Dynamical thermal dose models and dose time-profile effects.

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Supplemental material

S1  |  HEAT SHOCK RESPONSE NETWORK (HSRN)

S1.1  |  Mathematical description by mass action law of the HSRN

The description of the heat-shock response network (Fig. S1) involves the transcription factor Heat Shock Factor 1 (HSF1), the chaperone proteins of the Heat-Shock Protein (HSP) family, the sequestration complex (HSF1:HSP), the Misfolded Protein (MFP) the chaperone complex (MFP:HSP) and the cellular pool of protein (P).

Denaturation rate \( \kappa_d(T) \), is approximated in the 37–45°C range from [1] by:

\[
\kappa_d(T) = k_d \left( 1 - 0.4e^{37-T} \right) 1.4^{T-37}
\]  

where \( T \) is the temperature in °C. The protein refolding process is described by a Michaelis–Menten kinetics to stand for limited energy resources. The denaturation rate is the only input pathway of temperature in the network.

The HSRN involves diverse regulatory processes of transcription, degradation, multimerization, denaturation and renaturation which can be formulated as a set of biochemical reactions (Tab. S1), which lead to a system of ordinary differential equations with 6 variables and 17 parameters (Tab. S2)

In Tab. S1, the parameters \( \delta_u \) are the linear degradation rates, \( K_{\text{u}u} \) the kinetic constant for heterodimerization, \( \mu_u \) the basal transcription rates (\( u \) refers to any chemical species). The parameter \( P_0 \) defines the threshold of regulation and \( \mu_{\text{HSP}} + \lambda_{\text{HSP}} \) is the maximal transcription rate of \( \text{HSP} \).
S1.2  |  Cell population model

The hyperthermia impact on cellular viability is described via a population model in which the variable $N(t)$ denotes the number of viable and proliferating cells at time $t$. In a normal cell culture condition, $N$ is assumed to grow exponentially in time due to the proliferation rate $\gamma$. In a hyperthermia condition, these viable and proliferating cells can transit into a death or non-proliferating state with a rate $\kappa$:

$$\kappa(t) = \alpha \left( [\text{MFP}](t) + [\text{MFP} : \text{HSP}](t) \right) + \beta \left( 1 - \frac{[P](t)}{P_{\text{tot}}} \right)$$  \hfill (S2)

Eq. S2 is identical to Eq. 4 in the main text as $[P^*](t)$ is equal to the total MFP concentration $[\text{MFP}](t) + [\text{MFP} : \text{HSP}](t)$. The balance between proliferation and stress-induced cell-cycle arrest and death leads to the following ordinary differential equation governing the time evolution of the number of viable and proliferating cells:

$$\frac{dN}{dt} = \gamma N(t) - \kappa(t) N(t)$$  \hfill (S3)

The dynamical model of the survival response investigated in this paper consists of the population equation Eq. S3 and the HSRN model published in [2] and described in Tab. S1 with parameter values of Tab.S2. The survival probability due to hyperthermia is estimated by normalizing the number of viable and proliferating cells $N$ to the value obtained in cell culture condition (without hyperthermia) leading to Eq. 5 in the main text.
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FIGURE S2  Results of cell counting after a hyperthermia protocol. Cells were counted under microscope using a cell counting software, where viable cells (attached cells without PI and AV), PI cells, AV cells and PI+AV cells are distinguished. Time 0 refers to the moment before applying the hyperthermia protocol. Experiments were run in duplicates for three hyperthermia protocols: Control at 37°C (A), fast-rise/slow-decay hyperthermia protocol displayed as a black line in Fig. 2A (B), and slow-rise/fast-decay hyperthermia protocol displayed as a blue line in Fig. 2A (C).
We consider the following trapezoidal temperature profile \( T(t) = A_i t + B_i \), where \( i = 1, ..., 4 \) represent time intervals according to the following equation:

\[
T(t) = \begin{cases} 
\frac{T_{\text{max}} - 37}{t_1} t + 37 & 0 \leq t < t_1 \\
\frac{T_{\text{max}}}{\tau - t_2} t + \frac{T_{\text{max}} - 37 t_2}{\tau - t_2} & t_1 \leq t < t_2 \\
\frac{37 - T_{\text{max}}}{\tau - t_2} (t - t_2) & t_2 \leq t < \tau \\
37 & \text{otherwise}
\end{cases}
\]

(S4)

\( 0 \leq t_1 < t_2 < \tau \) are the times of the temperature signal that define intervals linearly increasing, being constant, and linearly decreasing, respectively. \( T_{\text{max}} \) is the maximum temperature and it is determined such that the equivalent CEM43 thermal dose is fixed regardless of the values \( t_1, t_2, \) and \( \tau \). Additionally, we introduce parameters \( \eta \) and \( \sigma \) to transform \( t_1 \) and \( t_2 \).

\[
\eta = \frac{t_1 + t_2}{2\tau}, \quad \sigma = \frac{t_2 - t_1}{\tau}
\]

(Figure S3) A general temperature profile and CEM43 dose. Black lines represent the temperature profile with maximum temperature \( T_{\text{max}} \), time duration \( \tau \), increasing time \( t_1 \), and decreasing time \( \tau - t_2 \). The gray shaded area is a corresponding CEM43 dose.

The CEM43 thermal dose value, e.g. integral

\[
\text{CEM43} = \int_{t=0}^{\tau_{\text{final}}} R^{T_c - T(t)} \, dt \quad \text{where} \quad \begin{cases} 
R = r_1 & T < T_c \\
R = r_2 & T > T_c 
\end{cases}
\]

is analytically solvable for the type of stress profile defined by S4:

\[
\text{CEM43} = \begin{cases} 
N_1^{T_{\text{max}}} \tau \sigma + N_2^{T_{\text{max}} - 37} \left( \frac{N_1^{T_{\text{max}} - 37} - N_1^{T_{\text{max}}}}{\ln r_1} \right) + N_3^{T_{\text{max}} - 37} (t - \tau) & T_{\text{max}} < 43 \\
N_2^{T_{\text{max}} - 37} \tau \sigma + N_3^{T_{\text{max}} - 37} \left( \frac{N_2^{T_{\text{max}} - 37} - N_2^{T_{\text{max}} - 37}}{\ln r_2} \right) + N_4^{T_{\text{max}} - 37} (t - \tau) & T_{\text{max}} \geq 43 
\end{cases}
\]

(S6)

For a fixed CEM43 dose and a fixed exposure duration \( \tau \), the maximum temperature \( T_{\text{max}} \) depends only on \( \sigma \). Therefore, parameter \( \sigma \) is a measure of the maximum temperature for a given CEM43, whereas parameter \( \eta \) is a measure of the speed of a temperature change. The smaller the \( \eta \), the faster the increase and the slower the decrease of the temperature.
DOSE-ASYMMETRY EFFECT DOES NOT SIGNIFICANTLY DEPEND ON \( \alpha \) AND \( \beta \) PARAMETER VALUE

**FIGURE S4** Dose-asymmetry effect does not significantly depend on \( \alpha \) and \( \beta \) parameter value (A) RMLSE value along the valley of the score landscape showed in Fig. 1C of the main text as a function of \( \alpha \) and \( \beta \) parameter, RMLSE value is indicated by the color bar. (B) Model predicted survival ratio, defined as the ratio of survival response between slow–fast (\( \sigma = 0, \eta = 1 \)) and fast–slow (\( \sigma = 0, \eta = 0 \)) thermal profile similar as Fig. 3C of the main text computed for 3 hours exposure heat stress (\( \tau = 3 \) h) triangular time profile having an identical cumulative equivalent minute at 43°C value of 180 CEM43 \( \tau = 3 \) h. Survival ratio value is indicated by the color bar.
FIGURE S5  Regulated transcription in HSRN plays no role in dose-asymmetry effect The survival ratio in the (CEM43,τ)–space computed numerically with the HSRN model where the active transcription process of HSP $\mu_{\text{HSP}} + \lambda_{\text{HSP}} \frac{[\text{HSF1}]^3}{[\text{HSF1}]}$ is fixed to its value in cell culture condition at 37°C. The active transcription process has no significant influence on the dose-asymmetry effect as the results are similar to those displayed in Fig. 3C of the main text.
Starting from HSRN model consisting of Tab. S1, we perform a model reduction by assuming in the first step that (i) all protein species except MFP have similar degradation rate e.g. \( \delta_f = \delta_{\text{HSF}1} = \delta_{\text{HSP}} = \delta_{\text{MFP}:\text{HSP}} \); (ii) HSF1 and HSP total protein pools are constant, in fact HSF1 does not vary significantly upon heat stress [2] and the regulated transcription of HSP does not significantly influence the survival response; (iii) the variation of the native protein pool due to heat induced misfolded proteins is negligible [2]; (iv) the renaturation rate is in a linear regime e.g. \( K_M \gg [\text{MFP} : \text{HSP}] \). Doing so, one arises with the following dynamical model

\[
\begin{align*}
\frac{d}{dt}[\text{MFP}] &= \kappa_d(T) - K^+_{\text{MFP}:\text{HSP}} \cdot [\text{HSP}] \cdot [\text{MFP}] - \delta_{\text{MFP}} [\text{MFP}]; \\
\frac{d}{dt}[\text{MFP} : \text{HSP}] &= K^+_{\text{MFP}:\text{HSP}} \cdot [\text{HSP}] \cdot [\text{MFP}] - \kappa_r \cdot \left[ \frac{[\text{MFP} : \text{HSP}]}{K_M} \right] \cdot [\text{MFP} : \text{HSP}]; \\
\frac{d}{dt}[\text{HSF}1] &= \mu_{\text{HSF}1} - K^+_{\text{HSF}1:\text{HSP}} \cdot [\text{HSP}] \cdot [\text{HSF}1] + \kappa_{\text{HSF}1:\text{HSP}} \cdot [\text{HSP}] \cdot [\text{HSF}1] - \delta [\text{HSF}1]; \\
\frac{d}{dt}[\text{HSP}] &= \kappa_{\text{HSP}} - K^+_{\text{HSF}1:\text{HSP}} \cdot [\text{HSP}] \cdot [\text{HSF}1] + \kappa^-_{\text{HSF}1:\text{HSP}} \cdot [\text{HSP}] \cdot [\text{HSF}1] - \delta \cdot [\text{HSP}].
\end{align*}
\]

(S7)

where \( \kappa_{\text{HSP}} \) is the constant synthesis rate of HSP protein.

The next step is to reduce the number of differential equations by assuming the conservation of the HSP and HSF1 number of copies. Let us denote by \([\text{HSF}1]_T, [\text{HSP}]_T, \text{ and } [\text{MFP}]_T \) the total concentration of HSF1, HSP, and MFP e.g.

\[
\begin{align*}
[\text{HSF}1]_T &= [\text{HSF}1] + [\text{HSP} : \text{HSF}1] \\
[\text{MFP}]_T &= [\text{MFP}] + [\text{HSP} : \text{MFP}] \\
[\text{HSP}]_T &= [\text{HSP}] + [\text{HSP} : \text{MFP}] + [\text{HSP} : \text{HSF}1].
\end{align*}
\]

then

\[
\begin{align*}
\frac{d}{dt}[\text{MFP}]_T &= \kappa_d(T) - \kappa_d(T^*) \cdot [\text{MFP} : \text{HSP}] - \delta_{\text{MFP}} [\text{MFP}]; \\
\frac{d}{dt}[\text{HSF}1]_T &= \mu_{\text{HSF}1} - \delta [\text{HSF}1]_T; \\
\frac{d}{dt}[\text{HSP}]_T &= \mu'_{\text{HSP}} - \delta [\text{HSP}]_T.
\end{align*}
\]

(S8)

For clarity, we introduce in Eq. S8 a new temperature parameter \( T^* \), defined such as \( \kappa_d(T^*) = \kappa_r/K_m \). In model S8, \([\text{HSF}1]_T \text{ and } [\text{HSP}]_T \) converge to a constant value that can be taken as a new constant parameter.

The last step is to derive analytical expressions of \([\text{MFP} : \text{HSP}] \) and \([\text{MFP}] \) as mathematical functions of \([\text{HSF}1]_T, [\text{HSP}]_T, \text{ and } [\text{MFP}]_T \) in order to obtain a closed system. Adiabatic elimination of dimer assembly and dis-assembly as detailed in the next paragraphs performs the last step.

Let A and B be two proteins, the equilibrium equation of reversible complex formation reaction \( A + B \leftrightarrow A : B \) is
written \([A : B] \equiv [A] \times [B]\) where \(k_0\) is a balance concentration. Straightforwardly, one gets

\[
[A : B] = \frac{a + b - k_0}{2} \left\lfloor 1 - \sqrt{1 - \frac{4ab}{(a + b + k_0)^2}} - 1 \right\rfloor
\]

where \(a = [A] + [A : B]\) and \(b = [B] + [A : B]\) stand for the total concentration of protein species A and B. If now the chemical species concentrations \(a\) and \(b\) dominate the equilibrium concentration \(k_0\) \((a + b \gg k_0)\), parameter free rational functions approximate the concentration at equilibrium:

\[
[A : B] \approx \frac{ab}{a + b}
\]

\[
[A] \approx \frac{a^2}{a + b}
\]

\[
[B] \approx \frac{b^2}{a + b}
\]

To reduce the mathematical model of the cellular heat shock response network, we consider that the hetero-dimer assembly and disassembly follow the equilibrium relation at equilibrium for a given pool of MFP, HSP, and HSF1, in an ordered reaction chain: firstly HSP binds MFP and secondly HSF1 binds the remaining free HSP pool.

Once applied to the dominant hetero dimer reaction \(HSP + MFP \rightarrow MFP : HSP\) the adiabatic elimination gives

\[
[MFP] = \frac{[MFP]^2_T}{[MFP]_T + [HSP]_T}
\]

\[
[MFP : HSP] = \frac{[MFP]_T [HSP]_T}{[MFP]_T + [HSP]_T}
\]

\[
[HSP] = \frac{[HSP]^2_T}{[MFP]_T + [HSP]_T}
\]

for the concentration of misfolded proteins in the free form, the hetero dimer MFP:HSP, and HSP in the free form before HSF1 binding.

Under those approximations, the HSRN is described by a single differential equation

\[
\tau_d \frac{dP^*}{dt} = f(T(t)) - P^* \frac{P^*(t)}{P^*(t) + 1} - f(T^*) \frac{P^*}{P^* + 1}
\]

(S9)

where \(P^* \equiv [MFP]_T/[HSP]_T\), and \(\tau_d \equiv 1/\delta_{MFP}\). We also simplify the mathematical expression of the temperature dependent denaturation rate by taking \(f(T(t)) = k_d 1.4^{T(t)-37}\) that coincides up to a scale factor with Eq. S1 when temperature increases.
In this section, we investigate a simplified model of the survival response, based on the reduced dynamical model of the HSRN Eq. S9. The aim is to identify the key mechanism accounting for dose-asymmetry effect in the survival response.

The simplified model S10 describes the dynamics of damage and damage accumulation, where damage stands for misfolded proteins, and damage accumulation leads to a cell death:

\[ \tau_d \frac{d}{dt} = f(T(t)) - \frac{d^2(t)}{d(t) + R} - f(T^*) \frac{d(t)}{d(t) + R} \]  \hspace{1cm} (S10)

\[ r = \frac{d}{dt} \]  \hspace{1cm} (S11)

\( d \) represents a damage variable, and \( r \) accumulation of damage. In Eq. S10 the first term on the right hand side corresponds to the damage production, second term corresponds to the degradation of the damaged proteins due to repair, and the third term corresponds to the renaturation of damaged proteins. Parameter \( \tau_d \) is the half life of the damage \( d \) and will be considered as a referent time scale e.g. \( \tau_d = 1 \), and parameter \( R \) determines the strength of the repair mechanism.

To facilitate analytical computation, the denaturation rate Eq. (S1) is replaced in Eq. S10 by the approximation:

\[ f(T(t)) = k_d 1.4^{T(t) - 37} \]  \hspace{1cm} (S12)

where \( T(t) \) represents temperature profile. \( k_d \) is a basal rate of damage production at \( T = 37^\circ C \); \( T^* \) is a threshold temperature and it is set to 42.5 in our model. \( k_d \) is set to 100.0 and \( R \) to 1 unless specified differently.

The model given by Eq.S10 includes two different fates for damage that come from misfolded protein degradation \( \left( \frac{d^2(t)}{d(t) + R} \right) \) or misfolded protein renaturation \( \left( f(T^*) \frac{d(t)}{d(t) + R} \right) \). To evaluate the respective contribution of these two processes, we also consider a model without a term for MFP degradation:

\[ \tau_d \frac{d}{dt} = f(T(t)) - f(T^*) \frac{d(t)}{d(t) + R} \]

\[ r = \frac{d}{dt} \]  \hspace{1cm} (S13)

In the following, all calculations are derived in the case of trapezoidal temperature profile (Eq S4). The accumulation of damages at time \( t : r(t) = \int_0^t d(u)du \) is taken as a measure of the lethal effect.
S7.1 | Dose–asymmetry effect in the reduced model

The accumulation of damage that determines the probability of the cell survival can strongly depend on \( \eta \) and \( \sigma \), see Fig. S6(A), which contradicts an assumption that the damage induced by stress is only dose dependent. In Figs. S6(A) and (B) we calculate \( r(\tau) \) for different \( \eta \) and \( \sigma \), fixed \( \tau = 180 \) min and 180CEM43. For each \( (\eta, \sigma) \)-pair \( T_{\text{max}} \) is calculated from analytical expression of CEM43.

Figure S6(A) depicts numerical results of damage accumulation obtained with the complete reduced system Eq. S10, whereas Fig. S6(B) depicts numerical results of damage accumulation obtained without the degradation term Eq. S13. Qualitatively, the two figures look similar, there is a maximum of \( r \) in the middle of the \( \eta = 2\sigma \) line with a gradual decrease toward the triangle corners. The values of damage accumulation are, expectedly, higher in the reduced system Eq. S13 than in the complete one Eq. S10 since there is no additional degradation.

\[ \int dt \text{ for } \tau = 180 \text{ min}, \ CEM43 = 180 \text{ min, and } \tau' = 2 \text{ weeks}. \]  
\[ \int dt \text{ different } \tau \text{ (points) and two different CEM43 values, 180 min (solid line) and 5 min (dashed line). Black curves correspond to the complete system Eq. S10, and red to Eq. S13. Stress durations for which the maximum is not at the (1/2,1) point are between 74 (90) min and 3594.6 min for 180 CEM43 complete system Eq. S10 (Eq. S13), and between 7.8 min and 99.0 min for 5 CEM43 (both models).}

Figure S6(C) depicts the position of the maximum for two values of cem43, 180 min (solid line) and 5 min (dashed line). Black lines correspond to the complete system, and red to the reduced. The maximum follows a trajectory originating in \( (\eta, \sigma) = (1/2, 1) \) (top of the triangle, a square pulse) and travels always across a left plane of the \( (\eta, \sigma) \)-space. Once it approaches \( (\eta, \sigma) = (1/2, 0) \) it goes rapidly back to \( (1/2, 1) \) point. The minimum is always on the \( \sigma = 2 - 2\eta \) line. The smaller the CEM43 the smaller the interval of \( \tau \) is needed for the maximum to go from \( (1/2, 1) \) and back to \( (1/2, 1) \), and the shorter the trajectory is, i.e. it covers a shorter path in \( (\eta, \sigma) \)-space, compare solid and dashed lines in Fig. S6(C).

Here again, we see similar behaviour of the complete Eq. S10 and degradation-free Eq. S13 systems. If we compare damage accumulation only for symmetric profiles, \( \sigma \) fixed and \( t_1 = \tau - t_2 \), there will always be more damage for faster increase of temperature.
In Fig. S7 we see how the patterns evolve by varying $\tau$ for a black solid line of the Fig. S6(C). Note that the difference between maximum and minimum value of $r$ depends on $\tau$. If the difference is very small, the asymmetry will not be experimentally measurable, and most likely biologically not relevant since even small noise or cell variability will make the asymmetry not visible. For small doses of CEM43, the difference in maximum and minimum is very small for all $\tau$, see Fig. S8. For larger doses, the difference can be significant. Both maximum and minimum reach the maximum value for $\tau$ a couple of times larger than $\tau = 60$ min. These are the typical values taken in experiments. The larger the $\tau$ the smaller the difference. This is due to the time scales, the slower the change of the temperature profile the easier it is for the system to adapt and reach the temporary fixed point which corresponds to the current value of $S$, i.e. if $S(\tau)$ changes slowly enough, $d$ follows the change exactly.

**FIGURE S7** Change of maximum of the damage accumulation as a function of stress duration $\tau$ in $(\eta, \sigma)$-space for a $180$ CEM43 doses. $\tau = 60, 168, 378, 1290, \text{and } 4410$ min, (A)-(E), respectively. This figures correspond to the solid black line in Fig. S6(C).

Beside the reduced system without degradation, we also made the same analysis for the reduced system with degradation but without renaturation. We find undetectable dependence on the shape of the stress profile, the difference of the maximum and minimum values is of the order of magnitude $10^{-6}$. 
FIGURE S8  Maximum and minimum curves as a function of pulse duration $\tau$ for low (A) and high (B) dose CEM43 and system S10, and low (C) and high (D) dose CEM43 and system S13. Small difference in the maximum and minimum of the damage accumulation will lead to an insignificant effect of asymmetry, only when the shaded area is large we expect an asymmetric effect that is measurable. The results indicate this is the case for $\tau$ up to two orders of magnitude larger than $\tau$ and CEM43 larger than 100 min.
Impact of non-linearity type on dose-asymmetry effect

As shown, the dose asymmetry effect is fully captured by the renaturation mechanism alone. The renaturation mechanism is here described as a non-linear saturation function to depict the finite number of chaperon protein copy. However, one can wonder whether the type of nonlinearity (saturation here) impacts or not the dose-asymmetry effect.

To answer this question, we perform a similar analysis on an even simpler model for damage induced by stress,

$$\tau_d \dot{d} = T(t) - d^e, \quad (S14)$$

where we assumed for simplicity a production of damage that is proportional to the temperature and a degradation of damage of the form $d^e$. The system of Eq. S14 is nonlinear for $e \neq 1$. In the case of a saturation–like nonlinearity ($e < 0.5$), the accumulated damage is in general smaller in the case of a fast increase than in the case of a slow increase of temperature, and then leads to higher survivability in the case of slow increase.

This is intuitively expected, if the time scales are of the same order of magnitude as $\tau$, fast increase doesn’t allow the system to reach the maximum temporary fixed point, while if the increase is gradual, the possibility exists. With the simplest possible type of nonlinearity, the asymmetry is generated only by competing time scales of the dynamics of $d$ and change of temperature $S$. To reproduce the asymmetry found in the detailed model and the experiments, it is necessary to have a special type of nonlinearity, a saturation function, commonly used in biological models, often derived from Michaelis-Menten kinetics.

However, using an exponential-like nonlinearity ($e > 1$), one finds an asymmetry effect but in the opposite direction than the one found in the detailed model and the experiments, e.g. the fast increase in temperature being less lethal than the slow one.
S7.3 | Doses–asymmetry for constant $T_{\text{max}}$

**FIGURE S9**  Ratio $\rho_S$ for the complete and the reduced system as a function of CEM43 or $T_{\text{max}}$ and $\tau$. There is a significant minimum for $\tau$ around $\tau$ and $T_{\text{max}} > T^*$. White lines in figures (A) and (C) represent constant $T_{\text{max}} = 46$ (solid) and 43 (dashed), and in figures (B) and (D) constant 180 CEM43 (solid) and 30 CEM43 (dashed).

The dose-asymmetry ratio $\rho_S$ develops a maximum where the duration of the pulse is of the same order of magnitude as the timescale of $d$ and $T_{\text{max}}$ close and above threshold temperature $T^*$ (Fig. S9(B)). The ($T_{\text{max}}, \tau$)-space can be divided in area where dose-asymmetry exist (measurable) and doesn’t exist (non measurable) (see Fig. S9(b)).

If $T_{\text{max}} < T^*$ then the reduced model Eq. S13 catches the dynamics of the complete system (Eq. S10) unless $\tau$ values are very small (Figs. S10(D)-(F)). In this last case, the thermal stress perturbs the system slightly out of the stationary state, less than the difference between the two systems, so the difference in the fixed points of the complete and the reduced systems doesn’t allow a good agreement. If $T_{\text{max}} > T^*$, we don’t expect agreement between the two systems. For $41 \lesssim T_{\text{max}} \lesssim 55$, the degradation and renaturation terms compete, degradation is still not strong enough to cancel the influence of the renaturation so the asymmetry becomes significant, Fig. S10(B).
**FIGURE S10** Time evolution of damage for the complete model Eq. S10 (black) and the degradation-free model model Eq. S13 (red) for three different $\tau$ value, $\tau = 10^{-5} \ll \tau_d$, $\tau = 1 = \tau_d$, and $\tau = 10^3 \gg \tau_d$. Top row corresponds to the maximum temperature $T_{\text{max}} = 45 > T^*$ and bottom row to $T_{\text{max}} = 39 < T^*$. Excellent agreement between the two systems are obtained for small $T_{\text{max}}$ and large enough $\tau$. 
S7.4 | Analytical expression for damage $d$

![Diagram of characteristic five regimes with respect to the dynamics of $d$ in the ($\tau - T_{\text{max}}$) space.](image)

**Figure S11** Diagram of the characteristic five regimes with respect to the dynamics of $d$ in the ($\tau - T_{\text{max}}$) space. Regime R4 (grey shaded area) corresponds to the parameter space where dose-asymmetry happens ($\rho_5$ significant greater than 1). Approximative analytical expressions of $d(t)$ are available for regimes R1, R3, and R5.

We identify five different regimes (Fig. S11) according to the dynamics of $d$:

- **R1:** $T_{\text{max}} \gg T^*$ and $\tau \ll \tau_d$, we separate the dynamics to the $\delta$-like pulse during $\tau$ and the exponential decay after;
- **R2:** $T_{\text{max}} \not\gg T^*$ and $\tau \ll \tau_d$, the dynamics after the pulse follows the exponential decay but the pulse is analytically overestimated and the asymmetry is present, but negligible;
- **R3:** $T_{\text{max}} < T^*$ and $\tau \geq \tau_d$, $d$ adapts to the change in temperature and follows a time dependent “fixed point”;
- **R4:** $T_{\text{max}} > T^*$ and $\tau \sim \tau_d$, strong asymmetry, dynamics cannot be analytically derived;
- **R5:** $T_{\text{max}} \gg T^*$ and $\tau \gg \tau_d$, the equations can be simplified by taking a $d \to \infty$ limit.
**S7.4.1 | Regime R1: \(T_{\text{max}} \gg T^*, \) and \(\tau \ll \tau_d\)**

In the \(\tau \to 0\) and \(T_{\text{max}} \to \infty\) limit we can assume that during the pulse the first term \(f(S(t))\) dominates. Taking the integral of the \(f(S(t))\) over the duration of the pulse \(\tau\) we can derive the value of \(d\) at time \(\tau\). After that, \(d\) decays exponentially to the fixed point at \(S = 37\). So the dynamics of \(d\) is

\[
d(t) = \frac{\tau k_d}{(T_{\text{max}} - 37) \ln(1.4)} \left(1.4^{T_{\text{max}} - 37} - 1\right) e^{-\lambda t} + d^*(S = 37)
\]

(S15)

where \(d^*(S = 37)\) is the fixed point of \(d\) at \(S = 37\) and \(\lambda\) its eigenvalue. Figure S12 shows excellent agreement of the numerical integration and analytically derived equation for \(d(t)\). Both ramps are shown, but the dynamics is identical so the curves overlap.

![Figure S12](image)

**Figure S12** Dynamics of the damage in the \(\delta\) function–like regime for \(\tau = 10^{-6}\) h and \(T_{\text{max}} = 60\). Numerical results are shown in black solid line and analytical, corresponding to the equation S15, are shown in dashed red.

**S7.4.2 | Regime R3: \(T_{\text{max}} < T^*, \) and \(\tau \geq \tau_d\)**

Below the threshold temperature \(T^*\), the change in the temperature is slow enough, even if \(\tau\) is of the same order of magnitude as \(\tau\), for \(d\) to adapt to the change, so for the two symmetrical stress profiles, the total accumulated damage is the same and the asymmetric effect is absent. For \(\tau \gg 1\), \(d\) always has enough time to adapt, regardless of the value of \(T_{\text{max}}\). This doesn’t hold for the reduced system since there is no degradation, for \(T_{\text{max}} > T^*\) the system diverges, there is no time dependent “fixed point” to which \(d\) can adapt.

The analytical expression for \(d\) is derived such that we assume that for every \(S(t)\) \(d\) reaches the value which corresponds to the fixed point \(d^*(S(t))\):

\[
f(S(t)) - \frac{d}{1 + d} (d + f(T^*)) = 0 \quad (\text{S16})
\]

\[
d^2 + d \left[T^* - f(S(t))\right] - f(S(t)) = 0 \quad (\text{S17})
\]

This gives

\[
d(t) = \frac{f(T^*) - f(S(t)) + \sqrt{[f(T^*) - f(S(t))]^2 + 4f(S(t))}}{2}
\]

(S18)

Agreement of the numerical and analytical results is presented in Fig. S13.
\textbf{FIGURE S13} Dynamics of the damage in the time dependent “fixed point” regime. Numerical results are shown in black solid line and analytical, corresponding to the equation S18, are shown in dashed red. For large $T_{\text{max}}$ and large $\tau$ the dynamics also agrees with a reduced equation S19.

\textbf{S7.4.3 | Regime R5: $T_{\text{max}} \gg T^*$, and $\tau \gg \tau_d$}

In the limit $T_{\text{max}} \gg T^*$, $\tau \gg 1$ the dynamics can be described with the previous analytical expression, or we can simplify the differential equation S10 assuming that for $T_{\text{max}} \gg T^* \rightarrow d \gg 1$. The simplified equation is

$$d = f(S(t)) - f(T^*) - d$$

(S19)

where we have taken the following limits

$$\frac{d}{1 + d} \xrightarrow{d \gg 1} 1 \quad \text{and} \quad \frac{d^2}{1 + d} \xrightarrow{d \gg 1} d$$

(S20)

For the decreasing ramp $t_1 = 0$ the solution of the equation S20 is

$$d(t) = Ce^{-t} - k_D \left(1.4^{T^* - 37} - \frac{1.4^{-\frac{T_{\text{max}} - 37}{T}}}{1 - \frac{T_{\text{max}} - 37}{T} \ln(1.4)}\right), \quad t \in \left(0, \frac{\tau}{2}\right).$$

(S21)

Integration constant $C$ is obtained from the initial conditions assuming $d(0) = d^*(T_{\text{max}})$.

$$C = d^*(T_{\text{max}}) + f(T^*) - \frac{f(T_{\text{max}})}{1 - \frac{T_{\text{max}} - 37}{T} \ln(1.4)}$$

(S22)

Solution for the increasing ramp is found by the time transformation $t \rightarrow \tau - t$. 

**FIGURE S14** Custom-built incubator. The incubator contains four culture dishes, one of the dishes hosts a thermistor immersed in culture medium to monitor the applied temperature. Inside the incubator humidity and atmosphere are regulated.
REFERENCES
