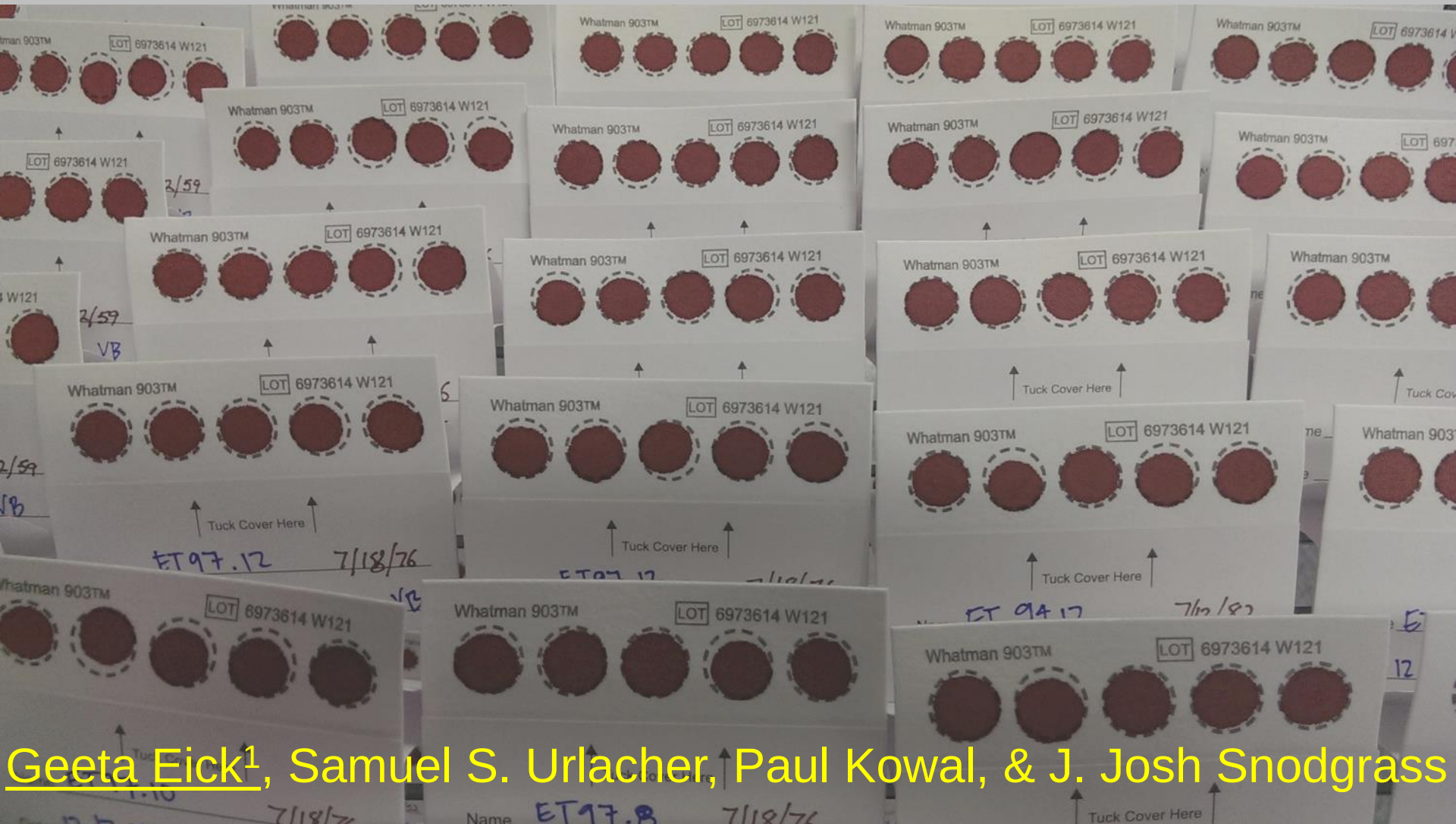
What a drop can(t) do: Methodological challenges associated with incorporating dried blood spot biomarkers into population-based research



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Methodological Advantages of DBS

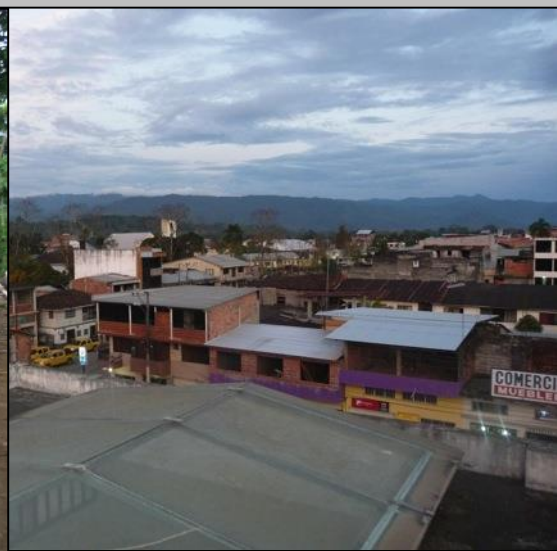
- Analysis of biomarkers from logistically-challenging areas and previously undersampled populations outside of a clinical setting
- Less invasive than venous blood draw
- Much smaller volume of blood required (~300 μl vs. 10 mL)
- No trained phlebotomist or sample centrifugation required
- DBS cards easy to store & transport
- Many analytes in blood more stable in filter paper matrix than in a liquid sample
- Numerous validated biomarker assays for DBS

Study on global AGEing and adult health (SAGE)

- Longitudinal study to generate comparable health data on older adults from South Africa, Ghana, India, Mexico, India, and the Russian Federation
- >38, 000 DBS samples collected
- Initial panel of markers for DBS analyses: C-reactive protein, glycated hemoglobin (HbA1c), hemoglobin, HIV, and Epstein-Barr virus (EBV) antibodies

Shaur Health & Life History Project

- Initiated in Ecuador in 2005 by Larry Sugiyama & Josh Snodgrass from the University of Oregon
- Shuar traditionally practiced a mixed subsistence pattern of hunting, fishing, and horticulture, but are currently experiencing a wide range of market integration across their territory.
- This project addresses how economic, social, and dietary changes associated with market integration affect life history tradeoffs, and how those tradeoffs affect health.
- Over 4000 DBS cards collected
- Biomarkers of interest: Immunoglobulin E, C-reactive protein, and antibodies to Epstein-Barr virus

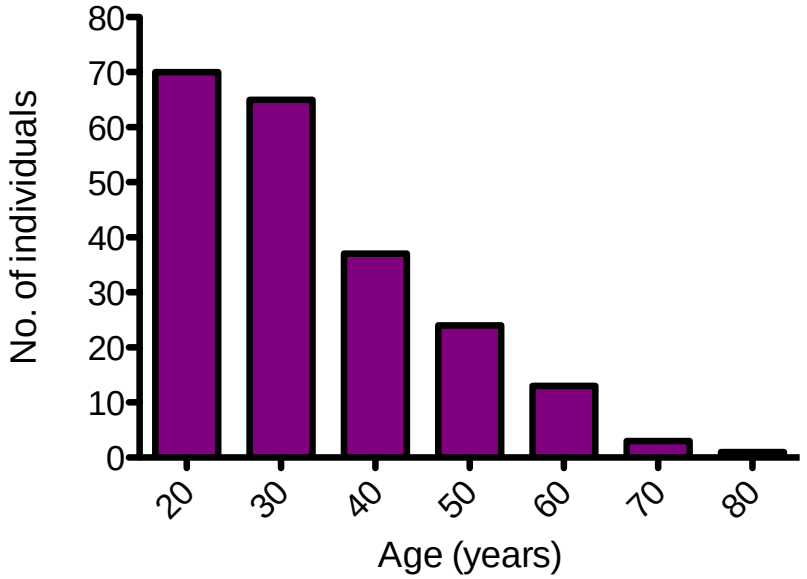


Analyte	DBS Validation Reference	Marker of	Dysregulation associated with
Epstein Barr Virus (EBV) antibodies	Currently being validated in Snodgrass lab	Cell-mediated immune system function	chronic psychosocial strength
C-reactive protein (CRP)	Currently being validated in Snodgrass lab	Systemic low-grade inflammation	coronary heart disease, ischemic stroke, vascular mortality
Hemoglobin	O'Broin and Gunter, 1999	Red blood cells	myocardial function, muscle density
Hemoglobin A1C	Hu et al., 2014; Williams *et al.* in prep	Blood sugar levels	diabetes and cardiovascular disease (CVD)
Immunoglobulin E	Currently being validated in Snodgrass lab	Immune health	High burden of helminth infection, allergic disease

Assay validation: The ‘Eugene200’

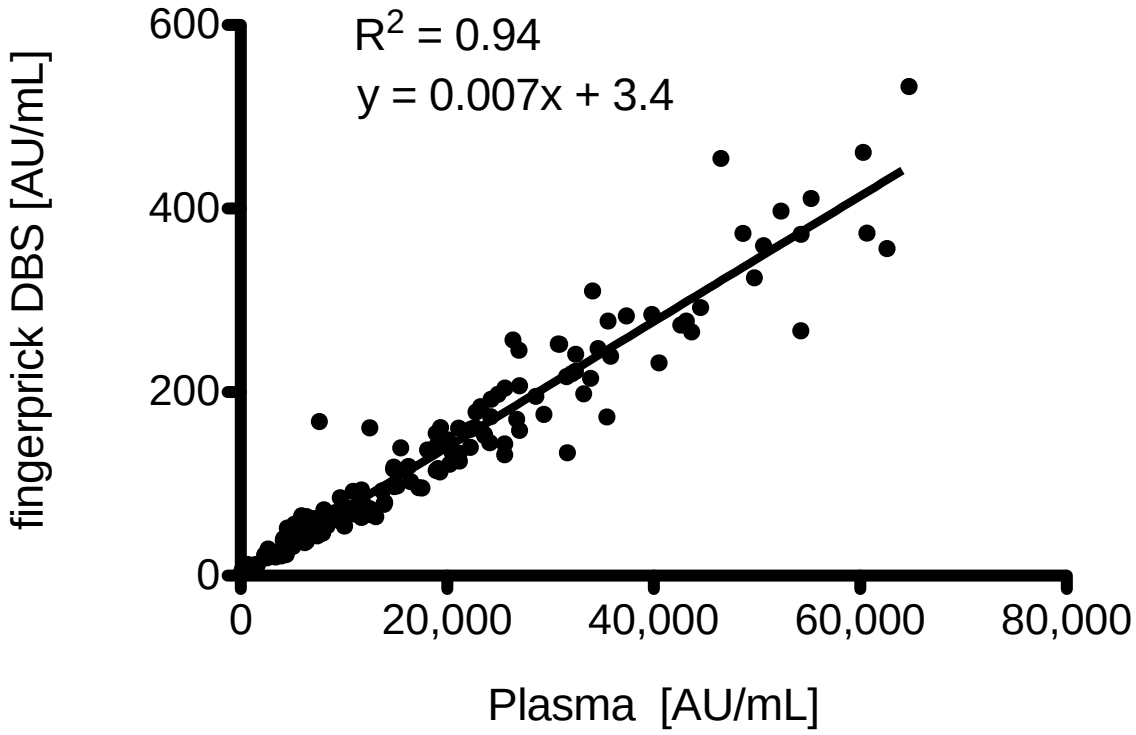
- Recruited 213 adults from Eugene & Springfield, OR from Nov 2014 - Feb 2015

- Collected matched fingerprick DBS, venous DBS, plasma, buffy coat, & saliva samples



DBS validation: Epstein-Barr virus antibodies

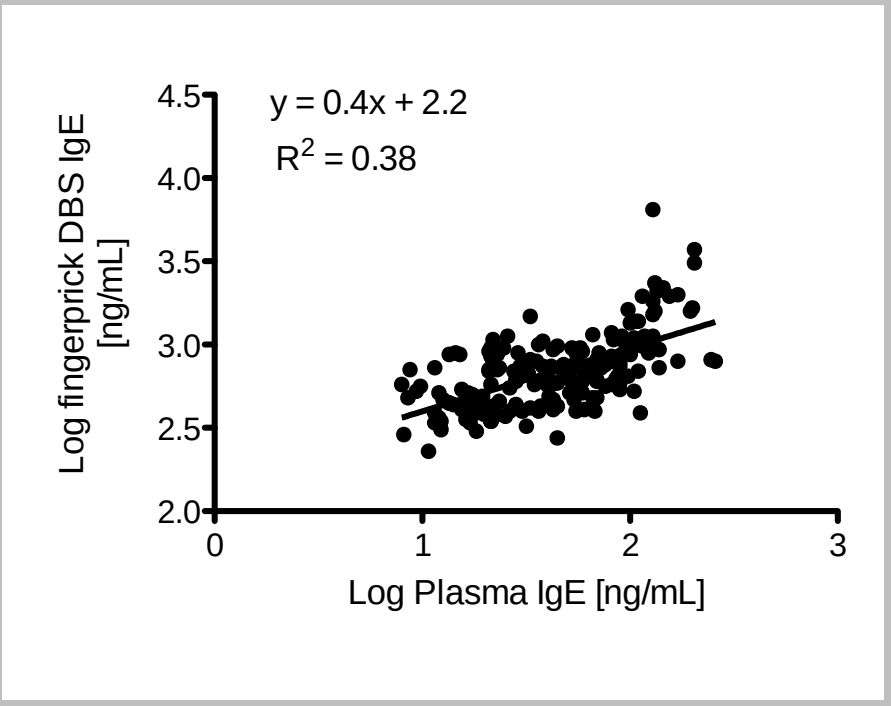
- Diasorin kit discontinued
- Many EBV kits available on the market – target EBV antibodies again diverse antigens of EBV
- Diamedix EBV VCA-IgG ELISA plate + Mikrogen *recom*well EBV VCA-IgG calibrator (in collaboration with Thom McDade, Northwestern)



- Intra-assay CV: 3.6%
- Inter-assay CV: 9.4%
- Lower limit of detection: 5 AU/mL
- DBS cut-off: 10 AU/mL

DBS validation: Immunoglobulin E

- Modification of the Tanner & McDade protocol (detection antibody optimized; required for each new lot of polyclonal coating and detection antibodies)



- Intra-assay CV: 2%
- Inter-assay CV: 6%
- Lower limit of detection: 154 ng/mL

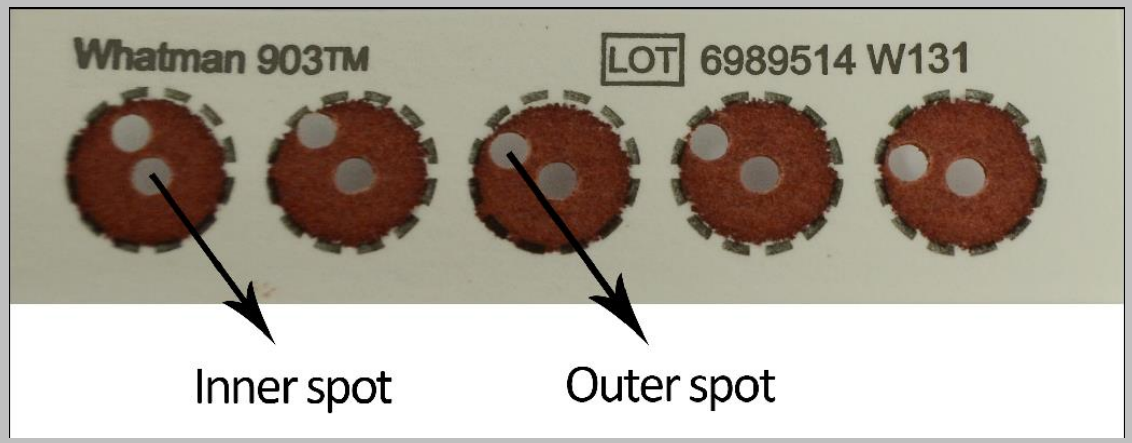
DBS Limitations

- Many assays dependent on the availability of manufactured kits that can disappear from the commercial market without notice (e.g. Diasorin's EBV VCA IgG kit)
- Assay validation very expensive ($5000 - $15,000 for reagents only) and time-consuming due to need to obtain matched plasma/DBS samples from a large number of individuals (100-200)
- No guidelines established for constructing DBS-based standard curves for ELISA-based DBS assays
- Cross-laboratory harmonization expensive and logistically challenging, especially across international borders
- Difficult to develop robust assays for biomarkers circulating at low concentrations (e.g. cyokines in the pg/mL range)

DBS Limitations contd…

Tyler Fording

- Concentration of analyte may vary across DBS (i.e. non-uniform distribution of analyte due to chromatographic effects)



  - Quantitated levels of C-reactive protein from inner and outer punches of venous DBS cards (5 spots per card; 25 or 50 μl spots) collected from five individuals and subjected to various numbers of freeze-thaw cycles and drying times
  - CRP concentrations consistently significantly lower in the inner vs. the outer 1/8” punch from spots

DBS Limitations contd…

• Effect of hematocrit (volume percentage red blood cells, ranging from 35 -50% for healthy individuals) on estimated biomarker concentration measured from a DBS punch, which is essentially a volumetric measurement, is untested and unknown

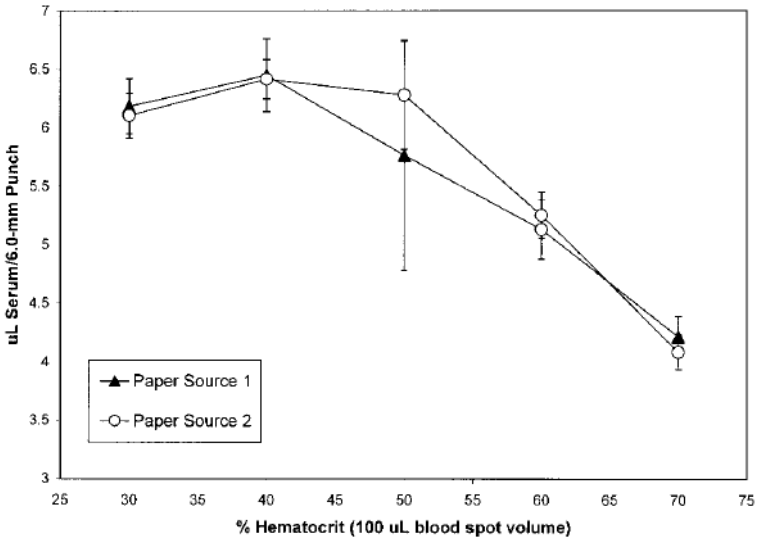


FIGURE 1 The effect of hematocrit (%) on the volume of serum (μl) in 6.0-mm (one-fourth-inch) punches. As the hemaocrit of the spotted blood increased, the amount of serum in the punch decreased. For each point, $n = 50$.

Two individuals with identical serum levels of an analyte of interest but different hematocrits are likely to have different DBS-based analyte values

Mei et al. 2001. Use of Filter Paper for the Collection and Analysis of Human Whole Blood Specimens. *Journal of Nutrition.*

What is the effect of this variation in hematocrit levels among DBS punches on the biomarker of interest?

Conclusions

- DBS collection is a minimally invasive, logistically convenient biological sampling methodology that has significantly promoted integrative, multi-level biodemographic and population-based research.

- Recent work in our lab and others, however, has indicated that the field was overly optimistic about how straightforward and inexpensive it would be to develop and validate DBS assays (McDade et al., 2007).

Conclusions contd…

- Key issues include difficulty of cross-laboratory harmonization, assay validation costs, translation of DBS values into serum/plasma equivalent levels, and the potentially confounding effect of hematocrit.
- Increased attention to methodological issues is critical to minimizing problems and maximizing the data that can be obtained.
- A more realistic perspective on use of DBS is required which appreciates methodological issues, costs, and need for a well-equipped and trained laboratory for conducting analyses.

Acknowledgements

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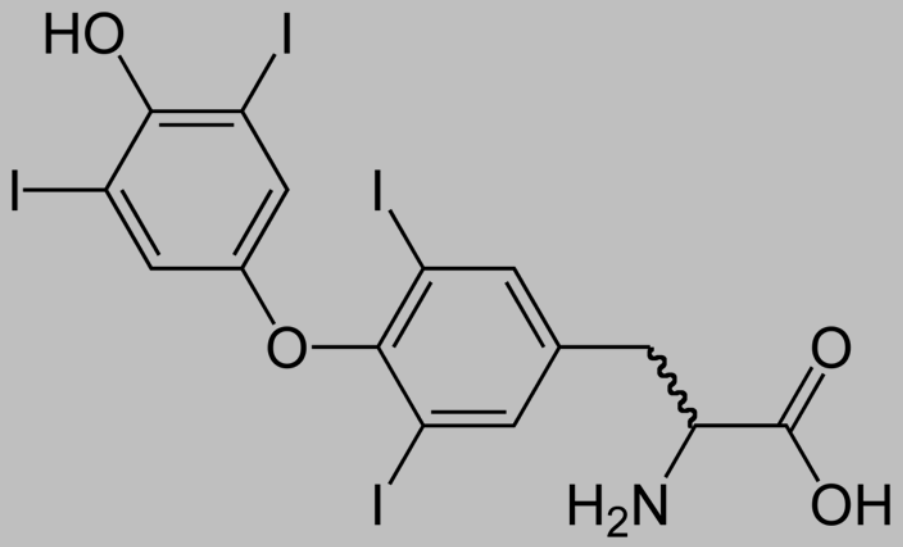
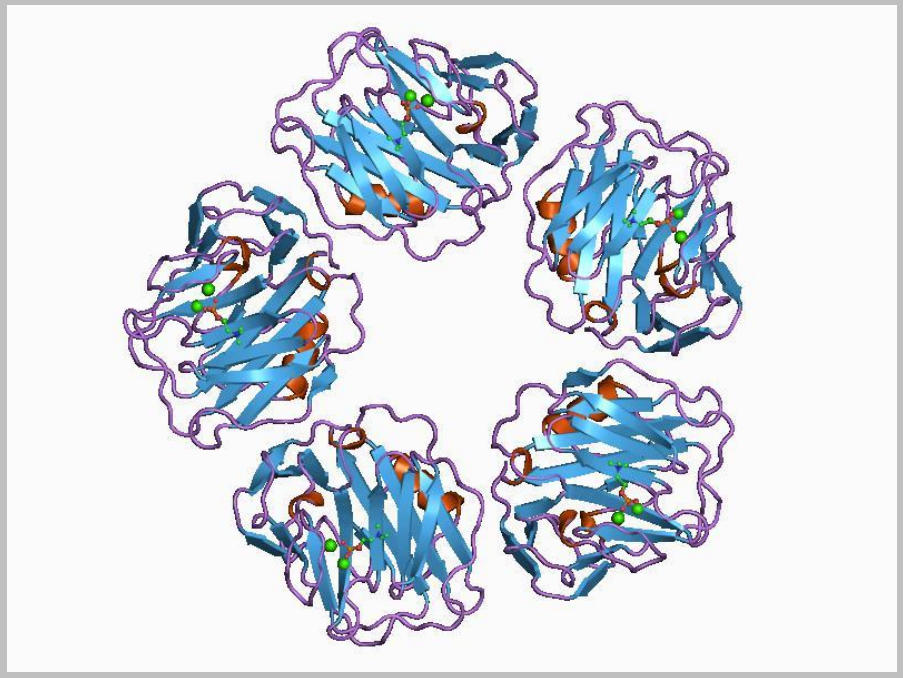


Thyroxine; MW = 776 Da



C-reactive protein; MW = 25 000 Da