

Supplementary material

Effect of Mutations on mRNA and Globin Stability: The Cases of Hb Bernalda/Groene Hart and Hb Southern Italy

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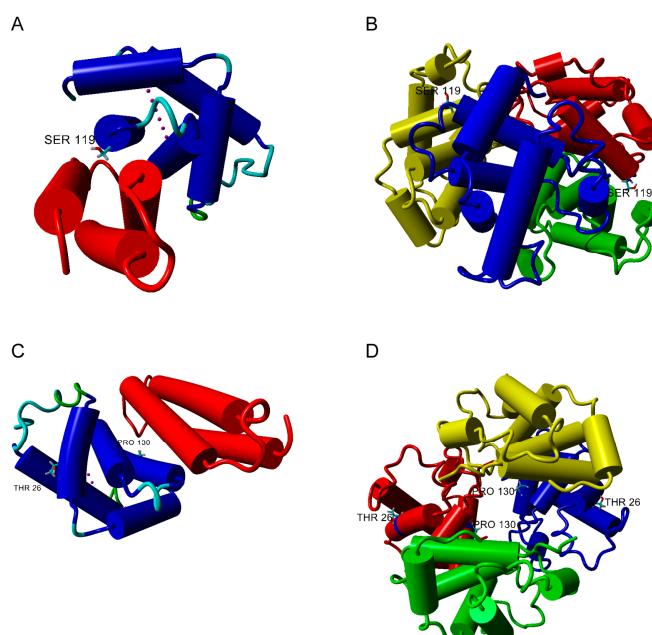


Figure S1. Position of mutations for Hb Bernalda/Groene Hart and Hb Southern Italy variants. (A) and (B) Positions of Ser119 in 1Y01 and 2HBB, respectively. (C) and (D) Positions of Thr26 and Pro130 in 1Y01 and 2HBB, respectively. The chains were in different colors. Highlighted residues are in stick representation. Pictures were obtained by the Yasara program.

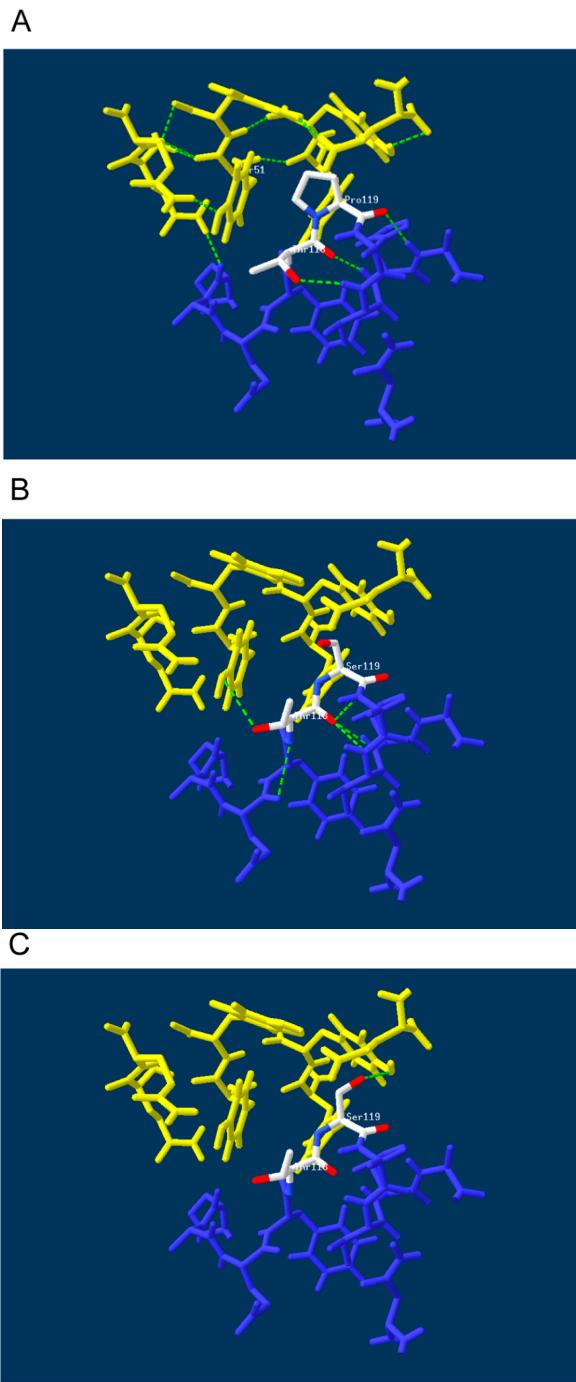


Figure S2. Hydrogen bonds between AHSP and α -globin chain highlighted with the Swiss PDB viewer program. (A) The chains interface in 1Y01 showing hydrogen bond interactions. (B) The mutant model highlighted with the hydrogen bond of Thr118 with Tyr51. (C) Hydrogen bond formed by the Ser119 rotamer with Tyr48.

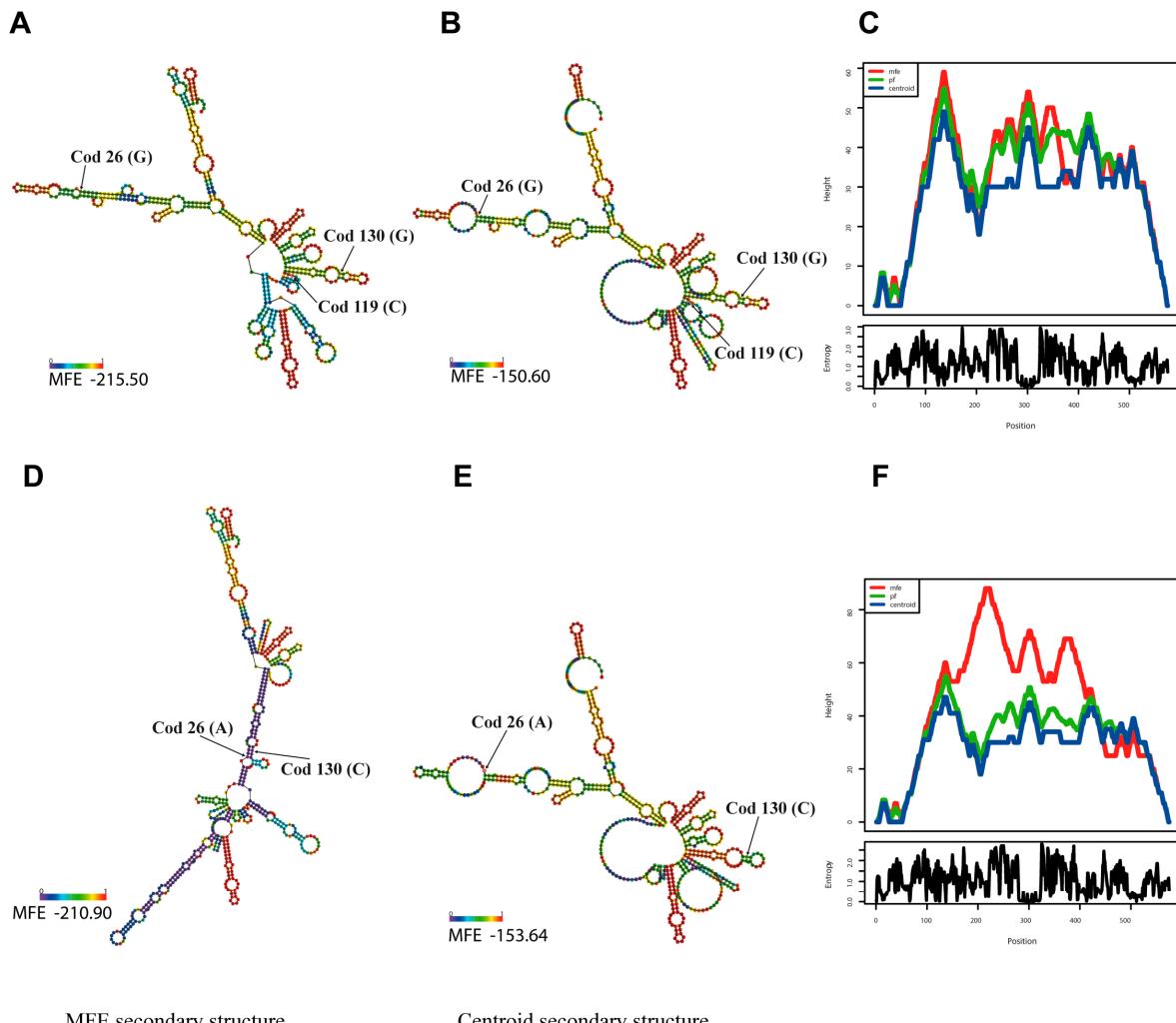


Figure S3. Secondary structure of α 2-globin mRNAs predicted by means of the RNAfold web server <http://rna.tbi.univie.ac.at/cgi-bin/RNAWebSuite/RNAfold.cgi> [26] (A,B,C), normal α 2-globin mRNA; (D,E,F) α 2 Hb Southern Italy mRNA. For each α -globin mRNA has been reported the minimum free energy (MFE) secondary structure (A,D), the Centroid secondary structure (B,E), with the corresponding values, and the mountain plot representation (C,F) of the MFE structure, the thermodynamic ensemble of RNA structures, and the centroid structure.

Table S1. Oligonucleotides used as Primers in the reported applications. The sequence and position of the primers were from GenBank Sequence *NG_000006.1.

Name	Direction, Sequence	Position from the Cap Site	Application	Used with Primer	Amplicon Length (bp)	References
A For	5'-GCCCTGAGCGACCTGCACCGC-3'	$\alpha 1, \alpha 2 +401/+421$	DGGE DNA	B	$\alpha 1$ 301; $\alpha 2$ 294	[20]
B Rev	5'-ACAGAAGCCAGGAACTTGTC-3'	$\alpha 1 +682/+701; \alpha 2 +675/+694$	DGGE DNA			[20]
C For	5'-TGGAGGGTGGAGACGTCTG-3'	$\alpha 1, \alpha 2 -202/-183$	DGGE DNA, PCR	D, F, G	$\alpha 1, \alpha 2$ 441; $\alpha 1$ 975; $\alpha 2$ 1106	[20]
D Rev	5'-AAGCAGACTGAGGGTGGGG-3'	$\alpha 1, \alpha 2 +220/+239$	DGGE DNA			[20]
E For	5'-TCGGGGCCGGCACTCTCTG-3'	$\alpha 1, \alpha 2 -12/+9$	Seq $\alpha 1, \alpha 2$			[22]
F Rev	5'-GAGGCCAAGGGCAAGAACAT-3'	$\alpha 1 +751/+773$	$\alpha 1$ Seq, RT-PCR			[13]
G Rev	5'-GTCTGAGACAGGTAAACACCTCAT-3'	$\alpha 2 +880/+904$	Seq $\alpha 2$, Common ARMS			[19]
H For	5'-CACAGACTCAGAGAGAAC-3'	$\alpha 1, \alpha 2 +15/+33$	RT-PCR $\alpha 1, \alpha 2$	F, I	$\alpha 1$ 493; $\alpha 2$ 261	[21]
I Rev	5'-CGTTGGCATGTCGTCAC-3'	$\alpha 2 +374/+392$	RT-PCR $\alpha 2$			[22]
J For	5'-CCTCCCCGGAGTCACCC-3'	$\alpha 2 +635/+654$	RE cDNA analysis	K	132 bp	p.a.
K Rev	5'-GGGAGGCCATGGCAGGAGAAC-3'	$\alpha 2 +744/+768$	RE cDNA analysis			[34]
L For	5'-GCGAAGAAGGTGGCCGAC-3'	$\alpha 1 +332/+349$	DGGE cDNA $\alpha 1$	B	$\alpha 1$ 221	[21]
M For, Hb Cas	5'GCACGCTGGCGAGTATGCTA-3'	$\alpha 1, \alpha 2 +97/+116$	ARMS	G	808	[13]
N For, cod26nor	5'-GCACGCTGGCGAGTATGCTG-3'	$\alpha 1, \alpha 2 +97/+116$	ARMS	G	808	[13]
O For, Hb Ber	5'-CCTCCCCGGAGTCAGCT-3'	$\alpha 1, \alpha 2 +642/+661$	ARMS	R	$\alpha 1$ 275	p.a.
P For, Hb S.P.	5'-CTCCCTGGACAAGTTCTGCG-3'	$\alpha 1, \alpha 2 +668/+687$	ARMS	G	237	[13]
Q For cod130nor	5'-CTCCCTGGACAAGTTCTGCG-3'	$\alpha 1, \alpha 2 +668/+687$	ARMS	G	237	[13]
R Rev	5'-TGTGTGCCCAGCTGCTCACGC-3'	$\alpha 1 +892/+916$	Common ARMS			[13]
S For	5'-AGGCTGTGGCGAGTCAGAAGA-3'	35943-35965 *	Control ARMS	T	714	[13]
T Rev	5'-CAATAGCTGAAACGGCTGGAG-3'	36656-36635 *	Control ARMS			[13]
U For	5'-CCCAGAGCCAGGTTGTTATCTG-3'	32840-32863 *	RFLP RsaI	G	1803	[21]
UGT1A1 For	5'-AACTGAACCTCTCTACCTT-3'	UGT1A1 -130/-110	PCR, Seq	UGT1A1 Rev	253	[24]
UGT1A1 Rev	5'-CCACTGGATCAACAGTATCT-3'	UGT1A1 +104/+124	PCR, Seq			[24]

Rev: Reverse; For: Forward; Seq: sequencing analysis; Hb Cas: Hb Caserta; Hb Ber: Hb Bernalda; Hb S.P.: Hb Sun Prairie; RE cDNA analysis: Restriction enzyme cDNA analysis; p.a.: present article.

Table S2. (A) Nucleotide triplets of the coding α -globin mRNA (NM_000517.6), starting from the ATG until the stop codon, below the relative amino acid present in the α -globin chain (NP_000508.1). (B) Type of amino acids present in the α -globin chain, the corresponding coding triplet, and number of times present in the α -globin mRNA.

		A																	
ATG	GTG	CTG	TCT	CCT	GCC	GAC	AAG	ACC	AAC	GTC	AAG	GCC	GCC	TGG	GGT	AAG	GTC	GGC	GCG
M	V	L	S	P	A	D	K	T	N	V	K	A	A	W	G	K	V	G	A
CAC	GCT	GGC	GAG	TAT	GGT	GCG	GAG	GCC	CTG	GAG	AGG	ATG	TTC	CTG	TCC	TTC	CCC	ACC	ACC
H	A	G	E	Y	G	A	E	A	L	E	R	M	F	L	S	F	P	T	T
AAG	ACC	TAC	TTC	CCG	CAC	TTC	GAC	CTG	AGC	CAC	GGC	TCT	GCC	CAG	GTT	AAG	GGC	CAC	GGC
K	T	Y	F	P	H	F	D	L	S	H	G	S	A	Q	V	K	G	H	G
AAG	AAG	GTG	GCC	GAC	GGC	CTG	ACC	AAC	GCC	GTG	GGC	CAC	GTG	GAC	GAC	ATG	CCC	AAC	GGC
K	K	V	A	D	A	L	T	N	A	V	A	H	V	D	D	M	P	N	A
CTG	TCC	GCC	CTG	AGC	GAC	CTG	CAC	GGC	CAC	AAG	CTT	CGG	GTG	GAC	CCG	GTC	AAC	TTC	AAG
L	S	A	L	S	D	L	H	A	H	K	L	R	V	D	P	V	N	F	K
CTC	CTA	AGC	CAC	TGC	CTG	CTG	GTG	ACC	CTG	GCC	GCC	CAC	CTC	CCC	GCC	GAG	TTC	ACC	CCT
L	L	S	H	C	L	L	V	T	L	A	A	H	L	P	A	E	F	T	P
GCG	GTG	CAC	GCC	TCC	CTG	GAC	AAG	TTC	CTG	GCT	TCT	GTG	AGC	ACC	GTG	CTG	ACC	TCC	AAA
A	V	H	A	S	L	D	K	F	L	A	S	V	S	T	V	L	T	S	K
TAC	CGT	TA																	
R Stop																			
		B																	
AA	n.	Codon (Time Present)																	
Ala, A	21	GCC(12), GCG(7), GCT(2), GCA(0)																	
Arg, R	3	CGT(1), CGG(1), AGG(1), CGC(0), CGA(0), AGA(0)																	
Asn, N	4	AAC(4), AAT(0)																	
Asp, D	8	GAC(8), GAT(0)																	
Cys, C	1	TGC(1), TGT(0)																	
Gln, Q	1	CAG(1), CAA(0)																	
Glu, E	4	GAG(4), GAA(0)																	
Gly, G	7	GGC(5), GGT(2), GGA(0), GGG(0)																	
His, H	10	CAC(10), CAT(0)																	
Ile, I	0	ATT(0), ATC(0), ATA(0)																	
Start	1	ATG(1), GTG(0)																	
Leu, L	18	CTG (14), CTC (2), CTT(1), CTA(1), TTA(0), TTG(0)																	
Lys, K	11	AAG(10), AAA(1)																	
Met, M	2	ATG (2)																	
Phe, F	7	TTC(7), TTT(0)																	
Pro, P	7	CCC(3), CCT(2), CCG(2), CCA(0)																	
Ser, S	11	TCC(4), AGC(4), TCT(3), TCA(0), TCG(0), AGT(0)																	
Thr, T	9	ACC(9), ACT(0), ACA(0), ACG(0)																	
Trp, W	1	TGG(1)																	
Tyr, Y	3	TAC(2), TAT(1)																	
Val, V	13	GTG(9), GTC(3), GTT(1), GTA(0)																	
Stop	1	TAG(0), TGA(0), TAA(1)																	

Table S3. (A) Nucleotide triplets of the coding α -globin mRNA (NM_000518.5), starting from the ATG until the stop codon, below the relative amino acid present in the α -globin chain (NP_000509.1). (B) Type of amino acids present in the α -globin chain, the corresponding coding triplet, and number of times present in the α -globin mRNA

		A
ATG	GTG	CAT CTG ACT CCT GAG GAG AAG TCT GCC GTT ACT GCC CTG TGG GGC AAG GTG AAC
M	V	H L T P E E K S A V T A L W G K V N
GTG	GAT	GAA GTT GGT GGT GAG GCC CTG GGC AGG CTG CTG GTG GTC TAC CCT TGG ACC CAG
V	D	E V G G E A L G R L L V V Y P W T Q
AGG	TTC	TTT GAG TCC TTT GGG GAT CTG TCC ACT CCT GAT GCT GTT ATG GGC AAC CCT AAG
R	F	F E S F G D L S T P D A V M G N P K
GTG	AAG	GCT CAT GGC AAG AAA GTG CTC GGT GCC TTT AGT GAT GGC CTG GCT CAC CTG GAC
V	K	A H G K K V L G A F S D G L A H L D
AAC	CTC	AAG GGC ACC TTT GCC ACA CTG AGT GAG CTG CAC TGT GAC AAG CTG CAC GTG GAT
N	L	K G T F A T L S E L H C D K L H V D
CCT	GAG	AAC TTC AGG CTC CTG GGC AAC GTG CTG GTC TGT GTG CTG GCC CAT CAC TTT GGC
P	E	N F R L L G N V L V C V L A H H F G
AAA	GAA	TTC ACC CCA CCA GTG CAG GCT GCC TAT CAG AAA GTG GTG GCT GGT GTG GCT AAT
K	E	F T P P V Q A A Y Q K V V A G V A N
GCC	CTG	GCC CAC AAG TAT CAC TAA
L	A	H K Y H Stop

		B
AA	n	Codon (n of Times Present)
Ala, A	15	GCC(9), GCT(6), GCA(0), GCG(0)
Arg, R	3	AGG(3), CGT(0), CGC(0), CGA(0), CGG(0), AGA(0)
Asn, N	6	AAC(5), AAT(1)
Asp, D	7	GAT(5), GAC(2)
Cys, C	2	TGT(2), TGC(0)
Gln, Q	3	CAG(3), CAA(0)
Glu, E	8	GAG(6), GAA(2)
Gly, G	13	GGC(8), GGT(4), GGG(1), GGA(0)
His, H	9	CAC(6), CAT(3)
Ile, I	0	ATT(0), ATC(0), ATA(0)
Start	1	ATG(1), GTG(0)
Leu, L	18	CTG(15), CTC(3), TTA(0), TTG(0), CTT(0), CTA(0)
Lys, K	11	AAG(8), AAA(3)
Met, M	1	ATG (1)
Phe, F	8	TTT(5), TTC(3)
Pro, P	7	CCT(5), CCA(2), CCC(0), CCG(0)
Ser, S	5	AGT(2), TCC(2), TCT(1), TCA(0), TCG(0), AGC(0)
Thr, T	7	ACT(3), ACC(3), ACA(1), ACG(0)
Trp, W	2	TGG(2)
Tyr, Y	3	TAT(2), TAC(1)
Val, V	18	GTG(13), GTT(3), GTC(2), GTA(0)
Stop	1	TAA(1), TAG(0), TGA(0)