Analytical ultracentrifugation (AUC) and size exclusion chromatography (SEC-HPLC) were used to assess TT30 molecular weight distribution. The sedimentation distribution of TT30 is shown in Fig. S1A. The inset panel is an enlarged view of the sedimentation pattern. The monomer TT30 was observed at a sedimentation coefficient of approximately 3 Svedbergs. The TT30 sample contained ≤ 0.3% of a high molecular weight species (visible in the inset panel). TT30 was also analyzed by size exclusion chromatography (SEC-HPLC). A SEC-HPLC chromatogram of TT30 is shown in Fig. S1B. The inset represents an enlarged view of the chromatogram. The TT30 monomer elutes in the partially included volume of the SEC-HPLC column.

Analysis of secondary structural elements of TT30 by circular dichroism revealed an ellipticity maximum at approximately 230 nm; this ellipticity maximum is similar to that reported previously for fH, confirming that the secondary structure of TT30 is similar to that of fH (Fig. S1C).

Analytical ultracentrifugation
An aliquot of TT30 was loaded into an AN-50Ti analytical ultracentrifugation rotor. The analysis was performed in a Beckman-Coulter ProteomeLab XL-I centrifuge. The temperature was 20°C, and the absorbance was monitored at 280 nm, the rotor speed was 45,000 rpm. Radial scans were recorded approximately every 5 minutes resulting in a total of 50 radial scans for each sample over a period of approximately 5 hours. The data analysis was performed using Sedfit (Beckman) applying a continuous c(s) distribution model.

Size-exclusion chromatography
TT30 sample was analyzed using a Tosoh Haas TSK Gel G3000SW_{XL} 7.8 mm × 30cm column at 1 mL/min and 280 nm detection.

Ellipticity by far UV circular dichroism
TT30 diluted to 0.3 mg/mL in PBS was analyzed using a 710 Spectropolarimeter by Jasco. Temperature was initially set to 20°C. Spectra was collected at 20, 30, 40, 50 and 60°C.

Manufacturing
TT30 is produced using fed-batch culture of Chinese hamster ovary (CHO) cells, and purified via a series of chromatography and membrane process steps.

Characterization
The theoretical molecular mass of TT30 is 62,088.5 Da, and the theoretical pI is 7.93. TT30 contains three consensus sites (Asn-X-Ser/Thr) for N-linked glycosylation: Asn_{101}, Asn_{107}, and Asn_{454}. The first two potential sites are within the CR2-derived sequence and the third is within the fH portion of TT30.

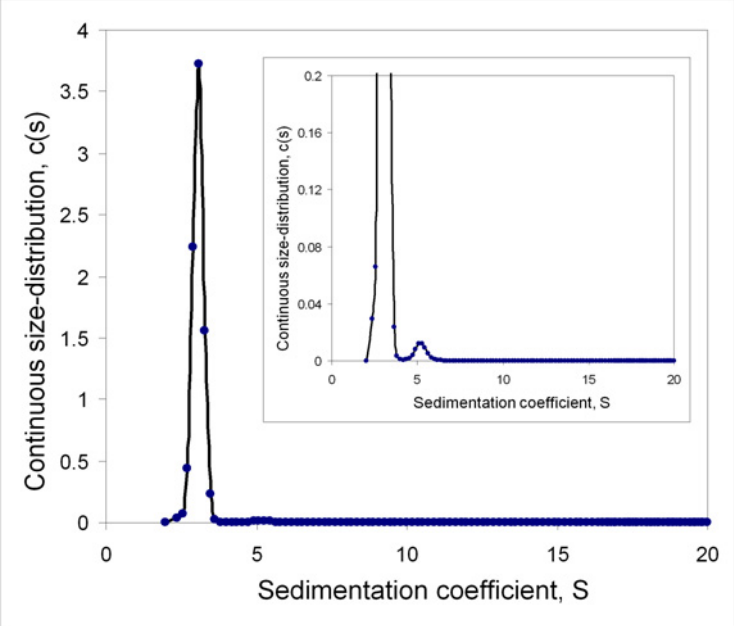
Molecular weight distribution by analytical ultracentrifugation (AUC) and size-exclusion chromatography (SEC)
The high-molecular-weight species content of TT30 was measured by sedimentation velocity using analytical ultracentrifugation. The sedimentation distribution of TT30 is shown in Fig. S1A. The monomer TT30 was observed at a sedimentation coefficient of approximately 3

Svedberg. The TT30 sample contained ≤ 0.3% of a high molecular weight species (visible in the inset panel), potentially aggregate, at a sedimentation coefficient of 5.53 Svedberg.

The SEC chromatogram contains a front eluting peak (0.51%) that may correspond to high molecular weight species or aggregate. (Shown in Fig. S1B) This result is consistent with the results obtained for the same batch from AUC.

Secondary structural elements by circular dichroism (CD)
The deconvoluted CD results indicate that TT30 contains 7% alpha helix, 51% beta sheets and 42% other structures. (Shown in Fig. S1C). The other structures are comprised of beta strands and beta turns, consistent with the expected structure of fH. These results (low alpha helix, mostly beta strands and beta sheets) are consistent with the published literature on the three-dimensional structures of the SCR modules from fH. (Ref. 1). Thus the TT30 results are consistent with those expected for a molecule comprising nine SCRs. TT30's denaturation temperature observed using CD showed good agreement with that of differential scanning calorimetry (58.8°C).

The thorough characterization of TT30 confirmed that the 1°, 2°, and higher order structures are in good agreement with the native structures of CR2 and Factor H.


Continuous size-distribution, c(s)
Sedimentation coefficient, S
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2
2.5
3
3.5
4
0
5
10
15
20
Continuous size-distribution, c(s)
Sedimentation coefficient, S
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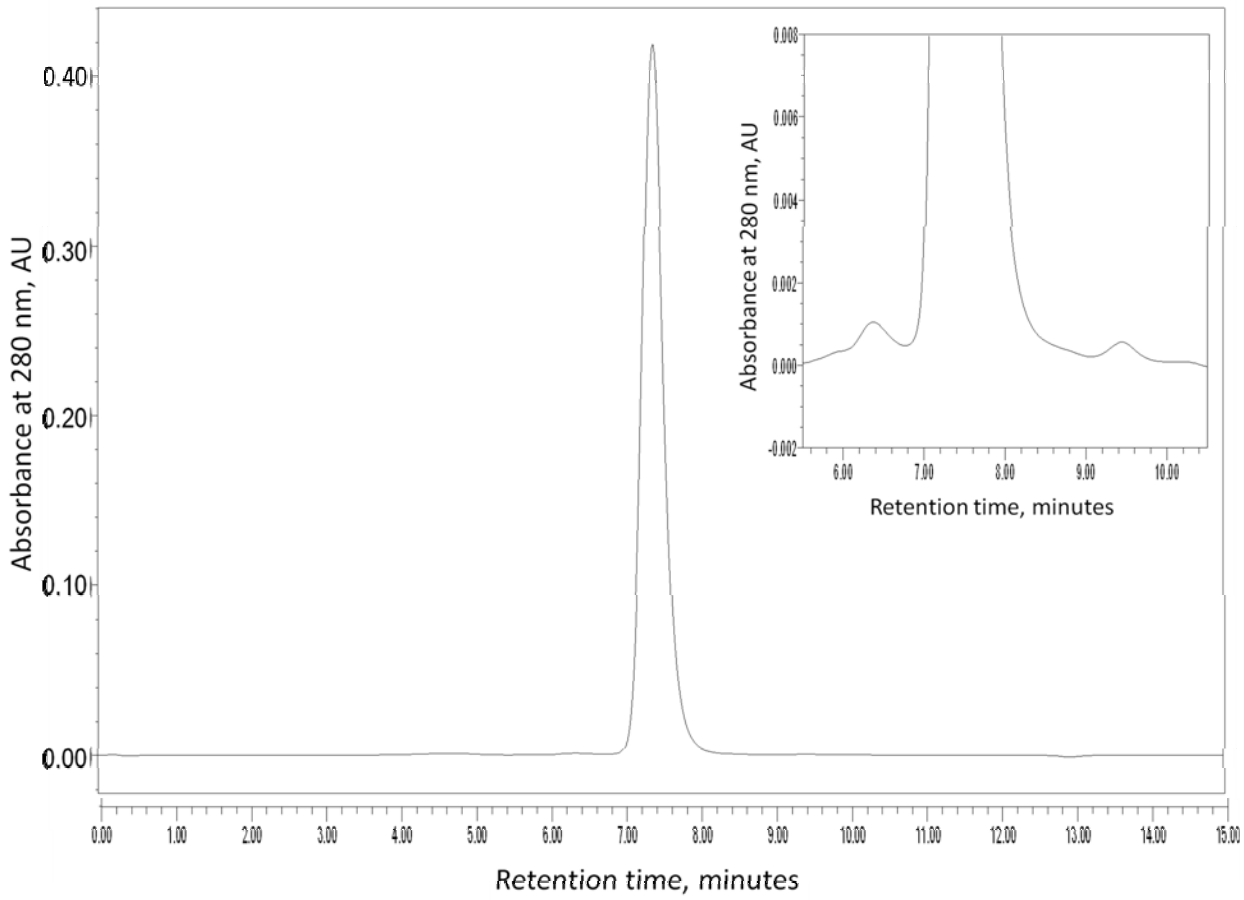
0.08
0.12
0.16
0.2
0
5
10
15
20

Figure S1A


Absorbance at 280 nm, AU
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0.20
0.10
0.00
0.00
1.00
2.00
3.00
4.00
5.00
6.00
7.00
8.00
9.00
10.00
11.00
12.00
13.00
14.00
15.00
Retention time, minutes
Absorbance at 280 nm, AU
0.008
0.006
0.004
0.002
0.000
-0.002
6.00
7.00
8.00
9.00
10.00
Retention time, minutes

Figure S1B

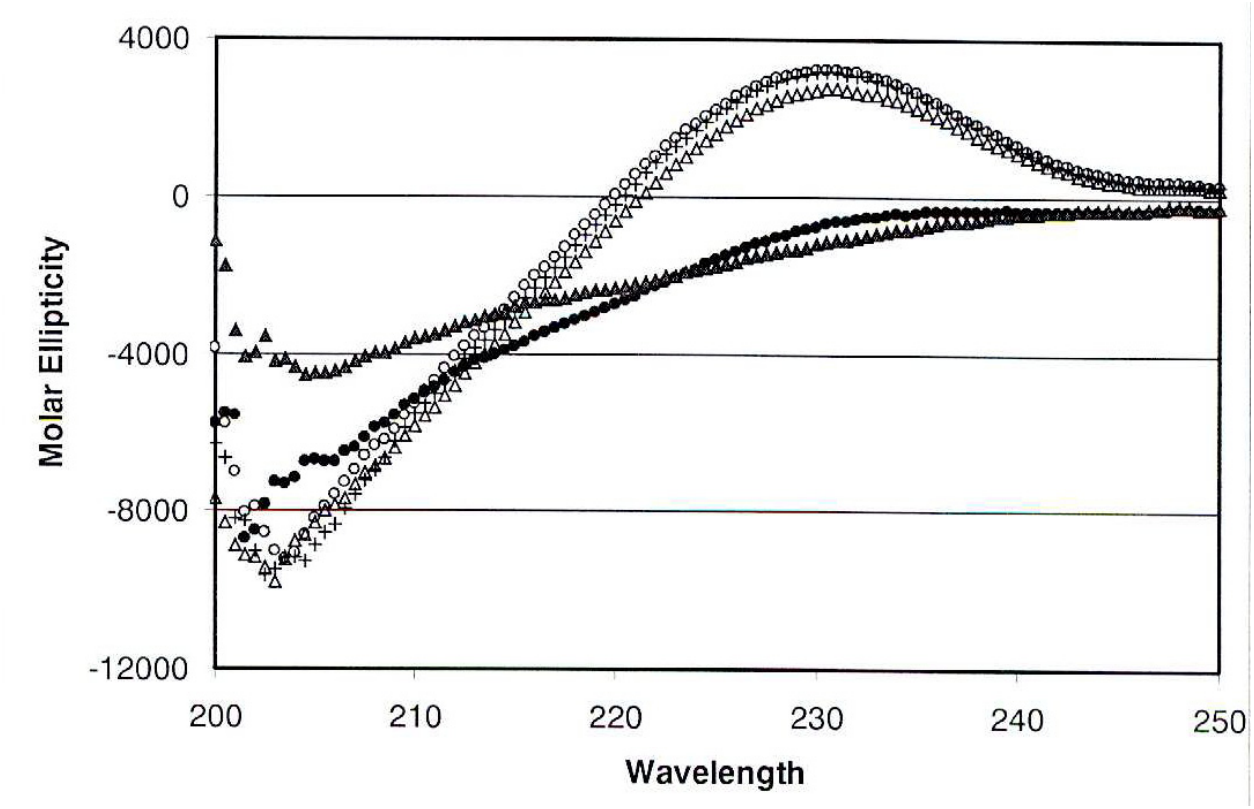
4000
0
-4000
-8000
-12000
Molar Ellipticity
200
210
220
230
240
250
Wavelength

Figure S1C

Figure S1. Size characterization of TT30: Purified TT30 demonstrates high degree of purity

(A) Analytical ultracentrifugation analysis using velocity sedimentation method. The analysis was performed in a Beckman-Coulter ProteomeLab XL-I centrifuge using an AN-50Ti rotor. TT30 concentration was approximately 0.8 mg/mL, the temperature was 20°C, and the absorbance was monitored at 280 nm. The rotor speed was 45,000 rpm. Radial scans were recorded approximately every 5 minutes resulting in a total of 50 radial scans for each sample over a period of approximately 5 hours. The data analysis was performed using Sedfit (Beckman) applying a continuous distribution model. The inset panel is an enlarged view of the sedimentation pattern. (B) Size-exclusion chromatography of TT30. Sample was analyzed using a Tosoh Haas TSK Gel G3000SW_{XL} 7.8 mm × 30cm column at 1 mL/min and 280-nm detection. The inset represents an enlarged view of the chromatogram. (C) CD of purified TT30 demonstrates appropriate pattern and substantial stability to heat denaturation. The maximum at 230 nm is characteristic to tryptophan in Complement Factor H. Increasing temperature decreased molar ellipticity at 230 nm, and increased helicity at 222 nm. Denaturation temperature of TT30 is approximately 58°C. Spectra were collected at 20 (empty circles), 30 (crosses), 40 (empty triangles), 50 (filled circles) and 60°C (filled triangles).

REFERENCES

Kask, L. et al. Structural stability and heat-induced conformational change of two complement inhibitors: C4b-binding protein and factor H. *Protein Sci*. 13, 1356–1364 (2004).