Neutron Reflectometry Studies of the Adsorbed Structure of the Amelogenin, LRAP

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Neutron Reflectivity Experimental

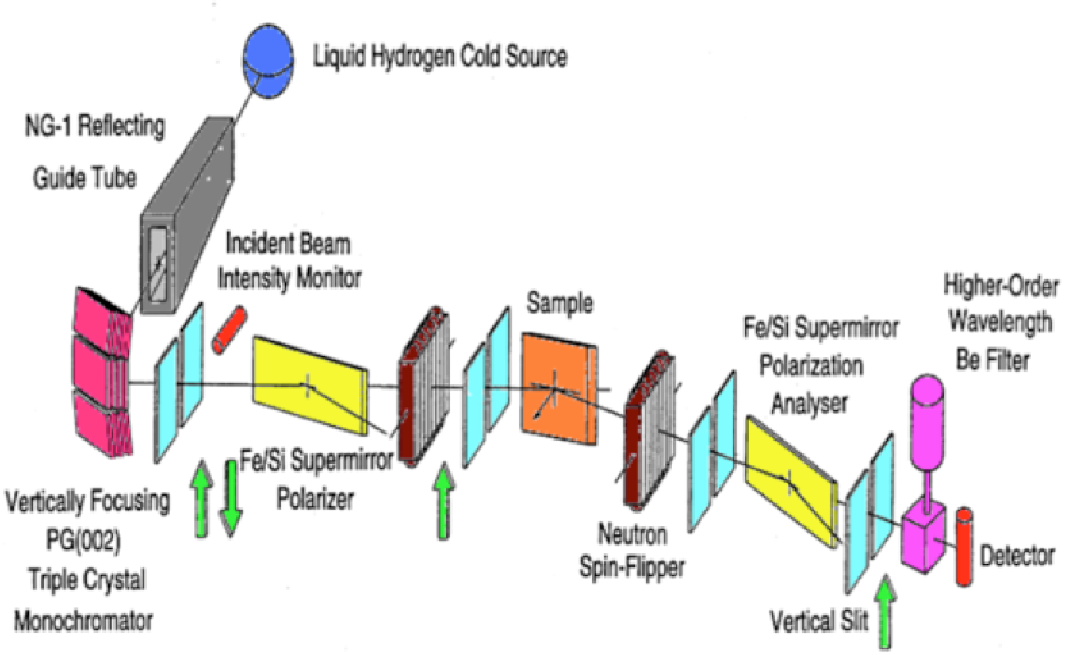

Figure S1. Schematic of NG-1 Reflectometer neutron focusing optics and detector

Sedimentation velocity equation relating sedimentation coefficient, s, to molecular mass, M

For spherical particles of radius R or molecular mass M the sedimentation coefficient, s, increases as R^2 or $M^{2/3}$, and is given exactly by

$$s = \frac{2R^2(1-\bar{\nu}\rho)}{9\bar{\nu}\eta} \quad \text{Equation 1}$$

$$s = \frac{M^{2/3}(1-\bar{\nu}\rho)}{6\pi\eta N_0 \sqrt[3]{\frac{3\bar{\nu}}{4\pi N_0}}} \quad \text{Equation 2}$$

where η is the solvent viscosity, N_0 is Avogadro's number, ρ is the solvent density, and $\bar{\nu}$ is the partial specific volume of the macromolecule (the inverse of the hydrated density)

Sedimentation velocity results

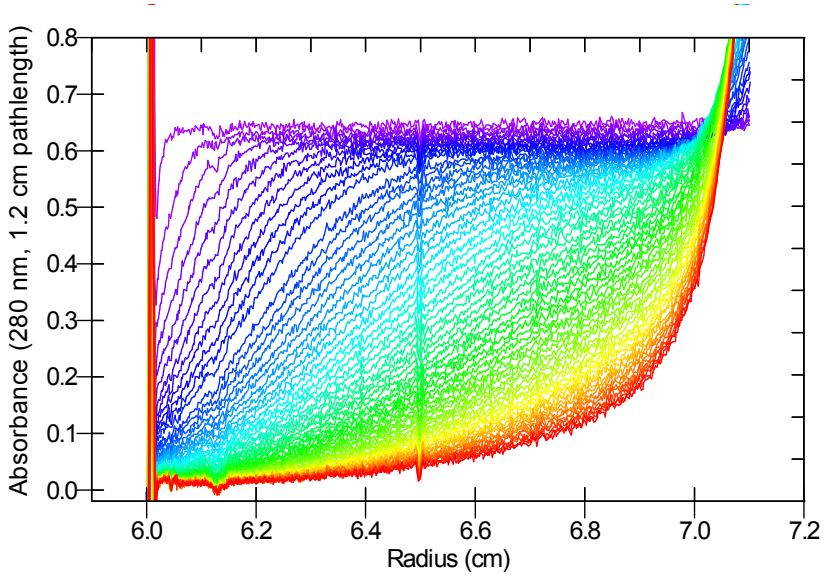

Figure S2. Raw data scans at 280 nm for sample LRAP in SCP at 60,000 rpm. The first scan and scans recorded approximately every 16 min thereafter are shown (~14.4 hr total run time). Only a single, very broad boundary is visible by eye, and late in the run (orange and red traces) there is little boundary movement because the sample is already approaching sedimentation equilibrium. Both of those observations indicate that the majority of this sample has a very low molar mass in solution.

Calculation of Protein SLDs for the modeling assuming 0% or 100% deuterium exchange from solvent

Protein SLDs were calculated using the NIST SLD Calculator (http://www.ncnr.nist.gov/resources/sldcalc.html) and are shown in Table S2. The calculator requires input of the atomic composition of the protein, protein density, and neutron beam wavelength. The atomic composition of the protein was determined assuming 0% deuterium exchange from solvent and 100% deuterium exchange from solvent.

The 100% exchange condition meant 100% of all possible labile protons. The possible labile protons in the side chains available for deuterium exchange from D_2O solvent were obtained by determining which protons were not ionized at pH 7.4 from published ionization contants.[1] Protons that were ionized at pH 7.4 were not available for exchange. It has been found that backbone amide protons that are not in secondary structure are most readily exchanged.[2] We assumed that all backbone amide protons were exchanged because LRAP has little secondary structure. Estimates of the exchangeable side chain protons and backbone protons in this way have been found to correspond well to experimentally determined exchange by elecctrospray ionization mass spectrometry.[3] The total number of backbone protons was the number of residues minus the proline residues. The LRAP sequence, labile protons in the side chains, and number of backbone protons are listed below:

1. LRAP sequence:
MPLPPHPGHPGYINFpSYEVLTPLKWYQSMIRHPPLPPMLPD**LPL**E**AW**P**A**TDKTKREEVD

Black – residues with site specific deuterium labeling

2. Numbers of labile protons in side chains:
MPLPPH_1PGH_1PGY_1IN_2F$pS_1Y_1E_0VLT_1PLK_3W_1Y_1Q_2S_1MIR_5H_1$PPLPPMLPD0**LPL**E**AW**P**A**T_1
$DK_3T_1K_3R_5$EEVD

3. Numbers of labile protons in amide backbone = 59-12 proline = 47. Number of possible protons deuterated by D2O exchange – 35 side chain and 47 backbone = 82

4. The "Inner" protein layer was composed of residues found close to surfaces by SSNMR.[4] The remaining part of the protein was called "outer" as listed below:

Inner – Inner N terminus (G8 to K24) + C terminus (42-59) – GHPGYINFpSYEVLTPLK + D**LPL**E**AWA**TDKTKREEVD (36 residues)

Outer – N-terminus M1-P7 – MPLPPHP + WYQSMIRHPPLPPMLP (23 residues)

The density of unlabeled LRAP was calculated by the density calculator used for the sedimentation velocity studies. Changes in density due to deuteration were calculated by taking the change in mass by deuteration divided by the unlabeled molecular weight times 1.338.

Table S2. Calculated SLDs for LRAP assuming 0 and 100% deuteration of possible labile protons. Residues containing labile protons are listed below.

Protein	Formula	MW (g)	Notes	Density (g/cm^3)	WL (Å)	SLD(x 10^6) A^{-2}
LRAPH	C314H471N77O86S3P1	6828	Unlabeled	1.338	5	1.81
LRAPD0	C314H447D24N77O86S3P1	6852	D labeled	1.343	5	2.16
LRAPD100	C314H366D105N77O86S3P1	6938	100% D exchange	1.359	5	3.13
Inner0	C184H257D24N45O61P1	4154.5	0% D exchange	1.346	5	2.31
Inner100	C184H171D79N45O61P1	4209.5	100% D exchange	1.356	5	3.82
Outer0	C130H196N32O28S3	2751.4	0% D exchange	1.338	5	1.81
Outer100	C130H170D26N32O28S3	2777.4	100% D exchange	1.351	5	2.55

REFERENCES

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(2) Dobson, C. M.; Evans, P. A.; Radford, S. E. *Trends in Biochemical Science* 1994, *19*, 31.

(3) Efimova, Y. M.; Well, A. A. v.; Hanefeld, U.; Wierczinski, B.; Bouwman, W. G. *Journal of Radioanalytical and Nuclear Chemistry* 2005, *264*, 271.

(4) Masica, D. L.; Gray, J. J.; Shaw, W. J. *Journal of Physical Chemistry C* 2011, *115*, 13775.