An Autoinhibited Coiled-Coil Design Strategy for Split-Protein Protease Sensors

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Supporting Information

Figure S1: Tabulation of all constructs used in these studies.

Figure S2: Sequences of all constructs utilized in these studies.

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Figure S7: Model for alternative assemblies for doubly inhibited coiled-coil sensors.

Reassembly Pair	Split Protein Fusion	Protease Cleavable Linker
B-NFluc CFluc-A	Firefly Luciferase(2-416) Firefly Luciferase(398-550)	TEV
B-NFluc CFlucA-TEV-B’	Firefly Luciferase(2-416) Firefly Luciferase(398-550)	TEV
B-NFluc CFlucA-TEV-B’$_{2A}$	Firefly Luciferase(2-416) Firefly Luciferase (398-550)	TEV
B-NFluc CFluc-A-TEVB’$_{4A}$	Firefly Luciferase(2-416) Firefly Luciferase(398-550)	TEV
A’-TEV-B-NFluc CFlucA-TEV-B’	Firefly Luciferase(2-416) Firefly Luciferase(398-550)	TEV
A’-TEV-B-NFluc CFluc-A-TEV-B’$_{2A}$	Firefly Luciferase(2-416) Firefly Luciferase(398-550)	TEV
A’-TEV-B-NFluc CFluc-A-TEVB’$_{4A}$	Firefly Luciferase(2-416) Firefly Luciferase(398-550)	TEV
A’-CASP3-B-NFluc CFluc-A-CASP3-B’	Firefly Luciferase(2-416) Firefly Luciferase(398-550)	CASPASE-3
RR-NFluc CFluc-EE	Firefly Luciferase(2-416) Firefly Luciferase(398-550)	TEV
RR-NFluc CFluc-EE-TEVRR’	Firefly Luciferase(2-416) Firefly Luciferase(398-550)	TEV
RR-NFluc CFluc-EE-TEV-RR’$_{3A}$	Firefly Luciferase(2-416) Firefly Luciferase (398-550)	TEV
RR-NFluc CFluc-EE-TEV-RR’$_{6A}$	Firefly Luciferase(2-416) Firefly Luciferase(398-550)	TEV
NβLac-A-TEV-B’ B-CβLac	βLactamase (26-196) βLactamase (198-290)	TEV
NβLac-A-TEV-B’$_{4A}$ B-CβLac	βLactamase (26-196) βLactamase (198-290)	TEV

Figure S1. Protein fusions and reporter fragments with protease cleavable linkers used in these studies.

Protein Construct	Sequence
CFluc-Acid	CFluc-AQLEKELQALEKKLAQLEWENQALE KELAQ
Base-NFluc	AQLKKKLQANKKELAQLKWKLQALKKKLAQ-NFluc
CFluc-Acid-TEV-Base	CFluc-AQLEKELQALEKKLAQLEWENQALE KELAQGGGGENLYFQGGKLGGGG AQLKKKLQANKKELAQLKWKLQALKKKLAQ
CFluc-Acid-TEV-Base2A	CFluc-AQLEKELQALEKKLAQLEWENQALE KELAQGGGGENLYFQGGKLGGGGAQAKKKAQANKKELAQLKWKLQALKKKLAQ
CFluc-Acid-TEV-Base4A	CFluc-AQLEKELQALEKKLAQLEWENQALE KELAQGGGGENLYFQGGKLGGGGAQAKKKAQANKKELAQLKWKLQAAKKKAAQ
Acid-TEV-Base-NFluc	AQLEKELQALEKKLAQLEWENQALE KELAQGGGGENLYFQGGKLGGGG AQLKKKLQANKKELAQLKWKLQALKKKLAQ-NFluc
CFluc-Acid-CASP3-Base	CFluc-AQLEKELQALEKKLAQLEWENQALE KELAQGGGGDEVDGGKLGGGG AQLKKKLQANKKELAQLKWKLQALKKKLAQ
Acid-CASP3-Base-NFluc	AQLEKELQALEKKLAQLEWENQALE KELAQGGGGDEVDGKLGGGG AQLKKKLQANKKELAQLKWKLQALKKKLAQ-NFluc
CFluc-EE	CFluc- LEIEAAFLEQENTALETEVAELEQEVQRLENIVSQYETRYGPL
RR-NFluc	LEIRAAFLRRRNTALRTRVAELRQRVQRLRNIVSQYETRYGPL-NFluc
CFluc-EE-TEV-RR	CFluc- LEIEAAFLEQENTALETEVAELEQEVQRLENIVSQYETRYGPLGGGGENLYFQGGKLGGGGLEIRAAFLRRRNTALRTRVAELRQRVQRLRNIVSQYETRYGPL
CFluc-EE-TEV-RR3A	CFluc- LEIEAAFLEQENTALETEVAELEQEVQRLENIVSQYETRYGPLGGGGENLYFQGGKLGGGGLEIRAAFARRRATAARTRVAELRQRVQRLRNIVSQYETRYGPL
CFluc-EE-TEV-RR6A	CFluc- LEIEAAFLEQENTALETEVAELEQEVQRLENIVSQYETRYGPLGGGGENLYFQGGKLGGGGLEIRAAFARRRATAARTRVAELRQRAQRARNIASQYETRYGPL
NβLac-Acid-TEV-Base	NβLac-AQLEKELQALEKKLAQLEWENQALE KELAQGGGGENLYFQGGKLGGGG AQLKKKLQANKKELAQLKWKLQALKKKLAQ
NβLac-Acid-TEV-Base 4A	NβLac-AQLEKELQALEKKLAQLEWENQALE KELAQGGGGENLYFQGGKLGGGGAQAKKKAQANKKELAQLKWKLQAAKKKAAQ
Base-CβLac	AQLKKKLQANKKELAQLKWKLQALKKKLAQ-CβLac

Figure S2.Amino acid sequences for various protein constructs used in the study, only coiled coil sequences are shown.

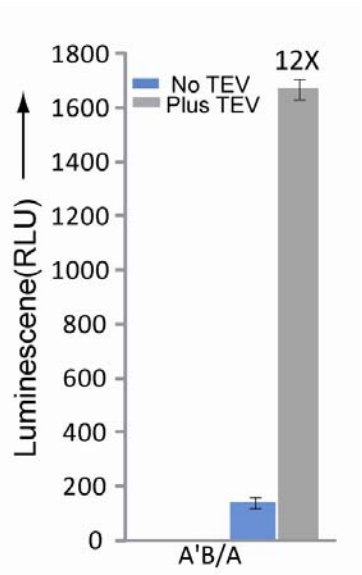

Figure S3. Singly inhibited A'B/A firefly luciferase system utilizing 1 pmol each of B-NFluc and CFluc-A mRNA with (grey) and without (blue) TEV protease.

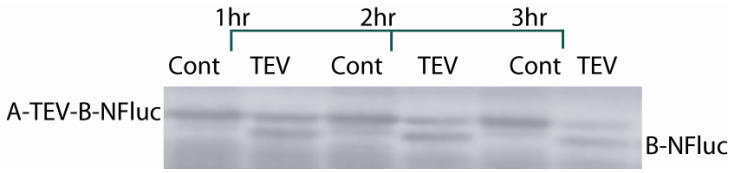

Figure S4. SDS-PAGE analysis of the TEV protease dependent cleavage of the ^{35}S labeled A-TEV-B-NFluc construct as imaged by auto-radiography.

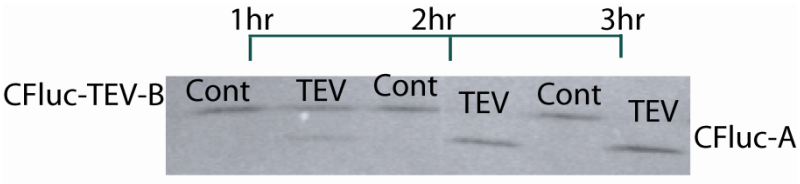

Figure S5. SDS-PAGE analysis of the TEV protease dependent cleavage of the ^{35}S labeled CFluc-A-TEV-B construct imaged by auto-radiography.

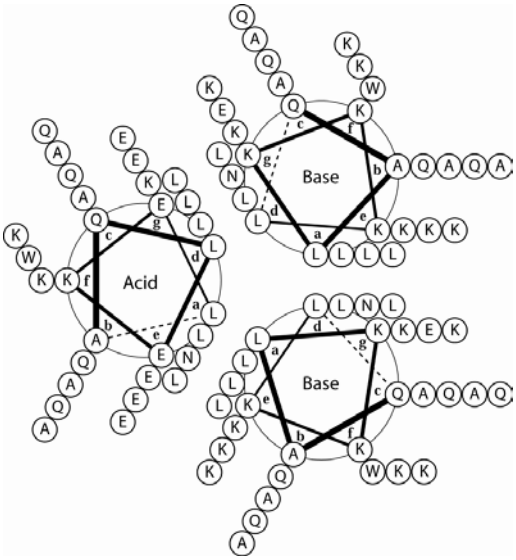

Figure S6. Helical wheel diagram representing a possible three helix bundle with the Acid and Base coiled coil system that could potentially be responsible for the background in the singly inhibited sensors.

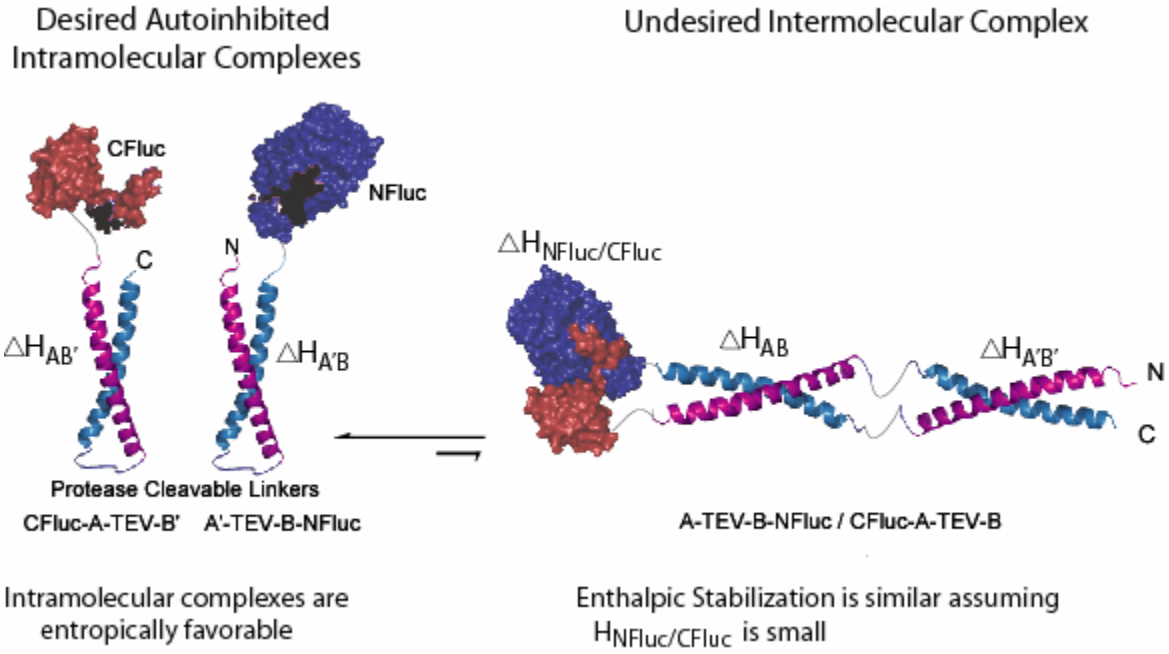

Figure S7. A model of the proposed intramolecularly inhibited coiled-coil system (left) and the extended coiled-coil assembly (right) described in the manuscript for the doubly inhibited firefly luciferase turn-on sensor. The complex to the right would be significantly disfavored entropically based on molecularity but possibly favored enthalpically over the intramolecular auto-inhibited coiled-coils (left). Based on the results (almost no signal from reassembled luciferase) it is likely that the entropic cost of the undesired complex (right) far outweighs any enthalpic benefit under our experimental conditions.