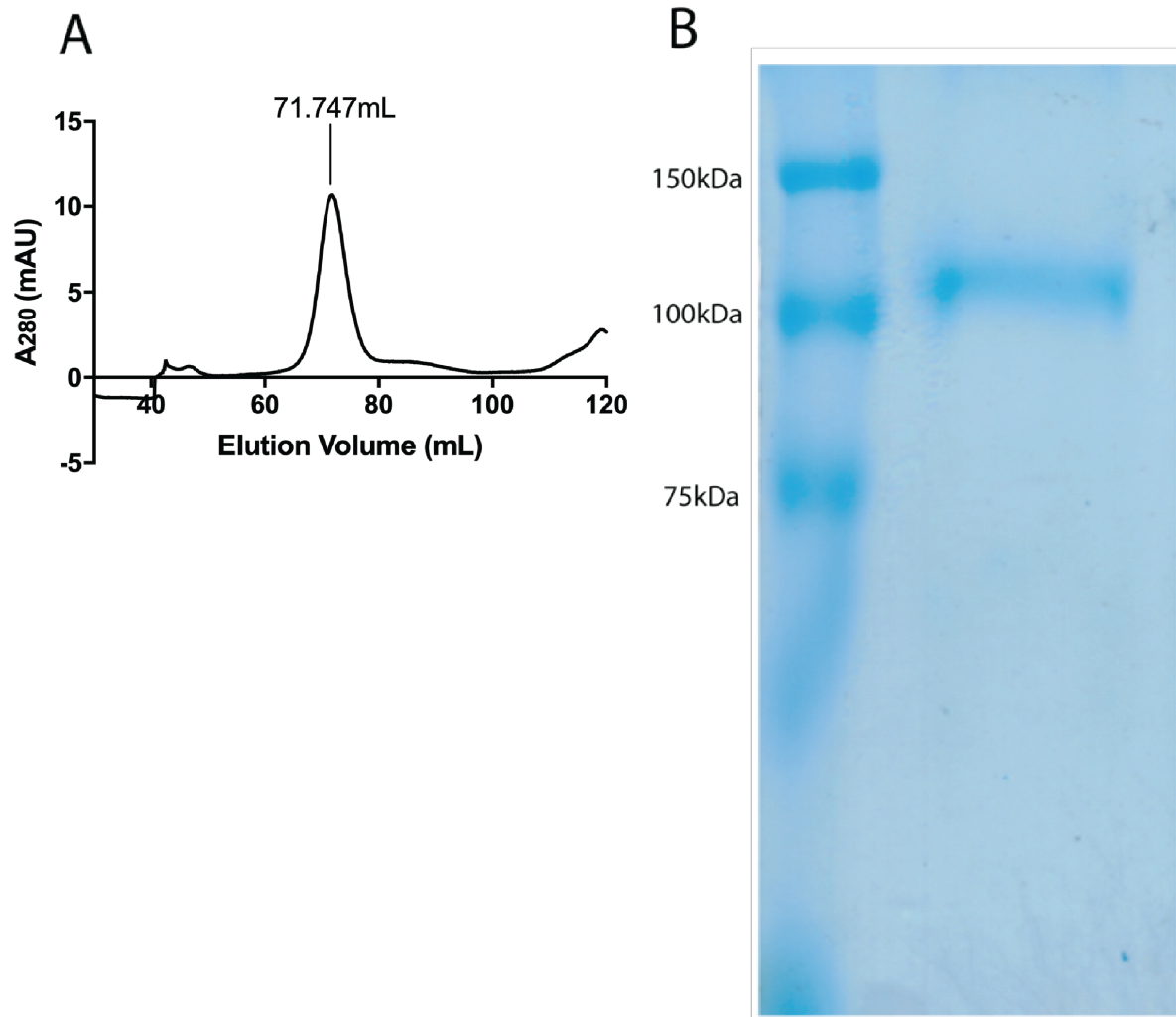


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**Supporting Information (SI)**

**Structural studies of thyroid peroxidase (TPO) show the monomer interacting with autoantibodies in thyroid autoimmune disease: implications for autoimmunity to TPO**

Daniel E. Williams, Sarah N. Le, David E. Hoke, Peter G. Chandler, Monika Gora,  
Marlena Godlewska, J. Paul Banga and Ashley M. Buckle



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13 **Figure S1 - Purification of the TPO construct  $\Delta$ proTPOe-GCN4** (A) A chromatogram  
14 from a Superdex S200 16/60 column, showing  $\Delta$ proTPOe-GCN4 eluting as a single  
15 major peak at 71.7mL, consistent with a 110 kDa protein. No other major large species  
16 appears to be present. (B) Reducing SDS-PAGE analysis of purified  $\Delta$ proTPOe-GCN4  
17 shows a major band at ~110 kDa.

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1	MRALAVLSVT	LVMACTEAF	PFISRGKELL	WGKPEESRVS	SVLEESKRLV
51	DTAMYATMQR	NLKKRGILSP	AQLLSFSKLP	EPTSGVIARA	AEIMETSIQA
101	MKRKVNLTQ	QSQHPTDALS	EDLLSIANM	SGCLPYMLPP	KCPNTCLANK
151	YRPITGACNN	RDHPRWGASN	TALARWLPPV	YEDGFSQPRG	WNPGLYNGF
201	PLPPVREVT	HVIQVSNEVV	TDDDRYSDLL	MAWGQYIDHD	IAFTPQSTSK
251	AAFGGGADCQ	MTCENQNPCF	PIQLPEEARP	AAGTACLPHY	RSSAACGTGD
301	QGALFGNLST	ANPRQQMNG	TSFLDASTVY	GSSPALERQL	RNWTSAEGLL
351	RVHARLRDSG	RAYLPFVPPR	APAACAPEPG	IPGETRGPCF	LAGDGRASEV
401	PSLTALHTLW	LREHNR	LAAA	LKALNAHWSA	DAVYQEAR
451	RDYIPRILGP	EAFQQYVG	PGPY	EGYDSTANPT	VSNVFSTAAF
501	VRRLDASFQE	HPDLPGLWLH	QAFFSPWTL	RGGGLDPLIR	GLLARPAKLQ
551	VQDQLMNEEL	TERLFVLSNS	STLDLASINL	QRGRDHGLPG	YNEWREFCGL
601	PRLETPADLS	TAIASRSVAD	KILDLYKHPD	NIDVWLGG	LA
651	PLFACLIGKQ	MKALRDGDWF	WWENSHVFTD	AQRRELEKHS	LSRVICDNTG
701	LTRVPMDAFQ	VGKFPEDEFES	CDSITGMNLE	AWRETFPQDD	KCGFPESVEN
751	GDFVHCEESG	RRVLVYSCRH	GYELQGREQL	TCTQEGWDFQ	PPLCKDVNEC
801	ADGAHPPCHA	SARCRNTKGG	FQCLCADPYE	LGDDGR	TCVD
851	LEDKVEELLS	KNYHLENEVA	RLKKLVGERG	TGSHHHHHHH	H

**Figure S2 – Mass spectrometry analysis of  $\Delta$ proTPOe-GCN4.** Sequence coverage was reported as 70% with a protein score of 19294, making  $\Delta$ proTPOe-GCN4 the most abundant species in the sample. Full length TPOe-GCN4 is in black lettering, with detected peptides highlighted in red. Note that residues 1 through 108 comprise the signal peptide and propeptide that are not incorporated into full length  $\Delta$ proTPOe-GCN4, though are included here to demonstrate their successful non-inclusion in our construct.

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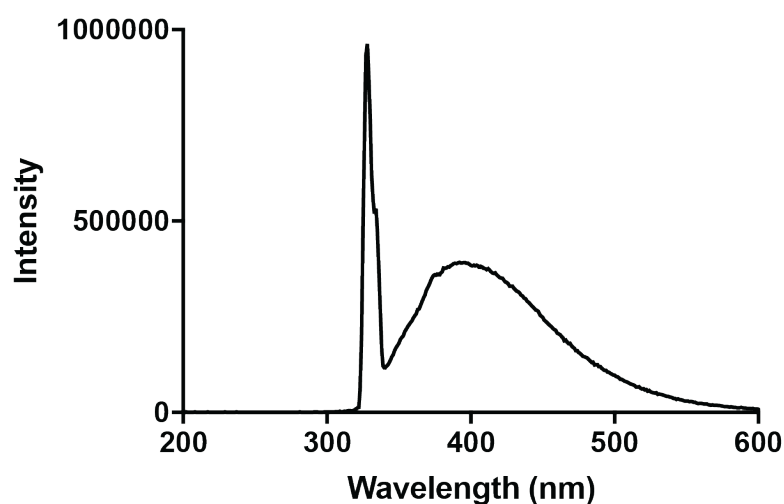
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51 DTAMYATMQR NLKKRGILSP AQLLSFSKLP EPTSGVIARA AEIMETSIQA
101 MKRKVNLKTQ QSQHPTDALS EDLLSIIANM SGCLPYMLPP KCPNTCLANK
151 YRPITGACNN RDHPRWGASN TALARWLPPV YEDGFSQPRG WNPGLYNGF
201 PLPPVREVTR HVIQVSNEVV TDDDRYSDLL MAWGQYIDHD IAFTPQSTSK
251 AAFGGGADCQ MTCENQNPCF PIQLPEEARP AAGTACLPHY RSSAACGTGD
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351 RVHARLRDSG RAYLPFVPPR APAACAPEPG IPGETRGPCF LAGDGRASEV
401 PSLTALHTLW LREHNRLAAA LKALNAHWSA DAVYQEARKV VGALHQIITL
451 RDYIPRILGP EAFQQYVGPy EGYDSTANPT VSNVFSTAAF RFGHATIHPL
501 VRRLDASFQE HPDLPLGLWLH QAFFSPWTLR RGGGLDPLIR GLLARPAKLQ
551 VQDQLMNEEL TERLFVLSNS STLDLASINL QGRGDHGLPG YNEWREFCGL
601 PRLETPADLS TAIASRSVAD KILDLYKHPD NIDVWLGGLA ENFLPRARTG
651 PLFACLIGKQ MKALRDGDWF WWENSHVFTD AQRRELEKHS LSRVICDNTG
701 LTRVPMDAFQ VGKFPEDFES CDSITGMNLE AWRETFPQDD KCGFPESVEN
751 GDFVHCEESG RRVLVYSCRH GYELQGREQL TCTQEGWDFQ PPLCKDVNEC
801 ADGAHPPCHA SARCRNTKGG FQCLCADPYE LGDDGRTQVD SGRLPRRMKQ
851 LEDKVEELLS KNYHLENEVA RLKKLVGERG TGSHHHHHHH H

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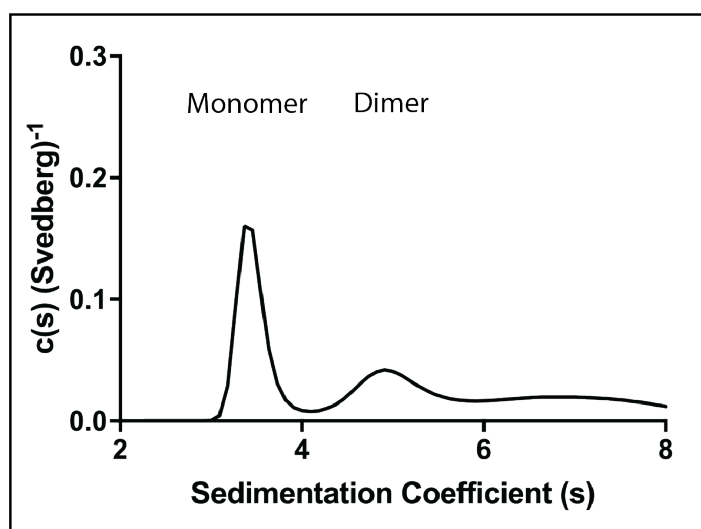
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32 **Figure S3 – Mass spectrometry analysis of suspected degraded ΔproTPOe-GCN4**  
33 **fragment.** Sequence coverage was reported as 63% with a protein score of 8710, making  
34 a degraded form of ΔproTPOe-GCN4 the most abundant species in the sample. Full  
35 length ΔproTPOe-GCN4 is in black lettering, with detected peptides highlighted in red.  
36 Note that residues 1 through 108 comprise the signal peptide and propeptide that are  
37 not incorporated into full length ΔproTPOe-GCN4, though are included here to  
38 demonstrate their successful non-inclusion in our construct.

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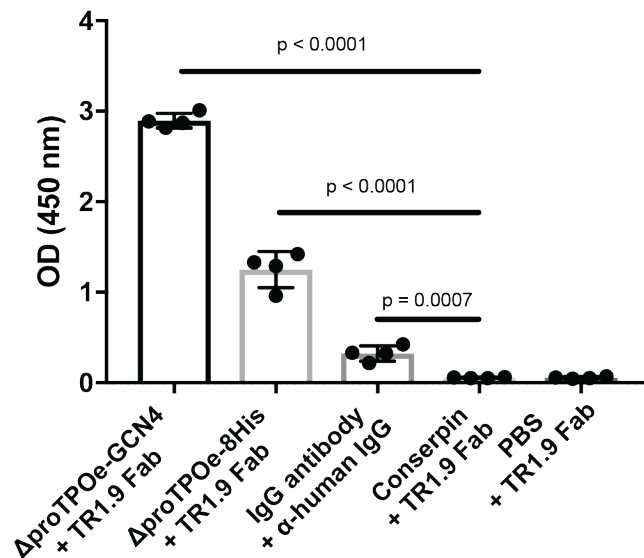


**Figure S4 – Characterisation of enzyme activity of  $\Delta$ proTPOe-GCN4.** Spectral scan of  $\Delta$ proTPOe-GCN4 after excitation using a wavelength of 330nm resulted in a Soret peak at 385nm, which is characteristic of hemoproteins. This indicates successful heme group incorporation into  $\Delta$ proTPOe-GCN4.

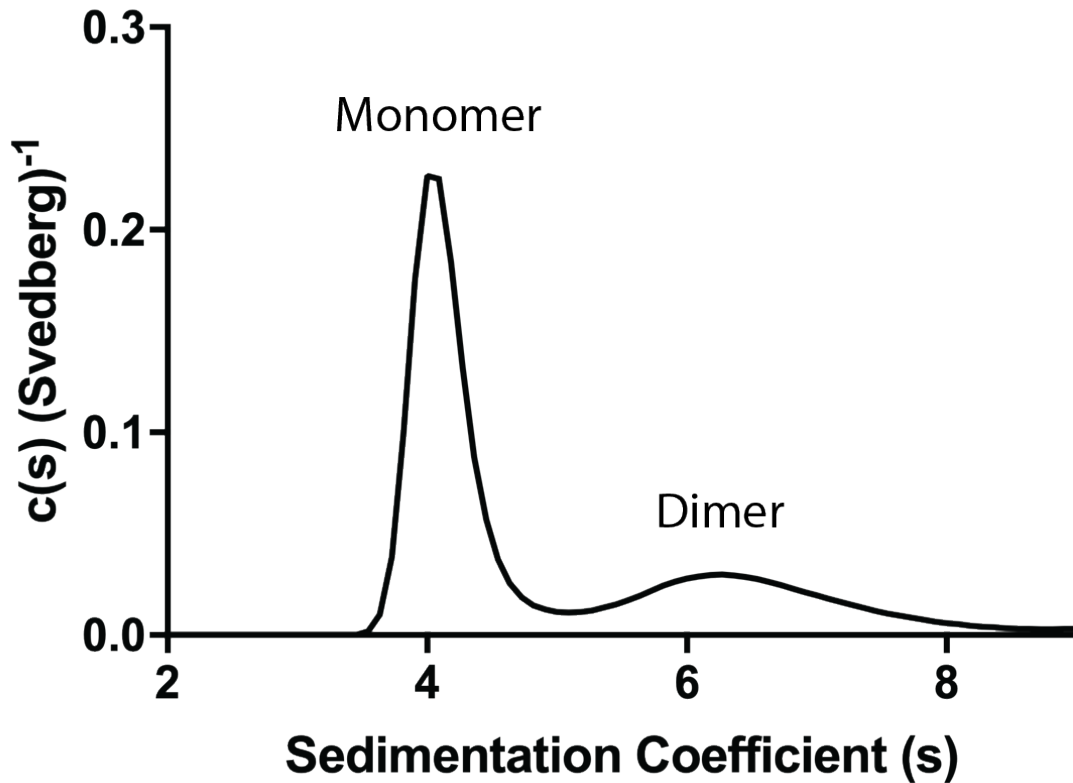


**Figure S5 – Sedimentation distribution of  $\Delta$ proTPOe-GCN4 alone.** Two distinct species are detected at a  $sw_{(20,w)}$  of 3.6 and 5.2 respectively. The molecular weights reported by c(M) analysis (not shown) in each case was 114 kDa and 201 kDa respectively, with a frictional ratio of 2.36. These molecular weights are consistent with a monomer (~110

kDa) and a dimer (~220 kDa) species. The relative abundance of each of the two species was analysed by SEDFIT (using area under the curve), with a monomer:dimer ratio of approximately 1.33:1, indicating the monomer is more abundant. The Stokes radii (Table S3) and frictional ratio of 2.36 suggest a non-spherical, elongated shape, consistent with our previous modelling (a frictional ratio of 1 would suggest a perfect sphere) (8).



**Figure S6 – ELISA results of TPO-Fab binding.** TR1.9 Fab shows statistically significant binding to both TPO constructs, with a p value less than 0.0001 compared to a non-specific protein that does not have the required epitope (conserpin (20), negative control), as well as a PBS blank. IgG antibody and anti-human IgG was used as a positive control. Error bars are standard deviation from the mean and statistical tests were performed with a two-tailed t-test with a 95% confidence interval. All samples were performed in quadruplicate.

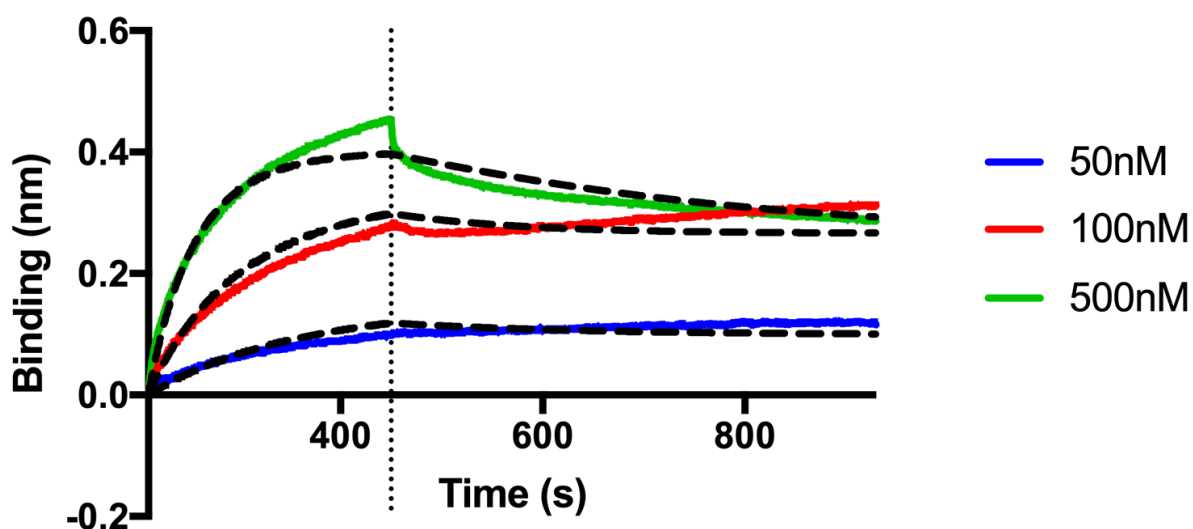


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70 **Figure S7 – Sedimentation distribution of  $\Delta\text{proTPOe-GCN4}$  bound to TR1.9 Fab.** Two  
 71 distinct species are detected at a  $sw_{(20,w)}$  of 4.1 and 6.6 respectively. AUC analysis of an  
 72 equimolar mixture of TR1.9 Fab and  $\Delta\text{proTPOe-GCN4}$  shows two peaks. The lack of a  
 73 peak at 2.6S suggests that none, or very little of the Fab, remained un-complexed.  
 74 Therefore, the two remaining peaks are most likely the TPO monomer/dimer peaks as  
 75 observed for  $\Delta\text{proTPOe-8His}$  (Fig. 5C). The observed shift in their standardised weight-  
 76 average sedimentation coefficients (4.1S, 6.6S, respectively) suggest a change in their  
 77 shape and mass, indicating Fab binding to both monomer and dimer  $\Delta\text{proTPOe-GCN4}$ .  
 78 The frictional ratio has also changed to 1.77 (from 2.36 with  $\Delta\text{proTPOe-GCN4}$  alone),  
 79 indicating that TPO has taken a more spherical shape upon TR1.9 Fab binding.  
 80 Importantly, the ratio of monomer and dimer has shifted to approximately 2:1.

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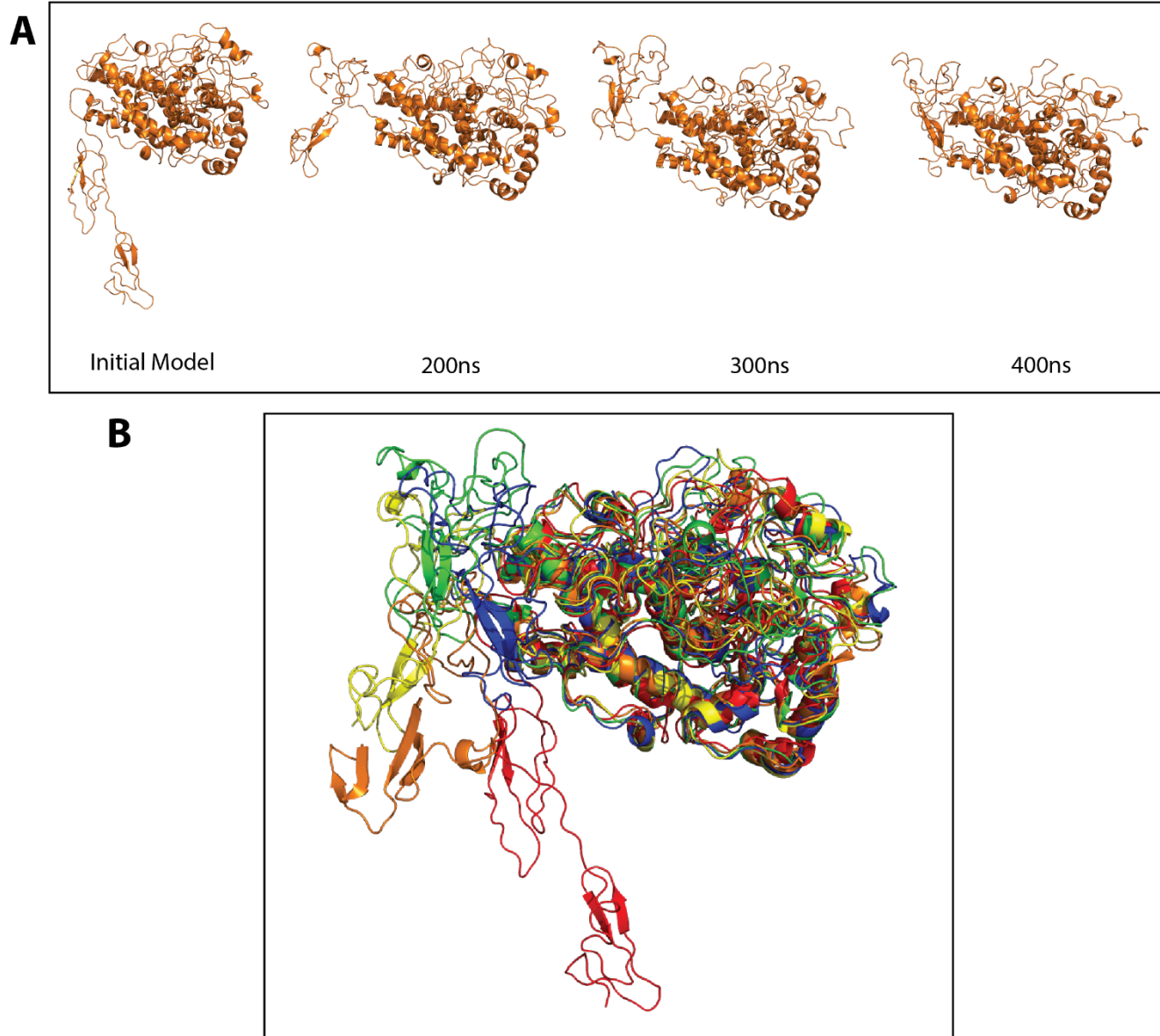
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**Figure S8 – Bio-layer interferometry (BLI) sensorgram data of  $\Delta$ proTPOe-8His binding to Fab.**

Sensorgram curves according to a TR1.9 Fab concentration range of between 0 and 500 nM.  $\Delta$ proTPOe-8His is immobilised on the biosensor surface. The data has been normalised against a blank run of buffer (1x PBS, pH 7.4). Vertical line at 450 s represents the end of the association phase. Dotted lines in black represent the fit calculated using a 1:1 binding model with global fitting within the BLItz Pro software.  $R^2$  values for the calculated fit were reported as 0.97.  $K_D$  was calculated as 20 nM.



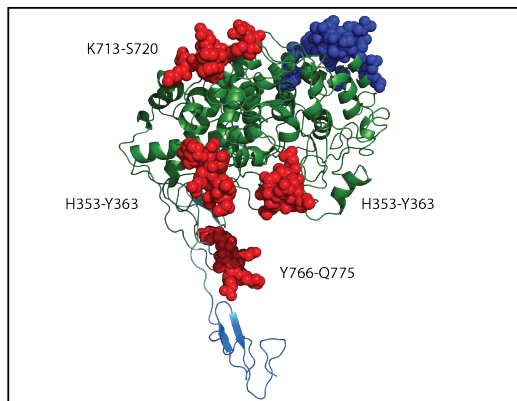


99

100 **Figure S9 – Snapshots from the *trans*  $\Delta$ proTPOe MD trajectory show TPO changing**  
 101 **conformation from extended to more compact structure.** Snapshots from the *trans*  $\Delta$ proTPOe  
 102 MD trajectory as presented in Figure 6. **(A)** Representation of the starting model from Le and co-  
 103 workers (1), as well as *trans*  $\Delta$ proTPOe after 200, 300 and 400 ns of simulation. **(B)** Structural  
 104 superpositions of the above snapshots with the starting *trans*  $\Delta$ proTPOe model in red. Orange  
 105 indicates *trans*  $\Delta$ proTPOe after 100 ns of simulation, yellow after 200 ns, green after 300 ns and  
 106 blue after 400 ns.

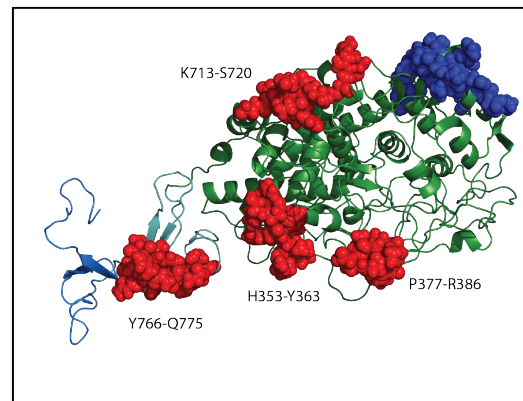
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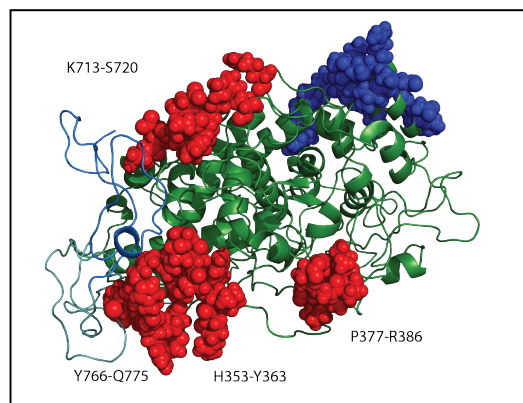
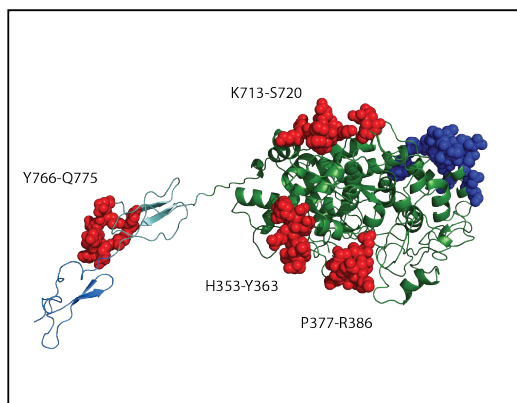


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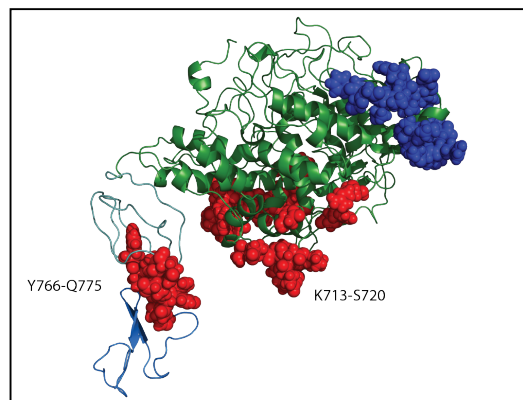
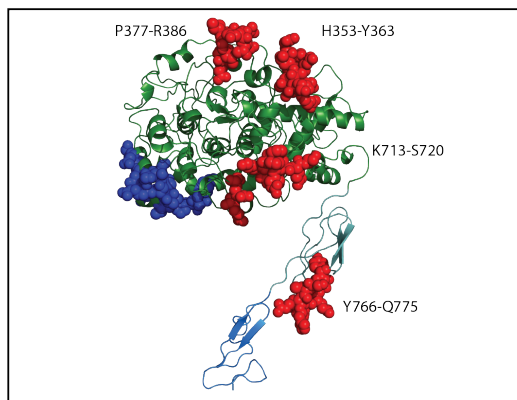
Cis



Extended



Trans



108

109 **Figure S10 – IDRs in context of the MD simulations with starting structures. (A)** TPO models  
 110 (simulation starting structures) with IDRs highlighted. **(B)** Representative structures taken from  
 111 the MD simulations for each of the *trans*, *cis* and *extended* forms of the  $\Delta$ proTPOe monomer, as  
 112 in Figure 7. IDR-A residues are highlighted by red spheres, and IDR-B residues by blue spheres.

The MPO-like domain, CCP-like domain and EGF-like domain are coloured in forest green, light teal and marine blue respectively (as in Figure 1).

**Table S1** – Published residues involved in IDRs of TPO

Antibody Involved	Number of Reported Epitopes	Epitopes	Study
<b>IDR-A</b>			
T13	4	H353-Y363, P377-R386, K713-S720, Y766-Q775	(2-6)
ICA1	1	H353-Y363	(2,3)
TR1.9	2	K713, K713-S720	(2,4,7)
126TO10	3	R225, R646, D707	(8,9)
126TP1	3	R225, R646, D707	(8,9)
126TP7	1	R225	(9)
<b>IDR-B</b>			
126TP5	5	D620, D624, K627, D630, F597-E604	(8-10)
126TP14	5	D620, D624, K627, D630, F597-E604	(8-10)
131TP7	1	K627	(9)
SP1.4	1	F597-E604	(10)
TR1.8	1	T611-V618	(10)
WR1.7	1	F597-E604	(10)

The epitopes that have been identified as making up the immunodominant regions (IDRs) of TPO, named IDR-A and IDR-B.

**Table S2 – Melting point data of ΔproTPOe-8His in different buffer conditions**

Buffer	pH	T <sub>m</sub> (°C)
50 mM HEPES, 250 mM NaCl	8.0	52.3
50 mM HEPES, 250 mM NaCl	7.0	55.2
50 mM Sodium Phosphate, 250 mM NaCl	6.0	54.7
50 mM Sodium Acetate, 250 mM NaCl	5.5	53.9
50 mM Glycine, 250 mM NaCl	4.0	53.6

**Table S3** – Theoretical and calculated Stokes Radii ( $R_s$ ) of TPO

Reference Dataset	Equation	Stokes Radius (Å)	
		Monomer	Dimer
Globular Folded Proteins	$R_s = (4.75)N^{0.29}$	32.52	39.75
Denatured Unfolded Proteins	$R_s = (2.21)N^{0.57}$	96.93	143.89
Analytical SEC of TPO with no TM domain		51.31	
AUC of $\Delta$ proTPOe-GCN4		75.7	91.4
AUC of $\Delta$ proTPOe-8His		64.9	77.1
AUC of $\Delta$ proTPOe-GCN4 with TR1.9		52.7	66.4
AUC of $\Delta$ proTPOe-8His with TR1.9		57.4	N/A

AUC, analytical ultracentrifugation; SEC, size exclusion chromatography; TM, transmembrane domain.

**Table S4 – Model Fit Percentages**

Model	Percentage of Molecules within the EM Map
Trans Monomer	73
Cis Monomer	72
Trans Dimer	54
Cis Dimer	59
Trans Monomer with Fab	53
Cis Monomer with Fab	55
Curled Monomer with Fab	58
Curled Monomer with scFv format of TR1.9	73
Trans Monomer with Fab sequentially fit*	68
Cis Monomer with Fab sequentially fit*	70
Trans Dimer with Fab	33
Cis Dimer with Fab	37

Fit percentages of various TPO models within the electron microscopy (EM) map. Asterisks (\*) indicates configurations where TR 1.9 Fab was fitted into the available space in the envelope without regard to its epitope's location, rather than in a realistic orientation in which the complementarity determining regions (CDR) face the published epitope of K713-S720.

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