the above modeling framework is equivalent to the zero-temperature dynamics of an asymmetric spin glass on a graph. Using this equivalence, we characterize an edge $i \to j$ in state $\{s_i\}$ as frustrated [15] if $J_{ij}s_is_j < 0$, i.e., if the values of node i and node j in that state do not follow the regulatory relationship between the two nodes. Then, the frustration of a state can be defined as the fraction of network edges that are frustrated in that state. If a network involves regulatory relationships that conflict with one another, all regulatory relationships cannot be satisfied in any state. Hence, such a network will have stable states with non-zero frustration.

Comparison of biological and random networks.— We determined the stable states of five biological networks taken from the literature [14, 16–19] (see Supplemental Material [20], section II (a)) and compared the frustration of the stable states of each of these networks with the frustration of the stable states of random networks with topological features similar to the biological network (each random network had the same total number of nodes and edges, node in- and out-degree distributions, and the total number of activating and inhibitory relationships between nodes in the network as the corresponding biological network) (Fig. 1; also see Supplemental Material [20], Fig. S1 and section II (b)-(c)). In the case of each biological network, most stable states had frustration comparable to the frustration of each of the stable states of the corresponding random networks. However, each biological network had a set of stable states with frustration much lower than the frustration of the stable states of random networks. Crucially, biological networks are highly likely to end up in one of these minimally frustrated stable states when their dynamics is simulated starting from random initial conditions (Fig. 1: blue (black in print) violins in each panel; also see Supplemental Material [20], Fig. S2). Minimally frustrated stable states are thus likely to be biologically significant, with most cells in a population exhibiting gene expression patterns corresponding to these states.

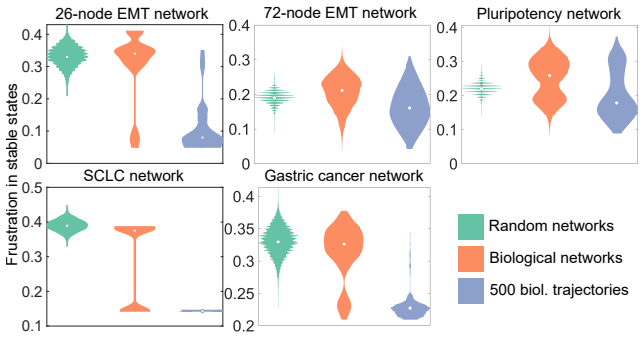

FIG. 1. (Color online) Distribution of frustration for the stable states of biological networks and random networks. The blue (black in print) violin in each panel shows the frustration of the states one ends up in when simulating the dynamics of the biological network starting from 500 random initial conditions. In the case of the 72-node EMT network and in the case of the pluripotency network, 5000 least frustrated stable states of each random network have been shown. The white circle in each violin indicates the median.

Relation between stables states and biological phenotypic states.— We next investigated if any structural patterns underlie the organization of stable states of biological networks. The distribution $P(q_{\alpha\beta})$ of the overlap between network states $q_{\alpha\beta} = \sum_i (s_i^\alpha s_i^\beta)/N$ was found to be very broad when α, β pairs are chosen randomly from the set of stable states of biological networks. This indicates a hierarchical organization of stable states [21]. However, when the pairs are sampled from among the minimally frustrated stable states, $P(q_{\alpha\beta})$ is bimodal with peaks near $+1$ and -1 (Fig. 2 (a) and 2 (d); also see Supplemental Material [20], Fig. S3 (a), S3 (c), and S3 (e)). Since $q_{\alpha\beta}$ is a measure of similarity between the states $\{s_i^\alpha\}$ and $\{s_i^\beta\}$, a collection of states with $q_{\alpha\beta}$ close to $+1$ for all pairs represents a collection of cells with reasonably similar gene expression profiles, to be associated with a distinct cell phenotype. A bimodal $P(q_{\alpha\beta})$ for minimally frustrated stable states of biological networks considered here thus suggests that these states constitute two stable cell phenotypes.

In the case of the network regulating epithelial-mesenchymal transition (EMT) [14], minimally frustrated stable states represent the two canonical phenotypic states, epithelial and mesenchymal (Fig. 2 (b)-(c); also see Supplemental Material [20], Fig. S3 (b)). In the case of the network regulating pluripotency and differentiation in human embryonic stem cells [18], minimally frustrated stable states define stem and differentiated cell types (Fig. 2 (e)-(f)). In contrast, high frustration stable states of biological networks involve co-presentation of molecular markers corresponding to conflicting biological behaviors. For instance, high frustration stable states of the network regulating EMT involve co-presentation of epithelial and mesenchymal markers (Fig. 2 (b)-(c); also see Supplemental Material [20], Fig. S3 (b)). Similarly, high frustration stable states of the network regulating the neuroendocrine-mesenchymal transition [16] involve co-presentation of neuroendocrine and mesenchymal markers (see Supplemental Material [20], Fig. S3 (d)). These stable states thus represent ambiguous cell fate choices. Such ambiguous phenotypic states have been reported in cancer cells across disease sub-types [16, 22], but appear to be suppressed in healthy tissue. To relate more directly to experimental data, we note that deletion of molecular species like GRHL2, OVOL2, and ΔNP63α from the 26-node EMT network can lower the frustration of network stable states and decrease the fraction of stable states with co-presentation of epithelial and mesenchymal markers (see Supplemental Material [20], Fig. S4). Indeed, loss of expression of such