in ensemble studies[3]. There are several possible rationales for this discrepancy, including a scenario in which many nascent aggregation reactions are efficiently neutralized by proteasome surveillance and thus never mature into macroscopic aggregates. Colby *et al.* also show that their experimentally determined kinetic parameters project to a reasonable approximation of the kinetics of decline in Huntington's disease for a htt concentration of 100 nM (the intracellular concentration of htt is, however, unknown). This ability to project time-dependent neuronal loss and hence Huntington disease age of onset purely on the basis of nucleation kinetics is an intriguing outcome of these experiments. Given the many added layers of complexity in cells as compared to *in vitro* experiments, the degree of agreement between the results of Colby *et al.* and the earlier ensemble measurements[2,3], with respect to both mechanism and projected ages of onset[2], is fairly astounding and gives us confidence in continuing to think of polyglutamine aggregation as a well-behaved and ultimately simple nucleation-dependent process.

At the same time, there is much to be learned before we have a full understanding of the aggregation mechanisms of disease-related polyglutamine proteins. For example, in contrast to simple polyglutamine peptides[7], htt fragments *in vitro* form oligomeric and protofibrillar aggregates in addition to amyloid-like fibrils[9]. Also, tissues from individuals with Huntington disease contain mixtures of both elongation-competent and elongation-incompetent polyglutamine aggregates[6]. Htt aggregation in the cell might thus occur by parallel pathways leading, with differing kinetics, to multiple aggregates of differing morphologies, functionalities and toxic activities[7].

In addition, studies of polyglutamine aggregates in disease and animal models often reveal several, and in some cases many, independent microaggregates in single cells[6,10,11]. Yet the simple nucleated growth models for htt aggregation discussed here suggest that, because nucleation is a very rare event, and because elongation is much more efficient than nucleation, each cell should never contain more than one aggregate (**Fig. 1**). How can we account for this discrepancy? Do multiple aggregates arise via coagulation kinetic mechanisms that do not depend on rare nucleation events, or, alternatively, from the breakdown of larger aggregates within the cell? Or are there aspects of the cellular environment (such as high viscosity, compartmentalization and perhaps others) that effectively create multiple, virtual reaction chambers each capable of sustaining an essentially independent nucleation and aggregation reaction? Further studies using approaches similar to that of Colby *et al.* may help to address some of these important issues.

Although the studies of Colby *et al.* do not address the issue of aggregate pathogenicity, and although they leave a number of important questions about the aggregation process to be answered by future experiments, the results confirm the small nucleus size and slow initiation of polyglutamine aggregation, and they also support the idea that treatments that either reduce the intracellular concentration of the expanded polyglutamine protein or target nascent aggregation nuclei may prove to be viable therapeutic approaches for this family of devastating diseases.

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Wanting contact: how to pick up a channel

Daniel L Minor, Jr

Because of their transmembrane nature, ion channels are notoriously difficult subjects for high-throughput screening approaches. A new method has been developed that provides a simple, elegant and rapid means for assaying channel function.

Assaying single-molecule behavior of ion channels by electrical measurements is a well-established, powerful approach[1]. Such analysis can yield deep insights into channel behavior and interactions with blockers; however, it poses serious technical challenges that have placed its implementation beyond the grasp of many researchers. Finding ways to speed the process, lower the technical barriers and change the method from a serial to a parallel measurement would have an enormous impact on the study of basic channel properties and on the development of methods for high-throughput screening of channel modulators. That is what makes the work of Holden *et al.*[2] reported on page 314 of this issue so exciting. They demonstrate a remarkably simple method for assaying channel function in which the channel proteins are mechanically transferred directly from bacterial colonies into a synthetic membrane and then assayed by single-channel recording. The whole procedure requires no protein purification and takes only minutes.

There are two widely used methods for studying single channels[1]. In one, the researcher brings a fire-polished glass pipette near the surface of a cell membrane. If the membrane cooperates, patience and a variety of tricks (such as sucking on the other end of the pipette and changing the electrical potential of the tip) cause the membrane to form a high-resistance seal over the open end of the pipette. With luck, the membrane patch contains a single channel of interest. From

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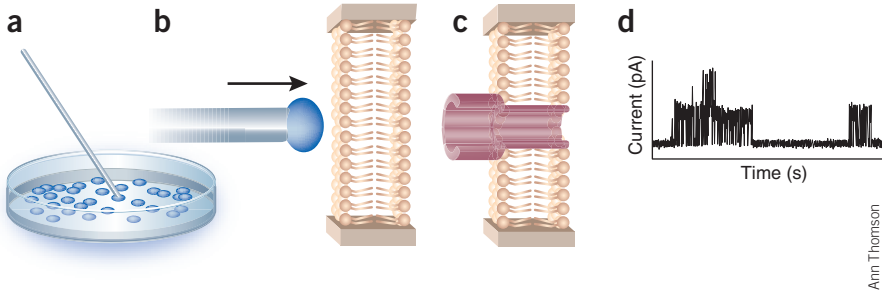

Figure 1 Schematic of the transfer of an αHL pore from a bacterial colony to a synthetic bilayer as described by Holden *et al.*[2]. (**a**) A bacterial colony expressing an ion channel is touched by a thin glass rod. (**b**) The end of the glass rod is brought in contact with a synthetic lipid bilayer. This procedure results in the insertion of ion channels from the rod tip into the bilayer. (**c**) Bilayer containing a single αHL pore. In order to reveal the transmembrane passage, not all of the subunits are shown. (**d**) Example of the type of data recorded from the activity of a single αHL pore, adapted from Figure 2a in Holden *et al.* Current (pA) is measured as a function of time (s). Figure is not drawn to scale.

this point, many different manipulations can be done to study the behavior of the channel in the patch[3]. By no means is this process high throughput. A talented electrophysiologist can record from several such patches in a day; however, it is not uncommon for hours or days to be spent in which no good seals form and no data are acquired. The other method for studying single ion channels is the biochemical approach. Here, the researcher must purify the channel protein, incorporate it into lipid vesicles and coax the vesicles to fuse with a lipid bilayer that has been painted over a small hole in the recording chamber. The technical demands in this approach are also high. One needs a channel protein that can be overexpressed, purified and reconstituted into an artificial bilayer. The fusion event must deliver only one active channel, and waiting for a single pore to enter the bilayer can be tortuously long.

These technical issues are what make the simplicity of the procedure that is reported in this issue so remarkable. Prior work from the Bayley group demonstrated that overexpressed, purified bacterial membrane proteins from both β-barrel and α-helical classes—α-hemolysin (αHL) and the KcsA potassium channel, respectively—could be applied to a cured glob of agarose on the end of a glass rod and then transferred to a synthetic bilayer for functional study[4]. Now, Holden *et al.* show that a similar method can be used to directly transfer active ion channels from bacterial colonies that are overproducing the channel of interest to an artificial bilayer[2]. The procedure is astoundingly simple (**Fig. 1**): take a glass rod having a tip 5–50 μm in radius, touch it to a bacterial colony that expresses the channel of interest, touch the rod to the bilayer, remove the rod and record from the transferred single channel. All of this can be accomplished in minutes.

Taking advantage of this speed, the authors screened 35 combinations of mutant heteromers of the αHL family members LukF and LukS for pores that have novel ligand-binding properties. As αHL proteins are already a favorite subject for engineering nanodevices that are sensors for a wide range of molecules (such as drugs[5], explosives[6], kinase activity[7], small proteins[8] and readouts of polynucleotide strands[9], including DNA at a single-base level[10]), there is no doubt the new approach will facilitate the development of many more diverse αHL sensors.

Many questions remain. For instance, what is the source of the proteins that end up in the bilayer? *Escherichia coli* are surrounded by a peptidoglycan cell wall that protects the outer and inner membranes. This barrier seems to make it unlikely that the channels are transferred directly from either of the cell membranes. The authors suggest that the channels originate from membrane fragments of lysed cells in the colony. For αHL-type proteins, this seems sensible, as αHL family members spend part of their lives as soluble subunits[11]. It is not farfetched to envision the glass tip picking up soluble subunits, which subsequently undergo the natural assembly process on the membrane following transfer. As far as anyone knows, the other subject, KcsA, does not live such a double life. Where, then, are these channels coming from?

Ion channels have been refractory to the high-throughput approaches that have worked so well for soluble proteins, and hence they remain a seriously underexploited drug target[12]. A mere 5% of drugs target ion channels. However, this small slice of pharmacology accounts for $8–10 billion annually in sales and touches a range of clinical problems that include hypertension, epilepsy and diabetes. Clearly, if the means could be devised for high- or even medium-throughput screening, there could potentially be a large gain in the development of new ion channel drug targets. One other problem that is square in the face of ion-channel drug development is drug-induced arrhythmias, which have led to the recent removal of several widely used drugs from the market[13,14]. A cardiac potassium channel known as HERG has an unusually high susceptibility to blockade by drugs and has been linked to the drug-induced arrhythmias. Consequently, all new drugs must be vetted against HERG, and there is strong motivation to weed HERG modifiers out early in drug-candidate development[13,14]. As drug company compound libraries are enormous (~10^5–10^6 different molecules)[12], finding a faster way to screen for adverse effects on HERG function would be extremely beneficial.

Is the method from Holden *et al.* likely to provide the screening system to open up targeting channels for drug discovery? Ion channels can be overexpressed in a variety of cell types that include yeast, insect cells and mammalian cells[3]. If this new method can transfer channels from these cell types, it will be transformative. If the method is instead restricted to channels that can be overexpressed in bacteria, there will still be a great potential in its application. In either case, one can imagine using it to evolve a wide range of new channels with unique properties.

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