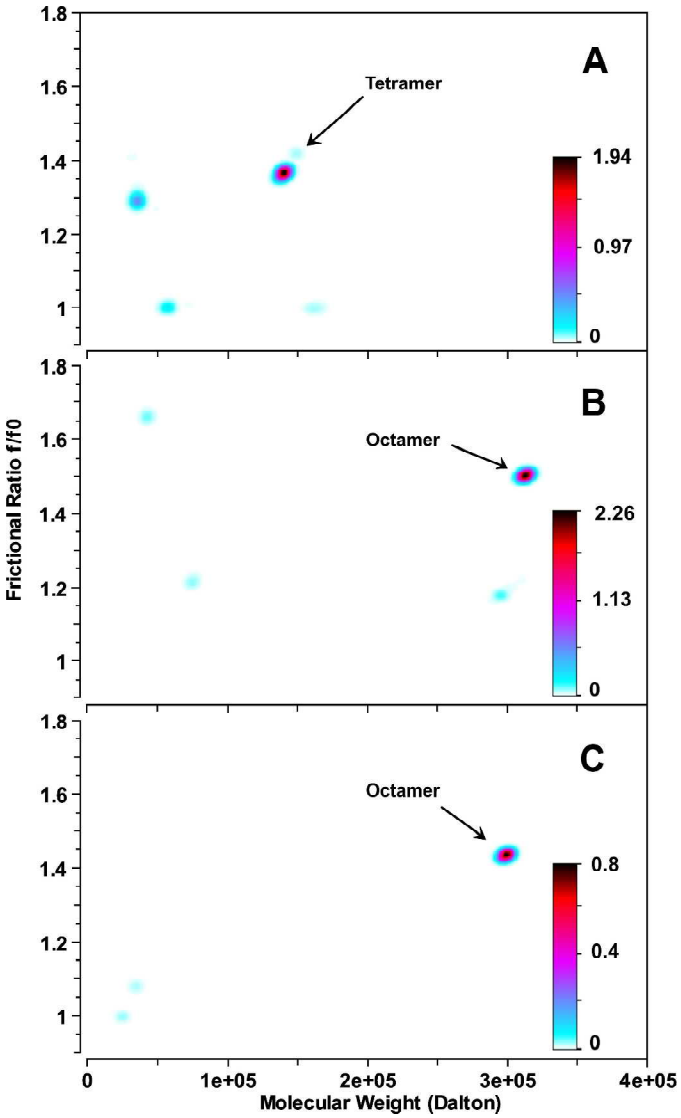
SUPPORTING INFORMATION

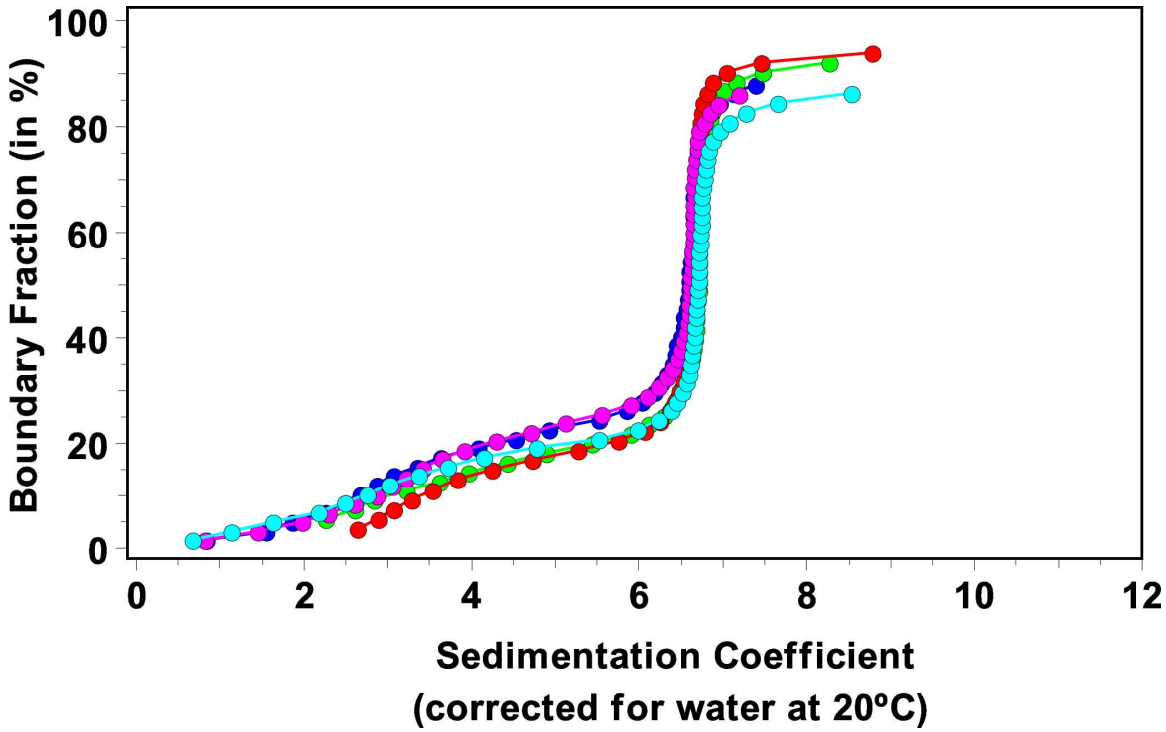


SUPPLEMENTARY FIGURE 1: Genetic algorithm - Monte Carlo analysis of sedimentation velocity data. Frictional ratios and molecular weights are shown for representative tetrameric ($^{-5}$IDH1^{G15D}/IDH2, Panel A) and octameric ($^{-5}$IDH1/IDH2, Panel B, and wild-type IDH1/IDH2, Panel C) forms of yeast IDH. The tetramer exhibits a slight increase in frictional ratio relative the octamer, but both values fall into the range expected for globular proteins (*1*). The molecular weights reported by the velocity experiments are in excellent agreement with expected molecular weights for tetramer (A) and octamer (B, C). Concentration is indicated in interference fringes (A, B) and absorbance at 280 nm (C) and represented by a color gradient where a darker solute spot indicates a higher partial concentration. Minor species are also visible at very low concentrations.

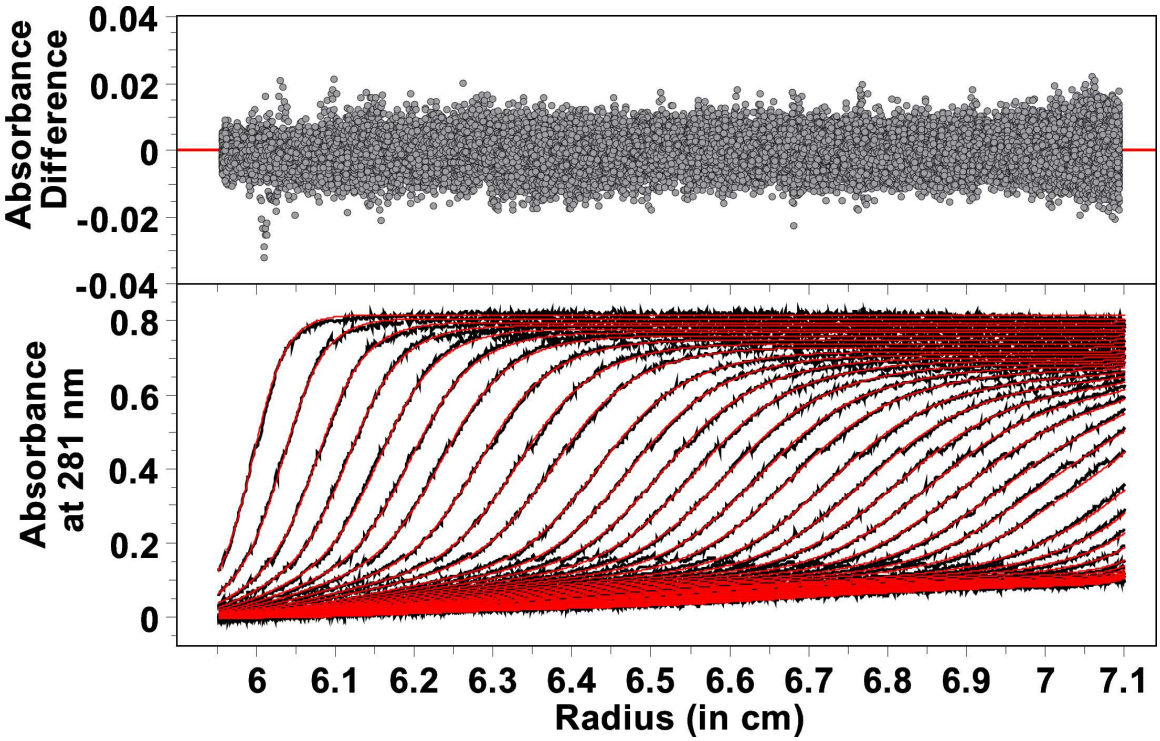
Supplementary Table 1: Detailed hydrodynamic results[a] for the major species in sedimentation velocity analyses of tetrameric and octameric forms of IDH.

Enzyme	M_r, kDa (95% confidence interval)	Sedimentation coefficient, x 10^{13} (95% confidence) interval)	% Boundary fraction
$^{-5}$IDH1/IDH2	310.8 (308.9 – 315.4)	10.55 (10.55 – 10.55)	82.4
$^{-5}$IDH1^{G15D}/IDH2	154.6 (154.0 – 155.4)	6.78 (6.78 – 6.78)	62.5
$^{-5}$IDH1^{D168K}/IDH2	141.1 (136.2 – 145.7)	6.81 (6.70-6.91)	72.8
IDH1/IDH2	299.1 (295.0 – 301.2)	10.71 (10.71 – 10.73)	92.4
IDH1^{G15D}/IDH2	164.8 (157.0 – 188.2)	6.71 (6.68 – 6.82)	68.0

[a]These values were determined with the genetic algorithm - Monte Carlo analysis (*3, 4*).



SUPPLEMENTARY FIGURE 2: van Holde - Weischet integral distribution plots for the IDH1^{G15D}/IDH2 enzyme using 3 different concentrations of enzyme [0.3 (blue), 0.6 (green) and 1.6 (red) absorbance units at 280 nm], and in the presence of a buffer containing ligands including 2 mM (magenta) and 6 mM (cyan) isocitrate. Sedimentation velocity experiments were conducted at 40,000 rpm. All conditions produce nearly identical diffusion-deconvoluted sedimentation coefficient distributions, suggesting that tetramers are not able to re-associate to octamers under these conditions.

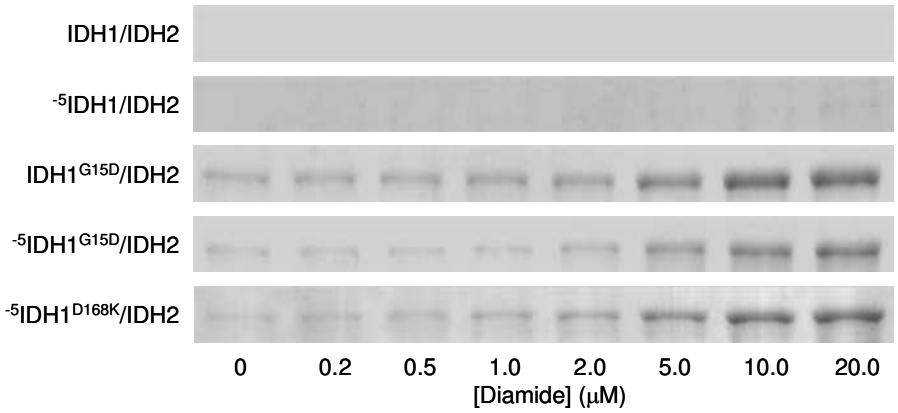


SUPPLEMENTARY FIGURE 3: A representative sedimentation velocity data set of the IDH1^{G15D}/IDH2 enzyme (in black), overlaid with the genetic algorithm - Monte Carlo analysis (in red), and the residuals of this fit (on top). The major boundary portion corresponds to a tetrameric species of the IDH1^{G15D}/IDH2 enzyme, and a low-concentration second boundary is apparent, corresponding to species with lower molecular weights, possibly dimers or monomers of the enzyme.

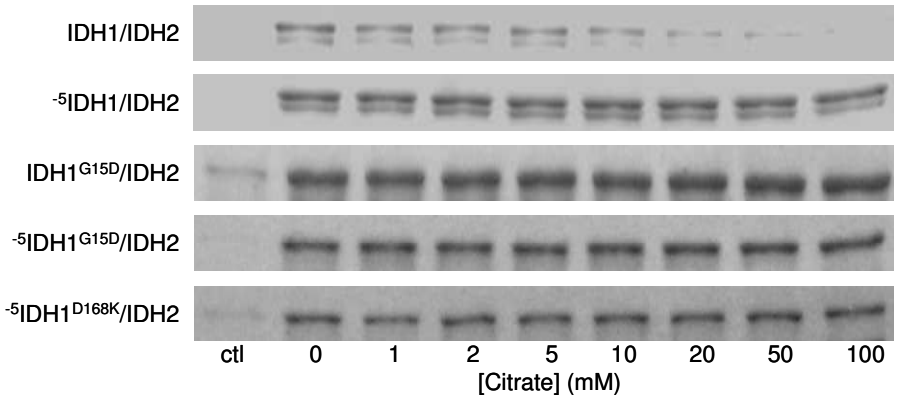
Supplementary Table 2: Detailed hydrodynamic results[a] for the major species in sedimentation velocity analyses of the IDH1^{G15D}/IDH2 mutant enzyme.

Buffer (enzyme concentration)	M_r, kDa (95% confidence interval)	Sedimentation coefficient, x 10^{13} (95% confidence) interval)	% Boundary fraction
Normal[b] (A_{280} = 0.3)	121.3 (117.9 - 131.9)	6.72 (6.71 – 6.72)	66.8
Normal (A_{280} = 0.6)	164.8 (157.0 – 188.2)	6.71 (6.68 - 6.82)	68.0
Normal (A_{280} = 1.8)	157.4 (156.9 - 157.8)	6.71 (6.71 - 6.71)	71.6
2 mM isocitrate[c] (A_{280} = 0.6)	143.7 (138.6 - 147.6)	6.76 (6.75 - 6.78)	63.5
6 mM isocitrate[d] (A_{280} = 0.6)	149.4 (147.4 - 153.4)	6.80 (6.79 - 6.81)	66.9

[a]These values were determined with the genetic algorithm - Monte Carlo analysis (*3*, *4*).
[b]Normal buffer conditions were 20 mM Tris-HCl (pH 7.4), 150 mM NaCl.
[c]The buffer was 20 mM Tris-HCl (pH 7.4), 150 mM NaCl, plus 4 mM $MgCl_2$, 100 μM AMP, and 2 mM isocitrate.
[d]The buffer was 20 mM Tris-HCl (pH 7.4), 150 mM NaCl plus 4 mM $MgCl_2$, 100 μM AMP, and 6 mM isocitrate.



SUPPLEMENTARY FIGURE 4: Disulfide-bond formation in response to incubation with low concentrations of diamide. Shown are bands corresponding to the IDH2 subunit containing a Cys-150 disulfide bond (*2*) (see Figure 7). The indicated enzymes were incubated for 2 h with increasing concentrations of diamide prior to determination of catalytic activity (see Figure 8) and electrophoresis of 3.5 μg samples on non-denaturing gels. Gels were stained with Coomassie blue. [Disulfide-bond formation in the octameric IDH1/IDH2 and $^{-5}$IDH1/IDH2 enzymes requires higher concentrations of diamide, as shown in Figure 8.]



SUPPLEMENTARY FIGURE 5: Effect of ligands on diamide-induced disulfide bond formation. Shown are bands corresponding to the IDH2 subunit containing a Cys-150 disulfide bond (*2*) (see Figure 7). Enzymes were pre-incubated with 4 mM $MgCl_2$, 100 μM AMP, and the indicated concentrations of citrate for 15 min prior to treatment with concentrations of diamide estimated to produce 50% reductions in catalytic activity (see Figure 9). Protein samples (3.5 μg) were electrophoresed using non-denaturing gels, and the gels were stained with Coomassie blue.

References

1. Demeler, B. (2010) Methods for the design and analysis of sedimentation velocity and sedimentation equilibrium experiments with proteins, *Curr Protoc Protein Sci Chapter 7*: Unit 7.13.
2. Garcia, J. A., Minard, K. I., Lin, A. P., and McAlister-Henn, L. (2009) Disulfide bond formation in yeast NAD^+-specific isocitrate dehydrogenase, *Biochemistry 48*, 8869-8878.
3. Brookes, E., and Demeler, B. (2007) Parsimonius Regularization using Genetic Algorithms Applied to the Analysis of Analytical Ultracentrifugation Experiments. GECCO Proceedings ACM 978-1-59593-697-4/07/0007.
4. Demeler, B., and Brookes, E. (2008) Monte Carlo analysis of sedimentation experiments, *Colloid Polym Sci 286*, 129-137.