

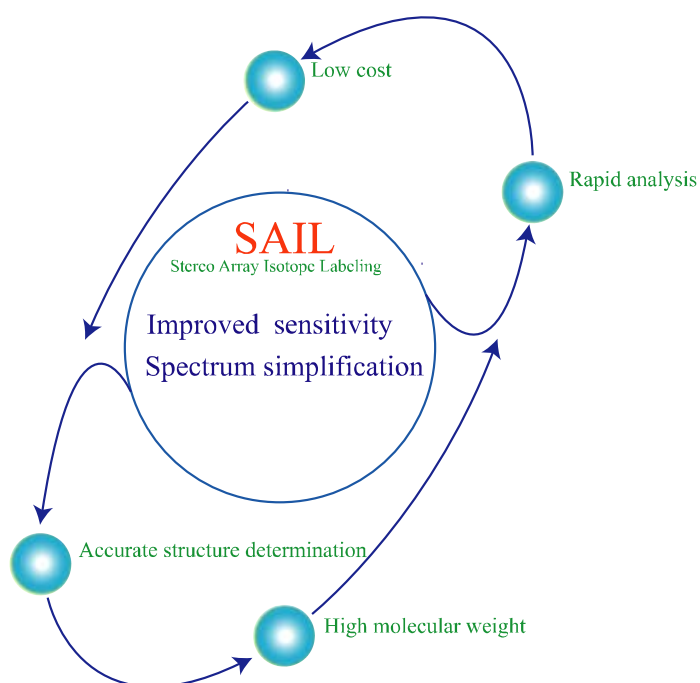
Introduction to the technology

1. Summary

The SAIL (Stereo-Array-Isotope-Labeling) method is an ingenious NMR approach for protein structure analysis. Developed by Professor Kainosho from Tokyo Metropolitan University, this novel approach is based on his experience accumulated over a lifetime of research on the development of stable isotope labeling for NMR analyses of biomolecules. SAIL utilizes the simple concept that certain stereospecific patterns of isotopes are optimal for protein NMR analysis and that these depend on the structures of the individual amino acids. SAIL amino acids are “designer molecules” engineered to provide all essential information and to minimize problems from unwanted spin-spin coupling and unfavorable relaxation mechanisms. This is achieved by building a particular pattern of isotopes into amino acids: ^1H or ^2H for hydrogen, ^{12}C or ^{13}C for carbon, and ^{14}N or ^{15}N for nitrogen. Once incorporated into a protein, these carefully designed SAIL amino acids enable the researcher to perform a three-dimensional NMR structural analysis rapidly and with high accuracy. SAIL labeling facilitates structural studies of proteins that are twice as large as those that can be analyzed by conventional labeling methods.

SAIL proteins are easily prepared from SAIL amino acids by cell-free protein synthesis. This approach ensures that the special labeling patterns of the amino acids are retained in the protein. Once prepared, NMR structures can be determined by conventional methods. Automation that accommodates the properties of the isotopes and their geometric configuration can make the structure determinations even more streamlined. By exploiting the features of the SAIL method, accurate structures can be determined quickly, even for proteins with higher molecular weights. The time savings and higher accuracy result from a number of factors.

1. It is much easier to identify NMR signals from SAIL proteins, because peaks are sharper and NMR spectra are less crowded.
2. Sharper peaks mean faster data collection.
3. NOESY spectra are simpler and can be collected at longer mixing times because direct relaxation is slower; this means that more distance constraints can be resolved, including those between pairs of protons that are more than 5 Å apart.
4. Since the amino acids are all chirally labeled, chiral assignments are unnecessary.
5. All of these factors support more reliable automated analysis. This reduced the enormous amounts of time and cost, typically required for a structure determination.

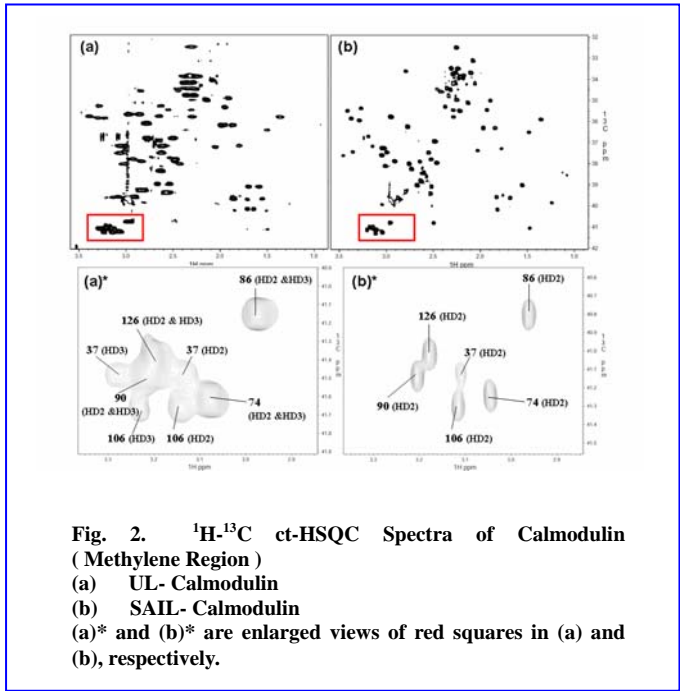
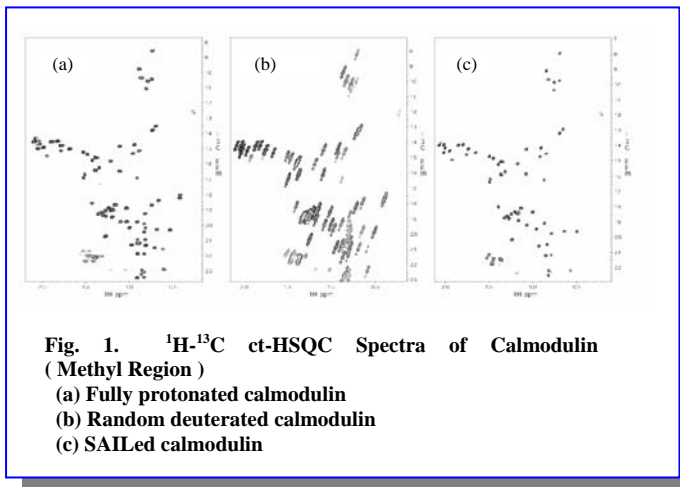


2. Features

1) Accurate structure determination

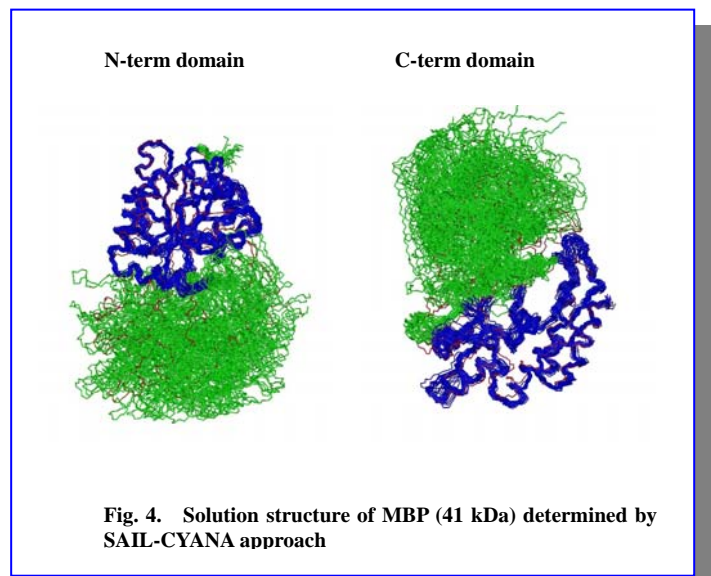
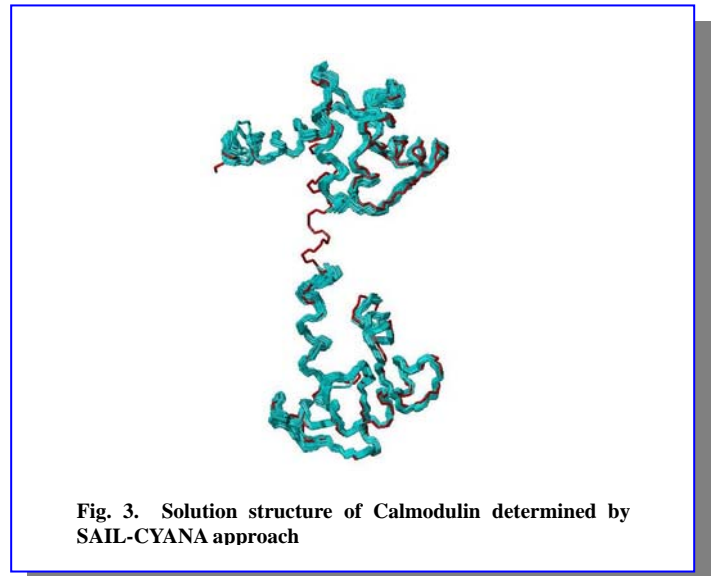
Signal simplification, achieved by lowering the proton density and eliminating the geminal coupling, increases identifiable NOE signals; the amount of information used for structural analysis is increased to provide a more accurate structure.

The stereoscopically-equivalent protons in the methylene groups are eliminated; therefore, the proton position can be determined more accurately. (Fig. 1, Fig. 2)



2) High molecular weight

Low proton density and improved signal sensitivity enables the researcher to increase the limit for molecular weight determination by at least two-fold (30 kDa \rightarrow 60 kDa) as compared to conventional NMR methods, where uniformly $^{13}\text{C}/^{15}\text{N}$ -double-labeled protein specimens are typically used. Thus, this broadens the range of proteins with structures that can be determined by NMR. (Fig. 3, Fig. 4)



3) Rapid analysis

A high, heavy hydrogen-labeling rate makes the NMR measurement time as short as about a week.

The speed of NMR spectrum analysis is about 4 times faster than the conventional method.

| | Usual method | SAIL method Manual assignment | SAIL method Automatic assignment |
|-----------------------|---|---|---|
| Sample Preparation | Cell free protein synthesis system 2 weeks | Cell free protein synthesis system 2 weeks | Cell free protein synthesis system 2 weeks |
| NMR experiment | Normal probe 2 weeks ~ 2 months | Cryo probe ~1 week | Cryo probe ~1 week |
| Assignment | Manual assignment 2 weeks ~ 2 months | Manual assignment 2 weeks ~ 2 months | Automatic assignment Hours ~ 2 days |
| Structure calculation | Manual 2 weeks ~ 2 months | CYANA (pc cluster) 30 min | CYANA (pc cluster) 30 min |
| | ~ 6 months | ~ 3 months | ~ 1 months |

*PC cluster system 8-node Xeon 2GHz x 16

4) Cost reduction

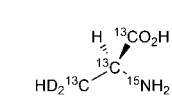
Amazing cost reduction as compared with that of conventional NMR measurements.

SAIL Technologies, Inc.

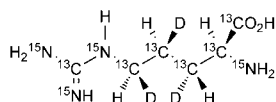
1-1-40 Suehiro-cho, Tsurumi-ku, Yokohama,
Kanagawa, Japan 230-0045

E-mail: info@sail-technologies.com

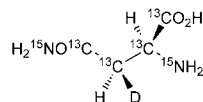
SAIL amino acids



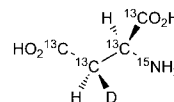
Ala



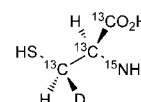
Arg



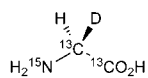
Asn



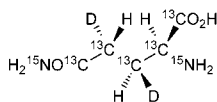
Asp



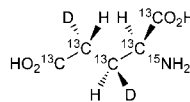
Cys



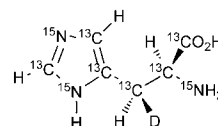
Gly



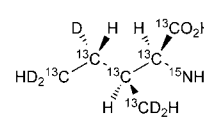
Gln



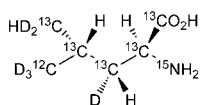
Glu



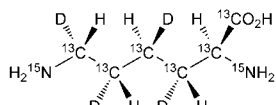
His



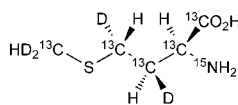
Ile



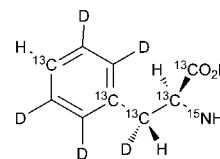
Leu



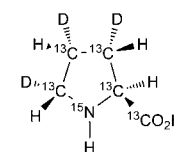
Lys



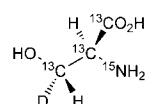
Met



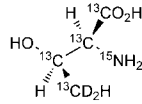
Phe



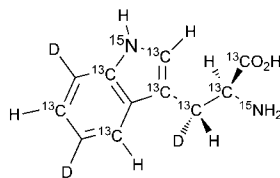
Pro



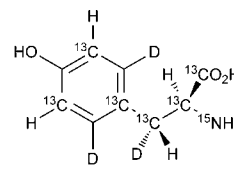
Ser



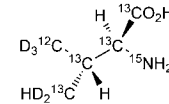
Thr



Trp



Tyr



Val