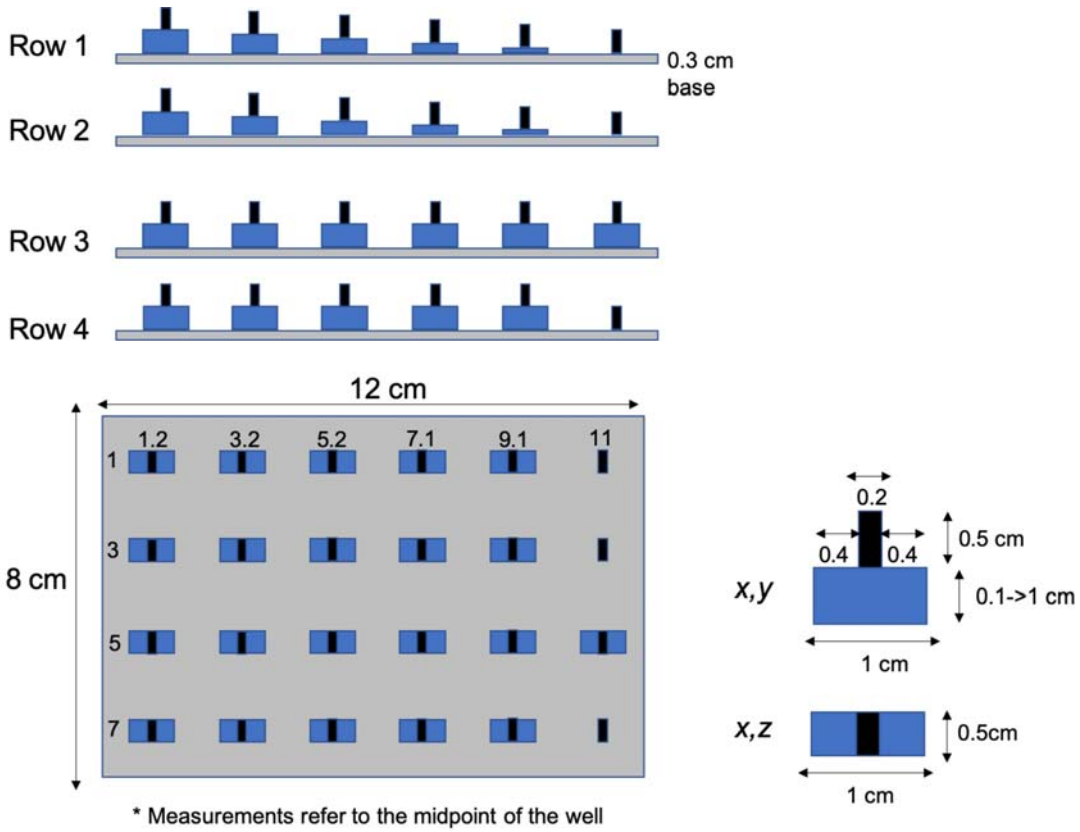
Biophysical Journal, Volume *120*

Supplemental Information

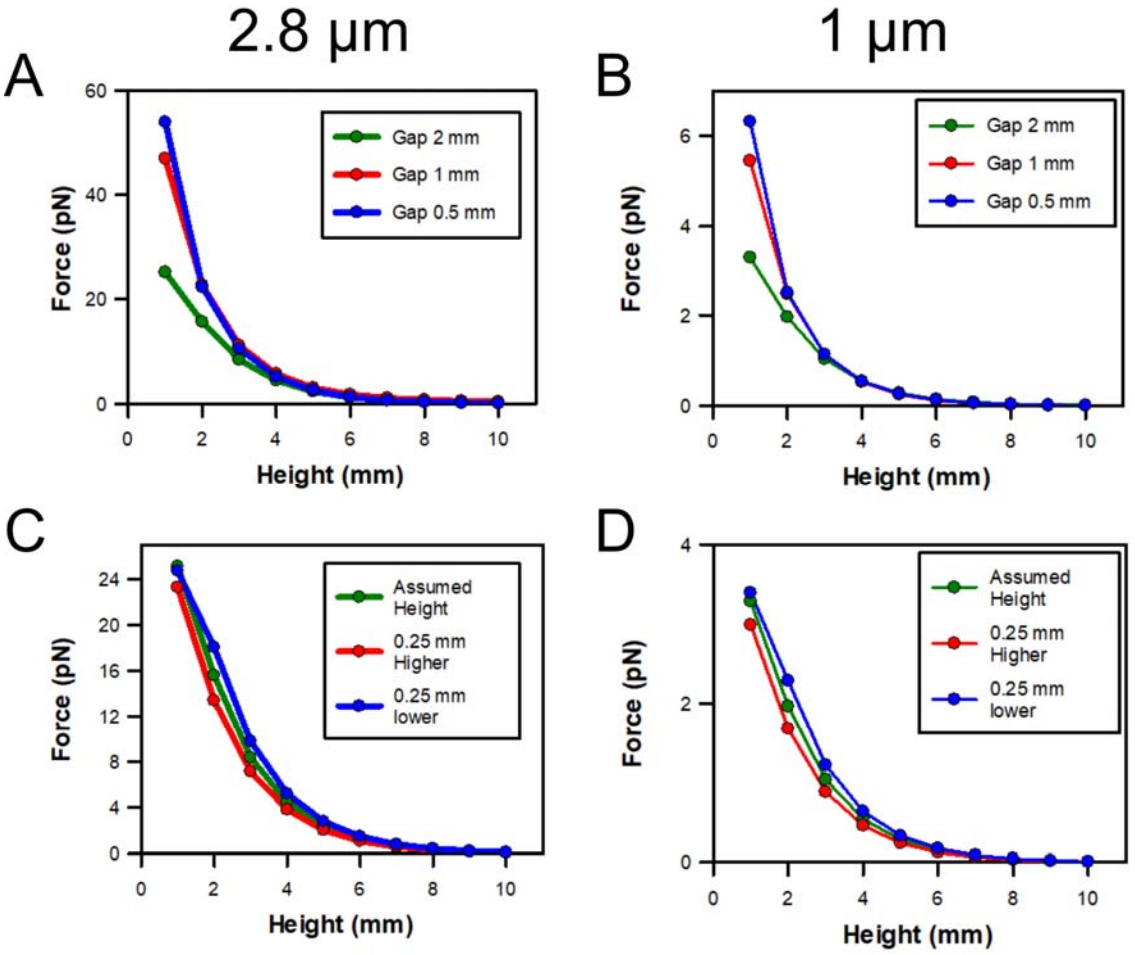
High-throughput mechanobiology: Force modulation of ensemble biochemical and cell-based assays

Ália dos Santos, Natalia Fili, David S. Pearson, Yukti Hari-Gupta, and Christopher P. Toseland



Supplementary Figure 1: Example 24 well microplate lid design.

The corresponding CAD file can be downloaded from https://github.com/cptoseland/MagPlate. Side and top-down projections are presented. The width of the divider (black) can be altered to change the space between the magnets which is labeled *x* in Figure 1A. The height of the spacer (blue) can be varied to adjust the distance between the magnets and the surface.



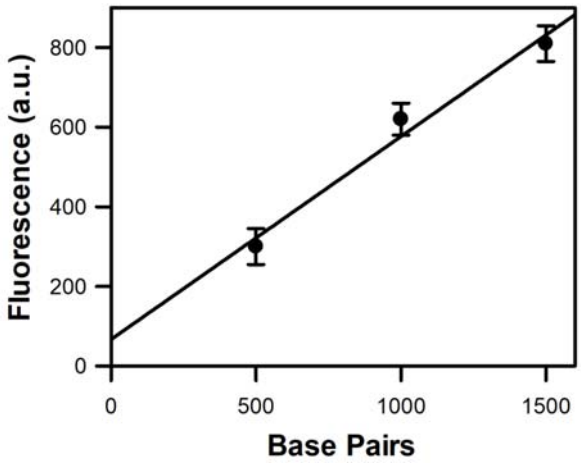
Supplementary Figure 2: Example 24 well microplate lid design.

The force exerted upon the biological sample is defined by the size of the paramagnetic particle, the distance of *Z* above the sample (noted as height) and the spacing between the magnets in *X* (noted as Gap). The force-distance relationship is plotted for the 2.8 µm **(A)** and 1 µm **(B)** particles used in these studies with Gap distances of 0.5, 1 and 2 mm, as calculated using Equations 1-6. (C) and (D) depict the force-distance relationship with a 2 mm Gap for 2.8 µm and 1 µm particles modelled assuming a +/- 0.25 mm error in spacer height.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1					0.26	0.24	0.24	0.25	0.25	0.26	0.26				
2				0.25	0.24	0.20	0.25	0.19	0.26	0.24	0.24	0.25			
3			0.24	0.26	0.24	0.21	0.26	0.18	0.24	0.22	0.25	0.26	0.25		
4		0.25	0.25	0.26	0.25	0.22	0.23	0.08	0.22	0.25	0.25	0.26	0.26	0.25	
5	0.23	0.28	0.24	0.25	0.25	0.25	0.20	0.05	0.18	0.23	0.25	0.26	0.26	0.26	0.27
6	0.26	0.27	0.24	0.25	0.24	0.24	0.07	0.06	0.11	0.21	0.24	0.26	0.26	0.26	0.25
7	0.25	0.26	0.24	0.24	0.25	0.23	0.07	0.04	0.09	0.22	0.24	0.25	0.26	0.28	0.26
8	0.26	0.25	0.25	0.25	0.24	0.22	0.07	0.05	0.05	0.25	0.26	0.25	0.25	0.28	0.27
9	0.27	0.24	0.26	0.25	0.25	0.23	0.08	0.03	0.06	0.26	0.26	0.26	0.25	0.28	0.25
10	0.26	0.26	0.26	0.24	0.26	0.27	0.18	0.08	0.13	0.25	0.26	0.26	0.25	0.27	0.26
11	0.25	0.28	0.25	0.24	0.26	0.24	0.22	0.18	0.18	0.22	0.25	0.27	0.27	0.25	0.26
12		0.25	0.24	0.24	0.25	0.28	0.23	0.22	0.25	0.24	0.25	0.27	0.26	0.26	
13			0.25	0.26	0.26	0.27	0.22	0.26	0.24	0.25	0.26	0.25	0.26		
14				0.25	0.26	0.26	0.23	0.25	0.26	0.26	0.26	0.27			
15					0.24	0.25	0.26	0.26	0.25	0.28	0.26				

Supplementary Figure 3: Example well scan depicting magnetic field effect.

The eGFP-Flagelliform(8*GPGGA)-mRFP FRET sensor was immobilised on the surface through an N-terminal biotinylation tag and was coupled to Protein-A Dynabeads through an antibody against GFP, as in Figure 2. A well scan measurement was performed across the microplate well at 1 mm intervals when 11 pN has been applied. The relative FRET signal was then calculated using Equation 7 and presented as a heat-plot.



Supplementary Figure 4: Helicase assay calibration.

To calibrate the fluorescence signal, three different lengths of DNA 500, 1000 and 1500 bp were used. The helicase assays were performed with1 μg DNA substrate pre-incubated for 10 min at room temperature with 500 nM RepD. 10 nM bioPcrA was added and unwinding was initiated with 1 mM ATP, supplemented with 100 nM MDCC-SSB. The reactions were performed at 25 C for 5 min and end-point MDCC fluorescence (ex. 430nm and em. 470 nm) was recorded. This time should enable complete unwinding of the DNA substrate. The fluorescence signal can then be plotted against DNA base pairs to provide a signal calibration.