present density functional calculations with gradient corrections give energy barriers of chemical reactions that are too low. A qualitative step forward is called for, similar to the step from the LDA to current gradient-corrected density functionals (5).

For these reasons, the design and optimization of high-performance electronic, optical, and magnetic materials benefits perhaps the most from the capabilities of first-principles quantum mechanical methods, because in these systems the key aspect is typically the relation between a static geometric structure and the corresponding electronic structure. A good example is related to the design of high-performance optical fibers, in which any impurity or defect is a potential source of scattering that can degrade performance. One such defect is caused by oxygen vacancies within the SiO_2 network. Starting from a crystal of α-quartz (see figure), the removal of an oxygen atom leaves two silicon atoms in the lattice with unsaturated bonds. It is not obvious how these atoms relax to minimize the total energy of the system. One possibility is that an internal Si–Si dimer forms, thus closing the microvoid created by the oxygen vacancy. Local density functional calculations (6) reveal that on removal of one oxygen atom the adjacent Si atoms spontaneously dimerize. However, a puckered conformation is found as another possible metastable conformation (see figure). For this structure, the calculations give a highly localized electronic state (see figure). The energy of this state falls into the optical gap of the perfect silica and thus may be a cause for light absorption. A key finding, which is being further investigated, is the fact that the puckered structure is stabilized by the trapping of a positive charge. In fact, with this detailed knowledge of the nature of the oxygen impurity and its sensitivity to the charge state, it is possible to design improved materials and processes that avoid this type of defect, thus leading to better high-performance silica materials for applications such as optical fibers.

These two examples demonstrate that current quantum mechanical methods allow the prediction of subtle structural features that can have significant effects on the properties of a material, such as its optical characteristics. Equipped with this type of predictive structural tool, materials scientists are now in a position to explore the "materials design space" in a much more creative and productive way than with a purely experimental approach. A large part of all possible combinations of atoms is still unknown, as can be seen from an analysis of the documented materials and phases. Whereas 83% of all possible binary systems have been experimentally characterized, less than 10% of all ternaries and less than 1% of quaternary systems are known (7). The majority of materials have yet to be synthesized and characterized. An important tool to this end is the reliable prediction of atomic structures, now possible with first-principles quantum mechanical approaches even when very subtle effects and dynamical aspects are involved.

References and Notes

1. J. Furthmüller, J. G. Kresse, J. Hafner, R. Stumpf, M. Scheffler, *Phys. Rev. Lett.* **74**, 5084 (1995).
2. Local density functional theory does not contain any arbitrary or adjustable parameters; hence, it can be called "first-principles" theory. In contrast, current gradient-corrected density functionals include some parametric aspects.
3. An overview of current atomistic computational methods is given, for example, by E. Wimmer, *J. Comput.-Aided Mater. Des.* **1**, 215 (1993).
4. R. Car and M. Parrinello, *Phys. Rev. Lett.* **55**, 247 (1985).
5. J. P. Perdew, Specialist Workshop on Density Functional Methods in Chemistry, Centre Européen des Calculs Atomiques et Moléculaires (CECAM), Lyon, France, 28 to 30 June 1995.
6. D. C. Allan and M. P. Teter, *J. Am. Ceram. Soc.* **73**, 3247 (1990).
7. P. Villars, A. Prince, H. Okamoto, *Handbook of Ternary Alloy Phase Diagrams* (ASM International, Materials Park, OH, 1995), vols. 1–10.

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Digging into Caveolae

Robert G. Parton and Kai Simons

Eukaryotic cells use stringent sorting mechanisms to maintain the lipid and protein composition of their intracellular organelles. This sorting process is best understood for the recruitment of certain transmembrane proteins into pits in the plasma membrane (defined by their coats of the protein clathrin). Concentration of these proteins generates heterogeneity within the plane of the membrane. The cell uses a different strategy to maintain other distinctive plasma membrane structures, the caveolae, a strategy that makes use of the biophysical properties of lipids rather than protein-protein interactions. A new purification technique for caveolae, reported in this issue of *Science*, demonstrates this in a striking way (*1*).

Caveolae ("small caves"), flask-shaped invaginations of the plasma membrane, are a prominent feature of many mammalian cells (*2*, *3*). They were originally defined by electron microscopy and are particularly abundant in adipocytes, smooth muscle, and endothelia. In the past 5 years, progress on the biochemistry of caveolae has been greatly facilitated by the characterization of VIP21-caveolin, a 21-kilodalton integral membrane protein proposed to be part of the "caveolar coat" (*4*, *5*). This unusual protein forms a hairpin structure in the membrane, with both the amino and carboxyl termini facing the cytoplasm (6). VIP21-caveolin is also present in the trans-Golgi network (TGN) and in both apical and basolateral exocytic vesicles of epithelial cells. It was isolated from such epithelial cells on the basis of its presence in low-density, detergent-insoluble complexes (*7*), which also contain glycosyl phosphatidylinositol (GPI)–linked proteins and other proteins implicated in signal transduction, including Src-family kinases and heterotrimeric guanosine triphosphate–binding proteins (G proteins) (8). The complexes are enriched in glycosphingolipids (GSLs), sphingomyelin, and cholesterol, but are depleted of phospholipids (9). These results suggested a link to earlier observations in lymphocytes showing that cross-linking of GPI-anchored proteins caused activation of Src-family kinase-mediated signaling pathways dependent both on the GPI-anchor and on the lipid modification (palmitoylation) of the Src kinase (*10*–*12*). Moreover, detergent treatment of lymphocytes extracted complexes enriched in GPI-anchored proteins and Src kinases (*13*). These findings led to the idea of microdomains of distinct lipid composition that mediate signal transduction.

However, the equivalence of detergent-insoluble complexes and caveolae has now been questioned (*14*), and it appears that at least in lymphocytes and neuroblastoma cells detergent-insoluble complexes can exist in the absence of caveolae, as shown by morphological criteria and by the absence of VIP21-caveolin (*15*, *16*). Clearly, signal transduction in these cells does not require VIP21-caveolin or caveolar invaginations. In addition, GPI-anchored proteins may not be greatly enriched in caveolae under steady-state conditions (*17*, *18*) and hence their detergent insolubility would not correlate with their presence in caveolae. Nevertheless, these points remain controversial. We therefore propose that the term "caveolae" be used for the classical morphological entity as originally defined and that these structures be differentiated from the de-

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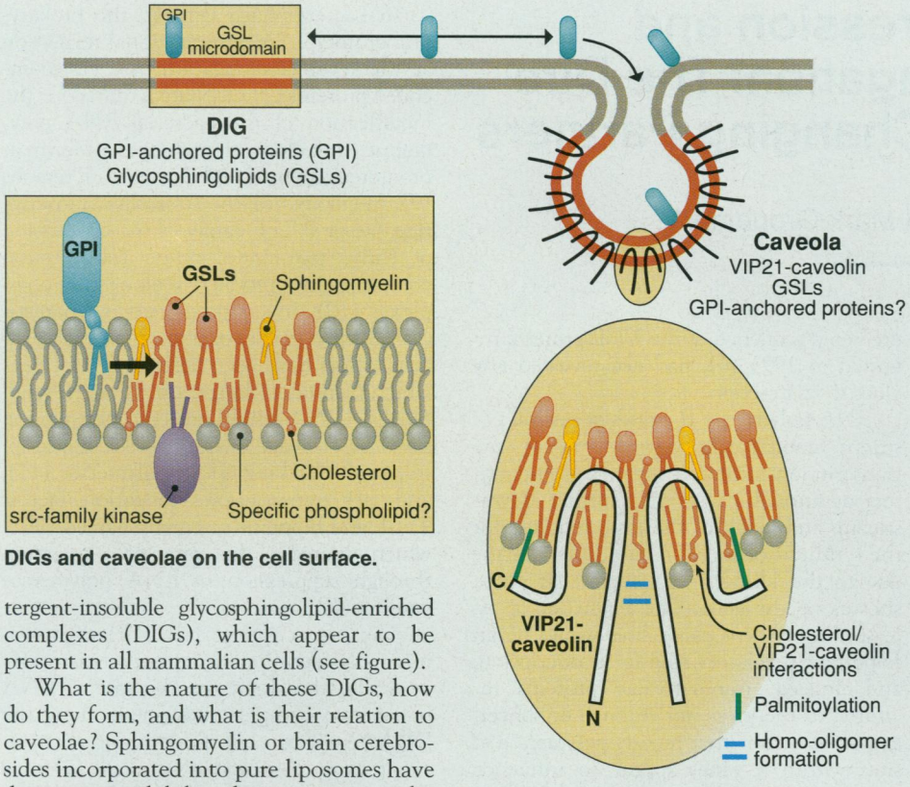

DIGs and caveolae on the cell surface.

tergent-insoluble glycosphingolipid-enriched complexes (DIGs), which appear to be present in all mammalian cells (see figure).

What is the nature of these DIGs, how do they form, and what is their relation to caveolae? Sphingomyelin or brain cerebrosides incorporated into pure liposomes have detergent-insolubility characteristics similar to those of the DIGs of intact cells (*19*). Cholesterol in the liposomes also becomes detergent-insoluble but is not essential for the insolubility of the lipids. GPI-anchored alkaline phosphatase incorporated into the same liposomes was also detergent-insoluble but, unlike sphingolipids, which were detergent-insoluble at all temperatures, alkaline phosphatase was soluble at higher temperatures. Thus, the insolubility of these molecules is not a fundamental feature of caveolae but is due to the physical properties of the lipid components. The basic insoluble core of the DIGs may in fact be due to the sphingolipids. Other components, such as cholesterol, would then interact with this basic core. GPI-anchored proteins may have lower affinity for these domains under normal conditions, but this affinity might be increased by experimental manipulations (such as cross-linking with antibodies, or detergent treatment) or regulated by physiological protein-protein interactions.

Similarly, the core of the caveolae may also be based on sphingolipids. The ganglioside G_{M1} can be seen within caveolae by electron microscopy, even without cross-linking (*20*). This point is now supported by biochemical evidence from a purification technique in which the endothelial cell surface is coated with colloidal silica in vivo (*1*). Remarkably, G_{M1}- and VIP21-caveolin–enriched domains were completely separated from fractions containing GPI-anchored proteins. The authors suggest that GPI-anchored proteins exist in a domain or annulus surrounding, but not within, the caveolae, a surprising suggestion in view of the postulated role of GPI-anchored proteins within caveolae (*3*) (although it is still possible that the cationic silica could cause some redistribution of the negatively charged GPI-anchored proteins from the caveolae interior).

Is the insolubility of VIP21-caveolin also due to an interaction with GSLs in caveolae? Possibly, because VIP21-caveolin interacts specifically with cholesterol in a detergent-resistant manner (*21*), and cholesterol is essential for the maintenance of caveolar morphology and function (*22*). VIP21-caveolin may therefore associate with the cholesterol surrounding the sphingolipids to form the caveolae. Few other components seem required to form the basic caveolar unit: Expression of VIP21-caveolin in lymphocytes causes de novo formation of plasma membrane invaginations indistinguishable from caveolae of other cells (*23*), perhaps requiring the self-association of VIP21-caveolin monomers (*24*).

We envisage the DIGs as rafts in which the constituent logs (lipids) are constantly moving back and forth, as do detergent monomers in a micelle. The DIGs would differ from caveolae in that the latter are invaginated and ready to be internalized from the surface like coated pits. Caveolae may also form around the DIGs, but we believe that even in cells with numerous caveolae, DIGs are not restricted to the caveolar domain. Characterization of the nature of the lipids on the cytoplasmic leaflet of the membrane will be revealing, as will reconstitution of the process in liposomes.

Finally, these results have implications for sorting of apically destined proteins in the TGN of epithelial cells. Buds of the TGN may be enriched in GSLs, VIP21-caveolin, and GPI-anchored proteins and function analogously to caveolae (*6*). Caveolae at the cell surface may act as traps for proteins with affinity for these membrane domains. This affinity might be modulated, for example, by processes that mimic cross-linking of GPI-anchored or transmembrane proteins (*25*). Palmitoylation, a reversible and regulatable modification, may influence the interaction of proteins with the DIGs (within or outside caveolae) on the cytosolic surface of the plasma membrane. Indeed, VIP21-caveolin (*26*), Src kinases (*27*), and heterotrimeric G proteins (*28*) are all palmitoylated. In this way the cell could build up a complex domain within the plasma membrane around an aggregate of lipids. The challenge in the future will be to understand how these interactions of lipids and proteins contribute to specificity in cellular function. Maybe the cell really does need the hundreds of lipid species that make up our cellular membranes.

References

1. J. E. Schnitzer, D. P. McIntosh, A. M. Dvorak, J. Liu, P. Oh, *Science* **269**, 1435 (1995).
2. N. J. Severs, *J. Cell Sci.* **90**, 341 (1988).
3. R. G. W. Anderson, *Curr. Opin. Cell Biol.* **5**, 647 (1993).
4. K. Rothberg *et al.*, *Cell* **68**, 673 (1992).
5. T. V. Kurzchalia, P. Dupree, S. Monier, *FEBS Lett.* **346**, 88 (1994).
6. P. Dupree, R. G. Parton, G. Raposo, T. V. Kurzchalia, K. Simons, *EMBO J.* **12**, 1597 (1993).
7. T. V. Kurzchalia *et al.*, *J. Cell Biol.* **118**, 1003 (1992).
8. M. Sargiacomo, M. Sudol, Z. Tang, M. P. Lisanti, *ibid.* **122**, 789 (1993).
9. D. A. Brown and J. K. Rose, *Cell* **68**, 533 (1992).
10. D. Brown, *Curr. Opin. Immunol.* **5**, 349 (1993).
11. S. A. Shenoy *et al.*, *J. Immunol.* **149**, 3535 (1992).
12. S. A. Shenoy, L. K. Gauen, J. Kwong, A. S. Shaw, D. M. Lublin, *Mol. Cell. Biol.* **13**, 6385 (1993).
13. T. Cinek and V. Horesji, *J. Immunol.* **149**, 2262 (1992).
14. T. V. Kurzchalia, E. Hartmann, P. Dupree, *Trends Cell Biol.* **5**, 187 (1995).
15. A. Fra, E. Williamson, K. Simons, R. G. Parton, *J. Biol. Chem.* **269**, 30745 (1994).
16. A. Gorodinsky and D. A. Harris, *J. Cell Biol.* **129**, 619 (1995).
17. S. Mayor, K. Rothberg, F. Maxfield, *Science* **264**, 1948 (1994).
18. R. G. Parton, B. Joggerst, K. Simons, *J. Cell Biol.* **127**, 1199 (1994).
19. R. Schroeder, E. London, D. Brown, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 12130 (1994).
20. R. G. Parton, *J. Histochem. Cytochem.* **42**, 155 (1994).
21. M. Murata *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, in press.
22. K. Rothberg, Y. Ying, B. A. Kamen, R. G. W. Anderson, *J. Cell Biol.* **111**, 2931 (1990).
23. A. M. Fra, E. Williamson, K. Simons, R. G. Parton, *Proc. Natl. Acad. Sci. U.S.A.*, in press.
24. S. Monier, R. G. Parton, F. Vogel, A. Henske, T. V. Kurzchalia, *Mol. Biol. Cell* **6**, 911 (1995).
25. K. Fiedler, R. G. Parton, R. Kellner, T. Etzold, K. Simons, *EMBO J.* **13**, 1729 (1994).
26. D. J. Dietzen, W. R. Hastings, D. M. Lublin, *J. Biol. Chem.* **270**, 6838 (1995).
27. S. A. Shenoy, D. J. Dietzen, J. Kwong, D. C. Link, D. M. Lublin, *J. Cell Biol.* **126**, 353 (1994).
28. S. M. Mumby, C. Kleuss, A. G. Gilman, *Proc. Natl. Acad. Sci. U.S.A.* **91**, 2800 (1994).