Article

Protein Structure in Context: The Molecular Landscape of Angiogenesis[S]

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Abstract

A team of students, educators, and researchers has developed new materials to teach cell signaling within its cellular context. Two nontraditional modalities are employed: physical models, to explore the atomic details of several of the proteins in the angiogenesis signaling cascade, and illustrations of the proteins in their cellular environment, to give an intuitive understanding of the cellular context of the pathway. The experiences of the team underscore the use of these types of materials as an effective mode for fostering students' understanding of the molecular world and the scientific method used to define it. © 2013 by The International Union of Biochemistry and Molecular Biology, 41(4):213–223, 2013

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He said science was going to discover the basic secret of life someday, the bartender put in. He scratched his head and frowned. *Didn't I read in the paper the other day where they'd finally found out what it was*?
[. . .]
What is the secret of life? I asked.
I forget, said Sandra.
Protein, the bartender declared. *They found out something about protein.*

Kurt Vonnegut, *Cat's Cradle*

Introduction

To appreciate the complexity and beauty of the inner workings of a living cell, students must have a solid understanding of what proteins are: what they look like, what they do, and how the former shapes the latter. Unfortunately, the beginning student rarely understands protein as anything more than something that belongs on a dinner plate. Therefore, a major goal of undergraduate educators in the molecular biosciences is to help their students develop a robust mental model of proteins and their biological activities that is accurate at both the molecular and cellular levels. At the molecular level, students need to understand the basic principles of chemistry and physics that drive a specific sequence of amino acids into their final functional form. Just as important, students must understand how individual proteins interact at a cellular level to perform the many processes needed for life.

What is the best way to ensure that our students go beyond simply memorizing amino acid structures and learning the basic connections between protein structure and function? How can we help our students truly

[S]*Additional Supporting Information may be found in the online version of this article.*

Abbreviations: *CREST, Connecting Researchers, Educators and STudents; ERK-2, extracellular signal-regulated kinase 2; DUSP-5, dual-specificity phosphatase 5; KEGG, Kyoto Encyclopedia of Genes and Genomes; MAPK, mitogen-activated protein kinase; MSOE, Milwaukee School of Engineering; PDB, Protein Data Bank; BMRB, Biological Magnetic Resonance Bank; VARK, Visual, Aural, Read-Write, Kinesthetic; VEGF, vascular endothelial growth factor*

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understand how proteins function at levels ranging from atomic to cellular? What would it take for students to appreciate their world as a manifestation of the molecular world? These were the questions considered by a team of researchers, educators and students participating in the CREST project at the MSOE (Milwaukee School of Engineering) Center for BioMolecular Modeling. The premise of the CREST project (Connecting Researchers, Educators and STudents) is that the collaborative efforts of these three stakeholders will result in innovative instructional tools that effectively translate the results of active research projects into the classroom. An important corollary to this outcome is that students will understand that the knowledge in textbooks is the direct product of a series of experiments performed by researchers. Speaking as a group of educators, students, and researchers, we believe that it is possible to provide students with a holistic view of biochemistry that is simultaneously accurate at the molecular level and scalable to the cellular and even macroscopic levels. The approach we describe here relies on the combined use of two new instructional tools: physical models of proteins and molecular landscapes, realistic illustrations of portions of cells at a molecular level of detail. The goal of the latter is to put the former in proper cellular context.

Proteins in the Classroom—A Problem of Perception

As proteins are not directly observable, the challenge of teaching protein structure and function is largely a problem of perception. Representations of proteins must be created that capture and present the features of interest without engendering misconceptions about function in the cellular context. Historically, concepts in biochemistry have been simplified and abstracted in order to make them accessible to students. Hence, double-stranded DNA is often represented as two parallel lines drawn on the white board or illustrated in a textbook, and proteins such as RNA polymerase are represented by brightly colored ovals. This is still the case today, as evidenced by the popularity of cartoon illustrations in biochemistry textbooks [1]. Although it has long been common practice conceptually to simplify and abstract proteins in this way—especially those proteins forming complex signaling cascades—the approach can be justifiably criticized for several reasons. Perhaps most importantly, it omits much of the information about the molecule, so important properties, such as the atomic basis of base pairing in DNA or the molecular driving forces for protein–ligand interactions, must be taken on faith instead of observed directly. Such abstracted representations of proteins are far removed from "real" life; it is therefore conceivable that the prevalence of this educational approach has contributed to public notions about science as an overly abstract field—a peculiar conclusion to be reached about a discipline with such a firm basis in the material world. One must conclude that we as educators are not adequately bridging the gap between the abstractions we use and the molecular reality in which proteins operate within cells. While there is likely to be no simple solution to this problem, we hypothesize that a contributing factor is educators' ignorance of the diversity of learning styles present in their biochemistry classrooms, which entails that a successful tool for one type of learner may have limited success with other learning types. Basic two-dimensional (2D) representations of a protein usually require students to have strong visual-spatial skills that enable them to synthesize the protein's intricate features in three dimensions. Students who are not visual learners may have difficulty with this task, and stand to benefit from the use of a more diverse set of educational tools for teaching protein structure.

2D representations of proteins, based on their atomic coordinates (from the Protein Data Bank (PDB) [2]) or other data (e.g. chemical shift data from Biological Magnetic Resonance Bank (BMRB) [3]), are also widely used, and computer software for 3D visualization of proteins has become more popular in the classroom in recent years [1, 4]. The trend toward increasing sophistication of our visual tools has great potential for the teaching of protein structure–function, with an eye toward providing a holistic molecular point of view. As observed by biomedical illustrator Linda Nye, "Humanity's ability to visualize objects and their transformations in space is a critical component of our intelligence. It makes possible the planning of actions and the anticipation of outcomes, which is the basis of science and the scientific method" [5]—this is indeed the ability required for comprehending an enzyme mechanism or protein signaling event. Visualizing the intricate details of biomolecular phenomena, such as interactions among residues in an enzyme's catalytic site, requires similarly intricate illustrations or models. It may seem that with every new application of realism to a visual model, at this molecular level of detail, the subject becomes more complex and thus more difficult to teach (for the educator) and to comprehend (for the student); however, students do not necessarily find realistic representations of biomolecules more intimidating than their schematic counterparts [4]. Conversely, students with lower visual-spatial skills may be daunted by more realistic representations: for these students, a 3D software program or high-resolution 2D drawing makes a protein appear not more realistic, but as a more complex abstraction [1, 6, 7].

Recently, the use of hand-held, physical models of proteins has emerged as a new modality to help students better grasp protein structure–function in contemporary biochemistry courses. Accurately rendered physical models of proteins have been made possible by recent developments in rapid prototyping technology, but the concept of 3D models in science is nothing new: Watson and Crick used 3D models (paper and other materials, such as metal) to discover the details of

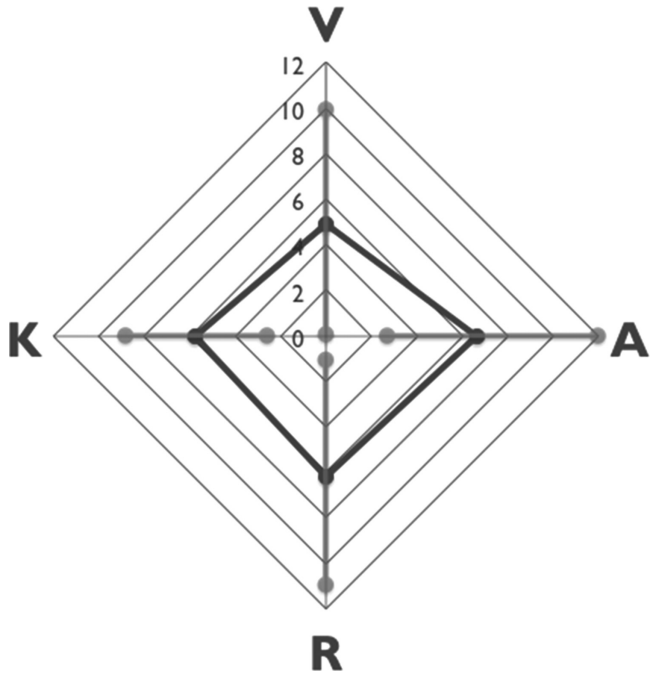


FIG 1 Biochemistry classroom VARK data. The average scores for student learning styles are illustrated by the dark blue plot, and the range of scores by the light blue lines on each axis. Students taking the VARK questionnaire are scored for their learning preferences in categories of Visual, Auditory, Read-write, or Kinesthetic. The possible range for each style is 0–16. The data indicate that the class is populated with students equally representing all learning styles, on average. Class size = 15 students. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

base pairing in DNA, and Linus Pauling discovered the basic secondary structures of proteins by experimenting with models. Assessments suggest that protein models facilitate mastery of basic protein structure–function, and students report feeling more connected to the subject when they use a tactile model [1, 6, 7]. It is also thought that students with low visual-spatial skills may learn more easily via kinesthetic interaction with the subject matter [6, 8]. Our own experience indicates that the biochemistry classroom is composed of students with a diversity of learning styles: we have quantified this observation using the VARK learning assessment (Fig. 1), a survey that measures students' preferences among Visual, Aural, Read/write, and Kinesthetic learning styles [8]. This being so, our VARK data do not indicate that any particular learning style preference puts students at an advantage for overall exam scores (see Supporting Information Fig. S1), although it appears that students who are weaker visual learners may improve their molecular visualization and reasoning skills after taking a course that employs multiple teaching modalities, including the use of protein physical models to complement traditional lectures (based on the molecular visualization assessment in Supporting Information Fig. S2). Our own preliminary data (from the course described, below) suggest this outcome; further studies in other, larger student groups are currently under way.

Studying Trees—Without Seeing the Forest

Building on our experiences and previously reported successes with using physical models in teaching [1, 6], we incorporated them into a university-level biochemistry course that is tailored to chemistry majors (with a class size of 15). These are more advanced students, who have already taken two semesters of organic chemistry. Lectures and homework assignments were centered on protein biochemistry and metabolic pathways with emphasis on mechanism and the nature of intermolecular interactions that lead to ligand binding (e.g. substrate, drug or protein partner). To complement the teaching of traditional biochemistry content, students worked in small groups (three to five students per group) on a semester-long, structure-based drug design project focused on vascular endothelial growth factor (VEGF) receptor signaling, a signaling cascade that leads to angiogenesis and is an important target for the development of anticancer drugs (Fig. 2, see Supporting Information for detailed pathway information).

Each group was assigned a different protein in this signaling pathway and then visited an academic research lab investigating that protein. After learning about the research project, groups used molecular visualization software (Discovery Studio Visualizer (Accelrys) and Jmol) and a rapid prototyping platform (at the MSOE Center for BioMolecular Modeling) to design and build 3D physical models of their proteins with features emphasizing structure and function, such as identifying and highlighting residues involved in ligand binding. Each group also designed a set of small molecules with shape and electronic complementarity to the active site pocket that in theory might inhibit the protein's activity. The purpose of this exercise is to help students: (a) learn how drugs are designed, and (b) better understand the intermolecular forces that lead to ligand binding. Typically, students started with a known ligand (substrate or inhibitor), and designed changes to it that retained the shape complementarity with the active site, and properly positioned hydrogen bond donors and acceptors (and charged groups, if present). If there was an open cavity in the active site proximal to the bound ligand, then students were told that additional functionality (e.g. methyl, ethyl, or even phenyl groups) should be added to their molecule. If there was an unsatisfied active site hydrogen bond donor (e.g. threonine OH) or acceptor (e.g. asparagine carbonyl) proximal to the bound ligand, then students would be counseled to add a complementary acceptor (e.g. fluorine; carbonyl) or donor (e.g. OH), respectively, to their molecule. As students are not used to being creative with

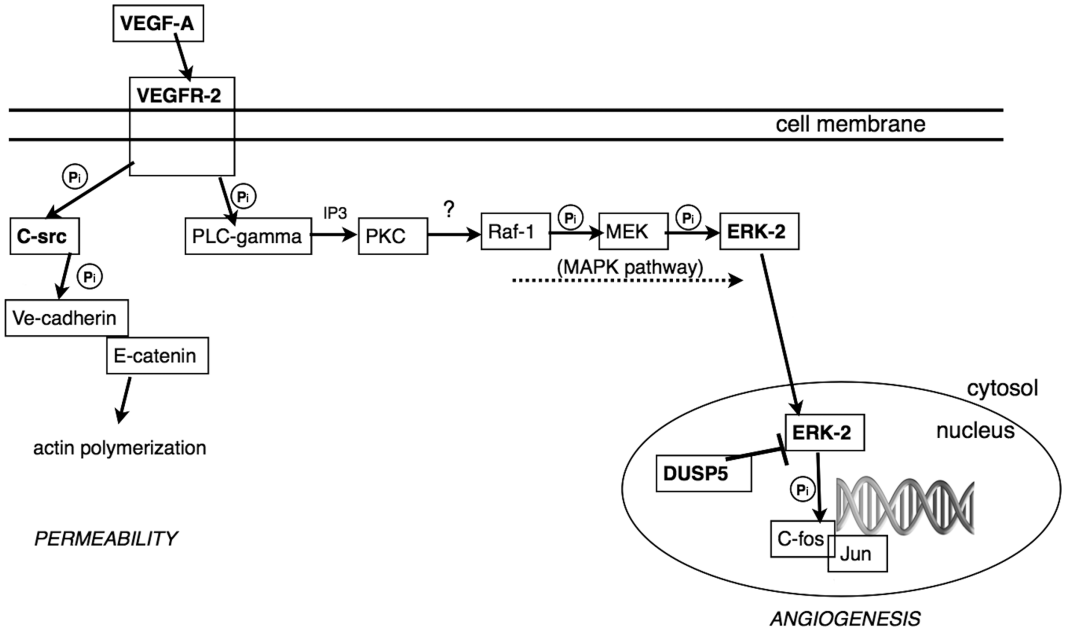


FIG 2 Schematic of the VEGF pathways to be depicted in the landscape. The DUSP-5/ERK-2 interaction that occurs in the nucleus (bottom right) is represented using physical models in Fig. 3. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

regard to organic chemistry, this required iterative rounds of the students making attempts at designing new ligands, and then getting feedback from the instructor on how to better approach the problem. The ligands that students designed were first drawn on paper, then using Spartan software (Wavefunction) and minimized using AM1 calculations. The molecules they designed were then manually positioned into the active site using Discovery Studio Visualizer software, using the original ligand to orient their ligand (i.e. overlay them), after which the original ligand was then deleted. Students then assessed the fit of their newly designed ligand into the active site. In effect, students were learning how to do rational drug design, with sequential rounds of guidance from the instructor. Students

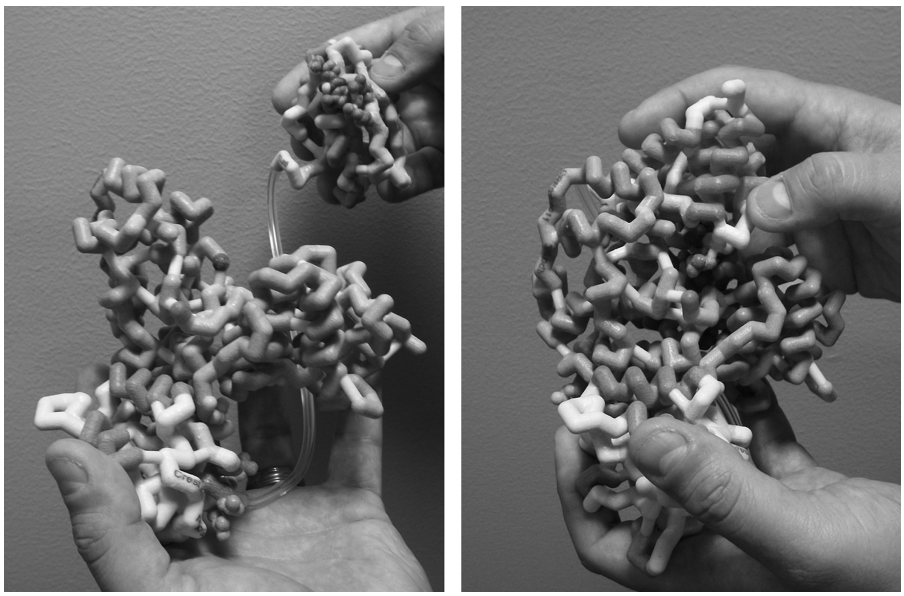


FIG 3 Physical models of DUSP-5 and ERK-2, demonstrating the binding interaction known to occur between these proteins. DUSP-5 in comprised of two domains, an ERK-binding domain and a phosphatase domain, connected by an unstructured linker region (plastic tubing in the model). The ERK-2 protein binds between these two domains of DUSP-5. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

then prepared and presented posters along with their physical models, showing their work and demonstrating their understanding of their protein's function, acquired based on discussions with their research mentors and their reading of the primary literature.

The drug design component of the final project encouraged students to think at atomic and molecular levels of interaction (protein–ligand) and narrowed their focus to a single protein within the signaling pathway. Lectures included an overview of the pathway and instruction on the interaction between two proteins in the pathway, phosphorylated extracellular signal-regulated kinase 2 (pERK-2) and dual-specificity phosphatase 5 (DUSP-5), which dephosphorylates pERK-2 (Fig. 2). This pairwise interaction was explored via molecular animations (e.g. using PyMOL software) and physical models (Fig. 3) and encouraged students to think about the molecular interactions (protein–protein) that constitute a signaling cascade. When students presented their projects, they demonstrated overall mastery of protein structure and function with regard to their protein member of the angiogenesis signaling cascade; however, their explanations of the signaling cascade itself tended to be qualitatively weak. Despite our best efforts to use all the learning tools available to us (computer visualization, animation, lectures, physical models, active learning via presentations), students still did not demonstrate a satisfactory appreciation of the network of protein–protein interactions that are behind signaling cascades, nor of the cellular context of these interactions. Although the physical models appeared to promote students' awareness of atomic-level function of the individual proteins, the "big picture" was lost. We decided to continue studying the effects of 3D models on student learning in different student populations, but also that subsequent work on the CREST project should focus on identifying and correcting this shortfall, by reinforcing the connection between all the proteins in the pathway. The further CREST work resulted in the design of a novel educational tool that in this article we propose for widespread use.

How Can Students Come to Appreciate the Forest?

To identify ways to help students better appreciate the cellular context in which individual proteins function in the VEGF-angiogenesis signaling pathway, we investigated the process by which most students learned about the pathway. A popular starting place for students trying to learn the pathway was an online pathway database, such as the Kyoto Encyclopedia of Genes and Genomes (KEGG) [9], which describes the networks of interacting proteins in signaling cascades and metabolic pathways. We found that students had trouble with two aspects of these diagrams. First, the diagrams have been developed primarily for the research community, and the conventions of the display may be difficult to comprehend by novices. They are often linear, glossing over details such as multiprotein complexes and subcellular compartmentalization; they often ignore the scale and population of the different protein players; and they often use coded descriptions for protein activities such as phosphorylation that may not be obvious to those outside the field. Second, and perhaps a greater problem, students were discouraged by the inconsistency among pathway diagrams from different sites, which is a natural outcome of research that is current and ongoing in developing fields, such as angiogenesis. Specifically, it is often difficult to discern between a pathway event that is strongly supported by the primary literature and an event that is less well-defined, perhaps because of ongoing scientific disputes. In short, the clarity and certainty with which textbooks present biochemistry leaves students ill-equipped to deal with the sometimes uncertain state of our understanding of many cellular processes, which the scientific process seeks to clarify; to borrow the phraseology of Donald Rumsfeld (February 12, 2002), science textbooks teach students that science is full of "known knowns," leaving them unprepared for the "known unknowns" and "unknown unknowns" that are pursued in active research projects.

Although the first problem may be addressed with improved diagrams, such as those described below, the second problem poses a greater conceptual challenge. We have found that the best way for students to appreciate the process of scientific discovery is to have a dialog with experts in the field. Exposure to active researchers in the first phase of the CREST project (before preparing posters) was a step in that direction, but that single conversation appears to have been insufficient to empower students to interpret the sometimes-conflicting literature. The need for students to have ongoing interactions with primary researchers must be balanced, of course, with the many other demands on the researchers' time. One of the researchers involved in the CREST project, and a coauthor of this article (RR), suggested that meetings held between students and researchers approximately every two weeks might strike a good balance between teaching the scientific process and respecting the time constraints of an active research program.

To help students better integrate molecular and cellular concepts, we have developed an approach to producing more realistic renderings of cellular processes. We refer to the resulting images as molecular landscapes. The goal of the approach is to create an illustration that accurately simulates a portion of a cell, using visualization methods that produce an intuitively comprehensible image. The illustration and its educational goals might draw a comparison with the Harvard BioVisions project, which has developed highly effective multimedia presentations of cellular scenes that beautifully convey concepts of dynamics and scale as they apply to cellular physiology [10]. According to the project's

illustrator, David Bolinsky, the animations were designed so that Harvard students "would have an internalized view of what a cell really is, in all of its truth and beauty, and be able to study with this view in mind" [11]. Although static, 2D molecular landscapes cannot convey molecular motions to the same extent as 3D molecular animations, they have the advantage of accurately portraying cellular crowding, something that in its entirety "would prevent the vista from happening" [11] and limit the intended effect of a 3D animation. In this sense, molecular landscapes and animations are complementary. Molecular landscapes attempt to simulate the environment of molecules inside living cells, with illustrations based on atomic structures of individual biomolecules, electron micrographs of cells, and biochemical studies of molecular concentrations, locations, and interactions [12–15]. These molecular landscapes provide a framework for intuitive understanding that prepares the student's mind to receive the onslaught of facts we provide about pathways, interactions, cellular context, protein dynamics, and so forth.

As part of one of the CREST team projects, we have created a molecular landscape of VEGF-receptor signaling at two million times magnification. In the landscape, all of the proteins are present at approximately accurate relative biological concentrations, with shapes that attempt to represent their observed domain architectures. Although the landscape presented here was made possible by collaboration with a skilled molecular artist and co-author of this article (DG), our team believes that any creative approach—regardless of skill level—to constructing a molecular landscape will be useful in very similar, if not the same, educational ways.

Construction of the Molecular Landscape

To integrate the cellular context effectively, it was necessary to go beyond the proteins connected with the class project in order to ensure that we had identified all the key protein players in the VEGF-angiogenesis pathway. To do so, we performed an initial KEGG inquiry into the VEGF-angiogenesis pathway, followed by primary literature searches. This work was coupled with meetings with the same researchers who participated in the in-class phase of the project. The meetings helped to sort out inconsistencies in the literature and to provide a realistic reflection of researchers' current understanding of the pathway. From the information gathered, we generated a pathway diagram (Fig. 2).

We then compiled characteristic information about each of the proteins involved in the pathway, including size, domain arrangement/shape, biological assembly, modifications, and their interactions/functions. These characteristics, along with sequence information from UniProt [16] and PDB ID numbers (where available), were used to generate a protein description table (Fig. 4). Based on the compiled information, a series of sketches were produced by the molecular artist (Fig. 5). Several revisions were made, based on our pedagogical goals and the constraints of producing the painting. During this process, our team met with researchers actively studying angiogenesis and VEGF pathways to iteratively refine the sketch of the signaling cascade. Meetings with the researchers over the course of the CREST project played a key role, serving the dual purpose of: (a) ensuring an accurate rendering of the proteins in the cascade, with proper relative scale, subcellular location, and interacting partners, and (b) teaching students the scientific process, including how much is unknown and where the gaps in our knowledge exist—in short, teaching why science is exciting, relevant, and dynamic in a way that is not well-conveyed in textbooks.

Some of the features that developed with successive sketches, literature searches, and discussions included the following:

1. In the pathway, molecules are shown in several states to give an idea of the process in action. This includes the VEGF receptor in monomeric unbound states and dimeric active states, various combinations of signaling proteins, and monomeric and heterodimeric versions of C-fos and Jun. Initial sketches of the pathway had many of the proteins assembled into a large complex; but, discussions with active researchers suggested that these complexes should be broken into smaller units.
2. The initial concept was to produce two panels, one of the cell surface and one of the nucleus, as the portion of the cell shown in a typical painting is not sufficient to show both. Fortunately, we found an electron micrograph of capillary endothelial cells, and these cells often have the nucleus tightly pressed to the cell surface near the junctions between cells. So, it was possible to develop a scientifically accurate sketch that showed the adherens junction, cell surface, and nucleus all in one panel (Fig. 6).
3. The initial sketches focused on a process where Hsp27 modifies the polymerization of actin. Discussion with those actively pursuing angiogenesis research, however, determined that this is a minor biological effect, so the focus was then changed to show two effects: (a) the disassembly of tight junctions through the Src pathway and the role of the released alpha-catenin in actin reorganization, and (b) the control of transcription through the MAPK (mitogen-activated protein kinase) pathway and phosphorylation of C-fos by pERK-2.

Once these details were finalized, a full sketch was created, filling in all of the other molecules (Fig. 7). This drew largely from the cellular panorama illustration from the Machinery of Life [17]. The cadherin/catenin structure of

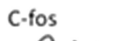
Protein	Activity to show	UniProt ID	Sequence	Monomer size	Attachments/ cofactors	Assembly	Sketch
VEGF-A	Binds to VEGFR-2	P15692	1 232	24 kDa	N-GlcNAc at 101	Antiparallel dimer; S-S linkage	
VEGFR-2	Binds VEGF; dimerizes; RTK autophosphorylation	P35968	20 1356 extracellular helical cytoplasmic	149 kDa	N-GlcNAc at 18 residues (46–721)	Inactive monomer; active dimer	
C-src	SH2 domain binds P-VEGFR-2, activating kinase domain	P12931	2 536 SH3 SH2 protein kinase	60 kDa	N-myristoyl Gly at 2	Monomer	
PLC-γ	SH2 domains bind P-VEGFR-2; PH domain interacts with PKC	P19174	2 1290 EF-hand PH2 SH2 2 PH2 C2 PH1 PI-PLC X-box SH2 1 SH3 PI-PLC Y-box	148 kDa	Ca^{2+} cofactor (165–176)	Monomer	
PKC	? Phosphorylates Raf-1	P17252	2 672 Zn finger Zn finger C2 protein kinase AGC-kinase	77 kDa	$3Ca^{2+}$ in C2 domain	Monomer	
Ve-cadherin	Phosphorylated by C-src; associates with E-catenin	P33151	48 784 extracellular TM cytoplasmic	83 kDa	N-GlcNAc at 7 residues (61–535);	Dimer	
E-catenin	Induces actin polymerization	P35221	2 906 homodimerization domain	100 kDa	None significant known	Dimer	
Raf-1	Phosphorylates MEK	P04049	1 648 Ras-binding domain Zn finger protein kinase	73 kDa	$2Zn^{2+}$ per subunit	Monomer	
MEK	Phosphorylates ERK-2	Q02750	2 393 protein kinase	43 kDa	None significant known	Monomer	
ERK-2	Phosphorylates transcription factor C-fos	P28482	2 360 protein kinase	41 kDa	Mg^{2+} cofactor	Dimer when active	
DUSP-5	Dephosphorylates and inhibits ERK-2	Q16690	1 384 rhodanese tyrosine-protein phosphatase	42 kDa	None significant known	Antiparallel dimer	
C-fos	Phosphorylated by ERK-2	P01100	1 380 basic motif leucine-zipper	41 kDa	None significant known	Heterodimer with Jun	
Jun	Dimerizes with C-fos; heterodimer binds DNA and activates transcription	P05412	1 331 basic motif leucine-zipper	37 kDa	None significant known	Heterodimer with C-fos	

FIG 4 Summary of the proteins in the VEGF-mediated angiogenesis signaling cascade (Fig. 2), including relevant structural information needed for drawing the molecular landscape.

the adherens junction was adapted from an earlier paper in the Oncologist on the same subject [18] and a recent paper on the process of VEGF signaling [19]. The color design of the painting was one of the challenging aspects of the project, as there were two conflicting goals that needed to be addressed with color: 1) to highlight the molecules in the two pathways, showing the order of signaling, and 2) to make the various compartments of the cell apparent. The

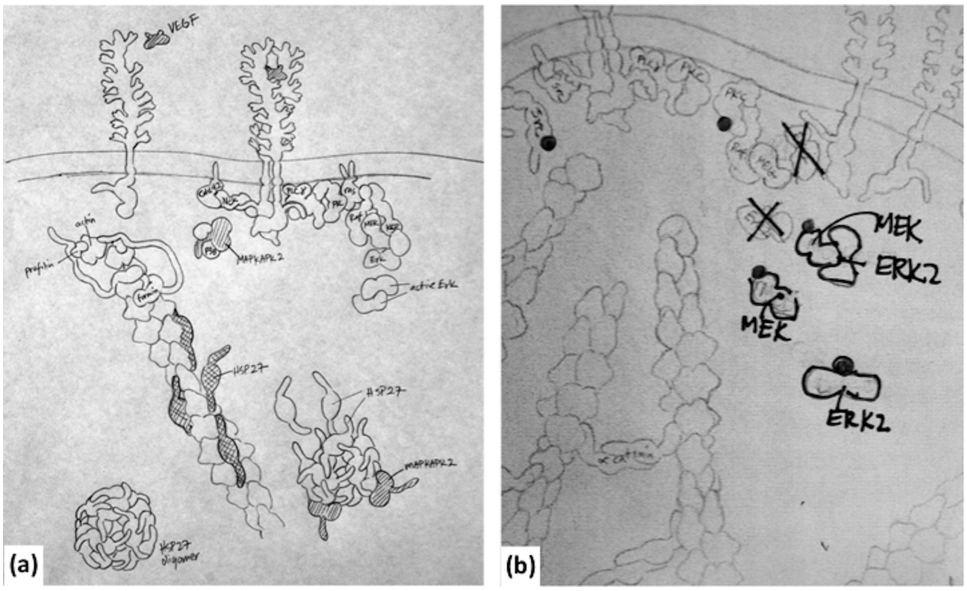


FIG 5

(*a*) An initial sketch for the molecular landscape painting depicting angiogenesis signaling, and (*b*) a sketch after three rounds of revisions, with markings suggesting further alterations. Relative protein sizes and shapes are based on the data compiled from Fig. 4. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

final color scheme uses a rainbow sequence of bright red-yellow-orange-magenta to convey the sequential action of molecules in the signaling cascade; blues, purples, and greens were used for the other molecules in the cell. This scheme may also be observed in the landscape's legends (Fig. 8).

Impact of the New Materials on Researchers, Educators, and Students

The collaborative creation of a series of physical models of interacting proteins and the molecular landscape of VEGF signaling described here has had a significant impact on every member of the team:

For students working on the project, the physical models of a protein facilitated meaningful conversations with the researchers regarding specific details of a protein's structure that were being investigated in the lab. At the same time, the planning of the landscape required a sophisticated synthesis of results from multiple research papers, leading to an understanding of how the destabilization of adherens junctions is a necessary prerequisite to angiogenesis, or to the transcriptional activation of a specific set of genes in the nucleus. In addition to this factual knowledge, the students were active participants in a modeling project in which the results of a wide range of experiments in different laboratories were synthesized into a conceptual model of how this signaling pathway leads to angiogenesis. This experience served as a clear example of how the concepts that are taught in a classroom are the direct product of experiments performed in a research lab. The students also had the rare experience of working closely with an educator outside the normal environment of a classroom. In the collaborative environment of this project, the "teacher" was transformed in the eyes of the students into a "scientist" who was working productively with a "researcher" to arrive at a deeper understanding of a molecular process.

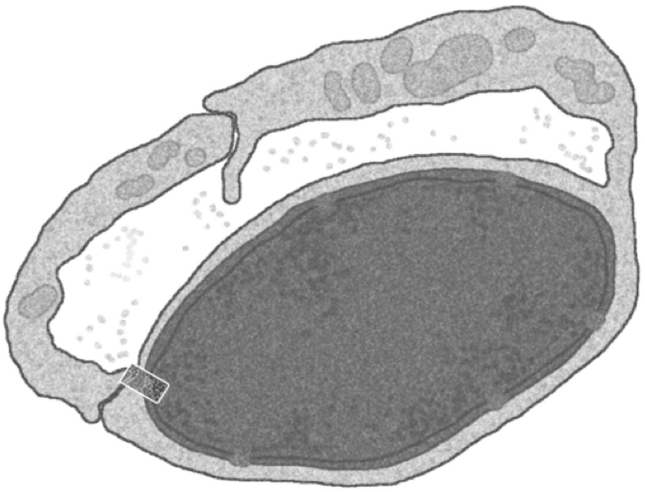


FIG 6

Simulated electron micrograph (based on ref. 20]) showing two cells from the vascular endothelium. The size and location of the portion of the vascular endothelium that is to be depicted in the molecular landscape painting is shown in color. This is the cellular context within which the angiogenesis signaling cascade is to be presented. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

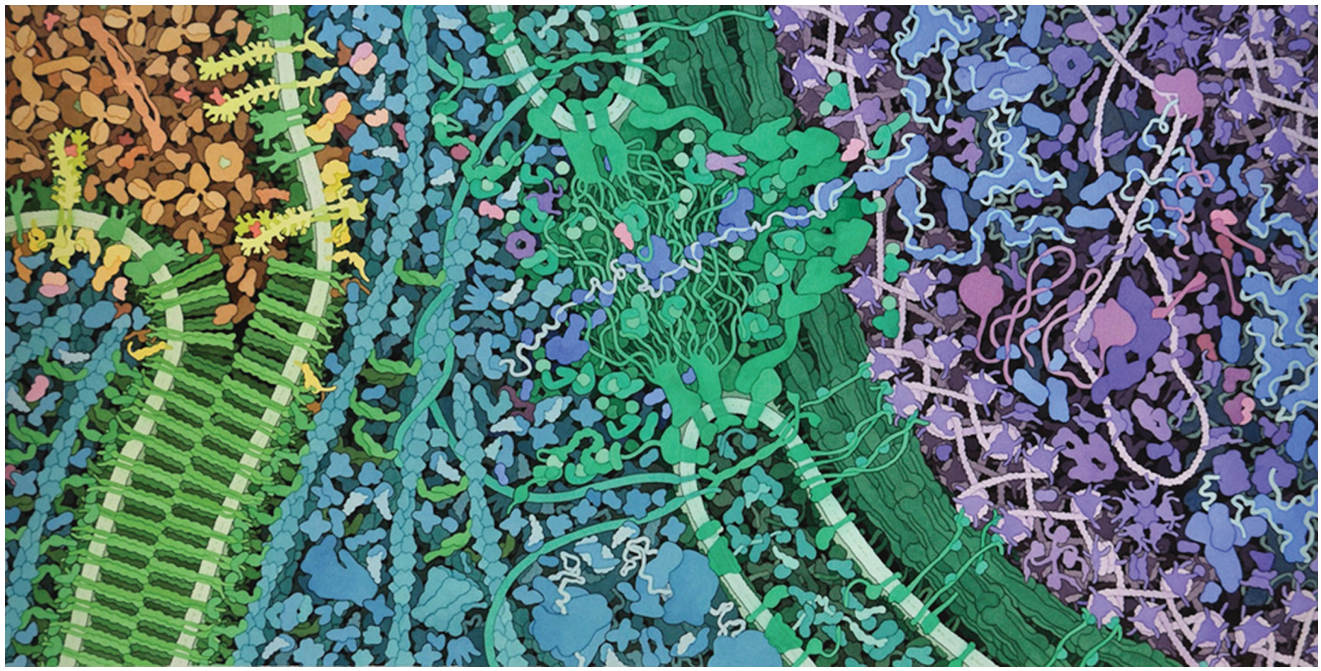


FIG 7

The final molecular landscape depicting VEGF signaling. Blood plasma is shown in tan at the upper left. The adherens junction between two cells is in green at left, with surface VEGF receptors shown in yellow. The cytoplasmic proteins are in turquoise, and the nuclear pore is at the center in green. The nucleus is at the right, with proteins shown in blues and purples.

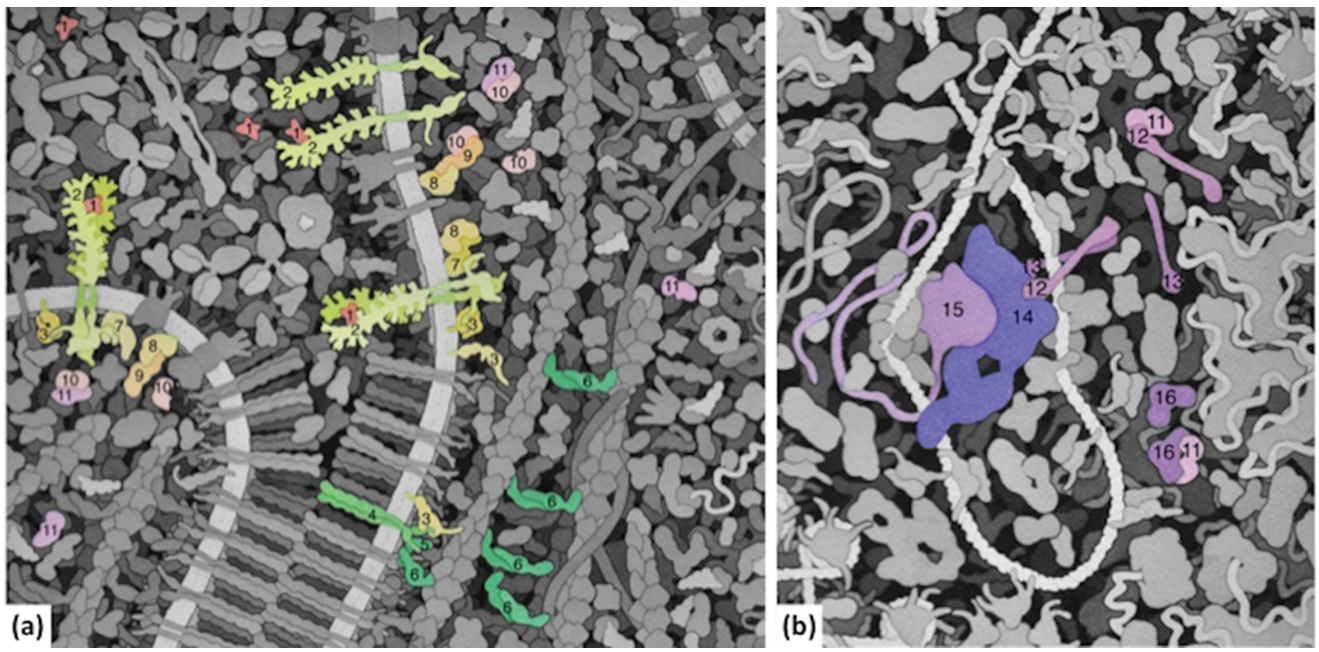


FIG 8

Expanded and color-coded views of the molecular landscape from Fig. 7. (*a*) VEGF signaling, across the cell membrane into the cytosol. VEGF-A (1) in blood plasma binds to VEGFR (2) and causes it to dimerize. This activates the VEGFR tyrosine kinase domains inside the cell. Two downstream pathways are shown. *VEGF Pathway-1*: C-src (3) is phosphorylated, causing it to open up and phosphorylate cadherins (4) in the adherens junctions, releasing alpha-catenin (6), which dimerizes and bundles actin. Beta-catenin (5), which is involved in the adherens junction structure, is also shown]. *VEGF Pathway-2*: VEGFR dimerization initiates a cascade of phosphorylation reactions through PLC-gamma (7), PKC (8), Raf-1 (9), MEK (10), and ultimately ERK-2 (11). Phosphorylated ERK-2 (pERK-2) is transported through the nuclear pore (the large structure at the center of the full painting). (*b*) Continuing the signaling cascade into the nucleus. pERK-2 (11) phosphorylates C-fos (12), causing it to form a heterodimer with Jun (13) and becoming active as a transcription factor effecting transcription of proteins needed for blood vessel growth. It is shown here in an enhancer binding to the transcription mediator (14) and RNA polymerase (15). Finally, DUSP-5 (16) terminates the process by dephosphorylating pERK-2. This final interaction is depicted with the physical models in Fig. 3.

The educators in this project also reported multiple beneficial outcomes, including new insights into the molecular mechanism of a specific signaling pathway, and a specific example they can use in their classrooms to demonstrate how reductionist biochemistry (details of interacting protein structures involved in ligand binding and subsequent activation of phosphorylation domains) combines with cell biology to explain the molecular process of how angiogenesis is activated in tumors. In addition, the educators reported a new appreciation for the talents and skills of the students involved in this project. In this and other modeling projects in the CREST program, educators report that the instructional materials that result from these collaborations simply would not have been developed without the efforts of students who readily embraced the use of new technologies in exploring the topic.

The researchers benefit directly from the final product of the project—they receive both a physical model of a protein and a molecular landscape they can use to communicate their research to others. The landscape can be used to introduce new students to the "big picture" of the lab's work, while the physical model of a protein can be used to discuss the specific details of the protein's structure that are believed to play a critical role in the process. These same tools can also be used to explain their work to colleagues visiting from other institutions. Researchers also report satisfaction from working with undergraduate students and contributing directly in the training of a future generation of researchers. A researcher's experience in working with a CREST modeling team is often the focus of the "broader impact" statement that is required of some research proposals.

It cannot be ignored that this work has had at its disposal cutting-edge resources and a network of innovative, skilled team members. This being so, the educational modalities we have created or applied are not constricted to groups with identical resources; in fact, we argue that the same educational goals, and perhaps others, may be attained through diverse means. Some may see a lack of access to rapid prototyping technology as a barrier to constructing physical protein models, but this is less and less the case: many schools, even some high schools, have 3D printers in their art and engineering departments, and in many cases these resources may be shared. If teachers do not have access to 3D printers, 3D Molecular Designs, LLC (affiliated with the MSOE Center for Biomolecular Modeling) can print models for a fee (cost varies depending on size and complexity, typically ranging from $100 to $400). In addition, the proficiency in Jmol (or other protein visualization software tools) required to design the models prior to 3D printing may be accessed through online tutorials. Finally, existing molecular landscapes may be used as tools in themselves; for example, they may be published online in clickable formats for student-users to actively learn the principles and content of signal transduction.

The process of constructing a molecular landscape arguably has even more value than using an existing landscape, and the process can take multiple forms. The artistic quality of the final painting is of secondary importance—the salient matter is the process of assembling the information needed to construct the image. For example, the landscape could be prepared as a crude sketch with pencil and paper; alternatively, established graphics design software could be used to build protein shapes approximating relative scale, cellular concentration, and location. One goal of the ongoing CREST project is to create an online Landscape Creator software package with which students can construct a landscape using a palette of images of commonly encountered proteins, membranes, and nucleic acids.

Other landscape modalities may succeed in other capacities that our team did not choose to focus on, such as the capacity for team-building, or for group discussion/learning: as an example, entire classes (small college or high school) could use an online landscape creator tool to build a chosen signaling pathway. In this setting, decision-making would have to occur as a group and could fuel interesting and valuable dialogue. Regardless of how the landscape is constructed, students will be confronted with design decisions that require them to learn not only specific information about the protein players and their interacting partners, but also the skill of critically engaging with scientific literature and databases. Besides learning difficult content, they will begin to develop the skills set that will make them strong scientific thinkers. This dual instructional mode endows molecular landscape projects with high educational value and makes them decidedly promising for further classroom use.

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