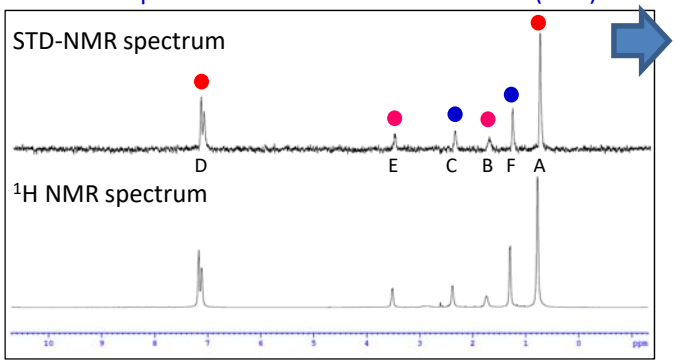
Physicochemical Analysis of Intermolecular Interactions in Pharmaceutical and Diagnostics Research

The pharmacological action of drugs is induced and detection by diagnostics is conducted based on intermolecular interactions between bimolecular such as antibodies and antigen. Physicochemical analysis of intermolecular interactions enables molecular level evaluation of interactions, and is applicable to the optimization of the structure and function of drugs and diagnostics, as well as quality control.

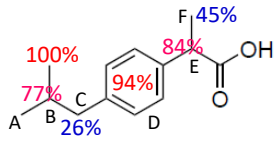
STD-NMR

Presence or absence of interaction between ligand and protein can be evaluated, and binding site information can be obtained.

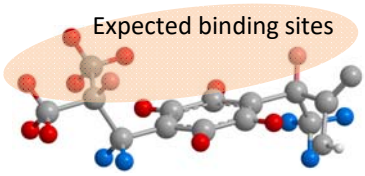
Ibuprofen and human serum albumin (HSA)



Ligand signals are observed. → Presence of interaction
Difference in signal intensity → Binding site information



Ibuprofen (MW: 206)

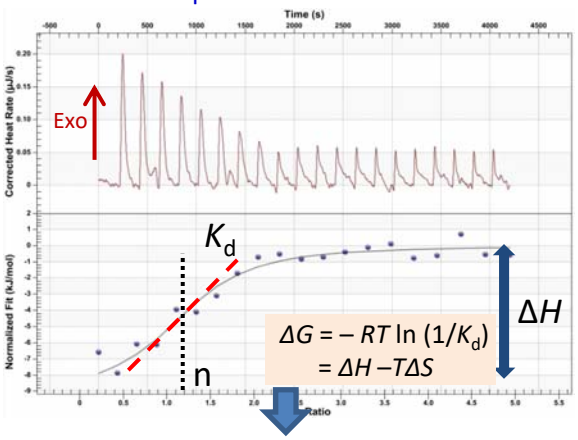


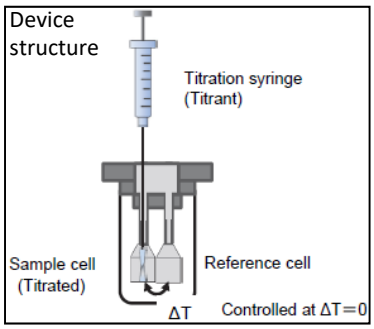
It's suggested that Ibuprofen interacts with HSA on the overall surface.

ITC (Isothermal titration calorimetry)

Information on interaction can be obtained from thermodynamic characterization by measuring minor heat transfer that is caused during bimolecular binding.

Ibuprofen titrated into HSA





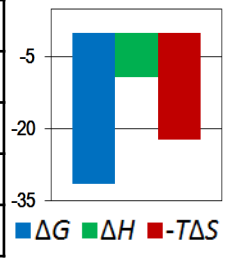
Analyte:
Small molecular drugs, peptides, nucleic acids, proteins, etc.

Application:
Bimolecular interactions,
Competitive bindings,
Enzyme kinetics,
Quality control of proteins,
Critical micelle concentration, etc.

Thermodynamic parameter

K_d (mol/L)	3.19×10^{-6}
n	1.19
ΔG (kJ/mol)	–31.4
ΔH (kJ/mol)	–9.2
$-T\Delta S$ (kJ/mol)	–22.2

(kJ/mol)



ΔH: Specific interactions
(Hydrogen bond, electrostatic binding)
$-T\Delta S$: Non-specific interactions
(Hydrophobic interactions)

Characterization of interaction
- Contributions of both TΔS and ΔH
- Larger contribution of $-T\Delta S$ than ΔH
- Presumably hydrophobic interactions with alkyl chains and benzene ring

Comparison of Analytical Methods

Method	SPR	ITC	STD-NMR	^{15}N-HSQC perturbation
Principles	Surface plasmon resonance	Heat transfer	Saturation transfer difference	Chemical shift perturbation
Immobiliza-tion, Levelling	Immobiliza-tion required	None	None	Labelling required
Molecular weight limitation	>100 Da	None	Low molecular weight	Low molecular weight
Affinity range	mM~nM	μM~nM	mM~μM	mM~μM
Information content	K_d, K_{on}, K_{off}	K_d, ΔH, ΔS, n	K_d, Ligand binding sites	K_d, Protein binding sites
Required amount of sample	Approx. 10 μg	1 mg or more	Approx. 1 mg	Approx. 1 mg

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