Table 1: The justification given by Liu et al for the study of the nanoparticles-diffuse-across-membranes Unicorn. References noted in brackets, e.g. [2-4], refer to Liu et al own bibliography.

Claim backed by references (except last one); quotes from Liu et al	**This may give the impression that:**	**Comment:**	**Relevance of the cited experimental work to the question of diffusion of nanoparticles across membrane:**
Tunable […] properties of […] nanomaterials enable engineering for a number of applications, such as ***drug delivery****,[2−4] …*	Nanomaterials are enabling drug delivery, or may do so soon.	Nanomaterials enable engineering for applications, but the cited articles are chemistry research articles making promises for the future. Ref 2 is so far from drug delivery that it does not even have cell biology experiments. Ref 3-4 are also mostly materials synthesis and characterization (some basic cell biology, no preclinical work).	Ref 2: None. Ref 3: None. The article report that the particles enter by endocytosis. They are supposed to escape later. No evidence, quantification or mechanism provided. Ref 4: None. The article reports that the particles enter by endocytosis.
Tunable […] properties of […] nanomaterials enable engineering for a number of applications, such as […], ***controlled release****,[3,5] …*	Nanomaterials are enabling controlled release, or may do so soon.	Ref 3, see above. Ref 5 is a highly-cited 2008 JACS communication. Mostly materials synthesis and characterisation with one figure showing cell viability and toxicity. 12 years later, the medical application of these materials (PEGylated Nanographene Oxide) does not seem any closer.	Ref 3: None. See above. Ref 5: The article reports that the particles enter by endocytosis.
Tunable […] properties of […] nanomaterials enable engineering for a number of applications, such as […], ***deep tissue imaging, and sensing of cellular behavior.[****6−10]*	Biologists and medical doctors are using those nanomaterials for deep tissue imaging, and sensing of cellular behaviour, or may do soon.	Ref 6-10 are chemistry papers largely describing the synthesis of new materials which could, according to their authors, be useful for deep tissue imaging etc. Some of these are 5+ years old but no biologist currently use these materials for their imaging or sensing needs.	Ref 6: No comment on mechanisms of uptake. Ref 7: None. See also PubPeer comment.[1] Ref 8: None. The article reports receptor-mediated endocytosis. Ref 9: None. Ref 10: None.

Nonetheless, over the years, a number of systems have been reported to give cytoplasmic access to biomacromolecules, most notably cell-penetrating peptides,[17] supercharged proteins,[18,19] and bacterial toxins and different types of NPs.[20–22]	Those strategies are an effective strategy for cytoplasmic access.	In fact, effective delivery to the cytosol remains a highly challenging task as some of these papers make clear. In the rare cases where cytosolic delivery is quantified, authors find that the proportion of nanomaterials reaching the cytosol is very small.[2] That is also our experience, e.g. with TAT.[3]	Ref 17: None. Entry into cells is by endocytosis. Endosomal escape is supposed to involve specific membrane disruption mechanisms. Ref 18: None. Entry into cells is by endocytosis. Endosomal escape is supposed to involve membrane fusion between the lipid-coated "super-charged protein" and the target cell. Ref 19: None. Entry into cells is by endocytosis. Endosomal escape is via a protein pore. Ref 20. None. It is a 20 years old review of synthetic DNA Delivery Systems. Ref 21: None. It is a review of extracellular vesicles for drug delivery. It includes very little on mechanisms of uptake and no suggestion it could be by diffusion through the membrane. Ref 22: None. It is a review of nanoparticulate drug delivery system. In the section on accessing intracellular targets, there is no mention of any potential role of diffusion through membranes.
For particles with the smallest dimension larger than the membrane thickness, approximately above 10−15 nm, the permeation is generally controlled by membrane deformation [23] and endocytosis [24].	General rules about what controls permeation for "particles with the smallest dimension above 10-15 nm" have been obtained.	Multiple levels of confusion. Ref 23 is about graphene sheets; their smallest dimension is well **below** 10-15 nm (and there is no reason to extrapolate any general rule from that particular study). Ref 24 is about endocytosis but the smallest dimension of the smallest particle tested is 100 nm so it cannot tell anything about a supposed threshold around 10-15 nm.	Ref 23: None. The experimental section of the article uses graphene oxide sheets, which are 200 to 700 nm in lateral size. There is no experimental evidence that such objects could diffuse through membranes. Ref 24: None. Entry into cells is indeed by endocytosis.
Smaller nanoparticles can instead cross the membrane by passive transport, that is, by displacing, sometimes irreversibly, the lipids or by diffusing in the hydrophobic region of the membrane and then on the other side.	Particles with the smallest dimension smaller than 10-15 nm can cross membranes by passive transport.	**No reference provided** for this essential claim. There is no serious evidence for its validity – and there is plenty of conflicting evidence. In fact, if true, most proteins would diffuse through membranes, which is obviously not the case.	N/A

1. Levy, Raphael. Comment on: 'Preparation of fluorescent graphene quantum dots from humic acid for bioimaging application'. https://pubpeer.com/publications/E8097D8C7A9D3CA3FED7F363B08110#1 (2015).
2. Gilleron, J. *et al.* Image-based analysis of lipid nanoparticle–mediated siRNA delivery, intracellular trafficking and endosomal escape. *Nat Biotechnol* **31**, 638–646 (2013).
3. Cesbron, Y., Shaheen, U., Free, P. & Lévy, R. TAT and HA2 facilitate cellular uptake of gold nanoparticles but do not lead to cytosolic localisation. *PLoS One* **10**, e0121683 (2015).