Supporting Information

Polyproline as a Minimal Antifreeze Protein Mimic That Enhances the Cryopreservation of Cell Monolayers

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Materials

L and D-proline, poly-L-proline mol wt 1,000-10,000 (PPro_{10-100}), ethyl (hydroxyimino) cyanoacetate (OxymaPure™), N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDCI), dichloromethane (DCM), phosphate-buffered saline preformulated tablets, and hydrochloric acid (37%) were purchased from Sigma Aldrich Co Ltd (Gillingham, UK) and used without further purification. Dialysis Membrane Spectra/Por 7 Flexible 38mm FWT 1000 MWCO 4.6 mL/cm was purchased from Fischer Scientific (Loughborough, UK) and used directly. Phosphate-buffered saline (PBS) solution was prepared using preformulated tablets in 200 mL of Milli-Q water (>18.2 Ω mean resistivity) to give [NaCl] = 0.138 M, [KCl] = 0.0027 M, and pH 7.4. PPro_{10} and PPro_{20} (>90%) were purchased bespoke from Peptide Protein Research Ltd (Fareham, UK) and were used without further purification. PPro_{10}: m/z (ESI) 988.0 (100%, -1); PPro_{20}: m/z (ESI) 491.0 (20%, +4), 654.3 (100%, +3), 981.0 (30%, +2).

Physical and analytical methods

SEC (size exclusion chromatography) was acquired a DMF Agilent 390-LC MDS instrument equipped with differential refractive index (DRI), viscometry (VS), dual angle light scatter (LS) and dual wavelength UV detectors. The system was equipped with 2 x PLgel Mixed D columns (300 x 7.5 mm) and a PLgel 5 µm guard column. The eluent is DMF with 5 mmol NH4BF4 additive. Samples were run at 1 mL/min at 50°C. Poly(methyl methacrylate) standards (Agilent EasyVials) were used for calibration. Analyte samples were filtered through a nylon membrane with 0.22 µm pore size before injection. Respectively, experimental molar mass (Mn,SEC) and dispersity (D) values of synthesized polymers were determined by conventional calibration (relative to poly(methyl methacrylate) standards) using Agilent GPC/SEC software. Refractive index recorded.
General procedure for the synthesis of polyproline peptides PPro

EDCI (0.50 g, 2.60 mmol) was dissolved in dry DCM (20 mL) and stirred at room temperature under a flow of nitrogen for 20 minutes, followed by cooling to 0 °C. Within 5 minutes of cooling, L-proline (0.30 g, 2.60 mmol, 1 eqv) and OxymaPure™ (0.37 g, 2.60 mmol, 1 eqv) were added together to the reaction mixture, resulting in an instantaneous colour change to yellow. The mixture was stirred on ice under nitrogen for 1 further hour, and then warmed to RT with stirring overnight. The dark yellow solution was condensed in vacuo, dissolved in Milli-Q water (10 mL) acidified to pH 3-4 with 3M HCl, and a minimum volume of methanol added until residual solids dissolved. Dialysis (> 1 kDa) for 48 hours was subsequently performed with regular water changes. The resulting solution was freeze dried, yielding an off-white solid. 31.4 mg (10.4%). The DL racemate, P(DL)Pro
, utilised a 1:1 ratio of L- and D-proline (2.60 mmol prolines).

Ice recrystallisation inhibition (splat) assay

Ice recrystallisation inhibition was measured using a modified splat assay. A 10 µL sample of polymer dissolved in PBS buffer (pH 7.4) was dropped 1.40 m onto a chilled glass coverslip, resting on a thin aluminium block placed on dry ice. Upon hitting the coverslip, a wafer with diameter of approximately 10 mm and thickness 10 µm was formed instantaneously. The glass coverslip was transferred onto the Linkam cryostage and held at -8°C under N₂ for 30 minutes. Photographs were obtained using an Olympus CX 41 microscope with a UIS-2 20x/0.45/∞/0-2/FN22 lens and crossed polarizers (Olympus Ltd, Southend-on-Sea, UK), equipped with a Canon DSLR 500D digital camera. Images were taken of the initial wafer (to ensure that a polycrystalline sample had been obtained) and again after 30 minutes. Image processing was conducting using Image J, which is freely available. In brief, five of the largest ice crystals in the field of view were measured and the single largest length in any axis recorded. The average
(mean) of these five measurements was then calculated to find the largest grain dimension along any axis. This was repeated for three individual wafers, and the average (mean) of these three values was calculated to give the mean largest grain size (MLGS). The average value was compared to that of a PBS buffer negative control.

**Surface hydrophobicity mapping of proteins**

NMR solution phase (AFP Sculpin) and X-Ray crystal structures of proteins and peptides of interest were acquired from the Protein Data Bank and other publically accessible sources, or computationally modelled in-house (PPro\textsubscript{10} and PGl\textsubscript{u}10). Structures were rendered in PyMOL (Schrödinger LLC, Cambridge, MA), which is freely available for educational use, and surfaces on the structures were displayed. An open source script “color\_h” was used to colour the protein surface according to the Eisenberg hydrophobicity scale of its constituent amino acids, from red (hydrophobic) to white (hydrophilic). For the homo-polypeptides where scaling is not possible, aliphatic hydrogen and carbon were defined as hydrophobic whilst oxygen, hydrogen and nitrogen as hydrophilic, utilising the same colour scheme. Due to the lack of hydrogen bond donors in a PPro\textsubscript{10} PPII helix, this was considered representative.

**Cell culture**

Human Caucasian lung carcinoma cells (A549) were obtained from the European Collection of Authenticated Cell Cultures (Salisbury, UK) and grown in 175 cm\textsuperscript{2} cell culture Nunc flasks (Corning Incorporated, Corning, NY). Standard cell culture medium was composed of Ham's F-12K (Kaighn's) Medium (F-12K) (Gibco, Paisley, UK) supplemented with 10% USA-origin foetal bovine serum (FBS) purchased from Sigma Aldrich Co Ltd (Gillingham, UK), 100 units/mL penicillin, 100 µg/mL streptomycin, and 250 ng/mL amphotericin B (PSA) (HyClone, Cramlington, UK). A549 cells were maintained in a humidified atmosphere of 5% CO\textsubscript{2} and 95% air at 37 °C and the culture medium was renewed every 3–4 days. The cells were
subcultured every 7 days or before reaching 90% confluency. To subculture, cells were dissociated using 0.25% trypsin plus 1 mM EDTA in balanced salt solution (Gibco) and reseeded at 1.87X10^5 cells per 175 cm^2 cell culture flasks.

**Cell solution preparation**

Solutions for cell incubation experiments were prepared by dissolving the individual compounds in F-12K supplemented with 10% FBS and 1X PSA (solutions used as freezing buffers did not contain PSA) and sterile filtered prior to use.

**Cryopreservation of A549 cell monolayers**

Cells to be frozen in the monolayer format were seeded at 0.4X10^6 cells per well in 500 µL of cell culture medium in 24-well plates (Corning Incorporated, Corning, NY). Plates had a total available volume of 3.4 mL with an approximate growth area of 1.9 cm^2, no coverslips were used and plates were used with the accompanying lid. Cells were allowed to attach to the entire free surface of the bottom of the well and formed a confluent layer not greater in height than one cell. Before experimental treatments, cells were allowed to attach for 2 h to the plates in a humidified atmosphere of 5% CO_2 and 95% air at 37 °C. The medium was exchanged against medium that was or was not supplemented with solutes as indicated in the figure. Control cells received no additional solutes and experimental cells were incubated with 23.1 mg/mL L-proline for 24 h in a humidified atmosphere of 5% CO_2 and 95% air at 37 °C. Following the incubation period, the culture medium was removed and cells were exposed for 10 min at room temperature to different concentrations of solutes dissolved in F-12K supplemented with 10% FBS and 10% DMSO. After 10 min, the freezing solutions were removed and the plates placed inside a CoolCell® MP plate (BioCision, LLC, Larkspur, CA), transferred to a -80 °C freezer and frozen at a rate of 1 °C/min. After 24 h at -80 °C, cells were rapidly thawed by addition of 500 µL cell culture medium warmed to 37 °C. Cells were placed in a humidified atmosphere
for 24 h and then dissociated using 0.25% trypsin plus 1 mM EDTA in balanced salt solution. The number of viable cells was then determined by counting with a haemocytometer (Sigma Aldrich Co Ltd) at room temperature after 1:1 dilution of the sample with 0.4% trypan blue solution (Sigma Aldrich Co Ltd). The initial cell medium was discarded such that any non-attached cells were not included in the assessment. The percentage of recovered cells was calculated by dividing the number of cells with intact membranes after freezing and thawing by the number of cells present prior to freezing (i.e. after application of pre-treatments), multiplied by 100.

Cytotoxicity Measurements

Cells were seeded at 6·10⁴ cells per well in 200 µL of cell culture medium with indicated concentrations of polyproline in 96-well plates (ThermoFisher). Cells were incubated for 24 h in a humidified atmosphere of 5% CO₂ and 95% air at 37 °C. Following the incubation period, resazurin sodium salt (Sigma Aldrich) was dissolved in phosphate buffered saline (Sigma Aldrich) and added to wells in an amount of 1/10th initial well volume. Absorbance was measured at 570/600 nm every 60 minutes until control cells reached ~70% reduction, and the viability reported relative to the control cells.

Haemolysis assay

Samples containing 250 µL ovine red blood cells (RBCs) and 250 µL of PPro solution (at indicated concentration) were incubated at 37 °C for 1 hour. After centrifugation, 10 µL of the supernatant was added to 90 µL of PBS buffer in a 96 well plate. The absorbance was measured at 450 nm and compared against a PBS buffer and deionised water (to lyse cells) controls to determine the % haemolysis relative to the controls.
**Statistical analyses**

Data were analysed with a one-way analysis of variance (ANOVA) on ranks followed by comparison of experimental groups with the appropriate control group (Holm–Sidak method) followed by Tukey’s post hoc test. Excel 2013 (Microsoft, Redmond, WA) and R (R Foundation for Statistical Computing, Vienna, Austria) were used for the analyses. Data sets are presented as mean ± (SEM).

**Confocal Microscopy sample preparation and measurements**

The solution of 20 mg/mL of poly(proline) in PBS was prepared as previously reported. This solution was stained with 60 µL/mL of 1 mM solution of Sulphorhodamine B in deionized water. The fluorophore absorbs the 552 nm wavelength laser light and emits in a wide band: The fluorescent light was integrated in the 575-625 nm range. Water at the liquid/solid phase transition expels all ions, such that the ice crystals will be constituted by pure water. In this way, we can highlight the ice crystals that upon freezing will grow in the solution as black bodies in the fluorescent liquid. The experimental setup for a similar experiment has been described elsewhere. A thin Hale-Shaw cell was built as follows: on a squared thin glass slide (20 x 20 mm², thickness \( \lambda \approx 170 \, \mu m, \) VWR) a 10 µl drop was deposited by using a pre-calibrated pipette. The sessile drop was carefully covered with a circular thin glass slide (Ø=15 mm, \( \lambda \approx 170 \, \mu m, \) VWR). The circular contour of such Hale-Shaw cell was sealed with nail polish (L'Oreal). With time, the solvent of the seal eventually evaporates, making the seal porous. The sample was therefore rapidly mounted on the cryostage and frozen only once, while the seal was still effective. The sample was discarded after the experiment.
Confocal Microscopy Measurement

To simulate – to a first approximation – the protocol of splat experiments reported elsewhere, the sample was brought as quickly as possible (a few minutes) at the minimum temperature $T_{\text{min}} \approx -22^\circ\text{C}$ that the cryostage can sustain. Nevertheless, this protocol is largely unable to provide the same crystallization feature of the splat experiments owing to the ice crystallization in a fraction of a second being more feasible with the latter. At a temperature below $-15^\circ\text{C}$ ice crystals burst from the cold side towards the hot side. The rapid imaging rate of the microscope is able to capture the growing ice crystal and the flow of fluorescent liquid.
Additional Data

Figure S1. Circular dichroism spectra. Synthesised proline polypeptides compared to a polyproline II helical reference (PPro (II) Helix) not corrected for concentration to enable comparison against reference standard.

Figure S2. SPLAT assays. A) PBS control; B) L-proline, 20 mg.mL⁻¹. Scale bar = 100 μm.
Figure S3. Hemolysis (1 hour at 37 °C) using PPro\textsubscript{10-25}. Values reported relative to a positive control for 100 % lysis. Errors bars represent the standard deviation from n = 3.
Figure S4. PPro_{10} ESI Mass Spec (Provided by Supplier)
Figure S5. PPro$_{20}$ ESI Mass Spec (Provided by Supplier)
Cytotoxicity of PPro upon extended exposure to A549 Cells.

The cryopreservation method introduced in this work only requires exposure of the IRI active polymer (in this case PPro) for 10 minutes (see experimental, above). After which time the media is removed, and the monolayer frozen, with minimal liquid (which in turn reduces the opportunities for unwanted ice growth). The cells are also thawed by addition of warm media, hence diluting the PPro. Considering this, the cells are only exposed to solutions of poly(proline) for short periods of time, and essentially at dilute conditions. To evaluate the cytotoxicity of polyproline, A549 cells were exposed to PPro (including concentration above that which is optimal for cryopreservation) for 24 hours, and the cell viability determined (Figure S5). Not that 5 mg.mL⁻¹ PPro lead to a reduction in viability, which indicates some cytotoxicity. It should be noted this is still better than what would be expected for DMSO solution and was only observed for these very long exposure times. These results will guide the development of this cryoprotectant in terms of optimising exposure times.

![Figure S6](image)

**Figure S6.** A549 Cell recovery after 24 hours exposure to PPRo as determined by alamar blue (resazurin) reduction. Error bars ± S.E.M from n=3; *P < 0.05 compared to control (0 mg.mL⁻¹ proline).
Data points for Figure 1

CD DATA (Figure 1A)

| λ (nm) | [θ] Pro\textsubscript{15} | λ (nm) | [θ] P(D)Pro\textsubscript{15} | λ (nm) | [θ] P(DL)Pro\textsubscript{14,24} |
|--------|----------------|--------|-------------------------------|--------|-------------------------|
| 260    | 550394.31     | 260    | 442165.5                      | 260    | 246849.8                |
| 259.8  | 582274.75     | 259.8  | 510005.7                      | 259.8  | 264495.4                |
| 259.6  | 596721.98     | 259.6  | 534083.9                      | 259.6  | 303294.1                |
| 259.4  | 566510.99     | 259.4  | 519574.4                      | 259.4  | 297284.2                |
| 259.2  | 505907.36     | 259.2  | 497586.1                      | 259.2  | 300964.2                |
| 259    | 454927.72     | 259    | 455692.4                      | 259    | 277777.9                |
| 258.8  | 410229.71     | 258.8  | 414608.3                      | 258.8  | 266765.5                |
| 258.6  | 341749.39     | 258.6  | 355809.1                      | 258.6  | 254649.1                |
| 258.4  | 294305.87     | 258.4  | 312592                        | 258.4  | 240835.3                |
| 258.2  | 262742.02     | 258.2  | 275324.3                      | 258.2  | 201215.5                |
| 258    | 227744.12     | 258    | 233517.1                      | 258    | 149104.9                |
| 257.8  | 170125.43     | 257.8  | 189089                        | 257.8  | 70944.19                |
| 257.6  | 135429.59     | 257.6  | 180836.9                      | 257.6  | 24049.49                |
| 257.4  | 146414.91     | 257.4  | 206591.4                      | 257.4  | 17041.87                |
| 257.2  | 122097.34     | 257.2  | 193249.7                      | 257.2  | -5892.44                |
| 257    | 141808.61     | 257    | 185244.9                      | 257    | -9680.93                |
| 256.8  | 128960.77     | 256.8  | 134643.3                      | 256.8  | -67076.1                |
| 256.6  | 103983.20     | 256.6  | 103988.4                      | 256.6  | -90315.3                |
| 256.4  | 81794.745     | 256.4  | 62922.87                      | 256.4  | -127594                 |
| 256.2  | 33956.786     | 256.2  | 50261.17                      | 256.2  | -158901                 |
| 256    | 20625.925     | 256    | 85558.88                      | 256    | -151209                 |
| 255.8  | -1639.2356    | 255.8  | 108190.9                      | 255.8  | -159768                 |
| 255.6  | 0             | 255.6  | 100738.8                      | 255.6  | -134455                 |
| 255.4  | 27955.243     | 255.4  | 104845.1                      | 255.4  | -114280                 |
| 255.2  | 48301.254     | 255.2  | 90398.9                       | 255.2  | -139461                 |
| 255    | 44177.972     | 255    | 47142.15                      | 255    | -162404                 |
| 254.8  | 77085.34      | 254.8  | 25633.41                      | 254.8  | -128463                 |
| 254.6  | 149172.191    | 254.6  | 44960.62                      | 254.6  | -83827.4                |
| 254.4  | 190383.04     | 254.4  | 79531.04                      | 254.4  | -38265.6                |
| 254.2  | 151891.232    | 254.2  | 49685.77                      | 254.2  | -32756.8                |
| 254    | 117403.855    | 254    | -2164.66                      | 254    | -45794.4                |
| 253.8  | 118977.117    | 253.8  | -12560.1                      | 253.8  | -21327.3                |
| 253.6  | 106699.653    | 253.6  | 29511.38                      | 253.6  | 16661.18                |
| 253.4  | 82690.886     | 253.4  | 42571.49                      | 253.4  | -8130.57                |
| 253.2  | 53692.972     | 253.2  | 45821.13                      | 253.2  | -47112.5                |
| 253    | 40658.979     | 253    | 82936.2                       | 253    | -48168                  |
| 252.8  | 60361.084     | 252.8  | 81673.13                      | 252.8  | -49672.6                |
| 252.6  | 42497.796     | 252.6  | 12590.32                      | 252.6  | -57747.7                |
| 252.4  | 48354.019     | 252.4  | 0                             | 252.4  | -54393.9                |
| 252.2 | 52651.512 | 252.2 | 8467.018 | 252.2 | -88725.5 |
| 252  | 57247.43  | 252  | 19634.64 | 252  | -109362  |
| 251.8 | 104930.901| 251.8 | 88325.32 | 251.8 | -75669.8 |
| 251.6 | 100547.6  | 251.6 | 121333.3 | 251.6 | -97280.3 |
| 251.4 | 73496.455 | 251.4 | 146685.3 | 251.4 | -62972.2 |
| 251.2 | 45569.065 | 251.2 | 144041.7 | 251.2 | -51501.4 |
| 251  | 88148.517 | 251  | 187288.1 | 251  | 0        |
| 250.8 | 156837.994| 250.8 | 285069.4 | 250.8 | 118206.7 |
| 250.6 | 149092.957| 250.6 | 340199.3 | 250.6 | 170051.4 |
| 250.4 | 87623.289 | 250.4 | 321998.7 | 250.4 | 120445   |
| 250.2 | 56966.305 | 250.2 | 332580.4 | 250.2 | 111415.7 |
| 250  | 59937.407 | 250  | 360014.7 | 250  | 151522.9 |
| 249.8 | 84503.061 | 249.8 | 351100   | 249.8 | 189975.6 |
| 249.6 | 28765.748 | 249.6 | 277396.9 | 249.6 | 120500.9 |
| 249.4 | -25167.521| 249.4 | 254481.3 | 249.4 | 70335.61 |
| 249.2 | -41124.868| 249.2 | 277227.3 | 249.2 | 93306.86 |
| 249  | -42493.644| 249  | 290166   | 249  | 135765.8 |
| 248.8 | -51087.419| 248.8 | 290015.5 | 248.8 | 155311.4 |
| 248.6 | -95344.452| 248.6 | 287382.4 | 248.6 | 155712.8 |
| 248.4 | -157497.99| 248.4 | 221704.7 | 248.4 | 107692.9 |
| 248.2 | -172823.19| 248.2 | 173467.1 | 248.2 | 67066.62 |
| 248  | -176830.22| 248  | 101054.3 | 248  | -17248.9 |
| 247.8 | -198612.65| 247.8 | 43082.36 | 247.8 | -56845   |
| 247.6 | -262209.18| 247.6 | -66126.5 | 247.6 | -134735  |
| 247.4 | -298743.32| 247.4 | -160436  | 247.4 | -210730  |
| 247.2 | -245082.18| 247.2 | -194064  | 247.2 | -230189  |
| 247  | -204655.21| 247  | -206929  | 247  | -223253  |
| 246.8 | -228166.24| 246.8 | -324574  | 246.8 | -303276  |
| 246.6 | -238049.73| 246.6 | -394431  | 246.6 | -388498  |
| 246.4 | -214855.62| 246.4 | -421596  | 246.4 | -435197  |
| 246.2 | -176171.09| 246.2 | -407873  | 246.2 | -396674  |
| 246  | -134956.78| 246  | -397147  | 246  | -347719  |
| 245.8 | -132472.67| 245.8 | -420594  | 245.8 | -376158  |
| 245.6 | -166716.12| 245.6 | -466126  | 245.6 | -458632  |
| 245.4 | -224375.81| 245.4 | -536364  | 245.4 | -531684  |
| 245.2 | -248872.61| 245.2 | -591264  | 245.2 | -580718  |
| 245  | -242390.3 | 245  | -602877  | 245  | -585566  |
| 244.8 | -261010.29| 244.8 | -629187  | 244.8 | -620908  |
| 244.6 | -233053.49| 244.6 | -593655  | 244.6 | -587096  |
| 244.4 | -188462.74| 244.4 | -578574  | 244.4 | -591500  |
| 244.2 | -143811.96| 244.2 | -564407  | 244.2 | -587875  |
| 244  | -142914.44| 244  | -548977  | 244  | -571003  |
| 243.8 | -148699.56| 243.8 | -537988  | 243.8 | -539456  |
| 243.6 | -155345.18| 243.6 | -522924  | 243.6 | -481024  |
| 243.4 | -156152.91 | 243.4 | -514000 | 243.4 | -411760 |
| 243.2 | -195474.43 | 243.2 | -550368 | 243.2 | -390002 |
| 243  | -219611.39 | 243  | -611583 | 243  | -388564 |
| 242.8 | -210669.02 | 242.8 | -690189 | 242.8 | -430933 |
| 242.6 | -151656.13 | 242.6 | -718030 | 242.6 | -447520 |
| 242.4 | -94874.584 | 242.4 | -748538 | 242.4 | -462033 |
| 242.2 | -60675.079 | 242.2 | -785934 | 242.2 | -491448 |
| 242  | -8431.3799 | 242  | -831279 | 242  | -520803 |
| 241.8 | 57767.468 | 241.8 | -862729 | 241.8 | -485509 |
| 241.6 | 63318.865 | 241.6 | -877280 | 241.6 | -495648 |
| 241.4 | 77563.339 | 241.4 | -890931 | 241.4 | -480916 |
| 241.2 | 84351.167 | 241.2 | -923867 | 241.2 | -452392 |
| 241  | 101002.763 | 241  | -998890 | 241  | -491229 |
| 240.8 | 86150.194 | 240.8 | -1069519 | 240.8 | -575948 |
| 240.6 | 114431.369 | 240.6 | -1171070 | 240.6 | -656496 |
| 240.4 | 162689.027 | 240.4 | -1237981 | 240.4 | -726409 |
| 240.2 | 277509.3 | 240.2 | -1282525 | 240.2 | -738351 |
| 240  | 420602.79 | 240  | -1297453 | 240  | -732557 |
| 239.8 | 526669.09 | 239.8 | -1297360 | 239.8 | -753659 |
| 239.6 | 580105.33 | 239.6 | -1308146 | 239.6 | -744075 |
| 239.4 | 633915.25 | 239.4 | -1352443 | 239.4 | -734993 |
| 239.2 | 725414.95 | 239.2 | -1394774 | 239.2 | -721731 |
| 239  | 790796.84 | 239  | -1447522 | 239  | -681803 |
| 238.8 | 876360.91 | 238.8 | -1486584 | 238.8 | -610225 |
| 238.6 | 905919.69 | 238.6 | -1538437 | 238.6 | -608456 |
| 238.4 | 934603.09 | 238.4 | -1591719 | 238.4 | -624712 |
| 238.2 | 967746.43 | 238.2 | -1711492 | 238.2 | -650820 |
| 238  | 1016082.63 | 238  | -1815514 | 238  | -707071 |
| 237.8 | 1051180.87 | 237.8 | -1909868 | 237.8 | -763308 |
| 237.6 | 1062417.22 | 237.6 | -1994517 | 237.6 | -784617 |
| 237.4 | 1136893.72 | 237.4 | -2099078 | 237.4 | -783056 |
| 237.2 | 1186191.8 | 237.2 | -2217739 | 237.2 | -799087 |
| 237  | 1254096.03 | 237  | -2293219 | 237  | -761592 |
| 236.8 | 1350553.91 | 236.8 | -2374460 | 236.8 | -766208 |
| 236.6 | 1451551.31 | 236.6 | -2472637 | 236.6 | -776464 |
| 236.4 | 1603320.75 | 236.4 | -2535505 | 236.4 | -698202 |
| 236.2 | 1729972.32 | 236.2 | -2599913 | 236.2 | -683371 |
| 236  | 1837830.9 | 236  | -2657903 | 236  | -659511 |
| 235.8 | 1924227.1 | 235.8 | -2748451 | 235.8 | -618893 |
| 235.6 | 1993634.7 | 235.6 | -2873098 | 235.6 | -655502 |
| 235.4 | 2109440.9 | 235.4 | -2970410 | 235.4 | -670547 |
| 235.2 | 2341693.4 | 235.2 | -3030891 | 235.2 | -604797 |
| 235  | 2541491.1 | 235  | -3078051 | 235  | -522217 |
| 234.8 | 2701377.7 | 234.8 | -3160226 | 234.8 | -490454 |
|   |     |     |     |     |     |
|---|-----|-----|-----|-----|-----|
| 234.6 | 2881661 | 234.6 | -3165416 | 234.6 | -373161 |
| 234.4 | 3068795.1 | 234.4 | -3197853 | 234.4 | -274220 |
| 234.2 | 3263627.7 | 234.2 | -3199254 | 234.2 | -119587 |
| 234 | 3456021 | 234 | -3157648 | 234 | 18089.52 |
| 233.8 | 3595130.3 | 233.8 | -3149327 | 233.8 | 81351.46 |
| 233.6 | 3637930.5 | 233.6 | -3248421 | 233.6 | 68245.14 |
| 233.4 | 3686301.3 | 233.4 | -3323884 | 233.4 | 121713 |
| 233.2 | 3748858.1 | 233.2 | -3385299 | 233.2 | 166836.9 |
| 233 | 3831777 | 233 | -3482006 | 233 | 190507.2 |
| 232.8 | 3933241.5 | 232.8 | -3581515 | 232.8 | 193263.7 |
| 232.6 | 3992822.7 | 232.6 | -3683430 | 232.6 | 153810.2 |
| 232.4 | 4030225.3 | 232.4 | -3760190 | 232.4 | 67833.44 |
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| 232 | 4154664.2 | 232 | -3863298 | 232 | 48639.71 |
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| 231.6 | 4247288.4 | 231.6 | -3908139 | 231.6 | 56946.16 |
| 231.4 | 4295884.1 | 231.4 | -3937584 | 231.4 | 31518.74 |
| 231.2 | 4282649.6 | 231.2 | -4016974 | 231.2 | -24384.1 |
| 231 | 4238292.4 | 231 | -4100290 | 231 | -76701.1 |
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| 230.4 | 4138333 | 230.4 | -4277944 | 230.4 | -261945 |
| 230.2 | 4148574.6 | 230.2 | -4233206 | 230.2 | -279096 |
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| 229.8 | 3891046.8 | 229.8 | -4277339 | 229.8 | -411633 |
| 229.6 | 3768441.7 | 229.6 | -4282529 | 229.6 | -502315 |
| 229.4 | 3673949.1 | 229.4 | -4189437 | 229.4 | -476121 |
| 229.2 | 3675748.3 | 229.2 | -4038149 | 229.2 | -432697 |
| 229 | 3682097.4 | 229 | -3926771 | 229 | -430620 |
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| 228.4 | 3152354.1 | 228.4 | -3578609 | 228.4 | -475141 |
| 228.2 | 2920914.7 | 228.2 | -3486573 | 228.2 | -545825 |
| 228 | 2718089.5 | 228 | -3286862 | 228 | -581452 |
| 227.8 | 2621244.1 | 227.8 | -3004110 | 227.8 | -553302 |
| 227.6 | 2478968.9 | 227.6 | -2686967 | 227.6 | -544778 |
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| 227.2 | 2072349.7 | 227.2 | -2094234 | 227.2 | -455630 |
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| 226 | 289069.16 | 226 | 212054.8 | 226 | -733799 |
| 225.8 | -206387.27 | 225.8 | 624076.7 | 225.8 | -865223 |
| 225.6 | -671861.07 | 225.6 | 1149182 | 225.6 | -835330 |
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| 223.8 | -5930318.9 | 223.8 | 7063140 | 223.8 | -889481 |
| 223.6 | -6674859 | 223.6 | 7878420 | 223.6 | -856053 |
| 223.4 | -7435297.8 | 223.4 | 8700654 | 223.4 | -838458 |
| 223.2 | -8100932.6 | 223.2 | 9596068 | 223.2 | -676662 |
| 223  | -8756775.6 | 223  | 10497069 | 223  | -474108 |
| 222.8 | -9523390.5 | 222.8 | 11516887 | 222.8 | -283033 |
| 222.6 | -10370952 | 222.6 | 12568554 | 222.6 | -852088 |
| 222.4 | -11147982 | 222.4 | 13643749 | 222.4 | 134360 |
| 222.2 | -11933350 | 222.2 | 14646907 | 222.2 | 234579.3 |
| 222  | -12795374 | 222  | 15554966 | 222  | 258338.3 |
| 221.8 | -13619546 | 221.8 | 16476780 | 221.8 | 296962.2 |
| 221.6 | -14422578 | 221.6 | 17520056 | 221.6 | 399167.3 |
| 221.4 | -15262856 | 221.4 | 18541621 | 221.4 | 474545.2 |
| 221.2 | -16155519 | 221.2 | 19675982 | 221.2 | 507642.2 |
| 221  | -16987441 | 221  | 20793389 | 221  | 538940.6 |
| 220.8 | -17994595 | 220.8 | 21975671 | 220.8 | 558014.5 |
| 220.6 | -18962876 | 220.6 | 23262272 | 220.6 | 580584.4 |
| 220.4 | -19912646 | 220.4 | 24491956 | 220.4 | 578351.1 |
| 220.2 | -20932308 | 220.2 | 25770080 | 220.2 | 578551.2 |
| 220  | -21907163 | 220  | 27083669 | 220  | 565657.4 |
| 219.8 | -23038064 | 219.8 | 28364215 | 219.8 | 447639.8 |
| 219.6 | -24170522 | 219.6 | 29542518 | 219.6 | 381873.6 |
| 219.4 | -25254367 | 219.4 | 30737775 | 219.4 | 417111.9 |
| 219.2 | -26314857 | 219.2 | 31891166 | 219.2 | 433881.2 |
| 219  | -27449391 | 219  | 33139707 | 219  | 393898 |
| 218.8 | -28559532 | 218.8 | 34550176 | 218.8 | 509197 |
| 218.6 | -29722784 | 218.6 | 36057179 | 218.6 | 624399.4 |
| 218.4 | -30944510 | 218.4 | 37383743 | 218.4 | 696246.8 |
| 218.2 | -32143919 | 218.2 | 38630208 | 218.2 | 680583.8 |
| 218  | -33295926 | 218  | 39993448 | 218  | 683509.4 |
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| 217.6 | -35609628 | 217.6 | 42917840 | 217.6 | 949587.2 |
| 217.4 | -36739491 | 217.4 | 44245788 | 217.4 | 1035929 |
| 217.2 | -37993049 | 217.2 | 45533427 | 217.2 | 1015648 |
|   |       |   |       |   |       |   |
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| 216.8| -40532170 | 216.8| 48014247 | 216.8| 1044032 |
| 216.6| -41717566 | 216.6| 49209158 | 216.6| 1145471 |
| 216.4| -42837914 | 216.4| 50521536 | 216.4| 1259006 |
| 216.2| -43979887 | 216.2| 51576317 | 216.2| 1265665 |
| 216| -45109750 | 216| 52810326 | 216| 1248012 |
| 215.8| -46112458 | 215.8| 54056791 | 215.8| 1328618 |
| 215.6| -47095790 | 215.6| 55275057 | 215.6| 1385543 |
| 215.4| -48175483 | 215.4| 56378278 | 215.4| 1418718 |
| 215.2| -49306211 | 215.2| 57637545 | 215.2| 1410022 |
| 215| -50441610 | 215| 59119809 | 215| 1499911 |
| 214.8| -51572338 | 214.8| 60429419 | 214.8| 1561863 |
| 214.6| -52631098 | 214.6| 61696990 | 214.6| 1704008 |
| 214.4| -53583636 | 214.4| 62823566 | 214.4| 1880512 |
| 214.2| -54473721 | 214.2| 64132830 | 214.2| 1955262 |
| 214| -55397195 | 214| 65285183 | 214| 2044679 |
| 213.8| -56318593 | 213.8| 66429059 | 213.8| 2188054 |
| 213.6| -57199336 | 213.6| 67567918 | 213.6| 2305106 |
| 213.4| -58131806 | 213.4| 68634463 | 213.4| 2526113 |
| 213.2| -59084690 | 213.2| 69795466 | 213.2| 2681639 |
| 213| -60048819 | 213| 71025150 | 213| 2742888 |
| 212.8| -60965719 | 212.8| 72383892 | 212.8| 2863799 |
| 212.6| -62019808 | 212.6| 73478290 | 212.6| 3003777 |
| 212.4| -63087737 | 212.4| 74456086 | 212.4| 3149505 |
| 212.2| -64141307 | 212.2| 75387172 | 212.2| 3299902 |
| 212| -64866004 | 212| 76351647 | 212| 3386037 |
| 211.8| -65551776 | 211.8| 77114577 | 211.8| 3361519 |
| 211.6| -66176825 | 211.6| 77645341 | 211.6| 3339278 |
| 211.4| -66733712 | 211.4| 78308277 | 211.4| 3371708 |
| 211.2| -67460312 | 211.2| 78908241 | 211.2| 3309033 |
| 211| -68106467 | 211| 79600668 | 211| 3261239 |
| 210.8| -68794142 | 210.8| 80420780 | 210.8| 3292864 |
| 210.6| -69409157 | 210.6| 81240627 | 210.6| 3296751 |
| 210.4| -70002201 | 210.4| 82042136 | 210.4| 3336495 |
| 210.2| -70640744 | 210.2| 82885511 | 210.2| 3353929 |
| 210| -71085008 | 210| 83668163 | 210| 3321890 |
| 209.8| -71372707 | 209.8| 84240966 | 209.8| 3374537 |
| 209.6| -71701580 | 209.6| 84597000 | 209.6| 3445170 |
| 209.4| -72111763 | 209.4| 84934004 | 209.4| 3435510 |
| 209.2| -72340296 | 209.2| 85502309 | 209.2| 3425344 |
| 209| -72558103 | 209| 86114902 | 209| 3377366 |
| 208.8| -72855490 | 208.8| 86765555 | 208.8| 3367223 |
| 208.6| -73208583 | 208.6| 86867279 | 208.6| 3521208 |
| 208.4| -73499915 | 208.4| 86891845 | 208.4| 3759419 |
|   | 208.2 | -73622226 | 208.2 | 87351852 | 208.2 | 3822324 |
|---|---|---|---|---|---|---|
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|   | 207.8 | -74000231 | 207.8 | 87768090 | 207.8 | 3975665 |
|   | 207.6 | -74182227 | 207.6 | 87629517 | 207.6 | 4017571 |
|   | 207.4 | -74349172 | 207.4 | 87372439 | 207.4 | 4037834 |
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|   | 207 | -73966496 | 207 | 86536849 | 207 | 4155364 |
|   | 206.8 | -73576727 | 206.8 | 86154692 | 206.8 | 4236508 |
|   | 206.6 | -73416010 | 206.6 | 85677212 | 206.6 | 4262107 |
|   | 206.4 | -73482096 | 206.4 | 85089185 | 206.4 | 4216107 |
|   | 206.2 | -73284530 | 206.2 | 84829339 | 206.2 | 4296791 |
|   | 206 | -73042157 | 206 | 84463098 | 206 | 4280921 |
|   | 205.8 | -72832308 | 205.8 | 83933372 | 205.8 | 4336972 |
|   | 205.6 | -72338047 | 205.6 | 83338771 | 205.6 | 4283497 |
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|   | 205.2 | -71037606 | 205.2 | 82309767 | 205.2 | 4076704 |
|   | 205 | -70477605 | 205 | 81533757 | 205 | 4120289 |
|   | 204.8 | -70238346 | 204.8 | 81540090 | 204.8 | 4162701 |
|   | 204.6 | -69873148 | 204.6 | 81033892 | 204.6 | 4147015 |
|   | 204.4 | -69310374 | 204.4 | 80265080 | 204.4 | 4145980 |
|   | 204.2 | -68689477 | 204.2 | 79500593 | 204.2 | 4109525 |
|   | 204 | -68002148 | 204 | 78703236 | 204 | 4095610 |
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|   | 203.6 | -66829554 | 203.6 | 77789277 | 203.6 | 3967684 |
|   | 203.4 | -66087384 | 203.4 | 77058179 | 203.4 | 3932402 |
|   | 203.2 | -65305251 | 203.2 | 76510807 | 203.2 | 3976355 |
|   | 203 | -64354097 | 203 | 76016027 | 203 | 3936335 |
|   | 202.8 | -63317481 | 202.8 | 75370737 | 202.8 | 3860527 |
|   | 202.6 | -62211319 | 202.6 | 74632027 | 202.6 | 3771954 |
|   | 202.4 | -61161555 | 202.4 | 73922208 | 202.4 | 3826372 |
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|   | 201.2 | -55855299 | 201.2 | 68421327 | 201.2 | 3951009 |
|   | 201 | -54789619 | 201 | 68104910 | 201 | 4025529 |
|   | 200.8 | -53836043 | 200.8 | 67587640 | 200.8 | 4096760 |
|   | 200.6 | -52934194 | 200.6 | 67034040 | 200.6 | 4183999 |
|   | 200.4 | -52223510 | 200.4 | 66103819 | 200.4 | 4145773 |
|   | 200.2 | -51377367 | 200.2 | 65294066 | 200.2 | 4169739 |
|   | 200 | -50567035 | 200 | 64401326 | 200 | 4244857 |
| 199.8 | -49579032 | 199.8 | 63141886 | 199.8 | 4210725 |
| 199.6 | -48742404 | 199.6 | 61949051 | 199.6 | 4272135 |
|   |       |       |       |       |
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| 199.4 | -47929131 | 199.4 | 60805694 | 199.4 | 4373542 |
| 199.2 | -47071743 | 199.2 | 59560786 | 199.2 | 4449304 |
| 199   | -46066440 | 199   | 58212943 | 199   | 4506896 |
| 198.8 | -45122725 | 198.8 | 57159546 | 198.8 | 4548273 |
| 198.6 | -44217243 | 198.6 | 56199396 | 198.6 | 4616169 |
| 198.4 | -43333559 | 198.4 | 55186481 | 198.4 | 4724039 |
| 198.2 | -42458179 | 198.2 | 53851613 | 198.2 | 4714471 |
| 198   | -41555125 | 198   | 52721750 | 198   | 4573228 |
| 197.8 | -40477502 | 197.8 | 51269934 | 197.8 | 4597171 |
| 197.6 | -39390716 | 197.6 | 49406032 | 197.6 | 4629049 |
| 197.4 | -38408768 | 197.4 | 47968229 | 197.4 | 4492107 |
| 197.2 | -37293610 | 197.2 | 46758440 | 197.2 | 4445210 |
| 197   | -36148177 | 197   | 45379457 | 197   | 4433848 |
| 196.8 | -35092704 | 196.8 | 43982302 | 196.8 | 4269536 |
| 196.6 | -34298980 | 196.6 | 42813002 | 196.6 | 3942108 |
| 196.4 | -33460622 | 196.4 | 41612036 | 196.4 | 3829684 |
| 196.2 | -32673645 | 196.2 | 40283223 | 196.2 | 3513204 |
| 196   | -31824734 | 196   | 39214083 | 196   | 3297441 |
| 195.8 | -31013018 | 195.8 | 38322614 | 195.8 | 3106748 |
| 195.6 | -30120511 | 195.6 | 37291707 | 195.6 | 2986435 |
| 195.4 | -29128010 | 195.4 | 36259762 | 195.4 | 2875851 |
| 195.2 | -28196405 | 195.2 | 35369331 | 195.2 | 2746085 |
| 195   | -27199752 | 195   | 34762966 | 195   | 2490923 |
| 194.8 | -26541660 | 194.8 | 34106950 | 194.8 | 2396830 |
| 194.6 | -26154170 | 194.6 | 33225688 | 194.6 | 2326266 |
| 194.4 | -25638254 | 194.4 | 32670012 | 194.4 | 2099599 |
| 194.2 | -24999192 | 194.2 | 32196511 | 194.2 | 2020060 |
| 194   | -24380371 | 194   | 31162663 | 194   | 1958997 |
| 193.8 | -24254254 | 193.8 | 29665694 | 193.8 | 1420620 |
| 193.6 | -24230726 | 193.6 | 28812631 | 193.6 | 905298.4 |
| 193.4 | -24299407 | 193.4 | 27991573 | 193.4 | 413155.9 |
| 193.2 | -24171560 | 193.2 | 27315662 | 193.2 | 134043.5 |
| 193   | -23562081 | 193   | 26821747 | 193   | 203461 |
| 192.8 | -23093597 | 192.8 | 26637848 | 192.8 | 267333.6 |
| 192.6 | -22214238 | 192.6 | 26088573 | 192.6 | 647585.7 |
| 192.4 | -21106865 | 192.4 | 25628566 | 192.4 | 931240.1 |
| 192.2 | -20454309 | 192.2 | 25317339 | 192.2 | 511835.1 |
| 192   | -19835661 | 192   | 23880401 | 192   | 414269.1 |
| 191.8 | -19184489 | 191.8 | 22406787 | 191.8 | 440719.1 |
| 191.6 | -18764964 | 191.6 | 21418438 | 191.6 | 255047 |
| 191.4 | -18193026 | 191.4 | 20897016 | 191.4 | -381377 |
| 191.2 | -17090964 | 191.2 | 20517454 | 191.2 | 44319.16 |
| 191   | -15484209 | 191   | 18808906 | 191   | 393484 |
| 190.8 | -14692613 | 190.8 | 17749800 | 190.8 | 791308.1 |
| 190.6 | -14041061 | 190.6 | 17319030 | 190.6 | 1502015 |
| 190.4 | -11112032 | 190.4 | 18516363 | 190.4 | 2240844 |
| 190.2 | -8905849.7 | 190.2 | 18620163 | 190.2 | 3204797 |
| 190 | -8738714.4 | 190 | 15273443 | 190 | 3073145 |
| 189.8 | -8898082 | 189.8 | 16586392 | 189.8 | 5868266 |
| 189.6 | -7323937.7 | 189.6 | 19336383 | 189.6 | 6663882 |
| 189.4 | -9986528.8 | 189.4 | 17514174 | 189.4 | 4003081 |
| 189.2 | -9373935.8 | 189.2 | 12649985 | 189.2 | 2348277 |
| 189 | -12986107 | 189 | 11203030 | 189 | 2322149 |
| 188.8 | -18413082 | 188.8 | 8979340 | 188.8 | 4183309 |
| 188.6 | -20081321 | 188.6 | 13082018 | 188.6 | 6261980 |
| 188.4 | -16418721 | 188.4 | 13285206 | 188.4 | 5085001 |
| 188.2 | -5518232.9 | 188.2 | 12184719 | 188.2 | 9972432 |
| 188 | -8390742.2 | 188 | 11304045 | 188 | 6027288 |
| 187.8 | -14113928 | 187.8 | 8158991 | 187.8 | 2956719 |
| 187.6 | -12283190 | 187.6 | 10165722 | 187.6 | 11381067 |
| 187.4 | -20430781 | 187.4 | 13814396 | 187.4 | 9119339 |
| 187.2 | -28004548 | 187.2 | 18274855 | 187.2 | 12090111 |
| 187 | -28706063 | 187 | 22038816 | 187 | 20206650 |
| 186.8 | -48261464 | 186.8 | 19144872 | 186.8 | 20142917 |
| 186.6 | -58922243 | 186.6 | 15093126 | 186.6 | 9812076 |
| 186.4 | -62774953 | 186.4 | 5522022 | 186.4 | -1637798 |
| 186.2 | -56822715 | 186.2 | 9386582 | 186.2 | 4718197 |
| 186 | -55715169 | 186 | 10862359 | 186 | 1362350 |
| 185.8 | -57923860 | 185.8 | 24323281 | 185.8 | 6731709 |
| 185.6 | -43837335 | 185.6 | 42044882 | 185.6 | 19081145 |
| 185.4 | -35808578 | 185.4 | 54249340 | 185.4 | 25842110 |
| 185.2 | -22204377 | 185.2 | 56859045 | 185.2 | 34812800 |
| 185 | -26073695 | 185 | 59040229 | 185 | 35990630 |
| 184.8 | -36469092 | 184.8 | 53041454 | 184.8 | 28445250 |
| 184.6 | -31906390 | 184.6 | 56200088 | 184.6 | 40191580 |
| 184.4 | -24378641 | 184.4 | 60140163 | 184.4 | 46489670 |
| 184.2 | -35957358 | 184.2 | 61401852 | 184.2 | 37349010 |
| 184 | -33385540 | 184 | 55510510 | 184 | 36446720 |
| 183.8 | -30890707 | 183.8 | 51395186 | 183.8 | 29684720 |
| 183.6 | -27235736 | 183.6 | 48053864 | 183.6 | 8749108 |
| 183.4 | -18466539 | 183.4 | 53925830 | 183.4 | 8162286 |
| 183.2 | -12400104 | 183.2 | 64554258 | 183.2 | 23405490 |
| 183 | -22487751 | 183 | 59962665 | 183 | 17420775 |
| 182.8 | -22976649 | 182.8 | 60747393 | 182.8 | 15840790 |
| 182.6 | -12764528 | 182.6 | 62327748 | 182.6 | 22918442 |
| 182.4 | -17044202 | 182.4 | 47164125 | 182.4 | 16274317 |
| 182.2 | -29108980 | 182.2 | 32040638 | 182.2 | 2135495 |
| 182 | -44019677 | 182 | 26467270 | 182 | -1.2E+07 |
| 181.8 | -52380594 | 181.8 | 32163987 | 181.8 | -1.6E+07 |
|-------|-----------|-------|---------|-------|-----------|
| 181.6 | -49937661 | 181.6 | 41231609 | 181.6 | -1258620  |
| 181.4 | -47278478 | 181.4 | 47707345 | 181.4 | 12820683  |
| 181.2 | -40097767 | 181.2 | 34361606 | 181.2 | -4362847  |
| 181   | -30717015 | 181   | 32062782 | 181   | -4984077  |
| 180.8 | -9468757.1| 180.8 | 44062408 | 180.8 | 17100385  |
| 180.6 | 4882976.9 | 180.6 | 52803579 | 180.6 | 23922990  |
| 180.4 | 6672696.5 | 180.4 | 63743234 | 180.4 | 38637700  |
| 180.2 | 5679382.4 | 180.2 | 66613304 | 180.2 | 53022590  |
| 180   | 18432631  | 180   | 66226822 | 180   | 65201780  |
### IRI Data (Figure 1B)

| PPro<sub>10-1000</sub>  | 5 mg/ml | PPro<sub>10-1000</sub>  | 10 mg/ml | PPro<sub>10-1000</sub>  | 20 mg/ml |
|-------------------------|---------|-------------------------|---------|-------------------------|---------|
| Avg Set 1               | 99.266  | Avg Set 1               | 103.716 | Avg Set 1               | 72.712  |
| Avg Set 2               | 99.448  | Avg Set 2               | 100.377 | Avg Set 2               | 74.805  |
| Avg Set 3               | 128.455 | Avg Set 3               | 108.021 | Avg Set 3               | 94.545  |
| Cum Average             | 109.0563| Cum Average             | 104.038 | Cum Average             | 80.68733|
| STD DEV                 | 19.13652| STD DEV                 | 8.717811| STD DEV                 | 17.58934|
| % MLGS                  | 79.02633| % MLGS                  | 75.38986| % MLGS                  | 58.46908|

| PPro<sub>10</sub>  | 5 mg/ml | PPro<sub>10</sub>  | 10 mg/ml | PPro<sub>10</sub>  | 20 mg/ml |
|---------------------|---------|---------------------|---------|---------------------|---------|
| Avg Set 1           | 100.582 | Avg Set 1           | 89.368  | Avg Set 1           | 97.569  |
| Avg Set 2           | 171.522 | Avg Set 2           | 91.748  | Avg Set 2           | 77.741  |
| Avg Set 3           | 116.791 | Avg Set 3           | 90.759  | Avg Set 3           | -       |
| Cum Average         | 129.6317| Cum Average         | 90.625  | Cum Average         | 87.655  |
| STD DEV             | 37.05471| STD DEV             | 13.5148 | STD DEV             | 13.7729 |
| % MLGS              | 93.93599| % MLGS              | 65.67029| % MLGS              | 63.51812|

| PPro<sub>20</sub>  | 5 mg/ml | PPro<sub>20</sub>  | 10 mg/ml | PPro<sub>20</sub>  | 20 mg/ml |
|---------------------|---------|---------------------|---------|---------------------|---------|
| Avg Set 1           | 105.192 | Avg Set 1           | 86.676  | Avg Set 1           | 96.27   |
| Avg Set 2           | 113.484 | Avg Set 2           | 147.657 | Avg Set 2           | 75.218  |
| Avg Set 3           | 105.151 | Avg Set 3           | 149.398 | Avg Set 3           | 101.992 |
| Cum Average         | 107.9423| Cum Average         | 127.9103| Cum Average         | 91.16   |
| STD DEV             | 10.63947| STD DEV             | 40.75635| STD DEV             | 13.22746|
| % MLGS              | 78.21908| % MLGS              | 92.68865| % MLGS              | 66.05797|
|            | PPro₁₅ 5 mg/ml |            | PPro₁₅ 10 mg/ml |            | PPro₁₅ 20 mg/ml |
|------------|----------------|------------|----------------|------------|----------------|
| Avg Set 1  | 91.391         | Avg Set 1  | 73.662         | Avg Set 1  | 46.842         |
| Avg Set 2  | 95.493         | Avg Set 2  | 89.935         | Avg Set 2a | 75.218         |
| Avg Set 3  | 95.687         | Avg Set 3  | 102.55         | Avg Set 2b | 71.791         |
| Cum Average| 94.19033       | Cum Average| 88.71567       | Cum Average| 64.617         |
| STD DEV    | 14.51387       | STD DEV    | 19.05693       | STD DEV    | 15.28502       |
| % MLGS     | 68.25386       | % MLGS     | 64.28671       | % MLGS     | 46.82391       |

|            | P(D)Pro₁₅ 5 mg/ml |            | P(D)Pro₁₅ 10 mg/ml |            | P(D)Pro₁₅ 20 mg/ml |
|------------|--------------------|------------|--------------------|------------|--------------------|
| Avg Set 1  | 82.376             | Avg Set 1  | 64.522             | Avg Set 1  | 61.31             |
| Avg Set 2  | 78.778             | Avg Set 2  | 88.988             | Avg Set 2  | 67.368             |
| Avg Set 3  | 75.119             | Avg Set 3  | 88.458             | Avg Set 3  | 80.032             |
| Cum Average| 78.75767           | Cum Average| 80.656             | Cum Average| 69.57             |
| STD DEV    | 10.18674           | STD DEV    | 16.25138           | STD DEV    | 13.22802           |
| % MLGS     | 57.07077           | % MLGS     | 58.44638           | % MLGS     | 50.41304           |

|            | P(DL)Pro₁₄₋₂₄ 5 mg/ml |            | P(DL)Pro₁₄₋₂₄ 10 mg/ml |            | P(DL)Pro₁₄₋₂₄ 20 mg/ml |
|------------|------------------------|------------|------------------------|------------|------------------------|
| Avg Set 1a | 90.729                 | Avg Set 1  | 84.264                 | Avg Set 1  | 68.397                 |
| Avg Set 2  | 90.254                 | Avg Set 2  | 93.582                 | Avg Set 2  | 66.639                 |
| Avg Set 1b | 91.995                 | Avg Set 3  | 81.885                 | Avg Set 3  | 79.321                 |
| Cum Average| 90.99267               | Cum Average| 86.577                 | Cum Average| 71.45233               |
| STD DEV    | 9.706077               | STD DEV    | 10.91247               | STD DEV    | 12.31481               |
| % MLGS     | 65.93671               | % MLGS     | 62.73696               | % MLGS     | 51.77705               |
References.

1. C. A. Knight, J. Jallett, A. L. DeVries, *Cryobiology*, 1988, **25**, 55-60

2. Protein Circular Dichroism Data Bank, 2016, CD0004553000

3. D. E. Mitchell, N. R. Cameron, and M. I. Gibson., *Chem. Commun.*, 2015, **51**, 12977 – 12980.

4. M. Marcellini, C. Noirjean, D. Dedovets, J. Maria, and S. Deville, *ACS Omega*, 2016, **1**, 1019 – 1026.