hERG channel function in LQT2 and drug induced conditions

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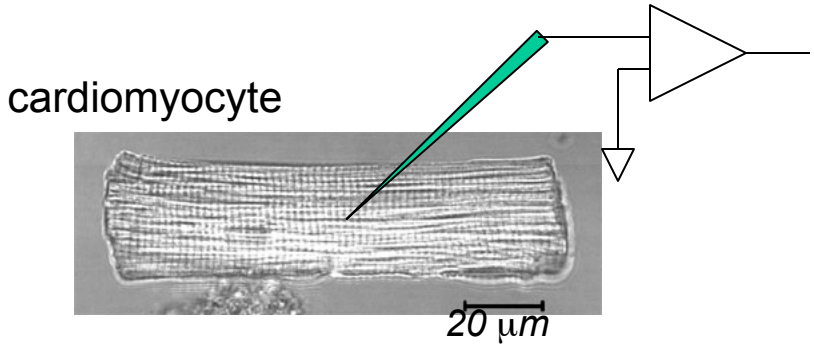
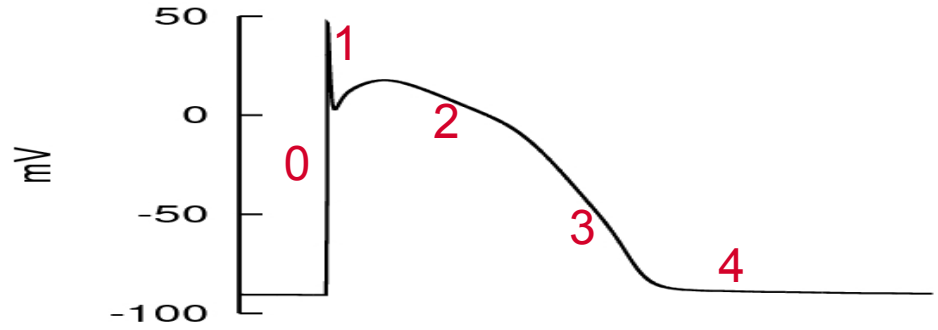
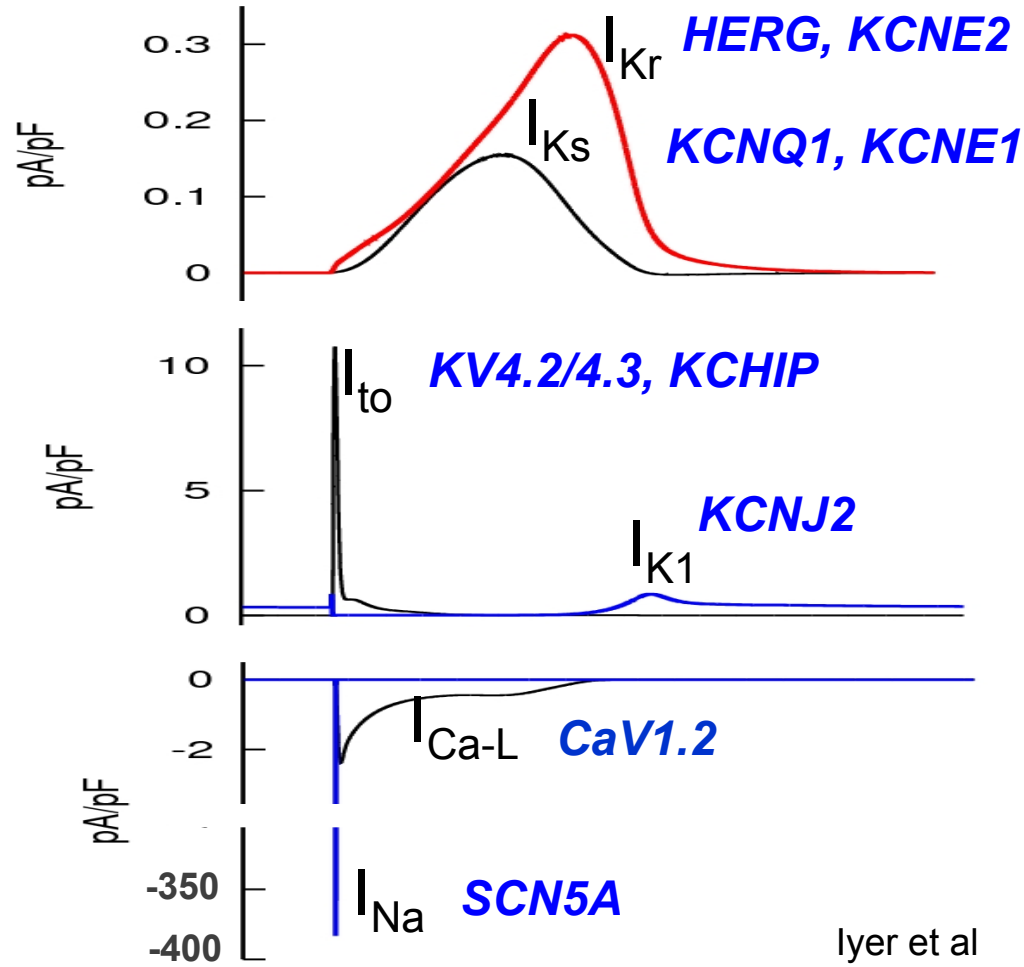




Multiple K^+ currents mediate repolarization of ventricular action potentials



Iyer et al

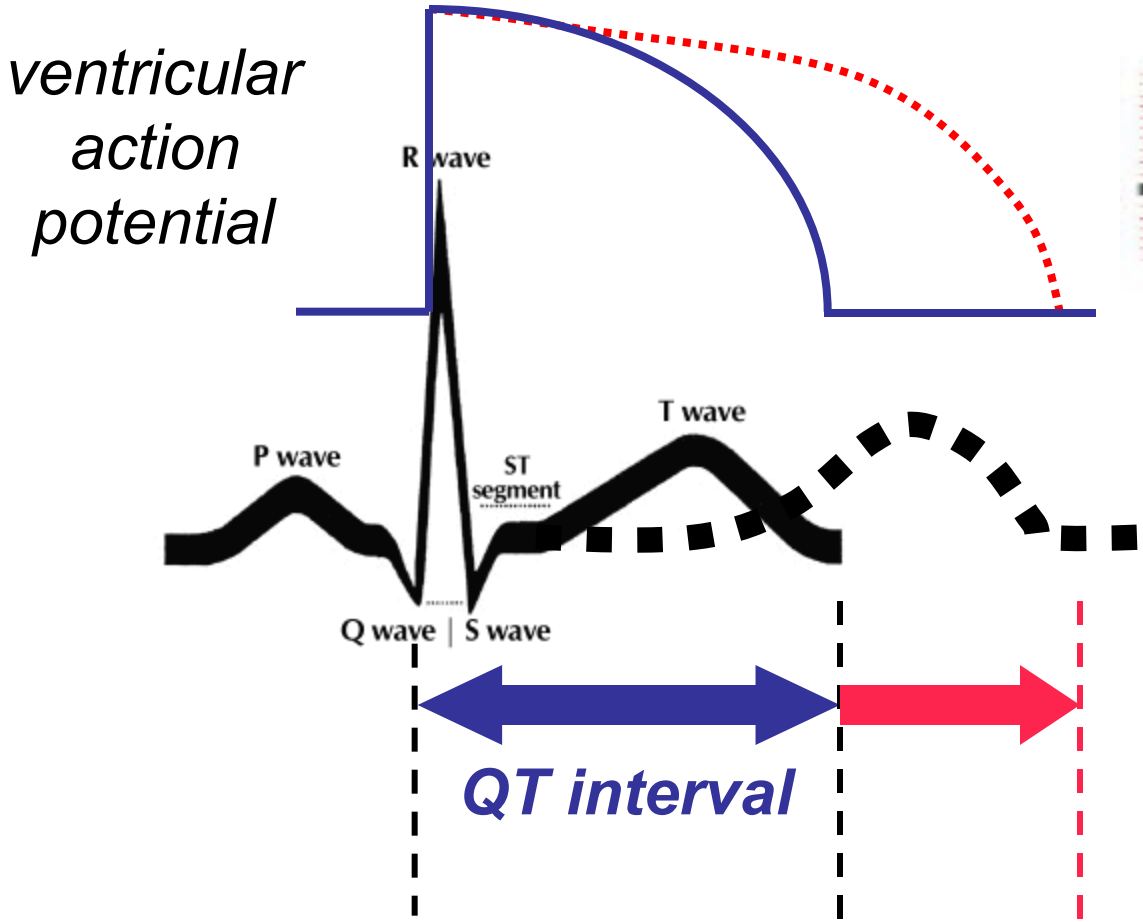
Long QT syndrome

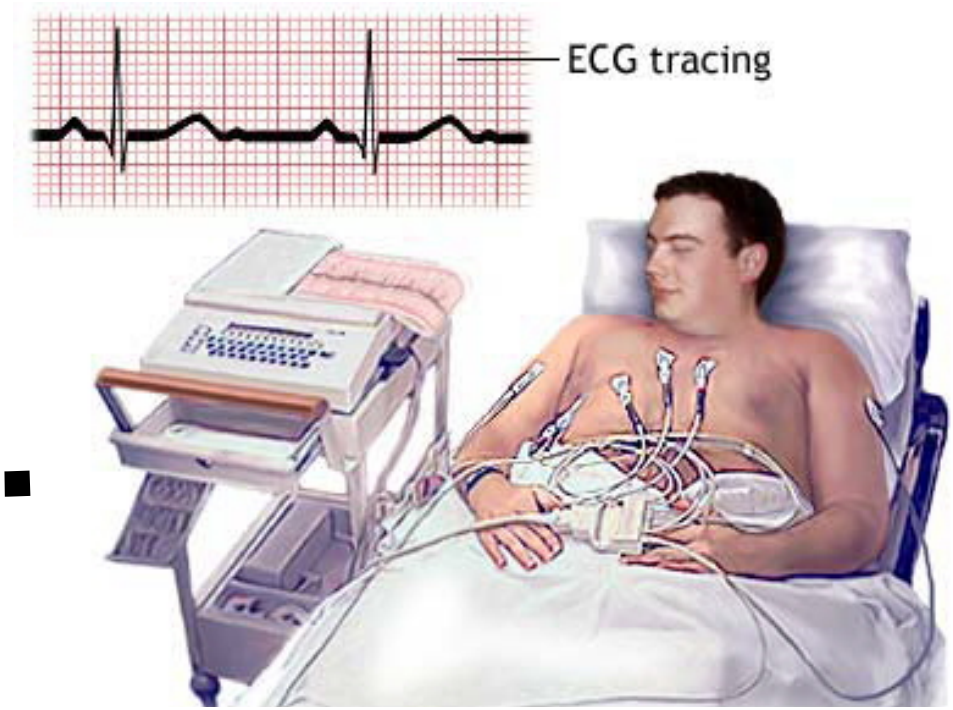
- **Inherited: uncommon** (~ 1/3000 people)
  (mutations in cardiac ion channel genes)

- **Acquired: common, but variable**
  (heart failure)
  (drug-induced: 1/20 – 1/10,000 patients)

QT interval: measure of ventricular repolarization

LONG QT SYNDROME: QT_c > 450 msec



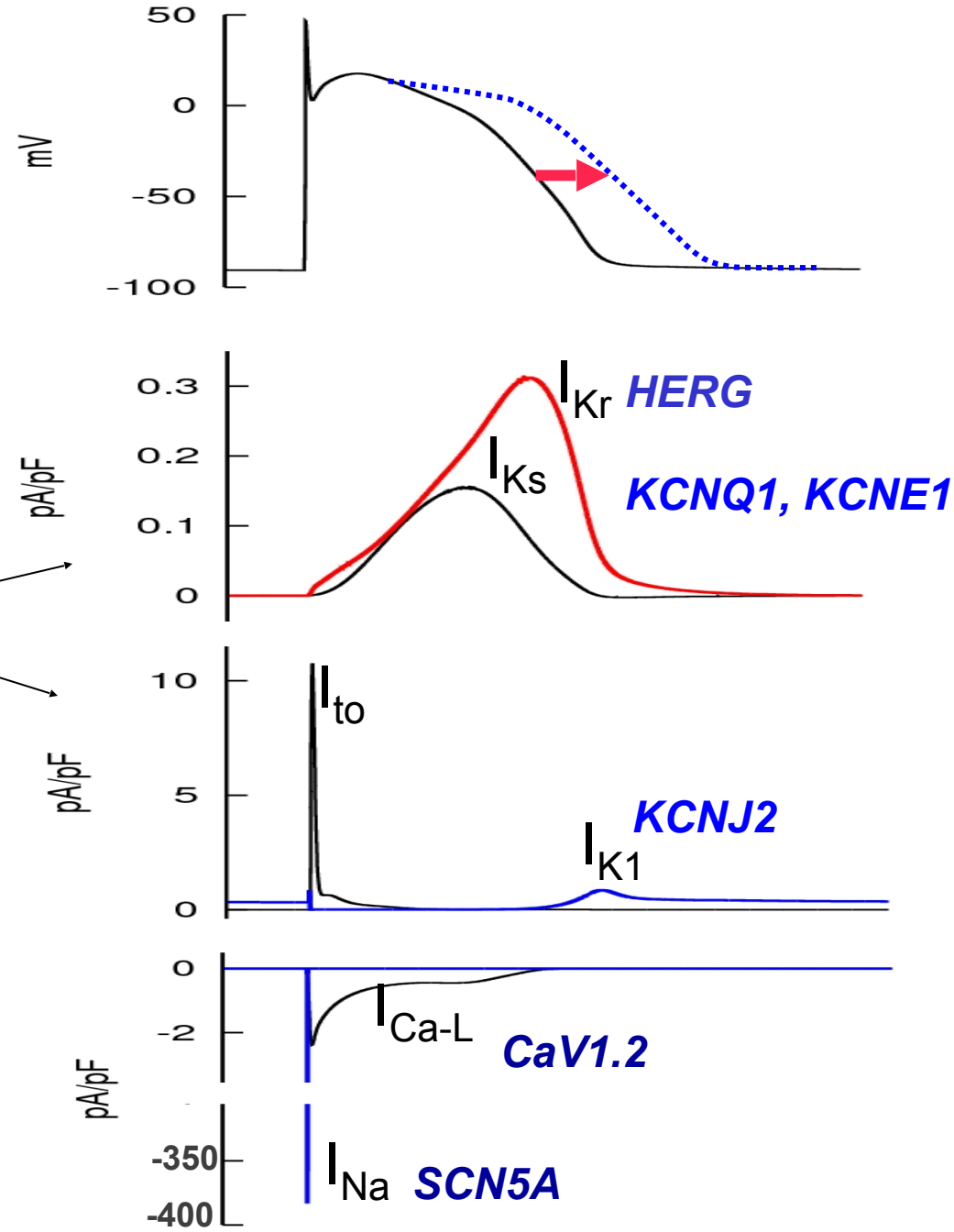


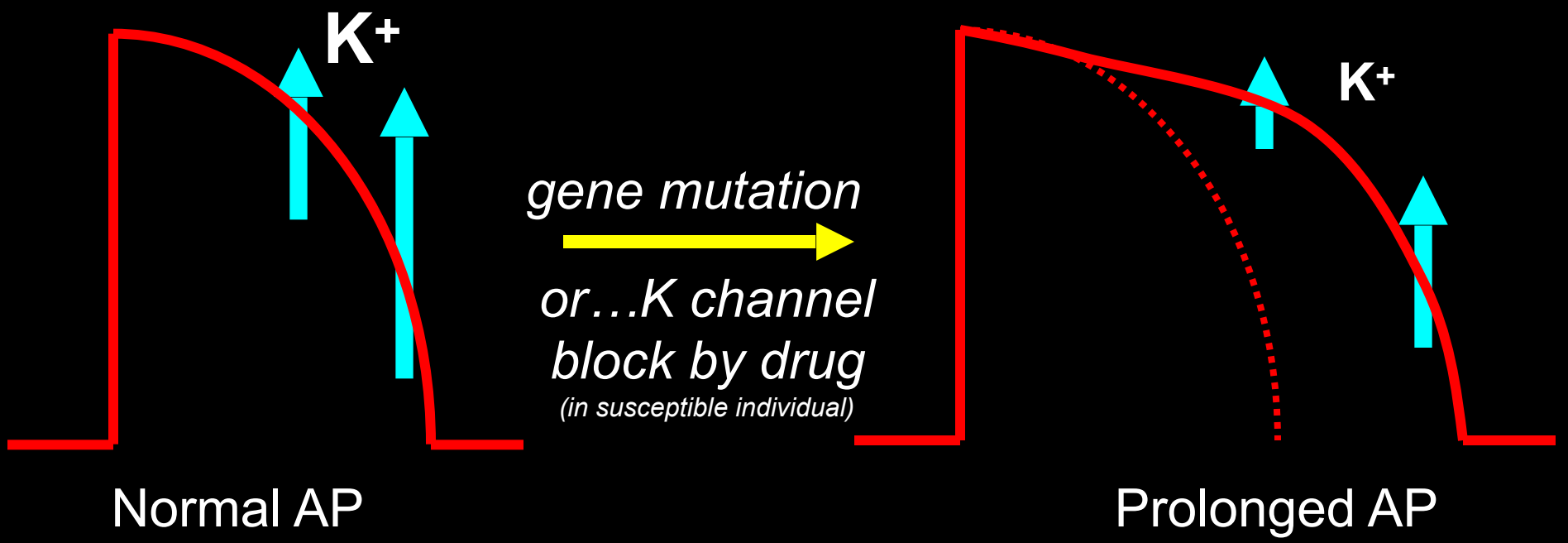
Long QT Syndrome:

prolonged ventricular repolarization caused by ion channel mutations

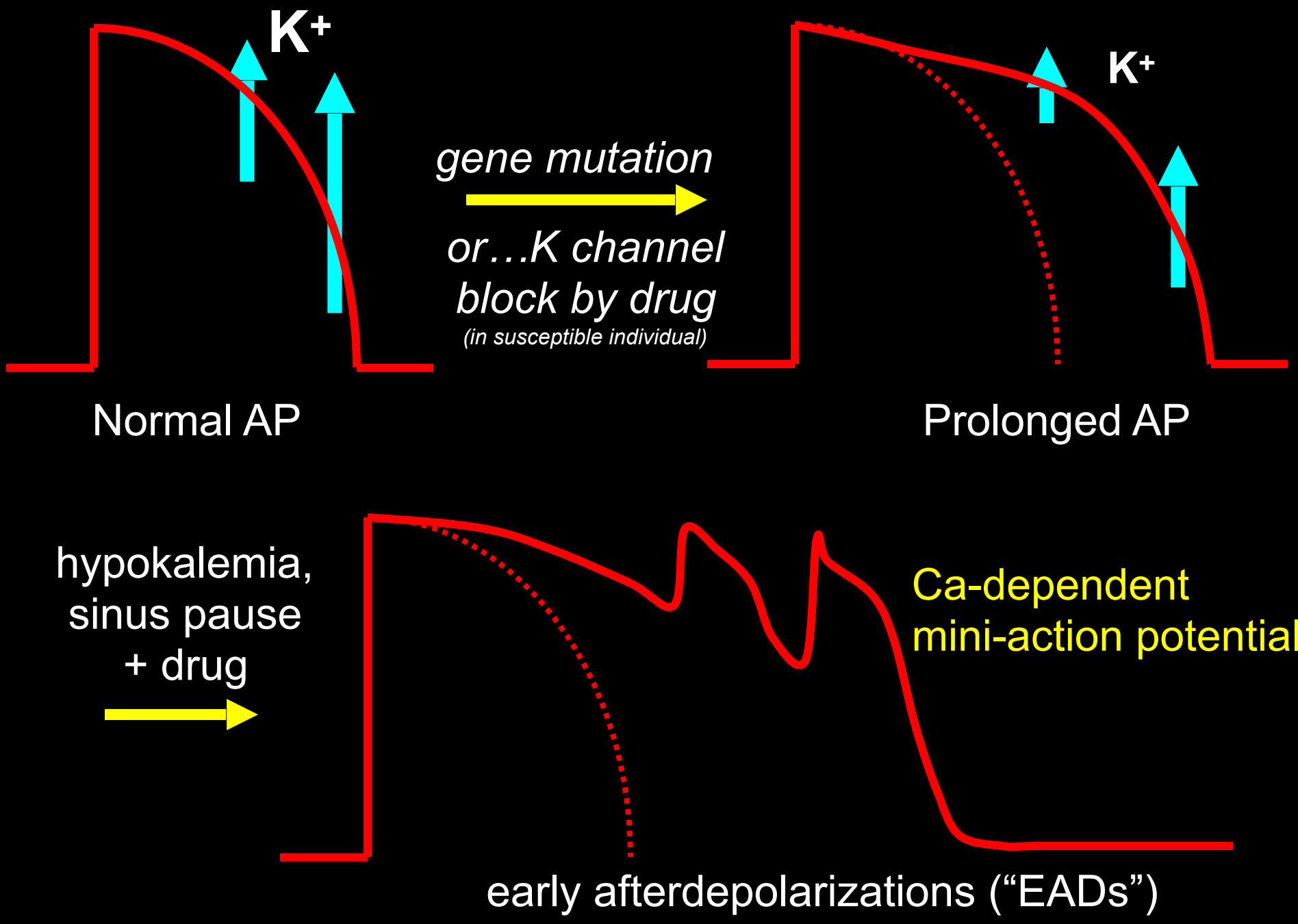
Loss of function mutations in **K^+** channel genes

Gain of function mutations in **Na^+** or **Ca^{2+}** channel genes



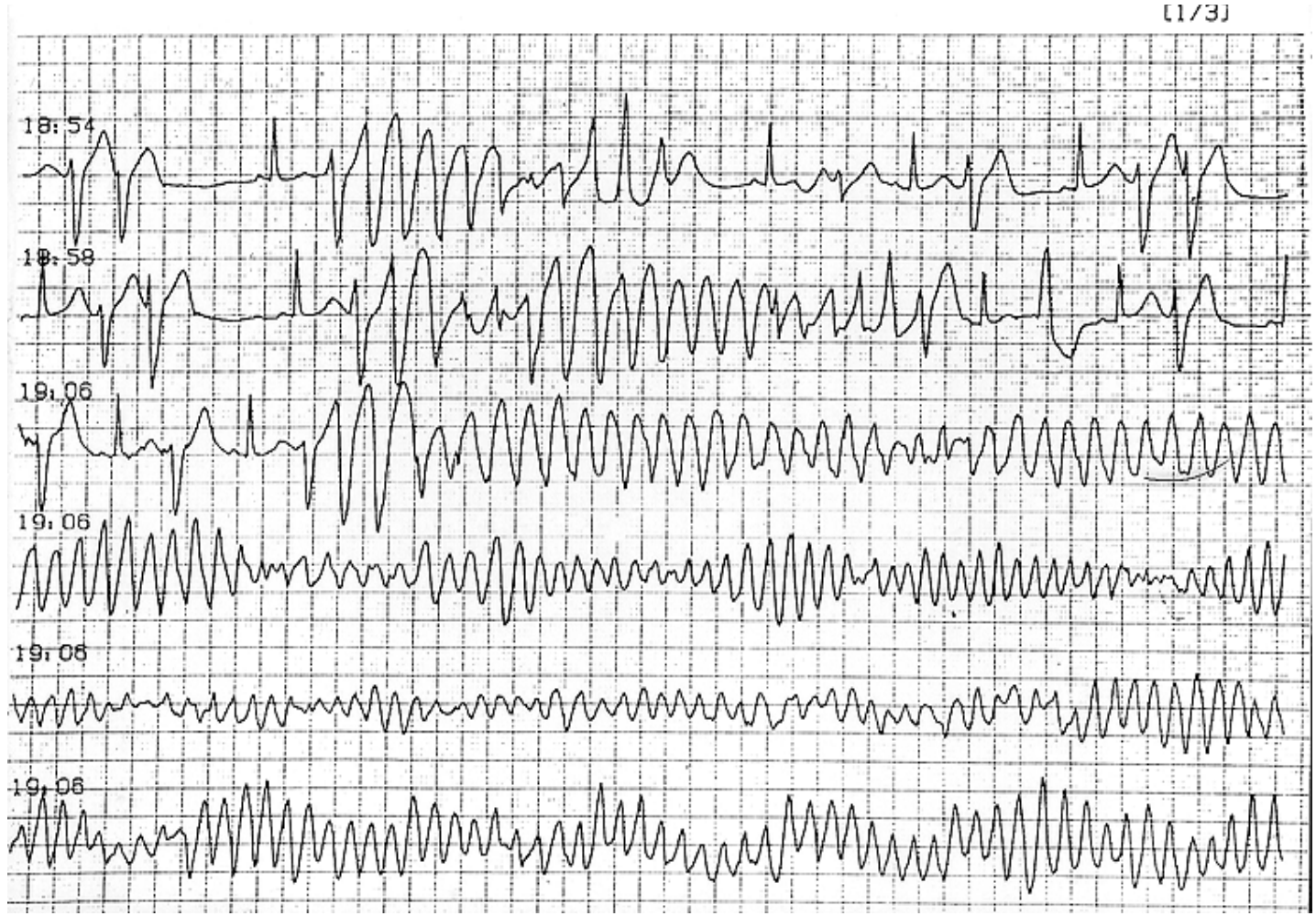


Cellular mechanism of long QT syndrome


K+
gene mutation
or…K channel block by drug
(in susceptible individual)
K+
Normal AP
Prolonged AP
hypokalemia, sinus pause + drug
Ca-dependent mini-action potentials
early afterdepolarizations (“EADs”)

Torsades de pointes: signature arrhythmia of long QT syndrome

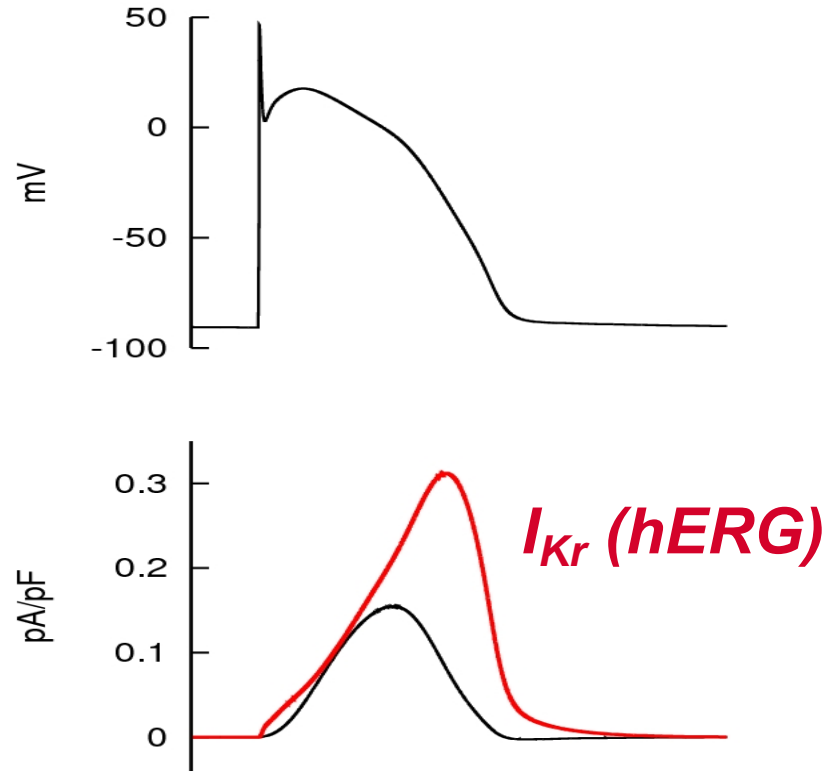
Continuous ECG tracing



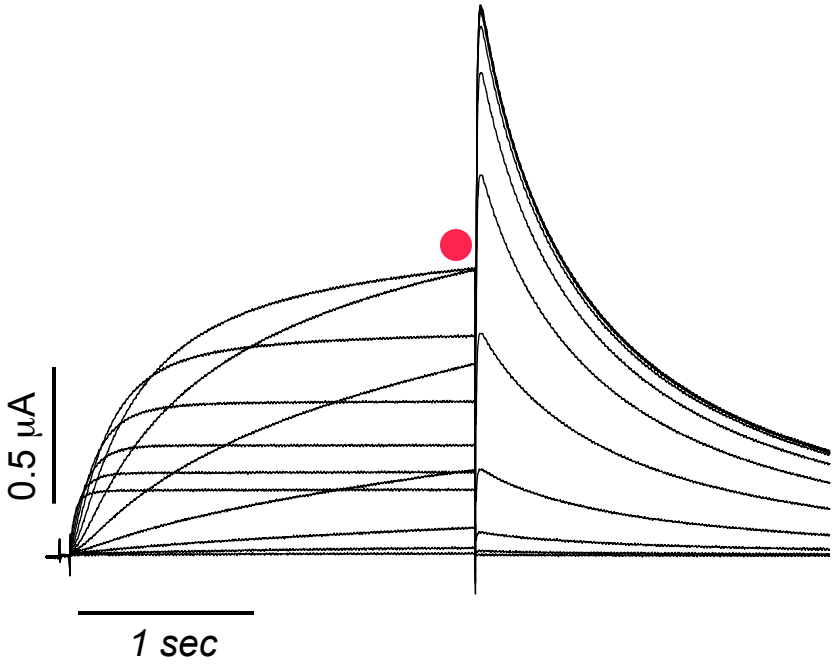
TdP

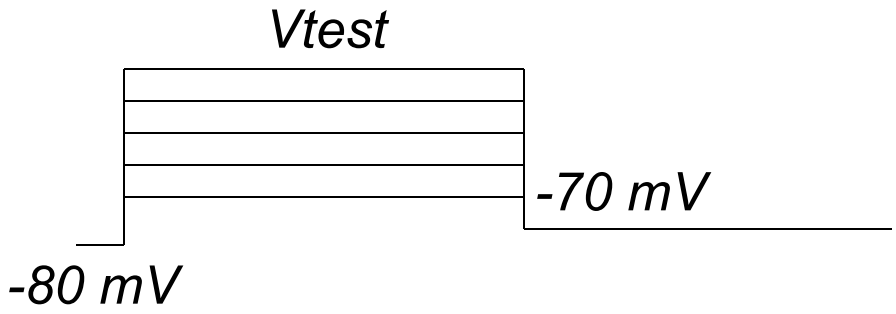
ventricular fibrillation --⟶ *sudden death*

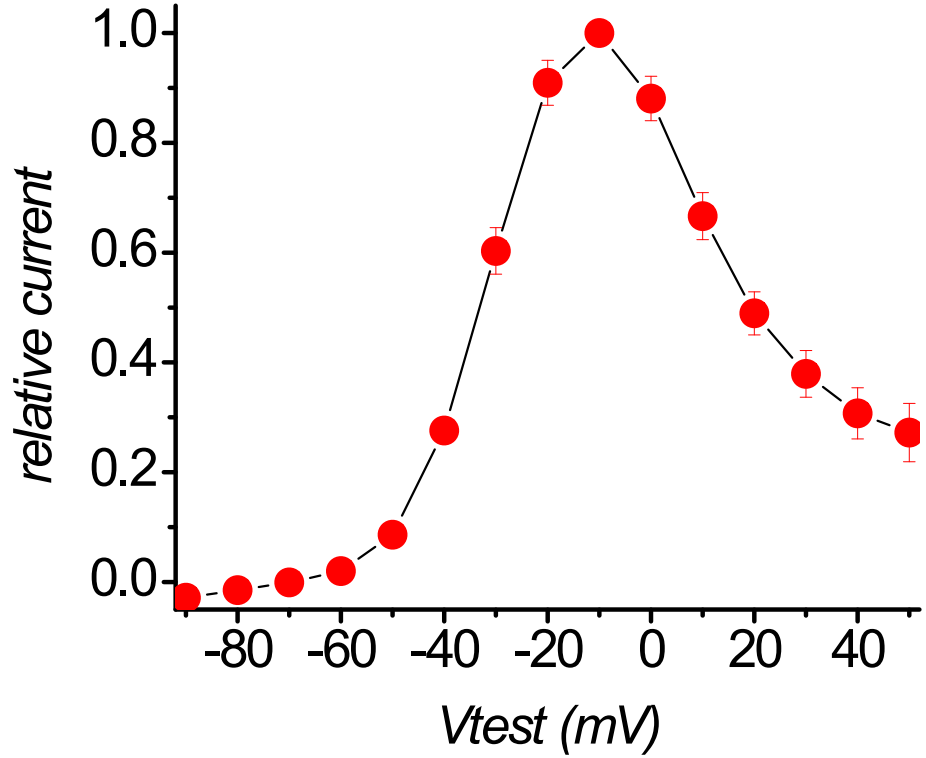
hERG channels and arrhythmia



Activation of hERG is voltage-dependent





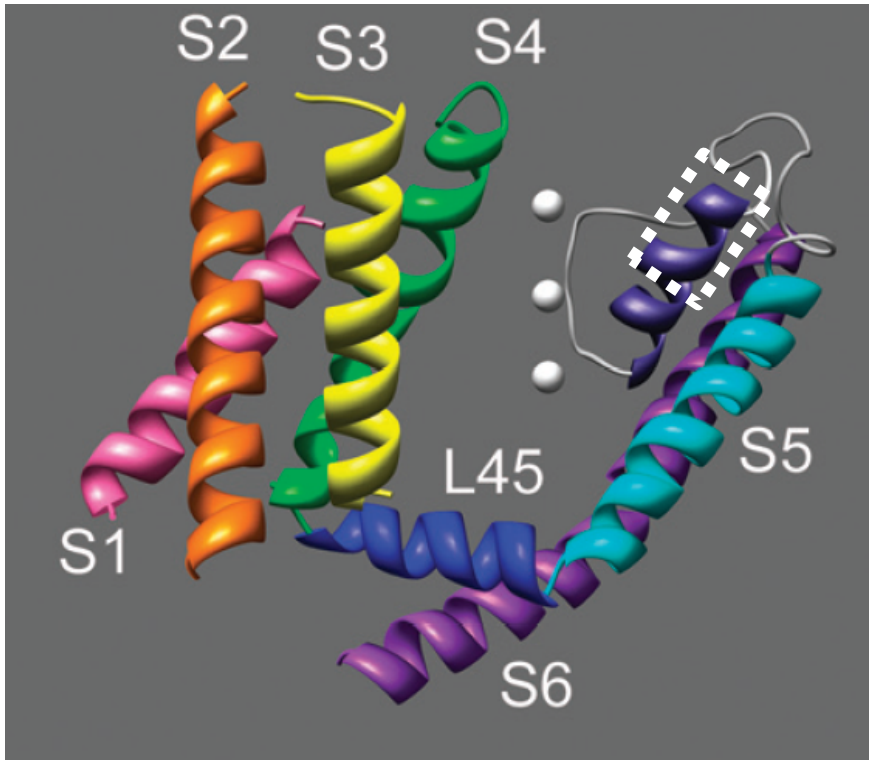


single channel:

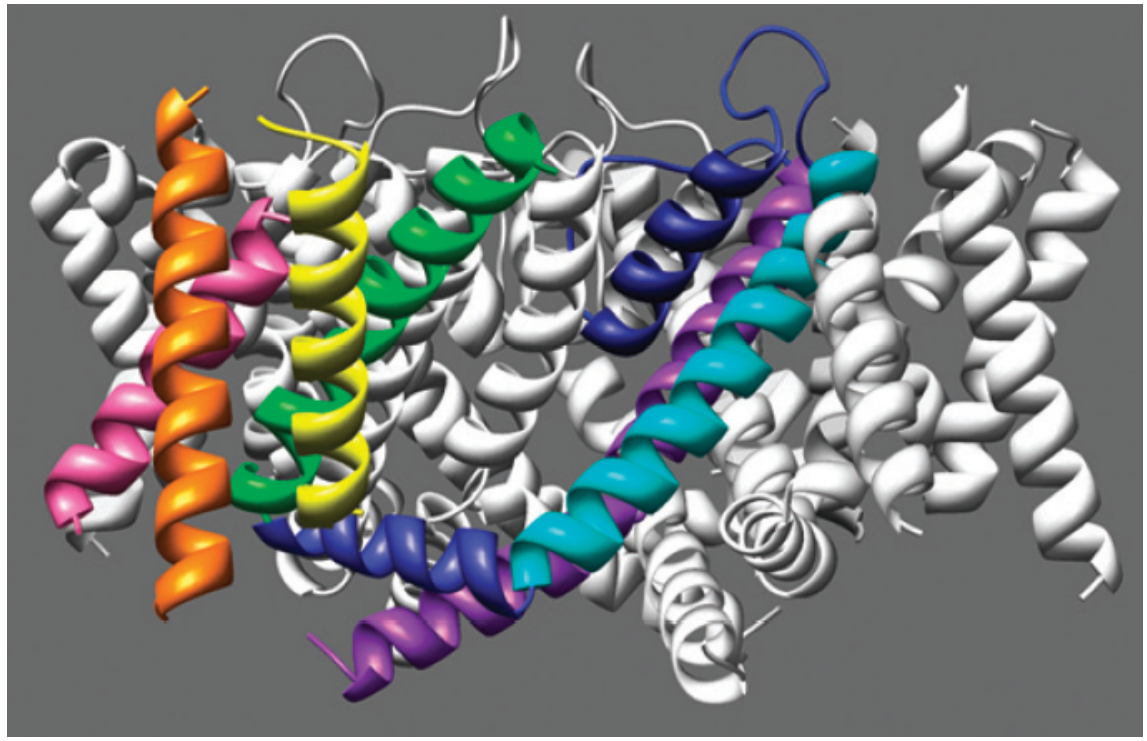
Closed → Open → Inactivated

Structure of voltage-gated K channels

one subunit



four subunits



Kv1.2 channel structure

Long et al (2005) Science 309:867

LQT2

- Inherited mutations in hERG1 (*aka KCNH2*)

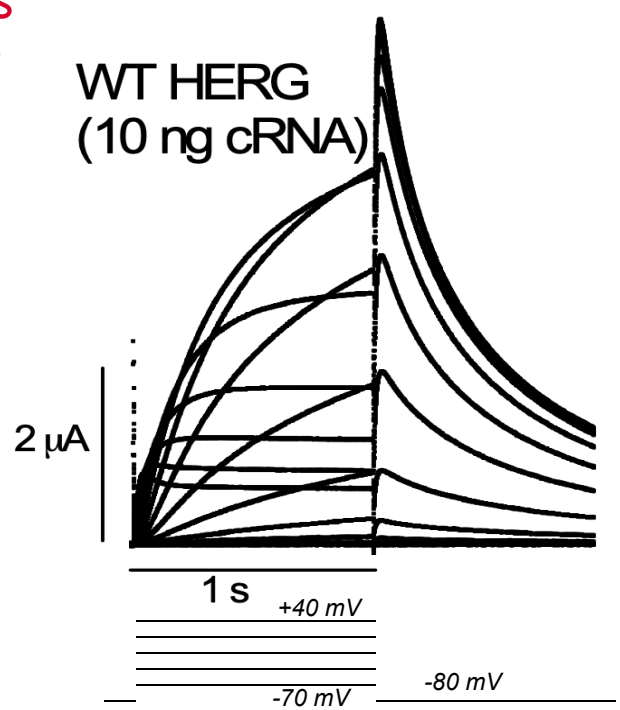
- Example: pore helix point mutations in hERG

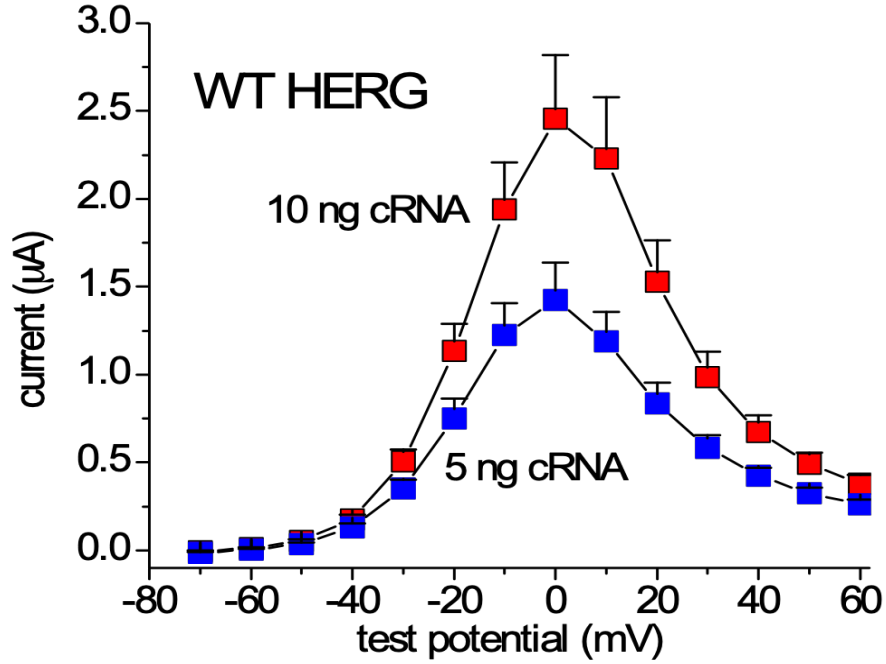
  Y611H, V612L, T613M, A614V, L615V

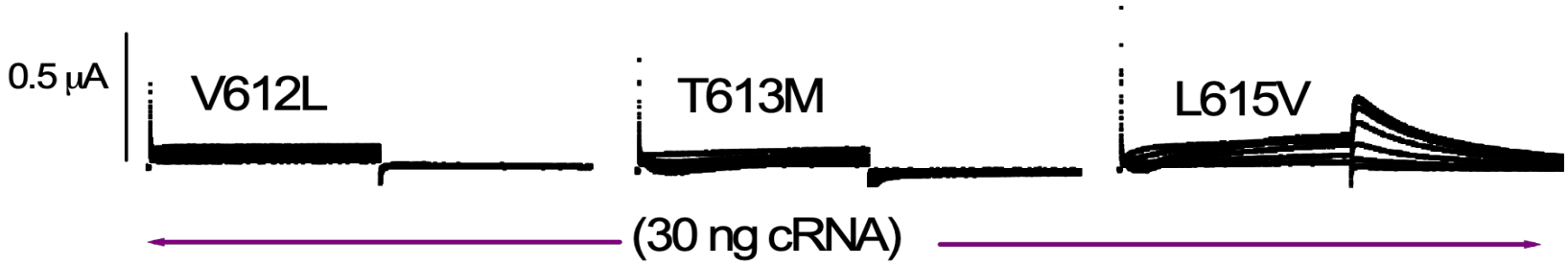
Inherited long QT syndrome

Example: hERG pore helix mutations

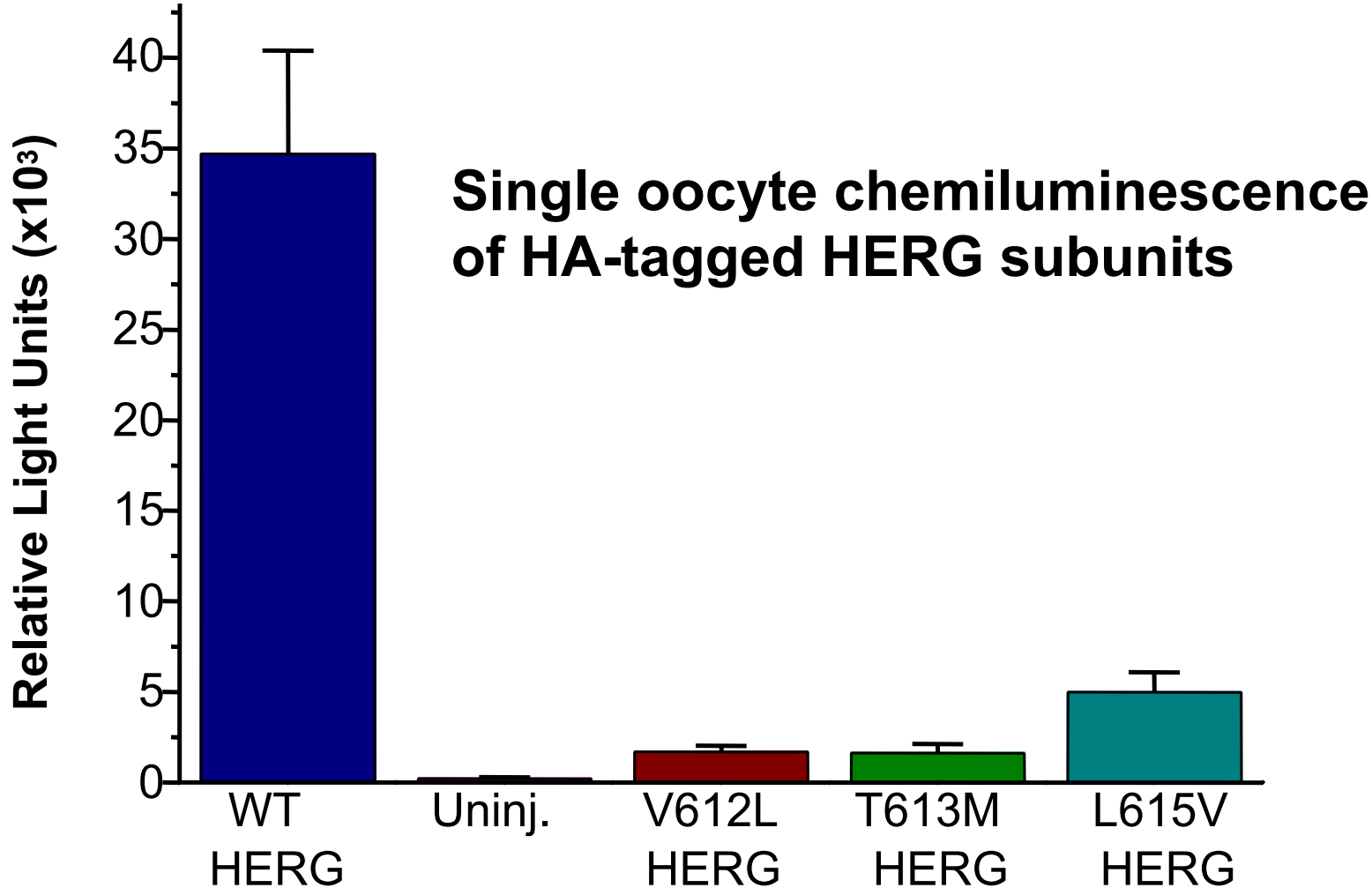
Xenopus oocytes



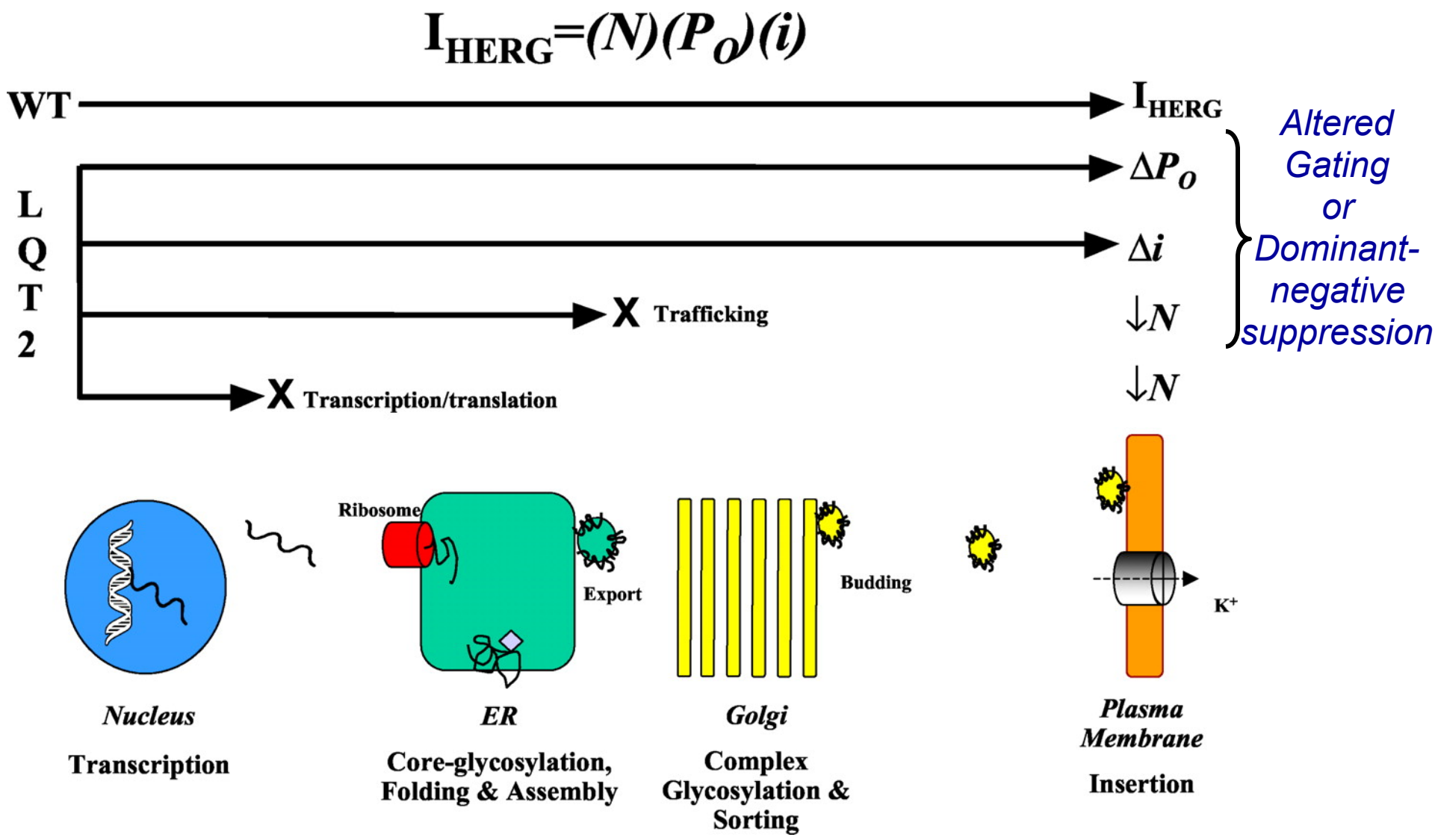




hERG pore helix mutations disrupt trafficking of channels to plasma membrane

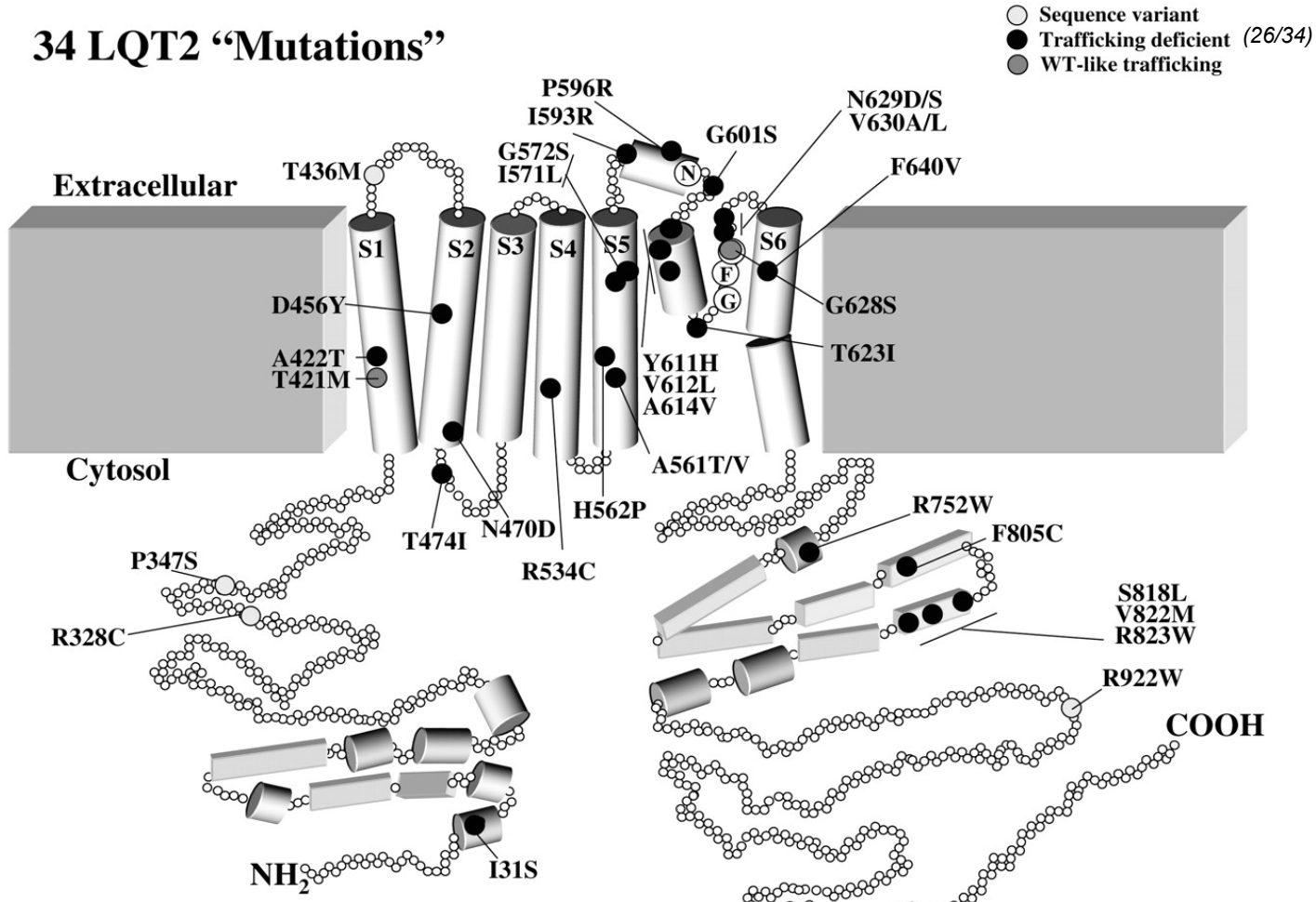


Molecular consequences of hERG mutations



Delisle, et al (2004) *Circ Res*

Most LQTS missense mutations in hERG cause defects in trafficking



Anderson et al (2006)
Circ. 113:365-373

Acquired Long QT syndrome
(drug-induced QT prolongation
& torsades de pointes arrhythmia)

in clinical practice:

Drug-induced torsades de pointes

- >100,000,000 prescriptions before risk of TdP and sudden death fully recognized.

- “Almost” all cases in the FDA database have identifiable risk factors (interacting drugs, overdose, liver disease)

Dan Roden

hERG ion channel

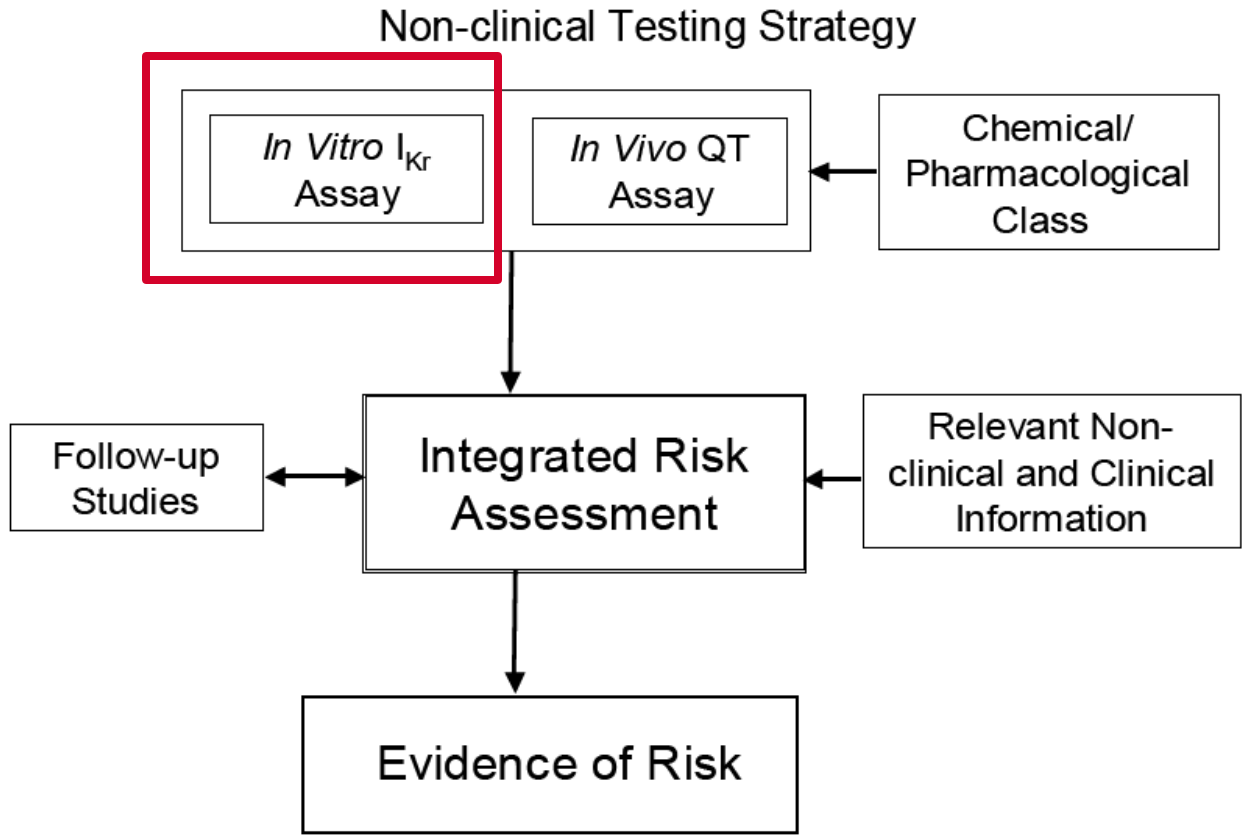
ICH

U.S. Food and Drug Administration
CENTER FOR BIOLOGICS EVALUATION AND RESEARCH

European Medicines Agency

Guidance for Industry

S7B Nonclinical Evaluation of the Potential for Delayed Ventricular Repolarization (QT Interval Prolongation) by Human Pharmaceuticals



Screening strategies

- **High throughput** (>100 compounds/day):
- **ligand binding assay**
  - Stably transfected cells (HEK293, CHO)
  - Radiolabeled dofetilide, astemizole, MK-499 or equivalent
  - Correlates reasonably well with electrophysiology
  - may not detect compounds that bind to atypical site(s)

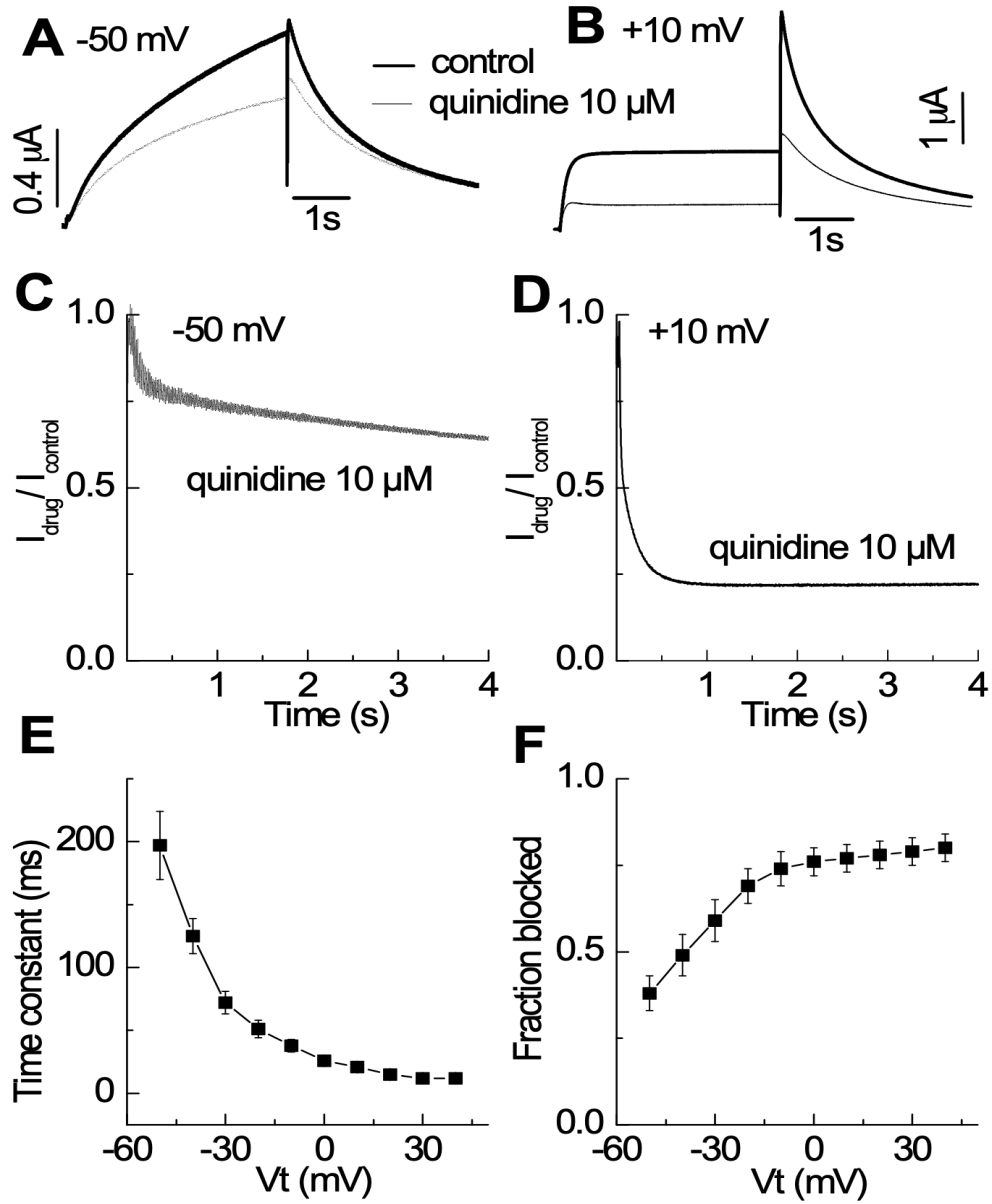
Screening strategies

- **Moderate throughput** (~10 compounds/day): **automated patch clamp**
  - PatchXpress (Mol Devices), Patchliner (Nanion), QPatch HT (Sophion)
  - can detect block due to binding at any site
  - Can detect voltage-dependent block
  - correlates with QT prolongation; but not necessarily so
    - (e.g., verapamil: Multi-channel block)
  - Interference with channel trafficking not detectable
  - Performed at hit to lead or lead optimization phase of drug discovery

48 channel

16 channel

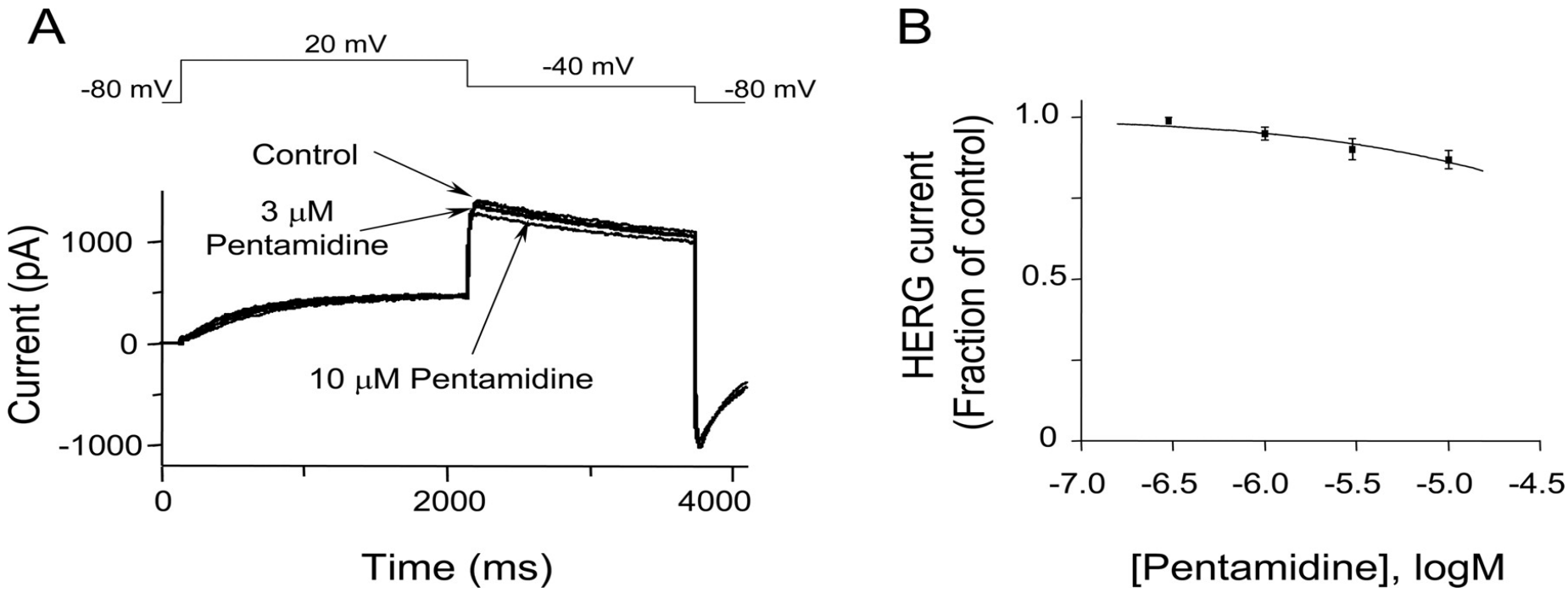
Block of hERG can be voltage-dependent



Sanchez-Chapula et al

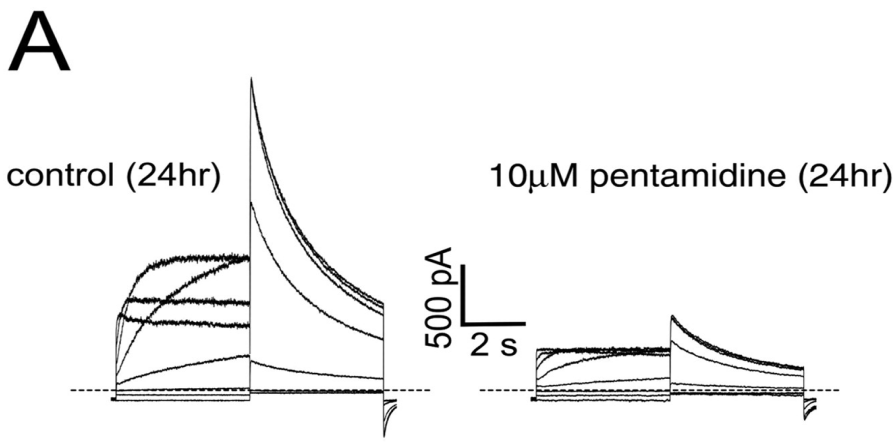
Some drugs do not block hERG channels, but decrease I_{Kr} over several days by inhibiting protein trafficking

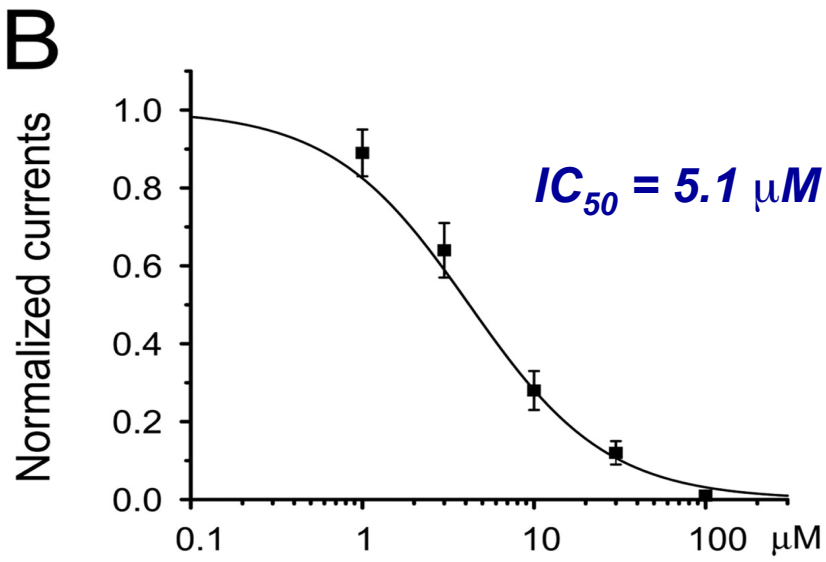
Inhibition of hERG trafficking
example: pentamidine

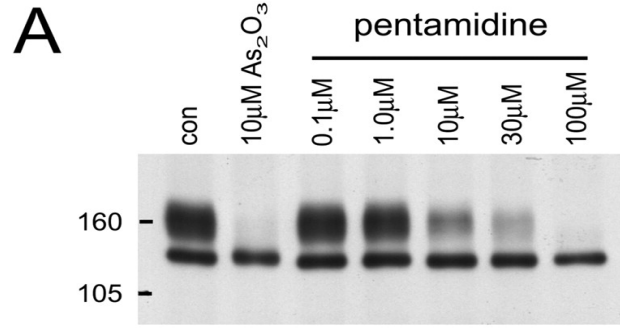


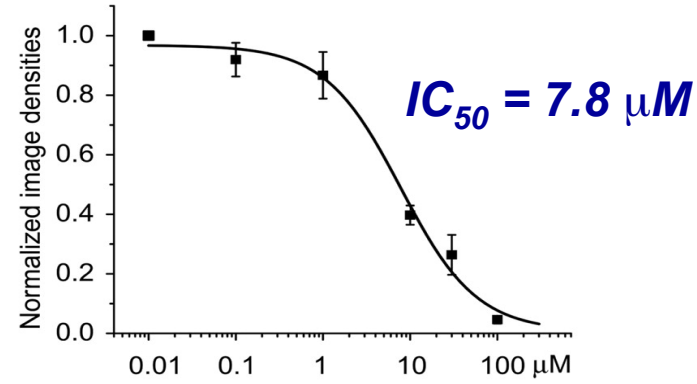
Kuryshev et al., 2005. JPET 312:316-323

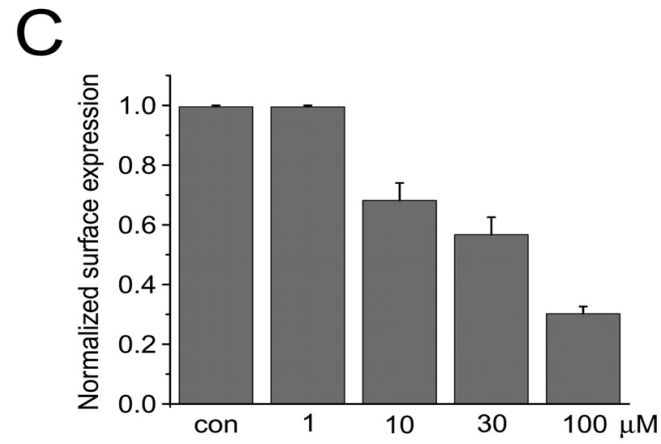
Inhibition of hERG trafficking











Kuryshev et al., 2005. JPET 312:316-323

Screening strategies (continued)

- **Low Throughput**: high cost; performed at candidate selection phase of drug discovery
- **Standard patch clamp assay**
  - Advantage: flexibility over automated patch clamp assay
- **Langendorff-perfused isolated heart preparation**
  - Monophasic action potentials
  - TRIaD (Hondeghem assay)
  - Advantage: monitors pro-arrhythmic activity other than prolonged APD
- **Action potential recordings of isolated tissues**
  - Usually dog Purkinje fibers
  - Advantages: more precise measurement of AP configuration
- **In vivo QT measurement**
  - Usually dogs (also rabbit-methoxamine sensitized, guinea pig, pig)
  - Conscious preferred over anesthetized animals
  - Advantages: other CV measures can be monitored at same time

Screening strategies (continued)

- Cardiac safety margin determination
  - IC_{50} receptor/IC_{50} hERG ~ 30 to 100
  - hERG block is common (30-60% of NCEs test positive within 30-fold criteria)
  - IC_{50} hERG varies widely
    - Low nM: sertindole, terfenadine
    - High μM: grepafloxacin, erythromycin

Drug-induced QT prolongation:
many therapeutic classes

- **ANTIARRYTHMICS:** **ajmaline, almokalant, amiodarone, aprindine, bretylium, dofetilide, ibutilide, procainamide, propafenone, quinidine, sematilide, sotalol**
- **CNS DRUGS: amitryptiline, chlorpromazine, desipramine, haloperidol, pimozide, sertindole, levomethadyl**
- **ANTIMICROBIALS/ANTIMALARIALS: amantadine, clarythromycin, chloroquine, erythromycin, halofantrine, quinine, sparfloxacine, grepafloxacin**
- **ANTIHISTAMINES: dephenhydramine, ebastine, hydroxyzine, loratadine, terfenadine, astemizole**
- **OTHER: budipine, ketanserine, probucol, cisapride, droperidol, terodiline, lidoflazine**

withdrawn from market, or use restricted

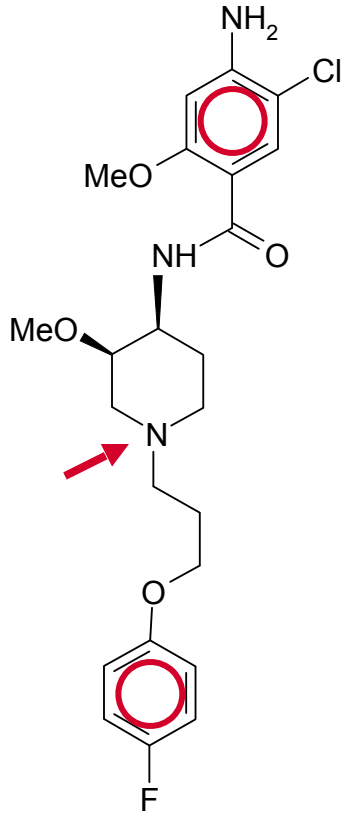
Pharmacophore models of hERG blockers

Pharmacophore/QSAR models

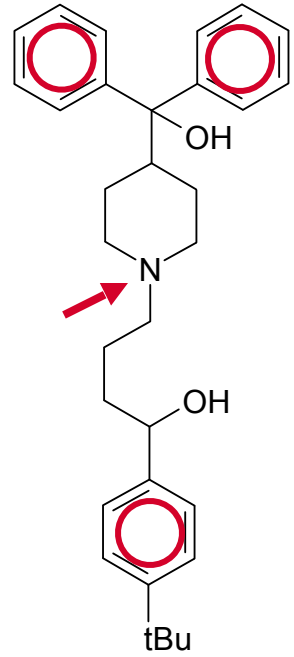
(In silico modeling using a ligand-based approach)

- 3D QSAR methodologies
  - CoMFA: comparative molecular field analysis
  - Catalyst (Molecular Simulations): does not require manual alignment of molecules
  - CoMSiA: comparative molecular similarity analysis
- Key pharmacophoric regions: several hydrophobes and one ionizable center
- Predictive value is limited, unless restricted to a limited chemical series

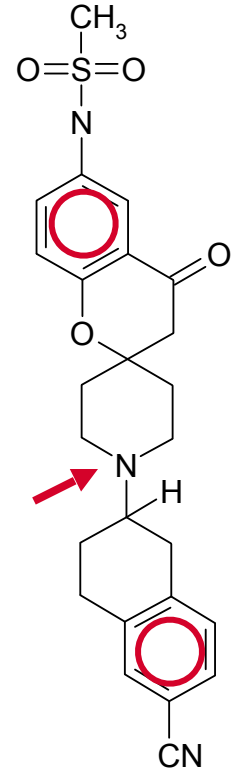
Structural diversity of hERG blockers



cisapride



terfenadine

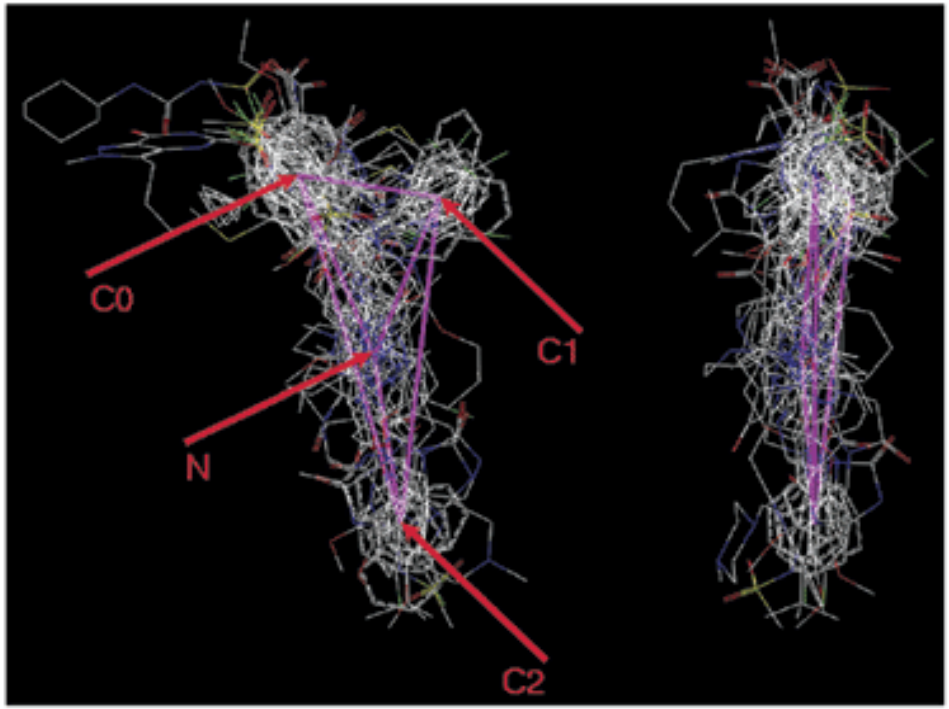


MK-499

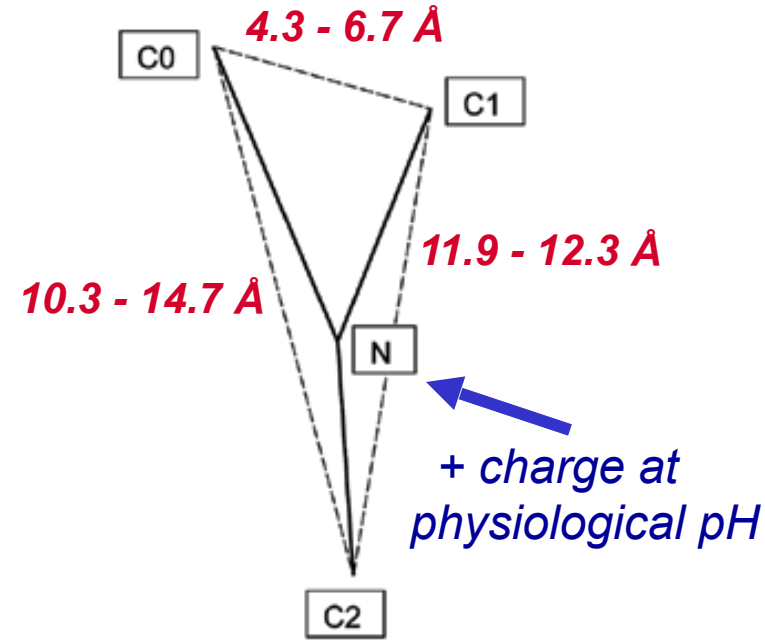
***potent* blockers have basic N …. and aromatic rings**

Pharmacophore model of hERG blockers

Cavalli, et al.(2002) J Med Chem. 45:3844



31 hERG blockers superimposed



Pharmacophore frame

N = Nitrogen atom
C0, C1, C2 = centroids (centers of mass)

CoMFA

(comparative molecular field analysis)

contour maps
shown in relation to
pharmacophore frame

Cavalli, et al.(2002)
J Med Chem. 45:3844

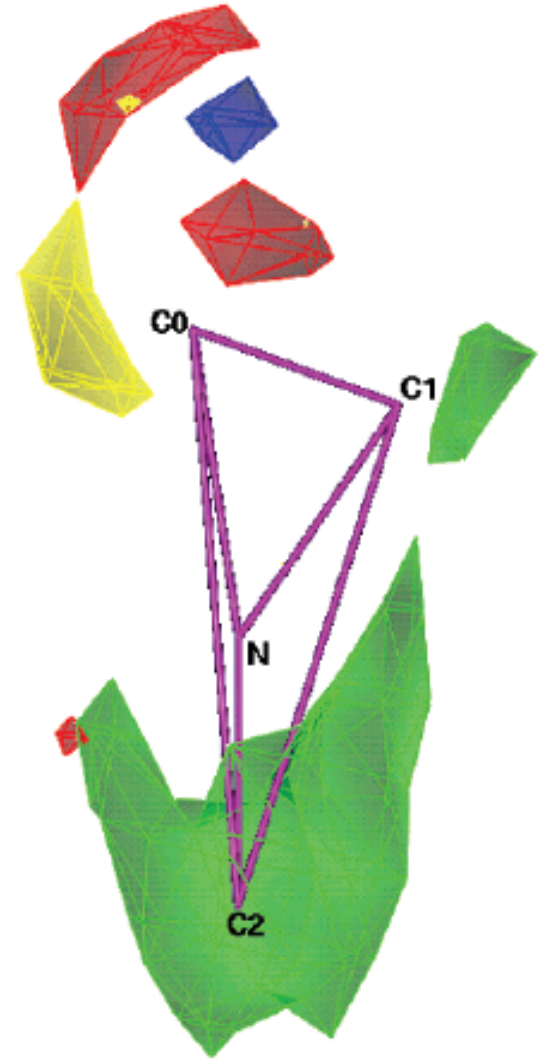
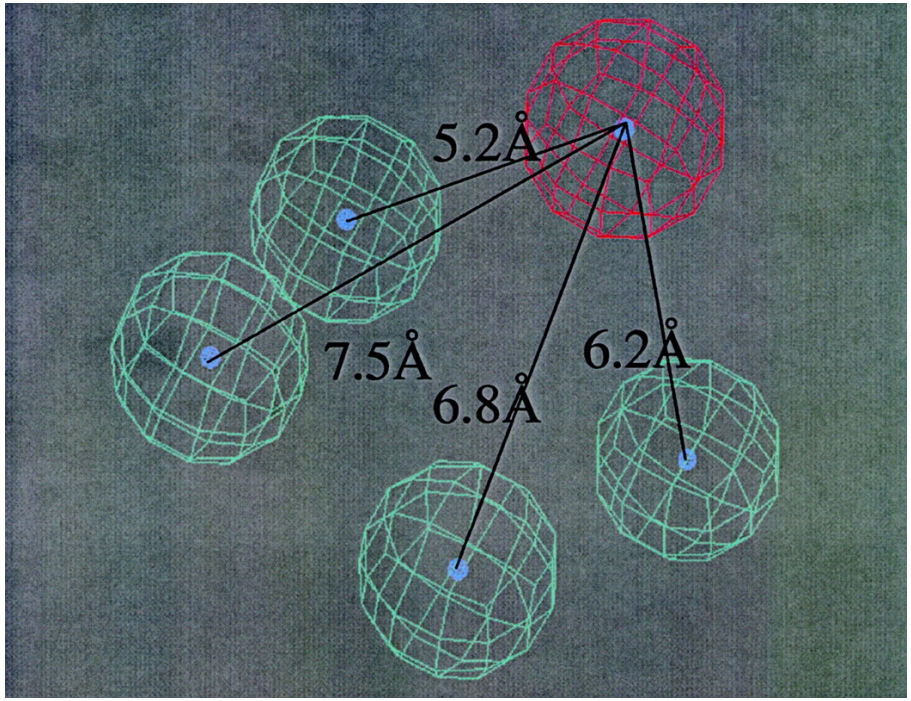


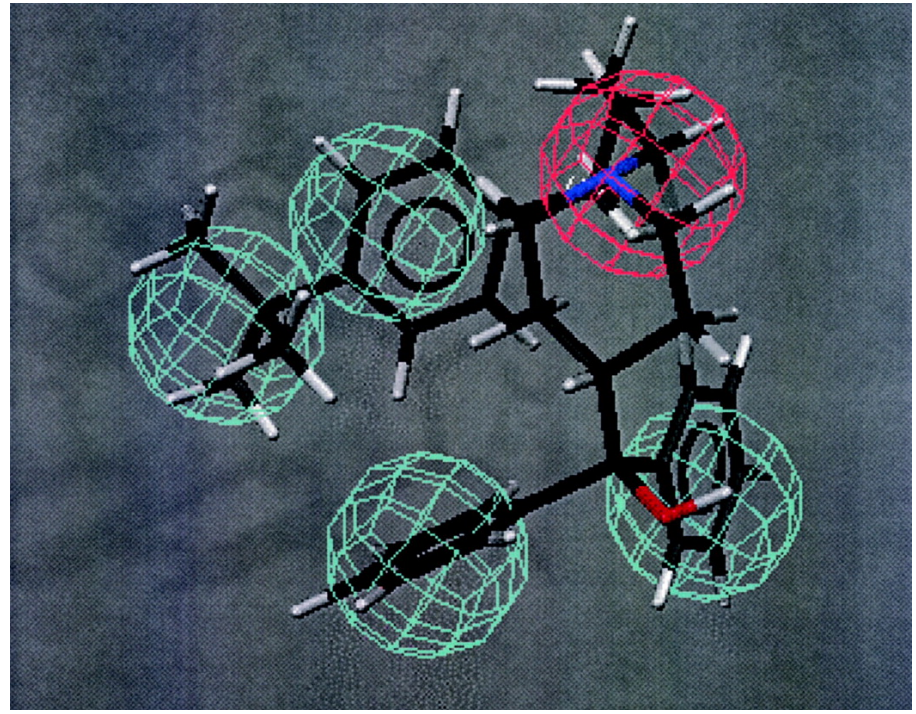
Figure 2. View of the steric and electrostatic CoMFA STDEV*COEFF contour maps. The regions where increasing the molecules' volume increases HERG K^+ channel blocking activity are green (0.028 level), and the region where increasing the volume decreases activity is yellow (−0.022 level). The electrostatic contours indicate an increase of activity with increasing positive (red, 0.010 level) and negative (blue, −0.012 level) charge, respectively. The pharmacophoric frame is shown for reference.

Catalyst-derived pharmacophore model

Ekins et al (2002) JPET 301:427



Catalyst General hERG pharmacophore generated with **15** molecules, showing hydrophobic (cyan) and positive ionizable features (red).



Terfenadine fitted to model

observed IC_{50} = 0.213 μM

predicted IC_{50} = 0.023 μM

CoMSiA

(comparative molecular similarity analysis)

Pearlstein et al (2003) Bioorg Med Chem Lett 13: 1829

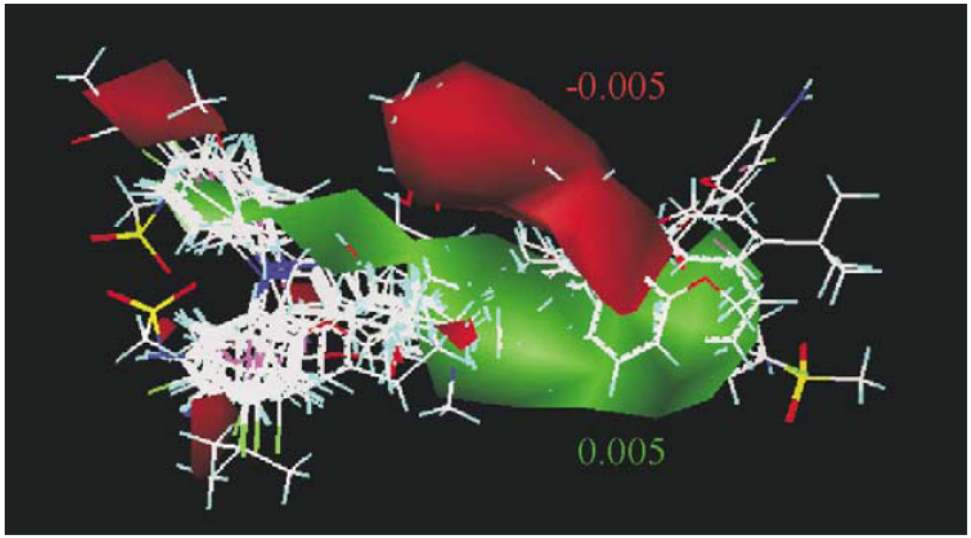


Figure 4. Superposition of the studied compounds showing the steric coefficients (coefficient values = ±0.005) from the CoMSiA. The contours of coefficients corresponding to the red region suggest desirable substitution sites aimed at decreasing HERG affinity.

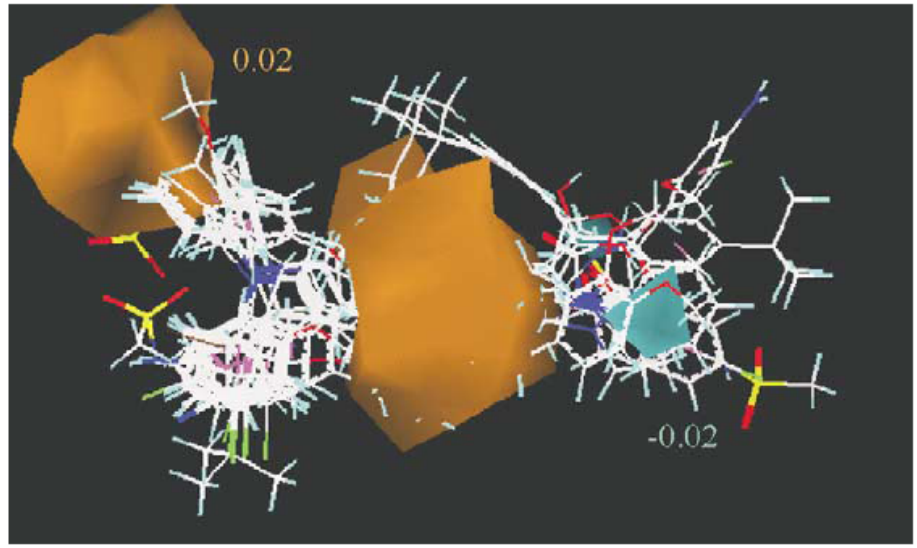


Figure 5. Superposition of the studied compounds showing the electrostatic contours of coefficients (coefficient values = ±0.02) from the CoMSiA. Modifications aimed at increasing the negative charge in the orange regions and the positive charge in the cyan region are predicted to decrease HERG affinity.

Determinants of hERG channel binding

Drug-induced long QT syndrome

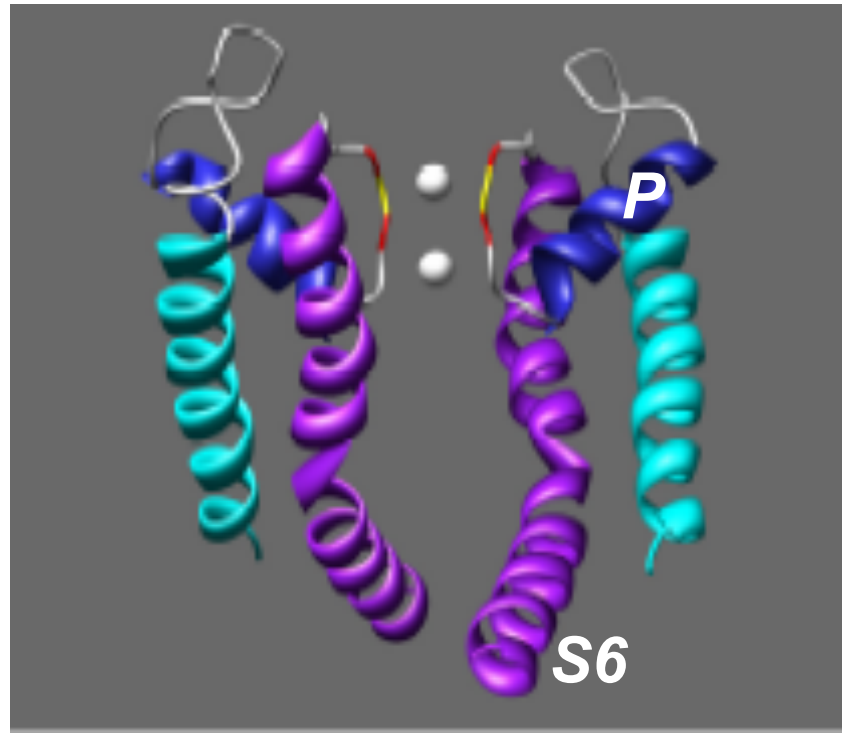
Clinical cases caused by block of
I_{Kr} (hERG), *not* other K channels

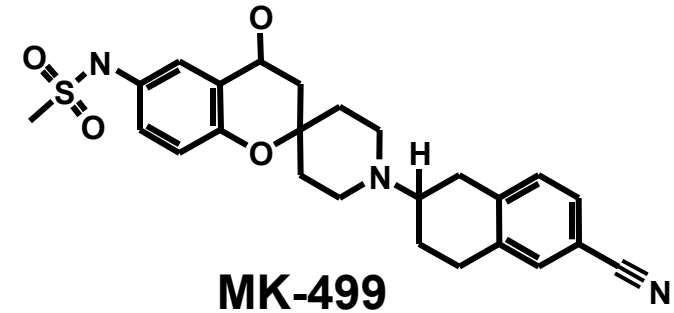
Obvious questions:

1) Structural basis of drug binding site?

2) Are features of the site unique to hERG?

Ala-scanning mutagenesis of residues near inner pore





MK-499

IC_{50} = 34 nM

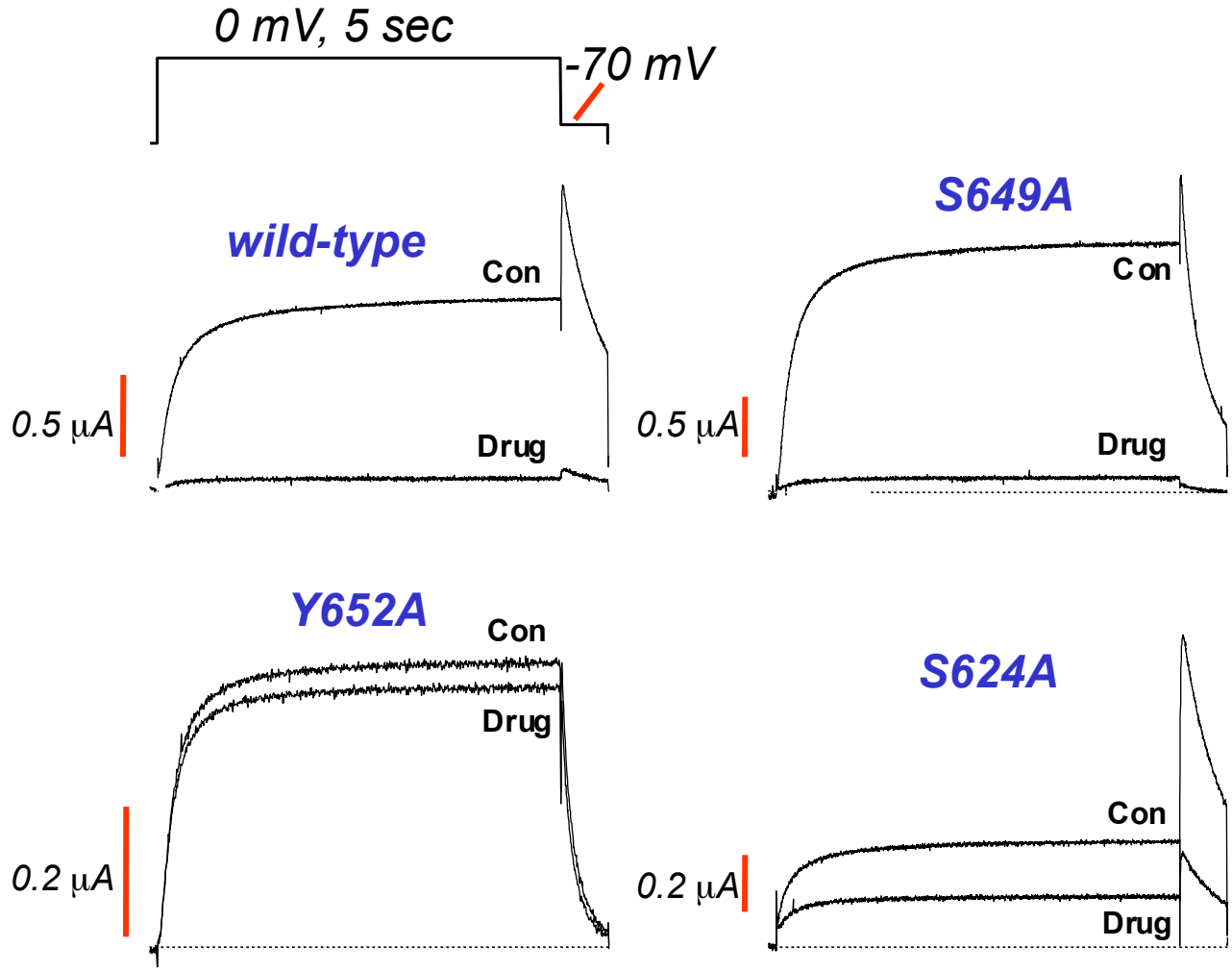
P S6

KcsA TYPRALWWSVETATTVGYGDLYPVTLWGRLVAVVVMVAGITSFGLVTAALATWFVGRE

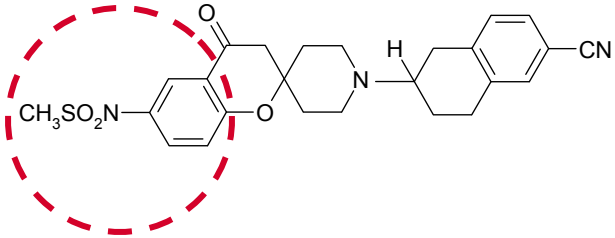
hERG KYVTALYFTFSSLTSVGFGNVSPNTNSEKIFSICVMLIGSLMYASIFGNVSAIIQRLY

Ala substitution

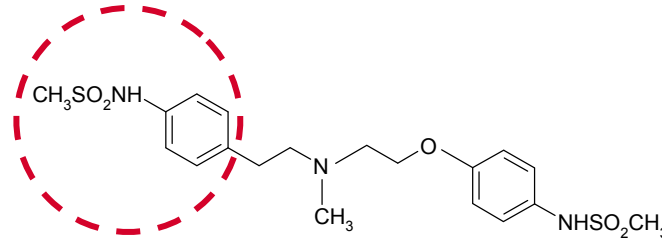
Variable sensitivity of mutated hERG channels to block by 0.3 μM MK-499



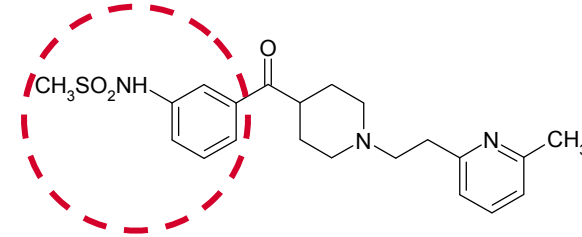
Ala scan: methanesulfonanilides



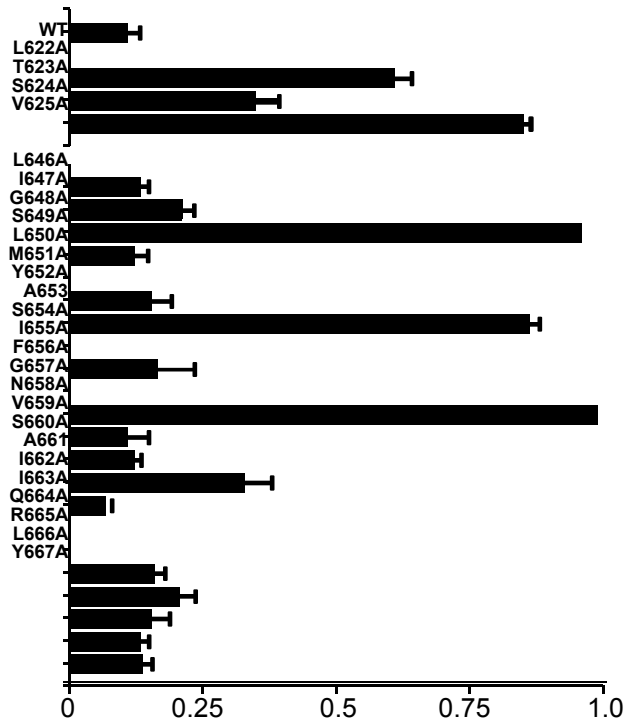
MK-499

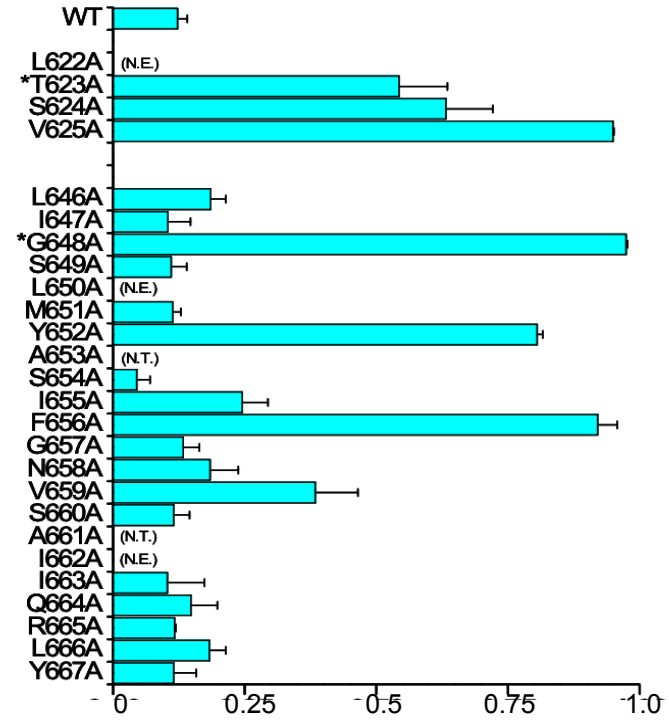


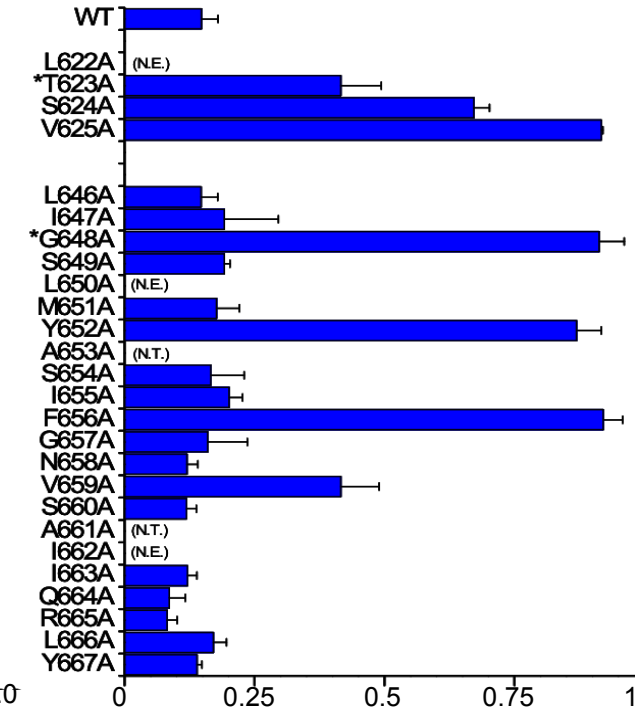
Dofetilide



E-4031



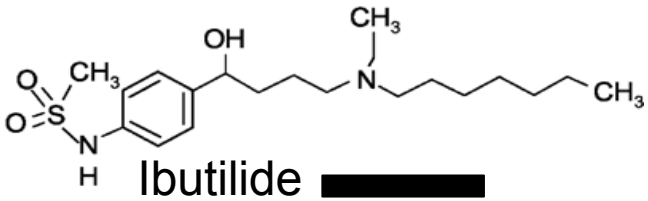




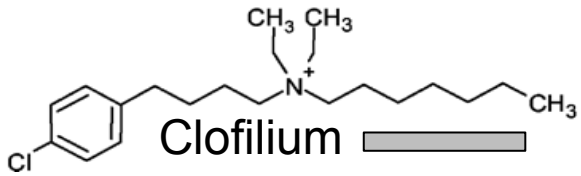
normalized current @ 0 mV ($I_{drug}/I_{control}$)

Kamiya et al, 2006 Mol Pharm

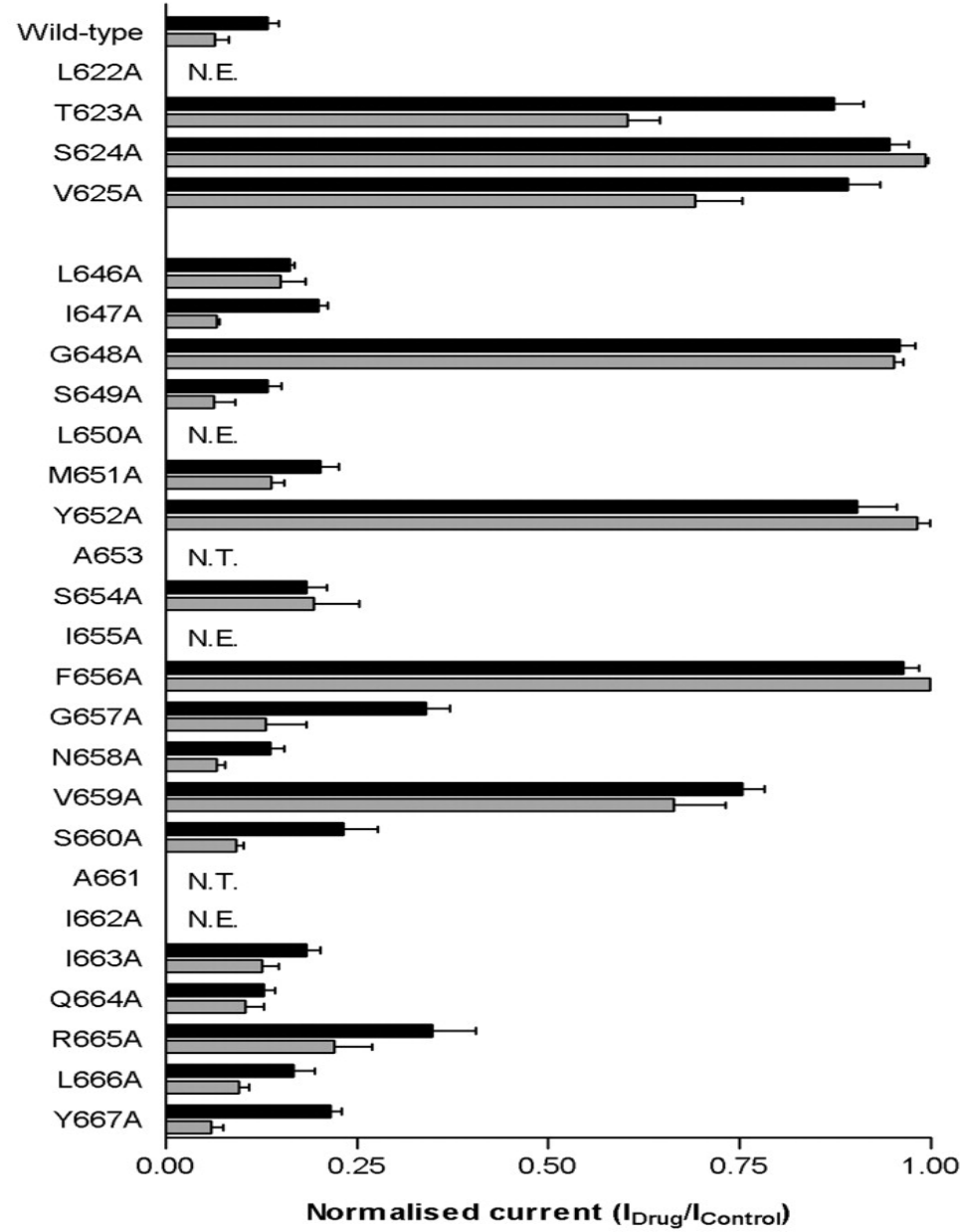
Ala scan: ibutilide and clofilium



Ibutilide

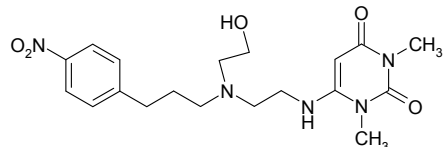


Clofilium

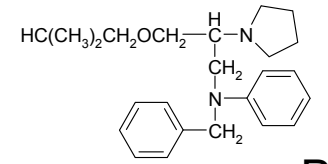


Perry et al, 2005 Mol Pharm

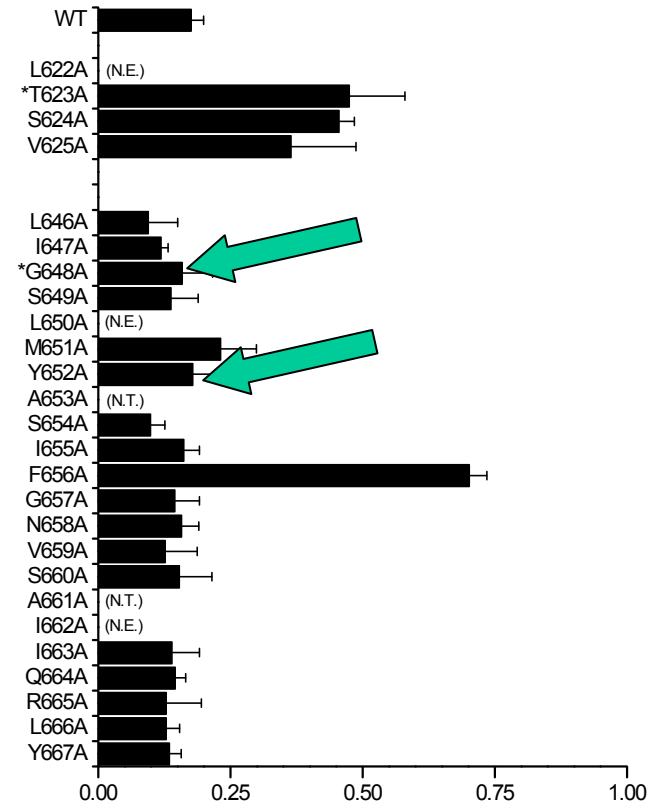
Ala scan: nifekalant and bepridil



Nifekalant



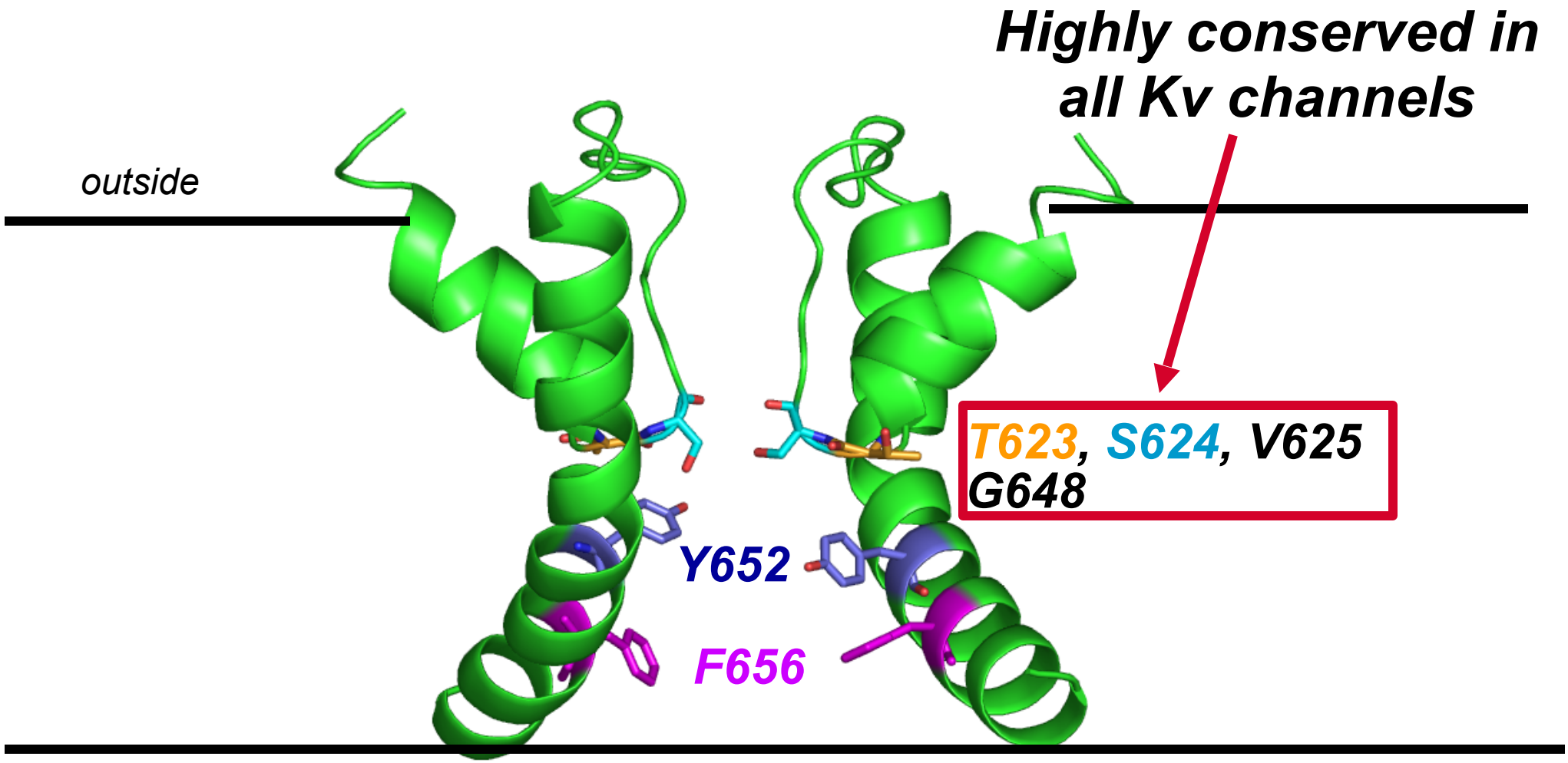
Bepridil



normalized current @ 0 mV ($I_{drug}/I_{control}$)

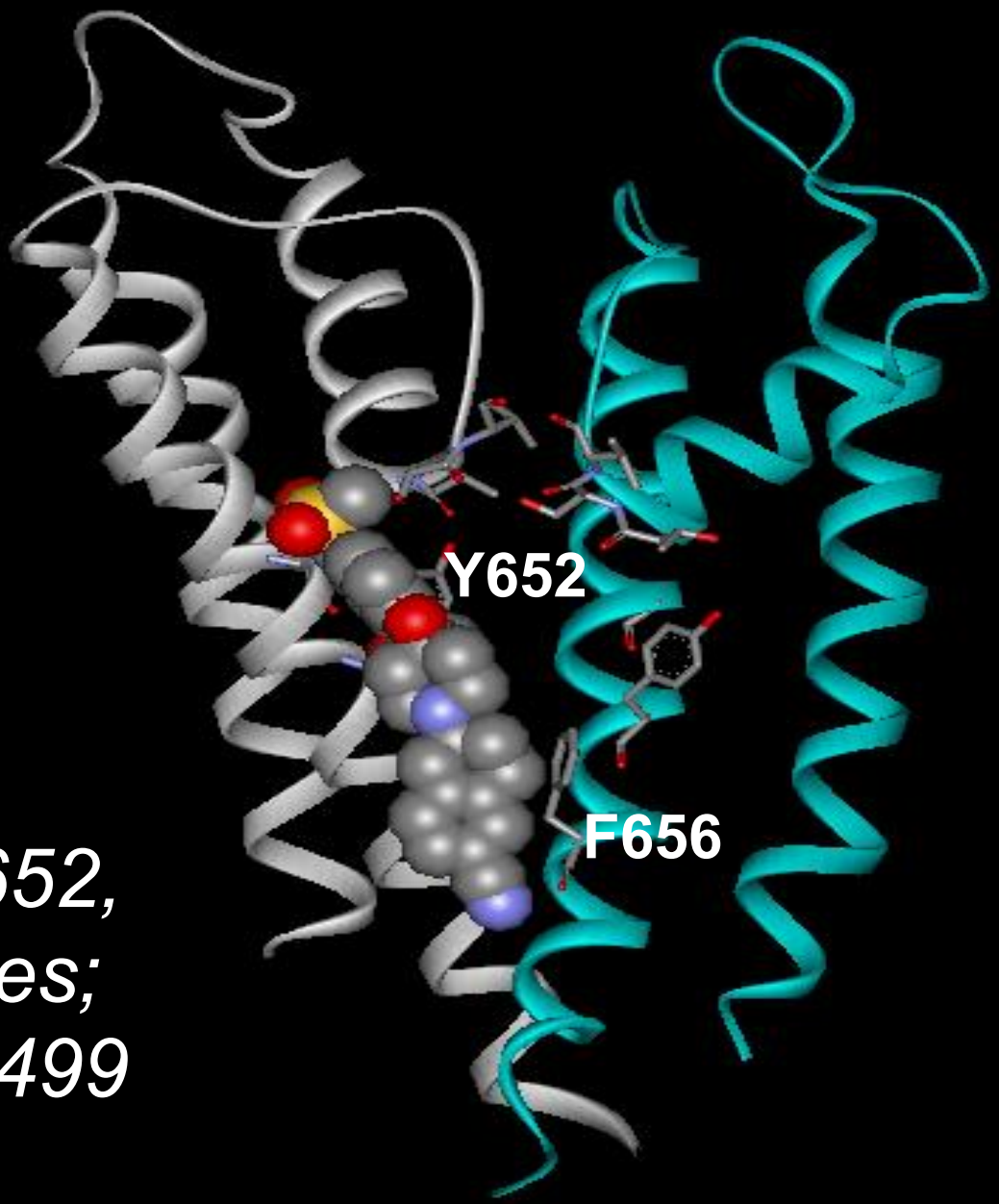
Kamiya et al (2006) Mol Pharm

hERG pore region



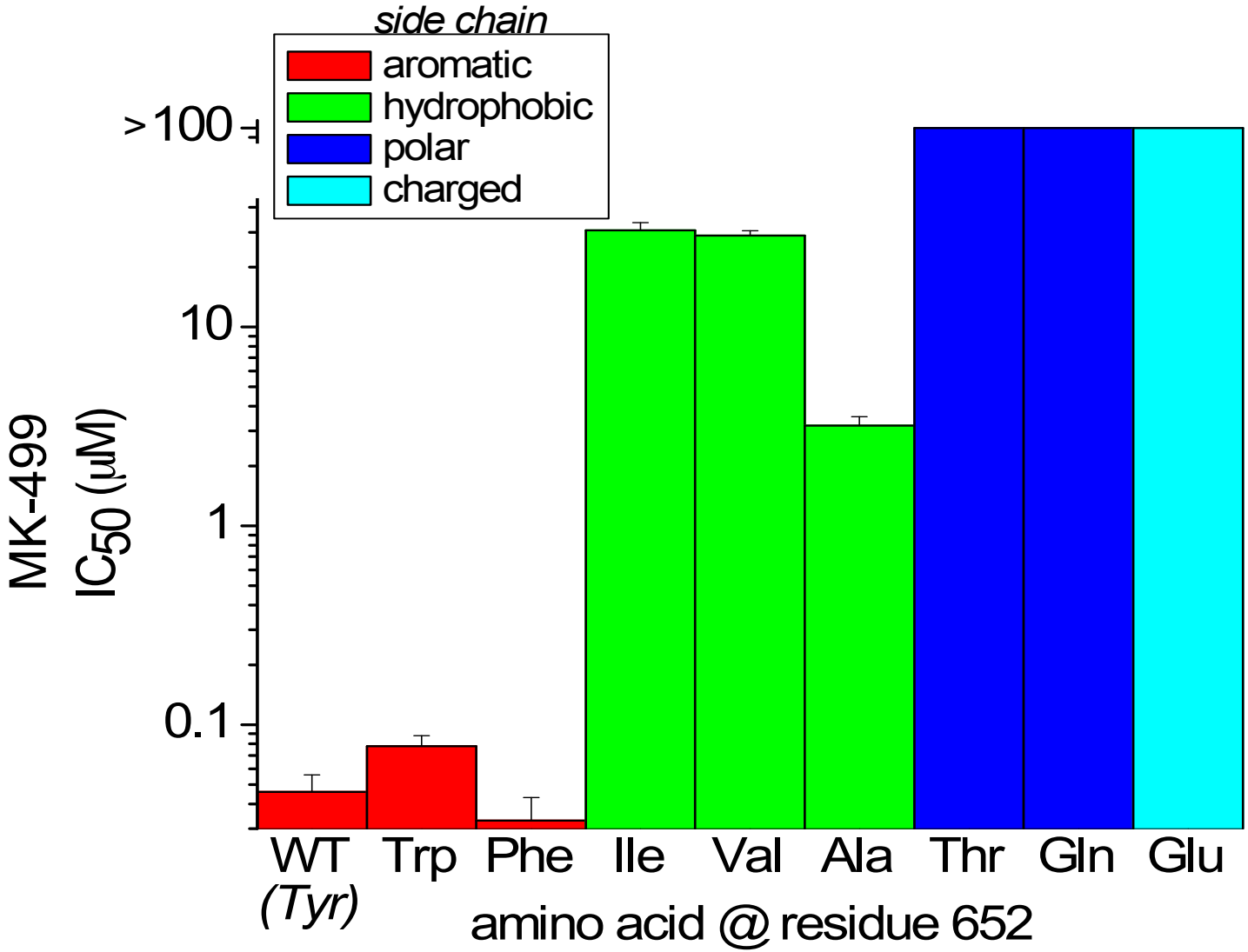
*Homology model based on **MthK** bacterial channel*

Are aromatic groups essential for high affinity block?



Approach: mutate Y652, F656 to other residues; determine IC_{50} of MK499

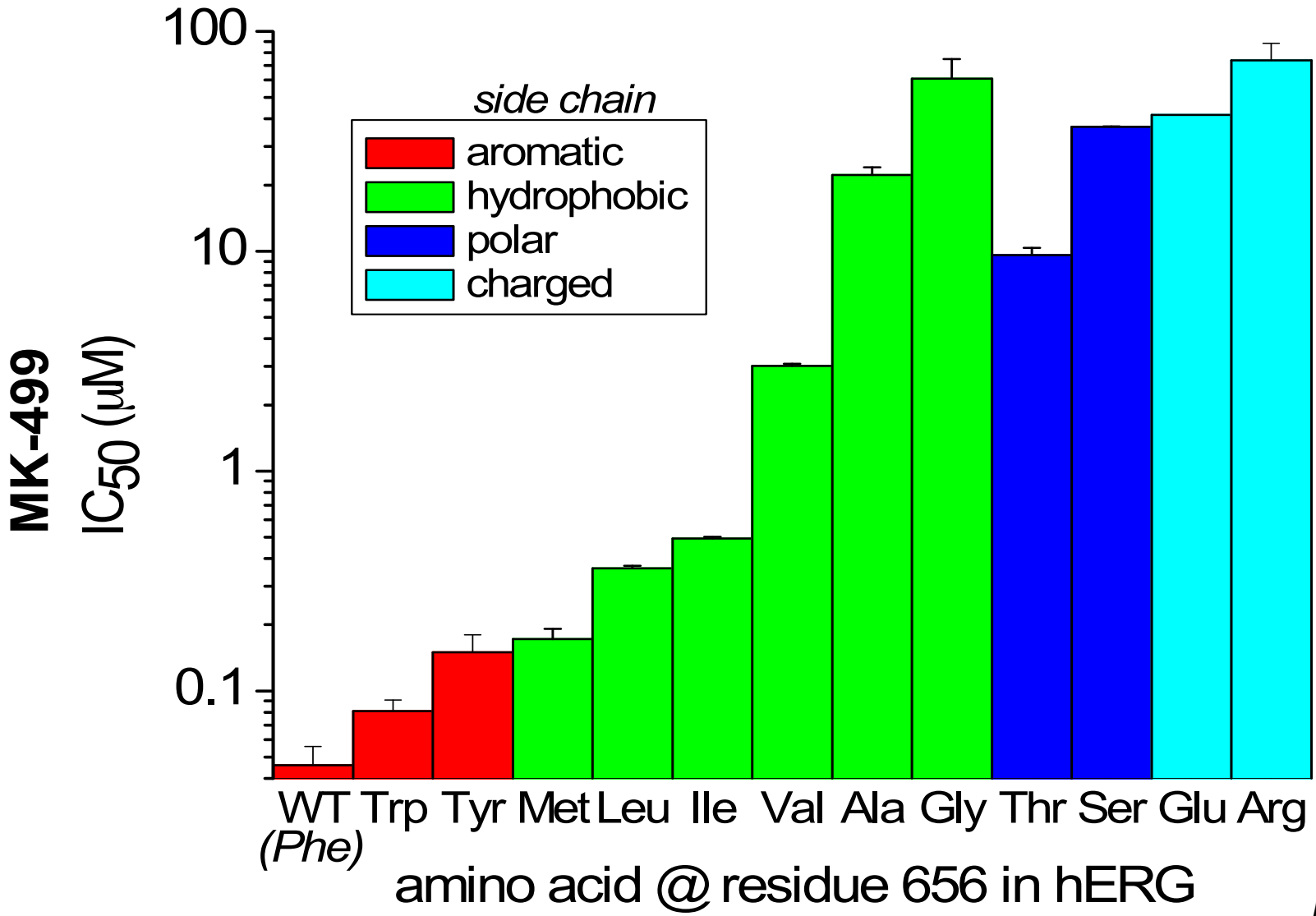
Residue 652: Aromatic aa required for block



similar results for cisapride, terfenadine

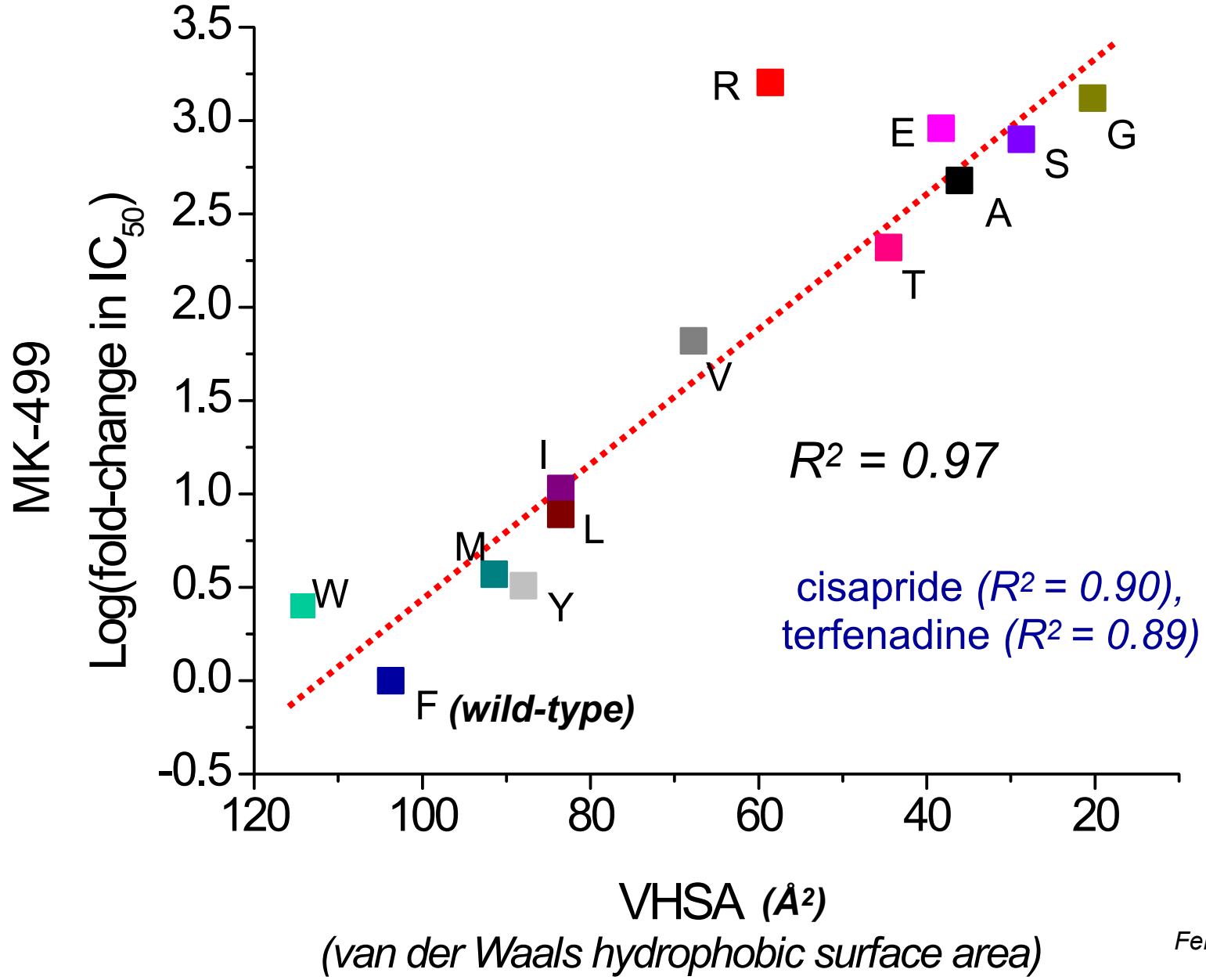
Fernandez et al (2004) J Biol Chem

Residue 656: hydrophobic aa required for block



Fernandez et al (2004)
J Biol Chem

hydrophobic residue at position 656 favors block



Fernandez et al (2004)
J Biol Chem

Strategies to reduce hERG block

(Jamieson et al 2006 J. Med Chem.)

(Organon Labs)

- Four strategies identified from literature review:
  - discrete structural modifications
  - control of log*P*
  - formation of zwitterions
  - control of p*K*a
- For clog*P* < 3.0, make discrete changes to structure
- For clog*P* > 3.0, attempt to reduce logP and establish correlation (*R*) between logP and hERG block within chem series (on average, 1 log reduction in clogP leads to 0.8 log unit reduction in hERG block)

Summary

- Loss of function mutations in hERG channels cause LQT2
- Drugs from diverse chemical & therapeutic classes prolong QTc
- In clinical practice, drug-induced QT prolongation and TdP is caused by block of hERG channels

- Most important feature of binding site for hERG *blockers* are aromatic residues in S6 domain (Tyr652 and Phe656)
- hERG pharmacophore and receptor models facilitate *in silico* screening of new compounds for assessment of potential arrhythmia risk
- Synthetic strategies to avoid hERG block are available, but limited, and best suited to discrete chemical series