Last Day(s) on RNA Engineering!

Life cycle of RNA

Logic gates

Modularity & Composition

Acuator Domains

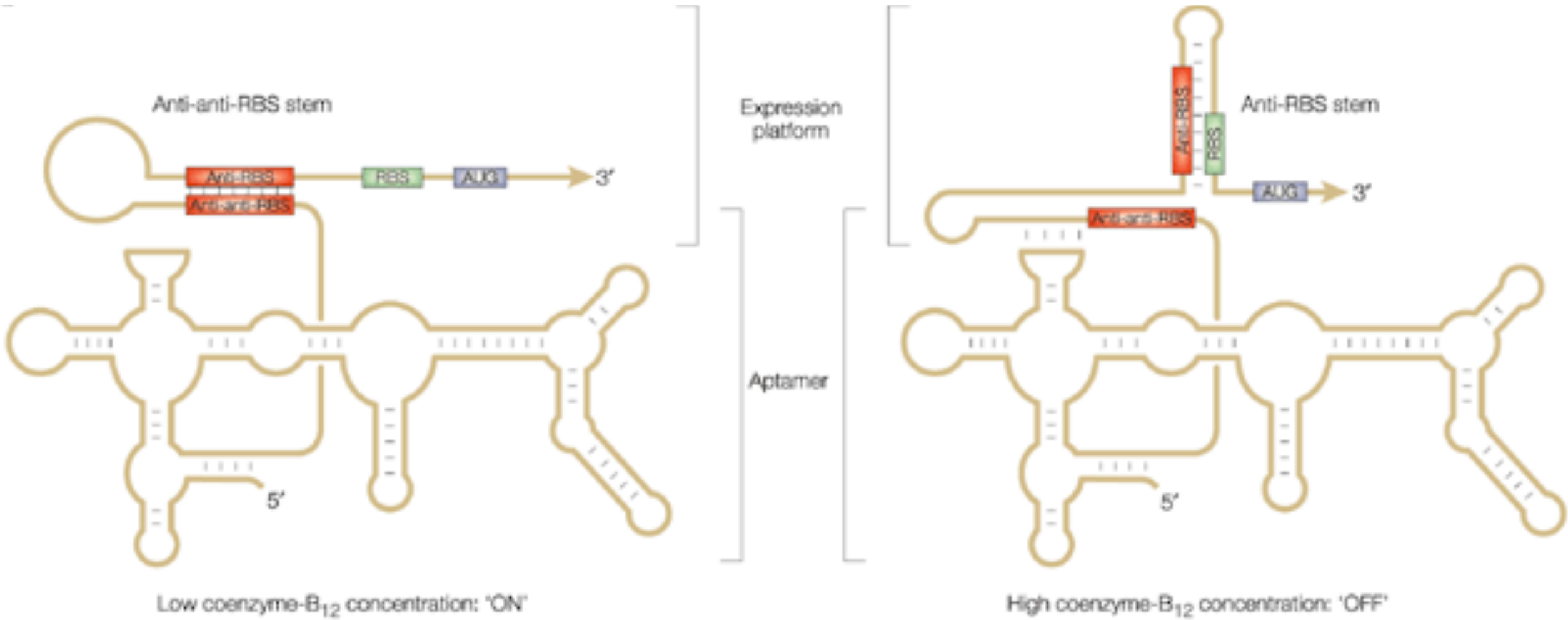
Transmitter / Computation Domains

Today

Natural versus Engineered Riboswitches

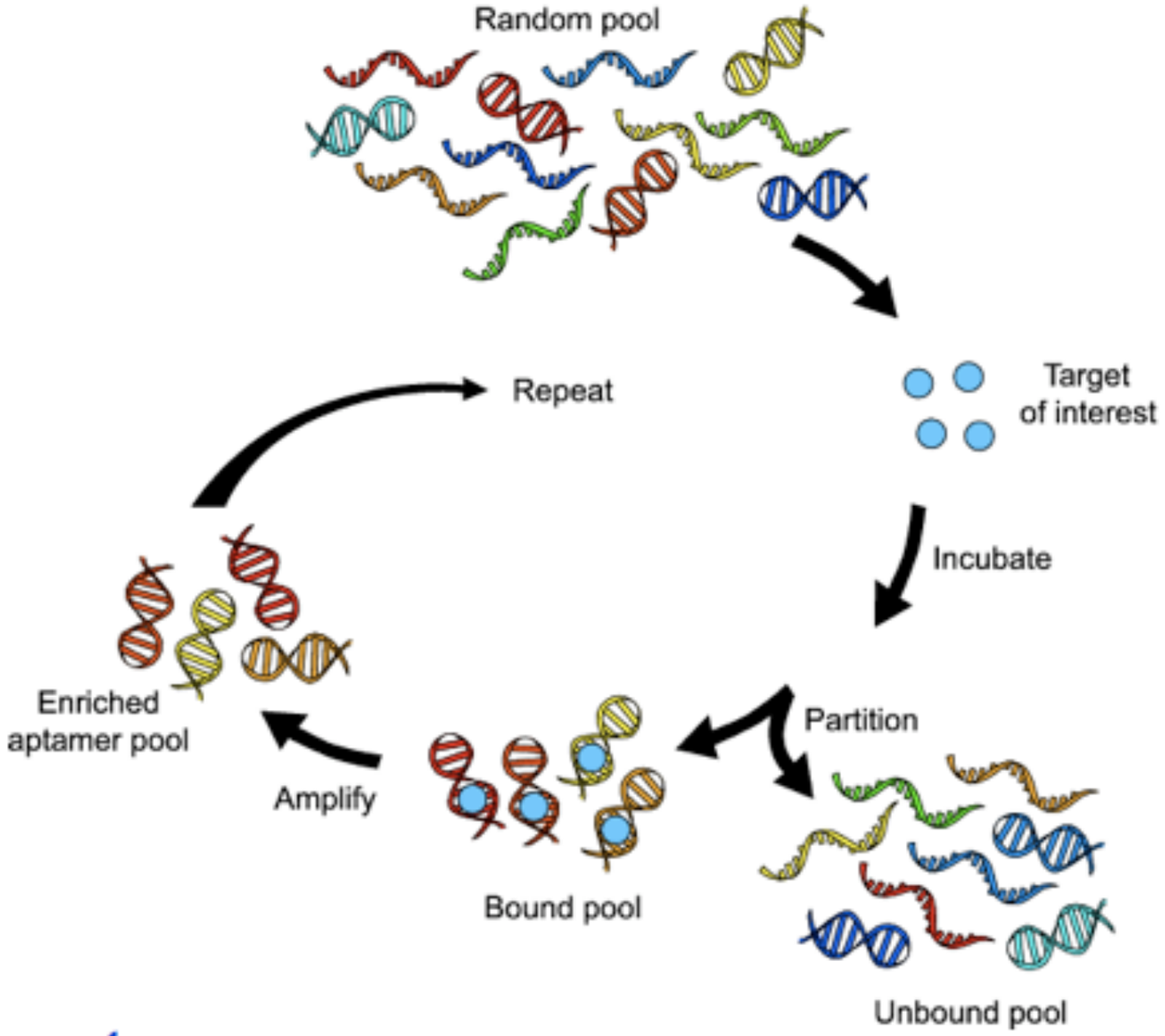
Aptamers / Sensors
(how to make?)
(how do they work?)

Natural coenzyme-B12 riboswitch



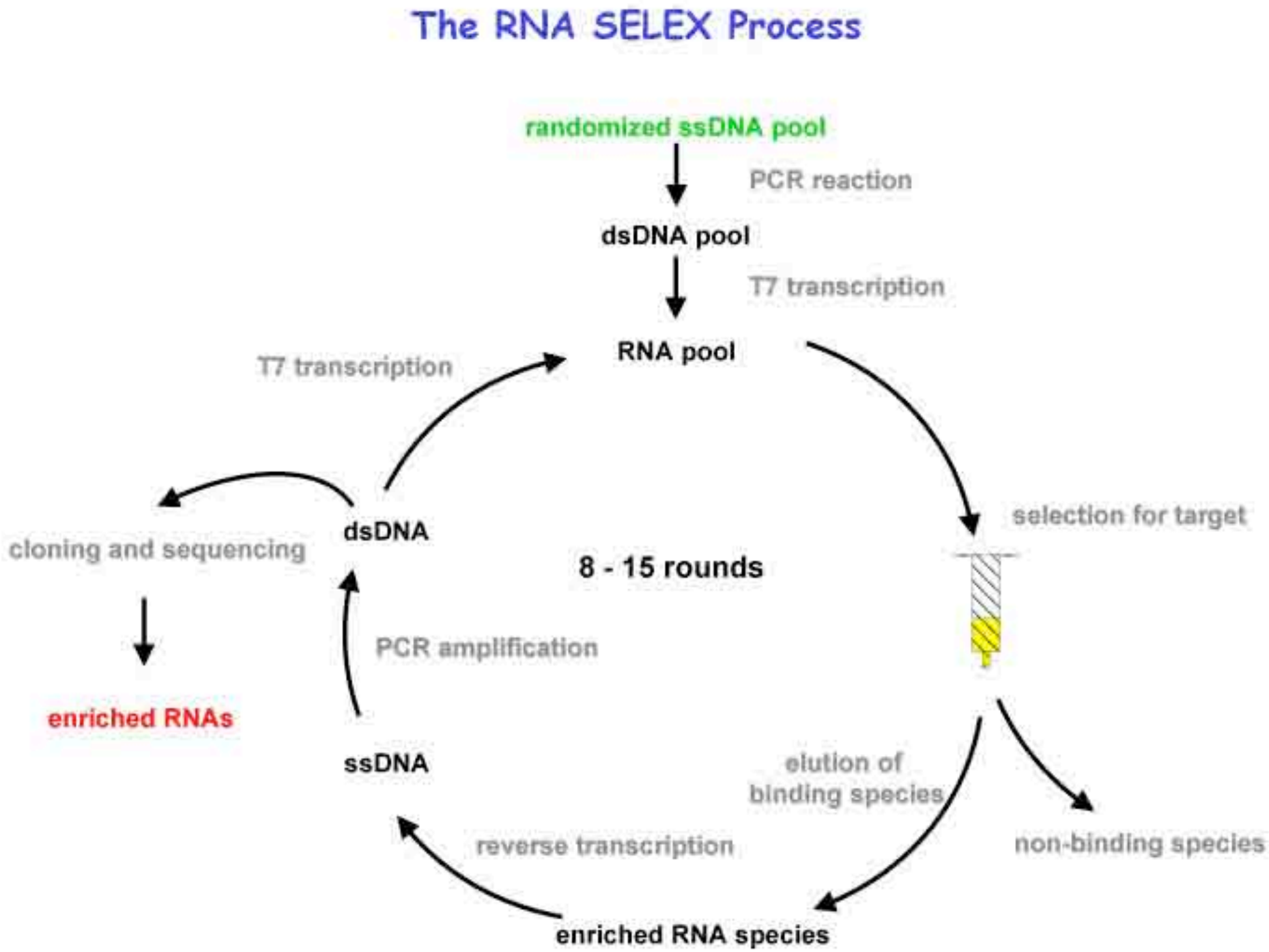
Mandal M, Breaker RR. 2004. *Nat Rev Mol Cell Biol.* 5: 451-63

How do we make the aptamer (sensors)?



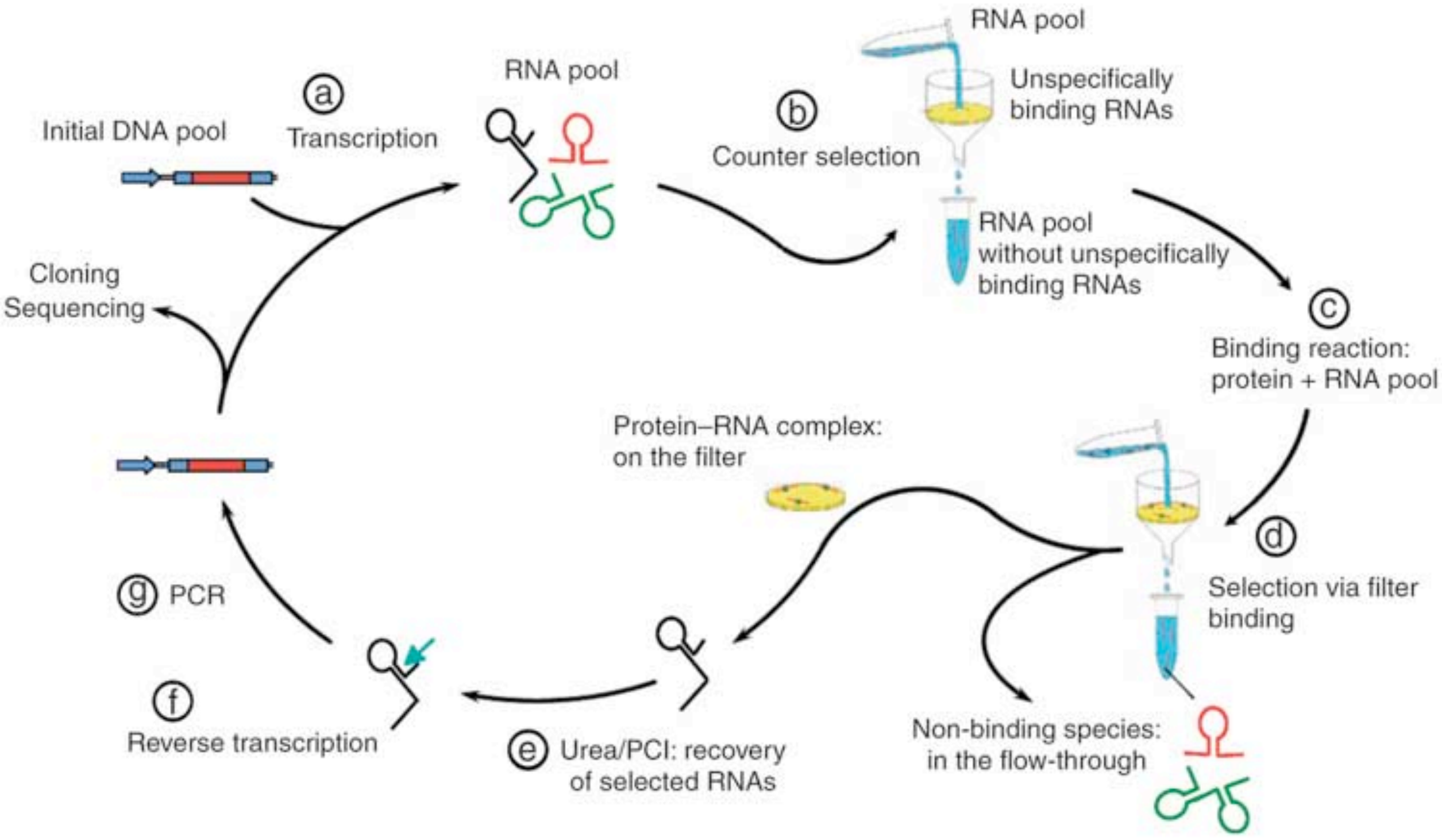
aptamer selection scheme

Systematic Evolution of Ligands by Exponential Enrichment (SELEX)



http://www.molmed.uni-luebeck.de/T.%20Restle/Bilder/SELEX.jpg

Systematic Evolution of Ligands by Exponential Enrichment (SELEX)



http://www.nature.com/nprot/journal/v1/n5/fig_tab/nprot.2006.372_F1.html

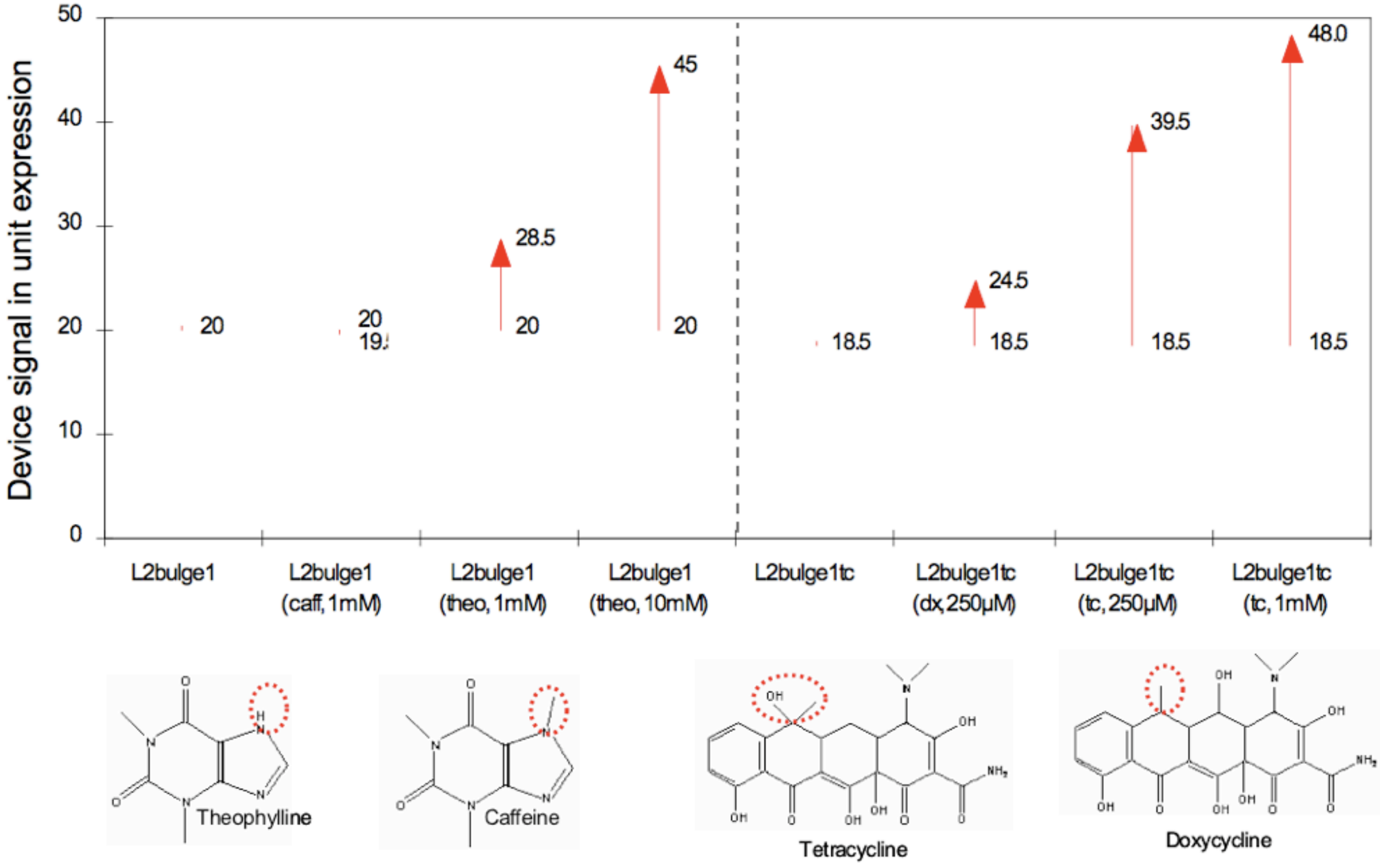
Table 1. Nucleic acid aptamers for which three-dimensional structures have been determined. ND, not determined.

Ligand	Nucleic acid*	Affinity K_d [μM]	3D structure†
Theophylline	RNA (*4*)	~0.3	NMR, 1EHT (*5*)
FMN	RNA (*6*)	~0.5	NMR, 1FMN (*7*)
AMP	DNA (*9*)	~6	NMR, 1AW4 (*12*)
	RNA (*8*)	~10	NMR, 1AM0, 1RAW (*10*, *11*)
Arginine	2 DNA (*15*)	~125	NMR, 1OLD, 2ARG (*18*, *20*)
	RNA (*16*)	~60	NMR, 1KOC (*19*)
Citrulline	RNA (*16*)	~65	NMR, 1KOD (*19*)
Tobramycin	2 RNA (*25*)	~ 0.009	NMR, 1TOB (*32*)
		~ 0.012	NMR, 2TOB (*33*)
Neomycin B	RNA (*26*)	~ 0.115	NMR, 1NEM (*34*)
HIV-1 Rev peptide	2 RNA (*40*)	~ 0.004	NMR, 1ULL, 484D (*41*, *42*)
HTLV-1 Rex peptide	RNA (*43*)	~ 0.025	NMR, 1C4J (*44*)
MS2 coat protein	3 RNA (*45*)	ND	X-ray, 5-7MSF (*45*, *46*)
Thrombin	DNA (*47*)	~ 0.025	NMR, 148D (*38*); x-ray, 1HAO (*39*)

*The number of different sequences that have been studied is indicated. †The structure determination method (e.g., NMR, nuclear magnetic resonance) and the Protein Data Bank entry for the atomic coordinates are given.

Adaptive recognition by nucleic acid aptamers. Science. 2000 Feb 4;287(5454):820-5. Hermann T, Patel DJ.

Impressively, aptamers can be selected for specificity!



c/o Smolke

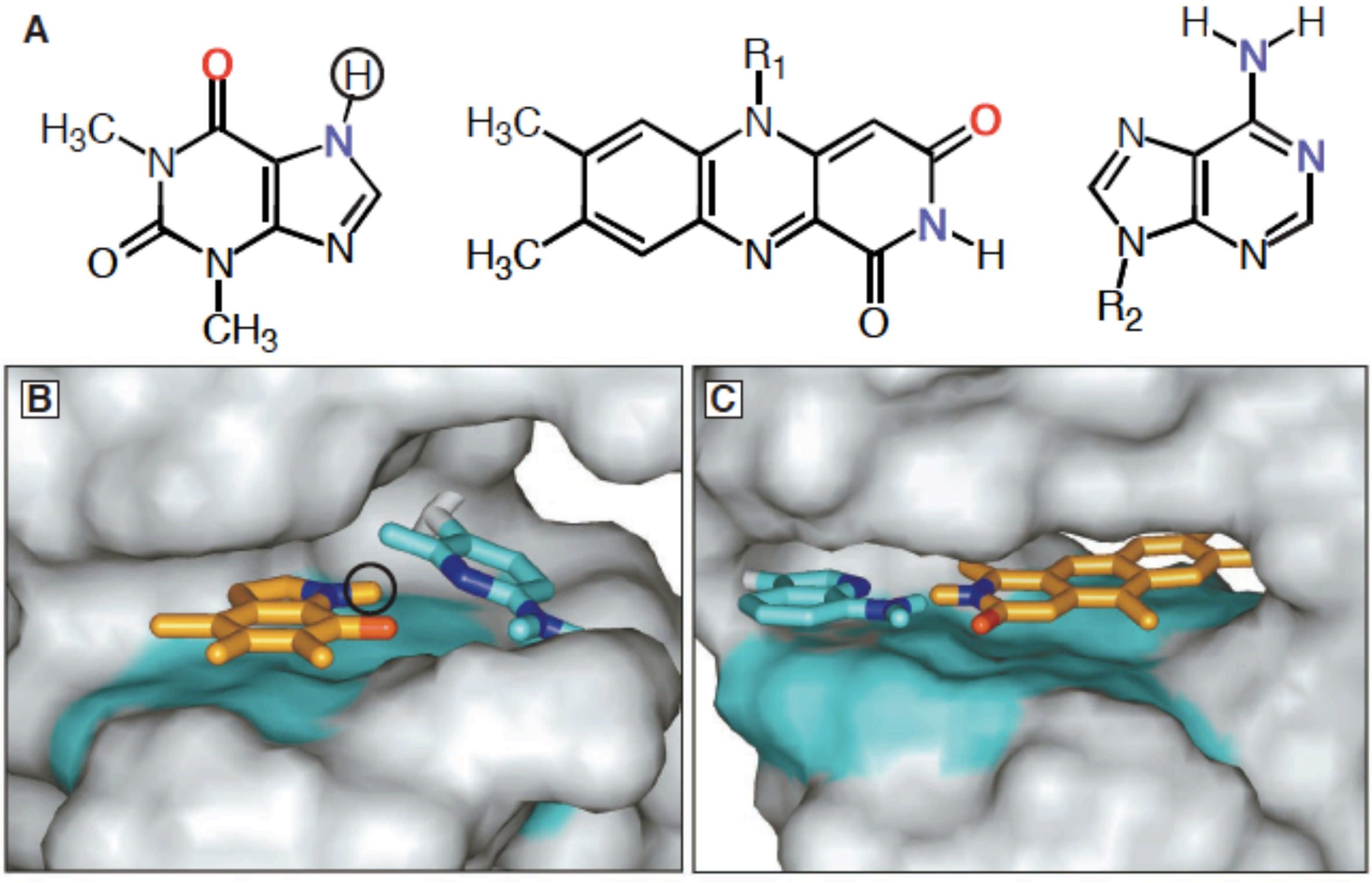


Fig. 1. Molecular recognition of flat aromatic ligands by nucleic acid aptamers. (**A**) (left to right) Theophylline (in caffeine, the encircled hydrogen is replaced by a methyl group); flavin mononucleotide (FMN), an isoalloxazine derivative; and adenosine monophosphate (AMP). The ligand-binding pockets are shown for the complexes of (**B**) a theophylline-RNA aptamer (*5*), (**C**) an

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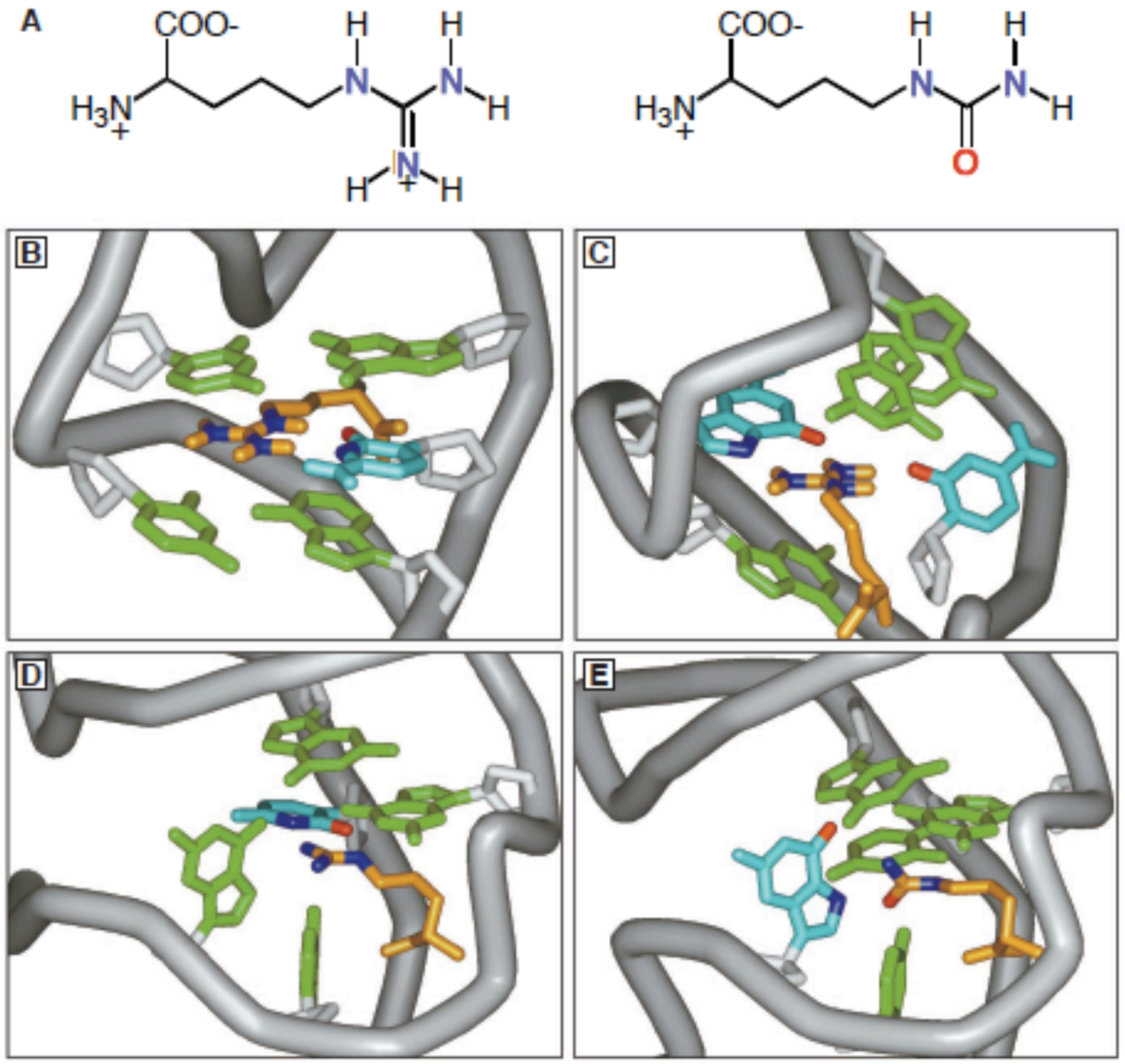

Fig. 2. Molecular recognition of the basic amino acids (**A**) arginine (left) and citrulline (right) by nucleic acid aptamers. The ligand-binding pockets are shown for arginine in complex with two different DNA aptamers (**B** and **C**) (*18*, *20*) and RNA-aptamer complexes (*19*) of arginine (**D**), and citrulline (**E**). In all four complexes, the positively charged amino acid side chain (orange) penetrates deeply into the nucleic acid fold where intermolecular hydrogen bonds are formed exclusively with bases (cyan). The ligand-binding pockets are lined by clusters of bases (green) excluding both the negatively charged phosphate backbone and solvent water. Polar nitrogen (blue) and oxygen (red) atoms participating in hydrogen bonds are marked.

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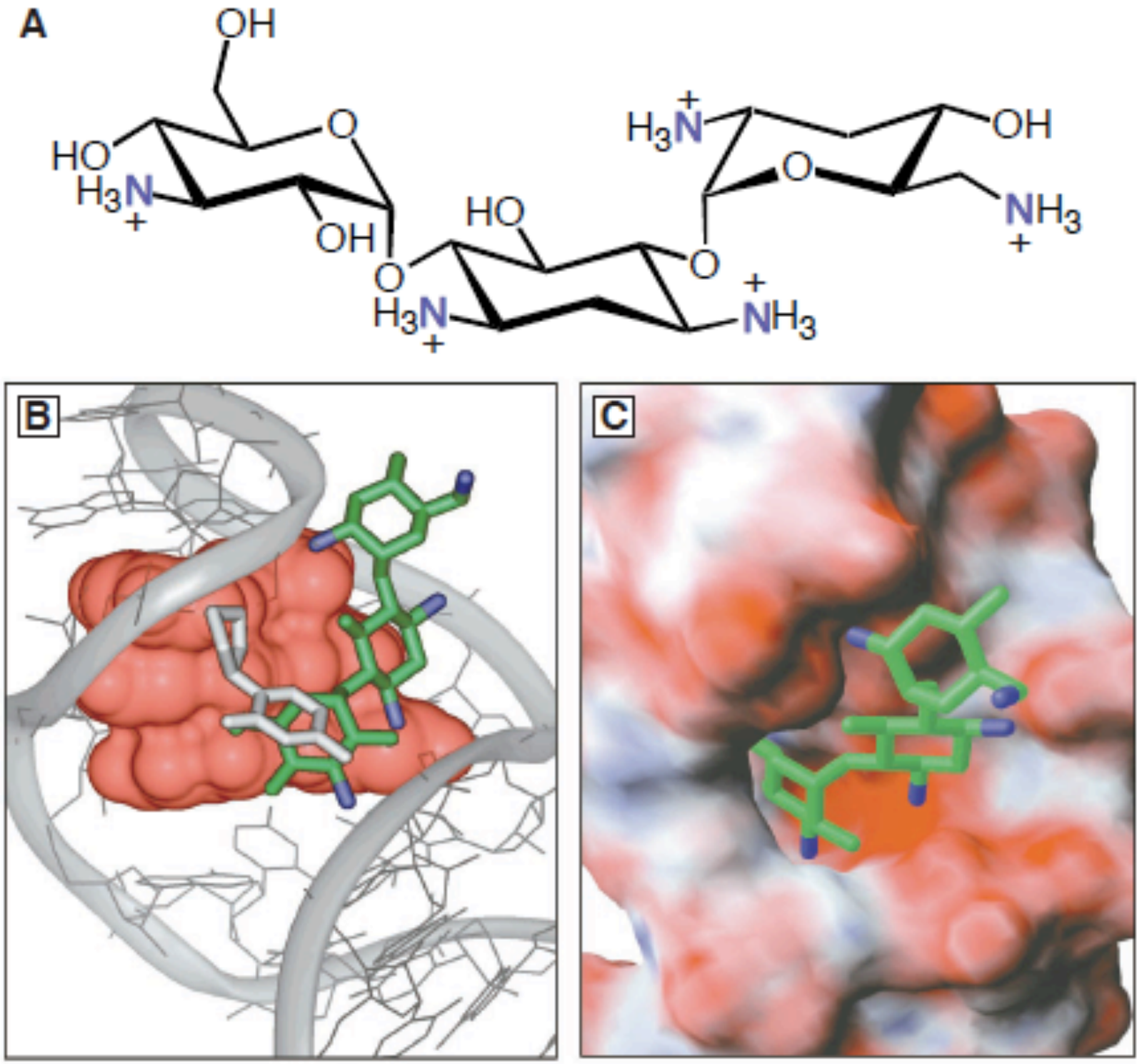

Fig. 3. Molecular recognition of the aminoglycoside antibiotic tobramycin (**A**) by an RNA aptamer. (**B**) In the aptamer complex (*33*), the RNA encapsulates the tobramycin ligand (green), which packs against the base edges (red) within the deep groove. A base flap (gray sticks) closes the groove above the bound drug. (**C**) The ligand-binding pocket provides a negatively charged environment displaying shape complementarity between electronegative sites (red) in the cavity and the positions of the cationic ammonium groups (blue) in the aminoglycoside. The RNA surface is colored according to the electrostatic potential, with red indicating negative charge and blue indicating positive charge (*73*).

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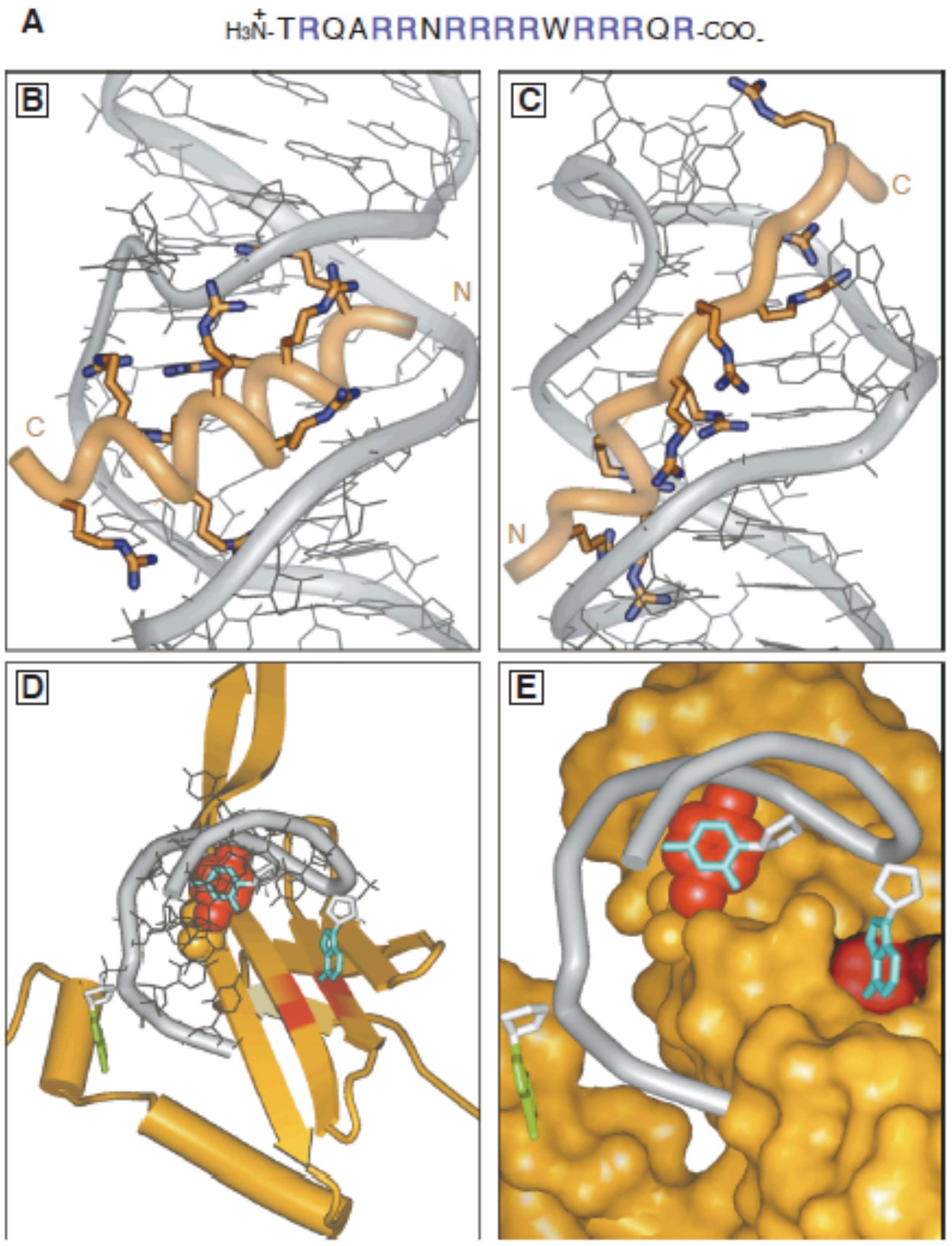

Fig. 4. Molecular recognition of peptides and proteins by RNA aptamers. The bound conformation of an arginine-rich peptide (**A**) from the human immunodeficiency virus (HIV-1) Rev protein is dictated by the nature of the RNA aptamer. (**B**) In one type of Rev aptamers, the bound peptide (orange) folds into an α-helical conformation within the widened deep groove of the RNA (*41*). (**C**) In a different aptamer, the peptide binds also within the widened deep groove, yet in an extended conformation (*42*). (**D** and **E**) An RNA aptamer (gray) recognizing the bacteriophage MS2 coat protein (orange) binds to the surface of antiparallel β sheets (*45*). Specific recognition of the protein by the RNA aptamer is provided by looped-out bases (cyan and green), which are inserted into cavities and involved in stacking interactions (red).

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