Molecular Simulation for Peptide Aptamer Immobilization in Hapten Capture

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BACKGROUND

- Haptens are small molecules, linked with many autoimmune & hypersensitive reactions that could lead to endocrine disruption & cancer risks. Sluggish development in detection & monitoring systems have hampered hapten regulation, putting public health at risk.[1]
- Aptamers like synthetic peptides can aid in hapten detection & monitoring. e.g., NFO4 for monitoring a mycotoxin like ochratoxin-A (OTA) (Fig. 1).[2]

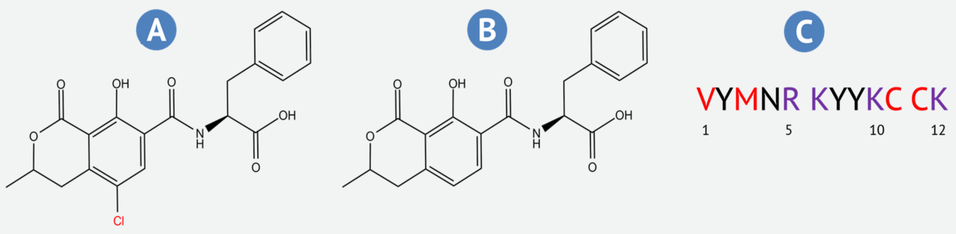


Fig.1: Chemical structures of (A) ochratoxin-A (OTA), (B) ochratoxin-B (OTB), & (C) primary sequence of NFO4 with hydrophobic residues in red, hydrophilic residues in black, charged residues in purple. OTA pose more serious health risks than OTB. NFO4 selectively binds to OTA as opposed to OTB.[2]

- Application of peptide aptamers as a cost-effective molecular recognition element however, require immobilization on solid surfaces via the addition of non-genetically encoded functional groups. Such modifications can affect the intrinsic bioactivity of an aptamer (Fig. 2). e.g. site preference of hexahis affinity tags affects NFO4 affinity to OTA.[1]

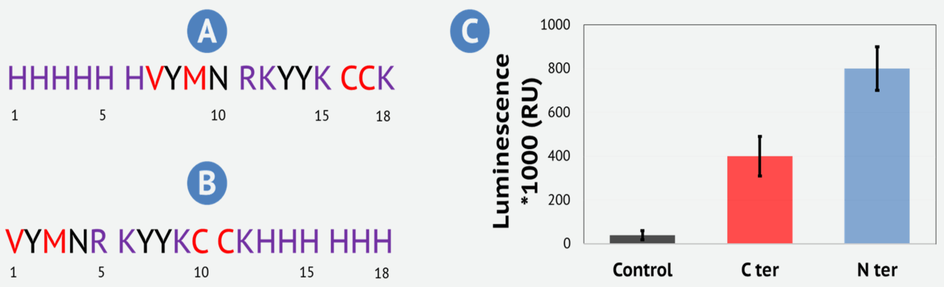


Fig.2: Chemical structures of HexaHis affinity tagged NFO4 at (A) N-terminus (N-ter, blue), (B) C-terminus (C-ter, red). (C) Influence of the aptamer modifications when immobilized at1000 µg L^{-1} on OTA detection. The control sample is the luminescence emitted with OTA–HRP without NFO4 peptide. Nter NFO4 binds more specifically to OTA as opposed to Cter NFO4.[1] Redrawn from Ref [1]

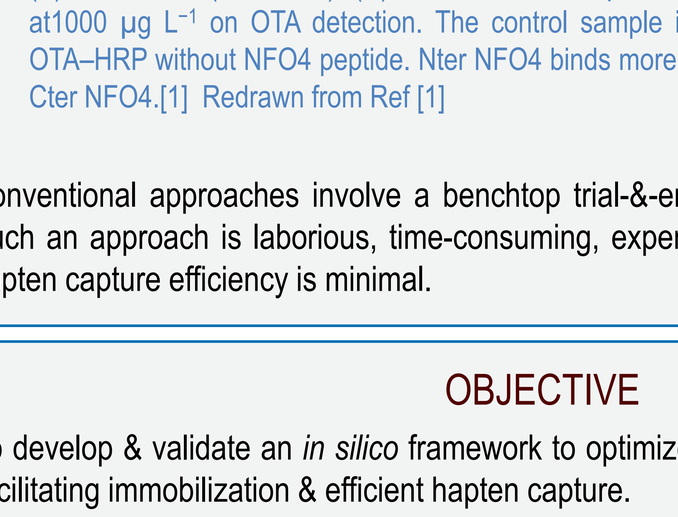
- Conventional approaches involve a benchtop trial-&-error testing of known modifications. Such an approach is laborious, time-consuming, expensive & the likelihood of an optimal hapten capture efficiency is minimal.

OBJECTIVE

- To develop & validate an *in silico* framework to optimize peptide aptamer modifications for facilitating immobilization & efficient hapten capture.

METHODS

- MD Simulations performed in 2 stages (Fig 3), (a) Generate low energy configurations, & (b) Derive kinetic & thermodynamic observables from configurations generated in stage 1



← Fig.3: Schematic overview of the protocol used to estimate the kinetic & thermodynamic observables in an aptmer-hapten system when starting from the respective structural files.

- Job Submission: Peptide folding and binding simulations were performed on ADA cluster provided by high performance computing (HPC) facility, TAMU. A 100 ns simulation spans 72 hours of wall clock time, with a typical job utilizing 20 cores, and an average memory of 1000 MB.
- Simulation System: NFO4-OTA & NFO4-OTB in a wine like solution pH(3-4) was used as the model aptamer-hapten system. Peptide structures were obtained from ProBuilder server. Initial coordinates of haptens were obtained from ZINC database. Ligand charges & topology were assigned using the automated topology & repository.[3] MD simulations were carried out in a GROMACS with PLUMED plugin [5] simulator using GROMOS 54A7 forcefield.[4] Markov State Model analysis provided with HTMD was used for equilibrated trajectory reconstruction & estimation of dynamic observables like free energy of binding ($\Delta G^{\circ}_{binding}$),

METHODS (Contd ..)

approach rate (k_{on}) and dissociation rate (k_{off}) using equations 1-3.[6]

$$\Delta G^{\circ}_{binding} = -kT \ln\left(\frac{\pi_{bound}}{\pi_{unbound}} \frac{V_{unbound}}{V_0}\right) \quad (1) \qquad k_{on} = \frac{1}{t_{unbound\rightarrow bound}} \frac{V_{unbound}}{V_0} \quad (2)$$

$$k_{off} = \frac{1}{t_{bound\rightarrow unbound}} \quad (3)$$

Where π_x represents stationary probability of the respective state, and $t_{unbound\rightarrow bound}$ & $t_{bound\rightarrow unbound}$ are the mean first passage times computed from the unbound to bound state and vice versa.

RESULTS AND DISCUSSION

Site-Specific Affinity Tags Influences Aptamer Folding

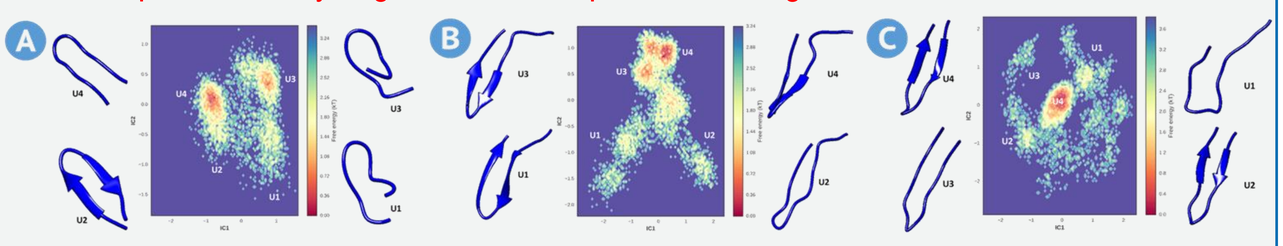
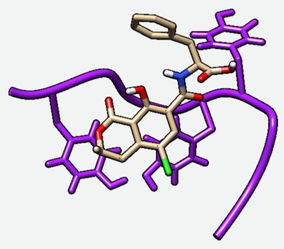


Fig.4: Free energy profile of (a) NFO4, (b) N-termini modified NFO4 and (c) C-termini modified NFO4. The four lowest energy profiles were identified by labels U1, U2, U3, & U4, with U4 being the most populated state.

Table 1: Structural preferences of different aptamer systems

Observables	NFO4	N-ter	C-ter
1° Structure (Binding Site Highlighted in red)	VYMNRKYYKCCK	HHHHHH VYMNRKYYKCCK	VYMNRKYYKCCK HHHHHH
2° Structure Preference	Disordered	β-sheet	β-sheet
H-bonds within 3Å	M3-C10, R5-K12	Y8-K15, N10-Y13, K12-N10, C17-H6	M3-H16, R5-H14, Y7-K12, C10-Y8, H18-V1

Site-Specificity of Affinity Tags Affect Hapten Capture



← Fig.5: Bound pose of OTA in the NFO4 active site.(purple). The carbon (pale brown), oxygen (red), nitrogen (blue) and chloride (green) groups in OTA is highlighted. The aromatic rings of Tyr residues within NFO4 were in contact with the ring structures and chloride group in OTA (< 4 Å)

Table 2: Simulation results of kinetic & thermodynamic parameters involved in hapten recognition by NFO4. Unmodified NFO4 had higher affinity & selectivity to OTA than OTB

Haptens	K_D (expt) nM	K_D (simln) nM	ΔG_{bind} (kcal/mol)	K_{on} (nM^{-1} s^{-1})	K_{off} (s^{-1})
OTA	7.92	5.83	-11.23	1.53	3
OTB	23.02	865	-8.27	0.36	787

Table 3. Predicted ΔG_{bind} (n= 5, 95% C.I.). Lower the ΔG_{bind}, better is the hapten affinity.

System	OTA (kcal/mol)	OTB (kcal/mol)
NFO4	-11.23 (1.29)	-8.27 (1.38)
N-ter NFO4	-7.88 (2.29)	-4.27 (1.27)
C-ter NFO4	-1.55 (1.25)	-7.04 (1.33)

- His tags competed with the native H-bonds in NFO4 peptide, altering binding site & molecular recognition
- Results show affinity tags modification at the Nter of NFO4 were better at OTA capture than Cter modifications.

CONCLUSION

- Developed & validated an *in silico* framework for assessing the impact of peptide modifications (including the type & site-preference) on the hapten capture efficiency of a peptide aptamer.
- Promising platform to accelerate the development of immobilized systems for a wider range of peptide-aptamer systems with an optimal hapten capture efficiency, that would eventually aid in mitigating potential public health risks.

REFERENCES: [1] R. Soleri et al., BIOSENS BIOELECTRON (2015), [2] I. Bazin et al., . BIOSENS BIOELECTRON (2013), [3] K. B. Koziara et al., J COMPUT AID MOL DES (2014), [4] M. Bonomi et al., COMPUT PHYS COMMUN (2009), [5] M. J. Abraham et al., SoftwareX (2015) [6] Doerr et al., J CHEM THEORY COMPUT (2016)