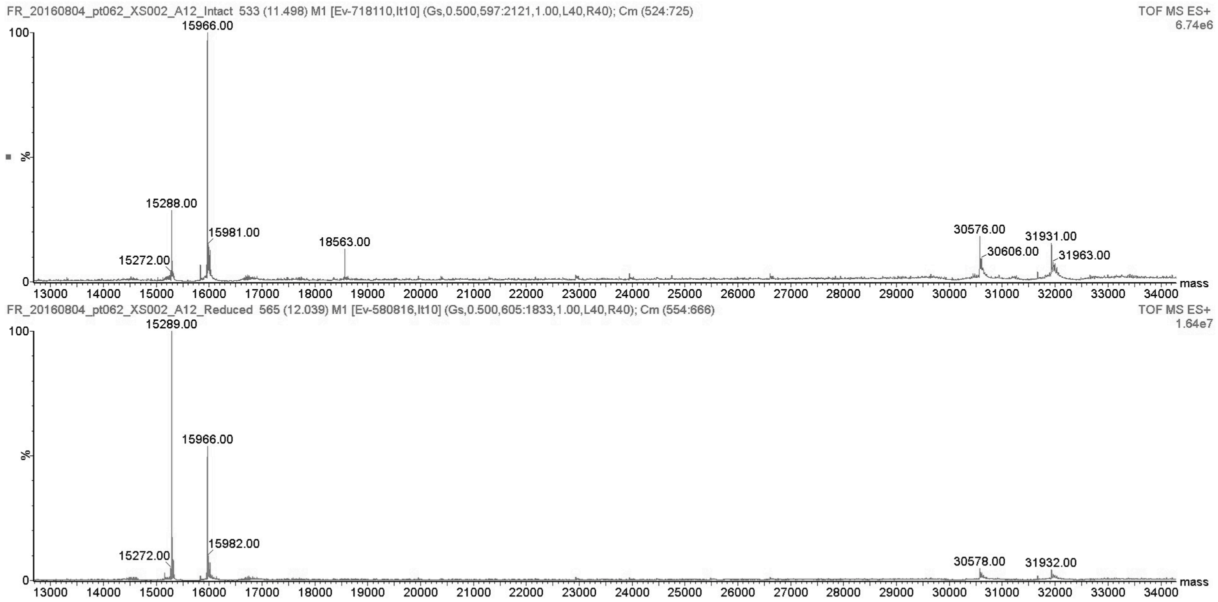


Figure S1 LC-MS Q-TOF of A12 mutant pre and post DTT treatment
Purified A12 Hb pre (top) and post (bottom) DTT treatment. For conditions see Materials and Methods. Note that the theoretical mass of the Hb subunits (with uncleaved N-terminal methionine) is α–subunit = 15289 Da and β-subunit = 15966 Da.

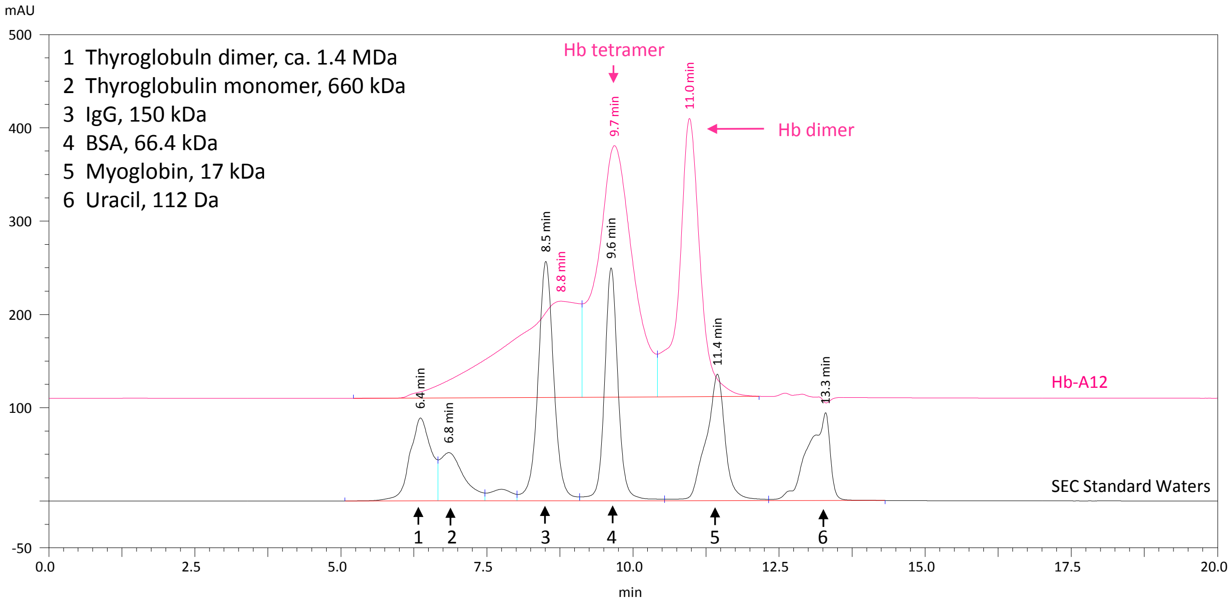


Figure S2 SEC of A12 versus SEC standards

For conditions see Materials and Methods. Note that compared to Figure 4, this particular sample retains some larger molecular weight contaminants

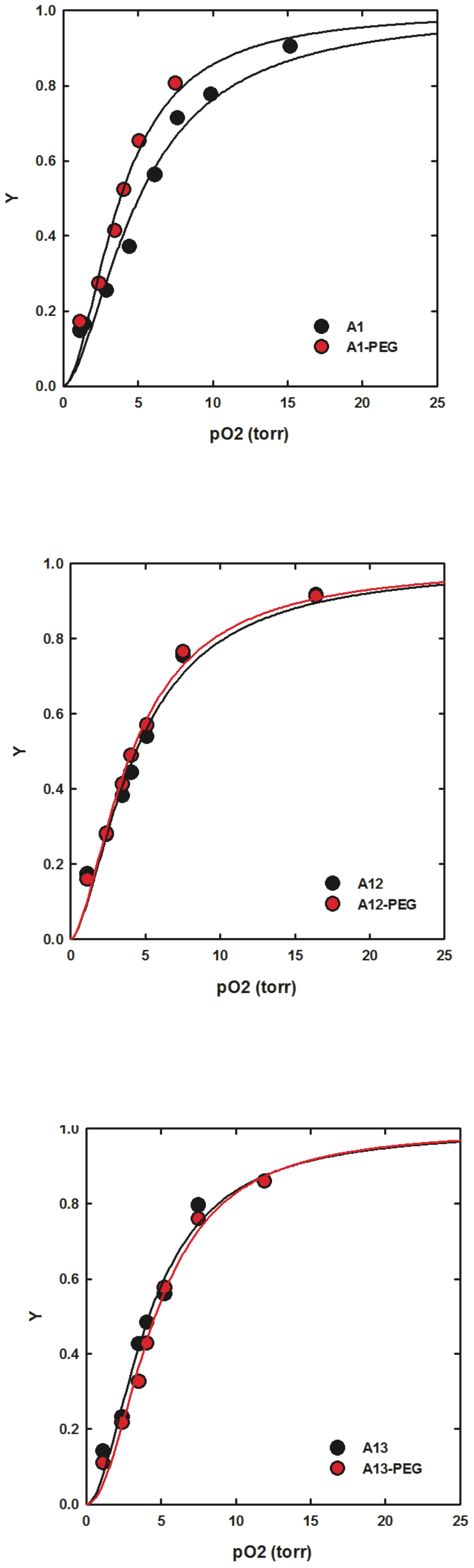
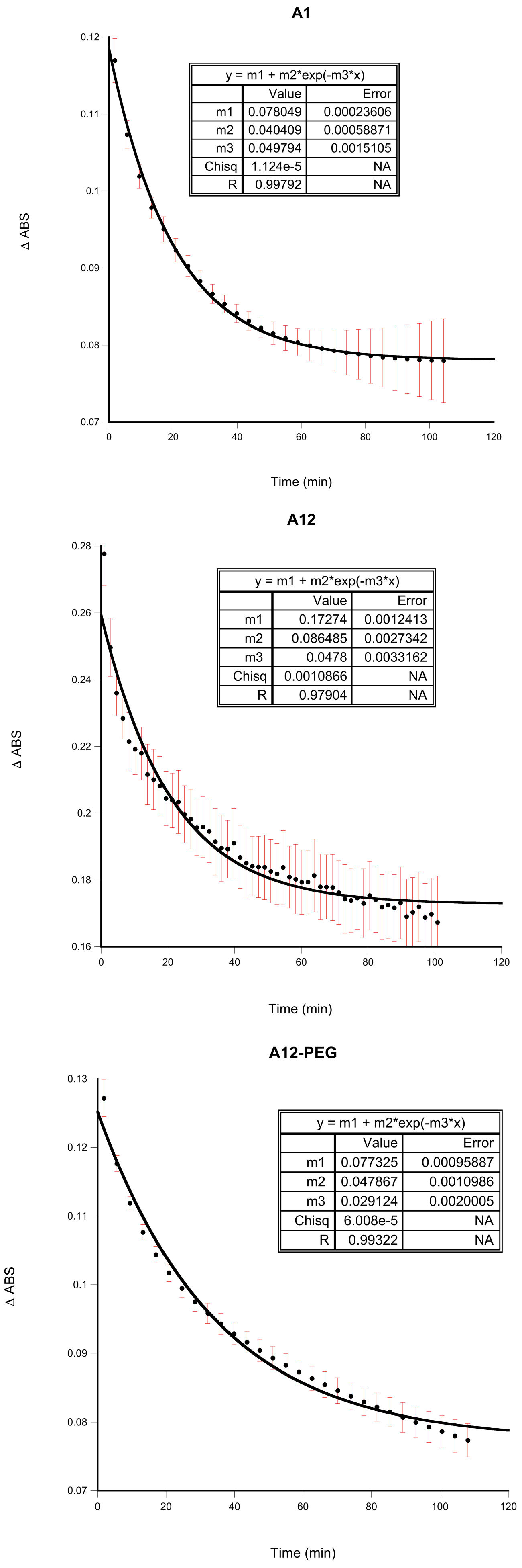


Figure S3 Oxygen equilibrium binding curves
Curve fit values presented in Table 1. For conditions see Materials and Methods.



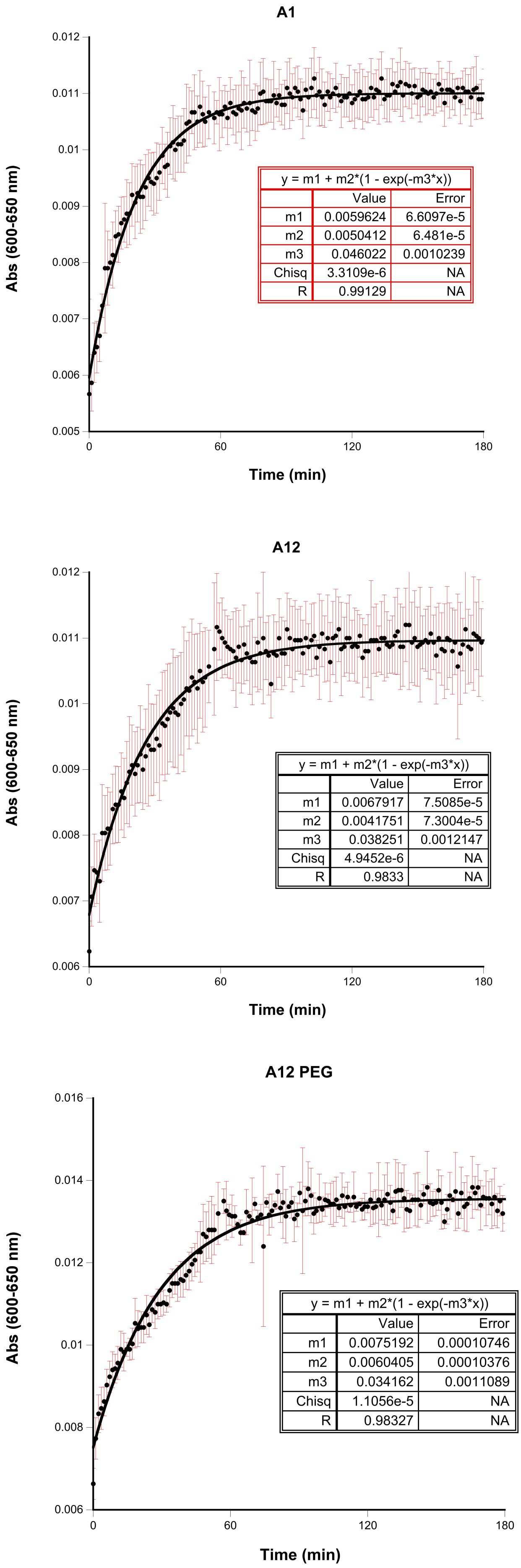
y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.078049	0.00023606
m2	0.040409	0.00058871
m3	0.049794	0.0015105
Chisq	1.124e-5	NA
R	0.99792	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.17274	0.0012413
m2	0.086485	0.0027342
m3	0.0478	0.0033162
Chisq	0.0010866	NA
R	0.97904	NA

y = m1 + m2*exp(-m3*x)		
	Value	Error
m1	0.077325	0.00095887
m2	0.047867	0.0010986
m3	0.029124	0.0020005
Chisq	6.008e-5	NA
R	0.99322	NA

Figure S4 Time course of autoxidation of oxyHb

For conditions see Materials and Methods. Data plotted are means ± SD for six measurements. Curve fits are non linear regression to single exponential decay. Note that for ease of presentation, the curve fits represented here are single fits ± SEM to the mean of the six averaged time courses, whereas those in Table 2 are the mean ± SD of the calculated fits to the six individual time courses. However, any differences in values for these two methods were trivial.



A1: y = m1 + m2*(1 - exp(-m3*x))

	Value	Error
m1	0.0059624	6.6097e-5
m2	0.0050412	6.481e-5
m3	0.046022	0.0010239
Chisq	3.3109e-6	NA
R	0.99129	NA

A12: y = m1 + m2*(1 - exp(-m3*x))

	Value	Error
m1	0.0067917	7.5085e-5
m2	0.0041751	7.3004e-5
m3	0.038251	0.0012147
Chisq	4.9452e-6	NA
R	0.9833	NA

A12 PEG: y = m1 + m2*(1 - exp(-m3*x))

	Value	Error
m1	0.0075192	0.00010746
m2	0.0060405	0.00010376
m3	0.034162	0.0011089
Chisq	1.1056e-5	NA
R	0.98327	NA

Figure S5 Time course of heme dissociation from metHb

For conditions see Materials and Methods. Data plotted are means ± SD for 3 measurements. Curve fits are non linear regression to single exponential. Note that for ease of presentation, the curve fits represented here are single fits ± SEM to the mean of the three averaged time courses, whereas those in Table 2 are the mean ± SD of the calculated fits to the three individual time courses. However, any difference in values for these two methods was trivial.