current blockades that might be caused by nucleic acids). In addition, they were also consistent with what one would expect for the mean residence time of a single molecule in a nanometer-scale pore. Specifically, nanopores are so short that Einstein's one-dimensional diffusion equation suggests that a single molecule—the size of a pore's length—would spend only ~100 ns in the pore—far too brief a time to be detected by any electrophysiology patch clamp amplifier[8–10] (without this understanding, one would assume that polymers longer than the pore, e.g., DNA oligonucleotides, would take somewhat longer to transit, but still barely be detectable, and details of their composition would be experimentally inaccessible). Furthermore, even if infinite bandwidth amplifiers were available, the flux of ions through the pore in that short time interval would be woefully inadequate to characterize the molecules at high resolution[10]. Importantly, we soon overcame that fundamental limitation with a striking experimental result, which we describe below.

In 1988, Krasilnikov and colleagues[11] reported a method to estimate the limiting aperture of the alpha-hemolysin ion channel using differently sized, non-electrolyte polymers[11]. Their results were particularly intriguing to us because the dependence of that pore's conductance on polymer size did not agree with theory. When we repeated those experiments in 1990, the very first result revealed that the mean residence time of a polymer in the pore was some 500 to 1,000 times greater than expected[12]! One of us (J.J.K.) had described those results to David Deamer and others during a workshop at NASA Ames (1991) and to Deamer personally at a Biophysical Society meeting in 1992. In their Historical Perspective, Deamer *et al.*[1] summarize this work as "Kasianowicz was also collaborating with Bezrukov to investigate the effect of polyethylene glycol on pore conductance and, consistent with earlier reports, found that a pore radius of ~1.1 nm accounted for their results." However, that summary misses the fundamental nature of our study (the importance of which was also missed by others 22 years ago; it took us several years to convince others that the work should be published). Its significance should not be lost today[10]. Nevertheless, the results gave one of us (J.J.K) experience-based confidence to detect individual molecules of single-stranded RNA and DNA with the alpha-hemolysin nanopore[13].

The possibility of passing DNA through nanopores was also plausible to others studying ion channels. For example, Zoratti and colleagues[14,15] used PCR to show that DNA could be transported through membranes containing either *Bacillus subtilis* or voltage-dependent anion channels (VDAC).

The authors also did not mention that Bayley and Oxford Nanopore Technology's (Oxford, UK) abandoned a particular nanopore-based DNA sequencing method. Specifically, in the early 2000s, they proposed the use of an exonuclease (attached adjacent to one of the pore's entrances) to cleave bases one at a time that would be 'read' by the nanopore, and they eventually published a paper suggesting the technique would be viable[16]. However, one of us (J.J.K.) subsequently demonstrated that this method would not work unless the substantial diffusion of cleaved mononucleotides away from the pore could be eliminated[17]. We point out this and our own setbacks[17] to illustrate how the seemingly smooth arrow of time in science, which is implicit in Deamer *et al.*[1] is often not the case. In our view, a frank discussion of what actually happens is important to those embarking on new careers in science and technology, and historians of science.

Finally, Deamer *et al.*[1] leave unanswered the important question of whether the alpha-hemolysin nanopore was sufficient to sequence DNA. Specifically, it is not at all clear whether the Oxford Nanopore Technology MinION device uses alpha-hemolysin, genetically engineered MspA[18,19] or alpha-hemolysin with lessons learned from MspA or Electronic BioSciences' (San Diego) alpha-hemolysin mutagenesis experiments. This information would obviously prove useful to both scientists in the field and those who invest in the technology.

COMPETING FINANCIAL INTERESTS
The authors declare competing financial interests: details are available in the online version of the paper (doi:10.1038/nbt.3570).

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Deamer, Akeson & Branton reply:

John Kasianowicz and Sergey Bezrukov[1] suggest that biophysicists in the 1990s would have accepted nanopore strand sequencing as obvious and plausible based on experimental evidence available at that time. In fact, most of our colleagues told us that it was not only implausible, but also—according to many grant review panel members—impossible.

The authors go on to describe their unpublished attempts to detect DNA with the voltage-dependent anion channel. We were not aware of these unpublished efforts until we received the Correspondence from Kasianowicz and Bezrukov.

Kasianowicz and Bezrukov complain that we did not reveal in our Historical Perspective[2] which nanopore channel is being used in the Oxford Nanopore Technology (Oxford, UK) MinION. This proprietary information has been closely held by the company, and it is only very recently that Oxford Nanopore Technologies has revealed its use of the CsgG pore (see note added in proof in Historical Perspective).

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