molecule have previously been described (25, 26). In contrast to these bispecific formats, the dual specific “two-in-one” antibody we describe has the molecular structure of a regular IgG (or Fab). It has all the favorable attributes of an IgG for therapeutic development, such as predictable pharmacokinetic properties, well established manufacturing protocols, choice of Fc-mediated effector functions, and bi-or mono-valencies (25).

In summary, we have demonstrated that an antigen binding site is capable of interacting with two unrelated protein antigens with high affinity. The dual specific antibodies reported here are derived from a monospecific antibody through mutations in the periphery of the antigen binding site in the LC CDRs. This strategy is a general one and can be applied to create dual specific antibodies against two distinct antigens. The mutational analysis of bH1 and bH1–44 (Fig. 2 and fig. S8) suggested that the dual specificity could be switched to monospecific binding to either antigen (10). Indeed, bH1–44 lost binding to VEGF but retained HER2 binding when mutating two LC residues. Similarly, two alanine mutations in the HC drastically reduced the affinity for HER2 while preserving tight binding for VEGF (fig. S9). This finding highlights how a limited number of mutations in the antigen binding site can alter specificity or add a distinct specificity. During development of the natural antibody repertoire, the antigen binding sites often undergo diversification by exchanging the VΔ that pairs with a VH (6). Somatic mutations also occur frequently, in particular among the residues in the periphery of the antigen binding site (27–30). Our studies reveal a mechanism by which one antibody can diverge into many antibodies with distinct specificity profiles. This mechanism may contribute to the large capacity of the natural antibody repertoire for diverse antigen recognition.

References and Notes
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31. We thank the Protein Engineering structure group, L. Haber, and D. McMahon for technical consultation and C. Eisenbut for helpful advice. We also thank members of the DNA synthesis, DNA sequencing, in vivo cell culture, and protein chemistry groups at Genentech. We are grateful for the support of R. Neutze and Chalmers University of Technology, Sweden. The Advanced Light Source is supported by the Director, Office of Science, Office of Basic Energy Sciences, of the U.S. Department of Energy under contract no. DE-AC02-05CH11231. The atomic coordinates of the bH1/HER2 and bH1/VEGF were deposited in the Protein Data Bank with accession numbers 3BDY and 3BE1, respectively. G.F. and J.B. are inventors of the patent application “Multispecific Antibodies” (Pub. App. No. 20080069820), which relates to this work. All authors were or were under the employment of Genentech and declare competing financial interests.

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Ankyrin-G Promotes Cyclic Nucleotide–Gated Channel Transport to Rod Photoreceptor Sensory Cilia

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Cyclic nucleotide–gated (CNG) channels localize exclusively to the plasma membrane of photosensitive outer segments of rod photoreceptors where they generate the electrical response to light. Here, we report the finding that targeting of CNG channels to the rod outer segment required their interaction with ankyrin-G. Ankyrin-G localized exclusively to rod outer segments, coimmunoprecipitated with the CNG channel, and bound to the C-terminal domain of the channel β1 subunit. Ankyrin-G depletion in neonatal mouse retinas markedly reduced CNG channel expression. Transgenic expression of CNG channel β-subunit mutants in Xenopus rods showed that ankyrin-G binding was necessary and sufficient for targeting of the β1 subunit to outer segments. Thus, ankyrin-G is required for transport of CNG channels to the plasma membrane of rod outer segments.

Cyclic nucleotide–gated (CNG) channels initiate the electrical responses to light in photoreceptors and to chemical stimuli in olfactory neurons (1). CNG channels are segregated to sensory cilia, where visual and olfactory signal transduction takes place. This precise intracellular localization is dependent on the channel’s β subunit (CNG-β1, CNGB1) in both classes of neurons (2–4). However, the molecular mechanism(s) of CNG channel targeting to the plasma membrane of sensory cilia, where this channel normally functions, are unclear.

Ankyrin-G is a versatile membrane adaptor involved in the formation and maintenance of diverse specialized membrane domains (5–9). Ankyrin-G is localized exclusively to rod outer segments (ROSs), where it was found along with CNG channels that have been localized to the ROS plasma membrane (10, 11) (Fig. 1, A and B). In contrast, the plasma membrane of the inner segment was lined with ankyrin-B, which is required for the coordinated expression of the Na+ and K+-dependent adenosine triphosphatase (ATPase), Na+ and Ca++ exchanger, and β2-spectrin (12) (Fig. 1A). Localization of ankyrin-G to the plasma membrane was evident in isolated mouse ROSs, but was better demonstrated in frog ROSs, which are three to four times as large in diameter (Fig. 1B). Ankyrin-G also localized in the olfactory sensory cilia and the principal piece of sperm flagella, together with CNG-β1 (4, 13) (fig. S1). We treated isolated bovine ROSs with a cleavable cross-linker, solubilized them in 0.1% SDS, and used ankyrin- or CNG-β1–specific antibodies for immunoprecipitation. We observed the reciprocal coimmunoprecipitation of CNG-β1 and ankyrin-G (Fig. 1C). The interaction between ankyrin-G and specific antibodies, because CNG-β1 was not precipitated by nonimmune or ankyrin-B-specific antibodies,

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and the major ROS-specific protein, rhodopsin, was not precipitated in either case (Fig. 1C).

The CNG channel binding to ankyrin-G was further evaluated using a HEK 293 cell-based assay for detecting ankyrin–membrane protein interactions (14). In this assay, overexpressed exogenous ankyrin-G fused to the C terminus of green fluorescent protein (GFP) (ankyrin-G–GFP), which is normally localized to the cytoplasm, is recruited to the plasma membrane when ankyrin binding partners, such as neurofascin, are coexpressed. CNG-α1 expressed in HEK 293 cells localized to the plasma membrane but did not recruit ankyrin-G–GFP (Fig. 1D), whereas CNG-β1 failed to localize to the plasma membrane of these cells when expressed by itself (fig. S2). Coexpression of CNG-α and CNG-β does yield functional heterotetrameric channels in the plasma membrane of HEK 293 cells (15). Such coexpression also resulted in efficient plasma membrane recruitment of ankyrin-G–GFP (Fig. 1E); complete recruitment was observed in 65% of cells coexpressing the three proteins (n = 50). Ankyrin-B–GFP was not recruited to the plasma membrane under similar conditions (Fig. 1E), which indicated that CNG-β1 interacts with ankyrin-G.

We next sought to evaluate whether ankyrin-G is required for localization of CNG channels to ROSs in vivo, using short hairpin RNA (shRNA) to deplete (knockdown) ankyrin-G expression in neonatal mouse retinas. We injected a mixture of a plasmid encoding shRNA targeting mouse ankyrin-G in 10-fold excess over a plasmid encoding GFP into the eyes of newborn pups followed by electroporation (16). Under these conditions, rods expressing GFP are typically co-transfected with the shRNA plasmid. Two weeks postinjection, photoreceptors transfected with control shRNA expressed GFP, displayed normal morphology, and were robustly immunostained with ankyrin-G (Fig. 2). In contrast, photoreceptors transfected with ankyrin-G shRNA (GFP-) displayed a major reduction in the ankyrin-G immunofluorescence in ROSs [20 to 30% of control, based on immunofluorescence intensity of samples on the same slide (fig. S3)], and their ROSs were significantly shortened [average length of 4.7 μm versus 15.5 μm in control rods (n = 25); compare rhodopsin-labeled sections]. The immunofluorescence levels of both CNG-β1 and CNG-α1 were also markedly reduced, to a degree comparable with the ankyrin-G reduction (Fig. 2 and fig. S3). In contrast, rhodopsin levels estimated by fluorescence intensities of samples on the same slide were similar for control ROSs and ROSs expressing ankyrin-G shRNA (Fig. 2 and fig. S3). The shortened ROS phenotype in ankyrin-G-depleted retina was more severe than reported for mice lacking the CNG-β1 subunit (2). Thus, ankyrin-G plays role(s) in either assembly and/or maintenance of ROSs in addition to localization of the CNG channel. This result is similar to the requirement of ankyrin-G for biogenesis of the lateral membrane in cultured columnar epithelial cells (17).

The reduction in CNG channel expression in ankyrin-G–depleted rods could be explained by a requirement for ankyrin-G in targeting the channel to ROS plasma membrane from the endoplasmic reticulum or Golgi located in the inner segment. Indeed, ankyrin-G is required for both post-Golgi transport and immobilization of its binding partner E-cadherin in epithelial cells (6). To test this hypothesis, we needed to identify CNG-β1 mutants lacking ankyrin-G binding. We first determined

Fig. 1. Ankyrin-G is restricted to photoreceptor outer segments and binds the rod CNG channel. (A) Colocalization of ankyrin-G (AnkG, red, left two columns) with CNG channel (CNG-β1, green) in ROSs and colocalization of ankyrin-B (AnkB, red, right two columns) with Na+– and K+–dependent ATPase (NKA) (green) in inner segments (IS). A schematic of a rod cell is shown to the right. (B) Ankyrin-G (red) localizes to the plasma membrane of isolated mouse and frog ROSs labeled with CNG-β1–specific antibody (green). ROS tangential sections are shown (top) and longitudinal sections (bottom). (C) Coimmunoprecipitation of ankyrin-G with CNG-β1 channels from bovine ROS extracts. Antibodies used for precipitations are indicated on the top (immunoglobulin G, IgG), antibodies used for protein detection are indicated on the left. (D) CNG-α1 (white) alone does not recruit ankyrin-G–GFP (green) to the plasma membrane of HEK 293 cells. (E) Ankyrin-G–GFP (green) is recruited to the plasma membrane of HEK 293 cells coexpressing CNG-β1 (red) and CNG-α1 (white). Scale bars: 5 μm in (A), 10 μm in (D) and (E).

Fig. 2. Ankyrin-G is required for ROS morphogenesis. Retinas of newborn mice were electroporated with either ankyrin-G shRNA or control pFIV (3 μg/μl) plasmid, each mixed with pCAGGS-GFP (0.3 μg/μl) (11). Ankyrin-G (AnkG) staining is shown in green. The staining of rhodopsin (left), CNG-β1 (center), and CNG-α1 (right) is shown in red. GFP staining is shown in white. Scale bars, 10 μm.
whether ankyrin-G bound to either the N- or C-terminal cytoplasmic domain of CNG-β1 (Fig. 3A). Ankyrin-G–GFP was coexpressed with protein constructs in which the ankyrin-binding domain of neurofascin was replaced with either the entire cytoplasmic N or C terminus of human CNG-β1 [NF-CNG-βN (amino acids 1 to 654) and NF-CNG-βC (amino acids 1041 to 1251), respectively (Fig. 3A)]. Ankyrin-G interacted only with the C-terminal domain of CNG-β1, both in the HEK 293–based plasma membrane recruitment assay (Fig. S4) and coimmunoprecipitation experiments (Fig. 3B). Immunoprecipitation experiments in HEK cells were performed in the absence of a cross-linking reagent.

A truncation of the C-terminal 28 residues of CNG-β1 is associated with retinitis pigmentosa (RP; hCNG-βA28) (Fig. 3A) (18). Indeed, neurofascin fused to the CNG-β1 C-terminal domain bearing this deletion failed to recruit or to coimmunoprecipitate ankyrin-G–GFP (Fig. S2A and Fig. 3B). Additional deletion mutagenesis (hCNG-βC1243 and hCNG-βC1236, Fig. 3C) (19) narrowed the interaction site to a seven–amino acid stretch in this region (Fig. 3C, underlined, and fig. S4B), and alanine-scanning mutagenesis revealed that the highly conserved residues Ile1237 and Leu1238 were essential for ankyrin-G binding (fig. S4B and Fig. 3C). Mutant CNG-β1 (RP deletion or IL1237AA mutation, in which alanine (A) replaced isoleucine (I) and leucine (L) at positions 1237 and 1238, respectively) coexpressed with CNG-α1 in HEK 293 cells failed to bind ankyrin-G without affecting the normal CNG-α1 and CNG-β1 association (Fig. 3D).

To test whether ankyrin-G binding was required for delivery of the channel to outer segments, human CNG-β1, either wild type or mutants unable to interact with ankyrin-G, were expressed in the rods of transgenic Xenopus laevis (20). We used a specific antibody against human CNG-β1 (21) to distinguish it from the endogenous Xenopus CNG-β1 (Fig. 4). Wild-type human CNG-β1 (WT) was found in ROSs in a distinctive pattern consistent with its plasma membrane localization (Fig. 4). In marked contrast, both the RP (Δ28) and IL1237AA mutants were confined to perinuclear sites within rod cell bodies and were completely absent from ROSs (Fig. 4).

We next tested whether an ankyrin-G–binding site from an unrelated protein was sufficient for targeting CNG-β1 to ROSs. The native site in CNG-β1 required for interaction with ankyrin-G was replaced with 14 amino acids from β-dystroglycan, which binds ankyrin-G directly (5) and has little sequence similarity with the CNG-β1 motif (Fig. 3C). The CNG-β1–dystroglycan (CNG-β-DAG) chimera associated with ankyrin-G when coexpressed with CNG-α in HEK 293 cells (fig. S5). When expressed in transgenic Xenopus, this chimera was targeted to the ROSs plasma membrane; however, the mutant CNG-β-DAG IIIF/AAA chimera lacking the ankyrin-binding site (fig. S5) (5) was retained in the photoreceptor cell body (Fig. 4). Because there is no retrograde movement of membrane proteins from ROSs back into the cell body (22), we conclude that ankyrin-G binding is both necessary and sufficient for trafficking CNG-β1 to the outer segment. The ankyrin-G pathway could intersect with the Rab11–Kif17/osm3 microtubule motor (23), which is found in ROSs in a distinctive pattern consistent with its plasma membrane localization (Fig. 4). Indeed, neurofascin fused to the CNG-β1 polypeptide fused to neurofascin. The ankyrin-G –GFP was coexpressed in HEK 293 cells with the chimeras shown in (A), cells were lysed, and proteins were immunoprecipitated by HA-specific antibodies. Immunoblots of samples from the starting material (left) and precipitated proteins (right) were probed with HA- or GFP-specific antibodies. Lane numbers correspond to the numbered chimeras in (A).

Fig. 3. The ankyrin-G–binding site resides in a C-terminal motif of CNG-β1. (A) Schematic diagrams of rod CNG-β1 (top) and HA-tagged neurofascin (HA-NF) chimeras with CNG-β1 (bottom). Numbers within parentheses indicate the amino acid ranges of the CNG-β1 polypeptide fused to neurofascin. The abilities of chimeras to recruit ankyrin-G–GFP to plasma membrane of HEK 293 cells is indicated by + or − (AnkBD, ankyrin-binding domain). (B) Ankyrin-G–GFP was coexpressed in HEK 293 cells with the chimeras shown in (A), cells were lysed, and proteins were immunoprecipitated by HA-specific antibodies. Immunoblots of samples from the starting material (left) and precipitated proteins (right) were probed with HA- or GFP-specific antibodies. Lane numbers correspond to the numbered chimeras in (A). (C) Sequence of the 28 C-terminal amino acids of human CNG-β1 and homologous regions from other vertebrates (29). Arrows indicate sites of C-terminal deletions hCNG-βC1243 and hCNG-βC1236 used to identify residues critical for ankyrin-G binding. Colored residues were mutated to alanine; those in red were critical for ankyrin-G binding and those in green were neutral. The human CNG-β1–β-dystroglycan chimera (hCNGβ-DAG) is shown at the bottom with the dystroglycan sequences marked in blue. (D) CNG-β1 (lane 1) CNG-β1Δ28 (lane 2) and CNG-β1 IL1237AA (lane 3) were coexpressed with CNG-α1 in HEK 293 cells and immunoprecipitated using the CNG-β1–specific antibodies. Each CNG-β1 mutant normally coprecipitated with CNG-α1, but failed to bind endogenous ankyrin-G. Starting material is shown on the left and immunoprecipitates on the right.

Fig. 4. Ankyrin-G binding is necessary and sufficient for CNG-β1 transport to ROSs of transgenic Xenopus. Retina sections in each panel are stained with CNG-β1–specific antibody (red) and TOTO-3 to label the nuclei. Nontransgenic tadpole control on the left demonstrates that this antibody does not recognize the endogenous channel. Other panels depict the localization of wild-type (WT) human CNG-β1 or its mutants (indicated above each panel; see results for abbreviations). Scale bar, 5 μm.

Ankyrin-G accomplishes two critical functions in photoreceptors: It is required for transport of CNG-β1 from its site of synthesis and the assembly and/or maintenance of ROSs. This...
The Surprising Power of Neighbory Advice

Daniel T. Gilbert, Matthew A. Killingsworth, Rebecca N. Eyre, Timothy D. Wilson

Two experiments revealed that (i) people can more accurately predict their affective reactions to a future event when they know how a neighbor in their social network reacted to the event than when they know about the event itself and (ii) people do not believe this. Undergraduates made more accurate predictions about their affective reactions to a 5-minute speed date (n = 25) and to a peer evaluation (n = 88) when they knew only how another undergraduate had reacted to these events than when they had information about the events themselves. Both participants and independent judges mistakenly believed that predictions based on information about the event would be more accurate than predictions based on information about how another person had reacted to it.

People make systematic errors when attempting to predict their affective reactions to future events, and these errors have social (1–3), economic (4–8), legal (9, 10), and medical (11–22) consequences. For example, people have been shown to overestimate how unhappy they will be after receiving bad test results (23), becoming disabled (14, 19–21), or being denied a promotion (24), and to overestimate how happy they will be after winning a price (6), initiating a romantic relationship (24), or taking revenge against those who have harmed them (3). Research suggests that the main reason people mispredict their affective reactions to future events is that they imagine those events inaccurately (25). For example, people tend to imagine the essential features of future events but not the incidental features (26–28), the early moments of future events but not the later moments (17, 24), and so on. When mental simulations of events are inaccurate, the affective forecasts that are based on them tend to be inaccurate as well.

Attempts to improve the accuracy of affective forecasting have generally concentrated on improving the accuracy of mental simulation, and the results have been disappointing (29–33). Some interventions have failed (16), and those that have successfully reduced forecasting errors in one situation have typically failed to reduce them in others (27, 29). But mental simulation is not the only way to make an affective forecast. The 17th century writer François de La Rochefoucauld suggested that rather than mentally simulating a future event, people should consult those who have experienced it. “Before we set our hearts too much upon anything,” he wrote, “let us first examine how happy those are who already possess it” (34). La Rochefoucauld was essentially suggesting that forecasters should use other people as surrogates for themselves, and the advantages of his “surrogation strategy” are clear: Because surrogation does not rely on mental simulation, it is immune to the many errors that inaccurate simulations produce.

The disadvantages of surrogation are also clear: Individuals differ, and thus, one person’s affective reaction is almost certainly an imperfect predictor of another’s. But there are at least two reasons to suspect that affective reactions are not as different as people may believe. First, affective reactions are produced in large part by physiological mechanisms that are evolutionarily ancient, which is why people the world over have very different beliefs and opinions but very similar affective reactions to a wide range of stimuli (35), preferring warm to cold, satiety to hunger, friends to enemies, winning to losing, and so on. An alien who knew all the likes and dislikes of a single human being would know a great deal about the entire species. Second, people tend to marry, befriend, work with, and live near those who share their preferences and personality traits (36, 37), and thus the people from whom they are especially likely to receive surrogation information—their neighbors in their social networks—are especially likely to share their affective reactions. In short, there is little disagreement among people about the sources of pleasure and pain, and even less disagreement among neighbors. These facts suggest that surrogation may be more powerful than people realize.

References and Notes


11. Materials and methods are available as supporting material on Science Online.


19. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.


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www.sciencemag.org/cgi/content/full/323/5921/1614/DC1

Materials and Methods

Fig. S1 to S6

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