Microrheological characterization of covalent adaptable hydrogel degradation in response to change in pH that mimics the gastrointestinal tract

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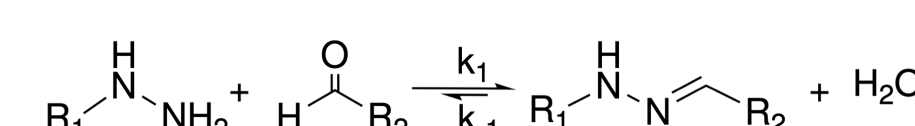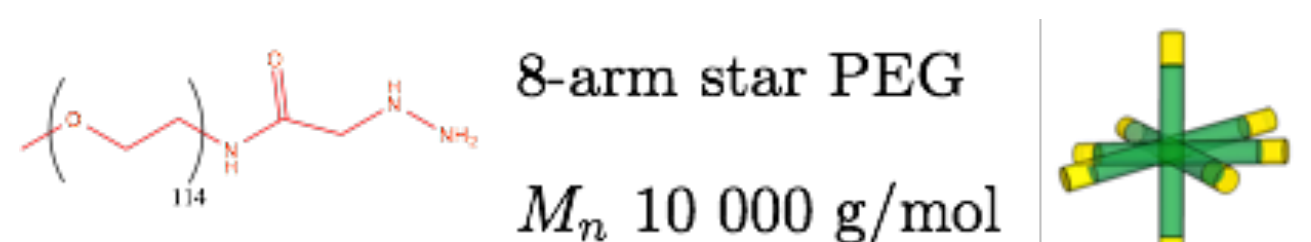
Abstract

Covalent adaptable hydrogels (CAHs) dynamically change their structure in response to external stimuli. These dynamic covalent bonds reversibly break and reform when they are pushed out of equilibrium. This dynamically evolving structure makes CAHs promising in biological applications, such as oral delivery of therapeutics and nutrients. To study the potential of using CAHs as a platform for oral delivery, we characterize scaffold degradation in response to changes in environmental pH which mimic the gastrointestinal (GI) tract. The CAH we are characterizing consists of 8-arm poly(ethylene glycol) (PEG)-hydrazine that covalently cross-links with 8-arm star PEG-aldehyde. μ^2rheology, multiple particle tracking microrheology (MPT) in a microfluidic device, is used to mimic pH-dependent degradation in the GI tract. In MPT, fluorescent probes particles are embedded into the sample and Brownian motion is measured. Our microfluidic device enables the fluid environment to be changed around a single sample with minimal sample loss. μ^2rheology is used to characterize degradation at a single pH (pH 4.3, 5.5 and 7.4), consecutive degradation with a single pH exchange (pH 4.3 to 7.4 and pH 7.4 to 4.3) and temporal pH changes that mimic the pH in the entire GI tract. We quantitatively determine the gel-sol transition during degradation by calculating the critical relaxation exponent, which is independent of degradation pH. In addition, we determine that degradation kinetics and material property evolution are not influenced by degradation history. However, the initial cross-link density of the scaffold at each pH exchange can be decreased by degradation history which reduces the time to the critical gel-sol transition. This result indicates degradation can be tuned by changing scaffold cross-link density which can be done by changing polymer concentration or the ratio of functional groups. This work will inform design of this scaffold for site-specific oral drug delivery.
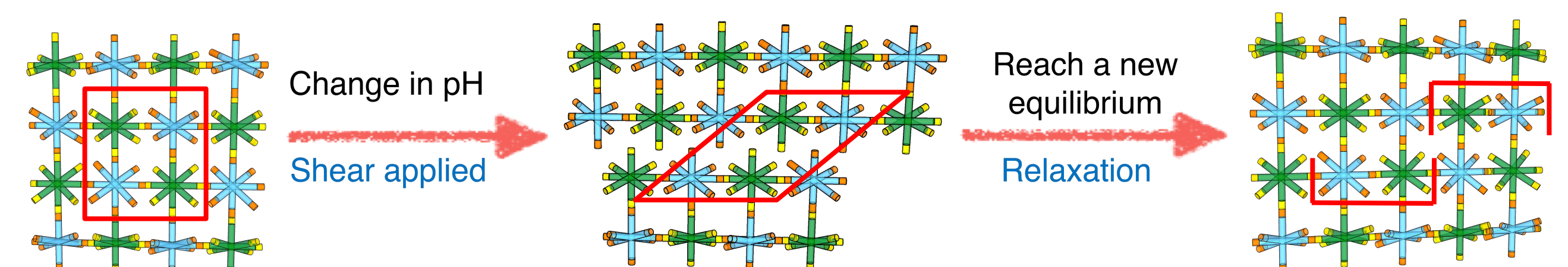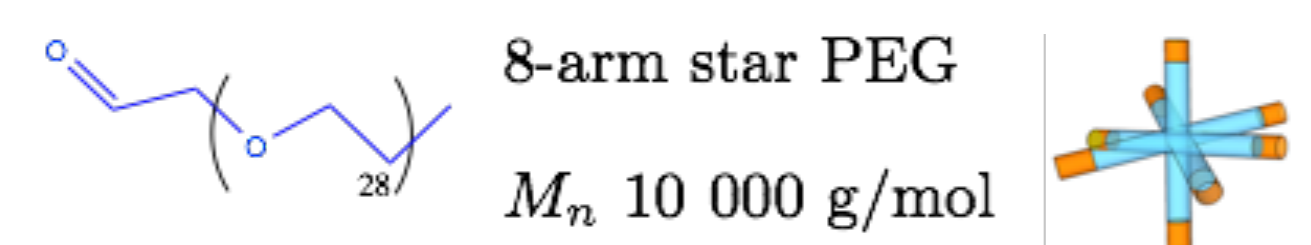
Poly(ethylene glycol) hydrazone hydrogel

Poly(ethylene glycol) (PEG)-hydrazine and PEG-aldehyde chemically cross-link into a covalent adaptable hydrazone-linked hydrogel

$$R_1 \overset{H}{\underset{}{N}}-NH_2^+ + R_2 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} R_1 \overset{H}{\underset{}{N}}-N \underset{}{=} R_2 + H_2O$$

Poly(ethylene glycol) hydrazine

8-arm star PEG
M_n 10 000 g/mol

Poly(ethylene glycol) aldehyde

8-arm star PEG
M_n 10 000 g/mol

Change in pH / Shear applied → Reach a new equilibrium / Relaxation

McKinnon et al., *Chem. Mater.*, 2014, 26, 2382
McKinnon et al., *Soft Matter*, 2014, 10, 9230
McKinnon et al., *Adv. Mater.*, 2014, 26, 865

Escobar, Anseth and Schultz, *Macromolecules*, 2017, 50, 7351
Wu and Schultz, *Soft Matter*, 2019, 15, 5921

Multiple particle tracking microrheology (MPT)

MPT characterizes the dynamic rheology by measuring the Brownian motion of embedded fluorescent particles
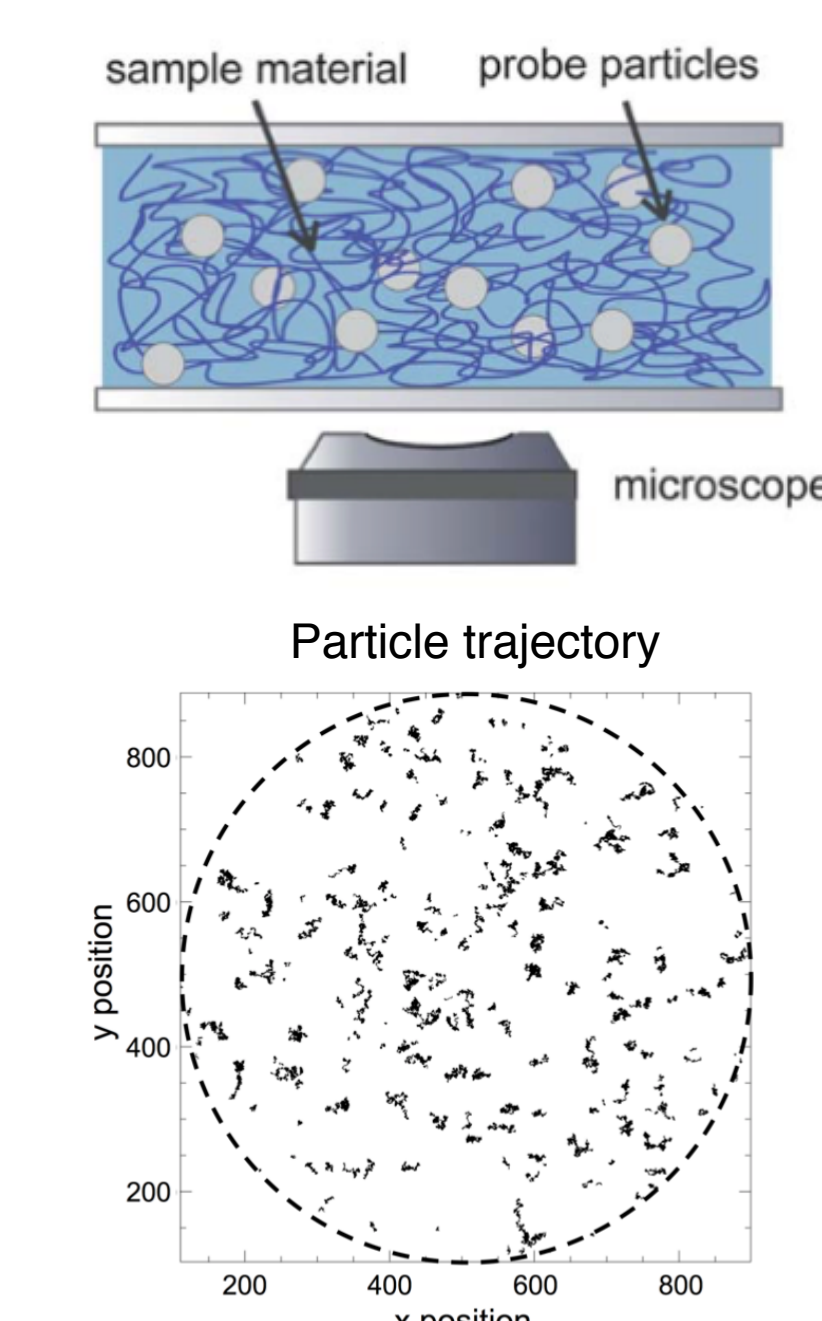
Mean squared displacement (MSD)
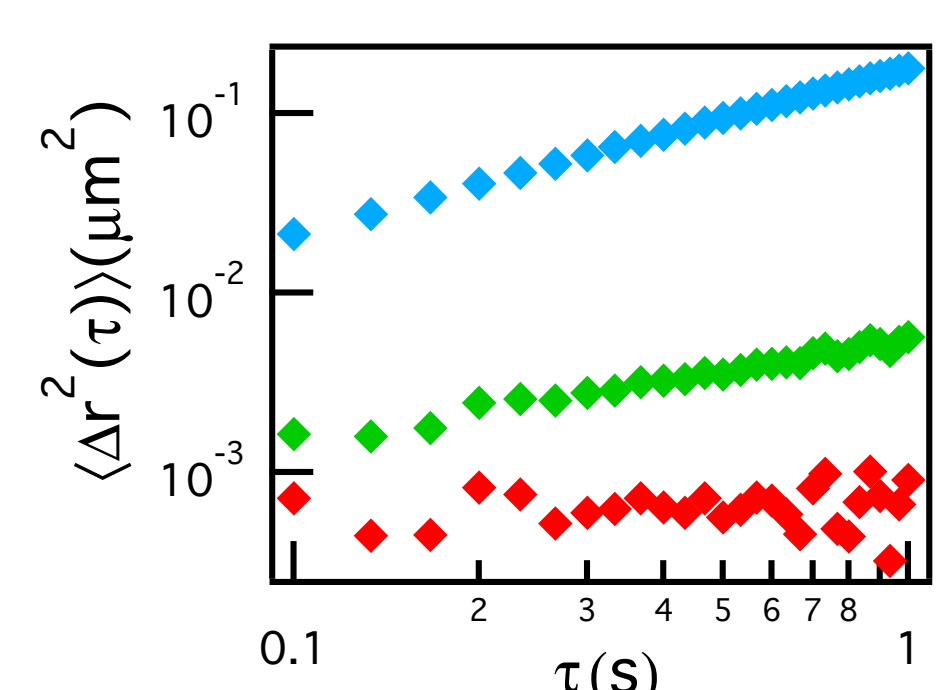
Generalized Stokes-Einstein Relation

$$\langle \Delta r^2(t) \rangle = \frac{k_B T}{\pi a} J(t)$$

Einstein Relation
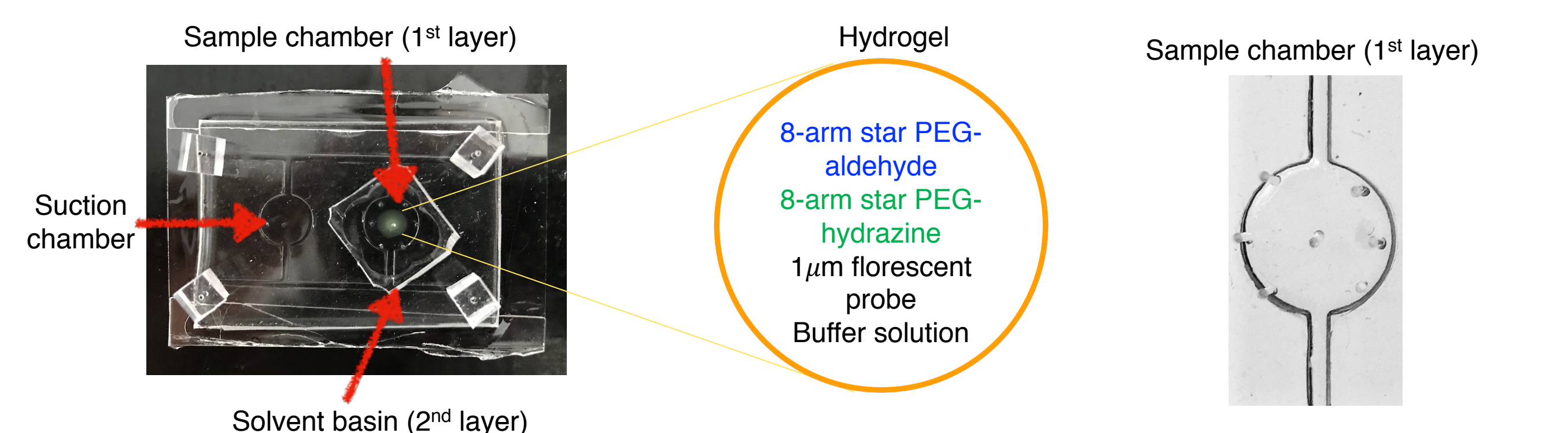
$$\langle \Delta r^2(t) \rangle = 2dD\tau^\alpha$$

The logarithmic slope of the mean-squared displacement

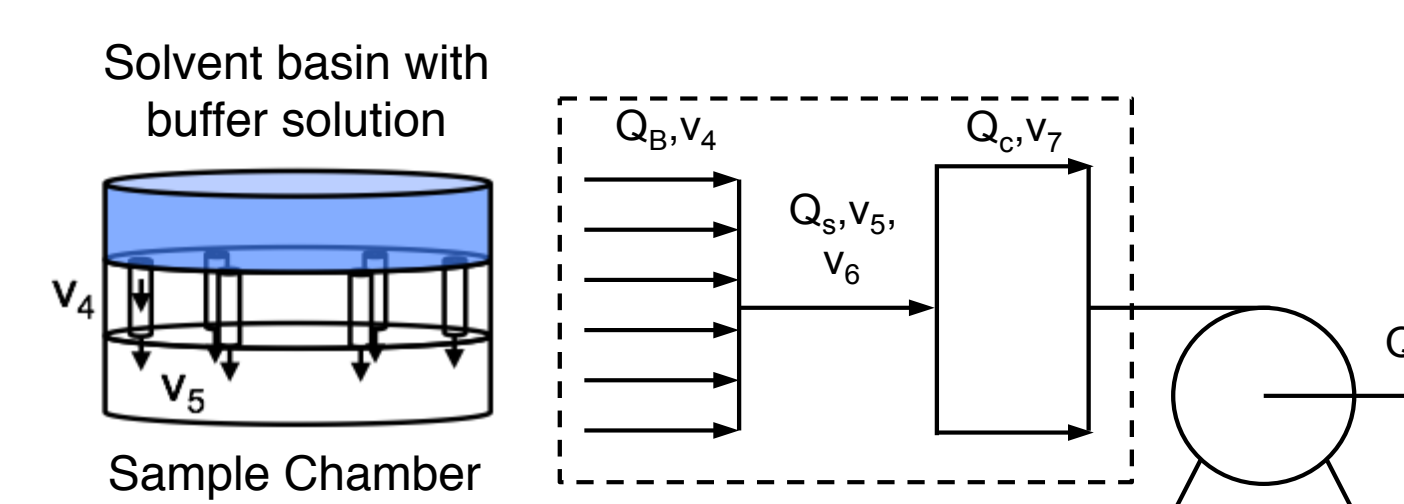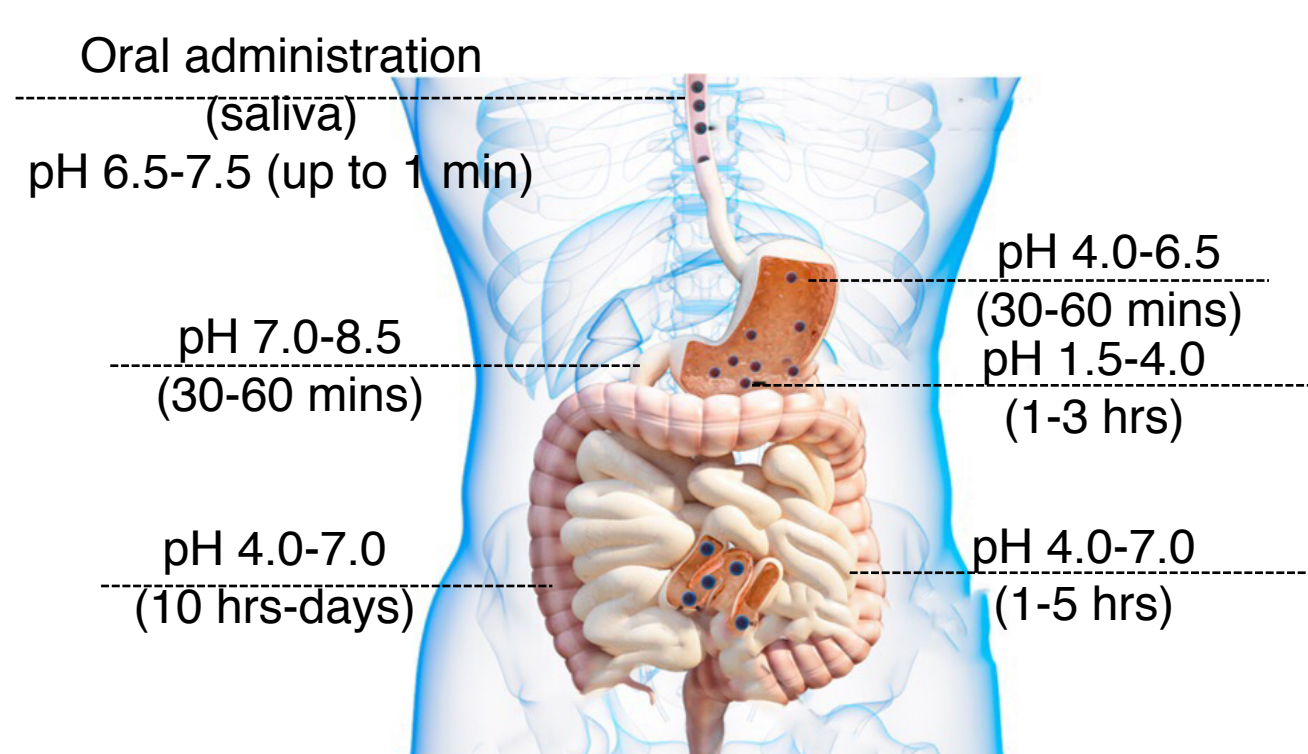$$\alpha = \frac{d \log \langle \Delta r^2(\tau) \rangle}{d \log \tau}$$

Liquid   Viscoelastic sol or gel   Gel

Crocker and Grier, *J. Coll. Inter. Sci.*, 1996,179, 298
Savin and Doyle, *Biophys. J.*, 2005, 88, 623
Squires and Mason, *Annu. Rev. Fluid Mech.*, 2010, 42, 413
Schultz and Furst, *Soft Matter*, 2012, 8, 6198

MacKintosh and Schmidt, *Curr. Opin. Colloid Interface Sci.*, 1999, 4, 300
Furst and Squires. *Microrheology*, 2017
Schultz, Ph.D. thesis, University of Delaware, 2011

Experimental design and apparatus

A previously designed two-layer microfluidic device enables changes in the fluid microenvironment around the sample with minimal sample loss to mimic pH changes in the digestive tract

Sample chamber (1st layer)
Suction chamber
Solvent basin (2nd layer)

Hydrogel
8-arm star PEG-aldehyde
8-arm star PEG-hydrazine
1 μm florescent probe
Buffer solution

Sample chamber (1st layer)

The average time and pH spends in each part of the digestive tract (GI tract)

Oral administration (saliva)
pH 6.5-7.5 (up to 1 min)

pH 7.0-8.5 (30-60 mins)
pH 4.0-6.5 (30-60 mins)
pH 1.5-4.0 (1-3 hrs)

pH 4.0-7.0 (10 hrs-days)
pH 4.0-7.0 (1-5 hrs)

Solvent basin with buffer solution

Sample Chamber (1st layer)

μ^2rheology experiment

EXP	pH formed	pH degraded	pH degraded	pH degraded	pH degraded
Single pH	4.3	7.4			
	7.4	4.3			
	7.4	5.5			
Single pH exchange	4.3	7.4	4.3		
	7.4	4.3	7.4		
Whole tract	7.4 (1 min)	5.5 (1 hr)	4.3 (3 hr)	7.4 (hr to day)	
	7.4 (1 min)	5.5 (1 hr)	4.3 (3 hr)	7.4 (1 hr)	4.3 (hr to day)

Wehrman et al., *Lab Chip*, 2017,17, 2085
Wehrman et al., *J Vis Exp*, 2018,134, e57429

McKinnon et al., *Soft Matter*, 2014, 10, 9230
McKinnon et al., *Adv. Mater.*, 2014, 26, 865

Escobar, Anseth and Schultz, *Macromolecules*, 2017, 50, 7351
Wu and Schultz, *Soft matter*, 2019, 15, 5921

Time-cure superposition

Time-cure superposition is the superposition of viscoelastic functions at different extents of degradation

$$a \sim \tau_L^{-1} \sim \left| \frac{P - P_C}{P_C} \right|^y \qquad b \sim \frac{1}{J_e^0} \sim \left(\frac{p - p_C}{p_C} \right)^z \qquad n = \frac{z}{y}$$

$n_{gel} = 0.79 \pm 0.13$
$n_{sol} = 0.73 \pm 0.04$
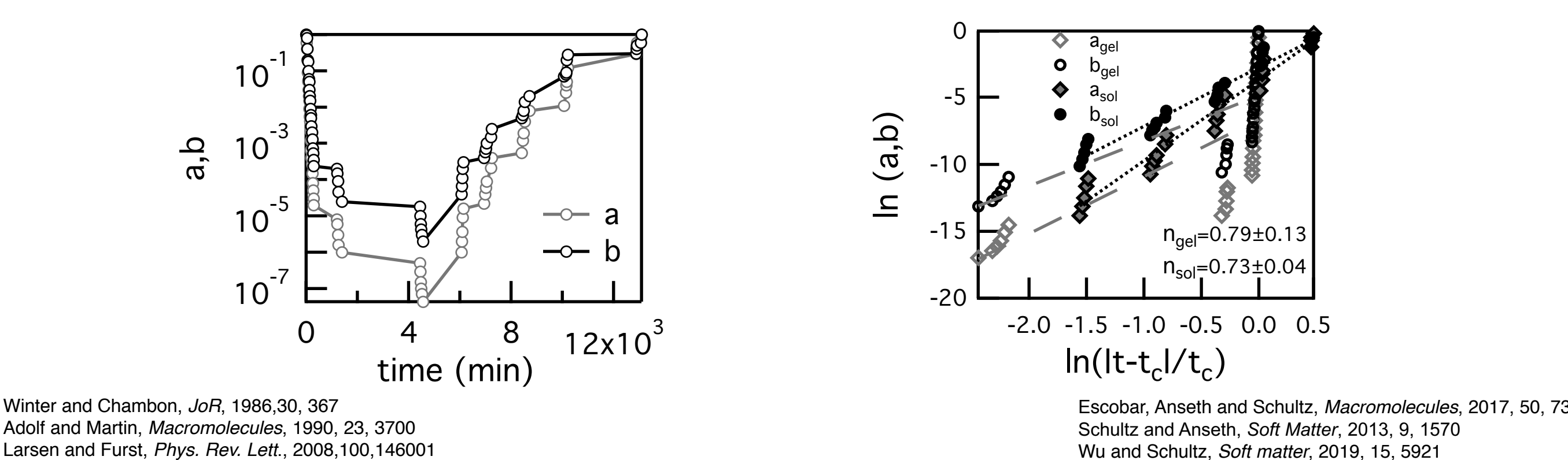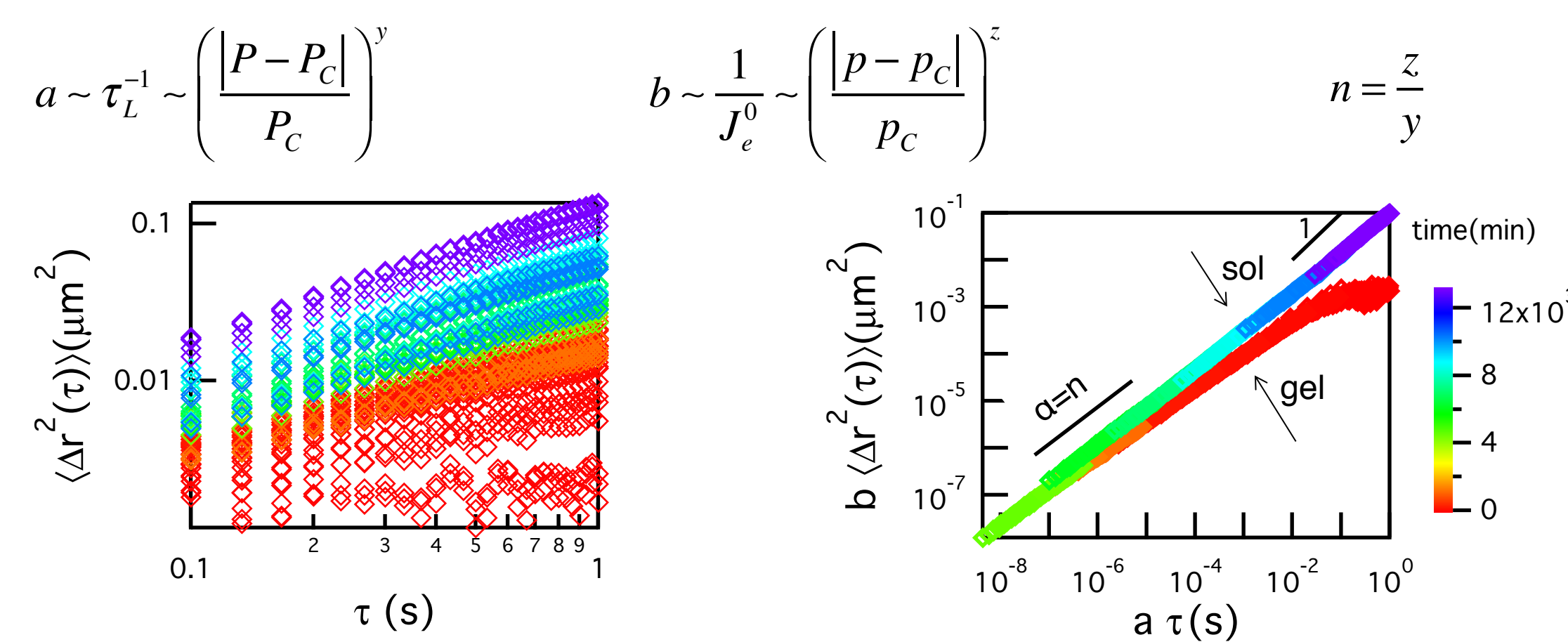
Winter and Chambon, *JoR*, 1986,30, 367
Adolf and Martin, *Macromolecules*, 1990, 23, 3700
Larsen and Furst, *Phys. Rev. Lett.*, 2008,100,146001

Escobar, Anseth and Schultz, *Macromolecules*, 2017, 50, 7351
Schultz and Anseth, *Soft Matter*, 2013, 9, 1570
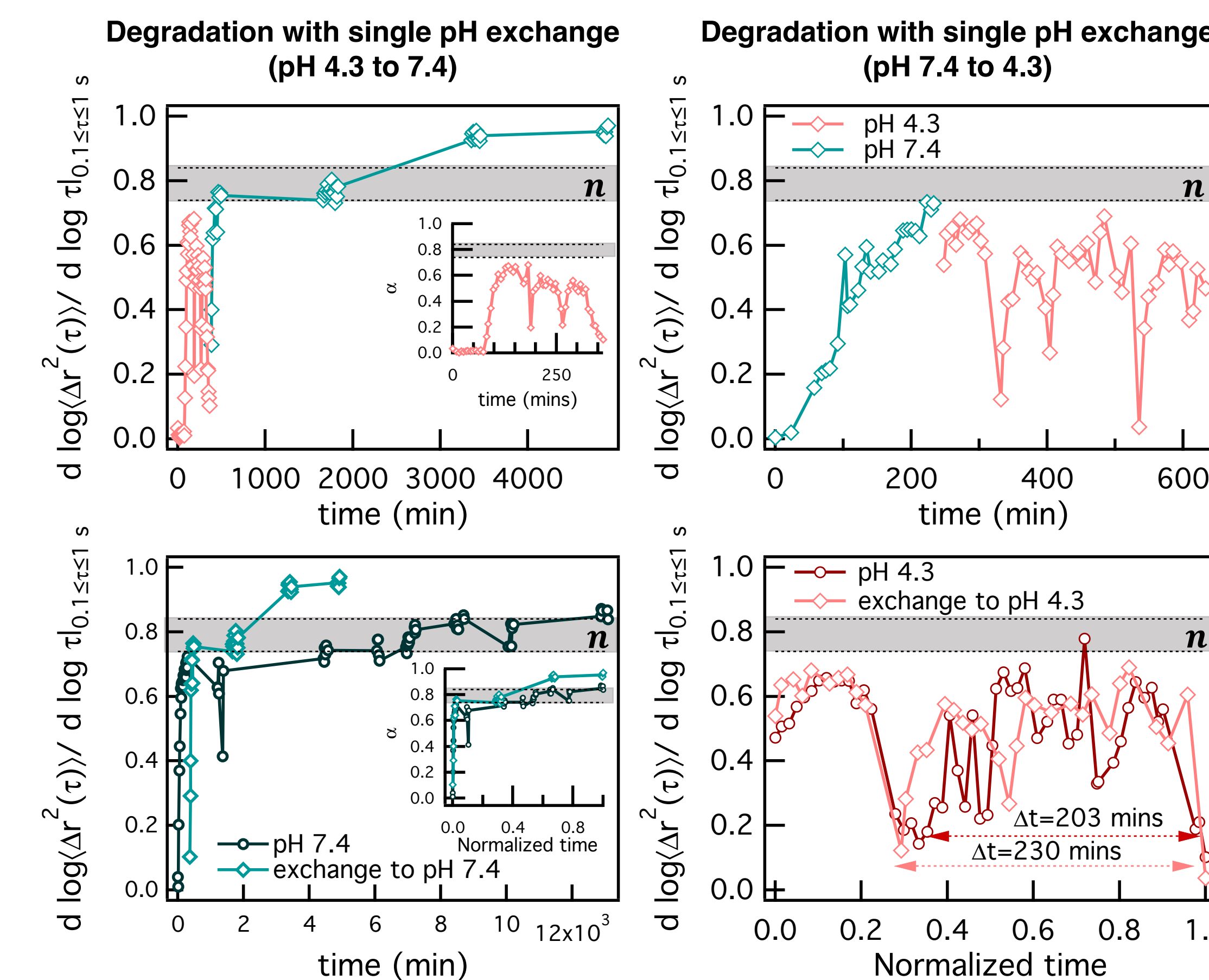Wu and Schultz, *Soft matter*, 2019, 15, 5921

Degradation at a single pH

Scaffold degraded at a single pH: pH 4.3, 5.5 and 7.4
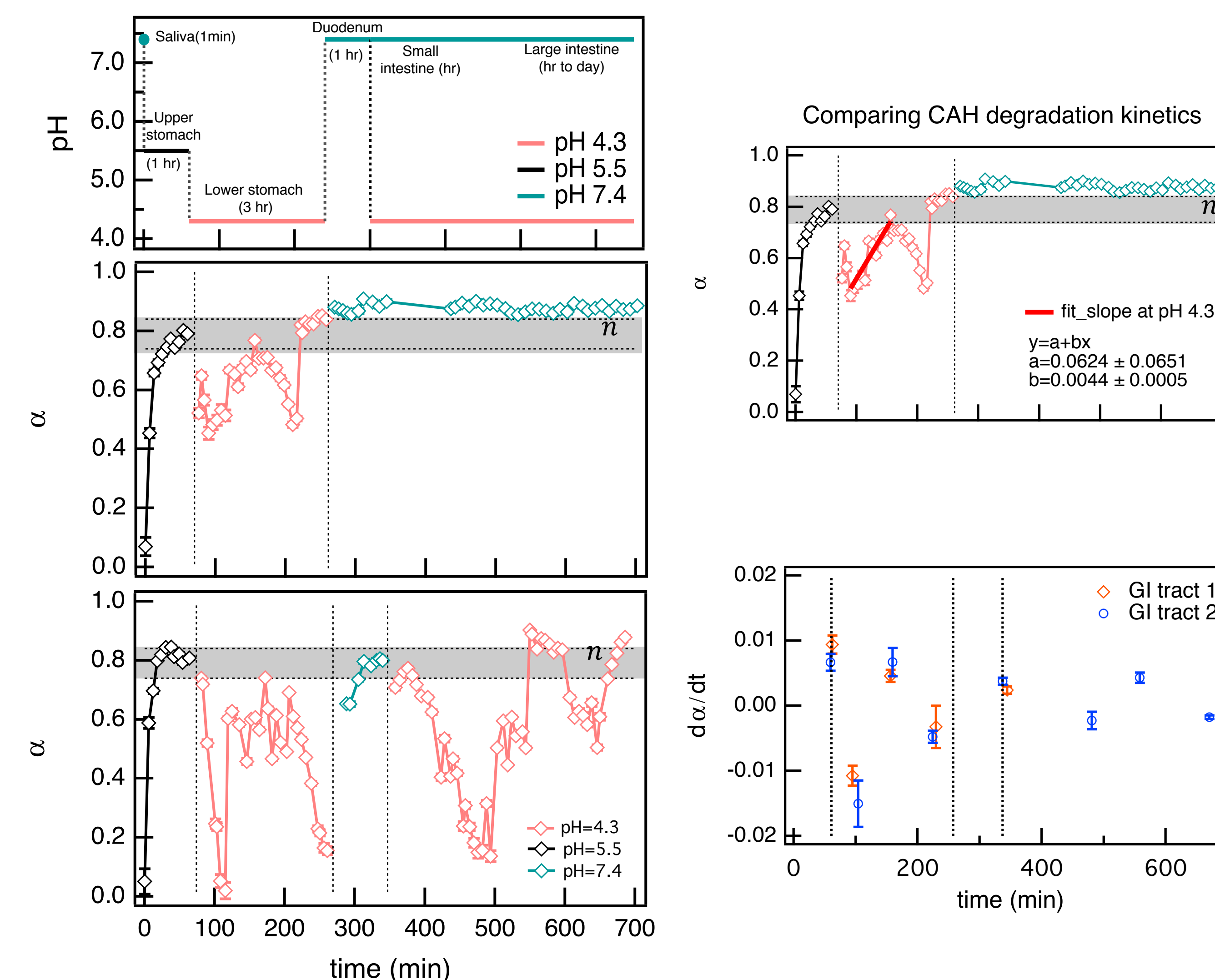
$n_{avg} = 0.79 \pm 0.06$
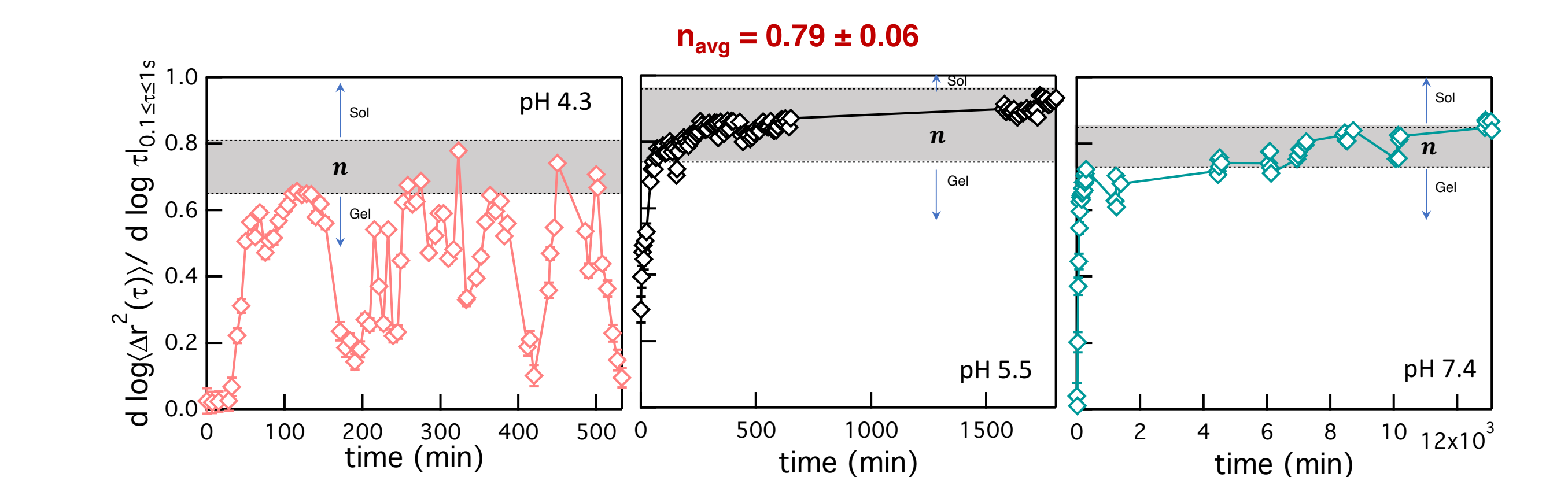
Consecutive degradation with pH exchange

The scaffold is consecutively degraded at different pHs that mimics pH-dependent degradation in the GI tract

Degradation with single pH exchange (pH 4.3 to 7.4)

Degradation with single pH exchange (pH 7.4 to 4.3)

pH 4.3
pH 7.4

exchange to pH 7.4

exchange to pH 4.3

$\Delta t = 203$ mins
$\Delta t = 230$ mins

Degradation induced by temporal pH changes through the entire GI tract

Saliva(1min)   Duodenum (1 hr)   Small intestine (hr)   Large intestine (hr to day)
Upper stomach (1 hr)
Lower stomach (3 hr)

pH 4.3
pH 5.5
pH 7.4

Comparing CAH degradation kinetics

fit_slope at pH 4.3
y=a+bx
a=0.0624 ± 0.0651
b=0.0044 ± 0.0005

GI tract 1
GI tract 2

Conclusions

- The gel-sol transition during degradation is quantitatively determined by the critical relaxation exponent, which is independent of degradation pH
- Degradation kinetics and material property evolution are not influenced by degradation history
- For scaffold degradation at two different pHs, the time scale of pH 7.4 degradation is reduced when the material is first degraded at pH 4.3, but there is no obvious time scale change when the material is first degraded at pH 7.4 then at pH 4.3
- The initial cross-link density of the scaffold at each pH exchange can be decreased by degradation history which reduces the time to the critical gel-sol transition
- μ^2rheology successfully mimics pH-dependent degradation in the digestive tract that enables consecutive degradation at native pH environments and future work will tether functional molecules in this CAH scaffold and investigate molecular release based on pH-induced degradation