

Guide for literature readings relating to "NMR Fingerprints of Proteins" lecture

Main reading:

Discovering high-affinity ligands for proteins: SAR by NMR

SB Shuker, PJ Hajduk, RP Meadows, SW Fesik, *Science* **274**, 1531 (1996).

optional additional reading:

A polar, solvent-exposed residue can be essential for native protein structure

RB Hill and WF DeGrado, *Structure* **8**, 471 (2000).

On Tuesday, January 28 or Thursday, January 30, we will spend some time discussing the Shuker article in class. Be prepared to discuss the questions below. The ppt notes should be a helpful companion in understanding a bit about what a 2D HSQC spectrum is, since this is the first time you will have seen a multidimensional NMR spectrum in this class. This paper is sort of a segue into our learning about multidimensional NMR, and for now you can think of a 2D HSQC as a fancier version of a 1D spectrum in which each peak represents the intersection of the chemical shifts of a group of two covalently bonded nuclei, rather than just a single chemical shift arising from a single nucleus. In essence, it's a "fingerprint" of a protein that is spread out in an extra dimension, making it easier to "read".

The Hill & DeGrado article is optional, and relates to one of the slides in the NMR Fingerprints ppt lecture. Although Shuker et al and Hill & DeGrado are very different studies, the two articles have in common the use of changes in simple 1D and 2D NMR spectra to detect changes in proteins due to structural alterations or ligand binding events.

Shuker article:

The "SAR by NMR" method produces high affinity ligands for a target protein using two separate NMR screening steps. These two steps involve binding of two different molecules to two different target sites, in each case with micromolar to millimolar affinity. The two molecules are then linked to each other to form a single ligand with nanomolar to picomolar affinity. To enable effective linkage using short connectors, the two binding sites must be nearby each other but essentially nonoverlapping

Why not just screen for a single molecule with nanomolar to picomolar affinity in the first place?

How do they make sure the two sites aren't overlapping?

How do they make sure the two sites are nearby each other? Why are ^{15}N - ^1H HSQC spectra such good tools for a method like this?

If you get to the Hill article, think about the following:

Hill & DeGrado article:

Rather than try to understand this article in its entirety and in depth, I'd like you to focus on how simple NMR spectra are being used to interpret the structural effects of mutations

in a designed protein. I'd like you to pay particular attention to the meaning of Figures 5, 6 and 9:

How do the authors interpret the differences in 1D NMR spectra for the position 7 mutants (Figure 5)?

What are some alternative explanations for the differences in 1D NMR spectra, and how do they rule out these alternatives?

What is the difference between Figures 5 and 6? Why do they include Figure 6?

What model for the structure of the E7V mutant is being implied by Figure 9?