ELECTRONIC SUPPLEMENTARY INFORMATION (ESI) for Manuscript

Design, Quality and Validation of the EU-OPENSCREEN Fragment Library Poised to a High-Throughput Screening Collection

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Supplementary Table S1. The 10 most frequent functional groups present in bioactive small molecules described in medicinal chemistry literature and its relative frequency found in current ChEMBL actives (pChEMBL > 5.0) and EFSL fragments. The R symbol indicates aliphatic or aromatic carbon. The analysis was done using the code provided in Sanchez-Cruz *et al*.[3]

Supplementary Table S2. Structures of the 17 fragment hits from the BLI screening of the EFSL. Marked with a * are the 4 hits binding to FabF C164A with K_D < 500 μM. Fragment hit EOS102727 (**1**) was repurchased for retesting and further validation using X-ray crystallography.

Supplementary Table S3. Structures of the 7 hits from the BLI screening of selected ECBL poised molecules. Fragment hits EOS69423 (**2**) and EOS21030 (**3**) were repurchased for retesting.

Supplementary Table S4. Data-collection and refinement statistics. Values in parentheses are for the highest resolution shell.

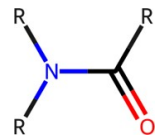
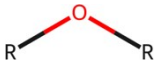
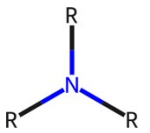
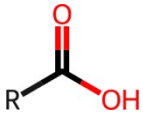
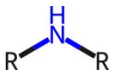
Supplementary Figure S1. Eight calculated descriptors comparing DSI-Poised, EFSL (EUOS) and FX2_BESSY Fragment Libraries

Supplementary Figure S2. Sensorgrams (left) and steady-state plots (right) for both FabF C164A (top) and w.t. (bottom) of the 17 fragment hits from the BLI screening of the EFSL. In bold, those samples with K_D values below 500 μM.

Supplementary Figure S3. Sensorgrams (left) and steady-state plots (right) for both FabF C164A of the 7 hits from the BLI screening of of selected ECBL poised molecules.

Supplementary Figure S4. Steady-state plots in triplicate of repurchased material of hits **2** (top) and **3** (bottom). Bars indicate range of responses for the same concentration.

Supplementary Table S1. The 10 most frequent functional groups present in bioactive small molecules described in medicinal chemistry literature and its relative frequency found in current ChEMBL actives (pChEMBL > 5.0) and EFSL fragments. The R symbol indicates aliphatic or aromatic carbon. Cal and Car indicate links to an aliphatic and an aromatic carbon, respectively. The analysis was done using the code provided in Sánchez-Cruz *et al*.[1]

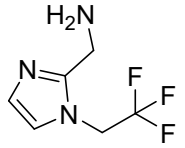
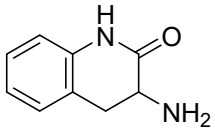
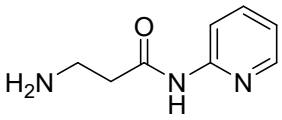
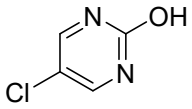
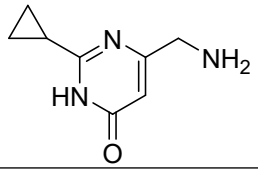
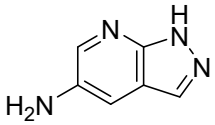
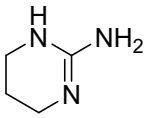
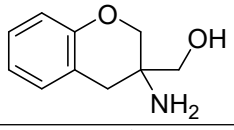
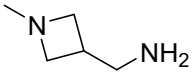
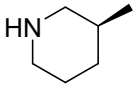
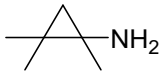
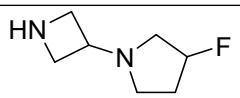
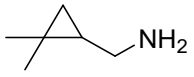
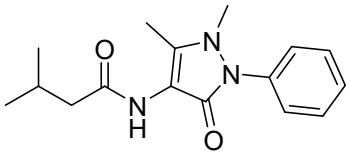
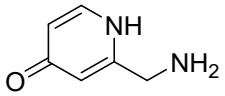
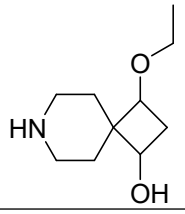
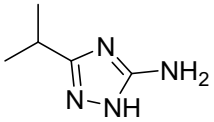
Functional Group	Ertl et al.[2,3]	ChEMBL Actives	EU-OS FBS Fragments		Functional Group	Ertl et al.[1,2]	ChEMBL Actives	EU-OS FBS Fragments
	40.2	41.9	32.3			19.6	18.7	4.2
	38.8	40.4	16.8			13.6	13.0	8.9
	29.5	26.6	4.8			13.1	11.0	5.0
	23.1	22.8	12.4			12.2	9.3	0.3
	19.6	18.2	23.3			10.3	9.6	7.1

(1) Sánchez-Cruz, N.; Pilón-Jiménez, B. A.; Medina-Franco, J. L. Functional Group and Diversity Analysis of BIOFACQUIM: A Mexican Natural Product Database. *F1000Research* **2019**, *8*, Chem Inf Sci-2071.

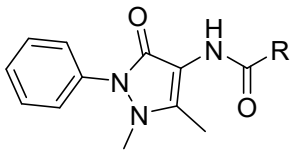
(2) Ertl, P.; Altmann, E.; McKenna, J. M. The most common functional groups in bioactive molecules and how their popularity has evolved over time. *J. Med. Chem.* **2020**, *63*, 8408-8418.

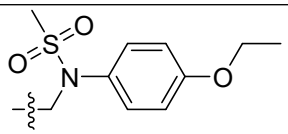
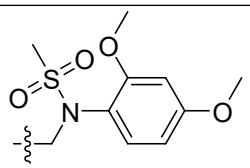
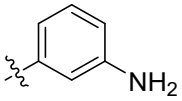
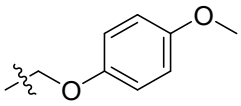
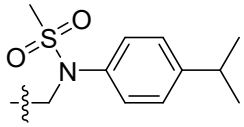
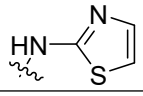
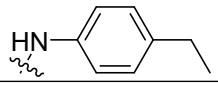
(3) Ertl, P.; Altmann, E.; Racine, S. The most common linkers in bioactive molecules and their bioisosteric replacement network. *Bioorg. Med. Chem.* **2023**, *81*, 117194.

Supplementary Table S2. Structures of the 17 fragment hits from the BLI screening of the EFSL. Marked with a * are the 4 hits binding to FabF C164A with K_D < 500 μM. Fragment hit EOS102727 (**1**) was repurchased for retesting and further validation using X-ray crystallography.

EOS ID	Fragment structure	EOS ID	Fragment structure
EOS102612		EOS102679	
EOS102836		EOS103073	
EOS102554		EOS102736	
EOS103477		EOS103128	
EOS103501		EOS103471	
EOS103478*		EOS103304	
EOS103499*		EOS102727* (**1**)	
EOS102855*		EOS102809	
EOS102663			

Supplementary Table S3. Structures of the 7 hits from the BLI screening of selected ECBL poised molecules. Fragment hits EOS69423 (**2**) and EOS21030 (**3**) were repurchased for retesting.

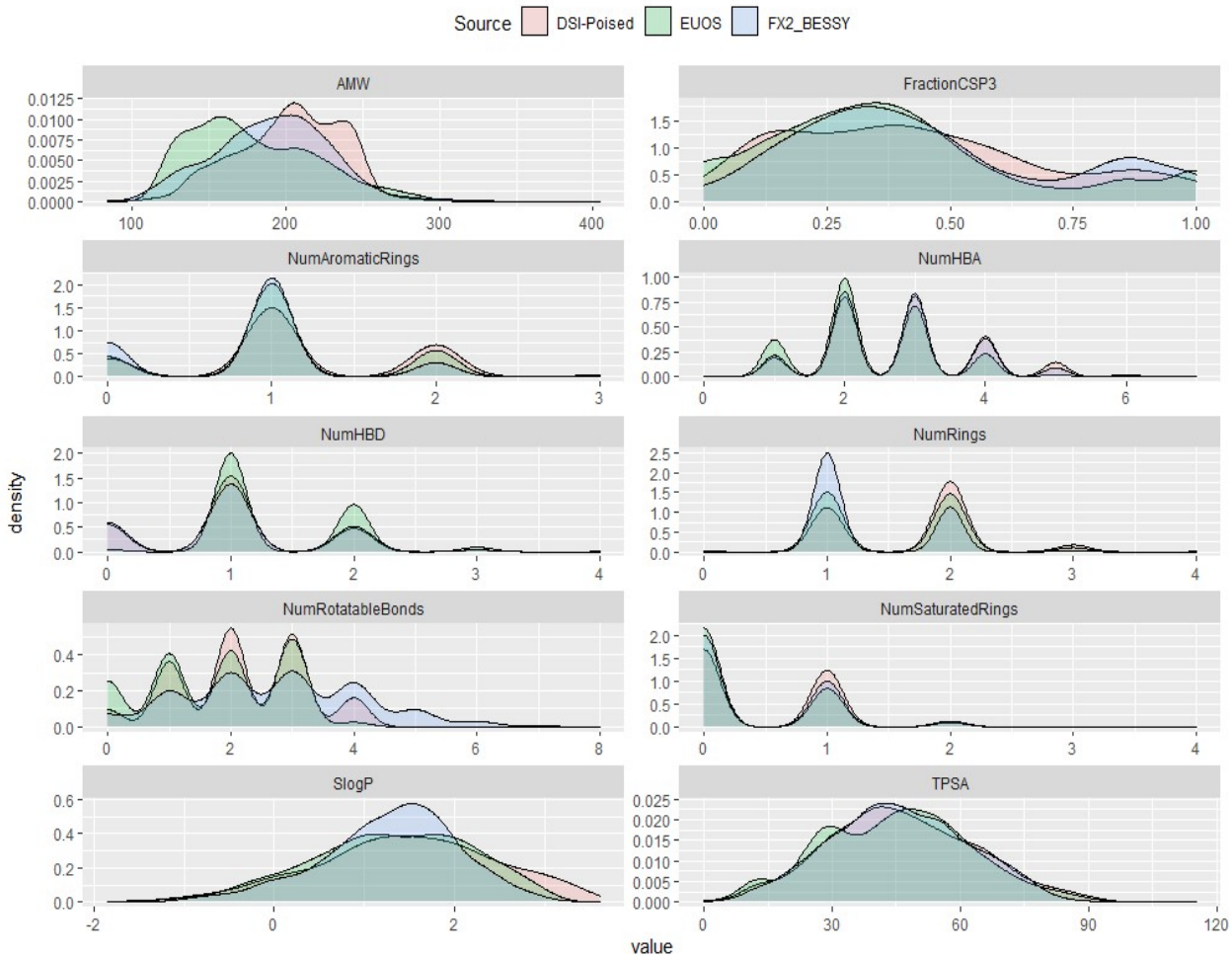


EOS ID	Structure
EOS11491	
EOS21049	
EOS69423 (**2**)	
EOS21030 (**3**)	
EOS11668	
EOS64113	
EOS21494	

Supplementary Table S4. Data-collection and refinement statistics. Values in parentheses are for the highest resolution shell.

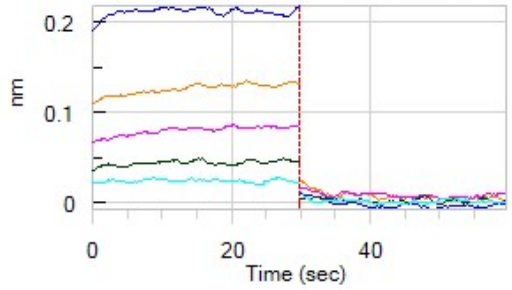
Structure	***Pa*FabF C164A-1**	***Pa*FabF C164A-2**	***Pa*FabF C164A-3**
PDB ID	**8PJ0**	**8ROI**	**8R1V**
Space group	P 1 21 1	C 2 2 21	C 2 2 21
a, *b*, *c* (Å) α, β, γ (°)	72.46 141.34 72.48 90.00 91.64 90.00	101.02 103.67 141.31 90.00 90.00 90.00	100.95 104.34 144.12 90.00 90.00 90.00
Solvent content (%)	41	42	43
Diffraction data			
Resolution range (Å)	48.83 - 1.70	72.35 – 1.51	72.66 – 2.09
Unique reflections	158061 (7870)	115834 (5652)	45462 (3420)
Multiplicity	7.1 (6.8)	6.8 (5.9)	6.7 (6.6)
R_{meas} (%)	0.07 (0.88)	0.08 (2.21)	0.25 (2.26)
Completeness (%)	99.0 (99.0)	99.9 (96.2)	99.8 (97.7)
I/sigI	14.8 (2.1)	10.8 (0.3)	6.2 (0.9)
CC ½	0.999 (0.998)	0.998 (0.293)	0.993 (0.436)
Refinement			
R_{work}/R_{free}	0.175/0.198	0.192/0.236	0.189/0.254
Quaternary structure	dimer	dimer	dimer
R.m.s.d.s			
Bonds (Å)	0.005	0.014	0.015
Angles (Å)	1.27	1.89	2.14
Ramachandran plot, residues in (%)			
Favoured regions	1612 (97%)	804 (97%)	793 (97%)
Allowed regions	53 (3%)	25 (3%)	28 (3%)
Outlier regions	-	1 (0.12%)	-
Mean *B* factors (Å²)			
Protein atoms	27.37	32.28	42.31
Ligands	51.31	36.70	64.69

Supplementary Figure S1. Eight calculated descriptors comparing DSI-Poised, EFSL (EUOS) and FX2_BESSY Fragment Libraries.

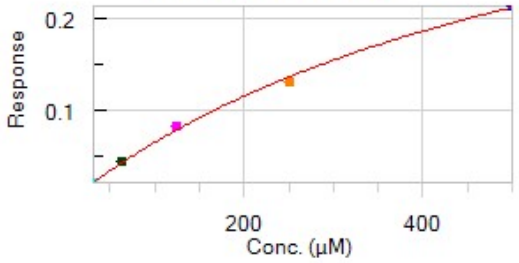


Supplementary Figure S2. Sensorgrams (left) and steady-state plots (right) for both FabF C164A (top) and w.t. (bottom) of the 17 fragment hits from the BLI screening of the EFSL.

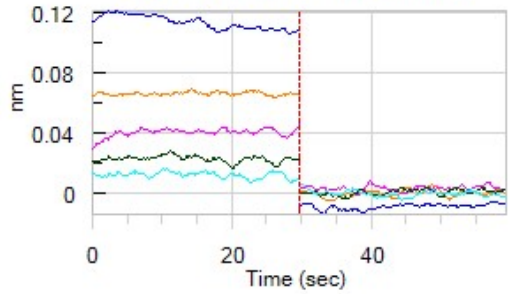
Sample ID: EOS102612; Sensor FabF C164A



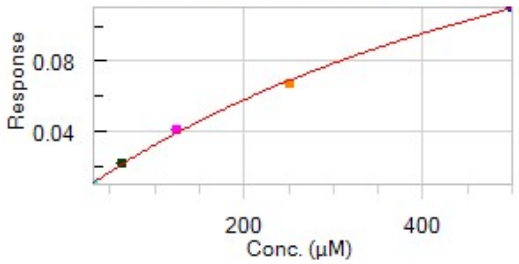
Sample ID: EOS102612; Sensor FabF C164A
K_D = 610 µM; R^2 = 0.9981



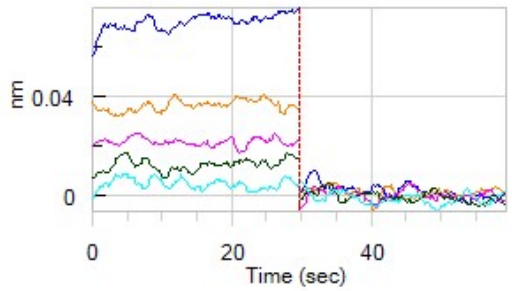
Sample ID: EOS102612; Sensor FabF w.t.



Sample ID: EOS102612; Sensor FabF w.t.
K_D = 730 µM; R^2 = 0.9987



Sample ID: EOS102836; Sensor FabF C164A



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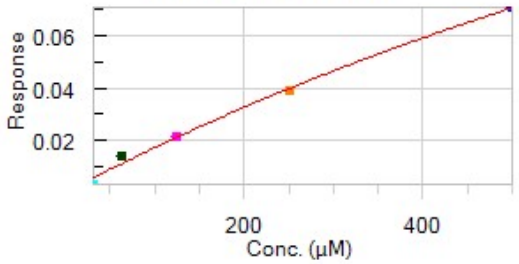
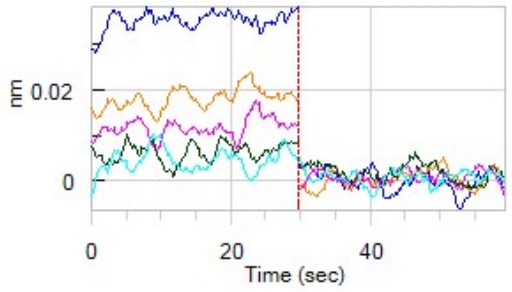


Sample ID: EOS102836; Sensor FabF w.t.



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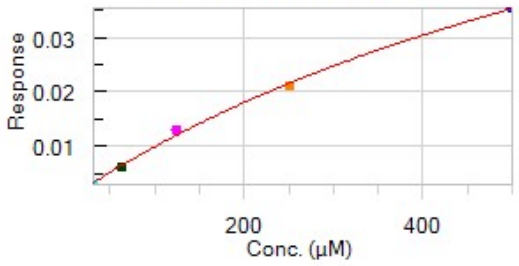
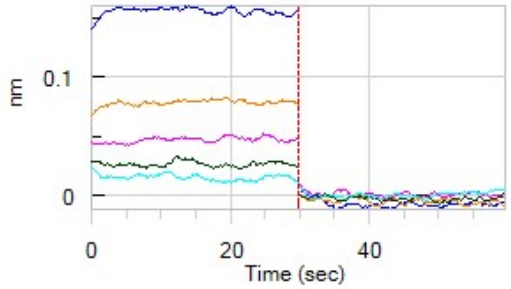
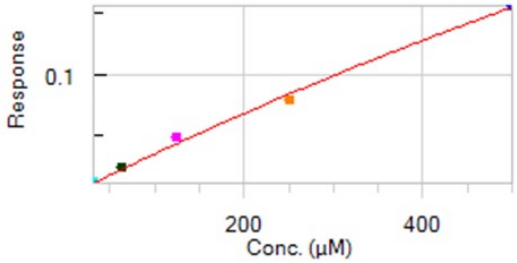


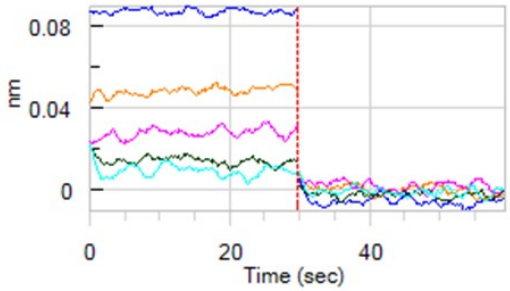
Sample ID: EOS102554; Sensor FabF C164A



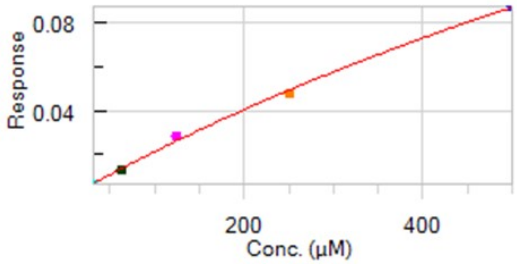
Sample ID: EOS102554; Sensor FabF C164A
K_D = 2700 µM; R^2 = 0.9956



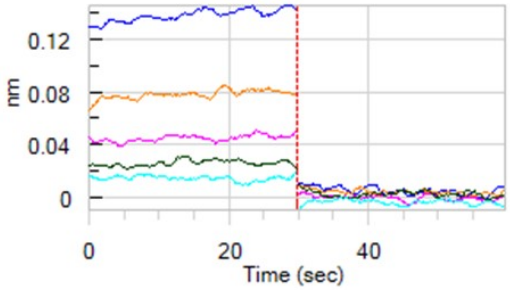
Sample ID: EOS102554; Sensor FabF w.t.



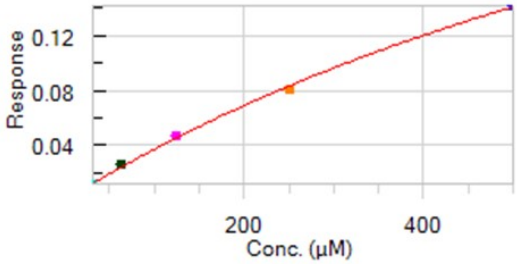
Sample ID: EOS102554; Sensor FabF w.t.
K_D = 1600 µM; R^2 = 0.9987



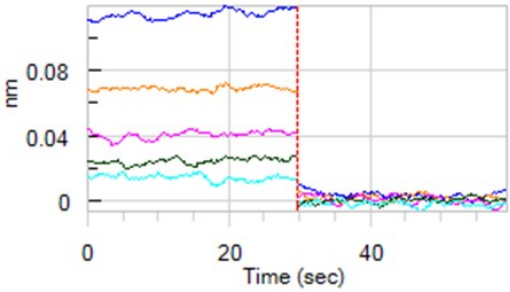
Sample ID: EOS103477; Sensor FabF C164A



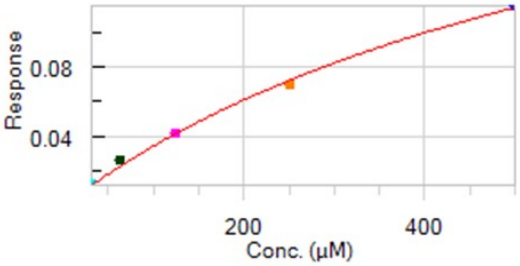
Sample ID: EOS103477; Sensor FabF C164A
K_D = 1100 µM; R^2 = 0.9984



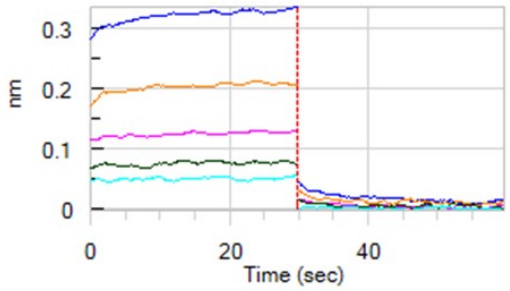
Sample ID: EOS102836; Sensor FabF w.t.



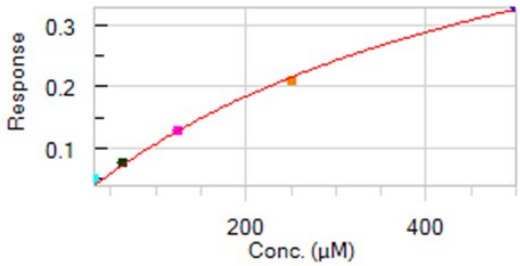
Sample ID: EOS103477; Sensor FabF w.t.
K_D = 690 µM; R^2 = 0.9964



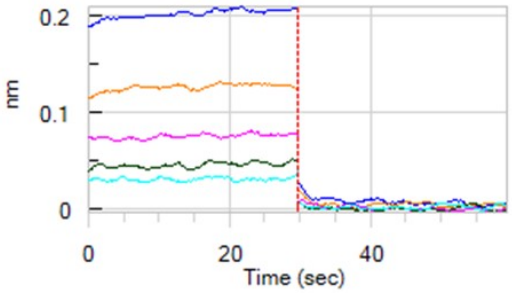
Sample ID: EOS103501; Sensor FabF C164A



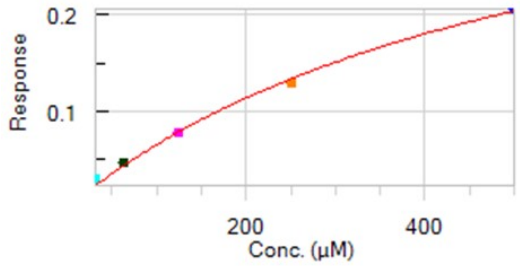
Sample ID: EOS10350; Sensor FabF C164A
K_D = 530 µM; R^2 = 0.9965



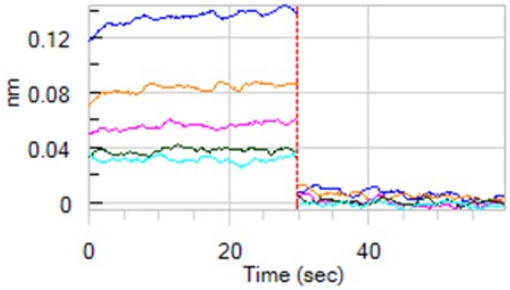
Sample ID: EOS103501; Sensor FabF w.t.



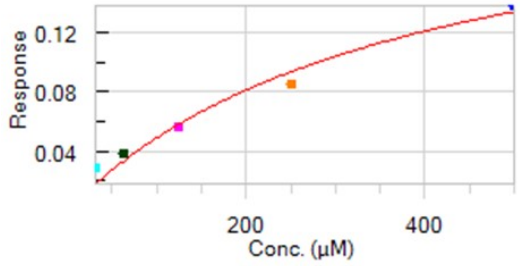
Sample ID: EOS10350; Sensor FabF w.t.
K_D = 560 µM; R^2 = 0.9955



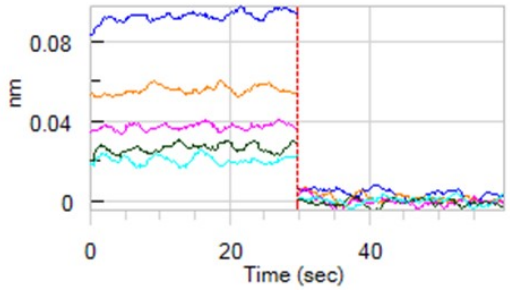
Sample ID: EOS103478; Sensor FabF C164A



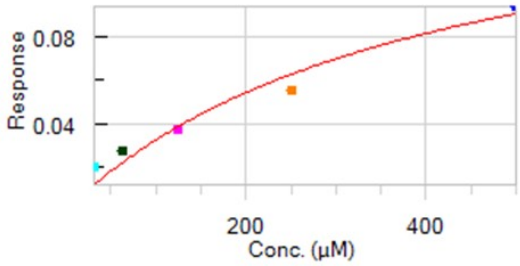
Sample ID: EOS103478; Sensor FabF C164A
K_D = 380 µM; R^2 = 0.9694



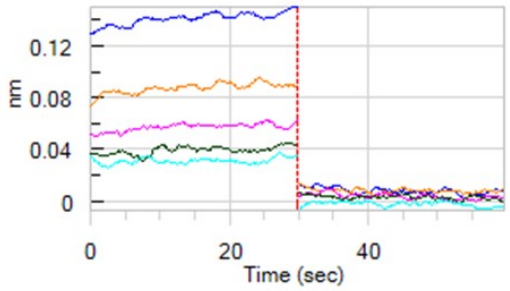
Sample ID: EOS103478; Sensor FabF w.t.



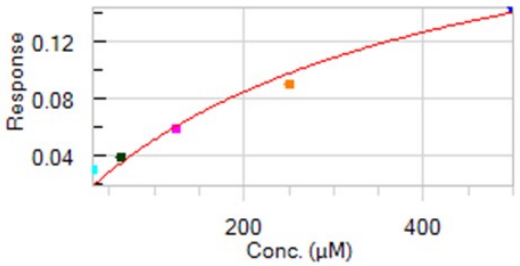
Sample ID: EOS103478; Sensor FabF w.t.
K_D = 400 µM; R^2 = 0.9543



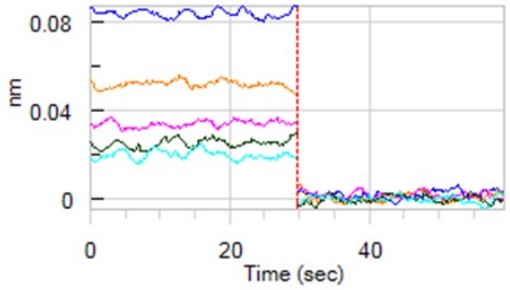
Sample ID: EOS103499; Sensor FabF C164A



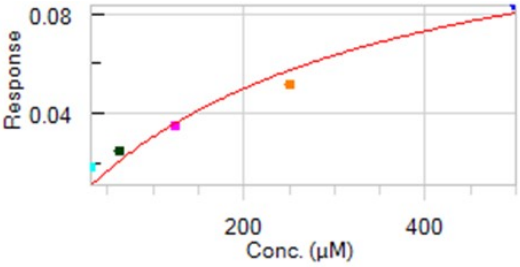
Sample ID: EOS103499; Sensor FabF w.t. K_D = 390 µM; R^2 = 0.9760



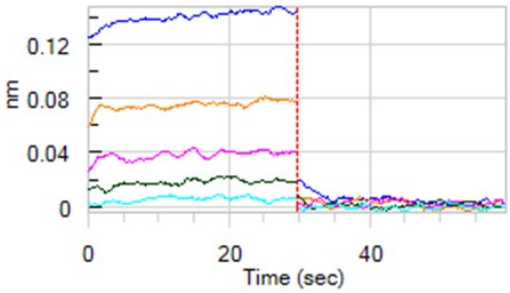
Sample ID: EOS103499; Sensor FabF w.t.



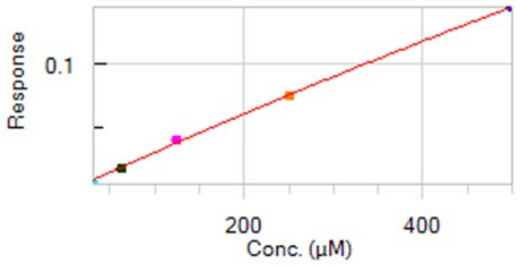
Sample ID: EOS103499; Sensor FabF w.t. K_D = 340 µM; R^2 = 0.9599



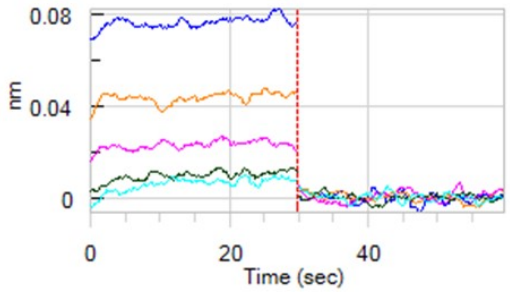
Sample ID: EOS102855; Sensor FabF C164A



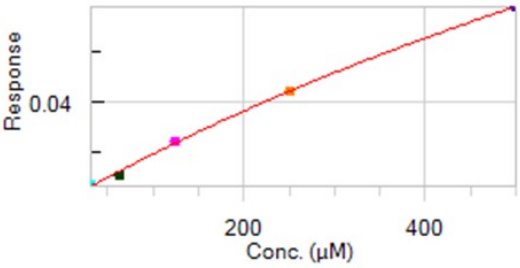
Sample ID: EOS102855; Sensor FabF C164A K_D = 450 µM; R^2 = 0.9989



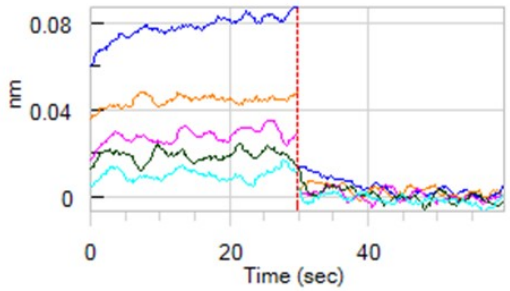
Sample ID: EOS102855; Sensor FabF w.t.



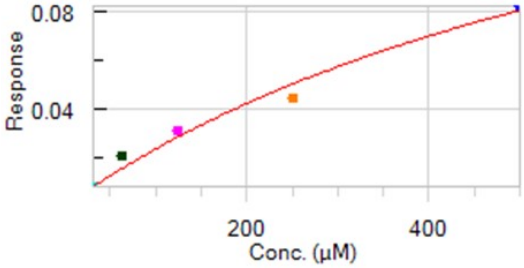
Sample ID: EOS102855; Sensor FabF w.t. K_D = 1600 µM; R^2 = 0.9985



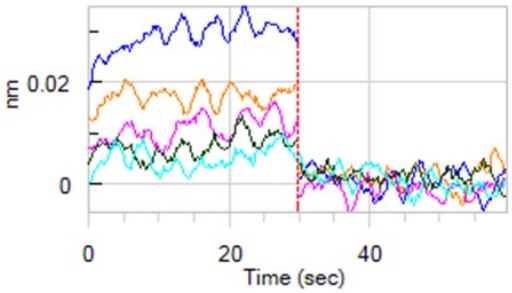
Sample ID: EOS102663; Sensor FabF C164A



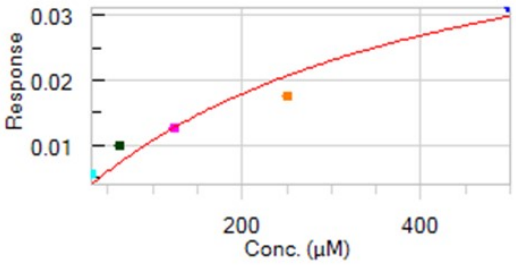
Sample ID: EOS102663; Sensor FabF C164A
K_D = 760 µM; R^2 = 0.9778



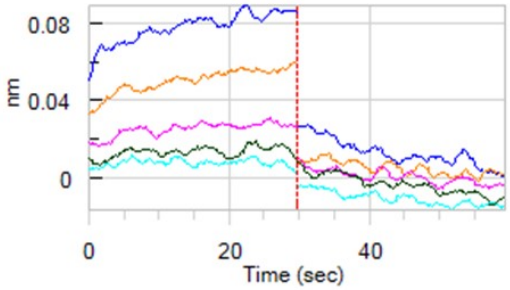
Sample ID: EOS102663; Sensor FabF w.t.



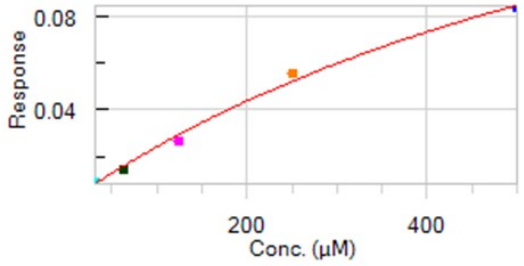
Sample ID: EOS102663; Sensor FabF w.t.
K_D = 400 µM; R^2 = 0.9478



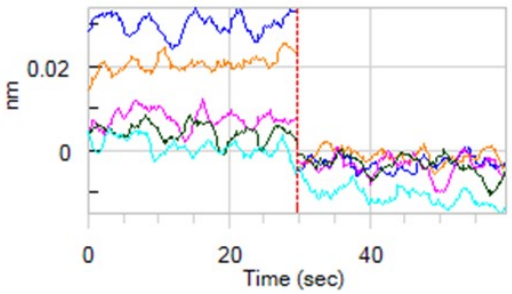
Sample ID: EOS102679; Sensor FabF C164A



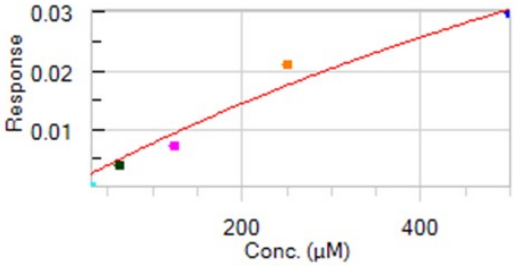
Sample ID: EOS102679; Sensor FabF C164A
K_D = 830 µM; R^2 = 0.9942



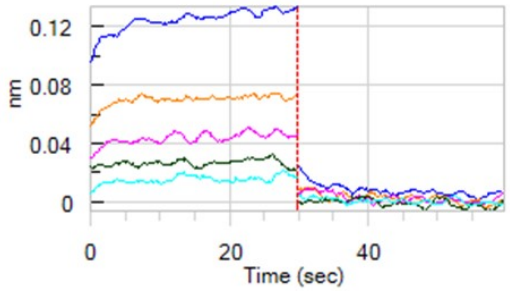
Sample ID: EOS102679; Sensor FabF w. t.



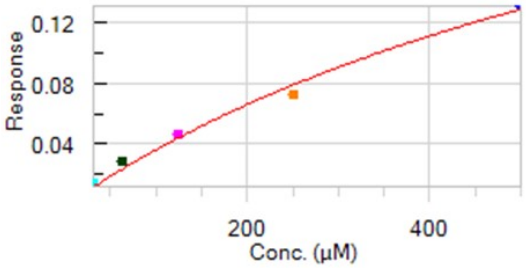
Sample ID: EOS102679; Sensor FabF w.t.
K_D = 1400 µM; R^2 = 0.9608



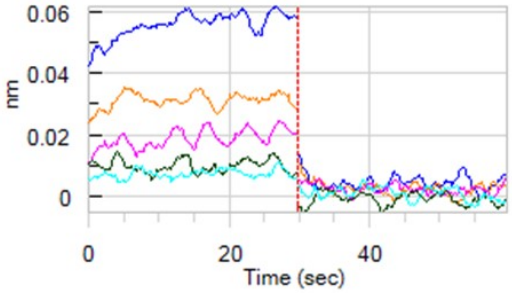
Sample ID: EOS103073; Sensor FabF C164A



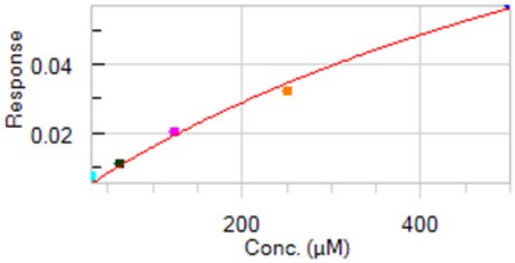
Sample ID: EOS103073; Sensor FabF C164A
K_D = 850 µM; R^2 = 0.9889



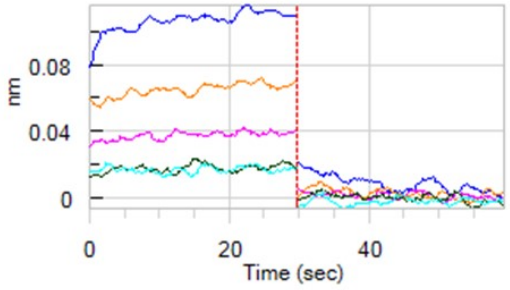
Sample ID: EOS103073; Sensor FabF w.t.



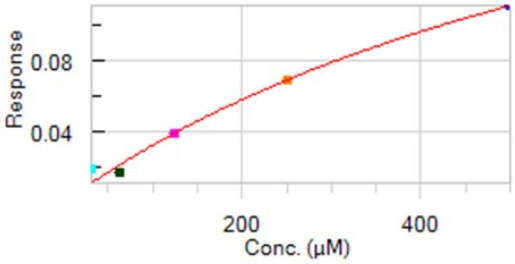
Sample ID: EOS103073; Sensor FabF w.t.
K_D = 870 µM; R^2 = 0.9924



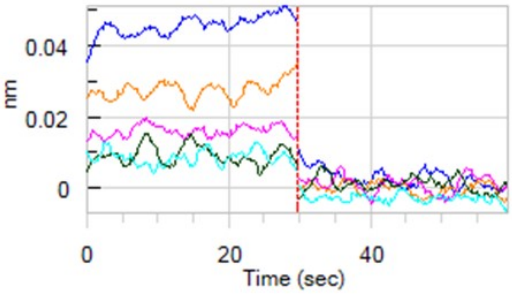
Sample ID: EOS102736; Sensor FabF C164A



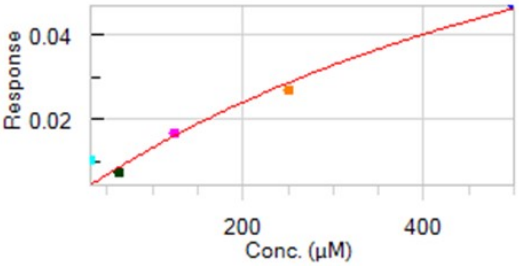
Sample ID: EOS102736; Sensor FabF C164A
K_D = 770 µM; R^2 = 0.9882



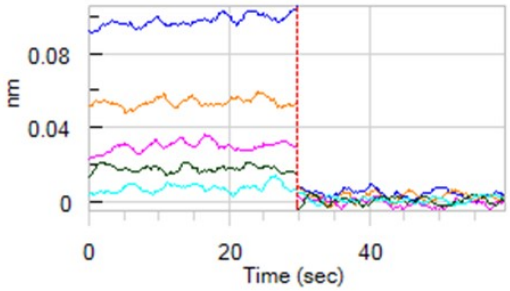
Sample ID: EOS102736; Sensor FabF w.t.



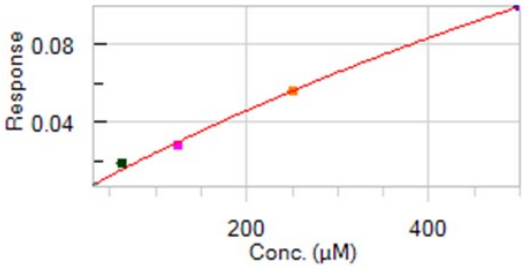
Sample ID: EOS102736; Sensor FabF w.t.
K_D = 790 µM; R^2 = 0.9628



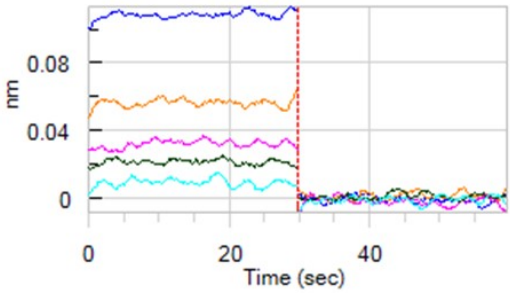
Sample ID: EOS103128; Sensor FabF C164A



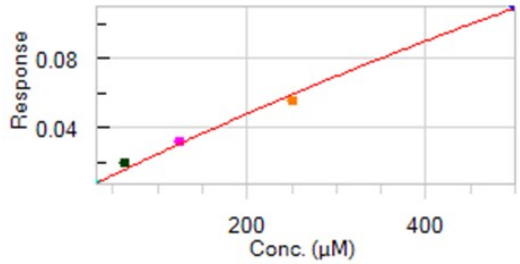
Sample ID: EOS103128; Sensor FabF C164A
K_D = 1700 µM; R^2 = 0.9972



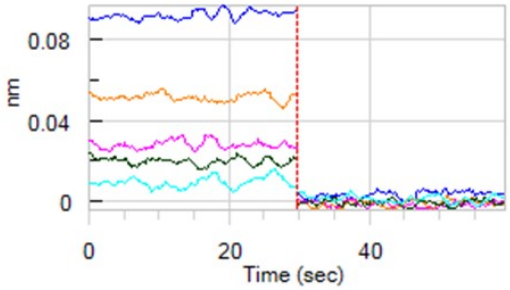
Sample ID: EOS103128; Sensor FabF w.t.



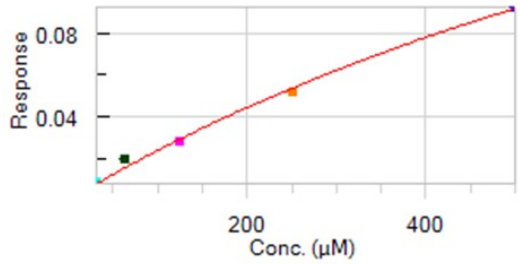
Sample ID: EOS103128; Sensor FabF w.t.
K_D = 2800 µM; R^2 = 0.9950



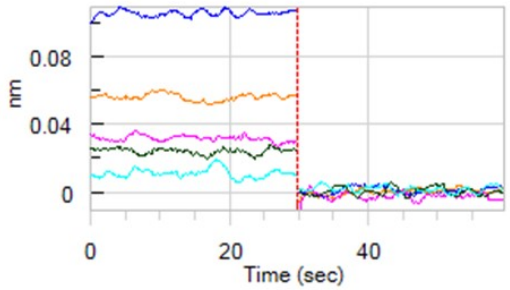
Sample ID: EOS103471; Sensor FabF C164A



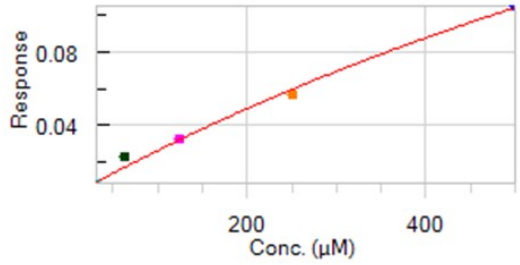
Sample ID: EOS103471; Sensor FabF C164A
K_D = 1200 µM; R^2 = 0.9937



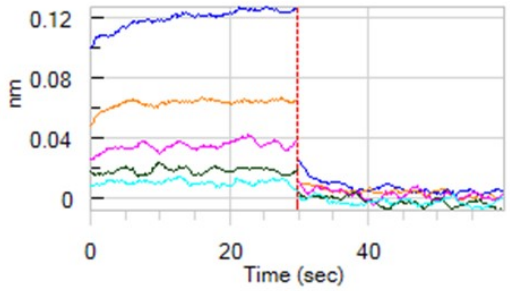
Sample ID: EOS103471; Sensor FabF w.t.



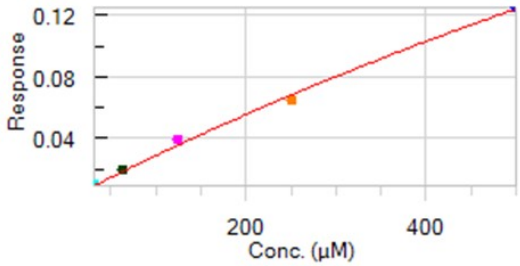
Sample ID: EOS103471; Sensor FabF w.t.
K_D = 1600 µM; R^2 = 0.9912



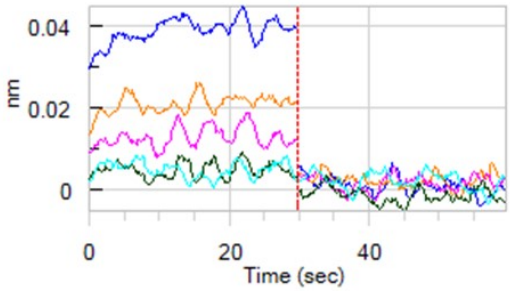
Sample ID: EOS103304; Sensor FabF C164A



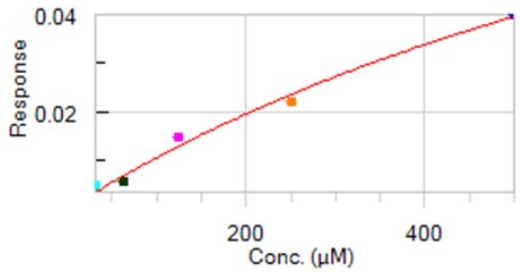
Sample ID: EOS103304; Sensor FabF C164A
K_D = 2300 µM; R^2 = 0.9963



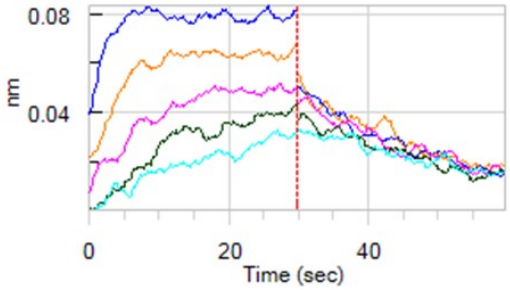
Sample ID: EOS103304; Sensor FabF w.t.



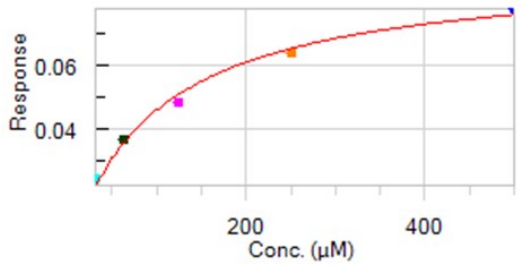
Sample ID: EOS103304; Sensor FabF C164A
K_D = 1100 µM; R^2 = 0.9885



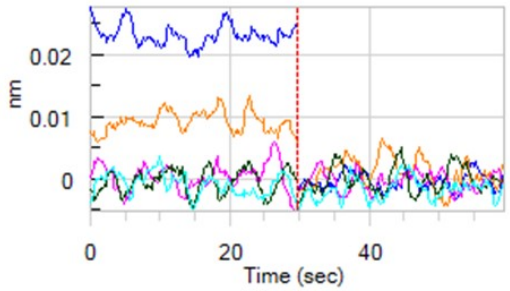
Sample ID: EOS102727; Sensor FabF C164A



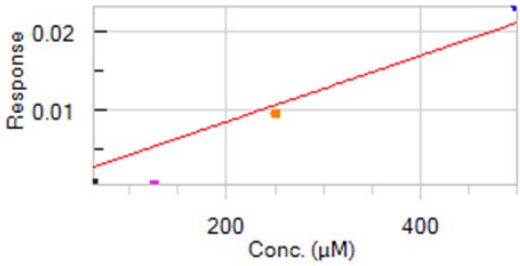
Sample ID: EOS102727; Sensor FabF C164A
K_D = 9.8 µM; R^2 = 0.9895



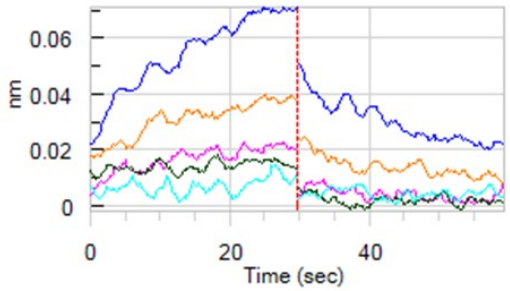
Sample ID: EOS102727; Sensor FabF w.t.



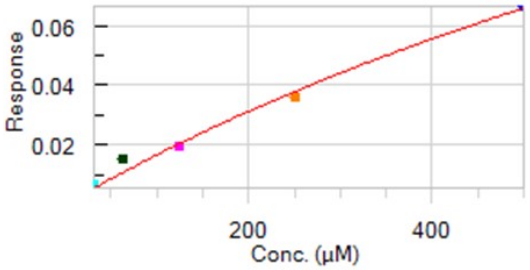
Sample ID: EOS102727; Sensor FabF w.t.
K_D and R^2 could not be determined.



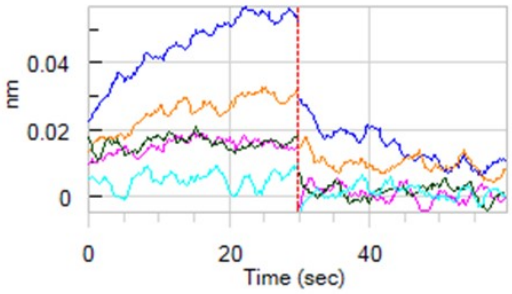
Sample ID: EOS102809; Sensor FabF C164A



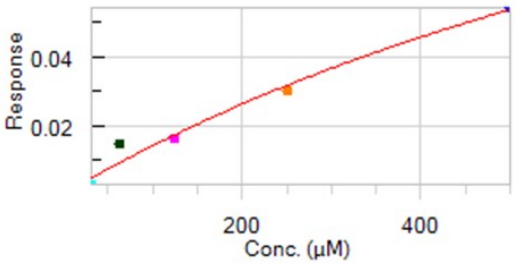
Sample ID: EOS102809; Sensor FabF C164A
K_D = 1400 µM; R^2 = 0.9868



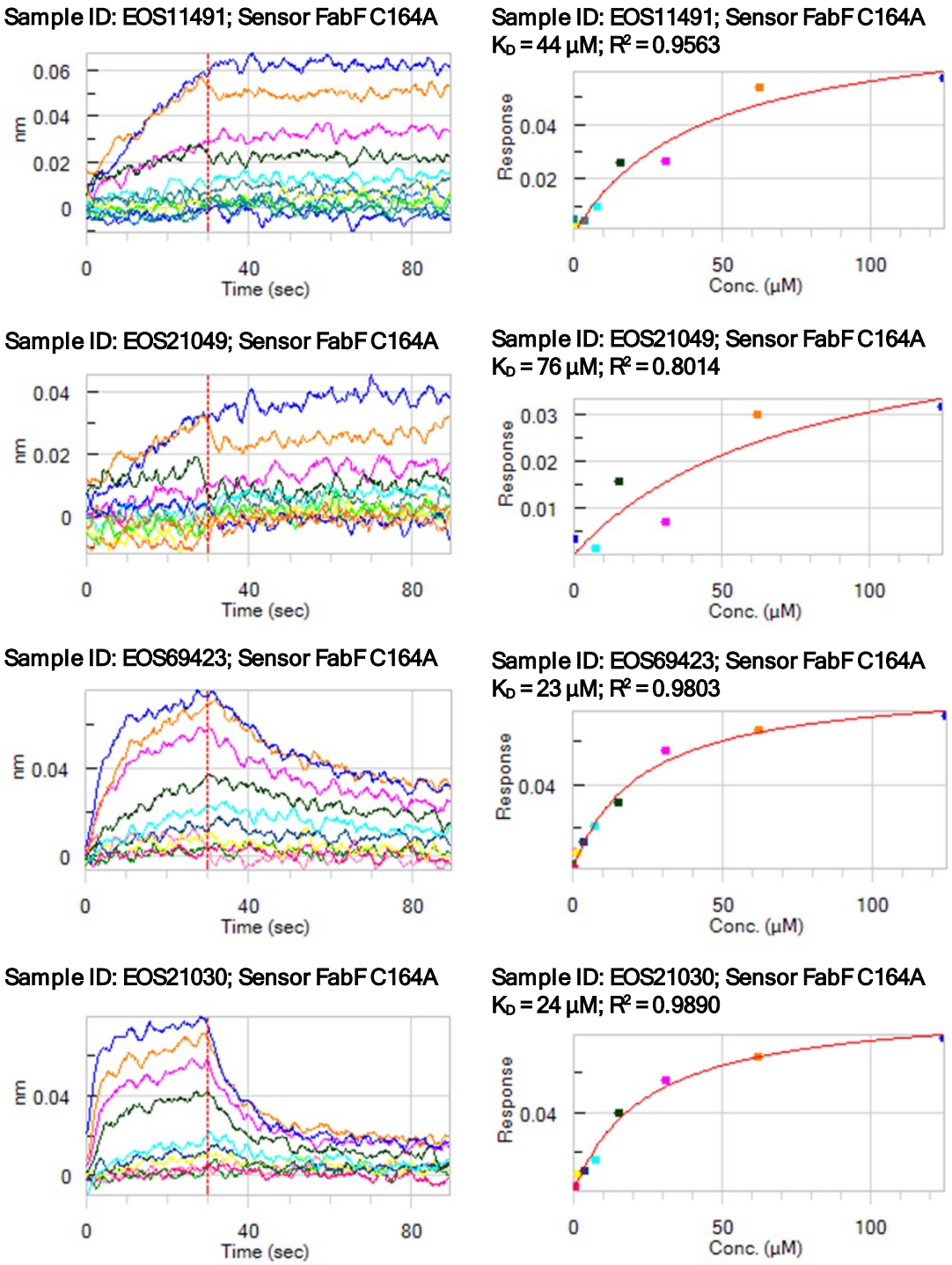
Sample ID: EOS102809; Sensor FabF w.t.



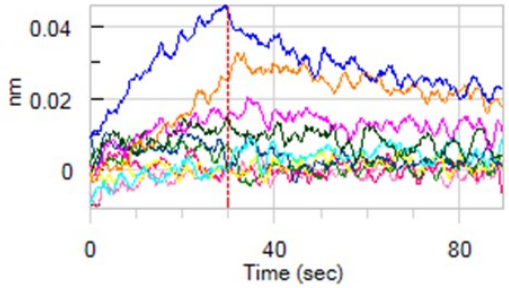
Sample ID: EOS102809; Sensor FabF w.t.
K_D = 1200 µM; R^2 = 0.9758



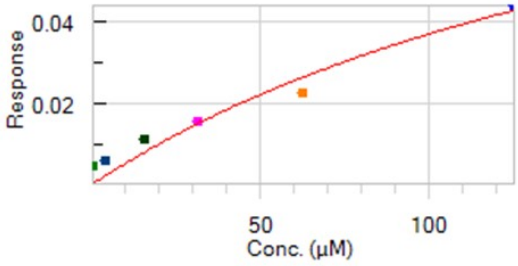
Supplementary Figure S3. Sensorgrams (left) and steady-state plots (right) for both FabF C164A of the 7 hits from the BLI screening of of selected ECBL poised molecules.



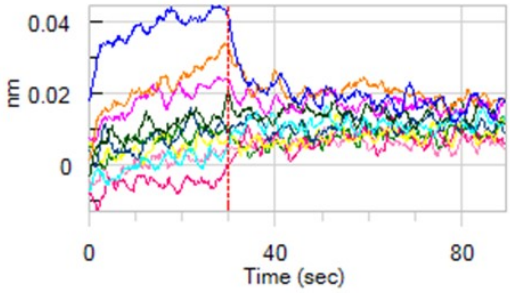
Sample ID: EOS11668; Sensor FabF C164A



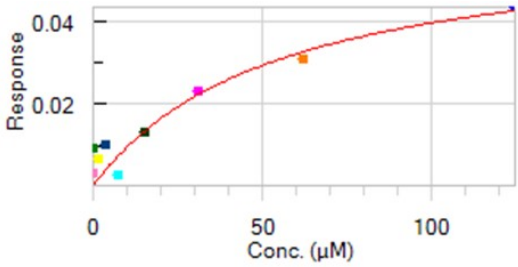
Sample ID: EOS11668; Sensor FabF C164A
K_D = 200 µM; R^2 = 0.9413



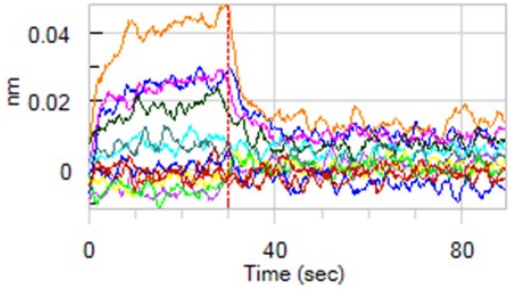
Sample ID: EOS64113; Sensor FabF C164A



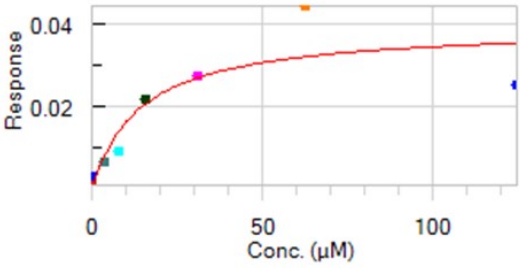
Sample ID: EOS64113; Sensor FabF C164A
K_D = 64 µM; R^2 = 0.8904



Sample ID: EOS21494; Sensor FabF C164A



Sample ID: EOS21494; Sensor FabF C164A
K_D = 15 µM; R^2 = 0.7782



Supplementary Figure S4. Steady-state plots in triplicate of repurchased material of hits **2** (top) and **3** (bottom). Bars indicate range of responses for the same concentration.