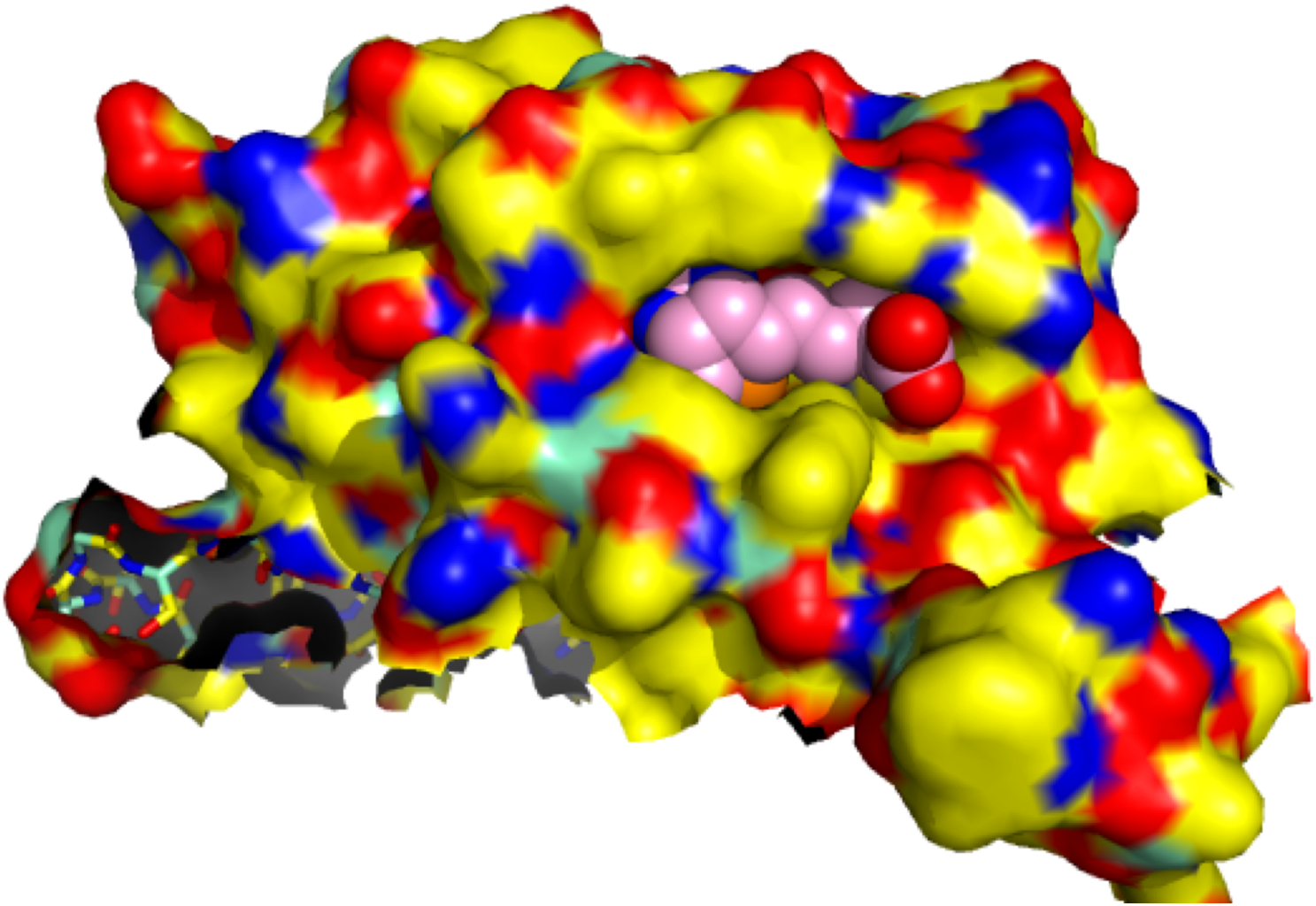
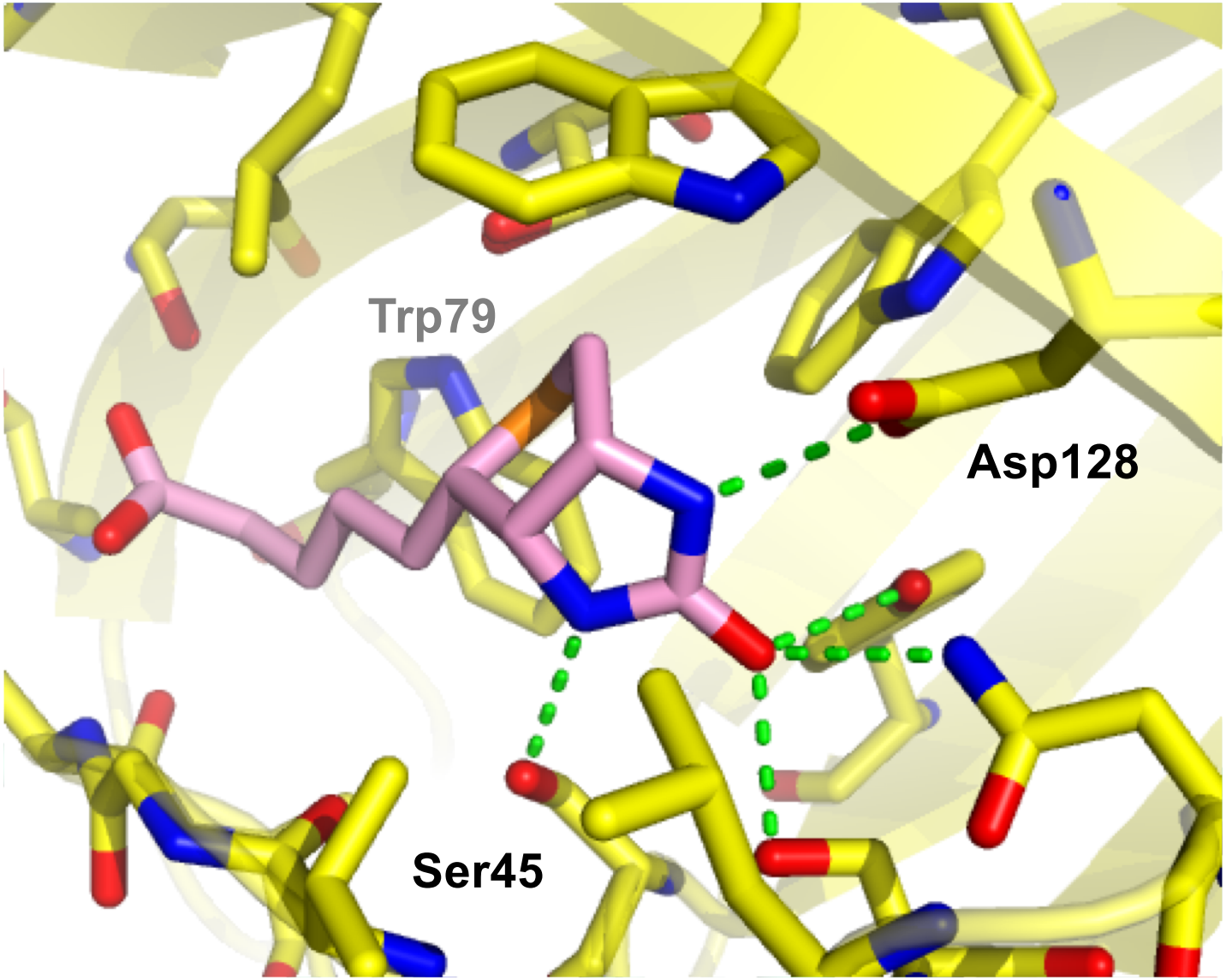
Biotin•Streptavidin

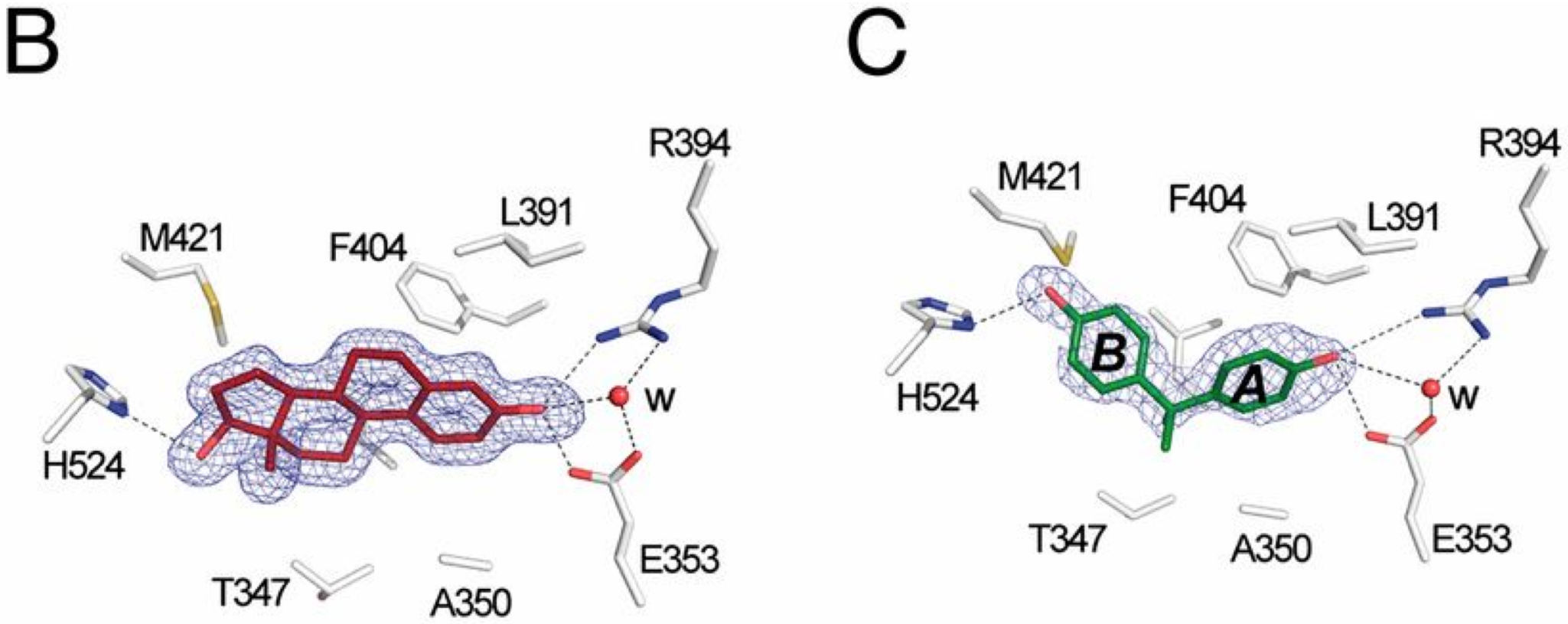


Biotin•Streptavidin



Protein Sci., **15**, 459.

Estrogen Receptor



Estradiol **Bisphenol A**

Nobels 2018! Directed Evolution of proteins

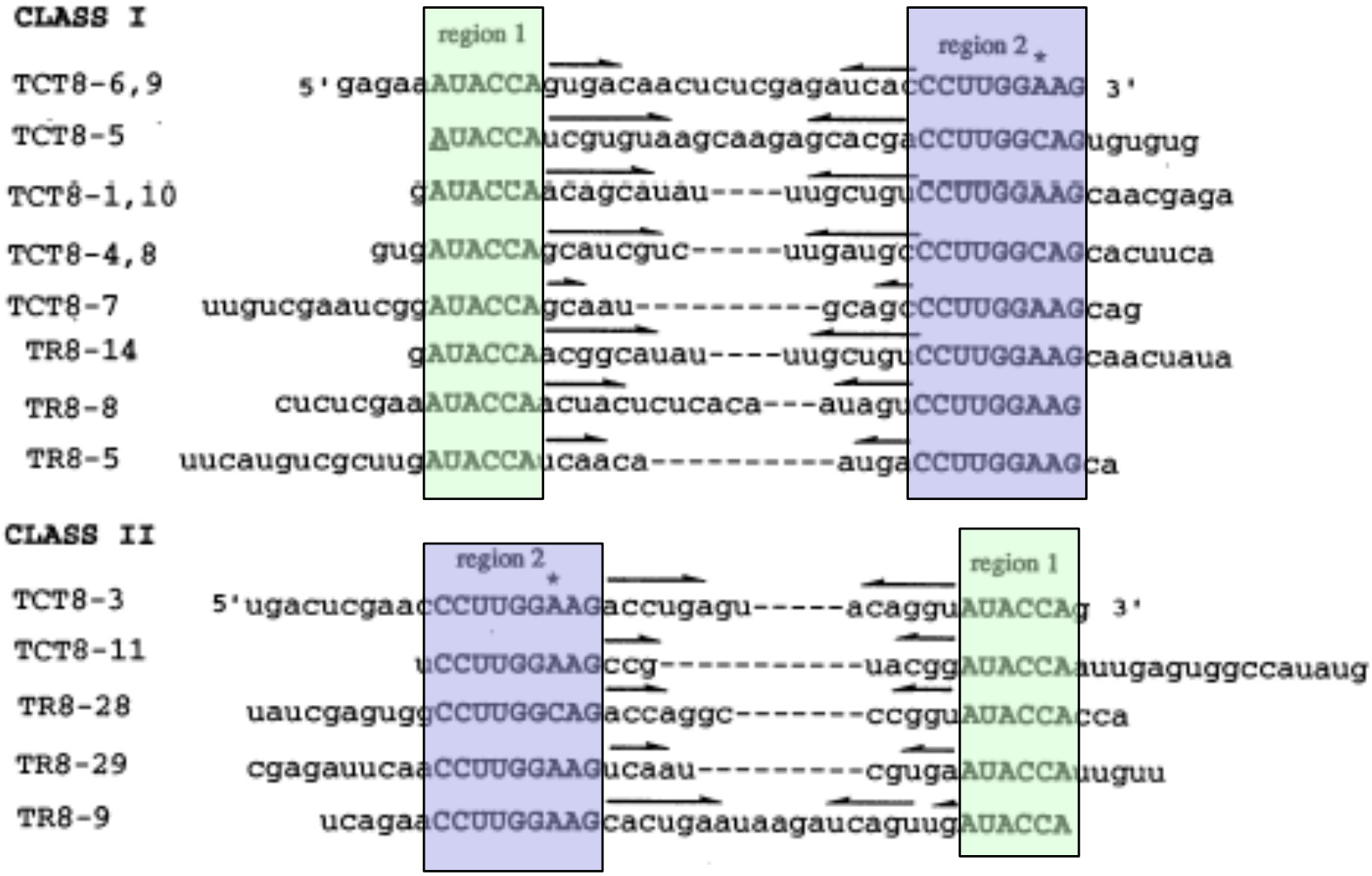
George Smith (US), Frances Arnold (US), Gregory Winter (UK)

Since 2006

- 2006 - RNA Polymerase (structure!)
- 2007 – Surface Chemistry
- 2008 – Green Fluorescent protein
- 2009 – Structure of the Ribosome (structure!)
- 2010 – Palladium coupling
- 2011 – Quasicrystals (structure! But not biochemical)
- 2012 – G-protein coupled receptors (structure!)
- 2013 - Computational modeling of proteins (kinda structure!)
- 2014 - Ultraresolution microscopy (another kinda structure!)
- 2015 – DNA repair
- 2016 – Molecular machines
- 2017 – Cryo-electron microscopy (structure!)
- 2018 – Directed Evolution

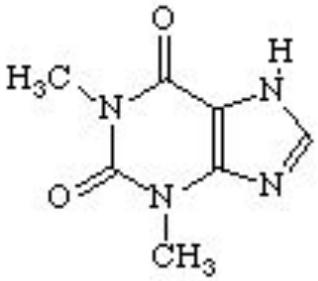
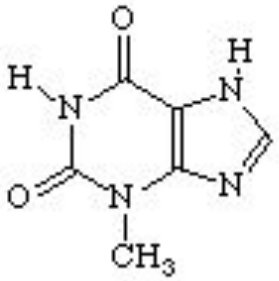
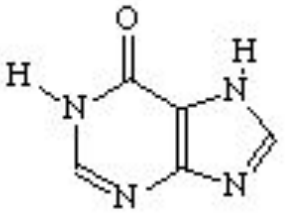
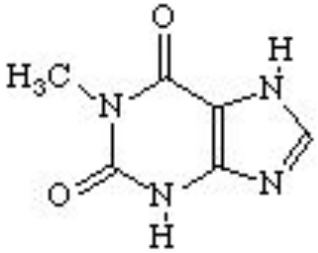
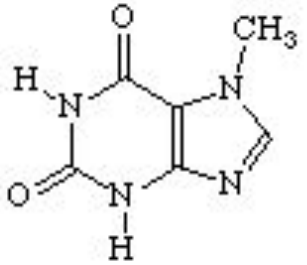
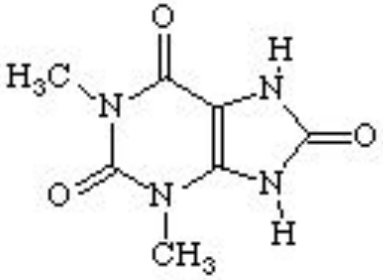
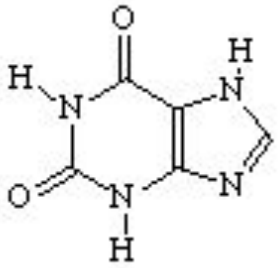
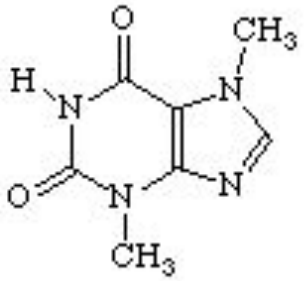
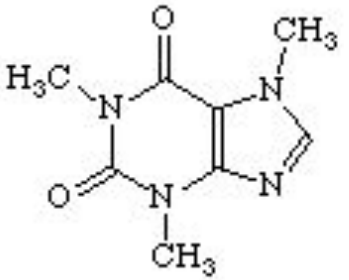
Conserved RNA Sequence:

AUACCA + CCUUGG$^{A}_{C}$AG



Properties of a Theophylline-Binding RNA Aptamer
(Jenison et al. (1994) *Science* **263**, 1425)

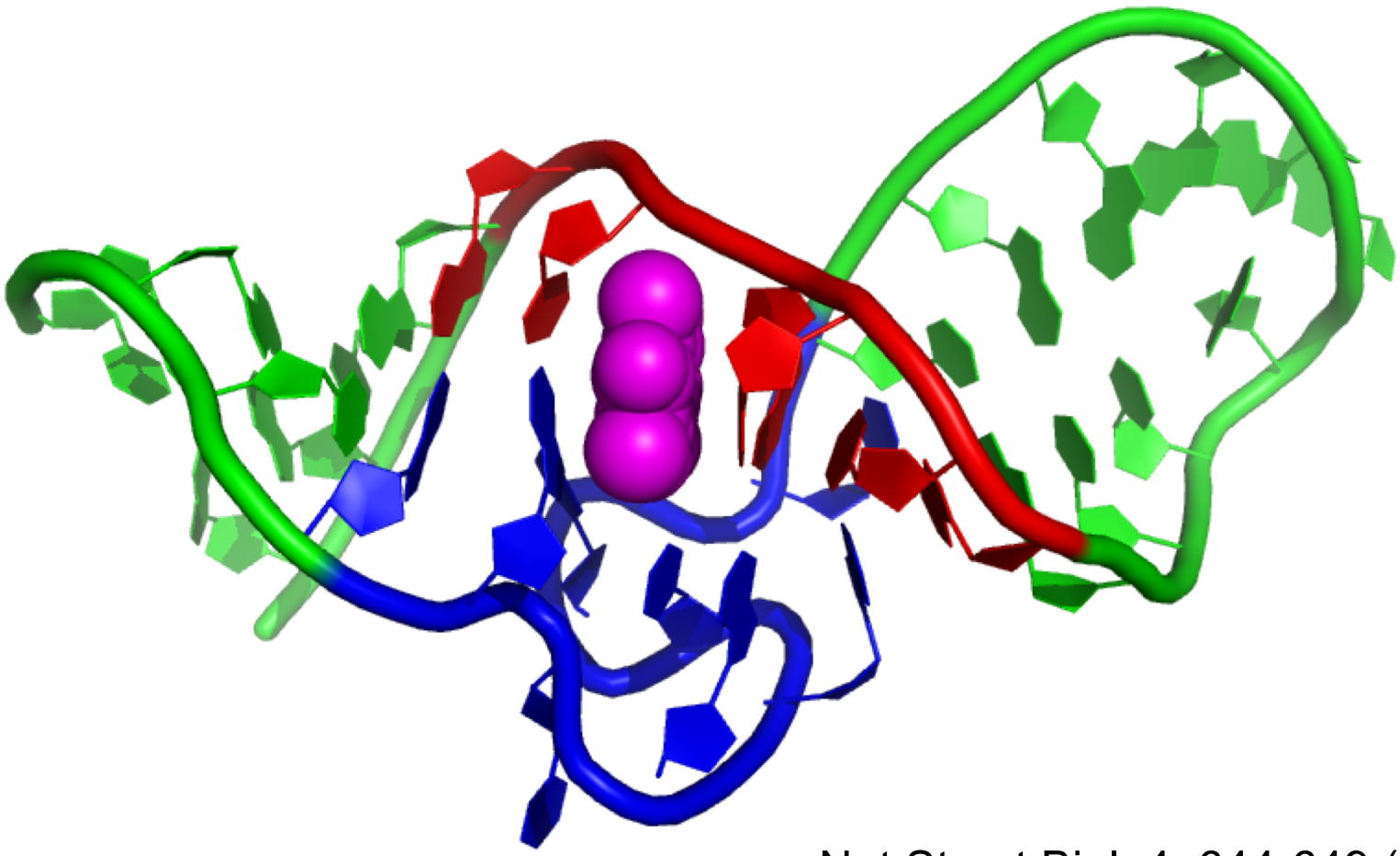
Theophylline

	K_d (μM)		K_d (μM)		K_d (μM)
	0.32		2.0		49.0
	9.0		>500		>1000
	8.5		>500		3500

Caffeine

Conserved RNA Sequence:

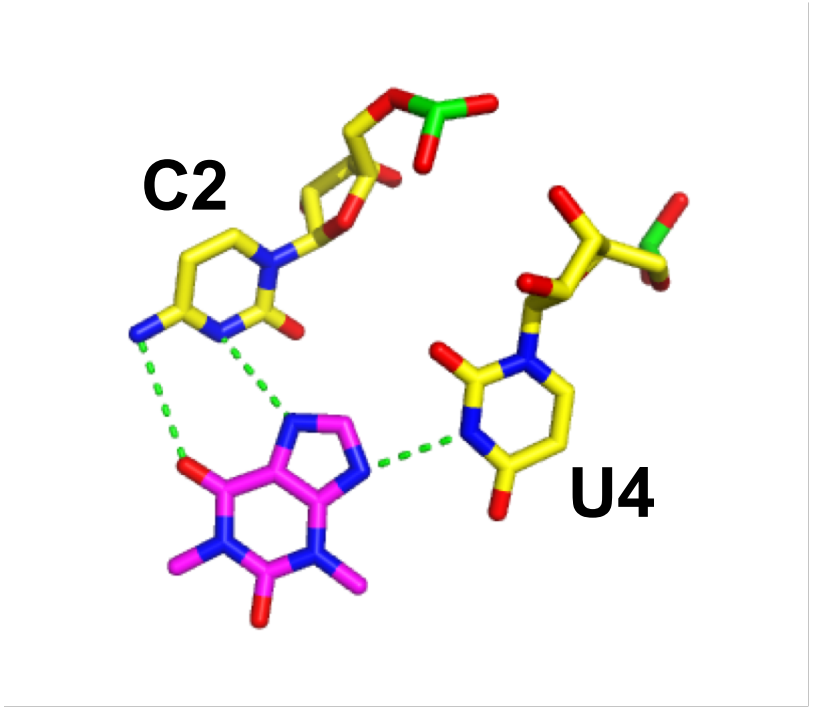
AUACCA + CCUUGG$^{A}_{C}$AG

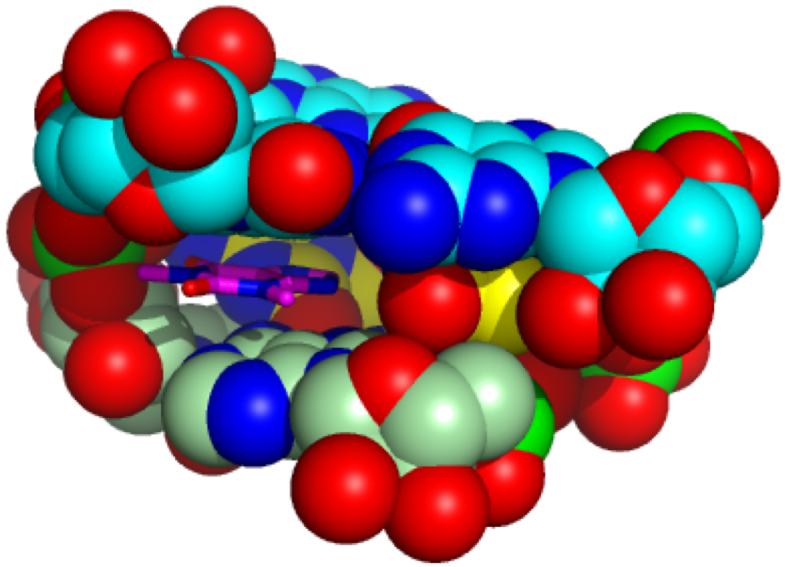


Nat.Struct.Biol. 4: 644-649 (1997)

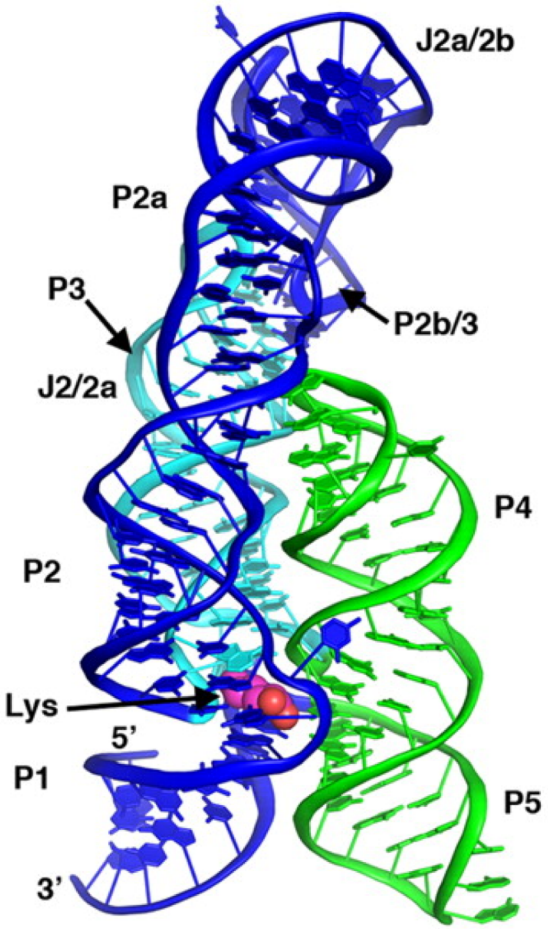
Conserved RNA Sequence:

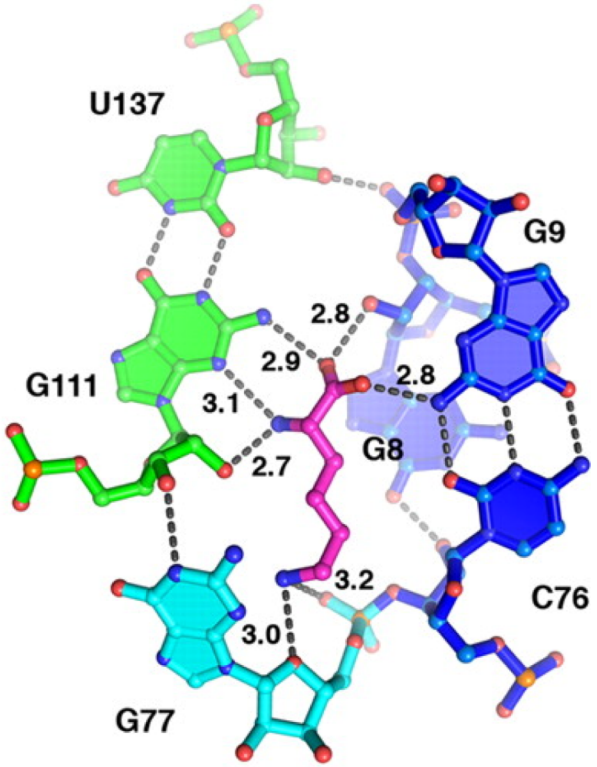
AUACCA + CCUUGG$^{A}_{C}$AG

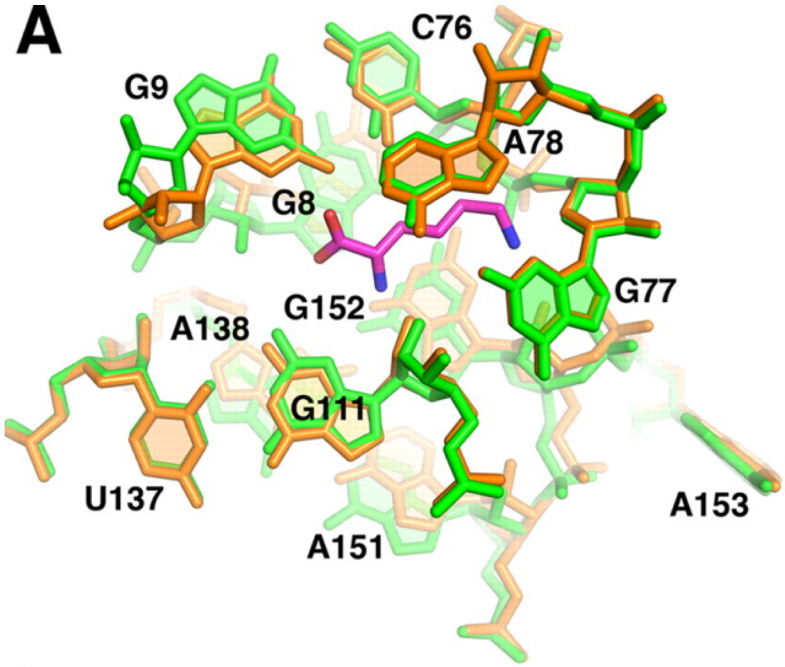




Lysine Riboswitch



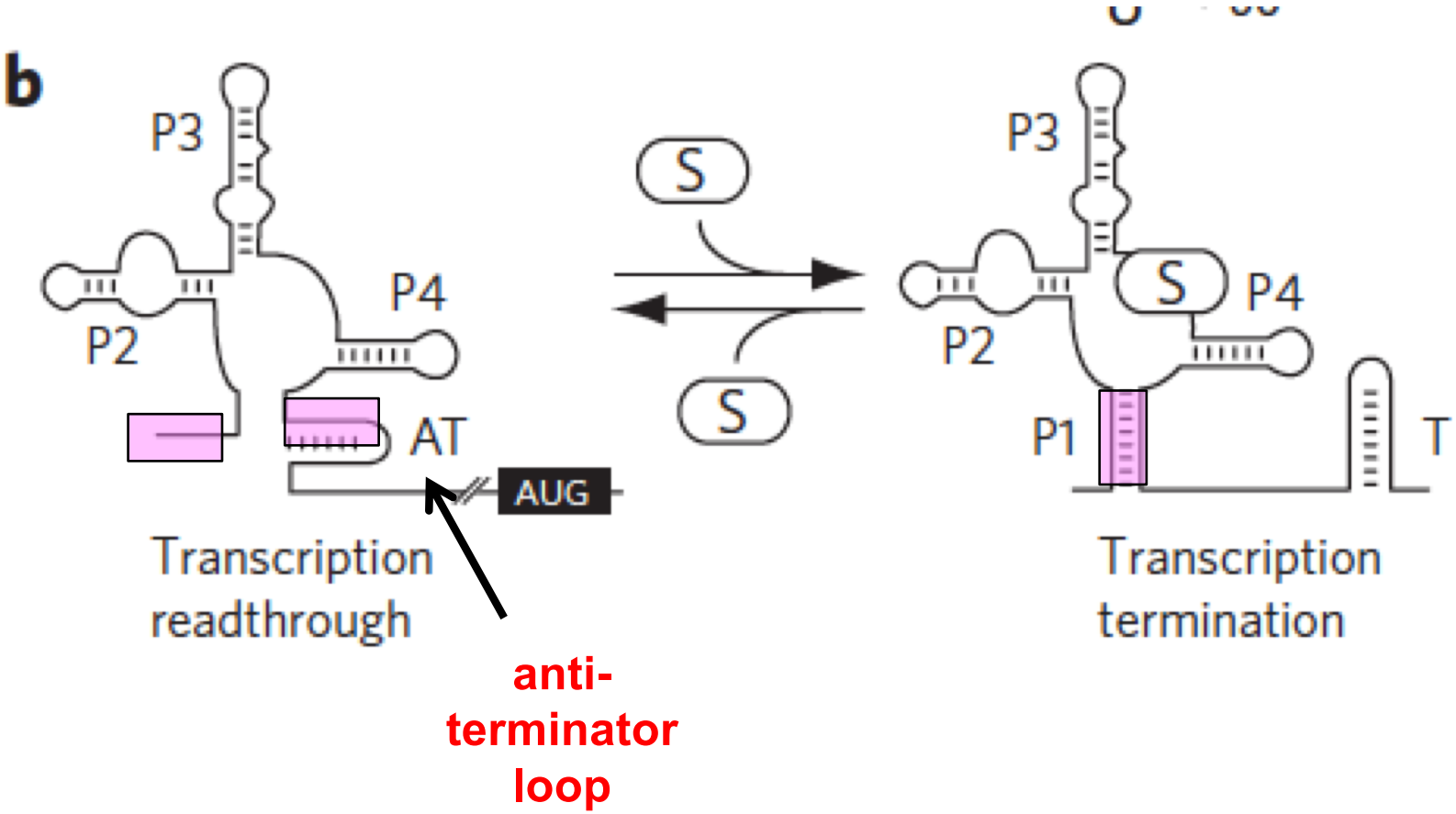




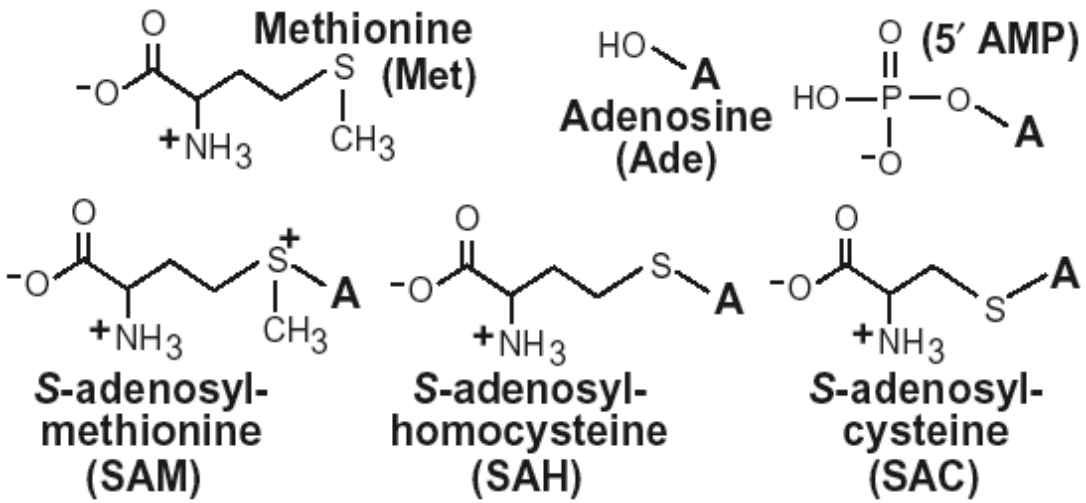
Green = with **Lys**
Gold = no Lys

Journal of Biological Chemistry, 283, 22347-22351 (2008).

SAM Riboswitch Transition



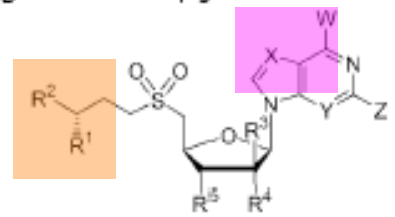
S Box Riboswitch is Specific



	K_d (nM)
SAM	~4
SAH	~400
SAC	~30,000
5′ AMP	>3,000,000
Met	>3,000,000

Need adenine & alpha carbon

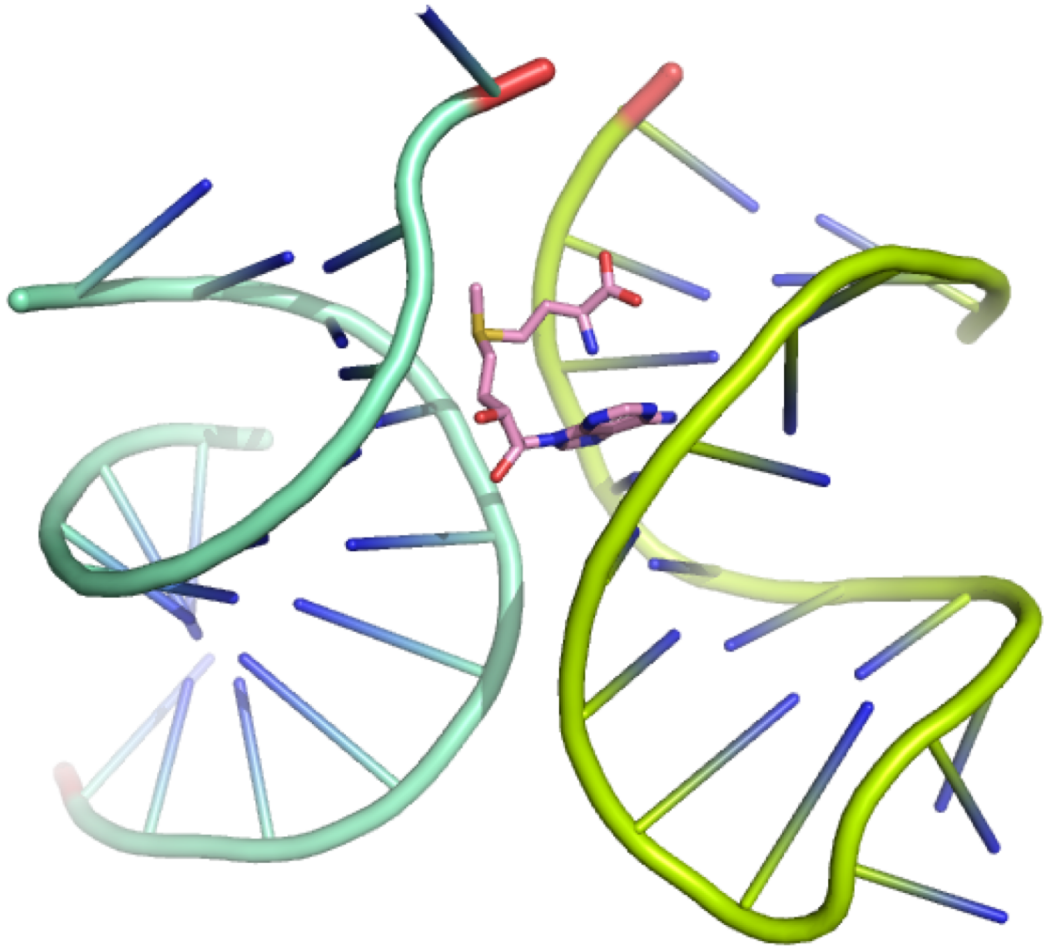
Table 1: Dissociation constant (K_d) values for SAM analogues with the *yitJ* SAM-I riboswitch aptamer from *B. subtilis*.



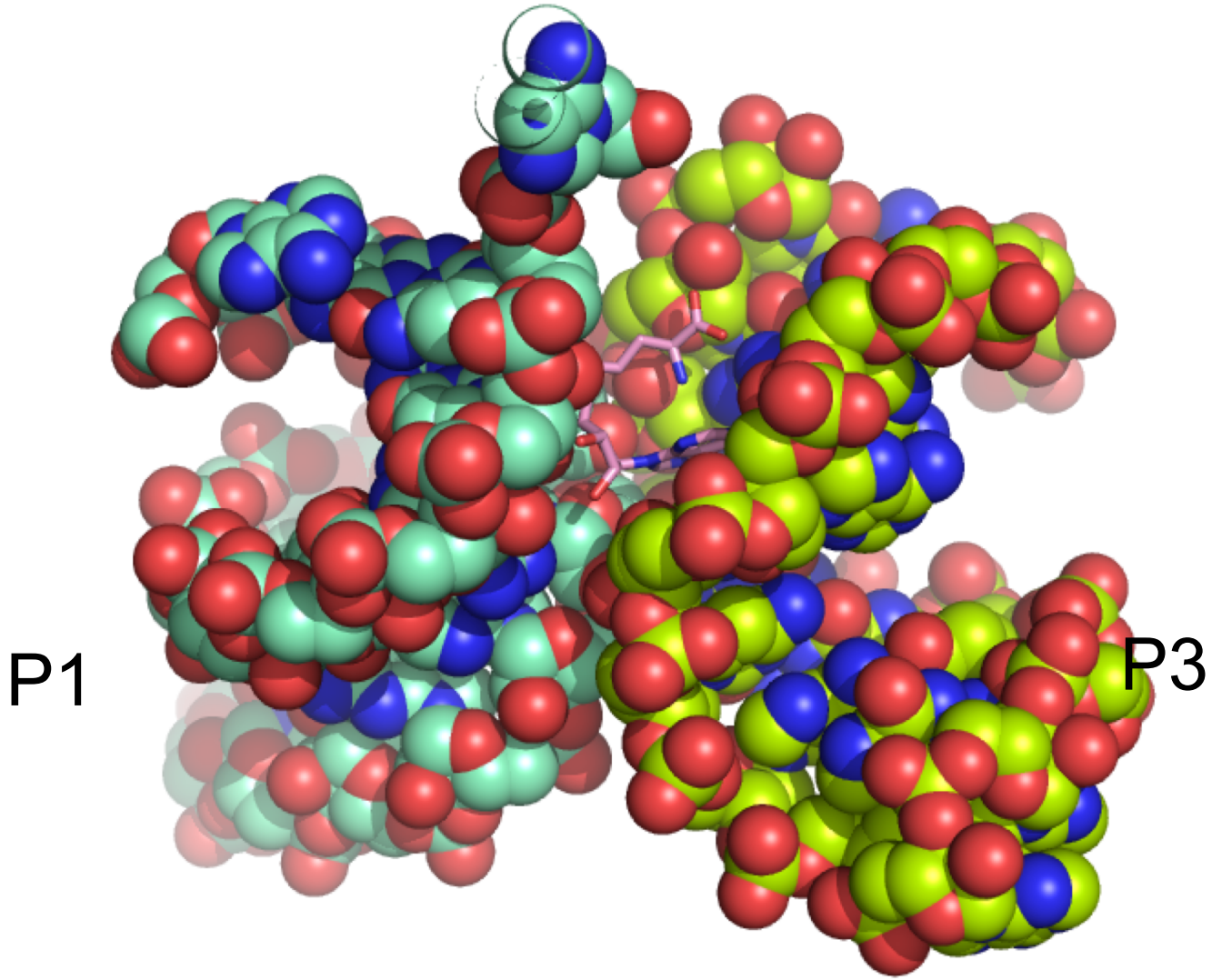
	R^1	R^2	R^3	R^4	R^5	W	X	Y	Z	K_d [μM]
4	NH_2	CO_2H	H	OH	OH	NH_2	N	N	H	3×10^{-2}
8	H	CH_2OH	H	OH	OH	NH_2	N	N	H	>1000
9	H	CO_2H	H	OH	OH	NH_2	N	N	H	>1000
10	NH_2	H	H	OH	OH	NH_2	N	N	H	>1000
11	NH_2	CO_2H	OH	H	OH	NH_2	N	N	H	40
12	NH_2	CO_2H	H	H	OH	NH_2	N	N	H	30
13	NH_2	CO_2H	H	OH	H	NH_2	N	N	H	5
14	NH_2	CO_2H	H	OH	OH	H	N	N	H	>1000
15	NH_2	CO_2H	H	OH	OH	OH	N	N	H	>1000
16	NH_2	CO_2H	H	OH	OH	NH_2	CH	N	H	>1000
17	NH_2	CO_2H	H	OH	OH	NH_2	N	CH	H	3
18	NH_2	CO_2H	H	OH	OH	NH_2	N	N	NH_2	2

Angew. Chem. Int. Ed. **2006**, *45*, 964 –968

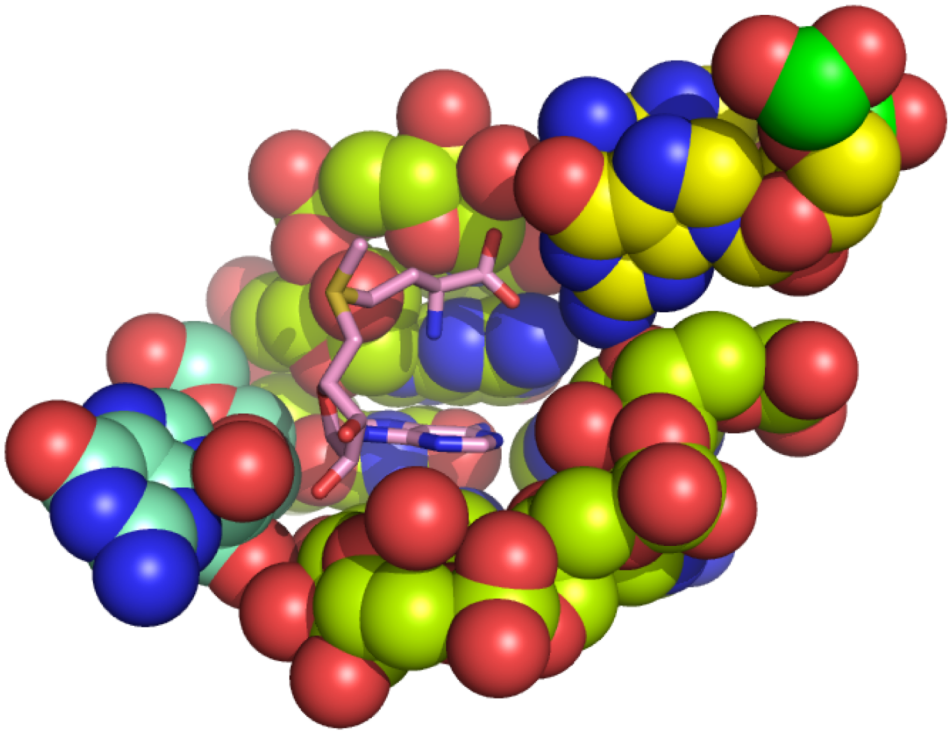
P1 & P3 Form Pocket

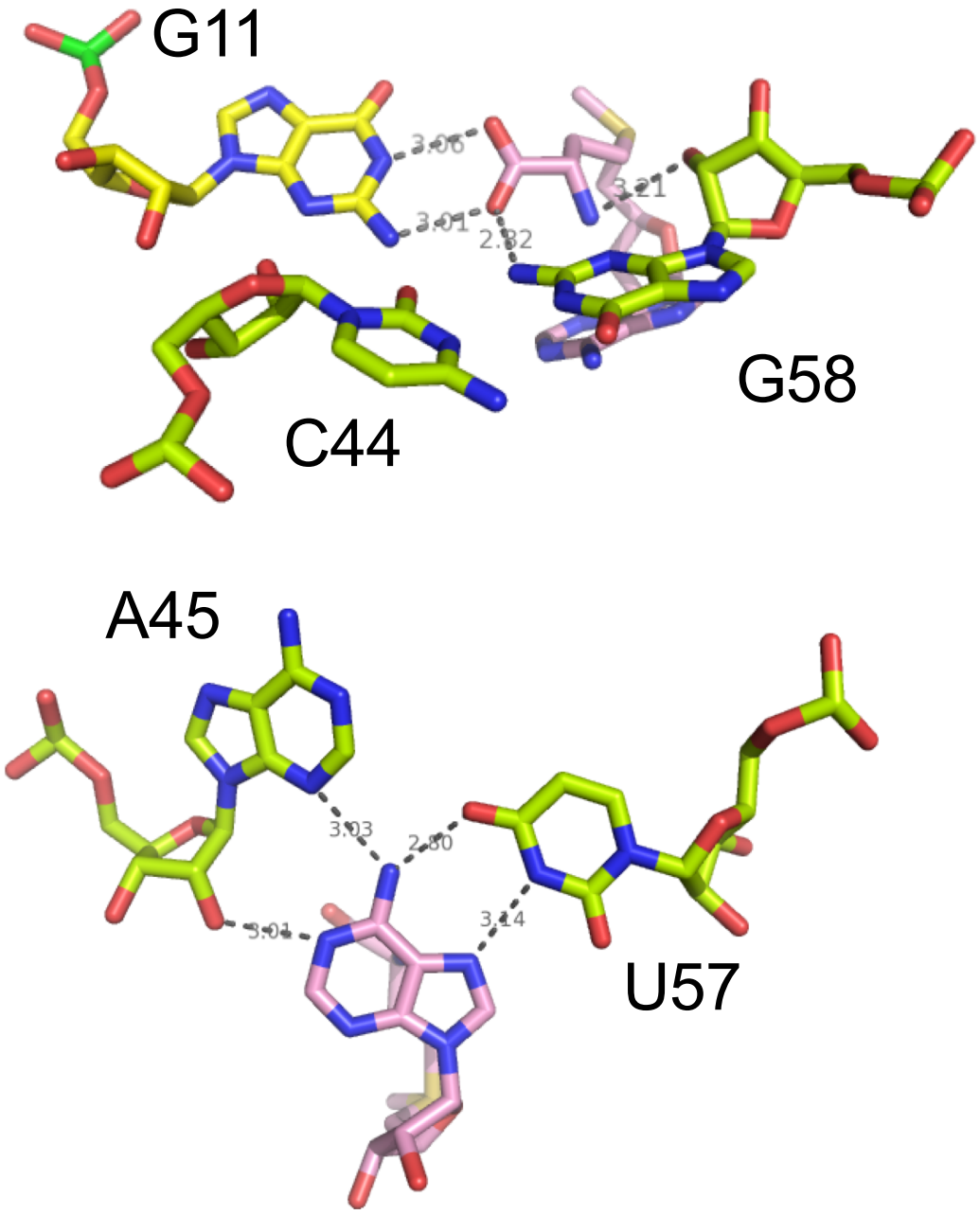


P1 & P3 Form Pocket

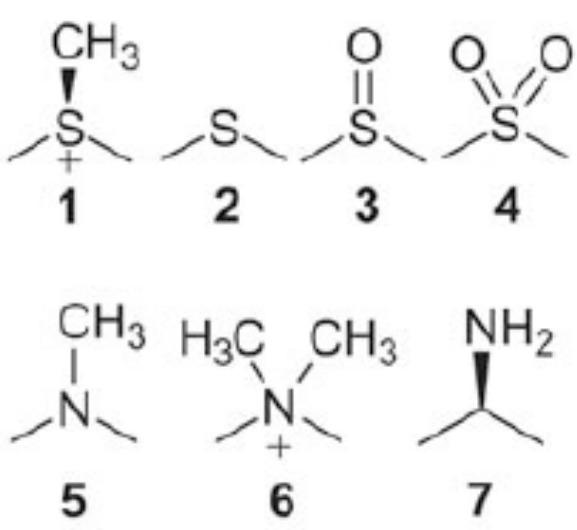


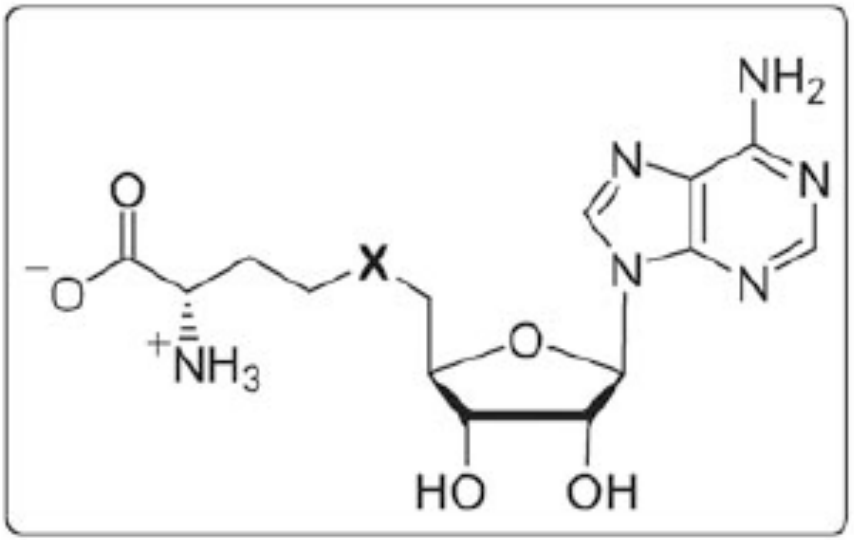
Base and α-Carbon Contacts





Specificity for Positive Sulfur





	SAM-I	
	K_d [mM]	$K_d/K_{d(SAM)}$ [a]
1	0.005	1
2	0.4	80
3	0.1	20
4	0.03	6
5	0.2	40
6	0.004	0.8
7	0.9	180

Angew. Chem. Int. Ed. **2006**, *45*, 964 –968

Sulfonium binds in minor groove of P1