Chem 560 module
Analysis of dynamic, equilibrating systems

Outline

A. Intro to dynamic systems and weak interactions
B. Stoichiometry and K_{assoc} by NMR
C. Thermodynamic parameters by NMR
D. Assembly kinetics by NMR
E. Other techniques — Student presentations
e.g. UV-Vis, Fluorescence, Kinetics by line shape analysis, Isothermal titration calorimetry, Stopped-flow, etc

Learning Aims

A1. Learn the fundamental nature of weak, non-covalent interactions
A2. Become familiar with dynamic systems and the meanings of the quantities used to characterize them.
B1. De-mystify the black-box of K_{assoc} determinations by all methods.
B2. Obtain in-depth understanding of the math and models for 1:1 binding equilibria.
B3. Understand the mathematics of the 1:1 binding isotherm, its applications, and its limitations.
B4. Gain a comprehensive understanding of how δ arises when looking at dynamic systems.
B5. Get practical, step-by-step instructions for determining stoichiometry and K_{assoc} by NMR.
C. Learn how NMR can be used to determine ΔH and ΔS for a given equilibrium
D. Achieve a beginner-level understanding of studying kinetics by NMR. The goal is to allow you to understand literature, not to teach you how to do the experiments.
E. Get a beginner-level understanding of other methods

Dynamic systems and supramolecular chemistry

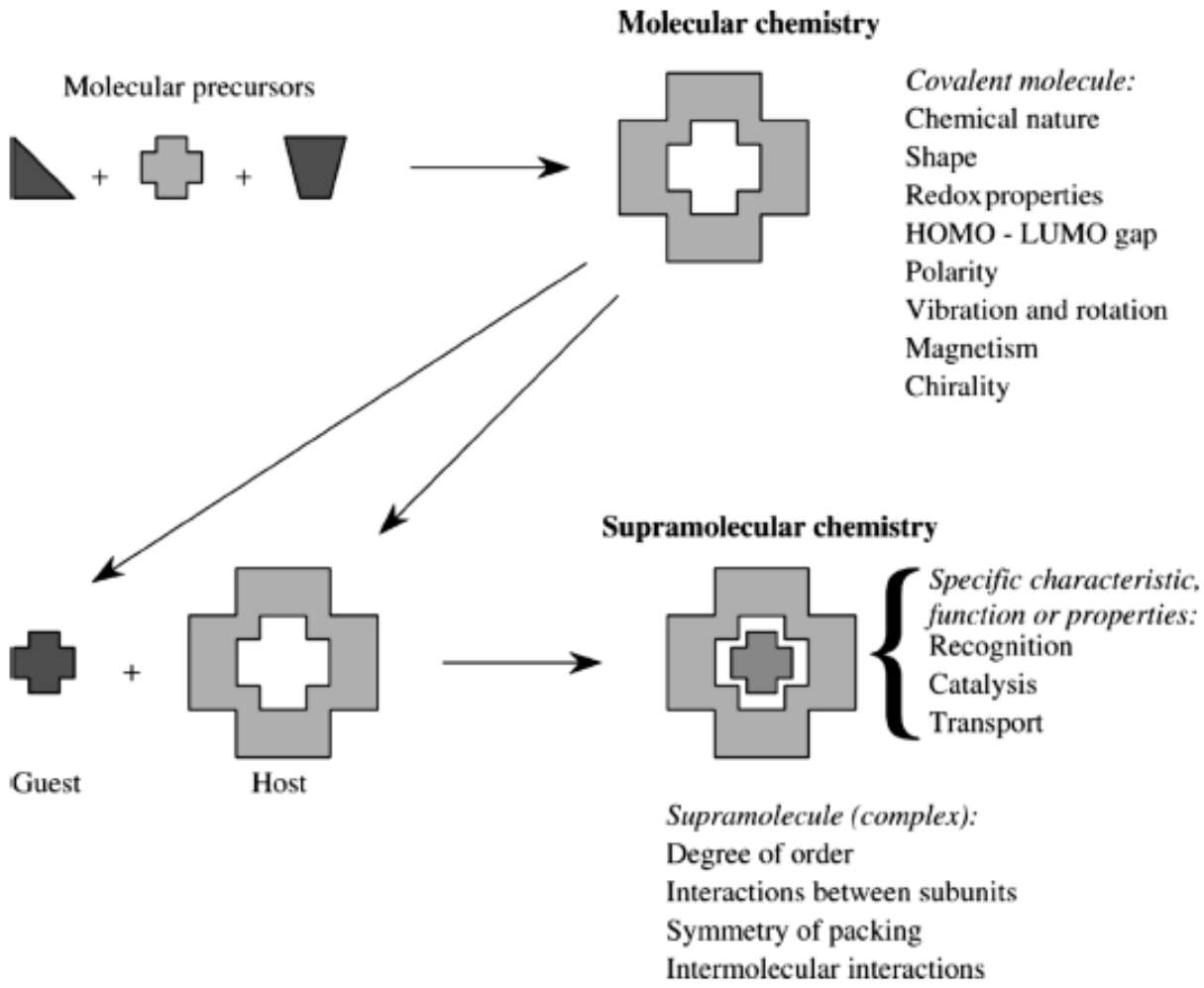
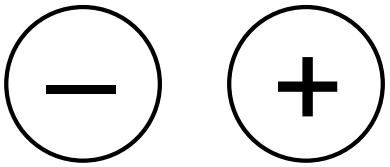
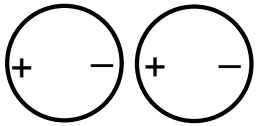


Figure 1.1 Comparison between the scope of molecular and supramolecular chemistry according to Lehn.[1]

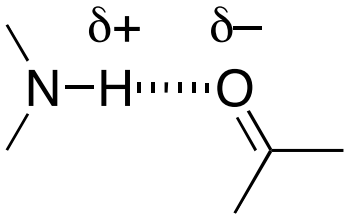
Dynamic systems are driven by weak interactions



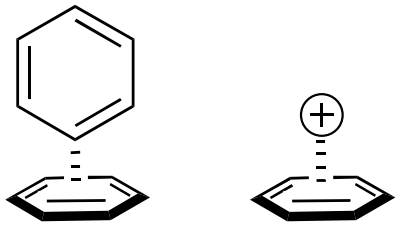
Electrostatic interactions



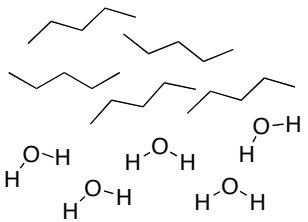
Dispersive forces



Hydrogen bonds

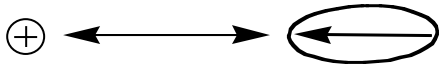


Aromatic-aromatic interactions and cation-pi interactions

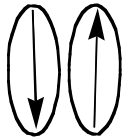


The hydrophobic effect

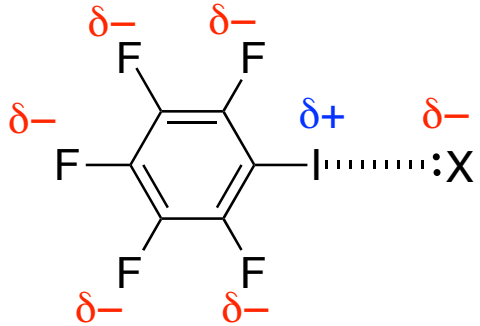
Ion–Dipole interactions



Dipole–Dipole interactions



Halogen bonds

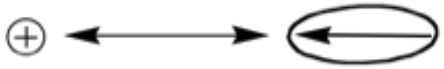


Two reminders: 1) Opposites attract. 2) Math is hard

Ion–Ion interactions

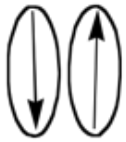
$$U = z_1 z_2 e^2 / 4\pi\varepsilon_0 \varepsilon r$$

Ion–Dipole interactions



$$U_{\text{ion-dipole}} = -\frac{1}{4\,\pi\,\varepsilon_o} \cdot \frac{z \cdot e \cdot \mu \cdot \cos\,\theta}{r^2}$$

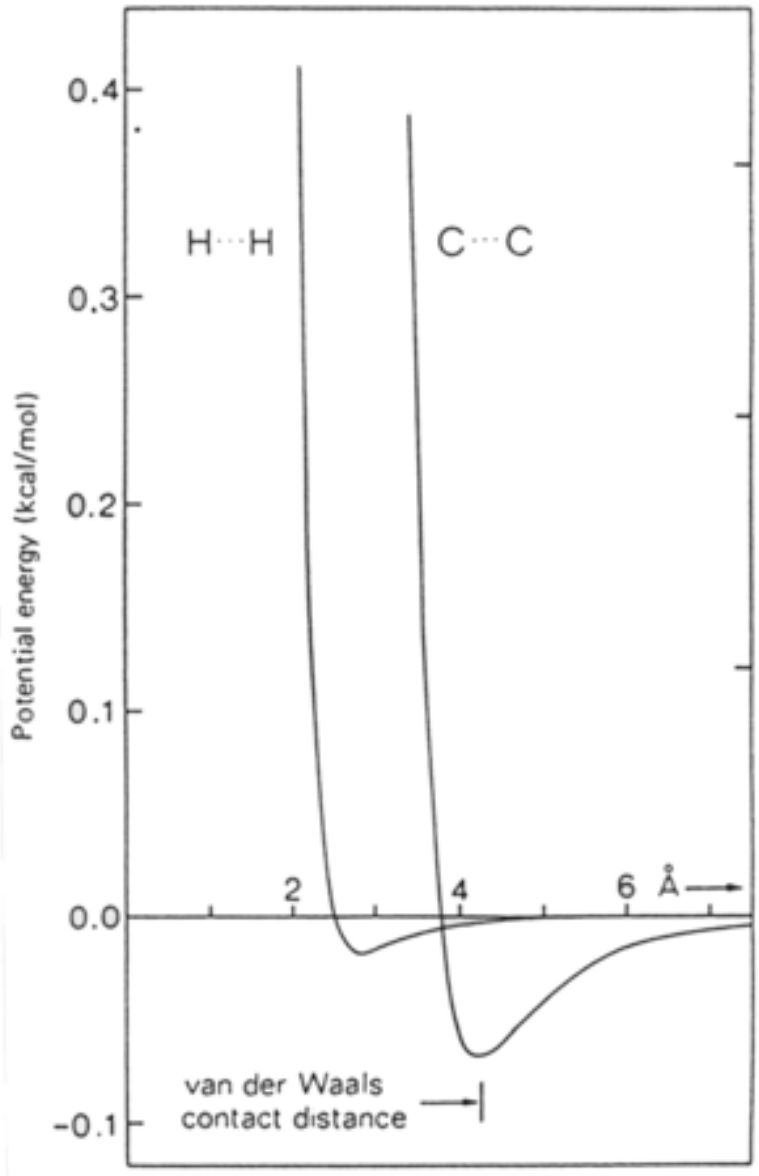
Dipole–Dipole interactions



$$U_{\text{dipole-dipole}} = \frac{f}{(4\,\pi \cdot \varepsilon_o)} \cdot \frac{\mu_1 \cdot \mu_2}{r^3}$$

$(f = 1 - 3\cos^2\theta)$

Van der Waals forces

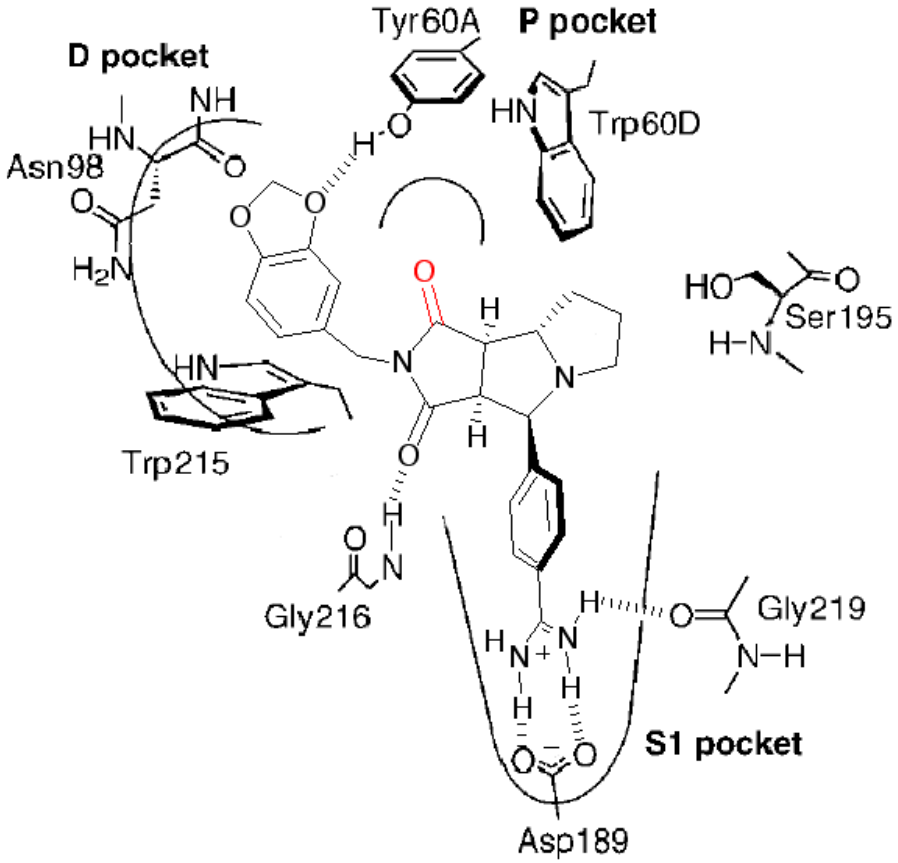


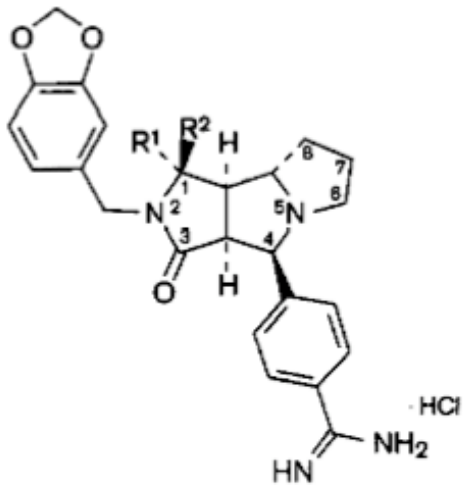
Van der Waals radii (Å)

H	1.20
C	1.70
N	1.55
O	1.52
S	1.80
F	1.47
Cl	1.75
Br	1.85
I	1.98

A. Bondi, J. Phys. Chem. **1964**, *68* (3), 441.

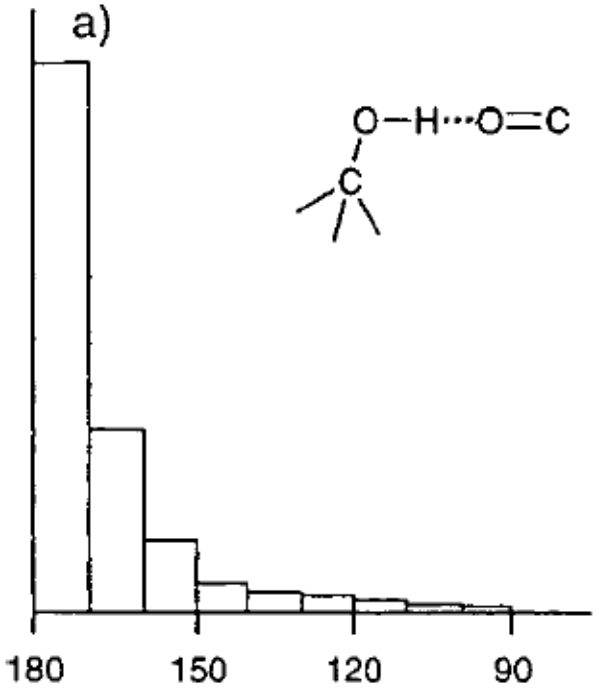
Dispersion forces: filling the P pocket of Thrombin



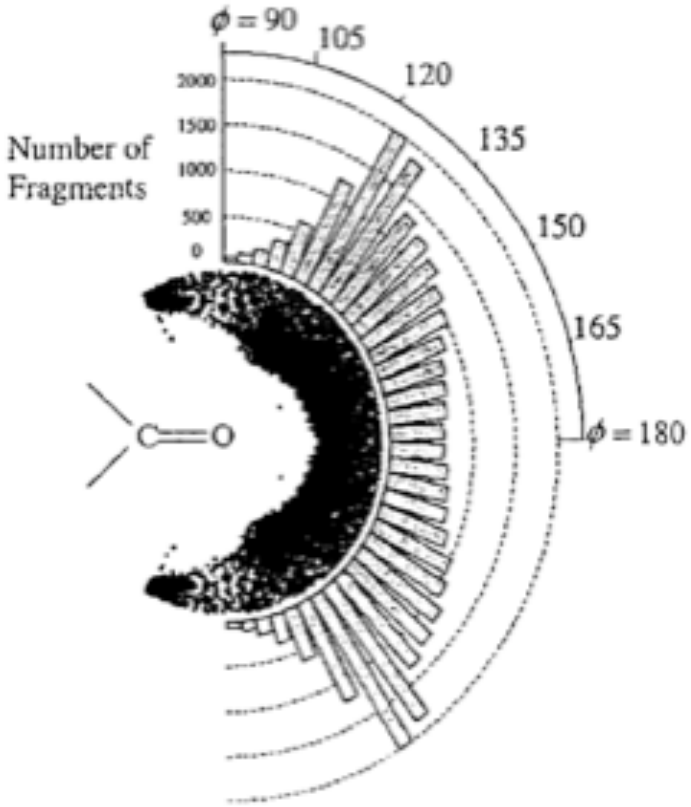


Compound	R^1	R^2	K_i (μM) Thrombin
rac-**1**[‡]	O		0.09
rac-**20b**	Ethyl	H	0.0081
rac-**20c**	Cyclopropyl	H	0.010
rac-**20d**	Isopropyl	H	0.013
rac-**20e**	Cyclohexyl	H	1.7
rac-**20f**	Phenyl	H	1.4

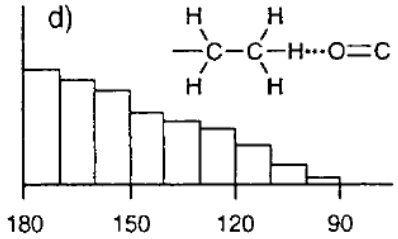
Hydrogen bonding angles: crystallographic survey



Geometry at donor

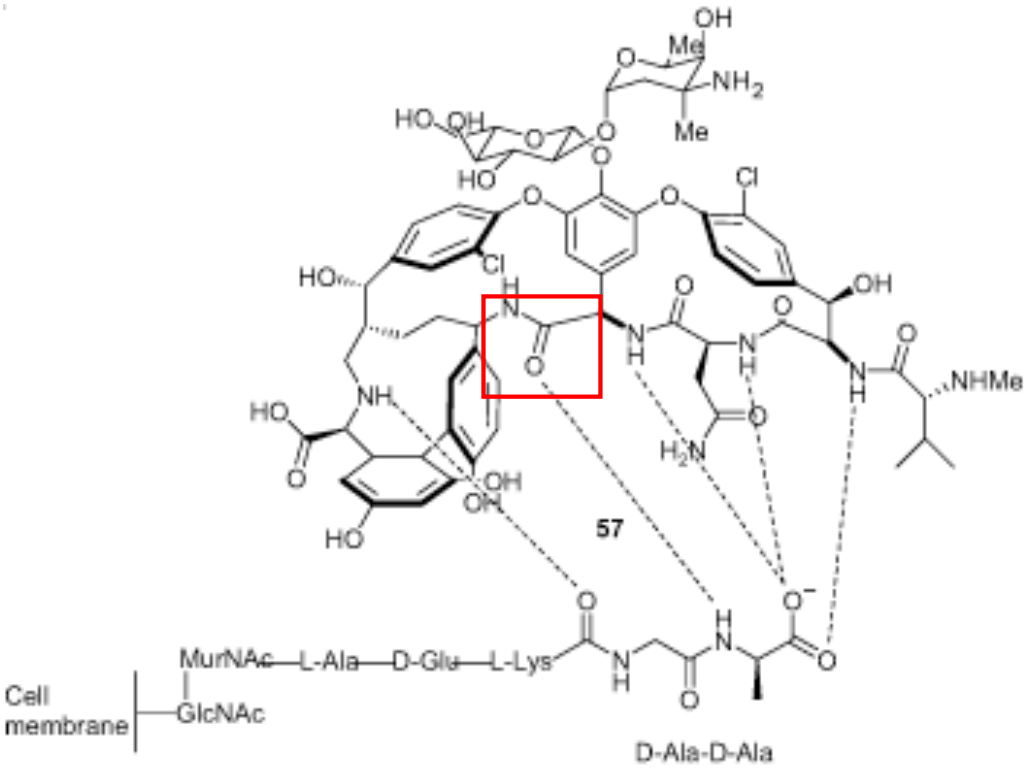


Geometry at acceptor

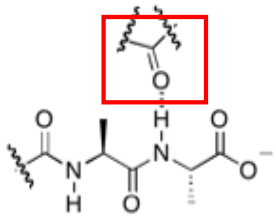


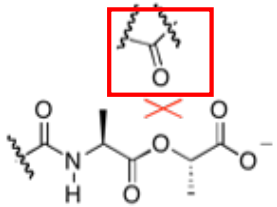
Steiner, Angew. Chem. Int. Ed. **2002**, *41*, 48–76.

Vancomycin hydrogen bonds to the bacterial cell wall precursor D-Ala-D-Ala



Vancomycin-resistant *Enterococcus* (VRE)

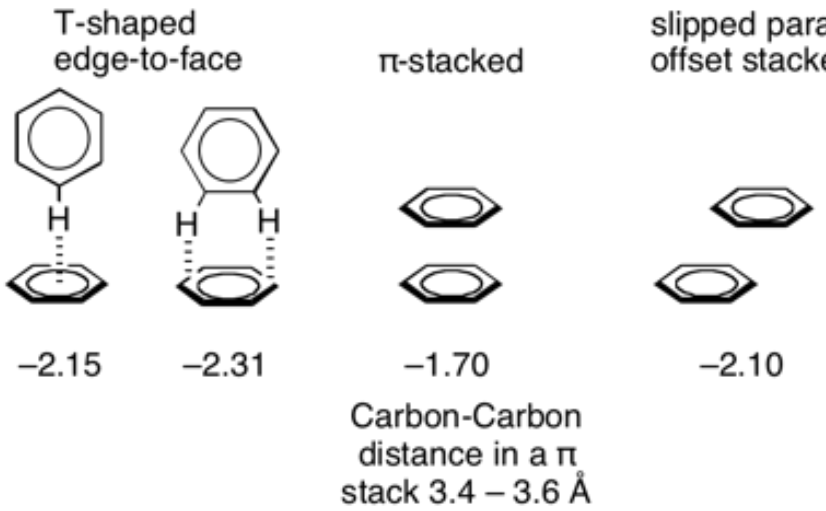




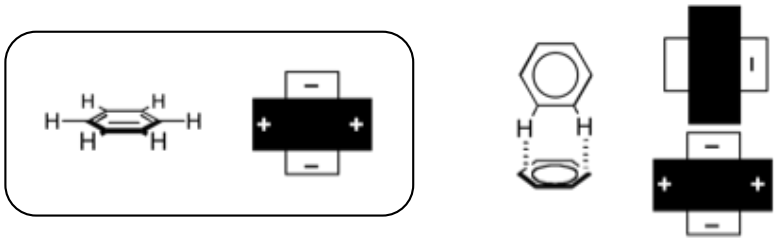
$\Delta K_{assoc.}$ = 1000x weaker to D-Ala-D-Lac

Aromatic-aromatic interaction geometries

Calculated binding energies (kcal/mol)



Electrostatic view of different geometries

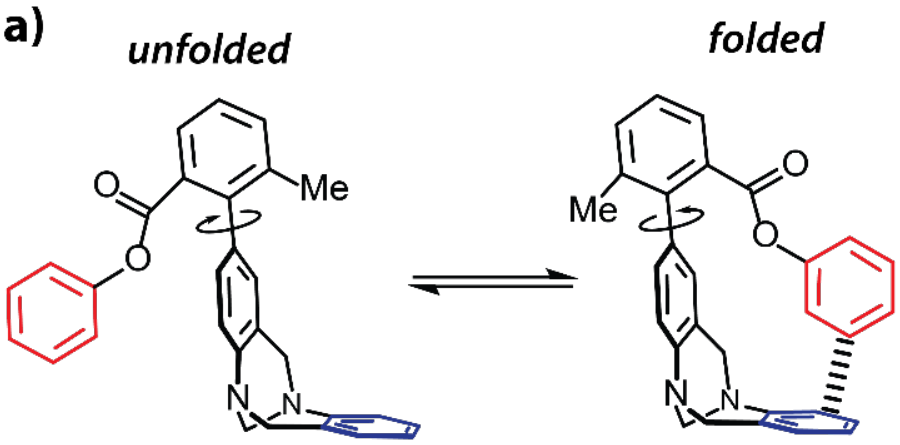


Meyer et al. *Angew. Chem. Int. Ed.* **2003**, *42*, 1210.

Dunitz, *ChemBioChem* **2004**, *5*, 614.

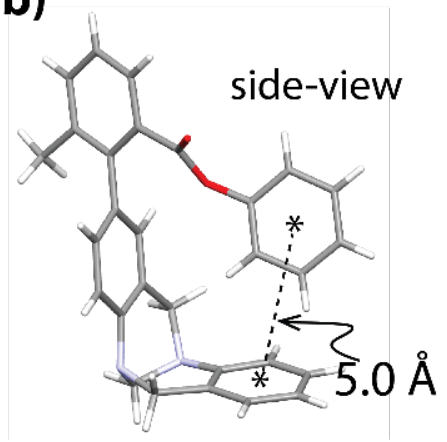
'Torsion Balances' for measuring interaction strengths

NMR integration measures conformational K_{eq}

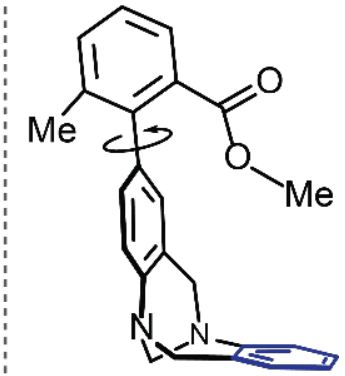


Craig Wilcox

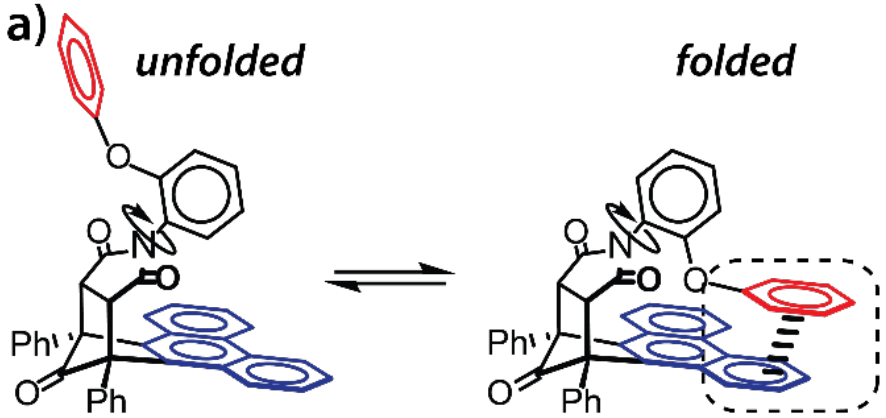
5



X-ray crystal structure of **5**

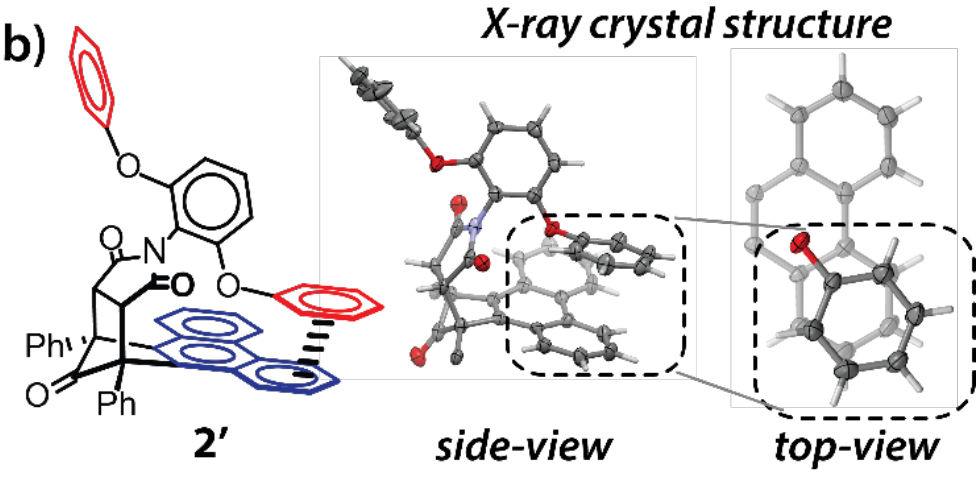


6



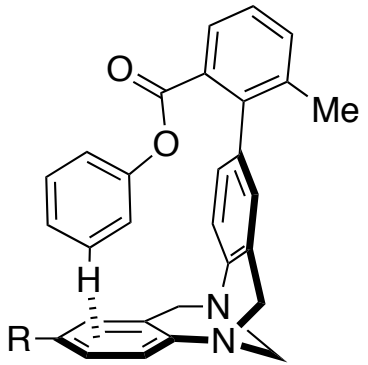
Ken Shimizu

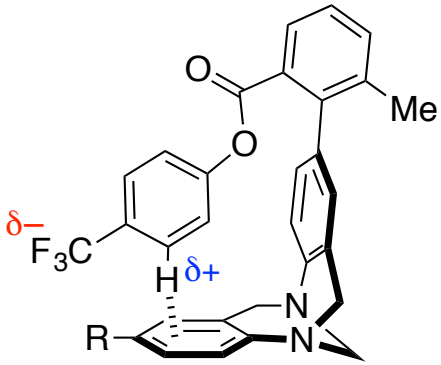
2



K. D. Shimizu,* P. Li, and J. Hwang Chapter in *Aromatic Interactions*, 2016. dx.doi.org/10.1039/9781782626626

Dispersion vs. Electrostatics: EDG/EWG substituents matter only if both rings are polarized





• Unpolarized Ar-H

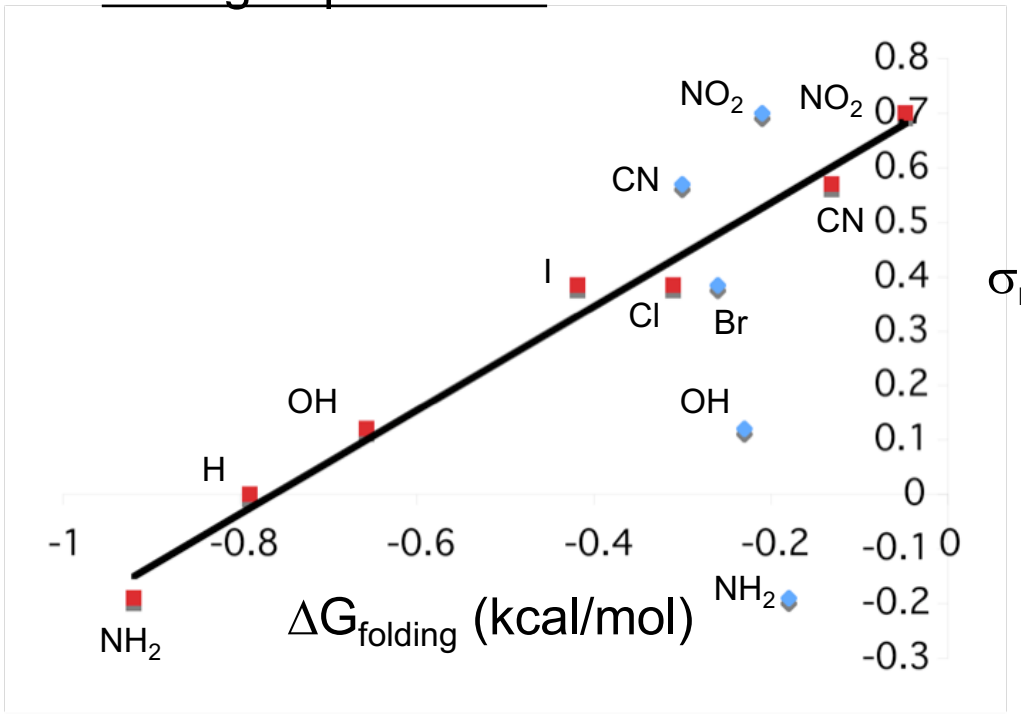
No dependence on R = EDG/EWG

■ Polarized Ar-H

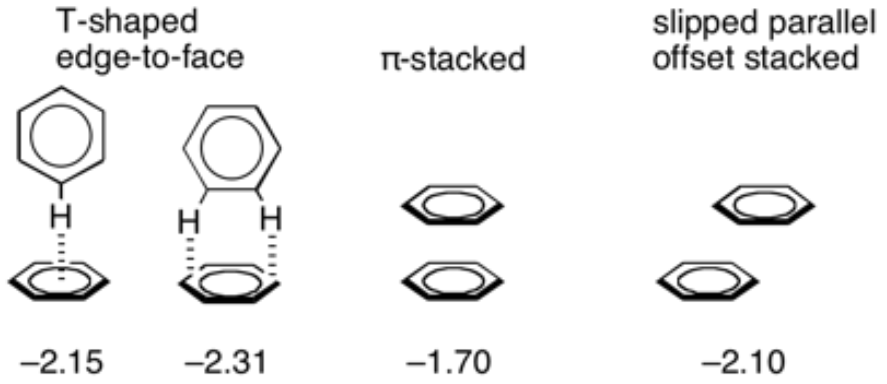
Strong dependence on R = EDG/EWG

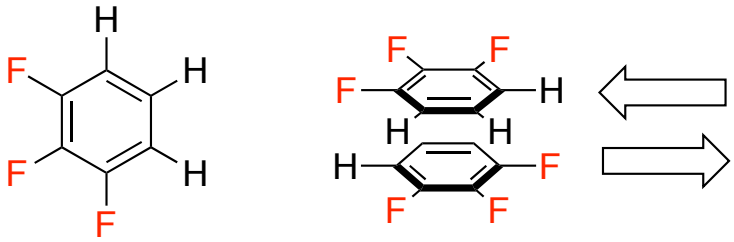
Linear free energy relationships:

$\Delta G_{folding}$ **vs.** σ_m (Hammett parameter; a measure of EW character)

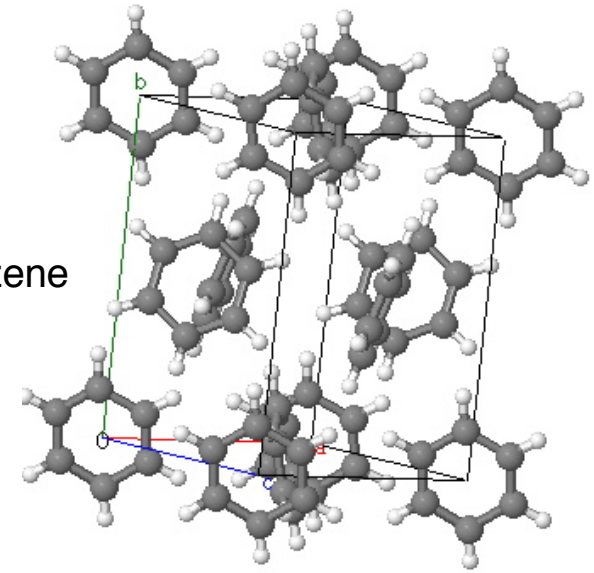


Dispersion vs. Electrostatics: EDG/EWG substituents matter only if rings are strongly polarized

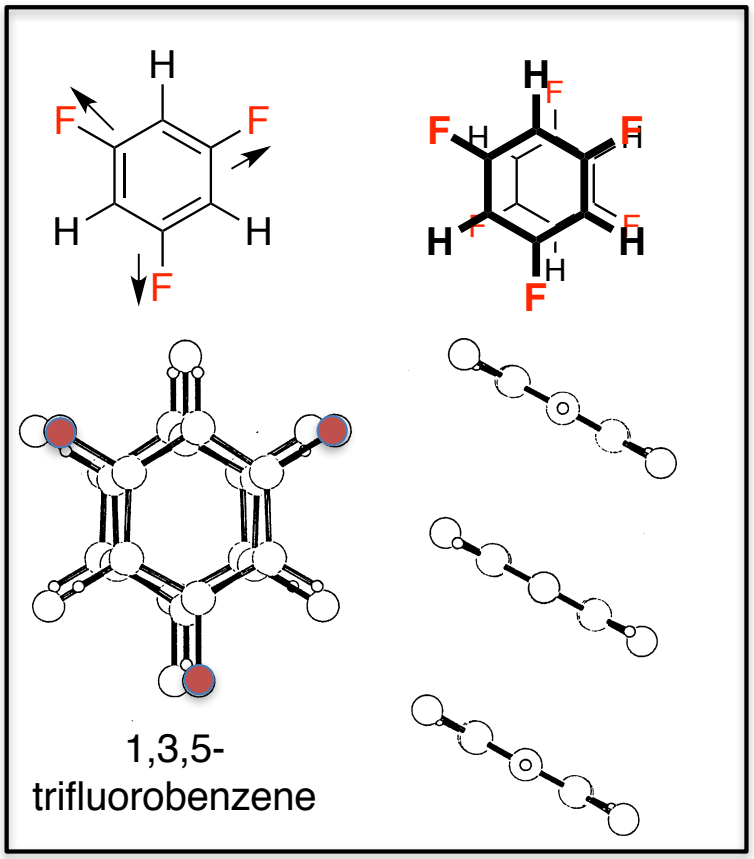




benzene

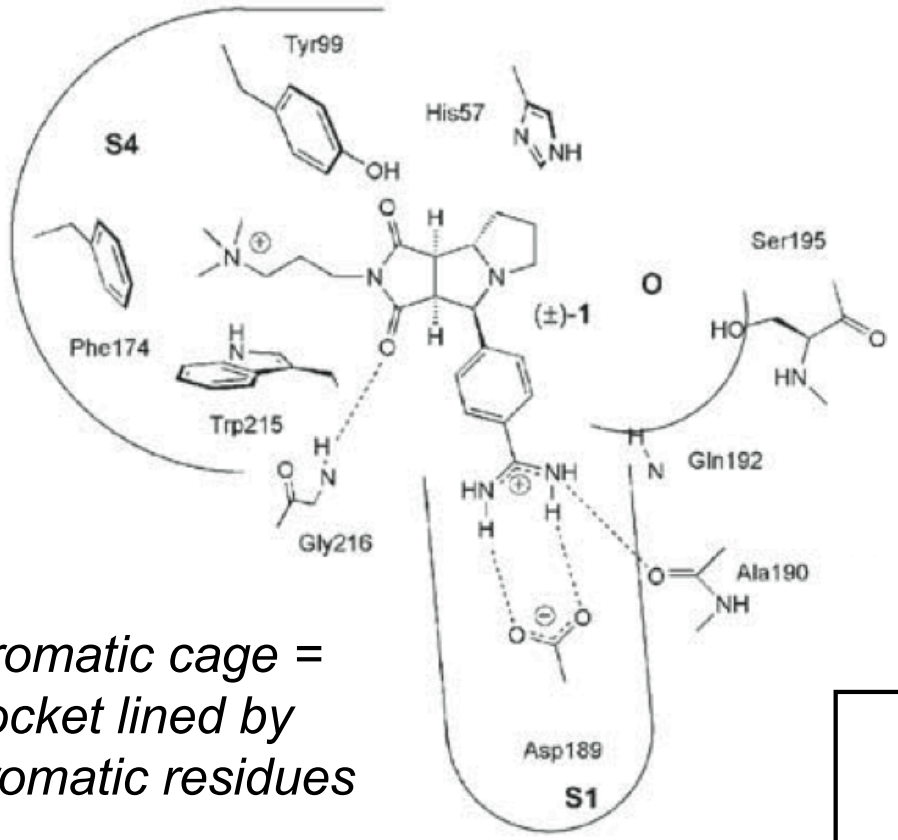


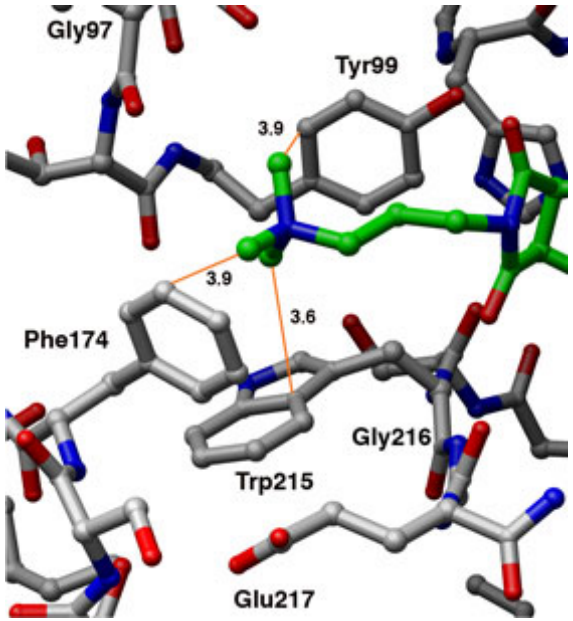
X-ray structures



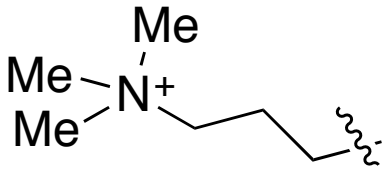
J. Dunitz, ChemBioChem, **2004**, 5, 614

Cation-π interactions: Isosteric inhibitors for the protein Factor Xa

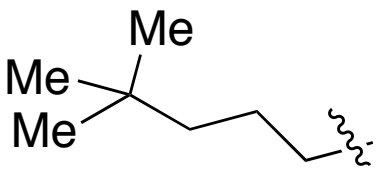




Aromatic cage = pocket lined by aromatic residues



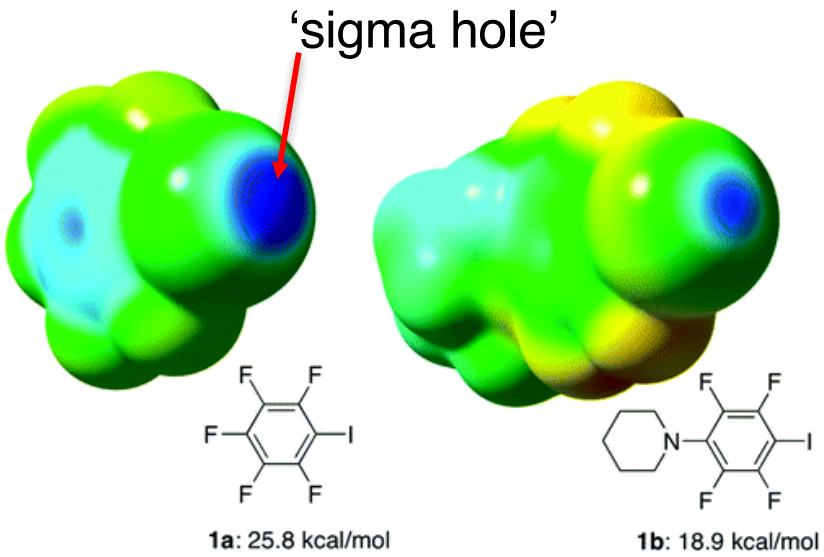
K_i = 0.28 μM

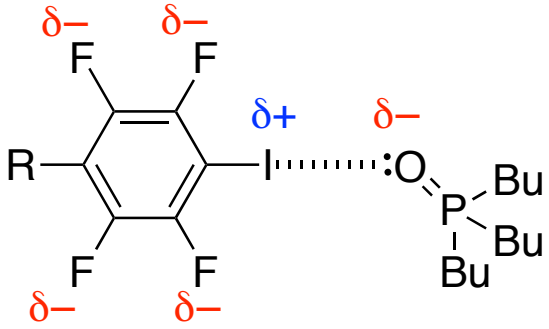


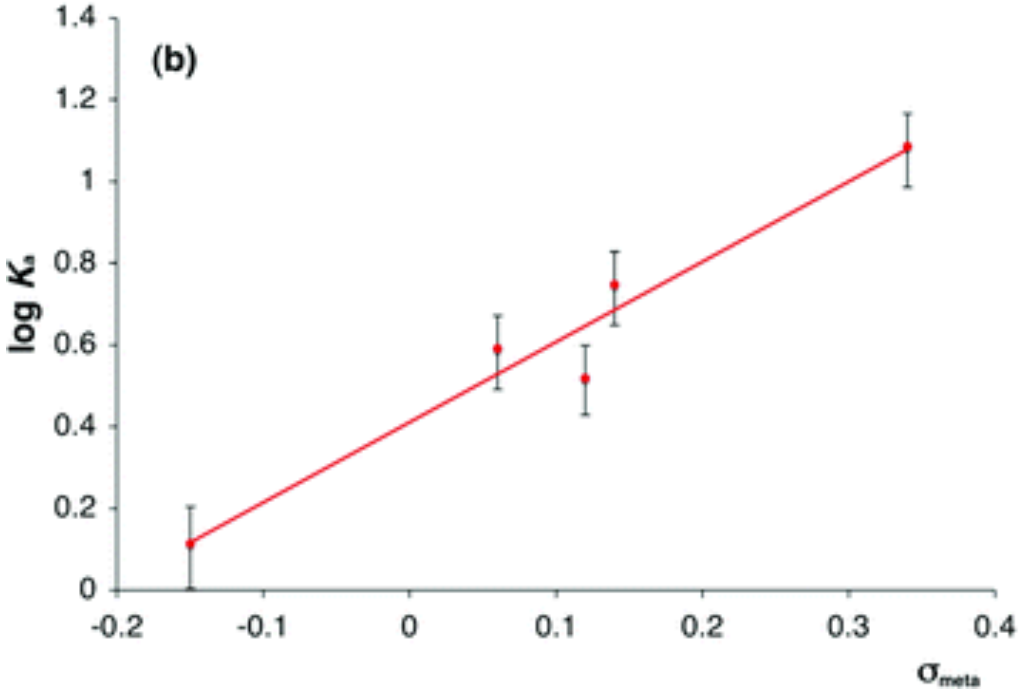
K_i = 29 μM

Angew Chem Int Ed Engl. 2005;44(28):4400

Halogen bonds… an unconventional (δ+)



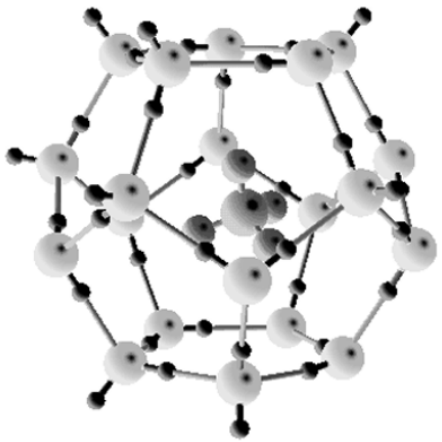


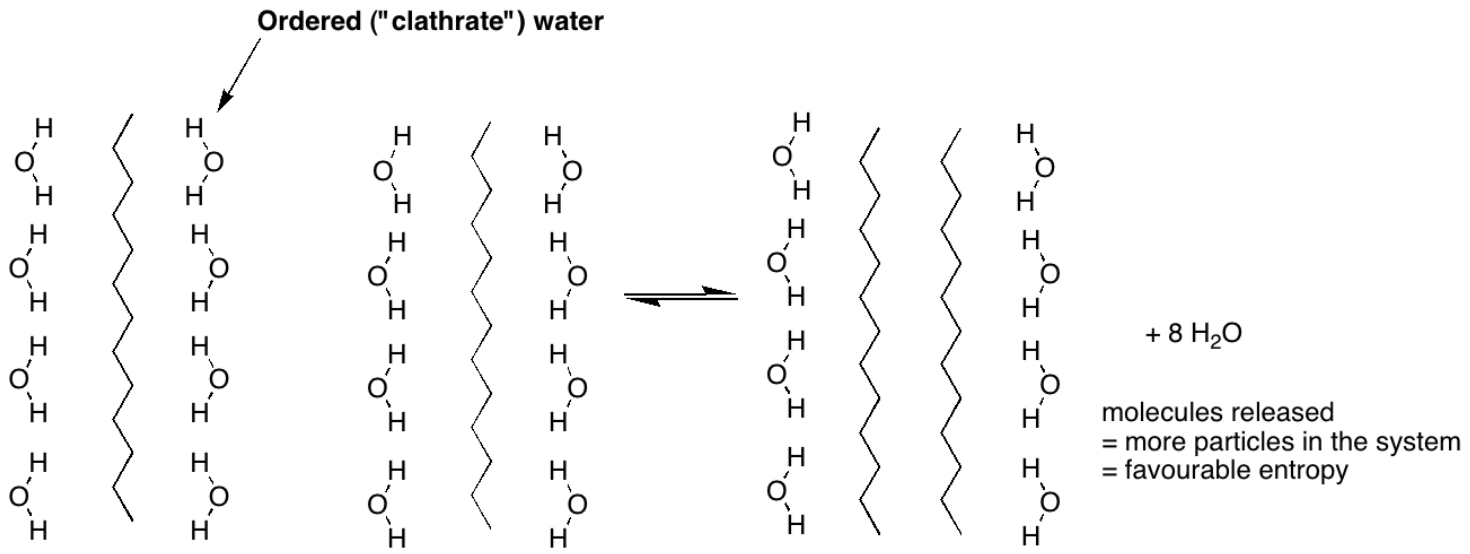


J. Am. Chem. Soc., **2010**, *132* (5), pp 1646–1653

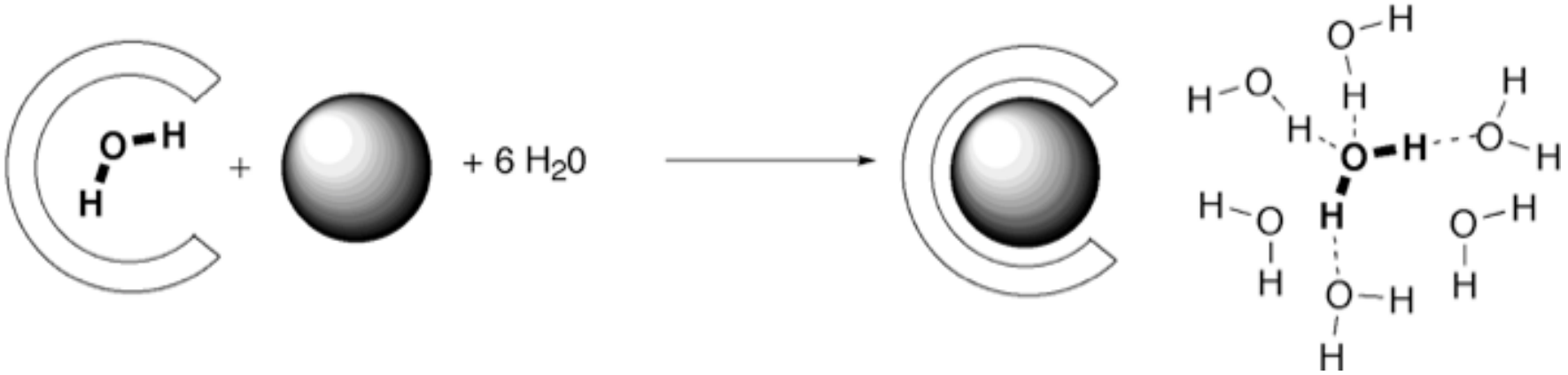
The clathrate model of the hydrophobic effect explains the entropic driving force

a methane-ice clathrate





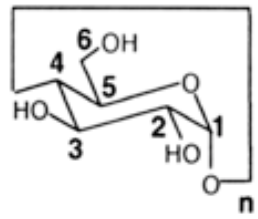
The "non-classical" model of the hydrophobic effect suggests an enthalpic driving force



Water in a small cavity can't form good H-bonds with neighbours.

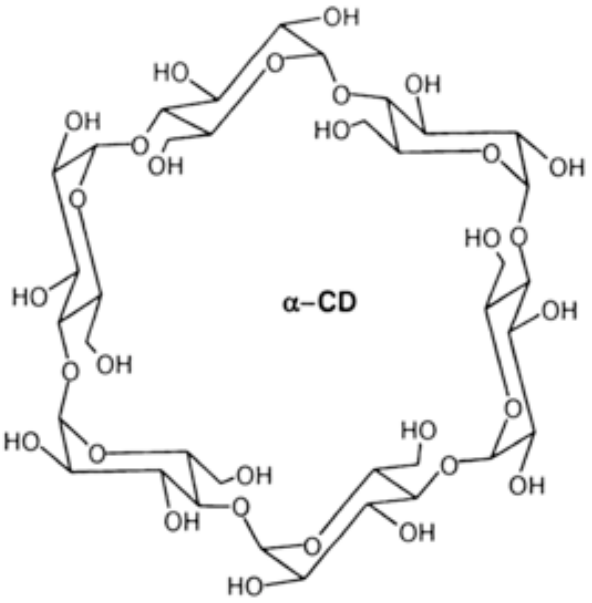
Water released to bulk solvent is free to form good H-bonds with neighbours.

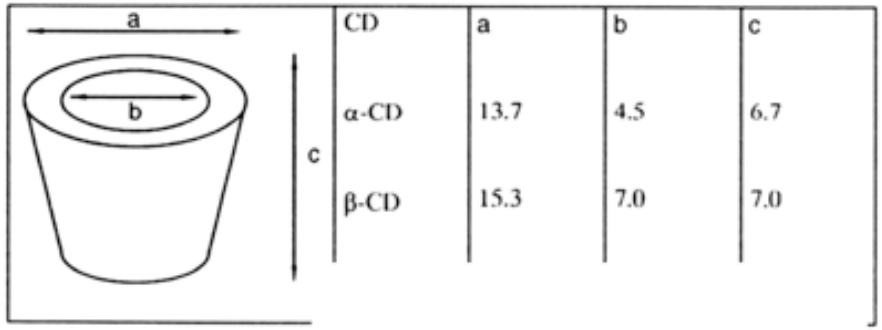
A hydrophobic binding event that's driven by ΔH



n = 6 α– CD

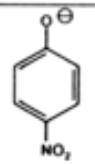
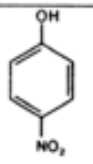
n = 7 β– CD





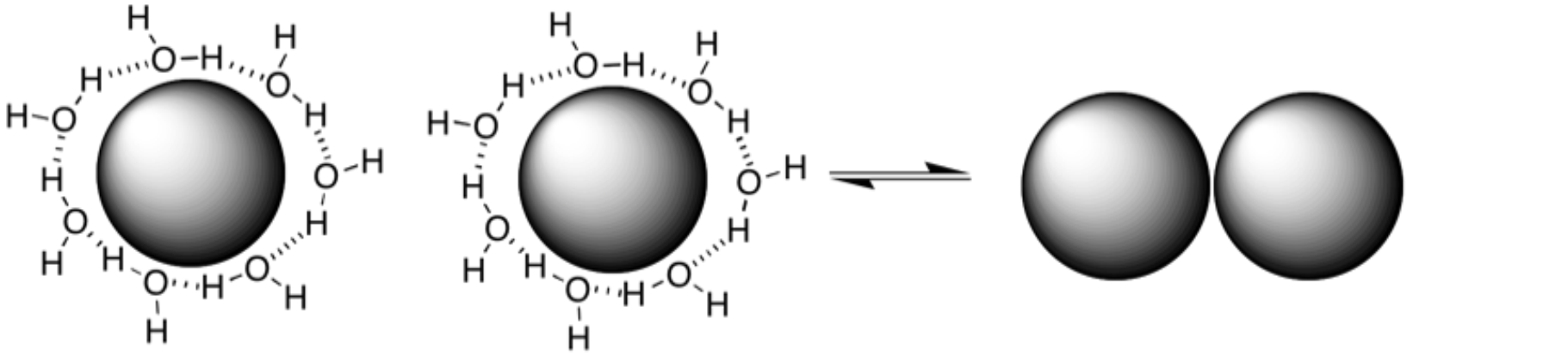
CD	a	b	c
α-CD	13.7	4.5	6.7
β-CD	15.3	7.0	7.0

Scheme B 20. α-,β-,γ-Cyclodextrins with cavity dimensions (Å).

guest:			
α-CD			
-ΔG	18.7	11.5	11.6
-ΔH	42.8	23.0	14.3
TΔS	-24.1	-11.5	-2.7
β-CD			
-ΔG	15.0	14.2	24.5
-ΔH	16.1	10.2	21.6
TΔS	-1.1	3.9	2.9

Scheme B 21. Thermodynamic data [kJ mol^{-1}] for selected cyclodextrin complexes.

Reality: interfacial water has limited H-bonds and orientations available



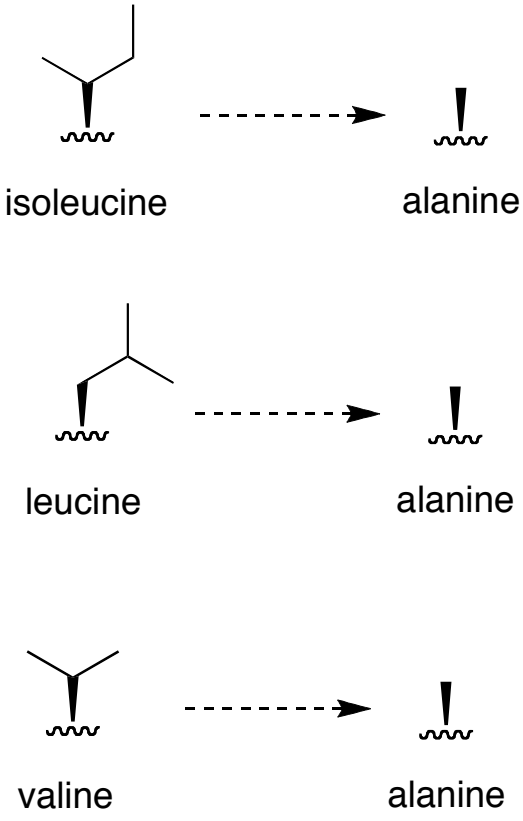
Interfacial water is limited in its possible orientations

Lower interfacial surface area means fewer water molecules are restricted

Lysozyme mutants fold more weakly when hydrophobic groups are shrunk down to Ala

Table 2. *Thermostabilities of mutant lysozymes*[a]

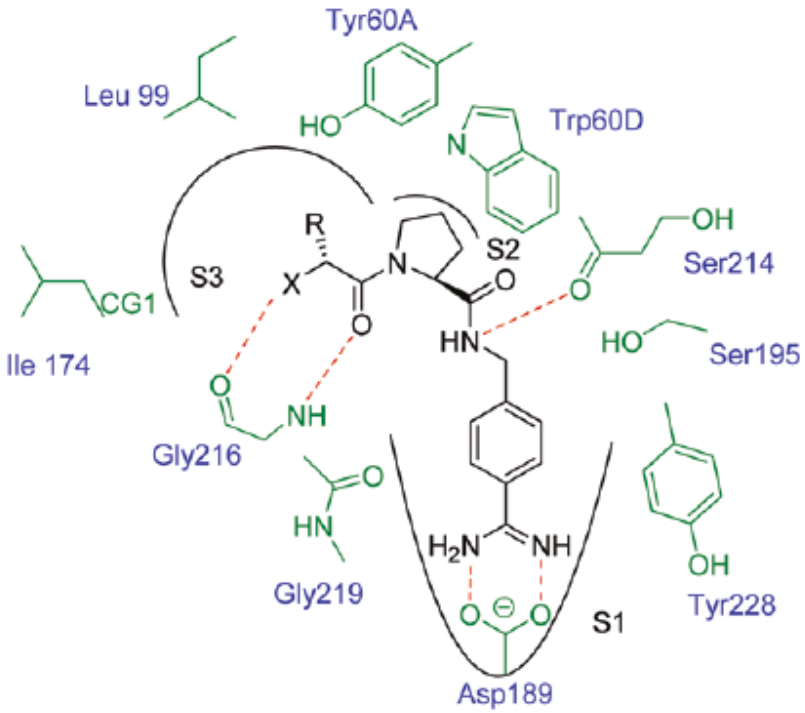
Proteins	ΔT_m (°C)	ΔH (kcal/mol)	$\Delta\Delta G$ (kcal/mol)
WT*	—	113	0
I17A	−8.4	87	−2.7
I27A	−10.1	76	−3.1
I29A	−8.2	85	−2.6
I50A	−5.8	94	−2.0
I58A	−10.4	80	−3.2
I78A	−4.7	105	−1.6
I100A	−10.7	85	−3.4
V71A	−4.7	108	−1.5
V87A	−4.9	102	−1.7
V94A	−5.0	94	−1.8
V103A	−6.6	94	−2.2
V111A	−3.7	100	−1.3
V149A	−11.0	66	−3.2
M6A	−5.7	95	−1.9
M106A	−7.1	89	−2.3
F67A	−5.7	101	−1.9
F104A	−9.7	82	−3.1
L7A	−8.1	90	−2.6
L33A	−12.3	67	−3.6
L66A	−13.4	69	−3.9
L84A	−13.4	67	−3.9
L91A	−9.7	85	−3.1



ΔΔG correlates with hydrophobic (interfacial) surface area!

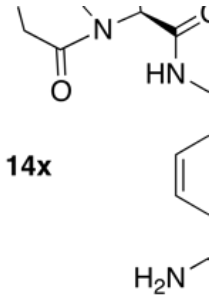
15-20 cal/mol for each Å^2

Cooperation between H-bonds and hydrophobicity (a non-linear addition of binding energies)

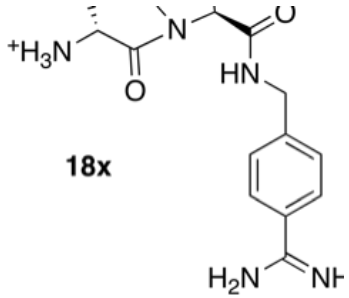


X = NH_3^+ (H-bond to Gly216)
or
X = H (no H-bond to Gly216)

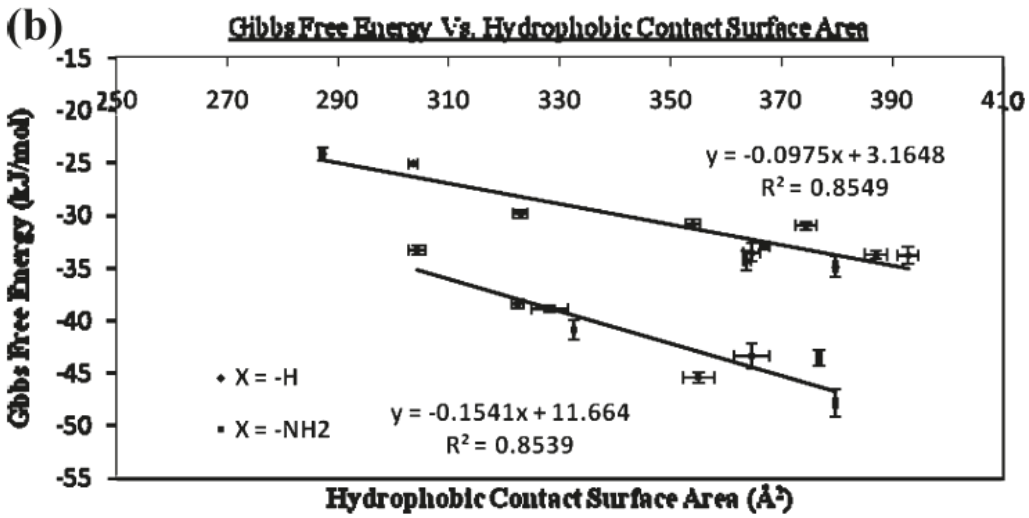
R = many sizes of hydrophobics for the S3 pocket (methyl through cyclohexyl and benzyl)



23 cal/mol for each Å^2 (hydrophobic surface area)



37 cal/mol for each Å^2



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A different way to look at a cooperativity effect (a non-linear addition of binding energies)

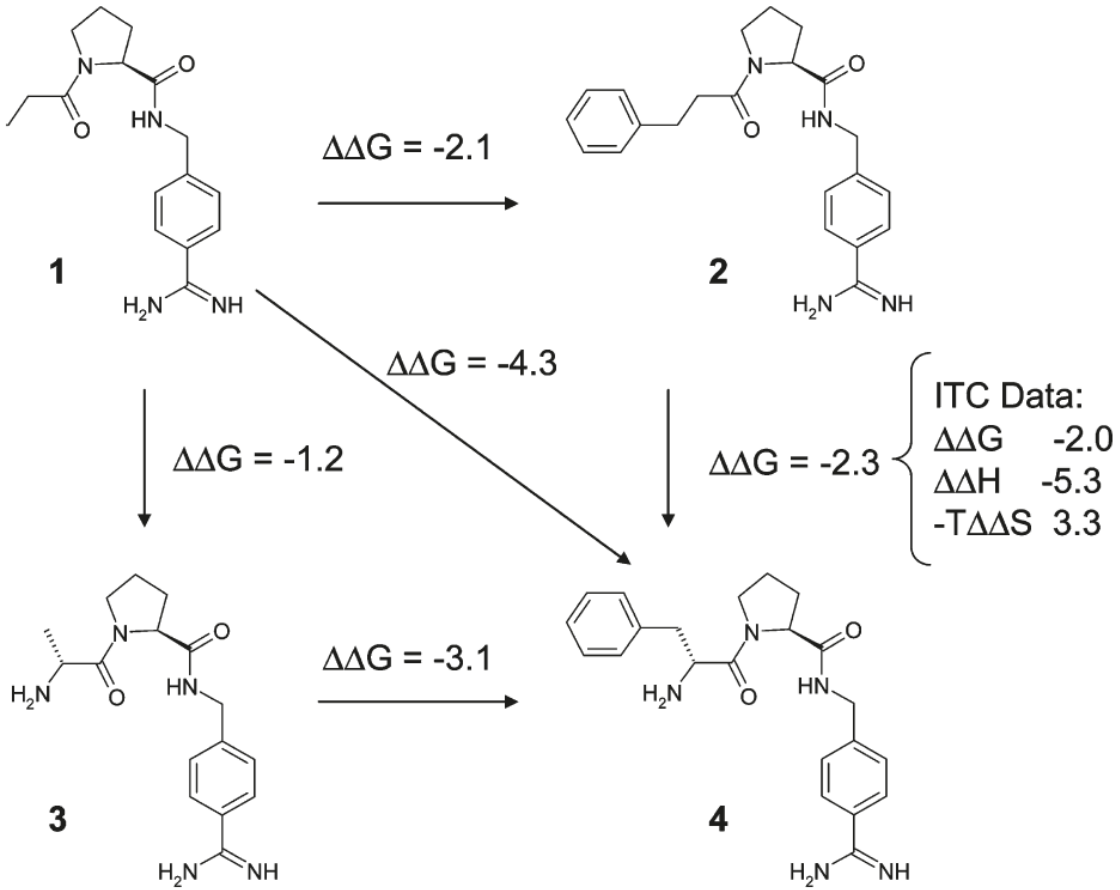

Figure 1. Cooperativity of hydrogen bond formation and hydrophobic contacts in a set of thrombin inhibitors. Extension of the lipophilic side chain alone increases affinity by 2.1 kcal/mol. Addition of the amino group increases affinity by 1.2 kcal/mol. Cooperativity therefore amounts to 4.3 − 2.1 − 1.2 = 1.0 kcal/mol. Data from refs 30 and 31 were converted to kcal/mol and rounded to 1 decimal place.

Enthalpy-entropy compensation

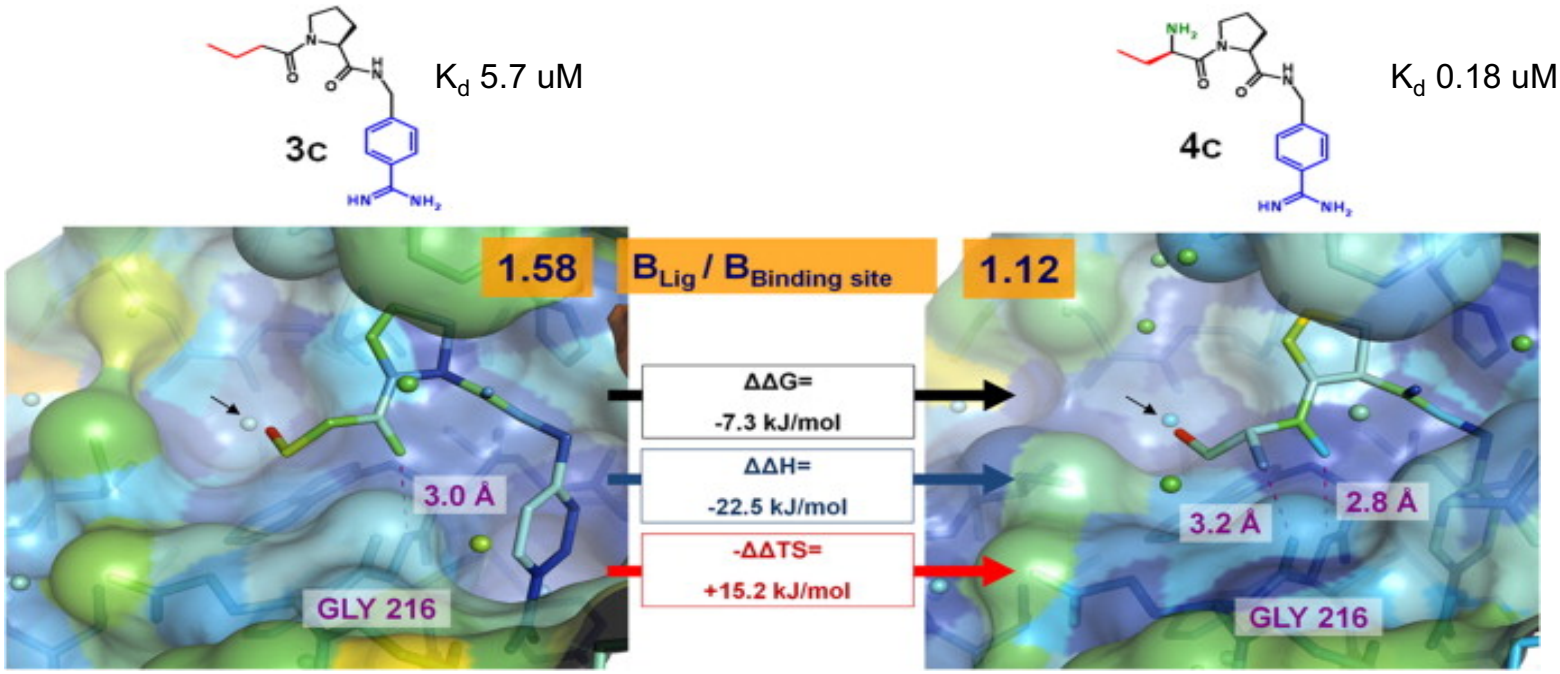

Fig. 4 The crystallographically determined binding mode of **3c** and **4c** in complex with thrombin.

Binding Thermodynamics (ITC)

(kcal/mol) **ΔG ΔH –TΔS**

3c –7.8–6.5–1.4

4c –9.6–9.2–0.3

Enthalpy-entropy compensation

As ΔH becomes more favorable, the complex binds more tightly, and entropy becomes more unfavorable.

Journal of Molecular Biology Volume 397, Issue 4 2010 1042 - 1054