23. M. H. Medema *et al.*, *Nucleic Acids Res.* **39**, W339–W346 (2011).
24. K. Jakobi, C. Hertweck, *J. Am. Chem. Soc.* **126**, 2298–2299 (2004).
25. J. T. Heap, O. J. Pennington, S. T. Cartman, G. P. Carter, N. P. Minton, *J. Microbiol. Methods* **70**, 452–464 (2007).
26. J. A. Imlay, *Mol. Microbiol.* **68**, 801–804 (2008).
27. A. Price-Whelan, L. E. P. Dietrich, D. K. Newman, *Nat. Chem. Biol.* **2**, 71–78 (2006).
28. L. E. Dietrich, T. K. Teal, A. Price-Whelan, D. K. Newman, *Science* **321**, 1203–1206 (2008).
29. N. R. Glasser, S. E. Kern, D. K. Newman, *Mol. Microbiol.* **92**, 399–412 (2014).

ACKNOWLEDGMENTS

We thank A. Perner for MS measurements, H. Heinecke for NMR measurements, U. Knüpfer and M. Cyrulies for anaerobic fermentation support, K. Martin for assistance in sample preparation, S. Linde for recording micrographs, L. D. Halder for recording confocal fluorescence microscope images, and T. Stinear and T. Seemann for assistance with DNA sequencing and bioinformatic analyses. Supported by an Alexander von Humboldt Foundation fellowship (S.J.P.) and Deutsche Forschungsgemeinschaft grant SFB 1127 ChemBioSys. G.S., K.I., S.J.P., C.H., and the Leibniz Institute for Natural Product Research and Infection Biology (Hans Knoell Institute) have filed a patent application (WO 2015/113761 A1) that relates to the structures of clostrubins A and B and their biological activities. Author contributions: G.S., K.I., and C.H. designed the research; G.S., K.I., S.J.P., and C.H. prepared the manuscript; G.S. performed anaerobic/aerobic bacterial culture experiments, potato tuber infection experiments, and antibacterial assays and constructed the Δ*clrA* mutant; K.I. and S.J.P. isolated clostrubins; K.I. elucidated structures; S.J.P. performed full genome sequencing and bioinformatic analyses and constructed the *C. beijerinckii* Δ*clrA* mutant; H.-M.D. performed FACS analyses; and M.R. designed and supervised anaerobic cultivation in fermenters. Supplement contains additional data.

SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/350/6261/670/suppl/DC1
Materials and Methods
Supplementary Text
Figs. S1 to S13
Tables S1 to S4
References (*30*–*42*)

10 July 2015; accepted 23 September 2015
10.1126/science.aac9990

OPHTHALMOLOGY

Pharmacological chaperone for α-crystallin partially restores transparency in cataract models

Leah N. Makley,[1]* Kathryn A. McMenimen,[1]* Brian T. DeVree,[3] Joshua W. Goldman,[2] Brittney N. McGlasson,[2] Ponni Rajagopal,[4] Bryan M. Dunyak,[1] Thomas J. McQuade,[5] Andrea D. Thompson,[1] Roger Sunahara,[3] Rachel E. Klevit,[4] Usha P. Andley,[2]† Jason E. Gestwicki[1,5]†

Cataracts reduce vision in 50% of individuals over 70 years of age and are a common form of blindness worldwide. Cataracts are caused when damage to the major lens crystallin proteins causes their misfolding and aggregation into insoluble amyloids. Using a thermal stability assay, we identified a class of molecules that bind α-crystallins (cryAA and cryAB) and reversed their aggregation in vitro. The most promising compound improved lens transparency in the R49C cryAA and R120G cryAB mouse models of hereditary cataract. It also partially restored protein solubility in the lenses of aged mice in vivo and in human lenses ex vivo. These findings suggest an approach to treating cataracts by stabilizing α-crystallins.

The mammalian lens grows by terminal differentiation of epithelial cells into elongated fiber cells. Protein synthesis and turnover in the mature fiber cell are halted, so that the proteins of the lens can remain soluble and transparent throughout life. This remarkable feat is accomplished, in part, by αA-crystallin (cryAA) and αB-crystallin (cryAB), which are molecular chaperones that belong to a family of small heat shock proteins (sHSPs) (*1*, *2*). Together, cryAA and cryAB constitute 30% of the protein content of the lens, where they help maintain the solubility of the other lens proteins, such as β- and γ-crystallins (*2*). Accumulated damage to these proteins over an individual's lifetime can lead to age-related nuclear cataracts, which are composed of aggregated crystallin proteins (*3*) (*2*, *4*–*6*). Clues to the mechanism(s) of age-associated cataracts come from hereditary forms of the disease, which are caused by mutations, such as R120G cryAB, that destabilize the lens proteins (*7*). For example, the R120G mutation disrupts ionic interactions that normally stabilize the cryAB dimer (fig. S1A). It is thought that the unstable protein then assembles into amyloid-like fibers, forming a physical barrier to light and also, in the case of cryAA or cryAB, reducing the available chaperone activity in the lens. Thus, one potential way to treat cataracts may be to identify molecules that bind and stabilize crystallins, favoring the soluble native forms (*8*).

Pharmacological chaperones (PCs) are small molecules that bind and stabilize a native state of a protein (*9*). A PC has been approved for clinical use in the treatment of transthyretin amyloidosis (*10*), and other PCs are being explored in late-stage clinical trials for use in treating a number of other misfolding diseases, such as Gaucher disease (*11*) and Anderson-Fabry disease (*12*). In the current work, we wanted to identify PCs that stabilize the soluble forms of cryAB to suppress its aggregation. Unlike previous targets for PC discovery (*11*), cryAB lacks natural substrates that are suitable as a starting point for drug design (*13*). Moreover, cryAB has no enzymatic activity, making it more difficult to envision a convenient high-throughput screen (HTS) strategy (*10*). These features place cryAB in a family of disease-associated proteins, including tau, myocilin, α-synuclein, and huntingtin, that are often considered to be "undruggable" (*14*, *15*).

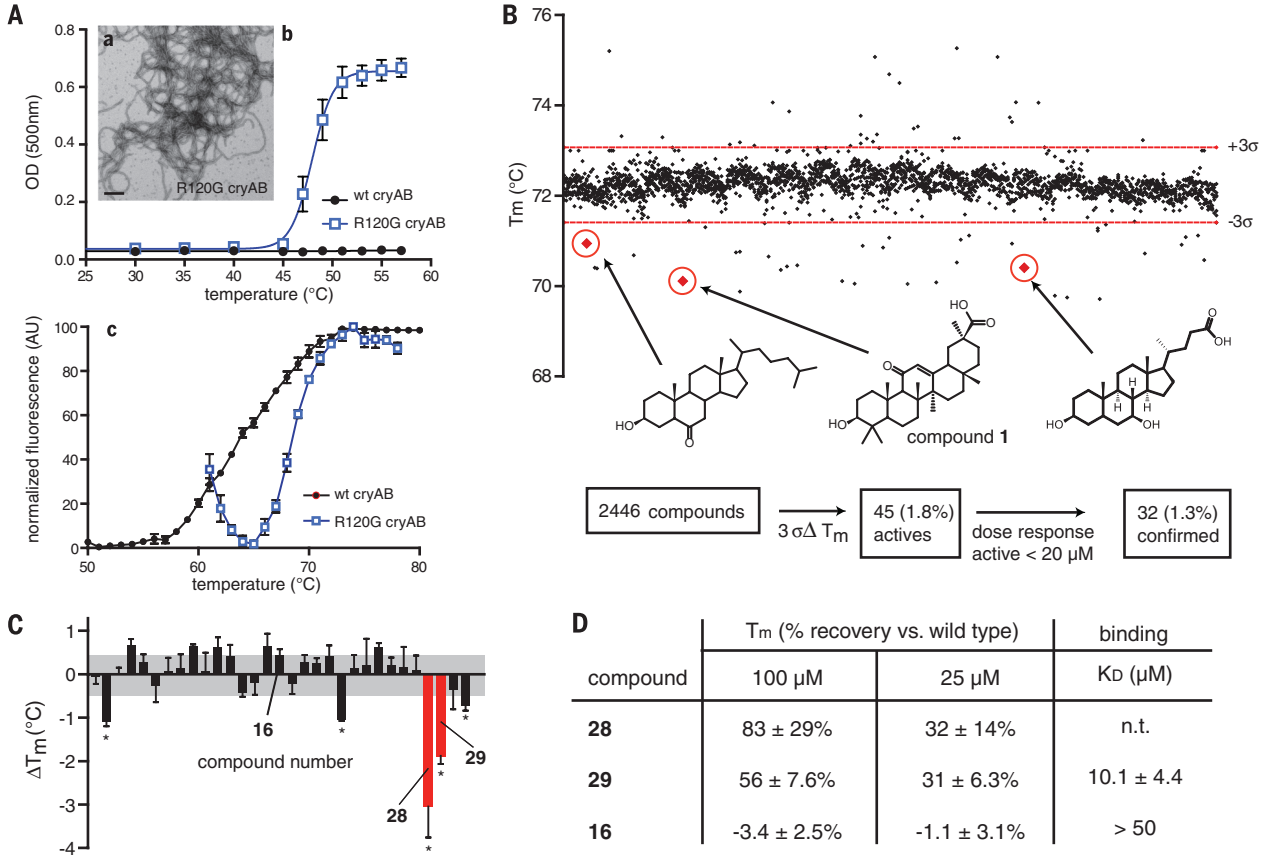
We wondered whether differential scanning fluorimetry (DSF) might provide a way to circumvent some of these challenges. In a typical DSF experiment, an apparent melting transition (T_m) of the protein target is measured in the presence of potential ligands (*16*). Binding of a ligand usually adds free energy to the state that it binds, shifting the apparent T_m. DSF has been used for decades in biochemical studies but has only recently been adapted for high throughput (*15*, *17*–*19*). To see if high-throughput DSF might be applicable to the discovery of PCs for cryAB, we first purified recombinant human R120G cryAB and wild-type cryAB and confirmed that both proteins were soluble and nonaggregated in solution (fig. S1B) (*20*). Upon gentle heating, R120G cryAB formed amyloids more readily than did wild-type cryAB, as measured by light scattering and electron microscopy (EM) (fig. S1, Aa and Ab). Because amyloids are relatively heat-resistant structures, we suspected that samples of R120G cryAB might be more difficult to melt. To investigate this possibility, R120G cryAB and wild-type cryAB were heated in the ThermoFluor DSF platform. Consistent with the model, the apparent T_m of wild-type cryAB was 64.1° ± 0.5°C, whereas the T_m of the R120G cryAB mutant was 68.3° ± 0.2°C (fig. S1Ac). Based on these observations, we hypothesized that molecules able to reduce the apparent T_m of R120G cryAB might be good candidates.

For the pilot screens, we turned to the model protein Hsp27. Hsp27 was used because, although it retains the highly conserved crystallin domain found in cryAA and cryAB (fig. S1C), we found

[1]Departments of Pathology, Biological Chemistry, and Medicinal Chemistry and the Life Sciences Institute, University of Michigan, Ann Arbor, MI, USA. [2]Department of Ophthalmology and Visual Sciences, Washington University School of Medicine, St. Louis, MO, USA. [3]Department of Pharmacology, University of Michigan, Ann Arbor, MI, USA. [4]Department of Biochemistry, University of Washington, Seattle, WA, USA. [5]Center for Chemical Genomics, University of Michigan, Ann Arbor, MI, USA.
*These authors contributed equally to this work. **†Corresponding author. E-mail: jason.gestwicki@ucsf.edu (J.E.G.); andley@vision.wustl.edu (U.P.A.)**

that it had a relatively high melting transition (T_m = 72°C by three independent biophysical methods; fig. S1D). This feature provided a superior signal:noise ratio in the high-throughput DSF platform, with Z′ factor values, a measure of assay robustness, between 0.5 and 0.8 and an average coefficient of variation of 8% at a final volume of only 7 μl in 384-well plates. Accordingly, we screened ~2450 compounds from the MS2000 and NCC collections at a screening concentration between 20 and 40 μM. These compound collections include both natural and synthetic molecules that are known to have activity in a wide variety of assays. The primary screen identified 45 compounds (1.8%) that decreased the apparent T_m by at least three standard deviations (±0.6°C) (Fig. 1B). All 45 of these "actives" were explored in dose-dependence experiments, revealing 32 (71%; 1.3% overall) that decreased the T_m at half-maximal concentrations less than 20 μM. Twelve of the 32 confirmed actives belonged to a single class of related sterols, so we selected this scaffold for further investigation. One of the weakly active sterols was lanosterol. While this manuscript was under review, Zhao *et al.* reported that lanosterol has anti-cataract activity (*21*). However, this compound has limited solubility, and it was only weakly active by HT-DSF, which inspired us to collect 32 additional sterols similar to compound 1 and screen them against R120G cryAB using the DSF procedure. Two compounds (28, 5α-cholestan-3b-ol-6-one; and 29, 5-cholesten-3b,25-diol) were at least two to three times more potent than sterol 1 (Fig. 1C), reducing the apparent T_m by at least 2°C (Fig. 1D and fig. S2, A and E). Many of the other sterols were inactive, suggesting a specific interaction. For example, compound 16 is structurally related to compounds 28 and 29 (fig. S2B), yet it was inactive (Fig. 1D). To confirm the direct interaction of compound 29 with R120G cryAB, we used biolayer interferometry. Immobilized R120G cryAB bound to compound 29 with a dissociation constant (K_d) of 10.1 ± 4.4 μM (Fig. 1D and fig. S2, C and D), whereas compound 16 did not bind (K_d > 50 μM). To examine where on R120G cryAB this interaction takes place, we used ^{15}N–heteronuclear single quantum coherence nuclear magnetic resonance (NMR) experiments to show that compound 29, but not compound 16, bound to the crystallin domain at the dimer interface (fig. S3, A to C). Based on the chemical shift perturbations, we docked compound 29 to cryAB, revealing that this compound fits into a groove that lies between the two protomers (Fig. 2B). In this configuration, compound 29 is predicted to make contacts with both cryAB subunits, suggesting that it might stabilize the native state.

To test whether compound 29 might block amyloid formation, we treated R120G cryAB (15 μM) with compounds 29 or 16 (100 μM), and the extent of aggregation was examined by EM. These studies confirmed that compound 29, but not 16 or the vehicle control, partially suppressed amyloid formation when added before the initiation of aggregation (Fig. 2A). To test whether 29 might also have an effect on pre-formed aggregates, we pre-generated R120G cryAB amyloids and then treated them with compound 29 or 16. Again, compound 29, but not 16, was able to partially reverse amyloid formation (Fig. 2A). The reversal took approximately 2 days, suggesting a slow equilibrium between the amyloid and the soluble forms of cryAB. To quantify these activities, we again treated R120G cryAB with 29 or 16, then



compound	Tm (% recovery vs. wild type) 100 μM	Tm (% recovery vs. wild type) 25 μM	binding KD (μM)
28	83 ± 29%	32 ± 14%	n.t.
29	56 ± 7.6%	31 ± 6.3%	10.1 ± 4.4
16	-3.4 ± 2.5%	-1.1 ± 3.1%	> 50

Fig. 1. A HT-DSF screen identifies pharmacological chaperones for a small HSP. (**A**) The R120G mutant of cryAB (blue) forms heat-resistant amyloids, as judged by EM (a), light scattering (b), and DSF (c). Wild-type (wt) cryAB (black) is more resistant to misfolding and has a lower melting transition by DSF, but will form amyloids under harsher conditions (fig. S1). Results are the average of triplicates, and error bars represent SEM. Microscopy results are representative of studies on at least three independent samples. Scale bar, 0.25 μm. (**B**) Summary of DSF screen against Hsp27. The structures of three active sterols are shown. (**C**) A collection of 32 sterols, based on compound 1, were screened at 20 μM for the ability to restore the T_m of R120G cryAB. Compounds 28 and 29 were two to three times more potent than the initial active molecule. (**D**) Compounds 28 and 29, but not the control compound 16, partially restored the T_m of R120G cryAB and bound to the protein. Results are the average of at least triplicates, and error is SEM.

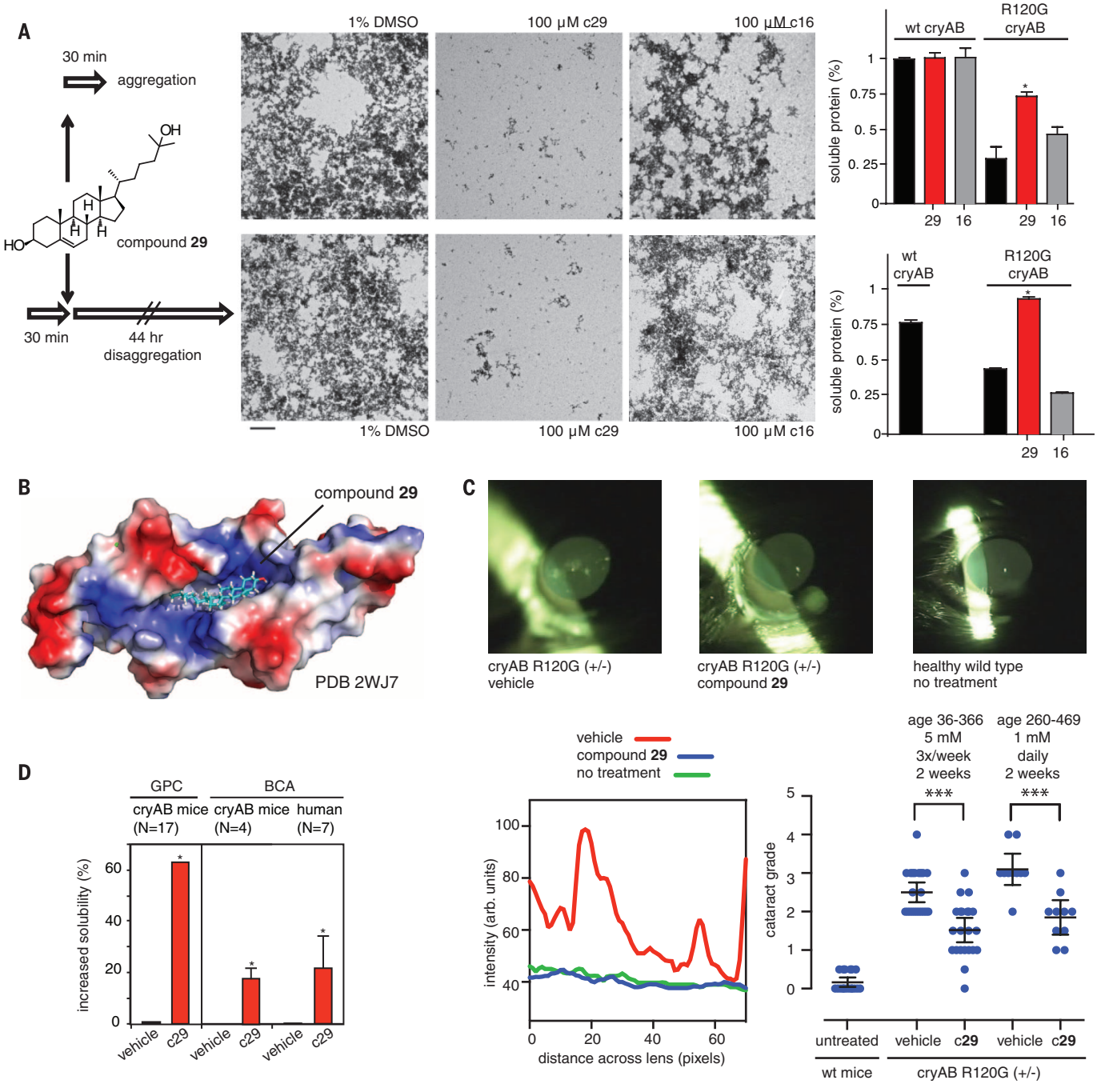


Fig. 2. Compound 29 reverses cataract formation in vitro and in the R120G cryAB knockin mouse. (**A**) Purified R120G cryAB (20 µM) was treated with compound 29 (100 µM), 16 (100 µM,) or a DMSO control (1%) and then aggregated at ambient temperature with shaking for 30 min for the aggregation study. For the disaggregation study, amyloid fibrils were formed using the conditions above (40 µM), and then the samples were treated with 100 µM compound. Aliquots were visualized at 44 hours after treatment. Samples were visualized by EM and then centrifuged to remove insoluble material. The levels of soluble and insoluble R120G cryAB were assessed by absorbance at 280 nm. Results are the average of independent triplicates, and the error bars represent SEM. Electron micrographs are representative of independent triplicates. Scale bar, 1 µm. (**B**) Docking of compound 29 to the crystallin domain of cryAB, based on NMR titrations and chemical shift perturbations (see the supplementary materials for details). (**C**) Summary of the treatment of R120G cryAB knockin mice with compound 29. Slit lamp images and corresponding densitometry plots are shown for wild-type mice aged 60 to 240 days; cryAB R120G heterozygotes aged 66 to 366 days, treated with vehicle control or 29 (5 mM) every other day for 2 weeks; and cryAB R120G heterozygotes aged 260 to 469 days treated with vehicle or compound 29 (1 mM) daily for 2 weeks. The transparency of the treated mice was measured using a LOCS III scoring system on masked images. In both dosing schedules, compound 29 significantly improved transparency ($P < 0.001$). (**D**) Treatment with compound 29 improved the solubility of mouse and human lens proteins, as measured by BCA assays.

separated the insoluble and soluble material by centrifugation and measured the amount of protein in these fractions using bicinchininic acid (BCA) assays. Consistent with the EM results, compound 29, but not 16 or the vehicle control, shifted cryAB into the soluble fraction when added either before or after aggregation (Fig. 2A). Together, these results show that compound 29 can block aggregation and partially reverse R120G cryAB insolubility in vitro. To test whether this activity was restricted to the R120G model, we next used the DSF platform to test whether compound 29 could restore the solubility of other cataract-associated proteins. We found that compound 29 could improve the T_m of three mutants in cryAA (R49C, F71L, and R116C) by at least 2°C (fig. S4). This effect might be expected, because cryAA and cryAB are highly conserved, especially in the region that binds compound 29 (fig. S1C). However, compound 29 had no effect on two mutants of the structurally unrelated lens protein γ-crystallin (P23T or W42R cryGD) (fig. S4), which lacks the α-crystallin domain. Thus, compound 29 appears to bind a specific region of cryAA and cryAB to restore solubility and partially reverse aggregation.

The R120G cryAB knockin mouse develops severe age-associated cataracts, with 100% showing lens opacities by 20 weeks of age (*22*). To test whether compound 29 can reverse this process, a single drop of compound 29 or vehicle (cyclodextrin) was delivered to the right eye of aged R120G cryAB knockin mice three times per week for 2 weeks. These mice had already developed severe cataracts before the treatments. Lens opacity was then assessed with a video slit lamp and scored using a five-grade scale adapted from the Lens Opacities Classification System III (LOCS III) cataract scoring system. In heterozygous R120G cryAB mice (age 36 to 366 days), we observed a substantial improvement in lens opacity grade (lens opacity grade 0.98 ± 0.46; n = 22; P < 0.001) (Fig. 2C). Compound 16 was inactive (lens opacity grade 2 ± 0; n = 3; P > 0.2). The effect of compound 29 was striking by visual inspection (Fig. 2C), quantification of the pixel intensities (Fig. 2C), or quantitative reconstructions of the slit lamp videos (fig. S5). To examine the durability of this effect, we examined the mice 4 weeks after ending treatment with compound 29. In these mice, the LOCS III cataract score was indistinguishable from the values taken immediately after treatment (lens opacity grade ~1.0). The cataracts in R120G cryAB mice become more dramatic with age. To test whether compound 29 could act on these cataracts, a second group of older heterozygotes was treated with a 1 mM solution three times per week for 2 weeks, revealing that lens opacity was significantly improved (lens opacity grade 1.25 ± 0.46; n = 10; P < 0.001) (Fig. 2C). Thus, compound 29 could even partially restore the transparency of older cryAB R120G heterozygous mice.

The R49C cryAA heterozygous knockin mouse also develops cataracts. To investigate the effect of compound 29 on this second model of hereditary cataract, we treated mice with 5 mM compound 29 or vehicle control three times per week for 4 weeks. We found that the lens transparency was significantly increased (lens opacity grade 1.11 ± 0.72; n = 14; P < 0.001) (fig. S6A). Similarly, a second group of R49C cryAA heterozygotes was treated with a lower dose (1 mM solution of compound 29) three times per week for 2 weeks, showing similar efficacy (lens opacity grade 1.3 ± 0.51; n = 5; P = 0.006) (fig.. S6A). Thus, compound 29 could partially restore transparency to two distinct models of hereditary cataract.

Although cataracts and the amount of total insoluble lens protein are only indirectly related (*1*), an improvement in overall protein solubility might be expected for compounds that have substantial anti-aggregation activity. To test whether compound 29 could restore protein solubility, we separated the soluble and insoluble fractions of treated lenses by centrifugation and measured the amount of crystallins (α, β, and γ) by gel permeation chromatography (GPC). We found that compound 29 improved the solubility of the α-crystallins (including cryAA and cryAB) by 63% (S6B). Moreover, the shape of this curve was similar to that of the untreated α-crystallins, suggesting that compound 29 favors a normal structure of the proteins. To verify this finding using a different method, we measured the total amount of soluble and insoluble protein in a subset of the treated lenses by BCA assays. Consistent with this model, compound 29 increased the ratio of soluble:insoluble protein by 16 ± 5% (Fig. 2D). Again, older heterozygous mice showed a more robust response (fig. S6C).

Hereditary cataracts are relatively rare in humans, so we next explored whether compound 29 might have an effect on the more prevalent, age-related cataracts. The exact mechanisms of age-associated cataracts are not clear, but are likely to involve oxidative and other damage to crystallin proteins, resulting in aggregation. Five C57BL/6J wild-type mice (aged 118 to 263 days) with spontaneous age-associated opacities were treated with compound 29 for 2 weeks and then examined by slit lamp biomicroscopy. We found that compound 29, but not vehicle control, improved transparency by at least one grade on the LOCS III scoring system in four of the five mice. Finally, we collected lens material from human patients (aged 63 to 80 years) with grade 1 to 4 cataracts and treated them with either vehicle or compound 29 for 6 days ex vivo. Compound 29, but not the vehicle, increased the amount of soluble protein by 18%, as assessed by BCA assays (Fig. 2D and fig. S6D). Thus, compound 29 may be a promising lead toward the nonsurgical treatment of both hereditary and age-associated cataracts. This molecule partially reversed protein aggregation by binding and stabilizing the more soluble forms of cryAA and cryAB, suggesting that it is a PC for these crystallins. Based on the in vitro, in vivo, and ex vivo studies, we suggest that compound 29 stabilizes the native forms of cryAB, which are in slow equilibrium with the amyloid forms. Once stabilized, the bound cryAB is less likely to misfold, a key feature of a PC. The GPC results also suggest that the treated cryAB is now partly capable of keeping other lens crystallin proteins soluble, suggesting that its chaperone activity is at least partially intact. Finally, and more broadly, these findings suggest that DSF-based HTS campaigns might be ideally suited for identifying PCs with activity against other "undruggable" targets.

REFERENCES AND NOTES

1. H. Bloemendal *et al.*, *Prog. Biophys. Mol. Biol.* **86**, 407–485 (2004).
2. M. Haslbeck, T. Franzmann, D. Weinfurtner, J. Buchner, *Nat. Struct. Mol. Biol.* **12**, 842–846 (2005).
3. H. M. Leibowitz *et al.*, *Surv. Ophthalmol.* **24**, 335–610 (1980).
4. S. Meehan *et al.*, *J. Biol. Chem.* **279**, 3413–3419 (2004).
5. S. Meehan *et al.*, *J. Mol. Biol.* **372**, 470–484 (2007).
6. A. Pande *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **98**, 6116–6120 (2001).
7. P. Vicart *et al.*, *Nat. Genet.* **20**, 92–95 (1998).
8. K. L. Moreau, J. A. King, *Trends Mol. Med.* **18**, 273–282 (2012).
9. F. E. Cohen, J. W. Kelly, *Nature* **426**, 905–909 (2003).
10. P. Hammarström, R. L. Wiseman, E. T. Powers, J. W. Kelly, *Science* **299**, 713–716 (2003).
11. A. R. Sawkar, W. D'Haeze, J. W. Kelly, *Cell. Mol. Life Sci.* **63**, 1179–1192 (2006).
12. J. Q. Fan, S. Ishii, *FEBS J.* **274**, 4962–4971 (2007).
13. A. Biswas, K. P. Das, *J. Biol. Chem.* **279**, 42648–42657 (2004).
14. L. N. Makley, J. E. Gestwicki, *Chem. Biol. Drug Des.* **81**, 22–32 (2013).
15. J. N. Burns *et al.*, *ACS Chem. Biol.* **5**, 477–487 (2010).
16. M. D. Cummings, M. A. Farnum, M. I. Nelen, *J. Biomol. Screen.* **11**, 854–863 (2006).
17. O. Fedorov, F. H. Niesen, S. Knapp, *Methods Mol. Biol.* **795**, 109–118 (2012).
18. L. L. Major, T. K. Smith, *Mol. Biol. Int.* **2011**, 389364 (2011).
19. J. J. Lavinder, S. B. Hari, B. J. Sullivan, T. J. Magliery, *J. Am. Chem. Soc.* **131**, 3794–3795 (2009).
20. Materials and methods are available as supplementary materials on *Science* Online
21. L. Zhao *et al.*, *Nature* **523**, 607–611 (2015).
22. U. P. Andley, P. D. Hamilton, N. Ravi, C. C. Weihl, *PLOS ONE* **6**, e17671 (2011).

ACKNOWLEDGMENTS

We thank J.–M. Fontaine for wild-type cryAB. This work was supported by NIH grants EY017370, EY05681, EY02687, UL1RR024986, and GM007767 and a predoctoral fellowship from the American Foundation for Pharmaceutical Education (L.N.M.). This work was also supported by a grant from Research to Prevent Blindness. J.E.G. is a co-founder of, and is on the Scientific Advisory Board of, Viewpoint Therapeutics, a biotechnology company developing treatments for cataract. L.N.M. is a co-founder and Chief Scientific Officer of Viewpoint Therapeutics. U.P.A. is on the Scientific Advisory Board of, and has stock options in, Viewpoint Therapeutics. The Regents of the University of Michigan hold U.S. Patent 13/944477 covering the method of using oxysterols to treat eye diseases. In addition, Viewpoint Therapeutics has filed provisional patent applications covering unrelated small molecules predicted to bind cryAB.

SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/350/6261/674/suppl/DC1
Materials and Methods
Figs. S1 to S6
References (*23–30*)

10.1126/science.aac9145

Pharmacological chaperone for α-crystallin partially restores transparency in cataract models

Leah N. Makley, Kathryn A. McMenimen, Brian T. DeVree, Joshua W. Goldman, Brittney N. McGlasson, Ponni Rajagopal, Bryan M. Dunyak, Thomas J. McQuade, Andrea D. Thompson, Roger Sunahara, Rachel E. Klevit, Usha P. Andley and Jason E. Gestwicki

(November 5, 2015)
Science **350** (6261), 674-677. [doi: 10.1126/science.aac9145]

Editor's Summary

A visionary approach to transparency

Cataracts are the most common cause of vision loss, especially in our ever-increasing elderly population. Cataracts arise when crystallin, a major protein component of the eye lens, begins to aggregate, which causes the lens to become cloudy. Makley *et al.* explored whether small molecules that reverse this aggregation might have therapeutic potential for treating cataracts, which normally require surgery (see the Perspective by Quinlan). They used a screening method that monitors the effect of ligands on temperature-dependent protein unfolding and identified several compounds that bind and stabilize the soluble form of crystallin. In proof-of-concept studies, one of these compounds improved lens transparency in mice.

Science, this issue p. 674; see also p. 636