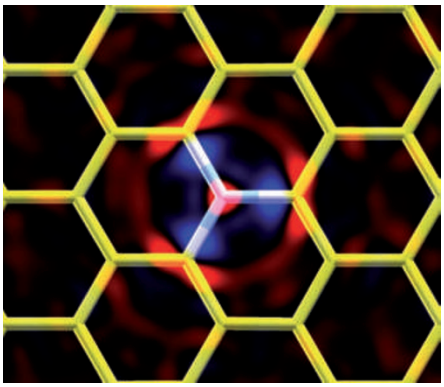

Figure 2 | DFT results for the changes in electron density due to bonding. The blue (red) colour shows a lower (higher) electron density than in the case in which the independent atomic model is used[1].

out the expected contrast difference. As a consequence, the two types of atom cannot be distinguished from each other, on physical grounds. This also shows that first-principle calculations should be used to take advantage of what sophisticated optics has to offer

The other popular concept in HRTEM is that of the 'ideally weak object'. As discussed in textbooks[12], a weak object is realized by a very thin sample consisting of atoms of low nuclear charge, which, owing to their weak electron scattering, induce only very weak phase shifts in the imaging electron-wave field. For such an idealized object, theory[13,14] yields phase-shift contrast that, as long as standard conditions are applied for acquiring an image, provides a direct representation of the scattering potential. This greatly aids image interpretation and has often served as an argument for deriving atomic-structure information from only a single acquisition, thus avoiding the painstaking effort needed to take image series and engage in complicated and time-consuming numerical procedures for electron-wavefunction reconstruction. Now, transmission electron microscopes do not transmit equally well all spatial frequencies that, in a Fourier representation, are required to describe an object structure correctly. For example, the microscope cuts spatial frequencies both in the high- and low-frequency range, thus acting as a low- and high-pass filter. Meyer and colleagues show that in the standard-conditions single acquisition mode, the contrast features induced by charge transfer are suppressed, making the bonding-induced effect unobservable. Yet single-layer graphene is, with respect to electron scattering, the weakest object so far available. Taking image series under defined variable-focus conditions allows expansion of the spatial-frequency characteristics of a microscope. This has to become part of the everyday routine of microscopy on the atomic scale.

To 'see' atoms and to measure the local electronic effect of integrating an atom into a solid is a long-standing dream in materials science. The recent work on graphene is a great step in this direction. In essence this means nothing less than an uncompromising application of quantum mechanics not only to the calculation of the electron structure in solids but also to the treatment of contrast formation in electron optics. ❐

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SYNTHETIC VACCINES

Immunity without harm

Multilamellar lipid vesicles with crosslinked walls carrying protein antigens in the vesicle core and immunostimulatory drugs in the vesicle walls generate immune responses comparable to the strongest live vector vaccines.

Abhinav P. Acharya and Niren Murthy

Preventive (prophylactic) vaccines have greatly helped the global eradication of some infectious viral diseases, for example poliomyelitis or smallpox[1]. However, vaccines used against pathogens such as the HIV and the hepatitis C virus (HCV) have for the most part failed. One problem is that vaccines based on attenuated viruses, despite generating protective immunity[2,3], are frequently too toxic for clinical use. On the other hand, non-viral vaccines, which typically have an excellent toxicity profile, are relatively ineffective at promoting immunity. It is therefore necessary to develop a vaccination strategy that can generate effective immunity and have low toxicity. As reported in *Nature Materials*, Irvine and colleagues have achieved an important step in this direction[4]: a non-viral vaccine carrier that provides immune responses comparable to viral vectors[5].

The vaccine carriers are multilamellar vesicles (liposomes) with crosslinked bilayers that entrap protein antigens in the vesicle core, and immunostimulatory Toll-like receptor (TLR) ligands in between bilayers (TLR is a protein that recognizes structural patterns). Irvine and colleagues showed that the crosslinked liposomes act as a controlled-release reservoir of protein antigen and can also target dendritic cells *in vivo*. Dendritic-cell targeting is a key part of the multi-step process by which vaccines activate antigen-specific memory T cells[6] — cells that recognize and rapidly clear pathogens that caused previous infections. In this process (Fig. 1), dendritic cells pick up the injected liposomes and present the delivered antigen and immunostimulatory molecules to cytotoxic T cells. The dendritic cells secrete cytokines — cell-signalling protein molecules — that help activate T cells against the pathogen-specific antigens. These cells then proliferate and circulate through the body, most of them dying off within a few days. However, a small subset of the population of cytotoxic (or killer) T cells survives in the long term (even for decades), giving rise to memory T cells. On

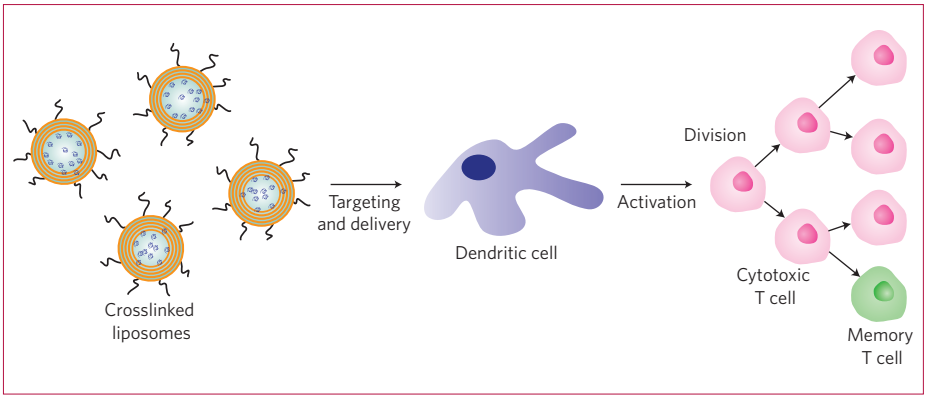

Figure 1 | Crosslinked liposomes target and deliver antigen and immunostimulatory drugs to dendritic cells to trigger the generation of memory T cells. Activated dendritic cells generate cytotoxic T cells, which then divide and clear the infection. Some of the cytotoxic T cells give rise to memory T cells, which survive long term.

infection by a pathogen with the known antigen, the antigen-specific memory T cells proliferate and differentiate into cytotoxic T cells, which clear the infection before it can gain a foothold. Hence, the presence of antigen-specific memory T cells greatly accelerates the timescale and magnitude of the immune response towards the infecting pathogen.

Although the detailed mechanism by which antigen-specific memory T cells are generated is not completely understood, key steps seem to be the presentation of the antigen to T cells, the secretion of cytokines by dendritic cells, and the controlled release of antigen. To secrete the cytokines needed for T cell activation, dendritic cells need to be stimulated by TLR ligands. Thus, several polymeric microparticles have been developed that can deliver both antigen and TLR ligands[7]. However, because these particles have difficulties in reaching the cytosol of dendritic cells, they tend to be less effective as vaccine carriers.

As an alternative, the delivery of vaccines loaded into liposomes is a very attractive strategy not only because the cytosol can be accessed, but also because both antigen and TLR ligands can be easily encapsulated within the same particle, owing to the presence of both aqueous and hydrophobic components. Furthermore, the synthesis of liposomes is simple, it can be performed on a small scale in an aqueous environment, and it provides high encapsulation of proteins. Despite the advantages, however, liposomes have previously not been critically successful as vaccine-delivery vehicles owing to their variable stability and unpredictable release profile[8]. Indeed, liposomes can be destabilized under the shear forces present in biological environments, resulting in structural disintegration and aggregation or fusion, and also have problems with chemical stability because of hydrolysis, oxidation and enzymatic degradation.

Irvine and co-authors avoided the usual problems in the stability and release profile of the liposomes by crosslinking their bilayers with thioether linkages (Fig. 2). Additionally, polyethylene glycol was conjugated on the surface of the resulting interbilayer crosslinked multilamellar vesicles (ICMVs) to enhance their *in vivo* performance. The authors showed that ICMVs have excellent stability in physiological solutions but rapidly break down intracellularly as a result of the presence of lipases — lipid-hydrolysing enzymes — within the cells[4]. They also showed that ICMVs provide both sustained release and intracellular delivery of the entrapped antigen, thus allowing efficient activation of both T and B cells, and the generation of memory T cells. B cells — cells that produce antibodies against antigens — are important because they neutralize pathogens that are circulating in the blood, and it is believed that effective vaccines for pathogens such as HIV will require activation of both T and B cells.

Although ICMVs have shown tremendous potential with the model antigen Ovalbumin, it needs to be seen if this approach shows similar levels of efficacy with protein antigens from pathogens such as HIV and HCV. Also, because the immune system of humans can be substantially different from that of a mouse, determining if ICMVs also have high efficacy in higher animal models is critical. All in all, the concept of crosslinking liposomes with dithiol linkages is a powerful way of delivering protein antigens and TLR ligands to immune cells, with the potential to greatly improve vaccine development and drug delivery. ❐

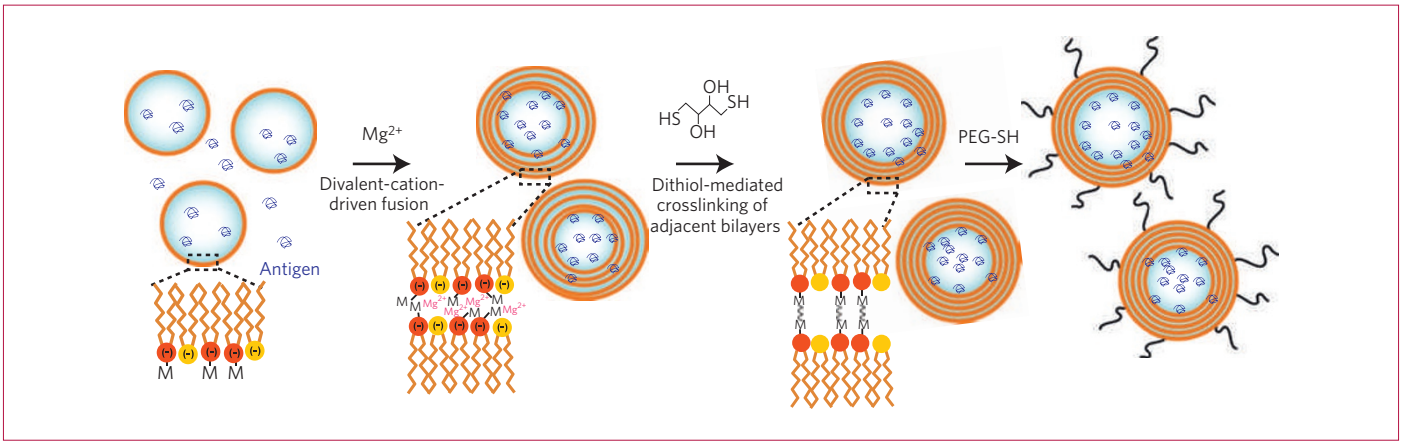

Figure 2 | Interbilayer-crosslinked multilamellar vesicles (ICMVs) encapsulating TLR ligands (not shown) and pathogen-specific antigens were synthesized[4] from dried liposomes by Mg^{2+}-induced fusion and by crosslinking lipid head groups from opposite lipid bilayers with bilayer-permeable dithiols. Thiol-terminated polyethylene glycol (PEG-SH) was then conjugated to the surface of the resulting ICMVs to enhance their performance *in vivo*.

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OXIDE ELECTRONICS

Interface takes charge over Si

The formation of a two-dimensional electron liquid at the interface between two insulating oxides, now extended to oxides on Si, joins a wealth of observations that reveal how electron transfer between layers is responsible for this unusual effect.

Darrell G. Schlom and Jochen Mannhart

Silicon, the backbone of modern electronics, is the most perfected, best understood and most heavily exploited electronic material of the information age we live in. The technological impact of new material systems with functional properties of relevance to semiconductor devices — properties that are not provided by silicon — relies on their successful integration with silicon. Writing in *Nature Communications*, Jae-Wan Park and colleagues report precisely this[1]: the integration of a functional oxide interface on silicon. This oxide system is known to show remarkable properties[1], including a transition from conducting to insulating states that can be reversibly written and erased locally with nanometre precision using techniques based on atomic force microscopy[2]. In addition to its possible role for semiconductor technology, this achievement provides important clues to the origin of the metallic conductivity that can be realized at the interface between these two oxides, $LaAlO_3$ and $SrTiO_3$, which are both insulators.

Whether this effect will have any relevance for silicon electronics depends strongly on whether this is an intrinsic electronic effect, or merely caused by defects. And indeed, this issue has been intensively investigated. Metallic conductivity is observed at the interface between the insulating oxides $LaAlO_3$ and $SrTiO_3$ when thin epitaxial $LaAlO_3$ films are grown on (001)-oriented $SrTiO_3$ single crystals that terminate with a TiO_2 layer[3], as schematically shown in Fig. 1a,b. In contrast, if SrO is the top layer of the $SrTiO_3$ (Fig. 1c), or if amorphous $LaAlO_3$ is grown on TiO_2-terminated $SrTiO_3$ (Fig. 1d), the resulting interfaces are insulating[3,4]. The origin of the interfacial conductivity has been attributed to electronic reconstruction[5], a process in which, owing to electron transfer, some of the Ti^{4+} in the TiO_2 layer at the interface with the $LaAlO_3$ is reduced to Ti^{3+}. In essence, when growing a (001)-oriented $LaAlO_3$ film on TiO_2-terminated $SrTiO_3$, an electrostatic voltage is built up because $LaAlO_3$ consists of charged, alternating planes of LaO^+ and AlO_2^- (ref. 5). The voltage grows with the thickness of the $LaAlO_3$ film to such large values that eventually electrons move from the surface of the $LaAlO_3$ film to the $LaAlO_3$/$SrTiO_3$ interface to occupy Ti 3*d* states there. These 3*d* states form two-dimensional electronic bands that extend parallel to the interface, and thereby provide interfacial

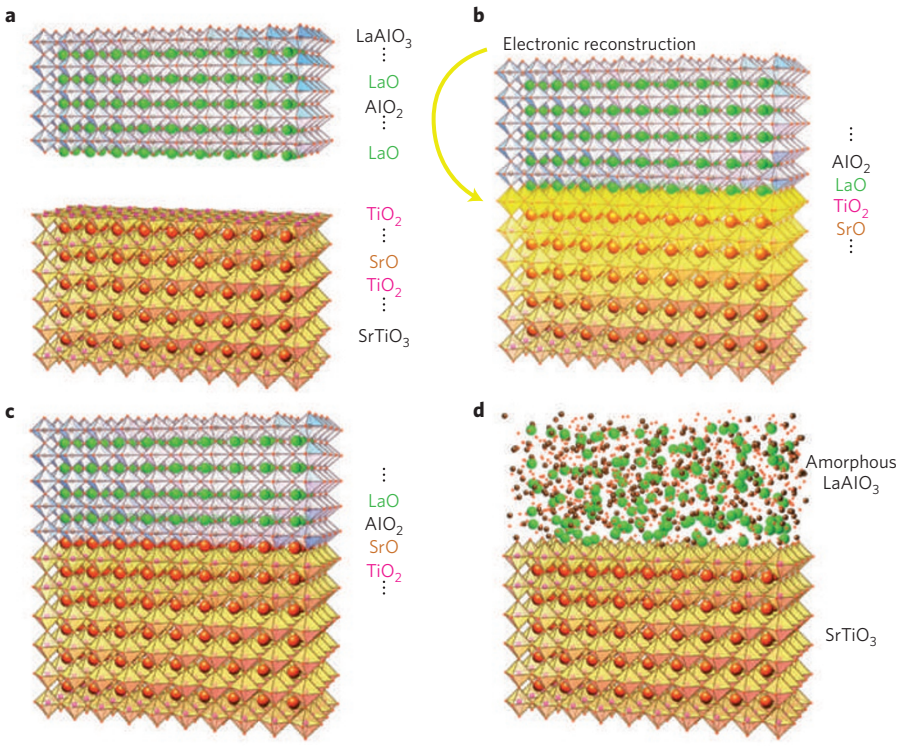

Figure 1 | Subtle differences between $LaAlO_3$/$SrTiO_3$ interfaces result in just one type of interface exhibiting interfacial conductivity. **a**, Schematic of a TiO_2-terminated (001) $SrTiO_3$ single crystal before contact with an epitaxial $LaAlO_3$ overlayer. **b**, Epitaxial $LaAlO_3$ on TiO_2-terminated $SrTiO_3$ is the only interface to exhibit interfacial conductivity, provided it is more than three unit cells thick. **c**, Epitaxial $LaAlO_3$ on SrO-terminated $SrTiO_3$ does not exhibit interfacial conductivity. **d**, Amorphous $LaAlO_3$ on TiO_2-terminated $SrTiO_3$ is also insulating. The interfaces shown are schematic and do not include atom rearrangements including octahedral rotations or the buckling of atomic planes.