

## A Day in the Life of Dr K. or How I Learned to Stop Worrying and Love Lysozyme: A Tragedy in Six Acts

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About the play: In modern drama, the agonizing nature of membrane protein work has not been adequately acknowledged. It is perhaps significant that the first attempt to bring this darker aspect of human existence into focus comes from a Scandinavian author, writing in the tradition of Ibsen and Strindberg but with a distinctly turn-of-the-millennium approach to the inner life of his characters: the despairing Dr K; the cynical Dr R with his post-modernistic life credo; the ambitious but unfeeling Dr C; the modern Übermensch, Dr B., with his almost Nietzschean view of human nature. This is a play that is brutally honest, yet full of empathy for the poor souls that get caught between the Scylla of unreachable scientific glory and the Charybdis of helpless mediocrity.

James Glib-Burdock, drama critic for *The Stratford Observer*.

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### The Cast

Dr K., a middle-aged biochemist with a certain interest in membrane proteins.

Dr R., another middle-aged biochemist with other things on his mind.

Dr C., a young, hungry crystallographer.

Dr B., a bioinformatics guru.

Dr H., over in molecular biology.

A class of medical school students.

### Act I

**Wherein Dr K. faces a class of reasonably arrogant second year medical students and loses a bet**

*K. enters the classroom*

K.: Morning class!

Class (*the more ambitious busying themselves with their Internal Medicine textbook; the rest with this morning's sports pages*): ...

K.: Ahem!

Student A (*lowering his paper after a carefully calculated pause*): Guys, I think the prof has something on his mind (*deep sigh*).

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Abbreviations used: GPCRs, G-protein-coupled reactions; EM, electron microscopy; HMM, hidden Markov models.  
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K.: Yes, well, I'm gonna talk about membrane proteins today. Now, I know proteins don't count much when you're preparing for an Internal Med. test, but they're in fact important even for medicine. Or rather, they're particularly important for medicine, since so much of what goes on in the body depends on membrane proteins doing their job. Take something trivial like local anesthesia: it works by inhibiting nerve conduction, which is a prime example of what membrane proteins do. Or think about psychoactive drugs, or the thousands of signaling pathways that depend on G-protein-coupled receptors (GPCRs). Speaking of which, did you know that the *Caenorhabditis elegans* genome codes for more than a thousand GPCRs (The *C. elegans* sequencing consortium, 1998) making up around 5% of all its genes, suggesting that our genome might contain maybe 3000 GPCR genes?

*At this point, a couple of students reluctantly start paying attention; after all, numbers are something that might appear in a test.*

K. (*continues, a little more bravely*): so you see, membrane proteins are not just any old enzymes, but lie at the heart of a whole range of really central biological functions. Like photosynthesis (*this is K.'s first big mistake and the three alert students immediately return to the Internal Medicine textbook*), perhaps the most important biochemical reaction on earth, and certainly the most abundant in terms of biomass. The photosynthetic reaction center (Deisenhofer *et al.*, 1985) (*the first slide flashes out, nobody notices; Figure 1*) is a perfect example of what we think that most integral membrane proteins look like: a bundle of long, hydrophobic transmembrane  $\alpha$ -helices, in this case arranged around a core of electron-conducting co-factors that carry out the charge separation that is the whole point of photosynthesis. In fact, solving the X-ray structure of the photosynthetic reaction center was considered such an important achievement that it was awarded the Nobel Prize in chemistry in 1988 (see [www.nobel.se](http://www.nobel.se))

*This last remark penetrates through to a few minds in the classroom. Slightly higher level of attention.*

Student B: Did you say Nobel Prize?

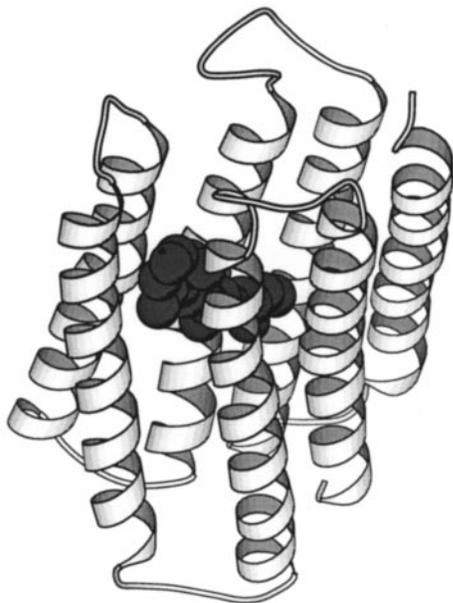
K.: Yes. To Deisenhofer, Huber, and Michel. This was the first really high-resolution structure of an integral membrane protein, though much earlier work by Henderson & Unwin (1975) on another light-driven enzyme (bacteriorhodopsin) had already established the idea of transmembrane  $\alpha$ -helices (*second slide; Figure 2*). And today we know the 3D structure of 15 or so integral membrane proteins (*another slide, this time with a list printed in an impossibly small font, Figure 3*). Most of these are helix bundles, though some are built on a totally different architecture which consists of a large, antiparallel  $\beta$ -barrel (*thankfully, another slide appears Figure 4*). Two examples are shown here: the smallest and the largest known. The eight-stranded OmpA  $\beta$ -barrel (Pautsch & Schulz, 1998) is thought to act simply as a membrane anchor that attaches another protein domain to the outer membrane of *Escherichia coli*, while the 22-stranded  $\beta$ -barrel in the FhuA protein (Ferguson *et al.*, 1998; Locher *et al.*, 1998) surrounds an internal "plug" that somehow can transport ferrichrome-iron complexes across the outer membrane.

Of course, both the helix bundle and  $\beta$ -barrel designs provide a large hydrophobic outer surface that can interface with the surrounding lipid molecules, and, equally important, satisfy all the backbone hydrogen bonds internally.

*Incredibly, there is a rather intelligent question at this point*



**Figure 1.** The photosynthetic reaction center. Electron-conducting co-factors are shown in space-filling mode. Plot made by MOLSCRIPT (Kraulis, 1991).



**Figure 2.** Bacteriorhodopsin. The light-absorbing retinal is shown in space-filling mode.

Student A: Are these all the possibilities? I mean, can't you have mixed  $\alpha/\beta$  structures? Or something completely different?

K. (*warming to his theme*): Good question! (*Student A gets ugly up-yours looks from the others.*) There have been many suggestions of this kind (Unwin, 1993; Hebert *et al.*, 1995), but no conclusive data so far. However, the recent structure of a bacterial  $K^+$  channel (Doyle *et al.*, 1998) is at least a step in this direction (*new slide - K. was prepared for this one...*; Figure 5). What it shows is that helix bundle proteins can be constructed with a rather large central channel, and that other parts of the polypeptide can fold back into this channel. Thus, the ion selectivity filter in the  $K^+$  channel is formed by four short, polar "helix-turn-extended chain" elements that pack together in the extracellular mouth of the helix bundle. One can easily imagine how such an internal globular structure could be made to span the entire width of the membrane. And I already pointed out to you that there is an internal globular domain in the FhuA iron transporter. So we may well be in for some surprises as more membrane protein structures are solved.

Student B: Well, I guess you can't ask about that in the test. So what do we really need to know about this stuff? I mean, which pages in the book?

K. (*brought back to reality with a crash*): I guess time is up. And I'm happy to say that this was my final lecture this semester. Hope you all fail.

*K. leaves the classroom. Over coffee, he meets R., a colleague who teaches basic enzymology. R. proposes a bet: if more than 75 % of the kids give correct answers to K.'s questions in the final exam, K. will buy R. beer for a week. Of course, K. loses the bet.*

#### Structures of integral membrane proteins

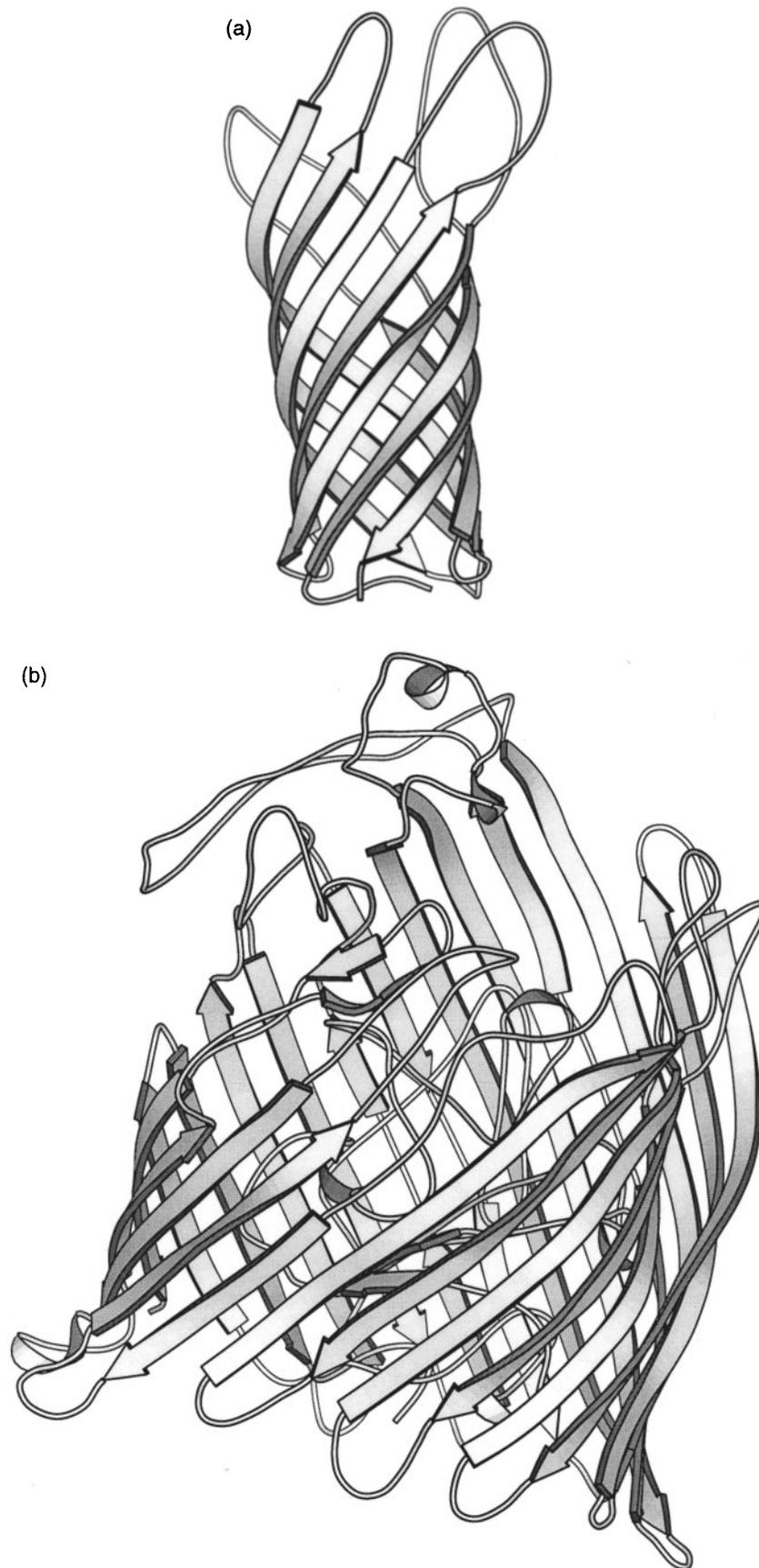
##### Helix bundle class

Bacteriorhodopsin (Essen *et al.*, 1998; Grigorieff *et al.*, 1996; Henderson *et al.*, 1990; Henderson & Unwin, 1975; Kimura *et al.*, 1997; Luecke *et al.*, 1998; Pebay-Peyroula *et al.*, 1997)  
 Photosynthetic reaction center (Allen *et al.*, 1987; Deisenhofer *et al.*, 1985; Yeates *et al.*, 1987)  
 MscL mechanosensitive channel (Chang *et al.*, 1998)  
 LHC II (Kühlbrandt & Wang, 1991; Kühlbrandt *et al.*, 1994)  
 LHC (McDermott *et al.*, 1995)  
 Cytochrome c oxidase (Iwata *et al.*, 1995; Tsukihara *et al.*, 1996)  
 Cytochrome b<sub>cl</sub> complex (Iwata *et al.*, 1998; Zhang *et al.*, 1998)  
 KcsA  $K^+$  channel (Doyle *et al.*, 1998)  
 Glycophorin A (MacKenzie *et al.*, 1997)

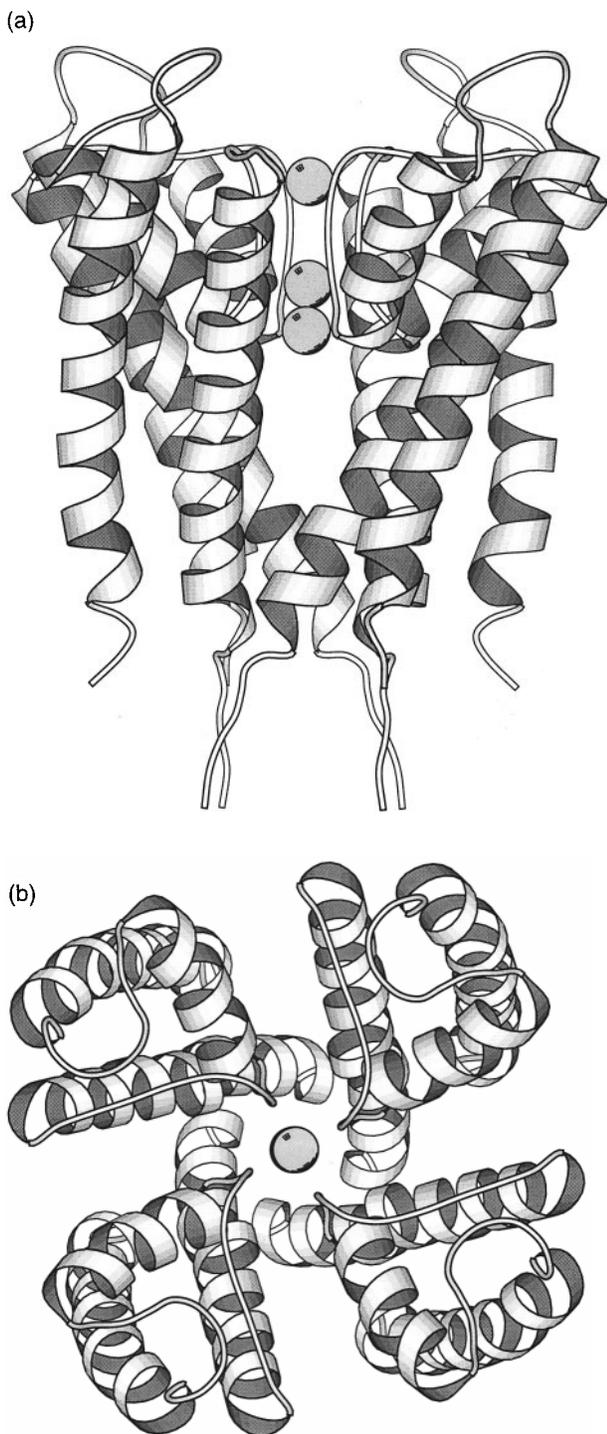
##### $\beta$ -barrel class

LamB porin (Meyer *et al.*, 1997; Schirmer *et al.*, 1995; Wang *et al.*, 1997)  
 R. blastica porin (Kreusch *et al.*, 1994)  
 R. capsulata porin (Weiss *et al.*, 1991a; Weiss *et al.*, 1991b)  
 OmpF porin (Cowan *et al.*, 1992)  
 PhoE porin (Cowan *et al.*, 1992)  
 FhuA siderophore transporter (Ferguson *et al.*, 1998; Locher *et al.*, 1998)  
 OmpA (Pautsch & Schulz, 1998)

**Figure 3.** High-resolution membrane protein structures.



**Figure 4.** Two  $\beta$ -barrel membrane proteins, (a) OmpA and (b) FhuA.



**Figure 5.** The KcsA  $K^+$  channel seen in (a) the plane of the membrane and (b) from above. Three  $K^+$  traversing the channel are shown as spheres.

## Act II

**Wherein K. tries to persuade his colleague R. that the molecular mechanisms of membrane protein assembly are worthy of his attention**

*K. and R. meet by the espresso machine.*

K.: Ever thought about how membrane proteins make it into the membrane?

*R. suspects a long lecture and pretends to be busy pushing various buttons.*

K.: I mean, you have this incredibly greasy piece of protein coming out of the ribosome, and you know it's supposed to somehow make it all the way into the ER membrane.

R.: Uh-uh.

K. (*undaunted*): Just saw some reviews (Rapoport *et al.*, 1996; Bibi, 1998); apparently, there are some really nifty things happening both in the ribosome and the ER translocation machinery when they are confronted with a membrane protein. As I understand it, membrane proteins are targeted to the ER translocon by the same SRP/SRP receptor pathway used by secretory proteins. This of course is old hat, but what's now coming out is that there is an intricate control mechanism to ensure that the hydrophobic transmembrane helices are properly inserted into the bilayer without making the translocation channel leaky to small molecules.

*R. (realizes that he cannot escape and tries a last diversion)*: This new espresso machine is really something. It costs more than our PCR block, but it's worth every cent!

K.: Yeah, so there's this amazing set of studies showing that when the ribosome first binds to the translocon, the protein-conducting channel opens up to the luminal space, allowing free passage to the nascent chain (Crowley *et al.*, 1994). Then, when the first hydrophobic transmembrane segment is polymerized on the ribosome, the whole ribosome-translocon complex reacts by first closing the luminal end of the channel, then immediately opening up an escape route for the nascent chain at the cytoplasmic ribosome-translocon junction (Liao *et al.*, 1997)!

R.: Slowly, please ...

K.: ... and that's not all, it even seems that the opening and closing of the luminal end of the channel is done by BiP, the luminal hsp70 (Hamman *et al.*, 1998). Look, I'll draw it for you (*K. picks up a napkin and makes a sketch*; Figure 6). And even that's not all, because then the transmembrane stretch, now caught in the closed translocation channel, is somehow moved out through the wall of the translocon in a series of steps, finally ending up spanning the lipid bilayer (Do *et al.*, 1996; Mothes *et al.*, 1997).

R.: Well, mighty interesting, but I have a meeting ...

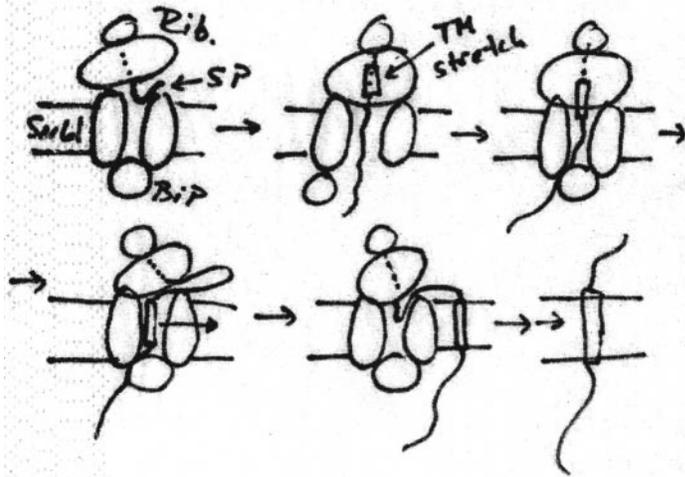


Figure 6. Assembly of a transmembrane protein into the ER membrane (felt-pen on napkin). Private collection.

K: I knew you'd like this! The translocon must be some machine! So far, there are only low-resolution EM pictures of it (Hanein *et al.*, 1996; Meyer *et al.*, 1999), but at least we can now see how the ribosome sits on top of the channel and how the nascent chain moves from the P-site through a passageway in the large subunit down into the translocon (Beckmann *et al.*, 1997).

K. jots down another sketch (Figure 7), and, blissfully forgetting his by now cold espresso, takes a quick breath and goes on.

I'll tell you something else: while there's a similar story in *E. coli* (de Gier *et al.*, 1997), although some small inner membrane proteins seem to make it into the membrane spontaneously (de Gier *et al.*, 1998), most mitochondria don't seem to have the same kind of translocon machinery (Glick & von Heijne, 1996). And sure enough, there are completely different but so far not well understood systems for inserting proteins into the mitochondrial inner membrane, both from the matrix side (Hell *et al.*, 1998) and from the inter-membrane space side (Koehler *et al.*, 1998a,b; Sirrenberg *et al.*, 1998). Just watch what's gonna come out in the next few years on this!

By now R. is getting desperate. Once he gets started, this guy K. is unstoppable. If he brings up protein insertion into the thylakoid membrane, R. will pull out his gun. But there must be a Divine Mercy after all. K. suddenly remembers ...

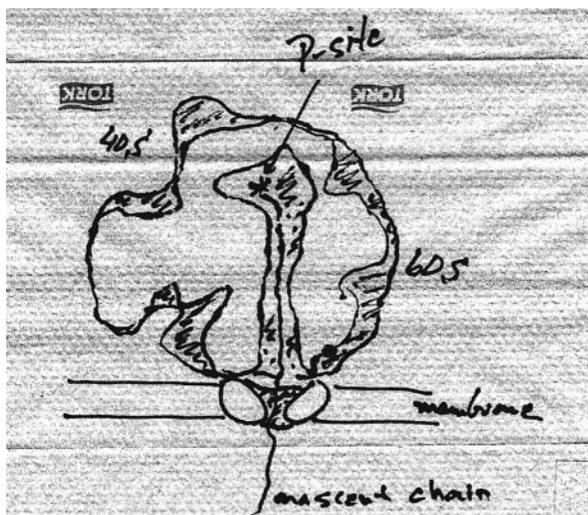


Figure 7. Yeast ribosome on top of Sec61 translocon (felt-pen on paper towel). After Beckmann *et al.* 1997). Private collection.

K.: Oh, dear! Sorry R., but I must run. Forgot to induce my culture; I have some really neat things on N-tail translocation brewing. Tell you more tomorrow!

K. leaves in a hurry. R. finally sits down with his coffee and the latest departmental PM on the cleaning of the centrifuge room. Peace and tranquility reigns.

### Act III

**Wherein K., after struggling for five years to purify 1 mg of his favorite membrane protein, is insulted by the Institute's newly hired crystallographer**

K., somewhat out of breath, rushes into the newly renovated office of, C., the department's latest recruit. Half-empty boxes and untidy heaps of books and papers cover the floor. In a corner, an SGI workstation is flashing its screen-saver.

K.: Hi! Welcome! I'm K., remember me from your interview? Great to have a crystallographer around the place at last!

C. (*looks up, tries in vain to remember K.'s face*): Yeees ... ?

K.: Don't want to rush you, but I happened to see you go into your office and I thought I would give you a chance to get started on some really interesting projects right away. Nothing more important for a young, ambitious guy than to get going, eh? And I've just gotten this great prep.

C.: Prep? Well, you know, it's a little hectic just now with the installation of the X-ray generator, and the fermentor guys coming tomorrow ...

K.: Yeah, so I've been working on this protein since 1995 or so, and really there's no-one else doing it, and we've finally come up with a way to produce it in huge amounts, and it is really pure on the Coomassie stain and all. So I thought ...

C. (*slightly more interested*): Done the mass-spec and light-scattering?

K.: Yeah, well, no. The mass-spec people for some reason weren't too keen, but I'm sure it's OK. Looks like a single band on the gel. And it's more or less a single peak on the HPLC.

C.: What expression system did you use?

K.: We've tried all kinds of vectors and hosts (Grisshammer & Tate, 1995), pET, pGEX, pBAD, Univectors (Liu *et al.*, 1998), BL21 cells, the Walker strains (Miroux & Walker, 1996), *Saccharomyces*, baculo ... you name it. And different media, different growth temperatures, different induction regimes. But finally we settled for a strain used successfully to overexpress various sugar transporters (Racher *et al.*, 1999), since, well I mean, at least we could see the protein then. His-tag purification, of course. And now we've got it pure, as I said.

C. (*not very impressed*): And what do you want me to do?

K.: Well, couldn't you get one of your students to pipet out one of these crystallization kits that I hear so much about? Throw in a few detergents for good measure, although we've got the protein nicely in Triton. Nothing much, for a start.

C. (*suddenly suspicious*): Detergents? Triton? What do you mean?

K.: I guess it's at least sort of OK in Triton, though we haven't been able to run a functional assay yet. But the inclusion bodies certainly dissolve nicely. So I would think there's no problem. Anyway, I thought you guys do these detergent exchanges all the time?

C.: You mean it's a refolded protein? But I still don't understand the detergents!

K.: Well, Triton seemed like a good choice since it was so cheap. No reason to spend money on stuff you don't need. But if you would prefer something else ...

C.: I don't prefer anything! This is too much! A band on a gel, Triton of all things, and I bet you'll tell me next it's a membrane protein! How much of this stuff do you have, by the way?

K.: You won't believe this - a milligram! Or nearly, at least. Maybe 100 µg. Anyway, it will last you forever. And you're right, you'll be one of the first to solve the structure of a membrane protein! It's all yours.

*At which point the Act ends prematurely to avoid an embarrassing collegial exchange of invectives.*

## Act IV

### Wherein K. approaches a bioinformatics whiz kid with a simple question

For some reason, hard-headed experimentalists like K. often lose all their senses when they find a bioinformatician to prey on. They actually seem to believe that one can predict anything with computers.

K. (*sneaks up on B. in the cafeteria lunch line*): Great! Here's the guy I've been looking for all week! Why don't we sit down over there, I've got something to ask you.

B. (*who has been in similar situations before tries pulling a fast one*):

Sorry, but I really have to get a quiet moment to figure out a way to align these two HMM profiles without spending two months of CPU time, actually, maybe you could help ...

*But of course it doesn't work.*

K.: Sure, but there's this membrane protein that I have been working on and I just need to get a handle on its 3D structure. I believe I read in some journal that membrane protein structure is really easy to predict (von Heijne, 1996), and, well, I'm not quite sure how to do it so I thought ...

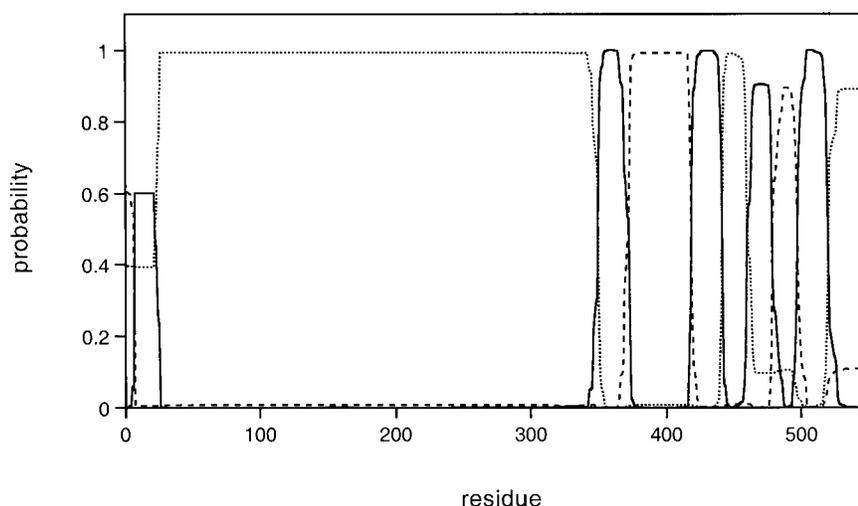
B.: That I could do it for you? No problem Mr K., I'll be more than happy. (*In fact, this kid is really sneaky.*) There are all these fail-safe programs out there, and I can personally guarantee that they'll give you what you want. So, let's see, do you have any idea how many times this protein spans the membrane?

K.: No, it's just a sequence and a band on a gel right now ...

B.: Great! That makes it even better! I'll first run it through ALOM (Klein *et al.*, 1985), a venerable old program that will no doubt predict that what you have is indeed a membrane protein. Then we'll do TMAP (Persson & Argos, 1996), TOPPRED (Claros & von Heijne, 1994), PhD (Rost *et al.*, 1996) and DAS (Cserzö *et al.*, 1997). They'll all tell you how many membrane spanning helices you have. And as a final check we'll run the latest, really fancy hidden Markov models TMHMM (Figure 8; Sonnhammer *et al.*, 1998) and HMMTOP (Tusnady & Simon, 1998) which produce these extremely neat plots.

K. (*duly impressed and not having a clue what a hidden Markov model is*): Hidden Markov models sounds perfect. I knew you would have the answer!

B.: No, that's not the answer. I mean, not the final answer. These programs will only give you the topology of the protein, not its 3D structure. For this you need a lot more, like the simulated annealing protocol



**Figure 8.** TMHMM analysis of the *E. coli* Oxa1p homologue (SwissProt entry P25714). The continuous line indicates the transmembrane helix probability, the dotted line the probability for periplasmic location, and the broken line the probability for cytoplasmic location. Note that the prediction is incorrect (Sääf *et al.*, 1998).

used to model the glycoporphin A dimer and the phospholamban pentamer (Adams *et al.*, 1995, 1996). And, considering that they got the glycoporphin A dimer right (*though only after an extensive mutagenesis study, editor's note*), I'm sure that your structure will come out just the way you want it. And if you're not happy I'll be glad to fiddle with the parameters until you're satisfied. Nothing you can't do with a computer these days.

K.: Would you really? Wow, this is something! I've always wanted my own structure to show at meetings, color slides and all. And now you're telling me that I don't even need to bother with all this crystallography rubbish. I never really liked these guys anyway. Too many *Nature* papers. I bet you these journals only take the structure papers for their cover pictures. When can I come by your lab?

B.: No need, just e-mail me the sequence and you'll get the coordinates back in a couple of days. No big deal. Always happy to help. Just promise me that you'll show the slides at the next Protein Society meeting. You don't even have to put me in the acknowledgments, this is really just five minutes work. Don't bother.

*But K. is already rushing towards his office.*

## Act V

**Wherein K., after a sleepless night brooding over mutually incompatible structure predictions, seeks salvation from his old buddy over in molecular biology**

K. (*stumbles into H.'s lab, hair on end and red eyes*): H., you've got to help. I'm going nuts!

H. (*in full attire, "James Dean Lives" T-shirt, tattered Levi's, and dirty Stan Smith sneakers*): Cool it, K., what's up?

K.: I can't make head or tail out of this prediction stuff. One printout says this, one says that. And with *P*-values and *E*-values and *Q*-values and I don't know what to back it up. Help!

H.: Hey, you must have been to see B.! Don't believe one word of what that guy says! The other day, he actually told me that he had found a previously undiscovered SH2 domain in hemoglobin. Of course, you don't see it in the X-ray structure, but what with prions and all, he thought maybe it can fold to a different structure during apoptosis or something. These computer nerds are total nuts, if you ask me. Waste of time and money. Stuff bioinformatics!

K. (*more relaxed*): OK, well, my problem is this membrane protein, you know. I have it in pure form, but don't know nothing about it really.

H. (*sensing a possibility to make a new convert*): Look here, K., I've always told you there's so much you can do with simple molecular biology techniques. Orientation in the membrane, number of transmembrane segments, tertiary interactions between transmembrane helices. No problem, as far as I'm concerned. Neat stuff like PhoA fusions or engineered glycosylation sites to map topology (Manoil *et al.*, 1990; van Geest *et al.*, 1999), saturation mutagenesis to find important residues (Wen *et al.*, 1996), or the heroic "Kaback Kamikaze Approach" where each new postdoc is forced to make ten single-site cysteine mutants until the whole protein is covered (Kaback, 1996). Then you can attach all kinds of probes to these cysteine residues and measure away to your heart's desire (Voss *et al.*, 1997; Wang & Kaback, 1999; Wu *et al.*, 1999). Great stuff and great numbers of papers to publish. Nothing you can't do with molecular biology.

K. (*slightly impressed*): I know that I could use a few more papers; there are not too many coming out since I started on this membrane protein project. You're telling me there's actually ways to publish in this field?

H.: Millions of ways, as long as your lab can put up with making all the necessary constructs and mutants. Just imagine: there are now ways to study how different residues affect the location of a transmembrane helix relative to the membrane (Monné *et al.*, 1998), how different residues are prone to induce tight turns between transmembrane helices (Nilsson & von Heijne, 1998; Monné *et al.*, 1999), to map precisely where a transmembrane helix exits the membrane (Nilsson *et al.*, 1998), to identify helix-helix interfaces (Lemmon *et al.*, 1992; Russ & Engelman, 1999), and a whole lot of similar problems just using good old-fashioned site-directed mutagenesis and no need to overexpress and purify your protein. Forget about NMR and X-ray crystallography, just go for the DNA and a convenient PCR-mutagenesis kit.

K.: I get this creeping feeling that maybe I started at the wrong end here ...

H.: You've got it! Haven't I always told you to stick with DNA and leave the messy proteins for the poor suckers who like to waste their life in front of an FPLC at +4 °C? Maybe their kind of work is what will really count in the end, but your life will be a whole lot more comfortable than theirs. And if the guy who dies with the largest numbers of papers on his CV wins, you're gonna be number one, no question.

K. (*upset at last*): Well now, H., that's a little stiff even for me! You molecular biologists always have all the answers, don't you! Me, I'd rather die with three good, solid protein biochemistry papers behind me than with a thousand easy-come mutants that in the end tell me nothing, thank you very much! Well, what can you expect when you let guys like Delbrück - a physicist! - wander off into biology. Sorry I came!

*K., enraged, stomps out. H. pulls out a beer from the lab fridge and turns up a Jimi Hendrix solo on his cassette deck. Curtain falls slowly.*

## Act VI

### Wherein K., tired and disappointed, laments in his study

K.: Dear Lord, why did you trick me into this membrane protein business? Haven't I served you well as a hard-working biochemist all my life? Haven't I done my share to reveal in my small way the glory of living things? What did I do wrong? Was it because I abused the students? Or because of those rats I butchered during my mitochondrial days? Or, yes it must be, because of that stupid name I suggested for this gene complex in hedgehogs that wakes them up after hibernation, the "resurrection complex"? I was just trying to make up a neat title for *Cell*, but of course they rejected it out of hand. Served me right.

But this? What do I have for all my toils? A band on a gel, a half-pure Triton prep, an amino acid sequence that tells me nothing. Tons of computer printouts, and some color slides that made me the laughing stock at the Society meeting. Dear Lord, I'm too old for this. I should have stayed with glycolysis and Michaelis-Menten. It wouldn't have made me famous, but at least I knew what I was doing then. Not leaving in disgrace, as now. It all seemed so simple, so well explained in all those reviews. Don't worry, they said, membrane proteins are nothing but a bunch of  $\alpha$ -helices packed into a membrane, much easier to figure out than soluble proteins. And they're on everybody's lips: G-protein-coupled receptors, multidrug resistance proteins, CFTR, ion channels, you name it, they're all membrane proteins. This is the future, they said.

Well, not for me. Not anymore. Nothing like good ol' lysozyme.

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