layers. While the three individual water-based ink-materials (TMDs, boron nitride and graphene) required to make a printed 2D transistor have been individually demonstrated, printing them sequentially to make a functional device has proved more difficult than expected compared with other nanomaterial inks made from carbon nanotubes or organic semiconductors. This printed 2D transistor challenge is likely a combination of several factors, chief among them the underlying flake-like nature of the 2D inks produced by ultrasonication, which means that a printed rectangular film of 2D transistor semiconductor channel, requires sufficiently overlapping flakes with good electrical continuity to realize a suitable transistor.

With the sustained research and development of 2D atomic materials over the past decade that shows no sign of fading anytime soon[1,8], further materials innovations are to be expected including size optimization of flake dispersions, increased studies on binder/linker additives, and exploration of numerous printing methods to address the printed 2D transistor challenge and other pressing concerns such as printing throughput. In addition, scale-up in printing more than four layers possibly up to a dozen or more will be an unprecedented tour de force. With this in mind, the new method of making biocompatible water-based 2D inks should find immediate interest for existing and emerging applications and serve as a platform for further advancements in printed 2D nanotechnology. ❐

Deji Akinwande is at the Microelectronics Research Center, The Department of Electrical and Computer Engineering, The University of Texas at Austin, Texas 78758, USA.
e-mail: deji@ece.utexas.edu

References

1. Ferrari, A. C. *et al. Nanoscale* **7,** 4598–4810 (2015).
2. Coleman, J. N. *et al. Science* **331,** 568–571 (2011).
3. Torrisi, F. *et al. ACS Nano* **6,** 2992–3006 (2012).
4. Secor, E. B. & Hersam, M. C. *J. Phys. Chem. Lett.* **6,** 620–626 (2015).
5. McManus, D. *et al. Nat. Nanotech.* **12,** 343–350 (2017).
6. Akinwande, D., Petrone, N. & Hone, J. *Nat. Commun.* **5,** 5678 (2014).
7. Bao, W. *et al. Nat. Commun.* **5,** 4224 (2014).
8. Choi, W. *et al. Mater. Today* http://doi.org/b4ks (2017).

NANOMEDICINE

Evolution of the nanoparticle corona

Understanding how complement proteins bind to nanoparticles and participate in their surface 'corona' can provide further insight into the relevance of the protein corona concept in medicine.

Marilena Hadjidemetriou and Kostas Kostarelos

Nanoparticles (NPs) administered in the body are rapidly modified once in contact with the biological milieu due to their interfacial interaction with various blood constituents, of which proteins have been most studied. The term 'protein corona' was proposed in 2007 to describe such spontaneous self-assembly and layering of proteins onto NP surfaces[1]. This surface 'bio-transformation' of nanomaterials modulates their overall pharmacological and toxicological profile and their potential therapeutic or diagnostic functionality in a rather unpredictable manner. Despite the mitigation of protein adsorption by NP surface functionalization strategies with high-molecular-weight hydrophilic groups (for example, PEGylation, glycosylation), currently there is no strategy able to completely eliminate protein corona formation.

The protein corona concept does not constitute a new discovery per se, but evolved from the pioneering 'protein adsorption' work by Bangham[2] and Vroman[3] in the 1950s and 60s. They were the first to show that protein adsorption plays an important role in overall biological interactions and responses to pristine surfaces and materials. Subsequent protein adsorption studies in the 80s and 90s, focused mainly on liposomes and polymeric NPs, aimed at the development of adsorption-resistant surfaces and nanomaterials to prevent their recognition, opsonization by cells and in the case of

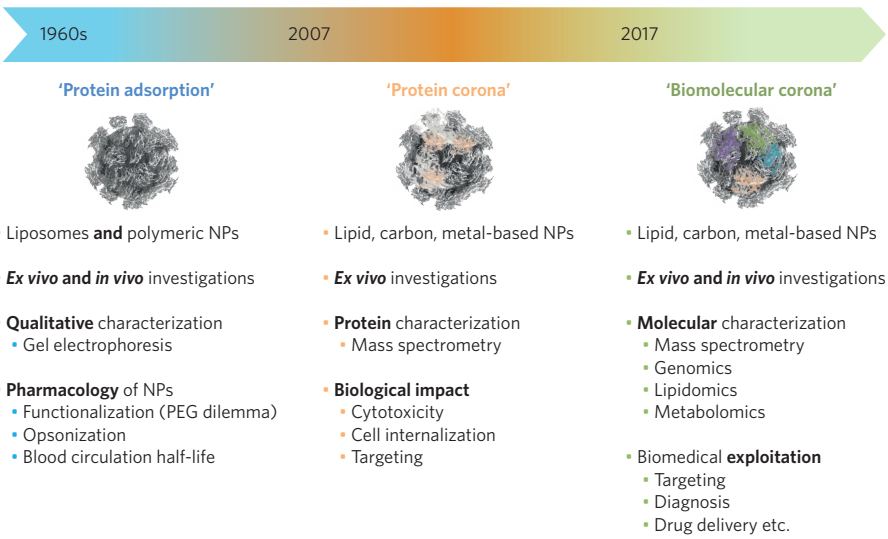

Figure 1 | The evolution of the nanoparticle corona concept. From the appreciation of 'protein adsorption' (in grey to indicate no molecular identification) studies in the 1960s, to the 'protein corona' (in orange and grey to indicate the beginning of protein molecule identification) and the most recent 'biomolecule corona' (multi-coloured to indicate the different types of biological molecules thought to constitute the corona). The differences and similarities in what has been mainly studied are shown in bold.

intravenously administered NPs to increase their blood circulation half-life. A major breakthrough was the surface-coating NPs with the hydrophilic polymer polyethylene glycol (PEG), which imparts steric stabilization and reduces the interaction of NPs with blood proteins[4]. This early work invariably referred to protein adsorption, and mostly used *in vivo* models with an emphasis on particle surface biocompatibility, pharmacology and pharmacokinetics (Fig. 1).

During the past decade, protein adsorption studies morphed into the nanoparticle protein corona literature that has emerged with the central aim of characterizing the adsorbed protein layers by high-throughput mass-spectrometry proteomics. The vast majority of protein corona studies so far have been performed *ex vivo* (primarily using extracted blood plasma) and much effort has been devoted to determine the different factors that govern the formation of protein corona and its correlation to the NP chemical composition and physicochemical properties[5]. Although some general principles have been revealed, a few fundamental issues still remain unresolved, such as the reversibility and displacement between the adsorbed proteins, the kinetics of corona formation and the prediction of protein corona composition based on the type and properties of the NPs. The multi-layered adsorption of proteins onto NP surfaces, and the distinction between 'hard' and 'soft' coronas, have been among the most controversial issues in the protein corona literature[6,7]. The prevailing hypothesis describes an inner layer of tightly bound proteins ('hard corona') and an outer rapidly exchanging layer of weakly bound proteins ('soft corona'). Some proposed models further suggest that hard corona proteins interact directly with the nanomaterial surface, while soft corona proteins associate with the hard corona via weak protein–protein interactions[8].

Writing in *Nature Nanotechnology*[9], Chen and colleagues provide an interesting insight into the mechanism of interaction of complement proteins with dextran-coated superparamagnetic iron oxide core–shell nanoworms after their *ex vivo* incubation in human plasma. Their results suggest that complement proteins, and specifically the third complement component (C3), were covalently bound to hard corona proteins (rather than the nanoworm surface), which accelerated the assembly of other complement components of the alternative pathway. Complement C3 adherence on NP-absorbed proteins rather than on the native NP surface reinforces the existence of softly bound proteins and underlines the requirement of new complement-modulating strategies. Since most of the work reported by Chen *et al.* is performed *ex vivo* (that is by incubation of nanoworms with human plasma) their observations about protein corona formation, as with all such studies, is highly dependent on the incubation conditions of the NPs with plasma, the purification protocols applied and the experimental approaches employed to characterize the protein corona. Although such *ex vivo* incubations of NPs with plasma proteins can improve our understanding of protein corona formation, their extrapolation to predict the fate of NPs or their immunotoxicity impact in physiological conditions should be made with extreme caution.

Chen *et al.* further attempted elucidation of the protein corona dynamics *in vivo*. To test whether the *ex vivo* formed corona proteins were replaceable *in vivo*, the dextran-coated superparamagnetic iron oxide core-shell nanoworms were pre-incubated in plasma that contained fluorescently-labelled proteins. The *ex vivo* corona-coated nanoworms were then intravenously injected in mice and recovered from the blood circulation by cardiac puncture. Gel electrophoresis of the recovered NP-associated proteins demonstrated a significant loss of the fluorescently labelled proteins, suggesting a kinetically unstable corona. The use of an *in vivo* model to interrogate the formation and dynamic exchange of an *ex vivo* protein corona is an illustration of the further evolution of the field from the study of the limited *ex vivo* protein corona studies to the richer and more physiologically relevant *in vivo* work needed.

To date, only a few studies describe the protein corona formed onto NPs *in vivo*. The first such demonstration has recently shown that the *in vivo* protein corona formed onto intravenously injected liposomes recovered from the bloodstream of mice differed in composition and morphology in comparison to the one formed *ex vivo*.[10] Even though the total amount of protein attached on the blood-circulating liposomes correlated with that observed from *ex vivo* plasma incubations,

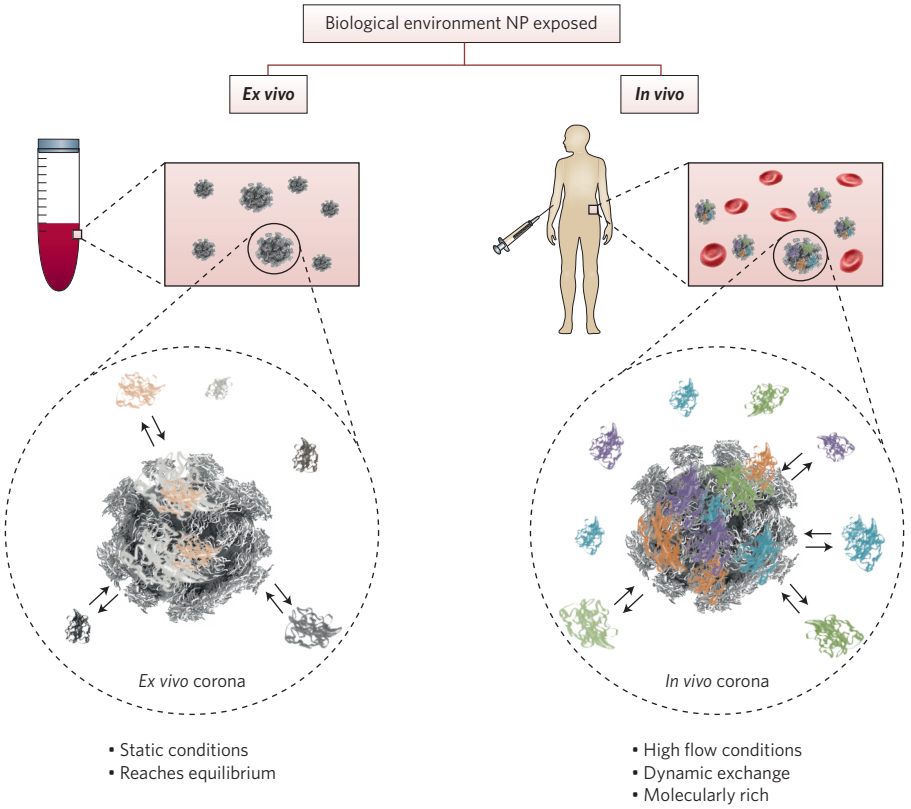

Figure 2 | The *ex vivo* and *in vivo* NP corona. The main differences between the protein coronas formed on NPs after their incubation in plasma or administration in living organisms are illustrated. In a static, *ex vivo* environment the protein corona rapidly adsorbs onto NPs and reaches equilibrium. *In vivo*, a molecularly richer protein corona is formed in flowing conditions within the blood circulation and by the dynamic exchange of proteins, it evolves over time.

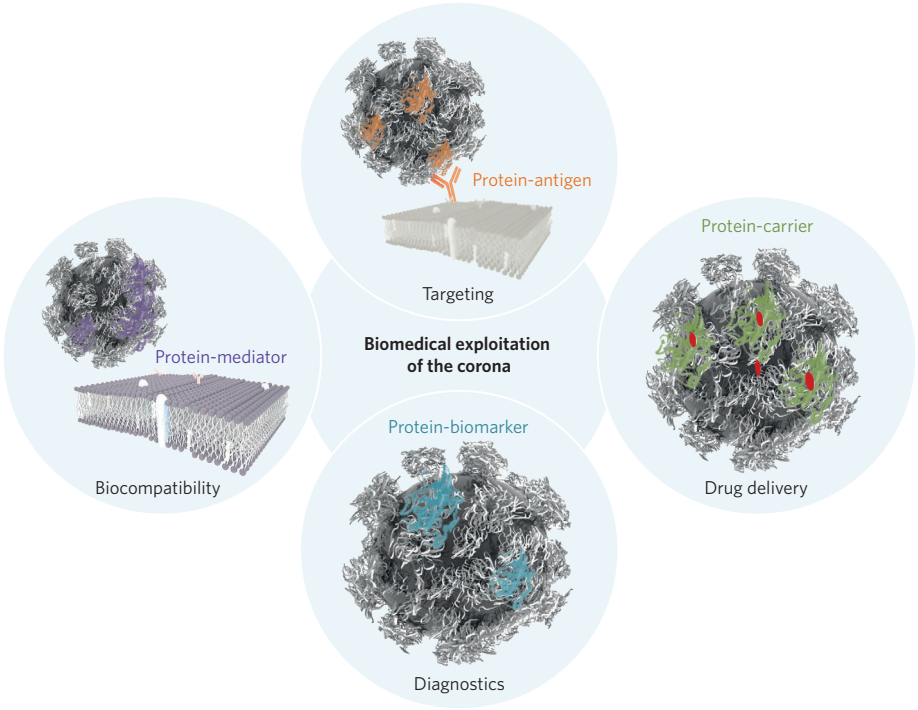

Figure 3 | The *in vivo* NP biomolecule corona and its potential applications in biomedicine. Potential exploitation of the biomolecule corona for improved NP biocompatibility and toxicity; cell targeting; increased drug payload NP capacity; and disease detection.

the variety of molecular species in the *in vivo* corona was considerably wider. Blood flow dynamics, interaction with circulating and endothelial lining cells, or immune responses triggered after injection of NPs are some of the factors that cannot be mimicked by *ex vivo* studies. *In vivo* investigations of the protein corona, although more reflective of the true biological environment that NPs will experience with all its complexity, they are unquestionably more challenging and limited by the amount of NPs recovered post-administration. Moreover, time evolution of the *in vivo* protein corona formation onto clinically used, PEGylated liposomes showed that protein adsorption is a highly dynamic process[11]. The abundance of corona proteins was found to fluctuate over time, indicating that competitive exchange processes are taking place. These observations have clearly indicated some important differences between coronas forming *ex vivo* and *in vivo* (Fig. 2).

The simultaneous characterization of different types of biomolecules — lipids, sugars, nucleic acids, hormones, metabolites — self-assembled onto NPs upon interaction with biofluids *in vivo* holds several experimental challenges, however should be considered the next frontier in the field. In view of the above, scepticism prevails as to whether the composition of the 'biomolecule corona' in complex *in vivo* physiological environments can be controlled as often suggested. Many more *in vivo* studies are needed to be able to realistically understand and determine the overall molecular identity of the adsorbed biomolecules onto the NP surface following their administration.

At the broader level, the key question lies on the overall impact of the 'biomolecule corona' on molecular recognition and its implication in various biological processes and consequently potential biomedical applications (Fig. 3). It is now reasonably accepted that the interaction of NPs with cells and their response to NP exposures (intended or unintentional) are greatly influenced by the biomolecules adsorbed onto their surfaces. Initial (*ex vivo*) work[12] reported that the binding specificity of targeted NPs can be lost in the presence of plasma proteins. That work stimulated a series of investigations aiming to provide mechanistic understanding of the effect of protein corona formation on cellular internalization, receptor targeting, cytotoxicity and immunotoxicity of NPs[13]. It has also resulted in attempts to exploit the biomolecular corona as a means to target specific cells. According to this strategy, NPs are thought to be surface-designed to interact with specific plasma proteins that will initiate targeted receptor-mediated cellular binding and internalization[14]. Of course, that would only be effective if target cells overexpressed the receptors specific to the corona-forming molecules. Along similar lines, the engineering of a biomolecular corona has been suggested as a potential strategy to mitigate the cytotoxicity of NPs[15]. Moreover, the utilization of the molecular fingerprint offered by the biomolecular corona for early disease detection has been recently theoretically postulated[16], even though such an approach remains largely unexplored and experimentally unproven.

Plenty of knowledge and understanding of the fundamental mechanisms governing biomolecular corona formation on NP surfaces is still missing, particularly outside the simplicity of an *ex vivo* biological (plasma) sample and into a complex, physiological environment. Nevertheless, a step-change has begun to occur in the way the biomolecule corona is perceived. Initially seen as an interfering obstacle that hinders the desired properties of nanomaterials, it is gradually seen as an engineering tool by which to modify the NP surface and potentially render greater therapeutic and diagnostic capability. In the interim, we urge for rigorous, systematic and thoroughly reported investigations under physiologically relevant *in vivo* conditions.

Marilena Hadjidemetriou and Kostas Kostarelos are at the Nanomedicine Lab, Faculty of Biology, Medicine & Health and the National Graphene Institute, AV Hill Building, The University of Manchester, Manchester M13 9PT, UK.
e-mail: kostas.kostarelos@manchester.ac.uk

References

1. Cedervall, T. *et al. Proc. Natl Acad. Sci. USA* **104,** 2050–2055 (2007).
2. Bangham, A. D., Pethica, B. A. & Seaman, G. V. *Biochem J.* **69,** 12–9 (1958).
3. Vroman, L. *Nature* **196,** 476–477 (1962).
4. Allen, T. M. *et al. Biochim. Biophys. Acta* **1066,** 29–36 (1991).
5. Walkey, C. D. *et al. ACS Nano* **8,** 2439–2455 (2014).
6. Casals, E., Pfaller, T., Duschl, A., Oostingh, G. J. & Puntes, V. *ACS Nano* **4,** 3623–3632 (2010).
7. Docter, D. *et al. Chem. Soc. Rev.* **44,** 6094–6121 (2015).
8. Lundqvist, M. *et al. Proc. Natl Acad. Sci. USA* **105,** 14265–14270, (2008).
9. Chen, F. et al. *Nat. Nanotech.* **12,** 387–393 (2017).
10. Hadjidemetriou, M. *et al. ACS Nano* **9,** 8142–8156 (2015).
11. Hadjidemetriou, M., Al-Ahmady, Z. & Kostarelos, K. *Nanoscale* **8,** 6948–6957 (2016).
12. Salvati, A. *et al. Nat. Nanotech.* **8,** 137–143 (2013).
13. Tenzer, S. *et al. Nat. Nanotech.* **8,** 772–781 (2013).
14. Zhang, Z. *et al. ACS Nano* **9,** 2405–2419 (2015).
15. Hu, W. *et al. ACS Nano* **5,** 3693–3700 (2011).
16. Hamad-Schifferli, K. *Nanomedicine* **10,** 1663–1674 (2015).