SUPPLEMENTAL METHODS

Tunable Protease-Activatable Virus Nanonodes

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Virus Titer and Heparin Affinity

Viruses titers (used to determine genomic multiplicity of infection, gMOI) were quantified by qPCR and heparin affinity chromatography performed as previously described. [29]

Silver Stains and Western Blots

For silver stains, virus samples were reduced, alkylated, denatured in LDS buffer, resolved on 12% NuPAGE gels, and stained with Silverquest staining kit according to manufacturer's instructions (Life Technologies, Carlsbad, CA). Due to their similarly large sizes, N-terminal fragments (Fig. S5e, f) required extended electrophoresis run times (~9 hours at 100 volts) to achieve sufficient resolution between large and small N-terminal VP3 fragments ($S_d(n)$ and $S_u(n)$ + $S_{ud}(n)$, respectively). Images were obtained with an Epson scanner, followed by background subtraction in Image J (National Institutes of Health, Bethesda, MD) and densitometric analysis in Image Studio Light (LI-COR Biosciences, Lincoln, NE). Western blots were performed as previously described. [29] Full-range rainbow marker (GE Healthcare, Pittsburgh, PA) was used to estimate apparent molecular weight in Fig. 1d and Fig. S2.

Kinetic Modeling and Transfer Function

To determine the transfer function of PAV activation in response to proteolytic cleavage, PAV1 (180 nM VP) was treated with MMP-9 (100 nM) and the reaction was stopped at different timepoints. The fraction of peptides removed from PAV1 was determined by quantitative silver stain (Figs. S5, S6). To measure virus activity A(UL), HEK293T cells were transduced (gMOI = 1000) with respective virus samples, and relative transduction index (rTI) was obtained by flow cytometry.

Since direct measurement of S_{ud} (UL) was not feasible, the quantified silver stain data was fit to a mathematical model of PAV-protease kinetics to obtain estimates of UL at each timepoint. The model consists of the following reactions:

$$E + S \underset{k_{b1}}{\overset{k_{f1}}{\rightleftharpoons}} ES_1 \xrightarrow{k_{c1}} E + S_u \tag{3}$$

$$E + S \underset{k_{b2}}{\overset{k_{f2}}{\rightleftharpoons}} ES_2 \xrightarrow{k_{c2}} E + S_d \tag{4}$$

$$E + S_u \underset{k_{b3}}{\overset{k_{f3}}{\rightleftharpoons}} ES_3 \xrightarrow{k_{c3}} E + S_{ud} \tag{5}$$

$$E + S_d \underset{k_{b4}}{\overset{k_{f4}}{\rightleftharpoons}} ES_4 \xrightarrow{k_{c4}} E + S_{ud} \tag{6}$$

where E and S represent the enzyme (protease) and substrate (PAV), respectively; S_u, S_d and S_{ud} represent the substrate cleaved at upstream, downstream and both sites, respectively. ES_i ($i \in 1\text{-}4$ and hereafter) represent the complexes between the enzyme and the different forms of the substrate. k_{fi}, k_{bi} and k_{ci} represent the forward, backward and catalytic rates of each reaction.

We assume that the various complexes in the model are at a quasi-steady state (*i.e.* their concentrations do not change in the time scale of product formation). This results in the following expression for the concentration of complexes:

$$(ES_i) = \frac{k_{fi}}{k_{bi} + k_{ci}}(E)(S_i) \tag{7}$$

The above biochemical reactions (3) – (6) give rise to the following differential equations:

$$\frac{d(S_u)}{dt} = k_{c1}(ES_1) - k_{f3}(E)(S_u) + k_{b3}(ES_3) \tag{8}$$

$$\frac{d(S_d)}{dt} = k_{c2}(ES_2) - k_{f4}(E)(S_d) + k_{b4}(ES_4) \tag{9}$$

$$\frac{d(S_{ud})}{dt} = k_{c3}(ES_3) + k_{c4}(ES_4) \tag{10}$$

$$\frac{d(S)}{dt} = -k_{f1}(E)(S) - k_{f2}(E)(S) + k_{b1}(ES_1) + k_{b2}(ES_2) \tag{11}$$

Substituting the expression for complexes from equation (7) gives rise to the following differential equations:

$$\frac{d(S_u)}{dt} = \frac{k_{c1}}{K_1}(E)(S) - \frac{k_{c3}}{K_3}(E)(S_u) \tag{12}$$

$$\frac{d(S_d)}{dt} = \frac{k_{c2}}{K_2}(E)(S) - \frac{k_{c4}}{K_4}(E)(S_d) \tag{13}$$

$$\frac{d(S_{ud})}{dt} = \frac{k_{c3}}{K_3}(E)(S_u) + \frac{k_{c4}}{K_4}(E)(S_d) \tag{14}$$

$$\frac{d(S)}{dt} = -\frac{k_{c1}}{K_1}(E)(S) + \frac{k_{c2}}{K_2}(E)(S) \tag{15}$$

where parenthesized terms represent concentration of various species, and $K_i = \frac{k_{bi} + k_{ci}}{k_{fi}}$ represents the Michaelis-Menten constants.

Furthermore, conservation of mass gives rise to the following relation for the total amount of enzyme in the system (E_0):

$$E_0 = (E) + (ES_1) + (ES_2) + (ES_3) + (ES_4) \tag{16}$$

Substituting the expressions for (ES_i) from equation (7) we obtain:

$$(E) = \frac{E_0}{1 + \frac{(S)}{K_1} + \frac{(S)}{K_2} + \frac{(S_u)}{K_3} + \frac{(S_d)}{K_4}} \tag{17}$$

We next converted the concentrations in our model (X) to a normalized form (x) by dividing them by the total amount of substrate in the system (S_0). This normalization also resulted in dimensionless parameters $K_i^* = \frac{K_i}{S_0}$ and $e_0 = \frac{E_0}{S_0}$.

The resulting mathematical model with 9 parameters (k_{ci}, K_i^* and e_0, $i \in 1\text{-}4$) was simulated with the initial condition ($s = 1, s_u = s_d = s_{ud} = 0$) using *ode15s* in MATLAB (The MathWorks Inc., Natick, MA). The simulated curves (lines) were fit to the experimental data (circles, Fig. S6a) by minimizing the sum of square of errors using particle swarm optimization (Soren Ebbesen et al., 2012). The results from the best fit (in yellow) and the top 100 fits are shown in Fig. S6a, b. The model prediction for fraction unlocked (s_{ud} , green curves) dynamics was used in conjunction with transduction index dynamics (cyan circles in Fig. S6c) to get the transfer function (brown circles in Fig. S6d). The transfer function for the best fit case (yellow circles in Fig. S6d) was fit with a Hill function.

Note that a Hill function is typically of the following form:

$$y = \frac{ax^n}{K^n + x^n} \tag{18}$$

where a is the maximum value that y can achieve, n is the Hill coefficient and K is the value of x at which y is half of its maximum value.

However, in our case by definition the maximum virus activity (A(UL)=1) is achieved only when all the peptide locks are cleaved (UL=1). Therefore, we constrained the above Hill function to pass through x=1, and y=1 to obtain:

$$a = K^n + 1 \tag{19}$$

Substituting this expression back into Equation (18) we arrive at the following Hill function with only two parameters, K and n:

$$y = \frac{(K^n + 1)x^n}{K^n + x^n} \tag{20}$$

The above Hill function was used for fitting the transfer function for the best fit case (yellow circles in Fig. S6d), where y = A(UL) and x = UL. The corresponding fit (black line in Fig. 3a) was obtained using the *fit* function in MATLAB and resulted in parameter values: $K = 0.85, n = 5.8.$

In the optimization, the various parameters in the model were picked from the bounds listed in the following table:

Parameter	Lower bound	Upper bound
$k_{ci}\,(i \in 1-4)$	0.01	100
$K_i^*\,(i \in 1-4)$	0.01	100
e_0	0.01	0.1

Methods Table 1. Parameter bounds for optimization.

Mosaic PAV Predictions

In Figure 4, to predict the relative activity of mosaic PAVs in response to different combinations of MMP -7 and MMP-9 concentrations, the experimentally determined activity response surface of the single subunit type PAVs were used to predict the fraction of unlocked peptides for each subunit type. This was done using the Hill function (Equation 2) with parameters fitted for PAV1 (above; K = 0.85, n = 5.8), assuming PAVs 10 and 12 followed a similar transfer function of PAV activity with respect to fraction unlocked. The total fraction of unlocked peptides (UL) for mosaic PAVs was then computed by equation 1 using the fraction unlocked of each subunit type as inputs I_i and appropriate weights. Using this adjusted fraction unlocked (UL), predicted relative PAV mosaic activity was then computed by Eqn 2.

Molecular Model of PAV

The PAV model was generated by submitting the AAV2 VP3 sequence, with the inserted peptide sequence included after residue 586, to the SWISS MODEL online 3D modeling program (Kiefer et al., 2009) with the AAV2 VP3 structure coordinates (PDB ID # 1LP3) supplied as a template. The resulting chimeric model was used to generate the 60-mer capsid coordinates by icosahedral matrix multiplication at the VIPERdb website (http://viperdb.scripps.edu/). The 60-mer coordinates was used to generate the figures using the PyMol program (http://www.pymol.org)

METHODS REFERENCE

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Kiefer F, Arnold K, Kunzli M, Bordoli L, Schwede T. 2009. The SWISS-MODEL repository and associated resources. Nucleic Acids Res. **37:**D387–392.