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Molecular Tug of War Reveals Adaptive Potential of an Immune Cell Repertoire

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The adaptive immune system constantly remodels its lymphocyte repertoire for better protection against future pathogens. Its ability to improve antigen recognition on the fly relies on somatic mutation and selective expansion of B lymphocytes expressing high-affinity antigen receptors. However, this rapid evolution inside an individual appears ineffective, hitting a modest ceiling of antigen-binding affinity. Only recently, experiments began to reveal that evolving B cells physically extract antigens from presenting cells using active forces, and that the extraction level dictates clonal fitness. These observations challenge the prevailing view that the equilibrium constant of receptor-antigen binding determines selective advantage of a B cell clone. Here, we present a theoretical framework to explore ways in which tug-of-war antigen extraction impacts the quality and diversity of an evolved B cell repertoire. We propose that the apparent "ineffectiveness" of *in vivo* selection can be a direct consequence of *nonequilibrium* antigen recognition. Our theory predicts, on the one hand, that the physical limits of antigen tether strength, under tugging forces, set the affinity ceiling. On the other hand, intriguingly, cells can use force heterogeneity to diversify binding phenotype without compromising fitness, thus remaining plastic under resource constraint. These results suggest that active probing of receptor quality—via a molecular tug of war during antigen recognition—limits the potency of response to current antigens, but confer adaptive benefit against future variants. By bridging physical mechanisms and evolved functions, this work reveals a multifaceted role of active forces in immune adaptation, which rationalizes key observations on repertoire dynamics.

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I. INTRODUCTION

The adaptive immune system protects living organisms against a vast and changing variety of microscopic invaders. Adaptive immunity relies on dynamic reorganization of populations of B and T lymphocytes that express unique antigen (Ag) receptors on their surface to recognize and remember encountered pathogens. The ability of an immune cell repertoire to learn from past encounters and improve antigen recognition on the fly—one of the hallmarks of natural immunity—is crucially dependent on somatic hypermutation [1] and selective expansion of B cells expressing high-affinity antigen receptors. Upon recognition of a pathogen, the process of affinity maturation [2]—a form of rapid Darwinian evolution within an individual—allows a diverse pool of naive (antigen-inexperienced) B cells to iteratively enhance the binding affinity of their receptors to the infecting antigen, generating a pool of memory cells with varied lifetimes and long-lived plasma cells that secrete antibodies [membrane-detached B cell receptors (BCRs)].

Since specific molecular interactions mediate immune recognition, existing models of affinity maturation (including our own) often assume that *equilibrium* binding affinity between B cell receptor and antigen determines the reproductive success of a B cell clone [3–7]. However, *in vivo* affinity maturation appears rather ineffective. First, it retains B cell clones with a wide variety of binding affinities for current antigen [8] and produces memory B cells of low specificity and high diversity [9,10]. Moreover, the B cell response exhibits a modest ceiling of antigen-binding affinity, characterized by an equilibrium dissociation constant $K_d > 0.1$ nM [11,12]. In contrast, *in vitro* evolved antibody mutants (via directed evolution) can achieve a monovalent binding affinity with $K_d \sim 50$ fM [13], more than 3 orders of magnitude above the *in vivo* affinity ceiling. The existence of these mutants demonstrates that BCRs are not intrinsically responsible for an affinity ceiling, pointing toward *in vivo* constraints not present in *in vitro* settings.

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In vitro measurements of binding affinity take place after equilibration is reached, and binding-curve fitting is based on the Langmuir isotherm [14]. Yet, *ex vivo* experiments have started to reveal that cells do their affinity measurements differently [15,16]: Evolving B cells exert mechanical pulling forces, generated by the actomyosin cytoskeleton, to physically extract antigens from the antigen presenting cells (APCs); extracted antigens are subsequently internalizated through endocytosis. In addition, antigens are attached to the APCs via protein tethers of various types: some are long and flexible and others short and stiff; even newly produced antibodies can serve as tethers by forming immune complexes with antigens [17,18]. Thus, B cells evolving *in vivo* actively probe their receptor quality during antigen recognition through a tug-of-war extraction process. As a result, both active forces and tether properties—factors extrinsic to BCRs—may influence the extraction outcome.

Furthermore, intravital imaging indicates an unexpected link from molecular interaction to clonal dynamics: the amount of antigen a B cell acquires and subsequently presents to T helper cells determines its number of offspring [19,20]. In this way, B cells translate antigen-binding affinity into clonal reproductive fitness, through the efficiency of antigen extraction. Connecting these intriguing observations across scales, we propose that the apparent "ineffectiveness" of *in vivo* B cell selection is not an artifact due to inevitable randomness but, rather, can be a direct, functional consequence of antigen recognition being *out of equilibrium*. This proposal challenges the prevailing view and convenient model assumption that equilibrium receptor-antigen binding largely governs B cell selection. Our goal is therefore to understand how active force usage by the cell, during antigen acquisition, shapes the evolution of repertoire responses.

Here, we present a theoretical framework to explore, for the first time, ways in which nonequilibrium antigen recognition may influence the quality and diversity of an evolved B cell repertoire. In particular, we develop a coarse-grained stochastic model of tug-of-war antigen extraction, which maps a multidimensional binding free-energy landscape—deformed by tugging forces—to extraction probability, based on kinetics of competitive bond rupture. This physical mapping provides the crucial link between molecular binding affinity and clonal fitness, which in turn forms the central piece of an iterative algorithm of affinity maturation *in silico*. By bridging the gap between physical mechanisms and evolved functions, our approach allows us to uncover physical principles of immune computation at and across different scales.

We argue that tug-of-war antigen extraction may represent an adaptive strategy for repertoire organization, which exploits active forces generated by individual cells to influence collective evolution. We find that physical limits of antigen tether strength, tunable by pulling force, may constrain evolvable antibody affinity *in vivo*, by causing a trade-off between response quality and magnitude. We further demonstrate that, by actively probing the free-energy landscape of coupled molecular bonds, cells can sense and adjust to the mechanical environment, communicating complex signals to induce evolutionary changes. Last but not least, our results suggest that physical extraction of signals using forces can promote adaptation to future challenges through phenotypic plasticity, while ensuring an effective protection against current infection, thus balancing response breadth and potency within a finite repertoire.

Our analytical theory of antigen extraction, mean field in nature, is able to rationalize multiple key observations and to generate falsifiable predictions. We show that, from the tug-of-war setting emerges a saddle point in the fitness landscape of B cell phenotype evolution. This saddle point topology suggests a common origin for the observed persistence of low-affinity clones [8–10] and widely differing rates of diversity loss among B cell populations [21]. Measurable consequences of the saddle point also include bimodality and a maximum variety of antibody quality at intermediate force heterogeneity, as well as correlations between force magnitude and evolved BCR-Ag bond lengths. These predictions can be tested by combining single-molecule force spectroscopy, sensitive tension probes, live-cell imaging, and antibody-antigen binding assays. Theory also predicts a logarithmic dependence of affinity ceiling on effective antigen tether strength; the latter can be tuned by varying force magnitude or surface concentrations of tethering receptors.

Our work is complementary to current modeling efforts in the field. Statistical models of immune systems have explored optimal repertoire organization, subject to resource constraints and functional trade-offs [22–32]. Here, from a statistical physics perspective, we elucidate *microscopic mechanisms* by which active forces and physical constraints on molecular scales can be harnessed to influence repertoire evolution. In a broader context, theoretical works on biological adaptation have focused on the impact of environment (statistics, correlation structures, and variational timescales) on adaptation strategies [33–42]. Our framework, instead, aims to characterize how physical dynamics of cells during signal acquisition shape the rapid evolution of adaptive responses.

II. MODEL

Affinity maturation is a Darwinian-like process by which protective antibodies evolve inside an individual. This rapid evolution takes place in germinal centers (GCs), transient structures that assemble soon after antigen exposure and dissolve when running out of fuel. Intravital imaging has uncovered a vivid picture of repertoire remodeling, highly dynamic in both space and time: Upon antigen recognition, a few founder B cells become activated and freely expand without mutation or selection to a population

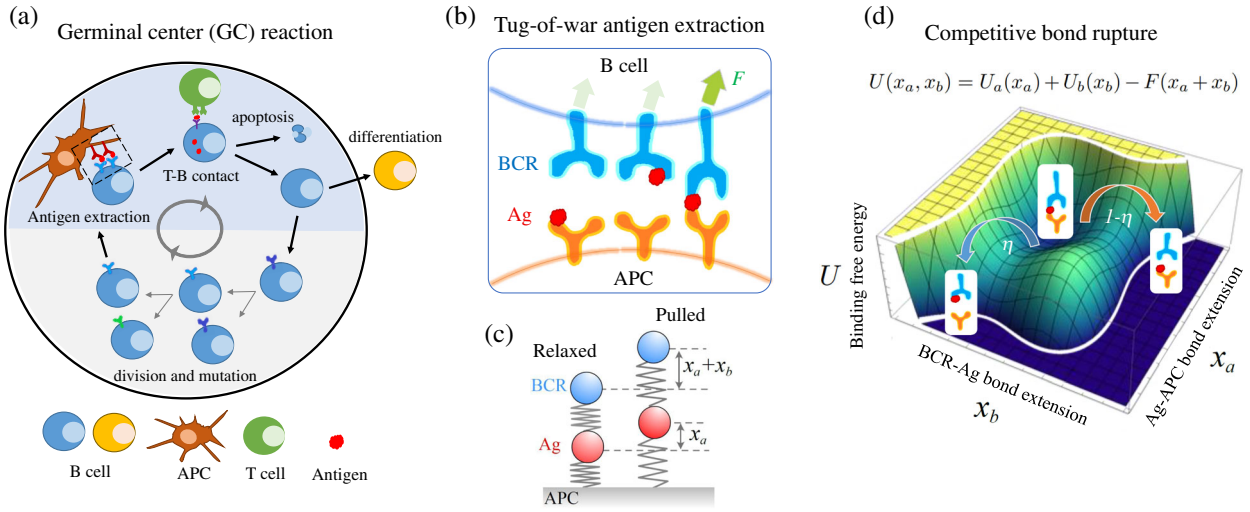

FIG. 1. Model overview. Evolutionary learning of a B cell repertoire crucially depends on physical extraction of antigen (Ag) via a molecular tug of war. (a) Affinity maturation takes place in germinal centers (GCs)—structured microenvironments where cellular players meet and interact. Iterative evolutionary learning proceeds through cycles of GC reaction: somatic hypermutations during proliferation cause changes in BCR properties (affinity and flexibility) that alter B cells' efficiency of acquiring antigen from the surface of antigen presenting cells (APCs) and subsequently presenting to helper T cells. This efficiency, in turn, determines the selective advantage of competing clones. Positively selected cells (avoiding apoptosis) either differentiate into memory or plasma cells and exit the GC or join the next cycle of reaction. (b) Central to this evolutionary program is the mapping from BCR-Ag binding characteristics to clonal reproductive fitness, arising from nonequilibrium antigen extraction under tugging forces. A BCR-Ag-APC complex coarse grains the protein chain linking a B cell to the APC. (c),(d) The essence of tug-of-war antigen extraction is competitive bond rupture under pulling force. System dynamics proceed on a combined free-energy surface $U(x_a, x_b)$ over a 2D space spanned by x_a and x_b, the extension of the Ag-APC and BCR-Ag bonds, respectively. With probability η, extraction occurs via thermally aided escape from the bound state over the activation barrier corresponding to Ag-APC bond rupture ($x_a = x_a^{\ddagger}$). BCR is B cell receptor.

of 10^2–10^4 cells. As enzymatic activation triggers somatic hypermutation in antibody-encoding genes, cycles of GC reaction ensue. Cyclic action of replication, mutation, acquisition of antigen and T cell help, death and differentiation or recycle leads to production of increasingly higher affinity antibodies and continuous export of memory and plasma cells [Fig. 1(a)]. Localized assembly of GCs allows frequent encounter of B cells and helper T cells amid space-spanning networks of follicular dendritic cells; these GC-resident APCs display antigens on their surface and modulate B cell antigen extraction through cell-cell contact.

While GC reaction has been modeled since the recent past to describe population dynamics [3,4,6,7,26,32,43], the nonequilibrium process of antigen extraction has not been studied and its evolutionary significance remains unknown. To fill this gap, we construct a physical theory of tug-of-war antigen extraction and subject the resulting phenotype to *in silico* affinity maturation, thereby bridging molecular and organismic scales.

A. Stochastic antigen extraction via a molecular tug of war

Evolving B cells extract antigen from APCs through transient cell-cell contact, in which modest BCR-Ag clusters form a multiplicity of contact foci [15]. Receptor clustering triggers B cell intracellular signaling that instructs generation of contractile forces in the acto-myosin cytoskeleton [44,45]. These active forces, in turn, pull on BCR-Ag clusters tethered to the APC [46]. Dynamic organization of cell-cell interfacial patterns is complex, reflecting an interplay of membrane elasticity, binding or unbinding kinetics, and cytoskeletal activity [47]. As a first step, we consider individual clusters as recognition units and assume independent BCR-Ag-APC complexes subject to equal pulling stress [Fig. 1(b)]. This mean-field picture is compatible with the observation that traction force applied to a BCR cluster scales with its size [44,48]. More importantly, as we show, this mean-field model can already capture multiple key features of antibody evolution, supporting molecular tug of war (prior to cellular modulation) as an essential microscopic underpinning of emergent repertoire responses.

BCR-Ag-APC complexes are a coarse-grained description of the protein chains linking a B cell to the APC. In particular, the Ag-APC bond (referred to as antigen tether) coarse grains potentially complex interactions, including multivalent binding of an antigen particle to tethering receptors and viscoelasticity of the APC membrane. Moreover, the weakest (shortest-lived) link in the antigen tether, under pulling force, limits its overall strength. While

rupture of molecular bonds with a single binding interface has been studied in depth to interpret single-molecule pulling experiments [49–51] and to understand cell adhesion [52–55], active ligand transfer between cells has not been treated before. The essence of tug-of-war antigen extraction is competitive rupture of the BCR-Ag and Ag-APC bonds, that is, a comparison of rupture *kinetics* between binding interfaces. A sooner breakage of the antigen tether results in successful extraction.

As pulling force applies to a BCR-Ag-APC complex, mechanical stress propagates and alters the extension of tugging and tethering bonds in the pulling direction. Since antigen movement couples bond extensions, system dynamics proceeds on a combined free-energy surface, deformed by pulling force, over a 2D state space spanned by x_a and x_b, the extension of the Ag-APC bond and the BCR-Ag bond, respectively [Figs. 1(c) and 1(d)]: $U(x_a, x_b) = U_a(x_a) + U_b(x_b) - F(x_a + x_b)$. Here, the last term describes landscape deformation caused by pulling force of magnitude F that can be time dependent. The intrinsic free-energy profile $U_a(x_a)$ [$U_b(x_b)$] has a potential well at zero extension and a barrier of height $\Delta G_a^{\ddagger}$ at the rupture length $x_a = x_a^{\ddagger}$ (of height $\Delta G_b^{\ddagger}$ at $x_b = x_b^{\ddagger}$). The combined surface has a minimum and two saddle points [Fig. 1(d)]. Applied force lowers both barriers and displaces the minimum and saddles. We use general linear-cubic potentials for $U_a(x_a)$ and $U_b(x_b)$ which permit an analytical understanding [see Supplemental Material (SM) [56]].

Antigen extraction is stochastic, because bond rupture occurs via activated dynamics, i.e., thermally assisted escape from the bound state over one of the activation barriers [Fig. 1(d)]. Extending the Kramers theory [57] to higher dimensions, we formulate extraction dynamics as a first-passage problem in a 2D state space with interfering absorbing boundaries at rupture lengths. The motion of antigen and BCR molecules, governed by force balance, is described by coupled Langevin equations in the overdamped limit:

$$\begin{aligned} \gamma_a \dot{x}_a &= -U_a'(x_a) + U_b'(x_b) + \xi_a, \\ \gamma_b(\dot{x}_a + \dot{x}_b) &= -U_b'(x_b) + F + \xi_b. \end{aligned} \tag{1}$$

Here, random forces, ξ_a and ξ_b, and frictional forces, $-\gamma\dot{x}_a$ and $-\gamma(\dot{x}_a + \dot{x}_b)$, both arise from collisions with particles in the ambient fluid and are related through $\langle \xi_i(t)\xi_j(t')\rangle = 2k_BT\gamma_i\delta_{ij}\delta(t-t')$, with $i, j = a, b$ and $\langle \xi_i \rangle = 0$. Damping coefficients γ_i set relaxation timescales.

Quantifying the intuition of competitive bond rupture, we calculate the extraction probability η as the chance by which the BCR-Ag bond persists longer than the Ag-APC bond. This amounts to comparing the first-passage time to reaching either absorbing boundary, i.e., exceeding one of the rupture lengths ($x_a^{\ddagger}$ and $x_b^{\ddagger}$). In the limit of high activation barriers (moderate pulling strength), a simple factorized form follows:

$$\eta = \int_0^{\infty} dt p_a(t) S_b(t). \tag{2}$$

Here, $S_b(t) = \int_t^{\infty} dt' p_b(t')$ is the survival probability of the BCR-Ag bond until at least time t when the Ag-APC bond breaks. $p_a(t)$ and $p_b(t)$ are distributions of bond lifetime governed by Eq. (1) (i.e., first-passage time to exceeding either rupture length treating the other boundary as reflective). Under modest constant pulling forces, bond lifetimes are nearly exponentially distributed and the extraction probability is simply $\eta = 1/(1 + \tau_a/\tau_b)$, depending only on the ratio of mean bond lifetimes: τ_a for the Ag-APC bond and τ_b for the BCR-Ag bond. This intuitive expression suggests that the tug-of-war extraction system implements a nonequilibrium ratio test of dynamic bond strengths; by counting successful events out of repeated extraction attempts, cells can measure the ratio of mean lifetimes between tugging and tethering bonds.

B. Germinal-center reaction implements evolutionary learning of a B cell repertoire

Affinity maturation appears to implement an iterative algorithm for optimizing molecular recognition *in vivo*. However, under natural conditions, selection on receptor binding affinity is only indirect; what determines the division capacity of a B cell is its ability to acquire membrane-tethered antigen using mechanical forces [19]. The proliferation rate is, therefore, a function not only of BCR-Ag affinity, but also of factors extrinsic to BCR, especially force magnitude and tether properties. It follows that these extrinsic physical factors can shape the selection pressure by modulating the extraction likelihood η. To characterize in what ways force usage influences evolutionary outcomes (e.g., receptor potency and diversity), we build a birth-death-mutation model of GC reaction and couple to it a physical theory of antigen extraction (Fig. 1). We implement the model using analytical theory and stochastic agent-based simulations (see SM [56]).

The *in vivo* observation of affinity-based B cell proliferation [10] motivates the key model ingredient: a proliferation rate $\lambda(\eta)$ that depends on binding affinity through extraction probability η:

$$\lambda(\eta) = \lambda_0 \frac{n_{\mathrm{Ag}}(\eta)}{n_0 + n_{\mathrm{Ag}}(\eta)}. \tag{3}$$

This sigmoidal dependence on the amount of extracted antigen n_{Ag} is chosen to approximate a global nonlinearity that summarizes intracellular processing of extracted antigen and subsequent acquisition for T cell help [58]. Assuming independent extraction events (mean-field assumption), we draw n_{Ag} from a binomial distribution

$n_{\mathrm{Ag}} \sim B(C_{\mathrm{Ag}}A, \eta)$, where C_{Ag} is the surface concentration of BCR-Ag-APC complexes and A the contact area between a B cell and the APC, both being treated as affinity independent. The extraction level n_0 leading to a half-maximum replication rate sets a threshold for B cell survival; cells with $n_{\mathrm{Ag}} \ll n_0$ fail to become activated and die, whereas cells having $n_{\mathrm{Ag}} \gg n_0$ proliferate at the maximum rate. To account for space and resource limitations, we assume an overall logistic growth at a rate $r(\eta) = \lambda(\eta)(1 - N/N_c)$, where N is the B cell count and N_c the carrying capacity. A death rate is assumed to be constrained and fixed, uniform among cells.

In our current model, we focus on force-modulated evolution of *off rates*, assuming that the force-free on rate has reached the diffusion limit and saturated. This assumption is backed by the fact that the observed on rate of antibody-antigen interaction [59] agrees with the theoretical value for diffusion-limited protein-protein association [60]; both lie within 10^4–10^6 $\mathrm{M}^{-1}\,\mathrm{s}^{-1}$. We discuss in SM [56] how and when the on-rate evolution (starting below saturation) might influence B cell selection.

Intrinsic parameters of a BCR—binding affinity $\Delta G_b^{\ddagger}$ and bond length $x_b^{\ddagger}$—characterize its binding free-energy surface for a given antigen in the absence of force. Different from past models of affinity maturation where only binding affinity evolves, we allow both $\Delta G_b^{\ddagger}$ and $x_b^{\ddagger}$ to alter. By opening a new dimension in phenotype space, an evolvable bond length permits a broader range of solutions capable of efficient antigen extraction. In fact, both traits can evolve through somatic mutations that introduce point changes to BCR-encoding gene segments [61,62]; typically, changes in the complementarity-determining regions directly influence antigen binding ($\Delta G_b^{\ddagger}$), whereas changes to the framework regions may alter BCR flexibility ($x_b^{\ddagger}$). We assume that upon mutation, each parameter picks up an increment according to a Gaussian distribution with a typical jump size σ:

$$\begin{aligned} \Delta G_{b,t+1}^{\ddagger} &= \Delta G_{b,t}^{\ddagger} + \sigma_{G_b}\eta_{t+1}, \\ x_{b,t+1}^{\ddagger} &= x_{b,t}^{\ddagger} + \sigma_{x_b}\xi_{t+1}, \end{aligned} \tag{4}$$

where $\langle \eta_t \rangle = \langle \xi_t \rangle = 0$ and $\langle \eta_t \eta_{t'} \rangle = \langle \xi_t \xi_{t'} \rangle = \delta_{t,t'}$. This form of mutation-induced changes allows both traits to continually evolve. Thus, if increases in affinity were to slow down within the model, it must be due to factors other than a lack of beneficial mutations.

Taken together, iterative cycles of GC reaction, driven by antigen-extraction-based clonal competition, implement repertoire learning. One learning cycle proceeds as follows: mutational changes in receptor traits [Eq. (4)] alter a cell's efficiency of acquiring antigen [Eqs. (1) and (2)], which in turn updates the selective advantage of competing clones [Eq. (3)], yielding a new generation of cells with modified binding characteristics. We simulate ensembles of polyclonal populations pulling at different strengths, obtain η analytically for each clone at each cycle, and examine features of population dynamics, evolution trajectories of surviving cells, and evolved repertoires.

III. RESULTS

The observation that maximum antibody affinities evolved in organisms are considerably lower than those achieved by directed evolution in the laboratory indicates the presence of *in vivo* factors that limit the efficacy of selection. We propose that tug-of-war antigen extraction holds the key to, at least in part, explaining this contrast by clarifying the impact of nonequilibrium recognition. In what follows, we identify the origin and determinants of affinity ceiling, evaluate to what extent active force usage influences selection, and propose ways in which cells may exploit physical constraints (e.g., antigen tethering) to their advantage.

A. Antigen tether strength sets affinity ceiling

In a tug-of-war configuration [illustrated in Figs. 1(b) and 1(c)], a B cell pulls on BCRs bound to antigens that are in turn tethered to the APC. Force propagates through the BCR-Ag-APC complexes, deforming coupled binding interfaces and modulating their lifetime distributions. The chance of antigen extraction η reflects the relative strength of the tugging and tethering bonds, measured by the ratio of expected lifetimes under force, τ_b and τ_a, respectively. Defining relative tether strength $s \equiv \tau_a/\tau_{b0}$, where τ_{b0} denotes a founder B cell's BCR-Ag bond lifetime under force, the extraction probability becomes $\eta = [1 + s/(\tau_b/\tau_{b0})]^{-1}$.

As one would expect, strong tethers (large s) suppress antigen extraction. This has indeed been observed in live B cells [16]. More importantly, this expression implies a limiting factor on affinity maturation: As τ_b increases well past τ_a, η tends toward saturation at η_{th}—an extraction threshold above which selectable differences among cells become too little to drive further improvement in binding quality. Thus, affinity maturation hits a ceiling.

To make this intuition quantitative, we estimate the ceiling affinity $\Delta G_b^{\ddagger}$ by inverting the relation $\eta[\tau_b(\Delta G_b^{\ddagger})] = \eta_{\mathrm{th}}$ and compare to the output of simulated GC reaction. When activation barriers are high and forces modest, ceiling affinity follows a logarithmic dependence on tether strength:

$$\Delta G_b^{\ddagger} \approx \Delta G_{b0}^{\ddagger} + k_B T\left[\ln\, s + \ln\left(\frac{\eta_{\mathrm{th}}}{1-\eta_{\mathrm{th}}}\right)\right], \tag{5}$$

where $\Delta G_{b0}^{\ddagger}$ is the founder affinity. As shown in Fig. 2, evolved affinities of simulated population ensembles (circles) match the prediction (dashed line) over a wide range of tether strengths, realized by systematically varying force magnitude (F, 0–30 pN) and tether bond length

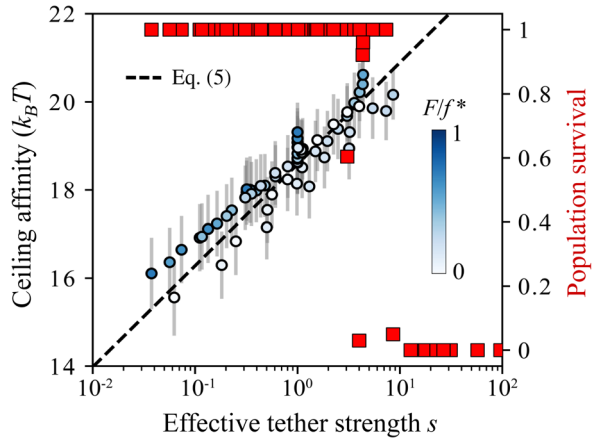

FIG. 2. Effective tether strength sets evolvable antibody affinity. Ceiling affinity—evolved activation energy or barrier height $\Delta G_b^\ddagger$—largely follows a logarithmic dependence on relative tether strength $s \equiv \tau_a/\tau_{b0}$, as estimated by Eq. (5) (dashed line). Symbols are obtained from simulations over a wide range of tether strengths realized by varying the tether rupture length ($x_a^\ddagger$, 0.5–4 nm) and force magnitude (F, 0–30 pN); each symbol results from 100 runs for a given pair of $x_a^\ddagger$ and F values. Modest deviation of symbols from the straight line arises due to relatively strong forces (darker blue circles) scaled by the critical force $f^* = \min\{3\Delta G_a^\ddagger/(2x_a^\ddagger), 3\Delta G_b^\ddagger/(2x_b^\ddagger)\}$ at which the barrier to rupture vanishes. While the ceiling affinity rises with tether strength (circles), the fraction of surviving populations (red squares) vanishes quickly above tether strengths a few-fold stronger than the founder BCR-Ag bond. Here, binding affinity $\Delta G_b^\ddagger$ evolves, while the bond length $x_b^\ddagger$ remains fixed. $\Delta G_a^\ddagger = \Delta G_{b0}^\ddagger = 14k_BT$, $x_b^\ddagger = 2$ nm. $\eta_{\rm th} = 0.97$. We used a linear-cubic free-energy profile to calculate extraction probability (see SM [56]) in all the results presented in this work.

($x_a^\ddagger$, 0.5–4 nm). Mild deviation arises under relatively strong forces [being a fraction of the critical force $f^* = \min\{3\Delta G_b^\ddagger/(2x_b^\ddagger), 3\Delta G_a^\ddagger/(2x_a^\ddagger)\}$ at which the barrier to rupture vanishes]; in this regime, considerable landscape deformation can cause a nonlinear reduction in the log escape times, an effect we neglect in our estimate.

Notably, force can modulate antigen tether strength and alter the affinity ceiling. In fact, force causes differential influences on coupled bonds, depending on their *relative* stiffness and affinity. To the leading order in F, $s \sim \exp[F(x_{b0}^\ddagger - x_a^\ddagger)/k_BT]$, which indicates that tugging forces enhance a stiff tether ($x_a^\ddagger < x_{b0}^\ddagger$) but weaken a soft tether ($x_a^\ddagger > x_{b0}^\ddagger$), yielding an elevated and a lowered ceiling, respectively (Fig. S1A in SM [56]). Intuitively, the same force, transmitted along a chain, more strongly impacts the softer bonds. As a consequence, pulling harder against stiff tethers or APCs will effectively strengthen the tether, suppress extraction, and therefore raise the ceiling. Yet, this also raises the risk of population collapse (Fig. S1B in SM [56]); strong tethers could lead to a deep population bottleneck (Fig. S1E in SM [56]) and vanishing population survival (Fig. 2). In line with current knowledge [63], our simulation begins with a clonal population that results from noncompetitive expansion of a few founder B cells; this sizable population is then subject to cycles of mutation and selection (see SM [56] for details). A population bottleneck, if any, occurs soon after GC reaction cycles begin; at the start of this competitive stage, the strength of antigen tethers may exceed that of founder BCR-Ag bonds, leading to faster B cell death than division (due to low extraction). The resulting population decline is halted as mutant clones better at acquiring antigen are produced and selectively expanded. Thus, the presence of strong antigen tethers can cause a deep population bottleneck and, in turn, frequent GC collapse. As a result, once tether strength exceeds a characteristic value, GC survival vanishes abruptly (Fig. 2, red squares).

These results are supported by *ex vivo* observations. First, GC B cells undergoing affinity maturation exert strong forces against stiff APCs, effectively enhancing antigen tethering; they indeed extract fewer antigens compared to naive cells that use weak forces [15]. Moreover, force usage was observed to increase discrimination stringency at the cost of absolute extraction [15,16]. The resulting trade-off between ceiling affinity and population survival yields an optimal tether strength a few-fold stronger than the founder BCR-Ag bond (Fig. 2).

Interestingly, ramping force can alleviate the trade-off and outperform constant-force schemes (see SM [56]). Under ramping force, the most probable rupture force of a molecular bond adjusts to binding affinity: When affinity is low, weak rupture force allows population survival. As affinity improves, increasing rupture force progressively elevates tether strength, hence selection pressure, yielding a ceiling higher than achievable by constant-force schemes that permit a similar survival rate.

We note that a precise prediction of the ceiling affinity requires full knowledge of tether properties, force magnitude, and founder affinity (Fig. S1C in SM [56]). Nonetheless, a good catch of the overall trend by Eq. (5) confirms that tether strength—a composite parameter summarizing features of molecular interactions and mechanical environments as well as the impact of force—indeed sets a bound to evolvable receptor affinity and at the same time reveals potential means to altering it.

B. Mismatch between B cell fitness and antibody quality reveals training-testing discrepancy

We now explore in what ways the ability of cells to generate force and sense stiffness might influence their course of evolution. Figure 3(a) demonstrates typical simulated trajectories (color coded for time) on a fitness landscape calculated from extraction probability (contour map) in the 2D trait space of BCR affinity $\Delta G_b^\ddagger$ and bond length $x_b^\ddagger$. Force magnitude governs the overall direction of

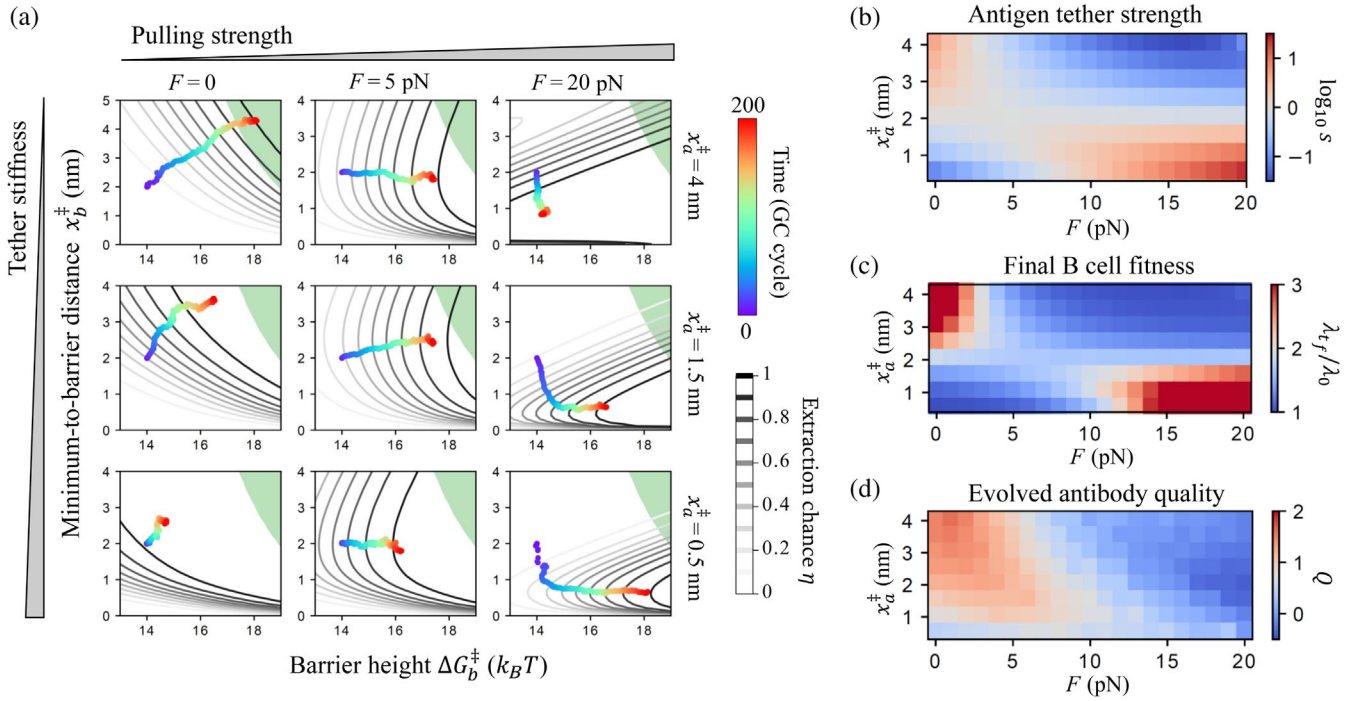

FIG. 3. Active force usage steers B cell evolution and causes discrepancy between B cell fitness and antibody quality. (a) Example evolutionary trajectories of population-mean binding affinity $\Delta G_b^\ddagger$ and bond length $x_b^\ddagger$, guided by fitness landscape (contour map of extraction probability η) and subject to stochasticity in mutation and reproduction. The green region indicates high intrinsic binding quality ($Q \geq \log_{10} 50$). Stronger pulling (left to right, $F = 0$, 5, 20 pN) selects for stiffer BCR-Ag bonds (smaller $x_b^\ddagger$), whereas varying tether stiffness (top to bottom, $x_a^\ddagger = 4$, 1.5, 0.5 nm) tunes the range and steepness of fitness gradient. Evolved BCRs appear to match the stiffness of antigen tethers; see main text for explanation. (b) Effective tether strength as a function of force magnitude F and tether bond length $x_a^\ddagger$, obtained from mean first-passage time calculation. Stronger forces strengthen stiff tethers ($x_a^\ddagger < x_b^\ddagger$) but weaken soft tethers ($x_a^\ddagger > x_b^\ddagger$), yielding maximum tether strengths at low force and soft tether or high force and stiff tether (red regions). (c) Dependence of evolved B cell fitness (fold increase in reproduction rate) on pulling force and tether stiffness largely follows the trend of tether strength (b). (d) Evolved antibody quality increases toward weak force and soft tether (upper left-hand corner) just as evolved fitness does (c). However, strong force and stiff tether (lower right-hand corner) lead to high B cell fitness yet low antibody quality. In (c) and (d), each pixel represents an average over 20 simulations, with equal mutation rates for $\Delta G_b^\ddagger$ and $x_b^\ddagger$; initial conditions are $\Delta G_{b0}^\ddagger = \Delta G_a^\ddagger = 14 k_B T$ and $x_{b0}^\ddagger = 2$ nm.

evolution: under vanishing force (left-hand column), populations evolve toward softer BCRs (larger $x_b^\ddagger$), because soft bonds have a longer intrinsic lifetime in the absence of force. In contrast, strong pulling (right-hand column) drives evolution toward stiffer BCRs (smaller $x_b^\ddagger$), since they are more resistant to force-induced barrier reduction, thus being longer-lived to support antigen extraction. For a given pulling strength, tether stiffness ($\sim 1/x_a^\ddagger$) tunes the steepness of fitness gradient. Most significant gradients occur at the corners—low force and soft tether (upper left) and high force and stiff tether (lower right)—consistent with maximum tether strengths [red corners in Fig. 3(b)], which lead to most pronounced fitness increases in surviving B cells [Fig. 3(c), red corners] but at the expense of population collapse (Fig. S2B in SM [56]).

Interestingly, it appears that BCRs evolve to match the tether or APC stiffness [Fig. 3(a), diagonal panels]: soft presenting receptors or membranes select for soft BCRs without force (upper left), whereas stiff substrates favor stiff BCRs under pulling (lower right). This "stiffness mimicking" behavior can be understood from mechanical feedback in combination with tug-of-war extraction. On the one hand, stiffer substrates promote generation of stronger pulling forces (Fig. S2A [56], red diagonal, preferred F increases as $x_a^\ddagger$ decreases). This feedback serves to maintain a fitness gradient toward higher affinity; as shown in Fig. 3(a), populations in diagonal panels evolve to larger $\Delta G_b^\ddagger$ compared to other combinations of F and $x_a^\ddagger$. This mechanosensing behavior was indeed observed *ex vivo* as B cells extract antigen from live APCs [16]. On the other hand, stronger forces favor the usage of stiffer BCRs to resist barrier reduction and support extraction (Fig. S2C [56], evolved $x_b^\ddagger$ decreases with increasing F). This prediction can potentially be tested by altering APC

stiffness, via cholesterol depletion or induced inflammation, and measuring changes in evolved BCR-Ag bond length through single-molecule pulling experiment.

Intuitively, one would expect that learning is only effective if training and testing are performed under similar conditions. However, B cells are trained to recognize membrane-bound antigens via nonequilibrium extraction, while antibody quality is tested through equilibrium binding to free antigens. We thus expect that this discrepancy, between the training objective (efficient antigen extraction) and testing criterion (strong equilibrium binding), would result in a mismatch between B cell fitness and antibody quality.

To characterize this mismatch, we define binding quality $Q \equiv \log_{10} \tilde{\tau}_b/\tilde{\tau}_{b0}$ that measures the fold change in intrinsic (i.e., force-free) lifetime of the BCR-Ag bond as a result of evolution and contrast it with B cell fitness λ [Eq. (3)]. Comparing Figs. 3(c) and 3(d), we see that pulling against stiff tethers or APCs—characteristic of evolving cells—indeed very effectively enhances clonal fitness [Fig. 3(c), lower right, red blob], but the resulting antibody quality is low [Fig. 3(d)]. Essentially, force alters the fitness landscape, "misleading" the population to turn away from the region of optimal binding quality [green corners in Fig. 3(a)]; see an example in the lower right-hand panel of Fig. 3(a). This result suggests a surprising possibility: force usage by evolving cells may not simply be optimizing receptor binding quality to the current target, because pulling may even reduce the relative fitness of intrinsically strong binders. Then, what might the immune system gain from the apparent loss in performance? In Sec. III D, we propose an unexpected answer, which reveals a basic trade-off and a possible balance.

C. Dynamic selection pressure improves response quality

As we show above, tether strength under force limits evolvable affinity (Sec. III A), whereas distinct conditions for training and testing may retain low-affinity clones (Sec. III B). One might wonder, can immune cells alleviate these constraints? We examine two biologically plausible strategies for their capacity to improve the response quality: renewable tether and dynamic force. Both schemes result in time-varying selection pressure.

Once BCR-Ag bonds become nearly as strong as the tethers, selection pressure vanishes and population affinity approaches saturation. Thus, key to lifting the affinity ceiling is the ability to strengthen the tether at a steady pace, neither too fast nor too slow, but best to match the rate at which BCR affinity improves. In this way, populations of cells adapt to the toughening environment at their best ability, not being slowed by saturating extraction or any severe population bottleneck. We test this idea *in silico*: at the end of each GC cycle, we sample from the high-affinity members (with top ranks in $\Delta G_b^{\ddagger}$) of a cumulative plasma cell population to form a pool of feedback candidates. From this pool, a random subset is drawn to supply antibodies as antigen tethers for the next cycle. This setting of antibody feedback is motivated by *in vivo* experiments showing that passively injected antibodies of high affinities can replace endogenous tethers to present antigens in mouse lymph nodes [17,18].

In Fig. 4(a) we compare the course of affinity maturation with and without antibody feedback. With fixed tethers (dashed lines), BCR affinity first rises then levels off. With renewing tethers (solid lines), however, contemporary clones receive a negative feedback from high-performing predecessors and achieve persistent adaptation. Importantly, overall tether strength (red solid) and average BCR affinity (black solid) indeed increase at a similar and steady rate, reflecting a restoring effect of antibody feedback: As effective tether strength becomes steady, extraction chance stabilizes, followed by a stable population size and selection strength, and hence a steady rate of adaptation. Eventually, antigen tethers might become so strong that the weakest link in the chain shifts to the membrane tube pulled out by the tugging force, which ultimately limits evolvable affinities; in this case, further increases in BCR-Ag affinity make no more difference in extraction levels, hence clonal fitness.

An apparent drawback of pulling against stiff APCs is that it inevitably drives selection of stiff BCR-Ag bonds that are short-lived without force [Fig. 4(b), top panel]—the condition under which antibodies detect pathogens. As a possible remedy, cells may attempt to evolve and maintain $x_b^{\ddagger}$ to near $x_a^{\ddagger}$. This is because with matching stiffness, extraction is almost independent of force (to the leading order, $\tau_a/\tau_b \sim \exp[F(x_b^{\ddagger} - x_a^{\ddagger})/k_BT]$), directly resolving the training-testing discrepancy. One way to achieve this is to apply pulling forces in an oscillatory manner: $F(t) = F_{\max}[1 + \cos(2\pi t/T_F)]/2$; the oscillation period T_F should be relatively short compared to relaxation timescales for "dynamic localization" to be effective [see an example trajectory in Fig. 4(b), lower left-hand panel]. Such periodic modulation of pulling strength is plausible, through cyclic resetting of the cytoskeletal contractile machinery or via coupling to circadian rhythms of other cellular and organismal processes.

In the fast-cycling limit, a population evolves in an effective fitness landscape that time averages those under varying force magnitudes. This effective landscape has an attractor at high affinity (large $\Delta G_b^{\ddagger}$) and matching stiffness ($x_b^{\ddagger} \approx x_a^{\ddagger}$), with a persistent gradient leading from low to high affinity [see contour map in Fig. 4(b), lower left-hand panel]. This is in stark contrast to the landscape under constant forces, for which affinity gradients are shallow and attractors biased toward extreme stiffness [Fig. 4(b), upper left-hand panel, $F = F_{\max}$]. Thus, as force increases in magnitude [Fig. 4(b), right], binding quality evolved under a constant force falls rapidly due to decreasing $x_b^{\ddagger}$ (black symbols). With cycling forces, however, response

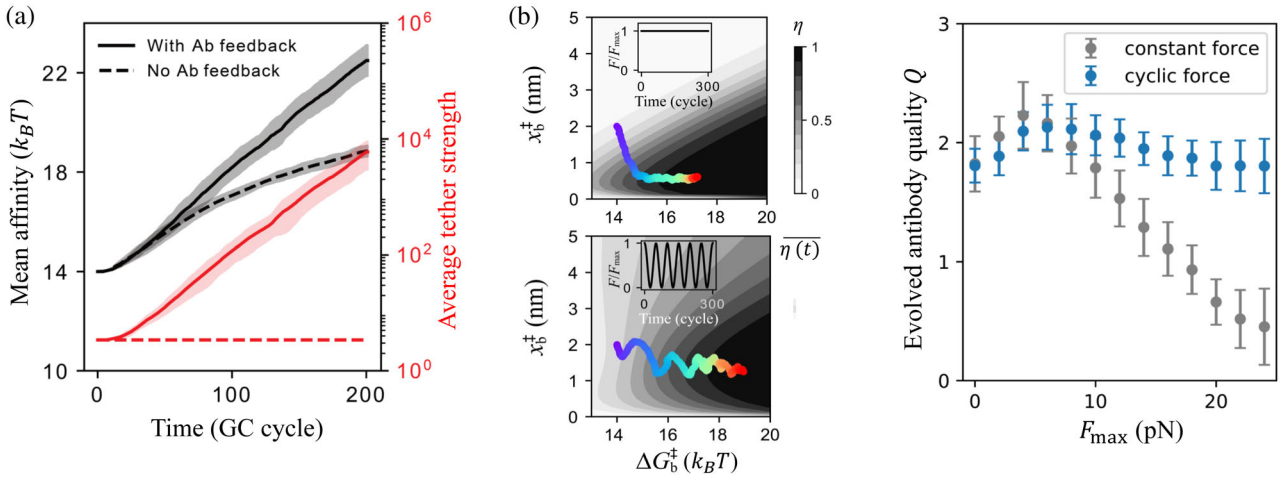

FIG. 4. Dynamic selection pressure sustains adaptation and improves evolved binding quality. (a) Evolutionary dynamics with (solid lines) and without (dashed lines) antibody feedback. With fixed antigen tethers (red dashed line), the rate of increase in BCR affinity slows down over time (black dashed line). With renewing tethers sampled from high-affinity plasma cells (top-K rank in $\Delta G_b^\ddagger$), the overall tether strength (red solid line) and BCR affinity (black solid line) improve at similar steady rates, indicating sustained adaptation. The shade shows variation among 10 simulations. $\Delta G_a^\ddagger = 14 k_B T$, $x_a^\ddagger = 1.5$ nm, $x_b^\ddagger = 2$ nm, $F = 20$ pN, $K = 100$. (b) Population-average evolution trajectories (left) and outcomes (right) under constant and oscillatory forces. Under constant forces, the fitness landscape (contour map of extraction probability) has a shallow affinity gradient and drives evolution toward stiff BCR-Ag bonds (upper left-hand panel), leading to a rapid fall in evolved binding quality with increasing force magnitude (right-hand panel, black symbols). Under not too slow oscillatory forces, populations evolve in an effective time-averaged fitness landscape (lower left-hand panel) that has an attractor at high affinity and matching stiffness ($x_b^\ddagger \sim x_a^\ddagger$), resulting in high quality over a wide range of force magnitude (right-hand panel, blue symbols). Constant force, $F = 20$ pN; oscillatory force, $F = F_{\max}[1 + \cos(2\pi t/T_F)]/2$, with $F_{\max} = 20$ pN, $T_F = 50$, $t_f = 300$. Error bars indicate variation among 20 simulations. $\Delta G_a^\ddagger = 14 k_B T$, $x_a^\ddagger = 1.5$ nm; $\Delta G_{b0}^\ddagger = 14 k_B T$, $x_{b0}^\ddagger = 2$ nm.

quality remains high regardless of force magnitude (blue symbols); clones forming too stiff ($x_b^\ddagger < x_a^\ddagger$) or too soft ($x_b^\ddagger > x_a^\ddagger$) BCR-Ag bonds are repeatedly removed during weak-force and strong-force periods, respectively, favoring the takeover by stiffness-matching clones ($x_b^\ddagger \sim x_a^\ddagger$) that remain fit under changing conditions.

D. Heritable force heterogeneity diversifies binding phenotype: Adaptive benefit of physical sensing

Lastly, we ask whether force usage during immune recognition can gain any advantage from the apparent loss in selected binding quality. Nongenetic variability among founder B cells has been observed [64] that results from intrinsic noise in molecular networks, accumulated during stem cell differentiation. We thus propose that heterogeneity in heritable force magnitude might be harnessed to generate diverse binding phenotypes. To test this hypothesis, we sample the force magnitude of founder cells from a distribution, assumed uniform for simplicity (with mean $F_{\rm av}$ and width σ_{F_0}), and analyze BCR traits resulting from evolution. Our predictions are robust to different choices of the distribution of founder force magnitude, as long as the values are limited to a finite range. One environmental cue capable of inducing variations in force magnitude is variability in APC stiffness, potentially caused by strain stiffening [65] under B cell contractility and/or regulated by inflammatory signals [66].

Figure 5(a) shows the fitness landscape (extraction probability as a proxy) as a function of force magnitude F and bond length $x_b^\ddagger$ at population-mean affinity. Examination of the fitness contours identifies a saddle point at $F \simeq 9$ pN and $x_b^\ddagger = 1.5$ nm. In its neighborhood, the fitness surface has a negative Gaussian curvature, with the ridge line (black dashed line) tracing the direction of the positive principal curvature. The presence of the saddle point is a direct consequence of the tug-of-war setting and reflects the fact that efficient antigen extraction is possible for two different regimes: either large extraction force and stiff bonds or small force and soft bonds; the latter has a higher absolute fitness.

The emergence of a saddle point in the F-$x_b^\ddagger$ space is a generic feature of the tug-of-war extraction system. Extraction probability exhibits a logistic nonlinearity: $\eta = \{1 + \exp[\beta \Delta\Delta G + \ln(\tau_{s,b}/\tau_{s,a})]\}^{-1}$, where the gap in activation barrier between the tugging and tethering bonds, $\Delta\Delta G = \Delta G_b^\ddagger (1 - F/f_b)^{3/2} - \Delta G_a^\ddagger (1 - F/f_a)^{3/2}$, and the ratio of time to leave the transition state, $\tau_{s,b}/\tau_{s,a} \propto \sqrt{(f_a - F)/(f_b - F)}$, have opposite dependence on bond length $x_b^\ddagger$ (via $f_b = 3\Delta G_b^\ddagger / 2 x_b^\ddagger$) and force magnitude F, respectively (see SM [56]); a saddle point occurs where the dependencies flip sign. Specifically, the F dependence changes sign at $x_b^\ddagger \simeq x_a^\ddagger$. Meanwhile, when force is weak, time to escape the transition state dominates

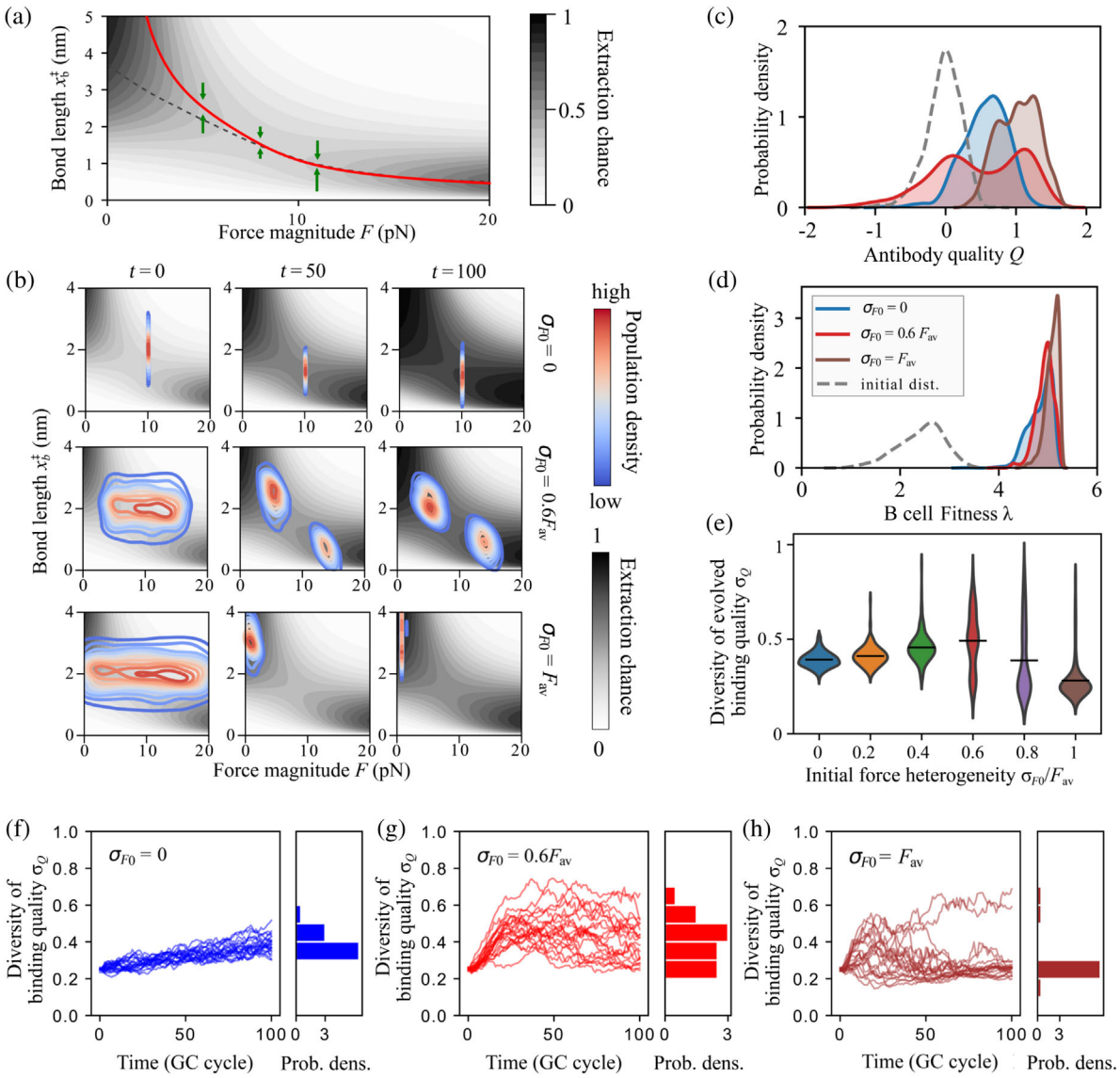

FIG. 5. An intermediate level of heritable force heterogeneity yields a broad range of binding quality and rate of diversity loss. (a) A saddle point in the fitness landscape (contour map showing extraction probability) is present at $F \simeq 9$ pN and $x_b^\dagger = 1.5$ nm. The ridge line (dashed black line) traces along the direction of the positive principal curvature near the saddle point. The line of steady states (solid red line), where the gradient in $x_b^\ddagger$ vanishes for a given F, deviates from the ridge line as force gets weaker, reflecting an asymmetry about the saddle point. (b) Snapshots of evolving population density (color coded) in the F-$x_b^\ddagger$ space, starting from different levels of force heterogeneity. Extraction contours are evaluated with population-mean affinity at each time point. At an intermediate force heterogeneity (middle row, $\sigma_{F_0} = 0.6F_{av}$), a population splits into two and remains bimodal for extended periods of time. Very small or very large heterogeneity (top and bottom rows) leads to a single crowd with similar force magnitudes. (c),(d) Distributions of the evolved antibody quality Q and B cell fitness λ for zero (blue), intermediate (red), and maximum (brown) initial force heterogeneity; dashed lines indicate initial distributions. B cell lineages with diverse binding qualities (c) can have similar fitness (d) and coexist. (e) A violin plot shows that an intermediate force heterogeneity results in a broad range of diversity σ_Q of evolved binding quality. Each violin is obtained from 20 simulations, with the horizontal bar indicating the ensemble average. (f)–(h) Evolution of the diversity of binding quality σ_Q at zero (f), intermediate (g), and high (h) initial force heterogeneity. The histogram on the side shows the final distribution of σ_Q. Parameters are $\Delta G_a^\ddagger = 14k_BT$, $x_a^\ddagger = 1.5$ nm, $F_{av} = 10$ pN, $t_f = 100$. Mean and width of initial distributions are $\Delta G_{b0}^\ddagger = 14k_BT$, $\sigma_{0,G_b} = 0.2k_BT$, $x_{b0}^\ddagger = 2$ nm, $\sigma_{0,x_b} = 0.5$ nm. Constant force, no antibody feedback.

the $x_b^\ddagger$ dependence, giving rise to the fitness optimum at soft bonds. Under strong force, activation barrier dominates the dependence in favor of stiff bonds, resulting in the competing fitness peak (Fig. S3 [56]).

Evolution of the antigen extraction system takes a surprising turn because of this saddle point. Since evolution primarily operates on the bond length and binding affinity rather than the extraction force, we assume that force

remains constant along lineages descending from the same founder cell, i.e., fully inheritable. Green arrows in Fig. 5(a) show examples of the direction and magnitude of fitness gradient under this restriction: if the initial state is above (below) the red line, then the bond becomes more rigid (flexible). This line of steady states (with vanishing gradient in $x_b^{\ddagger}$) is close to the ridge line under strong forces but above it under weak forces.

We now follow the evolution of cell populations on this landscape according to stochastic agent-based simulations. We start all populations with a certain amount of initial diversity in bond length and binding affinity, but vary the initial diversity of the extraction force σ_{F_0}. Figure 5(b) shows typical examples starting close to the saddle point. The fitness landscape changes over time due to the evolution of binding affinity, which does not significantly affect the saddle point topology. Under zero initial force diversity (top row), mean bond length shifts toward $x_a^{\ddagger}$ while the fitness increases. With intermediate force diversity (middle row), population distribution splits into two groups with comparable fitness, one with higher extraction force and shorter bonds and the other with lower force and longer bonds. This bimodal distribution is clearly a consequence of the presence of the saddle point in the fitness landscape. One might expect that this population split will be enhanced by increasing the initial force heterogeneity. As we increase σ_{F_0} by a considerable amount (from 6 to 10 pN), the split completely disappears (bottom row); there is a single population with a weak average force and a large mean bond length. The asymmetry of the saddle point is responsible for this: if the force distribution is sufficiently wide, cells with a force in the few pN range will rapidly evolve toward the fitness maximum, which then outcompete cells with large extraction force and small bond lengths. We note that the existence of the saddle point enables, rather than guarantees, a persistent diversity of binding phenotype. To best exploit the landscape topology for diversifying selection, populations should distribute their founder clones near the saddle point and away from any fitness peak [like in Fig. 5(b), middle row].

Figure 5(c) compares the distributions of intrinsic binding quality Q at the end of evolution for zero (blue), intermediate (red), and large (brown) force heterogeneity (dashed line showing the initial distribution). Note that the double-peaked distribution for intermediate heterogeneity extends toward very low intrinsic quality (negative Q indicating values lower than the initial mean). One would expect that the case of largest force heterogeneity produces B cells with highest fitness, because it most strongly expands the fittest subpopulation. Comparing the final fitness distributions for three cases [Fig. 5(d)], the brown curve indeed shows a higher peak at a slightly larger fitness than those of the red and blue curves. However, the widths of the three distributions exhibit a significant overlap. It appears that, because of the saddle point, lineages with diverse binding qualities can have similar fitness and are able to coexist. This thus provides an alternative explanation for the persistence of low-quality clones, even under strong selection.

Since B cell populations evolve in concurrent germinal centers, to what extent does the diversity of binding quality vary from one GC to another? Figure 5(e) compares the outcome of population ensembles evolved under varying initial force heterogeneity. Shown are probability densities of the diversity of final binding quality σ_Q in a violin plot. For zero initial force heterogeneity, the distribution is approximately Gaussian; σ_Q is narrowly distributed among populations. As force heterogeneity is increased, the distribution acquires a tail of populations with much larger diversity. Then, for intermediate force heterogeneity, the distribution becomes bimodal, with a very wide range of diversities. Among 1000 realizations, about 60% remain bimodal until the end, while nearly equal proportions of the rest become either fully low force and soft bonds or fully high force and stiff bonds, reflecting stochasticity in mutation and reproduction. When force heterogeneity is increased further, the distribution returns to approximately Gaussian, though still with a tail toward large diversity. Figures S4–S9 in SM [56] present temporal characteristics of coevolving subpopulations.

It is worth pointing out that heritability of force magnitude is essential for bimodality and the nonmonotonic dependence on initial force heterogeneity. If force is noninheritable (resampled from the initial distribution at each GC cycle), such that cells along a lineage may pull at different strengths, the evolved diversity of binding quality will remain low and insensitive to initial force heterogeneity (see SM text and Fig. S10 [56]). Indeed, Mitchell *et al.* [64] found that inheritable variability among founder cells contributes more to heterogeneity in B cell fate than intrinsic noise during proliferation.

Finally, we examine the evolution of the diversity distribution [see Figs. 5(f)–5(h) herein and Fig. S11 in SM [56]]. At zero force heterogeneity, the diversity distribution remains narrow until the end (histogram on the side). For intermediate force heterogeneity, the rate of diversity loss varies widely among populations (Fig. S11C [56]), resulting in a wide variety of final diversity of binding quality [Fig. 5(g)]; *in vivo* studies of GC dynamics have indeed reported widely disparate rates of diversity loss from GC to GC [21]. When starting with a very large diversity of extraction force, there is initially a steep rise in diversity, indicating population split, similar to the case of intermediate heterogeneity. But lineages with low force and soft bonds soon take over, leading to a rapid loss of diversity in binding quality.

Therefore, the presence of the saddle point *permits*—for intermediate force heterogeneity—evolution of B cell lineages with similar fitness but a very broad range of binding quality and a wide variety of force diversity.

The key is that, for long periods, the trajectories remain restricted to the saddle point region and this prevents evolution toward a single dominant peak in the fitness landscape. This result suggests a physical means by which energy-consuming microscopic processes can diversify cellular phenotype without compromising clonal reproductive fitness. In this sense, tug-of-war antigen extraction might have evolved to balance the trade-offs between the potency of response to the current pathogen and the breadth of coverage against future escape mutants or related pathogens. Such trade-offs may stem from resource constraints in support of GC reaction and immune memory formation, maintenance, and renewal.

IV. DISCUSSION

The adaptive immune system offers a unique opportunity for observing *in vivo* rapid evolution of molecular recognition: the process of affinity maturation iteratively alters the B cell repertoire and yields functional readouts on molecular, cellular, and organismic scales. While specificity and potency are desirable receptor traits to evolve, B cell selection is not simply favoring strong equilibrium binding to antigens. Rather, how many times a B cell divides upon activation reflects its ability to physically acquire antigen through active molecular processes. As an attempt to probe the limit and potential of immune adaptation, we present a theory that maps binding affinity to clonal fitness via antigen extraction, and use this mapping as the basis of B cell selection during *in silico* affinity maturation. This framework allows us to explore how active forces and physical constraints shape selection pressure, revealing alternative functional objectives.

Earlier models of affinity maturation offer useful insights into basic design features of mutation-selection cycles from the viewpoint of optimal control [3,43,67,68]. A recent revival of modeling effort was driven by a need for understanding antibody evolution in response to complex selection pressures, when diverse antigen variants are presented to the immune system as vaccines [4–7,69–71]. A primary goal of these studies was to devise strategies for eliciting broadly neutralizing antibodies against rapidly evolving pathogens. Nearly all existing models of affinity maturation assume that selection depends on equilibrium binding constants, in a similar spirit to how *in vitro* affinity measurements and directed evolution are conducted. One exception is a population dynamics model in which antigen acquisition is considered [32]. However, this work does not address the role of force and extraction dynamics in clonal selection. Our model provides a first coarse-grained microscopic description of nonequilibrium antigen recognition, highlighting the importance of kinetics and the comparative nature of affinity measurements *in vivo*. By coupling this physical theory to *in silico* competitive evolution, we are able to identify significant impact of active forces on evolved responses, with both limiting and enabling effect.

It has been speculated that *in vivo* antibody affinity should be limited to ensure response specificity [72] and to avoid autoimmunity [73]. Our theory suggests that *in vivo* affinity ceiling might be of a physical origin, stemming from force-modulated effective tether strength. Since tugging forces effectively strengthen stiff tethers, stronger pulling can serve to sustain selection pressure for improving BCR-Ag affinity. However, too strong forces may cause cell death due to failure of antigen extraction. This trade-off between response quality and magnitude thus sets a limit to evolvable antibody affinity. Our analysis further provides a quantitative prediction, namely, a logarithmic dependence of ceiling affinity on effective tether strength, which can be tested by varying force magnitude or tether characteristics and measuring the saturation affinity of evolved antibodies.

The enabling role of active force usage lies in how it influences the adaptive potential against future threats. Our model suggests that molecular noise in force generation can diversify binding phenotype (combination of force magnitude and bond length). By exploiting a saddle point in the fitness landscape emerging from the tug-of-war setting, competing clones assuming a wide variety of binding phenotypes and affinities may have similar fitness and hence coexist for extended time. Saddle point topology, combined with constrained heterogeneity of force magnitude, rationalizes multiple experiments, including retention of low-quality clones, coexistence of lineages with varying affinities, and diverging rates of diversity loss among B cell populations. We note that our proposal does not exclude other contributing mechanisms. For instance, in the case of complex antigens composed of multiple epitopes, synergistic interactions among coevolving B cell lineages, e.g., through binding to allosterically coupled epitopes, can also sustain low-affinity clones [70]. Another contribution may arise from responses to non-native antigen forms [9].

The emergence of phenotypic plasticity should bear multiple observable signatures, measurable using a combination of techniques, including single-molecule force spectroscopy (bond length), traction force spectroscopy or DNA-based tension sensors (pulling force magnitude), and antibody-antigen binding assays (antibody quality). First, the predicted correlation between force magnitude and bond length (high force and short bond or low force and long bond) can be sought among matured B cells exported from individual GCs. By collecting cells from multiple GCs at the start and end of a response, one can test for a nonmonotonic dependence of evolved diversity in binding quality on initial force heterogeneity. Further, if time series data can be obtained from longitudinal tracking of force diversity, slowest loss in diversity would be expected at intermediate levels of initial force heterogeneity.

A tug-of-war extraction system also allows cells to sense and adapt to mechanical cues in the physical environment, utilizing the tethering interaction to perform comparative measurements and create dynamic feedback. For example,

APCs can alter their stiffness in response to inflammatory signals from the innate immune system [66], or at a faster speed, via mechanical feedback such as strain stiffening [65] under B cell contractility. The model predicts a "stiffness-matching" behavior in which BCRs evolve to mimic the stiffness of the tethering complex, suggesting affinity discrimination as a potential functional objective, because most sensitive discrimination between similar BCR affinities is achieved when tugging and tethering bonds match in stiffness.

In contrast to the barrier height $\Delta G_b^\ddagger$ that increases considerably through affinity maturation, the extent and manner in which the bond length $x_b^\ddagger$ is altered due to evolution is less definite and strongly depending on the antigen involved. For some antigen, $x_b^\ddagger$ remains similar among antibody variants [74], whereas for a different antigen, $x_b^\ddagger$ changes in proportion to $\Delta G_b^\ddagger$ [61]; hence a higher affinity might be accompanied by a larger bond length. Recent studies have quantified antibody flexibility as a function of evolution and showed that, while mutations may act cooperatively to rigidify the protein [75,76], affinity maturation can generate a spectrum of changes in flexibility [61,62,77], indicating a diversity of biophysical mechanisms for increasing affinity. Our model predicts that rigid BCRs or antibodies evolve under strong tugging forces while flexible ones are selected under weak pulling. It will be interesting to search for the predicted force-bond length correlation among B cells or antibodies raised against antigens with different physical attributes (e.g., size, shape, charge). If proven, this correlation would imply an impact of force on clonal selection and suggest a mechanism for the diverse trend of bond-length variation through affinity maturation.

We identify an unexpected mismatch between conditions under which training and testing of antigen recognition are conducted, suggesting that active sensing by cells may not simply optimize receptor potency against current target. To construct alternative objectives in optimization schemes, a systematic understanding of the physical basis of immune sensing and adaptation is needed to characterize trade-offs between evolvable traits, such as force-stiffness and affinity-flexibility relations provided by our model. In addition, this training-testing discrepancy suggests an asymmetry between antigenicity and immunogenicity, i.e., distinction between B cell activation and antibody recognition. This asymmetry was predicted to play a large role in determining the course and fate of viral-immune coevolution [78].

Immune memory formation upon repeated exposure to pathogens or their antigens is a topic of lasting interest and long-standing debate. The phenomenon of immune imprinting [26], by which the immune repertoire is strongly directed toward the primary infecting strain even after the virus has drifted antigenically, appears to indicate highly effective reactivation of memory B cells. A recent prime-boost study in mice, however, showed that secondary responses are strongly restricted from reengaging the large diversity of memory B cells generated by priming, but are instead dominated by very few clones [79]. Our model suggests that variability in internal dynamics of cells might supply a persistent diversity of binding phenotype that compensates for the apparent loss in genetic diversity upon GC reseeding. We speculate that this phenotypic route of diversity generation and maintenance can be advantageous: It permits efficiency in limiting viral harm while circumventing the cost associated with responding *de novo*. Moreover, in synergy with restricted clonality, it may mitigate self-confinement of immune repertoire due to backboosting of existing memory clonotypes—the phenomenon of original antigen sin that limits the efficacy of vaccines [80]. It does so by providing temporary immune coverage as new memories evolve from naive ancestors. In fact, recent modeling work [7] suggests that loss in clonal diversity upon boosting can favorably support the expansion and dominance of cross-reactive clones, under serial exposure to distinct but related antigen variants.

One omitted characteristic in our theory is cellular organization of contact patterns, as evolving B cells collect antigens into clusters and extract them using forces. It will be interesting to study cluster extraction via synaptic contact, by incorporating tug-of-war dynamics to the active membrane model we earlier developed [47], and to examine potential trade-offs between speed and accuracy of affinity discrimination. This augmented framework will also allow us to investigate design principles of sensing structures and control algorithms for meeting potentially incompatible functional needs. Another future direction is to study how response breadth and potency depend on heritability of force magnitude and other traits, in both constant and fluctuating environments. We hope that our analysis of a minimal model of immune adaptation will motivate further work on conditions under which biological systems adapt by exploiting physical influences on function and evolution.

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