Hot off the Press

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In the *Hot off the Press* section of *Molecular BioSystems* members of the Editorial Board and their research groups highlight recent literature for the benefit of the community. This month the highlighted topics include molecular beacons for *in vivo* studies, fungus as an antibiotic source, and bioelectronic devices made by coupling microorganisms and nanoparticles.

Stable and specific molecular beacons for *in vivo* studies

First described in 1996, molecular beacons (MB), short hairpin oligonucleotide probes containing a fluorophore at one end and the quencher dye at the other end, found many applications in *in vitro* hybridisation assays. However, several problems arose when *in vivo* experiments, particularly the detection of gene expression, were attempted. Conventional MB was prone to degradation by DNAases and non-specific binding to DNA/RNA binding proteins, all of which led to false positives. Nuclease resistant MBs were synthesised but there was another set of new problems like toxicity and self-aggregation to be solved.

Now Steven Benner and Weihong Tan and their colleagues have designed a novel MB with remarkable properties which can offer a solution to all of the encountered problems (Fig. 1). They have prepared Locked Nucleic Acid MB (LNA-MB) with Cy3 at the 5′ and DABCYL at the 3′ end. LNAs usually contain one or more LNA nucleotide monomers with bicyclic furanose unit, where a methylene bridge is formed between the 2′ oxygen and the 4′ carbon of the sugar. LNA–MB had a 19-mer loop and a 6-mer stem, all containing repeating modified ribose units. Melting temperature experiments showed that LNA–MB does not open even at 95 °C as compared to conventional MB that has T_m at 60 °C indicating the high thermal stability of the novel beacon. When the target sequence was added to the solution, LNA–MB showed exceptionally strong affinity at 25 °C, but also functioned well at 95 °C. Additionally LNA–MB displayed superior selectivity than DNA–MB for a perfectly matched target sequence as compared to single base mismatched target.

The set of experiments was also aimed to check the stability of LNA–MB under *in vivo* conditions. Both beacons were subjected to DNAase I endonuclease treatment and also mixed with single stranded DNA binding protein (SSB). In both cases LNA–MB was superior to conventional MB not leading to false positives and demonstrating resistibility towards endonuclease degradation.

Finally, gene expression experiments in an intracellular environment showed that LNA–MB has longer lifetime and lower background and it could therefore be an excellent probe for gene expression analysis. The authors indicate that they are currently working on the use of LNA–MBs for *in vivo* monitoring of multiple genes so it would be interesting to follow their work in the near future.

L. Wang, C. J. Yang, C. D. Medley, S.A. Brenner, W. Tan, *J. Am. Chem. Soc.* 2005, **127**, 15664–15665

Reviewed by: Ljiljana Fruk, Universität Dortmund, Germany

A lung metastasis signature

Cancer metastasis, the spread of cancer from a primary site to other organs, is responsible for the majority of cancer-related deaths. Most metastases display an organ-specific pattern of spread; for example, breast carcinomas often metastasize to the bone and lungs. However, the nature of the small molecule and protein factors which promote organ specific metastasis is only beginning to be understood.

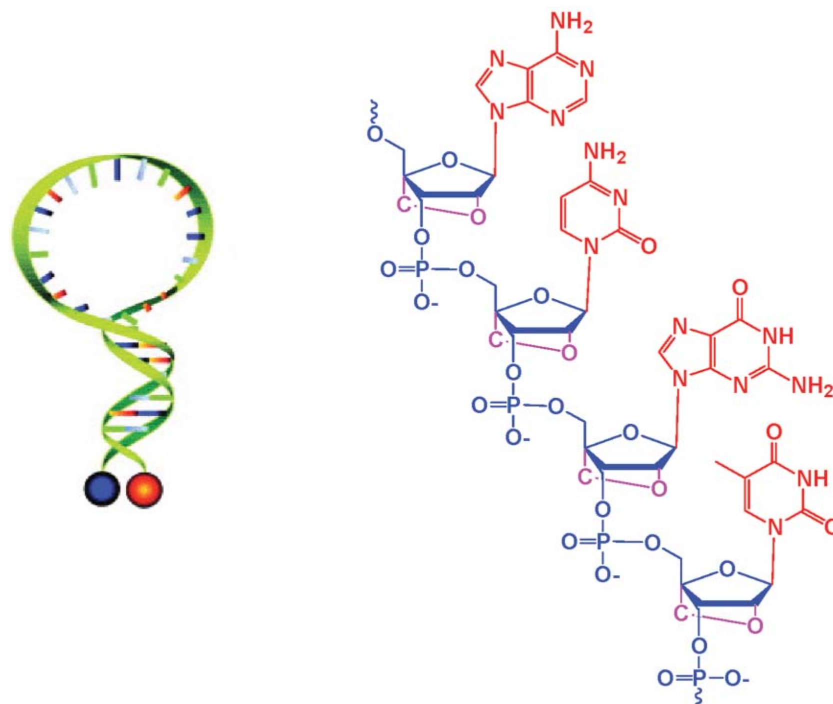

Fig. 1 Reprinted with permission from *J. Am. Chem. Soc.* 2005, **127**, 15664–15665. Copyright 2005 American Chemical Society.

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Towards this aim, Joan Massagué and colleagues used transcriptional microarray profiling of *in vivo*-derived highly aggressive lung metastatic breast cancer cell lines to identify genes which mediate lung metastasis. These lines were generated by repeated injection and retransplantation of the MDA-MB 231 breast cancer cell line first through the mouse left cardiac ventricle and then twice more through the tail vein.

Microarray analysis of the *in vivo*-derived cell lines revealed a set of 95 genes that displayed differential expression when compared to the parental cell line. This initial set of 95 genes was reduced to a final set of 54 genes by incorporating the microarray data from a group of parental-derived single cell-derived progeny which had varying degrees of ability to metastasize the lung and were referred to as the lung metastasis signature. The functional contribution of a small number of these genes, such as MMP1 (collagenase), SPARC, and EREG, to lung metastasis was tested either through stable over-expression studies in the parental cells or knock-down studies with short hairpin RNAi contructs in the *in vivo*-derived cell lines either individually or in groups of three to six. Such studies revealed that several of these genes either alone or in various combinations could significantly affect lung metastasis.

These studies further our understanding of genes involved in organ specific metastatic colonization, which may be the key to the development of therapies that target metastasis.

Andy J. Minn, Gaorav P. Gupta, Peter M. Siegel, Paula D. Bos, Weiping Shu, Dilip D. Giri, Agnes Viale, Adam B. Olshen, William L. Gerald and Joan Massagué, *Nature*, 2005, **436**, 518–524.

Reviewed by: Sherry Niessen, The Scripps Research Institute, California, USA

Peptide bond isomerization unlocks a channel gate

The $5HT_3$ receptor is a homopentameric ligand-gated ion channel, activated by serotonin. The extracellular ligand-binding domain is coupled to a transmembrane domain comprising α helices, four contributed by each subunit. Five M2 helices, one from each subunit, line the conductive pathway. In the closed state, five conserved Leu residues in the M2 helices block the channel near the intracellular entrance. How is serotonin binding to the extracellular domain coupled to channel opening?

A series of conventional mutagenesis experiments implicated a conserved Pro residue in the M2–M3 loop at the extracellular end of the transmembrane domain. By using nonsense suppression, Dennis Dougherty and co-workers substituted the Pro residue with various non-natural Pro analogs. The EC_{50} for serotonin was measured and found to be greatly reduced for $5HT_3$ receptors containing Pro analogs that favor the *cis* peptide bond conformation. Remarkably, a plot of $\Delta\Delta G(EC_{50})$ *versus* $\Delta\Delta G$(*cis*/*trans*) gave a straight line with a slope of 1 suggesting that the binding of serotonin is fully coupled to gating, which involves peptide-bond isomerization at the conserved Pro. Molecular modeling suggested that two loops in the ligand-binding domain hold the Pro residue in the *trans* form and release it upon serotonin binding.

As the authors point out, the work raises several new questions. For example the related acetylcholine receptor contains only two ligand-binding sites and opens more quickly than the $5HT_3$ receptor, more quickly than expected for a simple isomerization at Pro. Further, in other related receptors, the Pro residue is not conserved.

Sarah C. R. Lummis, Darren L. Beene, Lori W. Lee, Henry A. Lester, R. William Broadhurst and Dennis A. Dougherty, *Nature*, 2005, **438**, 248–252

Reviewed by: Hagan Bayley, University of Oxford, UK

PNA-encoded peptide libraries analyzed using DNA microarrays

For years, chemists have discussed the advantages of encoding split and mix chemical libraries with tags that would allow them to utilize the powerful tools of DNA technology to deconvolute the results of screening experiments. However, using DNA itself as an encoding molecule has proven to be impractical since it strictly limits the type of chemistry that one can use to make libraries. An interesting alternative, first developed by Jennifer Harris, Peter Schultz and their co-workers, was to use peptide nucleic acids (PNAs) as encoding molecules.

While PNAs cannot be amplified by PCR like native nucleic acids, they will hybridize tightly to complementary DNA sequences. This allows the use of DNA microarray technology for the analysis of libraries. In this experiment, a mixture of PNA-encoded molecules in solution is hybridized to a DNA microarray comprised of probes complementary to the encoding PNAs. Because of the very high affinity constants of PNAs for DNA, efficient capture of the PNA-containing molecules by their complementary immobilized DNA binding partners occurs. If the PNA-encoded molecule is also fluorescently tagged, then one can quantify the amount of any particular library molecule present in the original solution.

Mark Bradley and co-workers have now built on an earlier technical advance in which they developed cleanly orthogonal chemistries for the synthesis of split and mix peptide libraries and PNA encoding molecules. They used this protocol to create small libraries of PNA- and fluorescently-tagged peptides. These were used to probe the substrate preferences of a protease and a protein kinase (Fig. 2). For example, a library of 625 was prepared containing a fluorescent donor and quencher separated by the peptide. When the entire library is incubated with a protease, peptide cleavage increases the intensity of fluorescence greatly by separating the donor and acceptor. The mixture is then hybridized to a DNA microarray containing probes to the encoding PNAs for each member of the library and the fluorescence intensity is then read at each feature. This allows one to determine the efficiency of cleavage of each molecule in the library. This technique should be generally useful for various analyses of modest libraries, especially for the determination of the peptide substrate preference of enzymes that post-translationally modify other proteins.

Juan José Díaz-Mochón, Laurent Bialy, Lise Keinicke and Mark Bradley, *Chem. Commun.*, 2005, (11), 1384

Reviewed by: Thomas Kodadek, UT-Southwestern Medical Center, USA

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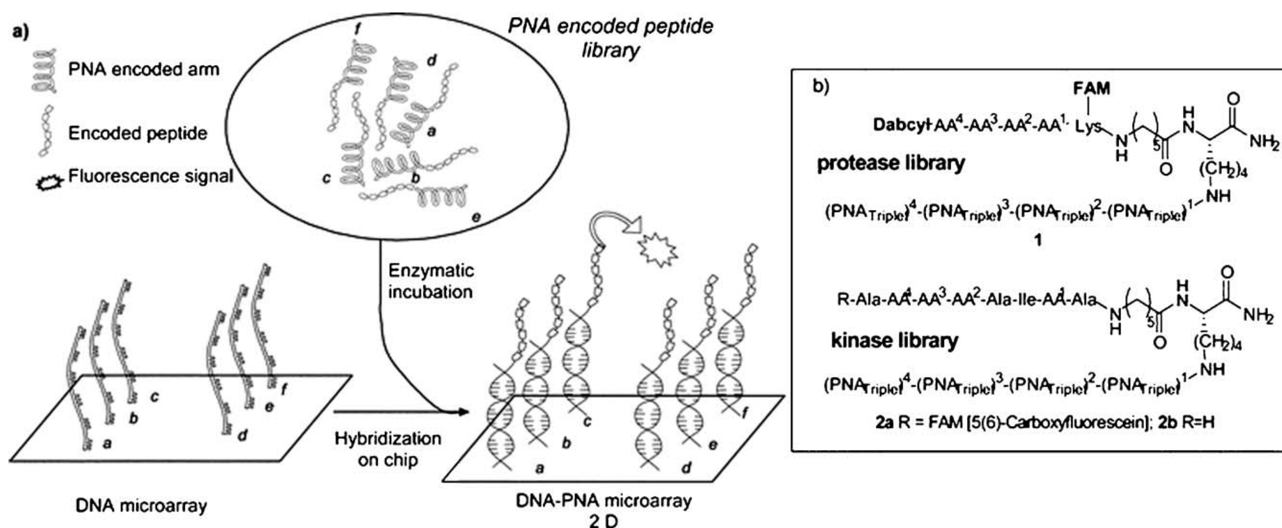

Fig. 2 (a) The general concept; (b) general structure of split and mix libraries. *Chem. Commun.*, 2005, (11), 1384. Reproduced by permission of The Royal Society of Chemistry.

Nanoparticles and bacteria: novel hybrids as tools in design of bioelectronic devices

Recently a great interest was awoken by the possibility of assembling hybrid structures of microorganisms and metal nanoparticles to design novel nanodevices. Although this can at first look like an odd pairing, microorganisms and nanoparticles could function very well together because of their complementing properties and could be the basis of powerful electronic devices. Metal nanoparticles, for example, have been shown to have interesting electronic properties, while on the other hand microorganisms represent an attractive template for nanoparticle deposition. However, some key problems have to be addressed, mainly, how to pattern nanoparticles without destroying the microorganism and how to connect the biological response to the electrical transport in nanoparticle devices.

Ravi Saraf and Vikas Berry from the University of Nebraska might have just found the right solution by assembling a microorganism—nanoparticle hybrid device in which electrical response is biologically controlled. They have deposited gold nanoparticles on the bacterium and used the humidity-induced changes in bacterial membrane to modulate the interparticle separation which subsequently leads to the change in tunnelling current.

Bacillus cereus, a Gram positive bacterium, was first deposited on a silicon substrate containing silica and gold electrode lines coated with poly(L-lysine). The time of deposition varied between 10 and 15 min and the bridges of bacteria spanning gold electrode lines were formed. Immersion of the chip in the solution of poly(L-lysine)-coated gold nanoparticles resulted in their deposition on the negatively charged bacterial surface. An important role in the final step is played by teichoic acid, a polyelectrolyte on the bacterium surface, which it thought to aid the deposition by wrapping over the positively charged Au particles.

The final test of the nanodevice function could be performed only if bacteria survive the above procedure. After that was confirmed, the difference in humidity level was used to induce a change in the hydrophilic bacterial membrane. This, in turn, caused the decrease in Au interparticle distance, which led to the increase in current flowing through the nanoparticle monolayer. Interestingly, the change of humidity from 20 to 0% (absolute change of interparticle distance of 0.2 nm) led to the 40-fold current increase proving that triggered changes in living organisms can be used to actuate electrical properties of nanoparticles.

These results show that bioelectronic devices based on simple chemistry can be devised by coupling microorganisms and nanoparticles and in the words of the authors, such hybrids could be the key to conceptually new electronic devices.

V. Berry, R. F. Saraf, *Angew. Chem. Int.Ed.*, 2005, **44**, 6668–6673.

Reviewed by: Ljiljana Fruk, Universität Dortmund, Germany

Inhibitors of *O*-GlcNAc transferase-elucidating its biological functions

O-GlcNAcylation, the modification of Ser and Thr residues of proteins by *O*-GlcNAc transferase (OGT), affects various cellular processes such as transcription, translation, organelle targeting, and protein–protein interactions. Indeed, the global impact of protein *O*-GlcNAcylation is probably comparable to that of protein phosphorylation. However, analytical and chemical tools to study this process lag far behind those available to study protein phosphorylation. In particular, the field would benefit greatly from specific inhibitors of OGT, which would facilitate studies of its various biological roles. Recently, Suzanne Walker and her colleagues at the Harvard Medical School have developed protocols to express large quantities of the catalytic domain of active OGT, which has allowed them to screen for inhibitors of OGT using a high-throughput donor displacement assay (Fig. 3).

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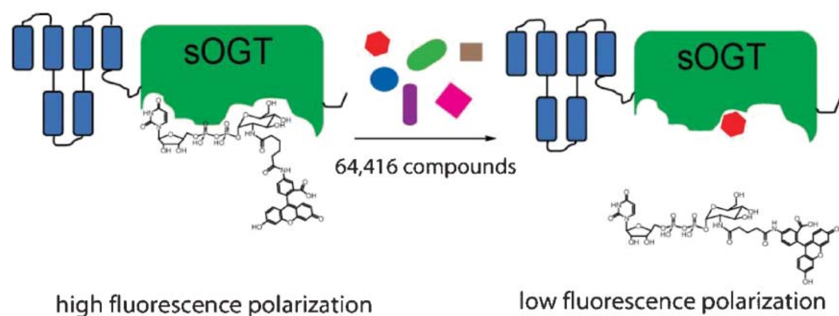

Fig. 3 Reprinted with permission from *J. Am. Chem. Soc.* 2005, **127**, 14588–14589. Copyright 2005 American Chemical Society.

At first, they made three OGT constructs based on the known splice variants of OGT, which in turn were expressed in *E. coli*. Among them, sOGT was obtained at much higher levels and showed the greatest specific activity. With the large amounts of OGT in hand, they explored high-throughput screening (HTS) to find inhibitors of OGT. The assay employed used fluorescence polarization to monitor displacement of a fluorescently labeled UDP-GlycNAc analogue from OGT by compounds from a commercial library of 64 416 compounds. Of the 102 hits initially observed, 19 compounds proved to inhibit sOGT by more than 40% at 25 μM. Interestingly, there was no overlap in the compounds identified in OGT and a similar screen conducted against MurG. While the compounds isolated in this study are themselves unlikely to be of practical utility, this communication provides a starting point on the road to the development pharmacologic inhibitors of the biologically important enzyme, OGT.

Benjamin J. Gross, Brian C. Kraybill, and Suzanne Walker, *J. Am. Chem. Soc.* 2005, **127**, 14588–14589

Reviewed by: Yong-Uk Kwon, Division of Translational Research, UT-Southwestern Medical Center, USA

Fungus: A new antibiotic source

Antibiotic resistance has been an emerging problem worldwide and there is a great deal of interest in the development of new antibiotics to treat deadly bacterial infections. Of particular importance is to identify new compounds with activity against strains resistant to current antibiotics. Hans-Henrik Kristensen and co-workers recently discovered a peptide antibiotic called plectasin from a fungus, which shows great potential as a therapeutic agent. This is the first antimicrobial defensin peptide to be identified in fungi. The 95 amino acid peptide has 50–55% sequence similarity to several invertebrate defensins but is not significantly similar to mammalian defensins.

Kristensen's research team tested the therapeutic potential of plectasin both *in vitro* and *in vivo*. Plectasin was an effective killing agent against *Streptococcus pneumoniae*, including several drug-resistant strains tested. *In vitro* results showed that plectasin selectively targets the bacteria over mammalian cells. *In vivo*, plectasin was as effective as vancomycin and penicillin against *S. pneumoniae*.

The major roadblock to the development of defensins as anti-infective therapeutics is their massive production with high purities. The significant advance in this study is the discovery of the first antimicrobial defensin peptide from a fungus, which can effectively be produced on an industrial scale using an economically viable fungal expression system. The researchers are currently investigating the mechanism of action of plectasin.

Per H. Mygind, Rikke L. Fischer, Kirk M. Schnorr, Mogens T. Hansen, Carsten P. Sönksen, Svend Ludvigsen, Dorotea Raventós, Steen Buskov, Bjarke Christensen, Leonardo De Maria, Olivier Taboureau, Debbie Yaver, Signe G. Elvig-Jørgensen, Marianne V. Sørensen, Bjørn E. Christensen, Søren Kjærulff, Niels Frimodt-Moller, Robert I. Lehrer, Michael Zasloff and Hans-Henrik Kristensen, *Nature*, 2005, **437**, 975–980

Reviewed by: M. Muralidhar Reddy, UT-Southwestern Medical Center, USA