.

The emergent properties arising from the multi-input/multi-output architecture of PROCISiR enable new types of synthetic cellular behaviors including graded and proportional control of two-or-more outputs in a temporally regulated manner. Using single or combined drug dosing, we can tune our output response from the switch-like response of bimolecular binding, to a more graded, linear response. Binary, switch-like responses allow digital logic operations to be performed, while graded, analog responses are useful for the fine-tuning of gene expression in metabolic engineering applications or studies of small changes in gene expression or signaling outputs[4,39]. Combination of multiple chemical inputs with our readers enabled multi-state control that could be used to create dynamic signaling or transcriptional programs centered around the NS3a receiver hub. We demonstrated combinatorial outputs in the form of proportional transcriptional and signaling control, a form of ratiometric computation that cells have been shown to perform in responding to competitive ligands such as galactose/glucose and BMP ligands[1,40]. When paired with single-cell transcriptomics or high-throughput microscopy, the fine-scale manipulations achieved with PROCISiR are well suited for addressing questions related to single-cell decision making. The ability to either orthogonally or proportionally control two outputs, as we demonstrate with Rac and Rho signaling, makes PROCISiR a versatile system for investigating and creating new regimes of signaling pathway cross-talk. We envision that the expanded post-translational control behaviors that can be achieved with PROCISiR will facilitate new investigations into basic cellular mechanisms and the engineering of unique cellular behaviors. Furthermore, PROCISiR is well suited for integration with engineered synthetic systems that respond to intrinsic inputs such as synNotch, a system that enables transcriptional control in response to recognition of extracellular ligands[41]. The combination of small molecule-based, extrinsic control offered by PROCISiR with intrinsic, cell-autonomous decision making is an attractive approach for enabling safer and more effective cell therapies. The robust, rapid, multi-input/multi-output control offered by PROCISiR makes it a useful addition to the growing set of tools for post-translational control of in vitro and in vivo mammalian synthetic biology.

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