Tuning conductivity whilst maintaining mechanical properties in perylene bisimide hydrogels at physiological pH

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SUPPORTING INFORMATION

1. Experimental Procedures

All chemicals were purchased from Merck and Alfa Aesar. All chemicals were used as received unless otherwise stated. Distilled water was used throughout.

PBI-H and PBI-L synthesis procedures

Both **PBI-L** and **PBI-H** were synthesised by Dr. Draper and have been reported previously.[1, 2]

Preparation of PBI solutions

Solutions of both **PBI-L** and **PBI-H** were prepared at different concentrations from 1 to 10 mg/mL of gelator. For comparative analysis the gels and solutions were prepared at 5 mg/mL of gelator unless otherwise stated.

To prepare a 10 mL solution of **PBI-L** or **PBI-H** at 5 mg/mL, 50 mg of the desired PBI is weighed into a vial. 1 molar equivalent of 0.1 M NaOH is then added and then topped up to 10 mL with distilled water. A background electrolyte of 0.1 M NaCl was then added to the solutions (this is for the electrochemical studies, but was in all solutions to ensure there was no effect on UV-vis, rheology etc). The resulting solution is then stirred with a magnetic stirrer overnight, or until the all the solid has visibly dissolved. The solutions were then adjusted to pH 7.4 using 1 µL at a time of 1 M HCl or 1 NaOH. Between each drop the solution was stirred for 2 minutes to allow for equilibration of the pH, until the desired pH is achieved.

Preparation of PBI metal salt gels

Gels using $MgCl_2$, $CaCl_2$ and $Ca(NO_3)_2$ were are prepared in the same way, but using a different container, depending on which method of analysis was being carried out. The metals salts were dissolved in distilled water at a concentration of 200 mg/mL. 50 µL of metal salt was then added per mL of gelator solution, so a 2 mL gel would contain 100 µL of metal salt. In each case after the metal salt had been added to the gelator solution it was left sealed 16 hours (overnight) at room temperature (20-25°C) to completely gel before being analysed. This time allows for all gels to be uniformly gelled through the dispersion of the salt throughout the system. From previous studies we know that gelation is complete after this time, and so all gels will be compared with other end-point gels. Any sample that visually did not look homogeneously gelled was not measured as it was assumed that the whole sample had not gelled and would not be an accurate comparison or measurement.

For rheology
2 mL of gels were prepared in 7 mL Sterilin vials. 2 mL of gelator solution was pipetted into the vials. Then 50 µL of metal salt was pipetted onto the top of the solution. Initially a dark spot of gelled material could be seen in the solution, which over time diffuses uniformly to gel the whole contents of the vial. For the aging experiments, gels were prepared in a repeat of 6 in Sterilin vials and left in the lab at room temperature for three weeks without being disturbed.

For UV-vis absorption

This was carried out using a 0.1 mm quartz demountable cuvette, or 1 mm cuvette as stated. The appropriate amount metal salt was pipetted dropwise to cover the surface of the cuvette and then the gelator added on top of this before the top of the cuvette was placed on top (the absolute amounts is determined by the volume of the cuvette, ensuring 0.05:1 mL of salt:gelator).

For SANS

40 µL of metal salt was first added to a 2 mm pathlength quartz cuvette, then 0.8 mL of gelator pipetted on top of the metal salt solution. Again, initially a dark spot at the bottom of the cuvette could be seen which diffuse and gel the whole cuvette.

For Cyclic Voltammetry and EIS measurements

CV was carried out using a custom-made cell, this is to increase the surface area of the working electrode and in order not to disturb the gels. Using this set up we also ensure we were measuring the gel rather than the solution surrounding the gel. The cell was made from two pieces of FTO TEC7 coated glass, with copper tape and a 4 cm diameter o-ring as a 1 mm spacer (see Figure S1 below). This set up uses both FTO as the working and counter as a two-electrode setup. There was no reference electrode (it is plugged into the counter electrode), due to this set up, instead we used the set up to run a ferrocene standard to compare the data to, and ensure the cell was working properly. The background electrolyte was already in the prepared solutions.

To prepare the gels in this set up, the o-ring was placed on the conductive side of one of the pieces of glass. The perylene solution (1 mL) was then pipetted into the o-ring ensuring it was full. Next the metal salt solution was then then added dropwise in regular interval over in the solution, to try and ensure an even diffusion. The second piece of FTO was then placed on top with the conductive side down, ensuring that the solution was in contact with both pieces of glass. The absolute volume was tested with water to ensure the right amount was added. The cell was then sealed with two bulldog clips on the edge of the glass not covered by copper tape and was allowed to gel overnight (16 hours). Uniformity of gelation could be seen by eye, and only uniform gels were measured.
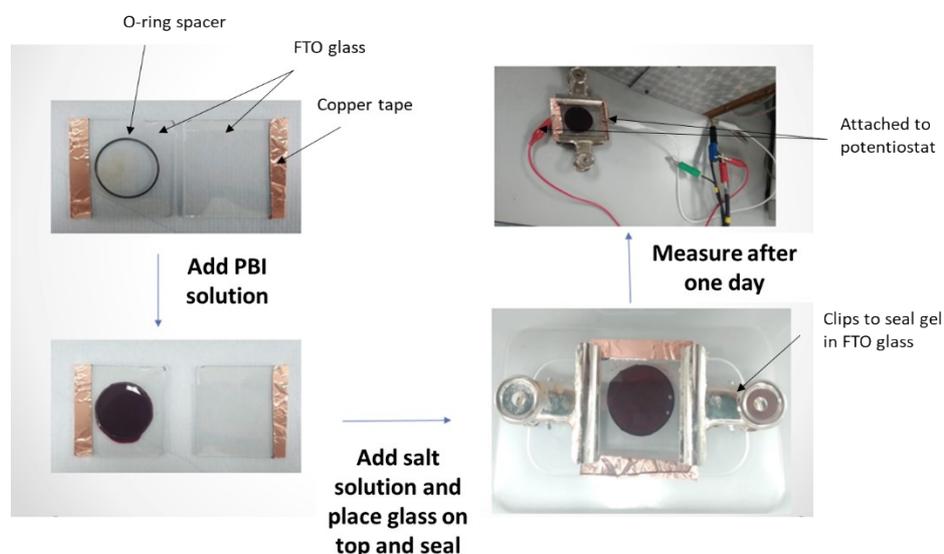
Figure S1. Diagram of the eChem set up for measuring CV of the gels.

Pre-cell culture

PBI-L and PBI-H solutions were prepared in H_2O with a pH of 7.40 at a concentration of 5.0 mg/mL. PBI Solutions were heat sterilized at 121°C. Gels were made using solutions of $CaCl_2$, $MgCl_2$, and $Ca(NO_3)_2$ at concentration of 200 mg/mL and were filter sterilized prior to use.

Cell Culture

C2C12 cells were grown and maintained in a 75 cm^2 cell-culture flask at 37°C, humidified atmosphere containing 5% CO_2 and in Dulbecco's Modified Eagle's Medium DMEM (21969, Gibco) containing 10% Foetal Bovine serum FBS (F9665, Sigma), and 2% Antibiotics containing Penicillin-Streptomycin (P0781, Sigma), Amphotericin B (15290026, Invitrogen), and L-Glutamine (G7513, Sigma).

Cell viability

For cytotoxicity assays, C2C12 cells were seeded at a density of 40,000 cell/mL in 96/48-well plate and allowed to attach for 24 hours before the addition of PBIs in solution and gel form. After a total incubation period of 20 hours, PBIs were removed, and cultures were rinsed thrice with 1x Dulbecco's phosphate buffered saline DPBS (2662059, Gibco).

For MTT assay, cytotoxicity was assessed by adding 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide MTT (M5655, Sigma-Aldrich) at a final concentration of 0.5 mg/mL. After 3.5 hours of incubation with MTT reagent, Dimethyl sulfoxide DMSO (67-68-5, Fisher scientific) was added to solubilize the precipitated formazan crystals. Absorbance was then measured at 570 nm using a microplate reader and quantification of cell viability was determined.

For Live/Dead assay, Cytotoxicity was assessed by using Live/Dead^{TM} Viability/Cytotoxicity kit (L3224, ThermoFisher) with Calcein AM and Ethidium homodimer-1 staining live and dead cells respectively. After washing with DPBS, a staining solution composed of 2 µM Calcein and 4 µM Ethidium homodimer-1 was prepared followed by an incubation with cells for 30 minutes at the dark at room temperature. Afterwards, cells were rinsed once with DPBS to remove residual staining solution before visualizing using EVOS M7000 microscope.

To assess statistical significance, all data was analysed using One-way analysis of variance (ANOVA) with Tukey's multiple comparison test. Significance was expressed as $* p < 0.05$, $* * p < 0.01$, $* * * p < 0.001$, and $* * * * p < 0.0001$. All analysis was performed using GraphPad Prism 10.0.2

2. Characterisation and analysis methods

pH Measurements

pH measurements were performed using a FC200 pH probe (HANNA Instruments) with a 6 mm x 10 mm conical tip. The accuracy of the pH measurements is quoted as ± 0.1.

Rheology

Rheological experiments were only carried out on samples that were stable to inversion. This was used as a screening for the minimum amount of gelator or metal salt required for gelation. Samples were then rheological measured in triplicate to confirm gelation. Yield point quoted at the point at which G' and G'' deviate from linearity. G' and G'' were taken from the mid point of the linear viscoelastic region (LVR).

Rheological experiments were performed on an Anton Paar Physica MCR301 or 101 rheometer vane (ST10-4V-8.8/97.5) and cup geometry to minimise loading issues. All measurements were carried out at 25°C.

Strain sweeps were performed first in order the frequency sweeps were carried out within the LVR. They were performed at 10 rad/s between 0.1-1000% stain.

Frequency sweeps were performed at 0.5% strain (again within the LVR determined above) between 1-100 rad/s.

SANS

For the SANS, a neutron beam, with a fixed wavelength of 6 Å and divergence of $\Delta\lambda/\lambda = 9\%$, allowed measurements over a large range in Q [$Q = 4\pi\sin(\theta/2)/\lambda$] of 0.001 to 0.3 Å-1, by using three sample-detector distances of 1.5 m, 8m, and 39 m. The cuvettes were housed in a temperature-controlled sample rack during the measurements. The data were reduced to 1D scattering curves of intensity vs. Q using the facility provided software LAMP. The electronic background was subtracted, the full detector images for all data were normalized and scattering from the empty cell was subtracted. The scattering from D_2O was also measured and subtracted from the data. The data were normalized to absolute units using a 1 mm thick water sample as secondary calibration standard, with a differential scattering cross section of 0.983 1/cm for the experimental settings used. Last, data were radially averaged to produce the 1D curves for each detector position. Experiment numbers 9-11-1964 and 9-12-598 at the ILL, Grenoble.

The instrument independent data were then fitted to the models discussed in the text using the SasView software package version 3.1.2.[3]

PBI-L and **PBI-H** were fitted using SLD values of 3.024x10-6 Å-2 and 3.698x10-6 Å-2 respectively calculated from the NIST website,[4] assuming a density of 1.55 g/cm3.

UV-vis absorption spectroscopy

UV- Vis absorption spectroscopy was carried out using an Agilent Technologies Cary 60 UV-Visible spectrophotometer. Absorption measurements were performed 0.1 mm quartz cuvettes.

CV

Cyclic voltammetry was carried out using a PalmSens4 potentiostat (Alvatek Ltd). Voltammograms were measured using 0.1 V/s scan rate. Measurements were collected using PSTrace software (Version 7.2). The samples were prepared as described above using FTO. A background electrolyte of 0.1 M NaCl was used. Blanks of water, electrolyte and metal salt was collected to ensure the reduction potential

were not from free metal salt in solution. Measurements were collected in triplicate and at different scans rates, the most representative data sets are shown below at the same scan rate for clarity.

Conductivity measurements
Electrochemical impedance spectroscopy (EIS) was employed for the measurements of the ionic conductivities of the prepared gel samples. The gel samples were sandwiched between two FTO glasses where the contact area and thickness of the gel were 1.2 cm^2 and 0.1 cm, respectively. The EIS data was obtained using a Palmsens4 potentiostat within a frequency range of 50 kHz to 1 Hz and a bias of 0.2 V. The ionic conductivities of the gels were calculated using Equation 1.

$$\sigma = \frac{d}{(Rb \times S)}$$

Equation (1)

Where σ, d, Rb and S represent the ionic conductivity, the gel thickness, volume resistance and the contact area respectively. The measurements were done in triplicate and the mean values of R_b obtained from the circuit fitting which correspond to the intercept of a straight line at high frequency was used to calculate the ionic conductivities.[5,6]

3. Supplementary Data

Table S1. Minimum gelation concentration for **PBI-H. N** indicates no gel upon inversion and **G** indicates a stable gel upon inversion.

PBI-H minimum gelation concentration at pH 7.4			
Concentration	**MgCl$_2$ Amount: 50 µL**	**CaCl$_2$ Amount: 50 µL**	**Ca(NO$_3$)$_2$ Amount: 50 µL**
1 mg/mL	N	N	N
2 mg/mL	N	N	G
3 mg/mL	N	N	G
4 mg/mL	G	N	G
5 mg/mL	G	G	G
10 mg/mL	G	G	G

Table S2. pH dependence for **PBI-H** using different amount of Ca(NO$_3$)$_2$ solution**. N** indicates no gel upon inversion and **G** indicates a stable gel upon inversion.

PBI-H			
Ca(NO$_3$)$_2$ Amount	**10 µL**	**30 µL**	**50 µL**
pH 5	G	G	G
pH 6	N	N	N
pH 7	G	G	G
pH 8	G	G	G
pH 9	N	N	N
pH 10	N	N	N

Table S3. pH dependence for **PBI-H** using different amount of $CaCl_2$ solution. **N** indicates no gel upon inversion and **G** indicates a stable gel upon inversion.

PBI-H			
$CaCl_2$ Amount	10 µL	30 µL	50 µL
pH 5	G	G	G
pH 6	N	G	G
pH 7	G	G	G
pH 8	G	G	G
pH 9	N	N	N
pH 10	N	N	N

Table S4. pH dependence for **PBI-H** using different amount of $MgCl_2$ solution. **N** indicates no gel upon inversion and **G** indicates a stable gel upon inversion.

PBI-H			
$MgCl_2$ Amount	10 µL	30 µL	50 µL
pH 5	G	G	N
pH 6	N	N	N
pH 7	G	G	G
pH 8	N	G	G
pH 9	N	G	G
pH 10	N	N	N

Table S5. Minimum gelation concentration for **PBI-L**. **N** indicates no gel upon inversion and **G** indicates a stable gel upon inversion.

PBI-L minimum gelation concentration at pH 7.4			
Concentration	$CaCl_2$ Amount: 50 µL	$MgCl_2$ Amount: 50 µL	$Ca(NO_3)_2$ Amount: 50 µL
1 mg/mL	N	N	N
2 mg/mL	G	G	G
3 mg/mL	G	G	G
4 mg/mL	G	G	G
5 mg/mL	G	G	G
10 mg/mL	G	G	G

Table S6. pH dependence for **PBI-L** using different amount of $Ca(NO_3)_2$ solution. **N** indicates no gel upon inversion and **G** indicates a stable gel upon inversion.

PBI-L pH dependence			
$Ca(NO_3)_2$ Amount	10 µL	30 µL	50 µL
pH 5	N	N	N
pH 6	G	G	G
pH 7	G	G	G
pH 8	G	G	G
pH 9	G	G	G

pH 10	G	G	G

Table S7. pH dependence for **PBI-HL**using different amount of CaCl$_2$ solution. **N** indicates no gel upon inversion and **G** indicates a stable gel upon inversion.

PBI-L pH dependence			
CaCl$_2$ Amount	**10 µL**	**30 µL**	**50 µL**
pH 5	N	N	N
pH 6	G	G	G
pH 7	G	G	G
pH 8	G	G	G
pH 9	G	G	G
pH 10	G	G	G

Table S8. pH dependence for **PBI-H** using different amount of MgCl$_2$solution. **N** indicates no gel upon inversion and **G** indicates a stable gel upon inversion.

PBI-L pH dependence			
MgCl$_2$ Amount	**10 µL**	**30 µL**	**50 µL**
pH 5	N	N	N
pH 6	G	G	G
pH 7	N	G	G
pH 8	N	G	G
pH 9	N	G	G
pH 10	N	G	G
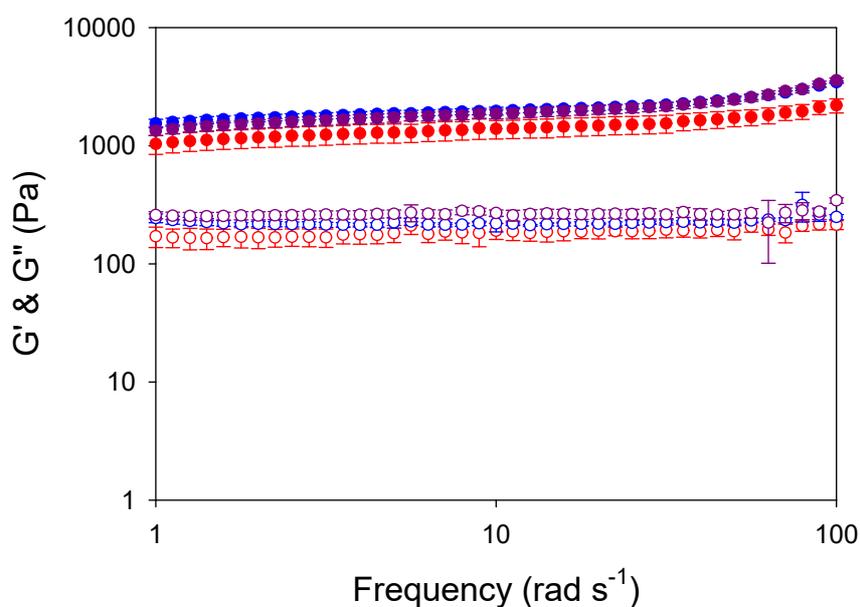
Figure S2. Rheological frequency sweeps for **PBI-L** at 5 mg/mL pH 7.4 with 50 µL of salt. Performed at 0.5% strain at 25°C. Filled shapes represent G' and open shapes represent G''. Measurements

performed in triplicate and error bars calculated from standard deviation. $MgCl_2$ gels data are purple, $CaCl_2$ gels data are blue and $Ca(NO_3)_2$ gels data are red.
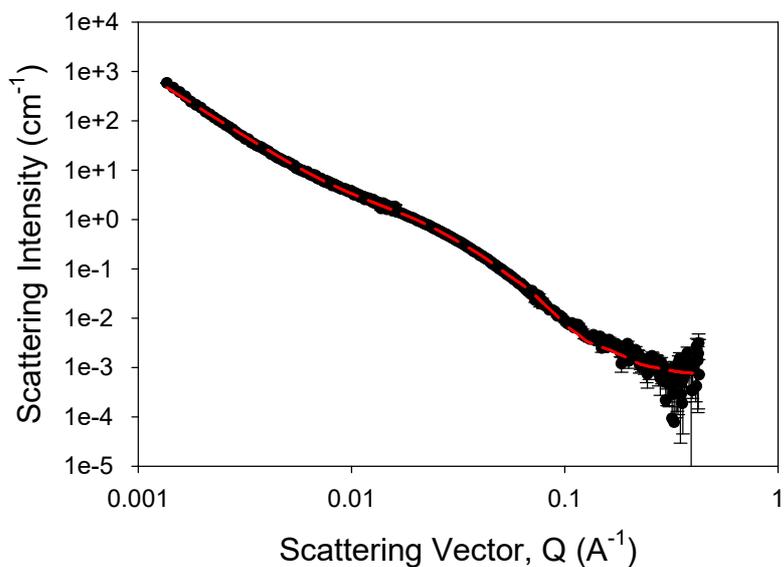
Figure S3. SANS and fit from a **PBI-L** solution at 5 mg/mL. The fit is shown in red and the collected scattering in black with error bars.
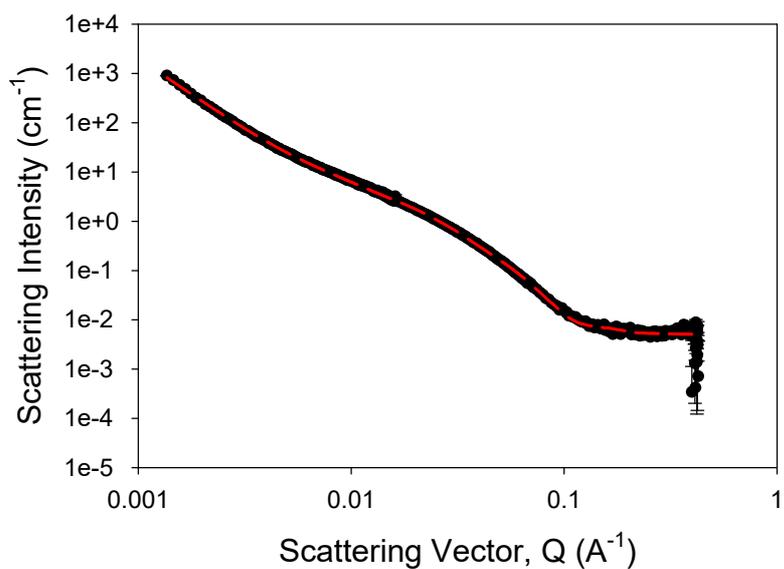
Figure S4. SANS and fit from a **PBI-L** $Ca(NO_3)_2$ gel at 5 mg/mL. The fit is shown in red and the collected scattering in black with error bars.
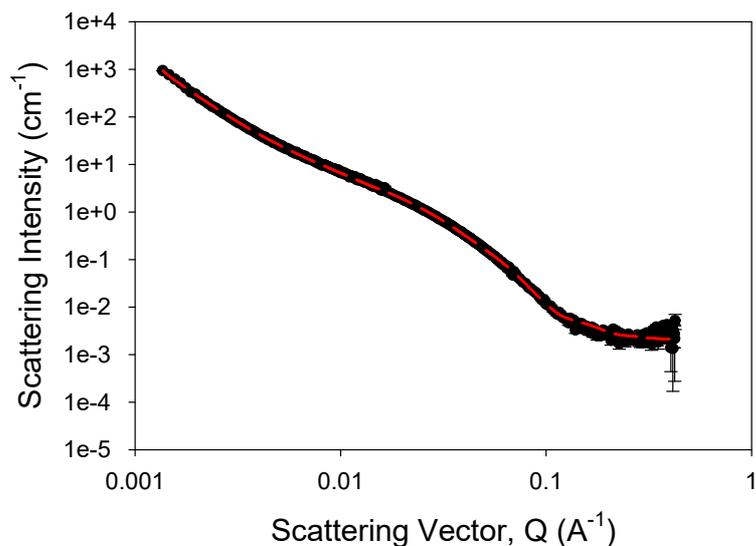
Figure S5. SANS and fit from a **PBI-L** CaCl$_2$ gel at 5 mg/mL. The fit is shown in red and the collected scattering in black with error bars.

Figure S6. SANS and fit from a **PBI-L** MgCl$_2$ gel at 5 mg/mL. The fit is shown in red and the collected scattering in black with error bars.

PBI-L fits best to an elliptical cylinder combined with a power law. Using a flexible elliptical cylinder requires the length to be >1038 for a good fit, which is outside the range of the equipment, and so combining an elliptical cylinder with a power law was used. The radius and axis ratio in both cases were very similar, suggesting that either fit is reasonable. The **PBI-L** gels can all be fitted to the same model. The MgCl$_2$ gel is different to the other two gels. It is necessary for a greater axis ratio for the best fit. Constraining the radius and/or axis ratio results in the fit becoming worse as determined visually and by an increase in chi squared.

Table S9. Table of SANS fit for **PBI-L**

	PBI-L solution	**PBI-L** CaCl$_2$ gel	**PBI-L** Ca(NO$_3$)$_2$ gel	**PBI-L** MgCl$_2$ gel
Scale (power law)	7.12x10^{-6} $\pm$ 1.83x10^{-7}	7.12x10^{-6} $\pm$ 2.16x10^{-7}	3.24x10^{-6} $\pm$ 1.08x10^{-7}	1.15x10^{-5} $\pm$ 3.35x10^{-7}
Power Law	2.73 $\pm$ 0.004	2.79 $\pm$ 0.005	2.93 $\pm$ 0.006	2.71 $\pm$ 0.005
Scale (EC)	8.58x10^{-4} $\pm$ 2.73x10^{-5}	1.63x10^{-3} $\pm$ 7.19x10^{-5}	1.67x10^{-3} $\pm$ 5.83x10^{-5}	1.32x10^{-3} $\pm$ 7.45x10^{-5}
Radius (Å)	28 $\pm$ 0.1	30 $\pm$ 0.1	29 $\pm$ 0.1	35 $\pm$ 0.1
Axis Ratio	2.9 $\pm$ 0.02	3.1 $\pm$ 0.02	3.3 $\pm$ 0.02	4.7 $\pm$ 0.03
Length (Å)	251 $\pm$ 2	540 $\pm$ 11	541 $\pm$ 5	458 $\pm$ 4
Chi Squared	5.4508	4.2661	5.0015	4.2661

Figure S7. Rheological strain sweeps performed at 10 rad/s at 25°C for. **PBI-H** gels formed at 5 mg/mL of gelator with MgCl$_2$, CaCl$_2$ and Ca(NO$_3$)$_2$ at pH 7.4. Filled shapes represent G' and open shapes represent G". Measurements performed in triplicate and error bars calculated from standard deviation. MgCl$_2$ gels data are purple, CaCl$_2$ gels data are blue and Ca(NO$_3$)$_2$ gels
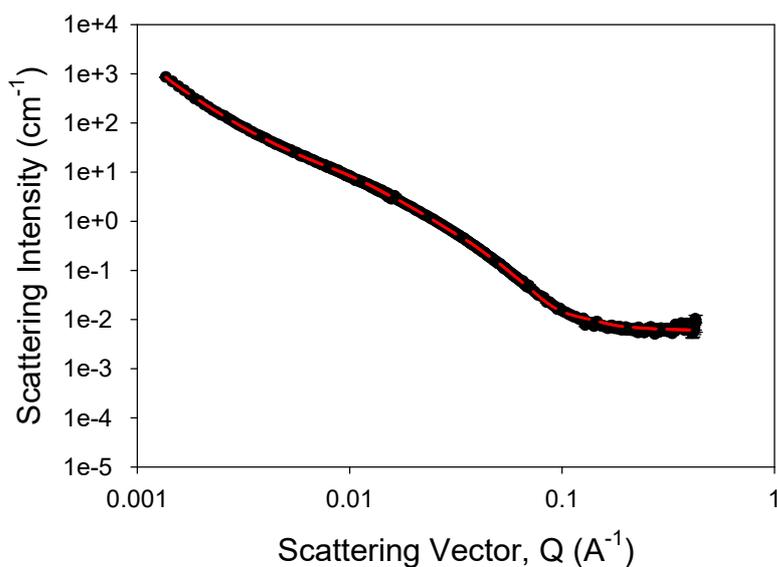
Figure S8. SANS and fit from a **PBI-H** solution at 5 mg/mL. The fit is shown in red and the collected scattering in black with error bars.
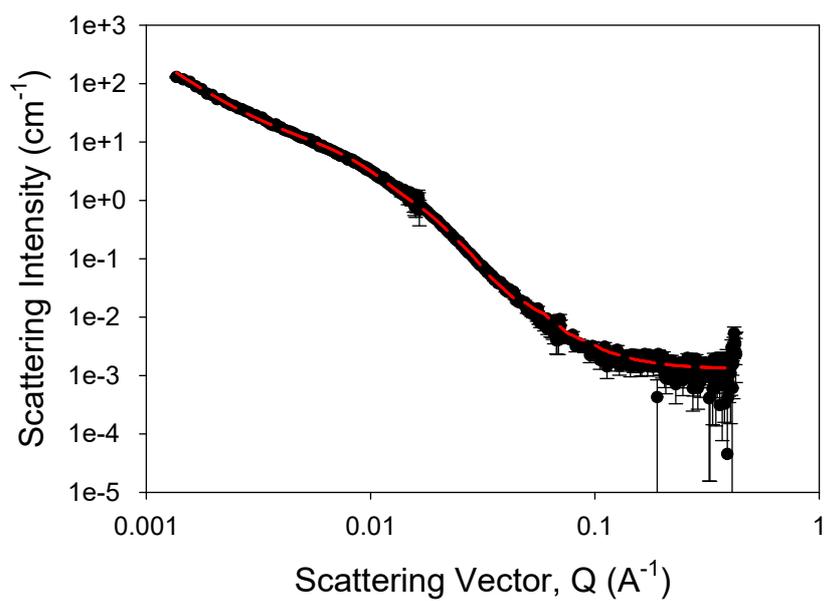
Figure S9. SANS and fit from a **PBI-H** $Ca(NO_3)_2$ gel at 5 mg/mL. The fit is shown in red and the collected scattering in black with error bars.
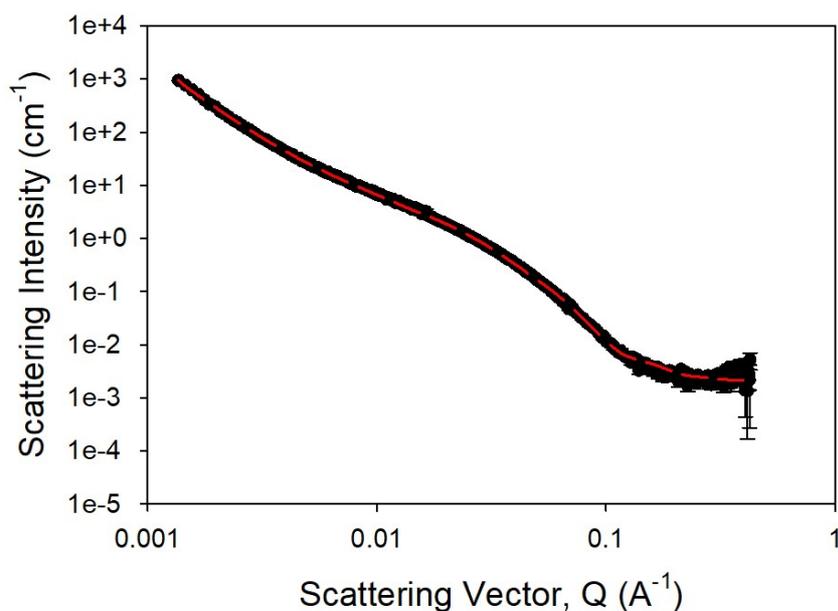
Figure S10. SANS and fit from a **PBI-H** CaCl$_2$ gel at 5 mg/mL. The fit is shown in red and the collected scattering in black with error bars.
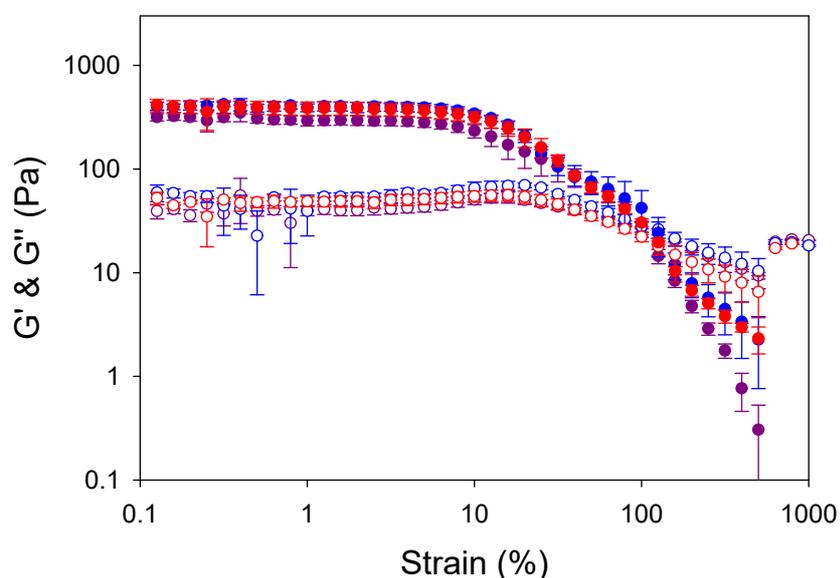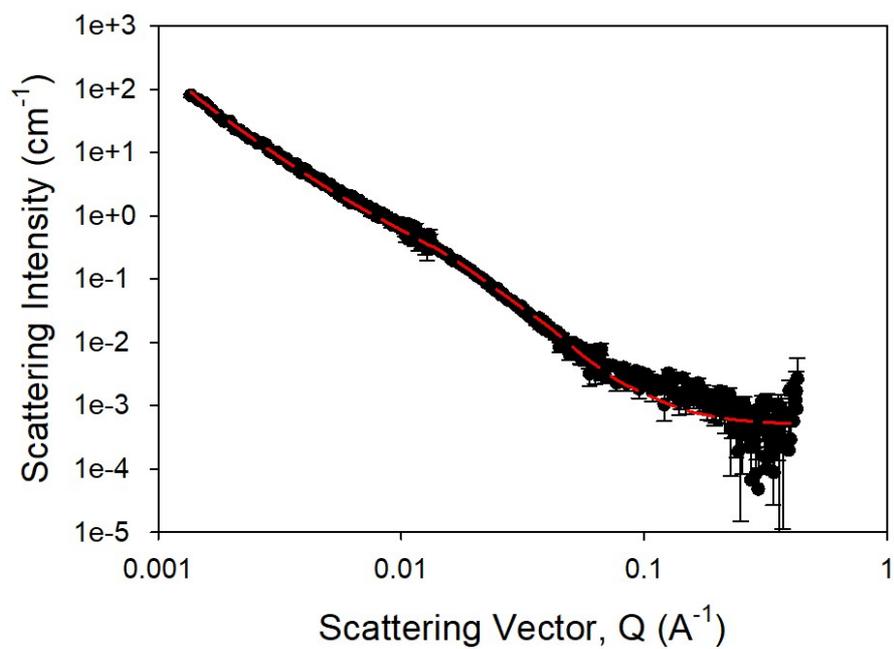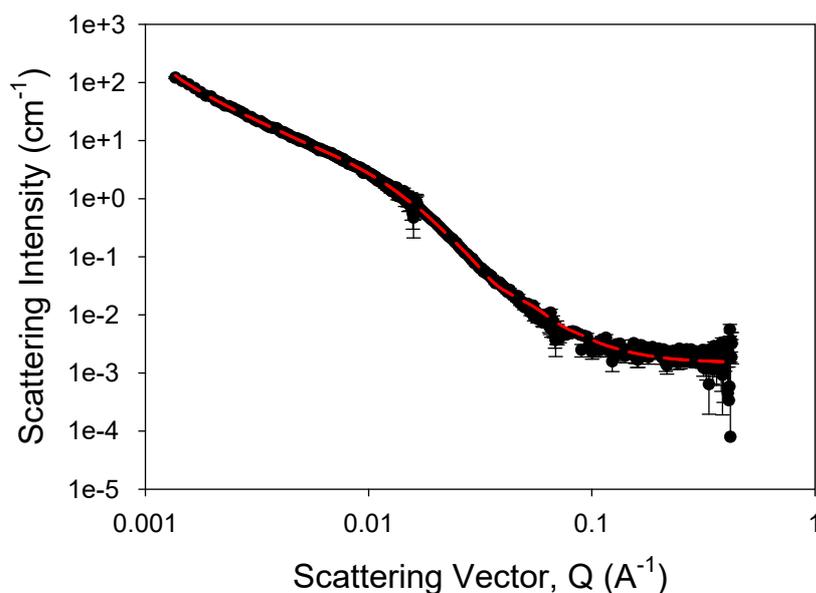

Figure S11. SANS and fit from a **PBI-H** MgCl$_2$ gel at 5 mg/mL. The fit is shown in red and the collected scattering in black with error bars.

The **PBI-H** gels and solutions can also be fitted using the same model as for **PBI-L** In this case, the MgCl$_2$ triggered gel is very similar to the Ca(NO$_3$)$_2$ triggered gel. The CaCl$_2$ triggered gel is however pretty similar. It looks like the gelation leads to a lateral association here as the radius really wants to be double that of the starting **PBI-H.**

Table S10. Table of SANS fit for **PBI-H**

	PBI-H solution	**PBI-H** CaCl$_2$ gel	**PBI-H** Ca(NO$_3$)$_2$	**PBI-H** MgCl$_2$ gel

			gel	
Scale (power law)	$2.16 \times 10^{-6} \pm 5.07 \times 10^{-8}$	$5.19 \times 10^{-6} \pm 1.52 \times 10^{-8}$	$3.46 \times 10^{-6} \pm 1.12 \times 10^{-7}$	$5.95 \times 10^{-5} \pm 1.851 0^{-7}$
Power Law	2.62 ± 0.004	2.57 ± 0.006	2.66 ± 0.006	2.51 ± 0.007
Scale (EC)	$7.41 \times 10^{-5} \pm 1.28 \times 10^{-6}$	$5.40 \times 10^{-4} \pm 2.45 \times 10^{-5}$	$3.91 \times 10^{-4} \pm 2.07 \times 10^{-5}$	$3.20 \times 10^{-4} \pm 1.99 \times 10^{-5}$
Radius (Å)	45 ± 1.0	94 ± 0.3	85 ± 0.4	89 ± 0.4
Axis Ratio	4.5 ± 0.4	2.4 ± 0.02	3.1 ± 0.05	2.31 ± 0.03
Length (Å)	>2000	1433 ± 75	591 ± 4	1449 ± 107
Chi Squared	1.9011	5.1393	2.741	1.6955
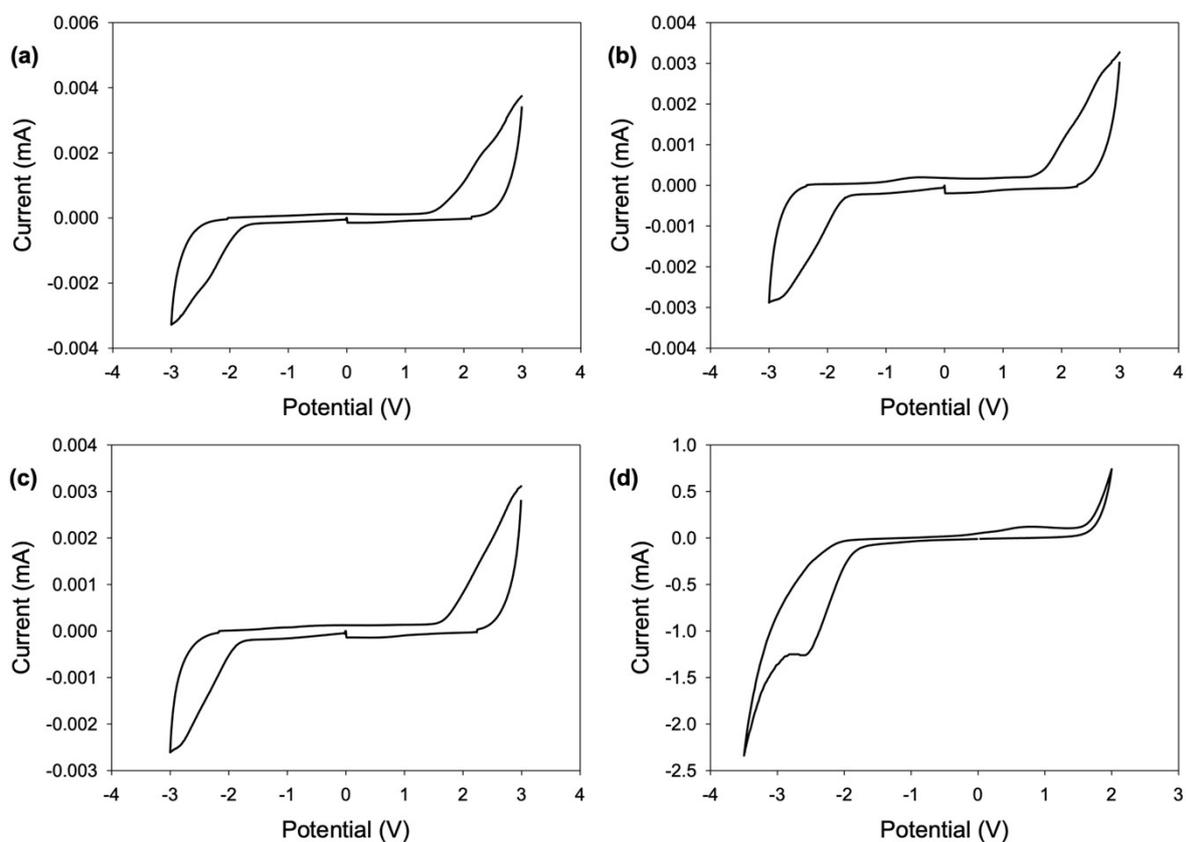
Figure S12. Blank cyclic voltammograms of (a) $Ca(NO_3)_2$ (b) $CaCl_2$ (c) $MgCl_2$ and (d) **PBI-H** solution at a scan rate of 0.10 V/s.
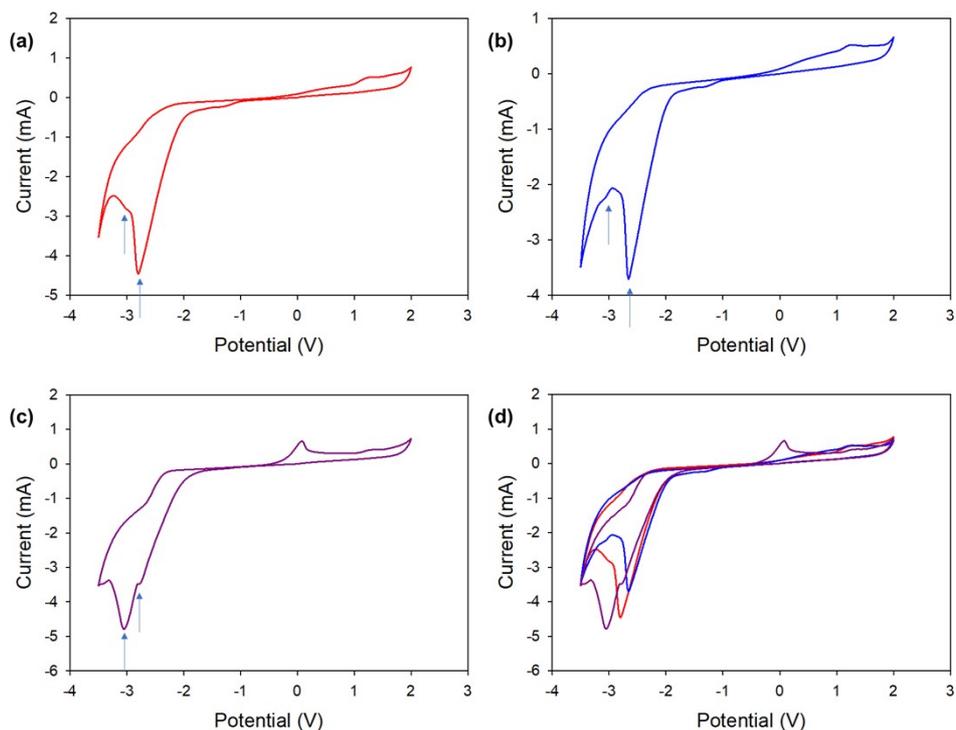
Figure S13. Cyclic voltammograms of **PBI-L** gels of (a) Ca(NO$_3$)$_2$ (b) CaCl$_2$ (c) MgCl$_2$ and (d) a comparison at a scan rate of 0.10 V/s.
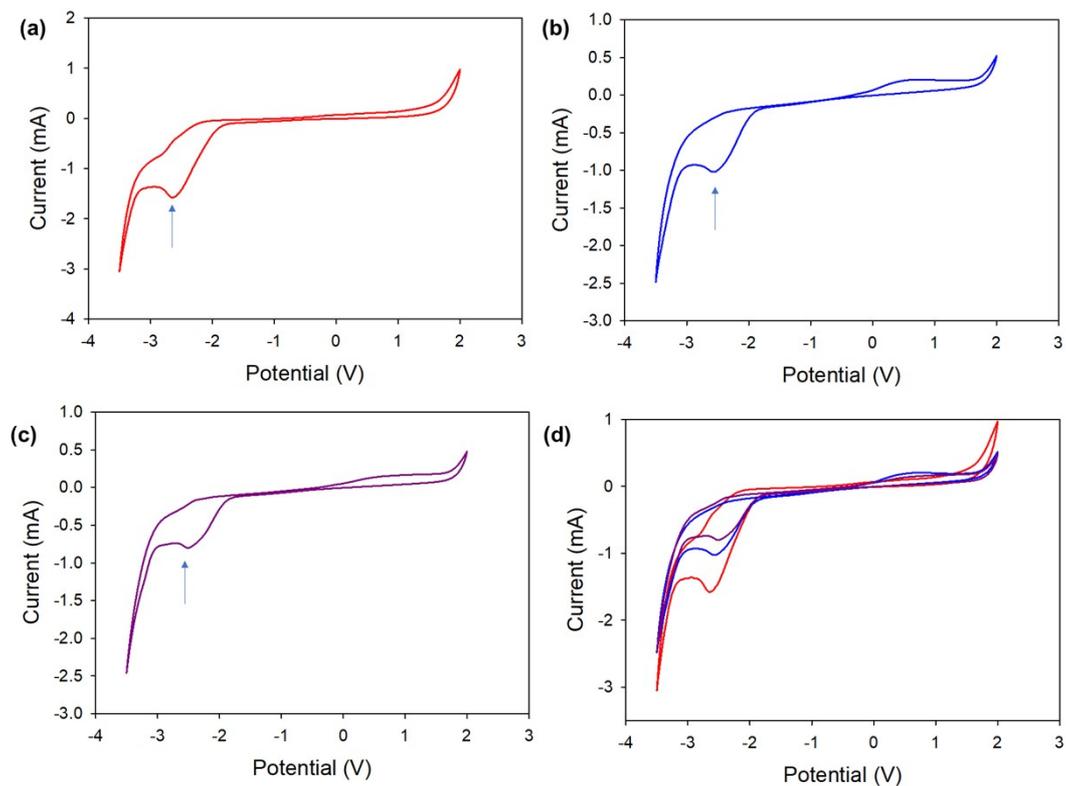
Figure S14. Cyclic voltammograms of **PBI-H** gels of (a) Ca(NO$_3$)$_2$ (b) CaCl$_2$ (c) MgCl$_2$ and (d) a comparison at a scan rate of 0.10 V/s.

Table S11. Reduction potentials for PBI gels

Salt	PBI-L reduction potentials (V)	PBI-H reduction potential (V)
$MgCl_2$	-2.7, -3.0	-2.5
$CaCl_2$	-2.5, -3.0	-2.6
$Ca(NO_3)_2$	-2.9, -3.0	-2.7

Table S12. Rb and ionic conductivity values for PBI gels

Gels	Rb (kΩ)	Ionic conductivity (x 10^{-5} S cm^{-1})
PBI-L $CaCl_2$	2.08 ±0.02	4.13
PBI-L $Ca(NO_3)_2$	2.29 ±0.01	3.63
PBI-L $MgCl_2$	1.87 ±0.01	4.45
PBI-H $CaCl_2$	1.87 ±0.02	4.45
PBI-H $Ca(NO_3)_2$	1.74 ±0.02	4.77
PBI-H $MgCl_2$	1.71 ±0.01	4.86
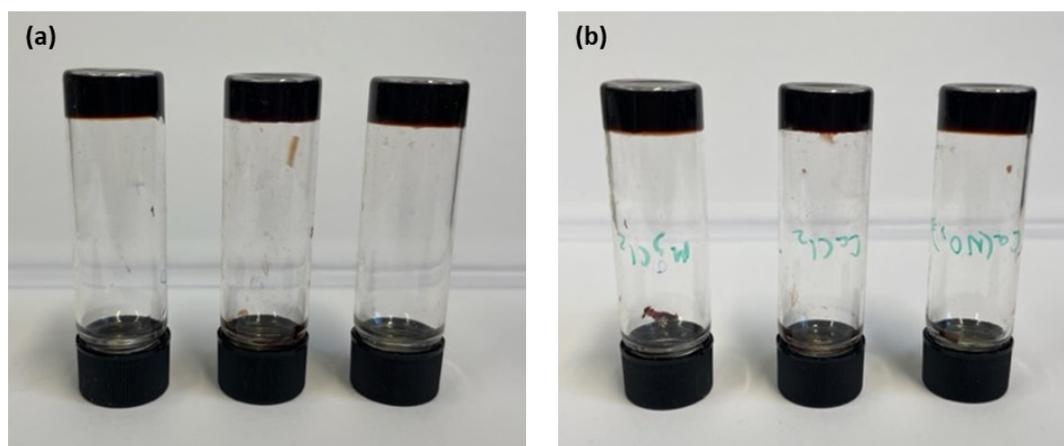
Figure S15. Photographs of (a) **PBI-H** and (b) **PBI-L** gels after 6 months aging.
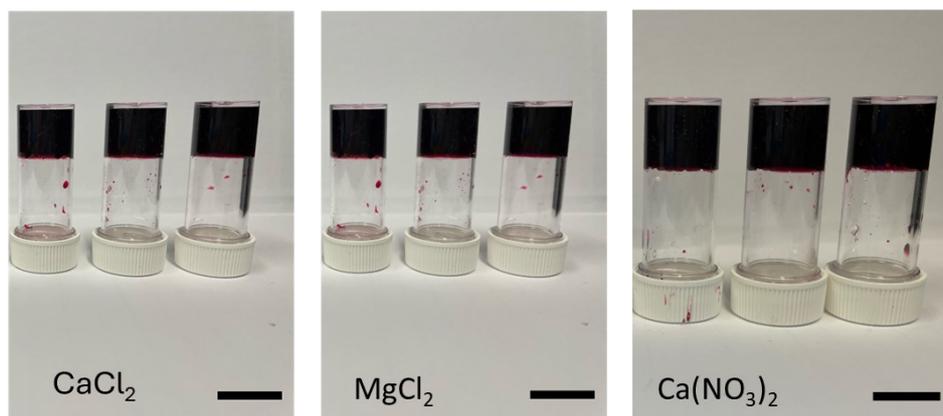
Figure S16. Photographs of **PBI-L** gels after 3 weeks.
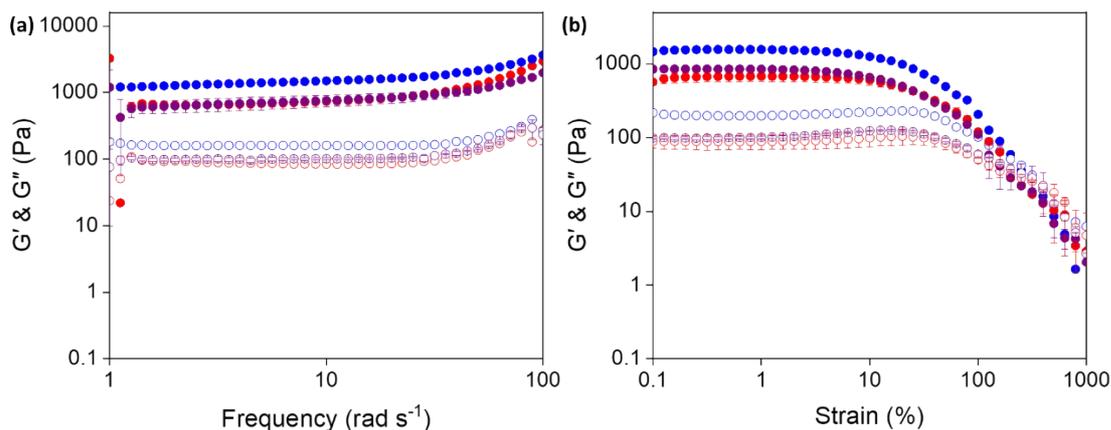
Figure S17. Rheological (a) frequency sweeps at 0.5% strain and (b) strain sweeps at 10 rad/s for **PBI-L** at 5 mg/mL pH 7.4 with 50 µL of salt after three weeks aging. Performed at 25°C. Filled shapes represent G' and open shapes represent G". Measurements performed in triplicate and error bars calculated from standard deviation. $MgCl_2$ gels data are purple, $CaCl_2$ gels data are blue and $Ca(NO_3)_2$ gels data are red.
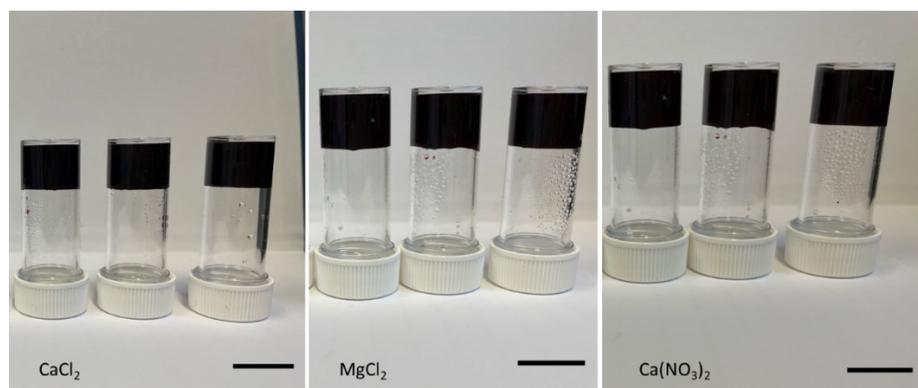

Figure S18. Photographs of **PBI-L** gels after 3 weeks.
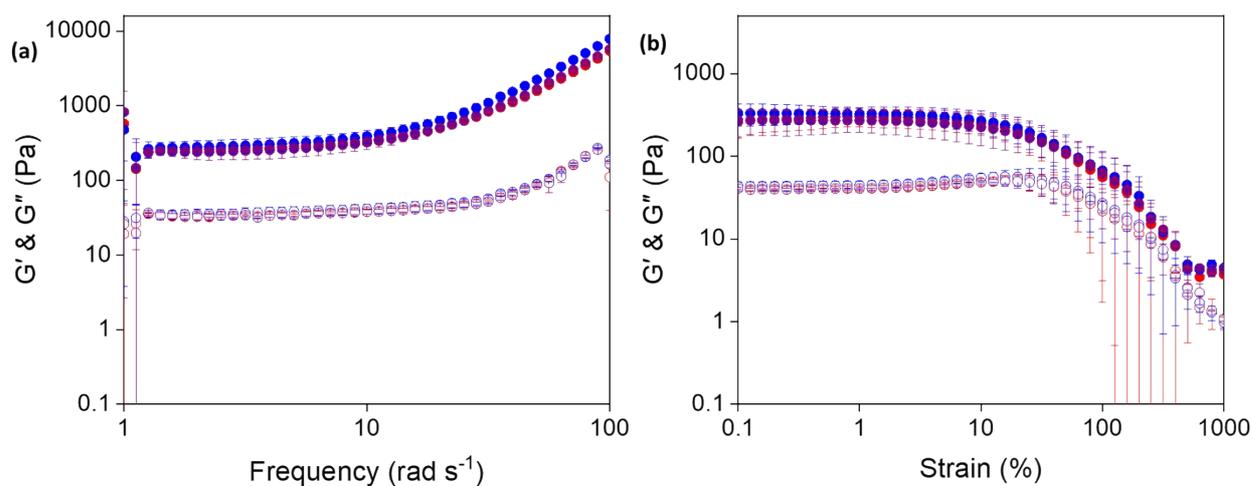
Figure S19. Rheological (a) frequency sweeps at 0.5% strain and (b) strain sweeps at 10 rad/s for **PBI-H** at 5 mg/mL pH 7.4 with 50 µL of salt after three weeks aging. Performed at 25°C. Filled shapes represent G' and open shapes represent G". Measurements performed in triplicate and error bars calculated from standard deviation. $MgCl_2$ gels data are purple, $CaCl_2$ gels data are blue and $Ca(NO_3)_2$ gels data are red.
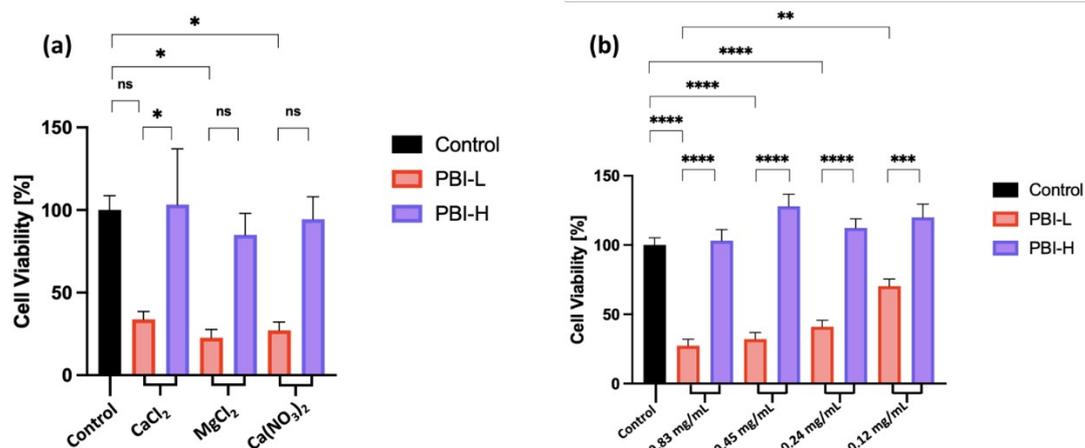
Figure S20. Effect of **PBI-L** and **PBI-H** on cell viability using MTT assay. (a) Cells were treated with **PBI-L** and **PBI-H** gels at 5 mg/mL using three inorganic salts as gelators, $CaCl_2$, $MgCl_2$, and $Ca(NO_3)_2$. (b) Cells treated with **PBI-L** and **PBI-H** solutions at concentration of 0.83, 0.45, 0.24, and 0.12 mg/mL. Data was obtained from 8 replicates and analysis was performed based on average of viability ± standard deviation.
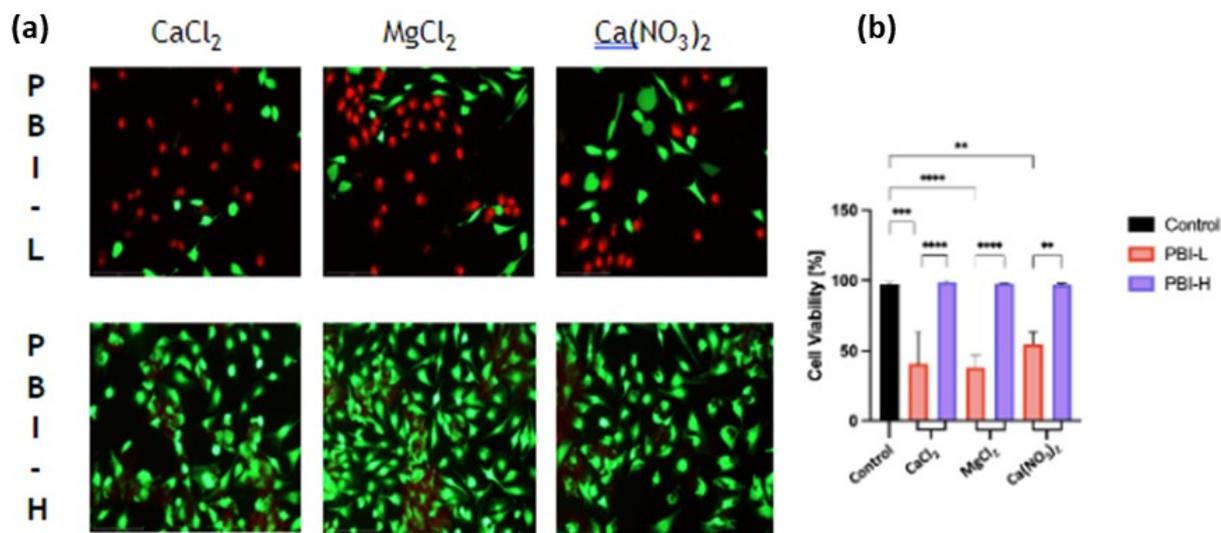
Figure S21. Live/Dead assay analysis of C2C12 cells treated with **PBI-L** and **PBI-H** hydrogels. (a) Representative images of cells labeled with Calcein as live (green) and Ethidium homodimer-1 as red (dead). (b) Quantification of viable cells. C2C12 cells were incubated for 20 hours with or without **PBI-L** and **PBI-H** hydrogels at 5 mg/mL gelled with $CaCl_2$, $MgCl_2$, and $Ca(NO_3)_2$ at 200 mg/mL. Viability represents the percentage of living cells counted at 3 independent positions from each well using Fiji software and analysis was performed based on average viability ± standard deviation. Scale bar is 150 μm.
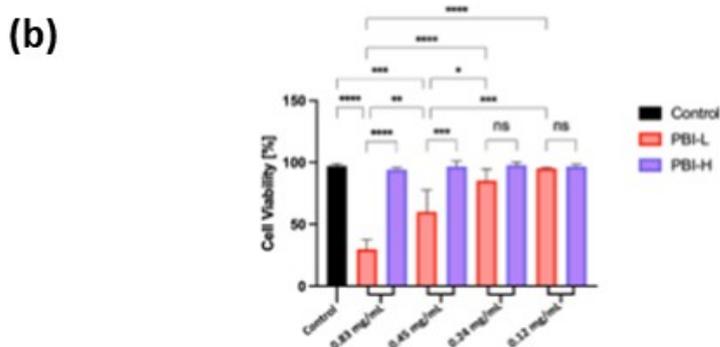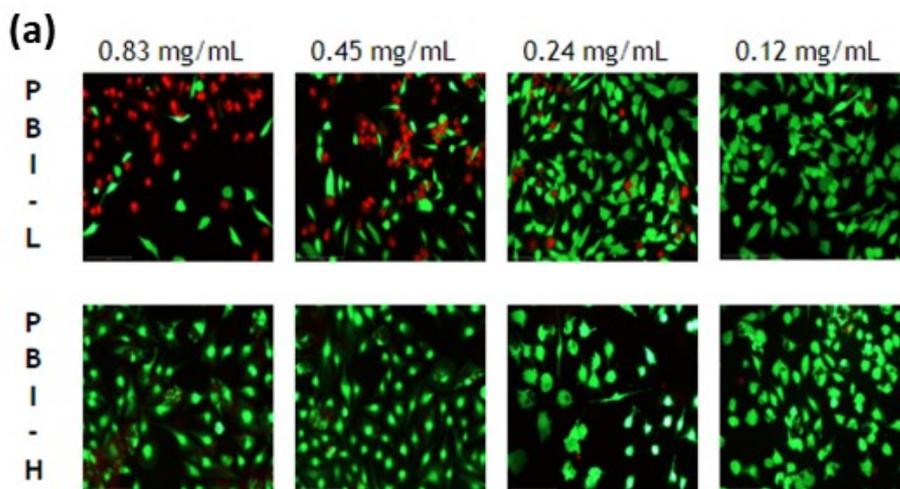
Figure S22. Live/Dead assay analysis of C2C12 cells treated with various concentrations of **PBI-L** and **PBI-H** solutions. (a) Representative images of cells labeled with Calcein as live (green) and Ethidium homodimer-1 as red (dead). (b) Quantification of viable cells. C2C12 cells were incubated for 20 hours with or without **PBI-L** and **PBI-H** solutions in a dose dependent manner. Viability represents the percentage of living cells counted at 3 independent positions from each well using Fiji software and analysis was performed based on average viability ± standard deviation. Scale bar is 150 μm.

4. Statistical analysis

For MTT:

Ordinary one-way ANOVA for gels

Table Analyzed	MTT-Gels				
Data sets analyzed	A-G				
ANOVA summary					
F	5.592				
P value	0.0002				
P value summary	***				
Significant diff. among means (P < 0.05)?	Yes				
R squared	0.4064				
Brown-Forsythe test					
F (DFn, DFd)	1.511 (6, 49)				
P value	0.1943				

P value summary	ns				
Are SDs significantly different (P < 0.05)?	No				
Bartlett's test					
Bartlett's statistic (corrected)	69.90				
P value	<0.0001				
P value summary	****				
Are SDs significantly different (P < 0.05)?	Yes				
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	0.9433	6	0.1572	F (6, 49) = 5.592	P=0.0002
Residual (within columns)	1.378	49	0.02811		
Total	2.321	55			
Data summary					
Number of treatments (columns)	7				
Number of values (total)	56				

Multiple comparisons:

Number of families	1							
Number of comparisons per family	21							
Alpha	0.05							
Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value			
Control vs. PBI-L Ca2+	0.2525	-0.005217 to 0.5102	No	ns	0.0583	A-B		
Control vs. PBI-H Ca2+	-0.01225	-0.2700 to 0.2455	No	ns	>0.9999	A-C		
Control vs. PBI-L Mg2+	0.2945	0.03678 to 0.5522	Yes	*	0.0157	A-D		
Control vs. PBI-H Mg2+	0.05763	-0.2001 to 0.3153	No	ns	0.9927	A-E		
Control vs. PBI-L NO3-	0.2775	0.01978 to 0.5352	Yes	*	0.0272	A-F		
Control vs. PBI-H NO3-	0.02150	-0.2362 to 0.2792	No	ns	>0.9999	A-G		
PBI-L Ca2+ vs. PBI-H Ca2+	-0.2648	-0.5225 to -0.007033	Yes	*	0.0405	B-C		
PBI-L Ca2+ vs. PBI-L Mg2+	0.04200	-0.2157 to 0.2997	No	ns	0.9987	B-D		
PBI-L Ca2+ vs. PBI-H Mg2+	-0.1949	-0.4526 to 0.06284	No	ns	0.2534	B-E		
PBI-L Ca2+ vs. PBI-L NO3-	0.02500	-0.2327 to 0.2827	No	ns	>0.9999	B-F		
PBI-L Ca2+ vs. PBI-H NO3-	-0.2310	-0.4887 to 0.02672	No	ns	0.1062	B-G		
PBI-H Ca2+ vs. PBI-L Mg2+	0.3068	0.04903 to 0.5645	Yes	*	0.0104	C-D		
PBI-H Ca2+ vs. PBI-H Mg2+	0.06988	-0.1878 to 0.3276	No	ns	0.9801	C-E		
PBI-H Ca2+ vs. PBI-L NO3-	0.2898	0.03203 to 0.5475	Yes	*	0.0183	C-F		
PBI-H Ca2+ vs. PBI-H NO3-	0.03375	-0.2240 to 0.2915	No	ns	0.9996	C-G		
PBI-L Mg2+ vs. PBI-H Mg2+	-0.2369	-0.4946 to 0.02084	No	ns	0.0906	D-E		
PBI-L Mg2+ vs. PBI-L NO3-	-0.01700	-0.2747 to 0.2407	No	ns	>0.9999	D-F		
PBI-L Mg2+ vs. PBI-H NO3-	-0.2730	-0.5307 to -0.01528	Yes	*	0.0314	D-G		
PBI-H Mg2+ vs. PBI-L NO3-	0.2199	-0.03784 to 0.4776	No	ns	0.1415	E-F		
PBI-H Mg2+ vs. PBI-H NO3-	-0.03613	-0.2938 to 0.2216	No	ns	0.9995	E-G		
PBI-L NO3- vs. PBI-H NO3-	-0.2560	-0.5137 to 0.001717	No	ns	0.0526	F-G		

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
Control vs. PBI-L Ca2+	0.5111	0.2586	0.2525	0.08384	8	8	4.259	49
Control vs. PBI-H Ca2+	0.5111	0.5234	-0.01225	0.08384	8	8	0.2066	49
Control vs. PBI-L Mg2+	0.5111	0.2166	0.2945	0.08384	8	8	4.968	49
Control vs. PBI-H Mg2+	0.5111	0.4535	0.05763	0.08384	8	8	0.9721	49
Control vs. PBI-L NO3-	0.5111	0.2336	0.2775	0.08384	8	8	4.681	49
Control vs. PBI-H NO3-	0.5111	0.4896	0.02150	0.08384	8	8	0.3627	49
PBI-L Ca2+ vs. PBI-H Ca2+	0.2586	0.5234	-0.2648	0.08384	8	8	4.466	49
PBI-L Ca2+ vs. PBI-L Mg2+	0.2586	0.2166	0.04200	0.08384	8	8	0.7085	49
PBI-L Ca2+ vs. PBI-H Mg2+	0.2586	0.4535	-0.1949	0.08384	8	8	3.287	49
PBI-L Ca2+ vs. PBI-L NO3-	0.2586	0.2336	0.02500	0.08384	8	8	0.4217	49
PBI-L Ca2+ vs. PBI-H NO3-	0.2586	0.4896	-0.2310	0.08384	8	8	3.897	49
PBI-H Ca2+ vs. PBI-L Mg2+	0.5234	0.2166	0.3068	0.08384	8	8	5.174	49
PBI-H Ca2+ vs. PBI-H Mg2+	0.5234	0.4535	0.06988	0.08384	8	8	1.179	49
PBI-H Ca2+ vs. PBI-L NO3-	0.5234	0.2336	0.2898	0.08384	8	8	4.888	49
PBI-H Ca2+ vs. PBI-H NO3-	0.5234	0.4896	0.03375	0.08384	8	8	0.5693	49
PBI-L Mg2+ vs. PBI-H Mg2+	0.2166	0.4535	-0.2369	0.08384	8	8	3.996	49
PBI-L Mg2+ vs. PBI-L NO3-	0.2166	0.2336	-0.01700	0.08384	8	8	0.2868	49
PBI-L Mg2+ vs. PBI-H NO3-	0.2166	0.4896	-0.2730	0.08384	8	8	4.605	49
PBI-H Mg2+ vs. PBI-L NO3-	0.4535	0.2336	0.2199	0.08384	8	8	3.709	49
PBI-H Mg2+ vs. PBI-H NO3-	0.4535	0.4896	-0.03613	0.08384	8	8	0.6094	49
PBI-L NO3- vs. PBI-H NO3-	0.2336	0.4896	-0.2560	0.08384	8	8	4.318	49

Descriptive statistics:

	Control	PBI-L Ca2+	PBI-H Ca2+	PBI-L Mg2+	PBI-H Mg2+	PBI-L NO3-	PBI-H NO3-
Number of values	8	8	8	8	8	8	8
Minimum	0.3760	0.2010	0.2880	0.1940	0.3280	0.1800	0.3410
25% Percentile	0.4368	0.2490	0.3258	0.1998	0.3605	0.2160	0.3575
Median	0.5145	0.2530	0.3940	0.2070	0.3995	0.2310	0.4620
75% Percentile	0.5733	0.2868	0.5265	0.2185	0.5128	0.2598	0.6208
Maximum	0.6460	0.2890	1.442	0.2880	0.7640	0.2780	0.7530
Mean	0.5111	0.2586	0.5234	0.2166	0.4535	0.2336	0.4896
Std. Deviation	0.08665	0.02933	0.3804	0.03006	0.1408	0.03178	0.1482
Std. Error of Mean	0.03064	0.01037	0.1345	0.01063	0.04978	0.01123	0.05240
Lower 95% CI	0.4387	0.2341	0.2053	0.1915	0.3358	0.2071	0.3657
Upper 95% CI	0.5836	0.2831	0.8414	0.2418	0.5712	0.2602	0.6135

Ordinary one-way ANOVA for solutions:

Table Analyzed	MTT-solution				
Data sets analyzed	A-I				
ANOVA summary					
F	28.47				
P value	<0.0001				
P value summary	****				
Significant diff. among means (P < 0.05)?	Yes				
R squared	0.7834				
Brown-Forsythe test					
F (DFn, DFd)	3.216 (8, 63)				
P value	0.0040				
P value summary	**				
Are SDs significantly different (P < 0.05)?	Yes				
Bartlett's test					
Bartlett's statistic (corrected)	48.25				
P value	<0.0001				

P value summary	****				
Are SDs significantly different (P < 0.05)?	Yes				
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	0.7259	8	0.09074	F (8, 63) = 28.47	P<0.0001
Residual (within columns)	0.2008	63	0.003187		
Total	0.9267	71			
Data summary					
Number of treatments (columns)	9				
Number of values (total)	72				

Multiple comparisons:

Number of families	1							
Number of comparisons per family	36							
Alpha	0.05							
Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value			
Control vs. PBI-L 20uL	0.1958	0.1051 to 0.2864	Yes	****	<0.0001	A-B		
Control vs. PBI-L 10uL	0.1833	0.09259 to 0.2739	Yes	****	<0.0001	A-C		
Control vs. PBI-L 5uL	0.1588	0.06809 to 0.2494	Yes	****	<0.0001	A-D		
Control vs. PBI-L 2.5uL	0.08038	-0.01028 to 0.1710	No	ns	0.1222	A-E		
Control vs. PBI-H 20uL	-0.008250	-0.09891 to 0.08241	No	ns	>0.9999	A-F		
Control vs. PBI-H 10uL	-0.07475	-0.1654 to 0.01591	No	ns	0.1881	A-G		
Control vs. PBI-H 5uL	-0.03275	-0.1234 to 0.05791	No	ns	0.9618	A-H		
Control vs. PBI-H 2.5uL	-0.05338	-0.1440 to 0.03728	No	ns	0.6222	A-I		
PBI-L 20uL vs. PBI-L 10uL	-0.01250	-0.1032 to 0.07816	No	ns	>0.9999	B-C		
PBI-L 20uL vs. PBI-L 5uL	-0.03700	-0.1277 to 0.05366	No	ns	0.9244	B-D		
PBI-L 20uL vs. PBI-L 2.5uL	-0.1154	-0.2060 to -0.02472	Yes	**	0.0038	B-E		
PBI-L 20uL vs. PBI-H 20uL	-0.2040	-0.2947 to -0.1133	Yes	****	<0.0001	B-F		
PBI-L 20uL vs. PBI-H 10uL	-0.2705	-0.3612 to -0.1798	Yes	****	<0.0001	B-G		
PBI-L 20uL vs. PBI-H 5uL	-0.2285	-0.3192 to -0.1378	Yes	****	<0.0001	B-H		
PBI-L 20uL vs. PBI-H 2.5uL	-0.2491	-0.3398 to -0.1585	Yes	****	<0.0001	B-I		
PBI-L 10uL vs. PBI-L 5uL	-0.02450	-0.1152 to 0.06616	No	ns	0.9939	C-D		
PBI-L 10uL vs. PBI-L 2.5uL	-0.1029	-0.1935 to -0.01222	Yes	*	0.0149	C-E		
PBI-L 10uL vs. PBI-H 20uL	-0.1915	-0.2822 to -0.1008	Yes	****	<0.0001	C-F		
PBI-L 10uL vs. PBI-H 10uL	-0.2580	-0.3487 to -0.1673	Yes	****	<0.0001	C-G		
PBI-L 10uL vs. PBI-H 5uL	-0.2160	-0.3067 to -0.1253	Yes	****	<0.0001	C-H		
PBI-L 10uL vs. PBI-H 2.5uL	-0.2366	-0.3273 to -0.1460	Yes	****	<0.0001	C-I		
PBI-L 5uL vs. PBI-L 2.5uL	-0.07838	-0.1690 to 0.01228	No	ns	0.1431	D-E		
PBI-L 5uL vs. PBI-H 20uL	-0.1670	-0.2577 to -0.07634	Yes	****	<0.0001	D-F		
PBI-L 5uL vs. PBI-H 10uL	-0.2335	-0.3242 to -0.1428	Yes	****	<0.0001	D-G		
PBI-L 5uL vs. PBI-H 5uL	-0.1915	-0.2822 to -0.1008	Yes	****	<0.0001	D-H		
PBI-L 5uL vs. PBI-H 2.5uL	-0.2121	-0.3028 to -0.1215	Yes	****	<0.0001	D-I		
PBI-L 2.5uL vs. PBI-H 20uL	-0.08863	-0.1793 to	No	ns	0.0603	E-		

		0.002034					F-E	
PBI-L 2.5uL vs. PBI-H 10uL	-0.1551	-0.2458 to -0.06447	Yes	****	<0.0001	E-G		
PBI-L 2.5uL vs. PBI-H 5uL	-0.1131	-0.2038 to -0.02247	Yes	**	0.0049	E-H		
PBI-L 2.5uL vs. PBI-H 2.5uL	-0.1338	-0.2244 to -0.04309	Yes	***	0.0004	E-I		
PBI-H 20uL vs. PBI-H 10uL	-0.06650	-0.1572 to 0.02416	No	ns	0.3259	F-G		
PBI-H 20uL vs. PBI-H 5uL	-0.02450	-0.1152 to 0.06616	No	ns	0.9939	F-H		
PBI-H 20uL vs. PBI-H 2.5uL	-0.04513	-0.1358 to 0.04553	No	ns	0.8019	F-I		
PBI-H 10uL vs. PBI-H 5uL	0.04200	-0.04866 to 0.1327	No	ns	0.8568	G-H		
PBI-H 10uL vs. PBI-H 2.5uL	0.02138	-0.06928 to 0.1120	No	ns	0.9976	G-I		
PBI-H 5uL vs. PBI-H 2.5uL	-0.02063	-0.1113 to 0.07003	No	ns	0.9981	H-I		
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
Control vs. PBI-L 20uL	0.4074	0.2116	0.1958	0.02822	8	8	9.808	63
Control vs. PBI-L 10uL	0.4074	0.2241	0.1833	0.02822	8	8	9.182	63
Control vs. PBI-L 5uL	0.4074	0.2486	0.1588	0.02822	8	8	7.954	63
Control vs. PBI-L 2.5uL	0.4074	0.3270	0.08038	0.02822	8	8	4.027	63
Control vs. PBI-H 20uL	0.4074	0.4156	-0.008250	0.02822	8	8	0.4134	63
Control vs. PBI-H 10uL	0.4074	0.4821	-0.07475	0.02822	8	8	3.745	63
Control vs. PBI-H 5uL	0.4074	0.4401	-0.03275	0.02822	8	8	1.641	63
Control vs. PBI-H 2.5uL	0.4074	0.4608	-0.05338	0.02822	8	8	2.674	63
PBI-L 20uL vs. PBI-L 10uL	0.2116	0.2241	-0.01250	0.02822	8	8	0.6263	63
PBI-L 20uL vs. PBI-L 5uL	0.2116	0.2486	-0.03700	0.02822	8	8	1.854	63
PBI-L 20uL vs. PBI-L 2.5uL	0.2116	0.3270	-0.1154	0.02822	8	8	5.781	63
PBI-L 20uL vs. PBI-H 20uL	0.2116	0.4156	-0.2040	0.02822	8	8	10.22	63
PBI-L 20uL vs. PBI-H 10uL	0.2116	0.4821	-0.2705	0.02822	8	8	13.55	63
PBI-L 20uL vs. PBI-H 5uL	0.2116	0.4401	-0.2285	0.02822	8	8	11.45	63
PBI-L 20uL vs. PBI-H 2.5uL	0.2116	0.4608	-0.2491	0.02822	8	8	12.48	63
PBI-L 10uL vs. PBI-L 5uL	0.2241	0.2486	-0.02450	0.02822	8	8	1.228	63
PBI-L 10uL vs. PBI-L 2.5uL	0.2241	0.3270	-0.1029	0.02822	8	8	5.155	63
PBI-L 10uL vs. PBI-H 20uL	0.2241	0.4156	-0.1915	0.02822	8	8	9.595	63
PBI-L 10uL vs. PBI-H 10uL	0.2241	0.4821	-0.2580	0.02822	8	8	12.93	63
PBI-L 10uL vs. PBI-H 5uL	0.2241	0.4401	-0.2160	0.02822	8	8	10.82	63
PBI-L 10uL vs. PBI-H 2.5uL	0.2241	0.4608	-0.2366	0.02822	8	8	11.86	63
PBI-L 5uL vs. PBI-L 2.5uL	0.2486	0.3270	-0.07838	0.02822	8	8	3.927	63
PBI-L 5uL vs. PBI-H 20uL	0.2486	0.4156	-0.1670	0.02822	8	8	8.368	63
PBI-L 5uL vs. PBI-H 10uL	0.2486	0.4821	-0.2335	0.02822	8	8	11.70	63
PBI-L 5uL vs. PBI-H 5uL	0.2486	0.4401	-0.1915	0.02822	8	8	9.595	63
PBI-L 5uL vs. PBI-H 2.5uL	0.2486	0.4608	-0.2121	0.02822	8	8	10.63	63
PBI-L 2.5uL vs. PBI-H 20uL	0.3270	0.4156	-0.08863	0.02822	8	8	4.441	63
PBI-L 2.5uL vs. PBI-H 10uL	0.3270	0.4821	-0.1551	0.02822	8	8	7.773	63
PBI-L 2.5uL vs. PBI-H 5uL	0.3270	0.4401	-0.1131	0.02822	8	8	5.668	63
PBI-L 2.5uL vs. PBI-H 2.5uL	0.3270	0.4608	-0.1338	0.02822	8	8	6.702	63
PBI-H 20uL vs. PBI-H 10uL	0.4156	0.4821	-0.06650	0.02822	8	8	3.332	63
PBI-H 20uL vs. PBI-H 5uL	0.4156	0.4401	-0.02450	0.02822	8	8	1.228	63
PBI-H 20uL vs. PBI-H 2.5uL	0.4156	0.4608	-0.04513	0.02822	8	8	2.261	63
PBI-H 10uL vs. PBI-H 5uL	0.4821	0.4401	0.04200	0.02822	8	8	2.104	63
PBI-H 10uL vs. PBI-H 2.5uL	0.4821	0.4608	0.02138	0.02822	8	8	1.071	63
PBI-H 5uL vs. PBI-H 2.5uL	0.4401	0.4608	-0.02063	0.02822	8	8	1.033	63

Descriptive statistics:

	Control	PBI-L 20uL	PBI-L 10uL	PBI-L 5uL	PBI-L 2.5uL	PBI-H 20uL	PBI-H 10uL	PBI-H 5uL	PBI-H 2.5uL
Number of values	8	8	8	8	8	8	8	8	8
Minimum	0.3730	0.1910	0.1900	0.2170	0.2920	0.3120	0.3100	0.3580	0.3200
25% Percentile	0.3735	0.2025	0.2088	0.2420	0.3035	0.3328	0.4228	0.3740	0.3630
Median	0.4110	0.2085	0.2235	0.2530	0.3255	0.4370	0.5255	0.4615	0.4725
75% Percentile	0.4385	0.2233	0.2440	0.2598	0.3365	0.4578	0.5313	0.4848	0.5515
Maximum	0.4400	0.2360	0.2550	0.2610	0.3950	0.5350	0.5700	0.5030	0.5890
Mean	0.4074	0.2116	0.2241	0.2486	0.3270	0.4156	0.4821	0.4401	0.4608
Std. Deviation	0.03066	0.01419	0.02122	0.01468	0.03160	0.07626	0.08576	0.05664	0.09744
Std. Error of Mean	0.01084	0.005018	0.007503	0.005189	0.01117	0.02696	0.03032	0.02003	0.03445

Lower 95% CI	0.3817	0.1998	0.2064	0.2364	0.3006	0.3519	0.4104	0.3928	0.3793
Upper 95% CI	0.4330	0.2235	0.2419	0.2609	0.3534	0.4794	0.5538	0.4875	0.5422

For Live-Dead

Ordinary one-way ANOVA for gels:

Table Analyzed	LD-Gels				
Data sets analyzed	A-G				
ANOVA summary					
F	25.09				
P value	<0.0001				
P value summary	****				
Significant diff. among means (P < 0.05)?	Yes				
R squared	0.9149				
Brown-Forsythe test					
F (DFn, DFd)	1.394 (6, 14)				
P value	0.2837				
P value summary	ns				
Are SDs significantly different (P < 0.05)?	No				
Bartlett's test					
Bartlett's statistic (corrected)					
P value					
P value summary					
Are SDs significantly different (P < 0.05)?					
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	15118	6	2520	F (6, 14) = 25.09	P<0.0001
Residual (within columns)	1406	14	100.4		
Total	16524	20			
Data summary					
Number of treatments (columns)	7				
Number of values (total)	21				

Multiple comparisons:

Number of families	1							
Number of comparisons per family	21							
Alpha	0.05							
Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value			
Control vs. PBI-L Ca2+	56.57	28.63 to 84.50	Yes	***	0.0001	A-B		
Control vs. PBI-H Ca2+	-1.800	-29.74 to 26.14	No	ns	>0.9999	A-C		
Control vs. PBI-L Mg2+	59.33	31.40 to 87.27	Yes	****	<0.0001	A-D		
Control vs. PBI-H Mg2+	-0.7000	-28.64 to 27.24	No	ns	>0.9999	A-E		
Control vs. PBI-L NO	42.40	14.46 to 70.34	Yes	**	0.0021	A-F		
Control vs. PBI-H NO	0.3333	-27.60 to 28.27	No	ns	>0.9999	A-G		
PBI-L Ca2+ vs. PBI-H Ca2+	-58.37	-86.30 to -30.43	Yes	****	<0.0001	B-C		

Comparison	Mean Diff.	95% CI	Significant?	Summary	Adjusted P Value	Code
PBI-L Ca2+ vs. PBI-L Mg2+	2.767	-25.17 to 30.70	No	ns	0.9998	B-D
PBI-L Ca2+ vs. PBI-H Mg2+	-57.27	-85.20 to -29.33	Yes	***	0.0001	B-E
PBI-L Ca2+ vs. PBI-L NO	-14.17	-42.10 to 13.77	No	ns	0.6083	B-F
PBI-L Ca2+ vs. PBI-H NO	-56.23	-84.17 to -28.30	Yes	***	0.0001	B-G
PBI-H Ca2+ vs. PBI-L Mg2+	61.13	33.20 to 89.07	Yes	****	<0.0001	C-D
PBI-H Ca2+ vs. PBI-H Mg2+	1.100	-26.84 to 29.04	No	ns	>0.9999	C-E
PBI-H Ca2+ vs. PBI-L NO	44.20	16.26 to 72.14	Yes	**	0.0014	C-F
PBI-H Ca2+ vs. PBI-H NO	2.133	-25.80 to 30.07	No	ns	>0.9999	C-G
PBI-L Mg2+ vs. PBI-H Mg2+	-60.03	-87.97 to -32.10	Yes	****	<0.0001	D-E
PBI-L Mg2+ vs. PBI-L NO	-16.93	-44.87 to 11.00	No	ns	0.4183	D-F
PBI-L Mg2+ vs. PBI-H NO	-59.00	-86.94 to -31.06	Yes	****	<0.0001	D-G
PBI-H Mg2+ vs. PBI-L NO	43.10	15.16 to 71.04	Yes	**	0.0018	E-F
PBI-H Mg2+ vs. PBI-H NO	1.033	-26.90 to 28.97	No	ns	>0.9999	E-G
PBI-L NO vs. PBI-H NO	-42.07	-70.00 to -14.13	Yes	**	0.0022	F-G

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
Control vs. PBI-L Ca2+	97.13	40.57	56.57	8.182	3	3	9.777	14
Control vs. PBI-H Ca2+	97.13	98.93	-1.800	8.182	3	3	0.3111	14
Control vs. PBI-L Mg2+	97.13	37.80	59.33	8.182	3	3	10.26	14
Control vs. PBI-H Mg2+	97.13	97.83	-0.7000	8.182	3	3	0.1210	14
Control vs. PBI-L NO	97.13	54.73	42.40	8.182	3	3	7.329	14
Control vs. PBI-H NO	97.13	96.80	0.3333	8.182	3	3	0.05762	14
PBI-L Ca2+ vs. PBI-H Ca2+	40.57	98.93	-58.37	8.182	3	3	10.09	14
PBI-L Ca2+ vs. PBI-L Mg2+	40.57	37.80	2.767	8.182	3	3	0.4782	14
PBI-L Ca2+ vs. PBI-H Mg2+	40.57	97.83	-57.27	8.182	3	3	9.898	14
PBI-L Ca2+ vs. PBI-L NO	40.57	54.73	-14.17	8.182	3	3	2.449	14
PBI-L Ca2+ vs. PBI-H NO	40.57	96.80	-56.23	8.182	3	3	9.720	14
PBI-H Ca2+ vs. PBI-L Mg2+	98.93	37.80	61.13	8.182	3	3	10.57	14
PBI-H Ca2+ vs. PBI-H Mg2+	98.93	97.83	1.100	8.182	3	3	0.1901	14
PBI-H Ca2+ vs. PBI-L NO	98.93	54.73	44.20	8.182	3	3	7.640	14
PBI-H Ca2+ vs. PBI-H NO	98.93	96.80	2.133	8.182	3	3	0.3687	14
PBI-L Mg2+ vs. PBI-H Mg2+	37.80	97.83	-60.03	8.182	3	3	10.38	14
PBI-L Mg2+ vs. PBI-L NO	37.80	54.73	-16.93	8.182	3	3	2.927	14
PBI-L Mg2+ vs. PBI-H NO	37.80	96.80	-59.00	8.182	3	3	10.20	14
PBI-H Mg2+ vs. PBI-L NO	97.83	54.73	43.10	8.182	3	3	7.450	14
PBI-H Mg2+ vs. PBI-H NO	97.83	96.80	1.033	8.182	3	3	0.1786	14
PBI-L NO vs. PBI-H NO	54.73	96.80	-42.07	8.182	3	3	7.271	14

Descriptive statistics:

	Control	PBI-L Ca2+	PBI-H Ca2+	PBI-L Mg2+	PBI-H Mg2+	PBI-L NO	PBI-H NO
Number of values	3	3	3	3	3	3	3
Minimum	95.70	23.00	98.00	29.40	97.30	48.00	95.60
25% Percentile	95.70	23.00	98.00	29.40	97.30	48.00	95.60
Median	96.90	31.70	98.80	36.90	98.00	51.60	97.40
75% Percentile	98.80	67.00	100.0	47.10	98.20	64.60	97.40
Maximum	98.80	67.00	100.0	47.10	98.20	64.60	97.40
Mean	97.13	40.57	98.93	37.80	97.83	54.73	96.80
Std. Deviation	1.563	23.30	1.007	8.884	0.4726	8.732	1.039
Std. Error of Mean	0.9025	13.45	0.5812	5.129	0.2728	5.042	0.6000
Lower 95% CI	93.25	-17.32	96.43	15.73	96.66	33.04	94.22
Upper 95% CI	101.0	98.45	101.4	59.87	99.01	76.43	99.38

Ordinary one-way ANOVA for solutions:

Table Analyzed	LD-Solutions				
Data sets analyzed	A-I				
ANOVA summary					
F	31.52				
P value	<0.0001				
P value summary	****				
Significant diff. among means (P < 0.05)?	Yes				
R squared	0.9334				
Brown-Forsythe test					
F (DFn, DFd)	2.088 (8, 18)				
P value	0.0928				
P value summary	ns				
Are SDs significantly different (P < 0.05)?	No				
Bartlett's test					
Bartlett's statistic (corrected)					
P value					
P value summary					
Are SDs significantly different (P < 0.05)?					
ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	13278	8	1660	F (8, 18) = 31.52	P<0.0001
Residual (within columns)	947.7	18	52.65		
Total	14226	26			
Data summary					
Number of treatments (columns)	9				
Number of values (total)	27				

Multiple comparisons:

Number of families	1							
Number of comparisons per family	36							
Alpha	0.05							
Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value			
Control vs. PBI-L 20uL	67.30	46.54 to 88.06	Yes	****	<0.0001	A-B		
Control vs. PBI-L 10uL	37.10	16.34 to 57.86	Yes	***	0.0002	A-C		
Control vs. PBI-L 5uL	11.77	-8.992 to 32.53	No	ns	0.5701	A-D		
Control vs. PBI-L 2.5uL	1.767	-18.99 to 22.53	No	ns	>0.9999	A-E		
Control vs. PBI-H 20uL	2.900	-17.86 to 23.66	No	ns	0.9999	A-F		
Control vs. PBI-H 10uL	0.4333	-20.33 to 21.19	No	ns	>0.9999	A-G		
Control vs. PBI-H 5uL	-0.5667	-21.33 to 20.19	No	ns	>0.9999	A-H		
Control vs. PBI-H 2.5uL	0.4333	-20.33 to 21.19	No	ns	>0.9999	A-I		
PBI-L 20uL vs. PBI-L 10uL	-30.20	-50.96 to -9.441	Yes	**	0.0019	B-C		
PBI-L 20uL vs. PBI-L 5uL	-55.53	-76.29 to -34.77	Yes	****	<0.0001	B-D		
PBI-L 20uL vs. PBI-L 2.5uL	-65.53	-86.29 to -44.77	Yes	****	<0.0001	B-E		
PBI-L 20uL vs. PBI-H 20uL	-64.40	-85.16 to -43.64	Yes	****	<0.0001	B-		

						F	
PBI-L 20uL vs. PBI-H 10uL	-66.87	-87.63 to -46.11	Yes	****	<0.0001	B-G	
PBI-L 20uL vs. PBI-H 5uL	-67.87	-88.63 to -47.11	Yes	****	<0.0001	B-H	
PBI-L 20uL vs. PBI-H 2.5uL	-66.87	-87.63 to -46.11	Yes	****	<0.0001	B-I	
PBI-L 10uL vs. PBI-L 5uL	-25.33	-46.09 to -4.575	Yes	*	0.0105	C-D	
PBI-L 10uL vs. PBI-L 2.5uL	-35.33	-56.09 to -14.57	Yes	***	0.0003	C-E	
PBI-L 10uL vs. PBI-H 20uL	-34.20	-54.96 to -13.44	Yes	***	0.0005	C-F	
PBI-L 10uL vs. PBI-H 10uL	-36.67	-57.43 to -15.91	Yes	***	0.0002	C-G	
PBI-L 10uL vs. PBI-H 5uL	-37.67	-58.43 to -16.91	Yes	***	0.0002	C-H	
PBI-L 10uL vs. PBI-H 2.5uL	-36.67	-57.43 to -15.91	Yes	***	0.0002	C-I	
PBI-L 5uL vs. PBI-L 2.5uL	-10.00	-30.76 to 10.76	No	ns	0.7461	D-E	
PBI-L 5uL vs. PBI-H 20uL	-8.867	-29.63 to 11.89	No	ns	0.8433	D-F	
PBI-L 5uL vs. PBI-H 10uL	-11.33	-32.09 to 9.425	No	ns	0.6143	D-G	
PBI-L 5uL vs. PBI-H 5uL	-12.33	-33.09 to 8.425	No	ns	0.5130	D-H	
PBI-L 5uL vs. PBI-H 2.5uL	-11.33	-32.09 to 9.425	No	ns	0.6143	D-I	
PBI-L 2.5uL vs. PBI-H 20uL	1.133	-19.63 to 21.89	No	ns	>0.9999	E-F	
PBI-L 2.5uL vs. PBI-H 10uL	-1.333	-22.09 to 19.43	No	ns	>0.9999	E-G	
PBI-L 2.5uL vs. PBI-H 5uL	-2.333	-23.09 to 18.43	No	ns	>0.9999	E-H	
PBI-L 2.5uL vs. PBI-H 2.5uL	-1.333	-22.09 to 19.43	No	ns	>0.9999	E-I	
PBI-H 20uL vs. PBI-H 10uL	-2.467	-23.23 to 18.29	No	ns	>0.9999	F-G	
PBI-H 20uL vs. PBI-H 5uL	-3.467	-24.23 to 17.29	No	ns	0.9995	F-H	
PBI-H 20uL vs. PBI-H 2.5uL	-2.467	-23.23 to 18.29	No	ns	>0.9999	F-I	
PBI-H 10uL vs. PBI-H 5uL	-1.000	-21.76 to 19.76	No	ns	>0.9999	G-H	
PBI-H 10uL vs. PBI-H 2.5uL	0.000	-20.76 to 20.76	No	ns	>0.9999	G-I	
PBI-H 5uL vs. PBI-H 2.5uL	1.000	-19.76 to 21.76	No	ns	>0.9999	H-I	
Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
Control vs. PBI-L 20uL	97.13	29.83	67.30	5.925	3	3	16.06	18
Control vs. PBI-L 10uL	97.13	60.03	37.10	5.925	3	3	8.856	18
Control vs. PBI-L 5uL	97.13	85.37	11.77	5.925	3	3	2.809	18
Control vs. PBI-L 2.5uL	97.13	95.37	1.767	5.925	3	3	0.4217	18
Control vs. PBI-H 20uL	97.13	94.23	2.900	5.925	3	3	0.6922	18
Control vs. PBI-H 10uL	97.13	96.70	0.4333	5.925	3	3	0.1034	18
Control vs. PBI-H 5uL	97.13	97.70	-0.5667	5.925	3	3	0.1353	18
Control vs. PBI-H 2.5uL	97.13	96.70	0.4333	5.925	3	3	0.1034	18
PBI-L 20uL vs. PBI-L 10uL	29.83	60.03	-30.20	5.925	3	3	7.209	18
PBI-L 20uL vs. PBI-L 5uL	29.83	85.37	-55.53	5.925	3	3	13.26	18
PBI-L 20uL vs. PBI-L 2.5uL	29.83	95.37	-65.53	5.925	3	3	15.64	18
PBI-L 20uL vs. PBI-H 20uL	29.83	94.23	-64.40	5.925	3	3	15.37	18
PBI-L 20uL vs. PBI-H 10uL	29.83	96.70	-66.87	5.925	3	3	15.96	18
PBI-L 20uL vs. PBI-H 5uL	29.83	97.70	-67.87	5.925	3	3	16.20	18
PBI-L 20uL vs. PBI-H 2.5uL	29.83	96.70	-66.87	5.925	3	3	15.96	18
PBI-L 10uL vs. PBI-L 5uL	60.03	85.37	-25.33	5.925	3	3	6.047	18
PBI-L 10uL vs. PBI-L 2.5uL	60.03	95.37	-35.33	5.925	3	3	8.434	18
PBI-L 10uL vs. PBI-H 20uL	60.03	94.23	-34.20	5.925	3	3	8.164	18
PBI-L 10uL vs. PBI-H 10uL	60.03	96.70	-36.67	5.925	3	3	8.753	18
PBI-L 10uL vs. PBI-H 5uL	60.03	97.70	-37.67	5.925	3	3	8.991	18
PBI-L 10uL vs. PBI-H 2.5uL	60.03	96.70	-36.67	5.925	3	3	8.753	18
PBI-L 5uL vs. PBI-L 2.5uL	85.37	95.37	-10.00	5.925	3	3	2.387	18
PBI-L 5uL vs. PBI-H 20uL	85.37	94.23	-8.867	5.925	3	3	2.117	18
PBI-L 5uL vs. PBI-H 10uL	85.37	96.70	-11.33	5.925	3	3	2.705	18
PBI-L 5uL vs. PBI-H 5uL	85.37	97.70	-12.33	5.925	3	3	2.944	18
PBI-L 5uL vs. PBI-H 2.5uL	85.37	96.70	-11.33	5.925	3	3	2.705	18
PBI-L 2.5uL vs. PBI-H 20uL	95.37	94.23	1.133	5.925	3	3	0.2705	18
PBI-L 2.5uL vs. PBI-H 10uL	95.37	96.70	-1.333	5.925	3	3	0.3183	18
PBI-L 2.5uL vs. PBI-H 5uL	95.37	97.70	-2.333	5.925	3	3	0.5570	18
PBI-L 2.5uL vs. PBI-H 2.5uL	95.37	96.70	-1.333	5.925	3	3	0.3183	18

PBI-H 20uL vs. PBI-H 10uL	94.23	96.70	-2.467	5.925	3	3	0.5888	18
PBI-H 20uL vs. PBI-H 5uL	94.23	97.70	-3.467	5.925	3	3	0.8275	18
PBI-H 20uL vs. PBI-H 2.5uL	94.23	96.70	-2.467	5.925	3	3	0.5888	18
PBI-H 10uL vs. PBI-H 5uL	96.70	97.70	-1.000	5.925	3	3	0.2387	18
PBI-H 10uL vs. PBI-H 2.5uL	96.70	96.70	0.000	5.925	3	3	0.000	18
PBI-H 5uL vs. PBI-H 2.5uL	97.70	96.70	1.000	5.925	3	3	0.2387	18

Descriptive statistics:

	Control	PBI-L 20uL	PBI-L 10uL	PBI-L 5uL	PBI-L 2.5uL	PBI-H 20uL	PBI-H 10uL	PBI-H 5uL	PBI-H 2.5uL
Number of values	3	3	3	3	3	3	3	3	3
Minimum	95.70	22.60	43.80	76.50	95.00	92.90	92.00	95.10	94.90
25% Percentile	95.70	22.60	43.80	76.50	95.00	92.90	92.00	95.10	94.90
Median	96.90	29.30	57.90	84.80	95.40	94.20	98.10	98.00	97.00
75% Percentile	98.80	37.60	78.40	94.80	95.70	95.60	100.0	100.0	98.20
Maximum	98.80	37.60	78.40	94.80	95.70	95.60	100.0	100.0	98.20
Mean	97.13	29.83	60.03	85.37	95.37	94.23	96.70	97.70	96.70
Std. Deviation	1.563	7.514	17.40	9.163	0.3512	1.350	4.180	2.464	1.670
Std. Error of Mean	0.9025	4.338	10.04	5.290	0.2028	0.7796	2.413	1.422	0.9644
Lower 95% CI	93.25	11.17	16.81	62.60	94.49	90.88	86.32	91.58	92.55
Upper 95% CI	101.0	48.50	103.3	108.1	96.24	97.59	107.1	103.8	100.8

5. References

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