

the clustering coefficients of the yeast two-hybrid interaction networks are lower bounds on the clustering coefficients of the complete interactomes and network topologies with significantly lower clustering coefficients than observed can be ruled out. These results suggest that interactomes are highly clustered, much more than randomly clustered networks of any topology (Supplementary Tables 1 and 2 online).

Because these results do not exclude a highly clustered topology different from a scale-free topology, we repeated the simulations described by Han *et al.* for exponential networks to determine at which coverage rates the transition from an exponential to a scale-free topology occurs. For all average degrees considered, coverage rates had to be lowered drastically to achieve this transition. Thus, to allow an exponential distribution in the original network, high error rates and consequently high original clustering coefficients would have to be assumed. If such a high degree of clustering and error appears unreasonable, the obvious conclusion is that the original interactome does in fact exhibit a scale-free topology, even though the associated power coefficient may be different from the one in the observed sample.

We conclude that limited sampling in large-scale experiments affects several aspects of the network topology apart from the degree distribution and thus additional characteristics have to be considered when evaluating sampling effects. Our simulations on the average clustering coefficient indicate that if a scale-free topology with high clustering coefficients is observed in a high-throughput experiment, the underlying network very likely also is scale-free and highly clustered.

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#### Marc Vidal and colleagues respond:

The distorting effect of limited sampling on the overall topology of interactome models, relative to the actual network they are supposed to represent, is a subject of great interest. Our paper showed that out of four different topological models examined, scale-free networks were most likely to produce

scale-free samples, although we could not formally exclude any of the other models by the criteria tested. Friedel and Zimmer suggest that limited sampling not only affects the degree distribution of a network, but can also distort its average local clustering coefficient (CC)<sup>1</sup>.

Their study concurs with our conclusions in two respects: first, partial networks can have different topological properties relative to the complete network they are derived from; and second, the power-law model is the most likely of the tested models. By demonstrating that the exact topology of the interactome could not be determined unambiguously from currently available data sets, our initial report is a reminder that the quality and coverage of interactome network maps needs to be improved. To question the generally accepted view that actual interactome networks are indeed scale-free, given the topology of the current maps, only one alternative model is needed and we proposed three of them. On the contrary, to conclude that the interactome can only be scale-free, as suggested by Friedel and Zimmer, would require proof by negation. Out of the large number of potential interactome topologies, how many can be excluded because they are inconsistent with the observations drawn from available maps, and what are the common properties of the remaining

ones? A high CC could be one of these characteristics, in which case some scale-free models would remain good candidates. However, many other possibilities have yet to be excluded before the issue is resolved.

We suggest that before concluding that “the underlying network very likely also is scale-free and highly clustered,” it would be appropriate to test whether other topological models with high CC could give rise to the topology observed in currently available interactome maps. For instance, is it sound to limit the rewiring of a scale-free model to generate a highly clustered scale-free model network so as to avoid “a deviation from the power-law behavior for nodes with low degrees”? Could the sampling of such networks result in sampled networks having topological properties consistent with those of the protein-protein interaction maps currently available?

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## On the path to TCR-directed therapeutics

### To the editor:

The prospect of developing T-cell receptors (TCRs) as therapeutic biomolecules has been hampered by the difficulties associated with the isolation of monoclonal T-cell lines that allow identification of clonotypic TCRs with defined antigen specificity and the low success rate of functional recombinant heterodimeric TCR production. In the November 2004 issue, Ramu Subbramanian *et al.* (*Nat. Biotechnol.* **22**, 1429–1434, 2004) describe a novel method to generate recombinant TCRs that promises to make investigations into the biotechnological



potential of these molecules much easier. By randomly expressing in insect cells all possible combinations of TCR  $\alpha$ - and  $\beta$ -chains amplified and cloned from an antigen-specific polyclonal CD8<sup>+</sup> T-cell population, the authors selected heterodimers of interest in flow cytometric binding assays with the cognate antigen. They then used these TCRs in soluble tetrameric form to characterize the proteins

further in both flow cytometric and functional assays. This technique therefore circumvents the need to isolate and expand single T cells and increases the likelihood of yielding

functional TCRs because a large number of  $\alpha$ - and  $\beta$ -chain pairs are screened before the recombinant protein expression step.

There remain, however, two major obstacles to the use of recombinant TCRs as antigen-targeting tools. First, T-cell recognition is inherently degenerate; thus, any given T-cell clonotype can potentially cross-react with a vast number of structurally distinct peptide-major histocompatibility (MHC) complexes, including self-derived antigens expressed on the surface of healthy cells<sup>1</sup>. Second, TCR/peptide-MHC interactions, in the periphery at least, are characterized by low affinities and rapid kinetics.

Although Subbramanian *et al.* satisfactorily assessed the MHC allelic restriction of their TCR tetramers, they did not report the more subtle peptide-dependent specificity of these reagents. We have used soluble TCR tetramers for the specific detection of target cells pulsed with cognate peptide, discrimination of quantitative changes in antigen display at the cell surface, identification of virus-infected cells, inhibition of antigen-specific cytotoxic T lymphocyte activation and identification of cross-reactive peptides<sup>2</sup>. We found that naturally occurring TCRs have the ability to cross-react with some self-derived peptide-MHC complexes to a degree that enables cellular targeting by tetrameric TCR<sup>2</sup>. Thus, the cross-reactivity that characterizes T-cell recognition at the cellular level extends to soluble recombinant forms of the corresponding antigen receptors.

To overcome the problem posed by the low affinity and rapid kinetics of TCR/peptide-MHC interactions, TCRs can either be multimerized, which reduces the composite dissociation rate, or mutagenized using yeast cell surface display technology to generate high-affinity TCRs<sup>3–6</sup>. Li *et al.*<sup>7</sup> have recently extended the latter approach, using phage display to produce human TCRs with affinities for cognate antigen that are several orders of magnitude greater than the parent receptor.

Direct comparison indicates that TCRs selected by phage display, as described by Li *et al.*<sup>7</sup>, are as biologically active as monomeric molecules and can exhibit substantially less relative cross-reactivity than the corresponding tetrameric parent TCR<sup>2</sup>. The cross-reactive nature of wild-type TCRs<sup>2</sup> led Subbramanian *et al.* to propose only limited applications for recombinant TCR technology, namely human leukocyte antigen (HLA) typing. In contrast, our results show that engineered high-affinity TCRs selected for improved binding to one particular ligand do not necessarily exhibit significantly

increased affinity for syngeneic mimic peptide-MHC molecules<sup>2</sup>.

Thus, a combination of two technologies recently described in *Nature Biotechnology*—those of Subbramanian *et al.* and Li *et al.*<sup>7</sup>—should allow the rapid selection of high-affinity TCRs without high levels of cross-reactivity and potentially holds the key to the development of these recombinant molecules for more ambitious applications<sup>8</sup>. Antibodies with TCR-like specificity also offer an attractive alternative to the targeting of peptide-MHCs<sup>9–11</sup>. Further work will be required to determine which reagents are best suited for antigen targeting *in vivo*.

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## Patenting pathways

### To the editor:

Many in the scientific community are concerned that access to crucial information will be constrained by the increasing number of patents covering basic research results<sup>1–3</sup>. In the December issue, Allarakhia and Wensley (*Nat. Biotechnol.* **23**, 1485–1488, 2005) argue that restricted access to patented research data is especially detrimental to systems biologists, who rely on data from basic research to analyze complex biological networks. Although it is true that an abundance of life science patents could stunt the advancement of scientific knowledge, we believe the article overstates the patentability of scientific research under current United States

and international patent law, leaving the impression that researchers can patent almost any information discovered in the laboratory. This is not the case, for the patent laws of every industrialized nation place clear limits on patenting biological subject matter.

To be patented in the United States, an invention must fulfill four requirements: it must consist of patentable subject matter, be useful, novel and nonobvious. Section

101 of the US Patent Act broadly defines patentable subject matter as “any new and useful process, machine, manufacture or composition of matter.” This has long been interpreted to permit patents only for scientific discoveries and inventions that do not occur in nature.

The Supreme Court applied this requirement in the landmark decision *Diamond v. Chakrabarty*, where it held that a genetically engineered microorganism designed to break down crude oil was patentable subject matter. In reaching this decision, the Court reiterated that “laws of nature, physical phenomena and abstract ideas” are not patentable<sup>4</sup>. Nevertheless, the justices concluded that

the “microorganism plainly qualifies as patentable subject matter” because rather than being “a hitherto unknown natural phenomenon, [it was] a non-naturally occurring manufacture or composition of matter—a product of human ingenuity—having a distinctive name, character [and] use”<sup>4</sup>.

Under this framework, systems biologists usually operate outside of the realm of patentable subject matter. Systems biology

