SURFing with Green Tea: What Neutrons Read in Tea Leaves

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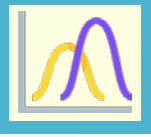
OUTLINE


BSA & Polyphenols


High Pressure

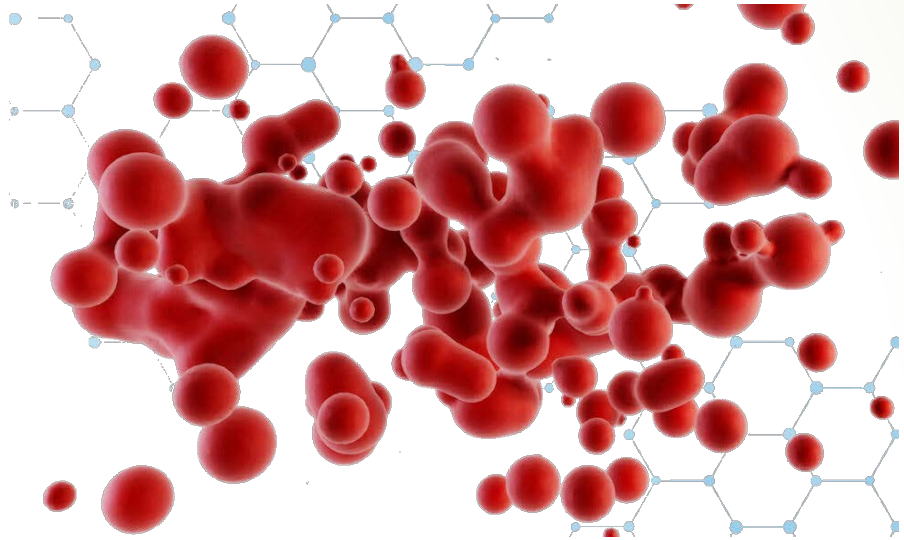

Neutron Scattering


Experimental Setup


Results


Conclusion & Future Directions

Bovine Serum Albumin



Electrostatic Potential (mV)

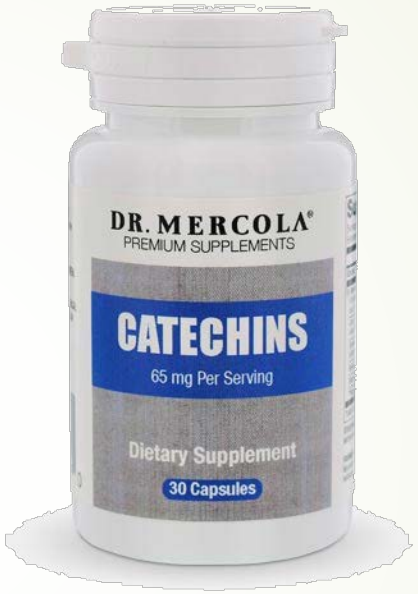
Calculated using Adaptive Poisson-Boltzmann Solver (APBS) at pH 7.

[Baker et al. (2001). PNAS 98, 10037-10041]

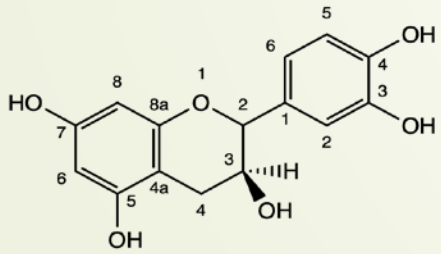
Green Tea Polyphenols

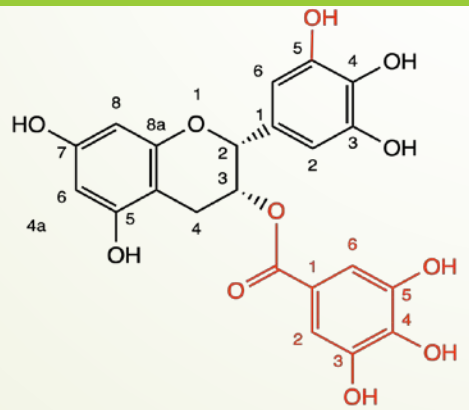
~84 Å

~47 Å



(+)-Catechin | **(-)-Epigallocatechin Gallate (EGCG)**





Computational docking analysis binding sites

Interactions

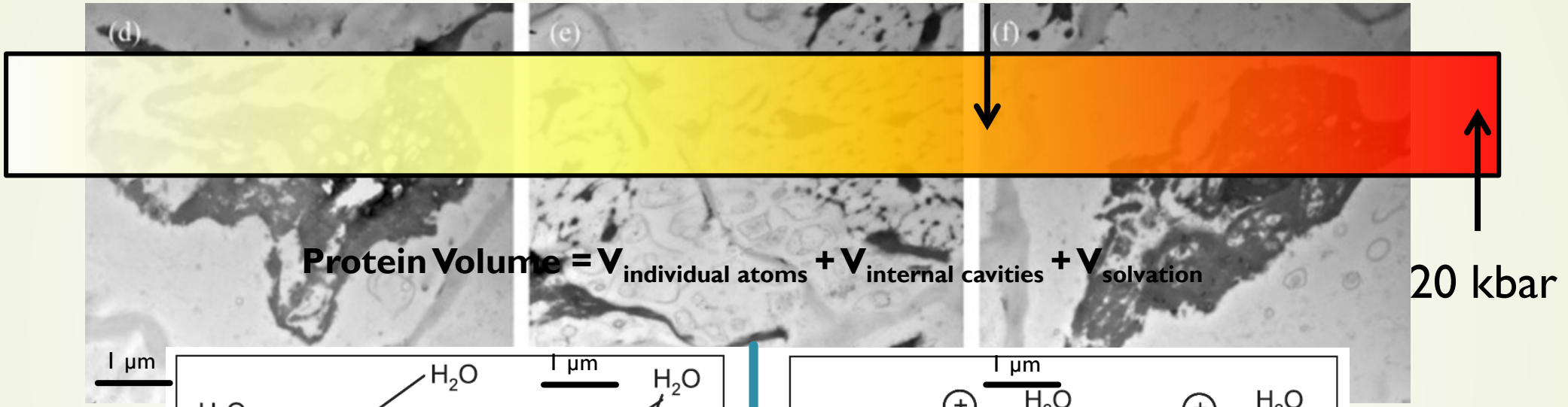
Hydrophobic (VDW + Nonpolar)	-25.11 kCal/mol (EGCG) -18.63 kCal/mol (Catechin)
Hydrogen bonding	7.49 (EGCG) 1.68 (Catechin)

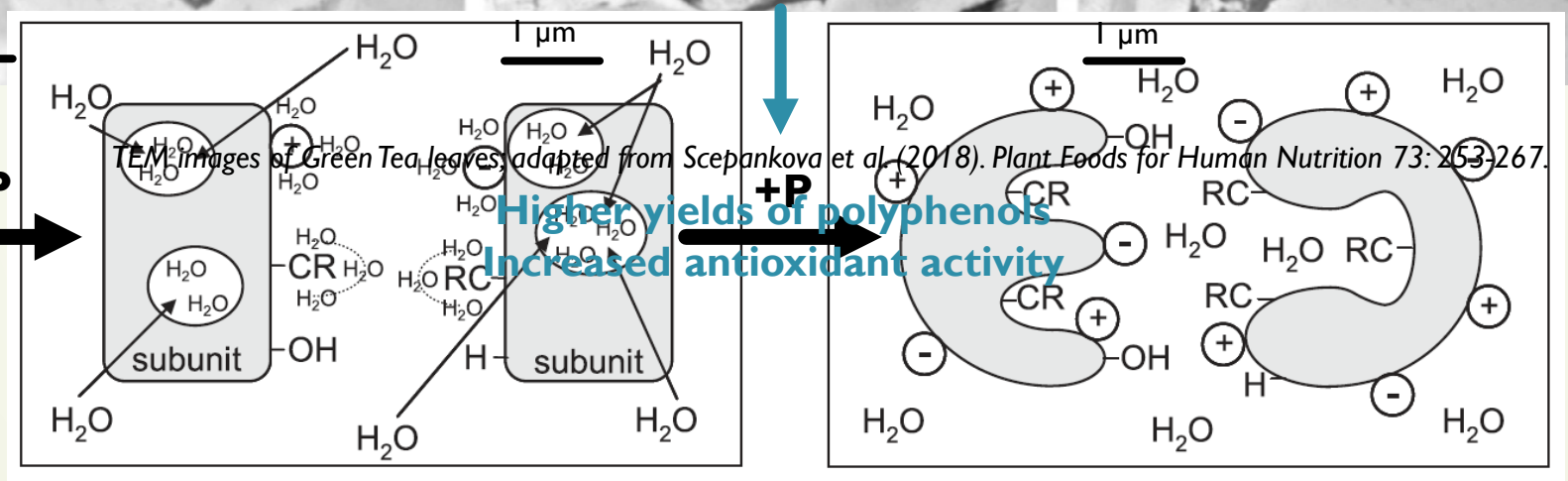
[Tang et al. (2017). J. Agric. Food Chem. 65: 656-665]

High Pressure Effects

No Treatment

High Pressure 4 kbar + High Temperature





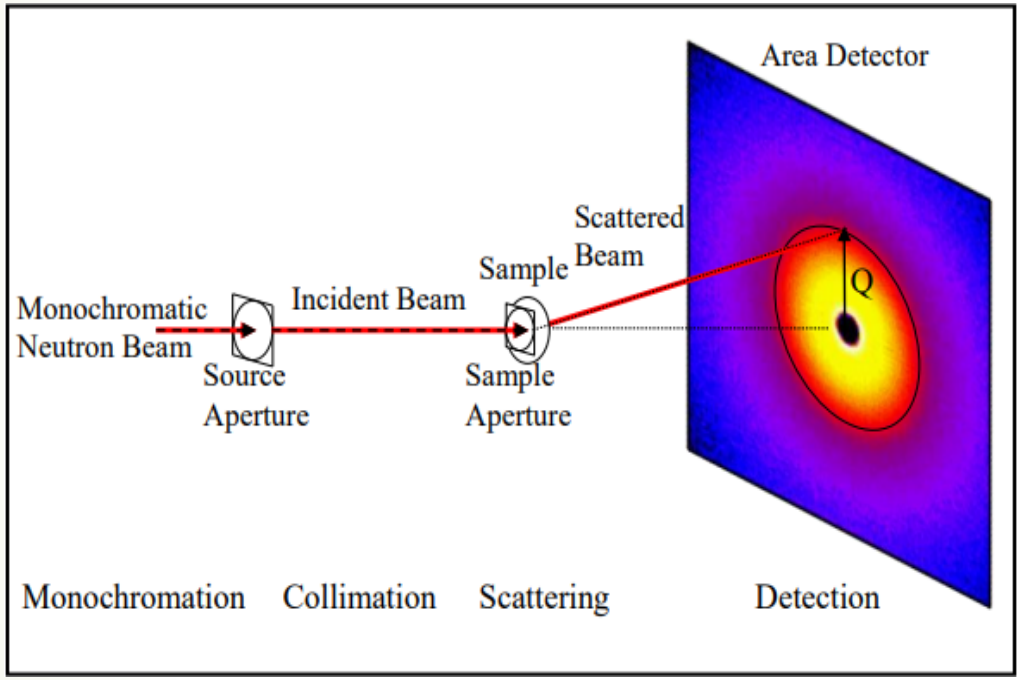
TEM images of Green Tea leaves; adapted from Scepankova et al. (2018). Plant Foods for Human Nutrition 73: 253-267.

Higher yields of polyphenols
Increased antioxidant activity

Adapted from: R. Winter et al. (2007). J. Non-Equilib. Thermodyn. 32, 41–97.

Why SANS?

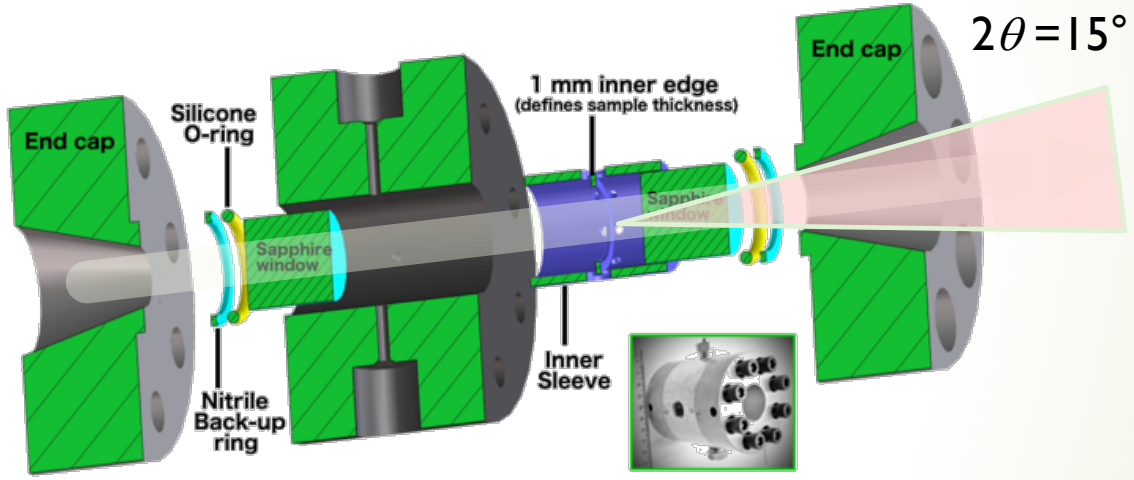
Structure, conformation of biomaterials ⇨ Function and behavior



Reciprocal space

Real space

Pressure: up to 3.5 kbar.
Temperature : -15°C to +65°C
q-Range: 0.001-0.3 $Å^{-1}$
Sample Volume: 2.5 mL per load.
Typical Sample: 3-10 mg/mL

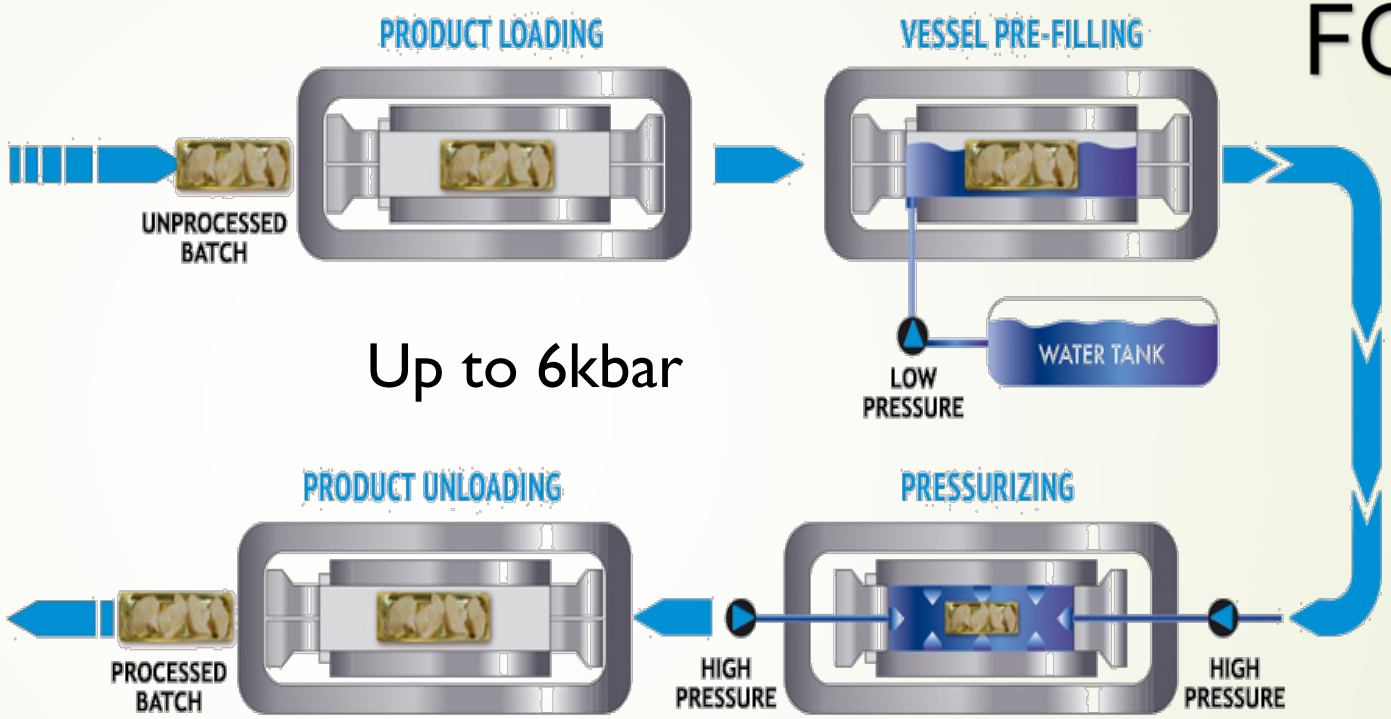


Adapted from Teixeira et al. (2018) J. Neutron Res. 20(1), 13-23.

GOALS

Investigate:

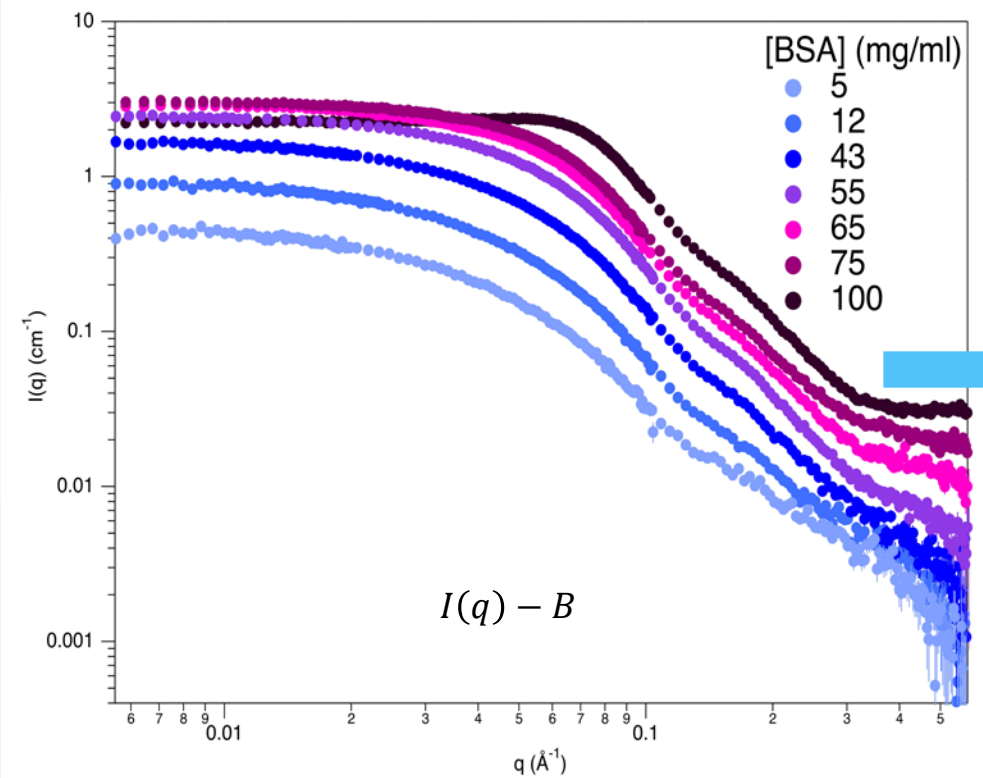
- Structure and stability of BSA-polyphenol complexes under conditions relevant to food processing
- Effects of pressure on conformation and binding of polyphenols to BSA

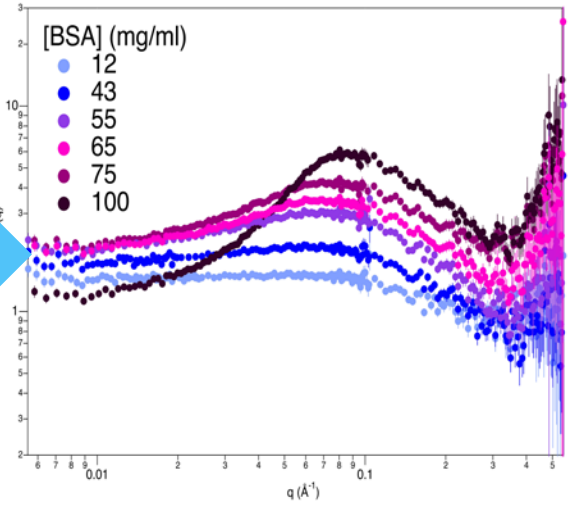


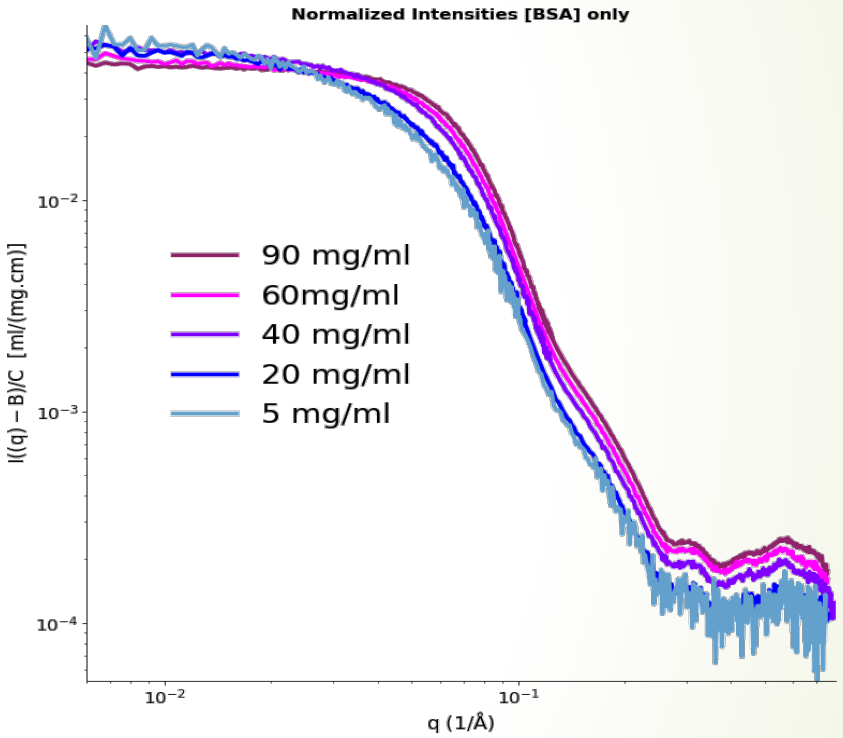
FOOD!

Adapted from Hiperbaric High Pressure Processing.

Concentration Screening & Structure Factor



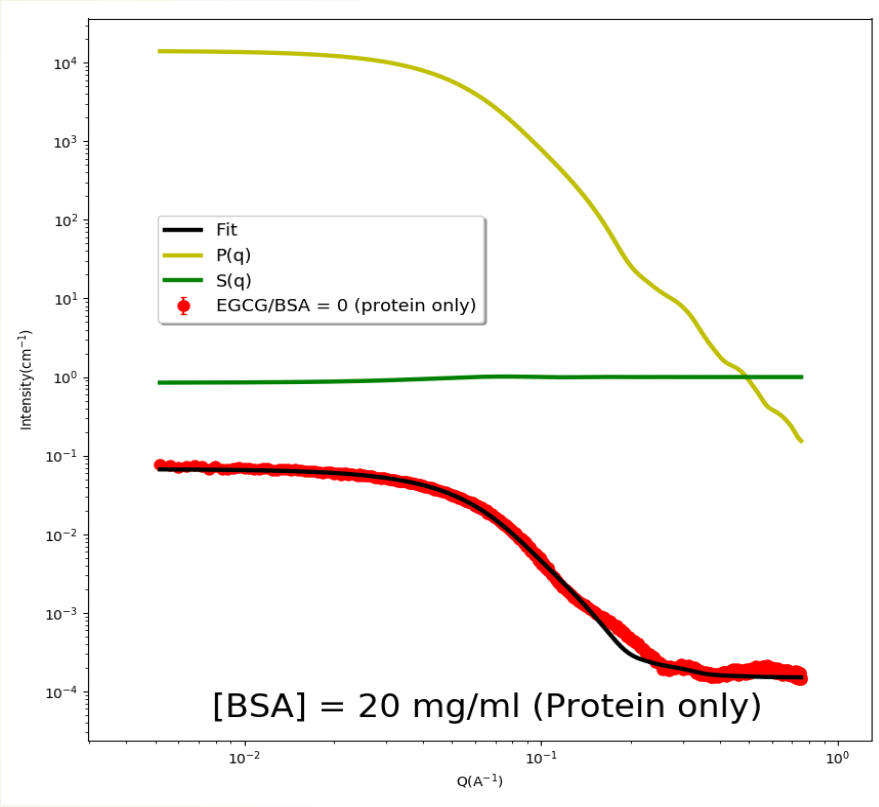


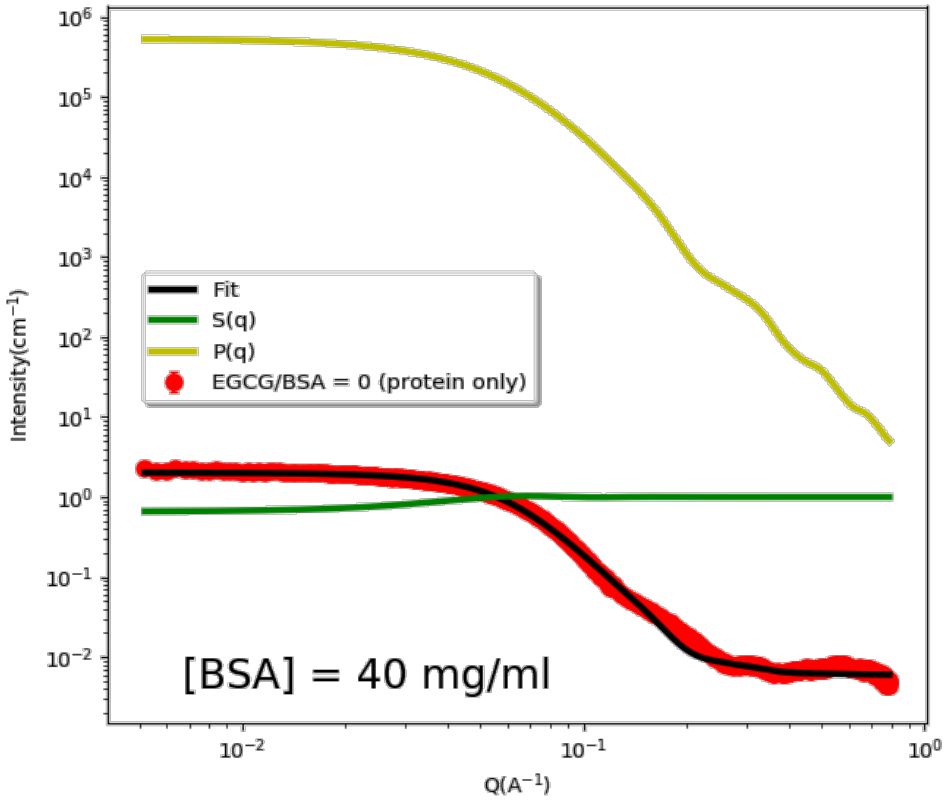


SANS (NG7 & NGB30 instruments)

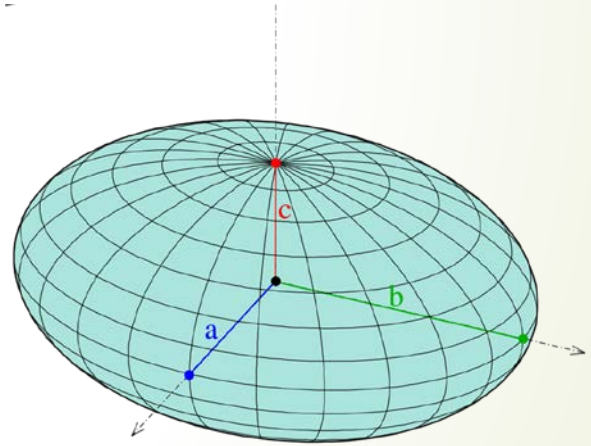
SAXS (IBBR In-House Source)

BSA Ellipsoid Model - Rough Fitting





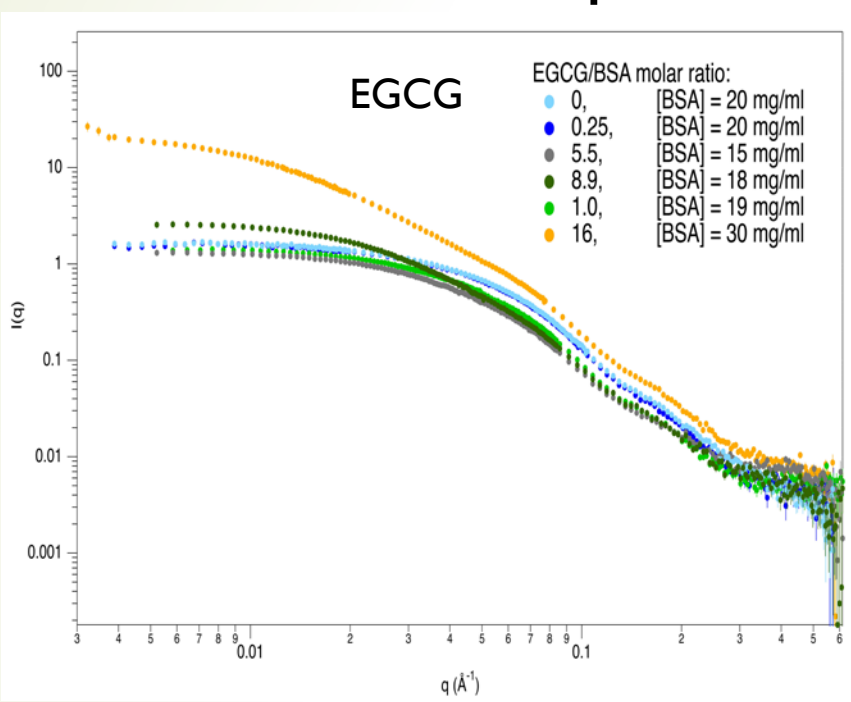
$$I(q) = P(q) * S(q)$$

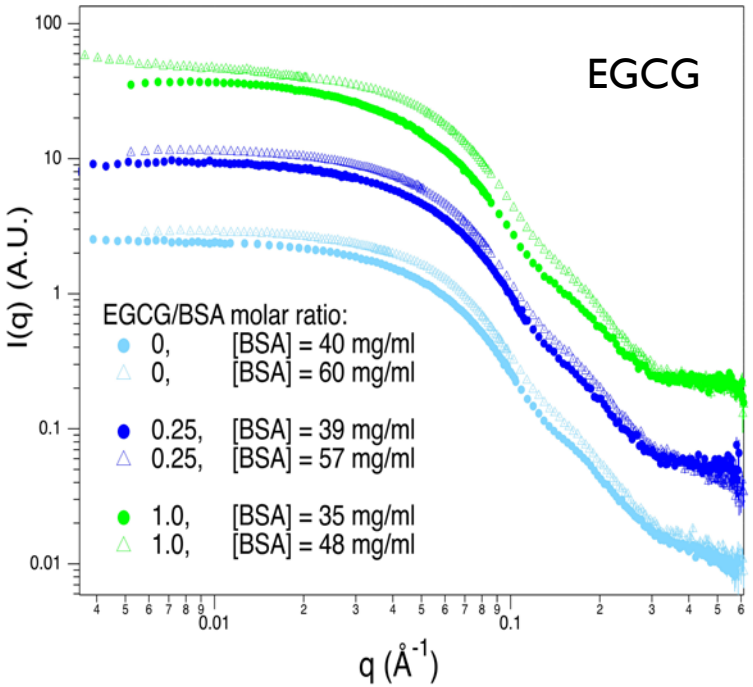


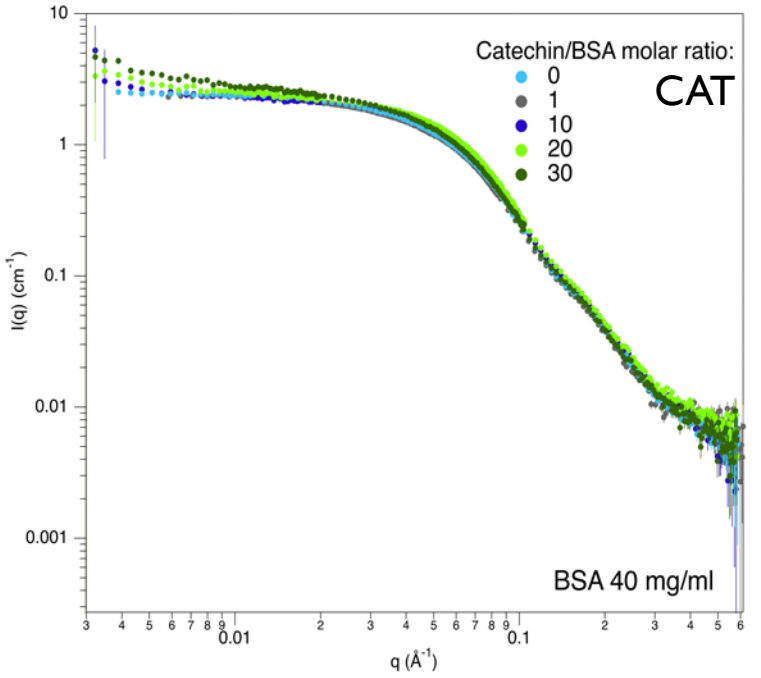
[Protein]	a Minor Eq. Radius (Å)	b Major Eq. Radius (Å)	c Polar Radius (Å)
20 mg/ml	42.6 ± 0.3	49.5 ± 0.3	17.7 ± 0.1
40 mg/ml	37.7 ± 0.1	64.9 ± 1.3	17.0 ± 0.1

Tri-axial model: Finnigan, J.A., Jacobs, D.J., 1971. J. Phys. D: Appl. Phys. 4, 72-77.
Hayter MSA Structure Factor model: J B Hayter & J Penfold, 1981. Molecular Physics 42, 109-118.

Complexes of Polyphenols/BSA – Parameters Probed

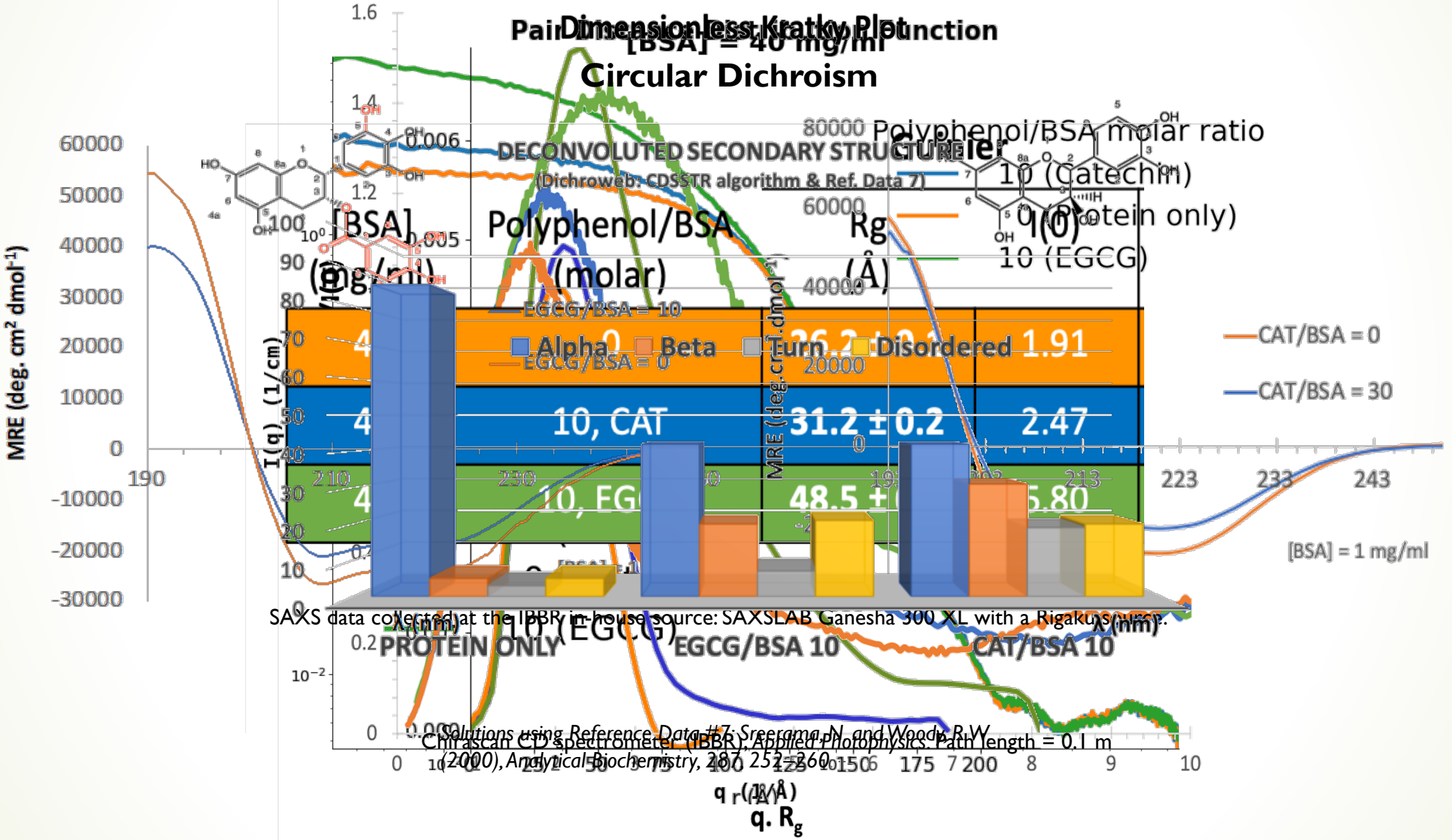






Parameters	
BSA Concentration	10 mg/mL – 60 mg/mL
Molar Ratio (Polyphenol: BSA)	0 – 30 mol
Time	2 hrs – 4 weeks
H_2O/D_2O Solvation	100% H_2O – 100% D_2O

Catechin vs EGCG Effects on BSA Structure – SAXS and CD Data

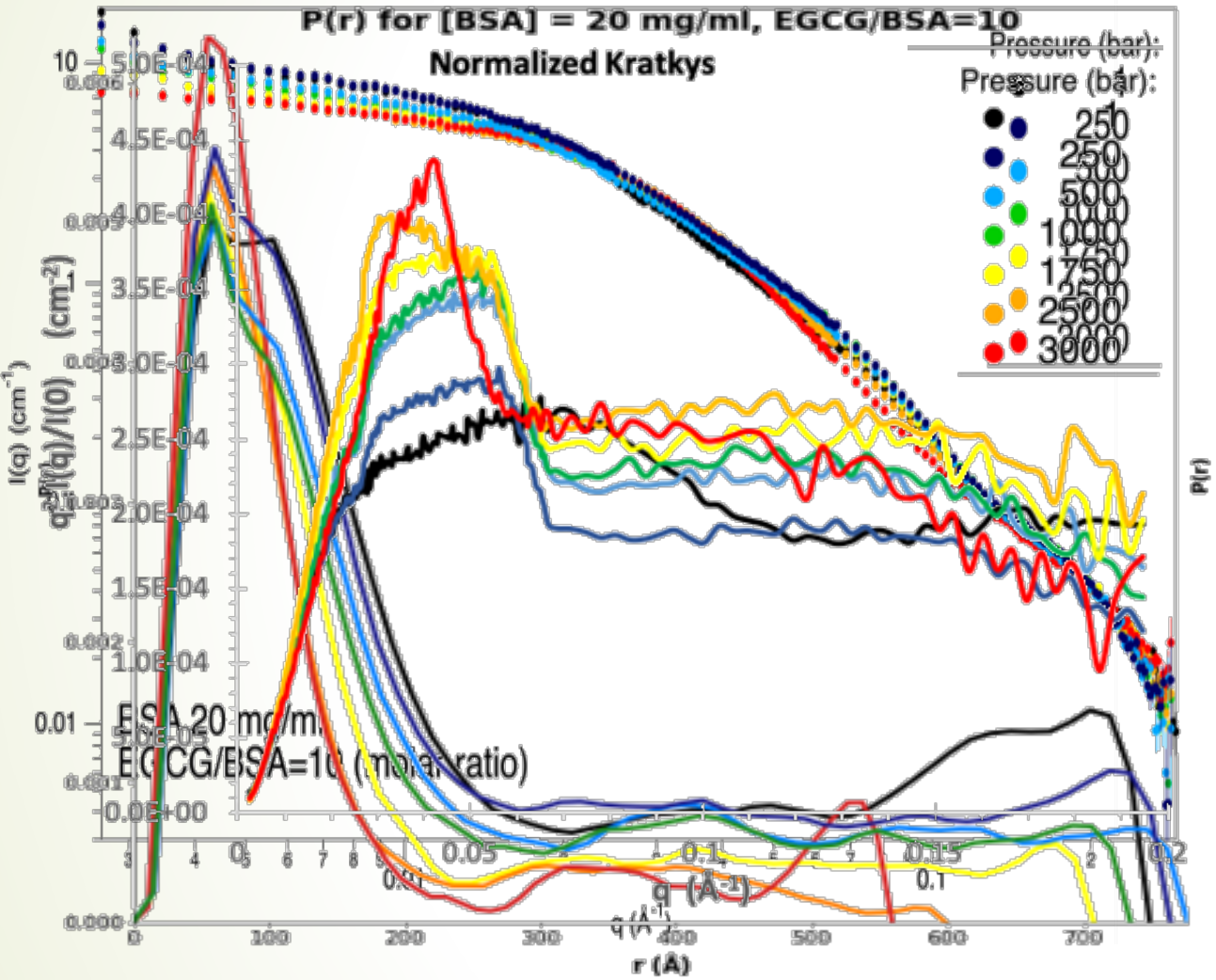


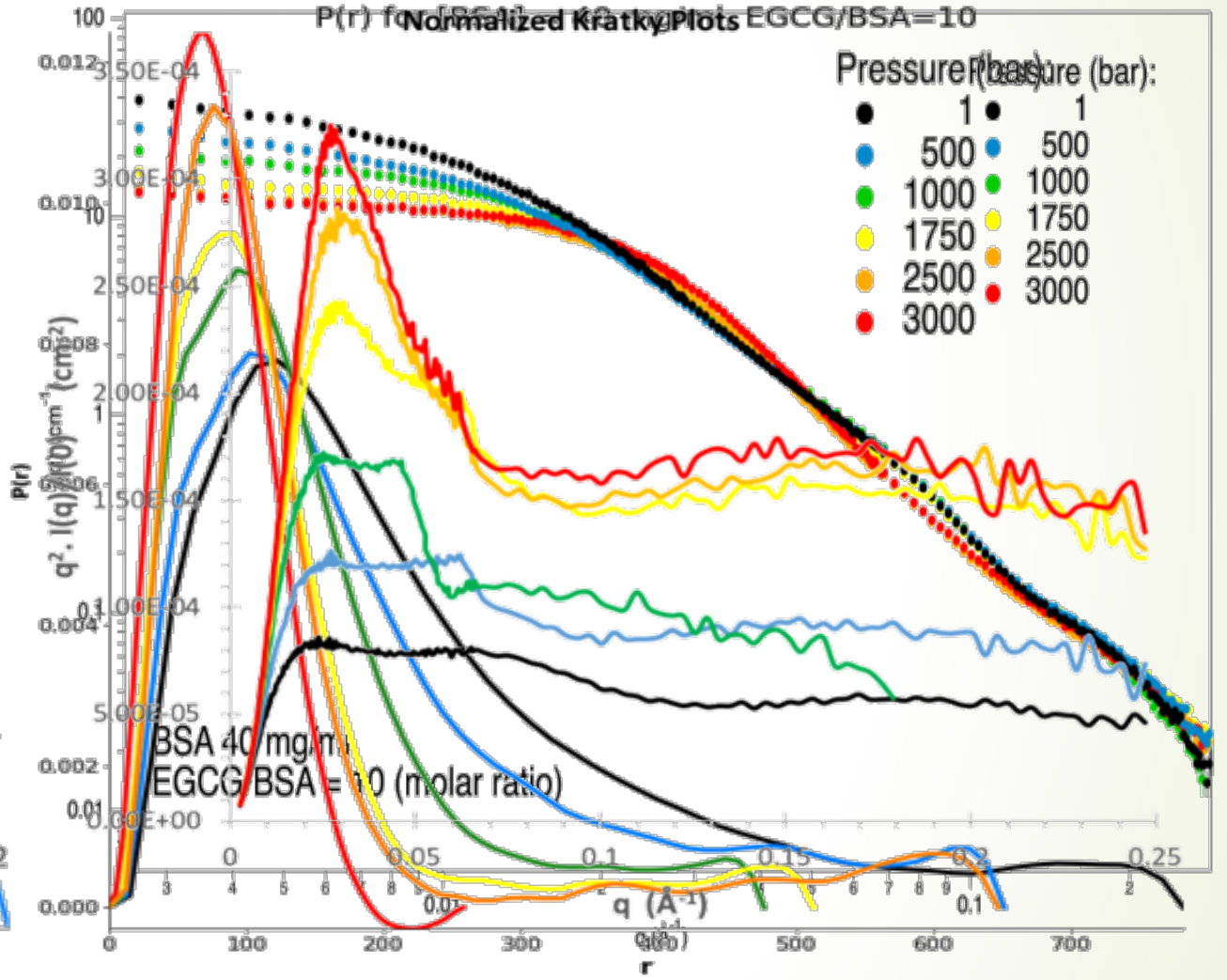
SAXS data collected at the IBBR in-house source: SAXSLAB Ganesha 300 XL with a Rigaku source.

Chirascan CD spectrometer (IBBR). Path length = 0.1 m

Solutions using Reference Data #7: Sreerama, N. and Woody, R.W. (2000), Analytical Biochemistry, 287, 252-260.

HP-SANS – 20 & 40 mg/mL BSA Complexed with EGCG





Conclusions

- Established the onset of S(q) for BSA solutions
- Established SAS profiles for BSA/polyphenol complexes
- Determined the effects of polyphenols on the secondary structure of BSA
- Determined the effects of HP on the complex structure
- Established the use of HP SANS as a viable tool to monitor the effects and their reversibility of HP *in situ*

Future Directions

- Molecular Dynamics (MD) on the BSA crystal structure to determine models for the solution structure of the protein at different pressures
- Near-UV (250-320 nm) CD profiles of complexes under HP-SANS conditions to monitor binding effects on specific types of amino acids and disulfide bonds
- Probe HP effects vs Temperature, pH

Acknowledgements

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THANK YOU!!!

CHRNS — Center for High Resolution Neutron Scattering — NSF — NIST

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