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leukocytes. Unlike the structurally related normal DHA dihydroxylated product obtained by the action of lipoxygenase on DHA, in which the 17-OH has the *S*-absolute configuration, the new product which is produced by the aspirin-acetylated COX-2 enzyme has a 17*R*-OH configuration (Figure 1). Complete structural characterization was carried out by elegant synthetic and analytical work as with the earlier identified neuroprotectins (Marcheselli et al., 2010). This new protectin has potent antiinflammatory actions and stimulates resolution by activating macrophages and assists in clearing sites of inflammation by stimulating macrophages to uptake dead leukocytes and other cellular debris.

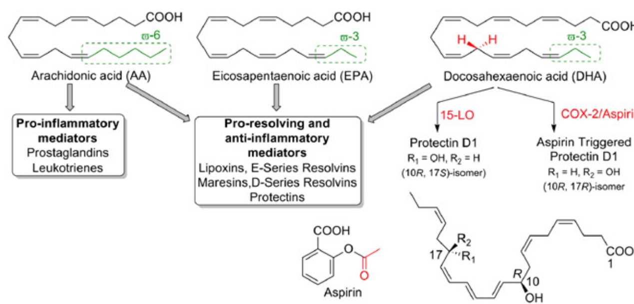

Figure 1. Products of Oxidative Transformations of Polyunsaturated Essential Fatty Acids

The structural characterization of these intriguing and very potent oxygenated lipid molecules coupled with the careful characterization of their immunomodulating properties opens the door for the development of novel therapeutics in the all-important field of inflammation. Characterization of the protein targets for these molecules is still at the early stages while correlation between their structures and activities is incomplete. Progress in these fronts should provide the basis for thoughtful target-based drug design to produce more biochemically stable and therapeutically targeted medications. Serhan's finding also suggests the combined use of DHA and aspirin as an attractive therapeutic avenue for the treatment of inflammatory conditions.

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PNA to DNA to Microarray Decoding Facilitates Ligand Discovery

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The development of a method for the amplification of PNA tags (Svensen et al., in this issue of *Chemistry & Biology*) should expand the range of biological targets amenable to screening using PNA-encoded combinatorial libraries and thus facilitate the discovery of new biologically useful agents.

The screening of libraries of molecules to identify those with desirable biological properties is fundamental to the drug discovery process and chemical biology studies in general. Solid-phase split-and-mix combinatorial synthesis techniques can rapidly generate libraries of vast numbers of compounds suitable for screening (Maclean et al., 1997; Harris and Winssinger, 2005; Díaz-Mochón et al., 2005). However, such libraries are obtained as complex mixtures, and determining the chemical identity of any individual biologically active member present is challenging. Toward this end, a number of chemical-encoding strategies have been developed where each molecule in the library becomes associated with (or incorporates) a unique tag encoding its unique synthetic history and thus molecular structure. This information can then

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be revealed by analyzing the tag via a suitable decoding process.

DNA encoding is arguably the most widely used tagging method for split-and-mix libraries, offering opportunities for parallel and quantitative decoding, either through the use of microarray platforms or sophisticated sequencing methods (Svensen et al., 2011b). Additionally, peptide nucleic acid (PNA) has been identified as valuable encoding alternative to DNA (Harris and Winssinger, 2005). PNA is a DNA mimic that has a high affinity for DNA, in principle, allowing the decoding of PNA libraries through their interface with organized DNA microarrays via sequence-specific hybridization (Svensen et al., 2011a). PNA is more resistant to both chemical and biological degradation than DNA; there are thus comparably less restrictions on the chemistries that can be used to build PNA-encoded libraries (which could allow a greater diversity of products to be generated) and PNA can exist in an environment with live cells for a longer period of time (Svensen et al., 2011a; Svensen et al., 2011b [this issue of *Chemistry & Biology*]). However, a fundamental problem with PNA encoding is that PNA cannot be amplified using standard nucleic acid amplification techniques; thus, if a screen yields a low concentration of PNA tags (for example, if the biological target is in relatively scarce amounts), which are below the level needed for microarray analysis, then decoding of any hits is not possible. Svensen et al. now report a novel method for the amplification of hits from a library of PNA-tagged peptides that addresses this key limitation. Their approach is based around the indirect DNA amplification of PNA tags, i.e., PNA to DNA decoding. The authors describe an overall screening strategy incorporating this novel amplification technique in the context of discovering new cell surface receptor ligands. This process involves the following main stages:

1. Incubation of a PNA-encoded peptide library against live cells overexpressing a cell surface receptor of interest
2. Extraction of the PNA tags
3. Hybridization of the isolated PNA with a single-stranded DNA (ssDNA) library complementary to the original PNA-encoded peptide library
4. Degradation of the nonhybridized ssDNA
5. Asymmetric PCR amplification of the DNA protected by the PNA with a fluorescent primer-generated ssDNA
6. Hybridization of the fluorescently labeled ssDNA with a complementary DNA microarray to allow decoding of the amplified ssDNA (and hence the PNA tags and their encoded peptides).

In proof-of-concept work the authors describe the screening of a 10,000 member PNA-encoded peptide library with live cells overexpressing members of two classes of pharmacologically relevant cell surface receptors, either integrins or the CCR6 receptor (a G protein coupled receptor). New peptide ligands for two integrins ($\alpha_\upsilon\beta_5$ and $\alpha_\upsilon\beta_5$) and CCR6 were discovered, demonstrating the feasibility of this approach. Indeed, this strategy may prove to be broadly applicable for the identification of ligands for a wide range of cell surface receptors, which has been a long-standing goal in medicinal chemistry research. The technology offers a tool for studying differences in membrane receptor expression between different cell types, including diseased states, which could have important implications for the identification of pharmaceutically relevant receptor drug targets.

The amplification technique developed by Svensen et al. represents a significant advancement in our ability to decode PNA-tagged libraries; as such, it is expected to have broad applications beyond the remit of discovering new cell surface receptor ligands. This technology may prove especially valuable when screening for hits against biological targets that are available only in limited amounts (e.g., due to limited number of cells or a target that is expressed only in small quantities), which has traditionally been viewed as challenging (or indeed impossible) to achieve using PNA-encoded libraries. There are several other significant aspects of this work. As screening is carried out prior to decoding and PNA is not degraded under normal biological conditions, the technology is suitable for both in vivo as well as in vitro applications. The use of DNA microarrays allows information on the interactions between active compounds and their targets to by extracted on a compound-by-compound basis. In principle, the new decoding strategy could be applied to the screening of libraries of nonpeptidic compounds, including small molecules, which would increase the chemical universe amenable to screening using PNA-encoding methods (Svensen et al., 2011b; Scheuermann et al., 2006). This is an important consideration given the growing demand for novel biological agents from unexplored regions of chemical space (Galloway et al., 2010). Overall the PNA tag amplification technique developed by Svensen et al. represents a significant advancement in the field of library screening using PNA encoding, which should facilitate the discovery of a wide-range of novel biologically useful molecules.

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