RESEARCH ARTICLE 11 NOVEMBER 2026

Reset kinetics shape HER2 capture during pulsed acid exposure

New York General Group, Inc.

Antibodies can be engineered to bind human epidermal growth factor receptor 2 (HER2) preferentially at acidic pH, and masking proteins can delay the recovery of antigen binding after acidification.[1,2] These observations raise a question that measurements at a fixed pH do not resolve: can an antibody distinguish continuous acid exposure from a series of shorter exposures of equal total duration? Here we examine this question using a kinetic simulation of a hypothetical anti-HER2 fragment that requires two successive activation steps before it can bind. We calibrated a one-step comparator so that both fragments had the same probability of capturing HER2 during 30 minutes of continuous acidity (32.33%). When exposure was divided into three 10-minute pulses and unbound fragments reset completely between pulses, capture fell to 8.79% for the two-step fragment and 17.33% for the comparator. With 10-minute neutral intervals, slowing the reset of the two-step fragment reversed this ranking. The decisive property was therefore not simply how long the fragment took to open, but how much progress towards opening it retained between pulses. The analysis identifies a measurable requirement for temporal discrimination in HER2 binding; it does not establish that tumour and non-malignant tissues supply distinguishable acid-exposure histories. (pmc.ncbi.nlm.nih.gov)

Sulea and colleagues engineered anti-HER2 antibodies with preferential binding at acidic pH and showed that an engineered antibody inhibited the growth of breast tumour spheroids at pH 6.4, with substantially less activity at physiological pH.[1] Biewenga and colleagues demonstrated a different mechanism: an engineered masking protein delayed the recovery of cell binding after acidification, including for masked trastuzumab at pH 6.0.[2] These studies distinguish two properties that need not coincide: the pH at which an antibody binds and the time it takes to become competent to bind. Neither study determines how far activation proceeds during a short acid pulse or how much of that progress survives a return to neutral pH. (pmc.ncbi.nlm.nih.gov)

We considered a hypothetical fragment, termed TD–HER2, that passes from a closed state through a binding-incompetent intermediate before its antigen-binding site becomes accessible. This intermediate has two opposing consequences. If it returns to the closed state between acid pulses, the next pulse must start the activation sequence again. If it persists, the next pulse starts with one step already complete. We asked which behaviour dominates and whether the resulting difference remains detectable when the comparator is matched for **antigen capture**, rather than merely for the fraction of binding sites open at the end of an exposure.

Matching sustained capture reveals a response to interruption

We compared one 30-minute acid exposure with three 10-minute acid pulses. Both fragments began closed. Once a binding site became accessible, it could capture HER2 at the same effective rate in both designs. We define *cumulative capture* as the fraction of the initial antibody fragments that had bound HER2 at least once. This quantity is distinct from both the fraction of fragments open at the endpoint and the fraction of receptors occupied.

For the principal comparison, we adjusted only the opening rate of the one-step fragment until its cumulative capture during continuous exposure matched that of TD–HER2. We then held this rate fixed. Both fragments captured HER2

with a probability of 32.33% during continuous exposure. Under complete reset between pulses, capture was 8.79% for TD–HER2 and 17.33% for the comparator: an absolute reduction of 8.54 percentage points, or 49.3% relative to the comparator (Table 1). The lower capture during pulsed exposure therefore cannot be attributed simply to a lower 30-minute continuous-exposure response. Matching this endpoint does not, however, imply that the two continuous binding curves coincide at earlier times.

Table 1 | Cumulative HER2 capture at matched continuous-exposure response

Acid exposure	Reset condition	Two-step TD–HER2	One-step comparator
One 30-min exposure	No intervening neutral interval	32.33%	32.33%
Three 10-min pulses	Complete reset of unbound fragments	8.79%	17.33%
Three 10-min pulses	10-min neutral intervals; reference reset rates	12.10%	19.08%
Three 10-min pulses	10-min neutral intervals; tenfold slower TD–HER2 reset	27.99%	19.08%

The HER2 capture rate was $b = 1/15$ min^{-1} throughout. The reference reset rate was 0.20 min^{-1} per closing step. In the last row, only the TD–HER2 reset rate was changed, to 0.02 min^{-1} per step. Previously captured fragments remained counted; capture during neutral intervals was excluded.

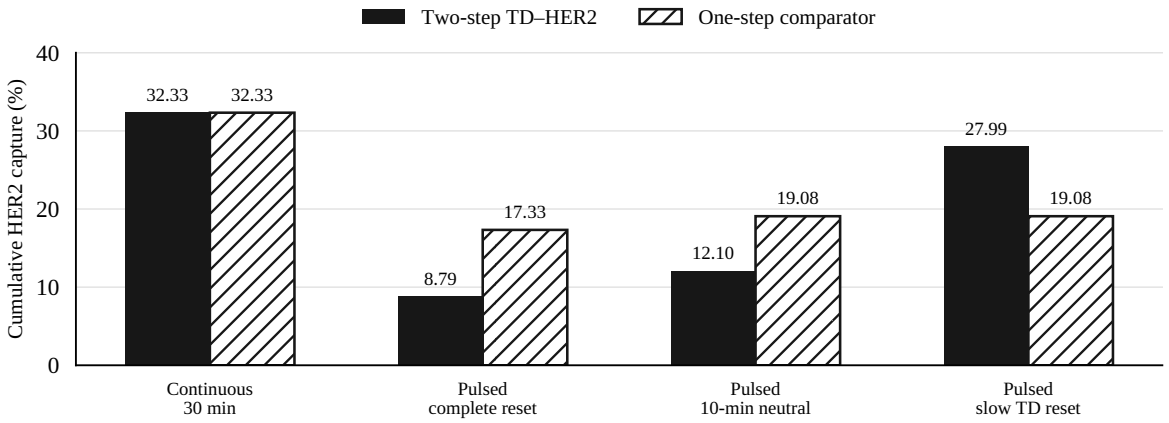


Fig. 1 | Simulated cumulative HER2 capture for the four schedules in Table 1.

The short-pulse behaviour explains the difference. Starting from a closed fragment, one-step activation followed by capture requires two successive events. At short times t, its capture probability is $qb\,t^2/2 + O(t^3)$, where q is the opening rate. TD–HER2 requires three events: two opening steps, each at rate k, followed by capture. Its probability is $k^2b\,t^3/6 + O(t^4)$. The additional power of t suppresses capture during short, freshly initiated pulses. It does not impose a sharp waiting time: even a short pulse has a non-zero probability of producing a binding event.

Closing the binding site is not the same as resetting the fragment

We next separated *accessibility* from capture by removing HER2 from the calculation. For this comparison, the one-step opening rate was recalibrated so that 59.40% of fragments in each design were open after 30 minutes of continuous acidity. Neutral intervals ranging from 2 to 20 minutes were then inserted between the three acid pulses (Table 2).

Table 2 | Binding-site accessibility after the final acid pulse

Neutral interval between pulses	Two-step TD–HER2	One-step comparator
0 min	59.40%	59.40%
2 min	43.79%	45.23%
5 min	29.96%	34.95%
10 min	20.02%	28.81%
20 min	15.27%	26.31%
Complete-reset limit	14.43%	25.95%

Both designs used the reference reset rate per step. Unlike Table 1, this comparison matches continuous **accessibility**, not continuous capture.

No receptor was present.

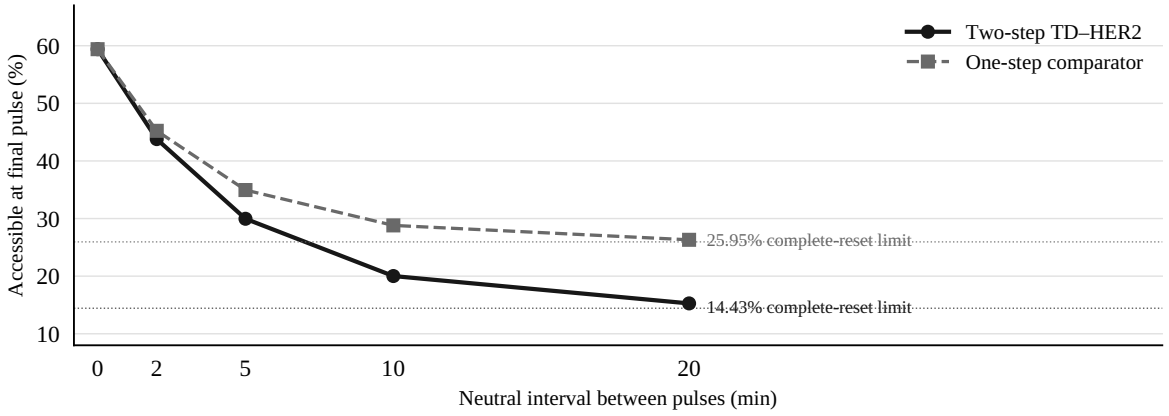


Fig. 2 | Receptor-free endpoint accessibility as a function of the neutral interval (Table 2).

With 2-minute interruptions, the difference in endpoint accessibility was only 1.44 percentage points. With 20-minute interruptions, it was 11.04 points, close to the complete-reset difference of 11.52 points. Thus, a long washout can make the designs appear readily distinguishable even when their responses to brief interruptions are nearly identical.

Slowing only the TD–HER2 reset rate tenfold produced a stronger effect. After three pulses separated by 10-minute neutral intervals, TD–HER2 accessibility rose from 20.02% to **50.78%**, whereas comparator accessibility remained at **28.81%**. To determine whether this reversal was restricted to the receptor-free readout, we repeated the finite-interval calculation with HER2 present and with the original **capture-matched** comparator. Cumulative TD–HER2 capture rose from 12.10% to **27.99%**, exceeding the unchanged comparator value of **19.08%** (Table 1). Thus, under the stated assumptions, retained activation could reverse the ranking for binding itself, not merely for accessibility.

The intermediate provides an experimental way to distinguish closure from reset. In a receptor-free acid challenge, let $A(t)$ and $I(t)$ denote the open and intermediate fractions. Immediately after acidification,

$$\frac{\mathrm{d}A}{\mathrm{d}t}(0^{+}) = kI(0).$$

A previously unexposed population starts entirely closed, so its initial opening slope is zero. A population that has lost accessibility during washout but retains intermediate-state occupancy has a positive initial slope on reacidification. Measuring the **second opening response**, rather than neutral-pH accessibility alone, would therefore test for residual activation.

Endpoint accessibility does not determine cumulative capture

The choice of calibration matters even when reset is complete. With the accessibility-matched comparator used in Table 2, both fragments were 59.40% open after 30 continuous minutes in the receptor-free calculation. Yet at $b = 1/15$ min^{-1}, continuous capture was 32.33% for TD–HER2 and 37.19% for the comparator (Table 3). Equal

final accessibility did not compensate for the additional time during which the one-step fragment had been available to bind.

Table 3 | Effect of capture rate when continuous accessibility is matched

Effective capture rate b	TD–HER2, continuous	One-step, continuous	TD–HER2, pulsed	One-step, pulsed
$1/30$ min^{-1}	20.52%	24.55%	4.84%	11.71%
$1/15$ min^{-1}	32.33%	37.19%	8.79%	20.40%
$1/5$ min^{-1}	49.19%	52.27%	18.96%	39.14%
Immediate capture on opening	59.40%	59.40%	37.34%	59.40%

Pulsed exposure comprises three 10-minute intervals with complete reset of unbound fragments between pulses. Capture rates are sensitivity parameters, not estimates from biochemical measurements.

The immediate-capture limit illustrates the distinction most clearly. After complete reset, 14.43% of TD–HER2 fragments were open at the end of a single 10-minute pulse in the absence of receptor. If every opening instead resulted immediately in capture, 37.34% of fragments would have bound across three pulses. The first number describes what is accessible **at the final measurement**; the second counts what was accessible **at least once**. An endpoint accessibility assay cannot substitute for the second measurement.

From molecular timing to tissue selectivity

The calculations do not assign continuous acidity to tumours or intermittent acidity to non-malignant tissues. To determine what such an assignment would require, let f_T and f_N denote the fractions of encounters following the continuous schedule in tumour and non-malignant tissue, respectively. Suppose that every other encounter follows the completely resetting pulsed schedule, and that delivery, receptor availability and binding kinetics are otherwise equal. Using the capture-matched results in Table 1, the additional tumour-minus-non-malignant capture difference attributable to the two-step design is

$$\Delta_{\text{two-step}} - \Delta_{\text{one-step}} \simeq 0.0854(f_T - f_N),$$

where capture is expressed as a fraction. Even if 60% of tumour encounters and only 40% of non-malignant encounters were continuous, the additional separation would be **1.71 percentage points**. If the exposure distributions were identical, it would be zero. These values are consequences of a two-schedule mixture, not estimates for either tissue.

The immediate experimental question is therefore whether a physical fragment can lose its activation history on the timescale of a relevant interruption. A paired acid challenge could answer it by measuring opening and HER2 capture separately, comparing the first and second responses over several neutral intervals, and matching the comparator for sustained capture before evaluating the pulsed condition. A faster second response after apparent closure would be evidence against complete reset. Even a successful molecular result would leave a separate biological question: whether fragments near accessible HER2 actually experience different acid-exposure histories in tumour and non-malignant tissue. Without that measurement, temporal discrimination is a property of the proposed binding mechanism, not evidence of therapeutic selectivity.

Methods

Kinetic assumptions

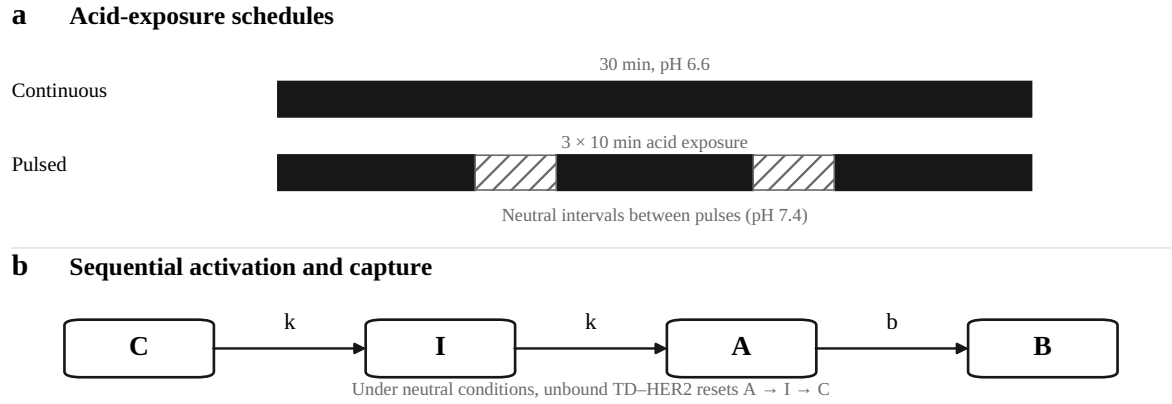


Fig. 3 | Acid-exposure schedules and the sequential TD–HER2 activation pathway.

TD–HER2 was described by the following first-order transitions during acid exposure:

$$C \xrightarrow{k} I \xrightarrow{k} A \xrightarrow{b} B,$$

where C is closed, I is binding-incompetent but partially activated, A is accessible and unbound, and B records a first HER2 capture. During a neutral interval, unbound fragments returned through $A \xrightarrow{r} I \xrightarrow{r} C$. The comparator followed $C \xrightarrow{q} A \xrightarrow{b} B$ during acid exposure and $A \xrightarrow{r_1} C$ during neutral exposure. All fragments started in C. Constant receptor availability made b a pseudo-first-order rate. No new binding was allowed during neutral intervals, and B was absorbing. The calculation therefore addresses first capture, not binding residence time, receptor occupancy, internalization or signalling.

Acid and neutral intervals were labelled pH 6.6 and 7.4, respectively. These labels specify the two simulated regimes; they do not constitute a measured pH–rate relationship. Rates were illustrative and were not fitted to the cited experiments. For elapsed-time-matched continuous controls, the neutral intervals were placed before the continuous acid exposure, when all fragments were closed.

Calibration and analytical checks

In the absence of receptor, the open fractions after uninterrupted exposure of duration t are

$$A_2(t) = 1 - e^{-kt}(1 + kt), \qquad A_1(t) = 1 - e^{-qt}.$$

At $k = 1/15$ min^{-1} and $T = 30$ min, matching $A_1(T) = A_2(T)$ gives

$$\begin{aligned} q_{\text{access}} &= k - \frac{\ln(1 + kT)}{T} \\ &= \frac{2 - \ln 3}{30\ \text{min}} \\ &\approx 0.03005\ \text{min}^{-1}. \end{aligned}$$

For the capture-matched comparison, $b = k$. The two-step fragment then requires three successive events of equal rate, giving the first-capture probability

$$P_2(t) = 1 - e^{-kt}\left(1 + kt + \frac{(kt)^2}{2}\right).$$

For the one-step comparator with $q \neq b$,

$$P_1(t) = 1 - \frac{be^{-qt} - qe^{-bt}}{b - q}.$$

Solving $P_1(T) = P_2(T)$ gives $q_{\text{capture}} \approx 0.0247856$ min^{-1}. As a direct check, $P_2(30\text{ min}) = 1 - 5e^{-2} \approx 0.32332$. For n identical pulses of duration τ with complete reset of unbound fragments, cumulative capture is $1-[1-P_j(\tau)]^n$ for design j. With $n = 3$ and $\tau = 10$ min, this gives 0.08793 for TD–HER2.

Finite-interval calculations

For each constant-pH interval of duration t, the state-probability column vector was updated as $\mathbf{p}(t) = \exp(Kt)\mathbf{p}(0)$. For a transition from state i to state j at rate v, the rate matrix K has v added to entry (j, i) and v subtracted from entry (i, i). The final distribution of each interval was used as the initial distribution of the next. Equal values of r and r_1 match the rates of individual closing steps, **not** the mean time to complete reset from A: those times are $2/r$ and $1/r_1$, respectively.

The calculations propagate probabilities directly. There were no sampled molecules, experimental replicates or fitted parameter uncertainties; confidence intervals and significance tests are therefore not applicable to the tabulated values. Their numerical precision should not be interpreted as precision about the behaviour of a physical antibody.

Data availability. All parameter values and numerical comparisons are reported above. No experimental or patient data were generated.

Code availability. The following reference implementation reproduces the complete- and finite-reset comparisons in Table 1. Setting $b = 0$ and reading the accessible fraction instead reproduces the receptor-free calculation.

```
import numpy as np
from scipy.linalg import expm
from scipy.optimize import brentq

def endpoint(steps, opening, capture, reset, pulses, gap):
    # States: closed, [intermediate], accessible, captured.
    size = steps + 2
    acid = np.zeros((size, size))
    neutral = np.zeros_like(acid)

    def add_rate(matrix, start, end, rate):
        matrix[end, start] += rate
        matrix[start, start] -= rate

    for i in range(steps):
        add_rate(acid, i, i + 1, opening)
        add_rate(neutral, i + 1, i, reset)
    add_rate(acid, steps, steps + 1, capture)

    acid_update = expm(acid * (30.0 / pulses))
    neutral_update = None if gap is None else expm(neutral * gap)
    p = np.zeros(size)
    p[0] = 1.0

    for pulse in range(pulses):
        p = acid_update @ p
        if pulse + 1 == pulses:
            break
        if neutral_update is None:  # complete reset of unbound fragments
            captured = p[-1]
            p[:] = 0.0
            p[0], p[-1] = 1.0 - captured, captured
        else:
            p = neutral_update @ p
    return p[-2], p[-1]          # accessible, captured

k = b = 1.0 / 15.0
r = 0.20
target = endpoint(2, k, b, r, 1, 0)[1]
q = brentq(lambda h: endpoint(1, h, b, r, 1, 0)[1] - target, 0.0, 0.1)

for pulses, gap, td_reset in [(1, 0, r), (3, None, r),
                              (3, 10, r), (3, 10, r / 10)]:
    td = endpoint(2, k, b, td_reset, pulses, gap)[1]
    one = endpoint(1, q, b, r, pulses, gap)[1]
    print(f"{100 * td:.2f}%  {100 * one:.2f}%")
```

References

1. Sulea, T. *et al.* Structure-based engineering of pH-dependent antibody binding for selective targeting of solid-tumor microenvironment. *mAbs* **12**, 1682866 (2020). (pmc.ncbi.nlm.nih.gov)

2. Biewenga, L., Vermathen, R., Rosier, B. J. H. M. & Merkx, M. A generic antibody-blocking protein that enables pH-switchable activation of antibody activity. *ACS Chem. Biol.* **19**, 48–57 (2024). (pure.tue.nl)