Supporting information

Characterization of DNA-conjugated Compounds Using Regenerable Chip

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Contents

1. Material

All chemicals, unless otherwise noted, were of reagent grade and purchased from Sigma-Aldrich. DNA oligonucleotides were synthesized using standard phosphoramidite synthesis; Standard DNA phosphoramidides, amino modifiers and other chemicals for oligonucleotide synthesis were obtained from Link Technologies, 5-amino-modifier-5 from Glen Research. EDC (1-Ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride) was purchased from Iris Biotec. NHS-Iminobiotin was purchased from Pierce, Thermo Scientific. Cyclosporin A was obtained from LC laboratories and Suc-Ala-Phe-Pro-Phe-pNA from Bachem. HisTrap HP 5ml column, Calmodulin-Sepharose 4B, HILOAD 16/60 Superdex 75PG were purchased from GE-Healthcare. NeutrAvidin® Agarose Resins(29200) were purchased from Thermo Scientific. *Escherichia coli* (*E. coli*) strain BL21(DE3) was purchased from Stratagene (La Jolla, CA, USA).

CypA plasmid (pQE-T7-1/CypA) was purchased from Qiagen. (The sequence of CypA translated from DNA sequencing is: MKHHHHHHMKQVNPTVFFDIAVDGEPLGRV SFELFADKVPKTAENFRALSTGEKGFGYKGSCFHRIIPGFMCQGGDFTRHNGTGGKSIYGEKFE DENFILKHTGPGILSMANAGPNTNGSQFFICTAKTEWLDGKHVVFGKVKEGMNIVEAMERFGSR NGKTSKKITIADCGQLE).

CypB plasmid (pQE-T7-1/CypB) was purchased from Qiagen. (The sequence of CypB translated from DNA sequencing is: MKHHHHHHMKQLLPGPSAADEKKKGPKVTV KVYFDLRIGDEDVGRVIFGLFGKTVPKTVDNFVALATGEKGFGYKNSKFHRVIKDFMIQGGDFT RGDGTGGKSIYGERFPDENFKLKHYGPGWVSMANAGKDTNGSQFFITTVKTAWLDGKHVVFGKV LEGMEVVRKVESTKTDSRDKPLKDVIIADCGKIEVEKPFAIAKE).

His-Cyp40 plasmid (pQE-T7-1/Cyp18) was purchased from Qiagen. (The sequence of His-Cyp40 translated from DNA sequencing is: HHHHHHMKQSHPSPQAKPSNPSNPRVFFDVDIGGERVGRIVLELFADIVPKTAENFRALCTGEK GIGHTTGKPLHFKGCPFHRIIKKFMIQGGDFSNQNGTGGESIYGEKFEDENFHYKHDREGLLSM ANAGRNTNGSQFFITTVPTPHLDGKHVVFGQVIKGIGVARILENVEVKGEKPAKLCVIAECGEL KEGDDGGIFPKDGSGDSHPDFPEDADIDLKDVDKILLITEDLKNIGNTFFKSQNWEMAIKKYAE VLRYVDSSKAVIETADRAKLQPIALSCVLNIGACKLKMSNWQGAIDSCLEALELDPSNTKALYR RAQGWQGLKEYDQALADLKKAQGIAPEDKAIQAELLKVKQKIKAQKDKEKAVYAKMFA)

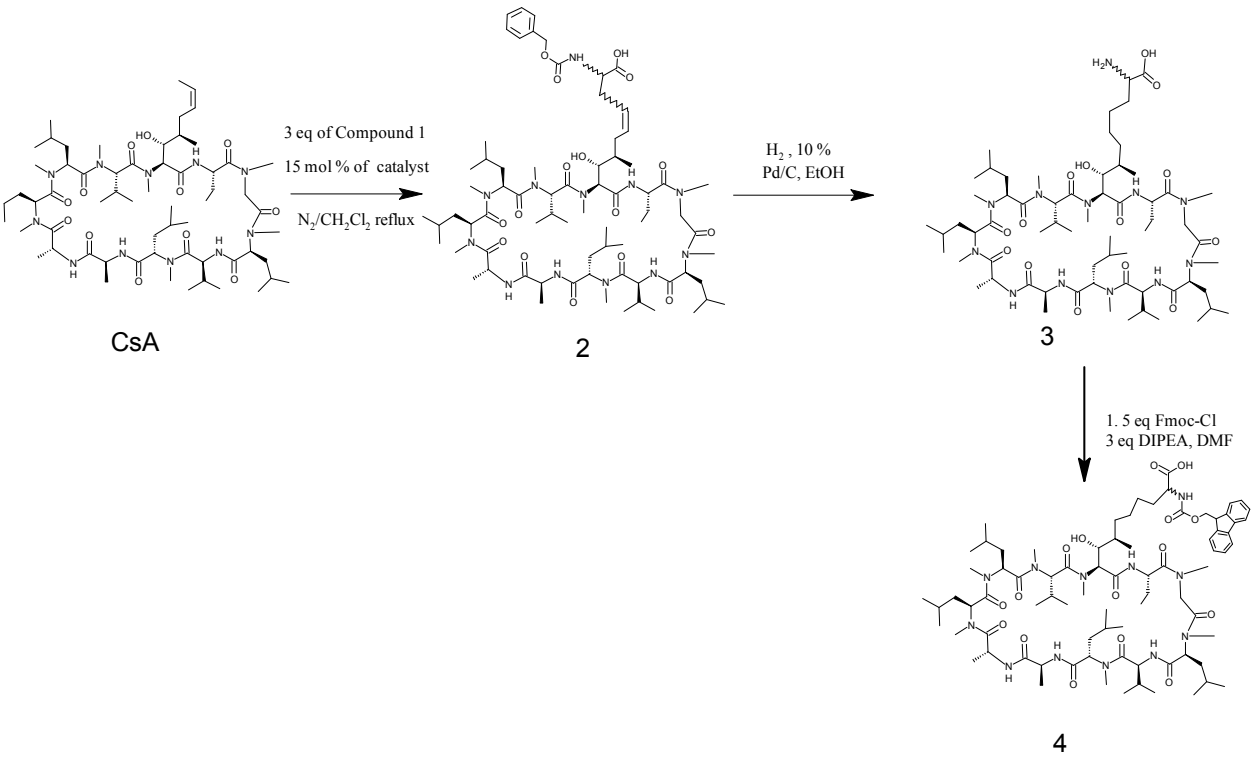
2. Fmoc-CsA-COOH synthesis, purification, and characterization

Waters e2695/Waters 2998 contains a Luna 100×10mm 5u C18 (S/No: 547035-2) column used for purification. For the purification of CsA derivatives, the column was heated up to 60 °C. The product was analyzed by waters ACQUITY UPLC Ultra Performance/Waters ACQUITY TQ Detector equipped with an analytical C18 column (ACQUITY UPLC BEH C18, bead size 1.7 µm, 50x2.1 mm, Waters, Milford Massachusetts, USA)

2.1 Synthesis of 2-{[(benzyloxy) carbonyl]amino}pent-4-enoic acid (Compound 1)

460 mg (4 mmol, 1.0 eq) DL-2-Allylglycine and 672 mg (8 mmol, 2 eq) sodium bicarbonate were dissolved in 30 ml ice cold H_2O under stirring conditions. To the stirred solution, 1.02 g (6 mmol, 1.5 eq) Benzyl chloroformate in 20 ml THF was added drop wise. The resulting mixture was stirred at 0 °C for 1 h and allowed to warm to room temperature overnight. The reaction mixture was acidified to pH 1 with 10% HCl and dried by a rotary evaporator. 50 ml of ethyl acetate was added to the powder. The mixture was filtered (paper) and dried by a rotary evaporator.

2.2 Synthesis Fmoc-CsA-COOH



Scheme S1. Synthesis Fmoc-CsA-COOH (4).

[Cbz amino acid-MeBMT] CsA (2): 602 mg (0.5 mmol, 1 eq) CsA, 403.5 mg (1.5 mmol, 3eq) compound 1 and 47 mg (0.075 mmol, 15 mol %) catalyst (Hoveyda-Grubbs Catalyst 2nd Generation) were dissolved in 2 ml in dry DCM in a 10 ml round flask. The solution was refluxed at 45 °C for 24 h under N_2. The solvent was evaporated by a rotary evaporator. 100 ml ethyl acetate was added and the organic layer was washed with H_2O and dried with Na_2SO_4. Dark brown product was observed after evaporation of the solvent. Without additional purification, the product was directly used for the next step. Mw, 1409.8; found, 1409.50

[Amino acid-MeBMT] CsA (3): The dark brown product (low purity of compound 2) was dissolved in 50 ml EtOH with 1 ml H_2O in a 250 ml round flask. Then 200 mg Palladium on carbon was added. The solvent was stirred under H_2 for 2 hours, avoiding the light. The mixture was filtered (paper) and evaporated. 100 ml ethyl acetate was added and the organic layer was washed with 1 mM HCl and dried with Na_2SO_4. The powder was dissolved in 1 ml 1 % TFA/DCM and the solvent was added dropwise to cold (-20 ºC) ether. The mixture was centrifuged and the precipitated product was dried under air and purified by HPLC. Lyophilization of the solvent gives 416 mg white powder (compound 3). Mw, 1277.7; Found, 1277.87

Fmoc-CsA-COOH (4): 416 mg (0.326 mmol, 1 eq) compound 3 dissolved in 1.5 ml DMF and 126.5 mg (0.489 mmol, 1 eq) Fmoc-Cl dissolved in 1 ml DMF were mixed in a 10 ml round flask at 0 °C. 435 µl 1.5 M DIPEA was added to the mixture dropwise under stirring. The resulting mixture is stirred at 0 °C for 1 h and allowed to warm to room temperature overnight. The solvent was evaporated and the pellet was dissolved in acetonitrile, purified by HPLC. Mw, 1499.9; Found, 1499.27

3. RhTC plasmid construction

The expression vector of RhTC was constructed using a QuikChange II site-directed mutagenesis kit (Agilent Technologies, Santa Clara, CA) using plasmid pQE-T7-1/CypA as a template. The forward (5' GTGTCAGGGTGGTAATTTTACCCATCATAATGGCACC 3') and reverse complement (5' GGTGCCATTATGATGGGTAAAATTACCACCCTGACAC 3') primers containing the base mismatches (indicated by red letters) were ordered from IBA Oligo Shop. (The sequence of CypA mutant (RhTC) translated from DNA sequencing is: MKHHHHHHMKQVNPTVFFDIAVDGEPLGRVSFELFADKVPKTAENFRALSTGEKGFGYKGSCFH RIIPGFMCQGGNFTHHNGTGGKSIYGEKFEDENFILKHTGPGILSMANAGPNTNGSQFFICTAK TEWLDGKHVVFGKVKEGMNIVEAMERFGSRNGKTSKKITIADCGQLE, the mutated amino acids are indicated in red).

4. Proteins purification

Recombinant CypA, RhTC, CypB and Cyp40 were expressed in BL21 (DE3). Single colonies of Escherichia coli were picked and inoculated into 20 ml of LB media containing kanamycin (50 μg/mL). The cultures were grown overnight at 37 °C with vigorous shaking. Subsequently 1 L of pre-warmed medium (2×YT, 50 μg/mL kanamycin) was inoculated with 15 mL of the overnight cultures, and incubated at 37 °C for 2 h with vigorous shaking until OD600 = 0.8-1. Expression was induced by adding IPTG to a final concentration of 300 μM and again incubating overnight at 25 °C (200 rpm shaking). Cells were harvested by centrifuging at 8000 rpm for 20 min and discarding the supernatant.

To purify the protein, the following method was used. Cells were resuspended in 50 ml buffer A (35 mM Hepes, 500 mM NaCl, 40 mM Imidazole) containing 1 mM DTT and 1 mM PMSF. This was followed by the lysis of cells by three passes through a French press (EmulsiFlex-C3, AVESTIN) at 4 °C under 1000 psi. The lysate obtained was centrifuged at 45,000 rpm, 4 °C for 1 h (Beckmann LE-80K ultracentrifuge, Beckmann, PaloAlto, California, USA, rotor SW 45Ti). The supernatant was collected and loaded on a Histrap HP column at 4 mL/min, followed by linear gradient change up to 100% Buffer B (35 mM Hepes, 500 mM NaCl, 500 mM Imidazole) over 30 min. The fractions showing peptidylprolyl isomerase (PPIase) activity were concentrated to 500 μL and then were injected on a Superdex 75. The fractions with PPIase activity were collected, concentrated, and analyzed by LC/MS.

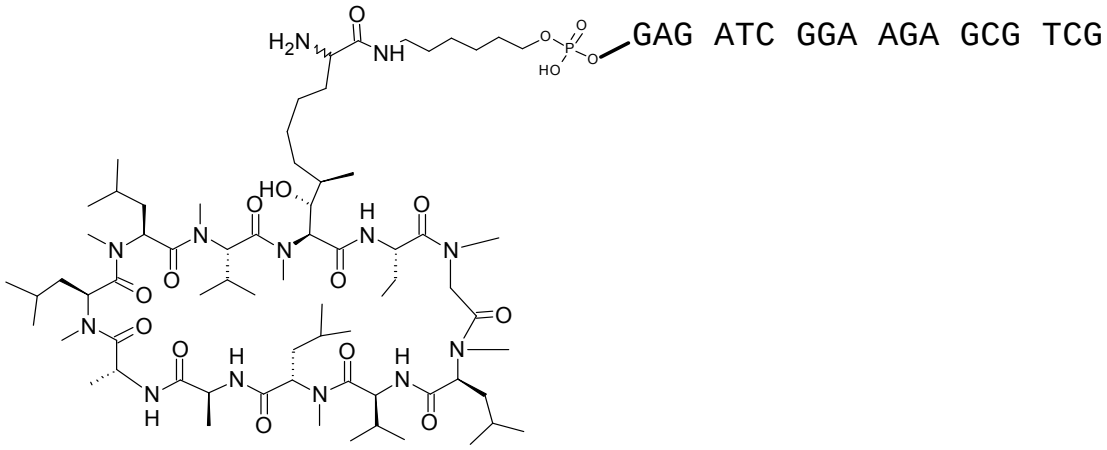
5. DNA oligonucleotide synthesis, purification, and characterization

DNA oligonucleotides were synthesized on a Polygen (Langen, Germany) 12-column DNA synthesizer using standard phosphoramidite protocols, cleaved at 50 ^{o}C overnight in 32 % NH_4OH, and purified on a C18 reverse–phase HPLC column (Phenomenex, Clarity 3u Oligo-RT) with aqueous 0.1 M triethylammonium acetate (TEAA)/CH_3CN gradient on Waters e2695/Waters 2998 systems. For all phosphoramidites with

unnatural bases, coupling time was extended to 300 seconds. Oligonucleotides with a 3'–amino group were synthesized using 3'–amino–modifier C7 CPG beads. 5'–amine was incorporated using 5-amino-modifier-5, 5-amino-modifier-C6, or 5-amino-C12-modifier. Oligonucleotides were quantitated by Nanodrop (Peqlab, Thermo Scientific) based on extinction coefficients at 260 nm. Waters ACQUITY UPLC Ultra Performance/Waters ACQUITY TQ Detector equipped with an analytical C18 column (ACQUITY UPLC OST C18, bead size 1.7 µm, 1x100 mm, Waters, Milford Massachusetts, USA) was used to analyze the mass of oligonucleotides. All DNA sequences are written in 5'- to 3'- orientation.

6. Ligand-DNA structures and Characterization

(a)CsA- 1':

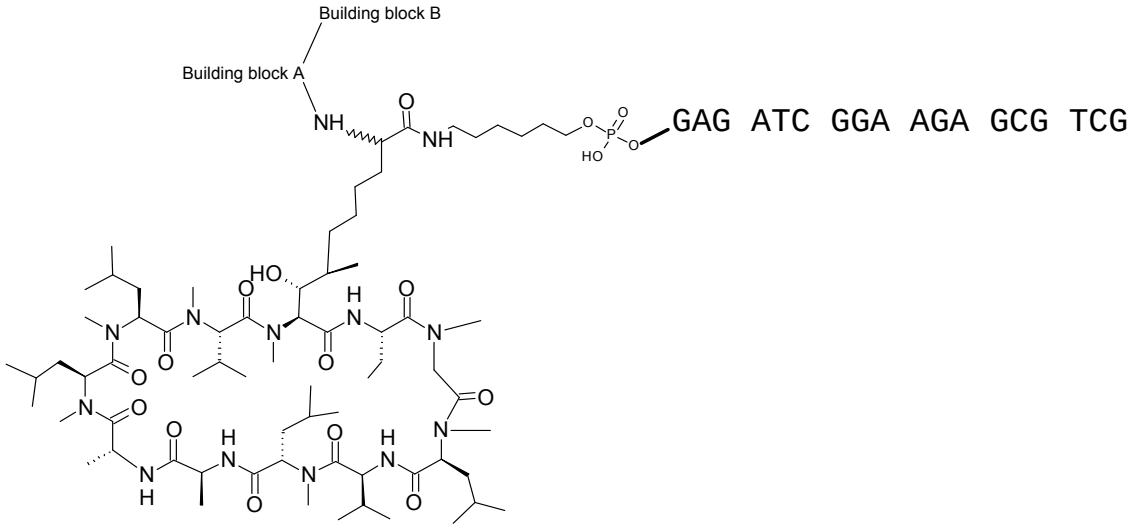


The CPG beads with sequence synthesized were washed with acetonitrile. The MMT group was removed by washing with 3% trichloroacetic acid in DCM until the supernatant was colorless and the trichloroacetic acid was then removed by additional 3 times DCM washes. The CPG beads were dried and ready to use. 5 µl of 200 mM Fmoc-CsA-COOH, 2 µl of 500 mM HOAt, and 1.9 µl of 500 mM HATU in dry DMF was agitated for 1 h. Then 9.2 µl of DMF was added and centrifuged at 13000 rpm for 10 min. The supernatant was transferred to a new eppendorf tube and 2 µl of 1500 mM triethylamine was added, then the reaction mixture was transferred to the eppendorf tube contained CPG beads and shaken overnight at 30 °C. The CsA conjugated oligonucleotide on CPG beads were purified as described previously.

Mw, 6825.2; Found, 6819.375±2

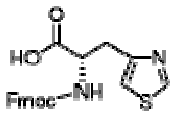
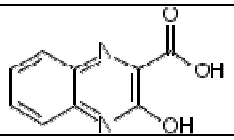
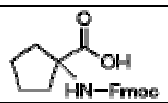
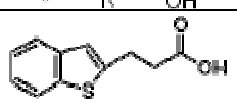
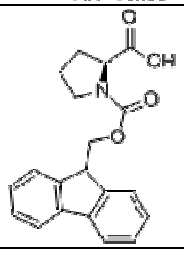
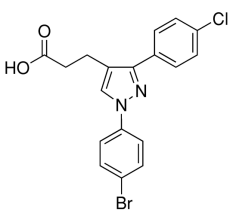
(b)Cs(n)-1'

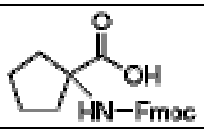
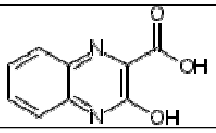
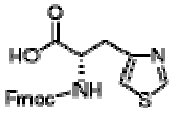
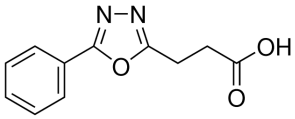
The structure of CypA/CsA complex shows that the side chain of MeVal-11(CsA) occupies the active site of CypA and the side chains of MeBmt-1(CsA) and Abu-2(CsA) contact the peptide segment Ala101-Asp102-Ala103 of CypA from both sides to strengthen the interaction.[1,2] As MeBmt-1 modification may change the affinity of CsA derivatives to CypA and cause different effects on various Cyps, MeBmt-1 was modified for DNA conjugation and different functional group introduction.



After the coupling of Fmoc-CsA-COOH, the CPG beads were washed 3 times with DMF , 3 times with 20 % piperidine in DMF(each time 5 min), 3 times with DMF, 3 times with 80 % acetic acid and 3 times with acetonitrile. The CPG beads were dried by a centrifugal evaporator and were ready for following synthesis. The coupling procedure was the same as Fmoc-CsA-COOH. The DNA conjugated CsA derivatives were cleaved from CPG beads. The products were purified by HPLC and analyzed with LC/MS as shown in table S1.

Table S1. List of Cs(n)-1' derivatives

CsA derivatives	Building blocks		MW	Found MW
	A	B		
Cs(1)-1'			7151	7147 ± 4
Cs(2)-1'			7124	7121 ± 4
Cs(3)-1'			7309	7302 ± 6

Cs(4)-1'			7108	7104 ± 5
Cs(5)-1'			7179	7174 ± 3

(c)1': CGACGCTCTTCCGATCTC, Mw, 5386.5; Found, 5380.0±4

(d)**Biotin-1'**: Biotin-C6- CGACGCTCTTCCGATCTC,
Mw, 5791.8; Found, 5787.49571±2.5
The biotin conjugated oligonucleotide was synthesized as CsA-1'

(e)SLF-1': SLF-5-amino-5-CGACGCTCTTCCGATCTC
Mw, 6161.2; Found, 6153.76±2.6
SLF-COOH: 12 mg SLF (1 eq.) and 7 mg Succinic anhydride (3 eq.) were dissolved in 100 µl DMF, then 28.7 µl of triethylamine was added, and the mixture was stirred overnight. The product was purified by HPLC and analyzed by LC/MS. Mw, 624.7; found, 624.3
The SLF-COOH conjugated oligonucleotide was synthesized as CsA-1'

(f)Benzamidine-1': Benzamidine-5-amino-5-CGACGCTCTTCCGATCTC
Mw, 5785.6; Found, 5778.5±3.2
100µl of 300 µM 5-amino-5-CGACGCTCTTCCGATCTC and 100 µl of 6% succinic anhydride in 0.2 M $NaHCO_3$ were mixed and the mixture was stirred at RT for 30 min. The resulted butanediotic acid conjugated oligonucleotide was purified by HPLC and analyzed by LC/MS. Mw, 5654.5; found, 5647.6±3.5
37.5 µl of 200 µM butanediotic acid-oligonucleotide was used to dissolve 6.25 mg EDC powder, then the mixture was transfer to a new Eppendorf tube contained 25 µl of 0.5 M 4-Aminobenzamidine dihydrochloride (dissolved in 0.1 M imidazole, pH 6.0), and the mixture vortex mixed. Then mixture was centrifuged at 12000 rpm for 5 min. 100 µl of 0.1 M imidazole (pH 6.0) was added to the mixture and stirred for 30 min. The product was purified by HPLC and analyzed by LC/MS.

(g)**1: (immobilized on chip, for single-pharmacophore)**: 5-amino-modifier-C6-GAGATC GGAAGAGCGTCG, Mw, 5792.7; Found, 5786.7±3

(h)**c1: (immobilized on chip, for dual-pharmacophore)**: 5-amino-modified-5 - GCAACC TCTCCCTGTTGTTA, Mw, 6186.1; Found, 6182.28±2.5

(i)**c1':** duplex of
CCTACGGATCGCACCATGCCTACTCGGACTCGTCATAACAACAGGGAGAGGTTGC and
TGACGAGTCCGAGTAGGCATGGTGCGATCCGTAGG.

(j)[**Iminobiotin coupled amino terminal oligonucleotides]** Iminobiotin was coupled to DNA by amide bond formation. 200 µl of 4 mM NHS-Iminobiotin in DMF was added to 100 µl of 100 µM 5' or 3' amino terminal oligonucleotide dissolved in triethylamine hydrochloride buffer (80 mM, pH 9) and the reaction mixture was stirred overnight at room temperature. The coupled compounds were purified and analyzed as described before.

Iminobiotin-5-amino-C12-modifier-CCTACGGATCGCACCATGCCTACTCGGACTCGTCATAACAACAGGGAGAGGTTGC, Mw, 17378.33; Found, 17373.39±3

TGACGAGTCCGAGTAGGCATGGTGCGATCCGTAGG-C7-amino-modifier-Iminobiotin, Mw, 11327.43; Found, 11326±2

7. Enzyme inhibition

The *cis-trans* peptidyl prolyl isomerase activity of CypA was measured with a protease-coupled assay with minor modifications[3]. The experiments were carried out in HEPES buffer, pH 7.8, at 7 °C, using succinyl-Ala-Phe-Pro-Phe-4-nitroanilide as substrate and α-chymotrypsin (Sigma-Aldrich) as the auxiliary protease. For the inhibition of CypA, 5 nM CypA and different concentrations of CsA derivatives and CsA were tested. The half maximal inhibitory concentration (IC_{50}) was determined by dose response fitting. The K_i was calculated according to the equation: $K_i = IC_{50} - \frac{[CypA]_4}{2}$, [CypA]represents the concentration of CypA.

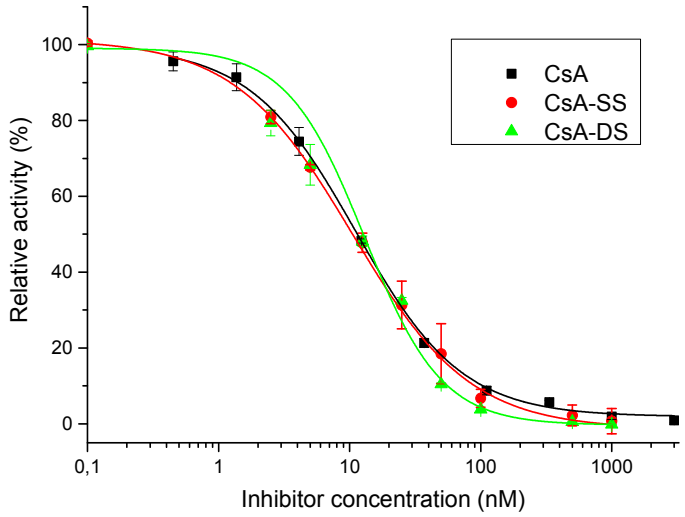


Figure S1. Dose-response curve for the inhibition of the PPIase activity of CypA by CsA (black square), CsA-SS (CsA conjugated to single strand DNA, red cicle), CsA-DS (CsA conjugated to double strand DNA, green up triangle). Activity was shown as the mean of triplicates ± S.D., and expressed as percentages of Cyclophilin activity relative to an inhibitor-free control.

8. Kinetic measurements on QCM (Attana A200 instrument)

8.1 Chip funzionalization with oligonucleotides

On an LNB-Carboxyl sensor chip (F213113 B), a terminal amino modified oligonucleotide (Q1 5' amino terminal, Q2 5' amino terminal) was immobilized by amide bond formation. The chip was flushed three times with milli-Q water and one time with 100 mM Acetic Acid, using a 200 µl pipette. A 200 µl fresh solution of 200 mM 1-ethyl-3-[3-dimethylaminopropyl]carbo-diimidehydrochloride (EDC) and 50 mM N-hydroxysulfosuccinimide (sulfo-NHS) was injected into the chip and pipetted several times avoiding bubbles in the chip. The chip was incubated for 10 min and flushed 3 times with 200 µl Milli-Q water. Then 10 µl of 400 µM terminal amine labeled oligonucleotide dissolved in 100 mM $NaHCO_3$ (pH 8.0) was injected into the chip and mixed for 1 min avoiding bubbles. The chip was flushed 3 times with 200 µl Milli-Q water after 1 h reaction. The cycles of activation and reaction were repeated three times. Residual free sulfo-NHS-activated carboxylic groups were quenched by a cycle of activation and reaction with 100 µl 1 M Ethanolamine. After 10 min, the chip was flushed three times with milli-Q water and ready to be installed into the QCM instrument.

8.2 Ligand coupled oligonucleotide immobilization on chip

The ligand coupled oligonucleotide was immobilized on the chip through hybridization reaction. The experiment was conducted in **HEPES buffer 1** (10 mM HEPES, 150 mM NaCl, pH 7.4, 0.05 % Tween 20) with a flow rate of 10 µl/min at 22 °C. 50 µl of 1 µM oligonucleotide with or without ligand conjugated in **HEPES buffer 1** were injected to the assay chip or referenced chip, separately. The flow rate was changed to 25 µl/min and a volume of 50 µl 1 % SDS was injected to both chips to removed unbound oligonucleotide. For cyclosporin-cyclophilin assays, 100 nM **CsA- 1'** and 900 nM **1'** oligonucleotides mixture was injected to the assay chip.

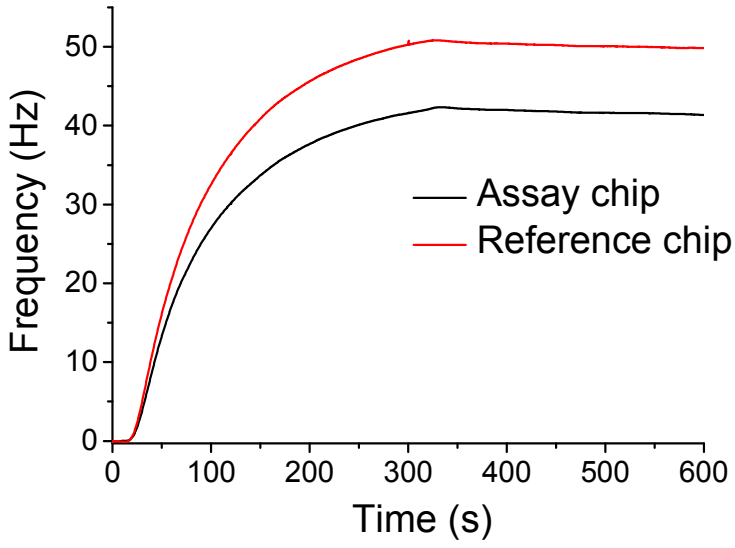


Figure S2. The frequency curves of 1 μM 1' oligonucleotide and 100 nM CsA- 1' combined with 900 nM 1' oligonucleotides injected to the reference chip and assay chip.

8.3 On-chip parameter measurements.

(A) Biotin-Streptavidin

The experiment was conducted in **HEPES buffer 1** with a flow rate of 25 μl/min at 22 °C. Then 35 μl 1 μM streptavidin was injected to both the assay chip and reference chip. Afterward, 50 μl 1% SDS was injected to both chips, then 50 μl 150 mM NaOH was injected to both chips to remove the oligonucleotide and oligonucleotide together with streptavidin.

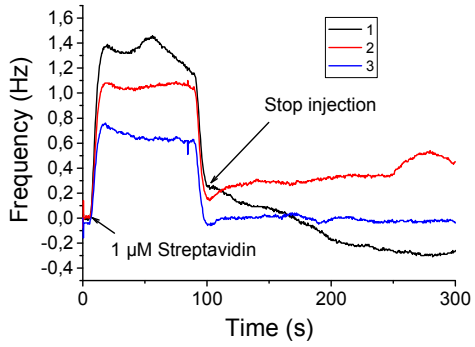


Figure S3. The frequency curves of streptavidin binding on reference chip (WITHOUT ligand) according to the procedure shown in figure 1d.

(B) Cyclosporin-Cyclophilin

The experiment was conducted on an Attana A200 instrument in **HEPES buffer 2** (10 mM HEPES, 1 M NaCl, pH 7. 4, 0.05 % Tween 20) with a flow rate of 25 μl/min at 22 °C. Differently concentrated cyclophilins in 35 μl **HEPES buffer 2 contained 1 mg/ml BSA** were injected and a waiting step of 300 s was used to monitor the dissociation rate (600 s for CypB). Between injections, 50 μl of 1 % SDS prepared in PBS was used to regenerate the chip. The kinetic parameters were observed by Attana evaluation software using mass transport limited and global fitting model.

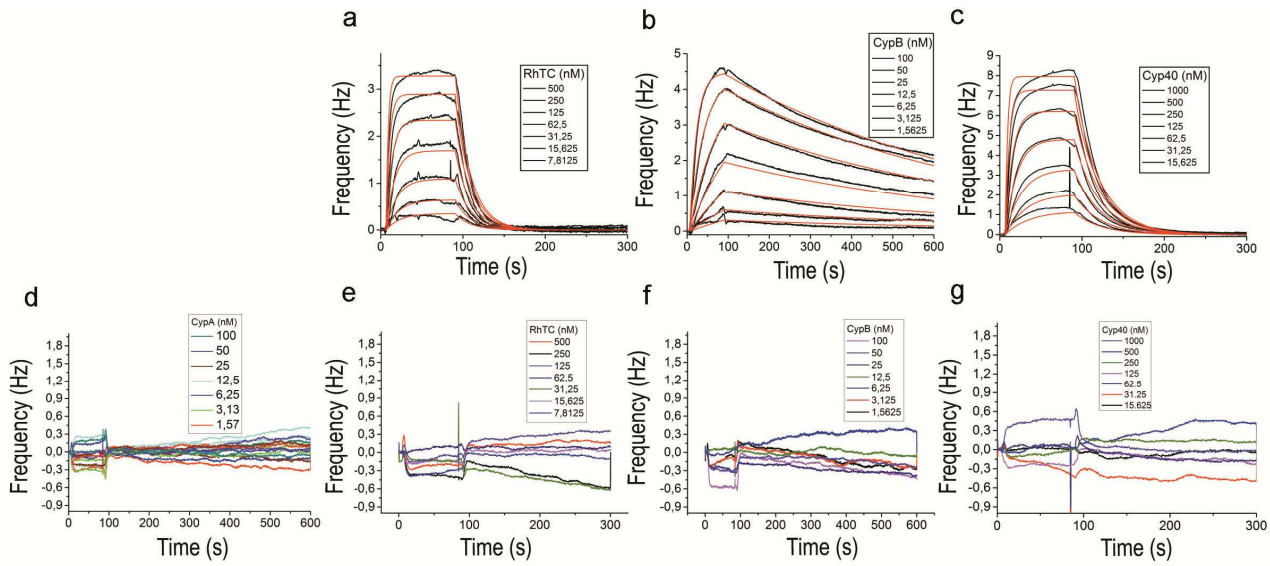


Figure S4. Reference-corrected binding and dissociation curves (black) of RhTC (a), CypB (b), Cyp40 (c) to CsA-1' annealing with DNA 1 on the chip. The data were globally fitted (red) using a kinetic model where a 1:1 complex is formed between Cyps and CsA. Mass transport limitations were considered during the fitting. And the curves of CypA, RhTC, CypB and Cyp40 from reference chip are d, e, f, and g, respectively.

(C) Iminobiotin-Streptavidin

An annealing step was performed before double strand coupled DNA immobilization. **$NaHCO_3$ buffer** (25 mM $NaHCO_3$, 150 mM NaCl, 0.005 % Tween20 pH 9.2) was used and the annealing was performed in a PCR machine by heating up the samples to 95 °C for 3 min and cooling down to 20 °C with a rate of -1 °C/min. The experiment was conducted in **$NaHCO_3$ buffer** with the flow rate of 25 µl/min at 22 °C. 25 µl amounts of differently concentrated streptavidin were injected and 50 µl of 1 % SDS prepared in PBS was used to regenerate the chip between injections.

For figure 2b, the annealed DNA double strand I_2-C1' (50 µl, 1 µM) was injected obtaining a high density surface of bivalent ligand on the chips. For figure 2c, a mixture of pre-annealed I_2-C1' and C1' (50 µl, 1 µM, ratio 2:8) was injected creating a low density surface of bivalent ligand. For figure 2d, the annealed DNA double strand I_1-C1' (50 µl, 1 µM) was injected to obtain a high density surface of monovalent ligand. For figure 2e, a mixture of pre-annealed I_1-C1' and C1' (50 µl, 1 µM, ratio 4:6) was injected creating a low density surface of monovalent ligand. In the reference chip, the annealed DNA double strand C1' (50 µl, 1 µM) was injected to create a surface without the ligand.

9. Kinetic measurement on multi-channel interferometer (Octet RED System)

9.1 Sensor funzionalization with oligonucleotide

AR2G - Amine Reactive 2nd Generation Sensors were used to covalent immobilize oligonucleotide. The experiment was performed under 1000 rpm shaking and the procedures were shown in table S2.

Table S2. The experiment procedures of sensor funzionalization with oligonucleotides

Steps	Reagent	Time (s)
1	Milli Q H_2O	50
2	0.1 M Acetic acid	50
3	200 mM EDC/50 mM sulfo-NHS in H_2O	600
4	Milli Q H_2O	50
5	100 μM DNA **1** in 0.1 M $NaHCO_3$	3600
6	Milli Q H_2O	50

9.2 Kinetic measurements of DNA conjugated CsA (CsA-1') in different sensors conducted on Octet Red System.

The Octet Red System allows 16 kinetic measurements to be performed simultaneously by loading 16 different ligands on corresponding sensor locations (from A_n to H_n and from A_{n+1} to H_{n+1} of a 96 well plate). In order to check the experiments reproducibility, **1'** was loaded on **A1** as reference sensor and **CsA-1'** was loaded from **B1** to **H1** in **HEPES buffer 3** (10 mM HEPES, 150 mM NaCl, pH 7.4, 1 % Tween 20). Then a dilution series of CypB (200 nM, 66.7 nM, 22.2 nM, 7.41 nM, 2.47 nM, 0.823 nM) in **HEPES buffer 2 containing 1 mg/ml BSA** was used to measure the kinetic parameters. Between injections, 1 % SDS prepared in PBS was used to regenerate the chip. The data were globally fitted (red) using a kinetic model where a 1:1 complex is formed between CypB and CsA.

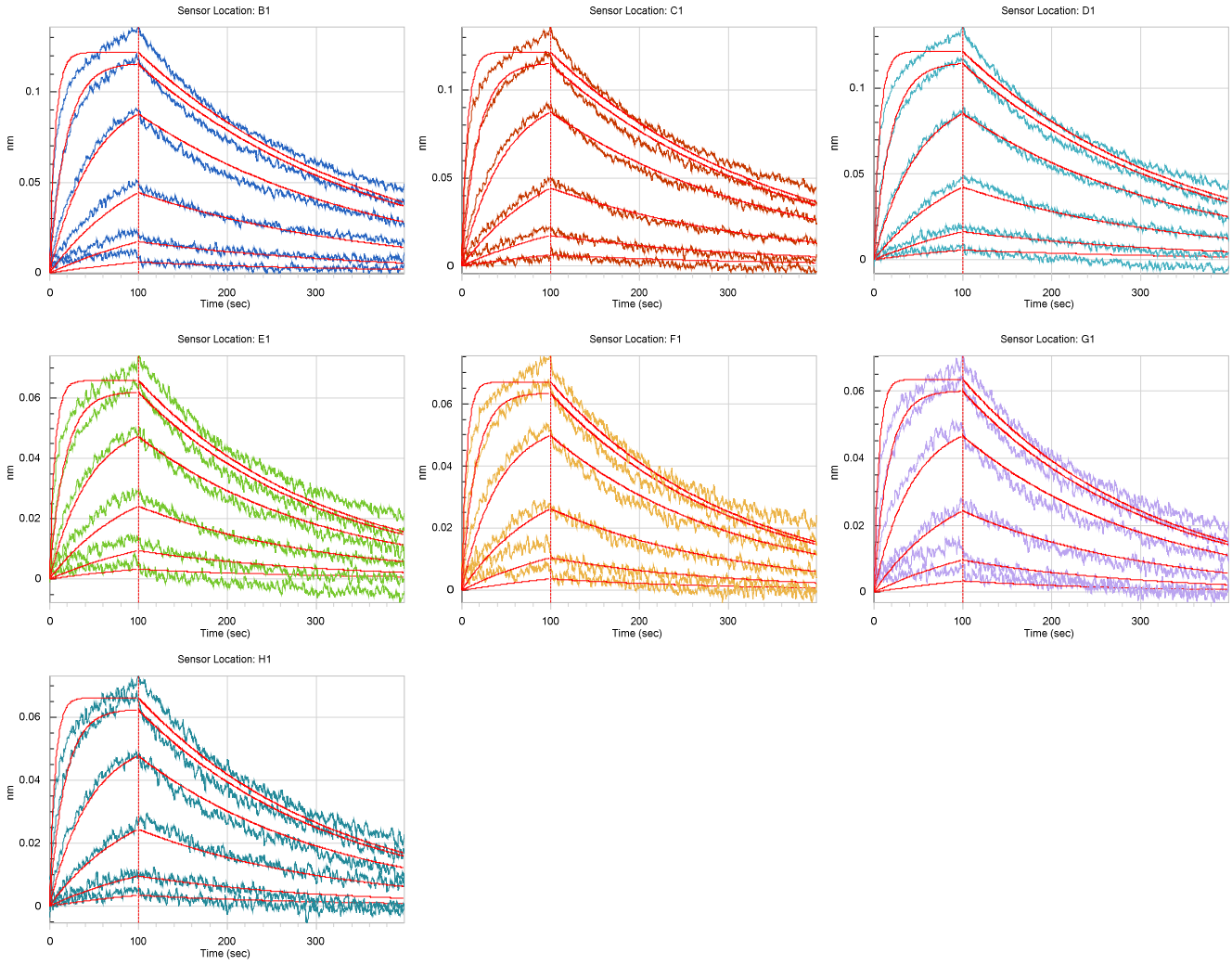


Figure S5. CsA-1' was loaded on different sensor locations, a dilution series of CypB (200 nM, 66.7 nM, 22.2 nM, 7.41 nM, 2.47 nM, 0.823 nM) was used to measure the kinetic parameters. The data were globally fitted (red) using a kinetic model where a 1:1 complex is formed between CypB and CsA

Table S3. Kinetics and binding affinity of CsA-1'/1 to CypB

Kinetic parameters	Sensor Location						
	B1	C1	D1	E1	F1	G1	H1
k_{on} (M^{-1} s^{-1} x 10^{5})	7.37 ± 0.05	7.42 ± 0.05	7.03 ± 0.04	7.79 ± 0.08	8.54 ± 0.08	8.23 ± 0.08	7.74 ± 0.06
k_{off} (s^{-1} x 10^{-3})	3.80 ± 0.02	4.03 ± 0.02	4.09 ± 0.02	4.78 ± 0.03	4.86 ± 0.03	4.80 ± 0.03	4.58 ± 0.03
K_d (nM) kinetic	5.16 ± 0.04	5.43 ± 0.04	5.81 ± 0.04	6.14 ± 0.08	5.69 ± 0.06	5.83 ± 0.07	5.91 ± 0.06

9.3 Kinetic measurements of DNA conjugated CsA derivatives to different cyclophilins

CsA-1' and Cs(n)-1' derivatives **(Table S1)** were loaded on separated oligonucleotide functionalized sensors and the kinetic experiments were performed parallel as described before. To avoid the strong base damage the glass sensor, 2.5 mM HCl was used to do the dehybridization.

a) CypA

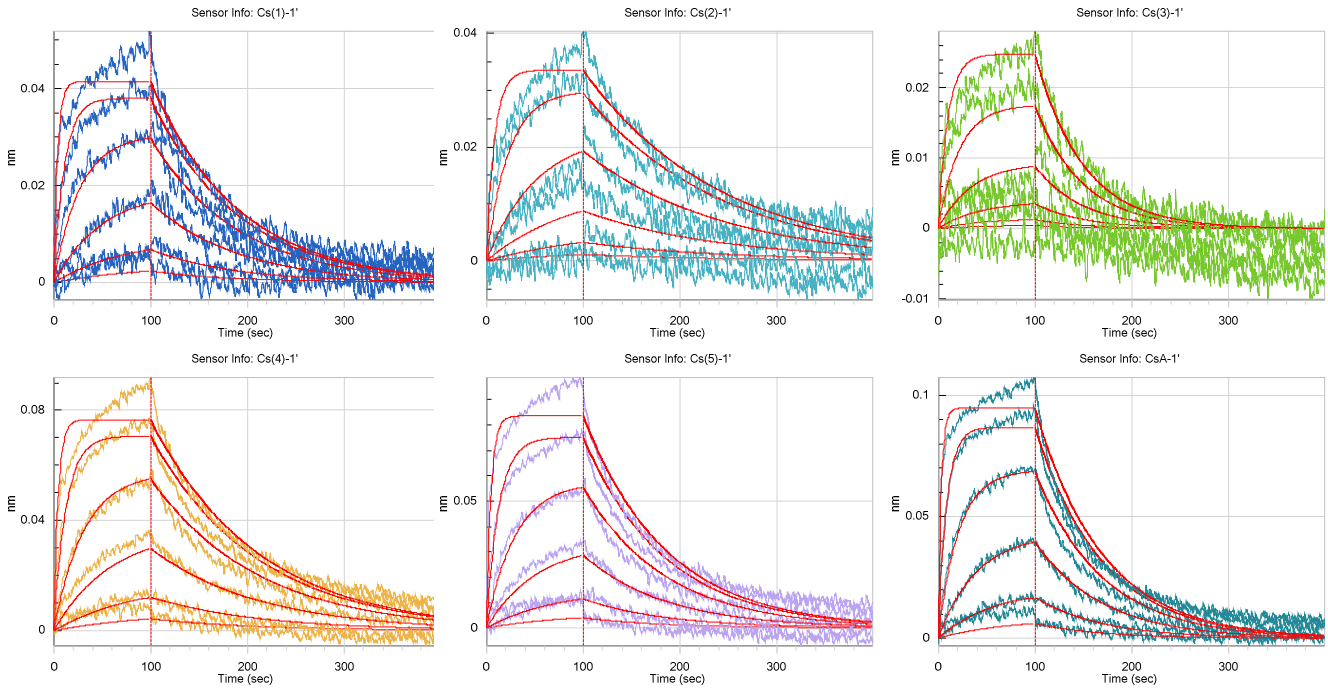


Figure S6. A dilution series of CypA (200 nM, 66.7 nM, 22.2 nM, 7.41 nM, 2.47 nM, 0.823 nM) was used to measure the kinetic parameters of CsA derivatives. The data were globally fitted (red) using a kinetic model where a 1:1 complex is formed between CypA and CsA

Table S4. Kinetics and binding affinity of CsA derivatives to CypA

Kinetic parameters	Cs(1)-1'	Cs(2)-1'	Cs(3)-1'	Cs(4)-1'	Cs(5)-1'	CsA-1'
k_{on} (M^{-1} s^{-1} x 10^5)	11.9 ± 0.16	5.51 ± 0.10	3.91 ± 0.20	10.4 ± 0.12	9.77 ± 0.13	14.9 ± 0.15
k_{off} (s^{-1} x 10^{-3})	11.2 ± 0.08	7.05 ± 0.07	20.2 ± 0.57	9.01 ± 0.56	11.7 ± 0.08	14.4 ± 0.08
K_d (nM) kinetic	9.41 ± 0.15	12.8 ± 0.26	51.6 ± 3.03	8.65 ± 0.11	12.0 ± 0.18	9.68 ± 0.11

b) RhTC

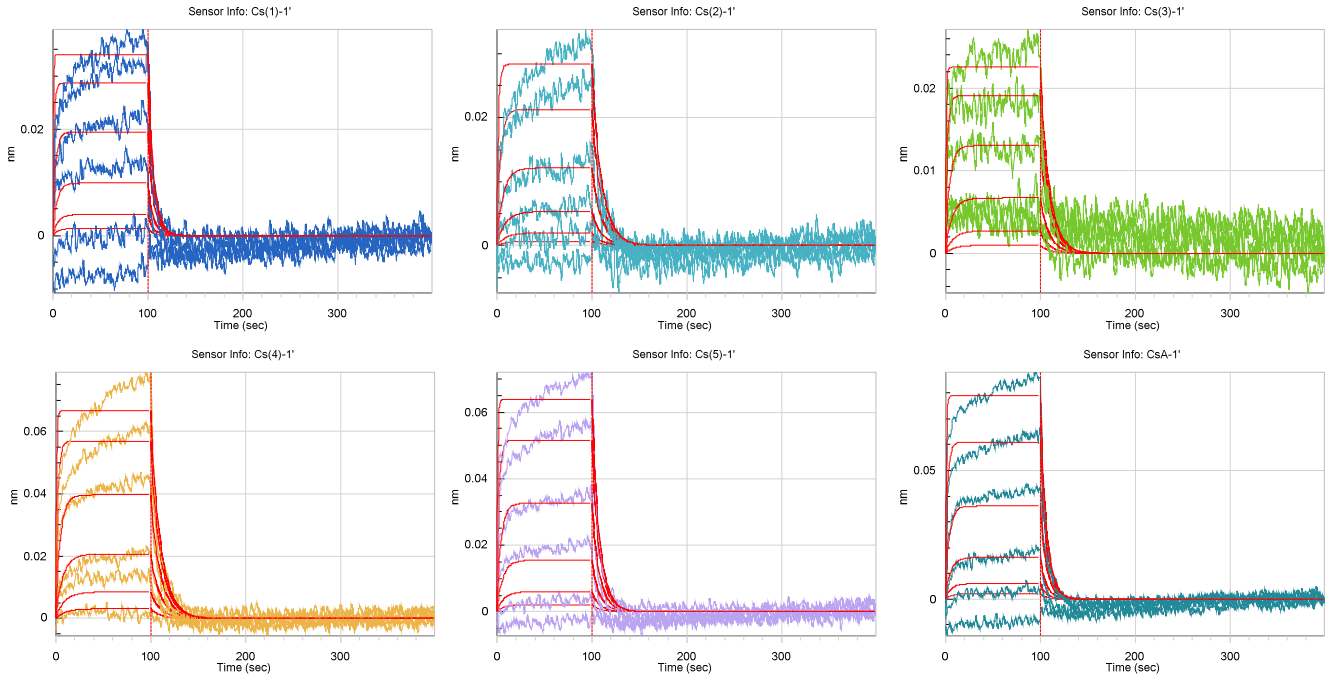


Figure S7. A dilution series of RhTC (1000 nM, 333.3 nM, 111.1 nM, 37 nM, 12.3 nM, 4.12 nM) was used to measure the kinetic parameters of CsA derivatives. The data were globally fitted (red) using a kinetic model where a 1:1 complex is formed between RhTC and CsA.

Table S5. Kinetics and binding affinity of CsA derivatives to RhTC

Kinetic parameters	Cs(1)-1'	Cs(2)-1'	Cs(3)-1'	Cs(4)-1'	Cs(5)-1'	CsA-1'
k_{on} (M^{-1} s^{-1} x 10^5)	20.5 ± 0.94	35.9 ± 2.86	76.1 ± 0.32	11.7 ± 0.94	13.1 ± 0.40	10.9 ± 0.36
k_{off} (s^{-1} x 10^{-3})	119 ± 4.50	75.1 ± 2.44	200 ± 14.2	79.6 ± 1.43	105 ± 2.60	119 ± 3.24
K_d (nM) kinetic	58.1 ± 3.46	98.8 ± 5.25	171 ± 18.3	68.2 ± 2.00	79.5 ± 3.14	110 ± 4.68

c) CypB

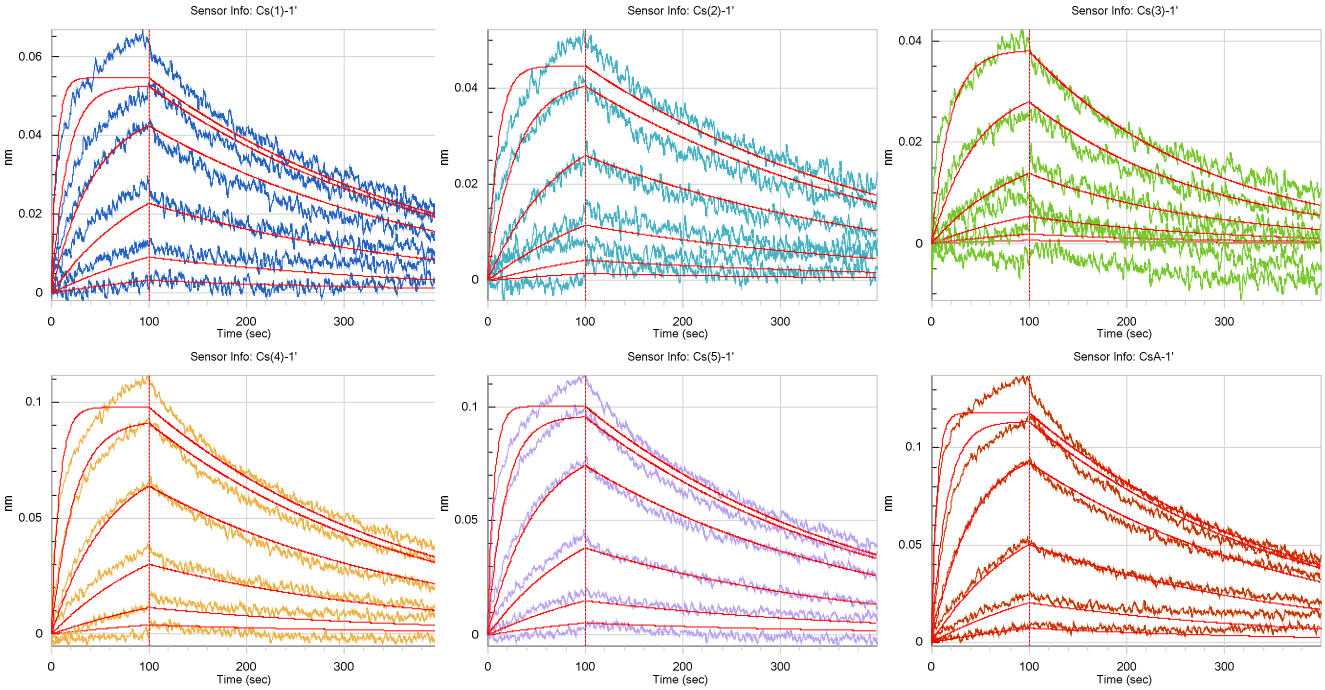


Figure S8. A dilution series of CypB (200 nM, 66.7 nM, 22.2 nM, 7.41 nM, 2.47 nM, 0.823 nM) was used to measure the kinetic parameters of CsA derivatives. The data were globally fitted (red) using a kinetic model where a 1:1 complex is formed between CypB and CsA.

Table S6. Kinetics and binding affinity of CsA derivatives to CypB

Kinetic parameters	Cs(1)-1'	Cs(2)-1'	Cs(3)-1'	Cs(4)-1'	Cs(5)-1'	CsA-1'
k_{on} (M^{-1} s^{-1} x 10^5)	8.68 ± 0.09	4.59 ± 0.05	2.39 ± 0.05	5.86 ± 0.05	7.69 ± 0.05	9.06 ± 0.06
k_{off} (s^{-1} x 10^{-3})	3.40 ± 0.03	3.08 ± 0.03	5.42 ± 0.07	3.68 ± 0.02	3.50 ± 0.02	3.65 ± 0.02
K_d (nM) kinetic	3.91 ± 0.06	6.72 ± 0.10	22.7 ± 0.58	6.27 ± 0.06	4.56 ± 0.04	4.02 ± 0.03

d) Cyp40 (1000 nM, 333.3 nM, 111.1 nM, 37 nM, 12.3 nM, 4.12 nM)

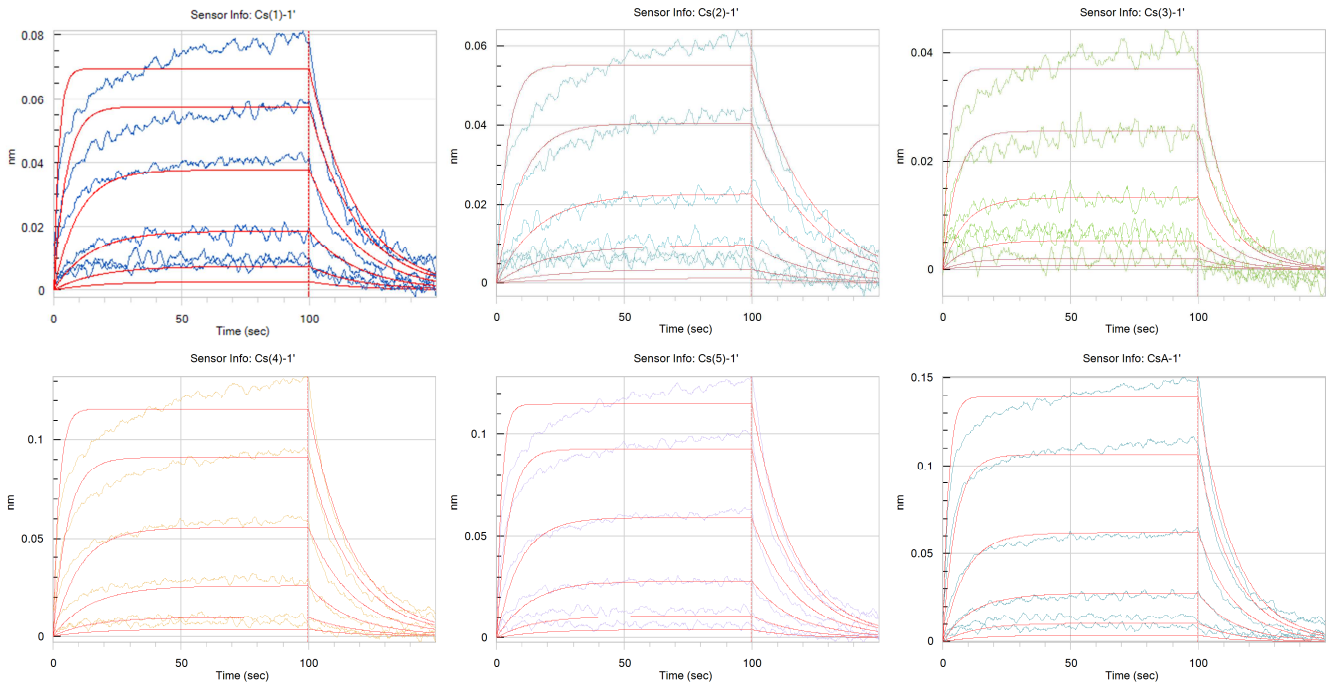


Figure S9. A dilution series of Cyp40 (1000 nM, 333.3 nM, 111.1 nM, 37 nM, 12.3 nM, 4.12 nM) was used to measure the kinetic parameters of CsA derivatives. The data were globally fitted (red) using a kinetic model where a 1:1 complex is formed between Cyp40 and CsA

Table S7. Kinetics and binding affinity of CsA derivatives to Cyp40

Kinetic parameters	Cs(1)-1'	Cs(2)-1'	Cs(3)-1'	Cs(4)-1'	Cs(5)-1'	CsA-1'
k_{on} (M^{-1} s^{-1} x 10^5)	4.51 ± 0.11	1.90 ± 0.05	2.97 ± 0.11	3.62 ± 0.08	4.46 ± 0.08	3.86 ± 0.06
k_{off} (s^{-1} x 10^{-3})	53.0 ± 0.99	42.4 ± 0.80	87.2 ± 2.45	56.9 ± 0.89	60.0 ± 0.84	70.3 ± 0.86
K_d (nM) kinetic	118 ± 3.67	223 ± 7.12	294 ± 13.6	157 ± 4.09	134 ± 3.12	183 ± 3.69

9.4 Kinetic measurements of DNA conjugated SLF and benzamidine to GST-FKBP and trypsin, respectively.

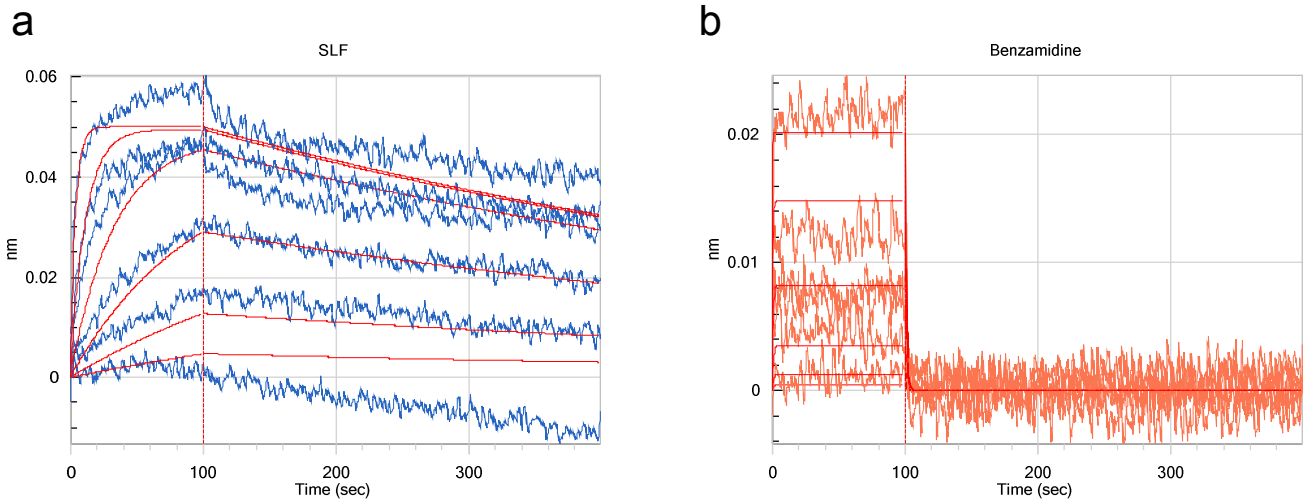


Figure S9. Dilution series of GST-FKBP (100 nM, 33.3 nM, 11.1 nM, 3.7 nM, 1.23 nM, 0.41 nM) and trypsin (500 µM, 166.7 µM, 55.6 µM, 18.5 µM, 6.2 µM, 2.1 µM) was used to measure the kinetic parameters of SLF-1' and benzamidine-1' to GST-FKBP (a) and trypsin (b), respectively. The data were globally fitted (red) using a kinetic model where a 1:1 complex is formed.

Kinetic parameters	SLF-1' & GST-FKBP	Benzamidine-1'
k_{on} (M^{-1} s^{-1})	$2.57 \pm 0.04 \times 10^{6}$	$8.43 \pm 1.06 \times 10^{3}$
k_{off} (s^{-1})	$1.45 \pm 0.03 \times 10^{-3}$	$9.31 \pm 1.13 \times 10^{-1}$
K_d kinetic	0.57 ± 0.02 nM	110 ± 19 µM

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(4) Copeland, R. A.; Lombardo, D.; Giannaras, J.; Decicco, C. P. *Bioorganic & Medicinal Chemistry Letters* **1995**, *5*, 1947.