Measuring and Using Terahertz Timescale Dynamics to Predict Solid-State Protein Stability

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Conclusions

- **Matrix Mobility Controls Protein Degradation in Dry, Glassy State**
  - β relaxation (ps – μs) are relevant motions, not α relaxation (ks – Ms)
  - We have developed a theoretical framework to make the connection between β relaxation and protein stability
  - β relaxation directly facilitates diffusion of small molecules (reactants)
  - β relaxation facilitates local fluctuations in protein conformation

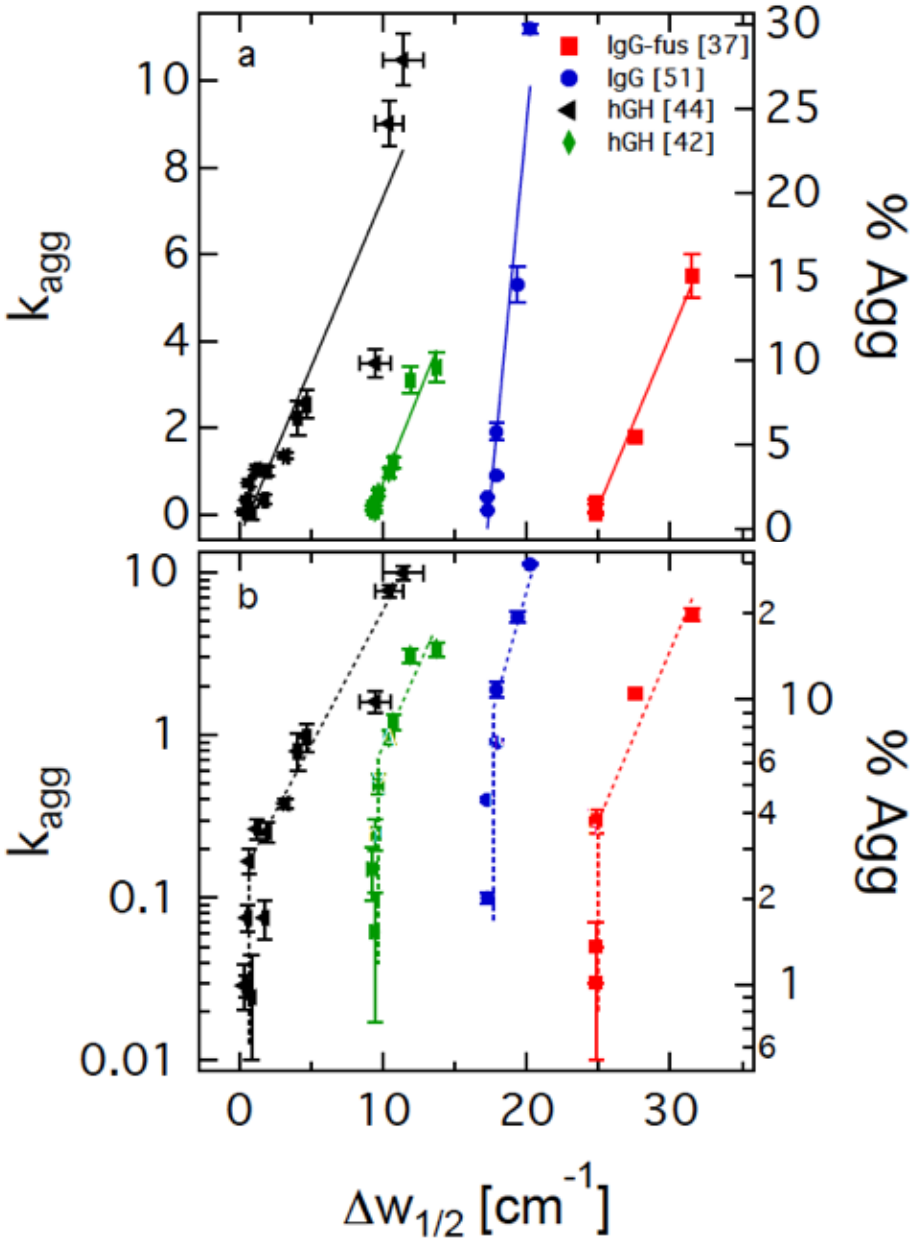
- **Low-Frequency Raman Scattering is Likely Able to Rank-Order Formulations to Within 10% of Relative Degradation Rates**
  - Estimation based on accumulated light scattering, neutron scattering, and protein degradation data

Protein Structure Appears to be a Fine First-Pass Indicator of Protein Stability, but…

Top panel: a correlation between protein secondary structure and protein stability in freeze-dried solids. (Frequently seen)

Bottom panel: this correlation breaks down completely for “good” formulations. Improvements in stability of > 10X were accompanied by no change in secondary structure.

Proteins still degrading when 2° structure is as good as it gets.

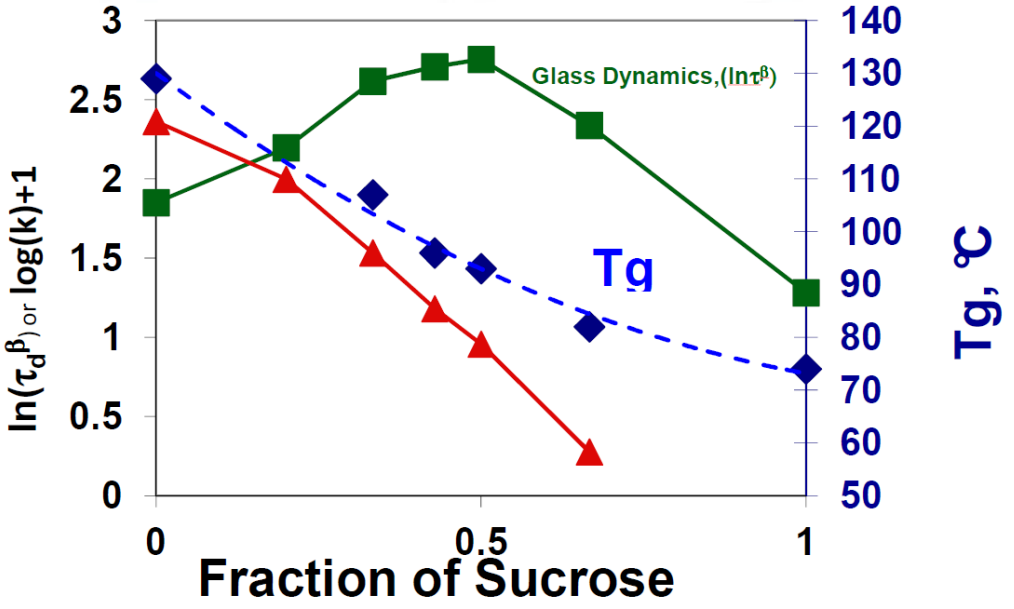


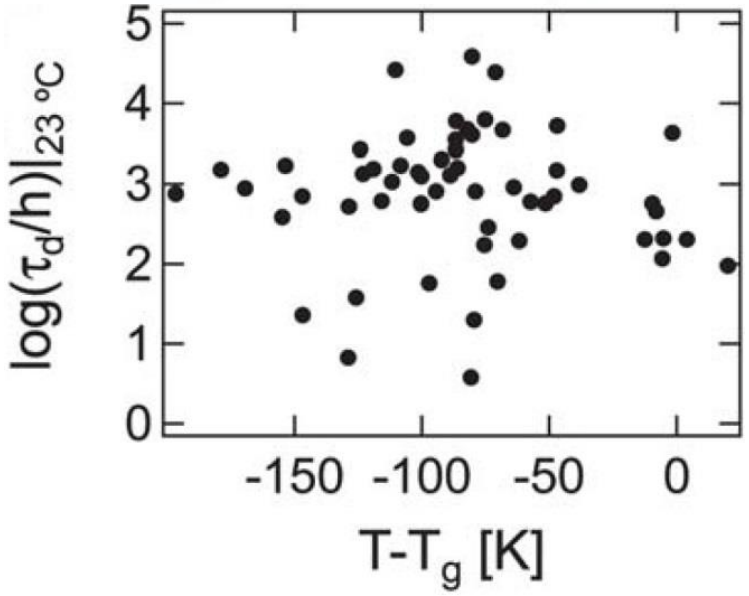
Cicerone et al. ADDR **93**, 14-24 (2015)

Neither T_g nor α relaxation (ks – Ms) are good predictors of stability

- Left Panel: Neither Tg nor α relaxation (enthalpy relaxation) tracks IgG aggregation in sucrose formulation

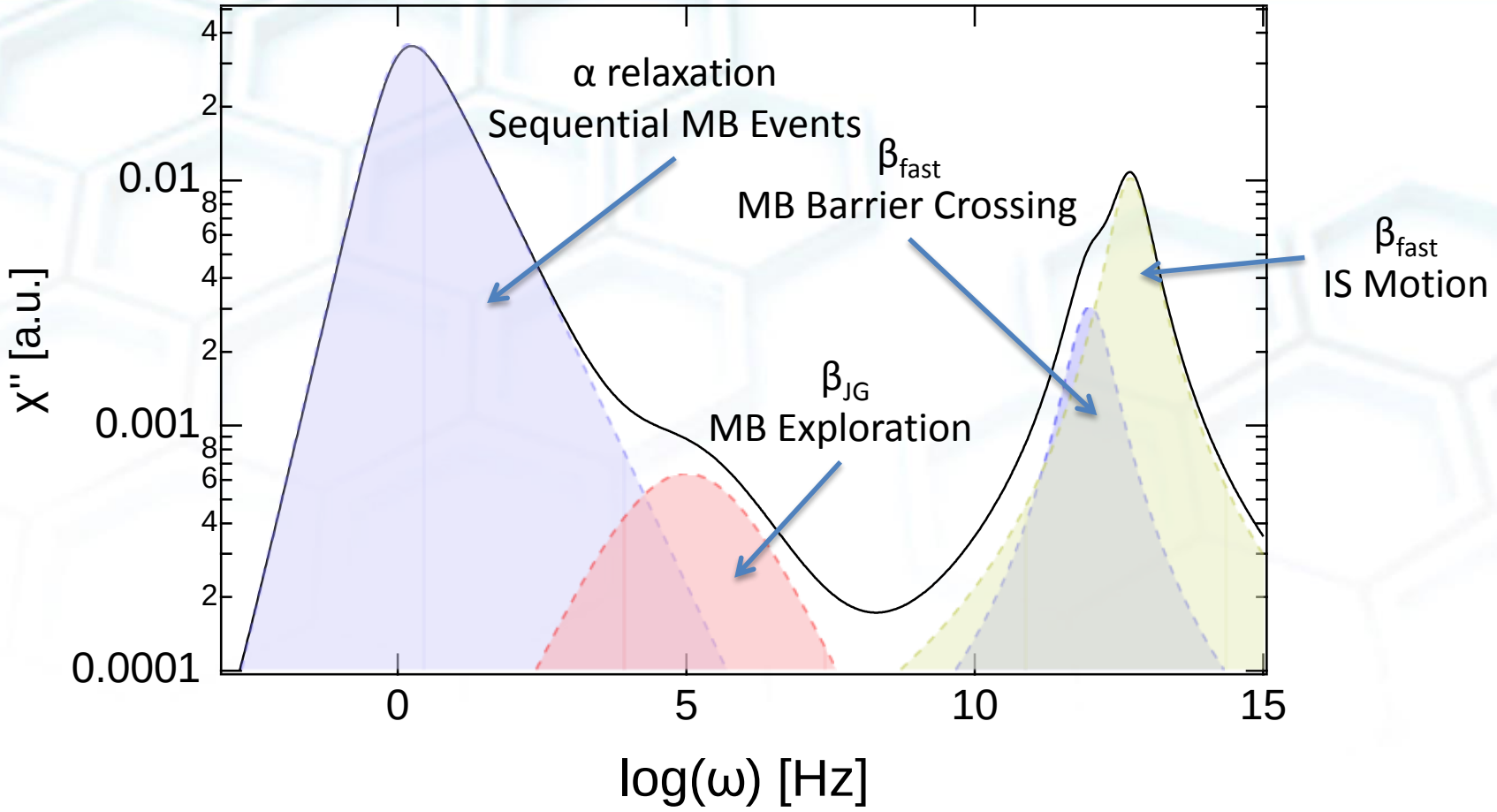
- Right Panel: Protein degradation rates can vary over a factor of 100,000 at a given matrix Tg value – there is no correlation between matrix Tg and protein stability





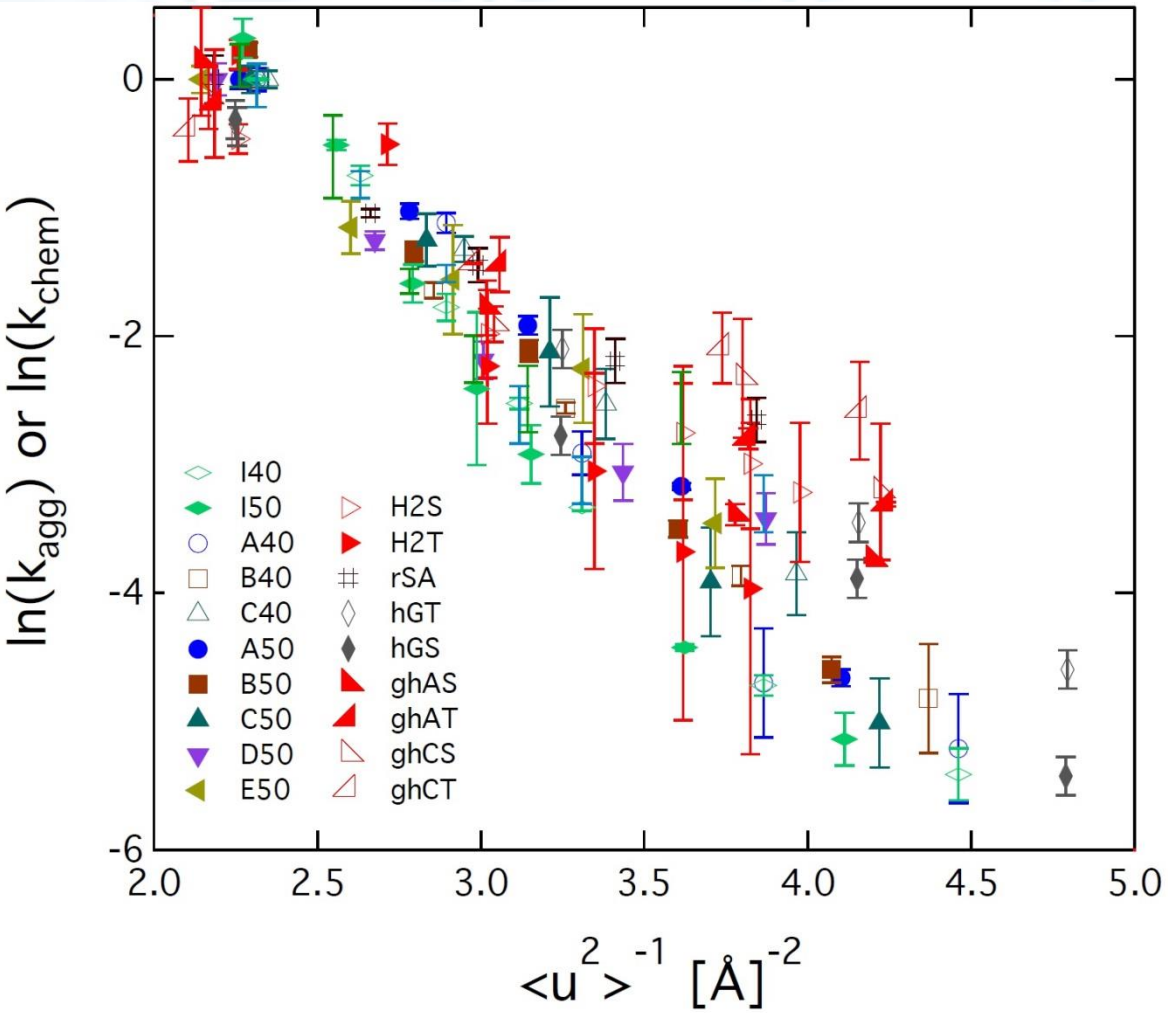
Chang et al. J Pharm Sci **94**:7, 1427 (2005)

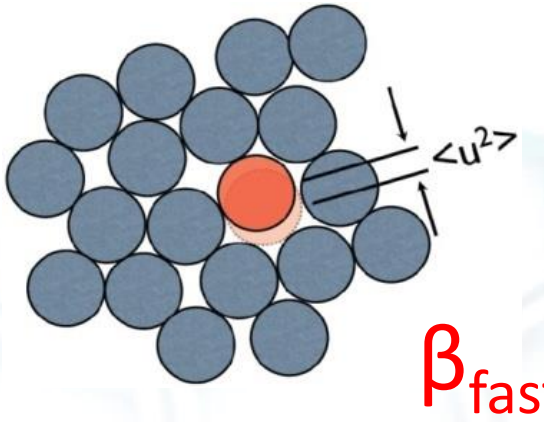
Cicerone et al. Soft Matter **8**:10, 2983 (2012)



- β_{JG} is directly related to transport and local relaxation, and reaction rates.
- $\langle u^2 \rangle$ or β_{fast} is fundamentally related to β_{JG}, but with slightly different δ for each material, leading to slight variations in $1/\langle u^2 \rangle$ vs $\log(k_{deg})$.

Protein stability controlled by β_{fast} relaxation? (ps – ns)

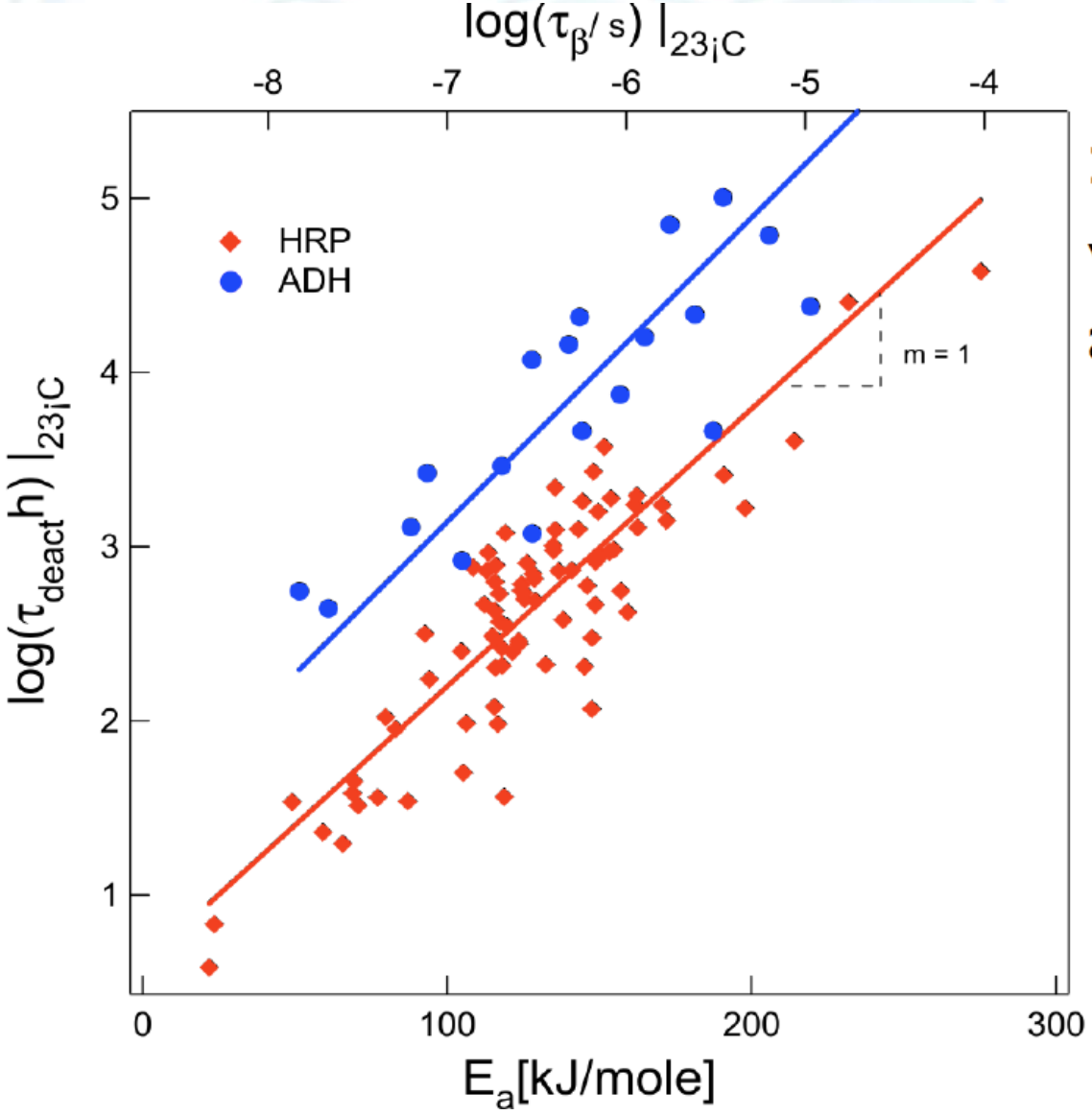




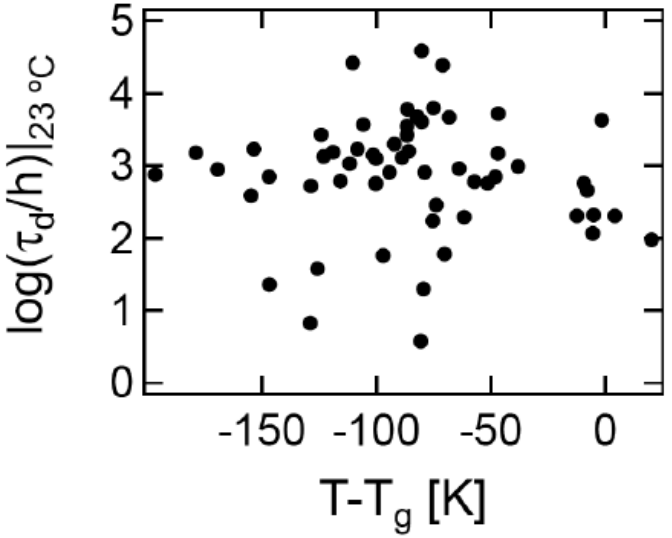
- Protein degradation rate (10^{6} s) tracks $\langle u^2 \rangle^{-1}$ (10^{-9} s)
- Mean-squared displacement (MSD), $\langle u^2 \rangle$, of H atoms obtained from neutron backscattering

Cicerone & Soles, Biophys. J. 2004; Cicerone & Douglas, Soft Matter 2012

Protein stability controlled by β_{JG} relaxation? (μs – ms)

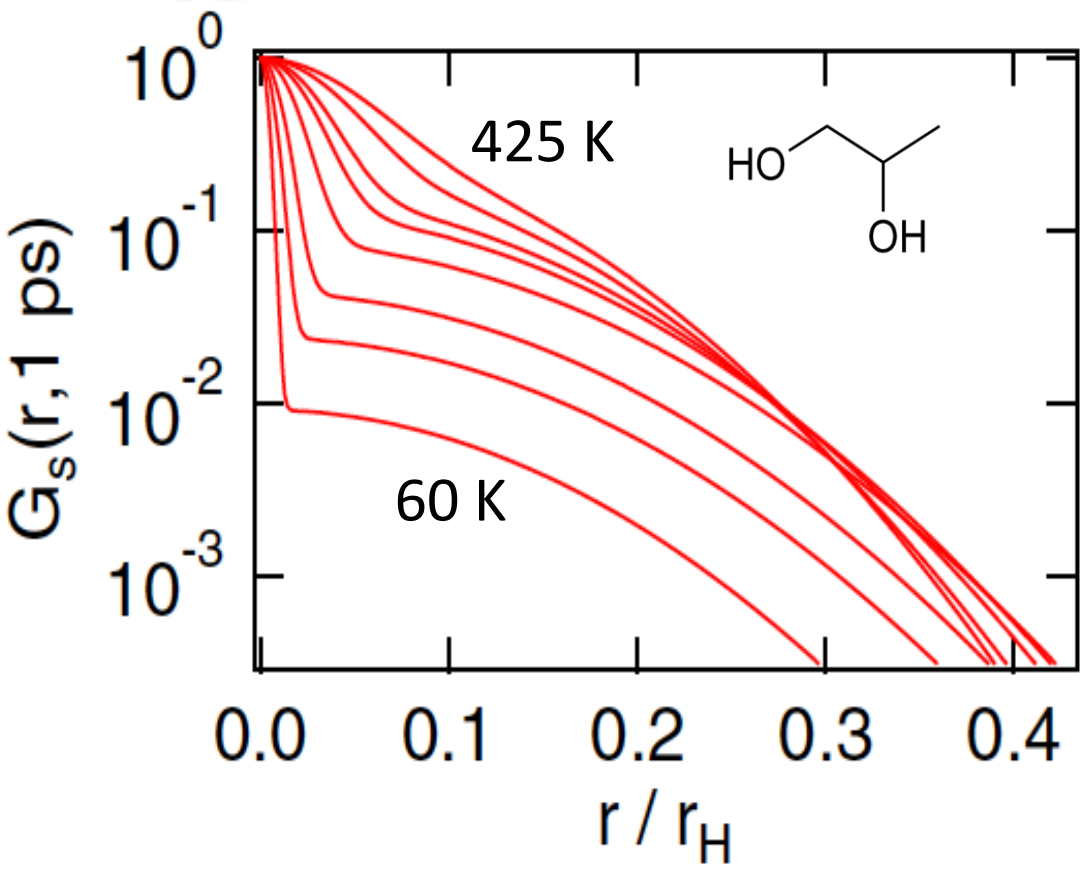


> 100 glasses of varying composition in antiplasticized series



Cicerone & Douglas, Soft Matter 2012

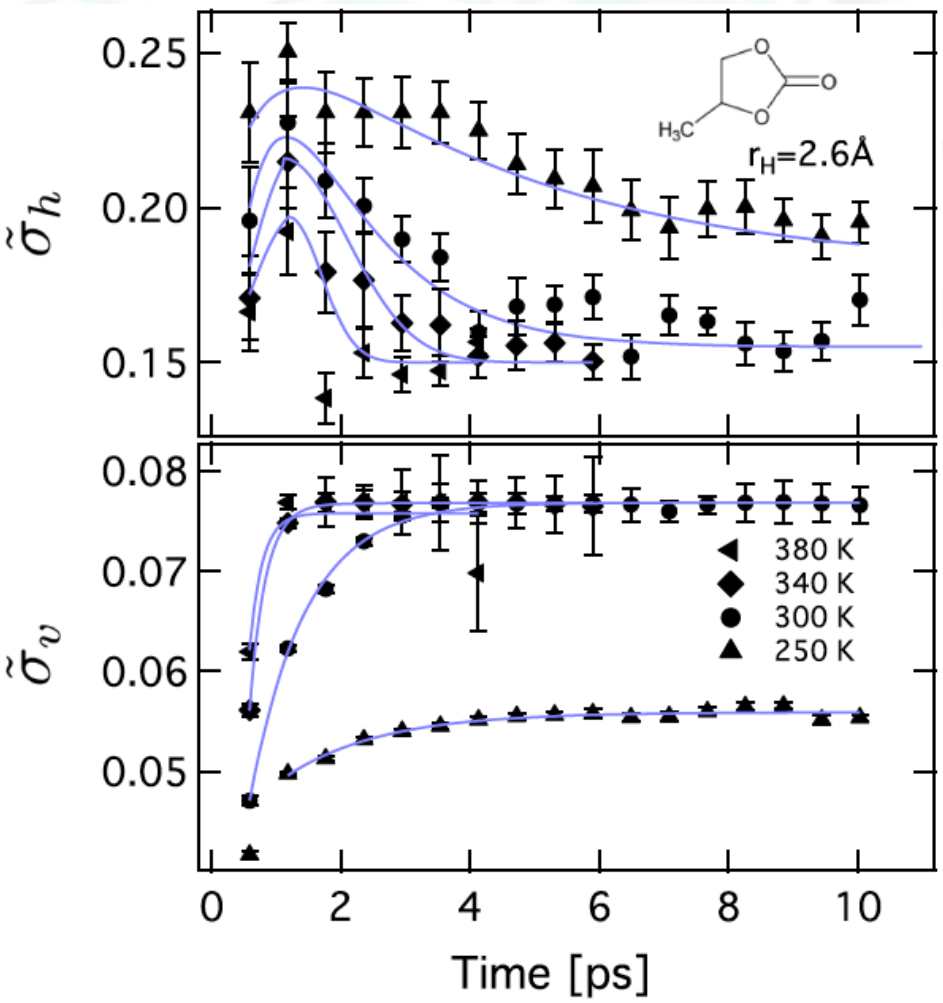
How Does Diffusion Occur in a Glass?



Spatially heterogeneous dynamics

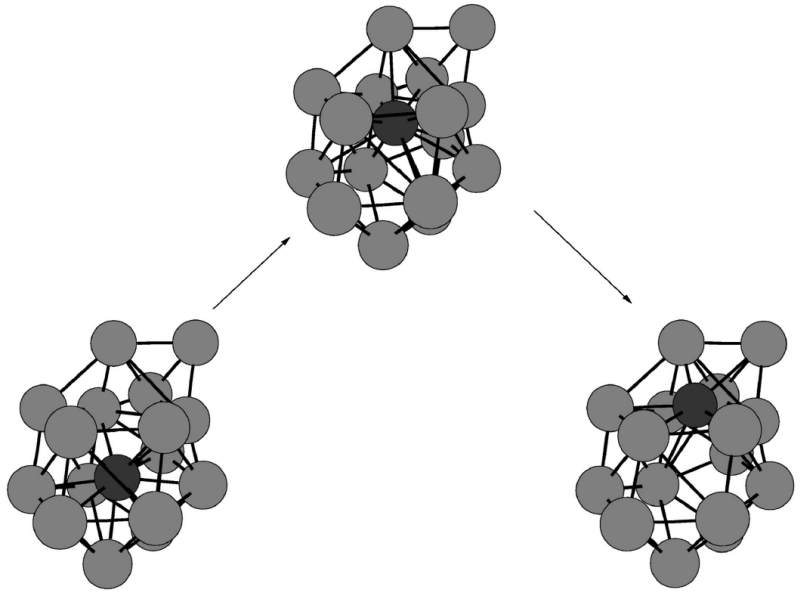
- Most molecules can move only ~1% of their radius on 1 ps timescale in a glassy state
- Some molecules can move (hop) ~20% of their radius in 1 ps, even at cryogenic temperatures

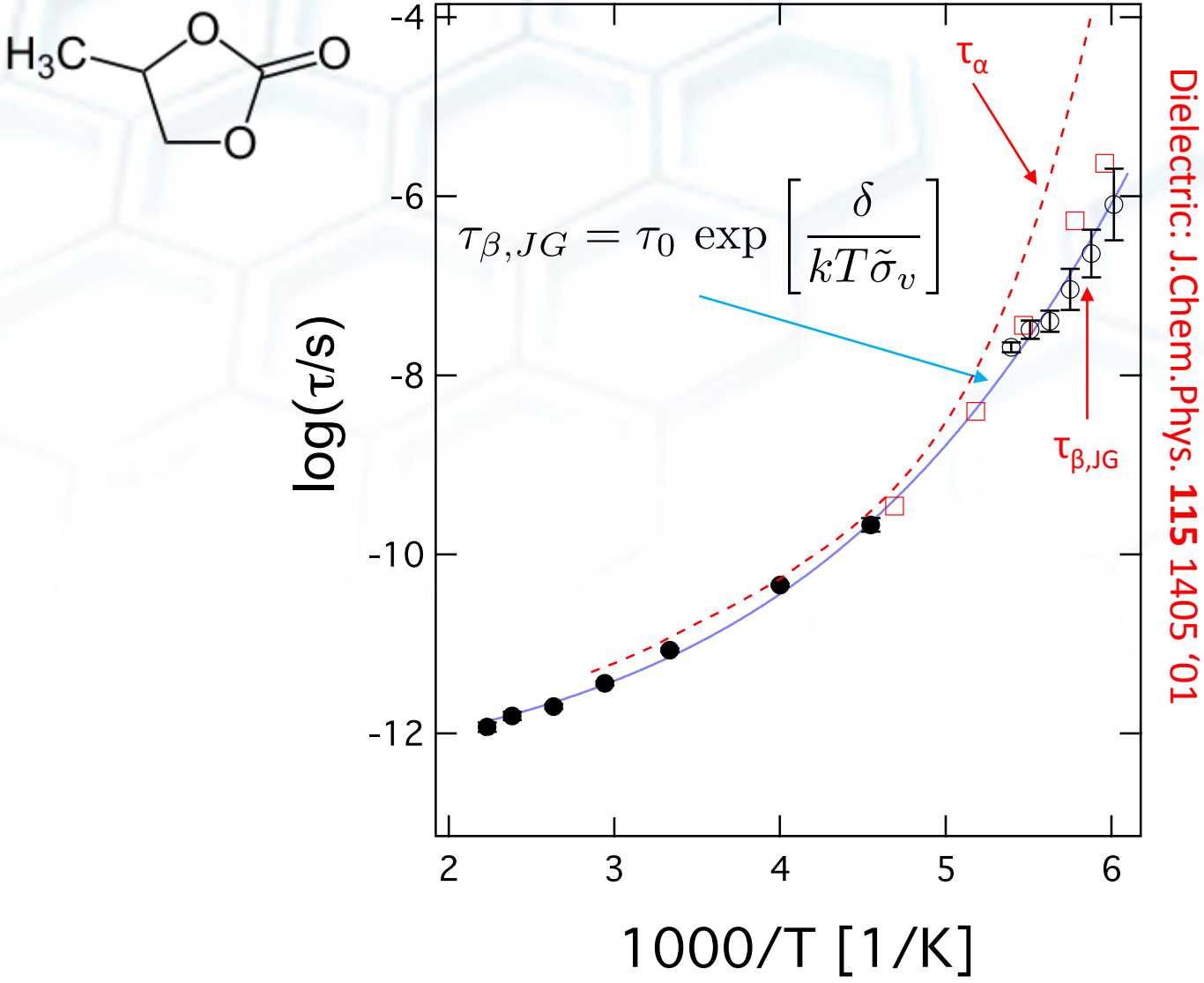
QENS – Propylene Carbonate



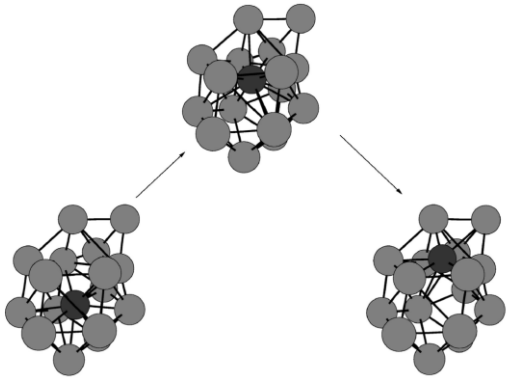
τ_h is MB barrier crossing time

τ_v is IS time




H_3C
O
O
O
$\tau_{\beta,JG} = \tau_0 \exp\left[\frac{\delta}{kT\tilde{\sigma}_v}\right]$
τ_α
$\tau_{\beta,JG}$
log(τ/s)
-4
-6
-8
-10
-12
2
3
4
5
6
1000/T [1/K]
Dielectric: J.Chem.Phys. **115** 1405 '01

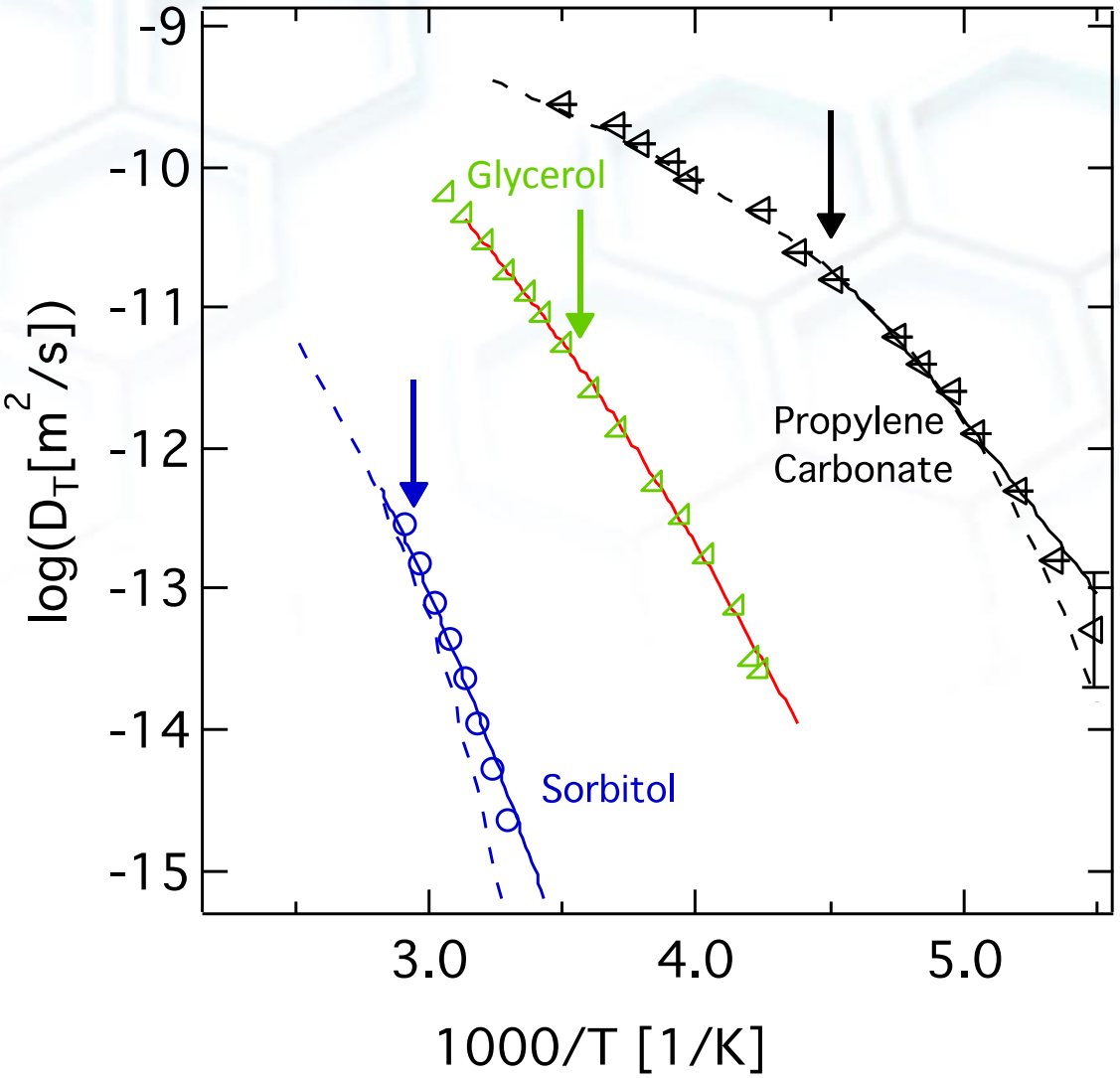
Quantitative Model



$$D_T = \frac{\sqrt{\pi}\Phi\sigma_h^2}{12g\tau_{\beta,\mathrm{JG}}}$$

$$\tau_{\beta,\mathrm{JG}} = \tau_0 \exp\left[\frac{\delta}{kT\tilde{\sigma}_v}\right]$$

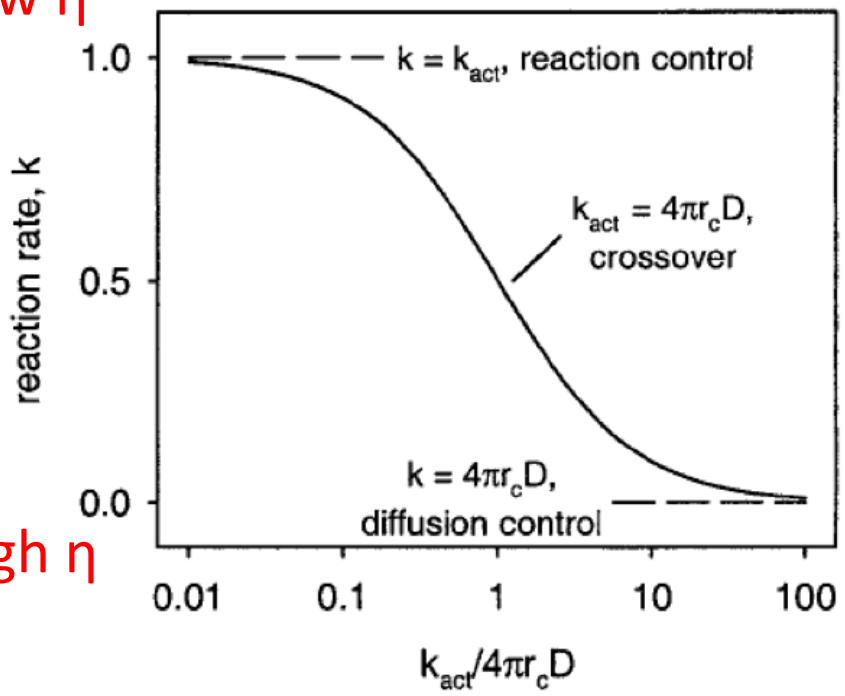
$$\delta = -25 + 8.6 \times 10^4 / T_m$$



Why is it that $log(\tau_{deg}) \propto log(\tau_{\beta JG}) \propto 1/\langle u^2 \rangle$?

Because reaction and crystallization rates in viscous liquids and glass are diffusion controlled (follow translational diffusion).

low η

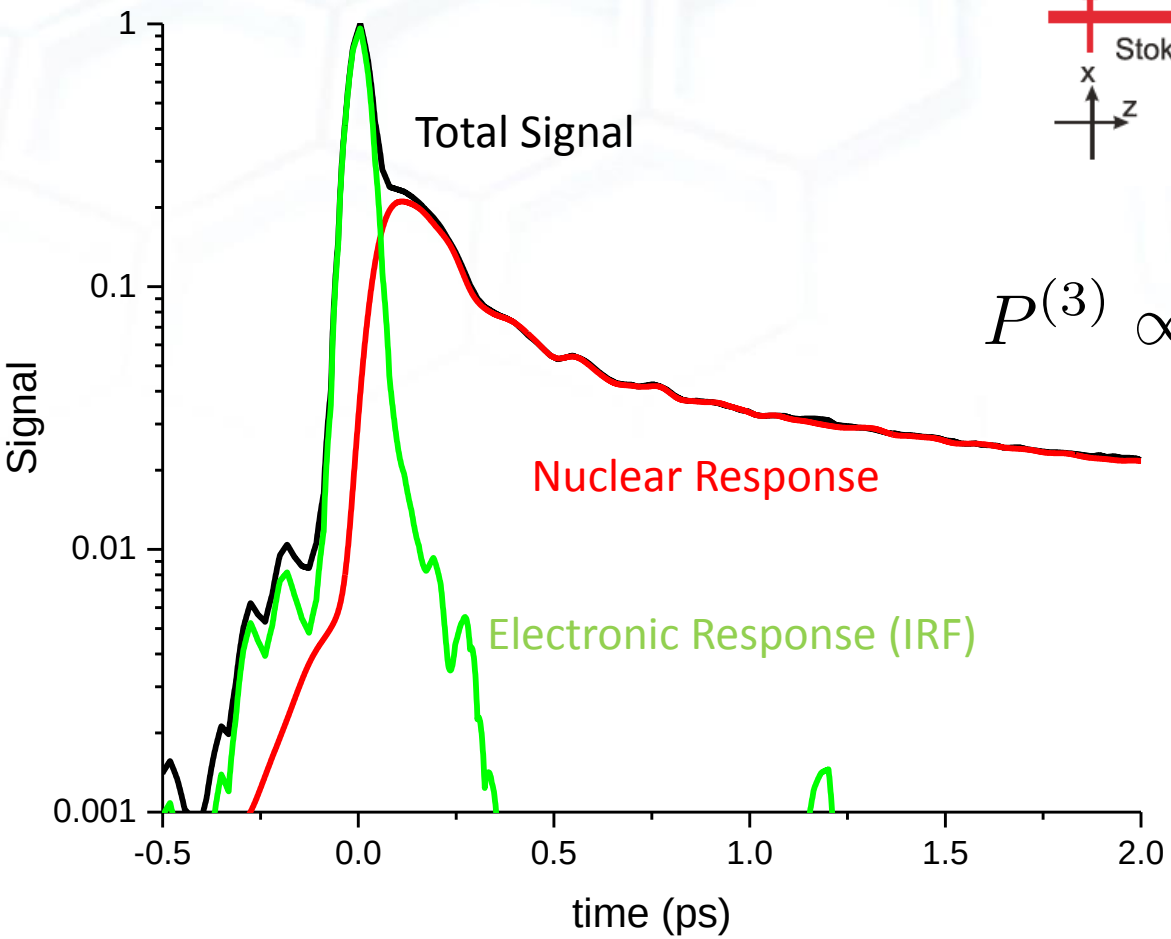


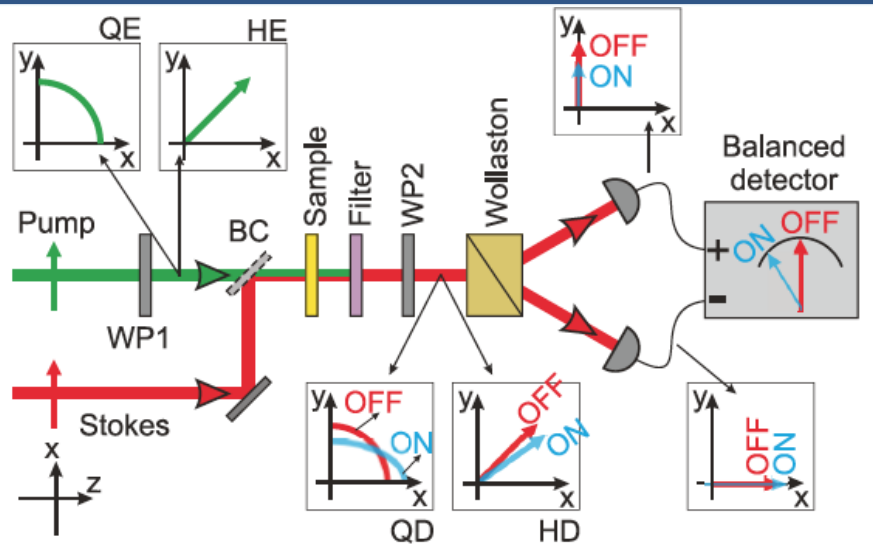
high η

And, $D_T = \frac{\sqrt{\pi}\Phi\tilde{\sigma}_h}{12\, g\tau_{\beta JG}}$ $\left(\tau_{\beta,\mathrm{JG}} = \tau_0 \exp\left[\frac{\delta}{kT\tilde{\sigma}_\mathrm{v}}\right], \tilde{\sigma}_v \propto \langle u^2 \rangle \right)$.

Optical Kerr Effect

Optical Kerr effect measures depolarized vibrations *and a time-dependent orientation correlation function*

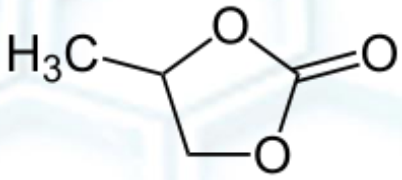


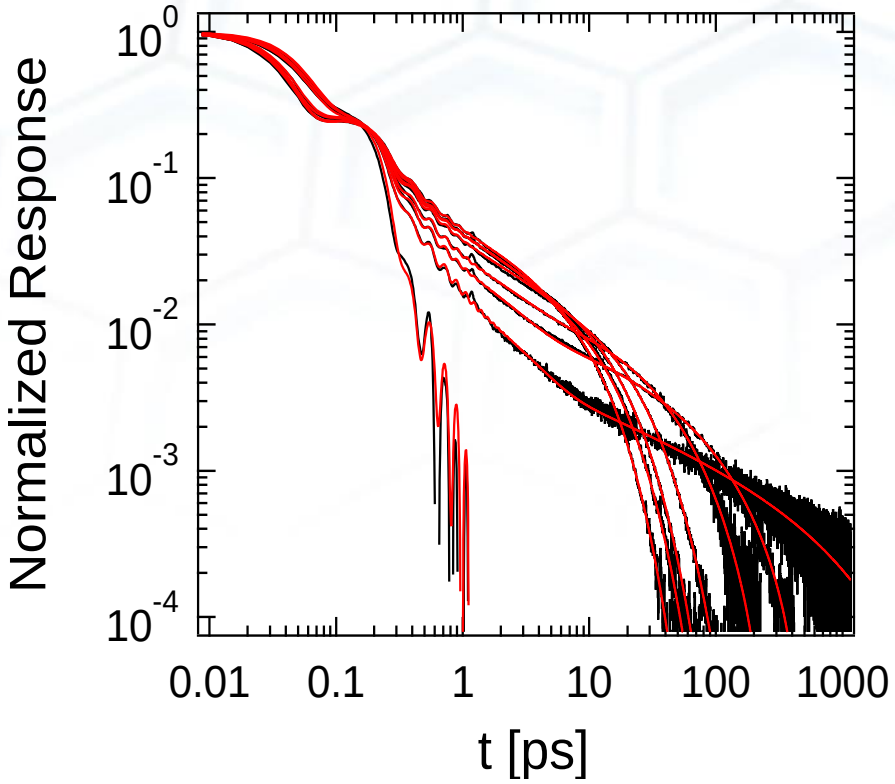


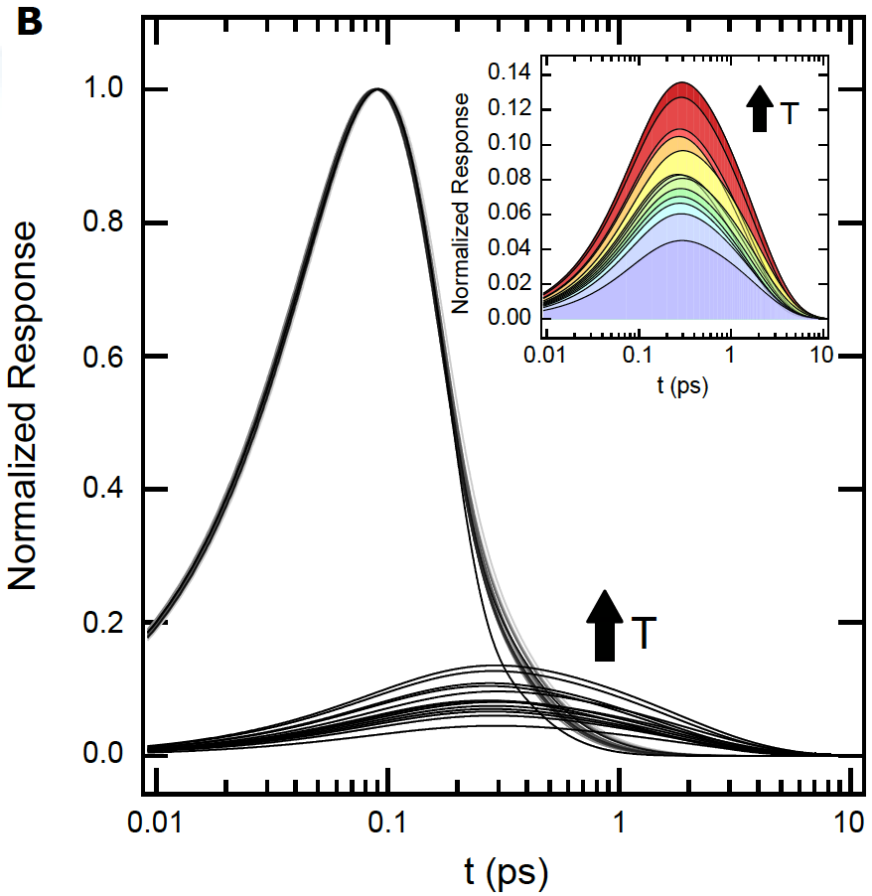
$$P^{(3)} \propto (\chi^{(3)}_{1122} + \chi^{(3)}_{1212})|E_p|^2 E_S$$

45° 0°

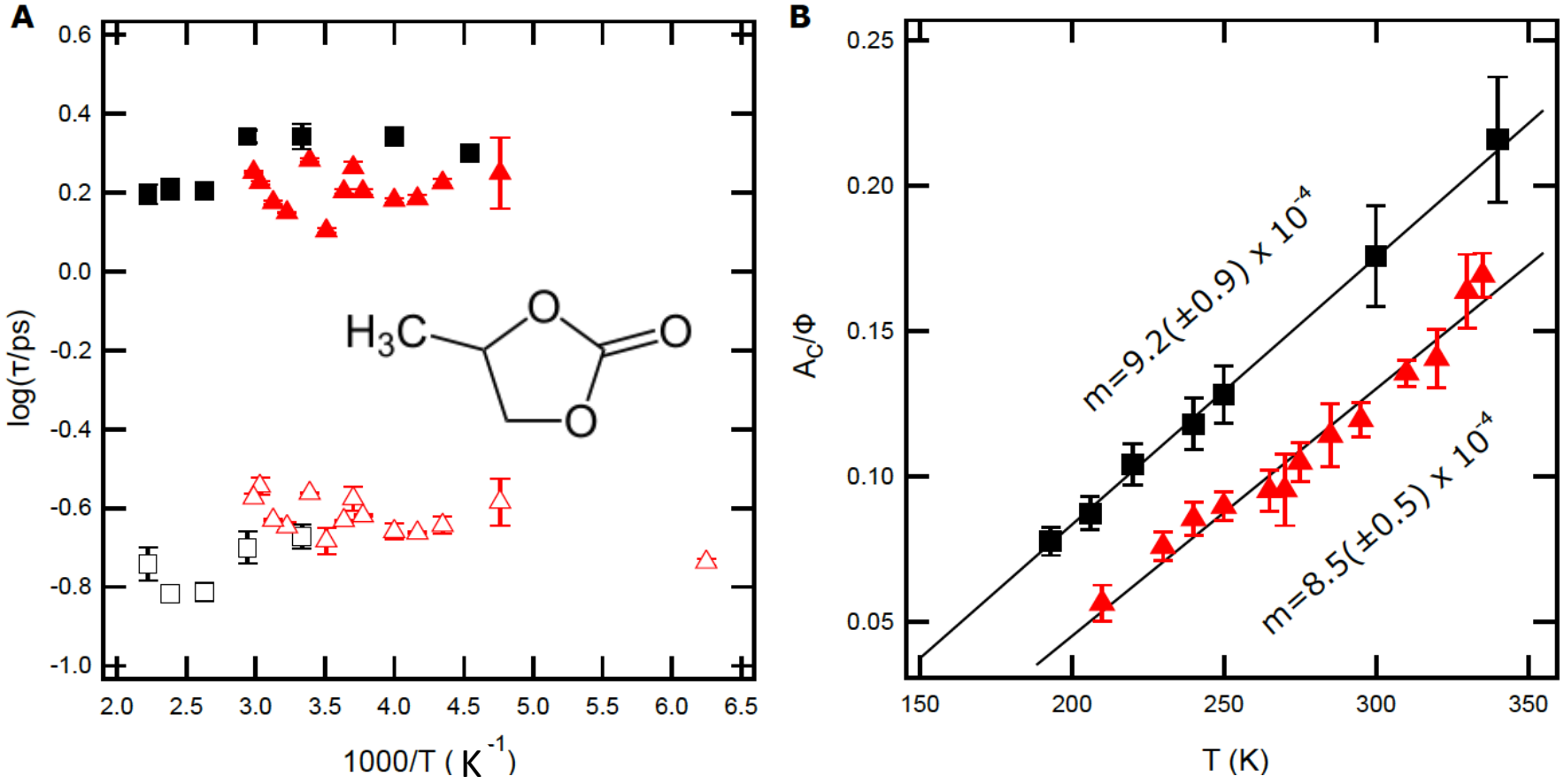
Optical Kerr Effect



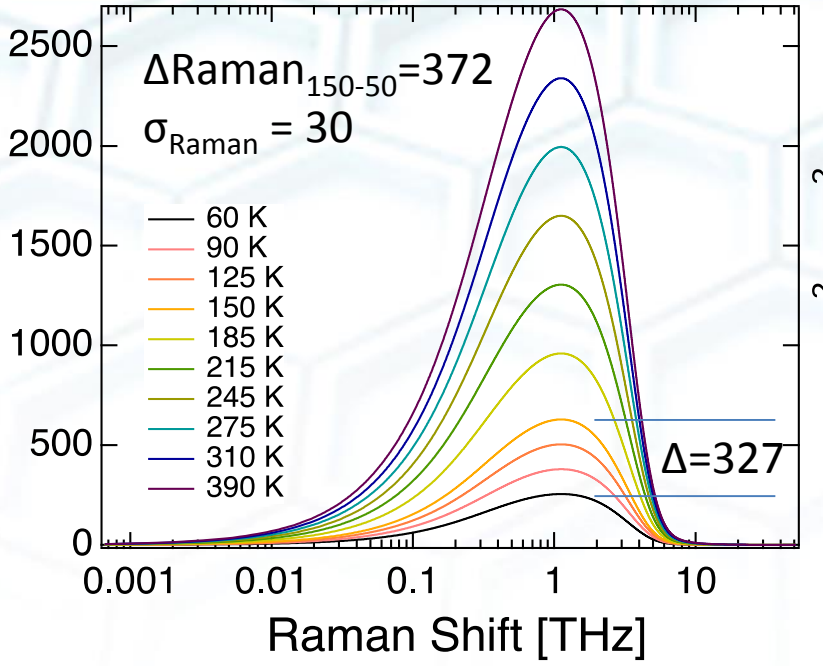


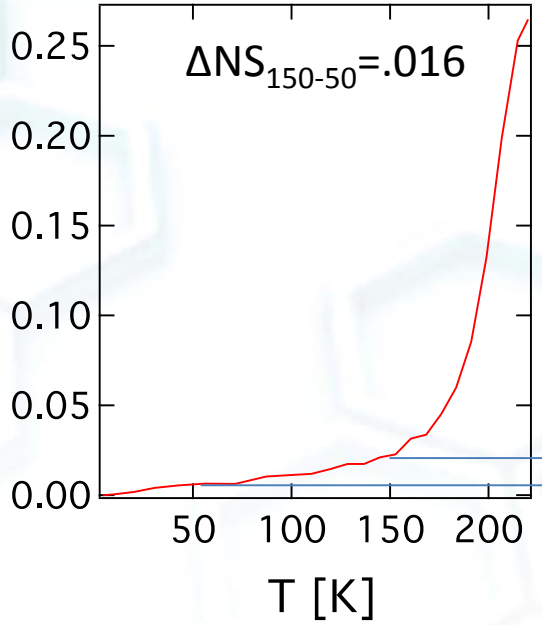


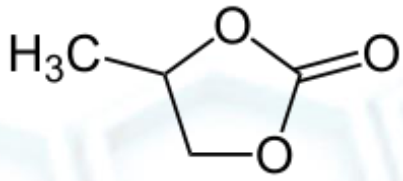
Timescales and Amplitudes: OKE and Neutron Scattering



- Timescales for IC and MB barrier crossing motion from NS correspond to librational and intermediate relaxation in OKE
- Temperature dependence of Φ (fraction of molecules instantaneously undergoing MB barrier crossing events) same as that of strength of intermediate relaxation

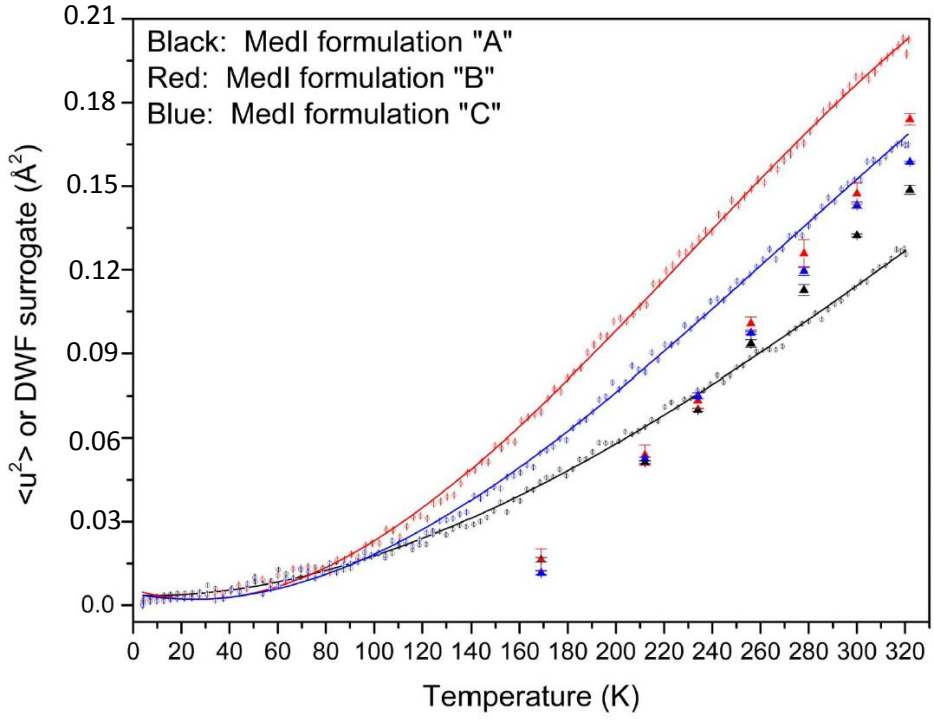


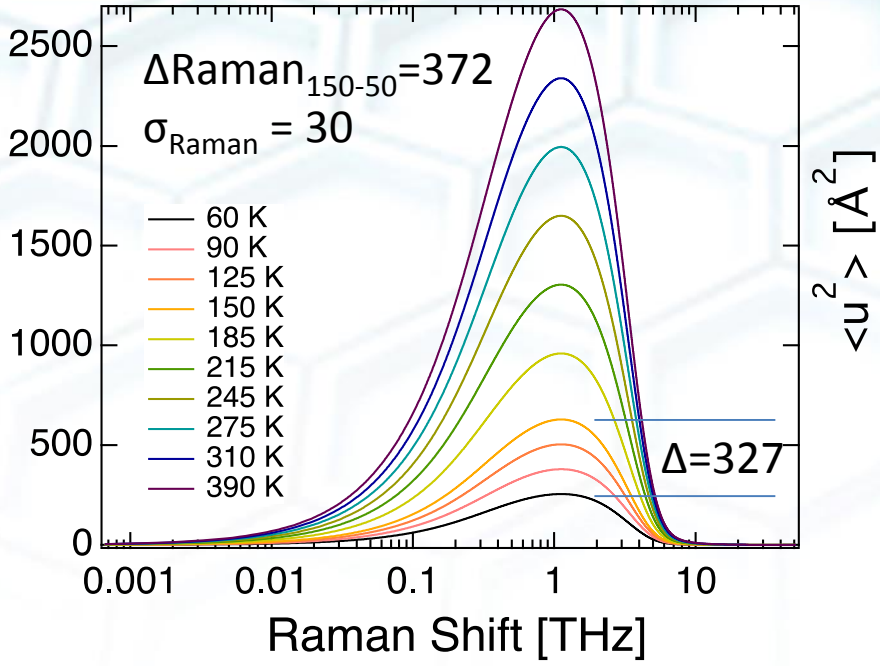


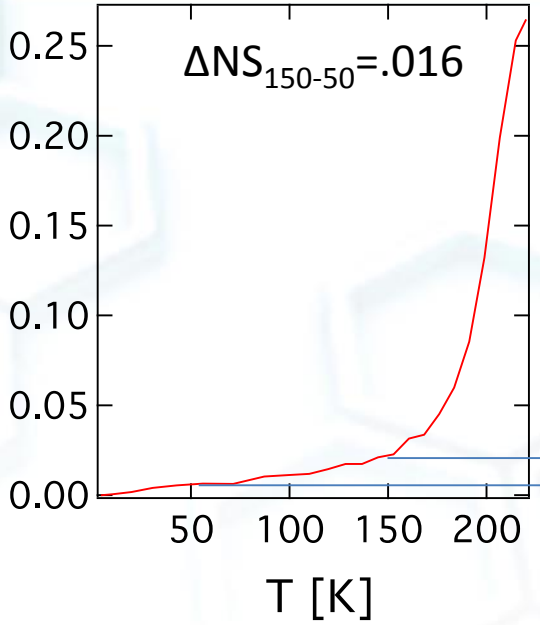


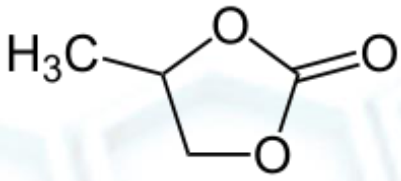
For 20 minute acquisition:
σ_{Raman} corresponds to σ_{NS}=.002

Low Frequency Raman should have sufficient sensitivity to distinguish even slightly different formulations.





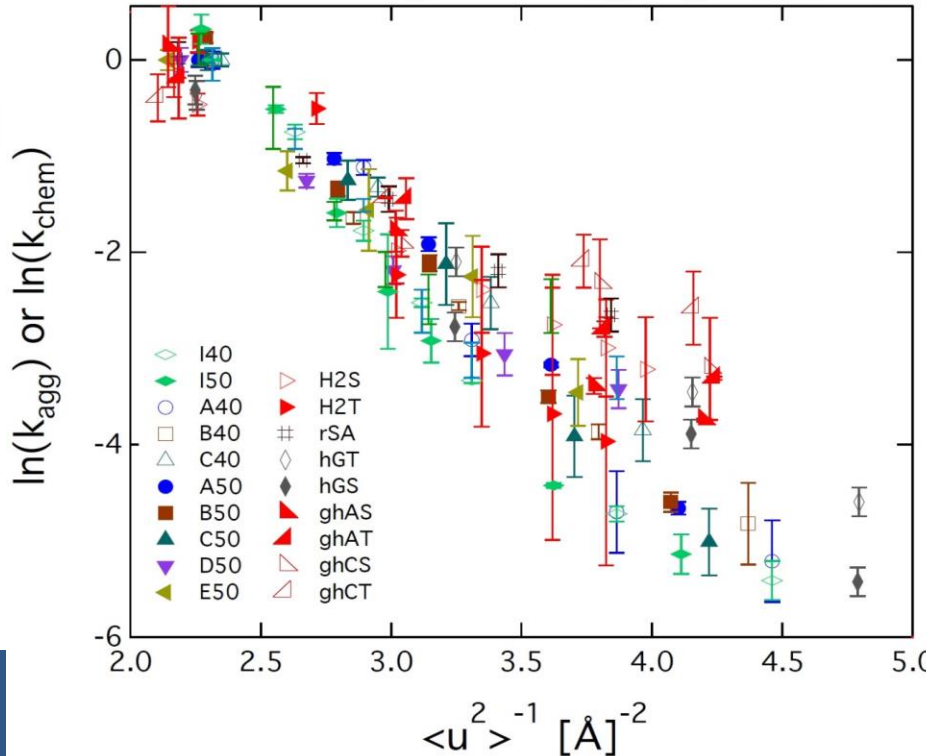




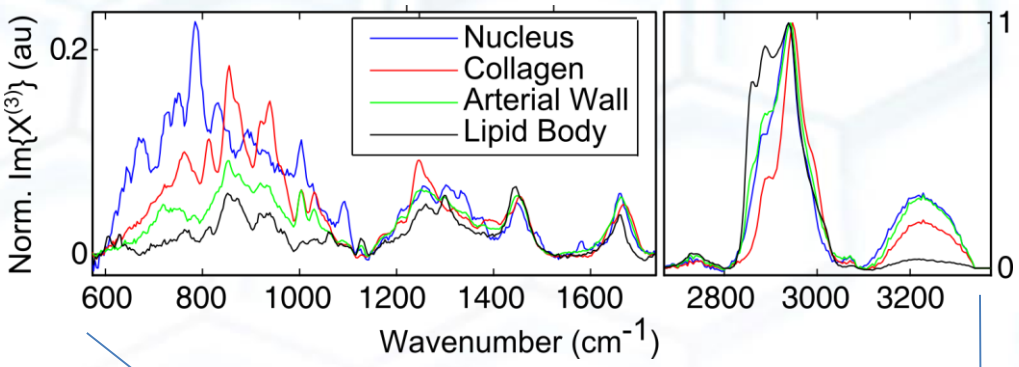
For 20 minute acquisition:

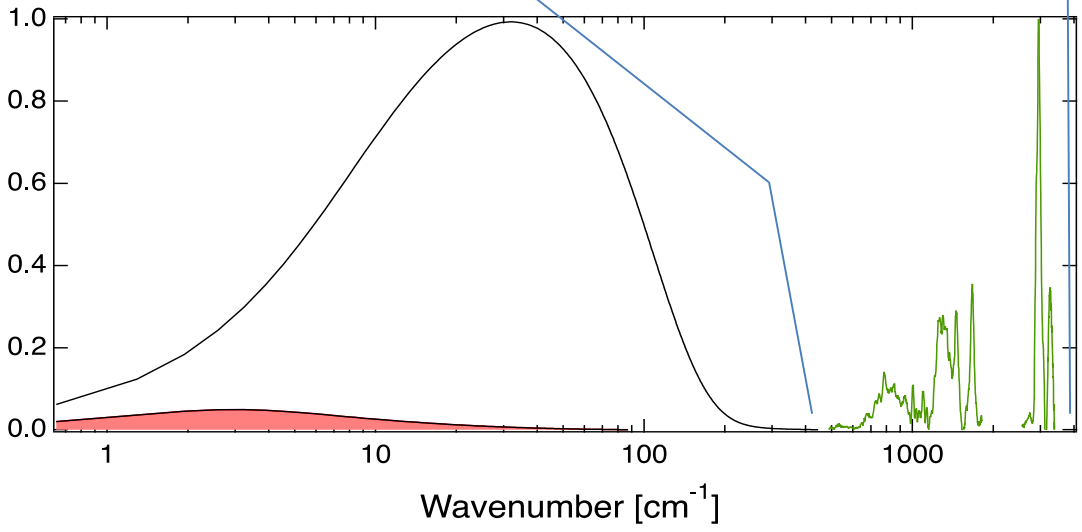
σ_{Raman} corresponds to σ_{NS}=.002

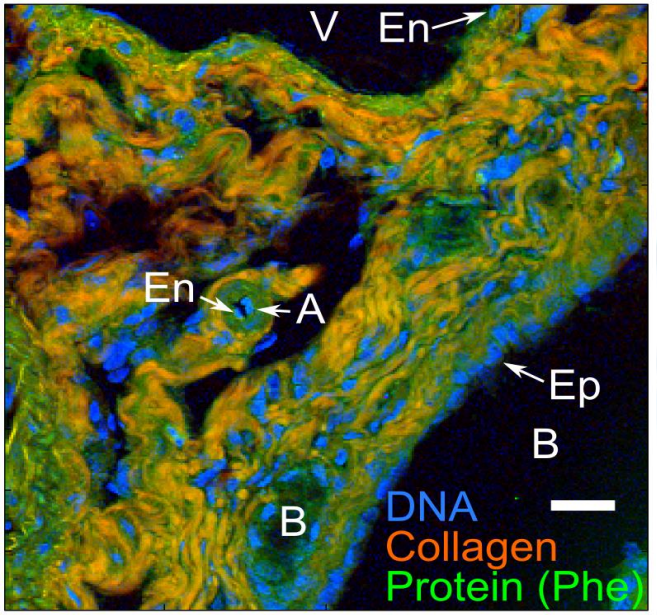
σ_{Raman} corresponds to $\sigma_{log(k)}$=.02, or +/- 10%



High-Resolution Functional Microscopy



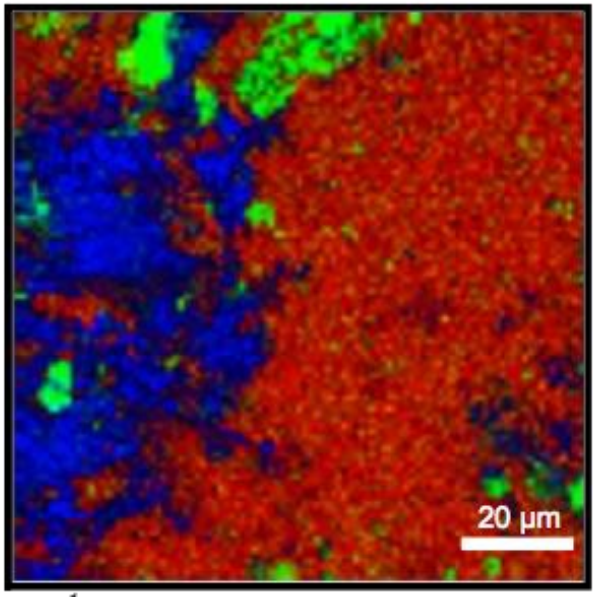




Camp Jr, *et al.*, Nat Photon **8** (2014)

V: Vein; **B**: Bile Duct; **A**: Artery; **L**: Lipid Droplet; **En**: Endothelial Cell; **Ep**: Epithelial Cell

25:75 γ/α-IMC



Hartshorn et al., Anal. Chem. (2013)

FDA Critical Path Document (2004)

The product development problems we are seeing today can be addressed, in part, through …collaborative efforts to create a new generation of performance standards and predictive tools.

The new tools will … build on knowledge delivered by recent advances in science, such as … imaging technologies and materials science.