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Electronic Supplementary Information

for

Treatment of kidney clear cell carcinoma, lung adenocarcinoma and glioblastoma cell lines with hydrogels made of DNA nanostars

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Section S1 – Gel formation and phase separation

We recall here some basic effects of the gel-formation process.

At the very dilute DNA concentration used in this article, gelation sets in only as a consequence of (and thus following) a phase-separation process. Such thermodynamic instability generates an inhomogeneous distribution of DNA nanostars in space. As a result, regions with a larger concentration of DNA NS coexist with regions with a very small concentration of DNA NS. In the dense regions, the DNA nanostars are sufficiently concentrated to pair the sticky-ends forming an extended network (i.e., a gel). Gel regions thus alternate with regions with a small NS concentration, providing a well-defined morphology to the arrested phase separated system. The spatial dimension of these differently concentrated regions is controlled by the underlying phase separation process.

In material science, the process by which a thermodynamically unstable system evolves toward equilibrium is named nucleation or spinodal decomposition and it is fairly well understood. For our porpoises, we recall that the characteristic length scale that modulate dense and loose regions is determined by the distance from the critical point, the so-called quench depth. This quantity measures how deep in the unstable region is the sample at the selected experimental conditions (temperature and concentration in our case). The morphology of the sample thus retains the information of the quench depth.

Fig. S1 provides a graphic explanation of the phase-separation process. It shows in the concentration-temperature plane the phase-separation curve, separating the region of thermodynamic stability, where the sample is a homogeneous solution of NSs, from the region of thermodynamic instability, where the sample is phase separated (i.e., it is spatially inhomogeneous).

By bringing the system from the stable to the unstable region (for example by abruptly changing the temperature), concentration fluctuations develop, resulting into regions of different local NSs concentration. The right panel schematically shows the resulting spatial pattern and the typical characteristic length scale ξ.

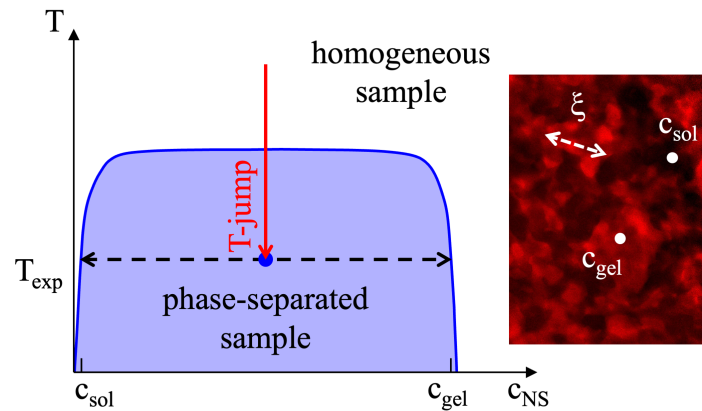

Fig. S1 - Schematic phase-separation diagram (left). The system is abruptly brought to the experimental temperature (T_{exp}) by a T-jump from a homogeneous state into the phase separation region, where it separates into regions with high (c_{gel}) and low (c_{sol}) concentration of DNA NSs. The right panel shows the resulting spatial pattern, with regions of characteristic size ξ.

The phase coexistence curve is influenced by the solvent physical properties (ionic strength, co-solute concentration, pH). All of them affect the critical temperature. For example, increasing ion concentration increases the critical temperature by screening the DNA-DNA electrostatic repulsive interaction, facilitating phase separation and thus gel-formation. Fig. S2 shows schematically the modification of the phase boundaries as a result of a modulation of the solvent properties. If the experimental T is fixed, modulation in the solvent essentially changes the quench depth.

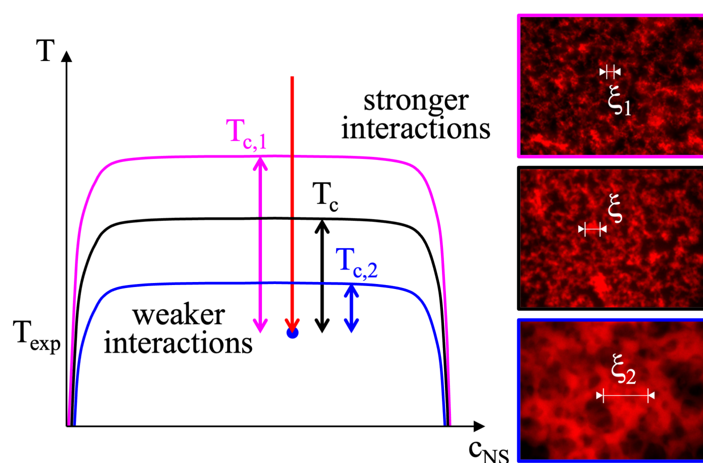

Fig. S2 - Effect of solvent modulation on the phase diagram (left). A solvent inducing stronger (weaker) interactions between NS will increase (decrease) the critical temperature of the system. As a result, the characteristic spatial size ξ of the concentrated regions will decrease (increase), as shown in the panels on the right.

The data reported in the article show that different concentrations of BSA give rise to different sample morphologies. Specifically, on increasing BSA concentration the characteristic size of the gel-rich regions increases. As we have discussed above, this indicates a progressively smaller quench depth (a lowering of the critical temperature). This behaviour is sketched in Fig. S3, which suggests that the net effect of adding BSA in the solution is a weakening of the attraction between the DNA NSs on increasing BSA concentration.

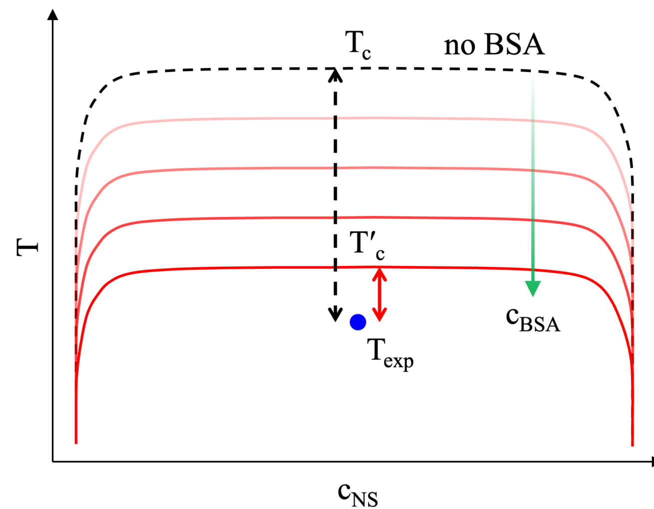

Fig. S3 - Effect of the addition of BSA on the phase diagram of the system. The dashed line is the coexistence curve in the absence of added BSA. As the concentration of BSA increases, the critical temperature T_c decreases, shifting the coexistence curve to lower temperatures (full lines) and closer to the experimental temperature.

Section S2 - Viability of cells after removal of DNA-GEL

Cell viability was analyzed in the same experiments of Section 3.4 of the main text by Trypan blue dye assay. The results collected are reported as percentage of viable cells during incubation with DNA-GEL and DNA-NoGEL (Fig. S4A) and after removal of DNA followed by incubation with fresh cell culture medium (Fig. S4B). As shown, incubation with DNA did not cause any evident cell death compared to untreated samples in all cell lines tested.

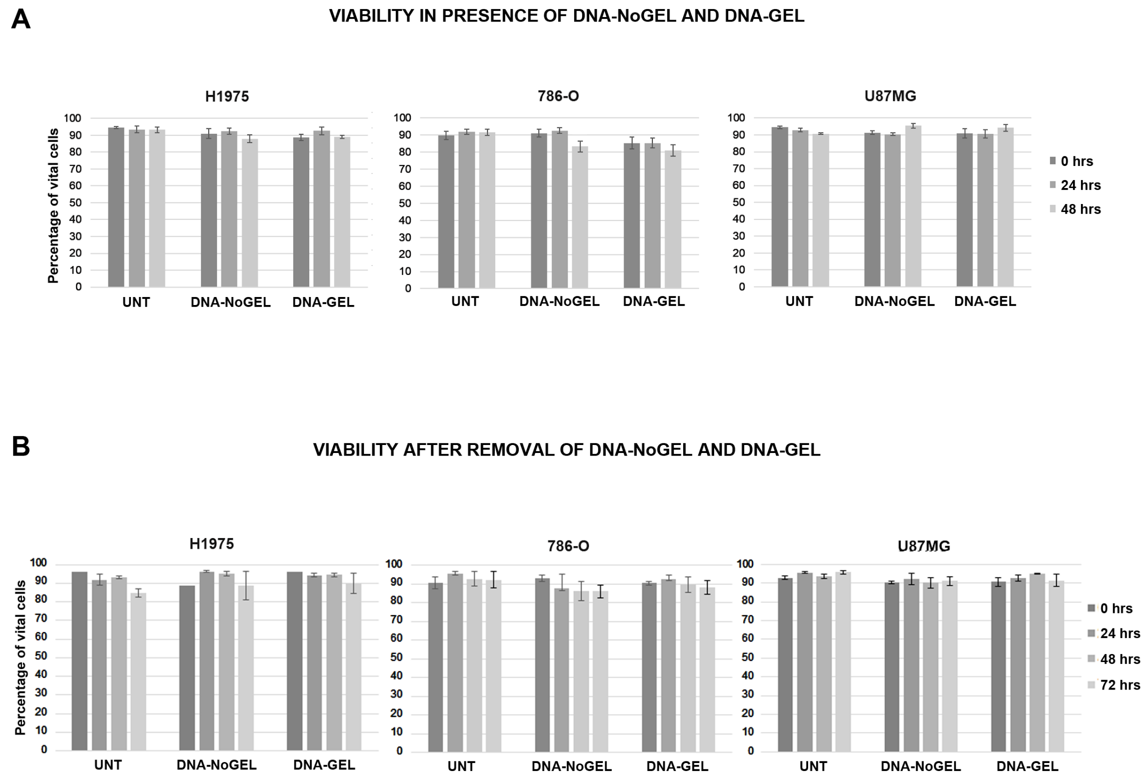

Fig. S4 - Cell viability was unchanged during incubation with DNA-GEL and DNA-NoGEL, and after removal of DNA. A) Cell viability was calculated as percentage of 786-O, U87MG and H1975 vital cells treated with DNA-GEL and No-GEL (35 μM) at different times, indicated by the labels. B) Cell viability found in cells treated with DNA-GEL and DNA-NoGEL for 48 h, then washed with PBS to remove the DNA-GEL and then incubated with fresh cell culture medium for 72h, as indicated by the labels.