

Supporting Information

Title: Stabilizing Silica Nanoparticles in Hydrogel: Impact on storage and polydispersity.

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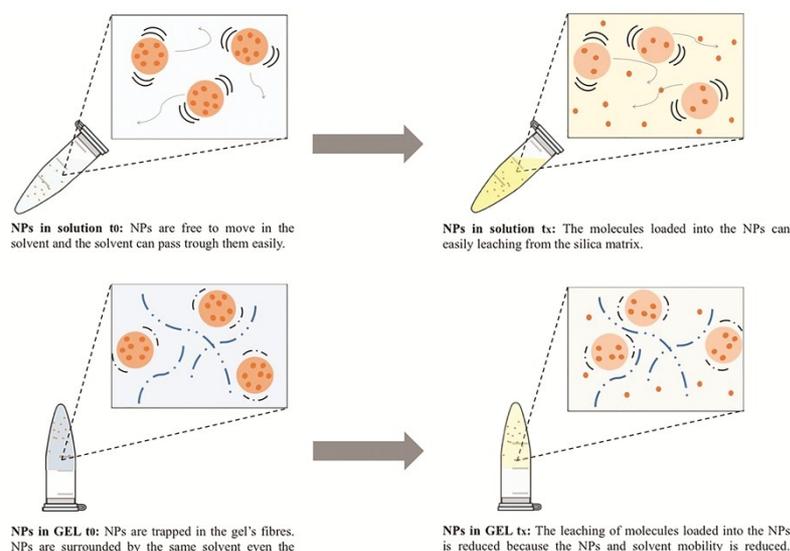
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1. Stability study

For each type of NPs two samples were prepared: NPs in solution and NPs in gel. The samples of NPs in gel were prepared by dispersion of NPs in a 2 mg/mL solution of Fmoc-gal. After sonication the gel was formed and its formation was confirmed by vial-inversion. Same procedure was used to form the samples with NPs in solution but using simple DI water as solvent. Both solution and gel samples were stored at 4°C for 8 days.

Our approach can be useful in order to reduce the leaching of the dye loaded into NPs. The following representation gives a better explanation in support of our idea (Scheme S.1).



Scheme S.1 Illustration of the dye leaching process when nanoparticles are stored in solution or in hydrogel

Figures S.1 shows the pictures of the samples after 1 day and 8 days of storage. Some important considerations can be made analyzing the samples with naked eye.

Considering NP-FITC, NP-A-FITC-NH₂, NP-B-FITC-NH₂ and NP-NaFlu-NH₂ by eye for 1 day, it was not possible to detect the presence of precipitates both in the solution-samples and in the gel-samples. However, differences in color were clearly evident when assessing the solution samples after 8 days. The change in color is likely attributed to the NP aggregation and the associated light scattering effect. Noteworthy, the difference in color between the gel samples analysed at day 1 and day 8 was almost negligible if not completely absent.

The results for NP-MB-NH₂ were remarkable. When stored in solution for 8 days, the particles aggregated massively. As a result, large blue particles were visibly floating in the solution. Such clear instability was not detectable when the NP-MB-NH₂ were stored in gel instead. The gel dramatically improved the colloidal stability of a sample, observable by naked eye.

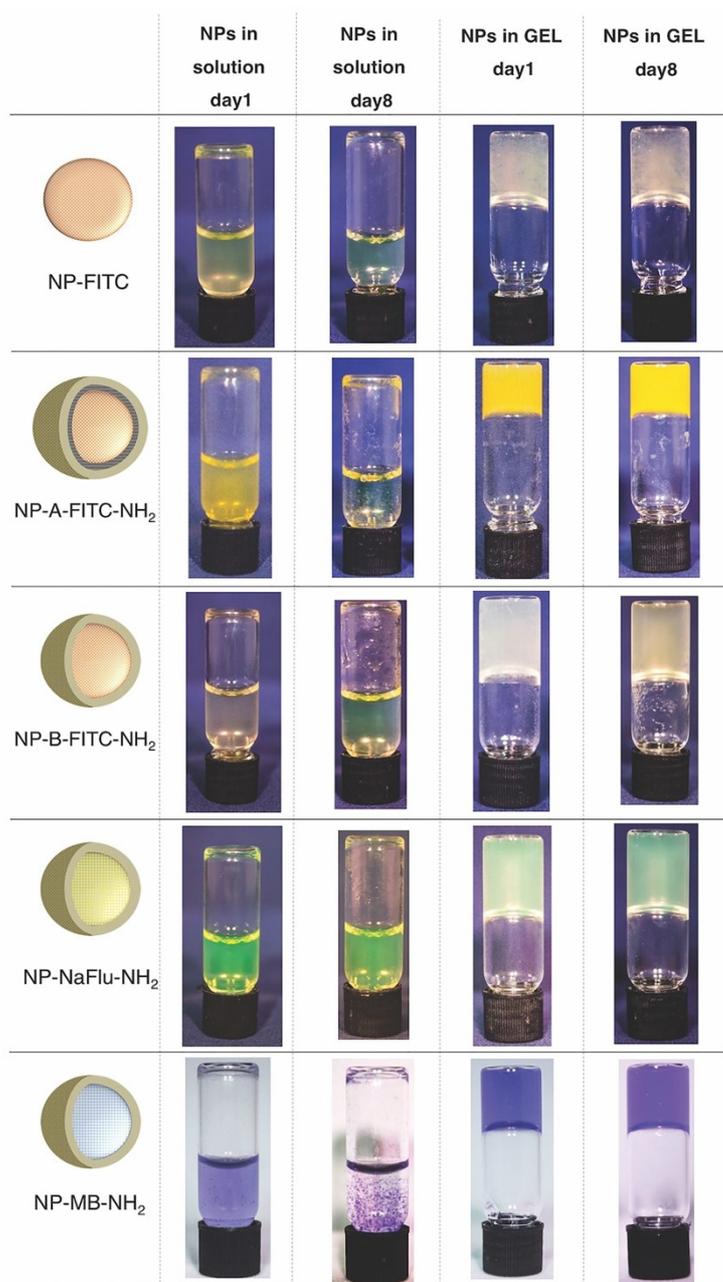
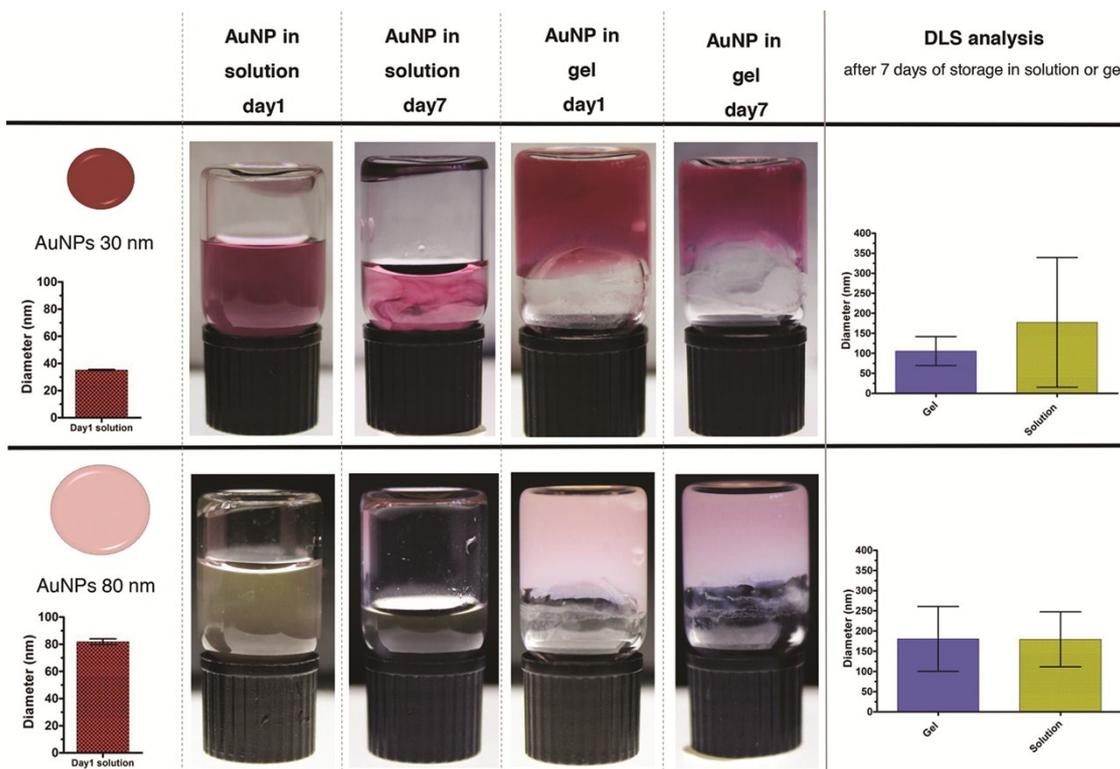


Figure S.1 Comparison between SiNPs in solution and SiNPs in gel the first day of the synthesis and after 8 days of storage at 4°C.

The AuNPs are characterized by a specific colour when monodispersed. Once aggregated, the characteristic colour changes. Figure S.2 shows the changes in colour when storing 30nm and 80nm AuNPs in solution or hydrogel.



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figure S.2 Comparison between AuNPs in solution and AuNPs in gel after storing it for day 1 and day 7 at 4°C. The DLS analysis of AuNPs in solution showed significant degree of aggregation. DLS analysis of AuNPs stored in solution was challenging due to the presence of gel fibres that remained in solution after the gel was converted to solution/suspension.

The monodispersity of AuNPs was monitored by NTA.

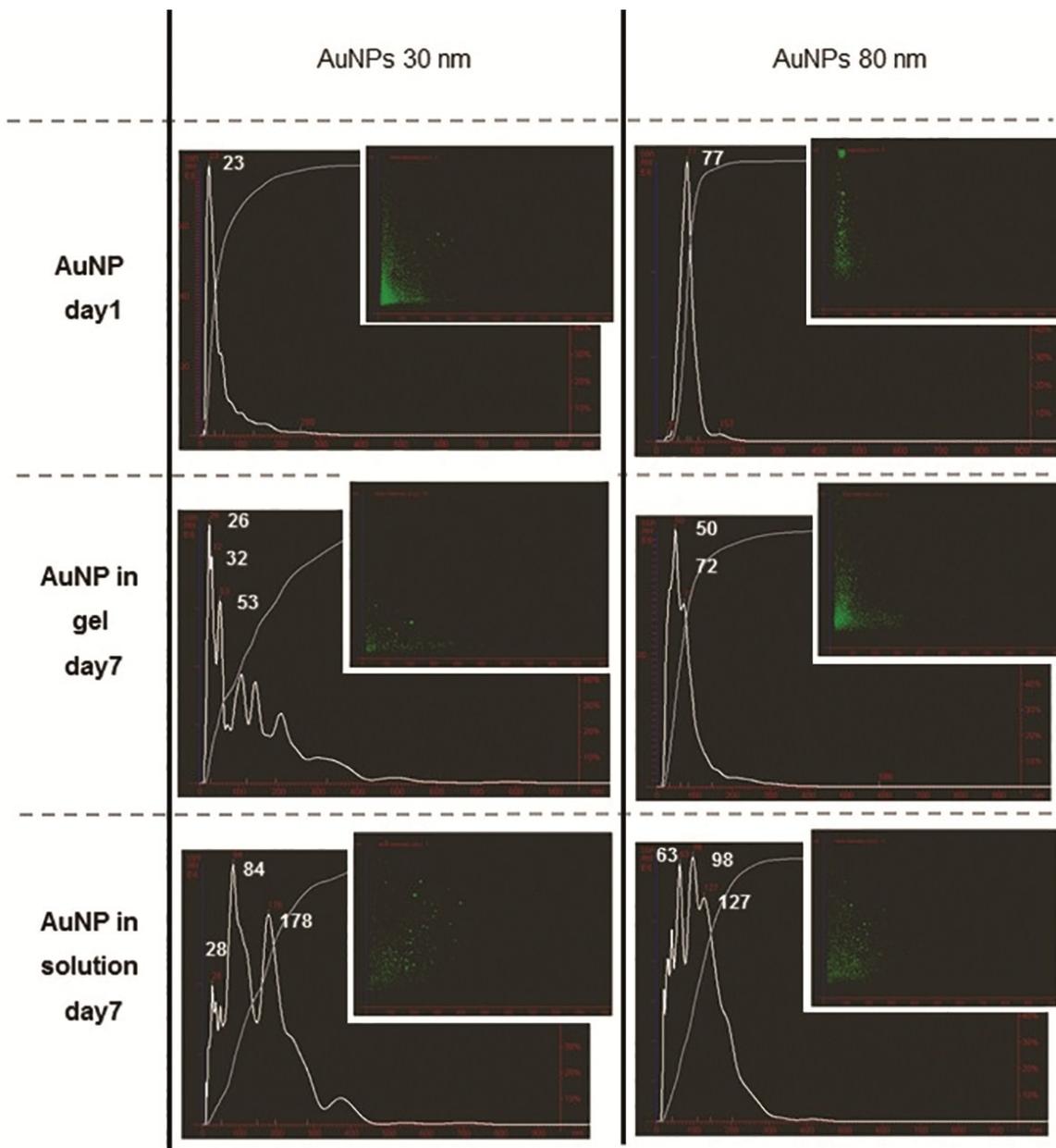


Figure S.3 NTA analysis of 30nm and 80nm AuNPs stored both in solution and hydrogel. The evolution of aggregates is clearly evident for samples stored in solution in 7 days. The AuNPs stored in hydrogel still show reasonably narrow size distribution of nanoparticles.

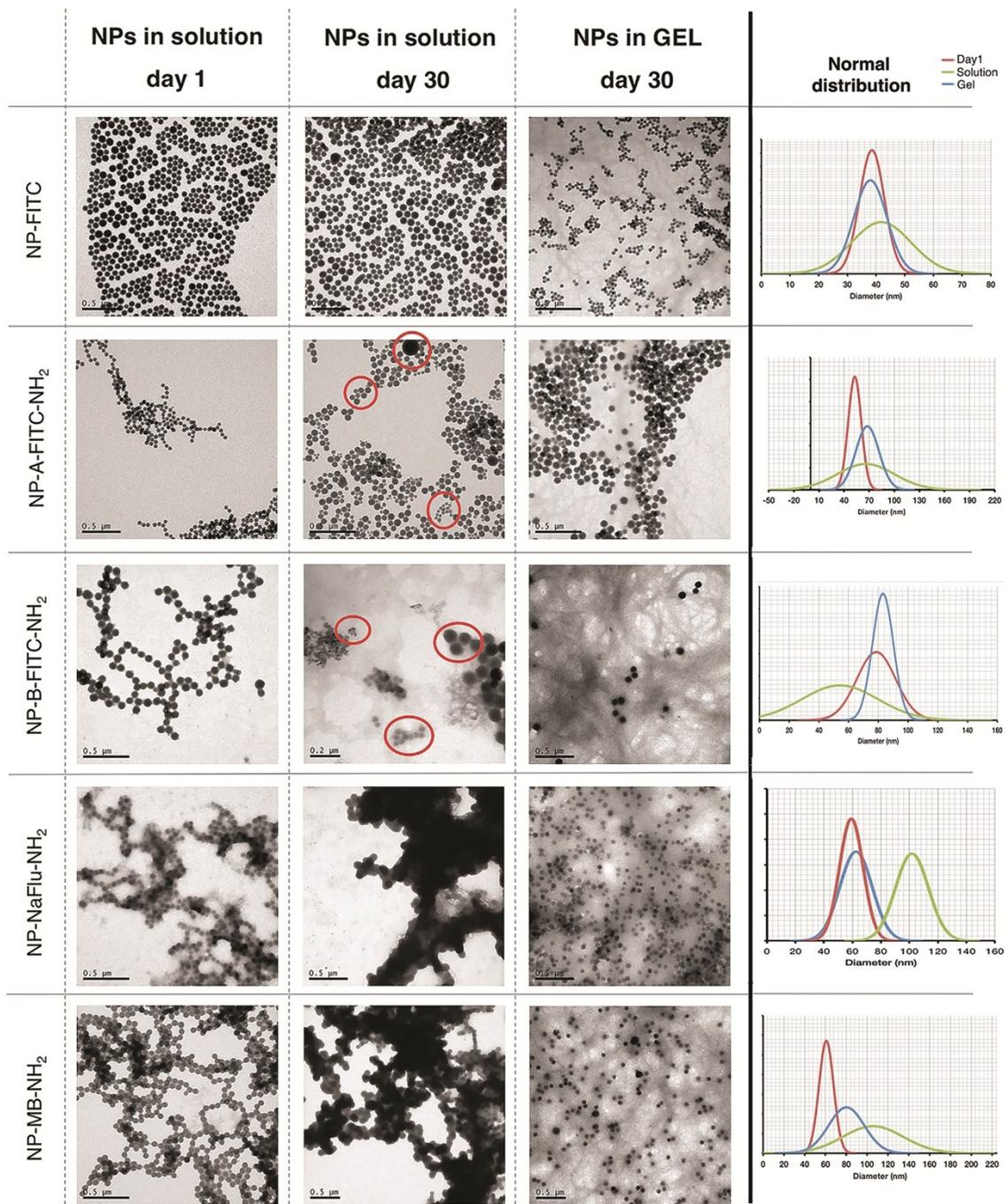


Figure S.4 TEM images showing the evolution of the SiNP morphologies and size distribution when stored in water for 1 day and 30 days (left and middle column) and in gel for 30 days. The TEM images were analysed and the Gaussian distribution is shown in the right column.

2. Dye-leaching study

Storing the SiNPs in gel slows down the leaching of the cargo loaded into the particles. NPs have been stored under different conditions and the amount of dye present in the supernatant and in the NPs were compared after different intervals of storage.

Firstly, we studied the capacity of NP-B-FITC-NH₂, NP-NaFlu-NH₂ and NP-MB-NH₂ to retain their cargo when stored in ethanol and water. While all three studied dyes were leaching relatively slowly when the NPs were stored in EtOH, the rate of leaching was significantly accelerated in water. The exception was methylene blue in NP-MB-NH₂, which as the most hydrophobic compound was not released into the aqueous environment.

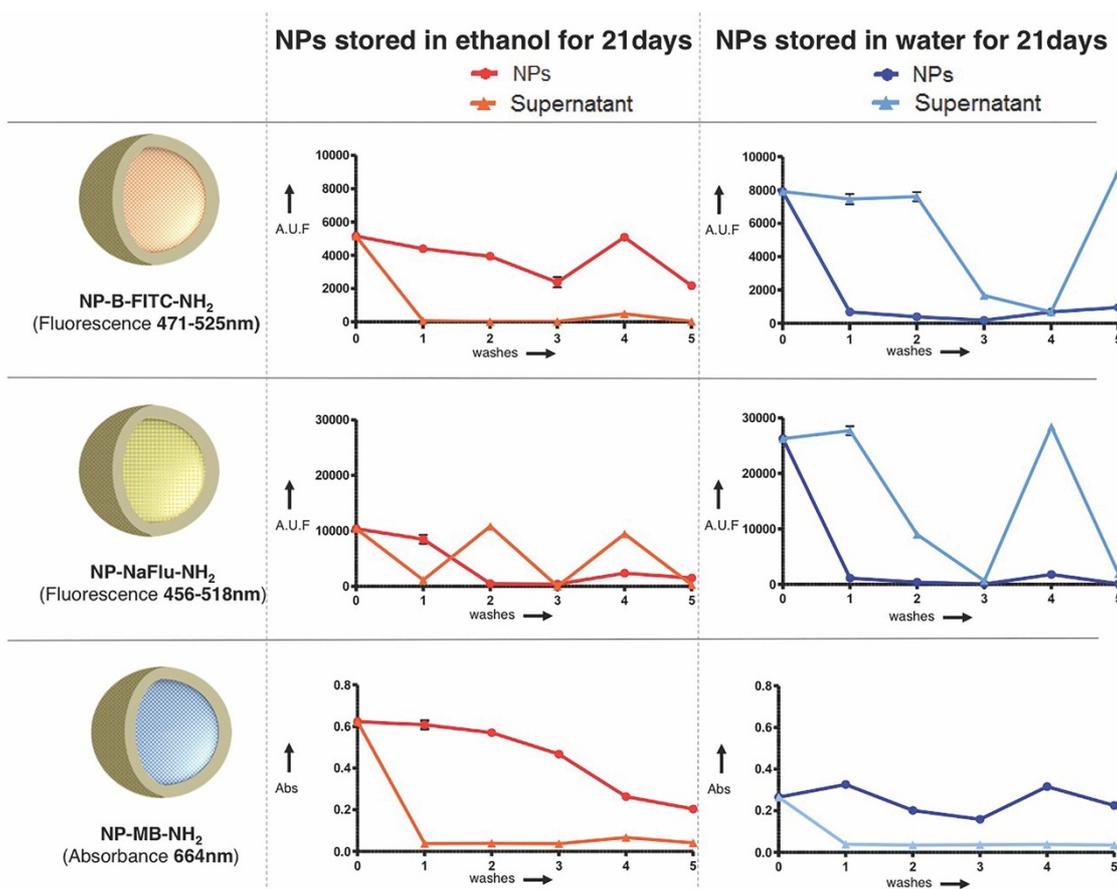


Figure S.5 Analysis of the relationship between the environmental characteristics and the leaching effect from dye-loaded NPs. In this study the leaching showed to be dependent on the environment in which particles are suspended and the physico-chemical characteristic of the model cargo (dye).

The accumulation of free dyes in the supernatant over time, measured by the fluorescence signal (absorbance in the case of methylene blue) was used as a simple mean to determine the degradation of silica and compare the rate of the release of the dyes from the NPs. The

dye release, as a consequence of silica matrix hydrolysis was very prominent both in water and PBS in the case of NP-B-FITC-NH₂ and NP-NaFlu-NH₂. Significant accumulation of FITC and the water-soluble fluorescein analogue (Na⁺Flu) in the supernatant was evident immediately after several minutes despite the fact that FITC was covalently attached to the silica matrix, while Na⁺Flu was physically entrapped in the silica core. The temperature and shaking applied in this study contributed to the dye-leaching effect. For example, the dye leaching was still relatively high in comparison with the result of the previous experiment which was conducted at 4°C in with no shaking or motion.

The release of methylene blue from NP-MB-NH₂ was slow in all solvents, PBS, water and ethanol.

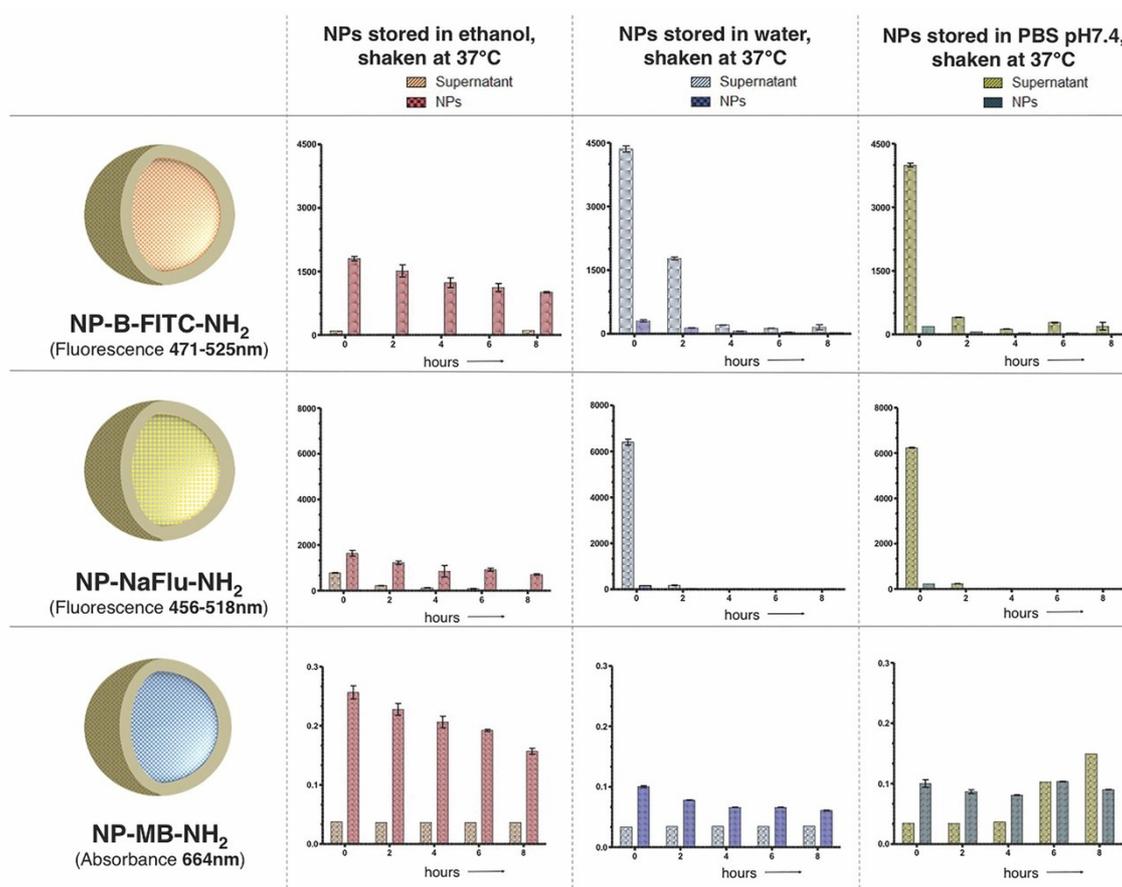


Figure S.6 Leaching effect has been analysed in an isotonic environment (PBS) and the results have been compared with the data collected measuring the effect in water and ethanol.

To demonstrate that the presented approach is useful to decrease the dye leaching from the NPs, NP-B-FITC-NH₂, NP-NaFlu-NH₂ and NP-MB-NH₂ were stored in water and in PBS as suspensions and in water-based and PBS-based gel. All samples were analysed after 30 days of storage at 4°C.

Considering NP-B-FITC-NH₂ and NP-NaFlu-NH₂ a difference in the color between the samples stored using the two difference approaches is clearly evident by eye and the visual analysis was then supported by the fluorescent measurement of the supernatant. Indeed, the result showed that FITC sodium salt leached out very quickly when stored in solution (water and PBS) as it is visible by the fading color of the samples. In the case NP-B-FITC-NH₂ the effect is less visible but the amount of FITC detected in the supernatant is high even after storage. Furthermore, some orange precipitates were visible when NP-B-FITC-NH₂ were stored in solution, but not if stored in gel!

A clear precipitation was visible with NP-MB-NH₂; even after dilution of the samples and shaking, the aggregates were still visibly floating in the suspension. Remarkably, when the same type of NPs was stored in gel, no aggregates were visible and the solution after dilution appeared clear.

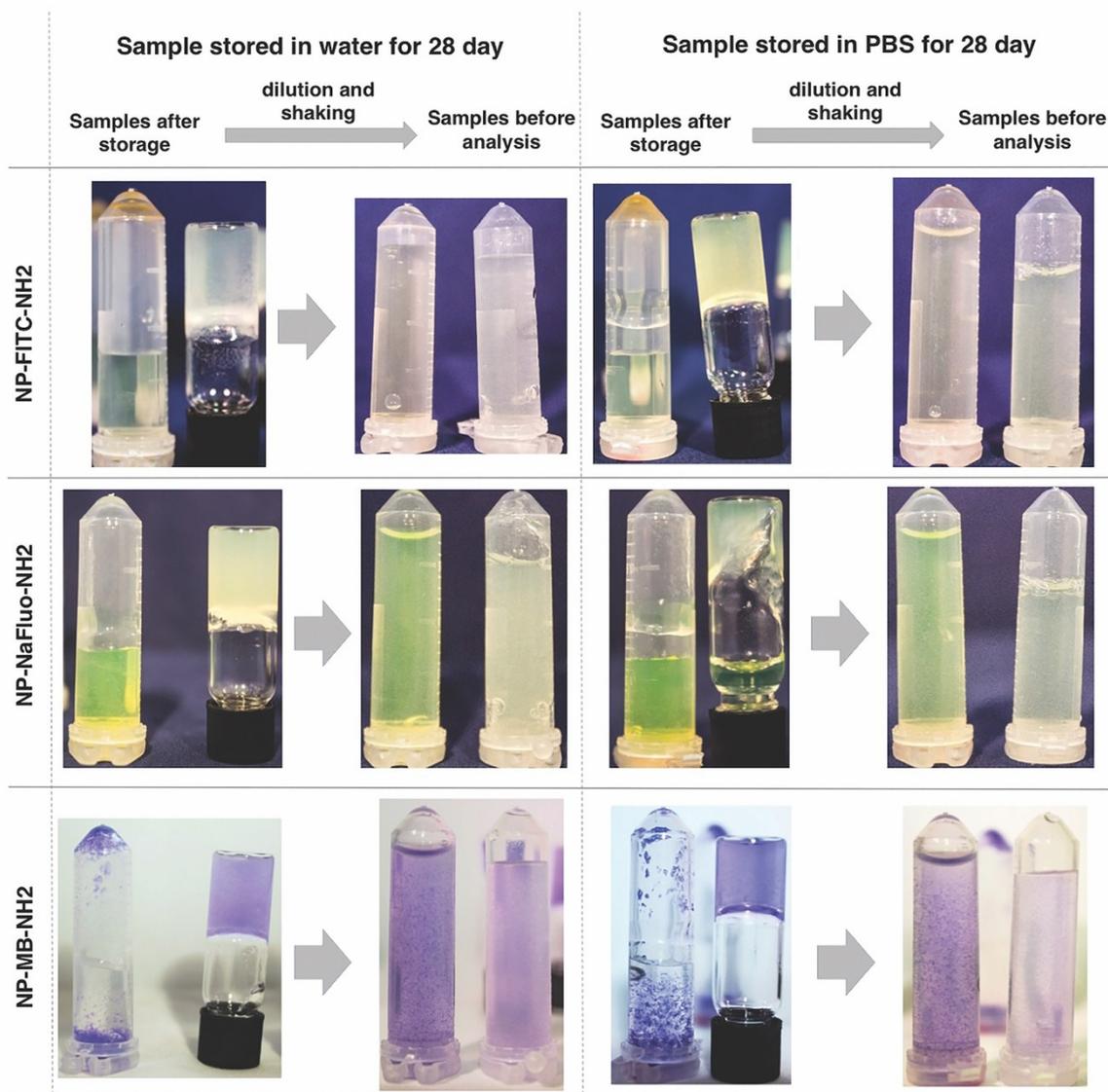


Figure S.7 Images showing the samples after 28 days of storage at 4°C. Every image has two vials – the one on the left represents NPs in solution, the one on the right represents NPs in gel.

3. Mechanical stress resistance analysis

We established that the prepared hydrogel is stable while being stored ‘still’ (no shaking or vibrations) at room temperature or at 4°C for longer term. For the hydrogel to ‘break’ into a solution, which could be further diluted to any desired concentrations, one needs to simply shake the sample by hand. We have formulated NP-B-FITC-NH₂, NP-NaFlu-NH₂ and NP-MB-NH₂ into gels and exposed them to vibrations using a simple orbital shaker.

We then qualitatively compared the conversion of the hydrogel into a solution as a function of shaking speed, time and the intrinsic properties of the NPs and their cargo. The results are summarized in pictures S.8-S.12. The more negative is the zeta potential of the SiNP, the lower is the resistance of the gel to a mechanical stress. The strength of the gel showed to be dramatically increased when a hydrophobic dye was loaded into particles.

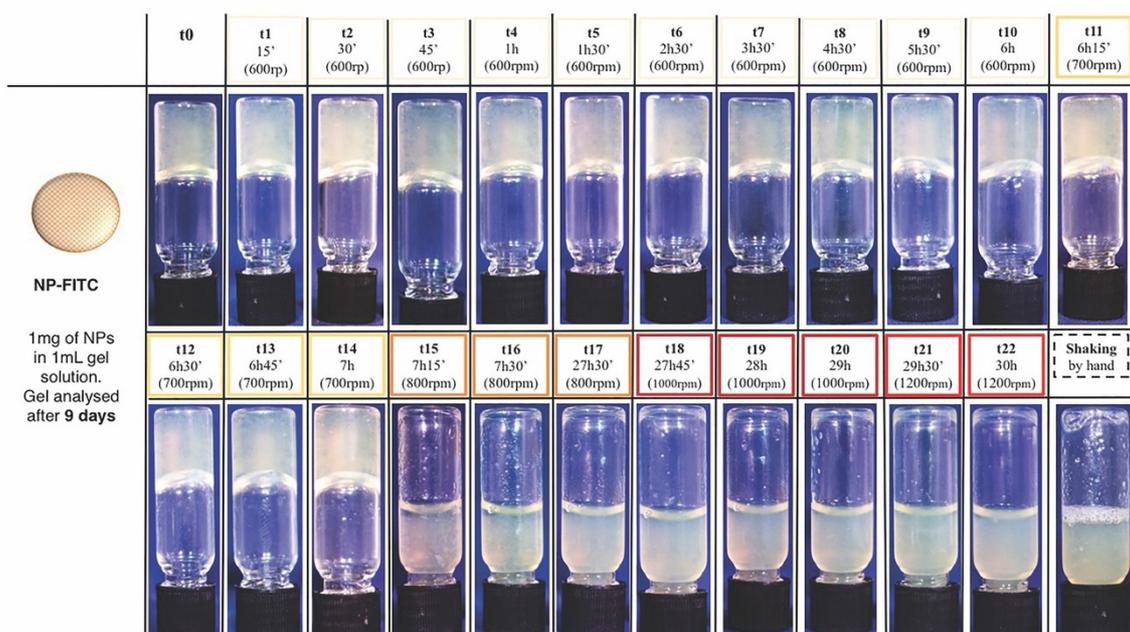


Figure S.8 NP-FICT were fully converted into solution when reached 800 rpm.

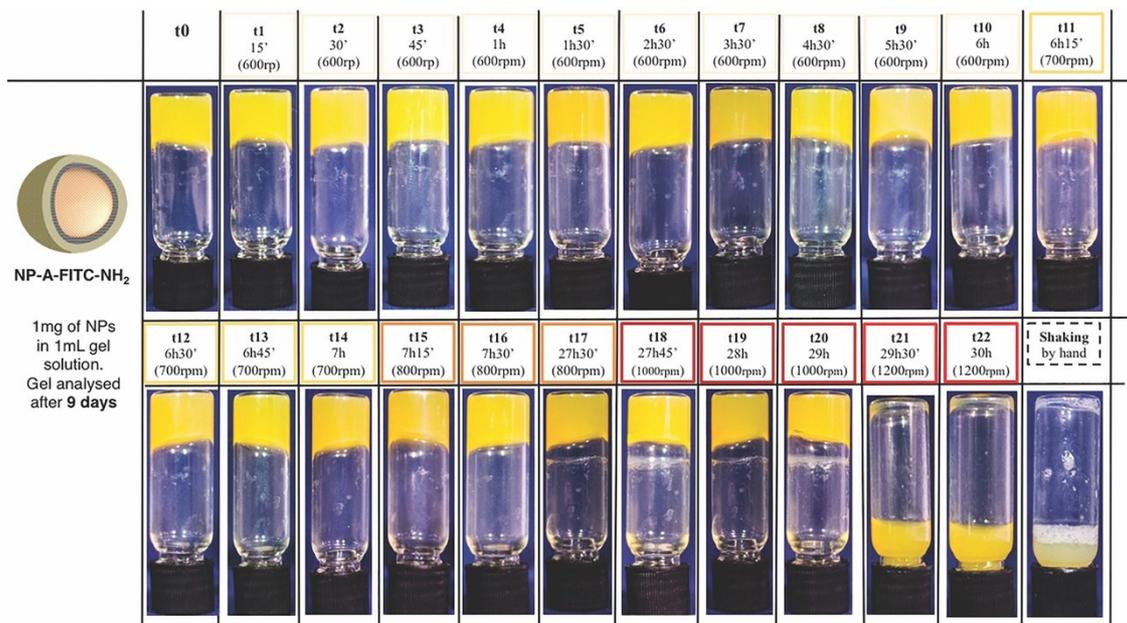


Figure S.9 NP-A-FITC-NH₂ were converted into solution only after 29h30 and only when reached 1200 rpm.

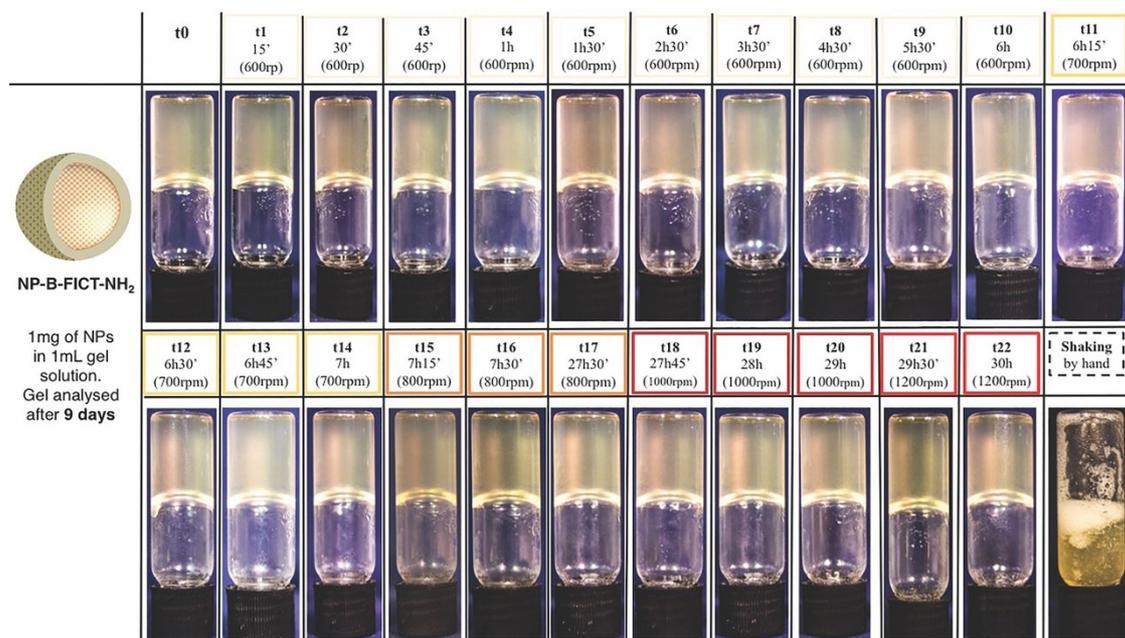


Figure S.10 NP-B-FITC-NH₂ showed to form a very strong gel: only by shaking the sample manually the gel could be converted into solution.

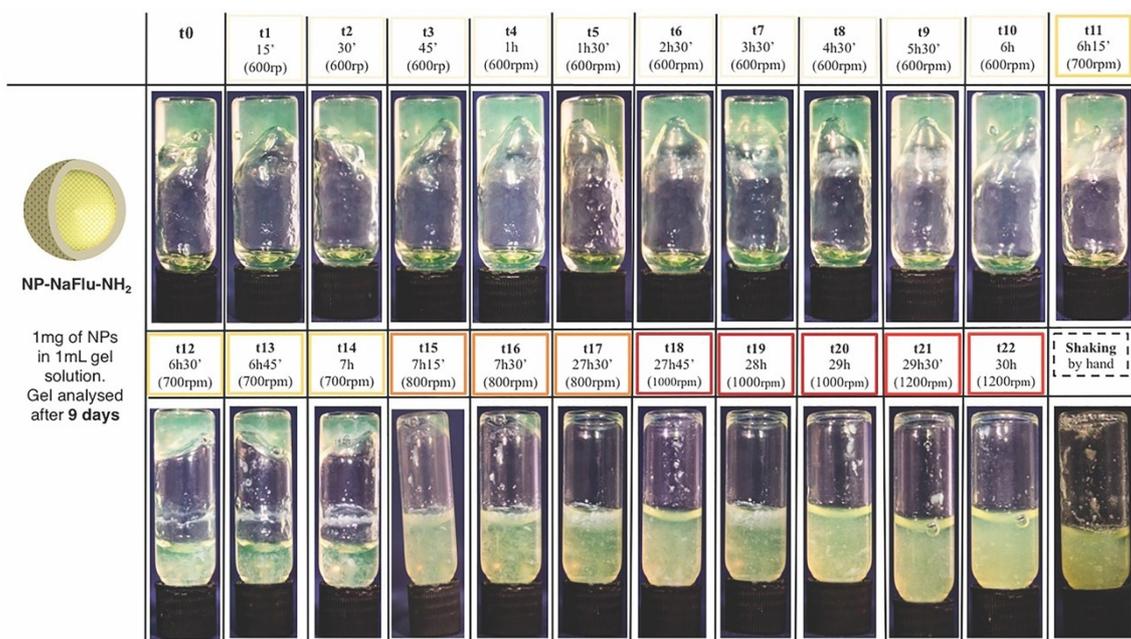


Figure S.11 The gel formed with NP-NaFlu-NH₂ showed to be unstable. We reason that NPs with highly water soluble cargo will require to be formulated in a gel with higher stiffness than the one reported here.



Figure S.12 NP-MB-NH₂ showed to form very strong gel which turned into solution only after manual shaking.

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- [3] L. S. Birchall, S. Roy, V. Jayawarna, M. Hughes, E. Irvine, G. T. Okorogheye, N. Saudi, E. De Santis, T. Tuttle, A. A. Edwards, R. V. Ulijn, *Chem. Sci.* **2011**, *2*, 1349.