Supplementary Information

Probing the Fibrate Binding Specificity of Rat Liver Fatty Acid Binding Protein

Sara Chuang, Tony Velkov, James Horne, Jerome Wielens, David K. Chalmers, Christopher J. H. Porter, Martin J. Scanlon

Contents

Supplementary Table 1: FABP sequence alignments

A

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Human      MSFSGKYQLQSQENFEAFMKAIGLPEELIQKGKDIKGVSEIVQNGKHFKFTITAGSKVIQ 60
Rat        MNFSGKYQVQSQENFEPFMKAMGLPEDLIQKGKDIKGVSEIVHEGKKVKLTITYGSKVIH 60
           *.******:*******.****:****:***************::**:.*:*** *****:

Human      NEFTVGEECELETMTGEKVKTVVQLEGDNKLVTTFKNIKSVTELNGDIITNTMTLGDIVF 120
Rat        NEFTLGEECELETMTGEKVKAVVKMEGDNKMVTTFKGIKSVTEFNGDTITNTMTLGDIVY 120
           ****:***************:**::*****:*****.******:*** ***********:

Human      KRISKRI 127
Rat        KRVSKRI 127
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B

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Heart      --MADAFVGTWKLVDSKNFDDYMKSLGVGFATRQVASMTKPTTIIEKNGDTITIKTHS-T 57
Adipocyte  --MCDAFVGTWKLVSSENFDDYMKEVGVGFATRKVAGMAKPNLIISVEGDLVVIRSES-T 57
Testis     --MIEPFLGTWKLVSSENFENYVRELGVECEPRKVACLIKPSVSISFNGERMDIQAGS-A 57
Epidermal  MASLKDLEGKWRLVESHGFEDYMKELGVGLALRKMGAMAKPDCIITLDNNNLTVKTES-T 59
Liver      ----MNFSGKYQVQSQENFEPFMKAMGLPEDLIQKGKDIKGVSEIVHEGKKVKLTITY-G 55
Ileal      ----MAFTGKYEFESEKNYDEFMKRLGLPDEVIERGRNFKIITEVQQDGENFTWSQSYSG 56
Intestinal ----MAFDGTWKVDRNENYEKFMEKMGINVVKRKLGAHDNLKLTITQEGNKFTVKESS-N 55
                : *.:..  ...:: ::. :*:     : .   :    :  :.. .

Heart      FKNTEISFQLGVEFDEVTADDRKVKSVVTLDGGKLVHVQKWD--GQETTLTRELSDGKLI 115
Adipocyte  FKNTEISFKLGVEFDEITPDDRKVKSIITLDGGVLVHVQKWD--GKSTTIKKRRDGDKLV 115
Testis     CRNTEISFKLGEEFEETTADNRKVKSLITFEGGSMIQIQRWL--GKQTTIKRRIVDGRMV 115
Epidermal  VKTTVFSCTLGEKFDETTADGRKTETVCTFTDGALVQHQKWE--GKESTITRKLKDGKMV 117
Liver      SKVIHNEFTLGEECELETMTGEKVKAVVKMEGD-NKMVTTFK--GIKSVT--EFNGDTIT 110
Ileal      GNIMSNKFTIGKECEMQTMGGKKFKATVKMEG--GKVVADFP--NYHQTS--EVVGDKLV 110
Intestinal FRNIDVVFELGVDFAYSLADGTELTGTWTMEGNKLVGKFKRVDNGKELIAVREISGNELI 115
            .       :* .       . :     .: .            .       .  .. :

Heart      LTLTHGNVVSTRTYEKEA 133
Adipocyte  VECVMKGVTSTRVYERA- 132
Testis     VECTMNNVVSTRTYERV- 132
Epidermal  VECVMNNAICTRVYEKVQ 135
Liver      NTMTLGDIVYKRVSKRI- 127
Ileal      EISTIGDVTYERVSKRVA 128
Intestinal QTYTYEGVEAKRIFKKE- 132
              .  .    *  ::
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(A) Sequence alignment of the primary structures of rat FABP proteins. (* = conservative residue, : = conserved substitution, . = semi-conserved substitution. Alignment conducted using Cluster W 2.0) (B) Comparison of human and rat L-FABP. Sequence alignment of the primary structures of L-FABP.

Supplementary Table 2. Binding site occupancy

Percentage occupancy at each site for rL-FABP (300 μM) at a 3-fold molar excess of each of the fibrate drugs used in the current study.

Ligand	% Occupancy 1st Site	2nd Site
Fenofibrate	100	100
Gemfibrozil	100	79
Bezafibrate	N/A	93
Ciprofibrate	100	85
Clofibrate	99	N/A
Fenofibric Acid	100	96

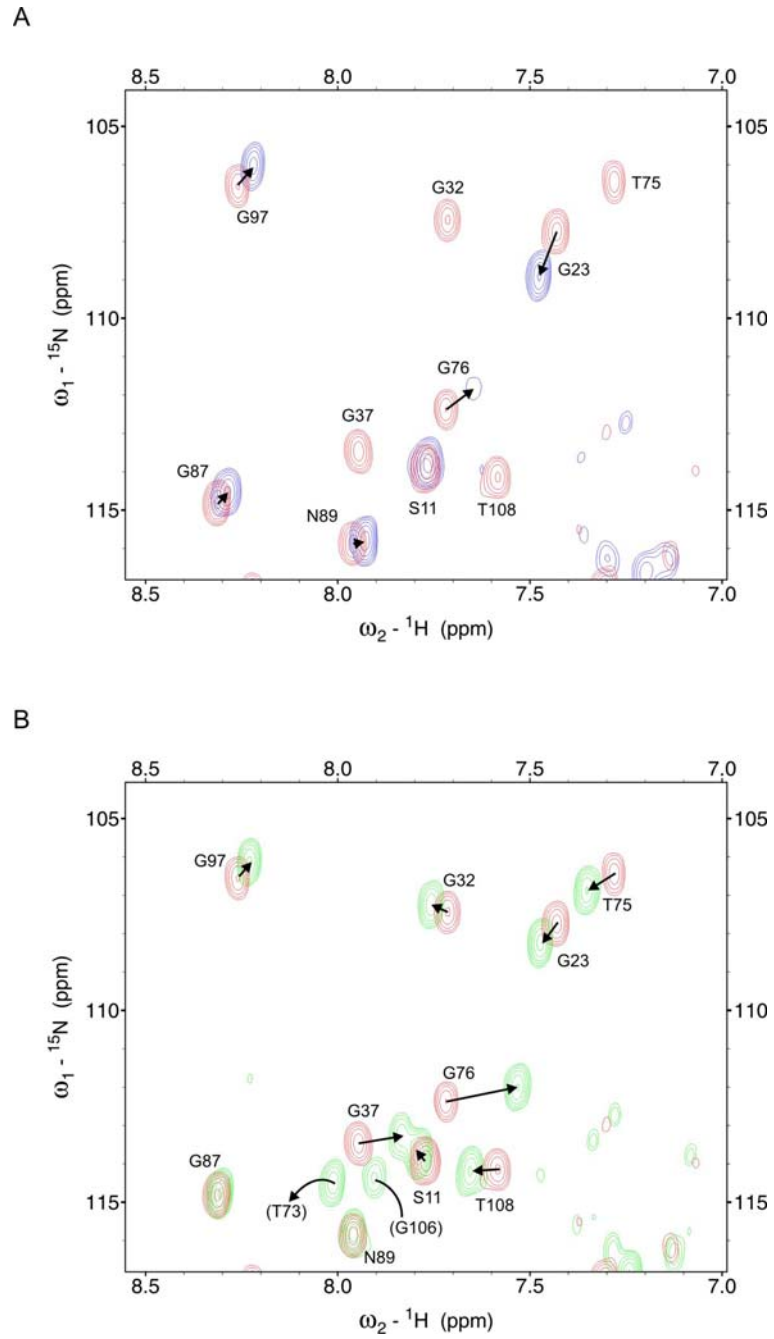
Supplementary Table 3. Temperature dependence of binding affinities

Binding affinity constants of fenofibric acid, fenofibrate and clofibrate for L-FABP measured from steady state fluoresecence at different temperatures, used for van't Hoff determination of thermodynamic parameters.

Temperature (°C)	Fenofibric Acid (μM)	Fenofibrate (μM)	Clofibrate (μM)
5	K_{d1}=0.094 ± 0.01 K_{d2}=18 ± 2.2	K_{d1}=0.018 ± 0.0020 K_{d2}=0.13 ± 0.020	K_{d1}=6.0 ± 2.9
10	K_{d1}=0.10 ± 0.02 K_{d2}=19 ± 1.9	K_{d1}=0.023 ± 0.0010 K_{d2}=0.15 ± 0.010	K_{d1}=6.7 ± 1.7
15	K_{d1}=0.16 ± 0.04 K_{d2}=20 ± 1.8	K_{d1}=0.024 ± 0.0020 K_{d2}=0.16 ± 0.020	K_{d1}=7.3 ± 1.4
20	K_{d1}=0.22 ± 0.030 K_{d2}=23 ± 2.0	K_{d1}=0.027 ± 0.0030 K_{d2}=0.20 ± 0.020	K_{d1}=7.8 ± 2.5
25	K_{d1}=0.34 ± 0.020 K_{d2}=24.0 ± 2.1	K_{d1}=0.032 ± 0.0040 K_{d2}=0.25 ± 0.020	K_{d1}=8.9 ± 2.8
30	K_{d1}=0.36 ± 0.040 K_{d2}=27 ± 2.5	K_{d1}=0.041 ± 0.0040 K_{d2}=0.27 ± 0.040	K_{d1}=9.6 ± 1.9
37	K_{d1}=0.42 ± 0.030 K_{d2}=28 ± 3.0	K_{d1}=0.062 ± 0.006 K_{d2}=0.32 ± 0.030	K_{d1}=11 ± 3.4
42	K_{d1}=ND K_{d2}=29 ± 3.5	K_{d1}=ND K_{d2}=0.36 ± 0.040	K_{d1}=12 ± 3.0

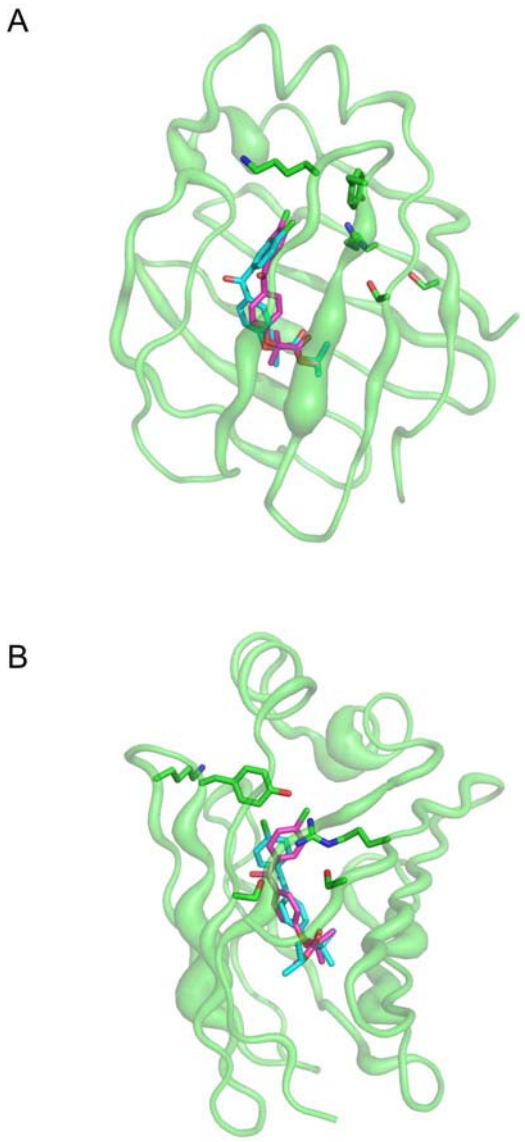
K_{d1} High affinity site; K_{d2} low affinity site; ND, Not detected; Affinity values are the mean ± the standard deviation of three independent measurements.

Supplementary Figure 1. ^{1}H-^{15}N HSQC data for rL-FABP



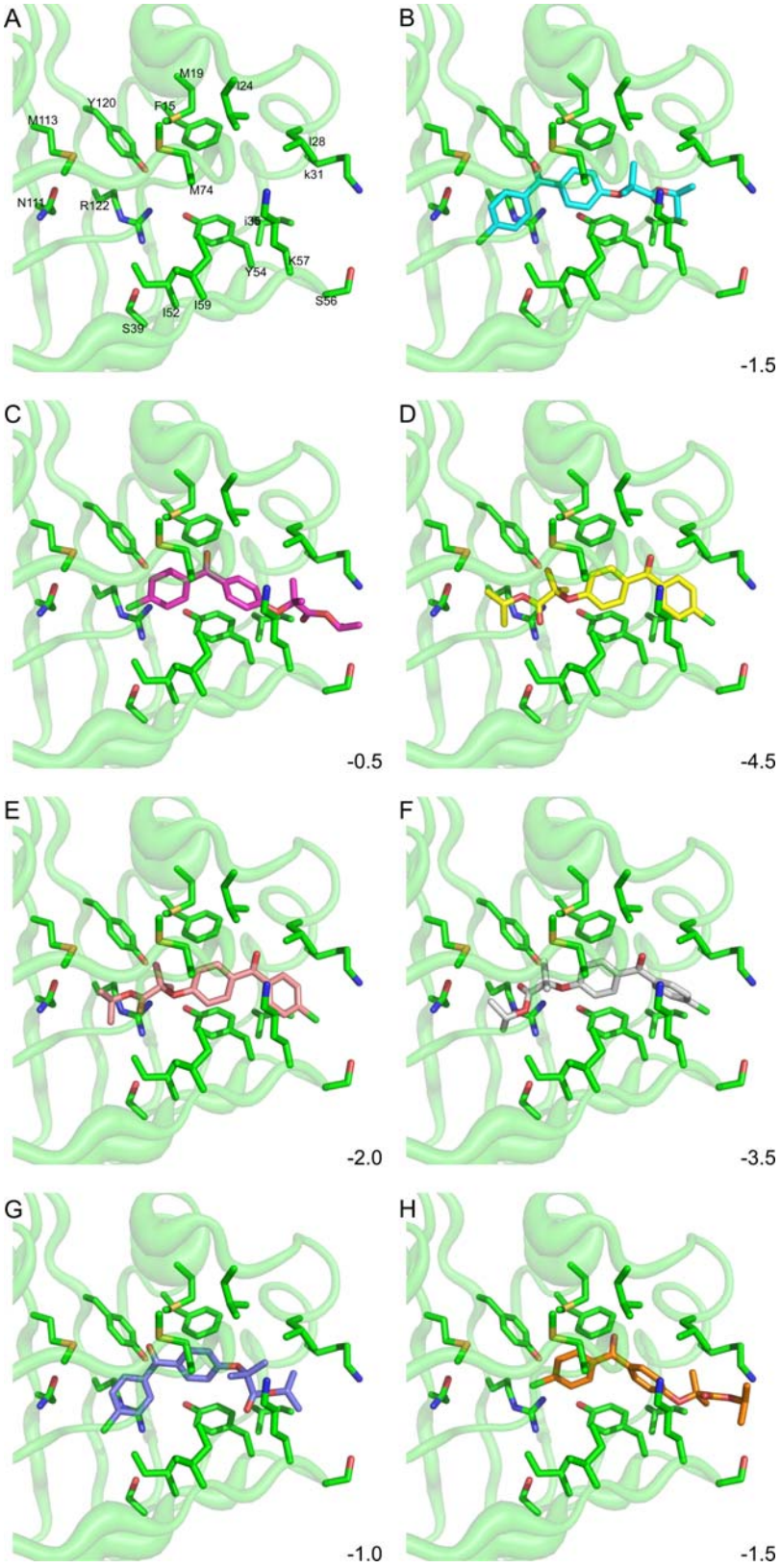
A portion of the HSQC spectrum of apo rL-FABP is shown in red and labeled with the assignment of each peak. (A) The HSQC spectrum of rL-FABP in the presence of three molar equivalents of clofibrate (green) is overlaid onto the spectrum of apo rL-FABP. The presence of clofibrate results in significant perturbation of several of the peaks. (B) The HSQC spectrum of rL-FABP in the presence of three molar equivalents of fenofibric acid (blue) is overlaid onto the spectrum of apo rL-FABP. Some peaks are perturbed upon addition of fenofibric acid, others are perturbed and demonstrate significant broadening (e.g. G76), whilst some are broadened beyond the limit of detection in the experiment (e.g. G32, G37, T75, T108).

Supplementary Figure 2. Docking of fenofibric acid methyl ester



A. A comparison of the docking clusters of fenofibrate (light blue carbon atoms) binding at the high affinity site of rL-FABP with those obtained for the methyl ester of fenofibric acid (magenta carbon atoms), which was docked using the same protocol. The poses for the two compounds are similar, suggesting that for these two compounds, the nature of the small aliphatic group in of the ester is not a dominating factor in driving the binding orientation. **B.** 90 degree rotation of the same.

Supplementary Figure 3. Docking clusters for fenofibrate



The seven docking clusters of fenofibrate along with their scores based on correlation with the CSPs observed in the NMR data.