

## Supplementary Materials

**Figure S1.** Multiple sequence alignment with predicted local (secondary) structures.

Human_PREPL	1	MQ <b>Q</b> KT <b>K</b> FL <b>L</b> Q <b>A</b> L <b>K</b> Y <b>S</b> I <b>P</b> H <b>L</b> G <b>K</b> C <b>M</b> Q <b>K</b> Q <b>H</b> L <b>N</b> H <b>Y</b> N <b>F</b> A <b>D</b> H <b>C</b> Y <b>N</b> R <b>I</b> K <b>L</b> K <b>K</b> Y <b>H</b> L <b>T</b> -	49
Human_POP	1	ML-----S <b>F</b> Q <b>Y</b> P <b>D</b> V <b>R</b> D <b>E</b> T <b>A</b> V <b>Q</b> D <b>Y</b> H <b>G</b> -	21
OPB_TB	1	-----M <b>Q</b> T <b>E</b> R <b>G</b> P <b>I</b> A <b>A</b> H <b>R</b> P <b>H</b> E <b>V</b> V <b>F</b> G <b>K</b> V	21
<u>Consensus ss:</u>		e <b>eeee</b>	
Human_PREPL	50	-----K <b>L</b> Q <b>N</b> K <b>P</b> K <b>I</b> S <b>E</b> L <b>A</b> R <b>N</b> I <b>P</b> S <b>R</b> S <b>F</b> S <b>C</b> K <b>D</b> L <b>Q</b> P <b>V</b> K <b>Q</b> E <b>N</b> E <b>K</b> P <b>L</b>	86
Human_POP	22	-----H <b>K</b> I <b>C</b> D <b>P</b> Y <b>A</b> W <b>L</b> E <b>D</b> P <b>D</b> -----S <b>E</b> Q <b>T</b> K <b>A</b> F <b>V</b> E <b>A</b> Q <b>N</b> K <b>I</b> T <b>V</b>	51
OPB_TB	22	E <b>G</b> E <b>D</b> R <b>G</b> A <b>N</b> P <b>M</b> D <b>P</b> P <b>R</b> K <b>V</b> D <b>P</b> L <b>F</b> W <b>L</b> R <b>D</b> D <b>N</b> -----R <b>A</b> D <b>P</b> E <b>V</b> L <b>A</b> H <b>L</b> H <b>L</b> E <b>K</b> D <b>Y</b> E	66
<u>Consensus ss:</u>		e <b>eee</b> <b>hhh</b> <b>hhhhhhhhhhhhhhhh</b>	
Human_PREPL	87	<b>P</b> E <b>N</b> M <b>---</b> D <b>A</b> F <b>E</b> K <b>V</b> R <b>T</b> K <b>L</b> E <b>T</b> Q <b>P</b> Q <b>E</b> E <b>Y</b> E <b>I</b> I <b>N</b> V <b>E</b> V <b>K</b> H <b>G</b> G <b>F</b> V <b>Y</b> Y <b>Q</b> E <b>G</b> C <b>---</b> C <b>L</b> V	130
Human_POP	52	<b>P</b> F <b>L</b> E <b>Q</b> C <b>-</b> P <b>I</b> R <b>G</b> L <b>Y</b> K <b>E</b> R <b>M</b> T <b>E</b> L <b>Y</b> D <b>Y</b> P <b>K</b> <b>--</b> Y <b>S</b> C <b>H</b> F <b>K</b> K <b>G</b> R <b>Y</b> F <b>Y</b> F <b>Y</b> N <b>T</b> G <b>L</b> Q <b>N</b> Q <b>R</b>	98
OPB_TB	67	<b>K</b> R <b>A</b> V <b>D</b> I <b>K</b> D <b>L</b> A <b>E</b> T <b>I</b> Y <b>Q</b> E <b>H</b> I <b>S</b> H <b>I</b> E <b>E</b> T <b>D</b> <b>--</b> M <b>S</b> A <b>P</b> Y <b>V</b> Y <b>D</b> R <b>F</b> L <b>Y</b> I <b>T</b> R <b>D</b> V <b>K</b> G <b>L</b> S <b>Y</b> K	114
<u>Consensus ss:</u>		<b>hhhh</b> <b>hhhhhhhhhhhhhh</b> <b>eee</b> <b>eeeeeee</b> <b>ee</b>	
Human_PREPL	131	<b>R</b> S <b>K</b> D <b>E</b> E <b>A</b> D <b>----</b> N <b>D</b> Y <b>E</b> V <b>L</b> F <b>N</b> L <b>E</b> L <b>K</b> L <b>D</b> Q <b>P</b> <b>---</b> F <b>I</b> D <b>C</b> I <b>R</b> V <b>A</b> P <b>D</b> E <b>K</b> Y <b>V</b> A <b>A</b>	172
Human_POP	99	V <b>L</b> Y <b>V</b> Q <b>D</b> S <b>L</b> <b>----</b> E <b>G</b> E <b>A</b> R <b>V</b> F <b>L</b> D <b>P</b> N <b>I</b> L <b>S</b> D <b>D</b> G <b>T</b> V <b>--</b> A <b>L</b> R <b>G</b> Y <b>A</b> F <b>S</b> E <b>D</b> G <b>E</b> Y <b>F</b> A <b>Y</b>	141
OPB_TB	115	L <b>H</b> C <b>R</b> V <b>P</b> A <b>G</b> K <b>T</b> P <b>G</b> E <b>G</b> E <b>D</b> E <b>I</b> V <b>L</b> D <b>E</b> N <b>K</b> L <b>A</b> E <b>G</b> K <b>S</b> F <b>C</b> V <b>V</b> G <b>C</b> V <b>A</b> P <b>A</b> P <b>P</b> E <b>H</b> A <b>L</b> V <b>A</b> Y	164
<u>Consensus ss:</u>		<b>eeeeee</b> <b>eee</b> <b>hhh</b> <b>ee</b> <b>eeeeeee</b> <b>eeee</b>	
Human_PREPL	173	<b>K</b> I <b>R</b> T <b>E</b> D <b>S</b> E <b>A</b> S <b>T</b> C <b>V</b> I <b>I</b> K <b>L</b> S <b>D</b> Q <b>P</b> V <b>M</b> E <b>A</b> S <b>F</b> P <b>N</b> V <b>S</b> S <b>F</b> E <b>W</b> V <b>K</b> D <b>E</b> E <b>D</b> E <b>D</b> V <b>L</b> F <b>Y</b> T <b>F</b> Q	222
Human_POP	142	<b>G</b> L <b>S</b> A <b>S</b> G <b>S</b> D <b>W</b> V <b>T</b> I <b>K</b> F <b>M</b> K <b>V</b> D <b>G</b> A <b>K</b> E <b>L</b> P <b>D</b> V <b>L</b> E <b>R</b> V <b>K</b> F <b>S</b> C <b>M</b> A <b>W</b> T <b>H</b> D <b>G</b> K <b>M</b> F <b>Y</b> N <b>S</b> Y <b>P</b>	191
OPB_TB	165	<b>S</b> V <b>D</b> Y <b>C</b> G <b>D</b> E <b>V</b> S <b>I</b> R <b>F</b> V <b>R</b> D <b>V</b> V <b>A</b> D <b>K</b> V <b>E</b> G <b>T</b> <b>----</b> N <b>G</b> S <b>V</b> V <b>W</b> G <b>P</b> N <b>A</b> E <b>C</b> F <b>F</b> Y <b>I</b> T <b>K</b> D	209
<u>Consensus ss:</u>		<b>ee</b> <b>eeeeeeee</b> <b>eeee</b> <b>eeeeee</b> <b>eeeeeeee</b>	
Human_PREPL	223	R <b>-----</b> N <b>L</b> R <b>C</b> H <b>D</b> V <b>R</b> A <b>T</b> F <b>G</b> D <b>N</b> K <b>R</b> <b>-</b> N <b>E</b> R <b>F</b> Y <b>T</b> E <b>K</b> D <b>-</b> P <b>S</b> Y <b>F</b> V <b>F</b> L <b>Y</b> L <b>T</b>	259
Human_POP	192	Q <b>Q</b> D <b>G</b> K <b>S</b> D <b>G</b> T <b>E</b> T <b>S</b> T <b>N</b> L <b>H</b> Q <b>K</b> L <b>Y</b> H <b>V</b> L <b>G</b> T <b>D</b> Q <b>S</b> E <b>D</b> I <b>L</b> C <b>A</b> E <b>F</b> P <b>D</b> E <b>P</b> K <b>W</b> M <b>G</b> G <b>A</b> E <b>L</b> S	241
OPB_TB	210	A <b>-----</b> S <b>K</b> R <b>D</b> N <b>K</b> V <b>R</b> H <b>I</b> I <b>G</b> Q <b>P</b> Q <b>S</b> E <b>D</b> V <b>C</b> L <b>Y</b> T <b>D</b> D <b>D</b> <b>-</b> P <b>L</b> F <b>S</b> V <b>G</b> V <b>G</b> K <b>S</b>	247
<u>Consensus ss:</u>		<b>eeeeeeee</b> <b>eeee</b> <b>eeeeeeee</b>	
Human_PREPL	260	<b>K</b> D <b>S</b> R <b>F</b> L <b>T</b> I <b>N</b> I <b>M</b> N <b>K</b> T <b>T</b> S <b>E</b> <b>--</b> V <b>W</b> L <b>I</b> D <b>G</b> L <b>S</b> P <b>W</b> D <b>P</b> V <b>L</b> I <b>Q</b> K <b>R</b> I <b>H</b> G <b>----</b> V <b>-</b> L <b>Y</b>	301
Human_POP	242	<b>D</b> D <b>G</b> R <b>Y</b> V <b>L</b> L <b>S</b> I <b>R</b> E <b>G</b> C <b>D</b> P <b>V</b> N <b>R</b> L <b>W</b> Y <b>C</b> D <b>L</b> Q <b>Q</b> E <b>S</b> S <b>G</b> I <b>A</b> G <b>I</b> L <b>K</b> W <b>V</b> K <b>L</b> I <b>D</b> N <b>F</b> E <b>G</b> E <b>Y</b> D	291
OPB_TB	248	<b>G</b> D <b>G</b> K <b>T</b> L <b>I</b> I <b>C</b> S <b>M</b> S <b>S</b> E <b>T</b> S <b>E</b> S <b>L</b> L <b>L</b> D <b>L</b> R <b>K</b> G <b>V</b> K <b>H</b> N <b>T</b> L <b>E</b> M <b>V</b> R <b>P</b> R <b>E</b> K <b>G</b> <b>----</b> V <b>R</b> Y <b>T</b>	292
<u>Consensus ss:</u>		<b>eeeeee</b> <b>eeeeee</b> <b>eeee</b> <b>ee</b>	
Human_PREPL	302	<b>Y</b> V <b>E</b> H <b>R</b> D <b>D</b> E <b>L</b> Y <b>I</b> L <b>T</b> N <b>V</b> G <b>E</b> P <b>T</b> E <b>F</b> K <b>L</b> M <b>R</b> T <b>A</b> A <b>D</b> T <b>P</b> A <b>I</b> M <b>N</b> W <b>D</b> L <b>F</b> F <b>T</b> M <b>K</b> R <b>N</b> T <b>K</b> V <b>I</b> D	351
Human_POP	292	<b>Y</b> V <b>T</b> N <b>E</b> G <b>A</b> V <b>F</b> T <b>F</b> K <b>T</b> N <b>R</b> Q <b>-</b> S <b>P</b> N <b>Y</b> R <b>V</b> I <b>N</b> I <b>D</b> F <b>R</b> D <b>P</b> E <b>S</b> K <b>W</b> K <b>V</b> L <b>V</b> P <b>E</b> H <b>E</b> K <b>D</b> V <b>L</b> E <b>W</b>	340
OPB_TB	293	<b>V</b> E <b>M</b> H <b>G</b> T <b>D</b> L <b>I</b> V <b>L</b> T <b>N</b> K <b>D</b> K <b>C</b> V <b>N</b> G <b>K</b> V <b>V</b> L <b>T</b> K <b>R</b> S <b>A</b> P <b>T</b> <b>-</b> D <b>W</b> G <b>T</b> V <b>L</b> I <b>P</b> H <b>D</b> D <b>K</b> V <b>T</b> I <b>D</b> D	341
<u>Consensus ss:</u>		<b>eee</b> <b>eeeeee</b> <b>eeeeeeee</b> <b>eeeeee</b> <b>eeeeee</b>	
Human_PREPL	352	<b>L</b> D <b>M</b> F <b>K</b> <b>-</b> D <b>H</b> C <b>V</b> L <b>F</b> L <b>K</b> H <b>S</b> N <b>L</b> L <b>Y</b> V <b>N</b> V <b>I</b> G <b>L</b> A <b>D</b> <b>----</b> D <b>S</b> V <b>R</b> S <b>L</b> K <b>L</b> P <b>P</b> W <b>----</b> A	390
Human_POP	341	<b>I</b> A <b>C</b> V <b>R</b> S <b>N</b> F <b>L</b> V <b>L</b> C <b>Y</b> L <b>H</b> D <b>V</b> K <b>N</b> I <b>L</b> Q <b>L</b> H <b>L</b> T <b>T</b> <b>----</b> G <b>A</b> L <b>L</b> K <b>T</b> F <b>P</b> L <b>D</b> V <b>----</b> G	380
OPB_TB	342	<b>V</b> A <b>V</b> F <b>A</b> <b>-</b> K <b>F</b> A <b>V</b> L <b>S</b> G <b>R</b> R <b>D</b> G <b>L</b> T <b>R</b> V <b>W</b> T <b>V</b> R <b>L</b> G <b>P</b> D <b>S</b> L <b>F</b> S <b>S</b> A <b>T</b> L <b>K</b> E <b>L</b> H <b>F</b> D <b>E</b> P <b>V</b> F <b>T</b> A <b>H</b>	390
<u>Consensus ss:</u>		<b>eeee</b> <b>eeeeeeee</b> <b>eeeeeeee</b> <b>eeeeee</b>	
Human_PREPL	391	<b>C</b> G <b>F</b> I <b>M</b> D <b>T</b> N <b>S</b> D <b>P</b> K <b>N</b> C <b>F</b> Q <b>L</b> C <b>S</b> P <b>I</b> R <b>P</b> P <b>K</b> Y <b>Y</b> T <b>Y</b> K <b>F</b> A <b>E</b> G <b>K</b> L <b>F</b> E <b>E</b> T <b>G</b> H <b>E</b> D <b>----</b> P	436
Human_POP	381	<b>S</b> I <b>V</b> G <b>Y</b> S <b>G</b> Q <b>K</b> K <b>D</b> T <b>E</b> I <b>F</b> Y <b>Q</b> F <b>T</b> S <b>F</b> L <b>S</b> P <b>G</b> I <b>I</b> Y <b>H</b> C <b>D</b> L <b>T</b> K <b>E</b> E <b>L</b> E <b>P</b> R <b>V</b> F <b>R</b> E <b>V</b> T <b>V</b> K <b>I</b> G	430
OPB_TB	391	<b>V</b> V <b>C</b> S <b>Q</b> M <b>K</b> T <b>Y</b> D <b>A</b> S <b>L</b> L <b>R</b> L <b>R</b> Y <b>S</b> S <b>M</b> T <b>T</b> P <b>T</b> V <b>W</b> Y <b>D</b> E <b>D</b> V <b>L</b> S <b>G</b> E <b>R</b> K <b>V</b> V <b>K</b> A <b>R</b> K <b>V</b> G <b>-</b> G <b>G</b> F	439
<u>Consensus ss:</u>		<b>eeeeee</b> <b>eeeeee</b> <b>eeeeee</b> <b>eeeeee</b>	
Human_PREPL	437	<b>I</b> T <b>K</b> T <b>S</b> R <b>V</b> L <b>R</b> L <b>E</b> A <b>K</b> S <b>K</b> D <b>G</b> K <b>L</b> V <b>P</b> M <b>T</b> V <b>F</b> H <b>K</b> T <b>D</b> S <b>E</b> D <b>L</b> Q <b>K</b> K <b>P</b> L <b>L</b> V <b>H</b> V <b>Y</b> G <b>A</b> Y <b>G</b> M <b>D</b> L	486
Human_POP	431	<b>D</b> A <b>S</b> D <b>Y</b> Q <b>T</b> V <b>Q</b> I <b>F</b> Y <b>P</b> S <b>K</b> D <b>G</b> T <b>K</b> I <b>P</b> M <b>F</b> I <b>V</b> H <b>K</b> S <b>I</b> K <b>L</b> D <b>G</b> S <b>H</b> P <b>A</b> F <b>L</b> Y <b>G</b> Y <b>G</b> F <b>N</b> I <b>S</b> I	480
OPB_TB	440	<b>Q</b> S <b>K</b> N <b>Y</b> V <b>C</b> R <b>R</b> E <b>L</b> A <b>T</b> A <b>P</b> D <b>G</b> T <b>K</b> V <b>P</b> I <b>S</b> L <b>V</b> Y <b>D</b> T <b>S</b> I <b>D</b> L <b>K</b> K <b>P</b> N <b>P</b> T <b>M</b> L <b>Y</b> G <b>Y</b> G <b>S</b> Y <b>G</b> I <b>C</b> I	489
<u>Consensus ss:</u>		<b>eeeeeeee</b> <b>eeeeeeee</b> <b>eeeeee</b>	
Human_PREPL	487	<b>K</b> M <b>N</b> F <b>R</b> P <b>E</b> R <b>R</b> V <b>L</b> V <b>-</b> D <b>D</b> G <b>W</b> I <b>L</b> A <b>Y</b> C <b>H</b> V <b>R</b> G <b>G</b> G <b>E</b> L <b>G</b> I <b>Q</b> W <b>H</b> A <b>D</b> <b>-</b> G <b>R</b> L <b>T</b> K <b>K</b> L <b>N</b> G <b>L</b> A <b>D</b>	534
Human_POP	481	<b>T</b> P <b>N</b> Y <b>S</b> V <b>S</b> R <b>L</b> I <b>F</b> V <b>R</b> H <b>M</b> G <b>G</b> I <b>L</b> A <b>V</b> A <b>N</b> I <b>R</b> G <b>G</b> E <b>Y</b> G <b>E</b> T <b>W</b> H <b>K</b> G <b>-</b> G <b>I</b> L <b>A</b> N <b>K</b> Q <b>N</b> C <b>F</b> D <b>D</b>	529
OPB_TB	490	<b>E</b> P <b>E</b> F <b>N</b> S <b>R</b> F <b>L</b> P <b>Y</b> V <b>-</b> D <b>R</b> G <b>M</b> I <b>Y</b> A <b>I</b> A <b>H</b> V <b>R</b> G <b>G</b> G <b>E</b> M <b>G</b> R <b>T</b> W <b>Y</b> E <b>V</b> G <b>G</b> K <b>Y</b> L <b>T</b> K <b>R</b> N <b>T</b> F <b>M</b> D	538
<u>Consensus ss:</u>		<b>hhhhhhh</b> <b>h</b> <b>eeee</b> <b>hhhhhh</b> <b>hhhh</b> <b>hhhh</b>	
Human_PREPL	535	<b>L</b> E <b>A</b> C <b>I</b> K <b>T</b> L <b>H</b> G <b>Q</b> G <b>F</b> S <b>Q</b> P <b>S</b> L <b>T</b> T <b>L</b> T <b>A</b> F <b>S</b> <b>*</b> <b>A</b> G <b>G</b> V <b>L</b> A <b>G</b> A <b>L</b> C <b>N</b> S <b>N</b> P <b>E</b> L <b>V</b> R <b>A</b> V <b>T</b> L <b>E</b> A <b>P</b>	584
Human_POP	530	<b>F</b> Q <b>C</b> A <b>E</b> Y <b>L</b> I <b>K</b> E <b>G</b> Y <b>T</b> S <b>P</b> K <b>R</b> L <b>T</b> I <b>N</b> G <b>S</b> <b>*</b> <b>N</b> G <b>L</b> L <b>V</b> A <b>A</b> C <b>A</b> N <b>Q</b> R <b>P</b> D <b>L</b> F <b>G</b> C <b>V</b> I <b>A</b> Q <b>V</b> G	579
OPB_TB	539	<b>F</b> I <b>A</b> C <b>A</b> E <b>H</b> L <b>I</b> S <b>S</b> G <b>L</b> T <b>T</b> P <b>A</b> Q <b>L</b> S <b>C</b> E <b>G</b> R <b>S</b> <b>*</b> <b>A</b> G <b>L</b> L <b>V</b> G <b>A</b> V <b>L</b> N <b>M</b> R <b>P</b> D <b>L</b> F <b>H</b> V <b>A</b> L <b>A</b> G <b>V</b> P	588

Consensus ss:	hhhhhhhhhh	hhheeeee	hhhhhhhhhh	eeeeeee								
Human_PREPL	585	FLDVLNTMMDTTLPL	LEELEEW	GNPSSDEKHKNYIKRYCPYQNIK	PQ--	632						
Human_POP	580	VMDMLKFHKYT---	IGHAWTTDY	GCS-DSKQHF	EVLVKYSPLHN	VKLPEA	625					
OPB_TB	589	FVDVMTTMC	DPSIPLT	TGEWEEW	GNP-NEYKFF	DYMNSYS	PIDN	VRAQ--	635			
Consensus ss:	hhh	hhhhhh	hhhhhhhh	hhhhhh								
		*										
Human_PREPL	633	---HYPSIHITAY	ENDERVP	LKGI	VSYTEKLKE	AIAE	HAKD	TGEGYQ	TPN	679		
Human_POP	626	DDIQYPSMLLLTA	DHDDR	VVPLH	SLKFIATLQY	IVGR	SRKQ	-----	SNP	669		
OPB_TB	636	---DYPHLMIQAG	LHDP	RVAY	WEP	AKW	AKLRE	LKT----	D-----	SNE	672	
Consensus ss:	eeeeee		hhhhhhhhhh						e			
		*										
Human_PREPL	680	IILDIQPGGNH	V----	IEDSH	KKIT	AQIK	FLYEEL	GLDST	SVFEDL	KKYL	725	
Human_POP	670	LLIHVDTKAGH	GAGKP	TAKV	IEEV	SDM	FAFIAR	CLNVD	WIP-----		710	
OPB_TB	673	VLLKMDLESCH	FSASD	RYKY	LE	RENA	IQQAF	VLKH	LNVR	QLL----	RK--	715
Consensus ss:	eeeeee			hhhhhhhhhhhhhhhh								
Human_PREPL	726	KF	727									
Human_POP		--										
OPB_TB		--										
Consensus ss:												

**Figure S1.** Multiple sequence alignment with predicted local (secondary) structures. Sequences of human PREPL (longer isoform), human POP and oligopeptidase B from *T. brucei* (OPB\_TB) were aligned at the PROMALS3D multiple sequence and structure alignment server (*PROMALS3D: a tool for multiple sequence and structure alignment. Jimin Pei, Bong-Hyun Kim and Nick V. Grishin. Nucleic Acids Res. 2008 36(7):2295-2300*). The  $\alpha$ -helix sections are shown in red letters and the  $\beta$ -strands in blue letters. For PREPL these secondary structural elements were predicted by the PROMALS3D whereas for POP they were based on the PDB 3EQ7 and for OPB\_TB on the PDB 4BP8. Asterisks are placed to show the residues of the conserved catalytic triad, which are also highlighted in gray. The region in which there are residues important to the substrate binding  $S_1$  sub-site of POP and OPB\_TB and consequently thought to form also the  $S_1$  sub-site of PREPL is boxed.

**Comments on Figure S1.** It is noteworthy that using only regular default sequence alignments (such as ClustalW), the histidine residue of the catalytic triad was incorrectly aligned: The PREPL His696 was incorrectly aligned with the His680 of POP and His683 of OPB\_TB, while, the correct residue of the catalytic triad of PREPL is the His690, as already experimentally verified by site-directed mutagenesis (Jaeken, J.; Martens, K.; François, I.; Eyskens, F.; Lecointre, C.; Derua, R.; Meulemans, S.; Sloomstra, J.W.; Waelkens, E.; De Zegher, F.; et al. *Deletion of PREPL, a gene encoding a putative serine oligopeptidase, in patients with hypotonia-cystinuria syndrome. Am. J. Hum. Genet. 2006, 78, 38–51.*). However, by making the alignment by PROMALS, considering the secondary structures, as showed above, the PREPL His690 is correctly aligned.

Figure S2. Comparison of the CD spectra obtained for PREPL and POP.

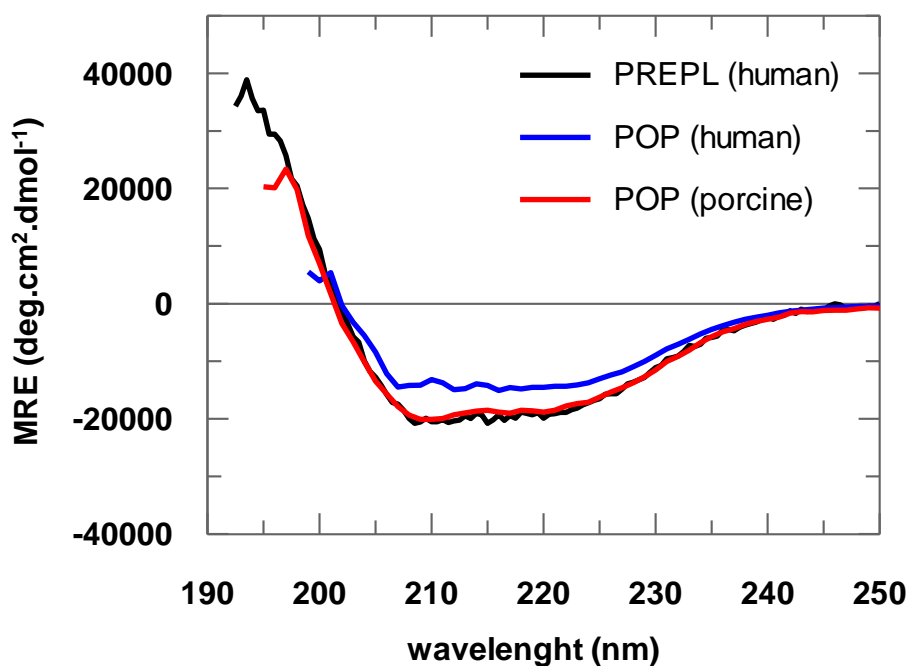
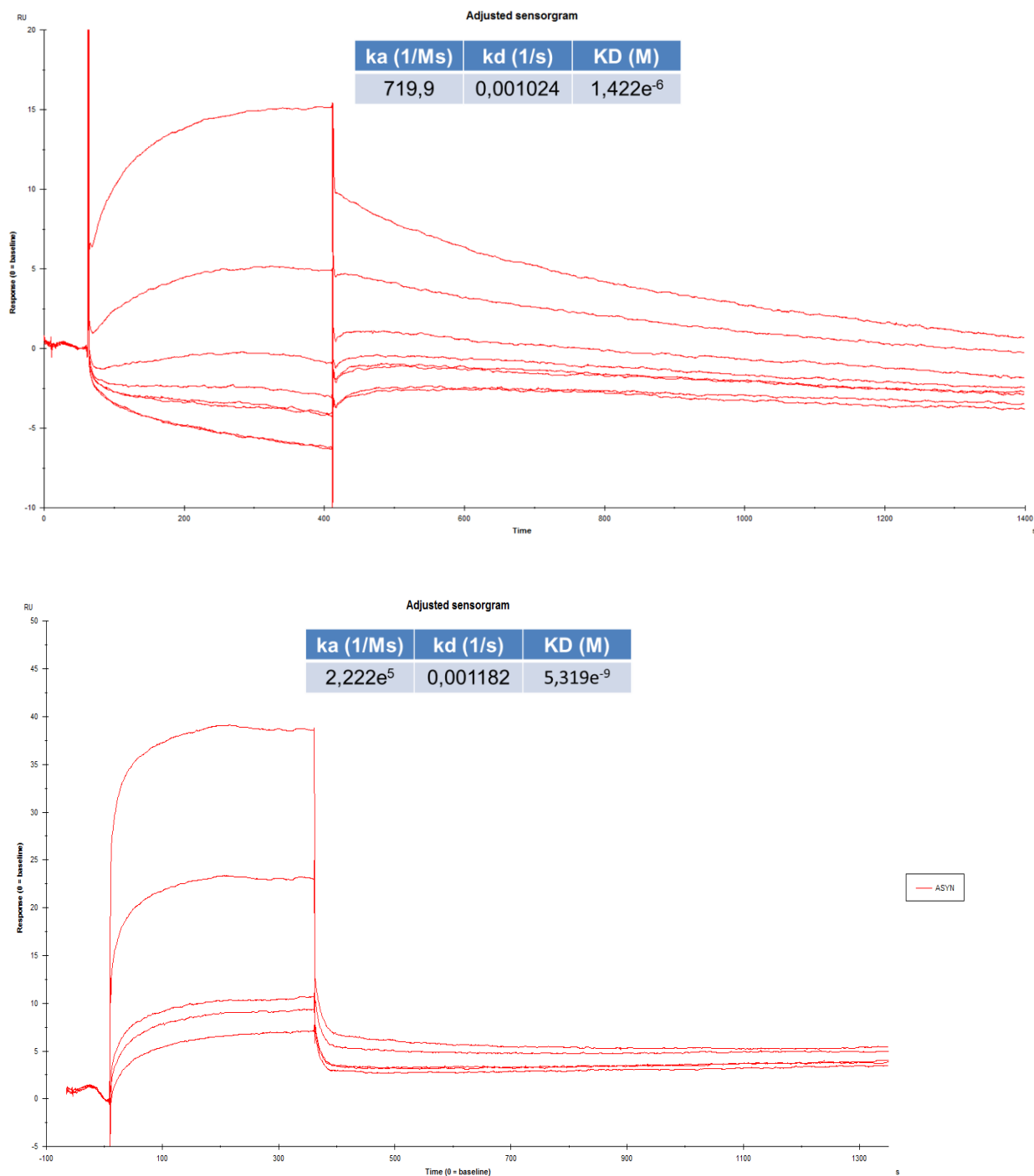


Figure S2. Comparison of the CD spectra obtained for *human* PREPL, *human* and *porcine* POP.

**Comments on Figure S2.** The spectrum obtained for human PREPL is more intense than the spectrum obtained for human POP. This most probably arise from the experimental errors intrinsic of the protein concentration measurements. However, in Figure 1S, it can be observed two helix sections predicted to exist in the PREPL structure that is not present in the structure of POP (one near the PREPL N-terminus, from residue number 3 to 16 or even to residue number 22, and another near the C-terminus, from residue number 715 to residue 725). These two extra helix sections may indicate that PREPL has a higher  $\alpha$ -helix content than POP. Therefore, in order to check such difference a parallel CD measurement with the porcine POP (which we have already prepared at our lab) was done. The CD spectra obtained for human PREPL and porcine POP superimposed perfectly. Confirming that the difference in the intensities of the CD spectra observed between the human PREPL and human POP probably is due to the errors intrinsic to our practical methodologies. An additional indication that this difference relies on practical manipulations reasons is that: we were able to use a 0.2 mm cuvette for the measurements with human PREPL and porcine POP what permitted the collection of reliable spectra (HT < 600V) at wavelengths as low as ~192 nm. However, we had to work with a 1 mm cuvette for samples of human POP to decrease the signal to noise ratio, but this larger pathlength increased the total absorbance of light and then we could just get a reliable CD signal until 199 nm.

**Figure S3. Adjusted sensorgrams obtained in the SPR assays with PREPL or POP immobilized and  $\alpha$ -Syn as the analyte injected at increasing concentrations.**



**Figure S3.** Adjusted sensorgrams obtained in the SPR assays with PREPL (upper panel) or POP (lower panel) immobilized and  $\alpha$ -Syn as the analyte injected at increasing concentrations.

**Comments on Figure S3.** Although we could not observe the saturation of the dose-response curve of the titrations with  $\alpha$ -Syn of the POP immobilized on the sensor chip, by the association constants obtained in the SPR analysis ( $k_a$  values shown in the Figure S3), it can be affirmed that the affinity of the  $\alpha$ -Syn for POP is higher than its affinity by PREPL, mainly because of a higher association constant.