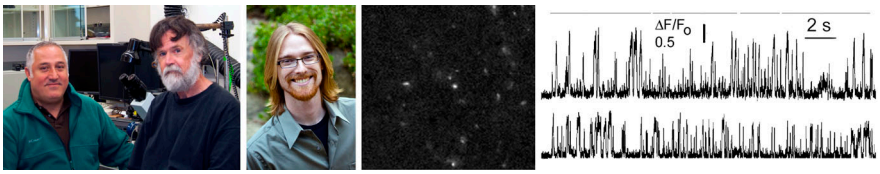
Text by Ben Short
bshort@rockefeller.edu

Imaging β amyloid's pore performance

Study visualizes Alzheimer's disease–related peptides forming toxic calcium channels in the plasma membrane.

Focal Point



PHOTOS COURTESY OF GEORGE DICKINSON

(Left to right) Angelo Demuro, Ian Parker, and Martin Smith use a high-resolution imaging technique called optical patch-clamping to monitor calcium influx through pores formed by the Alzheimer's disease–related peptide Aβ. The approach allows the properties of every channel in a population to be measured simultaneously—individual flashes of a calcium-sensitive fluorescent dye (second from right) are detected by TIRF microscopy and converted into traces of calcium conductance (far right) analogous to the measurements made by traditional patch-clamping methods. Individual Aβ pores have wildly variant properties, which may reflect differences in the peptide's oligomerization state. The results also show that Aβ oligomers can mediate calcium influx without activating endogenous channels or physically disrupting the plasma membrane.

Alzheimer's disease (AD) is triggered by the inappropriate processing of amyloid precursor protein to generate excess amounts of short peptide fragments called β amyloid (Aβ). For many years, the neurodegeneration associated with AD was thought to be caused by the aggregation of Aβ into insoluble, fibrous plaques. However, increasing suspicion now falls on smaller, soluble Aβ oligomers as the toxic form of the protein, partly through their ability to induce excess calcium influx into cells (1), which disrupts synaptic signaling and stimulates apoptosis. Demuro et al. use high-resolution imaging to reveal that Aβ oligomers elevate cytosolic calcium by forming calcium-permeable pores in the plasma membrane (2).

Aβ oligomers might induce calcium influx by physically disrupting the plasma membrane or by activating endogenous calcium channels (3, 4). But electrophysiological lipid bilayer and patch-clamp experiments have also shown that Aβ peptides can form calcium-permeable pores themselves in both artificial and cell membranes (5). A limitation of this technique, says Angelo Demuro, from the University of California, Irvine, is that it only monitors the activity of one or two channels at a time; and different groups have obtained disparate results regarding the properties of Aβ channels using this approach.

> "[β-amyloid pores] show a wide variety of behaviors."

Demuro and colleagues, however, have developed an alternative method to measure the activity of calcium channels in living cells (6). "We can simultaneously record the behavior of thousands of channels using an imaging technique we call optical patch-clamping," Demuro explains. In this approach, *Xenopus* oocytes are filled with a calcium-sensitive dye, and the cells are imaged by total internal reflection fluorescence (TIRF) microscopy, which visualizes only a small section of the cell closest to the coverslip. When plasma membrane channels open to admit calcium from the extracellular medium, small fluorescent flashes indicate the duration and extent of calcium influx at each individual pore.

Demuro et al. found that, just twenty minutes after Aβ oligomers were added to their bathing medium, oocytes displayed the flickering spots of fluorescence that typify calcium influx through single channels. This influx was unlikely to be through endogenous channels activated by Aβ oligomers because *Xenopus* oocytes barely express calcium channels of their own. Moreover, Aβ aggregates weren't simply disrupting the oocyte's plasma membrane, as the influx was inhibited by zinc ions, which block calcium-permeable pores.

Aβ oligomers therefore form calcium-permeable channels of their own in the oocyte plasma membrane. Demuro and colleagues characterized the properties of these pores by simultaneously imaging the activity of thousands of channels in a single membrane region. "They are all different," says Demuro. "[The pores] show a wide variety of behaviors." Most pores opened infrequently and only let in small amounts of calcium, but some opened more often and channeled large amounts of calcium into the cell. Though few in number, Demuro et al.'s measurements suggest that this latter type of pore may be largely responsible for the toxic increase in cytoplasmic calcium levels.

Differences in the properties of individual pores may be caused by differences in the number of Aβ peptides assembled into each channel, with higher-order oligomers forming the more active species of pore. "It would be nice to visualize how many Aβ peptides each pore has and whether this is related to the activity of the channel," Demuro says. If pore activity is affected by the oligomerization state of Aβ, it appears that Aβ peptides continue to aggregate after their insertion into membranes, as the pores became more active as oocytes were exposed to Aβ oligomers for longer periods. This increase in calcium conductance over time may be reflected in the gradual progression of AD symptoms.

Beyond Alzheimer's disease, Demuro et al.'s approach may help explain the pathogenesis of other neurodegenerative disorders like Parkinson's and Huntington's disease, where misfolded and aggregated proteins have also been reported to form calcium-permeable channels. "[Optical patch-clamping] could be easily applied to these diseases as well," Demuro says.

1. Demuro, A., et al. 2005. *J. Biol. Chem.* 280:17294–17300.
2. Demuro, A., et al. 2011. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201104133.
3. Sokolov, Y., et al. 2006. *J. Gen. Physiol.* 128:637–647.
4. Alberdi, E., et al. 2010. *Cell Calcium*. 47:264–272.
5. Arispe, N., et al. 2007. *Biochim. Biophys. Acta.* 1768:1952–1965.
6. Demuro, A., and I. Parker. 2005. *J. Gen. Physiol.* 126:179–192.