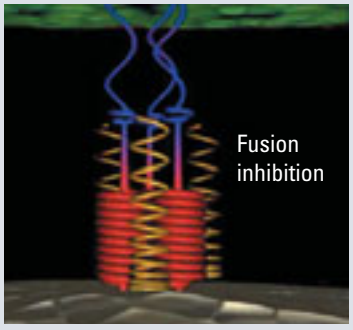
Fusion inhibitor approved

The first product in a new class of medications called fusion inhibitors was recently approved by the FDA for use in combination with other anti-HIV medications for the treatment of HIV-1 infections in adults and children.

The drug, a synthetic peptide called enfuvirtide (Fuzeon), received accelerated approval on the basis of six months of data from two ongoing clinical trials involving approximately 1000 patients. This data showed that the addition of enfuvirtide to a cocktail of other anti-HIV drugs reduced the load of HIV infection in the blood more than the cocktail of anti-HIV medications did alone.



Zip zap. Synthetic peptide fusion inhibitors like enfuvirtide (here shown as a gold spiral) are believed to bind to segments of gp41 (blue and red) extending from the HIV particle, thus preventing a zipping effect that would pull the virus into close proximity to the cell and eventually through its membrane. (Adapted with permission. Copyright 2001 Trimeris, Inc. Hoffman-La Roche, Ltd.)

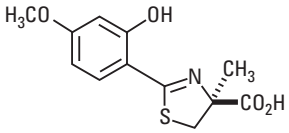
Enfuvirtide works by inhibiting the fusion of HIV to receptors on the surface of the CD4+ immune cells, thus blocking subsequent cellular infection. This novel extracellular mechanism of action—most anti-HIV medications work inside the cell—allows the drug to combat viral strains resistant to current drug regimes. This is imperative, as a large percentage of the estimated 850,000 to 950,000 people living with HIV in the United States have developed drug-resistant infections.

Because they remain outside the cell, fusion inhibitors such as enfuvirtide are also less likely to interact negatively with other drugs or to interfere with important biochemical processes, which reduces the likelihood of side effects.

The approved labeling for the new drug does, however, warn physicians to monitor patients for signs of pneumonia, because bacterial pneumonia was more frequent in those treated with enfuvirtide than in patients who did not receive this drug—however, pneumonia was uncommon in the clinical trial overall. In addition, enfuvirtide can cause both serious systemic allergic reactions and local skin reactions at the site of injection.

The drug was developed by Trimeris, Inc. (Durham, NC; *www.trimeris.com*) and was licensed to Roche Pharmaceuticals (Nutley, NJ; *www.roche.com*) for distribution. It is expected that demand will exceed initial supply, because, say the companies, enfuvirtide is "the most complex pharmaceutical molecule ever manufactured on a large scale." The partners have announced a progressive distribution program to ensure uninterrupted access of the drug to as many patients as possible during the period of limited supply.

—DAVID FILMORE



(*S*)-4′-(CH_3O)-DADFT

Iron ouster. (*S*)-4′-(CH_3O)-DADFT shows an iron-clearing efficiency of 24%.

Pumping iron

When it comes to iron, the human body is practically a sealed vault, with little of the metal being absorbed or excreted. This means that people who require frequent blood transfusions can suffer from a buildup of iron, which can react with naturally occurring hydrogen peroxide to form free radicals that can damage cells or generate carcinogens. Thus, medicinal chemists have worked to identify and characterize chelating compounds to clean the body of excess iron.

Microorganisms rely on a variety of low-molecular-weight chelators for iron clearing, such as desferrioxamine B (DFO), which is the ligand of choice for clinicians treating iron overload. Unfortunately, the low chelating efficiency of this compound—it only works at 5–7% of its potential—means that the drug requires almost daily subcutaneous injection in 8- to 12-h sessions, which decreases patient compliance. Desferrithiocin (DFT), by contrast, is orally active and more efficient than DFO (16% in primate models). However, patients receiving DFT exhibit severe nephrotoxicity. For this reason, Raymond Bergeron and colleagues at the University of Florida (Gainesville) are attempting to develop less toxic but equally efficient DFT analogues, and they recently reported some promising results (*J. Med. Chem.* **2003,** *46,* 1470–1477).

The researchers tried to determine the minimal structure that would maintain the iron-clearing efficiency of DFT and found that they could remove both the aromatic nitrogen and the thiazoline methyl and still maintain 12% efficiency. They used the resulting compound as a template for structure–activity studies. The researchers reduced toxicity by adding hydroxyl groups to the aromatic ring, but the new compounds were also less efficient (down to 4.2%). Methoxylating the aromatic rings, on the other hand, increased the efficiency to more than 24% in one case. The impact of methoxylation on toxicity, however, still needs to be assessed.

They speculated that the reduction in toxicity results from increased lipophilicity, which facilitates the compound's passing in the stool, reducing the chances of nephrotoxicity. The researchers have initiated preclinical toxicity trials with one of the methoxylated DFT analogues, (*S*)-4,5-dihydro-2-(2-hydroxy-4-methoxyphenyl)-4-methyl-4-thiazolecarboxylic acid [(*S*)-4′-(CH_3O)-DADFT, see figure].

—RANDALL C. WILLIS

The shorter the better

Peptides as short as two residues exhibited substantial antimicrobial activity in a recent study by John Svendsen and colleagues at the University of Tromsø (Norway), opening up new potential for the practical development of antibiotic candidates that have so far received sparing attention from large pharmaceutical companies, according to the Norwegian scientists.

Cationic antibacterial peptides contain both positively charged residues and lipophilic residues in almost equal parts and often form structures where the lipophilic residues are concentrated on one face of the molecule while the cationic groups are on the other. This confers a unique ability to target bacterial cell membranes, as opposed to the enzymatic targets of conventional antibiotics.

These peptides can be effective against bacteria that have developed cross-resistance to current antibiotics, and they also show a lower tendency to trigger resistance because they don't have to deal with enzymes that can mutate. Nonetheless, these compounds have not yet taken off in any big-time pharmaceutical development projects because of problems with bioavailability, toxicity, and high production costs.

A fix to these problems, say Svendsen and his team, would be shorter peptide chains. In their recent work, they set out to find the pharmacophore for antibacterial activity in cationic peptides (*J. Med. Chem.* **2003,** *75,* 1777–1785). They analyzed the activity of various peptide sequences containing four major residues or fewer against *Escherichia coli*, *Staphylococcus aureus*, and methicillin-resistant versions of *S. aureus* and *S. epidermidis*.

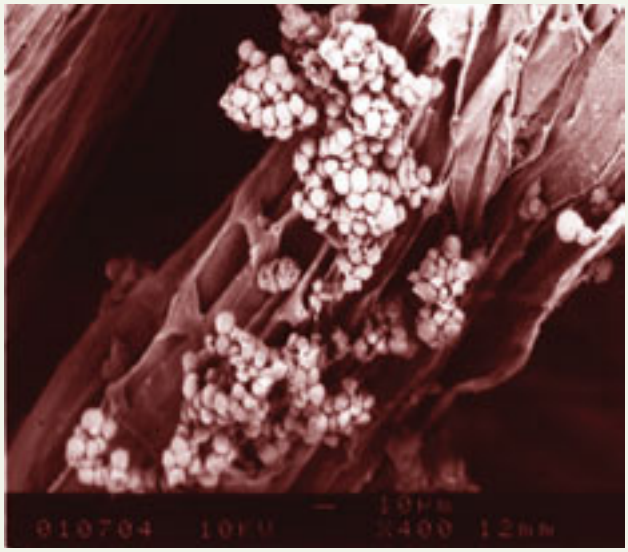
The latter three microbes were generally more susceptible to the peptides. The researchers found the minimum anti-staphylococcal motif to be a combination of two bulky lipophilic moieties and two charged entities. A notable example was RW-OBzl, a benzyl ester peptide derivative (where the lipophilic groups are tryptophan, W, and OBzl and the charged units are arginine, R, and the free N-terminal amino group), which had a very low minimal inhibitory concentration of 15 µg/mL. *E. coli*, on the other hand, required three lipophilic and two charged groups.

Natural cationic antibacterial peptides generally contain between 12 and 50 amino acids. Development of compounds of smaller size, the scientists say, would alleviate production costs of larger peptide chains and reduce the potential for immunogenicity (no toxicity data were presented). Furthermore, smaller peptides offer the possibility of oral administration because they can be carried across the epithelial membranes in the GI tract.

—DAVID FILMORE

Loofa livers

Jyh-Ping Chen and colleagues at Chang Gung University (Taoyuan, Taiwan), National Taiwan University (Taipei), and Chang Gung Memorial Hospital (Taoyuan) recently demonstrated the potential of a three-dimensional scaffold using natural loofa sponge as the medium for high-density culturing of human hepatocyte cells (C3A/HepG2) for liver-specific functions (*Biotechnol. Prog.* **2003,** *19,* 522–527). The researchers propose that this type of scaffold could be used in the development of bioartificial organs, specifically a liver, and other gene therapies. The loofa sponge has shown promise for acting as a carrier for cell immobilization, because its high porosity can sustain repetitive sterilization cycles and it is stable in a broad pH range.



Loofa lounge. SEM of hepatocyte cells shown after immobilization in a loofa sponge. (Adapted with permission from *Biotechnol. Prog.* **2003,** *19,* 522–527.)

To test the effectiveness of the loofa scaffold to express high levels of liver-specific functions in stationary and perfusion cultures, C3A/HepG2 cells were immersed in a culture medium, seeded onto a loofa sponge or cubes of polyurethane (PU), immobilized, and then analyzed by scanning electron microscopy (SEM).

The results show that the cell density of the loofa scaffold was greater than that of the PU scaffold, indicating that the loofa fibers could provide a populous site for cell attachment and growth. Also, the cells grew favorably on the indentations of the loofa, suggesting that the loofa surface seemed to support cell growth. SEM of the perfusion-immobilized cells indicated that the loofa had the greatest cell loading density. Similarly, the albumin concentration in stationary cultures was significantly higher in the loofa than in the PU (23.9 compared with 10.6 µg/10^6 cells/day) after 11 days and in perfusion cultures after 9 days (42.2 µg/10^6 cells/day).

The investigators believe this study has demonstrated loofa sponge to be an effective scaffold for high-density culturing of a human hepatocyte cell line. "A bioartificial liver device could be developed and is currently under study in our laboratories," they state in their report.

—KIMBERLY S. CLEAVES

Artificial glycoviral vectors

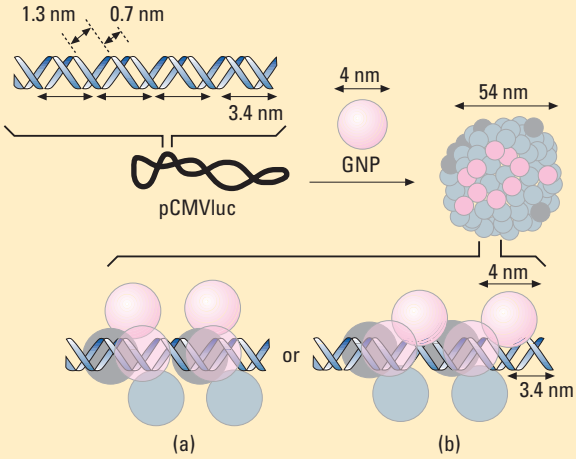
Researchers are continually attempting to develop simultaneously efficient and safe methods of delivering gene therapy to patients. Although viruses, by their very nature, are the most efficient means of getting genetic information into a cell, their history in gene therapy has been a checkered one, including the death of Jesse Gelsinger in 1999 from a reaction to the adenovirus vector during a clinical trial and the recent triggering of leukemia in children treated for "bubble-boy" syndrome using retroviruses.

Naked DNA, on the other hand, is extremely inefficient, even in tissue culture, and is almost useless in whole animals. Accessory compounds such as polycations added to the DNA improve uptake and transformation, but they often stimulate excess aggregation, producing particles whose sizes limit their utility.

To take advantage of the best of both worlds, Yashuro Aoyama and colleagues at Kyoto University (Japan) and Nagasaki University (Japan) reported on their development of an artificial virus construct that could be used to encapsulate DNA and transform cells in a safer and more efficient manner (*J. Am. Chem. Soc.* **2003,** *125,* 3455–3457). They created a macrocyclic glycocluster compound from the reaction between octaamines and cellobiose lactones, which in water forms micelle-like aggregates (or glycocluster nanoparticles, GNPs).

The GNPs produced were themselves nonaggregating but proved capable of binding to the phosphate backbone of DNA (in this case a pCMVluc plasmid containing a cytomegalovirus promoter and the luciferase recorder gene). As they encapsulated the DNA, the GNPs formed appropriate-sized, roughly spherical "glycoviruses" that could stimulate natural adsorption to cells and endocytosis.

The researchers demonstrated that these artificial glycoviruses contained only one molecule of efficiently packed DNA per particle and were capable of transfecting Hela cells and other cell types in serum-free and fetal bovine serum-containing media. Transfection with the glycoviruses, as determined by luciferase expression, was nearly 10-fold higher than that obtained with lipofectin, a common cationic-lipid vector. Viability assays determined the glycoviruses to be nontoxic.



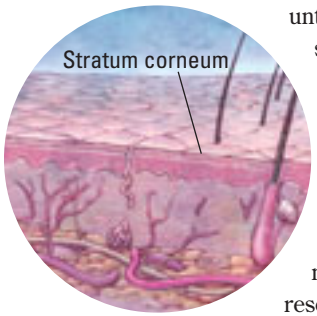
Glycocluster crunch. Illustration of GNP-induced compaction of supercoiled pCMVluc plasmid DNA into a 54-nm glycoviral particle having a stoichiometry of 2 GNPs per helical pitch. (Adapted with permission from *J. Am. Chem. Soc.* **2003,** *125,* 3455–3457.)

—MARK S. LESNEY

Penetrating the skin you're in

Transdermal drug delivery presents an appealing alternative to oral medications and injections. However, the skin's external tissue layer, stratum corneum (SC), acts as a relatively impermeable physical barrier. The SC "brick-and-mortar" layer is embedded in an extracellular lipid matrix, which acts as a physical and water-retaining barrier that controls permeability. Penetrating this barrier has been accomplished using a low-frequency ultrasound phenomenon called sonophoresis.

The pathway of transdermal drug delivery via sonophoresis is not well understood. However, R. Alvarez-Román and colleagues at the University of Geneva (Switzerland) and the University of Lyon (Archamps, France) recently demonstrated that low-frequency ultrasound increases transport across the skin by removing a significant fraction of SC lipids (*J. Pharm. Sci.* **2003**, *92,* 1138–1146).



To examine the effectiveness of sonophoresis, ultrasound was applied to porcine skin and the lipids were extracted. Using attenuated total reflectance IR, the researchers found that there was a decrease in the area under the curve (AUC) in the CH_2 absorbances in ultrasound-treated skin samples compared with the untreated samples, suggesting that lipids were removed from the SC. To further investigate these preliminary results, researchers reduced and extracted the entire membranes for lipid analysis. The AUCs of the CH_2 Fourier transform infrared absorbances of the ultrasound-extracted samples corresponded to ~30% of the untreated samples.

The researchers then treated the skin samples with fluorescent probes to look at the effects of ultrasound by laser-scanning confocal microscopy. The probe calcein offered the most helpful information. In the control samples, calcein was retained at the surface and unable to cross the SC; but upon sonophoresis, the permeation across the skin was significantly enhanced at discrete areas separated by regions of the SC that had not been visually affected by the ultrasound. This finding, the researchers say, lends support to the idea that cavitation is a key mechanism contributing to the action of sonophoresis.

—KIMBERLY S. CLEAVES

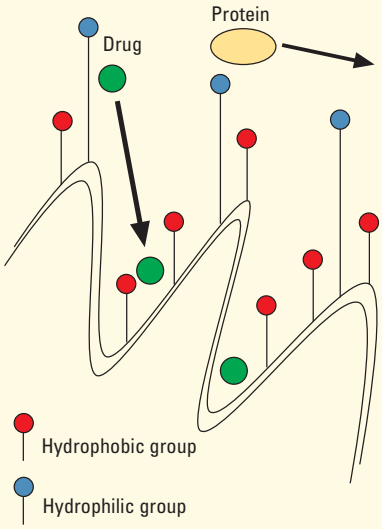
ILLUSTRATION: ARTVILLE

Sample prep school

Nowhere is the crunch for increasing sample throughput felt more strongly than in the pharmacokinetic analysis of plasma samples, which typically require tedious sample pretreatment to eliminate proteins that can clog an LC system. Often, researchers add compounds like acetonitrile to plasma and centrifuge the mixture to precipitate the proteins before applying the supernatant to a column. Unfortunately, such pretreatment steps can lead to the loss of the very trace metabolites that are being studied.

To address this problem, Jason Murphy and Medha Tomlinson from the Abbott Bioresearch Center (Worcester, MA) developed a new precolumn to eliminate plasma proteins from samples and presented their results at the 2003 Pittsburgh Conference in Orlando. Their column uses a mixed functional phase of hydrophilic polyoxyethylene groups and hydrophobic phenyl groups bonded to silica. Thus, when they directly inject a plasma sample into the precolumn, the proteins remain in the aqueous phase and are eliminated in the void volume, while the analytes interact with the hydrophobic phase and are retained on the column.

The researchers added an analytical column in series with the precolumn and a column-switching device. After they load a sample in aqueous buffer onto the precolumn and the proteins are eliminated, they can switch the column flow to the analytical column. Then a gradient of organic solvent elutes the retained compounds from the analytical column into a mass spectrometer or other detector.



With the in-crowd. Using a mixed functional-phase resin, researchers developed a method of eliminating plasma proteins from a sample while retaining metabolites.

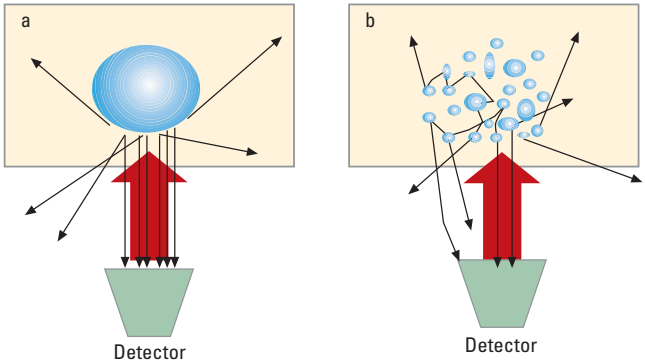
The researchers tested their system on a variety of plasma samples and compared the results with those of precipitated samples. They found that direct injection into the precolumn resulted in cleaner peaks than those from the precipitated samples. Furthermore, the precolumn was very durable, presenting comparable results on the 15th and 150th loadings.

—RANDALL C. WILLIS

Particle size on the fly

Drug particle milling to the nanometer size range has become an increasingly important approach to improving the bioavailability of poorly water-soluble compounds.

On-line process monitoring is also an increasing trend in drug production (see Rules and Regulators, p 51) that happens to bring particular benefit to nanoparticle milling—a process that usually lasts from 11 to 18 h—because of the significant costs of finding problems only after the fact. In this light, John Higgins and colleagues from Merck Research Laboratories (West Point, PA; *http://www.merck.com/mrl*) developed a near-infrared (NIR) spectroscopy method for real-time particle-size detection. According to the researchers, it is the first on-line spectroscopic approach that distinguishes size changes as small as 1–2 nm for particles with diameters less than 300 nm in dispersions with high solids content (*Anal. Chem.* **2003,** *75,* 1777–1785).



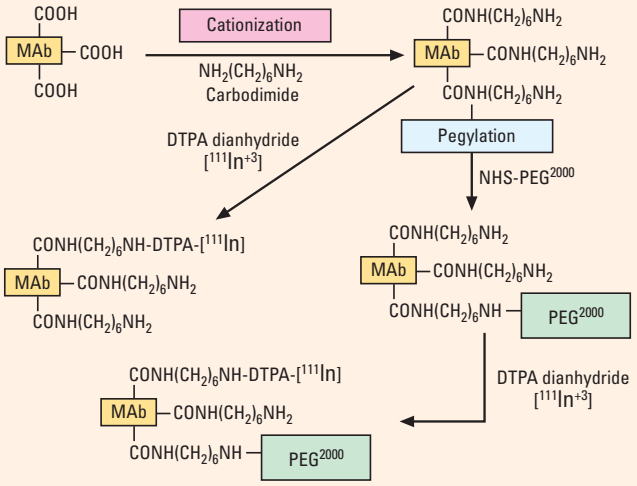
Scatter effect. Greater amounts of multiple scattering toward the end of the milling process (b), compared to the beginning (a), explains the proportionality between decreasing particle size and increasing NIR water absorptions. (Adapted with permission from *Anal. Chem.* **2003,** *75,* 1777–1785.)

The researchers monitored the aqueous-based milling process using NIR spectroscopy through fiber optics. Ruling out the occurrence of chemical changes with HPLC, they found that the resulting spectra had several absorption peaks that increased in intensity as a function of decreasing particle size. This was particularly evident for higher-wavelength peaks assigned as water vibrations. It turns out that, because the NIR light impinging on the dispersion undergoes greater multiple scattering as the drug particles get smaller (see figure), NIR photons have a greater probability of being absorbed by the surrounding water.

The researchers demonstrated changes in a specific NIR water band (near 1460 nm) to be very sensitive to the milling process. The peak's significant sensitivity even to very small size changes at the end of the process is crucial, because the milling process can require up to 5 h to reduce the particle size 5–10 nm once the total diameter is below 150 nm. Inability to monitor with this level of precision leads to wasted time, energy, and expense.

The absorption data for this peak was used to quantify particle sizes in a test run, and subsequent validation runs confirmed the method as a reproducible approach—one that the Merck team feels will offer a highly advantageous alternative to current off-line procedures.

—DAVID FILMORE



Marking mAb. Reaction scheme for the reformulation of 528 mAb for tumor imaging. (Adapted with permission from *Bioconjugate Chem.* **2003,** ASAP.)

Imaging with monoclonals

The major problem with using labeled monoclonals (mAbs) for imaging is their relative inability to travel easily across the microvascular endothelial barrier of the capillaries at the blood–tumor or blood–target organ interface.

To develop an improved method of single-photon-emission computed tomography, researchers Hwa Jeong Lee (Ewha Womans University, Seoul, South Korea) and William Pardridge (University of California at Los Angeles School of Medicine) examined the effects of a variety of chemical transformations of tumor-binding radiolabeled 528 murine mAb (*Bioconjugate Chem.* **2003,** ASAP). The 528 mAb binds to the human epidermal growth factor receptor (EGFR), a protein that is overexpressed in the majority of solid cancers. Two methods of radiolabeling (^{125}I and ^{111}In) and several methods of chemically modifying the antibodies to allow them to cross the capillary barrier were investigated.

The researchers demonstrated that cationization of the mAb with amino group ligands increased the uptake of the antibodies into the target tumor (because of absorptive-mediated endocytosis) as expected; they also maintained their affinity to the EGFR. This allowed rapid clearing of the labeled compound from the serum, decreasing the background noise. Unfortunately, the rapid shift of the label into small-molecular-weight metabolites within the cell allowed the ^{125}I to diffuse back into the bloodstream and nontarget tissues. Radiometal chelation with ^{111}In was examined to prevent this rapid breakdown. This required a conjugation step with the chelating moiety, diethylenetriaminepentaacetic acid (DTPA). In-labeled cationized mAb proved unable to rapidly cross the microvascular barriers—apparently because of low-molecular-weight serum factors that bind and inhibit the uptake. Pegylation, which is known to inhibit binding of serum proteins, was unable to eliminate the effect.

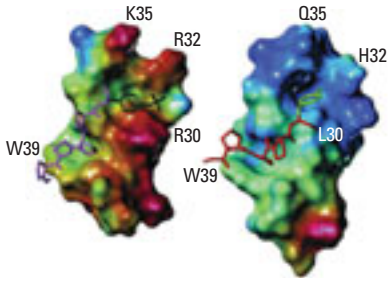
The researchers hope that this work will lead to further attempts to find an improved method for radiolabeling cationized mAbs.

—MARK S. LESNEY

Changing peptide partners

Protein synthesis technologies, both chemical and biological, have allowed researchers to expand the scope of proteins and peptides that can be screened in the search for therapeutics. Jen Schneider-Mergener and colleagues at Humboldt-Universität Berlin, Forschungsinstitut für Molekulare Pharmakologie, and Jerini AG (all in Berlin) recently screened almost 12,000 variants of a WW domain protein against a library of peptides, generating proteins with altered peptide specificity in the process (*Angew. Chem., Int. Ed.* **2003,** *42,* 1136–1140).

WW domains are 40-residue sequences that mediate protein–protein interactions in diseases such as Liddle's syndrome, muscular dystrophy, and Huntington's chorea. Largely β-sheet structures, WW domains bind to proline-rich sequences and, in the case of the human Yes-kinase-associated protein (hYAP) domain studied by the Berlin researchers, peptides containing a PPxY sequence (where x represents any L-amino acid).



In a groove. (Adapted with permission from *Angew. Chem., Int. Ed.* **2003**, *42,* 1136–1140. Copyright 2003 John Wiley & Sons.)

Previous structural work showed that tyrosine, essential to hYAP binding, fits into a groove formed by the protein residues L30, H32, and Q35 (see figure). Thus, the researchers synthesized two arrays of hYAP WW domains modified at these three positions. In one array, the residues were substituted with any of the 19 natural amino acids, excluding cysteine. The second array incorporated the 19 natural residues and 20 nonproteinogenic and phosphoamino acids.

The researchers compared the affinities of the variant proteins with both the native hYAP target peptide and its phosphotyrosine (pY) derivative. As expected, the normal WW domain bound the native peptide but did not bind the phosphopeptide. The researchers found, however, that a WW variant with residues R, R, and K at positions 30, 32, and 35, respectively, bound the phosphopeptide but not the native peptide.

The researchers then determined the NMR structure of the RRK variant complexed with the pY-peptide and compared it with the normal WW domain complexed with the native peptide. They found that although the bulk of the peptides were bound in a similar manner, there were several distinct electrostatic contacts in the RRK complex.

—RANDALL C. WILLIS

KEY TERMS: automation (p 16), cell biology (p 12), clinical (p 11), drug delivery (p 15), high throughput (p 19), imaging (p 19), medicinal chemistry (pp 11, 12, 19), process (p 16), proteomics (p 19), screening (pp 11, 12, 19), technique (p 16)